Pergamon

Bioorganic & Medicinal Chemistry Letters 9 (1999) 1949–1952

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

ISOLATION AND STRUCTURE OF SCH 351633: A NOVEL HEPATITIS C VIRUS (HCV) NS3 PROTEASE INHIBITOR FROM THE FUNGUS *PENICILLIUM GRISEOFULVUM*

Min Chu,* Ronald Mierzwa, Ling He, Arthur King, Mahesh Patel, John Pichardo, Andrea Hart, Nancy Butkiewicz and Mohindar S. Puar

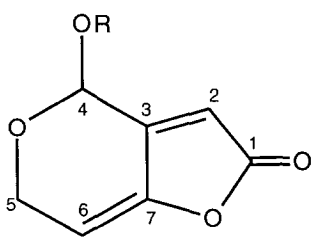
Schering-Plough Research Institute
2015 Galloping Hill Road, Kenilworth, NJ 07033, USA

Received 8 April 1999; accepted 25 May 1999

Abstract: A new hepatitis C virus (HCV) protease inhibitor designated as Sch 351633 (**1**) was isolated from the fungus, *Penicillium griseofulvum*. Structure elucidation of **1** was accomplished by analysis of spectroscopic data, which determined compound **1** to be a bicyclic hemiketal lactone. Compound **1** exhibited inhibitory activity in the HCV protease assay with an IC_{50} value of 3.8 μg/mL.

Hepatitis C virus (HCV) is responsible for viral infections such as non-A, non-B (NANB) hepatitis and cirrhoris, which is strongly associated with development of hepatocellular carcinoma.[1-3] Since a significant HCV infected population in the United States and around the world has been reported, there is a great interest in developing more effective therapeutic agents against HCV to complement currently available interferon therapy.[4] In the course of searching for novel HCV protease inhibitors,[5] a fungal metabolite, Sch 351633 (**1**) was isolated from the fungus, *Penicillium griseofulvum* (culture Mer-0442, designated as Schering Culture Collection Fungi, SCF-1704). The microorganism was isolated from a soil sample collected from a desert terrain in the state of Arizona, U.S.[6] Herewith we report the isolation and structure determination of **1**.

Figure 1. Structures of Sch 351633 (**1**) and Its Derivatives **2** and **3**



1. R=H
2. R=Ac
3. R=BrC_6H_4CO

An ethylacetate extract (3.3 g) from a 2 L fermentation broth of *P. griseofulvum* was dissolved in 150 mL of 40% aqueous MeOH, and partitioned with 100 mL of CH_2Cl_2. The HCV active aqueous MeOH portion (2.6 g) was subjected to reverse-phase HPLC purification (YMC ODS, S-5, 20 x 250 mm semi-preparative column

0960-894X/99/$ - see front matter
PII: S0960-894X(99)00321-2

with 20 x 50 mm guard column, 2-20% acetonitrile in H_2O with a linear gradient in 25 min, 12 mL/min, UV = 220 nm). A total of six replicate runs (50 mg per injection) yielded 100 mg of pure **1** which was obtained as jell-like material after lyophilization.

Table 1. ^1H and ^{13}C NMR Data of **1** and **2**[a]

	1 (Acetone-d_6)		2 (DMSO-d_6)	
#	^{13}C	^1H	^{13}C	^1H
1	169.4 s[b]	--	168.1 s	--
2	110.5 d	5.91 s	111.8 d	6.36 br.s
3	146.6 s	--	146.7 s	--
4	88.8 d	5.96 br.s	86.3 d	7.00 s
5	59.5 t	4.30 (dd 17.6, 3.5)[c]	60.1 d	4.51 (dd 17.0, 4.5)
		4.55 (br.d, 17.6)		4.62 (br.d 17.0)
6	108.8 d	5.96 br.s	108.5 d	6.29 (br.t 4.5)
7	151.9 s	--	144.5 s	--
$COCH_3$	--	--	168.8 s	--
$COCH_3$	--	--	20.6 q	2.10 s

a. Recorded on 400 MHz instrument.

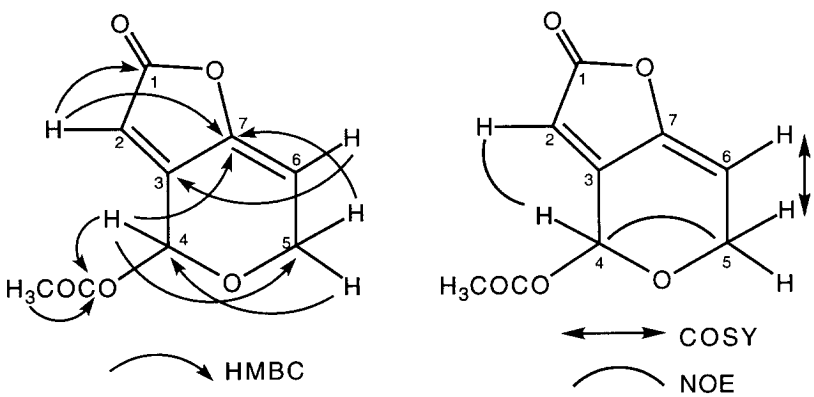
b. Multiplicity was determined by APT experiment.

c. Coupling constants in Hz.

The molecular weight of **1** was determined to be 154 by EI and CI-MS data that showed the molecular ion $M^+ = 154$ and protonated molecular ion $(M+H)^+$ at *m/z* 155, respectively. Molecular formula of $C_7H_6O_4$ for **1** was deduced based on elemental analysis (Calcd: C 54.55%, H 3.90%. Measured: C 54.32%, H 3.73%) as well as carbon NMR data. IR spectral data showed maximum absorption of 1741 cm^{-1} suggesting the presence of a conjugated lactone or ester functional group. UV maximal absorption of 285 nm indicated an extended conjugation system of **1**. ^1H NMR spectrum of **1** (Table 1) showed an AB quartet spin system at δ 4.30 and 4.55, in which each doublet had very small coupling with an adjacent proton reflecting an oxygenated allylic CH_2 group. A singlet at δ 5.91 and a broad singlet at δ 5.96 suggested two olefinic protons. It should be noted that the integration of broad singlet at δ 5.96 indicated two protons associated with this signal. ^{13}C NMR spectral data were consistent with ^1H NMR data showing one methylene carbon at δ 59.5, two olefinic methines at δ 108.8 and 110.5 and one dioxygenated methine at δ 88.8. In addition, one carbonyl at δ 169.4 and two olefinic quarternary carbons at δ 146.6 and 151.9 were observed the ^{13}C NMR experiments of **1**. Interestingly, the tautomeric (aldehyde-alcohol, ring cleavage) form of **1** was not observed in NMR spectra by using different solents (MeOH-d_6 and DMSO-d_6). The hemiacetal form presents predominantly perhaps due to the more favorable stabilization of forming a six ring in the molecule.

In order to obtain additional information and better quality of spectrum for assignments, compound **1** was acetylated with acetic anhydride-pyridine. Extensive 2-D NMR experiments and all detailed assignments, therefore, were performed on acetylation product **2**. Deuterated DMSO solvent was chosen instead of acetone for NMR experiments to avoid an overlap of the acetyl-CH_3 signal at ~2 ppm. COSY experiment clearly indicated direct connection of 5-CH_2 to 6-H. This connection was confirmed by observation of proton coupling and difference NOE experiments. Difference NOE experiments further revealed correlation of 2-H to 4-H, and 4-H to 5-CH_2. The carbon and proton connections were finally established based on HMBC experiments. The structure of **1** was proposed by analysis of long range carbon-proton coupling data from HMBC experiments, which are summarized and as shown in Figure 2. It should be noted that the stereochemistry of chiral center at 4-position was not determined by NMR spectroscopic data. A *p*-bromobenzoate derivative product **3** was made by esterification with *p*-bromobenzyl chloride/triethylamine.[7] However, several attempts of crystallization of **3** failed to provide suitable crystals for X-ray analysis.

Figure 2. 2-D NMR Data of Acetylated Derivative of **1**



Compound **1** demonstrated antiviral activity in the *in vitro* HCV protease scintillation proximity assay with the IC_{50} = 3.8 μg/mL.[8] *In vitro* antiviral activity for the acetylated product **2** and *p*-bromobenzoate derivative **3** were also evaluated. The IC_{50} values of **2** and **3** were shown to be 7.2 and 12.6 μg/mL, respectively.

Acknowledgments: The authors thank Mr. G. Torraca for IR and elemental analysis results, Mr. F. Gentile and Dr. J. Terracciano for fermentation work, Mr. R. Malchow and Mr. Y-H Ingfor MS data and Mrs. D. Scott for preparation of this manuscript.

References and Notes

1. Choo, Q.-L.; Kuo, G.; Weiner, A. J.; Overby, L. R.; Bradley, D. W.; Houghton, M. *Science* **1989**, *244*, 359.

2. Kuo, G.; Choo, Q.-L.; Alter, H. J.; Gitnick, G. L.; Redeker, A. G.; Purcell, R. H.; Miyamuza, T.; Dienstag, J. L.; Alter, M. J.; Stevens, C. E.; Tegtmeier, G. E.; Bonino, F.; Colombo, M.; Lee, W.-S; Kuo, C.; Berger, K.; Shuster, J. R.; Overby, L. R.; Bradley, D. W.; Houghton, M. *Science* **1989**, *244*, 362.
3. Alter, M. J.; Hadler, S. C.; Judson, F. N.; Mares, A.; Alexander, W. J.; Hu, P. J.; Miller, J. K.; Moyer, L. A.; Fields, H. A.; Bradley, D. W.; Margolis, H. S. *J. Am. Med. Assoc.* **1990**, *264*, 2231.
4. Bresters, D. In *Hepatitis C. Virus* Reesink, H. W. Ed.; Karger: Basel, 1994, pp 121~136.
5. Chu, M.; Mierzwa, R.; Truumees, I.; King, A,; Patel, M.; Berrie, R.; Hart, A.; Butkiewicz, N.; DasMahapatra, B.; Chan, T-M.; Puar, M. S. *Tetrahedron Lett.* **1996**, *37*, 7229.
6. The fungus was supplied by Mr. G. M. Luedemann.
7. To a mixture of Sch 351633 (100 mg, 0.65 mmol) and pyrdine (0.5 mL) in dry CH_2Cl_2 (10 mL) and acetonitrile (1 mL) solution was added *p*-bromobenzoyl chloride (150 mg, 0.68 mmol), and then stirred at room temperature overnight. The reaction was quenched by adding water (30 mL) followed by additional 15 mL of CH_2Cl_2. The CH_2Cl_2 layer was separated, dried over $NaSO_4$ and evaporated under nitrogen. The residue was redissolved in acetonitrile and yielded 65 mg of crude qacetonitrile soluble material. The crude material was purified on semi-preparative HPLC (YMC ODS 20 x 250 mm column with guard column, S-5, 20-100% ACN in H_2O with linear gradient in 30 min, 12 mL/min, UV = 220 nm) to afford 11 mg of pure **3**. CI-MS *m/z* 339 $(M+2)^+$; 337 $(M)^+$; ^1H NMR (400 MHz, $CDCl_3$) δ 4.53 (dd, *J* = 4.7, 17.5 Hz, 1H), 4.75 (br.d, *J* = 17.5 Hz, 1H), 5.97 (br.s, 1H), 6.08 (S, 1H), 7.16 (S, 1H), 7.56 (d, *J* = 8.7 Hz, 2H) 7.34 (d, *J* = 8.7 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 168.1, 164.1, 146.3, 145.8, 132.1, 132.1, 131.5, 131.5, 129.4, 127.7 112.7, 106.7, 88.1, 60.9.
8. Scintillation Proximity Assay (SPA) for HCV NS3 Protease.
Inhibition of proteolytic activity of HCV NS3 protease was determined by scitillation approximity assay (SPA). NS3 631/4A protease, purified to 95% homogeneity from a baculovirus expression system, was incubated at 10 nM concentration with the peptide substrate containing the HCV NS5A/5B cleavage site. This peptide substrate was biotinylated at the N-terminus and ^3H-labeled at the C-terminus. After incubation at 22 °C for 2 h, the reaction was stopped, and cleavage of the Cys-Ser bond was detected by the addition of streptavidin-coated SPA beads (Amersham), which can bind to the biotin-labeled peptide. Signal is detected as cpm using a Packard Topcount Model NXT instrument. Cleavage is indicated by reduction in signal caused by separation of the radiolabeled fragment from the bound biotinylated fragment, which is proportional to proteolytic activity.