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Benzobicyclooctanes as Novel Inhibitors of TNF-α Signaling

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Abstract—A novel series of TNF-α inhibitors based on a benzobicyclooctane scaffold is reported. The compounds display good potency in inhibiting TNF-α induced apoptosis and NFκB activation. Additionally, they are selective for TNF-α as they do not inhibit apoptosis induced by soluble Fas ligand. The compounds described here can act as leads for future medicinal chemistry efforts and may also be useful tools for elucidating the TNF-α signaling pathway. © 2002 Elsevier Science Ltd. All rights reserved.

Tumor necrosis factor-alpha (TNF-α) is a pleiotropic cytokine that has been shown to play an important role in the pathogenesis of many chronic inflammatory and autoimmune diseases.[1] Often referred to as a pro-inflammatory cytokine, TNF-α is secreted mainly by macrophages and monocytes in response to numerous inflammatory agents and immunological insults. It exerts its biological effects by interacting with either of two high affinity cell surface receptors of molecular weights 55 kDa (TNFR1 or p55) and 75 kDa (TNFR2 or p75). As a result of TNF-α binding to its receptors, a cascade of signaling events occurs within the cell. The nature and sequence of events are dependent upon cell type and receptor. Two of the more important physiological responses are the upregulation of new genes by activation of the transcription factor NFκB (predominantly by TNFR2) and induction of programmed cell death or apoptosis (predominantly by TNFR1).[2,3] The inhibition of either of these events by preventing the ligand from binding its receptors, or by interfering downstream in the signaling pathway, could lead to a reduction of undesired cell death and a decrease in circulating levels of harmful secondary inflammatory proteins. Application of such inhibitors could be a novel means for the treatment of diseases such as rheumatoid arthritis, sepsis, psoriasis, and inflammatory bowel disease. Additionally, they could act as useful tools for aiding in the biochemical dissection of TNF-α signaling pathways. We report herein the discovery of a novel series of dual TNF-α inhibitors based on a benzobicyclooctane scaffold that blocks both the apoptosis and NFκB cellular responses.

The synthesis of the benzobicyclooctane substrates begins with Diels–Alder product **1** obtained from the reaction of 2,7-dihydroxynaphthalene with maleic anhydride[4,5] (Scheme 1). The anhydride **1** is then treated with trimethylsily ethanol (TMSEOH) and DMAP to afford a 1:1 regioisomeric mixture of *cis* acid-esters **2** and **3**, which could be separated by chromatography. Synthesis of the *trans*-derivatives was accomplished by first converting the carboxylic acid group of **3** into an acyl azide at room temperature with diphenylphosphoryl azide (DPPA) and diisopropylethylamine (DIEA). Under the reaction conditions the *cis* isomer readily epimerizes and a very clean *cis–trans* isomerization is effected over the course of 4 h to afford acyl azide **4**. Conversion of **4** to esters **5** and amides **6** was accomplished by treatment with catalytic DMAP and alcohols and amines, respectively. Refluxing a solution of **4** in dioxane generated the isocyanate in situ, which could be trapped with alcohols and amines to afford carbamates and ureas **7** and **8**, respectively.

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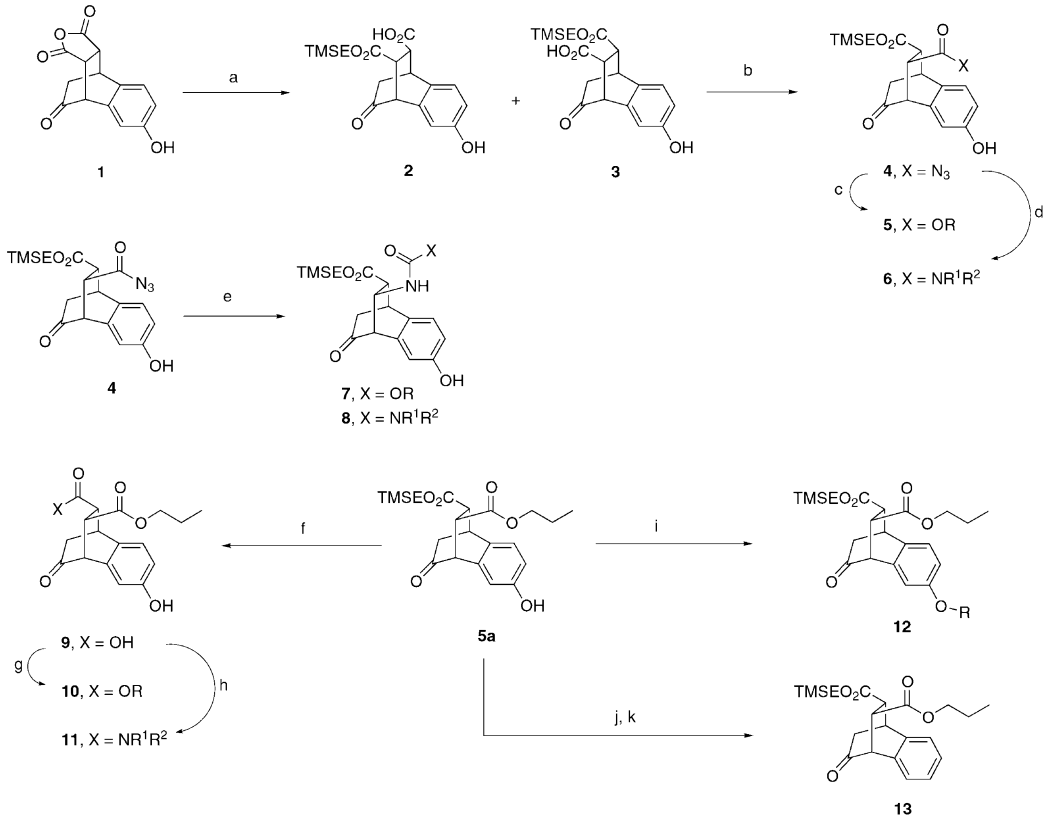


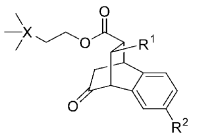
Scheme 1. Reagents and conditions: (a) TMSEOH, DMAP, CH_3CN, reflux, 29% **2**, 21% **3**; (b) DPPA, DIPEA, THF, rt, 36%; (c) ROH, DIPEA, DMAP, THF, rt, 30–62%; (d) HNR^1R^2, DIPEA, DMAP, THF, rt, 23–60%; (e) (i) dioxane, 100 °C; (ii) ROH, DMAP or HNR^1R^2, 11–64%; (f) 95% TFA/H_2O; (g) ROH, TSTU, NMM, DMF, rt, 38–87% from **5a**; (h) HATU, NMM, HNR^1R^2, DMF, rt, 19–55% from **5a**; (i) RX (X = Cl, Br, I), Cs_2CO_3, DMF, 8–82% or ROH, PPh_3, DEAD, THF, 30–54%; (j) $(CF_3SO_2)_2NPh$, DIEA, CH_2Cl_2; (k) $Pd(OAc)_2$, Et_3N, HCO_2H, DPPF, DMF, 59% from **5a**.

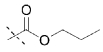
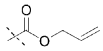
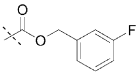
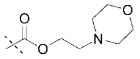
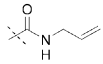
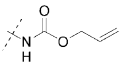
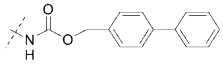
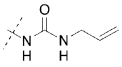
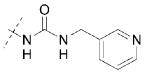
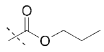
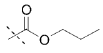
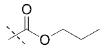
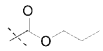
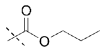
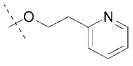
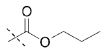
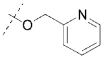
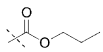
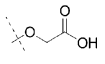
For the synthesis of the 'backside' carboxyl analogues, diester **5a** was treated with aqueous TFA to liberate carboxylic acid **9**. Standard coupling protocols using an amine and HATU[6] then afforded amides **11**. We found that esters **10** could not be formed with HATU but were readily prepared using TSTU[7] and an alcohol. The aryl ether analogues derived from diester **5a** were prepared either by alkylation with alkyl halides and Cs_2CO_3 in DMF or via reaction with an alcohol under Mitsunobu etherification conditions.[8,9] Removal of the aryl hydroxy group to afford **13** was accomplished by reduction of the corresponding aryl triflate using triethylammonium formate in the presence of $Pd(OAc)_2$ and DPPF.[10]

Initial screening efforts were focused on identifying compounds that could inhibit TNF-α induced apoptosis and utilized a version of a reported system that uses the A549 human lung carcinoma cell line.[11,12] Treatment of these cells with TNF-α in the presence of the de novo protein synthesis inhibitor actinomycin D for 18 h causes the cells to undergo apoptosis. A screening campaign revealed that the *trans*-carbamate **7a** was efficacious at inhibiting apoptosis with an $IC_{50} = 14\ \mu M$. This paved the way for initiating a lead optimization effort around the benzobicyclooctane scaffold, initially looking at inhibition of TNF-α induced apoptosis. A secondary assay using a gene reporter system subsequently allowed us to determine whether these compounds could inhibit NFκB activation following stimulation by TNF-α. For this secondary assay a stably transfected cell line derived from A549 cells was developed which uses an E-selectin promoter containing three NFκB recognition sites to drive luciferase enzyme production.[13]

Initial exploration focused on derivatization of the carboxylate group of acid **3**. The relative stereochemistry of the substituents on the 9,10-ethano bridge of the benzobicyclooctane unit was shown not to have a major impact on potency (data not shown). We therefore opted to pursue the *trans* isomers due to ease of synthesis, as the *cis* compounds are highly predisposed to epimerization and are difficult to isolate in pure form. A four-pronged parallel approach was taken to explore SAR around the 'frontside' carboxylate. In addition to examining a diverse set of pendant groups off the bicyclic scaffold, we were also interested in determining the optimum linker that connects the diversity element to the scaffold. A series of ureas, carbamates, esters, and amides was prepared (Table 1). The esters were the most potent of the series, the amides and ureas the least. A wide range of substituents are tolerated in the ester series for the inhibition of apoptosis, however for NFκB inhibition there is a slight preference for small aliphatic groups such as *n*-propyl and allyl. As a result of optimizing the frontside carboxyl we were able to identify the propyl ester **5a** as the best candidate for moving

Table 1. Representative inhibition of TNF-α induced apoptosis and NFκB activation by benzobicyclooctane derivatives



Compd	R^1	R^2	X	Apoptosis[a]	NFκB[a]
5a		OH	Si	3 μM	15 μM
5b		OH	Si	5 μM	21 μM
5c		OH	Si	12 μM	13%
5d		OH	Si	43%	18%
6a		OH	Si	18%	19%
7a		OH	Si	14 μM	34%
7b		OH	Si	0%	27%
8a		OH	Si	23%	28%
8b		OH	Si	11%	30%
10a		OH	C	NA	41 μM
10b		OH	$SiCH_2$	NA	40 μM
13		H	Si	38%	45 μM
12a			Si	10 μM	43%
12b			Si	4 μM	27 μM
12c			Si	47%	31%
12d			Si	13%	3%

[a]Values are listed as either percent inhibition at 20 μM or IC_{50}, and represent the average value of at least two experiments.
NA = Not Assayed

forward. A similar SAR strategy was used to explore diversity around the 'backside' carboxyl moiety, focusing on amide and ester derivatives. Interestingly, nearly all efforts to replace the trimethylsilylethyl ester group resulted in compounds with decreased potencies, including the all carbon analogue **10a** and the 3-trimethylsilylpropyl ester **10b**.

Options for exploring the SAR around the phenolic hydroxy group included preparing the des-hydroxy compound in addition to a variety of aryl ethers. The des-hydroxy compound **13** had significantly lower activity in both assays. In addition, most of the other aryl ether derivatives prepared demonstrated reduced potency. However, the 2-pyridinylethyl derivative **12b** maintained activity in the apoptosis assay and exhibited only a slight decrease in potency against NFκB activation as compared to **5a**. In contrast, the 2-pyridinylmethyl analogue **12c** was substantially less active than **5b** in both assays. For the aryl ether series, derivative **12b** seems to be a potentially suitable candidate for replacement of the phenolic alcohol.

Selectivity of the benzobicyclooctanes for TNF-α induced apoptosis was examined by screening a subset of compounds in a similar apoptosis assay employing A549 cells and 50 ng/mL soluble Fas ligand (sFasL).[14,15] The Fas receptor is a member of the TNF superfamily of cell surface receptors. When activated by the Fas ligand, the receptor initiates an apoptotic response via a signaling cascade which shares much of the same downstream cellular machinery as that of the TNFR1 receptor.[2,16] Thus, it was interesting to see that compounds **5a**, **5b**, and **7a** were completely ineffective at inhibiting sFasL induced apoptosis at 20 μM. This indicates that the benzobicyclooctane derivatives are interfering with the TNF-α induced apoptotic signaling mechanism prior to the point at which the sFasL and TNF-α pathways merge. Alternatively, the compounds could be disrupting a pathway not shared between the Fas and TNF-α receptors. To verify the inhibition of NFκB activation, esters **5a** and **5b** were examined for their ability to block IL-8 production in A549 cells using an ELISA. As expected, both compounds inhibited TNF-α induced IL-8 production with roughly the same potency as seen in the luciferase reporter assay, 14 and 21 μM, respectively.

The assay data indicate that there is a good correlation in the SAR of the substituted benzobicyclooctanes between the apoptosis and NFκB assays. It is tempting to suggest that this infers that the compounds are interacting with a single intracellular target involved in both pathways, however there is no direct evidence of this. If, in fact, the compounds are binding to a single intracellular target, then their greater potency against apoptosis induction relative to NFκB activation may reflect the relative importance of the binding target for each of the two pathways (i.e., the induction of apoptosis by TNF-α may be more reliant on the target protein than is the activation of NFκB).[17] The sFasL data indicate that the compounds are not interfering with caspase activity, a crucial part of the apoptotic response. To verify the role of caspases in the A549 apoptotic process, the assays were carried out in the presence of the known caspase inhibitors *Z*-Asp-CH_2-DCB and YVAD-CMK.[18] At 400 μM, YVAD-CMK, which inhibits caspase-1 and caspase-4, inhibited TNF-α and sFasL apoptosis by only 25%. *Z*-Asp-CH_2-DCB, which is selective for caspase-1 like proteases, showed complete inhibition at the same concentration, and a dose–response curve afforded IC_{50}'s of 107 and 63 μM for the TNF-α and sFasL induced apoptosis, respectively. Thus, the TNF-α and sFasL induced pathways respond identically to two different caspase inhibitors indicating the involvement of very similar, if not identical, caspase protease cascades for inducing apoptosis.

In conclusion, we have identified a novel series of TNF-α inhibitors that block apoptosis and de novo gene transcription via NFκB. The propyl ester **5a** is a low micromolar inhibitor of both of these events, and was shown to be highly selective for TNF-α over sFasL. In addition to acting as leads for further medicinal chemistry efforts, the compounds described here hold promise as tools for aiding in the elucidation of TNF-α signaling pathways, especially those events which occur immediately downstream of ligand–receptor interaction. In addition, simple modulation of these compounds could lead to substrates useful for the identification of their intracellular target(s).[19]

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and 100 μL of 0.5% crystal violet in 20% methanol was added. After 10 min the plates were rinsed with water to remove excess stain, air dried, and absorbance measured at 590 nm.
12. None of the compounds described here displayed activity in a TNF-α ligand binding assay available from MDS Pharma Services, Bothell, WA.
13. For the assay, 5×10^4 stably transfected cells were incubated in 96-well round bottom plates overnight in 100 μL of 10% FBS/RPMI medium at 37 °C in a 5% CO_2 atmosphere. The following morning the medium was removed and 90 μL of a 1% DMSO solution of compound solution was added and the plates incubated for 1 h. TNF-α (10 μL) was added to achieve a final assay concentration equal to its EC_{50} (normally 6 ng/mL) to each well and the plate incubated for 5 h. Luciferase buffer solution (100 μL) was added, and after 10 min the wells were assayed for luminescence.
14. sFasL was obtained from Alexis Biochemicals, San Diego, CA, USA. The sFasL assay protocol was similar to the TNF-α apoptosis assay except that actinomycin-D was omitted and the incubation time after addition of the agonist was 4 h.
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19. For example, see Rosania, G. R.; Chang, Y. T.; Perez, O.; Sutherlin, D.; Dong, H.; Lockhart, D. J.; Schultz, P. G. *Nature Biotech*. **2000**, *18*, 304.