Bioorganic & Medicinal Chemistry Letters 18 (2008) 5456–5459

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

'Reverse' α-ketoamide-based p38 MAP kinase inhibitors

Antonio Garrido Montalban *, Erik Boman, Chau-Dung Chang, Susana Conde Ceide, Russell Dahl, David Dalesandro, Nancy G. J. Delaet, Eric Erb, Andrew Gibbs, Jeff Kahl, Linda Kessler, Jan Lundström, Stephen Miller, Hiroshi Nakanishi, Ed Roberts, Eddine Saiah, Robert Sullivan, Zhijun Wang and Christopher J. Larson

Drug Discovery, Kemia, Inc., 5871 Oberlin Drive, Suite 100, San Diego, CA 92121, USA

ARTICLE INFO

Article history:
Received 22 June 2008
Revised 6 September 2008
Accepted 8 September 2008
Available online 11 September 2008

Keywords:
p38 MAP kinase
Antiinflammatory
SAR
Ketoamides
Cytokines
Drug-like

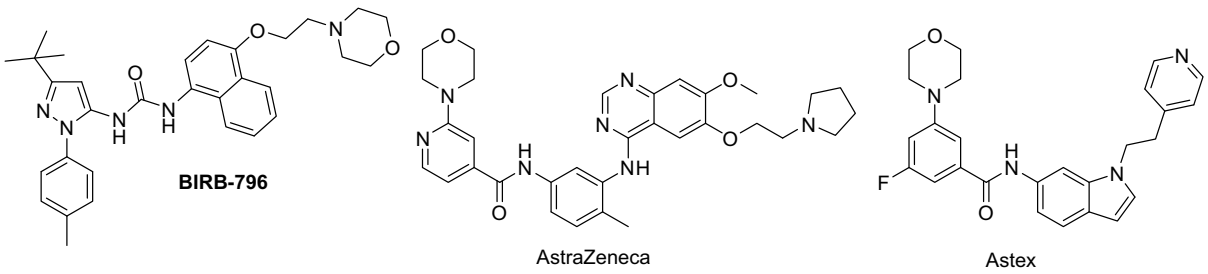
ABSTRACT

We have identified a second series of potent p38 inhibitors. As with our first generation series, these compounds are based on an α-ketoamide scaffold. The reversal of the ketoamide order, however, introduces more chemical flexibility and in addition results in improve potencies against p38.

The mitogen-activated protein (MAP) kinase p38 has been recognized as a highly attractive target for therapeutic intervention due to its role in the stress-activated signal transduction pathway leading to the release of proinflammatory cytokines such as tumour necrosis factor-α (TNF-α) and interleukin-1β (IL-1β).[1] It is well established that these cytokines play an important role in the pathogenesis of various inflammatory diseases.

After the ground breaking work on allosteric p38 inhibitors by BI that led to BIRB-796,[2] AstraZeneca[3] and Astex[4] followed with their own versions of p38 inhibitors that bind to an allosteric site (noncompetitive inhibitors).

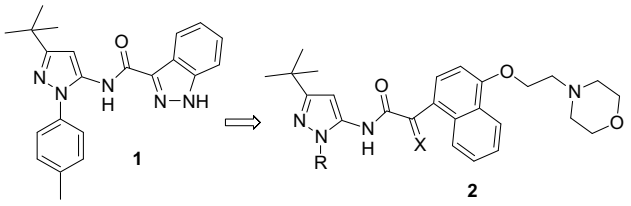
This limited number of noncompetitive, or allosteric, as compared to competitive, or orthosteric, p38 inhibitors led us to believe that it could be an attractive strategy to deliver a small molecule p38 therapeutic. We recently identified an indazol analogue (**1**) via high throughput screening (HTS) from which we derived a new structural class of potent, at least partially noncompetitive/allosteric, p38 inhibitors related to BIRB-796.[5] Herein, we now report that reversing the ketoamide order of our first generation α-ketoamide p38 inhibitors of type **2** not only leads to more diverse libraries but also results in drug-like compounds with improve potencies against p38. We again chose the cellular TNF-α inhibitory assay to guide our SAR efforts.



Reverse ketoamides **3–5**, **8** and **9** were assembled through reaction of the corresponding acid chlorides **17** and **23**, derived from α-ketoacids **16** and **22**, with aminonaphthalene **18**.[2] Two different

* Corresponding author. Tel.: +1 853 232 4672.
E-mail address: catalaba@hotmail.com (A.G. Montalban).

0960-894X/$ - see front matter © 2008 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2008.09.028



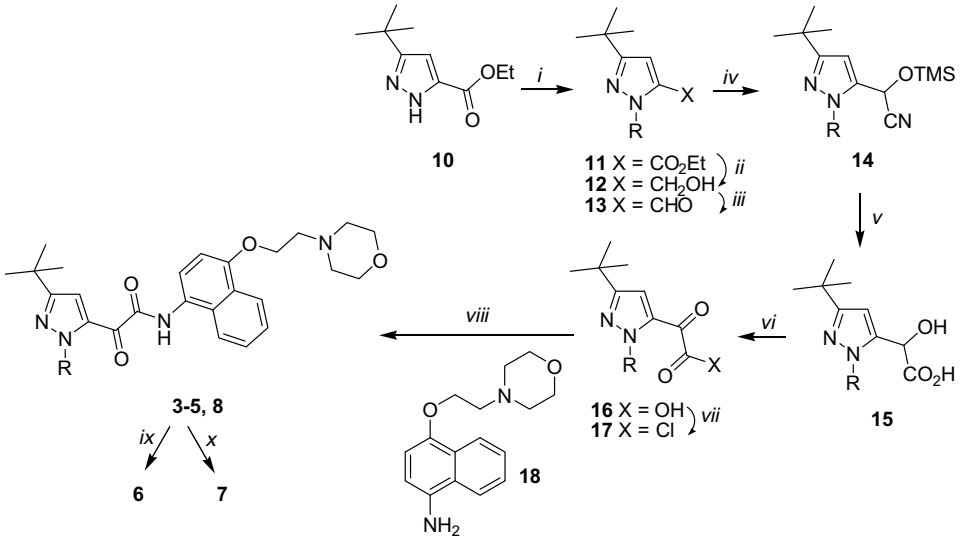
synthetic routes were explored for the synthesis of the requisite ketoacids as shown below. First, copper[6] catalyzed cross-coupling reaction of pyrazole **10** with the appropriate arylboronic acid gave the esters **11** which after reduction (**12**) followed by Dess–Martin oxidation yielded the aldehydes **13**. The aldehydes **13** were in turn reacted with TMSCN to give the cyanohydrins **14** which after concomitant deprotection and hydrolysis (**15**) were oxidized to give the pyrazol-α-ketoacids **16** (R = aryl).

The second route is exemplified for furan-α-ketoacid **22** (Scheme 2).[7] Treatment of the furan derivative **19** with (cyanomethylene)phosphorane in the presence of EDCI gave cyanoketophosphorane **20**. Dimethyldioxirane oxidation in MeOH converted **20** into α-ketoester **21** which after hydrolysis gave the requisite acid **22**.

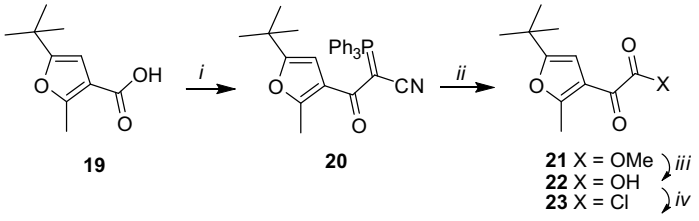
The oxime **6** was readily prepared as a mixture of geometrical isomers by treating **3** with hydroxylamine in the presence of pyridine (Scheme 1). Reduction of **4** with $NaBH_4$, on the other hand, gave the alcohol **7** (Scheme 1).

Our initial SAR survey is summarized in Table 1 and consistent (**3**–**7**) with the trends and potencies we reported previously for our first generation α-ketoamide p38 inhibitors (**2**),[5] for which we postulate that in addition to the ATP cavity of p38, these compounds also bind to an allosteric site. In this binding mode the *t*-butyl group occupies the exposed Phe169 hydrophobic pocket, the second ring of the naphthyl moiety resides deep within the kinase specificity pocket while the first one maintains the ideal edge-to-face interaction with Phe169 and the morpholino group forms a conserved hydrogen bond with the backbone amide hydrogen of Met109. In addition, we suggest that the improved potencies observed for the oximes (**2** X = NOH) are due to additional binding interactions in which the oxime O–H appears to hydrogen bond to one of the amide carbonyl groups of the conserve residue of Glu71. Although, docking results suggest that the amide in **2** simultaneously hydrogen-bonds to the backbone amide hydrogen of Asp168 and the carboxylate of Glu71 via the carbonyl oxygen and N–H, respectively, this could not be unambiguously confirmed for the new reverse ketoamide series through molecular modelling. Inhibition of p38 phosphorylation by its upstream kinase, however, proved supportive (see Table 3) of a similar binding mode since the conformation in which p38 is locked when inhibited allosterically (*vide supra*) is incompatible with ATP binding (Fig. 1).

Interestingly, the methylated derivative **8** (Table 1) was significantly more potent than its first generation ketoamide counterpart (**2** R = Me, X = O, TNF-α IC_{50} = 0.44 μM).[5] This was further confirmed by the activity of compound **9** in which the pyrazole moiety was replaced by a furan-ring. The same chemical change proved

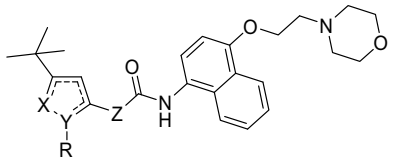


Scheme 1. Reagents and conditions: (i) CH_2Cl_2, pyridine, $ArB(OH)_2$, $Cu(OAc)_2$, 4 Å molecular sieves, rt, 16 h; (ii) THF, DIBAL-H, rt, 1 h; (iii) CH_2Cl_2, Dess–Martin periodinane, rt, 30 min; (iv) THF, TMSCN, *n*-BuLi(cat), 0 °C to rt, 16 h; (v) MeCN, cHCl, 80 °C, 16 h then MeOH, KOH, reflux, 2 h; (vi) CH_2Cl_2, Dess–Martin periodinane, rt, 30 min; (vii) CH_2Cl_2, DMF(cat), oxalyl chloride, rt, 1 h; (viii) EtOAc, 0.5 N $NaHCO_3$, rt, 16 h; (ix) EtOH, $NH_2OH{\cdot}HCl$, pyridine, 45 °C, 12 h; (x) EtOH, $NaBH_4$, rt, 12 h.



Scheme 2. Reagents and conditions: (i) CH_2Cl_2, (cyanomethylene)-phosphorane, EDCI, DMAP, rt, 16 h, 81%; (ii) CH_2Cl_2/MeOH, dimethyldioxirane, rt, 30 min, 92%; (iii) THF, 1 N LiOH, 50 °C, 1 h; CH_2Cl_2, DMF(cat), oxalyl chloride, rt, 1 h.

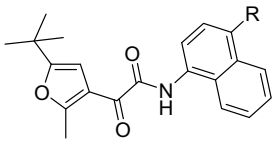
Table 1
P38-α inhibition data for reverse ketoamide derivatives

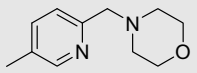
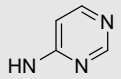
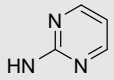


Compound	R	X	Y	Z	TNF-α IC_{50}[a] (μM)
3	*m*-Tolyl	N	N	CO	0.28
4	*p*-Tolyl	N	N	CO	0.33
5	3,4-Difluorophenyl	N	N	CO	0.18
6	*m*-Tolyl	N	N	CNOH	0.049
7	*p*-Tolyl	N	N	CHOH	3.3
8	Me	N	N	CO	0.070
9	Me	O	C	CO	0.061

[a] IC_{50} of LPS-stimulated TNF-α production in immortalized human cells of a monocytic lineage (THP-1). $IC_{50's}$ 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 2
P38-α inhibition data for furano ketoamide derivatives



Compound	R	TNF-α IC_{50}[a] (μM)
24		0.040
25		0.059
26		0.46

[a] IC_{50} of LPS-stimulated TNF-α production in immortalized human cells of a monocytic lineage (THP-1). $IC_{50's}$ 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 3
Comparison of IC_{50} data of selected compounds for the biochemical-, cell-based- and phospho-p38 assay

Compound	P38α IC_{50} (μM)	TNF-α IC_{50} (μM)	PP38-α IC_{50} (μM)
6	0.059	0.049	0.10
8	0.014	0.070	0.070
9	0.022	0.061	0.27
24	0.009	0.040	0.020

$IC_{50's}$ 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.

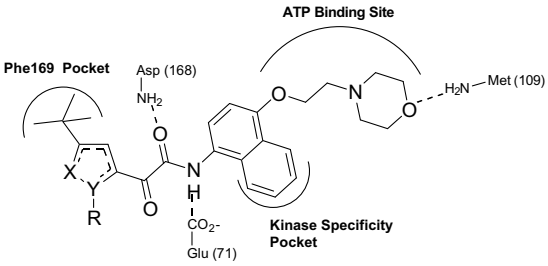
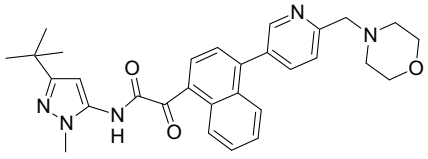


Figure 1. Simplified proposed binding mode of reverse α-ketoamides to p38α.

synthetically more challenging with the first generation ketoamide arrangement. In fact, we were not able to prepare any first generation ketoamide in which the pyrazole moiety was replaced by a furan-ring. We speculate, however, that the improved potencies of compounds **8** and **9** could be due to the repulsive interaction between the carbonyl group directly attached to the pyrazole or furan and the carboxylate of Glu71. As a result the carbonyl group and heteroaromatic ring may be more inclined to achieve coplanarity (which is less hindered by a smaller R-group) and, as our modelling[8] suggests and in contrast to our first generation ketoamides, adopt a conformation better suited for binding to the hydrophobic portion of the side chain of the conserved residue of Glu71. The oxygen in **2** (X = O), 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.[5]

Other changes that resulted in improved potencies over our first generation ketoamide series were replacement of the ethoxy- for more rigid-spacers (Table 2). The first generation ketoamide **27**, for example, inhibited TNF-α production with an IC_{50} of 0.85 μM whereas the related reverse ketoamide analogue **24** was 20-fold more potent. Similar potencies were obtained with an aminopyrimidine spacer whereby the pyrimidine nitrogen at the 4-position with respect to the amino linker appears to be better placed to interact with the amide hydrogen of Met109 than at the 2-position consistent with the docked structures of **25** and **26**, respectively. In contrast, compounds from our first generation ketoamide series containing an aminopyrimidine spacer trended towards reduced TNF-α inhibitory activity.

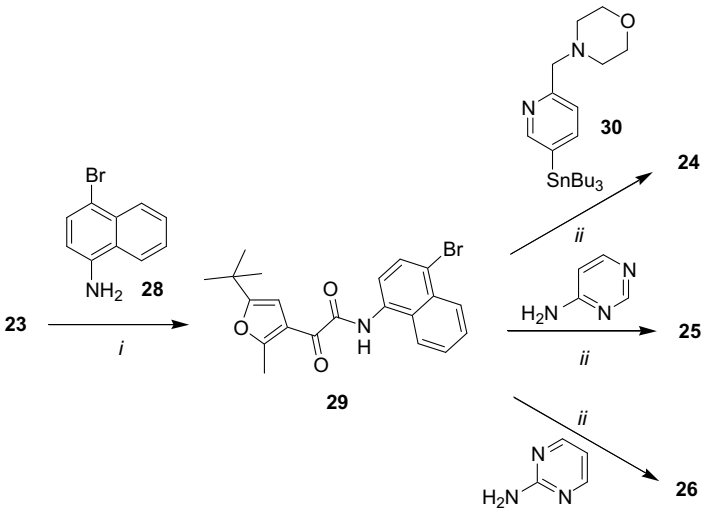


27

Compounds **24**, **25** and **26** were prepared via palladium catalyzed cross-coupling reaction of the common intermediate **29** with the organometallic reagent **30**,[9] and the corresponding aminopyrimidines, respectively (Scheme 3). Compound **29** was in turn obtained from reaction of the acid chloride **23** with the commercially available bromo-derivative **28**.

Activity in the THP-1 whole cell assay was confirmed for compounds **6**, **8**, **9** and **24** in a human p38α kinase assay (Table 3). The results from the p38α kinase assay was indistinguishable from the cell-based data when taking the different assay conditions and intra-assay variability into account.

The same set of compounds (Table 3) was tested in a p38 phosphorylation inhibition assay and the results suggest that they



Scheme 3. Reagents and conditions: (i) EtOAc, 0.5 N $NaHCO_3$, rt, 16 h, 87%; (ii) $Pd(OAc)_2$, BINAP, Cs_2CO_3, toluene, 80 °C, 16 h.

Table 4
Single dose plasma pharmacokinetic parameters of **6** following dosing in rats

	iv	po
Dose (mg/kg)	10	30
T_{max} (h)		1.5
C_{max} (μg/mL)		4.0
$T_{½}$ (h)		2.9
AUC (μg h/mL)		26
F%		54
Cl (L/h/kg)	0.61	
V_{dss} (L/kg)	1.5	

caused the necessary conformational change to inhibit p38α phosphorylation by its upstream kinase (MKK3/MKK6)[10] similar to our first generation series. These results support that our p38 inhibitors are likely to bind to the DFG-out conformation of the protein and, as with our first generation ketoamide series, we propose that these compounds inhibit at least partially via an allosteric/non-competitive mode rather than an orthosteric/competitive mode of binding in a manner similar to BIRB-796.[2]

In vitro ADME evaluation of our reverse α-ketoamide series was consistent with drug-like characteristics. This was further confirmed by in vivo rat snapshot PK studies of compound **6** for which the parameters are summarized in Table 4.

In summary, we have developed a second generation of potent α-ketoamide p38 inhibitors. In contrast to our first generation, reversing the α-ketoamide scaffold results in increase chemical flexibility and improved potencies against p38. We believe, based on computational modeling and a phospho p38α inhibition assay, that these compounds are novel allosteric p38 inhibitors. This series of inhibitors show potential for the development of an oral treatment of inflammatory conditions and further optimization of this novel scaffold will be reported in due course.

References and notes

1. (a) Han, J.; Lee, J.-D.; Tobias, P. S.; Ulevitch, R. J. *J. Biol. Chem.* **1993**, *268*, 25009; (b) Lee, J. C.; Kumar, S.; Griswold, D. E.; Underwood, D. C.; Votta, B. J.; Adams, J. L. *Immunopharmacology* **2000**, *47*, 185; (c) Pargellis, C.; Regan, J. *Curr. Opin. Invest. Drugs* **2003**, *4*, 566; (d) Kumar, S.; Boehm, J.; Lee, J. C. *Nat. Rev. Drug Discovery* **2003**, *2*, 717; (e) Chakravarty, S.; Dugar, S. *Annu. Rep. Med. Chem.* **2002**, *37*, 177; (f) Adams, J. L.; Badger, A. M.; Kumar, S.; Lee, J. C. *Prog. Med. Chem.* **2001**, *38*, 1.
2. Regan, J.; Breitfelder, S.; Cirillo, P.; Gilmore, T.; Graham, A. G.; Hickey, E.; Klaus, B.; Madwed, J.; Moriak, M.; Moss, N.; Pargellis, C.; Pav, S.; Proto, A.; Swinamer, A.; Tong, L.; Torcellini, C. *J. Med. Chem.* **2002**, *45*, 2994. references therein.
3. Cumming, J. G.; McKenzie, C. L.; Bowden, S. G.; Campbell, D.; Masters, D. J.; Breed, J.; Jewsbury, P. J. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 5389. references therein.
4. Gil, A. L.; Frederickson, M.; Cleasby, A.; Woodhead, S. J.; Carr, M. G.; Woodhead, A. J.; Walker, M. T.; Congreve, M. S.; Devine, L. A.; Tisi, D.; O'Reilly, M.; Seavers, L. C. A.; Davis, D. J.; Curry, J.; Anthony, R.; Padova, A.; Murray, C. W.; Carr, R. A. E.; Jhoti, H. *J. Med. Chem.* **2005**, *48*, 414.
5. Garrido Montalban, A.; Boman, E.; Chang, C.-D.; Conde Ceide, S.; Dahl, R.; Dalesandro, D.; Delaet, N. G. J.; Erb, E.; Ernst, J. T.; Gibbs, A.; Kahl, J.; Kessler, L.; Lundström, J.; Miller, S.; Nakanishi, H.; Roberts, E.; Saiah, E.; Sullivan, R.; Wang, Z.; Larson, C. *J. Bioorg. Med. Chem. Lett.* **2008**, *18*, 1772.
6. (a) Lam, P. Y. S.; Clark, C. G.; Saubern, S.; Adams, J.; Winters, M. P.; Chan, D. M. T.; Combs, A. *Tetrahedron Lett.* **1998**, *39*, 2941; (b) Huang, W.; Zhang, P.; Zuckett, J. F.; Wang, L.; Woolfrey, J.; Song, Y.; Jia, Z. J.; Clizbe, L. A.; Su, T.; Tran, K.; Huang, B.; Wong, P.; Sinha, U.; Park, G.; Reed, A.; Malinowski, J.; Hollenbach, S. J.; Scarborough, R. M.; Zhu, B.-Y. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 561.
7. Wong, M.-K.; Yu, C.-W.; Yuen, W.-H.; Yang, D. *J. Org. Chem.* **2001**, *66*, 3606.
8. Models of p38 in the DFG-out and DFG-in conformations were generated from the X-ray crystal structures of BIRB-796 and one of its analogues (pdb1kv1.ent and pdb1kv2.ent) and SB203580 (pdb1au9.ent) respectively, using the docking Software GLIDE (v4.0, Schrödinger, Portland, OR).
9. Moss, N.; Breitfelder, S.; Betageri, R.; Cirillo, P. F.; Fadra, T.; Hickey, E. R.; Kirrane, T.; Kroe, R. R.; Madwed, J.; Nelson, R. M.; Pargellis, C. A.; Qian, K. C.; Regan, J.; Swinamer, A.; Torcellini, C. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 4242.
10. Sullivan, J. E.; Holdgate, G. A.; Campbell, D.; Timms, D.; Gerhardt, S.; Breed, J.; Breeze, A. L.; Bermingham, A.; Pauptit, R. A.; Norman, R. A.; Embrey, K. J.; Read, J.; VanScyoc, W. S.; Ward, W. H. J. *Biochemistry* **2005**, *44*, 16475. references therein.