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Original article

CoMFA of benzyl derivatives of 2,1,3-benzo and benzothieno[3,2-*a*]thiadiazine 2,2-dioxides: clues for the design of phosphodiesterase 7 inhibitors

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Abstract – A CoMFA study of benzo- and benzothienothiadiazines derivatives as phosphodiesterase 7 inhibitors has been carried out in order to determine the factors required for the activity of these compounds and also for the selectivity versus other phosphodiesterase isoenzymes. This methodology is employed to gain clues on the design of new fused thiadiazines with improved activity and selectivity on phosphodiesterase 7. Using the information achieved from the three CoMFA models, new structures have been designed in silico and their inhibitory activity on phosphodiesterase 7 was predicted. © 2001 Éditions scientifiques et médicales Elsevier SAS

benzothienothiadiazine / benzothiadiazine / phosphodiesterase inhibitors / CoMFA

1. Introduction

Phosphodiesterases (PDEs) comprise a structural family of isoenzymes responsible for the hydrolysis of adenosine 3′,5′-monophosphate (c-AMP) involved in various biological processes [1]. At the moment, nine families of mammalian PDEs have been described, based on substrate specificity, affinity, sensitivity to cofactors, sequence similarity and sensitivity to inhibitory drugs [2]. To date, the search of selective PDE inhibitors has been focused, mainly, on PDE 4 inhibitors as drugs capable of inhibiting the c-AMP specific in pro-inflammatory leukocytes, as possible treatment for allergic diseases such as asthma [3]. However, the restricted tissular expression of PDE 7 detected in T-cell lines [4] suggests that selective inhibitors of this isoenzyme appear to be a potential promising approach to treat T-cell related diseases such as inflammatory and inmunological processes.

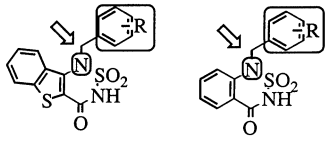

Figure 1. SAR conclusions for the benzo- and benzothiadiazine derivatives.

Recently, we have described a new family of compounds which appeared as the first heterocyclic family of PDE 7 inhibitors [5]. From this study, some preliminary structure–activity relationships could be concluded (*figure 1*): monosubstitution, lipophilic environment and the link between the heterocyclic system and the N1-substituents seem to be important factors for the PDE 7 activity of these compounds. Although some of these compounds showed PDE 7 inhibitory properties at micromolar level, others presented the same biological profile with concurrent inhibitory activity at PDE 4 and PDE 3.

Based on these previous results, we have carried out a 3D QSAR study. The goal of the present study was

Abbreviations: PDE, phosphodiesterase; c-AMP, adenosine 3′,5′-monophosphate.

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to attempt to gain clues for the design of selective inhibitors of PDE 7 using CoMFA method.

2. Results

For this study, we have chosen 19 thiadiazine derivatives which showed phosphodiesterase inhibitory properties against PDE 7, PDE 4 and PDE 3 (*figure 2*).

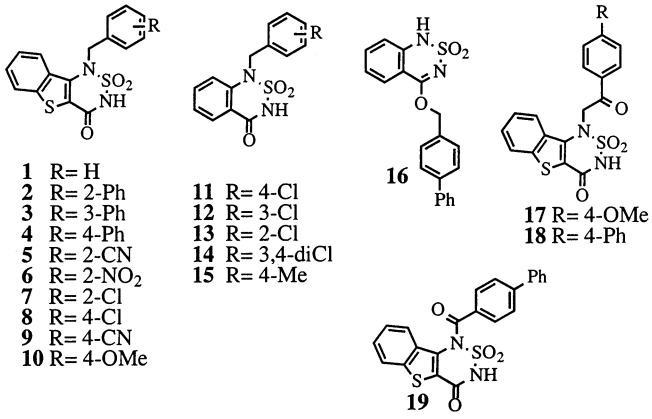


Figure 2. Benzo- and benzothiadiazine derivatives for training set.

All molecular modeling techniques and CoMFA studies described here were performed on a Silicon Graphics workstation using the Sybyl 6.4 molecular modelling software from Tripos, Inc., St. Louis, MO.

The compounds were built from fragments in the Sybyl database. Each structure was initially fully optimised using the standard Tripos molecular mechanics force field. With these initial structures a conformational analysis was carried out in order to define the low-energy conformations.

For conformational searching we employed GRIDSEARCH to rotate torsional angles defined in *figure 3* over 360° in 30° increments. The low-energy conformation obtained was fully geometrically optimised with the Tripos force field.

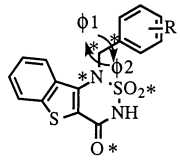


Figure 3. Torsional angles considered in conformational search.

Partial atomic charges required for calculation of the electrostatic interaction energies were calculated using fitting point charges to electrostatic potential at HF/6-31G* via ab initio level with GAUSSIAN-94 [6] which had been employed with success in previous CoMFA studies of related fused thiadiazines [7].

Prior to comparative molecular field analysis, thiadiazine **1** was used as template molecule on which to align the others using atoms defined in *figure 3*. Alignment was then performed using the minimum-energy conformation obtained as described above.

Due to the fact that electronic and steric fields, considered in traditional CoMFA, are not able to describe all binding forces appropriately [8], a molecular lipophilicity field has been taken into account in the present study [9]. This field encodes hydrogen-bonding and hydrophobic interactions, which are not sufficiently described by the steric and electrostatic fields.

In this study three independent variables are taken into account, one for PDE 7 activity and the other two for PDE 7 selectivity versus PDE 4 and PDE 3, respectively. As these compounds and their PDE 7 inhibition are the only tools available at the moment, we use the PDE inhibition percentage at 20 μM for each compound. So the scaled independent variable for each study was calculated as follows. The activity variable is calculated from the logarithmic function of PDE 7 inhibition percentage at 20 μM. The selectivity variables are calculated from the ratio between the logarithmic function of PDE 7 inhibition percentage at 20 μM/logarithmic function of PDE 4 inhibition percentage at 20 μM as a measure of PDE 7 selectivity versus PDE 4, and the ratio between the logarithmic function of PDE 7 inhibition percentage at 20 μM/logarithmic function of PDE 3 inhibition percentage at 20 μM as a measure of PDE 7 selectivity versus PDE 3.

Partial least squares (PLS) method was used to develop the relationship between the dependent values and the independent field potential values. Final non-cross-validated models were chosen on the basis of the best combinations of the mentioned three fields.

Finally, the graphical representations of CoMFA results indicated the regions where the variation in the steric, electrostatic and lipophilic properties of the molecules in the dataset is correlated with the variation in biological activity.

3. Discussion

QSAR studies were carried out to determine the factors required for the PDE 7 inhibition of these compounds and also for the selectivity versus other isoenzymes. Hence, the CoMFA method is employed to gain information on how modifications of specific regions of the thiadiazine ring are related to change or to improve activity in PDE 7 searching to enhance selectivity in other isoenzymes.

For the first point, PDE 7 inhibition is correlated with the descriptors obtained from the CoMFA study. For the second one, the ratio of PDE 7 inhibition and PDE 4 and PDE 3 inhibition, respectively, indicates the factors that improve the selectivity of this family and are correlated with the proposed fields.

For PDE 7 inhibition, the best model, from the different combinations of fields essayed, are those in which all fields are considered ($n = 3$, $r^2 = 0.976$, S.E.M. $= 0.063$, $F = 106.787$). The contribution of lipophilic field appears to be more important (44.2%) than steric (22.8%) or electrostatic (33.0%) ones, suggesting that activity in PDE 7 is mainly influenced by hydrogen-bond interactions. This fact is consistent with our previous results [5] in which lack of activity was found for disubstituted compounds.

We found similar results for selectivity where contributions of lipophilic, steric and electrostatic fields were 39.3:27.9:32.8 and 28.4:34.7:36.9 for PDE 7/PDE 4 and PDE 7/PDE 3, respectively.

Experimental values [5], quoted as log (% inhibition PDE 7 at 20 μM); log (% inhibition PDE 7 at 20 μM)/log (% inhibition PDE 4 at 20 μM); and log (% inhibition PDE 7 at 20 μM)/log (% inhibition PDE 3 at 20 μM), and the predicted values calculated from the three derived CoMFA models, are presented in *table I*. A good correlation between experimental and calculated PDE inhibition values was found (see *figure 4*).

Graphical representations of the models for PDE 7 activity together with representations of the corresponding models for PDE 7/PDE 4 and PDE 7/PDE 3 selectivity are shown in *figures 4–6*, using compound **17** as reference structure.

Similar to that shown in the previous SAR study, lipophilic contour maps for PDE 7 (*figure 5a*) showed an acceptor hydrogen region (white area) around N1, the non-substituted nitrogen atom of the heterocyclic system, suggesting that hydrogen-bonding interaction

Table I. Experimental versus calculated phosphodiesterase activity (inhibition percentage at 20 μM) values for thiadiazines in dataset.

No.	log (% inh. PDE 7)			log (% inh. PDE 7)/log (% inh. PDE 4)			log (% inh. PDE 7)/log (% inh. PDE 3)		
	Experimental	Calculated	Residual	Experimental	Calculated	Residual	Experimental	Calculated	Residual
1	1.14	1.26	−0.12	1.09	1.23	−0.14	0.84	1.02	−0.18
2	1.73	1.66	0.07	1.14	1.03	0.11	0.93	0.74	0.19
3	1.78	1.82	−0.04	0.98	0.97	0.01	0.94	0.92	0.02
4	1.80	1.81	−0.01	1.03	1.04	−0.01	0.96	0.98	−0.02
5	1.46	1.41	0.05	1.17	1.23	−0.06	0.84	0.92	−0.08
6	1.73	1.69	0.04	1.07	1.08	−0.01	0.93	0.92	0.01
7	1.59	1.60	−0.01	1.10	1.13	−0.03	0.90	0.77	0.13
8	1.44	1.37	0.07	1.26	1.20	0.06	0.93	0.98	−0.05
9	1.50	1.54	−0.04	2.17	2.17	0.00	0.97	1.02	−0.05
10	1.34	1.33	0.01	0.98	0.95	0.03	0.82	0.78	0.04
11	1.38	1.44	−0.06	1.53	1.45	0.08	1.77	1.55	0.22
12	1.54	1.44	0.10	1.71	1.70	0.01	1.71	1.91	−0.20
13	1.61	1.66	−0.05	1.69	1.59	0.10	2.68	2.53	0.15
14	1.32	1.33	−0.01	1.57	1.50	0.07	1.69	1.73	−0.04
15	1.82	1.77	0.05	1.07	1.24	−0.17	1.14	1.31	−0.17
16	1.72	1.75	−0.03	1.05	1.11	−0.06	1.07	1.04	0.03
17	1.80	1.83	−0.03	1.19	1.14	0.05	1.14	1.12	0.02
18	1.61	1.61	0.00	3.22	3.25	−0.03	1.37	1.40	−0.03
19	0.30	0.30	0.00	0.60	0.61	−0.01	0.29	0.30	−0.01

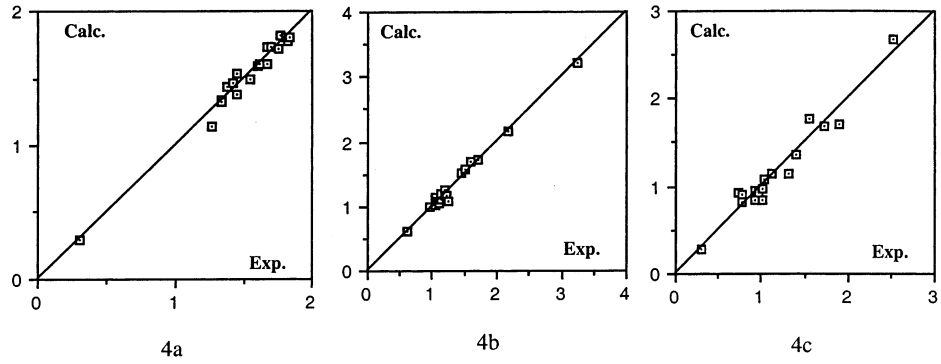


Figure 4. (a) log (% inh. PDE 7); (b) log (% inh. PDE 7)/log (% inh. PDE 4); (c) log (% inh. PDE 7)/log (% inh. PDE 3) calculated values versus experimental ones for the training set derived from the CoMFA models.

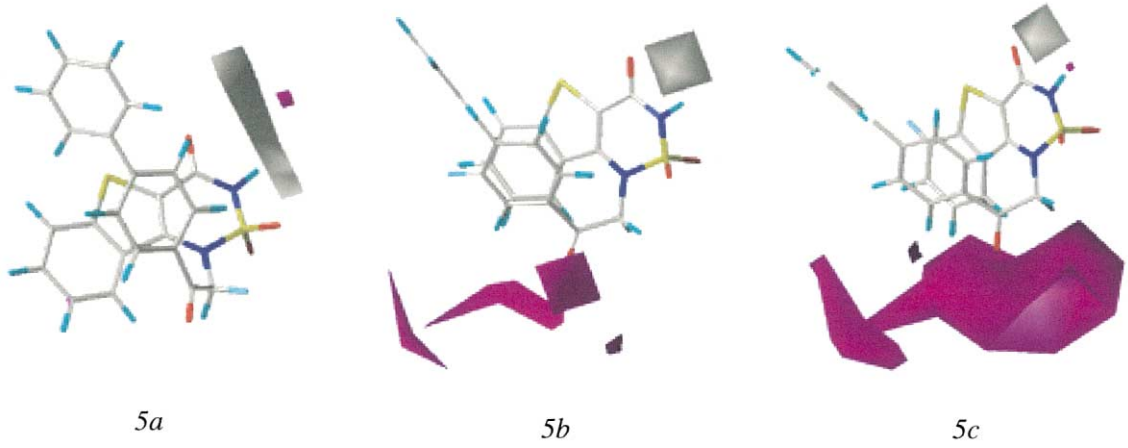


Figure 5. (a) CoMFA lipophilic contour plots for PDE 7; (b) PDE 7/PDE 4; and (c) PDE 7/PDE 3. For lipophilic contours plots, acceptor hydrogen bonds groups near white or donor hydrogen bonds groups near magenta is predicted to increase activity or selectivity.

is determining the activity of these compounds. Additionally, the donor hydrogen-bond region (magenta area) near the link between the heterocyclic system and the benzyl substituents were found for PDE 7/PDE 4 and PDE 7/PDE 3 contour maps (*figures 5b and c*). This might suggest that selectivity of this family could be enhanced establishing additional hydrogen-bonds interactions around the link.

Steric contour maps shown in *figure 6* indicated that the *m*-position of N1-alkylphenylsubstituents appears particularly important for PDE 7 inhibition while the *p*-position leads to selectivity over PDE 3 and PDE 4 isoenzymes. Thus a sterically allowed area (green area) was found for the PDE 7 activity near the *m*-position (*figure 6a*) together with the green area over both *p*- and *m*-position in the PDE 7/PDE 4 and PDE 7/PDE 3 contour plots (*figures 6b and c*). Therefore, compounds with bulk substituents in *m*-position should result in more PDE 7 active compounds whilst modulation of PDE 4 or PDE 3 selectivity would be driven by the size of *p*-substituent.

Electrostatic contour maps seem to suggest that PDE 3 selectivity may be enhanced through electronic effects over the heterocyclic framework together with substituents in the phenyl group attached to the N1 position of the heterocyclic system, while in the case of PDE 4 the same effect could be modulated by modifications in the link between the heterocyclic system and the phenyl group.

Thus, PDE 7/PDE 3 electrostatic contour plots (*figure 7c*) showed a large blue area around the heterocyclic system which confirms the selectivity found in the benzothiadiazine derivatives with regard to the benzothienothiadiazine ones. The CoMFA model for PDE 7/PDE 4 showed a large region favouring positive charge (blue region) around the linker between the heterocyclic system and the N1-substituent (*figure 7b*). This is consistent with the greater PDE 7/PDE 4 selectivity found in compounds **4** versus derivative **19**.

On the other hand, from the electrostatic contour map for PDE 7 (*figure 7a*) the electronic effects required for the activity are located around the link, finding a favouring positive charge in this area. Therefore, the link could modulate both activity and selectivity for PDE 7 and PDE 4.

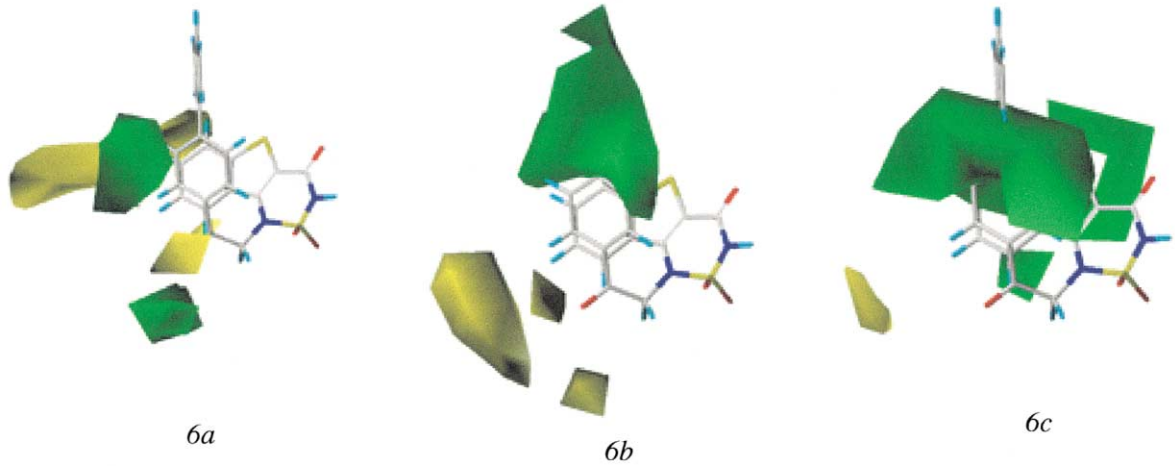


Figure 6. (a) CoMFA steric contour plots for PDE 7; (b) PDE 7/PDE 4; and (c) PDE 7/PDE 3. The green area represents the region where an increase of steric bulk is predicted to increase activity or selectivity. The yellow area represents the region where an increase of steric bulk is predicted to decrease activity or selectivity.

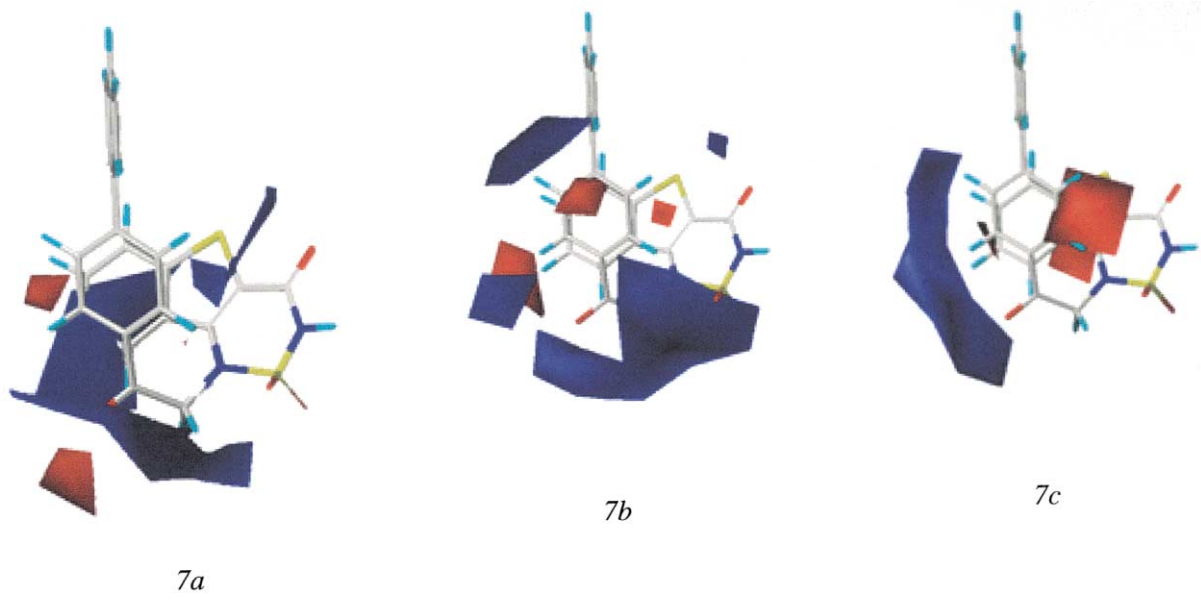
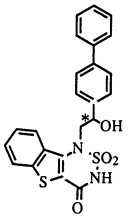


Figure 7. (a) CoMFA electrostatic contour plots for PDE 7; (b) PDE 7/log PDE 4; and (c) PDE 7/PDE 3. For electrostatic contours plots, more positive charge near blue or more negative charge near red is predicted to increase activity or selectivity.

Table II. Predicted values for designed benzothiadiazines at 20 μM.



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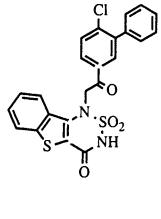

21

Comp.	% inh. PDE 7	% inh. PDE 4	% inh. PDE 3
20	74	11	16
21	79	24	23

Finally, in order to validate the proposed models and considering results from the three CoMFA models simultaneously, we designed and predicted the following structures (*table II*) as potential PDE 7 selective inhibitors by using the methodology previously indicated.

The predicted activity and selectivity values for PDE 7 (listed in *table II*) showed that the compounds designed were found to be more active than those previously synthesised (compounds **20** and **21** versus **15** or **17**).

These results are consistent with the conclusions obtained from the CoMFA models. The importance of additional hydrogen-bond interactions around the link is confirmed by hydroxy compound **20**. Steric requirements in N1-alkylphenyl substituents to modulate PDE 4 or PDE 3 selectivity is reflected in the theoretical biological data found for the disubstituted compound **21**.

4. Conclusions

A CoMFA study was conducted on a series of fused thiadiazines derivatives with PDE 7 inhibitory properties in order to determine alternative molecular regions which could be modified to improve both activity on PDE 7 and selectivity versus PDE 4 and/or PDE 3.

The main conclusion of this 3D-QSAR study revealed the importance of hydrogen-bond interactions for phosphodiesterase inhibition properties of these compounds. In addition, from contour plots some clues for the design of new PDE 7 inhibitors can be deduced suggesting which frameworks could be involved in activity/selectivity of this family of compounds.

New structures have been prepared and evaluated in silico using the information obtained from the three CoMFA models providing theoretically more potent and selective inhibitors.

Acknowledgements

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