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1-Phenyl-5-pyrazolyl Ureas: Potent and Selective p38 Kinase Inhibitors

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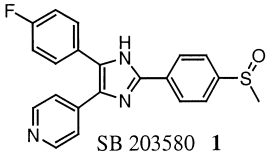
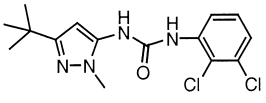
Abstract—Inhibitors of the MAP kinase p38 are potentially useful for the treatment of arthritis and osteoporosis. Several 2,3-dichlorophenyl ureas were identified as small-molecule inhibitors of p38 by a combinatorial chemistry effort. Optimization for cellular potency led to the discovery of a new class of potent and selective p38 kinase inhibitors, exemplified by the 1-phenyl-5-pyrazolyl urea **7** (IC_{50} = 13 nM).

Inhibitors of the MAP kinase p38[1,2] such as SB203580 (**1**, Fig. 1) provide novel approaches for the treatment of osteoporosis and inflammatory disorders.[3] As part of a combinatorial chemistry program, pyrazole **2** was identified as a new lead structure[4] (see preceding paper). This compound, as well as **1**, also inhibits TNF and IL-1 induced IL-6 production in SW1353 cells (human chondro-sarcoma).[4]



SB 203580 **1**
p38 IC_{50} = 20 nM
SW 1353 cells IC_{50} = 50 nM



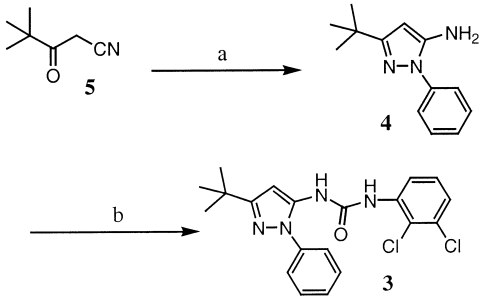
2
p38 IC_{50} = 53 nM
SW1353 cells IC_{50} = 820 nM

Figure 1. p38 Kinase inhibitors.

This communication describes our efforts in exploring the substitution of **2**, leading to the more potent 1-phenyl-5-pyrazolyl urea series, as well as optimizing the potency in the cellular functional assay.

Chemistry

1-Phenyl-5-pyrazolyl ureas, such as **3**, are easily accessible by the reaction of 1-phenyl-5-aminopyrazole **4** with 2,3-dichlorophenyl isocyanate. This reaction usually proceeds in very good yields (Fig. 2). The requisite 1-phenyl-5-aminopyrazoles are prepared by the condensation of phenylhydrazines with the commercially available ketonitrile **5**.



(a) $PhNHNH_2$, cat. AcOH, EtOH, reflux, 18 h, 60%. (b) 2,3-dichlorophenyl isocyanate, $PhCH_3$, 60 °C, 72 h, 62%.

Figure 2. General synthesis of phenylpyrazolyl ureas.

Reduction and acylation of the analogously formed 1-(3-nitrophenyl)pyrazole urea **6** leads to **7** and **8** respec-

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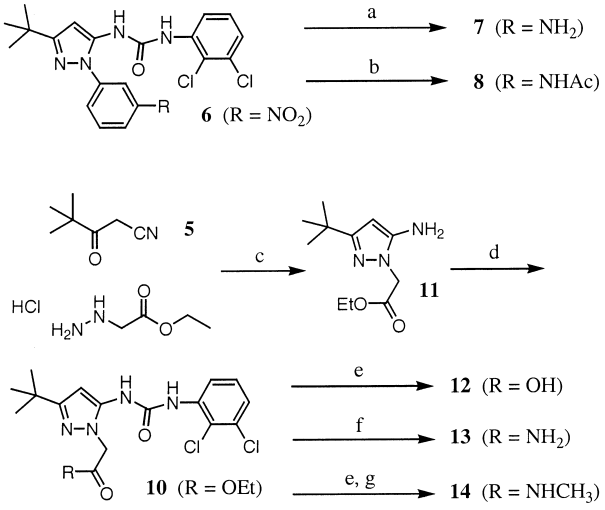


(a) Fe, AcOH, water, rt, 18 h, 88%. (b) Fe, AcOH, rt, then CH_3COCl, pyridine, CH_2Cl_2, 30 min, 82%. (c) EtOH, 45 °C, 5 h, 36%. (d) 2,3-dichlorophenyl isocyanate, $PhCH_3$, 50 °C, 18 h, 89%. (e) NaOH, EtOH, water, rt, 2h, 89%. (f) aq NH_3, reflux, 18 h, 52%. (g) DIEA, CDI, CH_2Cl_2, $CH_3NH_2.HCl$, rt, 45%.

Figure 3. Synthesis of substituted pyrazolyl ureas.

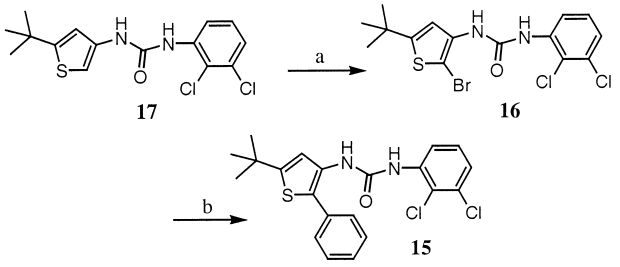
tively (Fig. 3). The *para*-substituted aniline **9** can be prepared in a similar fashion. The 2-pyrazolylacetates **12–14** are prepared by derivatization of the ester **10**, which is prepared from **11**.

The analogue in which the pyrazole ring is replaced by a thiophene is prepared by a different protocol (Fig. 4). Phenylthiophene **15** results from the palladium-catalyzed coupling of the corresponding bromide **16**, which is obtained by direct bromination of thienyl urea **17** (see preceding paper).

Results and Discussion

The right-hand side of **2** consists of a phenyl ring with two lipophilic substituents. A small set of analogues focusing on the substitution of this ring is shown in Table 1. Of these, only **22** and **23** retain nanomolar potency, albeit weaker than that of the lead. Pyrazole **2** is used as our reference compound for the variation of the pyrazole substituent (Table 2). Deletion of the methyl group of **2** (as in **28**) results in an equipotent compound in the kinase assay, but a dramatic loss of activity in the functional assay, suggesting a cellular permeability issue.

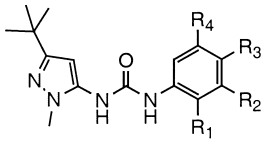
The 2-position of the pyrazole can accommodate a variety of substituents. Based on the hypothesis that this position of the pyrazole could interact with the sugar-binding pocket of the enzyme, we introduced a series of hydrogen bonding donors and acceptors. This hypothesis turned out to be unlikely, since potency decreases as hydrophilicity increases (compounds **10** and **12–14**). However, replacement of the methyl by a phenyl as in **3** results in a significant increase in biochemical and cellular potency. The substitution of the phenyl ring of **3** is further examined in Table 3.



(a) Br_2, chloroform, rt, 2.5 h, 93%. (b) $(CH_3)_3SnPh$, DMF, $Pd(PPh_3)_2Cl_2$ (10 mol%), 80 °C, 18 h, 10%.

Figure 4. Preparation of phenylthienyl ureas.

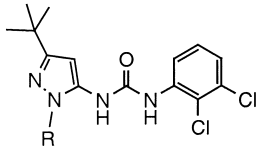
Table 1. Variation of the phenyl substituent



Compound	R_1	R_2	R_3	R_4	% Inhibition (500 nM)	p38 α2 IC_{50} (nM)	SW 1353[4] IC_{50} (nM)
2	Cl	Cl	H	H		53	820
18	H	H	CH_3	H	22[a]		
19	H	H	H	H	30[a]		
20	H	H	CF_3	H	47		
21	CF_3	H	H	H	30		
22	H	Br	H	Br		325	
23	H	H	CO_2Bu	H		290	
24	CH_3	CH_3	H	H	24		
25	H	Br	H	H	33		
26	H	CF_3	H	H	10		
27	H	Ph	H	H		797	

[a]Percent inhibition measured at 5 μM.

Table 2. Variation of the pyrazole substituent

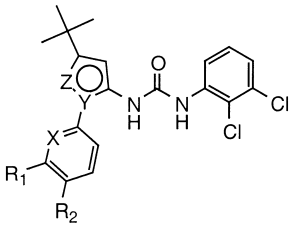


Compound	R	% Inhibition (500 nM)	p38 α2 IC_{50} (nM)	SW 1353[4] IC_{50} (nM)
2	CH_3		53	820
28	H	91	44	Inactive[a]
29	CH_2CH_2CN	81	180	Inactive[a]
30	CH_2CF_3	91	87	Inactive[a]
3	Ph	97	30	70
10	CH_2CO_2Et	76	105	Inactive[a]
12	CH_2CO_2H	0		
13	$CH_2C(O)NH_2$	48		
14	$CH_2C(O)NHCH_3$	61	250	
31	CH_2CH_2OH	75	130	Inactive[a]

[a]Less than 20% inhibition at 2.5 μM.

With the exception of the acetamide **8** (in which the introduction of a hydrophilic group tends to decrease potency), all analogues are potent inhibitors of p38. The more potent compounds of this set are the 3-nitro and the 3-amino analogues, **6** and **7**. These compounds are

Table 3. Structure–activity relationships of phenylpyrazolyl ureas



Compound	X	Y	Z	R_1	R_2	p38 α2 IC_{50} (nM)	SW 1353[4] IC_{50} (nM)
3	CH	N	N	H	H	30	70
15	CH	C	S	H	H	130	142
6	CH	N	N	NO_2	H	11	23
7	CH	N	N	NH_2	H	13	42
8	CH	N	N	NHAc	H	>500	
32	CH	N	N	H	*i*-Pr	110	Inactive[a]
33	CH	N	N	H	OCH_3	53	2000
34	CH	N	N	CF_3	H	56	209
35	CH	N	N	OCH_3	H	35	60
36	CH	N	N	H	NO_2	39	25
37	CH	N	N	H	SO_2CH_3	32	67
38	CH	N	N	H	Cl	42	324
39	CH	N	N	F	H	33	49
40	N	N	N	H	H	120	Inactive[a]
41	CCH_3	N	N	H	H	43	912
9	CH	N	N	H	NH_2	29	148

[a]Less than 20% inhibition at 2.5 μM.

also very potent in the functional cellular assay, although there is no good correlation between the activity against p38 and cellular potency (**33** vs **35**, **6** vs **36**). In general, analogues with an electron-withdrawing group in the *para* position of the phenyl moiety (such as pyrazolyl ureas **36** and **37**) show more activity in the functional assay. Finally, two analogues in which the phenylpyrazole conformation is modified, such as 2-pyridylpyrazolyl urea **40** (flat structure due to a strong internal hydrogen bond, as observed by ^{1}H NMR), or the non-planar *ortho*-substituted analogue **41**, are still active in the kinase assay, but very weak in cells. The biopharmaceutical properties of the best phenylpyrazole analogues are depicted in Table 4.

While molecular weights lie mostly within acceptable ranges for favorable oral availability, lipophilicities and aqueous solubilities appear to be suboptimal for many members of this new class.[5] Because of its superior aqueous solubility, 3-aminophenylpyrazolyl urea **7**[6] was selected for further pharmacological characterization. The selectivity of pyrazole **7** has been assessed against several cytosolic signaling kinases.[7] With the exception of the other p38 isoform, p38 β1 (IC_{50} = 52 nM), **7** is only moderately active against JNK-1 (IC_{50} = 850 nM) and abl (IC_{50} = 2.7 μM), and inactive against ERK-1 (0% inhibition at 5 μM). Additional data obtained from MDS Panlabs confirms this result.[8]

Table 4. Biopharmaceutical properties of selected phenylpyrazolyl ureas

Compound	p38 α2 IC_{50} (nM)	SW 1353 IC_{50} (nM)	MW (g/mol)	cLog P (daylight)	Aqueous solubility (μg/mL, pH 7.5)
3	30	70	403	5.96	24
6	11	23	448	6.06	18
7	13	42	418	4.76	594
35	35	60	433	5.99	46
36	39	25	448	6.06	66
37	32	67	481	4.74	79
39	33	49	421	6.27	17

In conclusion, we wish to report a new class of highly potent and selective p38 kinase inhibitors.[9] Replacement of the methyl group of **2** by a phenyl group leads to an improvement of the cellular potency. Results of pharmacological studies with the most promising analogue **7** will be reported in due course.

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5. Lipinski, C. A.; Lombardo, F.; Dominy, B. W.; Feeney, P. J. *Adv. Drug Delivery Rev*. **1997**, *23*, 3.
6. Preparation of **6** and **7**: A mixture of 4,4-dimethyl-3-oxopentanenitrile **5** (32.8 g, 0.26 mol), 3-nitrophenyl hydrazine hydrochloride (50 g, 1 equiv) and AcOH (6 mL) in EtOH (525 mL) is heated at the reflux temperature overnight, then cooled down to rt and concentrated under reduced pressure. The solid residue is washed with Et_2O, suspended in EtOAc, and treated with 300 mL of 1 M aq $NaHCO_3$ solution. The organic layer is separated, washed with brine, dried ($MgSO_4$), and concentrated. The solid residue is washed with 30% Et_2O in hexanes to afford 59.4 g (87% yield) of 2-(3-nitrophenyl)-3-amino-5-*tert*-butyl pyrazole as a yellow solid: ^{1}H NMR

(DMSO-d_6) δ 1.22 (s, 9H), 5.46 (s, 1H), 5.49 (bs, 2H), 7.72 (t, 1H, $J=8$ Hz), 8.08 (m, 2H), 8.44 (t, 1H, $J=2$ Hz). A mixture of the above material (13.07 g, 50 mmol) and 2,3-dichlorophenyl isocyanate (9.8 g, 1.04 equiv) in dry toluene (300 mL) is heated at 70 °C overnight, and cooled to rt. The reaction mixture is diluted with EtOAc, washed with water, brine, dried ($MgSO_4$), and concentrated. The solid residue is washed with toluene (100 mL) then with Et_2O/hexanes to afford **6** (16 g, 71% yield) as a white solid: ^{1}H NMR (DMSO-d_6) δ 1.29 (s, 9H), 6.44 (s, 1H), 7.30 (m, 2H), 7.81 (t, 1H, $J=8$ Hz), 7.97 (m, 1H), 8.05 (bd, 1H, $J=8$ Hz), 8.22 (bd, 1H, $J=8$ Hz), 8.36 (t, 1H, $J=2$ Hz), 8.76 (bs, 1H), 9.33 (bs, 1H); MS (FAB) m/z 448 ($M+H^+$, 12%). Anal. calcd C, 53.58; H, 4.27; N, 15.62. Found: C, 53.44; H, 4.35; N, 15.35. Iron powder (18.7 g) is added to a mixture of **6** (31.4 g, 70 mmol), AcOH (700 mL) and water (8 mL). The reaction mixture is stirred at rt overnight, then diluted with EtOAc and water. The pH is adjusted to 4 by slow addition of a 1 N aq NaOH solution. The organic layer is separated, washed with brine, dried ($MgSO_4$), and concentrated. The residue is purified by chromatography (SiO_2) (35% EtOAc/hexanes) to afford **7** (25.9 g, 88% yield) as a white solid (recrystallized from Et_2O/hexanes). ^{1}H NMR (DMSO-d_6) δ 1.25 (s, 9H), 5.40 (bs, 2H), 6.34 (s, 1H), 6.58 (m, 2H), 6.68 (bs, 1H), 7.13 (t, 1H, $J=8$ Hz), 7.30 (m, 2H), 8.09 (m, 2H), 8.86 (bs, 1H), 9.23 (bs, 1H); MS (FAB) m/z 418 ($M+H^+$, 70%). Anal. calcd C, 57.42; H, 5.06; N 16.74. Found: C, 57.39; H, 5.10; N, 16.53.

7. His-tagged JNK-1 was expressed in Sf9 cells and purified on imidazole sepharose. Abl kinase was purchased from Calbiochem. Activated ERK-1 was purchased from Upstate Biotechnology.

8. Testing of **7** against a panel of kinases at MDS Panlabs (Bothell, WA) showed modest potency against HER-2 (8.6 μM), $p56^{lck}$ (2.7 μM), and little effect against PKA, PKCα, PKCβ, PKCγ, EGF receptor kinase, and $p59^{fyn}$ (less than 50% inhibition at 10 μM).

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