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Inhibition of Tpl2 kinase and TNFα production with quinoline-3-carbonitriles for the treatment of rheumatoid arthritis

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Abstract—The synthesis and structure–activity studies of a series of quinoline-3-carbonitriles as inhibitors of Tpl2 kinase are described. Potent inhibitors of Tpl2 kinase with selectivity against a panel of selected kinases in enzymatic assays and specificity in cell-based phosphorylation assays in LPS-treated human monocytes were identified. Selected inhibitors with moderate activity in human whole blood assay effectively inhibited LPS/D-Gal induced TNFα release when administered intraperitoneally in mice.

Rheumatoid arthritis (RA) is an autoimmune disorder that induces chronic inflammation, leading to progressive joint destruction. It affects about 1% of the population with approximately 5 million people worldwide. Up to 50% of affected patients with RA are unable to work 10 years after diagnosis. Currently, there is no cure for this disease. Although the etiology is still under investigation, it is believed that tumor necrosis factor-α (TNFα), a cytokine responsible for the initiation and mediation of inflammation, initiates and maintains the disease development through the induction of effector proteins such as adhesion molecules and matrix metalloproteinases (MMPs). The control of TNFα, therefore, is a major goal in treating the progression of RA.[1] One way to inhibit TNFα production is through the signal transduction pathway that includes several members of the mitogen activated protein kinase (MAP kinase) family, notably p38, which has been the focus of several programs in the pharmaceutical industry.[2] Another important TNFα signal transduction pathway involves the kinases MEK and ERK and is controlled upstream by Tpl2 (Tumor Progression Loci-2) kinase.[3]

Tpl2 kinase is a serine/threonine MAP kinase with 467 amino acids. Sequencing studies of the Tpl2 protein indicate a very low homology to other kinases (~30 %). Furthermore Tpl2 is not inhibited by the 'pan kinase inhibitor,' staurosporine, and is therefore likely to have very unique structural features.

Tpl2 activates the translation of the TNFα messenger RNA and TNFα production through the phosphorylation of MEK/ERK. TNFα thus produced can then bind to the TNFα receptor thereby further activating the Tpl2 signaling pathway. Essentially, Tpl2 is important for both TNFα production and signaling, and a Tpl2 inhibitor would have the double benefit of blocking both.[4] The recent clinical success of anti-TNFα agents, notably the injectable protein ENBREL® (sTNFRα), has further validated TNFα as an important therapeutic target for RA. Despite the success of these biological agents, the development of an orally bioavailable, small molecule TNFα pathway inhibitor (e.g., a Tpl2 inhibitor) represents a highly desirable strategy for treating RA.

We recently disclosed a novel class of Tpl2 inhibitors, the 1,7-naphthyridine-3-carbonitriles (**1**, Fig. 1).[5] In this manuscript, we report on the synthesis, SAR, and in vitro and in vivo TNFα inhibition studies of a new related series of Tpl2 inhibitors with a quinoline-3-carbonitrile scaffold (**2**).

The synthesis (Scheme 1) of the quinoline-3-carbonitrile analogs **2** varied at R^1, R^2, headpiece, and tailpiece

Keywords: Tpl2 kinase; Quinoline-3-carbonitrile.
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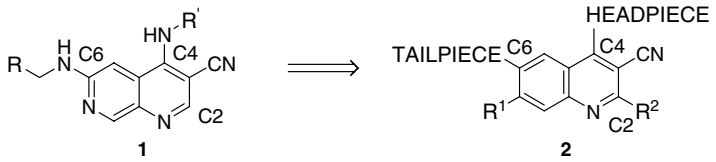
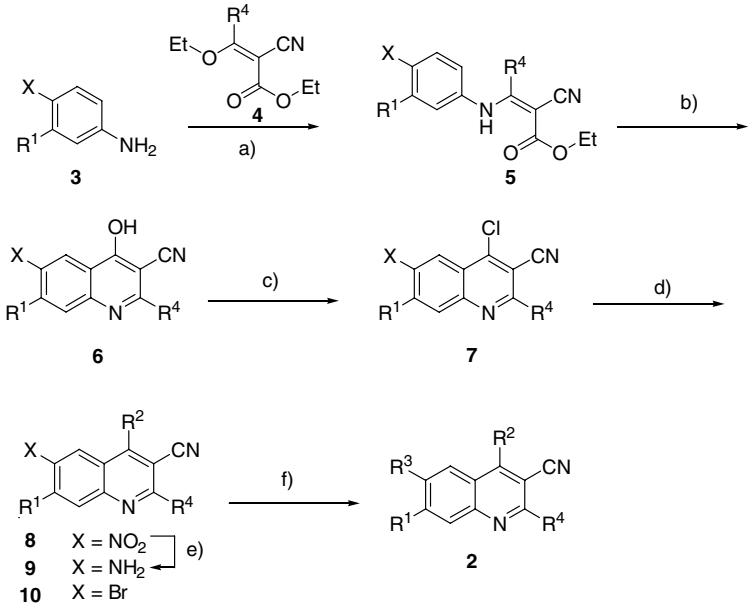


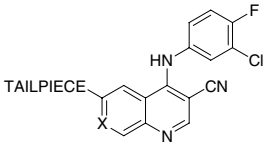
Figure 1. Optimization of quinoline-3-carbonitriles.

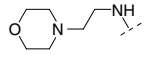
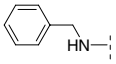
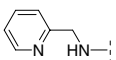


Scheme 1. Reagents and conditions: (a) Cs_2CO_3, DMF, rt, 2 h, 72–99%; (b) Dowtherm A, 260 °C, 4–5 h, 21–68%; (c) $(COCl)_2$, DMF (cat), DCE or $POCl_3$, reflux, 12 h, 85–92%; (d) RNH_2, EtOH, reflux, 12 h, 41–78%; or ROH, KOH, 100 °C, 2 h, 40%; or RSH, DMF; rt, 12 h, 86%; (e) $SnCl_2{\cdot}2H_2O$, EtOH, microwave (110 °C, 5 min), or Fe, NH_4CI, MeOH, H_2O, microwave (100 °C, 10 min), or Pd/C, H_2, EtOH, acetic acid, 12 h, 63–90%; (f) when X = NH_2, RCHO, $NaCNBH_3$, HOAc, EtOH, 3 h, 25–76%. For compound **20**, X = Br, i—PhCHCHSnBu_3, $Pd(PPh_3)_4$, toluene, microwave, 150 °C, 1 h, 45%; ii—H_2, Pd/C, THF, 6 d, 80%. For compound **21**, X = Br, aniline, $Pd(OAc)_2$, BINAP, Cs_2CO_3, microwave, 180 °C, 1.5 h, 9%. For compound **22**, X = Br, benzamide, K_3PO_4, CuI, *trans*-1,2-diaminocyclohexane, dioxane, 150 °C, 1 h, 33%. For compound **23**, X = NH_2, phenylsulfonyl chloride, DIEA, THF, 24 h, 13%.

(Fig. 1) commenced with substituted aniline **3**. Substituted aniline **3** was alkylated by Michael addition–elimination with the 2-cyano-3-ethoxyacrylate **4**. It is critical that compound **5** be void of any basic residue or moisture before the cyclization in Dowtherm to construct **6**. Additionally, this reaction is substrate-de-

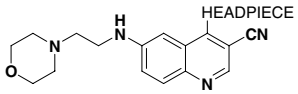
Table 1. Comparison of 1,7-naphthyridine-3-carbonitriles and quinoline-3-carbonitriles

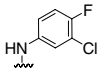
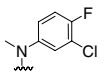
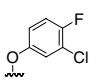
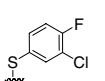


Tailpiece	X = N compound	Tpl2 IC_{50} (μM)	X = CH compound	Tpl2 IC_{50} (μM)
	**11**	1.2	**14**	1.0
	**12**	0.14	**15**	0.21
	**13**	0.13	**16**	0.09

X = N; 1,7-naphthyridine-3-carbonitrile series; X = CH, quinoline 3-carbonitrile series.

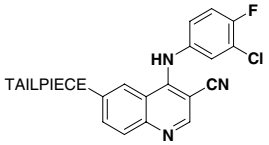
Table 2. Headpiece linker modification



Compound	Headpiece	Tpl2 IC_{50} (μM)
14		1.0
17[a]		>40
18		>40
19		>10

[a] 1,7-Naphthyridine-3-carbonitrile.

Table 3. Tailpiece modification



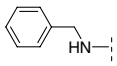
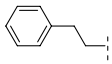
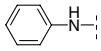
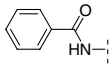
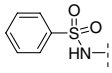
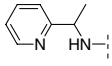
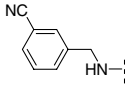
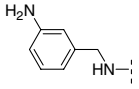
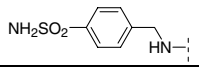
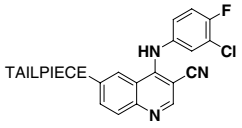
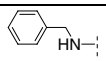
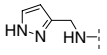
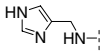
Compound	Tailpiece	Tpl2 IC_{50} (μM)
15		0.21
20		>10
21		>40
22		11
23		11.5
24		0.06
25		0.1
26		0.14
27		0.11

Table 4. Tailpiece modification: heterocycles



Compound	Tailpiece	Tpl2 IC_{50} (μM)
15		0.21
28		0.24
29		0.031
30		0.074
31		0.019
32		0.11
33		2.2
34		30
35[a]		0.18
36[a]		7

[a] 1,7-Naphthyridine-3-carbonitrile.

pendent and sensitive to reactant concentration and reaction time. Headpieces were installed by amine S_NAr displacement of the chloride **7**. The bromide intermediate **10** can be used for further derivatizations through Pd- or Cu-catalyzed chemistry to synthesize compounds **20**–**22**. The nitro intermediate **8** can be reduced using $SnCl_2$, or Fe/NH_4Cl, or hydrogenation to give C6-aniline **9** for reductive amination of an aldehyde or ketone.[6]

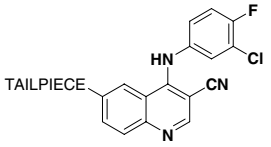
The inhibitors synthesized as shown in Scheme 1 were evaluated in vitro against Tpl2 kinase and the data are shown in Tables 1–6.[9] From the SAR investigation of previously reported 1,7-naphthyridine-3-carbonitrile Tpl2 inhibitors (**1**),[5] it was known that a large range of inhibitory activity depended on the length, hybridization, and stereochemistry of the C6-tailpiece moiety. This finding was also true for the quinoline-3-carbonitrile series. Interestingly, this C6-tailpiece position has had less influence on isolated kinase activity than the headpiece unit in other kinase programs previously reported.[7,8]

In general, quinoline-3-carbonitriles (**2**) have very similar headpiece and tailpiece SAR to that of the 1,7-naphthyridine-3-carbonitriles (**1**) (complete data not shown). For example, in Table 1, compounds **14**–**16** of the quinoline-3-carbonitrile scaffold have similar Tpl2 activity to their corresponding compounds **11**–**13** of the 1,7-naphthyridine-3-carbonitrile scaffold. The more readily accessible quinoline-3-carbonitrile chemistry[7] allowed us to better explore SAR at R^1, R^2, headpiece and tailpiece of the molecule (Scheme 1).

We next sought to investigate the importance of the C4–NH functionality. By methylating the nitrogen atom (**17**) or by replacing the NH with either oxygen (**18**) or sulfur (**19**), significant loss of activity was observed (Table 2). We believe that the C4–NH and C3–CN of the molecule act as hydrogen-bond donor–acceptor system for the ATP binding site and are therefore important for activity.

Some more detailed information on the tailpiece modifications on quinoline-3-carbonitriles is shown in Tables 3–5. Direct comparison of compounds **15** (benzylamine tail) and **20** (phenethyl tail) reveals that a tailpiece NH linkage is important for activity. If the methylene carbon unit is removed (**21**) or replaced with an amide (**22**) or a sulfonamide (**23**) linkage, the compounds

Table 5. Tailpiece modification: substituted imidazoles



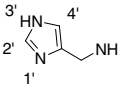
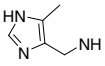
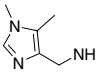
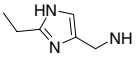
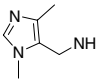
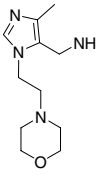
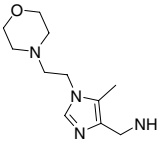
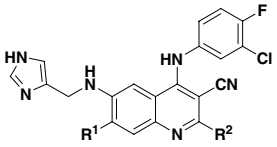
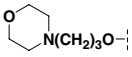
Compound	Tailpiece	Tpl2 IC_{50} (μM)
31		0.019
37		0.003
38		0.003
39		0.64
40		0.2
41		0.26
42		0.014

Table 6. R^1 or R^2-substitutions



Compound	R^1	R^2	Tpl2 IC_{50} (μM)
43	Me	H	>40
44	*i*-PrO	H	>40
45	CF_3O	H	>40
46		H	9.2
47	H	Me	19
48	H	OH	>40

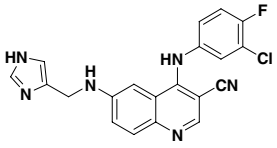
showed significant loss of activity. An α-methyl substitution (**24**) retained activity (cf. **16**).

The effect of a variety of substitutions on the phenyl ring of the tailpiece was also investigated. In general, SAR in this regard appears to be flat; for example, compounds **25**–**27** did not show improved activity when compared to their parent compound **15** (Table 3).

Interestingly, when five-membered heteroaromatic tailpieces were prepared (**28**–**31**, Table 4), we observed significantly improved activity. Thiazole (**29**), pyrazole (**30**), and imidazole (**31**) tails give a 3-, 7-, and 10-fold improvement of activity, respectively, when compared to compound **15**. Pyridine *N*-oxide **32** is 9-fold more active than parent compound **16**. However, compounds with saturated heterocyclic tails (**33**–**36**) showed decreased activity.

We then investigated the substitution effect on the imidazole tail (Table 5). A C4′ and/or N3′ substitution (**37**, **38**, and **42**) appears to improve activity while a C2′ substitution (**39**) gives reduced activity. Through this work, we were able to achieve single-digit nanomolar activity. Di-substitution on N1′ and C4′ (**40** and **41**) resulted in reduced activity. With the new imidazole tail in place, we then investigated substitutions at R^1 (methyl (**43**), alkoxyl (**44**-**45**), or solubilizing group (**46**)) or R^2 (methyl (**47**)

Table 7. In vitro selectivity profile (enzymatic and cell-based selectivity) for compound **31** (μM)



Tpl2	MEK	p38	Src	CAMKII	MK2	PKA	PKC	S6
0.019	2.40	30.0	IA	IA	IA	5.84	>10	>10
P-MEK[a]	P-p38[a]	P-MK2[a]	P-cJun[a]	P-lkBα[a]	EGFR[b]			
0.21	2.11	>5	5	>5	0.03			

IA, inactive.
[a] In human monocytes.
[b] In A431 cells.

Table 8. In vitro (μM) and in vivo (%) inhibition of TNFα production with selected inhibitors

Compound	Tpl2	Monocytes	HWB	LPS-TNF
29	0.031	0.6	>20	NT
31	0.019	0.46	3.3	70%[b]
32	0.011	1.7	7.8	54%[a]
37	0.003	0.4	7.9	84%[a]
38	0.003	0.4	15.8	NT
41	0.014	0.7	13.2	NT

Monocytes: TNF inhibition in LPS-treated human monocytes.
HWB: TNF inhibition in LPS-treated human whole blood.
LPS-TNF: LPS-induced TNF inhibition in mice (IP dosing): [a]50 mg/kg; [b]25 mg/kg; NT, not tested.

or OH (**48**)[9]) positions (Table 6). Both R^1 and R^2 substitutions are detrimental to activity.

Compound **31**[10] was screened for selectivity over a panel of kinases both in in vitro assays and in cellular assays (Table 7).[11] This compound is >120-fold selective against MEK and >300-fold selective against PKA while much more selective against other kinases such as p38, Src, CAMKII, MK2, PKC, and S6. In human monocytes, compound **31** selectively inhibits the phosphorylation of the downstream MEK kinase (P-MEK: 0.21 μM) over other MAP kinase signaling pathways, for example, P-p38, P-MK2, P-cJun, and P-lkBα. However, in A431 cells, compound **31** appears to also inhibit EGFR kinase (30 nM).

Selected compounds were evaluated for TNFα inhibitory activities in cells, blood, and in vivo (Table 8).[11] Compounds **29**, **31**, **32**, **37**, **38**, and **41** are all potent in LPS-treated human monocytes with compound **32** being the least potent, possibly due to low permeability of the charged *N*-oxide moiety in cells. In human whole blood, compound **31** is most potent with an IC_{50} of 3.3 μM. Compounds **32**, **31**, and **37** were tested intraperitoneally in a mouse model of LPS-stimulated production of TNFα.[12] As indicated in Table 8, all three compounds demonstrated in vivo efficacy on LPS/D-Gal induced TNFα release. The fact that compound **31** appears to be more potent than compounds **32** and **37** in the in vivo model (70% inhibition at 25 mg/kg) correlates with their respective whole blood activities.

In summary, we have successfully identified potent and selective Tpl2 inhibitors with a quinoline-3-carbonitrile scaffold. Compound **31** is selective against a panel of kinases in enzymatic assays, and it also selectively inhibits phospho-MEK (P-MEK) formation in LPS-treated human monocytes. Selected compounds with cellular and whole blood activity also effectively inhibited LPS/D-Gal-induced TNFα release when administered intraperitoneally in mice. Further studies to improve EGFR selectivity for this series will be reported in due course.

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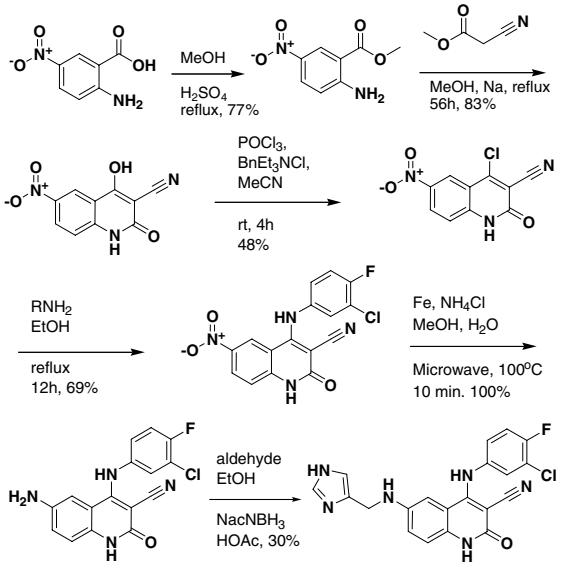
References and notes

1. (a) Choy, E. H. S.; Panayi, G. S. P. *N. Eng. J. Med.* **2001**, *344*, 907; (b) Feldmann, M.; Mani, R. *Ann. Rev. Immunol.* **2001**, *19*, 163; (c) Feldmann, M.; Brennan, F. M.; Foxwell, B. M.; Maini, R. N. *Curr. Dir. Autoimmun.* **2001**, *3*, 188.
2. Kumar, S.; Boehm, J.; Lee, J. C. *Nat. Rev. (Drug Discov.)* **2003**, *2*, 717.
3. (a) Miyoshi, J.; Higashi, T.; Mukai, H.; Ohuchi, T.; Kakunaga, T. *Mol. Cell. Biol.* **1991**, *11*, 4008; (b) Dumitru, C. D.; Ceci, J. D.; Tsatsanis, C.; Konoyiannis, D.; Stamatakis, K.; Lin, J. H.; Patriotis, C.; Jenkins, N. A.; Copeland, N. G.; Kollias, G.; Tsichlis, P. N. *Cell* **2000**, *103*, 1071; (c) Schlesinger, T. K.; Franger, G. R.; Yujiri, T.; Johnson, G. L. *Front. Biosci.* **1998**, *3*, D1181.
4. Chiarello, M.; Marinissen, M. J.; Gutkind, J. S. *Mol. Cell. Biol.* **2000**, *20*, 1747.
5. Gavrin, L. K.; Green, N.; Hu, Y.; Janz, K.; Kaila, N.; Li, H.-Q.; Tam, S. Y.; Thomason, J. R.; Gopalsamy, A.; Ciszewski, G.; Cuozzo, J. W.; Hall, J. P.; Hsu, S.; Telliez, J.-B.; Lin, L.-L. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 5288.
6. A typical preparation of target compound **2** through reductive amination is illustrated by the following procedure for **31**: in a 50 mL round-bottomed flask 6-amino-4-(3-chloro-4-fluoro-phenylamino)-quinoline-3-carbonitrile (200 mg, 0.64 mmol) and 4(5)-imidazole carboxaldehyde (147 mg, 1.53 mmol) were taken up in ethanol (10 mL). Then, acetic acid was added to bring the pH of the solution to 4, and the mixture was stirred for 15 min. $NaCNBH_3$ (48 mg, 0.77 mmol) was then added and the reaction mixture warmed to 30 °C for 2.5 h or until complete by TLC. The reaction mixture was stripped to dryness and the residue was purified via preparative HPLC and lyophilized to give the product as a yellow solid (166 mg, 66%): ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 4.26 (d, J = 4.80 Hz, 2H) 6.53 (t, J = 5.43 Hz, 1H) 7.05 (s, 1H) 7.20 (d, J = 2.53 Hz, 1H) 7.22–7.28 (m, 1H) 7.38 (dd, J = 8.97, 2.40 Hz, 1H) 7.43 (t, J = 9.09 Hz, 1H) 7.48 (dd, J = 6.57, 2.78 Hz, 1H) 7.62–7.70 (m, 2H) 8.15 (s, 2H) 9.36 (s, 1H); HRMS (ESI$^+$) calcd for $C_{20}H_{14}ClFN_6$ (MH^+) 393.10252, found 393.1019.
7. (a) Wissner, A.; Overbeek, E.; Reich, M. F.; Floyd, M. B.; Johnson, B. D.; Mamuya, N.; Rosfjord, E. C.; Discafani, C.; Davis, R.; Shi, Xi.; Rabindran, S. K.; Cruber, B. C.; Ye, F.; Hallett, W. A.; Nilakantan, R.; Shen, R.; Wang, Y.-F.; Greenberger, L. M.; Tsou, H.-R. *J. Med. Chem.* **2003**, *46*, 49; (b) Tsou, H.-R.; Overbeek-Klumpers, E. G.; Hallett, W. A.; Reich, M. F.; Floyd, M. B.; Johnson, B. D.; Michalak, R. S.; Nilakantan, R.; Discafani, C.; Golas, J.; Rabindran, S. K.; Shen, R.; Shi, X.; Wang, Y.-F.; Upeslacis, J.; Wissner, A. *J. Med. Chem.* **2005**, *48*, 1107.
8. (a) Smaill, J. B.; Showalter, H. D. H.; Zhou, H.; Bridges, A. J.; McNamara, D. J.; Fry, D. W.; Nelson, J. M.; Sherwood, V.; Vincent, P. W.; Roberts, B. J.; Elliott, W. L.; Denny, W. A. *J. Med. Chem.* **2001**, *44*, 429; (b) Hennequin, L. F.; Stokes, E. S. E.; Thomas, A. P.; Johnstone, C.; Plé, P. A.; Ogilvie, D. J.; Dukes, M.; Wedge, S. R.; Kendrew, J.; Curwen, J. O. *J. Med. Chem.*

2002, *45*, 1300; (c) Banerjee, R.; Rachid, Z.; McNamee, J.; Jean-Claude, B. J. *J. Med. Chem.* **2003**, *46*, 5546; (d) Plé, P. A.; Green, T. P.; Hennequin, L. F.; Curwen, J.; Fennell, M.; Allen, J.; Lambert-van der Brempt, C.; Costello, G. *J. Med. Chem.* **2004**, *47*, 871.

9. Synthesis of compound **48**:



10. Physicochemical properties for compound **31**: solubility: 26 μg/mL at pH 4.5; permeability (PAMPA at pH 7.4): 2.04×10^{-6} cm/s; Rat microsomal stability: $t_{1/2} > 30$ min. CypP450 % inhibition: 89% at 3 μM for 3A4.
11. For detailed assay conditions for the measurement of Tpl2 activity, selectivity, inhibition of TNFα in primary monocytes, and human blood, see Refs. 9, 13, and 14 in Ref. 5 herein.
12. LPS/D-Gal-induced acute TNFα production in mouse sera: Female C57Bl/6 mice, 8–10 weeks of age, were obtained from the Jackson Laboratory. The animals were fed food and water ad libitum, and all procedures were approved by the Institutional Animal Care and Use Committee. Compound at 25 or 50 mg/kg, or vehicle, was administered to the mice by the intraperitoneal (ip) route. One hour after the compound, LPS plus D-galactosamine in PBS was administered ip. Final LPS and D-gal concentrations in each animal were 2 and 160 ng/kg, respectively. The mice were euthanized with carbon dioxide 1.5 h after the LPS/D-Gal injection and the mice were bled by cardiac puncture. TNFα levels were measured in the serum samples using a TNFα ELISA.