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Development of *N*-2,4-pyrimidine-*N*-phenyl-*N*′-phenyl ureas as inhibitors of tumor necrosis factor alpha (TNF-α) synthesis. Part 1

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Abstract—A new class of tumor necrosis factor alpha (TNF-α) synthesis inhibitors based on an *N*-2,4-pyrimidine-*N*-phenyl-*N*′-phenyl urea scaffold is described. Many of these compounds showed low-nanomolar activity against lipopolysaccharide stimulated TNF-α production. X-ray co-crystallization studies with mutated p38α showed that these trisubstituted ureas interact with the ATP-binding pocket in a pseudo-bicyclic conformation brought about by the presence of an intramolecular hydrogen bonding interaction.

The over-expression of cytokines, such as tumor necrosis factor alpha (TNF-α) and interleukin-1β (IL-1β), has been implicated in a number of serious inflammatory disorders.[1] Consequently, agents that inhibit the proliferation of these proinflammatory cytokines could reduce inflammation and prevent further tissue destruction in diseases such as rheumatoid arthritis (RA), osteoarthritis (OA), and Crohn's disease. Several biological agents such as the soluble TNF-α receptor etanercept (Enbrel) and the TNF-α antibody infliximab (Remicade) have been shown to be a clinically effective treatment for these disorders.[2] However, universal acceptance of these medications has been limited by their high cost and need for intravenous or subcutaneous administration.

It has been well documented that potent inhibitors of p38 mitogen-activated protein (MAP) kinase attenuate TNF-α expression in vitro as well as in vivo. Many small molecule TNF-α production inhibitors that function by competitively binding to the ATP-binding pocket of p38 have been reported. SB203580[3](Fig. 1) represents a prototypical 4-aryl-5-pyridylimidazole-based inhibitor, although numerous other structural classes (e.g., pyrroles, pyrimidines, pyridines, pyrimidones, indoles, heteroindoles, ureas, and various fused bicyclic heterocycles) containing a variety of functionality have been reported to inhibit cytokine activity.[4] Several companies have progressed p38 inhibitors into clinical development for treatment of chronic inflammatory diseases, with varying degrees of success.[5] BIRB-796[6] (Fig. 1) was progressed into phase II clinical trials before development was halted due to reported liver toxicity.[7]

Recently we reported the synthesis and biological activity of a series of bicyclic pyrazolone-based inhibitors of TNF-α production exemplified by **1** (TNF-α IC_{50} = 22 nM, Fig. 1).[8] Pyrazolone **1** was a potent inhibitor of p38α (IC_{50} = 54 nM), while showing a good overall profile of kinase selectivity. Herein we wish to report a new structural class of trisubstituted ureas as cytokine inhibitors.

Synthesis of the described pyrimidine-ureas was accomplished in three steps from known starting materials. A representative procedure for this synthesis is shown in Scheme 1. Regioselective addition of variably substituted anilines to 2,4-dichloropyrimidine **2** gave the corresponding 6-anilino-4-chloropyrimidine **3**. A second nucleophilic addition of various alkyl amine derivatives to **3** gave the desired disubstituted pyrimidine **4**. Reaction of **4** with an appropriate isocyanate in dichloroethane (DCE) gave the corresponding trisubstituted ureas **5**–**6**.[9]

Keywords: Cytokine synthesis inhibitors; p38α kinase; Urea.
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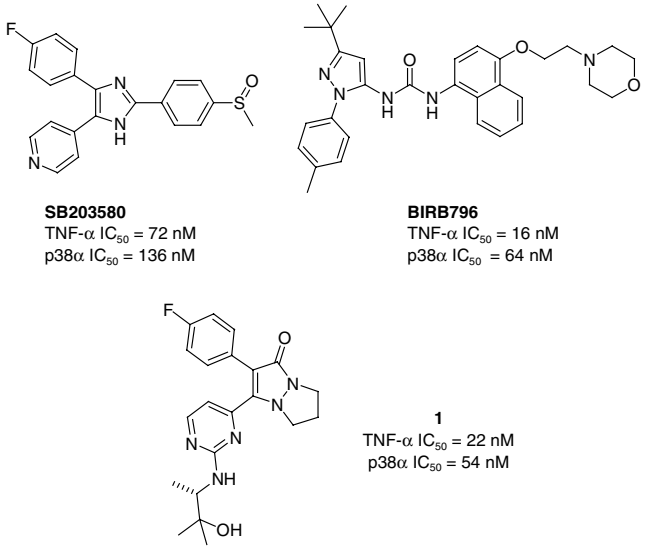
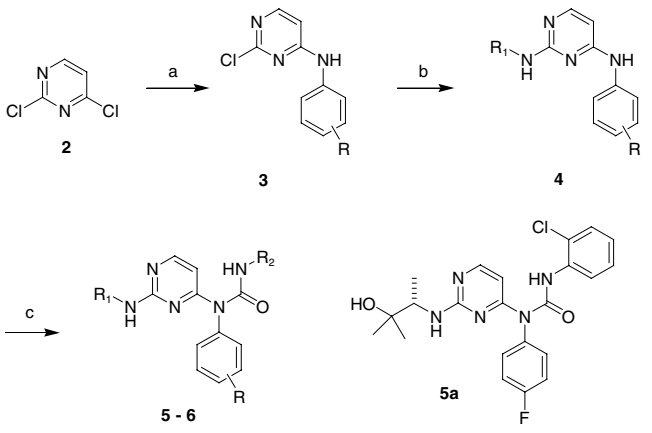


Figure 1. Small molecule proinflammatory cytokine inhibitors.



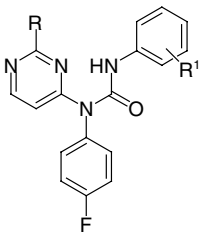
Scheme 1. Reagents and conditions: (a) EtOH, Na_2CO_3, $ArNH_2$; (b) NMP, $^iPr_2NEt$, R_1NH_2, 135 °C; (c) DCE, R_2NCO, rt to 80 °C.

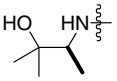
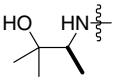
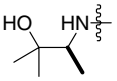
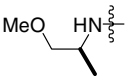
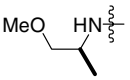
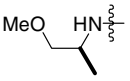
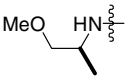
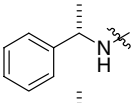
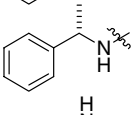
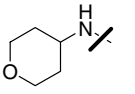
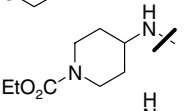
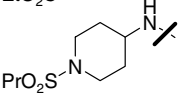
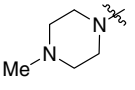
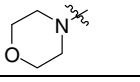
The urea analogs synthesized in this study were all tested for inhibition of TNF-α in an LPS stimulated human monocytic (THP-1) whole cell-based assay.[10]

When 2-chlorophenyl urea **5a** (Fig. 1) was tested for inhibition of TNF-α, the compound showed good potency (IC_{50} = 122 nM, Table 1). When the 2-chloro group was substituted with a 2-methyl substituent, the resulting analog **5b** proved to be virtually equipotent (IC_{50} = 176 nM). The 2,6-dichlorophenyl derivative **5c** showed somewhat reduced potency relative to the monosubstituted phenyl analogs. However, when the 2-pyrimidyl group was changed from (1*S*)-2-hydroxy-1,2-dimethylpropylamino to (1*S*)-2-methoxy-1-methylethylamino, the corresponding phenyl ureas **5d**, **5e**, and **5f** showed a greater than 10-fold loss in potency. The 4-substituted phenyl derivative **5g** was virtually inactive in the assay, indicating the need for 2-substitution on the *N*′-phenyl ring to maintain activity.

A series of other 2-pyrimidyl urea analogs (**5h–n**) were synthesized containing the N′-2-chlorophenyl

Table 1. TNF-α data for urea analogs containing an *N*-4-fluorophenyl group and an N′-substituted phenyl group



Compound	R	R^1	TNF-α IC_{50}[a] (μM)
5a		2-Cl	0.122
5b		2-Me	0.176
5c		2,6-Di-Cl	63%[b]
5d		2-Cl	3.10
5e		2-Me	3.65
5f		2-F	2.88
5g		4-CF_3	>5
5h		2-Cl	0.843
5i		2-Cl	>5
5j		2-Cl	0.539
5k		2-Cl	3.61
5l		2-Cl	1.42
5m		2-Cl	>5
5n		2-Cl	>5

[a] Standard deviation for the assay was ±30% of mean or less.
[b] Percentage inhibition at 1 μM.

substitution. The most active analog of this group was the tetrahydropyran-4-ylamino derivative **5j** which was nearly 5-fold less potent than **5a**. The chiral

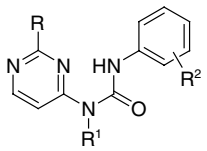
α-methylbenzylamino analog **5h** also showed sub-nanomolar activity, whereas the simple benzylamino analog **5i** produced an IC_{50} greater than 10 μM which suggests that alkyl branching adjacent to the amino linkage is key to the potency of these compounds. Additionally, the substituted aminopiperidine derivatives **5k** and **5l** both proved to be only low micromolar inhibitors of TNF-α production. Finally, the 2-pyrimidyl tertiary amino analogs **5m**–**n** were very poor inhibitors (IC_{50} > 10 μM), suggesting a need for the amino NH in maintaining activity.

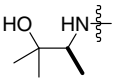
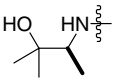
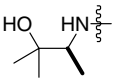
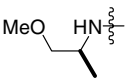
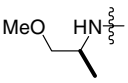
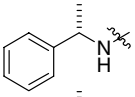
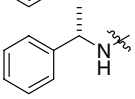
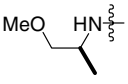
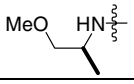
Unfortunately, this class of phenyl ureas proved to be chemically unstable. Although the DMSO stock solutions of inhibitors for biological testing were kept at 4 °C and showed no degradation over several weeks' storage, solutions (in methanol or DMSO) of compounds stored at room temperature would slowly decompose over several days. The decomposition was composed exclusively of hydrolysis back to the aniline precursor **4**. To address this issue, we first investigated adding electron density into the urea system by modifying the electron-withdrawing 4-fluorophenyl group. This work produced the series of phenyl urea analogs listed in Table 2. The first group of 4-methoxyphenyl ureas **6a** and **6b** were found to be nearly equipotent with the corresponding 4-fluorophenyl analogs **5a** and **5b**. These 4-methoxyphenyl analogs only proved to be slightly more stable than the 4-fluorophenyl derivatives, decomposing more slowly to the pyrimidyl aniline precursor. Replacement by the unsubstituted phenyl ring as in **6c** further reduced the potency by 6-fold versus **5a**. A similar loss in potency was observed when the dimethylhydroxypropylamino group was substituted with the methoxypropylamino group as in **6d**, in which this compound was nearly 3-fold less potent than **5e**. The 4-*N*,*N*-dimethylaminophenyl analogs **6e** and **6f** were even less active (IC_{50} > 10 μM).

Other modifications to the 4-fluorophenyl group were made to investigate not only effects on stability, but also potency. When the fluoro substituent was moved from the *para* position to the *meta* position to give compound **6g**, a near 10-fold loss of potency was observed compared to **5h**. The substitution of a phenyl group for an *N*-amino group as in **6h** resulted in complete loss of activity. Finally, replacement of the *N*-phenyl substituent with a methyl group (**6i**) also produced an inactive analog.

Due to an inability to significantly improve chemical stability while maintaining potency of the series, we were unable to progress these compounds into further pharmacokinetic or in vivo studies. However, we were still interested in the binding mode of these compounds in p38α. An X-ray crystal structure was obtained and solved for the co-crystal formed between the 2-chlorophenyl urea inhibitor **6a** and mutated p38α[13] (Fig. 2) to obtain additional information concerning the possible mechanism for TNF-α inhibition. This compound contains several of the necessary interactions for classical p38 inhibitors. Primary among these is the hydrogen bonding motif between the 2-aminopyrimidine

Table 2. TNF-α data for analogs of N′-phenyl-substituted ureas containing various N-substitutions



Compound	R	R^1	R^2	TNF-α IC_{50}[a] (μM)
6a		4-MeO-Ph	2-Cl	0.092
6b		4-MeO-Ph	2-Me	0.135
6c		Ph	2-Cl	0.718
6d		Ph	2-Me	1.00
6e		4-Me_2N-Ph	2-Cl	>5
6f		4-Me_2N-Ph	2-Me	>5
6g		3-F-Ph	2-Cl	6.16
6h		NH_2	2-Cl	>5
6i		Me	2-Me	>5

[a] Standard deviation for the assay was ±30% of mean or less.

functionality of the inhibitor and the Met-109 residue of the backbone of ATP-binding pocket. Additionally, the 4-MeO-phenyl group positions itself into the well-defined hydrophobic pocket defined by the 'gatekeeper' Thr-106 residue. Most notably, the conformation of the urea allows for the urea NH to form an intramolecular hydrogen bond with N-3 of the pyrimidine ring (2.69 Å N–N distance). This interaction creates a pseudo-bicyclic structure for this compound, which adds rigidity to this class of inhibitors. The 2-chlorophenyl group is positioned toward the exposed solvent pocket of the enzyme. As seen by the overlap of bicyclic pyrazolone **1**, the carbonyl of urea **6a** does not interact with the amino group of Lys-53, as was seen with the pyrazolone carbonyl.[8] This may explain the somewhat reduced potency of **5a** against TNF-α production versus pyrazolone **1**.

We have developed a new class of trisubstituted ureas as inhibitors of TNF-α production. First generation *N*-phenyl ureas showed good potency for inhibition of TNF-α, but proved to be chemically unstable. Attempts

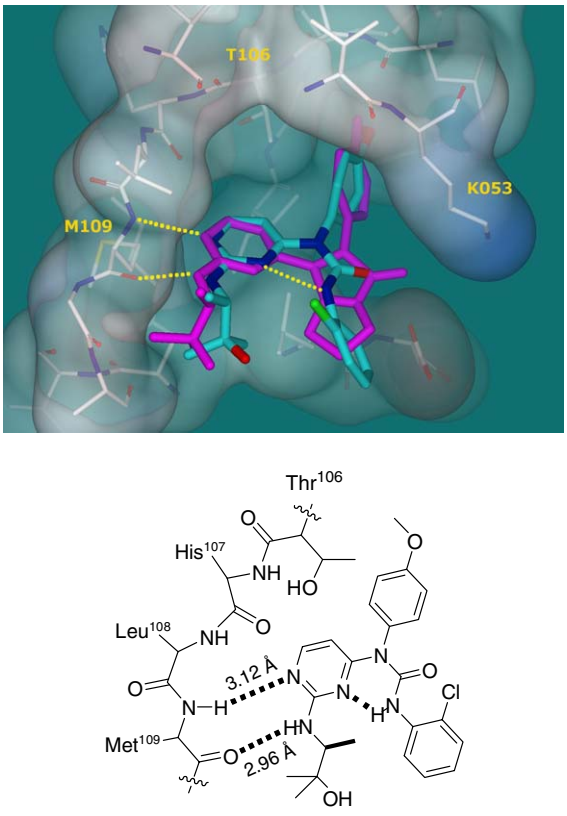


Figure 2. Co-crystal X-ray structure of **5b** (cyan) overlaid with **1** (magenta) in the ATP-binding site of mutated p38α.

to improve chemical stability by modifying the electronic properties of the various substitutions generally led to loss of potency with no significant improvement in stability. X-ray crystallography studies with mutated p38α showed a mode of binding for this class of urea inhibitors that mimics that of traditional vicinal bis-aryl MAP kinase inhibitors. Additionally, we observed that the urea adopts a pseudo-bicyclic orientation due to the compound's ability to form an internal hydrogen bond. Additional studies to improve chemical stability and test analogs from this class for in vivo anti-inflammatory activity will be reported in a separate communication.

Acknowledgments

We are grateful to M. Buchalova for supporting chemical stability studies, and R. L. Walter for X-ray co-crystallization studies. We thank A. G. Evdokimov and M. E. Pokross for cloning, expression, and purification of mp38 protein. We would like to acknowledge that X-ray data were collected at Southeast Regional Collaborative Access Team (SER-CAT) 22-ID (or 22-BM) beamline at the Advanced Photon Source, Argonne National Laboratory. Supporting institutions may be found at www.ser-cat.org/members.html. Use of the Advanced Photon Source was supported by the U.S. Department of Energy, Office of Science, Office of Basic Energy Sciences, under Contract No. W-31-109-Eng-38.

References and notes

1. For a recent review, see: Adams, J. L.; Badger, A. M.; Kumar, S.; Lee, J. C. In *Progress in Medicinal Chemistry*; King, F. D., Oxford, A. W., Eds.; Elsevier: Amsterdam, 2001; Vol. 39, pp 1–60.
2. Bolós, J. *Mini-Rev. Med. Chem.* **2005**, *5*, 857.
3. Badger, A. M.; Bradbeer, J. N.; Votta, B.; Lee, J. C.; Adams, J. L.; Griswold, D. E. *J. Pharmacol. Exp. Ther.* **1996**, *279*, 1453.
4. Boehm, J. C.; Adams, J. L. *Expert Opin. Ther. Patents* **2000**, *10*, 25.
5. Goldstein, D. M.; Gabriel, T. *Curr. Top. Med. Chem.* **2005**, *5*, 1017.
6. Pargellis, C. A.; Tong, L.; Churchill, L.; Cirillo, P.; Gilmore, T.; Graham, A. G.; Grob, P. M.; Hickey, E. R.; Moss, N.; Pav, S.; Regan, J. *Nat. Struct. Biol.* **2002**, *9*, 268.
7. Lee, M. R.; Dominguez, C. *Curr. Med. Chem.* **2005**, *12*, 2979.
8. Clark, M. P.; Laughlin, S. K.; Laufersweiler, M. J.; Bookland, R. G.; Brugel, T. A.; Golebiowski, A.; Sabat, M.; Townes, J. A.; VanRens, J. C.; Djung, J. F.; Natchus, M. G.; De, B.; Hsieh, L. C.; Xu, S. C.; Walter, R. L.; Mekel, M. J.; Heitmeyer, S. A.; Brown, K. K.; Juergens, K.; Taiwo, Y. O.; Janusz, M. J. **2004**, *47*, 2724.
9. All final compounds were characterized by ^{1}H NMR, HRMS, and HPLC analysis. Purity was assessed as >95%.
10. Duplicate cultures of human monocytic cells (THP-1)[11] (2.0×105/well) were incubated for 15 min in the presence or absence of various concentrations of inhibitor before the stimulation of cytokine release by the addition of lipopolysaccharide (final LPS concentration, 1 μg/ml). The amount of TNF-α released was measured 4 h later using an ELISA (R&D Systems, Minneapolis, MN). The viability of the cells after the 4 h incubation was measured using MTS assay[12] (Promega Co., Madison, WI).
11. Mohler, K. M.; Sleath, P. R.; Fitzner, J. N.; Cerretti, D. P.; Alderson, M.; Kerwar, S. S.; Torrance, D. S.; Otten-Evans, C.; Greenstreet, T.; Weerawarna, K.; Kronhelm, S. R.; Petersen, M.; Gerhart, M.; Kozlosky, C. J.; March, C. J.; Black, R. A. *Nature* **1994**, *370*, 218.
12. Barltrop, J. A.; Owen, T. C.; Cory, A. H.; Cory, J. G. *Bioorg. Med. Chem. Lett.* **1991**, *1*, 611.
13. The X-ray coordinates have been deposited with the Protein Data Bank, www.rcsb.org, as entry 2GHL. Mutated p38 co-crystallization/X-ray crystallography was conducted as follows: The mutated p38α herein described is a double mutant (S180A, Y182F) of murine p38α.[14] The mutant enzyme cannot be phosphorylated and, therefore, it is not competent for activation. Protein expression and purification were carried out as previously described for the murine enzyme.[15] For crystallization, mutated p38α was incubated overnight (12–16 h) with 1 mM compound. Co-crystals were grown by hanging drop vapor diffusion using PEG as a precipitating agent and overall protocols similar to those previously described for the human enzyme.[16] Crystals typically diffracted to 1.9 Å resolution and were of the previously reported space group: P212121; $a = 65.2$ Å, $b = 74.6$ Å, $c = 78.1$ Å (4′).
14. Han, J.; Lee, J. D.; Bibbs, L.; Ulevitch, R. J. *Science* **1994**, *265*, 808.
15. Wang, Z.; Harkins, P. C.; Ulevitch, R. J.; Han, J.; Cobb, M. H.; Goldsmith, E. J. *Proc. Natl. Acad. Sci. U.S.A.* **1997**, *94*, 2327.
16. Pav, S.; Whit, D. M.; Rogers, S.; Crane, K. M.; Cywin, C. L.; Davidson, W.; Hopkins, J.; Brown, M. L.; Pargellis, C. A.; Tong, L. *Protein Sci.* **1997**, *6*, 242.