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Novel DMARDs on the Basis of a New Concept of Dual Cytokine Regulation, TNF-α Suppression and IL-10 Augmentation

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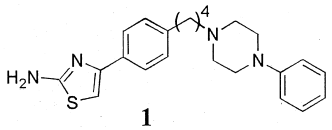
Abstract—A series of arylpiperazine derivatives was synthesized to obtain agents showing apparent therapeutic effects in a chronic inflammatory animal model, starting from a lead possessing potent dual cytokine regulatory activity in vivo. We found a pyrimidylpiperazine derivative **17c** showing the dual regulatory activity and an excellent therapeutic effect in an adjuvant-induced arthritis model. © 2000 Elsevier Science Ltd. All rights reserved.

Rheumatoid arthritis (RA) is a chronic and systemic autoimmune inflammatory disease, where immunological effects play a critical role in its pathogenesis. Recently, tumor necrosis factor-α (TNF-α), an inflammatory cytokine, has been suggested to be deeply associated with the development of RA.[1] Nonsteroidal anti-inflammatory drugs (NSAIDs) have been used as the primary drug for the treatment of RA but can provide only a symptomatic relief; therefore, disease-modifying anti-rheumatic drugs (DMARDs) that directly and selectively act on the abnormal immune system have been desired.[2] We have studied the development of DMARDs on the basis of a new dual cytokine regulation concept that suppression of TNF-α and augmentation of interleukin-10 (IL-10), an anti-inflammatory cytokine having anti-TNF-α activity as well, synergistically effect on TNF-α associated diseases. We have reported that studies based on this concept led to 2,4-difluorophenylpiperazine derivative **3** (Fig. 1) that had potent dual cytokine regulatory activity and demonstrated remarkable protective effects against shock where TNF-α is thought to be one of the major pathogenic factors.[3] Herein we describe further synthetic investigation aimed at the development of novel DMARDs.

Our first attempt to generate a lead **2** from the seed compound **1** that showed moderate dual cytokine regulatory activity but possessed high binding affinities for the central nervous system (CNS) receptors was performed aimed both at increase of the activity and reduction of the affinities for the receptors.[3] Thus, it proved that the aminothiazole moiety of **1** could be replaced by a more simplified group (i.e. acetoamido group); one methylene group was the most preferable for the distance between the acetamido group and the middle benzene group; and that one methylene length between the middle benzene group and the piperazine ring was critical for the deletion of the affinities for CNS receptors.



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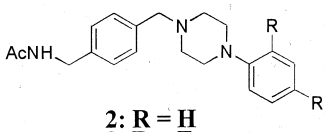

2: R = H
3: R = F

Figure 1. Phenylpiperazine derivatives having dual cytokine regulatory activity.[3]

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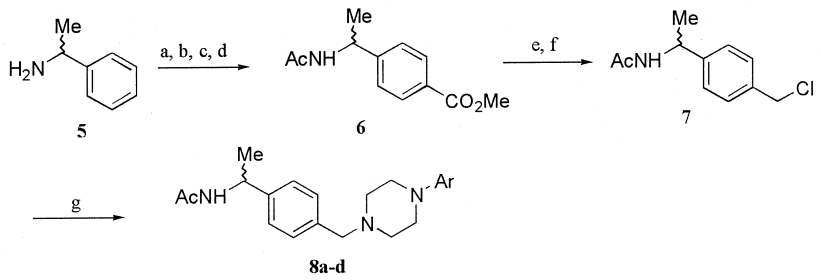
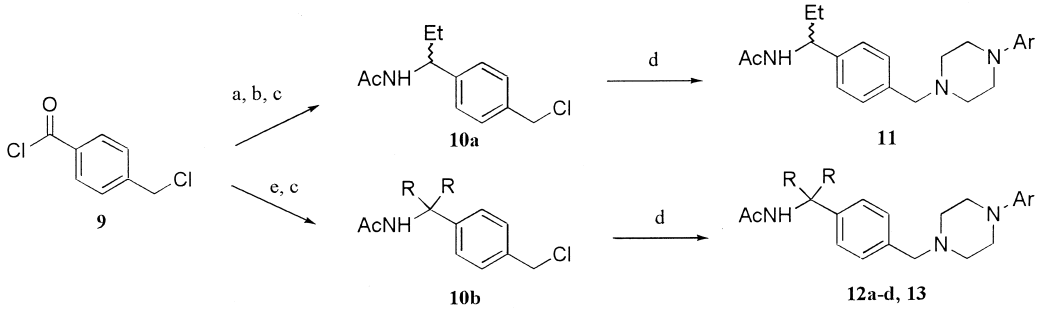
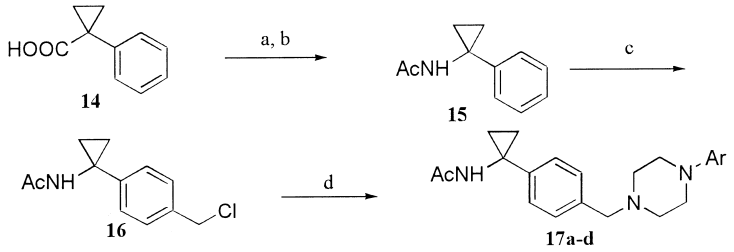


Scheme 1. Reagents and conditions: (a) Ac_2O, Et_3N, CH_2Cl_2, 0 °C–rt (87%); (b) AcCl, $AlCl_3$, dichloroethane, rt (40–51%); (c) Br_2, NaOH, dioxane/H_2O (44–75%); (d) cH_2SO_4, MeOH, reflux (52–68%); (e) $LiAlH_4$, THF 0 °C–rt (70–76%); (f) $SOCl_2$, $CHCl_3$, reflux (73–100%); (g) arylpiperazines, K_2CO_3, DMF, 80 °C (45–55%).



Scheme 2. Reagents and conditions: (a) EtMgBr (1.0 equiv), $Fe(acac)_3$ (0.03 equiv), THF, 0 °C (25%); (b) $NaBH_4$, MeOH, 0 °C (63%); (c) H_2SO_4, CH_3CN, rt (21–82%); (d) arylpiperazines, K_2CO_3, DMF, 80 °C (44–85%); (e) RMgBr, THF, 0 °C (26–27%).



Scheme 3. Reagents and conditions: (a) Curtius rearrangements (58–67%); (b) Ac_2O, Et_3N, CH_2Cl_2, 0 °C–rt (68–82%); (c) MOM-Cl, $TiCl_4$, dichloroethane, rt (38–65%); (d) arylpiperazines, K_2CO_3, DMF, 80 °C (35–64%).

On the basis of information mentioned above, we performed further modifications starting from the lead **2** focusing on the following two parts: 1. replacement of the methylene group between the acetamido group and the middle benzene group by other substituted methylene groups, 2. replacement of the terminal benzene group by other heteroaryl groups. Eventually we found a pyrimidylpiperazine derivative **17c** showing not only potent dual cytokine regulatory activity in lipopolysaccharide (LPS)-stimulated mice but also an excellent therapeutic effect in a rat adjuvant-induced arthritis model.

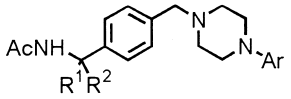
Chemistry

Arylpiperazine derivatives **4a–h** (see Table 1) were synthesized via a similar synthetic route to that reported in our previous paper.[3] Heteroarylpiperazine derivatives **8a–d**, **11**, **12a–d**, **13** and **17a**, were synthesized as shown in Schemes 1–3.[4] *N*-(4-Methoxycarbonylphenylethyl) acetamide (**6**) synthesized from racemic phenylethylamine (**5**) by acetylation, Friedel–Crafts reaction, haloform reaction, and esterification was converted into benzylchloride **7** by reduction and chlorination. The benzylchloride **7** was led to the heteroarylpiperazines **8a–d** in racemic form (Scheme 1). Benzylchloride (**10a**) prepared from 4-chloromethylbenzoylchloride (**9**) by substitution of one equivalent of Grignard reagent in the presence of a catalytic amount of $Fe(acac)_3$, sodium borohydride reduction, and Ritter reaction was converted into racemic **11**. Similarly compounds **12a–d** and **13** were prepared from **10b** which was synthesized from **9** by Grignard reaction and Ritter reaction (Scheme 2). 1-Phenyl-1-cyclopropanecarboxylic acid (**14**) was subjected to Curtius rearrangement followed by acetylation to afford acetamide **15**. Compound **15** was treated with chloromethyl methyl ether in the presence of $TiCl_4$ to afford benzylchoride **16**, which was converted into the heteroarylpiperazines **17a–d** (Scheme 3).

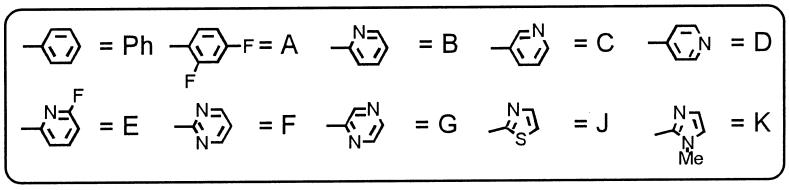
Biological assay

The effects of the synthesized compounds on the production of cytokines (TNF-α and IL-10) in LPS-stimulated

Table 1. Effect of synthesized compounds on the production of cytokines in LPS-stimulated mice (10 mg/kg, po)



				% Inhibition	% of control						% Inhibition	% of control	
	R^1	R^2	Ar	TNF-α	IL-10	Log P[5]		R^1	R^2	Ar	TNF-α	IL-10	Log P[5]
2	H	H	Ph	89	376	1.55	**4f**	H	H	G	76	334	−0.09
3	H	H	A	91	2293	1.83	**4g**	H	H	J	85	1150	1.23
8a	Me	H	Ph	87	270	2.08	**4h**	H	H	K	62	747	0.67
11	Et	H	Ph	82	222	2.59	**8b**[8]	Me	H	E	82	404	1.99
12a	Me	Me	Ph	88	561	2.83	**8c**	Me	H	F	78	298	0.93
13	Et	Et	Ph	81	321	3.35	**8d**	Me	H	J	84	621	1.76
17a	$-CH_2CH_2-$		Ph	90	712	1.90	**12b**	Me	Me	E	88	579	2.24
4a	H	H	B	88	642	0.88	**12c**	Me	Me	F	77	200	1.18
4b	H	H	C	61	236	0.49	**12d**	Me	Me	J	85	712	2.01
4c	h	H	D	40	198	0.51	**17b**	$-CH_2CH_2-$		E	84	454	1.80
4d	H	H	E	85	973	1.45	**17c**	$-CH_2CH_2-$		F	88	419	0.75
4e	H	H	F	82	713	0.40	**17d**	$-CH_2CH_2-$		J	84	646	1.58



mice were evaluated according to our reported method.[3] Anti-inflammatory effects in adjuvant arthritis were evaluated as follows. Briefly, male Lewis rats were sensitized by injecting Freund's complete adjuvant intradermally at the tail head on day 0. A test compound was orally administered once a day from day 15 up to day 20. The volumes of both hind paws were measured at days 15 and 21. The therapeutic effects were calculated as follows: 100×[(hind paws volume on day 21 (control)-hind paws volume on day 21 (experimental))/(hind paws volume on day 21-hind paws volume on day 15 (control))].

Results and Discussion

As reported in the preceding paper, a phenylpiperazine derivative **2** showed good dual cytokine regulatory activity in LPS-stimulated mice.[3] Compound **2**, however, does not have a significant therapeutic effect in a chronic inflammatory animal model using adjuvant arthritic rats presumably due to its metabolic instability. In order to obtain agents that have an apparent therapeutic effect in the chronic inflammatory model, we investigated chemical modifications of **2**. First, to investigate the effects of the substituent(s) at the methylene group between the acetamido group and the middle benzene group, five phenylpiperazine derivatives (**8a**, **11**, **12a**, **13**, **17a**) were synthesized. Monomethyl, dimethyl, and ethylene derivatives retained the activity although the activity of monoethyl and diethyl derivatives decreased, suggesting that the substituent(s) at this position may be sterically restricted to some extent for the activity. Secondly, acetamidomethyl derivatives ($R^1 = R^2 = H$) having various heteroaryl groups were synthesized. Only pyridin-2-yl, pyrimidin-2-yl, and thiazol-2-yl derivatives showed comparable activity to **2**. The activity of the other derivatives **4b**, **4c**, **4f** and **4h** decreased presumably due to their low absorption predicted based on their lower calculated log P value (0.49, 0.51, −0.09 and 0.67, respectively) compared with that of **4a**, **4d** and **4g** (0.88, 1.45 and 1.23, respectively).[5,6] The log P value of a pyrimidylpiperazine derivative **4e** is very low (0.40); in fact, the bioavailability of **4e** at a dose of 30 mg/kg in rats was estimated to be 17%.[7] Nonetheless, **4a** retained the activity probably because its intrinsic activity might be very high. Finally, nine derivatives (**8b–d**, **12b–d**, **17b–d**) having a monomethyl, dimethyl, or ethylene group(s) at the methylene group between the acetamido group and the middle benzene group and a 6-fluoropyridin-2-yl, pyrimidin-2-yl, or thiazol-2-yl group for the terminal phenyl ring of **2** were synthesized. Almost all the compounds retained the activity except **8c** and **12c**.

Next we selected 13 compounds showing high dual cytokine regulatory activity in vivo in the above-mentioned screening system and evaluated their therapeutic effects in a chronic inflammatory animal model (Table 2). Phenylpiperazine derivatives **2** and **12a** showed only a poor therapeutic effect presumably due to ease of oxidative metabolism at their terminal phenyl ring. Fluoropyridine derivative **4d** was much more effective than **4a** probably because **4d** (log $P = 1.45$) is absorbed more efficiently than **4a** (log $P = 0.88$) and/or **4d** is metabolically more stable than **4a** (prevention of the oxidative metabolism of the pyridine ring of **4d** by the fluorine atom). In the thiazolylpiperazine series (**4g**, **8d** and **12d**),

Table 2. Therapeutic effects of selected compounds in adjuvant arthritis

	Dose (mg/kg)	Therapeutic effect (%)		Dose (mg/kg)	Therapeutic effect (%)
2	30	35	**8b**	10	173
3	30	173	**8d**	10	101
12a	30	80	**12b**	10	142
17b	10	55	**12d**	10	131
4a	10	62	**17b**	10	55
4d	10	144	**17c**	10	163
4e	10	46	**17d**	10	133
4g	10	80			

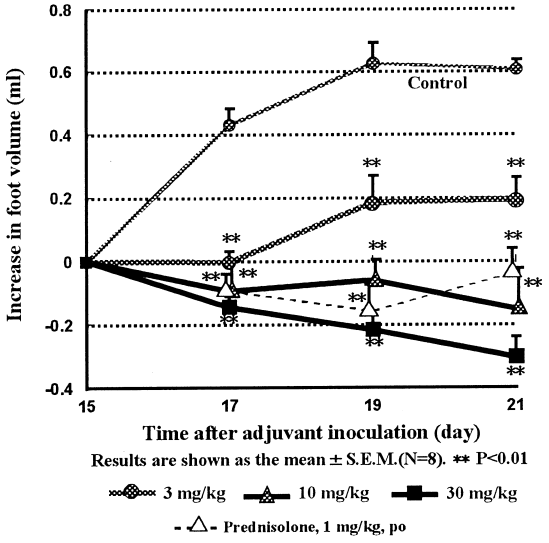


Figure 2. Dose-dependent therapeutic effect of **17c** in adjuvant arthritis.

therapeutic effectiveness increased upon increasing the log *P* values (1.23, 1.76, and 2.01, respectively). Compound **17c**, a cyclopropyl derivative of compound **4e**, shows a good therapeutic effect, suggesting that the modification of the methylene group between the acetamido group and the middle benzene group with the cyclopropyl group led to a significant increase in therapeutic effectiveness. The improved log *P* value (0.75) of **17c** compared with that of **4e** reflects its good bioavailability (76% at a dose of 30 mg/kg in rats). Compounds **8b**, **12b** and **17d** also demonstrated an excellent therapeutic effect in the model.

We chose compound **17c** for further pharmacological examination because it possessed the most favorable toxicological profile among the compounds (**3**, **4g**, **12b**, **12d**, **17c** and **17d**) that had shown a good therapeutic effect.[9] Compound **17c** was evaluated for its therapeutic effect in the above-mentioned model at three doses (3, 10 and 30 mg/kg, po) along with prednisolone (1 mg/kg, po)[10] as the positive control (Fig. 2). Compound **17c** significantly reduced the increased volumes of both hind paws of sensitized rats in a dose-dependent manner.

Conclusion

Our synthetic investigation to obtain anti-inflammatory agents effective in the chronic inflammatory animal model led to the discovery of the pyrimidylpiperazine derivative **17c**, which showed dual cytokine regulatory activity (TNF-α suppression and IL-10 augmentation) in LPS-stimulated mice and an excellent therapeutic effect in the adjuvant arthritis model. Compound **17c** is expected to be a promising drug candidate for the treatment of TNF-α associated diseases including RA.

Acknowledgements

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References and Notes

1. (a) Macnaul, K. L.; Hutchinson, N. I.; Parsons, J. N.; Bayne, E. K.; Tocci, M. J. *J. Immunol.* **1990**, *145*, 4154. (b) Brennan, F. M.; Gibbons, D. L.; Mitchell, T.; Cope, A. P.; Maini, R. N.; Feldmann, M. *Eur. J. Immunol.* **1992**, *22*, 1907.
2. Jackson, C. G.; Williams, H. J. *Drugs* **1998**, *56*, 337.
3. Hanano, T.; Adachi, K.; Aoki, Y.; Morimoto, H.; Naka, Y.; Hisadome, M.; Fukuda, T.; Sumichika, H. *Bioorg. Med. Chem. Lett. 10*, 875.
4. Compounds **2a–h**, **6a–e**, **11a–c**, **15a–c**, and **19a–c** gave satisfactory analytical and spectroscopic data in accord with their assigned structures.
5. The log *P* values were calculated using the well authorized program, PROLOGP contained in the PALLAS 1.2 package, a product of CompuDrug Chemistry Ltd (Budapest, Hungary).
6. It has been reported that there is a good relationship between membrane permeability and log *P* in the following paper. Komiya, I.; Park, J. Y.; Kamani, A.; Ho, N. F. H.; Higuchi, W. I. *Int. J. Pharm.* **1980**, *4*, 249.
7. Pharmacokinetic parameters were calculated according to our previous method.[3]
8. In order to investigate the effect of the absolute configuration of the racemic **8b** on the biological activity, we synthesized (*R*)-**8b** and (*S*)-**8b** starting from optically pure (*R*)- and (*S*)-phenethylamine, respectively [(*R*)-**8b**: $[\alpha]_D^{25}+85.9$ (c1.00, $CHCl_3$), (*S*)-**8b**: $[\alpha]_D^{25}-87.7$ (c1.00, $CHCl_3$)]. We found that the enantiomers have no significant difference in the activity [TNF-α (% inhibition) (*R*)-**8b**: 80%, (*S*)-**8b**: 79%; IL-10 (% of control) (*R*)-**8b**: 499%, (*S*)-**8b**: 413%)].
9. Compound **17c** showed no significant adverse effects at up to 100 mg/kg, po in toxicological studies using rats.
10. It is well known that steroids such as prednisolone have an excellent anti-inflammatory effect on various diseases but they often show adverse effects (e.g. rebound). It should be possible to use the dual cytokine regulators with steroids (at low doses where steroids do not show adverse effects) at the same time because these two agents have totally different mechanisms of action. For example, rebound has been observed in a collagen induced arthritis model in rats; see the following reference. Yamaki, K.; Nakagawa, H.; Tsurufuji, S. *Ann. Rheum. Dis.* **1987**, *46*, 543