New bioactive cyclopeptide alkaloids with rare terminal unit from the root bark of *Ziziphus cambodiana*†

Nattahaln Lomchoey, Panomwan Panseeta, Pornthip Boonsri, Nuttapon Apiratikul, Samran Prabpai, Palangpon Kongsaeere and Sunit Suksamrarn

Six new 14-membered ring cyclopeptide alkaloids, cambodines A–F (1–6), and two known compounds, frangufoline (7) and lotusanine B (8), were isolated from the root bark extract of *Ziziphus cambodiana* Pierre. Their structures and configurations were established based on 1D and 2D NMR, HRMS, ECD, and X-ray crystallographic data. Compounds 1 and 3 are rare 5(14)-type cyclopeptide alkaloids that possess an imidazolidin-4-one ring in the terminal unit. The cyclopeptides were tested for their in vitro antiplasmodial, antitubercular, and cytotoxic effects against three cancer cell lines. Compound 3 showed significant antimalarial activity against the malarial parasite *Plasmodium falciparum*, with an IC₅₀ value of 6.09 μM.

**Introduction**

*Ziziphus*, a genus of almost 100 species in the Rhamnaceae family, is found in the warmer parts of the world,¹ of which Thailand exhibits a diversity of 9 species.² *Ziziphus* plants are recognized to produce triterpenoids and their saponins,³–⁵ flavonoids⁶ and cyclopeptide alkaloids⁷–⁹ and have been used in indigenous medicine for the treatment of various diseases.¹⁰,¹¹ We previously reported the characterization of several bioactive natural products from Thai *Ziziphus* plants.¹²–¹⁴ *Z. cambodiana* Pierre is a thorny scandent that is commonly found in the north-east of Thailand and mainly in Southeast Asia and has traditionally been used for its anti-infective properties.¹⁵,¹⁶ The bioactive substances obtained from this plant include flavonoids and their glycosides with neuraminidase inhibitory activity.¹⁷ Triterpenoids with antiproliferative, antimycobacterial,¹⁸ and hedgehog/GLi-mediated transcriptional inhibitors¹⁹ were also reported. To date, no study has been published on the cyclopeptide alkaloids from this plant species. Herein, the isolation and structural elucidation of six new 14-membered ring cyclopeptides, cambodines A–F (1–6), along with two known compounds, 7¹⁹ and 8²⁰ from the root bark of *Z. cambodiana* are reported. Compounds 1 and 3 are additional representatives of the (5)14-membered ring cyclopeptide series, with a terminal imidazolidinone ring. Interestingly, these 8 cyclopeptides pose a challenging problem in the determination of the absolute configuration. Electronic circular dichroism (ECD) have been widely used in the determination the absolute configuration, and a great number of chiral compounds have been well investigated via theoretical methods.²¹–²⁶ Among the techniques employed to compute electronic transitions, TD-DFT is the most widely used and most efficient method for the study of excited states and the prediction of ECD spectra. It has been successfully applied for the description of the conformational structures and the electronic spectra of natural products, metal complexes and other organic molecules.²⁷–³¹ In the present work, the DFT and TD-DFT methods were applied to simulate the ECD spectra in order to confirm the structure assignment obtained from NMR spectroscopy and the observed ECD spectra. Finally, the *in vitro* antimalarial, antimycobacterial, and cytotoxic activities of the isolated compounds are also described.

**Results and discussion**

Chemical investigations of the EtOAc and MeOH extracts of *Z. cambodiana* root bark resulted in the isolation of three 14-membered ring cyclopeptide alkaloids, cambodines A (1), B (4), and C (2), and known compounds 7 and 8. The removal of tannins from the MeOH extract with NaCl solution (1% v/v) followed by partitioning with EtOAc yielded a detannified EtOAc-soluble fraction that was column chromatographed to
furnish three additional cyclopeptides: cambodines D (5), E (6), and F (3) (Fig. 1).

Several common chemical and spectroscopic characteristics were evident for compounds 1–8. They displayed a blue color upon staining with anisaldehyde-H2SO4 reagent on TLC.11 Their IR spectra showed absorption bands for amide (1632–1685 cm–1) and aryl ether (1221–1237 cm–1) functionalities. The ECD spectra of compounds 1–8 showed intense negative and weak positive Cotton effects in the 236–244 and 279–286 nm regions, respectively, consistent with the (S,S,8S,9S)-configurations.32 Their 1H and 13C NMR data agreed well with the published values for 5(14)-scutianine A-type (1–3), 4(14)-integerrine-type (4–6), 4(14)-frangulanine-type (7), and 5(14)-neutral-type cyclopeptide alkaloid (8).

Compound 1 was isolated as a colourless amorphous solid. The HRESITOFMS data showed a protonated ion at m/z 680.3808 [M + H]+, in accordance with a molecular formula C40H49N5O5. The 13C NMR and DEPT data in DMSO-d6 (Table 1) revealed 40 carbon resonances, which were classified as four methyls (δC 10.8, 12.0, 14.5, 15.4), an N-methyl (δC 40.5), four methylenes (δC 24.0, 24.3, 34.2, 66.3), 23 methines (six aliphatic C 35.0, 35.5, 53.4, 56.0, 57.4, 69.7, one oxygenated at δC 125.8 and 126.7 and 14 aromatic at δC 119.5, 121.8, 126.2, 127.6 × 2, 127.9 × 3, 128.4 × 2, 128.8 × 2, 129.5, 130.0), four amide carbonyls (δC 167.4, 168.9, 169.9, 170.7), three aromatic quaternary (δC 131.5, 136.7, and 137.8), and an oxygenated tertiary (δC 154.7). Interpretation of the 1H, 13C NMR, and 2D NMR spectroscopic data and the IR absorption frequencies at 3289, 1682, 1636, and 1241 cm–1 led to the conclusion that 1 was a 5(14)-type cyclopeptide alkaloid in which the cyclic part comprised a phenylalanine, an p-oxystyrylamine with a Z double bond, an isoleucine moiety, in addition to the coupled phenylalanine and N-methylisoleucine moiety as the terminal unit (Fig. 1). The assembly of these fragments was made possible on the basis of correlations in the COSY, HMBC, and NOESY experiments (Fig. 2). The HMBC spectrum showed correlations of the olefinic H-2 (δH 6.23, dd, J = 7.3, 4.5 Hz) to the C-4 carbonyl carbon resonance (δC 168.9) and of the oxygenated methine H-9 (δH 5.84, d, J = 8.1 Hz) and isoleucyl H-5 (δH 3.81, t, J = 8.5 Hz) to C-7 (δC 169.9), in addition to the NOESY cross-peaks between H-9 and the aromatic resonances H-12 (δH 7.14) and H-22 (δH 7.44, dd, J = 7.7, 2.2 Hz) established the linkage of the p-oxystyrylamine moiety to the phenylalanine unit and the location of this dimeric moiety in the macrocyclic ring. The H-8 methine signal at δH 4.78 (brt, J = 8.8 Hz) showed an HMBC cross-peak to the phenylalanine carbonyl C-26 (δC 167.4), indicating the connection between the phenylalanine units. A prominent fragment ion at m/z 622 and a base peak at m/z 155 in the EIMS spectrum of 1 (the molecular ion of which is presented as 1’ in Fig. 3) indicated the fragmentations of the corresponding N-methylimidazolidin-4-one to produce 1’a and the terminal residue 1’b,33,34 respectively (Fig. 3). The relatively low field chemical shifts of the two dia- stereotopic protons at δH 3.99 and 3.11 (each d, J = 4.4 Hz, H-41a and H-41b, respectively), δC 66.3 (C-41), an N-methyl (δH 2.13), a sec-butyl group (δH 1.23, m, H-36, δC 35.0; δH 0.99, m, H-33, 34, 35) and of the oxygenated methine H-9 (δH 5.84, d, J = 8.1 Hz) and isoleucyl H-5 (δH 3.81, t, J = 8.5 Hz) to C-7 (δC 169.9), in addition to the NOESY cross-peaks between H-9 and the aromatic resonances H-12 (δH 7.14) and H-22 (δH 7.44, dd, J = 7.7, 2.2 Hz) established the linkage of the p-oxystyrylamine moiety to the phenylalanine unit and the location of this dimeric moiety in the macrocyclic ring. The H-8 methine signal at δH 4.78 (brt, J = 8.8 Hz) showed an HMBC cross-peak to the phenylalanine carbonyl C-26 (δC 167.4), indicating the connection between the phenylalanine units. A prominent fragment ion at m/z 622 and a base peak at m/z 155 in the EIMS spectrum of 1 (the molecular ion of which is presented as 1’ in Fig. 3) indicated the fragmentations of the corresponding N-methylimidazolidin-4-one to produce 1’a and the terminal residue 1’b,33,34 respectively (Fig. 3). The relatively low field chemical shifts of the two dia-stereotopic protons at δH 3.99 and 3.11 (each d, J = 4.4 Hz, H-41a and H-41b, respectively), δC 66.3 (C-41), an N-methyl (δH 2.13), a sec-butyl group (δH 1.23, m, H-36, δC 35.0; δH 0.99, m, H-33, 34, 35) and of the oxygenated methine H-9 (δH 5.84, d, J = 8.1 Hz) and isoleucyl H-5 (δH 3.81, t, J = 8.5 Hz) to C-7 (δC 169.9), in addition to the NOESY cross-peaks between H-9 and the aromatic resonances H-12 (δH 7.14) and H-22 (δH 7.44, dd, J = 7.7, 2.2 Hz) established the linkage of the p-oxystyrylamine moiety to the phenylalanine unit and the location of this dimeric moiety in the macrocyclic ring. The H-8 methine signal at δH 4.78 (brt, J = 8.8 Hz) showed an HMBC cross-peak to the phenylalanine carbonyl C-26 (δC 167.4), indicating the connection between the phenylalanine units. A prominent fragment ion at m/z 622 and a base peak at m/z 155 in the EIMS spectrum of 1 (the molecular ion of which is presented as 1’ in Fig. 3) indicated the fragmentations of the corresponding N-methylimidazolidin-4-one to produce 1’a and the terminal residue 1’b,33,34 respectively (Fig. 3). The relatively low field chemical shifts of the two dia-stereotopic protons at δH 3.99 and 3.11 (each d, J = 4.4 Hz, H-41a and H-41b, respectively), δC 66.3 (C-41), an N-methyl (δH 2.13), a sec-butyl group (δH 1.23, m, H-36, δC 35.0; δH 0.99, m, H-
Table 1 1H and 13C NMR spectroscopic data for compounds 1–3

| No. | 1a | 2a | 2b | 3b |
|-----|-----|-----|-----|-----|
| 1   | 125.8 | 127.5 | 117.5 | 117.5 |
| 2   | 126.7 | 126.7 | 125.5 | 125.4 |
| 3   | 154.7 | 154.8 | 151.5 | 151.2 |
| 4   | 121.8 | 118.1 | 123.1 | 122.9 |
| 5   | 129.5 | 129.4 | 130.1 | 130.1 |
| 6   | 131.5 | 131.2 | 132.5 | 132.2 |
| 7   | 127.6 | 127.7 | 128.8 | 128.8 |
| 8   | 127.9 | 127.8 | 128.9 | 128.9 |
| 9   | 167.4 | 169.5 | 170.9 | 170.0 |
| 10  | 34.2  | 37.5  | 36.6  | 33.2  |
| 11  | 136.7 | 137.7 | 136.3 | 136.6 |
| 12  | 128.8 | 128.9 | 129.2 | 129.2 |
| 13  | 126.2 | 126.0 | 127.0 | 127.0 |
| 14  | 170.7 | 172.2 | 174.2 | 174.8 |
| 15  | 69.7  | 69.1  | 71.0  | 71.0  |
| 16  | 35.0  | 36.1  | 37.6  | 36.5  |
| 17  | 24.3  | 24.1  | 24.2  | 24.8  |
| 18  | 12.0  | 10.9  | 11.7  | 12.2  |
| 19  | 14.5  | 15.4  | 15.5  | 14.9  |
| 20  | 66.3  | 78.9  |       |       |

| No. | 1a | 2a | 2b | 3b |
|-----|-----|-----|-----|-----|
| 21  |    |    |    |     |
| 22  |    |    |    |     |
| 23  |    |    |    |     |
| 24  |    |    |    |     |
| 25  |    |    |    |     |
| 26  |    |    |    |     |
| 27  |    |    |    |     |
| 28  |    |    |    |     |
| 29  |    |    |    |     |
| 30  |    |    |    |     |
| 31  |    |    |    |     |
| 32  |    |    |    |     |
| 33  |    |    |    |     |
| 34  |    |    |    |     |
| 35  |    |    |    |     |
| 36  |    |    |    |     |
| 37  |    |    |    |     |
| 38  |    |    |    |     |
| 39  |    |    |    |     |
| 40  |    |    |    |     |

| a | | | |
|---|---|---|---|

Recorded in DMSO-d6. b Recorded in CDCl3.

37, δC 24.3; δH 0.67, t, J = 6.3 Hz, H-38, 12.0; and δH 0.41, d, J = 6.8 Hz, H-39, 14.5), and an amide carbonyl carbon at δC 170.7 (C-34) further supported the presence of a 3-substituted 5-(sec-butyl)-1-methylimidazolidin-4-one ring. Furthermore, the correlations of H-8, NH-25 (δH 8.23, d, J = 9.4 Hz), and H-27 (δH 4.58, dd, J = 10.7 and 5.1 Hz) to CO-26 (δC 167.4) in the HMBC spectrum along with the interactions of H-27 to H-30 (δH 7.02), of H-41a to H-28 (δH 2.61 m) and H-30, and of N–CH3 to H-41b in the NOESY experiments permitted the assignment of the linkage between both phenylalanine units to the imidazolidinone group. The HMBC cross-peaks of H-41a to CO-34, C-35 (δC 69.7), and N–CH3 and of H-35 to CO-34 and C-36 (δC 35.0) and the NOESY interaction between H-35 and N–CH3 were also observed. Thus, the structure of cyclopeptide 1, cambodine A, was deduced as a new member of the 5(14)-scutianine A-type cyclopeptides.

Compound 2 was also obtained as a colourless amorphous solid. Its molecular formula was determined by the HRESI-TOFMS ion at m/z 668.3807 [M + H]+ and 13C NMR spectroscopic data as C38H48N2O6. Its 1H and 13C NMR data in DMSO-d6 (Table 1) were similar to those of 1, except for the absence of the two geminal protons at C-41 in 1 and the presence of an amide...
proton NH-33 at $\delta_H 7.64$ (1H, d, $J = 7.9$ Hz) in 2, which are in agreement with the structural change in the terminal residue (Fig. 1). The NMR data of 2 recorded in CDCl$_3$ (Table 1) were similar to those observed in DMSO-$d_6$, except for the C-1 chemical shift. Information from the HMBC association of H-8 ($\delta_H 4.83$, dt, $J = 9.8$, 7.9 Hz) to CO-26 ($\delta_C 169.5$) and of NH-33 to CO-34 ($\delta_C 172.2$) in addition to the NOESY interactions of H-27 ($\delta_H 4.23$, dt, $J = 10.8$, 7.9 Hz) to NH-25 ($\delta_H 7.93$, d, $J = 8.9$ Hz) and H-30 ($\delta_H 6.99$) and of H-35 ($\delta_H 2.35$, brs) to NCH$_3$ ($\delta_H 1.79$, s) (Fig. 2) provided evidence for the exocyclic

Fig. 2 Selected COSY, HMBC and NOESY interactions for compounds 1, 3, 4 and 5.

Fig. 3 EI fragmentations of compounds 1 and 3.
phenylalanine moiety connecting to the macrocyclic ring at N-25 and the terminal N-methylisoleucine unit. The structure of 2, cambodine C, was therefore also defined as a new 5(14)-scutianine A-type compound.

Compound 3 displayed a sodium adduct molecular ion at m/z 716.3792 [M + Na]+ in the HRESITOFMS, corresponding to the molecular formula C41H51N5O5. Its 1H and 13C NMR data in CDCl3 (Table 1) showed signals similar to those observed for compounds 1 and 2 in the same NMR solvent, except for one fewer methylene carbon and the additional methyl (δC 19.5) and methine resonances (δC 78.9) observed in the DEPT spectra for 3. Similar to those of 1, the observed fragment peaks at m/z 636 and 169 in the EIMS data of 3 suggest the presence of phenylalanine and a 5-(sec-butyl)-1,2-dimethylimidazolidin-4-one as the respective exocyclic units (Fig. 3), which is supported by a molecular mass 14 amu greater than that of 1. The NOE associations of NH-25 (δH 8.73, δJ = 8.4 Hz) to H-9 (δH 6.17, δJ = 7.1 Hz) and H-28a (δH 2.29, brt, J = 12.6 Hz), of H-41 (δH 2.41, δJ = 5.4 Hz) to H-27 (δH 3.49, dd, J = 11.9, 4.4 Hz), H-30 (δH 6.93, dd, J = 7.6, 1.1 Hz), and N-CH3 (δH 1.85), and of the latter N-CH3 to H-35 (δH 2.57 brs), together with connectivities of CH3 to C-41 (δC 129.2), of N-CH3 to C-41 (δC 78.9) and C-35 (δC 71.0), and of H-35 to C-36 (δC 36.5) in the HMBC spectrum also supported the linkage between the acyclic part and the macrocyclic ring (Fig. 2). The NOESY associations of NH-6 (δH 5.92, δJ = 8.0 Hz)/H-8 and H-9/H-16 (δH 4.60, brs, J = 8.4, 2.1 Hz) confirmed the location of the phenylalanine unit next to the p-oxytryptamine moiety and the isoleucine fragments in the macrocyclic system. The significantly different C-27 (δC 63.0) and C-41 (δC 78.9) chemical shifts compared to those of 1 (C-27, δC 53.4 and C-41, 66.3) and 2 (C-27, δC 54.7) could be attributed to the presence of the C-41 methyl group. However, the existing data did not permit the establishment of the configuration at C-41. Thus, the structure of 3, cambodine F, was deduced as a new member of the 5(14)-scutianine A-type cyclodeptides possessing a 5-(sec-butyl)-1,2-dimethylimidazolidin-4-one moiety.

The J values of H-1 and H-2 ranging from 7.1–7.4 Hz accounted for the Z geometry of the double bond in compounds 1–3. The intense negative (236 nm) and weak positive (280 nm) Cotton effects present in the CD spectrum of 1, similar to those of 2 and 3, and indicative of the [5S,8S,9S,17S,27S,35S,36S]-1 configuration calculated for 1.17 The experimental ECD spectrum of 1 was in agreement with that of all 5 configuration on the amino acid residues. Calculation of the ECD spectra of individual epimer of 1 were also performed (Fig. 4B) and revealed that the major Cotton effect contribution with opposite sign observed at the band near 238 nm for 9S/9R and at around 245 nm for 8S/8R epimer pair. Similar Cotton effects were found for compounds 2 (negative 236 nm and positive 283 nm bands) and 3 (negative 236 nm and positive 282 nm bands) and comparable to the theoretical ECD ones (Fig. 4C) allowed the same absolute configuration assignment as for 1. From these evidences, including their negative specific rotations, the stereochemical structures of compounds 1–3 were then deduced.

Compound 4, a minor compound from the same fraction of compound 1, was isolated as a colourless amorphous solid and given a molecular formula C37H36N4O5, as deduced from its positive ion HRESITOFMS at m/z 617.2751 [M + H]+. The 13C NMR and DEPT spectra (CDCl3) disclosed 37 carbon resonances, consisting of an N-methyl (δC 39.8), two methylenes (δC 37.5, 67.1), 23 methines (two olefinic at δC 121.2, 125.2, two oxymethine at δC 73.1, 81.6, 19 aromatic at δC 121.9, 123.2, 126.3, 126.9 × 2, 128.2 × 4, 128.6 × 2, 128.7 × 3, 129.1 × 3, and 130.2), four aromatic quaternary (δC 131.9, 136.0, 138.1, 139.0), an oxygenated tertiary (δC 155.3), three amide carbonyls (δC 167.7, 167.8 and 172.1) and no signal observed in the aliphatic region (Table 2, Fig. 1). A 1D and 2D NMR extensive data analysis in addition to a comparison with previously described values evidenced the spin system of a p-oxytryptamine, a β-hydroxyphenylalanine, a phenylalanine, and a 1-methylimidazolidin-4-one, which is derived from the phenylalanine of the 4(14)-integerrime-type cyclodeptide alkaloid. Interestingly, a broad three-bond singlet at δH 1.59 was assigned to the tryptamine double bond H-1, H-2, and NH-3 by the HMBC of H-1 to C-2 (δC 125.5), C-14 (δC 131.9) and C-15 (δC 131.0) and of H-2 to C-14 and the NOESY of H-3 to H-5 (δH 4.51, brt, J = 8.1
Hz) connectivities was characterized by the OH absorption frequency at 3447 cm⁻¹ in the IR data, along with the correlations from the oxymethine doublet resonance H-17 (δH 4.94, J = 7.6 Hz) to a carbonyl carbon C-4 at δC 167.8 and an aromatic carbon at δC 126.9 (C-18) in the HMBC spectrum, in addition to the NOESY connectivities of H-17 to NH-6 (δH 6.37, brd, J = 8.5 Hz). As for other cyclopeptides, the HMBC correlations of H-1 to C-14 and C-15, of H-5 to CO-7 (δC 167.7), and of H-9 (δH 5.90, d, J = 7.3 Hz) to C-22 (δC 128.2) and of the NOESY interactions of H-5 to NH-3 and of H-9 to H-16 (δH 7.29) suggested that the phenylalanine and β-hydroxyphenylalanine were placed next to each other and attached to the oxytryptamine in the cyclic ring.

A 5-benzyl-1-methylimidazolidin-4-one group was determined by the analysis of the 1D and 2D NMR spectra of 4: the presence of the two diastereotopic protons at δH 3.66 and 2.70 (each d, J = 4.3 Hz, H-34a and H-34b), δC 67.1; an N-methyl (δH 1.64, s), a ring multiplet methine proton resonance at δH 2.69 (H-27), δC 66.5; a benzyl group (δH 2.67, m, H-28, δC 37.5; δH 2.74–7.47, ArH, δC 126.3–138.1) and an amide carbonyl at δC 172.1 (C-26). A series of connectivities was observed: a diastereotopic proton at δH 3.66 showed NOESY correlations to H-9 and H-27 and of H-27 to N-CH3, and the HMBC connections of H-9 (δH 4.87, d, J = 7.3 Hz) to carbonyl carbon C-26, of H-27 to C-29 and of H-28 to C-30 showed that the imidazolidin-4-one ring was connected to the macrocyclic ring at C-8 of the phenylalanine. The structure of 4, cambodine B, was thus elucidated as a new member of the 4(14)-integerrine-type cyclopeptides.

Compound 5 was also obtained as a colourless amorphous solid from the detamified EtOAc-soluble fraction of Z. cambodiana. Its molecular formula was deduced as C_{31}H_{40}N_{2}O_{4} from its positive ion HRESITOFMS at m/z 555.2929 [M + H]^+. A detailed analysis of the 1D, DEPT and 2D spectroscopic data suggested that 5 also possessed a 4(14)-type cyclopeptide containing the same macrocyclic ring and terminal unit as for 1 (Fig. 1). The main differences in their $^{13}$C NMR data (Table 2) are the absence of the intermediate phenylalanine resonances in 5 compared to that of 1. A series of correlations of H-8 (δH 5.09, d, J = 7.6 Hz) to C-26 (δC 172.8), of H-33a (δH 3.83, brd, J = 3.9 Hz) to C-27 (δC 70.4), and of H-27 (δH 2.48, d, J = 2.6 Hz) to CO-26, C-29 (δC 24.7) and C-31 (δC 14.7) displayed in the HMBC spectrum, together with the interactions of H-33a to H-9 (δH 5.99, d, J = 7.6 Hz), H-22 (δH 7.52, dd, J = 7.7, 1.9 Hz) and N-CH$_1$ (δH 2.11) and of H-27 to N-CH$_3$, H-9 (δH 1.08, m) and H-31 (δH 0.55, d, J = 6.8 Hz) observed in the NOESY spectrum indicated that the 5-(sec-butyl)-1-methylimidazolidin-4-one ring was the end fragment of the system (Fig. 2). The structure of 5, cambodine D, was thus established as an additional member of the 4(14)-integerrine-type cyclopeptides.

Compound 6 was isolated as colourless needles and displayed a sodium adduct molecular ion [M + Na]$^+$ at m/z 623.2606 in the HRESITOFMS, corresponding to a molecular formula of C$_{37}$H$_{44}$N$_4$O$_4$. Its $^1$H and $^{13}$C NMR data (Table 2, Fig. 1) were almost identical to those of compound 4, with the difference being the presence of the two geminal proton resonances at δH 3.40 (dd, J = 14.8 and 3.4 Hz, H-17a) and δH 2.61 (dd, J = 14.8 and 11.2 Hz, H-17b) (δC 36.7) of the ring-bound amino acid in 6, instead of the oxymethylene signal in 4. In the HMBC spectrum, the connectivities of the resonance at δH 2.61 to C-5 (δC 55.4) and a carbonyl carbon at δC 166.5 (C-4), of NH-6 at δH 6.40 (d, J = 8.1 Hz) to C-5, and of an aromatic signal at δH 7.09 (d, J = 8.7 Hz, H-18) to C-17 confirmed that the phenylalanine was bound to p-oxytryptamine and β-hydroxyphenylalanine in the cyclic structure. Its EIMS spectrum showed the base peak at m/z 509 and...
a fragment ion peak at \( m/z \) 227, indicating the existence of a 5-benzyl-1-methylimidazolidin-4-one unit as the end residue. This was supported by the presence of two geminal protons at \( \delta_H 3.56 \) (\( d, J = 4.7 \) Hz, H-34a) and \( \delta_H 2.43 \) (\( d, J = 4.7 \) Hz, H-34b), a singlet N-CH\(_3\) at \( \delta_H 1.48 \) (\( \delta_C 39.5 \)), and a multiplet methine resonance of H-27 at \( \delta_H 2.39 \) (\( \delta_C 66.1 \)), in addition to a carbonyl carbon signal at \( \delta_C 172.3 \) (C-26) and a benzyl group in its NMR data. Compound 6 exhibited similar 2D NMR (HMBC and NOESY) correlations, both at the cyclic and the terminal ring, to those of compound 4. A remarkable upfield-shifted N-CH\(_3\) resonance at \( \delta_H 1.48 \) in 6 could be due to an anisotropic effect arising from the benzyl group when compared with compound 5 (\( \delta_H 2.11 \)) that has a sec-butyl moiety. The structure of 6, cambodine E, was thus elucidated as an analogue of cyclopeptide 4.

The X-ray crystal structure of 6 supported a skeleton comprised of a Z-styrylamine, two phenylalanines and a 5-benzyl-1-methylimidazolidin-4-one subunit and also confirmed

![Fig. 5 ORTEP plot of the X-ray crystal structure for compound 6.](image)

### Table 2 ¹H and ¹³C NMR spectroscopic data for compounds 4–6 in CDCl\(_3\)

| No. | \( \delta_C \) | \( \delta_H \) |
|-----|---------------|---------------|
| 1   | 121.2         | 6.59, brs     |
| 2   | 125.2         | 6.59, brs     |
| 4   | 167.8         | 6.66, d (9.5, 7.6) |
| 5   | 57.5          | 4.51, brt (8.1) |
| 7   | 167.7         | 7.32, overlap  |
| 8   | 57.8          | 7.34, overlap  |
| 9   | 81.6          | 7.34, overlap  |
| 11  | 155.3         | 7.34, overlap  |
| 12  | 123.2         | 7.34, overlap  |
| 13  | 130.2         | 7.34, overlap  |
| 14  | 131.9         | 7.34, overlap  |
| 15  | 131.0         | 7.34, overlap  |
| 16  | 121.9         | 7.34, overlap  |
| 17  | 73.1          | 7.34, overlap  |
| 18a | 139.0         | 7.34, overlap  |
| 18,18' | 126.9       | 7.34, overlap  |
| 19,19' | 128.7       | 7.34, overlap  |
| 20  | 128.7         | 7.34, overlap  |
| 21  | 136.0         | 7.34, overlap  |
| 22,22' | 128.2       | 7.34, overlap  |
| 23,23' | 128.6       | 7.34, overlap  |
| 24  | 129.1         | 7.34, overlap  |
| 26  | 172.1         | 7.34, overlap  |
| 27  | 66.5          | 7.34, overlap  |
| 28  | 37.5          | 7.34, overlap  |
| 29  | 138.1         | 7.34, overlap  |
| 30  | 129.1         | 7.34, overlap  |
| 31  | 128.2         | 7.34, overlap  |
| 32  | 126.3         | 7.34, overlap  |
| 33  | 67.6          | a 3.83, br d (3.9) |
| 34  | 67.1          | b 3.22, br d (3.9) |
| 3-NH | 6.59         | 6.50, d (9.5)   |
| 6-NH | 5.81         | 5.40, d (8.1)   |
| NMe | 39.8          | 1.48, s         |
the trans arrangement between H-8 and H-9 displayed in the macrocyclic motif (Fig. 5). The ECD spectrum of 6, as well as the spectra of the other cyclopeptides indicated above, provided the $S_S$, $S_S$ and $S_S$ configuration assignments at the amino acid residues of the cyclic part. The imidazolidinone configuration at 27$S$ was then subsequently decisively assigned. The similarities in the NMR data on the terminal ring of 6 compared with those of 4 and 5, associated with the calculated ECD spectra of 4–6 (Fig. 6) were in accordance with those observed ECD value have led to the conclusion that cyclopeptides 4–6 contribute the same stereochemistry both at the macrocyclic ring and at the imidazolidinone unit.

The two cyclopeptides 7 and 8 were identified as the 4(14)-type cyclopeptide alkaloids, frangufoline$^{39}$ or daechuine $S_{12}$ or sanjoinine $A^{42}$ and lotusanine $B^{20}$ respectively, by detailed examinations of their 1D and 2D NMR and MS spectroscopic data along with comparison to their reported values (Fig. 1, ESI†). The $^{13}$C NMR resonances of the chiral carbons observed for 7 were in good agreement with the literature data for frangufoline whose stereochemistry was proven to be all $S$ configurations by the analysis of each amino acid residue in its acid hydrolysate and confirmed by total synthesis.$^{39,44}$ The ECD spectrum of 7 exhibited strong negative (239 nm) and positive (287 nm) Cotton effects that also supported the same $S_S$-conformation at the amino acid residues of the cyclic part. The imidazolidinone configuration at 27$S$ was then subsequently decisively assigned. The similarities in the NMR data on the terminal ring of 6 compared with those of 4 and 5, associated with the calculated ECD spectra of 4–6 (Fig. 6) were in accordance with those observed ECD value have led to the conclusion that cyclopeptides 4–6 contribute the same stereochemistry both at the macrocyclic ring and at the imidazolidinone unit.

Experimental section

General experimental procedure

Melting points were determined using a Griffin melting point apparatus and are uncorrected. Optical rotations at the sodium D line were measured on a JASCO-1020 digital polarimeter. ECD spectra were recorded on a Jasco J-810 spectropolarimeter. IR spectra were measured on a Perkin-Elmer FT-IR Spectrum BX spectrophotometer, with $r_{max}$ given in cm$^{-1}$. NMR spectra were measured at 300 MHz (1H) and 75 MHz ($^{13}$C) on a Bruker AVANCE 300 FT-NMR spectrometer using TMS or residual non-deuterated solvent signals as an internal standard (CDCl$_3$: $\delta_H$ 7.24, $\delta_C$ 77.00; DMSO-d$_6$: $\delta_H$ 2.49 and $\delta_C$ 39.5 for $^1$H and $^{13}$C NMR spectra, respectively). EIMS were recorded on a Thermo Finnigan Polaris Q mass spectrometer at 70 eV (probe). ESIMS were obtained on a Finnigan LC-Q mass spectrometer. The HRESI-TOFMS were measured on a Bruker microOTOF-QII mass spectrometer. The X-ray crystallographic data analysis was carried out with a Bruker-Nonius kappaCCD diffractometer with a graphite monochromator, MoKz radiation ($\lambda = 0.71073$ Å) at 298(2) K. Silica gel (finer than 0.063 mm, Merck) and Sephadex LH-20 (Pharmacia) were used for column chromatography. TLC analyses were carried out on plates that had been precoated with silica gel $F_254$ from Merck and visualized under a UV light at 254 or 365 nm and by spraying with 5% anisaldehyde-H$_2$SO$_4$ solution followed by heating.

Fig. 6 The superimpose of calculated ECD for 4–6 in comparison to the observed ECD spectra of 4.
Plant materials
Shade-dried root bark of *Z. cambodiana* was collected from Chamni District, Burirum Province, Thailand, in March 2007. The material was identified by James F. Maxwell of the Faculty of Science, Chiang Mai University, Thailand. A voucher specimen (Wichan Wisetsri 002) has been deposited at the Laboratory of the Natural Product Research Unit, Srinakarinwirot University, Thailand.

Extraction and separation
The powdered root bark of *Z. cambodiana* (10.0 kg) was extracted successively with EtOAc (20 L x 3) and MeOH (20 L x 3) at 50 °C for 48 h for each solvent. The combined extract was evaporated under reduced pressure at temperature 40–45 °C to yield EtOAc (144.7 g) and MeOH (1.4 kg) extracts. The crude extract was subjected to chromatographic separation, and only fractions that showed blue spots upon staining with anisaldehyde-H2SO4 reagent on TLC were selected for further separation and purification. A portion of the EtOAc soluble extract (80 g) was fractionated by quick column chromatography on silica gel (100), 227 (12); positive HRESITOFMS *m/z* 680.3807 [M + H]+ (calcd for C41H51N5O5, 680.3806).

Cambodine B (2). Colourless amorphous solid, mp 225–227 °C; [α]D25 –199 (c 0.2, MeOH); ECD (MeOH) (Δ) 280 (+16.44), 236 (–41.88), 220 (+15.46) nm; IR (KBr) νmax 3388, 2959, 2927, 2878, 1668, 1642, 1508, 1453, 1211, 1032, 701 cm−1; 31C NMR (CDCl3) spectroscopic data, see Table 1; EIMS *m/z* 694 (9), 693 (24), 636 (49), 243 (17), 169 (100), 135 (14); positive HRESITOFMS *m/z* 676.3807 [M + Na]+ (calcd for C39H37N4O5Na, 676.3811).

Cambodine C (3). Colourless amorphous solid, mp 216–218 °C; [α]D25 –131 (c 0.2, MeOH); ECD (MeOH) (Δ) 282 (+9.76), 236 (–46.29), 213 (–16.80) nm; IR (KBr) νmax 3447, 3031, 2924, 1635, 1507, 1455, 1337, 1233, 1012, 758, 699 cm−1; 31C NMR (CDCl3 and DMSO-d6) spectroscopic data, see Table 1; EIMS *m/z* 694 (9), 693 (24), 636 (49), 243 (17), 169 (100), 135 (14); positive HRESITOFMS *m/z* 716.3792 [M + Na]+ (calcd for C41H33N4O5Na, 716.3782).

Cambodine D (3). Colourless amorphous solid, mp 140–141 °C; [α]D25 –107 (c 0.2, MeOH); ECD (MeOH) (Δ) 279 (+6.67), 240 (–36.46), 224 (–9.30) nm; IR (film) νmax 3447, 3031, 2924, 1635, 1507, 1455, 1328, 1012, 758, 699 cm−1; 31C and 1H NMR (CDCl3) spectroscopic data, see Table 2; positive HRESITOFMS *m/z* 617.2751 [M + H]+ (calcd for C27H26N2O4, 617.2785).

Cambodine E (6). Colourless needles, mp 135–137 °C; [α]D25 –169 (c 0.2, MeOH); ECD (MeOH) (Δ) 286 (+4.25), 243 (–88.99), 220 (–14.47) nm; IR (film) νmax 3289, 1682, 1670, 1624, 1507, 1228, 752, 699 cm−1; 31C and 1H NMR (CDCl3) spectroscopic data, see Table 2; EIMS *m/z* 623 (1), 599 (1), 510 (38), 509 (100), 227 (12); positive HRESITOFMS *m/z* 623.2606 [M + Na]+ (calcd for C27H30N4O5Na, 623.2628).
**Frangufoline** (7). Colourless needles, mp 216–218 °C, lit:43 246–248 °C; [α]_D^28 = −219 (c 0.2, CHCl₃), lit:52 [α]_D^20 = −316 (c 1.25, CHCl₃), lit:⁸ [α]_D^20 = −299 (c 0.1, CHCl₃); ECD (MeOH) (Δε) 284 (+2.26), 237 (−21.24), 215 (−8.18) nm; ¹H and ¹³C NMR (300/75 MHz, CDCl₃, see ESI); negative ESIMS m/z 533 [M − H]⁻ (100), positive ESIMS m/z 535 [M + H]⁺ (100).

**Lotusanine B** (8). Colourless amorphous solid, mp 185–187 °C; [α]_D^24 = −118 (c 0.2, MeOH), lit:⁶⁰ [α]_D racemate; ECD (MeOH) (Δε) 283 (+1.80), 237 (−10.92), 220 (−5.92) nm; ¹H and ¹³C NMR (300/75 MHz, CDCl₃, see ESI); negative ESIMS m/z 619 [M − H]⁻ (100).

**Crystal data of 6.** C₁₇H₃₆N₄O₄.H₂O, Mₘ = 618.74, orthorhombic, dimensions: 0.20 × 0.15 × 0.10 mm, D = 1.273 g cm⁻³, space group P2₁2₁2₁, Z = 4, a = 8.3830(1), b = 9.5920(1), c = 16 938 7921, number of observations [I > 2σ(I)] 7250, final R indices [I > 2σ(I)]: R₁ = 0.0427, wR₂ = 0.0893. The structure was solved by the direct method using SIR97 and refined with a full-matrix least-squares calculation on F² using SHELXL-97. Crystallographic data have been deposited at the Cambridge Crystallographic Data Centre under the reference number CCDC 1469415.

**Calculation of ECD spectra**

Conformational analysis of all structures were carried out, the ground state geometries were computed at the M062X/6-311G level of theory. Excited states were performed by TD-DFT using M062X/6-311G(d,p) method. Geometry optimization and TD-DFT computations were both carried out with CPCM solvation model in MeOH solution. All quantum chemical calculations were performed with Gaussian 09 programs. The ECD spectra were simulated with overlapping Gaussian functions with sigma (σ) = 0.25 eV fitting parameter using Gauss Sum program. Analysis of the excited states was carried out with Gauss Sum program.

**Bioassay procedure**

The antimalarial activity was assayed against the parasite *Plasmodium falciparum* (K1, multidrug resistant strain), which was cultured continuously using the method of Trager and Jensen. An *in vitro* quantitative assessment of the antimalarial activity was performed by means of the microculture radioisotope technique based upon the method described by Desjardins. The inhibitory concentration that causes a 50% reduction in parasite growth was indicated by the *in vitro* uptake of ³H-hypoxanthine by *P. falciparum*. Under the same test system, the standard compound, dihydroartemisinin, showed an IC₅₀ value of 4.29 nM. By employing the microplate Alamar blue assay described by Collins and Franziab,⁷ the antimalarial activity was evaluated against *Mycobacterium tuberculosis* H₃₇Ra (purchased from ATCC). The standard drugs for the antimalarial assay, isoniazid and kanamycin sulfate, showed MICs of 0.44 and 4.29 μM, respectively. By using the previously described colorimetric method,⁴⁴ the cytotoxicity of the sample was determined. The reference substance, ellipticine, exhibited cytotoxic activity against human epidermod carcinoma (KB, ATCC CCL-87), human breast cancer (BC-1, ATCC11778), and human small cell lung cancer (NCI-H187, ATCC CRL-5804) cells, with IC₅₀ values of 5.39, 5.92, and 1.58 μM, respectively. The cytotoxicity against an African green monkey kidney (Vero) cell line was evaluated by green fluorescent protein (GFP) detection, and ellipticine was used as a positive control.⁵⁵

**Conclusions**

The present study revealed that the root bark of *Z. cambodiana* is a rich source of 14-membered ring cyclopeptide alkaloids. Six new 14-membered cyclopeptide alkaloids, cambodines A–F (1–6) along with two known cyclopeptides, frangufoline and lotusanine B were obtained. Compounds 1 and 3 are rare 5(14)-type alkaloids possessing an imidazolin-4-one ring in the terminal unit. Interestingly, the *in vitro* antimalarial assay disclosed that only the cyclopeptide with the 5-(sec-butyl)-1,2-dimethylimidazolidin-4-one exhibited significant activity. Some of the isolated alkaloids displayed antimycobacterial and cytotoxic properties, and most of them were nontoxic to Vero cells. Other bioactivity evaluations of these *Ziziphus* constituents are under active investigation.

**Conflicts of interest**

There are no conflicts of interest to declare.

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