1-Substituted 4-Aryl-5-pyridinylimidazoles: A New Class of Cytokine Suppressive Drugs with Low 5-Lipoxygenase and Cyclooxygenase Inhibitory Potency[#]

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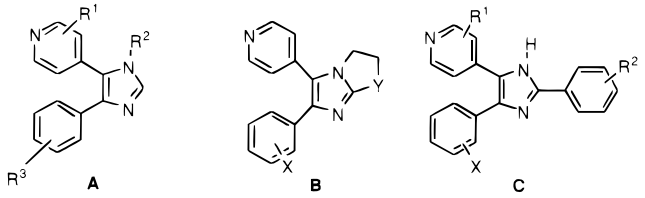
A series of 1-alkyl- or -aryl-4-aryl-5-pyridinylimidazoles (A) were prepared and tested for their ability to bind to a recently discovered protein kinase termed CSBP and to inhibit lipopolysaccharide (LPS)-stimulated TNF production in mice. The kinase, CSBP, appears to be involved in a signaling cascade initiated by a number of inflammatory stimuli and leading to the biosynthesis of the inflammatory cytokines IL-1 and TNF. Two related imidazole classes (B and C) had previously been reported to bind to CSBP and to inhibit LPS-stimulated human monocyte IL-1 and TNF production. The members of the earlier series exhibited varying degrees of potency as inhibitors of the enzymes of arachidonic acid metabolism, PGHS-1 and 5-LO. Several of the more potent CSBP ligands and TNF biosynthesis inhibitors among the present series of N-1-alkylated imidazoles (A) were tested as inhibitors of PGHS-1 and 5-LO and were found to be weak to inactive as inhibitors of these enzymes. One of the compounds, **9** (SB 210313) which lacked measureable activity as an inhibitor of the enzymes of arachidonate metabolism, and had good potency in the binding and *in vivo* TNF inhibition assays, was tested for antiarthritic activity in the AA rat model of arthritis. Compound **9** significantly reduced edema and increased bone mineral density in this model.

The NSAID class of antiinflammatory agents, acting through the inhibition of arachidonate metabolism, have become the cornerstone of modern arthritis therapy. Unfortunately these drugs are limited by toxicity and treat only the symptoms of pain and inflammation with no effect on the underlying disease process. Since the toxicity of the NSAIDs is associated with their mechanism of action, it may be difficult to dissociate efficacy from toxicity.[1] New classes of antiarthritic agents, acting through novel mechanisms, are needed to separate potent antiinflammatory effects from the adverse effects associated with the NSAIDs and to treat the disease process rather than simply ameliorating the symptoms of inflammation.

With that goal in mind our group has recently described a novel approach toward the treatment of inflammation by the disruption of the signaling pathway initiated by a variety of inflammatory stimuli.[4] Certain 4-aryl-5-pyridinylimidazoles were shown to bind to and inhibit a Ser/Thr protein kinase homologous to the MAP kinases. By photoaffinity labeling this protein with a radiolabeled 4-aryl-5-pyridinylimidazole ligand, the kinase was identified and partially sequenced. The sequence data then allowed for the cloning and sequencing of the full-length kinase, which was found to be the human homolog of the murine protein kinase p38.[5] The affinity of the 4-aryl-5-pyridinylimidazoles for the recombinant kinase correlated with the ability of the ligands to inhibit lipopolysaccharide (LPS)-induced synthesis of IL-1 in human monocytes. Furthermore, the inhibition of kinase activity in intact cells was correlated with the inhibition of cytokine biosynthesis.[4] Since the 4-aryl-5-pyridylimidazoles suppressed biosynthesis of inflammatory cytokines, they were termed CSAID drugs. The target kinase was thus named the CSAID binding protein (CSBP).

Chart 1. Structural Classes of Imidazole CSBP Ligands



The initial structural leads with which we demonstrated the inhibition of TNF and IL-1 biosynthesis in human monocytes were bicyclic imidazoles such as SK&F 86002 (Chart 1, B, Y = S, X = 4-F).[6,7] These compounds were reported to have an antiinflammatory effect in an adjuvant arthritic rat assay. Subsequently a generally more potent series of related analogs containing a third aryl group at the imidazole C-2 (C) demonstrated the ability to inhibit IL-1 production in intact human monocytes[8] and to be an effective inhibitor of disease severity in the collagen-induced model of arthritis in the mouse. Members of the triarylimidazole class (C) have also demonstrated activity in the adju-

[#] CSAID is a trademark of SmithKline Beecham.

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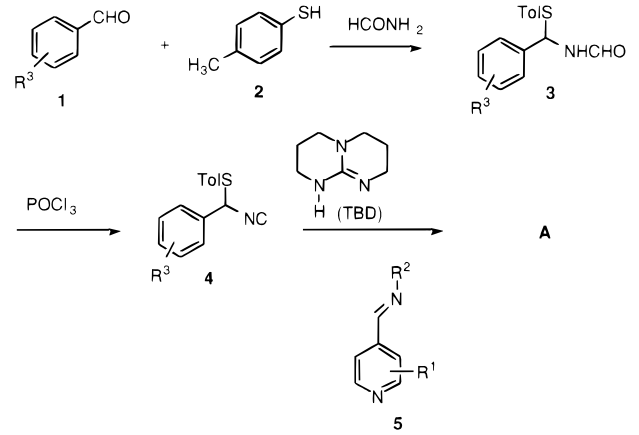
Scheme 1. Regioselective Syntheses of 1,4,5-Substituted Imidazole CSBP Ligands (Method A)



vant arthritis (AA) model in rats.[9] In addition to the ability of the imidazoles of general structures B and C to inhibit the biosynthesis of TNF and IL-1, they were also inhibitors of arachidonic acid metabolism.[7] However, no correlation was observed between suppression of IL-1 synthesis and 5-LO inhibition.[7,8]

We report here an additional class of CSBP ligands composed of 1-alkyl- or 1-aryl-4-aryl-5-pyridinylimidazoles (A). Selected members of this class of CSBP ligands have improved potencies compared to SK&F 86002 for binding to CSBP and as inhibitors of LPS-induced TNF production in the mouse. Improvements in potency in the later assay by the incorporation of structural changes which are expected to increase the metabolic stability of some of the inhibitors are reported. Several of the more potent N-1-substituted CSBP ligands are shown to be inactive as inhibitors of PGHS-1 and 5-LO. Antiinflammatory activity in the AA rat arthritis model is reported for one of these inhibitors of cytokine biosynthesis, thus supporting the proposal that modulation of inflammatory cytokine biosynthesis, through inhibition of CSBP kinase activity, produces an antiinflammatory effect which does not require direct inhibition of arachidonate metabolism.

Chemistry

The regioselective synthesis of the 1,4,5-substituted imidazoles utilized van Leusen's methodology for 1,3-dipolar cycloadditions of the anion of tosylmethyl or mercaptomethyl isocyanides to imines.[10] Since we encountered some difficulties with our initial attempt to prepare the aryl-substituted tosylmethyl isocyanides, we chose to use the less acidic tolyl sulfide isocyanide **4**. The general approach is depicted in Scheme 1 (method A). Of note is the need for a strong amine base to deprotonate the isonitrile in the cyclization step. Commercially available 1,5,7-triazabicyclo[4.4.0]dec-5-ene (TBD) met this need, and with this base cycloadditions could be effected in CH_2Cl_2 at 4 °C. The isonitrile was stable at −20 °C for months and was used as needed to regioselectively prepare a diverse group of 1,4,5-substituted imidazoles (Tables 1−3). The convergent nature of the route depicted in Scheme 1 permitted the rapid formation of numerous highly substituted imidazoles from readily available amines and aldehydes.

Several compounds were prepared from the 1-(chloropropyl)imidazole **6** by displacement of Cl with the desired nucleophile (Scheme 2). Compound **6** was prepared by method A. Compounds **12**−**16**, **18**, and **19** were prepared by direct displacement with the appropriate amine or alcohol in the presence of catalytic NaI (method B). Likewise the primary amine **17** was prepared by displacement with azide in the presence of NaI and reduction with LAH (method C). The sulfides **20** and **23** were prepared from the corresponding thiol in the same manner except that the catalytic NaI was omitted (method D).

All the sulfoxides reported were prepared by chemoselective oxidation of the sulfides with $K_2S_2O_8$ in H_2O/HOAc (method E). The sulfones were prepared from the sulfides with a slight excess of *m*-chloroperoxybenzoic acid in THF containing 2 equiv of TFA to minimize oxidation of the pyridyl nitrogen (method F). The carboxylic acid **27** was obtained by LiOH hydrolysis of the methyl ester (method G) which was itself prepared by method A using commercially available 3-carbomethoxypropylamine as the amine precursor to imine **5**.

A recently reported modification of van Leusen's methodology has been used to prepare 4,5-disubstituted imidazoles.[11] We applied this procedure toward the synthesis of **28** (Scheme 3) (method H). In this case the sulfone **8** rather than the sulfide isonitrile **4** was utilized.[12]

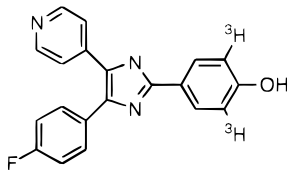
The aldehyde precursors to **41** and **42** (Table 2) were obtained by DIBAL reduction of the known 2-methyl- and 2,6-dimethyl-4-cyanopyridines.[13] The aldehyde precursor to **44** was prepared as reported.[14] Compound **44** served as starting material for the synthesis of **45** by displacement of the pyridyl chloride with hydrazine and subsequent hydrogenation over Raney nickel to afford the amine (method I).

Compounds **46**−**52** (Table 3) were prepared via formation of the required isonitrile from the appropriate aldehyde followed by imidazole formation by method A.

Synthesis of the triarylimidazoles (**55**−**57**, Table 4) has previously been reported.[8]

Biological Results

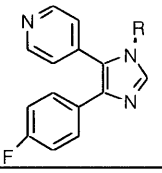
CSBP Binding. The *in vitro* binding assay utilizes a preparation of CSBP in THP-1 cytosol in which a radiolabeled triaryl CSBP ligand ([^{3}H]SB 202190) is displaced from the protein by unlabeled analogs.[4] (See the Experimental Section.)



^{3}H-**SB 202190**

At the time we initiated the study of this new class of CSBP ligands, an extensive series of compounds of class B and C (Chart 1) had already been evaluated as antiinflammatory agents, as inhibitors of IL-1 biosynthesis in human whole blood, and in the CSBP binding assay.[4,6,7,8] The potency of members of the earlier series as antiinflammatory agents and inhibitors of IL-1 biosythesis had correlated with CSBP binding potency. As a first hypothesis we assumed that the mode of binding for the new CSBP ligands would be similar to

Table 1. Synthesis, Yields, Physical Properties, and Initial Screening of CSBP Ligands from Class A: Imidazole N-1 Analogs

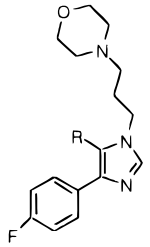


cmpd	R	method	mp	formula [a]	CSBP binding IC_{50} (uM)	% change in mouse TNF @ 50 mg/Kg
9	$-(CH_2)_3-N$ O	A	149 - 150	$C_{21}H_{23}FN_4O \cdot 1/4\ H_2O$	0.12	-55***
10	$-(CH_2)_2-N$ O	A	114 - 115	$C_{20}H_{21}FN_4O$	0.76	-25*
11	$-(CH_2)_4-N$ O	A	103 - 104	$C_{22}H_{25}FN_4O$	0.48	-40***
12	$-(CH_2)_3-N(Bn)(CH_3)$	B	90 - 91	$C_{25}H_{25}FN_4$	0.13	-28**
13	$-(CH_2)_3-N(Bn)(H)$	B	125 - 126	$C_{14}H_{23}FN_4 \cdot 1/10\ H_2O$	0.07	-43**
14	$-(CH_2)_3-N$	B	105 - 108	$C_{21}H_{25}FN_4 \cdot 1/5\ H_2O$	1.17	-26 NS
15	$-(CH_2)_3-N$	B	105 - 107	$C_{22}H_{23}FN_4 \cdot 1/4\ H_2O$	0.34	-17 NS
16	$-(CH_2)_3NEt_2$	B	94 - 95	$C_{21}H_{25}FN_4 \cdot 1/20\ H_2O$	0.27	-21 NS
17	$-(CH_2)_3NH_2$	C	81 - 82 (free base)	$C_{17}H_{17}FN_4 \cdot 3\ 1/4\ H_2O \cdot 2\ 1/2$ HCl [d]	0.15	nt
18	$-(CH_2)_3OEt$	B	85 - 85	$C_{19}H_{20}FN_3O$ [c]	0.10	-29***
19	$-(CH_2)_3OPh$	B	95 - 96	$C_{23}H_{20}FN_3O$ [c]	0.07	-14 NS
20	$-(CH_2)_3SCH_3$	D	85 - 86	$C_{18}H_{18}FN_3S$	0.19	-49
21	$-(CH_2)_3SOCH_3$	E	122 - 123	$C_{18}H_{18}FN_3OS$	1.40	-43
22	$-(CH_2)_3SO_2CH_3$	F	146 - 147	$C_{18}H_{18}FN_3O_2S$	0.51	nt
23	$-(CH_2)_3SPh$	D	98 - 99	$C_{23}H_{20}FN_3S$ [c]	0.08	-42***
24	$-(CH_2)_3SOPh$	E	146-148	$C_{23}H_{20}FN_3OS \cdot 1/4\ H_2O$	0.63	nt
25	$-(CH_2)_3SO_2Ph$	F	109 - 110	$C_{23}H_{20}FN_3SO_2$ [c]	1.81	-42***
26	$-(CH_2)_3CO_2CH_3$	A	69 - 70	$C_{19}H_{18}FN_3O_2 \cdot H_2O$	0.29	-33
27	$-(CH_2)_3CO_2H$	G	-	$C_{18}H_{15}FN_3O_2$ [c]	3.83	-47***
28	H	H	245 -246 (dec)	$C_{14}H_{10}FN_3 \cdot 1/10\ H_2O$	0.21	-82***
29	4-CH_3SPh-	A	172 - 173	$C_{21}H_{16}FN_3S \cdot 2/5\ H_2O$ [b]	1.47	-20*
30	4-CH_3SOPh-	E	202 - 203	$C_{21}H_{16}FN_3OS \cdot 1/4\ H_2O$	0.75	nt
31	3-CH_3SPh-	A	105 - 106	$C_{21}H_{16}FN_3S$	0.12	-23*
32	3-CH_3SOPh-	E	118 - 119	$C_{21}H_{16}FN_3OS$	0.52	-20**
33	2-CH_3SPh-	A	137 - 138	$C_{21}H_{16}FN_3S$	0.08	+29
34	2-CH_3SOPh-	E	165 - 166	$C_{21}H_{16}FN_3OS$	0.71	+40
35	i-Pr	A	59 - 60	$C_{17}H_{16}FN_3$	0.12	-56***
36	cyclo-Pr	A	129.0 - 129.5	$C_{17}H_{14}FN_3$	0.08	-79***
37	CH_2-cyclo-Pr	A	162.0 - 162.5	$C_{18}H_{16}FN_3$	0.09	-65***
38	t-Butyl	A	199 - 200	$C_{18}H_{16}FN_3$	4.74	-35

[a] C,H, N analysis within 0.4 of calculated except where noted. [b] C,H analysis within 0.4 of calculated; N analysis within 0.6 of calculated. [c] HRMS. [d] Cl analysis Calcd.: 25.05. Found: 24.71. *** statistically significant from controls at $p < 0.001$. ** statistically significant from controls at $p < 0.01$. * statisticaly significant from controls at $p < 0.05$. NS not statistically significant.

that of the earlier analogs and that the previous SAR would be useful in guiding our choice of new compounds. On this basis we designed the new analogs around the 4-aryl-5-(4-pyridyl)imidazole scaffolding which had been determined to be the core pharmacophore in the earlier CSBP ligands.

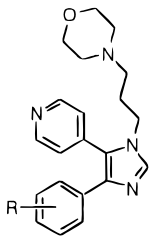
Table 2. Synthesis, Yields, Physical Properties, and Initial Screening of CSBP Ligands from Class A: Imidazole C-5 Analogs



compd	R	method	mp	formula[a]	CSBP binding, IC_{50} (μM)	% change in mouse TNF at 50 mg/kg
39	3-pyridyl	A	88.0−88.5	$C_{21}H_{23}FN_4O$	64.7	nt
40	2-pyridyl	A	90.0−90.5	$C_{21}H_{23}FN_4O$	64.6	nt
41	2-methyl-4-pyridyl	A	116−117	$C_{22}H_{25}FN_4O$	3.05	−37***
42	2,6-dimethyl-4-pyridyl	A	127−128	$C_{23}H_{27}FN_4O$	25.0	nt
43	4-quinoyl	A	139−140	$C_{25}H_{25}FN_4O$	9.0	−19*
44	2-chloro-4-pyridyl	A	97.0−97.5	$C_{21}H_{22}ClFN_4O$	1.89	nt
45	2-amino-4-pyridyl	I	186−187	$C_{21}H_{24}FN_4O\cdot{}^7/_{20}H_2O$[b]	1.18	−38

[a,b] See footnotes for Table 1.

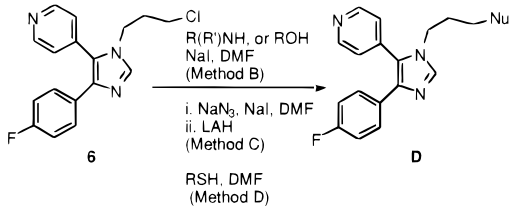
Table 3. Synthesis, Yields, Physical Properties, and Initial Screening of CSBP Ligands from Class A: Imidazole C-4 Analogs



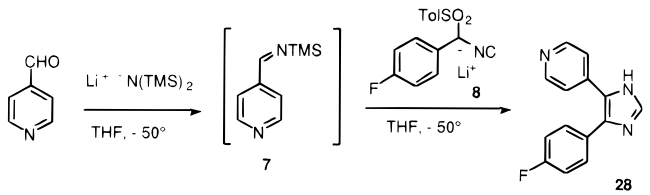
compd	R	method	mp (°C)	formula[a]	CSBP binding, IC_{50} (μM)	% change in mouse TNF at 50 mg/kg
46	3-chloro	A		$C_{21}H_{23}ClN_4O$[c]	0.21	−49**
47	3-methylthio	A	105−106	$C_{22}H_{26}N_4OS$	0.29	−10 NS
48	3,4-dichloro	A	101−102	$C_{21}H_{22}Cl_2N_4O$	0.32	−73***
49	3-trifluoromethyl	A		$C_{21}H_{23}F_3N_4O$[c]	0.46	nt
50	4-trifluoromethyl	A	133−134	$C_{21}H_{23}F_3N_4O$[c]	2.98	nt
51	3-methylsulfinyl	A	118−119	$C_{22}H_{26}N_4O_2S$	4.95	nt
52	3,5-bis(trifluoromethyl)	A	138−139	$C_{23}H_{22}F_6N_4O$	114	nt

[a,c] See corresponding footnote for Table 1.

Scheme 2. Synthesis of N-1-Alkylated Imidazole CSBP Ligands by Displacement

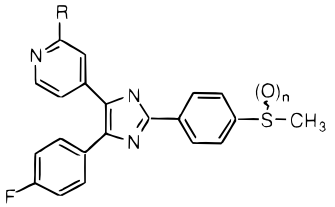


Scheme 3. Synthesis of the N-1 Protio Analog **28** (Method H)



The CSBP binding results for a variety of N-1-substituted CSAIDs based on this core structure are depicted in Table 1. The early lead, SK&F 86002 (B, Y = S, X = 4-F; CSBP binding IC_{50} = 0.40 μM) is used as a reference for comparison of the relative *in vitro* potencies of the new analogs. In series A the results show that acidic functionality is deleterious toward

Table 4. Triaryl CSBP Ligands; CSBP Binding and Murine TNF Inhibition



compd	R	*n*	CSBP binding, IC_{50} (μM)	% change in mouse TNF at 50 mg/kg
55	H	1	0.042	−83***
56	CH_3	1	0.37	−64***
57	H	0	0.34	−63***

binding (compare **27** and **26**). Also, generally, there is a loss in potency for the polar though neutral sulfoxides (**21**, **24**, **32**, and **34**) when compared to the related sulfides (**20**, **23**, **31**, and **33**). Incorporation of basic functionality maintains or increases potency relative to other class A analogs (**9**−**17**). The 3-carbon link in **9** affords marginally better results than the 2- or 4-carbon links in **10** or **11**, and primary or secondary amines (**13**, **17**) bind more strongly than the corresponding tertiary amines (**12**, **16**). Lipophillic substitution generally results in low IC_{50} values in the binding assay (**18**−**20**,

23, **35**−**38**). The one striking exception to this trend is the *tert*-butyl-substituted CSBP ligand **38**, which has relatively low affinity for CSBP.

Work with the bicyclic CSBP ligands B had established that a 4-pyridyl substituent was required at the imidazole C-5 for good potency as antiinflammatory agents[6] and as inhibitors of LPS-induced IL-1 biosynthesis.[7] The opposite regioisomers, with the pyridyl at the imidazole C-4, were significantly less potent in these assays. On the basis of our proposal that the binding mode of all of the classes of imidazole CSBP ligands is roughly the same, we prepared a number of pyridine replacements at the imidazole C-5 (Table 2). As previously observed with the bicyclic CSBP ligands,[7] attachment of the pyridyl through the pyridine C-4 is required for CSBP affinity (**9** compared to **39** and **40**). Replacement of the pyridyl with a 2-methylpyridyl results in a 25-fold decrease in binding affinity (**9** compared to **41**). This effect is also noted for the triaryl CSBP ligands **55** and **56** (Table 4) although in that case there is only an 8-fold loss in potency. Additional steric bulk around the pyridine (**42**, **43**) results in further loss in affinity.

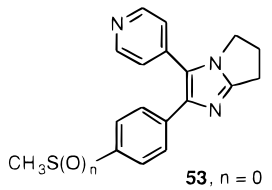
On the basis of the regiochemical requirement for a 4-pyridyl substituent, the pyridine nitrogen is expected to be a hydrogen bond acceptor. However, on the basis of the published pK_a values of the parent 5-substituents unattached to imidazole, the binding affinity of the present series of analogs is not exclusively correlated with basicity. For instance, the aminopyridine **45** binds more strongly than the less basic quinoline **43** but less strongly than the unsubstituted pyridine **9**, which is expected to have about the same pK_a as the quinoline **45**.[15]

The requirement for an aryl substituent at the imidazole C-4 in series B and C was previously established,[6,8] and it was demonstrated that a 4-(4-fluorophenyl) was more potent than the unsubstituted 4-phenyl analog.[16] In series C the 4-(3-chlorophenyl) was comparable to the 4-(4-fluorophenyl) as an inhibitor of IL-1 biosynthesis in human monocytes.[8] In the present study the 4-aryl analogs containing nonpolar substituents (**46**−**49**) (Table 3) were comparable in binding potency to **9**. However, with the addition of a bulky substituent at the 4-position on the phenyl (**50**) or the addition of a polar methyl sulfinyl substituent at the meta position (**51**), more than 1 order of magnitude loss in potency compared to **9** is observed. The 3,5-bis-(trifuoromethyl) analog **52** exhibited a 1000-fold loss of binding potency compared to that of **9**. It is likely that the dramatic loss in binding affinity for **52** results from steric constraints in the binding site which prevents entry of the bulky bis(trifluoromethyl)-substituted ring.

We have recently developed a homology model for CSBP based on related MAP kinases. The development of a binding hypothesis consistent with the SAR and the homology modeling is the subject of a recent publication.[16]

Murine TNF Inhibition. The preliminary *in vivo* assay involves the determination of the inhibition of LPS-induced TNFα production in Balb/c mice as previously described.[17]

Earlier SAR studies on sulfide and sulfoxide analogs in series B and C indicated that the relative *in vivo* and *in vitro* potencies of sulfides and sulfoxides varied with the location of the substituent. For instance in the bicyclic pyrroloimidazole series (B) the 4-(4-methylsulfinyl)phenyl analog **54** was shown to be a more potent antiinflammatory agent than the sulfide **53**, whereas



53, n = 0

54, n = 1

the sulfide was more potent as an inhibitor of IL-1 biosynthesis in whole cells.[18] This is reminiscent of the pharmacodynamic relationship of the sulfoxide cyclooxygenase inhibitor Sulindac with its sulfide form.[19] In contrast, the triaryl analogs **55** and **57** (Table 4) demonstrated that reduction of the sulfoxide **55** to the sulfide **57** is not required for binding to the target, as **55** is itself both a more potent CSBP ligand and *in vivo* TNF inhibitor in the mouse.

With these contrasting results in mind we prepared the series of N-1-substituted sulfides, sulfoxides, and sulfones **20**−**25** and **29**−**34** (Table 1). The results suggest that in this class of compounds the sulfoxides may serve as prodrugs for the sulfides. Although the sulfoxides are significantly less potent in the binding assay than the sulfides (except in one case where the sulfide (**29**) has relatively poor binding potency), there are negligible differences in the *in vivo* results between analogous sulfides and sulfoxides. This would be the expected result if the less potent sulfoxides are rapidly metabolized to the sulfides after absorption. Of note in the series of N-1 aryl-substituted analogs is the finding that the 2-aryl sulfide and sulfoxide (**33** and **34**) stimulate rather than inhibit TNF production.

The majority of compounds possessing an unbranched alkyl side chain (**9**−**27**) demonstrated *in vitro* binding potency equivalent to or better than that of SK&F 86002. However, with the exception of **9**, these compounds were weakly potent *in vivo* in comparison to the prototypic CSAID SKF 86002 (66% decrease in LPS induced mouse TNF at 50 mg/kg).

Since metabolic oxidation of *n*-alkyl and sulfur substituents directly attached to the imidazole in the pyrroloimidazole series B had previously been demonstrated,[20] we speculated that metabolic oxidation of the *n*-alkyl side chains on compounds **9**−**27** was limiting *in vivo* potency. As a way to improve metabolic stability and *in vivo* potency, we endeavored to sterically block the putative oxidation of the CH bonds on the methylene at the imidazole N-1.[21] This led to the synthesis of **29**−**38**.

Although no improvement in *in vivo* potency was noted for the 1-arylimidazoles, the branched alkyl analogs (except for the weak CSBP ligand **38**) exhibited increased *in vivo* potency. For example, unbranched lipophilic analogs such as **18** and **19**, though potent *in vitro*, were quite weak in the *in vivo* screen. In contrast, the lipophilic branched analogs **35**, **36**, and **37** were potent in both the *in vitro* and the *in vivo* screens. These results support the hypothesis that, all else being equal, hindering oxidative metabolism of the N-1 substituent improves *in vivo* potency.

PGHS-1 and 5-LO Assays. The earlier series of CSBP ligands displayed some degree of efficacy as inhibitors of the enzymes of arachidonic acid metabo-

Table 5. Effect of Potent N-1-Substituted CSBP Ligands on Cyclooxygenase and 5-Lipoxygenase Activities

	IC_{50} (μM)	
compd	PGHS-1 synthase	RBL-1 5-LO
naproxen	21	
zileuton		3.2
SK+F 86002	5.4	10
53	100	3
9	>100	>100
28	>100	>100
36	16	60
37	51	100
48	>100	>100

lism, PGHS-1, and 5-LO. Although there was no quantitative correlation of these activities with CSBP binding, the question remained whether the antiinflammatory effects of the compounds resulted from inhibition of these enzymes or resulted from CSBP inhibition. Six of the more potent N-1-substituted compounds in the binding and TNF inhibition assays were tested for their ability to inhibit prostaglandin H synthase-1 (PGHS-1) and 5-lipoxygenase (5-LO) (Table 5). The N-1-substituted CSBP ligands were weak to inactive in these assays.

Related to these results is the recent finding that the CSBP ligand **55** (SB 203580) which contains the 4-aryl-5-pyridinylimidazole pharmacophore has been tested as an inhibitor of PGHS-2 and proved to be inactive up to 100 μM.[23]

The Pharmacology of a Selective CSBP Ligand. Out of the group of N-1-substituted CSBP ligands which were assayed for PGHS-1 and 5-LO activity, compound **9** (SB 210313) was considered to have an attractive combination of *in vivo* and *in vitro* potency in the assay profile with no detectable activity as an inhibitor of the enzymes involved in arachidonic acid metabolism. This inhibitor of cytokine biosynthesis was tested in the AA rat arthritis model and produced a modest though statistically significant decrease in edema in the test animals (Figure 1). Furthermore, AA rats treated with compound **9** showed a significant increase in bone mineral density (Figure 2). When compared to a dosage of indomethicin which was chosen to match the effects of **9** on edema, **9** proved to exhibit a more significant effect on bone mineral density than indomethicin. Thus **9**, although inactive as an inhibitor of PGHS-1 and 5-LO, is efficacious in preserving bone mineral density.

In summary, a new series of CSBP ligands have been developed which include a number of potent inhibitors of TNF biosynthesis *in vivo*. One of the more potent compounds, **9** (SB 210313), was inactive as an inhibitor of 5-LO and PGHS-1 and decreased disease severity in the AA rat model of arthritis. These results suggest that the CSAID class of drugs may afford antiarthritic agents which treat the underlying disease process rather than providing only palliative relief.

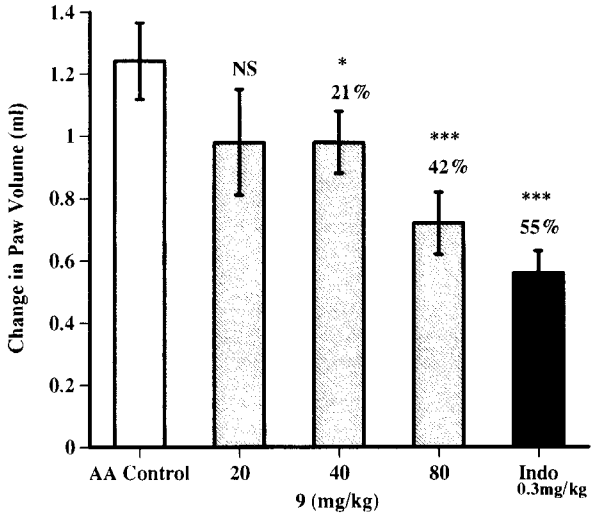


Figure 1. Suppression of hindpaw edema in the AA rat; treatment with 9 (day 0−23). Adjuvant arthritis (AA) was induced by a single injection of 0.75 mg of *Mycobacterium butyricum* (Difco, Detroit, MI) suspended in paraffin oil into the base of the tail of male Lewis rats. Hindpaw volumes were measured by water displacement at various time points following adjuvant injection. Compound **9** was homogenized in acidified aqueous 0.5% gum tragacanth and administered orally in a volume of 10 mL/kg. (Indo = indomethacin).

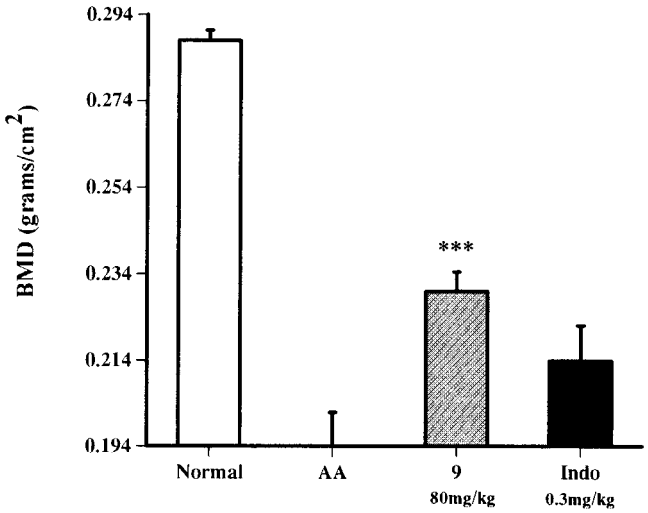


Figure 2. Effect of treatment with 9 on BMD in the AA rat. Bone mineral density (BMD) measurement was determined by dual-energy X-ray absorptiometry (DXA) using the Hologic QDR-1000 equipped with high-resolution scanning software. Scans were made of the distal tibia region of excised bones stored in 70% ethanol.

Experimental Section

Chemistry. ^{1}H NMR spectra were recorded on a Bruker WM 450 (400 MHz) instrument in $CDCl_3$ unless otherwise noted. NMR peak positions are reported in parts per million on the δ scale relative to tetramethylsilane as internal standard. Elemental analyses were performed in the Analytical and Physical Chemistry Department of SmithKline Beecham. Mass spectra were obtained by the Physical and Structural Chemistry Department of SmithKline Beecham. Flash chromatography utilized Merck silica gel 60 (230−400 mesh). THF was distilled from Na^0/benzophenone ketyl. Where other anhydrous solvents were required, Aldrich anhydrous solvents were used. Reactions requiring anhydrous and/or O_2 free conditions were conducted in flame-dried glassware under argon.

General Procedure for the Preparation of Formamides 3: [(4-Fluorophenyl)(tolylthio)methyl]formamide (3a). A solution of *p*-fluorobenzaldehyde (13.1 mL, 122 mmol), thiocresol (16.64 g, 122 mmol), formamide (15.0 mL, 445 mmol), and toluene (300 mL) was heated to toluene reflux with azeotropic removal of H_2O for 18 h, cooled, diluted with EtOAc (500 mL), and washed with saturated aqueous Na_2CO_3 (3 × 100 mL) and saturated aqueous NaCl (100 mL), dried (Na_2SO_4), and concentrated, and the residue was triturated with petroleum ether, filtered, and dried *in vacuo* to afford 28.50 g (85%) of **3a** as a white solid: mp 119−120 °C; ^{1}H NMR 8.03 (s, 1H), 7.71 (d, J = 8 Hz, 2H), 7.48 (m, 2H), 7.36 (d, J = 8 Hz, 2H), 7.11 (m, 2H), 6.30 (s, 1H), 2.46 (s, 3H).

General Procedure for the Preparation of Isocyanides 4: (4-Fluorophenyl)(tolylthio)methyl Isocyanide (4a). To a stirred, −30 °C solution of **2a** (25 g, 91 mmol) in CH_2Cl_2 (300 mL) was added dropwise $POCl_3$ (11 mL, 110 mmol) followed by the dropwise addition of Et_3N (45 mL, 320 mmol). The mixture was stirred at −30 °C for 30 min and 5 °C for 2 h, diluted with CH_2Cl_2 (300 mL), washed with 5% aqueous Na_2CO_3 (3 × 100 mL), dried (Na_2SO_4), and concentrated to 500 mL. This solution was filtered through 2 L of silica in a large sintered glass funnel with CH_2Cl_2 to afford 12.5 g (53%) of **4a** as a light brown, waxy solid: IR (CH_2Cl_2) 2130 cm^{-1}; ^1H NMR 7.39 (d, J = 8 Hz, 2H), 7.25 (m, 2H), 7.19 (d, J = 8 Hz, 2H), 7.10 (m, 2H), 5.76 (s, 1H), 2.37 (s, 3H).

General Procedure for the Preparation of Imines 5: Pyridine-4-carboxaldehyde (4-Morpholinylprop-3-yl)-imine (5a). Pyridine-4-carboxaldehyde (2.14 g, 20 mmoL), 4-(3-aminopropyl)morpholine (2.88 g, 20 mmol), toluene (50 mL), and $MgSO_4$ (2 g) were combined and stirred under argon for 18 h. The $MgSO_4$ was filtered off, and the filtrate was concentrated to afford 4.52 g (97%) of **5a** as a yellow oil containing less than 5% of aldehyde based on ^1H NMR 8.69 (d, J = 5 Hz, 2H), 8.28 (s, 1H), 7.58 (d, J = 5 Hz, 2H), 3.84 (m, 6H), 2.44 (m, 6H), 1.91 (m, 2H).

General Procedure for the Formation of 1,4,5-Substituted Imidazoles from 5 and 4 (Method A): 1-[3-(4-Morpholinyl)propyl]-4-(4-fluorophenyl)-5-(4-pyridyl)imidazole (9). To a 5 °C solution of **4a** (1.41 g, 5.5 mmol), **5a** (1.17 g, 5.0 mmol), and CH_2Cl_2 (10 mL) was added 1,5,7-triazabicyclo[4.4.0]dec-5-ene (TBD), (0.71 g 5.0 mmol), and the reaction mixture was kept at 5 °C for 16 h, diluted with EtOAc (80 mL), and washed with saturated aqueous Na_2CO_3 (2 × 15 mL). The EtOAc was extracted with 1 N HCl (3 × 15 mL), and the acid phases were washed with EtOAc (2 × 25 mL), layered with EtOAc (25 mL), and made basic by the addition of solid K_2CO_3 until pH 8.0 and then 10% NaOH until pH 10. The phases were separated, and the aqueous phase was extracted with additional EtOAc (3 × 25 mL). The extracts were dried (K_2CO_3) and concentrated, and the residue was crystallized from acetone/hexane to afford 0.94 g (51%) of **9**: mp 149−150 °C; ^1H NMR 8.71 (d, J = 4 Hz, 2H), 7.66 (s, 1H), 7.36 (m, 2H), 7.27 (d, J = 4 Hz, 2H), 6.93 (m, 2H), 3.99 (t, J = 7 Hz, 2H), 3.64 (m, 4H), 2.29 (m, 4H), 2.21 (t, 7 Hz, 2H), 1.68 (m, 2H); MS (ES^+) m/z 367 (MH^+). Anal. ($C_{21}H_{23}FN_4O\cdot{}^1/_4H_2O$) C, H, N.

General Procedures for the Formation of 1,4,5-Substituted Imidazoles from 1-(3-Chloropropyl)-4-(4-fluorophenyl)-5-(4-pyridyl)imidazole (6) (Methods B, C, and D): 1-(3-Chloropropyl)-4-(4-fluorophenyl)-5-(4-pyridyl)-imidazole (6). Pyridine-4-carboxaldehyde (3-Chloropropyl)imine (5b). To 3-chloropropylamine hydrochloride (15.1 g, 0.120 mol) and H_2O (100 mL) was added pyridine-4-carboxaldehyde (9.55 mL, 0.100 mol), then K_2CO_3 (8.28 g, 0.060 mol), and then CH_2Cl_2 (100 mL), and the mixture was stirred for 40 min. The phases were separated, the aqueous phase was extracted with CH_2Cl_2 (2 × 50 mL), and the organic phases were dried (Na_2SO_4) and concentrated to afford 17.1 g (94%) of **5b** as a light yellow oil: ^1H NMR 8.69 (d, J = 4 Hz, 2H), 8.32 (s, 1H), 8.28 (s, 1H), 7.58 (d, J = 4 Hz, 2H), 3.71 (m, 2H), 3.63 (t, J = 4 Hz, 2H), 2.24 (t, J = 6 Hz, 2H). The presence of 9% of the aldehyde was evident by ^1H NMR.

1-(3-Chloropropyl)-4-(4-fluorophenyl)-5-(4-pyridyl)imidazole (6). This was prepared from **5b** and **3a** by method A (38%): ^1H NMR 8.25 (d, J = 8 Hz, 2H), 7.69 (s, 1), 7.37 (d, J = 8 Hz, 2H), 7.26 (m, 2H), 6.94 (m, 2H), 4.12 (t, J = 7 Hz, 2H), 3.42 (t, J = 6 Hz, 2H), 1.97 (m, 2H): MS (ES^+) m/z = 318 (MH^+); mp 139−140 °C. Anal. ($C_{17}H_{15}ClFN_3$) C, H, N.

1-[3-[*N*-(Phenylmethyl)-*N*-methylamino]propyl]-4-(4-fluorophenyl)-5-(4-pyridyl)imidazole (12) (Method B). To a solution of **6** (0.17 g, 0.53 mmol) and DMF (10 mL) was added benzylmethylamine (0.10 mL, 0.80 mmol) and NaI (10 mg), and the mixture was heated to 90 °C for 30 h, cooled, and added to 5% aqueous Na_2CO_3 (20 mL) and extracted with EtOAc (3 × 25 mL). The combined extracts were washed with H_2O (3 × 25 mL) and flash chromatographed with 0−1% MeOH in CH_2Cl_2 to afford 90 mg (42%) of **12** as a white solid: ^1H NMR 8.70 (d, J = 8 Hz, 2H), 7.58 (s, 1H), 7.41−7.20 (m, 8H), 6.94 (m, 2H), 3.97 (t, J = 7 Hz, 2H), 2.30 (t, J = 6 Hz, 2H), 2.10 (s, 3H), 1.73 (m, 2H); MS (ES^+) m/z = 401 (MH^+); mp 139−140 °C. Anal. ($C_{17}H_{15}ClFN_3$) C, H, N.

1-(3-Aminopropyl)-4-(4-fluorophenyl)-5-(4-pyridyl)imidazole (17) (Method C). 1-(3-Azidopropyl)-4-(4-fluorophenyl)-5-(4-pyridyl)imidazole. The azide was prepared from **6** and NaN_3 by method B (100%): mp 64−65 °C; ^1H NMR 8.73 (d, J = 8 Hz, 2H), 7.66 (s, 1H), 7.38 (m, 2H), 7.67 (m, 2H), 6.93 (m, 2H), 4.01 (t, J = 7 Hz, 2H), 3.25 (t, J = 6 Hz, 2H), 1.78 (m, 2H); MS (ES^+) m/e = 323 (MH^+).

1-(3-Aminopropyl)-4-(4-fluorophenyl)-5-(4-pyridyl)imidazole (17). 1-(3-Azidopropyl)-4-(4-fluorophenyl)-5-(4-pyridyl)-imidazole (254 mg, 0.79 mmol), was dissolved in THF (2 mL) and added dropwise to a 0 °C solution of 1 M $LiAlH_4$ in THF (1.2 mL, 1.2 mmol), the mixture was stirred at 0 °C for 15 min, EtOAc (4 mL) was carefully added, and the mixture was added to ice cold 10% aqueous NaOH (15 mL), extracted with EtOAc (4 × 25 mL), dried (K_2CO_3), and concentrated. Flash chromatography (0−8% MeOH, 0−2% Et_3N) afforded a waxy solid (175 mg, 75%): mp 81−82 °C; ^1H NMR 8.71 (d, J = 8 Hz, 2H), 7.68 (s, 1H), 7.37 (m, 2H), 7.27 (d, J = 8 Hz, 2H), 6.93 (m, 2H), 4.00 (t, J = 7 Hz, 2H), 2.65 (t, J = 6 Hz, 2H), 1.70 (m, 2H); MS (ES^+) m/z = 297 (MH^+). The free amine was dissolved in a minimum of EtOH and treated with excess 1 M HCl in Et_2O. The precipitated salt was filtered off, dissolved in H_2O, and lyophilized to a hydroscopic solid: ($C_{17}H_{17}FN_4\cdot 3^1/_4HCl\cdot 2^1/_2H_2O$) C, H, Cl; N: calcd, 12.18; found, 11.31.

1-[3-Methylthio)propyl]-4-(4-fluorophenyl)-5-(4-pyridyl)-imidazole (20) (Method D). To a solution of **6** (0.39 g, 1.25 mmol) and DMF (25 mL) was added sodium thiomethoxide (0.17 g, 2.5 mmol), and the mixture was heated to 50 °C for 2 h. The cooled reaction mixture was added to 5% aqueous Na_2CO_3(20 mL) and extracted with EtOAc (3 × 25 mL). The extracts were washed with H_2O (3 × 25 mL), concentrated, and purified by trituration with hot hexane to afford 0.2 g (50%) of **20** as a white solid: ^1H NMR 8.72 (d, J = 8 Hz, 2H), 7.67 (s, 1H), 7.39 (m, 2H), 7.28 (d, J = 8 Hz, 2H), 6.93 (t, 2H), 4.03 (t, J = 7 Hz, 2H), 2.38 (t, J = 6 Hz, 2H), 2.00 (s, 3H), 1.82 (m, 2H); MS (ES^+) m/z = 328 (MH^+); mp 85−88 °C. Anal. ($C_{18}H_{18}FN_3S$) C, H, N.

General Procedure for the Preparation of Sulfoxides (Method E): 1-[3-(4-Morpholinyl)propyl]-4-[3-(methylsulfinyl)phenyl]-5-(4-pyridyl)imidazole (21). To a solution of **20** (200 mg, 0.49 mmol) in HOAc (4 mL) was added a solution of $K_2S_2O_8$ (151 mg, 0.56 mmol) in H_2O (4 mL). The solution was stirred for 16 h, poured into 10% aqueous NaOH (50 mL), and extracted with EtOAc (3 × 25 mL). The extracts were dried (K_2CO_3) and concentrated, and the residual oil was crystallized from acetone/hexane to afford 87 mg (42%) of a white solid: ^1H NMR 8.72 (d, J = 8 Hz, 2H), 7.68 (s, 1H), 7.37 (m, 2H), 7.28 (d, J = 8 Hz, 2H), 6.90 (m, 2H), 4.10 (t, J = 7 Hz, 2H), 2.50 (m, 5H), 2.05 (m, 2H); MS (ES^+) m/z = 344 (MH^+); mp 117−118 °C. Anal. ($C_{18}H_{18}FN_3OS$) C, H, N.

General Procedure for the Preparation of Sulfones (Method F): 1-[3-(Methylsulfonyl)propyl]-4(4-fluorophenyl)-5-(4-pyridinyl)imidazole (22). To a 0 °C solution of **20** (0.509 g, 1.48 mmol) in MeOH (8 mL) was added TFA (0.12 mL, 1.6 mmol), then *m*-chloroperoxybenzoic acid (0.23 g, 2.2 mmol) dissolved in CH_2Cl_2 (10 mL) was added dropwise, the mixture was stirred for 1 h, and the solvent was evaporated *in vacuo*. The residue was partitioned between H_2O and EtOAc, and the aqueous phase was made basic by the addition of 2 N NaOH. The organic phase was separated, dried ($MgSO_4$), and concentrated, and the residue was purified by flash chromatography (silica gel, 0−5% MeOH/CH_2Cl_2) to afford **22** as a white solid (0.37 g, 69%): ^1H NMR 8.72 (d, J = 8 Hz, 2H), 7.68 (s, 1H), 7.38 (m, 2H), 7.25 (d, J = 8 Hz, 2H), 6.90 (m, 2H), 4.13 (t, J = 7 Hz, 2H), 3.37 (m, 5H), 2.05 (m, 2H); MS (ES^+) m/z = 360.0 (MH^+); mp 146−147 °C. Anal. ($C_{18}H_{18}FN_3O_2S$) C, H, N.

1-(4-Carboxypropyl)-4-(4-fluorophenyl)-5-(4-pyridyl)-imidazole (27) (Method G). To a solution of **26** (100 mg, 0.29 mmol), CH_3OH (3 mL), and THF (1.5 mL) was added a solution of LiOH (62 mg, 1.5 mmol) in H_2O (1.5 mL), the resulting solution was stirred for 4 h, solvent was evaporated *in vacuo*, and the residue was dissolved in H_2O and chromatographed through HP-20 with H_2O until the eluates were neutral and then with 25% aqueous MeOH to afford the title

compound as the lithium salt (65 mg, 68%): ^{1}H NMR 8.35 (d, J = 5 Hz, 2H), 7.69 (s, 1H), 7.20 (d, J = 5 Hz, 2H), 7.09 (m, 2H), 6.81 (m, 2H), 3.80 (t, J = 7 Hz, 2H), 1.79 (t, J = 7 Hz, 2H), 1.54 (m, 2H); MS (ES$^+$) m/z = 326 (MH$^+$).

(4-Fluorophenyl)tosylmethyl Isocyanide (8). Toluenesulfinic acid sodium salt (150 g, 0.84 mol), H_2O (500 mL), and *tert*-butylmethyl ether (TBME) (250 mL) were vigorously stirred, and concentrated HCl (75 mL) was added dropwise. The resulting two phases were separated, and the aqueous phase was extracted with TBME (100 mL). The TBME phases were dried (Na_2SO_4) and concentrated to near dryness, and the white solid was combined with hexane (350 mL), filtered, and dried *in vacuo* to afford 96 g of toluenesulfinic acid.

The free acid (92.3 g, 0.62 mol), *p*-fluorobenzaldehyde (92.3 g, 0.744 mol), formamide (73.9 mL), and camphorsulfonic acid (14.4g, 0.062 mol) were combined, vigorously stirred, and heated to 65 °C for 16 h. The resulting white mass was cooled to 23 °C, triturated with CH_3OH (150 mL) and hexane (350 mL), filtered, and dried *in vacuo* to afford 88.35 g (46%) of (4-fluorophenyl)(tosylmethyl)formamide as a white solid: ^{1}H NMR 8.06 (s, 1H), 7.69 (d, J = 8 Hz, 2H), 7.43 (m, 2H), 7.32 (d, J = 8 Hz, 2H), 7.08 (m, 2H), 6.29 (s, 1H), 2.43 (s, 3H).

A solution of the formamide obtained above (20.2 g, 65.7 mmol) and anhydrous DME (330 mL) was cooled to −10 °C, and $POCl_3$ (18.4 mL, 197 mmol) was added dropwise. Triethylamine (45.8 mL, 329 mmol) in DME (30 mL) was added dropwise (T < 5 °C), and the reaction mixture was stirred at −5 °C for 2 h, then poured into H_2O (600 mL), and extracted with EtOAc (3 × 150 mL). The extracts were washed with saturated aqueous $NaHCO_3$, dried (Na_2SO_4), and concentrated. The colorless oil was triturated with hexane to afford a solid. Filtration and drying afforded 16.2 g (85%) of (4-fluorophenyl)-tosylmethyl isocanide (**8**) as a white solid: ^{1}H NMR 7.62 (d, J = 8 Hz, 2H), 7.34 (m, 4H), 7.10 (m, 2H), 5.59 (s, 1H), 2.48 (s, 3H).

4(5)-(4-Fluorophenyl)-5(4)-(4-pyridyl)imidazole (28) (Method H). Pyridine-4-carboxaldehyde (321 mg, 3.0 mmol) and THF (3 mL) were cooled to −50 °C, lithium bis(trimethylsilyl)amide (LIBSA) (1 M in THF) (3 mL, 3.0 mmol) was added dropwise (T < −40 °C), and the mixture was stirred for 45 min and warmed to −30 °C for 5 min to afford solution A.

THF (3 mL) and **8** (867 mg, 3.0 mmol) were cooled to −50 °C, LIBSA (1 M in THF) (3 mL, 3.0 mmol) was added dropwise at −50 °C, and the mixture was stirred for 30 min to afford solution B.

Solution A was cooled to −60 °C, and solution B was added dropwise. The resulting solution was stirred at −70 °C for 30 min, warmed to 23 °C over 4 h, stirred at 23 °C for 16 h, poured into 5% aqueous Na_2CO_3 (25 mL), extracted with EtOAc (4 × 25 mL), dried (Na_2SO_4), concentrated, and flash chromatographed (0−8% MeOH in CH_2Cl_2) to afford 251 mg (35%) of **28**. The product was crystallized from acetone/hexane: ^{1}H NMR 8.42 (m, 2H), 7.72 (s, 1H), 7.40 (m, 4H), 7.12 (m, 2H); MS (ES$^+$) m/z = 240 (MH$^+$); mp 245−245 °C (dec). Anal. ($C_{14}H_{10}FN_3 \cdot {}^1/_{10}H_2O$) C, H, N.

4-Formyl-2-methylpyridine. 4-Cyano-2-methylpyridine was prepared from 2,6-lutidine according to the literature procedure.[9] A solution of 4-cyano-2-methylpyridine (0.367 g, 3.11 mmoL) and toluene (3.5 mL) was cooled to −78 °C, and 1 M DIBAL in hexanes (3.6 mL, 3.6 mmoL) was added dropwise via syringe pump (T < −65 °C). The reaction mixture was warmed to 5 °C, stirred for 5 min, and recooled to −78 °C, and CH_3OH (3.5 mL) was added (T < −40 °C). The mixture was warmed to 5 °C and stirred for 5 min, then 25% aqueous Rochelle's salt (potassium sodium tartrate) was added. The mixture was stirred for 3 min and then acidified to pH <1.0 with 10% aqueous H_2SO_4. The aqueous solution was made basic by the addition of solid K_2CO_3 and extracted with EtOAc. The extracts were dried (Na_2SO_4), concentrated, and filtered through silica (2% MeOH in CH_2Cl_2) to afford 253 mg (84%) of aldehyde: ^{1}H NMR 10.05 (s, 1H), 8.74 (d, J = 7 Hz, 1H), 7.51 (s, 1H), 7.30 (d, J = 7 Hz, 1H), 2.68 (s, 3H).

4-Formyl-2,6-dimethylpyridine. This was prepared in the same manner as 4-formyl-2-methylpyridine:[9] ^{1}H NMR 10.01 (s, 1H), 7.37 (s, 2H), 2.64 (s, 6H).

1-[3-(4-Morpholinyl)propyl]-4-(4-fluorophenyl)-5-(2-chloropyridin-4-yl)imidazole (44). 2-Chloropyridine-4-carboxaldehyde was prepared as described in the patent literature.[10] This aldehyde was reacted by procedure A to afford **44**: ^{1}H NMR 8.46 (d, J = 5 Hz, 1H), 7.67 (s, 1H), 7.35 (m, 3H), 7.19 (m, 1H), 6.97 (m, 2H), 4.01 (t, J = 7 Hz, 2H), 3.67 (m, 4H), 2.32 (m, 4H), 1.95 (t, J = 7 Hz, 2H), 1.70 (m, 2H): MS (ES$^+$) m/z = 401 (MH$^+$); mp 97.0−97.5 °C. Anal. ($C_{21}H_{22}ClFN_4O$) C, H, N.

1-[3-(4-Morpholinyl)propyl]-4-(4-fluorophenyl)-5-(2-amino-4-pyridinyl)imidazole (45) (Method I). 1-[3-(4-Morpholinyl)propyl]4-(4-fluorophenyl)-5-(2-hydrazinyl-4-pyridinyl)imidazole (45a). Compound **44** (872 mg, 2.18 mmoL) and 98% hydrazine hydrate (9 mL) were heated to 115 °C for 14 h, cooled to 23 °C, combined with H_2O (20 mL), and extracted with EtOAc (3 × 25 mL). The combined extracts were washed with H_2O (2 × 20 mL), dried (Na_2SO_4), concentrated to a dry residue, and triturated with Et_2O to afford 547 mg (63%) of **45a** as a white solid: ^{1}H NMR 8.20 (d, J = 5 Hz, 1H), 7.62 (s, 1H), 7.45 (m, 2H), 6.94 (m, 2H), 6.71 (s, 1H), 6.63 (d, J = 5 Hz, 1H), 5.94 (m, 1H), 3.98 (t, J = 5 Hz, 2H), 3.82 (m, 2H), 3.66 (m, 4H), 2.30 (m, 4H), 2.23 (t, J = 5 Hz, 2H), 1.71 (m, 2H); MS (ES$^+$) m/z = 397 (MH$^+$); mp 150−151 °C. Anal. ($C_{21}H_{25}FN_6O$) C, H, N.

1-[3-(4-Morpholinyl)propyl]-4-(4-fluorophenyl)-5-(2-amino-4-pyridinyl)imidazole (45). 4-(4-Fluorophenyl)-1-[3-(4-morpholinyl)propyl]-5-(2-hydrazinyl-4-pyridinyl)imidazole (100 mg, 0.25 mmoL), absolute EtOH (15 mL), and Raney Ni (0.4 mL) were shaken under H_2 (45 psi) for 4 h. Flash chromatography with 0−8% CH_3OH in CH_2Cl_2 afforded 34 mg (37%) of **45** as a white solid: ^{1}H NMR 8.16 (d, J = 6 Hz, 2H), 7.61 (s, 1H), 7.43 (m, 2H), 6.94 (m, 2H), 6.62 (d, J = 6 Hz, 1H), 6.42 (s, 1H), 4.64 (m, 2H), 3.96 (t, J = 7 Hz, 2H), 3.66 (m, 4H), 2.23 (m, 4H), 2.22 (t, J = 7 Hz, 2H), 1.70 (m, 2H); MS (ES$^+$) m/z = 382 (MH$^+$); mp 186−187 °C. Anal. ($C_{21}H_{24}FN_5O \cdot 0.35H_2O$) C, H; N: calcd, 18.06; found, 17.48.

Biological Methods

CSBP Binding Assay. A buffer solution consisting of 20 mM Tris-HCl pH 7.4, 20 mM Hepes buffer, and 1 mM $MgCl_2$ (binding buffer) is used throughout the assay for dilution of the reagents. The total volume in each reaction tube is 120 μL, consisting of 100 μL of the cytosolic fraction of the human monocytic cell line, THP.1, which contains ca. 100 μg of CSBP, 10 μL of [^{3}H]SB-202190[4,18] to afford a concentration of 5 nM in the final 120 μL volume and 10 μL of varying concentrations of the test compounds. The resulting mixture is incubated for 15 min at room temperature. After incubation, the reaction mixtures (120 μL total volume) are chilled in an ice bath, and separation of free from bound [^{3}H]SB-202190 is performed by size exclusion chromatography at 4 °C as follows. The reaction mixtures are applied to G-10 Sephadex straw columns (1.5 in. bed volume), pre-equillibrated with 5 mL cold elution buffer (20 mM Tris-HCl, pH 7.4, and 50 μM 2-ME), the column is washed by another 400 μL of elution buffer, and the total eluent to this point is discarded, the bound ligand elutes in the protein-containing void volume with a final 500 μL elution. This 0.5 mL fraction is collected in 20 mL glass scintillation vials. After addition of 15 mL scintillation fluid, the vials are vortexed and the radioactivity accessed in a Beckman scintillation counter. Assay controls include binding of [^{3}H]SB-202190 to CSBP in the absence of competitor (total binding) and in the absence of cytosol (background). IC_{50}'s are determined by linear regression analysis for a compound's ability to inhibit 50% of the radioligand binding to THP.1 cytosol/CSBP.

Determination of the inhibition of LPS-induced TNFα production in Balb/C mice was preformed as previously described.[17]

RBL-1 5-Lipoxygenase Assay. The 5-LO activity was assessed by a continuous assay which monitored the consumption of O_2.[24] A 100000*g* cytosolic preparation enriched in 5-LO isolated from RBL-1 cells (ATCC no. CRL 1378), was prepared as described previously.[25] The 5-LO preparation (200 μL, 1 mg protein) was preincubated with the inhibitor or its vehicle in 25 mM Bis-Tris buffer (pH 7.0) containing 1 mM EDTA, 2 mM ATP, and 150 mM NaCl for 3 min at 20 °C (total volume 2.97 mL). The reaction was started by the addition of arachidonic acid (10 μM) and $CaCl_2$ (2 mM) (final volume 3 mL), and the decrease in O_2 concentration with time was

followed using a Clark-type electrode with a Yellow Springs model 53 O_2 monitor (Yellow Springs, OH) and an offset amplifier (Sigma Electronics, King of Prussia, PA). The optimal velocity was calculated from the steepest portion of the progress curves. The compound-mediated inhibition of 5-LO activity is described as the concentration of compound causing a 50% inhibition of the optimal velocity as determined for the vehicle-treated sample. All compounds were dissolved in dimethyl sulfoxide (final concentration 1%).

Prostaglandin H Synthase Type-1 (PGHS-1) Assay. The cyclooxygenase assay was modeled after a method described previously.[26] The assay was performed at 37 °C by monitoring O_2 uptake using a Clark-type electrode with a Yellow Springs model 53 oxygen monitor (Yellow Springs, OH) and an offset amplifier (Sigma Electronics, King of Prussia, PA). The assay mixture contained 0.5 mM phenol, 1.0 μM hematin, 10 μM arachidonic acid, and the inhibitor or its vehicle (DMSO) in 0.1 M Tris-HCl, pH 8.0 (final volume 2 mL). After a 1-min preincubation, the reaction was started by addition of 100 units (2.2 μg) of ram seminal vesicle PGHS-1 enzyme (Oxford Biomedical Research, Inc., Oxford, MI). The initial velocity was measured as a tangent to the decreasing O_2 concentration curve. The compound-mediated inhibition of PGHS-1 activity is described as the concentration of compound causing a 50% inhibition of the initial velocity as determined for the vehicle-treated sample.

Suppression of Hindpaw Edema in the AA Rat. Adjuvant arthritis (AA) was induced by a single injection of 0.75 mg of *Mycobacterium butyricum* (Difco, Detroit, MI) suspended in paraffin oil into the base of the tail of male Lewis rats. Hindpaw volumes were measured by water displacement at various time points following adjuvant injection. Compound **9** was homogenized in acidified aqueous 0.5% gum tragacanth and administered orally in a volume of 10 ml/kg.

Effect of Treatment with 9 on BMD in the AA Rat. Bone mineral density (BMD) measurement was determined by dual-energy X-ray absorptiometry (DXA) using the Hologic QDR-1000 equipped with high-resolution scanning software. Scans were made of the distal tibia region of excised bones stored in 70% ethanol.

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