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3D-QSAR Studies on Natural Acetylcholinesterase Inhibitors of *Sarcococca saligna* by Comparative Molecular Field Analysis (CoMFA)

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Abstract—We have derived a comprehensive structure–activity relationship (SAR) picture for a new series of natural acetylcholinesterase inhibitors isolated from *Sarcococca saligna*. A set of 32 previously isolated and tested pregnane-type steroidal alkaloids inhibitors were investigated with respect to their IC_{50} values (pIC_{50}) against the AChE enzyme in order to derive CoMFA models using atom-based alignment. A highly significant CoMFA model was obtained with r^2 value of 0.974. The q^2 (cross validation r^2) value also confirms the statistical significance of our model.

Introduction

Alzheimer's disease (AD) is the most common cause of dementia in the elderly population; it is a chronic, slowly progressive neurodegenerative disorder. The gradual loss of memory, decline in other cognitive functions and decrease in functional capacity result in death approximately 10 years after the onset of symptoms.[1] The enzyme acetylcholinesterase (AChE) has been targeted in treatments for Alzheimer's disease, myasthenia gravis, and glaucoma, and in the recovery of victims of nerve agent exposure.[2,3]

Interest in the discovery of novel AChE inhibitors is expected to continue in future since the current AChE inhibitors lack perfection. Recently the group of Atta-ur-Rahman and M. Iqbal Choudhary reported the isolation, characterization and biological evaluation of a series of pregnane-type steroidal alkaloids **1**–**32**, isolated from *Sarcococca saligna* based on the cyclopentanophenanthriene ring,[4–7] which were found to be inhibitors of acetylcholinesterase. A number of CoMFA models have already been published on AChE inhibitors,[8–11] but according to our knowledge no work on three-dimensional analysis of steroidal alkaloids as AChE inhibitors has been published yet. We have performed a 3D-QSAR study by comparative molecular field analysis (CoMFA),[12] which may serve as a useful tool to gain insight into the mechanism of inhibitory action and to predict the inhibitory activity of new compounds.

Data sets

A total of 32 steroidal alkaloids was used for the CoMFA studies. In each analysis, the training sets constitute 28 compounds and the remaining four compounds are part of the test set. The activity data and 2D structure of all steroidal alkaloids were taken from literature recently reported by Atta-ur-Rahman et al.[4–7] The structures of these compounds are given in Scheme 1 and their biological activities are listed in Table 1.

Structure generation

All structures were initially generated by Gaussian View[13] and minimization was performed with the Austin Model 1 (AM1) parameterization.[14] All geometric

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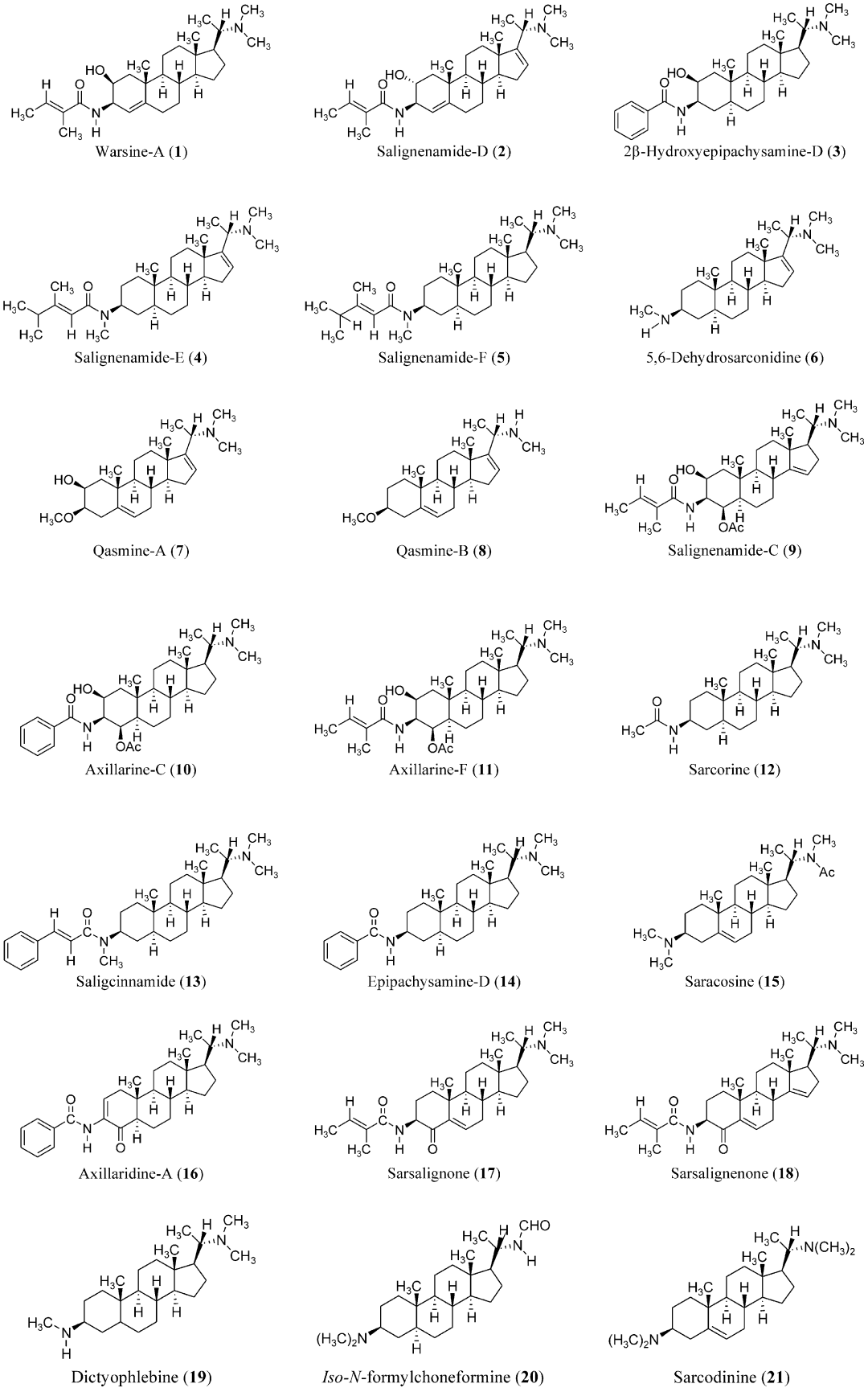
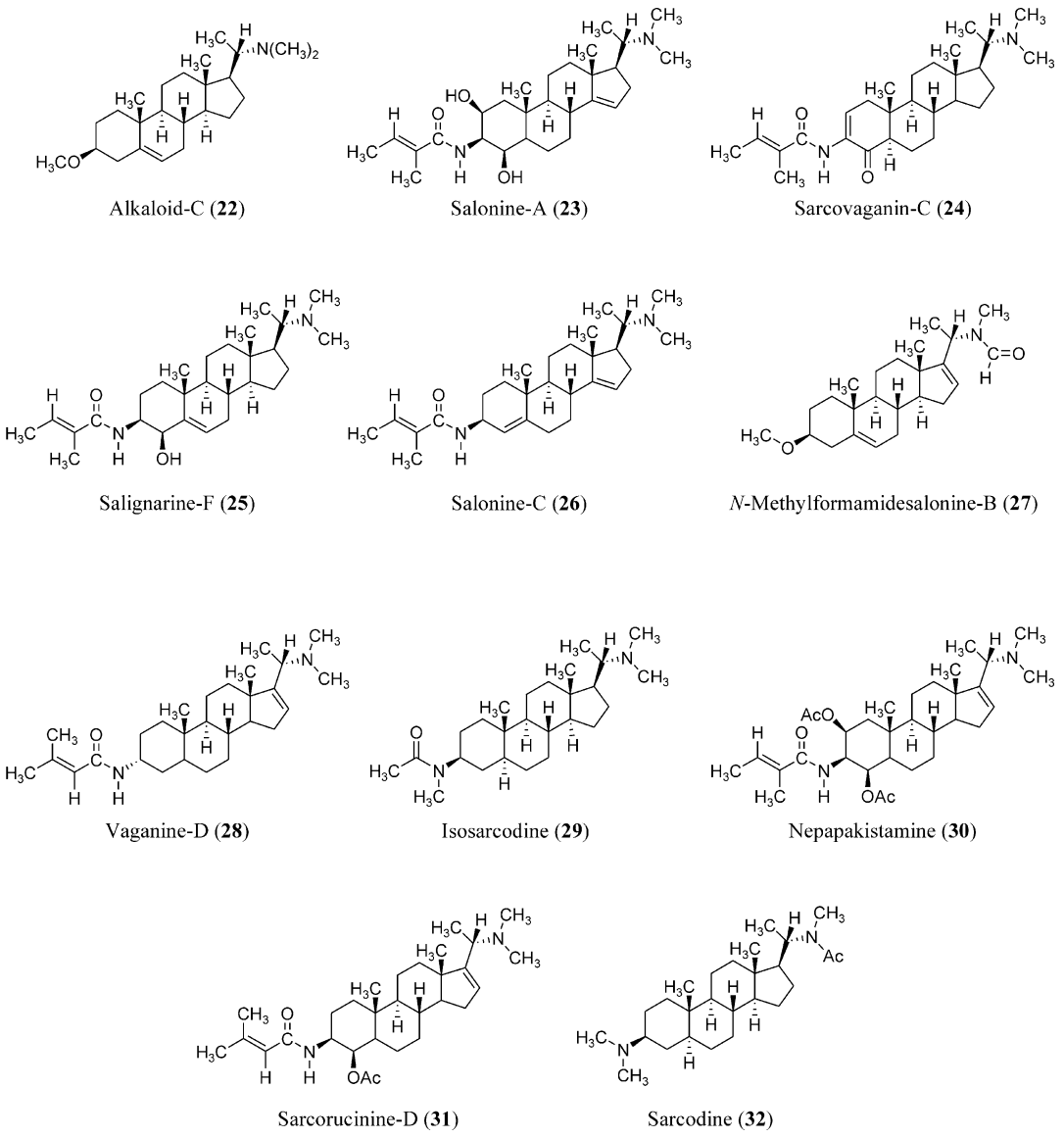


Scheme 1. Chemical structures of acetylcholinesterase inhibitors **1–32**.



Scheme 1. (*continued*).

variables were completely optimized for each compound and the lowest energy conformations were used in the CoMFA analysis. Atomic charges required for calculation of the electrostatic interaction energies for each molecule were calculated in Sybyl with Gasteiger charges.[15]

Alignment

The molecules were superimposed using the atom-based alignment in the SYBYL software.[15] The structure of compound **16** has been used as a template for a manual alignment of the inhibitors. The atoms C3, C5, C6, C13, C14 and C17 (see Fig. 1) of the reference structure have been used in the RMS fitting procedure in Sybyl for each molecule in the data base.

CoMFA study

The CoMFA studies described here were performed on a Silicon Graphics Workstation using the SYBYL molecular modeling Software from Tripos Inc., St. Louis, Mo, USA.[15] Steric and electrostatic fields are included in the analysis and different grid spaces were applied, leading to almost the same results for every case, with very minor differences. On the basis of higher cross-validated r^2 (r^2_{cv}) value and the minimum number of components, we decided to use a grid space of 2.0 Å. However model #4 has better q^2 but it exceeds the limit of components.[16] The Tripos force field[17] was used in field calculations, and the inverse logarithm of IC_{50} values was used as dependent variable in CoMFA. All of the 27 compounds for the training set were superimposed (see Fig. 2) onto a template using an atom-by-atom least-square fit, and one of the most active compounds (**16**) was used as the reference molecule. After alignment the molecules were put into a 3D grid with a spacing of 2.0 Å. The steric and electrostatic fields were then calculated using a 'sp^3' C-atom with +1 charge, with the seven selected atoms designated in Figure 1 as the fitting centers, and the default cutoff energy was set to 30 Kcal/mol. Regression analysis of the resulting field matrix was performed by Partial Least Squares (PLS)

for all compounds. Cross-validation in PLS was carried out using the leave-one-out method. The optimum number of PLS components for the final, non-validated analysis was chosen based on the smallest S_{PRESS} values from the cross-validated analysis. To obtain the 3D QSAR equations, PLS analysis was performed using each of the steric and electrostatic CoMFA fields alone and also in combination.

Results and Discussion

The initial cross-validated fit on all 28 molecules was characterized by a q^2 of 0.308 (with 4 components). A graphical inspection of the measured versus calculated $\log(1/IC_{50})$ values immediately indicated that the overall fit of the molecule was satisfactory except for molecules **2** and **28**, which behave as outlier. These are the only compounds that contain an α substituent at the C-2 and C-3 position respectively. This particular orientation could play a specific role in activity. Due to lack of other examples of such substituted compounds we could not verify this act, thus these molecules had to be omitted from further studies. After removing the outlier, new CoMFA models were obtained with q^2 values of 0.671 (with 6 components).

The results of the analyses are shown in Table 2, from which it is obvious that the best model is # 1; that is, the joint use of both fields gave the best statistical result.

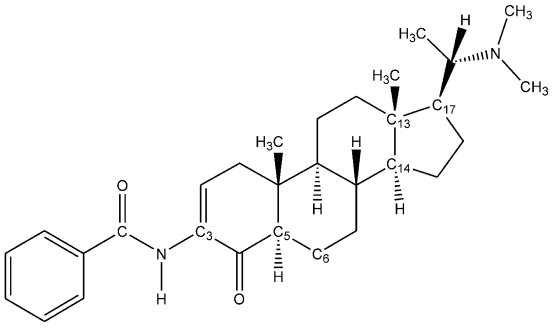


Figure 1. Axillaridine-A (**16**) was used as a template for alignment of data base.

Model-1, which uses both steric and electrostatic CoMFA fields, was thus chosen as the working CoMFA model, whose validity and predictability were assessed by the r^2 value of 0.974 and q^2 value of 0.671, respectively. The final predictability result and residual values are presented in Table 2 and Figure 3.

Having obtained a valid CoMFA model, it is possible to identify regions of space around the molecules where changes with altering substitution would lead to an increase or a decrease in biological activity. This has

Table 1. Experimental and calculated pIC_{50}[a] values for acetylcholinesterase inhibitors **1–32**

#	pIC_{50} (Exp.)	pIC_{50}(Calcld)	Residue
Training Set			
1	4.80	4.81	−0.01
3	4.11	4.15	−0.04
4	5.21	5.08	0.13
5	5.19	5.31	−0.12
6	4.69	4.87	−0.18
7	3.6	3.57	0.03
8	4.08	4.05	0.03
9	4.21	4.02	0.19
10	3.64	3.66	−0.02
11	3.74	3.91	−0.17
12	4.16	4.21	−0.05
13	4.71	4.71	0.00
14	4.54	4.59	−0.05
15	4.70	4.69	0.01
16	5.28	5.27	0.01
17	5.15	5.05	0.10
18	5.24	5.25	−0.01
19	5.21	5.07	0–14
20	5.19	5.24	−0.05
21	4.40	4.44	−0.04
22	4.37	4.32	0.05
23	4.48	4.51	−0.03
24	4.86	4.89	−0.03
25	4.52	4.54	−0.02
26	5.11	5.07	0.04
27	4.31	4.30	0.01
Test Set			
29	4.15	4.39	−0.24
30	4.30	4.29	0.01
31	5.28	4.93	0.35
32	4.30	4.76	−0.46

[a]The potency was defined as log (1/C (pIC_{50})), while C is the effective inhibitor concentration of a compound required to achieve 50% (IC_{50}) inhibition against acetylcholinesterase.

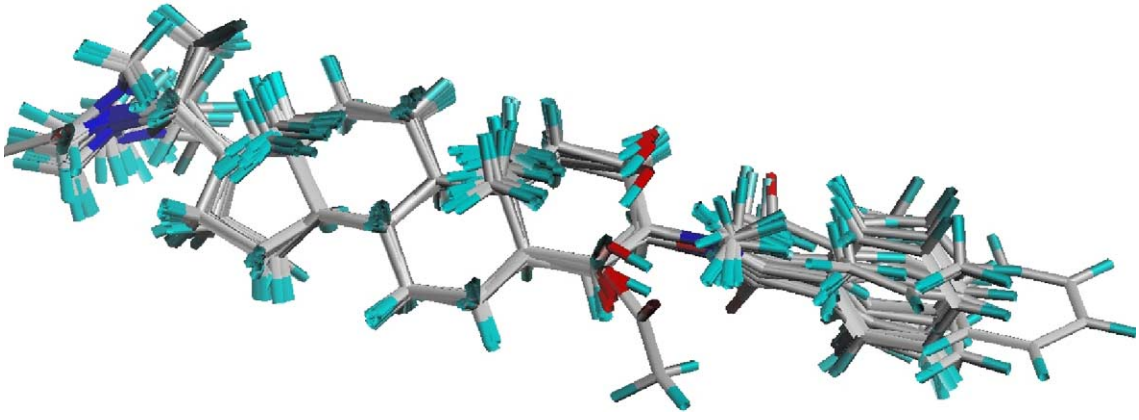


Figure 2. Stereoview of all superimposed cholinesterase inhibitors.

Table 2. Results of the CoMFA analyses of the training set

#	Type of field	Grid space Å	Leave one out q^2	Conventional r^2	Standard error	F-test values	No. of component
1	CoMFA	2.0	0.671	0.974	0.096	118.480	6
2	Steric	2.0	0.602	0.938	0.145	60.130	5
3	Electrostatic	2.0	0.521	0.875	0.200	36.769	4
4	CoMFA	1.5	0.712	0.981	0.084	133.885	7
5	CoMFA	1	0.668	0.968	0.106	96.270	6

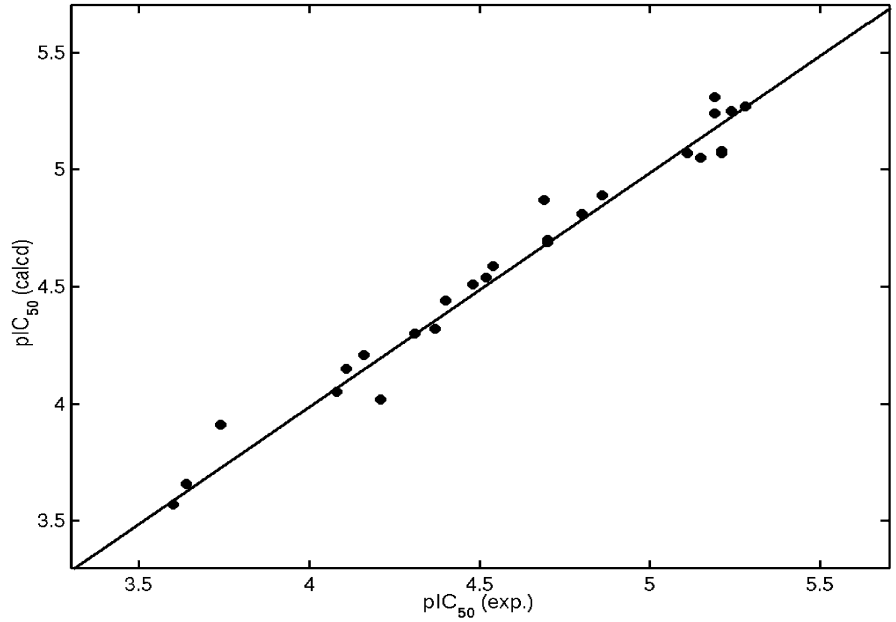


Figure 3. Experimental and calculated pIC_{50} values for acetylcholinesterase inhibitors.

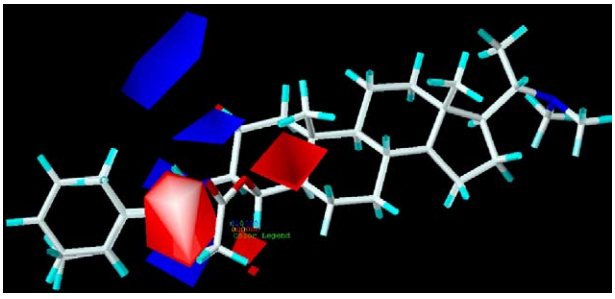


Figure 4. Stereoview of the CoMFA map for the electrostatic contribution. Red color (80 kcal mol^{-1}) indicates areas where more negative density favors activity. Blue color (20 kcal mol^{-1}) indicates areas where more positive density promotes the activity.

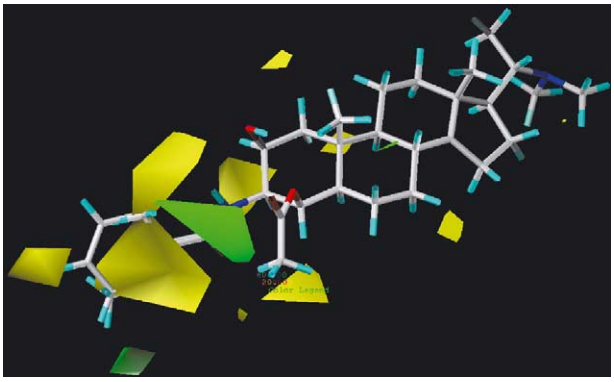


Figure 5. Stereoview of the CoMFA map for the steric contribution. Yellow color (80 kcal mol^{-1}) indicates areas where more bulky substituents favor activity, green color (20 kcal mol^{-1}) indicates areas where less bulky substituents promote the activity.

been achieved by using a CoMFA contour map for model 1, as shown in Figure 4 (electrostatic contributions) and in Figure 5 (steric contributions).

In Figure 4 it is possible to observe two well-defined zones (close to the substituent at position C-4 and side chain at C-3 position) in which the presence of negative density favors an increase of AChE inhibitory activity. Furthermore the presence of a double bond in ring B, between C-5 and C-6, also increases the activity. Small zones near C-2 have an opposite effect, that is, a negative density decreases the activity: for example, the only difference between compounds **3** and **14** is the presence of a hydroxyl group at C-2, which makes compound **3** less active than **14**, on the other hand a negative group at C-4 position makes compounds **16**, **17**, **18** and **31** the most active compounds in our study. Similarly, the presence of a double bond between C-5 and C-6 makes compound **15** more active than compound **32** which has an otherwise identical structure.

Figure **5** displays two big zones near position C-4 and near the side chain at C-3, where the presence of bulky substituents decreases the activity. The region between C14 and C15 is also not favorable for bulky groups,

which assigns a further role to the presence of the double bond in ring D as it means the lack of a further substitution. This difference can be easily seen by comparing compounds **17** and **18**.

Conclusion

Based on the result from CoMFA maps it is possible to assume that ring A of the steroid nucleus is the most important part for the activity of these series of alkaloids. Furthermore, substitution at C-3 position is one of the most influencing factors. The information obtained in this study provides tools for predicting the activity of related compounds, and for guiding further structural modification and synthesis of new potent cholinesterase inhibitors.

Acknowledgements

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