Bioorganic & Medicinal Chemistry Letters 21 (2011) 2547–2549

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

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

Synthesis and biological evaluation of 4-styrylcoumarin derivatives as inhibitors of TNF-α and IL-6 with anti-tubercular activity

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ARTICLE INFO

Article history:
Received 29 November 2010
Revised 22 January 2011
Accepted 4 February 2011
Available online 15 February 2011

Keywords:
4-Styrylcoumarin
Anti-inflammatory activity
TNF-α
IL-6
Anti-tubercular

ABSTRACT

A series of 4-styrylcoumarin have been synthesized by Knoevenagel condensation between substituted 4-methylcoumarin-3-carbonitrile and different heterocyclic or aromatic aldehydes. 4-Methylcoumarin-3-carbonitrile has been synthesized by the base catalyzed reaction between substituted 2-hydroxyacetophenone and ethyl cyanoacetate. The structures of the newly synthesized compounds were confirmed by ^{1}H NMR, IR and mass spectral analysis. All the compounds were evaluated for their anti-inflammatory activity (against TNF-α and IL-6) and anti-tubercular activity. Compounds **6a**, **6h** and **6j** exhibited promising activity against IL-6 with 72–87% inhibition and compound **6v** showed potent activity against TNF-α with 73% inhibition at 10 μM concentration. Whereas compounds **6n**, **6o**, 6r and **6u** showed very good anti-tubercular activity against *Mycobacterium tuberculosis* H37Rv strain at <6.25 μM.

Inflammation is the complex process due to which it causes a large number of diseases, among this some commonest are rheumatoid arthritis (RA), inflammatory bowel disease, psoriasis and multiple sclerosis.[1,2] Tumor necrosis factor-alpha (TNF-α) and Interleukin-6 (IL-6) are two major multifunctional proinflammatory mediators of a variety of autoimmune diseases such as pain and joint destruction characteristics of RA.[3] The inhibition of release of cytokines becomes a major focus of current drug development and an important method for evaluating the bioactivity of drugs.[4] It is a key cytokine in the inflammation cascade, causing the production and/or release of other cytokines and agents. Over-expression of TNF-α is responsible for a number of pathological conditions like Crohn's disease, ulcerative colitis,[5] diabetes,[6] multiple sclerosis,[7] atherosclerosis,[8] and stroke.[9] In spite of enormous efforts, no small molecule has yet been approved to specifically inhibit TNF-α activity. TNF-α inhibitor drugs in clinics are proteins (Etanercept, Infliximab, Adalimumab, and Anakinra) that display adverse effects such as aplastic anemia, pancytopenia, vasculitis, demyelination and congestive heart failure.[10]

Among pro-inflammatory cytokines, the IL-6 is a multifunctional cytokine produced by a variety of cells in response to infection, trauma, or immunological challenge. It appears to be the central mediator of anemia of chronic disease in a range of inflammatory diseases, including end-stage renal disease and rheumatoid arthritis.[11] The IL-6 inhibitors can be used in Alzheimer's disease, psychiatric disorders, cancer, diabetes, and depression.[12–14] Till date, designing the IL-6 inhibitory agents has remained a significant hope in the mainstream of anti-inflammatory drug development.

Tuberculosis (TB) is one of the leading causes of death and suffering worldwide among the infectious diseases. The ever-increasing drug resistance, toxicity and side effects of currently used anti-tuberculosis drugs and the absence of their bactericidal activity highlight the need for new, safer and more effective anti-tuberculosis drugs.[15–19] Generation of functional molecular diversity to probe biological activity space requires robust molecular scaffolds that are low in molecular weight and are easily modified to create a variety of chemically diverse, biologically active potential drugs.[20,21]

The coumarins have long been recognized to possess anti-inflammatory, antioxidant, antiallergic, hepatoprotective, antithrombotic, antiviral, and anticarcinogenic activities.[22] A series of 6- or 7-styrylcoumarin were synthesized by Song et al.[23] in order to find compounds of antitumor activities and several compounds showed different inhibitory effects on L-1210, HL-60, HCT-8, KB and Bel-7402 cell lines in vitro. In continuation with this study Su and co-workers have synthesized a series of 4-styryl coumarin and reported in vitro antitumor activity of on KB cell lines.[24] As an attempt to determine basic structural features required as antimicrobial agents Kawase et al.[25] have synthesized a series of polyfunctionalized 3-cyano-4-styrylcoumarin and suggested that five compounds shows moderate activity against *Staphylococcus aureus* NCTC 8530,

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doi:10.1016/j.bmcl.2011.02.016

S. aureus NCTC 8531, *S. aureus* ML 36, *Shigella dysenteriae* 1, *Escherichia coli Raw*, *Vibrio cholerae*945, *V. cholerae*946, *E. coli Raw* and *Shigella Sonnei* at MIC value of 25 μg/mL.

The 5′-chloro-2′-hydroxy acetophenone were synthesized by Fries rearrangement of *o*-acetyl ester derivative of 4-chlorophenol gave only single isomer as *para* position was already occupied with the substitution. This on Claisen condensation with ethyl cyanoacetate gave 6-chloro-3-cyano-4-methylcoumarin. The method, we earlier practiced suggested by Kendall and Axford method[71] was very drastic and time consuming. The current work presented a direct, yield efficient and operationally convenient approach to the synthesis of some styryl derivatives of the substituted 3-cyano-4-methyl coumarins as during the course of cyclization the rate of both the Aldol condensation and intramolecular Claisen condensation proceeds very smoothly and speedily at mild reaction conditions and the reaction outcome as high purity of the product. Thus, the 6-chloro-3-cyano-4-methylcoumarin obtained on Knoevenagel condensation with different substituted aromatic and hetero (aromatic) aldehydes, afforded the corresponding 6-chloro-3-cyano-4-styrylcoumarin derivatives (**6a–6v**) as per Scheme 1. All the newly synthesized compounds were well characterized by IR, NMR and mass spectral and elemental analysis. The physical constant and elemental analysis data of all the synthesized compounds were shown in Table 1.

All the synthesized compounds were evaluated for anti-inflammatory and anti-tubercular activity and the results are summarized in Table 2. All molecules of the series show moderate to good TNF-α and IL-6% inhibition and anti-tubercular activity.

Compound **6v** possessing methoxy group on phenyl ring at *m*-position shows good TNF-α inhibitory activity, while phenyl ring having electron withdrawing group as substitution makes molecules inactive (**6b–6h**). Molecule **6a** with unsubstituted phenyl group acquires good inhibitory activity of IL-6, while substitution weakens the inhibitory activity. Though, molecule **6j** containing hydroxyl group at *p*-position in phenyl ring induces the potency while bulky group makes molecule inactive.

We observed that molecules having bulky group shows good anti-tubercular activity, as **6n** and **6r** having 3,4,5 tri-OCH_3 phenyl and 9-anthracyl group improves the potency, respectively. Moreover, electron withdrawing groups attached at *m*-position of phenyl ring (**6d** and **6h**) demonstrates induced activity.

Pro-inflammatory cytokine production by lipopolysaccharide (LPS) in THP-1 cells was measured according to the method described by Hwang et al., 1933.[26] Briefly, THP-1 cells were cultured in RPMI 1640 culture medium (Gibco BRL, Pasley, UK) containing 100 μ/mL penicillin and 100 mg/mL streptomycin (100× solution), Sigma Chemical Co. St. Louis, MO) containing 10% fetal bovine serum (FBS, JRH). Cells were differentiated with phorbol myristate acetate (PMA, Sigma). Following cell plating, the test compounds or vehicle (0.5% DMSO) was added to each well and the plate was incubated for 30 min at 37 °C. Finally, LPS (*E. coli* 0127: B8, Sigma Chemical Co., St. Louis, MO) was added, at a final concentration of 1 mg/mL. Plates were incubated at 37 °C for 24 h, 5% CO_2. Supernatants were harvested and assayed for TNF-α and IL-6 by ELISA as described by the manufacturer (BD Biosciences). The cells were simultaneously evaluated for cytotoxicity using CCK-8 from Dojindo Laboratories. Percent inhibition of cytokine release compared to the control was calculated.

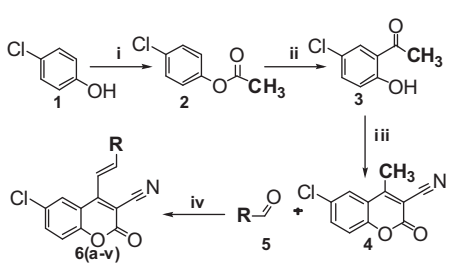
The primary screen is conducted at 6.25 μg/mL (or molar equivalents of highest molecular weight compound in a series of congeners) against *Mycobacterium tuberculosis* H37Rv (ATCC 27294) in BACTET 12B medium using Microplate Almar Blue Assay (MABA).[27] Compounds exhibiting fluorescence are tested in the BACTET 460-radiometric system.[27] Compounds effecting >99% inhibition in the primary screen (MIC <6.25 μg/mL) are generally evaluated further.

Table 1
Physical constants of 6-chloro-2-oxo-4-[-2-subst. ethenyl]-2*H*-chromene-3-carbonitriles

Entry	Substitution	Mp
6a	Phenyl	193–195
6b	4-F Phenyl	246–248
6c	2-Cl Phenyl	257–259
6d	3-Cl Phenyl	239–241
6e	4-Cl Phenyl	275–277
6f	3-Br Phenyl	235–237
6g	2-NO_2 Phenyl	139–141
6h	3-NO_2 Phenyl	236–238
6i	3-OH Phenyl	229–231
6j	4-OH Phenyl	160–162
6k	2-OCH_3 Phenyl	157–159
6l	4-OCH_3 Phenyl	>300
6m	3,4-DiOCH_3 phenyl	203–205
6n	3,4,5-TriOCh_3 phenyl	261–263
6o	3-OC_6H_5 Phenyl	221–223
6p	4-SCH_3 Phenyl	205–207
6q	2-Ethenyl phenyl	257–259
6r	9-Anthracyl	115–117
6s	2-Furyl	173–175
6t	3-Indolyl	158–160
6u	4-*N*,*N*-DiCh_3 amine-phenyl	156–158
6v	3- OCH_3Phenyl	202–204

Table 2
Anti-inflammatory (TNF-α and IL-6 Inhibition) and anti-tubercular activity of 6-chloro-2-oxo-4-[-2-subst. ethenyl]-2*H*-chromene-3-carbonitriles

Entry	Anti-inflammatory activity (%Inhibition at 10 μM concn)		Anti-tubercular activity (%Inhibition at <6.25 μM Concn) *Mycobacterium tuberculosis* H37Rv strain
	TNF-α	IL-6	
6a	32	87	55
6b	0	61	ND
6c	0	19	ND
6d	0	21	69
6e	0	4	ND
6f	0	2	23
6g	0	14	11
6h	0	72	69
6i	23	39	ND
6j	0	73	ND
6k	19	17	24
6l	ND	ND	33
6m	0	40	63
6n	38	8	87
6o	0	11	72
6p	0	15	ND
6q	52	0	ND
6r	ND	ND	81
6s	9	0	63
6t	31	0	ND
6u	33	0	79
6v	73	0	31



Scheme 1. Reagents and conditions: (i) acetic anhydride, cat. sulfuric acid, reflux, 2 h; (ii) anhyd $AlCl_3$, fusion 160° C, 6 h; (iii) ethyl cyanoacetate, sodium ethoxide, ethanol, reflux, 2 h; (iv) cat. piperidine, dichloromethane or chloroform, 1.5–3 h.

In summary, we have synthesized a new series of 4-styrylcoumarin derivatives and evaluated for their anti-inflammatory and anti-tubercular activity. Among them, molecules **6a**, **6h** and **6j** exhibited promising activity against IL-6 with 72–87% inhibition and molecule **6v** showed significant TNF-α inhibitory activity with 73% inhibition at 10 μM concentration. In addition, molecules **6n**, **6o**, 6r and **6u** showed 87%, 72%, 81% and 79% inhibition respectively against *M. tuberculosis* H37Rv strain at <6.25 μM. However, this structure activity relationship (SAR) might provide these compounds a potential suitable tool for designing new molecules having specific selectivity for TNF-α and IL-6 inhibition and anti-tubercular activity. Furthermore, the 4-styrylcoumarin moiety can be optimized as a lead molecule for anti-inflammatory or anti-tubercular agents.

Acknowledgments

The authors are thankful to Mrs. Asha Almeida, Piramal Life Sciences Ltd., Mumbai for screening of the compounds against TNF-α and IL-6 and Dr.Cecil Kwong, Antimicrobial Acquisition and Coordinating Facility (TAACF), Alabama, USA, for anti-tubercular activity screening and also to National Facility for Drug Discovery supported by DPRS at Saurashtra University, Rajkot.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2011.02.016.

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