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14. ABSTRACT Abstract: XXX aminopyrazoles, a new class of c-jun-N-terminal kinase (JNK) inhibitors, have been synthesized and the biochemical IC₅₀ has been determined for JNK3, JNK2, JNK1, and p38. In addition, these compounds have been tested in cell-based assays that monitor the inhibition of c-jun phosphorylation and some drug metabolism and pharmacokinetic (DMPK) properties have been measured. Moreover, two additional classes of JNK inhibitors have also been generated as backups. XXX compounds from the pyridopyrimidinone class have been synthesized and tested in biochemical and cell based assays, and XXX compounds from the amino acid transporter analog class have been made and tested in biochemical assays. The goal of this work is to find JNK3 isoform selective inhibitors. Eight novel aminopyrazoles have been developed with JNK3 selectivity > 20-fold, three novel compounds have been developed with JNK3 selectivity > 50-fold, one novel compound has been developed with JNK3 selectivity > 200-fold, and two compounds have cell-based IC₅₀s < 1 mM. SR-11935, a highly selective JNK2/3 isoform inhibitor from the aminopyrazoles class has been optimized for potency, selectivity, pharmacokinetics, and brain penetration and has been tested <i>in vitro</i> to see if it protects motor neurons from Tg SOD1^{G93A} mice from astrocyte-mediated toxicity. SR-11935 demonstrated near 100% protection of motor neurons from astrocyte-mediated toxicity at 50 nM indicating the high potency and <i>in vitro</i> efficacy of this JNK2/3 isoform selective inhibitor. In addition, SR-3306 and SR-11935 have been tested for efficacy <i>in vivo</i> in transgenic G93A SOD1 mice. Preliminary results show that SR-3306 and SR-11935, an aminopyrimidine and aminopyrazole, respectively, are well tolerated with no adverse effects after once daily dosing for 90 days at 30 mg/kg, and 40 mg/kg, respectively. Spinal cords and L4 ventral roots are being harvested for detailed histopathology studies to assess spinal MN loss, reactive gliosis, and numbers of myelinated axons. The tibialis anterior muscles will be likewise processed for neuromuscular junction denervation studies.		

15. SUBJECT TERMS ALS; aminopyrazoles; aminopyrimidines; aminopyrazoles; pyridopyrimidinones; c-jun; DMPK; JNK; SOD1					
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1. Table of Contents

Introduction: p. 1

Body p. 1-13: Table 1, p. 2-3; Table 2, p. 3; Table 3, p. 3-4; Table 4, p. 4-5; Table 5, p. 5-6; Table 6, p. 6; Table 7, p. 7-8; Table 8, p. 8; Table 9, p. 8; Table 10, p. 9; Table 11, p. 11; Figure 1, p. 10.

Key Research Accomplishments: p. 12

Reportable Outcomes: p. 12-13

Conclusions: p. 13

Publications: p. 13-14; Inventions, Patents and Licenses: p. 14

2. Introduction: The goal of this project is to test if JNK inhibitors are protective in *in vitro* and *in vivo* models of ALS based on mutations in SOD1. This will be done with JNK inhibitors generated from two different classes of compounds: amino pyrimidines and amino-pyrazoles. The amino pyrimidines are an existing class of compounds that allow for rapid initial *in vivo* analysis and the amino pyrazoles are a newly synthesized class of JNK inhibitors that have yet to be characterized. By utilizing two novel classes of compounds we aim to test if JNK inhibition can be effective in preventing neurodegeneration and motor deficits in ALS animal models. In addition, we went beyond the scope of the initial grant and generated two other back up classes of compounds: Pyridopyrimidinones and amino acid transporter analogs.

3. Body:

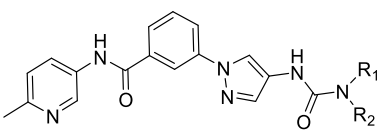
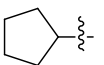
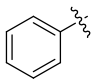
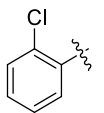
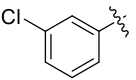
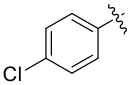
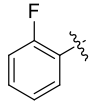
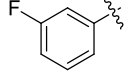
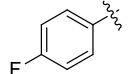
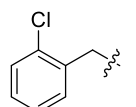
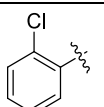
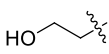
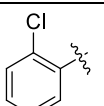
Aim-3: Synthesis, optimization, biochemical, cell biological and DMPK characterization of Amino-pyrazole JNK inhibitors, and Pyridopyrimidinone JNK Inhibitors

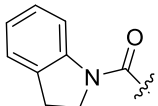
~ 250 novel amino pyrazoles have been synthesized in the past two years and these compounds have been tested in four different biochemical assays (JNK3, JNK2, JNK1, and p38). An HTRF biochemical assay was developed for are four enzymes described. In addition the cell-based potency of these compounds have been tested in SHSY5Y cells. This cell-based assay monitors the inhibition of c-jun phosphorylation in an In-cell Western assay format. In addition to this, we developed three other cell-based functional assays that monitor, mitochondrial membrane potential depolarization, mitochondrial ROS generation, and neurotoxin-induced cell death. Moreover, DMPK assays on select compounds have been executed for solubility, microsomal stability, and inhibition of four different cytochrome P450s.

Tables 1-6 present the biochemical IC₅₀ data for the key amino pyrazoles synthesized in the first and second year. The tables present data for JNK3, JNK2, JNK1, and p38. The IC₅₀ ± SE is presented along with the number of replicates (n) for each compound. In addition, the In-cell Western SHSY5Y cell-based IC₅₀ ± SE, the inhibition of 6-OHDA-induced cell death, and the

inhibition of 6-OHDA-induced mitochondrial membrane depolarization is presented along with the number of replicates (n) for each compound. Similarly, the cell-based IC₅₀, microsomal stability, the solubility, and CYP450 inhibition for all of the key JNK3 isoform selective compounds that represent the best-in-class aminopyrazole inhibitors are presented.

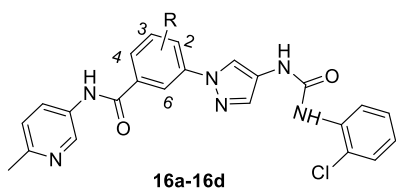
Table 1. Biochemical IC₅₀ values for JNK3 and JNK1 for SAR studies of the urea moiety

					
cmpd	R ₁	R ₂	JNK3 IC ₅₀ (nM)	JNK1 IC ₅₀ (nM)	JNK1/JNK3
8a	H		N/I ^b	N/I ^b	-
8b	H		115	138	-
8c	H		38	170	4.4
8d	H		596	N/I ^b	-
8e	H		1829	N/I ^b	-
8f	H		313	1607	5.1
8g	H		139	653	3.3
8h	H		162	400	2.5
8i	H		N/I ^b	N/I ^b	-
8j	Me		3063	N/I ^b	-
8k			N/I ^b	N/I ^b	-

8l		N/I ^b	N/I ^b	-
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^a IC₅₀ values are means of two or more experiments (with triplicate replicates for each experiment) with errors within 80% of the mean. ^b No inhibition up to 10 μM.

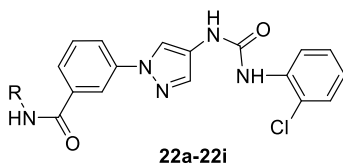
Table 2. Biochemical IC₅₀ values for JNK3 and JNK1 for SAR studies for the middle phenyl moiety

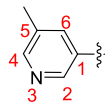
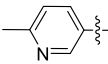
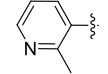
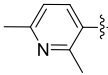


cmpd	R	JNK3 IC ₅₀ (nM)	JNK1 IC ₅₀ (nM)	JNK1/JNK3
16a	2-F	80	2369	29.6
16b	3-F	4588	N/I ^b	-
16c	4-F	71	180	2.5
16d	6-F	230	3691	16.1

^a IC₅₀ values are means of two or more experiments (with triplicate replicates for each experiment) with errors within 80% of the mean. ^b No inhibition up to 10 μM.

Table 3. Biochemical IC₅₀ values for JNK3 and JNK1 for SAR studies for the amide moiety^a



cmpd	R	JNK3 IC ₅₀ (nM)	JNK1 IC ₅₀ (nM)	JNK1/JNK3
SR-4326		117	2169	18.5
8c		38	170	4.4
22b		141	2689	19
22c		141	1028	7

22d		N/I ^b	N/I ^b	-
22e		5184	N/I ^b	-
22f		98	2740	28
22g		130	4544	35
22h		62	537	9
22i		311	7791	25

^a IC₅₀ values are means of two or more experiments (with triplicate replicates for each experiment) with errors within 80% of the mean. ^b No inhibition up to 10 μ M.

Table 4. Biochemical IC₅₀ values for JNK3 and JNK1 for SAR studies for the amide moiety

cmpd	R	JNK3 IC ₅₀ (nM)	JNK1 IC ₅₀ (nM)	JNK1/JNK3
26a		133	2057	16
26b		203	2625	28
26c		162	3162	20
26d		116	1284	11
26e		594	5385	9
26f		114	5046	44
26g		34	758	22

26h		21	1249	60
26i		23	1226	54
26j		23	679	30
26k		< 1	528	>500
26l		137	4495	33
26m		100	4728	47
26n		206	N/I ^b	>50
26o		169	N/I ^b	>60
26p		34	1873	55
26q		29	2756	94

^a IC₅₀ values are means of two or more experiments (with triplicate replicates for each experiment) with errors within 80% of the mean. ^b No inhibition at 10 μ M.

Table 5. Inhibitor selectivity and *in vitro* DMPK data

cmpd	R	JNK2 IC ₅₀ (nM)	p38 α IC ₅₀ (nM)	Microsomal stability T _{1/2} (min) ^b		Cyp-450 % inh. at 10 μ M 1A2/2C9/2D6/3A4	Solubility in 1% DMSO/buffer (μ M)	
				Human	Mouse		pH 3.5	pH 7.4
22f		2836	N/I ^c	32	26	17 / 60 / 25 / 44	23	0.2
22g		561	N/I ^c	28	6	10 / 19 / -25 / 26	23	0.2
22i		428	N/I ^c	11	17	17 / 48 / 83 / 49	29	0.9
16a^d		148	N/I ^c	50	86	19 / 76 / 93 / 10	9	0.5

26f		158	3623	39	37	15 / 49 / 54 / 16	2	1.4
26g		66	N/I ^c	35	4	-5 / 12 / 23 / -9	71	72
26j		25	N/I ^c	408	54	3 / 22 / 35 / -16	63	53
26k		210	N/I ^c	113	33	38 / 66 / 43 / 70	n.d ^b	n.d ^b
26l		N/I ^c	N/I ^c	40	6	-12 / -1 / 17 / 15	37	45
26n		311	N/I ^c	305	72	-8 / 1 / 8 / 4	151	79

^a IC₅₀ values are means of two or more experiments (with triplicate replicates for each experiment) with errors within 80% of the mean. ^b Not determined. ^c No inhibition up to 10 μ M. ^d With fluoro-substitution on the middle phenyl ring at Region B.

Table 6. Cytotoxicity and cell based potency data for selected compounds.

cmpd	Cytotoxicity in SHSY5Y cells after 48 hrs		In-cell Western ^a	Inhibition of 6-OHDA induced cell death	Inhibition of 6-OHDA induced mitochondrial membrane depolarization
	% cell viability at 10 μ M	% cell viability at 30 μ M	SHSY5Y IC ₅₀ (nM)	SHSY5Y IC ₅₀ (nM)	SHSY5Y IC ₅₀ (nM)
22f	7	5	866	2976	40
22g	24	5	2331	n.d ^b	n.d ^b
22i	80	56	905	13	17
26f	84	16	3250	n.d ^b	130
26j	91	98	1436	568	25
26g	105	97	N/I ^c	N/I ^c	N/I ^c
26k	112	97	N/I ^c	N/I ^c	31
26n	92	98	1895	281	4

^a IC₅₀ values are means of two or more experiments (with triplicate replicates for each experiment) with errors within 80% of the mean. ^b Not determined. ^c No inhibition up to 10 μ M.

These data are all in press in the *Journal of Medicinal Chemistry*.

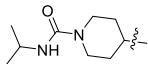
Zheng, Ke, Iqbal, Sarah, Hernandez, Pamela, Park, Hajeung, **LoGrasso, Philip V.**, and Feng, Yangbo. 2014.

Design and Synthesis of Highly Potent and Isoform Selective JNK3 Inhibitors: SAR Studies on Aminopyrazole Derivatives. *J. Medicinal Chemistry*. In Press.

We characterized the ~ 80 pyridopyrimidinones we synthesized in a similar manner to the aminopyrazoles. Table 7-10 present the biochemical IC₅₀ data for the key pyridopyrimidinones synthesized in the second year. The tables present data for JNK3, JNK2, JNK1, and p38. The IC₅₀ ± SE is presented along with the number of replicates (n) for each compound. In addition, the In-cell Western SHSY5Y cell-based IC₅₀ ± SE, the inhibition of 6-OHDA-induced cell death, and the inhibition of 6-OHDA-induced mitochondrial membrane depolarization is presented along with the number of replicates (n) for each compound. Similarly, the cell-based IC₅₀, microsomal stability, CYP450 inhibition, and pharmacokinetic parameters for all of the key compounds that represent the best-in-class pyridopyrimidinone inhibitors are presented.

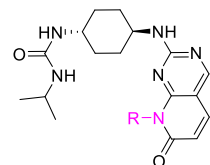
Table 7. SAR studies for the *trans*-cyclohexanol moiety.

cmpd	R	Biochemical Inhibition IC ₅₀ ^a (nM)		
		JNK3	JNK2	JNK1
1		58	18	18
7		532	241	318
8		N/I ^b	nd ^c	N/I ^b
9		N/I ^b	nd ^c	3480
10		201	nd ^c	80
11		31	29	48
12		57	26	23
13		15	66	21
14		81	65	40
15		321	143	171
16		37	61	31
17		162	160	400
18		54	114	85

19		28	nd ^c	36
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^aIC₅₀ values are the mean of two or more experiments with errors within 40% of the mean. ^bNo inhibition at 10 μM. ^cNot determined.

Table 8. SAR studies for the *N*-substitutions on pyridoamide moiety.



cmpd	<i>N</i> -R	Biochemical Inhibition IC ₅₀ ^a (nM)		
		JNK3	JNK2	JNK1
20	H	2166	3450	1988
21	Me	238	463	303
13		15	66	21
22		11	12	7
23		204	434	438

^aIC₅₀ values are the mean of two or more experiments with errors within 40% of the mean.

Table 9. Data for p450 inhibition, microsomal stability, and c-Jun phosphorylation for lead JNK inhibitors.

cmpd	CYP-450 % inh. ^a	Mic. stability t _{1/2} (min)		c-Jun phosphorylation IC ₅₀ (nM) ^b
	1A2/2C9/2D6/3A4	Human	Mouse	
11	11/28/18/33	44	19	1232
12	1/19/2/16	46	17	1015
13	10/-26/-58/14	76	22	1733
16	38/47/15/50	16	8	nd ^c
18	-12/10/-20/-2	65	14	990
22	-5/-7/-30/53	7	5	nd ^c

^a% inh. at 10 μM. ^bData were the average of ≥ 2 experiments performed in SHSY5Y cells. ^cNot determined.

Table 10. *In vivo* PK data in mice for selected lead JNK inhibitors^a

compd	C _{max} ^a (μM), i.v	AUC ^a (μM.h), i.v	t _{1/2} ^a (h), i.v	Cl (i.v) ^a mL/min.kg	V _d (i.v) ^a L/kg	%F ^a
11	1.0	0.7	0.5	33	1.3	66
12	0.6	0.3	0.3	72	1.9	100
13	0.9	0.8	1.1	28	1.8	87
18	1.1	0.7	0.4	33	1.0	43

^a Data were generated from three determinations, and dosed at 0.5 mg/kg for i.v and at 3 mg/kg for p.o.

These data have all been submitted to *ACS Med Chem Letters* and are under review.

Ke Zheng, Chul Min Park, Sarah Iqbal, Pamela Hernandez, HaJeung Park, Philip V. LoGrasso, Yangbo Feng 2014. *ACS Med Chem Letters* , Submitted.

Pyridopyrimidinone Derivatives as Potent and Selective JNK inhibitors

Aim-2: Preclinical testing of JNK inhibitors

Hypothesis

Our preliminary *in vitro* data demonstrate that pharmacological inhibition of the pan-stress activated protein kinases –JNKs- with the small molecule SR-3306 completely prevents ALS astrocyte-induced motor neuron (MN) death in a mouse model of familial ALS (fALS). Given this promising data, we hypothesized that fALS astrocytes kill MNs via a JNK-dependent signaling pathway and that inhibitors of the JNK cascade are promising neuroprotective agents for the treatment of ALS.

As SR-3306 has previously been validated and optimized in an *in vivo* model of Parkinson's disease, our first objective was to test the beneficial effects of this compound in an animal model of familial ALS, the transgenic mouse overexpressing mutant human superoxide dismutase-1 SOD1^{G93A} (Tg SOD1^{G93A}). We performed daily gavage on Tg SOD1^{G93A} mice with either SR3306 (30 mg/kg) or with the vehicle (1% hydroxypropylmethylcellulose in distilled water) from presymptomatic stage (postnatal day 50, P50) to end stage (~P150). Non-transgenic (NTg) littermate mice received a similar dosing regimen and we didn't observe any adverse side effects. Unfortunately, compared to the vehicle treated Tg SOD1^{G93A} mice, Tg SOD1^{G93A} animals treated with SR3306 didn't exhibit any signs of neuroprotection as attested by behavioral tests or histopathology analysis.

Our goals were then to 1) understand the lack of neuroprotective effect of SR3306 in Tg SOD1^{G93A} animals, 2) test in an *in vitro* model of fALS the neuroprotective property of another of JNK inhibitor, SR11935 (26n), and 3) optimize the delivery of SR3306 or S11935 (26n) in Tg SOD1^{G93A} mice and test their neuroprotective effects.

Key Accomplishments

1) Pharmacokinetic study of SR-3306 long time delivery by gavage in Tg SOD1^{G93A} mice.

Prior embarking into the study of SR-3306 delivery in Tg SOD1^{G93A} mice, we performed pharmacokinetic analysis on NTg mice. We found that daily gavage of mice with SR3306 (30 mg/kg/day) for 42 days resulted in 400 nM of the drug in the brain. Because of the lack of neuroprotective effect of SR-3306 in our preclinical study, we then compared the SR-3306 brain levels in Tg SOD1^{G93A} and NTg mice. We found that after 100 days of daily gavage with SR-3306 (30 mg/kg/day), the SR-3306 brain concentrations were~65% lower in Tg SOD1^{G93A} mice than in the NTg mice. We haven't clarified the difference in brain concentration between NTg and Tg SOD1^{G93A} mice, but it could explain the lack of neuroprotective effect of SR-3306 in transgenic animals.

2) *In vitro* testing of SR11935 in mouse model of fALS

In contrast to the ubiquitously expressed JNK1 and JNK2, JNK3 is almost exclusively expressed in the central nervous system, with very low level expression in the heart and testes. As SR-3306 is a modest inhibitor of JNK3 and as we had negative results with this drug administered orally, we decided to test in parallel a more selective JNK3 inhibitor with good brain penetration: SR-11935 (26n). We first tested SR-11935 in our *in vitro* model of fALS. In this model, astrocytes layers (or their supernatant (ACM)) originating from Tg SOD1^{G93A} mice induce selective death of wild-type motor neurons (MNs). We found that similarly to SR-3306, treatment of MN culture with 50 nM or 100 nM of SR-11935 abolishes the fALS astrocyte mediated toxicity (see figure 1)

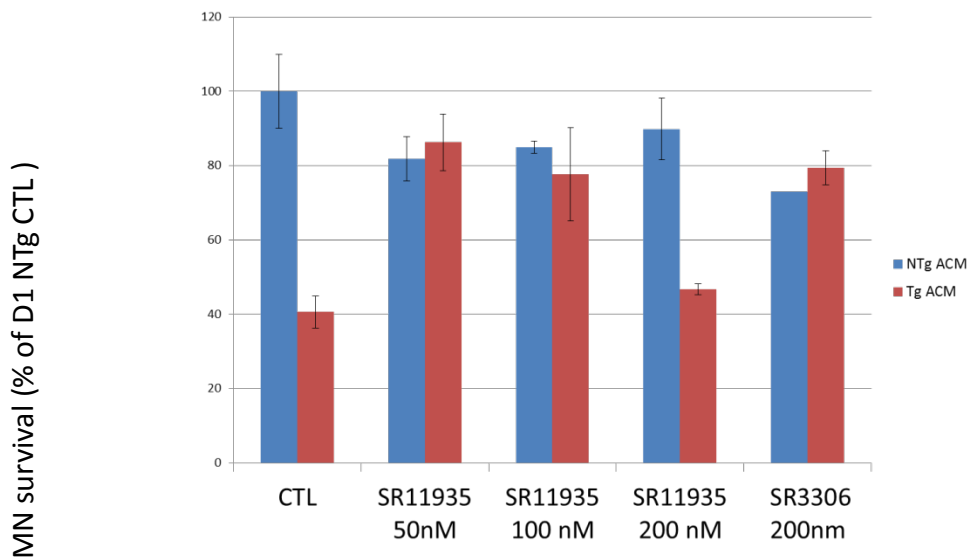


Figure 1: SR11935 protects motor neurons exposed to Tg SOD1^{G93A} astrocyte conditioned medium

3) Pharmacokinetic and neuroprotective studies of SR3306 and SR11935 (26n) long time delivery via subcutaneous pump in Tg SOD1^{G93A} mice

a) Pharmacokinetic study of SR-3306 and SR-11935 (26n) delivered via osmotic subcutaneous pumps in NTg mice.

As daily oral delivery failed to deliver significant levels of SR3306 in Tg SOD1^{G93A}, we decided to use another route of administration that would allow continuous drug delivery and bypass any potential intestinal absorption problem in Tg SOD1^{G93A} mice. For this purpose, we implanted subcutaneous osmotic pumps filled with SR3306 (83 mg/ml of 30 % hydroxybetacyclodextrin (OH-BCD) in distilled water) or SR-11935 (26n) (167 mg/ml of 30 % OH-BCD) in NTg mice for 4 weeks. After 4 weeks, we collected blood, brain, spinal cord and the content of the pumps. We found that after 1 month in the osmotic pump at 37°C, the concentration of SR-3306 in the pump decreased by only ~15% and that the concentration of SR-11935 remained stable, indicating that both drugs are stable and suitable for long term delivery via osmotic pumps. Plasma, brain and spinal cord concentrations are indicated in the table 1. Overall, this preliminary data indicate that both drugs reach the central nervous system and that their concentration level is compatible with an efficient JNK inhibition.

	<i>Plasma</i>		<i>Brain</i>		<i>Spinal Cord</i>	
	Conc.(ng/mL)	Conc. (in uM)	Conc.(ng/mL)	Conc. (in uM)	Conc.(ng/mL)	Conc. (in uM)
SR3306: 20mg/kg/day	36.4	0.07	27.6	0.06	55.8	0.11
SR11935: 40mg/kg/day	190.0	0.42	144.5	0.32	12.3	0.03

Table 11: Pharmacokinetic data of SR-3306 and SR-11935 delivery via subcutaneous osmotic pump for 1 month

b) Preclinical testing of SR-3306 and SR-11935 (26n) in transgenic SOD1^{G93A} mice

Osmotic subcutaneous pumps containing SR3306, SR11935 (26n) or the vehicle were surgically implanted in Tg SOD1^{G93A} mice. Transgenic SOD1^{G93A} mice received 30 mg/kg/day of SR3306 (n=8) or 40 mg/kg/day of SR11935 (n=8) or the vehicle (30% OH-BCD, n=9). Non transgenic littermate mice also received subcutaneous pumps filled vehicle. For each mouse, a pump was implanted from P30 to P58, and then replaced by a new pump until P100. A third pump is currently implanted in mice until they reach end stage (~P150) in ~2 weeks.

Body weight is monitored once a week and no adverse effects due to drug administration or repetitive surgeries were observed. Starting at P42, we perform once a week a grip strength test (loaded grid test).

At end stage, we will cut the tail of each mouse to check the genotype and the transgene copy number for which we have a validated RT-PCR protocol. An intracardiac blood draw will be performed for hematology and serum chemistry. Finally, mice will be perfused and the major

organs (heart, spleen, intestines, kidney, and liver) will be collected for clinical pathology. Spinal cords and L4 ventral roots will be harvested for detailed histopathology studies to assess spinal MN loss, reactive gliosis, and numbers of myelinated axons. The tibialis anterior muscles will be likewise processed for neuromuscular junction denervation studies.

Reportable Outcomes

Considerable progress is being made on the preclinical study of JNK inhibitors in the Tg SOD1^{G93A} mice.

- We have found that the pharmacokinetic of SR-3306 differs between Tg SOD1^{G93A} and NTg mice, resulting in lower SR3306 brain levels in Tg SOD1^{G93A} mice.
- We have identified that another JNK inhibitor targeting more selectively the JNK3 isoform, SR11935 (26n), was also conferring neuroprotection in our *in vitro* fALS model.
- We have performed pharmacokinetic study in NTg mice for the subcutaneous delivery of SR-3306 and SR-11935 (26n) via osmotic pumps. We are currently testing the neuroprotective effects of each of these drugs in Tg SOD1^{G93A} mice. So far, there is no statistical difference in behavioral scores between the three groups of Tg SOD1^{G93A} mice that received the SR3306, SR11935 or the vehicle. Until further investigation of the JNK inhibitors levels in the brain and spinal cord, it suggests that JNK inhibitor don't exert a neuroprotective effect on the neuromuscular junction. More in depth histological studies on muscles and spinal cords need to be completed at end stage in order to assess the level of muscle denervation and whether the JNK inhibitors could still prevent spinal motor neuron death in Tg SOD1^{G93A} mice.

4. Key research accomplishments and reportable outcomes for entire project: The key research accomplishments from the entire project are summarized below.

- > 50g of SR-3306 (an amino pyrimidine) has been synthesized for *in vivo* use
- > 1g SR-3562 (second amino pyrimidine) has been synthesized for *in vivo* use
- Synthesis of three other amino pyrimidines completed (SR-2502, SR-3058, and SR-4073)
- Eighteen novel amino pyrazoles have been designed with > 20-fold JNK3 selectivity
- Seven novel amino pyrazoles have been designed with > 50-fold JNK3 selectivity
- One novel amino pyrazoles have been designed with > 500-fold JNK3 selectivity
- five novel amino pyrazoles have been designed with functional activity in preventing mitochondrial dysfunction with cell-based potency < 50 nM
- Good DMPK properties designed into amino pyrazoles class

- assessment of amino pyrimidine JNK inhibitors and JNK2/3 isoform selective aminopyrazoles inhibitors show efficacy in protecting primary motor neurons *in vitro*
- SR-11935 (26n) is a selective JNK2/3 inhibitor with >50-fold selectivity over JNK1; it was found not to inhibit 464 other kinases at 10 μ M giving it one of the highest selectivity profiles of any kinase inhibitor for any class of compounds. In addition SR-11935 (26n) potently protected primary motor neurons from astrocyte-mediated cytotoxicity at concentrations as low as 50 nM. This dose gave near 100% protection of the motor neurons.
- *in vivo* assessment of one amino pyrimidine (SR-3306) and one aminopyrazoles (SR-11935, 26n) had no adverse effects after long term (> 90 days) dosing
- *in vivo* efficacy for both compounds nearing completion
- A second class of potent selective JNK inhibitors have been synthesized
- The pyridopyrimidinones are highly potent. SR-12519 had JNK3 IC_{50} = 15 nM and had good pharmacokinetic properties with oral bioavailability, %F= 87
- The pyridopyrimidinones (SR-12519) were potent inhibitors of mitochondrial dysfunction showing protection in the range of 40 nM.

5. Conclusions: Significant progress has been made during the funding period for this project. First, ~250 novel aminopyrazoles have been synthesized and tested in four different biochemical assays. In addition, many of the potent, selective compounds have been tested in four different cell-based assays. The JNK2/3 isoform selective inhibitors such as SR-11935 (26n) showed protection against functional mitochondrial loss, and protection against motor neuron death at concentrations of < 50 nM suggesting JNK2/3 isoform selective inhibitors are highly potent and efficacious in protecting motor neurons and mitochondrial function. Drug metabolism and pharmacokinetic properties have been optimized for JNK2/3 isoform selective inhibitors. The *in vivo* efficacy model has been established for amino pyrimidine pan JNK inhibitors and the aminopyrazole JNK2/3 selective inhibitors have been shown to be safe and well tolerated. Final analysis of efficacy will be established in the next 3 months.

6. Publications:

1.) Zheng, Ke, Iqbal, Sarah, Hernandez, Pamela, Park, Hajeung, LoGrasso, Philip V., and Feng, Yangbo. 2014.

Design and Synthesis of Highly Potent and Isoform Selective JNK3 Inhibitors: SAR Studies on Aminopyrazole Derivatives. *J. Medicinal Chemistry*. In Press.

2.) Park, Hajeung, Iqbal, Sarah, Hernandez, Pamela, Mora, Rudy, Zheng, Ke, Feng, Yangbo, and LoGrasso, Philip V. 2014.

Structural Basis and Biological Consequences for JNK2/3 Isoform Selective Aminopyrazoles. *Scientific Reports*. Under Revision.

3.) Ke Zheng, Chul Min Park, Sarah Iqbal, Pamela Hernandez, HaJeung Park, Philip V. LoGrasso, Yangbo Feng 2014.

Pyridopyrimidinone Derivatives as Potent and Selective JNK inhibitors. *ACS Med Chem Letters* , Submitted.

7. Inventions, Patents and Licenses: International Patent Application no. PCT/US2104/068333

“Novel Compounds as JNK Kinase Inhibitors”