Bioorganic & Medicinal Chemistry Letters 21 (2011) 3784–3787

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Novel diarylheptanoids as inhibitors of TNF-α production

Sameer Dhuru [a], Dilip Bhedi [a], Dnyaneshwar Gophane [a], Kiran Hirbhagat [a], Vijaya Nadar [a], Dattatray More [a], Sapna Parikh [b], Roda Dalal [b], Lyle C. Fonseca [b], Firuza Kharas [b], Prashant Y. Vadnal [b], Ram A. Vishwakarma [a], H. Sivaramakrishnan [a,*]

[a] Department of Medicinal Chemistry, Piramal Life Sciences Limited, 1 Nirlon Complex, Off Western Express Highway, Goregaon (E), Mumbai 400 063, India
[b] Department of Pharmacology, Piramal Life Sciences Limited, 1 Nirlon Complex, Off Western Express Highway, Goregaon (E), Mumbai 400 063, India

ARTICLE INFO

Article history:
Received 4 January 2011
Revised 22 March 2011
Accepted 12 April 2011
Available online 22 April 2011

Keywords:
Diarylheptanoids
Inflammation
TNF-α

ABSTRACT

Synthesis and anti-inflammatory activity of novel diarylheptanoids [5-hydroxy-1-phenyl-7-(pyridin-3-yl)-heptan-3-ones and 1-phenyl-7-(pyridin-3-yl)hept-4-en-3-ones] as inhibitors of tumor necrosis factor-α (TNF-α) production is described in the present article. The key reactions involve the formation of a β-hydroxyketone by the reaction of substituted 4-phenyl butan-2-ones with pyridine-3-carboxaldehyde in presence of LDA and the subsequent dehydration of the same to obtain the α,β-unsaturated ketones. Compounds **4i**, **5b**, **5d**, and **5g** significantly inhibit lipopolysaccharide (LPS)-induced TNF-α production from human peripheral blood mononuclear cells in a dose-dependent manner. Of note, the in vitro TNF-α inhibition potential of **5b** and **5d** is comparable to that of curcumin (a naturally occurring diarylheptanoid). Most importantly, oral administration of **4i**, **5b**, **5d**, and **5g** (each at 100 mg/kg) but not curcumin (at 100 mg/kg) significantly inhibits LPS-induced TNF-α production in BALB/c mice. Collectively, our findings indicate that these compounds may have potential therapeutic implications for TNF-α-mediated auto-immune/inflammatory disorders.

The key pro-inflammatory cytokine tumor necrosis factor-α (TNF-α) is mainly produced by the activated macrophages and monocytes, which further induces the production of several inflammatory cytokines such as interleukin-1β (IL-1β), interleukin-6 (IL-6) and granulocyte-macrophage colony-stimulating factor (GM-CSF)[1,2] thereby enhancing the inflammation in many auto-immune/inflammatory diseases such as rheumatoid arthritis (RA). In RA patients, TNF-α expression is particularly elevated in RA synovial fluid, serum and synovial fibroblasts, and, importantly, these enhanced levels of TNF-α correlate well with disease pathology and severity.[2] The treatment profile of RA has significantly changed after introduction of antibody based protein therapeutic agents such as the TNF soluble receptor (Enbrel™) and TNF antibody (Remicade™) which have shown excellent efficacy in RA patients.[2] However, these biopharmaceuticals and antibodies are expensive, difficult to administer orally and have major side effects (susceptibility to infections such as tuberculosis) on prolonged clinical use. Therefore, there is a great unmet medical need to discover small molecule agents to deal with higher levels of production of TNF-α. Many low molecular weight compounds have been reported to have inhibitory activity towards the TNF-α production.

Historically, natural products have provided the most consistent source of new scaffolds to design new therapeutic agents, and a number of naturally occurring phenolic compounds have been discovered as potent anti-inflammatory agents.[3] In many cases, the pronounced anti-inflammatory activity of natural products is mediated through the inhibition of the synthesis of nitric oxide, prostaglandins and leukotrienes, and the production and/or signaling of cytokines such as TNF-α, IL-1β, and IL-6. The antioxidants such as (−)-epigallocatechin-3-gallate,[4] resveratrol[5] and the flavonoids (epigenin and kaemferol)[6] have been reported to suppress nitric oxide (NO) production through inhibition of nuclear factor-κB (NF-κB).

The most widely used spice ginger (*Zinziber officinale*) and its major chemical constituents (gingerols, β-carotene, capsaicin, caffeic acid and curcumin) are reported to be robust inhibitors of immune cell activation and cytokine secretion.[7] Various formulations of ginger have also been reported as dual inhibitors of two key enzymes cyclooxygenase (COX) and lipooxygenase (LOX),[8] as well as the inhibitors of leukotriene synthesis.[9] Their anti-inflammatory activity has also been demonstrated in carrageenan-induced rat-paw edema model.[10] Closely related medicinal plants such as the greater-galangal (*Alpinia galanga*) and lesser-galangal (*Alpinia officinarum*) have been used for rheumatic diseases in Asian medicine producing linear diarylheptanoid group of compounds (general structure Ar-C_7-Ar) isolated,[11] responsible for anti-inflammatory activity. Prior studies have shown that the principal constituent (7-(4-hydroxy-3-methoxyphenyl)-1-phenylhept-4-en-3-one) isolated from *Alpinia officinarum* suppressed the

* Corresponding author.
E-mail address: h.sivaramakrishnan@piramal.com (H. Sivaramakrishnan).

0960-894X/$ - see front matter © 2011 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2011.04.040

lipopolysaccharide (LPS)-induced TNF-α production in human peripheral blood mononuclear cells (hPBMC), and attenuated LPS-induced production of NO, iNOS, and COX-2 in mouse RAW264.7 macrophages.[12] In order to investigate the structure–activity relationship of diarylheptanoid scaffold for the anti-inflammatory activity, we initiated the design and synthesis of diarylheptanoid analogues and evaluation of their potential in suppressing LPS-induced TNF-α production.[12–16]

The synthetic route designed for synthesis of β-hydroxyketones and subsequent α,β-unstaturated ketones is depicted in Figure 1.

Using this synthetic strategy, a number of substituted 4-phenyl-butan-2-ones (**3a–n**) were synthesized starting from various substituted benzaldehydes (**1a–n**, Table 1) in two steps. First step involved an aldol condensation of benzaldehydes with acetone in presence of 10% aqueous NaOH to yield 4-phenyl-but-3-en-2-ones (**2a–n**), which further on hydrogenation in presence of 10% Pd/C gave desired 4-phenyl-butan-2-ones (**3a–n**). Substituted 4-phenyl-butan-2-ones were subjected to aldol reaction with 3-(pyridin-3-yl)-propanal (**7**, prepared by the Swern oxidation of 3-(pyridin-3-yl)propan-1-ol **6** with DMSO and oxalyl chloride) in presence of LDA in THF at −78 °C to yield substituted 5-hydroxy-1-phenyl-7-(pyridin-3-yl)heptan-3-ones(**4a–n**, Table 2). These were further dehydrated using *p*-toluenesulfonic acid in toluene under reflux conditions to give the respective 1-phenyl-7-(pyridin-3-yl)hept-4-en-3-one derivatives (**5a–m**). In case of the reaction of 4-chloro substituted 4-phenyl-butan-2-one (**3l**) with 3-(pyridin-3-yl)-propanal (**7**), a side product **8** (Table 3) was also formed along with the desired product (**4l**). The structures of various synthesized compounds were assigned on the basis of different spectral data (experimental details are provided in the Supplementary data).

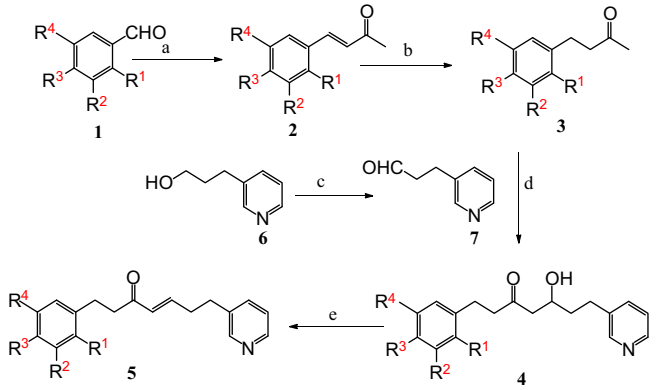


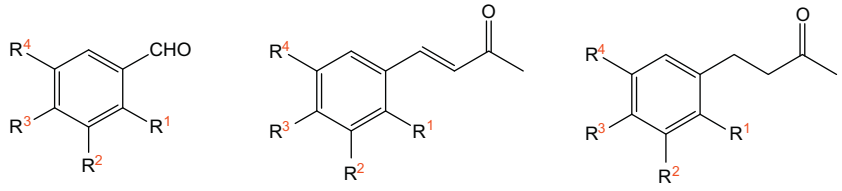
Figure 1. Synthetic scheme. Reagents and conditions: (a) Acetone/10% NaOH, 0 °C, rt; (b) H_2, Pd/C; (c) $(COCl)_2$, DMSO, Et_3N, DCM, −78 °C; (d) *i*-Pr_2NH, *n*-BuLi, THF, −78 °C; (e) *p*TSA, toluene, reflux.

The compounds **4a–n**, **5a–k**, **5m** (Table 2) and **8** (Table 3) were evaluated for their TNF-α inhibitory activity in whole blood cell culture system (WBCCS) assay[14] and the results are as shown in the Table 2. It was observed that compounds **4i, 5b, 5d**, and **5g** showed a dose dependent inhibition of TNF-α production in the WBCCS assay. Accordingly, these compounds were further studied for their ability to inhibit TNF-α production in the human peripheral blood mononuclear cells (hPBMC) assay.[17]

Compounds **5b** and **5d** significantly inhibited the production of TNF-α in a concentration dependent manner (IC_{50} = 2.0 and 2.5 μM, respectively) (Fig. 2). The inhibitory effects on the production of TNF-α were not due to non-specific toxicity since the viability of the cells determined by MTS was not influenced at any of the concentrations of the compound tested. The compound **4i** (IC_{50} = 5.7 μM) also inhibited the production of TNF-α, albeit at higher concentrations. The compound **5g** was the least active of the four with an IC_{50} of 24.1 μM. Of note, the TNF-α inhibition potential of **5b** and **5d** was found to be comparable to that of curcumin (a naturally occurring diarylheptanoid)—which inhibited LPS-induced TNF-α production in hPBMC with an IC_{50} of 2.71 μM (Figure 2). Of note, the IC_{50} values for TNF-α inhibition (for **5b, 5d, 4i** and **5g**) were far lower than the IC_{50} values for cytotoxicity (38, 80, >100 and >100 μM for **5b, 5d, 4i and 5g**, respectively).

To assess whether the diarylheptanoid-mediated marked inhibition of TNF-α production observed in vitro could be translated into a meaningful pharmacological effect in vivo, we used an acute model of inflammation. In these studies, oral administration of

Table 1
List of various substituted benzaldehydes (**1**), 4-phenyl-but-3-en-2-ones (**2**) and 4-phenyl-butan-2-ones (**3**)

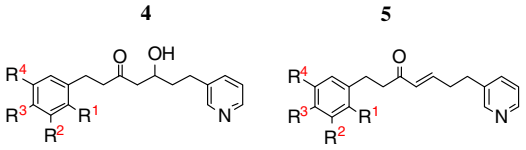


1 **2** **3**

1	2	3	R^1	R^2	R^3	R^4
1a	**2a**	**3a**	OMe	OMe	H	H
1b	**2b**	**3b**	OMe	H	OMe	H
1c	**2c**	**3c**	OMe	H	H	OMe
1d	**2d**	**3d**	H	OMe	OMe	H
1e	**2e**	**3e**	H	OMe	OMe	OMe
1f	**2f**	**3f**	H	Me	H	H
1g	**2g**	**3g**	H	OMe	H	H
1h	**2h**	**3h**	H	H	CN	H
1i	**2i**	**3i**	H	OPMB[a]	OMe	H
1j	**2j**	**3j**	H	H	H	H
1k	**2k**	**3k**	H	H	F	H
3l	**2l**	**3l**	H	H	Cl	H
1m	**2m**	**3m**	H	H	NMe_2	H
1n	**2n**	**3n**	H	H	OCH_2O	H

[a]PMB = *para*-methoxybenzyl.

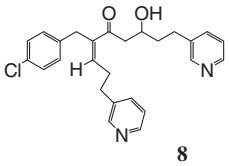
Table 2
Inhibition of TNF-α in the whole blood cell culture system assay by **4a–n**, **5a–k** and **5m**



Compd	R^1	R^2	R^3	R^4	% Inhibition of TNF-α at 10 μM
4a	OMe	OMe	H	H	10
4b	OMe	H	OMe	H	22
4c	OMe	H	H	OMe	0
4d	H	OMe	OMe	H	18
4e	H	OMe	OMe	OMe	12
4f	H	Me	H	H	11
4g	H	OMe	OPMB[a]	H	2
4h	H	H	CN	H	35
4i	H	OPMB	OMe	H	44
4j	H	H	H	H	3
4k	H	H	F	H	34
4l	H	H	Cl	H	16
4m	H	H	NMe_2	H	0
4n	H	H	O–CH_2–O		41
5a	OMe	OMe	H	H	33
5b	OMe	H	OMe	H	56
5c	OMe	H	H	OMe	0
5d	H	OMe	OMe	H	56
5e	H	OMe	OMe	OMe	23
5f	H	Me	H	H	25
5g	H	OMe	OPMB	H	40
5h	H	H	CN	H	9
5i	H	OPMB	OMe	H	19
5j	H	H	H	H	0
5k	H	H	F	H	35
5m	H	H	NMe_2	H	32

Human whole blood cells were treated with various compounds for 30 min. Following 30 min. incubation, cells were stimulated with LPS. After 5 h, supernatants were collected and TNF-α levels determined using ELISA.
[a] PMB = *para*-methoxybenzyl.

Table 3
Inhibition of TNF-α in whole blood cell culture system assay by (**8**)



8

% Inhibition of TNF-α at 10 μM
32

compounds **4i, 5b**, **5d**, and **5g** significantly inhibited LPS-induced production of TNF-α (from BALB/c mice) corroborating the in vitro findings (71%, 79%, 50% and 75% inhibition of TNF-α production with**4i, 5b, 5d** and **5g**, respectively; Figure 3). Interestingly, curcumin—dosed at similar levels—had no effect on in vivo LPS-induced TNF-α production (Fig. 3).

Our results clearly demonstrate that the synthesized novel diarylheptanoids significantly inhibit in vitro and in vivo production of TNF-α. This functional property of diarylheptanoids could translate in them showing efficacy in various animal models of arthritis (e.g., collagen induced arthritis model). Future studies are warranted to investigate this. In this context, it is important to note that earlier studies have demonstrated that TNF-α-deficient mice have reduced susceptibility for developing experimental arthritis,[18] and neutralization of TNF-α decreases the incidence and

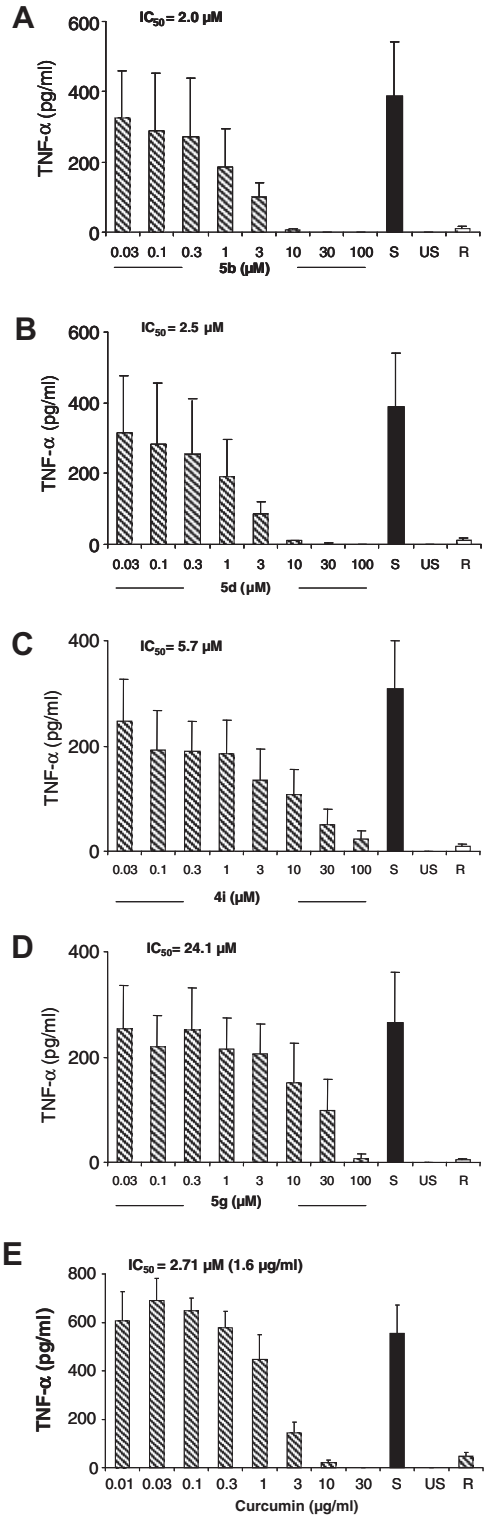


Figure 2. Effect of **5b**, **5d**, **4i**, **5g** and curcumin on LPS-induced production of TNF-α from hPBMC. S = LPS-stimulated cells treated with vehicle (DMSO), US = Unstimulated control, R = Rolipram (standard)-treated LPS-stimulated cells. Pre-treatment: 30 min. LPS-stimulation: 5 h. Values are average ± S.E.M. of three experiments.

severity of inflammatory collagen-induced arthritis.[19] Given that diaryl heptanoids inhibit in vitro and in vivo LPS-induced TNF-α production, combined with the fact that gram negative bacteria-mediated signal transduction pathway plays a crucial role in ulcerative colitis, it would be of interest to assess the effect of these compounds in the experimental models of colitis. In this regards,

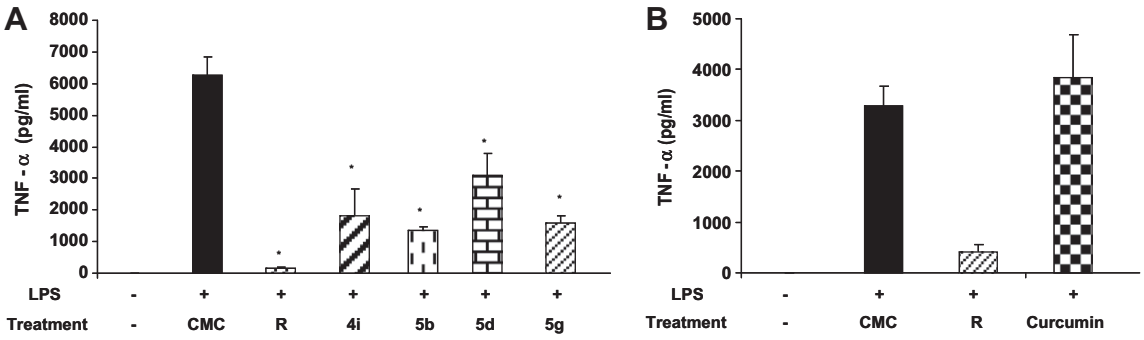


Figure 3. Effect of (A) **4i**, **5b**, **5d**, and **5g** and (B) curcumin in in vivo LPS-induced TNF-α production assay. Compounds **4i**, **5b**, **5d**, **5g** and curcumin (each at 100 mg/kg), rolipram (R) or 0.5% carboxymethylcellulose (CMC) were administered orally to BALB/c mice. After 1 h, mice were treated with LPS. Following 1.5 h. LPS treatment, blood was collected and, subsequently, plasma levels of TNF-α determined using ELISA. All values are average ± S.E.M. of at least three mice. * indicates p <0.05 compared to CMC-fed, LPS-treated group.

it is important to note that prior studies have demonstrated that (i) genetic deletion of TNF-α[20] protected mice against experimental colitis and (ii) blockade of TNF-α production attenuated experimental colitis.[21] Interestingly, a recent study demonstrated a critical role of TNF-α in experimental colitis-associated colon carcinogenesis. Thus, it is reasonable to speculate that these compounds may also have potential implications for inflammation-associated oncologic disorders. Clearly, future studies probing the in vivo efficacy of these compounds in various experimental models of disease are warranted.

In summary, we have designed and synthesized a novel series of diarylheptanoids. Compounds **4i**, **5b**, **5d**, and **5g** significantly inhibit in vitro TNF-α production from human cells. More importantly, oral administration of these compounds significantly inhibits TNF-α production in mice. These compounds may have potential therapeutic implications for TNF-α-mediated auto-immune/inflammatory diseases.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2011.04.040.

References and notes

1. Bemelmans, M. H. A.; Van Tits, L. J. H.; Buurmann, W. A. *Crit. Rev. Immunol.* **1996**, *16*, 1.
2. Feldmann, M.; Maini, S. R. *Immunol. Rev.* **2008**, *223*, 7.
3. Lin, Y. L.; Lin, J. K. *Mol. Pharmacol.* **1997**, *52*, 465.
4. Tsai, S. H.; Lin-Shiau, S. Y.; Lin, J. K. *Br. J. Pharmacol.* **1999**, *126*, 673.
5. Liang, Y. C.; Huang, Y. T.; Tsai, S. H.; Lin-Shiau, S. Y.; Chen, C. F.; Lin, J. K. *Carcinogenesis* **1999**, *20*, 1945.
6. Ageel, A. M.; Mossa, J. S.; Al-Yahya, M. A.; al-Said, M. S.; Tariq, M. *Drugs Exp. Clin. Res.* **1989**, *15*, 369.
7. Mustafa, T.; Srivastava, K. C.; Jensen, K. B. *J. Drug Dev.* **1993**, *6*, 25.
8. Kiuchi, F.; Iwakami, S.; Shibuya, M.; Hanaoka, F.; Sankawa, U. *Chem. Pharm. Bull.* **1992**, *40*, 387.
9. Mascolo, N.; Jain, R.; Jain, S. C.; Capasso, F. *J. Pharmacol. Exp. Ther.* **1989**, *27*, 129.
10. Jana, U.; Chattopadhayay, R. N.; Shaw, B. P. *Indian J. Pharmacol.* **1999**, *31*, 232.
11. Kato, N.; Hamada, Y.; Shioiri, T. *Chem. Pharm. Bull.* **1984**, 3223.
12. Yadav, P. N.; Liu, Z.; Rafi, M. M. *J. Pharmacol. Exp. Ther.* **2003**, *305*, 925.
13. Rafi, M. M.; Liu, L.; Rosen, R. T.; Rosen, S. L. US Patent 0215635 A1, 2005.
14. Wilson, B. M. G.; Severn, A.; Rapson, N. T.; Chana, J.; Hopkins, P. *J. Immunol. Methods* **1991**, *139*, 233.
15. Jin, W.; Cai, X. F.; Na, M.; Lee, J. J.; Bae, K. *Biol. Pharm. Bull.* **2007**, *30*, 810.
16. Lee, C. S.; Jang, E. R.; Kim, Y. J.; Lee, M. S.; Seo, S. J.; Lee, M. W. *Int. Immunopharmacol.* **2010**, *10*, 520.
17. Jansky, L.; Reymanova, P.; Kopecky, J. *Physiol. Res.* **2003**, *52*, 593.
18. Hata, H.; Sakaguchi, N.; Yoshitomi, H.; Iwakura, Y.; Sekikawa, K.; Azuma, Y.; Kanai, C.; Moriizumi, E.; Nomura, T.; Nakamura, T.; Sakaguchi, S. *J. Clin. Invest.* **2004**, *114*, 582.
19. Williams, R. O.; Feldmann, M.; Maini, R. N. *Proc. Natl. Acad. Sci. U.S.A.* **1992**, *89*, 9784.
20. Corazza, N.; Eichenberger, S.; Eugster, H. P.; Mueller, C. *J. Exp. Med.* **1999**, *190*, 1479.
21. Zhang, D. K.; Cheng, L. N.; Huang, X. L.; Shi, W.; Xiang, J. Y.; Gan, H. T. *Int. J. Colorectal Dis.* **2009**, *24*, 5.