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Antileukemic α-pyrone derivatives from the endophytic fungus *Alternaria phragmospora*

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Abstract

Four new (**1**–**4**) and two known (**5** and **6**) α-pyrone derivatives have been isolated from *Alternaria phragmospora*, an endophytic fungus from *Vinca rosea*, leaves. The isolated compounds were chemically identified to be 5-butyl-4-methoxy-6-methyl-2*H*-pyran-2-one (**1**), 5-butyl-6-(hydroxymethyl)-4-methoxy-2*H*-pyran-2-one (**2**), 5-(1-hydroxybutyl)-4-methoxy-6-methyl-2*H*-pyran-2-one (**3**), 4-methoxy-6-methyl-5-(3-oxobutyl)-2*H*-pyran-2-one (**4**), 5-(2-hydroxyethyl)-4-methoxy-6-methyl-2*H*-pyran-2-one (**5**), and 5-[(2*E*)-but-2-en-1-yl]-4-methoxy-6-methyl-2*H*-pyran-2-one (**6**). Compounds **2** and **4** showed moderate antileukemic activities against HL60 cells with IC_{50} values of 2.2 and 0.9 μM and against K562 cells with IC_{50} values of 4.5 and 1.5 μM, respectively.

Keywords

Antileukemic; α-Pyrone derivatives; *Alternaria phragmospora*; Endophytic fungi

Introduction

The endophytic fungi can be considered as an unexplored source for chemically novel and biologically active secondary metabolites in a wide variety of medical and agricultural areas.[1] The genus *Alternaria* was established in 1817, and contains 44 different species. In

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Supplementary data

Supplementary data (^{1}H, ^{13}C, and 2D NMR, spectra, and HR-ESI-MS of compounds **1**–**4**, X-ray supporting data for **1**, and the experimental section) associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.tetlet.2014.04.085.

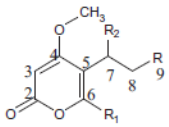
the last few decades more than 268 metabolites from different species of *Alternaria* fungi have been reported.[2] The isolated metabolites from *Alternaria* fungi were found to be belonging to several chemical classes including nitrogen-containing compounds,[3] steroids,[4] terpenoids,[5] pyranones,[6] quinones,[7] phenolics,[8] and various other classes.[2] The isolated metabolites showed different biological activities: antitumor, herbicide, antimicrobial, antimalarial, and antileishmanial.[2] However the endophytic fungus *Alternaria phragmospora* has not been examined phytochemically or biologically before. The crude ethyl acetate extract of the cultured endophytic fungus *A. phragmospora* showed antileukemic activities against Leukemic K562 cells and HL60 cells with IC_{50} values of 0.035 and 0.045 μg/ml, respectively. Consequently, it was chosen for further investigations seeking for isolation and identification of the secondary metabolites which may be responsible for this activity. Leukemic chronic K562 cells and acute HL60 cells have been chosen as models in this research. The K562 cell line is composed of undifferentiated blast cells that are rich in glycophorin and may be induced to produce fetal and embryonic hemoglobin in the presence of hemin, while the HL-60 is a promyelocytic leukemia cell line which in the presence of DMSO, matures into granulocytes and when exposed to phorbol esters, differentiate into nondividing mononuclear phagocytes.[9,10]

Results and discussion

In this study, phytochemical and biological investigations for the endophytic fungus are reported. The ethyl acetate extract was subjected to different chromatographic techniques which led to the isolation and structural elucidation of four new (**1**–**4**) and two known (**5** and **6**) α-pyrone derivatives. We herein report the isolation, structural elucidation, including relative and absolute configuration, and the results of some bioactivity tests of these compounds.

Compound **1** was isolated as white crystalline needles. The molecular formula was determined to be $C_{11}H_{16}O_3$ by HR-ESI-MS (+ve mode) showing molecular ion peak $[M+H]^+$ at *m/z* 197.1176 (calcd for $C_{11}H_{17}O_3$, 197.1178) indicating four degrees of unsaturation. The IR spectrum of **1** showed absorption bands at 1720, 1647, and 1563 cm^{-1} indicating the presence of a lactone group and α-pyrone ring. The ^{13}C NMR spectrum (Table 2) of compound **1** displayed 11 carbon resonances, while the DEPT 135 experiment sorted these signals into two methyl groups at (δ_C 13.8) and (δ_C 17.097), one methoxy group at (δ_C 55.9), three methylenes at (δ_C 22.4, 23.8, and 31.3), one methine at (δ_C 87.6), and four quaternary carbons signals, one of them was found to be an ester carbonyl at (δ_C 164.5) while the other three were found to be resonating at (δ_C 170.8, 157.8, and 111.6). By analysis of ^1H NMR (Table 1) and HMQC spectral data, compound **1** exhibited two signals for methyl groups [δ 0.86 (3H, t, (6.8), H-10) and δ 2.11 (s, 3H, H-11)], a signal of aromatic proton at δ 5.34 (s, H-3), a methoxy group signal at δ_H 3.72 (s), and three methylene group signals [δ 1.25 (m, 2H, H-9), 1.26 (m, 2H, H-8), and 2.21 (t, 7.6, 2H, H-7)]. The α-pyrone ring structure was confirmed by several HMBC correlations between H-3 and both of C-4 and C-5. HMBC spectrum confirmed the presence of a butyl side chain by showing some correlations between H-10 to both of C-9 and C-8, H-7 to both of C-8 and C-9. The attachment of butyl side chain at C-5 was confirmed from HMBC correlations of H-7 to C-4, C-5, and C-6. The attachment of the methoxy group was confirmed by a HMBC correlation

between the methoxy group and C-4. The structure of **1** was authenticated by 2D NMR experiments, giving pertinent COSY and HMBC correlations (Fig. 2). Compound **1** was suggested to be 5-butyl-4-methoxy-6-methyl-2*H*-pyran-2-one. The assigned chemical structure of **1** was confirmed by X-ray diffraction analysis. Crystals of **1** suitable for X-ray diffraction were obtained by slow evaporation of a solution of **1** in methanol–water (30:70). The final X-ray crystallographic model of **1** (Fig. 1) confirmed the structure of **1** as shown. The asymmetric unit contains two independent molecules, one of which (not shown) is disordered, with two conformations of the *n*-butyl substituent.



Compound	R	R1	R2
1	CH_2CH_3	CH_3	H
2	CH_2CH_3	CH_2OH	H
3	CH_2CH_3	CH_3	αOH
4	$COCH_3$	CH_3	H
5	OH	CH_3	H
6	$=CHCH_3$	CH_3	H

Compounds (**1**-**6**)

Compound **2** was isolated as yellowish needles. The molecular formula was determined to be $C_{11}H_{16}O_4$ by HR-ESI-MS (+ve mode) showing molecular ion peak $[M+H]^+$ at *m/z* 213.1133 (calcd for $C_{11}H_{17}O_4$, 213.1127) indicating four degrees of unsaturation. The IR spectrum of **2** absorption bands at 1720, 1647, and 1563 cm^{-1} indicated the presence of a lactone group and α-pyrone ring.

The ^1H and ^{13}C NMR spectral data (Tables 1 and 2) of compound **2** and those for **1** were found to be similar, with a difference observed in the chemical shift of carbon 11, the methyl group 11 in **1** of (δ_H 2.11, s, and δ_C 17.0) was replaced with oxygenated methylene (δ_H 4.41, s, and δ_C 58.5) in **2**. The structure of **2** was authenticated by 2D NMR experiments, giving pertinent COSY and HMBC correlations (Fig. 2). Compound **2** was identified to be 5-butyl-6-(hydroxymethyl)-4-methoxy-2*H*-pyran-2-one.

Compound **3** was isolated as white amorphous powder. The molecular formula was determined to be $C_{11}H_{16}O_4$ by HR-ESI-MS (+ve mode) showing molecular ion peak $[M+H]^+$ at *m/z* 213.1123 (calcd for $C_{11}H_{17}O_4$, 213.1127) indicating four degrees of unsaturation. The IR spectrum of **3** showed absorption bands at 1720, 1647, and 1563 cm^{-1} indicating the presence of a lactone group and α-pyrone ring. The ^1H and ^{13}C NMR spectral data (Tables 1 and 2) of compound **3** and those for **1** were found to be similar, with a difference observed in the chemical shift of carbon 7, The methylene group 7 in **1** of (δ_H 2.21, t, *J* 7.6, and δ_C 23.8) was replaced by oxygenated methine (δ_H 4.63, m and δ_C 68.6) in compound **3** indicating the oxygenation of C-7. The structure of **3** was authenticated by 2D NMR experiments, giving pertinent COSY and HMBC correlations (Fig. 2). The absolute configuration of **3** was assigned by application of the modified Mosher method.[11-13]

Treatment of **3** with (*S*)- and (*R*)-MTPA Cl afforded the (*R*)-MTPA ester (**3a**) and (*S*)-MTPA ester (**3b**), respectively. The difference in chemical shift values ($\Delta\delta$) ($\delta_S - \delta_R$) for the diastereomeric esters **3b** and **3a** was calculated in order to assign the absolute configuration at C-4. Calculation for all relevant signals suggested the (*R*) absolute configuration at C-7, as shown in Figure 3. Here in this research we report the first isolation for compound **3** from a natural source and the identification of its absolute configuration. Compound **3** was obtained before by a chemical reduction reaction for citrepyrone, an α-pyrone derivative isolated from *Penicillium citreoviride*[14] and another time by a chemical reduction reaction for pyrenocine C, a phytotoxin-related metabolite produced by onion pink root fungus, *Pyrenochaeta terrestris*.[15]

Compound **3** was identified to be (*R*)-5-(1-hydroxybutyl)-4-methoxy-6-methyl-*2H*-pyran-2-one.

Compound **4** was isolated as white amorphous powder. The molecular formula was determined to be $C_{11}H_{14}O_4$ by HR-ESI-MS (+ve mode) showing molecular ion peak $[M+H]^+$ at *m/z* 211.0966 (calcd for $C_{11}H_{15}O_4$, 211.0970) indicating four degrees of unsaturation. The IR spectrum of **4** absorption bands at 1720, 1647, and 1563 cm^{-1} indicated the presence of a lactone group and α-pyrone ring. The ^1H and ^{13}C NMR spectral data (Tables 1 and 2) of compound **4** were found to be similar to those of **1**, with a difference observed in the chemical shift of carbon 9. The methylene group 9 in **1** of (δ_H 1.25, m and δ_C 22.4) was replaced by a carbonyl group (δ_C 210.1) in compound **4**. The structure of **4** was authenticated by 2D NMR experiments, giving pertinent COSY and HMBC correlations (Fig. 2). Compound **4** was identified to be 4-methoxy-6-methyl-5-(3-oxobutyl)-2*H*-pyran-2-one.

Compound **5** was isolated as white amorphous powder and characterized by analysis of its spectroscopic data and by comparison with data reported in the literature[16] was found to be macommelinol [5-(2-hydroxyethyl)-4-methoxy-6-methyl-2*H*-pyran-2-one].

Compound **6** was isolated as white amorphous powder and identified by analysis of its spectroscopic data and by comparison with data reported in the literature[17] was found to be novae-zelandin A [5-[(2*E*)-but-2-en-1-yl]-4-methoxy-6-methyl-2*H*-pyran-2-one].

Compounds **2** and **4** showed moderate antileukemic activities against HL60 cells with IC_{50} values of 0.45 and 0.18 μg/ml and against K562 cells with IC_{50} values of 0.9 and 0.3 μg/ml, respectively, as shown in Table 3 and Figure 4

All the isolated compounds have been examined for antimicrobial, antimalarial, and antileishmania activities, but did not show any promising effects.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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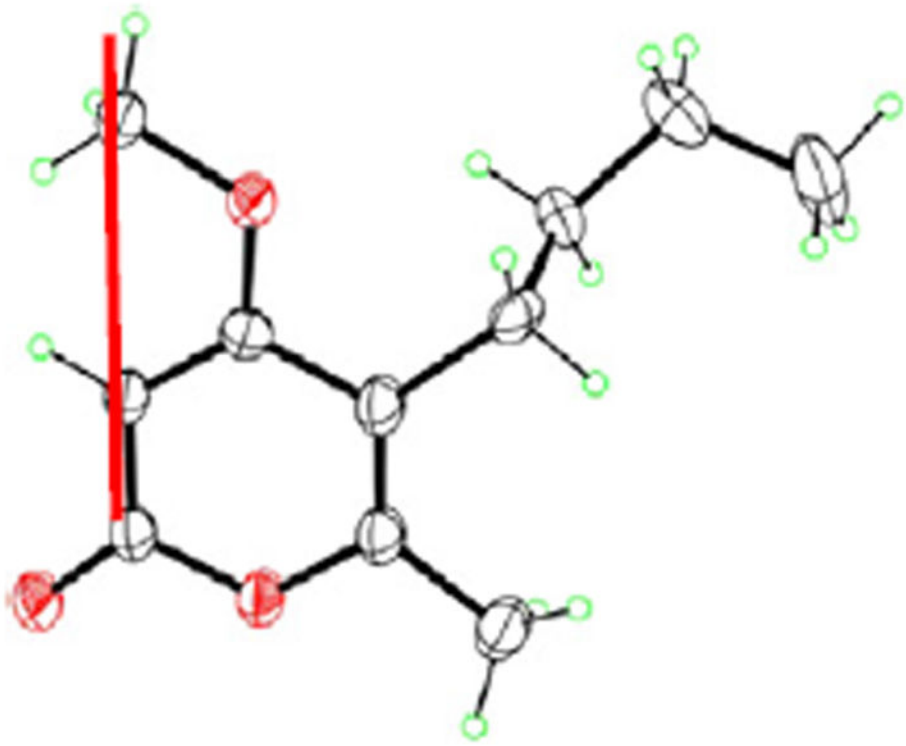

Figure 1.
X-ray structure of **1**.

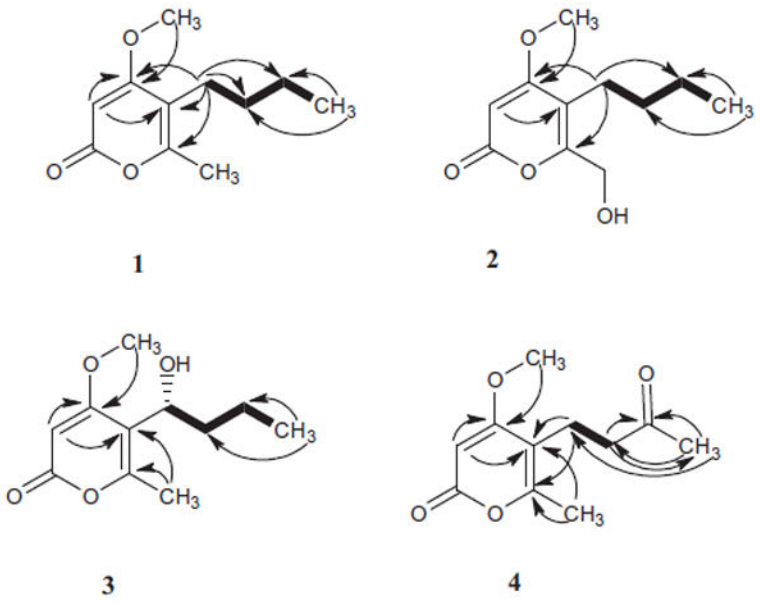

Figure 2.
Key HMBC ⌒ and H–H cosy ▬ correlations for (**1**–**4**).

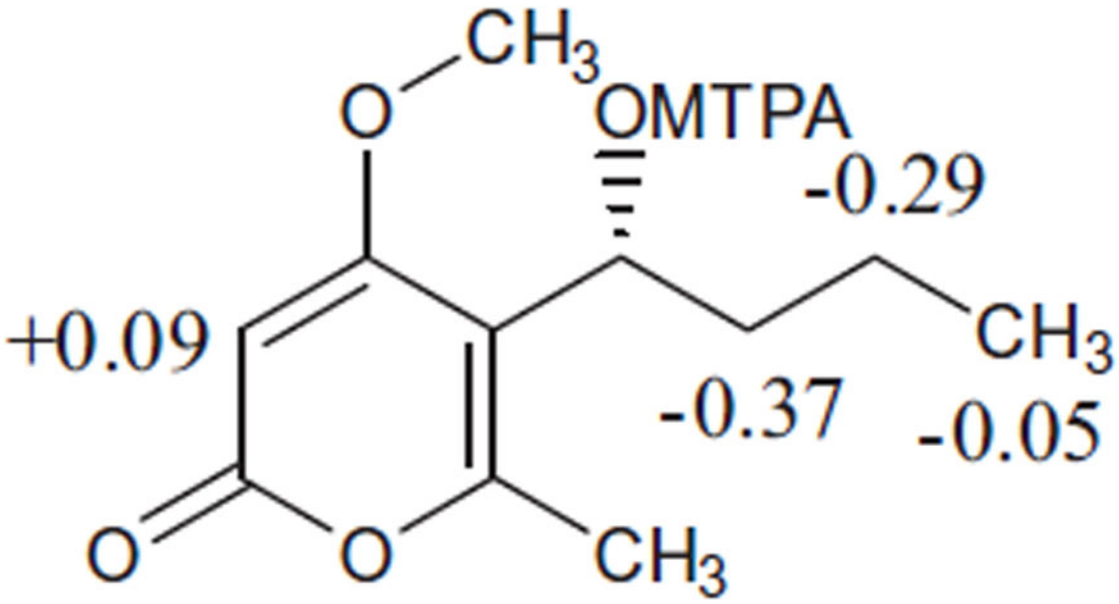

Figure 3.
$\Delta\delta\,(\delta_S - \delta_R)$ values (in ppm) for the MTPA ester of **3**.

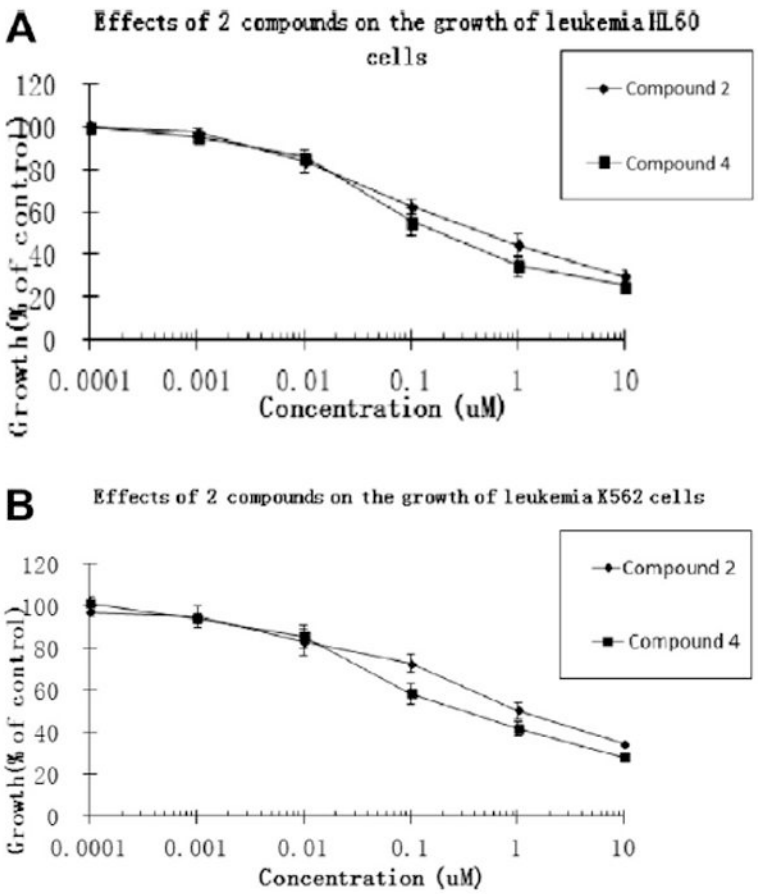

Figure 4.

The concentration-dependent effects of compounds **2** and **4** on the growth of acute leukemia HL60 (A) and chronic leukemia K562 (B) cells.

Table 1

^{1}H NMR spectroscopic data (400 MHz in $CDCl_3$) for (**1**–**4**)

Position	δ_H (*J* in Hz)			
	1	**2**	**3**	**4**
3	5.34, s	5.48, s	5.49, s	5.57, s
7	2.21, t (7.6)	2.35, t (7.6)	4.63, m	2.62, m
8	1.26, m	1.34, m	1.65, m	2.64, m
			8a: 1.84, m	
9	1.25, m	1.28, m	8b: 1.27, m	–
10	0.86, t (6.8)	0.86, t (7.2)	0.93, t (7.2)	2.14, s
11	2.11, s	4.41, s	2.27, s	2.28, s
O–CH_3	3.72, s	3.80, s	3.85, s	3.88, s

Table 2

^{13}C NMR spectroscopic data (400 MHz in $CDCl_3$) for (**1–4**)

Position	δ_C			
	1	**2**	**3**	**4**
2	164.5	164.8	163.8	167.0
3	87.6	89.1	89.0	88.2
4	170.8	170.9	170.4	172.8
5	111.6	113.4	113.1	112.0
6	157.8	158.1	159.0	160.1
7	23.8	23.3	68.6	19.4
8	31.3	32.0	39.2	42.6
9	22.4	22.4	19.4	210.1
10	13.8	13.8	13.4	29.5
11	17.0	58.5	17.8	17.0
O–CH_3	55.9	56.3	56.2	57.0

Table 3

Inhibitory effects of compounds **2** and **4** on the growth of human leukemia cells in vitro

Compounds	HL60 cells IC_{50} (μg/ml)	K562 cells IC_{50} (μg/ml)
2	0.45	0.9
4	0.18	0.3
Taxol	0.0003	0.0023