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# Chemical investigation of drug-like compounds from the Australian tree, *Neolitsea dealbata*

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ABSTRACT

Two of the four parameters in the 'rule of five', molecular weight and log *P*, which can be detected and predicted by mass spectrometry and compound retention on reversed-phase HPLC, were used as guidelines in natural product isolation. A new aporphine alkaloid, (6a*R*)-normecambroline (**1**), was isolated from the bark of *Neolitsea dealbata* (R. Br.) Merr. Its structure was determined on the basis of NMR, MS and CD analysis. It is the first time the absolute configuration of the roemerine-*N*-oxide was assigned for both roemerine-$N_{\alpha}$-oxide (**3**) and roemerine-$N_{\beta}$-oxide (**4**). Physico-chemical property evaluation demonstrated all alkaloids had no Lipinski violation. Compound **1** inhibited selectively against cervical cancer cells (HeLa) with an $IC_{50}$ of 4.0 μM.



Since the beginning of the 1990s, high throughput screening has emerged as an important lead identification method due to its ability to test large numbers of compounds efficiently.[1] However, Lipinski et al. [2] indicated that the solubility of compounds in aqueous media under thermodynamic equilibrating conditions in in vitro testing is a determinant which has an effect on the efficacy of biological assays in high throughput screening. They studied the solubility and permeability of drug candidates reaching the phase II clinical process, and proposed the 'rule of five' or Lipinski's rule as key predictors for oral bioavailability of a compound. According to this rule, an oral-acting drug-like molecule should have a molecular weight of <500 Da, an octanol/water partition coefficient (log *P*) of <5, <5 hydrogen bond donors (HBD) and <10 hydrogen bond acceptors (HBA).[2] If a compound fails two or more parameters, there is a high probability that the compound will have poor bioavailability. From this physico-chemical property analysis, attention in drug discovery shifted to the generation of smaller high-quality libraries using the drug-like properties as filters. More and more synthetic compound libraries based on natural product core structures have been developed for new screening campaigns in drug discovery.[3–5] Although combinatorial chemistry has the advantage of producing massive numbers of compounds, statistical analysis employed by Henkel[6] and Feher[7] showed that the physico-chemical properties of natural products and drugs are generally quite similar, and different to compounds from combinatorial synthesis. Moreover, possessing a diversity of chemical structures and a range of biological activities helps natural products become a potential source for the lead finding process.[8] Recently we have successfully generated a drug-like compound library[9] in which 85% of the isolated natural products had no Lipinski violation. The result showed that it is possible to address Lipinski's rule in front-loading a pure natural product library. Building on this initial success, the program of isolating drug-like natural products and screening for cytotoxicity is being pursued.

Mass spectroscopic data from an LC/MS analysis indicated that the bark of *Neolitsea dealbata* contains alkaloids with molecular weights around 300 Da. The elution of these compounds from a reversed-phase HPLC column with <70% methanol also reflected that they had log *P*'s <5,[10] satisfying Lipinski's rule. The genus *Neolitsea* encompasses more than 80 species in the world[11] many of which have been phytochemically studied, including *N. acuminatissima*,[12] *N. buisanensis*,[13] *N. cuipala*,[14] *N. parvigemma*,[15,16] *N. pubescens*,[17] *N. pulchella*,[18] *N. sericea*,[19] *N. villosa*,[20] *N. zeylanica*.[21] However, studies of the chemistry of *N. dealbata* are limited to the isolation of three triterpenoids (cycloneolitsin, taraxerone and taraxerol) and three sesquiterpenoids (linderadine, pseudoneolinderane and linderalactone).[22,23] Therefore, chemical investigation of this species is a high priority for the discovery of new bioactive secondary metabolites.

This Letter, reports the isolation and identification of 'drug-like' alkaloids from *N. dealbata* bark. A new aporphine alkaloid, (6a*R*)-normecambroline (**1**), was isolated, together with eight known alkaloids (Fig. 1). Their cytotoxicity was tested against a panel of human cell lines, including five cancer cell lines, lung adenocarcinoma cancer cells (A549), cervix adenocarcinoma cells (HeLa), breast cancer cells (MCF7), prostate cancer cells (PC3 and LNCaP) and two non-cancer cell lines, human embryonic kidney 293 (HEK 293) and neonatal foreskin fibroblast cells (NFF).

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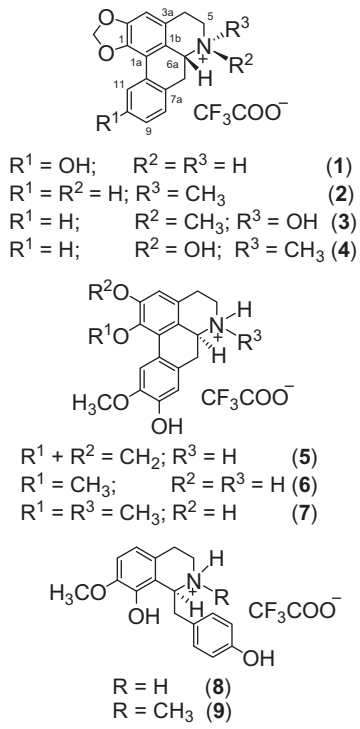


**Figure 1.** Chemical structures for natural products **1**–**9**.

The $CH_2Cl_2$/MeOH extract of the dried and ground bark of *N. dealbata* was initially chromatographed through polyamide gel. The resulting MeOH eluent was then loaded on $C_{18}$ bonded silica HPLC (MeOH/$H_2O$/0.1% TFA). Further purification was performed by pursuing the ion peaks of interest in (+)-LRESIMS at (+)-*m/z* 280, 282, 286, 296, 300, 312, 314 and 328. Nine alkaloids were isolated as their TFA salts. NMR analysis led to the characterisation of a new aporphine alkaloid, (6a*R*)-normecambroline (**1**), along with eight known alkaloids, (6a*R*)-roemerine (**2**),[24] (6*S*,6a*R*)-roemerine-$N_\alpha$-oxide (**3**), (6*R*,6a*R*)-roemerine-$N_\beta$-oxide (**4**), (6a*S*)-actinodaphnine (**5**),[25] (6a*S*)-laurolitsine (**6**),[26,27] (6a*S*)-boldine (**7**),[28] (1*S*)-norjuziphine (**8**)[29,30] and (1*S*)-juziphine (**9**).[30]

Compound **1** was isolated as a light brown amorphous solid. Its (+)-HRESIMS revealed a signal for $[M+H]^+$ at *m/z* 282.1135 (calcd 282.1125, $\Delta$3.5 ppm), corresponding to the molecular formula $C_{17}H_{15}NO_3$.[31] Its UV spectrum had intense absorptions at $\lambda$ 240, 265, 275 and 309 (shoulder) nm, which are characteristic of an aporphine skeleton.[32] Two geminal protons at $\delta$ 6.21 ppm (1H, d, $J$ = 1.2 *Hz*) and $\delta$ 6.06 ppm (1H, d, $J$ = 1.2 *Hz*) had HMBC correlations to C-1 ($\delta$ 142.5 ppm) and C-2 ($\delta$ 147.4 ppm), which was diagnostic for a methylenedioxy group at C-1 and C-2 in ring A of the aporphine system.[27] A singlet aromatic proton at $\delta$ 6.83 ppm was assigned to H-3 due to the HMBC correlations from H-3 to C-1, C-2 and C-4. The other three aromatic protons at $\delta$ 7.50 ppm (1H, d, $J$ = 2.4 Hz), $\delta$ 7.18 ppm (1H, d, $J$ = 7.8 Hz) and $\delta$ 6.72 ppm (1H, dd, $J$ = 8.4 and 2.4 Hz) had COSY correlations to each other suggesting these protons were in ring D of the aporphine skeleton. The low field proton at $\delta$ 7.50 ppm attached to carbon at $\delta$ 113.4 ppm exhibited the characteristic of the deshielded H-11.[27] The proton H-8 ($\delta$ 7.18 ppm) was assigned by the ROESY correlation between H-8 ($\delta$ 7.18 ppm) and H-7 ($\delta$ 3.00 ppm). The remaining aromatic proton signal at $\delta$ 6.72 ppm was assigned to H-9 in ring D. *J*-couplings of H-9 ($J$ = 8.4 and 2.4 Hz), H-8 ($J$ = 8.4 Hz) and H-11 ($J$ = 2.4 Hz) further supported this assignment. The location of quaternary carbons C-3a, C-7a, C-10, C-11a, C-1a and C-1b were deduced from the HMBC correlations. The lowest downfield resonance at C-10 ($\delta$ 156.4 ppm) and the upfield shifts of both C-9 ($\delta$ 115.0 ppm) and C-11 ($\delta$ 113.4 ppm) led to the assignment of a hydroxy group (broad signal at $\delta$ 9.53 ppm at C-10. The absolute stereochemistry at C-6a was determined by using the CD experiment. The observed maxima for a negative Cotton effect (CE) at 238 (−15.7) nm and two positive CEs at 275.0 (+4.6) and 207 (+7.7) nm (Fig. 2) as well as the measured optical rotation $[\alpha]_D^{25}$ of −284.4 (*c* 0.05, $C_2H_5OH$) indicated that compound **1** had an *R*-configuration at C-6a.[33]

Roemerine *N*-oxide was reported as a constituent of *Papaver glaucum*[34] and *Papaver gracile*[35] in the Papaveraceae family. Its structure was first suggested based on the comparison of its mass spectroscopic fragments with those of roemerine. Recently, roemerine *N*-oxide has been isolated from *Fissistigma acuminatissima* in the Annonaceae family and its structure was confirmed by NMR analysis.[36] However, absolute configurations of this compound have not been solved so far. Here two isomers of roemerine-*N*-oxides were isolated from *N. dealbata*. Their full absolute stereochemistries were determined for both roemerine-$N_\alpha$-oxide (**3**) and roemerine-$N_\beta$-oxide (**4**) using NMR and CD experiments.

Compound **3** was purified as a light yellow amorphous solid. The (+)-HRESIMS showed a signal for $[M+H]^+$ at *m/z* 296.1296 (calcd 296.1281, $\Delta$5.0 ppm).[31] Its $^1$H NMR spectrum was similar to that of roemerine (**2**) (Table 2). However, there was a notable downfield shift of both protons and carbon in an *N*-methyl group (from $\delta_H$ 3.07 ppm in roemerine to $\delta_H$ 3.72 ppm and from $\delta_C$ 40.6 ppm in roemerine to 54.7 ppm). The resonances of both protons and carbons at positions 5 and 6a shifted to lower field than those in roemerine ($\Delta\delta_{H5}$ = 0.33 and 0.62 ppm, $\Delta\delta_{H6}$ = 0.64 ppm, $\Delta\delta_{C5}$ = 11.1 ppm, $\Delta\delta_{C6}$ = 9.3 ppm). These differences could be due to the influence of the oxygen atom attached to the *N*-methyl group in an *N*-oxide aporphine derivative. This suggestion was supported by the 16 amu weight excess in the mass spectroscopic data. A sharp singlet signal at 12.45 ppm showed a HMBC correlation to C-6a suggesting this proton was in a hydroxy group bound directly to the nitrogen atom. Here the presence of this exchangeable proton was observed since trifluoroacetic acid (TFA) was used as a buffer in the HPLC resulting in the protonation of the *N*-oxide group. Compound **3**, therefore, was isolated as a TFA salt of roemerine-*N*-oxide. The observation of two positive CEs at 270 nm and 207 nm as well as a negative CE at 231 nm in the CD spectra (Fig. 2) indicated this compound had an *R*-configuration at C-6a.[33] A significant correlation was observed between H-6a and *N*-$CH_3$ indicating that an anti-arrangement must exist between H-6a and *N*-oxide. From this evidence, the absolute stereochemistry of this compound was assigned as (6*S*,6a*R*)-roemerine-$N_\alpha$-oxide.

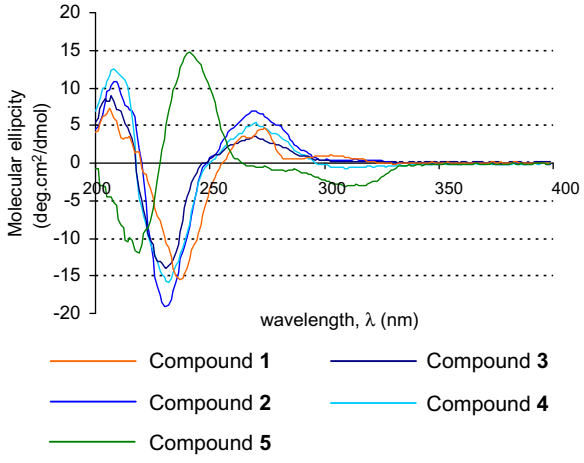


**Figure 2.** CD spectra of compounds **1**, **2**, **3**, **4** and **5**.

**Table 1**
NMR data for TFA salt of (6a*R*)-normecambroline (**1**) in DMSO-$d_6$ at 30 °C[a]

| Position | $\delta_C$[b] | $\delta_H$ (mult., *J*) | gCOSY (H No.) | ROESY (H No.) | gHMBC (C No.) |
|---|---|---|---|---|---|
| 1 | 142.5 | — | — | — | — |
| 2 | 147.4 | — | — | — | — |
| 3 | 107.4 | 6.83 (s) | — | 4 | 1, 1b, 2, 4, 1a |
| 4 | 24.7 | 3.07 (td. 12.0, 6.0) | 5 | 5 | 3a, 5, 1b |
| | | 2.90 (dd,16.8, 4.2) | 5 | 3, 5 | 1b, 3a, 3 |
| 5 | 40.3 | 3.62 (overlap $H_2O$) | 5b, 4 | 4 | 3a |
| | | 3.28 (overlap $H_2O$) | 5a | 4, 6a | 3a, 6a |
| 7 | 31.4 | 3.00 (dd, 14.4, 4.8) | 6a | 6a, 8 | 8, 11a, 1b, 6a |
| | | 2.80 (t, 14.4) | 6a | | 1b, 6a, 11a |
| 8 | 129.0 | 7.18 (d, 7.8) | 9 | 9, 7 | 10, 11a, 7 |
| 9 | 115.0 | 6.72 (dd, 8.4, 2.4) | 8, 11 | — | 7a, 10, 11 |
| 10 | 156.4 | — | — | — | — |
| 11 | 113.4 | 7.50 (d, 2.4) | 9 | - | 7a, 9, 10 |
| 3a | 124.3 | — | — | — | — |
| 6a | 51.9 | 4.38 (br t) | 7, NH | 5, 7 | — |
| 7a | 122.5 | — | — | — | — |
| 11a | 130.2 | — | — | — | — |
| 1a | 115.4 | — | — | — | — |
| 1b | 121.0 | — | — | — | — |
| –$OCH_2O$– | 101.0 | 6.21 (d, 1.2) | | — | 1, 2 |
| | | 6.06 (d, 1.2) | | — | 1, 2 |
| NH | — | 9.47 (d, 7.8) | 5, 6a | — | — |
| | | 9.02 (d, 10.2) | — | — | — |
| OH | — | 9.53 (br s) | — | — | — |

[a] $^1$H NMR at 600 MHz referenced to residual DMSO solvent ($\delta_H$ 2.5 ppm) and $^{13}$C NMR at 150 MHz referenced to DMSO ($\delta_C$ 39.5 ppm).
[b] $^{13}$C chemical shifts obtained from correlations observed in HSQC and gHMBC spectra.

**Table 2**
NMR data for TFA salt of compounds **2**, **3** and **4** in DMSO-$d_6$ at 30 °C[a]

| Position | (6a*R*)-Roemerine (**2**) | | (6*S*,6a*R*)-Roemerine-$N_\alpha$-oxide (**3**) | | (6*R*,6a*R*)-Roemerine-$N_\beta$-oxide (**4**) | |
|---|---|---|---|---|---|---|
| | $\delta_C$[b] | $\delta_H$ (mult., *J*) | $\delta_C$[b] | $\delta_H$ (mult., *J*) | $\delta_C$[b] | $\delta_H$ (mult., *J*) |
| 1 | 142.9 | — | 143.1 | — | 143.1 | — |
| 2 | 147.7 | — | 147.8 | — | 148.0 | — |
| 3 | 107.3 | 6.85 (s) | 107.2 | 6.88 (s) | 107.3 | 6.92 (s) |
| 4 | 25.3 | 3.22 (m) | 23.2 | 3.25 (overlap $H_2O$) | 25.7 | 3.30 (overlap $H_2O$) |
| | | 2.96 (dd, 17.5, 3.5) | | 3.01 (dd, 4.8, 18.0) | | 3.14 (dd, 4.8, 18.6) |
| 5 | 51.4 | 3.74 (dd, 12.0, 5.4) | 62.5 | 4.06 (td, 6.0, 12.6) | 64.1 | 4.11 (dd, 6.0, 11.4) |
| | | 3.40 (overlap $H_2O$) | | 4.02 (td, 4.8, 12.6) | | 4.07 (dd, 5.4, 13.2) |
| 7 | 30.0 | 3.55 (dd, 13.8, 4.2) | 27.6 | 3.58 (dd, 4.2, 14.4) | 27.5 | 3.44 (overlap $H_2O$) |
| | | 2.86 (t, 14.0) | | 3.13 (t, 14.4) | | 3.22 (t, 13.8) |
| 8 | 128.0 | 7.40 (d, 7.8) | 128.6 | 7.44 (d, 7.8) | 128.4 | 7.46 (d, 7.8) |
| 9 | 127.9 | 7.35 (t, 7.8) | 128.1 | 7.36 (td, 7.2, 1.2) | 128.2 | 7.35 (td, 7.8, 1.8) |
| 10 | 128.0 | 7.40 (t, 7.8) | 127.8 | 7.40 (t, 7.8) | 127.7 | 7.41 (t, 7.8) |
| 11 | 126.3 | 8.01 (d, 7.8) | 126.3 | 8.02 (d, 7.8) | 126.3 | 8.04 (d,7.8) |
| 3a | 124.4 | — | 123.5 | — | 122.2 | — |
| 6a | 60.5 | 4.43 (br s) | 69.8 | 5.08 (dd, 3.6, 13.8) | 71.7 | 5.09 (dd, 4.2, 14.4) |
| 7a | 132.5 | — | 131.9 | — | 131.7 | — |
| 11a | 132.5 | — | 131.4 | — | 130.9 | — |
| 1a | 115.2 | — | 115.6 | — | 115.3 | — |
| 1b | 121.1 | — | 119.3 | — | 119.6 | — |
| –$OCH_2O$– | 101.2 | 6.23 (s) | 101.1 | 6.24 (d, 0.6) | 101.4 | 6.24 (s) |
| | | 6.07 (s) | | 6.05 (d, 0.6) | | 6.05 (s) |
| *N*–$CH_3$ | 40.6 | 3.07 (d, 4.2) | 54.7 | 3.72 (s) | 46.9 | 3.39 (overlap $H_2O$) |
| NH | — | 10.65 (br s) | — | — | — | — |
| *N*–OH | — | — | — | 12.45 (s) | — | 12.87 (s) |

[a] $^1$H NMR at 600 MHz referenced to residual DMSO solvent ($\delta_H$ 2.5 ppm) and $^{13}$C NMR at 150 MHz referenced to DMSO ($\delta_C$ 39.5 ppm).
[b] $^{13}$C chemical shifts obtained from correlations observed in HSQC and gHMBC spectra.

Compound **4** was obtained as a light yellow amorphous solid. The (+)-HRESIMS showed a signal for $[M+H]^+$ at *m/z* 296.1295 (calcd 296.1281, $\Delta$4.7 ppm).[31] Compound **4** had the same molecular weight as compound **3** and its $^1$H NMR data were quite similar to those of **3**. Further NMR analysis revealed that there were small differences in chemical shifts of the neighbouring protons and carbons of the nitrogen atom in **4** and **3**, including the downfield shift of C-5 ($\Delta\delta_{C5}$ = 1.6 ppm) and H-5 ($\Delta\delta_{H5}$ = 0.05 ppm) as well as C-6 ($\Delta\delta_{C6}$ = 1.9 ppm) and H-6 ($\Delta\delta_{H6}$ = 0.01 ppm) and the upfield shift of *N*-$CH_3$ ($\Delta\delta_C$ = −7.8 ppm and $\Delta\delta_H$ = −0.33 ppm). All information was evident that **4** was isomeric to **3**. The *R*-configuration at C-6a was deduced since its CD spectrum was quite similar to that of **3** (Fig. 2). Due to the lack of a correlation between *N*-$CH_3$ and H-6a in the ROESY spectrum, roemerine-$N_\beta$-oxide was suggested for **4**. The absolute configuration of compound **4** was determined as (6*R*,6a*R*)-roemerine-$N_\beta$-oxide.

The identification of roemerine-*N*-oxides along with roemerine led to the hypothesis that these *N*-oxides could be artefacts of the parent compound since this plant was extracted in dichloromethane which can convert tertiary basic alkaloids to the *N*-oxide form.[37] To clarify this hypothesis, a fresh crude extract in methanol was prepared and subjected to LC/MS analysis instantly. The LC/MS

**Table 3**
In silico physico-chemical properties of **1**–**9** (in neutral forms)

| Compound | $M_W$ | *c* log *P* | HBA | HBD | PSA | NROT | Predicted bioavailability |
|---|---|---|---|---|---|---|---|
| **1** | 281.31 | 2.19 | 4 | 2 | 50.72 | 0 | ✓ |
| **2** | 279.33 | 3.32 | 3 | 0 | 21.70 | 0 | ✓ |
| **3** | 295.33 | 2.20 | 4 | 0 | 45.34 | 0 | ✓ |
| **4** | 295.33 | 2.20 | 4 | 0 | 45.34 | 0 | ✓ |
| **5** | 311.33 | 1.89 | 5 | 2 | 59.95 | 1 | ✓ |
| **6** | 313.35 | 1.63 | 5 | 3 | 70.95 | 2 | ✓ |
| **7** | 327.37 | 2.57 | 5 | 2 | 62.16 | 2 | ✓ |
| **8** | 285.34 | 2.53 | 4 | 3 | 61.72 | 3 | ✓ |
| **9** | 299.36 | 3.09 | 4 | 2 | 52.93 | 3 | ✓ |

data indicated that the ion peak (+) *m/z* 280 of roemerine eluted at 6.02 min while the peak (+) *m/z* 296 corresponding to roemerine-$N_\beta$-oxide and roemerine-$N_\alpha$-oxide eluted at 6.65 min and 6.93 min, respectively. This result confirmed these isolated roemerine-*N*-oxides were not artefacts produced by dichloromethane during the extraction.

The other known compounds (**5**–**9**) were characterised by comparison of their spectroscopic data (UV, NMR and MS) and optical rotations with the values in literatures.[24–30]

The four Lipinski properties of these compounds were evaluated using the Instant *J*-Chem 2.5.2 software (Table 3). It can be seen that molecular weights of these compounds range from 281 Da to 327 Da matching with the maximum molecular weight distribution of natural products reported by Henkel[3] and Feher[4] groups. The results of calculated log *P* and HBA are also consistent with Feher's analysis[4] in which log *P* of compounds from natural sources reached the maxima at 2–3 units while HBA distributed around 3–5 units. In comparison with Feher's report,[4] most isolated compounds have quite similar HBD to the majority of combinatorial synthetic products and increase noticeably in two donors as compared to the natural product database. However, all compounds comply with four properties of Lipinski's rule.

Besides the four parameters in the 'rule of five', Veber and co-workers[38] considered polar surface area (PSA) and number of rotatable bonds (NROT), as two more requirements for drug-like molecules. By observing the oral bioavailability in rat for over 1100 drug candidates, Veber's rule suggested that a compound which has PSA greater than 140 Å$^2$ and NROT greater than 10 will decrease the oral bioavailability. All isolated compounds (Table 3) also satisfy these two criteria with the PSA approximately distributing from 50 to 60 Å$^2$ and NROT ranging from 0 to 3 units. It is interesting that all these compounds not only have physico-chemical properties of drug-like molecules but also satisfy the requirements for lead-like compounds, including $M_W \leqslant 460$, $-4 \leqslant \log P \leqslant 4.2$, HBA $\leqslant 9$, HBD $\leqslant 5$ and NROT $\leqslant 10$.[39]

Cytotoxic screening was carried out on a panel of human cancer cell lines, including lung adenocarcinoma cancer cells (A549), cervical adenocarcinoma cells (HeLa), breast cancer cells (MCF-7) and prostate cancer cells (LNCaP and PC3) as well as two human non-cancer cell lines, human embryonic kidney 293 (HEK 293) and neonatal foreskin fibroblast cells (NFF). Although cytotoxic activities of compounds **2** and **5** have been reported,[24,25,40] their activities have not been evaluated against these cell lines previously. The screening result (Table 4) showed that only three compounds (**1**, **2** and **5**) out of the nine isolated alkaloids were active. The new compound (**1**) demonstrated the highest potency against HeLa cells ($IC_{50}$ of 4.0 μM) and was less active against the four other cancer cell lines with $IC_{50}$ values ranging from 13.8 to 28.2 μM. Interestingly, it had selective cytotoxicity against HeLa cells with approximately 10-fold higher potency than the two non-cancer cells HEK 293 and NFF. In comparison with **1**, **2** was less active against the cervical cancer cells (HeLa) with an $IC_{50}$ of 16.6 μM; but exhibited the most significant activity against lung cancer cells (A549) with an $IC_{50}$ of 3.4 μM which was eightfold more potent compared to **1**. Among the three active compounds, **5** showed moderate activities on the growth of the five cancer cell lines. However, it was selective against cancer cells due to having less inhibitory effect on the proliferation of non-cancer cells (HEK 293 and NFF). This compound has been reported to have other biological activities, including antibacterial and antifungal,[41] antiparasite,[28] antiplatelet.[42] Notably, when the 1,2-methylenedioxy substituent was absent from the structures of compounds **6** and **7**, there was no inhibition. This result again confirmed that the 1,2-methylenedioxy functional group was required for the expression of cytotoxicity of the aporphine alkaloids as suggested by Cordell and co-workers.[43] However, compounds **3** and **4** had no cytotoxicity although their structures contain the 1,2-methylenedioxy group and their parent compound, roemerine (**2**) demonstrated general cytotoxic activities against all cell lines tested. This phenomenon was also found previously in some biologically active compounds, such as codeine, ethylmorphine, morphine and thebaine of which the *N*-oxides derivatives were less toxic than the parent compounds.[44] From the physico-chemical properties (Table 3) the *N*-oxide analogues (compounds **3** and **4**) would be expected to have similar capacity to enter the cell as compounds **1** and **2**. The *N*-oxides may be involved in detoxification mechanisms or preventing DNA binding.

So far the knowledge about mechanism of aporphine alkaloids against cancer cells is limited with studies pointing to cell cycle arrest, DNA damage and inhibition of topoisomerase II as the cause of cytotoxicity.[45–47] As the result, the activity is usually greater in cell lines, which are faster growing. Results from the cytotoxicity studies here confirm this with the cancer cell lines HeLa and A549 being more susceptible than the slower growing NFF and HEK cells.

**Table 4**
Evaluation of cytotoxic potential of compounds **1**–**9**

| Compound | $IC_{50}$ (±SD) (μM) or % inhibition (±SD)[a] | | | | | | |
|---|---|---|---|---|---|---|---|
| | A549 | HeLa | MCF7 | LNCaP | PC3 | HEK | NFF |
| **1** | 28.2(±5.5) | 4.0(±0.5) | 27.4(±6.1) | 13.8(±3.1) | 23.0(±4.0) | 59.7(±2.2) | 42.6(±6.8) |
| **2** | 3.4(±0.3) | 16.6(±3.5) | 17.7(±4.8) | 12.7(±2.9) | 7.7(±1.4) | 15.9(±4.7) | 15.2(±2.9) |
| **3** | >100 | >100 | >100 | >100 | >100 | 61(±10)% [b] | 83(±14)% |
| **4** | >100 | >100 | >100 | >100 | >100 | 49(±6)% | 66(±9)% |
| **5** | 16.0(±4.9) | 28.1(±3.5) | 52(±9.2)% | 27.4(±5.1) | 36.4(±1.0) | 55(±10)% | >100 |
| **6** | >100 | >100 | >100 | >100 | >100 | >100 | >100 |
| **7** | >100 | >100 | >100 | >100 | >100 | >100 | >100 |
| **8** | >100 | >100 | >100 | >100 | >100 | >100 | >100 |
| **9** | >100 | >100 | >100 | >100 | >100 | >100 | >100 |
| Paclitaxel | 0.0038(±0.0008) | 0.0089(±0.0006) | 0.0044(±0.0016) | | | 0.0039(±0.0005) | 0.0047(±0.0010) |
| Vincristine sulphate | | | | 0.0029(±0.0006) | 0.0110(±0.0020) | | |

[a] Each $IC_{50}$ was determined as the mean ± SD of two independent experiments with triplicate determinations for each concentration.
[b] The inhibition was calculated at the concentration of 100 μM.

Typically it has been considered that the mechanism of action results from the intercalation of the molecule into DNA due to the compound planar structure.[40] However, here we have seen that the twisted biphenyl skeleton of aporphines supported by the typical intense CE from 230 to 240 nm in CD spectra[33] produces cytotoxicity and the lack of the 1,2-methylenedioxy group or the inclusion of *N*-oxide into the molecule cause the loss of activity. How these factors influence the cytotoxicity is something that warrants further investigation.

In conclusion, mass-guided identification of the pseudo-molecular ions at (+) *m/z* 280, 282, 286, 296, 300, 312, 314 and 328 from the $CH_2Cl_2$/MeOH extracts of *N. dealbata* bark led to the isolation of the new aporphine alkaloid (**1**) along with the eight known alkaloids (**2**–**9**). All compounds passed the 'rule of five' for oral bioavailability. The cytotoxic evaluation against a panel of seven human cell lines showed that normecambroline (**1**), roemerine (**3**) and actinodaphnine (**5**) were active. The new alkaloid (**1**) was selective against HeLa cells with an $IC_{50}$ of 4.0 μM compared with those of the two non-cancer cells, HEK 293 ($IC_{50}$ of 59.7 μM) and NFF ($IC_{50}$ of 42.6 μM).

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## Supplementary data

Supplementary data ($^1$H NMR and 2D NMR spectra for 6a*R*-normecambroline (**1**), $^1$H NMR spectra for roemerine (**2**), roemerine-$N_\alpha$-oxide (**3**) and roemerine-$N_\beta$-oxide (**4**), general experimental details, collection and identification of plant material, extraction and isolation procedures and cytotoxicity assay) associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2010.07.100.

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31. (a) *Compound* **1***:* light brown amorphous solid (1.0 mg, 0.01%); $[\alpha]_D^{25}$ −284.4 (*c* 0.05, $C_2H_5OH$); CD (MeOH) $\lambda_{max}$ ($\Delta\varepsilon$) 275.0 (5.5), 238 (−18.8), 207 (9.2) nm; UV (MeOH) $\lambda_{max}$ (log $\varepsilon$) 309 (4.6), 275 (4.7), 265 (sh) (4.7), 240 (4.7) nm; IR (film) $\nu_{max}$ 3386, 2925, 1679, 1460, 1203; (+)-HRESIMS *m/z* 282.1135 ([M+H]$^+$) (calcd 282.1125, $\Delta$3.5 ppm); $^1$H (600 MHz) and $^{13}$C (150 MHz) NMR data are summarized in Table 1. (b) *Compound* **2***:* light yellow amorphous solid; $[\alpha]_D^{25}$ −43.1 (*c* 0.10, $C_2H_5OH$) [lit. $[\alpha]_D^{25}$ −54.0 (c 0.10, $C_2H_5OH$)][24]; CD (MeOH) $\lambda_{max}$ ($\Delta\varepsilon$) 270 (7.2), 231 (−20.6) and 208 (13.1) nm; (+)-LRESIMS *m/z* 280 ([M+H]$^+$); $^1$H (600 MHz) and $^{13}$C (150 MHz) NMR data are summarized in Table 2; (c) *Compound* **3***:* light yellow amorphous solid; $[\alpha]_D^{25}$ −13.9 (*c* 0.04, $C_2H_5OH$); CD (MeOH) $\lambda_{max}$ ($\Delta\varepsilon$) 270 (5.9), 231 (−18.4) and 207 (12.5) nm; (+)-HRESIMS *m/z* 296.1296 ([M+H]$^+$) (calcd 296.1281, $\Delta$5.0 ppm); $^1$H (600 MHz) and $^{13}$C (150 MHz) NMR data are summarized in Table 2; *Compound* **4***:* light yellow amorphous solid; $[\alpha]_D^{25}$ −17.4 (*c* 0.04, $C_2H_5OH$); CD (MeOH) $\lambda_{max}$ ($\Delta\varepsilon$) 271 (6.8), 232 (−19.8) and 208 (15.9) nm; (+)-HRESIMS *m/z* 296.1295 ([M+H]$^+$) (calcd 296.1281, $\Delta$4.7 ppm); $^1$H (600 MHz) and $^{13}$C (150 MHz) NMR data are summarized in Table 2.
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