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SAR of 4-Hydroxypiperidine and Hydroxyalkyl Substituted Heterocycles as Novel p38 Map Kinase Inhibitors

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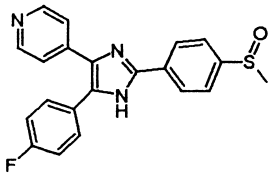
Abstract—The 4-hydroxypiperidine substituent was found to confer high p38 selectivity devoid of COX-1 affinity, when attached to a series of pyridinyl substituted heterocycles. Pyridinyloxazole **11** showed a promising in vivo profile with bioavailability of 64% and ED_{50} in rat collagen induced arthritis of 10 mg/kg po bid. In contrast to pyridinylimidazoles such as SB 203580, **11** did not inhibit human cytochrome P450 isoenzymes.

Introduction

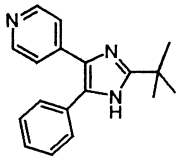
Inhibitors of p38 MAP kinase reduce the production of pro-inflammatory cytokines, e.g., IL-1, TNF-α, IFN-γ and IL-6, whose excessive production initiates events leading to inflammation and tissue destruction in diseases such as rheumatoid arthritis (RA).[1] p38 inhibitors not only block the synthesis but also the signal cascades induced by these cytokines;[2] in addition, p38 has been implicated in the induction of COX-2, the inducible prostaglandin cyclooxygenase.[3] The interest in the development of p38 MAP kinase inhibitors is based on the expectations, that p38 inhibiting drugs will treat the underlying cause of chronic inflammatory diseases and stop their progression. The present report describes efforts in the search, design and synthesis of p38 inhibitors and gives some results on biological activities.

Results and Discussion

Pyridinylimidazole SB 203580 is one of the best studied p38 inhibitors reported to date, with oral activity in several animal models of chronic inflammatory disease.[4] Presumably, modest bioavailability, toxicity and cytochrome P450 inhibition precluded its further development. As a predecessor of SB 203580, we claimed **1**[5] in 1972 as an anti-inflammatory agent, long before its target p38 was identified. Compound **1** is a moderately potent p38α inhibitor ($IC_{50} = 0.45$ μM), but shows COX-1 inhibition ($IC_{50} = 5$ μM) representing an unacceptable ulceration risk. Since pyridinylimidazoles including SB 203580[6] often block both enzymes, the design and synthesis of selective p38 inhibitors devoid of COX-1 inhibition became our primary goal.



SB 203580



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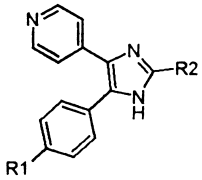
COX-1 activity appeared to be related to the lipophilicity of the substituent in position 2 of the imidazole ring. Replacement of one C-atom of the *tert* butyl group in **1** by a polar hydroxy functionality led to the selective p38 inhibitors **2** and **3** (Table 1). By increasing ring size and lipophilicity of the hydroxycycloalkyl group to 4-, 5-, 6-, or 7-membered rings, COX-1 inhibition gradually returned.



1-7

Although **3** in Table 1 showed the best p38α/COX-1 selectivity ratio, **6** was preferred for further optimisation

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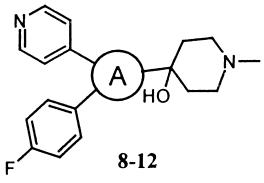
Table 1.

Compound	**1**	**2**	**3**	**4**	**5**	**6**	**7**
R_2							
R_1	H	F	F	F	F	F	F
p38α[7] IC_{50} (μM)	0.45	0.47	0.02	0.15	0.08	0.12	0.076
COX-1[8] IC_{50} (μM)	5.0	87.0	40.0	20.0	3.0	6.3	0.2

for two reasons: (a) polarity of **6** was easily enhanced by replacing one C-atom in the cyclohexanol moiety by an NMe group without introducing a chiral centre; (b) molecular modeling suggested that the piperidine nitrogen of the novel imidazole analogue **8** might form a salt bridge to Asp168 in p38α,[9] promising higher potency. Indeed, **8** was a better p38α inhibitor than **6** and was devoid of COX-1 activity (Table 2). **8** showed potent oral activity in the acute LPS induced TNF-α release model in mice, but was ineffective in the established rat adjuvant induced arthritis (AA) model. This prompted us to investigate related 5-membered piperidinol substituted heterocycles **A** in search of p38α inhibitors possessing useful in vivo activity in relevant models of RA. Table 2 summarizes in vitro and in vivo properties of five piperidinol-substituted heterocycles **A**. None of them showed COX-1 inhibition, while the 5-membered heterocycle had a prominent influence on in vitro and in vivo activities. Compounds **8**–**11** were acceptable p38α inhibitors, while oxazole **12** was rather inactive, pointing to the crucial importance of the nitrogen-position within the 5-membered heterocycle. Imidazole **8** and pyrrole **9** were nearly equipotent, but superior to thiazole **10** and oxazole **11**. Cellular TNF-α inhibition by **8**–**11** correlated well with enzyme inhibition, but much less with in vivo results. Only **8** and **11** gave TNF-α inhibitions in mice of >50% at a dose of 10 mg/kg po, while **9** and **10** were inactive. Unlike **8**, oxazole **11** proved to be effective in the AA-model and also potently inhibited swelling in the rat collagen induced arthritis (CIA). Absolute bioavailability of **11** amounted to 65% with maximal plasma levels of 1.96 μM at a dose of 10 mg/kg po.



8-12

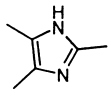
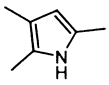
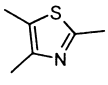
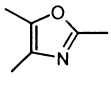
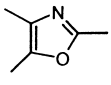
Pyridinyloxazole **11** was tested against 10 other kinases and showed sufficient selectivity to relate its in vivo effects to p38α inhibition (Table 3).

In contrast to SB 203580, which showed significant inhibition of human cytochrome P450 (Isoenzyme: IC_{50} (μM); CYP1A2: 3.8; CYP2C9: <2; CYP2D6: >100; CYP3A4: <2), **11** did not inhibit P450 (isoenzyme: IC_{50} (μM); CYP1A2: >100; CYP2C9: >100; CYP2D6; 21.7; CYP3A4: 100) in our test system.[18] Increased liver weight and significant elevations of hepatic P450 enzymes by SB 203580 were stated to be related to P450 inhibition and the latter to the presence of a pyridinyl group.[19] The pyridinyloxazole **11** is one representative of a class of compounds, whose pyridinyl group is selective for the amide NH of Met109[9] in the p38α

Table 3.

	p38β2[16]	p38δ[16]	JNK1[16]	JNK2[16]	PKCα[17]
11 IC_{50} (μM)	2.408	>10	>10	8.088	51.102
	ERK2[17]	MKK6b[17]	EGFR[17]	c-abl[17]	c-src[17]
11 IC_{50} (μM)	>10	>100	0.797	86.759	22.682

Table 2.

Compound	**8**	**9**	**10**	**11**	**12**
A					
p38α[7] IC_{50} (μM)	0.090	0.13	0.45	0.35	2
COX-1[8] IC_{50} (μM)	>100	>100	73	>100	n.t.[a]
TNFα release[10] IC_{50} (μM)	0.015	0.125	0.145	0.18	n.t.
TNFα release[11]% inhibition at 10 mg/kg po	77	18	0	50	n.t.
Bioavailability[12] (%)	23	n.t.	n.t.	65	n.t.
Cmax [13] (μM)	0.4	n.t.	n.t.	1.96	n.t.
AA[14] (% inhibition)	23	n.t.	n.t.	40	n.t.
CIA[15] ED_{50} po (mg/kg/day)	n.t.	n.t.	n.t.	2×10	n.t.

[a]n.t., not tested.

ATP-binding site without coordinating to the heme iron of P450.

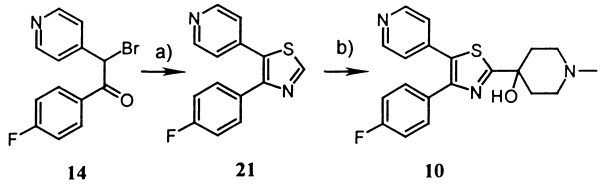
In conclusion, among a small series of heterocycles, oxazole **11** was the only compound with excellent pharmacokinetic properties and consequently good efficacy in relevant models of rheumatoid arthritis. Due to its lack of COX-1 and human cytochrome P450 inhibition, **11** is an interesting development candidate with a possibly low toxicological risk profile.

Chemistry

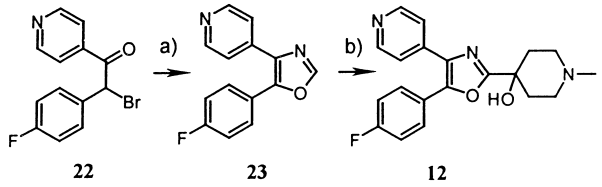
The synthesis of imidazoles **2**–**8** and oxazole **11** is summarized in Scheme 1. Ketone **13**[20] was brominated to yield bromoketone **14**,[21] which gave a 1:1 mixture of imidazole **15** and oxazole **16** following Bredereck's procedures.[22] Compound **15** was *N*-protected as diethoxyacetal **17**, deprotonated with *n*BuLi at −45 °C and reacted with ketones including *N*-methyl-4-piperidone to render imidazoles **2**–**8** in yields of 40–67%. Oxazole **16** was deprotonated with nBuLi at −40 °C and reacted with *N*-methyl-4-piperidone to yield **11**. The synthesis of pyrrole **9** is summarized in Scheme 2. Following the procedures of Severin et al.,[23] **13** was condensed with the monohydrazone of glyoxal to yield **18**, which was reduced by $Na_2S_2O_4$ and cyclised to pyrrole **19**. Bromination using NBS and subsequent SEM-protection yielded bromopyrrole **20**, which — after bromine-lithium exchange and reaction with *N*-methyl-4-piperidone, followed by SEM-deprotection — gave the desired pyrrole **9**.

The synthesis of thiazole **10** is described in Scheme 3. Bromoketone **14** and thioformamide generated in situ from formamide and P_2S_5[24] were converted to thiazole **21**. The lithium-salt of **21** was obtained via deprotonation with *n*BuLi at −40 °C and reacted with *N*-methyl-4-piperidone to the desired thiazole analogue **10**.

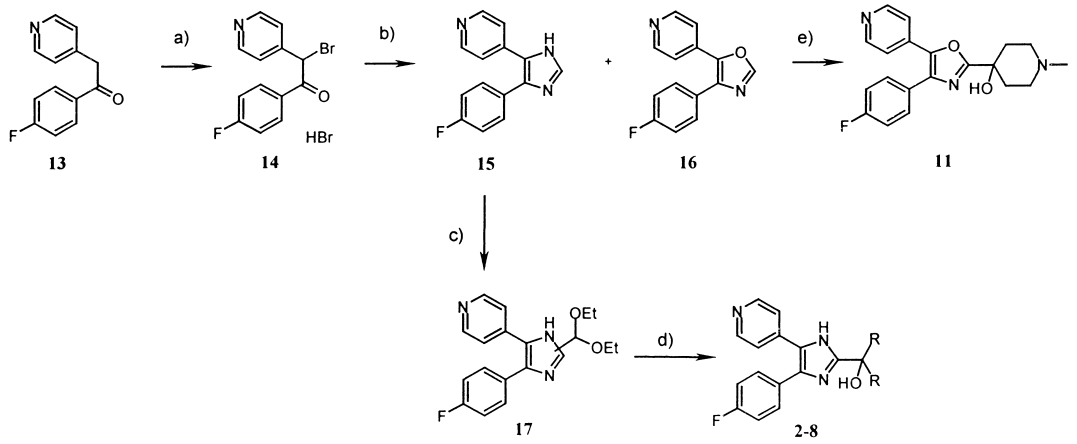
The synthesis of oxazole **12** is described in Scheme 4. Bromoketone **22**[25] was converted to oxazole **23** using formaldehyde and H_2SO_4.[22] Oxazole **23** was deprotonated with *n*BuLi and reacted with *N*-methyl-4-piperidone to yield the desired piperidinol **12**.



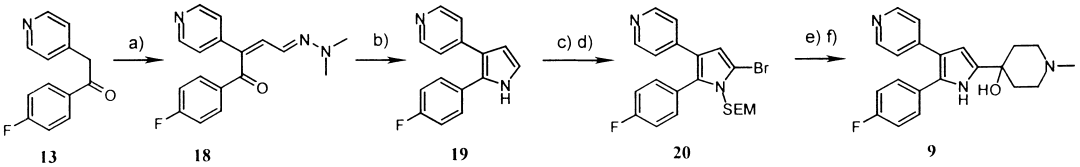
Scheme 3. (a) P_2S_5, $HCONH_2$, $NaHCO_3$, 90 °C, 30 min, 42%; (b) nBuLi, −40 °C, *N*-methyl-4-piperidone, 56%.



Scheme 4. (a) $HCONH_2$, H_2SO_4 concd 115 °C, 6 min, 30%; (b) *n*BuLi, THF, −40 °C, 15 min, then add *N*-methyl-4-piperidone, 18%.



Scheme 1. (a) Br_2, HOAc, 72%; (b) ammonium oxalate, $HCONH_2$, 200 °C, add **14** portionwise over 45 min. **15**: 26%, **16**: 31%; (c) $HC(OEt)_3$, pTsOH, reflux 2 h, 76%; (d) nBuLi, THF, −45 °C, ketone, 10 min, HCl, basic work up, 40–67%; (e) *n*BuLi, THF, −40 °C, *N*-methyl-4-piperidone, 62%.



Scheme 2. (a) Glyoxal-*N,N*-dimethyl monohydrazone, NaOEt, EtOH, reflux 3h, 93%; (b) $Na_2S_2O_4$, H_2O, tartaric acid, 75 °C, 10 min. 43%; (c) NBS, DMF, 0 °C, 95%; (d) DMF:THF (1:1), $KN(TMS)_2$, SEM-Cl, −78 °C, 52%; (e) *n*BuLi, THF, −78 °C, *N*-methyl-4-piperidone, 5 min., 57%; (f) Bu_4NF, 1 h, 60 °C, 86%.

References and Notes

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7. A phosphorylated form of His-p38α MAP kinase (10 ng/well) of murine origin was used and immobilised GST-ATF-2 as substrate in the presence of 120 μM cold ATP.
8. COX-1 from ram seminal vesicles (Nr. 60100; Cayman Chemical Company) was used and PGE2 determined by RIA.
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10. Human peripheral blood mononuclear cells from healthy volunteers were incubated with the inhibitor and stimulated with LPS/IFN-γ for 3 h. The supernatants were collected for TNF-α determination by ELISA.
11. Eight-week old female OF1 mice were dosed perorally by gavage with solutions of the compounds in DMSO/cornoil. One hour after dosing, LPS (20 mg/kg) was injected iv for stimulation of TNF-α release into plasma. One h later blood was collected and TNF-α was determined using a mouse specific ELISA.
12. Compound **8** was dosed at 1 mg/kg iv and 20 mg/kg po. Compound **11** was dosed at 3 mg/kg iv and 10 mg/kg po.
13. Cmax: maximal plasma concentration after oral dose (**8**: 20 mg/kg po. **11** mg/kg po).
14. AA: adjuvant induced arthritis. Female Wistar rats were immunized with Mycobacterium tuberculosis at day 0 and dosed with the compounds (2×25 mg/kg po per day) from day 14–20. Swelling of the joints was measured on day 20.
15. CIA:collagen induced arthritis. Female (WAG×BUF/F1) rats were immunized intradermally with bovine nasal septum type II collagen emulsified in Freund's incomplete adjuvant. Compound **11** was dosed twice daily for 10 days after the onset of swelling.
16. Phosphorylated forms of His-p38β2, His-p38δ, His-JNK1 and His-JNK2 MAP kinases of human origin phosporylated the immobilised substrate GST-ATF-2 in the presence of cold ATP (120 μM). Antibody detected phosphorylated GST-ATF-2.
17. A phosphorylated form of murine **ERK2** MAP kinase phosphorylated a peptide in the presence of cold and 0.1 μCi of (^{32}P)γ-ATP. **MKK6b**(EE) kinase: An active form of GST-MKK6b(EE) of human origin phosporylated the immobilised substrate GST-p38α(K >M) in the presence of cold ATP (12 μM). **EGFR** kinase: purified human EGFR-ICD, 400 nM cold ATP, poly (EY) as substrate and 0.1 μCi of (^{32}P)γ-ATP. **C-abl**: murine enzyme, cold ATP (5 μM), poly(AEKY) as substrate and 0.06 μCi of (^{32}P)γ-ATP. **C-src**: activated chicken kinase 20μM, cold ATP, poly (EY) as substrate and 0.07 μCi of (^{32}P)γ-ATP. **PKCα**: bovine kinase, 10 μM cold ATP, substrate protamine sulfate and 0.1 μCi of (^{32}P)γ-ATP.
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