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TITLE: Fluorenone Drug for Treatment of Combat-Related Traumatic Optic Neuropathy

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14. ABSTRACT

Traumatic optic neuropathy (TON) is a frequent cause of significant visual loss after combat-related frontal head trauma. There is no clinically proven therapy for improving visual outcomes in TON patients. Identification of new treatment options for TON is of highest priority. Our goal is to conduct studies needed to advance the proposed drug candidate, B-3(+), to clinical development for the TON indication. The following specific aims have been identified in order to attain the study objective: Develop a standard in vitro ADMET package required to support pre-clinical and clinical development of the drug candidate (Specific Aim 1); Conduct systemic and ocular pharmacokinetic studies in beagle dogs; use blood samples collected in PK studies for assessing drug nephrotoxicity (Specific Aim 2); Expand upon existing pre-clinical efficacy data and develop a robust efficacy package that can convincingly justify compound testing in patients with traumatic optic neuropathy (Specific Aim 3).

15. SUBJECT TERMS
None listed.

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1. INTRODUCTION:

Traumatic optic neuropathy (TON) is a frequent cause of significant visual loss after combat-related frontal head trauma. The optic nerve is divided in four anatomical segments: intraocular, intraorbital, intracanalicular and intracranial. In TON, the injury of the optic nerve originates from concussive forces to the head which are transmitted to the optic canal forcing it to move faster than the intracanalicular optic nerve, which thus induce the optic nerve damage. There is no clinically proven therapy for improving visual outcomes in TON patients, and identification of new treatment options for TON is of highest priority. Astrocyte swelling was shown to be the primary trigger inducing tissue damage after closed head injury. Injury-induced astrocyte swelling is assumed to be a significant factor in pathogenesis of TON. Prevention of swelling by inhibiting injury-induced edema in the intracanalicular portion of the optic nerve is a sound strategy for TON treatment. B-3(+) is a fluorenone drug that has demonstrated significant efficacy in preventing mortality in animal models of concussive brain injury and thromboembolic stroke. The overall objective of the proposed project is to conduct the studies needed to support regulatory filings required for our drug candidate to enter clinical development for TON indication.

2. KEYWORDS:

Traumatic optic neuropathy; Astrocyte swelling; Fluorenone drug

3. ACCOMPLISHMENTS:

What were the major goals of the project?

There is no clinically proven therapy for improving visual outcomes in TON patients. Identification of new treatment options for TON is of highest priority. Our goal is to conduct studies needed to advance the proposed drug candidate, B-3(+), to clinical development for the TON indication. The following specific aims have been identified in order to attain the study objective: Develop a standard in vitro ADMET package required to support pre-clinical and clinical development of the drug candidate (Specific Aim 1); Conduct pharmacokinetic studies in beagle dogs (Specific Aim 2); Expand upon existing pre-clinical efficacy data and develop a robust efficacy package that can convincingly justify compound testing in patients with traumatic optic neuropathy (Specific Aim 3).

What was accomplished under these goals?

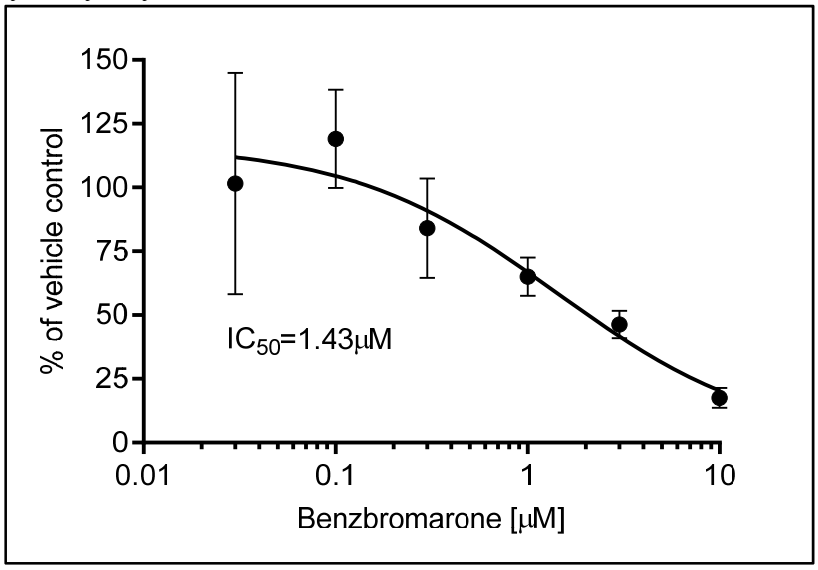
We have successfully completed all studies related to Major Task 1, which involved synthesizing the drug candidate and implementing in vitro ADMET assays. All objectives of Subtasks 1-5 were achieved, and we also performed additional characterization of compound activity in the URAT1 transporter assay during the grant period. The goals of major Task 1 were completed during the Year 3 grant period.

In Year 3, we made significant progress on objectives related to Major Task 2. We completed the last Subtasks of Major Task 2 (Subtasks 5 and 6) and compared pharmacokinetic data obtained in beagle dogs with historic datasets related to characterization of this compound in cats and rats. The goals of Major Task 2 were fully accomplished during this grant period.

We also made significant progress in the work on Major Task 3 (Define the range of efficacious drug concentrations in the animal model of optic nerve damage) by successfully completing parts of Subtask 1 related to guinea pig protocol approval and performing a set of optic nerve crush experiments on guinea pig optic nerve.

A. Completion of characterization of in vitro ADMET characteristics of the drug candidate:

One remaining question related to ADME characteristics of B-3(+) is the ability of this drug candidate to inhibit a group of major reabsorptive anion transporters. According to the literature, B-3(+) can act as an inhibitor of responsible for the transport of anions. Significant inhibition of reabsorptive transporters (such as URAT1 and members of the OAT family) would be considered a significant drug-drug interaction issue given that such trnapsorters are responsible for clearance major classes of drugs such as statins. Moreover, our evaluation of the available clinical data developed in 1980's for systemically administered B-3(+) indicates that this compound may be acting as a uricosuric agent which may be caused by inhibition of renal URAT1. URAT1 is a transporter protein that plays an important role in the regulation of uric acid levels in the body. URAT1 is responsible for reabsorbing uric acid from the urine and returning it to the bloodstream. We implemented the URAT1 (SLC22A12) Transporter Assay for characterization of the ability of B-3(+) to inhibit transport of urate anion. URAT1 belongs to the OAT transporter family. It is an anion exchanger that specifically reabsorbs uric acid from the proximal tubule in exchange for monovalent anions such as lactate, nicotinoate, acetoacetate, and hydroxybutyrate.



MDCK cell line expressing the URAT1 transporter was used in ourstudy, and we tested the effect of B-3(+) on the ability to inhibit transport of urate anion. In this study, the ability of B-3(+) to inhibit the transport of urate anions by URAT1 was evaluated. As shown in Fig. 1, the positive control, benzbromarone, inhibited transport of urate anion with IC50 of 1.43 μM.

Figure 1. Determination of IC50 value of benzbromarone against URAT1 mediated transport. Benzbromarone was studied in the concentration range of 0.02 - 10 μM. The probe substrate was 20 μM Uric acid. Data represent the mean and standard deviation of triplicate samples.

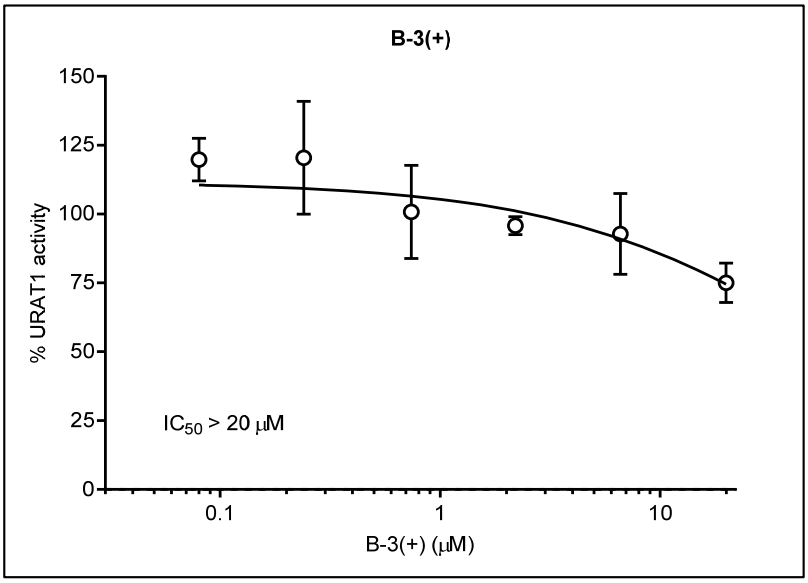
We evaluated the ability of B-3(+) for the ability to inhibit urate transport in the 0-20 μM concentration range . Table 1 shows individual data points of inhibition with each data point originated from three independent measurements.

Table 1

In vitro data for the determination of IC_{50} value of B-3(+) against the transport mediated by URAT1.

B-3(+) Concentration (μM)	Accumulation of Uric acid (pmol/min/cm²)			Inhibition (%)
	In URAT1 cells	In control cells	Net	
0	2.50 ± 0.266	0.546 ± 0.123	1.95 ± 0.266	0.00 ± 13.6
0.08	2.90 ± 0.152	0.565 ± 0.0365	2.34 ± 0.152	-19.8 ± 7.79
0.24	3.03 ± 0.401	0.681 ± 0.227	2.35 ± 0.401	-20.5 ± 20.6
0.74	2.56 ± 0.331	0.595 ± 0.0908	1.97 ± 0.331	-0.803 ± 17.0
2.2	2.63 ± 0.0642	0.760 ± 0.131	1.87 ± 0.0642	4.24 ± 3.29
6.6	2.43 ± 0.286	0.622 ± 0.0539	1.81 ± 0.286	7.19 ± 14.7
20	2.00 ± 0.140	0.537 ± 0.0278	1.46 ± 0.140	25.0 ± 7.19
10 μM benzbromarone	0.430 ± 0.0445	0.647 ± 0.0849	-0.217 ± 0.0445	111 ± 2.28

The probe substrate was 20 μM Uric acid.
Data represent the mean and standard deviation of triplicate samples.



As shown in Figure 2, the compound does not significantly inhibits the traffic of yrate mediated by the URAT1 transporter.

Figure 2. Determination of IC50 value of B-3(+) against URAT1 mediated transport. The compound was studied in the concentration range of 0 - 20 µM. The probe substrate was 20 µM Uric acid. Data represent the mean and standard deviation of triplicate samples.

As a result of conducted experiments we established that B-3(+) is not an inhibitor of URAT1. As discussed in the following section, this leaves the question on the mechanism of the uricosuric effect of B-3(+) in humans open

B. Analysis of historic data generated in 1980's reveals the uricosuric effect of B-3(+) in humans

In 1980's, intravenously administered B-3(+) was tested in human clinical trials for traumatic brain injury. Certain clinical records from those clinical trials became available to us from the FDA. We analyzed this available data for indications of potential clinical issues. One notable observation we made was the effect of the systemically administered compound on dynamics of uric acid in human plasma and in urine. Figure 3 indicates that systemic administration of the drug candidate induces a significant dose-dependent reduction in urate concentration in human serum.

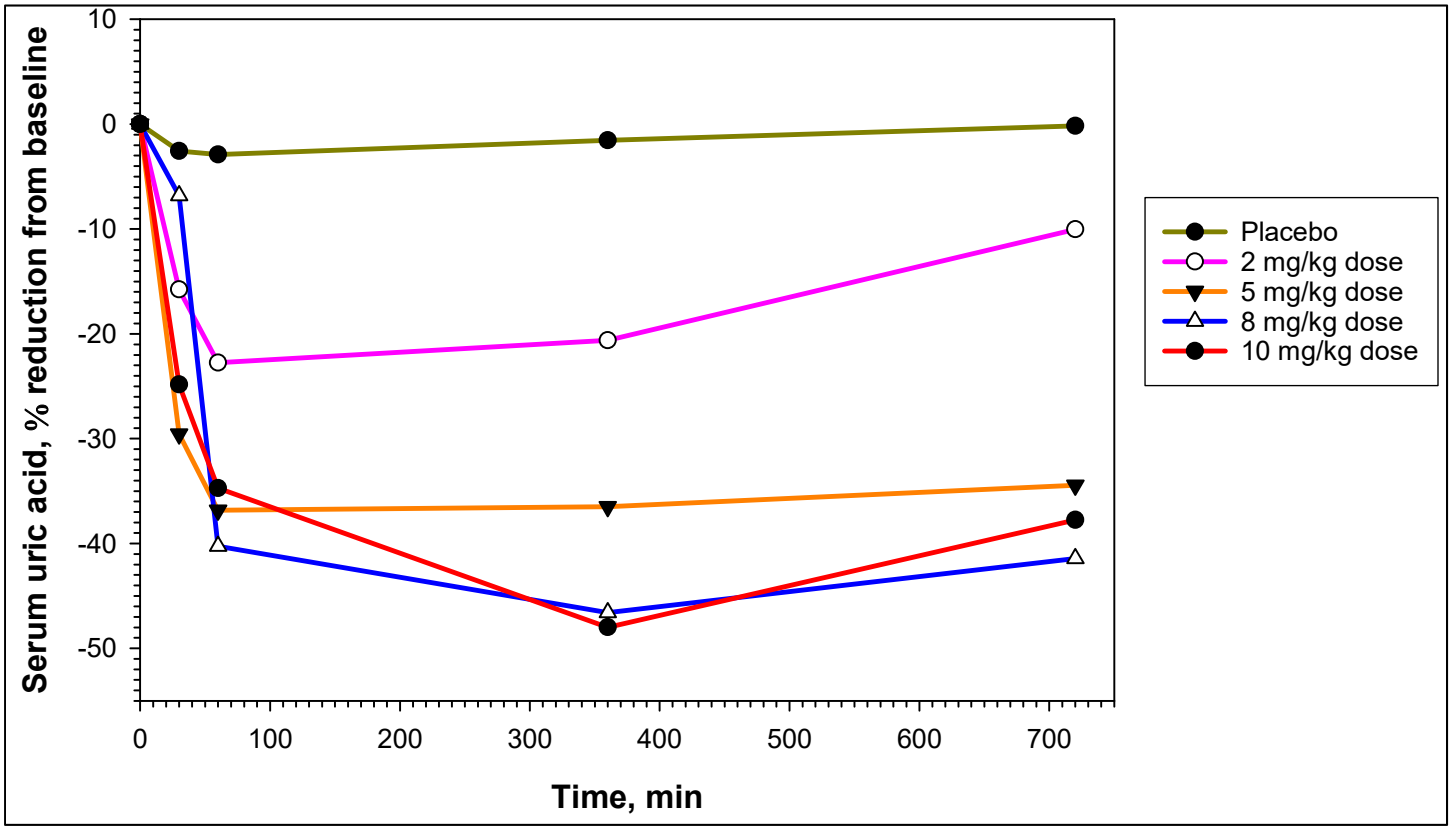


Figure 3. Dose-dependent reduction of serum uric acid by intravenous B-3(+) in 12 normal healthy volunteers

It was also evident from the data contained in the information package that B-3(+) induces increase in urinary uric acid output in humans, dose-dependently increasing uric acid excretion (Figure 4)

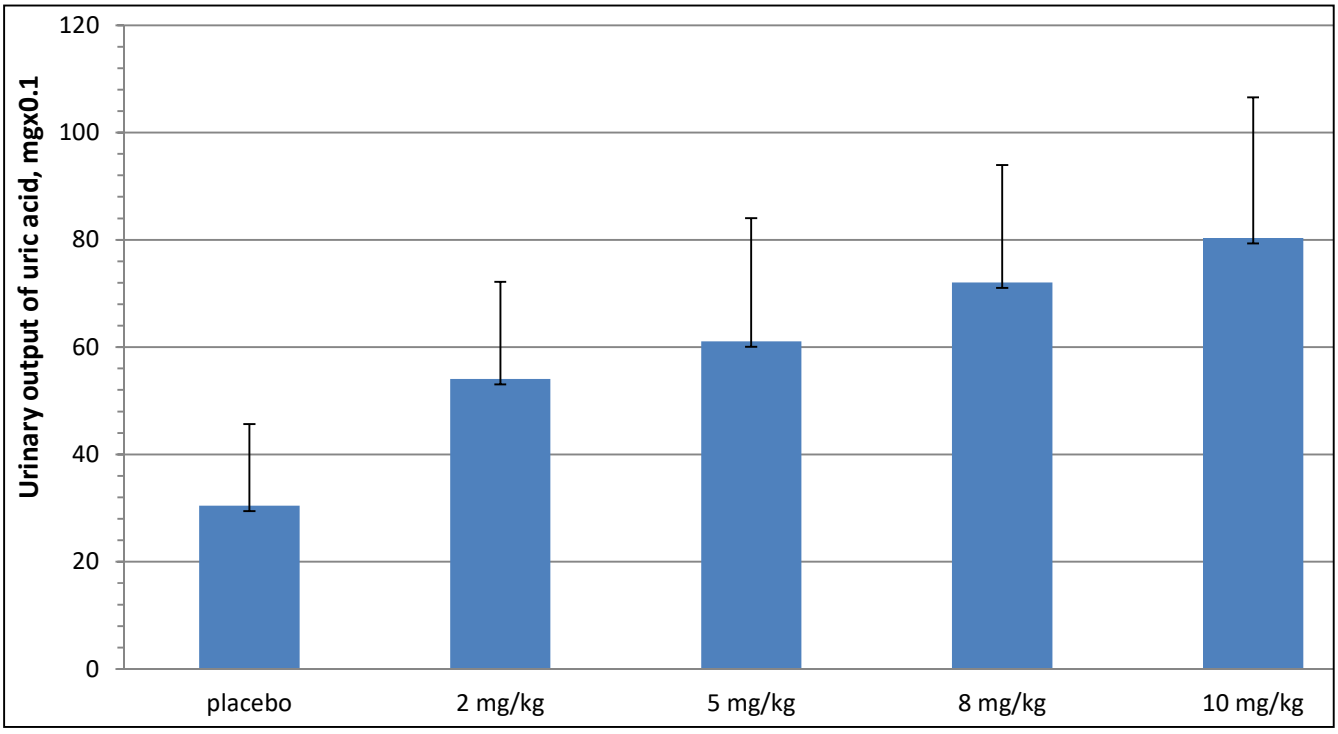


Figure 4. Dose-dependent increase in urinary excretion of uric acid in 12 normal healthy volunteers.

Overall, available data indicates that B-3(+) is a uricosuric compound that induces urinary uric acid excretion in humans. This effect, however, cannot be regarded as a negative attribute, given that the suggested route of administration in patients with traumatic optic neuropathy is intravitreal, not systemic. It should also be noted that according to our testing the compound does not inhibit URAT1. Thus, the mechanism of the uricosuric activity remains unknown. Even though elucidation of such mechanism is beyond the scope of the current study, our data indicates that B-3(+) may potentially be a novel therapy for gouty arthritis and other diseases associated with increase concentration of circulating uric acid.

C. Dog pharmacokinetic study and comparison of its outcomes with historic datasets.
Following the completion of the in-life portion of the pharmacokinetic study dog (Major Task 2) we conducted the detailed analysis of the obtained data (Major Task 2, Subtasks 4-7) comparing it with historic dataset available in the literature.

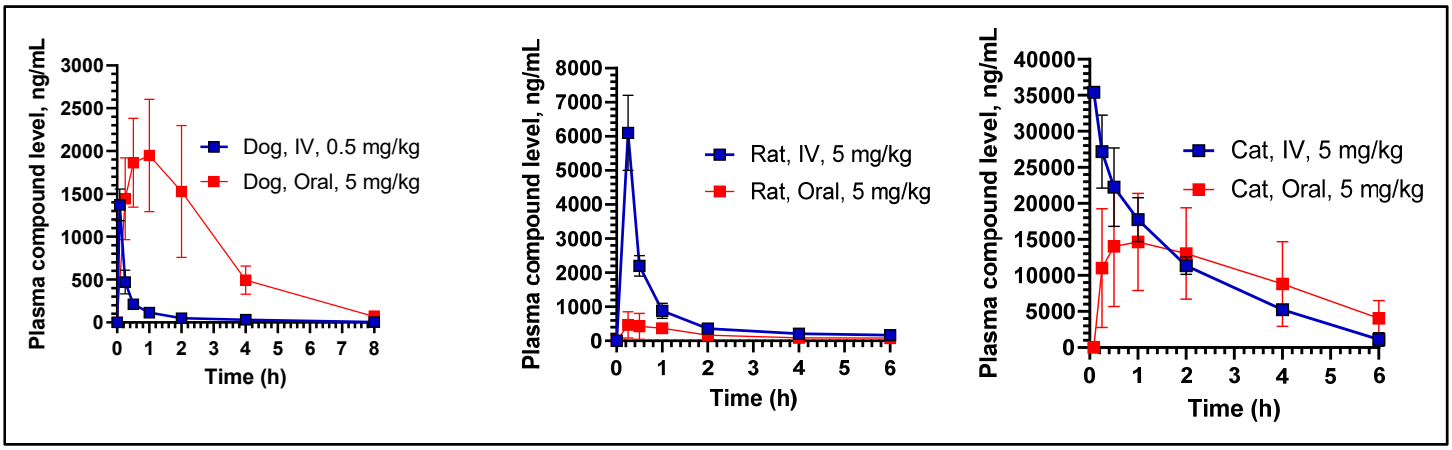


Figure 5. Pharmacokinetic properties of B-3(+) in beagle dog, rat and cat. Dog dataset was developed in the current study. Rat and Cat datasets were previously reported

Figure 5 illustrates the comparison of B-3(+) pharmacokinetic in three potential safety species: dog, cat and rat. While the dog dataset was developed in the current study, the plots related to rat and cat graphs were ploted from the data published in the literature or previously submitted to the FDA. As Fig 5 indicates, systemic exposure of the compound caries drastically in three species. To compare the exposure in three species, we analyzed dose-normalized AUC_{inf} values (AUC_{inf}/dose), as shown in Figure 6.

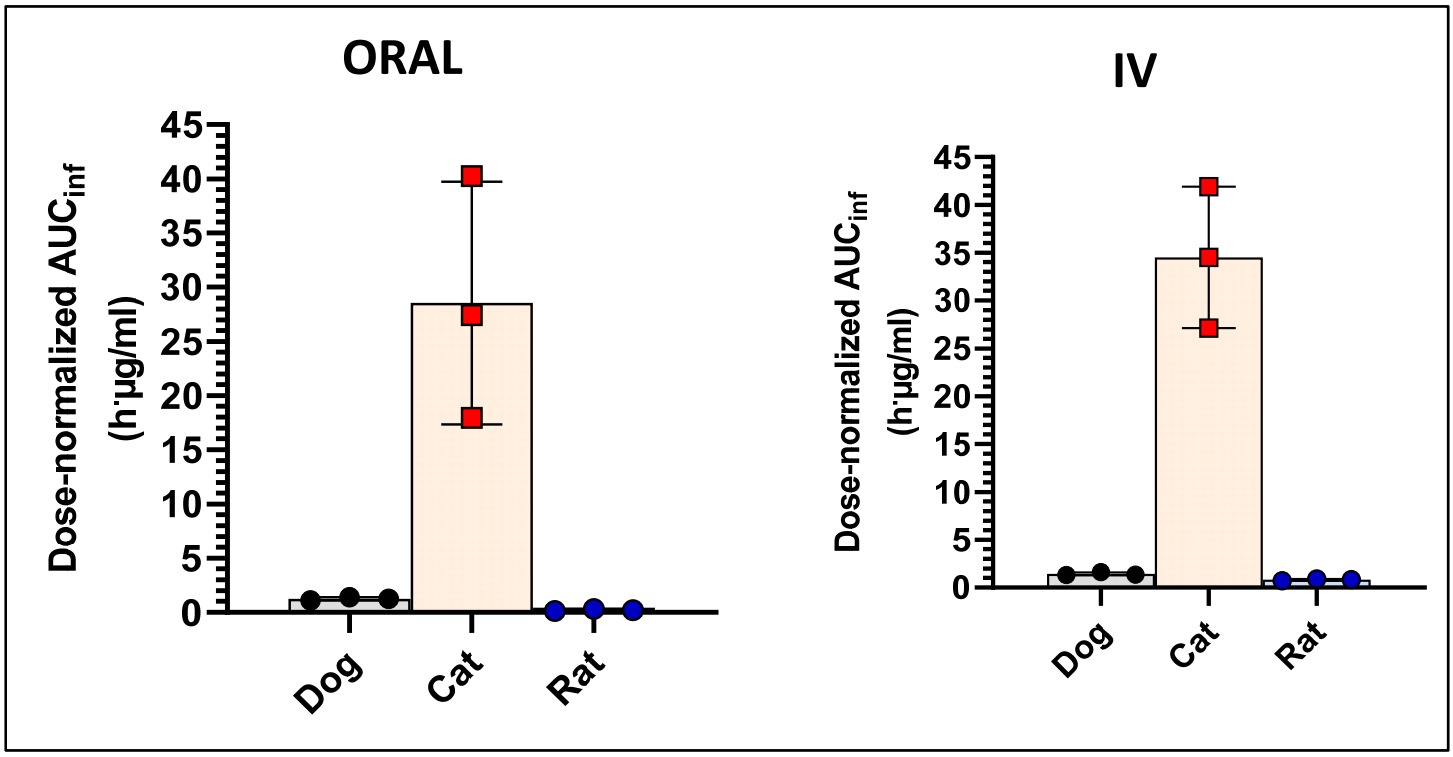


Figure 6. Comparison of dose-normalized exposure in three species after oral and IV administration of B-3(+). B-3(+) was orally or IV administered at the dose range of 0.5-5 mg/kg in dog, cat and rat. AUCinf, Area under the compound plasma concentration versus time curve from 0 to infinity normalized to the compound dose. Colored bars show data means; error bars show standard deviations. Each data point on the graph represents AUCinf determined for an individual animal.

We compared exposure in three species by analyzing dose-normalized AUCinf values. Dose-normalized AUC_{inf} values (AUC_{inf}/dose) were highest in cat (Figure 6) while significantly lower exposures were seen in rat and dog PK studies (Figure 6) after both oral and IV administration. It may be interesting note that the strongest evidence in support of B-3(+) efficacy in brain injury models was obtained in cat, which is consistent with the highest systemic (and, potentially, CNS) exposure after systemic administration.

D. Define the range of efficacious drug concentrations in the animal model of optic nerve damage (Major Task 3).

In the current grant period the guinea pig optic nerve crush animal protocol that was reviewed and approved by Columbia University IACUC committee was also approved by ACURO. This indicates that Major task 3 Subtask 1 was accomplished. Following the guinea pig animal protocol approvals by relevant committees, we conducted the optic nerve protection experiments in guinea pigs (Major Task 3, Subtasks 2-4). After performing pilot experiments, three guinea pig were anesthetize with isoflurane and optic crush model was surgically performed. Optic nerves were dissected and placed in cold 4% PFA for overnight incubation at 4 oC. On the next day, lipid fixation were performed using 1% osmium tetroxide in 0.01 M sodium-phosphate buffer for overnight at 4 oC. Prepared optic nerve samples were imbedded in paraffin and sectioned in 1-2 µm slices. In order to cut the thin 1-2µm sections from paraffin blocks, the blocks were pre-cooled for 30min at -20oC

followed by quick slicing at room temperature. To perform axon counting, we stained myelin in deparaffinized slices with 2% p- Phenylenediamine (PPD), at room temperature for 20 min. Prepared optic nerve slices will be imaged with 63X oil-immersion lenses.

What opportunities for training and professional development has the project provided?
Nothing to Report

How were the results disseminated to communities of interest?
Nothing to Report

What do you plan to do during the next reporting period to accomplish the goals?
We will continue to implement our research plan as outlined in the Statement of Work in order to accomplish the study objectives

4. IMPACT:

We successfully completed all objectives of Major Task 1 and Major Task 2 over the period of Grant Year 2. We built a strong foundation for successful completion of Major Tasks 3 a during the remaining grant period.

What was the impact on the development of the principal discipline(s) of the project?

We completed characterization of in vitro ADME properties of the drug candidate, identified its unusual effect on uric acid metabolism, and performed the comparative analysis of its pharmacokinetic properties in several species. We began the vivo efficacy experiments in the guinea pig optic nerve crush model.

What was the impact on other disciplines?
Nothing to Report

What was the impact on technology transfer?
Nothing to Report

What was the impact on society beyond science and technology?
Nothing to Report

5. CHANGES/PROBLEMS:

Changes in approach and reasons for change
Nothing to Report

Actual or anticipated problems or delays and actions or plans to resolve them
A delay in drafting and reviewing the animal protocol was encountered due to the general slowing of business operations in the first half of 2021. The work on the protocols has resumed and we will attempt to complete all remaining animal studies outlined in the proposal in a timely fashion.

Changes that had a significant impact on expenditures
Nothing to Report

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents
Nothing to Report

Significant changes in use or care of human subjects
Nothing to Report

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**
  - Nothing to Report

  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**
  Nothing to Report

- **Other Products**
  Nothing to Report

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Name:	Konstantin Petrukhin, Ph.D.
Project Role:	PI/PD
Researcher Identifier (eRA Commons):	KEPET4
Nearest person month worked:	2.2 (18% effort)
Contribution to Project:	Dr. Petrukhin has designed experiments and analyzed the data
Name:	Boglarka Racz, Ph.D.
Project Role:	Associate Research Scientist
Researcher Identifier (eRA Commons):	BORACZ
Nearest person month worked:	12.0 (100% effort)
Contribution to Project:	Dr. Racz was responsible for implementation of studies and participated in data analysis
Name:	Jeffrey Liebmann, M.D.
Project Role:	Co-Investigator
Researcher Identifier (eRA Commons):	LIEBMANNCU
Nearest person month worked:	0.6 (5% effort)
Contribution to Project:	Dr. Liebmann contributed to designing experiments and analyzing the data

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

Nothing to Report

What other organizations were involved as partners?

Nothing to Report

8. SPECIAL REPORTING REQUIREMENTS

Nothing to Report

9. APPENDICES:

Nothing to Report