Bioorganic & Medicinal Chemistry 16 (2008) 7599–7606

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry

journal homepage: www.elsevier.com/locate/bmc

In silico prediction of novel phosphodiesterase type-5 inhibitors derived from Sildenafil, Vardenafil and Tadalafil

João E. Antunes [a], Matheus P. Freitas [a,*], Elaine F. F. da Cunha [a], Teodorico C. Ramalho [a], Roberto Rittner [b]

[a] Departamento de Química, Universidade Federal de Lavras—UFLA, CP 3037, 37200-000 Lavras, MG, Brazil
[b] Chemistry Institute, State University of Campinas, P.O. Box 6154, 13084-971 Campinas, SP, Brazil

ARTICLE INFO

Article history:
Received 3 June 2008
Revised 8 July 2008
Accepted 9 July 2008
Available online 13 July 2008

Keywords:
PDE-5 inhibitors
MIA-QSAR
Docking studies
Erectile dysfunction

ABSTRACT

A series of drug-like compounds derived from Sildenafil, Vardenafil and Tadalafil analogues were modelled through the MIA-QSAR (multivariate image analysis applied to quantitative structure-activity relationships) ligand-based approach. A highly predictive model was achieved and novel compounds, miscellany of substructures of these three representative phosphodiesterase type-5 (PDE-5) inhibitors were predicted using the calibration parameters obtained through partial least squares (PLS) regression. The high bioactivities of eight promising compounds were corroborated by docking evaluation. Calculated ADME–Tox (absorption, distribution, metabolism, excretion and toxicity) profiles for such compounds suggest advantages of some of them over the currently available, most common drugs used for the treatment of erectile dysfunction.

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1. Introduction

Erectile dysfunction, an alteration in the normal process of penis erection, is manifested in ca. 30% of the economically active population, mainly in 40–69 years old people.[1] However, this problem is supposed to be larger/wider, since it is classified as a non-communicable 'lifestyle' disease by the World Healthy Organization. Organic and/or psychogenic factors are responsible for the inadequate functioning of blood supply in *corpus cavernosum* of penis after stimulus. Biochemically, nitric oxide (NO) is released from non-adrenergic, non-cholinergic neurons in the penis upon sexual stimulation. NO activates guanylyl cyclase, which produces cyclic guanosine 3′,5′-monophosphate (cGMP). In turn, cGMP initiates a protein phosphorylation cascade and decrease in intracellular calcium within *corpus cavernosum* smooth muscle cells, leading to vasodilatation and increasing flow and erection. Inhibition of phosphodiesterase type-5 (PDE-5), a cGMP-specific isozyme in the *corpus cavernosum*, increases the effective concentration of cGMP in the *corpus cavernosum*, enhancing the above-described effects.[2–4]

Treatment of erectile dysfunction has greatly advanced after introduction of Sildenafil (Viagra™),[5] despite its clinically significant side effects.[6] Syntheses of more potent and/or selective PDE-5 inhibitors then emerged, highlighting Tadalafil (Cialis™)[7] and Vardenafil (Levitra™),[8] Figure 1. The positive effects of a new PDE-5 inhibitor, that is, higher activity and/or reduced side effects (e.g., headache, indigestion, back pain, muscle aches, flushing, and stuffy or runny nose), may be potentialized by summing substructure contributions of these three representative compounds. This may be achieved by building a QSAR model for a congeneric series composed of Sildenafil, Vardenafil and Tadalafil analogues (they have fused rings as a common scaffold), and then predicting the activities of novel compounds, which can be a miscellany of substructures of the above commercial drugs. The bioactivities of such compounds were obtained by using similar methodologies, and the extracted enzymes (from human corpus cavernosal tissue, bovine aorta or rabbit platelet) were found to be essentially identical.

A convenient way to derive this model is by using the MIA-QSAR (multivariate image analysis applied to QSAR) method,[9–13] a 2D image-based approach which uses pixels of 2D chemical structures as descriptors (binaries), and that has shown to be a simple and highly predictive tool. MIA-QSAR was applied in this work and, in order to corroborate the results obtained through this ligand-based approach, docking studies were carried out for the most promising proposed drugs and for reference compounds. Also, an ADME–Tox (absorption, distribution, metabolism, excretion and toxicity) evaluation was carried out to search for the more suitable, predicted compounds.

2. Computational methods

2.1. MIA-QSAR

MIA descriptors are binaries obtained from pixels of 2D chemical structures, which must be drawn by using any appropriate drawing program. In this work, the 48 PDE-5 inhibitors (Fig. 2) ob-

* Corresponding author. Tel.: +55 35 3829 1891; fax: +55 35 3829 1271.
E-mail address: matheus@ufla.br (M.P. Freitas).

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doi:10.1016/j.bmc.2008.07.022

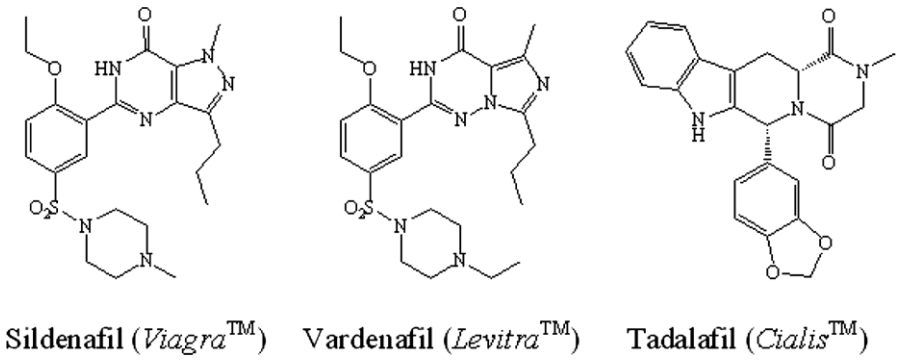


Figure 1. Structures of commercial PDE-5 inhibitors.

tained from the literature,[5,8,14] were drawn through ChemSketch program.[15] Each 2D chemical structure was systematically built, that is, the scaffold (fused six- and five-membered rings) was kept unaltered and the substituents of Figure 2 were added accordingly. The chemical structures were then transformed into bitmaps and saved in a 400 × 400 pixels workspace. Since the dataset used is a congeneric series of compounds, chemical structures possess a common substructure, which was superimposed for the entire series. This was achieved by taking a pixel in common among the whole series of compounds and fitting it in a given coordinate (200,100 coordinate) of the defined workspace. This 2D alignment is rapid and just requires a simple manual precision. The 48 2D images were read and converted into double arrays by using Matlab.[16] These 48 samples were then grouped to give a 48 × 400 × 400 three-way array and then unfolded to a two-way array (48 × 160,000). Two outliers were identified through Student's diagnostic (compounds **1** and **36**) and then removed from dataset, giving the **X**-matrix (46 × 160,000). The calibration was carried out through partial least squares (PLS) regression, and the model validation achieved through leave-one-out cross-validation (LOO CV). External validation has been strongly recommended in lieu of LOO CV [17]; thus, randomly selected samples, 20% from the total series of 46 compounds, were also used as external test set. Randomization was performed 10 times, and average predictions were considered. The predictive ability was statistically evaluated through the root mean square errors of calibration (RMSEC) and validation (RMSECV and RMSEP), as well as by the squared correlation coefficients of the regression line of experimental versus fitted/predicted activity values.

2.2. Docking studies

Crystal coordinates of PDE-5 enzyme in the bound state with Sildenafil and Tadalafil were taken from Protein Data Bank (PDB code: 1TBF and 1XOZ, respectively).[18] Sildenafil, Vardenafil, Tadalafil, **2–5**, **10–13**, **15**, **21–25, 27**, **29**, **31**, **33, 38**, **41–43** (Table 1) and **A–H** compounds were docked inside the PDE-5 active site. Three-dimensional (3D) structures of compounds were built in the PC Spartan program Pro/Builder module[19] using the Sildenafil and Vardenafil crystal structures bound to the PDE-5 as template. Subsequently, the overall geometry optimizations and partial atomic charge distribution calculations of the ligands were performed with the same program using the AM1 semi-empirical molecular orbital method.[20] Compounds were docked into the PDE-5 binding sites using the Molegro Virtual Docker (MVD),[21] a program for predicting the most likely conformation of how a ligand will bind to a macromolecule. The MolDock scoring function (MolDock Score) used by MVD program is derived from the PLP (Piecewise Linear Potential) and further extended in GEMDOCK (Generic Evolutionary Method for molecular DOCK) with a new hydrogen bonding term and new charge schemes.[21] The docking search algorithm used in MVD is based on interactive optimization techniques inspired by Darwinian evolution theory (evolutionary algorithms—EA). The potential binding site of PDE-5 receptor was calculated using the built-in cavity detection algorithm from program. Ligand molecules and a subset region composed of all amino acid residues (side chain) having at least one atom within 12 Å of the center of the Sildenafil are considered flexible during the docking simulation. We selected the conformation of each compound using their best spatial similarity with Sildenafil (compounds **2–5**, **10–13**, **15**, Table 1) and Tadalafil (compounds **21–25**, **27**, **29**, **31**, **33**, **38**, **41–43,** Table 1), which were represented by the structure with the most favorable interaction energy between the pose of ligand and the protein.[22–24]

2.3. ADME–Tox evaluation

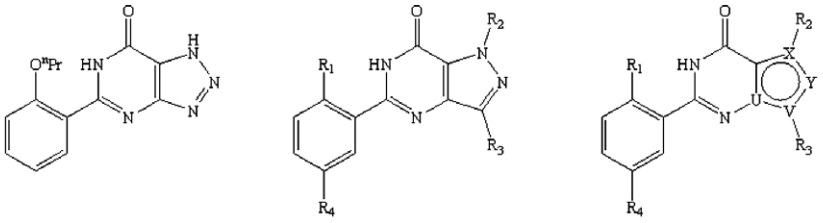
The pharmacokinetic and toxicity profiles of the proposed drugs were calculated through the ADME and Tox boxes of Pharma Algorithms.[25] The Lipinski's rule of five parameters and total polar surface area (TPSA), which have shown to correlate with drug absorption, were obtained by using the Molinspiration program.[26]

3. Results and discussion

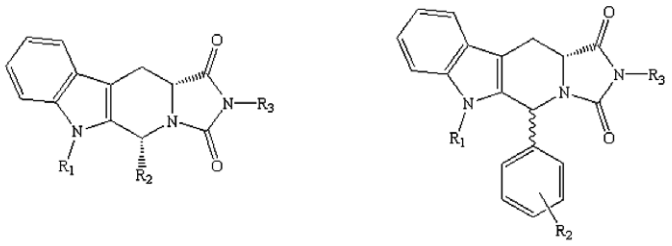
3.1. MIA-QSAR step

According to the MIA-QSAR protocol, the chemical structures of the 48 PDE-5 inhibitors were grouped in such a way that the coincident substructures (the basic scaffold) were all congruent, that is, only substituents accounted for the explained variance of data (99.7%)— the basis of a ligand-based QSAR method. MIA descriptors (pixels = binaries) were then regressed against the **Y**-block (the bioactivities column vector) by using PLS regression. The optimum number of latent variables (LVs) was chosen by jointly analyzing RMSEC and RMSECV; the number of PLS components used was the one in which errors did not decrease significantly after adding more factors (8 latent variables). Two outliers were identified by analyzing the large cross-validated residuals of compounds **1** and **36** ($pIC_{50(exp)}$—$pIC_{50(LOO\ CV)}$ = −1.67 and −2.25, respectively), and through Student's diagnostic (Fig. 3). The activity value of compound **1** (Zaprinast) is controversial in the literature,[5,7,8,14] and **36** is the unique compound in dataset containing a methyl group bonded to the indole nitrogen; thus it appears as an extrapolation during the cross-validation process. These two outliers were removed from dataset in order to proceed with calibration.

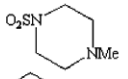
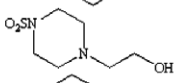
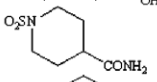
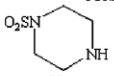
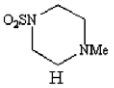
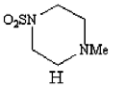
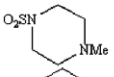
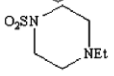
Calibration gave a high correlation between experimental and fitted pIC_{50}, that is, r^2 of 0.970 (RMSEC = 0.16). Many explana-



1 (Zaprinast) **2 – 13** **14 – 20**



21 – 25 **26 – 48**

Cpd	R_1	R_2	R_3	R_4	U	V	Y	X
1	O^{n}Pr	H		H				
2	OEt	Me	Me	H				
3	OEt	Me	^{n}Pr	H				
4	H	H	^{n}Pr	H				
5	H	Me	^{n}Pr	H				
6	OH	Me	^{n}Pr	H				
7	$OCH_2{}^c$Pr	Me	^{n}Pr	H				
8	NO_2	Me	^{n}Pr	H				
9	$NHSO_2Me$	Me	^{n}Pr	H				
10	OEt	Me	^{n}Pr					
11	OEt	Me	^{n}Pr					
12	OEt	Me	^{n}Pr					
13	OEt	Me	^{n}Pr					
14	OEt	Me	^{n}Pr	H	N	C	N	C
15	OEt	Me	^{n}Pr		N	C	N	C
16	OEt	Me	^{n}Pr	H	C	C	N	N
17	OEt	Me	^{n}Pr		C	C	N	N
18	O^{n}Pr		^{n}Pr	H	C	N	C	N
19	O^{n}Pr		^{n}Pr		C	N	C	N
20	OEt	Me	^{n}Pr		N	C	N	C
21	H	*cis*-4-pyridinyl	^{n}Bu					
22	H	*cis*-3-pyridinyl	^{n}Bu					
23	H	*cis*-3-thienyl	^{n}Bu					
24	H	*cis*-3-furanyl	^{n}Bu					

Cpd	R_1	R_2	R_3
25	H	*cis*-phenyl	^{n}Bu
26	H	(*trans*) H	^{n}Bu
27	H	(*cis*) 4-OMe	^{n}Bu
28	H	(*trans*) 4-OMe	^{n}Bu
29	H	(*cis*) 3-Cl	^{n}Bu
30	H	(*trans*) 3-Cl	^{n}Bu
31	H	(*cis*) 4-Cl	^{n}Bu
32	H	(*trans*) 4-Cl	^{n}Bu
33	H	(*cis*) 4-CN	^{n}Bu
34	H	(*trans*) 4-CN	^{n}Bu
35	H	(*trans*) 2-OMe	^{n}Bu
36	Me	(*trans*) 4-OMe	^{n}Bu
37	H	(*trans*) 4-OEt	H
38	H	(*cis*) 4-OEt	Me
39	H	(*cis*) 4-OEt	Et
40	H	(*trans*) 4-OEt	Et
41	H	(*cis*) 4-OEt	Bz
42	H	(*trans*) 4-OEt	Bz
43	H	(*cis*) 4-OEt	^{c}hexyl
44	H	(*trans*) 4-OEt	^{c}hexyl
45	H	(*trans*) 4-OEt	$CH_2CH_2NMe_2$
46	H	(*trans*) 4-OEt	CH_2-3-pyridinyl
47	H	(*trans*) 4-OEt	CH_2CH_2-2-pyridinyl
48	H	(*trans*) 4-OEt	CH_2CH_2-pyrrolidinyl

Figure 2. Structures of the PDE-5 inhibitors in the dataset.

tory variables may be correlated with target variables using PLS regression, but in order to assure that the high correlation obtained did not result from happenstance, the **Y**-block was randomized in such a way that compounds were not corresponding to their respective bioactivity values. This procedure gave a r^2 significantly smaller (0.74 ± 0.11, average of ten measurements) than the real r^2 at 8 LVs, confirming that calibration was not a fortuitous correlation and allowing to assess the robustness of the model. The model was validated through leave-one-out cross-validation (LOO CV), giving a q^2 of 0.694 (RMSECV = 0.53). In order to give another insight about the model validity, the training set was randomized and separated into five segments, and predictions were carried out for each segment using the regression parameters of the remaining samples. This procedure was repeated ten times and the average r^2_{test} obtained was 0.679 (RMSEP = 0.56).

Table 1
Experimental, fitted and predicted (LOO CV and leave-20%-out cross-validation) pIC_{50} for the series of PDE-5 inhibitors[a]

Compound	Exp	Fitted	LOO CV	Leave-20%-out CV
1 (**I**)	5.70	–	–	–
2 (**II**)	6.48	6.43	7.62	7.60
3 (**III**)	7.57	7.34	7.12	7.02
4 (**IV**)	7.09	7.28	7.57	7.51
5 (**V**)	5.35	5.59	6.23	6.32
6 (**VI**)	6.00	5.91	6.00	6.07
7 (**VII**)	6.02	5.94	6.94	6.99
8 (**VIII**)	5.36	5.25	5.57	5.64
9 (**IX**)	6.11	6.14	6.92	6.96
10 (**X**, Sildenafil)	8.44	8.33	8.24	8.32
11 (**XI**)	8.72	8.86	7.87	7.86
12 (**XII**)	8.68	8.59	8.42	8.38
13 (**XIII**)	8.24	8.31	8.57	8.37
14 (**8**)	8.30	8.54	7.87	7.78
15 (**9**)	9.22	9.13	8.77	8.70
16 (**10**)	7.40	7.34	7.21	7.08
17 (**11**)	8.18	8.33	8.44	8.45
18 (**12**)	8.00	7.94	7.55	7.53
19 (**13**)	8.00	7.93	8.12	7.97
20 (Vardenafil)	9.15	9.18	9.39	9.31
21 (**2**)	6.52	6.49	7.07	7.11
22 (*cis*-**6a**)	7.04	6.97	6.89	6.95
23 (*cis*-**6b**)	7.52	7.23	6.98	6.97
24 (*cis*-**6c**)	7.00	7.17	7.51	7.29
25 (*cis*-**6d**)	7.22	7.41	7.50	7.52
26 (*trans*-**6d**)	7.70	7.47	7.31	7.35
27 (*cis*-**6e**, Tadalafil analogue)	8.10	8.07	7.94	7.60
28 (*trans*-**6e**)	8.30	8.13	7.90	7.70
29 (*cis*-**6f**)	7.30	7.27	7.14	7.08
30 (*trans*-**6f**)	7.30	7.35	7.26	7.24
31 (*cis*-**6g**)	7.30	7.55	7.65	7.61
32 (*trans*-**6g**)	7.70	7.61	7.46	7.39
33 (*cis*-**6h**)	6.04	6.23	6.61	6.53
34 (*trans*-**6h**)	6.52	6.29	6.09	6.04
35 (*trans*-**6i**)	6.00	6.18	7.39	7.41
36 (*trans*-**7**)	5.70	-	-	-
37 (*trans*-**6j**)	7.70	7.56	7.15	7.11
38 (*cis*-**6k**)	8.00	7.98	7.98	7.97
39 (*cis*-**6l**)	8.00	8.06	8.10	8.04
40 (*trans*-**6l**)	8.15	8.13	8.01	7.91
41 (*cis*-**6m**)	8.40	7.98	7.55	7.56
42 (*trans*-**6m**)	7.74	8.02	8.21	8.20
43 (*cis*-**6n**)	8.15	8.19	8.38	8.33
44 (*trans*-**6n**)	8.52	8.32	8.11	8.15
45 (*trans*-**9a**)	7.52	7.78	8.02	7.93
46 (*trans*-**9b**)	7.70	7.89	8.09	7.98
47 (*trans*-**9c**)	8.22	8.17	7.89	7.94
48 (*trans*-**9d**)	7.00	7.05	8.04	8.01

[a] In parentheses, compound names/numbering found in the original references: **1**–**13**, Ref. 5; **14**–**20**, Ref. 13; **21**–**48**, Ref. 8.

The statistical results, together with the fitted (calibrated) and predicted (validated) values of Table 1, as illustrated in Figure 4, suggest that the QSAR model built is suitable to make useful predictions. Structures of new drug candidates (PDE-5 inhibitors) may then be achieved by using the regression parameters to predict the activities of compounds designed according to the following strategy: compounds owning the common scaffold of Sildenafil, Vardenafil and Tadalafil analogue (previously calibrated), plus the substructure contribution of at least two of them, may give rise to novel compounds with all pixels (descriptors) calibrated. According to this procedure, compounds were proposed, and six of them (Fig. 5) exhibited predicted bioactivities significantly large (predicted $pIC_{50} > 8$), as compared to the more active compounds of dataset. Thus, such compounds were submitted to docking evaluation, in order to confirm the predicted trends by comparing their affinities toward receptor with the reference compound, Sildenafil, Vardenafil and Tadalafil analogue. It is known that appropriate drugs are not only those having high biological activity; they should obey some pharmacological requisites, such as suitable absorption and low toxicity. Thus, ADME–Tox parameters were calculated for the proposed compounds to account for their suitability as useful drugs in the treatment of erectile dysfunction.

3.2. Docking studies

The MolDock scoring function was applied to evaluate the binding affinities between the PDE-5 and selected 22 inhibitors, Sildenafil, Tadalafil and Vardenafil. Table 2 lists the calculated interaction energy. Figure 6 shows the relationship between the calculated interaction energies (ΔE) and the inhibitory activities (pIC_{50} values) from the PDE-5 including Sildenafil and Tadalafil. A correlation was found between ΔEs and the pIC_{50}s *via* linear regression analysis ($r^2 = 0.56$). This rather good correlation demonstrates that pIC_{50} might be constructed from the modelled interaction energy values of the PDE-5 inhibitors. Thus, correlation between the theoretical and experimental data is satisfactory.

The MIA-QSAR model built for the series of 46 PDE-5 inhibitors of Table 1 was validated, and used to predict the activities of six novel compounds (**A**–**F**, Fig. 5). The accuracy of predictions and high potency of the proposed compounds was reinforced by comparing their affinities toward receptor with the corresponding value for Sildenafil, Tadalafil and Vardenafil (Table 3). The trends in affinity do not exactly correspond to the predicted pIC_{50} obtained through MIA-QSAR, although deviations are nearly within the RMSECV. However, the calculated affinities are at least similar (significantly larger in some cases) to that of Sildenafil, as predicted by

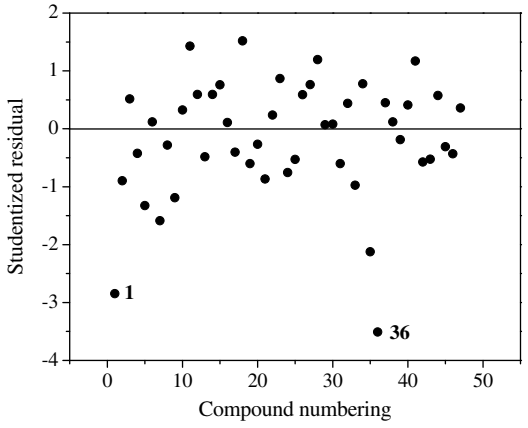


Figure 3. Student's residual diagnostic for outlier detection.

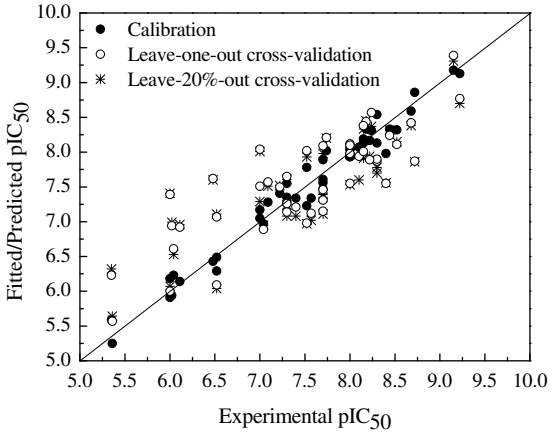


Figure 4. Plot of experimental versus fitted/predicted pIC_{50}.

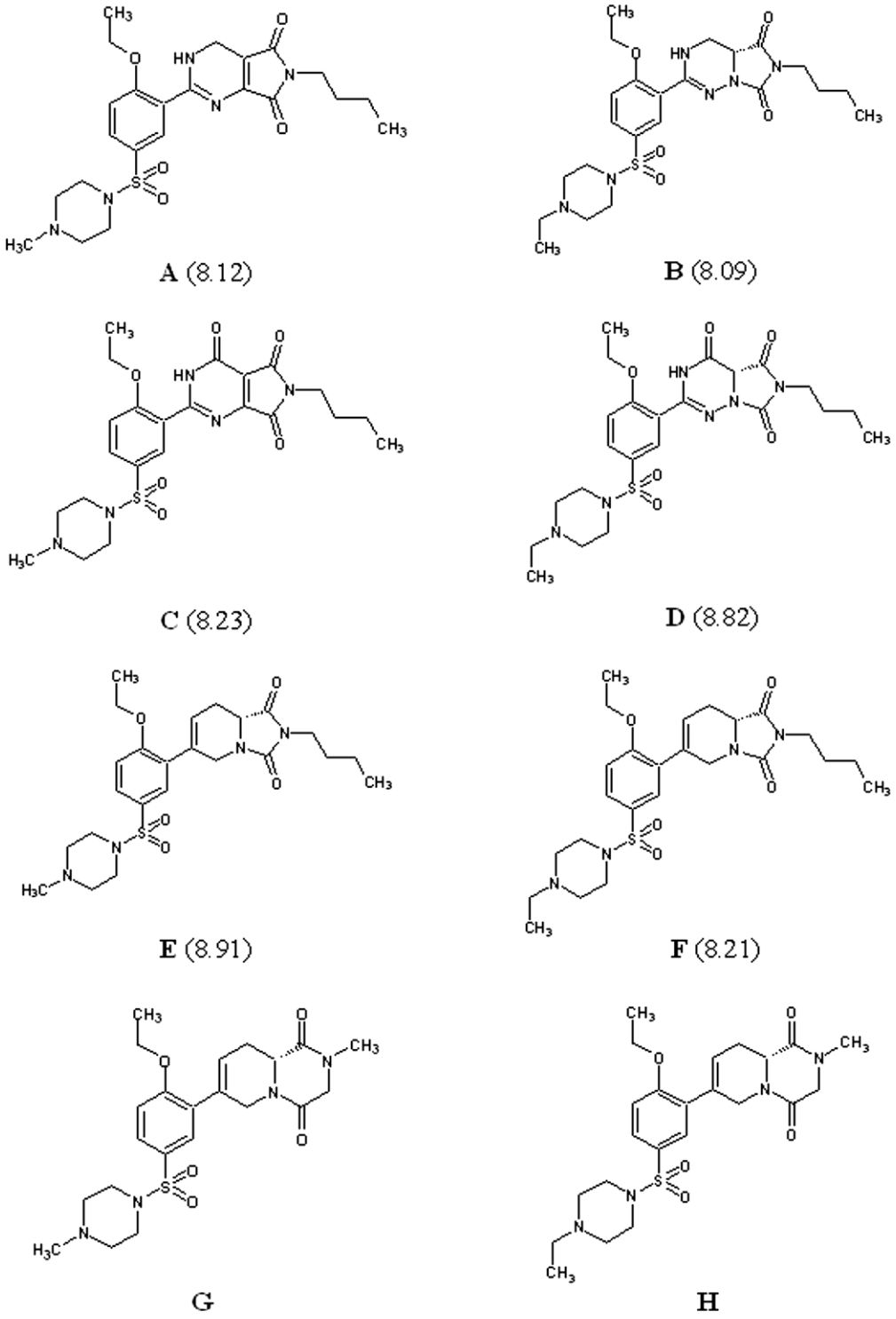


Figure 5. Proposed PDE-5 inhibitors (predicted pIC_{50} in parentheses).

the ligand-based approach. Thus, docking results corroborate the MIA-QSAR findings.

Two additional compounds, **G** and **H**, were subsequently docked into the receptor cavity, and the corresponding affinities were then measured. Activities of these compounds were not calculated by using the MIA-QSAR model because the fused five-membered ring of the common scaffold in training set was replaced by a piperazinedione moiety, which is not calibrated. This structural modification makes **G** and **H** more similar to Tadalafil, which presents important advantages over Sildenafil.[14] According to the docking evaluation, both **G** and **H** presented interaction energy values larger than Vardenafil. Given the satisfactory results for the eight proposed compounds, all of them were submitted to ADME–Tox evaluation.

The proposed compounds **A–H** are miscelany of the most representative compounds of training set: Sildenafil, Vardenafil and Tadalafil analogue. It has been found that polar groups bonded to the sulfonylpiperazine nitrogen, such as **11** (ethoxy group) and **12**

Table 2
Interaction energy values (kcal mol^{-1}) between PDE-5 and some inhibitors of Figure 2

Compound	*E* (docking)	Compound	*E* (docking)
2	−131.21	**23**	−144.49
3	−143.24	**24**	−151.28
4	−108.01	**25**	−152.94
5	−112.16	**27**	−163.09
10	−176.90	**29**	−171.19
11	−177.90	**31**	−159.50
12	−182.24	**33**	−153.02
13	−186.50	**38**	−159.95
15	−176.03	**41**	−181.43
21	−148.93	**42**	−185.27
22	−159.44	**43**	−173.63

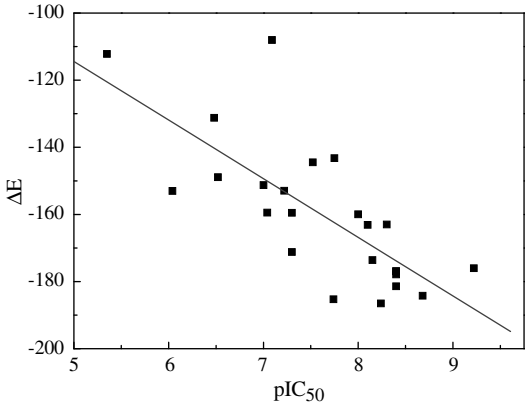


Figure 6. Correlation between intermolecular energies (ΔE_s, kcal mol^{-1}) and experimental bioactivities (pIC_{50}).

(amide group), potentialize bioactivity, due to an hydrophilic interaction with solvent. In Tadalafil, the piperazinedione moiety plays an important role for the high activity and selectivity of this compound. The main interaction governing the energy scores of Table 3 is the hydrogen bond between PDE-5 active site and ligands. The ethoxy group of **A**, **B**, **D**, **E** and **F** interacts with the PDE-5 active site by forming hydrogen bond with Gln817. Moreover, the imide carbonyl group of compounds **C** and **D** interacts in a similar manner with Gln817. Additional hydrogen bonds are observed between the corresponding carbonyl group of compounds **A**, **B**, **C**, **D** and **F**, and the Tyr612 residue. The pyrimidinone ring of compound **C** interacts with Tyr612 through hydrogen bond. Finally, compounds **G** and **H** interact with Gln817 through the carbonyl group. It should be kept in mind, however, that other intermolecular interactions may be important. In line with that, we observed π-staking interactions between fused ring of all compounds (**A**–**H**) and Phe820.

Table 3
Predicted pIC_{50}, interaction (*E*) and hydrogen bond energy values (kcal mol^{-1}) toward PDE-5 for Sildenafil, Tadalafil, Vardenafil and proposed compounds

Compound	pIC_{50} (MIA-QSAR)	*E* (docking)	H-bond
Sildenafil	8.44[a]	-161.63	-4.60
Tadalafil	8.30[a]	-163.01	-2.26
Vardenafil	9.15[a]	-160.69	-5.01
A	8.12	−169.70	−4.38
B	8.09	−167.56	−1.85
C	8.23	−175.70	−5.30
D	8.82	−172.25	−4.71
E	8.91	−163.90	−2.10
F	8.21	−165.60	−2.50
G		−161.09	−2.50
H		−162.23	−2.74

[a] Experimental value.

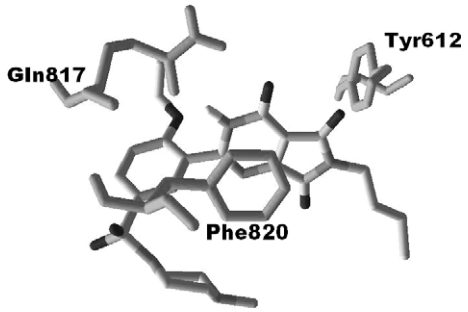


Figure 7. Docked structure of compound **C** into the PDE-5 active site. The residues shown are involved in hydrogen bonding (Gln817 and Tyr612) and π-stacking interaction (Phe820) with the proposed compound.

It is evident from Table 3 that the energy scores of the compounds decreases according to **C** > **D** > **A** > **B** > **F** > **E** > Tadalafil > **H** > Sildenafil > **G** > Vardenafil, that is, compound **C** interacts more strongly with PDE-5 than the remaining compounds. Figure 7 shows **C** inside the PDE-5 active site and the amino acid residues that interact through hydrogen bonds.

3.3. ADME–Tox evaluation

Lipinski et al.[27] have proposed a series of rules imposing limitations on log*P* (the logarithm of octanol/water partition coefficient), molecular weight, and the number of hydrogen bond acceptors and donors, known as 'rule of five'. The rule states that most 'drug-like' molecules have log$P \leqslant 5$, molecular weight $\leqslant$500, number of hydrogen bond acceptors $\leqslant$10, and number of hydrogen bond donors $\leqslant$5. Molecules violating more than one of these rules may have problems with bioavailability. Table 4 shows that reference compounds did not violate any rule, whilst the proposed compounds **B**, **C** and **D** have larger molecular weight and more hydrogen bond acceptors than required to fit them in a drug-like profile. Also, such compounds possess high TPSA values, suggesting that their oral bioavailability should be poorer than those of the remaining compounds of Table 4 (the oral bioavailability is inversely proportional to TPSA[10]). However, the predicted compounds **A**, **E** and **F**, did not violate any rule and exhibited low TPSA values (especially **E** and **F**), and thus are supposed to be more bioavailable than Sildenafil and Vardenafil. This is clearly a result of deletion of the carbonyl group and the nitrogen atom from the fused six-membered ring. Modification of the fused indole-1,3(2*H*)-dione system in **E** and **F**, by exchanging the five-membered ring by a piperazinedione moiety (such as in the Tadalafil structure—Fig. 1), gave compounds **G** and **H**, with similar 'rule of five' parameters and

Table 4
Results for the calculated Lipinski's rule of five and total polar surface area (TPSA)

Parameter	Sildenafil	Vardenafil	Tadalafil	**A**	**B**	**C**	**D**	**E**	**F**	**G**	**H**
log*P*	2.511	2.557	2.360	0.984	1.253	1.857	0.574	2.398	2.774	0.416	0.792
Molecular weight	474.6	488.6	389.4	489.6	506.6	503.6	520.6	490.6	504.6	462.6	476.6
Number of hydrogen bond acceptors	10	10	7	10	11	11	12	9	9	9	9
Number of hydrogen bond donors	1	1	1	1	1	1	1	0	0	0	0
TPSA	113.4	112.9	74.9	113.3	114.9	134.7	131.9	90.5	90.5	90.5	90.5

Table 5
ADME–Tox parameters calculated for the reference and proposed compounds

ADME–Tox[a]	Sildenafil	Vardenafil	Tadalafil	A	B	C	D	E	F	G	H
Solubility H_2O (mg mL^{-1})	0.43	0.86	0.088	0.069	0.023	0.17	0.086	0.18	0.10	0.51	0.32
Log sol. pH 1.7 (stomach)	−0.22	0.44	−3.65	−0.09	−0.45	−2.36	−0.82	−0.54	−0.74	−0.16	−0.32
Log sol. pH 4.6 (duodenum)	−1.15	−0.62	−3.65	−0.09	−0.44	−2.47	−1.59	−1.55	−1.49	−1.05	−1.00
Log sol. pH 6.5 (jejunum, ileum)	−2.74	−2.28	−3.65	−1.24	−2.94	−2.21	−3.31	−3.15	−3.21	−2.66	−2.70
Log sol. pH 7.4 (blood)	−2.93	−2.66	−3.65	−2.32	−3.92	−1.59	−3.69	−3.40	−3.59	−2.90	−3.08
Log sol. pH 8.0 (colon)	−2.81	−2.73	−3.65	−2.91	−4.20	−1.03	−3.75	−3.43	−3.66	−2.94	−3.15
% Oral bioavailability	30–70	<30	30–70	<30	<30	<30	<30	30–70	30–70	30–70	30–70
Absorption (cm s^{-1})	1.58×10^{-4}	1.23×10^{-4}	1.85×10^{-4}	1.9×10^{-5}	1.44×10^{-4}	0.62×10^{-4}	1.00×10^{-4}	3.61×10^{-4}	3.91×10^{-4}	1.09×10^{-4}	1.26×10^{-4}
Distribution (L kg^{-1})[b]	1.1 (93%)	1.9 (92%)	2.1 (93%)	2.4 (76%)	2.4 (73%)	0.3 (85%)	1.6 (71%)	1.9 (82%)	1.1 (94%)	1.4 (78%)	1.5 (81)
$LD_{50\ rat/mouse}$ (mg kg^{-1}, oral)	540/470	1100/950	570/1000	180/360	1300/990	400/490	1100/1200	900/850	950/880	570/720	760/740
$LD_{50\ rat/mouse}$ (mg kg^{-1}, intraperitoneal)	60/84	170/240	750/460	25/35	200/280	39/51	240/310	98/210	95/200	750/200	92/190
$LD_{50\ mouse}$ (mg kg^{-1}, intravenous)	45	72	120	20	84	27	100	66	61	74	68
$LD_{50\ mouse}$ (mg kg^{-1}, subcutaneous)	180	430	420	57	330	76	330	350	360	290	390
Ames test (genotoxicity, %)	0.014	0.006	0.001	0.002	0.002	0.003	0.002	0	0	0	0
Prob. of blood effect	0.55	0.53	0.49	0.91	0.44	0.92	0.45	0.48	0.46	0.78	0.77
Prob. of cardiovascular system	0.93	0.92	0.94	0.95	0.93	0.94	0.91	0.96	0.96	0.96	0.96
Prob. of gastrointestinal system	0.86	0.86	0.49	0.99	0.97	0.93	0.34	0.73	0.76	0.77	0.76
Prob. of kidney effect	0.13	0.13	0.98	0.32	0.09	0.30	0.08	0.31	0.31	0.34	0.33
Prob. of liver effect	0.40	0.39	0.99	0.76	0.49	0.74	0.47	0.38	0.38	0.37	0.37
Prob. of lung effect	0.65	0.74	0.14	0.79	0.52	0.85	0.22	0.63	0.61	0.43	0.42

[a] No compounds are predicted to be transported by intestinal peptide transporter (PepT1) and apical sodium-dependent bile acid transporter (ASBT).
[b] The binding to plasma protein is given in parentheses.

TPSA. This modification was found to be beneficial during the development of Tadalafil.[7] It is evident from Table 4 that the improved current drug, Tadalafil, exhibits molecular weight, number of hydrogen bond acceptors and TPSA significantly smaller than all compounds of Table 4, which supposedly contributes for its known advantages over Sildenafil and Tadalafil. In addition, its octanol/water partition coefficient is comparable to Sildenafil, Vardenafil, **E** and **F**, but larger than **A–D**, **G** and **H**; these latter compounds are farer from violating the log *P* rule, but they should have reasonable lipophilicity for permeating membrane. More specific analysis of pharmacological parameters may be scrutinized through a complete ADME–Tox evaluation.

Table 5 depicts some specific parameters related to absorption, distribution, metabolism, excretion and toxicity for the reference and predicted compounds. In general, all compounds presented advantages and disadvantages when compared to each other. No marked difference in health effects and in rodent toxicity profiles were observed among the compounds. However, the absorption-related parameters call for attention, since the promising compounds **E** and **F** are calculated to be at least as soluble as the reference compounds both in water and buffer, and is predicted to have oral bioavailability and absorption significantly larger than Sildenafil and Vardenafil. Also, these values are comparable (marginally inferior or superior) to those obtained for the last-generation PDE-5 inhibitor Tadalafil. Similar behaviour was observed for **G** and **H**.

4. Conclusions

The MIA-QSAR model built for the series of Sildenafil, Vardenafil and Tadalafil analogues was used to predict the activities of novel phosphodiesterase type-5 inhibitors, as attested by validation tests and docking studies. Chemical structures were proposed based on a combinatorial strategy, in which eight propositions resulted in highly potent compounds. The satisfactory ADME–Tox profiles to four of them, namely **E**, **F**, **G** and **H**, suggest that further synthesis should be driven in terms of such chemical structures.

Acknowledgement

FAPEMIG (Grant Nos. CEX 415/06, CEX 353/06, and CEX 97/07) and FAPESP (Grant No. 2005/59649-0) are gratefully acknowledged for financial support of this research, as is CNPq for the studentship (J.E.A.) and fellowships (M.P.F. and R.R.).

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