Correlation between the predicted and the observed biological activity of the symmetric and nonsymmetric cyclic urea derivatives used as HIV-1 protease inhibitors. A 3D-QSAR-CoMFA method for new antiviral drug design

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Abstract

The predicted inhibition constant (K_i) and the predicted inhibitor concentration (IC_{90}) of the HIV-1 protease (HIV-1 PR) inhibitors: symmetric and nonsymmetric - benzyl, ketone, oxime, pyrazole, imidazole, and triazole cyclic urea derivatives, were obtained by the 3D-CoMFA (Comparative Molecular Field Analysis) method. The CoMFA statistical parameters: cross-validate correlation coefficient (q^2), higher than 0.5, and the fitted correlation coefficient (r^2), higher than 0.90 validated the predicted biological activities. The best predictions were found for the trifluoromethyl ketoxime derivative ($\log 1/K_i$ predict = 8.42), the m-pyridineCH₂ pyrazole derivative ($\log 1/K_i$ predict = 9.77) and the 1,2,3 triazole derivative ($\log 1/K_i$ predict = 7.03). We attempted to design a new potent HIV-1 protease inhibitor by addition of o-benzyl to the (p-HOPhCH₂) pyrazole 12f derivative inhibitor. A favorable steric area surrounded the o-benzyl, suggesting a possible new potent HIV-1 protease inhibitor.

Keywords: 3D-QSAR-CoMFA • the predicted biological activity • cyclic urea derivatives • HIV-1 protease inhibitors

Introduction

Acquired immunodeficiency syndrome (AIDS) typically leads to opportunistic infections or malignancies associated with the underlying damage to the

immune system characterized by the progressive loss of CD4 helper T cells. The HIV genome encodes enzymes necessary for the viral replication such as protease, transcriptase and integrase [1, 3]. These proteins are translated as part of the larger polyprotein precursor whose proteolytic processing during virus assembly and maturation is performed by the protease (PR) [1, 3, 4]. Therefore, PR is an essential enzyme for HIV life-cycle, the inhibition

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of the HIV-1 protease *in vivo* leads to immature and noninfectious viral particles and represents a very attractive target for the synthesis of new antiviral drugs. HIV-1 PR is an aspartyl protease that is functional as a dimer of two identical subunits with 99 amino acid residue. The dimer has one active site region, situated at the interface between the two monomers, with one catalytic triad (Asp-Thr-Gly) from each monomer [1-4].

Currently, there are several FDA approved PR inhibitors in the clinical use saquinavir, indinavir, nelfinavir, zidovudine and zalcitabine [4]. Unfortunately, despite the success in the viral load reduction by existing anti-HIV inhibitors, the widespread use of these chemotherapeutics has resulted in the emergence of drug-resistant mutants that pose a continuing challenge to the design of new active compounds.

Many crystallographic [1-4] and energetic studies [5-9] about the HIV-1 PR, wild type and mutants, have made the enzyme an attractive target for the computer-aided drug design strategy [10-12]. The resistance of the HIV-1 PR mutants obliged us to apply very fast and precise techniques, able to predict the biological activity for the new HIV-1 PR inhibitors. QSAR (quantitative structure-activity relation) techniques [12-14], especially 3D-QSAR-CoMFA (Comparative Molecular Field Analysis) or 3D-QSAR-CoMSIA (Comparative Molecular Similarity Analysis) [15-20] proved to be very useful when the HIV-1 PR inhibitors potency are to be predicted. In the last years, a good correlation between the predicted and the observed biological activity for the HIV-1 PR cyclic urea inhibitors was reported [16, 20, 21]. In a previous work [20] we established a QSAR by the CoMFA method for a series of 45 cyclic urea derivatives used as HIV-1 PR inhibitors. A docking study [9] was performed by us for the binding of the cyclic urea inhibitor DMP323 to the HIV-1 PR.

In this study, the predicted inhibition constant, K_i , and the predicted inhibitor concentration IC_{90} , of the HIV-1 PR inhibitors were performed by 3D-QSAR-CoMFA using Sybyl 6.4 (Software Tools for Molecular Modeling & Visualization, Advanced Computation, NMR Analysis & Structure Generation, Bioinformatics & Structure-Based Design, Combinatorial Chemistry & Molecular Diversity, Pharmacophore Perception and QSAR, developed by Tripos, Inc., <http://www.tripos.com/>,

license used by the Faculty of Chemistry, Biology and Geography, West University, Timisoara) [22, 23]. The symmetric and nonsymmetric cyclic urea derivatives used as HIV-1 PR inhibitors were taken from the literature [24, 25]. The low inhibition constants of the HIV-1 PR inhibitors (for the most active inhibitor-6a, $K_i = 0.01 \text{ nM}$) was the reason for selecting these cyclic urea derivatives in our QSAR study.

The CoMFA method was used firstly to predict the biological activity ($\log 1/K_i$, respective $\log 1/IC_{90}$) of the symmetric and nonsymmetric: ketone, oxime, pyrazole, imidazole and triazole and benzyl (DMP450) cyclic urea derivatives used as HIV-1 PR inhibitors. Secondly, the CoMFA method was applied to predict the biological activity ($\log 1/K_i$) for the symmetric benzyl cyclic urea derivatives (the DMP450 is present, too), only, which are used as viral inhibitors.

The satisfactory statistical correlation coefficients: cross-validate correlation coefficient q^2 and fitted correlation coefficient r^2 allowed us to design a new, possible more potent HIV-1 PR inhibitor. So, we choose to add *o*-benzyl to *p*-HOPhCH₂-(12f HIV-1 PR inhibitor), because the inhibitor activity of the cyclic urea derivatives is improved if the induced hydrophobic effect ensured an easier passage of the HIV-1 PR inhibitors through the biological membrane. By displaying the favorable and unfavorable steric areas of the 12f derivative ($R = (\textit{o}-benzyl, *p*-HO)PhCH₂) (by color polyhedras) we detected the supplementary favorable steric area induced by *o*-benzyl. In this condition we suppose that a suitable steric interaction between the 12f pyrazole derivative ($R = (\textit{o}-benzyl, *p*-HO)PhCH₂) and the HIV-1 PR active site is possible.$$

Materials and methods

Molecular modeling of HIV-1 PR inhibitors

In our study we selected from the literature a set of symmetric and nonsymmetric cyclic urea derivatives used as HIV-1 PR inhibitors [24, 25]. The selection of these inhibitors followed some criteria: (i) the very low inhibition constants (e.g. the most active inhibitor, 6a, having $K_i = 0.01 \text{ nM}$), (ii) the existence of a symmetric and nonsymmetric substitution on the cyclic urea, taking in account that

nonsymmetric HIV-1 PR inhibitors are seldom used, because of the symmetric crystallographic structure of HIV-1 PR [1] and (iii) the great number of various possible substituents to cyclic urea (e.g. nitrobenzyl, aminobenzyl, cyanobenzyl, hydroxybenzyl, (benzyloxy)benzyl, ketone, oxime, pyrazole, imidazole and triazole). The HIV-1 PR inhibitors modeling was performed in Sybyl 6.4 [26].

From the HIV-1 PR wild type -cyclic urea derivative (DMP323) complex, obtained from PDB (<http://www.rcsb.org/pdb/>, Brookhaven National Laboratory, Protein Data Bank, file 1QBS) [27], the cyclic urea derivative DMP323 [24, 25, 28-30] was extruded and used as template for HIV-1 PR inhibitors modeling. We kept unchanged the common cyclic urea, and specific substituents were added.

Minimum energy

Energy optimization of HIV-1 PR inhibitors was run using the Maxim 2 minimization routine in Sybyl 6.4 [31] with Tripos force field [32], Conjugate-Gradient algorithm [32]. After the minimum energy was obtained, the Gasteiger-Marsili partial charges of the HIV-1 PR inhibitors [32, 33] were loaded on the chemical structures from the Sybyl 6.4 dictionary [31, 33].

CoMFA methodology

The superposition of C-a cyclic urea inhibitors on the C-a cyclic urea template molecule 6a, ($K_i = 0.01\text{nM}$), lead to the "common scaffold", which was obtained in Sybyl 6.4 [26, 34]. The steric and the electrostatic CoMFA fields energies of each HIV-1 PR inhibitor were calculated using an sp^3 carbon probe atom, with a +1 charge, at all the intersections in regularly spaced ($2\text{\AA}$) grids in a grid box (which simulated the active site and is automatically created in Sybyl), surrounding each molecule. The Lennard-Jones 6-12 potential and Coulomb potential functions within the Tripos force field [32] were used in calculation.

The regression analysis was performed using the Partial Least Squares [13, 26, 33, 34] (PLS) algorithm within Sybyl 6.4. The statistical parameters: PRESS (Predictable Residual Sum Squares), SD (standard deviation) and q^2 [13, 15, 34, 35]

were obtained by leave-one-out cross-validation technique [13, 15, 33]. We considered that the leave-one-out cross-validation technique, which is known to be more suitable than other statistical techniques, is more appropriate for calculation of statistical parameters in our CoMFA study, kept into account that the HIV-1 PR inhibitors present different chemical types of radicals. So, we avoid to used other technique such as leave-many-out cross validation which in our conception could leads to worse statistical parameters: PRESS, SD and q^2 . Further, the control criterions: r^2 coefficient [13, 34], SEE (standard error of estimate) [13, 33, 34] and F (Fisher) [13, 33, 34] were predicted in Sybyl CoMFA module by non-cross-validated method [13, 33, 34].

The favorable and unfavorable steric areas representation surrounding the 12f ($R = \text{p-HOPhCH}_2$) and its new derivative ($R = (\text{o-benzyl, p-HO})\text{PhCH}_2$) was performed in Sybyl 6.4, CoMFA module, by color polyhedras.

Results

Initial, the statistical correlation coefficients: $q^2 = 0.525$ and $r^2 = 0.981$ allow us to predict the inhibition constant, K_i , of the symmetric and nonsymmetric ketones (5c,d,f), oximes (6a-e), symmetric pyrazoles (10a, b), nonsymmetric pyrazoles (12a-g), imidazoles (10c,d), triazoles (10e, f) and DMP450 cyclic urea derivative used as HIV-1 PR inhibitors (Table 1).

Because, the CoMFA method needs electrostatic and steric descriptors for the biological activity prediction, their contributions were recorded. Table 1A shows that the steric descriptor (0.586) has a larger contribution than the electrostatic one (0.414). Starting from initial CoMFA model, by elimination of the 10c inhibitor ($R = 2\text{-imidazole}$) a new CoMFA model was obtain described by a significant improvement of the statistical parameters: $q^2 = 0.590$, $r^2 = 0.988$, $\text{SEE} = 0.096$ and Fisher test = 188.504 was noticed (Table 1B). The predicted and the observed biological activities (as $\log 1/K_i$) of the ketones, oximes, pyrazoles, imidazoles, triazoles and DMP450 cyclic urea derivative used as HIV-1 PR inhibitors, after elimination of the 10c derivative inhibitor, are shown in Table 2.

Table 1 The results of the CoMFA-PLS Analysis used to evaluate the prediction quality for the biological activity of the HIV-1 PR cyclic urea inhibitors: A, B when the predicted inhibition constants (K_i) of the ketone (5c,d,f), oxime (6a-e), symmetric pyrazole (10a,b), nonsymmetric pyrazole (12a-g), imidazole (10c,d), triazole (10e,f) and DMP450 were calculated before (A) and after (B) the elimination of 10c derivative inhibitor; C. when the predicted inhibitors concentration (IC_{90}) of the ketone (5a-e), oxime (6a-d), symmetric pyrazole (10a,b), nonsymmetric pyrazole (12a-g), imidazole (10d), triazole (10e,f) and DMP450 cyclic urea derivative inhibitors were calculated; D. when the predicted inhibition constants (K_i) of the benzyl cyclic urea derivative inhibitors were calculated.

Statistical parameters	Values			
	A	B	C	D
molecules number	22	21	22	21
q^2	0.525	0.590	0.627	0.536
r^2	0.981	0.988	0.984	0.982
Standard error of estimate(SEE)	0.116	0.096	0.091	0.128
Fisher	129.04	188.50	150.41	128.65
Electrostatic contribution	0.414	0.424	0.422	0.588
Steric contribution	0.586	0.576	0.578	0.412

The well predicted biological activities of the pyrazoles cyclic urea derivative (12a-g) allow us to design a new pyrazole cyclic urea derivative using as template the 12f inhibitor. In our opinion an important improvement of the inhibitory activity could be obtained if the HIV-1 PR inhibitors would have the possibility to cross easier through biological membranes. So, we decided to increase the number of hydrophobic groups of the pyrazole 12f derivative, by adding of o-benzyl to the p-HOPhCH₂. The predicted biological activity (A_i) of 12f derivative was obtained. We noticed its increase from $A_i = 10.72$ (12f) to $A_i = 11.10$ (for the 12f derivative). We extended our study by adding m-benzyl on the p-HOPhCH₂. As control criterion we looked for the favorable steric areas (by color polyhedras) which formed around the 12f derivatives. Fig 1 shows the favorable and unfavorable steric areas (by color polyhedras chosen by the worker) formed around the 12 f inhibitor and its derivative ($R = (o\text{-benzyl}, p\text{-HO})PhCH_2$).

For certain ketones (5a-e), oximes (6a-d), symmetric pyrazoles (10a, b), nonsymmetric pyrazoles (12a-g), imidazoles (10d), triazoles (10e, f) and DMP450 cyclic urea derivative used as HIV-1 PR inhibitors, a prediction of the inhibitor concentra-

Fig. 1 The CoMFA steric favorable and unfavorable areas are represented by color polyedras. a. 12f ($R = p\text{-HOPhCH}_2$) - shows steric favorable (green) and unfavorable (yellow) areas; b. 12f derivative ($R = (o\text{-benzyl}, p\text{-HO})PhCH_2$) has a supplementary favorable steric area (violet) formed around of o-benzyl, the unfavorable steric areas are represented by green. Image worked in Sybyl 6.4.

tion, IC₉₀, was performed. The statistical parameters: $q^2 = 0.627$, respective $r^2 = 0.984$ (Table 1C), obtained after elimination of 6e and 10c inhibitors, allow us to predict the biological activities (as $\log 1/IC_{90}$) for these HIV-1 inhibitors (Table 2). Must be mentioned that before selected the final CoMFA model we investigated other two CoMFA models

which, supplementary, contained outliers: oxime 6e and imidazole 10c inhibitors, that were eliminated successive from models. For these models the statistical q^2 was less than 0.4.

We used the CoMFA method also for the inhibition constant prediction of the symmetric benzyl-cyclic urea derivatives used as HIV-1 PR inhibitors.

Table 2 The predicted and the observed biological activity of the ketone (5a-f), oxime (6a-e), symmetric pyrazole (10a,b), nonsymmetric pyrazole (12a-g), imidazole (10c,d), triazole (10e,f) and benzyl (9b-s,v-x) cyclic urea derivative used as HIV-1 PR inhibitors

index	Substituents		$(\log 1/K_i)_{obs}$	$(\log 1/K_i)_{pred}$	$(\log 1/IC_{90})_{obs}$	$(\log 1/IC_{90})_{pred}$
	R	X				
5a	H	O	-	-	7.45	7.37
5b	Me	O	-	-	7.41	7.48
5c	Et	O	9.68	9.56	6.85	7.02
5d	nPr	O	8.86	8.99	5.85	5.84
5e	CF ₃	O	-	-	7.49	7.42
5f	tBu	O	8.45	8.47	-	-
6a	H	N(OH)	11.01	10.98	8.31	8.31
6b	Me	N(OH)	10.75	10.85	8.70	8.77
6c	Et	N(OH)	10.51	10.64	8.16	8.08
6d	nPr	N(OH)	10.51	10.33	7.20	7.18
6e	CF ₃	N(OH)	8.41	8.42	-	-
	dmp450		9.56	9.50	6.89	6.82

index	Substituents	$(\log 1/K_i)_{obs}$	$(\log 1/K_i)_{pred}$	$(\log 1/IC_{90})_{obs}$	$(\log 1/IC_{90})_{pred}$
10a	3-pyrazole	10.57	10.54	7.70	7.64
10b	4-pyrazole	9.21	9.19	7.50	7.56
10c	2-imidazole	9.79	-	6.63	-
10d	4-imidazole	9.73	9.75	7.08	7.02
10e	1,2,3 triazole	9.77	9.87	7.03	7.03
10f	1,2,4 triazole	10.29	10.24	6.90	6.96

index	Substituents	(log 1/K _i)obs	(log 1/K _i)pred	(log 1/IC ₉₀)obs	(log 1/IC ₉₀)pred
12a	H	9.59	9.56	7.18	7.29
12b	m-MeO ₂ C PhCH ₂	9.86	9.78	7.50	7.45
12c	m-HOH ₂ C PhCH ₂	9.80	9.87	6.64	6.88
12d	m-H ₂ NPhCH ₂	10.46	10.43	7.57	7.42
12e	m-pyridine CH ₂	9.77	9.77	7.03	7.04
12f	p-HOPhCH ₂	10.68	10.72	7.53	7.60
12g	m-(morpholine ethyl-NHCO) PhCH ₂	10.29	10.33	6.90	6.88

index	Substituents	(log 1/K _i)obs	(log 1/K _i)pred
9b	allyl	8.29	8.22
9c	n-propyl	8.10	8.41
9d	n-butyl	8.86	8.69
9e	3,3-dimethylallyl	8.80	8.75
9f	3-methylbutyl	7.93	7.97
9g	cyclopropylmethyl	8.68	8.64
9h	cyclobutylmethyl	8.89	8.81
9i	cyclopentylmethyl	8.37	8.38
9j	cyclohexylmethyl	7.44	7.45
9k	benzyl	8.53	8.57
9l	3-nitrobenzyl	8.56	8.58
9m	4-nitrobenzyl	7.50	7.58
9n	3-aminobenzyl (DMP450)	9.56	9.52
9o	4-aminobenzyl	8.96	9.16
9q	3-cyanobenzyl	8.53	8.50
9p	4-cyanobenzyl	7.29	7.15
9r	3-hydroxybenzyl	9.93	9.98
9s	4-hydroxybenzyl	9.93	9.90
9v	3-(hydroxymethyl)benzyl	9.86	9.81
9w	4-(hydroxymethyl)benzyl	9.47	9.44
9x	2-naphthylmethyl	9.51	9.44

Statistical parameters q^2 and r^2 were suitable ($q^2 = 0.536$, $r^2 = 0.982$) (Table 1D) when outliers: 9a ($P2 = \text{hydrogen}$), 9t ($P2 = 3\text{-(benzyloxy)benzyl}$) and 9u ($P2 = 4\text{-(benzyloxy)benzyl}$) were excluded from the training set. We mentioned that a lot of three CoMFA models were investigated before to chose the final CoMFA model. These models were obtained by successive elimination of outliers 9a, 9t and 9u. For all these models the statistical parameter was less than 0.4. Table 2 shows the predicted and observed biological activity of the HIV-1 PR benzyl cyclic urea derivative inhibitors.

Discussion

The predicted biological activity of the symmetric and nonsymmetric cyclic urea derivatives as very potent HIV-1 PR inhibitors was performed by the CoMFA method. The experimental data [25] show that the inhibitory potency of the ketone derivatives decreased while the size of the R- group increased (e.g. 5b ($R = \text{methyl}$), $K_i = 0.06\text{nM}$, 5c ($R = \text{ethyl}$), $K_i = 0.21\text{nM}$, 5d ($R = \text{n-propyl}$), $K_i = 1.4\text{nM}$, and 5f ($R = \text{t-butyl}$), $K_i = 3.6\text{nM}$). Contrary, for the oxime derivatives (6a-e) a highest inhibitory activity was detected (6a ($R = \text{H}$), $K_i = 0.01\text{nM}$ and 6b ($R = \text{Me}$), $K_i = 0.018\text{nM}$). The pyrazole (10a,b), imidazole (10c,d) and triazole (10e,f) cyclic urea derivatives are all tight binders of the HIV1-PR. Unfortunately, for these inhibitors a low oral bioavailability, despite an excellent chemical stability was found [25]. Starting from the 3-pyrazole derivative, 10a, which has the best K_i and IC_{90} among the heterocycles 10a-g, one has obtained the nonsymmetric cyclic urea (12a-g) for which an improvement of oral bioavailability was recorded [25].

When the predicted inhibition constants, K_i , of the symmetric and nonsymmetric ketone, oxime, pyrazole, imidazole and triazole cyclic urea derivative used as HIV-1 PR inhibitors were analyzed (i) the best correlation between the predicted and the observed biological activity was recorded for: 6e (trifluoromethyl ketoxime, residual value (the difference between the predicted and observed biological activity) = 0.01) and 12e ($R = \text{m-pyridineCH}_2$, residual value = 0.00), (ii) while 6d (n-propyl oxime) reached only the resid-

ual value of 0.18. Because an increase of the electrostatic descriptor contribution (from 0.414 to 0.424) and a decrease of the steric contribution (from 0.586 to 0.576) was found by 10c outlier elimination, we supposed that a certain unfavorable steric effect was induced by the 2-imidazole. Even if, the increase / decrease rate is not significant we must notice that, after outlier elimination, an improvement of q^2 and r^2 was recorded (see Table 1A,B).

Recent pharmacokinetic studies reported a high inhibitory potency of the nonsymmetric HIV-1 PR inhibitors [4, 16, 25]. In our study we obtained a good correlation between the predicted and the observed biological activity (as $\log 1/K_i$) of the nonsymmetric cyclic urea derivatives used as HIV-1 PR inhibitors (12a-g), the residual values range from 0.00 (12e) to 0.08 (12b). Basically, the hydrophobic effect is very important when the enzyme-inhibitors interactions are studied. The easy sliding of inhibitors through biological membranes together with a favorable electrostatic enzyme-inhibitor interaction provided an efficient HIV-1 PR inhibition. On the other hand we suggest that the hydrophobicity could represent an important factor when the viral resistant process is observed. The different hydrophobic parameters which described the viral mutations could determine a favorable or not fashion of enzymatic inhibition of the HIV-1 PR active, especially when the mutations are localized close of active site.

Below, we try to give a possible correlation between the hydrophobic parameters and inhibitory activities of the HIV-1 PR inhibitors symmetric and nonsymmetric - benzyl, ketone, oxime, pyrazole, imidazole, and triazole cyclic urea derivatives. Such as general observation, the presence of hydrophobic radicals (e.g. naphthylmethyl, methyl or isopropyl) belong to chemical structure of inhibitors induced a high enzymatic inhibition to HIV-1 PR active site. For HIV-1 PR benzyl cyclic urea derivative inhibitors can be notice that the presence of hydrophobic groups as: naphthylmethyl (9x, $A_i = 9.51$), 3-(hydroxymethylbenzyl) (9v, $A_i = 9.86$) or 4-hydroxybenzyl (9s, $A_i = 9.93$) can be correlated with a suitable biological activity while, the inhibitors having as radicals cyano (9q, $A_i = 7.29$) or nitro (9m, $A_i = 7.50$) groups are poorly inhibitors. Must be remark that in our CoMFA study we obtained a very good correlation

between the predicted and the observed biological activity for these compounds (see Table 2).

If a correlation between hydrophobic parameters and biological activity is done for the symmetric and nonsymmetric ketones, oximes, pyrazoles, imidazoles, triazoles and DMP450 cyclic urea derivative used as HIV-1 PR inhibitors can be notice that the inhibitors having as radicals: methyl (6b, $A_i = 10.75$), ethyl (5c, $A_i = 9.68$, 6c, $A_i = 10.51$) and n-propyl (5d, $A_i = 8.86$, 6d, $A_i = 10.63$) groups are very potent inhibitors. By this correlation we can show again that the hydrophobic parameter is very important for the enzymatic inhibition but if the goal of study is the enzymatic interaction the hydrophobic parameter must be evaluates near with the steric and van der Waals interactions of the HIV-1 PR and its inhibitors.

So, the appropriate correlation between the predicted and the observed biological activity of the 12f derivative, allow us to model new pyrazole derivatives by successive additions of o-benzyl, o,o'-benyl and m-benzyl to p-HOPhCH₂ (12f), for which the biological activity was predicted. Unfortunately, we found only for the pyrazole 12f derivative ($R = (\text{o-benzyl, p-HO})\text{PhCH}_2$) a higher predicted biological activity (11.10) than for 12f (10.72). The visualization of favorable and unfavorable steric areas (by color polyhedras) were useful in our trial to detect the supplementary favorable steric area formed around the (o-benzyl, p-HO)PhCH₂ radical (Fig. 1). For a complete study, the representation of favorable and unfavorable steric areas of 12f was done (Fig.1). The higher predicted biological activity of the 12f derivative ($R = (\text{o-benzyl, p-HO})\text{PhCH}_2$) could be explained by the favorable steric effect induced by the conjugate presence of the p-HOPhCH₂ and o-benzyl. Instead, an obvious unfavorable steric effect is induced when the m-benzyl substituent is taken into account (the predicted biological activity of the 12f derivative ($R = (\text{m-benzyl, p-HO})\text{PhCH}_2$) was less than 10.32). We have not a clear explanation for the fact that the addition of m-benzyl to p-HOPhCH₂ lead to a decrease of biological activity, but we suppose that the simultaneous presence of m-benzyl and p-HOPhCH₂ induced a strong steric unfavorable effect. So that, the 12f derivative ($R = (\text{m-benzyl, p-HO})\text{PhCH}_2$) is pushed out of the energetic grid box which simulated the active site and a decrease of the steric descriptor follows.

A progressive decrease of the predicted biological activity for the pyrazole 10b derivative (residual value = 0.02), triazole 10e derivative (residual value = 0.10) and triazole 10f derivative (residual value = 0.05) was correlated with the presence of the nitrogen aromatic ring. We suggest that the progressive increasing of the nitrogen atoms number in this compounds series explains the presence of the unfavorable steric effect.

For the HIV-1 PR ketone (5a-e), oxime (6a-d), symmetric pyrazole (10a,b), nonsymmetric pyrazoles (12a-g), imidazole (10d), triazole (10e,f) and DMP450 cyclic urea derivative inhibitors the biological activity was predicted as $A_i = \log 1/IC_{90}$. The results given in Table 1C shows that the q_2 and r_2 parameters are statistically better than the similar parameters described by the CoMFA model, obtained when the predicted biological activity was calculated as $\log 1/K_i$ (Table 1A). The correlation between the predicted and the observed biological activity allow us the following observations: (i) best correlations were obtained for 5d ($R = \text{n-propyl}$, $X = \text{O}$, residual value = 0.01), 6a ($R = \text{H}$, $X = \text{N(OH)}$, residual value = 0.00), 10e ($R = 1,2,3$ triazole, residual value = 0.00), 12e ($R = \text{m-pyridine}$, residual value = 0.01) while (ii) unsatisfactory correlation was recorded for 5c ($R = \text{ethyl}$, $X = \text{O}$, residual value = 0.17) and 12d ($R = \text{m-H}_2\text{NPhCH}_2$, residual value = 0.15) (Table 2)

For the HIV1-PR benzyl cyclic urea derivative inhibitors the best correlation between the predicted and observed biological activity is obtained for: 9i ($P_2 = \text{cyclopentylmethyl}$, residual value 0.01), 9j ($P_2 = \text{cyclohexylmethyl}$, residual value 0.01), 9l ($P_2 = 3\text{nitrobenzyl}$, residual value 0.02). Instead, the 9o ($P_2 = 4\text{aminobenzyl}$) recorded the highest residual value (0.20).

We compared the predicted biological activity for the HIV-1 PR benzyl cyclic urea derivative inhibitors having the substituted aromatic ring in *meta* and *para* positions. The p-substituted aromatic ring (e.g. 4nitrobenzyl (9m), 4 aminobenzyl (9o), 4 cyanobenzyl (9q) and 4 hydroxybenzyl (9s)) shows a worse prediction of the biological activity than similar substituted aromatic ring in m-position (e.g. 3nitrobenzyl (9l), 3aminobenzyl (9n), 3cyanobenzyl (9p) and 3hydroxybenzyl (9r)) (see Table 2). We suggested that the spatial orientation of the p-substituents out of hypermolecule could explain this fact.

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