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Quantitative structure–activity relationship studies on acetylcholinesterase enzyme inhibitory effects of Amaryllidaceae alkaloids

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Abstract

Quantitative structure–activity relationship (QSAR) studies on 24 Amaryllidaceae alkaloids, belonging to five ring types, as acetylcholinesterase inhibitors were carried out using physicochemical properties as descriptors. Multiple linear regression analysis of the data has shown that strain energy, heat of formation and substituents at both the aromatic ring and ring C play important roles in the development of the QSAR model. The contribution of substituents at ring C to the model was further supported when strain energy was omitted from the model and ring-type based QSAR analysis for crinine- and lycorine-type alkaloids were performed.

Keywords: Acetylcholinesterase enzyme; Amaryllidaceae; Alkaloids; QSAR

1. Introduction

Acetylcholinesterase enzyme (AChE) inhibitors from general chemical classes such as physostigmine, tacrine and heptylphysostigmine have been tested for the symptomatic treatment of Alzheimer's disease (AD) (Becker and Giacobini, 1988). Clinical studies have shown symptomatic improvements in some patients resulting in the approval of these compounds for the treatment of AD. However, non-selectivity of these drugs, their limited efficacy, poor bioavailability, adverse cholinergic side effects in the periphery, narrow therapeutic ranges and hepatotoxicity are among the severe limitations to their therapeutic success (Bores et al., 1996).

Recently, the Amaryllidaceae alkaloid galanthamine **17** was approved in many European countries for the treatment of Alzheimer's disease (Sramek et al., 2000). The long acting, selective, reversible, and competitive AChE inhibitory properties of galanthamine led to the search for other AChE inhibitors from the family Amaryllidaceae (Sweenly et al., 1989; Thompsen et al., 1990, 1991). The research focussed mainly on galanthamine-type alkaloids. Significant AChE inhibitory activity for Amaryllidaceae alkaloids other than galanthamine-type alkaloids such as the lycorine-type alkaloids assoanine **14**, oxoassoanine **15** and 1-*O*-acetyllycorine **10** has also been reported (López et al., 2002; Elgorashi et al., 2004). The higher activity of assoanine and oxoassoanine with respect to other lycorine-type alkaloids was attributed to the aromatisation of ring C (Fig. 1) which gives a certain planarity to those alkaloids (López et al., 2002). The fact that 1-*O*-acetyllycorine **10** lacks aromatisation at ring C and is 200-fold more potent than lycorine **9**, 2-*O*-acetyllycorine **11** and 1,2-*O*-diacetyllycorine **12**, prompted the search for other properties that affect the binding of the ligand to the active site of the enzyme.

Quantitative structure–activity relationship (QSAR) development provides a powerful tool to correlate the biological activities of compounds to their structural or physicochemical parameters and extends the correlated parameters for the prediction of new active ligands (Viswanadhan et al., 1989). The aim of this study was to uncover the relationship of the AChE inhibitory effects, expressed as IC_{50}, of Amaryllidaceae alkaloids and their physicochemical properties using stepwise multiple linear regression analysis.

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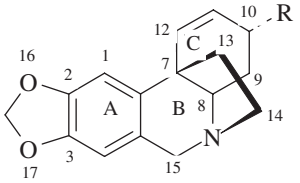
2. Experimental

The alkaloids used in this study as a training set were isolated from a number of Amaryllidaceae species (Fig. 1). Crinine (**1**), epibuphanisine (**2**), epivittatine (**3**), crinamidine (**6**), 1-*O*-acetyllycorine (**10**) and Cherylline (**20**) were isolated from *Crinum moorei* (Elgorashi et al., 2001a). 3-*O*-Acetylhamayne (**5**), crinamine (**7**), 6-hydroxycrinamine (**8**), 8α-ethoxyprecriwelline (**22**), *N*-desmethyl-8α-ethoxypretazettine (**23**) and *N*-desmethyl-8β-ethoxypretazettine (**24**) were isolated from *C. bulbispermum* (Elgorashi et al., 1999). Hamayne (**4**) and lycorine (**9**) were isolated from *C. macowanii* (Elgorashi et al., 2001b). Tazettine (**21**) was isolated from *Cyrtanthus falcatus* (Elgorashi and van Staden, 2003). 2-*O*-Acetyllycorine



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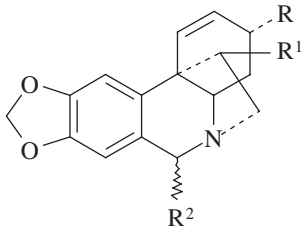
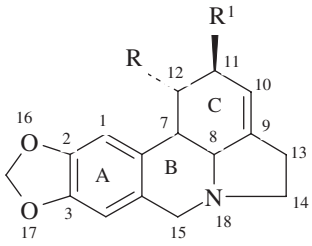
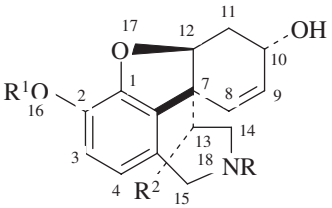
2 R = OMe



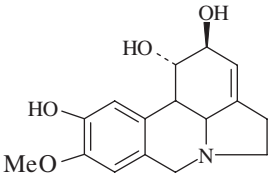
3 R = OH, $R^1 = R^2 = H$
4 $R = R^1 = OH$, $R^2 = H$
5 R = OAc, $R^1 = OH$, $R^2 = H$
7 R = OMe, $R^1 = OH$, $R^2 = H$
8 R = OMe, $R^1 = R^2 = OH$



9 $R = R^1 = OH$
10 R = OAc, R^1 =OH
11 R = OH, R^1 = OAc
12 $R = R^1$ = OAc



16 R = H, R^1 = Me, R^2 = H
17 $R = R^1$ = Me, R^2 = H
18 R = Me, $R^1 = R^2 = H$
19 $R = R^1$ = Me, R^2 = OH



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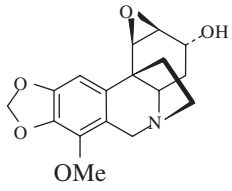

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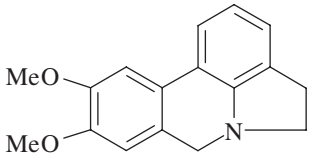

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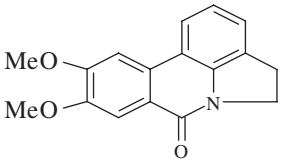

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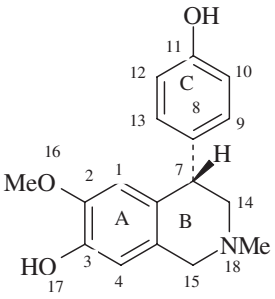
Fig. 1. Chemical structures of Amaryllidaceae alkaloids used for model development: crinine **1**, epibuphanisine **2**, epivittatine **3**, hamayne **4**, 3-*O*-acetylhamayne **5**, crinamidine **6**, crinamine **7**, 6-hydroxycrinamine **8**, lycorine **9**, 1-*O*-acetyllycorine **10**, 2-*O*-Acetyllycorine **11**, 1,2 di-*O*-acetyllycorine **12**, pseudolycorine **13**, assoanine **14**, oxoassoanine **15**, epinorgalanthamine **16**, galanthamine **17**, sanguinine **18**, 11-hydroxygalanthamine **19,** Cherylline **20**, tazettine **21**, 8α-ethoxyprecriwelline **22**, *N*-desmethyl-8α-ethoxypretazettine **23**, *N*-desmethyl-8β-ethoxypretazettine **24**. *Numbering of atoms does not follow the standard numbering of each ring type. It was carried out for illustrative purposes.



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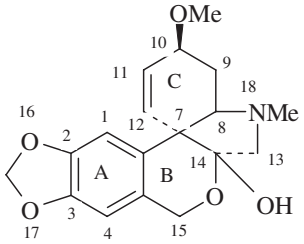

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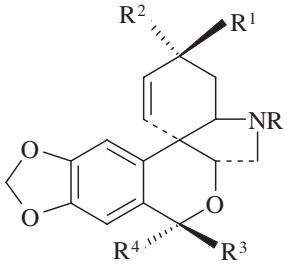

22 R = Me, $R^1 = R^3 = H$, R^2= OMe, R^4 = OEt

23 $R = R^2 = R^3 = H$, R^1 = OMe, R^4= OEt

24 $R = R^2 = R^4 = H$, R^1 = OMe, R^3= OEt

Fig. 1 (*continued*).

(**11**) and 1,2 di-*O*-acetyllycorine (**12**) were obtained by the acetylation of **9** and galanthamine (**17**) was obtained from Sigma-Aldrich. The purity of the above-mentioned alkaloids, assessed using GC–MS, were found to be > 95%. The procedure used for acetylcholinesterase inhibitory activity of the above-mentioned alkaloids is detailed elsewhere (Elgorashi et al., 2004). The IC_{50} values were determined by regression analysis and calculated from at least four individual determinations each performed in duplicate. AChE inhibitory effects of pseudolycorine (**13**), assoanine (**14**), oxoassoanine (**15**), epinorgalanthamine (**16**), sanguinine (**18**) and 11-hydroxygalanthamine (**19**) were obtained from literature (López et al., 2002) and correlated to experimental values obtained in our laboratory.

Advanced chemistry development's *ACD/ChemSketch*® program (ACD/ChemSketch® 4.54, 2000) was used to calculate molar refractivity (Å^3), molecular volume (cm^3), parachor (Å^3), density (g/cm^3), refractive index, surface tension (dyne/cm) and polarisability (Å^3). *ACD/Log P*® (ACD/LogP® 4.54, 2000) was used to calculate lipophilicity (log *P*), lipophilicity of the neutral form (log P_{NF}), log *D*, log $D_{7.4}$ and degree of ionisation (pKa).

Structural optimization was accomplished using MMFF94 (Merck Molecular Force Field) calculations in the PC Spartan Pro® modelling software (PC Spartan Pro® 1.0, 1999). MM+ and AM1 minimization models were used for molecular and electronic calculations. Strain energy (*E*; kcal/mol) was determined from molecular mechanics calculations and heat of formation (HF; kcal/mol), solvation energy (kcal/mol) and electrostatic potential from semi-empirical calculations.

Spartan Pro® 1.0 was further used to calculate the surface electrostatic potential map from semi-empirical calculations (AM1) as well for the alignment of the different alkaloids. For superpositions, 1-*O*-acetyllycorine and galanthamine molecules in their minimum-energy conformation were used as references. The compounds are all of similar molecular size and relatively rigid and hence atoms 1–3 in the aromatic ring of the reference and the test compound were selected for superpositioning. Stepwise multiple regression analysis of the QSAR data was carried out using Statistica (Statistica data analysis software system® 6.0, 2003).

3. Results and discussion

The physicochemical properties of the 24 Amaryllidaceae alkaloids and their AChE inhibitory effects expressed as IC_{50} are presented in Table 1. Single linear regression analysis of the IC_{50} of AChE inhibitory effects of these alkaloids and their physico-chemical properties did not reveal significant correlations between the individual descriptors and the IC_{50}.

Stepwise multiple linear regression analysis of all data resulted in a five-component model (Eq. (1)). Strain energy (*E*), heat of formation (HF), electrostatic potential (E_P) at carbons 3 and 12, and at oxygen 16 were found to be the major descriptors of the of AChE inhibitory (Log IC_{50}) effects of these Amaryllidaceae alkaloids. Omission of strain energy (Eq. (2)) revealed electrostatic potential at carbons 3, 7 and 12 together with oxygen 16 and the heat of formation as

Table 1
Physicochemical properties of the different Amaryllidaceae alkaloids investigated

Alkaloid	IC_{50} (μM)	MW	MR	MV	Para	RI	ST	*D*	Pol	Log *P*	Log P_{NR}	Log *D*	Log $D_{7.4}$	pKa	*E*	HF	SE
1	461	271.311	73.64	188.9	544.8	1.707	69.1	1.43	29.19	1.23	1.34	1.65	1.02	14.177	70.68	−47.37	−14.05
2	547	285.338	78.47	213.8	588.2	1.654	57.2	133	31.11	1.98	2.09	1.61	1.84	7.302	80.05	−42.38	−11.17
3	239	271.311	73.64	188.9	544.8	1.707	69.1	1.43	29.19	1.23	1.34	0.77	1.02	14.177	67.17	−53.34	−12.37
4	553	287.311	75.15	186.9	559.7	1.737	80.4	1.53	29.79	0.06	0.17	0.1	0.14	14.551	107.82	−94.66	−15.08
5	594	329.347	84.74	224.9	648.4	1.677	69.0	1.46	33.59	0.94	1.05	1.01	1.03	14.228	108.78	−127.43	−14.50
6	300	317.337	79.75	205.9	602.6	1.701	73.2	1.54	31.61	0.61	1.08	0.74	0.91	14.128	96.68	−101.96	−15.24
7	697	301.337	79.99	211.8	603.1	1.679	65.7	1.42	31.71	0.81	0.92	0.87	0.9	14.278	102.3	−88.17	−12.70
8	490	317.337	81.5	209.8	618.1	1.704	75.3	1.51	32.31	−0.3	−0.19	−0.19	−0.19	14.418	83.68	−129.08	−15.41
9	213	287.311	74.89	168.9	557.7	1.733	79.1	1.53	29.69	1.02	1.02	0.98	1.00	14.58	97.70	−100.26	−14.78
10	0.960	329.347	84.48	225.0	646.3	1.674	68.0	1.46	33.49	1.15	1.15	1.14	1.15	13.416	69.29	−128.33	−13.29
11	169	329.347	84.48	225.0	646.3	1.674	68.0	1.46	33.49	1.45	1.45	1.43	1.44	13.464	89.56	−128.09	−13.52
12	211	371.384	94.06	262.9	735.0	1.634	77.1	1.41	37.29	2.52	2.52	2.51	2.51	5.211	78.90	−154.17	−14.51
13	152	289.326	76.73	197.0	583.8	1.706	55.5	1.46	30.41	0.12	0.12	0.11	0.11	14.66	99.05	−123.30	−14.21
14	3.87	267.322	77.86	211.2	576.8	1.658	61.0	1.26	30.86	3.51	3.51	3.51	3.51	6.673	82.43	−5.96	−8.18
15	47.2	281.306	77.97	208.2	582.2	1.672	57.9	1.35	30.91	2.88	2.88	2.87	2.87	−1.971	70.66	−42.25	−8.49
16	9.60	273.327	75.45	208.8	576.2	1.642	56.6	1.3	29.91	1.24	1.24	−0.51	−0.14	13.978	56.88	−72.41	−11.13
17	1.90	287.354	80.31	223.9	614.3	1.636	67.8	1.28	31.83	1.59	1.59	0.61	0.94	13.981	70.998	−64.14	−9.08
18	0.100	273.327	75.47	198.9	570.9	1.683	64.6	1.37	29.92	0.78	0.82	−0.22	0.11	14.059	54.30	−71.52	−11.86
19	1.61	303.353	81.82	221.9	629.3	1.658	50.6	1.36	32.43	0.62	0.66	0.31	0.49	14.049	103.65	−103.81	−11.79
20	407	285.338	81.58	232.3	619.7	1.619	64.0	1.227	32.34	1.77	1.77	0.81	1.14	10.267	71.19	−71.53	−12.04
21	705	331.363	86.39	233.9	661.9	1.66	53.2	1.41	34.24	1.8	1.54	1.2	1.33	12.116	136.45	−122.79	−11.66
22	1150	359.416	95.8	275.8	745.0	1.611	53.9	1.3	37.98	2.33	2.07	1.29	1.59	7.107	157.02	−67.71	−9.66
23	234	345.39	90.94	260.8	706.8	1.614	53.9	1.32	36.05	1.72	1.46	0.32	0.67	7.614	100.22	−133.14	−10.99
24	419	345.39	90.94	260.8	706.8	1.614	53.9	1.32	36.05	1.72	1.46	0.32	0.67	7.614	100.74	−133.92	−10.80

Alkaloid	(E_P)															
	IC_{50} (μM)	C2	C3	C4	C7	C8	C9	C10	C11	C12	C13	C14	C15	O16	O17	N18
1	461	0.208	0.277	−0.321	0.16	0.17	−0.162	0.236	−0.188	−0.181	−0.272	0.163	0.121	−0.376	−0.387	−0.614
2	547	0.207	0.279	−0.303	0.13	0.256	−0.287	0.304	−0.261	−0.145	−0.273	0.151	0.116	−0.374	−0.381	−0.626
3	239	0.2	0.307	−0.365	0.206	0.124	−0.125	0.198	−0.195	−0.206	−0.219	0.044	0.117	−0.379	−0.387	−0.577
4	553	0.213	0.281	−0.305	0.017	0.137	−0.122	0.245	−0.213	−0.123	0.181	−0.006	0.107	−0.377	−0.384	−0.571
5	594	0.206	0.281	−0.299	0.019	0.092	−0.175	0.311	−0.192	−0.145	0.167	0.045	0.133	−0.385	−0.393	−0.574
6	300	0.268	0.105	0.174	0.26	0.173	−0.262	0.207	−0.029	−0.092	−0.286	0.181	0.159	−0.372	−0.372	−0.610
7	697	0.226	0.249	−0.273	0.059	0.03	−0.051	0.152	−0.145	−0.165	0.179	−0.058	0.063	−0.377	−0.377	−0.543
8	490	0.275	0.213	−0.202	0.069	−0.011	−0.004	0.14	−0.153	−0.157	0.144	0.055	0.497	−0.386	−0.376	−0.598
9	213	0.231	0.246	−0.248	−0.056	0.209	0.082	−0.332	0.193	0.174	−0.175	0.177	0.289	−0.384	−0.386	−0.677
10	0.960	0.25	0.203	−0.165	−0.568	0.495	−0.102	−0.143	−0.006	0.597	−0.171	0.102	0.341	−0.389	−0.386	−0.623
11	169	0.235	0.223	−0.176	−0.491	0.454	−0.14	−0.088	−0.065	0.342	−0.097	0.039	0.269	−0.379	−0.389	−0.589
12	211	0.259	0.22	−0.162	0.325	0.297	−0.123	−0.12	0.165	0.296	−0.136	0.081	0.294	−0.385	−0.385	−0.568
13	152	0.214	0.173	−0.159	−0.102	0.221	0.102	−0.356	0.231	0.168	−0.23	0.237	0.368	−0.453	−0.339	−0.689
14	3.87	0.187	0.159	−0.125	−0.219	0.295	−0.018	−0.134	−0.158	−0.037	−0.182	0.199	0.409	−0.368	−0.363	−0.630
15	47.2	0.269	0.126	−0.065	−0.077	0.1	0.017	−0.145	−0.102	−0.104	−0.166	0.028	0.729	−0.360	−0.282	−0.331
16	9.60	0.232	−0.188	−0.136	0.379	−0.241	−0.209	0.248	−0.157	−0.007	−0.196	0.249	0.21	−0.346	−0.306	−0.799
17	1.90	0.205	−0.183	−0.177	0.31	−0.252	−0.214	0.273	−0.172	0.065	−0.165	0.243	0.041	−0.337	−0.330	−0.564
18	0.100	0.227	−0.167	−0.188	0.21	−0.172	−0.264	0.314	−0.188	0.175	−0.223	0.255	0.063	−0.480	−0.376	−0.591
19	1.61	0.187	−0.235	−0.043	−0.132	−0.054	−0.247	0.287	−0.164	0.227	0.154	0.177	0.110	−0.340	−0.392	−0.408
20	407	0.183	0.189	−0.157	−0.294	−0.06	−0.017	0.295	0.365	−0.251	−0.105	0.321	0.318	−0.335	−0.451	−0.646
21	705	0.268	0.231	−0.230	0.182	0.295	−0.205	0.333	−0.250	−0.142	0.301	0.192	0.247	−0.392	−0.382	−0.690
22	1150	0.271	0.238	−0.208	0.009	0.393	−0.177	0.338	−0.431	0.018	0.258	−0.016	0.610	−0.386	−0.385	−0.723
23	234	0.257	0.255	−0.237	0.096	0.266	−0.232	0.403	−0.357	−0.016	0.290	0.001	0.575	−0.396	−0.401	−0.816
24	419	0.261	0.259	−0.224	−0.087	0.243	−0.182	0.352	−0.287	−0.003	0.273	0.117	0.689	−0.383	−0.386	−0.805

MW=Molecular weight, MR=Molar refractivity, MV=Molar volume, Para=Parachor, RI=Refractive index, ST=Surface tension, *D*=Density, Pol=Polarizability, Log *P*=Lipophilicity, Log P_{NF}=Lipophilicity of the neutral form, pKa=Degree of ionisation, *E*=Strain energy, HF=Heat of formation, SE=Solvation energy.

the prime predictors of the AChE inhibitory effects of these alkaloids.

$$\log IC_{50} = 1.92 + 3.7\ E_P\ (C3) - 2.46\ E_P(C12) - 0.008\ HF + 0.009\ E + 5.67\ E_P(O1) \times \left(n = 24, R^2 = 0.87,\ p < 0.00000\right) \quad (1)$$

$$\log IC_{50} = 2.98 + 4.5\ E_P\ (C3) - 2.13\ E_P\ (C12) - 0.009\ HF + 6.91\ E_P(O1) + 0.67\ E_P(C7)\left(n = 24,\ R^2 = 0.85,\ p < 0.00000\right) \quad (2)$$

Omission of strain energy resulted in a slightly weaker correlation of Log IC_{50} with the other physicochemical properties (R^2=0.85). The effect of an energy descriptor such as the strain energy on the linearity of the model could be attributed to the importance of conformational requirements for binding of these molecules to the active site of the enzyme (Zah et al., 2003).

The electrostatic potential of atoms in ring C (Fig. 1) and the aromatic ring (ring A) contributed strongly to the linearity of the model. Atomic charge is a good measure of the electrostatic forces which govern the interaction of the ligand at a specific region of the enzyme (Ghose and Crippen, 1987). This effect of electrostatic potential on these atoms also emphasizes the importance of substituents on both ring C and the aromatic ring (ring A).

Amaryllidaceae alkaloids are classified into different ring-types such as crinine-, lycorine-, galanthamine-, cherylline- and tazettine-type alkaloids. Including ring type-based regression analysis further supports the effects of substituents on the activity. Electrostatic potential on C7 and log D were the prime descriptors of the Log IC_{50} of crinine-type alkaloids irrespective of inclusion or omission of energy (Eq. (3), compound **1**–**8**) while that on C2 and energy were the important predictors of lycorine-type alkaloids (Eq. (4), compound **9**–**15**). Omission of energy indicated that C7 and molar refractivity (MR) were the main predictors of Log IC_{50} (Eq. (5)). In both cases an increase in electron density leads to an increase in activity (Eqs. (3) and (4)).

$$\log IC_{50} = 2.78 - 1.6\ E_P(C7) + 0.085\ \log D\ \left(n = 8,\ R^2 = 0.79,\ p < 0.018\right) \quad (3)$$

$$\log IC_{50} = -11.005 + 0.08\ E + 24.85\ E_P(C2)\ \left(n = 7,\ R^2 = 0.77,\ p < 0.052\right) \quad (4)$$

$$\log IC_{50} = 6.78 + 3.44\ E_P(C7) - 0.059\ MR\ \left(n = 7,\ R^2 = 0.84,\ p < 0.025\right) \quad (5)$$

The regression analyses showed that log D has a relatively small effect on the linear regression power of Log IC_{50} of lycorine-type alkaloids and their physicochemical properties. Log D, the effective partition coefficient for dissociative systems, is closely related to log P which is the octanol–water distribution coefficient for neutral species. Almost all of the alkaloids have positive log P values and therefore are relatively hydrophobic (Table 1). Hydrogen bonding appears to play a small role in the binding of these alkaloids to the active site of the enzyme. This is in line with literature reports where only two classical hydrogen bonds appear to be formed when the X-ray crystal structure of galanthamine **17**, bound in the active site of *Torpedo californica* acetylcholinesterase (TcAChE), was examined. These hydrogen bonds are between the hydroxyl group of the inhibitor and the oxygen of the methoxy group of the protein. The rest of the interactions involve either non-classical hydrogen bonding, between the *N*-methyl group of the inhibitor and the protein, or non-polar interactions (Greenblatt et al., 1999).

The molar refractivity contributed positively to the linearity of lycorine-type alkaloid model when the strain energy was omitted (Eq. (5)) and an increase in molar refractivity is accompanied by a decrease in log IC_{50} within this range. This is not surprising as molar refractivity is one of the parameters related to the shape and size of the drug necessary for the effective binding to its target site (Thomas, 2000). Above a certain critical point, the ligand becomes too bulky to fit into the active site.

The surface electrostatic potential was calculated to investigate the correlation between the surface charge distribution

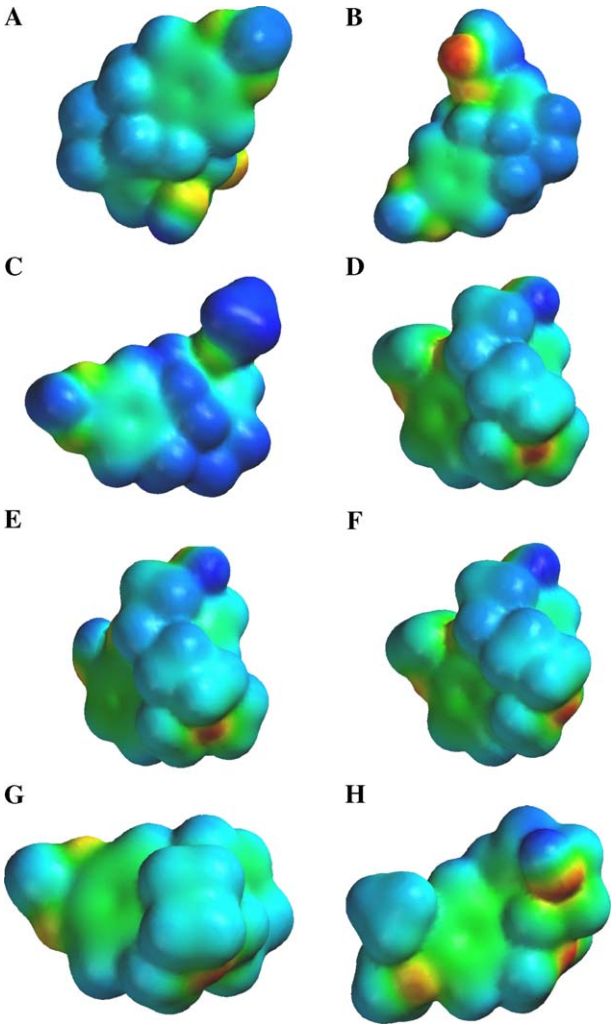

Fig. 2. Surface electrostatic potential map of: (A) 1-*O*-acetyllycorine; (B) 2-*O*-acetyllycorine; (C) 1, 2-*O*-diacetyllycorine; (D) galanthamine; (E) sanguinine; (F) epinorgalanthamine; (G) crinine; (H) maritidine (included for comparison, did not show AChE inhibitory activity). Red indicates negative charges, blue represents positive charges. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

of the alkaloids and their IC_{50} values. Interestingly, the surface charge distribution of the most active group of the Amaryllidaceae alkaloids, namely galanthamine **17**, sanguinine **18** and epinorgalanthamine **19** were found to be similar (Fig. 2). The surface electrostatic potential of 1-*O*-acetyllycorine **10**, the second most active alkaloid in the whole group, is also much closer to that of the galanthamine-type alkaloids than to those of the related lycorine-type alkaloids such as 2-*O*-acetyllycorine **11** and 1,2-*O*-diacetyllycorine **12**. These differences in surface charge distribution within the lycorine-type alkaloids further highlight the effect of substituents on the activity of a particular alkaloid within the group.

Superpositioning of galanthamine **17** on 1-*O*-acetyllycorine **10** (Fig. 3B) indicated that the 1-*O*-acetyl group and the nitrogen atom of the later superimpose on the hydroxyl group and the nitrogen atom of galanthamine, respectively. This superpositioning confirms the possibility of the hydrogen bonding capacity of 1-*O*-acetyllycorine. The log *P* values of almost all of the compounds are comparable to that of galanthamine and hence sufficient to enable them to cross the blood–brain barrier.

It was also reported that the double bond of the cyclohexene ring of galanthamine **17** stacks against the indole–ring binding site while the *O*-methyl group of galanthamine occupies the acetyl-binding pocket of acetylcholine (Greenblatt et al., 1999). Again, from the analysis and superpositioning of 1-*O*-acetyllycorine **10** and other related lycorine-type alkaloids on galanthamine (Table 2), it appears that the methoxy group of galanthamine partially aligns with

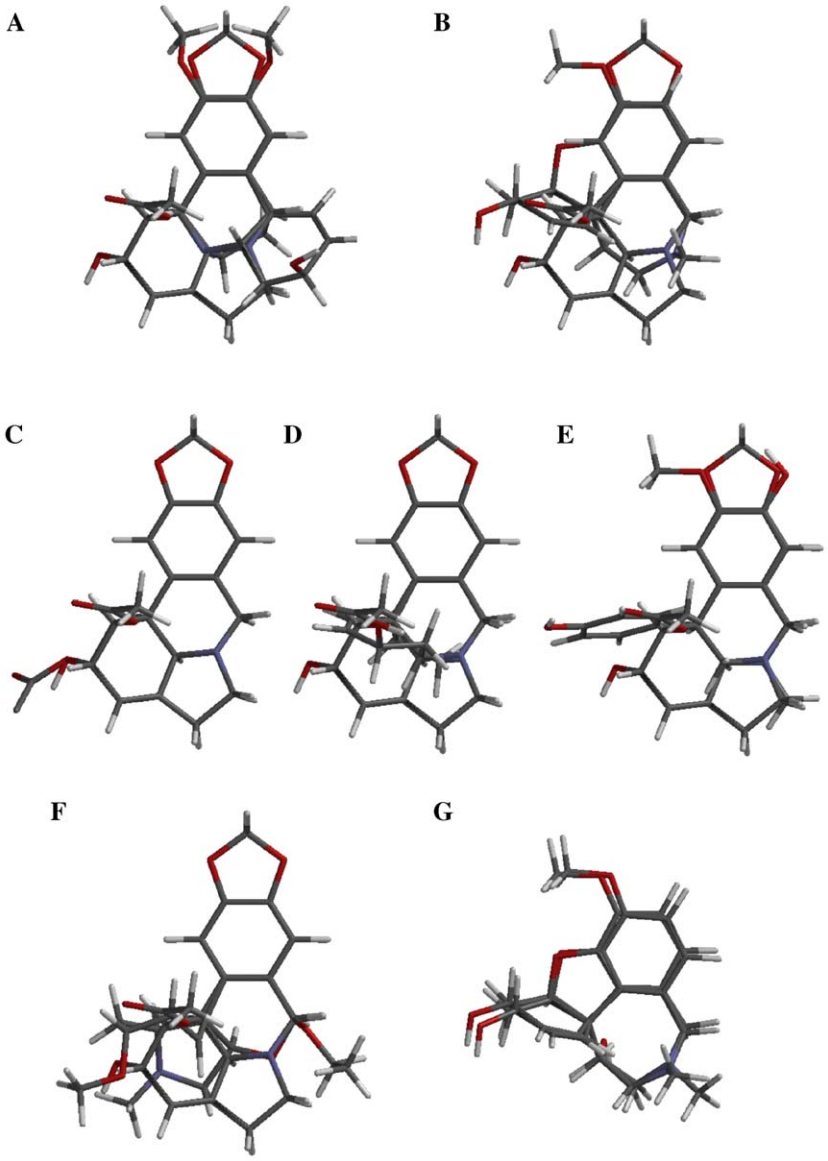

Fig. 3. Superpositioning of: (A) 1-*O*-acetyllycorine and maritidine (B) 1-*O*-acetyllycorine and galanthamine. (C) 1-*O*-acetyllycorine and 2-*O*-acetyllycorine (D) 1-*O*-acetyllycorine and crinine (E) 1-*O*-acetyllycorine and cherylline (F) 1-*O*-acetyllycorine and 8α-ethoxyprecriwelline (G) galanthamine and 11-hydroxygalanthamine. C = gray, O = red, N = blue, H = white. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 2
Atoms-based description of the molecular superpositions[a]

Compounds	Atom numbers
1-*O*-Acetyllycorine/ maritidine	1/1, 2/2, 3/3, 4/4, 5/5, 6/6, 7/7, 8/–, 9/–[b], 10/–, 11/–, 12/–, 13/–, 14/–, 15/15, 16/0, 17/0, 18/18
1-*O*-Acetyllycorine/ galanthamine	1/1, 2/2, 3/3, 4/4, 5/5, 6/6, 7/7, 8/–, 9/–, 10/–, 11/–, 12/–, 13/–, 14/–, 15/15, 16/16, 17/–, 18/18, 11-OH/–, 12-OAc/–
1-*O*-Acetyllycorine/ 2-*O*-Acetyllycorine	1/1, 2/2, 3/3, 4/4, 5/5, 6/6, 7/7, 8/8, 9/9, 10/10, 11/11, 12/12, 13/13, 14/14, 15/15, 16/16, 17/17, 18/18, 12-OAc/12OAc, 11-*O*H/11-*O*COMe
1-*O*-Acetyllycorine/ crinine	1/1, 2/2, 3/3, 4/4, 5/5, 6/6, 7/7, 8/–, 9/–, 10/–, 11/–, 12/–, 13/–, 14/–, 15/15, 16/16, 17/17, 18/18
1-*O*-Acetyllycorine/ cherylline	1/1, 2/2, 3/3, 4/4, 5/5, 6/6, 7/7, 8/8, 9/–, 10/–, 11/–, 12/–, 13/–, 14/–, 15/–, 16/16, 17/0, 18/18
1-*O*-Acetyllycorine/ 8α-ethoxyprecriwelline	1/1, 2/2, 3/3, 4/4, 5/5, 6/6, 7/7, 8/8, 9/–, 10/–
Galanthamine/ 11-hydroxygalanthamine	1/1, 2/2, 3/3, 4/4, 5/5, 6/6, 7/7, 8/8, 9/9, 10/10, 11/11, 12/12, 13/13, 14/14, 15/15, 16/16, 17/17, 18/18, 10-OH/10-OH, 2-OMe/2-OMe, –/13-OH

1-*O*-acetyllycorine and galanthamine are used as reference compounds. The superpositioning atoms are given in the form X/Y, where X is the atom of the reference and Y is the corresponding superposed atom of the test alkaloid. [b]No corresponding superposed atom of the test alkaloid. All RMS values were <1 indicating favourable fit on the selected compound.

the methylene dioxy group of the lycorine-type alkaloids, while the double bond of the cyclohexene ring of galanthamine does not align with any part of the lycorine-type alkaloids. This indicates that the mechanism of binding of 1-*O*-acetyllycorine to AChE enzyme might not be the same as that of galanthamine.

4. Conclusions

The regression analysis based on the physicochemical properties of all the alkaloids (Eqs. (1) and (2)) shows that strain energy, heat of formation, and substituents at ring C and the aromatic ring play significant roles in the activity against AChE. Good linear regression was obtained when ring-type based models were established for crinine-type (Eq. (3), $R^2=0.79$) and lycorine-type (Eqs. (4) and (5), $R^2=0.77$ and 0.84) alkaloids. The latter models also supported the effect of electrostatic potential on the aromatic ring and ring C and hence the effect of substituents on these rings. It should be noted, however, that for these models (Eqs. (3)–(5)) a smaller number of alkaloids ($n=7$ and 8) was used. The study also revealed that log *P* of most of the alkaloids investigated is comparable to that of galanthamine and this would probably facilitate their passage through the brain–blood barrier.

Alignment of representative alkaloids with galanthamine revealed that the active binding site of 1-*O*-acetyllycorine might be different from that of galanthamine. However, the surface energy potential showed close similarities in charge distribution between 1-*O*-acetyllycorine and galanthamine-type alkaloids.

The predictive potential of the models established in this study is however limited to this class of compounds and the physicochemical parameters investigated.

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References

ACD/ChemSketch® 4.54, 2000. Advanced Chemistry Development Inc., Toronto, Canada.

ACD/LogP® 4.54, 2000. Advanced Chemistry Development Inc., Toronto, Canada.

Becker, R.E., Giacobini, E., 1988. Mechanisms of cholinesterase inhibition in sensile dementia of the Alzheimer type: clinical, pharmacological, and therapeutic aspects. Drug Development Research 12, 163–195.

Bores, G.M., Huger, F.P., Petko, W., Mutalib, A.E., Camacho, F., Rush, D.K., Selk, D.E., Wolf, V., Kosley, R.W., Davis, L., Vargas, H.M.J., 1996. Pharmacological evaluation of novel Alzheimer's disease therapeutics: acetylcholinesterase inhibitors related to galanthamine. American Society Journal of Pharmacology and Experimental Therapeutics 277, 728–738.

Elgorashi, E.E., van Staden, J., 2003. Alkaloids from *Cyrtanthus falcatus*. South African Journal of Botany 69, 593–594.

Elgorashi, E.E., Drewes, S.E., van Staden, J., 1999. Alkaloids from *Crinum bulbispermum*. Phytochemistry 53, 533–536.

Elgorashi, E.E., Drewes, S.E., van Staden, J., 2001a. Alkaloids from *Crinum moorei*. Phytochemistry 56, 637–640.

Elgorashi, E.E., Drewes, S.E., van Staden, J., 2001b. Alkaloids from *Crinum macowanii*. Biochemical Systematics and Ecology 29, 749–750.

Elgorashi, E.E., Stafford, G.I., van Staden, J., 2004. Acetylcholinesterase enzyme inhibitory effects of Amaryllidaceae alkaloids. Planta Medica 70, 260–262.

Ghose, A.K., Crippen, G.M., 1987. Atomic physicochemical parameters for three-dimensional-structure-directed quantitative structure–activity relationships: 2. Modelling disperse and hydrophobic interactions. Journal of Chemical Information and Computer Sciences 27, 21–35.

Greenblatt, H.M., Kryger, G., Lewis, T., Silman, I., Sussman, J.L., 1999. Structure of acetylcholinesterase complexed with (−)-galanthamine at 2.3 Å Å^3 resolution. FEBS Letters 463, 321–326.

López, S., Bastida, J., Viladomat, F., Codina, C., 2002. Acetylcholinesterase inhibitory activity of some Amaryllidaceae alkaloids and *Narcissus* extracts. Life Science 71, 2521–2529.

PC Spartan Pro® 1.0, 1999. Wavefunction, Irvine.

Sramek, J.J., Frackiewics, E.J., Cutler, N.R., 2000. Review of the acetylcholinesterase inhibitor galanthamine. Expert Opinion on Investigational Drugs 9, 2393–2402.

Statistica data analysis software system® 6.0, 2003. Statsoft Inc., California.

Sweenly, J.E., Puttfarcken, P.S., Coyle, J.T., 1989. Galanthamine, an acetylcholinesterase inhibitor: a time course of the effects on performance and neurochemical parameters in mice. Pharmacology, Biochemistry and Behaviour 34, 129–137.

Thomas, G., 2000. Medicinal Chemistry. An Introduction. John Wiley & Sons, Chichester, pp. 49–59.

Thompsen, T., Bicker, U., Fischer, J.P., Kewitz, H., 1990. Galanthamine hydrobromide in along-term treatment of Alzheimer's disease. Dementia 1, 46–51.

Thompsen, T., Kaden, B., Fisher, J.P., 1991. Inhibition of acetylcholinesterase activity in human brain tissue and erthrocytes by galanthamine, physostigmine and tacrine. European Journal of Clinical Chemistry and Clinical Biochemistry 29, 487–492.

Viswanadhan, V.N, Ghose, A.K., Revankar, G.R., Robins, R.K., 1989. Atomic physicochemical parameters for three dimensional structure directed quantitative structure–activity relationships: 4. Additional parameters for hydrophobic and dispersive interactions and their application for an automated superposition of certain naturally occurring nucleoside antibiotics. Journal of Chemical Information and Computer Science 29, 163–172.

Zah, J., Terre'Blanche, G., Erasmus, E., Malan, S.F., 2003. Physicochemical prediction of a brain–blood distribution profile inpolycyclic amines. Bioorganic and Medicinal Chemistry 11, 3569–3578.