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Highly Potent Inhibitors of TNF-α Production. Part 1: Discovery of Chemical Leads

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Abstract—The discovery of 2-acylamino-2-phenylethyl disodium phosphates **1** and **2** as structurally novel inhibitors of TNF-α production is reported. Structure–activity relationships (SARs) are also discussed.

Introduction

Tumor necrosis factor-α (TNF-α),[1,2] which was first purified and characterized in the mid-1980s,[3] is a critical pro-inflammatory cytokine released as one of the mediators of the biological response to bacterial infection and inflammation. Among the many cytokines responsible for regulating cellular physiology, TNF-α has been proven to play a dominant role both in normal immune function and in disturbances leading to autoimmune disease. Overexpression of TNF-α can lead to a variety of pathological conditions including rheumatoid arthritis (RA), multiple sclerosis, cachexia, sepsis, ulcerative colitis, congestive heart failure and Crohn's disease. To date, much research has been directed toward the inhibition of TNF-α production, the antagonism of TNF-α and the shedding of TNF-α from the cell surface.[4–6] Thus, TNF-α has received a considerable amount of attention as a molecular target for the treatment of the diseases mentioned above. For example, phosphodiesterase type IV (PDE IV) inhibitors,[4–6] the agonists of adenosine,[7,8] matrix metalloproteinase inhibitors[4–6] and thalidomide[4] all have been thought to act through inhibition of TNF-α production. TNF-α antagonists have provided some of the greatest successes to date. Monoclonal antihuman TNF-α antibody (infliximab) has been approved for the treatment of Crohn's disease and RA.[9–12] Soluble TNF p75 receptor fusion protein (etanercept) has been approved for the treatment of RA.[9,10,13] Thus inhibition of TNF-α production with a small molecule could be one approach to the treatment of diseases in which an overexpression of TNF-α is involved. In this article, we report the discovery of a series of phosphates as structurally novel inhibitors of TNF-α production.

Results and Discussion

Much attention has been paid to small molecules which demonstrate activity to inhibit the production of TNF-α because of their expected therapeutic potential. In the course of screening for inhibitors of TNF-α production, we discovered a new class of compounds, 2-acylamino-2-phenylethyl disodium phosphate derivatives **1** ($ID_{50}=3.0$ mg/kg, iv, in rats) and **2** ($ID_{50}=0.26$ mg/kg, iv, in rats), to be highly potent (Fig. 1). A series of phosphates was synthesized and their ability to inhibit LPS-induced plasma TNF-α production in mice and rats biologically evaluated. Our chemical modifications started with optimization of the *N*-acyl chain of **1** followed by the introduction of an alkoxy group into the *meta*-position of the phenyl moiety. Biological evaluation was carried out in mice in the earlier stage of this project, while it was carried out in rats in the later stage because of the effectiveness of the screening. Compound **2** was nearly 10 times more active than **1**. The corresponding enantiomers **34** and **35** were nearly 3 times less and nearly 6 times less potent than **1** and **2**, respectively.

As described in Table 1, good ID_{50} values were obtained for **6**, **1**, **12a**–**b** and **13**. An important role for the *N*-acyl

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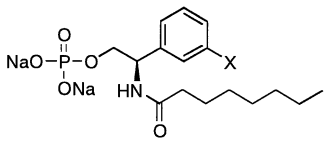
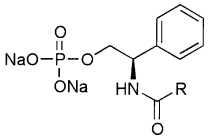


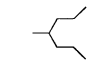
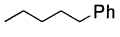
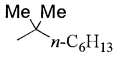
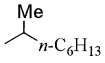
1 : X = H (ID_{50} 3.0 mg/kg, iv in rats)

2: X = OMe (ID_{50} 0.26 mg/kg, iv in rats)

Figure 1. New inhibitors of TNF-α production.

Table 1. Optimization of the *N*-acyl moiety



Compd	R	Inhibition of TNF-α production ID_{50} (mg/kg, iv)	
		Mice[a]	Rats[b]
3	Me	(28)[c]	(21)[d]
4	*n*-Pr	(17)[c]	(37)[d]
5	*n*-C_5H_{11}	30	50
6	*n*-C_6H_{13}	2.8	4.5
1	*n*-C_7H_{15}	0.8	3.0
7	*n*-$C_{10}H_{21}$	(50)[c]	N.T.[e]
8	—Ph	(32)[c]	N.T.
9		(36)[d]	N.T.
10		(27)[c]	N.T.
11		(56)[c]	N.T.
12a (less polar)		4.2	8.0
12b (more polar)		2.4	1.7
13	–O–*n*-C_6H_{13}	1.1	3.2
14	–NH–*n*-C_6H_{13}	(58)[c]	N.T.

[a]LPS from *Escherichia coli* strain 055 B5 (Difco Laboratories) and the test compounds were dissolved in saline. Male BALB/c mice aged 8 weeks ($n=5$) were injected intravenously with the test compounds (0.01–0.1 mg/10 mL/kg), and then immediately given an intraperitoneal injection of LPS (5 mg/10 mL/kg). Plasma TNF-α production was determined by ELISA using a commercial kit (GENZYME) at 90 min after the LPS challenge. ID_{50} values were determined. ID_{50} = The dosage required to inhibit plasma TNF-α production by 50%.

[b]Male Sprague–Dawley (CD)/IGS rats (Charles River Inc., Japan) aged 6–8 weeks ($n=5$) were injected intravenously with the test compounds (0.01–0.1 mg/10 mL/kg), and then immediately given an intraperitoneal injection of LPS (30 μg/kg). Plasma TNF-α production was determined by ELISA using a commercial kit (R&D Systems) at 90 min after the LPS challenge. ID_{50} values were determined. ID_{50} = The dosage required to inhibit plasma TNF-α production by 50%. % Inhibition = 100−(C−S)/(L−S) × 100. C, Plasma TNF-α concentration of LPS-treated animals pretreated with a test compound. L, Plasma TNF-α concentration of LPS-treated animals pretreated with saline. S, Plasma TNF-α concentration of saline-treated animals pretreated with saline.

[c]Inhibition (%) at 10 mg/kg, iv.

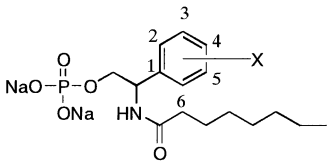
[d]Inhibition (%) at 30 mg/kg, iv.

[e]N.T., not tested.

moiety in the inhibitory activity of these compounds was revealed by the marked reduction in the potency of **3**–**4** and **8**–**10**. As illustrated in **3**–**7**, the length of the *N*-acyl side chain was optimized at the *N*-octanoyl moiety. Compounds **8**, **9** and **10** possessing benzoyl moiety, branched alkanoyl and 5-phenylpentanoyl moieties exhibited a marked reduction in inhibitory activity compared with **1**. To elucidate the effect on the inhibitory activity of blocking the predicted metabolic hydrolysis of the *N*-acyl chain, compounds **11** and **12a**–**b** were synthesized and evaluated biologically. Introduction of a 2,2-dimethyl group into the optimized *N*-octanoyl moiety of **1** afforded **11** with reduced inhibitory activity. Introduction of a 2-monomethyl group into the same position afforded two diastereomers, **12a** (less polar isomer) and **12b** (more polar isomer). The inhibitory activity of the more polar isomer **12b** was greater than that of **12a**. Also, that of **12b** was 3 times less potent in mice, nearly 2 times more potent in rats than **1**. Thus the SAR in mice tended to be maintained in the rats. *N*-Hexanoyloxy carbonyl derivative **13** showed nearly the same potency as **1** in the evaluation with both mice and rats. Replacement of the urethane moiety of **13** with a urea moiety afforded **14** with a reduction in potency. According to the data, the ID_{50} values of these compounds tended to be lower in the evaluation with mice than rats.

Optimization of the substituents on the phenyl moiety was carried out using racemic compounds as illustrated in Table 2. Introduction of a methoxy group into the phenyl moiety of (±)-**1** afforded **15**–**17**. Of these compounds, the *meta*-substituted derivative **16** exhibited the most potent inhibitory activity in the evaluation with rats. Replacement of the methoxy group with a *meta*-methlythio group afforded **18** with no change in the ID_{50} value. The inhibitory activity of the *ortho*-isomer **15** seemed to be more potent than that of the *para*-isomer **17**. Introduction of a methyl group into the phenyl moiety of (±)-**1** instead of a methoxy group afforded **19**–**21**. Among these compounds, the *ortho*-substituted **19** and *meta*-substituted **20** had lower ID_{50} values than the *para*-substituted **21**, which showed 44% inhibition at 10 mg/kg, iv. In the evaluation of methyl-substituted compounds **19**–**21**, the *ortho*-isomer **19** showed much more activity (ID_{50} = 2.0 mg/kg, iv) than the *meta*-isomer **20**. Introduction of a chloro group into the phenyl moiety of (±)-**1** provided **22**–**24**. Of these compounds, the *meta*-substituted derivative **23** again exhibited the most potent activity. The *ortho*-substituted isomer **22** showed weak activity (46% inhibition at 10 mg/kg, iv) while the *para*-substituted isomer **24** did not show any inhibitory activity at 10 mg/kg, iv. Based on these experimental results, introduction of *meta*-alkoxy or *meta*-alkylthio substitutions was suggested to be a promising chemical modification towards optimization. For further optimization of the *meta*-alkoxy moiety, compounds **25**–**33** were synthesized and evaluated pharmacologically. Of these compounds, the *meta*-ethoxy derivative **26** and *meta*-isopropyloxy derivative **28** had the most potent ID_{50} values, 0.2 and 0.1 mg/kg, respectively. As expected, the isopropylthio derivative **29** was nearly as potent as **28**. Removal of the methyl

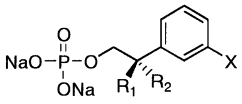
Table 2. Optimization of the subsutituent on the aromatic moiety



Compd	X	Inhibition of TNF-α production[a] ID_{50} (mg/kg, iv) rats
15	2-OMe	(55)[b]
16	3-OMe	0.5
17	4-OMe	(34)[b]
18	3-SMe	0.56
19	2-Me	2.0
20	3-Me	53
21	4-Me	(44)[b]
22	2-Cl	(46)[b]
23	3-Cl	5.2
24	4-Cl	(−118)[b]
25	3-OH	1.4
26	3-OEt	0.2
27	3-O^{n}Pr	0.7
28	3-O^{i}Pr	0.1
29	3-S^{i}Pr	0.21
30	3-O^{i}Bu	33
31		0.9
32		(7)[b]
33	3,5-OMe	3.8

[a]Biological evaluation was performed as described in the footnotes to Table 1.
[b]Inhibition (%) at 10 mg/kg, iv.

Table 3. Biological evaluation of the optically active forms



Compd	R_1	R_2	X	Inhibition of TNF-α production[a] ID_{50} (mg/kg, iv) rats
1	–NHCO–*n*-C_7H_{15}	H	H	3.0
2	–NHCO–*n*-C_7H_{15}	H	OMe	0.26
34	H	–NHCO–*n*-C_7H_{15}	H	10.0
35	H	–NHCO–*n*-C_7H_{15}	OMe	1.6

[a]Biological evaluation was performed as described in the footnotes to Table 1.

group of the *meta*-methoxy moiety of **16** provided *meta*-hydroxy derivative **25** with nearly 3 times less potency. *meta*-*n*-Propyloxy and *meta*-cyclobutyloxy derivatives **27** and **31** showed potent activity while *meta*-cyclopentyloxy derivative **32** exhibited markedly reduced activity, presumably because of the bulkiness of its cyclopentyl moiety. Isobutyloxy derivative **30** was nearly 6 times less potent and the introduction of another *meta*-methoxy group into the phenyl moiety of **16** afforded **33**, which was nearly 8 times less active than **16**.

As described in Table 3, the optically active derivatives **1**–**2** and **34**–**35** were synthesized and evaluated biologically. The newly discovered chemical lead **1** exhibited activity to inhibit production of TNF-α with an ID_{50} value of 3.0 mg/kg, iv, in rats. The *meta*-methoxy derivative **2** had nearly a 10 times lower ID_{50} value (0.26 mg/kg, iv, in rats) than **1**. Their corresponding enantiomers **34** and **35** had nearly 3 and 6 times higher ID_{50} values, respectively. With regard to oral dosing, much higher dose compared with iv dosing was needed for the compound **1** to be effective ($ID_{50} = 103$ mg/kg, po).

In summary, we have discovered a new series of inhibitors of TNF-α production. A number of the compounds, 2-acylamino-2-phenylethyl disodium phosphate derivatives, most notably **2**, **16**, **18**, **26**, **28** and **29**, were excellent inhibitors. Biological evaluation of the optically active derivatives clearly shows that (2*R*)-enantiomers **1** and **2** are more potent inhibitors than their corresponding (2*S*)-enantiomers **34** and **35**, respectively. The findings from the present study will be useful for further optimization of the newly discovered chemical lead **2**, which will be reported in the following paper in this journal. Full details including chemistry and mechanism of action will be reported in *Bioorganic & Medicinal Chemistry* very soon.

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