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Selective inhibitors of tumor progression loci-2 (Tpl2) kinase with potent inhibition of TNF-α production in human whole blood

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ABSTRACT

Tpl2 (cot/MAP3K8) is an upstream kinase of MEK in the ERK pathway. It plays an important role in Tumor Necrosis Factor-α (TNF-α) production and signaling. We have discovered that 8-halo-4-(3-chloro-4-fluoro-phenylamino)-6-[(1*H*-[1,2,3]triazol-4-ylmethyl)-amino]-quinoline-3-carbonitriles **(4)** are potent inhibitors of this enzyme. In order to improve the inhibition of TNF-α production in LPS-stimulated human blood, a series of analogs with a variety of substitutions around the triazole moiety were studied. We found that a cyclic amine group appended to the triazole ring could considerably enhance potency, aqueous solubility, and cell membrane permeability. Optimization of these cyclic amine groups led to the identification of 8-chloro-4-(3-chloro-4-fluorophenylamino)-6-((1-(1-ethylpiperidin-4-yl)-1*H*-1,2,3-triazol-4-yl)methylamino)quinoline-3-carbonitrile (**34**). In a LPS-stimulated rat inflammation model, compound **34** showed good efficacy in inhibiting TNF-α production.

Studies on the complex biology of Tumor Necrosis Factor-α (TNF-α) have revealed that this cytokine is involved in the inflammation process in several disease states such as rheumatoid arthritis (RA), psoriasis, inflammatory bowel disease, and multiple sclerosis.[1] TNF-α also stimulates the production of other pro-inflammatory cytokines such as IL-1 and IL-6, and induces the activity of degradative enzymes such as the matrix metalloproteases (MMPs).[2] Inhibition of TNF-α with a number of biological agents such as etanercept (Enbrel),[3] infliximab (Remicade),[4] and adalimumab (Humira)[5] has been a major therapeutic advance in treating RA. These protein therapeutics have changed the treatment paradigm and improved the quality of life of RA patients. Small molecule drug can usually be less expensive and more conveniently administered (orally available) than proteins. Our desire for such therapeutics prompted our investigation of small molecules to inhibit TNF-α production.

Tpl2 (cot/MAP3K8), a serine/threonine kinase, plays a pivotal role in TNF-α production through the MEK-Erk pathway. Studies with Tpl2 knockout mice demonstrated that Tpl2 is essential for lipopolysaccharide (LPS) induced TNF-α production.[6] Furthermore, Tpl2 kinase is also required for TNF-α signaling.[7] Inhibition of Tpl2 thus could potentially have the double benefits of blocking both TNF-α production and signaling.

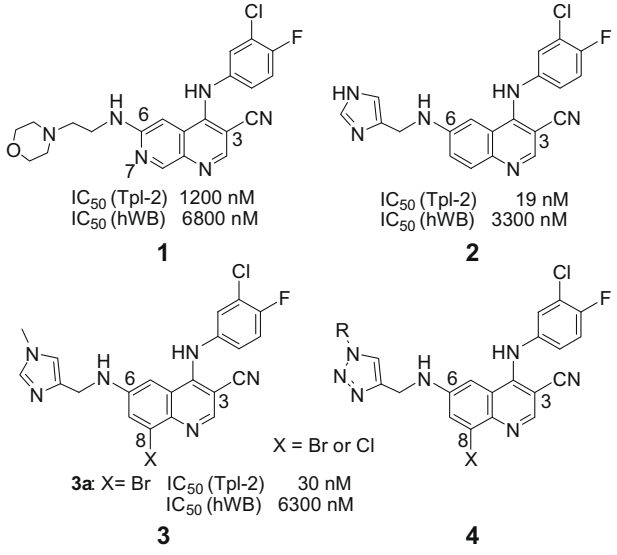

Figure 1. Tpl2 kinase inhibitors.

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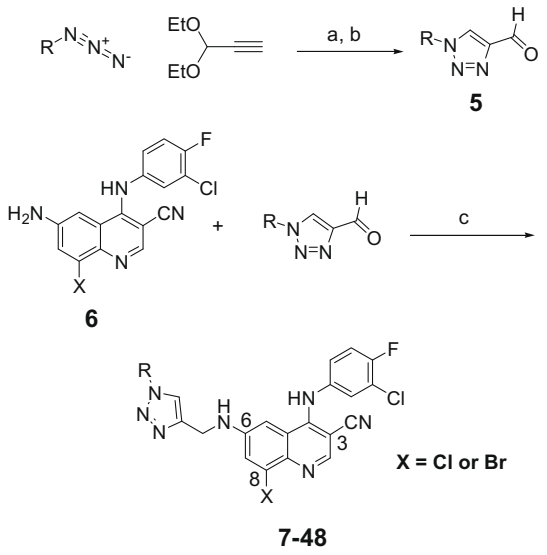


Scheme 1. Reagents and conditions: (a) sodium L-ascorbate, copper(II) sulfate; (b) hydrochloric acid; (c) sodium triacetoxyborohydride, 1,2-dichloroethane, rt, 4 h, 20–80%.

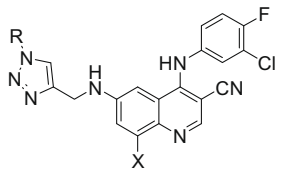
Previous studies in our laboratories have shown that 1,7-naphthyridine-3-carbonitriles (**1**, Fig. 1)[8] and 4,6-diaminoquinoline-3-carbonitriles (**2**)[9] were potent inhibitors of Tpl2 kinase. We subsequently reported improved selectivity and in vivo efficacy with 8-substituted-4-anilino-6-aminoquinoline-3-carbonitriles such as **3** in models of inflammation.[10] 8-Bromo-4-anilino-6-aminoquinoline-3-carbonitriles (**3a**) were over 1000-fold selective against a panel of serine–threonine and tyrosine kinases; however, these compounds show similar potency as compounds **1** and **2** in the inhibition of TNF-α production from LPS-stimulated human whole blood (hWB). As part of our continuing efforts toward the optimization of Tpl2 inhibitors, we herein report on a series of 8-halo-4-anilino-6-[(1*H*-[1,2,3]triazol-4-ylmethyl)-amino]-quinoline-3-carbonitriles (**4**) with improved potency in human blood.

Our preparation of 8-halo-4-anilino-6-[(1*H*-[1,2,3]triazol-4-ylmethyl)-amino]-quinoline-3-carbonitriles employed the synthetic route outlined in Scheme 1. Aldehyde **5** was synthesized through a [3+2] dipolar cyclo-addition of 3,3-diethoxyprop-1-yne and an organic azide,[11] which was either purchased commercially or prepared from the azide displacement of an alkyl halide. Under reductive alkylation conditions, aldehyde **5** reacted with 6-amino-8-halo-4-(3-chloro-4-fluorophenylamino)-quinoline-3-carbonitrile **6**, which was prepared through a previously reported multi-step synthesis,[10] to give the target compounds **7–48**.

All compounds prepared for this study were tested for inhibition of Tpl2 kinase using an ELISA assay that measures the amount of direct phosphorylated substrate MEK kinase.[8,9] Functional inhibition of TNF-α was measured using LPS-stimulated human monocytes and LPS-stimulated human whole blood. As illustrated in Table 1, compounds with an unsubstituted triazole at the 6-position (**7** and **8**) are potent in Tpl2 inhibition (IC_{50} of 40 nM and 18 nM, respectively). They inhibit TNF-α production in LPS-stimulated human whole blood with IC_{50}s of 4.2 μM, and 7.0 μM, respectively. Compared to **3**, these compounds show no significant improvement in hWB potency. In addition, **7** and **8** suffer from very low aqueous solubility and permeability.

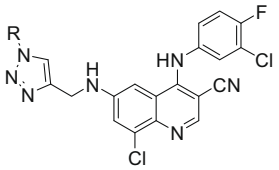
In order to improve the potency and physical properties of **7** and **8**, a variety of groups were introduced to the triazole ring (Table 1). The in vitro data suggest that incorporation of hydrophobic groups on the triazole moiety is not beneficial to the binding activity. For example, in both the C-8 chloride and bromide series, compounds with a hydrophobic group (**9–11** and **15–16**) demonstrated more than three-fold loss of potency in Tpl-2 binding affinity; in human blood assay, the IC_{50}s of these compounds were greater than 20 μM in hWB. However, when an acidic group such as a carboxylic acid was employed, the resulting analogs were highly potent in Tpl2 inhibition as indicated by **12** and **17**, which showed Tpl2 inhibition IC_{50}s of 9.6 nM and 7.0 nM, respectively, four-fold more potent than **7** and **8**. In addition, both compounds also have much improved aqueous solubility and **17** exhibited high permeability in PAMPA assay (1.8×10^{-6} cm/s). Unfortunately these improved potencies and physical properties did not translate into increased potency in hWB as neither **12** nor **17** had IC_{50}s of below 20 μM.

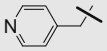
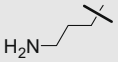
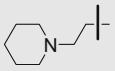
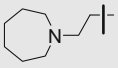
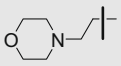
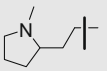
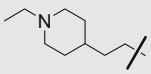
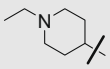
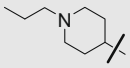
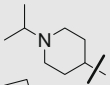
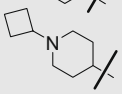
Table 1
Inhibition of Tpl2 kinase by compounds with N1-substitutions at the triazole tail



Compound	X	R	Inhibition of LPS-induced TNF-α production			Solubility (μg/mL, pH 7.4)	PAMPA ($\times 10^{-6}$ cm/s)
			Tpl2 IC_{50} (nM)	IC_{50} (monocytes) (μM)	IC_{50} (hWB) (μM)		
7	Cl	H	40	0.39	7.0	0	0
8	Br	H	18	1.8	4.2	0	0
9	Cl	*t*-Butyl	170	0.69	>20	1.0	0.1
10	Cl	Phenyl	120	3.9	>20	1.0	0
11	Cl	Benzyl	140	5.0	>20		
12	Cl	CH_2COOH	9.6	5.0	>20	73	0.04
13	Cl	2-Pyrrolidinylethyl	40	0.27	4.4	28.8	0.57
14	Cl	3-Pyridinylmethyl	10	0.05	5.5	34.7	0.18
15	Br	*t*-Butyl	61	1.8	>20		
16	Br	Phenyl	40,000	NA	NA	0	1.2
17	Br	CH_2COOH	7.0	5.0	>20	64	1.8
18	Br	2-Pyrrolidinylethyl	23	0.32	5.9	35.7	0.72
19	Br	3-Pyridinylmethyl	20	0.22	3.9	28.2	0.13

Table 2
Inhibition of Tpl2 kinase by compounds with N1-substitutions at the triazole tail



Compounds	R	IC_{50} (nM) Tpl2	IC_{50} (μM) (hWB)
20		4	2.8
21		2.0	9
22		7.6	>20
23		3	>20
24		17	19
25		20	12.8
26		13	10.0
27		22	5.4
28		28	5.7
29		8.0	7.1
30		22	3.1
31		18	5.5
32		5.3	3.9
33		2.2	4.1
34		1.6	0.3
35		8.2	0.9
36		9.1	1.1
37		10	0.4

On the other hand, compounds with groups containing a basic nitrogen atom showed comparable or slightly increased potency over **7** and **8** in both the enzymatic assay and secondary assays. Specifically, when a pyridine moiety was applied, compounds tended to show better aqueous solubility and cell permeability profiles (**14** and **19**). For example, while compound **7** has very poor solubility and permeability, **14**, with a 3-pyridinylmethyl group at the triazole, is moderately soluble in aqueous buffer (34.7 μg/mL at pH 7.4) and showed moderate cell permeability (0.18×10^{-6} cm/s in PAMPA). In addition, **14** is four-fold more potent than **7** in the enzymatic assay of Tpl2 inhibition. Further optimization of **14** led to **20** (Table 2), which showed a 10-fold increase in Tpl2 inhibition and two-fold improvement in human whole blood activity. However, in a pharmacokinetic study, **20** showed high clearance (88 mL/min/kg) in rats. One of the metabolic soft spots was the benzylic methylene between the pyridyl and triazole rings. To avoid the methylene linker, the pyridine ring was directly connected to the triazole ring and **23** was prepared. Without the methylene between the pyridine ring and triazole, **23** showed lower clearance (28 mL/min/kg) in rats than **20**, however, this compound showed diminished activity in human monocytes and hWB, despite the improved potency (IC_{50} = 3.0 nM) in the enzymatic assay.

We then turned our attention to amine groups as substitutions on the triazole. A series of compounds with a cyclic or non-cyclic amine moiety at the triazole were synthesized and studied. Some representative examples (compounds **24–37**) are listed in Table 2. We found that compounds with an acyclic amine substitute on the triazole had reduced activity in hWB (for example, IC_{50}s for **24**, **25**, and **26** are greater than 10 μM). On the other hand, cyclic amine analogs (**27–37)** were generally more potent in both assays. Some of them showed significantly improved potency in hWB (**34–37**). Specifically, **34**, with a 1-ethyl-4-piperidinyl substitution at the triazole, was 25-fold more potent than **7** in Tpl-2 binding in the enzymatic assay (IC_{50} 1.6 nM) and 23-fold more potent in hWB (IC_{50} 0.3 μM). In addition, compared to **7**, this series of compounds (**31–37**) exhibited moderate to significant improvements in aqueous solubility and cell membrane permeability (Table 3). For example, **35**, with a 4-piperidinyl group at the triazole, is quite soluble in aqueous buffer (50 μg/mL at pH 7.4) and showed moderate cell permeability (0.48×10^{-6} cm/s in PAMPA).

Several active compounds from Table 2 were tested in a panel of kinases, including some known to be involved in TNF-α production (e.g., p38 and MK2) (Table 4). All the compounds reported here are highly selective against these kinases. It is noteworthy to mention that even though **7** weakly inhibits EGFR with an IC_{50} of 1.0 μM, compounds with N-1 substitutions at the triazole ring showed much improved selectivity (10–30-fold improvement) between Tpl2 and this receptor tyrosine kinase. The selectivity of Tpl2 against EGFR for compound **34** is 6875-fold.

In a pharmacokinetics study, compound **34** was orally administered in rats with 100 mg/kg dosing and showed a C_{max} of 517 ng/mL (0.89 μM) and AUC of 4841 ng h/mL, above its IC_{50} in hWB. The compound was then tested in the LPS-induced TNF-α production model in female Sprague-Dawley rats. With a 25 mg/kg po dose, **34** inhibited LPS-induced TNF-α production by 83% compared to the vehicle group.

Table 3
Physical properties of selected 8-bromo-4-(3-chloro-4-fluoro-phenylamino)-6-{[1-substituted-1-[1,2,3]triazol-4-ylmethyl]-amino}-quinoline-3-carbonitriles

Compound	Solubility[a] (μg/mL, pH 4.5)	Solubility[a] (μg/mL, pH 7.4)	PAMPA ($\times 10^{-6}$ cm/s)
7	3.0	0	0
32	>100	50	0.03
33	N/A	1.0	0.39
34	N/A	4.0	0.36
35	>100	50	0.48
37	22	1.0	0.43

[a] The compound to be tested was dissolved in DMSO (20 mg/ml) and a small portion of the solution was added to an aqueous buffer at either pH of 4.5 or 7.4. The mixture was incubated for 18 h at ambient temperature prior to quantitation using a UV plate reader.[12]

Table 4
In vitro selectivity profile of selected 8-bromo-4-(3-chloro-4-fluoro-phenylamino)-6-{[1-substituted-1-[1,2,3]triazol-4-ylmethyl]-amino}-quinoline-3-carbonitriles

	EGFR (μM)	P38 (μM)	Src (μM)	CAMKII (μM)	MK2 (μM)	PKA (μM)	PKC (μM)
8	1.0	4	41	>100	49	>100	>100
34	11	16	53	39	63	>200	>200
35	30	>200	72	2.6	>200	171	>200
36	22	4.7	35	75	>200	>200	>200
37	22	16	110	70	131	116	>200

In summary, through exploring substitutions at the N1 position of triazole, we have discovered a series of potent and selective Tpl2 inhibitors. Incorporation of a basic amine group gave a number of compounds with moderate improvements in physicochemical properties and improved potency in human blood, for example, **34** is more than 20-fold more potent than **7**. Compound **34** was orally efficacious in reducing TNF-α production in a LPS-induced rat model. This series of inhibitors demonstrates potential as orally available compounds for the treatment of RA and other inflammatory diseases.

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