Bioorganic & Medicinal Chemistry Letters 20 (2010) 3925–3929

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Reducing ion channel activity in a series of 4-heterocyclic arylamide FMS inhibitors

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ARTICLE INFO

Article history:
Received 25 March 2010
Revised 4 May 2010
Accepted 7 May 2010
Available online 12 May 2010

Keywords:
FMS
CSF-1R
M-CSF
Colony stimulating factor-1
Macrophages
Anti-inflammatory activity

ABSTRACT

During efforts to improve the bioavailability of FMS kinase inhibitors **1** and **2**, a series of saturated and aromatic 4-heterocycles of reduced basicity were prepared and evaluated in an attempt to also improve the cardiovascular safety profile over lead arylamide **1**, which possessed ion channel activity. The resultant compounds retained excellent potency and exhibited diminished ion channel activity.

Colony stimulating factor-1 (CSF-1 or macrophage colony stimulating factor-1, M-CSF) binds to the CSF-1 receptor (FMS) on the cell surface of the monocyte/macrophage lineage, thus controlling their growth and differentiation.[1] Increased numbers of macrophages within a variety of human tissues portend a link to other types of disease progressions such as cancer,[2] rheumatoid arthritis[3] and immune nephritis.[4] In addition, osteoclasts, whose formation requires the presence of receptor activator of nuclear factor ligand κB (RANK) and CSF-1, mediate bone erosions leading to pain and fracture in metastatic bone disease and osteoporosis as well as deformity in rheumatoid arthritis.

The inhibition of CSF-1 for treating diseases such as rheumatoid arthritis and metastatic cancer of the bone, where osteoclasts and macrophages are pathogenic, is supported by biological studies conducted with CSF-1-deficient mice.[5] As a result, considerable effort continues to be invested in the discovery and optimization of a variety of structural classes of FMS small molecule inhibitors.[6,7]

In contrast, it is noteworthy that recombinant CSF-1 (Lanimostim/MacroTac™) is used clinically in bone marrow transplantation patients who suffer from fungal and bacterial infections due to the lack of myeloid cells.[8]

Previous Letters[6] detailed the genesis of the potent CSF-1 inhibitors **1** and **2** (Fig. 1) in the arylamide series from initial leads that were discovered[6a,b] using Thermofluor® screening technology and whose potencies were optimized utilizing structure-based design.[6c,f] As these compounds were further scrutinized it was found that both **1** and **2** had poor bioavailability (F = 12% and 15%, respectively) while **1** had a very high volume of distribution in mice (32 L/kg). Furthermore, both **1** and **2** had activity in sodium and calcium ion channel binding assays, which may foreshadow undesired cardiovascular effects.[9,10] It was found that both of these compounds had poor cardiovascular safety profiles in the guinea pig right atrium (GPRA) assay (Table 1).[11] The compounds result in a moderate (at the lowest concentration) to a very strong (at the highest concentration) reduction in both the rate and force of contraction. For example, **1** at 10 μM resulted in a severe reduction

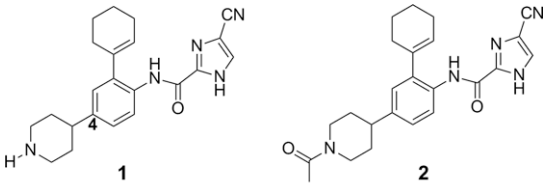

Figure 1. Piperidinyl 4-arylamides **1** and **2**. [a]Percent inhibition of reference compound binding at 10 μM.

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doi:10.1016/j.bmcl.2010.05.013

Table 1
Guinea pig right atrium assay results for **1** and **2**

Compound	Rate of contraction (% of initial value)			Force of contraction (% of initial value)		
	1 μM	3 μM	10 μM	1 μM	3 μM	10 μM
1	84.9	54.6	23.2	76.4	40.0	21.0
2	92.1	86.3	37.6	79.4	65.3	42.1
95%PI[a]	95–103	93–106	94–110	80–95	68–91	61–90

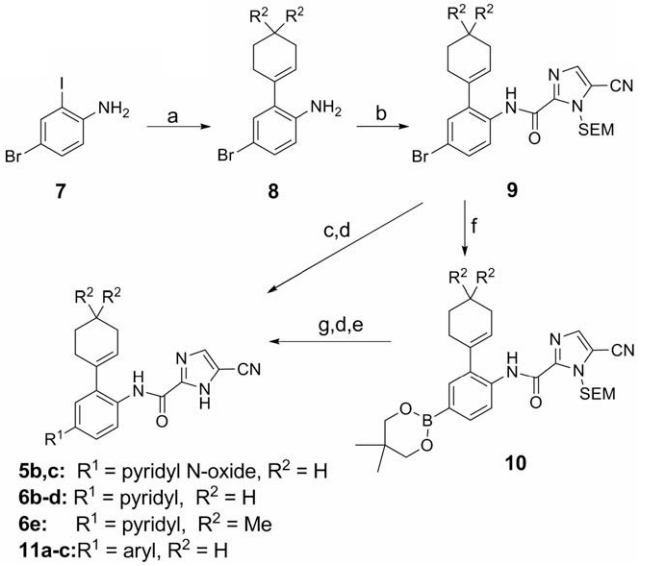
[a] The 95% prediction interval is the expected range of values for the control in the presence of solvent.

in the force of contraction to only 21% of the values measured in the baseline period of the experiment.

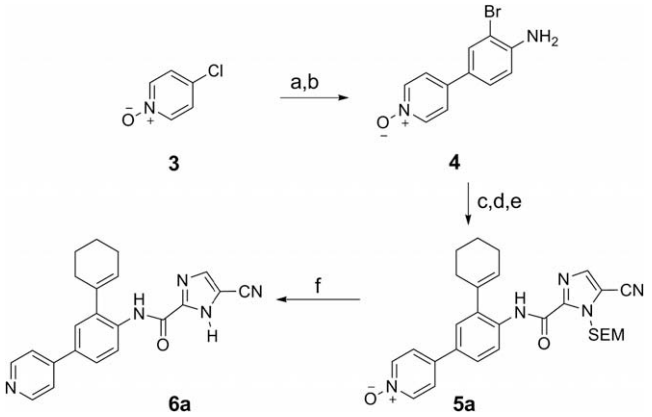
Having reported on the X-ray crystallography and structure-based design of this class of compounds[6c,f] it was evident that the 4-position of the scaffold was a permissive region for substitution (Fig. 1). Thus, given the range of substituents allowable, a series of saturated and unsaturated six-membered, nitrogen-containing heterocycles were prepared to improve both the pharmacokinetic properties and cardiovascular safety profile of the arylamides.

Based on the cardiovascular properties of **1** and **2**, less-basic and non-basic non-piperidine-containing compounds were targeted on the premise that this would lead to decreased sodium[9] and calcium[10] ion channel activity and possibly reduced cardiovascular safety liability. Recognizing that complete loss of basicity could negatively impact the solubility of these analogues, compounds of reduced basicity were prioritized.

First prepared were pyridines and non-basic aromatic groups, such as pyridine N-oxides and substituted aryls, as surrogates for the 4-piperidyl ring found in **1** and **2**, the introduction of which necessitated three strategically different approaches. It was found that **5a** was best prepared by introducing the pyridyl-N-oxide moiety early in the synthesis (Scheme 1). All attempts to introduce this regioisomer at a later stage in the synthesis (Scheme 2) resulted in very poor yields. The other regioisomeric *N*-oxo-pyridines **5b** and **5c** were prepared using the methodology shown in Scheme 2, by coupling the boronate **10** with 3-bromo- and 2-bromo-pyridine N-oxides, respectively. The pyridines **6a–c** were synthesized via Fe reduction of their corresponding N-oxides. Amino and methoxy-substituted pyridines **6d**, **e** were prepared by the Suzuki coupling reaction using the requisite chloro- or bromopyridine and substrate **10**. The aryl derivatives **11a–c** were easily prepared by



Scheme 2. Reagents and conditions: (a) cyclohexen-1-yl boronic acid or 4,4-dimethylcyclohexen-1-yl boronic acid, $Pd(PPh_3)_4$, toluene, EtOH, 2 M Na_2CO_3, 80 °C; (b) potassium-4-cyano-1-(2-trimethylsilanyl-ethoxymethyl)-1*H*-imidazole-2-carboxylate, PyBrOP, DIEA, CH_2Cl_2; (c) for **11a**: 4-(*N*,*N*-dimethylsulfonamido)-boronic acid, $Pd(PPh_3)_4$, toluene/EtOH, 2 M Na_2CO_3, 80 °C; for **11b**: 4-aminophenylboronic acid, $Pd(PPh_3)_4$, toluene/EtOH, 2 M Na_2CO_3, 80 °C; for **11c**: phenylboronic acid, $Pd(PPh_3)_4$, toluene/EtOH, 2 M Na_2CO_3, 80 °C; (d) TFA, CH_2Cl_2; (e) for N-oxide reduction of **5b**, **c** to **6b**, **c**: Fe, NH_4Cl, EtOH–water; (f) bis(neopentylglycolato)-diboron, $Pd(dppf)Cl_2$, KOAc, dioxane, 80 °C; (g) for **5b**, **c**: 3- or 2-bromopyridyl-N-oxide respectively, $Pd(PPh_3)_4$, dioxane, 2 M Na_2CO_3, 80 °C; for **6d**: 5-bromo-2-aminopyridine, 2 M Na_2CO_3, dioxane 80 °C; for **6e**: 5-bromo-2-methoxypyridine, 2 M Na_2CO_3, dioxane 80 °C.



Scheme 1. Reagents and conditions: (a) 4-aminophenyl boronic acid, $Pd(PPh_3)_4$, toluene/EtOH, 2 M Na_2CO_3, 80 °C; (b) NBS, CH_2Cl_2; (c) cyclohexen-1-yl-boronic acid, $Pd(PPh_3)_4$, toluene/EtOH, 2 M Na_2CO_3, 80 °C; (d) potassium-4-cyano-1-(2-trimethylsilanyl-ethoxymethyl)-1*H*-imidazole-2-carboxylate[6c], PyBrOP, DIEA, CH_2Cl_2; (e) TFA, CH_2Cl_2; (f) Fe, NH_4Cl, EtOH, water.

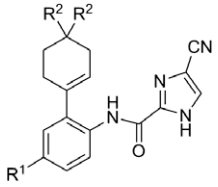
Suzuki coupling using the requisite boronic ester and bromide **9**, followed by TFA-promoted SEM deprotection (Scheme 2).

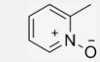
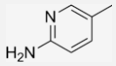
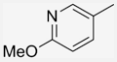
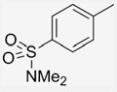
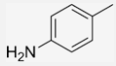
With the pyridyl derivatives **5** and **6** and the aryl derivatives **11** in hand, their respective binding activities were evaluated in an in vitro FMS kinase assay and a cell-based, bone marrow-derived macrophage proliferation (BMDM) FMS activity assay (Table 2).[6d]

As X-ray structures had predicted, excellent in vitro activity in the FMS kinase assay was retained by **5a** and **6a**. This is not surprising, in that the placement of the nitrogen in the rings mimics **1** and **2**. Indeed, all of the pyridyl derivatives (**5a–c** and **6a–c**) were well tolerated as the *N*-oxo-pyridyl compounds **5b**, **c** and pyridines **6b**, **c** also had single digit activity in the FMS kinase inhibitory assay.

The amino-substituted pyridine **6d** also retained good FMS inhibitory activity. Because the 4-methoxy analogue **6e** had the poorest FMS inhibitory activity of all the pyridyl compounds, further testing was not warranted. Unsubstituted phenyl **11c** lost activity (>60 nM), while sulfonamide **11a** and amine **11b** were modestly potent (20 and 23 nM, respectively). Despite most of the aryl derivatives having good FMS IC_{50} values (except **11c**), only **5a** and **6a** had good cellular activity. Unfortunately **6a** (a potential

Table 2
Biological activities of 4-aryl/pyridyl imidazoyl amides



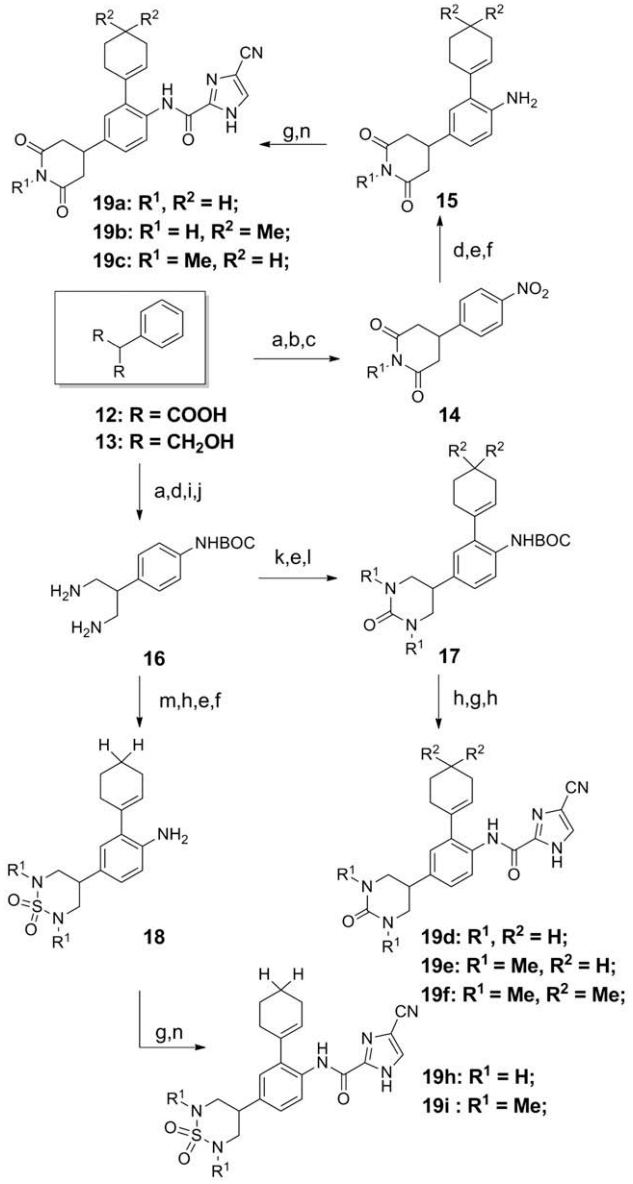
Compound	R^1	R^2	FMS (nM)	BMDM IC_{50} (nM)	Ca^{+2}/Na^{+}[a] (%)
5a		H	1.5	6.6	n.d.[b]
6a		H	4.5	9.2	12/25
5b		H	1.4	26.7	15/18
6b		H	2.3	15.5	13/27
5c		H	7.1	131	n.d.
6c		H	11	>100	12/25
6d		H	4.5	65	n.d.
6e		Me	41	n.d.	n.d.
11a		H	20	23	n.d.
11b		H	23	66	n.d.
11c		H	>60	n.d.	n.d.

[a] Percent inhibition of reference compound binding at 10 μM.
[b] Not determined.

metabolite of **5a**) also displayed strong cytochrome P450 inhibition (2C9 IC_{50} = 3 nM, 3A4 IC_{50} = 87 nM)—a potential liability for both compounds.

Nevertheless, the pyridines **6a–c** and the N-oxide **5b** were evaluated for ion channel activity. All four compounds had significantly lower Ca channel binding compared to lead compounds **1** and **2**, and equal or lower Na channel binding, thereby supporting the hypothesis that by lowering the basicity of the arylamides, their ion channel liability could be reduced.

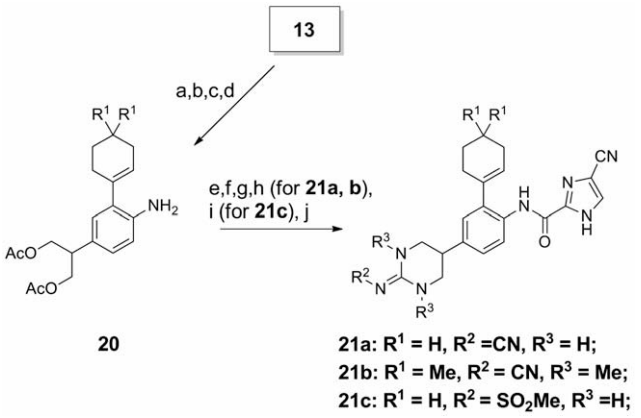
Attention was then focused on the preparation of saturated nitrogen-containing heterocycles which retain more of the lipophilic character of the piperidine rings in **1** and **2**. The series of heterocycles **19** were made according to the synthesis outlined in Scheme 3. A variety of heterocycles could be prepared from the commercially available scaffolds **12** and **13**. These included glutarimides **19a–c** (from **12**), cyclic ureas **19d–g** (from **13**), and cyclic sulfonyl ureas **19h**, **i** (from **18**).[12] The cyclohexenyl rings (R^2 = H or Me) in the compound series **15**, **17**, and **18** were introduced using Suzuki coupling. Unlike series **17**, which could be prepared using standard aqueous Suzuki conditions, substrates **15** and **18** were synthesized using Buchwald's non-aqueous Suzuki conditions after ortho bromination of their respective precursor amines.[13] The hydrolytic propensity of the aforementioned glutarimide and sulfonyl ureas compounds also mandated different deprotection conditions to remove the SEM group. Instead of using



Scheme 3. Synthesis of saturated heterocyclic arylamides. Reagents and conditions: (a) HNO_3/H_2SO_4 (for **16** use **13**, for **14** use **12**; (b) Ac_2O, heat; (c) R-NH_2; (d) Pd–C/H_2, MeOH; (e) NBS, CH_2Cl_2; (f) cyclohexen-1-yl boronic acid pinacol ester, $Pd(OAc)_2$, K_3PO_4, dicyclohexyl-biphenylphosphine, toluene/dioxane; (g) potassium-4-cyano-1-(2-trimethylsilanyl-ethoxymethyl)-1*H*-imidazole-2-carboxylate, PyBrOP, DIEA, CH_2Cl_2; (h) TFA, CH_2Cl_2; (i) BOC_2O; (j) MsCl, NEt_3, then NaN_3; Pd–C/H_2, one step; (k) (1) bis-4-nitrophenylcarbonate, DCE, reflux; (2) for **17** (R^1 = methyl): K_2CO_3, MeI, DMF, heat or K_2CO_3, NBu_4Br, Me_2SO_4, DCE; (l) cyclohexen-1-yl boronic acid pinacol ester or 4,4-dimethyl-cyclohexen-1-yl boronic acid pinacol ester or, for **19g**, cyclohepten-1-yl boronic acid, $Pd(PPh_3)_4$, toluene, EtOH; (m) (1) sulfamide, pyridine, reflux; (2) For **18** (R^1 = methyl): K_2CO_3, NBu_4Br, Me_2SO_4, DCE; (n) TBAF, DMF, 60 °C.

our standard SEM deprotection conditions (TFA, CH_2Cl_2), **19a–c** and **19h**, **i** were deprotected using TBAF in DMF with mild heating. Both deprotection methods generally gave >80% yields from their respective substrates.

To complete the set of targeted heterocycles, the cyclic cyanoguanidines **21a–c** were prepared using the diol **13** (Scheme 4). Alkylation was performed after amide formation for the N-alkylated derivative **21b**, in contrast to the saturated heterocycles **19e–g**, **i** which were alkylated prior to amide coupling (see **17** and **18**).



Scheme 4. Synthesis of 4-substituted cyclic ureas. Reagents and conditions: (a) HNO_3/H_2SO_4; (b) AcO_2, pyridine; (c) Pd–C, H_2; (d) (i) NBS; (ii) CH_2Cl_2, cyclohexen-1-yl boronic acid pinacol ester, $Pd(PPh_3)_4$, toluene, EtOH, 2 M Na_2CO_3; (e) 2 M NaOH, *i*PrOH; (f) MsCl, NEt_3, then NaN_3; Zn, NH_4Cl; (g) potassium-4-cyano-1-(2-trimethylsilanyl-ethoxymethyl)-1*H*-imidazole-2-carboxylate, PyBrOP, DIEA, CH_2Cl_2; (h) (i) dimethyl-*N*-cyanodithioiminocarbonate, CH_2Cl_2, reflux; (ii) K_2CO_3, $(n\text{-}Bu)_4NBr$, methyl iodide, DCE; (i) *N*-(bis-methylsulfanyl-methylene)-methanesulfonamide, DCE, reflux; (j) TFA, CH_2Cl_2.

Remarkably, **19a–i** and **21a–c** all possessed FMS inhibitory IC_{50} values of less than 3 nM, with many in the set having picomolar activities (Table 3). It is noteworthy that the three compounds **19b**, **19f**, and **21b** where R^2 = Me are especially potent. This is consistent with prior observations gleaned through X-ray crystallography that this space is well-suited to fit 1–2 Me groups at this position.[6c] A cycloheptenyl group is also well tolerated (**19g**).

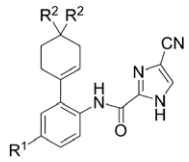
Excellent activities were also observed in the cellular assay, with eight of the thirteen compounds having single digit nanomolar activity. Upon examination of the BMDM assay results of compound sets **19** and **21**, it is clear that the compounds having two exchangeable hydrogens (**19d**, **19h**, **21a**, **21c**) have poor cellular activity. Replacing these hydrogens with methyl groups (**19d** to **19e** and **19h** to **19i**) restored single digit nanomolar potency, although this trend did not extend to the cyano-guanidines **21a** and **21b.**

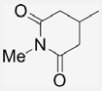
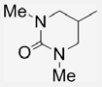
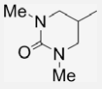
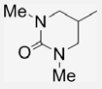
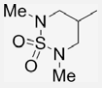
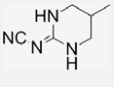
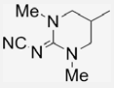
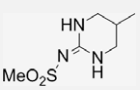
Five compounds (**19a**, **c**, **e**, **f**, **i**) were then selected for the calcium and sodium channel binding assay. Consistent with the hypothesis regarding the effects of basicity or the presence of a piperidine ring in this region on ion channel binding, low values of binding inhibition were observed with the exception of **19e** (60% Na channel binding). With five candidates to choose from, **19c**, **e**, **f** were eliminated from further consideration due to metabolic instability. Finally, **19a**, despite its apparent in vitro metabolic stability, was eliminated due to chemical instability during formulation.

The pharmacokinetic behavior of compound **19i** was then assessed in rat.[14] Plasma concentrations reached a mean C_{max} of 839 ng/ml. The compound showed a low volume of distribution of 564 ml/kg (compared to rat total body water volume of 668 ml/kg), and low clearance (4 ml/min/kg, compared to the rat liver blood flow rate of 55 ml/min/kg). This compound had a measured half-life of 113 min after iv administration. Unfortunately, the mean bioavailability (F = 15%) was unimproved over **1** and **2**.

Thus, utilizing our previous knowledge of the FMS binding site, we were able to replace the piperidine ring in **1** and **2** with a series of less-basic and nonbasic heterocycles which maintain a low potential for undesired cardiovascular effects as a result of their reduced ion channel binding inhibition. Although improvements in the bioavailability of these compounds were not attained, they were consistently very potent in both kinase and cell assays. Compound **19b** modeled into the FMS binding pocket clearly demonstrates the spatial allowance of groups that can be substituted for the piperidine ring in **1** and **2** (Fig. 2). Further exploration of the SAR in this region will be reported in due course.

Table 3
Biological activities of nitrogen-containing saturated heterocycles



Compound	R^1	R^2	FMS IC_{50} (nM)	BMDM IC_{50} (nM)	Ca^{+2}/Na^{+}[a] (%)	Metabolic[b] stability HLM/RLM
19a		H	1.0	~5.9	0/11	87/67
19b		Me	0.8	7	n.d.[c]	65/46
19c		H	0.5	3.6	<10/ <10	18/49
19d		H	2.3	75	n.d.	87/78
19e		H	0.5	2.2	21/ 60	4/40
19f		Me	~0.3	2.6	20/ 30	19/27
19g		H[d]	0.9	4.3	n.d.	2/27
19h		H	2.9	47	n.d.	92/85
19i		H	1.7	3.6	0/38	58/80
21a		H	2.5	42	n.d.	88/100
21b		Me	0.4	40	n.d.	n.d.
21c		H	1.9	100	n.d.	93/98

[a] Percent inhibition of reference compound binding at 10 μM.
[b] Microsomal stability percent remaining at 10 min.
[c] Not determined.
[d] **19g** possesses a cyclohepten-1-yl group instead of a cyclohexen-1-yl group.

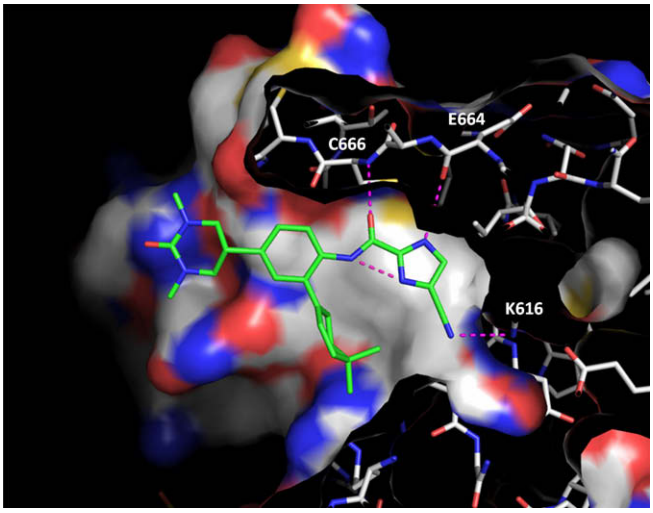


Figure 2. Compound **19b** bound to FMS binding site.

Acknowledgments

The author would like to thank Bruce Damiano, An Herman (GPRA assay), and Michael Kolpak (analytical).

References and notes

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