Natural indole butyrylcholinesterase inhibitors from *Nauclea officinalis*

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**Abstract**

Nine monoterpenoid indole alkaloids; naucletine (1), angustidine (2), nauclefine (3), angustine (4), naucleine (5), angustoline (6), harmane (7), 3,14-dihydroangustoline (8), strictosamide (9) and one quinoline alkaloid glycoside; pumiloside (10) from *Nauclea officinalis* were tested for cholinesterase inhibitory activity. All the alkaloids except for pumiloside (10) showed strong to weak BChE inhibitory effect with IC\(_{50}\) values ranging between 1.02–168.55 \(\mu\)M. Angustidine (2), nauclefine (3), angustine (4), angustoline (6) and harmane (7) showed higher BChE inhibiting potency compared to galanthamine. Angustidine (2) was the most potent inhibitor towards both AChE and BChE. Molecular docking (MD) studies showed that angustidine (2) docked deep into the bottom gorge of AChE and formed hydrogen bonding with Ser 198 and His 438. Kinetic study of angustidine (2) on BChE suggested a mixed inhibition mode with an inhibition constant \((K_i)\) of 6.12 \(\mu\)M.

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**Article Info**

Article history:
Received 23 June 2014
Revised 12 September 2014
Accepted 12 November 2014
Available online xxx

**Keywords:**
*Nauclea officinalis*
Angustidine
Cholinesterase
Molecular docking
Kinetic study

**Abbreviations**

BChE - butyrylcholinesterase
AChE - acetylcholinesterase
MD - molecular docking
*N. officinalis* - *Nauclea officinalis*
AD - Alzheimer’s disease
ACh - acetylcholine
ChEIs - cholinesterase inhibitors
CH\(_2\)Cl\(_2\) - dichloromethane
MeOH - methanol
DEB - dichloromethane extract of bark
DEL - dichloromethane extract of leaves
MEL - methanol extract of leaves
HPLC - high performance liquid chromatography
PTLC - preparative thin layer chromatography
TeAChE - *Torpedo californica* acetylcholinesterase
hBChE - human butyrylcholinesterase
LB - Lineweaver–Burk
\(K_i\) - inhibition constant

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http://dx.doi.org/10.1016/j.phymed.2014.11.003
0944-7113/© 2014 Published by Elsevier GmbH.

Please cite this article as: S.Y. Liew et al., Natural indole butyrylcholinesterase inhibitors from *Nauclea officinalis*, Phytomedicine (2014), http://dx.doi.org/10.1016/j.phymed.2014.11.003
(angustidine 2) and BChE (angustidine 2 and nauclefine 3) by MD, together with the kinetic studies of the most potent compound (angustidine 2) were also communicated.

49 Materials and methods

50 General procedures and plant materials

51 The general procedures were same as previously described (Liew et al. 2012). The plant materials of N. officinalis were collected at Hutan Simpan Madek, Keluang, Johor, Malaysia. The voucher specimens (KL 5655) of these plants were deposited at the Herbarium of the Department of Chemistry, University of Malaya, Kuala Lumpur, Malaysia.

52 Extraction and isolation

53 Compounds from dichloromethane extract of the bark (DEB) (2.0 kg) of N. officinalis were isolated as previously described (Liew et al. 2012). The extraction procedures for the leaves (1.9 kg) were same as the bark but with the additional acid-base extraction to obtain dichloromethane extract of the leaves (DEL). Methanol extract of the leaves (MEL) was subjected to HPLC for purification of polar compounds.

54 Purification of compounds

55 DEB gave compounds 1–6. Purification of fractions 6 and 7 by preparative thin layer chromatography (PTLC) yielded 1 (118.8 mg, CH₂Cl₂:MeOH; 99:1) and 4 (15.2 mg, CH₂Cl₂:MeOH; 98:2) respectively. Both compounds 2 (5.5 mg, CH₂Cl₂:MeOH; 98:2) and 3 (8.5 mg, CH₂Cl₂:MeOH; 98:2) were obtained after purification by PTLC from fraction 12 while 5 (12.8 mg, CH₂Cl₂:MeOH; 97:2) and 6 (4.6 mg, CH₂Cl₂:MeOH; 97:3) were obtained from fractions 14 and 15, respectively. DEL yielded 7 (3.2 mg, CH₂Cl₂:MeOH; 96:4) and 8 (2.3 mg, CH₂Cl₂:MeOH; 95:5) by purification of fractions 10 and 12 respectively using PTLC.

56 Purification of MEL by HPLC was carried out as follows: A Waters HPLC system equipped with a C18 reversed phase column (10 mm × 150 mm × 5 μM) was employed with a mobile phase flow-rate of 3 ml/min. Gradient elution system: water + formic acid (A) and methanol + formic acid (B) as the mobile phase: 0–2 min, 30–30% B; 2–32 min, 30–100% B; 32–35 min, 100–100% B; 35–37 min, 100–30% B; 37–40 min 30–30% B. Injection volume was 200 μl and the UV spectrum range was 210–400 nm. The respective retention times of 10 and 9 were 17.22 min and 23.64 min.

57 Chemicals and enzymes

58 All the chemicals and enzymes used were same as described by Jamila et al. (2014).

59 In vitro cholinesterase inhibitory assay and MD studies

60 Cholinesterase inhibitory activity and MD studies were carried out as described by Jamila et al. (2014).

61 BChE kinetic study

62 Kinetic studies of BChE inhibition was determined by constructing Lineweaver–Burk (LB) plots; reciprocal plots of 1/V versus 1/[S] at different concentrations of substrate S-butyrylthiocholine chloride (1.75–14.00 mM) in the absence and presence of two different concentrations of inhibitors (33.2 and 66.4 μM). The Kᵢ value was estimated from the replots of the slope of the individual LB plots versus the inhibitor concentrations.

Results and discussion

Cholinesterase inhibition studies

DEB and MEL from N. officinalis showed moderate BChE inhibition; IC₅₀ values of 97.33 ± 5.07 μg/ml and 132 ± 12.7 μg/ml, respectively. Therefore, the phytochemicals from both the extracts were isolated and tested for both BChE and AChE. The determination of cholinesterase inhibition of AChE and BChE were evaluated according to colorimetric Ellman’s method (Ellman et al. 1961). Compounds 1–6 were obtained from DEB while compounds 7 and 8 were isolated from DEL. Compounds 9 and 10 were obtained from MEL. All of the alkaloids except for pumiloside (10) displayed strong to weak BChE inhibitory effect with the IC₅₀ values ranging between 1.02 and 168.55 μM while three compounds (angustidine 2, angustoline 6 and pumiloside 10) showed moderate to weak AChE inhibition with IC₅₀ values between 21.71 and 261.89 μM (Table 1). Interestingly, angustidine (2), angustine (4), nauclefine (3), angustoline (6) and harmarine (7) were more potent BChE inhibitors compared to galanthamine. Angustidine (2) (Fig. 1) was found to be the most potent inhibitor of both AChE and BChE with a higher selectivity towards BChE (selectivity index = 21.09).

Despite being 23 times less potent than galanthamine against AChE, upon a molar basis comparison, angustidine (2) was 28 times more potent as an inhibitor of BChE compared to galanthamine. Both enzymes complement one another for their role in cholinergic neurotransmission. Based on recent studies, it was postulated that progressive decline in activity of AChE in certain brain regions during the progression of AD, is replaced by progressive increase in BChE activity, which may act as a compensatory mechanism for ACh hydrolysis (Çokuğraş 2003). Owing to the growing importance of BChE, an agent that inhibits BChE would be beneficial especially during the later stages of AD.

The alkaloids isolated in the present study showed comparable inhibitory activity with previously reported alkaloids (Ahmed et al. 2013), however these isolated alkaloids were more BChE selective. To the knowledge of the authors, this is the first report on the BChE inhibitory activity, which may act as a compensatory mechanism for ACh hydrolysis (Çokuğraş 2003). Owing to the growing importance of BChE, the last being the most potent. 135

136 MD of angustidine (2) and nauclefine (3)

137 In order to understand the binding mechanisms of these compounds with cholinesterase enzymes, MD studies were performed on the most potent inhibitor; angustidine (2) and nauclefine (3). MD of angustine (4) was not performed as it was reported before (Passos et al. 2013). The findings indicated that angustidine (2) docked deep into the bottom gorge of hBChE, forming hydrogen bonds with Ser 198 and His 438 (Table 2). The hydrogen bonds formed with the amino acid residues at the catalytic site could be responsible for the potency of angustidine (2) as a BChE inhibitor. On the other hand, angustidine (2) was well accommodated, forming π–π stacking interaction with
Table 1
Cholinesterase inhibitory activities of alkaloids from *N. officinalis*.

<table>
<thead>
<tr>
<th>Compounds</th>
<th>% inhibition at 100 μg/ml</th>
<th>IC50 AChE μg/ml</th>
<th>IC50 BChE μM</th>
<th>Selectivity</th>
<th>IC50 AChE μg/ml</th>
<th>IC50 BChE μM</th>
<th>Selectivity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>AChE</td>
<td>BChE</td>
<td>AChE</td>
<td>BChE</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Naucletine (1)</td>
<td>34.19 ± 7.06</td>
<td>62.02 ± 1.37</td>
<td>ND</td>
<td>ND</td>
<td>20.78 ± 3.06</td>
<td>63.14</td>
<td>–</td>
</tr>
<tr>
<td>Angustidine (2)</td>
<td>82.62 ± 2.35</td>
<td>95.88 ± 0.50</td>
<td>6.54 ± 0.37</td>
<td>21.72</td>
<td>0.31 ± 0.07</td>
<td>1.03</td>
<td>0.05</td>
</tr>
<tr>
<td>Nauclefine (3)</td>
<td>34.61 ± 4.84</td>
<td>75.31 ± 16.61</td>
<td>ND</td>
<td>ND</td>
<td>2.21 ± 0.03</td>
<td>7.70</td>
<td>–</td>
</tr>
<tr>
<td>Angustine (4)</td>
<td>40.19 ± 0.65</td>
<td>83.97 ± 1.35</td>
<td>ND</td>
<td>ND</td>
<td>1.56 ± 0.05</td>
<td>4.98</td>
<td>–</td>
</tr>
<tr>
<td>Naucleine (5)</td>
<td>35.27 ± 4.74</td>
<td>82.47 ± 1.10</td>
<td>ND</td>
<td>ND</td>
<td>12.17 ± 2.23</td>
<td>38.25</td>
<td>–</td>
</tr>
<tr>
<td>Angustoline (6)</td>
<td>77.53 ± 4.40</td>
<td>82.5 ± 0.67</td>
<td>86.72 ± 5.41</td>
<td>261.89</td>
<td>8.31 ± 1.25</td>
<td>25.10</td>
<td>0.10</td>
</tr>
<tr>
<td>Harmane (7)</td>
<td>58.42 ± 4.98</td>
<td>96.72 ± 1.53</td>
<td>54.75 ± 0.88</td>
<td>300.68</td>
<td>2.4 ± 0.13</td>
<td>13.18</td>
<td>0.04</td>
</tr>
<tr>
<td>3,14-Dihydroangustoline (8)</td>
<td>38.55 ± 5.94</td>
<td>72.82 ± 1.60</td>
<td>ND</td>
<td>ND</td>
<td>16.58 ± 1.35</td>
<td>49.77</td>
<td>–</td>
</tr>
<tr>
<td>Strictosamide (9)</td>
<td>34.76 ± 3.27</td>
<td>56.01 ± 0.93</td>
<td>ND</td>
<td>ND</td>
<td>83.97 ± 0.61</td>
<td>168.54</td>
<td>–</td>
</tr>
<tr>
<td>Pumiloside (10)</td>
<td>54.96 ± 12.15</td>
<td>17.38 ± 5.47</td>
<td>60.62 ± 4.98</td>
<td>118.36</td>
<td>8.12 ± 0.61</td>
<td>28.29</td>
<td>30.10</td>
</tr>
<tr>
<td>Galanthamine (standard)</td>
<td>–</td>
<td>–</td>
<td>0.27 ± 0.07</td>
<td>0.94</td>
<td>2.02 ± 0.13</td>
<td>6.68</td>
<td>0.04</td>
</tr>
</tbody>
</table>

Data presented as mean ± SD (n = 3). ND = not determined.

a Selectivity for AChE is defined as IC50(BChE)/IC50(AChE).
b Selectivity for BChE is defined as IC50(AChE)/IC50(BChE).

Table 2
Binding interaction data for bioactive alkaloids from *N. officinalis* docked into active site gorge of AChE and BChE.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Enzyme</th>
<th>Binding energy (kcal)</th>
<th>Interacting site</th>
<th>Residue Type of interaction</th>
<th>Distance (Å)</th>
<th>Ligand interacting</th>
</tr>
</thead>
<tbody>
<tr>
<td>Angustidine (2)</td>
<td>hBChE</td>
<td>−10.14</td>
<td>CBS</td>
<td>Trp 84 Hydrophobic</td>
<td>3.47</td>
<td>Aromatic ring</td>
</tr>
<tr>
<td></td>
<td>TcAChE</td>
<td>−11.53</td>
<td>CS</td>
<td>Ser 198 Hydrogen</td>
<td>1.83</td>
<td>C-19</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>His 438 Hydrophobic</td>
<td>2.76</td>
<td>C-19</td>
</tr>
<tr>
<td>Nauclefine (3)</td>
<td>hBChE</td>
<td>−10.15</td>
<td>CS</td>
<td>His 438 Hydrophobic</td>
<td>3.44</td>
<td>Aromatic ring</td>
</tr>
<tr>
<td></td>
<td>TcAChE</td>
<td>−11.53</td>
<td>CS</td>
<td>His 438 Hydrophobic</td>
<td>4.09</td>
<td>Aromatic ring</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Gly 116 Hydrogen</td>
<td>5.21</td>
<td>Aromatic ring</td>
</tr>
</tbody>
</table>

* Choline binding site.

** Catalytic site.

*** Oxyanion hole.

Fig. 2. Binding interaction of angustidine (2) with active site residue (a) hBChE and (b) TcAChE.

Trp 84 at the choline binding site, which anchored this compound to the TcAChE active site gorge (Fig. 2). Meanwhile, nauclefine (3) formed hydrogen bond with Gly 116 at the oxyanionic hole and π–π interactions were observed between His 438 of hBChE and the aromatic ring of nauclefine (3). Since the active site of BChE has many of its channel-lining aromatic residues replaced by residues with aliphatic side chains, it is able to accommodate bulkier compounds compared to AChE. Due to space availability, both angustidine (2) and nauclefine (3) were able to accommodate and docked completely into the base of the active site and was held in place by the hydrogen bond. In contrast, please cite this article as: S.Y. Liew et al., Natural indole butyrylcholinesterase inhibitors from *Nauclea officinalis*, *Phytomedicine* (2014), http://dx.doi.org/10.1016/j.phymed.2014.11.003.
Fig. 3. Lineweaver–Burk (LB) plot of BChE activity over a range of substrate concentration (1.75–14.0 μM) for angustidine (2). The K_i value (6.12 μM) for BChE inhibition by angustidine (2) was estimated by plotting the slopes of LB plots versus inhibitor concentrations.

Passos et al. (2013) reported that angustine (4) was mainly stabilized by hydrophobic interactions involving its aromatic moieties with Trp82, Trp 231, Leu 286 and Phe 329 residues of BChE.

Enzyme kinetic study was performed for the most potent inhibitor; angustidine (2) on BChE. Graphical analysis of the Lineweaver–Burk (LB) plot constructed for BChE suggested a mixed type inhibition (Fig. 3) with K_i value of 6.12 μM.

Conflict of interest

No conflict to disclose.

Acknowledgement

This work was supported by University of Malaya research grants (UM.C/625/1/HIR/SC37 and PV050/2012A) and Centre National de la Recherche Scientifique (CNRS) grant (57-02-03-1007). The authors thank D.M. Nor and R. Syamsir for collection of plant material and T. L. Eng for botanical identification. The plant collection was carried out within the framework of an official agreement between the CNRS and the University of Malaya (Malaysia).

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