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Novel p38 inhibitors with potent oral efficacy in several models of rheumatoid arthritis

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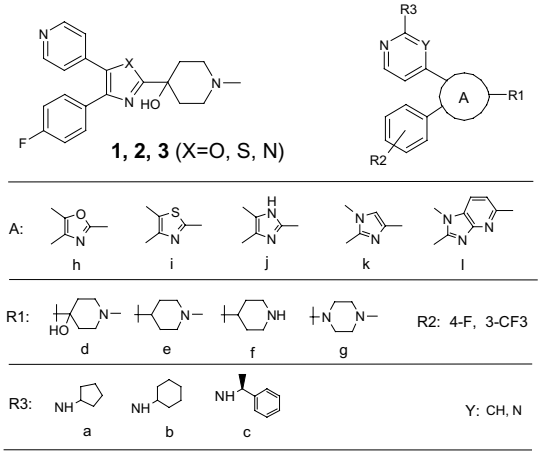
Abstract—A library of trisubstituted oxazoles, thiazoles, imidazoles (1,2,4- and 2,4,5-substituted) and imidazo[1,2-*b*]pyridines was prepared and evaluated in vitro as p38α inhibitors and in vivo in several models of rheumatoid arthritis. Four structures—**32**, **37**, **45** and **59**—were identified as potent inhibitors of p38α with high efficacy in the LPS induced TNFα release model in the mouse, the adjuvant induced arthritis and the collagen induced arthritis in the rat with ED_{50}s between 1.0 and 9.5 mg/kg p.o.

Inhibition of p38α has become one of the major targets in developing anti-inflammatory drugs, which is due to the prominent role in regulating inflammatory cytokines like TNFα and IL-1.[1] The benefits of treating rheumatoid arthritis patients with TNFα inhibitors (Enbrel® and Remicade®) or IL-1 inhibitors (Kineret®) has raised the desire to develop orally available medication. Blockade of p38α is a very attractive option, since p38α inhibitors are of low molecular weight, downregulate production and signalling of TNFα, IL-1 and in addition inhibit COX-2 induction. The value of COX-2 inhibitors (Celebrex®, Vioxx®) has been proved by their successful use in arthritic diseases.

We have previously disclosed[2] the anti-inflammatory properties of 4-hydroxypiperidine substituted pyridinyloxazoles, -imidazoles and -thiazoles **1**, **2**, **3** (Scheme 1) as moderately potent p38α inhibitors. Here we wish to report on the extension of this work directed towards a series of p38α inhibitors with increased in vitro potency and oral efficacy in several animal models of rheumatoid arthritis. The novel series (Scheme 1) was assembled around the heterocyclic template A, which represents a diverse set of five scaffolds h–l. Alkylamine substituents R3 (a–c) attached to the pyridinyl and pyrimidinyl rings (Y = CH, N) were chosen to reach into a lipophilic binding pocket of p38α not utilized by ATP itself, while



Scheme 1.

the basic piperidinyl- and piperazinyl substituents R1 were expected to form a salt bridge with Asp168.[3]

4-Hydroxypiperidinyl substituted oxazoles, thiazoles and imidazoles **22**–**26** were prepared according to Scheme 2, starting from bromoketones[4] **4** and **5**, which were reacted at elevated temperature with formamide/H_2SO_4 for oxazoles **6** and **10**, formamide/P_2S_5 for thiazole **9** or ammonium formate/formamide/formic acid providing imidazoles **7** and **11**. SEM-protection of imidazoles, deprotonation by *n*BuLi at −100 to −40 °C and treatment with *N*-methyl-4-piperidone converted **6**, **8**, **9**, **10** and **12** into the 4-hydroxypiperidinyl derivatives

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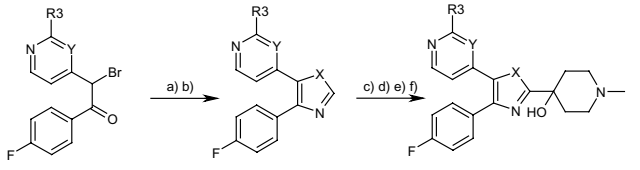


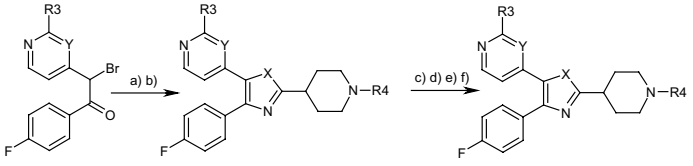
Scheme 2. Reagents and conditions: (a) Formamide, H_2SO_4 cat., 150 °C, 15 min, 25–50% **6**, **10**. Ammonium formate, formamide, formic acid, 165 °C, 20 min, 30–45% **7**, **11**. Formamide, P_2S_5, 30 min, 90 °C, 44% **9**. (b) $KN(TMS)_2$, THF/DMF (1:2), SEM-Cl, −78 °C to rt, 54–59% **8**, **12**. (c) *n*BuLi, THF, *N*-methyl-4-piperidone, −100 or −78 or −40 °C depending upon substrate; 20–60% **13**–**17**. (d) *m*CPBA, CH_2Cl_2/HOAc (1:2) 0 °C, 10 min, 60–70% **18**, **19**. (e) *S*-(−)-1-Phenylethylamine, 140–190 °C, 30 min to 4 h; 35–70% **20**–**24**. (f) EtOH/HCl concd (1:1) rt, 20 min, 80–90% **25**, **26**.

13–**17**. The R3 group in **20**–**24** was introduced by heating sulfoxides **18**, **19** and fluorides **13**–**15** with *S*-(−)-1-phenylethylamine. Removing the SEM-protecting group from imidazoles **20** and **21** delivered **25** and **26**.

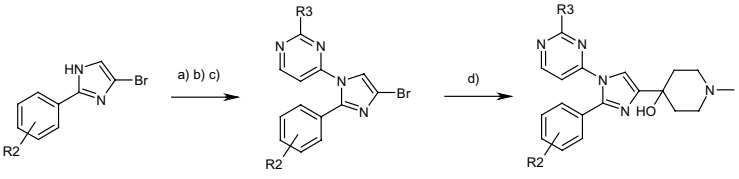
Piperidinyl substituted oxazoles **32**, **34** and thiazoles **36**, **37** were obtained according to Scheme 3 by condensing bromoketone[4] **4** or **5** with the amide or thioamide[4] of N-protected piperidine-4-carboxylic acid. Thiazole **28** was available in 70% yield, while the yields for oxazoles **27** and **29** were lower with 11% and 40%. Fluorides **27**, **28** and sulfoxide **30** heated with cyclohexylamine or *S*-(−)-1-phenylethylamine provided oxazoles **31**, **33** and thiazole **35**. Removing the protecting groups generated the piperidinyl-NH substituted oxazoles **32**, **34** and thiazole **36**. N-methylation of **36** rendered thiazole **37**.

1,2,4-Substituted imidazoles **44** and **45** were prepared according to Scheme 4, starting from 2-aryl-4-bromoimidazoles[5] **38** and **39**, which were regioselectively converted into 4-imidazol-1-ylpyrimidines **40** and **42**. The corresponding sulfoxides were subsequently heated with *S*-(−)-1-phenylethylamine and cyclopentylamine to provide pyrimidines **41** and **43**. Br/Li exchange and treatment with *N*-methyl-4-piperidone at low temperature gave the desired 4-hydroxy-1-methylpiperidine substituted imidazoles **44** and **45**.

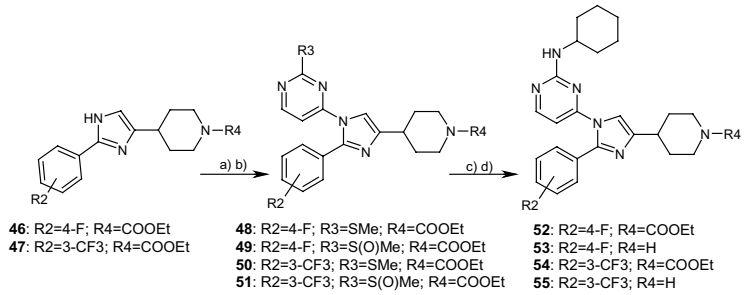
1,2,4-Substituted imidazoles **53** and **55** were prepared according to Scheme 5, starting with the regioselective pyrimidinylation of 2-phenyl-4-(4-piperidinyl)-imidazoles[5] **46** and **47** to generate **48** and **50**. The corresponding sulfoxides **49** and **51** were heated with cyclohexylamine and provided imidazol-1-yl pyrimidines **52** and **54**. Removal of the ethylcarbamate pro-



Scheme 3. Reagents and conditions: (a) 4-Carbamoylpiperidine-1-carboxylic acid *tert* butyl ester, melt at 160 °C, 20 min. (ii) EtOH/HCl concd (1:1) rt, 10 min. (iii) BOC_2O, THF, rt, 1 h, 11% **27**. 4-Thiocarbamoylpiperidine-1-carboxylic acid ethyl ester,[4] DMF, 60 °C, 30 min, 70% **28**. DMPU, 4-carbamoylpiperidine-1-carboxylic acid *tert* butyl ester, 150 °C, 15 min, 40% **29**. (b) *m*CPBA, CH_2Cl_2/HOAc (1:2) 0 °C, 10 min, 90% **30**. (c) (i) Cyclohexylamine, 200 °C, 70 min. (ii) BOC_2O, THF, rt, 1 h, 79% **31**. Cyclohexylamine, 110 °C, 1 h, 65% **33**. *S*-(−)-1-Phenylethylamine, 195 °C, 5 h, 67% **35**. (d) EtOH/HCl concd (1:1), 10 min, rt, 70–80% **32**, **34**. (e) $CHCl_3$, TMSI, 4.5 h, 60 °C, 80% **36**. (f) $LiAlH_4$, THF, reflux, 30 min, 80% **37**.



Scheme 4. Reagents and conditions: (a) 4-Chloro-2-methylthio pyrimidine, toluene, $KN(TMS)_2$, 24 h, 80 °C, 60–90% **40**, **42**. (b) *m*CPBA, CH_2Cl_2, 0 °C, 15 min, 90%. (c) *S*-(−)-1-Phenylethylamine, 5 min, 120 °C, 78% **41**. Cyclopentylamine, 30 min, 60 °C, 80% **43**. (d) *n*BuLi, THF, *N*-methyl-4-piperidone, −100 °C (for **45**) or −78 °C (for **44**), 36–42%.

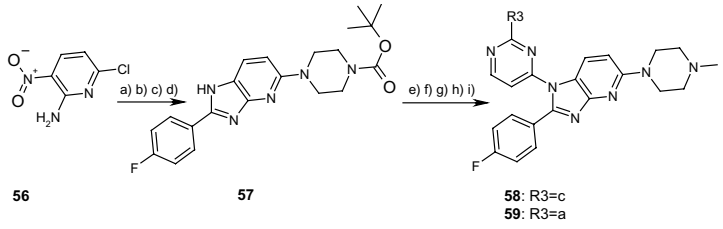


Scheme 5. Reagents and conditions: (a) 4-Chloro-2-methylthio pyrimidine, DMF/toluene (2:1), $KN(TMS)_2$, 8 h, 80 °C, 70–80% **48**, **50**. (b) *m*CPBA, CH_2Cl_2/HOAc (1:2) 0 °C, 10 min, 90% **49**, **51**. (c) Cyclohexylamine, 1 h, 110 °C, 80% **52**, **54**. (d) TMSI, $CHCl_3$, 3.5 h, 60 °C, 75% **53**, **55**.

tecting group by treating with TMSI yielded the NH-piperidines **53** and **55**.

Piperazine substituted imidazo[4,5-*b*]pyridines **58** and **59** were prepared according to Scheme 6, starting from 2-amino-6-chloro-3-nitropyridine[6] **56**. Chlorine in **56** was substituted by mono-protected piperazine, the nitro group hydrogenated, and the resulting 2,3-diamino-pyridine condensed with 4-fluorobenzoic acid in polyphosphoric acid (PPA) to provide **57**. Regioselective pyrimidinylation of **57** with 2-methylthio-4-iodopyrimidine,[7] oxidation of sulfide to sulfoxide and introduction of alkylamine side chains, removing the Boc-protecting group and reductive amination gave the *N*-methylpiperazines **58** and **59**.

Table 1 summarizes the p38α[8] and cellular IC_{50} values[9] and the % inhibition of LPS induced TNFα release in mice upon oral administration.[10] Compared to the lead structures **1**–**3**, the novel compounds **22**–**59** in Table 1 showed a 45–150-fold increase in binding and cellular activity, some with excellent oral efficacies. p38α affinities showed little dependency of the central scaffold A, which implies that scaffolds h–l are bioisosters, capable



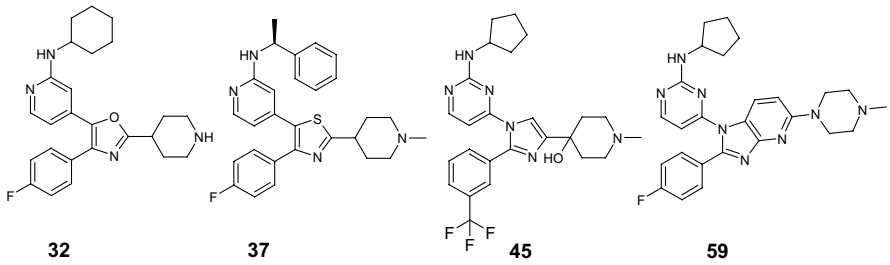
Scheme 6. Reagents and conditions: (a) Piperazine-1-carboxylic acid ethyl ester, isopropanol reflux 2–3 h, 75%. (b) Pd/C, H_2, EtOH, 85%. (c) 4-Fluorobenzoic acid, PPA, 130–150 °C, 1–5 h, 55%. (d) BOC_2O, EtOAc, Na_2CO_3, rt, 2 h, 70%. (e) $KN(TMS)_2$, 2-methylthio-4-iodopyrimidine, DMF, 2 h, 155 °C, 55%. (f) *m*CPBA, CH_2Cl_2/HOAc (1:1), 0 °C, 30 min, 65%. (g) *S*-(−)-1-Phenylethylamine or cyclopentylamine, 1 h, 120 °C, 70–80%. (h) EtOH/HCl concd (1:1) rt, 1 h, 90%. (i) $NaBH_4$, 40% aq formaldehyde, 2 h, rt, 80–90%.

Table 1

Compound	A	R1	R2	R3	Y	p38α[a,8]	TNFα/hPBMC[a,9]	TNFα/mouse[10] (%)
1	h	d	4-F	H	CH	0.350	0.18	50
2	i	d	4-F	H	CH	0.450	0.145	0
3	j	d	4-F	H	CH	0.090	0.015	77
22	h	d	4-F	c	CH	0.009	0.003	21
23	h	d	4-F	c	N	0.011	0.008	19
24	i	d	4-F	c	CH	0.005	0.001	94
25	j	d	4-F	c	CH	0.007	0.003	42
26	j	d	4-F	c	N	0.002	0.0001	83
32	h	f	4-F	b	CH	0.015	0.0002	90
34	h	f	4-F	b	N	0.011	0.009	48
36	i	f	4-F	c	CH	0.004	n.t.	63
37	i	e	4-F	c	CH	0.018	0.0006	88
44	k	d	4-F	c	N	0.004	n.t.	94
45	k	d	3-CF3	a	N	0.029	0.001	94
53	k	f	4-F	b	N	0.005	0.0008	29
55	k	f	3-CF3	b	N	0.008	0.002	77
58	l	g	4-F	c	N	0.006	0.001	83
59	l	g	4-F	a	N	0.015	0.001	94

[a] IC_{50} (μM).

Table 2



32 **37** **45** **59**

	32	37	45	59
LPS/TNFα[10] ED_{50} (mg/kg p.o.)	2.29	2.06	1.07	1.41
AIA[11] % swelling (25 mg/kg b.i.d. p.o.)	64	57	58	55
CIA[12] ED_{50} (mg/kg p.o. q.d.)	1.83	7	9.51	4.22
F (%)	50.1	20.3	23.2	72.8
Half life (h)	21.2	12.5	3.5	4.1
Log *D* (pH 7.4)	2.9	6.0	2.1	2.0

to position their aromatic, heteroaromatic and heterocyclic substituents in a spacial relationship favourable to interact with the p38α binding pocket. While the new series are potent in vitro inhibitors (p38α IC_{50}s: 0.02–0.002 μM; hPBMC IC_{50}s: 0.036–0.0001 μM) their in vivo efficacies vary in a broad range between inactive to highly potent upon oral administration. Among the four oxazoles (A = h) **22**, **23**, **32** and **34**, only **32** showed >80% inhibition in the acute TNFα mouse model. Among the six imidazoles (A = j, k) **25**, **26**, **44**, **45**, **53** and **55**, only **26** and **45** showed excellent oral efficies in spite of low bioavailability (*F*: 10% for **26**; 23% for **45**). Among the three thiazoles (A = i) **36**, **37** and **24**, only the latter two showed >80% inhibition in the TNFα/mouse model. Imidazo[1,2-*b*]pyridines (A = l) **58** and **59** were both potent upon oral administration.

From eight compounds in Table 1 with >80% inhibition in the acute LPS/TNFα model, five also showed good efficacy in the subchronic adjuvant induced arthritis (AIA)[11] model in the rat with swelling inhibited by 55–64% at a dose of 25 mg/kg b.i.d. p.o. Compounds demonstrating low body weight increase in AIA, low bioavailability, inhibition of cytochrome P450 isoenzymes[13] or COX-1 inhibition[14] or genotoxicity[15] were dropped from further profiling, leaving four structures to be evaluated in the collagen induced arthritis (CIA)[12] model in the rat: **32**, **37**, **45** and **59**. Table 2 summarizes the in vivo data and reveals the high potency of all four compounds in three relevant models of rheumatoid arthritis. LPS induced TNFα release in mice was inhibited with ED_{50}s between 1.07 and 2.29 mg/kg p.o., while the ED_{50}s to inhibit swelling in rat CIA[12] were between 1.83 and 9.51 mg/kg p.o. Histological evaluation of hind paws from CIA-rats (not shown here) revealed a dose-dependent protective activity on bone apposition, loss of proteoglycans, cartilage damage and infiltration of cells, pointing to a disease modifying potential of **32**, **37**, **45** and **59** in rheumatoid arthritis. The range of half lives between 3.5 and 21.2 h, and oral bioavailabilities (*F*) between 20.3% and 72.8% offered interesting pharmacokinetic profiles. **45** appeared to be the most selective analogue and exhibited a >160-fold selectivity against a panel of 13 kinases,[16] while **32**, **37** and **59** had submicromolar affinities against kinases like JNK2, EGFR or HER-1.[16]

In summary, our pilot library outlined in Scheme 1 comprising 18 compounds delivered four structurally diverse and novel p38α inhibitors with impressive enzymatic, cellular and in vivo activities in three models of rheumatoid arthritis.

Acknowledgements

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8. A phosphorylated form of His-p38α MAP kinase (10 ng/well) of murine origin was used and immobilized GST-ATF-2 as substrate in the presence of 120 μM cold ATP.
9. Human peripheral blood mononuclear cells from healthy volunteers were incubated with the inhibitor and stimu-

lated with LPS/IFN-γ for 3 h. The supernatants were collected for TNF-α determination by ELISA.
10. Eight-week old female OF1 mice were dosed perorally by gavage with solutions of the compounds (10 mg/kg) in DMSO/cornoil. One hour after dosing, LPS (20 mg/kg) was injected iv for stimulation of TNF-α release into plasma. One hour later blood was collected and TNF-α was determined using a mouse specific ELISA.
11. AIA: adjuvant induced arthritis. Female Wistar rats were immunized with Mycobacterium tuberculosis at day 0 and dosed with the compounds (2×25 mg/kg p.o. per day) from day 14 to 20. Swelling of the joints was measured on day 20.
12. CIA: Collagen induced arthritis. Female (WAGxBUF/F1) rats were immunized intradermally with bovine nasal septum type II collagen emulsified in Freund's incomplete adjuvant. Swelling started ~10 days after immunization. Dosing of compounds started on day 13, when swelling was nearly maximal. Compounds were dosed once (q.d.) daily for 10 days.
13. Profiling continued, if $IC_{50} > 2\,\mu M$ for human P450 isoenzymes CYP1A2, CYP2C9, CYP2D6, CYP3A4.
14. Profiling continued, if $IC_{50} > 80\,\mu M$ for COX-1.
15. Profiling continued, if Ames assay and the Comet assay (in vitro with human lymphocytes) were negative.
16. Selectivity profiles were determined in house as described.[17] Kinase (IC_{50} (μM) for **32/37/45/59**): hJNK1 (0.15/1.9/>10/1.5); hJNK2 (0.03/0.261/4.66/0.047); CDK1 (>10/>10/>10/>10); HER-1 (1.1/0.28/>10/>10); c-Abl (>10/>10/>10/>10); c-Src (>10/8.0/>10/>10); Kdr (1.4/1.7/>10/>10); c-Met (>10/>10/>10/>10); c-Kit (>10/>10/>10/>10); IGF1R (>10/>10/>10/>10); HER-2 (2.0/1.1/>10/>10); c-Raf (8.7/2.1/>10/>10); hEGFR (1.1/0.7/>10/8.7).
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