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Synthesis of novel anilinoquinolines as *c-fms* inhibitors

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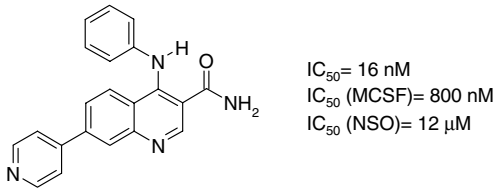
Abstract—A novel series of potent substituted anilinoquinolines were discovered as *c-fms* inhibitors. The potency could be manipulated upon modification of the C4 aniline and C7 aryl functionality. Pharmacokinetic analysis identified a metabolically stable analog suitable for further investigative work.

The role of inflammation in the progression of human diseases is well documented.[1] Osteoarthritis in particular has been shown to be caused in part by the over-production of macrophages in the synovial fluid of joints, leading to inflammation, cartilage loss, and pain.[2] The discovery of progenitors of inflammation has sparked a great deal of interest from the scientific community in regards to potential treatments for diseases. Macrophage colony stimulating factor (MCSF) has been implicated as playing a role in several diseases, including inflammation.[3] Most notable is the role of MCSF in cancer, particularly angiogenesis. Due to its ability to promote tumor growth and survival, research into down-regulating MCSF has been an area of intense interest.[4] The receptor for MCSF activation, *c-fms*, is a potential target for drug discovery efforts.[5]

Our interest in *c-fms* inhibitors began with the identification of compound **1** through a high throughput screening effort of the GSK compound collection (Fig. 1). The measured in vitro potency in the binding assay is excellent, with an IC_{50} = 16 nM.[6] In our cell assays the potency was not quite as good, showing an IC_{50} = 800 nM against a cell line expressing MCSF and 14-fold selectivity when compared to a non-MCSF expressing cell line (NSO), which was used as a measure of toxicity. In addition, pharmacokinetic (PK) experiments indicated that **1** was cleared rapidly from plasma (Cl = 39 mL/min/kg). In vivo tests in the rat paw inflammation model (carrageenan challenge) showed that **1** produced a 95% inhibition of inflammation, as measured by paw size, relative to controls when dosed at 40 mg/kg IV. While these results were promising, we wanted to increase the potency in the cell assay while maintaining or improving the binding potency. We were also interested in improving the in vivo exposure of **1** by substituting the C4 aryl group to prevent metabolic degradation arising from oxidative processes and by replacing the C7 pyridine, which can potentially generate an undesirable *N*-oxide.



1

Figure 1. Structure and assay data for **1**.

The synthesis of the anilinoquinolines is depicted in Scheme 1.[7] 3-Iodoaniline is treated with diethyl ethoxymethylenemalonate to afford enamine **2**. Cyclization occurred upon heating in phenyl ether and the product (**3**) was isolated by filtration upon cooling. Saponification of the ester was followed by conversion to the 4-chloro-7-iodoquinoline-3-carboxylic acid chloride with $SOCl_2$. Treatment of the acid chloride with NH_3 gave **4**. Nucleophilic displacement of the 4-chloro moiety with substituted anilines was accomplished in refluxing dioxane to provide compounds of type **5**. Subsequent

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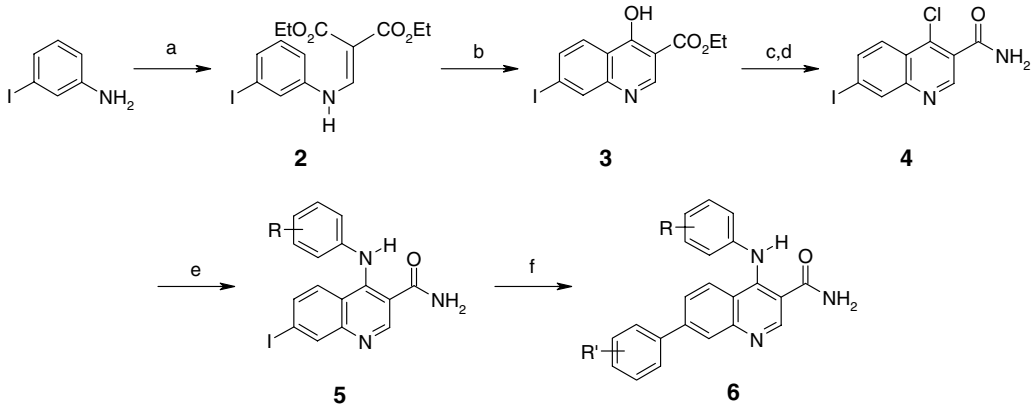


Scheme 1. Reagents and conditions: (a) diethyl ethyoxymethylenemalonate, EtOH, 100%; (b) Ph_2O, 250 °C, 90%; (c) NaOH, MeOH, reflux, 95%; (d) $SOCl_2$, reflux, then $NH_{3(g)}$, 67%; (e) substituted aniline, dioxane, reflux; (f) arylboronic acid, 2 M aq K_2CO_3, $PdCl_2(PPh_3)_2$, $PhCH_3$, EtOH, reflux.

Suzuki coupling with substituted arylboronic acids gave the desired compounds (**6**).

We identified two areas of diversity that would allow for rapid analog synthesis, the C4 aniline and the C7 aryl positions. Computational analysis of **1** bound to the active site of *c-fms* suggested that the C4 aryl group resides in a lipophilic pocket while the C7 group points toward the protein/solvent interface (Fig. 2).[8] As a result compounds containing lipophilic residues were targeted as potential C7 derivatives, while substituents containing more hydrophilic residues were proposed as potential C4 targets.

Several analogs differing at C4 were made and the results are shown in Table 1. In general, introduction of lipophilic substituents at the C4 aryl group gave compounds with excellent binding potency but reduced cell potency (**7c–e**, **7n–p**). We were excited to discover that the 3,4-dimethyl analog (**7f**) exhibited excellent cell potency and selectivity. The potent binding activity of

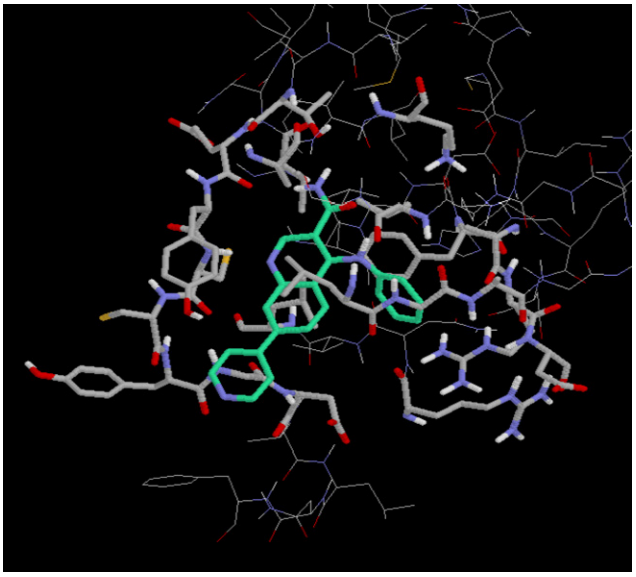
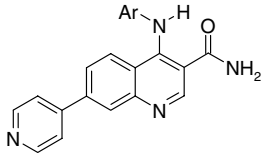


Figure 2. Stick model of **1** (in green) bound in the active site of *c-fms*.

Table 1. Structure–activity relationships of **1** with modification of the C4 aniline[a]



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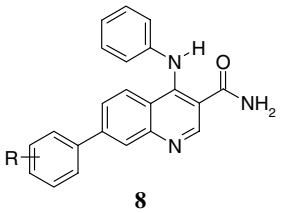
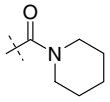
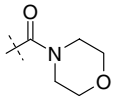
Ar	IC_{50} (μM)	IC_{50} MCSF (μM)	IC_{50} NSO (μM)	Ar	IC_{50} (μM)	IC_{50} MCSF (μM)	IC_{50} NSO (μM)
H (**1**)	0.016	0.80	12	3-Cl, 4-Me (**7j**)	0.040	0.16	> 32
4-OMe (**7a**)	0.063	0.80	16	3-Me, 4-Cl (**7k**)	0.16	2.0	32
4-F (**7b**)	0.10	0.80	>40	3-F, 4-OMe (**7l**)	0.040	0.32	32
4-SMe (**7c**)	0.13	10	>50	3-Cl, 4-OMe (**7m**)	0.032	0.20	>50
4-NMe2 (**7d**)	0.063	6.3	10	3-Cl, 4-OCF_3 (**7n**)	0.032	>20	>32
4-*i*-PrO (**7e**)	0.63	40	>50	3-F, 4-OEt (**7o**)	0.10	4.0	>16
3,4-di-Me (**7f**)	0.080	0.1	>50	3-F, 4-O-*i*-Pr (**7p**)	1.0	8.0	13
3,4-di-F (**7g**)	0.032	2.5	>32	3-Isoxazolyl (**7q**)	3.2	>50	40
3-F, 4-Me (**7h**)	0.080	5.0	>20	3-Benzopyrazolyl (**7r**)	0.32	0.80	>50
3-Me, 4-F (**7i**)	0.050	1.0	>50	4-Benzopyrazolyl (**7s**)	0.16	1.30	>32

[a] All results are reported as an average of at least two separate assays.

Table 2. Structure–activity relationships of **1** with modification of the C7 aryl group[a]



8

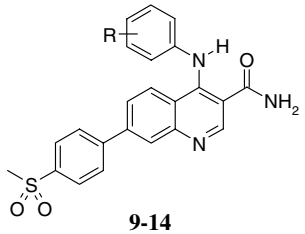
R	IC_{50} (μM)	IC_{50} MCSF (μM)	IC_{50} NSO (μM)	R	IC_{50} (μM)	IC_{50} MCSF (μM)	IC_{50} NSO (μM)
H (**8a**)	0.25	10	32	4-$NHSO_2Me$ (**8l**)	0.063	2.0	10
4-Me (**8b**)	0.050	32	25	4-C(O)-*c*-C_3H_5 (**8m**)	0.10	4.0	10
4-OMe (**8c**)	0.025	6.3	8.0	4-C(O)-*c*-C_5H_9 (**8n**)	0.032	>20	>20
4-F (**8d**)	0.032	25	25	3-CH_2SO_2Me (**8o**)	0.025	5.0	>20
4-CF_3 (**8e**)	0.13	16	20	4-CH_2SO_2Me (**8p**)	0.050	1.6	8.0
4-Br (**8f**)	0.016	50	50	4-SO_2Et (**8q**)	0.20	0.80	8.0
4-CN (**8g**)	0.05	4.0	32	 (**8r**)	0.016	0.40	8.0
3-CN (**8h**)	0.32	8.0	10	 (**8s**)	0.080	0.20	8.0
4-NH_2 (**8i**)	0.16	1.6	10	4-SO_2NH_2 (**8t**)	0.080	3.2	13
4-NMe_2 (**8j**)	0.040	6.3	32	4-SO_2Me (**8u**)	0.016	0.10	20
4-NHAc (**8k**)	0.080	1.3	4.0				

[a] All results are reported as an average of at least two separate assays.

the 3,4-difluoro analog **7g** led to the synthesis of a series of analogs having a combination of a halogen with a methyl group (**7h–j**) showing good potency. Unfortunately those compounds were significantly less potent in the cell assay. A methoxy group at the 4-position was also well tolerated in the series (**7l**, **7m**), however increasing the bulk to 4-ethoxy (**7o**) or 4-isopropoxy (**7p**) led to a loss in potency. Heterocyclic replacements of the benzene ring (**7q–s**) provided compounds with little or no advantage over **1**.

Upon optimizing the C4 position, we turned our attention to the C7 analogs. The results are summarized in Table 2. Initially several substitutions were made with small lipophilic groups (**8a–f**), which gave compounds with reasonable binding potency, but limited cell potency. Based on this data we proposed that lack of compound solubility could be a key factor in cell potency, and the synthesis of a series of compounds with more hydrophilic residues at C7 was initiated. As such, substitution of an amino group in the *para* position (**8i**) provided some cell activity that was reduced upon methylation (**8j**). In addition, we saw a gradual increase in cell potencies in compounds with increasing hydrophilicity, culminating with the synthesis of the piperidine (**8r**) and morpholine (**8s**) amides. Interestingly, the *para*-substituted methyl sulfonyl analog (**8u**) was very active in both the binding and cell assays and had acceptable cell selectivity. Substitution at the *meta*-position of the aryl ring provided compounds that were equipotent in the binding assay but less potent in the cell assay. Based

Table 3. Optimization of C4 Anilines in C7 methylsulfonyl-containing compounds[a]



9-14

	R	IC_{50} (μM)	IC_{50} MCSF (μM)	IC_{50} NSO (μM)
9	3,4-di-Me	0.008	0.10	10
10	3-F-4-Me	0.063	3.2	>20
11	3,4-di-F	0.20	2.0	>20
12	4-F	0.16	0.40	10
13	3-Cl-4-OMe	ND	0.10	13
14	3-F-4-OMe	0.10	1.0	20

[a] All assays are reported as an average of at least two separate assays.

on the data observed and accounting for any potential metabolic liability of the amides, we selected the 4-methylsulfonyl substituent as the optimal C7 substituent.

With both positions, C4 and C7, independently optimized we devised a strategy to maximize the cell potency of our inhibitors by combining the most active substituents at both positions. The striking feature that the most potent substituent at C7 was the 4-methylsulfonyl moiety led us to retain that group and modify the C4 position with substituents from Table 1. The results of this exercise are summarized in Table 3. Of the C4 substituents tested, **9** was the most interesting as the binding potency and cell potency were the greatest, with 100-fold selectivity. The cell potencies of **10**, **11**, and **14** were not enough to elicit any further interest in these compounds. The binding potencies of **12** and **13** were lower than that of **7**, but the cell potencies and selectivities were comparable. From these results, we chose to assess the in vivo PK profile of compounds **9** and **12**.[9] When dosed in rats at 3 mg/kg IV, compound **9** showed a plasma clearance of 51 mL/min/kg while **12** showed a clearance of 4.5 mL/min/kg.

In conclusion a series of potent anilinoquinoline inhibitors of *c-fms* have been discovered. Modification of the C4 aryl group showed that lipophilic substituents provided an increase in potency. Modification of the C7 aryl group with potential H-bond acceptors gave compounds with increased cell potency (~5–10 times) than those without H-bond acceptors. By optimizing the C4 substituent while retaining the C7 methylsulfonyl moiety, we were able to generate compounds that possessed nanomolar binding and cell potency, along with up to 100-fold selectivity for the *c-fms*-expressing cell line. Identification of a lead compound for in vivo studies was made by in vivo PK experiments, and compound **12** is currently being evaluated as a treatment for osteoarthritis.

References and notes

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5. The results described herein were previously presented at the 230th ACS National Meeting. Smalley, T. L.; Mills, W. Y.; Chamberlain, S. D.; Kuyper, L.; Randhawa, S. A.; Tay, J. A.; Samano, V.; Frick, L. Abstract of Papers, 230th National American Chemical Society meeting, MEDI-376.
6. Compounds were assayed in the primary binding assay using activated *c-fms* protein and ^{33}P-labeled ATP as a substrate in 96-well plates. Inhibition was determined by scintillation counting. The cell assays were conducted in mouse myeloid M-NSF-60 cells containing 20 ng/mL of mouse MCSF and a MCSF-independent mouse myeloid NSO cell line. Activity was determined by the difference in cell growth upon treatment with inhibitors between the cell lines. For detailed descriptions of the assays, please see: 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.
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9. Although compound **13** showed excellent cell potency and acceptable cell selectivity, this compound was not included in the PK study due to the anticipated metabolic liability of the 4-methoxy group at C4.