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Discovery and structure–activity relationship of coumarin derivatives as TNF-α inhibitors

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Abstract—The discovery and structure–activity relationship of a novel series of coumarin-based TNF-α inhibitors is described. Starting from the initial lead **1a**, various derivatives were prepared surrounding the coumarin core structure to optimize the in vitro inhibitory activity of TNF-α production by human peripheral blood mononuclear cells (hPBMC), stimulated by bacterial lipopolysaccharide (LPS). Selected compounds also demonstrated in vivo inhibition of TNF-α production in rats.

Tumor necrosis factor α (TNF-α) is a proinflammatory cytokine secreted by a variety of cells, including monocytes and macrophages, in response to many inflammatory stimuli or external cellular stress.[1] It is a key cytokine in the inflammation cascade, causing the production and/or release of other cytokines and agents. TNF-α exerts its biological effects through interaction with one of two ubiquitously expressed cell surface receptors, TNFR1 (p55) and TNFR2 (p75). Binding of TNF-α to its receptors initiates a series of parallel protein serine/threonine kinase cascades, which lead to the activation of members of the MAP kinase superfamily. It also causes activation of the transcription factors NFκB and Jun Kinase.[2] NFκB in turn regulates the production of many proinflammatory cytokines including TNF-α and related proteins that are elevated in immunoinflammatory diseases.[3] TNF-α levels and NFκB transcriptional activity are controlled by a reciprocal feedback loop. Since excessive or unregulated TNF-α production has been implicated in mediating or exacerbating a number of disease states, for example, cachexia and sepsis,[4] decreasing TNF-α levels or inhibiting NFκB transcriptional activation constitute valuable therapeutic strategies for the treatment of many inflammatory, infectious, immunological, or malignant diseases such as rheumatoid arthritis, psoriasis, and inflammatory bowel disease.[5]

Three macromolecular TNF-α inhibitors, such as Enbrel®, a soluble TNF-α receptor (TNFR2) coupled to Fc portion of IgG,[6a] mouse human chimeric anti-human TNF-α antibody[6b,c,d] Remicade® and human anti-human TNF-α antibody Adalimumab[7] have been shown to be useful for the treatment of inflammatory and autoimmune diseases. They are approved for reducing the signs and symptoms of moderate to severe rheumatoid arthritis, psoriatic arthritis, and Crohn's disease. The efficacy of a number of small molecules[7,8] with anti-inflammatory activity has also been linked to their ability to lower TNF-α levels.

As part of our continuing search for novel and potent TNF-α inhibitors,[9] we recently identified a coumarin-based compound, dimethyl-carbamic acid 3-benzyl-4-methyl-2-oxo-2*H*-chromen-7-yl ester (**1a**), as a moderate inhibitor of TNF-α. Compound **1a** inhibits NFκB and AP-1 in a transfected cell based reporter gene assay as well as TNF-α production in LPS-challenged human peripheral blood mononuclear cells (**1a**: IC_{50} 1.8 μM). The aim of the present study was to establish the structure–activity relationships for this class of inhibitor and to identify derivatives with improved TNF-α inhibitory activity.

Keywords: Coumarin; TNF-α.
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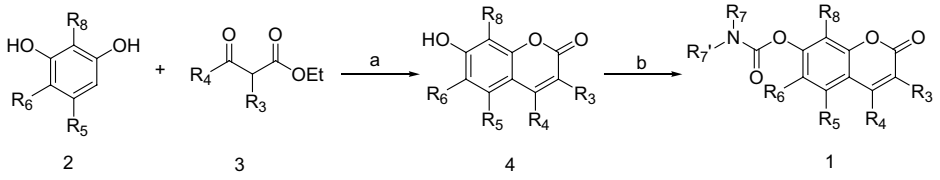
Synthesis of derivatives of compound **1** is straightforward. As shown in Scheme 1, the key intermediates, C-7 hydroxy coumarins **4**, were prepared from substituted or unsubstituted resorcinols and β-ketoesters according to literature procedures.[10] Thus, 2-arylmethyl β-ketoesters **3** reacted with resorcinols **2** in 70% sulfuric acid to provide 3-arylmethyl-4-substituted coumarins **4**. Upon treatment with *N*,*N*-dimethylcarbamoyl chlorides in the presence of NaH, the C-7 hydroxylcoumarins **4** were readily converted into the desired *N*,*N*-dimethylcarbamates (**1**) in good to excellent yields (Scheme 1). Using this procedure, a series of compounds with different substituents, in particular halogen, cyano, nitro, lower alkyl, trifluoromethyl, lower alkoxyl groups at C-5, C-6, and C-8 positions, and alkyl/substituted alkyl groups at C-3 and C-4 positions were prepared using a variety of β-ketoesters and substituted resorcinols.

Alternatively, C-4 modified analogs could be synthesized by elaboration of 4-methyl-courmain intermediate **4a** ($R_4 = Me$) as shown in Scheme 2. Deprotonation of 4-methyl-7-hydroxylcoumarin **4a** with LiHMDS, followed by treatment of the resultant anion with an aldehyde, an alkyl halide, or an ester gave rise to the corresponding hydroxyl, alkyl, or ketone products of the general structure **5**. Compound **5** was subsequently converted into its corresponding *N*,*N*-dimethylcarbamate **1** under the same reaction condition as shown in Scheme 1.

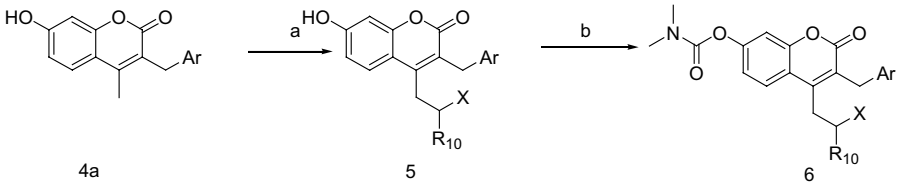
Scheme 3 describes the synthesis of C-3 benzyl substituted analogs. 3-Nitrobenzylcoumarin derivatives **7** were converted into their corresponding anilines by hydrogenation. Reaction of the resultant aniline derivatives **8** with an acyl chloride or an isocyanate provided the final products **9**.

Coumarin derivatives shown above were evaluated in LPS-challenged human peripheral blood mononuclear cells (PBMC) for their ability to inhibit TNF-α production, using an ELISA assay. The cells were cultured in RPMI1640 medium supplemented with 5% heat-inactivated fetal calf serum and antibiotics. PBMC (5×10^5 cells/mL) in 0.2 mL aliquots were pretreated with the test compound in DMSO for 60 min at 37 °C in 96 well round-bottomed tissue culture plates. Thereafter, the PBMC in the presence or absence of compound were stimulated with 1 μg/mL lipopolysaccharide (LPS) from *Salmonella minnesota* R595 at a final concentration of 100 ng/mL. After overnight culture, the supernatants were harvested and assayed immediately for TNF-α. The concentration of TNF-α in the supernatant is determined using a human TNF-α ELISA Kit. A secondary assay using a reporter gene system confirmed that these compounds inhibit NFκB and AP-1 activation (data not shown).

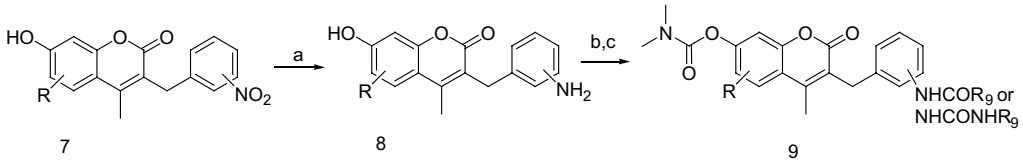
Modifications of the core coumarin structure of initial hit **1a**, such as removal of the 2-carbonyl group or 3,4-double bond and replacement of 1-oxygen atom with an NH group, did not generate active analogs. The *N*,*N*-dimethylcarbamate at C-7 position could be replaced by *N*,*N*-dimethylthiocarbamate or *N*,*N*-diethylcarbamate. Other carbamates abolished the activity completely (data not shown). Therefore the SAR studies were focused on the substitutions around the coumarin core with *N*,*N*-dimethylcarbamate at C-7 position. The influence of substituents at the C-3 to C-8 positions of coumarin was investigated. As shown in Table 1, a hydrophobic group at C-3 appears to be preferred. For example, compounds with a proton or a methyl group at C-3 position did not show any TNF-α inhibitory



Scheme 1. Reagents and conditions: (a) 70% H_2SO_4, 100 °C; (b) NaH, THF, $R_7R_{7'}NCOCl$, rt.

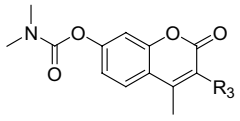


Scheme 2. Reagents and conditions: (a) LiHMDS, $R_{10}CHO$, THF; (b) NaH, THF, Me_2NCOCl.



Scheme 3. Reagents and conditions: (a) H_2/Pd–C, EtOH, rt; (b) R_9COCl or R_9NCO, THF; (c) Me_2NCOCl, NaH, THF.

Table 1. In vitro inhibitory activity of C-3 modified coumarins on TNF-α production



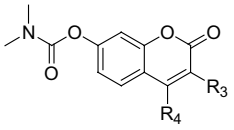
Compounds	R_3	IC_{50} (μM)
1a	Bn	1.8
10	4-Fluorobenzyl	0.32
11	H	>50
12	Me	⩾50
13	*n*-Butyl	24.7
14	*n*-Pentyl	22.8
15	*n*-Hexyl	25.4
16	*c*-Hexylmethyl	7.2
17	1-Piperidinylmethyl	>50
18	4-Morpholinylmethyl	>50
19	Ph	>50
20	Phenethyl	>50
21	2-Pyridinylmethyl	6.5
22	3-Pyridinylmethyl	2.7
23	4-Pyridinylmethyl	1.3
24	*o*-NH_2Ph–	2.1
25	*m*-NH_2Ph–	2.7
26	*p*-NH_2Ph–	5.2
27	*o*-MeOPh–	7.5
28	*m*-MeOPh–	3.2
29	*p*-MeOPh–	5.6
30	*o*-CF_3Ph–	⩾50
31	*m*-CF_3Ph–	>50
32	*p*-CF_3Ph–	>50
33	*m*-MeO_2CPh–	1.7
34	*p*-MeO_2CPh–	⩾50
35	*m*-AcNHPh–	1.1
36	*m*-EtNHCONHPh	2.4

activity. The activity appears to increase with the size of the alkyl group at this position, especially with carbocycles (e.g., compound **16** with cyclohexylmethyl substitution). However, its activity is five times lower than the aromatic counterpart **1a**. Non-carbocyclic analogs such as 4-morpholinylmethyl or 1-piperidinylmethyl groups were not tolerated at the C-3 position.

An aryl group greatly enhanced TNF-α inhibitory activity, when attached to the C-3 position via a methylene link. Removal (**19**) or extension of this methylene link to an ethylene (**20**) both abolished the activity. A fivefold increase in TNF-α inhibitory activity was seen when a fluoro atom was introduced to the *para*-position of the C-3 benzyl group. However, a trifluoromethyl group, a carboxylic acid, or an ester group at this position was not tolerated. On the other hand, it seems that modification of the *meta*-position of C-3 benzyl group was well tolerated. Compounds bearing an acetamido (**35**) or an ethyl ureido (**36**) group at the *meta*-position were as potent as the parent compound **1a**. Although the 4-pyridinylmethyl analog is the best TNF-α inhibitor among the three possible regioisomers, all showed low micromolar inhibition, similar to a benzyl group.

A number of C-4 analogs (Table 2) were prepared and tested. It appears that a small aliphatic group at the C-4 position is optimal for activity. Elimination of C-4

Table 2. In vitro inhibitory activity of C-4 modified coumarins on TNF-α production



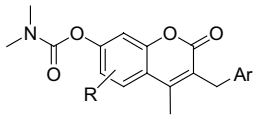
Compounds	R_3	R_4	IC_{50} (μM)
37	Bn	H	>50
38	Bn	Et	1.7
39	Bn	*n*-Pr	0.78
40	*p*-Fluorobenzyl	*n*-Pr	1.2
41	Bn	$HOCH_2CH_2$	2.1
42	Bn	COOH	>50
43	*p*-Fluorobenzyl	CF_3	>50
44	*p*-Fluorobenzyl	Ph	1.7
45	Bn	*o*-F–Ph	4.9
46	Bn	*p*-F–Ph	41.7
47	Bn	*m*-CF_3–Ph	>50
48	Bn	*p*-CF_3–Ph	>50
49	Bn	2-Pyridinyl	18.7
50	Bn	4-Pyridinyl	4.9
51	Bn	$PhCH_2CH_2$–	>50
52	Bn	*p*-CNPhCH(OH)CH_2–	28.3
53	Bn	2-Thiazolyl-CH(OH)CH_2–	16.5
54	Bn	*c*-Hexyl-CH(OH)CH_2–	>50
55	Bn	CH_2=$CH(CH_2)_2$CH(OH)CH_2–	48.4

methyl group (**37**) completely abolished the activity. Compounds with C-4 ethyl (**38**), *n*-propyl (**39**, **40**) or hydroxyethyl (**41**) groups showed comparable activity to the parent compound **1a**. Replacement of C-4 methyl group with a carboxylic acid (**42**) or a trifluoromethyl (**43**) group resulted in inactive compounds. A phenyl group is tolerated at this position (**44**: IC_{50} 1.7 μM). However, a substituent, such as a fluoro or trifluoromethyl group, at the *para*-position of the phenyl ring dramatically decreased activity. Similarly, pyridinyl groups (**49**, **50**) were not good surrogates of a methyl or ethyl group at this position.

Although a hydroxyethyl group (**41**) appeared to be tolerated at the C-4 position, all attempts to introduce other moieties at this position failed to generate more potent TNF-α inhibitors. Those analogs with a phenyl (**51**), substituted phenyl (**52**), heterocyclic (**53**), cyclohexyl (**54**), or a linear aliphatic group (**55**) all showed a decrease or complete loss of TNF-α inhibitory activity.

The most dramatic increase in TNF-α inhibitory activity was observed for those compounds with C-6 substitutions, especially with 6-halo substituted derivatives (Table 3). For example, the 6-halo coumarin compounds (**63**–**65**) prepared from 4-haloresorcinol was 20–30 times more potent than the corresponding nonsubstituted counterpart **1a**. Alkyl groups except methyl group (**56**) at this position tended to decrease the activities. No clear trend was observed for electronic effects of the substituents. Electron withdrawing group such as CHO (**60**), CN (**61**), NO_2 (**62**), and COOH (**59**) either improved or diminished the TNF-α inhibitory activities. Electron donating group like MeO (**66**) increased

Table 3. In vitro inhibitory activity of C-5/C-8 modified coumarins on TNF-α production



Compounds	R	Ar	IC_{50} (μM)
1a	6-H	Ph–	1.8
56	6-Me	Ph–	0.4
57	6-Et	Ph–	4.6
58	6-*i*Pr	Ph–	40.1
59	6-COOH	Ph–	≫50
60	6-CHO	Ph–	9.8
61	6-CN	Ph–	0.85
62	6-NO_2	Ph–	0.12
63	6-Cl	Ph–	0.09
64	6-Br	Ph–	0.06
65	6-I	Ph–	0.06
66	6-OMe	Ph–	0.58
67	6-Cl	3-Pyridinyl	0.45
68	6-Cl	4-Pyridinyl	0.13
69	5-OMe	Ph	≫50
70	5-F	Ph–	0.44
71	8-Me	Ph–	10.3
72	6-Cl	*m*-$EtOCOCH_2CONHPh$–	0.05
73	6-Cl	*m*-AcNHPh–	0.31

activity by threefold, similar to the CN group. For comparison, compounds with substitution at other positions of the coumarin ring than C-6 were also prepared. A C-8 methyl derivative (**71**) is 25 less potent than the corresponding C-6 derivative (**56**). Similarly, a methoxy group at C-5 position completely abolished the TNF-α inhibitory activity. Only a small substituent like fluorine (**70**) is tolerated at C-5 position.

Improvement in potency was also observed for C-3 pyridinylmethyl or other substituted benzyl groups when a halogen such as chlorine was present at the C-6 position. Pyridine derivatives **67** and **68** were about 6–10 times more potent than the corresponding nonsubstituted counterparts. Compound **73** (IC_{50} 0.31 μM), which possesses a C-6 chloro and substitutions at C-3 benzyl group, is a better TNF-α inhibitor than **35** (IC_{50} 1.1 μM).

Representative compounds that possess excellent in vitro TNF-α inhibitory activity were tested in vivo by subcutaneous (sc) administration at 100 mg/kg (Table 4). TNF-α inhibitory activity was assessed by in vivo inhibition of serum TNF-production in the rats.[11] As a result, compounds **10** and **63**, which possess a C-3 benzyl group with IC_{50} at 0.32 and 0.09 μM, respectively, showed only moderate inhibition of TNF-α production in vivo. However, those derivatives with a 4-pyridinylmethyl group at C-3 position (**22**: IC_{50} 2.7 μM; **67**: IC_{50} 0.45 μM) demonstrated higher in vivo efficacy, even though their in vitro activities are not as potent as **10** or **63**. These results clearly indicated the importance of solubility, along with the compound potency for the in vivo TNF-α production inhibition.

Table 4. In vivo inhibition of TNF-α production by coumarin compounds

Compounds	Inhibition (%) @ 100 mg/kg sc
Control	—
10	42
22	86
63	57
67	86
Dexamethasone	99[a]

[a] At 100 μg/kg.

In summary, a novel series of coumarin-based carbamates was synthesized, which exhibited potent inhibitory activity of TNF-α production in human PBMC cells stimulated by bacterial lipopolysaccharide (LPS). Substitution at C-6 position of the coumarin ring system was found to most dramatically influence TNF-α inhibitory activity. This observation led to the discovery of 6-halo derivatives with low nanomolar activity. Several of the coumarin derivatives (e.g., **22**, **67**) also inhibited TNF-α production in rats. Several types of coumarins have been reported in the literatures to inhibit TNF-α production via different mechanism of action.[12] For example, cloricromene, a semi-synthetic coumarin derivative, inhibited NFκB activation and subsequent TNF-α neosynthesis, by scavenging reactive oxygen species.[12a,b] More detailed mechanism of action and pharmacological investigations of these compounds may afford novel anti-TNF-α agents for the treatment of autoimmunoinflammatory diseases.

References and notes

1. Locksley, R. M.; Killeen, N.; Lenardo, M. J. *Cell* **2001**, *104*, 487.
2. Hsu, H.; Huang, J.; Shu, H. B.; Baichwal, V.; Goeddel, D. V. *Immunity* **1996**, *4*, 387.
3. Palanki, M. S. S.; Erdman, P. E.; Gayo-Fung, L. M.; Shevlin, G. I.; Sullivan, R. W.; Suto, M. J.; Goldman, M. E.; Ransone, L. J.; Bennett, B. L.; Manning, A. M. *J. Med. Chem.* **2000**, *43*, 3995.
4. *Tumor Necrosis Factor*; Beutler, B., Ed.; Raven: New York, 1992.
5. Black, R. A.; Bird, T. A.; Mohler, K. M. *Annu. Rep. Med. Chem.* **1997**, *30*, 241.
6. (a) Moreland, L. W. *Exp. Opin. Invest. Drugs* **1999**, *8*, 1443; (b) Mikuls, T. R.; Moreland, L. W. *Exp. Opin. Pharmacother.* **2001**, *2*, 75; (c) Blam, M. E.; Stein, R. B.; Lichtenstein, G. R. *Am. J. Gastroenterol.* **2001**, *96*, 1977; (d) Assche, V. G.; Rutgeerts, P. *Exp. Opin. Invest. Drugs* **2000**, *9*, 103.
7. Palladino, M. A.; Bahjat, F. R.; Emmanuel, A.; Theodorakis, E. A.; Moldawer, L. L. *Nature Rev.: Drug Discov.* **2003**, *2*, 736.
8. Thalimide and analogs: (a) Sampaio, E. P.; Sarno, E. N.; Galilly, R.; Cohn, Z. A.; Kaplan, G. J. *Exp. Med.* **1991**, *173*, 699; (b) Niwayama, S.; Turk, B.; Liu, J. *J. Med. Chem.* **1996**, *39*, 3044; (c) Miyachi, H.; Azuma, A.; Hiioki, E.; Iwasaki, S.; Kobayashi, Y.; Hashimoto, Y. *Biochem. Biophys. Res. Commun.* **1996**, *226*, 439; (d) PDE-IV inhibitors and MMP inhibitors: Nelson, F. C.; Zask, A. *Exp. Opin. Invest. Drugs* **1999**, *8*, 383; (e) Lowe, C. *Exp.*

Opin. Ther. Pat. **1998**, *8*, 1309; (f) Adenosine agonists: Sajjadi, F. G.; Takabayashi, K.; Foster, A. C.; Domingo, R. C.; Firestein, G. S. *J. Immunol.* **1996**, *156*, 3435; (g) Parmely, M. J.; Zhou, W. W.; Edwards, C. K.; Borcherding, D. R.; Silverstein, R.; Morrison, D. C. *J. Immunol.* **1993**, *151*, 389.

9. Cheng, J.-F.; Ishikawa, A.; Ono, Y.; Arrhenius, T.; Nadzan, A. *Bioorg. Med. Chem. Lett.* **2003**, *13*(21), 3647–3650.
10. Usgaonkar, R. N.; Thakor, V. M.; Jadhav, G. V.; Shab, R. C. *J. Indian Chem. Soc.* **1953**, *30*, 743.
11. The in vivo TNF-α inhibition assay was conducted with male SD rats ($n = 3$). LPS (*E. coli* B055/B5, 0.1 mg/kg) challenge was performed 2 h before the subcutaneous (sc) administration of test compounds, which was generally dissolved in a mixture solvent of 20% EtOH and 80% PEG400. The pyridine-containing compounds, for example, **22** and **27**, were dissolved in saline as hydrochloric acid salt formed in situ. The rats were sacrificed and bled 90 min later and the serum TNF-α activity was measured using ELISA kits. Percent inhibition of TNF-α production at 100 mg/kg was determined by comparison of yield with a control to which no test compound was added. See also: Wadsworth, S. A.; Cavender, D. E.; Beers, S. A.; Lalan, P.; Schafer, P. H.; Malloy, E. A.; Wu, W.; Fahmy, B.; Olini, G. C.; Davis, J. E.; Pellegrino-Gensey, J. L.; Wachter, M. P.; Siekierka, J. J. *J. Pharmacol. Exp. Ther.* **1999**, *291*, 680.
12. (a) Corsini, E.; Lucci, L.; Binaglia, M.; Viviani, B.; Bevilacqua, C.; Monastra, G.; Marinovich, M.; Galli, C. L. *Eur. J. Pharmacol.* **2001**, *418*, 231–237; (b) Squadrito, F.; Altavilla, D.; Zingarelli, B.; Ioculano, M.; Calapai, G.; Campo, G. M.; Miceli, A.; Prosdocimi, M.; Caputi, A. P. *Life Sci.* **1993**, *53*, 341–355; (c) Heguy, A.; Cai, P.; Meyn, P.; Houck, D.; Russo, S.; Michitsch, R.; Pearce, C.; Katz, B.; Bringmann, G.; Feineis, D.; Taylor, D. L.; Tyms, A. S. *Antiviral Chem. Chemother.* **1998**, *9*, 149–155.