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# Highly Potent Inhibitors of TNF-α Production. Part 2: Identification of Drug Candidates

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**Abstract**—Metabolic stabilization of the chemical lead **1**, which is a structurally novel inhibitor of TNF-α production, was accomplished by introducing a (1*S*)-methyl group into the optically active backbone. As a result, **2**, **3** and **4** were identified as drug candidates and evaluated pharmacologically. The analysis of an active conformer was also carried out. 

## Introduction

Tumor necrosis factor-α (TNF-α) is a dominant mediator of the cytokine cascade that causes inflammation and autoimmune diseases such as rheumatoid arthritis (RA), multiple sclerosis, cachexia, sepsis, ulcerative colitis, congestive heart failure and Crohn's disease.[1] In fact, two biological TNF-α inhibitors have been approved for RA and Crohn's disease, which has validated TNF-α inhibition as a clinical treatment. New classes of compounds which block the activity of TNF-α have been under investigation for their therapeutic potential.[2] Results of recent clinical trials have improved our understanding of the major role played by TNF-α.[3] Thus inhibitors of TNF-α production are expected to be therapeutically useful for the diseases mentioned above.[4] In the preceding paper, we reported a new class of compounds, 2-acylamino-2-phenylethyl disodium phosphates, as highly potent inhibitors of TNF-α production. Since our goal is to identify a clinically useful drug candidate, we next focused on blocking the expected rapid metabolism of the phosphate moiety of the newly discovered chemical lead. In this article, we report the metabolic stabilization of the chemical lead **1** to increase inhibitory activity, and the identification of drug candidates **2**–**4**.

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## Results and Discussion

According to our experimental results, one of the chemical leads, **12** ($ID_{50}$ for LPS-induced TNF-α production in rats = 3.0 mg/kg, iv), was unstable to the metabolic hydrolysis. As outlined in Scheme 1, **12** was converted to an inactive metabolite **20** by the rapid metabolic hydrolysis of its phosphate moiety on treatment with tissue homogenates,[5] while compound **21**, the phosphate moiety of which was hindered with a methyl group, showed good resistance to metabolic hydrolysis by the tissue homogenates and maintained potent inhibitory activity for TNF-α production ($ID_{50}$ = 5.6 mg/kg, iv, in rats).[6] Design and synthesis of metabolically stabilized inhibitors were started with the introduction of a methyl group into the optically active backbone (Fig. 1).

As shown in Table 1, two classes of four isomers (**5**, **6**, **8**, **10** and **2**, **7**, **9**, **11**) were synthesized and evaluated individually. The (*S*)-configuration of the newly introduced methyl group and (*R*)-configuration of the *N*-acyl moiety were needed for potent inhibitory activity, as illustrated in **5** and **2**. Introduction of a *meta*-methoxy group into the phenyl moiety of **5** afforded **2** with a marked increase in activity. The same tendency was observed upon the chemical modification of **6** to **7**, while **6** exhibited weak inhibitory activity. A marked reduction of inhibitory activity was observed in all the (*R*)-methyl derivatives **8**–**11**. Therefore, the configuration of the methyl group was thought to have a dominant effect on the activity to inhibit TNF-α production. The con-

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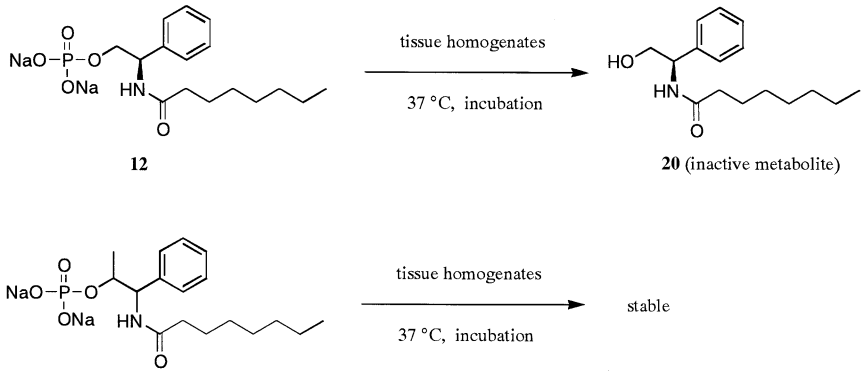



**Scheme 1.** Metabolic hydrolysis of the phophates **12** and **21**.

figuration of the *N*-acyl moiety plays an important role, though it seems not to be as critical as that of the methyl group, as illustrated in **5** and **7**. Introduction of a *meta*-methoxy group also increased the inhibitory activity as determined based on the marked potency observed upon the chemical modification of **5** to **2** and of **6** to **7**. Replacement of the *meta*-methoxy group of **2** with a *meta*-isopropyloxy group provided **3** with the highly potent activity. Replacement of the *N*-octanoyl moiety of **2** with an *N*-hexyloxy carbonyl moiety afforded **4**, also with strong activity to inhibit the production of TNF-α. Thus, the (1*S*,2*R*)-configuration was found to be a structural requirement in this series of compounds.

As shown in Table 2, further chemical modification was continued to identify another substituent which is acceptable instead of the (1*S*)-methyl group. The synthesized compounds **14**–**16** were evaluated biologically and their potentials compared with the chemical leads **5** and **12**. Introduction of another methyl group at the geminal position of **2** afforded **13** with a marked reduction in activity because of the presumed bulkiness of the newly constructed 2,2-dimethyl moiety, while a less hindered 2,2-trimethylene derivative **14**, which was evaluated as a DL-mixture, had an $ID_{50}$ value of 9.5 mg/kg, iv, in mice. Replacement of the methyl group of **5** with a hydroxymethyl group provided **15** with a decrease in activity. Introduction of a phenyl moiety to **5** instead of a methyl group afforded **16** with a marked reduction in activity. Thus, (1*S*)-methyl was concluded to be the most optimized partial structure.

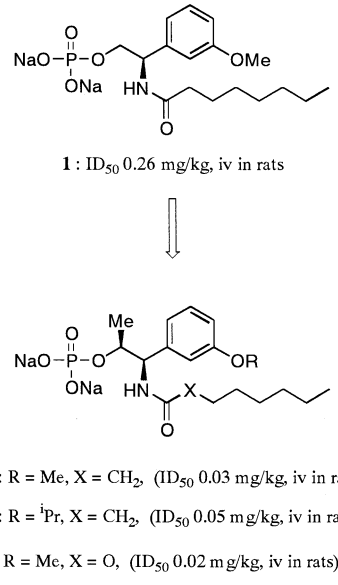


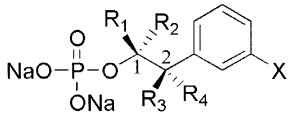

**Figure 1.** Design of metabolically stabilized molecules.

To elucidate the three-dimensional active structure, compounds **17**, **18** and **19**, in which free rotations are restricted and/or blocked, were synthesized as the optically active forms and evaluated biologically. Interestingly, the *trans*-isomer **18** was much more potent than the *cis*-isomer **17**. The *trans*-isomer **18** was estimated to occupy a three-dimensional structure similar to the real active conformer. This SAR strongly suggested an active conformation of the optimized compounds **2**, **3** and **4**.

As illustrated in Figure 2, the more active compound **2** is able to occupy the favored conformer more easily than the less active compound **9** because of a less hindered intramolecular repulsion between the methyl group and the *ortho*-hydrogen of the phenyl moiety. *N*-Methyl derivative **19** maintained quite good levels of activity, as shown in Table 3. The *N*-methyl group did not appear to prevent **19** from occupying the favored conformation. The dephosphorylated compound **20** did not show any activity at 10 mg/kg, iv, in mice.

The efficacy in disease models and safety of **2**, **3** and **4** were evaluated. As described in Table 4, the minimum effective doses (MEDs) of compounds **2**, **3** and **4** in the LPS-induced shock model in mice were 0.1, 0.3 and 0.1 mg/kg, iv, respectively. The minimum effective doses (MEDs) of **2**, **3** and **4** in the D-(+)-galactosamine/LPS-induced hepatitis model in rats were 0.3, 0.3 and 0.1 mg/kg, iv, respectively. With respect to the safety concern, the minimum lethal dose (MLD) of **3** in rats was 100 mg/kg, iv, while the MLDs of **2** and **4** were more than 100 mg/kg, iv. All three compounds **2**, **3** and **4** demon-

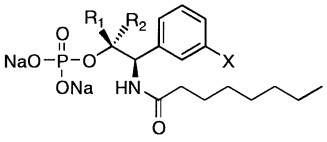
**Table 1.** Biological evaluation of the metabolically stabilized derivatives



| Compd | $R_1$ | $R_2$ | $R_3$ | $R_4$ | X | Inhibition of TNF-α production[a] $ID_{50}$ (mg/kg, iv) rats |
|---|---|---|---|---|---|---|
| **5** | Me | H | –NHCO–*n*-$C_7H_{15}$ | H | H | 4.5 |
| **2** | Me | H | –NHCO–*n*-$C_7H_{15}$ | H | OMe | 0.03 |
| **6** | Me | H | H | –NHCO–*n*-$C_7H_{15}$ | H | (17)[c] |
| **7** | Me | H | H | –NHCO–*n*-$C_7H_{15}$ | OMe | 3.7 |
| **8** | H | Me | –NHCO–*n*-$C_7H_{15}$ | H | H | (23)[c] |
| **9** | H | Me | –NHCO–*n*-$C_7H_{15}$ | H | OMe | (−13)[b] |
| **10** | H | Me | H | –NHCO–*n*-$C_7H_{15}$ | H | (5)[c] |
| **11** | H | Me | H | –NHCO–*n*-$C_7H_{15}$ | OMe | (38)[b] |
| **3** | Me | H | –NHCO–*n*-$C_7H_{15}$ | H | O$^i$Pr | 0.05 |
| **4** | Me | H | –NHCOO–*n*-$C_6H_{13}$ | H | OMe | 0.02 |

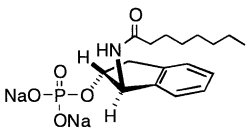
[a]Biological evaluation was performed according to the procedure used in the preceding paper.
[b]Inhibition (%) at 10 mg/kg, iv.
[c]Inhibition (%) at 30 mg/kg, iv.

**Table 2.** Attempt to discover a substitute for the (1*S*)-methyl group

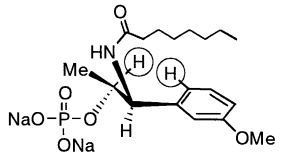


| Compd | $R_1$ | $R_2$ | X | Inhibition of TNF-α production[a] $ID_{50}$ (mg/kg, iv) rats |
|---|---|---|---|---|
| **12** | H | H | H | 3.0 |
| **5** | Me | H | H | 4.5 |
| **13** (DL) | Me | Me | OMe | (12)[b] |
| **14** (DL) | –$CH_2$–$CH_2$–$CH_2$– | | H | 9.5[c] |
| **15** | $CH_2OH$ | H | H | (42)[b] |
| **16** |  | H | H | (3)[b] |

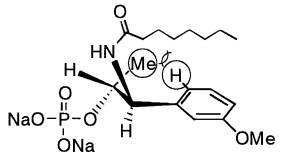
[a]Biological evaluation was performed according to the procedure used in the preceding paper.
[b]Inhibition (%) at 10 mg/kg, iv.
[c]Tested in mice.



**18** (*trans*-isomer: more active than *cis*-isomer **17**)



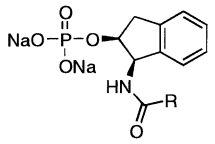
**2** (1*S*, 2*R*)



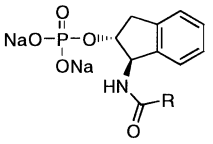
**9** (1*R*, 2*R*)

**Figure 2.** Proposed active conformation based on the more active *trans*-isomer **18**.

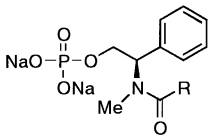
**Table 3.** Analysis of the structural requirements for the inhibitory activity



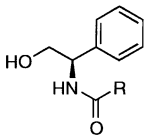
**17**



**18**



**19**



**20**

| Compd | R | Inhibition of TNF-α production[a] $ID_{50}$ (mg/kg, iv) rats |
|---|---|---|
| **17** | $C_7H_{15}$ | (34)[b] |
| **18** | $C_7H_{15}$ | 2.4 |
| **19** | $C_7H_{15}$ | 9.8 |
| **20** | $C_7H_{15}$ | (14)[c] |

[a]Biological evaluation was performed according to the procedure used in the preceding paper.
[b]Inhibition (%) at 10 mg/kg, iv.
[c]Inhibition (%) at 10 mg/kg, iv, in mice.

**Table 4.** Biological evaluation of **2**, **3** and **4**

| Compd | LPS-induced shock model in mice ($n=18$ or 20)[a] MED[c] mg/kg, iv (survival rate) | D-(+)-Galactosamine/LPS-induced hepatitis model in rats ($n=18$)[b] MED[c] mg/kg, iv (survival rate) | Safety in rats ($n=3$) MLD[d] mg/kg, iv (mortality rate) |
|---|---|---|---|
| **2** | 0.1 (10/18) | 0.3 (7/18) | >100 (0/3) |
| **3** | 0.3 (10/18) | 0.3 (8/18) | 100 (1/3) |
| **4** | 0.1 (14/20) | 0.1 (6/18) | >100 (0/3) |

[a]LPS from *Escherichia coli* strain 055 B5 (Difco Laboratories, Detroit, MI, USA) and the test compounds were dissolved in saline. Female BALB/c mice (Charles River Inc., Japan) aged 7–8 weeks were injected intravenously with the test compounds and then immediately given an intraperitoneal injection of LPS (5 mg/kg). The survival rate of the mice was evaluated after 96 h. Predonisolone (10 mg/kg, iv), which was used as a positive control, demonstrated an efficacy (survival rate: 13/20) equivalent to **4** (survival rate 15/20 at 0.3 mg/kg, iv) in this model. Survival rate of the controls which were dosed with saline was 2/20.
[b]D-(+)-Galactosamine/LPS and the test compounds were dissolved in saline. Male Sprague–Dawley rats (Charles River Inc., Japan) aged 6 weeks were injected intravenously with the test compounds and then immediately given an intraperitoneal injection of D-(+)-galactosamine/LPS (1 g/7.5 μg/5 mL/kg). The survival rate of the rats was evaluated after 96 h. Predonisolone (10 mg/kg, iv), which was used as a positive control, demonstrated an efficacy (survival rate: 13/18) equivalent to **4** (survival rate: 12/18 at 0.3 mg/kg, iv) in this model. Survival rate of the controls which were dosed with saline was 0/18.
[c]MED (minimum effective dose): survival rates are described in parentheses.
[d]MLD (minimum lethal dose): at least one of the tested animals died at this dose. Mortality rates are described in parentheses.

strated a sufficient margin of safety for pharmacological evaluation. With regard to oral dosing, much higher dose compared with iv dosing was needed for the compounds **2**, **3** and **4** to be effective ($ID_{50}$ 15.9, 9.4 and 4.1 mg/kg, po, respectively).

In summary, we have discovered the drug candidates **2**, **3** and **4** for the treatment of diseases caused by the overexpression of TNF-α through the design and synthesis of the metabolically stabilized inhibitors of TNF-α production. These three compounds demonstrated efficacy in animal models of disease and are expected to be clinically useful while specialized clinical uses are not yet intended. Full details including chemistry and mechanism of action will be reported in *Bioorganic & Medicinal Chemistry* very soon.

## References and Notes

1. Tracey, K. J.; Cerami, A. *Annu. Rev. Med.* **1994**, *45*, 491.
2. Marriott, J. B.; Westry, M.; Dalgleish, A. G. *Drug Discov. Today* **1997**, *2*, 273.
3. Camussi, G.; Lupia, E. *Drugs* **1998**, *55*, 613.
4. Lowe, C. *Exp. Opin. Ther. Pat.* **1998**, *8*, 1309.
5. Percentage of compound **12** remaining (determined based on HPLC analysis): 15% in mouse liver homogenates (after 15 min); 17% in mouse small intestine homogenates (after 15 min); 67% in mouse plasma (after 120 min); 27% in rat plasma (after 120 min); 0% in rat liver homogenates (after 5 min); 88% in human plasma (after 120 min).
6. Percentage of compound **21** remaining (determined based on HPLC analysis): 80% in mouse liver homogenates (after 120 min); 89% in mouse small intestine homogenates (after 120 min); 88% in mouse plasma (after 120 min); 97% in rat plasma (after 120 min); 74% in rat liver homogenates (after 120 min); 100% in human plasma (after 180 min).