Journal Pre-proofs

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Graphical Abstract

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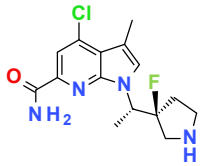

KG1 EC_{50} = 61 nM
pS6 EC_{50} = 71 nM
TGI 87%

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Discovery of SARxxxx92, a pan-PIM kinase Inhibitor, efficacious in a KG1 tumor model

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ABSTRACT

*N-substituted azaindoles were discovered as potent pan-PIM inhibitors. Lead optimization, guided by structure and focused on physico-chemical properties allowed us to solve inherent hERG and permeability liabilities, and provided compound **27**, which subsequently impacted KG-1 tumor growth in a mouse model.*

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PIM kinases (PIM1, PIM2 and PIM3) with roles in the regulation of signal transduction.1 The oncogenic activity is a consequence of the regulation of MYC transcriptional activity, the regulation of cell cycle progression and by phosphorylation and inhibition of pro-apoptotic proteins such as BAD, MAP3K5, FOXO3. PIM2 specifically was discovered as being critically important for growth and survival of multiple myeloma cancers.3 However, PIM2 has proven to be the most challenging to inhibit with ATP competitive inhibitors. This is most likely due to the high affinity of PIM2 for ATP.2 PIM kinases regulate several signaling pathways that are fundamental to cancer development and progression. For example, they promote cell survival, proliferation and drug resistance. As such, the PIM kinase family is an attractive set of targets for pharmacological inhibition as a cancer therapy.

PIM kinases are critical downstream effectors of the ABL, JAK2, and Flt-3 oncogenes and are required to promote tumorigenesis.4 Over-expression of PIM kinases has been reported in several hematological and solid tumors for PIM1, myeloma, lymphoma, leukemia for PIM2 and adenocarcinomas for PIM3.5 The absence of a regulatory domain confirms that these proteins are constitutively active once transcribed. The PIM family is also involved in inflammatory processes6 with data reported on the role of these kinases in human and mouse CD4+ T cell activation and inflammatory bowel disease.7

The compelling biological rationale for inhibiting PIM kinases has prompted numerous research groups to engage in small molecule inhibitor programs. Triple-PIM knockout mice are viable and fertile with a slightly deficient growth response and a normal life span.9 Crystallography studies have revealed that, unlike other family members, PIM kinases possess a hinge region with a unique binding pocket for ATP, namely Pro-123, with insertion of Pro-125 and Val-126.8 Several PIM inhibitors have been reported in the literature with two successfully advancing to the clinic.10-11

In previous reports12, we highlighted the optimization challenges encountered with the azaindole chemical series. Our initial efforts culminated with the discovery of C2 substituted compounds (Figure 1) which showed minimal tumor growth inhibition. Figure 1 summarizes the properties associated with these molecules; highlighting attempts to increase target potencies only led to adverse drug properties. With this in mind, we revisited our strategy to address the challenges.

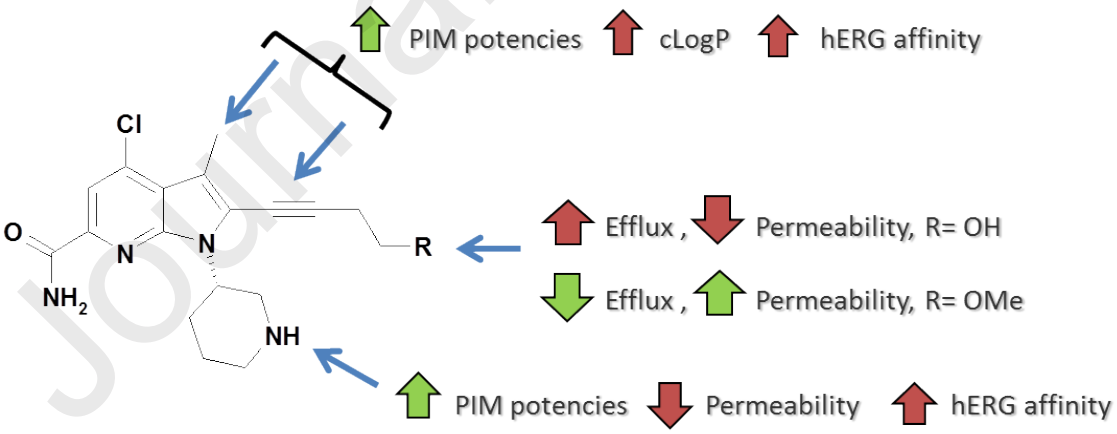
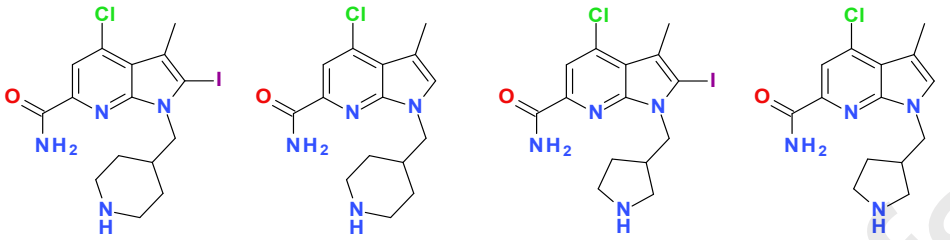


Fig. 1. C2-alkynyl-substituted series summary

We looked back in our original dataset to find plausible alternatives to resolve these eADME issues before deciding on stopping efforts on intractable chemical matter. We focused our attention identifying compounds with desired indexes between cellular potency, eADME liabilities and with good LE.

Ana LE >0.4 at the expense of reduced potency (Fig. 2). To recover potency while maintaining LE, we returned to the X-ray crystal structure of compound **A** in complex with PIM 1 (Fig 3) to search for ways to modify compounds binding efficiently to the protein.



1
PIM1 IC_{50} = 5 nM
PIM2 IC_{50} = 62 nM
KG1 EC_{50} = 250 nM
LE = -0.45

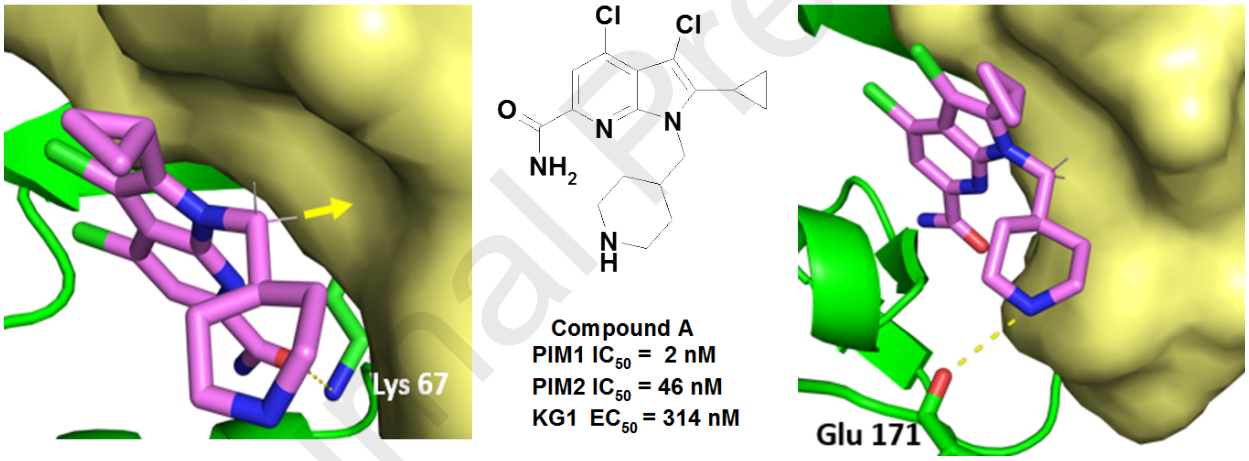
2
PIM1 IC_{50} = 20 nM
PIM2 IC_{50} = 560 nM
KG1 EC_{50} = 643 nM
LE = -0.41

3
PIM1 IC_{50} = 2 nM
PIM2 IC_{50} = 36 nM
KG1 EC_{50} = ND
LE = -0.49

4
PIM1 IC_{50} = 14 nM
PIM2 IC_{50} = 250 nM
KG1 EC_{50} = ND
LE = -0.46

Fig. 2. Early prototypes in the azaindole series.

As indicated with the yellow arrow in **Figure 3**, a small hydrophobic pocket defined by Val-52, Phe-49, Gly-45, Leu-44 was present with a good vector off the methylene (Pro-*S*) of the 4-piperididyl moiety. Occupying this pocket was an opportunity to bring back potency, compensating for loss of the C2 alkynyl moieties.



Compound A
PIM1 IC_{50} = 2 nM
PIM2 IC_{50} = 46 nM
KG1 EC_{50} = 314 nM

Fig. 3. Compound A (PDB: 6VRU), close analog of compounds **1**-**2** bound to PIM1

Introduction of an α–Methyl and separation of enantiomers led to compound **5** which, gratifyingly provided much improved potency and LE[1] (Fig 4). To note, the stereochemistry was initially assigned arbitrarily for both **5** and **6**. Compound **5** though showed sufficient safety against the cardiac hERG ion channel but was poorly permeable. Adjusting the basic amine pKa is a standard approach to modulating permeability. To that end, we targeted positioning a fluorine atom two carbons away from the basic nitrogen for optimal effect. 13

[1] LE (Kcal/Mol) = 1.4(-log Pim2 IC50)/nbr atom non H

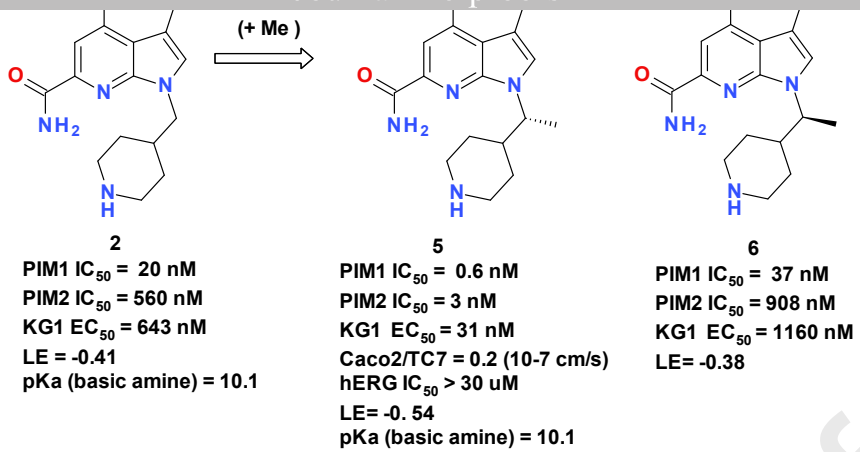
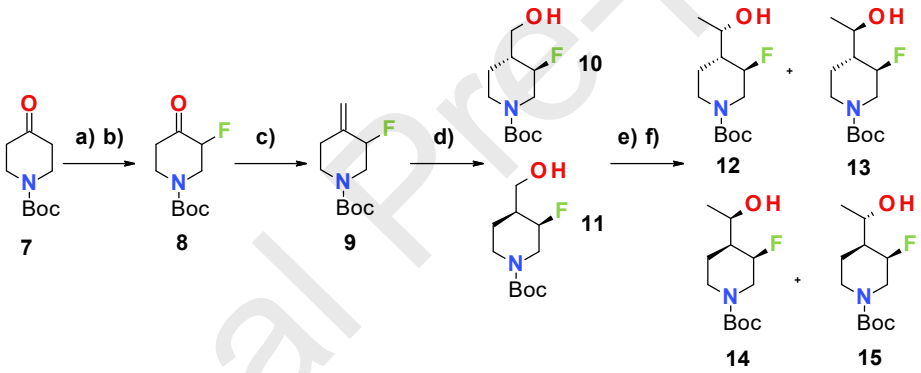


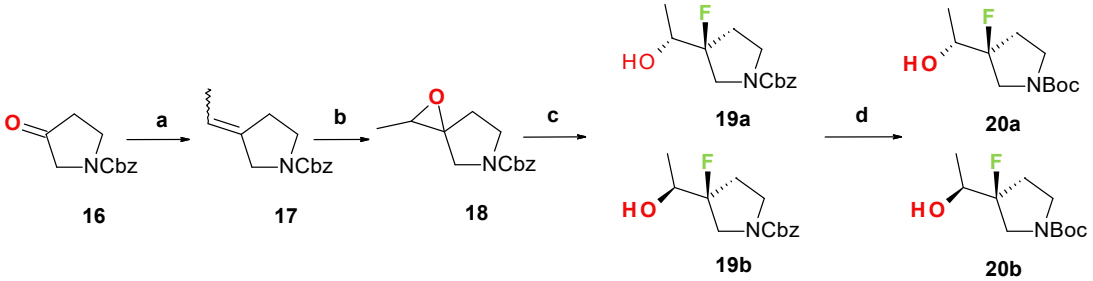
Fig. 4. Effect of the methyl on PIM1/2 enzymes

Fluoropiperidines derived from **5** consisted of 4 diastereomers and it was anticipated that each would have unique properties. We aimed to access each isomer via chromatography in conjunction with chiral separation. We carried out the synthesis of these compounds as shown in **Scheme 1** and decided to work with diastereomers. We envisioned the synthesis of fluoro-piperidine chiral building blocks **12-15** as described in **Scheme 1**.



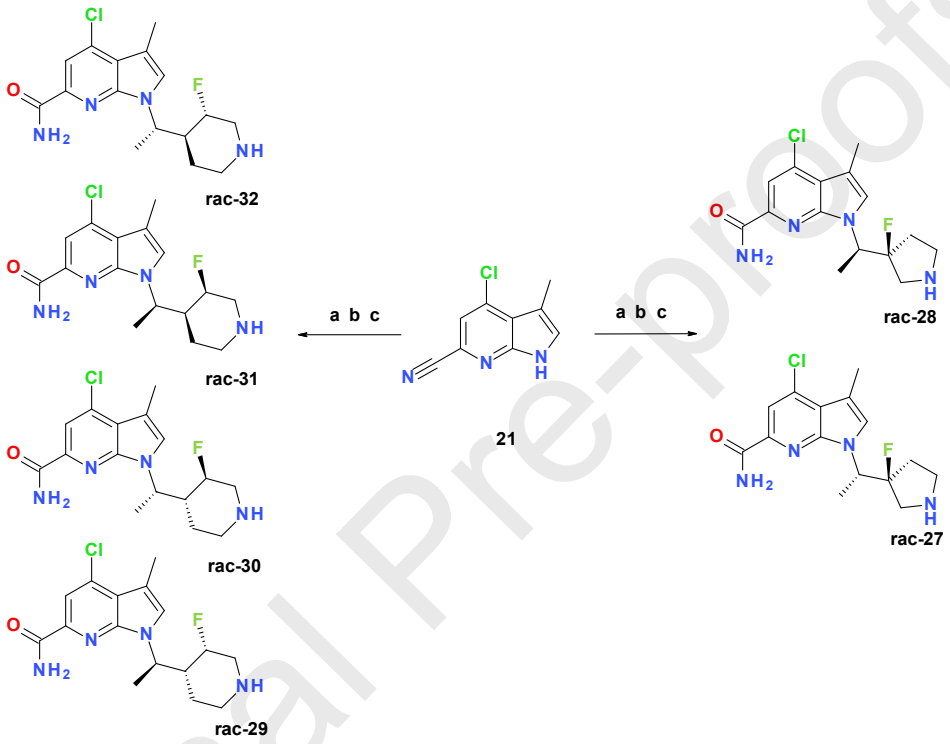
Scheme 1. a) TMSCl, Et_3N; b) Selectfluor, 87%, ref.14; c) Ph_3PMeBr, nBuLi, THF 69%; d) BH_3.THF, **10** (24%), **11** (51%). e) Dess-Martin periodinane, DCM; f) MeMgBr, THF; **12** (26%), **13** (41%), **14** (13%), **15** (7%).

Boc-protected piperidinone **7** was mono-fluorinated in a two-step procedure to give **8** in excellent yield14. Homologation of **8** via Wittig reaction gave exo-olefin **9** which upon hydroboration gave access to the first pair of diastereomers **10-11,** readily separated by chromatography on silica gel. Each diastereomer was separately subjected to oxidation to the corresponding aldehyde followed by methyl Grignard addition to give mixture **12-13** from **10** and **14-15** from **11**. Careful separation of each led to all four diastereomeric alcohols building blocks **12-15**, albeit in low isolated yields.



Scheme 2. a) BuLi, $EtPPh_3Br$, THF, 62%. b) mCPBA, DCM, 80%. c) HF.Pyr, DCM, 60%. d) H_2, Pd/C, $(Boc)_2O$, MeOH, 92%.

Closely related building blocks, fluoro-pyrrolidines alcohols **20a** and **20b,** were synthesized according to **Scheme 2.** N-protected pyrrolidone **16** was transformed to **17** under Wittig conditions providing a mixture of olefin stereoisomers. Epoxidation of the alkene using mCPBA afforded **18** in good overall conversion. Regioselective epoxide ring opening proceeded with HF.Pyr solution in a Teflon flask produced alcohols **19a** and **19b**, which were then separated using silica gel chromatography. Exchange of protecting group from Cbz (used as a chromophore) to Boc on each isomer separately was accomplished in one pot to deliver **20a** and **20b** as racemates.

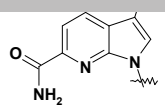
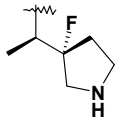
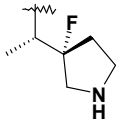
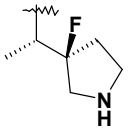
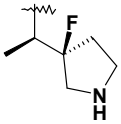
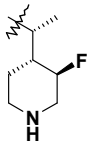
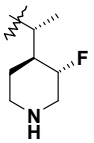
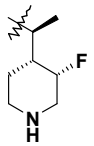
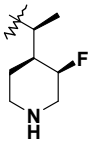


Scheme 3. a) Bu_3PCHCN, Toluene, 65%. b) Acetamidoxime, $RhCl(PPh_3)_3$, toluene, 75%. c) TFA, DCM, 99%.

To complete the synthesis of target analogs, a general protocol was utilized to convert cyano pyrrolopyridine **21** to compounds **25-32** (**Table 1**) resulting from the chiral separation of racemic compounds **27-28** and **29-32**. Accordingly, carbonitrile **21** was coupled to alcohols **20a-20b** under Mitsunobu conditions at 90°C to provide adduct in good yields. Hydration of nitrile proceeded smoothly using the Wilkinson's catalyst to afford carboxamides. Acid-mediated Boc-deprotection provided final target compounds as mixture of enantiomers. In each case, pyrrolidine or piperidine series, further chiral separation using SFC chromatography gave access to pure enantiomers.

Target compounds (**25-32**) were first evaluated for their ability to inhibit enzymatic and cellular PIM related activity and further cascaded down numerous assays to assess drug-like properties as shown in **Table 1**. In the fluoro-piperidine series, only the 4 isomers potent on enzyme (PIM1/2) and cells were progressed in eADME and are displayed in the table below.

Table 1. Comparison and characterization of new N1-fluoropyrrolidine/piperidine 7-Azaindole isomers.

Entry		Pim1[a] IC_{50} (nM)	Pim2[a] IC_{50} (nM)	p38[a] EC_{50} KG1 (nM)	KG1[a] EC_{50} viability (nM)	Caco2/TC7[b]	hERG[c] IC_{50} (uM)	HLM[d]	MLM[d]	RLM[d]
1* (25)		254	1780	ND	7230	ND	ND	ND	ND	ND
2* (26)		14	160	876	808	ND	ND	ND	ND	ND
3* (27)		1	6	71	61	23	22	5	31	10
4* (28)		68	515	2030	9070	18	30	5	4	18
5* (29)		1	2	52	51	71	7	9	93	32
6* (30)		1	3	24	20	120	6	24	89	22
7* (31)		0.7	6	6	17	15	10	5	83	3
8* (32)		2	22	115	195	17	10	3	81	0

*Unknown absolute configuration, randomly assigned stereochemistry.

[a]IC_{50} are measured in nM. Data represent an average of at least two separate determinations.

[b]Caco2/TC7 permeability measurement (10^{-7}cm/sec) using a 5uM stock.

[c]hERG data is an IC50 (uM) measurement patch clamp at RT.

[d]Liver Microsome clearance data (% extraction) using a 5uM stock.

As projected, the introduction of a fluorine atom on pyrrolidines and piperidines provided a shift in the pKa range going from 10.1 for compounds such as **4** to 8.1 for fluoro-pyrrolidines like **27**. The decrease in basicity translated into improved Caco2/TC7 permeability for all entries. Compound **27** (Entry 3) was the most cellular potent analog with EC50's <100nM in the pyrrolidine sub-series. Furthermore, it had a favorable overall in vitro ADME profile with very good thermodynamic aqueous solubility of 6.8 mM at pH7.4. Most gratifying, **27** had low affinity for the hERG ion channel that was previously difficult to overcome in this chemotype.

Also, a trend towards lower intrinsic metabolism was favorable with pyrrolidines (Entries 1-4), with piperidines showing extensive intrinsic clearance in mouse liver microsomes. hERG was also a differentiating factor between the two series where most of the piperidines were between 1-10 uM in the patch clamp assay.

We were successful in solving the X-ray crystal structure of **27** bound to PIM1 with a resolution 1.74 Å. For the first time, we were able to assign the absolute stereochemistry as being 1(S) and 3(S) for compound **27**. Some notable binding interactions included the carboxamide with Lys-67 and Asp-186 and furthermore, interactions of the engineered pyrrolidine NH with Asp-128 and Asp171 which explained the weak enzyme activity for the 3 other isomers (**25, 26, 28**).

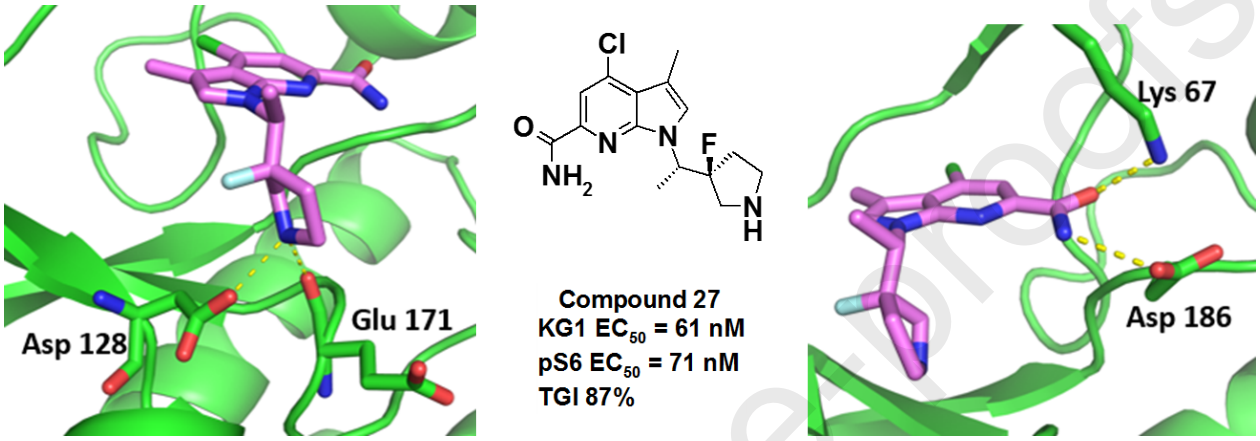


Fig. 5. Crystal structure of **27** bound to PIM1 (PDB: 6VRV).

Selectivity of compound **27** was determined against an internal panel of 192 kinases. **27** displayed a very favorable selectivity profile showing only 8 other kinases with a percentage of inhibition above 50% at 1uM. The off-target kinases were RSK 1/2/3/4 with 55%, 84%, 87%, 53% inhibition respectively, LOK (87%), IKKβ (86%), PKCη (73%), PKCε (57%).

Pharmacokinetic measurements were conducted in rat at 3 mpk IV and 10 mpk PO doses. Compound **27** dosed intravenously displayed a low Cmax of 174 ng/mL corresponding to about 0.5 uM and a short half-life of 2.7 hours due to high clearance (4.5 L/h/kg) and high volume of distribution (9.9 L/Kg). The oral bioavailability was a respectable 59%. The high clearance in rat was inconsistent with *in vitro* intrinsic clearance data so we investigated partitioning of "drug" between plasma and red blood cells. A ratio of 2 was obtained which indicated that the compound was moderately cleared in rat accounting for some of the discrepancy.

Given compound **27** was orally bioavailable, a pharmacokinetic-pharmacodynamic (PK/PD) was performed in tumor-bearing mice using a single oral dose of 25 mpk with the mouse unbound fraction measured to be 5.5%. At 8h and 24h, we observed plasma free drug concentration of 240 nM and 54 nM, respectively. Conversely, tumor free drug at 8h was 720 nM and 120 nM at 24h, consistent with the high volume of distribution for **27**. PD portion of the experiment provided a similar pattern of pBAD saturation at 8h with 65% pBAD reduction which decreased to 55% at 24h. This data indicated that a BID dosing protocol would be more suitable for a chronic efficacy study in a mouse disease model.

Acc ice a day at 50, 100, 150 mpk BID, with 8 mice in each dose group for 3 weeks and tumor volume was measured throughout the study. **Figure 6** shows a plot of tumor volume versus time with four plots (vehicle and 3 dose groups). At lower doses, the compound had marginal effect on the tumor volume vs vehicle but at the top dose of 150 mpk, T/C of 13.1%, representing a TGI of 87% was observed. In addition, based on body weight changes, the compound was well tolerated at all doses although 2 of 8 mice in the 150 mpk group showed >15% body weight loss but were generally healthy and mobile. After the 2 animals rested for 2 days without dosing on day 32-33, the treatment resumed, and the animals recovered as shown in the body curve.

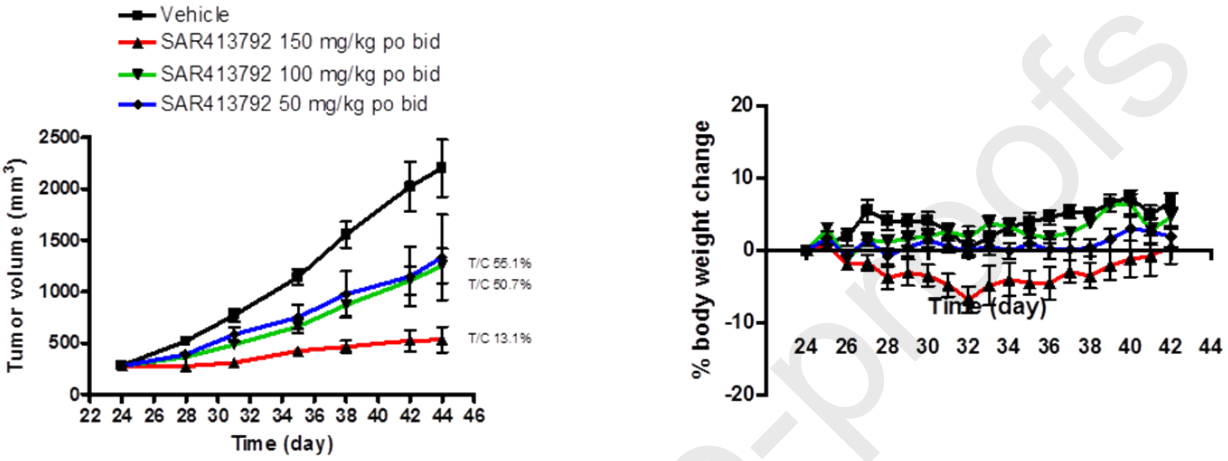


Fig. 6. Dosing **27** into a KG1 mouse xenograph model with tolerance

In summary, investigations of 7-azaindole containing fluoropyrrolidines/piperidines to increase LE, led to compound **27** with good potency and increased ligand efficiency compared to previous C2-analogs. Subsequently, **27** showed significant efficacy, excellent kinase selectivity and was devoid of any significant hERG activity.

Acknowledgments

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References and notes

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2. PIM1,2,3 ATP Km = 67, 5.4, 97 uM using a radioactive filter-binding assay. For IC50 calculation using full length Bad, PIM1/2 ATP Km = 336/8 uM.

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