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Synthesis and biological activity of quinolinone and dihydroquinolinone p38 MAP kinase inhibitors

Meng-Hsin Chen,[a,*] Patricia Fitzgerald,[a] Suresh B. Singh,[a] Edward A. O'Neill,[b] Cheryl D. Schwartz,[b] Chris M. Thompson,[b] Stephen J. O'Keefe,[b] Dennis M. Zaller[b] and James B. Doherty[a]

[a]*Department of Medicinal Chemistry, Merck Research Laboratories, PO Box 2000, Rahway, NJ 07065, USA*
[b]*Department of Inflammation Research, Merck Research Laboratories, PO Box 2000, Rahway, NJ 07065, USA*

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Abstract—Synthesis and biological activities of some quinolinone and dihydroquinolinone p38 MAP kinase inhibitors are reported. Modifications to the dihydroquinolinone pharmacophore revealed that dihydroquinolinone may be replaced with a quinolinone pharmacophore and lead to enhanced p38 inhibitory activity. From a study of C-7 substitutions by amino acid side chains, a very potent series of compounds in the p38 enzyme assays was identified. Translation of the in vitro activity into reasonable whole blood activity can be improved in this series of compounds by judicious modification of the physical properties at appropriate regions of the lead.

The enzyme p38 is an intracellular mitogen-activated protein (MAP) kinase which regulates the release and the actions of pro-inflammatory mediators such as TNF-α and IL-β[1]. Once activated, p38 initiates a signal cascade leading to the synthesis and amplification of these mediators. Clinical studies have shown that inhibition of these inflammatory mediators individually is beneficial for treating rheumatoid arthritis. Therefore, inhibition of p38 enzyme, conceptually a concerted inhibition of these mediators, may have considerable therapeutic advantage.

The discovery of series of triaryl-imidazoles as p38 inhibitors, exemplified by SB203580[2,3] (Fig. 1), was seminal. Subsequent investigation by Vertex scientists led to a structurally diverse set of p38α inhibitors, exemplified by VX-745 (Fig. 1). This compound exhibited high selectivity for p38α over a variety of other closely related kinases[4]. The Vertex work encouraged us to design several new series of compounds related to VX-745. In this communication, we wish to report the synthesis and evaluation of substitution with various functional groups at C-3, C-5, and C-7 in a series of quinolinone and dihydroquinolinone compounds that are closely related to VX-745 structure.

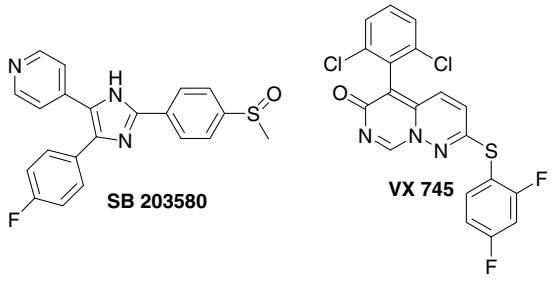

Figure 1. Two p38α inhibitors SB203580 and VX-745.

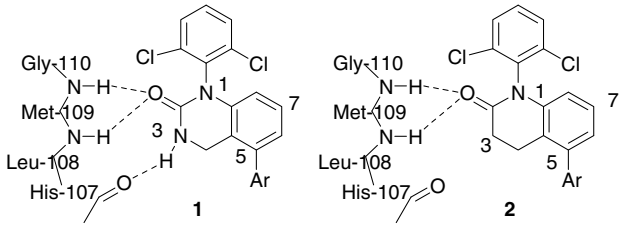

Figure 2. Relative binding orientation of dihydroquinazolinone **1** and dihydroquinolinone **2** to p38α.

Keywords: p38; MAP Kinase inhibitors.
* Corresponding author. Tel.: +1 732 594 3304; fax: +1 732 594 9556; e-mail: meng_hsin_chen@merck.com

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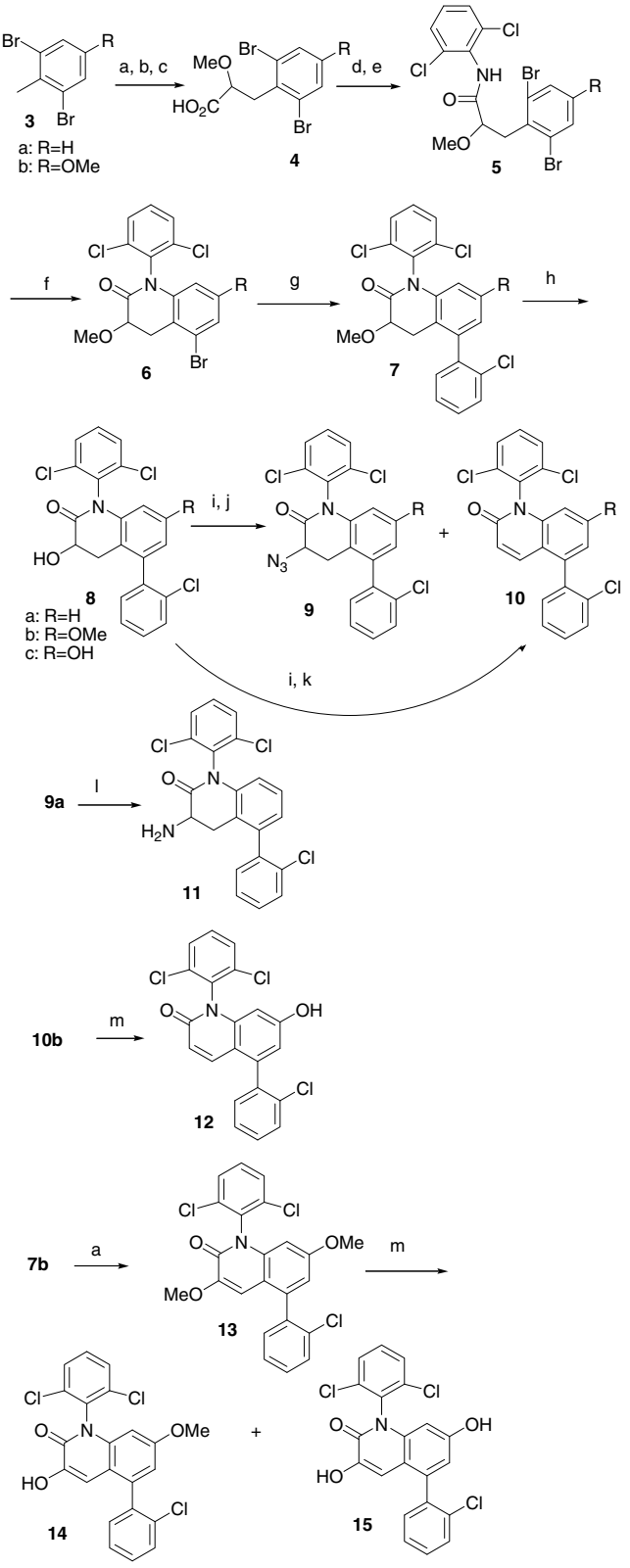
Recently, Merck scientists have published on several scaffold variants of VX-745 and demonstrated that these inhibitors were highly selective toward p38 against closely related kinases, had good potency (p38α, TNFα, and in human whole blood), and were readily orally bioavailable[5]. The key ligand–enzyme interactions of the dihydroquinazolinone series of inhibitors with the enzyme were clearly discerned in X-ray crystallographic studies[6]. Three hydrogen bonds (H bonds) were observed in the dihydroquinazolinone (**1**) series with the p38α enzyme (Fig. 2). Two of the H bonds result from the carbonyl oxygen of **1** interacting with the N–H bonds of Met-109 and Gly-110 of the enzyme backbone; a third H bond is formed between the cyclic urea N–H bond of **1** and His-107 carbonyl oxygen. In contrast, in the dihydroquinolinone (**2**)-series this third H bond with His-107 is absent (see Fig. 2). Our working hypothesis was that this missing third H bond may explain why, in general, the activity of the dihydroquinazolinone series (**1**) was consistently 3- to 5-fold more potent than that of the dihydroquinolinone series (**2**) (data not shown). In this report, compounds were designed in order to create a third H bond between the dihydroquinolinone compounds (**2**) and His-107 in the enzyme backbone through introduction of an H bond donor attached to C-3. The syntheses of these designed p38α inhibitors are summarized in Scheme 1.

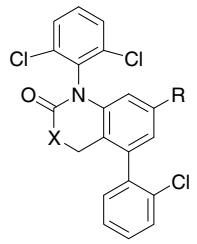
Bromination of 2,6-dibromotoluene **3** with NBS gave the expected benzylic bromide in quantitative yield and reaction of this derived benzyl bromide with methyl methoxyacetate anion followed by saponification afforded the carboxylic acid **4**. The cyclization precursor **5** was obtained from conversion of the carboxylic acid **4** into the corresponding acid chloride and then reaction of the acid chloride with 2,6-dichloroaniline. The resultant amide **5** was converted to dihydroquinolinone **6** in good yield using Ullmann reaction conditions[7]. A Suzuki reaction[8] between dihydroquinolinone **6** and 2-chlorophenyl boronic acid gave the desired biaryl coupling product **7**. The latter was then subjected to treatment with boron tribromide. This led to the anticipated demethylation product **8**, and the alcohol was converted to the azide **9** via the mesylate. The quinolinone **10** was obtained from a 1,2-elimination reaction on the mesylate intermediate just mentioned; this transformation was effected very smoothly by DBU. The azide **9a** on exposure to Ra-Ni led to the formation of the amino analog **11**. The methoxy quinolinone **13** was prepared by a bromination and elimination reaction sequence on compound **7b**. Demethylation with boron tribromide followed by careful chromatographic separation gave the monohydroxyquinolinone **14** and dihydroxyquinolinone **15**.

The designed inhibitors were tested in the p38 enzyme assay and whole blood assay[5a]. The effects on the inhibition of p38 MAP kinase from the various substituents attached on C-3 and on C-7 of the quinolinone and dihydroquinolinone scaffold are shown in Table 1 below. The five hydroxylated analogs (**8a–c**, **14**, and **15**) were all more potent than the three methoxy-ether analogs (**7a**, **7b**, and **13**), suggesting that the hydroxyl group

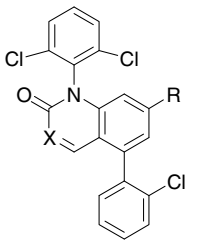


Scheme 1. Reagents and conditions: (a) NBS, cat. $(BzO)_2$, CCl_4, quant.; (b) methyl methoxyacetate, $LiN(TMS)_2$; (c) NaOH 66% for the two steps; (d) oxalyl chloride, cat. DMF, benzene; (e) 2,6-dichloroaniline, TEA, −78 °C to rt, 78% for the two steps; (f) CuI, K_2CO_3, DMF, 150 °C, [R = H (73%), R = OMe (84%)]; (g) 2-chlorophenylboronic acid, $PdCl_2(PPh_3)_2$, 2 N Na_2CO_3, [R = H (71%), R = OMe (78%)]; (h) BBr_3, −78 °C, CH_2Cl_2 [(R = H (75%), R = OMe (65%), R = OH (15%)]; (i) MsCl, TEA, 0 °C; (j) NaN_3, DMSO; (k) DBU, DMSO, 80 °C, (93%) over the two steps; (l) Ra-Ni/H_2 R = H 55%; (m) BBr_3, (93%, **14** 65%, **15** 28%).

Table 1. Substituent effects on p38α inhibition at C-3 and C-7 on dihydroquinolinone (A) and quinolinone (B) analogs



template (A)

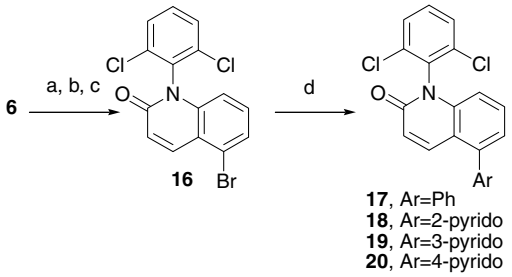


template (B)

Compound	Template	X	R	p38α IC_{50} (nM)
7a	A	CH–OMe	H	Inactive
8a	A	CH–OH	H	27.5
9	A	CH–N_3	H	15% at 100
10a	B	CH	H	6
11	A	CH–NH_2	H	Inactive
7b	A	CH–OMe	OMe	63% at 1250
8b	A	CH–OH	OMe	9
10b	B	CH	OMe	1.5
8c	A	CH–OH	OH	4
12	B	CH	OH	0.7
13	B	C–OMe	OMe	29% at 100
14	B	C–OH	OMe	28
15	B	C–OH	OH	5

which can serve as a hydrogen-bond donor to His-107 interacts with p38α much more effectively than the methoxy group which lacks this hydrogen bonding capacity. Alternatively, it could be that the size of the binding pocket around C-3 is such that the hydroxyl group is well tolerated but not the methoxy group, resulting in weaker interactions overall for the latter. Intriguingly, from inspection of the binding data in Table 1, it appears that an amino group at C-3 (compound **11**) is not tolerated. We surmise that although the amine is a small and capable of acting as an H bond donor group, in the binding pocket the amine moiety may be binding to the enzyme in its protonated form. The presence of a cationic charge located near position C-3 of the quinolone may be detrimental for potent p38α binding.

Interestingly, the simple quinolinone analogs **10a**, **10b**, and **12** (hydrogen atoms attached at C-7) were the most potent p38α inhibitors in the set of compounds investigated in Table 1. A possible explanation for this increase in potency of the quinolinone analogs is that this increase is due to the increased electron density on the carbonyl oxygen in **10a**, **10b**, and **12** and so stronger H bonds with Met-109 and Gly-110 N–H on the p38α enzyme are formed compared with the dihydroquinolinone analogs. Similar increases in potency were also seen when electron-donating groups such as a methoxy or a hydroxyl group were attached at position C-7 of the quinolinone (**10b** and **12**). These functional groups at C7 also can increase the electron density of the carbonyl oxygen and therefore result in more potent p38α inhibitors when compared with those compounds that were unsubstituted at C-7 (**10a**). Even though the quinolinones **10a**, **10b**, and **12** had high potency in the p38α enzyme inhibition assay, none of the three compounds were particularly potent in the p38α assay containing whole blood. For example, one of the most active against p38α in the enzyme assay was compound (**10b**) (IC_{50} = 1.5 nM), but it was only moderately active (75% inhibition at 10 μM) in the whole blood assay. The observed lower potency in the whole blood assay may be accounted for by the high lipophilicity[9] and therefore leading to high plasma binding.

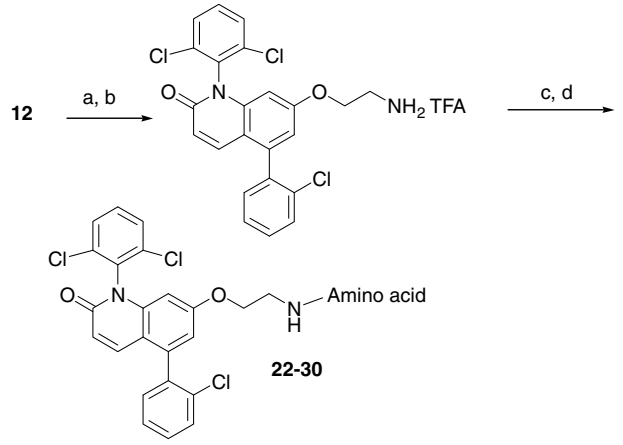


Scheme 2. Reagents and conditions: (a) BBr_3, −78 °C, 90%; (b) MsCl, TEA, 0 °C; (c) DBU, DMSO, 80 °C, 93% over the two steps; (d) Ar–$B(OH)_2$, $PdCl_2$ $(PPh_3)_2$, 2 N Na_2CO_3, 80 °C, 25–50%.

To address the loss of potency in the whole blood assay, another series of compounds was crafted in order to try to reduce lipophilicity and increase water solubility through modification of the substituents attached at C-5 and at C-7. These designed compounds were prepared in the following way. The bromide **16** was prepared from compound **6** as shown in Scheme 2 and this bromide **16** then served as a convenient intermediate for palladium(0)-mediated Stille or Suzuki cross-coupling arylation reactions at position C-5 of the quinolone.

Table 2. Substituent effects on p38α inhibition at C-5 on quinolinone analogs

Compound	Ar	P38α IC_{50} (nM)
17	Phenyl	13.4
18	2-Pyrido	350
19	3-Pyrido	460
20	4-Pyrido	58% at 1000



Scheme 3. Reagents and conditions: (a) K_2CO_3, *N-Boc*-2-chloroethylamine, DMF, 80 °C; (b) TFA; (c) *N-Boc* amino acids, EDC, HOBt, TEA; (d) HCl, EtOAc.

Table 3. The effects on binding from varying the amino acid in compounds **22**–**30** in the p38α inhibition and whole blood assay

Compound	Amino acid	P38α IC_{50} (nM)	WB IC_{50} (nM)
22	L-Pro	0.75	87
23	D-Pro	0.9	73
24	1-Amino-1-cyclopropane-carboxylic acid	1.5	1010
25	Gly	0.3	80
26	Sar	0.5	200
27	α-Methyl-Ala	0.12	28
28	L-Ala	0.16	32
29	D-Ala	0.2	47
30	β-Ala	0.57	130

Comparison between the 2-chlorophenyl analog (**10a**) with the simple phenyl analog **17**, the former was marginally more potent, but when the C-5 phenyl substitution was replaced with pyridine, all three pyridyl-isomers (**18**, **19**, and **20**) were at least 10-fold less active than the original phenyl analogs (**17**) in the p38α enzyme assays (Table 2). This replacement by a pyridine for phenyl ring resulted in weaker hydrophobic interactions than those encountered by the phenyl group in this segment of the molecule. This approach did not lead to a practical solution to the problem and so another tack was taken.

A series of papers from Merck have outlined that attachment of a piperidinyl moiety at the C-7 position of naphthyridinone-, quinolinone-, dihydroquinazolinone-, and dihyropyridopyrimidone-derived p38α inhibitors is optimal[5]. By studying the crystal structures obtained for the four series of inhibitors with p38α, the piperidinyl group was located in the kinase enzyme in a phosphate-binding area, under a glycine-rich loop that participates with an extensive number of water molecules to form a hydrogen binding network with the enzyme[6]. The intriguing conjecture that appropriate substituents at the C-7 position could change the physical properties of these p38α inhibitors has been raised. Indeed, this seems reasonable if one considers the published data on the interactions between several series of p38α inhibitors with the enzyme[6]. These data suggest that piperidinyl at the C-7 position binds well to the enzyme at least partly by virtue of their participation with surrounding water molecules in a H-bonding network with the enzyme. We followed this line of investigation by preparing a series of amino acid tethered to an aminoethoxy moiety at C-7, conceptually reminiscent of piperidinyl, as shown in Scheme 3. Compound **12** was converted to **22**–**30** through alkylation, deprotection, standard peptide coupling reactions[10], and removal of the BOC-protecting group. Biological evaluation of this series of compounds on p38α is shown in Table 3.

Gratifyingly this amino acid series of derived inhibitors was indeed very potent in the enzyme assays regardless of the substituents, configuration of the stereocenters or basicity of the amino acid attached.[11,12] The amino acid derivative **24** however was considerably shifted in its potency in the whole blood assay with respect to compound **27**. Possibly the poor whole blood activity of **24** may be attributed to the physical properties of this compound, for compound **24** possesses a less basic amino group and this may lead to a higher plasma binding for analog **24** than the compound **27**. On the other hand, the glycine analog **25** and β-alanine analog **30** had similar potency in both the p38α enzyme and whole blood assays. It does appear that the physical properties of p38α inhibitors seem to play an important role in translating in vitro enzyme inhibition into functional whole blood activity. Modification of different physical properties of p38α inhibitors on the various related pharmacophores is still under active investigation.

Conclusion. A series of quinolinone and dihydroquinolinone analogs related to VX-745 structure were prepared. The five hydroxylated analogs **8a–c**, **14**, and **15**, all capable of hydrogen bonding to the p38α enzyme, were potent but the methoxy analogs **7a**, **7b**, and **13** were less potent and the amino analog **11** was essentially inactive. The most potent compounds in the in vitro assay, the simple quinolinones **10a**, **10b**, and **12**, lost activity considerably in the whole blood assays. Introduction of the pyridyl substituents (**18**, **19**, and **20**) for phenyl at C-5 in the quinolinone series attempting to lower lipophilicity was fruitless. However, incorporation of amino acids on a 2-aminoethoxy sidechain at C-7 (**22**–**30**) did lead to active compounds in both the p38α in vitro and also in the whole blood assay. Translation of the in vitro activity into reasonable whole blood activity can be improved in this series of compounds by judicious modification of the physical properties at appropriate regions of the lead.

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9. The calculated log *D* (pH 7.4) values for compounds **10a**, **10b**, and **12** are 6.34, 6.29, and 6.05, respectively, according to the ACD program in the ISIS database.
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11. The calculated log *D* (pH 7.4) values for compounds **22** and **23** is 2.74; **24** is 4.04; **25** is 3.79; **26** is 3.98; **27** is 4.03; **28** and **29** is 3.78; and **30** is 2.94 according to the ACD program in the ISIS database.
12. The amino group present in compounds **22**–**30** is protonated at pH 7.4, with the exception of compound **24**, according to calculations using the ISIS ACD program. The estimated p*K*a value for compounds **22** and **23** is 9.43; while the estimated p*K*a of **24** is 6.71; **25** is 7.72; **26** is 6.71; **27** is 8.30; **28** and **29** is 8.18; and **30** is 8.91.