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Cytotoxic Alkaloids from the Whole Plants of *Zephyranthes candida*

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Abstract

Seven new alkaloids, *N*-methylhaemanthidine chloride (**1**), *N*-methyl-5,6-dihydroplicane (**5**), *O*-methylnerinine (**6**), *N*-ethoxycarbonylethylcrinasiadine (**7**), *N*-ethoxycarbonylpropylcrinasiadine (**8**), *N*-phenethylcrinasiadine (**9**) and *N*-isopentylcrinasiadine (**10**), together with eight known alkaloids, haemanthamin (**2**), 3-epimacronine (**3**), (+)-tazettine (**4**), *N*-methylcrinasiadine (**11**), trisphaeridine (**12**), 5,6-dihydrobicolorine (**13**), lycorine (**14**), and nigragillin (**15**), were isolated from the whole plants of *Zephyranthes candida*. The structures of the new compounds were established by spectroscopic data interpretation, with single-crystal X-ray diffraction analysis performed on **1**. The absolute configuration of 3-epimacronine (**3**) was determined by single-crystal X-ray diffraction analysis with CuK_{α} irradiation. Compounds **1–15** were evaluated for their in vitro cytotoxicity against five human cancer cell lines and the Beas-2B immortalized (non-cancerous) human bronchial epithelial cell line. Compounds **1**, **2**, **9**, and **14** exhibited cytotoxicity with IC_{50} values ranging from 0.81 to 13 μM with selectivity indices as high as 10 when compared to the Beas-2B cell line.

Plants of the family Amaryllidaceae comprise ca. 85 genera and 1100 species that are distributed widely in tropical regions of the world. More than 500 Amaryllidaceae alkaloids representing 18 skeletal types have been isolated and reported to have acetylcholinesterase (AChE) inhibitory, analgesic, antibacterial, antifungal, antimalarial, antitumor, antiviral, and cytotoxic activities.[1] *Zephyranthes candida* (Lindl.) Herb. is an amaryllidaceous bulbous herb native to South America. The plant is widely cultured as an ornamental flower and used

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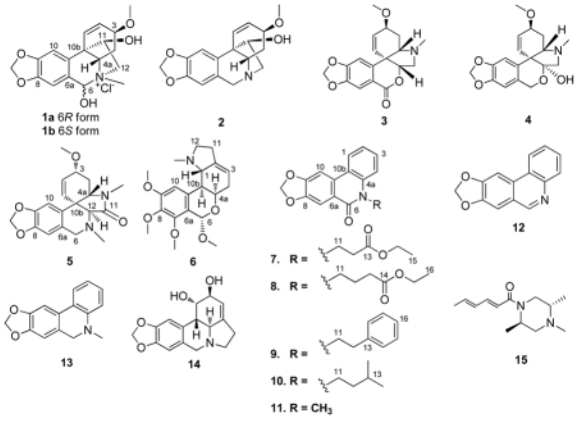
Supporting Information Available. (+)-HR-ESIMS, 1D and 2D NMR spectra for compounds **1**, **5**–**10**, CD for compound **1**, crystal packing of compounds **1** and **3**, X-ray crystallographic data (CIF files) for compounds **1** and **3**, as well as cytotoxicity data of compounds **1**–**15** against five cancer cell lines and one non-cancerous human Beas-2B cell line. This material is available free of charge via the Internet at http://pubs.acs.org.

as a medicinal plant in mainland China. The whole plants of *Z. candida* are used to treat infantile convulsions, epilepsy, and tetanus.[2] Previous phytochemical studies on *Z. candida* have focused on the bulbs, leading to reports of four ceramides[3,4] and nine alkaloids.[5–9] Among these, *trans*-dihydronarciclasine was obtained as a cytostatic agent with an IC_{50} value of 3.2 ng/mL.[9] Expecting the other plant parts to be chemically distinct from the previously well-studied bulbs, the whole plant extract of *Z. candida* was investigated, leading to the isolation of seven new alkaloids, compounds **1** and **5**–**10**, and eight known alkaloids (**2**–**4**, **11**–**15**). Herein, we report the isolation, structure determination, and cytotoxic activities of compounds **1**–**15**.



RESULTS AND DISCUSSION

The air-dried whole plants of *Z. candida* were ground and extracted with acidic aqueous EtOH at room temperature. After evaporation of EtOH under reduced pressure, the residue was dissolved in 2% HCl, and extracted with $CHCl_3$ three times. The acidic aqueous phase was adjusted to pH 7 with aqueous NH_4OH and then extracted with $CHCl_3$. The resulting $CHCl_3$-soluble fraction was subjected to silica gel, RP (reversed-phase) C_{18}, and Sephadex LH-20 column chromatography, and repeated RP C_{18} HPLC, to afford compounds **1**–**15**.

Compound **1** was isolated as colorless cubic crystals, mp 237 °C, and exhibited a positive Dragendorff's test, indicating it to be an alkaloid. The molecular formula for the quaternary base of **1** was determined to be $C_{18}H_{22}NO_5^+$ by (+)-HRESIMS of the $[M - Cl]^+$ ion at *m/z* 332.1488 (calcd 332.1493). The IR spectrum showed the presence of hydroxy group (3239 cm^{-1}), aromatic (3003, 1509 and 1489 cm^{-1}), and methylenedioxy (937 cm^{-1}) absorption. The UV absorption at 245 and 292 nm was consistent with a compound containing a methylenedioxy-substituted benzene ring.[10] The NMR spectra (Tables 1 and 2) indicated that compound **1** was isolated as a mixture (ca. 3:2) of two isomers (**1a** and **1b**) at C-6. The ^1H NMR spectrum of **1** (Table 1) exhibited signals for an *O*-methyl (δ_H 3.40, s), a *N*-methyl (δ_H 3.23, s), a methylenedioxy group (δ_H 6.03, s), two sets of singlet aromatic protons (δ_H 6.92/6.86, s; 7.03/7.07, s), and two olefinic protons (δ_H 6.42, m; 6.41, m). In turn, the ^{13}C NMR and DEPT spectra (Table 2) showed the presence of an *O*-methyl (δ_C 57.2), a *N*-methyl (δ_C 43.8/44.4), a methylenedioxy (δ_C 103.6), two methylenes (δ_C 62.9/68.7, 26.2/25.7), four methines bearing heteroatoms (71.9/65.4, 72.5/72.6, 78.1/78.2, 96.2/96.9), four sp^2 methines (δ_C 104.5, 109.2/110.0, 126.8/126.7; 131.3/131.4), a sp^3 quaternary carbon (δ_C 53.4/53.9), and four sp^2 quaternary carbons (δ_C 124.3/125.4, 134.1, 149.3, 150.9/149.3). The NMR data of **1** were similar to those of compound **2**, which was identified as haemanthamin,[5] and previously isolated from the bulbs of *Z. candida*.[3,5] The major difference found was the chemical shift of C-6 (δ_C 96.2/96.9), which in **1** was shifted downfield by almost 32 ppm in comparison to that of **2** (δ_C 64.0), indicating that C-6 in **1**

bears two heteroatoms. Furthermore, an additional *N*-methyl group in **1** was deduced from the HMBC correlations from *N*-CH_3 (δ_H 3.23, s, 3H) to C-6 (δ_C 96.2/96.9), C-4a (δ_C 71.9/65.4), and C-12 (δ_C 62.9/68.7). The planar structures of **1a** and **1b** were deduced unambiguously from analysis of the 2D-NMR spectroscopic data, including ^{1}H-^{1}H COSY, HSQC, and HMBC experiments, and the relative configurations of **1a** and **1b** were determined from the NOESY spectrum. In the NOESY spectrum of **1**, the cross peak of *O*CH_3-3 and H-4a indicated the *syn*-relationship of these groups, which were assigned in a β-orientation, the same as those in **2**. The NOESY correlations of H-3/H-4α/H-12 suggested the *N*-ethyl bridged ring between C-10b and *N*-5 is α-oriented. The NOESY correlations of H-6 with H-12 in **1a**, and H-6 with H-4a in **1b**, suggested H-6 in **1a** and **1b** are in an α and a β-orentation, respectively. This is consistent with the structures of (+)-6-hydroxycrinamine, yemenines B and C,[10] and haemanthidine[11] which all have a similar epimeric C-6 relationship, while the structure of (+)-6-hydroxycrinamine has been confirmed by single-crystal X-ray diffraction.[12] The characteristic Cotton effects [250 nm ([θ], −4686), 289 nm ([θ], +5931) in MeOH] in the circular dichroism (CD) spectrum of **1** were used to determine that the absolute configuration of the 5α,10b-ethanophenanthridine system[10] is the same as that of haemanthamin (**2**). A suitable crystal of **1** was obtained for single-crystal X-ray diffraction analysis, and the result revealed that **1** was obtained in the form of a chloride salt, and confirmed the structure deduced. In the crystal structure (Figure 1), the ratio of the two epimers **1a** and **1b** was determined as 75:25. Importantly, the presence of the chlorine atom allowed a determination of the chirality of this structure using the Flack coefficient.[13] The coefficient obtained [−0.04 (12)] for the given coordinates indicated that **1a** and **1b** possess the same absolute configurations as both haemanthamin (**2**), ignoring C-6, and the parent structure haemanthidine, which was isolated from the bulbs of *Z. candida*.[5] It is possible that the chloride atom in **1** was introduced by the addition of HCl during the extraction procedures. Therefore, compound **1** is determined to be *N*-methylhaemanthidine chloride.

Compound **5** was obtained as yellow amorphous powder, and its HRESIMS showed quasimolecular ion peaks at *m/z* 343.1688 $[M + H]^+$ (calcd for $C_{19}H_{23}N_2O_4^+$, 343.1652), 685.3244 $[2M + H]^+$ (calcd for $C_{38}H_{45}N_4O_8^+$, 685.3232), and 707.3064 $[2M + Na]^+$ (calcd for $C_{38}H_{44}N_4NaO_8^+$, 707.3051), corresponding to the molecular formula, $C_{19}H_{22}N_2O_4$, and indicating ten degrees of unsaturation. The UV spectrum showed absorption maxima at 250 and 290 nm, again consistent with the presence of a methylenedioxy-substituted benzene ring. The NMR data of compound **5** (Tables 1 and 2) were similar to those of tazettine (**4**).[14] However, three differences were evident: (1) the oxygen atom at position C-5 in compound **4** is replaced by a *N*-Me group (δ_H 2.78, s, 3H; δ_C 43.7) in compound **5**; (2) C-11 is oxidized from a methylene (δ_H 3.28, d, J = 10.4 Hz, 1H, H-6α; 2.66, d, J = 10.4 Hz, 1H, H-6β; δ_C 65.7) in compound **4** to a carbonyl (δ_C 173.9) in compound **5**; (3) the quaternary carbon (δ_C 102.3) bearing two heteroatoms at C-12 in compound **4** is replaced with a methine (δ_H 3.56, s, 1H; δ_C 68.9) bearing a nitrogen atom in compound **5**. The planar structure of **5** was deduced from its ^{1}H-^{1}H COSY, HSQC, and HMBC data, and the relative configuration was determined from the NOESY spectrum. The α-orientation of the methoxy group (δ_H 3.49, s, 3H) at C-3 was inferred from the coupling constant of $J_{2,3}$ = 4.9 Hz[15,16] and the NOE correlation of *O*CH_3-3 to H-10 (δ_H 6.77, s). The cross peaks between H-12 (δ_H 3.56, s) and H-1 (δ_H 5.87, d, J = 10.0 Hz) and H-4α (δ_H 1.73, ddd, J = 13.4, 10.8, 3.5 Hz) in the NOESY spectrum of **5** indicated H-12, H-1, and H-4α to be in the same orientation. Literature investigation showed that **5** has the same molecular skeleton as plicamine,[16] plicane,[17] and obliquine,[18] where there is a nitrogen atom, instead of an oxygen atom at position C-5 of the basic tazettine nucleus. To our knowledge, compound **5** (*N*-methyl-5,6-dihydroplicane) is the fourth example of a plicamine-type Amaryllidaceae alkaloid.

Compound **6**, a colorless oil, was found to be optically active, $[\alpha]^{25}_{D}$ +140. The molecular formula of this compound was established as $C_{20}H_{28}NO_5$ by HRESIMS at *m/z* 362.1965 $[M + H]^+$ (calcd for $C_{20}H_{28}NO_5^+$, 362.1962), in combination with the ^1H NMR and ^{13}C NMR data. The ^1H NMR spectra showed a *N*-CH_3 signal at δ_H 2.03 (s, 3H), four *O*-CH_3 signals at δ_H 3.50 (s, 3H), 3.81 (s, 3H), 3.86 (s, 3H), and 3.88 (s, 3H), an aromatic proton signal at δ_H 6.85 (s, 1H, H-10), and an olefinic proton signal at 5.51 (m, 1H, H-3). The ^{13}C NMR, DEPT, and HSQC spectra indicated the presence of a *N*-CH_3 (δ_C 44.4), four *O*-CH_3 (δ_C 55.8, 56.7, 61.3, 61.6), three methylenes (δ_C 32.7, C-4; 29.0, C-11; 57.9, C-12), and three methines (δ_C 98.0, C-6; 67.3, C-4a; 44.8, C-10b). The NMR data (Tables 1 and 2) suggested compound **6** has a similar structure to nerinine,[8,19] which was first isolated from the bulbs of *Z. candida* collected in Japan. The major difference was an additional methoxy group (δ_H 3.50, s, 3H; δ_C 55.8) in **6**. In the HMBC spectrum of **6**, the correlations of the additional methoxy group (δ_H 3.50, s, 3H) to C-6 (δ_C 98.0) and H-6 (δ_H 5.52, s, 1H) to the *O*-Me (δ_C 55.8), supported the methoxy group being located at C-6. A series of 2D-NMR experiments including ^1H-^1H COSY, HSQC, and HMBC allowed assignment of the planar structure of compound **6**. The larger coupling constant $J_{1,10b}$ = 9.6 Hz of H-1 (δ_H 2.79, br d) indicated that H-1 and H-10b are in an *anti*-relationship, while the smaller coupling constant $J_{4a,10b}$ = 2.0 Hz of H-10b (δ_H 2.41, dd, *J* = 9.6, 2.0 Hz) suggested the *syn*-relationship of H-4a and H-10b. The NOESY correlations of H-4a, H-10b, and *O*CH_3-6 reflected the same orientation. All of the above information suggested compound **6** (*O*-methylnerinine) had the same configuration as that of nerinine.

Compound **7** was isolated as a gray amorphous powder. The molecular formula was deduced as $C_{19}H_{17}NO_5$ on the basis of the ion peaks at *m/z* 340.1185 $[M + H]^+$ (calcd for $C_{19}H_{18}NO_5^+$, 340.1180) and 701.2100 $[2M + Na]^+$ (calcd for $C_{38}H_{34}N_2NaO_{10}^+$, 701.2106) in the HRESIMS. The ^1H NMR (Table 3) and ^{13}C NMR (Table 4) spectra of **7** resembled those of **11**, *N*-methylcrinasiadine, [20] except that the *N*-Me in **11** was replaced by a *N*-ethoxycarbonylethyl in **7**. The ^1H NMR spectrum showed characteristic signals for *O*-CH_2CH_3 at δ_H 4.16 (q, *J* = 7.2 Hz, 2H, H-14) and 1.24 (t, *J* = 7.2 Hz, 3H, H-15), and for *N*-CH_2CH_2-at δ_H 4.67 (t, *J* = 7.9 Hz, 2H, H-11) and 2.79 (t, *J* = 7.9 Hz, 2H, H-12). The signals at δ_C 38.9 (t, C-11), 32.4 (t, C-12), 171.6 (s, C-13), 61.1 (t, C-14), 14.4 (q, C-15) in the ^{13}C NMR and DEPT spectra confirmed the presence of the *N*-ethoxycarbonylethyl group. In the HMBC spectrum, the correlating peaks between H-11, H-12, and H-14 to C-13 (δ_C 171.6) further supported the above deduction. In addition, the HMBC correlations of H-11 to C-4a (δ_C 136.4) and C-6 (δ_C 160.9) suggested that the *N*-ethoxycarbonylethyl group was located at *N*-5. The structure was confirmed by the analysis of the HMBC, HSQC, and ^1H-^1H COSY spectra. Therefore, compound **7** was assigned to be *N*-ethoxycarbonylethylcrinasiadine.

Compound **8** was isolated as a white amorphous powder. Its HRESIMS displayed a quasimolecular ion peak at *m/z* 354.1340 $[M + H]^+$ (calcd for $C_{20}H_{20}NO_5^+$, 354.1336), corresponding to the molecular formula $C_{20}H_{19}NO_5$. The ^1H NMR (Table 3) and ^{13}C NMR (DEPT) (Table 4) spectra of compound **8** exhibited resonances closely related to those of compound **7**, except for the appearance of signals for an additional methylene (δ_H 2.10, m, 2H, H-12; δ_C 22.7, C-12). The cross peaks of this methylene to H-11 (δ_H 4.43, t, *J* = 7.9 Hz, 2H) and H-13 (δ_H 2.51, t, *J* = 7.0 Hz, 2H) in the ^1H-^1H COSY spectrum indicated it to be inserted between C-11 and C-13. This confirmed the presence of a partial structure, -CH_2-CH_2-CH_2-. HMBC correlations of H-12 to C-11 (δ_C 42.3), C-13 (δ_C 31.7), and C-14 (173.4), and H-15 (δ_H 4.15, q, *J* = 7.1 Hz, 2H) to C-14 suggested the presence of a *N*-ethoxycarbonylpropyl group. The location of this substituent was concluded from the HMBC cross-peaks of H-11 to C-4a (δ_C 136.7), C-6 (δ_C 161.0), C-12, and C-13. The structure of compound **8** was assigned as *N*-ethoxycarbonylpropylcrinasiadine.

Compound **9** was isolated as a white amorphous powder. Its HRESIMS indicated a molecular formula of $C_{22}H_{17}NO_3$, based on the ion peaks at *m/z* 344.1282 $[M + H]^+$ (calcd for $C_{22}H_{18}NO_3^+$, 344.1281) and 687.2474 $[2M + H]^+$ (calcd for $C_{44}H_{35}N_2O_6^+$, 687.2490). Comparison of their NMR data (Tables 3 and 4) showed that compounds **9** and **7** have the same parent ring system. However, signals for a mono-substituted benzene ring (δ_H 7.34, m, 2H; 7.38, m, 2H; 7.25, m, 1H; δ_C 138.8, 129.1, 128.9, 126.9) were apparent in compound **9**, but signals for the ethoxycarbonyl group were absent in compound **7**. The position of the substituent of the mono-substituted benzene ring was determined from the cross-peaks of H-12 (δ_H 3.06, t, J = 8.4 Hz) to C-13 (δ_C 138.8) and C-14 (δ_C 129.1) in the HMBC spectrum. HMBC correlations of H-11 (δ_H 4.57, t, J = 8.4 Hz, 2H) with C-4a (δ_C 136.6), C-6 (δ_C 160.8), and C-12 (δ_C 33.9), together with the chemical shifts of H-11 and C-11 suggested the phenethyl is located at position *N*-5. Accordingly, the structure of **9** was assigned as *N*-phenylethylcrinasiadine.

The molecular formula of compound **10** was determined as $C_{19}H_{19}NO_3$ by HRESIMS analysis [*m/z* 310.1434 $[M + H]^+$ (calcd for $C_{19}H_{20}NO_3^+$, 310.1438), 619.2790 $[2M + H]^+$ (calcd for $C_{38}H_{39}N_2O_6^+$, 619.2803)]. The ^{1}H NMR, ^{13}C NMR, and DEPT spectra of **10** exhibited signals for two methyls (δ_H 1.04, d, J = 6.6 Hz, 6H; δ_C 22.8, C-14/15), two methylenes (δ_H 1.66, m, 2H, δ_C 36.3, C-12; δ_H 4.37, 2H, t, J = 8.1 Hz, δ_C 41.7, C-11), and a methine (δ_H 1.81, m, 1H; δ_C 26.9, C-13). It was concluded from the ^{1}H-^{1}H COSY, HSQC, and HMBC spectra of **10** that the above signals could be attributed to an isopentyl group. In consideration of the chemical shifts of H-11 and C-11, the isopentyl group could be connected to a nitrogen atom. Compound **10** had similar NMR spectra (Tables 3 and 4) compared with compound **11**, except that the signals for *N*-CH_3 in **11** were absent and replaced by signals for *N*-isopentyl group in **10**. The cross peaks of H-11 to C-4a (δ_C 136.8) and C-6 (δ_C 160.8) in the HMBC spectrum of **10** indicated that the isopentyl group was located at *N*-5. Detailed ^{1}H-^{1}H COSY, HSQC, and HMBC spectroscopic analysis was used to assign compound **10** as *N*-isopentylcrinasiadine.

Compounds **2**–**4**, **11**–**15** were identified as haemanthamin (**2**),[5] 3-epimacronine (**3**),[21] (+)-tazettine (**4**),[14] *N*-methylcrinasiadine (**11**),[20] trisphaeridine (**12**),[22] 5,6-dihydrobicolorine (**13**),[21] lycorine (**14**),[23] and nigragillin (**15**),[24] respectively, on the basis of spectroscopic data analysis and comparison with those reported in the literature. The known amaryllidaceous alkaloids **3**, **11**, and **13** were isolated from *Z. candida* for the first time. Compound **15** was originally isolated from the fungus *Aspergillus niger*,[24] and is reported from a plant for the first time, although it is possibly biosynthesized by an endophytic fungus of *Z. candida*. For compounds **7** and **8**, it cann't be ruled out that they are artifacts of isolation, resulting from esterification of the corresponding carboxylic acids, which presently are unknown.

The relative configuration of 3-epimacronine (**3**) has been confirmed by single-crystal X-ray diffraction analysis irradiated by MoK_α at 173 K,[25] but the absolute configuration of 3-epimacronine is still unknown. In order to determine its absolute configuration, compound **3** was recrystallized in MeOH containing a small amount of H_2O, and the crystal was subjected to single-crystal X-ray diffraction analysis on a Bruker APEX-II diffractometer equipped with graphite-monochromatized CuK_α radiation (λ = 1.54178 Å) at 100 (2) K. The results indicated that the molecule crystallized in the enantiomorphic $P2_1$ space group (No. 4), and should have optical properties. The Flack parameter, associated with the absolute configuration, was calculated to be −0.01 (12), which confirmed the absolute configuration of compound **3**. The crystal structure of compound **3** (3-epimacronine) is shown in Figure 2, and its absolute configuration was determined as 3*S*, 10b*S*, 5*S*, 11*R*.

Since Amaryllidaceae alkaloids are reported to exhibit cytotoxic properties,[1,26–33] compounds **1**–**15** were evaluated for their cytotoxic activities against five human cancer cell lines, namely HL-60 and K562 human myeloid leukemia, A549 lung cancer, HepG2 hepatocellular carcinoma, and HT-29 colon cancer, as well as the immortalized non-cancerous Beas-2B human bronchial epithelial cell line. The results (Supporting Information, Table S1) showed compounds **1**, **2**, **9**, and **14** exhibited more potent cytotoxicity than one of the positive controls (*cis*-platin), while compounds **3**–**8**, **10**–**13**, and **15** did not show obvious cytotoxicity (IC_{50} >10 μM). The known compound **2** (haemanthamin) strongly inhibited the proliferation of five cancer cells with IC_{50} values of 1.4, 2.5, 2.5, 4.8, and 2.1 μM, respectively, which are in agreement with those reported in the literature.[26,29] Compound **2** has been reported to be cytostatic, not cytotoxic, and its antiproliferative effects result from its complex formation with RNA.[26] The new compound **1** showed more potent cytotoxicity than compound **2** against the same cell lines with IC_{50} values of 0.91, 1.0, 1.1, 1.5 and 1.2 μM, respectively. The mechanism by which compound **1** exhibits cytotoxicity is likely similar to that of **2**, due to their structural similarity. The new compound **9** showed the strongest cytotoxicity against HL-60, K562, A549, HepG2, and HT-29 cells with IC_{50} values of 0.81, 0.70, 13, 1.4, and 2.3 μM, respectively. Compound **14** (lycorine) strongly inhibited cancer cell proliferation with IC_{50} values of 1.6, 2.3, 1.9, 3.7, and 3.2 μM, respectively, which was consistent with previous reports,[29,30] and it was reported that effects of lycorine (**14**) on HL-60 cells was via arresting cell cycle and inducing apoptosis,[31] due to the down-regulation of Mcl-1 in human leukemia cells.[32] Interestingly, compounds **1**, **2**, **7**–**9**, and **14** exhibited higher IC_{50} values against the non-cancerous Beas-2B cell line (3.7, 5.0, > 40, 36, 7.3, and 4.9 μM, respectively) than those against the five cancer cell lines, showing these compounds are selective for the cancer cell lines used (see Supporting Information, Table S1). Therefore, understanding the molecular and cellular mechanism of these compounds in affecting cell proliferation or possible apoptosis may be relevant to the design of therapeutic strategies.

EXPERIMENTAL SECTION

General Experimental Procedures

Melting points (uncorrected) were determined on a Beijing Tech X-5 microscopic melting point apparatus. Optical rotations were determined in $CHCl_3$ or MeOH, as indicated, on a Perkin-Elmer 341 polarimeter. The CD spectra were obtained on a JASCO J-810 spectrometer. UV and FT-IR spectra were determined using Varian Cary 50 and Bruker Vertex 70 instrument, respectively. NMR spectra were recorded on a Bruker AM-400 spectrometer, and the ^1H and ^{13}C NMR chemical shifts were referenced to the solvent peaks for $CDCl_3$ at δ_H 7.24 and δ_C 77.23 or for CD_3OD at δ_H 3.31 and δ_C 49.15. Electrospray time-of-flight mass spectra (ESI-TOF-MS) (accurate mass) were measured in the positive-ion mode on an Agilent MSD-TOF mass spectrometer, while low-resolution and multidimensional electrospray ion trap mass spectra (ESIMS and ESIMS/MS) were measured in the positive-ion mode on a Thermo Finnigan LCQ Deca XP Max mass spectrometer. The crystallographic data were obtained on a Bruker SMART CCD area-detector diffractometer equipped with graphite-monochromatized MoK_α radiation (λ = 0.71073 Å), and a Bruker SMART APEX-II CCD diffractometer equipped with graphite-monochromatized CuK_α radiation (λ = 1.54178 Å). HPLC was conducted on an Agilent 1200 instrument with detection at 210 nm using a C_{18} column (5 μm, 250 × 10 mm, YMC-pack ODS-A) and MeOH–H_2O as the mobile phase at 2.0 mL/min flow rate. Column chromatography (CC) was carried out on silica gel (100–200 mesh, 200–300 mesh, and 400 mesh, Qingdao Haiyang Chemical Industry Co. Ltd, P. R. China), RP-C_{18} silica gel (ODS-A-HG, YMC Co. Ltd, Japan), and Sephadex LH-20 (GE Healthcare Bio-Sciences AB, Sweden). Thin-layer chromatography (TLC) was performed with silica gel 60 F254 (Yantai

Chemical Industry Research Institute, P. R. China) and RP-C_{18} F254 plates (Merck, Germany). MPLC was carried out with a EZ Purifier III chromatography system.

Plant Material

The whole plants of *Zephyranthes candida* were collected at Shiyan, Hubei Province, People's Republic of China, in June, 2010. The plant material was identified by Professor Changgong Zhang of the School of Pharmacy, Tongji Medical College, Huazhong University of Science and Technology. A voucher specimen (No. 20100601) has been deposited at the Hubei Key Laboratory of Natural Medicinal Chemistry and Resource Evaluation, School of Pharmacy, Tongji Medical College, Huazhong University of Science and Technology.

Extraction and Isolation

The whole dried plants of *Z. candida* (10 kg) were extracted four times with 25 L each of 95% aqueous EtOH with 2% HCl at room temperature. The filtrates were combined and concentrated under vacuum to afford 1150 g of crude extract, which was then partitioned between $CHCl_3$ and 2% aqueous HCl (3 L each), followed by re-extracting the aqueous phase three additional times with $CHCl_3$ (3 L). After the aqueous phase was adjusted to pH 7 with NH_4OH, it was partitioned between $CHCl_3$ (4 × 1.5 L) for the second time. On evaporation, the $CHCl_3$ phase (18 g) was chromatographed over silica gel by MPLC and eluted with a MeOH–$CHCl_3$ gradient to give five fractions, A–E. Fraction A was separated into three subfractions, A1, A2, and A3, by CC on Sephadex LH–20 eluting with MeOH-CH_2Cl_2 (50:50). Subfraction A2 was subjected to CC on silica gel using a gradient system of cyclohexane–$CHCl_3$ (33:67) providing two fractions, A2A and A2B. Fraction A2B was further separated by semi-preparative HPLC (70:30 MeOH-H_2O mobile phase) to yield compound **11** (5.7 mg, t_R 38.4 min). Subfraction A3 was subjected to CC on silica gel using a gradient system of cyclohexane–$CHCl_3$ (25:75) providing two fractions, A3A and A3B. A3B was further separated by semi-preparative HPLC (80:20 MeOH-H_2O) to yield compounds **7** (2.0 mg, t_R 19.2 min), **8** (3.8 mg, t_R 24.5 min), **9** (2.3 mg, t_R 45.6 min), and **10** (2.4 mg, t_R 48.0 min). Similar to fraction A, fraction B was separated into three subfractions, B1, B2, and B3. B2 was subjected to separation on RP-C_{18} using a gradient system of MeOH–H_2O (50:50–100:0) to provide three subfractions, B2A, B2B, and B2C. Compound **3** (8.6 mg, mp 132 °C) was crystallized from subfraction B2B. Fraction B3 was purified by semi-preparative HPLC (80:20 MeOH-H_2O) to yield compounds **13** (6.4 mg, t_R 15.3 min) and **12** (5.9 mg, t_R 19.4 min). Fraction C was separated into two subfractions (C1 and C2) by Sephadex LH–20 using MeOH-CH_2Cl_2 (50:50). Subfraction C2 was subjected to separation on RP-C_{18} using a gradient system of MeOH–H_2O (20:80–100:0) to provide three further subfractions, C2A, C2B, and C2C. Fraction C2B was subjected to separation over silica gel using a gradient system of MeOH–$CHCl_3$ (10:90–100:0), to provide three subfractions, C2BA, C2BB, and C2BC. Compounds **5** (16.2 mg, t_R 15.9 min), **6** (7.6 mg, t_R 36.1 min), and **15** (2.1 mg, t_R 14.5 min) were isolated from two subfractions, C2BB and C2BC, using semi-preparative HPLC (70:30 MeOH-H_2O), and compound **4** (34.9 mg, mp 210 °C) was crystallized from fraction C2C. Fraction D was separated into two subfractions, D1 and D2, by passage over Sephadex LH–20. Subfraction D2 was subjected to purification on RP-C_{18} using stepwise gradient elution with MeOH–H_2O, to furnish two subfractions, D2A and D2B. Granules of compound **2** (15.7 mg) appeared in fraction D2B. Fraction E was separated on a RP–C_{18} column using a MeOH-H_2O gradient to yield three fractions, E1, E2, and E3. Compound **14** (5.4 mg) was afforded from fraction E2, and compound **1** (63.8 mg, mp 237 °C) was crystallized from fraction E3.

***N*-Methylhaemanthidine chloride (1)—**Colorless cubes; mp 237 °C; $[\alpha]^{25}_D$ +5 (c 0.75, CH_3OH); UV (MeOH) λ_{max} (log ε) 210 (4.27), 245 (3.47), 292 (3.52) nm ; CD (MeOH)

209 ([θ], +18842), 250 ([θ], −4686), 289 ([θ], +5931) nm; IR (KBr) ν_{max} 3239, 3003, 1509, 1489, 1252, 1132, 1030, 937, 825 cm^{-1}; ^1H NMR data, see Table 1; ^{13}C NMR data, see Table 2; (+)-HRESIMS *m/z* 332.1488 $[M - Cl]^+$ (calcd for $C_{18}H_{22}NO_5^+$, 332.1493).

***N*-Methyl-5,6-dihydroplicane (5)—**Yellow amorphous powder; $[\alpha]^{25}_D$ +100 (*c* 0.063, CH_3OH); UV (MeOH) λ_{max} (log ε) 210 (4.31), 245 (3.70), 292 (3.51) nm; IR (KBr) ν_{max} 2935, 1607, 1489, 1341, 1048, 1025 cm^{-1}; ^1H NMR data, see Table 1; ^{13}C NMR data, see Table 2; HRESIMS *m/z* 343.1688 $[M + H]^+$ (calcd for $C_{19}H_{23}N_2O_4^+$, 343.1652), 685.3244 $[2M + H]^+$ (calcd for $C_{38}H_{45}N_4O_8^+$, 685.3232), 707.3064 $[2M + Na]^+$ (calcd for $C_{38}H_{44}N_4NaO_8^+$, 707.3051).

***O*-Methylnerinine (6)—**Colorless oil; $[\alpha]^{25}_D$ + 140 (*c* 0.22, CH_3OH); UV (MeOH) λ_{max} (log ε) 214 (4.42), 240 (3.90), 284 (3.51) nm; IR ν_{max} 2939, 2909, 1600, 1493, 1460, 1337, 1097, 1051, 1022 cm^{-1}; ^1H NMR data, see Table 1; ^{13}C NMR data, see Table 2; HRESIMS *m/z* 362.1965 $[M + H]^+$ (calcd for $C_{20}H_{28}NO_5^+$, 362.1962).

***N*-Ethoxycarbonylethylcrinasiadine (7)—**Gray amorphous powder; UV ($CHCl_3$) λ_{max} (log ε) 240 (5.72), 311(4.66), 339 (4.62), 368 (4.26), 371 (4.31), 665 (3.66) nm; IR (KBr) ν_{max} 2922, 2853, 1730, 1644, 1627, 1601, 1510, 1484, 1462, 1313, 1037, 751 cm^{-1}; ^1H NMR data, see Table 3; ^{13}C NMR data, see Table 4; HRESIMS *m/z* 340.1185 $[M + H]^+$ (calcd for $C_{19}H_{18}NO_5^+$, 340.1180), 701.2100 $[2M + H]^+$ (calcd for $C_{38}H_{34}N_2NaO_{10}^+$, 701.2106).

***N*-Ethoxycarbonylpropylcrinasiadine (8)—**White amorphous powder; UV ($CHCl_3$) λ_{max} (log ε) 252 (4.58), 257 (4.52), 270 (4.15), 308 (3.14), 318 (3.99), 341 (3.83), 371 (3.17), 668 (2.22) nm; IR (KBr) ν_{max} 2976, 2926, 1892, 1729, 1647, 1626, 1601, 1461, 1199, 1176,1036, 753 cm^{-1}; ^1H NMR, see Table 3; ^{13}C NMR, see Table 4; HRESIMS *m/z* 354.1340 $[M + H]^+$ (calcd for $C_{20}H_{20}NO_5^+$, 354.1336), 729.2395 $[2M + Na]^+$ (calcd for $C_{40}H_{38}N_2NaO_{10}^+$, 729.2419).

***N*-Phenylethylcrinasiadine (9)—**White amorphous powder; UV ($CHCl_3$) λ_{max} (log ε) 270 (4.08), 313 (3.91), 341 (3.76), 372 (3.13) nm; IR (KBr) ν_{max} 2957, 2924, 2855, 1747, 1631, 1600, 1462, 1310, 1039, 751 cm^{-1}; ^1H NMR, see Table 3; ^{13}C NMR, see Table 4; HRESIMS *m/z* 344.1282 $[M + H]^+$ (calcd for $C_{22}H_{18}NO_3^+$, 344.1281), 687.2474 $[2M + H]^+$ (calcd for $C_{44}H_{35}N_2O_6^+$, 687.2490).

***N*-Isopentylcrinasiadine (10)—**Colorless solid; UV ($CHCl_3$) λ_{max} (log ε) 270 (3.96), 312 (3.80), 341 (3.68), 371 (3.30) nm; IR (KBr) ν_{max} 2924, 2856, 1720, 1639, 1601, 1459, 1312, 1035, 749 cm^{-1}; ^1H NMR, see Table 3; ^{13}C NMR, see Table 4; HRESIMS *m/z* 310.1434 $[M + H]^+$ (calcd for $C_{19}H_{20}NO_3^+$, 310.1438), 619.2790 $[2M + H]^+$ (calcd for $C_{38}H_{39}N_2O_6^+$, 619.2803).

Crystallographic Data and X-ray Structure Analysis of 1

A suitable colorless crystal of **1** was obtained by slow evaporation from MeOH at room temperature. Diffraction intensity data were obtained on a Bruker SMART CCD area-detector diffractometer equipped with graphite-monochromatized MoK_α radiation (λ = 0.71073 Å) at 298(2) K. Collection was conducted using the multirun procedure in the Bruker SMART software. Data reduction was subsequently performed with Bruker SAINT. Structure solution and refinement were performed with the SHELXTL program package. The final structure model obtained for **1** is shown in Figure 1. Crystal data and experimental details: $C_{18}H_{22}ClNO_5$, formula weight 367.82, crystal size 0.20 × 0.20 × 0.20 mm^3, crystal system tetragonal, space group *P*4 (1), *a* = 10.0493(12) Å, *b* = 10.0493(12) Å, *c* = 17.167(4)

Å, $\alpha = \beta = \gamma = 90°$, $V = 1733.7(5)$ Å^3, $Z = 4$, D_c 1.409 Mg/m^3, $F(000) = 776$, absorption coefficient 0.249 mm^{-1}. A total of 11307 reflections was collected in the range $2.03° < \theta < 25.99°$, with 3406 independent reflections [$R_{(int)} = 0.1008$], completeness to θ_{max} was 100.0 %; no absorption correction applied; full-matrix least-squares refinement on F^2; the number of data/restraints/parameters were 3406/3/241; goodness-of-fit on $F^2 = 1.065$; Final R indices [$I > 2\sigma(I)$] $R_1 = 0.0745$, $wR_2 = 0.1339$; R indices (all data) $R_1 = 0.1170$, $wR_2 = 0.1496$; absolute structure parameter −0.04 (12); largest difference peak and hole 0.227 and −0.179 e.Å^{-3}. Crystallographic data for the structure of **1** have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication CCDC 94570. Copies of these data can be obtained free of charge at www.ccdc.cam.ac.uk/conts/retrieving.html (or from the Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB21EZ, UK; fax: (+44) 1223-336-033; or e-mail: deposit@ccdc.cam.ac.uk).

Crystallographic Data and Single-Crystal X-ray Absolute Configuration Analysis of 3

A suitable light-yellow crystal of **3** was obtained by slow evaporation from MeOH at room temperature. Diffraction intensity data were obtained on a Bruker APEX-II diffractometer equipped with a graphite-monochromatized CuK_α radiation ($\lambda = 1.54178$ Å) at 100 (2) K. Collection was conducted using the multirun procedure in the Bruker APEX-II software. Data reduction was subsequently performed with Bruker SAINT. Structure solution and refinement were performed with the SHELXTL program package. The final structure model obtained for **3** is shown in Figure 2. Crystal data and experimental details: empirical formula $C_{18}H_{19}NO_5 \cdot 0.13H_2O$, formula weight 331.68, crystal size 0.80 × 0.60 × 0.25 mm^3, crystal system monoclinic, space group $P2_1$, $a = 8.1308$ (2) Å, $b = 17.5736$ (3) Å, $c = 11.0262$ (2) Å, $\alpha = 90°$, $\beta = 94.9400$ (10)°, $\gamma = 90°$, $V = 1569.65$ (5) (2) Å^3, $Z = 4$, D_c 1.404 Mg/m^3, $F(000) = 716$, absorption coefficient 0.857 mm^{-1}. A total of 13397 reflections was collected in the range $4.02° < \theta < 69.46°$, with 5191 independent reflections [$R_{(int)} = 0.0274$], completeness to θ_{max} was 95.2 %; semi-empirical from equivalents absorption correction with SADABS applied; full-matrix least-squares refinement on F^2; the number of data/restraints/parameters were 5191/1/447; goodness-of-fit on $F^2 = 1.047$; final R indices [$I > 2\sigma(I)$] $R_1 = 0.0282$, $wR_2 = 0.0732$; R indices (all data) $R_1 = 0.0282$, $wR_2 = 0.0732$; absolute structure parameter 0.01 (10); largest difference peak and hole 0.238 and −0.191 e.Å^{-3}. Crystallographic data for the structure of **3** have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication CCDC 94569. Copies of these data can be obtained free of charge at www.ccdc.cam.ac.uk/conts/retrieving.html (or from the Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB21EZ, UK; fax: (+44) 1223-336-033; or e-mail: deposit@ccdc.cam.ac.uk).

Cytotoxicity Assays

Five human cancer cell lines were used, namely, HL-60 and K562 human myeloid leukemias, A549 lung cancer, HepG2 hepatocellular carcinoma, and HT-29 colon cancer, together with one non-cancerous cell line, Beas-2B human bronchial epithelial. Cells were cultured in RPMI-1640 or in DMEM medium (Hyclone, Logan, UT, USA), supplemented with 10% fetal bovine serum (Hyclone) in 5% CO_2 at 37 °C. The cytotoxicity assay was performed using a MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) method in 96-well microplates, as reported previously, with slight modification.[34,35] Briefly, 100 μL of adherent cells were seeded into each well of the 96-well culture plates and allowed to adhere for 12 h before adding the test compounds, while suspended cells were seeded into wells at a density of 1×10^5 cells/mL just prior to the addition of the test compounds. Each tumor cell line was exposed to the test compound at concentrations of 0.0625, 0.32, 1.6, 8, and 40 μM in triplicates for 48 h. Wells with DMSO were used as negative controls, and DDP (*cis*-platin, Sigma, St. Louis, MO, USA) was used as a positive control. After compound treatment, cell viability was detected by a Bio-Rad 680 at λ = 595

nm and a cell growth curve was graphed. IC_{50} values were calculated by the method described by Reed and Muench.[36]

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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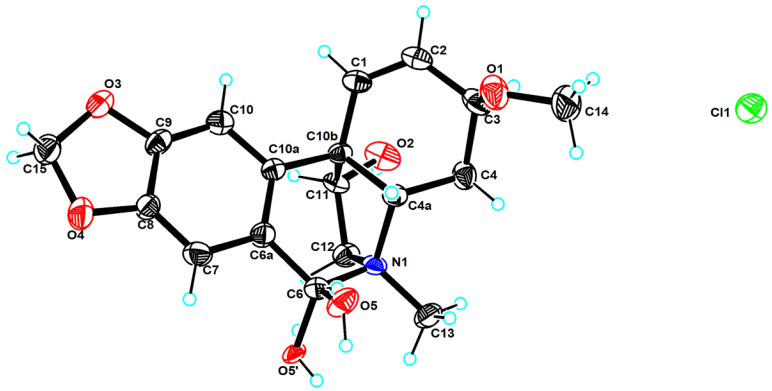

Figure 1.
X-ray ORTEP drawing of compound **1** with ellipsoid probability of 30% by MoK$_\alpha$ radiation at 298 (2) K.

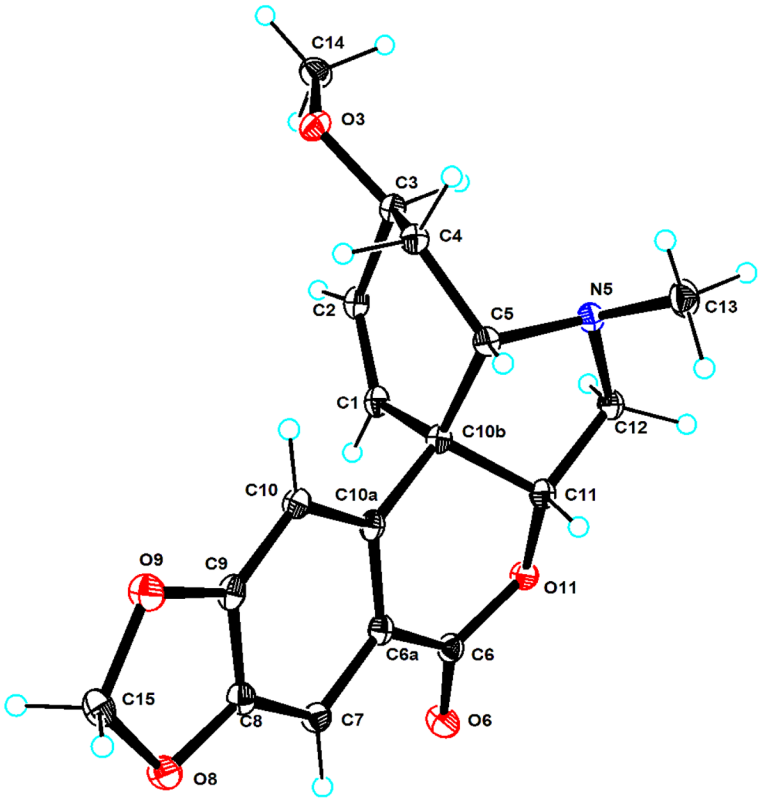

Figure 2.
X-ray ORTEP drawing of compound **3** with ellipsoid probability of 30% by CuK_{α} radiation at 100 (2) K.

Table 1

^{1}H NMR [δ, mult, (J in Hz)] Data for Compounds 1, 5, and 6 in CD_3OD (400 MHz)

position	1a	1b	5	6
1	6.42, overlap	6.42, overlap	5.87, d (10.0)	2.79, br d (9.6)
2	6.41, overlap	6.41, overlap	6.13, dd (10.0, 4.9)	
3	4.04, overlap	4.04, overlap	3.94, ddd (4.9, 4.0, 3.5)	5.51, overlap
4α	2.56, ddd (15.6, 13.3, 2.8)	2.56, ddd (15.6, 13.3, 2.8)	1.73, ddd (13.4, 10.8, 3.5)	2.66, m
4β	2.46, dd (15.6, 4.6)	2.34, dd (15.6, 4.6)	2.41, ddd (13.4, 4.4, 4.0)	2.28, m
4a	4.16, dd (13.3, 4.6)	4.16, dd (13.3, 4.6)	3.79, dd (10.8, 4.4)	4.38, br d (6.4)
6α	6.05, s		3.84, d (15.3)	
6β		5.64, s	3.55, d (15.3)	5.52, s
7	6.92, s	6.86, s	6.54, s	
10	7.03, s	7.07, s	6.77, s	6.85, s
10b				2.41, dd (9.6, 2.0)
11	4.02, m, overlap	4.02, m, overlap		2.50, m
12α	3.25, dd (12.7, 2.4)	3.63, d (13.7)	3.56, s	3.15, ddd (10.0, 7.5, 2.9)
12β	4.58, dd (12.7, 6.3)	4.20, m, overlap		2.30, q (10.0)
OCH_2O	6.03, s	6.03, s	5.90, dd (4.1, 1.0)	
*O*Me-3	3.40, s	3.40, s	3.49, s	
*N*Me-5	3.23, s	3.23, s	2.78, s	
*O*Me-6				3.50, s
*O*Me-7				3.88, s
*O*Me-8				3.81, s
*O*Me-9				3.86, s
*N*Me-13			2.75, s	2.03, s

Table 2

^{13}C NMR Data for Compounds 1, 5, and 6 in CD_3OD (100 MHz)

position	1a	1b	5	6
1	126.8	126.7	136.4	69.1
2	131.3	131.4	126.8	141.3
3	72.5	72.6	72.7	117.6
4	26.2	25.7	30.4	32.7
4a	71.9	65.4	63.6	67.3
6	96.2	96.9	53.7	98.0
6a	124.3	125.4	129.5	134.6
7	109.2	110.0	107.0	153.2
8	149.3	149.3	148.4	142.9
9	150.9	151.2	148.5	154.8
10	104.5	104.5	109.4	110.3
10a	134.1	134.1	131.2	121.8
10b	53.4	53.9	45.9	44.8
11	78.1	78.2	173.9	29.0
12	62.9	68.7	68.9	57.9
OCH_2O	103.6	103.6	102.6	
*O*Me-3	57.2	57.2	57.0	
*N*Me-5	43.8	44.4	43.7	
*O*Me-6				55.8
*O*Me-7				61.6
*O*Me-8				61.3
*O*Me-9				56.7
*N*Me-13			28.1	44.4

Table 3

^{1}H NMR [δ, mult, (J in Hz)] Data for Compounds 7–10 in $CDCl_3$ (400 MHz)

position	7	8	9	10
1	8.10, dd (8.1, 1.1)	8.08, br d (8.0)	8.12, br d (8.0)	8.09, dd (8.0, 1.2)
2	7.28, ddd (8.1, 7.0, 1.1)	7.27, t (7.5)	7.29, ddd (8.0, 7.0, 1.2)	7.27, ddd (8.0, 7.1, 1.0)
3	7.50, ddd (8.4, 7.0, 1.2)	7.52, t (7.5)	7.52, ddd (8.4, 7.0, 1.1)	7.49, ddd (8.2, 7.1, 1.2)
4	7.42, br d (8.4)	7.58, br d (8.6)	7.46, br d (8.4)	7.37, br d (8.2)
7	7.87, s	7.88, s	7.91, s	7.89, s
10	7.61, s	7.61, s	7.63, s	7.61, s
OCH_2O	6.12, s	6.11, s	6.12, s	6.11, s
11	4.67, t (7.9)	4.43, t (7.9)	4.57, t (8.4)	4.37, t (8.1)
12	2.79, t (7.9)	2.10, m	3.06, t (8.4)	1.66, m
13		2.51, t (7.0)		1.81, m
14	4.16, q (7.2)		7.34, m	1.04, d (6.6)
15	1.24, t (7.2)	4.15, q (7.1)	7.38, m	1.04, d (6.6)
16		1.26, t (7.1)	7.25, m	
17			7.38, m	
18			7.34, m	

Table 4

^{13}C NMR Data for Compounds 7–10 in $CDCl_3$ (100 MHz)

position	7	8	9	10
1	123.6	123.3	123.5	123.4
2	122.7	122.5	122.5	122.3
3	129.3	129.3	129.2	129.1
4	114.8	115.5	115.0	115.2
4a	136.4	136.7	136.6	136.8
6	160.9	161.0	160.8	160.8
6a	130.8	130.8	130.7	130.7
7	107.1	107.1	107.1	107.1
8	148.7	148.6	152.5	148.6
9	152.6	152.5	148.7	152.4
10	100.7	100.6	100.7	100.6
10a	119.7	119.6	119.7	119.7
10b	121.3	121.3	121.5	121.5
OCH_2O	102.2	102.2	102.2	102.1
11	38.9	42.3	44.6	41.7
12	32.4	22.7	33.9	36.3
13	171.6	31.7	138.8	26.9
14	61.1	173.4	129.1	22.8
15	14.4	60.8	128.9	22.8
16		14.5	126.9	
17			128.9	
18			129.1	