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The development of new bicyclic pyrazole-based cytokine synthesis inhibitors

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Abstract—4-Aryl-5-pyrimidyl-based cytokine synthesis inhibitors of TNF-α production, which contain a novel bicyclic pyrazole heterocyclic core, are described. Many of these inhibitors showed low nanomolar activity against LPS-induced TNF-α production in a THP-1 cell-based assay and against human p38α MAP kinase in an isolated enzyme assay. The X-ray crystal structure of a bicyclic pyrazole inhibitor co-crystallized with mutated p38 (mp38) is presented.

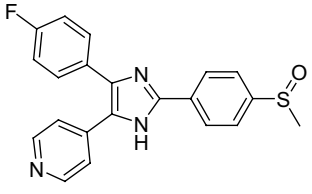
1. Introduction

Tumor necrosis factor-α (TNF-α) is a key pro-inflammatory cytokine produced in response to immunostimulants primarily by activated monocytes/macrophages to help clear infections and damaged cells from injured tissues. However, the overexpression of TNF-α has been implicated in a number of serious inflammatory disorders[1] such as rheumatoid arthritis,[2] inflammatory bowel disease (IBD),[3] osteoarthritis,[4] and Crohn's disease. Furthermore, TNF-α is a potent inducer of other pro-inflammatory cytokines such as IL-1, IL-6, and IL-8.[5] Accordingly, agents that inhibit TNF-α production can diminish the levels of these proinflammatory cytokines resulting in a reduction of inflammation and prevention of further tissue destruction.

Our research strategy involves designing kinase inhibitors that will result in the inhibition of TNF-α formation. There are multiple points at which one may effectively inhibit TNF-α synthesis since several MAP kinases exist in the signaling cascade. With this in mind, our primary assay entails screening analogs for inhibition of TNF-α production in LPS-stimulated human monocytic cells (THP-1). Although we are interested in the efficacy of our inhibitors against the overall cascade that leads to TNF-α formation, inhibition of p38 kinase may be one possible pathway through which the intended cellular effect is being demonstrated.

Various p38 inhibitors have been reported containing a common 4-aryl-5-pyridinyl (or 5-pyridyl)-based motif fused to a five or six-membered heterocyclic core,[6] such as SB203580[7] (Fig. 1), which represents a prototypical pyridyl imidazole-based inhibitor.

In a previous paper, we have reported the development of a class of cytokine inhibitors, which contains a vicinal bis-aryl five-membered heterocyclic core (Fig. 2).[8] In our continued effort towards the development of disease modifying treatments for osteoarthritis, we began to



SB203580
TNF-α IC_{50} = 72 nM
p38 kinase IC_{50} = 136 nM

Figure 1. Imidazole-based pro-inflammatory cytokine inhibitor.

Keywords: Bicyclic pyrazole; TNF-α; p38; Anti-inflammatory.
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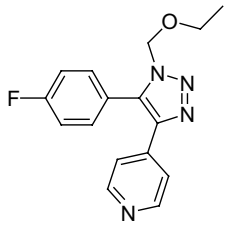


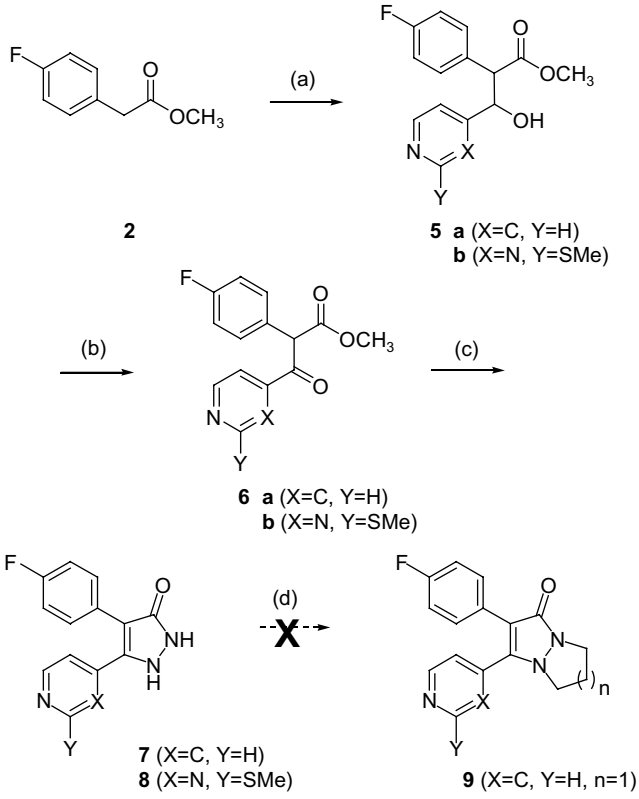
1
TNF-α IC_{50} = 490 nM

Figure 2. Vicinal bis-aryl five-membered heterocyclic cytokine inhibitor.

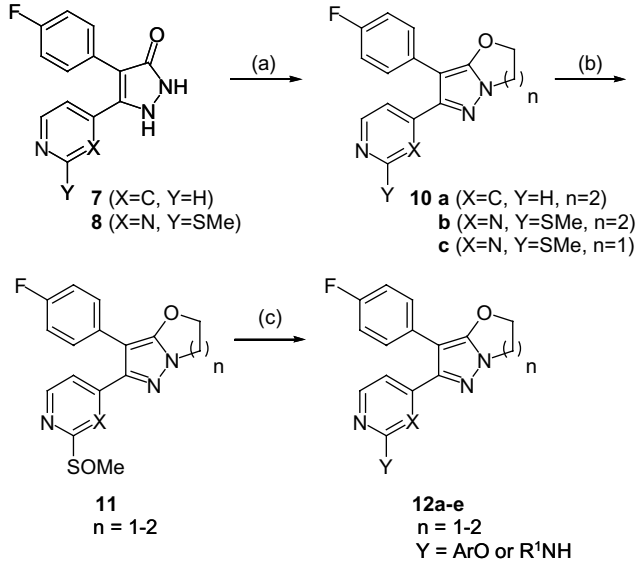
investigate other vicinal bis-aryl five-membered heterocycles as potential TNF-α inhibitors and these efforts led to the development of a new structural class of cytokine inhibitors, which contain a novel bicyclic pyrazole core. Herein we wish to report the synthesis and structure–activity relationships for this developing series.

2. Chemistry

Based upon the success of the triazole series (Fig. 2),[8] we began to explore similar heterocycles as potential TNF-α inhibitors such as the pyrazolone series. The unsubstituted parent pyrazolone **7** was synthesized in four steps from commercially available starting materials. Aldol reaction of methyl 4-fluorophenylacetate (**2**, Scheme 1) with 4-pyridine-carboxaldehyde (**3**) or 2-methylsulfanyl-pyrimidine-4-carbaldehyde (**4**) using LDA gives β-hydroxyester products **5a**–**b**. Alcohols **5a**–**b** are oxidized with chromium trioxide/pyridine to provide β-ketoesters **6a**–**b** containing the desired 4-fluorophenyl and either pyridyl or pyrimidyl functionality. The pyrazolone core is installed through a thermal cyclization reaction between β-ketoesters **6a**–**b** and semicarbazide hydrochloride (EtOH, 78 °C) to give 1,2-dihydropyrazolones **7** and **8**. We envisioned further functionalization of **7** via alkylation with dihaloalkanes to provide bicyclic pyrazolone **9**. However, alkylation of the 1,2-dihydropyrazolone **7** with 1,3-dibromopropane in the presence of NaH gives bicyclic pyrazole **10a** (Scheme 2) instead of the anticipated bicyclic pyrazolone **9**.[9] Alkylation of the 1,2-dihydropyrazolone **8** with either 1,2-dibromopropane or 1,2-dibromoethane in the presence of NaH gives **10b**–**c** as either a [5,6]- or [5,5]-bicyclic pyrazole, respectively. The 2-substituted pyrimidyl bicyclic pyrazole series provides an added benefit of a second site for functionalization. Oxidation of the thiomethylether group in **10b**–**c** to the corresponding sulfoxide **11** is performed using mCPBA in $CHCl_3$. Finally, displacement of sulfoxide **11** with either amines (R^1NH_2, toluene, 120 °C) or phenol (ArOH, NaH, THF) gives the corresponding amino- and phenoxy-bicyclic pyrazoles.



Scheme 1. Synthesis of pyrazolones **2** and **8**. Reagents and conditions: (a) LDA, 4-pyridine-carboxaldehyde **3** or 2-methylsulfanyl-pyrimidine-4-carbaldehyde **4**, THF, −78 °C, 76%; (b) CrO_3, pyridine, CH_2Cl_2, 43%; (c) Semicarbazide hydrochloride, EtOH, 78 °C, 81%; (d) $Br(CH_2)_nBr$, NaH, DMF, rt.

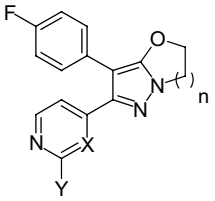


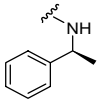
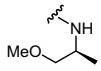
Scheme 2. Synthesis of the bicyclic pyrazole series. Reagents and conditions: (a) $Br(CH_2)_nBr$, NaH, DMF, rt, 44%; (b) mCPBA, $CHCl_3$, 0 °C, quant.; (c) R^1NH, toluene, 120 °C or ArOH, NaH, THF.

3. Results and discussion

Table 1 summarizes the results from our screening of seven novel bicyclic pyrazoles for inhibiting the production of TNF-α in LPS-stimulated human monocytic cells (THP-1)[10] and for the inhibition of human p38 MAP kinase.[13] The initial [5,6]-bicyclic pyrazole **10a** demonstrated similar effectiveness as an inhibitor of

Table 1. TNF-α inhibition data for the bicyclic pyrazole derivatives



Compound	*n*	X	Y	TNF-α IC_{50} (μM)	Human p38 MAP Kinase IC_{50} (μM)
1	—	—	—	0.49	0.31
7	—	C	H	3.1	0.32
10a	2	C	H	3.1	0.46
12a	2	N		>10	6.6
12b	1	N		0.30	2.2
12c	1	N		>2.0	11.2
12d	1	N		1.7	0.28
12e	1	N		0.08	0.36

both TNF-α synthesis and human p38 MAP kinase as the unsubstituted parent pyrazolone **7**. In the pyrimidyl series, the [5,5]-bicyclic pyrazole inhibitor **12b** proved to be a more potent inhibitor of both TNF-α production and human p38 MAP kinase than its [5,6]-bicyclic pyrazole counterpart **12a**. The X-ray crystal structure[14] of bicyclic pyrazole **12b** co-crystallized with mutated p38 (mp38) is shown in Figure 3. The mutated p38α herein described is a double mutant (S180A, Y182F) of murine p38α.[17] The phenoxy-substituted inhibitor **12b** orients itself in the mp38 enzyme such that the 4-fluorophenyl ring lies in the hydrophobic (specificity) pocket (Thr_{106}). The inhibitor has a direct interaction between the oxygen of the pyrazole ring and Lys_{53} as well as an interaction between the nitrogen of the pyrimidine ring and the Met_{109} N–H. However, the oxygen of the phenoxy-substituent suffers from a possible repulsive interaction with the carbonyl of Met_{109}.

Various amine analogs were then synthesized in the hope of obtaining a more favorable interaction between the amine substituent and the carbonyl of the Met_{109} residue. However, sulfoxide displacement with a benzylamine (**12c**) resulted in a less potent inhibitor. Smaller alkyl amines such as 2-methoxypropyl amine (**12d**) resulted in a modest improvement in potency against TNF-α production. Bicyclic pyrazole **12e** containing a

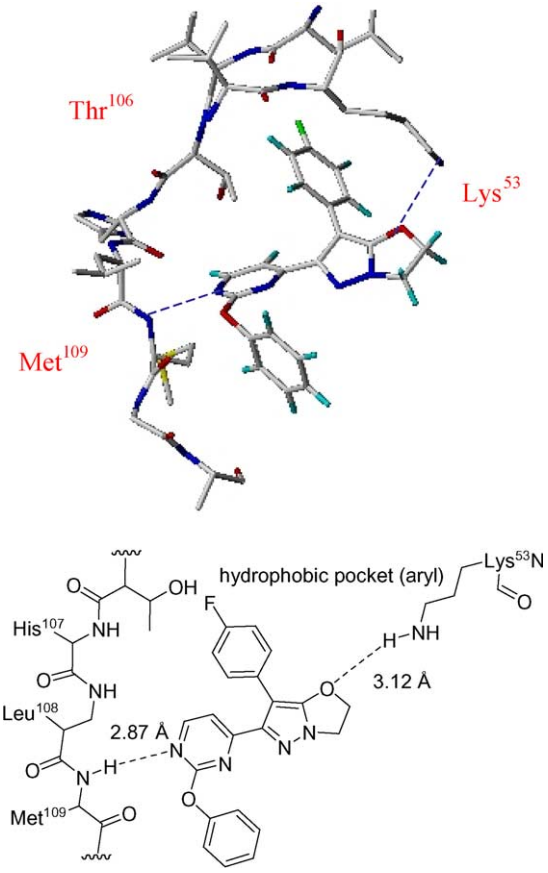


Figure 3. X-ray crystal structure of bicyclic pyrazole **12b** co-crystallized with mutated p38 (mp38) enzyme.

sec-butylamine substitution on the 2-position of the pyrimidine ring demonstrated a pronounced improvement in potency against TNF-α formation (IC_{50} = 0.08 μM) as compared to the other analogs in this series. Presumably, the improvement in activity seen with **12e** is due to the amine providing an additional interaction with the carbonyl of Met_{109} as compared to **12b**. Additionally, bicyclic pyrazoles **12d** and **12e** proved to be as effective as the initial pyrazolone lead **7** against human p38 MAP kinase.

The discrepancies between the human p38 MAP kinase data and the TNF-α inhibition data suggest that the bicyclic pyrazole series is possibly inhibiting TNF-α production via multiple mechanisms. Recent literature reports[18] have described known p38 inhibitors to be even more effective at inhibiting the RICK (Rip-like interacting caspase-like apoptosis-regulatory protein [CLARP] kinase/Rip2/CARDIAK) kinase, which is a signal transducer of inflammatory responses.

In conclusion, we have reported the synthesis of a series of novel bicyclic pyrazole inhibitors of TNF-α synthesis. Beginning from a modestly active lead **10a**, we have identified bicyclic pyrazole **12e** (IC_{50} = 0.08 μM) as a potent inhibitor of TNF-α formation in an in vitro assay against LPS-stimulated production of TNF-α. We hope to report further progress in developing this series of bicyclic pyrazoles in the near future.

Acknowledgements

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14. The mutant enzyme cannot be phosphorylated and, therefore, it is homologous to the inactive form of murine p38α. 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. Cocrystals 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.[17] Crystals typically diffracted to better than 2.0 Å resolution and were of the previously reported space group: $P2_12_12_1$; $a = 65.2$ Å, $b = 74.6$ Å, $c = 78.1$ Å.[16] X-ray data were collected at beamline 17-BM in the facilities of the Industrial Macromolecular Crystallography Association Collaborative Access Team (IMCA-CAT) at the Advanced Photon Source. These facilities are supported by the companies of the Industrial Macromolecular Crystallography Association through a contract with Illinois Institute of Technology (IIT), executed through IIT's Center for Synchrotron Radiation Research and Instrumentation. Use of the Advanced Photon Source was supported by the US Department of Energy, Basic Energy Sciences, Office of Science, under Contract No. W-31-109-Eng-38.
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