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Six new monoterpenoid indole alkaloids from the aerial part of *Gelsemium elegans*

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ABSTRACT

Six new monoterpenoid indole alkaloids along with four known analogues were isolated from the aerial part of *Gelsemium elegans*. Their structures with absolute configurations were elucidated by NMR, HRESIMS, X-ray diffraction, CD spectra, and molecular modeling calculation. Among them, gelselenidine (**1**) is a new gelsedine-type alkaloid with a 2,3-epoxybutane unit. Gelseziridine (**2**) is the first example of monoterpenoid indole alkaloids with an oxaziridine residue. Compounds **6** and **7** are a pair of N_4 epimers of humantenine N_4-oxide. A plausible biogenetic pathway for compounds **1**–**4** was also proposed.

1. Introduction

Gelsemium elegans (Loganiaceae), widely distributed in Southeast Asia, is a well known toxic plant. The roots of this plant have been used as a traditional Chinese medicine in the treatment of cancer, furuncle, and carbuncle.[1] Previous phytochemical studies of this plant had led to the isolation of more than 70 alkaloids,[2,3] which demonstrated cytotoxic,[4] analgesic, and anti-inflammatory[5] activities. These alkaloids attracted much attention of organic chemists and pharmacologists due to their complex structural features and multiple biological effects. In our studies on the bioactive alkaloids from the aerial parts of *G. elegans* growing in Guangdong province of China, six new monoterpenoid indole alkaloids gelselenidine (**1**), gelseziridine (**2**), gelsemolenines A and B (**3** and **4**), (4*R*)-gelsevirine N_4-oxide (**5**), and (4*R*)-humantenine N_4-oxide (**6**), together with four known alkaloids (**7**–**10**) were isolated (Fig. 1). In this paper, we describe the structure elucidation of these new alkaloids by means of NMR, HRESIMS, X-ray diffraction, circular dichroism spectroscopy, and molecular modeling calculation.

2. Results and discussion

2.1. Structural elucidation of alkaloids 1–7

Compound **1** was found to have the molecular formula $C_{21}H_{24}N_2O_4$ from its HRESIMS data (*m*/*z* 369.1816 $[M+H]^+$). The ^1H NMR spectrum displayed some readily assignable signals due to the known gelsenicine (**8**) portion,[6] such as four aromatic protons [δ_H 7.53 (1H, d, *J*=7.3 Hz, H-9), 7.26 (1H, dd, *J*=7.6, 7.6 Hz, H-11), 7.07 (1H, dd, *J*=7.6, 7.3 Hz, H-10), 6.88 (1H, d, *J*=7.7 Hz, H-12)], an N_1-methoxy group [δ_H 3.93 (3H, s)], and three oxygenated protons [δ_H 4.27 (2H, m, H_2-17), 3.72 (1H, d, *J*=4.6 Hz, H-3)]. In addition, signals for a methyl [δ_H 1.49 (3H, d, *J*=5.4 Hz)] and an oxygenated methine [δ_H 3.40 (1H, q, *J*=5.4 Hz)] were observed. The ^{13}C NMR spectrum of **1** demonstrated signals similar to those of gelsenicine (**8**),[6] except for the changes at C-18 and C-19, as well as two additional signals at δ_C 14.1 and 58.8 were observed. The assignment of the additional signals could be deduced by ^1H, ^1H COSY, and HMBC experiments (Fig. 2). Hence, the ^1H, ^1H correlation between H_3-24 (δ_H 1.49) and H-23 (δ_H 3.40), as well as the HMBC cross peaks between H_3-24 (δ_H 1.49) and C-19 (δ_C 59.1), between H-23 (δ_H 3.40) and C-20 (δ_C 182.2), between H_3-18 (δ_H 1.70) and C-20, as well as between H_3-18 and C-23 (δ_C 58.8) revealed the presence of a four-carbon side chain (Fig. 2). According to the chemical shifts of C-19,

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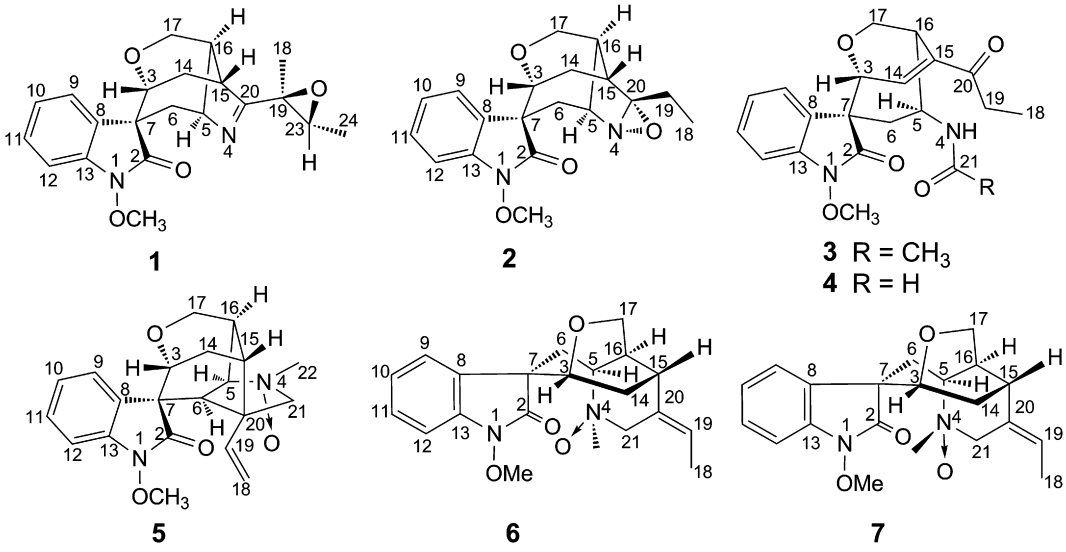


Fig. 1. Structures of alkaloids **1**–**7**.

C-23, and H-23, as well as the degree of unsaturation and the characteristic IR absorptions at 1248 and 885 cm^{-1} for epoxy,[7] a C-19, C-23 epoxy group was assigned.

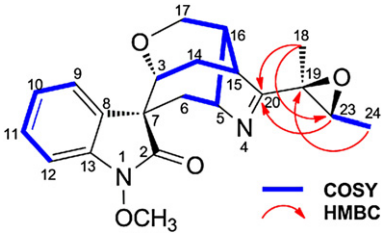


Fig. 2. Key $^{1}H-^{1}H$ COSY and HMBC correlations of **1**.

The configuration of the epoxide at C-19 and C-23 was deduced by ROESY experiment and molecular mechanics calculation. The NOE correlation between H_3-18 and H_3-24 indicated the presence of a *trans*-epoxide. Furthermore, the molecular modelings of 19*R*,23*R* and 19*S*,23*S* isomers were, respectively, submitted to a conformational analysis using the Tripos force field based on molecular modeling software package SYBYL 7.0.[8] The corresponding minimum geometries were further fully optimized by using DFT at the B3LYP/6-31G(d) level as implemented in the Gaussian 09 program package (Fig. 3). The optimized structure of the 19*R*,23*R* isomer was fully consistent with the corresponding ROESY data. The calculated distances from H-23 to H-14α, H-14β, and H-15 were 2.29, 2.96, and 2.45 Å, consistent with the ROESY correlations between H-23 and H-14α, H-14β and H-15 of **1** (Fig. 3). On the other hand, the distances calculated from H-23 to H-14α and H-14β of the 19*S*,23*S* isomer were too large (>4.0 Å) to produce NOE correlations. Thus, the configuration (19*R*,23*R*) of 19, 23-epoxy group was determined. The CD spectrum of **1** showed the same Cotton effects as the known gelsedine-type alkaloids, such as gelsenicine (**8**) and 19-oxo-gelsenicine,[9] indicating the presence of *S* configuration at C-7 (Fig. 4). Therefore, the structure including the absolute configuration of **1** was established as shown in Fig. 1 and named as gelselenidine. It is noteworthy that gelselenidine (**1**) is the first example of gelsedine-type alkaloid bearing a 2,3-epoxybutane residue, which represents a rare class of Gelsemium alkaloids with an additional C_2 unit.[10]

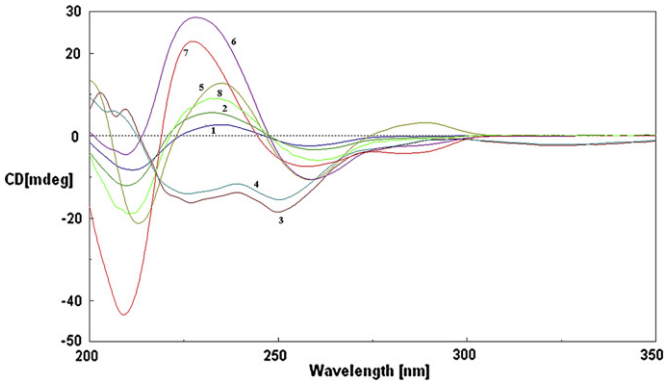


Fig. 4. CD spectra for **1**–**8** (in MeOH).

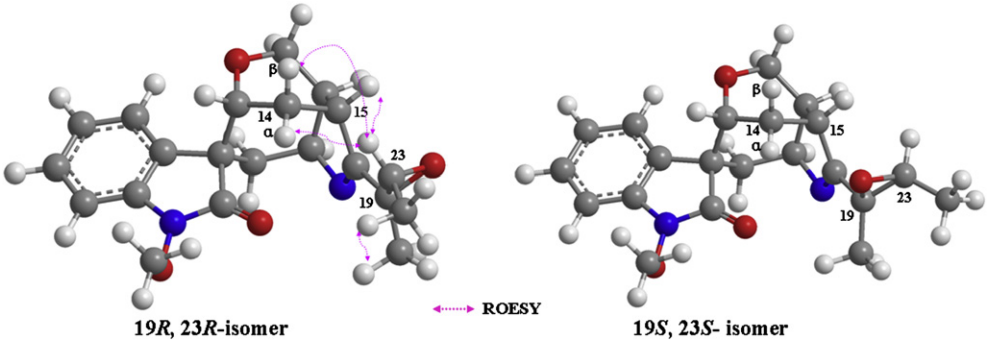


Fig. 3. Two DFT-optimized structures (19*R*,23*R*- and 19*S*,23*S*-isomers) and key ROESY correlations for **1**.

The molecular formula of **2** was established as $C_{19}H_{22}N_2O_4$ by a quasi-molecular ion at *m/z* 343.1659 $[M+H]^+$ (calcd *m/z* 343.1652) in its HRESIMS. The IR spectrum showed absorptions due to carbonyl (1729 cm^{-1}) and aromatic ring (1616, 1466 cm^{-1}). The UV spectrum of **2** displayed maxima absorptions at 208 and 250 nm, characteristic for an indolin-2-one chromophore. The ^1H NMR spectrum of **2** showed signals for an *ortho*-disubstituted benzene ring [δ_H 7.47 (1H, d, *J*=7.4 Hz), 7.28 (1H, dd, *J*=7.7, 7.7 Hz), 7.09 (1H, dd, *J*=7.7, 7.4 Hz), 6.93 (1H, d, *J*=7.7 Hz)], a methoxyl [δ_H 3.99 (3H, s)], and an isolated ethyl group [δ_H 1.06 (3H, t, *J*=7.5 Hz), 2.50 (1H, overlapped), 2.11 (1H, dq, *J*=14.8, 7.5 Hz)]. The ^{13}C NMR and DEPT spectra of **2** displayed 19 signals, including two methyls, four methylenes, eight methines, one carbonyl, and four quaternary carbons, suggesting the presence of a monoterpenoid indole alkaloid. Comparison of the NMR data of **2** with those of gelsedine[6] revealed that their chemical shifts were similar, except for the absence of signals for H-20 and the presence of an oxygenated quaternary carbon signal at δ_C 93.4 ppm, indicating an oxygen atom was attached to C-20. The downfield shift (+8.8 ppm) for C-5 in **2** relative to that in gelsedine[6] and the presence of 10° of unsaturation suggested that the oxygen atom was also connected to N_4, forming an oxaziridine ring.[11] This conclusion was further confirmed by the IR absorption band for oxaziridine ring at 1318 cm^{-1}[12,13] and by the high intensity fragment ion peak at *m/z* 327.1709 in HRESIMS, which was formed by eliminating a neutral oxygen atom (calcd 327.1703).[13] A comprehensive analysis of the $^1H-^1H$ COSY, HSQC, and HMBC spectra allowed the assignment of NMR data of **2** as shown in Tables 1 and 2.

The relative configuration of **2** could be deduced from the ROESY experiment and molecular modeling calculations (Gaussian 09). In the ROESY spectrum, the correlations of H-3/H_2-14, H-5/H-16, H-5/H_2-17, H-15/H_2-17, and H-15/H_3-18 indicated that the relative configuration of C-3, C-5, C-15, and C-16 in **2** is identical to that in gelsedine (Fig. 5).[6] The α-configuration of the epoxy located at $C_{20}-N_4$ was indicated by the *γ-gauche* effect from the epoxy group to C-16 and C-18 (Fig. 5),[14] which resulted in significant upfield shifts of C-16 (−8.1 ppm) and C-18 (−3.6 ppm) compared with gelsedine. The CD spectrum of **2** showed negative and positive Cotton effects at 209 and 232 nm, respectively (Fig. 4), revealed the presence of *S* configuration at C-7. Therefore, the structure of **2** was determined as shown in Fig. 1 and named as gelseziridine. To the best of our knowledge, gelseziridine (**2**) is the first example of monoterpenoid indole alkaloids bearing an oxaziridine unit. From a biogenetic point of view, gelseziridine (**2**) may be a biogenetic precursor of 18,19-nor-gelsedine-type alkaloids (Scheme 1).[10,15]

The HRESIMS of **3** showed a quasi-molecular ion at *m/z* 385.1763 $[M+H]^+$, consistent with the molecular formula $C_{21}H_{24}N_2O_5$ (calcd 385.1758), indicating that **3** has two carbon atoms more than the common gelsedine-type alkaloids. The ^1H NMR spectrum of **3** showed signals for four aromatic protons [δ_H 7.47 (1H, d, *J*=7.4 Hz), 7.35 (1H, ddd, *J*=7.8, 7.6, 1.1 Hz), 7.15 (1H, ddd, *J*=7.6, 7.4, 1.0 Hz),

Table 2
^{13}C (100 MHz) NMR data for compounds **1**–**7** in $CDCl_3$

Position	1	2	3	4	5	6	7
2	171.3	171.5	171.7	171.5	171.8	173.6	173.6
3	74.7	74.6	72.0	71.9	68.8	72.5	72.0
5	73.1	68.5	47.1	45.9	86.0	77.6	77.1
6	37.7	32.2	35.4	35.4	49.5	27.6	30.3
7	55.9	55.1	53.0	53.0	51.4	54.9	55.7
8	132.0	131.9	126.8	126.5	126.4	130.3	127.8
9	124.6	124.8	126.4	126.4	128.0	125.5	125.7
10	123.4	123.5	123.7	123.7	123.1	123.4	123.5
11	128.1	128.3	128.7	128.8	128.8	128.4	128.7
12	106.6	106.9	107.5	107.6	107.4	107.4	107.6
13	138.0	138.1	139.0	139.1	139.6	138.4	138.7
14	28.1	23.8	137.5	137.6	22.5	26.1	27.1
15	37.7	34.1	139.7	139.4	35.2	34.3	33.0
16	39.9	33.6	38.1	38.1	37.5	34.2	30.7
17	62.0	62.0	67.5	67.4	60.5	64.6	65.6
18	14.2	8.4	8.2	8.2	115.3	13.0	12.9
19	59.1	22.5	30.6	30.6	134.8	126.0	125.9
20	182.2	93.4	201.2	201.2	52.2	133.0	131.5
21/23	58.8	—	169.1	160.0	81.3	60.0	59.7
22/24	14.1	—	23.3	—	—	—	—
N_1–OMe	63.3	63.4	63.5	63.6	63.5	63.4	63.5
N_4–Me	—	—	—	—	53.4	56.5	57.1

Table 1
^1H (400 MHz) NMR data for compounds **1**–**7** in $CDCl_3$[a]

Position	1	2	3	4	5	6	7
3	3.72 (d, 4.6)	3.68 (d, 3.9)	4.28 (d, 6.5)	4.29 (d, 6.2)	3.81 (s)	3.58 (d, 7.5)	3.62 (d, 7.1)
5	4.49 (m)	3.96 (m)	4.41 (m)	4.51 (m)	4.01 (br s)	4.18	3.80 (m)
6	α: 2.33 (d, 15.3); β: 2.43 (dd, 15.5, 4.9)	2.29 (2H, m)	α: 2.04 (dd, 14.0, 5.2); β: 1.61 (dd, 14.0, 10.1)	α: 2.05 (dd, 14.0, 5.3); β: 1.44 (dd, 14.0, 10.1)	3.53 (s)	α: 2.37; β: 3.44 (dd, 17.6, 4.8)	α: 2.11 (m); β: 2.44 (dd, 15.3, 9.0)
9	7.53 (d, 7.3)	7.47 (d, 7.4)	7.47 (d, 7.4)	7.46 (d, 7.5)	7.37 (d, 7.6)	7.34 (d, 7.6)	7.34
10	7.07 (dd, 7.6, 7.3)	7.09 (dd, 7.7, 7.4)	7.15 (ddd, 7.6, 7.4, 1.0)	7.15 (ddd, 7.6, 7.6, 1.0)	7.04 (dd, 7.6, 7.6)	7.10 (ddd, 7.6, 7.6, 1.0)	7.11 (dd, 7.6, 7.6)
11	7.26 (dd, 7.6, 7.6)	7.28 (dd, 7.7, 7.7)	7.35 (ddd, 7.8, 7.6, 1.1)	7.37 (ddd, 7.8, 7.6, 1.0)	7.27 (dd, 7.7, 7.7)	7.30 (ddd, 7.7, 7.7, 1.0)	7.32
12	6.88 (d, 7.7)	6.93 (d, 7.7)	7.00 (d, 7.8)	7.01 (d, 7.8)	6.93 (d, 7.7)	6.98 (d, 7.7)	6.99 (d, 7.7)
14	α: 2.29 (d, 15.1); β: 2.11 (ddd, 14.9, 9.5, 4.8)	α: 2.43 (d, 15.4); β: 2.18 (m)	7.30 (dd, 6.1, 1.8)	7.32 (dd, 6.1, 1.7)	α: 2.90 (dd, 14.7, 2.4); β: 2.05 (ddd, 14.7, 5.6, 2.8)	α: 2.22 (ddd, 15.4, 11.7, 7.6); β: 2.37	α: 2.19 (m); β: 2.27 (m)
15	2.92 (t, 9.1)	2.77 (dd, 10.4, 7.6)	—	—	2.43 (m)	2.67 (m)	2.71 (m)
16	2.56 (t, 8.1)	2.50	3.31 (br s)	3.31 (br s)	2.54 (d, 8.0)	2.49 (m)	3.71 (m)
17	4.27 (2H, m)	4.22 (2H, m)	α: 4.26 (d, 8.9); β: 3.71 (dd, 9.3, 3.1)	α: 4.27 (dd, 9.8, 1.0); β: 3.72 (dd, 9.8, 3.1)	α: 3.99 (br d, 11.3); β: 4.10 (br d, 11.4)	α: 4.00; β: 4.18	4.14 (2H, m)
18	1.70 (s)	1.06 (t, 7.5)	1.21 (t, 7.3)	1.21(t, 7.3)	a: 5.22 (d, 11.0); b: 5.02 (d, 17.8)	1.73 (d, 6.9)	1.68 (d, 6.9)
19	—	a: 2.50; b: 2.11 (dq, 14.8, 7.5)	a: 3.02 (dq, 17.5, 7.3); b: 2.77 (dq, 17.5, 7.3)	a: 3.03 (dq, 17.5, 7.2); b: 2.78 (dq, 17.5, 7.2)	6.18 (dd, 17.8, 11.0)	5.70 (q, 6.9)	5.68 (q, 6.9)
21/23	3.40 (q, 5.4)	—	—	8.07 (s)	α: 3.23 (d, 12.1); β: 3.55 (d, 12.4)	α: 4.00; β: 4.78 (d, 14.6)	α: 4.02 (d, 14.3); β: 4.42 (d, 14.3)
22/24	1.49 (d, 5.4)	—	1.94 (s)	—	—	—	—
N_1–OMe	3.93 (s)	3.99 (s)	3.99 (s)	3.99 (s)	3.96 (s)	3.98 (s)	3.95 (s)
N_4–Me	—	—	—	—	3.31 (s)	3.31 (s)	3.19 (s)
NH	—	—	6.46 (d, 7.8)	6.55 (d, 7.7)	—	—	—

[a] Overlapped signals were reported without designating multiplicity.

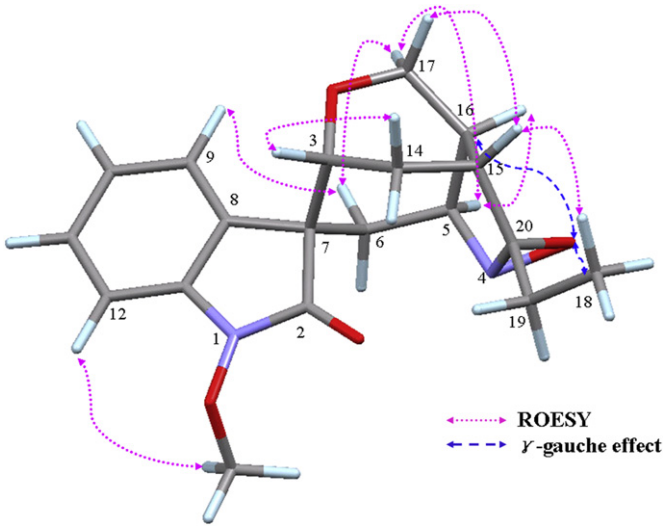


Fig. 5. Key ROESY correlations and the *γ-gauche* effect of epoxy group of **2**.

7.00 (1H, d, *J*=7.8 Hz)], an oxygenated methylene [δ_H 4.26 (1H, d, *J*=8.9 Hz), 3.71 (1H, dd, *J*=9.3, 3.1 Hz)], an oxygenated methine [δ_H 4.28 (1H, d, *J*=6.5 Hz)], a N_1-methoxyl [δ_H 3.99 (3H, s)], and an ethyl [δ_H 1.21 (3H, t, *J*=7.3 Hz), 3.02 (1H, dq, *J*=17.5, 7.3 Hz), 2.77 (1H, dq, *J*=17.5, 7.3 Hz)], suggesting the presence of a monoterpenoid indole alkaloid. The NMR data at δ_C 137.5 (C-14), 139.7 (C-15), 201.2 (C-20) and δ_H 7.30 (H-14) are characteristic for an α,β-unsaturated ketone unit. In the HMBC spectrum, correlations between H-16 (δ_H 3.31) and C-14 (δ_C 137.5), C-15 (δ_C 139.7), and C-20 (δ_C 201.2), between H-14 (δ_H 7.30) and C-20, as well as between H-18 (δ_H 1.21) and C-20 suggested that **3** was a N_4/C-20 seco-oxindole alkaloid,[15] which was further confirmed by comparison of the NMR data of **3** with those of gelseiridone.[15] The remaining NMR signals at δ_C 169.1 and 23.3, as well as δ_H 1.94 (3H, s) could be assigned to an acetyl group. The linkage between the acetyl group and N_4 atom was demonstrated by HMBC cross peak between H-5 (δ_H 4.41) and C-21 (δ_C 169.1). The relative stereochemistry of **3** was deduced by analysis of its ROESY spectrum.[15] The absolute configuration of the spiro-center at C-7 was determined to be *S* by comparison of its CD spectrum with that of gelseiridone (Fig. 4).[15] Based on the above evidences, the structure of **3** was fully established and named as gelsemolenine A.

The molecular formula of **4** was displayed as $C_{20}H_{22}N_2O_5$ by its HRESIMS (*m*/*z* 393.1422 $[M+Na]^+$, calcd 393.1421). The ^1H and ^{13}C NMR data of **4** were very similar to those of **3**, except for some differences occurred for the signals due to the group attached to N_4 position. The absence of signals for acetyl group and the presence of an aldehyde proton [δ_H 8.07 (1H, s)] in **4** implied that the acetyl group in **3** was replaced by a formyl residue in **4**, which was further confirmed by the HMBC correlation between H-21 (δ_H 8.07) and C-5 (δ_C 45.9). With the aid of ^1H, ^1H COSY, HSQC, HMBC, and ROESY experiments, the ^1H and ^{13}C NMR data of **4** were assigned as shown in Tables 1 and 2. The absolute configuration of **4** was identical to that of **3**, since they showed similar Cotton effects in CD measurement (Fig. 4). Thus, the structure of **4** was determined and named as gelsemolenine B. To the best of our knowledge, gelsemolenines A (**3**) and B (**4**) are the first examples of monoterpenoid indole alkaloids possessing an additional acetyl or formyl unit at N_4 position.

Compound **5** was obtained as colorless crystal. The molecular formula of **5** was established as $C_{21}H_{24}N_2O_4$ by the quasi-molecular ion at *m*/*z* 369.1810 $[M+H]^+$ (calcd 369.1809) in its HRESIMS. The IR spectrum showed absorptions for carbonyl (1721 cm^{-1}) and aromatic ring (1615, 1592, and 1463 cm^{-1}). The UV spectrum of **5** exhibited maxima absorptions at 209, 256, and 341 nm, characteristic for an indolin-2-one chromophore. The ^1H NMR spectrum of **5** showed signals for an *ortho*-disubstituted benzene ring [δ_H 7.37 (1H, d, *J*=7.6 Hz), 7.27 (1H, dd, *J*=7.7, 7.7 Hz), 7.04 (1H, dd, *J*=7.6, 7.6 Hz), 6.93 (1H, d, *J*=7.7 Hz)], a terminal double bond [δ_H 6.18 (1H, dd, *J*=17.8, 11.0 Hz), 5.22 (1H, d, *J*=11.0 Hz), 5.02 (1H, d, *J*=17.8 Hz)], and a N_1-methoxy group [δ_H 3.96 (3H, s)]. The ^{13}C NMR spectrum of **5** displayed twenty-one signals. The signals for a carbonyl at δ_C 171.8 and two quaternary carbons at δ_C 51.4 and 52.2 were characteristic for gelsemine type alkaloid. Comparison of the NMR data of **5** with those of the known compound gelsevirine (**9**)[9,16] revealed that their NMR signals were similar, except for the resonances for N_4–CH_3 (δ_H 3.31, δ_C 53.4), CH-5 (δ_H 4.01, δ_C 86.0), and CH_2-21 (δ_H 3.55, 3.23, δ_C 81.3) were downfield shifted in **5**. Taking into account that **5** had an oxygen atom more than the known compound gelsevirine (**9**), **5** was proposed to be a N_4-oxide derivative of gelsevirine (**9**). With the aid of ^1H–^1H COSY, HSQC, HMBC, and ROESY experiments, the ^1H and ^{13}C NMR data of **5** were assigned (Tables 1 and 2). Furthermore, the structure with relative configuration of **5** was unequivocally confirmed by an X-ray diffraction analysis (Fig. 6). The CD spectrum of **5** displayed negative, negative, and positive cotton effects at 212, 258, and 235 nm, respectively, indicating the absolute configuration of C-7 was also *S* (Fig. 4).[3] Based on the above evidences, the structure of **5** was determined as (4*R*)-gelsevirine N_4-oxide.

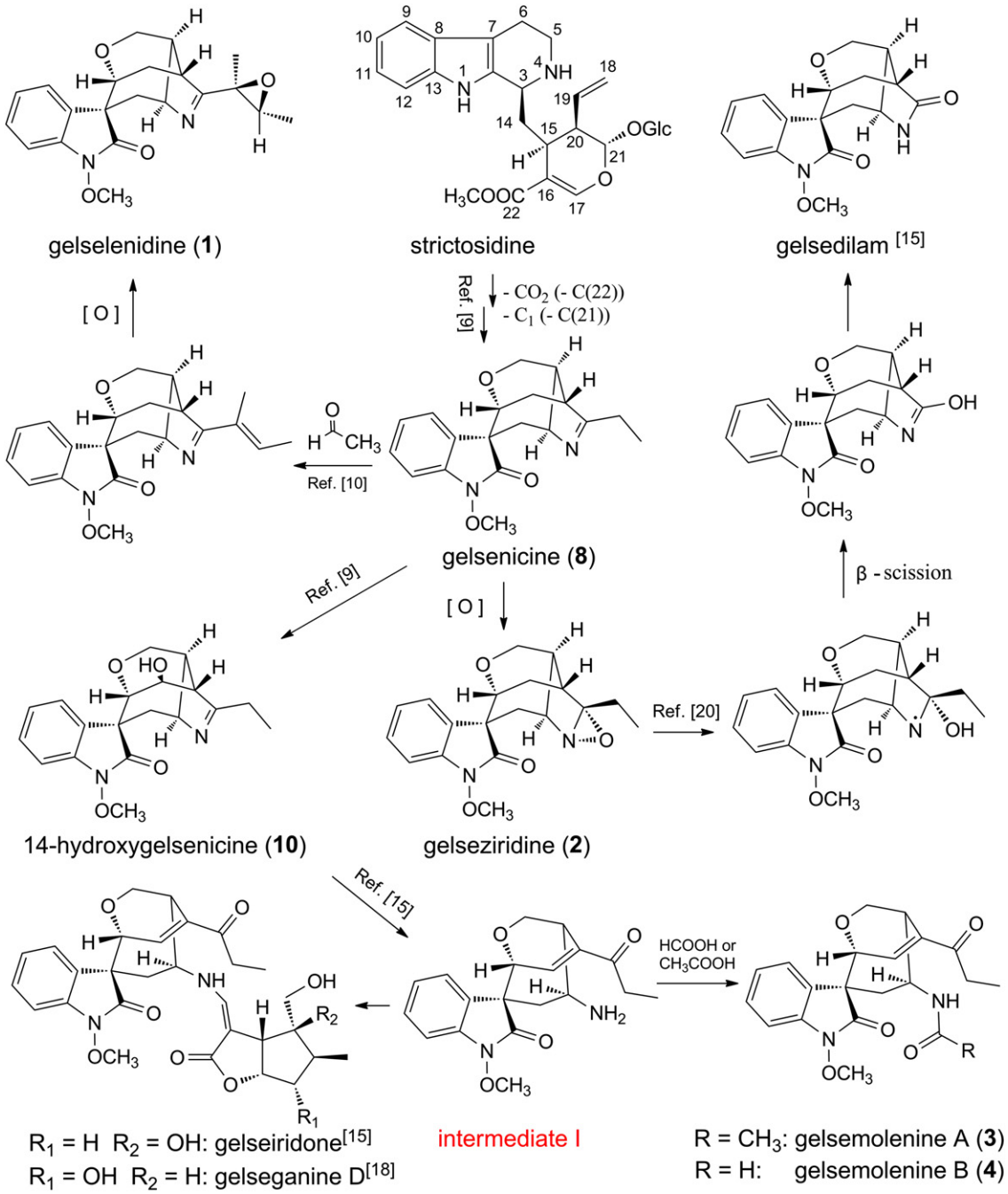
Compound **6** displayed the molecular formula $C_{21}H_{26}N_2O_4$ by its HRESIMS (*m*/*z* 371.1966 $[M+H]^+$). The ^1H and ^{13}C NMR data of **6** were similar to those of humantenine,[17] except for the chemical shifts around the N_4 atom, which suggested that **6** is an N_4-oxide derivative of humantenine. This conclusion was confirmed by the extensive analysis of ^1H–^1H COSY, HSQC, HMBC, and ROESY spectra. The *S* configuration at C7 was deduced from the CD spectra of **6** (Fig. 4).[17] The configuration at the N_4 position was determined to be *R* by the lower-field-shifted H-6 interpreted from the anisotropy effect of the oxygen atom on N_4, as well as the ROESY cross peaks between N_4–CH_3 (δ_H 3.31) and H-5 (δ_H 4.18) and H-16 (δ_H 2.49) (Fig. 7). Therefore, the structure of **6** was elucidated as (4*R*)-humantenine N_4-oxide.

Compound **7** showed the same molecular formula as **6** by its HRESIMS $C_{21}H_{26}N_2O_4$ (*m*/*z* 393.1780 $[M+Na]^+$). The ^1H and ^{13}C NMR data of **7** were identical to those of humantenine N_4-oxide.[18] However, the absolute configuration of N_4 was not clearly defined in the previous work. The CD spectrum of **7** displayed similar Cotton effects as that of **6**, indicated the presence of *S* configuration at C-7 in **7** (Fig. 4). In the ^1H NMR spectrum, the signals for H-16 was downfield (+1.40 ppm) compared with that of humantenine,[17] which was attributable to anisotropy of $N\rightarrow O$ function.[9] Furthermore, the ROESY correlations between N_4–Me and H-6α/H-6β were observed. Thus, the absolute configuration of N_4 was assigned as *S*. The structure of **7** was determined as (4*S*)-humantenine N_4-oxide.

The known compounds gelsenicine (**8**),[6] gelsevirine (**9**),[9,16] and 14-hydroxygelsenicine (**10**)[9] were also isolated and identified on the basis of their physical and spectroscopic data.

2.2. Possible biogenetic pathway for alkaloids 1–4

On the basis of literatures and our research results, a plausible biosynthetic pathway for new alkaloids **1**–**4** was proposed (Scheme 1). The alkaloid gelsenicine (**8**), a major component of *Gelsemium* plants, was originated from strictosidine.[9] Addition with acetaldehyde[10] and subsequent oxidation of the olefinic bond would afford gelselenidine (**1**). On the other hand, after oxidation of the imine N_4/C-20[19] of **8**, gelseziridine (**2**) could be yielded. Compound **2** might be a biogenetic precursor of 18,19-nor-gelsedine-type alkaloids, such as gelsedilam,[15] which is characterized by the oxaziridine cleavage and β-scission.[20,21] 14-Hydroxygelsenicine



Scheme 1. Plausible biosynthetic pathway for compounds **1**–**4**.

(**10**) was also derivatized from strictosidine.[9] Hydrolytic cleavage of the imine part in **10** and dehydration at C-14 and C-15 positions would afford a ketoamine intermediate **I**, which was further acetylated or formylated to yield gelsemolenines A (**3**) or B (**4**).

The unusual N_4/C-20 seco-oxindole alkaloids possessing an N_4-iridoid residue, such as gelseiridone[15] and gelseganine D[18] had been recently isolated from *G. elegans*. In the possible biogenetic pathway of these novel alkaloids, intermediate **I** played an important role[15,18] (Scheme 1). However, the existence of the key intermediate **I** have not been proved. Gelsemolenines A (**3**) and B (**4**) are obviously biogenetically related to **I**, which could rationalize the existence of **I** and further support the proposed biosynthetic pathway for the related alkaloids in this plant.[15,18]

2.3. Conclusions

Six new monoterpenoid indole alkaloids were isolated from the aerial parts of *G. elegans*. Among them, gelselenidine (**1**) is a new gelsedine-type alkaloid with a 2,3-epoxybutane residue; gelseziridine (**2**) has a rare oxaziridine unit at the skeleton of monoterpenoid indole alkaloid; gelsemolenines A (**3**) and B (**4**) are the first examples of Gelsemium alkaloids with an additional acetyl or formyl unit at N_4 position. It is very interesting that a pair of N_4 epimers of humantenine N_4-oxide were co-existed in the same plant. In addition, gelseziridine (**2**) was proposed to be a biogenetic precursor of 18,19-nor-gelsedine-type alkaloids. The presence of gelsemolenines A (**3**) and B (**4**) could rationalize the biosynthetic pathway proposed for the related alkaloids in this plant.

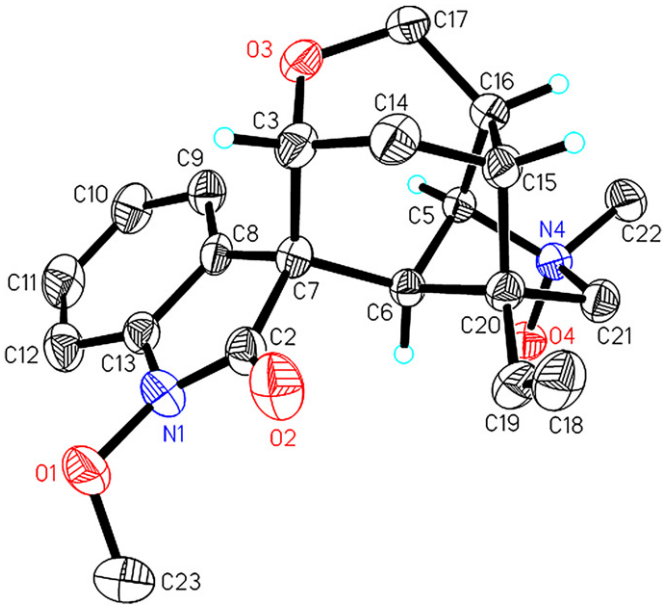


Fig. 6. Perspective drawing of X-ray structure of **5**.

3.2. Plant material

The aerial parts of *G. elegans* were collected in Shantou city, Guangdong province of P.R. China, in May of 2009, and authenticated by Prof. Guang-Xiong Zhou (College of Pharmacy, Jinan University). A specimen (No. 2009052301) was deposited in the Institute of Traditional Chinese Medicine and Natural Products, Jinan University, People's Republic of China.

3.3. Extraction and isolation

The air-dried leaves and stems of *G. elegans* (4.5 kg) were percolated five times with 95% EtOH (50 L) at room temperature for 4 days. The combined EtOH solution was concentrated in vacuo to afford a residue (650 g), which was dissolved in 2% hydrochloric acid and then successively extracted with petroleum ether and EtOAc. The remained acidic layer was basified with aqueous ammonia to around pH 10 and extracted with $CHCl_3$. The $CHCl_3$ solution was concentrated to afford total alkaloids (60 g). The alkaloid extract was subjected to silica gel column chromatography eluting with $CHCl_3$/MeOH (100:1→2:1) to afford five major fractions (A–E). Fraction A (16 g) was resubjected to silica gel column chromatography (cyclohexane/EtOAc=100:0→0:100) to afford **8** (680 mg). Fraction C (24 g) was subjected to column chromatography over silica gel (cyclohexane/EtOAc/Et_2NH=100:2:1→100:100:2) to give five fractions (C1–C5). Fraction C1 was further purified by preparative HPLC (MeOH/H_2O/Et_2NH=60:40:0.01, 8 mL min^{-1}) to give **1** (8 mg), **2** (11 mg), and **9** (12 mg). Fraction C2 was purified by preparative HPLC (MeOH/H_2O/Et_2NH=48:52:0.01, 8 mL min^{-1}) to afford **3** (8 mg) and **4** (5 mg). Fractions C3 and C5 were purified by preparative HPLC to give **10** (10 mg) (MeOH/H_2O/Et_2NH=58:42:0.01, 8 mL min^{-1}) and **5** (12 mg) (MeOH/H_2O/Et_2NH=48:52:0.01, 8 mL min^{-1}), respectively. Fraction C4 was subjected to column chromatography with C18 reversed-phase silica gel (MeOH/H_2O=30:70→100:0). The 50% MeOH eluent was further purified by preparative HPLC (MeOH/H_2O/Et_2NH=38:62:0.01, 8 mL min^{-1}) to give **6** (9 mg) and **7** (8 mg).

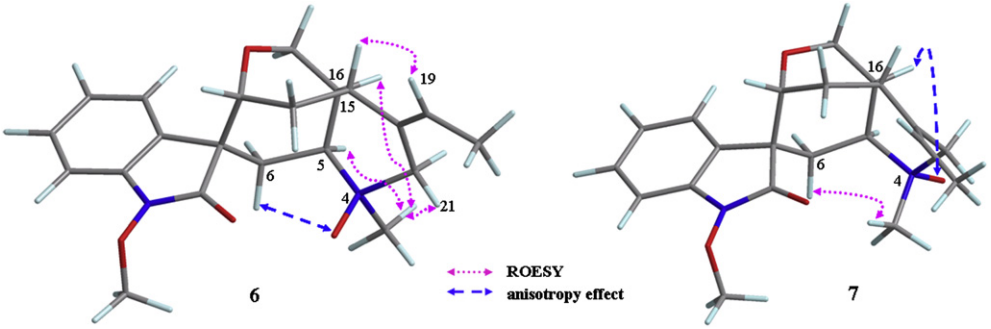


Fig. 7. Key ROESY correlations and the anisotropy effect of *N*→*O* function of **6** and **7**.

3. Experimental

3.1. General

Melting points were measured on an XT-5 micro melting point apparatus. Optical rotations were measured by a Jasco P-1020 digital polarimeter at room temperature. UV spectra were recorded on a Jasco-V-550 UV–vis spectrophotometer. CD spectra were obtained on a Jasco J-810 spectropolarimeter at room temperature. IR spectra were conducted on a Jasco FT/IR-480 plus Fourier transform infrared spectrometer. HRESIMS were performed on an Agilent 6210 ESI/TOF mass spectrometer. 1D NMR (^1H, ^{13}C, and DEPT) and 2D (^1H–^1H COSY, HSQC, HMBC, and ROESY) NMR spectra were recorded on a Bruker AV-400 spectrometer with TMS as internal standard, and chemical shifts were expressed in δ values (ppm). Silica gel (200–300 mesh) (Qingdao, P.R. China), Sephadex LH-20 (Pharmacia Biotec AB), and RP-18 (Merck, Darmstadt, Germany) were used for column chromatographies (CC). Preparative high-performance liquid chromatography (HPLC) was carried on a Varian chromatograph equipped with a Prostar 215 pump and a Prostar 325 UV–vis detector with a C_{18} reversed-phase column (Cosmosil, 30×250 mm, 5 μm). All solvents used in column chromatography were of analytical grade (Tianjin Damao Chemical Plant, Tianjin, P.R. China).

3.4. Characteristics of alkaloids 1–7

*3.4.1. Gelselenidine (**1**).* Colorless gum; $[\alpha]_D^{25}$ −22.9 (*c* 0.380, MeOH); CD (*c* 0.815 mmol/L, MeOH, 22 °C) $\Delta\varepsilon$ (λ, nm): 0 (297), −0.91 (258), 0 (246), 1.00 (235), 0 (224), −3.07 (211); UV (MeOH): λ_{max} nm (log ε)=208 (7.45), 255 (6.79); IR (KBr): ν_{max} 3417, 2924, 2852, 1723, 1617, 1466, 1382, 1318,1248, 1113, 1041, 980, 885, 826, 750 cm^{-1}; ^1H NMR: see Table 1; ^{13}C NMR: see Table 2; HRESIMS: 369.1816 $[M+H]^+$ (calcd for $C_{21}H_{24}N_2O_4$, 369.1809).

*3.4.2. Gelseziridine (**2**).* Amorphous powder; $[\alpha]_D^{25}$ −24.5 (*c* 0.363, MeOH); CD (*c* 0.848 mmol/L, MeOH, 22 °C) $\Delta\varepsilon$ (λ, nm): 0 (298),

−1.22 (259), 0 (248), 2.01 (232), 0 (221), −4.32 (209); UV (MeOH): λ_{max} nm (log ε)=208 (7.55), 250 (6.90); IR (KBr): ν_{max} 3421, 2923, 2853, 1729, 1616, 1466,1318, 1222, 1118, 1040, 953, 882, 750 cm^{-1}; ^1H NMR: see Table 1; ^{13}C NMR: see Table 2; HRESIMS: 343.1659 $[M+H]^+$ (calcd for $C_{19}H_{22}N_2O_4$, 343.1652).

*3.4.3. Gelsemolenine A (**3**).* Amorphous powder; $[\alpha]_D^{25}$ −19.4 (*c* 0.310, MeOH); CD (*c* 0.807 mmol/L, MeOH, 22 °C) $\Delta\varepsilon$ (λ, nm): 0 (378), −0.86 (325), −0.30 (294), −6.83 (250), −5.25 (239), −5.99 (227), 0 (213); UV (MeOH): λ_{max} nm (log ε)=206 (7.24), 251 (6.71), 341 (6.14); IR (KBr): ν_{max} 3441, 3249, 3076, 2971, 2921, 2874, 1726, 1677, 1635, 1563, 1460, 1191, 1113, 763 cm^{-1}; ^1H NMR: see Table 1; ^{13}C NMR: see Table 2; HRESIMS: 385.1763 $[M+H]^+$ (calcd for $C_{21}H_{24}N_2O_5$, 385.1758).

*3.4.4. Gelsemolenine B (**4**).* Amorphous powder; $[\alpha]_D^{25}$ −10.0 (*c* 0.251, MeOH); CD (*c* 0.676 mmol/L, MeOH, 22 °C) $\Delta\varepsilon$ (λ, nm): 0 (373), −0.93 (324), −0.39 (298), −6.80 (250), −5.36 (237), −6.21 (226), 0 (212); UV (MeOH): λ_{max} nm (log ε)=206 (7.26), 268 (6.23); IR (KBr): ν_{max} 3314, 2975, 2941, 2874, 1719, 1671, 1617, 1462, 1192, 1077, 749 cm^{-1}; ^1H NMR: see Table 1; ^{13}C NMR: see Table 2; HRESIMS: 393.1422 $[M+Na]^+$ (calcd for $C_{20}H_{22}N_2O_5$, 393.1421).

*3.4.5. (4R)-Gelsevirine N_4-oxide (**5**).* Colorless crystals (MeOH). Mp: 125–126 °C; $[\alpha]_{D^{25}}$ −22.3 (*c* 0.269, MeOH); CD (*c* 0.733 mmol/L, MeOH, 22 °C) $\Delta\varepsilon$ (λ, nm): 0 (309), 1.34 (289), 0 (274), −4.42 (258), 0 (247), 5.33 (235), 0 (223), −8.93 (212); UV (MeOH): λ_{max} nm (log ε)=209 (6.79), 256 (6.20), 341(5.23); IR (KBr): ν_{max} 3403, 2925, 1721, 1615, 1592, 1463, 1384, 1080, 754 cm^{-1}; ^1H NMR: see Table 1; ^{13}C NMR: see Table 2; HRESIMS: 369.1810 $[M+H]^+$ (calcd for $C_{21}H_{24}N_2O_4$, 369.1809).

*3.4.6. (4R)-Humantenine N_4-oxide (**6**).* Amorphous powder; $[\alpha]_D^{25}$ −13.2 (*c* 0.317, MeOH); CD (*c* 0.865 mmol/L, MeOH, 22 °C) $\Delta\varepsilon$ (λ, nm): 0 (305), −3.73 (259), 0 (248), 10.06 (228), 0 (214), −1.62 (209); UV (MeOH): λ_{max} nm (log ε)=207 (7.97), 255 (7.19), 341(5.71); IR (KBr): ν_{max} 3412, 2924, 1719, 1617, 1465, 1384, 1120, 1072, 750 cm^{-1}; ^1H NMR: see Table 1; ^{13}C NMR: see Table 2; HRESIMS: 371.1966 $[M+H]^+$ (calcd for $C_{21}H_{26}N_2O_4$, 371.1965).

*3.4.7. (4S)-Humantenine N_4-oxide (**7**).* Amorphous powder; $[\alpha]_D^{25}$ −11.5 (*c* 0.301, MeOH); CD (*c* 0.811 mmol/L, MeOH, 22 °C) $\Delta\varepsilon$ (λ, nm): 0 (305), −2.80 (257), 0 (244), 8.64 (227), 0 (219), −16.53 (208); UV (MeOH): λ_{max} nm (log ε)=207 (7.19), 255 (6.51), 341(4.25); IR (KBr): ν_{max} 3407, 2937, 1720, 1618, 1466, 1386, 1330, 1209, 1122, 1065, 944, 886, 752 cm^{-1}; ^1H NMR: see Table 1; ^{13}C NMR: see Table 2; HRESIMS: 393.1780 $[M+Na]^+$ (calcd for $C_{21}H_{26}N_2O_4$, 393.1785).

*3.4.8. Crystal data for (4R)-gelsevirine N_4-oxide (**5**).* $C_{21}H_{24}N_2O_4$, monoclinic, space group $P2_1$, *a* 9.33100(1), *b* 21.0564(3), *c* 10.9924(2) Å, *V* 2159.75(6) $Å^3$, *Z* 2, D_{calcd} 1.256 g cm^{-1}, *T* 293 K, *R* 0.0461, R_W 0.1248. CCDC-809159 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via http://www.ccdc.cam.ac.uk/data_request/cif.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tet.2011.05.026. These data include MOL files and InChIKeys of the most important compounds described in this article.

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