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The Development of New Triazole Based Inhibitors of Tumor Necrosis Factor-α (TNF-α) Production

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Abstract—4-Aryl-5-pyridyl and 4-aryl-5-pyrimidyl based inhibitors of TNF-α production, which contain a novel triazole 5-member heterocyclic core, are described. Many pyridyl triazoles containing either an alkyl ether or a substituted aryl side chain on the triazole core showed sub-micromolar activity against LPS-induced TNF-α, while pyrimidyl triazoles containing an ethoxymethyl side chain exhibited even better inhibitory activity. Secondary screening data are presented for the pyrimidyl triazoles. Triazole **14e** combined excellent potency with good oral bioavailability in the rat.

In our continuing efforts toward the development of disease modifying treatments for osteoarthritis, we are targeting the development of novel cytokine synthesis inhibitors. The overexpression of cytokines, such as TNF-α and IL-1β, has been implicated in a number of serious inflammatory disorders. Consequently, agents that inhibit the production of TNF-α can decrease levels of these proinflammatory cytokines, and thereby reduce inflammation and prevent further tissue destruction in diseases such as rheumatoid arthritis (RA),[1] osteoarthritis (OA)[2] and Crohn's disease.

SB203580[3] represents a prototypical pyridyl imidazole-based inhibitor, although numerous structural classes (e.g., imidazoles (Fig. 1), pyrroles, pyrimidines, pyridines, pyrimidones, indoles, heteroindoles, ureas, and various fused bicyclic heterocycles) containing a variety of functionality have been reported to inhibit TNF-α production.[4] Herein we wish to report a new structural class of 4-aryl-5-heteroaryl based TNF-α inhibitors which contain a novel triazole core.

Syntheses of the described pyridyl and pyrimidyl triazoles were accomplished in 3 and 4 steps, respectively, from readily available starting materials. In addition, both syntheses are amenable to large scale preparation and have been performed on 100+ g scale. Coupling of 4-fluorophenyl acetylene (**1**) (Scheme 1) with 4-bromopyridine (**2**) as described by Mangalagiu and co-workers[5] gives alkyne **3**, which is converted to triazole **4** via 3+2 cycloaddition with sodium azide. While the unsubstituted parent triazole **4** itself possessed only modest activity (TNF-α $IC_{50}=12.5$ μM), numerous possibilities existed for further functionalization of compound **4**: alkylation with alkyl halides; acylation with acid chlorides, carbamoyl chlorides and isocyanates; sulfonylation with sulfonyl chlorides; and arylation with aryl boronic acids. In practice, alkyl chlorides, acyl chlorides and isocyanates reacted with **4** in the presence of Et_3N and 1,2-dichloroethane at either ambient temperature or 80 °C. Arylation was accomplished using the corresponding aryl boronic acid, $Cu(OAc)_2$ and pyridine.[6] Structural assignments were made on the basis of X-ray crystallography, and in all cases the predominate isomer proved to have N2 substitution. Table 2 summarizes a broad preliminary survey of different appendages to the triazole. We have made amides, carbamates, sulfonamides, alkylamines, and ureas.

The related pyrimidyl triazole series, which was made analogously to the pyridyl triazoles, provided an added benefit of a second site of diversity. Coupling of

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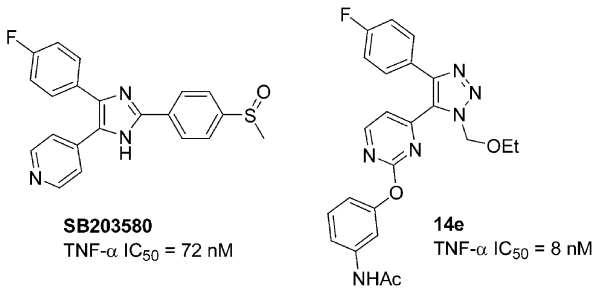
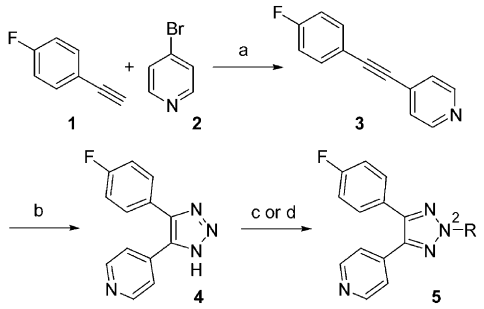
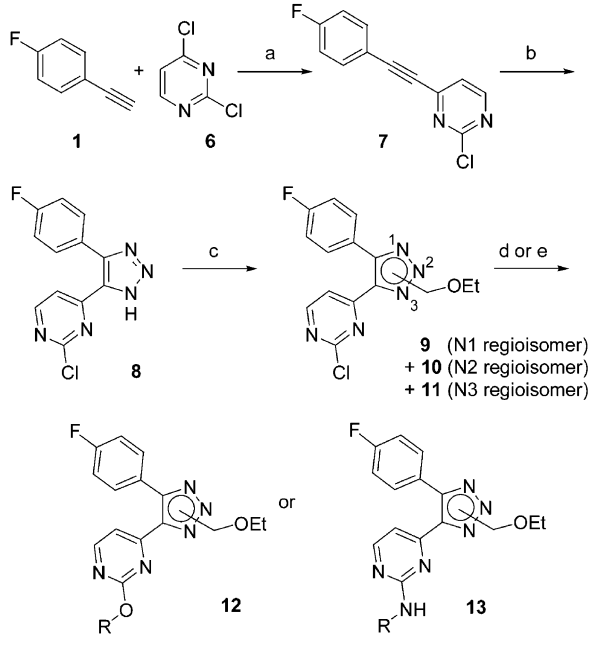


Figure 1. Vicinal aryl/pyridin (pyrimidin)-4-yl TNF-α inhibitors.



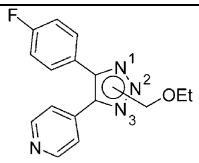
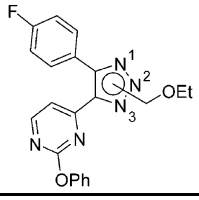
Scheme 1. Reagents and conditions: (a) ref 7; (b) NaN_3, DMA, 100 °C; (c) R-Cl, Et_3N, DCE; (d) Ar-$B(OH)_2$, ref 8.

4-fluorophenyl acetylene **1** (Scheme 2) with 2,4-dichloropyrimidine **6** yielded alkyne **7**,[5] which underwent [3 + 2] cycloaddition to give triazole **8**. Based on the survey of triazole substituents performed on the pyridyl series, the ethoxymethyl substituent was chosen to be held constant while a series of substitutents on the pyrimidine ring was examined. Reaction of triazole **8** with 2-chloromethyl ethyl ether and Et_3N at ambient temperature gave a 4:5:1 mixture of regioisomers **9**:**10**:**11** which were separable by HPLC and identified



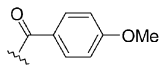
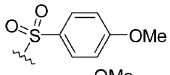
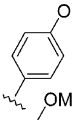
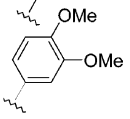
Scheme 2. Reagents and conditions: (a) ref 7; (b) NaN_3, DMF, 75 °C; (c) $EtOCH_2Cl$, Et_3N, CH_2Cl_2; (d) R-OH or R-NH_2, NaH, THF; (e) R-NH_2 neat, Δ.

Table 1. Comparative activity of selected regioisomers[a]

Entry	Structure	TNF-α IC_{50} (nM) N1	N2	N3
1		490	1069	N/A
2		611	4023	74

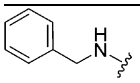
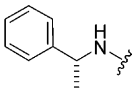
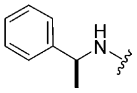
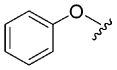
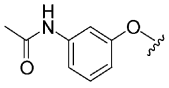
[a]Standard Deviation for enzyme assays were typically ±30% of the mean or less.

Table 2. TNF-α inhibition data for pyridyl triazole derivatives substituted on N2[a]

Compd	R	TNF-α IC_{50} (nM)
5a		3303
5b		4803
5c		1315
5d		744
5e		3832
5f		3716
5g		1924
5h		> 10,000
5i		7591
5j		490
5k		3756

[a]Standard Deviation for enzyme assays were typically ±30% of the mean or less.

Table 3. TNF-α inhibition data for N1 and N3-ethoxymethyl-substituted pyrimidyl triazole derivatives

XR	N3-substituted compd	IC_{50} (nM)[a]	N1-substituted compd	IC_{50} (nM)[a]
	**14a**	100	**15a**	557
	**14b**	46	**15b**	1602
	**14c**	54	**15c**	306
	**14d**	74	**15d**	164
	**14e**	8	**15e**	189

[a]Standard Deviation for enzyme assays were typically ±30% of the mean or less.

by X-ray crystallography. Reaction of chloropyrimidines **9–11** with alcohols and amines under either basic conditions (NaH, THF) or neat amine at elevated temperature yielded the desired substituted derivatives (e.g., **12** and **13**). In practice, derivatives of **10** proved to be 1-2 orders of magnitude less active than derivatives of **9**, while limited quantities prevented a systematic survey of derivatives of **11**. Table 3 summarizes several promising substituents of types **12** and **13** for the more potent triazole inhibitors.

All compounds were tested for the inhibition of TNF-α production using lipopolysaccharide (LPS) stimulated human monocytic cells (THP-1).[7] Table 1, which compares the three possible sites for substitution, reveals a general trend in TNF-α IC_{50} values that holds for the pyridyl and pyrimidyl triazoles: N2 > N1 > N3.

Table 2 summarizes a broad preliminary survey of pyridyl triazole substituents: amides, sulfonamides, carbamates, ureas, alkyl ethers, and aryl groups. In general, examples from each substituent class were found to exhibit modest inhibitory properties, even though the absolute potencies of the inhibitors were quite sensitive to even minor structural variations. Methoxyphenyl substituents linked with either a carbonyl or sulfonyl group (**5a** and **5b**) showed modest activity, but removal of the linker altogether resulted in analogues with activity around 1 μM (**5c** and **5d**). Far more pronounced substituent effects were observed for ureas and alkyl ethers. *N*-methyl-*N*-phenyl urea **5g** showed moderate activity, but the closely related analogue **5h** lost all TNF-α activity. Similarly, ethoxymethyl ether **5j** showed 500 nM potency, but even min changes resulted in substantial loss of TNF-α activity (e.g., **5i** and **5k**). Representing the most active substituent in the pyridyl triazole series, the ethoxymethyl group was then used for the investigation of the pyrimidyl triazole series.

Table 3 displays a comparison between N1 and N3-ethoxymethyl-substituted pyrimidyl triazole inhibitors. The N3-substituted inhibitors showed better inhibition than the corresponding N1-substituted analogues, mirroring the trend observed in the pyridyl triazole series. The benzylamino pyrimidine triazole **14a** showed good potency (100 nM). An improvement in inhibitory activity was seen with both of the methylbenzylaminopyrimidine triazoles (**14b** and **14c**). Phenoxypyrimidine **14d** showed good activity, while substitution of the phenoxy group with electron-withdrawing substituents, such as an acetamido group, on the 3-position increased the potency dramatically to provide one of our most potent inhibitors (**14e**). The 3-acetamido analogue **14e** displayed an excellent pharmacokinetic profile. We achieved good oral bioavailablity (F = 56%) in the rat while maintaining moderate half-life ($t_{1/2}$ = 2.2 h) and clearance (36.5 mL/min/kg) values. Finally, triazole **14e** showed positive oral efficacy (25 mg/kg) in the rat iodoacetate in-vivo model.[10] Currently, we are continuing to optimize characteristics of the triazole inhibitors to provide more in-vivo activity.

We have reported a novel series of pyridyl and pyrimidyl triazole TNF-α production inhibitors. Several of the pyridyl triazole inhibitors showed sub-μM activity in the LPS-induced TNF-α assay, while the N3-substituted pyrimidyl triazoles highlighted in Table 3 showed excellent potency at or below 100 nM. The 3-acetamidophenoxy-pyrimidinyl triazole **14e** also has shown an excellent pharmacokinetic profile and was orally efficacious in the rat iodoacetate model.

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7. Duplicate cultures of human monocytic cells (THP-1)[8] cells (2.0×10^5/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 (LPS, 2 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[9] (Promega Co., Madison, WI).

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9. Barltrop, J. A.; Owen, T. C.; Cory, A. H.; Cory, J. G. *Bioorg. Med. Chem. Lett.* **1991**, *1*, 611.
10. Sprague–Dawley male rats (200–225 g) from Harlan (Oregon, WI) under anesthesia were injected in the patellar ligament region of the left leg (flexed 90°at the knee) with 20 μL of a 10 mg/mL concentration of monosodium iodoacetate (IA) (Aldrich Chemical, Milwaukee, WI). Animals (groups of 15) were dosed for 7 days BID (∼every 12 h) with the potential inhibitor (25 mg/kg) or Vehicle (2.5 mL/kg). Animals were sacrificed on day 22 and the left joint was disarticulated and fixed in 10% formalin for 24–48 h prior to capturing the image. An image of the tibial cartilage surface was captured using an Optimas (Optimas, Media Cybernetics LP, Silver Springs, MA) image analysis system. Three independent observers assessed the damage in a blinded manner using a scale of 0–4 of increasing severity (0 = normal; 4 = maximum severity).[11]

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