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ScienceDirect
Bioorganic & Medicinal Chemistry Letters
Bioorganic & Medicinal Chemistry Letters 17 (2007) 5523–5524

Synthesis of 3-*O*-methylviridicatin analogues with improved anti-TNF-α properties

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Received 12 July 2007; revised 15 August 2007; accepted 15 August 2007
Available online 21 August 2007

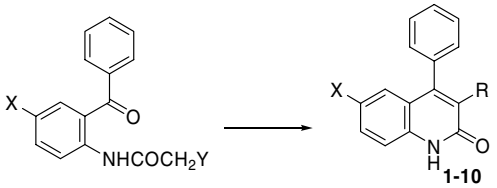
Abstract—We synthesized 3-*O*-methylviridicatin **1** and several analogues of this fungal metabolite. We showed that replacement of the methoxy moiety by a thiomethyl enhanced dramatically its ability to inhibit TNF-α secretion. These results strongly suggest that 4-phenyl-3-methylthioquinolinone **3** may provide the basis for the development of new anti-inflammatory agents.

Overproduction of tumour necrosis factor-alpha (TNF-α) plays a key role in the onset and progression of a number of inflammatory diseases such as diabetes, multiple sclerosis, rheumatoid arthritis, psoriatic arthritis, tumorigenesis and inflammatory bowel diseases.[1] In spite of enormous efforts, no small molecule has yet been approved to specifically inhibit TNF-α activity. The only available drugs in clinics are proteins (Etanercept, Infliximab, Adalimumab and Anakinra) that display adverse effects such as aplastic anaemia, pancytopenia, vasculitis, demyelination and congestive heart failure.[2] Therefore, there is a continuing interest in the search of small molecules that can block TNF-α signalling without these side effects and the general disadvantages associated with protein drugs. As recently reviewed, natural products offer promising opportunities to develop new treatments of inflammatory diseases by inhibiting synthesis rather than the activity of TNF-α.[3]

In 1964, Austin and Myers isolated 3-*O*-methylviridicatin **1** from the fungus *Penicillium puberulum*.[4] However, the biological activity remained unexplored until 1998, when Heguy and coll. reported that it inhibits the replication of the HIV virus induced by TNF-α with an IC_{50} of 2.5 μM.[5] These authors suggested that **1** inhibits the signalling of NF-κB. Due to the critical involvement of this transcription factor in inflammation, we hypothesized that **1** may prevent the secretion of TNF-α and serve as a promising lead for the development of new anti-inflammatory drugs. We report herein the first study on the structure–activity relationships of **1** and the identification of analogues that display enhanced anti-inflammatory properties.

We synthesized 3-*O*-methylviridicatin **1** by acylation of 2-amino-benzophenone with methoxyoacetyl chloride followed by a cyclisation under alkaline conditions (KOH in ethanol, method a, Scheme 1).[6]

Carbaisostere **2** was prepared similarly using propionyl chloride as acylating agent. Compounds **3**–**10** were prepared by acylation of an amino-benzophenone with bromoacetyl bromide to give bromoamides **11c** and



11a : X=H, Y=OMe
11b: X=H, Y=Et
11c : X=H, Br
11d : X=NO_2, Br

conditions:
a) **11a** or **11b**, KOH, EtOH, reflux
b) **11c**, MeSNa, MeOH, rt
c) i) **11c**, amine, CH_3CN, rt; ii) tBuOK, EtOH, reflux
d) **11c**, $MeNH_2$, CH_3CN, rt; ii) Boc_2O, DMAP, CH_2Cl_2; iii) tBuOK, EtOH, reflux; iv) HCl, THF
e) i) pyridine, reflux; ii) $NH_2NH_2.H_2O$, EtOH, reflux

Scheme 1. Synthesis of 3-*O*-methylviridicatin **1** and its analogues **2**–**10.**

Keywords: Inflammation; TNF-α.
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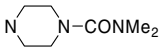
doi:10.1016/j.bmcl.2007.08.036

Table 1. Inhibition of TNF-α secretion by THP-1 cells and human PBMCs[a]

Entry	Compounds (method, yield)	X	R	% Inhibition of TNF-α release[a]			
				THP-1		PBMC	
				10 μM	50 μM	10 μM	50 μM
1	**1** (a, 38%)	H	OMe	7	10	24	67
2	**2** (a, 31%)	H	Et	3	80	32	50
3	**3** (b, 31%)	H	SMe	49	93	78	95
4	**4** (c, 78%)	H	NMe_2	0	83	ND	ND
5	**5** (d, 10%)	H	NHMe	50	97	36	67
6	**6** (c, 66%)	H	$N(CH_2CH_2OMe)_2$	0	59	ND[b]	ND
7	**7** (c, 54%)	H	$NMeCH_2CH_2OH$	0	21	ND	ND
8	**8** (c, 43%)	H		21	43	ND	ND
9	**9** (e, 61%)	H	NH_2	ND	ND	29	79
10	**10** (b, 59%)	NO_2	SMe	ND	ND	86	94

[a] Cells were incubated for 24 h with 5 μg/ml LPS in the presence of the tested compound or DMSO alone as a control. Concentrations of TNF-α were assessed by ELISA in culture supernatants (for a detailed assay description, see Ref. 9).
[b] ND, not determined.

11e. Displacement of the bromide by an amine followed by an alkaline cyclisation afforded compounds **4**, **6–8** (method c).[7] Synthesis of the methylamino **5** required a transient protection with a Boc (method d).

Interestingly, reaction of **11c** and **11d** with sodium methanethiolate gave an intermediate that cyclised spontaneously to afford the thio-isosteres **3** and **10** (method b).[8] Compound **10** was synthesized by alkylation of pyridine by bromoamide **11c**, followed by hydrazinolysis according to established procedures (method e).[9]

First, we assayed the anti-inflammatory activity of 3-*O*-methylviridicatin **1** and its analogues **2–8** in an inhibition model of TNF-α secretion by human monocytic THP-1 cells after human LPS activation (Table 1). Then, we confirmed these data on human LPS-activated peripheral blood mononuclear cells (PBMCs).[10] PBMCs, unlike THP-1 cells, do not extrude drugs outside due to multidrug-resistant pumps, and represent therefore a better model to study anti-inflammatory drugs.

3-*O*-Methylviridicatin **1** weakly inhibits the production of TNF-α in vitro by THP-1 and PBMCs (7% and 24% inhibition, respectively, at a 10 μM concentration) (Table 1, entry 1). Replacement of the 3-methoxy by an ethyl did not significantly modify the activity (entry 2), but replacement by a thiomethyl dramatically increased the inhibition of TNF-α liberation (entry 3, IC_{50} = 1 μM). This might be due to the enhanced ability of vinyl-thioethers substituted by a carbonyl to trap radicals by captodative effect.[11] In a previous study, we have shown that celastrol, which displays similar features, exhibits also potent anti-inflammatory activity.[10] All together these data suggest that at least a part of the mechanism of action of these drugs may involve trapping radicals.

Substitution by a bulky amine led to a dramatic loss of activity (entries 4, 6–8), but substitution by NH_2 or NHMe retained an activity similar to that of 3-*O*-methylveridicatin **1** (entries 5 and 9), indicating that this position is very sensitive to steric hindrance.

Introduction of a nitro group at the 6 position was well tolerated (entry 10, IC_{50} = 3.5 μM), suggesting that functionalization of this position should be considered in further studies.

In summary, we performed the first study on the structure–activity relationships of 3-*O*-methylviridicatin and we identified its thio-isosteres **3** and **10** as lead compounds to develop new anti-inflammatory drugs.

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