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TITLE: A Gut-Restricted Small-Molecule Therapeutic for a Novel Target to Treat Inflammatory Bowel Disease

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14. ABSTRACT The purpose of this proposal is the creation of an intestinal epithelial targeted small molecule inhibitor of PAI-1 for moderate to severe inflammatory bowel disease patients. The rationale for this molecular target is that PAI-1 is elevated in areas of mucosal injury in patients with both major forms of inflammatory bowel disease (IBD), Crohn's disease and ulcerative colitis. Our predictive algorithms supported a driver role for PAI-1 in maintaining dysregulated epithelium and immune activity. Our preclinical work supported this model and further suggested the cellular target of excess PAI-1 was wound associated epithelial (WAE) cells; excess PAI-1 inhibits tPA which is required for WAE cell migration across wound beds. Thus, a program aimed to inhibit PAI-1 would promote tissue repair in IBD. We have designed small molecules to inhibit PAI-1 and target the gut epithelium by oral delivery. This project was designed to choose a lead compound and perform the pre-IND studies for it. We entered this program with a lead compound in hand, CCF104. Our initial studies evaluating toxicity and PK in rodents are supportive of continuing to move forward with this compound into year 2. We also have produced 90 additional analogue compounds to screen for a backup PAI-1inhibitor. We have performed binding assays and we are currently evaluating their activity in an in vitro tPA activity assay. Based on these findings, our goal is to evaluate top candidates based on binding activity for in vivo activity testing using in vivo injury models. This activity we expect will produce at least one backup compound for this program.					
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1. INTRODUCTION: A group of chronic intestinal diseases characterized by inflammation of the large and small intestine known as inflammatory bowel disease (IBD) affects over 3.1 million of the population and is not rare entity among soldiers and can decrease mission capability along with unit readiness and cohesion. Patients with IBD often require intensive and expensive medical interventions. Current medical treatment approaches, dominated by biologic agents, have failed to maintain the disappearance of the symptoms of IBD for approximately 40% of the patients. Furthermore, all the above biologic medicines are administered by injection (IV or subcutaneously), which limits accessibility and medication adherence. Recently approved Janus kinase (JAK) inhibitors that can be taken by mouth are exciting small molecule therapeutics in IBD, however, they carry safety concerns for coagulation/clotting of the blood, limiting the potential for the entire class. We have developed PAI-1 as a novel target for IBD therapeutics. We have created an inhibitor compound (CCF104) that is gut restricted and showed efficacy in chemical-induced animal models of IBD. We plan to perform pre-IND in vitro and vivo toxicity studies of our lead candidate as well as to identify and develop a back-up compound to our current lead. The role of our novel therapy would provide an oral therapeutic option with a favorable safety profile and efficacy that improves individual and unit readiness. This proposal aligns with DOD areas of research interest (inflammatory bowel disease research strategies that target epithelial health and function, including patients who are refractory to standard care) and also useful in a dual-capacity to benefit civilian population and also address a military need. On the overall, with a minimal side effect profile and gut-restricted efficacy, service members would be able to maintain deployability and be retained on active duty service; overall improvement in medical readiness.

2. KEYWORDS:

Inflammatory bowel disease
CCF104
Genotoxicity
Cardiac toxicity
Scale-up
Plasma protein binding
Off-target
PK studies
ADME

3. ACCOMPLISHMENTS:

What were the major goals of the project?

- i) Perform preclinical development studies of CCF104, our current lead
- ii) Further pharmacological assessment of CCF104, and
- iii) Identification of a second-generation PAI-1 inhibitor (backup candidate to CCF104)

What was accomplished under these goals?

Specific Aim 1: Preclinical development studies of CCF104

Major task 1 Scale up of CCF104 to evaluate drug-drug interactions and identify metabolites
Subtask 1. ACURO approval of protocol number PR211463.e001(1-3 months). Achieved (letter Feb 2023).

Subtask 3. Scale up of lead candidate CCF104 (1-3 months). Achieved.

Optimize the synthetic route and synthesize 100 grams of GLP material for preclinical testing: Initially, we optimized the synthetic procedure for CCF104 scale-up. To date, we synthesized ~75 grams of CCF-104 sodium salt, which involves 4 steps, and ~20 grams of parent CCF-104, which involves 3 steps. Both these compounds were synthesized with a >98% chemical purity. These compounds have been used for preclinical testing.

Subtask 4. Assessing the drug-drug interactions of CCF104 (3-4 months). Achieved.

Obtain human cytochrome P450 inhibition data for assessing the potential drug-drug interactions: Evaluate whether CCF104 is a substrate of any of these cytochromes. CCF104 was assessed for its capability to inhibit cytochrome p450 (CYP) enzymes. These isoforms include CYP1A2, CYP2A6, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, CYP2E1 and CYP3A4. These assays were done at eight different concentrations using appropriate positive controls (known inhibitors of a particular CYP isoform) for comparison. CCF104 showed IC₅₀s (the half-maximal concentration) >30 µM against all these CYP enzymes, except CYP2C8 and CYP2C9, whose IC₅₀s are 3.61 and 7.09 µM, respectively (**Appendix 1**)

Subtask 5. Stability of CCF104 in the presence of microsomes and hepatocytes and metabolite identification (3-6 months). Partially Achieved.

CCF104 was incubated with rat and human liver microsomes for 60 minutes and it showed half-life (t_{1/2}) of 210 min and >145 minutes respectively. The calculated intrinsic clearance is <0.96 and 11.6 µL/mL/mg for rat and human microsomes respectively. We plan to do metabolite identification once we are assured that CCF104 will be our likely IND candidate. This latter analysis is more costly and can wait until this decision has been made. We have budgeted to do this assay on one compound.

Subtask 6. Assess Plasma protein binding of CCF104 (3-4 months). Achieved.

Determine protein binding of rat, dog, non-human primate and human serum: Plasma protein binding (PPB) of a compound influences drug distribution in the body as well as the availability of the free compound to access the active sites in the cell. We assessed the PPB of CCF104 to various species, including rats, dogs, non-human primates, and humans. The unbound fraction is 0.2 to 0.5% and species are not significant (**Appendices 2 and 3**).

Major task 2. Evaluating CCF104 for potential in vitro geno-, cardio-, off-target and general toxicities

Subtask 1. Assess the potential cardio-toxicity of CCF104 (4-7 months). Achieved.

Evaluate CCF104 in hERG ion channel patch clamp assay and also in radio ligand binding assays for potassium, sodium and calcium channels for an initial evaluation of potential cardiac-toxicity: It

is important to assess the cardiac-toxicity potential of the test compound early in the drug discovery process. CCF104 was evaluated at a single concentration and % of inhibition was calculated against the core potassium, sodium, and calcium channels as well as in the multi-electrode assay (MEA) using human-derived pluripotent cardiomyocytes and appropriate positive controls. CCF104 was tested at 20 µg/mL (concentration was selected based on the solubility in the assay buffer) against HEK-hNav1.5, CHO-hERG, and, HEK-hCav1.2 channels and showed 4 -7% inhibition in these assays. CCF104 was tested in the multi-electrode assay at 200 µg/mL; the beat period of 19% change from the baseline, 24% change in the field potential duration, and a spike amplitude of -36% indicating that CCF104 has minimal cardio-toxicity in these assays. Later in the process, we will also evaluate this compound in the appropriate animal model. Four more analogs of this series were also tested in these assays and they also showed minimal potential for cardio-toxicity (**Appendix 5**).

*Subtask 2. Assess the potential geno-toxicity of CCF104 (4-7 months). **Achieved.***

Evaluate CCF104 for its potential geno-toxicity in the Ames test (with and without S9 activation) and in chromosomal aberration assay: The Ames test is a common, initial assay that uses bacteria to test if a compound can cause DNA mutations in a test organism. CCF104 was assessed in *Salmonella typhimurium* strains TA98, TA100, TA1535, and TA1537 in the presence and absence of an exogenous metabolic activation system (S9). At first, CCF was also investigated for bacterial cytotoxicity at eight different concentrations (100, 50, 25, 10, 5, 1.2, and 0.6 µM) and showed no cytotoxicity at these concentrations. CCF104 was tested at four different concentrations (100, 50, 10, and, 5 µM) in the Ames assay. The results indicate that CCF104 is not mutagenic at these concentrations. CCF104 was also assessed in in vitro micronucleus assay, to detect any chromosomal aberrations, using CHG-K1 cells at different concentrations (1000, 500, 250, 130, 62, 31, 16, and 8 µM) and didn't show any toxicity at or below 130 µM. CCF104 didn't show any chromosomal aberrations or damage below 130 µM. These results indicate that CCF104 has minimal genotoxicity potential (**Appendix 6**).

*Subtask 3. Evaluating CCF104 in CEREP panel of assays for any off-target activity as well as toxicity in primary intestinal epithelial cells (4-7 months). **Achieved.***

i) *Off-target activity studies:* In the drug discovery process, it is imperative to profile drug candidates against a broad range of targets so that safety-related attribution will be reduced in the development phase. We have evaluated CCF104 against a panel of 167 assays (SpectrumScreen Panel) at 10 µM concentration. The targets for these assays include several GPCRs, Chemokines, Ion channels, kinases, etc. CCF104 showed 54% and 62% inhibition against adenosine A3 receptor and γ-hydroxybutyric acid (GHB) receptor at 10 µM concentration and less than <50% inhibition against all other targets. These results indicate a very low potential for off-target activities for CCF104 (**Appendix 7**).

ii) *General Toxicities. **Achieved.***

In vitro pharmacology study: CCF104 in various concentrations, with a top concentration of 30 µM, was tested in cytotoxicity assays using human foreskin fibroblasts (BJ), rat hepatoma cells (H4-II-E-C3), human embryonic kidney cells (HEK-293), human hepatocellular carcinoma cells (HepG2), human renal glomerulomesangial cells (HK2), human vascular endothelial cells (HUV-EC-C), human T-cells leukemia (Jurkat), human lung fibroblasts (MRC-5), mouse neuroblastoma cells (Neuro-2a), mouse embryonic fibroblasts (NIH/3T3), rat kidney proximal tubule cells (NRK-52E), human neuroblastoma cells (SH-SY5Y) and, human neuroblastoma cells (SK-N-SH). CCF104

belongs to class 4 for BJ, H4-II-E-C3, HEK-293, HepG2, Jurkat, MRC-5, Neuro-2a, NRK-52E, SH-SY5Y and SK-N-SH cell line. It is considered to have no significant cytotoxic effect on the tested models. CCF104 belongs to class 3 for HK2 and HUV-EC-C cell lines. It is considered to have a cytotoxic effect of more than 25% only at the highest tested concentration on the tested models. CCF104 belongs to class 1 for NIH/3T3 cell line with CC50 of 23 μ M. It displays full response curve and is considered to have significant cytotoxic effect at the tested concentration on the tested models. In these assays tamoxifen was used as a positive control and it showed cytotoxicities at low micro molar levels, as expected (**Appendix 8**).

*De-risking CCF-104, based on the mechanism of action, for potential bleeding. **Achieved.***

Since PAI-1 is a master regulator of the fibrinolytic system, it is imperative to de-risk any PAI-1 inhibitor for potential bleeding. CCF104 was evaluated in in vitro platelet assays. We also evaluated CCF104 in three in vivo experiments, in rat model at three different doses (10, 30 and 100 mg/kg), to address this potential issue. Drug levels of CCF104 at three different are discussed in detail in Major task 3 (below). One issue is that we could not achieved bleeding in these assays with CCF104 at a maximum dose of 100 mg/kg (positive control, Plavix increased bleeding). This level exceeds the dose that would be given to a patient (Appendices 9-11).

a) Platelet aggregation assays: Initially, CCF104 was tested at three different concentrations (0.5, 1 and, 30 μ M) in platelet aggregation assays using TRAP6 (for PAR1), U46619 (for thromboxane) and, ADP (for P2Y12) activators. The results from these experiments showed that CCF104 blocks platelet aggregation through PAR1 in platelet rich plasma. CCF104 has no effect on platelet PAR4, probably not modulating thrombin directly. However, in biochemical assays CCF104 did not show any antagonist effect on PAR1 and PAR2. Therefore, any effect on PAR1 may be an indirect one.

b) Clot growth and fibrin production ex vivo assay: The effect of CCF104 on clot growth in platelet-deplete plasma, in and ex vivo assay, was measured. CCF104 has a mild/no-effect on fibrin production in this assay and on exogenous tPA activity.

c) Coagulation Study in murine animals: CCF104 was administered in β -hydroxypropyl-cyclodextrin (HPBCD) in buffer orally (PO, 10, 30 and 100 mg/kg) to Sprague Dawley rats and blood was collected via orbital after 4h, 8h, and 24h post dose on Day1. Plasma was separated by centrifugation at 2500g for 5 minutes at 4°C for coagulation analysis by using Stago Emo Express. Thrombin time (TT), Prothrombin time (PT), activated partial thromboplastin time (APTT), and concentration of fibrinogen were determined and compared with the vehicle group, where no drug was administered. Statistical analysis was performed by one-way ANOVA. The results showed that these parameters in CCF104 administered groups are similar to the vehicle, indicated no bleeding risk.

d) Assessing CCF104 in murine Venous Thrombosis model: The conventional inferior vena cava (IVC) ligation stasis model was used to induce thrombogenesis. In this experiment, total of six groups of SD rats were used; three groups of CCF104 (at three different doses, 10, 30 and 100 mg/kg), a positive control group (clopidogrel (Plavix®)) and two vehicle groups one with thrombosis and other group without thrombosis. Briefly, prior to surgery, study rats were anesthetized with isoflurane. A midline laparotomy was performed and the IVC exposed by blunt dissection. The back branches and the IVC itself, just caudal to the renal veins were ligated by

suture. The abdominal muscle and skin were closed in two layers. All animals were euthanized at 6h post-surgery and thrombosis were collected for weight and length measurements. Thrombus weights for animals dosed with three different doses of CCF104 are similar to the vehicle group with thrombosis, whereas clopidogrel administered group showed 75% less thrombosis weight. Thrombus lengths for animals dosed with three different doses of CCF104 were ~25% less compared to the vehicle group with thrombosis, whereas clopidogrel group showed 60% less thrombus length. These results demonstrate that CCF104 even at 100 mg/kg dose doesn't possess any bleeding risk.

e) Assessing CCF104 in rat tail bleeding model: This rat model is one of the standard murine models to assess the bleeding risk of any compound. In this experiment, total of five groups of SD rats were used; three groups of CCF104 (at three different doses, 10, 30 and 100 mg/kg), a positive control group (clopidogrel (Plavix®)) and one vehicle group. Based on our PK studies (see below in Major task 3 section), CCF-104 was given 3h before the experiment. Then, the rats were anesthetized, rat tail was sterilized with 70% ethanol and rat tail was cut off with a sterile blade at 5 mm from the end. Using filter paper for absorbing the blood and the timing was started. The filter paper containing the blood was changed every 2 minutes and its weight was recorded. The bleeding was terminated when the no blood points were visible to the naked eye. The bleeding times, measured in seconds, of CCF104 administered rats were similar to that of vehicle group, whereas clopidogrel administered group had 8x higher bleeding time compared to the vehicle group. The bleeding volumes, measured in μL , were 2x higher for CCF-104 administered groups, whereas clopidogrel administered group had 16x higher bleeding volumes. It interesting to note that there is no increase of bleeding volumes when CCF-104 dose is increased. These results indicate that CCF104 even at 100 mg/kg dose does not possess any bleeding risk, at the most it could be slightly anti-thrombolytic.

Major task 3. Pharmacokinetic studies of CCF104

*Subtask 1. PK studies of CCF104. PK studies of CCF104 in two different animal species (rat and dog) and evaluate bio-distribution of CCF104 in different organs in rats (5-9 months). **Partially Achieved.***

PK studies of CCF104 was performed in three different animals, namely rats, beagle dogs and cynomolgus monkeys. CCF104 was administered in β -hydroxypropyl-cyclodextrin (HPBCD) in buffer either intravenously (IV, 1 mg/kg) and orally (PO, 10 mg/kg) to Sprague Dawley rats, beagle dogs and cynomolgus monkeys and drug levels were measured at eight different time points up to 24 hours. The pharmacokinetic parameters, including bioavailability, were measured using standard calculations. CCF104 showed oral bioavailability of $25 \pm 11\%$, $42 \pm 4\%$ and, $24 \pm 6\%$ in rats, dogs, and monkeys respectively. A previous study from rats demonstrated that CCF104 enriches the gut versus systemic circulation. We will complete the biodistribution study (measuring the amount the PAI-1 inhibitor in each organ) once we confirm the lead status of CCF014. This is a costly study and we have budgeted to do this on one compound.

*Subtask 2. Dose-range finding non-GLP toxicology study (single dose) (6-11 months). **Partially Achieved.***

CCF104 was administered in β -hydroxypropyl-cyclodextrin (HPBCD) in buffer orally (PO, 10, 30 and 100 mg/kg) to Sprague Dawley rats and blood was collected at 0.25, 0.5, 1, 2, 4, 6, 8 and 24h post dose on Day 1. Blood samples were collected via orbital into EDTA-K2 coated centrifuge tubes. Plasma was separated from whole blood by centrifugation at 4000 ref for 5 minutes and drug levels were assessed. C_{max} were 0.94, 4.1, and 12 $\mu\text{g/mL}$ at 10, 30 and 100 mg respectively; whereas $AUC_{0-\infty}$ were 5.8, 26.5 and 90.4 $\mu\text{g/hr/mL}$ at 10, 30 and 100 mg respectively indicated that the drug levels are increasing proportionately with dosage of CCF104 administered. This key data are extremely useful in interpreting efficacy as well as toxicity (PK/PD).

Since CCF104 is a PAI-1 inhibitor, our key focus in the last six months is to de-risk CCF104 for potential bleeding. That's why we set aside dose-range study. However, having the above mentioned encouraging preliminary results on hand, we plan to initiate this study soon, preferably in this quarter. We already have sufficient amounts of CCF104 on-hand to conduct this study.

Subtask 3. Dose-range finding non-GLP toxicology study (repeat dose) (13-18 months). We plan to do this study in rats at three different doses. ***Not yet started.***

Specific Aim 2 Further pharmacological assessment of CCF104

Major Task 4. Assess CCF104 and new analogs in acute model of colitis

Subtask 1. DSS- and TNBS-induced mouse colitis model (4-20 months) Partially Achieved

To assess CCF104 and new candidate leads (from Aim 3) in DSS- and TNBS- induced acute IBD models. (CCF IACUC protocol #2019-2281). We have validated preliminary morphological results with CCF104 in the DSS injury model and begun to evaluate 6 new potential lead candidates that were identified by SPR (with higher binding affinity than CCF104) (**Appendix 12**). Histologic readouts of wound associated epithelial (WAE) cell coverage of wounds works well when comparing CCF104 to vehicle. However, incremental differences are more challenging to discern using this assay, because of sampling of the wound (see variation in WAE coverage).

To improve our assay and collect data with less variation (allowing for better comparison of the effects of different PAI-inhibitors), we are developing new tools to evaluate WAE cell coverage of mucosal injury sites using whole mount staining for WAE/epithelial cell markers. We have used this method in the past and our initial experiments in this system look promising. (**Appendix 13**)

Looking ahead to treating humans, we will not be able to rely solely on histopathologic evaluation of WAE cells which may be challenging to collect and evaluate by repeat biopsy. As PAI-1 inhibition activates tPA/plasmin, we are developing a molecular marker of the protein degradome of the wound beds in mice treated with DSS. The wound bed is collected from mice treated with DSS for 12 days and additionally treated with CCF104 versus vehicle for 6 days. The mucosal samples are labeled to identify free amino termini of all peptides and the entire sample is analyzed by mass spectroscopy. As a positive control, we found that labeled peptides for fibrinogen were enriched in CCF104 treated mice suggesting increased tPA activity (as expected) (**Appendix 14**). This result supports what we see histologically in wound repair in the colonic mucosa; initial clots are replaced by WAE cells and fibrinogen degradation products would be expected at these sites. We are also validating peptides from additional proteins that were identified in this experiment. We expect one or more of these peptides could be a marker of PAI-1 activity and WAE formation in response to

PAI-1 inhibition. This work was additionally funded by the Crohn's Colitis Foundation given their funding for the initial phase of this project and their desire to see this to completion.

*Subtask 2. Evaluate select compounds in IL10KO mice (6-20 months) **Partially Achieved***

Evaluate CCF104 and new candidate leads in IL10KO mice model of colitis using anti-TNF agent as a negative control. (CCF IACUC protocol #2019-2281) We have expanded our IL-10KO colony and are now engaged in testing CCF104 in this model +/- antiTNF.

*Subtask 3. Evaluate CCF104 and new candidate leads in SERPINE1-/- mouse models of colitis (6-20 months) **Partially Achieved***

(CCF IACUC protocol #2019-2281). We have tested CCF104 in the SERPINE1-/- mouse and found that a similar phenotype was present in these mice as compared to CCF104 when treated with DSS. To test the role of epithelial expressed PAI-1, we have developed a mouse model with SERPINE1 deleted in the intestinal epithelium by breeding mice with a floxed allele of SERPINE1 with Villin - Cre. This mouse model will be useful to evaluate if there are multiple molecular targets of CCF104 (or other candidate leads) in mouse colonic injury.

*Subtask 4. Evaluate CCF104 and new candidate leads in repair of focal colonic biopsy injuries (7-9 months) **Not started***

(CCF IACUC protocol #2019-2281). These experiments will be performed with new lead candidates that outperform CCF104 in DSS and TNBS models. These experiments are best done in our top leads that emerge from DSS and TNBS models.

Specific Aim 3. Identification of a second generation of PAI-1 inhibitor

Major Task 5. Identification of a second generation of PAI-1 inhibitor

*Subtask 1. Design and synthesize 125 novel PAI-1 inhibitor analogues (1-20 months). **Partially Achieved.***

Currently, the synthesis of novel PAI-1 inhibitors and the medicinal chemistry campaign is ongoing. To date, we have developed synthetic routes for chemically diverse analogs, based on our initial lead of CCF104. So far, we synthesized ~90 analogs. The synthesis of additional analogs is in progress. We are in process of synthesizing 30 additional analogues.

*Subtask 2. Evaluate novel compounds for their PAI-1 inhibitory activity (3-20 months). **Partially Achieved.***

i) *Establishing and testing compounds in Surface Plasmon Resonance (SPR) assay.* We have established an SPR assay for this project in a 96-well format using biotinylated recombinant human PAI-1 protein. This is the first time using SPR assay in a 96-well format for measuring PAI-1 binding affinities. We also used this assay to evaluate our previously synthesized analogs. Each compound was tested at 6 different concentrations and binding affinity (K_d) values were determined. Till to date, we have identified several analogs with better binding affinities compared CCF104. We will confirm the activities of these analogs with tPA assay.

PAI-1 spontaneously converts into unreactive latent form and it poses challenges to do its biology as well as structural determination. The first stable mutants of PAI-1 crystallized in the active conformation were PAI-1-N150H-K154T-Q319L-M354I (quadruple mutant). This mutant has higher $t_{1/2}$ (145h) compared to the WT PAI-1. Before we initiate structural determination of

CCF104 and its analogs binding to active PAI-1, we need to establish binding affinity to quadruple mutant PAI-1. We utilized SPR assay to determine the binding affinity of select compounds. Currently, we have been optimizing this assay. This data would be useful to perform cryo-EM or X-ray crystal studies so that the results shed light on the structural information on CCF-104 and its analogs binding to PAI-1 active protein.

ii) Establishing a tPA assay for PAI-1 inhibition. In general, biochemical assays are highly reproducible and have been used in drug discovery, wherever they are applicable. We have been developing a biochemical assay that is useful to evaluate our compounds, using human recombinant tissue plasminogen activator (tPA) protein, human recombinant PAI-1 protein, and a fluorogenic substrate. Recently we established this assay in a 96-well format. Currently, we are evaluating all of our compounds in this assay. This project will utilize the above two assays to select compounds for further evaluation including ADME, PK, and in vivo testing (**Appendix 15**).

*Subtask 3. Evaluate novel compounds for their ADME properties (5-22 months). **Partially Achieved.*** A subset of compounds that meet our criteria will be evaluated for PK properties and in vivo efficacy in mouse colitis model (see Major task 4).

We have evaluated ~10 compounds for their absorption, distribution, metabolism and, elimination (ADME) properties in vitro and three compounds for their pharmacokinetic (PK) properties in Sprague Dawley rats. These compounds showed ADME properties similar to CCF104 and gut-enrichment in rats.

What opportunities for training and professional development has the project provided?

We have had outstanding technical assistance in this project. Ingrid Owasu was an important team member until 8/23 when she began a program as a doctor of osteopathy at Akron University. The training and experience of this program was a key element of her development. Collin Wong has recently taken over for Ingrid. He is a recent graduate of Case Western Reserve University. His goal is also to go to medical school after two years working on this project.

How were the results disseminated to communities of interest?

We routinely report to the Crohn's and Colitis Foundation who supported the early phase of this program (which produced CCF104 our current lead). Ted Espenschied is a third-year post doc in the lab who has handled the presentations to this group with the guidance of Drs. Stappenbeck and Josyula. We have disclosure agreement in place with Crohn's and Colitis Foundation for these confidential conversations. We have not presented this project to any other group.

What do you plan to do during the next reporting period to accomplish the goals?

For CCF104, we will complete all advanced pre-clinical IND studies described in the SOW. We don't anticipate any major hurdles.

We will also continue our backup compound program. We expect to identify additional candidates that have a superior profile compared to CCF104 regarding in vitro and in vivo potency.

4. IMPACT: What was the impact on the development of the principal discipline(s) of the project?

- To date, CCF104 studies show it is a viable lead candidate for further studies as a PAI-1 inhibitor.
- We have optimized a medium-throughput SPR assay in 96-well format for determining binding affinity of small molecule therapeutics and novel compounds to PAI-1 protein.
- We have optimized a medium throughput tPA activity assay in a 96-well format for determining the activity of PAI-1 inhibitors.
- We have optimized the in vivo determination of WAE coverage of wounds in the intestine using in vivo injury models.

What was the impact on other disciplines?

We have tested CCF104, our lead compound in a mouse model of lung fibrosis with Dr. Mitch Olman at the Cleveland Clinic. We found that it shows efficacy in a bleomycin fibrosis model of idiopathic pulmonary fibrosis (IPF). We are developing methods to deliver an inhaled formulation as a delivery option for patients with IPF. As treatment will be chronic, delivery to the lung is advantageous.

We have also tested CCF104 in a model of lye ingestion. There are 5,000 cases a year of lye ingestion in the US. The consequences are devastating. If the subject survives, they are plagued by chronic esophageal fibrosis that needs frequent dilation to allow for food passage. This is an orphan disease with no therapy. We found in our pilot experiments using mouse model of lye ingestion, CCF104 rescued the fibrosis. Treatment was only required for a short period (several days) after the injury in this model so CCF104 was delivered intraperitoneally. We did these experiments in collaboration with Dr. Julia Liu at Morehouse School of Medicine.

What was the impact on technology transfer?

We plan to file INDs for CCF104 (or a better lead if identified) for use in IBD, IPF and lye ingestion. Our goal with the lye ingestion indication will be to expedite use in humans for compassionate use once safety is established.

What was the impact on society beyond science and technology?

Nothing to Report because nothing has been publicly disclosed.

5. CHANGES/PROBLEMS:

Changes in approach and reasons for change

We have only made minor alterations to the project, mostly to optimize assays to improve data collection and analysis. These alterations do not affect our budget or timeline.

1. In order to optimize/efficiently filter our compounds through our screening funnel, we developed SPR and tPA assays in 96-well plate format. These assays together with ADME properties will guide the program for selecting better compounds, especially a 2nd generation PAI-1 inhibitor.
2. We added a whole mount assay for WAE cell coverage in the injured mouse colon to improve quantification. This will facilitate discrimination of PAI-1 inhibitor compounds in vivo.
3. We are pursuing a molecular marker of WAE formation (in response to PAI-1 inhibition) in the colonic wound bed based on protein degradomics. This approach is now additionally funded by the Crohn's and Colitis Foundation and the findings we expect will be synergistic with the DOD application and provide a new marker for PAI-1 activity that could potentially translate to humans.

Actual or anticipated problems or delays and actions or plans to resolve them.

A few assays were delayed based on waiting for data from other experiments. This has not delayed the overall project.

Changes that had a significant impact on expenditures

We have delayed a few expensive assays for CCF104 to wait for de-risking experiments and to confirm that CCF104 will be our lead. We expect to move forward on these assays in the next quarter.

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents

Significant changes in use or care of human subjects

<i>Nothing to Report</i>

Significant changes in use or care of vertebrate animals

Nothing to Report

Significant changes in use of biohazards and/or select agents

Nothing to Report

6. PRODUCTS:

- **Publications, conference papers, and presentations**

Journal publications.

Nothing to Report

Books or other non-periodical, one-time publications.

Nothing to Report

Other publications, conference papers and presentations.

Nothing to Report

- **Website(s) or other Internet site(s)**

Nothing to Report

- **Technologies or techniques**

Nothing to Report

- **Inventions, patent applications, and/or licenses**

- **Other Products**

Nothing to Report

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Name: Thaddeus Stappenbeck MD, PhD
Project Role: PI
Nearest person month worked: 1.8

Contribution to Project: Dr. Stappenbeck oversees the entire project.

Name: Vara Prasad Josyula, PhD
Project Role: Co-I
Nearest person month worked: 4.6

Contribution to Project: Dr. Josyula oversees the med chem and CRO projects.

Name: Julie Zhou, PhD
Project Role: Post Doctoral Fellow
Nearest person month worked: 11

Contribution to Project: Dr. Zhou helped oversee the in vivo based approaches to test new PAI-1 inhibitors.

Name: Ingrid Owusu
Project Role: Tech
Nearest person month worked: 4

Contribution to Project: Ms. Owusu performed the in vivo based approaches to test new PAI-1 inhibitors.

Name: Pat Kessler
Project Role: Senior Research Tech
Nearest person month worked: 3.2

Contribution to Project: Ms. Kessler performed the in vitro based approaches to test new PAI-1 inhibitors.

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?

See updated Other Support for Drs. Stappenbeck and Josyula in Appendix 16.

What other organizations were involved as partners?

Crohn's Colitis Foundation
New York, NY
Financial support to identify a molecular marker of WAE cell activity (protein fragment).

8. SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS:

QUAD CHARTS:

9. APPENDICES:

Appendix 1

Study Report

Report Name	CYP Inhibition of CYP1A2, CYP2A6, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, CYP2E1, CYP3A-M and CYP3A-T by 2 CCF Compounds in Human Liver Microsomes
Pharmaron Study Number	ADME-CCF-230217-2
Pharmaron Report Number	ADME-CCF-CYP Inhibition-20230228
Submitted to	Cleveland Clinic Foundation
Submitted by	<i>in vitro</i> ADME laboratory, Pharmaron
Study Start Date	02/22/2023
Report Submission Date	02/28/2023
Study Director	Jianhua Jiao
Assay Scientist	Shu Wang
QC for The Key Steps of The Experiment	Shan Liu
QC for Raw Data and Data Analysis	Shan Liu
QC for Report	Jianhua Jiao
Report Approval	Xiaoting Wang

Data Summary

Table 1. IC₅₀ values for test compounds and known inhibitors against CYP1A2, CYP2A6, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, CYP2E1, CYP3A-M and CYP3A-T

Compound	CYP1A2			CYP2A6			CYP2B6			CYP2C8			CYP2C9			CYP2C19			CYP2D6			CYP2E1			CYP3A-M			CYP3A-T		
	Replicate 1	Replicate 2	Mean	Replicate 1	Replicate 2	Mean	Replicate 1	Replicate 2	Mean	Replicate 1	Replicate 2	Mean	Replicate 1	Replicate 2	Mean	Replicate 1	Replicate 2	Mean	Replicate 1	Replicate 2	Mean	Replicate 1	Replicate 2	Mean	Replicate 1	Replicate 2	Mean	Replicate 1	Replicate 2	Mean
Furafylline	2.34	2.36	2.35	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Tranlycypromine	-	-	-	0.14	0.14	0.14	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Ketoconazole	-	-	-	-	-	-	2.65	2.31	2.48	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Quercetin	-	-	-	-	-	-	-	-	-	3.17	3.79	3.48	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Sulfaphenazole	-	-	-	-	-	-	-	-	-	-	-	-	0.33	0.32	0.33	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Tranlycypromine	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	8.05	9.52	8.78	-	-	-	-	-	-	-	-	-	-	-	-
Quinidine	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.029	0.027	0.028	-	-	-	-	-	-	-	-	-
Quercetin	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	18.14	20.12	19.13	-	-	-	-	-	-
Ketoconazole	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.026	0.027	0.026	-	-	-
Ketoconazole	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.018	0.018	0.018
CCF2201	>30	>30	>30	>30	>30	>30	>30	>30	>30	3.95	3.26	3.61	6.50	7.67	7.09	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30
CCF8205	>30	>30	>30	>30	>30	>30	>30	>30	>30	17.59	18.64	18.11	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30

Table 2. The remaining activity (%) of test compounds and control compound against CYP1A2

Isoform	Compound	Pre-incubation	Remaining% compared to vehicle control								
			Vehicle Control	Test Conc. 1	Test Conc. 2	Test Conc. 3	Test Conc. 4	Test Conc. 5	Test Conc. 6	Test Conc. 7	Test Conc. 8
CYP1A2	Furafylline	Replicate 1	100	96.45	91.96	95.80	86.15	72.92	41.86	19.98	8.74
		Replicate 2	100	99.47	93.31	94.53	88.12	70.48	43.67	20.00	8.61
		Mean	100	97.96	92.64	95.17	87.14	71.70	42.76	19.99	8.68
	CCF2201	Replicate 1	100	99.90	103.15	101.86	104.54	97.55	99.11	93.41	87.59
		Replicate 2	100	102.85	103.18	99.60	100.98	101.75	100.43	100.29	89.51
		Mean	100	101.38	103.16	100.73	102.76	99.65	99.77	96.85	88.55
	CCF8205	Replicate 1	100	94.29	95.84	101.06	100.37	98.11	98.59	108.21	109.74
		Replicate 2	100	104.86	105.88	103.16	103.79	103.71	104.76	111.88	117.16
		Mean	100	99.57	100.86	102.11	102.08	100.91	101.68	110.04	113.45

Table 3. The remaining activity (%) of test compounds and control compound against CYP2A6

Isoform	Compound	Pre-incubation	Remaining% compared to vehicle control								
			Vehicle Control	Test Conc. 1	Test Conc. 2	Test Conc. 3	Test Conc. 4	Test Conc. 5	Test Conc. 6	Test Conc. 7	Test Conc. 8
CYP2A6	Tranlycypromine	Replicate 1	100	96.66	98.76	88.49	71.95	49.00	26.08	6.91	1.93
		Replicate 2	100	97.65	97.33	88.73	70.77	49.99	26.57	7.08	2.13
		Mean	100	97.15	98.05	88.61	71.36	49.50	26.33	7.00	2.03
	CCF2201	Replicate 1	100	97.34	102.65	101.22	98.55	100.57	99.66	93.48	86.28
		Replicate 2	100	99.22	101.83	106.34	101.83	104.38	100.92	96.49	90.12
		Mean	100	98.28	102.24	103.78	100.19	102.47	100.29	94.99	88.20
	CCF8205	Replicate 1	100	95.96	98.01	97.57	100.00	94.12	100.08	96.05	88.76
		Replicate 2	100	94.71	101.23	97.49	100.00	97.82	100.87	99.03	92.29
		Mean	100	95.33	99.62	97.53	100.00	95.97	100.48	97.54	90.52

Table 4. The remaining activity (%) of test compounds and control compound against CYP2B6

Isoform	Compound	Pre-incubation	Remaining% compared to vehicle control								
			Vehicle Control	Test Conc. 1	Test Conc. 2	Test Conc. 3	Test Conc. 4	Test Conc. 5	Test Conc. 6	Test Conc. 7	Test Conc. 8
CYP2B6	Ketoconazole	Replicate 1	100	93.61	95.71	93.04	86.72	73.80	47.18	22.47	7.04
		Replicate 2	100	96.43	89.61	94.39	81.38	71.39	44.88	21.58	7.11
		Mean	100	95.02	92.66	93.71	84.05	72.60	46.03	22.02	7.07
	CCF2201	Replicate 1	100	101.87	98.17	100.00	100.41	100.48	99.96	99.15	82.28
		Replicate 2	100	99.60	102.30	99.67	100.47	99.78	101.22	100.33	88.38
		Mean	100	100.73	100.24	99.83	100.44	100.13	100.59	99.74	85.33
	CCF8205	Replicate 1	100	98.35	100.08	101.28	100.58	105.02	101.33	113.46	109.64
		Replicate 2	100	104.13	103.18	101.39	101.97	102.63	102.02	111.90	118.20
		Mean	100	101.24	101.63	101.34	101.28	103.83	101.67	112.68	113.92

Table 5. The remaining activity (%) of test compounds and control compound against CYP2C8

Isoform	Compound	Pre-incubation	Remaining% compared to vehicle control								
			Vehicle Control	Test Conc. 1	Test Conc. 2	Test Conc. 3	Test Conc. 4	Test Conc. 5	Test Conc. 6	Test Conc. 7	Test Conc. 8
CYP2C8	Quercetin	Replicate 1	100	90.36	88.25	98.61	89.91	75.88	52.96	24.94	6.00
		Replicate 2	100	99.96	90.98	95.08	98.31	83.19	56.06	25.25	6.11
		Mean	100	95.16	89.62	96.85	94.11	79.54	54.51	25.09	6.06
	CCF2201	Replicate 1	100	105.38	112.49	101.28	101.92	90.55	57.07	21.65	8.05
		Replicate 2	100	103.98	101.29	96.23	94.09	82.56	52.01	19.61	7.35
		Mean	100	104.68	106.89	98.76	98.01	86.55	54.54	20.63	7.70
	CCF8205	Replicate 1	100	99.11	98.94	97.22	93.66	83.89	88.91	62.16	36.91
		Replicate 2	100	97.64	109.47	113.11	101.64	95.64	82.86	65.15	39.16
		Mean	100	98.38	104.21	105.16	97.65	89.76	85.89	63.66	38.03

Table 6. The remaining activity (%) of test compounds and control compound against CYP2C9

Isoform	Compound	Pre-incubation	Remaining% compared to vehicle control								
			Vehicle Control	Test Conc. 1	Test Conc. 2	Test Conc. 3	Test Conc. 4	Test Conc. 5	Test Conc. 6	Test Conc. 7	Test Conc. 8
CYP2C9	Sulfaphenazole	Replicate 1	100	101.63	98.97	97.07	87.52	66.86	39.46	20.19	7.77
		Replicate 2	100	103.99	99.61	96.22	86.80	64.79	39.73	19.21	7.96
		Mean	100	102.81	99.29	96.64	87.16	65.83	39.60	19.70	7.86
	CCF2201	Replicate 1	100	90.39	98.85	95.80	94.63	85.23	71.50	38.85	15.96
		Replicate 2	100	98.81	101.15	104.82	103.43	92.41	75.02	41.19	17.61
		Mean	100	94.60	100.00	100.31	99.03	88.82	73.26	40.02	16.78
	CCF8205	Replicate 1	100	100.22	103.42	108.90	99.14	100.18	91.48	78.17	56.20
		Replicate 2	100	100.18	105.18	108.07	99.18	103.29	90.65	78.21	56.09
		Mean	100	100.20	104.30	108.49	99.16	101.74	91.07	78.19	56.15

Table 7. The remaining activity (%) of test compounds and control compound against CYP2C19

Isoform	Compound	Pre-incubation	Remaining% compared to vehicle control								
			Vehicle Control	Test Conc. 1	Test Conc. 2	Test Conc. 3	Test Conc. 4	Test Conc. 5	Test Conc. 6	Test Conc. 7	Test Conc. 8
CYP2C19	Tranlycypromine	Replicate 1	100	96.36	97.38	98.34	101.73	97.56	70.87	43.45	20.82
		Replicate 2	100	100.07	105.60	115.04	106.21	102.37	74.09	48.21	22.46
		Mean	100	98.22	101.49	106.69	103.97	99.97	72.48	45.83	21.64
	CCF2201	Replicate 1	100	95.44	93.56	90.57	92.89	94.75	89.35	86.16	68.25
		Replicate 2	100	103.27	97.10	92.52	96.65	98.39	91.33	92.77	76.54
		Mean	100	99.36	95.33	91.54	94.77	96.57	90.34	89.47	72.39
	CCF8205	Replicate 1	100	87.28	89.61	90.49	91.20	90.59	92.27	97.40	96.89
		Replicate 2	100	80.80	89.04	90.16	90.38	87.92	94.67	95.35	96.12
		Mean	100	89.04	89.33	89.51	90.68	90.49	90.10	96.04	96.12

Table 8. The remaining activity (%) of test compounds and control compound against CYP2D6

Isoform	Compound	Pre-incubation	Remaining% compared to vehicle control								
			Vehicle Control	Test Conc. 1	Test Conc. 2	Test Conc. 3	Test Conc. 4	Test Conc. 5	Test Conc. 6	Test Conc. 7	Test Conc. 8
CYP2D6	Quinidine	Replicate 1	100	101.23	101.08	96.07	82.23	64.62	36.74	17.86	8.03
		Replicate 2	100	95.92	100.22	93.15	85.46	60.61	35.92	17.34	8.00
		Mean	100	98.57	100.65	94.61	83.85	62.62	36.33	17.60	8.02
	CFZ201	Replicate 1	100	102.84	105.60	104.04	104.94	100.04	92.74	87.27	81.27
		Replicate 2	100	104.20	101.79	101.56	104.43	102.44	102.83	102.49	91.61
		Mean	100	103.57	103.69	102.25	102.21	101.41	100.43	103.13	89.39
	CFR205	Replicate 1	100	98.48	98.73	101.49	96.35	99.76	101.49	112.39	119.77
		Replicate 2	100	97.49	98.12	98.11	99.79	96.45	98.43	107.59	107.59
		Mean	100	97.98	98.43	99.80	98.07	97.61	101.49	109.99	119.45

Materials

1. Test compounds were provided by the sponsor. The compound information is listed in Table 12.

Table 12. Compound information

ClientID	FW(g/mol)	Purity
CCF2201	425.86	>98.00%
CCF8205	471.44	>98.00%

2. The microsomes were stored at -80°C prior to use, and the liver microsome information is listed in Table 13.

Table 13. Liver microsome information

Species	Vendor	Cat. No.	Lot. No.	Strain & Gender
Human	BD Gentest	452117	38297	Pooled, Mixed Gender

Study Design

1. Equipment, materials and reagents

1) Preparation of compound working solutions
To prepare 10 mM test compound stock solutions, in separate vials weigh required amounts of powder and add the corresponding volumes of DMSO. Then make a serial dilution to obtain the solutions of each compound at the nominal concentrations of 0, 2, 6, 20, 60, 200, 600, 2000 and 6000 µM in DMSO. The final concentrations of the compounds in the assay are 0, 0.01, 0.03, 0.1, 0.3, 1, 3, 10 and 30 µM, and the final percentage of organic solvent introduced by test compound or the positive control inhibitors is 0.5%. The final concentrations of positive control inhibitors are listed in Table 14.

Table 14. Positive control inhibitor final concentrations of each isoform

CYP Isoform	Positive Inhibitors	Final Concentration (µM)
CYP1A2	Furafylline	0, 0.01, 0.03, 0.1, 0.3, 1, 3, 10, 30
CYP2A6	Tranilcypromine	0, 0.0015, 0.005, 0.015, 0.05, 0.15, 0.5, 1.5, 5
CYP2B6	Ketoconazole	0, 0.01, 0.03, 0.1, 0.3, 1, 3, 10, 30
CYP2C8	Quercetin	0, 0.01, 0.03, 0.1, 0.3, 1, 3, 10, 30
CYP2C9	Sulfaphenazole	0, 0.0015, 0.005, 0.015, 0.05, 0.15, 0.5, 1.5, 5
CYP2C19	Tranilcypromine	0, 0.01, 0.03, 0.1, 0.3, 1, 3, 10, 30
CYP2D6	Quinidine	0, 0.0015, 0.005, 0.015, 0.05, 0.15, 0.5, 1.5, 5
CYP2E1	Quercetin	0, 0.05, 0.15, 0.5, 1.5, 5, 15, 50, 150
CYP3A	Ketoconazole	0, 0.00015, 0.0005, 0.0015, 0.005, 0.015, 0.05, 0.15, 0.5

2) Preparation of substrate stock solution

The stock solution of substrate of each isoform is listed in Table 15. The substrate stock solutions are stored in a -20°C freezer. Prior to use, remove the substrate solution from the freezer and allow it to warm up to room temperature.

Table 15. Preparation of substrate stock solution

CYP Isoform	Substrate	Working Conc.(µM)	Final Conc.(µM)	Incubation Time
CYP1A2	Phenacetin	800	40	20 min
CYP2A6	Coumarin	40	2	5 min
CYP2B6	Bupropion	800	40	20 min
CYP2C8	Amodiaquine	20	1	5 min
CYP2C9	Diclofenac	120	6	5 min
CYP2C19	Mephenytoin	1000	50	20 min
CYP2D6	Dextromethorphan	40	2	20 min
CYP3A-M	Midazolam	20	1	5 min
CYP3A-T	Testosterone	800	40	10 min

ACN Acetonitrile

3) Phosphate buffer (100 mM, pH 7.4)

Analytically pure disodium hydrogen phosphate and potassium dihydrogen phosphate are purchased from local supplier. To prepare the Solution A, accurately weigh 7.098 g of disodium hydrogen phosphate and add into 500 mL of pure water, then sonicate to dissolve the content. To prepare the Solution B, accurately weigh 3.400 g of potassium dihydrogen phosphate and add into 250 mL of pure water, then sonicate to dissolve the content. Place Solution A on a stirrer and slowly add Solution B into Solution A until the pH reaches 7.4.

4) 10 mM NADPH Solution

NADPH (MW: 833.4 g/mol) purchased from Solarbio. Dissolve at 8.334 mg/mL in phosphate buffer (100 mM, pH 7.4).

5) Human Liver Microsomes (HLM)

HLMs are stored in a -80°C freezer. Prior to use, remove the pooled HLM from the freezer and allow it to thaw in a 37 °C water bath and then stored on wet ice.

2. Assay procedure

1) Preparation of master solution

Make a master solution according to table 16 below.

Table 16. Preparation of master solution

Buffer	Stock Concentration	Volume	Final Concentration
Microsomes	20 mg/mL	2 µL	0.2 mg/mL
Phosphate buffer	100 mM	167 µL	100 mM

2) Incubation

The incubation is carried out in 96 deep well plates. Dispense the following volumes into each well of the incubation plate: 169 µL of the master solution and 1 µL of the compound working solution or vehicle (acetonitrile). The incubation plate is placed into the water bath and pre-warmed at 37 °C for 5 minutes. Add 10 µL of substrate to the Incubation Plate, mix the incubation mixture on a whirly mixer for 15 seconds, and then add 20 µL of 10 mM NADPH solution to start the reaction at the final concentration of 1 mM. The assay is performed in duplicate.

2.3. Reaction Quenching

Quench the reaction by the addition of 1.5 volume (300 µL) of cold ACN containing 3% formic acid and 200 nM Tolbutamide, 200 nM aprozolam and 200 nM Labalol. Centrifuge the plate at 3,220 g for 40 minutes. Transfer 150 µL of the supernatant to a new plate. The supernatant may be diluted with 150 µL pure water. Mix well and analyze samples using UPLC-MS/MS.

3. Data Processing

The automatic peak integration areas are checked for all of the samples. The Analyte peak area and internal standard peak area are exported into excel spread sheet. The inhibition of each P450 enzyme in human liver microsomes is measured as the percentage decrease in the activity of marker metabolite formation compared to non-inhibited controls (= 100% activity). The IC50 values are calculated with remaining activity (%) and logarithm of inhibitor concentrations.

Calculate the percentage of remaining activity as follows:

Area Ratio = Peak Area_{Analyte}/ Peak Area_{Internal Standard}

Remaining Activity (%) = Area Ratio_{test compound}/ Area Ratio_{vehicle} *100%

Calculate IC₅₀ value (test compound concentration which produces 50% inhibition) by using Excel XLfit 5.3.1.3.

Bioanalytical Method

1. Chromatographic conditions

LC system: Shimadzu
MS analysis : Triple Quad™ 5500+ instrument from AB Inc with an ESI interface for
Column temperature: 40°C
Column: Xselect® HSS T3 2.5 µm (2.1×50mm)
Mobile phase: 0.1% formic acid in water (A) and 0.1% formic acid in acetonitrile (B)
Elution rate: 0.7 mL/min
Injection volume: 10 µL
LC gradient conditions

CYP1A2

Time (min)	0	0	0.8	1.0	1.2	1.4
%B	5	5	100	100	100	5

CYP2A6 and CYP2E1

Time (min)	0	1.1	1.2	1.7	1.9	2.5
%B	30	50	95	95	30	30

CYP2B6, CYP2C19 and CYP2D6

Time (min)	0	0.3	0.8	1.2	1.5	2
%B	5	5	95	95	5	5

CYP2C8

Time (min)	0	0.3	0.8	1.2	1.3	1.5	1.7	2	2.2	2.5
%B	5	5	95	95	5	5	95	95	5	5

CYP2C9

Time (min)	0	1.1	1.2	1.5	1.6	2
%B	30	50	95	95	30	30

CYP3A-M

Time (min)	0	0.1	0.8	0.8	1.0
%B	5	100	100	5	5

CYP3A-T

Time (min)	0	2	2.2	2.4	2.6	3
%B	5	50	95	95	5	5

2. MS parameters

Ion source: Turbo spray
Ionization model: ESI
Scan type: MRM
Collision gas: 9 L/min
Curtain gas: 40 L/min
Nebulize gas: 55 L/min
Auxiliary gas: 55 L/min
Temperature: 500°C
Ionspray voltage:+ 5500 V/-4500 V

CYP Isoform	Compound ID	Q1 (m/z)	Q3 (m/z)	DP (v)	CE (v)
1A2	Acetaminophen	152.1	110	80	25
2A6	7-OH-coumain	160.8	133	-60	-27
2B6	OH Bupropion	256.2	238.201	30	20
2C8	N-Desethylamodiaquine	328.2	283.1	75	35
2C9	4-OH-Diclofenac	312	231.1	80	25
2C19	4-OH-Mephenytoin	235.1	150.1	70	30
2D6	Dextrophan	258.1	157.1	60	50
2E1	6-OH-chlozoxazone	183.8	119.8	-80	-20
3A-M	1-OH-Midazolam	342.3	203	95	35
3A-T	6β-OH-Testosterone	305.2	269.3	75	20

Appendix 2

Study number	20034-22002
Test article	CCF 22-01
Purpose	To determine PK profiles of CCF 22-01 after IV & PO administrations to beagle dog
GLP compliance	Non-GLP
Sponsor	The Cleveland Clinic Foundation
Study director	Xiaomei Ren
In-life scientists	Yufeng Meng
Formulation	Chenchen Qi
Bioanalytical	Wei Zheng
Effective date	6 th Jun 2022

		HL	Lambda z(h)	Tmax(h)	Cmax(ng/mL)	UClast(h*ng/mL)	CINF obs(h*ng/m	MRTlast(h)	MRTINF obs(h)
G1	Administratio	Dose Level	$T_{1/2}$	T_{max}	C_{max}	$AUC_{(0-t)}$	$AUC_{(0-\infty)}$	$MRT_{(0-t)}$	$MRT_{(0-\infty)}$
Animal No.	n Route	mg/kg	h	h	ng/mL	h*ng/mL	h*ng/mL	h	h
101	IV	1.0	1.57	0.083	2875.13	1499.67	1523.13	1.20	1.34
102	IV	1.0	1.97	0.083	2172.50	1045.84	1065.88	0.83	1.02
103	IV	1.0	3.77	0.083	3759.53	2614.41	2634.27	2.17	2.38
n		3	3	3	3	3	3	3	3
G1-Mean		1.0	2.44	0.083	2935.72	1719.97	1741.09	1.40	1.58
G1-SD		0.0	1.17	0.00	795.25	807.16	806.59	0.69	0.71

Peak Name: Tolbutamide (IS)
Use as Internal Standard
Q1/Q3 Masses: 271.10/172.00 Da

Peak Name: CCF22-01-2
Internal Standard: Tolbutamide (IS)
Q1/Q3 Masses: 426.10/171.00 Da

Fit	Linear	Weighting 1 / (x * x)	Iterate	No
Intercept	0.00118			
Slope	0.00193			
Correlation coefficient	0.9994			
Use Area				

Sample Name	Sample Type	Dilution Factor	Analyte Peak	Analyte Concentration	Retention Time	Area	Ratio	IS Peak	Ar	IS Retention	Use	Record	Record	Mc	Calculated	Accuracy (%)
Blank	Double Blank	1	0.00E+00	0	0	#DIV/0!	0.00E+00	0						0	N/A	N/A
Blank+IS	Blank	1	0.00E+00	0	0	0.00E+00	4.51E+05	1.31						0	N/A	N/A
STD1-01	Standard	1	2.28E+03	2	1.44	5.14E-03	4.44E+05	1.3			1	0	2.05399	102.699		
STD1-02	Standard	1	4.48E+03	5	1.44	1.02E-02	4.40E+05	1.31			1	0	4.67283	93.4565		
STD1-03	Standard	1	8.76E+03	10	1.44	2.02E-02	4.34E+05	1.31			1	0	9.87515	98.7515		
STD1-04	Standard	1	4.41E+04	50	1.45	9.99E-02	4.41E+05	1.31			1	0	51.2528	102.506		
STD1-05	Standard	1	8.71E+04	100	1.44	2.00E-01	4.35E+05	1.31			1	0	103.472	103.472		
STD1-06	Standard	1	4.25E+05	500	1.45	9.60E-01	4.43E+05	1.31			1	0	498.107	99.6214		
STD1-07	Standard	1	8.49E+05	1000	1.45	1.93E+00	4.41E+05	1.31			1	0	999.536	99.9536		
STD1-08	Standard	1	1.72E+06	2000	1.45	3.83E+00	4.47E+05	1.31			1	0	1990.8	99.5398		
WASH	Unknown	1	9.64E+03	N/A	1.45	#DIV/0!	0.00E+00	0				0	#DIV/0!	N/A		
WASH	Unknown	1	2.79E+03	N/A	1.45	#DIV/0!	0.00E+00	0				0	#DIV/0!	N/A		
WASH	Unknown	1	1.93E+03	N/A	1.45	#DIV/0!	0.00E+00	0				0	#DIV/0!	N/A		
QCL1	Quality Control	1	5.80E+03	6	1.45	1.28E-02	4.53E+05	1.31			1	0	6.03011	100.502		
QCM1	Quality Control	1	1.38E+05	160	1.45	3.04E-01	4.53E+05	1.32			1	0	157.449	98.4053		
QCH1	Quality Control	1	1.31E+06	1600	1.45	2.99E+00	4.37E+05	1.32			1	0	1553.16	97.0728		
DQC1(DL10)	Quality Control	10	8.52E+05	10000	1.46	1.91E+00	4.45E+05	1.32			1	0	9928.52	99.2852		
WASH	Unknown	1	8.07E+03	N/A	1.45	#DIV/0!	0.00E+00	0				0	#DIV/0!	N/A		
WASH	Unknown	1	2.56E+03	N/A	1.45	#DIV/0!	0.00E+00	0				0	#DIV/0!	N/A		
WASH	Unknown	1	1.92E+03	N/A	1.45	#DIV/0!	0.00E+00	0				0	#DIV/0!	N/A		
101-0.083H(DL10)	Unknown	10	2.53E+05	N/A	1.45	5.55E-01	4.55E+05	1.31				0	2875.13	N/A		
101-0.25H(DL10)	Unknown	10	9.75E+04	N/A	1.45	2.20E-01	4.42E+05	1.32				0	1138.33	N/A		
101-0.5H(DL10)	Unknown	10	3.34E+04	N/A	1.45	7.24E-02	4.61E+05	1.32				0	369.919	N/A		
101-1H	Unknown	1	1.34E+05	N/A	1.45	2.87E-01	4.66E+05	1.32				0	148.557	N/A		
101-2H	Unknown	1	1.19E+05	N/A	1.45	2.67E-01	4.45E+05	1.32				0	138.038	N/A		
101-4H	Unknown	1	6.89E+04	N/A	1.45	1.53E-01	4.51E+05	1.32				0	78.6295	N/A		
101-8H	Unknown	1	9.70E+03	N/A	1.45	2.12E-02	4.58E+05	1.32				0	10.3737	N/A		
101-24H	Unknown	1	0.00E+00	N/A	0	0.00E+00	4.61E+05	1.32				0	No Peak	N/A		
102-0.083H(DL10)	Unknown	10	1.89E+05	N/A	1.45	4.20E-01	4.50E+05	1.32				0	2172.5	N/A		
102-0.25H(DL10)	Unknown	10	6.98E+04	N/A	1.45	1.59E-01	4.39E+05	1.32				0	819.156	N/A		
102-0.5H(DL10)	Unknown	10	3.82E+04	N/A	1.45	8.57E-02	4.46E+05	1.32				0	438.895	N/A		
102-1H	Unknown	1	1.29E+05	N/A	1.45	2.82E-01	4.58E+05	1.31				0	145.71	N/A		
102-2H	Unknown	1	5.34E+04	N/A	1.45	1.15E-01	4.65E+05	1.32				0	59.0018	N/A		
102-4H	Unknown	1	2.39E+04	N/A	1.45	5.29E-02	4.51E+05	1.32				0	26.8752	N/A		
102-8H	Unknown	1	6.56E+03	N/A	1.46	1.47E-02	4.45E+05	1.32				0	7.04312	N/A		
102-24H	Unknown	1	1.22E+03	N/A	1.45	2.59E-03	4.71E+05	1.31				0	0.731116	N/A		
103-0.083H(DL10)	Unknown	10	3.29E+05	N/A	1.44	7.25E-01	4.54E+05	1.3				0	3759.53	N/A		
103-0.25H(DL10)	Unknown	10	1.67E+05	N/A	1.45	3.77E-01	4.43E+05	1.31				0	1950.29	N/A		
103-0.5H(DL10)	Unknown	10	7.54E+04	N/A	1.45	1.69E-01	4.47E+05	1.31				0	869.38	N/A		
103-1H	Unknown	1	2.57E+05	N/A	1.45	5.64E-01	4.56E+05	1.31				0	292.119	N/A		
103-2H	Unknown	1	1.75E+05	N/A	1.45	3.87E-01	4.52E+05	1.31				0	200.444	N/A		
103-4H	Unknown	1	9.36E+04	N/A	1.45	2.12E-01	4.42E+05	1.31				0	109.348	N/A		
103-8H	Unknown	1	2.88E+04	N/A	1.45	6.25E-02	4.60E+05	1.31				0	31.8652	N/A		
103-24H	Unknown	1	3.67E+03	N/A	1.44	8.20E-03	4.47E+05	1.31				0	3.64665	N/A		
201-0.25H(DL10)	Unknown	10	1.77E+05	N/A	1.44	3.93E-01	4.51E+05	1.3				0	2035.9	N/A		
201-0.5H(DL10)	Unknown	10	1.13E+05	N/A	1.44	2.48E-01	4.54E+05	1.3				0	1283.93	N/A		
201-1H(DL10)	Unknown	10	9.87E+04	N/A	1.44	2.24E-01	4.41E+05	1.3				0	1155.36	N/A		
201-2H(DL10)	Unknown	10	4.58E+04	N/A	1.45	1.04E-01	4.39E+05	1.31				0	535.803	N/A		
201-4H(DL10)	Unknown	10	2.93E+04	N/A	1.44	6.51E-02	4.51E+05	1.3				0	331.887	N/A		
201-6H	Unknown	1	2.39E+05	N/A	1.44	5.27E-01	4.53E+05	1.31				0	273.135	N/A		
201-8H	Unknown	1	2.36E+05	N/A	1.45	5.18E-01	4.56E+05	1.31				0	268.503	N/A		
201-24H	Unknown	1	4.86E+04	N/A	1.45	1.05E-01	4.64E+05	1.31				0	53.7468	N/A		
202-0.25H(DL10)	Unknown	10	1.30E+05	N/A	1.45	2.84E-01	4.57E+05	1.31				0	1468.51	N/A		
202-0.5H(DL10)	Unknown	10	8.68E+04	N/A	1.45	1.93E-01	4.49E+05	1.32				0	996.664	N/A		
202-1H(DL10)	Unknown	10	9.26E+04	N/A	1.45	2.08E-01	4.45E+05	1.31				0	1074.61	N/A		
202-2H(DL10)	Unknown	10	6.34E+04	N/A	1.45	1.40E-01	4.52E+05	1.32				0	721.229	N/A		
202-4H(DL10)	Unknown	10	3.84E+04	N/A	1.44	8.65E-02	4.44E+05	1.31				0	442.845	N/A		
202-6H	Unknown	1	4.44E+05	N/A	1.45	9.78E-01	4.53E+05	1.32				0	507.478	N/A		

202-8H	Unknown	1	3.07E+05	N/A	1.45	6.76E-01	4.55E+05	1.31	0	350.208	N/A
202-24H	Unknown	1	2.85E+04	N/A	1.45	6.18E-02	4.61E+05	1.31	0	31.4634	N/A
203-0.25H(DL10)	Unknown	10	9.84E+04	N/A	1.44	2.18E-01	4.51E+05	1.31	0	1127.59	N/A
203-0.5H(DL10)	Unknown	10	9.33E+04	N/A	1.45	2.08E-01	4.48E+05	1.31	0	1075.74	N/A
203-1H(DL10)	Unknown	10	7.99E+04	N/A	1.45	1.80E-01	4.44E+05	1.31	0	927.94	N/A
203-2H(DL10)	Unknown	10	3.62E+04	N/A	1.45	7.94E-02	4.55E+05	1.31	0	406.226	N/A
203-4H(DL10)	Unknown	10	3.89E+04	N/A	1.45	8.59E-02	4.53E+05	1.31	0	439.76	N/A
203-6H	Unknown	1	3.65E+05	N/A	1.45	7.83E-01	4.66E+05	1.31	0	405.859	N/A
203-8H	Unknown	1	3.31E+05	N/A	1.45	7.33E-01	4.52E+05	1.32	0	379.794	N/A
203-24H	Unknown	1	9.51E+03	N/A	1.45	2.05E-02	4.64E+05	1.32	0	10.0372	N/A
WASH	Unknown	1	0.00E+00	N/A	0	#DIV/0!	0.00E+00	0	0	#DIV/0!	N/A
WASH	Unknown	1	0.00E+00	N/A	0	#DIV/0!	0.00E+00	0	0	#DIV/0!	N/A
WASH	Unknown	1	0.00E+00	N/A	0	#DIV/0!	0.00E+00	0	0	#DIV/0!	N/A
QCL2	Quality Control	1	4.66E+03	6	1.45	1.06E-02	4.39E+05	1.32	1	0	4.9056 81.76
QCM2	Quality Control	1	1.27E+05	160	1.45	2.86E-01	4.46E+05	1.32	1	0	147.773 92.3582
QCH2	Quality Control	1	1.25E+06	1600	1.45	2.80E+00	4.48E+05	1.32	1	0	1455.11 90.9442
DQC2(DL10)	Quality Control	10	8.00E+05	10000	1.45	1.76E+00	4.55E+05	1.32	1	0	9119.59 91.1959

Group 1

Test Article: CCF 22-01

Vehicle:5% DMSO+95% (10% HP-b-CD in saline)

Route:IV

Dosage Level:1mg/kg

Dosage Volume:2mL/kg

Theoretical Conc.:0.5mg/mL

Ingredient	ID Number	Supposed Quantity	Practical Quantity	Preparation			
CCF 22-01	PK221635	33.21mg	33.27mg	Correct calculation		60*0.5*447.85/42	
DMSO	20210302	3mL	3mL	Vortex	NA	Sonicate	5min
				other	Stir: 2min		
10% HP-b-CD in saline	220601-07	57mL	57mL	Vortex	NA	Sonicate	NA
				other	Stir: 2min		

The PH was measured using PH paper as/pH: ~7

Amount of Preparation: 60 mL

The physical appearance was recorded as: colorless clear solution

Group 2

Test Article: CCF 22-01

Vehicle:5% DMSO+95% (10% HP-b-CD in saline)

Route:PO

Dosage Level:10mg/kg

Dosage Volume:5mL/kg

Theoretical Conc.:2mg/mL

Ingredient	ID Number	Supposed Quantity	Practical Quantity	Preparation			
CCF 22-01	PK221635	332.1mg	333.0mg	Correct calculation		150*2*447.85/42	
DMSO	20210302	7.5mL	7.5mL	Vortex	NA	Sonicate	5min
				other	Stir: 2min		
10% HP-b-CD in saline	220601-07	142.5mL	142.5mL	Vortex	NA	Sonicate	NA
				other	Stir: 2min		

Analytical method

Instrument	LC-MS/MS-12 (TQ5500)		
Matrix	Dog Plasma		
Analyte(s)	CCF22-01		
Internal standard(s)	Tolbutamide (IS)		
MS conditions	ESI: Positive		
	MRM detection		
	CCF22-01:	Q1/Q3 Masses: 426.10/171.00 Da	
	IS:	Q1/Q3 Masses: 271.10/172.00 Da	
HPLC conditions	Mobile phase:		
	Mobile phase A: 0.1%Formic acid in water		
	Mobile phase B: 0.1%Formic acid in acetonitrile		
	weak wash:50% MeOH/Water(v:v=1:1)		
	strong wash:IPA:ACN:MeOH:0.1%FA in water(v:v:v:v,1:1:1:1)		
	Time (min)	Moblie phase B (%)	
	0.01	10	
	0.20	40	
	0.60	40	
	1.20	90	
	1.50	90	
	1.51	10	
	1.80	stop	
	Column: ACQUITY UPLC BEH Phenyl 1.7um 2.1*50mm		
	Oven: 40°C		
	Flow rate: 0.60 mL/min		
	Retention time:		
CCF22-01:	1.45min		
IS:	1.32min		

Oven: 40°C
Flow rate: 0.60 mL/min
Retention time:
CCF22-01: 1.45min
IS: 1.32min

Sample preparation An aliquot of 40 µL plasma sample was protein precipitated with 400 µL MeOH in which contains 100 ng/mL IS. The mixture was vortexed for 1 min and centrifuged at 18000g for 7 min. Transfer 400 µL supernatant to 96 well plates.An aliquot of 1 µL supernatant was injected for LC-MS/MS analysis.

Calibration curve 2-2000ng/mL for CCF22-01 in Dog Plasma samples

SD curves of CCF 22-01 in dog plasma			
SD sample	Anal. Conc. (ng/mL)	Calculated Conc. (ng/mL)	Accuracy (%)
STD1-01	2.00	2.05	102.70
STD1-02	5.00	4.67	93.46
STD1-03	10.00	9.88	98.75
STD1-04	50.00	51.25	102.51
STD1-05	100.00	103.47	103.47
STD1-06	500.00	498.11	99.62
STD1-07	1000.00	999.54	99.95
STD1-08	2000.00	1990.80	99.54

*: The calculated value that was not within 80% to 120% of the theoretical value was excluded from calibration curve

QC samples of CCF 22-01 in dog plasma			
SD sample	Anal. Conc. (ng/mL)	Calculated Conc. (ng/mL)	Accuracy (%)
QCL1	6.00	6.03	100.50
QCM1	160.00	157.45	98.41

QCH1	1600.00	1553.16	97.07
DQC1(DL10)	10000.00	9928.52	99.29
QCL2	6.00	4.91	81.76
QCM2	160.00	147.77	92.36
QCH2	1600.00	1455.11	90.94
DQC2(DL10)	10000.00	9119.59	91.20

*: The calculated value that was not within 80% to 120% of the theoretical value was excluded from calibration curve

Description of Test Articles

TA	Lot/Batch No	Storage Condition	MW of Free Form	MW of Salt Form	Purity	Salt, Ester or Free Form
CCF 22-01	1	4°C	425.87	447.85	>95%	sodium salt of carboxylic acid

Note: the test article is prepared as free form concentration. The purity and salt factor need to adjustment.

Test Article Preparation

Formulation	IV	5% DMSO+95% (10% HP-β-CD in saline)
	PO	

Test System

Species and Strain: Beagle dog (Non-naïve)

Source: Testing Facility Colony, 999M-004(non-naïve).

Original source: Beijing Marshall Biotechnology Co. Ltd, Beijing, China

Number of Animals, transferred, 7 Male dog; **used, 6 Male dog.**

Selection for Study: No formal randomization will be required.

Study Design

Group	TA	No.	Dose Level	Dose Conc.	Dose Volume	Route of Dosing	Collection
		Male**	(mg/kg)	(mg/mL)	(mL/kg)		
1	CCF 22-01	3	1	0.5	2	IV	Plasma
2		3	10	2	5	PO*	Plasma

** n=3. * The animals will be fasted overnight (10-18 hours) to PO dosing. Notably, food supply to the animals will be resumed 4 hours post-dose.

Route of Administration

The test articles will be administered via intravenous injection or oral administration.

Collection Intervals

IV: post-dose at 0.083 h, 0.25 h, 0.5 h, 1 h, 2 h, 4 h, 8 h and 24 h.

PO: post-dose at 0.25 h, 0.5 h, 1 h, 2 h, 4 h, 6 h, 8 h and 24 h.

Blood Sample Collection and Procedure

Blood samples (approximately 1 mL/sample at each time point) will be collected from the jugular vein or other suitable vein. 1.0 mL/time point.

Sample will be placed in tubes containing **K2-EDTA** and stored on ice until centrifuged. The blood samples will be centrifuged at 2200 g for 10 minutes at 2-8°C within 1h after collected and stored frozen at approximately -80°C.

Sample Analysis and Data Processing

The analytical results will be confirmed using quality control samples for intra-assay variation. The accuracy of >66.7% of the quality control samples should be between 80 - 120% of the known value(s).

Standard set of parameters including Area Under the Curve ($AUC_{(0-t)}$ and $AUC_{(0-\infty)}$), elimination half-live ($T_{1/2}$), maximum plasma concentration (C_{max}), time to reach maximum plasma concentration (T_{max}) will be calculated using noncompartmental analysis modules in FDA certified pharmacokinetic program Phoenix WinNonlin 7.0 (Pharsight, USA) by the Study Director.

Disposition of Remaining Compounds and Remaining Biological Samples

The remaining compounds and remaining biological samples will be discarded after one month unless requested.

Appendix 3

Study number	20034-22003
Test article	CCF 22-01
Purpose	To determine PK profiles of CCF 22-01 after IV & PO administrations to Cynomolgus Monkey
GLP compliance	Non-GLP
Sponsor	The Cleveland Clinic Foundation
Study director	Xiaomei Ren
In-life scientists	Yufeng Meng
Formulation	Chenchen Qi
Bioanalytical	Wei Zheng
Effective date	22 th Jun 2022

		HL	Lambda z(h)	Tmax(h)	Cmax(ng/mL)	UClast(h*ng/mL)	CINF obs(h*ng/m	MRTlast(h)	MRTINF obs(h)
G1	Administratio	Dose Level	T _{1/2}	T _{max}	C _{max}	AUC _(0-t)	AUC _(0-∞)	MRT _(0-t)	MRT _(0-∞)
Animal No.	n Route	mg/kg	h	h	ng/mL	h*ng/mL	h*ng/mL	h	h
101	IV	1.0	9.25	0.083	3353.24	1201.55	1279.97	1.92	4.09
102	IV	1.0	19.75	0.083	5632.33	1869.21	2098.94	1.63	7.20
103	IV	1.0	10.39	0.083	6395.74	2093.27	2155.82	1.04	2.14
n		3	3	3	3	3	3	3	3
G1-Mean		1.0	13.13	0.083	5127.10	1721.35	1844.91	1.53	4.48
G1-SD		0.0	5.76	0.00	1582.92	463.88	490.08	0.45	2.55

Peak Name: Verapamil (IS)
Use as Internal Standard
Q1/Q3 Masses: 455.30/165.20 Da

Peak Name: CCF 22-01_2
Internal Standard: Verapamil (IS)
Q1/Q3 Masses: 426.10/171.00 Da

Fit Linear Weighting 1 / (x * x) Iterate No
Intercept 0.000637
Slope 0.00127
Correlation coefficient 0.9989
Use Area

Sample Name	Sample Type	Dilution Factor	Analyte Peak	Analyte Concentration	Retention Time	Area	Ratio	IS Peak Area	IS Retention Time	Use Record	Record	Mean	Calculated	Accuracy (%)
Blank	Double Blank	1	0.00E+00	0	0	#DIV/0!	0.00E+00	0				0	N/A	N/A
Blank+IS	Blank	1	0.00E+00	0	0	0.00E+00	1.85E+06	0.821				0	N/A	N/A
STD1-01	Standard	1	5.54E+03	2	0.995	3.07E-03	1.80E+06	0.822		1	0	1.92045	96.0225	
STD1-02	Standard	1	1.36E+04	5	0.994	7.46E-03	1.83E+06	0.822		1	0	5.38242	107.648	
STD1-03	Standard	1	2.59E+04	10	0.995	1.40E-02	1.85E+06	0.82		1	0	10.5171	105.171	
STD1-04	Standard	1	1.16E+05	50	1	6.29E-02	1.84E+06	0.825		1	0	49.0919	98.1838	
STD1-05	Standard	1	2.34E+05	100	0.995	1.26E-01	1.86E+06	0.821		1	0	98.762	98.762	
STD1-06	Standard	1	1.12E+06	500	0.994	6.14E-01	1.82E+06	0.819		1	0	483.668	96.7337	
STD1-07	Standard	1	2.21E+06	1000	1	1.23E+00	1.80E+06	0.829		1	0	969.619	96.9619	
STD1-08	Standard	1	4.57E+06	2000	1	2.55E+00	1.79E+06	0.828		1	0	2010.34	100.517	
wash	Unknown	1	1.55E+04	N/A	0.994	#DIV/0!	0.00E+00	0			0	#DIV/0!	N/A	
wash	Unknown	1	5.83E+03	N/A	1	#DIV/0!	0.00E+00	0			0	#DIV/0!	N/A	
wash	Unknown	1	2.21E+03	N/A	0.994	#DIV/0!	0.00E+00	0			0	#DIV/0!	N/A	
QCL1	Quality Control	1	1.44E+04	6	1	8.01E-03	1.79E+06	0.827		1	0	5.81457	96.9094	
QCM1	Quality Control	1	1.78E+06	800	0.998	9.81E-01	1.82E+06	0.824		1	0	773.4	96.675	
QCH1	Quality Control	1	3.59E+06	1600	1	1.98E+00	1.82E+06	0.83		1	0	1560.22	97.5141	
DQC1(DL10)	Quality Control	10	1.15E+06	5000	0.999	6.32E-01	1.82E+06	0.825		1	0	4980.53	99.6105	
wash	Unknown	1	6.85E+03	N/A	0.996	#DIV/0!	0.00E+00	0			0	#DIV/0!	N/A	
wash	Unknown	1	2.61E+03	N/A	0.995	#DIV/0!	0.00E+00	0			0	#DIV/0!	N/A	
wash	Unknown	1	1.91E+03	N/A	0.999	#DIV/0!	0.00E+00	0			0	#DIV/0!	N/A	
101-0.083h(DL10)	Unknown	10	7.69E+05	N/A	0.998	4.26E-01	1.81E+06	0.823			0	3353.24	N/A	
101-0.25h(DL10)	Unknown	10	1.39E+05	N/A	1	7.53E-02	1.84E+06	0.826			0	589.011	N/A	
101-0.5h(DL10)	Unknown	10	3.44E+04	N/A	0.999	1.89E-02	1.82E+06	0.826			0	143.801	N/A	
101-1h	Unknown	1	8.53E+04	N/A	1	4.66E-02	1.83E+06	0.829			0	36.2341	N/A	
101-2h	Unknown	1	6.33E+04	N/A	0.996	3.46E-02	1.83E+06	0.823			0	26.7721	N/A	
101-4h	Unknown	1	6.04E+04	N/A	1	3.33E-02	1.81E+06	0.827			0	25.7918	N/A	
101-8h	Unknown	1	2.06E+04	N/A	0.996	1.12E-02	1.83E+06	0.822			0	8.3662	N/A	
101-24h	Unknown	1	1.53E+04	N/A	1	8.08E-03	1.89E+06	0.826			0	5.87299	N/A	
102-0.083h(DL10)	Unknown	10	1.33E+06	N/A	1	7.15E-01	1.86E+06	0.827			0	5632.33	N/A	
102-0.25h(DL10)	Unknown	10	2.68E+05	N/A	1	1.47E-01	1.82E+06	0.826			0	1154.84	N/A	
102-0.5h(DL10)	Unknown	10	4.98E+04	N/A	1	2.75E-02	1.81E+06	0.829			0	212.132	N/A	
102-1h	Unknown	1	1.27E+05	N/A	1	6.80E-02	1.87E+06	0.826			0	53.1514	N/A	
102-2h	Unknown	1	7.84E+04	N/A	0.995	4.24E-02	1.85E+06	0.822			0	32.9632	N/A	
102-4h	Unknown	1	4.21E+04	N/A	0.998	2.25E-02	1.87E+06	0.824			0	17.2653	N/A	
102-8h	Unknown	1	3.06E+04	N/A	0.998	1.65E-02	1.85E+06	0.824			0	12.5472	N/A	
102-24h	Unknown	1	2.10E+04	N/A	1	1.09E-02	1.93E+06	0.827			0	8.06232	N/A	
103-0.083h(DL10)	Unknown	10	1.51E+06	N/A	1	8.12E-01	1.86E+06	0.826			0	6395.74	N/A	
103-0.25h(DL10)	Unknown	10	4.35E+05	N/A	0.997	2.35E-01	1.85E+06	0.823			0	1852.11	N/A	
103-0.5h(DL10)	Unknown	10	6.92E+04	N/A	1	3.74E-02	1.85E+06	0.827			0	289.867	N/A	
103-1h	Unknown	1	1.67E+05	N/A	1	8.98E-02	1.87E+06	0.826			0	70.2909	N/A	
103-2h	Unknown	1	1.19E+05	N/A	0.997	6.47E-02	1.84E+06	0.823			0	50.5479	N/A	
103-4h	Unknown	1	4.34E+04	N/A	1	2.39E-02	1.81E+06	0.826			0	18.3522	N/A	
103-8h	Unknown	1	2.25E+04	N/A	0.994	1.21E-02	1.86E+06	0.82			0	9.05524	N/A	
103-24h	Unknown	1	1.14E+04	N/A	0.995	5.93E-03	1.93E+06	0.822			0	4.17356	N/A	
201-0.25h	Unknown	1	1.26E+06	N/A	1	6.95E-01	1.82E+06	0.826			0	547.285	N/A	
201-0.5h	Unknown	1	1.19E+06	N/A	0.994	6.44E-01	1.85E+06	0.821			0	507.357	N/A	
201-1h	Unknown	1	4.44E+05	N/A	0.995	2.42E-01	1.84E+06	0.821			0	190.259	N/A	
201-2h	Unknown	1	1.78E+05	N/A	0.997	9.63E-02	1.85E+06	0.823			0	75.4162	N/A	
201-4h	Unknown	1	4.02E+05	N/A	1	2.24E-01	1.79E+06	0.828			0	176.507	N/A	
201-6h	Unknown	1	6.21E+05	N/A	0.999	3.39E-01	1.83E+06	0.825			0	266.901	N/A	
201-8h	Unknown	1	3.87E+05	N/A	1	2.11E-01	1.84E+06	0.826			0	165.619	N/A	
201-24h	Unknown	1	3.40E+04	N/A	0.999	1.80E-02	1.89E+06	0.824			0	13.6598	N/A	
202-0.25h	Unknown	1	1.32E+06	N/A	0.993	7.11E-01	1.85E+06	0.819			0	560.022	N/A	
202-0.5h	Unknown	1	1.00E+06	N/A	0.999	5.58E-01	1.80E+06	0.825			0	439.168	N/A	
202-1h	Unknown	1	7.04E+05	N/A	0.999	3.87E-01	1.82E+06	0.826			0	304.317	N/A	
202-2h	Unknown	1	7.69E+05	N/A	1	4.19E-01	1.83E+06	0.828			0	329.921	N/A	
202-4h	Unknown	1	9.91E+05	N/A	0.998	5.39E-01	1.84E+06	0.823			0	424.585	N/A	
202-6h	Unknown	1	3.50E+05	N/A	0.995	1.90E-01	1.85E+06	0.821			0	149.136	N/A	

202-8h	Unknown	1	4.83E+05	N/A	1	2.63E-01	1.84E+06	0.826	0	207.185	N/A
202-24h	Unknown	1	5.73E+04	N/A	0.996	3.01E-02	1.91E+06	0.822	0	23.1993	N/A
203-0.25h	Unknown	1	5.83E+05	N/A	1	3.19E-01	1.83E+06	0.826	0	250.954	N/A
203-0.5h	Unknown	1	3.05E+03	N/A	1	#DIV/0!	0.00E+00	0	0	#DIV/0!	N/A
203-1h	Unknown	1	8.94E+05	N/A	0.995	4.86E-01	1.84E+06	0.82	0	382.672	N/A
203-2h	Unknown	1	5.07E+05	N/A	1	2.80E-01	1.81E+06	0.827	0	220.214	N/A
203-4h	Unknown	1	1.11E+06	N/A	0.998	6.00E-01	1.86E+06	0.829	0	472.604	N/A
203-6h	Unknown	1	6.60E+05	N/A	1	3.57E-01	1.85E+06	0.83	0	280.811	N/A
203-8h	Unknown	1	6.67E+05	N/A	0.997	3.60E-01	1.85E+06	0.826	0	283.797	N/A
203-24h	Unknown	1	1.19E+05	N/A	0.994	6.24E-02	1.91E+06	0.821	0	48.7088	N/A
wash	Unknown	1	2.66E+03	N/A	1	#DIV/0!	0.00E+00	0	0	#DIV/0!	N/A
wash	Unknown	1	1.19E+03	N/A	0.99	#DIV/0!	0.00E+00	0	0	#DIV/0!	N/A
wash	Unknown	1	9.69E+02	N/A	0.999	#DIV/0!	0.00E+00	0	0	#DIV/0!	N/A
QCL2	Quality Control	1	1.45E+04	6	0.994	7.89E-03	1.84E+06	0.822	1	0	5.72331 95.3885
QCM2	Quality Control	1	1.85E+06	800	0.995	1.00E+00	1.84E+06	0.823	1	0	790.382 98.7978
QCH2	Quality Control	1	3.51E+06	1600	0.994	2.00E+00	1.75E+06	0.82	1	0	1579.06 98.6914
DQC2(DL10)	Quality Control	10	1.12E+06	5000	0.995	6.18E-01	1.82E+06	0.821	1	0	4871.92 97.4385

Peak Name: Verapamil (IS)
 Use as Internal Standard
 Q1/Q3 Masses: 455.30/165.20 Da

Peak Name: CCF 22-01_2
 Internal Standard: Verapamil (IS)
 Q1/Q3 Masses: 426.10/171.00 Da

Fit	Linear	Weighting 1 / (x * x)	Iterate	No
Intercept	0.000741			
Slope	0.00128			
Correlation coefficient	0.999			
Use Area				

Sample Name	Sample Type	Dilution	Fc	Analyte Pe	Analyte Cc	Analyte Rc	Area Ratio	IS Peak Ar	IS Retentic	Use	Record	Record	Mc	Calculated	Accuracy (%)
Blank	Double Blank	1	0.00E+00	0	0	#DIV/0!	0.00E+00	0					0	N/A	N/A
Blank+IS	Blank	1	0.00E+00	0	0	0.00E+00	1.79E+06	0.822					0	N/A	N/A
STD2-01	Standard	1	5.76E+03	2	1	3.23E-03	1.78E+06	0.826		1	0	1.93828	96.9139		
STD2-03	Standard	1	2.59E+04	10	0.993	1.44E-02	1.80E+06	0.82		1	0	10.6148	106.148		
STD2-05	Standard	1	2.34E+05	100	1	1.33E-01	1.76E+06	0.83		1	0	103.072	103.072		
STD2-07	Standard	1	2.29E+06	1000	0.996	1.27E+00	1.79E+06	0.822		1	0	991.698	99.1698		
wash	Unknown	1	4.62E+03	N/A	0.991	#DIV/0!	0.00E+00	0			0	#DIV/0!	N/A		
wash	Unknown	1	2.21E+03	N/A	1	#DIV/0!	0.00E+00	0			0	#DIV/0!	N/A		
wash	Unknown	1	0.00E+00	N/A	0	#DIV/0!	0.00E+00	0			0	#DIV/0!	N/A		
203-0.5h	Unknown	1	1.71E+05	N/A	1	9.36E-02	1.83E+06	0.828			0	72.3011	N/A	Average	
203-0.5h	Unknown	1	1.73E+05	N/A	1	9.25E-02	1.87E+06	0.828			0	71.4502	N/A	71.87565	
wash	Unknown	1	1.73E+03	N/A	1	#DIV/0!	0.00E+00	0			0	#DIV/0!	N/A		
wash	Unknown	1	0.00E+00	N/A	0	#DIV/0!	0.00E+00	0			0	#DIV/0!	N/A		
wash	Unknown	1	1.40E+03	N/A	1.01	#DIV/0!	0.00E+00	0			0	#DIV/0!	N/A		
STD2-02	Standard	1	1.37E+04	5	0.993	7.47E-03	1.83E+06	0.82		1	0	5.23845	104.769		
STD2-04	Standard	1	1.18E+05	50	0.995	6.35E-02	1.86E+06	0.822		1	0	48.8594	97.7189		
STD2-06	Standard	1	1.15E+06	500	0.998	6.17E-01	1.87E+06	0.825		1	0	480.266	96.0533		
STD2-08	Standard	1	4.53E+06	2000	1	2.47E+00	1.83E+06	0.828		1	0	1923.1	96.1548		

Group 1

Test Article: CCF 22-01

Vehicle:5% DMSO+95% (10% HP-b-CD in saline)

Route:IV

Dosage Level:1mg/kg

Dosage Volume:2mL/kg

Theoretical Conc.:0.5mg/mL

Ingredient	ID Number	Supposed Quantity	Practical Quantity	Preparation			
CCF 22-01	PK221874	19.93mg	20.09mg	Correct calculation		36*0.5*447.85/42	
DMSO	20210302	1.8mL	1.8mL	Vortex	1min	Sonicate	10min
				other	NA		
10% HP-b-CD in saline	220619-01	34.2mL	34.2mL	Vortex	1min	Sonicate	NA
				other	NA		

The PH was measured using PH paper as/pH: ~7

Amount of Preparation: 36 mL

The physical appearance was recorded as: colorless clear solution

Group 2

Test Article: CCF 22-01

Vehicle:5% DMSO+95% (10% HP-b-CD in saline)

Route:PO

Dosage Level:10mg/kg

Dosage Volume:5mL/kg

Theoretical Conc.:2mg/mL

Ingredient	ID Number	Supposed Quantity	Practical Quantity	Preparation			
CCF 22-01	PK221874	185.97mg	186.53mg	Correct calculation		84*2*447.85/425	
DMSO	20210302	4.2mL	4.2mL	Vortex	1min	Sonicate	10min
				other	NA		
10% HP-b-CD in saline	220619-01	79.8mL	79.8mL	Vortex	1min	Sonicate	NA
				other	NA		

Analytical method

Instrument	LC-MS/MS-DESKTOP-C8081MD (TQ6500)		
Matrix	Cynomolgus Monkey Plasma		
Analyte(s)	CCF 22-01		
Internal standard(s)	Verapamil (IS)		
MS conditions	ESI: Positive		
	MRM detection		
	CCF 22-01	Q1/Q3 Masses: 426.10/171.00 Da	
	IS:	Q1/Q3 Masses: 455.30/165.20 Da	
HPLC conditions	Mobile phase:		
	Mobile phase A: 0.1%FA in H2O		
	Mobile phase B: 0.1%FA in ACN		
	weak wash:50% MeOH/Water(v:v=1:1)		
	strong wash:IPA:ACN:MeOH:0.1%FA in water(v:v:v:v,1:1:1:1)		
	Time (min)	Moblie phase B (%)	
	0.01	10	
	0.60	90	
	1.10	90	
	1.11	10	
	1.40	10	
	Column: ACQUITY UPLC BEH C18 1.7um 2.1*50mm		
	Oven: 40°C		
	Flow rate: 0.60 mL/min		
	Retention time:		
	CCF 22-01	1.00min	
	IS:	0.82min	
Sample preparation	An aliquot of 50 µL plasma sample was protein precipitated with 500 µL MEOH in which contains 10 ng/mL IS. The mixture was vortexed for 1 min and centrifuged at 18000g for 7 min. Transfer 500 µL supernatant to 96 well plates. An aliquot of 1 µL supernatant was injected for LC-MS/MS analysis		
Calibration curve	2-2000 ng/mL for CCF 22-01 in Cynomolgus Monkey Plasma samples		

SD curves of CCF 22-01 in monkey plasma			
SD sample	Anal. Conc. (ng/mL)	Calculated Conc. (ng/mL)	Accuracy (%)
STD1-01	2.00	1.92	96.02
STD1-02	5.00	5.38	107.65
STD1-03	10.00	10.52	105.17
STD1-04	50.00	49.09	98.18
STD1-05	100.00	98.76	98.76
STD1-06	500.00	483.67	96.73
STD1-07	1000.00	969.62	96.96
STD1-08	2000.00	2010.34	100.52

*: The calculated value that was not within 80% to 120% of the theoretical value was excluded from calibration curve

QC samples of CCF 22-01 in monkey plasma			
SD sample	Anal. Conc. (ng/mL)	Calculated Conc. (ng/mL)	Accuracy (%)
QCL1	6.00	5.81	96.91
QCM1	800.00	773.40	96.68
QCH1	1600.00	1560.22	97.51

DQC1(DL10)	5000.00	4980.53	99.61
QCL2	6.00	5.72	95.39
QCM2	800.00	790.38	98.80
QCH2	1600.00	1579.06	98.69
DQC2(DL10)	5000.00	4871.92	97.44

*: The calculated value that was not within 80% to 120% of the theoretical value was excluded from calibration curve

SD curves of CCF 22-01 in monkey plasma			
SD sample	Anal. Conc. (ng/mL)	Calculated Conc. (ng/mL)	Accuracy (%)
STD2-01	2.00	1.94	96.91
STD2-02	5.00	5.24	104.77
STD2-03	10.00	10.61	106.15
STD2-04	50.00	48.86	97.72
STD2-05	100.00	103.07	103.07
STD2-06	500.00	480.27	96.05
STD2-07	1000.00	991.70	99.17
STD2-08	2000.00	1923.10	96.15

*: The calculated value that was not within 80% to 120% of the theoretical value was excluded from calibration curve

Description of Test Articles

TA	Lot/Batch No	Storage Condition	MW of Free Form	MW of Salt Form	Purity	Salt, Ester or Free Form
CCF 22-01	1	4°C	425.87	447.85	>95%	sodium salt of carboxylic acid

Note: the test article is prepared as free form concentration.The purity and salt factor need to adjustment.

Test Article Preparation

Formulation	IV	5% DMSO+95% (10% HP-β-CD in saline)
	PO	

Test System

Species and Strain: Cynomolgus Monkey(non-naïve)
Source: Medicilon Colony: 999M-014.
Number of Animals,transferred, 7 male ; **used, 6 male.**
Selection for Study:No formal randomization will be required.

Study Design

Group	TA	No.	Dose Level	Dose Conc.	Dose Volume	Route of Dosing	Collection
		Male**	(mg/kg)	(mg/mL)	(mL/kg)		
1	CCF 22-01	3	1	0.5	2	IV	Plasma
2		3	10	2	5	PO*	Plasma

** n=3. * The animals will be fasted overnight (10-18 hours) to PO dosing. Notably, food supply to the animals will be resumed 4 hours post-dose.

Route of Administration

The test articles will be administered via intravenous injection or oral administration.

Collection Intervals

IV: post-dose at 0.083 h, 0.25 h, 0.5 h, 1 h, 2 h, 4 h, 8 h and 24 h.
PO: post-dose at 0.25 h, 0.5 h, 1 h, 2 h, 4 h, 6 h, 8 h and 24 h.

Blood Sample Collection and Procedure

The blood will be taken via femoral vein or other suitable vein, 1.0 mL/time point. Sample will be placed in tubes containing **K2-EDTA** and stored on ice until centrifuged. The blood samples will be centrifuged at 2200 g for 10 minutes at 2-8°C within 1h after collected and stored frozen at approximately -80°C.

Sample Analysis and Data Processing

The analytical results will be confirmed using quality control samples for intra-assay variation. The accuracy of >66.7% of the quality control samples should be between 80 - 120% of the known value(s).

Standard set of parameters including Area Under the Curve (AUC_(0-t) and AUC_(0-∞)), elimination half-live (T_{1/2}), maximum plasma concentration (C_{max}), time to reach maximum plasma concentration (T_{max}) will be calculated using noncompartmental analysis modules in FDA certified pharmacokinetic program Phoenix WinNonlin 7.0 (Pharsight, USA) by the Study Director.

Disposition of Remaining Compounds and Remaining Biological Samples

The remaining compounds and remaining biological samples will be discarded after one month unless requested.

Appendix 4

REPORT

Study Period | 15 Dec 2022 - 10 Feb 2023

STUDY OBJECTIVE:

Client Name: Cleveland Clinic Foundation

Compound Names: CCF9002, CCF8201, CCF8205, CCF8209, CCF8208

Tested in IonChannelProfiler™ CardiacProfiler Core Panel

STUDY INFORMATION:

Study code#: US034-0018738

Revised Report

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STUDY
APPROVAL

I certify that this report accurately reflects all relevant data collected in this study.



Audrey Rizor, Associate Scientist

I certify that the results presented in this report were generated using materials and methods mentioned and that these results accurately reflect the raw data.



Jennifer Wesley, Lab Supervisor
Quality statement

Compliance
Statement

Eurofins Discovery adheres to industry best practices for data quality and integrity. This study was conducted within a non-GLP framework and laboratory.

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COMPOUND INFORMATION

Test compounds

Eurofins Compound I.D.	Client Compound I.D.	Client Reference I.D.	MW + Salt	Volume (μ L)	Concentration (mg/mL)	Received condition
US034-0018738-1	CCF9002			10	200	Liquid
US034-0018738-2	CCF8201			10	200	Liquid
US034-0018738-3	CCF8205			10	200	Liquid
US034-0018738-4	CCF8209			100	40	Liquid
US034-0018738-5	CCF8208			5	200	Liquid

Reference compounds

In each experiment and if applicable, the respective reference compounds were tested concurrently with the test compounds, and the data were compared with historical values determined at Eurofins. The experiment was accepted in accordance with Eurofins Standard Operating Procedure on assay validation.

SUMMARY

Ion Channels tested

Panel Part Number: CYL8004QB2, CYL8051QP2, CYL8038QB2

Voltage-Gated Sodium: HEK-Nav1.5

Voltage-Gated Calcium: HEK-Cav1.2

Voltage-Gated Potassium: CHO-hERG

Study objective

Electrophysiological assays conducted to profile five (5) compounds for activities on the ion channel targets specified above using the Qube electrophysiological platform.

Measurements

Methods employed in this study have been developed and validated with reliability and reproducibility. Assays were performed under conditions described in the accompanying "Materials and Methods" section of this report.

Where presented, IC_{50} values were determined by a non-linear, least squares regression analysis. Reference standards were run as an integral part of each assay to ensure the validity of the results obtained.

Significant results

Results showing an inhibition greater than 50% are considered to represent significant effects of test compounds and listed in the following tables with individual calculation results.

EXPERIMENTAL RESULTS

CardiacProfiler Core Panel Data Tables

Qube Assay Data Tables

hNav1.5

Compound ID	Client Compound ID	Batch Number	Concentration	% inhibition		
				n1	n2	mean
US034-0018738-1	CCF9002		20µg/ml	3.04	4.06	3.55
US034-0018738-2	CCF8201		7µg/ml	4.12	3.64	3.88
US034-0018738-3	CCF8205		67µg/ml	5.67	3.79	4.73
US034-0018738-4	CCF8209		45µg/ml	3.50	6.27	4.88
US034-0018738-5	CCF8208		67µg/ml	4.85	7.03	5.94
Time-Matched Vehicle Control	0.33% DMSO Addition 1			3.16	7.35	5.26
Positive Reference Control	Tetracaine		0.003µM	2.06	4.58	3.32
Positive Reference Control	Tetracaine		0.03µM	12.20	12.55	12.38
Positive Reference Control	Tetracaine		0.3µM	39.25	36.78	38.01
Positive Reference Control	Tetracaine		3µM	79.38	77.62	78.50
Positive Reference Control	Tetracaine		30µM	92.04	90.87	91.46
Positive Reference Control	Tetracaine		300µM	99.50	97.62	98.56

hERG

Compound ID	Client Compound ID	Batch Number	Concentration	% inhibition		
				n1	n2	mean
US034-0018738-1	CCF9002		20µg/ml	-0.42	-0.63	-0.53
US034-0018738-2	CCF8201		7µg/ml	1.81	6.30	4.05
US034-0018738-3	CCF8205		67µg/ml	4.12	5.42	4.77
US034-0018738-4	CCF8209		45µg/ml	4.66	0.38	2.52
US034-0018738-5	CCF8208		67µg/ml	-1.01	-1.10	-1.05
Time-Matched Vehicle Control	0.33% DMSO Addition 1			4.33	6.93	5.63
Positive Reference Control	Verapamil		0.01µM	5.23	10.96	8.10
Positive Reference Control	Verapamil		0.03µM	12.60	19.83	16.22
Positive Reference Control	Verapamil		0.1µM	19.37	24.62	21.99
Positive Reference Control	Verapamil		0.3µM	36.10	42.45	39.27
Positive Reference Control	Verapamil		1µM	56.62	63.39	60.00
Positive Reference Control	Verapamil		3µM	79.80	88.01	83.90

QPatch Assay Data Table

hCav1.2

Compound ID	Client Compound ID	Batch Number	Concentration	% inhibition		
				n1	n2	mean
US034-0018738-1	CCF9002		20µg/ml	-5.28	-8.49	-6.89
US034-0018738-2	CCF8201		7µg/ml	10.30	4.89	7.59
US034-0018738-3	CCF8205		67µg/ml	-12.32	-15.92	-14.12
US034-0018738-4	CCF8209		45µg/ml	9.47	-10.34	-0.44
US034-0018738-5	CCF8208		67µg/ml	-15.66	-6.87	-11.27
Time-Matched Vehicle Control	0.33% DMSO Addition 1			0.24	7.40	3.82
Positive Reference Control	Nifedipine		0.001µM	-9.00	-3.47	-6.23
Positive Reference Control	Nifedipine		0.01µM	10.74	9.10	9.92
Positive Reference Control	Nifedipine		0.1µM	41.59	31.15	36.37
Positive Reference Control	Nifedipine		1µM	66.02	61.88	63.95
Positive Reference Control	Nifedipine		10µM	75.65	68.50	72.08
Positive Reference Control	Nifedipine		100µM	87.63	86.76	87.19

REFERENCE COMPOUND RESULTS

CardiacProfiler Core Reference Compound Panel

ITEM	Assay Name	Mode	Reference Compound	Estimated IC ₅₀ (μM)
CYL8004QB2	hNav1.5 Sodium Channel Assay Qube APC (6 conc.)	Antagonist	Tetracaine	0.56
CYL8051QP2	Cav1.2 (L-type) Human Calcium Ion Channel Cell Based QPatch CiPA Assay	Antagonist	Nifedipine	0.80
CYL8038QB2	hERG Human Potassium Channel Assay Qube APC (6 conc.)	Antagonist	Verapamil	0.49

DATA CALCULATION AND ANALYSIS

CardiacProfiler Core Panel Qube Assays

All compounds were tested in the presence of 0.1% Pluronic F-68 Non-Ionic Surfactant for all assays.

CYL8004QB2 hNav1.5 Sodium Channel Assay – Qube APC

The parameters measured were difference between the peak inward current on stepping to -15mV (i.e. peak of the current) and the leak current. All data were filtered for seal quality, seal drop, and current amplitude. The peak current amplitude was calculated before and after compound addition and the amount of block was assessed by dividing the Test compound current amplitude by the Control current amplitude. Control data is the mean Nav1.5 current amplitude collected 10 seconds at the end of the vehicle control application; Test compound data is the mean Nav1.5 current amplitude collected 10 seconds at the end of test concentration application for each concentration.

CYL8038QB2 hERG Potassium Channel Assay - Qube APC

The parameters measured were the maximum tail current evoked on stepping to 40mV and ramping back to -80mV from the test pulse. All data were filtered for seal quality, seal drop, and current amplitude. The peak current amplitude was calculated before and after compound addition and the amount of block was assessed by dividing the Test compound current amplitude by the Control current amplitude. Control data is the mean hERG current amplitude collected 15 seconds at the end of the vehicle control period; Test compound data is the mean hERG current amplitude collected 15 seconds at the end of test concentration application for each concentration.

CardiacProfiler Core Panel QPatch Assay

CYL8051QP2DR Cav1.2 (L-type) Human Calcium Ion Channel Cell Based QPatch CiPA Assay

The parameters measured were the maximum inward current evoked on stepping to 10mV from the test pulse. All data were filtered for seal quality, seal drop, and current amplitude. The peak current amplitude was calculated before and after compound addition and the amount of block was assessed by dividing the Test compound current amplitude by the Control current amplitude. Control is the mean hCav1.2 current amplitude collected 15 seconds at the end of the control; Test Compound is the mean hCav1.2 current amplitude collected in the presence of test compound at each concentration.

MATERIALS AND METHODS

CardiacProfiler Core Qube Assay Protocols

CYL8004QB2 hNav1.5 Sodium Channel Assay – Qube APC

After whole cell configuration is achieved, the cells are held at -80mV. Onset and steady state block of peak Nav1.5 current is measured using a pulse pattern consisting of a hyperpolarizing pulse to -120mV for a 200ms duration, depolarization to -15mV amplitude for a 40ms duration, followed by step to 40mV for 200ms and finally a 100ms ramp (1.2 V/s) to a holding potential of -80 mV. Peak current is measured during the step to -15 mV. This paradigm is delivered once every 5s to monitor the current amplitude.

CYL8038QB2 hERG Potassium Channel Assay - Qube APC

After whole cell configuration is achieved, the cells are held at -80 mV. Cells are held at this voltage for 50 ms to measure the leak current, which is subtracted from the hERG current on-line. The cells are depolarized to +40mV for 500ms and then to -80mV over a 100ms ramp to elicit the hERG tail current. This paradigm is delivered once every 5s to monitor the current amplitude.

CardiacProfiler Core Panel QPatch Assay Protocol

CYL8051QP2DR Cav1.2 (L-type) Human Calcium Ion Channel Cell Based QPatch CiPA Assay

After whole cell configuration is achieved, the human Cav1.2 currents are evoked from a holding potential of -90mV. Currents are evoked following a 100ms pulse to -60mV followed by a 50ms pulse to +10mV before returning to the holding potential. This paradigm is delivered three times once every 20 s to monitor the current amplitude for each compound concentration. Cells are held at -90mV with a 5s pulse to -60mV every 20s for a total of 120s between each set of three pulses. Up to three (3) test concentrations are applied sequentially on each cell.

STORAGE AND RETENTION OF RECORDS

Documents generated during the performance of the study will be archived by Eurofins Discovery for a period of time after study completion (this period of time is dependent on each individual site policy). The access to the archives is restricted to authorized employees only.

Appendix 5

REPORT

Study Period	15 Dec 22 – 19 Jan 23
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STUDY OBJECTIVE:

Client Name: Cleveland Clinic Foundation

Compound Names: Five (5) compounds

Tested in: IonChannelProfiler™ Multi Electrode Array Assay

STUDY INFORMATION:

Study Number: US034-0018738

SPONSOR
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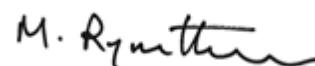
STUDY
APPROVAL

*I certify that this report accurately reflects all
relevant data collected in this study.*



Lori Forster, PhD
Scientist

*I certify that the results presented in this report
were generated using materials and methods
mentioned and that these results accurately
reflect the raw data.*



Study Director
Quality statement

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COMPOUND INFORMATION

Test compounds

Eurofins Compound I.D.	Client Compound I.D.	Client Reference I.D.	[Stock] Solvent (mg/ml)	Volume Received (μ l)	Reference
US034-0018738-1	CCF9002		200	10	
US034-0018738-2	CCF8201		200	10	
US034-0018738-3	CCF8205		200	10	
US034-0018738-4	CCF8209		200	100	
US034-0018738-5	CCF8208		200	5	

compounds

In each experiment and if applicable, the respective reference compounds were tested concurrently with the test compounds, and the data were compared with historical values determined at Eurofins. The experiment was accepted in accordance with Eurofins Standard Operating Procedure on assay validation.

SUMMARY

Assay tested

Cardiac extracellular field potential measurement by Multi Electrode Array

Cells: iCell² Cardiomyocytes Kit, 01434

Catalog number: R1017

Study objective

The Multi-electrode Array (MEA) assay was conducted to profile five (5) compounds for their effect on the extracellular field potential (FP) generated from spontaneously beating hiPSC derived cardiomyocytes, specifically whether these compounds prolong Field Potential Duration (FPD), an *in vitro* parameter indicating *in vivo* QT prolongation. Perturbations to the recorded FP waveform can be used as an unbiased method to distinguish a pure hERG blocker from balanced ion channel blocker that is a part of the Comprehensive *in vitro* Proarrhythmia assay (CiPA).

Measurements

Methods employed in this study have been developed and validated with reliability and reproducibility. Assays were performed under conditions described in the accompanying "Materials and Methods" section of this report. Reference standards were run as an integral part of each assay to ensure the validity of the results obtained.

EXPERIMENTAL RESULTS

Positive Controls Data Table

Treatment/ID	Percent Change from Baseline			
	Beat Period	FPD	FPDc (Fridericia)	Spike Amplitude
Vehicle control	16.58	-8.53	-13.09	-8.17
Vehicle control	338.96	71.10	4.50	-26.57
Vehicle control	299.21	54.76	-2.44	-10.06
Mean	218.25	39.11	-3.68	-14.93
SD	175.78	42.05	8.86	10.12
SEM	101.49	24.28	5.11	5.84
E-4031 [0.03 μ M]	342.88	153.03	54.08	-16.79
E-4031 [0.03 μ M]	500.49	553.74	259.67	-30.31
E-4031 [0.03 μ M]	256.28	176.57	81.08	-19.32
Mean	366.55	294.44	131.61	-22.14
SD	123.81	224.86	111.72	7.19
SEM	71.48	129.82	64.50	4.15
Diltiazem [3 μ M]	-13.45	-48.01	-45.44	-33.31
Diltiazem [3 μ M]	-52.34	-38.36	-21.08	-24.71
Diltiazem [3 μ M]	-54.27	-43.98	-27.28	-23.40
Mean	-40.02	-43.45	-31.27	-27.14
SD	23.03	4.85	12.66	5.38
SEM	13.30	2.80	7.31	3.11
Mexiletine [3 μ M]	245.84	42.60	-5.70	-29.74
Mexiletine [3 μ M]	303.89	56.61	-1.66	-49.95
Mexiletine [3 μ M]	338.70	78.68	9.15	-32.83
Mean	296.14	59.30	0.60	-37.50
SD	46.91	18.19	7.68	10.89
SEM	27.08	10.50	4.43	6.29

1. Beat Period is measured as the duration between the initial peaks of sequential beats.
2. The initial peak or spike indicates depolarization (spike amplitude).
3. The secondary deflection indicates repolarization or "T wave".
4. The field potential duration is taken as the duration between depolarization and repolarization (FPD).
5. Flat indicates that the repolarization peak was flat and FPD could not be measured but beat period could be measured from successive depolarization peaks.
6. Quiescent indicates cardiomyocytes have stopped beating.

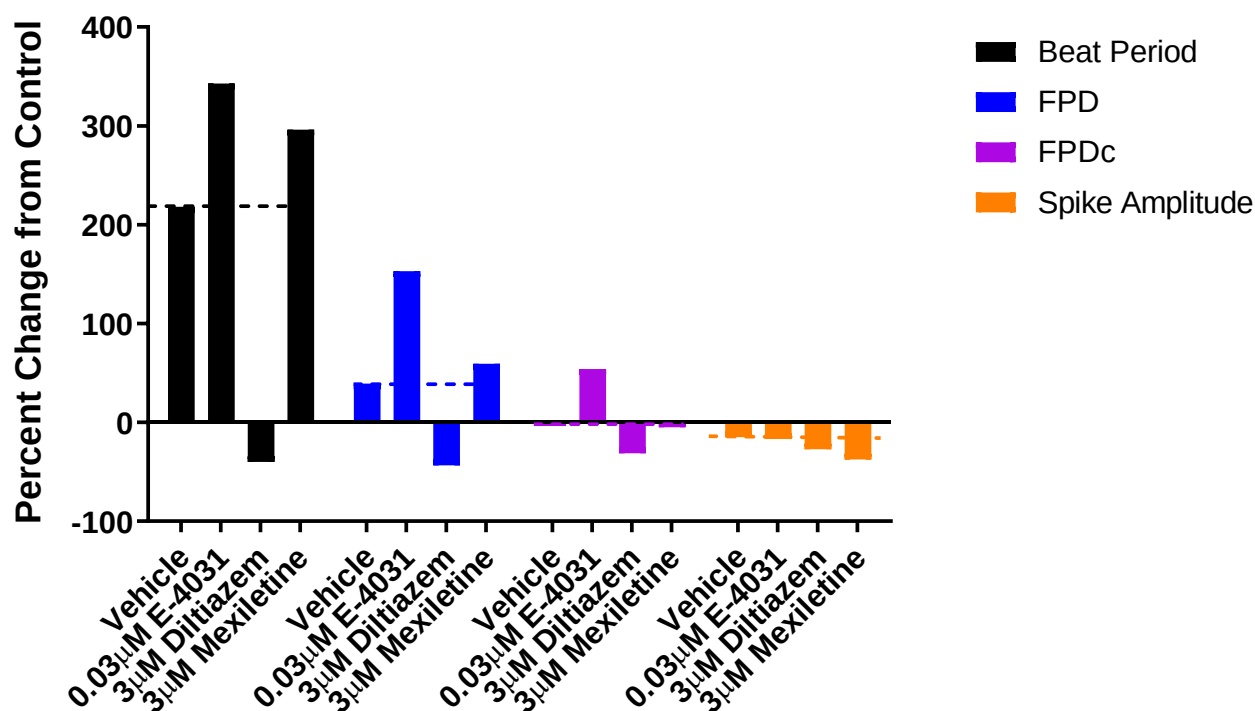
Test Compound Data Tables

Compounds were tested at their solubility limit in the Maintenance medium.

Glossary of Terms				
Quiescent indicates cardiomyocyte stopped beating				
Flat indicates "T"wave can not be seen. and measured				
Percent Change from Baseline				
Treatment/ID	Beat Period	FPD	FPDc (Fridericia)	Spike Amplitude
CCF9002 [200 µg/ml]	16.61	27.63	21.25	-35.54
CCF9002 [200 µg/ml]	28.87	33.33	22.52	-64.20
CCF9002 [200 µg/ml]	10.80	31.12	26.72	-8.54
Mean	18.76	30.69	23.50	-36.10
SD	9.23	2.88	2.86	27.84
SEM	5.33	1.66	1.65	16.07
CCF8201 [67 µg/ml]	25.01	42.42	32.21	-63.14
CCF8201 [67 µg/ml]	5.09	43.53	41.17	-44.20
CCF8201 [67 µg/ml]	18.67	37.14	29.54	-40.96
Mean	16.26	41.03	34.31	-49.43
SD	10.18	3.41	6.09	11.98
SEM	5.88	1.97	3.52	6.92
CCF8205 [200 µg/ml]	219.00	-17.97	-44.27	-57.49
CCF8205 [200 µg/ml]	318.48	-11.86	-45.30	-72.41
CCF8205 [200 µg/ml]	275.58	-4.41	-38.50	-59.28
Mean	271.02	-11.41	-42.69	-63.06
SD	49.90	6.79	3.67	8.14
SEM	28.81	3.92	2.12	4.70
CCF8209 [40 µg/ml]	21.75	12.71	5.55	-12.69
CCF8209 [40 µg/ml]	346.48	23.62	-24.93	-69.97
CCF8209 [40 µg/ml]	200.04	13.63	-14.39	-40.40
Mean	189.42	16.65	-11.26	-41.02
SD	162.63	6.05	15.48	28.65
SEM	93.89	3.49	8.94	16.54
CCF8208 [200 µg/ml]	-18.50	-4.59	2.14	-66.84
CCF8208 [200 µg/ml]	-0.42	9.95	10.11	-71.02
CCF8208 [200 µg/ml]	-0.24	5.75	5.84	-41.00
Mean	-6.39	3.70	6.03	-59.62
SD	10.49	7.49	3.99	16.26
SEM	6.06	4.32	2.30	9.39

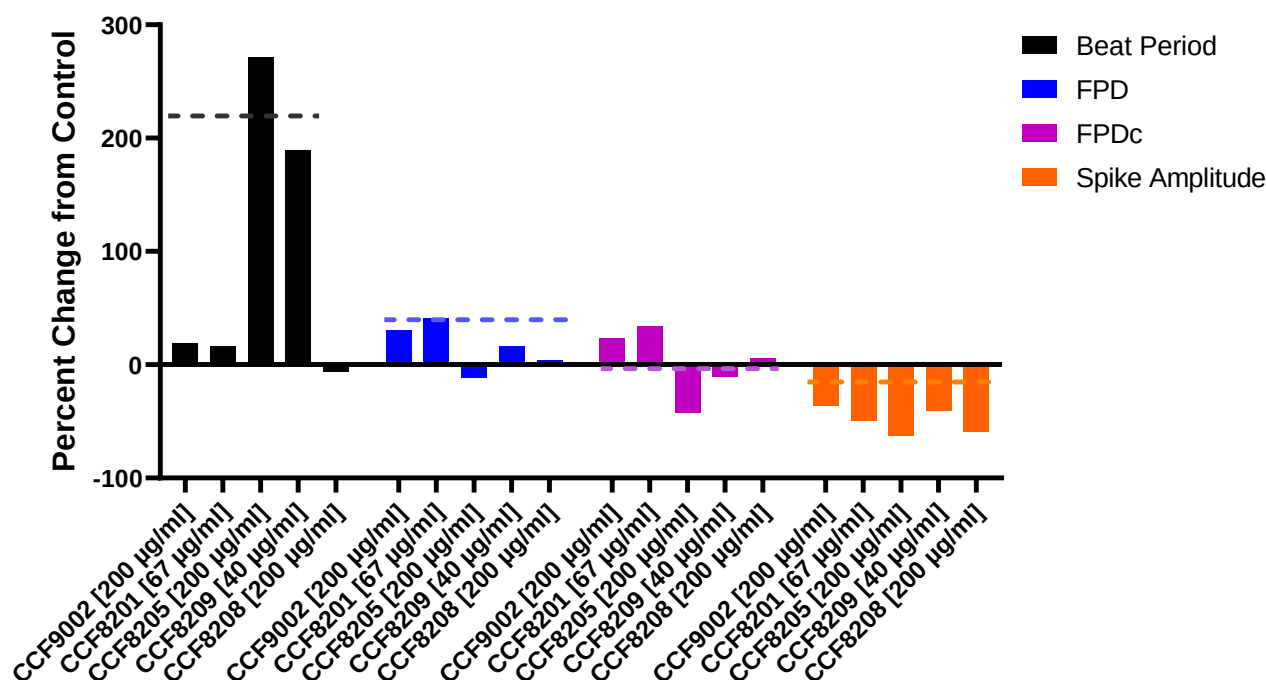
1. Beat Period is measured as the duration between the initial peaks of sequential beats.
2. The initial peak or spike indicates depolarization (spike amplitude).
3. The secondary deflection indicates repolarization or "T wave".
4. The field potential duration is taken as the duration between depolarization and repolarization (FPD).
5. Flat indicates that the repolarization peak was flat and FPD could not be measured but beat period could be measured from successive depolarization peaks.
6. Quiescent indicates cardiomyocytes have stopped beating.

Reference Compound Graphs



- Time-matched vehicle control that matched the duration of positive controls, and test article concentration applications, was used to illustrate the time-dependent changes of cardiomyocyte functional parameters from baseline values.
- Beat Period (s): Vehicle control = 218% (black dashed-line), E-4031 = 342.9%, Diltiazem = -40.0%, Mexiletine = 296.1%. E-4031 (hERG channel blocker) and Mexiletine (Na channel blocker) increased beat period significantly compared to the vehicle control value. Diltiazem as a Ca channel blocker shortened the duration of beat period compared to the vehicle control value.
- FPD (ms): Vehicle control = 39.1% (blue dashed-line), E-4031 = 294.4%, Diltiazem = -43.5%, Mexiletine = 59.3%. E-4031 (hERG channel blocker) and Mexiletine (Na channel blocker) increased FPD compared to the vehicle control value. Diltiazem as a Ca channel blocker shortened the duration of FPD compared to the vehicle control value.
- FPDc (ms): FPD values are inversely related to the beat period; Therefore, FPD values were corrected for the beat period using the Fridericia equation. Vehicle control = -3.68% (Purple dashed-line), E-4031 = 131.6%, Diltiazem = -31.3%, Mexiletine = 0.6%. E-4031 (hERG channel blocker) and Mexiletine (Na channel blocker) increased FPDc compared to the vehicle control value. Diltiazem as a Ca channel blocker shortened the duration of FPDc compared to vehicle control value.
- Spike Amplitude (mV): Vehicle control = -14.9% (Orange dashed-line), E-4031 = -22.1%, Diltiazem = -27.1%, Mexiletine = -37.5%. E-4031 reduced the spike amplitude but not much from vehicle control values. Diltiazem as a Ca channel blocker decreased spike amplitude due to increased cardiac beat rate. Mexiletine (Na channel blocker) decreased spike amplitude compared to the vehicle control value.

Test Compound Graphs



- Beat Period (s). Vehicle control (218%) is the black dashed-line. CCF9002, CCF8201 and CCF8208 decreased the beat period relative to the vehicle control, while CCF8208 showed a slight decrease from vehicle control. CCF8205 showed an increase in beat period compared to control.
- FPD results (ms). Vehicle control (39%) is the blue dashed-line. CCF9002 showed a slight decrease from vehicle control. CCF8201 FPD was not changed compared to vehicle control, where the FPD for CCF8205, CCF8209 and CCF8208 were decreased from vehicle control.
- FPDc results (ms). FPD values are inversely related to beat period, therefore, FPD values were corrected for the beat period using the Fridericia equation. Vehicle control (-3.6%) is the purple dashed-line. CCF9002 and CCF8201 showed an increase compared to vehicle control. CCF8205 showed a decrease compared to vehicle control, while CCF8209 and CCF8208 showed a slight decrease and increase in FPDc compared to vehicle control, respectively.
- Spike amplitude (mV). Vehicle control (-14.9%) is the orange dashed-line. All compounds showed a decrease in spike amplitude compared to vehicle control.

SCIENTIFIC RATIONALE

The QT interval is a sensitive biomarker of drug-induced hERG potassium channel block and potential torsade de pointes risk; however, it is not specific. Characterization of the effects of additional inward current block (INaL and ICa) on cardiac repolarization may mitigate the risk assessment because inward current block can offset the proarrhythmic effects of hERG potassium channel (IKr) block as illustrated in the figure below.

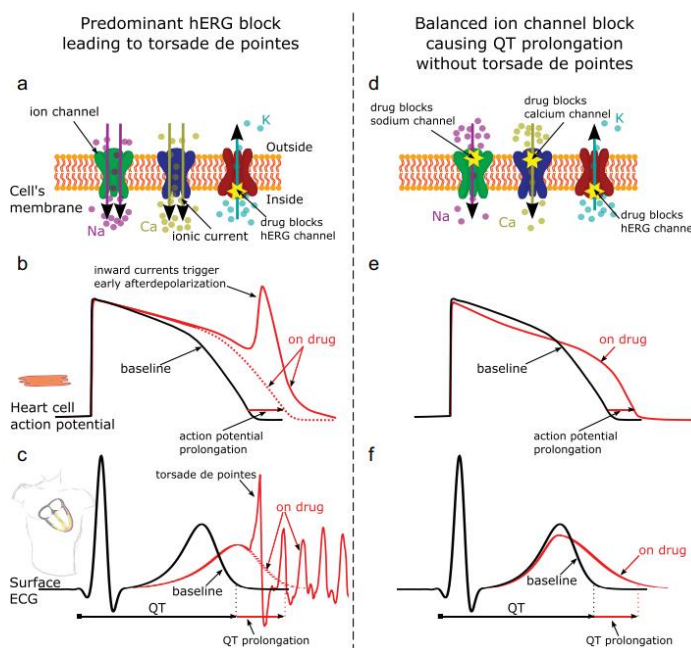


Illustration of predominant hERG block leading to torsade de pointes (left column) vs. balanced ion channel block causing QT prolongation without torsade de pointes (right column).

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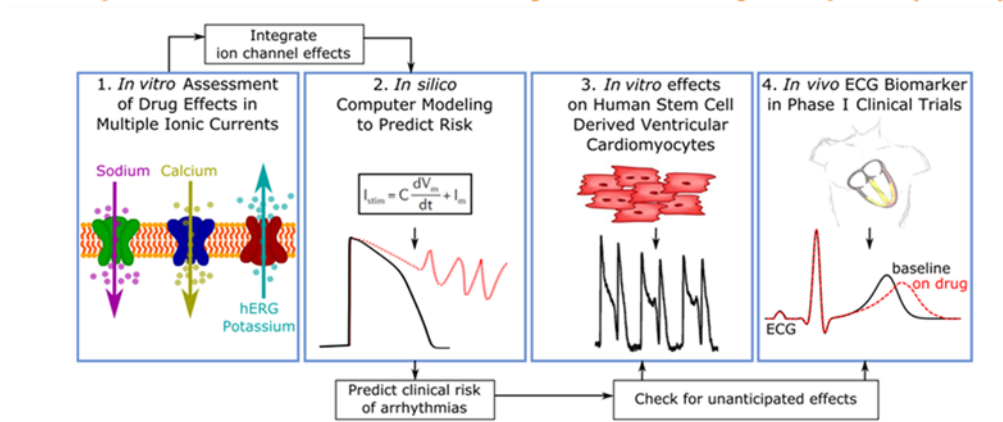
Predominant hERG block reduces the hERG potassium channel current (panel a), which delays repolarization and prolongs the action potential duration of cardiomyocytes (panel b, red dotted line) and the QT interval on the ECG (panel c, red dotted line). Prolonged repolarization can result in early after depolarizations (panel b, red solid line), which are caused by inward currents through sodium and calcium channels (panel a, purple and yellow arrows) and can trigger torsade de pointes (panel c, solid red line).

In addition to causing hERG block, balanced ion channel-blocking drugs block the L-type calcium and/or late sodium currents (panel d). While balanced ion channel block can prolong both the action potential duration of cardiomyocytes (panel e) and the QT interval on the ECG (panel f), the block of inward currents (calcium, late sodium) prevents the occurrence of early after depolarizations and has antiarrhythmic effects. In addition, balanced ion channel block causes different morphology changes in the action potential (panel e, red solid line) and the ECG (panel f, red solid line) than predominant hERG block (panels b and c, respectively).

The goal of the MEA study is to show that it can differentiate predominant hERG-blocking drugs from balanced ion channel-blocking drugs. Na, sodium ions; Ca, calcium ions; K, potassium ions.

Human induced pluripotent stem cells (hiPSC) derived cardiomyocytes grown on multi-electrode array plates (multi well format) offer an integrated test system for evaluating drug effects on cardiac electrical activity (extracellular field potential mimicking ECG in beating cardiomyocytes) and is part of Comprehensive In Vitro Proarrhythmia assay (CiPA).

Comprehensive *In Vitro* Proarrhythmia Assay Proposal (CiPA)



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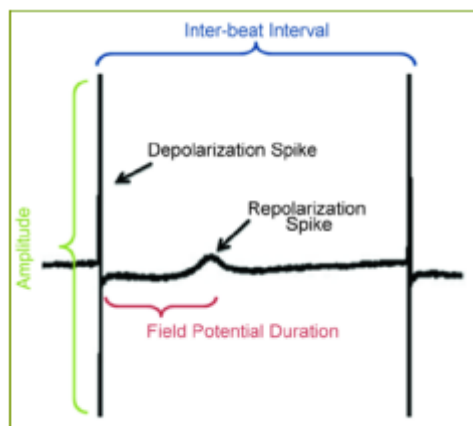
Under CiPA, early assessment of drug effects on multiple ion channels using high-throughput systems coupled with the *in silico* model predictions and outcomes from human induced pluripotent stem cell (iPSC) derived cardiomyocytes assays could inform lead identification/optimization, candidate drug selection. CiPA's mechanistic approach will provide additional information that could influence the ECG monitoring decision process in clinical drug development and drug labeling in marketing materials.

The Multi-electrode Array (MEA) assay is an electrophysiology-based technique to measure the extracellular field potential (voltage changes from inward currents, i.e., I_{NaL} and I_{Ca} currents, and outward cardiac currents, i.e., I_{Kr} and I_{Ks} currents, see figure below) generated from spontaneously beating hiPSC derived cardiomyocyte. Perturbations to the recorded Field Potential Duration (FPD) waveform can be used as an unbiased method of predicting the identity of ion channel(s) impacted on drug exposure (see figure above).

DATA CALCULATION AND ANALYSIS

PCiPAMEA CiPA Human iPSC-derived Cardiomyocytes Multi-Electrode Array assay

The waveform recorded by each electrode on the MEA plate reflects the field potential at that electrode relative to ground electrodes. Raw voltage signals from the MEA plate show easily identifiable features corresponding to the depolarization and repolarization phases of the cardiomyocyte action potential. The following figure illustrates key characteristics of the MEA waveforms and performance of iCell² Cardiomyocytes.



MEA Cardiac Field Potential and Analysis Parameters

The components of the field potential waveform represent distinct electrical behavior. The initial peaks indicate depolarization due to Na current or Spike Amplitude while the secondary deflection indicates repolarization due to K current or "T wave". The field potential duration is taken as the duration between depolarization and repolarization (FPD). Inter spike interval or beat Period (the inverse of frequency) is measured as the duration between the initial peaks of sequential beats.

Field Potential Duration was corrected (FPDc) for beat rate changes using Fridericia equation $FPDc = FPD / \sqrt[3]{ISI}$

Raw MEA data from all recording groups were acquired and analyzed using AxIS Software and exported to Microsoft Excel or MATLAB software for calculation. Data from each compound tested, vehicle control and positive control groups are calculated as percent change from baseline data of the respective wells that were recorded before the application of the test compound concentrations.

MATERIALS AND METHODS

PCiPAMEA CiPA Human iPSC-derived Cardiomyocytes Multi-Electrode Array assay

Cardiac beating activity typically stabilizes 5 days after post-plating cardiomyocytes in the MEA plate, at which point the monolayers are suited for data acquisition. Electrical activity on the Axion Maestro MEA system is acquired using AxIS Software. The Maintenance Medium was replaced 1 - 4 hours before compound application. Test compounds were prepared in maintenance Medium first at 100X dilution and then to 1X the final concentration to keep the DMSO concentration at 0.1%. The plate containing the 1X compound solutions was kept in a cell culture incubator at 37°C, 5% CO₂ for 10 to 15 minutes. A baseline period was recorded before the addition of compounds and compounds were then added to the wells. The media from the MEA plates were removed 1 row at a time and the 1X compound solutions were quickly transferred from the cell culture plate to the MEA plate. Then, the MEA plate was placed in a cell culture incubator at 37°C, 5% CO₂ for 30 minutes. After 30 min incubation, the MEA plate was removed from cell culture incubator and placed on the Axion Maestro System to measure spontaneous electrical activity.

STORAGE AND RETENTION OF RECORDS

Documents generated during the performance of the study will be archived by Eurofins Discovery for a period of time after study completion (this period of time is dependent on each individual site policy). The access to the archives is restricted to authorized employees only.

Appendix 6

ADME-TOX - Study of CCF104

STUDY ID: US034-0017052

STUDY NUMBER
100062770

May 26, 2022

1. STUDY REFERENCES

Study title	ADME-TOX - Study of CCF104	
Study number	100062770	FINAL REPORT May 26, 2022
Study ID	US034-0017052	
Experimental period	May 10, 2022 - May 24, 2022	
PO number	CCF22306755	

2. PERSONS INVOLVED IN THE STUDY

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Study sponsor	Cleveland Clinic Foundation The Cleveland Clinic Lerner Research Institute 9500 Euclid Avenue, ND2-51 Cleveland, OH 44195 U.S.A.	Vara Prasad JOSYULA Patent Agent, Senior Director

3. APPROVAL

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I certify that this report accurately reflects all relevant data collected in this study.

Date 27 May 2022

Signature 

Quality Control Review

Our quality Control Designee assures that data results presented in this report were generated using appropriate materials and controlled methods and that the final results accurately reflect the raw data.

Date 27 May 2022

Signature 

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5. PURPOSE OF THE STUDY

The purpose of this study was to test CCF104 in genetic toxicity assays.

6. COMPOUNDS

6.1. Test Compounds

Client Compound ID	Compound ID	Reference Number	Batch Number	FW	MW	Purity	Received Form	Stock solution	Flag
CCF104	100062770-1	-	22-01	447.85	447.85	-	Powder	1.E-01 M DMSO	-

FW: Formula Weight - MW: Molecular Weight

6.2. Reference Compounds

In each experiment and if applicable, the respective reference compound was tested concurrently with CCF104, and the data were compared with historical values determined at Eurofins. The experiment was accepted in accordance with Eurofins validation Standard Operating Procedure.

7. RESULTS

7.1. ADME-Tox: Genetic Toxicity

7.1.1. Test Compound Results

Compound I.D.	Client Compound I.D.	Test Concentration	1 st	2 nd	%Control 3 rd	Mean %Control	Cytotoxicity (% of control)
Bacterial cytotoxicity (TA98 - S9)							
100062770-1	CCF104	6.0E-07 M	98.7	99.6	97.8	98.7	99
100062770-1	CCF104	1.2E-06 M	96.0	98.7	94.2	96.3	96
100062770-1	CCF104	2.5E-06 M	100.6	100.6	96.0	99.0	99
100062770-1	CCF104	5.0E-06 M	106.0	97.8	99.6	101.2	101
100062770-1	CCF104	1.0E-05 M	107.8	102.4	106.9	105.7	106
100062770-1	CCF104	2.5E-05 M	121.3	115.0	118.6	118.3	118
100062770-1	CCF104	5.0E-05 M	120.4	128.5	125.8	124.9	125
100062770-1	CCF104	1.0E-04 M	118.6	133.9	120.4	124.3	124
Bacterial cytotoxicity (TA100 - S9)							
100062770-1	CCF104	6.0E-07 M	116.1	100.7	98.1	105.0	105
100062770-1	CCF104	1.2E-06 M	96.9	104.6	100.7	100.7	101
100062770-1	CCF104	2.5E-06 M	113.5	108.4	111.0	111.0	111
100062770-1	CCF104	5.0E-06 M	120.0	111.0	105.8	112.3	112
100062770-1	CCF104	1.0E-05 M	139.2	139.2	132.8	137.1	137
100062770-1	CCF104	2.5E-05 M	155.9	140.5	146.9	147.8	148
100062770-1	CCF104	5.0E-05 M	157.2	107.1	134.1	132.8	133
100062770-1	CCF104	1.0E-04 M	194.4	180.3	198.2	190.9	191
Bacterial cytotoxicity (TA1535 - S9)							
100062770-1	CCF104	6.0E-07 M	107.9	97.7	104.5	103.4	103
100062770-1	CCF104	1.2E-06 M	103.4	107.9	104.5	105.3	105
100062770-1	CCF104	2.5E-06 M	107.9	107.9	107.9	107.9	108
100062770-1	CCF104	5.0E-06 M	113.6	110.2	123.8	115.8	116
100062770-1	CCF104	1.0E-05 M	144.2	104.5	110.2	119.6	120
100062770-1	CCF104	2.5E-05 M	166.8	145.3	141.9	151.3	151
100062770-1	CCF104	5.0E-05 M	175.8	240.4	199.6	205.3	205
100062770-1	CCF104	1.0E-04 M	234.7	210.9	181.5	209.1	209
Bacterial cytotoxicity (TA1537 - S9)							
100062770-1	CCF104	6.0E-07 M	108.9	99.2	99.2	102.5	102
100062770-1	CCF104	1.2E-06 M	103.1	102.1	95.4	100.2	100
100062770-1	CCF104	2.5E-06 M	112.8	107.9	97.3	106.0	106
100062770-1	CCF104	5.0E-06 M	107.0	115.7	110.8	111.2	111
100062770-1	CCF104	1.0E-05 M	120.5	109.9	104.1	111.5	111
100062770-1	CCF104	2.5E-05 M	143.6	130.1	134.0	135.9	136
100062770-1	CCF104	5.0E-05 M	140.8	139.8	144.6	141.7	142
100062770-1	CCF104	1.0E-04 M	152.3	148.5	155.2	152.0	152

Notes:

1. Cytotoxicity is presented as % of control growth.
2. A cytotoxicity value of less than 60 % is flagged, and the compound is considered as toxic at the respective concentration.

7.1.2. Reference Compound Results

Compound I.D.	IC ₅₀ (M)	nH
Bacterial cytotoxicity (TA98 - S9)		
Mitomycin C	1.6E-08 M	1.1
Bacterial cytotoxicity (TA100 - S9)		
Mitomycin C	1.0E-08 M	0.9

Compound I.D.	IC ₅₀ (M)	nH
Bacterial cytotoxicity (TA1535 - S9)		
Mitomycin C	3.4E-08 M	>3
Bacterial cytotoxicity (TA1537 - S9)		
Mitomycin C	1.3E-08 M	>3

CYTOX: Test compound appears to have cytotoxic effect.

7.1.3. Background Individual Results

Compound I.D.	Count (# of wells)
Ames fluctuation test (TA98 - S9)	
Background	0
Ames fluctuation test (TA98 + S9)	
Background	2
Ames fluctuation test (TA100 - S9)	
Background	0
Ames fluctuation test (TA100 + S9)	
Background	6
Ames fluctuation test (TA1535 - S9)	
Background	0
Ames fluctuation test (TA1535 + S9)	
Background	1
Ames fluctuation test (TA1537-S9)	
Background	0
Ames fluctuation test (TA1537 + S9)	
Background	0

7.1.4. Reference Compound Results

Compound I.D.	Test Concentration	Count (# of wells)	Positive Significance (- to +++)	Fisher Exact Test (p-value)	Count Flag
Ames fluctuation test (TA98 - S9)					
2-Aminoanthracene	5.0E-06 M	3	-	0.1211	
9-Aminoacridine	1.0E-05 M	0	-	1.0000	
Quercetin	2.0E-05 M	13	+++	0.0000	
Streptozotocin	2.5E-06 M	5	+	0.0280	
Ames fluctuation test (TA98 + S9)					
2-Aminoanthracene	2.0E-06 M	46	+++	0.0000	
9-Aminoacridine	1.0E-05 M	1	-	1.0000	-
Quercetin	2.0E-05 M	33	+++	0.0000	
Streptozotocin	2.5E-06 M	2	-	0.5000	
Ames fluctuation test (TA100 - S9)					
2-Aminoanthracene	5.0E-06 M	0	-	1.0000	
9-Aminoacridine	1.0E-05 M	0	-	1.0000	
Quercetin	2.0E-05 M	1	-	0.5000	
Streptozotocin	2.5E-06 M	6	+	0.0132	
Ames fluctuation test (TA100 + S9)					
2-Aminoanthracene	1.0E-06 M	19	++	0.0023	
9-Aminoacridine	1.0E-05 M	0	-	0.0132	<
Quercetin	2.0E-05 M	14	+	0.0384	
Streptozotocin	5.0E-06 M	18	++	0.0043	

Compound I.D.	Test Concentration	Count (# of wells)	Positive Significance (- to +++)	Fisher Exact Test (p-value)	Count Flag
Ames fluctuation test (TA1535 - S9)					
2-Aminoanthracene	5.0E-06 M	0	-	1.0000	
9-Aminoacridine	1.0E-05 M	0	-	1.0000	
Quercetin	2.0E-05 M	0	-	1.0000	
Streptozotocin	2.5E-06 M	43	+++	0.0000	
Ames fluctuation test (TA1535 + S9)					
2-Aminoanthracene	2.0E-06 M	13	+++	0.0000	
9-Aminoacridine	1.0E-05 M	0	-	1.0000	-
Quercetin	2.0E-05 M	1	-	0.5000	
Streptozotocin	2.5E-06 M	48	+++	0.0000	
Ames fluctuation test (TA1537-S9)					
2-Aminoanthracene	5.0E-06 M	0	-	1.0000	
9-Aminoacridine	1.0E-05 M	48	+++	0.0000	
Quercetin	2.0E-05 M	1	-	0.5000	
Streptozotocin	2.5E-06 M	5	+	0.0280	
Ames fluctuation test (TA1537 + S9)					
2-Aminoanthracene	2.0E-06 M	7	++	0.0062	
9-Aminoacridine	1.0E-05 M	48	+++	0.0000	
Quercetin	2.0E-05 M	2	-	0.2474	
Streptozotocin	2.5E-06 M	2	-	0.2474	

Notes:

1. Weak positive, if $p < 0.05$, denoted as "+"
Strong positive, if $p < 0.01$, denoted as "++"
Very strong positive, if $p < 0.001$, denoted as "+++"
 2. When possible, compounds which score significantly below background are flagged. This may indicate low level cytotoxicity undetectable by the growth assay. The compounds are flagged as described below.
if $p < 0.05$, flagged as "<"
if $p < 0.01$, flagged as "<<"
if $p < 0.001$, flagged as "<<<"
3. Hyphens (-) indicate negative results.

7.1.5. Test Compound Results

Compound I.D.	Client Compound I.D.	Test Concentration	Count (# of wells)	Positive Significance (- to +++)	Fisher Exact Test (p-value)	Count Flag
Ames fluctuation test (TA98 - S9)						
100062770-1	CCF104	5.0E-06 M	1	-	0.5000	
100062770-1	CCF104	1.0E-05 M	2	-	0.2474	
100062770-1	CCF104	5.0E-05 M	2	-	0.2474	
100062770-1	CCF104	1.0E-04 M	1	-	0.5000	
Ames fluctuation test (TA98 + S9)						
100062770-1	CCF104	5.0E-06 M	1	-	1.0000	-
100062770-1	CCF104	1.0E-05 M	1	-	1.0000	-
100062770-1	CCF104	5.0E-05 M	0	-	0.5000	-
100062770-1	CCF104	1.0E-04 M	2	-	0.5000	
Ames fluctuation test (TA100 - S9)						
100062770-1	CCF104	5.0E-06 M	0	-	1.0000	
100062770-1	CCF104	1.0E-05 M	0	-	1.0000	
100062770-1	CCF104	5.0E-05 M	0	-	1.0000	
100062770-1	CCF104	1.0E-04 M	0	-	1.0000	
Ames fluctuation test (TA100 + S9)						
100062770-1	CCF104	5.0E-06 M	7	-	0.5000	
100062770-1	CCF104	1.0E-05 M	9	-	0.2876	
100062770-1	CCF104	5.0E-05 M	14	+	0.0384	
100062770-1	CCF104	1.0E-04 M	5	-	0.5000	-

Compound I.D.	Client Compound I.D.	Test Concentration	Count (# of wells)	Positive Significance (- to +++)	Fisher Exact Test (p-value)	Count Flag
Ames fluctuation test (TA1535 - S9)						
100062770-1	CCF104	5.0E-06 M	0	-	1.0000	
100062770-1	CCF104	1.0E-05 M	0	-	1.0000	
100062770-1	CCF104	5.0E-05 M	0	-	1.0000	
100062770-1	CCF104	1.0E-04 M	0	-	1.0000	
Ames fluctuation test (TA1535 + S9)						
100062770-1	CCF104	5.0E-06 M	2	-	0.2474	
100062770-1	CCF104	1.0E-05 M	3	-	0.1211	
100062770-1	CCF104	5.0E-05 M	6	+	0.0132	
100062770-1	CCF104	1.0E-04 M	6	+	0.0132	
Ames fluctuation test (TA1537-S9)						
100062770-1	CCF104	5.0E-06 M	0	-	1.0000	
100062770-1	CCF104	1.0E-05 M	0	-	1.0000	
100062770-1	CCF104	5.0E-05 M	0	-	1.0000	
100062770-1	CCF104	1.0E-04 M	0	-	1.0000	
Ames fluctuation test (TA1537 + S9)						
100062770-1	CCF104	5.0E-06 M	0	-	1.0000	
100062770-1	CCF104	1.0E-05 M	0	-	1.0000	
100062770-1	CCF104	5.0E-05 M	1	-	0.5000	
100062770-1	CCF104	1.0E-04 M	0	-	1.0000	

Notes:

1. Weak positive, if $p < 0.05$, denoted as "+"
Strong positive, if $p < 0.01$, denoted as "++"
Very strong positive, if $p < 0.001$, denoted as "+++"
 2. When possible, compounds which score significantly below background are flagged.
This may indicate low level cytotoxicity undetectable by the growth assay.
The compounds are flagged as described below.
if $p < 0.05$, flagged as "<"
if $p < 0.01$, flagged as "<<"
if $p < 0.001$, flagged as "<<<"
 3. Hyphens (-) indicate negative results.

7.1.6. Test Compound Results

Compound I.D.	Client Compound I.D.	Test Concentration	Scored Cells	% Cytotoxicity CBPI Index	% Cytotoxicity Cell Numbers	% Micronucleated Cells	t-Test p-value	Test Result	Flags
Micronucleus (CHO + S9, HCA)									
100062770-1	CCF104	8.0E-06 M	2069	-0.6	12.1	0.29	0.4819	-	
100062770-1	CCF104	1.6E-05 M	2098	1.8	31.9	0.14	0.2709	-	
100062770-1	CCF104	3.1E-05 M	2059	4.8	31.7	0.34	0.4373	-	
100062770-1	CCF104	6.2E-05 M	2122	17.1	68.3	0.23	0.3947	-	
100062770-1	CCF104	1.3E-04 M	2036	26.7	74.8	0.19	0.3419	-	
100062770-1	CCF104	2.5E-04 M							CYTO
100062770-1	CCF104	5.0E-04 M							CYTO
100062770-1	CCF104	1.0E-03 M							CYTO
Micronucleus (CHO - S9, HCA)									
100062770-1	CCF104	8.0E-06 M	2166	2.0	13.3	0.09	0.0729	-	
100062770-1	CCF104	1.6E-05 M	2151	-0.8	-3.9	0.19	0.3456	-	
100062770-1	CCF104	3.1E-05 M	2157	-5.9	6.8	0.27	0.3670	-	
100062770-1	CCF104	6.2E-05 M	2146	-7.4	30.9	0.19	0.3434	-	
100062770-1	CCF104	1.3E-04 M	2068	18.7	65.9	1.16	0.0098	+	
100062770-1	CCF104	2.5E-04 M							CYTO
100062770-1	CCF104	5.0E-04 M							CYTO
100062770-1	CCF104	1.0E-03 M							CYTO

Notes:

- '+' $p < 0.05$ by t-test and % of micronucleated cells at least 3-fold higher than background levels.

Compound I.D.	Client Compound I.D.	Test Concentration	Scored Cells	% Cytotoxicity CBPI Index	% Cytotoxicity Cell Numbers	% Micronucleated Cells	t-Test p-value	Test Result	Flags
'+' p < 0.05 by t-test and % of micronucleated cells at least 2-fold higher than background levels.									
'-' p > 0.05 by t-test and % of micronucleated cells less than 2-fold higher than background levels.									

CYTO: High cytotoxicity resulting in an insufficient number of scorable cells (>80% cytotoxicity).

7.1.7. Reference Compound Results

Compound I.D.	Test Concentration	Scored Cells	% Cytotoxicity CBPI Index	% Cytotoxicity Cell Numbers	% Micronucleated Cell	t-Test p-value	Test Result
Micronucleus (CHO + S9, HCA)							
Control +S9	0 M	4422	0.9	38.6	0.30		
Cyclophosphamide	3.6E-05 M	2069	2.8	64.2	3.56	0.0005	+
Micronucleus (CHO - S9, HCA)							
Control -S9	0 M	4545	0.1	0.0	0.24		
Mitomycin C	1.5E-07 M	2066	1.9	40.3	3.78	0.0000	+

Notes:

'+' p < 0.05 by t-test and % of micronucleated cells at least 3-fold higher than background levels.

'-' p > 0.05 by t-test and % of micronucleated cells less than 2-fold higher than background levels.

NA: Non-applicable.

8. MATERIALS AND METHODS

8.1. Experimental Conditions

Minor variations to the experimental protocol described below may have occurred during the testing, they have no impact on the quality of the results obtained.

8.1.1. ADME-Tox: Genetic Toxicity

Assay	Source	Technique	Incubation	Detection Method	Bibl.
Toxicity					
Bacterial cytotoxicity (TA98 - S9)	Reverted <i>Salmonella typhimurium</i> (TA98)	Optical density	96 hr 37°C	Photometry	341
Bacterial cytotoxicity (TA100 - S9)	Reverted <i>Salmonella typhimurium</i> (TA100)	Optical density	96 hr 37°C	Photometry	341
Bacterial cytotoxicity (TA1535 - S9)	Reverted <i>Salmonella typhimurium</i> (TA1535)	Optical density	96 hr 37°C	Photometry	341
Bacterial cytotoxicity (TA1537 - S9)	Reverted <i>Salmonella typhimurium</i> (TA1537)	Optical density	96 hr 37°C	Photometry	341
Ames fluctuation test (TA98 - S9)	<i>Salmonella typhimurium</i> (TA98)	Fluctuation test	96 hr 37°C	Photometry	341
Ames fluctuation test (TA98 + S9)	<i>Salmonella typhimurium</i> (TA98) and rat liver S9	Fluctuation test	96 hr 37°C	Photometry	341
Ames fluctuation test (TA100 - S9)	<i>Salmonella typhimurium</i> (TA100)	Fluctuation test	96 hr 37°C	Photometry	341
Ames fluctuation test (TA100 + S9)	<i>Salmonella typhimurium</i> (TA100) and rat liver S9	Fluctuation test	96 hr 37°C	Photometry	341
Ames fluctuation test (TA1535 - S9)	<i>Salmonella typhimurium</i> (TA1535)	Fluctuation test	96 hr 37°C	Photometry	341
Ames fluctuation test (TA1535 + S9)	<i>Salmonella typhimurium</i> (TA1535) and rat liver S9	Fluctuation test	96 hr 37°C	Photometry	341
Ames fluctuation test (TA1537 - S9)	<i>Salmonella typhimurium</i> (TA1537)	Fluctuation test	96 hr 37°C	Photometry	341
Ames fluctuation test (TA1537 + S9)	<i>Salmonella typhimurium</i> (TA1537) and rat liver S9	Fluctuation test	96 hr 37°C	Photometry	341
Micronucleus (CHO + S9, HCA)	CHO-K1 cell line and rat liver S9	High content analysis (HCA)	4 hr 37°C	Fluorescent Image Analysis	908
Micronucleus (CHO - S9, HCA)	CHO-K1 cell line	High content analysis (HCA)	24 hr 37°C	Fluorescent Image Analysis	908

8.2. Analysis and expression of results

8.2.1. ADME-Tox: Genetic Toxicity

Bacterial Cytotoxicity

The results for cytotoxicity are expressed as percent of control growth (OD₆₅₀). Compounds with growth of less than 60 % of control are flagged and considered cytotoxic.

Ames Tests

Wells that displayed bacteria growth due to the reversion of the histidine mutation (as judged by the ratio of OD₄₃₀/OD₅₇₀ being greater than 1.0) are counted and recorded as positive counts. The significance of the positive counts between the treatment (in the presence of test compound) and the control (in the absence of test compound) are calculated using the one-tailed Fisher's exact test. Three significance levels are reported as follows:

Weak positive, if $0.01 \leq p < 0.05$, denoted as "+"

Strong positive, if $0.001 \leq p < 0.01$, denoted as "++"

Very strong positive, if $p < 0.001$, denoted as "+++"

in vitro Micronucleus

The percent of micronucleated cells is calculated. A marginally-positive result ("-/+") is defined as a value significantly higher than controls (t-test, $p < 0.05$), and at least 2-fold higher than controls. A positive result ("++") is defined as a value significantly higher than controls (t-test, $p < 0.05$) and at least 3-fold higher than controls. Cytotoxicity indexes are also provided to aid in the interpretation of the results.

A "scorable" cell is a bi-nucleated cell contained within the field boundary, in which both nuclei are of similar size (the maximum area ratio of the largest nucleus versus the smallest nucleus within a cell must be lower than 1.5).

Cytokinesis Block Proliferation Index (CBPI) % Cytotoxicity uses a modified version of the (CBPI). This method takes advantage of the fact that cytotoxicity very often induces cell cycle arrest, which is reflected in a decreased ratio of bi-nucleated to mononucleated cells when using cytochalasin B. A CBPI of 1 is equivalent to 100% cytotoxicity.

The % cytotoxicity based on the CBPI is calculated as follows:

$$\text{Cytotoxicity(\%)} = 100 - \left(\frac{\text{CBPI}_t - 1}{\text{CBPI}_c - 1} * 100 \right)$$

where:

CBPI_t = CBPI of treated cells

CBPI_c = CBPI of control cells

CBPI is defined as:

$$\frac{\text{MoNC} + 2 * \text{BiNC} + 3 * \text{MuNC}}{\text{Total number of cells}}$$

Since most multinucleated cells are bi-nucleated, our approximation is:

$$\text{CBPI} = \frac{\text{MoNC} + 2 * \text{MuNC}}{\text{Total number of cells}}$$

where

MoNC = number of mononucleated cells

BiNC = number of binucleated cells

MuNC = number of multinucleated cells

The Cell Number % Cytotoxicity is an index based on cell numbers, in which:

$$\text{Cytotoxicity(\%)} = 100 - \frac{\text{Number of treated cells} * 100}{\text{Number of control cells}}$$

For both indexes, the control cell population is defined as the cells exposed to medium containing 1% DMSO. For the S9-treated cells, the control cell population is also exposed to medium containing 1% DMSO.

In most cases, the cell number index of cytotoxicity is more sensitive than the CBPI index. This is probably because the cell number index accounts for both cell proliferation and cell death, whereas the CBPI index is only based on cell proliferation. In addition, we find the cell number index especially useful in detecting aneugens, since aneugens can inhibit cytokinesis and they might not induce a drop in CBPI even at very cytotoxic concentrations.

The % Micronucleated Cells is the percentage of scorable cells that contain at least one micronucleus within their cytoplasmic domain. The largest allowed ratio of a micronucleus radius to the average nuclei radius within a scorable cell is 0.33.

9. BIBLIOGRAPHY

341. Maron, D.M. and Ames, B.N. (1983), *Mutation Res.*, 113: 173-215.
908. Diaz, D. et al. (2007), *Mutat Res.* 630(1-2):1-13.

Appendix 7

Data Report for Pharmacology Services

Cleveland Clinic Foundation

The Cleveland Clinic Lerner Research Institute 950, 0 Euclid Avenue, ND2-51, Cleveland, OH 44195, United States

Vara Prasad Josyula

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Study #: TW04-0013389

Quote #: TW04-0013389-Q02

Client Study #:

Study Date: Nov 17, 2022 - Dec 01, 2022

PO #: Pending

Compound Information

Panlabs Code: CCF-9

Panlabs #: 1271304

Alt. Code 1: CCF2201

Alt. Code 2: Batch# 22-01

Alt. Code 3:

M.W.: -

F.W.: 447.45

Eurofins Panlabs Discovery Services Taiwan, Ltd.

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Cerep and Eurofins Panlabs are two companies of the Eurofins Group

Services Performed

SpectrumScreen (Total # of Assays: 167)

Study Objectives

To evaluate, in SpectrumScreen, the activity of test compound CCF2201 (Panlabs # 1271304).

Study Signatures



Yao-Chieh Chou

Study Director for Binding Assays

"This study was conducted according to the procedures described in this report. All data presented are authentic, accurate and correct to the best of our knowledge."

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Summary

STUDY OBJECTIVE

To evaluate, in Radioligand Binding assays, the activity of compound CCF2201 (CCF-9, PT# 1271304).

METHODS

Methods employed in this study have been adapted from the scientific literature to maximize reliability and reproducibility. Reference standards were run as an integral part of each assay to ensure the validity of the results obtained. Assays were performed under conditions described in the accompanying "Methods" section of this report.

Where presented, IC_{50} values were determined by a non-linear, least squares regression analysis using MathIQ™ (ID Business Solutions Ltd., UK). Where inhibition constants (K_i) are presented, the K_i values were calculated using the equation of Cheng and Prusoff (Cheng, Y., Prusoff, W.H., Biochem. Pharmacol. 22:3099-3108, 1973) using the observed IC_{50} of the tested compound, the concentration of radioligand employed in the assay, and the historical values for the K_D of the ligand (obtained experimentally at **Eurofins Panlabs, Inc.**). Where presented, the Hill coefficient (n_H), defining the slope of the competitive binding curve, was calculated using MathIQ™. Hill coefficients significantly different than 1.0, may suggest that the binding displacement does not follow the laws of mass action with a single binding site. Where IC_{50} , K_i , and/or n_H data are presented without Standard Error of the Mean (SEM), data are insufficient to be quantitative, and the values presented (K_i , IC_{50} , n_H) should be interpreted with caution.

RESULTS

A summary of results meeting the significance criteria is presented in the following sections. Complete results are presented under the section labeled "Experimental Results". Individual responses, if requested, are presented in the section labeled "Individual Responses".

SUMMARY/CONCLUSION

Significant results are displayed in the following table(s) in rank order of potency for estimated IC_{50} and/or K_i values.

Summary of Significant Results

Biochemical assay results are presented as the percent inhibition of specific binding or activity throughout the report. All other results are expressed in terms of that assay's quantitation method.

- For primary assays, only the lowest concentration with a significant response judged by the assays' criteria, is shown in this summary.
- Where applicable, either the secondary assay results with the lowest dose/concentration meeting the significance criteria or, if inactive, the highest dose/concentration that did not meet the significance criteria is shown.
- Unless otherwise requested, primary screening in duplicate with quantitative data (e.g., $IC_{50} \pm SEM$, $K_i \pm SEM$ and n_H) are shown where applicable for individual requested assays. In screening packages, primary screening in duplicate with semi-quantitative data (e.g., estimated IC_{50} , K_i and n_H) are shown where applicable (concentration range of 4 log units); available secondary functional assays are carried out (30 mM) and MEC or MIC determined only if active in primary assays >50% at 1 log unit below initial test concentration.

Significant responses ($\geq 50\%$ inhibition or stimulation for Biochemical assays) were noted in the primary assays listed below:

Cat #	Assay Name	Species	Conc.	% Inh.	IC_{50}^*	K_i	n_H
200720	Adenosine A ₃	hum	10 μ M	54			
231650	γ -Hydroxybutyric Acid (GHB) Receptor	rat	10 μ M	62			

* A standard error of the mean is presented where results are based on multiple, independent determinations.
gp=Guinea pig; ham=Hamster; hum=Human; rm=Rhesus Macaque

Experimental Results

Cat #	Assay Name	Batch*	Spec.	Rep.	Conc.	% Inh.	IC ₅₀ *	K _i	n _H	R
Compound: CCF2201, PT #: 1271304										
200510	Adenosine A ₁	496528	hum	2	10 µM	27				
200610	Adenosine A _{2A}	496528	hum	2	10 µM	11				
200650	Adenosine A _{2B}	496623	hum	2	10 µM	4				
200720	Adenosine A ₃	496532	hum	2	10 µM	54				
203110	Adrenergic α _{1A}	496425	hum	2	10 µM	3				
203210	Adrenergic α _{1B}	496425	hum	2	10 µM	6				
203400	Adrenergic α _{1D}	496606	hum	2	10 µM	-7				
203630	Adrenergic α _{2A}	496670	hum	2	10 µM	-7				
203710	Adrenergic α _{2B}	496432	hum	2	10 µM	-4				
203810	Adrenergic α _{2C}	496450	hum	2	10 µM	4				
204010	Adrenergic β ₁	496550	hum	2	10 µM	5				
204110	Adrenergic β ₂	496552	hum	2	10 µM	5				
204200	Adrenergic β ₃	496551	hum	2	10 µM	1				
206000	Androgen (Testosterone)	496458	hum	2	10 µM	14				
210030	Angiotensin AT ₁	496433	hum	2	10 µM	1				
210120	Angiotensin AT ₂	496433	hum	2	10 µM	1				
210720	APJ	496539	hum	2	10 µM	3				
211600	Bombesin BB1	496541	hum	2	10 µM	7				
211700	Bombesin BB2	496540	hum	2	10 µM	-5				
211800	Bombesin BB3	496541	hum	2	10 µM	-3				
212520	Bradykinin B ₁	496450	hum	2	10 µM	-4				
212620	Bradykinin B ₂	496607	hum	2	10 µM	1				
299009	Calcitonin	496629	hum	2	10 µM	-8				
214020	Calcitonin Gene-Related Peptide CGRP ₁	496549	hum	2	10 µM	3				
214510	Calcium Channel L-Type, Benzothiazepine	496426	rat	2	10 µM	4				
214600	Calcium Channel L-Type, Dihydropyridine	496553	rat	2	10 µM	2				
215000	Calcium Channel L-Type, Phenylalkylamine	496531	rat	2	10 µM	2				
216000	Calcium Channel N-Type	496533	rat	2	10 µM	7				
217050	Cannabinoid CB ₁	496352	hum	2	10 µM	-5				
217100	Cannabinoid CB ₂	496378	hum	2	10 µM	14				
217510	Chemokine CCR1	496517	hum	2	10 µM	-17				
217550	Chemokine CCR2B	496374	hum	2	10 µM	-3				
217730	Chemokine CCR5 (Rhesus Macaque)	496373	rm	2	10 µM	5				

Note: Items meeting criteria for significance (≥50% stimulation or inhibition) are highlighted.

* Batch: Represents compounds tested concurrently in the same assay(s).

gp=Guinea pig; ham=Hamster; hum=Human; rm=Rhesus Macaque

R=See Remarks (if any) at end of this section.

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Experimental Results

Cat #	Assay Name	Batch*	Spec.	Rep.	Conc.	% Inh.	IC ₅₀ *	K _i	n _H	R
244600	Chemokine CX3CR1	496656	hum	2	10 µM	-1				
244420	Chemokine CXCR1 (IL-8R _A)	496373	hum	2	10 µM	-1				
244500	Chemokine CXCR2 (IL-8R _B)	496376	hum	2	10 µM	-8				
244550	Chemokine CXCR4	496655	hum	2	10 µM	16				
218020	Cholecystokinin CCK ₁ (CCK _A)	496524	hum	2	10 µM	16				
218130	Cholecystokinin CCK ₂ (CCK _B)	496391	hum	2	10 µM	-15				
219100	Colchicine	496652	rat	2	10 µM	-2				
219150	Corticotropin Releasing Factor CRF ₁	496541	hum	2	10 µM	-2				
299003	CysLT ₂ (LTC ₄)	496662	hum	2	10 µM	1				
219500	Dopamine D ₁	496453	hum	2	10 µM	9				
219700	Dopamine D _{2S}	496455	hum	2	10 µM	1				
219800	Dopamine D ₃	496452	hum	2	10 µM	-4				
220000	Dopamine D _{4.4}	496454	hum	2	10 µM	-4				
220200	Dopamine D ₅	496452	hum	2	10 µM	6				
224010	Endothelin ET _A	496512	hum	2	10 µM	5				
224110	Endothelin ET _B	496377	hum	2	10 µM	-3				
225510	Epidermal Growth Factor (EGF)	496374	hum	2	10 µM	-7				
226010	Estrogen ER _α	496354	hum	2	10 µM	-7				
226050	Estrogen ER _β	496354	hum	2	10 µM	-14				
226810	GABA _A , Chloride Channel, TBOB	496654	rat	2	10 µM	37				
226600	GABA _A , Flunitrazepam, Central	496519	rat	2	10 µM	3				
226500	GABA _A , Muscimol, Central	496653	rat	2	10 µM	3				
228610	GABA _{B1A}	496355	hum	2	10 µM	10				
228710	GABA _{B1B}	496355	hum	2	10 µM	1				
230000	Gabapentin	496356	rat	2	10 µM	-1				
231650	γ-Hydroxybutyric Acid (GHB) Receptor	496357	rat	2	10 µM	62				
231680	Glucagon	496358	hum	2	10 µM	0				
232030	Glucocorticoid	496456	hum	2	10 µM	17				
232600	Glutamate, AMPA	496534	rat	2	10 µM	0				
232710	Glutamate, Kainate	496435	rat	2	10 µM	-7				
236400	Glutamate, Metabotropic, mGlu ₂	496359	hum	2	10 µM	15				
237000	Glutamate, Metabotropic, mGlu ₅	496734	hum	2	10 µM	7				
232810	Glutamate, NMDA, Agonism	496520	rat	2	10 µM	6				
232910	Glutamate, NMDA, Glycine	496520	rat	2	10 µM	6				

Note: Items meeting criteria for significance (≥50% stimulation or inhibition) are highlighted.

* Batch: Represents compounds tested concurrently in the same assay(s).

gp=Guinea pig; ham=Hamster; hum=Human; rm=Rhesus Macaque

R=See Remarks (if any) at end of this section.

Cleveland Clinic Foundation

Study #: TW04-0013389, Compound Code: CCF2201 (1271304)

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Experimental Results

Cat #	Assay Name	Batch*	Spec.	Rep.	Conc.	% Inh.	IC ₅₀ *	K _i	n _H	R
233000	Glutamate, NMDA, Phencyclidine	496521	rat	2	10 µM	7				
234000	Glutamate, NMDA, Polyamine	496545	rat	2	10 µM	-7				
239000	Glycine, Strychnine-Sensitive	496438	rat	2	10 µM	-10				
239200	Gonadotropin-Releasing Hormone	496376	hum	2	10 µM	-3				
239300	Growth Hormone Secretagogue (GHS, Ghrelin)	496532	hum	2	10 µM	0				
239610	Histamine H ₁	496518	hum	2	10 µM	0				
239710	Histamine H ₂	496535	hum	2	10 µM	6				
239820	Histamine H ₃	496548	hum	2	10 µM	-18				
299035	Histamine H ₄	496671	hum	2	10 µM	-12				
241000	Imidazoline I ₂ , Central	496546	rat	2	10 µM	14				
242500	Inositol Trisphosphate IP ₃	496360	rat	2	10 µM	17				
243000	Insulin	496459	rat	2	10 µM	1				
243530	Interleukin IL-1 R1	496544	hum	2	10 µM	-4				
244100	Interleukin IL-6	496597	hum	2	10 µM	3				
299004	IP (PGI ₂)	496603	hum	2	10 µM	17				
250460	Leukotriene, Cysteinyl CysLT ₁	496384	hum	2	10 µM	27				
251010	Melanin-Concentrating Hormone MCH1 (SLC1)	496430	hum	2	10 µM	3				
251100	Melanocortin MC ₁	496439	hum	2	10 µM	7				
251300	Melanocortin MC ₃	496449	hum	2	10 µM	3				
251350	Melanocortin MC ₄	496439	hum	2	10 µM	-1				
251400	Melanocortin MC ₅	496449	hum	2	10 µM	14				
251600	Melatonin MT ₁	496361	hum	2	10 µM	-5				
251700	Melatonin MT ₂	496362	hum	2	10 µM	-7				
252200	Motilin	496363	hum	2	10 µM	-7				
252610	Muscarinic M ₁	496380	hum	2	10 µM	3				
252710	Muscarinic M ₂	496381	hum	2	10 µM	19				
252810	Muscarinic M ₃	496382	hum	2	10 µM	10				
252910	Muscarinic M ₄	496383	hum	2	10 µM	2				
253010	Muscarinic M ₅	496383	hum	2	10 µM	-3				
254500	Muscarinic, Oxotremorine M	496657	rat	2	10 µM	-9				
257010	Neuropeptide Y Y ₁	496624	hum	2	10 µM	-7				
257110	Neuropeptide Y Y ₂	496364	hum	2	10 µM	3				
258010	Neurotensin NT ₁	496658	hum	2	10 µM	2				

Note: Items meeting criteria for significance (≥50% stimulation or inhibition) are highlighted.

* Batch: Represents compounds tested concurrently in the same assay(s).

gp=Guinea pig; ham=Hamster; hum=Human; rm=Rhesus Macaque

R=See Remarks (if any) at end of this section.

Cleveland Clinic Foundation

Study #: TW04-0013389, Compound Code: CCF2201 (1271304)

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Experimental Results

Cat #	Assay Name	Batch*	Spec.	Rep.	Conc.	% Inh.	IC ₅₀ *	K _i	n _H	R
258700	Nicotinic Acetylcholine α 1, Bungarotoxin	496440	hum	2	10 μ M	0				
258730	Nicotinic Acetylcholine α 3 β 4	496441	hum	2	10 μ M	-1				
258750	Nicotinic Acetylcholine α 4 β 2, Cytisine	496659	rat	2	10 μ M	-5				
258630	Nicotinic Acetylcholine α 7, Bungarotoxin	496365	rat	2	10 μ M	0				
258660	Nicotinic Acetylcholine α 7, Methyllycaconitine	496366	hum	2	10 μ M	1				
260130	Opiate δ 1 (OP1, DOP)	496625	hum	2	10 μ M	-3				
260210	Opiate κ (OP2, KOP)	496451	hum	2	10 μ M	15				
260410	Opiate μ (OP3, MOP)	496379	hum	2	10 μ M	2				
299030	Orexin OX ₁	496542	hum	2	10 μ M	-41				1
299002	Orexin OX ₂	496543	hum	2	10 μ M	-9				
260600	Orphanin ORL ₁	496607	hum	2	10 μ M	0				
260900	Oxytocin	496392	hum	2	10 μ M	-3				
264500	Phorbol Ester	496552	mouse	2	10 μ M	1				
299037	Platelet Activating Factor (PAF)	496445	hum	2	10 μ M	2				
265200	Platelet-Derived Growth Factor (PDGF)	496367	mouse	2	10 μ M	-2				
265510	Potassium Channel [K _A]	496624	rat	2	10 μ M	4				
265600	Potassium Channel [K _{ATP}]	496448	ham	2	10 μ M	19				
265800	Potassium Channel [SK _{CA}]	496368	rat	2	10 μ M	1				
265910	Potassium Channel hERG, [³ H]Dofetilide	496442	hum	2	10 μ M	0				
299005	Progesterone PR-B	496460	hum	2	10 μ M	1				
268030	Prostanoid CRTH2	496392	hum	2	10 μ M	25				
268060	Prostanoid DP	496735	hum	2	10 μ M	41				
268110	Prostanoid EP ₁	496603	hum	2	10 μ M	-6				
268200	Prostanoid EP ₂	496385	hum	2	10 μ M	47				
299006	Prostanoid EP ₃	496385	hum	2	10 μ M	-3				
268420	Prostanoid EP ₄	496385	hum	2	10 μ M	39				
268510	Prostanoid FP	496603	hum	2	10 μ M	37				
299036	Purinergic P2X	496431	rat	2	10 μ M	-3				
268820	Purinergic P2Y, Non-Selective	496523	rat	2	10 μ M	34				
270000	Rolipram	496524	rat	2	10 μ M	22				
270300	Ryanodine RyR3	496631	rat	2	10 μ M	4				
271110	Serotonin (5-Hydroxytryptamine) 5-HT _{1A}	496519	hum	2	10 μ M	-3				
271200	Serotonin (5-Hydroxytryptamine) 5-HT _{1B}	496630	rat	2	10 μ M	-1				
271700	Serotonin (5-Hydroxytryptamine) 5-HT _{2B}	496428	hum	2	10 μ M	3				

Note: Items meeting criteria for significance ($\geq 50\%$ stimulation or inhibition) are highlighted.

* Batch: Represents compounds tested concurrently in the same assay(s).

gp=Guinea pig; ham=Hamster; hum=Human; rm=Rhesus Macaque

R=See Remarks (if any) at end of this section.

Cleveland Clinic Foundation

Study #: TW04-0013389, Compound Code: CCF2201 (1271304)

Friday, December 2, 2022

Experimental Results

Cat #	Assay Name	Batch*	Spec.	Rep.	Conc.	% Inh.	IC ₅₀ *	K _i	n _H	R
271800	Serotonin (5-Hydroxytryptamine) 5-HT _{2C}	496447	hum	2	10 µM	16				
271910	Serotonin (5-Hydroxytryptamine) 5-HT ₃	496739	hum	2	10 µM	-6				
272000	Serotonin (5-Hydroxytryptamine) 5-HT ₄	496660	gp	2	10 µM	21				
272100	Serotonin (5-Hydroxytryptamine) 5-HT _{5A}	496429	hum	2	10 µM	0				
272200	Serotonin (5-Hydroxytryptamine) 5-HT ₆	496525	hum	2	10 µM	2				
272320	Serotonin (5-Hydroxytryptamine) 5-HT ₇	496392	hum	2	10 µM	-1				
299034	Sigma σ ₁	496525	hum	2	10 µM	6				
278200	Sigma σ ₂	496429	rat	2	10 µM	6				
279510	Sodium Channel, Site 2	496444	rat	2	10 µM	6				
282510	Somatostatin sst1	496618	hum	2	10 µM	10				
282700	Somatostatin sst2	496617	hum	2	10 µM	2				
282530	Somatostatin sst3	496786	hum	2	10 µM	-17				
282910	Somatostatin sst4	496461	hum	2	10 µM	9				
283010	Somatostatin sst5	496619	hum	2	10 µM	29				
255520	Tachykinin NK ₁	496536	hum	2	10 µM	-8				
255620	Tachykinin NK ₂	496535	hum	2	10 µM	7				
285900	Thyroid Hormone	496599	rat	2	10 µM	12				
286010	Thyrotropin Releasing Hormone (TRH)	496369	hum	2	10 µM	-5				
286200	Transforming Growth Factor-β (TGF-β)	496370	mouse	2	10 µM	-3				
202000	Transporter, Adenosine	496514	gp	2	10 µM	31				
219010	Transporter, Choline	496379	hum	2	10 µM	-23				
220320	Transporter, Dopamine (DAT)	496427	hum	2	10 µM	17				
226400	Transporter, GABA	496547	rat	2	10 µM	27				
239100	Transporter, Glycine	496620	rat	2	10 µM	-18				
204410	Transporter, Norepinephrine (NET)	496427	hum	2	10 µM	32				
274030	Transporter, Serotonin (5-Hydroxytryptamine) (SERT)	496671	hum	2	10 µM	3				
252030	Transporter, Vesicular Monoamine (Non-Selective)	496552	hum	2	10 µM	10				
286700	Urotensin II	496371	hum	2	10 µM	3				
287010	Vasoactive Intestinal Peptide VIP ₁	496372	hum	2	10 µM	-18				
287100	Vasoactive Intestinal Peptide VIP2/PACAP VPAC2	496373	hum	2	10 µM	2				
287530	Vasopressin V _{1A}	496430	hum	2	10 µM	-6				
287560	Vasopressin V _{1B}	496661	hum	2	10 µM	-3				
287610	Vasopressin V ₂	496548	hum	2	10 µM	-3				

Note: Items meeting criteria for significance (≥50% stimulation or inhibition) are highlighted.

* Batch: Represents compounds tested concurrently in the same assay(s).

gp=Guinea pig; ham=Hamster; hum=Human; rm=Rhesus Macaque

R=See Remarks (if any) at end of this section.

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Experimental Results

Eurofins Panlabs, Inc. has an exclusive, worldwide limited use license from Synaptic Pharmaceutical Corporation to perform these assays: Adrenergic Alpha 1D, Adrenergic Alpha 2B, and Dopamine D5 for safety and selectivity profiling. Eurofins Panlabs' license excludes performing those assays in connection with drug discovery or development activities where the principal therapeutic mechanism of action of the test compound involves selective binding to a licensed receptor. Customers may contact Synaptic directly if they believe they need a broader license.

Remarks

1) Substantial negative inhibition can be caused by physical properties or insolubility of compound in final reaction conditions.

Note: Items meeting criteria for significance ($\geq 50\%$ stimulation or inhibition) are highlighted.

* Batch: Represents compounds tested concurrently in the same assay(s).

gp=Guinea pig; ham=Hamster; hum=Human; rm=Rhesus Macaque

R=See Remarks (if any) at end of this section.

Cleveland Clinic Foundation

Study #: TW04-0013389, Compound Code: CCF2201 (1271304)

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Methods

■ 200510 Adenosine A₁

Source:	Human recombinant CHO-K1 cells	Ligand:	1.0 nM [³ H] DPCPX
Vehicle:	1.0% DMSO	Non-Specific Ligand:	100 μM R(-)-PIA
Incubation Time/Temp:	90 minutes @ 25°C	Specific Binding:	85% *
Incubation Buffer:	20 mM HEPES, pH 7.4, 10 mM MgCl ₂ , 100 mM NaCl	Quantitation Method:	Radioligand Binding
Kd:	1.40 nM *	Significance Criteria:	≥50% of max stimulation or inhibition
		Bmax:	2.70 pmole/mg Protein *

■ 200610 Adenosine A_{2A}

Source:	Human recombinant HEK-293 cells	Ligand:	0.05 μM [³ H] CGS-21680
Vehicle:	1.0% DMSO	Non-Specific Ligand:	50.0 μM NECA
Incubation Time/Temp:	90 minutes @ 25°C	Specific Binding:	85% *
Incubation Buffer:	50 mM Tris-HCl, pH 7.4, 10 mM MgCl ₂ , 1 mM EDTA, 2 U/mL Adenosine Deaminase	Quantitation Method:	Radioligand Binding
Kd:	0.064 μM *	Significance Criteria:	≥50% of max stimulation or inhibition
		Bmax:	7.0 pmole/mg Protein *

■ 200650 Adenosine A_{2B}

Source:	Human recombinant HEK-293 cells	Ligand:	1.60 nM [³ H] MRS1754
Vehicle:	1.0% DMSO	Non-Specific Ligand:	100 μM NECA
Incubation Time/Temp:	90 minutes @ 25°C	Specific Binding:	77% *
Incubation Buffer:	50 mM Tris-HCl, pH 6.5, 5 mM MgCl ₂ , 1 mM EDTA, 0.01% Bacitracin	Quantitation Method:	Radioligand Binding
Kd:	0.77 nM *	Significance Criteria:	≥50% of max stimulation or inhibition
		Bmax:	6.20 pmole/mg Protein *

■ 200720 Adenosine A₃

Source:	Human recombinant CHO-K1 cells	Ligand:	0.5 nM [¹²⁵ I] AB-MECA
Vehicle:	1.0% DMSO	Non-Specific Ligand:	1.0 μM IB-MECA
Incubation Time/Temp:	60 minutes @ 25°C	Specific Binding:	83% *
Incubation Buffer:	25 mM HEPES, pH 7.4, 5 mM MgCl ₂ , 1 mM CaCl ₂ , 0.1% BSA	Quantitation Method:	Radioligand Binding
Kd:	5.90 nM *	Significance Criteria:	≥50% of max stimulation or inhibition
		Bmax:	1.80 pmole/mg Protein *

* Historical Values

Cleveland Clinic Foundation

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Methods

■ 203110 Adrenergic α_{1A}

Source:	Human recombinant Chem-1 cells	Ligand:	0.6 nM [^{3} H] Prazosin
Vehicle:	1.0% DMSO	Non-Specific Ligand:	10.0 μ M Phentolamine
Incubation Time/Temp:	60 minutes @ 25°C	Specific Binding:	90% *
Incubation Buffer:	50 mM HEPES, pH 7.4, 5 mM MgCl ₂ , 1 mM CaCl ₂ , 0.2% BSA	Quantitation Method:	Radioligand Binding
Kd:	0.57 nM *	Significance Criteria:	≥50% of max stimulation or inhibition
		Bmax:	8.70 pmole/mg Protein *

■ 203210 Adrenergic α_{1B}

Source:	Human recombinant Chem-1 cells	Ligand:	0.2 nM [^{3} H] Prazosin
Vehicle:	1.0% DMSO	Non-Specific Ligand:	10.0 μ M Phentolamine
Incubation Time/Temp:	60 minutes @ 25°C	Specific Binding:	90% *
Incubation Buffer:	50 mM HEPES, pH 7.4, 10 mM MgCl ₂ , 1 mM EDTA	Quantitation Method:	Radioligand Binding
Kd:	0.13 nM *	Significance Criteria:	≥50% of max stimulation or inhibition
		Bmax:	6.20 pmole/mg Protein *

■ 203400 Adrenergic α_{1D}

Source:	Human recombinant HEK-293 cells	Ligand:	0.6 nM [^{3} H] Prazosin
Vehicle:	1.0% DMSO	Non-Specific Ligand:	10.0 μ M Phentolamine
Incubation Time/Temp:	60 minutes @ 25°C	Specific Binding:	80% *
Incubation Buffer:	50 mM Tris-HCl, pH 7.4	Quantitation Method:	Radioligand Binding
Kd:	0.58 nM *	Significance Criteria:	≥50% of max stimulation or inhibition
		Bmax:	0.17 pmole/mg Protein *

■ 203630 Adrenergic α_{2A}

Source:	Human recombinant CHO-K1 cells	Ligand:	1.50 nM [^{3} H] Rauwolscine
Vehicle:	1.0% DMSO	Non-Specific Ligand:	10.0 μ M WB-4101
Incubation Time/Temp:	60 minutes @ 25°C	Specific Binding:	95% *
Incubation Buffer:	50 mM Tris-HCl, pH 7.4, 1 mM EDTA	Quantitation Method:	Radioligand Binding
Kd:	1.50 nM *	Significance Criteria:	≥50% of max stimulation or inhibition
		Bmax:	16.0 pmole/mg Protein *

* Historical Values

Cleveland Clinic Foundation

Study #: TW04-0013389, Compound Code: CCF2201 (1271304)

Friday, December 2, 2022

Methods

■ 203710 Adrenergic α_{2B}

Source:	Human recombinant CHO-K1 cells	Ligand:	2.50 nM [^{3} H] Rauwolscine
Vehicle:	1.0% DMSO	Non-Specific Ligand:	10.0 μ M Prazosin
Incubation Time/Temp:	60 minutes @ 25°C	Specific Binding:	90% *
Incubation Buffer:	50 mM Tris-HCl, pH 7.4, 1 mM EDTA, 12.5 mM MgCl ₂ , 0.2% BSA	Quantitation Method:	Radioligand Binding
Kd:	2.10 nM *	Significance Criteria:	$\geq 50\%$ of max stimulation or inhibition
		Bmax:	2.10 pmole/mg Protein *

■ 203810 Adrenergic α_{2C}

Source:	Human recombinant CHO-K1 cells	Ligand:	0.5 nM [^{3} H] Rauwolscine
Vehicle:	1.0% DMSO	Non-Specific Ligand:	10.0 μ M WB-4101
Incubation Time/Temp:	60 minutes @ 25°C	Specific Binding:	95% *
Incubation Buffer:	50 mM Tris-HCl, pH 7.4, 1 mM EDTA	Quantitation Method:	Radioligand Binding
Kd:	0.4 nM *	Significance Criteria:	$\geq 50\%$ of max stimulation or inhibition
		Bmax:	12.0 pmole/mg Protein *

■ 204010 Adrenergic β_1

Source:	Human recombinant CHO-K1 cells	Ligand:	0.03 nM [^{125} I] Cyanopindolol
Vehicle:	1.0% DMSO	Non-Specific Ligand:	100 μ M S(-)-Propranolol
Incubation Time/Temp:	2 hours @ 25°C	Specific Binding:	95% *
Incubation Buffer:	50 mM Tris-HCl, pH 7.4, 1.4 mM Ascorbic Acid, 0.001% BSA, 5 mM EDTA, 1.5 mM CaCl ₂ , 120 mM NaCl	Quantitation Method:	Radioligand Binding
Kd:	0.041 nM *	Significance Criteria:	$\geq 50\%$ of max stimulation or inhibition
		Bmax:	0.072 pmole/mg Protein *

■ 204110 Adrenergic β_2

Source:	Human recombinant CHO cells	Ligand:	0.2 nM [^{3} H] CGP-12177
Vehicle:	1.0% DMSO	Non-Specific Ligand:	10.0 μ M ICI-118551
Incubation Time/Temp:	60 minutes @ 25°C	Specific Binding:	95% *
Incubation Buffer:	50 mM Tris-HCl, pH 7.4, 0.5 mM EDTA, 5.0 mM MgCl ₂ , 120 mM NaCl	Quantitation Method:	Radioligand Binding
Kd:	0.44 nM *	Significance Criteria:	$\geq 50\%$ of max stimulation or inhibition
		Bmax:	0.44 pmole/mg Protein *

* Historical Values

Cleveland Clinic Foundation

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Methods

■ 204200 Adrenergic β_3

Source:	Human recombinant HEK-293 cells	Ligand:	0.5 nM [^{125} I] Cyanopindolol
Vehicle:	1.0% DMSO	Non-Specific Ligand:	1.0 mM Alprenolol
Incubation Time/Temp:	90 minutes @ 25°C	Specific Binding:	80% *
Incubation Buffer:	20 mM Tris-HCl, pH 7.4, 120 mM NaCl, 2 mM MgCl ₂ , 284 μ M L-Ascorbic acid	Quantitation Method:	Radioligand Binding
Kd:	1.50 nM *	Significance Criteria:	$\geq 50\%$ of max stimulation or inhibition
		Bmax:	0.55 pmole/mg Protein *

■ 206000 Androgen (Testosterone)

Source:	Human LNCaP clone FGC cells	Ligand:	0.5 nM [^{3} H] Methyltrienolone
Vehicle:	1.0% DMSO	Non-Specific Ligand:	1.0 μ M Testosterone
Incubation Time/Temp:	20 hours @ 4°C	Specific Binding:	75% *
Incubation Buffer:	25 mM HEPES, pH 7.4, 10% Glycerol, 1 mM EDTA, 10 mM Molybdate	Quantitation Method:	Radioligand Binding
Kd:	0.71 nM *	Significance Criteria:	$\geq 50\%$ of max stimulation or inhibition
		Bmax:	0.25 pmole/mg Protein *

■ 210030 Angiotensin AT₁

Source:	Human recombinant CHO-K1 cells	Ligand:	20.0 pM [^{125} I] (Sar ¹ , Ile ⁸)-Angiotensin II
Vehicle:	1.0% DMSO	Non-Specific Ligand:	10.0 μ M Angiotensin II
Incubation Time/Temp:	3 hours @ 37°C	Specific Binding:	90% *
Incubation Buffer:	50 mM Tris-HCl, pH 7.4, 5 mM MgCl ₂ , 0.1% BSA, 1 mM EDTA	Quantitation Method:	Radioligand Binding
Kd:	0.056 nM *	Significance Criteria:	$\geq 50\%$ of max stimulation or inhibition
		Bmax:	16.0 pmole/mg Protein *

■ 210120 Angiotensin AT₂

Source:	Human recombinant CHO-K1 cells	Ligand:	0.05 nM [^{125} I] CGP-42112A
Vehicle:	1.0% DMSO	Non-Specific Ligand:	10.0 μ M (Sar ¹ , Ile ⁸)-Angiotensin II
Incubation Time/Temp:	3 hours @ 37°C	Specific Binding:	80% *
Incubation Buffer:	50 mM Tris-HCl, pH 7.4, 5 mM MgCl ₂ , 1 mM EDTA, 0.1% BSA	Quantitation Method:	Radioligand Binding
Kd:	0.04 nM *	Significance Criteria:	$\geq 50\%$ of max stimulation or inhibition
		Bmax:	0.33 pmole/mg Protein *

* Historical Values

Cleveland Clinic Foundation

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Methods

■ 210720 APJ

Source:	Human recombinant Chem-1 cells	Ligand:	0.05 nM [¹²⁵ I] (Glp ⁶⁵ , Nle ⁷⁵ , Tyr ⁷⁷)-Apelin-13
Vehicle:	1.0% DMSO	Non-Specific Ligand:	1.0 μM (Pyr ¹)-Apelin-13
Incubation Time/Temp:	2 hours @ 25°C	Specific Binding:	95% *
Incubation Buffer:	50 mM HEPES, pH 7.4, 5 mM MgCl ₂ , 1 mM CaCl ₂ , 0.2% BSA	Quantitation Method:	Radioligand Binding
Kd:	0.05 nM *	Significance Criteria:	≥50% of max stimulation or inhibition
		Bmax:	20.0 pmole/mg Protein *

■ 211600 Bombesin BB1

Source:	Human recombinant CHO-K1 cells	Ligand:	0.05 nM [¹²⁵ I] (Tyr ⁴)-Bombesin
Vehicle:	1.0% DMSO	Non-Specific Ligand:	1.0 μM Neuromedin B
Incubation Time/Temp:	60 minutes @ 25°C	Specific Binding:	95% *
Incubation Buffer:	25 mM HEPES-KOH, pH 7.4, 5 mM MgCl ₂ , 0.2% BSA	Quantitation Method:	Radioligand Binding
Kd:	0.045 nM *	Significance Criteria:	≥50% of max stimulation or inhibition
		Bmax:	6.60 pmole/mg Protein *

■ 211700 Bombesin BB2

Source:	Human recombinant HEK-293 cells	Ligand:	0.05 nM [¹²⁵ I] (Tyr ⁴)-Bombesin
Vehicle:	1.0% DMSO	Non-Specific Ligand:	1.0 μM GRP (human)
Incubation Time/Temp:	60 minutes @ 25°C	Specific Binding:	96% *
Incubation Buffer:	25 mM HEPES-KOH, pH 7.4, 5 mM MgCl ₂ , 0.2% BSA, 1 mM EDTA	Quantitation Method:	Radioligand Binding
Kd:	0.062 nM *	Significance Criteria:	≥50% of max stimulation or inhibition
		Bmax:	13.0 pmole/mg Protein *

■ 211800 Bombesin BB3

Source:	Human recombinant Chem-1 cells	Ligand:	0.1 nM [¹²⁵ I] (D-Tyr ⁶ ,β-Ala ¹¹ ,Phe ¹³ ,Nle ¹⁴)-BN (6-14)
Vehicle:	1.0% DMSO	Non-Specific Ligand:	1.0 μM (D-Phe ⁶ ,β-Ala ¹¹ ,Phe ¹³ ,Nle ¹⁴)-BN (6-14)
Incubation Time/Temp:	60 minutes @ 25°C	Specific Binding:	87% *
Incubation Buffer:	25 mM HEPES-KOH, pH 7.4, 5 mM MgCl ₂ , 0.01% Trypsin Inhibitor, 0.2% BSA, 0.1% Bacitracin, 0.1 mM AEBSF	Quantitation Method:	Radioligand Binding
Kd:	0.21 nM *	Significance Criteria:	≥50% of max stimulation or inhibition
		Bmax:	1.60 pmole/mg Protein *

* Historical Values

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Methods

■ 212520 Bradykinin B₁

Source:	Human recombinant CHO-K1 cells	Ligand:	0.3 nM [³ H] (Des-Arg ¹⁰ , Leu ⁹)-Kallidin
Vehicle:	1.0% DMSO	Non-Specific Ligand:	10.0 μM (Des-Arg ⁹ , Leu ⁸)-Bradykinin
Incubation Time/Temp:	60 minutes @ 25°C	Specific Binding:	90% *
Incubation Buffer:	20 mM HEPES, 140 μg/ml Bacitracin, pH 7.4	Quantitation Method:	Radioligand Binding
Kd:	0.3 nM *	Significance Criteria:	≥50% of max stimulation or inhibition
		Bmax:	0.2 pmole/mg Protein *

■ 212620 Bradykinin B₂

Source:	Human recombinant Chem-1 cells	Ligand:	0.5 nM [³ H] Bradykinin
Vehicle:	1.0% DMSO	Non-Specific Ligand:	5.0 μM Bradykinin
Incubation Time/Temp:	60 minutes @ 25°C	Specific Binding:	90% *
Incubation Buffer:	50 mM HEPES, pH 7.4, 0.2% BSA, 1 mM CaCl ₂ , 5 mM MgCl ₂ , 0.05 mM Bacitracin	Quantitation Method:	Radioligand Binding
Kd:	0.85 nM *	Significance Criteria:	≥50% of max stimulation or inhibition
		Bmax:	9.40 pmole/mg Protein *

■ 299009 Calcitonin

Source:	Human T-47D cells	Ligand:	0.05 nM [¹²⁵ I] Calcitonin (salmon)
Vehicle:	1.0% DMSO	Non-Specific Ligand:	0.5 μM Calcitonin (salmon)
Incubation Time/Temp:	60 minutes @ 37°C	Specific Binding:	73% *
Incubation Buffer:	PBS, pH 7.4, 0.2% BSA	Quantitation Method:	Radioligand Binding
Kd:	0.045 nM *	Significance Criteria:	≥50 of max stimulation or inhibition
		Bmax:	

■ 214020 Calcitonin Gene-Related Peptide CGRP₁

Source:	Human recombinant Chem-1 cells	Ligand:	0.03 nM [¹²⁵ I] CGRP (human)
Vehicle:	1.0% DMSO	Non-Specific Ligand:	1.0 μM α-CGRP (human)
Incubation Time/Temp:	90 minutes @ 25°C	Specific Binding:	80% *
Incubation Buffer:	50 mM HEPES, pH 7.4, 5 mM MgCl ₂ , 1 mM CaCl ₂ , 0.2% BSA	Quantitation Method:	Radioligand Binding
Kd:	0.11 nM *	Significance Criteria:	≥50% of max stimulation or inhibition
		Bmax:	8.10 pmole/mg Protein *

* Historical Values

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Methods

■ 214510 Calcium Channel L-Type, Benzothiazepine

Source:	Wistar Rat brain (minus cerebellum)	Ligand:	2.0 nM [³ H] Diltiazem
Vehicle:	1.0% DMSO	Non-Specific Ligand:	10.0 μM Diltiazem
Incubation Time/Temp:	3 hours @ 4°C	Specific Binding:	73% *
Incubation Buffer:	50 mM Tris-HCl, pH 7.4, 0.1% BSA	Quantitation Method:	Radioligand Binding
Kd:	16.0 nM *	Significance Criteria:	≥50% of max stimulation or inhibition
		Bmax:	0.21 pmole/mg Protein *

■ 214600 Calcium Channel L-Type, Dihydropyridine

Source:	Wistar Rat cerebral cortex	Ligand:	0.1 nM [³ H] Nitrendipine
Vehicle:	1.0% DMSO	Non-Specific Ligand:	1.0 μM Nitrendipine
Incubation Time/Temp:	90 minutes @ 25°C	Specific Binding:	91% *
Incubation Buffer:	50 mM Tris-HCl, pH 7.4	Quantitation Method:	Radioligand Binding
Kd:	0.18 nM *	Significance Criteria:	≥50% of max stimulation or inhibition
		Bmax:	0.23 pmole/mg Protein *

■ 215000 Calcium Channel L-Type, Phenylalkylamine

Source:	Wistar Rat cerebral cortex	Ligand:	0.4 nM [³ H] (-)-Desmethoxyverapamil (D-888)
Vehicle:	1.0% DMSO	Non-Specific Ligand:	10.0 μM Methoxyverapamil (D-600)
Incubation Time/Temp:	60 minutes @ 25°C	Specific Binding:	80% *
Incubation Buffer:	50 mM HEPES, pH 7.4	Quantitation Method:	Radioligand Binding
Kd:	14.0 nM *	Significance Criteria:	≥50% of max stimulation or inhibition
		Bmax:	1.60 pmole/mg Protein *

■ 216000 Calcium Channel N-Type

Source:	Wistar Rat frontal brain	Ligand:	10.0 pM [¹²⁵ I] ω-Conotoxin GVIA
Vehicle:	1.0% DMSO	Non-Specific Ligand:	0.1 μM ω-Conotoxin GVIA
Incubation Time/Temp:	30 minutes @ 4°C	Specific Binding:	96% *
Incubation Buffer:	20 mM Tris-HCl, pH 7.4, 0.5% BSA	Quantitation Method:	Radioligand Binding
Kd:	0.051 nM *	Significance Criteria:	≥50% of max stimulation or inhibition
		Bmax:	0.88 pmole/mg Protein *

* Historical Values

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Methods

■ 217050 Cannabinoid CB₁

Source:	Human recombinant Chem-1 cells	Ligand:	2.0 nM [³ H] SR141716A
Vehicle:	1.0% DMSO	Non-Specific Ligand:	10.0 μM CP-55,940
Incubation Time/Temp:	60 minutes @ 37°C	Specific Binding:	78% *
Incubation Buffer:	50 mM HEPES, pH7.4, 5 mM MgCl ₂ , 1 mM CaCl ₂ , 0.2% BSA	Quantitation Method:	Radioligand Binding
Kd:	18.0 nM *	Significance Criteria:	≥50% of max stimulation or inhibition
		Bmax:	15.0 pmole/mg Protein *

■ 217100 Cannabinoid CB₂

Source:	Human recombinant CHO-K1 cells	Ligand:	2.40 nM [³ H] WIN-55,212-2
Vehicle:	1.0% DMSO	Non-Specific Ligand:	10.0 μM R(+)-WIN-55,212-2
Incubation Time/Temp:	90 minutes @ 37°C	Specific Binding:	80% *
Incubation Buffer:	20 mM HEPES, pH 7.0, 0.5% BSA	Quantitation Method:	Radioligand Binding
Kd:	4.90 nM *	Significance Criteria:	≥50% of max stimulation or inhibition
		Bmax:	1.70 pmole/mg Protein *

■ 217510 Chemokine CCR1

Source:	Human recombinant Chem-2 cells	Ligand:	0.1 nM [¹²⁵ I] MIP-1α
Vehicle:	1.0% DMSO	Non-Specific Ligand:	0.3 μM MIP-1β
Incubation Time/Temp:	3 hours @ 25°C	Specific Binding:	72% *
Incubation Buffer:	50 mM HEPES, pH 7.4, 5 mM MgCl ₂ , 1 mM CaCl ₂ , 0.2% BSA, 260 mM NaCl	Quantitation Method:	Radioligand Binding
Kd:	0.46 nM *	Significance Criteria:	≥50% of max stimulation or inhibition
		Bmax:	1.10 pmole/mg Protein *

■ 217550 Chemokine CCR2B

Source:	Human recombinant CHO-K1 cells	Ligand:	0.1 nM [¹²⁵ I] MCP-1
Vehicle:	1.0% DMSO	Non-Specific Ligand:	0.03 μM MCP-1
Incubation Time/Temp:	60 minutes @ 25°C	Specific Binding:	80% *
Incubation Buffer:	25 mM HEPES, pH 7.4, 1 mM CaCl ₂ , 0.5% BSA, 5 mM MgCl ₂	Quantitation Method:	Radioligand Binding
Kd:	0.063 nM *	Significance Criteria:	≥50% of max stimulation or inhibition
		Bmax:	0.25 pmole/mg Protein *

* Historical Values

Cleveland Clinic Foundation

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Methods

■ 217730 Chemokine CCR5 (Rhesus Macaque)

Source:	Rhesus Macaque recombinant Chem-1 cells	Ligand:	0.1 nM [¹²⁵ I] MIP-1α
Vehicle:	1.0% DMSO	Non-Specific Ligand:	0.1 μM MIP-1β
Incubation Time/Temp:	90 minutes @ 25°C	Specific Binding:	85% *
Incubation Buffer:	50 mM HEPES, pH 7.4, 1 mM CaCl ₂ , 5 mM MgCl ₂ , 0.2% BSA	Quantitation Method:	Radioligand Binding
Kd:	0.31 nM *	Significance Criteria:	≥50% of max stimulation or inhibition
		Bmax:	0.98 pmole/mg Protein *

■ 244600 Chemokine CX3CR1

Source:	Human recombinant Chem-1 cells	Ligand:	20.0 pM [¹²⁵ I] Fractalkine
Vehicle:	1.0% DMSO	Non-Specific Ligand:	10.0 nM Fractalkine
Incubation Time/Temp:	2 hours @ 25°C	Specific Binding:	74% *
Incubation Buffer:	50 mM HEPES, pH 7.4, 1 mM CaCl ₂ , 5 mM MgCl ₂ , 0.5% BSA, 0.1% NaN ₃	Quantitation Method:	Radioligand Binding
Kd:	0.07 nM *	Significance Criteria:	≥50% of max stimulation or inhibition
		Bmax:	4.60 pmole/mg Protein *

■ 244420 Chemokine CXCR1 (IL-8R_A)

Source:	Human recombinant Chem-1 cells	Ligand:	15.0 pM [¹²⁵ I] IL-8
Vehicle:	1.0% DMSO	Non-Specific Ligand:	10.0 nM IL-8
Incubation Time/Temp:	90 minutes @ 25°C	Specific Binding:	90% *
Incubation Buffer:	50 mM HEPES, pH 7.4, 1 mM CaCl ₂ , 5 mM MgCl ₂ , 0.2% BSA	Quantitation Method:	Radioligand Binding
Kd:	13.0 pM *	Significance Criteria:	≥50% of max stimulation or inhibition
		Bmax:	6.0 pmole/mg Protein *

■ 244500 Chemokine CXCR2 (IL-8R_B)

Source:	Human recombinant CHO-K1 cells	Ligand:	15.0 pM [¹²⁵ I] IL-8
Vehicle:	1.0% DMSO	Non-Specific Ligand:	10.0 nM IL-8
Incubation Time/Temp:	60 minutes @ 25°C	Specific Binding:	80% *
Incubation Buffer:	25 mM HEPES, pH 7.4, 2 mM CaCl ₂ , 1 mM MgCl ₂ , 0.2% BSA	Quantitation Method:	Radioligand Binding
Kd:	12.0 pM *	Significance Criteria:	≥50% of max stimulation or inhibition
		Bmax:	0.28 pmole/mg Protein *

* Historical Values

Cleveland Clinic Foundation

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■ 244550 Chemokine CXCR4

Source:	Human recombinant Chem-1 cells	Ligand:	0.03 nM [¹²⁵ I] SDF-1α
Vehicle:	1.0% DMSO	Non-Specific Ligand:	0.03 μM SDF-1α
Incubation Time/Temp:	90 minutes @ 25°C	Specific Binding:	70% *
Incubation Buffer:	50 mM HEPES, pH 7.4, 5 mM MgCl ₂ , 1 mM CaCl ₂ , 0.2% BSA	Quantitation Method:	Radioligand Binding
Kd:	0.096 nM *	Significance Criteria:	≥50% of max stimulation or inhibition
		Bmax:	2.70 pmole/mg Protein *

■ 218020 Cholecystokinin CCK₁ (CCK_A)

Source:	Human recombinant 1321-N1 cells	Ligand:	0.8 nM [³ H] L-364,718
Vehicle:	1.0% DMSO	Non-Specific Ligand:	1.0 μM Devazepide (L-364,718)
Incubation Time/Temp:	60 minutes @ 4°C	Specific Binding:	90% *
Incubation Buffer:	50 mM Tris-HCl, pH 7.4, 5 mM MgCl ₂ , 0.2% BSA	Quantitation Method:	Radioligand Binding
Kd:	0.28 nM *	Significance Criteria:	≥50% of max stimulation or inhibition
		Bmax:	22.0 pmole/mg Protein *

■ 218130 Cholecystokinin CCK₂ (CCK_B)

Source:	Human recombinant Chem-1 cells	Ligand:	0.05 nM [¹²⁵ I] CCK-8
Vehicle:	1.0% DMSO	Non-Specific Ligand:	1.0 μM Sincalide
Incubation Time/Temp:	3 hours @ 25°C	Specific Binding:	90% *
Incubation Buffer:	50 mM HEPES, pH 7.4, 0.1% BSA, 1 mM CaCl ₂ , 5 mM MgCl ₂	Quantitation Method:	Radioligand Binding
Kd:	0.069 nM *	Significance Criteria:	≥50% of max stimulation or inhibition
		Bmax:	9.40 pmole/mg *

■ 219100 Colchicine

Source:	Wistar Rat brain	Ligand:	2.50 nM [³ H] Colchicine
Vehicle:	1.0% DMSO	Non-Specific Ligand:	100 μM Colchicine
Incubation Time/Temp:	3 hours @ 37°C	Specific Binding:	97% *
Incubation Buffer:	10 mM Sodium Phosphate, pH 6.7, 10 mM MgCl ₂ , 1 mM EGTA, 0.1 mM GTP	Quantitation Method:	Radioligand Binding
Kd:	1.20 μM *	Significance Criteria:	≥50% of max stimulation or inhibition
		Bmax:	0.073 pmole/mg Protein *

* Historical Values

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Methods

■ 219150 Corticotropin Releasing Factor CRF₁

Source:	Human recombinant CHO-K1 cells	Ligand:	0.05 nM [¹²⁵ I] Tyr ⁰ -CRF (ovine)
Vehicle:	1.0% DMSO	Non-Specific Ligand:	0.1 μM Urocortin (human)
Incubation Time/Temp:	60 minutes @ 25°C	Specific Binding:	93% *
Incubation Buffer:	50 mM Tris-HCl, pH 7.4, 10 mM MgCl ₂ , 2 mM EDTA, 0.1% BSA	Quantitation Method:	Radioligand Binding
Kd:	0.47 nM *	Significance Criteria:	≥50% of max stimulation or inhibition
		Bmax:	16.0 pmole/mg Protein *

■ 299003 CysLT₂ (LTC₄)

Source:	Human recombinant HEK-293 cells	Ligand:	1.0 nM [³ H] LTD ₄
Vehicle:	1.0% DMSO	Non-Specific Ligand:	1.0 μM LTD ₄
Incubation Time/Temp:	60 minutes @ 25°C	Specific Binding:	85% *
Incubation Buffer:	50 mM Tris-HCl, pH 7.4, 10 mM CaCl ₂ , 10 mM MgCl ₂ , 0.1% BSA	Quantitation Method:	Radioligand Binding
Kd:	1.50 nM *	Significance Criteria:	≥50 of max stimulation or inhibition
		Bmax:	1.0 pmole/mg *

■ 219500 Dopamine D₁

Source:	Human recombinant CHO cells	Ligand:	1.40 nM [³ H] SCH-23390
Vehicle:	1.0% DMSO	Non-Specific Ligand:	10.0 μM (+)-Butaclamol
Incubation Time/Temp:	2 hours @ 37°C	Specific Binding:	90% *
Incubation Buffer:	50 mM Tris-HCl, pH 7.4, 1.4 mM Ascorbic Acid, 0.001% BSA, 150 mM NaCl	Quantitation Method:	Radioligand Binding
Kd:	1.40 nM *	Significance Criteria:	≥50% of max stimulation or inhibition
		Bmax:	0.63 pmole/mg Protein *

■ 219700 Dopamine D_{2s}

Source:	Human recombinant CHO cells	Ligand:	0.16 nM [³ H] Spiperone
Vehicle:	1.0% DMSO	Non-Specific Ligand:	10.0 μM Haloperidol
Incubation Time/Temp:	2 hours @ 25°C	Specific Binding:	90% *
Incubation Buffer:	50 mM Tris-HCl, pH 7.4, 1.4 mM Ascorbic Acid, 0.001% BSA, 150 mM NaCl	Quantitation Method:	Radioligand Binding
Kd:	0.09 nM *	Significance Criteria:	≥50% of max stimulation or inhibition
		Bmax:	1.60 pmole/mg Protein *

* Historical Values

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Methods

■ 219800 Dopamine D₃

Source:	Human recombinant CHO cells	Ligand:	0.7 nM [³ H] Spiperone
Vehicle:	1.0% DMSO	Non-Specific Ligand:	25.0 μM S(-)-Sulpiride
Incubation Time/Temp:	2 hours @ 37°C	Specific Binding:	85% *
Incubation Buffer:	50 mM Tris-HCl, pH 7.4, 1.4 mM Ascorbic Acid, 0.001% BSA, 150 mM NaCl	Quantitation Method:	Radioligand Binding
Kd:	0.36 nM *	Significance Criteria:	≥50% of max stimulation or inhibition
		Bmax:	1.10 pmole/mg Protein *

■ 220000 Dopamine D_{4.4}

Source:	Human recombinant CHO-K1 cells	Ligand:	1.20 nM [³ H] Spiperone
Vehicle:	1.0% DMSO	Non-Specific Ligand:	10.0 μM Haloperidol
Incubation Time/Temp:	2 hours @ 25°C	Specific Binding:	85% *
Incubation Buffer:	50 mM Tris-HCl, pH 7.4, 1.4 mM Ascorbic Acid, 0.001% BSA, 150 mM NaCl	Quantitation Method:	Radioligand Binding
Kd:	0.46 nM *	Significance Criteria:	≥50% of max stimulation or inhibition
		Bmax:	0.63 pmole/mg Protein *

■ 220200 Dopamine D₅

Source:	Human recombinant CHO cells	Ligand:	2.0 nM [³ H] SCH-23390
Vehicle:	1.0% DMSO	Non-Specific Ligand:	10.0 μM Flupentixol
Incubation Time/Temp:	2 hours @ 37°C	Specific Binding:	85% *
Incubation Buffer:	50 mM Tris-HCl, pH 7.4, 1.4 mM Ascorbic Acid, 0.001% BSA, 150 mM NaCl	Quantitation Method:	Radioligand Binding
Kd:	0.73 nM *	Significance Criteria:	≥50% of max stimulation or inhibition
		Bmax:	0.47 pmole/mg Protein *

■ 224010 Endothelin ET_A

Source:	Human recombinant CHO-S cells	Ligand:	0.03 nM [¹²⁵ I] Endothelin-1
Vehicle:	1.0% DMSO	Non-Specific Ligand:	0.1 μM Endothelin-1
Incubation Time/Temp:	2 hours @ 37°C	Specific Binding:	90% *
Incubation Buffer:	50 mM Tris-HCl, pH 7.4, 0.1% BSA, 0.5 mM CaCl ₂	Quantitation Method:	Radioligand Binding
Kd:	0.048 nM *	Significance Criteria:	≥50% of max stimulation or inhibition
		Bmax:	0.35 pmole/mg Protein *

* Historical Values

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■ 224110 Endothelin ET_B

Source:	Human recombinant CHO-K1 cells	Ligand:	0.1 nM [¹²⁵ I] Endothelin-1
Vehicle:	1.0% DMSO	Non-Specific Ligand:	0.1 μM Endothelin-1
Incubation Time/Temp:	2 hours @ 25°C	Specific Binding:	75% *
Incubation Buffer:	50 mM HEPES, pH 7.4, 1 mM CaCl ₂ , 5 mM MgCl ₂ , 0.5% BSA	Quantitation Method:	Radioligand Binding
Kd:	0.085 nM *	Significance Criteria:	≥50% of max stimulation or inhibition
		Bmax:	4.30 pmole/mg Protein *

■ 225510 Epidermal Growth Factor (EGF)

Source:	Human A431 cells	Ligand:	0.08 nM [¹²⁵ I] EGF (human)
Vehicle:	1.0% DMSO	Non-Specific Ligand:	0.1 μM EGF (human)
Incubation Time/Temp:	60 minutes @ 25°C	Specific Binding:	90% *
Incubation Buffer:	50 mM HEPES, pH 7.4, 0.1% BSA, 1.2 mM CaCl ₂ , 5 mM KCl, 1.2 mM MgCl ₂ , 138 mM NaCl	Quantitation Method:	Radioligand Binding
Kd:	0.17 nM *	Significance Criteria:	≥50% of max stimulation or inhibition
		Bmax:	5.50 pmole/mg Protein *

■ 226010 Estrogen ERα

Source:	Human recombinant insect Sf9 cells	Ligand:	0.5 nM [³ H] Estradiol
Vehicle:	1.0% DMSO	Non-Specific Ligand:	1.0 μM Diethylstilbestrol
Incubation Time/Temp:	2 hours @ 25°C	Specific Binding:	85% *
Incubation Buffer:	10 mM Tris-HCl, pH 7.4, 0.1% BSA, 10% Glycerol, 1 mM DTT	Quantitation Method:	Radioligand Binding
Kd:	0.2 nM *	Significance Criteria:	≥50% of max stimulation or inhibition
		Bmax:	1400 pmole/mg Protein *

■ 226050 Estrogen ERβ

Source:	Human recombinant insect Sf9 cells	Ligand:	0.5 nM [³ H] Estradiol
Vehicle:	1.0% DMSO	Non-Specific Ligand:	1.0 μM Diethylstilbestrol
Incubation Time/Temp:	2 hours @ 25°C	Specific Binding:	90% *
Incubation Buffer:	10 mM Tris-HCl, pH 7.4, 10% Glycerol, 1 mM DTT, 0.1% BSA	Quantitation Method:	Radioligand Binding
Kd:	0.13 nM *	Significance Criteria:	≥50% of max stimulation or inhibition
		Bmax:	3000 pmole/mg Protein *

* Historical Values

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■ 226810 GABA_A, Chloride Channel, TBOB

Source:	Wistar Rat brain (minus cerebellum)	Ligand:	3.0 nM [³ H] TBOB
Vehicle:	1.0% DMSO	Non-Specific Ligand:	200 μM Picrotoxin
Incubation Time/Temp:	2 hours @ 25°C	Specific Binding:	78% *
Incubation Buffer:	50 mM Potassium Phosphate, pH 7.4, 200 mM NaCl	Quantitation Method:	Radioligand Binding
Kd:	0.026 μM *	Significance Criteria:	≥50% of max stimulation or inhibition
		Bmax:	2.10 pmole/mg Protein *

■ 226600 GABA_A, Flunitrazepam, Central

Source:	Wistar Rat brain (minus cerebellum)	Ligand:	1.0 nM [³ H] Flunitrazepam
Vehicle:	1.0% DMSO	Non-Specific Ligand:	10.0 μM Diazepam
Incubation Time/Temp:	60 minutes @ 25°C	Specific Binding:	91% *
Incubation Buffer:	50 mM Phosphate Buffer, pH 7.4	Quantitation Method:	Radioligand Binding
Kd:	4.40 nM *	Significance Criteria:	≥50% of max stimulation or inhibition
		Bmax:	1.20 pmole/mg Protein *

■ 226500 GABA_A, Muscimol, Central

Source:	Wistar Rat brain (minus cerebellum)	Ligand:	1.0 nM [³ H] Muscimol
Vehicle:	1.0% DMSO	Non-Specific Ligand:	0.1 μM Muscimol
Incubation Time/Temp:	10 minutes @ 4°C	Specific Binding:	90% *
Incubation Buffer:	50 mM Tris-HCl, pH 7.4	Quantitation Method:	Radioligand Binding
Kd:	3.80 nM *	Significance Criteria:	≥50% of max stimulation or inhibition
		Bmax:	1.80 pmole/mg Protein *

■ 228610 GABA_{B1A}

Source:	Human recombinant CHO-K1 cells	Ligand:	4.0 nM [³ H] CGP-54626
Vehicle:	1.0% DMSO	Non-Specific Ligand:	3.0 mM GABA
Incubation Time/Temp:	3 hours @ 25°C	Specific Binding:	90% *
Incubation Buffer:	50 mM Tris-HCl, pH 7.4, 2.5 mM CaCl ₂ , 0.1% BSA	Quantitation Method:	Radioligand Binding
Kd:	3.30 nM *	Significance Criteria:	≥50% of max stimulation or inhibition
		Bmax:	48.0 pmole/mg Protein *

* Historical Values

Cleveland Clinic Foundation

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Methods

■ 228710 GABA_{B1B}

Source:	Human recombinant CHO-K1 cells	Ligand:	4.0 nM [³ H] CGP-54626
Vehicle:	1.0% DMSO	Non-Specific Ligand:	3.0 mM GABA
Incubation Time/Temp:	3 hours @ 25°C	Specific Binding:	90% *
Incubation Buffer:	50 mM Tris-HCl, pH 7.4, 2.5 mM CaCl ₂ , 0.1% BSA	Quantitation Method:	Radioligand Binding
Kd:	3.60 nM *	Significance Criteria:	≥50% of max stimulation or inhibition
		Bmax:	13.0 pmole/mg Protein *

■ 230000 Gabapentin

Source:	Wistar Rat brain cortex	Ligand:	20.0 nM [³ H] Gabapentin
Vehicle:	1.0% DMSO	Non-Specific Ligand:	100 μM Gabapentin
Incubation Time/Temp:	30 minutes @ 25°C	Specific Binding:	76% *
Incubation Buffer:	10 mM HEPES, pH 7.4	Quantitation Method:	Radioligand Binding
Kd:	0.038 μM *	Significance Criteria:	≥50% of max stimulation or inhibition
		Bmax:	6.80 pmole/mg Protein *

■ 231650 γ-Hydroxybutyric Acid (GHB) Receptor

Source:	Wistar Rat brain cortex	Ligand:	20.0 nM [³ H] NCS382
Vehicle:	1.0% DMSO	Non-Specific Ligand:	100 μM NCS382
Incubation Time/Temp:	2 hours @ 4°C	Specific Binding:	85% *
Incubation Buffer:	50 mM KH ₂ PO ₄ /K ₂ HPO ₄ , pH 6.0	Quantitation Method:	Radioligand Binding
Kd:	0.63 μM *	Significance Criteria:	≥50% of max stimulation or inhibition
		Bmax:	39.0 pmole/mg Protein *

■ 231680 Glucagon

Source:	Human recombinant Chem-1 cells	Ligand:	0.1 nM [¹²⁵ I] Glucagon
Vehicle:	1.0% DMSO	Non-Specific Ligand:	1.0 μM [des-His ¹ -Glu ⁹]-Glucagon (1-29) amide
Incubation Time/Temp:	90 minutes @ 25°C	Specific Binding:	90% *
Incubation Buffer:	50 mM Tris-HCl, pH 7.4, 5 mM MgCl ₂ , 1 mM CaCl ₂ , 0.2% BSA	Quantitation Method:	Radioligand Binding
Kd:	0.37 nM *	Significance Criteria:	≥50% of max stimulation or inhibition
		Bmax:	24.0 pmole/mg Protein *

* Historical Values

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■ 232030 Glucocorticoid

Source:	Human recombinant Insect cells	Ligand:	5.0 nM [³ H] Dexamethasone
Vehicle:	1.0% DMSO	Non-Specific Ligand:	10.0 μM Dexamethasone
Incubation Time/Temp:	1 day @ 4°C	Specific Binding:	97% *
Incubation Buffer:	1.5 mM KH ₂ PO ₄ , 8 mM Na ₂ HPO ₄ , pH7.4, 137 mM NaCl, 2.7 mM KCl, 0.2% BSA	Quantitation Method:	Radioligand Binding
Kd:	4.60 nM *	Significance Criteria:	≥50% of max stimulation or inhibition
		Bmax:	1.0 pmole/mg *

■ 232600 Glutamate, AMPA

Source:	Wistar Rat cerebral cortex	Ligand:	5.0 nM [³ H] AMPA
Vehicle:	1.0% DMSO	Non-Specific Ligand:	1.0 mM L-Glutamic acid
Incubation Time/Temp:	90 minutes @ 4°C	Specific Binding:	90% *
Incubation Buffer:	50 mM Tris-HCl, pH 7.4, 200 mM KSCN	Quantitation Method:	Radioligand Binding
Kd1:	18.0 nM *	Significance Criteria:	≥50% of max stimulation or inhibition
Kd2:	0.99 μM *	Bmax1:	0.62 pmole/mg Protein *
		Bmax2:	17.0 pmole/mg Protein *

■ 232710 Glutamate, Kainate

Source:	Wistar Rat brain cortex	Ligand:	6.0 nM [³ H] Kainic acid
Vehicle:	1.0% DMSO	Non-Specific Ligand:	1.0 mM L-Glutamic acid
Incubation Time/Temp:	2 hours @ 4°C	Specific Binding:	80% *
Incubation Buffer:	50 mM Tris-HCl, pH 7.4	Quantitation Method:	Radioligand Binding
Kd:	5.40 nM *	Significance Criteria:	≥50% of max stimulation or inhibition
		Bmax:	0.21 pmole/mg Protein *

■ 236400 Glutamate, Metabotropic, mGlu₂

Source:	Human recombinant Chem-1 cells	Ligand:	2.0 nM [³ H] LY341495
Vehicle:	1.0% DMSO	Non-Specific Ligand:	5.0 μM LY-354740
Incubation Time/Temp:	60 minutes @ 25°C	Specific Binding:	90% *
Incubation Buffer:	10 mM KH ₂ PO ₄ , 100 mM KBr, pH 7.6 (pH adjust with KOH)	Quantitation Method:	Radioligand Binding
Kd:	2.0 nM *	Significance Criteria:	≥50% of max stimulation or inhibition
		Bmax:	38.0 pmole/mg Protein *

* Historical Values

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■ 237000 Glutamate, Metabotropic, mGlu₅

Source:	Human recombinant CHO-K1 cells	Ligand:	0.03 μ M [³ H] Quisqualic acid
Vehicle:	1.0% DMSO	Non-Specific Ligand:	1.0 mM L-Glutamic acid
Incubation Time/Temp:	2 hours @ 25°C	Specific Binding:	85% *
Incubation Buffer:	25 mM HEPES, pH 7.4, 1 mM MgCl ₂ , 2.5 mM CaCl ₂	Quantitation Method:	Radioligand Binding
Kd:	0.026 μ M *	Significance Criteria:	≥50% of max stimulation or inhibition
		Bmax:	0.68 pmole/mg Protein *

■ 232810 Glutamate, NMDA, Agonism

Source:	Wistar Rat cerebral cortex	Ligand:	2.0 nM [³ H] CGP-39653
Vehicle:	1.0% DMSO	Non-Specific Ligand:	1.0 mM L-Glutamic acid
Incubation Time/Temp:	20 minutes @ 4°C	Specific Binding:	70% *
Incubation Buffer:	50 mM Tris-HCl, pH 7.4	Quantitation Method:	Radioligand Binding
Kd:	19.0 nM *	Significance Criteria:	≥50% of max stimulation or inhibition
		Bmax:	2.30 pmole/mg Protein *

■ 232910 Glutamate, NMDA, Glycine

Source:	Wistar Rat cerebral cortex	Ligand:	0.33 nM [³ H] MDL 105,519
Vehicle:	1.0% DMSO	Non-Specific Ligand:	10.0 μ M MDL 105,519
Incubation Time/Temp:	30 minutes @ 4°C	Specific Binding:	85% *
Incubation Buffer:	50 mM HEPES, pH 7.7	Quantitation Method:	Radioligand Binding
Kd:	6.0 nM *	Significance Criteria:	≥50% of max stimulation or inhibition
		Bmax:	3.70 pmole/mg Protein *

■ 233000 Glutamate, NMDA, Phencyclidine

Source:	Wistar Rat cerebral cortex	Ligand:	4.0 nM [³ H] TCP
Vehicle:	1.0% DMSO	Non-Specific Ligand:	1.0 μ M Dizocilpine ((+)-MK-801)
Incubation Time/Temp:	45 minutes @ 25°C	Specific Binding:	94% *
Incubation Buffer:	10 mM Tris-HCl, pH 7.4	Quantitation Method:	Radioligand Binding
Kd:	8.40 nM *	Significance Criteria:	≥50% of max stimulation or inhibition
		Bmax:	0.78 pmole/mg Protein *

* Historical Values

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■ 234000 Glutamate, NMDA, Polyamine

Source:	Wistar Rat cerebral cortex	Ligand:	2.0 nM [³ H] Ifenprodil
Vehicle:	1.0% DMSO	Non-Specific Ligand:	10.0 μM Ifenprodil
Incubation Time/Temp:	2 hours @ 4°C	Specific Binding:	80% *
Incubation Buffer:	50 mM Tris-HCl, pH 7.4	Quantitation Method:	Radioligand Binding
Kd:	0.026 μM *	Significance Criteria:	≥50% of max stimulation or inhibition
		Bmax:	1.10 pmole/mg Protein *

■ 239000 Glycine, Strychnine-Sensitive

Source:	Wistar Rat spinal cord	Ligand:	10.0 nM [³ H] Strychnine
Vehicle:	1.0% DMSO	Non-Specific Ligand:	1.0 mM Glycine
Incubation Time/Temp:	10 minutes @ 4°C	Specific Binding:	77% *
Incubation Buffer:	50 mM Potassium Phosphate, pH 7.1, 200 mM NaCl	Quantitation Method:	Radioligand Binding
Kd:	13.0 nM *	Significance Criteria:	≥50% of max stimulation or inhibition
		Bmax:	0.35 pmole/mg Protein *

■ 239200 Gonadotropin-Releasing Hormone

Source:	Human recombinant Chem-1 cells	Ligand:	0.05 nM [¹²⁵ I] [D-Trp ⁶]-LH-RH
Vehicle:	1.0% DMSO	Non-Specific Ligand:	1.0 μM [D-Trp ⁶]-LH-RH
Incubation Time/Temp:	60 minutes @ 25°C	Specific Binding:	85% *
Incubation Buffer:	50 mM HEPES, pH 7.4, 5 mM MgCl ₂ , 1 mM CaCl ₂ , 0.2% BSA	Quantitation Method:	Radioligand Binding
Kd:	0.31 nM *	Significance Criteria:	≥50% of max stimulation or inhibition
		Bmax:	0.61 pmole/mg Protein *

■ 239300 Growth Hormone Secretagogue (GHS, Ghrelin)

Source:	Human recombinant CHO-K1 cells	Ligand:	0.03 nM [¹²⁵ I] Ghrelin (human)
Vehicle:	1.0% DMSO	Non-Specific Ligand:	0.1 μM Ghrelin (human)
Incubation Time/Temp:	60 minutes @ 25°C	Specific Binding:	90% *
Incubation Buffer:	25 mM HEPES, pH 7.4, 5 mM MgCl ₂ , 1 mM CaCl ₂ , 0.1 % BSA	Quantitation Method:	Radioligand Binding
Kd:	0.038 nM *	Significance Criteria:	≥50% of max stimulation or inhibition
		Bmax:	9.40 pmole/mg Protein *

* Historical Values

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■ 239610 Histamine H₁

Source:	Human recombinant CHO-K1 cells	Ligand:	1.20 nM [³ H] Pyrilamine
Vehicle:	1.0% DMSO	Non-Specific Ligand:	1.0 μM Pyrilamine
Incubation Time/Temp:	3 hours @ 25°C	Specific Binding:	94% *
Incubation Buffer:	50 mM Tris-HCl, pH 7.4, 5 mM MgCl ₂	Quantitation Method:	Radioligand Binding
Kd:	1.10 nM *	Significance Criteria:	≥50% of max stimulation or inhibition
		Bmax:	6.70 pmole/mg Protein *

■ 239710 Histamine H₂

Source:	Human recombinant CHO-K1 cells	Ligand:	0.1 nM [¹²⁵ I] Aminopotentidine
Vehicle:	1.0% DMSO	Non-Specific Ligand:	3.0 μM Tiotidine
Incubation Time/Temp:	2 hours @ 25°C	Specific Binding:	90% *
Incubation Buffer:	50 mM Phosphate, pH 7.4, 0.2% Gelatin	Quantitation Method:	Radioligand Binding
Kd:	0.45 nM *	Significance Criteria:	≥50% of max stimulation or inhibition
		Bmax:	6.90 pmole/mg Protein *

■ 239820 Histamine H₃

Source:	Human recombinant CHO-K1 cells	Ligand:	0.4 nM [³ H] N-α-Methylhistamine (NAMH)
Vehicle:	1.0% DMSO	Non-Specific Ligand:	1.0 μM R(-)-α-Methylhistamine (RAMH)
Incubation Time/Temp:	2 hours @ 25°C	Specific Binding:	90% *
Incubation Buffer:	50 mM Tris-HCl, pH 7.4, 5 mM MgCl ₂ , 0.1% BSA	Quantitation Method:	Radioligand Binding
Kd:	0.38 nM *	Significance Criteria:	≥50% of max stimulation or inhibition
		Bmax:	2.0 pmole/mg Protein *

■ 299035 Histamine H₄

Source:	Human recombinant HEK-293 cells	Ligand:	10.0 nM [³ H] Histamine
Vehicle:	1.0% DMSO	Non-Specific Ligand:	1.0 μM Imetit
Incubation Time/Temp:	60 minutes @ 25°C	Specific Binding:	90% *
Incubation Buffer:	50 mM Tris-HCl, pH 7.4, 2 mM EDTA	Quantitation Method:	Radioligand Binding
Kd:	7.60 nM *	Significance Criteria:	≥50% of max stimulation or inhibition
		Bmax:	0.61 pmole/mg Protein *

* Historical Values

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■ 241000 Imidazoline I₂, Central

Source:	Wistar Rat brain (minus cerebellum)	Ligand:	2.0 nM [³ H] Idazoxan
Vehicle:	1.0% DMSO	Non-Specific Ligand:	1.0 μM Idazoxan
Incubation Time/Temp:	30 minutes @ 25°C	Specific Binding:	85% *
Incubation Buffer:	50 mM Tris-HCl, pH 7.4, 0.5 mM EDTA	Quantitation Method:	Radioligand Binding
Kd:	4.0 nM *	Significance Criteria:	≥50% of max stimulation or inhibition
		Bmax:	0.14 pmole/mg Protein *

■ 242500 Inositol Trisphosphate IP₃

Source:	Wistar Rat cerebellum	Ligand:	2.50 nM [³ H] 1,4,5-IP ₃
Vehicle:	1.0% DMSO	Non-Specific Ligand:	1.0 μM 1,4,5-IP ₃
Incubation Time/Temp:	10 minutes @ 25°C	Specific Binding:	85% *
Incubation Buffer:	50 mM Tris-HCl, pH 8.3, 1 mM EDTA, 0.1% BSA	Quantitation Method:	Radioligand Binding
Kd:	0.066 μM *	Significance Criteria:	≥50% of max stimulation or inhibition
		Bmax:	3.50 pmole/mg Protein *

■ 243000 Insulin

Source:	Wistar Rat liver	Ligand:	0.03 nM [¹²⁵ I] Insulin
Vehicle:	1.0% DMSO	Non-Specific Ligand:	1.0 μM Insulin
Incubation Time/Temp:	16 hours @ 4°C	Specific Binding:	89% *
Incubation Buffer:	10 mM Phosphate Buffer, pH 7.4, 85 μg/ml PMSF, 6 μg/ml Bacitracin, 2.5 mM MgCl ₂	Quantitation Method:	Radioligand Binding
Kd:	0.47 nM *	Significance Criteria:	≥50% of max stimulation or inhibition
		Bmax:	0.13 pmole/mg Protein *

■ 243530 Interleukin IL-1 R1

Source:	Human recombinant HEK 293 cells	Ligand:	0.1 nM [¹²⁵ I] Interleukin-1β
Vehicle:	1.0% DMSO	Non-Specific Ligand:	10.0 nM Interleukin-1β
Incubation Time/Temp:	20 hours @ 4°C	Specific Binding:	80% *
Incubation Buffer:	PBS, 0.02% NaN ₃ , 0.5 mg/ml Protein A YSi SPA beads, 0.1% BSA	Quantitation Method:	Radioligand Binding
Kd:	1.90 nM *	Significance Criteria:	≥50% of max stimulation or inhibition
		Bmax:	690 pmole/mg Protein *

* Historical Values

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■ 244100 Interleukin IL-6

Source:	Human U266 cells TIB-196	Ligand:	0.08 nM [¹²⁵ I] IL-6
Vehicle:	1.0% DMSO	Non-Specific Ligand:	10.0 nM IL-6
Incubation Time/Temp:	16 hours @ 4°C	Specific Binding:	80% *
Incubation Buffer:	RPMI 1640, 10% BSA, 25 mM HEPES, pH 7.1	Quantitation Method:	Radioligand Binding
Kd:	0.047 nM *	Significance Criteria:	≥50% of max stimulation or inhibition
		Bmax:	1100 R/cell Receptors/ cell *

■ 299004 IP (PGI₂)

Source:	Human recombinant HEK-293 cells	Ligand:	6.0 nM [³ H] Iloprost
Vehicle:	1.0% DMSO	Non-Specific Ligand:	10.0 μM Iloprost
Incubation Time/Temp:	60 minutes @ 25°C	Specific Binding:	95% *
Incubation Buffer:	10 mM HEPES, pH 7.4 (adjust with KOH), 1 mM EDTA, 10 mM MnCl ₂	Quantitation Method:	Radioligand Binding
Kd:	8.0 nM *	Significance Criteria:	≥50 of max stimulation or inhibition
		Bmax:	3.0 pmole/mg *

■ 250460 Leukotriene, Cysteinyl CysLT₁

Source:	Human recombinant CHO-S cells	Ligand:	0.3 nM [³ H] LTD ₄
Vehicle:	1.0% DMSO	Non-Specific Ligand:	0.3 μM LTD ₄
Incubation Time/Temp:	30 minutes @ 25°C	Specific Binding:	93% *
Incubation Buffer:	50 mM Tris-HCl, pH 7.4, 5 mM CaCl ₂ , 5 mM MgCl ₂ , 100 μg/ml Bacitracin, 1 mM Benzamidine, 0.1 mM PMSF	Quantitation Method:	Radioligand Binding
Kd:	0.21 nM *	Significance Criteria:	≥50% of max stimulation or inhibition
		Bmax:	3.0 pmole/mg Protein *

■ 251010 Melanin-Concentrating Hormone MCH1 (SLC1)

Source:	Human recombinant CHO-K1 cells	Ligand:	0.05 nM [¹²⁵ I] Tyr-S36057
Vehicle:	1.0% DMSO	Non-Specific Ligand:	1.0 μM MCH (human, mouse, rat)
Incubation Time/Temp:	2 hours @ 25°C	Specific Binding:	85% *
Incubation Buffer:	25 mM HEPES, pH 7.4, 10 mM MgCl ₂ , 1 mM EDTA, 0.2% BSA	Quantitation Method:	Radioligand Binding
Kd:	0.1 nM *	Significance Criteria:	≥50% of max stimulation or inhibition
		Bmax:	26.0 pmole/mg Protein *

* Historical Values

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■ 251100 Melanocortin MC₁

Source:	Human recombinant CHO-K1 cells	Ligand:	0.04 nM [¹²⁵ I] NDP-α-MSH
Vehicle:	1.0% DMSO	Non-Specific Ligand:	1.0 μM NDP-α-MSH
Incubation Time/Temp:	2 hours @ 37°C	Specific Binding:	90% *
Incubation Buffer:	25 mM HEPES-KOH, pH 7.0, 100 mM NaCl, 1 mM 1,10-Phenanthroline, 1.5 mM CaCl ₂ , 1 mM MgSO ₄ , one complete TM protease inhibitor tablet/100 ml	Quantitation Method:	Radioligand Binding
Kd:	0.037 nM *	Significance Criteria:	≥50% of max stimulation or inhibition
		Bmax:	0.53 pmole/mg Protein *

■ 251300 Melanocortin MC₃

Source:	Human recombinant HEK-293 cells	Ligand:	0.035 nM [¹²⁵ I] NDP-α-MSH
Vehicle:	1.0% DMSO	Non-Specific Ligand:	3.0 μM NDP-α-MSH
Incubation Time/Temp:	60 minutes @ 37°C	Specific Binding:	80% *
Incubation Buffer:	25mM HEPES,-KOH pH 7.0, 100 mM NaCl, 1mM 1,10-Phenanthroline, 1.5 mM CaCl ₂ , 1 mM MgSO ₄ , one complete TM protease inhibitor tablet/100 ml	Quantitation Method:	Radioligand Binding
Kd:	0.24 nM *	Significance Criteria:	≥50% of max stimulation or inhibition
		Bmax:	1.30 pmole/mg Protein *

■ 251350 Melanocortin MC₄

Source:	Human recombinant HEK-293 cells	Ligand:	20.0 pM [¹²⁵ I] NDP-α-MSH
Vehicle:	1.0% DMSO	Non-Specific Ligand:	3.0 μM NDP-α-MSH
Incubation Time/Temp:	2 hours @ 37°C	Specific Binding:	90% *
Incubation Buffer:	25 mM HEPES-KOH, pH 7.0, 100 mM NaCl, 1 mM 1,10-Phenanthroline, 1.5 mM CaCl ₂ , 1 mM MgSO ₄ , one complete TM protease inhibitor tablet/100 ml	Quantitation Method:	Radioligand Binding
Kd:	0.5 nM *	Significance Criteria:	≥50% of max stimulation or inhibition
		Bmax:	3.90 pmole/mg Protein *

* Historical Values

Cleveland Clinic Foundation

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Methods

■ 251400 Melanocortin MC₅

Source:	Human recombinant HEK-293 cells	Ligand:	0.035 nM [¹²⁵ I] NDP-α-MSH
Vehicle:	1.0% DMSO	Non-Specific Ligand:	3.0 μM NDP-α-MSH
Incubation Time/Temp:	60 minutes @ 37°C	Specific Binding:	85% *
Incubation Buffer:	25 mM HEPES-KOH, pH 7.0, 100 mM NaCl, 1 mM 1,10-Phenanthroline, 1.5 mM CaCl ₂ , 1 mM MgSO ₄ , one complete™ protease inhibitor tablet/100 ml	Quantitation Method:	Radioligand Binding
Kd:	0.53 nM *	Significance Criteria:	≥50% of max stimulation or inhibition
		Bmax:	6.0 pmole/mg Protein *

■ 251600 Melatonin MT₁

Source:	Human recombinant CHO-K1 cells	Ligand:	0.05 nM [¹²⁵ I] 2-Iodomelatonin
Vehicle:	1.0% DMSO	Non-Specific Ligand:	1.0 μM 6-Chloromelatonin
Incubation Time/Temp:	3 hours @ 25°C	Specific Binding:	97% *
Incubation Buffer:	25 mM HEPES, pH 7.4, 5 mM MgCl ₂ , 1 mM CaCl ₂ , 0.5% BSA	Quantitation Method:	Radioligand Binding
Kd:	0.054 nM *	Significance Criteria:	≥50% of max stimulation or inhibition
		Bmax:	3.50 pmole/mg Protein *

■ 251700 Melatonin MT₂

Source:	Human recombinant CHO-S cells	Ligand:	0.05 nM [¹²⁵ I] 2-Iodomelatonin
Vehicle:	1.0% DMSO	Non-Specific Ligand:	1.0 μM 6-Chloromelatonin
Incubation Time/Temp:	4 hours @ 25°C	Specific Binding:	98% *
Incubation Buffer:	25 mM HEPES, pH 7.4, 5 mM MgCl ₂ , 1 mM CaCl ₂ , 0.1% BSA	Quantitation Method:	Radioligand Binding
Kd:	0.05 nM *	Significance Criteria:	≥50% of max stimulation or inhibition
		Bmax:	0.93 pmole/mg Protein *

■ 252200 Motilin

Source:	Human recombinant HEK-293 cells	Ligand:	0.1 nM [¹²⁵ I] Motilin (human, porcine)
Vehicle:	1.0% DMSO	Non-Specific Ligand:	1.0 μM Motilin (human, porcine)
Incubation Time/Temp:	2.5 hours @ 25°C	Specific Binding:	95% *
Incubation Buffer:	50 mM Tris, pH 7.4, 10 mM MgCl ₂ , 0.5% BSA	Quantitation Method:	Radioligand Binding
Kd:	0.1 nM *	Significance Criteria:	≥50% of max stimulation or inhibition
		Bmax:	9.80 pmole/mg Protein *

* Historical Values

Cleveland Clinic Foundation

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Methods

■ 252610 Muscarinic M₁

Source:	Human recombinant CHO-K1 cells	Ligand:	0.8 nM [³ H] N-Methylscopolamine
Vehicle:	1.0% DMSO	Non-Specific Ligand:	1.0 μM Atropine
Incubation Time/Temp:	2 hours @ 25°C	Specific Binding:	95% *
Incubation Buffer:	50 mM Tris-HCl, pH 7.4, 10 mM MgCl ₂ , 1 mM EDTA	Quantitation Method:	Radioligand Binding
Kd:	0.26 nM *	Significance Criteria:	≥50% of max stimulation or inhibition
		Bmax:	2.0 pmole/mg Protein *

■ 252710 Muscarinic M₂

Source:	Human recombinant CHO-K1 cells	Ligand:	0.8 nM [³ H] N-Methylscopolamine
Vehicle:	1.0% DMSO	Non-Specific Ligand:	1.0 μM Atropine
Incubation Time/Temp:	2 hours @ 25°C	Specific Binding:	95% *
Incubation Buffer:	50 mM Tris-HCl, pH 7.4, 10 mM MgCl ₂ , 1 mM EDTA	Quantitation Method:	Radioligand Binding
Kd:	0.58 nM *	Significance Criteria:	≥50% of max stimulation or inhibition
		Bmax:	5.10 pmole/mg Protein *

■ 252810 Muscarinic M₃

Source:	Human recombinant CHO-K1 cells	Ligand:	0.8 nM [³ H] N-Methylscopolamine
Vehicle:	1.0% DMSO	Non-Specific Ligand:	1.0 μM Atropine
Incubation Time/Temp:	2 hours @ 25°C	Specific Binding:	95% *
Incubation Buffer:	50 mM Tris-HCl, pH 7.4, 10 mM MgCl ₂ , 1 mM EDTA	Quantitation Method:	Radioligand Binding
Kd:	0.75 nM *	Significance Criteria:	≥50% of max stimulation or inhibition
		Bmax:	5.40 pmole/mg Protein *

■ 252910 Muscarinic M₄

Source:	Human recombinant CHO-K1 cells	Ligand:	0.8 nM [³ H] N-Methylscopolamine
Vehicle:	1.0% DMSO	Non-Specific Ligand:	1.0 μM Atropine
Incubation Time/Temp:	2 hours @ 25°C	Specific Binding:	95% *
Incubation Buffer:	50 mM Tris-HCl, pH 7.4, 10 mM MgCl ₂ , 1 mM EDTA	Quantitation Method:	Radioligand Binding
Kd:	0.22 nM *	Significance Criteria:	≥50% of max stimulation or inhibition
		Bmax:	1.60 pmole/mg Protein *

* Historical Values

Cleveland Clinic Foundation

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Methods

■ 253010 Muscarinic M₅

Source:	Human recombinant CHO-K1 cells	Ligand:	0.8 nM [³ H] N-Methylscopolamine
Vehicle:	1.0% DMSO	Non-Specific Ligand:	1.0 μM Atropine
Incubation Time/Temp:	2 hours @ 25°C	Specific Binding:	95% *
Incubation Buffer:	50 mM Tris-HCl, pH 7.4, 10 mM MgCl ₂ , 1 mM EDTA	Quantitation Method:	Radioligand Binding
Kd:	1.30 nM *	Significance Criteria:	≥50% of max stimulation or inhibition
		Bmax:	5.80 pmole/mg Protein *

■ 254500 Muscarinic, Oxotremorine M

Source:	Wistar Rat cerebral cortex	Ligand:	5.0 nM [³ H] Oxotremorine M
Vehicle:	1.0% DMSO	Non-Specific Ligand:	10.0 μM Oxotremorine M
Incubation Time/Temp:	30 minutes @ 37°C	Specific Binding:	90% *
Incubation Buffer:	50 mM Tris-HCl, pH 7.4, 2 mM MgCl ₂	Quantitation Method:	Radioligand Binding
Kd:	4.80 nM *	Significance Criteria:	≥50% of max stimulation or inhibition
		Bmax:	0.2 pmole/mg Protein *

■ 257010 Neuropeptide Y Y₁

Source:	Human SK-N-MC cells	Ligand:	15.0 pM [¹²⁵ I] Peptide YY
Vehicle:	1.0% DMSO	Non-Specific Ligand:	1.0 μM Neuropeptide Y (human, rat)
Incubation Time/Temp:	60 minutes @ 37°C	Specific Binding:	80% *
Incubation Buffer:	25 mM HEPES, pH 7.4, 1 mM MgCl ₂ , 2.5 mM CaCl ₂ , 0.1% BSA, 0.01% Bacitracin	Quantitation Method:	Radioligand Binding
Kd:	0.24 nM *	Significance Criteria:	≥50% of max stimulation or inhibition
		Bmax:	0.58 pmole/mg protein *

■ 257110 Neuropeptide Y Y₂

Source:	Human KAN-TS cells	Ligand:	10.0 pM [¹²⁵ I] Peptide YY
Vehicle:	1.0% DMSO	Non-Specific Ligand:	1.0 μM Neuropeptide Y (13-36) (porcine)
Incubation Time/Temp:	2 hours @ 37°C	Specific Binding:	90% *
Incubation Buffer:	25 mM HEPES, pH 7.4, 2.5 mM CaCl ₂ , 1 mM MgCl ₂ , 0.1% Bacitracin	Quantitation Method:	Radioligand Binding
Kd:	12.0 pM *	Significance Criteria:	≥50% of max stimulation or inhibition
		Bmax:	0.5 pmole/mg Protein *

* Historical Values

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Methods

■ 258010 Neurotensin NT₁

Source:	Human recombinant HEK-293 cells	Ligand:	20.0 pM [¹²⁵ I] Neurotensin
Vehicle:	1.0% DMSO	Non-Specific Ligand:	1.0 μM Neurotensin
Incubation Time/Temp:	45 minutes @ 25°C	Specific Binding:	90% *
Incubation Buffer:	50 mM Tris-HCl, pH 7.4, 1 mM MgCl ₂ , 0.1% BSA, 0.8 mM 1,10-Phenanthroline	Quantitation Method:	Radioligand Binding
Kd:	0.082 nM *	Significance Criteria:	≥50% of max stimulation or inhibition
		Bmax:	4.40 pmole/mg Protein *

■ 258700 Nicotinic Acetylcholine α1, Bungarotoxin

Source:	Human RD cells	Ligand:	0.6 nM [¹²⁵ I] α-Bungarotoxin
Vehicle:	1.0% DMSO	Non-Specific Ligand:	1.0 μM α-Bungarotoxin
Incubation Time/Temp:	2 hours @ 25°C	Specific Binding:	85% *
Incubation Buffer:	147 mM NaCl, 4 mM KCl, 2.2 mM CaCl ₂	Quantitation Method:	Radioligand Binding
Kd:	1.10 nM *	Significance Criteria:	≥50% of max stimulation or inhibition
		Bmax:	1.0 pmole/mg Protein *

■ 258730 Nicotinic Acetylcholine α3β4

Source:	Human recombinant CHO-K1 cells	Ligand:	0.05 nM [¹²⁵ I] Epibatidine
Vehicle:	1.0% DMSO	Non-Specific Ligand:	10.0 μM Epibatidine
Incubation Time/Temp:	60 minutes @ 25°C	Specific Binding:	90% *
Incubation Buffer:	50 mM Tris-HCl, pH 7.4	Quantitation Method:	Radioligand Binding
Kd:	0.13 nM *	Significance Criteria:	≥50% of max stimulation or inhibition
		Bmax:	1.50 pmole/mg Protein *

■ 258750 Nicotinic Acetylcholine α4β2, Cytisine

Source:	Rat brain	Ligand:	2.0 nM [³ H] Cytisine
Vehicle:	1.0% DMSO	Non-Specific Ligand:	10.0 μM (-)-Nicotine
Incubation Time/Temp:	2 hours @ 4°C	Specific Binding:	75% *
Incubation Buffer:	50 mM Tris-HCl, pH 7.4, 2 mM CaCl ₂ , 5 mM KCl, 1 mM MgCl ₂ , 120 mM NaCl	Quantitation Method:	Radioligand Binding
Kd:	0.86 nM *	Significance Criteria:	≥50% of max stimulation or inhibition
		Bmax:	0.039 pmole/mg Protein *

* Historical Values

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■ 258630 Nicotinic Acetylcholine $\alpha 7$, Bungarotoxin

Source:	Wistar Rat brain (minus cerebellum)	Ligand:	0.6 nM [^{125} I] α -Bungarotoxin
Vehicle:	1.0% DMSO	Non-Specific Ligand:	1.0 μ M α -Bungarotoxin
Incubation Time/Temp:	2.5 hours @ 37°C	Specific Binding:	85% *
Incubation Buffer:	50 mM Potassium Phosphate, pH 7.4, 1 mM EDTA, 0.1 mM PMSF	Quantitation Method:	Radioligand Binding
Kd:	2.10 nM *	Significance Criteria:	$\geq 50\%$ of max stimulation or inhibition
		Bmax:	0.11 pmole/mg Protein *

■ 258660 Nicotinic Acetylcholine $\alpha 7$, Methyllaconitine

Source:	Human recombinant SH-SY5Y Cells	Ligand:	2.0 nM [^{3} H] Methyllaconitine
Vehicle:	1.0% DMSO	Non-Specific Ligand:	10.0 μ M Methyllaconitine
Incubation Time/Temp:	2 hours @ 25°C	Specific Binding:	90% *
Incubation Buffer:	50 mM Phosphate buffer (40 mM $K_2HPO_4 \cdot 3H_2O$ + 10 mM KH_2PO_4), pH 7.4, 0.1% BSA	Quantitation Method:	Radioligand Binding
Kd:	1.70 nM *	Significance Criteria:	$\geq 50\%$ of max stimulation or inhibition
		Bmax:	0.99 pmole/mg Protein *

■ 260130 Opiate δ_1 (OP1, DOP)

Source:	Human recombinant HEK-293 cells	Ligand:	1.30 nM [^{3} H] Naltrindole
Vehicle:	1.0% DMSO	Non-Specific Ligand:	1.0 μ M Naltrindole
Incubation Time/Temp:	60 minutes @ 25°C	Specific Binding:	95% *
Incubation Buffer:	50 mM Tris-HCl, pH 7.4, 1 mM EDTA, 10 mM $MgCl_2$	Quantitation Method:	Radioligand Binding
Kd:	0.053 nM *	Significance Criteria:	$\geq 50\%$ of max stimulation or inhibition
		Bmax:	1.40 pmole/mg Protein *

■ 260210 Opiate κ (OP2, KOP)

Source:	Human recombinant HEK-293 cells	Ligand:	0.6 nM [^{3} H] Diprenorphine
Vehicle:	1.0% DMSO	Non-Specific Ligand:	10.0 μ M Naloxone
Incubation Time/Temp:	60 minutes @ 25°C	Specific Binding:	90% *
Incubation Buffer:	50 mM Tris-HCl, pH 7.4	Quantitation Method:	Radioligand Binding
Kd:	0.4 nM *	Significance Criteria:	$\geq 50\%$ of max stimulation or inhibition
		Bmax:	1.10 pmole/mg Protein *

* Historical Values

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■ 260410 Opiate μ (OP3, MOP)

Source:	Human recombinant CHO-K1 cells	Ligand:	0.6 nM [^{3} H] Diprenorphine
Vehicle:	1.0% DMSO	Non-Specific Ligand:	10.0 μ M Naloxone
Incubation Time/Temp:	60 minutes @ 25°C	Specific Binding:	90% *
Incubation Buffer:	50 mM Tris-HCl, pH 7.4	Quantitation Method:	Radioligand Binding
Kd:	0.41 nM *	Significance Criteria:	$\geq 50\%$ of max stimulation or inhibition
		Bmax:	3.80 pmole/mg Protein *

■ 299030 Orexin OX₁

Source:	Human recombinant CHO-S cells	Ligand:	0.1 nM [^{125} I] Orexin A
Vehicle:	1.0% DMSO	Non-Specific Ligand:	1.0 μ M SB 334867
Incubation Time/Temp:	60 minutes @ 25°C	Specific Binding:	90% *
Incubation Buffer:	25 mM Hepes/NaOH, pH7.4, 2.5 mM MgCl ₂ , 2.5 mM CaCl ₂ , 0.5 mM EDTA, 0.25 mg/ml Bacitracine	Quantitation Method:	Radioligand Binding
Kd:	10.0 nM *	Significance Criteria:	≥ 50 of max stimulation or inhibition
		Bmax:	2.40 pmole/mg *

■ 299002 Orexin OX₂

Source:	Human recombinant HEK-293 cells	Ligand:	0.04 nM [^{125} I] Orexin A
Vehicle:	1.0% DMSO	Non-Specific Ligand:	1.0 μ M Orexin B
Incubation Time/Temp:	3 hours @ 25°C	Specific Binding:	85% *
Incubation Buffer:	25 mM Hepes/NaOH, pH7.4, 1 mM MgCl ₂ , 2.5 mM CaCl ₂ , 0.5% BSA	Quantitation Method:	Radioligand Binding
Kd:	0.2 nM *	Significance Criteria:	≥ 50 of max stimulation or inhibition
		Bmax:	0.21 pmole/mg *

■ 260600 Orphanin ORL₁

Source:	Human recombinant HEK-293 cells	Ligand:	0.6 nM [^{3} H] Nociceptin
Vehicle:	1.0% DMSO	Non-Specific Ligand:	1.0 μ M Orphanin-FQ
Incubation Time/Temp:	60 minutes @ 25°C	Specific Binding:	85% *
Incubation Buffer:	50 mM HEPES, pH 7.4, 1 mM EDTA, 10 mM MgCl ₂ , 0.01% Bacitracin	Quantitation Method:	Radioligand Binding
Kd:	1.0 nM *	Significance Criteria:	$\geq 50\%$ of max stimulation or inhibition
		Bmax:	4.60 pmole/mg Protein *

* Historical Values

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■ 260900 Oxytocin

Source:	Human recombinant Chem-1 cells	Ligand:	0.8 nM [³ H] Oxytocin
Vehicle:	1.0% DMSO	Non-Specific Ligand:	1.0 μM Oxytocin
Incubation Time/Temp:	2 hours @ 25°C	Specific Binding:	90% *
Incubation Buffer:	50 mM Tris-HCl, pH 7.4, 5 mM MgCl ₂ , 0.1% BSA	Quantitation Method:	Radioligand Binding
Kd:	0.39 nM *	Significance Criteria:	≥50% of max stimulation or inhibition
		Bmax:	4.0 pmole/mg Protein *

■ 264500 Phorbol Ester

Source:	ICR Mouse brain (minus cerebellum)	Ligand:	3.0 nM [³ H] PDBu
Vehicle:	1.0% DMSO	Non-Specific Ligand:	1.0 μM PDBu
Incubation Time/Temp:	60 minutes @ 25°C	Specific Binding:	80% *
Incubation Buffer:	20 mM Tris-HCl, pH 7.4, 5 mM CaCl ₂	Quantitation Method:	Radioligand Binding
Kd:	8.70 nM *	Significance Criteria:	≥50% of max stimulation or inhibition
		Bmax:	26.0 pmole/mg Protein *

■ 299037 Platelet Activating Factor (PAF)

Source:	Human recombinant CHO-K1 cells	Ligand:	1.0 nM [³ H] PAF
Vehicle:	1.0% DMSO	Non-Specific Ligand:	10.0 μM C16-PAF
Incubation Time/Temp:	3 hours @ 25°C	Specific Binding:	80% *
Incubation Buffer:	25 mM HEPES, pH 7.4, 10 mM MgCl ₂ , 0.3% BSA	Quantitation Method:	Radioligand Binding
Kd:	1.50 nM *	Significance Criteria:	≥50% of max stimulation or inhibition
		Bmax:	0.78 pmole/mg *

■ 265200 Platelet-Derived Growth Factor (PDGF)

Source:	Mouse 3T3-SWISS cells	Ligand:	20.0 pM [¹²⁵ I] PDGF
Vehicle:	1.0% DMSO	Non-Specific Ligand:	10.0 nM PDGF
Incubation Time/Temp:	45 minutes @ 25°C	Specific Binding:	88% *
Incubation Buffer:	DMEM (Dulbecco's Modified Eagle Medium, Low glucose), pH 7.4, 10% FBS	Quantitation Method:	Radioligand Binding
Kd:	12.0 pM *	Significance Criteria:	≥50% of max stimulation or inhibition
		Bmax:	3100 R/cell Receptors/cell *

* Historical Values

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Methods

■ 265510 Potassium Channel [K_A]

Source:	Wistar Rat cerebral cortex	Ligand:	5.30 pM [¹²⁵ I] α-Dendrotoxin
Vehicle:	1.0% DMSO	Non-Specific Ligand:	10.0 nM α-Dendrotoxin
Incubation Time/Temp:	60 minutes @ 37°C	Specific Binding:	90% *
Incubation Buffer:	50 mM Tris-HCl, pH 7.4, 20 mM NaCl, 0.3% BSA	Quantitation Method:	Radioligand Binding
Kd:	4.60 pM *	Significance Criteria:	≥50% of max stimulation or inhibition
		Bmax:	1.0 pmole/mg Protein *

■ 265600 Potassium Channel [K_{ATP}]

Source:	Hamster pancreatic HIT-T15 beta cells	Ligand:	5.0 nM [³ H] Glyburide
Vehicle:	1.0% DMSO	Non-Specific Ligand:	1.0 μM Glyburide
Incubation Time/Temp:	2 hours @ 25°C	Specific Binding:	90% *
Incubation Buffer:	50 mM MOPS, pH 7.4, 0.1 mM CaCl ₂	Quantitation Method:	Radioligand Binding
Kd:	0.64 nM *	Significance Criteria:	≥50% of max stimulation or inhibition
		Bmax:	1.0 pmole/mg Protein *

■ 265800 Potassium Channel [SK_{CA}]

Source:	Wistar Rat brain	Ligand:	5.0 pM [¹²⁵ I] Apamin
Vehicle:	1.0% DMSO	Non-Specific Ligand:	0.1 μM Apamin
Incubation Time/Temp:	60 minutes @ 4°C	Specific Binding:	90% *
Incubation Buffer:	25 mM Tris-HCl, pH 7.4, 10 mM KCl, 0.1% BSA	Quantitation Method:	Radioligand Binding
Kd:	0.14 nM *	Significance Criteria:	≥50% of max stimulation or inhibition
		Bmax:	0.067 pmole/mg Protein *

■ 265910 Potassium Channel hERG, [³H]Dofetilide

Source:	Human recombinant HEK-293 cells	Ligand:	3.0 nM [³ H] Dofetilide
Vehicle:	1.0% DMSO	Non-Specific Ligand:	10.0 μM Dofetilide
Incubation Time/Temp:	60 minutes @ 25°C	Specific Binding:	95% *
Incubation Buffer:	50 mM Tris-HCl, pH 7.4, 10 mM KCl, 1 mM MgCl ₂	Quantitation Method:	Radioligand Binding
Kd:	6.60 nM *	Significance Criteria:	≥50% of max stimulation or inhibition
		Bmax:	8.10 pmole/mg Protein *

* Historical Values

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Methods

■ 299005 Progesterone PR-B

Source:	Human T-47D cells	Ligand:	0.5 nM [³ H] Progesterone
Vehicle:	1.0% DMSO	Non-Specific Ligand:	1.0 μM R-5020
Incubation Time/Temp:	20 hours @ 4°C	Specific Binding:	91% *
Incubation Buffer:	5 mM Na ₂ HPO ₄ /NaH ₂ PO ₄ , pH 7.4, 10 % Glycerol, 10 mM Monothioglycerol, 20 mM Sodium molybdate	Quantitation Method:	Radioligand Binding
Kd:	2.0 nM *	Significance Criteria:	≥50 of max stimulation or inhibition
		Bmax:	0.0002 fmole/cell *

■ 268030 Prostanoid CRTH2

Source:	Human recombinant CHO-K1 cells	Ligand:	1.0 nM [³ H] Prostaglandin D ₂ (PGD ₂)
Vehicle:	1.0% DMSO	Non-Specific Ligand:	1.0 μM Prostaglandin D ₂ (PGD ₂)
Incubation Time/Temp:	2 hours @ 25°C	Specific Binding:	88% *
Incubation Buffer:	50 mM Tris-HCl, pH 7.4, 40 mM MgCl ₂ , 0.1% BSA, 0.1% NaN ₃	Quantitation Method:	Radioligand Binding
Kd:	4.10 nM *	Significance Criteria:	≥50% of max stimulation or inhibition
		Bmax:	13.0 pmole/mg Protein *

■ 268060 Prostanoid DP

Source:	Human recombinant Chem-1 cells	Ligand:	2.0 nM [³ H] Prostaglandin D ₂ (PGD ₂)
Vehicle:	1.0% DMSO	Non-Specific Ligand:	1.0 μM Prostaglandin D ₂ (PGD ₂)
Incubation Time/Temp:	2 hours @ 25°C	Specific Binding:	80% *
Incubation Buffer:	25 mM HEPES, pH 7.4, 5 mM MnCl ₂ , 1 mM EDTA	Quantitation Method:	Radioligand Binding
Kd:	10.0 nM *	Significance Criteria:	≥50% of max stimulation or inhibition
		Bmax:	1.90 pmole/mg Protein *

■ 268110 Prostanoid EP₁

Source:	Human recombinant HEK-293 cells	Ligand:	1.0 nM [³ H] Prostaglandin E ₂ (PGE ₂)
Vehicle:	1.0% DMSO	Non-Specific Ligand:	10.0 μM Prostaglandin E ₂ (PGE ₂)
Incubation Time/Temp:	60 minutes @ 25°C	Specific Binding:	93% *
Incubation Buffer:	10 mM MES, pH 6.0 (adjust with KOH), 1 mM EDTA, 10 mM MgCl ₂	Quantitation Method:	Radioligand Binding
Kd:	5.20 nM *	Significance Criteria:	≥50% of max stimulation or inhibition
		Bmax:	2.60 pmole/mg Protein *

* Historical Values

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■ 268200 Prostanoid EP₂

Source:	Human recombinant HEK-293 cells	Ligand:	4.0 nM [³ H] Prostaglandin E ₂ (PGE ₂)
Vehicle:	1.0% DMSO	Non-Specific Ligand:	10.0 μM Prostaglandin E ₂ (PGE ₂)
Incubation Time/Temp:	2 hours @ 25°C	Specific Binding:	80% *
Incubation Buffer:	10 mM MES, pH 6.0, 1 mM EDTA, 10 mM MgCl ₂	Quantitation Method:	Radioligand Binding
Kd:	3.10 nM *	Significance Criteria:	≥50% of max stimulation or inhibition
		Bmax:	1.40 pmole/mg Protein *

■ 299006 Prostanoid EP₃

Source:	Human recombinant HEK-293 cells	Ligand:	0.5 nM [³ H] Prostaglandin E ₂ (PGE ₂)
Vehicle:	1.0% DMSO	Non-Specific Ligand:	1.0 μM Prostaglandin E ₂ (PGE ₂)
Incubation Time/Temp:	2 hours @ 25°C	Specific Binding:	93% *
Incubation Buffer:	10 mM MES, pH 6.0 (adjust with KOH), 1 mM EDTA, 10 mM MgCl ₂	Quantitation Method:	Radioligand Binding
Kd:	0.8 nM *	Significance Criteria:	≥50 of max stimulation or inhibition
		Bmax:	4.10 pmole/mg *

■ 268420 Prostanoid EP₄

Source:	Human recombinant Chem-1 cells	Ligand:	1.0 nM [³ H] Prostaglandin E ₂ (PGE ₂)
Vehicle:	1.0% DMSO	Non-Specific Ligand:	10.0 μM Prostaglandin E ₂ (PGE ₂)
Incubation Time/Temp:	2 hours @ 25°C	Specific Binding:	90% *
Incubation Buffer:	10 mM MES, pH 6.0, 1 mM EDTA, 10 mM MgCl ₂	Quantitation Method:	Radioligand Binding
Kd:	0.69 nM *	Significance Criteria:	≥50% of max stimulation or inhibition
		Bmax:	4.30 pmole/mg Protein *

■ 268510 Prostanoid FP

Source:	Human recombinant HEK-293 cells	Ligand:	1.0 nM [³ H] PGF ₂ α
Vehicle:	1.0% DMSO	Non-Specific Ligand:	1.0 μM Cloprostenol
Incubation Time/Temp:	60 minutes @ 25°C	Specific Binding:	94% *
Incubation Buffer:	10 mM MES, pH 6.0, 1 mM EDTA, 10 mM MnCl ₂	Quantitation Method:	Radioligand Binding
Kd:	2.40 nM *	Significance Criteria:	≥50% of max stimulation or inhibition
		Bmax:	0.48 pmole/mg Protein *

* Historical Values

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Methods

■ 299036 Purinergic P2X

Source:	Wistar Rat urinary bladder	Ligand:	3.0 nM [³ H] α, β-Methylene-ATP
Vehicle:	1.0% DMSO	Non-Specific Ligand:	10.0 μM α, β-Methylene-ATP
Incubation Time/Temp:	2 hours @ 4°C	Specific Binding:	90% *
Incubation Buffer:	50 mM Tris-HCl, pH 7.0, 1 mM EDTA	Quantitation Method:	Radioligand Binding
Kd:	2.60 nM *	Significance Criteria:	≥50% of max stimulation or inhibition
		Bmax:	3.65 pmole/mg Protein *

■ 268820 Purinergic P2Y, Non-Selective

Source:	Wistar Rat brain (minus cerebellum)	Ligand:	0.1 nM [³⁵ S] dATPαS
Vehicle:	1.0% DMSO	Non-Specific Ligand:	10.0 μM ATP
Incubation Time/Temp:	3 hours @ 25°C	Specific Binding:	98% *
Incubation Buffer:	50 mM Tris-HCl, pH 7.4, 4 mM EDTA	Quantitation Method:	Radioligand Binding
Kd:	0.38 nM *	Significance Criteria:	≥50% of max stimulation or inhibition
		Bmax:	3.38 pmole/mg Protein *

■ 270000 Rolipram

Source:	Wistar Rat brain	Ligand:	1.80 nM [³ H] Rolipram
Vehicle:	1.0% DMSO	Non-Specific Ligand:	10.0 μM Rolipram
Incubation Time/Temp:	60 minutes @ 4°C	Specific Binding:	90% *
Incubation Buffer:	50 mM Tris-HCl, pH 7.4	Quantitation Method:	Radioligand Binding
Kd:	1.0 nM *	Significance Criteria:	≥50% of max stimulation or inhibition
		Bmax:	0.31 pmole/mg Protein *

■ 270300 Ryanodine RyR3

Source:	Wistar Rat cerebral cortex	Ligand:	3.0 nM [³ H] Ryanodine
Vehicle:	1.0% DMSO	Non-Specific Ligand:	10.0 μM Ryanodine
Incubation Time/Temp:	2 hours @ 37°C	Specific Binding:	80% *
Incubation Buffer:	10 mM HEPES, pH 7.2, 1 M KCl, 100 μM CaCl ₂	Quantitation Method:	Radioligand Binding
Kd:	2.0 nM *	Significance Criteria:	≥50% of max stimulation or inhibition
		Bmax:	0.11 pmole/mg Protein *

* Historical Values

Cleveland Clinic Foundation

Study #: TW04-0013389, Compound Code: CCF2201 (1271304)

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Methods

■ 271110 Serotonin (5-Hydroxytryptamine) 5-HT_{1A}

Source:	Human recombinant CHO-K1 cells	Ligand:	1.50 nM [³ H] 8-OH-DPAT
Vehicle:	1.0% DMSO	Non-Specific Ligand:	10.0 μM Metergoline
Incubation Time/Temp:	60 minutes @ 25°C	Specific Binding:	75% *
Incubation Buffer:	50 mM Tris-HCl, pH 7.4, 0.1% Ascorbic Acid, 0.5 mM EDTA, 10 mM MgSO ₄	Quantitation Method:	Radioligand Binding
Kd:	2.0 nM *	Significance Criteria:	≥50% of max stimulation or inhibition
		Bmax:	1.30 pmole/mg Protein *

■ 271200 Serotonin (5-Hydroxytryptamine) 5-HT_{1B}

Source:	Wistar Rat cerebral cortex	Ligand:	10.0 pM [¹²⁵ I] Cyanopindolol
Vehicle:	1.0% DMSO	Non-Specific Ligand:	10.0 μM Serotonin (5-HT)
Incubation Time/Temp:	90 minutes @ 37°C	Specific Binding:	70% *
Incubation Buffer:	50 mM Tris-HCl, pH 7.4, 154 mM NaCl, 10 μM Pargyline, 30 μM Isoprenaline	Quantitation Method:	Radioligand Binding
Kd:	0.19 nM *	Significance Criteria:	≥50% of max stimulation or inhibition
		Bmax:	0.14 pmole/mg Protein *

■ 271700 Serotonin (5-Hydroxytryptamine) 5-HT_{2B}

Source:	Human recombinant CHO-K1 cells	Ligand:	1.20 nM [³ H] Lysergic acid diethylamide (LSD)
Vehicle:	1.0% DMSO	Non-Specific Ligand:	10.0 μM Serotonin (5-HT)
Incubation Time/Temp:	60 minutes @ 37°C	Specific Binding:	80% *
Incubation Buffer:	50 mM Tris-HCl, pH 7.4, 4 mM CaCl ₂ , 0.1% Ascorbic Acid	Quantitation Method:	Radioligand Binding
Kd:	2.10 nM *	Significance Criteria:	≥50% of max stimulation or inhibition
		Bmax:	1.10 pmole/mg Protein *

■ 271800 Serotonin (5-Hydroxytryptamine) 5-HT_{2C}

Source:	Human recombinant CHO-K1 cells	Ligand:	1.0 nM [³ H] Mesulergine
Vehicle:	1.0% DMSO	Non-Specific Ligand:	1.0 μM Mianserin
Incubation Time/Temp:	60 minutes @ 25°C	Specific Binding:	90% *
Incubation Buffer:	50 mM Tris-HCl, pH 7.4, 0.1% Ascorbic Acid, 10 μM Pargyline	Quantitation Method:	Radioligand Binding
Kd:	1.10 nM *	Significance Criteria:	≥50% of max stimulation or inhibition
		Bmax:	4.90 pmole/mg Protein *

* Historical Values

Cleveland Clinic Foundation

Study #: TW04-0013389, Compound Code: CCF2201 (1271304)

Friday, December 2, 2022

Methods

■ 271910 Serotonin (5-Hydroxytryptamine) 5-HT₃

Source:	Human recombinant HEK-293 cells	Ligand:	0.69 nM [³ H] GR-65630
Vehicle:	1.0% DMSO	Non-Specific Ligand:	10.0 μM MDL 72222
Incubation Time/Temp:	60 minutes @ 25°C	Specific Binding:	90% *
Incubation Buffer:	50 mM Tris-HCl, pH 7.4, 1 mM EDTA, 5 mM MgCl ₂	Quantitation Method:	Radioligand Binding
Kd:	0.2 nM *	Significance Criteria:	≥50% of max stimulation or inhibition
		Bmax:	11.0 pmole/mg Protein *

■ 272000 Serotonin (5-Hydroxytryptamine) 5-HT₄

Source:	Duncan Hartley derived Guinea pig striatum	Ligand:	0.7 nM [³ H] GR-113808
Vehicle:	1.0% DMSO	Non-Specific Ligand:	100 μM RS-23597-190
Incubation Time/Temp:	30 minutes @ 25°C	Specific Binding:	80% *
Incubation Buffer:	50 mM Tris-HCl, pH 7.4	Quantitation Method:	Radioligand Binding
Kd:	0.14 nM *	Significance Criteria:	≥50% of max stimulation or inhibition
		Bmax:	0.13 pmole/mg Protein *

■ 272100 Serotonin (5-Hydroxytryptamine) 5-HT_{5A}

Source:	Human recombinant CHO-K1 cells	Ligand:	1.70 nM [³ H] Lysergic acid diethylamide (LSD)
Vehicle:	1.0% DMSO	Non-Specific Ligand:	100 μM Serotonin (5-HT)
Incubation Time/Temp:	60 minutes @ 37°C	Specific Binding:	80% *
Incubation Buffer:	50 mM Tris-HCl, pH 7.4, 10 mM MgCl ₂ , 0.5 mM EDTA	Quantitation Method:	Radioligand Binding
Kd:	1.80 nM *	Significance Criteria:	≥50% of max stimulation or inhibition
		Bmax:	4.30 pmole/mg Protein *

■ 272200 Serotonin (5-Hydroxytryptamine) 5-HT₆

Source:	Human recombinant HeLa cells	Ligand:	1.50 nM [³ H] Lysergic acid diethylamide (LSD)
Vehicle:	1.0% DMSO	Non-Specific Ligand:	5.0 μM Serotonin (5-HT)
Incubation Time/Temp:	2 hours @ 37°C	Specific Binding:	90% *
Incubation Buffer:	50 mM Tris-HCl, pH 7.4, 150 mM NaCl, 2 mM Ascorbic Acid, 0.001% BSA	Quantitation Method:	Radioligand Binding
Kd:	1.30 nM *	Significance Criteria:	≥50% of max stimulation or inhibition
		Bmax:	1.70 pmole/mg Protein *

* Historical Values

Cleveland Clinic Foundation

Study #: TW04-0013389, Compound Code: CCF2201 (1271304)

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Methods

■ 272320 Serotonin (5-Hydroxytryptamine) 5-HT₇

Source:	Human recombinant CHO-K1 cells	Ligand:	5.50 nM [³ H] Lysergic acid diethylamide (LSD)
Vehicle:	1.0% DMSO	Non-Specific Ligand:	10.0 μM Serotonin (5-HT)
Incubation Time/Temp:	2 hours @ 25°C	Specific Binding:	90% *
Incubation Buffer:	50 mM Tris-HCl, pH 7.4, 10 mM MgCl ₂ , 0.5 mM EDTA	Quantitation Method:	Radioligand Binding
Kd:	7.40 nM *	Significance Criteria:	≥50% of max stimulation or inhibition
		Bmax:	0.95 pmole/mg Protein *

■ 299034 Sigma σ₁

Source:	Human Jurkat cells	Ligand:	15.0 nM [³ H] Pentazocine
Vehicle:	1.0% DMSO	Non-Specific Ligand:	10.0 μM Haloperidol
Incubation Time/Temp:	2 hours @ 37°C	Specific Binding:	90% *
Incubation Buffer:	50 mM Tris-HCl, pH 8.0	Quantitation Method:	Radioligand Binding
Kd:	16.0 nM *	Significance Criteria:	≥50% of max stimulation or inhibition
		Bmax:	1.08 pmole/mg Protein *

■ 278200 Sigma σ₂

Source:	Wistar Rat brain (minus cerebellum)	Ligand:	3.0 nM [³ H] Ifenprodil
Vehicle:	1.0% DMSO	Non-Specific Ligand:	10.0 μM Ifenprodil
Incubation Time/Temp:	60 minutes @ 37°C	Specific Binding:	85% *
Incubation Buffer:	50 mM Tris-HCl, pH 7.4	Quantitation Method:	Radioligand Binding
Kd:	4.80 nM *	Significance Criteria:	≥50% of max stimulation or inhibition
		Bmax:	1.30 pmole/mg Protein *

■ 279510 Sodium Channel, Site 2

Source:	Wistar Rat brain (minus cerebellum)	Ligand:	5.0 nM [³ H] Batrachotoxinin
Vehicle:	1.0% DMSO	Non-Specific Ligand:	100 μM Veratridine
Incubation Time/Temp:	60 minutes @ 37°C	Specific Binding:	77% *
Incubation Buffer:	50 mM HEPES, 50 mM Tris-HCl, pH 7.4, 130 mM Choline Chloride, 5.4 mM KCl, 0.8 mM MgCl ₂ , 5.5 mM Glucose, 40 μg/ml LqTX	Quantitation Method:	Radioligand Binding
Kd:	0.052 μM *	Significance Criteria:	≥50% of max stimulation or inhibition
		Bmax:	0.7 pmole/mg Protein *

* Historical Values

Cleveland Clinic Foundation

Study #: TW04-0013389, Compound Code: CCF2201 (1271304)

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Methods

■ 282510 Somatostatin sst1

Source:	Human recombinant CHO-S cells	Ligand:	0.1 nM [¹²⁵ I] Tyr ¹¹ -Somatostatin 14
Vehicle:	1.0% DMSO	Non-Specific Ligand:	1.0 μM Somatostatin-14
Incubation Time/Temp:	2 hours @ 25°C	Specific Binding:	93% *
Incubation Buffer:	25 mM HEPES, pH 7.4, 5 mM MgCl ₂ , 1 mM CaCl ₂ , 0.1% BSA	Quantitation Method:	Radioligand Binding
Kd:	0.48 nM *	Significance Criteria:	≥50% of max stimulation or inhibition
		Bmax:	5.80 pmole/mg Protein *

■ 282700 Somatostatin sst2

Source:	Human recombinant CHO-K1 cells	Ligand:	0.03 nM [¹²⁵ I] Tyr ¹¹ -Somatostatin 14
Vehicle:	1.0% DMSO	Non-Specific Ligand:	1.0 μM Somatostatin-14
Incubation Time/Temp:	4 hours @ 25°C	Specific Binding:	90% *
Incubation Buffer:	25 mM HEPES, pH 7.4, 5 mM MgCl ₂ , 1 mM CaCl ₂ , 0.1% BSA	Quantitation Method:	Radioligand Binding
Kd:	0.034 nM *	Significance Criteria:	≥50% of max stimulation or inhibition
		Bmax:	11.0 pmole/mg Protein *

■ 282530 Somatostatin sst3

Source:	Human recombinant CHO-K1 cells	Ligand:	0.1 nM [¹²⁵ I] Tyr ¹¹ -Somatostatin 14
Vehicle:	1.0% DMSO	Non-Specific Ligand:	1.0 μM Somatostatin-14
Incubation Time/Temp:	2 hours @ 25°C	Specific Binding:	80% *
Incubation Buffer:	25 mM HEPES, pH 7.4, 5 mM MgCl ₂ , 1 mM CaCl ₂ , 0.1 % BSA	Quantitation Method:	Radioligand Binding
Kd:	0.052 nM *	Significance Criteria:	≥50% of max stimulation or inhibition
		Bmax:	5.50 pmole/mg Protein *

■ 282910 Somatostatin sst4

Source:	Human recombinant Chem-1 cells	Ligand:	0.1 nM [¹²⁵ I] Tyr ¹¹ -Somatostatin 14
Vehicle:	1.0% DMSO	Non-Specific Ligand:	1.0 μM Somatostatin-14
Incubation Time/Temp:	2 hours @ 25°C	Specific Binding:	85% *
Incubation Buffer:	25 mM HEPES, pH 7.4, 5 mM MgCl ₂ , 1 mM CaCl ₂ , 0.1% BSA	Quantitation Method:	Radioligand Binding
Kd:	0.9 nM *	Significance Criteria:	≥50% of max stimulation or inhibition
		Bmax:	27.5 pmole/mg Protein *

* Historical Values

Cleveland Clinic Foundation

Study #: TW04-0013389, Compound Code: CCF2201 (1271304)

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Methods

■ 283010 Somatostatin sst5

Source:	Human recombinant Chem-1 cells	Ligand:	0.1 nM [¹²⁵ I] Tyr ¹¹ -Somatostatin 14
Vehicle:	1.0% DMSO	Non-Specific Ligand:	1.0 μM Somatostatin-14
Incubation Time/Temp:	2 hours @ 25°C	Specific Binding:	90% *
Incubation Buffer:	25 mM HEPES, pH 7.4, 5 mM MgCl ₂ , 1 mM CaCl ₂ , 0.1% BSA	Quantitation Method:	Radioligand Binding
Kd:	0.13 nM *	Significance Criteria:	≥50% of max stimulation or inhibition
		Bmax:	5.20 pmole/mg Protein *

■ 255520 Tachykinin NK₁

Source:	Human recombinant CHO cells	Ligand:	0.8 nM [³ H] Substance P
Vehicle:	1.0% DMSO	Non-Specific Ligand:	10.0 μM L-703,606
Incubation Time/Temp:	90 minutes @ 4°C	Specific Binding:	90% *
Incubation Buffer:	20 mM HEPES, pH 7.4, 1 mM MnCl ₂ , 0.1% BSA	Quantitation Method:	Radioligand Binding
Kd:	2.10 nM *	Significance Criteria:	≥50% of max stimulation or inhibition
		Bmax:	1.70 pmole/mg Protein *

■ 255620 Tachykinin NK₂

Source:	Human recombinant CHO cells	Ligand:	20.0 pM [¹²⁵ I] Neurokinin A
Vehicle:	1.0% DMSO	Non-Specific Ligand:	3.0 μM MEN-10,376
Incubation Time/Temp:	2 hours @ 25°C	Specific Binding:	90% *
Incubation Buffer:	20 mM HEPES, pH 7.4 with NaOH, 1 mM MnCl ₂ , 0.1% BSA	Quantitation Method:	Radioligand Binding
Kd:	0.18 nM *	Significance Criteria:	≥50% of max stimulation or inhibition
		Bmax:	1.30 pmole/mg Protein *

■ 285900 Thyroid Hormone

Source:	Wistar Rat liver	Ligand:	0.03 nM [¹²⁵ I] Triiodothyronine
Vehicle:	1.0% DMSO	Non-Specific Ligand:	1.0 μM Triiodothyronine
Incubation Time/Temp:	18 hours @ 4°C	Specific Binding:	77% *
Incubation Buffer:	20 mM Tris-HCl, pH 7.6, 50 mM NaCl, 10% Glycerol, 2 mM EDTA, 5 mM DTT	Quantitation Method:	Radioligand Binding
Kd:	0.034 nM *	Significance Criteria:	≥50% of max stimulation or inhibition
		Bmax:	0.16 pmole/mg Protein *

* Historical Values

Cleveland Clinic Foundation

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Methods

■ 286010 Thyrotropin Releasing Hormone (TRH)

Source:	Human recombinant CHO-K1 cells	Ligand:	4.0 nM [³ H] Me-TRH
Vehicle:	1.0% DMSO	Non-Specific Ligand:	10.0 μM TRH
Incubation Time/Temp:	2 hours @ 4°C	Specific Binding:	92% *
Incubation Buffer:	50 mM Tris-HCl, pH 7.4, 0.3% BSA	Quantitation Method:	Radioligand Binding
Kd:	2.50 nM *	Significance Criteria:	≥50% of max stimulation or inhibition
		Bmax:	29.0 pmole/mg Protein *

■ 286200 Transforming Growth Factor-β (TGF-β)

Source:	Mouse 3T3-SWISS cells CCL-92	Ligand:	12.5 pM [¹²⁵ I] TGF-β ₁
Vehicle:	1.0% DMSO	Non-Specific Ligand:	10.0 nM TGF-β ₁
Incubation Time/Temp:	45 minutes @ 25°C	Specific Binding:	66% *
Incubation Buffer:	DMEM (Dulbecco's Modified Eagle Medium, Low glucose), pH 7.4, 10% FBS	Quantitation Method:	Radioligand Binding
Kd:	7.50 pM *	Significance Criteria:	≥50% of max stimulation or inhibition
		Bmax:	1300 R/cell Receptors/cell *

■ 202000 Transporter, Adenosine

Source:	Duncan Hartley derived Guinea pig cerebral cortex	Ligand:	0.5 nM [³ H] Nitrobenzylthioinosine
Vehicle:	1.0% DMSO	Non-Specific Ligand:	5.0 μM Nitrobenzylthioinosine
Incubation Time/Temp:	30 minutes @ 25°C	Specific Binding:	90% *
Incubation Buffer:	50 mM Tris-HCl, pH 7.4	Quantitation Method:	Radioligand Binding
Kd:	0.26 nM *	Significance Criteria:	≥50% of max stimulation or inhibition
		Bmax:	0.24 pmole/mg Protein *

■ 219010 Transporter, Choline

Source:	Human recombinant CHO-S cells	Ligand:	3.0 nM [³ H] Hemicholinium-3
Vehicle:	1.0% DMSO	Non-Specific Ligand:	1.0 μM Hemicholinium-3
Incubation Time/Temp:	60 minutes @ 25°C	Specific Binding:	90% *
Incubation Buffer:	50 mM Glycylglycine, 200 mM NaCl, pH 7.8	Quantitation Method:	Radioligand Binding
Kd:	4.20 nM *	Significance Criteria:	≥50% of max stimulation or inhibition
		Bmax:	1.10 pmole/mg Protein *

* Historical Values

Cleveland Clinic Foundation

Study #: TW04-0013389, Compound Code: CCF2201 (1271304)

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Methods

■ 220320 Transporter, Dopamine (DAT)

Source:	Human recombinant CHO-S cells	Ligand:	0.15 nM [¹²⁵ I] RTI-55
Vehicle:	1.0% DMSO	Non-Specific Ligand:	10.0 μM Nomifensine
Incubation Time/Temp:	3 hours @ 4°C	Specific Binding:	90% *
Incubation Buffer:	50 mM Tris-HCl, pH 7.4, 100 mM NaCl, 1 μM Leupeptin, 10 μM PMSF	Quantitation Method:	Radioligand Binding
Kd:	0.58 nM *	Significance Criteria:	≥50% of max stimulation or inhibition
		Bmax:	0.047 pmole/mg Protein *

■ 226400 Transporter, GABA

Source:	Wistar Rat brain (minus cerebellum)	Ligand:	6.0 nM [³ H] GABA
Vehicle:	1.0% DMSO	Non-Specific Ligand:	10.0 μM NO-711
Incubation Time/Temp:	20 minutes @ 25°C	Specific Binding:	80% *
Incubation Buffer:	10 mM HEPES, pH 7.5, 120 mM NaCl, 4 mM Ca(CH ₃ COO) ₂ , 10 μM Isoguvacine, 10 μM S(-)-Baclofen	Quantitation Method:	Radioligand Binding
Kd:	0.3 μM *	Significance Criteria:	≥50% of max stimulation or inhibition
		Bmax:	60.0 pmole/mg Protein *

■ 239100 Transporter, Glycine

Source:	Wistar Rat brain cortex	Ligand:	0.05 μM [³ H] Glycine
Vehicle:	1.0% DMSO	Non-Specific Ligand:	10.0 mM Glycine
Incubation Time/Temp:	15 minutes @ 37°C	Specific Binding:	90% *
Incubation Buffer:	20 mM HEPES, pH 7.4, 150 mM NaCl, 5 mM KCl, 1 mM CaCl ₂ , 1 mM MgCl ₂ , 10 mM Glucose	Quantitation Method:	Radioligand Binding
Kd:	320 μM *	Significance Criteria:	≥50% of max stimulation or inhibition
		Bmax:	680 pmole/mg Protein *

■ 204410 Transporter, Norepinephrine (NET)

Source:	Human recombinant MDCK cells	Ligand:	0.2 nM [¹²⁵ I] RTI-55
Vehicle:	1.0% DMSO	Non-Specific Ligand:	10.0 μM Desipramine
Incubation Time/Temp:	3 hours @ 4°C	Specific Binding:	75% *
Incubation Buffer:	50 mM Tris-HCl, pH 7.4, 100 mM NaCl, 1 μM Leupeptin, 10 μM PMSF	Quantitation Method:	Radioligand Binding
Kd:	0.024 μM *	Significance Criteria:	≥50% of max stimulation or inhibition
		Bmax:	2.50 pmole/mg Protein *

* Historical Values

Cleveland Clinic Foundation

Study #: TW04-0013389, Compound Code: CCF2201 (1271304)

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Methods

■ 274030 Transporter, Serotonin (5-Hydroxytryptamine) (SERT)

Source:	Human recombinant HEK-293 cells	Ligand:	0.4 nM [³ H] Paroxetine
Vehicle:	1.0% DMSO	Non-Specific Ligand:	10.0 μM Imipramine
Incubation Time/Temp:	60 minutes @ 25°C	Specific Binding:	95% *
Incubation Buffer:	50 mM Tris-HCl, pH 7.4, 120 mM NaCl, 5 mM KCl	Quantitation Method:	Radioligand Binding
Kd:	0.078 nM *	Significance Criteria:	≥50% of max stimulation or inhibition
		Bmax:	4.40 pmole/mg Protein *

■ 252030 Transporter, Vesicular Monoamine (Non-Selective)

Source:	Human platelets	Ligand:	2.0 nM [³ H] Dihydrotetrabenazine
Vehicle:	1.0% DMSO	Non-Specific Ligand:	10.0 μM Ro4-1284
Incubation Time/Temp:	60 minutes @ 25°C	Specific Binding:	80% *
Incubation Buffer:	50 mM HEPES, pH 8.0, 300 mM Sucrose	Quantitation Method:	Radioligand Binding
Kd:	2.50 nM *	Significance Criteria:	≥50% of max stimulation or inhibition
		Bmax:	0.52 pmole/mg Protein *

■ 286700 Urotensin II

Source:	Human recombinant CHO-S cells	Ligand:	0.1 nM [¹²⁵ I] Urotensin II
Vehicle:	1.0% DMSO	Non-Specific Ligand:	1.0 μM Urotensin II
Incubation Time/Temp:	2 hours @ 37°C	Specific Binding:	85% *
Incubation Buffer:	20 mM Tris-HCl, pH 7.4, 5 mM MgCl ₂ , 0.5% BSA	Quantitation Method:	Radioligand Binding
Kd:	0.2 nM *	Significance Criteria:	≥50% of max stimulation or inhibition
		Bmax:	0.42 pmole/mg Protein *

■ 287010 Vasoactive Intestinal Peptide VIP₁

Source:	Human HT29 colon adenocarcinoma cells	Ligand:	10.0 pM [¹²⁵ I] VIP
Vehicle:	1.0% DMSO	Non-Specific Ligand:	1.0 μM VIP
Incubation Time/Temp:	60 minutes @ 25°C	Specific Binding:	80% *
Incubation Buffer:	60 mM HEPES, pH 7.4, 0.1% BSA, 0.1% Bacitracin	Quantitation Method:	Radioligand Binding
Kd:	0.024 nM *	Significance Criteria:	≥50% of max stimulation or inhibition
		Bmax:	0.032 pmole/mg Protein *

* Historical Values

Cleveland Clinic Foundation

Study #: TW04-0013389, Compound Code: CCF2201 (1271304)

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Methods

■ 287100 Vasoactive Intestinal Peptide VIP2/PACAP VPAC2

Source:	Human recombinant Chem-1 cells	Ligand:	0.03 nM [¹²⁵ I] PACAP27
Vehicle:	1.0% DMSO	Non-Specific Ligand:	1.0 μM VIP
Incubation Time/Temp:	90 minutes @ 25°C	Specific Binding:	80% *
Incubation Buffer:	50 mM HEPES, pH 7.4, 1 mM CaCl ₂ , 5 mM MgCl ₂ , 0.2% BSA	Quantitation Method:	Radioligand Binding
Kd:	0.65 nM *	Significance Criteria:	≥50% of max stimulation or inhibition
		Bmax:	33.0 pmole/mg Protein *

■ 287530 Vasopressin V_{1A}

Source:	Human recombinant HEK-293 cells	Ligand:	0.03 nM [¹²⁵ I] PhenylacetylTyr(Me)PheGlnAsnArgProArgTyr
Vehicle:	1.0% DMSO	Non-Specific Ligand:	1.0 μM (Arg ⁸)-Vasopressin
Incubation Time/Temp:	2 hours @ 25°C	Specific Binding:	85% *
Incubation Buffer:	50 mM Tris-HCl, pH 7.4, 5 mM MgCl ₂ , 0.1% BSA	Quantitation Method:	Radioligand Binding
Kd:	7.40 pM *	Significance Criteria:	≥50% of max stimulation or inhibition
		Bmax:	2.80 pmole/mg Protein *

■ 287560 Vasopressin V_{1B}

Source:	Human recombinant CHO-K1 cells	Ligand:	1.0 nM [³ H] (Arg ⁸)-Vasopressin
Vehicle:	1.0% DMSO	Non-Specific Ligand:	1.0 μM (Arg ⁸)-Vasopressin
Incubation Time/Temp:	2 hours @ 25°C	Specific Binding:	92% *
Incubation Buffer:	50 mM Tris-HCl, pH 7.4, 5 mM MgCl ₂ , 0.1% BSA	Quantitation Method:	Radioligand Binding
Kd:	0.21 nM *	Significance Criteria:	≥50% of max stimulation or inhibition
		Bmax:	3.60 pmole/mg Protein *

■ 287610 Vasopressin V₂

Source:	Human recombinant CHO-K1 cells	Ligand:	4.0 nM [³ H] (Arg ⁸)-Vasopressin
Vehicle:	1.0% DMSO	Non-Specific Ligand:	1.0 μM (Arg ⁸)-Vasopressin
Incubation Time/Temp:	2 hours @ 25°C	Specific Binding:	74% *
Incubation Buffer:	50 mM Tris-HCl, pH 7.4, 10 mM MgCl ₂ , 0.1% BSA	Quantitation Method:	Radioligand Binding
Kd:	3.20 nM *	Significance Criteria:	≥50% of max stimulation or inhibition
		Bmax:	3.20 pmole/mg Protein *

* Historical Values

Literature References

Cat # Reference

- 200510 Libert F, Sande JV, Lefort A, Czernilofsky A, Dumont JE, Vassart G, Ensinger HA and Mendla KD (1992)**
Cloning and functional characterization of a human A₁ adenosine receptor. *Biochem Biophys Res Commun.* 187(2): 919-926.
- Olah ME, Ren H, Ostrowski J, Jacobson KA and Stiles GL (1992)**
Cloning, expression and characterization of the unique bovine A₁ adenosine receptor. *J Biol Chem.* 267: 10764-10770.
- 200610 Varani K, Gessi S, Dalpiaz A and Borea PA (1996)**
Pharmacological and biochemical characterization of purified A_{2A} adenosine receptors in human platelet membranes by [³H]CGS21680 binding. *Br J Pharmacol.* 117:1693-1701.
- 200650 Ji X, Kim YC, Ahern DG, Linden J and Jacobson KA (2001)**
[³H]MRS 1754, a selective antagonist radioligand for A_{2B} adenosine receptors. *Biochem Pharmacol.* 61(6): 657-663.
- 200720 Olah ME, Gallo-Rodriguez C, Jacobson KA and Stiles GL (1994)**
¹²⁵I-4-Aminobenzyl-5'-N-methylcarbox-amidoadenosine, a high affinity radioligand for the rat A₃ adenosine receptor. *Mol Pharmacol.* 45(5): 978-982.
- Salvatore CA, Jacobson MA, Taylor HE, Linden J and Johnson RG (1993)**
Molecular cloning and characterization of the human A₃ adenosine receptor. *Proc Natl Acad Sci USA.* 90(21): 10365-10369.
- 202000 Verma A and Marangos PJ (1985)**
Nitrobenzylthioinosine binding in brain; an interspecies study. *Life Sci.* 36(3): 283-290.
- 203110 Ford AP, Daniels DV, Chang DJ, Gever JR, Jasper JR, Lesnick JD and Clarke DE (1997)**
Pharmacological pleiotropism of the human recombinant α_{1A}-adrenoceptor: implications for α₁-adrenoceptor classification. *Br J Pharmacol.* 121(6): 1127-1135.
- Sato S, Hatanaka T, Yuyama H, Ukai M, Noguchi Y, Ohtake A, Taguchi K, Sasamata M and Miyata K (2012)**
Tamsulosin potently and selectively antagonizes human recombinant α_{1A/1D}-adrenoceptors: slow dissociation from the α_{1A}-adrenoceptor may account for selectivity for α_{1A}-adrenoceptor over α_{1B}-adrenoceptor subtype. *Biol Pharm Bull.* 35(1): 72-77.
- 203210 Ford AP, Daniels DV, Chang DJ, Gever JR, Jasper JR, Lesnick JD and Clarke DE (1997)**
Pharmacological pleiotropism of the human recombinant α_{1A}-adrenoceptor: implications for α₁-adrenoceptor classification. *Br J Pharmacol.* 121(6): 1127-1135.
- Sato S, Hatanaka T, Yuyama H, Ukai M, Noguchi Y, Ohtake A, Taguchi K, Sasamata M and Miyata K (2012)**
Tamsulosin potently and selectively antagonizes human recombinant α_{1A/1D}-adrenoceptors: slow dissociation from the α_{1A}-adrenoceptor may account for selectivity for α_{1A}-adrenoceptor over α_{1B}-adrenoceptor subtype. *Biol Pharm Bull.* 35(1): 72-77.
- 203400 Kenny BA, Chalmers DH, Philpott PC and Naylor AM (1995)**
Characterization of an α_{1D}-adrenoceptor mediating the contractile response of rat aorta to noradrenaline. *Br J Pharmacol.* 115: 981-986.
- 203630 Gleason MM and Hieble JP (1991)**
Ability of SK&F 104078 and SK&F 104856 to identify alpha-2 adrenoceptor subtypes in NCB20 cells and guinea pig lung. *J Pharmacol Exp Ther.* 259(3): 1124-1132.
- Lalchandani SG, Lei L, Zheng W, Suni MM, Moore BM, Liggett SB, Miller DD and Feller DR (2002)**
Yohimbine dimers exhibiting selectivity for the human alpha 2C-adrenoceptor subtype. *J Pharmacol Exp Ther.* 303(3): 979-984.

Literature References

Cat #	Reference
203710	Uhlen S, Dambrova M, Nasman J, Schioth HB, Gu Y, Wikberg-Matsson A and Wikberg JE (1998) [³ H]RS79948-197 binding to human, rat, guinea pig and pig α_{2A} -, α_{2B} - and α_{2C} - adrenoceptors comparison with MK912, RX821002, rauwolscine and yohimbine. Eur J Pharmacol. 343 (1): 93-101.
203810	Gleason MM and Hieble JP (1992) The α_2 -adrenoceptors of the human retinoblastoma cell line (Y79) may represent an additional example of the α_{2C} -adrenoceptor. Br J Pharmacol. 107(1): 222-225. Lalchandani SG, Lei L, Zheng W, Suni MM, Moore BM, Liggett SB, Miller DD and Feller DR (2002) Yohimbine dimers exhibiting selectivity for the human alpha 2C-adrenoceptor subtype. J Pharmacol Exp Ther. 303(3): 979-984.
204010	Feve B, Elhadri K, Quignard-Boulange A and Pairault J (1994) Transcriptional down-regulation by insulin of the β_3 -adrenergic receptor expression in 3T3-F442A adipocytes: A mechanism for repressing the cAMP signalling pathway. Proc Natl Acad Sci U S A. 91: 5677-5681.
204110	McCrea KE and Hill SJ (1993) Salmeterol, a long-acting β_2 -adrenoceptor agonist mediating cyclic AMP accumulation in a neuronal cell line. Br J Pharmacol. 110: 619-626.
204200	Feve B, Elhadri K, Quignard-Boulange A and Pairault J (1994) Transcriptional down regulation by insulin of the β_3 -adrenergic receptor expression in 3T3-F442A adipocytes: a mechanism for repressing the cAMP signaling pathway. Proc Natl Acad Sci U S A. 91: 5677-5681.
204410	Galli A, DeFelice LJ, Duke B-J, Moore KR and Blakely RD (1995) Sodium-dependent norepinephrine-induced currents in norepinephrine-transporter transfected HEK-293 cells blocked by cocaine and antidepressants. J Exp Biol. 198(Pt 10):2197-2212.
206000	Traish AM, Muller RE and Wotiz HH (1986) Binding of 7 alpha, 17 alpha-dimethyl-19-nortestosterone (mibolerone) to androgen and progesterone receptors in human and animal tissues. Endocrinology. 118(4): 1327-1333.
210030	Webb ML, Monshizadegan H, Dickinson KEJ, Serafino R, Moreland S, Michel I, Seiler SM and Murphy TJ (1993) Binding and signal transduction of the cloned vascular angiotensin II (AT 1a) receptor cDNA stably expressed in Chinese hamster ovary cells. Regul Pept. 44(2): 131 - 139.
210120	Lee S, Kramer CM, Mankad S, Yoo SE and Sandberg K (2001) Combined angiotensin converting enzyme inhibition and angiotensin AT ₁ receptor blockade up-regulates myocardial AT ₂ receptors in remodeled myocardium post-infarction. Cardiovasc Res. 51(1): 131-139. Scheidegger KJ, Butler S and Witztum JL (1997) Angiotensin II increases macrophage-mediated modification of low density lipoprotein via a lipoxygenase-dependent pathway. J Biol Chem. 272(34): 21609-21615.
210720	Katugampola SD, Maguire JJ, Matthewson SR and Davenport AP (2001) [¹²⁵ I](Pyr ¹)-Apelin-13 is a novel radioligand for localizing the APJ orphan receptor in human and rat tissues with evidence for a vasoconstrictor role in man. Br J Pharmacol. 132(6): 1255 - 1260. Kleinz MJ and Davenport AP. (2005) Emerging roles of apelin in biology and medicine. Pharmacol Ther. 107(2): 198-211, 2005.
211600	Ryan RR, Katsuno T, Mantey SA, Pradhan TK, Weber HC, Coy DH, Battey JF and Jensen RT (1999) Comparative pharmacology of the nonpeptide neuromedin B receptor antagonist PD 168368. J Pharmacol Exp Ther. 290(3): 1202 - 1211. Sainz E, Akeson M, Mantey SA, Jensen RT and Battey JF (1998) Four amino acid residues are critical for high affinity binding of neuromedin B to the neuromedin B receptor. J Biol Chem. 273(26): 15927 - 15932.

Literature References

Cat # Reference

- 211700 Benya RV, Kusui T, Pradhan TK, Battey JF and Jensen RT (1995)**
Expression and characterization of cloned human bombesin receptors. *Mol Pharmacol.* 47(1): 10 - 20.
- 211800 Mantey SA, Weber HC, Sainz E, Akesson M, Ryan RR, Pradhan TK, Searles RP, Spindel ER, Battey JF, Coy DH and Jensen RT (1997)**
Discovery of a high affinity radioligand for the human orphan receptor, bombesin receptor subtype 3, which demonstrates that it has a unique pharmacology compared with other mammalian bombesin receptors. *J Biol Chem.* 272(41): 26062 - 26071.
- 212520 Horlick RA, Ohlmeyer MH, Stroke IL, Strohl B, Pan G, Schilling AE, Paradkar V, Quintero JG, You M, Riviello C, Thorn MB, Damaj B, Fitzpatrick VD, Dolle RE, Webb ML, Baldwin JJ and Sigal NH (1999)**
Small molecule antagonists of the bradykinin B₁ receptor. *Immunopharmacology.* 43(2-3): 169-177.
- Phagoo SB, Reddi K, Anderson KD, Leeb-Lundberg LM and Warburton D (2001)**
Bradykinin B₁ receptor up-regulation by interleukin-1beta and B₁ agonist occurs through independent and synergistic intracellular signaling mechanisms in human lung fibroblasts. *J Pharmacol Exp Ther.* 298(1): 77-85.
- 212620 Luo SF, Tsai CT, Wu WB, Pan SL, Tsai YJ and Yang CM (1996)**
Pharmacological and functional characterization of bradykinin receptors in canine cultured tracheal epithelial cells. *Br J Pharmacol.* 119(2): 439-445.
- Paquet JL, Luccarini JM, Fouchet C, Defrene E, Loillier B, Robert C, Belichard P, Cremers B and Pruneau D (1999)**
Pharmacological characterization of the bradykinin B₂ receptor: inter-species variability and dissociation between binding and functional responses. *Br J Pharmacol.* 126(5): 1083-1090.
- 214020 Elshourbagy NA, Adamou JE, Swift AM, Disa J, Mao J, Ganguly S, Bergsma DJ and Aiyar N (1998)**
Molecular cloning and characterization of the porcine calcitonin gene-related peptide receptor. *Endocrinology.* 139(4): 1678-1683.
- Zimmermann U, Fischer JA and Muff R (1995)**
Adrenomedullin and calcitonin gene-related peptide interact with the same receptor in cultured human neuroblastoma SK-N-MC cells. *Peptides.* 16(3): 421-424.
- 214510 Schoemaker H and Langer SZ (1985)**
[³H]Diltiazem binding to calcium channel antagonists recognition sites in rat cerebral cortex. *Eur J Pharmacol.* 111(2):273-277.
- 214600 Ehlert FJ, Roeske WR, Itoga E and Yamamura HI (1982)**
The binding of [³H]nitrendipine to receptors for calcium channel antagonists in the heart, cerebral cortex, and ileum of rats. *Life Sci.* 30(25):2191-2202.
- Gould RJ, Murphy KMM and Snyder SH (1982)**
[³H]nitrendipine-labeled calcium channels discriminate inorganic calcium agonists and antagonists. *Proc Natl Acad Sci USA.* 79(11):3656-3660.
- 215000 Reynolds IJ, Snowman AM and Snyder SH (1986)**
(-)-[³H]Desmethoxyverapamil labels multiple calcium channel modulator receptors in brain and skeletal muscle membranes: Differentiation by temperature and dihydropyridines. *J Pharmacol Exp Ther.* 237:731-738.
- 216000 Moresco RM, Govoni S, Battaini F, Trivulzio S and Trabucchi M (1990)**
Omegaconotoxin binding decreases in aged rat brain. *Neurobiol Aging.* 11(4):433-436.
- 217050 Jung M, Calassi R, Rinaldi-Carmona M, Chardenot P, Le Fur G, Soubrie P and Oury-Donat F (1997)**
Characterization of CB1 receptors on rat neuronal cell cultures: binding and functional studies using the selective receptor antagonist SR 141716A. *J Neurochem.* 68(1):402-409.
- Melck D, De Petrocellis L, Orlando P, Bisogno T, Laezza C, Bifulco M and Di Marzo V (2000)**
Suppression of nerve growth factor Trk receptors and prolactin receptors by endocannabinoids leads to inhibition of human breast and prostate cancer cell proliferation. *Endocrinology.* 141(1):118-126.

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Literature References

Cat #	Reference
217100	Munro S, Thomas KL and Abu-Shaar M (1993) Molecular characterization of a peripheral receptor for cannabinoids. <i>Nature</i> 365:61-65.
217510	Hesselgesser J, Ng HP, Liang M, Zheng W, May K, Bauman JG, Monahan S, Islam I, Wei GP, Ghannam A, Taub DD, Rosser M, Snider RM, Morrissey MM, Perez HD and Horuk R (1998) Identification and characterization of small molecule functional antagonists of the CCR1 chemokine receptor. <i>J Biol Chem.</i> 273(25): 15687-15692. Pease JE, Wang J, Ponath PD and Murphy PM (1998) The N-terminal extracellular segments of the chemokine receptors CCR1 and CCR3 are determinants for MIP-1α and eotaxin binding, respectively, but a second domain is essential for efficient receptor activation. <i>J Biol Chem.</i> 273(32): 19972-19976.
217550	Gong X, Gong W, Kuhns DB, Ben-Baruch A, Zack Howard OM and Wang JM (1997) Monocyte chemotactic protein-2 (MCP-2) uses CCR1 and CCR2B as its functional receptors. <i>J Biol Chem.</i> 272(18): 11682 - 11685. Moore UM, Kaplow JM, Pleass RD, Castro SW, Naik K, Lynch CN, Daly S, Roach AG, Jaye M and Williams RJ (1997) Monocyte chemoattractant protein-2 is a potent agonist of CCR2B. <i>J Leukoc Biol.</i> 62(6):911 - 915.
217730	Blanpain C, Migeotte I, Lee B, Vakili J, Doranz BJ, Govaerts C, Vassart G, Doms RW and Parmentier M (1999) CCR5 binds multiple CC-chemokines: MCP-3 acts as a natural antagonist. <i>Blood.</i> 94(6): 1899-1905. Colin P, BQnureau Y, Staropoli I, Wang Y, Gonzalez N, Alcamí J, Hartley O, Brelot A, Arenzana-Seisdedos F and Lagane B (2013) HIV-1 exploits CCR5 conformational heterogeneity to escape inhibition by chemokines. <i>Proc Natl Acad Sci U S A.</i> 110(23): 9475-9480.
218020	Jensen RT, Qian JM, Lin JT, Mantey SA, Pisegna JR and Wank SA (1994) Distinguishing multiple CCK receptor subtypes. Studies with guinea pig chief cells and transfected human CCK receptors. <i>Ann N Y Acad Sci.</i> 713: 88-106.
218130	Bignon E, Bachy A, Boigegrain R, Brodin R, Cottineau M, Gully D, Herbert JM, Keane P, Labie C, Molimard JC, Olliero D, Oury-Donat F, Peterreau C, Prabonnaud V, Rockstroh MP, Schaeffer P, Servant O, Thurneyssen O, Soubrie P, Pascal M et al. (1999) SR146131: a new potent, orally active, and selective nonpeptide cholecystokinin subtype 1 receptor agonist. I. In vitro studies. <i>J Pharmacol Exp Ther.</i> 289(2): 742-751. Kawasaki D, Emori Y, Eta R, Iino Y, Hamano H, Yoshinaga K, Tanaka T, Takei M and Watson SA (2008) Effect of Z-360, a novel orally active CCK-2/gastrin receptor antagonist on tumor growth in human pancreatic adenocarcinoma cell lines in vivo and mode of action determinations in vitro. <i>Cancer Chemother Pharmacol.</i> 61(5): 883-892.
219010	Elizabeth A Ennis, Jane Wright, Cassandra L Retzlaff, Owen B McManus, Zhinong Lin, Xiaofang Huang, Meng Wu, Min Li, J Scott Daniels, Craig W Lindsley, Corey R Hopkins, Randy D Blakely (2015) Identification and characterization of ML352: a novel, noncompetitive inhibitor of the presynaptic choline transporter. <i>ACS Chem Neurosci.</i> 6(3):417-27. K Sandberg and J T Coyle (1985) Characterization of [^{3}H]hemicholinium-3 binding associated with neuronal choline uptake sites in rat brain membranes. <i>Brain Res.</i> 348(2):321-30.
219100	Tahir SK, Kovar P, Rosenberg SH and Ng SC (2000) Rapid colchicine competition-binding scintillation proximity assay using biotin-labeled tubulin. <i>BioTechniques.</i> 29(1): 156 - 160.

Literature References

Cat # Reference

- Weisenberg RC, Borisy GG and Taylor EW (1968)**
The colchicine-binding protein of mammalian brain and its relation to microtubules. *Biochemistry*. 7(12): 4466 - 4479.
- 219150 Lewis K, Li C, Perrin MH, Blount A, Kunitake K, Donaldson C, Vaughan J, Reyes TM, Gulyas J, Fischer W, Bilezikjian L, Rivier J, Sawchenko PE and Vale WW (2001)**
Identification of urocortin III, an additional member of the corticotropin-releasing factor (CRF) family with high affinity for the CRF₂ receptor. *Proc Natl Acad Sci USA*. 98(13): 7570 - 7575.
- Sutton SW, Behan DP, Lahrchi SL, Kaiser R, Corrigan A, Lowry P, Potter E, Perrin MH, Rivier J and Vale WW (1995)**
Ligand requirements of the human corticotropin-releasing factor-binding protein. *Endocrinology*. 136(3): 1097 - 1102.
- 219500 Dearth A, Gingrich JA, Falardeau P, Fremeau Jr RT, Bates MD and Caron MG (1990)**
Molecular cloning and expression of the gene for a human D₁ dopamine receptor. *Nature*. 347(6288):72-76.
- Zhou Q-Y, Grandy DK, Thambi L, Kushner JA, Van Tol HHM, Cone R, Pribnow D, Salon J, Bunzow JR and Civelli O (1990)**
Cloning and expression of human and rat D₁ dopamine receptors. *Nature*. 347(6288):76-80.
- 219700 Grandy DK, Marchionni MA, Makam H, Stofko RE, Alfano M, Frothingham L, Fischer JB, Burke-Howie KJ, Bunzow JR, Server AC and Civelli O (1989)**
Cloning of the cDNA and gene for a human D₂ dopamine receptor. *Proc Natl Acad Sci USA*. 86(24):9762-9766.
- Hayes G, Biden TJ, Selbie LA and Shine J (1992)**
Structural subtypes of the dopamine D₂ receptor are functionally distinct: Expression of the cloned D_{2A} and D_{2B} subtypes in a heterologous cell line. *Mol Endocrinol*. 6(6):920-926.
- 219800 Sokoloff P, Giros B, Martres M-P, Bouthenet M-L and Schwartz J-C (1990)**
Molecular cloning and characterization of a novel dopamine receptor (D₃) as a target for neuroleptics. *Nature*. 347(6289):146-151.
- 220000 Van Tol HHM, Bunzow JR, Guan H-C, Sunahara RK, Seeman P, Niznik HB and Civelli O (1991)**
Cloning of the gene for a human dopamine D₄ receptor with high affinity for the antipsychotic clozapine. *Nature*. 350(6319):610-614.
- Van Tol HHM, Wu CM, Guan H-C, Ohara K, Bunzow JR, Civelli O, Kennedy J, Seeman P, Niznik HB and Jovanovic V (1992)**
Multiple dopamine D₄ receptor variants in the human population. *Nature*. 358(6382):149-152.
- 220200 Sunahara RK, Guan H-C, O'Dowd BF, Seeman P, Laurier LG, Ng G, George SR, Torchia J, Van Tol HHM and Niznik HB (1991)**
Cloning of the gene for a human D₅ receptor with higher affinity for dopamine than D₁. *Nature*. 350(6319):614-619.
- Weinshank RL, Adham N, Macchi M, Olsen MA, Branchek TA and Hartig PR (1991)**
Molecular cloning and characterization of a high affinity dopamine receptor (D_{1β}) and its pseudogene. *J Biol Chem*. 266(33):22427-22435.
- 220320 Giros B and Caron MG (1993)**
Molecular characterization of the dopamine transporter. *Trends Pharmacol Sci*. 14(2): 43-49.
- Gu H, Wall SC and Rudnick G (1994)**
Stable expression of biogenic amine transporters reveals differences in inhibitor sensitivity, kinetics, and ion dependence. *J Biol Chem*. 269(10):7124-7130.
- 224010 Mihara S-I, Nakajima S, Matumura S, Kohnnoike T and Fujimoto M (1994)**
Pharmacological characterization of a potent nonpeptide endothelin receptor antagonist, 97-139. *J Pharmacol Exp Ther*. 268(3): 1122-1128.

Literature References

Cat # Reference

- 224110 Cain MJ, Garlick RK and Sweetman PM (1991)**
Endothelin-1 receptor binding assay for high throughput chemical screening. J Cardiovasc Pharmacol. 17 Suppl. 7: S150-151.
- Chiou WJ, Magnuson SR, Dixon D, Sundy S, Opgenorth TJ and Wu-Wong JR (1997)**
Dissociation characteristics of endothelin receptor agonists and antagonists in cloned human type-B endothelin receptor. Endothelium. 5(3): 179-189.
- 225510 Dittadi R, Gion M, Brazzale A and Bruscagnin G (1990)**
Radioligand binding assay of epidermal growth factor receptor: Causes of variability and standardization of the assay. Clin Chem. 36(6): 849-854.
- Massague J (1983)**
Epidermal growth factor-like transforming growth factor: II. Interaction with epidermal growth factor receptors in human placenta membranes and A431 cells. J Biol Chem. 258(22): 13614-13620.
- 226010 Obourn JD, Koszewski NJ and Notides AC (1993)**
Hormone- and DNA-binding mechanisms of the recombinant human estrogen receptor. Biochemistry 32(24): 6229 - 6236.
- 226050 Obourn JD, Koszewski NJ and Notides AC (1993)**
Hormone- and DNA-binding mechanisms of the recombinant human estrogen receptor. Biochemistry 32(24): 6229 - 6236.
- 226400 Shank RP, Baldy WJ, Mattucci LC and Villani FJ Jr (1990)**
Ion and temperature effects on the binding of gamma-aminobutyrate to its receptors and the high-affinity transport system. J Neurochem. 54:2007-2015.
- 226500 Enna SJ and Snyder SH (1977)**
Influences of ions, enzymes and detergents on gamma-aminobutyric acid-receptor binding in synaptic membranes of rat brain. Mol Pharmacol. 13:442-453.
- Martinin C, Rigacci T and Lucacchini A (1983)**
[³H]muscimol binding site on purified benzodiazepine receptor. J Neurochem. 41:1183-1185.
- 226600 Damm HW, Muller WE, Schlafer U and Wollert U (1978)**
[³H]flunitrazepam: Its advantages as a ligand for the identification of benzodiazepine receptors in rat brain membranes. Res Commun Chem Pathol Pharmacol. 22(3):597-600.
- Speth RC, Wastek GJ and Yamamura HI (1979)**
Benzodiazepine receptors: temperature dependence of [³H]flunitrazepam binding. Life Sci. 24(4):351-357.
- 226810 Lewin AH, de Costa BR, Rice KC and Skolnick P (1989)**
Meta- and para-isothiocyanato-t-butylbicycloorthobenzoate: Irreversible ligands of the γ-aminobutyric acid-regulated chloride ionophore. Mol Pharmacol. 35:189-194.
- Schwartz RD and Mindlin MC (1988)**
Inhibition of the GABA receptor-gated chloride ion channel in brain by noncompetitive inhibitors of the nicotinic receptor-gated cation channel. J Pharmacol Exp Ther. 244:963-970.
- 228610 Green A, Walls S, Wise A, Green RH, Martin AK and Marshall FH (2000)**
Characterization of [³H]-CGP54626A binding to heterodimeric GABA(B) receptors stably expressed in mammalian cells. Br J Pharmacol. 131(8): 1766-1774.
- Kaupmann K, Huggel K, Heid J, Flor PJ, Bischoff S, Mickel SJ, McMaster G, Angst C, Bittiger H, Froestl W and Bettler B (1997)**
Expression cloning of GABA_B receptors uncovers similarity to metabotropic glutamate receptors. Nature. 386 (6622): 239-246.

Literature References

Cat #	Reference
228710	Green A, Walls S, Wise A, Green RH, Martin AK and Marshall FH (2000) Characterization of [³H]-CGP54626A binding to heterodimeric GABA_B receptors stably expressed in mammalian cells. <i>Br J Pharmacol.</i> 131(8): 1766 - 1774. Kaupmann K, Huggel K, Heid J, Flor PJ, Bischoff S, Mickel SJ, McMaster G, Angst C, Bittiger H, Froestl W and Bettler B (1997) Expression cloning of GABA_B receptors uncovers similarity to metabotropic glutamate receptors. <i>Nature.</i> 386 (6622): 239 - 246.
230000	Gee NS, Brown JP, Dissanayake VU, Offord J, Thurlow R and Woodruff GN (1996) The novel anticonvulsant drug, gabapentin (Neurontin), binds to the alpha2delta subunit of a calcium channel. <i>J Biol Chem.</i> 271(10): 5768-5776. Suman-Chauhan N, Webdale L, Hill DR and Woodruff GN (1993) Characterisation of [³H]gabapentin binding to a novel site in rat brain: homogenate binding studies. <i>Eur J Pharmacol.</i> 244(3): 293-301.
231650	Carter LP, Wu H, Chen W, Matthews MM, Mehta AK, Hernandez RJ, Thomson JA, Ticku MK, Coop A, Koek W and France CP. (2005) Novel γ-hydroxybutyric acid (GHB) analogs share some, but not all, of the behavioral effects of GHB and GABA_B receptor agonists. <i>J Pharmacol Exp Ther.</i> 313(3): 1314-1323. Wellendorph P, Hog S, Greenwood JR, de Lichtenberg A, Nielsen B, Frolund B, Brehm L, Clausen RP and Brauner-Osborne H. (2005) Novel cyclic γ-hydroxybutyrate (GHB) analogs with high affinity and stereoselectivity of binding to GHB sites in rat brain. <i>J Pharmacol Exp Ther.</i> 315(1): 346-351.
231680	Mason JC and Tager HS. (1985) Identification of distinct receptor complexes that account for high- and low-affinity glucagon binding to hepatic plasma membranes. <i>Proc Natl Acad Sci U. S. A.</i> 82(20): 6835-6839.
232030	Honer C, Nam K, Fink C, Marshall P, Ksander G, Chatelain RE, Cornell W, Steele R, Schweitzer R and Schumacher C (2003) Glucocorticoid receptor antagonism by cyproterone acetate and RU486. <i>Mol Pharmacol.</i> 63(5): 1012-1020. Mulatero P, Panarelli M, Schiavone D, Rossi A, Mengozzi G, Kenyon CJ, Chiandussi L and Veglio F (1997) Impaired cortisol binding to glucocorticoid receptors in hypertensive patients. <i>Hypertension.</i> 30(5): 1274-1278.
232600	Honore T, Lauridsen J and Krogsgaard-Larsen P (1982) The binding of [³H]AMPA, a structural analogue of glutamic acid, to rat brain membranes. <i>J Neurochem.</i> 38(1):173-178. Olsen RW, Szamraj O and Houser CR (1987) [³H]AMPA binding to glutamate receptor subpopulations in rat brain. <i>Brain Res.</i> 402(2):243-254.
232710	Smith AL and McIlhinney RA (1992) Effects of acromelic acid A on the binding of [³H]-kainic acid and [³H]-AMPA to rat brain synaptic plasma membranes. <i>Br J Pharmacol.</i> 105(1): 83-86. Zhou LM, Gu ZQ, Costa AM, Yamada KA, Mansson PE, Giordano T, Skolnick P and Jones KA (1997) (2S,4R)-4-methylglutamic acid (SYM 2081): a selective, high-affinity ligand for kainate receptors. <i>J Pharmacol Exp Ther.</i> 280(1): 422-427.
232810	Sills MA, Fagg G, Pozza M, Angst C, Brundish DE, Hurt SD, Wilusz EJ and Williams M (1991) [³H]CGP 39653: a new N-methyl-D-aspartate antagonist radioligand with low nanomolar affinity in rat brain. <i>Eur J Pharmacol.</i> 192: 19-24.

Literature References

Cat #	Reference
232910	Siegel BW, Sreekrishna K and Baron BM (1996) Binding of the radiolabeled glycine site antagonist [³ H]MDL105,519 to homomeric NMDA-NR1a receptors. Eur J Pharmacol. 312(3):357-365.
233000	Goldman ME, Jacobson AE, Rice KC and Paul SM (1985) Differentiation of [³ H] phencyclidine and (+)-[³ H]SKF-10047 binding sites in rat cerebral cortex. FEBS Lett. 190:333-336.
234000	Schoemaker H, Allen J and Langer SZ (1990) Binding of [³ H]ifenprodil, a novel NMDA antagonist, to a polyamine-sensitive site in the rat cerebral cortex. Eur J Pharmacol. 176(2):249-250.
236400	Johnson BG, Wright RA, Arnold MB, Wheeler WJ, Ornstein PL and Schoepp DD (1999) [³ H]-LY341495 as a novel antagonist radioligand for group II metabotropic glutamate (mGlu) receptors: characterization of binding to membranes of mGlu receptor subtype expressing cells. Neuropharmacology 38(10): 1519-1529. Wright RA, Arnold MB, Wheeler WJ, Ornstein PL and Schoepp DD (2000) Binding of [³ H](2S,1'S,2'S)-2-(9-xanthylmethyl)-2-(2'-carboxycyclopropyl) glycine ([³ H]LY341495) to cell membranes expressing recombinant human group III metabotropic glutamate receptor subtypes. Naunyn Schmiedeberg's Arch Pharmacol. 362(6): 546-554.
237000	Mutel V, Ellis GJ, Adam G, Chaboz S, Nilly A, Messer J, Bleuel Z, Metzler V, Malherbe P, Schlaefer EJ, Roughley BS, Fauli RL and Richards JG (2000) Characterization of [³ H]Quisqualate binding to recombinant rat metabotropic glutamate 1a and 5a receptors and to rat and human brain sections. J Neurochem. 75(6): 2590-2601.
239000	Young AB and Snyder SH (1974) Strychnine binding in rat spinal cord membranes associated with the synaptic glycine receptor: cooperativity of glycine interactions. Mol Pharmacol. 10(5):790-809.
239100	Atkinson BN, Bell SC De Vivo M, Kowalski LR, Lechner SM, Ognyanov VI, Tham CS, Tsai C, Jia J, Ashton D and Klitenick MA (2001) ALX 5407: a potent, selective inhibitor of the hGlyT1 glycine transporter. Mol Pharmacol. 60(6): 1414 - 1420.
239200	Halmos G, Schally AV, Pinski J, Vadillo-Buenfil M and Groot K. (1996) Down-regulation of pituitary receptors for luteinizing hormone-releasing hormone (LH-RH) in rats by LH-RH antagonist Cetrorelix. Proc Natl Acad Sci U S A. 93(6): 2398-2402.
239300	Katugampola SD, Pallikaros Z and Davenport AP (2001) [¹²⁵ I-His(9)]-ghrelin, a novel radioligand for localizing GHS orphan receptors in human and rat tissue: up-regulation of receptors with atherosclerosis. Br J Pharmacol. 134(1): 143 - 149. Muccioli G, Papotti M, Locatelli V, Ghigo E and Deghenghi R (2001) Binding of [¹²⁵ I]-labeled ghrelin to membranes from human hypothalamus and pituitary gland. J Endocrinol Invest. 24(3): RC7 - 9.
239610	De Backer MD, Gommeren W, Moereels H, Nobels G, Van Gompel P, Leysen JE and Luyten WH (1993) Genomic cloning, heterologous expression and pharmacological characterization of a human histamine H ₁ receptor. Biochem Biophys Res Commun 197(3): 1601-1608.
239710	Ruat M, Traiffort E, Bouthenet ML, Schwartz JC, Hirschfeld J, Buschauer A and Schunack W (1990) Reversible and irreversible labeling and autoradiographic localization of the cerebral histamine H ₂ receptor using [¹²⁵ I]iodinated probes. Proc Natl Acad Sci USA. 87(5): 1658-1662.
239820	Krueger KM, Witte DG, Ireland-Denny L, Miller TR, Baranowski JL, Buckner S, Milicic I, Esbenshade TA and Hancock AA (2005) G protein-dependent pharmacology of histamine H ₃ receptor ligands: evidence for heterogeneous active state receptor conformations. J Pharmacol Exp Ther. 314(1): 271-281.

Literature References

Cat #	Reference
241000	Brown CM, MacKinnon AC, McGrath JC, Spedding M and Kilpatrick AT (1990) α_2 -Adrenoceptor subtypes and imidazoline-like binding in the rat brain. <i>Br J Pharmacol.</i> 99:803-809.
242500	Worley PF, Baraban JM, Supattapone S, Wilson VS and Snyder SH (1987) Characterization of inositol trisphosphate receptor binding in brain. Regulation by pH and calcium. <i>J Biol Chem.</i> 262(25):12132-12136.
243000	Koch R and Weber U (1981) Partial purification of the solubilized insulin receptor from rat liver membranes by precipitation with concanavalin A. <i>Hoppe-Seyler's Z Physiol Chem</i> 362(3):347-351.
243530	Akeson AL, Woods CW, Hsieh LC, Bohnke RA, Ackermann BL, Chan KY, Robinson JL, Yanofsky SD, Jacobs JW, Barrett RW and Bowlin TL (1996) AF12198, a novel low molecular weight antagonist, selectively binds the human type I interleukin (IL)-1 receptor and blocks in vivo responses to IL-1. <i>J Biol Chem.</i> 271(48):30517-30523. Slack J, McMahan CJ, Waugh S, Schooley K, Spriggs MK, Sims JE and Dower SK (1993) Independent binding of interleukin-1 alpha and interleukin-1 beta to type I and type II interleukin-1 receptors. <i>J Biol Chem.</i> 268(4):2513-2524.
244100	Cornfield LJ and Sills MA (1991) High affinity interleukin-6 binding sites in bovine hypothalamus. <i>Eur J Pharmacol.</i> 202(1): 113-115. Taga T, Kawanishi Y, Hardy RR, Hirano T and Kishimoto T (1987) Receptors for B cell stimulatory factor 2. Quantitation, specificity, distribution, and regulation of their expression. <i>J Exp Med.</i> 166(4): 967-981.
244420	Hipkin RW, Deno G, Fine J, Sun Y, Wilburn B, Fan X, Gonsiorek W and Wiekowski MT (2004) Cloning and pharmacological characterization of CXCR1 and CXCR2 from <i>Macaca fascicularis</i> . <i>J Pharmacol Exp Ther.</i> 310(1): 291-300.
244500	Ahuja SK and Murphy PM (1996) The CXC chemokines growth-regulated oncogene (GRO) α , GRO β , GRO γ , neutrophil-activating peptide-2, and epithelial cell-derived neutrophil-activating peptide-78 are potent agonists for the type B, but not type A, human interleukin-8 receptor.
244550	Valenzuela-Fernandez A, Planchenault T, Baleux F, Staropoli I, Le-Barillec K, Leduc D, Delaunay T, Lazarini F, Virelizier JL, Chignard M, Pidard D and Arenzana-Seisdedos F. (2002) Leukocyte elastase negatively regulates Stromal cell-derived factor-1 (SDF-1)/CXCR4 binding and functions by amino-terminal processing of SDF-1 and CXCR4. <i>J Biol Chem.</i> 277(18):15677-15689.
244600	Combadiere C, Salzwedel K, Smith ED, Tiffany HL, Berger EA and Murphy PM. (1998) Identification of CX3CR1. A chemotactic receptor for the human CX3C chemokine fractalkine and a fusion coreceptor for HIV-1. <i>J Biol Chem.</i> 273(37): 23799-23804. McDermott DH, Fong AM, Yang Q, Sechler JM, Cupples LA, Merrell MN, Wilson PW, D'Agostino RB, O'Donnell CJ, Patel DD and Murphy PM. (2003) Chemokine receptor mutant CX3CR1-M280 has impaired adhesive function and correlates with protection from cardiovascular disease in humans. <i>J Clin Invest.</i> 111(8): 1241-1250.
250460	Martin V, Sawyer N, Stocco R, Unett D, Lerner MR, Abramovitz M and Funk CD (2001) Molecular cloning and functional characterization of murine cysteinyl-leukotriene 1 (CysLT(1)) receptors. <i>Biochem Pharmacol.</i> 62(9): 1193-1200.
251010	Audinot V, Lahaye C, Suply T, Beauverger P, Rodriguez M, Galizzi JP, Fauchere JL and Boutin JA (2001) [¹²⁵ I]-S36057: a new and highly potent radioligand for the melanin-concentrating hormone receptor. <i>Br J Pharmacol</i> 133(3): 371-378.

Literature References

Cat # Reference

- Maulon-Feraille L, Della Zuana O, Suply T, Rovere-Jovene C, Audinot V, Levens N, Boutin JA, Duhault J and Nahon JL (2002)**
Appetite-boosting property of pro-melanin-concentrating hormone(131-165) (neuropeptide-glutamic acid-isoleucine) is associated with proteolytic resistance. *J Pharmacol Exp Ther.* 302(2): 766-773.
- 251100 Yang Y, Dickinson C, Haskell-Luevano C and Gantz I (1997)**
Molecular basis for the interaction of [Nle⁴,D-Phe⁷]melanocyte stimulating hormone with the human melanocortin-1 receptor. *J Biol Chem.* 272(37): 23000 - 23010.
- 251300 Schioth HB, Muceniece R, Wikberg JES and Chhajlani V (1995)**
Characterisation of melanocortin receptor subtypes by radioligand binding analysis. *Eur J Pharmacol.* 288: 311 - 317.
- 251350 Schioth HB, Muceniece R and Wikberg JES (1996)**
Characterisation of the melanocortin 4 receptor by radioligand binding. *Pharmacol Toxicol.* 79: 161 - 165.
- Schioth HB, Muceniece R, Mutulis F, Prusis P, Lindeberg G, Sharma SD, Hruby VJ and Wikberg JES (1997)**
Selectivity of cyclic [D-Nal⁷] and [D-Phe⁷]substituted MSH analogues for the melanocortin receptor subtypes. *Peptides.* 18(7): 1009-1013.
- 251400 Schioth H B, Muceniece R, Wikberg JES and Chhajlani V (1995)**
Characterisation of melanocortin receptor subtypes by radioligand binding analysis. *Eur J Pharmacol.* 288: 311 - 317.
- 251600 Beresford IJ, Browning C, Starkey SJ, Brown J, Foord SM, Coughlan J, North PC, Dubocovich ML and Hagan RM (1998)**
GR196429: a nonindolic agonist at high-affinity melatonin receptors. *J Pharmacol Exp Ther.* 285(3): 1239-1245.
- Paul P, Lahaye C, Delagrange P, Nicolas JP, Canet E and Boutin JA (1999)**
Characterization of 2-[¹²⁵I]iodomelatonin binding sites in Syrian hamster peripheral organs. *J Pharmacol Exp Ther.* 290(1):334-340.
- 251700 Dubocovich ML, Yun K, Al-Ghoul WM, Benloucif S and Masana MI (1998)**
Selective MT₂ melatonin receptor antagonists block melatonin-mediated phase advances of circadian rhythms. *FASEB J.* 12(12): 1211-1220.
- Gerdin MJ, Masana MI, Ren D, Miller RJ and Dubocovich ML (2003)**
Short-term exposure to melatonin differentially affects the functional sensitivity and trafficking of the hMT₁ and hMT₂ melatonin receptors. *J Pharmacol Exp Ther.* 304(3): 931-939.
- 252030 Cesura AM, Bertocci B and Da Prada M (1990)**
Binding of [³H]dihydrotetraabenazine and [¹²⁵I]azidoiodoketanserin photoaffinity labeling of the monoamine transporter of platelet 5-HT organelles. *Eur J Pharmacol.* 186(1): 95-104.
- Zucker M, Weizman A and Rehavi M (2001)**
Characterization of high-affinity [³H]TBZOH binding to the human platelet vesicular monoamine transporter. *Life Sci.* 69(19): 2311-2317.
- 252200 Feighner SD, Tan CP, McKee KK, Palyha OC, Hreniuk DL, Pong SS, Austin CP, Figueroa D, MacNeil D, Cascieri MA, Nargund R, Bakshi R, Abramovitz M, Stocco R, Kargman S, O'Neill G, Van Der Ploeg LH, Evans J, Patchett AA, Smith RG and Howard AD (1999)**
Receptor for motilin identified in the human gastrointestinal system. *Science* 284(5423): 2184 - 2188.
- 252610 Buckley NJ, Bonner TI, Buckley CM and Brann MR (1989)**
Antagonist binding properties of five cloned muscarinic receptors expressed in CHO-K1 cells. *Mol Pharmacol.* 35(4): 469-476.
- Luthin GR and Wolfe BB (1984)**
Comparison of [³H]pirenzepine and [³H]quinuclidinylbenzilate binding to muscarine cholinergic receptors in rat brain. *J Pharmacol Exp Ther.* 228(3):648-655.

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Literature References

Cat # Reference

- 252710 Buckley NJ, Bonner TI, Buckley CM and Brann MR (1989)**
Antagonist binding properties of five cloned muscarinic receptors expressed in CHO-K1 cell. *Mol Pharmacol.* 35(4):469-476.
- Luthin GR and Wolfe BB (1984)**
Comparison of [³H]pirenzepine and [³H]quinuclidinylbenzilate binding to muscarine cholinergic receptors in rat brain. *J Pharmacol Exp Ther.* 228(3): 648-655.
- 252810 Buckley NJ, Bonner TI, Buckley CM and Brann MR (1989)**
Antagonist binding properties of five cloned muscarinic receptors expressed in CHO-K1 cell. *Mol Pharmacol.* 35(4):469-476.
- Luthin GR and Wolfe BB (1984)**
Comparison of [³H]pirenzepine and [³H]quinuclidinylbenzilate binding to muscarinic cholinergic receptors in rat brain. *J Pharmacol Exp Ther.* 228(3): 648-655.
- 252910 Buckley NJ, Bonner TI, Buckley CM and Brann MR (1989)**
Antagonist binding properties of five cloned muscarinic receptors expressed in CHO-K1 cells. *Mol Pharmacol.* 35 (4): 469-476.
- Luthin GR and Wolfe BB (1984)**
Comparison of [³H]pirenzepine and [³H]quinuclidinylbenzilate binding to muscarine cholinergic receptors in rat brain. *J Pharmacol Exp Ther.* 228(3): 648-655.
- 253010 Buckley NJ, Bonner TI, Buckley CM and Brann MR (1989)**
Antagonist binding properties of five cloned muscarinic receptors expressed in CHO-K1 cell. *Mol Pharmacol.* 35(4):469-476.
- Luthin GR and Wolfe BB (1984)**
Comparison of [³H]pirenzepine and [³H]quinuclidinylbenzilate binding to muscarine cholinergic receptors in rat brain. *J Pharmacol Exp Ther.* 228(3): 648-655.
- 254500 Birdsall NJM, Burgen ASV and Hulme EC (1978)**
The binding of agonists to brain muscarinic receptors. *Mol Pharmacol.* 14:723 - 736.
- 255520 Patacchini R and Maggi CA (1995)**
Tachykinin receptors and receptor subtypes. *Arch Int Pharmacodyn.* 329:161-184.
- 255620 Rupniak NMJ, Perdona E, Griffante C, Cavallini P, Sava A, Ricca DJ, Thor KB, Burgard EC and Corsi M (2018)**
Affinity, potency, efficacy, and selectivity of neurokinin A analogs at human recombinant NK2 and NK1 receptors. *PLoS One.* 13(10):e0205894.
- Warner FJ, Comis A, Miller RC and Burcher E (1999)**
Characterization of the [¹²⁵I]-neurokinin A binding site in the circular muscle of human colon. *Br J Pharmacol.* 127(5):1105-1110.
- 257010 Fuhlendorff J, Gether U, Aakerlund L, Langeland-Hohansen N, Thogersen H, Melberg SG, Olsen UB, Thastrup O and Schwartz TW (1990)**
[Leu³¹,Pro³⁴]neuropeptide Y: a specific Y₁ receptor agonist. *Proc Natl Acad Sci USA* 87:182-186.
- Sheikh SP, O'Hare MMT, Tortora O and Schwartz TW (1989)**
Binding of monoiodinated neuropeptide Y to hippocampal membranes and human neuroblastoma cell lines. *J Biol Chem.* 264(12):6648-6654.
- 257110 Rose PM, Fernandes P, Lynch JS, Frazier ST, Fisher SM, Kodukula K, Kienzle B and Seethala R (1995)**
Cloning and functional expression of a cDNA encoding a human type 2 neuropeptide Y receptor. *J Biol Chem.* 270(39):22661-22664.

Literature References

Cat # Reference

- 258010 Martin S, Botto JM, Vincent JP and Mazella J (1999)**
Pivotal Role of an Aspartate Residue in Sodium Sensitivity and Coupling to G Proteins of Neurotensin Receptors. *Mol Pharmacol.* 55(2) : 210-215.
- Vita N, Laurent P, Lefort S, Chalon P, Dumont X, Kaghad M, Gully D, Le Fur G, Ferrara P and Caput D (1993)**
Cloning and expression of a complementary DNA encoding a high affinity human neurotensin receptor. *FEBS Lett.* 317(1-2) : 139-142.
- 258630 Lukas RJ (1984)**
Properties of curare-mimetic neurotoxin binding sites in the rat central nervous system. *Biochemistry.* 23(6): 1152-1160.
- Sharples CG, Kaiser S, Soliakov L, Marks MJ, Collins AC, Washburn M, Wright E, Spencer JA, Gallagher T, Whiteaker P and Wonnacott S (2000)**
UB-165: a novel nicotinic agonist with subtype selectivity implicates the $\alpha 4\beta 2^*$ subtype in the modulation of dopamine release from rat striatal synaptosomes. *J Neurosci.* 20(8): 2783-2791.
- 258660 Davies AR, Hardick DJ, Blagbrough IS, Potter BV, Wolstenholme AJ and Wonnacott S. (1999)**
Characterisation of the binding of [^3H]methyllycaconitine: a new radioligand for labelling $\alpha 7$ -type neuronal nicotinic acetylcholine receptors. *Neuropharmacology.* 38(5): 679-690.
- Nuutinen S, Ekokoski E, Lahdensuo E and Tuominen RK (2006)**
Nicotine-induced upregulation of human neuronal nicotinic $\alpha 7$ -receptors is potentiated by modulation of cAMP and PKC in SH-EP1- $\alpha 7$ cells. *Eur J Pharmacol.* 544(1-3): 21-30.
- 258700 Lukas RJ (1986)**
Characterization of curare-mimetic neurotoxin binding sites on membrane fractions derived from the human medulloblastoma clonal line, TE671. *J Neurochem.* 46(6): 1936-1941.
- Sine SM (1988)**
Functional properties of human skeletal muscle acetylcholine receptors expressed by the TE671 cell line. *J Biol Chem.* 263(34): 18052-18062.
- 258730 Michelmore S, Croskery K, Nozulak J, Hoyer D, Longato R, Weber A, Bouhelal R and Feuerbach D (2002)**
Study of the calcium dynamics of the human $\alpha 4\beta 2$, $\alpha 3\beta 4$ and $\alpha 1\beta 1\gamma$ nicotinic acetylcholine receptors. *Naunyn-Schmiedeberg's Arch Pharmacol.* 366(3): 235-245.
- Zaveri N, Jiang F, Olsen C, Polgar W and Toll L (2010)**
Novel $\alpha 3\beta 4$ nicotinic acetylcholine receptor-selective ligands. Discovery, structure-activity studies, and pharmacological evaluation. *J Med Chem.* 53(22): 8187-8191.
- 258750 Pabreza LA, Dhawan S and Kellar KJ (1991)**
 [^3H]cytisine binding to nicotinic cholinergic receptors in brain. *Mol Pharmacol.* 39(1): 9-12.
- 260130 Clark MJ, Emmerson PJ, Mansour A, Akil H, Woods JH, Portoghese PS, Remmers AE and Medzihradsky F (1997)**
Opioid efficacy in a C6 glioma cell line stably expressing the delta opioid receptor. *J Pharmacol Exp Ther.* 283(2): 501-510.
- Martin NA and Prather PL (2001)**
Interaction of co-expressed μ - and δ -opioid receptors in transfected rat pituitary GH₃ cells. *Mol Pharmacol.* 59(4): 774-783.
- 260210 Maguire P, Tsai N, Kamal J, Cometta-Morini C, Upton C and Loew G (1992)**
Pharmacological profiles of fentanyl analogs at μ , δ , and κ opiate receptors. *Eur J Pharmacol.* 213(2): 219-225.

Literature References

Cat # Reference

- Simonin F, Gaveriaux-Ruff C, Befort K, Matthes H, Lannes B, Micheletti G, Mattei M-G, Charron G, Bloch B and Kieffer B (1995)**
Kappa-opioid receptor in humans: cDNA and genomic cloning, chromosomal assignment, functional expression, pharmacology, and expression pattern in the central nervous system. *Proc Natl Acad Sci USA*. 92(15): 7006-7010.
- 260410 Wang JB, Johnson PS, Persico AM, Hawkins AL, Griffin CA and Uhl GR (1994)**
Human mu opiate receptor: cDNA and genomic clones, pharmacologic characterization and chromosomal assignment. *FEBS Lett*. 338:217-222.
- 260600 Ardati A, Henningsen RA, Higelin J, Reinscheid RK, Civelli O and Monsma Jr FJ (1997)**
Interaction of [³H]orphanin FQ and [¹²⁵I]Tyr¹⁴-orphanin FQ with the orphanin FQ receptor: kinetics and modulation by cations and guanine nucleotides. *Mol Pharmacol*. 51:816-824.
- 260900 Pettibone DJ, Woyden CJ and Totaro JA. (1990)**
Identification of functional oxytocin receptors in lactating rat mammary gland in vitro. *Eur J Pharmacol*. 188(4-5): 235-241.
- Tahara A, Tsukada J, Tomura Y, Wada K, Kusayama T, Ishii N, Yatsu T, Uchida W and Tanaka A. (200)**
Pharmacologic characterization of the oxytocin receptor in human uterine smooth muscle cells. *Br J Pharmacol*. 129(1): 131-139, 2000.
- 264500 Ashendel CL (1985)**
The phorbol ester receptor: a phospholipid-regulated protein kinase. *Biochim Biophys Acta*. 822:219-242.
- 265200 Williams LT, Tremble PM, Lavin MF and Sunday ME (1984)**
Platelet-derived growth factor receptors form a high affinity state in membrane preparations. Kinetics and affinity cross-linking studies. *J Biol Chem*. 259(8): 5287-5294.
- 265510 Rehm H and Lazdunski M (1988)**
Purification and subunit structure of a putative K⁺ channel protein identified by its binding properties for dendrotoxin I. *Proc Natl Acad Sci USA*. 85:4919-4923.
- Sorensen RG and Blaustein MP (1989)**
Rat brain dendrotoxin receptors associated with voltage-gated potassium channels: dendrotoxin binding and receptor solubilization. *Mol Pharmacol*. 36(5): 689-698,
- 265600 Gaines KL, Hamilton S and Boyd III AE (1988)**
Characterization of the sulfonylurea receptor on beta cell membranes. *J Biol Chem*. 263(6):2589-2592.
- 265800 Mourre C, Hugues M and Lazdunski M (1986)**
Quantitative autoradiographic mapping in rat brain of the receptor of apamin, a polypeptide toxin specific for one class of Ca²⁺-dependent K⁺ channels. *Brain Res*. 382:239-249.
- 265910 Finlayson K, Turnbull L, January CT, Sharkey J, Kelly JS (2001)**
[³H]dofetilide binding to HERG transfected membranes: a potential high throughput preclinical screen. *European Journal of Pharmacology*. 430(1): 147-148.
- Huang XP, Mangano T, Hufeisen S, Setola V and Roth BL (2010)**
Identification of human Ether-a-go-go related gene modulators by three screening platforms in an academic drug-discovery setting. *Assay Drug Dev Technol*. 8(6): 727-742.
- 268030 Sugimoto H, Shichijo M, Iino T, Manabe Y, Watanabe A, Shimazaki M, Gantner F and Bacon KB. (2003)**
An orally bioavailable small molecule antagonist of CRTH2, ramatroban (BAY u3405), inhibits prostaglandin D2-induced eosinophil migration in vitro. *J Pharmacol Exp Ther*. 305(1): 347-352.
- 268060 Sharif NA, Williams GW and Davis TL (2000)**
Pharmacology and autoradiography of human DP prostanoid receptors using [³H]-BWA868C, a DP receptor-selective antagonist radioligand. *Br J Pharmacol*. 131(6): 1025-1038.

Literature References

Cat # Reference

- Wright DH, Metters KM, Abramovitz M and Ford-Hutchinson AW (1998)**
Characterization of the recombinant human prostanoid DP receptor and identification of L-644,698, a novel selective DP agonist. *Br Pharmacol.* 123(7): 1317-1324.
- 268110 Abramovitz M, Adam M, Boie Y, Carriere M, Denis D, Godbout C, Lamontagne S, Rochette C, Sawyer N, Tremblay NM, Belley M, Gallant M, Dufresne C, Gareau Y, Ruel R, Juteau H, Labelle M, Ouimet N and Metters KM (2000)**
The utilization of recombinant prostanoid receptors to determine the affinities and selectivities of prostaglandins and related analogs. *Biochim Biophys Acta.* 1483(2): 285-293.
- Funk CD, Furci L, FitzGerald GA, Grygorczyk R, Rochette C, Bayne MA, Abramovitz M, Adam M and Metters KM (1993)**
Cloning and expression of a cDNA for the human prostaglandin E receptor EP₁ subtype. *J Biol Chem.* 268(35): 26767-26772.
- 268200 Bastien L, Sawyer N, Grygorczyk R, Metters KM and Adam M (1994)**
Cloning, functional expression, and characterization of the human prostaglandin E₂ receptor EP₂ subtype. *J Biol Chem.* 269(16): 11873-11877.
- Boie Y, Stocco R, Sawyer N, Slipetz DM, Ungrin MD, Neuschafer-Rube F, Puschel GP, Metters KM and Abramovitz M (1997)**
Molecular cloning and characterization of the four rat prostaglandin E₂ prostanoid receptor subtypes. *Eur J Pharmacol.* 340(2-3): 227-241.
- 268420 Davis TL and Sharif NA (2000)**
Pharmacological characterization of [³H]-prostaglandin E₂ binding to the cloned human EP₄ prostanoid receptor. *Br J Pharmacol.* 130(8): 1919-1926.
- 268510 Abramovitz M, Boie Y, Nguyen T, Rushmore TH, Bayne MA, Metters KM, Slipetz DM and Grygorczyk R (1994)**
Cloning and expression of a cDNA for the human prostanoid FP receptor. *J Biol Chem.* 269(4): 2632-2636.
- 268820 Schachter JB and Harden TK (1997)**
An examination of deoxyadenosine 5'(α -thio)triphosphate as a ligand to define P₂Y receptors and its selectivity as a low potency partial agonist of the P₂Y₁ receptor. *Br J Pharmacol.* 121(2):338-344.
- Schafer R and Reiser G (1997)**
Characterization of [³⁵S]-ATP α S and [³H]- α , β -MeATP binding sites in rat brain cortical synaptosomes: regulation of ligand binding by divalent cations. *Br J Pharmacol.* 121(5):913-922.
- 270000 Duplantier AJ, Biggers MS, Chamber RJ, Cheng JB, Cooper K, Damon DB, Eggler JF, Kraus KG, Marfat A, Masamune H, Pillar JS, Shirley JT, Umland JP and Watson JW (1996)**
Biarylcarboxylic acids and -amides: inhibition of phosphodiesterase type IV versus [³H]rolipram binding activity and their relationship to emetic behavior in the ferret. *J Med Chem.* 39:120-125.
- 270300 El-Hayek R, Lokuta AJ, Arevalo C and Valdivia HH. (1995)**
Peptide probe of ryanodine receptor function. Imperatoxin A, a peptide from the venom of the scorpion *Pandinus imperator*, selectively activates skeletal-type ryanodine receptor isoforms. *J Biol Chem.* 270(48):28696-28704.
- Padua RA, Wan WH, Nagy JI and Geiger JD. (1991)**
[³H]Ryanodine binding sites in rat brain demonstrated by membrane binding and autoradiography. *Brain Res.* 542(1): 135-140.
- 271110 Martin GR and Humphrey PPA (1994)**
Receptor for 5-hydroxytryptamine: current perspectives on classification and nomenclature. *Neuropharmacol.* 33(3-4):261-273.
- May JA, McLaughlin MA, Sharif NA, Hellberg MR and Dean TR (2003)**
Evaluation of the ocular hypotensive response of serotonin 5-HT_{1A} and 5-HT₂ receptor ligands in conscious ocular hypertensive cynomolgus monkeys. *J Pharmacol Exp Ther.* 306(1): 301-309.

Literature References

Cat #	Reference
271200	Hoyer D, Engel G and Kalkman HO (1985) Characterization of the 5-HT_{1B} recognition site in rat brain: binding studies with (-)[¹²⁵I]iodocyanopindolol. Eur J Pharmacol. 118(1-2):1-12. Pazos A, Hoyer D and Palacios JM (1985) Mesulergine, a selective serotonin-2 ligand in the rat cortex, does not label these receptors in porcine and human cortex: evidence for species differences in brain serotonin-2 receptors. Eur J Pharmacol. 106(3):531-538.
271700	Bonhaus DW, Bach C, DeSouza A, Salazar FHR, Matsuoka BD, Zuppan P, Chan HW and Eglen RM (1995) The pharmacology and distribution of human 5-hydroxytryptamine_{2B} (5-HT_{2B}) receptor gene products: comparison with 5-HT_{2A} and 5-HT_{2C} receptors. Br J Pharmacol. 115: 622 - 628.
271800	Wolf WA and Schutz LJ (1997) The serotonin 5-HT_{2C} receptor is a prominent serotonin receptor in basal ganglia: evidence from functional studies on serotonin-mediated phosphoinositide hydrolysis. J Neurochem. 69(4):1449-1458.
271910	Boess FG, Steward LJ, Steele JA, Liu D, Reid J, Glencorse TA and Martin IL (1997) Analysis of the ligand binding site of the 5-HT₃ receptor using site directed mutagenesis: importance of glutamate 106. Neuropharmacology. 36(4-5): 637-647. Miller K, Weisberg E, Fletcher PW and Teitler M (1992) Membrane-bound and solubilized brain 5-HT₃ receptors: improved radioligand binding assays using bovine area postrema or rat cortex and the radioligands ³H-GR65630, ³H-BRL43694, and ³H-LY278584. Synapse. 11(1):58-66.
272000	Grossman CJ, Kilpatrick GJ and Bunce KT (1993) Development of a radioligand binding assay for 5-HT₄ receptors in guinea-pig and rat brain. Br J Pharmacol. 109:618-624.
272100	Rees S, den Daas I, Foord S, Goodson S, Bull D, Kilpatrick G and Lee M (1994) Cloning and characterization of the human 5-HT_{5A} serotonin receptor. FEBS Lett. 355:242-246.
272200	Monsma FJ Jr, Shen Y, Ward RP, Hamblin MW and Sibley DR (1993) Cloning and expression of a novel serotonin receptor with high affinity for tricyclic psychotropic drugs. Mol Pharmacol. 43:320-327.
272320	Roth BL, Craigo SC, Choudhary MS, Uluer S, Monsma Jr FJ, Shen Y, Meltzer HY and Sibley DR (1994) Binding of typical and atypical antipsychotic agents to 5-hydroxytryptamine-6 and 5-hydroxytryptamine-7 receptors. J Pharmacol Exp Ther. 268:1403-1410. Shen Y, Monsma Jr FJ, Metcalf MA, Jose PA, Hamblin MW and Sibley DR (1993) Molecular cloning and expression of a 5-hydroxytryptamine 7 serotonin receptor subtype. J Biol Chem. 268: 18200 - 18204.
274030	Shearman LP, McReynolds AM, Zhou FC and Meyer JS (1998) Relationship between [¹²⁵I]RTI-55-labeled cocaine binding sites and the serotonin transporter in rat placenta. Am J Physiol. 275(6 Pt 1): C1621-1629. Wolf WA and Kuhn DM (1992) Role of essential sulfhydryl groups in drug interactions at the neuronal 5-HT transporter. Differences between amphetamines and 5-HT uptake inhibitors. J Biol Chem. 267(29): 20820-20825.
278200	Hashimoto K and London ED (1993) Further characterization of [³H]ifenprodil binding to σ receptors in rat brain. Eur J Pharmacol. 236:159-163.
279510	Catterall WA, Morrow CS, Daly JW and Brown CB (1981) Binding of batrachotoxinin A 20-α-benzoate to a receptor site associated with sodium channels in synaptic nerve ending particles. J Biol Chem. 256(17):8922-8927.

Literature References

Cat #	Reference
282510	Liapakis G, Fitzpatrick D, Hoeger C, Rivier J, Vandlen R and Reisine T (1996) Identification of ligand binding determinants in the somatostatin receptor subtypes 1 and 2. <i>J Biol Chem.</i> 271(34): 20331 - 20339. Patel YC and Srikant CB (1994) Subtype selectivity of peptide analogs for all five cloned human somatostatin receptors (hsstr 1-5). <i>Endocrinology.</i> 135 (6):2814 - 2817.
282530	Liapakis G, Fitzpatrick D, Hoeger C, Rivier J, Vandlen R and Reisine T (1996) Identification of ligand binding determinants in the somatostatin receptor subtypes 1 and 2. <i>J Biol Chem.</i> 271(34): 20331 - 20339. Patel YC and Srikant CB (1994) Subtype selectivity of peptide analogs for all five cloned human somatostatin receptors (hsstr 1-5). <i>Endocrinology.</i> 135 (6):2814 - 2817.
282700	Liapakis G, Fitzpatrick D, Hoeger C, Rivier J, Vandlen R and Reisine T (1996) Identification of ligand binding determinants in the somatostatin receptor subtypes 1 and 2. <i>J Biol Chem.</i> 271(34): 20331 - 20339. Patel YC and Srikant CB (1994) Subtype selectivity of peptide analogs for all five cloned human somatostatin receptors (hsstr 1-5). <i>Endocrinology.</i> 135 (6):2814 - 2817.
282910	Engstrom M, Tomperi J, El-Darwish K, Ahman M, Savola JM and Wurster S (2005) Superagonism at the human somatostatin receptor subtype 4. <i>J Pharmacol Exp Ther.</i> 312(1): 332-338. Smalley KS, Koenig JA, Feniuk W and Humphrey PP (2001) Ligand internalization and recycling by human recombinant somatostatin type 4 (h sst(4)) receptors expressed in CHO-K1 cells. <i>Br J Pharmacol.</i> 132(5):1102-1110.
283010	Buscail L, Esteve JP, Saint-Laurent N, Bertrand V, Reisine T, O'Carroll AM, Bell GI, Schally AV, Vaysse N and Susini C (1995) Inhibition of cell proliferation by the somatostatin analogue RC-160 is mediated by somatostatin receptor subtypes SSTR2 and SSTR5 through different mechanisms. <i>Proc Natl Acad Sci USA.</i> 92(5):1580-1584. Shimon I, Taylor JE, Dong JZ, Bitonte RA, Kim S, Morgan B, Coy DH, Culler MD and Melmed S (1997) Somatostatin receptor subtype specificity in human fetal pituitary cultures. Differential role of SSTR2 and SSTR5 for growth hormone, thyroid-stimulating hormone, and prolactin regulation. <i>J Clin Invest.</i> 99(4):789-798.
285900	Inoue A, Yamakawa J, Yukioka M and Morisawa S (1983) Filter-binding assay procedure for thyroid hormone receptors. <i>Anal Biochem.</i> 134(1): 176-183.
286010	Engel S, Skoumbourdis AP, Childress J, Neumann S, Deschamps JR, Thomas CJ, Colson AO, Costanzi S and Gershengorn MC. (2008) A virtual screen for diverse ligands: discovery of selective G protein-coupled receptor antagonists. <i>J Am Chem Soc.</i> 130(15): 5115-5123.
286200	Massague J (1987) Identification of receptors for type-β transforming growth factor. <i>Methods Enzymol.</i> 146:174-195.
286700	Nothacker HP, Wang Z, McNeill AM, Saito Y, Merten S, O'Dowd B, Duckles SP and Civelli O (1999) Identification of the natural ligand of an orphan G-protein-coupled receptor involved in the regulation of vasoconstriction. <i>Nat Cell Biol.</i> 1(6):383-385.
287010	Couvineau A, Rousset M and Laburthe M (1985) Molecular identification and structural requirement of vasoactive intestinal peptide (VIP) receptors in the human colon adenocarcinoma cell line, HT-29. <i>Biochem J.</i> 231:139-143.

Literature References

Cat #	Reference
287100	Yung SL, Dela Cruz F, Hamren S, Zhu J, Tsutsumi M, Bloom JW, Caudle M, Rocznik S, Todd T, Lemoine L, MacDougall M, Shanafelt AB and Pan CQ (2003) Generation of highly selective VPAC2 receptor agonists by high throughput mutagenesis of vasoactive intestinal peptide and pituitary adenylate cyclase-activating peptide. <i>J Biol Chem.</i> 278(12): 10273-10281.
287530	Thibonnier M, Auzan C, Madhun Z, Wilkins P, Berti-Mattera L. and Clauser E (1994) Molecular cloning, sequencing, and functional expression of a cDNA encoding the human V _{1a} vasopressin receptor. <i>J. Biol. Chem.</i> 269(5): 3304-3310.
287560	Tahara A, Saito M, Sugimoto T, Tomura Y, Wada K, Kusayama T, Tsukada J, Ishii N, Yatsu T, Uchida W and Tanaka A (1998) Pharmacological characterization of the human vasopressin receptor subtypes stably expressed in Chinese hamster ovary cells. <i>Br J Pharmacol.</i> 125(7): 1463 - 1470.
287610	Tahara A, Saito M, Sugimoto T, Tomura Y, Wada K, Kusayama T, Tsukada J, Ishii N, Yatsu T, Uchida W and Tanaka A (1998) Pharmacological characterization of the human vasopressin receptor subtypes stably expressed in Chinese hamster ovary cells. <i>Br J Pharmacol.</i> 125(7): 1463 - 1470.
299002	Wieland HA1, Soll RM, Doods HN, Stenkamp D, Hurnaus R, Lammle B and Beck-Sickinger AG. (2002) The SK-N-MC cell line expresses an orexin binding site different from recombinant orexin 1-type receptor. <i>Eur. J. Biochem.</i> , 269(4): 1128-1135.
299003	Hui Y, Yang G, Galczenski H, Figueroa DJ, Austin CP, Copeland NG, Gilbert DJ, Jenkins NA and Funk CD (2001) The murine cysteinyl leukotriene 2 (CysLT ₂) receptor. cDNA and genomic cloning, alternative splicing, and in vitro characterization. <i>J Biol Chem.</i> 276(50): 47489-47495.
299004	Abramovitz M1, Adam M, Boie Y, Carriere M, Denis D, Godbout C, Lamontagne S, Rochette C, Sawyer N, Tremblay NM, Belley M, Gallant M, Dufresne C, Gareau Y, Ruel R, Juteau H, Labelle M, Ouimet N and Metters KM (2000) The utilization of recombinant prostanoid receptors to determine the affinities and selectivities of prostaglandins and related analogs. <i>Biochem. Biophys. Acta.</i> , 1483(2): 285-293.
299005	Sarup JC, Rao KV, Williams RE and Fox CF (1988) Resolution of high and low affinity progesterone receptors from human breast carcinoma T47D cells. <i>J Biol Chem</i> , 263(12):5624-5633.
299006	Abramovitz M, Adam M, Boie Y, Carriere M, Denis D, Godbout C, Lamontagne S, Rochette C, Sawyer N, Tremblay NM, Belley M, Gallant M, Dufresne C, Gareau Y, Ruel R, Juteau H, Labelle M, Ouimet N and Metters KM (2000) The utilization of recombinant prostanoid receptors to determine the affinities and selectivities of prostaglandins and related analogs. <i>Biochem. Biophys. Acta.</i> , 1483(2): 285-293.
299009	Kurokawa M, Michelangeli VP and Findlay DM. (1991) Induction of calcitonin receptor expression by glucocorticoids in T47D human breast cancer cells. <i>J Endocrinol.</i> 130: 321-326
299030	Langmead CJ, Jerman JC, Brough SJ, Scott C, Porter RA and Herdon HJ (2004) Characterisation of the binding of [3H]-SB-674042, a novel nonpeptide antagonist, to the human orexin-1 receptor. <i>Br J Pharmacol.</i> 141(2): 340-346.
299034	Ganapathy ME, Prasad PD, Huang W, Seth P, Leibach FH and Ganapathy V (1999) Molecular and ligand-binding characterization of the σ -receptor in the Jurkat human T lymphocyte cell line. <i>J Pharmacol Exp Ther.</i> 289(1): 251-260.
299035	Liu C, Wilson SJ, Kuei C and Lovenberg TW (2001) Comparison of human, mouse, rat, and guinea pig histamine H ₄ receptors reveals substantial pharmacological species variation. <i>J Pharmacol Exp Ther.</i> 299(1): 121 - 130.

Literature References

Cat #	Reference
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299036	Bo XN and Burnstock G (1990)
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	High- and low-affinity binding sites for [³ H]-alpha, beta-methylene ATP in rat urinary bladder membranes. Br J Pharmacol. 101(2): 291-296.
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299037	Fukunaga K, Ishii S, Asano K, Yokomizo T, Shiomi T, Shimizu T and Yamaguchi K (2001)
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	Single nucleotide polymorphism of human platelet-activating factor receptor impairs G-protein activation. J Biol Chem. 276(46): 43025-43030.
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Reference Compounds

Cat #	Assay Name	Reference Compound	Historical			Concurrent	
			IC ₅₀ *	K _i	n _H	Batch *	IC ₅₀ *
200510	Adenosine A ₁	R(-)-PIA	0.83 µM	0.49 µM	0.90	496528	0.41 µM
200610	Adenosine A _{2A}	CGS-21680	0.13 µM	0.079 µM	1.0	496528	0.15 µM
200650	Adenosine A _{2B}	DPCPX	0.25 µM	0.081 µM	0.80	496623	0.30 µM
200720	Adenosine A ₃	IB-MECA	0.78 nM	0.72 nM	0.80	496532	1.75 nM
203110	Adrenergic α _{1A}	Prazosin	0.87 nM	0.42 nM	0.90	496425	0.80 nM
203210	Adrenergic α _{1B}	Prazosin	0.25 nM	0.098 nM	1.0	496425	0.24 nM
203400	Adrenergic α _{1D}	Prazosin	0.88 nM	0.43 nM	0.70	496606	0.54 nM
203630	Adrenergic α _{2A}	Yohimbine	1.80 nM	0.9 nM	0.80	496670	1.69 nM
203710	Adrenergic α _{2B}	Yohimbine	14.0 nM	6.40 nM	1.0	496432	13.3 nM
203810	Adrenergic α _{2C}	Yohimbine	2.0 nM	0.89 nM	1.0	496450	2.04 nM
204010	Adrenergic β ₁	S(-)-Propranolol	2.50 nM	1.40 nM	0.80	496550	1.11 nM
204110	Adrenergic β ₂	S(-)-Propranolol	0.78 nM	0.54 nM	1.20	496552	0.56 nM
204200	Adrenergic β ₃	Alprenolol	0.18 µM	0.13 µM	0.5	496551	0.13 µM
206000	Androgen (Testosterone)	Testosterone	2.10 nM	1.20 nM	1.10	496458	1.42 nM
210030	Angiotensin AT ₁	Saralasin	0.92 nM	0.68 nM	0.90	496433	0.48 nM
210120	Angiotensin AT ₂	Saralasin	0.35 nM	0.16 nM	1.0	496433	0.48 nM
210720	APJ	(Pyr ¹)-Apelin-13	0.19 nM	0.095 nM	0.70	496539	0.18 nM
211600	Bombesin BB1	Neuromedin B	17.0 pM	8.10 pM	0.90	496541	15.1 pM
211700	Bombesin BB2	GRP (human)	0.027 nM	15.0 pM	1.10	496540	0.048 nM
211800	Bombesin BB3	(D-Phe ⁶ ,β-Ala ¹¹ ,Phe ¹³ ,Nle ¹⁴)-Bombesin (6-14)	3.40 nM	2.30 nM	0.90	496541	2.07 nM
212520	Bradykinin B ₁	(Des-Arg ¹⁰)-Kallidin	0.29 nM	0.15 nM	1.10	496450	0.22 nM
212620	Bradykinin B ₂	Bradykinin	1.80 nM	1.10 nM	1.0	496607	0.60 nM
299009	Calcitonin	Calcitonin (salmon)	0.5 nM	0.24 nM	1.0	496629	0.47 nM
214020	Calcitonin Gene-Related Peptide CGRP ₁	α-CGRP (human)	0.069 nM	0.054 nM	1.30	496549	0.082 nM
214510	Calcium Channel L-Type, Benzothiazepine	Diltiazem	0.036 µM	0.032 µM	0.90	496426	15.6 nM
214600	Calcium Channel L-Type, Dihydropyridine	Nitrendipine	0.3 nM	0.19 nM	0.90	496553	0.21 nM
215000	Calcium Channel L-Type, Phenylalkylamine	Methoxyverapamil (D-600)	0.022 µM	0.021 µM	0.60	496531	0.039 µM
216000	Calcium Channel N-Type	ω-Conotoxin GVIA	13.0 pM	11.0 pM	1.50	496533	6.39 pM
217050	Cannabinoid CB ₁	CP 55,940	0.033 µM	0.03 µM	0.70	496352	0.033 µM
217100	Cannabinoid CB ₂	R(+)-WIN-55,212-2	5.80 nM	3.90 nM	1.10	496378	6.58 nM
217510	Chemokine CCR1	MIP-1β	15.0 nM	12.0 nM	1.20	496517	5.86 nM
217550	Chemokine CCR2B	MCP-1	0.077 nM	0.03 nM	0.90	496374	0.16 nM
217730	Chemokine CCR5 (Rhesus Macaque)	MIP-1β	0.12 nM	0.091 nM	1.0	496373	0.23 nM
244600	Chemokine CX3CR1	Fractalkine	0.078 nM	0.061 nM	1.40	496656	0.11 nM
244420	Chemokine CXCR1 (IL-8R _A)	IL-8	0.11 nM	0.051 nM	1.0	496373	0.1 nM
244500	Chemokine CXCR2 (IL-8R _B)	IL-8	0.18 nM	0.08 nM	0.90	496376	0.095 nM
244550	Chemokine CXCR4	SDF-1α	0.12 nM	0.091 nM	1.0	496655	0.19 nM

* Batch: Represents compounds tested concurrently in the same assay(s).

Cleveland Clinic Foundation

Study #: TW04-0013389, Compound Code: CCF2201 (1271304)

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Reference Compounds

Cat #	Assay Name	Reference Compound	Historical		n _H	Concurrent	
			IC ₅₀ *	K _i		Batch *	IC ₅₀ *
218020	Cholecystokinin CCK ₁ (CCK _A)	Devazepide (L-364,718)	0.18 nM	0.047 nM	0.90	496524	0.34 nM
218130	Cholecystokinin CCK ₂ (CCK _B)	Sincalide	0.064 nM	0.037 nM	1.10	496391	0.054 nM
219100	Colchicine	Colchicine	2.80 μM	2.80 μM	1.30	496652	2.17 μM
219150	Corticotropin Releasing Factor CRF ₁	Sauvagine (frog)	0.85 nM	0.77 nM	0.60	496541	1.12 nM
299003	CysLT ₂ (LTC ₄)	LTC ₄	7.20 nM	4.30 nM	0.90	496662	7.25 nM
219500	Dopamine D ₁	R(+)-SCH-23390	1.40 nM	0.7 nM	0.90	496453	1.51 nM
219700	Dopamine D _{2S}	Spiperone	0.25 nM	0.089 nM	1.0	496455	0.20 nM
219800	Dopamine D ₃	Spiperone	0.51 nM	0.17 nM	1.0	496452	0.98 nM
220000	Dopamine D _{4.4}	Spiperone	0.76 nM	0.21 nM	0.80	496454	1.02 nM
220200	Dopamine D ₅	R(+)-SCH-23390	1.50 nM	0.4 nM	0.90	496452	2.62 nM
224010	Endothelin ET _A	Endothelin-1	0.065 nM	0.04 nM	1.0	496512	0.045 nM
224110	Endothelin ET _B	Endothelin-1	0.13 nM	0.06 nM	0.90	496377	0.071 nM
225510	Epidermal Growth Factor (EGF)	EGF (human)	0.2 nM	0.14 nM	1.10	496374	0.26 nM
226010	Estrogen ERα	Diethylstilbestrol	0.77 nM	0.22 nM	1.0	496354	0.74 nM
226050	Estrogen ERβ	Diethylstilbestrol	0.61 nM	0.13 nM	1.20	496354	1.09 nM
226810	GABA _A , Chloride Channel, TBOB	Picrotoxin	0.27 μM	0.24 μM	1.0	496654	0.46 μM
226600	GABA _A , Flunitrazepam, Central	Diazepam	16.0 nM	13.0 nM	0.80	496519	0.03 μM
226500	GABA _A , Muscimol, Central	GABA	0.032 μM	0.026 μM	0.90	496653	0.032 μM
228610	GABA _{B1A}	CGP-54626	6.40 nM	2.90 nM	1.0	496355	10.1 nM
228710	GABA _{B1B}	CGP-54626	6.30 nM	3.0 nM	1.10	496355	8.27 nM
230000	Gabapentin	Gabapentin	0.04 μM	0.026 μM	0.90	496356	0.068 μM
231650	γ-Hydroxybutyric Acid (GHB) Receptor	NCS382	0.5 μM	0.48 μM	0.70	496357	0.23 μM
231680	Glucagon	[des-His ¹ -Glu ⁹]-Glucagon (1-29) amide	5.0 nM	3.90 nM	1.0	496358	4.55 nM
232030	Glucocorticoid	Dexamethasone	3.80 nM	1.80 nM	0.90	496456	4.92 nM
232600	Glutamate, AMPA	L-Glutamic acid	0.32 μM	0.25 μM	1.0	496534	0.23 μM
232710	Glutamate, Kainate	L-Glutamic acid	0.16 μM	0.076 μM	0.80	496435	0.32 μM
236400	Glutamate, Metabotropic, mGlu ₂	LY-354740	0.23 μM	0.12 μM	1.20	496359	0.19 μM
237000	Glutamate, Metabotropic, mGlu ₅	L-Glutamic acid	1.50 μM	0.7 μM	0.90	496734	0.88 μM
232810	Glutamate, NMDA, Agonism	L-Glutamic acid	0.41 μM	0.37 μM	0.90	496520	0.18 μM
232910	Glutamate, NMDA, Glycine	MDL 105,519	8.0 nM	7.60 nM	1.0	496520	5.43 nM
233000	Glutamate, NMDA, Phencyclidine	Dizocilpine ((+)-MK-801)	5.10 nM	3.40 nM	0.70	496521	2.60 nM
234000	Glutamate, NMDA, Polyamine	Ifenprodil	0.023 μM	0.021 μM	1.10	496545	18.2 nM
239000	Glycine, Strychnine-Sensitive	(-)-Strychnine	8.90 nM	5.0 nM	1.0	496438	16.9 nM
239200	Gonadotropin-Releasing Hormone	[D-Trp ⁶]-LH-RH	0.12 nM	0.1 nM	1.20	496376	0.24 nM
239300	Growth Hormone Secretagogue (GHS, Ghrelin)	Ghrelin (human)	0.13 nM	0.073 nM	0.80	496532	0.13 nM
239610	Histamine H ₁	Pyrilamine	3.30 nM	1.60 nM	1.0	496518	3.47 nM
239710	Histamine H ₂	Tiotidine	0.022 μM	18.0 nM	1.10	496535	0.028 μM
239820	Histamine H ₃	R(-)-α-Methylhistamine (RAMH)	2.30 nM	1.10 nM	1.10	496548	1.73 nM

* Batch: Represents compounds tested concurrently in the same assay(s).

Cleveland Clinic Foundation

Study #: TW04-0013389, Compound Code: CCF2201 (1271304)

Friday, December 2, 2022

Reference Compounds

Cat #	Assay Name	Reference Compound	Historical			Concurrent	
			IC ₅₀ *	K _i	n _H	Batch *	IC ₅₀ *
299035	Histamine H ₄	Imetit	4.40 nM	1.90 nM	1.0	496671	5.36 nM
241000	Imidazoline I ₂ , Central	Idazoxan	12.0 nM	8.0 nM	1.0	496546	11.5 nM
242500	Inositol Trisphosphate IP ₃	1,4,5-IP ₃	0.023 μM	0.022 μM	1.30	496360	0.020 μM
243000	Insulin	Insulin	1.10 nM	1.0 nM	0.90	496459	1.34 nM
243530	Interleukin IL-1 R1	IL-1β	1.80 nM	1.70 nM	1.70	496544	1.80 nM
244100	Interleukin IL-6	IL-6	0.077 nM	0.029 nM	1.10	496597	0.047 nM
299004	IP (PGI ₂)	Iloprost	0.022 μM	13.0 nM	1.10	496603	7.21 nM
250460	Leukotriene, Cysteinyl CysLT ₁	LTD ₄	0.5 nM	0.21 nM	1.0	496384	0.96 nM
251010	Melanin-Concentrating Hormone MCH1 (SLC1)	MCH (human, mouse, rat)	0.67 nM	0.45 nM	1.10	496430	1.03 nM
251100	Melanocortin MC ₁	NDP-α-MSH	0.055 nM	0.026 nM	1.0	496439	0.060 nM
251300	Melanocortin MC ₃	NDP-α-MSH	0.15 nM	0.13 nM	0.90	496449	0.31 nM
251350	Melanocortin MC ₄	NDP-α-MSH	0.22 nM	0.21 nM	1.10	496439	0.14 nM
251400	Melanocortin MC ₅	NDP-α-MSH	0.48 nM	0.45 nM	0.90	496449	0.38 nM
251600	Melatonin MT ₁	Melatonin	0.21 nM	0.11 nM	0.70	496361	0.13 nM
251700	Melatonin MT ₂	4-P-PDOT	0.23 nM	0.12 nM	0.90	496362	0.52 nM
252200	Motilin	Motilin (human, porcine)	0.54 nM	0.27 nM	0.90	496363	0.64 nM
252610	Muscarinic M ₁	4-DAMP	4.50 nM	1.10 nM	1.0	496380	5.44 nM
252710	Muscarinic M ₂	4-DAMP	0.055 μM	0.023 μM	1.0	496381	0.036 μM
252810	Muscarinic M ₃	4-DAMP	5.10 nM	2.50 nM	1.10	496382	3.04 nM
252910	Muscarinic M ₄	4-DAMP	15.0 nM	3.20 nM	1.0	496383	16.6 nM
253010	Muscarinic M ₅	4-DAMP	3.20 nM	2.0 nM	1.0	496383	1.69 nM
254500	Muscarinic, Oxotremorine M	Oxotremorine M	15.0 nM	7.40 nM	0.90	496657	13.7 nM
257010	Neuropeptide Y Y ₁	Neuropeptide Y (human, rat)	0.22 nM	0.21 nM	1.10	496624	0.20 nM
257110	Neuropeptide Y Y ₂	Neuropeptide Y (13-36) (porcine)	0.21 nM	0.12 nM	0.90	496364	0.25 nM
258010	Neurotensin NT ₁	Neurotensin	0.7 nM	0.57 nM	0.70	496658	0.25 nM
258700	Nicotinic Acetylcholine α ₁ , Bungarotoxin	α-Bungarotoxin	1.10 nM	0.72 nM	1.10	496440	1.40 nM
258730	Nicotinic Acetylcholine α _{3β4}	Epibatidine	0.099 nM	0.072 nM	1.0	496441	0.053 nM
258750	Nicotinic Acetylcholine α _{4β2} , Cytisine	(-)-Nicotine	17.0 nM	5.10 nM	0.70	496659	17.9 nM
258630	Nicotinic Acetylcholine α ₇ , Bungarotoxin	α-Bungarotoxin	1.0 nM	0.77 nM	1.0	496365	2.94 nM
258660	Nicotinic Acetylcholine α ₇ , Methyllycaconitine	Methyllycaconitine	4.0 nM	1.80 nM	1.0	496366	4.89 nM
260130	Opiate δ ₁ (OP1, DOP)	Naltrindole	2.60 nM	0.1 nM	1.0	496625	2.83 nM
260210	Opiate κ (OP2, KOP)	U-69593	16.0 nM	6.40 nM	0.5	496451	5.81 nM
260410	Opiate μ (OP3, MOP)	DAMGO	20.0 nM	8.10 nM	0.60	496379	0.050 μM
299030	Orexin OX ₁	Orexin A	0.49 nM	0.49 nM	1.10	496542	0.46 nM
299002	Orexin OX ₂	Orexin B	0.21 nM	0.18 nM	1.0	496543	0.12 nM
260600	Orphanin ORL ₁	Orphanin-FQ	0.59 nM	0.37 nM	1.20	496607	1.18 nM
260900	Oxytocin	Oxytocin	0.95 nM	0.31 nM	0.90	496392	0.56 nM
264500	Phorbol Ester	PMA	0.79 nM	0.59 nM	1.0	496552	1.52 nM

* Batch: Represents compounds tested concurrently in the same assay(s).

Cleveland Clinic Foundation

Study #: TW04-0013389, Compound Code: CCF2201 (1271304)

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Reference Compounds

Cat #	Assay Name	Reference Compound	Historical		n _H	Concurrent	
			IC ₅₀ *	K _i		Batch *	IC ₅₀ *
299037	Platelet Activating Factor (PAF)	C16-PAF	2.20 nM	1.30 nM	0.80	496445	2.0 nM
265200	Platelet-Derived Growth Factor (PDGF)	PDGF	0.05 nM	19.0 pM	0.70	496367	0.14 nM
265510	Potassium Channel [K _A]	α-Dendrotoxin	2.70 pM	1.30 pM	1.10	496624	3.11 pM
265600	Potassium Channel [K _{ATP}]	Glyburide	5.70 nM	0.65 nM	0.80	496448	3.53 nM
265800	Potassium Channel [SK _{CA}]	Apamin	8.80 pM	8.50 pM	1.10	496368	6.38 pM
265910	Potassium Channel hERG, [³ H]Dofetilide	Dofetilide (MK-499)	2.50 nM	1.70 pM	1.0	496442	6.58 nM
299005	Progesterone PR-B	R-5020	0.49 nM	0.39 nM	2.20	496460	0.43 nM
268030	Prostanoid CRTH2	Prostaglandin D ₂ (PGD ₂)	4.40 nM	3.50 nM	0.70	496392	5.53 nM
268060	Prostanoid DP	BW 245C	2.90 nM	2.40 nM	0.80	496735	1.80 nM
268110	Prostanoid EP ₁	Prostaglandin E ₂ (PGE ₂)	4.40 nM	3.70 nM	0.70	496603	3.01 nM
268200	Prostanoid EP ₂	Prostaglandin E ₂ (PGE ₂)	6.60 nM	2.90 nM	1.0	496385	3.89 nM
299006	Prostanoid EP ₃	Sulprostone	1.0 nM	0.62 nM	1.10	496385	1.61 nM
268420	Prostanoid EP ₄	Prostaglandin E ₂ (PGE ₂)	1.10 nM	0.45 nM	0.90	496385	2.58 nM
268510	Prostanoid FP	PGF2α	1.0 nM	0.71 nM	1.0	496603	2.15 nM
299036	Purinergic P2X	α, β-Methylene ATP	11.0 nM	5.0 nM	1.10	496431	10.1 nM
268820	Purinergic P2Y, Non-Selective	2-Methylthio-ATP	0.085 μM	0.067 μM	1.10	496523	0.098 μM
270000	Rolipram	Rolipram	5.70 nM	2.10 nM	1.0	496524	3.78 nM
270300	Ryanodine RyR3	Ryanodine	2.70 nM	1.10 nM	1.0	496631	4.10 nM
271110	Serotonin (5-Hydroxytryptamine) 5-HT _{1A}	Metergoline	4.10 nM	2.30 nM	0.90	496519	2.29 nM
271200	Serotonin (5-Hydroxytryptamine) 5-HT _{1B}	Serotonin (5-HT)	5.70 nM	5.40 nM	0.80	496630	5.76 nM
271700	Serotonin (5-Hydroxytryptamine) 5-HT _{2B}	Ketanserin	0.29 μM	0.18 μM	0.60	496428	0.85 μM
271800	Serotonin (5-Hydroxytryptamine) 5-HT _{2C}	RS 102221	2.0 nM	1.0 nM	1.30	496447	2.08 nM
271910	Serotonin (5-Hydroxytryptamine) 5-HT ₃	MDL 72222	11.0 nM	2.50 nM	0.80	496739	17.6 nM
272000	Serotonin (5-Hydroxytryptamine) 5-HT ₄	RS-23597-190	0.25 μM	0.042 μM	0.70	496660	0.29 μM
272100	Serotonin (5-Hydroxytryptamine) 5-HT _{5A}	Methiothepin	2.90 nM	1.50 nM	0.90	496429	3.01 nM
272200	Serotonin (5-Hydroxytryptamine) 5-HT ₆	Methiothepin	2.80 nM	1.30 nM	1.10	496525	1.77 nM
272320	Serotonin (5-Hydroxytryptamine) 5-HT ₇	Methiothepin	0.39 nM	0.22 nM	0.90	496392	0.54 nM
299034	Sigma σ ₁	Haloperidol	2.0 nM	1.0 nM	0.90	496525	2.80 nM
278200	Sigma σ ₂	Ifenprodil	8.0 nM	4.90 nM	0.70	496429	9.55 nM
279510	Sodium Channel, Site 2	Dibucaine	0.61 μM	0.55 μM	0.90	496444	1.13 μM
282510	Somatostatin sst1	Somatostatin-14	0.25 nM	0.21 nM	0.90	496618	0.42 nM
282700	Somatostatin sst2	Somatostatin-14	9.10 pM	4.80 pM	1.20	496617	15.7 pM
282530	Somatostatin sst3	Somatostatin-14	0.066 nM	0.023 nM	0.80	496786	0.13 nM
282910	Somatostatin sst4	Somatostatin-14	0.56 nM	0.5 nM	1.20	496461	0.44 nM
283010	Somatostatin sst5	Somatostatin-14	0.43 nM	0.24 nM	0.80	496619	0.44 nM

* Batch: Represents compounds tested concurrently in the same assay(s).

Cleveland Clinic Foundation

Study #: TW04-0013389, Compound Code: CCF2201 (1271304)

Friday, December 2, 2022

Reference Compounds

Cat #	Assay Name	Reference Compound	Historical		n _H	Concurrent	
			IC ₅₀ *	K _i		Batch *	IC ₅₀ *
255520	Tachykinin NK ₁	L-703,606	3.60 nM	2.60 nM	1.0	496536	1.46 nM
255620	Tachykinin NK ₂	MEN-10,376	3.70 nM	3.30 nM	1.20	496535	2.28 nM
285900	Thyroid Hormone	Triiodothyronine	0.034 nM	18.0 pM	1.0	496599	0.041 nM
286010	Thyrotropin Releasing Hormone (TRH)	TRH	0.045 μM	17.0 nM	0.90	496369	0.020 μM
286200	Transforming Growth Factor-β (TGF-β)	TGF-β ₁	0.025 nM	9.0 pM	0.90	496370	0.035 nM
202000	Transporter, Adenosine	Nitrobenzylthioinosine	0.85 nM	0.29 nM	1.0	496514	0.54 nM
219010	Transporter, Choline	Hemicholinium-3	5.0 nM	2.90 nM	0.90	496379	7.04 nM
220320	Transporter, Dopamine (DAT)	GBR-12909	1.70 nM	1.30 nM	0.90	496427	0.75 nM
226400	Transporter, GABA	NO-711	0.2 μM	0.2 μM	1.10	496547	0.27 μM
239100	Transporter, Glycine	Sarcosine	85.0 μM	85.0 μM	0.5	496620	114 μM
204410	Transporter, Norepinephrine (NET)	Desipramine	0.93 nM	0.92 nM	0.60	496427	0.79 nM
274030	Transporter, Serotonin (5-Hydroxytryptamine) (SERT)	Fluoxetine	8.60 nM	1.40 nM	0.90	496671	4.06 nM
252030	Transporter, Vesicular Monoamine (Non-Selective)	Tetrabenazine	4.0 nM	2.20 nM	0.90	496552	5.78 nM
286700	Urotensin II	Urotensin II	0.41 nM	0.27 nM	1.0	496371	0.64 nM
287010	Vasoactive Intestinal Peptide VIP ₁	VIP	0.023 nM	16.0 pM	0.90	496372	0.041 nM
287100	Vasoactive Intestinal Peptide VIP ₂ /PACAP VPAC ₂	PACAP ₂₇	1.10 nM	1.10 nM	0.70	496373	1.88 nM
287530	Vasopressin V _{1A}	(Arg ⁸)-Vasopressin	0.33 nM	0.065 nM	0.80	496430	0.55 nM
287560	Vasopressin V _{1B}	(Arg ⁸)-Vasopressin	0.66 nM	0.11 nM	1.0	496661	0.72 nM
287610	Vasopressin V ₂	(Arg ⁸)-Vasopressin	4.50 nM	2.0 nM	1.0	496548	4.20 nM

* Batch: Represents compounds tested concurrently in the same assay(s).

Cleveland Clinic Foundation

Study #: TW04-0013389, Compound Code: CCF2201 (1271304)

Friday, December 2, 2022

Appendix 8

In Vitro Pharmacology
Study of CCF2201

STUDY ID: FR095-0036305

STUDY NUMBER
100065480

November 21, 2022

1. STUDY REFERENCES

Study title	<i>In Vitro</i> Pharmacology Study of CCF2201	
Study number	100065480	FINAL REPORT November 21, 2022
Study ID	FR095-0036305	
Experimental period	November 15, 2022 - November 18, 2022	
PO number	CCF22773426	

2. PERSONS INVOLVED IN THE STUDY

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Study sponsor	Cleveland Clinic Foundation Technology Proof-of Concept Office, Center for The rapeutic Discovery The Cleveland Clinic Lerner Research Institute 950,0 Euclid Avenue, ND2-51,... Cleveland, OH 44195 U.S.A.	Vara Prasad JOSYULA Patent Agent, Senior Director

3. APPROVAL

Head of laboratory statement

This study was conducted according to the procedures described in this report.

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Signature



Quality assurance statement

This study was inspected by Eurofins Cerep Quality Control Unit, the results and methods presented in this report accurately reflect the methods used and the data collected for this study.

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Principal scientist statement

This report accurately reflects all relevant data collected in this study.

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5. SUMMARY

The purpose of this study was to test CCF2201 in cytotoxicity assays.

5.1. Study Design

CCF2201 was tested at several concentrations for IC₅₀ determination.

5.2. Measurements

Compound cytotoxicity effect was calculated as % dead cells.

5.3. Results

Results showing an inhibition or stimulation higher than 50% are considered to represent significant effects of the test compounds.

Assay Compound I.D.	Client Compound I.D.	IC50	Flags	Cytotoxicity class
cytotoxicity profile (BJ)				
100065480-1	CCF2201		N.C.	4
cytotoxicity profile (H4-II-E-C3)				
100065480-1	CCF2201		N.C.	4
cytotoxicity profile (HEK-293)				
100065480-1	CCF2201		N.C.	4
cytotoxicity profile (HepG2)				
100065480-1	CCF2201		N.C.	4
cytotoxicity profile (HK2)				
100065480-1	CCF2201		> 3.0E-05 M	3
cytotoxicity profile (HUV-EC-C)				
100065480-1	CCF2201		> 3.0E-05 M	3
cytotoxicity profile (Jurkat)				
100065480-1	CCF2201		N.C.	4
cytotoxicity profile (MRC-5)				
100065480-1	CCF2201		N.C.	4
cytotoxicity profile (Neuro-2a)				
100065480-1	CCF2201		N.C.	4
cytotoxicity profile (NIH/3T3)				
100065480-1	CCF2201	2.3E-05 M		1
cytotoxicity profile (NRK-52E)				
100065480-1	CCF2201		N.C.	4
cytotoxicity profile (SH-SY5Y)				
100065480-1	CCF2201		N.C.	4
cytotoxicity profile (SK-N-SH)				
100065480-1	CCF2201		N.C.	4

5.4. Conclusions

CCF2201 belongs to class 4 for BJ, H4-II-E-C3, HEK-293, HepG2, Jurkat, MRC-5, Neuro-2a, NRK-52E, SH-SY5Y and SK-N-SH cell line. It is considered to have no significant cytotoxic effect on the tested models.

CCF2201 belongs to class 3 for HK2 and HUV-EC-C cell lines. It is considered to have a cytotoxic effect of more than 25% only at the highest tested concentration on the tested models.

CCF2201 belongs to class 1 for NIH/3T3 cell line. It displays full response curve and is considered to have significant cytotoxic effect at the tested concentration on the tested models.

6. COMPOUNDS

6.1. Test Compounds

Client Compound ID	Compound ID	Reference Number	Batch Number	FW	MW	Purity	Received Form	Stock solution	Flag
CCF2201	100065480-1	-	22-01	447.85	-	100.0	Powder	1.E-02 M DMSO	-

FW: Formula Weight - MW: Molecular Weight

6.2. Reference Compounds

In each experiment and if applicable, the respective reference compound was tested concurrently with CCF2201, and the data were compared with historical values determined at Eurofins. The experiment was accepted in accordance with Eurofins validation Standard Operating Procedure.

7. RESULTS

7.1. *In Vitro* Pharmacology: Cytotoxicity Profile Assays

7.1.1. IC₅₀ Determination: Test Compound Results

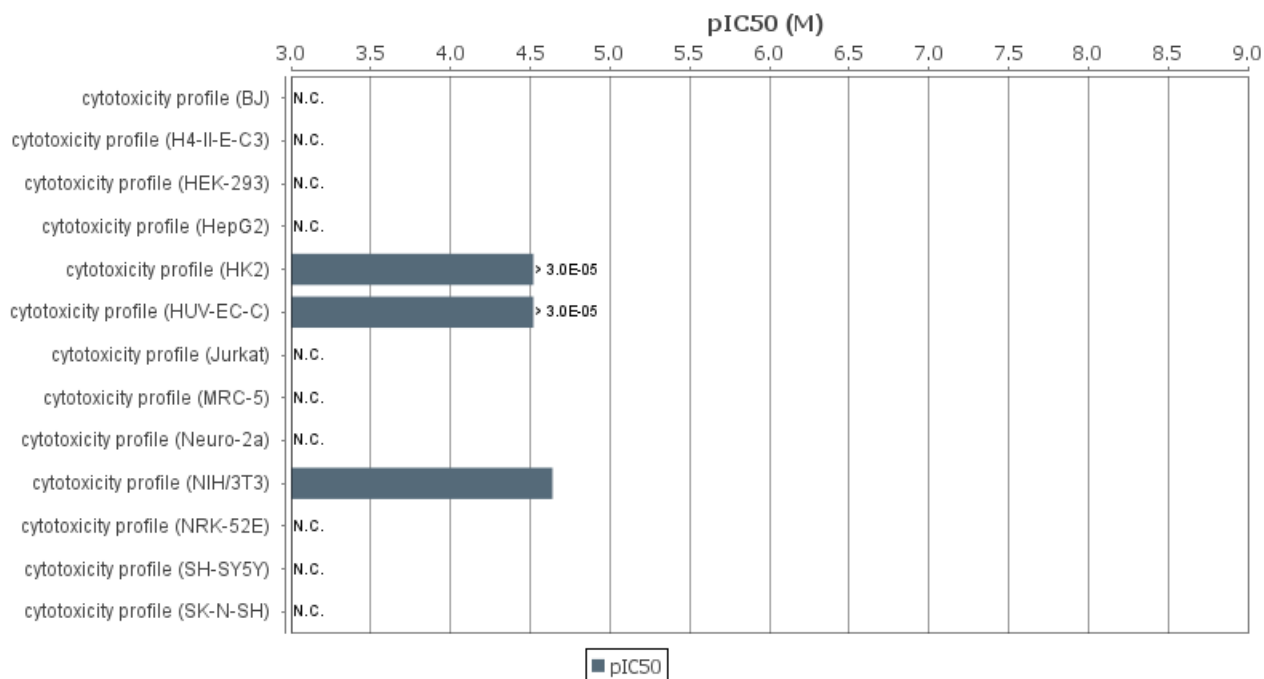


Figure 1. Histogram for CCF2201

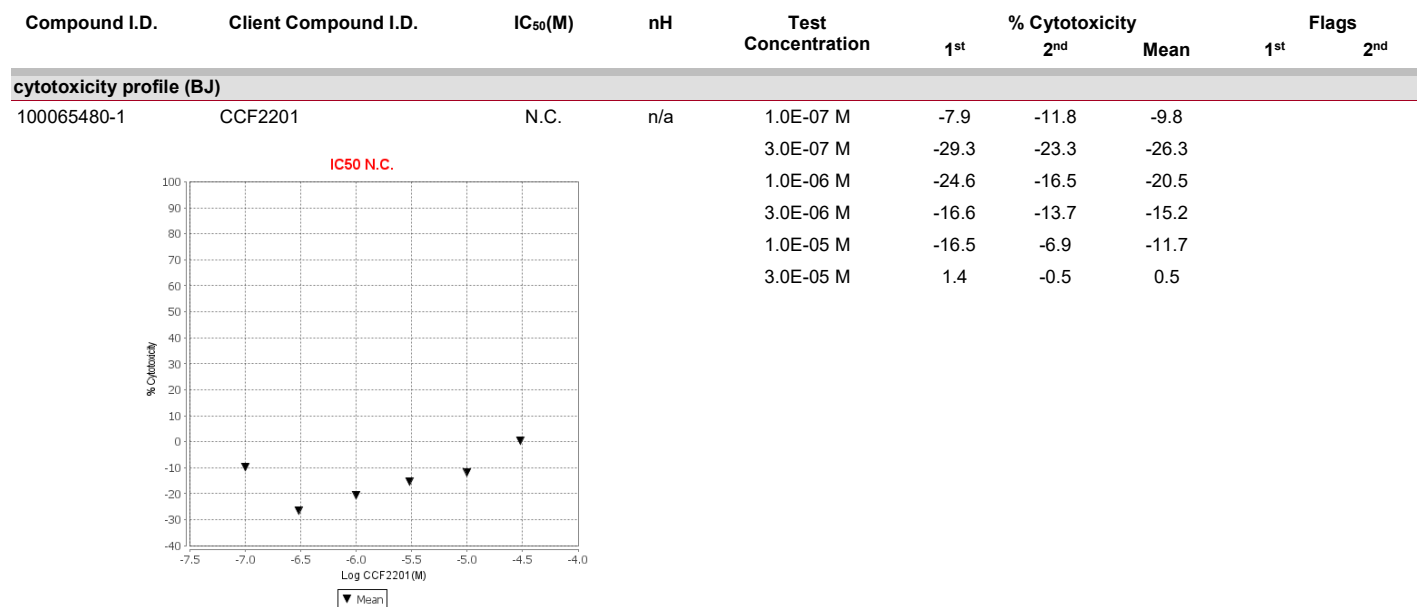


Figure 2. CCF2201 on cytotoxicity profile (BJ)

Compound I.D.	Client Compound I.D.	IC ₅₀ (M)	nH	Test Concentration	% Cytotoxicity			Flags	
					1 st	2 nd	Mean	1 st	2 nd
cytotoxicity profile (H4-II-E-C3)									
100065480-1	CCF2201	N.C.	n/a	1.0E-07 M	-22.2	-17.4	-19.8		
				3.0E-07 M	-39.7	-26.6	-33.2		
				1.0E-06 M	-28.0	-24.2	-26.1		
				3.0E-06 M	-10.6	-11.7	-11.2		
				1.0E-05 M	-18.4	-3.3	-10.8		
				3.0E-05 M	-0.9	-3.3	-2.1		

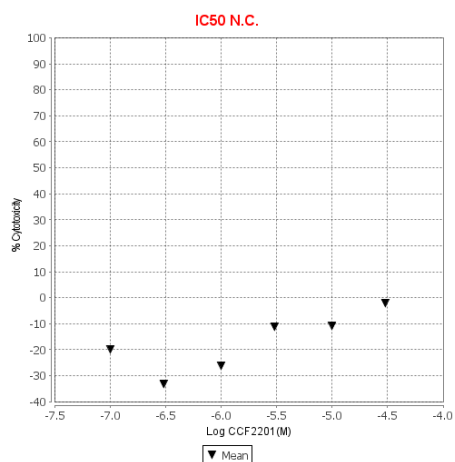


Figure 3. CCF2201 on cytotoxicity profile (H4-II-E-C3)

cytotoxicity profile (HEK-293)									
100065480-1	CCF2201	N.C.	n/a	1.0E-07 M	-16.0	-9.9	-12.9		
				3.0E-07 M	-25.0	-17.8	-21.4		
				1.0E-06 M	-16.2	-11.8	-14.0		
				3.0E-06 M	-3.6	-11.5	-7.6		
				1.0E-05 M	-5.0	-4.5	-4.8		
				3.0E-05 M	5.9	3.5	4.7		

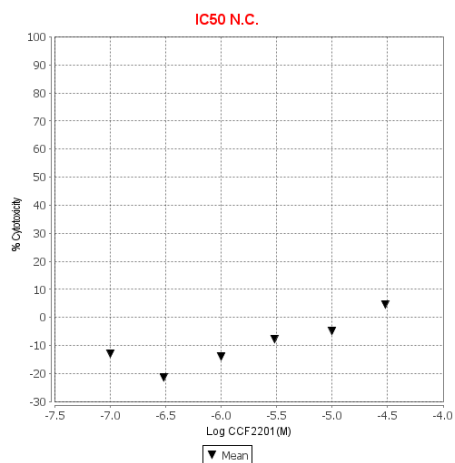


Figure 4. CCF2201 on cytotoxicity profile (HEK-293)

Compound I.D.	Client Compound I.D.	IC ₅₀ (M)	nH	Test Concentration	% Cytotoxicity			Flags	
					1 st	2 nd	Mean	1 st	2 nd
cytotoxicity profile (HepG2)									
100065480-1	CCF2201	N.C.	n/a	1.0E-07 M	-24.0	-21.2	-22.6		
				3.0E-07 M	-29.1	-27.5	-28.3		
				1.0E-06 M	-25.7	-18.1	-21.9		
				3.0E-06 M	-24.0	-14.3	-19.1		
				1.0E-05 M	-17.7	-15.1	-16.4		
				3.0E-05 M	-15.0	-12.0	-13.5		

100

90

80

70

60

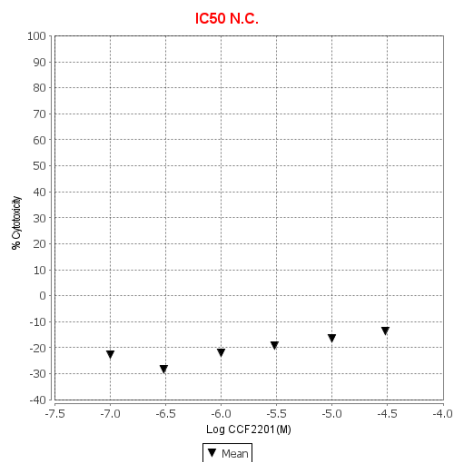


Figure 5. CCF2201 on cytotoxicity profile (HepG2)

cytotoxicity profile (HK2)									
100065480-1	CCF2201	> 3.0E-05	n/a	1.0E-07 M	-10.9	-6.2	-8.6		
				3.0E-07 M	-9.5	-13.5	-11.5		
				1.0E-06 M	-12.6	-3.9	-8.2		
				3.0E-06 M	2.2	-3.5	-0.6		
				1.0E-05 M	8.7	15.1	11.9		
				3.0E-05 M	26.2	30.8	28.5		

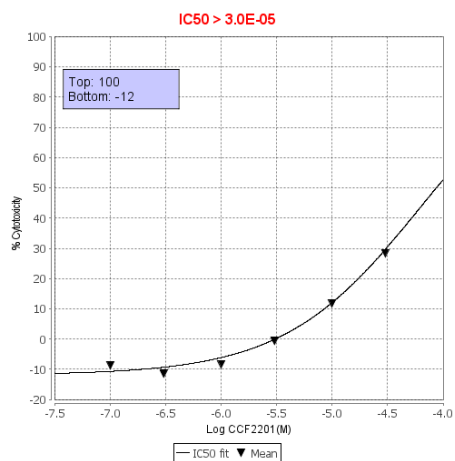


Figure 6. CCF2201 on cytotoxicity profile (HK2)

Compound I.D.	Client Compound I.D.	IC ₅₀ (M)	nH	Test Concentration	% Cytotoxicity			Flags	
					1 st	2 nd	Mean	1 st	2 nd
cytotoxicity profile (HUV-EC-C)									
100065480-1	CCF2201	> 3.0E-05	n/a	1.0E-07 M	-13.6	-8.6	-11.1		
				3.0E-07 M	-6.5	-8.9	-7.7		
				1.0E-06 M	-3.3	-9.9	-6.6		
				3.0E-06 M	3.1	-1.0	1.1		
				1.0E-05 M	6.1	8.3	7.2		
				3.0E-05 M	39.7	38.5	39.1		

IC50 > 3.0E-05

Top: 100

Bottom: -8

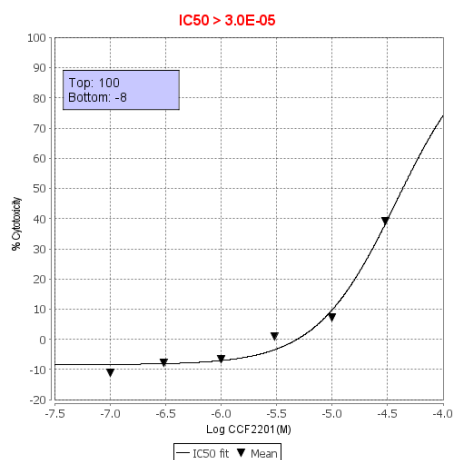


Figure 7. CCF2201 on cytotoxicity profile (HUV-EC-C)

cytotoxicity profile (Jurkat)									
100065480-1	CCF2201	N.C.	n/a	1.0E-07 M	-17.4	-19.0	-18.2		
				3.0E-07 M	-29.7	-34.4	-32.1		
				1.0E-06 M	-29.4	-22.8	-26.1		
				3.0E-06 M	-22.6	-17.7	-20.2		
				1.0E-05 M	-17.4	-10.7	-14.1		
				3.0E-05 M	5.7	3.9	4.8		

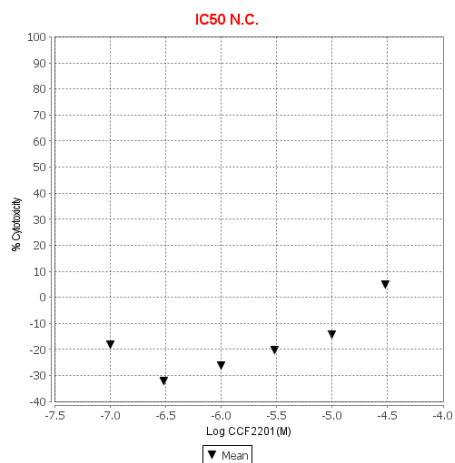


Figure 8. CCF2201 on cytotoxicity profile (Jurkat)

Compound I.D.	Client Compound I.D.	IC ₅₀ (M)	nH	Test Concentration	% Cytotoxicity			Flags	
					1 st	2 nd	Mean	1 st	2 nd
cytotoxicity profile (MRC-5)									
100065480-1	CCF2201	N.C.	n/a	1.0E-07 M	-13.9	-10.9	-12.4		
				3.0E-07 M	-26.1	-27.5	-26.8		
				1.0E-06 M	-22.6	-13.8	-18.2		
				3.0E-06 M	-11.0	-10.2	-10.6		
				1.0E-05 M	-18.3	-6.1	-12.2		
				3.0E-05 M	-5.6	-2.1	-3.9		

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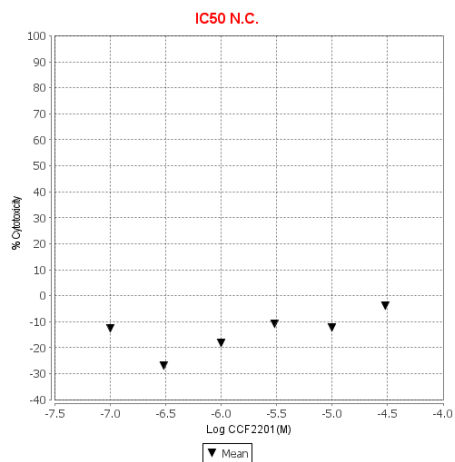


Figure 9. CCF2201 on cytotoxicity profile (MRC-5)

cytotoxicity profile (Neuro-2a)									
100065480-1	CCF2201	N.C.	n/a	1.0E-07 M	-17.9	-7.2	-12.6		
				3.0E-07 M	-23.1	-16.7	-19.9		
				1.0E-06 M	-30.3	-17.1	-23.7		
				3.0E-06 M	-19.1	-9.9	-14.5		
				1.0E-05 M	-21.3	1.4	-9.9		
				3.0E-05 M	-4.0	-1.7	-2.9		

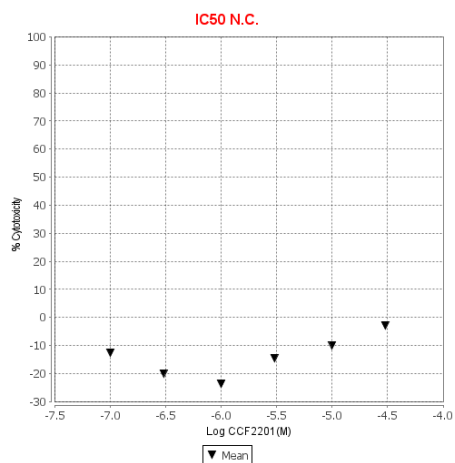


Figure 10. CCF2201 on cytotoxicity profile (Neuro-2a)

Compound I.D.	Client Compound I.D.	IC ₅₀ (M)	nH	Test Concentration	% Cytotoxicity			Flags	
					1 st	2 nd	Mean	1 st	2 nd
cytotoxicity profile (NIH/3T3)									
100065480-1	CCF2201	2.3E-05 M	n/a	1.0E-07 M	-3.3	-4.0	-3.7		
				3.0E-07 M	-11.7	-12.2	-11.9		
				1.0E-06 M	-11.2	-8.9	-10.0		
				3.0E-06 M	-5.4	-7.6	-6.5		
				1.0E-05 M	-3.4	0.3	-1.5		
				3.0E-05 M	77.1	60.3	68.7		

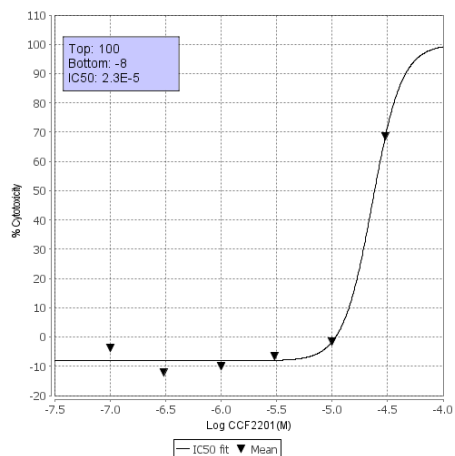


Figure 11. CCF2201 on cytotoxicity profile (NIH/3T3)

cytotoxicity profile (NRK-52E)									
100065480-1	CCF2201	N.C.	n/a	1.0E-07 M	-23.3	-20.8	-22.1		
				3.0E-07 M	-27.6	-23.6	-25.6		
				1.0E-06 M	-34.8	-22.2	-28.5		
				3.0E-06 M	-21.0	-17.7	-19.4		
				1.0E-05 M	-19.1	-17.7	-18.4		
				3.0E-05 M	-8.2	-10.7	-9.5		

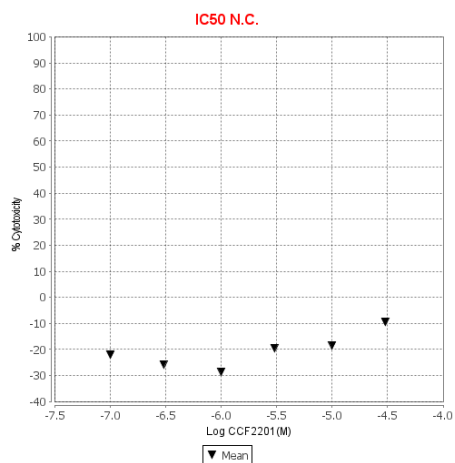


Figure 12. CCF2201 on cytotoxicity profile (NRK-52E)

Compound I.D.	Client Compound I.D.	IC ₅₀ (M)	nH	Test Concentration	% Cytotoxicity			Flags	
					1 st	2 nd	Mean	1 st	2 nd
cytotoxicity profile (SH-SY5Y)									
100065480-1	CCF2201	N.C.	n/a	1.0E-07 M	-21.2	-19.4	-20.3		
				3.0E-07 M	-21.9	-13.3	-17.6		
				1.0E-06 M	-18.6	-15.0	-16.8		
				3.0E-06 M	-12.7	-10.2	-11.5		
				1.0E-05 M	-8.9	-2.5	-5.7		
				3.0E-05 M	3.0	7.0	5.0		

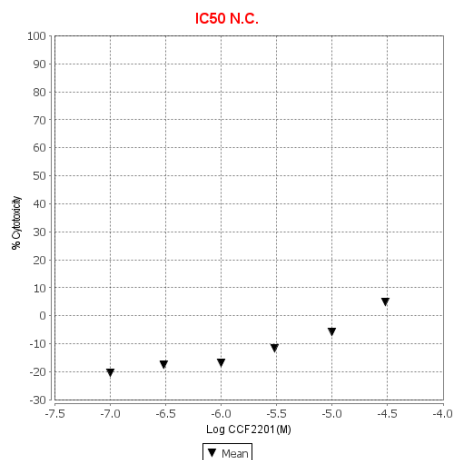


Figure 13. CCF2201 on cytotoxicity profile (SH-SY5Y)

cytotoxicity profile (SK-N-SH)									
100065480-1	CCF2201	N.C.	n/a	1.0E-07 M	-18.3	-16.6	-17.5		
				3.0E-07 M	-23.0	-19.8	-21.4		
				1.0E-06 M	-16.3	-14.5	-15.4		
				3.0E-06 M	-15.3	-11.0	-13.1		
				1.0E-05 M	-8.6	-11.7	-10.1		
				3.0E-05 M	-8.7	-5.7	-7.2		

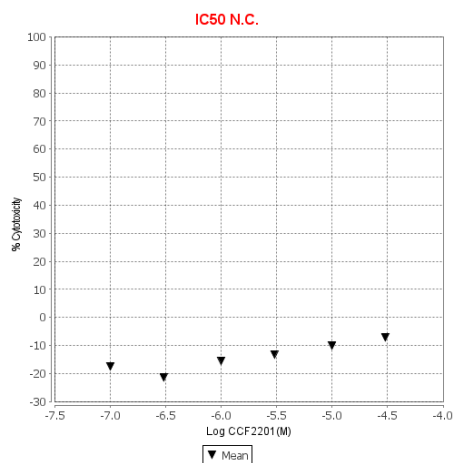


Figure 14. CCF2201 on cytotoxicity profile (SK-N-SH)

N.C.: IC₅₀ value not calculable. Concentration-response curve shows less than 25% effect at the highest validated testing concentration.

> Conc.: IC₅₀ value above the highest test concentration. Concentration-response curve shows less than 50 % effect at the highest validated testing concentration

7.1.2. Reference Compound Results

Compound I.D.	IC ₅₀ (M)	nH
cytotoxicity profile (BJ)		
tamoxifen	4.1E-06 M	n/a
cytotoxicity profile (H4-II-E-C3)		
tamoxifen	1.0E-05 M	n/a
cytotoxicity profile (HEK-293)		
tamoxifen	1.4E-05 M	n/a
cytotoxicity profile (HepG2)		
tamoxifen	5.8E-06 M	n/a
cytotoxicity profile (HK2)		
tamoxifen	5.9E-06 M	n/a
cytotoxicity profile (HUV-EC-C)		
tamoxifen	8.3E-06 M	n/a
cytotoxicity profile (Jurkat)		
tamoxifen	7.7E-06 M	n/a
cytotoxicity profile (MRC-5)		
tamoxifen	1.2E-05 M	n/a
cytotoxicity profile (Neuro-2a)		
tamoxifen	9.4E-06 M	n/a
cytotoxicity profile (NIH/3T3)		
tamoxifen	1.1E-05 M	n/a
cytotoxicity profile (NRK-52E)		
tamoxifen	4.4E-06 M	n/a
cytotoxicity profile (SH-SY5Y)		
tamoxifen	5.5E-06 M	n/a
cytotoxicity profile (SK-N-SH)		
tamoxifen	3.8E-06 M	n/a

8. RESULTS INTERPRETATION GUIDE

In Vitro Pharmacology

Results showing an inhibition (or stimulation for assays run in basal conditions) higher than 50% are considered to represent significant effects of the test compounds. 50% is the most common cut-off value for further investigation (determination of IC_{50} or EC_{50} values from concentration-response curves) that we would recommend.

Results showing an inhibition (or stimulation) between 25% and 50% are indicative of weak to moderate effects (in most assays, they should be confirmed by further testing as they are within a range where more inter-experimental variability can occur).

Results showing an inhibition (or stimulation) lower than 25% are not considered significant and mostly attributable to variability of the signal around the control level.

Low to moderate negative values have no real meaning and are attributable to variability of the signal around the control level. High negative values ($\geq 50\%$) that are sometimes obtained with high concentrations of test compounds are generally attributable to non-specific effects of the test compounds in the assays. On rare occasion they could suggest an allosteric effect of the test compound.

9. MATERIALS AND METHODS

9.1. Experimental Conditions

Minor variations to the experimental protocol described below may have occurred during the testing, they have no impact on the quality of the results obtained.

9.1.1. *In Vitro* Pharmacology: Cytotoxicity Profile Assays

Assay	Source	Substrate/Stimulus/Tracer	Incubation	Measured Component	Detection Method	Bibl.
Specialized cellular assays						
cytotoxicity profile (BJ)	human foreskin fibroblasts (BJ)	Beetle luciferin + ATP	48 h 37°C	Oxyluciferin + AMP	Luminescence	1144
cytotoxicity profile (H4-II-E-C3)	rat hepatoma cells (H-4-II-E)	Beetle luciferin + ATP	48 h 37°C	Oxyluciferin + AMP	Luminescence	1144
cytotoxicity profile (HEK-293)	human embryonic kidney cells (HEK-293)	Beetle luciferin + ATP	48 h 37°C	Oxyluciferin + AMP	Luminescence	1144
cytotoxicity profile (HepG2)	human hepatocellular carcinoma cells (HepG2)	Beetle luciferin + ATP	48 h 37°C	Oxyluciferin + AMP	Luminescence	1144
cytotoxicity profile (HK2)	human renal glomeruli mesangial cells (HK2)	Beetle luciferin + ATP	48 h 37°C	Oxyluciferin + AMP	Luminescence	1144
cytotoxicity profile (HUV-EC-C)	human vascular endothelial cells (HUV-EC-C)	Beetle luciferin + ATP	48 h 37°C	Oxyluciferin + AMP	Luminescence	1144
cytotoxicity profile (Jurkat)	human T cells leukemia (Jurkat)	Beetle luciferin + ATP	48 h 37°C	Oxyluciferin + AMP	Luminescence	1144
cytotoxicity profile (MRC-5)	human lung fibroblasts (MRC-5)	Beetle luciferin + ATP	48 h 37°C	Oxyluciferin + AMP	Luminescence	1144
cytotoxicity profile (Neuro-2a)	mouse neuroblastoma cells (Neuro-2a)	Beetle luciferin + ATP	48 h 37°C	Oxyluciferin + AMP	Luminescence	1144
cytotoxicity profile (NIH/3T3)	mouse embryonic fibroblasts (NIH/3T3)	Beetle luciferin + ATP	48 h 37°C	Oxyluciferin + AMP	Luminescence	1144
cytotoxicity profile (NRK-52E)	rat kidney proximal tubule cells (NRK-52E)	Beetle luciferin + ATP	48 h 37°C	Oxyluciferin + AMP	Luminescence	1144
cytotoxicity profile (SH-SY5Y)	human neuroblastoma cells (SH-SY5Y)	Beetle luciferin + ATP	48 h 37°C	Oxyluciferin + AMP	Luminescence	1144
cytotoxicity profile (SK-N-SH)	human neuroblastoma cells (SK-S-SH)	Beetle luciferin + ATP	48 h 37°C	Oxyluciferin + AMP	Luminescence	1144

9.2. Analysis and expression of results

9.2.1. *In Vitro* Pharmacology: Cytotoxicity Profile Assays

The results are expressed as a percent of control specific activity

$$\frac{\text{measured specific activity}}{\text{control specific activity}} * 100$$

and as a percent inhibition of control specific activity

$$100 - \left(\frac{\text{measured specific activity}}{\text{control specific activity}} * 100 \right)$$

obtained in the presence of CCF2201.

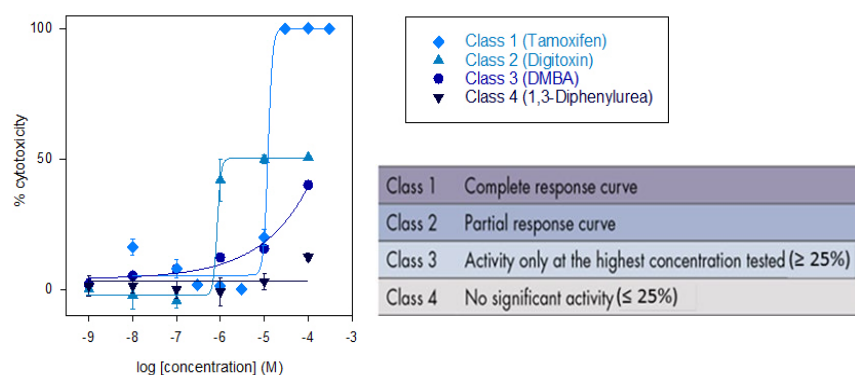
The IC₅₀ values (concentration causing a half-maximal inhibition of control specific activity), EC₅₀ values (concentration producing a half-maximal increase in control basal activity), and Hill coefficients (nH) were determined by non-linear regression analysis of the inhibition/concentration-response curves generated with mean replicate values using Hill equation curve fitting

$$Y = D + \left[\frac{A - D}{1 + (C/C_{50})^{nH}} \right]$$

where Y = specific activity, A = left asymptote of the curve, D = right asymptote of the curve, C = compound concentration, C₅₀ = IC₅₀ or EC₅₀, and nH = slope factor.

This analysis was performed using software developed at Cerep (Hill software) and validated by comparison with data generated by the commercial software SigmaPlot® 4.0 for Windows® (© 1997 by SPSS Inc.).

Definition of cytotoxicity classes:



| 10. BIBLIOGRAPHY

1144. Xia M. et al. (2008), *Environ. Health Perspect.*, 116:284-291

Appendix 9

Title:The effect of compounds on tail bleeding in Sprague-Dawley Rats
Quote No.:PH-CCF-IVP-2023-02

Pharmaron Study #	Report Submitted	Analysis
IVP-CCF-Tail bleeding-04132023	2023.07.4	In Vivo Pharmacology

In Life Design

Species/strain: Sprague Dawley (SD) rats
Supplier: Vital River Laboratory Animal Technology Co.
Age: 6 to 8 weeks
Body Weight: 246-265g
Animal Number: 8 animals / Group, Total 40 animals
Date to Start(In Vivo): 6/28/2023
Date to End(In Vivo): 6/28/2023

Group	Treatment	Dose(mg/kg)	Dosing Volume (mL/kg)	Concentration (mg/mL)	Route	Frequency	Animals
1	Vehicle	——	5	——	PO	Single dose	8
2	Clepidogrel	10	5	2	PO		8
3	CCF104	10	5	2	PO		8
4	CCF104	30	5	6	PO		8
5	CCF104	100	5	20	PO		8
Note: The compound CCF104 and Clepidogrel was given 3 h before the tail bleeding Modelmodel							

Notebook #: A19560
Study director Zhao Ma
In vivo scientists: Qing li, Jian Yin, Rufang Gao, Yang Yang
Study and reported by: Zhao Ma
Report QC by: Feng Liang

Department Head Jingqi Huang

Sunday	Monday	Tuesday	Wednesday	Thursday	Friday	Saturday
2023.06.25	2023.06.26	2023.06.27	2023.06.28	2023.06.29	2023.06.30	2023.07.01
			Day1			
			1.Body weight 2.Group 3.Dose 4.Tail bleeding			

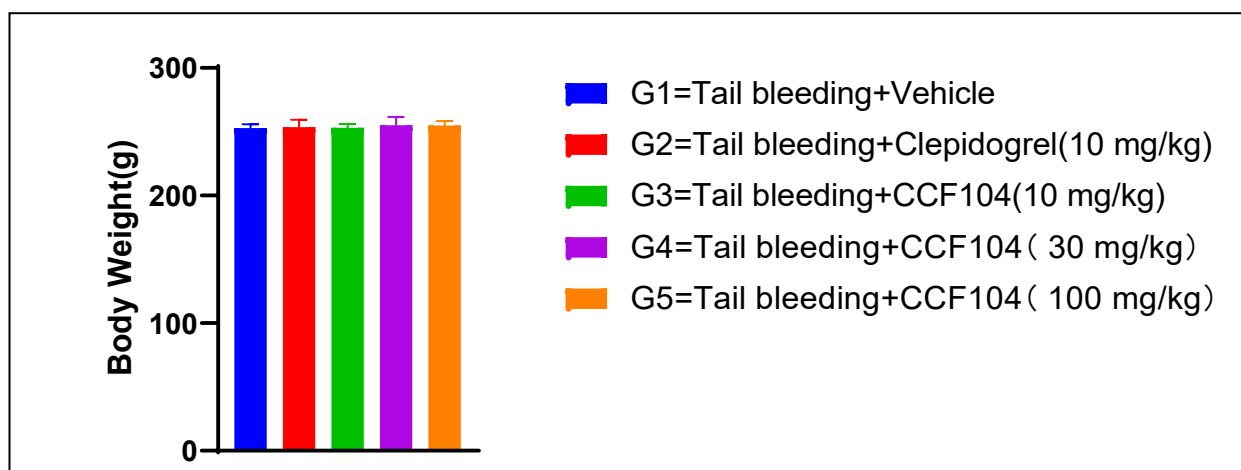
PH-CCF-IVP-2023-02		
Group	ID	Body weight (g)
G1=Tail bleeding+Vehicle	3	250
	9	251
	16	251
	21	258
	22	255
	32	249
	42	255
	49	253
G2=Tail bleeding+Clepidogrel(10 mg/kg)	10	254
	13	253
	20	248
	23	254
	26	253
	31	246
	34	256
	46	265
G3=Tail bleeding+CCF104(10 mg/kg)	2	248
	11	253
	12	253
	36	255
	40	252
	41	251
	47	257
	50	256
G4=Tail bleeding+CCF104 (30 mg/kg)	6	260
	8	254
	15	257
	18	248
	25	250
	33	260
	39	265
	45	246
G5=Tail bleeding+CCF104 (100 mg/kg)	1	261
	5	258
	7	253
	14	249
	17	256
	19	254
	27	253
	48	255

Mean	Body weight (g)
G1=Tail bleeding+Vehicle	253
G2=Tail bleeding+Clepidogrel(10 mg/kg)	254
G3=Tail bleeding+CCF104(10 mg/kg)	253
G4=Tail bleeding+CCF104 (30 mg/kg)	255

G5=Tail bleeding+CCF104 (100 mg/kg)	255
-------------------------------------	-----

SD	Body weight (g)
G1=Tail bleeding+Vehicle	3
G2=Tail bleeding+Clepidogrel(10 mg/kg)	6
G3=Tail bleeding+CCF104(10 mg/kg)	3
G4=Tail bleeding+CCF104 (30 mg/kg)	7
G5=Tail bleeding+CCF104 (100 mg/kg)	4

Mean±SD	Body weight (g)
G1=Tail bleeding+Vehicle	253±3
G2=Tail bleeding+Clepidogrel(10 mg/kg)	254±6
G3=Tail bleeding+CCF104(10 mg/kg)	253±3
G4=Tail bleeding+CCF104 (30 mg/kg)	255±7
G5=Tail bleeding+CCF104 (100 mg/kg)	255±4
Compared with Group 1, statistical analysis was performed using the T.TEST method, *P<0.05, **P<0.01.	



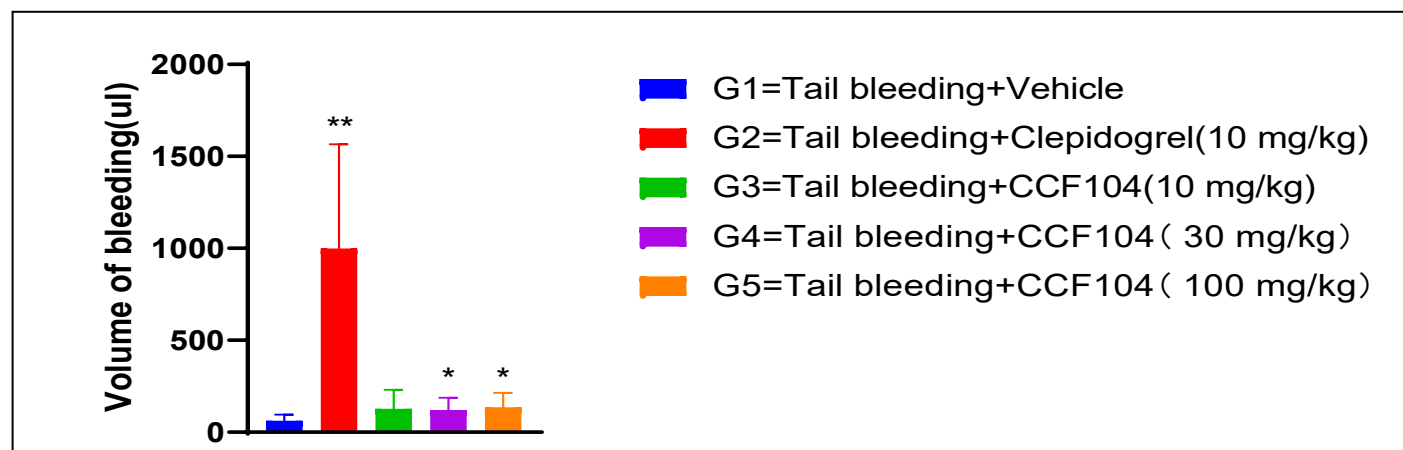
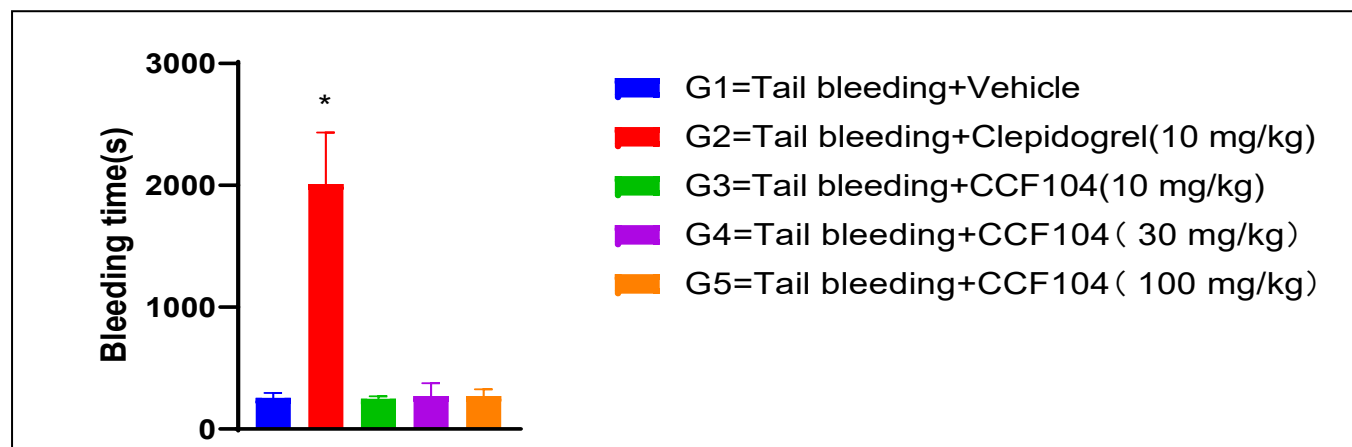
PH-CCF-IVP-2023-02			
Group	ID	Bleeding time(s)	Volume of bleeding(ul)
G1=Tail bleeding+Vehicle	3	240	115
	9	240	81
	16	240	45
	21	360	25
	22	240	65
	32	240	99
	42	240	45
	49	240	21
G2=Tail bleeding+Clepidogrel(10 mg/kg)	10	2160	1935
	13	2160	915
	20	2160	901
	23	2160	847
	26	960	331
	31	2160	357
	34	2160	1022
	46	2160	1687
G3=Tail bleeding+CCF104(10 mg/kg)	2	240	44
	11	300	122
	12	240	18
	36	240	271
	40	240	222
	41	240	51
	47	240	41
	50	240	245
G4=Tail bleeding+CCF104 (30 mg/kg)	6	240	22
	8	240	201
	15	120	145
	18	360	125
	25	480	171
	33	240	177
	39	240	37
	45	240	89
G5=Tail bleeding+CCF104 (100 mg/kg)	1	360	37
	5	240	132
	7	240	92
	14	240	47
	17	360	264
	19	240	196
	27	240	145
	48	240	178

Mean	Bleeding time(s)	Volume of bleeding(ul)
G1=Tail bleeding+Vehicle	255	62
G2=Tail bleeding+Clepidogrel(10 mg/kg)	2010	999
G3=Tail bleeding+CCF104(10 mg/kg)	248	127
G4=Tail bleeding+CCF104 (30 mg/kg)	270	121
G5=Tail bleeding+CCF104 (100 mg/kg)	270	136

SD	Bleeding time(s)	Volume of bleeding(ul)
G1=Tail bleeding+Vehicle	42	34
G2=Tail bleeding+Clepidogrel(10 mg/kg)	424	567
G3=Tail bleeding+CCF104(10 mg/kg)	21	104
G4=Tail bleeding+CCF104 (30 mg/kg)	106	66
G5=Tail bleeding+CCF104 (100 mg/kg)	56	77

Mean±SD	Bleeding time(s)	Volume of bleeding(ul)
G1=Tail bleeding+Vehicle	255±42	62±34
G2=Tail bleeding+Clepidogrel(10 mg/kg)	2010±424**	999±567**
G3=Tail bleeding+CCF104(10 mg/kg)	248±21	127±104
G4=Tail bleeding+CCF104 (30 mg/kg)	270±106	121±66*
G5=Tail bleeding+CCF104 (100 mg/kg)	270±56	136±77*

Note: The timing is stopped when the tail blears for more than 36 minutes; Compared with Group 1, statistical analysis was performed using the T.TEST method, *P<0.05, **P<0.01.



Treatment	Preparation	Concentration (mg/mL)	Prepared Frequency
Vehicle	5% DMSO+ 95% (10% hydroxypropyl -b-cyclodextrin in saline)	—	Prepared freshly
CCF104 @ 10 mg/kg	Dissolve 13.99 mg of CCF104 (Purity: 98.0%) in 0.343 mL of DMSO, then added 6.512 mL of 10% hydroxypropyl -b-cyclodextrin with vortex to obtain solution. The final concentration was 2 mg/mL.	2	
CCF104 @ 30 mg/kg	Dissolve 38.42 mg of CCF104 (Purity: 98.0%) in 0.314 mL of DMSO, then added 5.961 mL of 10% hydroxypropyl -b-cyclodextrin with vortex to obtain solution. The final concentration was 6 mg/mL.	6	
CCF104 @ 100 mg/kg	Dissolve 126.43 mg of CCF104 (Purity: 98.0%) in 0.310 mL of DMSO, then added 5.885 mL of 10% hydroxypropyl -b-cyclodextrin with vortex to obtain solution. The final concentration was 20 mg/mL.	20	
Clepidogrel @ 10 mg/kg	Dissolve 14.36 mg of Clepidogrel in 0.718 mL of DMSO, then added 6.462 mL of 20% SBE-β-CD with vortex to obtain suspension. The final concentration was 2 mg/mL.	2	

Vehicle	Supplier	Lot#
DMSO	Sigma-Aldrich	220105016
hydroxypropyl -b-cyclodextrin	Shanghai yuanye Bio-Technology Co., Ltd	O22HS198750
SBE-β-CD	Shandong Binzhou Zhiyuan Bio-Technology Co., Ltd.	HP20220223

Test Compound	Supplier	Purity	lot number
CCF104	Sponsor	>98.0%	120S

Appendix 10

Title:PD and PK study in Sprague-Dawley Rats
Quote No.:PH-CCF-IVP-2023-01

Pharmaron Study #	Report Submitted	Analysis
IVP-CCF-PDPK-04132023	6/8/2023	In Vivo Pharmacology

In Life Design

Species/strain: Sprague Dawley (SD) rats
Supplier: SPF (Beijing) Laboratory Animal Technology Co. Ltd.
Age: 6 to 8 weeks
Body Weight: 228-257 g
Animal Number: 6 animals / Group, Total 24 animals
Date to Start(In Vivo): 6/1/2023
Date to End(In Vivo): 6/2/2023

Group	Treatment	Concentration (mg/mL)	Dosing Volume (mL/kg)	Dose(mg/kg)	Route	Frequency	Animals	Sample Collection
1	Vehicle	——	5	——	PO	Single dose	6	1#-3# For PK Analysis: 0.25, 0.5, 1, 2, 4, 6, 8 h post dose 4#-6# For Coagulation Analysis: 4h, 8h post dose
2	CCF104	2	5	10	PO		6	
3	CCF104	6	5	30	PO		6	
4	CCF104	20	5	100	PO		6	

Notebook #: A17377
Study director Lixia Hao
In vivo scientists: Xin Qian, Yuying Kang, Bowen Zheng, Yanan Zhang, Xiaolan Zhang, Qing Li, Rufang Gao, Jian Yin
Study and reported by: Lixia Hao
Report QC by: Feng Liang

Department Head Jingqi Huang

Sunday	Monday	Tuesday	Wednesday	Thursday	Friday	Saturday
2023.05.28	2023.05.29	2023.05.30	2023.05.31	2023.06.01	2023.06.02	2023.06.03
				Day1	Day2	
				1.Body weight 2.Group 3.Clinical observation 4.Dose 5.Clinical observation 6.Plasma samples collection (1#-3# For PK Analysis: 0.25, 0.5, 1, 2, 4, 6, 8 h post dose. 4#-6# For Coagulation Analysis: 4h, 8h post dose)	1.Body weight 2.Plasma samples collection (1#-3# For PK Analysis: 24h post dose. 4#-6# For Coagulation Analysis: 24 h post dose)	

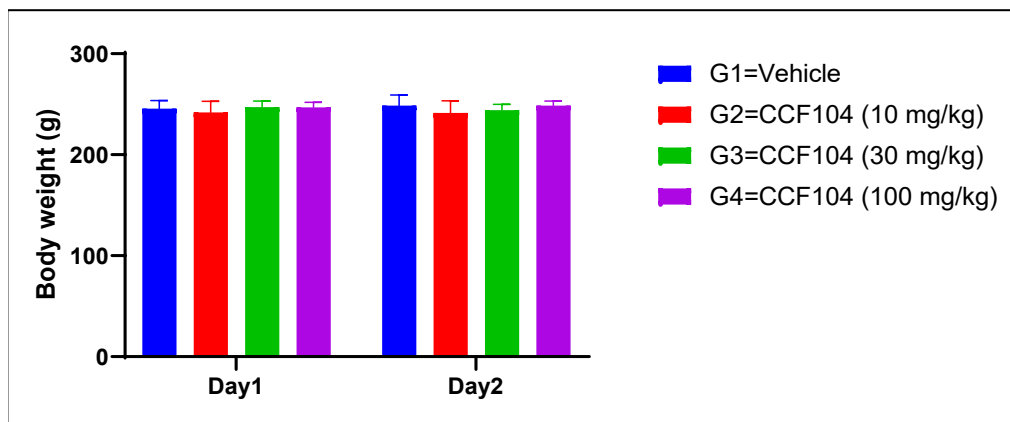
PH-CCF-IVP-2023-01			
Group	ID	Body weight (g)	
		Day1	Day2
G1=Vehicle	2	241	252
	3	257	263
	7	249	258
	9	251	242
	20	237	239
	24	238	237
G2=CCF104 (10 mg/kg)	8	251	246
	12	229	222
	13	251	250
	18	241	246
	22	228	231
	25	251	253
G3=CCF104 (30 mg/kg)	1	257	247
	11	252	246
	16	242	233
	21	242	246
	23	243	242
	27	246	250
G4=CCF104 (100 mg/kg)	4	243	241
	6	249	250
	10	256	253
	14	246	252
	15	243	245
	17	244	250

Mean	Body weight (g)	
	Day1	Day2
G1=Vehicle	246	246
G2=CCF104 (10 mg/kg)	242	241
G3=CCF104 (30 mg/kg)	247	244
G4=CCF104 (100 mg/kg)	247	249

SD	Body weight (g)	
	Day1	Day2
G1=Vehicle	8	11
G2=CCF104 (10 mg/kg)	11	12
G3=CCF104 (30 mg/kg)	6	6
G4=CCF104 (100 mg/kg)	5	5

Mean±SD	Body weight (g)	
	Day1	Day2
G1=Vehicle	246±8	246±11
G2=CCF104 (10 mg/kg)	242±11	241±12

G3=CCF104 (30 mg/kg)	247±6	244±6
G4=CCF104 (100 mg/kg)	247±5	249±5
Compared with Group1 , Statistical analysis was performed separately by one-way ANOVA, * P<0.05, **P<0.01.		



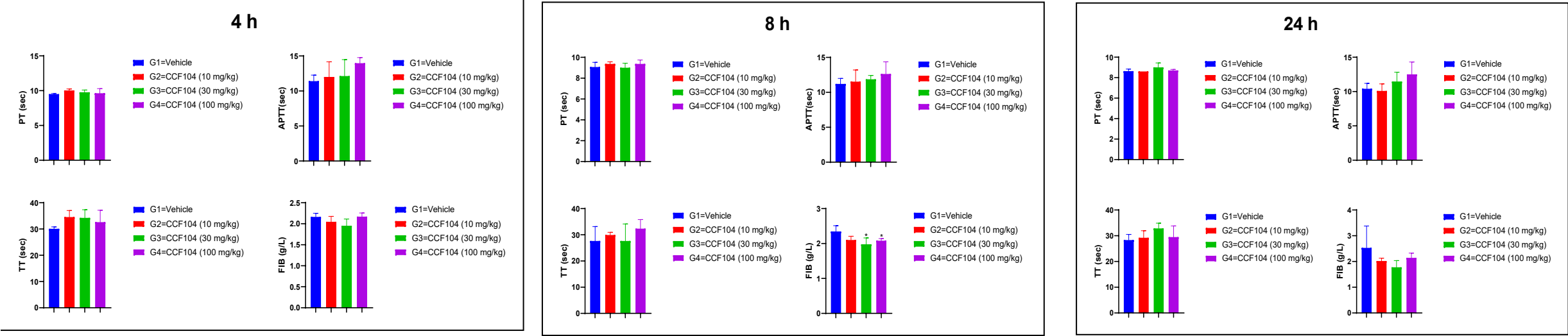
Group	ID	4 h				8 h				24 h			
		PT (sec)	APTT(sec)	TT (sec)	FIB (g/L)	PT (sec)	APTT(sec)	TT (sec)	FIB (g/L)	PT (sec)	APTT(sec)	TT (sec)	FIB (g/L)
G1=Vehicle	9	9.4	12.0	30.5	2.26	8.6	10.3	22.0	2.52	8.4	11.2	26.1	3.51
	20	9.5	11.8	29.2	2.10	9.1	11.5	27.9	2.33	8.8	10.4	28.3	2.04
	24	9.6	10.4	30.5	2.13	9.5	11.8	33.0	2.19	8.7	9.6	30.5	2.04
G2=CCF104 (10 mg/kg)	18	10.1	10.9	32.1	2.19	9.6	12.5	31.1	2.22	8.6	9.2	28.3	2.13
	22	9.8	10.6	34.4	1.94	9.2	9.6	29.1	2.07	8.6	9.9	32.3	1.91
	25	10.2	14.5	37.1	2.01	9.3	12.5	29.5	2.01	8.6	11.2	27.1	2.01
G3=CCF104 (30 mg/kg)	21	10.0	11.2	36.6	1.82	9.5	11.6	34.8	1.86	9.5	12.2	35.2	1.49
	23	9.4	10.4	30.7	1.91	8.7	12.5	22.1	1.89	8.7	9.9	31.6	1.82
	27	9.9	14.8	35.4	2.13	8.8	11.5	26.0	2.19	8.8	12.3	31.7	2.01
G4=CCF104 (100mg/kg)	14	10.4	14.9	36.3	2.07	9.8	14.6	35.3	2.01	8.7	14.5	24.7	1.96
	15	9.2	13.5	27.5	2.22	9.1	11.3	28.5	2.10	8.8	12.0	33.2	2.33
	17	9.3	13.5	34.0	2.22	9.2	12.0	33.2	2.13	8.6	11.0	30.5	2.13

Mean	4 h				8 h				24 h			
	PT (sec)	APTT(sec)	TT (sec)	FIB (g/L)	PT (sec)	APTT(sec)	TT (sec)	FIB (g/L)	PT (sec)	APTT(sec)	TT (sec)	FIB (g/L)
G1=Vehicle	9.5	11.4	30.1	2.16	9.1	11.2	27.6	2.35	8.6	10.4	28.3	2.5
G2=CCF104 (10 mg/kg)	10.0	12.0	34.5	2.05	9.4	11.5	29.9	2.10	8.6	10.1	29.2	2.0
G3=CCF104 (30 mg/kg)	9.8	12.1	34.2	1.95	9.0	11.9	27.6	1.98	9.0	11.5	32.8	1.8
G4=CCF104 (100 mg/kg)	9.6	14.0	32.6	2.17	9.4	12.6	32.3	2.08	8.7	12.5	29.5	2.1

SD	4 h				8 h				24 h			
	PT (sec)	APTT(sec)	TT (sec)	FIB (g/L)	PT (sec)	APTT(sec)	TT (sec)	FIB (g/L)	PT (sec)	APTT(sec)	TT (sec)	FIB (g/L)
G1=Vehicle	0.1	0.9	0.8	0.09	0.5	0.8	5.5	0.17	0.2	0.8	2.2	0.8
G2=CCF104 (10 mg/kg)	0.2	2.2	2.5	0.13	0.2	1.7	1.1	0.11	0.0	1.0	2.7	0.1
G3=CCF104 (30 mg/kg)	0.3	2.3	3.1	0.16	0.4	0.6	6.5	0.18	0.4	1.4	2.1	0.3
G4=CCF104 (100 mg/kg)	0.7	0.8	4.6	0.09	0.4	1.7	3.5	0.06	0.1	1.8	4.3	0.2

Mean±SD	4 h				8 h				24 h			
	PT (sec)	APTT(sec)	TT (sec)	FIB (g/L)	PT (sec)	APTT(sec)	TT (sec)	FIB (g/L)	PT (sec)	APTT(sec)	TT (sec)	FIB (g/L)
G1=Vehicle	9.5±0.1	11.4±0.9	30.1±0.8	2.16±0.09	9.1±0.5	11.2±0.8	27.6±5.5	2.35±0.17	8.6±0.2	10.4±0.8	28.3±2.2	2.5±0.8
G2=CCF104 (10 mg/kg)	10.0±0.2	12.0±2.2	34.5±2.5	2.05±0.13	9.4±0.2	11.5±1.7	29.9±1.1	2.10±0.11	8.6±0.0	10.1±1.0	29.2±2.7	2.0±0.1
G3=CCF104 (30 mg/kg)	9.8±0.3	12.1±2.3	34.2±3.1	1.95±0.16	9.0±0.4	11.9±0.6	27.6±6.5	1.98±0.18*	9.0±0.4	11.5±1.4	32.8±2.1	1.8±0.3
G4=CCF104 (100 mg/kg)	9.6±0.7	14.0±0.8	32.6±4.6	2.17±0.09	9.4±0.4	12.6±1.7	32.3±3.5	2.08±0.06*	8.7±0.1	12.5±1.8	29.5±4.3	2.1±0.2

Note: Compared with Group1 , Statistical analysis was performed separately by one-way ANOVA, * P<0.05, **P<0.01.



Parameters for Hematology Analysis			
PT	APTT	TT	FIB
Prothrombin time	Activated Partial	Thrombin Time	Fibrinogen Activity

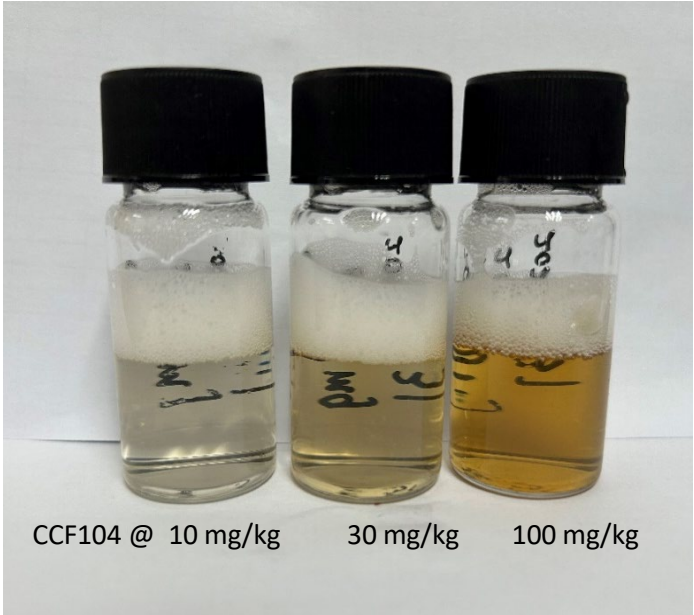
PH-CCF-IVP-2023-01			
Group	ID	Clinical observation	
		Day1	Day2
G1=Vehicle	2	-	-
	3	-	-
	7	-	-
	9	-	-
	20	-	-
	24	-	-
G2=CCF104 (10 mg/kg)	8	-	-
	12	-	-
	13	-	-
	18	-	-
	22	-	-
	25	-	-
G3=CCF104 (30 mg/kg)	1	-	-
	11	-	-
	16	-	-
	21	-	-
	23	-	-
	27	-	-
G4=CCF104 (100 mg/kg)	4	-	-
	6	-	-
	10	-	-
	14	-	-
	15	-	-
	17	-	-

Note: "-" means that no obvious abnormality was observed during the experiment.

Treatment	Preparation	Concentration (mg/mL)	Prepared Frequency
Vehicle	5% DMSO+ 95% (10% hydroxypropyl -b-cyclodextrin in saline)	—	Prepared freshly
CCF104 @ 10 mg/kg	Dissolve 21.80 mg of CCF104 (Purity: 98.0%) in 0.534 mL of DMSO, then added 10.148 mL of 10% hydroxypropyl -b-cyclodextrin with vortex to obtain solution. The final concentration was 2 mg/mL.	2	
CCF104 @ 30 mg/kg	Dissolve 64.79 mg of CCF104 (Purity: 98.0%) in 0.529 mL of DMSO, then added 10.053 mL of 10% hydroxypropyl -b-cyclodextrin with vortex to obtain solution. The final concentration was 6 mg/mL.	6	
CCF104 @ 100 mg/kg	Dissolve 215.88 mg of CCF104 (Purity: 98.0%) in 0.529 mL of DMSO, then added 10.049 mL of 10% hydroxypropyl -b-cyclodextrin with vortex to obtain solution. The final concentration was 20 mg/mL.	20	

Vehicle	Supplier	Lot#
DMSO	Sigma-Aldrich	220105016
hydroxypropyl -b-cyclodextrin	Shanghai yuanye Bio-Technology Co., Ltd	O22HS198750

Test Compound	Supplier	Purity	lot number
CCF104	Sponsor	>98.0%	120S



Appendix 11

Title:PD and PK study in Sprague-Dawley Rats
Quote No.:PH-CCF-IVP-2023-03

Pharmaron Study #	Report Submitted	Analysis
IVP-CCF-Thrombosis-04132023	2023.06.19	In Vivo Pharmacology

In Life Design

Species/strain: Sprague Dawley (SD) rats
Supplier: Vital River Laboratory Animal Technology Co.
Age: 6 to 8 weeks
Body Weight: 224-245ng
Animal Number: 10 animals / Group, Total 60 animals
Date to Start(In Vivo): 6/14/2023
Date to End(In Vivo): 6/14/2023

Group	Treatment	Dose(mg/kg)	Dosing Volume (mL/kg)	Concentration (mg/mL)	Thrombosis	Route	Frequency	Animals
1	Vehicle	——	5	——	No	PO	Single dose	10
2	Vehicle	——	5	——	Yes	PO		10
3	CCF104	10	5	2	Yes	PO		10
4	CCF104	30	5	6	Yes	PO		10
5	CCF104	100	5	20	Yes	PO		10
6	Clepidogrel	15	5	3	Yes	PO		10
Note:The compound CCF104 will be given 0.5 h before the thrombus model and Clepidogrel will be given 1 h before the thrombus model								

Notebook #: A19551
Study director Zhao Ma
In vivo scientists: Qing li, Jian Yin, Rufang Gao, Yang Yang
Study and reported by: Zhao Ma
Report QC by: Feng Liang

Department Head Jingqi Huang

Sunday	Monday	Tuesday	Wednesday	Thursday	Friday	Saturday
2023.06.11	2023.06.12	2023.06.13	2023.06.14	2023.06.15	2023.06.16	2023.06.17
			Day1			
		1.Fasting for 8 hours before thrombus modeling	1.Body weight 2.Group 3.Dose 4.Thrombosis 5.All animals will be euthanized at 6 hours post-surgery and thrombosis will be collected for weight and length measurement			

PH-CCF-IVP-2023-03		
Group	ID	Body weight (g)
G1=Vehicle	12	230
	14	232
	25	240
	27	240
	37	238
	39	224
	40	236
	52	243
	54	238
	58	234
G2=Thrombosis+Vehicle	6	236
	8	229
	9	240
	18	229
	28	234
	30	232
	34	228
	35	232
	42	242
	45	237
G3=Thrombosis+CCF104 (10 mg/kg)	1	228
	11	236
	21	236
	24	237
	47	230
	49	237
	55	240
	57	230
	67	234
	70	232
G4=Thrombosis+CCF104 (30 mg/kg)	3	233
	7	232
	20	237
	36	241
	41	232
	44	232
	46	240
	51	240
	59	236
	69	230
G5=Thrombosis+CCF104 (100 mg/kg)	10	238
	29	233
	32	225
	33	233
	43	233
	50	232
	61	245
	62	235
	63	236
	68	236
	2	239
	4	235

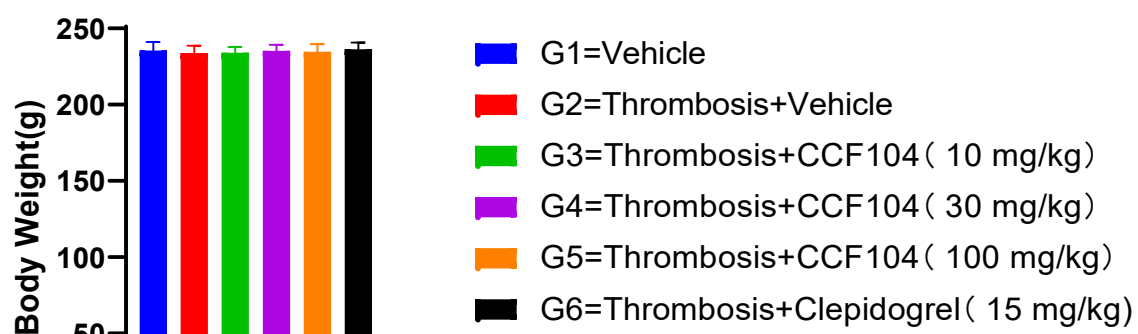
G6=Thrombosis+Clepidogrel (15 mg/kg)	19	229
	26	235
	31	231
	38	238
	48	238
	53	240
	56	234
	60	244

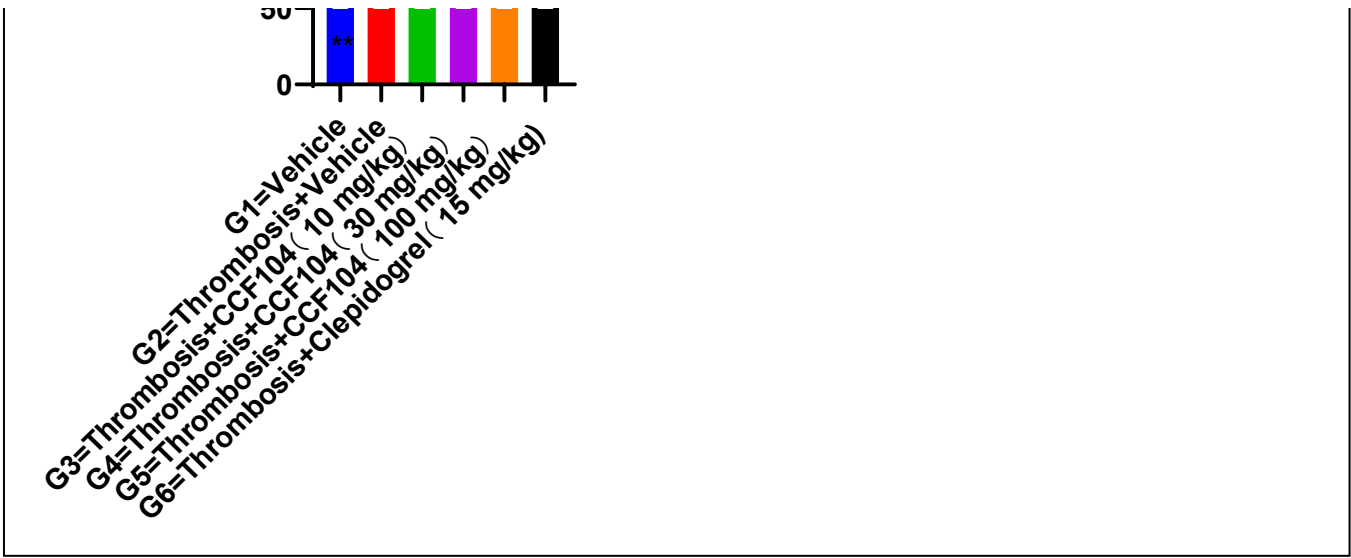
Mean	Body weight (g)
G1=Vehicle	236
G2=Thrombosis+Vehicle	234
G3=Thrombosis+CCF104 (10 mg/kg)	234
G4=Thrombosis+CCF104 (30 mg/kg)	235
G5=Thrombosis+CCF104 (100 mg/kg)	235
G6=Thrombosis+Clepidogrel (15 mg/kg)	236

SD	Body weight (g)
G1=Vehicle	6
G2=Thrombosis+Vehicle	5
G3=Thrombosis+CCF104 (10 mg/kg)	4
G4=Thrombosis+CCF104 (30 mg/kg)	4
G5=Thrombosis+CCF104 (100 mg/kg)	5
G6=Thrombosis+Clepidogrel (15 mg/kg)	4

Mean±SD	Body weight (g)
G1=Vehicle	236±6
G2=Thrombosis+Vehicle	234±5
G3=Thrombosis+CCF104 (10 mg/kg)	234±4
G4=Thrombosis+CCF104 (30 mg/kg)	235±4
G5=Thrombosis+CCF104 (100 mg/kg)	235±5
G6=Thrombosis+Clepidogrel (15 mg/kg)	236±4

Compared with Group 2, statistical analysis was performed using the T.TEST method, *P<0.05, **P<0.01.





PH-CCF-IVP-2023-03			
Group	ID	Thrombosis length(mm)	Thrombosis weight (mg)
G1=Vehicle	12	0.00	0
	14	0.00	0
	25	0.00	0
	27	0.00	0
	37	0.00	0
	39	0.00	0
	40	0.00	0
	52	0.00	0
	54	0.00	0
	58	0.00	0
G2=Thrombosis+Vehicle	6	6.07	17
	8	6.31	17
	9	6.84	22
	18	11.91	43
	28	6.87	16
	30	4.84	15
	34	6.78	31
	35	7.00	24
	42	8.59	20
	45	5.14	18
G3=Thrombosis+CCF104 (10 mg/kg)	1	2.89	3
	11	2.00	0
	21	6.70	22
	24	2.66	2
	47	7.23	30
	49	7.02	24
	55	7.03	32
	57	4.46	18
	67	3.96	17
	70	8.02	47
G4=Thrombosis+CCF104 (30 mg/kg)	3	2.32	0
	7	8.01	49
	20	1.48	2
	36	5.73	27
	41	4.64	23
	44	7.86	40
	46	6.80	19
	51	8.03	49
	59	3.41	8
	69	3.20	9
G5=Thrombosis+CCF104 (100 mg/kg)	10	4.41	27
	29	5.80	24
	32	3.84	14
	33	4.41	27
	43	2.97	4
	50	4.02	8
	61	7.49	45
	62	5.54	19
	63	6.74	33
	68	0.50	0
G6=Thrombosis+Clepidogrel (15 mg/kg)	2	7.12	12
	4	3.12	3
	19	5.50	9
	26	0.00	0
	31	4.48	12
	38	1.49	0
	48	1.88	0
	53	0.00	0
	56	0.00	0
	60	4.24	11

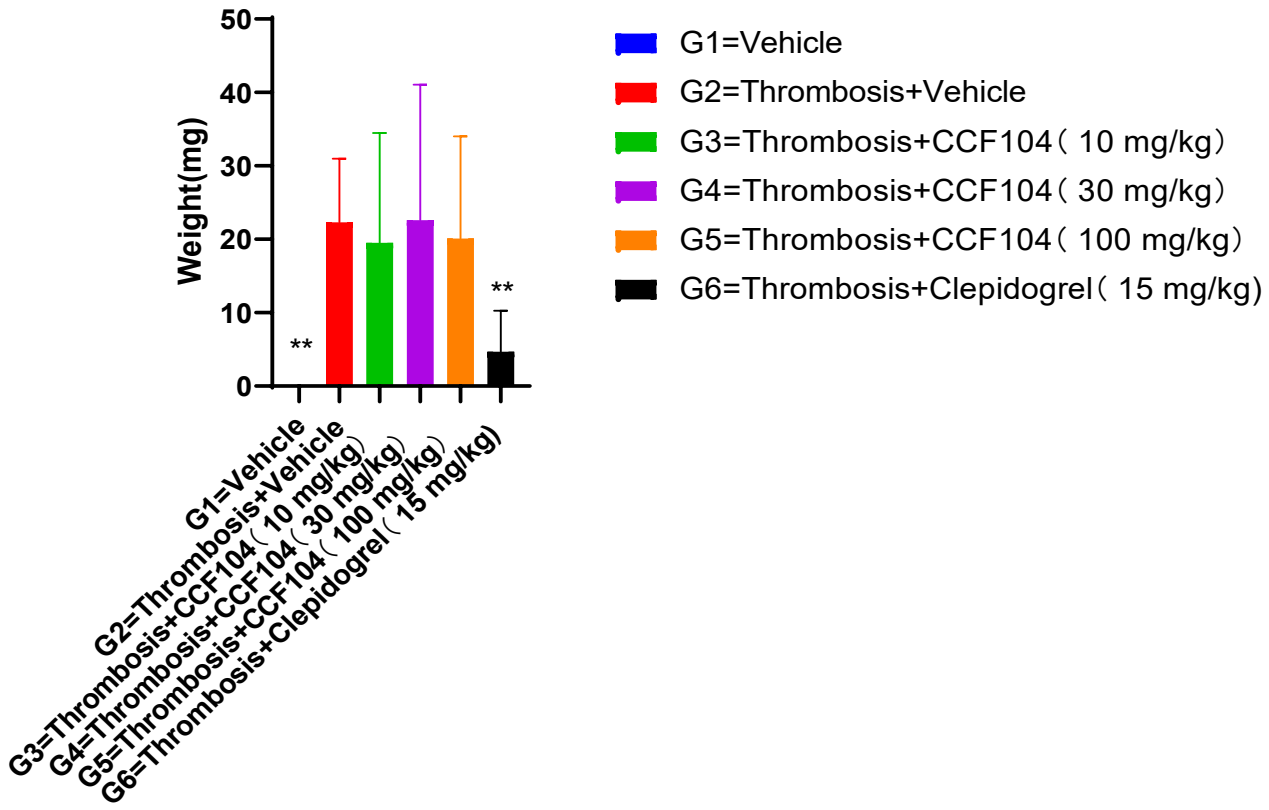
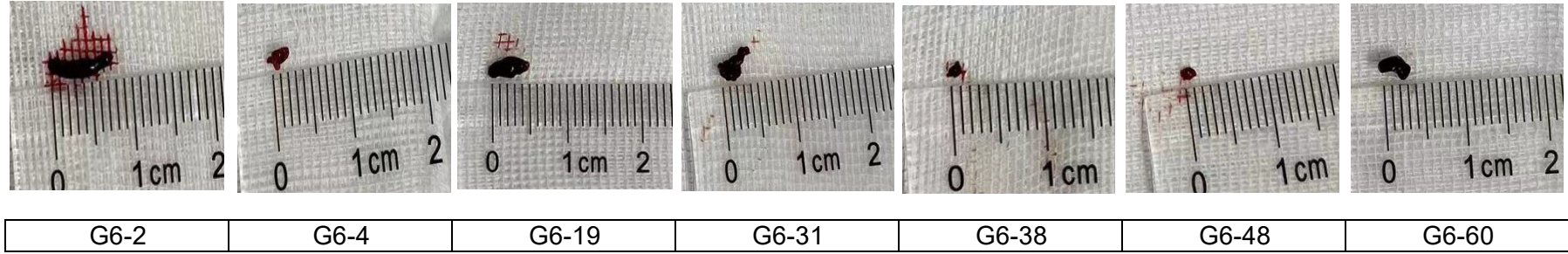
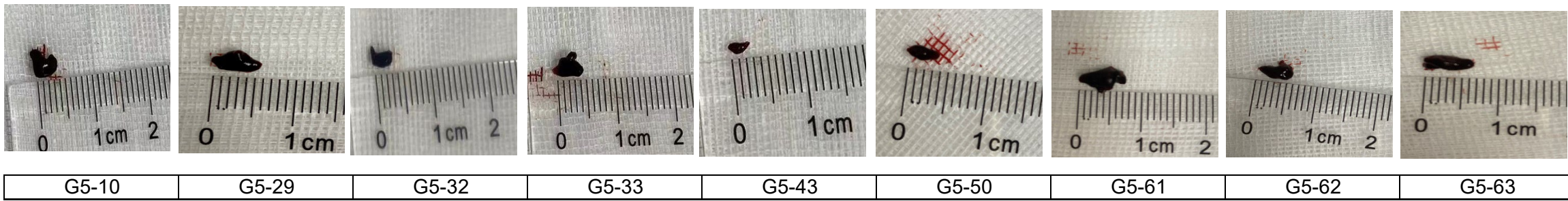
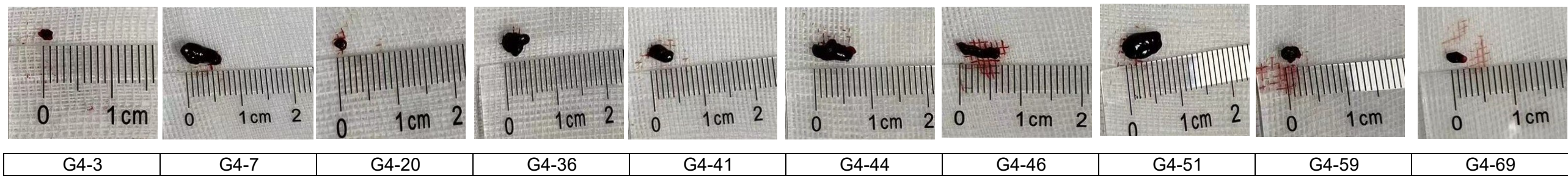
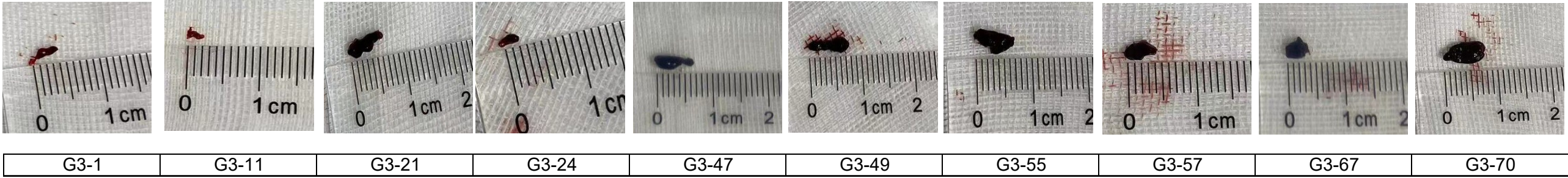
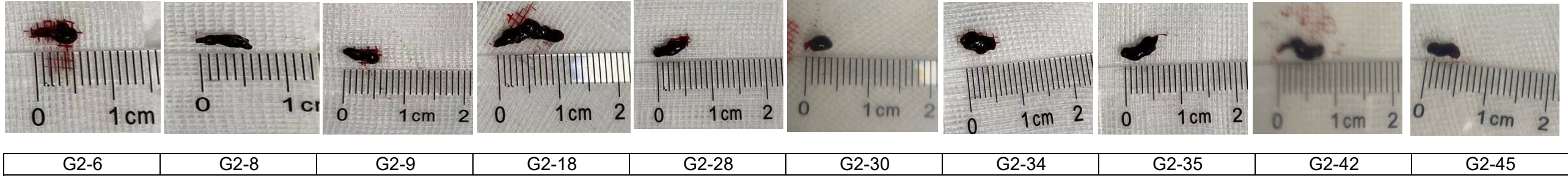
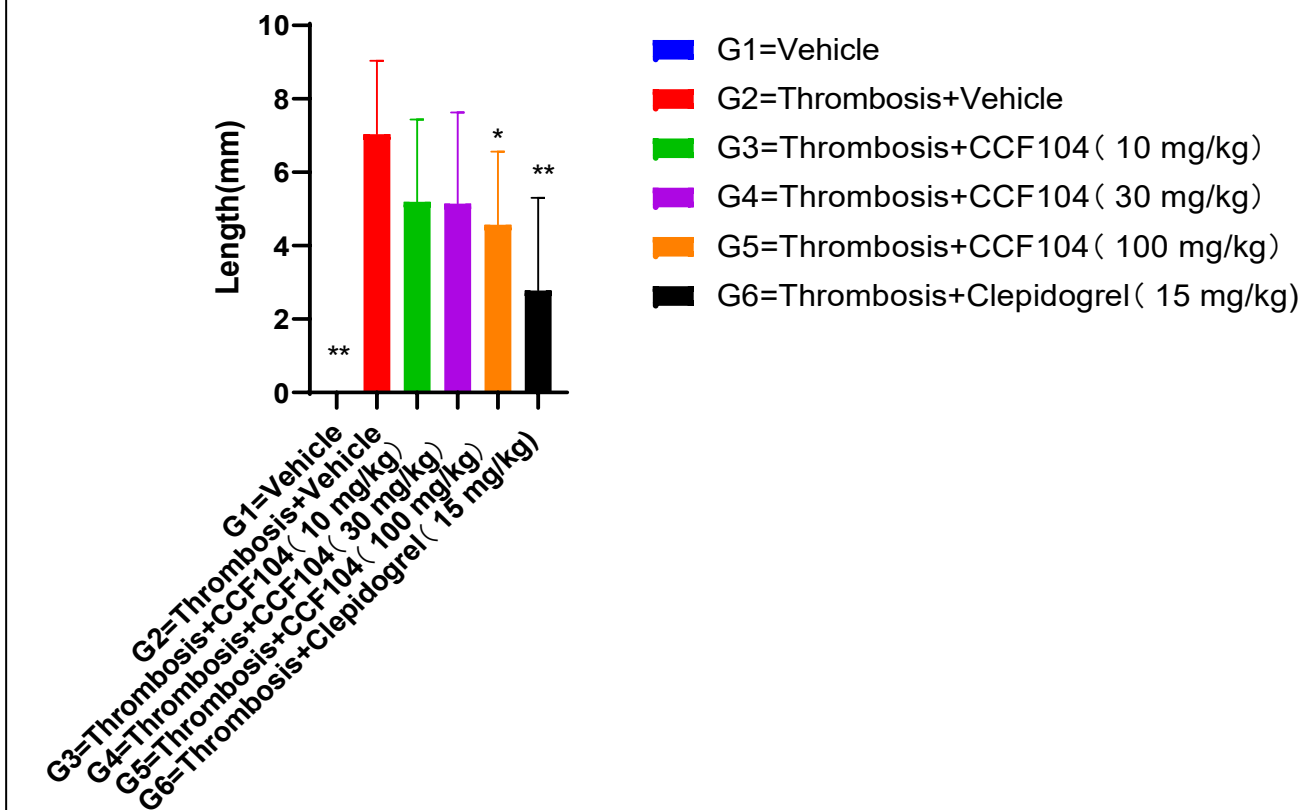
Mean	Thrombosis length(mm)	Thrombosis weight (mg)
G1=Vehicle	0.00	0
G2=Thrombosis+Vehicle	7.04	22
G3=Thrombosis+CCF104 (10 mg/kg)	5.20	20
G4=Thrombosis+CCF104 (30 mg/kg)	5.15	23
G5=Thrombosis+CCF104 (100 mg/kg)	4.57	20
G6=Thrombosis+Clepidogrel (15 mg/kg)	2.78	5

SD	Thrombosis length(mm)	Thrombosis weight (mg)
G1=Vehicle	0.00	0
G2=Thrombosis+Vehicle	2.01	9
G3=Thrombosis+CCF104 (10 mg/kg)	2.24	15
G4=Thrombosis+CCF104 (30 mg/kg)	2.48	18
G5=Thrombosis+CCF104 (100 mg/kg)	1.99	14
G6=Thrombosis+Clepidogrel (15 mg/kg)	2.52	6

Mean±SD	Thrombosis length(mm)	Thrombosis weight (mg)
G1=Vehicle	0.00±0.00	0±0
G2=Thrombosis+Vehicle	7.04±2.01	22±9
G3=Thrombosis+CCF104 (10 mg/kg)	5.20±2.24	20±15
G4=Thrombosis+CCF104 (30 mg/kg)	5.15±2.48	23±18
G5=Thrombosis+CCF104 (100 mg/kg)	4.57±1.99	20±14
G6=Thrombosis+Clepidogrel (15 mg/kg)	2.78±2.52	5±6

Mean±SD	Thrombosis length(mm)	Thrombosis weight (mg)
G1=Vehicle	0.00±0.00**	0±0**
G2=Thrombosis+Vehicle	7.04±2.01	22±9
G3=Thrombosis+CCF104 (10 mg/kg)	5.20±2.24	20±15
G4=Thrombosis+CCF104 (30 mg/kg)	5.15±2.48	23±18
G5=Thrombosis+CCF104 (100 mg/kg)	4.57±1.99*	20±14
G6=Thrombosis+Clepidogrel (15 mg/kg)	2.78±2.52**	5±6**

Compared with Group 2, statistical analysis was performed using the T.TEST method, *P<0.05, **P<0.01.



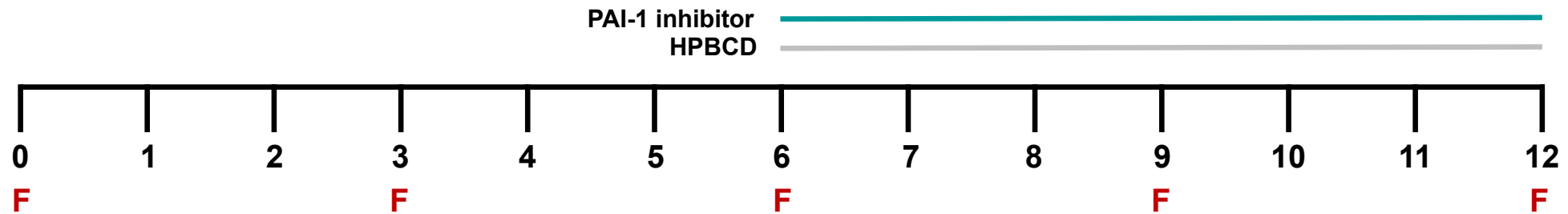
Treatment	Preparation	Concentration (mg/mL)	Prepared Frequency
Vehicle	5% DMSO+ 95% (10% hydroxypropyl -b-cyclodextrin in saline)	—	Prepared freshly
CCF104 @ 10 mg/kg	Dissolve 14.93 mg of CCF104 (Purity: 98.0%) in 0.366 mL of DMSO, then added 6.950 mL of 10% hydroxypropyl -b-cyclodextrin with vortex to obtain solution. The final concentration was 2 mg/mL.	2	
CCF104 @ 30 mg/kg	Dissolve 44.78 mg of CCF104 (Purity: 98.0%) in 0.366 mL of DMSO, then added 6.948 mL of 10% hydroxypropyl -b-cyclodextrin with vortex to obtain solution. The final concentration was 6 mg/mL.	6	
CCF104 @ 100 mg/kg	Dissolve 149.26 mg of CCF104 (Purity: 98.0%) in 0.366 mL of DMSO, then added 6.948 mL of 10% hydroxypropyl -b-cyclodextrin with vortex to obtain solution. The final concentration was 20 mg/mL.	20	
Clepidogrel @ 15 mg/kg	Dissolve 26.89 mg of Clepidogrel in 0.893 mL of DMSO, then added 8.067 mL of 20% SBE-β-CD with vortex to obtain suspension. The final concentration was 3 mg/mL.	3	

Vehicle	Supplier	Lot#
DMSO	Sigma-Aldrich	220105016
hydroxypropyl -b-cyclodextrin	Shanghai yuanye Bio-Technology Co., Ltd	O22HS198750
SBE-β-CD	Shandong Binzhou Zhiyuan Bio-Technology Co., Ltd.	HP20220223

Test Compound	Supplier	Purity	lot number
CCF104	Sponsor	>98.0%	120S

Appendix 12

**Primary in vivo colonic injury model:
12 days continuous DSS (2-3% w/v in drinking water)
Oral delivery of test compounds (5 mg kg⁻¹ bw) for last 6 days**



Experiment:

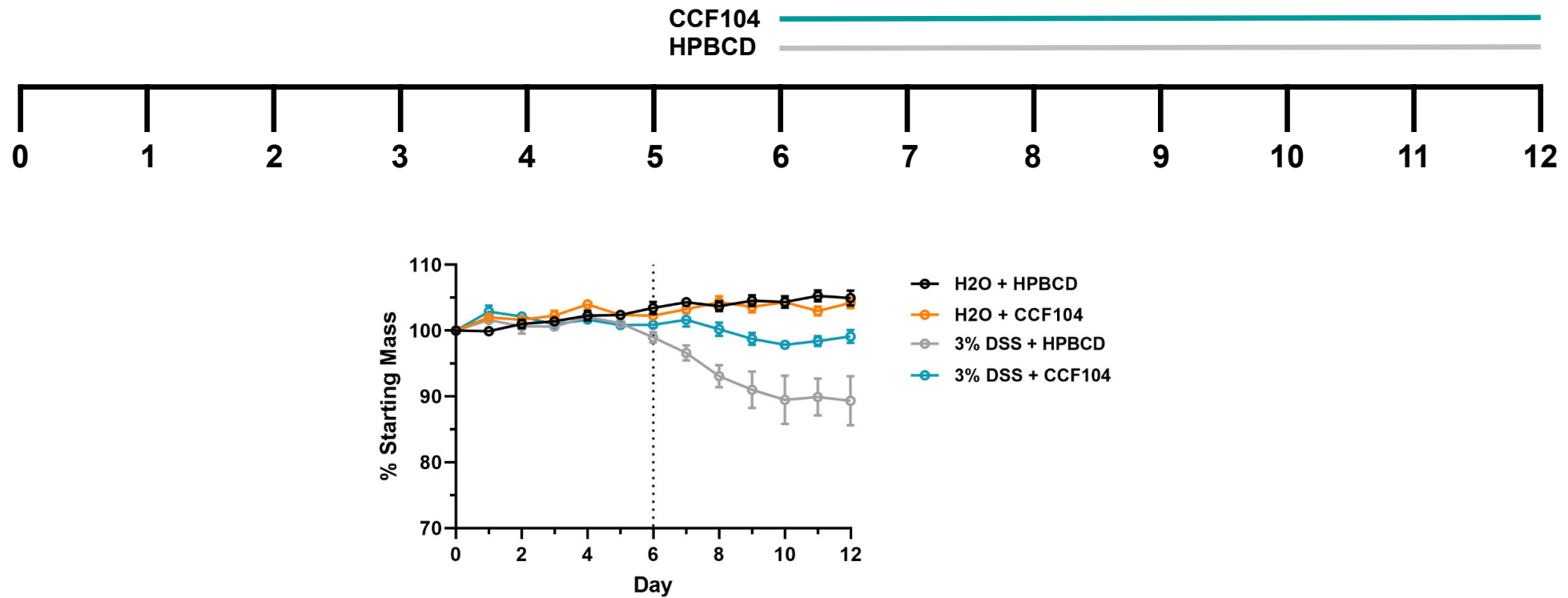
- 1 week acclimation
- Move to cages on static rack
- Weigh daily
- Cage change at exp day 6
- CCF104 (or vehicle) administered by oral gavage daily (100 µL bolus)
- Fecal samples collected at experimental days 0, 3, 6, 9, 12

Endpoint:

- Plasma
- Spleen (snap frozen)
- Liver (snap frozen)
- Colon: histopathology

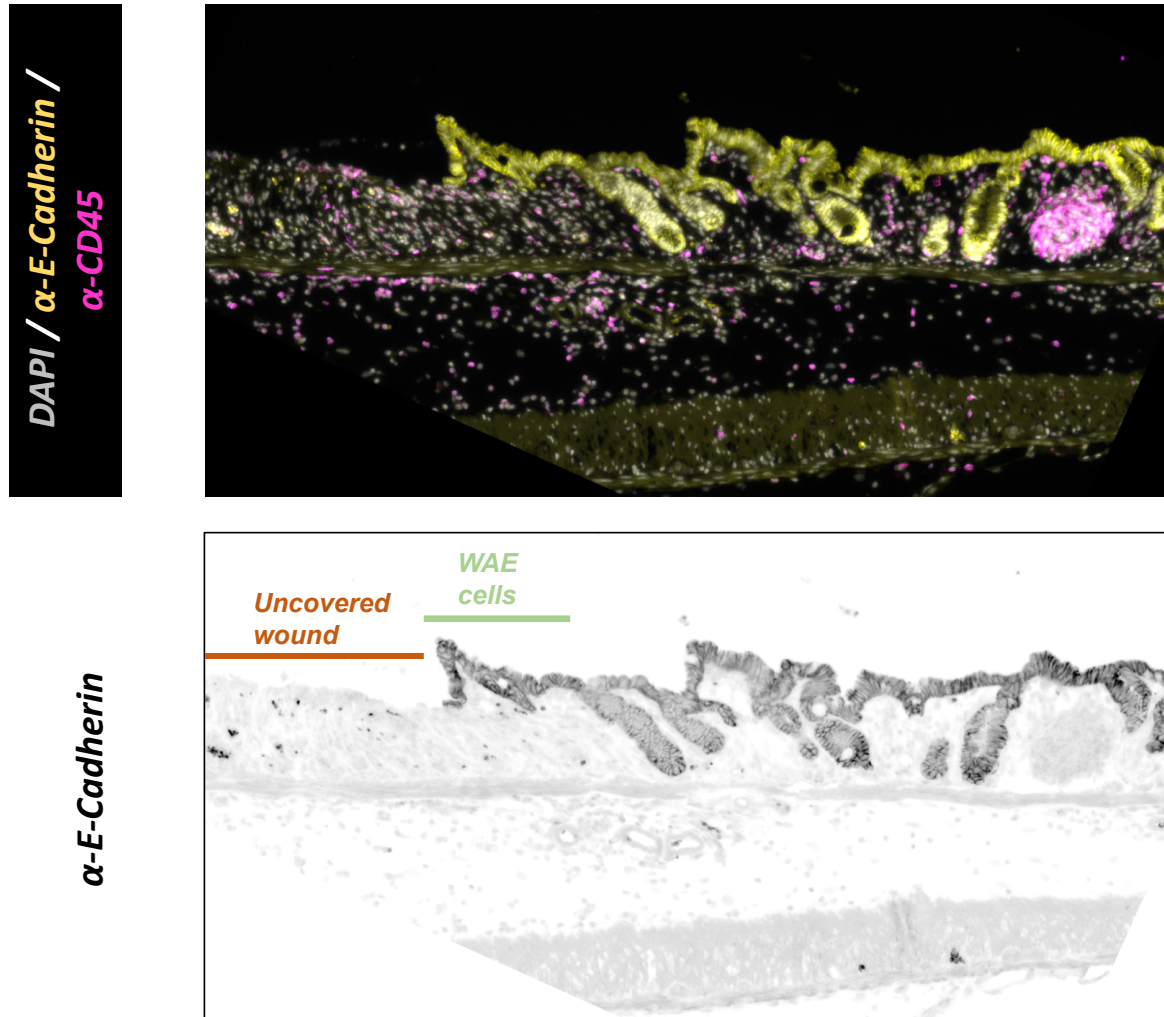
N.b. this paradigm used for all subsequent experiments

Oral delivery of CCF104 is associated with improved response in mouse model of continuous intestinal injury

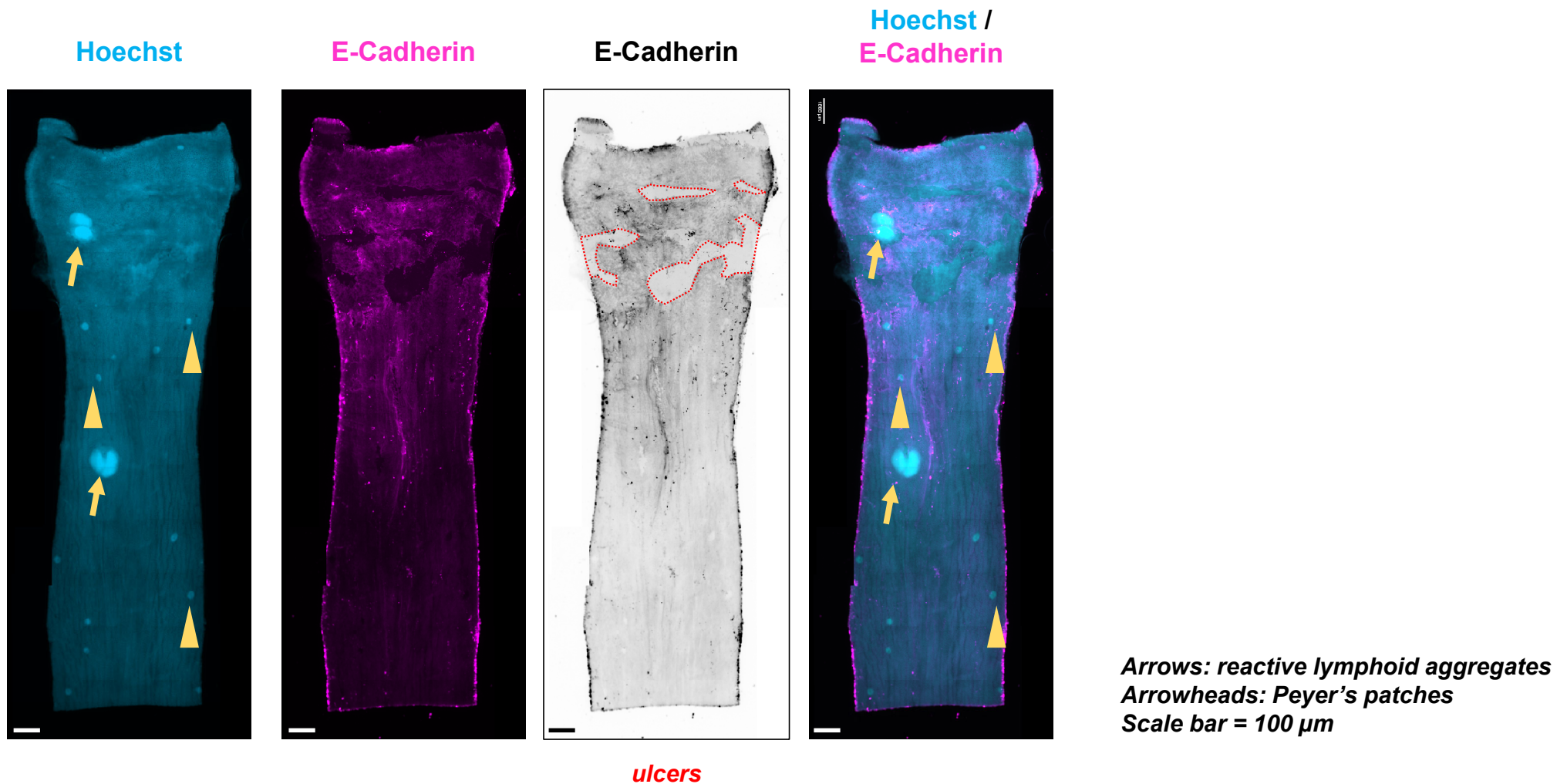


Appendix 13

Immunofluorescence and histopathologic analysis of colonic sections are limited by sampling



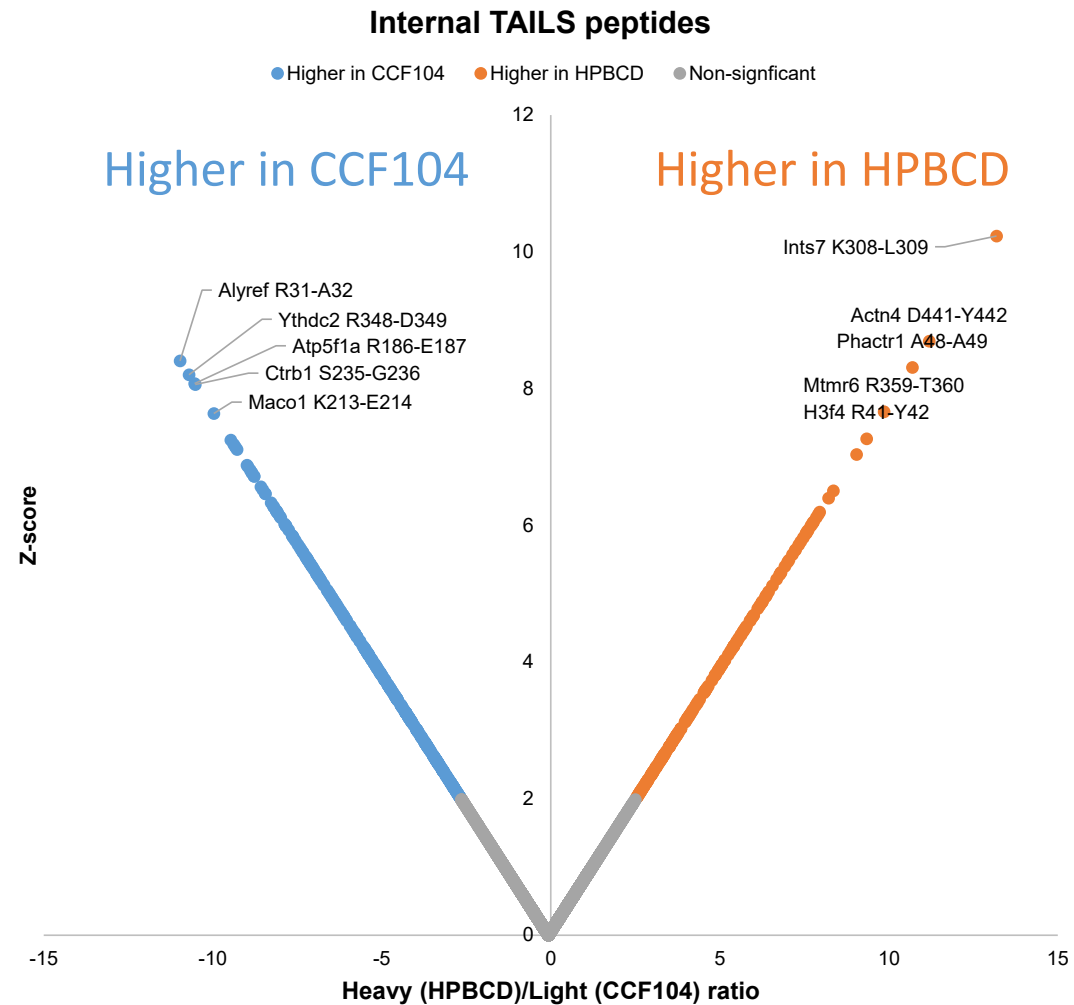
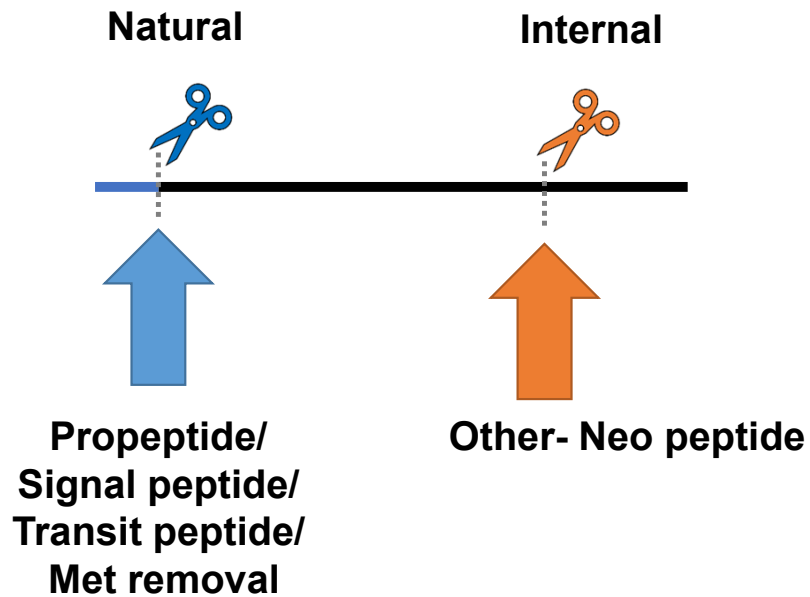
Tissue-level analysis of epithelial damage following colonic injury (DSS) using high-resolution whole-mount immunofluorescence microscopy



Appendix 14

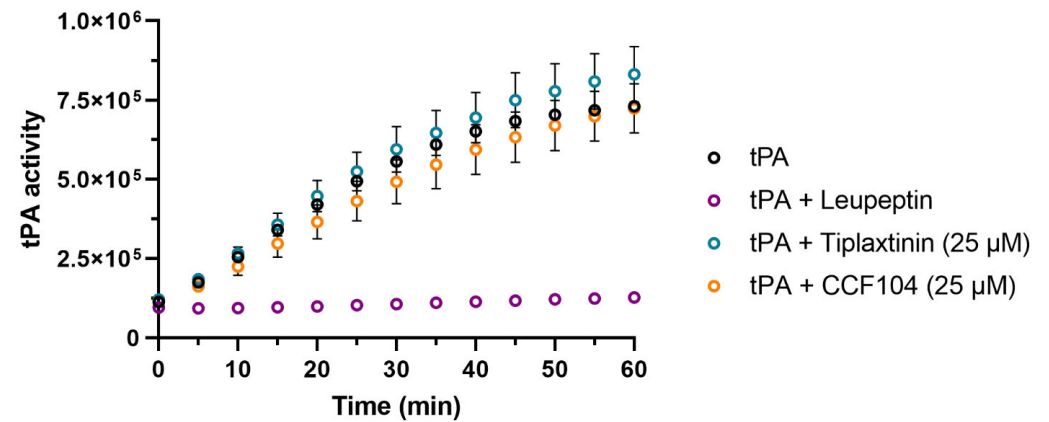
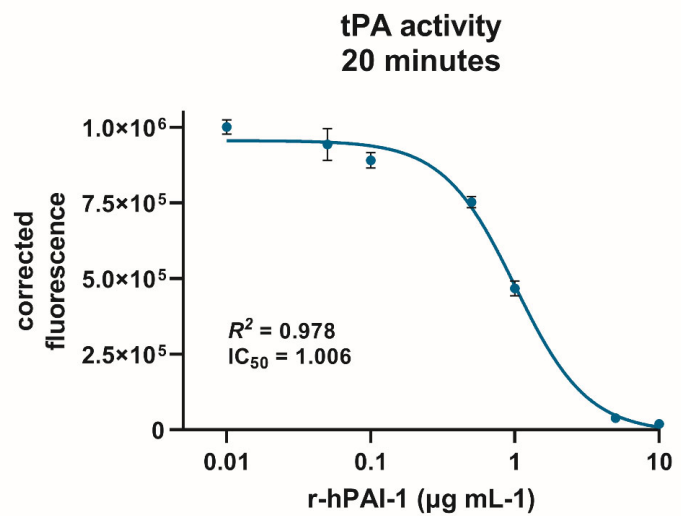
“Degradomics” analysis of isolated colonic epithelium from mice treated with CCF104 or vehicle identifies differentially proteolyzed peptides in the damaged colon (12 days DSS)

Peptide processing



Appendix 15

Optimization of a biochemical tPA activity assay for screening candidate PAI-1 inhibitors



Appendix 16

OTHER SUPPORT – PROJECT/PROPOSAL

Name of Individual: STAPPENBECK, THADDEUS
Commons ID: STAPPENBECK

ACTIVE

Title: Supplemental Funding to Support Project 733314: Developing PAI-1 Inhibitors for Inflammatory Bowel Disease Therapy

Major Goals: The major goal of this project is to establish and validate an in vivo assay to measure target engagement of our lead PAI-1 inhibitor compounds in IBD patients treated with this drug.

Status of Support: Active

Project Number: 733314

Name of PD/PI: MPI: Stappenbeck, T.; Josyula, V.

Source of Support: Crohn's & Colitis Foundation (CCFA)

Primary Place of Performance: Lerner Research Institute, Cleveland Clinic

Project/Proposal Start and End 01/01/2023-12/31/2023

Date: Total Award Amount: Person

Months/Budget Period: Year

Person Months

1. 2023 0.60 calendar

Title: Pre-clinical Development of Inhaled PAI-1 Inhibitors for the Treatment of Pulmonary Fibrosis

Major Goals: The major goal of this project is to reformulate a small molecule PAI-1 inhibitor into nanoparticles for aerosol use to test efficacy in pulmonary fibrosis. No salary support requested.

Status of Support: Active

Project Number: Shark Tank Innovations Award

Name of PD/PI: Stappenbeck, T.; Olman, M.; Josyula, V.

Source of Support: Cleveland Clinic

Primary Place of Performance: Lerner Research Institute, Cleveland Clinic

Project/Proposal Start and End 12/01/2022-11/30/2023

Date: Total Award Amount: Person

Months/Budget Period: Year

Person Months

1. 2023 0.00 calendar

Title: Understanding genetic architecture and host-microbiome interactions in Inflammatory bowel disease in under-represented minority populations and in patients with unmet medical need.

Major Goals: The major goal of the Stappenbeck lab for this proposal is to evaluate the function of rare variants identified in patients with inflammatory bowel disease. They will use epithelial cells derived from iPSCs and primary intestinal epithelial stem cells derived from patients with these mutations to determine function. Role:

Subaward PI

Status of Support: Active

Project Number: 2 U01 DK062413-22

Name of PD/PI: McGovern, D.

Source of Support: NIDDK/Cedars Sinai Medical Center

Primary Place of Performance: Lerner Research Institute, Cleveland Clinic

Project/Proposal Start and End Date:

Subaward start: 09/30/2022-06/30/2027

Total Subaward Amount:

Person Months/Budget Period:

Year	Person Months
2. 2024	1.2 calendar
3. 2025	1.2 calendar
4. 2026	1.2 calendar
5. 2027	1.2 calendar

Title: Precision IBD via genetics and genomics: integrating International and multi-omic datasets, expanding studies in diverse populations, and defining mechanisms of unmet clinical needs in IBD

Major Goals: The NIDDK IBD Genetics Consortium's (IBDGC) mission is to characterize the genetic architecture of IBD phenotypes, particularly in populations currently underrepresented in IBD genomic studies, and to elucidate the biological mechanisms by which genetic variants influence IBD pathophysiology, phenotypes and clinical course.

Status of Support: Active

Project Number: 2 U24 DK062429-24

Name of PD/PI: Cho, J.

Source of Support: NIDDK/ICAHN School of Medicine at Mount Sinai

Primary Place of Performance: Lerner Research Institute, Cleveland Clinic

Project/Proposal Start and End Date: 09/30/2022-06/30/2027

Total Award Amount: Person Months/Budget Period:

Year	Person Months
2. 2024	0.24 calendar
3. 2025	0.24 calendar
4. 2026	0.24 calendar
5. 2027	0.24 calendar

Title: Molecular Dissection of Cytokine Crosstalk in the Tumor Microenvironment

Major Goals: The major goals of this project are to: 1) determine the mechanisms by which IL-17/TGF β /STING-IFN-I regulate the mesenchymal/stem-like tumor phenotype and chemoresistance; and 2) determine the mechanisms by which IL17/TGF β /STING-IFN-I shape the physical and immune barriers against anti-tumor immunity and response to immunotherapy. Role: Co-I

Status of Support: Awarded

Project Number: 1 P01 CA272161-01

Name of PD/PI: Stark, G.

Source of Support: NCI

Primary Place of Performance: Lerner Research Institute, Cleveland Clinic

Project/Proposal Start and End 09/13/2022-08/31/2027

Date: Total Award Amount: Person

Months/Budget Period: Year

Person Months

1. 2023 0.6 calendar
2. 2024 0.6 calendar
3. 2025 0.6 calendar
4. 2026 0.6 calendar
5. 2027 0.6 calendar

Title: A gut-restricted small molecule therapeutic for a novel target to treat inflammatory bowel disease

Major Goals: This proposal will focus on the development of a gut-restricted, small molecule therapeutic addressing a novel target for patients suffering from inflammatory bowel diseases (IBD). The target is upregulated in the damaged epithelial cells near areas of mucosal damage. Inhibition of the target promotes wound repair, often defective in IBD. The major goals of this project are to: 1) Perform preclinical development studies of CCF104, 2) Further pharmacological assessment of CCF104 and 3) Identify a second generation of PAI-1 inhibitors.

Status of Support: Active

Project Number: W81XWH2210675

PRMP Therapeutic Developmental Award

Name of PD/PI: Stappenbeck, T.

Source of Support: Department of Defense

Primary Place of Performance: Lerner Research Institute, Cleveland Clinic

Project/Proposal Start and End 08/01/2022-07/31/2024

Date: Total Award Amount: Person

Months/Budget Period: Year

Person Months

2. 2024 1.80 calendar

Title: Novel cytoskeletal mechanisms of pathogenic bacteria interactions with intestinal epithelium

Major Goals: The major goals of this project are to: 1) to determine the roles of NM II in regulating intestinal epithelial cell interactions with attaching and effacing bacteria; 2) to dissect the mechanisms of NM II-driven internalization of invading bacteria into intestinal epithelial cells. Role: Co-I

Status of Support: Active

Project Number: 5 R01 DK131550-02

Name of PD/PI: Ivanov, A.

Source of Support: NIDDK

Primary Place of Performance: Lerner Research Institute, Cleveland Clinic

Project/Proposal Start and End 07/15/2022-04/30/2027

Date: Total Award Amount: Person

Months/Budget Period: Year

Person Months

2. 2024 0.6 calendar

3. 2025 0.6 calendar

4. 2026 0.6 calendar

5. 2027 0.6 calendar

Title: PACS GI Dysmotility

Major Goals: This funding will support supplies to support a Long Covid Study. The major goal of this project is to identify serum markers/autoantibodies that can track the consequences and severity of post-acute COVID-19 syndrome (PACS) on the gastro-intestinal tract motility which is characterized in a variety symptoms including by dysphagia, dyspepsia, constipation, diarrhea and abdominal pain, all indicative of defects in intestinal motility.

No salary support requested.

Status of Support: Active

Project Number: IF110482

Name of PD/PI: Stappenbeck, T.

Source of Support: Global Center for Pathogen and Human Health Research

Primary Place of Performance: Lerner Research Institute, Cleveland Clinic

Project/Proposal Start and End Date: 07/01/2022-12/31/2023

Total Award Amount: Person Months/Budget Period:

Year Person Months

1. 2023 0.00 calendar

Title: Effects of inflammaging on intestinal epithelial cells and aspirin chemoprevention

Major Goals: The major goal of this project is to evaluate the effects of old age and low dose aspirin on the repertoire and function of intestinal epithelial stem cells through lineage tracing in vivo, primary intestinal epithelial cell culture and single cell transcriptomic analysis.

Status of Support: Active

Project Number: 5 R01 CA257523-03

Name of PD/PI: MPI: Chan, A. (contact), Stappenbeck, T., Yilmaz, O.

Source of Support: NCI/Massachusetts General Hospital

Primary Place of Performance: Lerner Research Institute, Cleveland Clinic

Project/Proposal Start and End 04/05/2021-03/31/2026

Date: Total Award Amount: Person

Months/Budget Period: Year

Person Months

3. 2024 1.2 calendar

4. 2025 1.2 calendar

5. 2026 1.2 calendar

Title: Gut microbiota modulation of Chikungunya Virus infection and pathogenesis

Major Goals: The overall goal of this project is to understand whether specific gut bacteria and their microbial constituents modulate CHIKV pathogenesis by regulating antiviral type I IFN and inflammatory responses in pDCs and monocytes. The major goals will be to address: 1) which immune cells coordinate the rapid systemic IFN response following CHIKV infection (e.g., pDCs), 2) what immune cues limit viral infection in circulating immune cells (e.g., monocytes), 3) how does the gut microbiota regulate pDC IFN production and trafficking of circulating immune cells, and 4) which constituents (e.g., metabolites) of the microbiota regulate antiviral and inflammatory responses. Role: Co-I

Status of Support: Active

Project Number: 5 R01 AI152484-03

Name of PD/PI: Diamond, M. (contact), Fischbach, M., Handley, S., Stappenbeck, T.

Source of Support: NIAID/Washington University

Primary Place of Performance: Lerner Research Institute, Cleveland Clinic

Project/Proposal Start and End Date: 04/01/2021-03/31/2026

Total Award Amount: Total Award

Amount (to PI): Person Months/Budget

Period:

Year Person Months

3. 2024 0.6 calendar

4. 2025 0.6 calendar

5. 2026 0.6 calendar

Title: Unconventional myosins and the regulation of gut barrier integrity and restitution during inflammation

Major Goals: The goals of this project are to 1) determine the roles of Myo18A in the regulation of intestinal epithelial cell migration and ECM adhesion; 2) delineate the mechanisms of Myo18A-dependent assembly of the epithelial barrier and establishment of the apico-basal cell polarity; and 3) examine the roles of Myo18A in regulating disruption and restitution of the intestinal epithelial barrier in vivo. Role: Co-I

Status of Support: Active

Project Number: 5 R01 DK126702-03

Name of PD/PI: Ivanov, A.

Source of Support: NIDDK

Primary Place of Performance: Lerner Research Institute, Cleveland Clinic

Project/Proposal Start and End Date: 09/15/2020-07/31/2024 (no-cost extension)

Total Award Amount: Person

Months/Budget Period: Year

Person Months

3./NCE. 2024 0.6 calendar

Title: Creeping fat and Crohn's disease associated strictures

Major Goals: The major goals of this project are to: 1) define the mechanisms of creeping fat-induced smooth muscle cell hyperplasia; 2) determine the role of HIMC-derived ECM molecules in integrin-mediated adipocyte migration; and 3) investigate the effect of mesenteric fat deletion and mitochondrial muscle metabolism on murine intestinal smooth muscle hyperplasia in vivo. Role: Co-I

Status of Support: Active

Project Number: 5 R01 DK123233-04

Name of PD/PI: Rieder, F

Source of Support: NIDDK

Primary Place of Performance: Lerner Research Institute, Cleveland Clinic

Project/Proposal Start and End 07/15/2020-03/31/2025

Date: Total Award Amount: Person

Months/Budget Period: Year

Person Months

4. 2024 0.36 calendar

5. 2025 0.36 calendar

Title: Systemic Neurotropic virus infection effects on GI Dysmotility

Major Goals: The major goals of this project are to: 1) determine how CD8+ T cell responses to WNV infection alter GI motility; 2) determine how WNV infection alters the gut microbiota to affect GI motility; and 3) define how neuroendocrine intervention modulates WNV-mediated GI dysmotility.

Status of Support: Active

Project Number: 5 R01 DK122790-04

Name of PD/PI: Stappenbeck, T., Diamond, M., Handley, S.

Source of Support: NIDDK

Primary Place of Performance: Lerner Research Institute, Cleveland Clinic

Project/Proposal Start and End 07/01/2020-04/30/2024

Date: Total Award Amount: Person

Months/Budget Period: Year

Person Months

4. 2024 1.2 calendar

Title: Computational and Experimental Resources for Virome Analysis in Inflammatory Bowel Disease (CERVAID)

Major Goals: The overall goal of this project is to develop novel computational and experimental tools to address challenges in understanding the role of viruses in inflammatory bowel disease. Role: Co-I

Status of Support: Active

Project Number: 5 RC2 DK116713-04

Name of PD/PI: Wang, D.

Source of Support: NIDDK/Washington University

Primary Place of Performance: Lerner Research Institute, Cleveland Clinic

Project/Proposal Start and End 09/18/2019-08/31/2024

Date: Total Award Amount: Person

Months/Budget Period: Year

Person Months

4. 2023 0.6 calendar

5. 2024 0.6 calendar

Title: Tunable therapeutic modulation of the gut microbiome by engineered probiotics

Major Goals: The major goals of this project are to identify gene pathways and develop synthetic regulatory circuits that, when engineered into probiotics, robustly improve duration and tunability of therapeutic production by probiotics in the gut, and apply these technologies to the treatment of phenylketonuria.

Status of Support: Active

Project Number: 5 R01 AT009741-05

Name of PD/PI: MPI: Dantas, G. (contact), Moon, T., Stappenbeck, T.

Source of Support: NCCIH/Washington University

Primary Place of Performance: Lerner Research Institute, Cleveland Clinic

Project/Proposal Start and End 08/10/2018-07/31/2024 (no-cost extension)

Date: Total Award Amount: Person

Months/Budget Period: Year

Person Months

5./NCE 2024 0.96 calendar

Title: Primary Immunoprevention of Triple Negative Breast Cancer

Major Goals: The major goals of this project are to 1) complete a phase Ia clinical trial designed to determine dosage and safety of alpha-lactalbumin vaccination in subjects with high-risk TNBC; and 2) complete a phase Ib clinical trial designed primarily to determine safety of alpha-lactalbumin vaccination in healthy, cancer-free subjects undergoing elective mastectomy as prophylaxis for breast cancer risk.

Status of Support: Active

Project Number: W81XWH-17-1-0592

Name of PD/PI: Stappenbeck, T. (effective 01/18/2023)

Source of Support: Department of Defense

Primary Place of Performance: Lerner Research Institute, Cleveland Clinic

Project/Proposal Start and End 09/30/2017-09/29/2024 (no-cost extension)

Date: Total Award Amount: Person

Months/Budget Period: Year

Person Months

2nd NCE. 2023 0.60 calendar

3rd NCE. 2024 0.60 calendar

PENDING

Title: Role of fungal organisms in non-healing mucosal ulcers and fistulizing disease of Black CD patients

Major Goals: The major goal for this project is to test Koch's postulates in Black CD subjects due to the severity of the disease in this population. First, we will determine the prevalence of *D. hansenii* in non-healing ulcers and fistulae of Crohn's patients from Blacks in two locations (Atlanta, GA, and Jackson, MS) and we will collect yeast isolates from these patients with a focus on *D. hansenii* strains. Second, we will test these yeast isolates by infection of macrophages in vitro and mice with colonic injury in vivo. No salary support requested, funds for supplies. Role: Co-PI

Status of Support: Pending

Project Number: COCCI2203TS

Name of PD/PI: MPI: Stappenbeck, T.; Liu, J.; Glover, S.

Source of Support: Color of Crohn's & Chronic Illness (CCOCI)

Primary Place of Performance: Lerner Research Institute, Cleveland Clinic

Project/Proposal Start and End 09/01/2023-04/30/2024

Date: Total Award Amount: Person

Months/Budget Period: Year

Person Months

1. 2023 0.12 calendar

Title: Actin methylation as a novel mechanism that regulates gut barrier integrity and repair

Major Goals: The goals of this project are to 1) delineate the roles of SETD3 in establishment of the intestinal epithelial barrier and assembly of apical junctions, 2) determine the roles of SETD3 in regulating intestinal epithelial cell migration, and 3) examine the roles of SETD3 in regulating disruption and restitution of the intestinal epithelial barrier in vivo. Role: Collaborator

Status of Support: Pending

Project Number: 1 R01 DK137822-01

Name of PD/PI: Ivanov, A.

Source of Support: NIDDK

Primary Place of Performance: Lerner Research Institute, Cleveland Clinic

Project/Proposal Start and End 09/01/2023-08/31/2028

Date: Total Award Amount: Person

Months/Budget Period: Year

Person Months

- | | |
|---------|---------------|
| 1. 2024 | 0.60 calendar |
| 2. 2025 | 0.60 calendar |
| 3. 2026 | 0.60 calendar |
| 4. 2027 | 0.60 calendar |
| 5. 2028 | 0.60 calendar |

Title: Innate immune responses to tissue infection by intestinal fungi inhibit wound repair

Major Goals: The goals of this project are to 1) test the hypothesis that *D. hansenii* spores infect and persist in macrophages due to reduced immune recognition; 2) test the hypothesis that spore germination leads to an enhanced type I IFN response; and 3) test the hypothesis that antifungal clearance of persistent *D. hansenii* infection restores wound repair.

Status of Support: Pending

Project Number: 1 R01 AI168411-01A1

Name of PD/PI: MPI: Stappenbeck, T.; Hogan D.

Source of Support: NIH

Primary Place of Performance: Lerner Research Institute, Cleveland Clinic

Project/Proposal Start and End 09/01/2023-08/31/2028

Date: Total Award Amount: Person

Months/Budget Period: Year

Person Months

- | | |
|---------|---------------|
| 1. 2024 | 2.40 calendar |
| 2. 2025 | 2.40 calendar |
| 3. 2026 | 2.40 calendar |
| 4. 2027 | 2.40 calendar |
| 5. 2028 | 2.40 calendar |

Title: Elucidating mechanisms of efficacy and resistance to cabozantinib and checkpoint blockade for rational multi-drug immunotherapy in bladder cancer

Major Goals: The goals of this project are to 1) determine the clinical utility of combined cabozantinib and anti-PD-L1 immune checkpoint blockade therapy, and define the molecular determinants of response; 2) characterize the adaptive response of tumors and the microenvironment to anti-PD-L1 therapy alone and in combination with cabozantinib therapy; and 3) elucidate mechanisms of acquired resistance to cabozantinib plus avelumab or anti-PD-L1 therapy alone in urothelial cancers. Role: Co-I

Status of Support: Pending

Project Number: 1 R01 CA287855-01

Name of PD/PI: Chan, T.; Gupta, S.

Source of Support: NCI

Primary Place of Performance: Lerner Research Institute, Cleveland Clinic

Project/Proposal Start and End 12/01/2023-11/30/2028

Date: Total Award Amount: Person

Months/Budget Period: Year

Person Months

- | | |
|---------|---------------|
| 1. 2024 | 0.60 calendar |
| 2. 2025 | 0.60 calendar |
| 3. 2026 | 0.60 calendar |
| 4. 2027 | 0.60 calendar |
| 5. 2028 | 0.60 calendar |

Title: Microbial drivers of penetrating fibrosis in colonic Crohn's disease

Major Goals: Our hypothesis is that a subset of the CD microbiome can stimulate the repetitive inflammatory injury that is a nidus for fibrogenic activity in the mucosa that then can over time penetrate the distal colon leading to ano-rectal fistulae. This project will define the specific set of microbes that drive the early phases of fibrosis. We propose these microbes will be new therapeutic targets to prevent ano-rectal fistulae in all CD patients. We created a system to identify microbes that trigger fibrosis by adapting our deep expertise in gut-on-a-chip technology to model the effects of mucosal microbes from CD patients on human colonic epithelial cells. This project will use our system to screen for microbes that generate CD epithelial signatures, use additional co-culture with intestinal fibroblasts to screen for pathogenic pathways in these cells such as IL-11 overexpression, and, using microbes identified in the screen, test them for their ability to trigger penetrating fibrosis both in vitro and in vivo, the latter using a mouse model of repetitive injury. We will obtain samples from three centers (Morehouse, Tulane and Cleveland Clinic) that care for substantial numbers of Black CD patients, which is the demographic group at highest risk for this complication.

Status of Support: Pending

Project Number: 27697, Biology of Fibrosis in IBD

Name of PD/PI: MPI: Stappenbeck, T.; Kim, H.J.; Liu, J.; Glover, S.; Awoniyi, O.

Source of Support: Crohn's & Colitis Foundation

Primary Place of Performance: Lerner Research Institute, Cleveland Clinic

Project/Proposal Start and End 01/01/2024-12/31/2026

Date: Total Award Amount: Person

Months/Budget Period: Year

Person Months

- | | |
|---------|---------------|
| 1. 2025 | 1.20 calendar |
| 2. 2026 | 1.20 calendar |

Title: Role of Stress in the degradation of intestinal IgA and intestinal health

Major Goals: The goals of this project are to 1) define microbes and their proteases that degrade IgA in mice in response to chronic stress and 2) determine bacteria that degrade IgA in humans in response to chronic stress.

Status of Support: Pending

Project Number: PR230937

Name of PD/PI: Stappenbeck, T.

Source of Support: Department of Defense

Primary Place of Performance: Lerner Research Institute, Cleveland Clinic

Project/Proposal Start and End 08/01/2024-07/31/2028

Date: Total Award Amount: Person

Months/Budget Period: Year

Person Months

- | | |
|---------|---------------|
| 1. 2024 | 1.80 calendar |
| 2. 2025 | 1.80 calendar |
| 3. 2026 | 1.80 calendar |
| 4. 2027 | 1.80 calendar |

Title: Cabozantinib and anti-PD-L1 combination therapy in bladder cancer: mechanisms and molecular drivers of response

Major Goals: The major goal(s) of this project are to: 1) Determine the clinical utility of combined cabozantinib and anti-PD-L1 immune checkpoint blockade therapy, and define the molecular determinants of response. 2) Characterize the adaptive response of tumors and the microenvironment to anti-PD-L1 therapy alone and in combination with cabozantinib therapy. 3) Elucidate mechanisms of acquired resistance to cabozantinib plus avelumab or anti-PD-L1 therapy alone in urothelial cancers.

Role: Department Chair

Status of Support: Pending

Project Number: PCRP12 Translational Science Award

Name of PD/PI: Chan, T.

Source of Support: Department of Defense

Primary Place of Performance: Lerner Research Institute, Cleveland Clinic

Project/Proposal Start and End Date:

09/01/2024-08/31/2028 Total Award Amount:

Person Months/Budget Period:

Year Person Months

- | | |
|---------|---------------|
| 1. 2025 | 0.60 calendar |
| 2. 2026 | 0.60 calendar |
| 3. 2027 | 0.60 calendar |
| 4. 2028 | 0.60 calendar |

IN-KIND

Title: IgA determinants in human fecal samples from a healthy cohort (MTA)

Summary of In-Kind Contribution: No salary support requested. Seres will be sending the Stappenbeck lab de-identified human stool samples from a healthy cohort for project. Number of samples may be in the hundreds over a period of time. The Stappenbeck lab will analyze the IgA level in the stool samples. Results will be shared back with Seres to generate publications and for use as preliminary data for a grant submission.

Status of Support: Pending Final Contract 4287844

Primary Place of Performance: Lerner Research Institute, Cleveland Clinic

Project/Proposal Start and End Date: 09/01/2023-03/31/2024

Person Months/Budget Period:

Year (YYYY)	Person Months (##.##)
1. 2024	0 calendar

Estimated Dollar Value of In-Kind Information: ~\$50/sample

Title: Pre-clinical Development of Inhaled PAI-1 Inhibitors for the Treatment of Pulmonary Fibrosis

Summary of In-Kind Contribution: Shark Tank Innovations Award. Title: Pre-clinical Development of Inhaled PAI-1 Inhibitors for the Treatment of Pulmonary Fibrosis. The major goal of this project is to reformulate a small molecule PAI-1 inhibitor into nanoparticles for aerosol use to test efficacy in pulmonary fibrosis. No salary support requested.

Status of Support: Active

Primary Place of Performance: Lerner Research Institute, Cleveland Clinic

Project/Proposal Start and End Date: 12/01/2022-11/30/2023

Person Months/Budget Period:

Year (YYYY)	Person Months (##.##)
1. 2023	0 calendar

Estimated Dollar Value of In-Kind Information:

Summary of In-Kind Contribution: TUM-IAS Hans Fischer Senior Fellow (Germany), Technical University of Munich - Institute for Advanced Study: This fellowship supports collaborative studies with Prof. Dirk Haller.

Status of Support: Active

Primary Place of Performance: Lerner Research Institute, Cleveland Clinic

Project/Proposal Start and End Date: 2021-2024

Person Months/Budget Period:

Year (YYYY)	Person Months (##.##)
3. 2024	0.62 calendar

Estimated Dollar Value of In-Kind Information:

Overlap:

- If pending COCCI2203TS, R01 DK137822-01, and R01 AI168411-01A1 are all awarded, Dr. Stappenbeck will reduce effort on all awards by ~20% as to not exceed 12 calendar months total effort.
- If pending R01 CA287855-01 and CCFA 27697 are later awarded, Dr. Stappenbeck will reduce effort on all awards by an allowable amount (up to 25%) and additionally reduce effort on R01 AT009741, which is in a no-cost extension, to 0.6 calendar months in order to not exceed 12 calendar months total effort.
- If pending DOD PR230937 is later awarded, a 10% reduction on all active awards at that time will maintain Dr. Stappenbeck within 12 calendar months total effort.

I, PD/PI or other senior/key personnel, certify that the statements herein are true, complete and accurate to the best of my knowledge, and accept the obligation to comply with Public Health Services terms and conditions if a grant is awarded as a result of this application. I am aware that any false, fictitious, or fraudulent statements or claims may subject me to criminal, civil, or administrative penalties.

Signature:

PI PREVIOUS/CURRENT/PENDING SUPPORT

Josyula, Vara Prasad

PREVIOUS SUPPORT (past 5 years)

Project Title Rapid Diagnostics of SARS-CoV-2/COVID-19 of People Wishing to Return to Work or School
RADx Tech Project 5146

Project Number: 3U54HL119810-07S1

Level of Effort (percentage or calendar months): 23.5%

Performance Period (mm/dd/yy to mm/dd/yy): 09/01/2020 – 08/31/2021

Supporting Agency: NIH

Supporting Agency Point of Contact (name and contact information): John Diggs, diggsj@nhlbi.nih.gov, 301-827-8028

Specific Aims/Tasks: Simultaneous detection of viral antigen, IgG and IgM at once in single testing. To procure specific potential covid-19 antibodies to detect viral antigens (i) Nucleocapsid protein (N antigen) (ii) Spike protein (S antigen). In parallel, attempts will be made to screen DNA aptamers against selected viral antigens from designed oligonucleotide library. The specificity and affinity of procured/synthesized bioreceptors will be evaluated using ELISA and other biochemical tests. Immobilization of viral antibodies and anti-viral antibodies onto the integrated micro cantilevers via suitable optimized chemistry for IgG, IgM and antigen testing respectively. Testing and validation of assay with real samples (swab/saliva).

Brief Description of the Project's Goals: Establish a proof-of-concept with a novel rapid, hand-held, multiplex (antigen and antibody) diagnostic system that is ready for clinical testing with sensitivity, specificity, and accuracy of >90% based on saliva and nasal; swab sampling.

CURRENT

(Awarded since JIT)

Project Title: Supplemental Funding to Support Project 733314: Developing PAI-1 Inhibitors for Inflammatory Bowel Disease Therapy

Project Number: 733314

Level of Effort (percentage or calendar months): 0.6 calendar

Performance Period: 01/01/2023-12/31/2023

Level of Funding:

Supporting Agency: Crohn's & Colitis Foundation (CCFA)

Supporting Agency POC: Andrés Hurtado-Lorenzo (ahurtadolorenzo@crohnsandcolitisfoundation.org)

Brief Description of the Project's Goals: The major goal of this project is to establish and validate an in vivo assay to measure target engagement of our lead PAI-1 inhibitor compounds in IBD patients treated with this drug.

Potential Overlap with this DOD Proposal: None.

Project Title: A gut-restricted small molecule therapeutic for a novel target to treat inflammatory bowel disease

Project Number: W81XWH2210675 PR211463 (PRMP Therapeutic Developmental Award)

Level of Effort (percentage or calendar months): 4.80 calendar

Performance Period (mm/dd/yy to mm/dd/yy): 08/01/2022-07/31/2024

Level of Funding:

Supporting Agency: Department of Defense

Supporting Agency POC: Amanda Carrera (Amanda.C.Carrera.civ@mail.mil), Jonathan E. Ryder (jonathan.e.ryder2.civ@mail.mil)

Specific Aims/Tasks: The major goals of this project are to: 1) Perform preclinical development studies of CCF104, 2) Further pharmacological assessment of CCF104 and 3) Identify a second generation of PAI-1 inhibitors.

Brief Description of the Project's Goals: This proposal will focus on the development of a gut-restricted, small molecule therapeutic addressing a novel target for patients suffering from inflammatory bowel diseases (IBD). The target is upregulated in the damaged epithelial cells near areas of mucosal damage. Inhibition of the target promotes wound repair, often defective in IBD.

This DoD Proposal

PENDING

None.