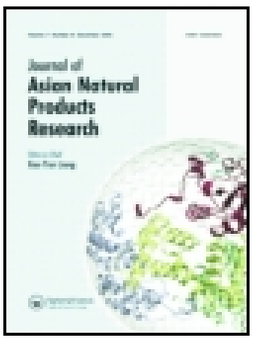

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The first total syntheses of (±)-norphoebine, dehydrophoebine, oxophoebine, dehydrocrebanine, oxocrebanine and uthongine and their cytotoxicity against three human cancer cell lines

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ABSTRACT

The first total syntheses of (±)-norphoebine, dehydrophoebine, oxophoebine, dehydrocrebanine, oxocrebanine and uthongine have been achieved. The crucial step involved the formation of ring C by a microwave-assisted direct biaryl coupling to produce the aporphine skeleton in high yields. The synthetic alkaloids were evaluated for their cytotoxicity against three human cancer cell lines MCF7, KB and NCI-H187. The results showed that uthongine was the best candidate of the series and it exhibited cytotoxicity against a human breast cancer MCF7 line with an IC_{50} = 3.05 μM

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Oxophoebine; oxocrebanine; uthongine; cytotoxic activity; total syntheses

1. Introduction

The aporphine alkaloids possess a tetracyclic skeleton derived by bonding between rings A and D of the benzylisoquinoline structure. More than 500 aporphine alkaloids have been isolated from a large number of plant families and they play a crucial role in the field of drug discovery research due to a broad range of pharmacological properties including cytotoxic [1], antioxidant [2], antimalarial [3], antimicrobial [2,4], acetylcholinesterase inhibiting [5], anti-HIV [6], antiparkinsonian [7] activities and anti-platelet aggregation [8]. Unfortunately, natural aporphines occur in extremely minute quantities, making the study of their biological activity an impossible undertaking. Thus, a number of syntheses of aporphine alkaloids and their derivatives have been undertaken in order to study the structure activity relationship and also to discover new drugs [5,7,9].

As part of our search for new anticancer drugs from natural sources, phoebine and crebanine-type natural alkaloids have attracted our attention because several compounds in these series showed promising anticancer activity. Oxophoebine has been identified as a DNA topoisomerase inhibitor [10] and has been shown to have cytotoxicity against GLC-82 and HCT cell lines [11]. Dehydrocrebanine was shown to have strong activity against several cancer cell lines (HL-60, HUCCA-1, BC and MOLT-3) [4] whereas crebanine was

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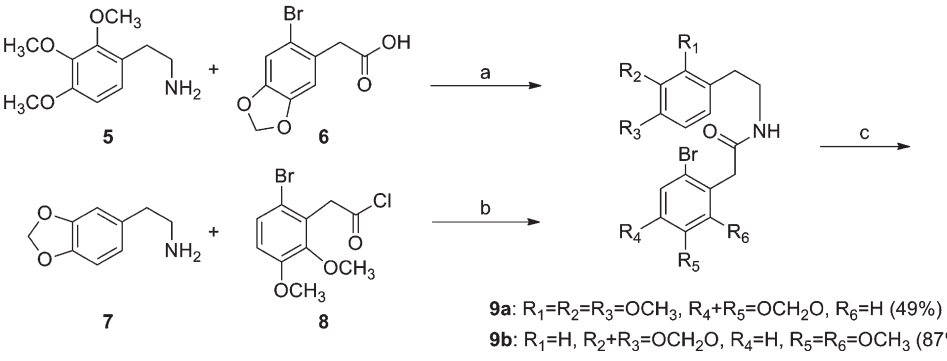
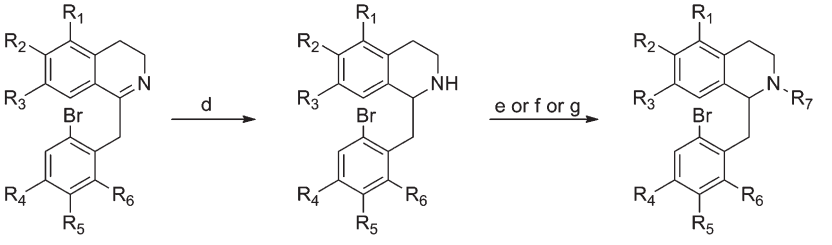
found to significantly inhibit the proliferation of human leukemic cells (HL-60, U937 and K526), human fibrosarcoma cells (HT1080) and cervix cancer cell lines (KB-3-1 and KB-V1) [12,13]. However, some alkaloids in the phoebine and crebanine series isolated from natural sources still have not been evaluated for their cytotoxicity. In order to explore their structure activity relationship, we decided to undertake the first total syntheses of six natural aporphine alkaloids; (±)-norphoebine, dehydrophoebine, oxophoebine, dehydrocrebanine, oxocrebanine and uthongine and a non-natural aporphine alkaloid norcrebanine in which the formation of ring C was achieved via a microwave-assisted direct biaryl coupling reaction and the evaluation of cytotoxicity against three human cancer cell lines (MCF7, KB and NCI-H187) was tested. Our investigation will be a preliminary study for the further synthesis of aporphine alkaloid derivatives which will lead to the discovery of novel and effective anticancer drugs.

2. Results and discussion

The syntheses of phoebine (**1a**), norphoebine (**1a′**), crebanine (**1b**) and 8,9-dimethoxy-1, 2-methylenedioxynoraporphine (norcrebanine, **1b′**) (Scheme 1) were based on the construction of ring C of the aporphine skeleton by palladium-catalyzed direct biaryl coupling reaction under microwave radiation, recently developed by Chaudhary et al. [14]. Prior to the report of Chaudhary et al., the formation of ring C of a number of aporphine alkaloids was achieved by radical cyclization using a radical initiator and tributyltin hydride. However, this methodology has limited applicability with low to moderate yields for concomitant formation of the reduction products [15]. On the other hand, the microwave-assisted direct biaryl coupling using palladium as a catalyst represents a promising route for this process and has previously been applied successfully for several alkaloid syntheses [9]. Therefore, we decided to further apply this method for the synthesis of a number of aporphine alkaloids with different oxygenation in order to study the scope and limitation of this process. The isoquinolines (**12**) required for this crucial step were synthesized as follows. Condensation of 2,3,4-trimethoxyphenethylamine (**5**) [16] with 6-bromo-3,4-methylenedioxyphenylacetic acid (**6**) and 3,4-methylenedioxyphenethylamine (**7**) [17] with 6-bromo-2,3-dimethoxyphenylacetyl chloride (**8**) [18] afforded, respectively, amides **9a** and **9b** which were converted by a Bischler–Napieralski reaction to give dihydroisoquinolines **10a** and **10b** in good yields. Sodium borohydride reduction then produced the tetrahydroisoquinolines **11a** and **11b**. Subsequent protection of compounds **11a** and **11b** as methyl carbamate, trifluoacetamide and formamide gave isoquinolines (**12a**, **12a′**, **12b** and **12b′**) in reasonable yields. With the desired precursors in hand, the palladium-catalyzed biaryl coupling of **12a**, **12b** and **12b′** under microwave irradiation at 850 W for 5 min afforded aporphines (**13a**, **13b** and **13b′**) in 81–96% yields. However, under the same reaction condition, compound **13a′** was obtained in a low yield (19%). Several attempts to optimize the reaction conditions to improve the yields were fruitless. The cause of this was not further investigated and remains obscure. The structures of **13a** and **13a′** were supported by the presence of singlets at δ_H 7.90 and 7.91 due to the proton at C-11 of the aporphine skeleton. Similarly, the H-11 signal of **13b′** exhibited a doublet at δ_H 7.86 (J = 8.7 Hz) whereas that of **13b** was observed as two doublets at δ_H 7.85 (J = 8.7 Hz) and 7.84 (J = 8.7 Hz) of the two conformers. Reduction of the carbomethoxy group in **13a** and the formyl group in **13b** with lithium aluminum hydride gave phoebine (**1a**, 84% yield) and crebanine (**1b**, 97% yield), respectively. On the other



9a: R_1=R_2=R_3=OCH_3, R_4+R_5=OCH_2O, R_6=H (49%)
9b: R_1=H, R_2+R_3=OCH_2O, R_4=H, R_5=R_6=OCH_3 (87%)

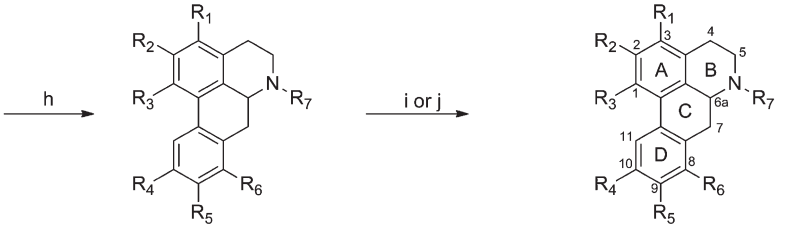


10a (72%)
10b (96%)

11a (46%)
11b (94%)

12a: R_1=R_2=R_3=OCH_3, R_4+R_5=OCH_2O, R_6=H, R_7=$COOCH_3$ (63%)
12a': R_1=R_2=R_3=OCH_3, R_4+R_5=OCH_2O, R_6=H, R_7=$COCF_3$ (45%)
12b: R_1=H, R_2+R_3=OCH_2O, R_4=H, R_5=R_6=OCH_3, R_7=CHO (45%)
12b': R_1=H, R_2+R_3=OCH_2O, R_4=H, R_5=R_6=OCH_3, R_7=$COCF_3$ (42%)

10a, 11a: R_1=R_2=R_3=OCH_3, R_4+R_5=OCH_2O, R_6=H
10b, 11b: R_1=H, R_2+R_3=OCH_2O, R_4=H, R_5=R_6=OCH_3



13a: R_1=R_2=R_3=OCH_3, R_4+R_5=OCH_2O, R_6=H, R_7=$COOCH_3$ (96%)
13a': R_1=R_2=R_3=OCH_3, R_4+R_5=OCH_2O, R_6=H, R_7=$COCF_3$ (19%)
13b: R_1=H, R_2+R_3=OCH_2O, R_4=H, R_5=R_6=OCH_3, R_7=CHO (94%)
13b': R_1=H, R_2+R_3=OCH_2O, R_4=H, R_5=R_6=OCH_3, R_7=$COCF_3$ (81%)

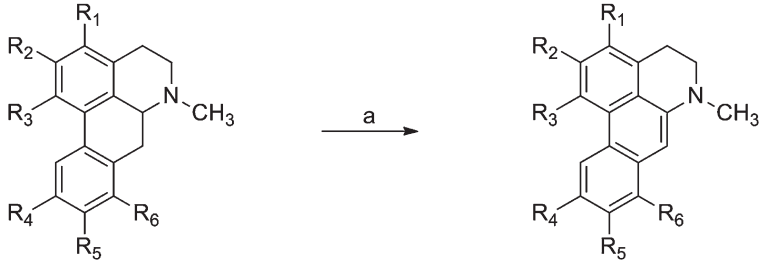
1a: R_1=R_2=R_3=OCH_3, R_4+R_5=OCH_2O, R_6=H, R_7=CH_3 (**Phoebine**, 84%)
1a': R_1=R_2=R_3=OCH_3, R_4+R_5=OCH_2O, R_6=H, R_7=H (**Norphoebine**, 99%)
1b: R_1=H, R_2+R_3=OCH_2O, R_4=H, R_5=R_6=OCH_3 R_7=CH_3 (**Crebanine**, 97%)
1b': R_1=H, R_2+R_3=OCH_2O, R_4=H, R_5=R_6=OCH_3 R_7=H (**Norcrebanine**, 99%)

Scheme 1. Syntheses of phoebine (**1a**), norphoebine (**1a'**), crebanine (**1b**) and norcrebanine (**1b'**). Reagents and conditions: (a) reflux, xylene; (b) 10% $NaHCO_3$, $CHCl_3$; (c) $POCl_3$, benzene, reflux; (d) $NaBH_4$, EtOH, reflux; (e) CH_3CO_2Cl, Et_3N, $CHCl_3$ (**11a**→**12a**); (f) $(CF_3CO)_2O$, Et_3N, CH_2Cl_2 (**11a**→**12a'**, **11b**→**12b'**); (g) formic acid, DCC, CH_2Cl_2 (**11b**→**12b**); (h) Pd $(OAc)_2$, pivalic acid, di-*tert*-butyl(methyl) phosphonium tetrafluoroborate, K_2CO_3, DMA, microwave, 850 W, 5 min; (i) $LiAlH_4$, reflux, THF (**13a**, **13b**→**1a**, **1b**); (j) Na_2CO_3, reflux, 90% MeOH-H_2O (**13a'**, **13b'**→**1a'**, **1b'**).

hand, removal of the trifluoroacetyl groups from **13a′** and **13b′** was achieved using aqueous sodium carbonate to yield norphoebine (**1a′**) and norcrebanine (**1b′**) in excellent yields.

Dehydrophoebine (**2a**) and dehydrocrebanine (**2b**) were synthesized by treatment of phoebine (**1a**) and crebanine (**1b**) with 10% palladium on carbon in acetonitrile under reflux in 90% and 99% yields, respectively (Scheme 2). Oxidation of norphoebine (**1a′**) and norcrebanine (**1b′**) with a saturated solution of Fremy's salt at room temperature for 6 h gave oxophoebine (**3a′**) and oxocrebanine (**3b′**) in 81 and 86% yields (Scheme 3). Finally, treatment of oxocrebanine with iodomethane gave uthongine (**4**) with a 91% yield (Scheme 4). The ^{1}H and ^{13}C-NMR spectral data of synthetic (±)-norphoebine, dehydrophoebine, oxophoebine, dehydrocrebanine, oxocrebanine and uthongine were identical in all respects with those previously reported for the natural alkaloids [19–23].

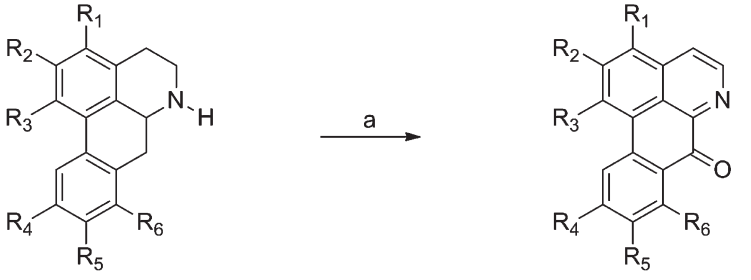
All synthetic compounds (**1–4**) were screened for their cytotoxicity against NCI-H187 (human small cell lung cancer), KB (human carcinoma of the nasopharynx) and MCF7



1a: R_1=R_2=R_3=OCH_3, R_4+R_5=OCH_2O, R_6=H (**Phoebine**)
1b: R_1=H, R_2+R_3=OCH_2O, R_4=H, R_5=R_6=OCH_3 (**Crebanine**)

2a: R_1=R_2=R_3=OCH_3,R_4+R_5=OCH_2O, R_6=H (**Dehydrophoebine**, 90%)
2b: R_1=H, R_2+R_3=OCH_2O, R_4=H, R_5=R_6=OCH_3 (**Dehydrocrebanine**, 99%)

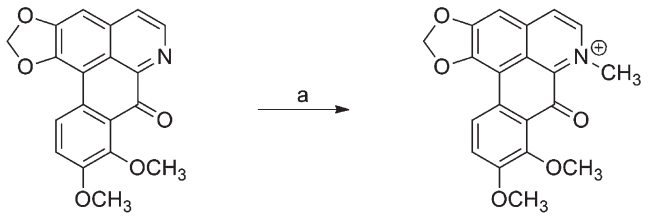
Scheme 2. Syntheses of dehydrophoebine (**2a**) and dehydrocrebanine (**2b**). Reagent and conditions: (a) 10% Pd-C, acetonitrile, reflux.



1a': R_1=R_2=R_3=OCH_3, R_4+R_5=OCH_2O, R_6=H (**Norphoebine**)
1b': R_1=H, R_2+R_3=OCH_2O, R_4=H, R_5=R_6=OCH_3 (**Norcrebanine**)

3a': R_1=R_2=R_3=OCH_3,R_4+R_5=OCH_2O, R_6=H (**Oxophoebine**, 81%)
3b': R_1=H, R_2+R_3=OCH_2O, R_4=H, R_5=R_6=OCH_3 (**Oxocrebanine**, 86%)

Scheme 3. Syntheses of oxophoebine (**3a′**) and oxocrebanine (**3b′**). Reagent and conditions: (a) Fremy's salt, methanol, rt.



Scheme 4. Synthesis of uthongine (**4**). Reagent and conditions: (a) CH_3I, acetonitrile, reflux.

Table 1. Cytotoxic activities of the aporphine alkaloids (**1–4**) against MCF7, KB and NCI-H187 cancer cell lines.

	Cytotoxicity (IC_{50}, μM)		
Compounds	MCF7	KB	NCI-H187
Phoebine (**1a**)	>50	>50	>50
Norphoebine (**1a′**)	>50	29.40	>50
Dehydrophoebine (**2a**)	27.30	22.62	>50
Oxophoebine (**3a′**)	>50	>50	>50
Crebanine (**1b**)	>50	>50	>50
Norcrebanine (**1b′**)	>50	>50	>50
Dehydrocrebanine (**2b**)	10.34	8.63	13.04
Oxocrebanine (**3b′**)	>50	>50	>50
Uthongine (**4**)	3.05	4.77	26.29
Ellipticine[a]	NT[b]	4.30	7.55
Doxorubicin[a]	15.23	1.36	0.20

[a]Positive control.
[b]Not tested.

(human breast cancer) cell lines using MTT assays reported by Skehan et al. [24] and Plumb et al. [25]. The results of their cytotoxic activities are expressed in IC_{50} (median inhibitory concentration) and are summarized in Table 1. In particular, uthongine (**4**) exhibited the most potent activity against MCF7 cell line with an IC_{50} value of 3.05 μM which is significantly more potent than doxorubicin (IC_{50} = 15.23 μM) which was used as the positive control. Moreover, uthongine also showed cytotoxic activity against KB cell line with an IC_{50} value (4.77 μM) comparable with that of ellipticine (4.30 μM) and exhibited weak cytotoxicity against NCI-H187 cell line with an IC_{50} value of 23.29 μM. Dehydrocrebanine exhibited moderate cytotoxic activities against all three cell lines with IC_{50} values of 8.36 (KB), 10.34 (MCF7) and 13.04 (NCI-H187) μM while dehydrophoebine showed weak activity against KB (IC_{50} = 22.62 μM) and MCF7 (IC_{50} = 27.30 μM) cell lines. Norphoebine also showed weak activity against a KB cell line with an IC_{50} value of 29.40 μM. Unfortunately, the rest of the alkaloids (phoebine, oxophoebine, crebanine, norcrebanine and oxocrebanine) did not display significant cytotoxic effects (IC_{50} > 50 μM).

In conclusion, we have achieved the first total syntheses of (±)-norphoebine, dehydrophoebine, oxophoebine, dehydrocrebanine, oxocrebanine and uthongine. The successful cyclization of the isoquinolines (**12**) was achieved using the palladium-catalyzed biaryl coupling under microwave radiation as a key step. All synthetic alkaloids were evaluated for *in vitro* cytotoxicity against three human cancer cell lines. Our investigation revealed that the most potent compound in the series is uthongine (**4**), which showed the highest

cytotoxicity against MCF7 breast cancer cell line. It is interesting to note that the quaternary oxoaporphine salt (uthongine) appears to enhance a greater degree of anticancer activity than its core structure (oxocrebanine). In addition, the presence of an aromatic moiety on ring C (dehydrocrebanine and dehydrophoebine) is possibly crucial in the enhancement of the cytotoxic activity of these molecules.

3. Experimental

3.1. General experimental procedures

Reagents were obtained from commercial sources (Sigma-Aldrich Corp., St. Louis, MO, USA; Merck KGaA, Darmstadt, Germany; Acros Organics, Geel, Belgium) and were used without further purification. All solvents were reagent grade and were dried by distillation from the appropriate drying reagents immediately prior to use. Tetrahydrofuran was distilled from sodium and benzophenone under an argon atmosphere. Triethylamine and dichloromethane were distilled from calcium hydride under argon. Starting materials (compounds **5**, **7** and **8**) were synthesized according to literature procedures [16–18]. Analytical thin-layer chromatography (TLC) was performed on Merck precoated silica gel 60 F_{254} plates (Merck KGaA, Darmstadt, Germany). Compounds were visualized under UV light and/or by heating the plate after spraying with a 1% solution of vanillin in 0.1 M sulfuric acid in methanol. Column chromatography (CC) was carried out on silica gel 60 (70–230 mesh or 230–400 mesh, Merck KGaA, Darmstadt, Germany). Melting points were measured on a Stuart Scientific SMP 2 melting point apparatus (Bibby Scientific US, Burlington, NJ, USA) and are uncorrected. Optical rotations were measured on a JASCO P1010 digital polarimeter (JASCO, Tokyo, Japan). Ultraviolet (UV) spectra were recorded on a Hewlett Packard 8453 UV–vis spectrometer (Agilent Technologies Inc., Waldbronn, Germany). Infrared (IR) spectra were obtained on a Perkin Elmer GX FT-IR spectrophotometer (Perkin Elmer Inc., Waltham, MA, USA). 1D and 2D nuclear magnetic resonance (NMR) experiments were recorded on a Bruker AVANCE 300 MHz spectrometer (Bruker AG, Fällanden, Germany) operating at 300 MHz for proton and 75 MHz for carbon. Mass spectra were recorded on a Bruker microTOF LC mass spectrometer (Bruker Daltonik GmbH, Bremen, Germany) and the lock mass calibration was applied for the determination of accurate masses.

3.2. Preparation

3.2.1. 2-(2-Bromo-4,5-methylenedioxyphenyl)-N-(2,3,4-trimethoxyphenethyl) acetamide (9a)

A solution of **5** (14.70 g, 69.58 mmol) and **6** (17.30 g, 66.78 mmol) in xylene (150 ml) was refluxed for 2 h with water removal using a Dean-Stark trap. The xylene was then removed under reduced pressure to give a residue which was dissolved in chloroform (200 ml). The chloroform layer was washed with 5% hydrochloric acid (3 × 200 ml), water (300 ml), 10% sodium carbonate (3 × 200 ml) and then dried over anhydrous sodium sulfate. Removal of the solvent under reduced pressure gave a residue which was recrystallized from ethanol to give **9a** as white crystals (14.90 g, 49%), mp 136–137 °C. IR (film) υ_{max} cm^{-1}: 3318, 2940, 1650, 1602, 1495, 1481, 1233, 1100, 1036, 924, 799, 674. ^{1}H-NMR ($CDCl_3$) δ: 7.02 (1H, s, ArH), 6.78 (1H, s, ArH), 6.75 (1H, d, J = 8.4 Hz, ArH), 6.57 (1H, d, J = 8.4 Hz, ArH), 5.99

(2H, s, OCH_2O), 5.82 (1H, br s, NH), 3.85 and 3.83 (9H, 2s, 3xOCH_3), 3.57 (2H, s, CH_2), 3.43 (2H, apparent q, J = 6.6 Hz, CH_2), 2.72 (2H, t, J = 6.6 Hz, CH_2). ^{13}C-NMR ($CDCl_3$) δ: 169.7, 152.6, 151.7, 147.7, 147.6, 142.2, 127.7, 124.7, 124.5, 115.3, 112.8, 111.0, 107.4, 101.9, 60.9, 60.7, 55.9, 43.7, 40.8, 29.5. HRESIMS: *m/z* 452.0716 $[M + H]^+$ (calcd for $C_{20}H_{23}BrNO_6$, 452.0709).

3.2.2. 2-(6-Bromo-2,3-dimethoxyphenyl)-N-(3,4-methylenedioxyphenethyl) acetamide (9b)

A solution of **8** (13.15 g, 44.80 mmol) in ethanol-free chloroform (150 ml) was added to a mixture of **7** (7.40 g, 44.80 mmol) in ethanol-free chloroform (35 ml) and 10% sodium hydrogen carbonate (150 ml). The mixture was stirred at room temperature for 2 h. The chloroform layer was washed with 10% sodium hydrogen carbonate (3 × 200 ml), water (3 × 200 ml), 10% hydrochloric acid (3 × 200 ml) and water (3 × 200 ml) and then dried over anhydrous sodium sulfate. Removal of the solvent under reduced pressure gave a residue which was recrystallized from ethanol to give **9b** as a pale yellow solid (13.84 g, 87%), mp 164–166 °C. IR (film) υ_{max} cm^{-1}: 3282, 2881, 1636, 1546, 1472, 1246, 1007, 924, 799, 595. ^{1}H-NMR ($CDCl_3$) δ: 7.28 (1H, d, J = 8.7 Hz, ArH), 6.76 (1H, d, J = 8.7 Hz, ArH), 6.65 (1H, d, J = 7.8 Hz, ArH), 6.58 (1H, d, J = 1.5 Hz, ArH), 6.51 (1H, dd, J = 7.8, 1.5 Hz, ArH), 5.91 (2H, s, OCH_2O), 5.51 (1H, br s, NH), 3.86 (3H, s, OCH_3), 3.80 (3H, s, OCH_3), 3.75 (2H, s, CH_2), 3.41 (2H, apparent q, J = 6.6 Hz, CH_2), 2.66 (2H, t, J = 6.6 Hz, CH_2). ^{13}C-NMR ($CDCl_3$) δ: 169.4, 152.3, 148.3, 147.7, 146.0, 132.6, 129.4, 128.0, 121.7, 115.8, 112.8, 109.1, 108.3, 100.8, 60.9, 55.9, 40.9, 38.2, 35.3. HRESIMS: *m/z* 422.0624 $[M + H]^+$ (calcd for $C_{19}H_{21}BrNO_5$, 422.0603).

3.2.3. 1-(2-Bromo-4,5-methylenedioxybenzyl)-5,6,7-trimethoxy-3,4-dihydroisoquinoline (10a)

A solution of **9a** (22.60 g, 49.97 mmol) and phosphorus oxychloride (67.80 g, 442.18 mmol) in benzene (770 ml) was refluxed for 3 h. The excess reagent and solvent were removed under reduced pressure and the residue was dissolved in chloroform (770 ml). The mixture was basified by addition of solid sodium carbonate followed by dropwise addition of water. The chloroform layer was washed with water (300 ml) and then dried over anhydrous sodium sulfate. Removal of the solvent under reduced pressure gave a yellow solid which was recrystallized from ethanol to give **10a** as yellow crystals (15.70 g, 72%), mp 135–137 °C. IR (film) υ_{max} cm^{-1}: 2942, 1675, 1613, 1478, 1238, 1119, 1037, 949, 789, 605. ^{1}H-NMR ($CDCl_3$) δ: 7.02 (H, s, ArH), 6.83 (1H, s, ArH), 6.79 (1H, s, ArH), 5.93 (2H, s, OCH_2O), 4.20 (2H, s, $ArCH_2$), 3.91 (3H, s, OCH_3), 3.84 (3H, s, OCH_3), 3.81 (3H, s, OCH_3), 3.64 (2H, t, J = 7.2 Hz, CH_2), 2.73 (2H, t, J = 7.2 Hz, CH_2). ^{13}C-NMR ($CDCl_3$) δ: 164.0, 151.9, 151.3, 150.0, 147.6, 145.0, 133.0, 124.4, 121.7, 114.1, 113.3, 111.0, 107.0, 102.6, 61.0, 60.6, 60.4, 56.2, 47.9, 18.5. HRESIMS: *m/z* 434.0598 $[M + H]^+$ (calcd for $C_{20}H_{21}BrNO_5$, 434.0603).

3.2.4. 1-(6-Bromo-2,3-dimethoxybenzyl)-6,7-methylenedioxy-3,4-dihydroisoquinoline (10b)

By the same procedure used to prepare **10a**, compound **10b** was obtained in 96% yield as a yellow solid, mp 84–86 °C. IR (film) υ_{max} cm^{-1}: 2940, 1637, 1601, 1473, 1268, 1007, 935, 737. ^{1}H-NMR ($CDCl_3$) δ: 7.27 (1H, d, J = 8.7 Hz, ArH), 7.12 (1H, s, ArH), 6.73 (1H, d, J = 8.7 Hz, ArH), 6.68 (1H, s, ArH), 5.98 (2H, s, OCH_2O), 4.23 (2H, s, $ArCH_2$), 3.83 (3H,

s, OCH_3), 3.79 (3H, s, OCH_3), 3.58 (2H, t, J = 7.2 Hz, CH_2), 2.62 (2H, t, J = 7.2 Hz, CH_2). ^{13}C-NMR ($CDCl_3$) δ: 164.0, 152.1, 149.0, 148.4, 146.4, 133.3, 132.3, 127.5, 123.3, 116.2, 112.0, 107.9, 105.6, 101.3, 60.8, 55.7, 46.7, 36.8, 26.3. HRESIMS: m/z 404.0456 $[M + H]^+$ (calcd for $C_{19}H_{19}BrNO_4$, 404.0497).

3.2.5. 1-(2-Bromo-4,5-methylenedioxybenzyl)-5,6,7-trimethoxy-1,2,3,4-tetrahydroisoquinoline (11a)

Sodium borohydride (2.00 g, 52.86 mmol) was added portionwise to a stirred solution of **10a** (16.20 g, 37.30 mmol) in ethanol (560 ml) and the mixture was refluxed for 1 h. Removal of the ethanol under reduced pressure gave a residue which was shaken with chloroform (500 ml) and water (500 ml). The chloroform layer was washed with brine and then dried over anhydrous sodium sulfate. Removal of the solvent gave a yellow solid which was recrystallized from ethanol to give **11a** as a yellow solid (7.40 g, 46%), mp 130–131 °C. IR (film) υ_{max} cm^{-1}: 3319, 2939, 1603, 1476, 1238, 1117, 1037, 930, 837. ^{1}H-NMR ($CDCl_3$) δ: 7.05 (1H, s, ArH), 6.79 (1H, s, ArH), 6.62 (1H, s, ArH), 5.98 (2H, s, OCH_2O), 4.19 (1H, dd, J = 9.9, 2.3 Hz, CH), 3.88 and 3.84 (9H, 2s, 3xOCH_3), 3.30–3.20 (2H, m, CH_2), 3.01–2.68 (4H, m, 2xCH_2). ^{13}C-NMR ($CDCl_3$) δ: 151.4, 151.1, 147.3, 147.2, 140.5, 134.1, 131.7, 121.6, 114.8, 112.8, 111.3, 105.6, 101.7, 60.8, 60.4, 56.0, 55.4, 42.7, 39.6, 23.8. HRESIMS: m/z 436.0748 $[M + H]^+$ (calcd for $C_{20}H_{23}BrNO_5$, 436.0759).

3.2.6. 1-(6-Bromo-2,3-dimethoxybenzyl)-6,7-methylenedioxy-1,2,3,4-tetrahydroisoquinoline (11b)

By the same procedure used to prepare **11a**, compound **11b** was obtained in 94% yield as a yellow solid, mp 103–105 °C. IR (film) υ_{max} cm^{-1}: 3332, 2938, 1648, 1471, 1247, 1079, 1039, 1010, 934, 800, 737, 612. ^{1}H-NMR ($CDCl_3$) δ: 7.29 (1H, d, J = 8.7 Hz, ArH), 6.86 (1H, s, ArH), 6.73 (1H, d, J = 8.7 Hz, ArH), 6.56 (1H, s, ArH), 5.89 (2H, s, OCH_2O), 4.25 (1H, dd, J = 10.5, 2.4 Hz, CH), 3.85 (3H, s, OCH_3), 3.83 (3H, s, OCH_3), 3.66–2.62 (6H, m, 3xCH_2). ^{13}C-NMR ($CDCl_3$) δ: 152.2, 148.6, 145.9, 145.7, 133.2, 131.9, 128.3, 128.0, 115.8, 112.0, 108.7, 106.8, 100.6, 60.7, 55.8, 55.5, 39.3, 37.1, 29.8. HRESIMS: m/z 406.0642 $[M + H]^+$ (calcd for $C_{19}H_{21}BrNO_4$, 406.0654).

3.2.7. 1-(2-Bromo-4,5-methylenedioxybenzyl)-2-carbomethoxy-5,6,7-trimethoxy-1,2,3,4-tetrahydroisoquinoline (12a)

Methyl chloroformate (18.50 g, 195.77 mmol) was added dropwise to a stirred solution of **11a** (13.30 g, 30.48 mmol) and triethylamine (26.30 g, 259.91 mmol) in chloroform (370 ml) at 0–10 °C. Stirring was continued at room temperature for 3 h. The chloroform layer was washed with 10% hydrochloric acid (3 × 200 ml), water (200 ml) and brine and then dried over anhydrous sodium sulfate. Removal of the solvent under reduced pressure gave the crude product which was recrystallized from ethanol to give **12a** as pale yellow crystals (9.50 g, 63%), mp 131–132 °C. IR (film) υ_{max} cm^{-1}: 2940, 1698, 1604, 1477, 1408, 1245, 1230, 1112, 1037, 929, 838, 653. ^{1}H-NMR ($CDCl_3$) δ: 7.02 and 6.98 (total 1H, 2s, ArH of both conformers), 6.65 and 6.56 (total 1H, 2s, ArH of both conformers), 6.46 and 6.29 (total 1H, 2s, ArH of both conformers), 5.94 (2H, s, OCH_2O), 5.34–5.31 (1H, m, CH), 4.32–4.24 and 3.97–3.90 (total 1H, m, H-5α), 3.86 and 3.85 (6H, 2s, 2xOCH_3), 3.80 and 3.72 (total 3H, 2s, OCH_3 of both conformers), 3.65 and 3.41 (total 3H, 2s, CO_2CH_3 of both conformers), 3.48–2.67 (5H, m, H-5β, 2xCH_2). ^{13}C-NMR ($CDCl_3$) δ: 155.8, 151.8, 151.1, 147.1, 147.0,

140.9, 131.8, 130.7, 120.7, 115.2, 112.4, 111.1, 105.9, 101.7, 60.9, 60.5, 56.0, 54.1, 52.3, 42.5, 37.2, 22.3. HRESIMS: *m/z* 494.0785 $[M + H]^+$ (calcd for $C_{22}H_{25}BrNO_7$, 494.0814).

3.2.8. 2-Trifluoroacetyl-1-(2-bromo-4,5-methylenedioxybenzyl)-5,6,7-trimethoxy-1,2,3,4-tetrahydroisoquinoline (12a′)

Trifluoroacetic anhydride (24.00 g, 114.60 mmol) was added dropwise to a stirred mixture of **11a** (5.00 g, 11.46 mmol) and triethylamine (6.96 g, 68.76 mmol) in dichloromethane (130 ml) at 0–10 °C. Stirring was continued at room temperature for 3 h. Dichloromethane (100 ml) was added and the organic solution was washed with 10% sodium hydrogen carbonate (3 × 100 ml), water (100 ml), 10% hydrochloric acid (3 × 100 ml) and water (100 ml). The organic layer was separated and then dried over anhydrous sodium sulfate. Removal of the solvent gave a red-brown viscous oil which was recrystallized from ethanol to give **12a′** as a pale yellow solid (2.75 g, 45%), mp 126–127 °C. IR (film) υ_{max} cm^{-1}: 2940, 1690, 1604, 1478, 1351, 1232, 1196, 1142, 1122, 1038, 933, 846, 660. ^{1}H-NMR ($CDCl_3$) δ: 7.00 and 6.99 (total 1H, 2s, ArH of both conformers), 6.64 and 6.54 (total 1H, 2s, ArH of both conformers), 6.39 and 6.29 (total 1H, 2s, ArH of both conformers), 5.97 and 5.95 (total 2H, 2s, OCH_2O of both conformers), 5.73–5.68 (1H, m, CH), 4.08–3.62 (2H, m, CH_2), 3.88 (3H, s, OCH_3), 3.86 (3H, s, OCH_3), 3.77 (3H, s, OCH_3), 3.34–2.73 (4H, m, 2xCH_2). ^{13}C-NMR ($CDCl_3$) δ: 155.6, 152.3, 150.9, 147.5, 147.4, 141.2, 130.2, 129.4, 119.4, 118.4, 115.5, 112.7, 110.6, 106.1, 101.8, 60.9, 60.6, 56.0, 54.2, 41.5, 39.9, 23.4. HRESIMS: *m/z* 532.0576 $[M + H]^+$ (calcd for $C_{22}H_{22}BrF_3NO_6$, 532.0582).

3.2.9. 2-Trifluoroacetyl-1-(2-bromo-5,6-dimethoxybenzyl)-6,7-methylenedioxy-1,2,3,4-tetrahydroiso quinoline (12b′)

By the same procedure used to prepare **12a′**, compound **12b′** was obtained in 42% yield as pale yellow crystals, mp 140–141 °C. IR (film) υ_{max} cm^{-1}: 2943, 1690, 1483, 1472, 1280, 1238, 1196, 1140, 1080, 1039, 1009, 940, 864, 799, 752, 651. ^{1}H-NMR ($CDCl_3$) δ: 7.23 and 7.22 (total 1H, 2d, J = 8.7 Hz, ArH of both conformers), 6.81 (1H, s, ArH), 6.73 and 6.71 (total 1H, 2d, J = 8.7 Hz, ArH of both conformers), 6.58 (1H, s, ArH), 5.94 and 5.93 (total 2H, 2s, OCH_2O of both conformers), 5.87 (1H, dd, J = 10.8, 3.6 Hz, CH), 3.90 (3H, s, OCH_3), 3.84 (3H, s, OCH_3), 4.04–3.83 (2H, m, CH_2), 3.42–2.75 (4H, m, 2xCH_2). ^{13}C-NMR ($CDCl_3$) δ: 156.0, 151.8, 149.0, 146.8, 146.5, 130.7, 128.8, 127.4, 126.1, 116.0, 113.1, 108.3, 107.1, 106.7, 101.1, 60.7, 56.0, 53.6, 40.0, 36.5, 29.4. HRESIMS: *m/z* 502.0479 $[M + H]^+$ (calcd for $C_{21}H_{20}BrF_3NO_5$, 502.0477).

3.2.10. 1-(2-Bromo-5,6-methoxybenzyl)-2-formyl-6,7-methylenedioxy-1,2,3,4-tetrahydroisoquinoline (12b)

A solution of *N*,*N*′-dicyclohexylcarbodiimide (DCC, 8.23 g, 39.89 mmol) in dichloromethane (88 ml) was added to a stirred solution of **11b** (14.57 g, 35.86 mmol) and formic acid (1.80 g, 39.11 mmol) in dichloromethane (220 ml). The resulting mixture was stirred at room temperature for 2 h. The mixture was filtered and the filtrate was washed with 5% hydrochloric acid (3 × 250 ml), water (3 × 250 ml), 5% sodium hydrogen carbonate (3 × 250 ml) and water (250 ml). The organic layer was dried over anhydrous sodium sulfate. Removal of the solvent gave a brown viscous oil which was recrystallized from ethanol to give **12b** as a pale yellow solid (7.00 g, 45%), mp 134–136 °C. IR (film) υ_{max} cm^{-1}: 2941, 2840, 1669, 1483, 1471, 1273, 1240, 1217, 1078, 1037, 1008, 931, 801, 643. ^{1}H-NMR ($CDCl_3$) δ: 7.92

and 7.51 (total 1H, 2s, CHO of both conformers), 7.26 (1H, d, J = 8.7 Hz, ArH), 6.83 (1H, s, ArH), 6.74 (1H, d, J = 8.7 Hz, ArH), 6.60 (1H, s, ArH), 5.93 (2H, s, OCH_2O), 4.80 (1H, dd, J = 10.8, 2.4 Hz, CH), 4.43–4.37 (1H, m, H-5α), 3.85 (3H, s, OCH_3), 3.84 (3H, s, OCH_3), 3.44–2.71 (5H, m, H-5β, 2xCH_2). ^{13}C-NMR ($CDCl_3$) δ: 160.8, 152.1, 148.7, 146.8, 146.3, 130.9, 129.0, 127.8, 127.4, 115.4, 112.8, 108.7, 106.6, 101.0, 60.6, 56.8, 55.9, 37.4, 34.7, 28.2. HRESIMS: m/z 434.0636 $[M + H]^+$ (calcd for $C_{20}H_{21}BrNO_5$, 434.0603).

3.2.11. Methyl 1,2,3-trimethoxy-9,10-methylenedioxynoraporphine-6-carboxylate (13a)

To a solution of **12a** (100.0 mg, 0.20 mmol) in dimethylacetamide (DMA, 4 ml), were added palladium(II) acetate (4.5 mg, 0.02 mmol), di-*tert*-butyl(methyl) phosphonium tetrafluoro borate (9.9 mg, 0.04 mmol), potassium carbonate (82.9 mg, 0.60 mmol) and pivalic acid (6.1 mg, 0.06 mmol). The mixture was irradiated in a microwave reactor for 5 min with the power level at 850 W under an argon atmosphere. After cooling to room temperature, the reaction mixture was filtered over Celite and the solvent was removed under reduced pressure to give the crude product which was purified by column chromatography with 25% ethyl acetate-hexane to give **13a** as a white solid (80.1 mg, 96%), mp 184–185 °C. IR (film) υ_{max} cm^{-1}: 2940, 1700, 1459, 1414, 1338, 1236, 1197, 1150, 1092, 1038, 933, 877. ^{1}H-NMR ($CDCl_3$) δ: 7.90 (1H, s, H-11), 6.76 (1H, s, H-8), 5.98 (2H, AB q, J = 0.9 Hz, OCH_2O), 4.71–4.65 (1H, m, H-6a), 4.45–4.41 (1H, m, H-5α), 3.95 (3H, s, OCH_3), 3.89 (3H, s, OCH_3), 3.76 (3H, s, OCH_3), 3.73 (3H, s, CO_2CH_3), 3.01–2.50 (5H, m, H-5β, 2xCH_2). ^{13}C-NMR ($CDCl_3$) δ: 155.9, 149.8, 149.6, 146.6, 146.3, 145.4, 130.6, 128.9, 125.1, 123.7, 123.4, 108.64, 108.6, 100.9, 61.1, 60.9, 60.4, 52.7, 51.9, 38.6, 34.9, 23.8. HRESIMS: m/z 414.1539 $[M + H]^+$ (calcd for $C_{22}H_{24}NO_7$, 414.1553).

3.2.12. 1,2,3-Trimethoxy-9,10-methylenedioxy-6-trifluoroacetylnoraporphine (13a')

By the same procedure used to prepare **13a**, compound **13a′** was obtained in 19% yield as a white solid, mp 184–185 °C. IR (film) υ_{max} cm^{-1}: 2944, 1689, 1461, 1415, 1340, 1237, 1187, 1144, 1094, 1046, 921, 758. ^{1}H-NMR ($CDCl_3$) δ: 7.91 (1H, s, H-11), 6.75 (1H, s, H-8), 5.98 (2H, AB q, J = 1.2 Hz, OCH_2O), 4.98 (1H, dd, J = 13.8 and 4.2 Hz, H-6a), 4.24–4.20 (1H, m, H-5α), 3.96 (3H, s, OCH_3), 3.91 (3H, s, OCH_3), 3.75 (3H, s, OCH_3), 3.30–2.57 (5H, m, H-5β, 2xCH_2). ^{13}C-NMR ($CDCl_3$) δ: 156.1, 150.4, 149.5, 146.8, 146.6, 145.7, 129.6, 127.2, 124.7, 123.5, 122.4, 118.3, 108.8, 108.5, 101.0, 61.1, 61.0, 60.5, 52.4, 41.0, 33.3, 24.2. HRESIMS: m/z 452.1344 $[M + H]^+$ (calcd for $C_{22}H_{21}F_3NO_6$, 452.1321).

3.2.13. 6-Formyl-8,9-dimethoxy-1,2-methylenedioxynoraporphine (13b)

By the same procedure used to prepare **13a**, compound **13b** was obtained in 94% yield as a white solid, mp 133–134 °C. IR (film) υ_{max} cm^{-1}: 2959, 2875, 1645, 1492, 1408, 1278, 1239, 1180, 1078, 1034, 933, 810. ^{1}H-NMR ($CDCl_3$) δ: 8.41 and 8.28 (total 1H, 2s, CHO of both conformers), 7.85 and 7.84 (total 1H, 2d, J = 8.7 Hz, H-11 of both conformers), 6.92 and 6.89 (total 1H, 2d, J = 8.7 Hz, H-10 of both conformers), 6.58 and 6.55 (total 1H, 2s, H-3 of both conformers), 6.09 and 5.97 (total 2H, 2s, OCH_2O of both conformers), 4.98 (total 1H, dd, J = 13.8, 4.2 Hz, H-6a of both conformers), 4.56–4.48 (total 1H, m, H-5α of both conformers), 3.92 and 3.91 (total 3H, 2s, OCH_3 of both conformers), 3.85 and 3.82 (total 3H, 2s, OCH_3 of both conformers), 3.66–2.40 (total 5H, m, H-5β, 2xCH_2 of both conformers). ^{13}C-NMR ($CDCl_3$) δ: 162.1, 152.5, 147.0, 146.3, 142.7, 129.8, 128.9, 127.6, 126.5,

124.0, 123.4, 110.5, 106.9, 100.9, 60.7, 55.8, 49.2, 42.1, 31.0, 26.9. HRESIMS: *m/z* 354.1344 $[M + H]^+$ (calcd for $C_{20}H_{20}NO_5$, 354.1341).

3.2.14. 8,9-Dimethoxy-1,2-methylenedioxy-6-trifluoroacetylnoraporphine (13b′)

By the same procedure used to prepare **12a** (but the reaction mixture was recrystallized from ethanol), compound **13b′** was obtained in 81% yield as a white solid, mp 198–199 °C. IR (film) υ_{max} cm^{-1}: 2900, 1679, 1461, 1272, 1188, 1083, 915, 817, 650. ^{1}H-NMR ($CDCl_3$) δ: 7.86 (1H, d, J = 8.7 Hz, H-11), 6.90 (1H, d, J = 8.7 Hz, H-10), 6.57 (1H, s, H-3), 6.10 and 5.98 (2H, 2s, OCH_2O), 5.05 (1H, dd, J = 13.5, 3.9 Hz, H-6a), 4.25–4.18 (1H, m, H-5α), 3.91 (3H, s, OCH_3), 3.86 (3H, s, OCH_3), 3.56 (1H, dd, J = 14.4, 4.2 Hz, H-5β), 3.40–2.49 (4H, m, 2xCH_2). ^{13}C-NMR ($CDCl_3$) δ: 156.1, 152.6, 147.1, 146.3, 142.8, 129.4, 126.4, 123.8, 123.6, 118.3, 117.4, 114.5, 110.7, 106.7, 101.0, 60.8, 55.8, 52.3, 41.4, 30.4, 26.3. HRESIMS: *m/z* 422.1239 $[M + H]^+$ (calcd for $C_{21}H_{19}F_3NO_5$, 422.1215).

3.2.15. Phoebine (1a)

A mixture of **13a** (0.94 g, 2.27 mmol) and lithium aluminum hydride (0.78 g, 20.48 mmol) in dry tetrahydrofuran (50 ml) was refluxed for 2 h. Water (15 ml) was added dropwise followed by dilute ammonium hydroxide (5 ml). The pale yellow granular residue was filtered off and washed with chloroform. The organic phase was separated and then dried over anhydrous sodium sulfate. Removal of the solvent under reduced pressure gave a pale brown solid which was chromatograped over silica gel using ethyl acetate as eluent to give **1a** as a yellow solid (0.71 g, 84%), mp 91–92 °C. UV (MeOH) λ_{max}/nm (log ε) 220.7 (4.55), 282.2 (4.12), 313.8 (4.14). IR (film) υ_{max} cm^{-1}: 2936, 1485, 1458, 1413, 1373, 1338, 1241, 1096, 1043, 936, 877. ^{1}H-NMR ($CDCl_3$) δ: 7.81 (1H, s, H-11), 6.74 (1H, s, H-8), 5.96 (2H, AB q, J = 1.2 Hz, OCH_2O), 3.94 (3H, s, OCH_3), 3.89 (3H, s, OCH_3), 3.73 (3H, s, OCH_3), 2.52 (3H, s, NCH_3), 3.10–2.78 and 2.46–2.38 (7H, 2 m, H-6a, 3xCH_2). ^{13}C-NMR ($CDCl_3$) δ: 149.7, 149.4, 146.5, 146.0, 145.2, 130.8, 130.1, 125.4, 122.8, 122.6, 108.4, 108.2, 100.7, 62.8, 60.9, 60.6, 60.4, 53.0, 44.0, 34.8, 23.8. HRESIMS: *m/z* 370.1633 $[M + H]^+$ (calcd for $C_{21}H_{24}NO_5$, 370.1654).

3.2.16. Crebanine (1b)

By the same procedure used to prepare **1a**, compound **1b** was obtained in 97% yield as a yellow-brown solid, mp 114–115 °C. UV (MeOH) λ_{max}/nm (log ε) 217.6 (4.62), 242.5 (4.21), 279.3 (4.44), 291.0 (4.37), 322.2 (3.77). IR (film) υ_{max} cm^{-1}: 2927, 1601, 1496, 1413, 1282, 1236, 1129, 1073, 1036, 987, 937, 818. ^{1}H-NMR ($CDCl_3$) δ: 7.81 (1H, d, J = 8.7 Hz, H-11), 6.88 (1H, d, J = 8.7 Hz, H-10), 6.52 (1H, s, H-3), 5.99 (2H, AB q, J = 0.9 Hz, OCH_2O), 3.90 (3H, s, OCH_3), 3.81 (3H, s, OCH_3), 3.68 (1H, dd, J = 14.7, 4.2 Hz, H-6a), 3.18–2.25 (6H, m, 3xCH_2), 2.58 (3H, s, NCH_3). ^{13}C-NMR ($CDCl_3$) δ: 152.0, 146.5, 145.8, 142.0, 129.7, 126.6, 126.5, 124.6, 123.1, 116.5, 110.2, 106.8, 100.6, 61.9, 60.6, 55.7, 53.6, 43.9, 29.2, 26.9. HRESIMS: *m/z* 340.1582 $[M + H]^+$ (calcd for $C_{20}H_{22}NO_4$, 340.1549).

3.2.17. Norphoebine (1a′)

Compound **13a′** (0.14 g, 0.31 mmol) was dissolved in 90% methanol-water (10 ml). Sodium carbonate (0.067 g, 0.63 mmol) was added and then the mixture was refluxed for 2 h. The solvent was then removed under reduced pressure and water (20 ml) and 10% sodium bicarbonate (20 ml) were added to the residue followed by extraction with dichloromethane

(3 × 20 ml). The organic layer was then dried over anhydrous sodium sulfate. Removal of the solvent under reduced pressure gave crude norphoebine which was chromatograped over silica gel using ethyl acetate as eluent to give **1a′** as a purple-brown solid (0.12 g, 99%), mp 112–113 °C. UV (MeOH) λ_{max}/nm (log ε) 219.8 (4.54), 238.7 (4.27), 282.4 (4.12), 309.9 (4.12). IR (film) υ_{max} cm^{-1}: 3313, 2936, 1734, 1619, 1462, 1411, 1337, 1241, 1199, 1133, 1094, 1039, 934, 878. ^{1}H-NMR ($CDCl_3$) δ: 7.84 (1H, s, H-11), 6.70 (1H, s, H-8), 5.94 (2H, s, OCH_2O), 3.93 (3H, s, OCH_3), 3.89 (3H, s, OCH_3), 3.73 (3H, s, OCH_3), 3.76–3.70 (1H, m, H-6a), 3.40–3.34 (1H, m, H-5α), 2.95–2.57 (5H, m, H-5β, 2xCH_2). ^{13}C-NMR ($CDCl_3$) δ: 150.2, 149.5, 146.5, 146.0, 145.4, 131.3, 129.7, 125.5, 122.8, 122.4, 108.5, 108.2, 100.8, 61.0, 60.6, 60.4, 54.0, 42.7, 37.1, 23.7. HRESIMS: *m/z* 356.1526 [M + H]$^+$ (calcd for $C_{20}H_{22}NO_5$, 356.1498).

3.2.18. Norcrebanine (1b′)

By the same procedure used to prepare **1a′** (but the mixture was refluxed for 6.5 h and the product was purified by recrystallization from ethanol), compound **1b′** was obtained in 99% yield as yellow crystals, 101–102 °C. UV (MeOH) λ_{max}/nm (log ε) 218.0 (4.55), 243.8 (4.18), 279.8 (4.39), 289.8 (4.34), 321.4 (3.74). IR (film) υ_{max} cm^{-1}: 3302, 2956, 1602, 1495, 1413, 1272, 1233, 1059, 939, 836, 713. ^{1}H-NMR ($CDCl_3$) δ: 7.78 (1H, d, J = 8.7 Hz, H-11), 6.82 (1H, d, J = 8.7 Hz, H-10), 6.47 (1H, s, H-3), 5.92 (2H, AB q, J = 1.2 Hz, OCH_2O), 3.86 (3H, s, OCH_3), 3.79 (3H, s, OCH_3), 3.82–2.31 (7H, m, H-6a, 3xCH_2), 2.17 (1H, br s, NH). ^{13}C-NMR ($CDCl_3$) δ: 152.0, 146.7, 145.7, 141.9, 129.3, 127.0, 126.4, 124.6, 123.2, 116.1, 110.2, 107.2, 100.5, 60.6, 55.7, 53.1, 43.2, 29.4, 29.0. HRESIMS: *m/z* 326.1378 [M + H]$^+$ (calcd for $C_{19}H_{20}NO_4$, 326.1392).

3.2.19. Dehydrophoebine (2a)

To a solution of **1a** (0.11 g, 0.30 mmol) in acetonitrile (10 ml) was added 10% palladium on carbon (0.088 g, 0.83 mmol). The mixture was heated to reflux for 2 h under an argon atmosphere. The reaction mixture was filtered and the filtrate was concentrated under reduced pressure to give the crude product which was recrystallized from ethanol to give **2a** as a green solid (0.099 g, 90%), mp 155–156 °C. UV (MeOH) λ_{max}/nm (log ε) 216.4 (4.55), 241.9 (4.76), 256.2 (4.85), 271.1 (4.74), 297.9 (4.29), 335.4 (4.16). IR (film) υ_{max} cm^{-1}: 2936, 1597, 1458, 1402, 1337, 1219, 1141, 1091, 1042, 940, 853, 796. ^{1}H-NMR ($CDCl_3$) δ: 8.90 (1H, s, H-11), 7.03 (1H, s, H-8), 6.60 (1H, s, H-7), 6.01 (2H, s, OCH_2O), 4.06 (3H, s, OCH_3), 3.93 (6H, s, OCH_3), 3.27–3.21 (4H, m, 2xCH_2), 3.02 (3H, s, NCH_3). ^{13}C-NMR ($CDCl_3$) δ: 150.1, 147.3, 146.8, 145.7, 145.1, 142.2, 130.6, 122.3, 121.2, 120.3, 119.2, 105.4, 104.0, 103.5, 100.7, 61.2, 60.8, 60.2, 49.9, 40.5, 24.2. HRESIMS: *m/z* 368.1469 [M + H]$^+$ (calcd for $C_{21}H_{22}NO_5$, 368.1498).

3.2.20. Dehydrocrebanine (2b)

By the same procedure used to prepare **2a**, compound **2b** was obtained in 99% yield as a brown solid, mp 139–140 °C. UV (MeOH) λ_{max}/nm (log ε) 214.1 (4.25), 247.4 (4.19), 271.0 (4.58), 335.2 (3.87). IR (film) υ_{max} cm^{-1}: 2940, 2831, 1604, 1492, 1400, 1303, 1278, 1219, 1105, 1059, 1038, 985, 821, 743. ^{1}H-NMR ($CDCl_3$) δ: 8.63 (1H, d, J = 9.0 Hz, H-11), 7.00 (1H, d, J = 9.0 Hz, H-10), 6.86 (1H, s, H-3), 6.84 (1H, s, H-7), 6.16 (2H, s, OCH_2O), 3.97 (3H, s, OCH_3), 3.96 (3H, s, OCH_3), 3.34 (2H, t, J = 6.0 Hz, CH_2), 3.18 (2H, d, J = 6.0 Hz, CH_2), 3.11 (3H, s, NCH_3). ^{13}C-NMR ($CDCl_3$) δ: 150.2, 145.1, 144.1, 141.6, 141.5, 129.5, 127.5,

123.4, 118.6, 118.3, 117.4, 108.5, 106.7, 100.8, 93.8, 60.5, 56.2, 50.5, 40.5, 30.8. HRESIMS: *m/z* 338.1358 $[M + H]^+$ (calcd for $C_{20}H_{20}NO_4$, 338.1392).

3.2.21. Oxophoebine (3a′)

A saturated solution of Fremy's salt in 4% sodium bicarbonate was added dropwise to a solution of **1a′** (0.11 g, 0.31 mmol) in methanol (10 ml) and the mixture was stirred at room temperature for 6 h. Dichloromethane (20 ml) was added to the mixture followed by extraction with water. The organic phase was separated and then dried over anhydrous sodium sulfate. Removal of the solvent under reduced pressure gave a red viscous oil which was recrystallized from ethanol to give **3a′** as a red amorphous solid (92.2 mg, 81%), UV (MeOH) λ_{max}/nm (log ε) 210.9 (4.39), 250.6 (4.22), 278.0 (4.38), 319.9 (3.84). IR (film) υ_{max} cm^{-1}: 2923, 1644, 1582, 1463, 1402, 1363, 1268, 1116, 1031, 955, 897. ^{1}H-NMR ($CDCl_3$) δ: 8.93 (1H, d, J = 5.4 Hz, H-5), 8.56 (1H, s, H-11), 8.17 (1H, d, J = 5.4 Hz, H-4), 7.93 (1H, s, H-8), 6.12 (2H, s, OCH_2O), 4.19 (3H, s, OCH_3), 4.08 (3H, s, OCH_3), 4.07 (3H, s, OCH_3). ^{13}C-NMR ($CDCl_3$) δ: 180.6, 156.2, 153.2, 148.2, 147.9, 147.5, 144.9, 144.0, 131.3, 131.1, 127.7, 121.9, 119.0, 115.7, 107.5, 107.1, 102.1, 61.9, 61.5, 61.1. HRESIMS: *m/z* 366.0937 $[M + H]^+$ (calcd for $C_{20}H_{16}NO_6$, 366.0977).

3.2.22. Oxocrebanine (3b′)

By the same procedure used to prepare **3a′**, compound **3b′** was obtained in 86% yield as an orange solid, mp 101–102 °C. UV (MeOH) λ_{max}/nm (log ε) 207.4 (4.40), 249.5 (4.38), 272.5 (4.30), 375.2 (3.55). IR (film) υ_{max} cm^{-1}: 2934, 1651, 1567, 1463, 1243, 1037, 968, 833. ^{1}H-NMR ($CDCl_3$) δ: 8.76 (1H, d, J = 5.4 Hz, H-5), 8.22 (1H, d, J = 9.0 Hz, H-11), 7.61 (1H, d, J = 5.4 Hz, H-4), 7.11 (1H, d, J = 9.0 Hz, H-10), 6.98 (1H, s, H-3), 6.29 (2H, s, OCH_2O), 4.00 (3H, s, OCH_3), 3.93 (3H, s, OCH_3). ^{13}C-NMR ($CDCl_3$) δ: 180.9, 153.4, 152.3, 150.8, 146.9, 144.7, 143.3, 135.9, 126.3, 124.9, 123.9, 121.7, 117.3, 117.2, 107.9, 102.7, 102.0, 61.3, 56.1. HRESIMS: *m/z* 336.0813 $[M + H]^+$ (calcd for $C_{19}H_{14}NO_5$, 336.0872).

3.2.23. Uthongine (4)

Compound **3b′** (48.3 mg, 0.14 mmol) was dissolved in acetonitrile (5 ml). Iodomethane (20.5 mg, 0.14 mmol) was added and then the mixture was refluxed for 6 h. The solvent was removed under reduced pressure. The resulting residue was shaken with water (50 ml) and dichloromethane (50 ml). The aqueous layer was separated and water was then removed under vacuum to give **4** as a purple amorphous solid (44.8 mg, 91%), UV (MeOH) λ_{max}/nm (log ε) 219.1 (4.37), 261.0 (4.23), 291.6 (4.14), 382.1 (3.57). IR (film) υ_{max} cm^{-1}: 2925, 1667, 1594, 1492, 1404, 1271, 1032, 985, 844. ^{1}H-NMR (CD_3OD) δ: 9.16 (1H, d, J = 6.0 Hz, H-5), 8.53 (1H, d, J = 6.0 Hz, H-4), 8.44 (1H, d, J = 8.4 Hz, H-11), 7.57 (1H, s, H-3), 7.44 (1H, d, J = 8.4 Hz, H-10), 6.64 (2H, s, OCH_2O), 4.85 (3H, s, NCH_3), 4.08 (3H, s, OCH_3), 4.04 (3H, s, OCH_3). ^{13}C-NMR (CD_3OD) δ: 178.4, 157.4, 154.5, 153.2, 150.6, 150.2, 141.1, 139.6, 126.4, 125.3, 124.1, 123.7, 123.1, 118.9, 109.2, 104.8, 102.8, 61.7, 56.4, 48.9. HRESIMS: *m/z* 350.0999 $[M]^+$ (calcd for $C_{20}H_{16}NO_5$, 350.1023).

3.3. Cytotoxicity bioassays

NCI-H187 (Human small cell lung carcinoma, ATCC CRL-5804) was determined by a MTT assay [25]. Briefly, the cells were diluted to 10^5 cells/ml. The test compounds were

diluted in distilled water and added to microtiter plates in a total volume of 100 µl. The plates were incubated at 37 °C, 5% CO_2 for 5 days. Fifty microliters of a 2 µg/µl MTT solution (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide; Thiazolyl blue) was added to each well of the plate. The plates were wrapped with aluminum foil and incubated for 4 h. After the incubation period, the microplates were spun down at 200 x g for 5 min. MTT was then removed from the wells and the formazan crystals were dissolved in 200 µl of 100% DMSO and 25 µl of Sorensen's glycine buffer. The OD was read in a microtiter plate reader at a wavelength of 570 nm. MCF7 (Human breast adenocarcinoma, ATCC HTB-22) and KB (Human carcinoma of the nasopharynx, ATCC CCL-17) were determined by a colorimetric cytotoxicity assay that measured the level of cell growth from the cellular protein content according to literature [24]. Ellipticin and doxorubicin were used as the positive controls. DMSO was used as the negative control. Briefly, the cells in a logarithmic growth phase were harvested and diluted to 10^5 cells/ml with fresh medium and mixed gently. The test compounds were diluted in distilled water and placed into microtiter plates in a total volume of 200 µl. The plates were incubated at 37 °C, 5% CO_2 for 72 h. After the incubation period, the cells were fixed by 50% trichloroacetic acid. The plates were incubated at 4 °C for 30 min, washed with tap water and air-dried at room temperature. The plates were stained with 0.05% sulforhodamine B (SRB) dissolved in 1% acetic acid for 30 min. After the staining period, SRB was removed with 1% acetic acid. The plates were air-dried before the bound dye was dissolved with 10 mM Tris base for 5 min on a shaker. The OD was read on a microtiter plate reader at the wavelength of 510 nm.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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