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Novel Phenylpiperazine Derivatives as Dual Cytokine Regulators with TNF-α Suppressing and IL-10 Augmenting Activity

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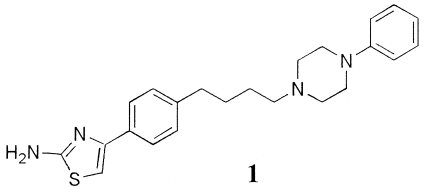
Abstract—Phenylpiperazine derivatives were synthesized as dual cytokine regulators with TNF-α suppressing and IL-10 augmenting activity. Lead optimization led to compound **5k** having the potent regulatory activity and demonstrating remarkable protective effects against the lethal challenge of LPS in mice, suggesting that **5k** would be a promising drug candidate for the treatment of TNF-α associated diseases including septic shock. © 2000 Elsevier Science Ltd. All rights reserved.

Tumor necrosis factor-α (TNF-α) is an inflammatory cytokine with a multitude of biological activities linked to pathology of autoimmune diseases such as rheumatoid arthritis (RA),[1] Crohn's disease,[2] systemic lupus erythematosus,[3] and multiple sclerosis;[4] septic shock;[5] and AIDS.[6] Clinical studies have shown that an anti-TNF-α chimera antibody is effective for the treatment of RA and Crohn's disease.[7] Low molecular weight compounds such as thalidomide derivatives,[8] imidazole derivatives,[9] adenosine derivatives,[10] and phosphodiesterase type IV (PDE-IV) inhibitors[11] are known to inhibit the production of TNF-α. On the other hand, interleukin-10 (IL-10) is an anti-inflammatory cytokine having various biological activities.[12] A recombinant human IL-10 has been reported to be clinically effective for steroid-resistant Crohn's disease and RA.[13] IL-10 is also known to suppress the production of TNF-α;[14] therefore, it was expected that agents capable of regulating both TNF-α and IL-10 at the same time would have a synergistic effect in the treatment of TNF-α associated diseases. Herein we describe the synthesis and biological evaluation of novel phenylpiperazine derivatives having oral potential to suppress TNF-α production as well as to augment IL-10 production.

Initially we screened our library compounds that had proved to have anti-inflammatory activity for their ability to regulate the cytokines (TNF-α and IL-10) in lipopolysaccharide (LPS)-stimulated mice in vivo to find a lead compound **1** possessing modest dual cytokine regulatory activity; however, **1** showed high affinities for receptors of central nervous system (CNS) because of its being originally synthesized for a CNS agent (Fig. 1).



1

LPS-stimulated mice		Binding affinty			
% inhibition	% of control	Ki (nM)			
TNF-α	IL-10	D_2	5-HT_{1A}	5-HT_2	α_1
87	382	5.5	3.8	37.0	7.9

Figure 1. Cytokine regulatory activities (10 mg/kg, po) and CNS receptor binding affinities[15] of **1**.

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Structural optimization of the lead compound **1** was aimed both at increase of the dual cytokine regulatory activity and at reduction of the binding affinities for CNS receptors in order to avoid CNS side effects, leading to the optimized compound **5k** showing potent dual cytokine regulatory activity without any significant binding affinities for CNS receptors.

Chemistry

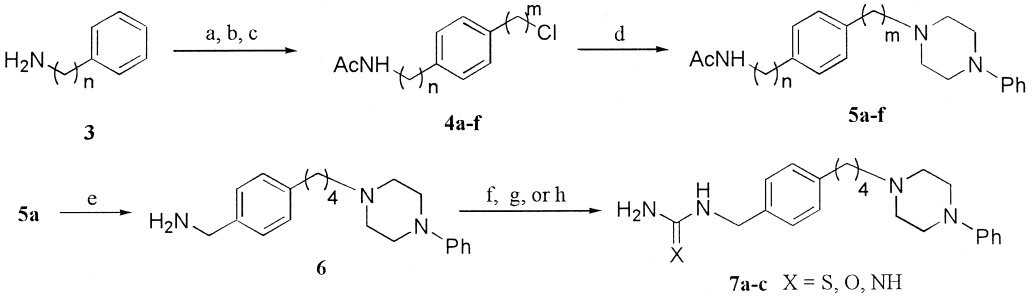
Phenylpiperazine derivatives **5a–k** and **7a–c** (m = 2, 3, 4) were synthesized as shown in Schemes 1 and 2.[16] Phenylalkylchlorides **4a–f** were synthesized from phenylalkylamines **3** by acetylation, Friedel–Crafts reaction, and reduction of ketone. Condensation of **4a–f** with phenylpiperazine gave the target phenylpiperazine derivatives **5a–f**. Other derivatives (thiourea **7a**, urea **7b**, and guanidine **7c**) were prepared from amine **6** obtained by acidic hydrolysis of **5a** (Scheme 1). Benzylpiperazine derivatives **5g–k** were synthesized from 4-(aminomethyl) benzoic acid **8** as shown in Scheme 2. Thus, the methyl benzoate **9** was prepared from the 4-aminomethylbenzoic acid **8** by acetylation and esterification. Subsequently, the benzoate **9** was converted to the chloride derivative **4g** by reduction of ester and chlorination. The benzyl chloride **4g** was led to the target arylpiperazine derivatives **5g–k**.

Biological assay

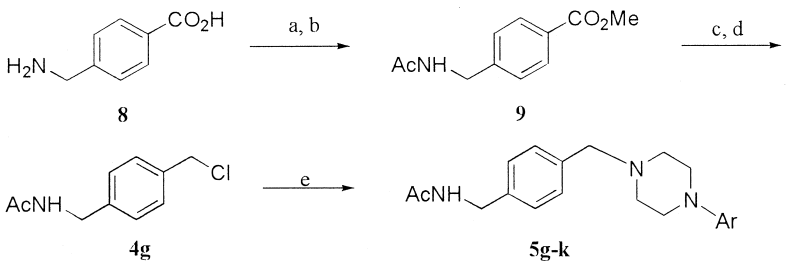
The effects of the synthesized compounds on the production of cytokines (TNF-α and IL-10) were evaluated according to a literature method.[17] Briefly, test compounds (1, 3, or 10 mg/kg) were orally administered to female BALB/c mice 30 min prior to LPS (*Escherichia coli* 0111:B4) injection (0.5 mg/kg, ip). Blood samples were collected 90 min after LPS injection and analyzed by enzyme-linked immunosorbent-assay (ELISA) for TNF-α and IL-10.[18] The potencies of TNF-α inhibition were expressed as percent inhibition calculated as follows: 100×(1-(TNF-α (experimental)/TNF-α (control))). The potencies of IL-10 augmentation were expressed as percent of control calculated as follows: 100×(IL-10 (experimental)/IL-10 (control)).

Results and Discussion

Optimization of **1** started with modification of the aminothiazole moiety (Table 1). Complete removal of the moiety resulted in a moderate loss of the activity (**2**).[19] Ring-opening of the thiazole ring led to thiourea derivative **7a**, which narrowly retained the activity. Replacement of the sulfur atom of the thiourea moiety in **7a** with oxygen and nitrogen gave urea derivative **7b** and guanidine derivative **7c**, respectively. The latter almost lost the activity although the former displayed comparable activity to **1**. Conversion of the ureidomethyl group into the acetamidomethyl group dramatically enhanced the activity. The amide proton of the acetamidomethyl group in **5a** seems to be critical for the activity because *N*-methylacetamidomethyl and ethylaminomethyl derivatives showed no activity (data not shown). For optimization of the distance between the acetamide group and the middle benzene group, another three **5a** analogues (**5b**, **5c** and **5d**) were synthesized. Compound **5a**

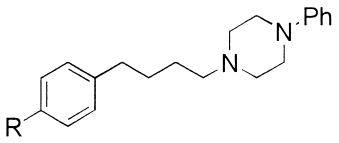


Scheme 1. Reagents and conditions: (a) AcCl, NaOH, CH_2Cl_2/H_2O, rt (92%); (b) ω-chloroaliphatic acid chlorides, $AlCl_3$, dichloroethane, rt (17–79%); (c) Et_3SiH, TFA, 40 °C (63–95%); (d) phenylpiperazine, K_2CO_3, DMF, 80 °C (49–58%); (e) 10% HCl reflux (95%); (f) EtOCONCS, acetone, reflux, then NaOH, $EtOH/H_2O$, reflux (63%, 2 steps); (g) urea, $cHCl/H_2O$/AcOH reflux (44%); (h) MeSC(=NZ)NHZ, MeOH, rt, then HBr/AcOH, rt (30%, 2 steps).



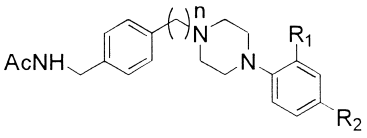
Scheme 2. Reagents and conditions: (a) Ac_2O, NaOH, H_2O, rt (87%); (b) cH_2SO_4, MeOH, reflux (72%); (c) $LiAlH_4$, THF, 0 °C–rt (98%); (d) $SOCl_2$, $CHCl_3$, reflux (91%); (e) arylpiperazines, K_2CO_3, DMF, 80 °C (49–58%).

Table 1. Effect of **2**, **5a–d** and **7a–c** on the production of cytokines in LPS-stimulated mice (10 mg/kg, po)



Compound	R	TNF-α % inhibition	IL-10 % of control
2	H	77	337
7a	$H_2N(CS)NHCH_2$-	69	129
7b	$H_2N(CO)NHCH_2$-	85	253
7c	$H_2N(CNH)NHCH_2$-	39	124
5a	$AcNHCH_2$-	94	1337
5b	AcNH-	88	585
5c	$AcNH(CH_2)_2$-	76	292
5d	$AcNH(CH_2)_3$-	68	146

Table 2. Effects of **5a** and **5e–k** on the production of cytokines in LPS-stimulated mice and binding affinities for CNS receptors



				10 mg/kg, po		Binding affinity K_i (nM)			
Compound	*n*	R_1	R_2	TNF-α	IL-10	D_2	5-HT_{1A}	5-HT_2	α_1
5a	4	H	H	94	1337	49	6	48	4.6
5e	3	H	H	97	1805	340	33	160	6.4
5f	2	H	H	96	2784	170	2.9	130	69
5g	1	H	H	89	376	>1000	140	>1000	>1000
5h	1	H	F	87	1494	>1000	>1000	>1000	>1000
5i	1	H	Cl	65	361	>1000	>1000	>1000	>1000
5j	1	H	OMe	74	449	>1000	>1000	>1000	>1000
5k	1	F	F	91	2293	>1000	>1000	>1000	>1000

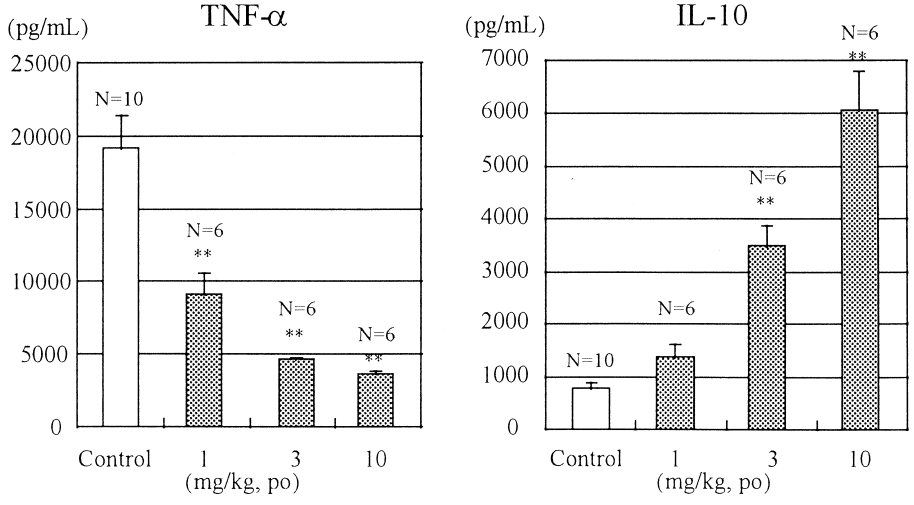
showed the most potent activity among them, suggesting that the acetamidomethyl group is the most suitable as a substituent on the benzene ring.

Next, we synthesized **5a** analogues (**5e**, **5f** and **5g**) to optimize the methylene length of the central linker between the piperazine group and the middle benzene group (Table 2). The analogues demonstrated comparable activity to **5a**. On the other hand, the binding affinities of **5g** for CNS receptors dramatically decreased while those of the others did not change significantly, suggesting that the middle benzene group of **5g** may be much more effective in interrupting the interaction between G-protein coupled receptors (GPCRs)[20] and the piperazine group than that of the others.

Finally we investigated the effects of substituent(s) on the terminal benzene ring. Four derivatives (**5h–k**) with only one methylene unit as the central linker were synthesized. As expected, none of the derivatives (**5h–k**) showed binding affinities for CNS receptors. 2,4-Difluorophenylpiperazine derivative **5k**[21] showed the most potent activity among them. Compound **5k** inhibited TNF-α production and enhanced IL-10 production in a dose-dependent manner in LPS-stimulated mice (Fig. 2).

Compound **5k** was tested for its ability to protect mice from lethal challenge of LPS (Fig. 3).[22] Compound **5k** dose-dependently protected mice from death. Surprisingly, it almost perfectly prevented mice from shock death at a dose of 10 mg/kg, po; 8 out of 10 mice survived while all the control mice died at day 3.

Pharmacokinetics of **5k** was also investigated using female rats.[23] Compound **5k** proved to have good pharmacokinetic properties: an excellent oral bioavailability (95.5%) was observed at a dose of 30 mg/kg.



** P<0.01 vs control (Dunnett method)

Figure 2. Dose-dependent cytokine regulation **5k** in LPS-stimulated mice.

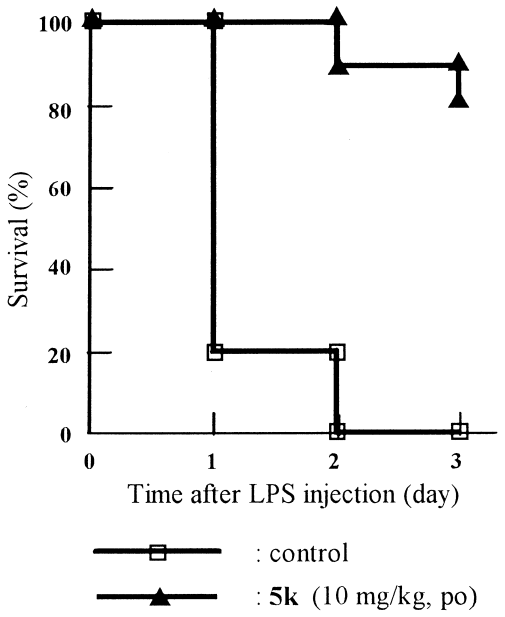


Figure 3. Protection by **5k** of mice from LPS-induced shock.[22]

Conclusion

Optimization of the lead compound **1** having modest dual cytokine regulatory activity but showing CNS receptor binding affinities led to a potent dual cytokine regulator **5k** without showing any significant affinities for the receptors.[24] The compound **5k** dose-dependently regulated both TNF-α and IL-10 production in LPS-stimulated mice. Furthermore, **5k** demonstrated potent protective effects against the lethal challenge of LPS in mice, suggesting that **5k** would be a promising drug candidate for the treatment of TNF-α associated diseases including septic shock.

Acknowledgements

We are grateful to Dr. Takashi Horikawa for pharmacokinetic studies.

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18. In control mice, TNF-α plasma concentration reached maximum 90 min after LPS injection while IL-10 plasma concentration reached maximum within the range of 90–120 min after LPS injection. Our preliminary examination using anti IL-10 antibody showed that both TNF-α and IL-10 may be independently regulated since pretreatment of mice with anti IL-10 antibody did not affect the degree of TNF-α suppression by active compounds in LPS-injected mice.
19. Compound **2** was readily prepared from 1-phenylpiperazine and (4-bromobutyl)benzene synthesized starting from commercially available (4-hydroxybutyl)benzene.
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21. Analytical and spectroscopic data for **5k**: mp 94–95 °C; ^{1}H NMR (270 MHz, $CDCl_3$) δ 2.02 (s, 3H), 2.61 (dd, $J = 5.3$, 4.6

Hz, 4H), 3.04 (dd, $J=5.3$, 4.6 Hz, 4H), 3.56 (s, 2H), 4.42 (d, $J=5.9$ Hz, 2H), 5.71 (brs, 1H), 6.73–6.93 (m, 3H), 7.24 (d, $J=7.9$ Hz, 2H), 7.32 (d, $J=7.9$ Hz, 2H); IR (KBr) 3307, 2939, 2821, 1645, 1556 cm^{-1}; MS (EI) 359 (M^+). Anal. calcd for $C_{20}H_{23}F_2N_3O$: C, 66.84; H, 6.45; N, 11.69%. Found: C, 66.84; H, 6.43; N, 11.66%.

22. The effect of **5k** on shock was studied as follows. Compound **5k** was orally administered (10 mg/kg) to female BALB/c mice ($n=10$/group) 30 min prior to intraperitoneal injection of LPS (*Escherichia coli* 055:B5) at a dose of 15 mg/kg. Survival was monitored 72 h after LPS injection.

23. The pharmacokinetic behavior of **5k** was evaluated in female SD rats. Briefly, groups of rats ($n=5$/group) received either a 10 mg/kg intravenous dose or a 30 mg/kg oral dose. Blood samples were collected 0.083 (iv only), 0.25 (po only), 0.5, 1, 2, 4, 6, and 24 h after dosing and analyzed by reverse phase (C_8) HPLC. The obtained C_{max}, t_{max}, AUC_{0-24h}, and BA of **5k** were 9.26 μg/ml, 3.80 h, 123.22 μgh/mL, and 95.5%, respectively.

24. Compound **5k** showed no inhibitory activity for PDE-IV up to 10^{-6} M in vitro.