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A New Fungal Metabolite, Sch 202596, with Inhibitory Activity in the Galanin Receptor GALR1 Assay

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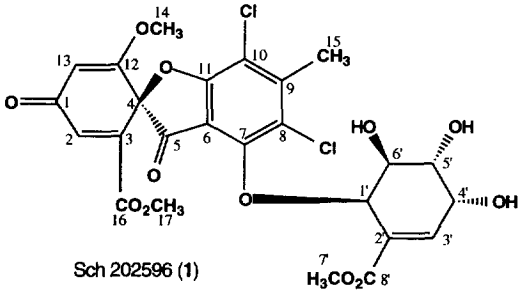
Abstract: A novel spirocoumaranone, Sch 202596 (**1**), was isolated from the fermentation broth of *Aspergillus* sp. The isolation, structure elucidation and stereochemistry of **1** are described.

The neuropeptide galanin (30 amino acids in humans) is widely distributed in the body and is often found colocalized with substances which mediate intercellular signaling such as neurotransmitters.[1,2] Studies of galanin function in the central nervous system (CNS) include feeding behavior, modulation of acetylcholine release, decreased firing of noradrenergic neurons, regulation of growth hormone secretion and inhibition of spinal reflexes. Peripheral aspects of galanin function include inhibition of glucose stimulated insulin secretion and contraction of gastric smooth muscle. The pharmacology and molecular biology of galanin receptors has recently begun to shed light on the mechanisms of galanin induced cellular responses. A galanin receptor (GALR1) has been cloned from a Bowes human melanoma cell. The sequence of the receptor, and extensive pharmacological studies have indicated that this receptor is a member of the G-protein coupled superfamily of receptors. The galanin receptor is considered an important target for development of therapeutic agents in the area of eating disorders.[3,4]

As part of our natural product program to search for galanin receptor agonists and/or antagonists, a large number of samples from various fungal or bacterial fermentations were screened. A novel fungal metabolite, Sch 202596 (**1**) was discovered from the fermentation of a fungal culture *Aspergillus* sp. (culture Belt-1498). The fungus, *Aspergillus* sp. was isolated from the tailing piles of an abandoned uranium mine in Tuolemene County, California.[5] Compound **1** was shown by spectroscopy to be a new spirocoumaranone, related to the griseofulvin family of compounds. In this paper we report the isolation, structure elucidation and IC_{50} value in the galanin receptor assay of **1**.



Sch 202596 (**1**)

The crude ethyl acetate extract of fermentation culture broth (4 L) was partitioned by a centrifugal partition chromatography (CPC) with hexane:EtOAc:MeOH:H_2O (4:5:4:5) biphasic solvent system. The active fractions from CPC were combined and subjected to further purification on reverse phase HPLC (YMC ODS semi-preparative column, 20 x 250 mm, S-5 120Å, 50-90% MeOH in H_2O with a linear gradient in 25 min, 12 mL/min., UV=220 nm) to obtain 15 mg of pure **1** as pale yellow solid, mp 136-138°C, $[\alpha]^{22}_D$=+235.4° (*c* 0.1, MeOH).

Table 1. NMR Assignments and HMBC Data of **1**[a,b]

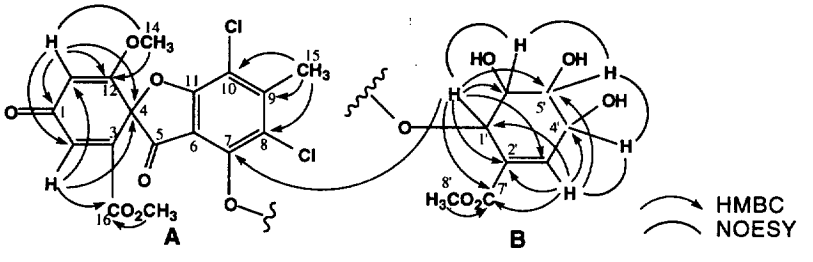
No.	^{13}C (δ)	^{1}H(δ)	HMBC
1	185.1 s	--	
2	137.4 d	7.13 (d, 1.4)	C-4, C-13, C-16
3	138.0 s	--	--
4	84.4 s	--	--
5	192.1 s	--	--
6	112.7* s	--	--
7	151.4 s	--	--
8	112.6* s	--	--
9	146.1 s	--	--
10	127.7 s	--	--
11	167.2 s	--	--
12	168.3 s	--	--
13	104.3 d	5.80 (d, 1.4)	C-1, C-2, C-4, C-12
14	57.1 q	3.70 (s)	C-12
15	19.1 q	2.59 (s)	C-8, C-9, C-10
16	163.5 s	--	--
17	53.1 q	3.78 (s)	C-16
1'	80.9 d	5.38 (d, 1.6)	C-7, C-2', C-3', C-5', C-6', C-7'
2'	128.9 s	--	--
3'	141.3 d	7.21, (d, 2.6)	C-1', C-2', C-4', C-5', C-7'
4'	66.2 d	4.37 (br. t, 2.6)	--
5'	67.1 d	4.18 (br. s)	--
6'	73.6 d	4.68 (br. t, 1.6)	C-1', C-2', C-4'
7'	166.1 s	--	--
8'	52.1 q	3.60 (s)	C-7'

a. Recorded in $CDCl_3$ on 400 MHz instrument
b. Multiplicity was determined by APT and HETCOR data. Coupling constants in (Hz)
* The assignment for these two carbons could be exchangeable.

Electrospray ionization (ESI) mass spectral analyses of **1** showed molecular ions at m/z 585, $(M+H)^+$ and 607 $(M+Na)^+$ in positive mode, and m/z 583 $(M-H)^-$ in negative mode, respectively. Furthermore, a chlorine containing ion cluster was observed (with the intensity ratio of m/z 585, 587 and 589: 100/65/11, suggesting the presence of two chlorine atoms). The molecular formula was deduced as $C_{25}H_{22}O_{12}Cl_2$ based on HRFAB-MS (Calcd: 585.0566. Found: 585.0564 for $C_{25}H_{23}O_{12}Cl_2$) and ^{13}C NMR data. Characteristic maximum UV absorptions at 284 and 350 nm and IR absorption bands at 3440 (hydroxyl), 1735 (cyclopentanyl), 1665 (quinone carbonyl) and 1610 cm^{-1} (conjugated ester carbonyl) indicated a typical profile of spirocoumaranone class of compounds. A total of 25 carbons were observed in ^{13}C NMR spectrum of **1** (Table 1). In the APT spectrum the following types of carbons were present: one methyl, three methoxyls, four oxygenated methines, one oxy-quaternary, three olefinic/aromatic methines, nine olefinic / aromatic

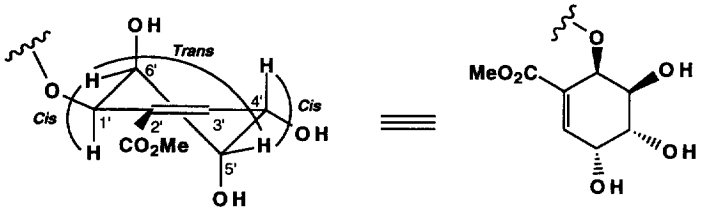
quaternaries, two conjugated ester carbonyls, one quinone and one conjugated ketone. ^{1}H NMR data (Table 1) displayed two of the three olefinic methine doublets with the same small coupling constant of J=1.4 Hz and the third with coupling constant of J=2.6 Hz. One of four oxygenated methine protons appeared as a doublet with J=1.6 Hz while another two appeared as broad triplets and one broad singlet suggesting the presence of three secondary hydroxyl groups. In addition, a methyl singlet at δ 2.59 indicated its connectivity with an aromatic ring. Detailed assignments of each proton and carbon were accomplished by analysis of 2D NMR data including COSY, NOESY, HETCOR and HMBC experiments. As shown in Fig. 1, both correlations of H-2 and H-13 to C-4 strongly suggested that the cyclohexadienone was linked to the aromatic ring through the formation of a five-membered ring with a spiro-junction at the quaternary C-4. The assignment of a spiro-tricyclic ring system for A was also supported by its unsaturation in order to fit the molecular formula of **1**. The B unit, a cyclochexene ring as shown in Fig. 1, was established based on ^{1}H-^{1}H and ^{1}H-^{13}C correlation observed in NOESY and HMBC experiments, respectively. The connectivity of partial structure A and B was determined by the correlation of H-1' to C-7 in HMBC experiments, which was the only cross peak detected from fragment B to A.

Fig. 1 Some Important NOESY and HMBC Correlations of **1**



The stereochemistry of the chiral center at C-4 of **1** was determined based on studies of the circular dichroic (CD) spectrum. In the CD spectrum of **1**, a negative Cotton effect at 205~230 nm region and a positive Cotton effect at 230~275 nm region, which reflected the dissymetric chromophore of spirotricyclic moiety,[6] were very similar to griseofulvin. Therefore, the same stereochemistry at C-4 was assigned as the **S** configuration for **1** since the absolute stereochemistry of griseofulvin was established by X-ray crystallography.[7] The optical rotation of griseofulvin with a large positive value, $[\alpha]_D$ =+370° ($CHCl_3$), further supported the above assignment. The relative stereochemistry of the cyclohexene ring of **1**, which does not exist in other related compounds, was established by the interpretation of NMR spectral data. Based on the analysis of Dreiding models, a pseudo-chair conformation appeared to be the most stable conformation. Since the correlations of 1,3-diaxial interactions for H-1' to H-5' and H-4' to H-6' were absent in the NOESY spectrum, several possible isomers with this interaction were eliminated. Only two arrangements of these four methine protons, *cis-trans-cis* and *trans-cis-trans* configurations were plausible. The small coupling constants of H-1' (J=1.6 Hz), H-4' (J=2.6 Hz), H-5' (The J value can only be estimated as ~ 2 Hz due to the broadness of this signal) and H-6' (J=1.6 Hz) further revealed that these four methine protons should have either pseudoequatorial-equatorial or pseudoequatorial-axial relationships, but not pseudoaxial-axial arrangements.

Fig. 2 The Relative Stereochemistry of the Cyclohexene Ring of **1**



Therefore, the *cis-trans-cis* configuration was the only one to meet the above criteria as shown in Fig. 2. However, the absolute stereochemistry of the cyclohexene ring was unable to be assigned by NMR methods due to the remote distance between this ring and C-4.

In the *in vitro* galanin receptor GALR1 assay[8] compound **1** exhibited inhibitory activity with an IC_{50} value at 1.7 μM.

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8. Bowes melanoma cell membranes were purchased from Receptor Biologies Inc. and stored in small aliquots at -80°C. Binding of human [^{125}I]-galanin (2200 Ci/mmol, NEN) to the membranes was performed in a buffer contaiing 10 mM HEPES (pH 7.4), 0.05% bovine serum albumin (RIA grade), 0.1% bacitracin, and 0.5 mM EDTA. Nonspecific binding was assessed by including 500 nM unlabeled galanin. Compound inhibition studies were in a total volume of 200 μl, containing 5 μg of membrane protein and 0.1 nM [^{125}I] galanin. Incubations were performed at room temperature for 1 hour and terminated by rapid vacuum filtration through Multiscreen FB Filter Plates (Millipore, Bedford, MA) which had been pretreated with 0.3% polyethylenimine. Each incubation was then washed 3 times with 100 μl of phosphate buffered saline (pH 7.4). Radioactivity on the filters was then assessed using a 96 well scintillation counter.

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