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Short communication

Two new substituted polychiral 5, 6-dihydro-α-pyrones from *Orthosiphon diffusus* and molecular docking studies

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ABSTRACT

Chemical investigation on *Orthosiphon diffusus* lead to isolation of two new substituted polychiral 5, 6-dihydro-α-pyrones, orthodiffenes E-F (**5-6**) which were characterized from the detailed studies of their 1D and 2D NMR spectra. Isolated molecules orthodiffenes E-F (**5-6**) along with previously isolated, reported orthodiffene A-D (**1-4**) molecules were subjected to *in silico* studies and analysed for anticancer target Topoisomerse I-B − DNA complex. Orthodiffene D and E are giving noteworthy observations while all isolated molecules are showing a pattern of binding in active pocket similar to each other however, different from Camptothecin, an anticancer agent. Orthodiffene A-C have comparable cytotoxicity to Camptothecin, which we have earlier reported.

1. Introduction

Orthosiphon is a genus of the family Lamiaceae. The species belong to this genus are annual or perennial herbs, distributed in different parts of India, particularly found in the Eastern and Western Ghats (Ambasta, 1986; Zaheer et al., 1966) of India. Various species of this genus are known for their medicinal properties like for the treatment of diseases like diabetes, hypertension, rheumatism, tonsillitis, menstrual disorder *etc.*, but the chemical studies on these species are limited (Ambasta, 1986). A small number of staminane type, migrated pimarane type and isopimarane type diterpenoids and flavonoids are mainly reported from *Orthosiphon stamineus* and *Orthosiphon aristatus* (Awale et al., 2002; Guerin et al., 1989; Indonesia, 1995; Masuda et al., 1992; Nguyen et al., 2004; Ohashi et al., 2000; Tailor and Wright, 1971; Tezuka et al., 2000). Earlier, we carried out the chemical investigation on *Orthosiphon diffusus* Benth. and reported rare novel polychiral furanopyrans, Orthodiffenes A-D (**1-4**) (Fig. 1) (Holla et al., 2011). In the same series, we found relatively less bioavailable two new polar substituted polychiral 5, 6-dihydro-α-pyrones, (**5** & **6**) (Fig. 1). Chemical investigation on this plant species is vital in identifying new class of anticancer molecules (Holla et al., 2011).

2. Result and discussion

The phytochemical studies in present work were conducted on whole plant parts, which after being dried and powdered extracted with (1:1) $CHCl_3$-MeOH. This extract was further fractionated and resulted in isolation of two newly substituted polychiral alpha pyrones. These molecules along with earlier reported four molecules (A-D) were subjected to molecular modelling to analyse their respective interaction with TOPO-I-DNA complex in comparison to its standard inhibitor Camptothecin.

2.1. Orthodiffene e (5)

Orthodiffene E (**5**) was isolated as viscous mass. Its molecular formula was derived to be $C_{22}H_{26}O_8$ from its LRESIMS (*m/z* 417.6 $[M]^+$, 435.9 $[M+NH_4]^+$) and analytical data. The IR spectrum indicated the presence of hydroxyl (3428 cm^{-1}) and carbonyl (1725 cm^{-1}) functionalities in the molecule. The structure of the compound was established from detailed analysis of its ^1H and ^{13}C NMR spectra including 2D NMR (^1H–^1H COSY, HSQC, HMBC and NOESY) experiments and mass spectral data. The ^1H NMR spectrum (Table 1) displayed signals for one secondary methyl (δ 1.32, 3H, d, J = 7.0 Hz), two *trans* olefinic protons (δ 5.90, 1H, dd, J = 16.0, 5.1 Hz and δ 5.88, 1H, dd, J = 16.0, 5.1 Hz) and two *cis* olefinic cinnamoyl type protons (δ 6.93, 1H, ddd,

Abbreviations: TOPO-I-DNA complex, Topoisomerase-I - DNA complex; LRESIMS, low resolution electrospray ionization mass spectrometry; NMR, nuclear magnetic resonance; COSY, ^1H-^1H correlation spectroscopy; HSQC, heteronuclear single-quantum correlation; NOESY, nuclear over hauser effect spectroscopy; HMBC, heteronuclear multiple bond correlation

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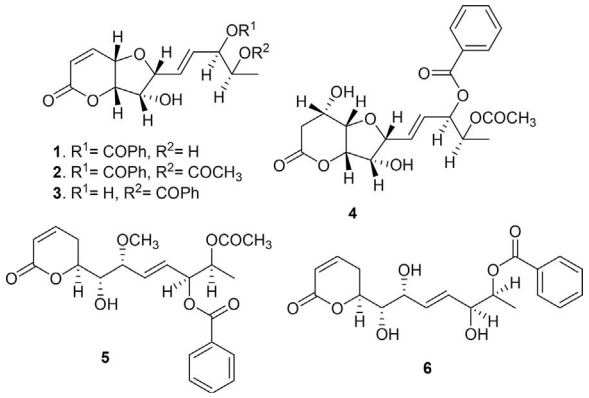

Fig. 1. Structures of isolated Orthodiffene A-D (**1-4**) & 5, 6-dihydro-α-pyrones, Orthodiffene E–F (**5-6**).

Table 1
NMR data for Orthodiffene E (**5**) and F (**6**) in $CDCl_3$.[a]

C/H	Orthodiffene (E) 5		Orthodiffene (F) 6	
	δH (mult, *J*, Hz)	δC	δH (mult, *J*, Hz)	δC
2	–	163.4	–	163.8
3	6.03 (dd, 9.6, 1.5)	121.1	5.99 (dd, 10.0, 1.5)	120.8
4	6.93 (ddd, 9.7, 5.8, 3.0)	145.3	6.9 (dd, 10.0, 4.1)	145.8
5a	2.5 (dt, 10.2, 4.4)	25.8	2.5 (m)	25.6
5b	2.56 (dt, 10.6, 4.6)			
6	4.48 (ddd, 11.0, 7.6, 4.0)	76.4	4.42 (m)	77.2
7	3.64 (br d, 6.2)	74.5	3.66 (br d)	74.8
8	4.03 (dd, 5.9, 3.0)	78.6	4.38 (m)	70.2
9	5.88 (dd, 16.0, 5.1)	131.4	5.92 (dd, 15.8, 4.9)	132.2
10	5.90 (dd, 16.0, 5.1)	129.1	5.95 (dd, 15.7, 4.9)	131.2
11	5.61 (dd, 5.0, 3.0)	75.2	4.30 (t, 3.2)	74.4
12	5.23 (m)	70.6	5.21 (m)	74.2
13	1.32 (d, 7.0)	15.3	1.36 (d, 7.0)	15.8
14	–	165.4	–	166.3
15	–	170.3	–	–
16	2.07 (s)	21.1	–	–
17	3.34 (s)	56.9	–	–
1′	–	130.3	–	130.0
2′, 6′	8.04 (d, 8.0)	129.6	8.02 (d, 7.9)	129.8
3′, 5′	7.46 (t, 8.0)	128.4	7.44 (t, 8.0)	128.2
4′	7.58 (t, 7.8)	133.2	7.56 (t, 7.8)	133.5

[a] Spectra were recorded at 300 MHz (^{1}H NMR) and 75 MHz (^{13}C NMR).

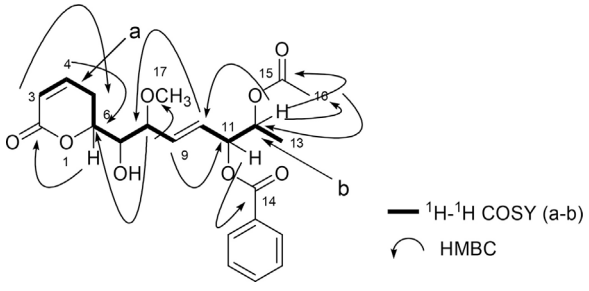

Fig. 2. ^{1}H-^{1}H COSY and HMBC correlations for **5**.

J = 9.7, 5.8, 3.0 Hz and *δ* 6.03, 1H, dd, *J* = 9.6, 1.5 Hz), five phenyl protons (*δ* 8.04, 2H, d, *J* = 8.0 Hz; 7.58, 1H, t, *J* = 7.8 Hz and *δ* 7.46, 2H, t, *J* = 8.0 Hz), two diastereotopic methine protons (*δ* 2.5, 1H, dt, *J* = 10.2, 4.4 Hz & *δ* 2.56, 1H, dt, *J* = 10.6, 4.6 Hz) and five methine protons (*δ* 3.64, 1H, br d, *J* = 6.2 Hz; 4.03, 1H, dd, *J* = 3.0 Hz; 4.48, 1H, ddd, *J* = 11.0, 7.6, 4.0 Hz; 5.23, 1H, m; 5.61, 1H, dd, *J* = 5.0, 3.0 Hz). These five methine protons are attached to heteroatom oxygen, which was confirmed by their corresponding ^{13}C signals at *δ* 74.5, 78.6, 76.4, 70.6, 75.2 and HSQC experiment. The ^{13}C signals *δ* 163.4 165.4, 170.3 revealed possibility of the presence of, three ester carbonyls carbons in the compound. The spectral data suggested that the structure of **5** was closely related to Orthodiffene B (**2**) (polychiral furanopyran compounds) (Fig. 1) except for the opening of the furan ring at C-5 *i.e.* the presence of only 5, 6 -dihydropyrone skeleton with substitution at C-6 (Fig. 2).

From the detailed examination of the ^{1}H NMR, COSY and HMBC it was possible to establish the skeleton of compound **5** as polychiral pyrone skeleton. From the ^{1}H-^{1}H COSY it was possible to establish two proton sequences H-3 to H-9 (a) and H-10 to H-13 (b) (Fig. 2). The HMBC experiment was used to connect the sequences. The HMBC experiment showed a clear correlation of H-6 with C-2 (*δ* 163.4), H-3 with C-5 (*δ* 25.8) and H-4 with C-6 (*δ* 76.4), which confirmed the presence of δ- lactone ring *i.e.* 5, 6-dihydro pyrone portion. Further, correlations of H-10 with C-8 (*δ* 78.6) and H-9 with C-7 (*δ* 74.5) confirmed C-8 and C-7 as allylic and homoallylic carbons, while C-9 (*δ* 131.4) & C-10 (*δ* 129.1) were confirmed to be *trans* olefinic carbons from their ^{13}C values, coupling constants and respective HMBC correlations from H-8. The correlation of H-8 with C-6 (*δ* 76.4) confirmed the attachment of aliphatic chain to δ- lactone skeleton at C-6, while correlation with C-17 (*δ* 56.9) confirmed the attachment of methoxy group at C-8. This much information by HMBC, ^{13}C value for C-5 (*δ* 25.8) and presence of methoxy functionality at C-8 proved compound **5** to be 5, 6- dihydro-α-pyrone with substitution by aliphatic carbon chain at C-6.

Further HMBC experiment depicted the correlations of H-9 with C-11 (*δ* 75.2) and H-11 with C-14 (*δ* 165.4) *i.e.* benzoyl carbonyl, which confirmed the attachment of benzoyl ester functionality at C-11 which was allylic carbon in the side chain. Correlations of H-12 with C-13 (*δ* 15.3) and C-10 (*δ* 129.1) confirmed the attachment of H-12 to homoallylic carbon in the side chain. H-12 also showed a correlation with C-15 (*δ* 170.3) *i.e.* acetate carbonyl, and acetate CH_3 protons showed correlation with C-12 (*δ* 70.6), which has justified the position of acetate functionality at C-12. The hydroxyl functionality at C-7 & C-8 was confirmed by the characteristic oxymethine ^{13}C values and justifying mass values.

Assigning the relative stereochemistry for Orthodiffene E (**5**) was a demanding task with multiple chiral centres, poorly resolved signals and limited bioavailability of pure isolated compound. Probable relative stereochemistry at C-6, C-7 and C-8 is proposed based on coupling values and literature precedent. Comparison of the coupling constant values for H-6, H-7 & H-8 ($J_{6,5}$ = 11.0, $J_{6,7}$ = 7.6, $J_{8,7}$ = 3.0) with the reported 6-substituted 5, 6- dihydro-α-pyrones by Pereda-miranda et al., supported a pseudo-equatorial orientation for the side chain at C-6, with H-6 clearly occupying pseudo axial orientation with $J_{6,5}$ = 11 Hz (Pereda-Miranda et al., 1990). The coupling constant values between pseudo-axial H-6 and H-7 ($J_{6,7}$ = 7.6 Hz) clearly indicated *anti* conformation, while the coupling constant value of ($J_{7,8}$ = 3 Hz) between H-7 & H-8 was suggestive of *syn* conformation with a probable dihedral angle of either 50° or 130° as per Karplus equation (Pereda-Miranda et al., 1990, 1993; Davies-Coleman and Rivett, 1987). The NOESY experiments (Fig. 3) were unable to provide concrete evidence for the relative stereochemistry, as H-6, H-7 & H-8 showed mutual correlations. The NOESY experiments (Fig. 3) also showed clear correlation of H-9 with H-11 and H-10 with H-12. Again H-11 and H-12 proposed to be *syn* based on their coupling constant

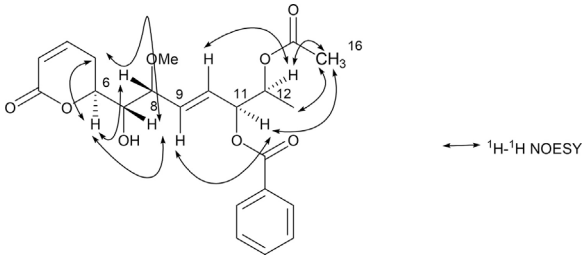

Fig. 3. NOESY correlations of **5**.