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SCH 207278: A NOVEL FARNESYL PROTEIN TRANSFERASE INHIBITOR FROM AN UNIDENTIFIED FUNGUS

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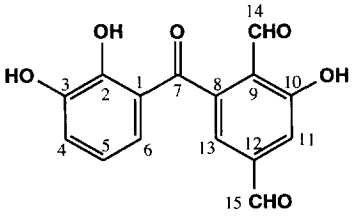
Abstract: Novel aromatic dialdehyde, Sch 207278 (**1**), was isolated from an unidentified fungus as an inhibitor of farnesyl protein transferase (FPTase). The structure of **1** was elucidated by spectroscopic methods. Compound **1** exhibited an IC_{50} value of 3.5 μM against FPTase and 70 μM against geranylgeranyl protein transferase-1 (GGPTase-1), respectively. © 1997 Elsevier Science Ltd.

Farnesyl-protein transferase (FPTase) catalyses the farnesylation of Ras (p21) proteins on a cysteine residue of the CAAX sequence (C: a conserved cystine, AA: two aliphatic amino acids, X: a carboxy-terminal residue).[1-3] This post-translational modification of Ras proteins is necessary for their association with plasma membrane and oncogenic activity.[4] Therefore, inhibitors of FPTase could suppress tumor growth induced by Ras. Since Ras oncogenes are frequently found in human tumors including 50% of colon and 90% of pancreatic carcinomas,[5] inhibition of Ras farnesyl-protein transferase is a potentially attractive therapeutic target for new anticancer agents. Recently, several natural product inhibitors of FPTase have been reported including cembranolide diterpene,[6] fusidienol,[7] chaetomellic acids,[8] and pepticinnamin.[9] Our microbial products screening efforts have now led to the isolation of a new inhibitor of FPTase, Sch 207278 (**1**) produced by an unidentified fungus collected from Equador (culture MYCO-2139).[10] This paper describes the assay-guided isolation, structure elucidation and biological activity of **1**.

Figure 1 Structure of Sch 207278 (**1**)



The fermentation broth (10 L) was extracted with ethyl acetate at harvest pH (6.8). The residue from EtOAc extraction was subjected to a modified Kupchan partition described as follows: EtOAc extract residue was dissolved in 90% aqueous MeOH (~25 g/L concentration), the solution was partitioned with an equal volume of hexane. The lower aqueous MeOH layer was separated to which 1/8 volume of H_2O (12.5%) was added. The aqueous MeOH solution was then partitioned with an equal volume of CH_2Cl_2. The lower CH_2Cl_2 layer was separated. To the aqueous MeOH layer was added 3/8 volume of H_2O (37.5%) and re-extracted with an equal volume of CH_2Cl_2. The combined active CH_2Cl_2 solution was purified by high speed centrifugal partition chromatography (CPC) with a solvent system of hexane:EtOAc:MeOH:H_2O (1.2:0.8:1:1)

to obtain pure **1**[11] as a white powder with mp 201~203 °C (dec.).

Fast atom bombardment (FAB) and chemical ionization (CI) mass spectral data of **1** showed a protonated molecular ion $(M+H)^+$ at *m/z* 287. The molecular formula of $C_{15}H_{10}O_6$ was deduced by HR-FABMS (Calcd: *m/z* 287.0556 for $C_{15}H_{11}O_6$. Found: *m/z* 287.0554). Absorption bands in the UV spectrum at 256 and 335 nm suggested a substituted benzene structure. IR absorption at 3428, 1686 and 1645 cm^{-1} indicated the presence of hydroxyl, conjugated/aromatic carbonyl and bisaromatic carbonyl, respectively. In the ^1H NMR spectrum of **1** (Table 1), two distinctive aldehyde proton singlets were observed at δ 9.59 and 9.94. Five proton resonances were found in the downfield aromatic region at δ 6.99~7.55. In addition, a broad exchangeable singlet at δ 3.40 indicated the presence of three hydroxyl groups based on the integration measurement. The ^{13}C NMR data showed three carbonyl signals at δ 200.8, 193.8 and 190.2, which represent one ketone and two aldehyde functionalities, respectively, based on the APT experiment. Observations of five aromatic methine signals at δ 119.3~135.9 was consistent with ^1H NMR data. The remaining seven aromatic quaternary carbons at δ 118.0~162.5 suggested the presence of two benzene units, **A** and **B**, to match the unsaturation of **1**.

Table 1. ^1H (400 MHz) & ^{13}C (100 Hz) NMR Data for **1** & **2**[a]

	1[b]		**2**	
No.	^{13}C (δ)	^1H (δ)	^{13}C (δ)	^1H (δ)
1	118.0 s[c]	--	128.1 s	--
2	162.5 s	--	151.5 s	--
3	140.7 s	--	141.8 s	--
4	119.9 d	6.93 (d, 8.0)	126.4 d	7.35 (d, 7.8)
5	135.9 d	7.55 (t, 8.0)	134.2 d	7.69 (d, 7.8)
6	119.3 d	7.13 (d, 8.0)	126.3 d	7.37 (d, 7.8)
7	200.8 s	--	192.0 s	--
8	128.1 s	--	134.1 s	--
9	147.3 s	--	146.1 s	--
10	156.6 s	--	144.1 s	--
11	120.7 d	7.35 (d, 1.9)	127.4 d	7.96 (d, 1.9)
12	119.5 s	--	132.4 s	--
13	128.2 d	7.51 (d, 1.9)	130.0 d	8.11 (d, 1.9)
14	190.2 d	9.94 (s)	188.0 d	10.10 (s)
15	193.8 d	9.59 (s)	189.3 d	99.9 (s)
$\underline{C}OCH_3$	--	--	169.0 s	--
$\underline{C}OCH_3$	--	--	167.6 s	--
$\underline{C}OCH_3$	--	--	167.5 s	--
$CO\underline{C}H_3$	--	--	20.85 q	2.40 (s)
$CO\underline{C}H_3$	--	--	20.60 q	2.32 (s)
$CO\underline{C}H_3$	--	--	19.95 q	2.01 (s)

a. Recorded in $CDCl_3$, chemical shift in PPM from TMS, coupling constants (Hz).

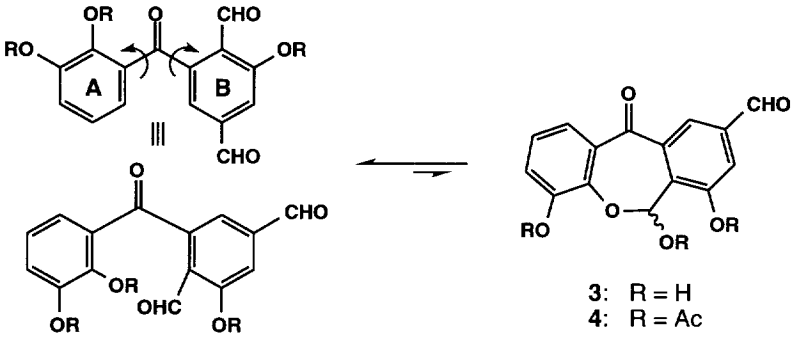
b. A small amount of CD_3OD was used as a cosolvent for **1**.

c. Multiplicity was determined based on APT data.

It should be noted that the NMR spectra of **1** often showed as a mixture of tautomers plausibly due to the equilibrium of aldehyde and hemiacetal forms. In order to prove this hypothesis and determine the exact

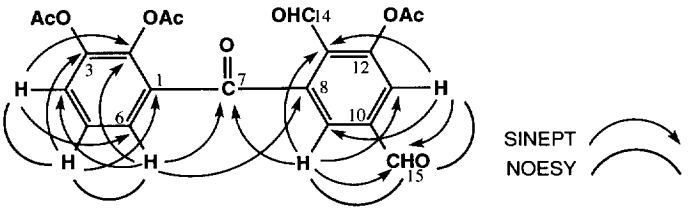
number of hydroxyl groups, acetylation of **1** was carried out with acetic anhydride and pyridine. As predicted both triacetylated products **2** and **4** (ratio 4:1) were obtained from the reaction mixture.[12]

Figure 2 Equilibration of Aldehyde and Hemiacetal Tautomers



Extensive NMR studies including HETCOR, NOESY, and selective INEPT were focused on triacetate **2** since it provided better spectral quality. In the NOESY spectrum, correlations of H-4 to H-5 and H-5 to H-6 revealed that these three protons are adjacent to each other. NOESY correlations of H-15 to H-11 and H-13 suggested that one aldehyde was located between two *meta* protons. This assignment was also supported by the observation of *meta* couplings (J = 1.9 Hz) for H-11 and H-13. Selective INEPT data not only confirmed the assignments of all five aromatic protons by NOESY experiments, but also indicated that H-4, H-5 and H-6 protons were attached to the **A** ring with two acetates, and H-11 and H-13 protons were attached to the **B** ring with one acetate and two aldehydes. As depicted in Figure 2, analysis of ^{1}H-^{13}C long range correlation obtained from selective INEPT experiments permitted the establishment of the regiochemistry of each functionality. The SINEPT correlations of H-6 to C-7 and C-8, H-9 to C-7 further established the connectivities of the two benzene rings, **A** and **B**, through the ketone carbonyl at position-1 and position-8, respectively. Thus, the structure elucidation of **1** was completed.

Figure 3 Some Important NOESY and Selective INEPT Correlations of **3**



Both compound **1** and its triacetate **2** exhibited in vitro inhibitory activity against recombinant human

FPTase with IC_{50}s of 3.5 and 2.4 μM, respectively. In addition, **1** and **2** displayed IC_{50}s of 70 and 85 μM against geranylgeranyl protein transferase-1 (GGPTase), a closely related isoprenyl protein transferase enzyme to FPTase.[13] Thus both compounds **1** and **2** showed approximately 20 and 35-fold selectivity for FPTase, respectively.

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11. The purity of **1** was analyzed by normal phase HPLC under conditions as follows: YMC PVA-Sil column, 4.6 x 150 mm, S-5, 120 Å, 40% EtOAc in hexane, isocratic, 1 mL/min, UV detection at 260 nm, 95% purity by area integration normalization.
12. To a mixture of **1** (60 mg, 0.21 mmol), pyridine (1 mL) and CH_2Cl_2 (8 mL) at room temperature was added acetic anhydride (0.75 mL, 68 mmol). The reaction mixture was stirred overnight and solvents was removed in vacuo. The residue was purified by HPLC (YMC semi-preparative PVA-Sil column, 20 x 250 mm, S-5, 20~50% EtOAc in hexane with a linear gradient in 30 min, 12 mL/min, UV detection at 270 nm) to afford 20 mg of **2** (see Table 1 for spectral data) and 5 mg of **4**. The spectroscopic data of **4**: CI-MS (relative intensity) *m/z* 413 (49, MH^+), 371 (62), 353 (100), 329 (9), 311 (34). ^1H NMR ($CDCl_3$, 400 MHz) δ 1.95 (s, 3H, $COCH_3$), 2.38 (s, 3H, $COCH_3$), 2.43 (s, 3H, $COCH_3$), 7.41 (d, 1H, J = 8.0 Hz, H-4), 7.60 (t, 1H, J = 8.0 Hz, H-5), 7.73 (s, 1H, H-14), 7.82 (d, 1H, J = 2.1 Hz, H-11), 7.87 (d, 1H, J = 8.0 Hz, H-6), 8.55 (d, 1H, J = 2.1 Hz, H-13), 10.00 (s, 1H, CHO). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 20.4 (q), 20.5 (q), 20.9 (q), 88.2 (d), 125.7 (s), 126.6 (d), 127.5 (d), 127.7 (s), 127.8 (d), 131.3 (d), 132.5 (d), 139.6 (s), 143.3 (s), 146.9 (s), 149.8 (s), 150.8 (s), 167.9 (s), 168.5 (s), 168.8 (s), 188.1 (s), 189.5 (d).
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