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Indolin-2-one p38α inhibitors III: Bioisosteric amide replacement

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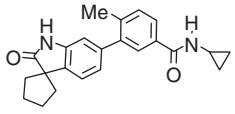
ABSTRACT

Crystallographic structural information was used in the design and synthesis of a number of bioisosteric derivatives to replace the amide moiety in a lead series of p38α inhibitors which showed general hydrolytic instability in human liver preparations. Triazole derivative **13** was found to have moderate bioavailability in the rat and demonstrated potent in-vivo activity in an acute model of inflammation.

In recent communications,[1] the design, synthesis and preliminary optimization of a potent series of indolin-2-one p38α inhibitors, as exemplified by lead **1** (Fig. 1), was described.



1: IC_{50} p38α = 1.3 nM

Figure 1. Lead molecule of the indolin-2-one p38α inhibitor series.

The preliminary compounds from this series were found to be metabolically labile when subjected to both rat and human hepatic microsomes. Of particular note is that amide bond hydrolysis occurred to a significant extent under non-oxidative metabolism conditions, particularly upon incubation with human liver microsome preparations.[1a] Subsequently it was demonstrated that both oxidative and non-oxidative metabolic pathways could be attenuated by the use of metabolic blocking groups and by tuning the physiochemical properties of the series.[1b]

Herein are described additional studies that were aimed at the definitive elimination of the hydrolytic metabolic pathway via replacement of the amide moiety by stable bioisosteres. Two distinct approaches (Fig. 2) were envisioned to achieve this goal:

(a) *Bioisosteric replacement of the amide by a heterocyclic moiety*:[2] it was surmised that derivatives of type A (Fig. 2), in which a heterocyclic moiety could maintain, in whole or in part, the hydrogen bonding interactions seen between the amide of **1** and residues Glu71/Asp168 in the p38α co-crystal structure,[3] could serve as hydrolytically stable amide replacements.[4]

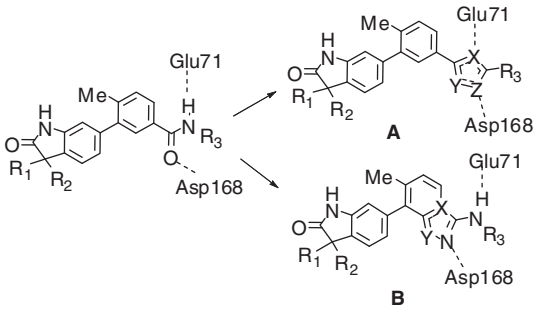


Figure 2. Design of amide bioisosteres.

(b) *Preparation of bicyclic derivatives of type B* (Fig. 2): in this case a heterocyclic amine could potentially fulfill the same functions as the amide moiety given that (i) the crystal structure of **1** bound in p38α[3] shows that only one lone pair of the oxygen of the carbonyl group is used to form the hydrogen bond with Asp168 and (ii) the dihedral angle between the tolyl ring of **1** and the carbonyl group of the amide was reasonably acute (31°) suggesting that conformation restriction onto to the proximal aromatic ring may be tolerated.[5]

Synthetic routes to compounds of type A are given in Schemes 1–5. Key heterocyclic intermediates (**2**–**7**) were prepared

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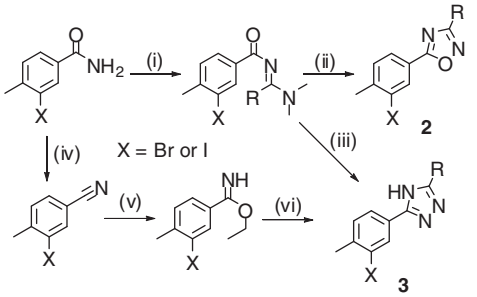
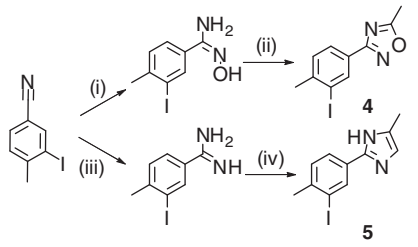
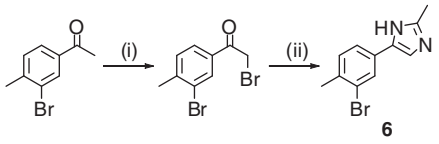


Scheme 1. Reagents and conditions: (i) $RC(OMe)_2NMe_2$, 120 °C; (ii) $NH_2OH{\cdot}HCl$, NaOAc, AcOH, 100 °C; (iii) $NH_2NH_2{\cdot}H_2O$, AcOH, 90 °C; (iv) $(CF_3CO)_2O$, Et_3N, CH_2Cl_2, 0 °C to rt; (v) HCl, EtOH, 0 °C; (vi) $RCONHNH_2$, MeOH, 75 °C then $POCl_3$, 110 °C.



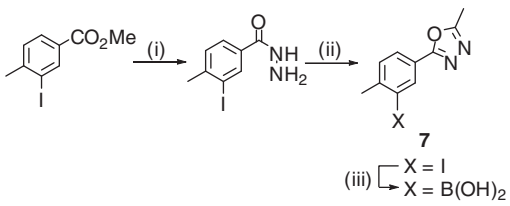
Scheme 2. Reagents and conditions: (i) $NH_2OH{\cdot}HCl$, NaOAc, EtOH, H_2O, 65 °C, 60%; (ii) $(CH_3CO)_2O$, 130 °C; (iii) LiHMDS, THF, 10 °C then 3 M HCl (aq), 25%; (iv) CH_3COCH_2Cl, $KHCO_3$, THF, H_2O, reflux, 22%.



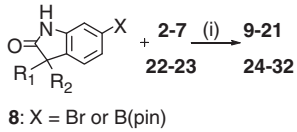
Scheme 3. Reagents and conditions: (i) Br_2, $CHCl_3$, 0 °C to rt, 95%; (ii) $CH_3C(=NH)NH_2{\cdot}HCl$, $KHCO_3$, THF, H_2O, reflux, 5%.

using standard synthetic chemistry (Schemes 1–4).[6] Suzuki coupling of intermediates **2**–**7** (Scheme 5) with indolin-2-one fragments **8**[1a] gave rise to final compounds **9**–**21** (Tables 1 and 2).

Biological results are presented in Tables 1 and 2.[7] Of the three isomeric oxadiazoles prepared (**9**–**11**), only the 1,2,4-isomer **9** showed respectable potency, albeit substantially less than the amide derivative **1**. All changes made to the alkyl substituent on



Scheme 4. Reagents and conditions: (i) $NH_2NH_2{\cdot}H_2O$, EtOH, reflux, 86%; (ii) $MeC(OEt)_3$, AcOH, 150 °C, 78%; (iii) $^{i}PrMgCl$, $B(O^{i}Pr)_3$, THF, −10 °C to rt then HCl (aq), 66%.

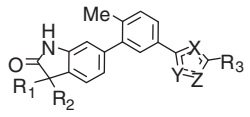


Scheme 5. Reagents and conditions: (i) $PdCl_2dppf{\cdot}CH_2Cl_2$, 2 M Cs_2CO_3 (aq), 1,4-dioxane, 100–110 °C.

the oxadiazole heterocycles (data not shown) gave rise to less potent compounds with poor and erratic whole blood potencies and high metabolic turnover. However, compounds containing a 1,2,4-triazole core ring system (**12**, **13**) were, in general, found to be potent enzyme inhibitors whilst possessing moderate whole blood potencies. The corresponding imidazole derivatives **14** and **15** which would be protonated at physiological pH were considerably less potent and no further derivatives were synthesized. As expected, the replacement of the amide furnished, in all cases, hydrolytically stable molecules as ascertained by incubation with both rat and human liver microsomes in the absence of the co-oxidant NADPH (data not shown).

From this selection of potential heterocyclic amide bioisosteres, the specific hydrogen-bonding requirements and overall polarity was seemingly best met by a 1,2,4-triazole and a further set of compounds was prepared that contained this ring system (Table 2). Methyl was found to be the optimal substituent for the triazole ring albeit from a very limited set of compounds (**12**, **16**, **17**). Changes to the spirocyclic portion of the molecule were then investigated. Replacement of the spirocycle with a gem-dimethyl substituent (**19**) resulted in a drop in potency of several fold. A reduction in ring size to give the cyclobutyl derivative **18** gave a compound which was potent in the enzymatic assay but showed a large shift in the whole blood assay. However, increasing the ring size to six

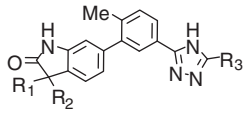
Table 1
Biological activities of heterocyclic derivatives[a,7]

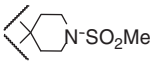


Compd	R_1, R_2	R_3	X	Y	Z	p38α IC_{50} (nM)	Whole blood IC_{50} (nM)
1	$-(CH_2)_4-$	—	—	—	—	1.3 ± 0.05	18 ± 2
9	$-(CH_2)_4-$	Me	O	N	N	32 ± 10	172 ± 10
10	$-(CH_2)_4-$	Me	N	N	O	583 ± 149	ND
11	$-(CH_2)_4-$	Me	N	O	N	741 ± 318	ND
12	$-(CH_2)_4-$	Me	NH	N	N	7.4 ± 0.7	127 ± 13
13		Me	NH	N	N	7.6 ± 2.7	93 ± 6
14		Me	NH	N	C	173 ± 8	ND
15		Me	NH	C	N	880 ± 190	ND

[a] IC_{50} values are reported as the mean of at least two experiments. ND = Not Determined.

Table 2
Biological activities of triazole derivatives[a,7]



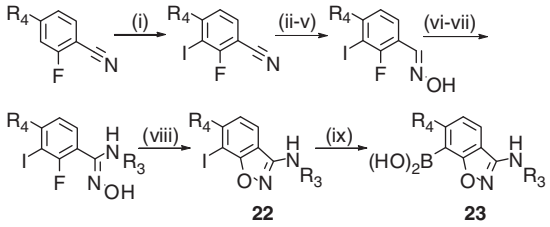
Compd	R_1, R_2	R_3	p38α IC_{50} (nM)	Whole Blood IC_{50} (nM)
12	$-(CH_2)_4-$	Me	7.4 ± 0.7	127 ± 13
16	$-(CH_2)_4-$	H	9.0 ± 2.0	253 ± 64
17	$-(CH_2)_4-$	cyc-Pr	135 ± 12	ND
18	$-(CH_2)_3-$	Me	18 ± 1	624 ± 150
19	Me, Me	Me	87 ± 16	ND
13		Me	7.6 ± 2.7	93 ± 6
20		Me	2 ± 0.5	98 ± 43
21		Me	4.7 ± 0.4	50 ± 6

[a] IC_{50} values are reported as the mean of at least two experiments unless otherwise stated. ND = Not Determined.

members with concomitant introduction of heteroatoms, with the aim of attenuating oxidative metabolism, furnished a number of interesting compounds (**13**, **20**, **21**) with good potency in the enzymatic assay and that were reasonably potent in the whole-blood assay.

Following the second strategy to replace the amide, a number of bicylic compounds of type B (Fig. 2) were then prepared—representative synthetic routes to key intermediates are given in Scheme 6.[6a] Benzisoxazole intermediates **22** and **23** were accessed in several synthetic steps from commercially available benzonitriles, key steps being ortho lithiation and trapping with iodine to prepare the key iodides and microwave-assisted cyclization to give the benzisoxazole nuclei **22**. As before, palladium-catalyzed coupling of **22** or **23** with the appropriate indolin-2-ones **8** (Scheme 5) gave compounds **24**–**32**. Biological activities and structures of final compounds are presented in Table 3.

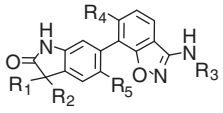
It was pleasing to find that the first benzisoxazole derivative prepared (**24**) had low nanomolar potency in both the isolated enzyme and whole blood assays. From the limited number of derivatives prepared the following trends were observed: (i) a



Scheme 6. Reagents and conditions: (i) LiTMP, THF, I_2, −78 °C to rt; (ii) H_2SO_4, dioxane, 115 °C; (iii) $BH_3{\cdot}Me_2S$, $B(OMe)_3$, THF, 0 °C to rt; (iv) MnO_2, CH_2Cl_2, 50 °C; (v) $NH_2OH{\cdot}HCl$, EtOH, rt; (vi) NCS, DMF, 55 °C; (vii) R_3NH_2, THF, 0 °C to rt; (viii) DBU, THF, 150 °C, microwaves; (ix) ^iPrMgCl, $B(O^iPr)_3$, THF, −10 °C to rt then HCl (aq).

substituent in either R_4 or R_5 (see Table 3), predisposing the two biaryl rings in an almost orthogonal arrangement,[8] is necessary for optimal enzymatic potency (derivatives **24**–**26**), although removal of the R_4 substituent led to compounds with poor whole blood potency (**25**, **26**); (ii) the five-membered spirocyclic ring can be replaced by either a gem-dimethyl group or a

Table 3
Biological activities of bicyclic aromatic derivatives[a,7]



Compd	R_1, R_2	R_3	R_4	R_5	p38α IC_{50} (nM)	Whole blood IC_{50} (nM)
24	$-(CH_2)_4-$	cyc-Pr	Me	H	0.9 ± 0.2	2.0 ± 0.1
25	$-(CH_2)_4-$	cyc-Pr	H	H	38 ± 14	1257 ± 577
26	$-(CH_2)_4-$	cyc-Pr	H	Cl	4.0 ± 1.5	438 ± 29
27	Me, Me	cyc-Pr	Me	H	0.4 ± 0.1	50 ± 11
28		cyc-Pr	Me	H	0.16 ± 0.03	8.4 ± 0.4
29	 Me, Me	cyc-Pr	Me	H	0.06 ± 0.01	10.7 ± 4.9
30	Me, Me	H	Me	H	38 ± 11	731 ± 199
31		H	Me	H	5.0 ± 2.0	145 ± 4
32		H	Me	H	1.2 ± 0.2	137 (n = 1)

[a] IC_{50} values are reported as the mean of at least two experiments unless otherwise stated.

six-membered ring (vide supra) giving rise to derivatives that are extremely potent in the enzymatic assay (**27**–**29**); (iii) the high intrinsic potency of the six-membered ring analogues allows for the removal of the amine substituent whilst maintaining compounds of good enzymatic potency (**30**–**32**).

Other bicylic nuclei other than the benzisoxazole were briefly investigated (Fig. 3). The indazole **33**[9] proved to be inactive (cf. the corresponding benzisoxazole **31**, IC_{50} = 5.0 nM). The triazolopyridine **34**,[10] however, was found to be a potent (IC_{50} = 34 nM) inhibitor in the enzymatic assay; unfortunately this compound was found to lose almost two orders of magnitude of potency in the whole blood assay and no further examples were prepared.

The in-vitro metabolic turnover in hepatic microsomes, permeability in a Caco-2 cell line and cytotoxicity in a Chinese hamster ovarian (CHO) cell line were determined for a selection of the most interesting compounds and results are given in Table 4.

In general, compounds presented good to excellent apical to basal flux in the Caco-2 assay apart from compound **21** which appears to have transporter-compromised permeability issues as determined by the significantly large BA/AB ratio. Metabolic turnover (NADPH dependent) was found to be low to moderate in both rat and human microsomes and, in contrast to the amide lead, no metabolism was seen in the absence of NADPH in the human preparation (data not shown). In addition, compounds did not show any significant cytotoxicity towards CHO cells with the exception of compound **20**.

On the basis of the in-vitro results, the intravenous and oral rat pharmacokinetic profiles were determined for compounds selected from both the triazole and benzisoxazole series and the results are presented in Tables 5 and 6.

Compound **13** was found to have a good intravenous half life in rat, as a result of low clearance and a moderate volume of distribution, and this coupled with a moderate oral bioavailability and good blood levels triggered further profiling of this derivative. Bicycle **28** was also found to have a good intravenous pharmacokinetic profile with both low clearance and a moderate volume of distribution contributing to a good half-life—however, upon oral dosing a poor oral bioavailability was noted.

Compound **13** was tested in-vivo in an acute model of inflammation in the rat based on LPS-induced TNFα production.[7] In this model, compounds were dosed orally one hour prior to LPS administration and the amount of TNFα in plasma was measured 1.5 h later (coinciding with the peak TNFα production). Compound **13** dose-dependently inhibited TNFα production with an ED_{50} = 0.69 mg/kg (95% CI: 0.4–1.0) performing better than the clinically relevant reference compound BIRB-796 (ED_{50} = 7.9 mg/kg, 95% CI:3–22).

In order to determine general selectivity, **13** was tested at a concentration of 10 μM in a panel of 51 representative kinases (Upstate) and a panel of 54 proteins which included GPCRs, phosphodiesterases, hormone receptors, ion channels and transporters (CEREP 'ExpresSProfile'). Overall, **13** demonstrated an excellent selectivity profile.[11] In relation to potential cardiovascular toxicity, **13** proved to be a relatively weak inhibitor of the the human I_{Kr} (hERG) ion channel (49 ± 8% block @ 10 μM) as deter-

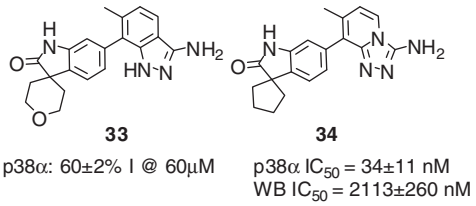


Figure 3. Miscellaneous bicyclic nuclei.

Table 6
Pharmacokinetic profiles in rat (10 mg/kg oral)[a]

Compd	C_{max} (ng/mL)	$AUC_{0-\infty}$ (ng h/mL)	F (%)
1	367	2644	10
13	1105	11,683	29
21	632	1371	13
28	479	3372	6
31	141	961	8

[a] Formulations: Compds **1**,**13**, **21**, **31**: 0.5% methylcellulose + 0.1% Tween 80; **28**: 40% PEG + 0.4% HCl 1 N + 0.5% DMSO.

Table 4
In-vitro profiles of selected derivatives

Compd	Rat/human metabolism (+NADPH)[a]	Caco-2 (AB/BA) Papp[b] (10^{-6} cm/s)	Cytotoxicity CHO IC_{50}[c] (μM)
1	44/55	18/10	26
12	15/13	ND	53% @ 100 μM
13	23/<5	18/27	48% @ 200 μM
20	5/<5	ND	20
21	<5/6	2/45	100
28	18/26	26/16	49
29	25/32	10/4	56% @ 200 μM
31	9/3	28/16	16% @ 200 μM

[a] % Turnover after a 30 min incubation period at 37 °C of a 5 μM solution of test compound with hepatic microsomes (1 mg/mL).
[b] Passive permeability through a Caco-2 monolayer determined using 12.5 μM test compound. ND = Not Determined.
[c] For assay details see Ref. 1a.

Table 5
Pharmacokinetic profiles in rat (1 mg/kg iv)[a]

Compd	$t½$ (h)	C_{max} (ng/mL)	Cl (mL/min/kg)	$AUC_{0-\infty}$ (ng h/mL)	V_{ss} (L/kg)	MRT (h)
1	5.3	2002	6.6	2532	0.8	2.1
13	3.1	1719	4.2	4063	1.0	4
21	3.8	1295	17.2	1030	1.75	1.8
28	2.9	1475	3.2	5271	0.8	4.6
31	1.9	752	13.8	1230	2.0	2.5

[a] Formulations: Compds **1**: 40% PEG; **13**, **21**, **31**: 40% PEG + 0.4% HCl 1 N; **28**: 40% PEG + 0.4% HCl 1 N + 0.5% DMSO.

mined in human embryonic kidney (HEK-293) cells. In addition, compound **13** did not inhibit any of the most relevant human cytochrome P450 (CYP) isoforms (IC_{50} >25 μM for CYPs 1A2, 3A4, 2C9, 2C19 and 2D6).

In summary, the replacement of the metabolically unstable amide moiety in a chemical lead series by a variety of mono and bicyclic heterocyclic bioisosteres has led to potent and metabolically stable compounds. A representative example (compound **13**) demonstrated moderate bioavailability in the rat and potent in-vivo activity in an acute model of inflammation.

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1. (a) Eastwood, P.; González, J.; Gómez, E.; Vidal, B.; Caturla, F.; Roca, R.; Balagué, C.; Orellana, A.; Domínguez, M. *Bioorg. Med. Chem. Lett.* **2011**, *21*, 4310; (b) Eastwood, P.; González, J.; Gómez, E.; Caturla, F.; Balagué, C.; Orellana, A.; Domínguez, M. *Bioorg. Med. Chem. Lett.* **2011**, *21*, 5270.
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3. The crystallographic binding mode of compound **1** has been published in Ref. 1a.
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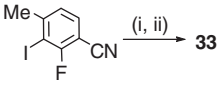
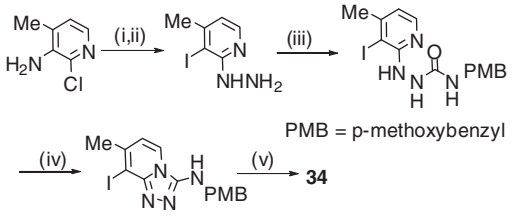
5. A similar strategy was adopted independently in another series of p38α inhibitors: Pettus, L. H.; Xu, S.; Cao, G.-Q.; Chakrabarti, P. P.; Rzasa, R. M.; Sham, K.; Wurz, R. P.; Zhang, D.; Middleton, S.; Henkle, B.; Plant, M. H.; Saris, C. J. M.; Sherman, L.; Wong, L. M.; Powers, D. A.; Tudor, Y.; Yu, V.; Lee, M. R.; Syed, R.; Hsieh, F.; Tasker, A. S. *J. Med. Chem.* **2008**, *51*(20), 6280.
6. (a) Full synthetic details can be found in the following patents: Eastwood, P. R.; González, J.; Matassa, V. PCT Int. Appl. WO2009132774, 2009.; (b) Eastwood, P. R.; González, J.; Vidal, B.; Aguilar, N. PCT Int. Appl. WO2009124692, 2009.
7. Full details of all biological assays and models used in this article can be found in: Lumeras, W.; Caturla, F.; Vidal, L.; Esteve, C.; Balagué, C.; Orellana, A.; Domínguez, M.; Roca, R.; Huerta, J. M.; Godessart, N.; Vidal, B. *J. Med. Chem.* **2009**, *52*, 5531. and Ref. 1a.
8. The torsion angle between these two rings in the bound conformation of compound **1** is 71° as ascertained from the crystal structure (Ref. 1a).
9. Compound **33** was accessed by the following synthetic route:



Reagents and conditions: (i) $NH_2NH_2{\cdot}H_2O$, EtOH, 100 °C, 78%; (ii) **8** [X = B(pin)], $PdCl_2dppf{\cdot}CH_2Cl_2$, 2 M Cs_2CO_3 (aq), 1,4-dioxane, 110 °C, 29%.

10. Compound **34** was accessed by the following synthetic route:



Reagents and conditions: (i) $NaNO_2$, HCl, H_2O, 0 °C then KI, 0 °C to rt, 58%; (ii) $NH_2NH_2{\cdot}H_2O$, pyridine, 115 °C, 78%; (iii) *p*-methoxybenzylisocyanate, CH_2Cl_2, rt, 81%; (iv) $POCl_3$, CH_3CN, 75 °C, 39%; (v) **8** [X = B(pin)], $PdCl_2dppf{\cdot}CH_2Cl_2$, 2 M Cs_2CO_3 (aq), 1,4-dioxane, 110 °C then TFA, CH_2Cl_2, rt 20%.

11. IC_{50} values were determined for relevant inhibitory values (>50% inhibition @ 10 μM) with the following findings: 5-HT_{1B}: IC_{50} ~10 μM, cRaf: IC_{50} = 1.6 μM, TrkA(h): IC_{50} = 7.7 μM.