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Structure-based optimization of a potent class of arylamide FMS inhibitors

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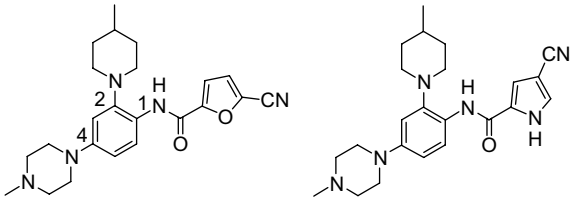
Abstract—An anti-inflammatory 1,2,4-phenylenetriamine-containing series of FMS inhibitors with a potential to form reactive metabolites was transformed into a series with equivalent potency by incorporation of carbon-based replacement groups. Structure-based modeling provided the framework to efficiently effect this transformation and restore potencies to previous levels. This optimization removed a risk factor for potential idiosyncratic drug reactions.

The macrophage colony-stimulating factor (CSF-1) is the primary growth factor for the macrophage lineage and it specifically binds to FMS, a type III receptor tyrosine kinase expressed by macrophages and their progenitor cells.[1] The binding of FMS by CSF-1 initiates an intracellular signaling cascade resulting in survival, proliferation, and differentiation of these cells.[2] As macrophages are known to play an important role in the inflammatory process, the inhibition of CSF-1-dependent macrophage proliferation might be of therapeutic value in intercepting an inflammatory process.[3] This hypothesis has also been validated by various animal studies.[4,5] CSF-1-deficient mice are reported to be resistant to collagen-induced arthritis (CIA). In a CIA model, CSF-1 was shown to increase the severity of disease while a neutralizing anti-CSF-1 antibody had the opposite effect.

In recent publications,[6] the discovery of the potent FMS inhibitors **1a** and **1b** (Fig. 1) was described and results demonstrating the anti-inflammatory activity of **1b** in a mouse model of collagen-induced arthritis were presented.[6b]



1a IC_{50} = 0.001 μM **1b** IC_{50} = 0.0008 μM

Figure 1. Anti-inflammatory FMS inhibitors **1a** and **1b**.

Despite the excellent potency and in vivo efficacy of this series, the 1,2,4-phenylenetriamine core structure of **1a** and **1b** was considered a liability due to its potential to form reactive quinonediimine metabolites, which might haptenize proteins and deplete glutathione, leading to tissue damage (Fig. 2).

In general, there are two types of adverse drug reactions that can occur. Type A adverse drug reactions are predictable, dose-dependent, and related to the pharmacology of the compound. Type B are idiosyncratic drug reactions (IDRs) and are of low incidence, human-specific, have a delayed onset, and account for a significant percentage of New Chemical Entity (NCE) attrition during development. The mechanisms of idiosyncratic toxicity may encompass one or more of the following: the formation of reactive metabolites in certain patients

Keywords: FMS; cFMS; CSF-1R; M-CSF; Colony-stimulating factor-1; Macrophages; Anti-inflammatory activity; Idiosyncratic drug reactions.
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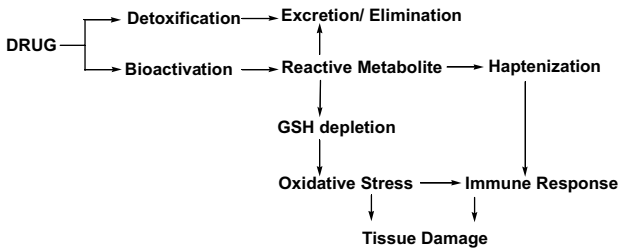


Figure 2. Idiosyncratic toxicity due to reactive metabolite formation.

due to the presence of polymorphs in drug-metabolizing enzymes, immune-mediated responses to the drug or its metabolites, the combination of drugs with low-level inflammatory reactions or drug-induced mitochondrial toxicity. Clinical manifestations include hepatotoxicity, anaphylaxis, blood dyscrasias, and skin reactions.[7]

We hypothesized that the 1,2,4-phenylenetriamine core structure of **1a** and its congeners could result in reactive metabolite formation and pose a risk factor for IDRs. As shown in Figure 3, the parent compound **2** could generate reactive quinonediimine-type intermediates (**2b** and **2c**) with or without cleavage of the amide bond that may then react with nucleophilic sites of endogenous proteins. These haptenized proteins (**2d** and **2e**) could potentially act as antigens, thereby generating an IDR.

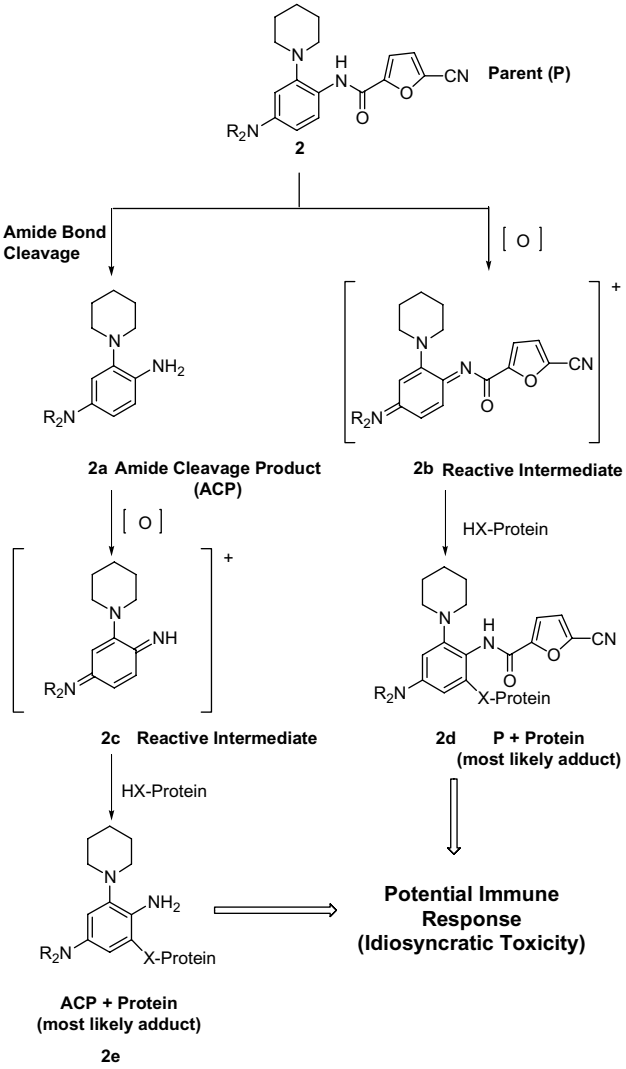


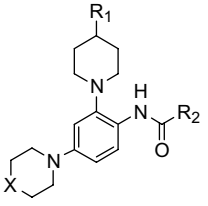
Figure 3. Potential mechanisms of idiosyncratic toxicity of a 1,2,4-phenylenetriamine-containing arylamide scaffold.

In order to quickly assess the potential to produce protein adducts, the propensity to form glutathione conjugates in vitro was evaluated with representative compounds. The test compound at 20 or 40 μM was incubated with NADPH (1 mM), GSH (5 mM) in PBS (0.1 mM), and 1 mg/mL of mouse liver microsomes at 37 °C for 1 h. The products were then analyzed by LC/MS for the glutathione conjugate of the parent (P+GSH), the amide bond cleavage product (ACP+GSH), and their fragments. Several compounds containing the 1,2,4-phenylenetriamine core formed GSH conjugates under these conditions. Some examples are shown in Table 1.

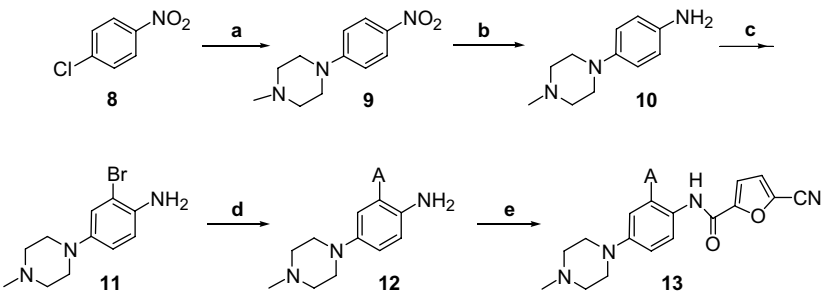
In light of these results, we directed our lead optimization efforts to address this potential problem with the aid of structure-based drug design. The primary focus was the replacement of the nitrogen substituents at the C-2 and C-4 positions with carbon substituents in order to minimize the possibility of reactive intermediate formation through in vivo oxidation. This was approached in a stepwise fashion at the C-2 and then the C-4 positions of the benzenoid core.

The synthesis of C-2 carbon-substituted, C-4 piperazinyl arylamides is shown in Scheme 1. The *N*-methylpiperazine at C-4 was first introduced by aromatic nucleophilic substitution of 4-chloronitrobenzene and the product **9** was reduced to the corresponding aniline **10**. The electrophilic bromination of **10** installed the bromine *ortho* to the amino function to yield product **11**. The *ortho*-bromoaniline **11** was then employed in a Suzuki coupling reaction to introduce the C-2 carbocycle. The

Table 1. In vitro GSH conjugate formation of 1,2,4-phenylenetriamine-containing arylamides



Compound		P+GSH	ACP+GSH
3	R_1 = H, X = N-CH_3 R_2 = 5-cyanofuranoyl	Yes	Yes
4	R_1 = CH_3, X = N-CH_3 R_2 = 5-cyanofuranoyl	Yes	Yes
5	R_1 = H, X = N-CH_3 R_2 = 4-cyanopyrrolyl	Yes	Yes
6	R_1 = CH_3, X = N-CH_3 R_2 = 4-cyanopyrrolyl	Yes	Yes
7	R_1 = CH_3, X=CH_2 R_2 = 4-cyanopyrrolyl	Yes	Yes

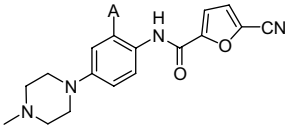


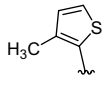
Scheme 1. Synthesis of C-2 carbon-substituted derivatives. Reagents: (a) 4-methylpiperazine; (b) Pd/H_2; (c) NBS/CH_2Cl_2; (d) $A\text{-}B(OH)_2$ or boronate esters (see text)/$Pd(Ph_3P)_4$; (e) 5-cyanofurancarbonyl chloride/Et_3N.

amino function of the resulting product **12** was then utilized for amide bond formation with 5-cyanofuranoyl chloride to obtain **13**.

FMS IC_{50} values of the C-2 piperidine substituent replacements are shown in Table 2. These replacement substituents were chosen based on their ability to mimic the conformation of the piperidine ring of the parent compound. Although related in size to piperidine, an unsubstituted phenyl ring at C-2 was predicted by modeling to adopt a conformation nearly coplanar with the central ring and quite different from the nearly orthogonal conformation of the piperidine, while addition of an *ortho* group on the phenyl should favorably bias the conformation toward that of the piperidine. Nevertheless, the selected *ortho*-substituted aromatic ring substituents at C-2 drastically reduced the FMS enzyme inhibitory activity (**13a–d**). Cyclohexyl was previously shown to be an inadequate replacement for piperidine (data not shown) since the ring cannot adopt the partially planar nature imposed by the piperidine nitrogen. However, both the predicted partial planarity and conformation of a cycloalkene ring appeared promising by modeling. Although the cycloalkene substituents (compounds **13e–g**) were also less potent than **1**, the loss of potency was much less profound. The 1-cyclohexenyl substituted analogue **13f** exhibited the highest FMS enzyme potency while the methyl substituent on the 'pseudo ortho' position of the cyclohexene (compound **13g**) further reduced the inhibitory potency, presumably owing to undesirable conformational changes in the cyclohexene ring system. Unfortunately, the 1-cyclohexenyl substituent (**13f**) was still 18-fold less potent than the parent compound **1a**.

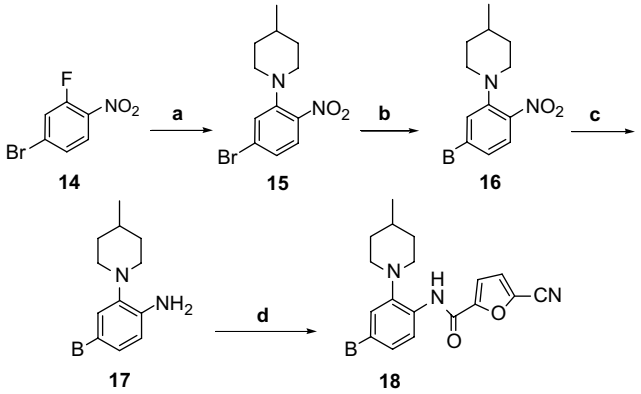
Table 2. FMS potency of C-4 methylpiperazinyl, C-2 carbon-substituted analogues



Compound	A	IC_{50}[a] (μM)
13a		0.63
13b		0.89
13c		0.47
13d		0.94
13e		0.060
13f		0.018
13g		0.054

[a] Reported IC_{50} values are the means of three experiments. Inter-assay variance was <20%. For assay details see Ref. 6a.

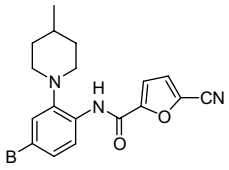
The general synthetic sequence for the synthesis of C-4 carbon-substituted, C-2 piperidinyl arylamides is shown in Scheme 2. 4-Methylpiperidine was first introduced by a selective nucleophilic substitution reaction on 4-bromo-2-fluoronitrobenzene to furnish intermediate **15**. This product was then subjected to a metal-catalyzed coupling reaction to introduce the C-4 carbon substituent to yield **16**. The intermediates for compounds **18a** and **18b** were made by Suzuki and Heck coupling reactions of intermediate **15** with 4-pyridylboronic acid and *tert*-butyl acrylate, respectively. Compound **18c** was made by acid-catalyzed hydrolysis of the *tert*-butyl ester of compound **18b**. To introduce acetylenic substituents, the intermediate **15** was subjected to Sonogoshira coupling reaction with propargyl alcohol and the resulting intermediate was converted to the corresponding mesylate. This mesylate was then reacted with dimethylamine and morpholine to obtain intermediates for the synthesis of compounds **18d** and **18f**, respectively, and these intermediates were then employed in the reduction step (c) using Fe/NH_4Cl as the reducing agent. Compound **18e** was made in an analogous manner using methylamine as the nucleophile followed by methanesulfonylation prior to the Fe/NH_4Cl reduction of the nitro group. The intermediate **16** for compound **18g** was made by a Suzuki coupling reaction of intermediate **15** with *N*-Boc-protected 1,2,3,6-tetrahydropyridine-4-pinacolbor-

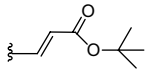
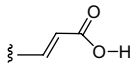
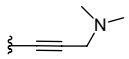
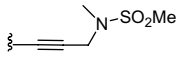
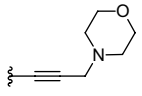
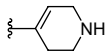
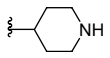
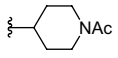


Scheme 2. Synthesis of C-4 carbon-substituted derivatives. Reagents: (a) 4-methylpiperidine; (b) BL/Pd(Ph_3P)$_4$; (c) [H]; (d) 5-cyanofurancarbonyl chloride/Et_3N.

onate. Pd/H_2 reduction of this intermediate provided the intermediate necessary for the synthesis of compound **18h** and Fe/NH_4Cl reduction gave the required intermediate for the synthesis of compound **18h**. The amine intermediate **17** was acylated with 5-cyanofuranoyl chloride, which provided the product **18**. Compound **18i** was

Table 3. FMS potency of C-2 piperidinyl, C-4 carbon-substituted analogues



Compound	B	IC_{50}[a] (μM)
18a		0.01
18b		0.038
18c		0.002
18d		0.015
18e		0.016
18f		0.005
18g		0.0008
18h		0.0003
18i		0.0008

[a] Reported IC_{50} values are the means of three experiments. Inter-assay variance was <20%. For assay details see Ref. 6b.

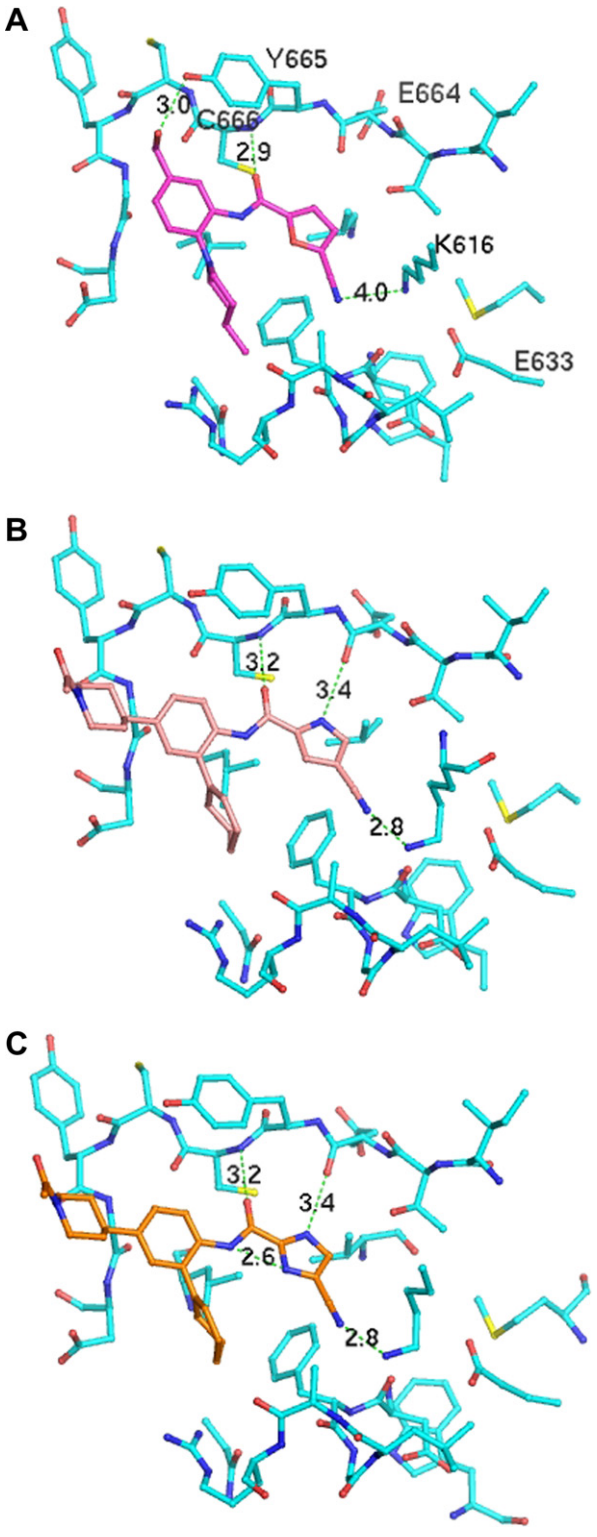
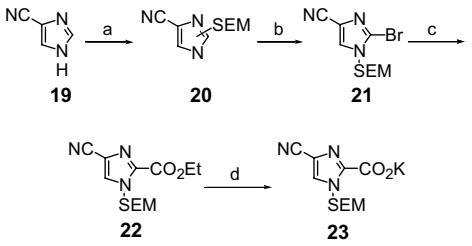


Figure 4. (A) Crystal structure of FMS, cyan carbons, with furan-containing compound, magenta carbons (PDB ID 2i0y)[8]; (B) model of FMS, cyan carbons, with pyrrole-containing compound, salmon carbons; (C) model of FMS, cyan carbons, with imidazole-containing compound, orange carbons. Hydrogen bonds between the inhibitor and FMS as well as inhibitor intramolecular hydrogen bonds are shown as green dashed lines. Figures were prepared with PyMol.[9]



Scheme 3. Synthesis of cyanoimidazole carboxylic acid potassium salt, **23**. Reagents and conditions: (a) SEMCl/K_2CO_3/acetone; (b) NBS/AIBN, CCl_4/60 °C; (c) *i*-PrMgCl/$CNCO_2Et$; (d) KOH/EtOH.

synthesized by the acetylation of **18h**. With compounds **18g** and **18h**, the Boc groups were then removed with TFA to obtain the final products.

The FMS inhibitory activity of C-4 carbon-substituted analogues shown in Table 3 suggests that the C-4 position of the 2-piperidinyl aryl amide scaffolds has tolerance for a wide range of substituents. The C-4, 4-pyridyl analogue **18a** was 10-fold less active than the parent **1a**. The acyclic substituents such as substituted propargyl, acrylate (**18b**, **d**–**f**) and other aliphatic amines (not shown) were also less potent than the parent compound, while **18c** was the notable exception. However, the replacement of the C-4 piperazine ring structure with piperidine or tetrahydropyridine (**18g**–**i**) maintained the extreme potency of the parent compound.

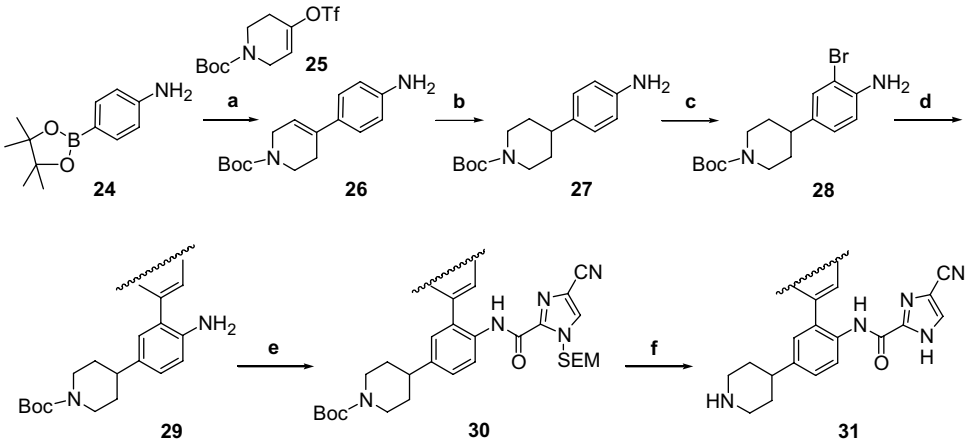
Although these efforts led to the discovery of potent inhibitors containing suitable replacement C-4 substituents, the most active analogues still retained the C-2 piperidine ring structure. The best C-2 carbon substituent, 1-cyclohexenyl, was nearly 20-fold less active than the parent compound.

The discovery that 5-cyanofuranoyl and 4-cyanopyrrolyl analogues **1a** and **1b** were equipotent[6b] led to a close examination of the co-crystal structure of FMS kinase with a 5-cyanofuran[8] (Fig. 4A). The structure indicated that the active conformation of the inhibitor was stabilized by an internal hydrogen bond between the furan oxygen and the amide NH. This interaction cannot be made by the 4-cyanopyrrole. However, a model of an inhibitor containing cyanopyrrole bound to FMS indicated the presence of an additional intermolecular hydrogen bond between the pyrrole nitrogen and the carbonyl of Glu 663 (Fig. 4B) and this alternate intermolecular interaction likely compensated for the absence of the intramolecular hydrogen bond. Based on these observations, we reasoned that an inhibitor that could make both of these interactions should be more potent and this notion thus led to the design of 4-cyanoimidazole-containing inhibitors (Fig. 4C).

The required 4-cyanoimidazole carboxylic acid was synthesized in SEM-protected form as its potassium salt **23** in four steps from 4-cyanoimidazole as shown in Scheme 3.

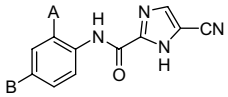
The route to the synthesis of this series of compounds (Scheme 4) incorporates the Suzuki coupling of 4-anilinopinacolboronate with the enoltriflate of *N*-Boc-piperidinone to obtain intermediate **26** followed by hydrogenation to obtain **27**. *Ortho*-bromination with NBS followed by a second Suzuki reaction with a cycloalkenyl boronic acid or boronate introduced the C-2 cycloalkene. The resulting compound **29** was coupled to the SEM-protected cyanoimidazole potassium salt **23** using PyBroP as the coupling agent. The final product **31** was obtained by acid-induced deprotection of intermediate **30**. The selective acylation of the final product produced the acylated piperidines **31e**–**g**.

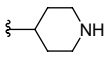
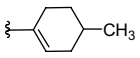
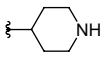
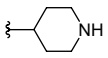
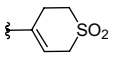
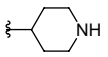
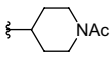
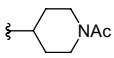
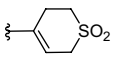
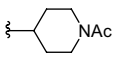
The inhibitory activities of this final series of compounds are shown in Table 4. As the modeling predicted, the 4-cyanoimidazole replacement of 5-cyanofuran/4-cyanopyrrole enhanced the enzyme potency of C-2 carbon-substituted derivatives by 10-fold (e.g., **31e** vs **13f**) thus bringing the potency back into the range of the original leads. Although the cyclopentene **31c** was eightfold less active than the 6-membered ring **31a**, 7-membered cycloalkenes (data not shown) displayed similar activity to **31a**. The presence of small substituents such as mono-



Scheme 4. General synthetic route to cyanoimidazole analogues. Reagents and conditions: (a) $Pd(Ph_3P)_4/Na_2CO_3$/dioxane; (b) 10% Pd/H_2, EtOH; (c) NBS/CH_2Cl_2/0 °C; (d) cycloalkenylboronic acid or boronate/$Pd(Ph_3P)_4/Na_2CO_3$/dioxane; (e) *N*-SEM-4-cyanoimidazolecarboxylic acid potassium salt (**23**)/PyBroP/DIEA/CH_2Cl_2; (f) TFA/EtOH/CH_2Cl_2 (1/0.1/3).

Table 4. Biological activities of C-2, C-4 dicarbon-substituted analogues



Compound	A	B	IC_{50} (μM)	BMDM assay IC_{50}[a] (μM)
1a			0.001	0.002
1b			0.0008	0.002
31a			0.001	0.001
31b			0.0004	0.0036
31c			0.008	0.03
31d			0.06	>1
31e			0.002	0.008
31f			>0.15	>1
31g			0.06	0.08

[a] Reported IC_{50} values are the means of three experiments. Inter-assay variance was <20%. For assay details see Ref. 6b.

methyl **31b** at the C-4′ position of cyclohexene also did not affect the FMS enzyme potency. However, polar moieties in the ring (**31d**, **31f**, and **31g**) decreased the enzyme potency as well as the cellular potency. All highly potent compounds also showed comparable cellular activity in a bone marrow-derived macrophage (BMDM) proliferation assay.[6a]

Having successfully identified carbon-based replacements for the C-2 and C-4 nitrogen substituents in parent compounds **1a** and **1b**, the more potent new analogues **31a**, **31b**, and **31e** were evaluated for their potential to exhibit IDRs as measured in the GSH conjugation assay. No GSH adduct formation was detected under the assay conditions described previously.

In conclusion, with the aid of structure-based drug design, the lead arylamide series, represented by **1a** and **1b**, was successfully optimized and improved addressing a potential IDR issue to generate novel, potent FMS inhibitors, which did not have this liability. The replacement substitutions required at C-2 resulted in a loss of potency that was restored by incorporating the complementary hydrogen-bonding characteristics of both **1a** and **1b**. The resulting imidazole analogues confirmed the hypothesis and were approximately 10-fold more potent than the corresponding analogues containing either a furan or pyrrole. The newly optimized series, exemplified by compounds **31a**, **31b**, and **31e**, is equipotent to the lead series in both enzyme and cell-based assays and is being examined as potential drug candidates.

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