Design, Synthesis, and Activity of 2-Imidazol-1-ylpyrimidine Derived Inducible Nitric Oxide Synthase Dimerization Inhibitors

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Received November 14, 2006

By the screening of a combinatorial library for inhibitors of nitric oxide (NO) formation by the inducible isoform of nitric oxide synthase (iNOS) using a whole-cell assay, 2-(imidazol-1-yl)pyrimidines were identified. Compounds were found to inhibit the dimerization of iNOS monomers, thus preventing the formation of the dimeric, active form of the enzyme. Optimization led to the selection of the potent, selective, and orally available iNOS dimerization inhibitor, **21b**, which significantly ameliorated adjuvant-induced arthritis in a rat model. Analysis of the crystal structure of the **21b**−iNOS monomer complex provided a rationalization for both the SAR and the mechanism by which **21b** blocks the formation of the protein−protein interaction present in the dimeric form of iNOS.

Introduction

Nitric oxide synthase (NOS[a]) is a dimeric enzyme that catalyzes the production of NO from arginine using heme, biopterin, and NADPH as cofactors. The three isoforms of NOS, iNOS, endothelial (ecNOS), and neuronal (bcNOS) differ in their tissue distribution and biological role but are similar in that they are only active in the dimeric form. iNOS is up-regulated during an immune response and has been implicated in the pathogenesis of numerous inflammatory diseases, including multiple sclerosis and rheumatoid arthritis. In contrast, bcNOS and ecNOS are constitutively expressed in mammals and are required for normal function. Therefore, a potent inhibitor of iNOS could ameliorate disease; however, high selectivity would be needed to avoid inhibiting the essential functions of the bcNOS and ecNOS isoforms. The potential therapeutic benefit of an iNOS inhibitor has prompted a major effort in the pharmaceutical industry to discover a potent, selective iNOS inhibitor.[1−3]

Our initial search for iNOS inhibitors focused on direct enzyme inhibition, specifically compounds that could mimic the substrate, arginine, or those that could directly interact with the heme. The heme binding approach led to the finding that 1-phenylimidazoles were selective but were only moderately potent inhibitors of iNOS.[4−6] To further explore the phenylimidazole template, a library was prepared using ECLIPS[7] technology (Figure 1) and screened for direct enzyme inhibitors, but no compounds were identified. The library was screened a second time using a whole-cell assay with NO production as the readout, and several library compounds, exemplified by **1**, significantly decreased levels of NO production.[8] On further evaluation, these compounds were found to permeate the cell and to inhibit formation of the enzymatically active dimer. Other groups have identified imidazole-containing compounds that also block NO formation by this mechanism.[9−11] In this paper, we describe the optimization of the initial hits, identified by screening the library, into orally available, potent, selective iNOS dimerization inhibitors.[7,12]

Chemistry

Compounds were originally prepared by an efficient method, which required a difficult separation of regioisomeric pyrimidines (Scheme 1). Reaction of 2,4-dichloropyrimidine with imidazole gave a mixture of chloro(imidazol-1-yl)pyrimidines, **2** and **3**, which were separated. The monochloropyrimidines were subsequently reacted with secondary amines to obtain the desired analogues. Because of problems in obtaining sufficient quantities of the desired regioisomers, an alternative synthetic strategy using 2-sulfonopyrimidines as an intermediate was developed.

The 2-sulfonopyrimidines were prepared under standard conditions (Scheme 2). Alkylation of the pyrimidinethione, **4**, with methyl iodide in the presence of base gave methylmercaptan, **5**. Reaction of the resulting hydroxypyrimidine with $POCl_3$ resulted in the formation of the corresponding chloropyrimidine, **6**, which was oxidized with mCPBA to give the sulfone, **7**. The sulfones are not stable and were best used soon after preparation or stored under anhydrous conditions.

The pyrimidinesulfone was reacted with the protected amines, and the intermediate sulfone, whose isolation is unnecessary but possible, was subsequently reacted with imidazole (Scheme 3). In general, by addition of the secondary amine to the chloropyrimidinesulfone and subsequently reaction of the intermediate with imidazole, the desired regioisomer was obtained with >98% regioselectivity. Under identical conditions, reaction of primary amines with the chloropyrimidinesulfone gave regioisomeric mixtures with addition of the amine at the 4 position predominating (>6:1). Derivatization of the ester or piperazine nitrogen was performed sequentially under standard conditions by initial deprotection of the ester or amine, depending on the group to be modified, to give the desired compounds. The regioisomeric piperazines, **19**, were prepared in a similar manner by starting with the alternatively protected starting piperazine.

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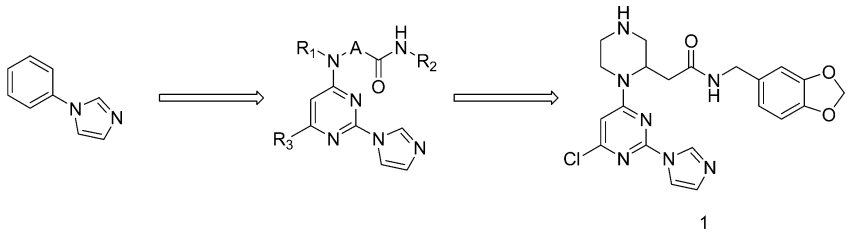
[a] Abbreviations: NOS, nitric oxide synthase; NO, nitric oxide; iNOS, inducible NOS; ecNOS, endothelial constitutive NOS; bcNOS, brain constitutive NOS.

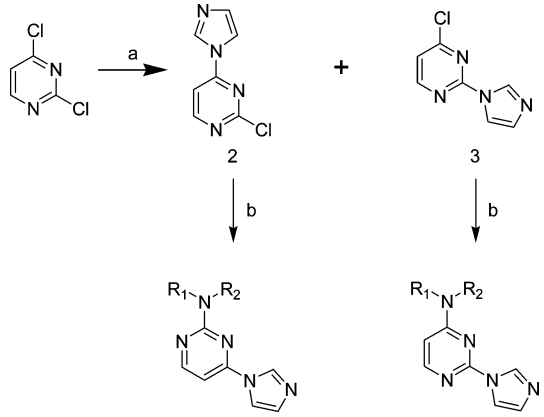
10.1021/jm061319i CCC: $37.00
Published on Web 02/23/2007



1

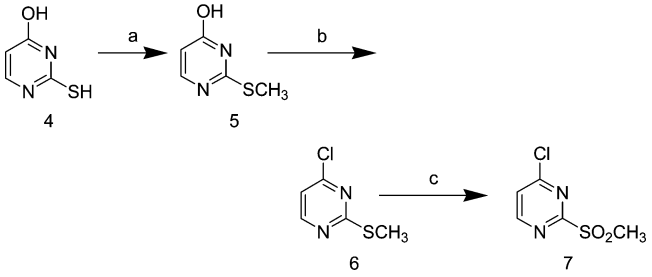
Figure 1. Discovery of dimerization inhibitors.

Scheme 1. Original Synthesis of Disubstituted Pyrimidines[a]



[a] Reagents and conditions: (a) imidazole; (b) amine, K_2CO_3, DMSO, heat.

Scheme 2. Synthesis of 2-Pyrimidinesulfone[a]



[a] Reagents and conditions: (a) MeI, K_2CO_3, DMSO; (b) $POCl_3$, heat; (c) mCPBA, CH_2Cl_2.

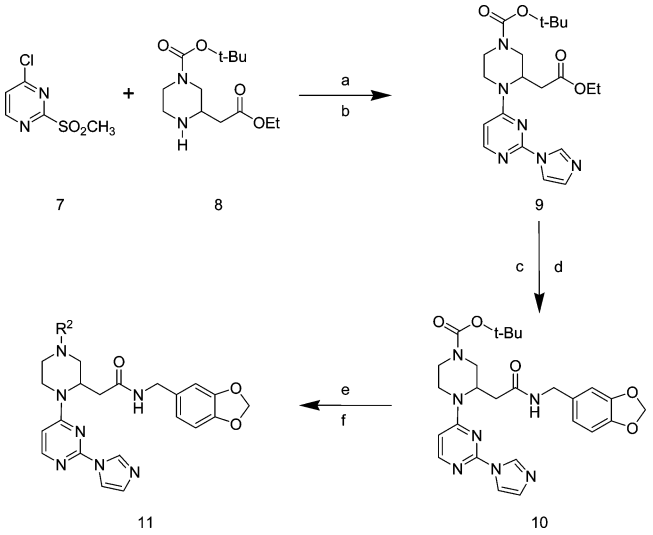
The regioselective piperazine synthesis has been described.[13] Reaction of the ethyl ester of piperazine-2-acetic acid with BOCON places a BOC group on the less hindered piperazine nitrogen. A synthesis of the benzyl protected piperazine has also been described.[14] BOC protection of the benzyl protected piperazine gives the diprotected compound. Selective removal of either protecting group gives the selected regioisomer that can be used in the synthesis of any of the piperazine substituted pyrimidines.

The synthesis of optically active **11d** and **19f** was accomplished by separating a precursor on a chiral column. The syntheses were completed by standard methods. Absolute stereochemistry was determined by an X-ray crystal structure of **11d** and an analogue of **19f** bound to the enzyme, and all other stereochemistry is inferred. The absolute stereochemistry of **11d** corresponds to that of the D-amino acid used as starting material in the syntheses of the more active compounds shown in the pyrrolidine series illustrated in Table 7.

Results and Discussion

Compounds were initially tested for inhibition of active iNOS formation in a whole-cell assay using human A172 cells induced with cytokines. NO formation was monitored spectrophotometrically with the Griess reagent.[15] All selected compounds were inactive in a standard noncellular iNOS enzyme assay up to 1 μM in which the enzyme had been isolated.[16] Selectivity between the NOS isoforms was determined using BSC-1 cells transfected with a vaccinia virus system or SF9 cells transfected with a tetracycline-induced system that expressed one of the three NOS isoforms. In these assays, NOS activity was monitored by measuring the production of radiolabeled citrulline from radiolabeled arginine. The two selectivity assays (vaccinia virus and tetracycline) gave similar results for iNOS potency but different results for ecNOS and bcNOS potency. The relevance of these differences is unknown. ecNOS was found to be more potent in the tetracycline induced system, while bcNOS was found to be more potent in the vaccinia system. Empirically, the tetracycline induced system gives us the ability to monitor ecNOS selectivity while the vaccinia system indicated the inhibitors to be very selective against ecNOS. The IC_{50} values for iNOS in the A172 cells and the two selectivity assays differed, possibly because of differences in NO production between the different cell types. It was difficult to make a good correlation concerning the selectivity with the less active compounds because the maximum compound concentrations were 10 μM.

Scheme 3. Synthesis of Piperazine Analogues[a]



[a] (a) K_2CO_3, heat; (b) imidazole, heat; (c) LiOH, THF; (d) carbonyldiimidazole, piperonylamine; (e) TFA, CH_2Cl_2; (f) standard methods.

Our optimization strategy was to first modify the different parts of the template independently and then to combine the best substituents into a single compound that would have an improved overall profile. The template was partitioned into the amide (R^1), piperazine substitution (R^2), and pyrimidine substitution (R^3) (Figure 2). In addition, different attachment loci of the piperazine side chain, chain lengths, and alternatives to the piperazine were tested.

Modification of the group on the piperazine nitrogen resulted in a series that had only modest differences in potency and NOS

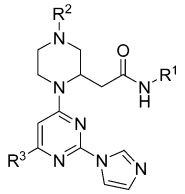
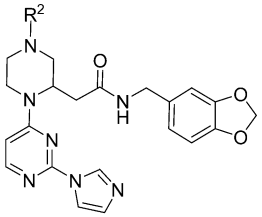


Figure 2. General optimization strategy.

Table 1. Substitution at the 4-Position of the Piperazine[15]



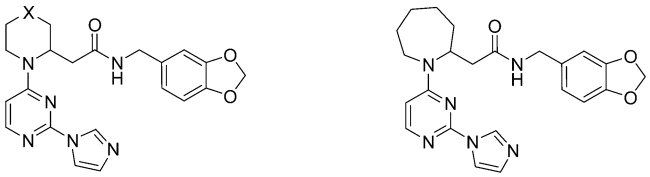
11

compd	R^2	IC_{50} [a] (nM) A172	IC_{50} [a] (nM) vaccinia	vaccinia selectivity ratio[a] b/i	vaccinia selectivity ratio[a] e/i
11a	$COCH_3$	1	320	3	>10
11b	CH_2Ph	1	13	7	770
11c	COPh	0.5	85	4	220
11d	$COOCH_3$	0.38	24	5	1700
11e	$CONHCH_3$	1	450	4	>250
11f	SO_2CH_3	0.7	74	12	>1000
11g	H	0.55	70	7	1200
11h	CH_3	0.48	11	2	1300
11i	CH_2-1-naphthalene	69	120	7	40
11j	CONHPh	0.87	25	3	350
11k	CONHBn	0.96	72	4	490
11l	SO_2Ph	2.7	34	9	1100
11m	CH_2-furan	0.58	13	2	1100
11n	$COCH_2OBn$	0.50	28	11	1220
11o	CO-2-furan	0.67	48	6	1000
11p	CO_2Ph	1.4	16	5	700
11q	COOBn	0.8	18	7	660
11r	$(CH_2)_2CHMe_2$	1.0	10	6	1000
11s	COO^iPr	1.0	24	4	800
11t	6-F-2-pyridine	0.28	10	6	850
11u	CH_2COOEt	0.93	4.9	5	1700
11v	C(=NH)Me	33	370	>10	350
11w	dansyl	1.1	NT	NT	NT

[a] NT: not tested.

selectivity (Table 1). Amides, amines, carbamates, ureas, and sulfonamides were well tolerated (e.g., **11a**, **11b**, **11d**, **11e**, and **11f**, respectively), with the unsubstituted compound, **11g**, as potent as any other compound. Steric bulk may have some effect on potency, with the large group (seen in **11i**) being the least potent compound, but other compounds with large substituents, such as benzoyl (**11q)** and dansyl (**11w)**, gave compounds with good activity. The basicity of the piperazine nitrogen did not seem to have much of an effect on activity in comparing **11b** with **11c**, **11m** with **11o**, or **11r** with **11s**. Although the highly basic amidine **11v** was less potent than the isosteric **11a**, the aminopyridine **11t** was highly active. Isosteric carbamates and ureas had similar activity (**11d** vs **11e**, **11j** vs **11p**, and **11k** vs **11q**), as well as amides and sulfonamides (**11a** vs **11f** and **11c** vs **11l**). In the vaccinia virus induced systems, these compounds demonstrated similar selectivity, good selectivity against ecNOS, and little selectivity against bcNOS.

Since we saw little effect of substituents on the piperazine nitrogen, we explored the effect of modulating ring size and replacement of the nitrogen (Figure 3). Incorporation of oxygen

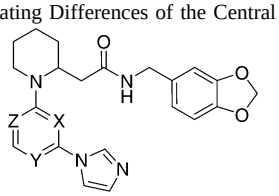


X = NH **11g**; 0.55 nM
X = CH_2 **12a**; 0.5 nM
X = O **13**; 0.5 nM

14; 2 nM

Figure 3. Comparison of piperazine alternatives in the A172 assay.

Table 2. Data Illustrating Differences of the Central Aromatic Ring[15]



12a,15-17

compd	X	Y	Z	IC_{50} [a] (nM) A172	IC_{50} [a] (nM) vaccinia	vaccinia selectivity ratio[a] b/i	vaccinia selectivity ratio[a] e/i
12a	N	N	CH	0.5	7	5	520
15	N	CH	N	52	220	6	>500
16	CH	N	N	10	100	19	>1000
17	CH	CH	CH	77	NT	NT	NT

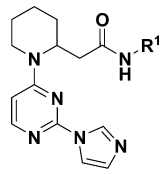
[a] NT: not tested.

or carbon to give morpholine (**13**) or piperidine (**12a**) based systems gives highly potent compounds with activity similar to the activity of the piperazines (**11**). For faster synthetic access, the piperidines were utilized to explore subsequent modifications. The perhydroazepine (**14**) was also a highly potent compound.

Early in the optimization, we looked at the difference in activity between the three possible pyrimidine regioisomers (Table 2). Previous results from libraries had indicated a potency preference for the compounds with the imidazole in the 2 position of the pyrimidine, and this was borne out. Comparison of the three regioisomers clearly shows that the 2-imidazolepyrimidine, **12a**, is the most potent. A possible explanation for this is that the imidazole needs to be nearly planar with the pyrimidine in the active site, and this is the only regioisomer that has no hydrogens flanking the imidazole on the pyrimidine, allowing the imidazole to be more planar. This hypothesis was further supported by the relatively weak iNOS activity shown by **17**, where the pyrimidine is replaced by a carbocyclic aromatic ring. In addition to trends in the SAR, the planar relationship of the imidazole and pyrimidine is demonstrated in the crystal structure (Figure 6). In the vaccinia virus induced systems, these compounds demonstrated similar overall selectivity, which can be described as good selectivity against ecNOS and as poor selectivity against bcNOS.

To examine the effect of side chain modifications, the corresponding piperidines, **12**, were prepared (Table 3). Unlike the modifications on the piperazine, different side chains gave compounds with very different activity. The initial hits from the library showed a clear preference for oxygen-containing substitution on an aromatic moiety and a specific chain length between the pyrimidine and the aromatic moiety. Placement of the methoxy group was important with a rank order of para, meta, and ortho (**12b** > **12c** > **12d**). The 3,4-disubstituted compound **12e** has a similar potency compared to the 4-substituted compound, **12b**, but the 3,4,5-trisubstituted analogue,

Table 3. Variation of the Amide Moiety[15]



12

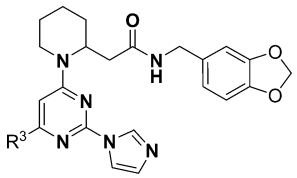
compd	R^1	IC_{50} [a] (nM) A172	IC_{50} [a] (nM) vaccinia	vaccinia selectivity ratio[a] b/i	vaccinia selectivity ratio[a] e/i
12a	CH_2-benzodioxolane	0.5	7	5	520
12b	CH_2Ph-4-OCH_3	0.8	23	2	200
12c	CH_2Ph-3-OCH_3	3.7	230	4	>430
12d	CH_2Ph-2-OCH_3	283	500	56	>200
12e	CH_2Ph-3,4-OCH_3	1.5	110	1	900
12f	CH_2Ph-3,4,5-OCH_3	>100	NT	NT	NT
12g	CH_2Ph	25	440	6	90
12h	CH_2Ph-4-Cl	5.7	160	8	270
12i	CH_2Ph-4-F	3.0	210	5	490
12j	CH_2Ph-4-OCF_3	19	140	5	220
12k	CH_2Ph-4-CH_3	23	274	3	48
12l	CH_2Ph-4-NH_2	270	850	>12	120
12m	CH_2Ph-4-$NHSO_2CH_3$	>100	>1000		
12n	CH_2Ph-4-$N(CH_3)_2$	66	245	29	86
12o	CH_2Ph-4-CF_3	150	260	3	70
12p	CH_2Ph-4-CN	>100	>1000		
12q	CH_2Ph-4-NO_2	168	940	>10	74
12r	CH_2Ph-4-SO_2NH_2	>10000	>330		
12s	CH_2Ph-4-SO_2CH_3	17	940	>13	110
12t	CH_2-2-furan	63	260	13	140
12u	CH_2-2-pyridine	539	NT	NT	NT
12v	$(NCH_3)CH_2Ph$	885	NT	NT	NT
12w	$CHPh_2$	1805	NT	NT	NT
12x	CH_3	>100			
12y	2-benzimidazole	11	1100	47	16
12z	benzodioxolane	>100	NT	NT	NT
12aa	Ph-3,4-OCH_3	66	1800	1	>55
12bb	$(CH_2)_2Ph$-3,4-OCH_3	12	680	1	120
12cc	$(CH_2)_2Ph$-4-OCH_3	3.5	42	8	>1000

[a] NT: not tested.

12f, lost significant potency. The potency difference between the isoelectronic **12a** and **12e** possibly indicates a steric interaction that is borne out in the crystal structure (Figure 6). Since the unsubstituted **12g** lost a significant amount of activity and the 4-substituted compound **12b** was the most potent monosubstituted compound, we further explored substituents at the 4 position but found no better substituent. The sterically similar **12h** and **12i** are potent, having activity less than 10 nM, but **12j**−**n** were all much less active. Compounds with electron-withdrawing groups, **12o**−**r**, were much less potent, but the sulfonamide **12s** has an IC_{50} of 17 nM. Some of the differences may be explained by a preference for a more electron-rich aromatic moiety (**12b** vs **12j** and **12k** vs **12o**), but the anilines (**12l**−**n**) do not fit this pattern. The monocyclic heterocycles tested, **12t** and **12u**, were less active than the unsubstituted **12g**. Substitution of the nitrogen (**12v** vs **12g**) and benzylic position (**12w** vs **12g**) both decreased activity. Loss of the aromatic moiety as in the methylamide, **12x**, gave a compound with little activity. In the vaccinia virus selectivity assays, the more active compounds (IC_{50} < 20 nM) demonstrated similar selectivity, good selectivity against ecNOS, and little selectivity against bcNOS.

Potency was very sensitive to the distance between the piperidine and the aromatic moiety (Table 3). Shortening the chain caused a dramatic decrease in activity; compare **12a** with **12z** or **12e** with **12aa**. Lengthening the chain caused a modest decrease in activity; compare **12e** with **12bb** or **12b** with **12cc**.

Table 4. Variation of the 6-substituent of the Pyrimidine[15]



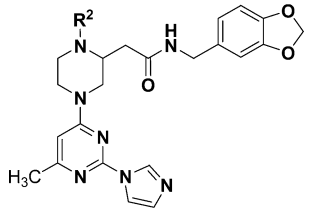
18

compd	R^3	IC_{50} (nM) A172	IC_{50} (nM) vaccinia	vaccinia selectivity ratio b/i	vaccinia selectivity ratio e/i
12a	H	0.5	7	5	520
18a	Cl	0.49	9	5	1100
18b	Ph	67	450	4	36
18c	Me	4.1	16	5	1200
18d	CF_3	180	320	>31	>310
18e	imidazole	35	515	13	130

Substitution of the pyrimidine 6 position indicated a preference for smaller groups when comparing potency, although the trend between hydrogen and methyl substitution was inconsistent (Table 4; additional compounds were prepared in this series, but the trends are consistent with those shown). The phenyl (**18b**) and imidazole (**18e**) substituted pyrimidines were less active than the corresponding unsubstituted compound, **12a**. The trifluoromethyl substituted analogue (**18d**) had the worst activity, but the methyl analogue (**18c)** was highly active. The differences in activity between the methyl and hydrogen substituted analogues were dependent on the amide substituent. When the benzodioxolane was the amide substituent, the hydrogen-containing compound **12a** was more potent than the methyl-containing compound **18c**, but with other substituted benzylamides, the hydrogen-containing compound was the same or was less active than the methyl-containing compound (data not shown). While the activity of 6-chloropyrimidines was consistently high, as exemplified by **18a**, the potential for displacement of the chlorine from these analogues was a concern and the analogues were not pursued. The selectivity of the compounds was similar to those already discussed.

By screening secondary libraries, we found that the alternatively substituted piperazines, **19**, had improved bcNOS selectivity but somewhat decreased activity. Further studies bore out this observation. Analogues were prepared (Table 5), and although most of the SAR was similar to that of the initially prepared piperazines, differences did appear. Of the few amide analogues prepared, the benzodioxalanemethyl substituent was found to be the most active. Substitution of the 6 position of the pyrimidine showed a clear preference for either methyl or chloro (data not shown). Unlike the initially substituted piperazine, substitution of the 4 position of these analogues resulted in large differences in activity, depending on the substituent. The unsubstituted analogue, **19a**, was the least active among the analogues. The lower activity did not seem to be due to the basicity of the compound, because the methyl and benzyl substituted compounds (**19b** and **19c**, respectively) were more active than the unsubstituted analogue, but may be due to the ability of the side chain to have freedom to rotate over the piperazine. The isosteric sulfonamide **19d** and acetamide **19e** have the same potency as **19b**. In general, the different placement of the side chain had an effect on bcNOS selectivity. The isobutyramide **19f** was the most active compound prepared in this series, and the bcNOS selectivity in the vaccinia system

Table 5. Piperazine Regioisomers[15]



19

compd	R^2	IC_{50} (nM) A172	IC_{50} (nM) vaccinia	vaccinia selectivity ratio b/i	vaccinia selectivity ratio e/i
19a	H	100	>1000		
19b	CH_3	12	340	72	>350
19c	Bn	25	160	130	350
19d	SO_2CH_3	12	>1000		
19e	Ac	8.5	340	>32	>320
19f	$COCH(CH_3)_2$	1.9	80	80	>300
19g	CO_2CH_3	5.4	420	50	>200

Table 6. Comparison of Enantiomers[15]

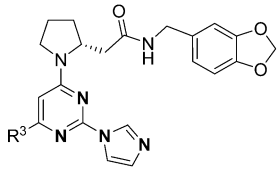
compd	IC_{50} (nM) A172	IC_{50} (nM) vaccinia	vaccinia selectivity ratio b/i	vaccinia selectivity ratio e/i	IC_{50}[a] (nm) tet	tet selectivity ratio[a] b/i	tet selectivity ratio[a] e/i
11d	0.38	24	5	1700	12	25	82
(*S*)-**11d**	0.24	18	6	1100	10	16	32
(*R*)-**11d**	34	>1000			NT	NT	NT
19f	1.9	80	80	>300	46	120	240
(*R*)-**19f**	0.75	350	29	>400	130	80	74
(*S*)-**19f**	300	>1000			NT	NT	NT

[a] NT: not tested.

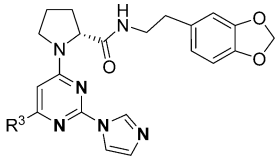
was improved compared to the alternatively substituted piperazines, while the ecNOS selectivity remained the same.

Two compounds were chosen for synthesis of their respective stereoisomers, **11d** and **19f.** The compounds were prepared by separating a common intermediate using chromatography on a chiral support and continuing on to the final compound. A large difference was found in the activity of the two isomers (Table 6), with, as expected, the more active isomer having activity and selectivity similar to those of the mixture. The activity of the less active isomer should be interpreted with caution, as the analytical methods would not discern a small impurity (<2%), which could easily explain all of the activity. The absolute stereochemistry of **11d** was determined by X-ray crystallography of the compound in complex with the protein and is consistent with the stereochemistry of compounds prepared from D-proline (Table 7). The absolute stereochemistry of **19d** is inferred from a related analogue, for which a structure was obtained.[17] Conveniently, the active isomers of **11d** and **19f** have different stereochemistry because they each arose from different isomers of the starting material.

Table 7. Pyrrolidine Analogues Arising from D-Proline[15]



20



21

compd	R^3	IC_{50}[a] (nM) A172	IC_{50}[a] (nM) tet	tet selectivity ratio[a] b/i	tet selectivity ratio[a] e/i	IC_{50}[a] (nm) vaccinia	vaccinia selectivity ratio[a] b/i	vaccinia selectivity ratio[a] e/i
20a	H	0.28	5.5	6	47	5.6	5	2900
20b	Me	0.12	4.5	27	91	5.9	8	1000
20c	Et	0.13	7.1	18	65	7.5	15	7800
21a	H	0.48	11	74	181	25	19	>2000
21b	Me	0.29	14	327	540	20	62	>5000
21b[b]	Me	6400	NT	NT	NT	NT	NT	NT
21c	Et	0.52	16	280	170	25	67	>3800

[a] NT: not tested. [b] Arose from L-proline.

In order to avoid separating isomers, we tested commercially available starting materials that contained the desired stereocenter. Analogues of proline were prepared and found to be highly active, some showing unexpectedly high bcNOS selectivity (Table 7). Again, keeping the distance between the pyrimidine and benzodioxolane group to six atoms was important, as changes caused a significant decrease in activity (compounds not shown). Moving the amide function in the side chain had no effect on potency but resulted in a change in selectivity for both isoforms (**20** vs **21**). Having the amide adjacent to the pyrrolidine, **21**, gave the most selective compounds identified in this series. The analogues arising from D-proline were significantly more potent than the analogues arising from L-proline and may better reflect the difference in activity of the stereoisomers (**21b** vs **21b***) than the earlier described pairs.

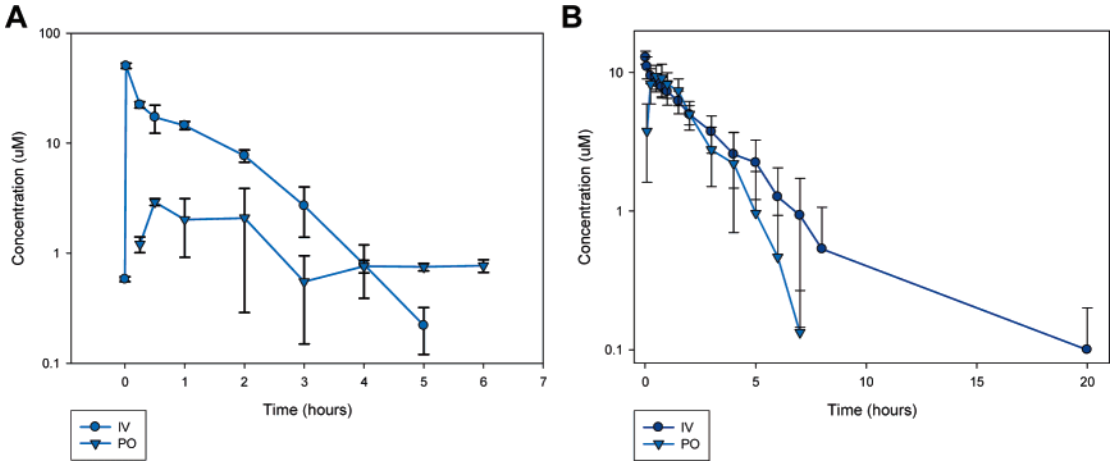


Figure 4. (A) Pharmacokinetics profile in rats. Error bars represent ±SEM; $n = 3$ rats per route. (B) Pharmacokinetics profile in dogs. Error bars represent ±SEM; $n = 3$ dogs per route.

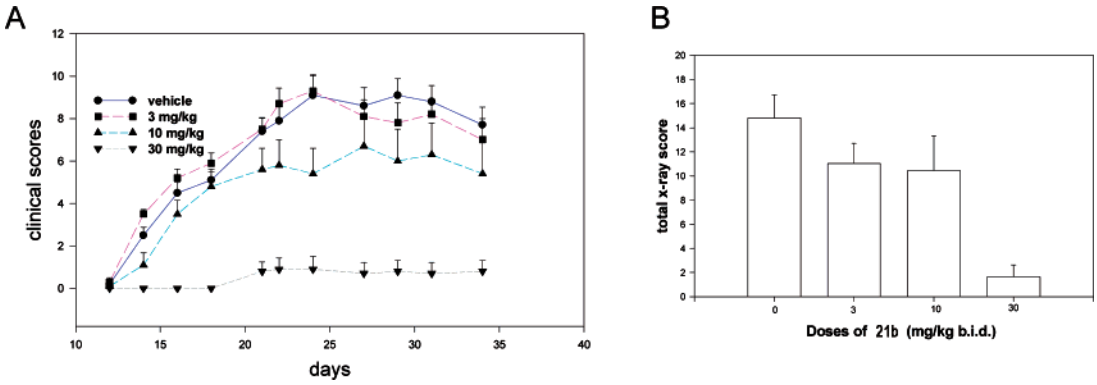


Figure 5. Effects of **21b** in a rat adjuvant-induced arthritis model. (A) Clinical scoring. Each limb was graded on a scale of 0−4 for (with increasing degrees of redness) gross swelling and distortion of the paw and joint fusion. The sum of the scores for each limb was totaled and designated as the clinical score. (B) Radiological scoring. Both hind limbs were graded on a scale of 0−3 for each of the following parameters: soft tissue swelling, cartilage loss, erosion, and heterotopic ossification. The sum of these scores for each limb was totaled and was designated as the radiological score.

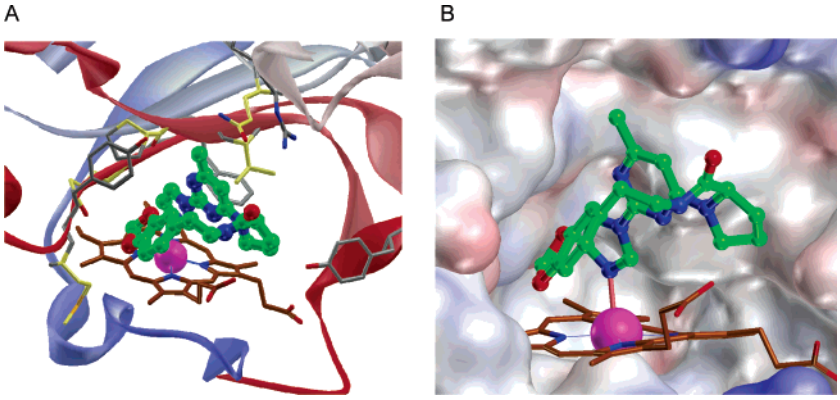


Figure 6. Compound **21b** (green carbon atoms) bound to murine iNOS monomer. The porphyrin is shown in brown and the iron in magenta: (A) a ribbon diagram with the residues that make contact with the inhibitor (<4.0 Å); (B) the solvent accessible surface (PDB entry 2ORO).

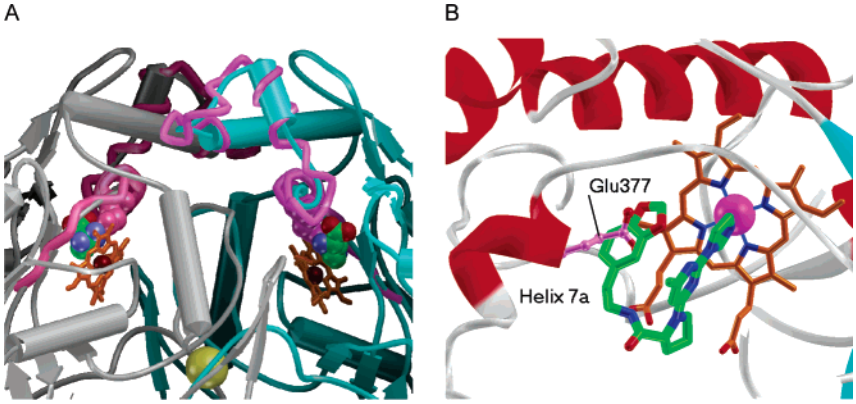


Figure 7. (A) Complex formed with L-Arg (green) and the iNOS dimer. The purple tube denotes the residues that become disordered in the iNOS−**21b** complex. (B) **21b** superimposed on the iNOS dimer complex (1NSI). In 1NSI, Glu377 forms a salt bridge to the guanidine of the arginine substrate. The benzodioxolane of **21b** occupies the same space as Glu377. This presumably disrupts the folding of helix 7a.

We selected **21b** for in vivo characterization because of its high potency and high selectivity versus the two other isoforms. The compound had good oral availability in both rats and dogs. In rats, the compound was dosed both intravenously and orally at 10 mg/kg, and although the half-life after iv dosing was short (0.7 h), the clearance and oral availability were reasonable (10 mL min^{-1} kg^{-1} and 21%, respectively, Figure 4A). The compound was administered to dogs at 3 mg/kg. The half-life after iv dosing was better (2.5 h), and the clearance and oral availability were improved (4.5 mL min^{-1} kg^{-1} and 71%, respectively; Figure 4B).

We then tested the ability of **21b** to inhibit adjuvant-induced arthritis in a rat model. The compound was dosed subcutaneously at 3, 10, and 30 mg/kg twice a day, and the rats were monitored and scored for arthritis using two methods, clinical signs and radiological scores (Figure 5). Compared to vehicle, both the 10 and 30 mg/kg dose resulted in a significantly lower clinical score. The 30 mg/kg dose also resulted in significantly

lower radiological scores. For rats dosed with 30 mg/kg, neither the clinical scores nor the radiological scores were significantly different from naive animals by analysis of both methods and at least 6 of the 10 animals in this high-dose group were free of disease. No adverse effects were observed for any of the doses tested.

Structural Analysis

An analysis of the structure of **21b** bound to monomeric iNOS oxygenase domain explains much of the SAR previously described (Figure 6).[18] The compound occupies the arginine binding pocket. The imidazole coordinates to the Fe, setting the inhibitors into the protein. The distal group of the piperazine (R^2) would point into the solvent and consequently does not have much effect on the activity of the resulting compounds. The substituent off the amide points back toward the porphyrin, and the benzodioxolane fills the pocket. The linker between the benzodioxolane and imidazole is critical because sufficient flexibility and distance between the two groups are needed to accommodate the bend between the two aromatics and to accommodate the ability of the compound to bind to the enzyme. The saturated nitrogen heterocycle probably aides the bend by predisposing the molecule to a β turn. We attempted to crystallize the less active enantiomer bound to the protein to better understand the binding, but the resulting crystals all contained the more potent isomer, which was presumably present as an undetected impurity (up to 2% would not be detected by our analytical methods).

A comparison of the inhibitor−monomer structure with a structure of the dimer helped rationalize the mechanism of inhibiting dimer formation seen with the compounds (Figure 7). The protein−protein interactions necessary for dimer formation are shown in Figure 7A. Compound **21b** displaces helix 7a (red) side chains from the Arg binding site and disrupts part of the dimer interface (Figure 7B), a disruption resulting in the disordering of the residues starting at helix 7a and up to the proximal half of helix 8.

Conclusion

A series of substituted pyrimidines containing an imidazole have been discovered that interrupts protein−protein interactions, displayed by inhibiting the dimerization of the iNOS monomers. This series has been optimized for their potency, selectivity against other NOS isoforms, and oral availability. Compound **21b** is a potent selective compound that shows efficacy in a model of rheumatoid arthritis. Future publications will illustrate the utilization of this template and the crystal structure in the design of novel templates.

Experimental Section

All starting materials not described below were purchased from commercial sources. All reagents and solvents were used as received from commercial sources without additional purification. Elemental analyses were performed by Robertson Microlit Laboratories (Madison, NJ), and results were within ±0.4% of the calculated values. NMR spectra were recorded with a Varian XL-300 spectrometer and were consistent with the assigned structures. Reverse-phase HPLC was performed with a Rainin SD-1 Dynamax system and a C-18 reverse-phase Dynamax 60A column using a gradient of acetonitrile (0.1% TFA) in water (0.1% TFA).

6-Methyl-2-(methylthio)pyrimidin-4-one (5). To a solution of 4-hydroxy-2-mercapto-6-methylpyrimidine (100 g, 0.70 mol) in DMSO (1 L) was added potassium carbonate (106 g, 0.77 mol), followed by iodomethane (48 mL, 0.77 mol), and the mixture was stirred at room temperature for 24 h. The reaction mixture was poured into 4 L of water with stirring. The resulting solids were filtered and washed with water, acetonitrile, and ether to afford 79 g (72% yield) of a white solid.

4-Chloro-6-methyl-2-(methylthio)pyrimidine (6). To phosphorus oxychloride (300 mL) under nitrogen was added 6-methyl-2-methylthiopyrimidin-4-one (79 g, 0.51 mol), and the mixture was heated at 80 °C for 5 h. The mixture was concentrated under vacuum, and the residue was poured onto 1 kg of ice and extracted with dichloromethane. The extract was washed with saturated potassium carbonate, dried over basic alumina, charcoal-treated, filtered, and concentrated under vacuum to give 81 g (91% yield) of a pale-yellow liquid. ^{1}H NMR ($CDCl_3$) δ 6.8 (s, 1H), 2.5 (s, 3H), 2.4 (s, 3H).

4-Chloro-6-methyl-2-(methylsulfonyl)pyrimidine (7). A solution of 3-chloroperoxybenzoic acid (240 g, 77%, 0.97 mol) in dichloromethane (2.5 L) was washed with saturated NaCl (500 mL), dried over magnesium sulfate, transferred to a 3 L flask with mechanical stirring, and cooled to −5 °C. A solution of 4-chloro-6-methyl-2-(methylsulfonyl)pyrimidine (81 g, 0.46 mol) in CH_2Cl_2 (100 mL) was added over 30 min. The mixture was allowed to warm to room temperature overnight. The solids were filtered and washed with cold CH_2Cl_2 (500 mL). The filtrate was washed with 10% K_2CO_3 (2 L), dried over basic alumina, charcoal-treated, filtered, and concentrated under vacuum. The residue was crystallized from hexanes (1 L) to afford 80 g (83% yield) of a white solid, which was stored under N_2 at 0 °C. ^{1}H NMR ($CDCl_3$) δ 7.45 (s, 1H), 3.35 (s, 3H), 2.65 (s, 3H).

Methyl 4-[(1,1-Dimethylethoxy)carbonyl]-2-piperazineacetate (8). Methyl 1,4-dibenzylpiperazine-2-acetate[14] (26.8 g, 79 mmol) was dissolved in MeOH (500 mL) and 0.5 N HCl (aqueous, 400 mL, 0.2 mol). Pd/C (10%, 9.5 g) was added, and the mixture was hydrogenated with H_2 (50 psi) for 24 h. The mixture was filtered through Celite, and the filtrate was concentrated in vacuo to remove most of the MeOH. The residue was made basic with K_2CO_3 (~16 g) to pH 9−10, and THF (300 mL) was added. The mixture was cooled to 0 °C, and BOC-ON (19.5 g, 79 mmol) dissolved in THF (100 mL) was added dropwise. The mixture was stirred at 0 °C for 1 h, allowed to warm to room temperature, and stirred for 2 h. Some solvent was removed in vacuo, and the residue was extracted with ethyl acetate. The combined extracts were treated with 100 mL of 1 M HCl (aqueous). The aqueous layer was separated, washed with ethyl acetate, basified with K_2CO_3, and extracted with ethyl acetate. The combined organic layers were washed with brine, dried (Na_2SO_4), and concentrated in vacuo to give 16.6 g (81%) of **8**. ^{1}H NMR ($CDCl_3$) δ 3.88 (b, 2H), 3.75 (s, 3H), 2.69−3.02 (m, 4H), 2.55 (b, 1H), 2.37 (dd, 1H), 2.31 (dd, 1H), 1.4 (s, 9H).

Methyl 1-[2-(1*H*-Imidazol-1-yl)pyrimidin-4-yl]-4-[(1,1-dimethylethoxy)carbonyl]-2-piperazineacetate (9). Methyl 4-[(1,1-dimethylethoxy)carbonyl]piperazine-2-acetate (**8**, 11.7 g, 45 mmol), 4-chloro-2-methylsulfonylpyrimidine (8.5 g, 44 mmol), and K_2CO_3 (13 g, 94 mmol) were dissolved in CH_3CN (250 mL) and heated to 35 °C for 18 h. Imidazole (15 g, 220 mmol) was added, and the mixture was heated to 65 °C for 24 h. The solvent was removed in vacuo, and the residue was slurried in CH_2Cl_2 and washed with water. The organic layer was separated, dried ($MgSO_4$), and concentrated in vacuo. The residue was chromatographed on silica (3% MeOH/CH_2Cl_2) to give 14.5 g (82%) of **9**.

***N*-[(1,3-Benzodioxol-5-yl)methyl]-1-[2-(1*H*-imidazol-1-yl)pyrimidin-4-yl]-4-[(1,1-dimethylethoxy)carbonyl]-2-piperazineacetamide (10).** To methyl 1-[2-(1*H*-imidazol-1-yl)pyrimidin-4-yl]-4-[(1,1-dimethylethoxy)carbonyl]piperazine-2-acetate (14.5 g, 36 mmol) dissolved in THF (250 mL) and water (70 mL) was added lithium hydroxide hydrate (7 g, 0.16 mol). The mixture was stirred for 18 h, and some of the solvent was removed in vacuo. Water (200 mL) was added, and the material was washed with ether. The aqueous solution was acidified with acetic acid, and the resulting precipitate was collected by filtration and washed with water, CH_3CN, and ether to give 10 g of a white solid. The solid was suspended in DMF (100 mL), and carbonyldiimidazole (4.4 g, 27 mmol) was added. The mixture was stirred for 3 h and became homogeneous. Piperonylamine (3.5 mL, 28 mmol) was added, and the mixture was stirred for another 1 h. The mixture was poured into 0.1 N

KOH and extracted with CH_2Cl_2. The extract was washed with water and dried ($MgSO_4$), and the solvent was removed in vacuo to give 13 g (69%) of **10** as a colorless oil.

4-(Acetyl)-*N*-[(1,3-benzodioxol-5-yl)methyl]-1-[2-(1*H*-imidazol-1-yl)pyrimidin-4-yl]-2-piperazineacetamide (11a). ^{1}H NMR (DMSO-d_6) δ 8.5 (s, 1 H), 8.35 (br, 1 H), 8.2 (m, 1 H), 7.85 (s, 1 H), 7.03 (s, 1 H), 6.6 (m, 4 H), 5.9 (s, 2 H), 5.3 (br, 1 H), 4.6 (br, 1 H), 4.25 (m, 1 H), 4.1 (m, 2 H), 3.8 (m, 1 H), 3.4 (m, 2 H), 3.0 (m, 1 H), 2.5 (m, 2 H), 1.95 and 2.05 (2 s, 3 H). Anal. ($C_{23}H_{25}N_7O_4\cdot 0.3H_2O$) C, H, N.

***N*-[(1,3-Benzodioxol-5-yl)methyl]-1-[2-(1*H*-imidazol-1-yl)pyrimidin-4-yl]-4-(phenylmethyl)-2-piperazineacetamide (11b).** ^{1}H NMR (DMSO-d_6) δ 8.5 (s, 1 H), 8.4 (br, 1 H), 8.2 (d, 1 H), 7.85 (s, 1 H), 7.3 (m, 5 H), 7.03 (s, 1 H), 6.8 (m, 3 H), 6.5 (d, 1 H), 5.9 (s, 2 H), 5.3 (br, 1 H), 4.6 (br, 1 H), 4.1 (m, 2 H), 3.95 (dd, 1 H), 355 (d, 1 H), 3.45 (d, 1 H), 2.9 (d, 1 H), 2.75 (d, 1 H), 2.7 (br, 1 H), 2.1 (m, 2 H). Anal. ($C_{28}H_{29}N_7O_3$) C, H, N.

***N*-[(1,3-Benzodioxol-5-yl)methyl]-1-[2-(1*H*-imidazol-1-yl)pyrimidin-4-yl]-4-(phenylcarbonyl)-2-piperazineacetamide (11c).** ^{1}H NMR (DMSO-d_6) δ 8.45 (s, 1 H), 8.2 (d, 1 H), 7.95 (br, 1 H), 7.85 (s, 1 H), 7.45 (s, 5 H), 7.03 (s, 1 H), 6.7 (m, 4 H), 5.9 (s, 2 H), 5.0 (m, 1 H), 4.3 (d, 1 H), 4.1 (m, 4 H), 3.4 (m, 2 H), 3.3 (m, 1 H), 2.6 (m, 2 H). Anal. ($C_{28}H_{27}N_7O_4$) C, H, N.

***N*-[(1,3-Benzodioxol-5-yl)methyl]-1-[2-(1*H*-imidazol-1-yl)pyrimidin-4-yl]-4-[(methoxy)carbonyl]-2-piperazineacetamide (11d).** Compound **11g** (5.04 g, 12 mmol) was slurried in CH_2Cl_2 (100 mL). Methyl chloroformate (1.03 mL, 13 mmol) and 1 N NaOH (12.5 mL) was added, and the mixture was stirred vigorously overnight. The clear solutions were separated, and the organic layer was dried (Na_2SO_4) and the solvent removed in vacuo. The residue was chromatographed (5% MeOH in CH_2Cl_2) to give 5.19 g (90%). ^{1}H NMR (DMSO-d_6) δ 8.45 (s, 1 H), 8.2 (d, 1 H), 8.0 (br, 1 H), 7.85 (s, 1 H), 7.03 (s, 1 H), 6.7 (m, 4 H), 5.9 (s, 2 H), 4.95 (br, 1 H), 4.15 (d, 1 H), 4.15 (d, 2 H), 3.95 (d, 2 H), 3.65 (s, 3 H), 3.3 (m, 2 H), 3.15 (dd, 1H), 2.5 (m, 2 H). Anal. ($C_{23}H_{25}N_7O_5$) C, H, N.

***N*-[(1,3-Benzodioxol-5-yl)methyl]-1-[2-(1*H*-imidazol-1-yl)pyrimidin-4-yl]-4-[(methylamino)carbonyl]-2-piperazineacetamide (11e).** ^{1}H NMR (DMSO-d_6) δ 8.45 (s, 1 H), 8.2 (d, 1 H), 8.0 (br, 1 H), 7.8 (s, 1 H), 7.03 (s, 1 H), 6.7 (m, 4 H), 6.15 (br, 1 H), 5.9 (s, 2 H), 4.9 (br, 1 H), 4.2 (m, 3 H), 3.9 (d, 2 H), 3.25 (dt, 1 H), 3.1 (dd, 1H), 3.0 (m, 1 H), 2.6 (s, 3 H), 2.5 (m, 2 H). Anal. ($C_{23}H_{26}N_8O_4\cdot 0.5H_2O$) C, H, N.

***N*-[(1,3-Benzodioxol-5-yl)methyl]-1-[2-(1*H*-imidazol-1-yl)pyrimidin-4-yl]-4-(methylsulfonyl)-2-piperazineacetamide (11f).** ^{1}H NMR (DMSO-d_6) δ 8.45 (s, 1 H), 8.2 (d, 1 H), 8.0 (br, 1 H), 7.8 (s, 1 H), 7.03 (s, 1 H), 6.7 (m, 4 H), 5.85 (s, 2 H), 5.1 (br, 1 H), 4.45 (d, 1 H), 4.1 (m, 2 H), 3.6 (m, 2 H), 3.3 (dt, 1 H), 3.05 (dd, 1H), 2.9 (m, 1H), 2.9 (s, 3H), 2.65 (d, 2H). Anal. ($C_{22}H_{25}N_7O_5S\cdot 0.25CH_2Cl_2$) C, H, N.

***N*-[(1,3-Benzodioxol-5-yl)methyl]-1-[2-(1*H*-imidazol-1-yl)pyrimidin-4-yl]-2-piperazineacetamide (11g).** *N*-[(1,3-Benzodioxol-5-yl)methyl]-1-[2-(1*H*-imidazol-1-yl)pyrimidin-4-yl]-4-[(1,1-dimethylethoxy)carbonyl]piperazine-2-acetamide (13 g, 24 mmol) was dissolved in CH_2Cl_2 (200 mL) and cooled to 0 °C. Trifluoroacetic acid (100 mL) was added, and the mixture was allowed to warm to room temperature. The solvent was removed in vacuo and the residue dissolved in EtOAc (200 mL). The organic layer was washed with saturated K_2CO_3 and dried ($MgSO_4$), and the solvent was removed in vacuo. Crystallization from ether gave 6 g (57%) of a white solid, mp 160−162 °C. ^{1}H NMR ($CDCl_3$) δ 8.5 (s, 1 H), 8.18 (d, 1 H), 7.8 (s, 1 H), 7.1 (s, 1 H), 6.7 (m, 3 H), 6.55 (m, 1H), 6.2 (t, 1H), 5.9 (s, 2 H), 4.8 (br, 1 H), 4.3 (m, 2 H), 2.8−3.2 (m, 7 H), 2.55 (dd, 1 H). Anal. ($C_{21}H_{23}N_7O_3\cdot 0.3H_2O$) C, H, N.

***N*-[(1,3-Benzodioxol-5-yl)methyl]-1-[2-(1*H*-imidazol-1-yl)pyrimidin-4-yl]-4-methyl-2-piperazineacetamide (11h).** ^{1}H NMR ($CDCl_3$) δ 8.45 (s, 1 H), 8.15 (d, 1 H), 7.78 (s, 1 H), 7.03 (s, 1 H), 6.4−6.7 (m, 4 H), 5.85 (s, 2 H), 4.25 (m, 2 H), 3.1 (m, 1 H), 2.8 (m, 4 H), 2.5 (dd, 1H), 2.25 (s, 3 H), 2.2 (m, 1 H), 2.05 (t, 1 H). Anal. ($C_{22}H_{26}N_7O_3\cdot 0.2CH_2Cl_2$) C, H, N.

***N*-[(1,3-Benzodioxol-5-yl)methyl]-1-[2-(1*H*-imidazol-1-yl)pyrimidin-4-yl]-4-[(1-naphthylenyl)methyl]-2-piperazineacetamide (11i).** ^{1}H NMR ($CDCl_3$) δ 8.55 (s, 1 H), 8.45 (s, 1 H), 8.15 (d, 1 H), 7.8 (m, 3 H), 7.4 (m, 4 H), 7.1 (s, 1 H), 6.65 (d, 1 H), 6.55 (m, 1H), 6.4 (m, 2 H), 5.9 (s, 2 H), 4.6 (m, 1H), 4.3 (m, 2 H), 4.0 (dd, 1H), 3.65 (m, 1 H), 3.1 (m, 3 H), 2.65 (m, 2 H), 2.4 (t, 1 H), 2.1 (m, 2 H). Anal. ($C_{32}H_{31}N_7O_3\cdot HCl\cdot 0.3H_2O$) C, H, N.

***N*-(1,3-Benzodioxol-5-ylmethyl)-1-[2-(1*H*-imidazol-yl)-4-pyrimidinyl]-4-(phenylamino)carbonyl]-2-piperazineacetamide (11j).** ^{1}H NMR ($CDCl_3$) δ 8.25 (m, 1H), 8.20 (m,1H), 7.80 (m, 1H), 7.50 (m, 2H), 7.30 (m, 2H), 7.00 (m, 1H), 6.80 (m, 4H), 6.40 (d, 1H), 5.80 (s, 2H), 5.0 (m, 1H), 4.35−4.65 (m, 4H), 3.60 (m, 1H),3.30 (m, 1H), 3.20 (m, 1H), 3.00 (m, 1H), 2.80 (m, 1H), 2.30 (d, 1H), 1.80 (m, 2H). Anal. ($C_{28}H_{28}N_8O_4\cdot 0.5H_2O$) C, H, N.

***N*-(1,3-Benzodioxol-5-ylmethyl)-1-[2-(1*H*-imidazol-yl)-4-pyrimidinyl]-4-[[(phenylmethyl)amino]carbonyl]-2-piperazineacetamide (11k).** ^{1}H NMR ($CDCl_3$) δ 8.40 (s, 1H), 8.20 (m,1H), 7.70 (m, 1H), 7.30 (m, 5H), 6.95 (s, 1H), 6.80 (m, 3H), 6.45 (m, 1H), 5.90 (s, 2H), 4.20−4.50 (m, 8H), 3.20 (m, 3H), 2.70 (m, 1H), 2.20 (m, 1H), 1.90 (m, 2H). Anal. ($C_{29}H_{30}N_8O_4\cdot 0.8H_2O$) C, H, N.

***N*-(1,3-Benzodioxol-5-ylmethyl)-1-[2-(1*H*-imidazol-yl)-4-pyrimidinyl]-4-(phenylsulfonyl)-2-piperazineacetamide (11l).** ^{1}H NMR ($CDCl_3$) δ 8.50 (s, 1H), (8.20 d, 1H), 7.50−7.80 (m, 6H), 7.00 (m, 1H),6.70 (m, 3H), 6.50 (m, 2H), 5.90 (s, 2H), 4.30 (m, 2H), 3.90 (m, 1H), 3.70 (m, 1H), 3.20 (m, 1H), 2.95 (m, 1H), 2.50 (m, 3H), 2.0 (m, 2H). Anal. ($C_{27}H_{27}N_7O_5S\cdot 0.7H_2O$) C, H, N.

***N*-[(1,3-Benzodioxol-5-yl)methyl]-1-[2-(1*H*-imidazol-1-yl)pyrimidin-4-yl]-4-(2-furylmethyl)-2-piperazineacetamide (11m).** ^{1}H NMR ($CDCl_3$) δ 8.45 (s, 1 H), 8.1 (d, 1 H), 7.78 (s, 1 H), 7.35 (s, 1 H), 7.03 (s, 1 H), 6.6 (m, 4 H), 6.3 (s, 1 H), 6.2 (s, 1 H), 5.85 (s, 2 H), 4.6 (br, 1 H), 4.25 (dd, 1 H), 4.15 (dd, 1 H), 4.0 (m, 2 H), 3.55 (AB, 2 H), 3.15 (m, 1 H), 2.8 (m, 3 H), 2.55 (dd, 1H), 2.2 (m, 2 H). Anal. ($C_{26}H_{27}N_7O_4\cdot 0.7H_2O$) C, H, N.

***N*-[(1,3-Benzodioxol-5-yl)methyl]-1-[2-(1*H*-imidazol-1-yl)pyrimidin-4-yl]-4-(methylsulfonyl)-2-piperazineacetamide (11n).** ^{1}H NMR (DMSO-d_6) δ 8.45 (s, 1 H), 8.2 (d, 1 H), 7.9 (br, 1 H), 7.83 (s, 1 H), 7.35 (m, 4 H), 7.3 (m, 1 H), 7.03 (s, 1 H), 6.7 (m, 4 H), 5.9 (s, 2 H), 4.95 (m, 1 H), 4.6 (s, 2 H), 4.25 (m, 4 H), 4.15 (m, 3 H), 3.35 (m, 2 H), 3.2 (m, 1 H), 2.55 (m, 2 H). Anal. ($C_{30}H_{31}N_7O_5\cdot 0.5H_2O$) C, H, N.

***N*-(1,3-Benzodioxol-5-ylmethyl)-4-(2-furanylcarbonyl)-1-[2-(1*H*-imidazolyl)-4-pyrimidinyl]-2-piperazineacetamide (11o).** ^{1}H NMR ($CDCl_3$) δ 8.50 (m, 1 H), 8.20 (m, 1H), 7.80 (m, 1H), 7.50 (m, 1H), 7.00 (m, 2H), 6.70 (m, 4H), 6.50 (m, 1H), 5.90 (s, 2H), 4.50 (m, 2H), 4.30 (m, 2H), 3.20 (m, 4H), 2.70 (m, 1H), 2.40 (m, 1H), 2.05 (m, 2H). Anal. ($C_{28}H_{25}N_7O_5\cdot 0.2CH_2Cl_2$) C, H, N.

3-[2-[(1,3-Benzodioxol-5-ylmethyl)amino]-2-oxoethyl]-4-[2-(1*H*-imidazolyl)-4-pyrimidinyl]-1-piperazinecarboxylic Acid, Phenyl Ester (11p). ^{1}H NMR ($CDCl_3$) δ 8.50 (m, 1 H), 8.20 (m, 1H), 7.80 (m, 1H), 7.35 (m, 1H), 7.20 (m, 1H), 7.10 (m, 4H), 6.60 (m, 5H), 5.85 (s, 2H), 4.30 (m, 4H), 3.30 (m, 3H), 2.50 (m, 2H), 2.00 (m, 2H). Anal. ($C_{28}H_{27}N_7O_5\cdot 0.1CH_2Cl_2$) C, H, N.

***N*-[(1,3-Benzodioxol-5-yl)methyl]-1-[2-(1*H*-imidazol-1-yl)pyrimidin-4-yl]-4-[(phenylmethoxy)carbonyl]piperazine-2-acetamide (11q).** ^{1}H NMR ($CDCl_3$) δ 8.45 (s, 1 H), 8.2 (d, 1 H), 7.8 (s, 1 H), 7.35 (m, 5 H), 7.05 (s, 1 H), 6.6 (m, 4 H), 5.85 (s, 2 H), 5.15 (m, 2 H), 4.2 (m, 5 H), 3.1 (m, 3 H), 2.5 (m, 2 H), 2.0 (m, 1 H). Anal. ($C_{29}H_{29}N_7O_5\cdot 0.5H_2O$) C, H, N.

***N*-[(1,3-Benzodioxol-5-yl)methyl]-1-[2-(1*H*-imidazol-1-yl)pyrimidin-4-yl]-4-(3-methylbutyl)-2-piperazineacetamide (11r).** ^{1}H NMR ($CDCl_3$) δ 8.5 (s, 1 H), 8.15 (d, 1 H), 7.8 (s, 1 H), 7.05 (s, 1 H), 6.65 (m, 3 H), 6.5 (m, 1 H), 6.25 (m, 1 H), 5.9 (s, 2 H), 4.7 (br, 1 H), 4.25 (m, 2 H), 3.15 (m, 1 H), 2.85 (m, 3 H), 2.5 (dd, 1 H), 2.3 (m, 2 H), 2.1 (m, 3 H), 1.6 (m, 1 H), 1.3 (m, 2 H), 0.9 (m, 6 H). Anal. ($C_{26}H_{33}N_7O_3\cdot 0.5H_2O$) C, H, N.

3-[2-[(1,3-Benzodioxol-5-ylmethyl)amino]-2-oxoethyl]-4-[2-(1*H*-imidazolyl)-4-pyrimidinyl]-1-piperazinecarboxylic Acid, 1-Methyl Ethyl Ester (11s). ^{1}H NMR ($CDCl_3$) δ 8.5 (s, 1 H), 8.2 (m,1H), 7.80 (m, 1H), 7.05 (m, 1H), 6.90 (m, 1H), 6.60 (m, 4H), 5.90 (s, 2H), 5.00 (m, 2H), 4.40 (m, 1H), 4.20 (m, 3H), 3.10 (m,

2H), 2.60 (m, 1H), 2.40 (m, 1H), 1.95 (s, 2H), 1.30 (m, 6H). Anal. ($C_{25}H_{29}N_7O_5 \cdot 0.5H_2O$) C, H, N.

***N*-[(1,3-Benzodioxol-5-yl)methyl]-1-[2-(1*H*-imidazol-1-yl)pyrimidin-4-yl]-4-(6-fluoropyridin-2-yl)-2-piperazineacetamide (11t).** ^{1}H NMR (DMSO-d_6) δ 8.5 (s, 1 H), 8.4 (m, 1 H), 8.2 (d, 1 H), 7.9 (s, 1 H), 7.65 (m, 1H), 7.05 (s, 1 H), 6.75 (d, 1 H), 6.7 (m, 2 H), 6.6 (m, 1 H), 6.3 (m, 1 H), 5.9 (s, 2 H), 4.0 (m, 3 H), 3.3 (m, 4 H). Anal. ($C_{26}H_{25}N_8O_3F \cdot 0.5H_2O$) C, H, N.

***N*-[(1,3-Benzodioxol-5-yl)methyl]-1-[2-(1*H*-imidazol-1-yl)pyrimidin-4-yl]-4-(2-furylmethyl)-2-piperazineacetamide (11u).** ^{1}H NMR (DMSO-d_6) δ 8.45 (s, 1 H), 8.35 (br, 1 H), 8.15 (d, 1 H), 7.8 (s, 1 H), 7.0 (s, 1 H), 6.6 (m, 4 H), 5.85 (s, 2 H), 4.0 (m, 5 H), 3.3 (m, 2 H), 3.2 (q, 2 H), 2.9 (d, 1 H), 2.8 (d, 1 H), 2.6 (m, 2H), 2.4 (d, 1 H), 2.25 (m, 1 H), 1.15 (t, 3 H). Anal. ($C_{25}H_{29}N_7O_5 \cdot 0.4H_2O$) C, H, N.

***N*-[(1,3-Benzodioxol-5-yl)methyl]-1-[2-(1*H*-imidazol-1-yl)pyrimidin-4-yl]-4-(1-iminoethyl)-2-piperazineacetamide, Hydrochloride (11v).** ^{1}H NMR (DMSO-d_6) δ 9.5 (m, 1 H), 8.9 (s, 1 H), 8.6 (m, 2 H), 8.25 (m, 1 H), 7.9 (m, 1 H), 7.05 (s, 1 H), 6.7 (m, 4 H), 5.9 (s, 2 H), 4.1 (m, 2 H), 4.0 (m, 3 H), 3.1−3.8 (m, 4 H), 2.6 (m, 2 H), 2.35 and 2.25 (2s, 3 H). MS *m*/*z* 463 (M + 1). Anal. ($C_{23}H_{26}N_8O_3 \cdot H_2O$) C, H, N.

***N*-[(1,3-Benzodioxol-5-yl)methyl]-4-[(5-dimethylaminonaphthyl)sulfonyl]-1-[2-(1*H*-imidazol-1-yl)pyrimidin-4-yl]-2-piperazineacetamide (11w).** ^{1}H NMR ($CDCl_3$) δ 8.6 (d, 1 H), 8.5 (s, 1 H), 8.3 (d, 1 H), 8.2 (d, 1 H), 8.1 (d, 1 H), 7.8 (s, 1 H), 7.6 (m, 2 H), 7.2 (d, 1 H), 7.1 (s, 1 H), 6.8 (m, 3 H), 6.6 (br s, 1 H), 6.0 (s, 2 H), 4.3 (m, 1 H), 4.2 (m, 1 H), 4.1 (d, 1 H), 3.7 (d, 1 H), 3.2 (br s, 1 H), 2.9 (s, 6 H), 2.8 (m, 4 H), 2.3 (dd, 1 H). Anal. ($C_{33}H_{34}N_8O_5S \cdot CH_2Cl_2$) C, H, N.

***N*-[(1,3-Benzodioxol-5-yl)methyl]-1-[2-(1*H*-imidazol-1-yl)-4-pyrimidinyl]-2-piperidineacetamide (12a).** ^{1}H NMR ($CDCl_3$) δ 8.45 (s, 1 H), 8.1 (d, 1 H), 7.85 (s, 1 H), 7.05 (s, 1 H), 6.7 (s, 1 H), 6.5 (m, 4 H), 5.85 (m, 2 H), 5.1 (br, 1 H), 4.2 (m, 3 H), 3.0 (m, 1 H), 2.7 (dd, 1 H), 2.55 (dd, 1 H), 1.6−1.8 (m, 5 H), 1.5 (m, 1 H). Anal. ($C_{22}H_{24}N_6O_3 \cdot 0.7CH_2Cl_2$) C, H, N.

1-[2-(1*H*-Imidazol-1-yl)-4-pyrimidinyl]-*N*-[(4-methoxyphenyl-)methyl]-2-piperidineacetamide (12b). ^{1}H NMR ($CDCl_3$) δ 8.45 (s, 1 H), 8.1 (d, 1 H), 7.75 (s, 1 H), 7.1 (s, 1 H), 7.0 (d, 2 H), 6.7 (d, 2 H), 6.5 (s, 1 H), 6.25 (m, 1 H), 5.1 (br, 1 H), 4.25 (m, 3 H), 3.75 (s, 3 H), 2.59 (m, 1 H), 2.65 (dd, 1 H), 2.55 (dd, 1 H), 1.3−1.8 (m, 6 H). Anal. ($C_{22}H_{26}N_6O_2 \cdot 0.8H_2O$) C, H, N.

1-[2-(1*H*-Imidazol-1-yl)pyrimidin-4-yl]-*N*-[(3-methoxyphenyl-)methyl]-2-piperidineacetamide (12c). ^{1}H NMR ($CDCl_3$) δ 8.4 (s, 1 H), 8.0 (m, 1 H), 7.75 (s, 1 H), 7.3 (m, 1 H), 7.05 (t, 1 H), 6.95 (s, 1 H), 6.6 (m, 3 H), 6.6 (m, 1 H), 5.0 (br, 1 H), 4.25 (m, 3 H), 3.65 (s, 3 H), 2.9 (m, 3 H), 2.6 (m, 2 H), 1.3−1.8 (m, 6 H). Anal. ($C_{22}H_{26}N_6O_2 \cdot 0.3H_2O$) C, H, N.

1-[2-(1*H*-Imidazol-1-yl)pyrimidin-4-yl]-*N*-[(2-methoxyphenyl-)methyl]-2-piperidineacetamide (12d). ^{1}H NMR ($CDCl_3$) δ 8.4 (s, 1 H), 8.0 (d, 1 H), 7.8 (s, 1 H), 7.1 (m, 2 H), 6.7 (m, 2 H), 6.4 (d, 1 H), 6.3 (m, 1 H), 5.0 (br s, 1H), 4.3 (m, 2 H), 3.8 (s, 3 H), 2.9 (m, 1 H), 2.8 (m, 2 H), 2.0 (m, 1 H), 1.7 (m, 5 H), 1.5 (m, 1 H). Anal. ($C_{22}H_{26}N_6O_2 \cdot H_2O$) C, H, N.

1-[2-(1*H*-Imidazol-1-yl)pyrimidin-4-yl]-*N*-[(3,4-dimethoxyphenyl)methyl]-2-piperidineacetamide (12e). ^{1}H NMR (DMSO-d_6) δ 8.5 (s, 1 H), 8.4 (m, 1 H), 8.15 (d, 1 H), 7.85 (s, 1 H), 7.05 (s, 1 H), 6.7 (m, 3 H), 6.6 (d, 1 H), 4.1 (m, 2 H), 3.6 (2 s, 6 H), 3.1 (m, 1 H), 3.0 (m, 2 H), 1.3−1.8 (m, 6 H). Anal. ($C_{23}H_{28}N_6O_3 \cdot H_2O$) C, H, N.

1-[2-(1*H*-Imidazol-1-yl)pyrimidin-4-yl]-*N*-[(3,4,5-trimethoxyphenyl)methyl]-2-piperidineacetamide (12f). ^{1}H NMR ($CDCl_3$) δ 8.5 (s, 1 H), 8.1 (d, 1 H), 7.8 (s, 1 H), 7.1 (s, 1 H), 6.5 (m, 1 H), 6.3 (s, 2 H), 6.2 (br, 1 H), 5.15 (br, 1 H), 4.4 (dd, 1 H), 4.2 (m, 1 H), 3.82 (s, 3 H), 3.77 (s, 6 H), 3.0 (m, 1 H), 2.7 (dd, 1 H), 2.55 (dd, 1 H), 1.3−1.8 (m, 6 H). Anal. ($C_{24}H_{30}N_6O_4$) C, H, N.

1-[2-(1*H*-Imidazol-1-yl)pyrimidin-4-yl]-*N*-(phenylmethyl)-2-piperidineacetamide (12g). ^{1}H NMR ($CDCl_3$) δ 8.4 (s, 1 H), 8.05 (d, 1 H), 7.75 (s, 1 H), 7.0−7.3 (m, 6 H), 6.7 (br, 1 H), 6.5 (m, 1 H), 5.15 (br, 1 H), 4.3 (m, 1 H), 2.95 (m, 1 H), 2.65 (dd, 1 H), 2.55 (dd, 1 H), 1.3−1.8 (m, 6 H). Anal. ($C_{24}H_{24}N_6O \cdot 0.2H_2O$) C, H, N.

***N*-[(4-Chlorophenyl)methyl]-1-[2-(1*H*-imidazol-1-yl)pyrimidin-4-yl]-2-piperidineacetamide (12h).** ^{1}H NMR ($CDCl_3$) δ 8.65 (br, 1 H), 8.05 (d, 1 H), 7.8 (s, 1 H), 7.4 (br, 1 H), 7.0 (m, 5 H), 6.5 (m, 1 H), 5.2 (br, 1 H), 4.2 (m, 2 H), 3.65 (m, 1H), 3.05 (m, 1 H), 2.75 (dd, 1 H), 2.6 (dd, 1 H), 1.3−1.8 (m, 6 H). Anal. ($C_{21}H_{23}N_6OCl \cdot 0.8H_2O$) C, H, N.

***N*-[(4-Fluorophenyl)methyl]-1-[2-(1*H*-imidazol-1-yl)pyrimidin-4-yl]-2-piperidineacetamide (12i).** ^{1}H NMR ($CDCl_3$) δ 8.4 (s, 1 H), 8.05 (d, 1 H), 7.75 (s, 1 H), 7.0 (m, 3 H), 6.8 (m, 2 H), 6.7 (m, 1 H), 6.5 (m, 1 H), 5.1 (br, 1 H), 4.25 (m, 3 H), 3.0 (m, 1 H), 2.7 (dd, 1 H), 2.55 (dd, 1 H), 1.3−1.8 (m, 6 H). Anal. ($C_{21}H_{23}N_6OF \cdot 0.8H_2O$) C, H, N.

1-[2-(1*H*-Imidazol-1-yl)-4-pyrimidinyl]-*N*-[[4-(trifluoromethoxy)phenyl]methyl]-2-piperidineacetamide (12j). ^{1}H NMR ($CDCl_3$) δ 8.5 (s, 1 H), 8.1 (d, 1 H), 7.8 (s, 1 H), 7.1 (m, 4 H), 6.5 (d, 1 H), 6.4 (br s, 1 H), 5.2 (br s, 1 H), 4.3 (m, 2 H), 3.0 (m, 1 H), 2.6 (m, 2 H), 1.8 (m, 6 H, partially obscured by water), 1.6 (m, 1 H). Anal. ($C_{22}H_{23}F_3N_6O_2 \cdot H_2O$) C, H, N.

1-[2-(1*H*-Imidazol-1-yl)pyrimidin-4-yl]-*N*-[(4-methylphenyl)-methyl]-2-piperidineacetamide (12k). ^{1}H NMR ($CDCl_3$) δ 8.4 (s, 1 H), 8.03 (d, 1 H), 7.75 (s, 1 H), 6.95 (m, 5 H), 6.8 (s, 1 H), 6.45 (s, 1 H), 5.0 (br, 1 H), 4.25 (m, 3 H), 2.9 (m, 1 H), 2.6 (m, 1 H), 2.5 (m, 1 H), 2.2 (s, 3 H), 1.3−1.8 (m, 6 H). Anal. ($C_{23}H_{28}N_6O_3 \cdot H_2O \cdot 0.6H_2O$) C, H, N.

***N*-[(4-Aminophenyl)methyl]-1-[2-(1*H*-imidazol-1-yl)-4-pyrimidinyl]-2-piperidineacetamide (12l).** ^{1}H NMR ($CDCl_3$) δ 8.4 (s, 1 H), 8.1 (d, 1 H), 7.7 (s, 1 H), 7.0 (s, 1 H), 6.8 (d, 2 H), 6.5 (m, 3 H), 6.0 (br s, 1 H), 5.1 (s, 1 H), 4.2 (m, 2 H), 2.9 (m, 1 H), 2.6 (m, 2 H), 1.7 (m, 6 H), 1.5 (m, 1 H). Anal. ($C_{21}H_{25}N_7O \cdot CH_2Cl_2$) C, H, N.

1-[2-(1*H*-Imidazol-1-yl)-4-pyrimidinyl]-*N*-[[4-(methylsulfonylamino)phenyl]methyl]-2-piperidineacetamide (12m). ^{1}H NMR (DMSO-d_6) δ 9.6 (s, 1 H), 8.5 (s, 1 H), 8.4 (m, 1 H), 8.1 (d, 1 H), 7.8 (s, 1 H), 7.1 (m, 5 H), 6.7 (m, 1 H), 4.2 (m, 1 H), 4.1 (m, 1 H), 3.0 (br s, 1 H), 2.9 (s, 3 H), 2.5 (m, 4 H, partially obscured by DMSO), 1.7 (m, 5 H), 1.4 (m, 1 H). Anal. ($C_{22}H_{27}N_7O_3S \cdot CH_2Cl_2$) C, H, N.

***N*-[(4-Dimethylaminophenyl)methyl]-1-[2-(1*H*-imidazol-1-yl)-pyrimidin-4-yl]-2-piperidineacetamide (12n).** ^{1}H NMR ($CDCl_3$) δ 8.5 (s, 1 H), 8.1 (d, 1 H), 7.8 (s, 1 H), 7.1 (s, 1 H), 7.0 (d, 2 H), 6.5 (d, 2 H), 6.1 (s, 1 H), 5.1 (br s, 1 H), 4.2 (m, 2 H), 2.9 (m, 1 H), 2.8 (s, 6 H), 2.6 (m, 1 H), 2.5 (m, 1 H), 2.0 (s, 1 H), 1.8 (m, 5 H), 1.5 (m, 1 H). Anal. ($C_{23}H_{29}N_7O \cdot CH_2Cl_2$) C, H, N.

1-[2-(1*H*-Imidazol-1-yl)-4-pyrimidinyl]-*N*-[[4-(trifluoromethyl)phenyl]methyl]-2-piperidineacetamide (12o). ^{1}H NMR ($CDCl_3$) δ 8.4 (s, 1 H), 8.1 (d, 1 H), 7.8 (s, 1 H), 7.5 (br s, 1 H), 7.4 (d, 2 H), 7.2 (d, 2 H), 7.0 (s, 1 H), 6.5 (s, 1 H), 5.1 (br s, 1 H), 4.4 (m, 2 H), 3.0 (m, 1 H), 2.6 (m, 2 H), 2.5 (br s, 1 H), 1.7 (m, 5 H), 1.5 (m, 1 H). Anal. ($C_{22}H_{23}F_3N_6O \cdot CH_2Cl_2$) C, H, N.

***N*-[(4-Cyanophenyl)methyl]-1-[2-(1*H*-imidazol-1-yl)pyrimidin-4-yl]-2-piperidineacetamide (12p).** ^{1}H NMR ($CDCl_3$) δ 8.4 (s, 1 H), 8.1 (d, 1 H), 7.8 (s, 1 H), 7.4 (d, 2 H), 7.2 (d, 2 H), 7.0 (s, 1 H), 6.8 (m, 1 H), 6.5 (m, 1 H), 5.2 (br s, 1 H), 4.4 (m, 2 H), 3.0 (m, 1 H), 2.8 (dd, 1 H), 2.6 (dd, 1 H), 1.8 (m, 5 H), 1.6 (m, 1 H). Anal. ($C_{22}H_{23}N_7O \cdot CH_2Cl_2$) C, H, N.

1-[2-(1*H*-Imidazol-1-yl)pyrimidin-4-yl]-*N*-[(4-nitrophenyl)-methyl]-2-piperidineacetamide (12q). ^{1}H NMR ($CDCl_3$) δ 8.4 (s, 1 H), 8.1 (d, 1 H), 8.0 (d, 2 H), 7.8 (s, 1 H), 7.2 (d, 2H), 7.1 (s, 1 H), 6.5 (d, 1 H), 5.2 (br s, 1H), 4.5 (dd, 1 H), 4.3 (dd, 1 H), 3.1 (m, 1 H), 2.8 (dd, 1 H), 2.6 (dd, 1 H), 1.8 (m, 4 H), 1.6 (m, 3 H, partially obscured by water). Anal. ($C_{21}H_{23}N_7O_3 \cdot CH_2Cl_2$) C, H, N.

***N*-[(4-Aminosulfonylphenyl)methyl]-1-[2-(1*H*-imidazol-1-yl)-pyrimidin-4-yl]-2-piperidineacetamide (12r).** ^{1}H NMR (DMSO-d_6) δ 8.6 (s, 2 H), 8.2 (d, 1 H), 7.8 (s, 1 H), 7.7 (d, 2 H), 7.3 (m, 4 H), 7.1 (s, 1 H), 6.7 (d, 1 H), 4.2 (m, 2 H), 3.0 (m, 1 H), 2.5 (m, 3 H, partially obscured by DMSO), 2.3 (m, 1 H), 1.6 (m, 5 H), 1.4 (m, 1 H). Anal. ($C_{21}H_{25}N_7O_3$) C, H, N.

1-[2-(1*H*-Imidazol-1-yl)-4-pyrimidinyl]-*N*-[[4-(methylsulfonyl)-phenyl]methyl]-2-piperidineacetamide (12s). ^{1}H NMR ($CDCl_3$) δ 8.4 (s, 1 H), 8.1 (d, 1 H), 7.8 (s, 1 H), 7.6 (d, 2 H), 7.4 (s, 1 H), 7.2 (d, 2 H), 7.0 (s, 1 H), 6.5 (s, 1 H), 5.1 (br s, 1 H), 4.4 (m, 2 H), 3.0 (s, 4 H), 2.7 (m, 2 H), 2.4 (s, 1 H), 1.8 (m, 5 H), 1.5 (m, 1 H). Anal. ($C_{22}H_{26}N_6O_3S\cdot CH_2Cl_2$) C, H, N.

1-[2-(1*H*-Imidazol-1-yl)-4-pyrimidinyl]-*N*-[(2-furanyl)methyl]-2-piperidineacetamide (12t). ^{1}H NMR ($CDCl_3$) δ 8.45 (s, 1 H), 8.05 (d, 1 H), 7.8 (s, 1 H), 7.2 (s, 1 H), 7.05 (s, 1 H), 6.7 (m, 1 H), 6.5 (m, 1H), 6.2 (s, 1H), 6.05 (s, 1H), 5.1 (br s, 1 H), 4.3 (m, 3 H), 2.95 (m, 1 H), 2.6 (m, 2 H), 1.7 (m, 5 H), 1.5 (m, 1 H). Anal. ($C_{19}H_{22}N_6O_2\cdot 0.5H_2O$) C, H, N.

1-[2-(1*H*-Imidazol-1-yl)-4-pyrimidinyl]-*N*-[(2-pyridinyl)methyl]-2-piperidineacetamide (12u). ^{1}H NMR ($CDCl_3$) δ 8.45 (s, 1 H), 8.4 (m, 1H), 8.05 (d, 1 H), 7.8 (s, 1 H), 7.55 (t, 1 H), 7.0−7.2 (m, 4 H), 6.5 (m, 1 H), 5.1 (br s, 1 H), 4.4 (m, 2 H), 4.4 (br, 1H), 3.0 (m, 1 H), 2.6 (m, 2 H), 1.7 (m, 5 H), 1.5 (m, 1 H). Anal. ($C_{20}H_{23}N_7O\cdot 0.4H_2O$) C, H, N.

1-[2-(1*H*-Imidazol-1-yl)-4-pyrimidinyl]-*N*-methyl-*N*-phenyl-2-piperidineacetamide (12v). ^{1}H NMR (DMSO-d_6) δ 8.45 (m, 1 H), 8.1 (m, 1H), 7.8 (m, 1 H), 7.2 (m, 3 H), 7.05 (m, 3 H), 6.7 (dd, 1 H), 4.6 (m, 1 H), 4.45 (m, 2 H), 2.85 (m, 3 H), 2.9 and 2.75 (2 s, 3 H), 1.4−1.8 (m, 6 H). Anal. ($C_{20}H_{23}N_7O\cdot 0.4H_2O$) C, H, N.

1-[2-(1*H*-Imidazol-1-yl)-4-pyrimidinyl]-*N*-[(diphenyl)methyl]-2-piperidineacetamide (12w). ^{1}H NMR ($CDCl_3$) δ 8.45 (s, 1 H), 8.05 (d, 1 H), 7.75 (s, 1 H), 6.9−7.4 (m, 12 H), 6.45 (m, 1 H), 6.2 (d, 1H), 5.1 (br s, 1 H), 4.4 (br, 1H), 3.0 (m, 1 H), 2.8 (dd, 1 H), 2.55 (dd, 1H), 1.7 (m, 5 H), 1.5 (m, 1 H). Anal. ($C_{27}H_{28}N_6O\cdot 0.5H_2O$) C, H, N.

1-[2-(1*H*-Imidazol-1-yl)pyrimidin-4-yl]-*N*-methyl-2-piperidineacetamide (12x). ^{1}H NMR (DMSO-d_6) δ 8.45 (s, 1 H), 8.15 (d, 1 H), 7.85 (s, 1 H), 7.55 (br, 1 H), 7.05 (s, 1 H), 6.65 (d, 1 H), 5.0 (br, 1 H), 4.25 (m, 1 H), 2.4 (m, 2 H), 1.3−1.8 (m, 6 H). Anal. ($C_{15}H_{20}N_6O\cdot 0.1CH_2Cl_2\cdot 0.9H_2O$) C, H, N.

***N*-[(2-Benzimidazolyl)methyl]-1-[2-(1*H*-imidazol-1-yl)pyrimidin-4-yl]-2-piperidineacetamide (12y).** ^{1}H NMR (DMSO-d_6) δ 8.7 (m, 1 H), 8.5 (s, 1 H), 8.15 (d, 1 H), 7.85 (s, 1 H), 7.45 (m, 2 H), 7.15 (m, 2 H), 7.05 (s, 1 H), 6.7 (d, 2 H), 5.3 (br, 2 H), 4.6 (br 1 H), 4.4 (m, 2 H), 3.0 (m, 1 H), 2.6 (m, 1 H), 2.5 (m, 1 H), 1.7 (m, 5 H), 1.4 (m, 1 H). Anal. ($C_{22}H_{24}N_8O$) C, H, N.

***N*-(1,3-Benzodioxol-5-yl)-1-[2-(1*H*-imidazol-1-yl)pyrimidin-4-yl]-2-piperidineacetamide (12z).** ^{1}H NMR (DMSO-d_6) δ 9.9 (s, 1 H), 8.5 (s, 1 H), 8.15 (d, 1 H), 7.85 (s, 1 H), 7.15 (s, 1 H), 7.05 (m, 1 H), 6.75 (m, 3 H), 5.9 (s, 2 H), 3.1 (m, 1 H), 2.65 (m, 2 H), 1.7 (m, 5 H), 1.4 (m, 1 H). Anal. ($C_{21}H_{28}N_6O_3\cdot H_2O$) C, H, N.

***N*-(3,4-Dimethoxyphenyl)-1-[2-(1*H*-imidazol-1-yl)pyrimidin-4-yl]-2-piperidineacetamide (12aa).** ^{1}H NMR (DMSO-d_6, 100 °C) δ 9.3 (s, 1 H), 8.5 (s, 1 H), 8.15 (d, 1 H), 7.85 (s, 1 H), 7.1 (s, 1 H), 7.0 (m, 2 H), 6.85 (d, 1 H), 6.7 (d, 1 H), 5.2 (s, 1 H), 4.3 (d, 1 H), 3.8 (s, 3 H), 3.75 (s, 3H), 3.1 (m, 1 H), 2.7 (m, 2 H), 1.3−1.8 (m, 6 H). Anal. ($C_{22}H_{26}N_6O_3\cdot 0.2CH_2Cl_2$) C, H, N.

1-[2-(1*H*-Imidazol-1-yl)-4-pyrimidinyl]-*N*-[2-(3,4-dimethoxyphenyl)ethyl]-2-piperidineacetamide (12bb). ^{1}H NMR ($CDCl_3$) δ 8.5 (s, 1 H), 8.1 (d, 1 H), 7.8 (s, 1 H), 7.1 (s, 1 H), 6.8 (s, 1 H), 6.6 (m, 2 H), 6.5 (m, 1 H), 5.95 (br, 1H), 5.0 (br, 1 H), 3.8 (2 s, 6 H), 3.4 (m, 2 H), 2.9 (m, 1 H), 2.65 (m, 2 H), 2.45 (m, 2 H), 1.7 (m, 6 H). Anal. ($C_{24}H_{30}N_6O_3\cdot 0.3H_2O\cdot 0.15C_4H_{10}O$) C, H, N.

1-[2-(1*H*-Imidazol-1-yl)-4-pyrimidinyl]-*N*-[2-(4-methoxyphenyl)ethyl]-2-piperidineacetamide (12cc). ^{1}H NMR ($CDCl_3$) δ 8.5 (s, 1 H), 8.1 (d, 1 H), 7.8 (s, 1 H), 7.1 (s, 1 H), 7.0 (d, 2 H), 6.8 (d, 2 H), 6.5 (br s, 1 H), 6.3 (m, 1 H), 5.0 (br s, 1 H), 3.8 (s, 3 H), 3.4 (m, 2 H), 2.9 (m, 1 H), 2.7 (m, 2 H), 2.4 (m, 3 H), 1.7 (m, 6 H). Anal. ($C_{23}H_{28}N_6O_2\cdot CH_2Cl_2$) C, H, N.

***N*-[(1,3-Benzodioxol-5-yl)methyl]-4-[2-(1*H*-imidazol-1-yl)-4-pyrimidinyl]-3-morpholineacetamide (13).** ^{1}H NMR ($CDCl_3$, 300 MHz) δ 2.45−2.55 (dd, 1H), 2.75−2.90 (dd, 1H), 3.20 (bt, 1H), 3.55−3.80 (m, 2H), 3.85−4.20 (m, 4H), 4.20−4.40 (dd, 2H), 5.90 (d, 2H), 6.10 (bs, 1H), 6.25−6.75 (m, 4H), 7.10 (m, 1H), 7.80 (m, 1H), 8.20 (d, 1H), 8.50 and 8.75 combined (d, 1H). MS *m*/*z* 423.5 $(M + H)^+$.

***N*-[(1,3-Benzodioxol-5-yl)methyl]hexahydro-1-[2-(1*H*-imidazol-1-yl)-4-pyrimidinyl]-1*H*-azepine-2-acetamide (14). 14** was prepared as described above from 1-(dimethylethoxycarbonyl)-hexahydro-1*H*-azepine-2-acetic acid.[19] ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.10−3.00 (m, 11H), 3.20−3.62 (m, 1H), 4.05−4.50 (m, 3H), 5.00−5.30 (m, 1H), 5.80−6.10 (m, 2H), 6.25−6.90 (m, 4H), 7.05 (d, 1H), 7.80 (d, 1H), 8.10 (d, 1H), 8.50 (d, 1H). MS *m*/*z* 435.3 $(M + H)^+$.

***N*-[(1,3-Benzodioxol-5-yl)methyl]-1-[4-(1*H*-imidazol-1-yl)-2-pyrimidinyl]-2-piperidineacetamide (15).** ^{1}H NMR ($CDCl_3$) δ 8.3 (s, 1 H), 8.15 (m, 1 H), 7.6 (s, 1 H), 7.15 (s, 1 H), 6.95 (br, 1 H), 6.6 (m, 3 H), 6.4 (d, 1 H), 5.9 (m, 2 H), 5.25 (m, 1 H), 4.7 (d, 1 H), 4.3 (dd, 1 H), 4.15 (dd, 1 H), 3.0 (t, 1 H), 2.85 (dd, 1 H), 2.55 (dd, 1 H), 1.6−1.8 (m, 5 H), 1.5 (m, 1 H). Anal. ($C_{22}H_{24}N_6O_3$) C, H, N.

***N*-[(1,3-Benzodioxol-5-yl)methyl]-1-[4-(1*H*-imidazol-1-yl)-6-pyrimidinyl]-2-piperidineacetamide (16).** ^{1}H NMR (DMSO-d_6) δ 8.5 (s, 1 H), 8.4 (m, 2 H), 7.95 (s, 1 H), 7.1 (s, 1 H), 7.0 (s, 1 H), 6.7 (m, 2 H), 6.6 (d, 1 H), 5.9 (s, 2 H), 5.1 (br, 1 H), 4.4 (br, 1 H), 4.05 (m, 2 H), 3.03 (t, 1 H), 2.5 (m, 2 H), 1.5−1.8 (m, 5 H), 1.3 (m, 1 H). Anal. ($C_{22}H_{24}N_6O_3\cdot 0.5H_2O$) C, H, N.

***N*-(1,3-Benzodioxol-5-ylmethyl)-1-[3-(1*H*-imidazol-1-yl)phenyl]-2-piperidineacetamide (17).** A mixture of 1-(3-aminophenyl)-imidazole[20] (755 mg, 4.7 mmol), ethyl 7-chloro-3-oxoheptanoate[21] (982 mg, 4.7 mmol), Na_2HPO_4 (667 mg, 4.7 mmol), iodine (60 mg, 0.23 mmol), and 500 mg of powdered 4 Å molecular sieves in 20 mL of dry benzene is refluxed for 5 h. An additional 60 mg of iodine is added, and the reaction mixture is refluxed overnight. After filtration of the reaction mixture, the filtrate is evaporated and the residue is purified by chromatography (silica gel, methylene chloride/methanol, 98:2) to yield 120 mg (8%) of [1-[3-(1*H*-imidazol-1-yl)phenyl]-2-piperedinylidine]ethanoic acid, ethyl ester. ^{1}H NMR ($CDCl_3$) δ 7.85 (s, 1H), 7.05−7.50 (m, 6H), 4.80 (s, 1H), 4.15 (q, 2H), 3.45 (t, 2H), 2.4 (t, 2H), 1.50−1.80 (m, 6H), 1.30 (t, 3H). The ester (120 mg, 0.38 mmol) is dissolved in methanol (20 mL), and 10% Pd/C (60 mg) is added. The mixture is hydrogenated at 1 atm for 60 h. The catalyst is filtered, and the filtrate is evaporated. The residue is partially purified (silica gel, hexane/ethyl acetate, 1:1), and the slightly impure product (24 mg, 0.076 mmol) is stirred in 5% methanolic NaOH overnight. After acidification with methanolic HCl, the contents are evaporated to dryness and the residue is coupled with piperonylamine using 2 equiv of Hunig's base and 1 equiv of HATU in dry DMF. After aqueous workup the residue is purified by chromatography (silica gel, ethyl acetate/methanol, 98:2) to yield 6 mg (19%) of the desired product. ^{1}H NMR ($CDCl_3$) δ 7.80 (s, 1H), 7.10−7.30 (m, 4H), 6.50−6.70 (m, 5H), 5.90 (s, 2H), 4.20−4.35 (m, 2H), 3.85 (bs, 1H), 3.50 (t, 2H), 2.95 (d, 1 H), 2.35−2.55 (m, 2H), 1.40−1.90 (m, 6H). MS *m*/*z* 419 $(M + H)^+$.

***N*-(1,3-Benzodioxol-5-ylmethyl)-1-[6-chloro-2-(1*H*-imidazol-1-yl)-4-pyrimidinyl]-2-piperidineacetamide (18a).** ^{1}H NMR (DMSO-d_6) δ 8.45 (s, 1 H), 8.4 (br, 1H), 7.85 (s, 1 H), 7.05 (s, 1 H), 6.85 (br, 1 H), 6.6 (m, 3 H), 5.9 (s, 2 H), 4−4.6 (m, 4H), 3.1 (m, 1 H), 2.56 (m, 2 H), 1.3−1.7 (m, 6 H). Anal. ($C_{22}H_{23}N_6O_3Cl$) C, H, N, Cl.

***N*-(1,3-Benzodioxol-5-ylmethyl)-1-[2-(1*H*-imidazol-1-yl)-6-phenyl-4-pyrimidinyl]-2-piperidineacetamide (18b).** ^{1}H NMR (DMSO-d_6) δ 8.5 (s, 1 H), 8.4 (s, 1H), 8.2 (d, 1 H), 7.95 (d, 2 H), 7.47 (m, 2 H), 7.2 (s, 1 H), 7.05 (s, 1 H), 6.5−6.8 (m, 3 H), 5.9 (s, 2 H), 4−4.2 (m, 4H), 3.1 (m, 1 H), 2.56 (m, 2 H), 1.3−1.7 (m, 6 H). Anal. ($C_{28}H_{28}N_6O_3\cdot 0.5C_3H_7NO\cdot 0.05C_3H_4N_2$) C, H, N.

***N*-(1,3-Benzodioxol-5-ylmethyl)-1-[2-(1*H*-imidazol-1-yl)-6-methyl-4-pyrimidinyl]-2-piperidineacetamide (18c).** ^{1}H NMR (DMSO-d_6) δ 9.6 (brd, 1 H), 8.5 (s, 1H), 8.25 (d, 1 H), 7.7 (m, 2 H), 6.5−6.8 (m, 3 H), 5.9 (s, 2 H), 4−4.2 (m, 4H), 3.1 (m, 1 H), 2.6 (m, 2 H), 2.2 (s, 3H), 1.3−1.7 (m, 6 H). Anal. ($C_{23}H_{26}N_6O_3\cdot 0.8H_2O\cdot 2.4C_2HF_3O_2$) C, H, N.

***N*-(1,3-Benzodioxol-5-ylmethyl)-1-[2-(1*H*-imidazol-1-yl)-6-(trifluoromethyl)-4-pyrimidinyl]-2-piperidineacetamide (18d).** ^{1}H NMR (DMSO-d_6) δ 9.4 (brd, 1 H), 8.5 (brd, 1H), 8.3 (s, 1 H), 7.5 (m, 2 H), 6.5−6.8 (m, 3 H), 5.9 (s, 2 H), 5.36 (s, 1H), 4.61 (m,

1H), 4.02 (m, 1H), 3.1 (m, 1H), 1.3−1.7 (m, 6 H). Anal. ($C_{23}H_{23}F_3N_6O_3 \cdot 0.5H_2O \cdot 1.5C_2HF_3O_2$) C, H, N, F.

***N*-(1,3-Benzodioxol-5-ylmethyl)-1-[2,6-di(1*H*-imidazol-1-yl)-4-pyrimidinyl]-2-piperidineacetamide (18e).** ^{1}H NMR (DMSO-d_6) δ 10.3 (br, 1 H), 9.9 (s, 1H), 8.78 (s, 1 H), 8.63 (m, 3 H), 7.95 (m, 2H), 7.41 (d, 1H), 6.5 (m, 2 H), 5.8 (s, 2H), 4.9 (m, 1H), 4.03 (m, 3H), 3.5 (t, 1H), 3.1 (m, 1H), 1.3−1.7 (m, 6 H). Anal. ($C_{25}H_{26}N_8O_3 \cdot 1.2H_2O \cdot 3C_2HF_3O_2$) C, H, N, F.

***N*-[(1,3-Benzodioxol-5-yl)methyl]-4-[2-(1*H*-imidazol-1-yl)-6-methylpyrimidin-4-yl]-2-piperazineacetamide (19a).** ^{1}H NMR ($CDCl_3$) δ 8.55 (s, 1 H), 7.8 (s, 1 H), 7.1 (s, 1 H), 6.75 (m, 4 H), 6.25 (s, 1 H), 5.95 (s, 2 H), 4.4 (m, 2 H), 4.35 (m, 2 H), 3.2 (m, 3 H), 2.85 (m, 2 H), 2.4 (m, 3 H), 2.4 (s, 3 H). Anal. ($C_{22}H_{25}N_7O_3 \cdot 0.1CH_2Cl_2 \cdot 0.8H_2O$) C, H, N.

***N*-[(1,3-Benzodioxol-5-yl)methyl]-4-[2-(1*H*-imidazol-1-yl)-6-methylpyrimidin-4-yl]-1-methyl-2-piperazineacetamide (19b).** ^{1}H NMR ($CDCl_3$) δ 8.5 (s, 1 H), 7.8 (s, 1 H), 7.7 (m, 1 H), 7.05 (s, 1 H), 6.75 (m, 3 H), 6.2 (s, 1 H), 5.9 (s, 2 H), 4.35 (m, 2 H), 3.9−4.2 (m, 2 H), 3.25 (m, 2 H), 2.85 (d, 1 H), 2.4−2.7 (m, 4 H), 2.4 (2 s, 6 H). Anal. ($C_{23}H_{27}N_7O_3 \cdot 0.2H_2O$) C, H, N.

***N*-[(1,3-Benzodioxol-5-yl)methyl]-4-[2-(1*H*-imidazol-1-yl)-6-methylpyrimidin-4-yl]-1-(phenylmethyl)-2-piperazineacetamide (19c).** ^{1}H NMR ($CDCl_3$) δ 8.5 (s, 1 H), 7.8 (s, 1 H), 7.2 (m, 6 H), 7.1 (s, 1 H), 6.75 (m, 3 H), 6.22 (s, 1 H), 5.97 (s, 2 H), 4.4 (dd, 1 H), 4.3 (dd, 1 H), 3.95 (d, 1 H), 3.8 (m, 3 H), 3.6 (m, 1 H), 3.45 (d, 1 H), 3.15 (m, 1 H), 2.75 (m, 1 H), 2.4−2.7 (m, 3 H), 2.4 (s, 3 H). Anal. ($C_{29}H_{31}N_7O_3 \cdot 0.1CH_2Cl_2 \cdot 0.4H_2O$) C, H, N.

***N*-(1,3-Benzodioxol-5-ylmethyl)-4-[2-(1*H*-imidazol-1-yl)-6-methyl-4-pyrimidinyl]-1-(methylsulfonyl)-2-piperazineacetamide (19d).** ^{1}H NMR (DMSO-d_6) δ 9.7 (s, 1H), 8.4 (m, 2 H), 7.8 (s, 1H), 6.6 (m, 3H), 5.95 (s, 2H), 4.45 (m, 4H), 4.3 (m, 3H), 3.62 (m, 2H), 3.1 (m, 1H), 3 (s, 3H), 2.45 (m, 3H), 2.1 (s, 3H). Anal. ($C_{23}H_{27}N_7O_5S \cdot 0.5H_2O \cdot 2.6C_2HF_3O_2$) C, H, N.

***N*-[(1,3-Benzodioxol-5-yl)methyl]-4-[2-(1*H*-imidazol-1-yl)-6-methylpyrimidin-4-yl]-1-(methylcarbonyl)-2-piperazineacetamide (19e).** ^{1}H NMR (DMSO-d_6, 100 °C) δ 8.45 (s, 1 H), 7.85 (m, 1 H), 7.8 (s, 1 H), 7.05 (s, 1 H), 6.7 (m, 3 H), 6.55 (s, 1 H), 5.95 (s, 2 H), 4.7 (m, 1H), 4.0−4.5 (m, 5 H), 3.35 (m, 1 H), 3.15 (m, 1 H), 2.4 (s, 3 H), 2.1 (s, 3 H). Anal. ($C_{26}H_{31}N_7O_4 \cdot 0.2CH_2Cl_2 \cdot 0.1H_2O$) C, H, N.

***N*-[(1,3-Benzodioxol-5-yl)methyl]-4-[2-(1*H*-imidazol-1-yl)-6-methylpyrimidin-4-yl]-1-[(1-methylethyl)carbonyl]-2-piperazineacetamide (19f).** ^{1}H NMR (DMSO-d_6, 100 °C) δ 8.45 (s, 1 H), 7.85 (m, 2 H), 7.05 (s, 1 H), 6.7 (m, 3 H), 6.55 (s, 1 H), 5.95 (s, 2 H), 4.8 (m, 1H), 4.45 (d, 1 H), 4.3 (d, 1H), 4.15 (m, 3 H), 3.35 (dd, 1 H), 3.2 (m, 2 H), 2.9 (m, 1 H), 2.55 (m, 1 H), 2.4 (m, 1 H), 2.35 (s, 3 H), 1.05 (2 s, 6 H). Anal. ($C_{26}H_{31}N_7O_4$) C, H, N.

2-[2-[(1,3-Benzodioxol-5-ylmethyl)amino]-2-oxoethyl]-4-[2-(1*H*-imidazol-1-yl)-6-methyl-4-pyrimidinyl]-1-piperazinecarboxylic Acid, Methyl Ester (19g). ^{1}H NMR (DMSO-d_6) δ 9.4 (s, 1H), 8.2 (m, 2 H), 7.6 (s, 1H), 6.7 (m, 3H), 5.95 (s, 2H), 4.6 (m, 2H), 3.9−4.41 (m, 4H), 3.62 (s, 3H), 3.1−3.4 (m, 3H), 2.6 (m, 4H), 2.3 (s, 3H). Anal. ($C_{24}H_{27}N_7O_5 \cdot 0.5H_2O \cdot 1.4C_2HF_3O_2$) C, H, N.

***N*-[(1,3-Benzodioxol-5-yl)methyl]-1-[2-(1*H*-imidazol-1-yl)-4-pyrimidinyl]-2-pyrrolidineacetamide (20a).** ^{1}H NMR ($CDCl_3$) δ 8.5 (s, 1 H), 8.1 (d, 1 H), 7.8 (s, 1 H), 7.0 (s, 1 H), 6.7 (m, 3 H), 6.1 (m, 4 H), 4.7 (br s, 1 H), 4.3 (m, 2 H), 3.4 (m, 2 H), 2.8 (br s, 1 H), 2.3 (br s, 1 H), 2.1 (m, 4 H). Anal. ($C_{21}H_{22}N_6O_3 \cdot CH_2Cl_2$) C, H, N.

***N*-[(1,3-Benzodioxol-5-yl)methyl]-1-[2-(1*H*-imidazol-1-yl)-6-methyl-4-pyrimidinyl]-2-pyrrolidineacetamide (20b).** ^{1}H NMR ($CDCl_3$) δ 8.4 (m, 1 H), 7.7 (m, 1 H), 7.0 (s, 1 H), 6.7 (m, 3 H), 6.0 (m, 4 H), 4.6 (br s, 1 H), 4.3 (m, 2 H), 3.4 (m, 2 H), 2.8 (br s, 1 H), 2.2 (m, 8 H). Anal. ($C_{22}H_{24}N_6O_3 \cdot CH_2Cl_2$) C, H, N.

***N*-[(1,3-Benzodioxol-5-yl)methyl]-1-[6-ethyl-2-(1*H*-imidazol-1-yl)-4-pyrimidinyl]-2-pyrrolidineacetamide (20c).** ^{1}H NMR ($CDCl_3$) δ 8.6 (s, 1 H), 7.8 (s, 1 H), 7.0 (s, 1 H), 6.7 (m, 3 H), 6.1 (m, 1 H), 5.9 (s, 2 H), 5.8 (m, 1 H), 4.7 (br s, 1 H), 4.4 (dd, 1 H), 4.3 (m, 1 H), 3.4 (m, 2 H), 2.8 (br s, 1 H), 2.6 (q, 2H), 2.4 (br s, 1 H), 2.1 (m, 4 H), 1.3 (t, 3H). Anal. ($C_{23}H_{26}N_6O_3 \cdot CH_2Cl_2$) C, H, N.

***N*-[2-(1,3-Benzodioxol-5-yl)ethyl]-1-[2-(1*H*-imidazol-1-yl)-4-pyrimidinyl]-2-pyrrolidinecarboxamide (21a).** ^{1}H NMR ($CDCl_3$) δ 8.4 (s, 1 H), 8.2 (d, 1 H), 7.7 (br s, 1 H), 7.1 (s, 1 H), 6.4 (m, 5 H), 5.8 (m, 2 H), 4.6 (br s, 1 H), 3.4 (m, 4 H), 2.8 (m, 2 H), 2.1 (m, 4 H). Anal. ($C_{21}H_{22}N_6O_3$) C, H, N.

***N*-[2-(1,3-Benzodioxol-5-yl)ethyl]-1-[2-(1*H*-imidazol-1-yl)-6-methyl-4-pyrimidinyl]-2-pyrrolidinecarboxamide (21b).** ^{1}H NMR ($CDCl_3$) δ 8.5 (s, 1 H), 7.7 (s, 1 H), 7.1 (s, 1 H), 6.5 (m, 4 H), 6.1 (br s, 1 H), 5.8 (m, 2 H), 4.6 (br s, 1 H), 3.5 (m, 4 H), 2.6 (m, 2 H), 2.4 (m, 4H), 2.1 (br s, 2 H), 1.3 (s, 1 H). Anal. ($C_{22}H_{24}N_6O_3$) C, H, N.

***N*-[(1,3-Benzodioxol-5-yl)ethyl]-4-[2-(1*H*-imidazol-1-yl)-6-ethylpyrimidin-4-yl]-2-pyrrolidinecarboxamide (21c).** ^{1}H NMR ($CDCl_3$) δ 8.5 (s, 1 H), 7.8 (s, 1 H), 7.1 (s, 1 H), 6.5 (m, 4 H), 6.1 (br s, 1 H), 5.8 (m, 2 H), 4.6 (br s, 1 H), 3.5 (m, 4 H), 2.6 (m, 4 H), 2.4 (br s, 1H), 2.1 (br s, 3 H), 1.3 (t, 3H). Anal. ($C_{23}H_{26}N_6O_3$) C, H, N.

Optically Active *N*-[(1,3-Benzodioxol-5-yl)methyl]-1-[2-(1*H*-imidazol-1-yl)pyrimidin-4-yl]-4-[(methoxy)carbonyl]-2-piperazineacetamide (11d*). *N*-[(1,3-Benzodioxol-5-yl)methyl-4-[(methoxy)carbonyl]-1-[(dimethylethoxy)carbonyl)]piperazine-2-acetamide was separated on a Chiralpak AD column (Chiral Technologies Inc.) using hexanes/isopropanol (4:1). After separation, the two enantiomers were treated identically by manners previously described to give the final compounds. Initial treatment with trifluoroacetic acid deprotected the piperazine. The piperazine was added to 4-chloro-2-methylsulfonylpyrimidine, and subsequent reaction with imidazole gave the title compound. The earlier eluting enantiomer resulted in the more potent final product, which was identified as the *S*-enantiomer by analysis of the crystal structure.

Assay To Determine Potency Inhibiting NO Formation. A172 cells were obtained from the American Type Culture Collection and were cultured routinely in DMEM without phenol red or sodium pyruvate but containing high glucose (Gibco BRL), supplemented with 10% (v/v) fetal bovine serum (Gibco BRL), in a humidified atmosphere of 5% CO_2 in air at 37 °C. Cells were harvested and plated at 100 000 cells/well into 96-well tissue culture dishes in a total of 100 μL of culture medium. After 18−24 h, inducible nitric oxide synthase (iNOS) activity was induced by the addition of 222 U/mL human interferon-γ, 22 ng/mL of human tumor necrosis factor-α, and 2.2 ng/mL of human interleukin 1-β. All cytokines were purchased from Boehringer Mannheim. Concomitant with cytokine addition, the appropriate concentration of the compound was also added. Compound stock solutions were prepared in DMSO, and vehicle was added to control wells. The final concentration of DMSO in the incubations was less than 0.2% and had no influence on iNOS induction or activity measurements. Incubations were continued for 18−24 h, at which time an aliquot of the culture medium was removed and tested for nitrite concentration. A 100 μL aliquot of the culture medium was removed and mixed with 150 μL of the Griess reagent (5% v/v phosphoric acid containing 2% w/v sulfanilamide plus 0.2% w/v naphthylethylenediamine) in a separate 96-well plate. The plates were read within 15 min at 550 nm in a SpectraMax spectrophotometer. The inhibition of NO formation by compound resulted in a decrease in the OD_{550} of the medium. IC_{50} values were calculated from a log−logit analysis of the data. Inhibition curves with Hill slopes of less than 0.5 or greater than 1.5 were rejected. Control experiments showed that no significant conversion of nitrite to nitrate occurred over the course of an experiment. Cells incubated in the absence of cytokines produced no measurable nitrite.

Assays To Determine Selectivity. BSC-1 cells (American Type Culture Collection) were maintained in DMEM containing 10% fetal bovine serum. Cells were plated into 96-well tissue culture plates at 30 000 cells per well. After 6 h, recombinant vaccinia virus strains encoding human inducible, endothelial, and neuronal NOS isoforms were added, along with inhibitor or vehicle. After 17 h, the medium was aspirated and 20 μL of 40 mM Tris, pH 7.5,

containing 0.1% NP-40, 5 mg/mL aprotinin, 1 μg/mL leupeptin, 1 μg/mL pepstatin, and 24 μg/mL of Pefabloc SC was added. The plates were then frozen at −80 °C. NOS activity in cell lysates was determined by measuring the conversion of arginine into citrulline. The assay mixture contained 40 mM Tris, pH 7.5, 3 mM dithiothreitol (DTT), 4 μM each of H_4B, FAD, and FMN, 0.5 μM calmodulin, 15 mM calcium chloride, 1 mM NADPH, and 3 μM [^{14}C]arginine (300 Ci/mol). NOS activity in Superdex 200 fractions was measured using the same protocol except with 4 μM [^{14}C]-arginine and 16 μM unlabeled arginine in the assay mixture.

Rat Adjuvant Arthritis Model.[22,23] Adult male Lewis rats were injected with 0.1 mL of *Mycobacterium butyricum* in incomplete Freund's adjuvant (10 mg/mL, intradermally) into the proximal quarter of the tail. Drug treatment started on the day following immunization. Compound (30, 10, and 3 mg/kg) or vehicle was administered subcutaneously twice daily (12 h intervals). Naive rats received no treatment. Rats were weighed and observed for clinical signs of arthritis 3 times per week. All rats were sacrificed 34−35 days after immunization. Each dosing group consisted of 10 animals. Clinical scoring was done on a scale of 0−4 for each limb, with increasing degrees of redness, gross swelling and distortion of the paw, and joint fusion. The sum of these scores for each limb was totaled and was designated as the clinical score. Radiological (X-ray) scoring was done by grading both hind limbs of the sacrificed animals on a scale of 0−3 for each of the following parameters: soft tissue swelling, cartilage loss, erosion, and heterotopic ossification. The sum of these scores for each limb was totaled and was designated as the radiological score.

Supporting Information Available: Results from elemental analysis. This material is available free of charge via the Internet at http://pubs.acs.org.

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JM061319I