Design of Selective PAK1 Inhibitor G-5555:

Improving Properties by Employing an Unorthodox Low-pk_a Polar Moiety

Ndubaku et al. *ACS Med. Chem. Lett.* **2015**, 6, 1241-1246

Celeste Alverez
Current Literature
January 16, 2015

PAK1

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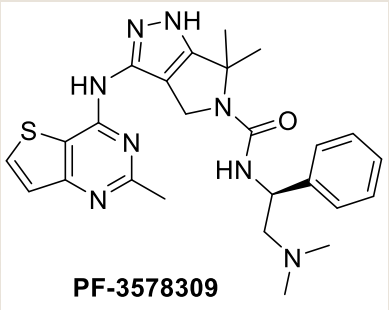
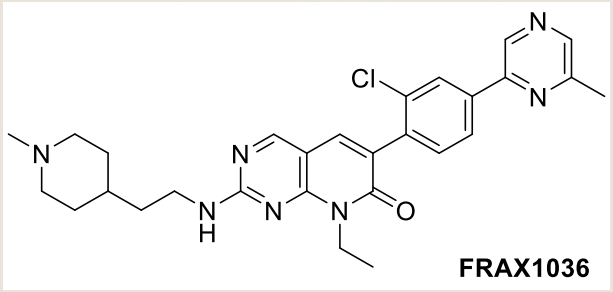
- p21-activated kinases (PAK)
- *Group I* and group II (determined by structural similarity)
- Downstream in many signal transduction pathways
- Play role in cell migration, proliferation, and survival
- PAK1 overexpression has been linked to poor prognosis in some types of breast cancer

- It has been shown that in combination with docetaxel it lead to increased apoptosis *in vitro*
- Previously 1 PAK1 inhibitor in clinical trials (phase 1)
  - No longer active: no proven efficacy/toxicity



PF-3578309

FRAX1036



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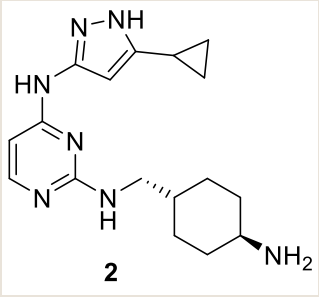
- Found in literature/in house
  - Appealing potency (22 nM)
  - Moderate kinase selectivity, including against group II PAKs (100-fold)
- Crystal structure showed the piperidine *N* makes no productive interactions
- 222 nM cellular activity
- 89% hERG inhibition at 10 uM

- Previous work showed that by targeting amine at Asp393/Asn394 in ribose binding site of active site increased potency and selectivity over group II
  - Need to change basic amine location from FRAX1036



Structural Modification Theory

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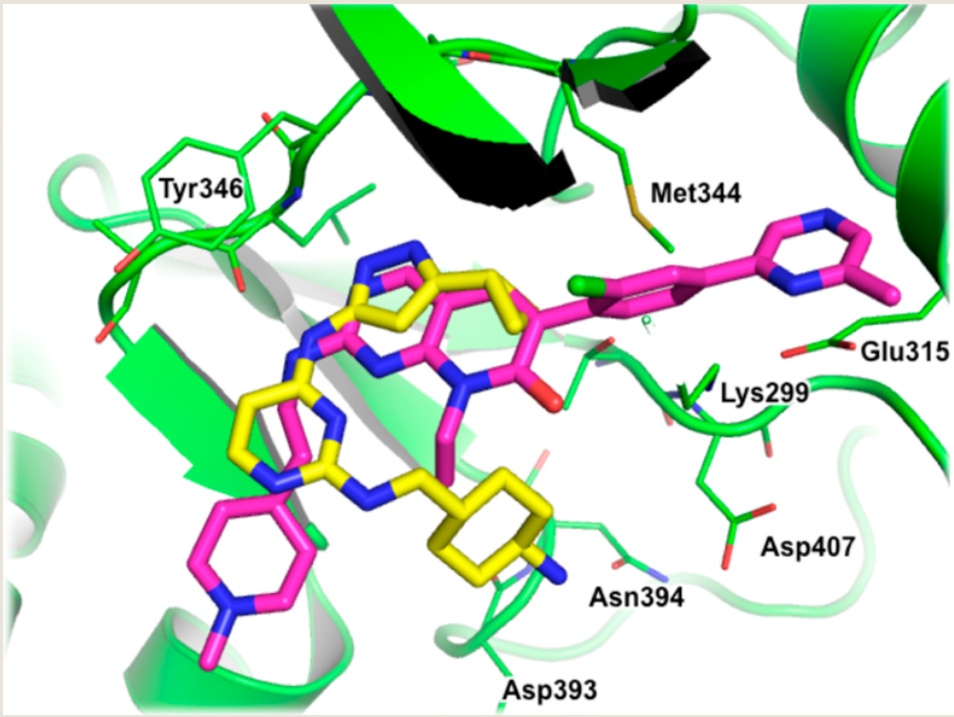
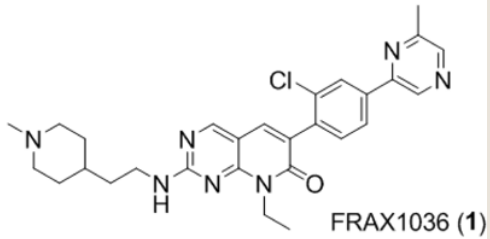
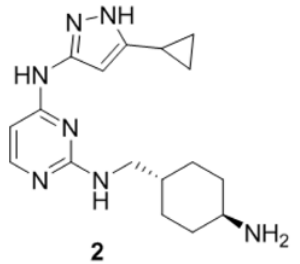


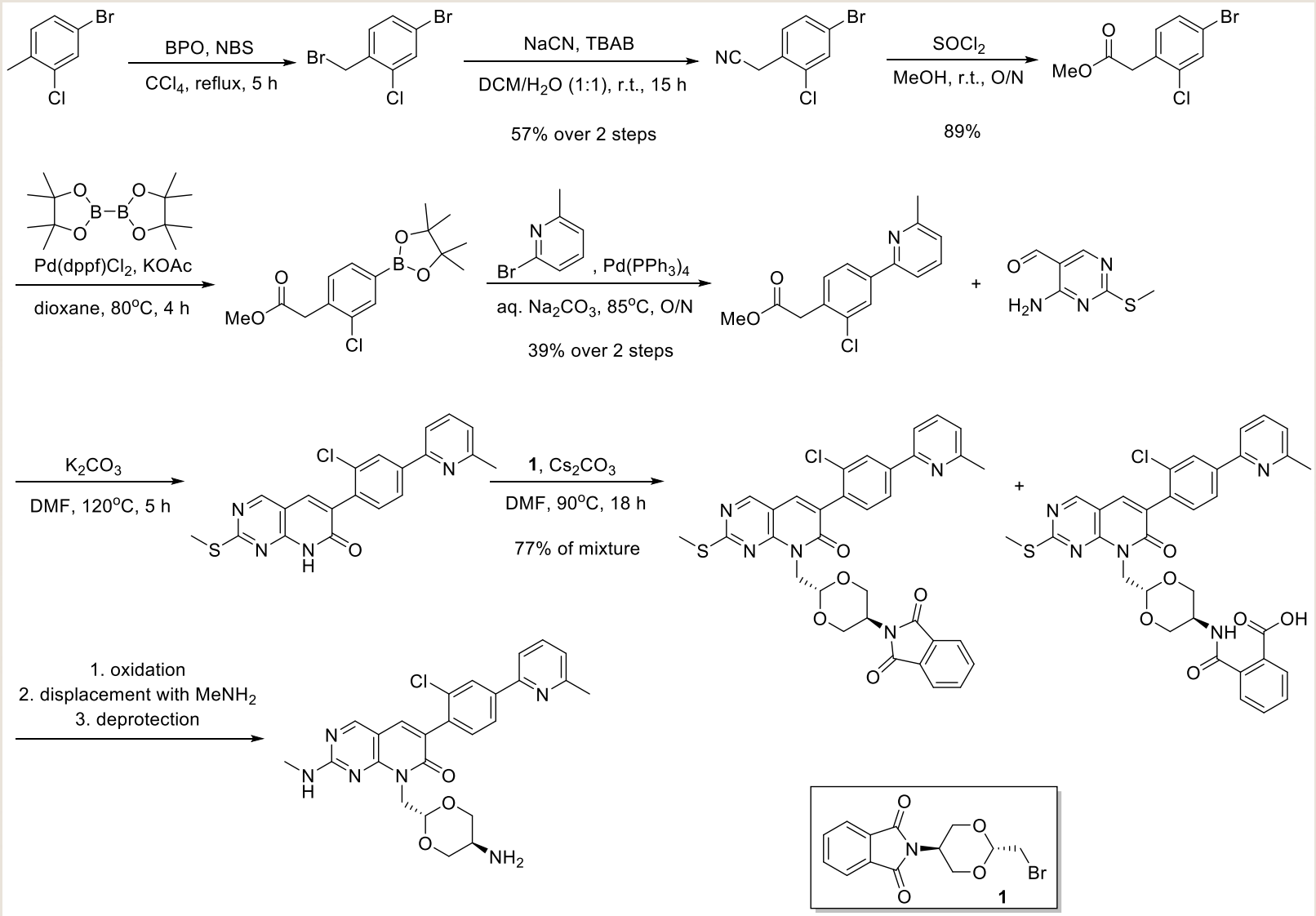


FRAX1036 (**1**)



2

Synthesis



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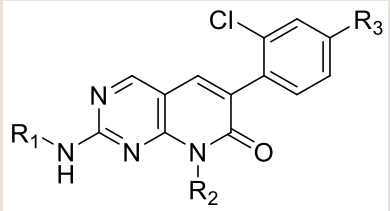

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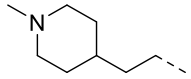
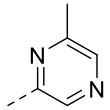
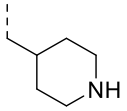
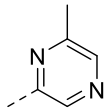
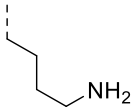
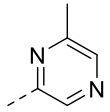
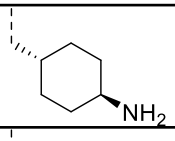
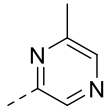
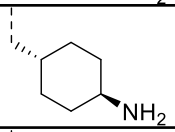
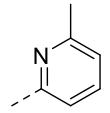
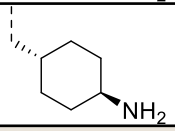
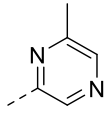
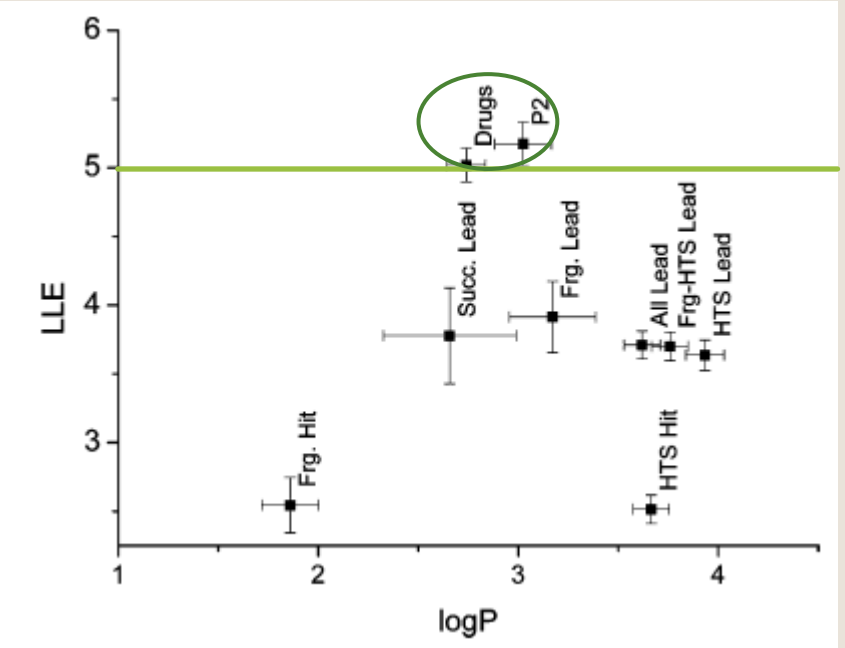
1		Et		22/2.8	222	4.9	2.7	89
3	Et			6.0/4.3	145	3.9	0.9	ND
4	Et			6.1/4.7	147	3.5	1.1	84
5	Et			1.9/4.2	124	4.5	0.4	58
6	Et			2.1/3.1	45	5.6	1.2	59
7	Me			7.4/4.1	341	4.0	0.4	33

Lipophilic Ligand Efficiency (LLE)

- Lipophilicity is an important molecular parameter to consider in drug development
  - Solubility
  - Permeability (passive)
  - Metabolism
  - Off-target effects/toxicity
- Describes the contribution of lipophilicity to potency
  - $\log P/D$ /–log(potency: Kd, Ki, IC5



J. Med. Chem. **2012**, *55*, 1252–1260

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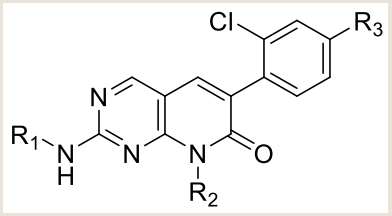
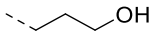
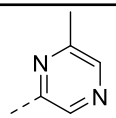
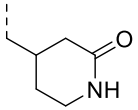
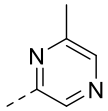
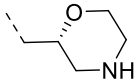
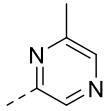
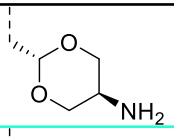
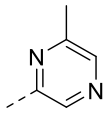
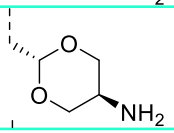
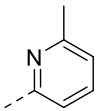
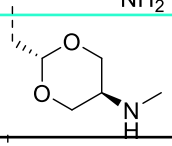
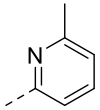
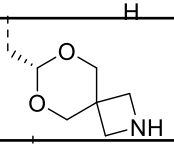
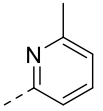
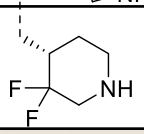
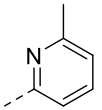


Cmpd	R_1	R_2	R_3	PAK1 Ki (nM)/LLE	Cellular IC_{50} (nM)	cpKa	cLogP	MDCK P_{app}	hERG %inhib @ 10 uM
8	Et			64/3.8	980	4.0	3.4	21.0	22
9	Et			66/4.3	813	3.9	2.9	10.6	12
10	Me			19/5.5	399	6.9	2.2	3.5	21
11	Et			8.0/5.7	148	7.7	2.4	1.7	11
12	Me			3.7/5.5	69	7.7	2.9	2.4	45
13	Me			1.9/5.5	57	8.4	3.2	0.4	60
14	Me			4.3/5.3	73	9.0	3.1	1.0	82
15	Me			7.9/3.6	53	7.6	4.5	ND	30

Structural Data

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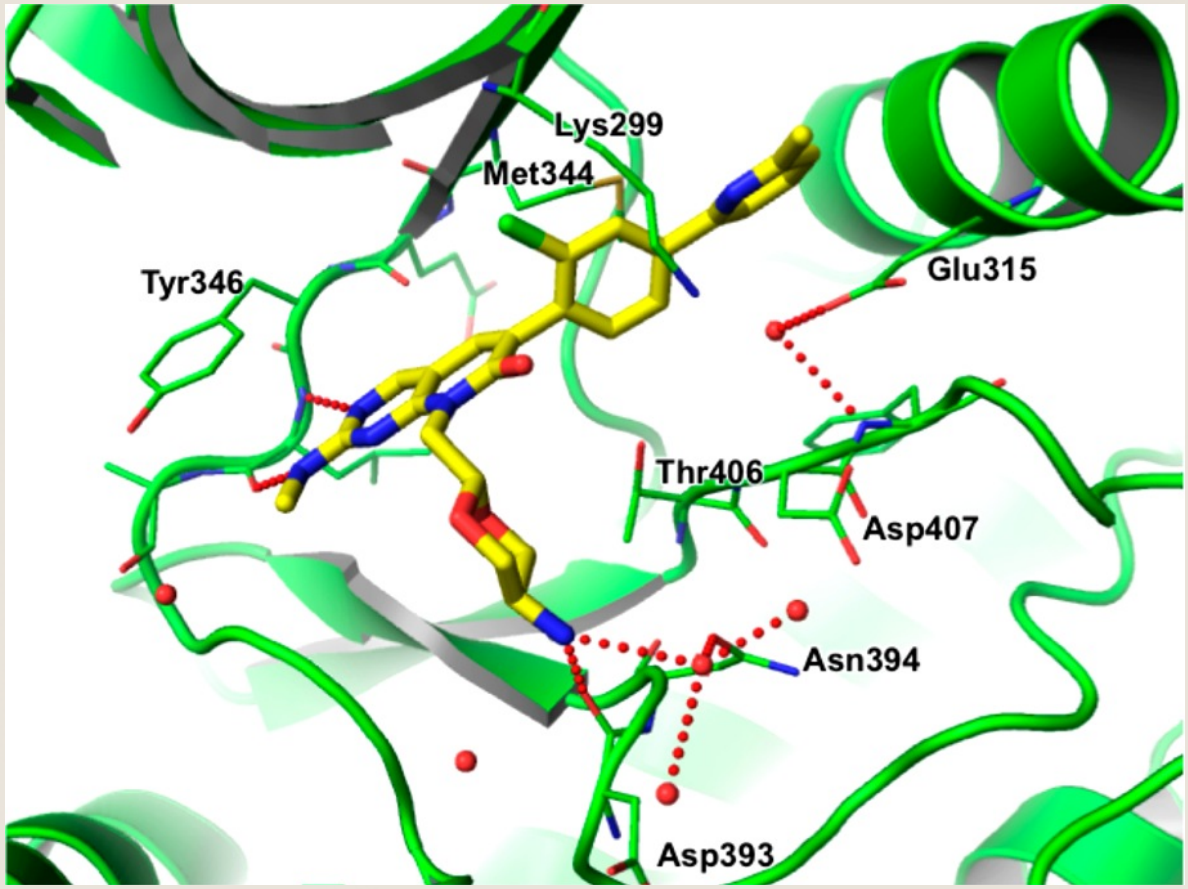
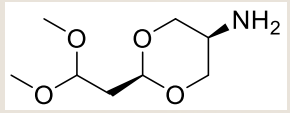


5-Amino-1,3-dioxane

- Known to have anti-inflammatory properties
  - In mouse model, ear edema showed 73.6% anti-inflammatory effects at 20 mg/kg



- Used in somatostatin analogs since 1999 to reduce basicity and improve solubility– improved bioavailability
  - Up to ~13-fold
- Typically avoided due to perceived instability
  - However found to be more stable due to amine
    - Protonate amine first, sparing acetal

Bioorg. Med. Chem. **2006** , *14*, 1339-1347
Bioorg. Med. Chem. Lett. **1999**, 9, 491-496

Stability of Compound **11**

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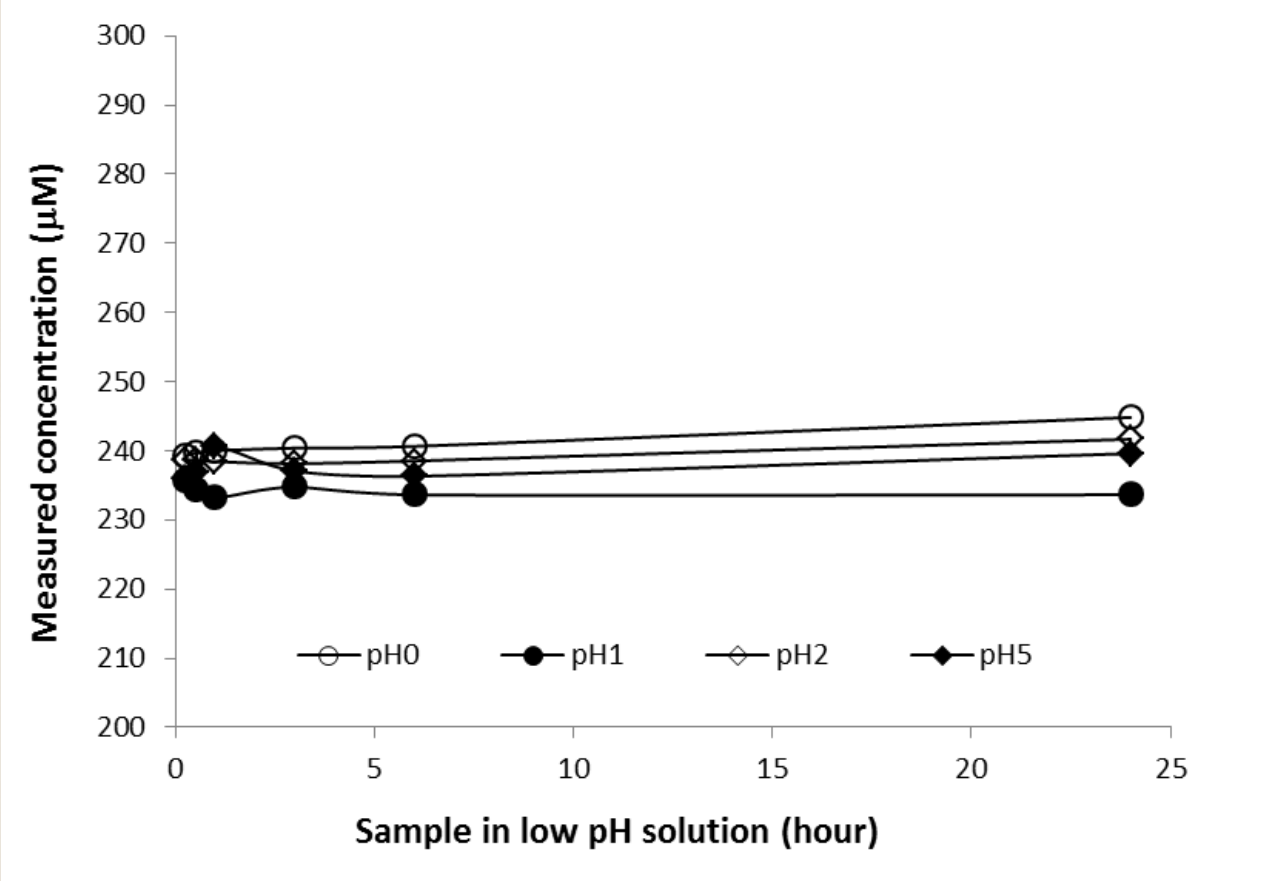

Stability

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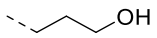
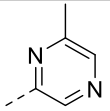
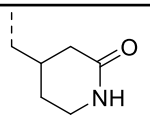
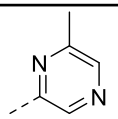
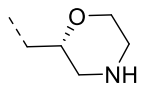
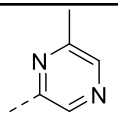
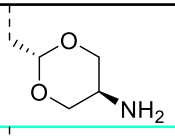
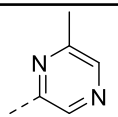
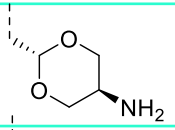
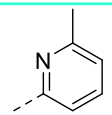
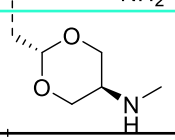
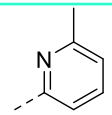
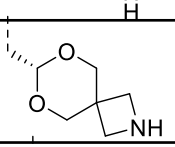
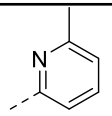
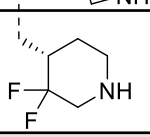
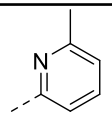
Cmpd	R_1	R_2	R_3	HLM Cl_{hep} (mL/min/kg)
8	Et			14.7
9	Et			12.5
10	Me			10.7
11	Et			9.6
12	Me			11.6
13	Me			13.8
14	Me			11.6
15	Me			17.8

Selectivity of **12**

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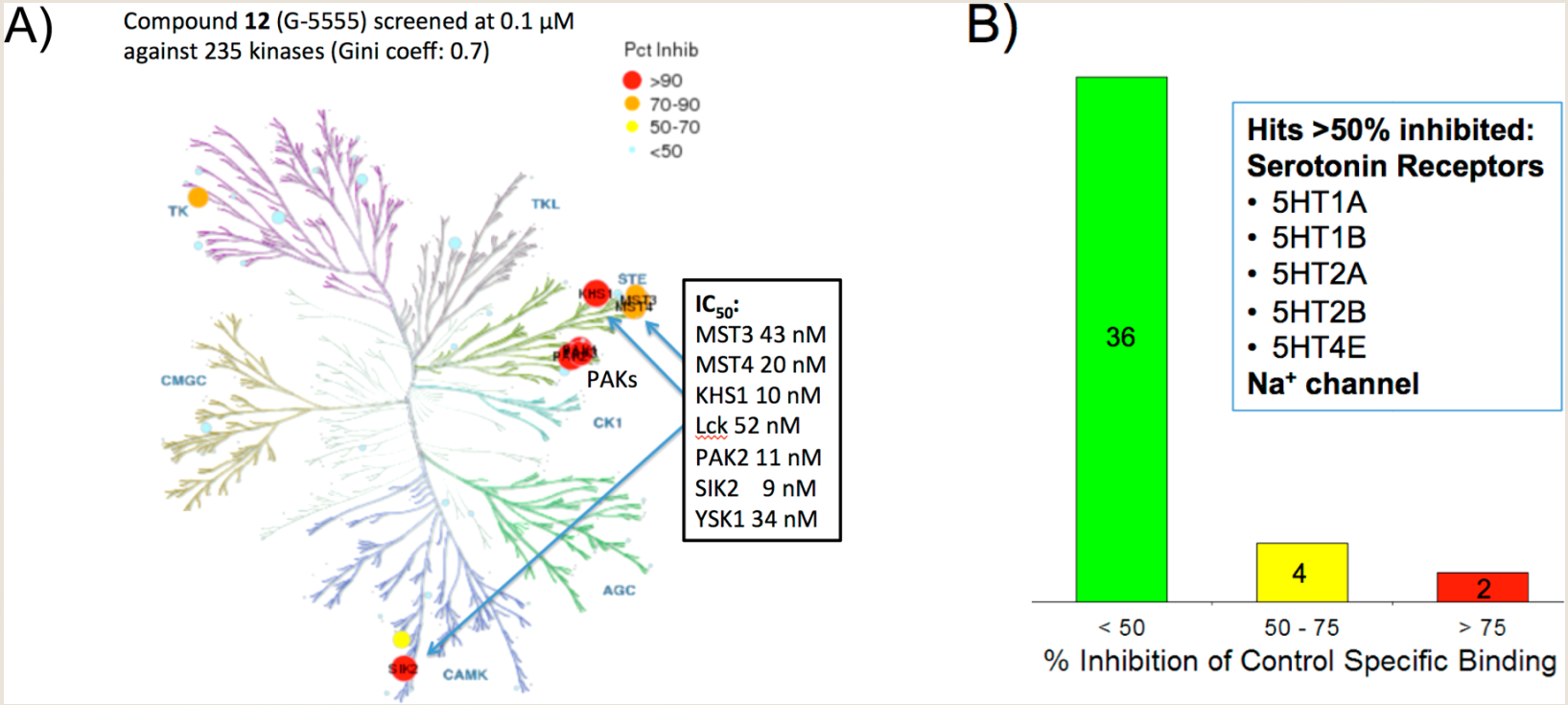
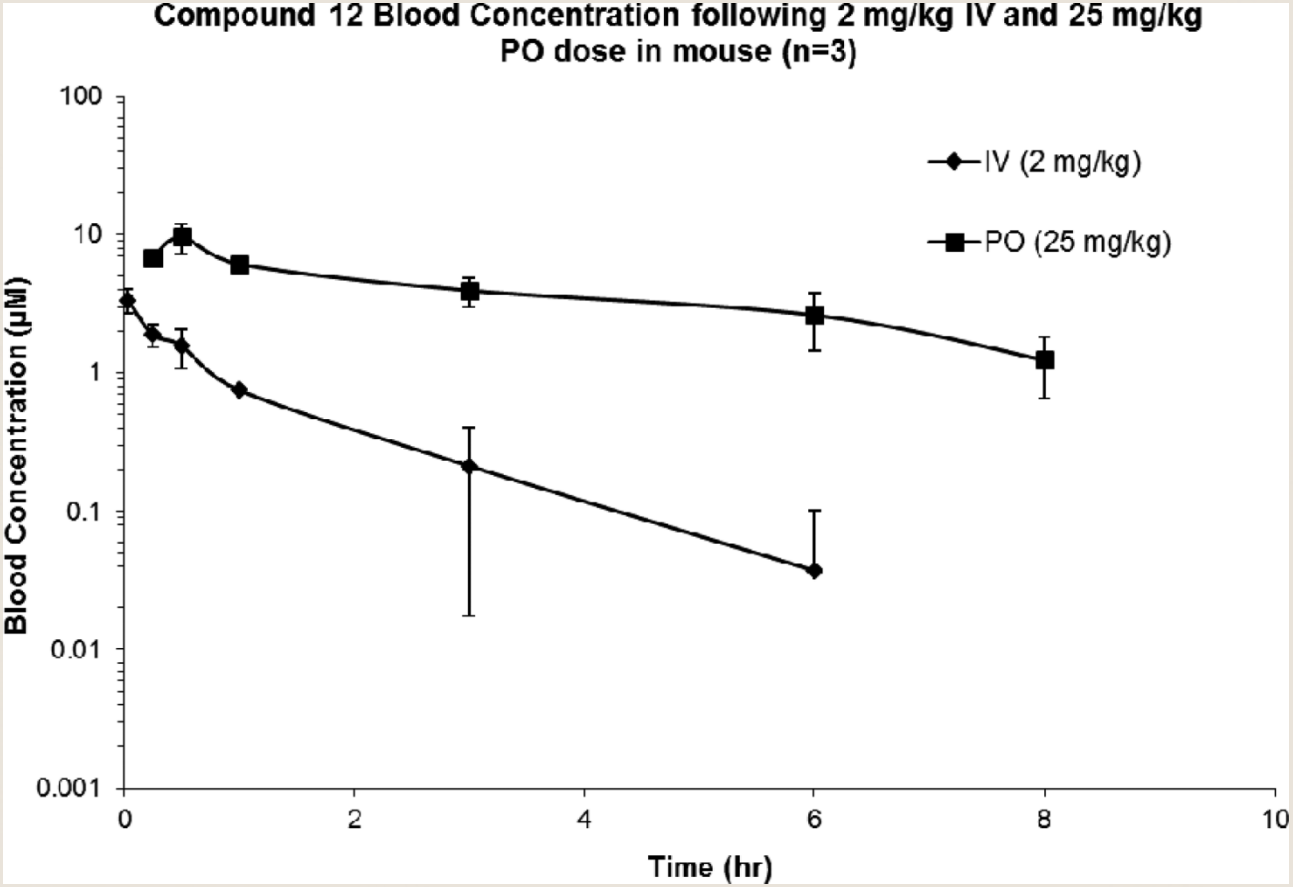


PK


Compound 12 Blood Concentration following 2 mg/kg IV and 25 mg/kg PO dose in mouse (n=3)
Blood Concentration (µM)
100
10
1
0.1
0.01
0.001
0
2
4
6
8
10
Time (hr)
IV (2 mg/kg)
PO (25 mg/kg)

PK

Table S1. Pharmacokinetic Parameters of G-5555 following 2 mg/kg IV Bolus Administration to Female Mice

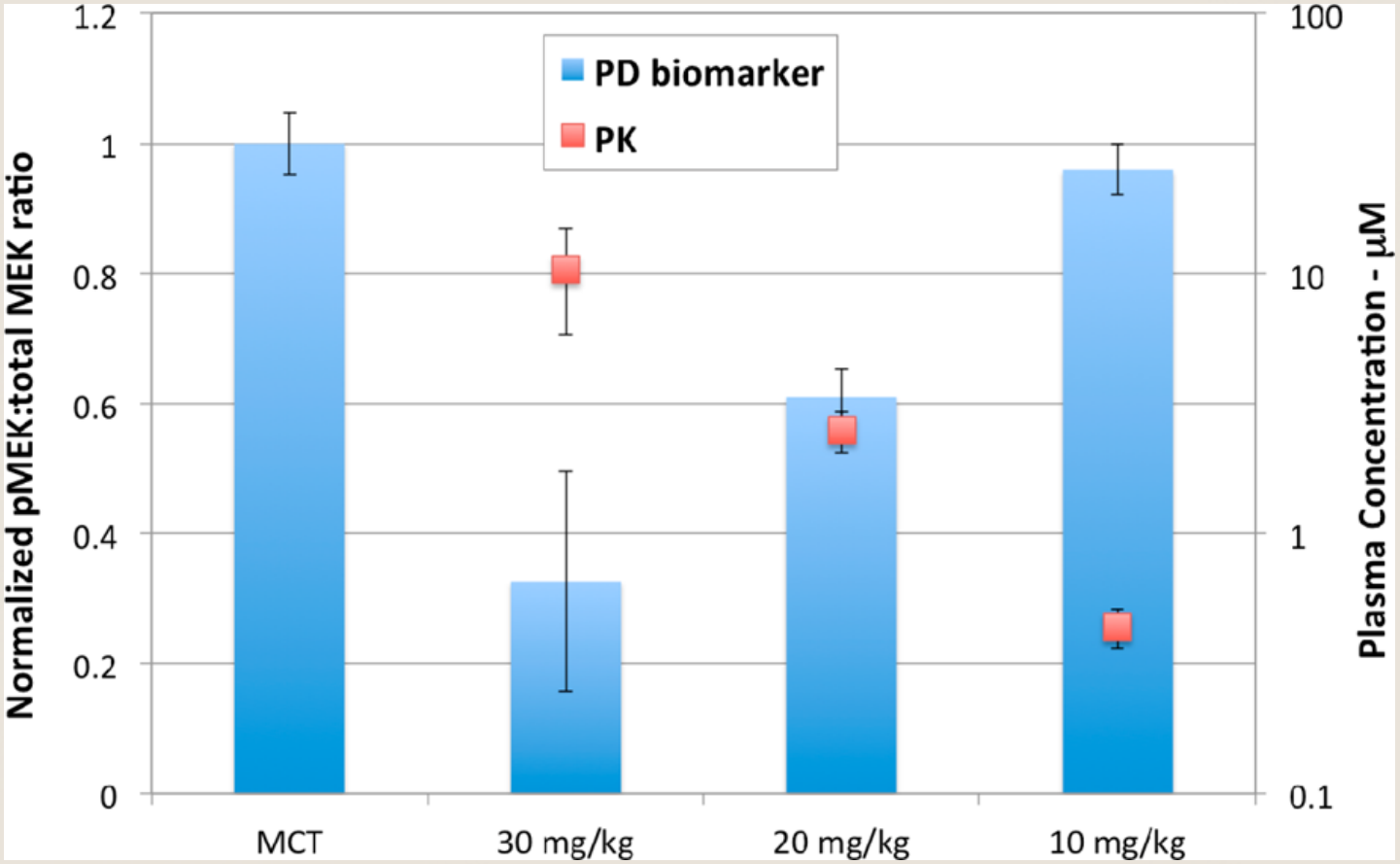
Animal ID	$AUC_{0\text{-tlast}}$ (μM • hr)	AUC_{inf} (μM • hr)	CL (mL/min/kg)	$t_{1/2}$ (hr)	V_{ss} (L/kg)
1	4.86	5.07	13.3	1.30	1.39
2	2.10	2.22	30.5	0.723	1.84
3	2.26	2.34	28.9	0.637	1.41
Mean	3.07	3.21	24.2	0.886	1.55
SD	1.55	1.61	9.48	0.359	0.252

Table S2. Pharmacokinetic Parameters of G-5555 following 25 mg/kg PO Administration to Female Mice

Animal ID	$AUC_{0\text{-tlast}}$ (μM • hr)	C_{max} (μM)	t_{max} (hr)	F (%)
4	30.0	8.64	0.5	84.7
5	33.8	12.3	0.5	84.2
6	25.8	7.96	0.5	69.4
Mean	29.9	9.63	0.5	79.5
SD	4.00	2.33	0.00	8.69

- In cynomolgus monkey:
  - CL_p = 3.4 mL/min/kg
  - F = 72%

Efficacy


PD biomarker
PK
Normalized pMEK:total MEK ratio
Plasma Concentration - µM
1.2
1
0.8
0.6
0.4
0.2
0
100
10
1
0.1
MCT
30 mg/kg
20 mg/kg
10 mg/kg

Conclusions

- Structural modification of known FRAX1036 guided by previous studies by authors and crystal structures lead to improved binding interactions and potency
- Able to balance potency, permeability, and hERG related toxicity
- Incorporation of the 5-amino-1,3-dioxanyl group lead to reduced pK_a and logP while improving potency and hERG activity
  - Showed via degradation studied the stability of the 5-amino-1,3-dioxanyl group
- New compound shows promising PK/PD characteristics that could translate well to the clinic

- *Need to show efficacy via effects on tumor growth*

Bromide Synthesis

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1/16/2016