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Design and Synthesis of Potent, Orally Bioavailable Dihydroquinazolinone Inhibitors of p38 MAP Kinase

John E. Stelmach,[a,*] Luping Liu,[a] Sangita B. Patel,[a] James V. Pivnichny,[a] Giovanna Scapin,[a] Suresh Singh,[a] Cornelis E. C. A. Hop,[b] Zhen Wang,[b] John R. Strauss,[c] Patricia M. Cameron,[d] Elizabeth A. Nichols,[d] Stephen J. O'Keefe,[d] Edward A. O'Neill,[d] Dennis M. Schmatz,[d] Cheryl D. Schwartz,[d] Chris M. Thompson,[d] Dennis M. Zaller[d] and James B. Doherty[a]

[a]*Department of Medicinal Chemistry, Merck Research Laboratories, PO Box 2000, Rahway, NJ 07065, USA*
[b]*Department of Drug Metabolism, Merck Research Laboratories, PO Box 2000, Rahway, NJ 07065, USA*
[c]*Department of Comparative Medicine, Merck Research Laboratories, PO Box 2000, Rahway, NJ 07065, USA*
[d]*Department of Inflammation/Rheumatology, Merck Research Laboratories, PO Box 2000, Rahway, NJ 07065, USA*

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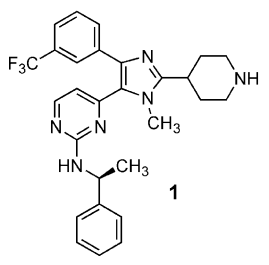
Abstract—The development of potent, orally bioavailable (in rat) and selective dihydroquinazolinone inhibitors of p38α MAP kinase is described. These analogues are hybrids of a pyridinylimidazole p38α inhibitor reported by Merck Research Laboratories and VX-745. Optimization of the C-5 phenyl and the C-7 piperidinyl substituents led to the identification of **15i** which gave excellent suppression of TNF-α production in LPS-stimulated whole blood (IC_{50} = 10 nM) and good oral exposure in rats (F = 68%, AUCn PO = 0.58 μM h).

The biological agents infliximab and etanercept are TNF-α sequestrants and are effective for the treatment of rheumatoid arthritis (RA).[1] Inhibitors of p38α MAP kinase suppress the production of proinflammatory cytokines TNF-α and IL1-β and have excellent potential for the treatment of inflammatory disorders.[2] Many of the published p38α inhibitors possess a vicinal aryl/4-pyridinyl-heterocycle arrangement exemplified by the pyridinylimidazole SB-203580 and compound **1**.[3] Crystal structures of p38-inhibitor complex have shown that this class of compounds utilizes two key binding interactions.[4] The pyridine nitrogen forms a hydrogen bond with the main chain N–H of Met 109 and the aryl substituent penetrates into a hydrophobic pocket not accessed by ATP. Recently, a new class of inhibitors has emerged that utilize similar interactions to bind to p38α but which possess a carbonyl hydrogen bond acceptor instead of pyridine nitrogen.[3] Of particular interest Vertex has reported the development of VX-745, which was recently in phase II clinical trials for the treatment of RA.[5] VX-745 was reported to be more than 1000-fold selective for p38α over the kinases ERK2, JNK1, JNK3, lck, Src, and MAPKAP-2.[6] Selectivity for p38α over other MAP kinases has been an issue with the pyridinylimidazole inhibitors. This new class of p38α inhibitors is of interest because of its inherent selectivity, novel structure, unique binding and potential for improved pharmacology. We have described the



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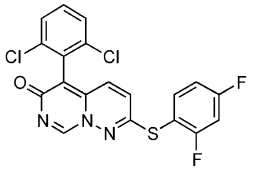
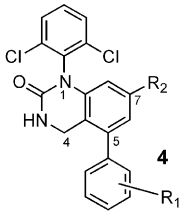


VX-745



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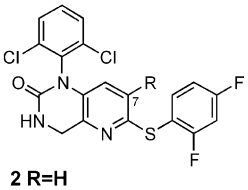
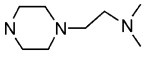


2 R=H

3 R= 

*Corresponding author. Fax: +1-732-594-8080; e-mail: john_stelmach@merck.com

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development of compound **2** which is a reduced-isomeric analogue of VX-745.[7] In addition, we have shown that polar amine substituents at C-7 (e.g., **3**) improves the potency and physical properties.

The rationale for the design of hybrid compound **3** was based on molecular modeling and by analogy to the potent pyridinylimidazole **1** reported by Merck.[8] This analysis also suggested that the p38α hydrophobic pocket, which is accessed by the C-6 thioether in **2**, could also be accessed by a C-5 phenyl substituent. We report in this paper the preparation, p38α inhibitory activity and rat pharmokinetic (PK) properties of dihydroquinazolinones **4**.

In order to confirm the predicted binding mode for the dihydroquinazolinone compounds the structure of p38α in complex with **14e** was solved to 2.4 Å resolution.[9] The binding orientation of **14e** relative to the binding orientation of **1** is shown in Figure. 1. The structure of **1** is taken from its crystallographic complex with p38α.[10] The urea carbonyl oxygen in **14e** and the pyrimidine ring nitrogen in **1** form hydrogen bond(s) interactions with the backbone of the hinge region. In contrast to **1**, which forms a single hydrogen bond with Met109, **14e** forms three hydrogen bond interactions (Leu107, Met109 and Gly110). The 2,6-dichlorophenyl ring of **14e** superimposes with the phenyl ring of the α-methylbenzyl ring of **1**. The 3-trifluoromethylphenyl ring in **1**, the critical hydrophobic substituent, overlays quite well with the 2,4-difluorophenyl ring in **14e**.

The preparation of quinazolinones **9** and **10** is summarized in Scheme 1. Urea **7** (R = CO_2Me) was prepared in three steps beginning with the benzylic bromination of methyl 3,5-dibromo-4-methylbenzoate. Alkylation of the product benzyl bromide with 4-methoxy benzyl amine gave **6**, which was then treated with 2,6-dichloroisocyanate. Initial attempts to cyclize compounds similar to **7** using palladium mediated conditions resulted in extended reaction times and the isolation of considerable amounts of debrominated starting material and debrominated cyclized product.[11] In contrast, cyclization of urea **7** using conventional Ullmann coupling conditions cleanly gave compound **8**.[12] The PMB protecting group was removed by refluxing in TFA and the C-5 phenyl substituents were attached by Suzuki coupling.

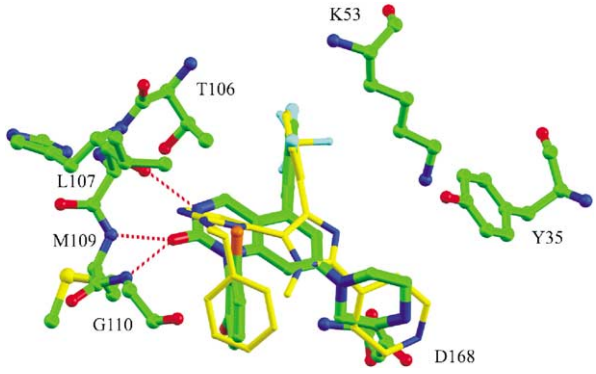
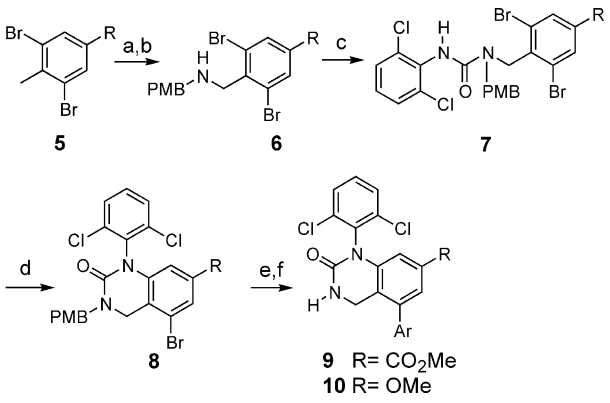


Figure 1. Relative binding orientations of **1** (yellow) and **14e** (green).

In the pyridinylimidazole series of p38 inhibitors aryl substituents bearing small alkyl or halide groups were optimal for the p38 hydrophobic pocket. Molecular modeling suggested that the C-5 phenyl substituent in compound **9** penetrates deeper into the p38α hydrophobic pocket than the 3-trifluoromethylphenyl substituent in compound **1**. The p38α inhibition of compounds **9** and **10** bearing a series of C-5 phenyl substituents is shown in Table 1. Consistent with our p38α binding model, compounds **9a** and **9b**, which lack the critical hydrophobic substituent, were inactive. In contrast, compound **9c** which has a C-5 phenyl substituent was very potent against p38α. Substitution with a fluoride or chloride at the 3-position resulted in a 2-fold decrease in potency (**9e** and **9h**, respectively). Substitution at the 3-position with larger substituents gave an even greater attenuation in potency. Fluoride,



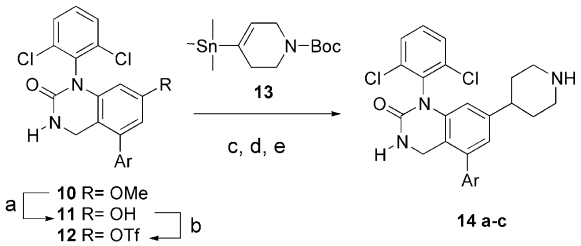
Scheme 1. Reagents and conditions: (a) NBS, Bz_2O_2; (b) $PMBNH_2$; (c) 2,6-diCl-PhNCO; (d) CuI, K_2CO_3, 150 °C DMF, 15 min; (e) TFA reflux; (f) $ArB(OH)_2$, $Pd(PPh_3)_4$.

Table 1. p38α inhibition of **9** and **10**

Compd	Ar	p38α IC_{50} (nM)
9a	Br	0% @ 1000 nM
9b	H	0% @ 1000 nM
9c	Phenyl	30
9d	2-Fl-Phenyl	7
9e	3-Fl-Phenyl	79
9f	4-Fl-Phenyl	22
9g	2-Cl-Phenyl	1
9h	3-Cl-Phenyl	67
9i	4-Cl-Phenyl	47
9j	2-CF_3-Phenyl	50% @ 890 nM
9k	3-CF_3-Phenyl	170
9l	4-CF_3-Phenyl	130
9m	2-CH_3-Phenyl	11
9n	3-CH_3-Phenyl	320
9o	4-CH_3-Phenyl	44
9p	2,4-di Fl-Phenyl	7
9q	2,6-di Fl-Phenyl	44
9r	2-CH_3-4-Fl-Phenyl	11
9s	-S-2,4-di Fl-Phenyl	48% @ 1000
10a	2-Cl-4-Fl-Phenyl	3
10b	2,4-di Fl-Phenyl	11
10c	2-Cl-Phenyl	6

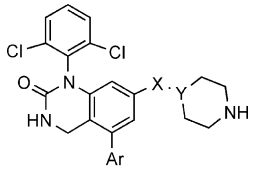
chloride and methyl substituents at the 2-position were optimal. Compound **9g**, which possesses a 2-chlorophenyl substituent, was 10-fold more potent than the corresponding unsubstituted phenyl. A fluoride at the 4-position gave a slight increase in potency. Other groups at the 4-position attenuated potency. Compound **9s**, which has the VX-745 hydrophobic substituent (i.e., 2,4-difluorothiophenyl ether), had modest activity.[13] While we were satisfied with the achieved p38α enzyme inhibition we found that none of the compounds in Table 1 gave satisfactory inhibition of TNF-α production in LPS-stimulated monocyte or THP-1 cells. For example, at 2 μM concentration, **9g** ($IC_{50} = 1$ nM p38α) inhibited the production of TNF-α in monocytes by a modest 33%. We attributed the poor cellular activity to the low aqueous solubility, high lipophilicity and anticipated high protein binding of these compounds.

The piperidinyl moiety of compound **1** confers aqueous solubility and increased p38α inhibition. The increased potency is presumed to arise from a salt bridge interaction between the protonated piperidine nitrogen and Asp168. This is a binding interaction not attained in VX-745. The superposition of **1** and **14e** (Fig. 1) indicates that the C-7 position is the optimal attachment point for a piperidinyl-like group. The preparation of analogues possessing a directly attached 4-piperidine is summarized in Scheme 2. The C-7 methyl ether of **10** was cleaved with boron tribromide and phenol product **11** converted to the triflate **12**. Stille coupling of **12** with the vinyl tin reagent **13** gave the desired coupled product. The trisubstituted double bond was hydrogenated and the BOC group removed by treatment with acid to give analogues **14a–c**.



Scheme 2. Reagents and conditions: (a) BBr_3; (b) $PhN(Tf)_2$; (c) $Pd(PPh_3)_4$, LiCl, **13**; (d) H_2, PtO_2; (e) TFA.

Table 2. p38α inhibition of **14**



14

Compd	Ar	X	Y	p38α IC_{50} (nM)	TNF-α Cell IC_{50} (nM)
14a	2-Cl-Phenyl	—	CH	0.9	2.0[a]
14b	2,4-di Fl-Phenyl	—	CH	0.2	1.3[a]
14c	2-Cl-4-Fl-Phenyl	—	CH	0.1	1.0[a]
14d	2-Cl-Phenyl	—	N	1.4	2.9[a]
14e	2,4-di Fl-Phenyl	—	N	2.6	7.8[a]
14f	2-Cl-Phenyl	O	CH	0.2	1.6[a]
14g	2-Cl-4-Fl-Phenyl	O	CH	0.1	0.7[b]
14h	2-Cl-Phenyl	NH	CH	0.5	4.4[b]
14i	2,4-di Fl-Phenyl	NH	CH	0.6	4.5[a]
14j	2-Cl-Phenyl	CH_2	N	0.1	1.4[a]
14k	2-Cl-4-Fl-Phenyl	CH_2	N	0.13	0.7[a]
14l	2-Cl-Phenyl	CO	N	1.5	11.4[a]
14m	2,4-di Fl-Phenyl	CO	N	2.4	26.8[a]
14n	2-Cl-4-Fl-Phenyl	CO	N	1.1	3.8[a]

[a]Monocyte cells.
[b]THP-1 cells.

In addition, analogues containing a C-7 piperidine or piperazine moiety directly attached to the dihydroquinazolinone or separated by one atom were also prepared (see Table 2). Analogues **14d**, **e**, **h**, and **i** were prepared by Buchwald–Hartwig coupling with triflate **12** and the appropriate amine. Analogues **14f** and **14g** were prepared by Mitsunobu coupling with phenol **11**. Compounds **14j–14n** were derived from **9** by reductive amination or by carbodiimide mediated carboxylic acid/amine coupling. Piperidine and piperazine C-7 substituents were well tolerated in the p38α enzyme assay. In most cases sub-nanomolar inhibition of p38α was observed. More importantly, these compounds are excellent inhibitors of TNF-α production in LPS-stimulated monocytes and THP-1 cells. Consistent with the anticipated selectivity of these p38α inhibitors, compound **14b** had insignificant activity against ERK1, JNK3, FYN, JAK1, JAK2, JAK3, lck, lyn, tyk2, and src kinases (i.e., $IC_{50} > 10$ μM).

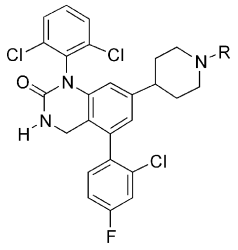
We evaluated the rat PK properties of these promising inhibitors (Table 3). Unfortunately, this series of compounds had poor rat PK properties characterized by high clearance and low oral bioavailability. Only compound **14d** had a modest PK profile. HPLC/MS/MS analysis of **14c** incubated with rat liver microsomes indicated extensive oxidation of the piperidine moiety. We reasoned that the metabolism, whether occurring by electron transfer from the piperidine nitrogen or by a hydrogen atom abstraction proximal to the nitrogen, would be suppressed by bulky nitrogen substituents.

Analogues of **14c** containing alkyl substituents on the piperidine nitrogen were investigated (Table 4). With the exception of **15i**, all compounds were prepared by standard alkylation or reductive amination of **14c** with the appropriate alkyl halide or aldehyde.[14] Piperidine nitrogen alkyl substituents were well tolerated in the p38α enzyme assay. These analogues also had excellent inhibition of TNF-α production in LPS-stimulated

Table 3. Rat pharmokinetic data for compound **14**

Compd	IV $t_{1/2}$ (h)	Vd (L/kg)	Clp (mL/min/kg)	AUCn PO (μM h)	F (%)
14a	2.5	21.4	181.0	0	0
14b	3.3	13.6	58.6	0.02	4.3
14c	2.0	7.8	72.3	0	0
14d	1.6	4.3	34.4	0.22	22.7
14f	3.0	21.0	115.5	0.02	6.3
14k	2.8	18.6	139.5	0.04	15.7
14m	2.3	12.8	94.7	0	1.5

Table 4. p38α inhibition and rat pharmokinetic data for compound **15**



15

Compd	R	p38α IC_{50} (nM)	TNF-α cell IC_{50} (nM)	TNF-α WB IC_{50} (nM)	IV $t_{1/2}$ (h)	Vd (L/kg)	Clp (mL/min/kg)	AUCn PO (μM h)	F (%)
15a	Methyl	0.5	1.3[a]	—	1.5	5.3	49.8	0.06	9.2
15b	Ethyl	1.2	0.7[b]	4.0	2.2	7.5	56.3	0.11	20.0
15c	*i*-Propyl	0.6	0.2[b]	5.6	1.9	12.2	84.8	0.06	16.0
15d	Cyclopropyl	1.1	16.6[a]	—	2.5	8.1	51.7	0.39	59.3
15e	Methyl Cyclopropyl	0.5	0.3[b]	7.6	1.9	6.3	50.9	0.15	22.6
15f	Ethyl 1-Cyclopropyl	0.3	—	7.0	2.0	4.7	31.1	0.36	38.0
15g	Cyclobutyl	0.4	0.5[b]	12.2	1.7	6.6	53.7	0.14	24.7
15h	Methyl Cyclobutyl	0.6	—	27.1	1.6	2.5	24.2	0.14	11.6
15i	*t*-butyl	0.2	0.6[b]	10.1	2.2	6.0	35.2	0.58	67.7

[a]Monocyte cells.
[b]THP-1 cells.

monocytes, THP-1 cells and human whole blood (WB). Small *N*-alkyl substituents (i.e., methyl, ethyl, *i*-propyl, **15a–c**) gave a modest improvement in rat PK.[15] In contrast, a bulky *N*-*t*-butyl substituent (**15i**) markedly improved the rat PK (F = 68%).

In summary, we have described the design and optimization of dihydroquinazolinone p38α inhibitors, which can be considered hybrids of the pyridinylimidazole **1** and VX-745. Dihydroquinazolinone analogues possessing a C-7 piperidine or piperazine moiety effectively suppressed the production of TNF-α in monocyte cells, THP-1 cells and whole blood. These analogues had high clearance and low oral bioavailability in rats. However, substitution of the piperidine nitrogen with a bulky *t*-butyl substituent dramatically improved the clearance and oral exposure in rats.

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12. The copper mediated cyclizations of ureas **7** lacking the internal PMB protecting group were not clean.
13. Palladium mediate coupling of **8** with 2,4-di-FlPhSSnBu_3 gave **9s**.
14. Analogue **15i** was prepared via the Stille coupling of **12c** with the corresponding *N*-*t*-butyl analogue of **13**.
15. HPLC/MS/MS analysis of **15c** incubated with rat hepatocytes indicated oxidative *N*-dealkylation of the piperidine and oxidation/conjugation of the C-5 phenyl and the C-4 benzylic carbon proximal to the urea moiety.