Available online at www.sciencedirect.com
ScienceDirect
Bioorganic & Medicinal Chemistry Letters 17 (2007) 6070–6074
Bioorganic & Medicinal Chemistry Letters

Potent 2′-aminoanilide inhibitors of cFMS as potential anti-inflammatory agents

Raymond J. Patch, Benjamin M. Brandt, Davoud Asgari, Nand Baindur, Naresh K. Chadha, Taxiarchis Georgiadis, Wing S. Cheung, Ioanna P. Petrounia, Robert R. Donatelli, Margery A. Chaikin and Mark R. Player*

Drug Discovery, Johnson and Johnson Pharmaceutical Research and Development, L.L.C., 8 Clarke Drive, Cranbury, NJ 08512, USA

Received 22 August 2007; revised 13 September 2007; accepted 14 September 2007
Available online 19 September 2007

Abstract—A series of 2′-aminoanilides have been identified which exhibit potent and selective inhibitory activity against the cFMS tyrosine kinase. Initial SAR studies within this series are described which examine aroyl and amino group substitutions, as well as the introduction of hydrophilic substituents on the benzene core. Compound **47** inhibits the isolated enzyme (IC_{50} = 0.027 μM) and blocks CSF-1-induced proliferation of bone marrow-derived macrophages (IC_{50} = 0.11 μM) and as such, serves as a lead candidate for further optimization studies.

cFMS is a type III receptor tyrosine kinase that is selectively expressed on macrophages and their progenitor cells and serves as the exclusive receptor for colony-stimulating factor (CSF-1), the primary growth factor for the macrophage lineage.[1] Upon binding to CSF-1, cFMS undergoes autophosphorylation and dimerization, and ultimately induces the phosphorylation of downstream signaling proteins, thereby driving the differentiation and activation of these cells.[2]

The expression of macrophages is significantly induced at sites of inflammation; this over-expression propagates a chronic inflammatory response, which is in part due to a CSF-1/FMS-driven mechanism.[3] Thus, ligation of cFMS by CSF-1 results in activation and proliferation of macrophages and their subsequent release of inflammatory mediators. These in turn stimulate neighboring fibroblasts to release additional CSF-1, thereby perpetuating an inflammatory cycle. Inhibitors of cFMS would represent a likely means of interrupting this cycle and would therefore be expected to result in an anti-inflammatory response.[4] Support for this hypothesis is provided by various animal studies. For example, in collagen-induced arthritis (CIA) models, CSF-1 treatment was shown to exacerbate the disease,[5] whereas anti-CSF-1 treatment resulted in a reduced severity of disease.[6] Furthermore, CSF-1 deficient (*op*/*op*) mice were shown to be completely resistant to CIA.[5]

Thus, the prominent role of the CSF-1/cFMS signaling in the activation and proliferation of macrophages makes this transduction pathway an attractive target for therapeutic intervention.[7] We therefore sought to identify selective inhibitors of cFMS as a potential treatment for macrophage-related diseases.

Amongst the active chemotypes identified from a focused screening effort, aroylanilide **1** was chosen for further evaluation and hit-to-lead optimization. This report describes initial structure–activity relationships (SAR) within this series, which led to the identification of an advanced analogue that proved to be suitable for X-ray crystallographic studies (Fig. 1).

It was apparent from the primary screening set that an aroyl-capped ortho-amino anilide structure represented the basic pharmacophoric unit for cFMS inhibition. Additionally, a preliminary examination of aroyl groups revealed a preference for N- and O-containing heterocycles. Moreover, nitroaromatics generally had greater affinity for cFMS than their unsubstituted counterparts. Of these, the 5-nitrofuroyl moiety was found to afford a

Keywords: cFMS; Colony-stimulating factor-1 receptor; CSF-1; M-CSF; 2′-Aminoanilides.
* Corresponding author. Tel.: +1 610 458 6980; e-mail: mplayer@prdus.jnj.com

0960-894X/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2007.09.057

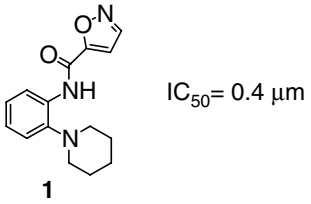


Figure 1. High-throughput screening hit: aroylanilide **1**.

10-fold improvement in inhibitory potency relative to isoxazole **1**. While we recognized the potential metabolic and genotoxic liability introduced by this functionality,[8] at this stage of the project, it served as a useful control for assessing SAR of the 2-amino substituent. Our initial follow-up strategy was therefore based on a two-point diversity scan against a broader array of aroyl and amino groups (Fig. 2).

2′-Aminoanilides were readily prepared by a standard S_NAr reaction of a 2-fluoronitrobenzene derivative with various amines, followed by nitro group reduction and capping with an aroyl chloride (Scheme 1).[9]

Compounds were initially assessed in an in vitro enzyme assay for their abilities to inhibit ATP-induced autophosphorylation of cFMS (Table 1).[10] The importance of maintaining an aliphatic cyclic amine structure at the 2-position was apparent in that replacement of piperidine with acyclic amines (**5**–**7**) led to a dramatic loss of cFMS inhibitory activity.[11] Ring size also affected activity, with optimal inhibition being derived from the 6-membered piperidine system of **2**, and decreasing with smaller (**8**, **9**) and larger (**10**) ring systems. Activity was also diminished by the incorporation of heteroatoms in the ring, as was observed with morpholine and piperazine analogues, **3** and **4**, respectively. When evaluated in a cellular kinase assay,[12] inhibitory activities largely paralleled those obtained in the isolated enzyme system, being roughly 5- to 10-fold less active.

Substituent effects appeared to be largely influenced by steric factors, being sensitive to both size and ring position. Thus, incorporation of small substituents, such as methyl, (**14**), hydroxyl (**17**), hydroxymethyl (**18**), and hydroxyethyl (**19**), were best tolerated at the 4-position of the piperidine ring. In contrast, hydroxy and hydroxymethyl substituents rendered pyrrolidino homologue **9** inactive (**20** and **21**, respectively). Whereas methyl substitution was tolerated on the 4-piperazinyl nitrogen (**15**), activity was lost upon phenyl substitution

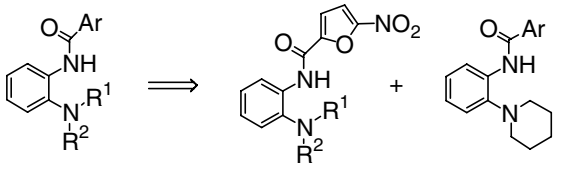
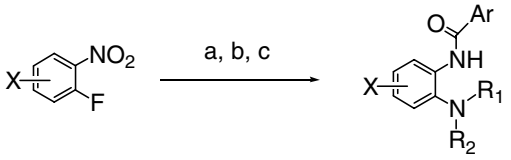
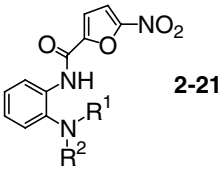


Figure 2. Two-point diversity strategy.



Scheme 1. Synthesis of 2′-aminoanilides. Reagents and conditions: (a) HNR^1R^2, PS-morpholine, $CHCl_3$, 50 °C, 2 h; (b) H_2, Pd/C, HOAc/MeOH/EtOAc (1 atm.); (c) ArCOCl, PS-morpholine, dioxane, 70 °C, 2 h.

Table 1. cFMS inhibitory activities for 2′-amino-5-nitrofuroylanilides **2**–**21**



Compound	NR^1R^2	Auto-P_i IC_{50}[a] (μM)	HEK 293 IC_{50}[b] (μM)
2	Piperidino	0.053	0.36
3	Morpholino	1.1	
4	Piperazine	>10	
5	*N*-Ethyl-*N*-propylamino	>10	
6	*N*,*N*-Dipropylamino	>10	
7	Anilino	>10	
8	Azetidino	>10	
9	Pyrrolidino	1.9	
10	Azepino	0.26	2.0
11	2-Methylpiperidino	0.72	
12	3-Methylpiperidino	0.38	2.2
13	3,5-Dimethylpiperidino	5.3	
14	4-Methylpiperidino	0.078	0.3
15	4-Methylpiperazino	0.39	1.4
16	4-Phenylpiperazino	>10	
17	4-Hydroxypiperidino	0.14	0.37
18	4-Hydroxymethylpiperidino	0.13	1.4
19	4-(2-Hydroxyethyl)piperidino	0.043	0.23
20	3-Hydroxypyrrolidino	>10	
21	3-Hydroxymethylpyrrolidino	>10	

[a] Reported IC_{50} values are means of three experiments. Inter-assay variance was <10%.

[b] Dose–response data are the average of at least two replicates per dose.

(**16**). Methyl group incorporation at the 3-position of the piperidine ring was also tolerated (**12**), though to a lesser extent than at the 4-position. Appending an additional methyl group at the 5-position further attenuated inhibitory activity (**13**).

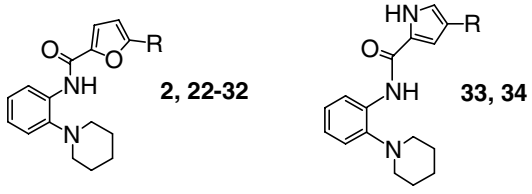
Stability studies with microsomal preparations revealed a significant metabolic liability with early series analogues. From metabolite ID studies, it was determined that the 4-position of the piperidine ring was susceptible to microsomal oxidation. However, this liability could be overcome simply by methylation (as in **14**), a tactic that would extend to later series analogues as well. For example, following a 10-min incubation period with

human liver microsomes (HLMs), 41% of piperidinyl **2** remained intact; methylpiperidine **14** was completely stable to these conditions (100% remaining).

A more directed study was undertaken to identify suitable heteroaroyl groups that could replace the nitrofuroyl system. At this time, the mechanism(s) by which the nitro group enhanced inhibitory activities in this series was unclear. And so, in order to determine whether this might be due to a specific favorable protein interaction or indirectly by way of favorable electronic modulation of the aroyl rings, a number of furan derivatives were evaluated (Table 2).

From this study, it would appear that both electronic and steric factors contribute to the activity-enhancing properties of the nitro group. The presence of electron withdrawing groups on the furan provided improved activities to various degrees as compared with unsubstituted **22**. In contrast, aminofuroyl analogues **23** and **24** were completely inactive. The diminished activity of carboxy analogue **29** relative to **2** may reflect a lack of involvement of a specific charge interaction between the 5-substituent and the enzyme. At first glance, this might imply that electronic effects of the substituents exert a predominating influence on activity; however, the inactivity of trifluoromethyl derivative **31** suggests that this property alone does not suffice. Sterics and hybridization likely also contribute to adventitious substituent effects, a notion that is supported by the improved activity of cyanofuroylanilide **32**.

Table 2. Aroyl ring substituent effects upon cFMS binding/inhibitory activities for 2′-(piperidin-1-yl)anilides **2**, **22**–**34**



Compound	R	Auto-P_i IC_{50}[a] (μM)	HEK 293 IC_{50}[b] (μM)
22	H	>10	
2	NO_2	0.053	0.36
23	NH_2	>10	
24	NHPh	>10	
25	Ph	>10	
26	Br	4.4	
27	Cl	1.9	
28	CHO	1.1	
29	CO_2H	>10	
30	$CONH_2$	>10	
31	CF_3	>10	
32	CN	0.036	0.37
33	NO_2	0.047	0.18
34	CN	0.047	0.15

[a] Reported IC_{50} values are means of three experiments. Inter-assay variance was <10%.

[b] Dose–response data are the average of at least two replicates per dose.

An expanded search for additional heterocyclic replacements led to the identification of nitropyrroloyl (**33**) and cyanopyrroloyl (**34**) analogues as particularly potent inhibitors of cFMS activity. Other isomeric azoles, however, proved much less active in these assays, demonstrating the sensitivity of heteroatom positioning in this ring (data not shown).

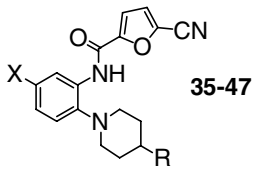
Examination of substituent effects on the benzene ring revealed a range of functionality that could be accommodated at the 5-position (Table 3). With the exception of a carboxyl group (**50**), polar substituents were particularly adventitious, not only affording increased inhibitory potency in several instances, but also providing a solubilizing handle for this chemotype. Exceptions to this were underivatized phenol **35** and guanidinylmethyl **46**, whose cellular activities were ~100-fold less than their activities against the isolated enzyme, possibly a reflection of limited cellular uptake. Hydroxy-containing analogues **37**, **44**, and **47** were amongst the most effective inhibitors of cFMS kinase activity. Again, the 4-methyl substituent in **47** imparted significant microsomal stability (83% remaining) to an otherwise metabolically susceptible analogue, **37** (25% remaining after 10-min incubation with HLMs).

Kinase selectivity of lead analogue **47** was assessed against a standard diverse panel of kinases (Table 4). At a concentration of 1 μM, only the neurotrophic tyrosine kinase receptor, type 1 (TRKA) was inhibited at above 50%.

In order to evaluate the functional activities of cFMS inhibitors, an assay was developed based upon CSF-1 driven proliferation of bone marrow-derived macrophages.[13] Following deprivation of CSF-1, cultured macrophages derived from mouse bone marrow can be driven into S-phase upon re-stimulation with CSF-1. Bromodeoxyuridine (BrDU) is incorporated into DNA of S-phase cells and can be quantitated in an ELISA format.[14] In this assay, **47** inhibited CSF-1 induced incorporation of BrDU into mouse macrophages with an IC_{50} of 0.11 μM.

In addition to its lead characteristics, **47** aided in the crystallization of a chimeric kinase domain of cFMS, the co-crystal structure of which was recently reported.[15] The interaction of **47** with cFMS occurs in the hinge region of the ATP pocket and involves a critical hydrogen bond between the aroylamide carbonyl and the backbone NH of Cys666 (Fig. 3). Interestingly, the ring oxygen of the furan is not involved in direct binding to a specific cFMS residue; rather, it shares an intramolecular hydrogen bond with the amide NH, thereby stabilizing a flat conformation of the aroylamide core. The methylpiperidine occupies the ATP sugar pocket; the hydroxymethyl extends away from this region, making a hydrogen bond with the phenol of Tyr665. Finally, this crystallographic information has provided the structural basis for further lead optimization studies within this series.

Table 3. Benzene ring substituent effects for 2′-(piperidin-1-yl)anilides **35–47**



Compound	X	R	Auto-P_i IC_{50}[a] (μM)	HEK 293 IC_{50}[b] (μM)
35	HO	H	0.017	2.7
36	MeO	H	0.09	0.96
37	Hydroxymethyl	H	0.025	0.19
38	Aminomethyl	H	0.2	3.1
39	CHO	H	0.037	0.33
40	CO_2H	H	>2	>2
41	CN	H	0.12	0.75
42	Piperidin-1-yl	H	0.017	0.076
43	4-Methylpiperazinomethyl	H	0.08	1.01
44	2,3-Dihydroxypropoxymethyl	H	0.019	0.17
45	Methylsulfonamidomethyl	H	0.037	0.31
46	Guanidinylmethyl	H	0.034	2.6
47	Hydroxymethyl	Me	0.024	0.25

[a] Reported IC_{50} values are means of three experiments. Inter-assay variance was <10%.
[b] Dose–response data are the average of at least two replicates per dose.

Table 4. Kinase selectivity screen for compound **47**

Kinase	% Inh[a]
Abl	−15
Akt1	0
Arg	−15
BTK	9
Blk	−4
CDK1/cyclinB	−3
CDK2/cyclinA	2
CHK1	2
CaMKIIα	0
EphB4	−2
FES	1
Fgr	2
Flt3	25
Fyn	10
GSK3β	−9
IGF-1R	0
IRAK4	1
Lck	5
PKA	−18
PKCθ	0
Rsk2	−1
Src	12
TRKA	54
Yes	5

[a] Compound **47** was tested at single concentration of 1 μM; ATP concentration was 100 μM. Values are averages of duplicate experiments.

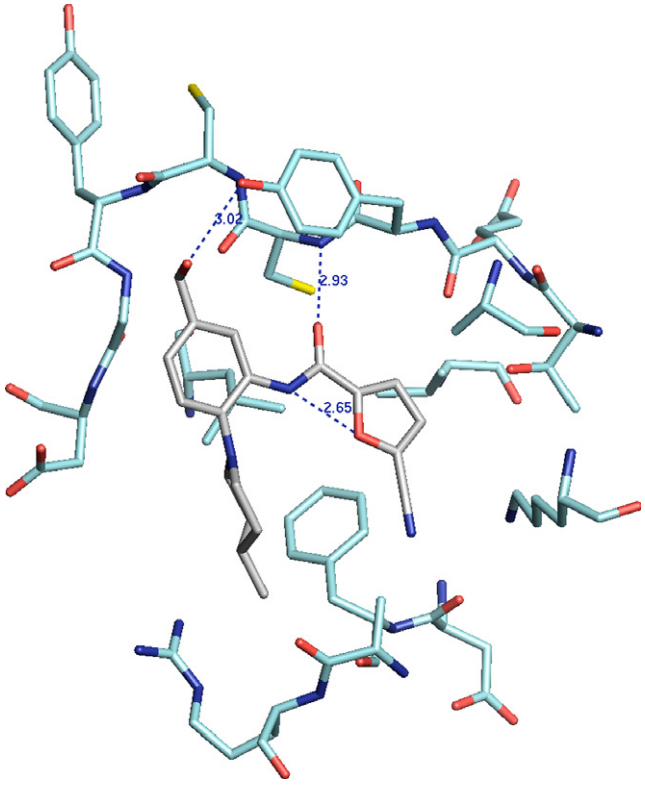


Figure 3. Cut-away illustration of the complex between cFMS (teal) and furoylanilide **47** (gray). Hydrogen bonds (blue dashes) are shown along with interatomic distances between heavy atoms.

References and notes

1. Sherr, C. J. *Biochim. Biophys. Acta* **1988**, *948*, 225.
2. (a) Pixley, F. J.; Stanley, E. R. *Trends Cell Biol.* **2004**, *14*, 628; (b) Sherr, C. J.; Roussel, M. F.; Rettenmier, C. W. *J. Cell Biochem.* **1988**, *38*, 179.
3. (a) Yanni, G.; Whelan, A.; Feighery, C.; Bresnihan, B. *Ann. Rheum. Dis.* **1994**, *53*, 39; (b) Yang, N.; Isbel, N. M.; Nikolic-Paterson, D. J.; Li, Y.; Ye, R.; Atkins, R. C.; Lan, H. Y. *Kidney Int.* **1998**, *54*, 143; (c) Isbel, N. M.; Nikolic-

Patterson, D. J.; Hill, P. A.; Dowling, J.; Atkins, R. C. *Nephrol. Dial. Transplant.* **2001**, *16*, 1638.
4. (a) Hamilton, H. A. *Trends Immunol.* **2002**, *23*, 403; (b) Conway, J. G.; McDonald, B.; Parham, J.; Keith, B.; Rusnak, D. W.; Shaw, E.; Jansen, M.; Lin, P.; Payne, A.; Crosby, R. M.; Johnson, J. H.; Frick, L.; Lin, M.-H. J.; Depee, S.; Tadepalli, S.; Votta, B.; James, I.; Fuller, K.; Chambers, T. J.; Kull, F. C.; Chamberlain, S. D.; Hutchins, J. T. *Proc. Natl. Acad. Sci. U.S.A.* **2005**, *102*, 16078.
5. Campbell, I. K.; Rich, M. J.; Bischof, R. J.; Hamilton, J. A. *J. Leukocyte Biol.* **2000**, *68*, 144.
6. Cook, A. D.; Braine, E. L.; Campbell, I. K.; Rich, M. J.; Hamilton, J. A. *Arthritis Res.* **2001**, *3*, 293.
7. Lawlor, K. E.; Campbell, I. K.; Metcalf, D.; O'Donnell, K.; van Nieuwenhuijze, A.; Roberts, A. W.; Wicks, I. P. *Proc. Natl. Acad. Sci. U.S.A.* **2004**, *101*, 11398.
8. (a) Jung, R.; Le, J. Y.; Wengenmayer, F.; Wolf, E.; Kramer, M. *Biomed. Biochim. Acta* **1985**, *44*, 485; (b) McCalla, D. R. *Environ. Mutagen.* **1983**, *5*, 745; (c) Schmid, A. *Tierarztl. Prax.* **1985**, *13*, 471.
9. Polymer-bound morpholine (PS-morpholine) was obtained from Sigma–Aldrich Chemical Co.
10. *cFMS autophosphorylation assay* Schalk-Hihi, C.; Ma, H. C.; Struble, G. T.; Bayoumy, S.; Williams, R.; Devine, E.; Petrounia, I. P.; Mezzasalma, T.; Zeng, L.; Schubert, C.; Grasberger, B.; Springer, B. A.; Deckman, I. C. *J. Biol. Chem.* **2007**, *282*, 4085.
11. That this cyclic amine should be aliphatic was determined from the initial screening set data. For example, the *N*-pyrrolo analogue of **1** was found to be inactive.
12. *HEK293 phosphorylation assay* Baumann, C. A.; Zeng, L.; Donatelli, R. R.; Maroney, A. C. *J. Biochem. Biophys. Methods* **2004**, *60*, 69.
13. Kaye, P. M. In *Macrophages*; Paulnock, D. M., Ed.; Oxford University Press: Oxford, 2000; pp 93–113.
14. *BMDM Proliferation Assay.* Bone marrow-derived macrophages (BMDM) were plated at a density of 5000 cells/well into Costar 96-well microtiter plates and incubated overnight in macrophage culture media without CSF-1. The following day, wells were adjusted to contain 5 ng/ml CSF-1, and graded concentrations of **47**. After a 24-h culture period, wells were further adjusted to contain bromodeoxyuridine (BrDU) for additional 6 h. The incorporation of BrDU into the DNA of proliferating macrophages was quantified using a specific ELISA (Exalpha Corp. Watertown, MA) and concentrations of **47** that inhibited BrDU incorporation by fifty percent (IC_{50}) were calculated using GraphPad Prism® software and a four parameter logistics equation.
15. Schubert, C.; Schalk-Hihi, C.; Struble, G. T.; Ma, H. C.; Petrounia, I. P.; Brandt, B.; Deckman, I. C.; Patch, R. J.; Player, M. R.; Spurlino, J. C.; Springer, B. A. *J. Biol. Chem.* **2007**, *282*, 4094, PDB: 2I0Y.