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Synthesis of tricyclic analogs of stephaoxocanidine and their evaluation as acetylcholinesterase inhibitors

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Abstract—The synthesis of simplified analogs of the novel isoquinoline alkaloid stephaoxocanidine, carrying the oxazaphenalene ABC-ring system of the natural product, and their activity as inhibitors of the enzyme acetylcholinesterase, are reported. 5,6-Dimethoxy-7*H* -8-oxa-1-aza-phenalen-9-one (**5**) was as active as a *Narcissus* extract enriched in galantamine.

The critical role of acetylcholine in cognitive function and the fact that cholinergic stimulation enhances performance of cognitive tasks in man and animals,[1] suggested that therapy with cholinomimetic agents may improve cognitive and memory deficits observed in Alzheimer's disease. Accordingly, to date cholinesterase inhibitors are the only class of compounds with proven efficacy in the treatment of the cognitive and functional symptoms of this disease, and became the cornerstone of its therapy.[2]

Galantamine (**1**), a natural benzazepine alkaloid,[3] and tacrine (**2**), a synthetic quinoline derivative, are among the first four medications approved by the FDA for the symptomatic treatment of mild to moderate Alzheimer's disease.

The stephaoxocanes (Fig. 1) are a small family of isoquinoline alkaloids recently uncovered by Japanese,[4] Chinese[5] and Brazilian [6] scientists, which share the tetracyclic skeleton **4a**. To date, only five members are known: stephaoxocanidine (**4b**) and stephaoxocanine (**4f**) isolated from *Stephania cepharantha* Hayata,[4] excentricine (**4d**) and *N*-methylexcentricine (**4e**), from

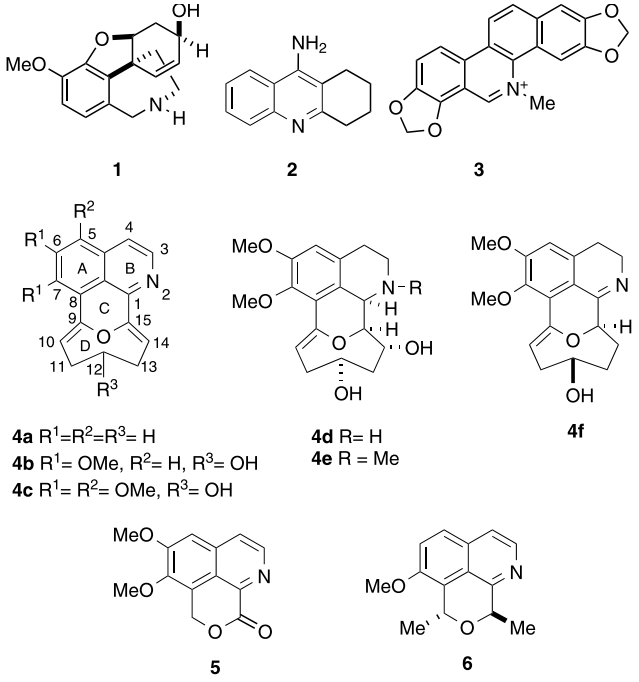

Figure 1.

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S. excentrica[5] and eletefine (**4c**) isolated from *Cissampelos pareira*.[6] These are Menispermaceae which have long found use in folk medicine.

The roots of *Cissampelos* species are widely used in indigenous and popular medical systems to cure heart, genital and urinary illnesses as well as respiratory diseases such as cold and asthma,[7] while *S. cepharantha* Hayata has been used in Chinese medicine for the treatment of diseases such as parotiditis, gastric ulcer and leukopenia.[8]

The genus *Stephania* is prolific in bioactive compounds. *S. cepharantha* has been recorded to produce cepharanthine and cycleanine, with activity on acetylcholine receptors.[9] Recently, interesting acetylcholinesterase inhibitory activity was found in *S. suberosa* Forman extracts, employed in Thai traditional neurotonic and rejuvenating medicine,[10] while *Stephania rotunda* has been used in Oriental medicine as treatment for dysautonomia (abnormal functioning of the autonomic nervous system). Furthermore, root extracts of *S. venosa*, a Thailand prescription for memory improvement in elderly, strongly inhibited acetylcholinesterase (90% inhibition with a 0.1 mg/ml extract)[11] and bisbenzyl-isoquinolines from *Stephania tetrandra* have also shown acetylcholinesterase inhibitory properties.[12]

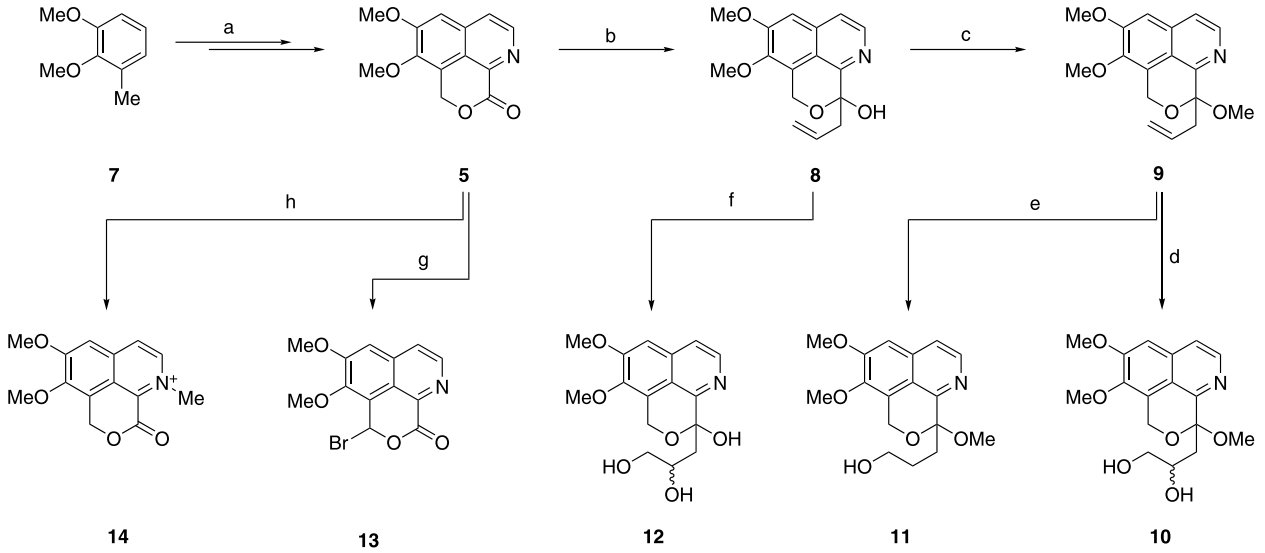
Interestingly, besides galantamine other alkaloids such as isoquinoline derivatives from Amarillidaceae[13] as well as protoberberines,[14c] and quaternary benzophenanthridine and isoquinoline alkaloids[14] including sanguinarine (**2**)[14b–d] and *N*-alkyl carneginium salts, have been shown to display acetylcholinesterase inhibitory activity.[15]

In retrospect, the use of natural products as templates has been the single most successful strategy in the discovery of novel medicines and in recent years the use of traditional medicine information on drug development has received considerable interest.[16] The chemistry and biological activity of the stephaoxocanes is an unexplored area; thus, we have developed two different approaches for the elaboration of the ABC ring system of stephaoxocanidine[17] and prepared compounds **5** and **6**.

Herein, we report the synthesis of tricyclic simplified analogs of stephaoxocanidine, some of which bear functionalized alkyl chains in place of its oxocane ring D, and their in vitro activity as inhibitors of the enzyme acetylcholinesterase. The synthesis was straightforward starting from the known oxazaphenalene lactone **5**, easily available from 2,3-dimethoxy toluene (**7**). Addition of allylmagnesium bromide at −20 °C furnished 85% of hemiacetal **8**, which was treated with trimethyl orthoformate under tosic acid catalysis, furnishing the corresponding methyl acetal **9** in 83% yield (see Scheme 1).

The use of CH_2Cl_2 at −20 °C as reaction condition for Grignard addition to **5** is remarkably unusual; nevertheless, this is a result of the poor solubility of lactone **5** in THF and Et_2O as well as in aromatics such as toluene, which prevented their use as solvents in this transformation. The lactone was only sparingly soluble in CH_2Cl_2 at −20 °C and the reaction did not proceed at temperatures below −35 °C due to its insolubility. Interestingly, however, yields of addition product were high in spite of the use of more than one equivalent of Grignard reagent, probably due to the insolubility of the resulting alkoxide in the reaction medium, while running the reaction at temperatures above −10 °C drastically reduced product yields.

Catalytic dihydroxylation of **8** and **9** furnished highly polar diols **10** and **12** in moderate to good yields, without spiroketalized products,[18] while exposure of **8** to an



Scheme 1. Reagents and conditions: (a) See Refs. 17b,c; (b) $CH_2{=}CHCH_2MgBr$, CH_2Cl_2, −20 °C (85%); (c) $HC(OMe)_3$, TsOH (cat.), MeOH–$CHCl_3$, rt, overnight (83%); (d) OsO_4 (cat.), NMO (1.25 equiv), acetone–H_2O (1:2, v/v), 0 °C → rt, overnight (74%); (e) (1) BH_3·THF, THF, 0 °C, (2) PCC/Al_2O_3, CH_2Cl_2, rt, (3) $NaBH_4$, MeOH, 0 °C (25%) or $AlCl_3$, $NaBH_4$ (27%); (f) OsO_4 (cat.), NMO (1.25 equiv), acetone–H_2O (1:2, v/v), 0 °C → rt, overnight (53%); (g) MeI, MeCN, reflux, 3 h (100%); (h) NBS, AIBN (cat.), CCl_4, reflux, 2 h (47%).

hydroboration with BH_3. THF in THF followed by solvent change and oxidation with pyridinium chlorochromate supported on alumina and in situ reduction of the resultant aldehyde with sodium borohydride in MeOH furnished **11**, albeit in only 25% yield. Unfortunately, submission of the starting allyl derivative **9** to the aluminum chloride–sodium borohydride reagent[19] did not meet with better success, providing **11** in meager 27% yield.

On the other hand, radical bromination of oxazaphenalenone **5** with NBS in refluxing carbon tetrachloride to which catalytic amounts of AIBN were added, gave 47% of bromo derivative **13** and quaternarization of the starting oxazaphenalenone with methyl iodide in refluxing acetonitrile furnished quantitative yield of methyl isoquinolinium iodide **14**.[20,21]

The thus synthesized simplified analogs of stephaoxocanidine were submitted to evaluation of their ability to inhibit acetylcholinesterase[22] by the method of Ellman (modified),[23] with the results collected in Table 1.

It was observed that lactone **5** exhibited an IC_{50} of 19.6 μM, a remarkable activity which is comparable to that of a *Narcissus* extract enriched in galantamine; however, the activity diminished to the half in 8-bromo derivative **13** and it was less than a quarter of the original in quaternary isoquinolinium compound **14**.

Compounds **8**–**12** are analogs in which their side chains represent part of the oxocane-ring of the stephaoxocanes. The simplest allyl derivative **8** was also the less active one; however, a notorious improvement of enzyme inhibition was detected when its free hydroxyl was converted to the corresponding methyl acetal **9**.

Compounds **10**–**12** were prepared taking into account that stephaoxocanes bear a C-12 hydroxyl. Glycols **10** and **12** were more active that their olefinic precursors and, as observed before, the acetal outperformed the hemiacetal.[25] Curiously, however, compound **11** which best resembles the structure of stephaoxocanidine exhibited a poor performance and none of the alcohols represented an improvement over **5**. Overall, the set of tested compounds were 2–3 orders of magnitude less active than therapeutically approved acetylcholinesterase inhibitors such as galantamine (**1**) and tacrine (**2**).

Table 1. Inhibition of acetylcholinesterase by stephaoxocanidine analogs

Entry	Compound	IC_{50} (μM)	IC_{50} (μg/ml)
1	**5**	19.6	4.8
2	**8**	174	50
3	**9**	100	30
4	**10**	96	32
5	**11**	137	43.8
6	**12**	109	35
7	**13**	46	15
8	**14**	105	40.7
9	Tacrine (**2**)[24]	0.20	0.04
10	Physostigmine[24]	0.03	0.008
11	Galantamine (**1**)[13]	1.07	0.29

In conclusion, seven ABC-ring analogs of stephaoxocanidine have been synthesized and their activity as inhibitors of acetylcholinesterase was tested. Lactone **5**, the most potent compound of this series, exhibited an activity similar to that found in *Narcissus* extracts enriched in galantamine. The lactone moiety does not seem to be the main responsible for the inhibition, but it may contribute to the effect found in **5**, since structural modifications of the latter with retention or transformation of the lactone moiety furnished less active compounds but did not abolish the acetylcholinesterase inhibiting activity. Unexpectedly, however, introduction of a functionalized side chain partially resembling ring D of the tetracyclic natural products did not improve the activity.

Acknowledgements

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20. All new compounds were fully characterized by IR, ^{1}H and ^{13}C NMR, as well as by high resolution mass spectra.
21. Compound **8**: White solid, mp: 159–161 °C (hexane–EtOAc); IR (KBr, *v*): 3450, 2950, 1630, 1475, 1425, 1350 1280, 1240, 1200, 1040 and 875 cm^{-1}; ^{1}H NMR (200 MHz, $CDCl_3$, δ): 1.28 (s, 1H, O*H*), 2.99 (dd, 1H, *J* = 5.6 and 13.8 Hz, CH_2CH=CH_2), 3.28 (dd, 1H, *J* = 8.9 and 13.8 Hz, CH_2CH=CH_2), 3.90 (s, 3H, OCH_3), 4.00 (s, 3H, OCH_3), 5.16 (d, 1H, *J* = 15.5 Hz, ArCH_2O), 5.34 (d, 1H, *J* = 15.5 Hz, ArCH_2O), 5.35 (bd, 1H, *J* = 12.7 Hz, CH$_2$CH=CH_2), 5.36 (bd, 1H, *J* = 9.2 Hz, CH$_2$CH=CH_2), 5.90–6.10 (m, 1H, CH$_2$C*H*=CH$_2$), 7.04 (s, 1H, H-4), 7.48 (d, 1H, *J* = 5.7 Hz, H-3) and 8.40 (d, 1H, *J* = 5.7 Hz, H-2); ^{13}C NMR (50 MHz, $CDCl_3$, δ): 42.71 (*C*H$_2$CH=CH$_2$), 55.65 (OCH$_3$-5), 57.12 (Ar*C*H$_2$O), 60.75 (OCH$_3$-6), 96.22 (C-9), 103.68 (C-4), 117.24 (C-6b), 119.26 (C-3), 120.59 (CH$_2$CH=*C*H$_2$), 125.18 (C-6a), 132.18 (CH$_2$*C*H=CH$_2$), 134.20 (C-6), 141.72 (C-2), 143.77 (C-3a), 154.05 (C-5) and 155.67 (C-9a). HRMS Calcd for $C_{16}H_{17}NO_4$ *m*/*z* 287.1158; obsd *m*/*z* 287.1155. Compound **9**: Oil; IR (film, *v*): 2950, 1620, 1550, 1460, 1400, 1350, 1275, 1225, 1175, 1150, 1025, 850 and 640 cm^{-1} ; ^{1}H NMR (200 MHz, $CDCl_3$, δ): 3.03 (ddt, 1H, *J* = 1.2, 7.2 and 14.0 Hz, CH_2CH=CH$_2$), 3.36 (ddt, 1H, *J* = 1.4, 6.5 and 14.0 Hz, CH_2CH=CH$_2$), 3.47 (s, 3H, OCH_3-acetal), 3,90 (s, 3H, OCH_3), 3.99 (s, 3H, OCH_3), 5.10 (d, 1H, *J* = 15.4 Hz, ArCH_2O), 5.21 (d, 1H, *J* = 15.4 Hz, ArCH_2O), 4.95 (ddd, 1H, *J* = 1.2, 1.4 and 10.1 Hz, CH$_2$CH=CH_2), 5.12 (ddd, 1H, *J* = 1.2, 1.4 and 16.7 Hz, CH$_2$CH=CH_2), 5.50–6.00 (m, 1H, CH$_2$C*H*=CH$_2$), 7.02 (s, 1H, H-4), 7.46 (d, 1H, *J* = 5.8 Hz, H-3) and 8.43 (d, 1H, *J* = 5.8 Hz, H-2); ^{13}C NMR(50 MHz, $CDCl_3$, δ): 38.19 (*C*H$_2$CH=CH$_2$), 49.66 (OCH$_3$-acetal), 55.63 (OCH$_3$-6), 57.43 (Ar*C*H$_2$O), 60.72 (OCH$_3$-5), 100.24 (C-9), 103.66 (C-4), 117.60 (CH$_2$CH=*C*H$_2$), 118.96 (C-3), 124.97 (C-6b), 132.95 (2 × C; C-6a and CH$_2$*C*H=CH$_2$), 134.16 (C-6), 141.87 (C-2), 143.65 (C-3a), 153.44 (C-5) and 155.53 (C-9a). HRMS Calcd for $C_{17}H_{19}NO_4$ *m*/*z* 301.1314; obsd *m*/*z* 301.1316. IR (film, *v*): 3400, 2975, 1620, 1580, 1480, 1430, 1350, 1290, 1240, 1130, 970, 880, 740 and 660 cm^{-1}. Compound **10**: Oil containing a ≅1:1 mixture of diastereomers. Diastereomer 1: ^{1}H NMR (200 MHz, $CDCl_3$, δ): 2.00–2.75 (m, 4H, CH_2CHOHCH$_2$OH and 2 × O*H*), 3.45–3.75 (m, 2H, CH$_2$CHOHCH_2OH), 3.38 (s, 3H, OCH_3-acetal), 3.70–3.95 (m, 1H, CH$_2$C*H*OH–CH$_2$OH), 3.92 (s, 3H, OCH_3), 4.01 (s, 3H, OCH_3), 5.14 (d, 1H, *J* = 15.6 Hz, ArCH_2O), 5.21 (d, 1H, *J* = 15.6 Hz, ArCH_2O), 7.05 (s, 1H, H-4), 7.50 (d, 1H, *J* = 5.8 Hz, H-3) and 8.32 (d, 1H, *J* = 5.8 Hz, H-2); ^{13}C NMR (50 MHz, $CDCl_3$, δ): 37.38 (*C*H$_2$CHOHCH$_2$OH), 49.59 (OCH$_3$-acetal), 55.71 (OCH$_3$-6), 57.88 (Ar*C*H$_2$O), 60.72 (OCH$_3$-5), 66.68 (CH$_2$CHOH-*C*H$_2$OH), 66.92 (CH$_2$*C*HOHCH$_2$OH), 100.70 (C-9), 103.91 (C-4), 117.28 (C-6b), 119.88 (C-3), 124.65 (C-6a), 134.43 (C-6), 141.11 (C-2), 144.02 (C-3a), 153.58 (C-5) and 155.91 (C-9a). Diastereomer 2: ^{1}H NMR (200 MHz, $CDCl_3$, δ): 2.00–2.75 (m, 4H, CH_2CHOHCH$_2$OH and 2 × O*H*), 3.45 (s, 3H, OCH_3-acetal), 3.45–3.75 (m, 2H, CH$_2$CHOHCH_2OH), 3.70–3.95 (m, 1H, CH$_2$C*H*OH–H$_2$OH), 3.92 (s, 3H, OCH_3), 4.01 (s, 3H, OCH_3), 5.14 (d, 1H, *J* = 15.6 Hz, ArCH_2O), 5.21 (d, 1H, *J* = 15.6 Hz, ArCH_2O), 7.05 (s, 1H, H-4), 7.51 (d, 1H, *J* = 5.8, H-3) and 8.36 (d, 1H, *J* = 5.8 Hz, H-2); ^{13}C NMR (50 MHz, $CDCl_3$, δ): 38.00 (*C*H$_2$CHOHCH$_2$OH), 49.95 (OCH$_3$-acetal), 55.71 (OCH$_3$-6), 57.70 (Ar*C*H$_2$O), 60.72 (OCH$_3$-5), 66.92(CH$_2$CHOH*C*H$_2$OH), 68.10 (CH$_2$*C*HOHCH$_2$OH), 100.60 (C-9), 103.91 (C-4), 117.54 (C-6b), 119.73 (C-3), 124.80 (C-6a), 134.59 (C-6), 140.25 (C-2), 143.96 (C-3a), 153.40 (C-5) and 155.91 (C-9a). Compound **11**: Oil; IR (film, *v*): 2975, 1620, 1580, 1480, 1430, 1360, 1280, 1230, 1190, 1150, 1110, 1020, 960, 850 and 640 cm^{-1}; ^{1}H NMR (200 MHz, $CDCl_3$, δ): 1.40–1.80 (m, 3H, CH_2CH$_2$OH and OH), 2.20–2.40 (m, 1H, CH_2CH$_2$CH$_2$OH), 2.55–2.75 (m, 1H, CH_2CH$_2$CH$_2$OH), 3.40–3.65 (m, 2H, CH$_2$CH_2OH), 3.42 (s, 3H, OCH_3-acetal), 3.91 (s, 3H, OCH_3), 4.01 (s, 3H, OCH_3), 5.10 (d, 1H, *J* = 15.6 Hz, ArCH_2O), 5.20 (d, 1H, *J* = 15.6 Hz, ArCH_2O), 7.04 (s, 1H, H-4), 7.49 (d, 1H, *J* = 5.7 Hz, H-3) and 8.40 (d, 1H, *J* = 5.7 Hz, H-2); ^{13}C NMR (50 MHz, $CDCl_3$, δ): 26.97 (*C*H$_2$CH$_2$OH), 29.34 (*C*H$_2$CH$_2$OH), 49.58 (OCH$_3$-acetal), 55.72 (OCH$_3$-6), 57.37 (Ar*C*H$_2$O), 60.81 (OCH$_3$-5), 62.40 (CH_2OH), 100.78 (C-9), 103.77 (C-4), 117.62 (C-6b), 119.28 (C-3), 125.04 (C-6a), 134.36 (C-6), 141.78 (C-2), 143.79 (C-3a), 153.63 (C-5) and 155.79 (C-9a). HRMS Calcd. for $C_{17}H_{21}NO_5$ *m*/*z* 319.1420; obsd *m*/*z* 319.1419. Compound **12**: Oil containing a 3:1 mixture of diastereomers. IR (film, *v*): 3300, 2950, 1620, 1580, 1475, 1425, 1350, 1280, 1240, 1120, 1040, 980, 870 and 740 cm^{-1}. *Major diastereomer*: ^{1}H NMR (200 MHz, $CDCl_3$, δ): 2.20 (dd, 1H, *J* = 2.1 and 14.9 Hz, CH_2CHOH), 2.74 (dd, 1H, *J* = 10.3 and 14.9 Hz, CH_2CHOH), 3.50–3.80 (m, 2H, CH$_2$CH_2OH), 3.90 (s, 3H, OCH_3), 4.00 (s, 3H, OCH_3), 4.20 (br s, 1H, 20 Hz, O*H*), 4.30–4.40 (m, 3H, C*H*OH and 2 × O*H*), 5.17 (d, 1H, *J* = 15.5 Hz, ArCH_2O), 5.40 (d, 1H, *J* = 15.5, ArCH_2O), 7.03 (s, 1H, H-4), 7.48 (d, 1H, *J* = 5.8 Hz, H-3) and 8.32 (d, 1H, *J* = 5.8 Hz, H-2); ^{13}C NMR (50 MHz, $CDCl_3$, δ):

40.37 ($CH_2CHOH–CH_2OH$), 55.65 (OCH_3-6), 56.93 ($ArCH_2O$), 60.72 (OCH_3-5), 66.68 ($CH_2CHOHCH_2OH$), 68.46 ($CH_2CHOHCH_2OH$), 97.18 (C-9), 103.04 (C-4), 117.00 (C-6b), 119.55 (C-3), 125.52 (C-6a),134.47 (C-6), 140.63 (C-2), 143.65 (C-3a), 154.50 (C-5) and 155.51 (C-9a). *Minor diastereomer*: ^{1}H NMR (200 MHz, $CDCl_3$, δ): 2.29 (dd, 1H, J = 2.0 and 15.0 Hz, CH_2CHOH), 2.72 (dd, 1H, J = 10.3 and 15.0 Hz, CH_2CHOH), 3.50–3.80 (m, 2H, CH_2CH_2OH), 3.91 (s, 3H, OCH_3), 4.00 (s, 3H, OCH_3), 4.20 (bs, 1H, 20 Hz, O*H*), 4.30–4.40 (m, 3H, C*H*OH and 2 × O*H*), 5.15 (d, 1H, J = 15.5 Hz, $ArCH_2O$), 5.39 (d, 1H, J = 15.5, $ArCH_2O$), 7.03 (s, 1H, H-4), 7.48 (d, 1H, J = 5.8 Hz, H-3) and 8.32 (d, 1H, J = 5.8 Hz, H-2); ^{13}C NMR (50 MHz, $CDCl_3$, δ): 41.27 ($CH_2CHOHCH_2OH$), 55.65 (OCH_3-6), 56.93 ($ArCH_2O$), 60.23 (OCH_3-5), 66.36 ($CH_2CHOHCH_2OH$), 68.63 ($CH_2CHOHCH_2OH$), 97.18 (C-9), 103.04 (C-4), 117.00 (C-6b), 119.55 (C-3), 125.52 (C-6a), 134.47 (C-6), 140.63 (C-2), 143.65 (C-3a), 154.50 (C-5) and 155.51 (C-9a). Compound **13**: White solid; mp: >300 °C, dec. (EtOAc). IR (KBr, ν): 2900, 1730, 1620, 1580, 1480, 1350, 1280, 1190, 1030, 990, 875, 750 and 680 cm^{-1}; ^{1}H NMR (200 MHz, $CDCl_3$, δ): 3.97 (s, 3H, OCH_3), 4.03 (s, 3H, OCH_3), 7.26 (s, 1H, H-4), 7.49 (s, 1H, H-7), 7.76 (d, 1H, J = 5.5 Hz H-3) and 8.77 (d, 1H, J = 5.5 Hz, H-2); ^{13}C NMR (50 MHz, $CDCl_3$, δ): 56.04 (OCH_3-5), 60.99 (OCH_3-6), 74.80 ($ArCHBr$), 106.03 (C-4), 117.35 (C-6b), 122.95 (C-3), 133.22 (C-3a and C-6a), 144.19 (3 × C; C-2, C-6 and C-9a), 155.82 (C-5) and 174.39 (C-9). HRMS Calcd for $C_{13}H_{10}BrNO_4$ m/z 322.9797; obsd m/z 322.9793.

22. *Assay for measuring acetylcholinesterase activity*: some 50 μl of acetylcholinesterase solution (0.25 U/ml) in phosphate buffer (8 mM K_2HPO_4, 2.3 mM NaH_2PO_4, 0.15 M NaCl, 0.05% Tween 20, pH 7.6) and 50 μl of the sample dissolved in the same buffer were added to the wells of a microplate. The plate was incubated for 30 min at room temperature before the addition of 100 μl of the substrate solution [0.1 M Na_2HPO_4, 0.5 M 5,5′-dithiobis(2-nitrobenzoic acid) and 0.6 mM acetylthiocholine iodide in distilled water, pH 7.5]. The absorbance was read in a Bio-Tek Instruments microplate reader at 405 nm after 3 min. Enzyme activity was calculated as a percentage compared to a control using buffer and enzyme solution only. The IC_{50} values were calculated from three individual determinations.
23. Ellman, G. L.; Lourtney, D. K.; Andres, V.; Gmelin, G. *Biochem. Pharmacol.* **1961**, *7*, 88.
24. Otogwo, K.; Kuno, F.; Omura, S. *Pharmacol. Therapeut.* **1997**, *76*, 45.
25. Compounds **10** and **12** were tested as diastereomeric mixtures. No further efforts were done toward separation in view of their rather low activity.