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PYRIMIDINYLIMIDAZOLE INHIBITORS OF CSBP/P38 KINASE DEMONSTRATING DECREASED INHIBITION OF HEPATIC CYTOCHROME P450 ENZYMES

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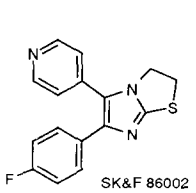
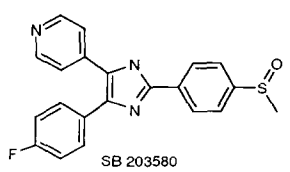
Abstract: Pyrimidine analogs of the pyridinylimidazole class of CSBP/p38 kinase inhibitors were prepared in an effort to reduce the potent inhibition of hepatic cytochrome P450 observed for the pyridinyl compounds. The substitution of pyrimidin-4-yl, 2-methoxypyrimidin-4-yl, or 2-methylaminopyrimidin-4-yl for pyridin-4-yl effectively dissociates CSBP/p38 kinase from P450 inhibition for this series and furthermore achieves an increase in oral activity. © 1998 Elsevier Science Ltd. All rights reserved.

Introduction

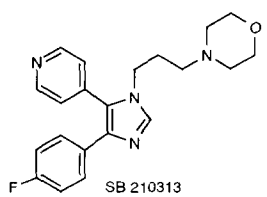
The pyridinylimidazoles (e.g., SK&F 86002 and SB 203580) are representative of a novel class of antiinflammatory agents.[1] This class of compounds selectively inhibits the stress-activated p38/CSBP MAP kinase and subsequently blocks the synthesis of several proinflammatory cytokines (e.g., IL-1 and TNF).[2] This inhibition of proinflammatory cytokine biosynthesis is believed to be the primary mechanism responsible for the potent in vitro and in vivo antiinflammatory activity of the pyridinylimidazoles. Hence, selective inhibition of the CSBP/p38 MAP kinase pathway may be an attractive target for the development of therapeutic agents to treat chronic inflammatory diseases, such as rheumatoid arthritis and inflammatory bowel disease.



SK&F 86002



SB 203580



SB 210313

Previous communications from our laboratories have established the importance of the 4-(4-pyridinyl)-5-phenylimidazole substructure for potent CSBP/p38 MAP kinase inhibition.[3] Recently published X-ray crystallographic studies of these inhibitors with CSBP/p38 MAP kinase have revealed the molecular basis for much of the observed SAR.[4] These studies locate the 4-pyridinyl group in the adenine binding pocket of ATP with a hydrogen bond between the pyridinyl nitrogen and the amide NH of Met109. The importance of the hydrogen bonding interaction in this otherwise lipophilic environment is illustrated by the >100-fold loss in

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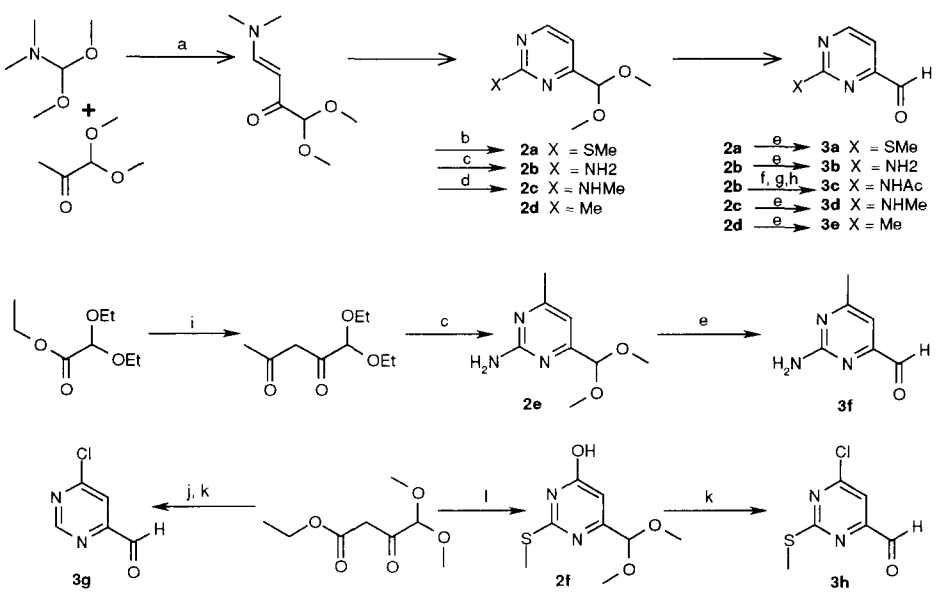
affinity resulting from substitution with a 2- or 3-pyridinyl or a phenyl.[3a]

The pyridinylimidazoles, SK&F 86002 and SB 203580, also potently inhibit human liver P450 isozymes.[5] The pyridine and imidazole ring systems are known to be good ligands for the ferric, heme iron of cytochrome P450 and compounds possessing these heterocyclic rings are often potent inhibitor of P450 enzymes.[6] The pyridine and not the more sterically congested α,α'-disubstituted imidazole was presumed to be the more likely coordinating ligand responsible for P450 inhibition. Subsequently, acquired SAR has provided further support for this hypothesis.[7] Thus the 4-pyridinyl group, in addition to being required for binding to CSBP/p38, is also an important factor contributing to potent P450 inhibition.

Scheme 1[8]



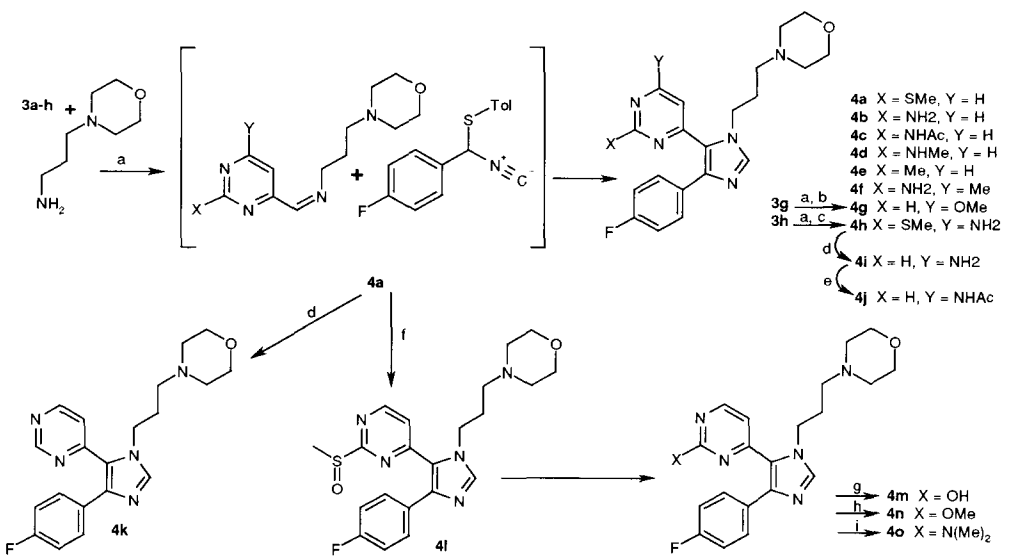
(a) 100 °C, 18 h; (b) $H_2NC(SMe)=NH$, Δ; (c) guanidine-HCl, $NaOH/H_2O$; (d) N-methylguanidine-HCl, $NaOH/H_2O$; (e) 3 N HCl, 45–50 °C for 1–3 h; (f) Ac_2O, 60 °C, 18 h; (g) Ac_2O, H_2SO_4; (h) NaOH/MeOH, 1 h; (i) NaH, acetone/benzene, 0 °C; (j) formamidine acetate, KOH/EtOH, Δ; (k) $POCl_3$; (l) $MeSC(=NH)NH_2$-H_2SO_4, $NaOH/H_2O$.

Inhibitors of human hepatic cytochrome P450 can potentially cause drug–drug interactions or lead to other hepatic changes such as P450 enzyme induction. In 10 day rat dose-ranging toxicological studies with SK&F 86002 and SB 203580 increased liver weight and significant elevations of hepatic P450 enzymes were noted.[9] In this communication we report the efficacy of substituted pyrimidines as pyridine replacements that retain affinity for CSBP/p38 and demonstrate markedly reduced cytochrome P450 inhibition.

Chemistry

The regioselective synthesis of 1,4,5-trisubstituted imidazoles (Scheme 2) was achieved using an imine-isonitrile cycloaddition (the van Leusen reaction).[10] Our previously described adaptation of this reaction included the synthesis of **1a–c**.[3a] The synthesis of the pyrimidine aldehydes **3a–h** required to prepare **4a–o** is outlined in Scheme 1 and follows the general procedure published by Bredereck.[11] Careful attention must be exercised in the hydrolysis of the acetals (**2**) and subsequent isolation of the water soluble aldehydes, especially **3b** that underwent an irreversible oligomerization upon standing. Also notable is the one step transformation of the hydroxy-acetals to the chloropyrimidine aldehydes (**3g** and **3h**) using $POCl_3$. Reaction of the crude imines formed by mixing aldehydes **3a–h** and 4-(3-aminopropyl)morpholine with α-(*p*-toluenesulfonyl)-4-fluorobenzylisontirile was initiated with 1,5,7-triazabicyclo[4.4.0]dec-5-ene (TBD) to afford the imidazoles **4a–f**. The 2-thiomethylpyrimidine **4a** proved to be a versatile intermediate. Raney nickel reduction produced the unsubstituted pyrimidine **4k**, whereas the sulfoxide **4l** was readily displaced by both oxygen (**4m and 4n**) and amine nucleophiles (**4o**).

Scheme 2



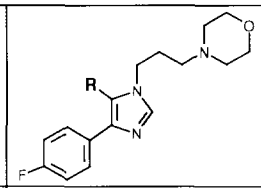
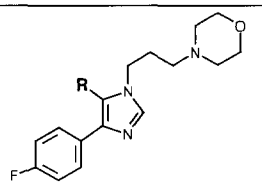
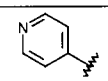
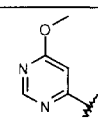
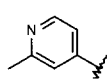
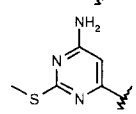
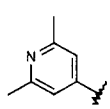
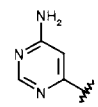
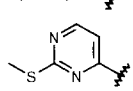
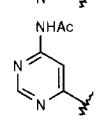
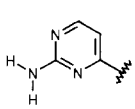
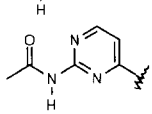
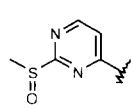
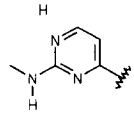
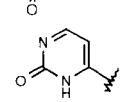
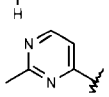
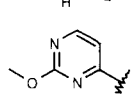
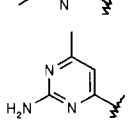
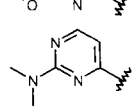
(a) TBD/CH_2Cl_2, 20 °C; (b) NaOMe/MeOH; (c) NH_4OH, 120 °C; (d) Raney Ni, EtOH, Δ; (e) Ac_2O, 60 °C, 48 h; (f) $K_2S_2O_8$, H_2O/HOAc, 72 h; (g) aq NaOMe; (h) NaOMe/MeOH; (i) dimethyl amine, Δ.

Results and Discussion

Pyridinylimidazole SB 210313 is an orally active CSBP/p38 kinase inhibitor devoid of dual 5-LO/COX-1 activity.[3a] An additional property of SB 210313 that distinguishes it from its predecessors, SK&F 86002 and SB 203580, is a markedly reduced interaction with cytochrome P450s 1A2, 2C9, 2C19 and 3A4 (< 50%

inhibition at 10 uM), but an increased inhibition of CYP2D6 (86% inhibition @ 10 uM). In general, P450 enzymes prefer lipophilic compounds as substrates/inhibitors. Hence, the decreased inhibition demonstrated by SB 210313 for cytochrome P450s 1A2, 2C9, 2C19 and 3A4 is attributed to a decrease in lipophilicity resulting from introduction of the basic polar morpholinylpropyl side chain. However, the presence of the morpholinylpropyl may explain the enhanced inhibition of CYP2D6 since this isozyme has a basic amine binding pocket located some 5–7 angstroms distal from an aromatic binding pocket.[12]

Table1. Inhibition of CSBP/p38 MAP Kinase by Pyridin-4-yl Group Replacements

compound		CSBP/p38 IC_{50}, uM	compound		CSBP/p38 IC_{50}, uM
1a **SB 210313**		1.3	**4g**		>17
1b		2.1	**4h**		>17
1c		>17	**4I**		>17
4a		2.0	**4j**		3.5
4b		0.48	**4k**		0.22
4c		0.46	**4l**		2.2
4d		1.9	**4m**		5.5
4e		1.3	**4n**		0.30
4f		>17	**4o**		3.6

Assuming that both CSBP/p38 and P450 binding required the pyridinyl nitrogen lone pair, a variety of SB 210313 analogs were prepared possessing a 4-azaheteroaromatic group of differing electronic or steric features in order to identify CSBP/p38 inhibitors devoid of significant interaction with cytochrome P450. Our initial approach was to hinder access of the pyridinyl nitrogen to P450 heme by introduction of sterically demanding alkyl groups. However, CSBP/p38 kinase inhibition also proved sensitive to steric effects, as potency decreased with the introduction of successive methyl groups (**1a** vs. **1b** and **1c**). Substitution of a pyrimidine for the pyridine was considered an attractive alternative as (1) both hydrogen bonding ability and placement of the 4-pyridinyl nitrogen is retained and (2) pyrimidine was known to be a weak P450 inhibitor relative to pyridine.[13].

The majority of the pyrimidine analogs were equivalent to or better than SB 210313, **1a**, as inhibitors of CSBP/p38 (Table 1). The most successful pyridine replacements were the 2-amino-, 2-methoxy- and 2-unsubstituted-pyrimidines (**4b**, **4k**, and **4n**). Whereas previous SAR established the requirement for a 4-azaheterocycle, the data in Table 1 demonstrate that CSBP/p38 inhibition can be influenced by both steric and electronic effects. The loss of CSBP/p38 inhibition with the introduction of α,α-disubstitution (**4f** and **4h**) parallels the SAR for **1a**–**1c** and suggests a steric constraint in the CSBP/p38 binding site.

Table 2. Effect of Pyridin-4-yl Group Replacement on P450 and Oral Activity

compound	CYP2D6 inhibition[a]	murine ED_{50} for TNFα[b]	compound	CYP2D6 inhibition[a]	murine ED_{50} for TNFα[b]
1a	86	42 mg/kg	**4d**	11	19 mg/kg
1b	51	37 % ***	**4e**	19	65 % ***
4a	47	43 mg/kg	**4k**	34	12 mg/kg
4b	47	5.2 mg/kg	**4n**	7	14 mg/kg

[a]percent inhibition of human cytochrome 2D6 at 10 uM of test compound; [b]the assay was conducted in Balb/c mice using a modification of the published protocol in which TNF levels were determined in the plasma;[14] data are presented as ED_{50} in mg/kg or % inhibition at the screen dose of 50 mg/kg; ***statistically significant from controls at $p < 0.001$.

Compounds demonstrating CSBP/p38 inhibition equivalent to or better than **1a** were examined for inhibition of human cytochrome P450 2D6 (CYP2D6) and oral activity in the mouse (Table 2).[15] Introduction of a methyl group adjacent to the pyridinyl nitrogen reduced inhibition of CYP2D6 (**1b**), but proved of limited benefit as oral activity also decreased. However, several of the pyrimidines (**4b**, **4d**, **4k**, **4n**) demonstrated a reduction in CYP2D6 inhibition along with increased oral activity. Particularly notewothy is the eight fold increase in oral activity achieved with the 2-aminopyrimidine (**4b**) and the lack of significant CYP2D6 interactions seen with the 2-(methylamino)pyrimidine (**4d**) and the 2-methoxypyrimidine (**4n**). The reduction in cytochrome P450 interactions achieved with these analogs is consistent with the proposed interaction of the 4-pyridinyl group with the heme iron of P450. Further optimization of the pyrimidinylimidazoles is therefore warranted.

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5. The potential of compounds to inhibit P450 was determined using isoform selective P450 assays based on the method reported by Bloomer, J.C. Clarke, S.E., and Chenery, R.J. *Xenobiotica* **1995**, 9, 917. The assays were performed with substrates present at their Km concentration, test compounds at 10 uM and used heterologously expressed enzymes. Measured inhibition of human cytochrome P450s for compounds at 10 uM is given as enzyme (% inhibition): SK&F 86002 - 1A2(85), 2C9(80), 2C19(64), 3A4(19) and 2D6(22); SB 203580, 1A2(61), 2C9(75), 2C19(85), 3A4(61) and 2D6(67).

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7. Unpublished observations and data reported in Table 2.

8. Compound **2d** was acquired from an outside supplier.

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15. Not included in Table 2 is compound **4c**, which failed to demonstrate statistically significant activity in the mouse LPS-induced TNF assay when dosed orally at 50 mg/kg (12% inhibiton). Additional compounds tested in the mouse model at a dose of 50 mg/kg were **4j** (21% inh.), **4l** (37% inh.) and **4m** (33% inh).