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SAR of benzoylpyridines and benzophenones as p38α MAP kinase inhibitors with oral activity

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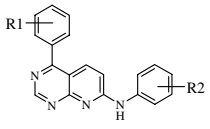
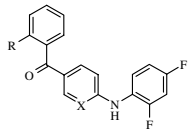
Abstract—Benzoylpyridines and benzophenones were synthesized and evaluated in vitro as p38α inhibitors and in vivo in several models of rheumatoid arthritis. Oral activity was found to depend upon substitution: 1,1-dimethylpropynylamine substituted benzophenone **10b** (IC_{50}: 14 nM) and pyridinoyl substituted benzimidazole **17b** (IC_{50}: 21 nM) showed highest efficacy and selectivity with ED_{50}s of 9.5 and 8.6 mg/kg po in CIA.

TNFα inhibitors (Etanercept, Infliximab) and IL-1 inhibitors (Anakinra) have demonstrated clinical efficacy in the treatment of rheumatoid arthritis and have raised the desire to develop small molecules as inhibitors of TNFα and IL-1. Blockade of p38α[1] is a very attractive option for this purpose, since p38α inhibitors 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.

Since the discovery of the pyridinylimidazoles,[2] several novel structural classes of p38 inhibitors have been reported.[3] During our own efforts directed towards the synthesis of p38 inhibitors distinct from the pyridinylimidazoles, we discovered that pyrido[2,3-*d*]pyrimidine **A** was a weak inhibitor, while the ring-opened benzoylpyridines and benzophenones[4] **B** were much more potent. We now wish to report on the in vivo and in vitro SAR of **B**.



A



B (X = C, N)

Benzoylpyridines **6a–c** and **7a,b** (Scheme 1) were prepared from 2,5-dibromopyridine **1**,[5] which was reacted with 2,4-difluoroaniline to render **2**,[6] employing the Buchwald[7] coupling conditions.

Combining the dilithium anion of **2**[6] and aldehydes **3a–c** yielded alcohols **4a–c**. Jones[8] oxidation generated the benzoylpyridines **5a–c**. Sonogashira[9] coupling of bromides **5b,c** with 1,1-dimethylpropynylamine or 1,1-dimethylpropynol gave rise to **6a–c**. Hydrogenation of **6a** and **6c** over Pd/C gave 1,1-dimethylpropylamine **7a** and 1,1-dimethylpropanol **7b**.

Benzophenones **10a**, **10b**, **11–13** (Scheme 2) were prepared from aldehydes **3a–d**, which were first reacted at −78 °C with 1-bromo-4-lithiobenzene to provide the corresponding alcohols. Jones[8] oxidation of the latter provided the bromobenzophenones **8a–d**. The dibromides **8c,d** reacted regioselectively under Buchwald[7] coupling conditions with 2,4-difluoroaniline to provide the bromides **9c,d**. Sonogashira[9] coupling of **9c,d** with 1,1-dimethylpropynylamine yielded propargyl amines **10a** and **10b**. Hydrogenation of **10b** over Pd/C gave

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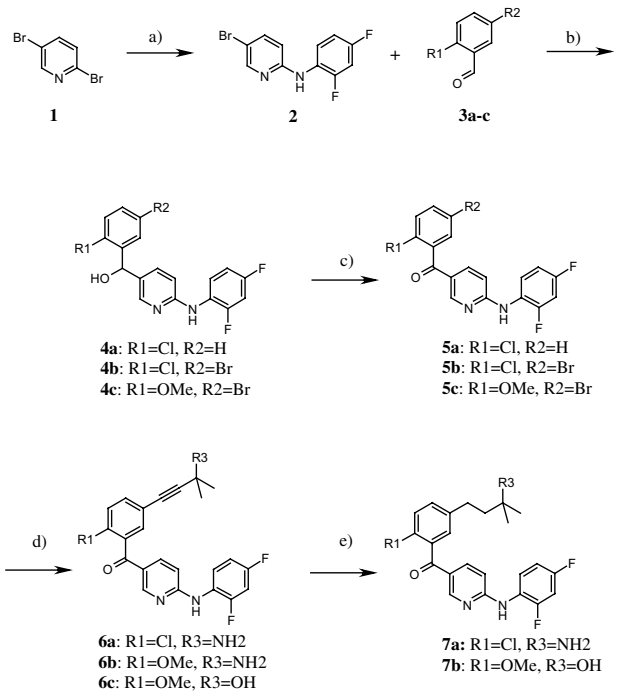


Scheme 1. (a) $Pd(OAc)_2$, NaO*t*Bu, R-(+)-BINAP, 2,4-difluoroaniline, 1,4-dioxane, reflux 1 h, 57%; (b) −78 °C, *n*BuLi, (2.2 equiv), **2**,[6] then add aldehyde **3a**–**c**; 50–60%; (c) CrO_3, H_2SO_4, acetone, room temperature, 10 min, 48–85%; (d) **6a**: $PdCl_2(PPh_3)_2$, CuI, 1,1-dimethylpropynylamine, reflux in Et_3N 1 h, 63%. **6b**: $PdCl_2(PPh_3)_2$, CuI, Cs_2CO_3, 1,1-dimethylpropynylamine, reflux in DIPEA, 30 min, 84%. **6c**: $PdCl_2(PPh_3)_2$, CuI, 1,1-dimethylpropynol, Et_3N, DMF, 100 °C, 1.5 h, 70%. (e) Pd/C, H_2, EtOH, 15 min, room temperature, **7a**: 45%, **7b**: 85%.

propylamine **11**. The 4-hydroxy-4-vinylpiperidine substituted benzophenone **12** was obtained by coupling bromide **9d** with vinylstannane **14**[6] under Stille[10] conditions. Hydrogenation of **12** gave **13**.

Pyridinoylbenzimidazoles **17a**,**b** (Scheme 3) were prepared from aldehydes **15a**,**b**.[6] Combining the latter with the dilithium anion of **2**[6] provided alcohols **16a**,**b**, which upon Jones[8] or MnO_2[11] oxidation rendered the desired benzimidazoles **17a**,**b**.

Benzoylbenzimidazoles **19a**,**b** (Scheme 4) were prepared from aldehydes **15a**,**b**,[6] which reacted at −78 °C with 1-bromo-4-lithiobenzene to the expected alcohols. The latter were oxidized with MnO_2[11] to the corresponding ketones. As Buchwald[7] reactions failed using the unprotected imidazoles, a SEM protecting group was introduced first. Buchwald reaction of **18a**,**b** with 2,4-difluoroaniline followed by SEM-deprotection yielded **19a** and **19b**.

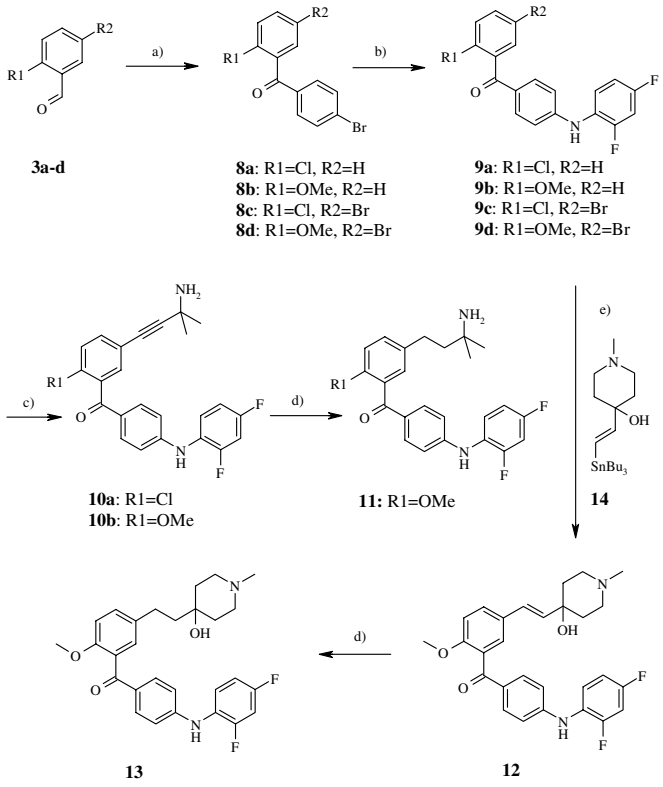
Table 1 summarizes the p38α[12] IC_{50} values and the % inhibition of LPS induced TNFα release in mice upon oral administration. Bulky R_2 substituents such as bromine in **5b** were not favoured, whereas the rigid 1,1-dimethylpropynylamino group in **6a** and **6b** increased p38α inhibitory potency 5–10 fold to $IC_{50} = 19$ and 8 nM. One may speculate that the NH_2 group in R_2 increases potency by interacting with the acidic Asp168 of p38α.[13] The 1,1-dimethylpropynol derivative **6c** was seven times weaker than its NH_2-analogue **6b**. Saturation of the triple bonds in **6a** and **6c** led to the 1,1-dimethylpropylamine **7a** and 1,1-dimethylpropanol **7b** with three freely rotatable C–C bonds. The effect of increased flexibility in the side chain resulted in an 8-fold loss in potency, with the amine **7a** still being three times more potent than the alcohol **7b**. Benzophenones and benzoylpyridines showed similar SAR; benzophenones were slightly more potent, when comparing **5a** and **6a** with **9a** and **10a**. 1,1-Dimethylpropynylamine substituted benzophenones **10a** and **10b** showed IC_{50}s of 6 and 14 nM, close to the benzoylpyridine analogues **6a**,**b**. Saturation of the triple bond of **10b** to **11** resulted in an 8-fold loss in activity. Introduction of the 1-methyl-4-hydroxy-4-vinylpiperidine group yielded the highly potent p38α inhibitor **12** ($IC_{50} = 1$ nM). Reduction of the double bond in **12** produced **13** with increased flexibility of the side chain and an expected (50-fold) loss in affinity.

Pyridinoyl substituted benzimidazoles **17a** and **17b** as well as the benzoyl substituted benzimidazoles **19a** and **19b** were strong p38α inhibitors, **19a** being the most potent of the series with $IC_{50} = 0.7$ nM. As above, the benzoyl analogues **19a** and **19b** were 4–10 times more potent than their pyridinoyl analogues **17a** and **17b**.

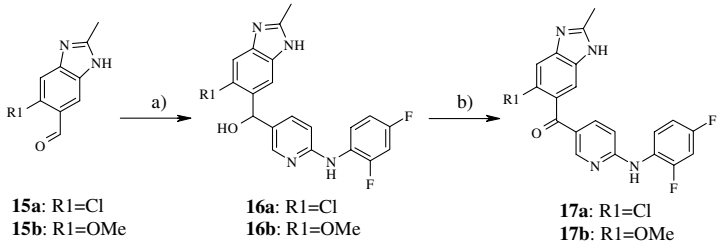
Compounds with $IC_{50} < 120$ nM against p38α were further tested in vivo in the acute LPS induced TNFα release model in the mouse.[14] Unsubstituted benzophenones ($R_2 = H$) **9a** and **9b** were orally inactive (Table 1), possibly due to their high lipophilicity. The hydrophilicity increasing groups 1,1-dimethylpropynylamine, 1,1-dimethylpropynol and 1-methyl-4-hydroxy-4-vinylpiperidine conferred potent oral activity in **6a**–**c**, **10a**,**b** and **12**, which inhibited TNFα by 50–95% at 20 mg/kg po. Benzimidazoles **17a**,**b** and **19a**,**b** were equally potent, inhibiting TNFα release by 57–93% at 20 mg/kg po. The saturated analogues **11** and **13** were devoid of oral efficacy.

From the 10 compounds with oral activity in the acute LPS/TNFα model,[14] nine also showed good efficacy in the subchronic adjuvant induced arthritis (AIA)[15] model in the rat at a dose of 25 mg/kg b.i.d. po; swelling was inhibited by 36–70% (Table 2). At this stage, compounds demonstrating low body weight increase in the AIA model or inhibition of cytochrome P450 isoenzymes[16] or COX-1 inhibition[17] or genotoxicity[18] were dropped from further profiling, leaving three structures for further investigation in the collagen induced arthritis (CIA)[19] model in the rat: **10b**, **17a** and **17b**. While **17a** proved ineffective in CIA, **10b** and **17b** had ED_{50}s of 9.5 mg/kg qd and 8.6 mg/kg po qd. The ED_{50} values compared favourably with two non-pyridinylimidazoles currently in clinical trials.[20,21]

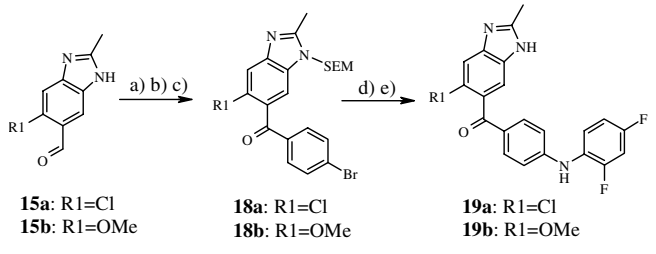
Pharmacokinetic profiles of **10b** and **17b** in the rat showed marked differences in their volumes of distribution (Vss) and the maximal plasma concentrations



Scheme 2. (a) 1. *n*BuLi, 1,4-dibromobenzene, THF, −78 °C, 5 min, then add aldehyde **3a**–**d**; 70–85%; 2. CrO_3, H_2SO_4, acetone, water, room temperature, 10 min, 50–83%. (b) **9a**–**c**: $Pd(OAc)_2$, NaO*t*Bu, R-(+)-BINAP, 2,4-difluoroaniline, 1,4-dioxane, reflux 20 min; 10–40%. **9d**: $Pd(OAc)_2$, Cs_2CO_3, R-(+)-BINAP, 2,4-difluoroaniline, 1,4-dioxane, reflux 2.5 h, 65%. (c) **10a**: $PdCl_2(PPh_3)_2$, CuI, 1,1-dimethylpropynylamine, reflux in Et_3N, 1 h, 86%. **10b**: $PdCl_2(PPh_3)_2$, CuI, Cs_2CO_3, 1,1-dimethylpropynylamine, reflux in DIPEA/diglyme (2:1) 3.5 h, 76%. (d) Pd/C, H_2, EtOH, 1 h, room temperature, 72%. (e) $Pd(PPh_3)_4$, toluene, **9d**, **14**,[6] 100 °C, 12 h, 33%.



Scheme 3. (a) **2**,[6] *n*BuLi (2.2 equiv), −78 °C, THF, 20 min, then add aldehyde (0.5 equiv), 10 min, −70 °C, 46–76%. (b) **17a**: MnO_2, acetone, 30 min, 35 °C, 36%. **17b**: CrO_3, H_2SO_4, acetone, water, room temperature, 10 min, 71%.



Scheme 4. (a) *n*BuLi (3.3 equiv), 1,4-dibromobenzene (3.0 equiv), THF, −78 °C, 15 min, add aldehyde (1 equiv), 10 min, −70 °C, 80–90% of alcohol. (b) MnO_2, acetone, reflux 10 min, 80–90%. (c) $KN(TMS)_2$, THF, −80 °C, 5 min, add SEM-Cl, 0 °C, 5 min, **18a**,**b**: 75–80% (mixture of regioisomers). (d) $Pd(OAc)_2$, Cs_2CO_3, R-(+)-BINAP, 2,4-difluoroaniline, 1,4-dioxane, reflux 15 min, 77–87%. (e) EtOH/HCl conc. (1:1) 5 min, 60 °C, **19a**,**b**: 80–90%.

(C_{max}), while oral bioavailabilities with 46% and 54% as well as terminal half lives with 7 and 4.4 h were similar. Compound **10b** showed a high Vss of 9 L kg^{-1} and a low C_{max} (@ 1 mg/kg po) of 59 nmol, while **17b** had a low Vss of 1.1 L kg^{-1} and a high C_{max} (@ 1 mg/kg po) of 607 nmol pointing to high plasma protein binding of **17b**. Both compounds had a satisfactory selectivity profile[22] (**17b** was $\geqslant$1000-fold, **10b** $\geqslant$100-fold selective against a panel of 13 kinases).

In summary, we discovered a series of novel benzoylpyridines and benzophenones as p38α inhibitors. Appropriate substitution of these structures leads to compounds with potent oral efficacy in disease models of rheumatoid arthritis.

Table 1

Benzoylpyridines			Benzophenones			Benzimidazoles		
Nr	p38α[a]	TNFα[b]	Nr	p38α[a]	TNFα[b]	Nr	p38α[a]	TNFα[b]
5a	101	n.t.	**9a**	11	6	**17a**	8	91
5b	300	n.t.	**9b**	38	0	**17b**	21	57
6a	19	50	**10a**	6	85	**19a**	0.7	93
6b	8	91	**10b**	14	95	**19b**	5	90
6c	54	88	**11**	113	0			
7a	160	n.t.	**12**	1	68			
7b	479	n.t.	**13**	51	0			

[a] IC_{50}(nM).[12]
[b] % Inhibition of LPS induced TNFα release in mice at 20 mg/kg po.[14]

Table 2

Benzoylpyridines			Benzophenones			Benzimidazoles		
Nr	AIA[a]	CIA[b]	Nr	AIA[a]	CIA[b]	Nr	AIA[a]	CIA[b]
6a	36	n.t.	**10a**	51	n.t.	**17a**	40	[c]
6b	52	n.t.	**10b**	45	9.5	**17b**	70	8.6
6c	23	n.t.	**12**	47	n.t.	**19a**	46	n.t.
						19b	50	n.t.

[a] % Inhibition of swelling in adjuvant induced arthritis rats (AIA)[15] at 25 mg/kg po b.i.d.
[b] CIA: collagen induced arthritis in rats.[19] ED_{50} (mg/kg po).
[c] 10 mg/kg po: 10% inhibition of swelling.

References and notes

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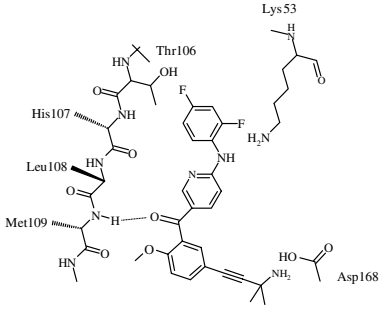
12. A phosphorylated form of His-p38α MAP kinase (10 ng/well) of murine origin was used and immobilized GST-ATF-2 as substrate in the presence of 120 μM cold ATP.
13. The proposed binding mode of benzoylpyridines and benzophenones to p38α implies, that the carbonyl oxygen forms a crucial H-bridge to the NH of Met109 and the difluorophenyl ring accommodates in the lipophilic binding pocket near Thr106. Reduction of the ketone to an alcohol or to a methylene group renders molecules inactive (data not shown).



Proposed binding mode of **6b** to p38α

14. Eight-week old female OF1 mice were dosed orally by gavage with solutions of the compounds in DMSO/cornoil. One hour after dosing, LPS (20 mg/kg) was injected iv for stimulation of TNFα release into plasma. One hour later blood was collected and TNFα was determined using a mouse specific ELISA.
15. AIA: Adjuvant induced arthritis. Female Wistar rats were immunized with Mycobacterium tuberculosis at day 0 and dosed with the compounds twice (b.i.d.) 25 mg/kg po per day from day 14–20. Swelling of the joints was measured on day 20.
16. Profiling continued, if $IC_{50} > 2\,\mu M$ for human P450 isoenzymes CYP1A2, CYP2C9, CYP2D6, CYP3A4.
17. Profiling continued, if $IC_{50} > 80\,\mu M$ for COX-1.
18. Profiling continued, if Ames assay and the Comet assay (in vitro with human lymphocytes) were negative.
19. 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 (qd) daily for 10 days.

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22. Selectivity profiles were determined in house as described.[23] Kinase (IC_{50} or % inhibition at 10 μM for **10b**/**19b**): JNK2 (>10 μM/>10 μM); CDK1: (−32%/0%); HER-1 (8.8 μM/−10%); c-Abl (−36%/−15%); c-Src (−10%/0%); Kdr (−33%/−13%); c-Met (1.5 μM/−34%); FGFR (−16%/0%); c-Kit (−12%/0%); IGF1R (0%/0%); HER-2 (4.4 μM/−24%); Flt-3 (−11%/n.t.); c-Raf (0%/n.t.).
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