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Sch 65676: A Novel Fungal Metabolite with the Inhibitory Activity Against the Cytomegalovirus Protease

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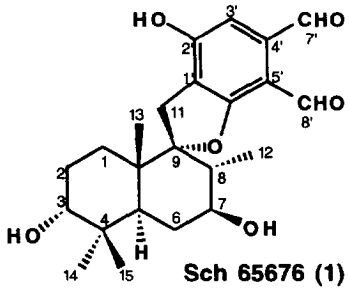
Abstract: A new secondary metabolite, Sch 65676 (1), was isolated from the fermentation broth of a fungal culture. The structure of 1 was elucidated based on comprehensive NMR studies including COSY, NOE and HMBC experiments. Compound 1 displays inhibitory activity against the cytomegalovirus (CMV) protease. Copyright © 1996 Elsevier Science Ltd

Human cytomegalovirus (CMV), a β-herpes virus, is an ubiquitous opportunistic pathogen which causes clinically important disease in congenitally infected infants and in immunocompromised adults, including patients with AIDS and recipients of organ or bone marrow transplants. Current therapies include nucleoside analogues whose use is limited due to accompanying toxicities. Thus, a medical need exists for an antiviral agent for CMV infection. Activity of the CMV protease is targeted to develop antiviral agents due to its critical role in the process of viral assembly.[1,2] The CMV UL 80 open reading frame encodes an 80 kDal precursor polyprotein whose N'-terminal 256 amino acid domain is a protease. The protease cleaves a specific peptide bond that results in its own release from the UL 80 precursor polyprotein, as well as a peptide bond near the C'-terminus of the viral assembly protein. This latter cleavage is required for subsequent encapsidation of the viral genomic DNA and maturation of the viral capsid.[3,4] Biochemical analyses indicate that the CMV protease is a member of the serine family of proteases.[5]

In order to discover the CMV protease inhibitors, a large number of fungal extracts have been screened in a biochemical assay for the viral enzyme. Briefly, the catalytic domain of CMV protease was recombinantly produced in *E. coli*, purified and used to cleave a synthetic peptide substrate based on the sequence of the assembly protein.[6] We found that, Sch 65676 (**1**), isolated from an unidentified fungus (SCF-1657),[7] which was collected from a humid deciduous forest located 40 km east of Trivandrum, Kerala State, India, inhibited the CMV protease activity. This report describes the isolation, structure determination and bioactivity of **1**.



Sch 65676 (1)

Centrifugal partition chromatography (CPC) of ethyl acetate extracts from a fermentation broth (4L) followed by PVA-SIL normal phase HPLC[8] afforded pure **1** (35.1 mg) which was crystallized from EtOAc-hexane to obtain a pale yellow solid with mp 233-235°C (dec.) and $[\alpha]^{23}_{D}$ - 20.8° (*c* 0.13, MeOH).

Electron impact (EI) and fast atom bombardment (FAB) mass spectra indicated a molecular ion of **1** at m/z 402 (M^+), 403 $(M+H)^+$ and 425 $(M+Na)^+$, respectively. The molecular weight was further confirmed by negative-mode FABMS technique showing deprotonated ion peak at m/z 401 $(M-H)^-$. The molecular formula was established by HR-FABMS as $C_{23}H_{30}O_6$ (Calcd: 403.2121. Measured: 403.2103 for $C_{23}H_{31}O_6$) which was supported by ^{13}C NMR data. Absorption bands of the UV spectrum of **1** at 245, 305 and 351 nm revealed a benzoic conjugation system. The IR absorptions at 3436, 1677 and 1589 cm^{-1} showed the presence of hydroxyl, conjugated or aromatic carbonyl and substituted phenolic functionalities, respectively. ^{13}C NMR and APT spectral data (Table 1)

Table 1. NMR Assignments and HMBC data of **1**[a]

Position	^{13}C (δ)	^{1}H (δ)	HMBC
1	24.93 t[b]	1.97 (m) 2.34 (m)	-- --
2	26.06 t	1.18 eg (dt, 13.8, 3.2[c]) 1.77 ax (dq, 13.8, 3.2)	--
3	74.51 d	3.64 (br.s)	C-2, C-4
4	38.19 s	--	--
5	38.54 d	2.78 (dd, 13.6, 2.2)	C-4, C-6, C-10, C-13, C-15
6	31.98 t	1.88 ax (dt, 13.6 2.2) 2.36 eq (m)	C-5, C-7, C-8, C-10
7	72.28 d	4.12 (dt, 10.2, 5.0)	C-8, C-12
8	45.78 d	2.09 (m)	C-7, C-12
9	101.93 s	--	--
10	42.89 s	--	--
11	32.08 t	3.18, 3.49 (ABq, 17.2)	C-2', C-3', C-5', C-6', C-8, C-9, C-10
12	11.73 q	1.34 (d, 6.2)	C-7, C-8, C-9
13	16.41 q	1.08 (s)	C-1, C-5, C-9, C-10
14	29.16 q	1.28 (s)	C-3, C-4, C-5, C-15
15	22.69 q	0.96 (s)	C-3, C-4, C-5, C-14
1'	111.49 s	--	--
2'	160.13 s	--	--
3'	109.07 d	7.30 (s)	C-1', C-2', C-4', C-5', C-7'
4'	139.29 s	--	--
5'	119.70 s	--	--
6'	167.94 s	--	--
7'	192.94 d	10.67 (s)	C-2', C-3', C-4'
8'	187.80 d	11.00 (s)	C-1', C-4', C-5', C-6'

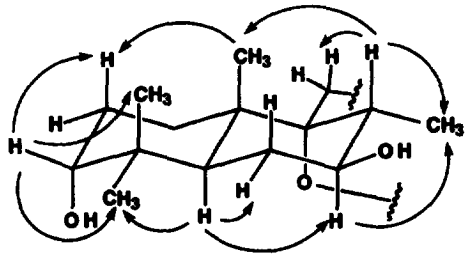
a. Recorded in pyridine-d_5 on 400 MHz instrument

b. Multiplicity was determined by APT data

c. Coupling constants in Hz

further indicated the presence of a total of 23 carbons, including five aromatic, one oxygenated and two aliphatic quaternary carbons, two aldehyde, one aromatic, two oxygenated and two aliphatic methine carbons, four methylenes and four methyl carbons. Analyses of the ^1H NMR spectrum (Table 1) was consistent with ^{13}C NMR data showing two aldehyde singlets at δ 10.67 and 11.00, and one aromatic singlet δ 7.30. Two oxygenated proton signals represented two secondary hydroxyl groups, one appeared as a broad singlet at δ 3.64 and the other displayed as a doublet of triplet at δ 4.12. One distinctive methylene AB quartet at δ 3.18 and 3.49 reflected its connection to two quaternary carbons which are asymmetrical. One methine doublet of doublet at δ 2.78 suggested the coupling to adjacent methylene protons. A methyl doublet at δ 1.34 connected to a methine, and the other three methyl groups appeared as singlets at δ 0.96, 1.08 and 1.28, respectively. The remaining three methylene groups were also observed. Partial structures of C1-C2-C3 and C5- C6-C7-C8-C12 were determined by COSY experiments. Structure elucidation of **1** was finally completed by HMBC experiments (Table 1). Correlations of the methine H-5 to C-4, C-6, C-10, C-13 and C-15, the methyl H-13 to C-1, C-5, C-9 and C-10, the oxy-methine H-3 to C-2 and C-4, together with the oxy-methine H-7 to C-8 and C-12 established a decaline ring system. The second fragment, a highly substituted benzene ring, was assigned based on correlation of the aromatic H-3' to C-1', C-2', C-4', C-5' and C-7', the aldehyde H-8' to C-4', C-5', C-6' and C-2' (weak) observed in HMBC spectrum. A spiro-joint connectivity of two ring fragments by forming a dihydrofuran ring was determined due to the correlation of methylene H-11 to C-4', C-5', C-6', C-8, C-9 and C-10, which was the only position showing a cross linkage between the decaline and benzene.

Fig.1 Some Important NOE Data of 1



The establishment of stereochemistry of **1** was acomplished by the analysis of difference NOE data. As shown in Fig. 1, NOE correlations from H-3 to H-2ax, H-13 to H-2ax and H-8 to H-13 demonstrated the same orientations of H-3, H-8 and CH_3-12 at the β-position. Attachments of H-5, H-7 and CH_3-12 were assigned to the α-position due to their NOE correlations. Therefore, **1** is illustrated as a novel member of stachybatrydial family,[9] with a hydroxyl substitution at C-7.

Compound **1** exhibited inhibitory activity against CMV maturational protease *in vitro* with an IC_{50} value of 9.8 μg/ml. To our knowledge, **1** is the first compound in this class reported as a CMV protease inhibitor.

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6. CMV protease assay: Standard protease assays were initiated by the addition of 0.06 μM purified CMV protease to 50 μM substrate peptide (Ala-Gly -Val-Val-Asp-Ala-Ser-Cys-Arg-Leu-Ala) in a 120 μl volume containing 50 mM of HEPES pH 7.5, 1 mM DTT, 0.1 mM EDTA, 20% glycerol, 2% DMSO, followed by incubation for 90 min at 30°C. Compounds to be tested were added prior to the addition of the protease. Reactions were terminated by the addition of 10% TFA (final concentration 0.1%). Cleavage products were analyzed in a reversed phase HPLC C-18 column and were estimated by area under the curve.
7. The fungus was supplied by Dr. B. Katz from MYCOsearch Lab.
8. CPC Conditions: Ito multilayer coil planet centrifuge instrument, 350 mL coil, EtOAc:hexane:MeOH: H_2O (5 : 3 : 3 : 5) solvent system, up layer used as mobile phase, 10 mL/min. HPLC Conditions: YMC PVA-SIL 20 x 250 mm semi-preparative column, S-5, 50-90% EtOAc in hexane with a linear gradient in 20 min, 12 mL/min, UV = 260 nm.
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