Pergamon

Bioorganic & Medicinal Chemistry Letters, Vol. 4, No. 12, pp. 1539-1542, 1994

0960-894X/94 $7.00+0.00

0960-894X(94)00185-5

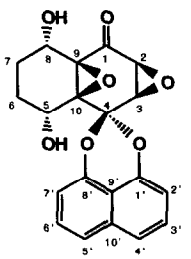
TWO NEW PHOSPHOLIPASE D INHIBITORS, SCH 49211 AND SCH 49212, PRODUCED BY THE FUNGUS *NATTRASSIA MANGIFERAE*

Min Chu*, Imbi Truumees, Mahesh G. Patel, Vincent P. Gullo, Jin-Keon Pai, Pradip R. Das and Mohindar S. Puar

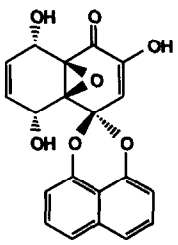
Schering-Plough Research Institute
2015 Galloping Hill Road, Kenilworth, NJ 07033

Abstract: *Sch 49211 (2) and Sch 49212 (3) have been isolated from the fermentation of the fungus Nattrassia mangiferae. The strucutres of 2 and 3 were determined by analysis of NMR spectroscopic data. Both 2 and 3 exhibit inhibitory activity (IC_{50} = 11 and 12 μM, respectively) in the phospholipase D (PLD) assay.*

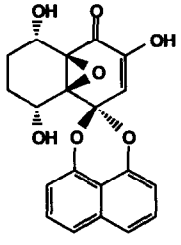
Previous studies of the fungus, *Nattrassia mangiferae*;[1] as potential source of new natural products with antitumor effects and other bioactivities led to the isolation of Sch 49210 (**1**) and its related analogues,[2,3] of which contain undescribed polycyclic keto-epoxide ring systems. Further studies of *N. mangiferae* metabolites enable us to discover two additional novel compounds, Sch 49211 (**2**) and Sch 49212 (**3**), with inhibitory activity in the phospholipase D (PLD) assay. In this paper we describe the isolation, structure determination and bioactivity of **2** and **3**.



Sch 49210(1)



Sch 49211(2)



Sch 49212(3)

The ethyl acetate extract from a 40 L fermentation broth was subject to XAD-16 chromatography eluting with gradient aqueous MeOH. The combined active fractions (80-100% MeOH eluate) were consecutively chromatographed over HP-20 column with gradient aqueous MeOH, and CH_3CN, respectively, to obtain pure **1** (95 mg) and a complex containing **2** and **3**. The complex was further purified by semi-preparative reversed-phase HPLC[4] to afford pure **2** (24 mg) and **3** (17 mg) as white solids with mp = 117-119°C and 114-115°C, respectively.

The molecular formula of $C_{20}H_{16}O_7$ for **3** was determined by HREIMS (calcd : 368.0896, found =

368.0899) and ^{13}C NMR data, which is identical to that of **1**.[2] Both UV (max. absorptions 225 and 298 nm) and IR (3449 and 1710 cm^{-1}) spectra of **3** were found to be resemble to **1** indicating the presence of a naphthalene unit, as well as hydroxyl and carbonyl functionalities. As expected, the ^{1}H NMR data (Table 1)

Table 1. ^{1}H (300 MHz) and ^{13}C (75 MHz) NMR data for **2** and **3**[a]

	2		**3**	
position	^{1}H	^{13}C	^{1}H	^{13}C
1	--	186.0 s[b]	--	183.6 s
2	--	133.7 s	--	133.6 s
3	6.79 (s)	133.9 d	6.73 (s)	133.5 d
4	--	96.81 s	--	96.89 s
5	5.54 (m)	60.20 d	5.06 (m)	60.26 d
6	5.86 (dd, 2.1, 10.5)	123.6 d	1.58-1.96 (m)	21.35 t
7	5.88-5.99 (m)[c]	128.3 d	1.55-2.10 (m)	23.18 t
8	5.07 (m)	60.34 d	4.80 (m)	61.20 d
9	--	60.59 s	--	61.30 s
10	--	63.51 s	--	64.40 s
1'	--	143.9 s	--	144.3 d
2'	7.61 (d, 8.5)	122.0 d	7.60 (d, 8.5)	121.5 d
3'	7.49 (t, 8.0)	127.2 d	7.47 (t, 7.8)	127.1 d
4'	7.02 (d, 7.2)	109.7 d	6.99 (d, 7.1)	109.4 d
5'	7.16 (d, 7.2)	110.6 d	7.14 (d, 7.1)	110.3 d
6'	7.53 (t, 8.0)	127.3 d	7.53 (t, 7.8)	127.1 d
7'	7.64 (d, 8.5)	121.5 d	7.62 (d, 8.5)	121.2 d
8'	--	145.0 s	--	145.2 s
9'	--	112.4 s	--	112.3 s
10'	--	132.1 s	--	131.9 s

a. Recorded in $CDCl_3$, chemical shifts in ppm from TMS, coupling constant (Hz).

b. Multiplicity was determined by APT or DEPT data.

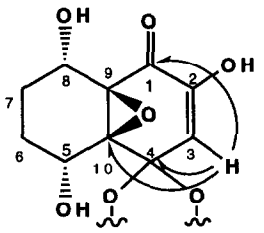
c. After CD_3OD exchange, the multiplet was identified as (dddd, 2.1, 4.8, 10.5).

revealed close similarities between the two compounds due to the same pattern of proton signals, including the six naphthalene protons at δ 6.99-7.62, two hydroxyl methine resonances at δ 4.80 and 5.06, and four methylene protons at δ 1.55-2.10. A difference, however, should be noted that two epoxide proton doublets of **1** were replaced by a vinyl proton singlet at δ 6.73 in **3**. This evidence strongly suggested the formation of a vinyl hydroxyl functional group by opening the epoxide ring at position-2,3 in **3**. The assignment was further supported by ^{13}C NMR data based on the observation of two olefinic carbons at δ 133.5 and 133.6 in the absence of two oxymethine carbons. However, these data did not provide sufficient information for an unambiguous assignment of the position of hydroxyl group. To establish the regiochemistry, selective INEPT experiments were performed. Selective INEPT correlations of the vinyl proton at δ 6.73 with signals for carbons 1, 4 and 10 indicated that the proton must be located at position-3 shown in Fig. 1. The remaining

carbon NMR assignments (Table 1) were determined with the aid of APT and selective INEPT experiments. The stereochemistry of **3** was established based on NOESY experiment (see Fig. 2) by the observation of a weak correlation from H-8 to H-5 due to the 1,4-diaxial interaction between these two protons. The configuration of H-8 was presumed to be analogous to that of **1**.

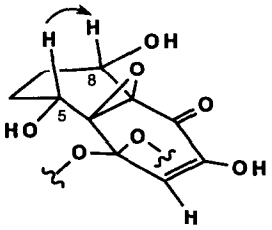
The molecular weight of **2** was found to be 366, two units less than **3**, based on CIMS data (m/z 367, $M+H^+$). The molecular formula, $C_{20}H_{14}O_7$, was deduced from HREIMS (calcd: 366.0740, found: 366.0743) and ^{13}C NMR data. Besides the identical UV and IR spectra, ^1H and ^{13}C NMR spectra of **2** contained signals that matched closely with those for **3**. The only differences were the appearence of two vinyl proton multiplets at δ 5.86-5.99 in **2** to replace four methylene protons of **3**, and the presence of two olefinic carbon resonances at δ 123.6 and 128.3 instead of two upfield signals accounting for methylene carbons. These observations suggested the formation of an unsaturated 1,4-diol system at position-6,7 in **2** by comparison to the saturated 1,4-diol of **3**.

Fig.1 The regiochemistry of **3** as revealed by SINEPT experiments



3

Fig. 2 Important NOESY data for **3**



3

Compounds **2** and **3** were tested for inhibition of (PLD) activation in HL-60 cells. *In vitro* bioactivity study showed the IC_{50} values of **2** and **3** to be 11 and 12 μM in the *f*MLP-stimulated PLD assay,[5] respectively. Since the major component **1** also demonstrated potent *in vitro* antitumor activity in the invasion chamber assay against HT 1080 human tumor cells, both compounds will be further evaluated in tumor biology. Detailed biological activity will be reported elsewhere.[6]

Acknowledgements:

Authors thank Mr. F. Gentile for fermentation work, Mr. Y. H. Ing for MS data, Ms. E. Dobek-Frank for the PLD assay data, Dr. C. Blood and Ms. J. Hailey for the invasion chamber assay data.

References and Notes:

1. The fungus was supplied by Dr. B. Katz from MYCOsearch Lab.
2. Chu, M.; Truumees, I.; Patel, M. G.; Gullo, V.P.; Blood, C.; King, I.; Pai, J-K. and Puar, M. S. *Tetrahedron Lett.* **1994**, *35*,1343~1346.
3. Chu, M.; Truumees, I.; Patel, M. G.; Gullo, V.P. and Puar, M. S. *J. Org. Chem.* **1994**, *59*,1222~1223.
4. HPLC conditions: YMC-ODS 20 x 500 mm column, irregular 15 μ particles, linear gradient 60-90% aqueous MeOH in 20 min, 12 mL/min flow rate, UV detection at 320 nm. The optical rotations for **2** and **3** were observed as $[\alpha]^{23}_D$ + 431.0°(c 0.2, $CHCl_3$) and $[\alpha]^{23}_D$ + 298.5° (c 0.2, $CHCl_3$), respectively.
5. A protocol of the PLD assay is described as follows: the assay mixtures, containing 10^7 prelabeled cell, 1.5 mM $CaCl_2$ and 5 μM cytochalasin B in a total volume of 450 μL Hepes-saline BSA buffer, were incubated (37°C) for 5 min before initiating the reaction by adding fMLP (100 nM) in a volume of 50 μL. After 2 min incubation, the reaction was stopped by adding chloroform:methanol:acetic acid mixture (100:200:40, by volume). The phases were separated by the procedure of Bligh and Dyer (Cf. Bligh, E. G. and Dyer, W. J. A rapid method of total lipid extraction and purification. *Canadian Journal of Biochemistry and Physiology*, **1959**, *37*, 911). One mL of water phase was transfered to vials and counted for radioactivity. Recovery of [^{3}H]-choline in the water phase indicates the induction of PLD activity. fMLP and drugs were initially dissolved in dimethylsulfoxide and then diluted with buffer to appropriate concentrations. The final concentration of dimethylsulfoxide in the assay did not exceed 0.1%, which had no discernible effects on the parameters measured.
6. A full paper detailing the taxonomy, fermentation, isolation and biological activity of keto-epoxide components is in preparation.

(*Received in USA* 18 *April* 1994; *accepted* 12 *May* 1994)