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Optimization of α-ketoamide based p38 inhibitors through modifications to the region that binds to the allosteric site

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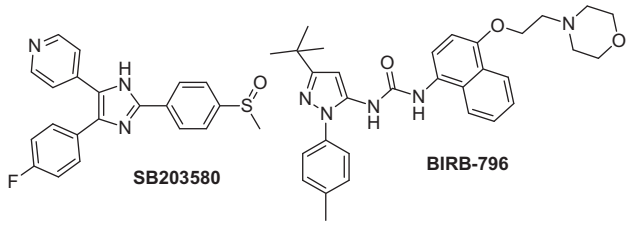
ABSTRACT

We have optimized a novel series of potent p38 MAP kinase inhibitors based on an α-ketoamide scaffold through structure based design that due to their extended molecular architecture bind, in addition to the ATP site, to an allosteric pocket. In vitro ADME, in vivo PK and efficacy studies show these compounds to have drug-like characteristics and have resulted in the nomination of a development candidate which is currently in phase II clinical trials for the oral treatment of inflammatory conditions.

After the ground breaking work by SmithKline Beecham on ATP-competitive or orthosteric (Type I) p38 inhibitors that led to SB203580,[1] and on allosteric (Type II) p38 inhibitors by BI that led to BIRB-796,[2] considerable research has been devoted to the identification of additional inhibitors of these two distinct classes.[3] This notion stems from the fact that p38 has been recognized as a highly attractive target for therapeutic intervention. It is well established that the proinflammatory cytokines tumor necrosis factor-α (TNF-α) and interleukin-1β (IL-1β) play an important role in the pathogenesis of various inflammatory diseases and that the stress-activated signal transduction pathway leading to these cytokines is in part regulated by p38. Blockade of TNF-α by the biologics Enbrel®, Remicade®, and Humira® in the treatment of deseases such as rheumatoid arthritis, Crohn's disease and psoriasis has led to clinical and commercial success.[4] Consequently, the pharmaceutical[5] and medicinal chemistry[6] communities anticipate that p38 inhibitors will display a similar therapeutic benefit but with the convenience of oral administration. Despite the advancement of a number of p38 inhibitors into the clinic, however, a small molecule therapeutic utilizing this mechanism of action still remains elusive.[7]



Type II inhibitors interact, in addition to the active site, with a region on the kinase that is spatially distinct from the ATP pocket. Furthermore, they inhibit p38 MAP kinase via a conformation (DFG-out) that is incompatible with ATP binding. As the DFG-in, active conformational states of all kinases must be competent to bind ATP, pharmacophores optimized to bind this structural motif in one kinase may have some cross-reactivity with this structural motif in other kinases, since both binding pockets must bind ATP sufficiently to support catalysis. However, there is no comparable selective pressure on the allosteric binding pocket of any two kinases adopting a DFG-out conformation to bind similar pharmacophores. Thus, binding to and stabilization of the DFG-out, inactive conformation should, in principle, lead to improve selectivity across the kinome.[8] In addition, selectivity for ATP site binders

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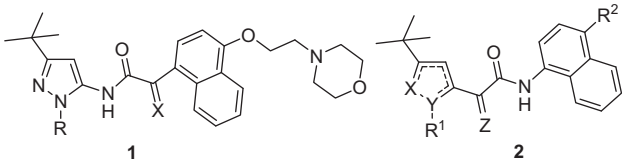
under physiological conditions may be quite different from what is observed in vitro due to ATP having different affinities for kinases and being present at high concentrations.[9]

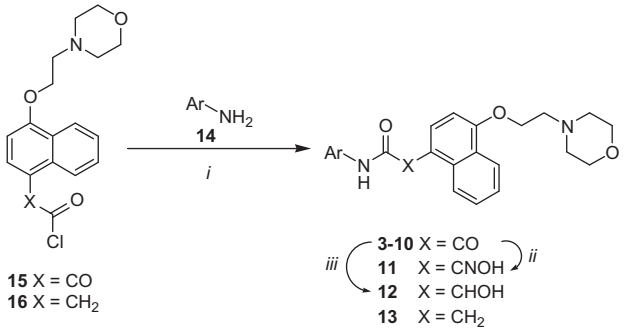
The above considerations led us to believe that it could be an attractive strategy to deliver a small molecule p38 therapeutic. We recently reported a new structural class of potent, Type II, p38 inhibitors based on an α-ketoamide scaffold (**1** X = O)[10] related and that bind in a similar manner to BIRB-796. Furthermore, we showed that reversing the ketoamide order as in **2** (Z = O), adds benefits such as chemical flexibility and improved potencies while retaining the allosteric binding mode.[11] Herein, we now report that maintaining the pharmacophore group as in **1** but embedding the moiety that occupies the exposed Phe169 pocket into a six-membered-ring increases activity against p38. In addition, we show that, as with our second generation series **2**, potency is not compromised when replacing the ethoxy- for more rigid-spacers/pharmacophores. The overall favorable drug-like properties of this new family of potent allosteric α-ketoamide p38 inhibitors ultimately led to the selection of a development candidate which is currently in phase II clinical trials for the treatment of various autoimmune deseases. During the course of our studies, BI published a similar approach and SAR studies around the BIRB-796 class of compounds.[2b]



1 **2**

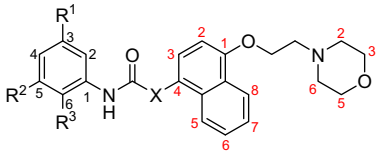
Ketoamides **3**–**10** were synthesized, as shown in Scheme 1, through reaction of the corresponding aniline derivatives **14** with the acid chloride **15**,[10] whereas **13** was prepared from acid chloride **16**.[10] Oxime **11** (X = C=NOH) was readily prepared as a mixture of geometrical isomers by treating **9** with hydroxylamine in the presence of pyridine. Reduction of **9**, on the other hand, gave the alcohol **12** (X = CHOH).

As expected from the SAR trends we established previously for our first and second generation α-ketoamide p38 inhibitors,[10,11] an unsubstituted phenyl- (Ar = Ph), saturated six-membered-ring or simple alkyl groups in place of Ar resulted in no significant inhibition of p38α (data not shown). Introducing the *t*-butyl group into the 3-position of the aniline, however, produced a compound (**3**, Table 1) within the same potency range of our first generation p38 inhibitors (e.g., **1** R = *p*-tolyl, X = O, p38α IC_{50} = 0.32 μM).[10] Molecular modelling[12] suggests that, similarly to BIRB-796,[2] the *t*-butyl group in **3** occupies the exposed Phe169 hydrophobic pocket and is essential for allosteric binding. Consistent with our first generation series,[10] modifications of the *t*-butyl group resulted in less p38α inhibitory activity confirming again that it has about



Scheme 1. Reagents and conditions: (i) EtOAc, 0.5 N $NaHCO_3$, 60 °C, 16 h; (ii) $NH_2OH{\cdot}HCl$, EtOH, pyridine, 45 °C, 12 h; (iii) MeOH, $NaBH_4$, rt, 1 h.

Table 1
P38α inhibition data for 6-membered-ring α-ketoamide derivatives



Compound	R^1	R^2	R^3	X	P38α IC_{50}[a] (μM)
3	*t*-Bu	H	H	CO	0.25
4	*t*-Bu	CN	H	CO	0.033
5	Morpholinyl	CN	H	CO	0.028
6	*t*-Bu	CN	OMe	CO	0.042
7	*t*-Bu	H	OMe	CO	0.25
8	*t*-Bu	Cl	OMe	CO	0.070
9	*t*-Bu	$NHSO_2R^4$	OMe	CO	0.059[b]
10	*t*-Bu	$CONHR^4$	OMe	CO	0.071[c]
11	*t*-Bu	$NHSO_2Me$	OMe	CNOH	0.20
12	*t*-Bu	$NHSO_2Me$	OMe	CHOH	0.39
13	*t*-Bu	$NHSO_2Me$	OMe	CH_2	0.19

[a] IC_{50} for inhibition of p38 kinase enzymatic activity. Data were generated either by ELISA using commercially available human p38 and myelin basic protein as a substrate, or using an Invitrogen Z'lyte kit with its proprietary substrate. $IC_{50's}$ given represent the means of a minimum of two, and generally three or more, independent experiments. Standard deviation for assays typically ±30% of the mean or less.
[b] R^4 = Me.
[c] R^4 = cyclopropyl.

the right size for the Phe169 hydrophobic pocket. With small alkyl groups (e.g., Me, CF_3) or larger alkyl groups (e.g., norbornyl, CMe_2R (R = Et, Pr or Ph), cyclohexyl) inhibition of TNFα production was generally decreased by more than 30%. Since efficacy and $IC_{50's}$ of compounds tested in both, the cellular TNFα and P38α inhibitory assays showed good correlation, we used efficacy in the cellular TNFα inhibitory assay as a first pass for our compound progression. In addition, the *t*-butyl group seems to be optimally placed within the six-membered-ring, since analogues with the *t*-butyl group in the 2- or 4-instead of the 3-position of the aniline, as defined here, were substantially less efficacious (again, generally by more than 30% less inhibition of TNFα production). Docking studies of **3** also revealed that when binding allosterically to p38, the 5-position of the aniline ring is in close proximity to an arginine-rich region of the protein. We reasoned that placing a hydrogen bond donor and/or acceptor moiety near that region could result in additional binding interactions. Indeed, the introduction of a cyano group into the 5-position of the six-membered ring (**4**), for example, resulted in a 7–8-fold improvement in potency over the parent compound **3**. When the *t*-butyl group was replaced for an unsaturated heterocyclic five- or six-membered-ring potency was retained as exemplified in compound **5**. A clear advantage of a phenyl versus an amino substituted five-membered heteroaromatic-ring as in **1** and **2** is chemical variability and accessibility. Replacement of the *t*-butyl group as above, for example, proved synthetically much more challenging with our first and second generation series as did taking advantage of the binding interaction with the arginine-rich region of the protein. By analogy with BIRB-796[2] and as our modeling suggested, the group at position one (**1**, R) of the pyrazole ring was hypothesized to be in close proximity and could, therefore, favorably interact with the hydrophobic portion of the side chain of the conserved residue Glu71.[10] When we introduced a methoxy group into the 6-position of the aniline, however, no further improvement in potency was obtained (**6**) although it was expected to project into the same region of the protein, namely the conserved residue of Glu71, according to our docking studies. The compound lacking the cyano group (**7**), on the other hand, had a similar potency to the parent compound **3**, indicating that

a substituent at the 6-position is at least tolerated. Small hydrophobic groups in conjunction with the methoxy substituent such as in **8** confirmed the hypothesis of a small group at the 6-position of the aniline been tolerated. Nevertheless, substitution at the 6-position while maintaining the hydrogen bond donor and/or acceptor group at the 5-position proved very useful for the adjustment of drug-like properties, such as for example improved aqueous solubility, other than potency. Only small groups, however, about the size of a methoxy group were generally preferred. For example, substituting the methoxy group for OEt, OiPr or OiBu generally decreased the inhibition of TNFα production by 30–50%. Other combinations of hydrogen bond donor/acceptor groups at the 5-position in conjunction with the methoxy substituent at the 6-position that retained high potency against p38α were, for example, the sulfonamide and amide derivatives **9** (R^4 = Me) and **10** (R^4 = cyclopropyl), respectively. With both pharmacophore moieties, only small hydrophobic groups (e.g., methyl, ethyl or cyclopropyl) at the R^4 position were accepted into this pocket of the protein. Reversing the sulfonamide or amide order at the 5-position of the aniline of **9** and **10** did not compromise potency greatly. Substitution of the 5-position with $-SO_2NMe_2$ or –NHCOMe, for example, resulted in compounds with similar potencies to **9** and **10**, respectively. Interestingly, oximes such as **11** generally trended to decreased p38α inhibition when compared to their respective keto-analogues. In contrast, the equivalent change in the first and second generation series consistently gave 5–10-fold more potent compounds (e.g., **1** R = *p*-tolyl, X = NOH, p38α IC_{50} = 0.023 μM and **2** XY = N, R^1 = *m*-tolyl, Z = NOH, R^2 = ethoxy morpholino, p38α IC_{50} = 0.059 μM).[10,11] This improvement in potency was postulated to be due to the hydrogen bond interaction observed in the docked structures of the oximes O–H with one of the amide carbonyl groups of the conserve residue of Glu71. On the other hand, computational models suggests that the extended (as the example in Fig. 1 illustrates) molecular architecture in which **11** is locked does not allow for the sulfonamide at position five of the aniline and the oxime O–H to simultaneously optimally interact with their respective regions of the protein. Reduction to the alcohol (**12**) or removal of the carbonyl group (**13**) resulted in reduced potencies and was again consistent with the results we obtained previously with our first and second generation series.[10,11] Other changes that resulted in decreased potencies and followed the same SAR trends as our first and second generation series[10,11] were substitution at the 6- and/or 7-positions (e.g., analog of **9** (R^4 = Me) with Cl in the 6-position of the naphthyl-ring, TNF-α IC_{50} = 0.22 μM) or replacement of the naphthyl- for a phenyl-ring (with or without substitution, e.g., plain phenyl analog of **9** (R^4 = Me), p38α IC_{50} = 7.3 μM), alkylation of the amide nitrogen (inhibition of TNF-α production generally dropped below 10%), removal (e.g., simple OMe derivative of **9**, TNF-α IC_{50} = 3.1 μM) or changing the position of the ethoxymorpholino group (e.g., moving the ketomamide moiety to the 2-position of the naphthyl-ring with respect to the ethoxymorpholino group would generally decrease inhibition of TNF-α production to ~10%), homologation of the ethyl linker (generally, >50-fold decrease in potency with respect to p38α), substitution at the 3- and/or 5-positions (generally, 2–10-fold less potent on p38α) and replacement of the morpholino group for other heterocyclic- (generally, 6–100-fold less potent on p38α) and ring-open-analogues (generally, 5–10-fold less potent on p38α). In summary, these results are consistent with an allosteric mode of binding similar to BIRB-796[2] in which the *t*-butyl group occupies the exposed Phe169 hydrophobic pocket and the amide simultaneously hydrogen bonds via the carbonyl with the backbone amide hydrogen of Asp168 and through the N–H with the carboxylate of Glu71. The second carbonyl group, on the other hand, acts as a hydrogen bond acceptor for the amide hydrogen of the conserved Lys53 residue which would otherwise form a salt-bridge with the carboxylate of Glu71. The substituted ring of the naphthyl maintains the ideal edge-to-face interaction with Phe169 while the second one resides deep within the kinase specificity pocket. The morpholino group, on the other hand, forms a conserved hydrogen bond with the backbone amide hydrogen of Met109. In addition, the substituent at position 5 of the aniline ring is hypothesized to interact with an arginine-rich region of the protein and the group at the 6-position with the hydrophobic portion of the side chain of the conserved residue of Glu71. Figure 1 shows the docked structure of compound **10** superimposed over BIRB-796.[13]

Consistent with our second generation ketoamide p38 inhibitors,[11] more rigid-spacers/pharmacophores within the ATP binding site (Table 2) gave sub 100 nM compounds comparable to the subseries having the ethoxy linker (see Table 1). Again, we established that the nitrogen at the 4-position of the pyrimidine ring, as defined here, is responsible for the hydrogen bond interaction with the backbone amide hydrogen of Met109 (**17**). This was further confirmed by the fact that the pyridine analogues **18** (Y = CH) and **19** (Y = CH) were as potent, whereas the pyrimidine regioisomer with the nitrogen at the 6- instead of the 4-position was significantly less active against p38α (this change generally resulted in compounds which were

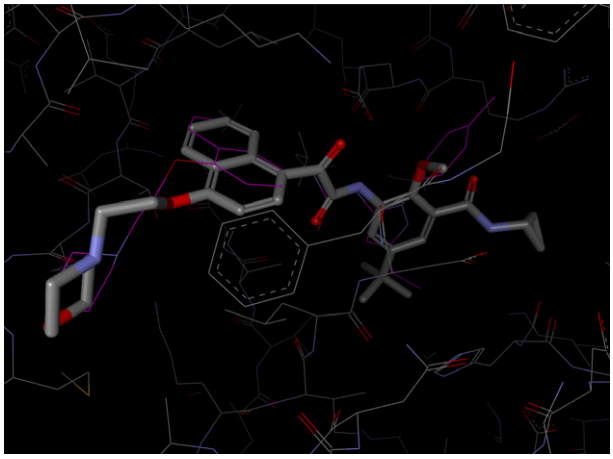
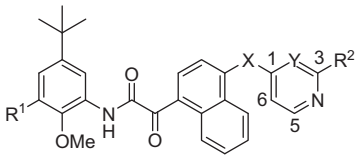


Figure 1. Docked structure of compound **10** in thick tube drawing to p38α using the induced fit docking protocol based on the X-ray structure of p38α cocrystalled with BIRB-796 (pdb1kv2.ent). Only residues within 5 Å from compound **10** are shown for clarity. BIRB-796 is shown in magenta.

Table 2
P38α inhibition data for six-membered-ring α-ketoamide derivatives with heteroatom linked rigid-spacers/phamacophores in the ATP binding site

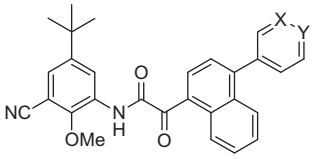


Compound	R^1	X	Y	R^2	P38αIC$_{50}$[a] (μM)
17	$NHSO_2Me$	NH	N	H	0.072
18	$NHSO_2Me$	NH	CH	H	0.025
19	CN	NH	CH	H	0.051
20	$NHSO_2Me$	NH	N	NHMe	0.008[b]
21	$NHSO_2Me$	O	N	morpholinyl	0.038

[a] IC_{50} for inhibition of p38 kinase enzymatic activity. Data were generated either by ELISA using commercially available human p38 and myelin basic protein as a substrate, or using an Invitrogen Z'lyte kit with its proprietary substrate. $IC_{50's}$ given represent the means of a minimum of two, and generally three or more, independent experiments. Standard deviation for assays typically ±30% of the mean or less.

[b] IC_{50} of LPS-stimulated TNF-α production in immortalized human cells of a monocytic lineage (THP-1).

Table 3
P38α inhibition data for six-membered-ring α-ketoamide derivatives with directly linked rigid-spacers/pharmacophores in the ATP binding site



Compound	X	Y	P38αIC$_{50}$[a] (μM)
22	N	CH	0.078
23	CH	N	0.060
24	N	CF	0.19
25	CMe	N	0.022

[a] IC_{50} for inhibition of p38 kinase enzymatic activity. Data were generated either by ELISA using commercially available human p38 and myelin basic protein as a substrate, or using an Invitrogen Z'lyte kit with its proprietary substrate. $IC_{50's}$ given represent the means of a minimum of two, and generally three or more, independent experiments. Standard deviation for assays typically ±30% of the mean or less.

Table 4
Comparison of IC_{50} data of selected compounds for the biochemical- and phospho-p38 assay

Compound	P38α IC$_{50}$[a] (μM)	PP38-αIC$_{50}$[a] (μM)
BIRB-796	0.044	0.017
SB203580	0.039	6.0
3	0.25	0.31
9	0.059	0.038
10	0.071	0.14
13	0.19	0.090
17	0.072	0.001
18	0.025	0.002
20	0.008[b]	0.021
21	0.038	0.013

[a] $IC_{50's}$ given represent the means of a minimum of two, and generally three or more, independent experiments. Standard deviation for assays typically ±30% of the mean or less.

[b] IC_{50} of LPS-stimulated TNF-α production in immortalized human cells of a monocytic lineage (THP-1).

~10-fold less potent). Molecular modeling was also in agreement with this hypothesis. Small substituents at the 3-position of the pyrimidine- or pyridine-ring, which for synthetic reasons limited us to primary-, secondary- and tertiary-amines, were generally well tolerated (**20**) with no difference between the heteroatom linking the naphthyl- to the six-membered-heteroaromatic-ring (**21**). Compounds in which the naphthyl- was directly attached to the six-membered-heteroaromatic-ring were also similarly potent (Table 3). The nitrogen was tolerated in both, the 3(X)- and 4(Y)-positions of the pyridine ring as exemplified with compounds **22** and **23**, respectively. Not surprisingly, decreasing the electron density of the pyridine nitrogen responsible for the hydrogen bond interaction with Me109, such as in **24**, trended towards decreased potency whereas activity was retained with electron donating groups such as in **25**. Again, small auxophore groups were preferred. Interestingly, replacing the nitrile group of **22** with a sulfonamide resulted in a 5–10-fold decrease in potency. Substituting the 4-position of the pyridine ring with a hydrogen bond acceptor moiety (see Fig. 2, e.g., $n = 0–1$, NR^1R^2 = morpholinyl, NH-pyranyl, NHMe), however, restored the sub 100 nM potency. As was the case with the oxime **11** (vide supra), molecular modeling suggests that the sulfonamide and the nitrogen at the 3-position of the pyridine ring are not in a suitable geometric arrangement with respect to one another to allow them to simultaneously interact favorably with their respective re-

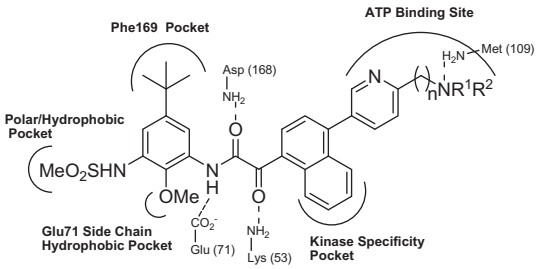
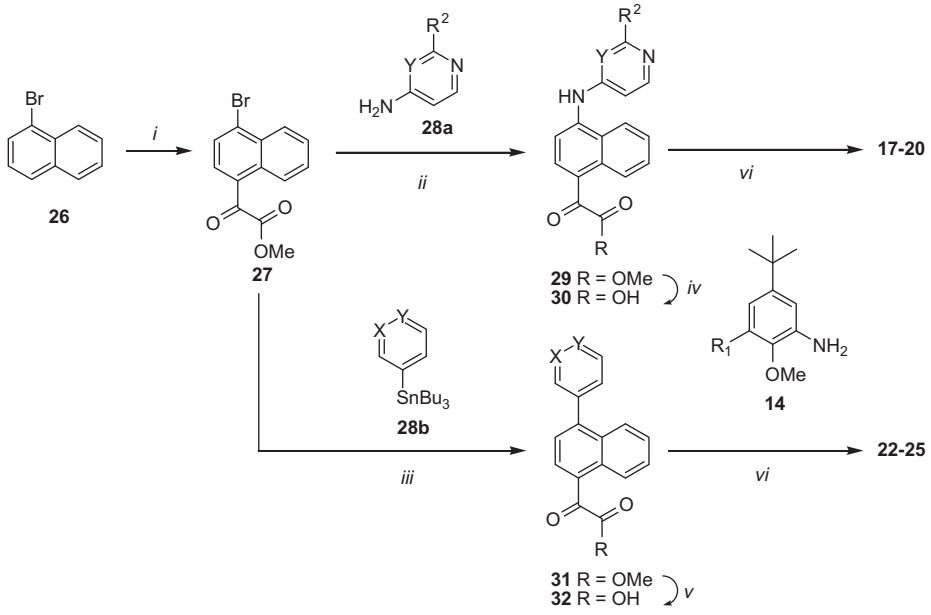
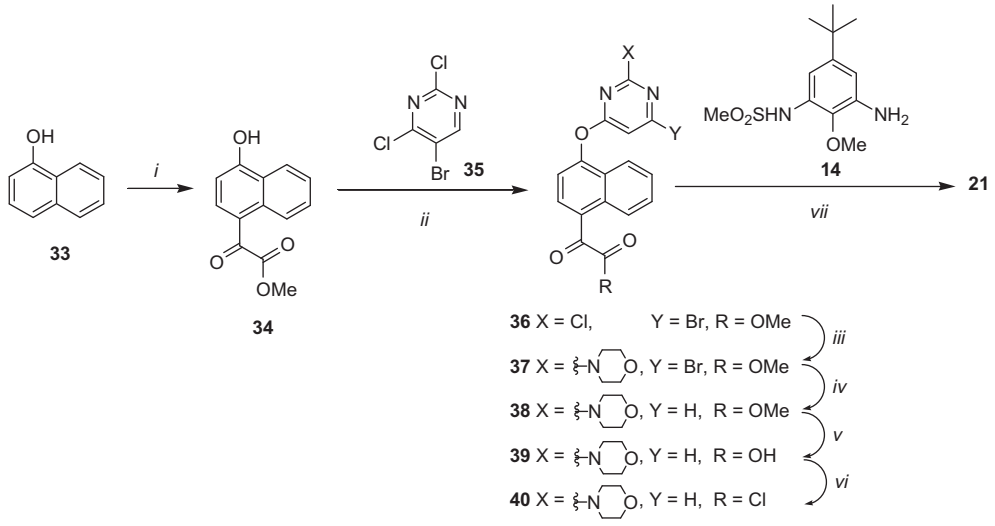


Figure 2. Simplified proposed binding mode of sulfonamide six-membered-ring α-ketoamides with directly linked rigid-spacers/pharmacophores in the ATP binding site.



Scheme 2. Reagents and conditions: (i) CH_2Cl_2, $AlCl_3$, rt then methyl oxalyl chloride, 16 h, 37%; (ii) $Pd(OAc)_2$, BINAP, $CsCO_3$, toluene, 100 °C, 24 h; (iii) $Pd(PPh_3)_4$, dioxane, microwave, 150 °C, 10 min; (iv) CH_2Cl_2, BBr_3, 0 °C, 35 min; (v) EtOAc, 40% aq KF, 1 h; (vi) PyBOP, HOBt, DIEA, DMF, rt, 16 h.



Scheme 3. Reagents and conditions: (i) CH_2Cl_2, $AlCl_3$, rt then methyl oxalyl chloride, 2 h, 38%; (ii) acetone, K_2CO_3, 60 °C, 5 h, 75%; (iii) THF/toluene, morpholine, DIEA, 80 °C, 16 h, 80%; (iv) MeOH/CH_2Cl_2, 10% Pd/C, H_2 (1 bar), rt, 50 min, 67%; (v) CH_2Cl_2, BBr_3, 0 °C to rt, 10 min; (vi) CH_2Cl_2, DMF (cat), oxalyl chloride, rt, 2 h; (vii) CH_2Cl_2, DIEA, rt, 12 h.

gions of the protein. It appears, however, that by moving the nitrogen further away from the pyridine ring, the protein can adjust slightly so that the hydrogen bond acceptor moiety can form a good hydrogen bond interaction within the hinge region while maintaining the favorable binding interaction of the sulfonamide with the arginine-rich area of p38α. The proposed allosteric binding mode is shown in Figure 2 and generally consistent with data from our p38 phosphorylation inhibition assay.[14] Table 4 summarizes some of the data which suggest that this new family of α-ketoamides can cause the necessary conformational change (DFG-in→DFG-out) to inhibit phosphorylation of p38 by its upstream kinase (MKK3/MKK6)[15] similar to BIRB-796[2] and our first and second generation series.[10,11] BIRB-796 and SB203580 are included for Ref. 10.

Ketoamides **17**–**20** and **22**–**25** were synthesized, as shown in Scheme 2, through reaction of the respective anisidine derivatives **14** (R^1 = $NHSO_2Me$[16] or CN) with the ketoacids **30** and **32**. The ketoacids **30** and **32**, on the other hand, were prepared through palladium catalyzed cross-coupling reaction of the correspondingly substituted amino-pyrimidines/pyridines **28a** and **28b**[17] with the bromo derivative **27** after subsequent hydrolysis of the ketoester intermediates **29** and **31**, respectively. Compound **27** was in turn obtained via acylation of commercially available bromonaphthol **26**.

Compounds with an oxygen spacer linking the naphthyl- to the six-membered-heteroaromatic-ring were generally prepared as shown in the reaction sequence bellow for the final compound **21** (Scheme 3). Thus, acylation of naphthol **33** to give **34**, followed by nucleophilic substitution of the commercially available pyrimidine derivative **35** gave the ketoester **36**. Compound **36** was in turn reacted with morpholine to give **37** which after reductive dehalogenation (**38**), hydrolysis (**39**) and treatment with oxalyl chloride gave the requisite acid chloride **40**. In the final step, the acid chloride **40** was reacted with **14** to give **21**.

Compounds exemplary for their whole blood and rodent PK properties are shown. Potent proinflammatory cytokine inhibition for this series was demonstrated in vitro using a THP-1 whole cell assay and ex vivo in rat and human whole blood. Compounds **19** and **20**, for example, inhibited TNF-α production in vitro with an IC_{50} = 8 nM, and ex vivo in human whole blood with an IC_{50} = 150 and 100 nM, respectively.

In vitro ADME evaluation of this new series of α-ketoamides yielded plasma stability, HLM, CYP, Caco-2, and kinetic solubility properties consistent with drug like characteristics. This was further confirmed by in vivo rat snapshot PK studies for which two examples are given in Table 5. In addition, in vivo anti-inflammatory activity for this class of p38 inhibitors was confirmed using a mouse contact hypersensitivity model[18] and in mouse and rat models of acute and chronic inflammation.[14]

Table 5
Single dose plasma pharmacokinetic parameters of **6** and **9** following dosing in rats

Compound	**6**	**9**
Dose (mg/kg) iv	10	10
Dose (mg/kg) po	30	30
T_{max} (h)	0.5	3.0
C_{max} (μg/mL)	2.4	1.3
$T_{½}$ (h)	9.8	3.5
AUC (μg?h/mL)	15	14
po *F*%	57	77
Cl (L/h/kg)	1.1	1.6
V_{dss} (L/kg)	4.9	5.2

In summary, we have optimized a novel class of potent p38 inhibitors based on an α-ketoamide scaffold that finally yielded an orally available compound that is currently undergoing phase II clinical trials in inflammatory conditions. We established SAR trends that are consistent with the related BIRB-796 series and achieved potencies in the double digit nanomolar range in both p38α and cytokine inhibition. In addition, we showed good TNF-α ex vivo inhibition in human whole blood and anti-inflammatory efficacy in animal models. We further believe, based on the above results, computational modeling and a phospho-p38α inhibition assay, that these compounds inhibit p38, at least partially, via an allosteric mode rather than an orthosteric/competitive mode of binding.

References and notes

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13. (a) A refined receptor model of p38 MAP kinase, which includes the activation loop missing in the X-ray crystal structure (pdb1kv2.ent) bound to BIRB796, was built with the PRIME comparative modeling software package (v.1.2, Schrödinger, LLC, New York, USA) using the above X-ray crystal structure as a template. Compound **10** was then docked using the induced fit protocol (GLIDE v4.0 and PRIME v1.5, Schrödinger, LLC, New York, USA).; (b) Sherman, W.; Day, T.; Jacobson, M. P.; Friesner, R. A.; Farid, R. *J. Med. Chem.* **2006**, *49*, 534.
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