NEW CYTOTOXIC BENZOPYRANS SYNTHESIZED BY MICHAEL TYPE AND NITRATION REACTIONS FROM MALLOAPELTA B

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ABSTRACT

Four new derivatives of Malloapelta B 8-[1'-oxo-3'(R)-methoxy-butyl]-5,7-dimethoxy-2,2-dimethyl-2H-1-benzopyran (1), 8-[1'-oxo-3'(R)-ethoxy-butyl]-5,7-dimethoxy-2,2-dimethyl-2H-1-benzopyran (2), 8-[1'-oxo-3'(R)-propoxy-butyl]-5,7-dimethoxy-2,2-dimethyl-2H-1-benzopyran (3), 8-[1'-oxo-3'(R)-isopropoxy-butyl]-5,7-dimethoxy-2,2-dimethyl-2H-1-benzopyran (4) have been synthesized based on Michael type reaction, and a new compound 8-(1'-oxo-2'-en-butyl)-5,7-dimethoxy-3-nitro-2,2-dimethyl-2H-1-benzopyran (5) was synthesized rely on Nitration reaction from Malloapelta B. Their structures were determined by detailed analysis of the1D- and 2D-NMR such as ^{1}H-, ^{13}C-NMR, DEPT 90, DEPT 135, HSQC, HMBC, and by the Electronspray Ionization (ESI) mass spectrum. Compound 1-5 ixhibited considerable cytotoxic effect against two human cancer cell lines as human hepatocellular carcinoma (Hep-2) and rhabdosarcoma (RD) with the 50% inhibition concentration (IC_{50}) values in the range 0.62-4.32 μg/ml by in vitro assay.

I. INTRODUCTION

In previous papers, we have reported the isolation of the new inhibitor of NF-κB activation Malloapelta B (**1**) from a Vietnamese traditional medicinal plant *Mallotus apelta* (Lour.) Muel.-Arg. (Kiem *et al.*, 2005a; Minh *et al.*, 2005). On going to investigate the relationships between the structures and their cytotoxicities, we report herein the synthesis of five new benzopyran derivatives base on Michael type and nitration reaction, and the determination of their structures. The *in vitro* cytotoxic efficacy of these compounds against two human cancer cell lines, namely, human hepatocellular carcinoma and rhabdosarcoma was investigated.

II. MATERIALS AND METHODS

Material

Malloapelta B was isolated from *Mallotus apelta* (Lour.) Muel.-Arg. The reagents were purchased from Aldrich Co. Solvents were distilled prior to use.

General Experimental Procedures

The Electron Spray Ionization (ESI) mass spectrum was obtained using a AGILENT 1100 LC-MSD Trap spectrometer. The ^{1}H-NMR (500 MHz) and ^{13}C-NMR (125 MHz) spectra were recorded on a Bruker AM500 FT-NMR spectrometer using TMS as the internal standard. Column chromatography (CC) was performed on silica gel (Kieselgel 60, 70-230 mesh and 230-400 mesh, Merck). TLC was performed with Thin layer Art 5562 DC-Alurolle Kieselgel made by Merck Co.. Optical rotations were determined on a JASCO DIP-1000 KUY polarimeter.

General method for the synthesis of compounds 1-4

The mixture of 0.5 mmol malloapelta B and 0,6 mmol of the corresponding alcohols (methanol, ethanol, propanol, isopropanol) were added in a 40ml round-flask with a magnetic stirrer. 6 gram of the dried and powdered catalyst mixture (3 gram KF and 3 gram Al_2O_3) together with 1 ml diethylamine were added slowly in the reaction. After two-hours stirring, the mixture was maintained at room temperature for 2h, filtered the catalyst and removed the solvent to afford the residue, that was then purified by column chromatography over silica gel eluted with *n*-hexane-acetone (4:1) to give corresponding compounds **1** (135 mg, 82%), **2** (130 mg, 76%), **3** (116 mg, 65%) and **4** (152 mg, 85%) as colorless oils.

Method for the synthesis of compound 5

150 mg (0.5 mmol) of malloapelta B was dissolved into 5 ml $CHCl_3$ in a 25 ml round flask. The nitration reagent, a mixture of 52 µl HNO_3 68% and 57 µl H_2SO_4 98% were dissolved in 5 ml $CHCl_3$, which was then dropped slowly into the reaction mixture and maintained at 0 – 5°C by ice water. The reaction mixture was further stirred one hour at room temperature, then was extracted with $NaHCO_3$ 10% (3 x 5 ml). The $CHCl_3$ extract was dried by Na_2SO_4. After removing the solvent, a residue was obtained, which was then purified by column chromatography over silica gel eluted with the n-hexane : ethyl acetate (4:1) solvent system to give compound **5** (51 mg, yield 30%) as yellow crystals.

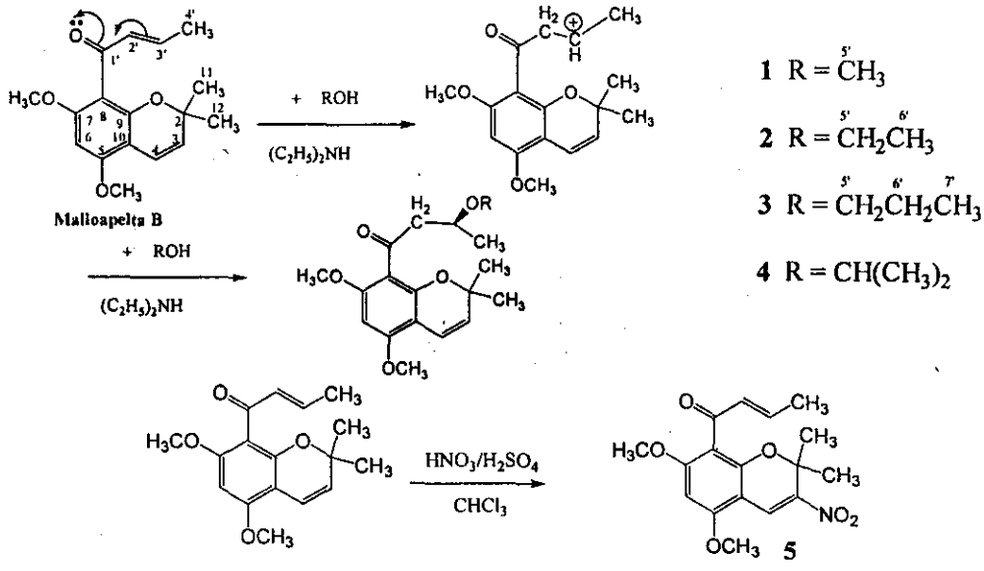

Fig. 1. The synthesis processings of compounds 1-5

8-[1'-oxo-3'(*R*)-methoxy-butyl]-5,7-dimethoxy-2,2-dimethyl-2H-1-benzopyran (1).

Colourless oil, $[\alpha]_D^{25}$ - 5.7° ($CHCl_3$, *c* 0.5); positive ESI-MS (*m/z*) 320 $[M+H]^+$ ($C_{18}H_{25}O_5$); ^{1}H-NMR (500MHz, $CDCl_3$), δ (ppm): 5.54 (d, *J* = 10.0 Hz, H-3), 6.56 (d, *J* = 10.0 Hz, H-4), 6.29 (s, H-6), 1.38 (s, 6H, H-11, H-12), 2.69 (dd, *J* = 16.0, 7.5 Hz, H_a-2'), 3.01 (dd, *J* = 16.0, 5.0 Hz, H_b-2'), 3.78 (m, *J* = 3'), 1.15 (d, *J* = 6.5 Hz, H-4'), 3.23 (s, H-5'), 3.81 (s, 7-OCH_3), 3.88 (s, 5-OCH_3).

^{13}C-NMR (125MHz, $CDCl_3$), δ (ppm): 77.4 (C-2), 127.4 (C-3), 117.2 (C-4), 157.5 (C-5), 89.3 (C-6), 158.7 (C-7), 114.4 (C-8), 151.9 (C-9), 104.6 (C-10), 27.8 (C-11, C-12), 200.6 (C-1'), 52.6 (C-2'), 73.8 (C-3'), 20.1 (C-4'), 56.1 (C-5'), 56.2 (5-OCH_3), 56.3 (7-OCH_3).

8-[1'-oxo-3'(*R*)-ethoxy-butyl]-5,7-dimethoxy-2,2-dimethyl-2H-1-benzopyran (2)

Colourless oil, $[\alpha]_D^{25}$ - 8.4° ($CHCl_3$, *c* 0.5); positive ESI-MS (*m/z*) 335 $[M+H]^+$ ($C_{19}H_{27}O_5$). ^{1}H-NMR (500MHz, $CDCl_3$), δ (ppm): 5.44 (d, *J* = 10.5 Hz, H-3), 6.56 (d, *J* = 10.5 Hz, H-4), 6.19 (s, H-6), 1.39 (6H, s, H-11, H-12), 3.11 (dd, *J* = 16.5, 5.5 Hz, H_a-2'), 2.84 (dd, J =16.5, 7.0 Hz, H_b-2'), 4.00 (m, H-3'), 1.23 (d, *J* = 6.0 Hz, H-4'), 3.48 (m, H-5'), 1.16 (t, *J* = 6.5 Hz, H-6'), 3.79 (s, 7-OCH_3), 3.83 (s, 5-OCH_3).

^{13}C-NMR (125MHz, $CDCl_3$), δ (ppm): 76.8 (C-2), 126.6 (C-3), 116.3 (C-4), 156.7 (C-5), 87.8 (C-6), 157.8 (C-7), 111.7 (C-8), 151.7 (C-9), 104.2 (C-10), 27.8 (C-11, C-12), 201.8 (C-1'), 52.1 (C-2'), 71.7 (C-3'), 20.5 (C-4'), 63.8 (C-5'), 15.6 (C-6'), 55.7 (5-OCH_3), 55.8 (7-OCH_3).

8-[1'-oxo-3'(*R*)-propoxy-butyl]-5,7-dimethoxy-2,2-dimethyl-2H-1-benzopyran (3)

Colourless oil, $[\alpha]_D^{25}$ - 8.1° ($CHCl_3$, *c* 0.5); positive ESI-MS (*m/z*) 349 $[M+H]^+$ ($C_{20}H_{29}O_5$). ^{1}H-NMR (500MHz, $CDCl_3$), δ (ppm): 5.44 (d, *J* = 10.5 Hz, H-3), 6.55 (d, *J* = 10.5 Hz, H-5), 5.99 (s, H-6), 1.39 (6H, s, H-11, H-12), 3.13 (dd, *J* = 16.5, 5.5 Hz, H_a-2'), 2.81 (dd, J =16.5, 7.0 Hz, H_b-2'), 3.99 (m, H-3'), 1.21 (d, *J* = 6.5 Hz, H-4'), 3.37 (2H, m, H-5'), 1.52 (2H, m, H-6'), 0.88 (3H, s, H-7'), 3.77 (s, 7-OCH_3), 3.82 (s, 5-OCH_3).

^{13}C-NMR (125MHz, $CDCl_3$), δ (ppm): 76.9 (C-2), 126.6 (C-3), 116.3 (C-4), 157.8 (C-5), 87.8 (C-6), 156.7 (C-7), 113.4 (C-8), 151.7 (C-9), 104.1 (C-10), 27.8 (C-11), 27.7 (C-12), 201.7 (C-1'), 52.1 (C-2'), 71.8 (C-3'), 20.5 (C-4'), 70.4 (C-5'), 23.3 (C-6'), 10.6 (C-7'), 55.7 (5-OCH_3), 55.8 (7-OCH_3).

8-[1'-oxo-3'(*R*)-isopropoxy-butyl]-5,7-dimethoxy-2,2-dimethyl-2H-1-benzopyran (4)

Colourless oil, $[\alpha]_D^{25}$ - 12.8° ($CHCl_3$, *c* 0.5); positive ESI-MS (*m/z*) 349 $[M+H]^+$ ($C_{20}H_{29}O_5$). ^{1}H-NMR (500MHz, $CDCl_3$), δ (ppm): 5.44 (d, *J* = 10.5 Hz, H-3), 6.56 (d, *J* = 10.5 Hz, H-5), 5.99 (s, H-6), 1.39 (6H, s, H-11, H-12), 3.08 (dd, *J* = 16.5, 5.5 Hz, H_a-2'), 2.85 (dd, J =16.5, 7.0 Hz, H_b-2'), 4.09 (m, H-3'), 1.21 (d, *J* = 6.5 Hz, H-4'), 3.69 (2H, m, H-5'), 1.11 (d, *J* = 6.0 Hz, H-6'), 1.34 (d, J 6.0 Hz, H-7'), 3.77 (s, 7-OCH_3), 3.82 (s, 5-OCH_3).

^{13}C-NMR (125MHz, $CDCl_3$), δ (ppm): 76.7 (C-2), 126.6 (C-3), 116.3 (C-4), 157.8 (C-5), 87.8 (C-6), 156.7 (C-7), 113.5 (C-8), 151.7 (C-9), 104.2 (C-10), 27.8 (C-11, C-12), 201.9 (C-1'), 52.8 (C-2'), 69.3 (C-3'), 21.5 (C-4'), 69.2 (C-5'), 22.8 (C-6', C-7'), 55.7 (5-OCH_3), 55.8 (7-OCH_3).

8-(1'-oxo-2'-en-butyl)-5,7-dimethoxy-3-nitro-2,2-dimethyl-2H-1-benzopyran (5)

Yellow crystals, mp. 256-257°C, $[\alpha]_D^{25}$ - 47.2° ($CHCl_3$, *c* 0.5); positive ESI-MS (*m/z*) 334 $[M+H]^+$ ($C_{17}H_{20}NO_6$). ^{1}H-NMR (500MHz, DMSO-d_6), δ (ppm): 7.87 (s, H-4), 6.45 (s, H-6), 1.26 (6H, s, H-11, H-12), 6.27 (dd, *J* = 16.0, 1.5 Hz, H-2'), 6.58 (dq, *J* = 16.0, 7.0 Hz), 1.87 (dd, J =7.0, 1.5 Hz), 3.32 (s, 6H, 5-OCH_3, 7-OCH_3).

^{13}C-NMR (125MHz, DMSO-d_6), δ (ppm): 78.6 (C-2), 142.7 (C-3), 124.2 (C-4), 159.2 (C-5), 90.0 (C-6), 162.5 (C-7), 110.8 (C-8), 151.3 (C-9), 101.2 (C-10), 25.3 (C-11, C-12), 191.6 (C-1'), 133.5 (C-2'), 146.8 (C-3'), 17.9 (C-4'), 56.4 (7-OCH_3), 56.5 (5-OCH_3).

III. RESULTS AND DISCUSSION

Compound **1-4** were obtained as colorless oils from the Michael type reaction with corresponding alcohols (methanol, ethanol, propanol, isopropanol, respectively) after being purified by column chromatography over silica gel. Compound **5** was obtained as yellow crystals from the nitration reaction. The synthesis processings (Son *et al.*, 1976; Chau, 2003) were illustrated in Fig. 1.

The NMR spectra of compounds **1-4** were similar to those of malloapelta B (Kiem *et al.*, 2005a), except for the NMR data of the carbonyl moiety. The absence of the double bond at δ_C 134.8 /144.8 and δ_H 6.38/6.69 together with the appearance of the signals, which correspond to methanol, ethanol, propanol, isopropanol, respectively, suggesting the methoxyl, ethoxyl, propanoxyl and isopropanoxyl groups were attached to the double bond. The ^{1}H-NMR of **1-4** exhibited the same two doublet of doublet signals at δ_H 2.69 / 3.01 (for **1**), 2.84/3.11 (for **2**), 2.81/3.12 (for **3**) and 2.85/3.08 corresponding to the H-2', and the multiplets at δ_H 3.78, 3.48, 3.99 and 4.09 of the methine as well as the doublets at δ_H 1.15, 1.23, 1.21 and 1.21 (J = 6.5 Hz) confirmed a partial structure of $-CH_2-CH(CH_3)-$. In addition, the appearances of the methoxyl [δ_H 3.23 (s)/δ_C 56.1 (q)], ethoxyl [δ_H 3.48 (m), 1.16 (t)/ δ_C 63.8 (t), 15.6 (q)], propanoxyl [δ_H 3.37(m) , 1.52 (m), 0.88 (t)/δ_C 70.4 (t), 23.3 (t), 10.6 (q)] and isopropanoxyl [δ_H 3.69 (m), 1.11 (d), 1.34 (d)/ δ_C 69.2 (d), 2 x 22.8 (q)] signals in the NMR spectra of **1-4**, respectively, as well as the spin-coupling type of the protons H-2', H-3', H-4' confirmed that the methoxyl, ethoxyl, propanoxyl and isopropanoxyl groups must be connected to C-3' of **1-4**, respectively. Furthermore, the positive ESI-MS pectra of **1-4** showed the quasi ion peaks at *m/z* 320 $[M+H]^+$, 335 $[M+H]^+$, 349 $[M+H]^+$ and 349 $[M+H]^+$, correspond to the molecular formula of $C_{18}H_{24}O_5$, $C_{19}H_{26}O_5$, $C_{20}H_{28}O_5$, $C_{20}H_{28}O_5$ of compounds **1-4**, respectively. The stereochemistry at C-3' of **1-4** were suggested to be (*R*) by comparing the chemical shifts and proton coupling constants of **1-4** with those of 6-(methyl 1'-oxo-3'-hydroxy-buthyl ete)-5,7-dimethoxy-2,2-dimethyl-2H-1-benzopyran and 6-(1'-oxo-3'-hydroxy-butyl)-5,7-dimethoxy-2,2-dimethyl-2H-1-benzopyran (Kiem *et al.*, 2005b), and with the similar structure reported in the literature (Hori *et al.*, 1990). Obviously, the structures of **1-4** were determined to be 8-[1'-oxo-3'(*R*)-methoxy-butyl]-5,7-dimethoxy-2,2-dimethyl-2H-1-benzopyran (**1**), 8-[1'-oxo-3'(*R*)-ethoxy-butyl]-5,7-dimethoxy-2,2-dimethyl-2H-1-benzopyran (**2**), 8-[1'-oxo-3'(*R*)-propoxy-butyl]-5,7-dimethoxy-2,2-dimethyl-2H-1-benzopyran (**3**), 8-[1'-oxo-3'(*R*)-isopropoxy-butyl]-5,7-dimethoxy-2,2-dimethyl-2H-1-benzopyran (**4**).

Comparison the NMR spectra of **5** with those of malloapelta B (Kiem *et al.*, 2005a) showed the differences at C-3 and C-4. The absence of the H-3 proton signal together with the resonance of H-4 proton at δ 7.87 as a singlet suggested the NO_2 group attached to C-3. To confirm this, the HMQC and HMBC spectra were taken. In the HMBC, the H-C long-range correlations were observed between H-4 (δ 7.87) and carbons C-10 (δ 151.3)/C-5 (δ 159.2) confirming the position of H-4 proton, and that the NO_2 group must be attached to C-3. Morever, the positive ESI-MS spectrum of **5** showed a quasi ion peak at *m/z* 334 $[M+H]^+$, correspond to the molecular formula of $C_{17}H_{19}NO_6$. Thus, the structure of **5** was determined to be 8-(1'-oxo-2'-en-butyl)-5,7-dimethoxy-3-nitro-2,2-dimethyl-2H-1-benzo- pyran.

Cytotoxicity

The cytotoxic activities of compounds **1-5** were assayed on Hep-2 (human hepatocellular carcinoma) and RD (rhabdosarcoma) cells by SRB method (Lee *et al.*, 2003; Likhitwitayawuid *et al.*, 1993). As a result, compounds **1-5** showed significant cytotoxic activities with the IC_{50} values of 1.23 μg/ml, 2.81 μg/ml, 3.01 μg/ml, 4.22 μg/ml and 0.87 μg/ml against cancer cell line Hep-2, respectively, and with the IC_{50} values of 0.75 μg/ml, 1.78 μg/ml, 2.34 μg/ml, 3.75 μg/ml and 0.62 μg/ml against cancer cell line RD, respectively. However, all these compounds showed the higher IC_{50} values comparing with those of malloapelta B (Hep-2, IC_{50}: 0.49 μg/ml and RD, IC_{50}: 0.54 μg/ml). The observed results proposed that the lost conjunction effect between the carbonyl group and the double bond at C-2'/C-3' may reduce the cytotoxic activities, and that, the double bond at C-3/C-4 may not effect to the cytotoxic activities of these compounds.

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