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Design, synthesis and optimization of novel Alk5 (activin-like kinase 5) inhibitors

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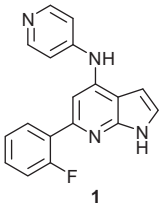
ABSTRACT

Using SBDD, a series of 4-amino-7-azaindoles were discovered as a novel class of Alk5 inhibitors that are potent in both Alk5 enzymatic and cellular assays. Subsequently a ring cyclization strategy was utilized to improve ADME properties leading to the discovery of a series of 1*H*-imidazo[4,5-*c*]pyridin-2(3*H*)-one drug like Alk5 inhibitors.

Transforming growth factor β (TGF- β) belongs to the TGF- β protein superfamily involved in cellular proliferation, cellular differentiation and other regulatory cell functions. It plays a role in immunity, cancer, heart disease and fibrosis.[1,2] TGF-β signals through a family of transmembrane serine/threonine kinase receptors. These receptors can be divided into two classes, the type I or activin-like kinase (Alk5) receptors and type II receptors.[3,4]

Signaling through these receptors starts when TGF-β ligand first binds to a type II receptor which then recruits and phosphorylates a type I receptor, Alk5. The Alk5 receptor then recruits and phosphorylates cytoplasmic protein SMADs which form heteromeric complexes that enter the cell nucleus to affect gene transcription.[5,6] Modulating Alk5 is thought to have therapeutic potential for metastatic cancer[7] and fibrotic disease.[8] Therefore we sought to develop a series of small molecule inhibitors of this target.

Screening of our internal compound collection afforded the early hit molecule **1** which displayed good Alk5 enzymatic potency as well as strong cellular activity (Fig. 1). The compound however, had poor drug like properties including elevated CYP inhibitory activity and metabolic instability. A discovery program was initiated to expand the SAR and improve the drug-likeness of **1**. These efforts are the focus of this publication.



1

Alk5 pKd = 8
Alk5 pEC_{50} = 7.3
CYP1A2 pIC_{50} = 6.14
$Cl_{int(rat)}$ (mL/min/kg) = 642
Eh (rat) = 0.9

Figure 1. Screening hit **1**.

A synthetic sequence to access compound **1** was developed and used to explore this series (Scheme 1). Starting with commercially available Boc protected bromo azaindole **1a**, Buchwald amination with 4-amino pyridine afforded **1b**. Further Suzuki coupling of this intermediate with 2-fluorophenyl boronic acid gave compound **1**. Variations of this scheme were then used to access a range of differentially substituted analogs.

Modeling of compound **1** into a crystal structure of the active site of the Alk5 kinase domain[9] (Fig. 2) suggested that the compound was anchored to the kinase hinge via a hydrogen bond between the ligand's pyridyl nitrogen and the backbone NH of His-283. Additionally the adjacent carbon CH forms a pseudo hydrogen bond[10] to the backbone carbonyl of Asp-281. The azaindole nitrogens appear to insert into a hydrogen bond network

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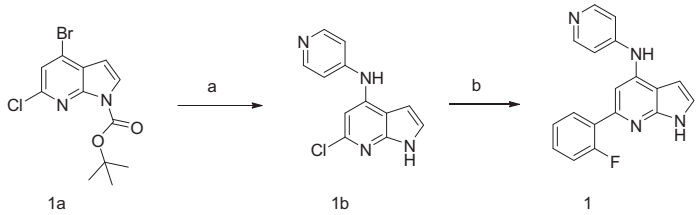


Scheme 1. Reagents and conditions: (a) pyridin-4-amine, sodium *t*-butoxide, tris(dibenzylideneacetone)dipalladium(0), Xantophos, 1,4-dioxane, 95 °C; (b) 2-fluorophenylboronic acid, bis(triphenylphosphine)palladium chloride, sodium carbonate (2 M aq), DMF, 175 °C.

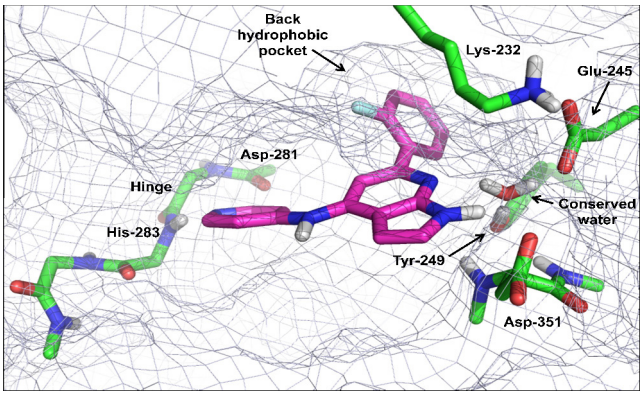


Figure 2. Model of compound **1** in the active site of Alk5.

defined by the conserved salt bridge residues Lys-232, Asp-351, Glu-245, Tyr-249 through a conserved water. Finally, the 2-fluorophenyl group is directed into the back hydrophobic pocket of the Alk5 enzyme which is delineated by Tyr-249, Phe-262, Leu-278, and gatekeeper Ser-280.

With this model, the hydrophobic pocket was initially targeted using key moieties previously disclosed within Alk5 literature.[11] This was accomplished by varying the group at position C-6 of the azaindole core while holding the aminopyridine group at position C-4 constant (Table 1).

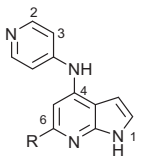
Installation of 2,4-difluorophenyl group at position C6 on the azaindole core gave good enzymatic potency (compound **2**). However exchange of 2,4 for 3,4-difluoro (compound **3**) was detrimental to enzymatic activity. This suggested that ortho substitution aided the biaryl structure adopting a favorable conformation. Incorporation of 6-methylpyridin-2-yl, 5-chloro-2-fluorophenyl or 5-chloro-2,4-difluorophenyl groups at position C6 on the azaindole core led to potent analogs **4**, **5** and **6**. Compounds **2** and **6** showed good enzymatic and cellular potency with moderate in vitro clearance (rat). Subsequent PK studies indicated that compound **2** had good bioavailability (F = 65%) while compound **6** had low bioavailability (F = 13%). However compound **2** and **6** suffered from elevated CYP inhibitory activity which may at least partly attributed to the unblocked basic pyridine site. Attempts to replace the pyridine ring with the pyrimidine ring afforded only a modest decrease in CYP2C8 and CYP3A4 inhibition (data not shown). Blocking pyridine ring at C-2 position resulted in loss of potency significantly (data not shown).

To decrease CYP inhibition, we have investigated incorporation of electron withdrawing group (EWG) at the C-3 position of the pyridine ring (Table 2). Starting with commercially available compound **7a** (Scheme 2), the reaction with cyclopropylamine afforded **7b**, followed by Buchwald amination with *tert*-butyl 4,6-dichloro-1*H*-pyrrolo[2,3-*b*]pyridine-1-carboxylate to give **7c**. Suzuki coupling of this intermediate with 2,4-difluorophenyl boronic acid yielded **7d**, which upon treatment with TFA afforded target molecule **7**.

Installation of electron withdrawing group R^1 at C-3 of the pyridine ring dramatically decreased CYP inhibition as shown in analogs **7**–**12** while the potency was maintained (Table 2). This was attributed to the decreased basicity of the pyridine and a lower overall lipophilicity (Compound **9**: calculated basic pK_a 6.7, *c*Log*D* 1.7; compound **2**: calculated basic pK_a 7.9, *c*Log*D* 2.7). 5-Chloro-2-fluorophenyl group was also explored at R^2 on position C-6 of azaindole ring (compound **10**).

Based on the cellular potency, CYP profile and in-vitro clearance, compound **10** was selected to be further studied for PK in rats. Unfortunately this molecule had low bioavailability (F = 17%). So we further explored the ring cyclization strategy in hope of identifying a better molecule, which gave analogs **13** to

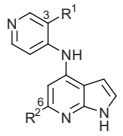
Table 1
Selected data for pyrrolopyridine analogs

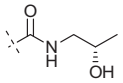
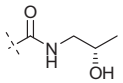
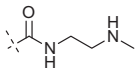
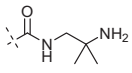


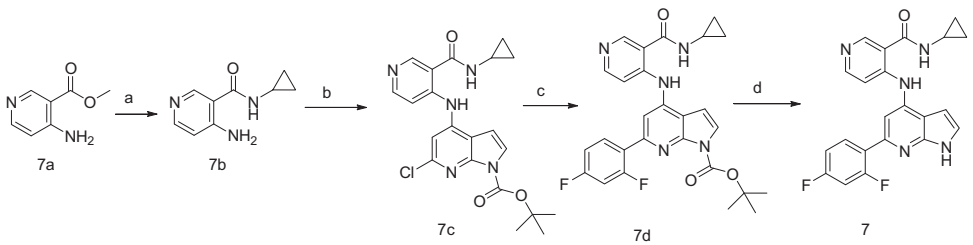
Compd	R	pIC$_{50}$[12]	pEC$_{50}$[13]	CYP1A2 pIC$_{50}$	CYP2C8 pIC$_{50}$	CYP3A4 pIC$_{50}$	Cl$_{int(rat)}$* (mL/min/kg)
1	2-Fluorophenyl	–	7.3	6.1	<4.3	5.6	642
2	2,4-Difluorophenyl	7.5	7.6	6.5	7.1	5.9	41
3	3,4-Difluorophenyl	4.2	–	6.7	6.2	5.7	–
4	6-Methylpyridin-2-yl	7.1	6.8	<4.3	5.4	4.6	1188
5	5-Chloro-2-fluorophenyl	8.5	7.7	–	–	–	118
6	5-Chloro-2,4-difluorophenyl	7.4	7.7	6.0	7.0	<6.1	29

* In vitro intrinsic clearance (microsomes).

Table 2
Selected analogs with electron withdrawing group R^1 at position C-3 of pyridine ring

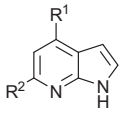


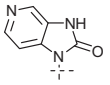
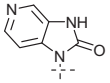
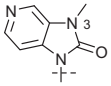
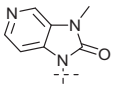
Compd	R^1	R^2	pIC_{50}	pEC_{50}	CYP1A2 pIC_{50}	CYP3A4 pIC_{50}	Cl_{int} (rat) (mL/min/kg)
2	H	2,4-Difluorophenyl	7.5	7.62	6.5	5.9	41
7		2,4-Difluorophenyl	7.9	7.78	<4.3	<4.3	78
8		5-Chloro-2-fluorophenyl	7.7	7.65	<4.3	4.8	70
9		2,4-Difluorophenyl	7.8	7.75	<4.3	<4.3	80
10		5-Chloro-2-fluorophenyl	7.9	8.04	<4.3	<4.3	37
11		2,4-Difluorophenyl	7.9	7.29	<4.3	<4.3	117
12		2,4-Difluorophenyl	6.4	–	<4.3	<4.3	34

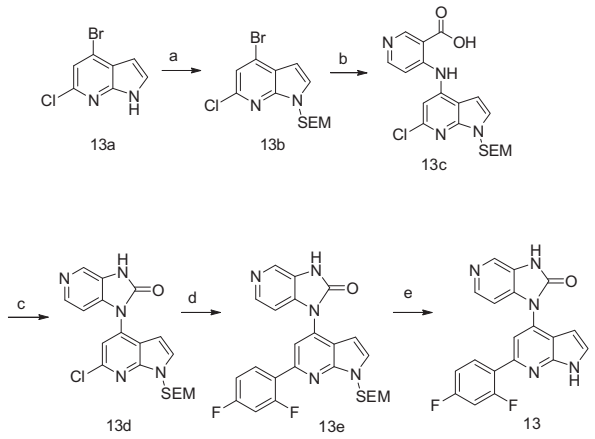


Scheme 2. Reagents and conditions: (a) $AlMe_3$, cyclopropanamine, DCE, 0°-70 °C; (b) *tert*-butyl 4,6-dichloro-1*H*-pyrrolo[2,3-*b*]pyridine-1-carboxylate, dicyclohexyl(2′,4′,6′-triisopropyl-[1,1′-biphenyl]-2-yl)phosphine, $Pd_2(dba)_3$, potassium carbonate, *t*-BuOH, 100 °C; (c) 2,4-difluorophenylboronic acid, bis(triphenylphosphine)palladium chloride, sodium carbonate (2 M aq), DMF, 175 °C; (d) TFA, DCM.

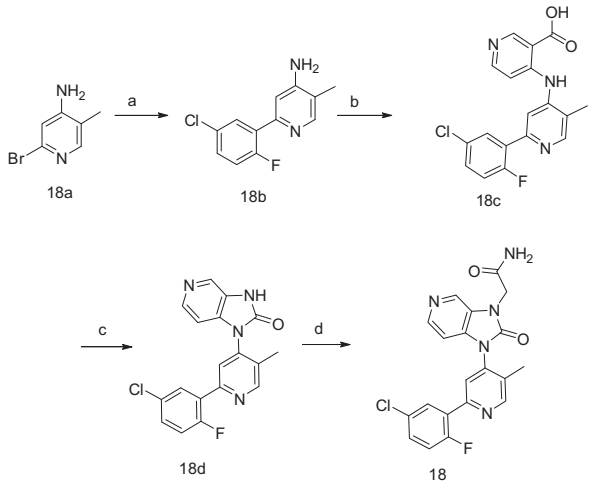
Table 3
Selected data for ring cyclized pyrrolopyridine analogs



Compd	R^1	R^2	pIC_{50}	pEC_{50}	CYP1C2 pIC_{50}	CYP3A4 pIC_{50}	Cl_{int} (rat) (mL/min/kg)
2	H	2,4-Difluorophenyl	7.5	7.62	6.5	5.9	41
13		2,4-Difluorophenyl	7.9	7.75	<4.3	<4.3	160
14		5-Chloro-2-fluorophenyl	8.4	8.02	5.2	5.5	197
15		2,4-Difluorophenyl	7.7	–	4.7	5.4	272
16		5-Chloro-2-fluorophenyl	8.2	–	5.8	6	204



Scheme 3. Reagents and conditions: (a) (2-(chloromethoxy)ethyl)trimethylsilane, sodium hydride, DMF, 0 °C–rt; (b) methyl 4-aminonicotinate, Tris(dibenzylideneacetone)dipalladium(0), (9,9-dimethyl-9*H*-xanthene-4,5-diyl)bis(diphenylphosphine), sodium *t*-butoxide, 1,4-dioxane; 130 °C; (c) DPPA, TEA, Toluene, reflux; (d) 2,4-difluorophenylboronic acid, sodium carbonate, Bis(triphenylphosphine)palladium(II) dichloride, 1,4-dioxane, 130 °C; (e) TBAF, ethylene diamine, THF, 130 °C.



Scheme 4. Reagents and conditions: (a) (5-chloro-2-fluorophenyl)boronic acid, sodium carbonate, Bis(triphenylphosphine)palladium(II) dichloride, 1,4-dioxane, 95 °C; (b) 4-chloronicotinic acid, Tris(dibenzylideneacetone)dipalladium(0), (9,9-dimethyl-9*H*-xanthene-4,5-diyl)bis(diphenylphosphine), sodium t-butoxide, 1,4-dioxane; 95 °C; (c) diphenyl phosphorazidate, triethylamine, toluene, reflux; (d) 2-chloroacetamide, cesium carbonate, DMF, 120 °C.

16 (Table 3). The synthesis of these analogs is described in Scheme 3. The commercially available 4-bromo-6-chloro-1*H*-pyrrolo[2,3-*b*]pyridine (**13a**) was protected with SEM group first and then subjected to Buchwald amination with methyl 4-aminonicotinate to provide **13c**. A Curtius rearrangement then afforded the imidazolidinone ring (**13d**). Suzuki coupling with 2,4-difluorophenyl boronic acid, followed by SEM deprotection with TBAF gave the target **13**.

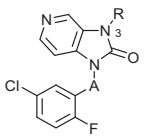
Compounds **13**–**16** showed high enzymatic and cellular potency. However, they suffered from high clearance.[14] Based on the activities of compounds **15** and **16**, a methyl group at 3 position on imidazopyridinone ring was tolerated. This provides a vector for ADME property optimization. Molecular modeling study also predicted that there was sufficient space to explore this area.

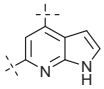
In an attempt to reduce MW, ring count, HBD and TPSA the azaindole core of compound **16** was replaced with a methyl pyridine moiety (compound **17**). Further introduction of a carboxamide group significantly improved metabolic stability (compound **18**) (Table 4).

Chemistry access to compound **18** is described in Scheme 4. Suzuki coupling of commercially available **18a** with (5-chloro-2-fluorophenyl)boronic acid gave the intermediate **18b**. Buchwald amination of **18b** with 4-chloronicotinic acid gave **18c**. Intermediate **18c** was converted to **18d** through Curtius rearrangement and cyclization. Alkylation of **18d** with 2-chloroacetamide afforded target **18**.

Compound **18** was selected as our lead molecule because of its overall favorable profiles. It showed a clean CYP profile, low clearance, good enzymatic and cellular potency, excellent bioavailability in rat PK ($F = 62\%$) and low hERG liability (21% inhibition @ 10 μM). Additionally compound **18** possessed good kinase and ALK5 family isozyme selectivity.[15] Molecular modeling suggests

Table 4
Selected analogs of imidazopyridinone



Compd	A	R	pIC_{50}	pEC_{50}	CYP1C2 pIC_{50}	CYP3A4 pIC_{50}	Cl_{int} (rat) (mL/min/kg)
16		Methyl	8.2	—	5.8	6	204
17		Methyl	7.5	6.34	5.7	5.8	105
18		$-CH_2CONH_2$	7.6	6.63	4.3	<4.3	29
19		Ethyl	7.9	6.48	5.1	>5.8	110

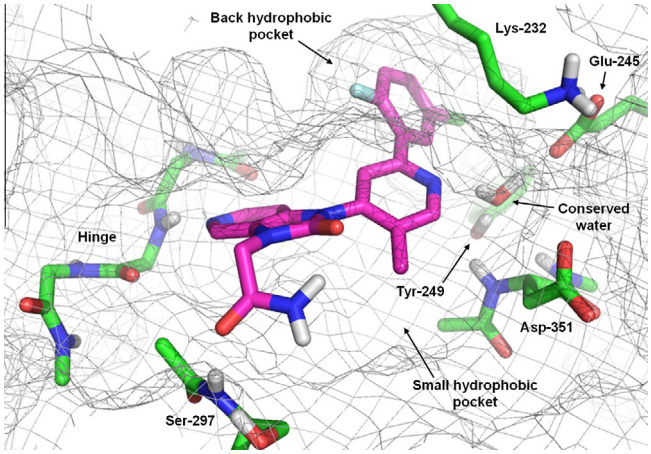


Figure 3. Model of compound **18** in the active site of Alk5.

Table 5
Rat toxicity study of compound **18**

Group	Test article and dosage[†]	Hearts evaluated	Incidence	Severity[**]
1	0.5%MC vehicle control	6	0	—
2	Compound **18** 50 mg/kg/day	6	0	—
3	Compound **18** 150 mg/kg/day	6	6	Minimal
4	Compound **18** 500 mg/kg/day	6	6	Moderate

[**] Severity grade represents an average grade of all lesions from all affected valves per animal. There was no apparent valve predilection.
[†] All rats treated with compound **18** daily for 5 consecutive days groups 3 and 4 developed discernable heart valve lesions.

that compound **18** maintains the hinge and back hydrophobic pocket interactions of compound **1** (Fig. 3). The addition of an acetamide group does generate a hydrogen bond to the backbone NH of Ser-287. It is not surprising that potency is not increased (relative to compound **17**) as it is solvent exposed.

At this point we were apprised of a potential cardiac toxicity associated with modulating Alk5.[16] The team wanted to determine if compound **18** had such liability. In mice, dosing of compound **18** at the 50 and 200 mg/kg modulated pSMAD for up to 8 hours triggering our decision to advance the compound into rat toxicity studies. The oral administration of our lead molecule (**18**) in a fully empowered exploratory toxicology study revealed an induced cardiovalvulopathy in all study rats at both the medium and high dose animal groups (Table 5). The observed cardiovascular toxicity was characterized by valvular interstitial cell proliferation, neutrophil presence, hemorrhage and fibrin deposition in the heart valves of the treated animals.[17]

This toxicity was deemed to have severe functional implications resulting in potential permanent structural and functional changes to heart valves. The toxicity was also fast onset and irreversible. These findings in conjunction with literature reports[16] led the project team to terminate the program and disclose our findings to the scientific community.

In summary, a novel series of Alk5 inhibitors has been identified and developed. Starting from screening hit **1**, by using SBDD and medicinal chemistry knowledge, metabolic stability and bioavailability were successfully optimized, culminating in the identification of lead compound **18**. However, inhibition of Alk5 with compound **18** produced significant cardiac toxicity and led to the program's termination.

Acknowledgments

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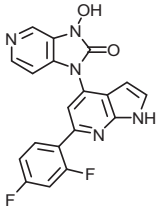
12. *Enzymatic activity assay summary:* Inhibition of ALK5 activity was tested by the use of a LanthaScreen™ activity assay. TGFBR1 ALK5, fluorescein-labeled Peptide substrate, and ATP were allowed to react for 90 minutes at room temperature with a concentration gradient of test compounds. Then EDTA (to stop the reaction) and terbium-labeled phospho-specific antibody (to detect phosphorylated peptide product) were added. The antibody association with the phosphorylated fluorescein labeled substrate resulted in an increased TR-FRET value. ALK5 activity was quantified by measuring an increase in TR-FRET on a BMG LABTECH PHERAstar plus instrument.
13. *Cell assay summary:* Alk5 inhibitor activity was tested in CellSensor® SBE-*bla* HEK 293T (Thermo Fisher Scientific) cells contain a beta-lactamase reporter gene under control of the Smad binding element (SBE) which was stably integrated into HEK 293T cells. Cells were plated and treated with compounds and stimulated with TGFb1. Cells were then incubated with LiveBLAzer FRET B/G CCF4-AM (Thermo Fisher Scientific) and read in Spectramax.
14. MS metabolic soft spot analysis of compound **13** in rat liver microsome samples was conducted. The poor metabolic stability of compound **13** (high clearance in rat, 160 mL/min/kg) was attributed to formation of the oxidation product as below.



15. *Compound **18** at 1 μM concn was profiled against the following kinase panel:* ABL1, AKT1 (PKB alpha), AURKA (Aurora A), BRAF, CDK1/cyclin B, CDK2/cyclin A, CDK5/p25, CHEK1 (CHK1), CHEK2 (CHK2), CLK1, CLK2, CSK, EGFR (ErbB1), ERBB2 (HER2), GSK3B (GSK3 beta), IGF1R, IKBKB (IKK beta), INSR, IRAK1, JAK2, KDR (VEGFR2), LCK, LIMK2, LYN A, MAP2K1 (MEK1), MAP2K2 (MEK2), MAP2K6 (MKK6), MAP3K10 (MLK2), MAP3K2 (MEKK2), MAP3K3 (MEKK3), MAP3K5 (ASK1), MAP3K9 (MLK1), MAPK1 (ERK2), MAPK10 (JNK3), MAPK12 (p38 gamma), MAPK14 (p38 alpha), MAPK3 (ERK1), MAPK8 (JNK1), MAPK9 (JNK2), MAPKAPK2, MAPKAPK5 (PRAK), MET (cMet), PAK1, PAK4, PAK6, PDK1 Direct, PRKACA (PKA), PRKCA (PKC alpha), PRKCB1 (PKC beta I), PRKCD (PKC delta), PRKCG (PKC gamma), RPS6KB1 (p70S6K), SGK (SGK1), SRC, STK3 (MST2), STK4 (MST1). Compound **18** was inactive against all (showing ⩽16% inhibition (est. IC_{50} ⩾5 μM) except MAP3K2 (MEKK2) showing 31% inhibition at 1 μM (est. IC_{50} = 2.2 μM).
*Compound **18** ALK5 family isozyme profiling selectivity:* ALK4 is 43-fold, ALK2 and ALK3 (BMPR1a) are each >200-fold, based on 2pt screening done at 10 and 1 μM, and compared to the in-house determined of ALK5 IC_{50} value.

16. Anderton, M. J.; Mellor, H. R.; Bell, A.; Sadler, C.; Pass, M.; Powell, S.; Steele, S. J.; Roberts, R. R. A.; Heier, A. *Toxicol. Pathol.* **2011**, *39*, 916.

17. *Experimental design:* Six female Wistar rats from Harlan (10 weeks of age)/group were used for the main study groups; the TK groups consisted of $N = 2$ rats/dosage. Rats were administered a po (QD) dose of the compound for 5 consecutive days. The rats were observed daily for their survival and signs of overt toxicity. The dose volume was 10 mL/kg and was based on body weight. The body weight range of test animals at the initiation of dosing will be between 150 and 190 g. Rats were held in the study room for approximately 7 days prior to the initiation of dosing.
Rats were assigned to treatment groups in a non-selective manner designed to balance body weights between groups. Each rat was identified by a unique number located on their tail.
Clinical observations: Rats were observed once prior to the start of dosing on Study Day 1. On Day 1–5, each rat was observed continuously for 30 min after dosing and again at approximately 4 h post-dosing for changes in general appearance and behavior. For each observation period, the observer was not be blinded as to the animals' treatment group. The rats were terminated on Day 6 (24 h after last dose).
Heart Perfusion & Collection: Rats were anesthetized, perfused through the left ventricle with ~100 mL ice cold PBS and subsequently 10% formalin fixed using 200–300mLs of ice cold formalin. Hearts and surrounding vasculature were dissected and placed into labeled formalin jars for delivery to embedding facility.
Microscopic examination: Mitral, Tricuspid, and Pulmonic valves were examined microscopically by 2 pathologists independently.