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Discovery of 2-(α-methylbenzylamino) pyrazines as potent Type II inhibitors of FMS

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ABSTRACT

A series of 2-(α-methylbenzylamino) pyrazines have shown to be potent inhibitors of the FMS tyrosine receptor kinase. Details of SAR studies, modeling and synthesis of compounds within this series are reported.

Macrophage or monocyte colony stimulating factor (CSF-1) interacts with cells through its one specific trans-membrane receptor FMS (also known as CSF-1R)—a receptor tyrosine kinase. Signal transduction through the CSF-1/FMS ligand–receptor complex leads to the differentiation and proliferation of cells of the monocyte/macrophage lineage.[1] Over-expression of CSF-1 and/or FMS has been implicated in a number of disease states including the growth and metastasis of particular cancers,[2] in promoting osteoclast proliferation in bone osteolysis,[3] in inflammatory diseases such as rheumatoid arthritis,[4] atherosclerosis,[5] and Crohn's disease[6] and in renal allograft rejection.[7] Strong evidence supports the major role that tumor associated macrophages (TAM) play in the microtumor environment.[8,9] A monoclonal antibody to CSF-1 developed by Pfizer, PD-0360324, has recently entered Phase 1 clinical trials for rheumatoid arthritis.[10] Therefore, small molecule FMS inhibitors are expected to be valuable therapeutic tools targeting various cancer and inflammatory diseases.

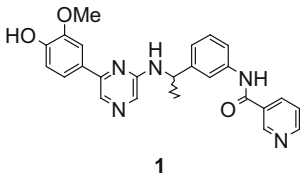
Several chemotypes have been identified as displaying highly potent FMS inhibitory activity including 2,4-diaminopyrimidines,[11] aminoindazoles,[12] dimethoxyquinazolines,[13] quinoline ureas,[4] 2-quinolones,[14] pyridopyrimidinones,[15] 2,4-disubstituted aryl amides,[16] and anilinoquinolines.[17] A recent paper has also drawn attention to the potential use of thiazolyl bisamides as FMS inhibitors in the treatment of cancer.[18]

Focused screening of compounds from Cytopia's internal small molecule library identified the racemic α-methylbenzylamino pyrazine derivative **1** (Fig. 1) as a potent (IC_{50} 22 nM) inhibitor of FMS.[19]

As well as being a potent inhibitor of FMS in biochemical assays, compound **1** also completely blocked CSF-1 induced survival in primary murine bone marrow-derived macrophages (BMM).[19]

Subsequently, we found that the *S* enantiomer of **1** was over 35 times more potent in the enzyme assay compared with its antipode (*S* enantiomer IC_{50} 11 nM, *R* enantiomer IC_{50} 416 nM).[20] We therefore focused all subsequent SAR investigations on the *S* enantiomeric series.

Initially, we discovered that replacement of the aromatic ring directly attached to the pyrazine ring of **1** by a simple halide al-



1

Figure 1. Initial screening hit **1**.

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lowed us to retain sub-micromolar activity in biochemical assays.[20] We therefore investigated the SAR of the aryl amide bond using the 2-chloropyrazine scaffold. We confirmed that both the presence of the amide bond proton and carbonyl were required for potency (Table 1).

To probe the range of terminal moieties tolerated on the amide bond, we prepared a series of derivatives, selected examples of which are displayed in Table 2.

Substitution of the pyridyl ring *meta* to the amide bond (Table 2 **8–10**) greatly increased potency in the biochemical assay. We also found that the pyridyl ring could be exchanged for a mono- (**12**, **14**, and **15**) or disubstituted (**13**, **16**) phenyl ring without losing potency, thus removing potentially deleterious interactions with metabolizing enzymes. Replacement of the pyridyl ring with other heteroaromatic or heterocyclic rings (**17**, **18**) led to a loss of potency.

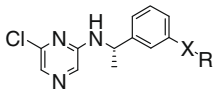
Whereas both **9** and **15** had high predicted clearance values based on human microsomal data, **15** proved to have a reasonable in vivo rat PK profile when dosed iv at 5 mg/kg: $t_{1/2}$ 5.9 h, blood CL_{TOT} 12.7 mL/min/kg and V_z 30.2 L/kg.

Counterscreening of **15** across a diverse panel of more than 300 kinases showed high potency against a limited number of enzymes, including the closely related Type III receptor tyrosine kinases shown in Table 3, DDR1 and FRK.[21] A K_d of greater than 10 μM was seen against most other kinases. Compound **15** also inhibited FMS dependent proliferation of the murine cell line MNFS 60 and CSF-1 dependent growth of murine BMM, as shown in Table 4, without showing general cellular cytotoxicity against human kidney and liver cell lines HEK293 and HepG2, respectively.[22]

We prepared compounds of the type shown in Tables 1 and 2 via the procedure shown in Scheme 1. Thus, a catalytic Leuckart–Wallach type reductive amination[23] of commercially available 3-nitroacetophenone **19** gave the racemic amine. Resolution with L-tartaric acid provided the *m*-nitro-substituted *S*-α-methylbenzylamine **20**. Following reduction of the nitro group and base-promoted coupling to 2,6-dichloropyrazine, we generated a series of amides via conventional coupling reactions with suitable carboxylic acids.[24]

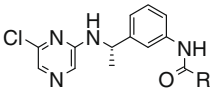
Homology models of the FMS kinase, based on a crystal structure of its most closely structurally related kinase cKit (PDB code 1T46) allowed us to perform docking studies on this series.[26] Initially, docking experiments involving **9** indicated that the compound interacted with the DFG-out (inactive) conformation of

Table 1
Alterations to the amide bond



Compound	X	R	FMS IC_{50} (nM)
2	NHCO		857
3	NH_2	—	>5000
4	N(Me)CO		>5000
5	$NHCH_2$		>5000
6	$NHSO_2$		>5000

Table 2
Alterations to the terminal position of the amide bond



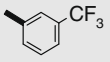
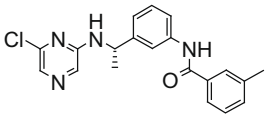
Compound	R	FMS IC_{50} (nM)
2		857
7		40
8		15
9		5
10		4
11		40
12		10
13		9
14		9
15		2
16		2
17		2000
18		5000

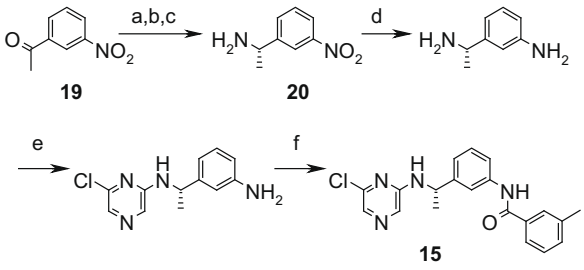
Table 3
Kinase selectivity of **15**



Enzyme	K_d (nM)	Enzyme	K_d (nM)
FMS	1.9	RET	95
cKit	2.2	Src	1300
PDGFRβ	2.3	AURKB	>10,000
PDGFRα	3	EGFR	>10,000
VEGFR-1	5.7	FGFR1,2,3	>10,000
DDR1	7.8	Flt3	>10,000
VEGFR-2	9.1	JAK3	>10,000
FRK	12	bRAF	>10,000
VEGFR-3	27	TIE2	>10,000

Table 4
Cellular activity of **15**

Cell line	MNFS 60	BMM	HEK293	HepG2
IC_{50} (nM)	211	107	>20,000	>20,000



Scheme 1. Reagents and conditions: (a) ammonium formate, $[RhCp^*Cl_2]_2$, MeOH, 70 °C, 5 h; (b) chiral resolution with L-tartaric acid[25]; (c) NaOH (aq); 25% over 3 steps; (d) H_2, Pd/C, EtOH, 30 psi, 30 min, 100%; (e) 2,6-dichloropyrazine, dioxane, K_2CO_3, 120 °C, 72 h, 85%; (f) *m*-toluic acid, EDAC, 4-pyrrolidinopyridine, NEt_3, CH_2Cl_2, rt, 48 h, 83%.

the enzyme in two possible binding modes as shown in Figure 2. In *binding mode A* the pyridine N of **9** is involved in a hinge binding interaction with Cys666 whereas in *binding mode B* it is the 4-N of the pyrazine ring that initiates this contact. In both cases there are interactions with the DFG motif which is in the 'out' configuration.

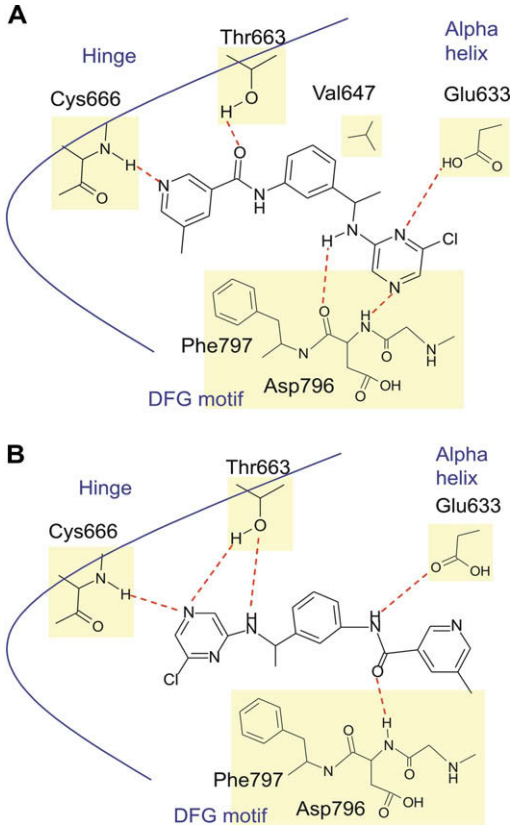
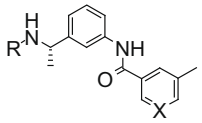


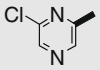
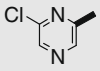
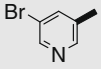
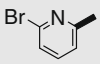
Figure 2. Two potential binding modes of **9** in the ATP binding pocket of FMS. Hinge interactions are with Cys666 and Thr663 (gatekeeper residue). In binding mode A, hinge interactions are postulated for the pyridine N and amide carbonyl. In binding mode B, hinge interactions are postulated for 4-N of the pyrazine and the secondary amine. Putative hydrogen bonds are indicated by a dashed line.

To assess the validity of the two binding modes, we modified compound **9** as shown in Table 5. Thus, when the pyridine N of **9** was exchanged with a CH to give compound **15** there was no change in inhibitory activity on FMS. When we replaced the pyrazine ring of **9** with a 3,5-disubstituted pyridine (compound **21**) there was again minimal change in activity. However, when we replaced the pyrazine with a 2,6-disubstituted pyridine, **22**, we lost all inhibitory activity. This observation confirmed binding mode B (Fig. 2): loss of the pyridine N from **9** does not affect activity while loss of the pyrazine 4-N in this series does.

Hence, as shown in Figure 3, the chloropyrazine moiety of **15** interacts with the adenine-hinge binding region of the ATP pocket while the amide bond sits over the DFG motif in the 'allosteric site'.

Table 5
Inhibitory activity of modified pyridine and pyrazine ring compounds in FMS biochemical assays



Compound	R	X	FMS IC_{50} (nM)
9		N	5
15		CH	2
21		N	5
22		N	>5000

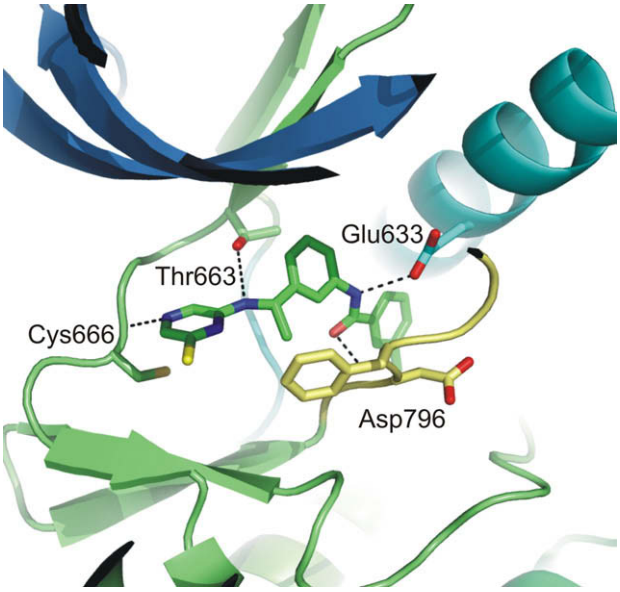


Figure 3. Model of **15** complexed with FMS in the DFG-out conformation.[26] Proposed hydrogen bonds in black are shown between 4-N of the pyrazine and hinge (Cys666 backbone), between the secondary amine and gatekeeper (Thr663 hydroxy), between the amide carbonyl and DFG motif (Asp796 backbone) and between the amide NH and α-helix (Glu633 carboxylate). The 3-methylbenzamide moiety extends into the allosteric site.

The 3-methylbenzamide moiety of **15** further extends into the hydrophobic region of the allosteric site. Overlay of our homology model with a subsequently published crystallographic structure of FMS, PDB code 3BEA,[15] produced an RMSD of 1.5 Å over Cα atoms, validating our FMS modeling approach. Compounds that bind to the inactive conformation of kinases in this manner are commonly referred to as Type II kinase inhibitors.[27]

In summary, we have developed a new series of Type II FMS inhibitors with excellent biochemical and cellular potency. Our ongoing efforts to improve the PK profile of this series will be reported in due course.

Acknowledgments

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21. KINOMEscan™ screen performed by AMBIT Biosciences, San Diego. See: Karaman, M. W.; Herrgard, S.; Treiber, D. K.; Gallant, P.; Atteridge, C. E.; Campbell, B. T.; Chan, K. W.; Ciceri, P.; Davis, M. I.; Edeen, P. T.; Faraoni, R.; Floyd, M.; Hunt, J. P.; Lockhart, D. J.; Milanov, Z. V.; Morrison, M. J.; Pallares, G.; Patel, H. K.; Pritchard, S.; Wodicka, L. M.; Zarrinkar, P. P. *Nat. Biotechnol.* **2008**, *26*, 127.
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24. All compounds were characterized by ^{1}H NMR and reverse phase LCMS and their purity determined to be >95%. *Compound **15**:* A mixture of (*S*)-*N*-[1-(3-aminophenyl)ethyl]-6-chloropyrazin-2-amine (11.45 g, 46 mmol), *m*-toluic acid (9.4 g, 69 mmol), *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride (17.6 g, 92 mmol), 4-pyrrolidinopyridine (1.36 g, 9.2 mmol) and triethylamine (25.7 mL, 184 mmol) in dichloromethane (400 mL) was stirred at room temperature for 48 hours. After this time the mixture was washed successively with saturated aqueous sodium hydrogen carbonate (2 x 100 mL) and brine (100 mL), dried ($MgSO_4$) and concentrated under reduced pressure to give an orange oil which was purified by flash chromatography (silica, ethyl acetate/hexanes) to give (*S*)-*N*-{3-[1-(6-chloropyrazin-2-ylamino)ethyl]phenyl}-3-methylbenzamide (14 g, 83%) as a pale yellow solid. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.58 (d, *J* 6.6 Hz, 3H), 2.42 (s, 3H), 4.85 – 4.94 (m, 1H), 5.16 (d, *J*6.6 Hz, 1H), 7.13 (d, *J*7.8 Hz, 1H), 7.31 – 7.37 (m, 3H), 7.49 – 7.52 (m, 1H), 7.61 – 7.65 (m, 2H), 7.67 (br s, 1H), 7.73 (br s, 1H), 7.78 (s, 1H), 7.86 (br s, 1H).
25. One recrystallisation of the racemic tartaric acid salt followed by liberation of the free base gave the (*S*)-benzylamine with an ee of 90–92%. This material was used in subsequent steps.
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