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Novel Chromene Derivatives as TNF-α Inhibitors

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Abstract—A novel series of chromene-based TNF-α inhibitors is described. These chromene derivatives inhibit bacterial lipopolysaccharide (LPS) stimulated production of TNF-α from human peripheral blood mononuclear cells (PBMC). Additionally, these compounds inhibit NF-kB mediated transcription activation.

Tumor Necrosis Factor α, or TNF-α, is a pro-inflammatory 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/theroine kinase cascades which lead to the activation of members of the MAP kinase superfamily. It also causes activation of the transcription factors NF-kB and Jun Kinase.[2] NF-kB in turn regulates the production of many proinflammatory cytokines including TNF-α and related proteins that are elevated in immunoinflammatory diseases.[3] TNF-α level and NF-kB 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 NF-kB 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]

Macromolecular TNF-α inhibitors, such as soluble TNF-α receptor[6] Enbrel® and the TNF-α specific monoclonal antibody[7] Remicade® have been shown to be useful for the treatment of inflammatory and autoimmune diseases. They were approved for reducing the sign and symptom of moderate to severe rheumatoid arthritis, psoriatic arthritis, and Crohn's disease. The efficacy of a number of small molecules[8] with anti-inflammatory activity has been linked to their ability to lower TNF-α level. Herein, we report a novel series of chromene-based TNF-α inhibitors.

As part of our effort in searching for small molecule TNF-α inhibitors, we have identified a novel series of chromene derivatives as potential HTS leads. The general synthesis of these chromene derivatives is shown in Scheme 1. Knoevenagel condensation of an appropriately substituted 2-hydroxyl acetophenone **1**, which was obtained either from commercially available sources or prepared according to the literature procedure,[9] with aryladehyde **2** in the presence of piperidine and acetic acid provides benzopyrone products **3** in fair to good yields.[10] When $R_1 = H$, the condensation is carried out using lithium hexamethyldisilylamide as a base. Reduction of the benzopyrone **3** with $NaBH_4$ followed by acid-promoted dehydration of the resultant alcohol intermediate give the desired chromene derivatives **4**. It was found that the substitution pattern in the chromene benzene ring has an important influence on the ability of the molecules to block TNF-α production. A methoxyl group at the C-7 position and a 3,4,5-trimethoxyphenyl group at the C-2 position are the preferred substituents (data not shown).

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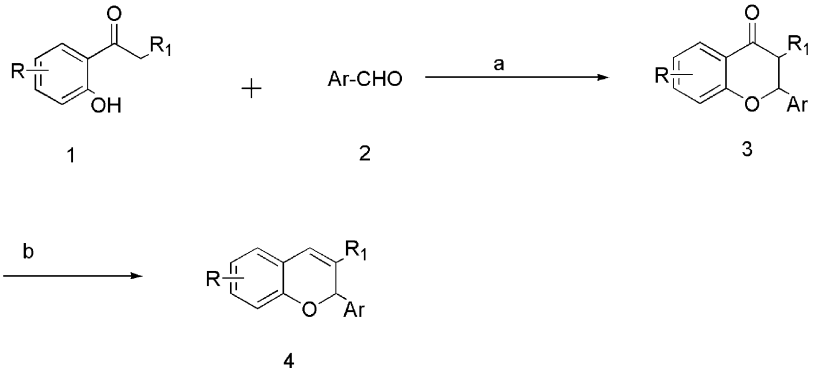
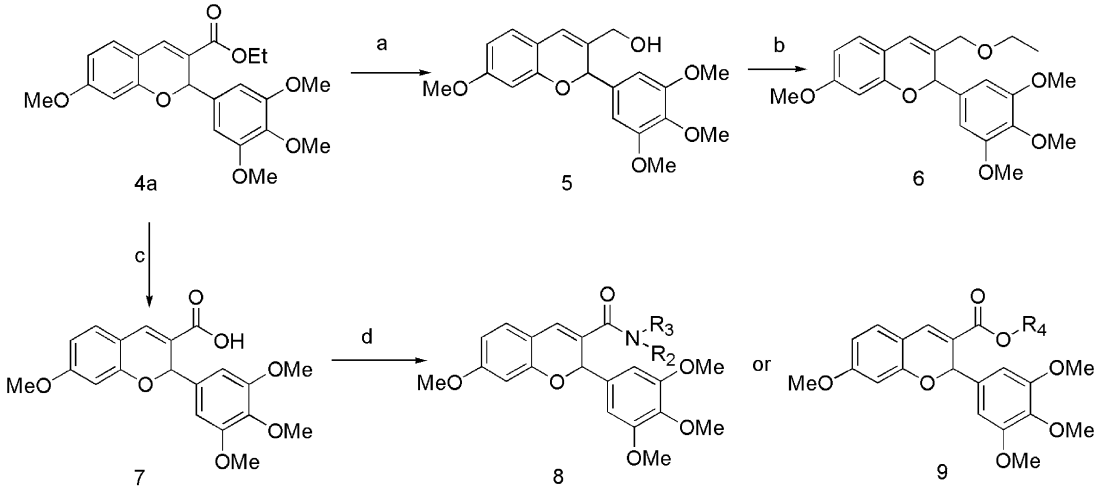
The synthesized compounds were evaluated in LPS-challenged human peripheral blood mononuclear cells (PBMC) for their ability to inhibit TNF-α production using an ELISA assay. The cells were cultured in RPMI1640 medium supplemented with 5% heat-inactivated fetal calf serum and antibiotics. PBMC (5×10^5 cells/mL) in 0.2 mL aliquot are pretreated with drugs 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 are stimulated with 1 mg/mL lipopolysaccharide (LPS) from *Salmonella minnesota* R595 at a final concentration of 100 ng/mL. After overnight culture, the supernatants are harvested and assayed immediately for TNF-α level. 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 NF-kB activation.[11] However, a clear correlation between NF-kB inhibition and TNF-α inhibition was not observed.

Starting from compound **4a**, a moderate TNF-α inhibitor with an IC_{50} about 7 μM, a series of analogues are prepared through the carboxylic acid intermediate **7** or by direct reduction of the ester functionality (Scheme 2). Reduction of the ester **4a** with $LiAlH_4$ affords the corresponding alcohol derivative **5**. Subsequent treatment with EtBr in the present of NaH provides the ethyl ether **6**. Both compounds showed improved inhibitory activity relative to the parent compounds (Table 1). Amide or ester formation of the carboxylic acid **7** are accomplished using the method of Yamaguchi.[12] As can be seen from Table 1, large groups in the ester or amides portion are not tolerated. Primary amide (**8a**) and methyl ester (**9a**) are the two best compounds in this series. Interestingly, the carboxylic acid compound **7** itself is not active in the TNF-α inhibition assay.

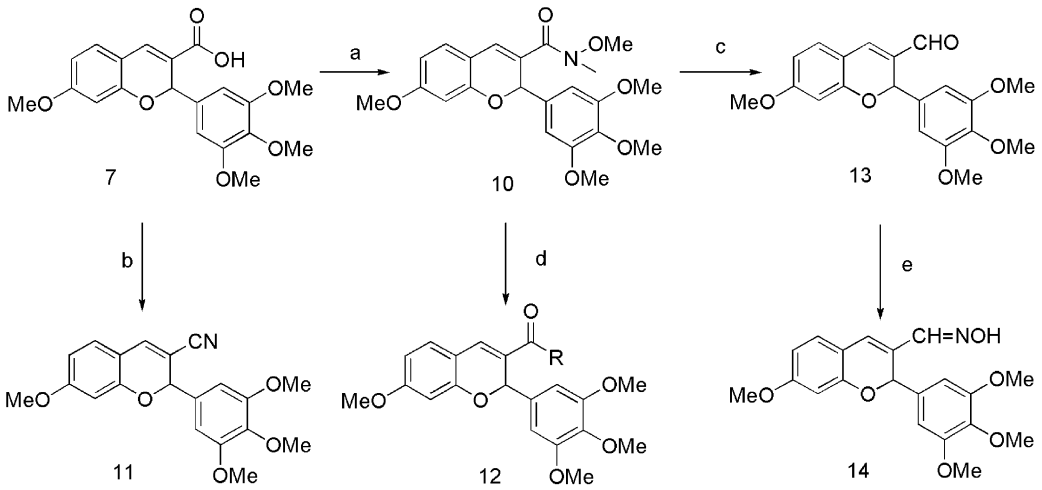
Based on the results obtained from the amide and ester series, the carboxyl acid was converted into other small groups as shown in Scheme 3. Weinreb amide **10** is prepared from the carboxylic acid **7**. Reduction of the Weinreb amide with DIBAL affords the corresponding 3-aldehyde derivative **13**, which is subsequently converted into its oxime derivative. Similarly, reaction of Weinreb amide **10** with organolithium reagents or Gringard reagent furnish the corresponding ketone derivatives **12**. On the other hand, reaction of carboxylic acid compound **7** with $ClSO_2NCO$ affords the corresponding 3-cyano compound **11**. The latter is about ten



Scheme 1. General synthesis of chromenes. Reagents and conditions: (a) piperidine/HOAc or lithium hexamethyldisilylamide, THF; (b) (i) $NaBH_4$/MeOH, rt; (ii) HCl.

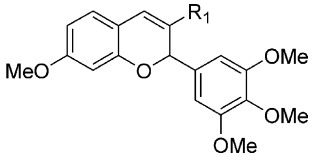


Scheme 2. Synthesis of chromene amides and esters. Reagents and conditions: (a) $LiAlH_4$, Ether, rt; (b) EtBr, NaH, THF, rt; (c) NaOH aq rt; (d) 2,4,6-tricholorobenzoyl chloride, R_2R_3NH or R_4OH.



Scheme 3. Synthesis of 3-substituted chromene derivatives. Reagents and conditions: (a) $(COCl)_2$, DMF, CH_2Cl_2; (b) $ClSO_2NCO$, toluene, reflux; (c) DIBAL, toluene, −78 °C–rt; (d) RLi or RMgX, THF, rt; (e) NH_2OHHCl, MeOH, Et_3N, rt.

Table 1. Representative chromene derivatives as TNF-α inhibitors



Compd	R	IC_{50} (μM) (NF-kB)	IC_{50} (μM) (TNF-α)
4a	–COOEt	25	7
10	–CON(Me)OMe	11.7	31.1
12a	–COMe	14.1	0.1
12b	–COPr-*n*	11.7	4.7
11	–CN	3.4	0.6
13	–CHO	13.5	0.6
14	–CH=NOH	14.1	4.1
5	$–CH_2OH$	> 50	2.4
6	$–CH_2OEt$	21.3	1.1
4b	–H	17.7	11.6
9a	–COOMe	13.8	2
9b	–COOPr-*i*	11.8	12
9c	–COOBu-*t*	14.1	13
9d	$–COOCH_2CH_2Ph$	16.2	> 50
8a	$–CONH_2$	> 50	0.6
8b	–CONHMe	> 50	8.1
8c	–CONHEt	> 50	17.9
8d	–CONHPr-*i*	> 50	41.6
8e	–CONHBu-*n*	25.5	31.3
8f	–CONHBu-*t*	28.4	⩾50
8g	–CONHPh	17.5	> 50
8h	–CO–N–Piperidinyl	> 50	48
8i	$–CONMe_2$	> 50	> 50
7	–COOH	> 50	> 50

times more active than the ester **4a** or **9**. Compound **12a** (R = Me) is identified as the most potent compound in the chromene series.

In conclusion, we have identified a novel series of chromene derivatives, which are potent inhibitors of LPS-induced TNF-α production in human blood peripheral monocytes and of activation of NF-kB gene transcription. It is clear that the small substitution at C-3 position is preferred provided that the C-2 and C-7 positions of the chromene core are fixed as shown in the above examples. A hydrogen bond acceptor may also contribute to the enhancement of the activity at this position (e.g., **4b** vs **13**). It will be interesting to see how the chirality at C-2 position affects the potency. Given the roles of TNF-α in inflammatory, infectious, immunological or malignant diseases, these compounds may serve as leads for novel therapeutic agents. Antioxidant such as 10,11-dihydroxyapporphine[13] and caffeic acid ester[14] have long been known to inhibit the activation of transcription factors including NF-kB as well as TNF-α production. These chromene derivatives, with structural features similar to some of the known antioxidants may also be useful for elucidating the TNF-α signaling pathway.

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