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Quantitative Structure-Activity Relationship Study on Phthalimide Derivatives as HIV-1 Reverse Transcriptase Inhibitors

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ABSTRACT Quantitative structure-activity relationships (QSAR) was performed to correlate the physicochemical properties, ie, electronic, hydrophobic, and steric properties with the HIV-1 reverse transcriptase inhibitory activity of phthalimide derivatives. Multiple linear regression (MLR) analysis was used for the study. The best QSAR model was obtained with correlation coefficient (r) of 0.974 and cross-validated r^2 (q^2) of 0.785. This model indicates that the inhibitory activity correlated to the electronic properties, ie, partial atomic charge at carbon 2, the highest occupied molecular orbital (HOMO), and the dipole moment. This activity also correlated to the steric property, ie, molecular volume (MV) and the hydrophobic property, log P.

KEYWORDS: QSAR, HIV-1 reverse transcriptase, phthalimide derivatives, non-nucleoside reverse transcriptase inhibitors.

INTRODUCTION

The virus-encoded reverse transcriptase (RT) plays an important role in the life cycle of the human immunodeficiency virus type 1 (HIV-1), the causative agent of AIDS. RT is a multifunctional enzyme having RNA-directed DNA polymerase and DNA-directed DNA polymerase activities as well as RNase H activity.[1] This enzyme is an attractive target for developing anti-HIV drugs for the treatment of AIDS. Several compounds targeted against HIV-RTI have been shown to be active in clinical trials.[2-5] So far, nine drugs from two classes have been approved by the US FDA, ie, AZT, ddI, ddC, d4T, 3TC, abacavir, nevirapine, delavirdine, and efavirenz. The first six drugs are members of the nucleoside class and the others belong to the non-nucleoside class. Treatment by these drugs usually leads to the development of resistant HIV-1 mutants. The emergence of resistant strains has necessitated the continuation of research to find new inhibitors. Recently, non-nucleoside HIV-1 RT inhibitors (NNRTIs)[4,5] have played an important role in the treatment of HIV infections, and several of them have been investigated for use in alternative or combination therapy. These compounds are highly active against HIV-1, but inactive against HIV-2 or any other retrovirus. This unique specificity of the NNRTIs for HIV-1 RT is due to the presence in HIV-1 RT, but not in other reverse transcriptases, of a flexible, highly hydrophobic pocket.[6,7] The studies of amino acids responsible for drug-resistance of HIV-1 RT suggested that this pocket determines the binding of the NNRTIs with their target site. These amino acid residues are Leu 100, Val 106, Val 108, Val 179, Tyr 181, Tyr 188, Gly 190, Met 230, and Pro 236. The binding of the NNRTIs to the hydrophobic pocket of the HIV-1 RT does not interfere with the binding of the dNTPs (deoxynucleotide triphosphates) but slows down the rate of incorporation of the dNTPs into the DNA product.[8]

Previous investigations in our research group have identified the phthalimide derivatives as a new class of non-nucleoside HIV-1 RT inhibitors.[9] The synthesized compounds were tested *in vitro* for their HIV-1 reverse transcriptase inhibitory activity at concentration of 200 μg/mL by radiometric assay using polyadenylic acid (poly A) as template, oligodeoxythymidylic acid (oligo dT) as primer, and radiolabeled thymidine triphosphate ([^{3}H]dTTP) as substrate. The activity was measured corresponding to the degree of inhibition of incorporation of

[^{3}H]dTTP into a polymer fraction by the synthesized compounds. The inhibitory activity was reported as % inhibition as shown in Table 1. The most potent activity, IC_{50}, 60.90 μg/mL, was obtained with compound 5, whereas compounds 11 and 2 were less potent with IC_{50} 98.10 μg/mL and 120.75 μg/mL respectively.[9] These three compounds exhibited IC_{50} value lower than that of delavirdine (IC_{50} = 502.22 μg/mL) and AZT in HIV-1 strain M48, ddI-resistant strain (IC_{50} = 184.69 μg/mL, using poly rI as template, oligo dC -primer and [^{3}H]dCTP as substrate).[10-11]

Quantitative structure-activity relationships (QSAR) was investigated based on the fact that the biological activity of a compound is a function of its physicochemical properties.[12-14] Physicochemical parameters which are representatives of structural features of compounds are, for examples, electronic effects, hydrophobicity (lipophilicity) and steric properties. In this study, the QSAR analysis of the compounds in this phthalimide series was performed based on the assumption of linear additive contributions of the different physicochemical properties mentioned above to the HIV-1 RT inhibitory activity. The most commonly used statistical and mathematical method, multiple linear regression (MLR) analysis, was applied to create QSAR models and to obtain statistical data values, ie, correlation coefficient (r), standard deviation (s), F-test, cross-validated correlation coefficient (q^2), and sum of square standard error (S_{PRESS}). These statistical data were used to evaluate the obtained QSAR models. The best-derived QSAR model was used to predict activity of the untested compounds and to suggest structural feature(s) which should be modified in order to improve activity.

Materials and Methods

Biological Data

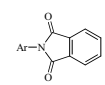
The previously synthesized phthalimide compounds (Table 1) were tested for their HIV-1 reverse transcriptase inhibitory activity by a radiometric assay.[9, 15-17] The results of the inhibitory activity were reported as present inhibition and were used as dependent variables in this QSAR study.

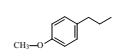
Generating the molecular structures and conformational analysis

The molecular structures of phthalimide derivatives were modeled with SYBYL 6.5 molecular modeling program (Tripos Associates, Saint Louis, MO) on an Indigo Elan workstation (Silicon Graphics Inc, Mountain View, CA) using the sketch approach. The fragment libraries in the SYBYL database containing small molecules were also used as building blocks for the construction of larger ones. Each structure was first energy minimized using the standard Tripos force field (Powell method and 0.05 kcal/mol*Å energy gradient convergence criteria) and electrostatic charge was assigned by the Gasteiger-Hückel method.

Since compounds in the training set possess a high degree of conformational flexibility, geometry optimization was performed by using a systematic conformational search. In the systematic conformational search process, the rotatable bonds of compounds (Figure 1) were rotated by 2 degree increments for all possible combinations to achieve the global minimum energy conformations. These conformations were used as starting geometries for the following optimization using the MOPAC 6.0 (PM3) interface. Finally, these molecules were further minimized by the standard Tripos force field (Powell method and 0.01 kcal/mol*Å energy gradient convergence, the same criteria used in the initial step of energy minimization). The conformations obtained for all molecules in the training set were used in the following QSAR study.

Table 1. *In vitro* RT inhibitory activity of phthalimide derivatives at concentration 200 μg/ml.



Cpd	Ar	% inhibition[a]	Cpd	Ar	% inhibition[a]
1		62 ± 11.6	7		11 ± 9.1
2		84 ± 2.1	8		8 ± 3.7
3		21 ± 4.6	9		41 ± 1.5
4		32 ± 6.9	10		20 ± 6.9
5		76 ± 2.0	11		78 ± 7.9
6		3 ± 4.3			

[a] The values represent the mean ± standard deviation of three separate determinations.

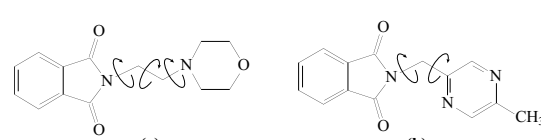


Fig 1. The rotatable bonds of molecules used in the systematic conformational search procedure: (a) three rotatable bonds and (b) two rotatable bonds.

QSAR Study

Calculation of physicochemical properties

The physicochemical properties used in this study were electronic, hydrophobic and steric properties. The representatives of electronic properties were partial atomic charges, electrostatic energy (ES), dipole moment, frontier orbital, ie, the highest occupied molecular orbital (HOMO) and the lowest unoccupied molecular orbital (LUMO), and ionization potentials. The partial atomic charges of carbon 1 (C1), carbon 2 (C2) and nitrogen 1 (N1) (as shown in Figure 2) were used for electronic descriptors. Partition coefficient (log P) and lipophilic potential (LP) descriptors were used as representatives of hydrophobic properties. Molar refractivity (MR), molecular weight (MW), molecular surface area (MS), and molecular volume (MV) were used for steric properties.

Log P, MR, MW, MS, and MV were calculated by Hyper Chem Release 3.0 for Windows. LP was calculated by the MOLCAD option in SYBYL 6.5 on the Silicon Graphic Workstation. Partial atomic charges were calculated by the Gasteiger-Hückel method and MOPAC 6.0-PM3 method in SYBYL 6.5. ES was calculated by using SYBYL 6.5 (Gasteiger-Hückel method). HOMO, LUMO energies, ionization potential, and dipole moment were calculated by MOPAC 6.0-PM3 option in SYBYL 6.5.

Multiple linear regression analysis

Multiple linear regression (MLR) analysis was used to investigate the correlation between biological activity and physicochemical properties. The MLR was performed by using the SPSS for Windows Release 7.0 package by the stepwise method. The highest correlation of independent variables with dependent variable was chosen for deriving the QSAR model. The statistical values, multiple correlation coefficient (r), standard errors (s), cross-validation r^2 (q^2) and standard error of prediction (S_{PRESS}) were used to evaluate the obtained QSAR models. Several combinations of independent variables were firstly attempted using three variables (one representative from each property) for individual models, and then, more variables were added in order to optimize the statistical values but not more than five independent variables were used. The best model derived from the MLR analysis was used to predict the inhibitory activity of the phthalimide compounds (structure as shown in Figure 3) which were not included in the training set.

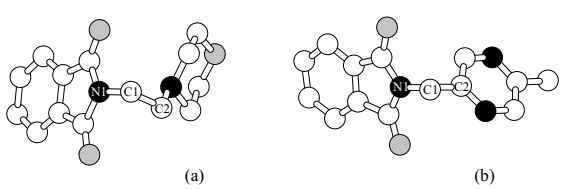


Fig 2. Structure of phthalimide compounds showing atoms used for atomic charge calculation: (a) phthalimide derivatives with two carbon atoms between the phthalimide and heterocyclic moieties and (b) one carbon atom between the two moieties.

Results and Discussion

The QSAR models were generated from the percent inhibition of phthalimide compounds (Table 1) as dependent variable. The calculated electronic, hydrophobic, and steric descriptors as shown in Table 2 were used as independent variables. All possible combinations of the physicochemical descriptors were investigated in order to obtain the best QSAR model. It should be mentioned that more models were obtained from the analysis, but they were ruled out by the MLR stepwise procedure. The stepwise MLR was used to select each independent variable for deriving a QSAR model by considering the correlation between each variable with the dependent variables. The MLR equation used for the QSAR model developed is as follows:

$y = a_1x_1 + a_2x_2 + a_3x_{3\ ...}\ a_nx_n + b$

y = dependent variable (% inhibition)

a_1, a_2, a_3,…a_n = the regression coefficients of independent variables

x_1, x_2, x_3,…x_n = independent variables

b = the regression constant obtained from the fit

To avoid self correlation between the variables used for the derivation of the QSAR model, the correlation matrix was calculated and the result shown in Table 3. The best QSAR model obtained from the MLR analysis is shown as the following equation:

% inhibition = -0.803 (± 0.700) MV + 18.326 (± 11.570) logP – 93.052 (± 79.267) HOMO – 422.111 (± 373.031) $C2_{GH}$ – 85.981 (± 74.882) Dipole – 51.972 (± 1.716)

(n = 11, r = 0.974, s = 9.683, F = 18.664, q^2 = 0.785, S_{PRESS} = 14.090)

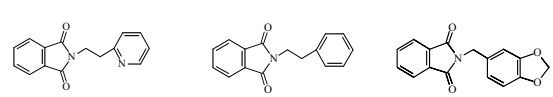


Fig 3. The phthalimide compounds not included in the training set.

Table 2. The calculated physicochemical descriptors values of phthalimide compounds used in QSAR models.

Cpd	% inhi	Molecular descriptors																
		Hydrophobic		Steric				Electronic										
	-bition	logP[a]	LP[b]	MR[a]	MW[a]	MS[a]	MV[a]	$N1_{GH}$[c]	$C1_{GH}$[c]	$C2_{GH}$[c]	$N1_{PM3}$[d]	$C1_{PM3}$[d]	$C2_{PM3}$[d]	Dipole[d]	ES[c]	Ioniz[d]	HOMO[d]	LUMO[d]
1	62	4.45	0.032	43.15	260.29	342.53	743.34	-0.183	0.069	0.023	-0.334	-0.014	-0.095	2.62	0.005	9.295	-9.2946	-1.0069
2	84	5.79	0.049	22.41	253.26	365.09	733.49	-0.170	0.108	0.078	-0.335	0.041	-0.111	2.99	-1.663	9.770	-9.7698	-1.1390
3	21	6.41	0.076	30.72	281.31	381.05	811.22	-0.183	0.065	0.000	-0.348	-0.007	-0.141	2.38	-2.052	8.804	-8.8035	-1.0023
4	32	5.88	0.082	19.28	227.22	318.06	647.97	-0.170	0.199	0.122	-0.354	0.056	-0.069	3.44	-1.913	9.107	-9.1067	-1.1420
5	76	6.05	0.071	22.31	269.26	362.19	742.93	-0.174	0.083	-0.360	-0.349	0.031	-0.119	4.19	-3.892	8.850	-8.8500	-1.1276
6	3	6.30	0.073	27.07	283.28	337.41	772.34	-0.184	0.064	-0.002	-0.349	-0.007	-0.144	2.94	-5.289	8.728	-8.7284	-1.0666
7	11	5.18	0.055	21.89	253.26	340.10	728.58	-0.173	0.091	-0.001	-0.352	0.033	-0.179	3.88	0.466	9.554	-9.5537	-1.1557
8	8	6.36	0.076	29.98	295.29	331.10	793.29	-0.183	0.064	-0.002	-0.350	-0.007	-0.146	2.86	-1.196	8.813	-8.8129	-1.0588
9	41	6.14	0.067	23.40	252.27	373.99	740.78	-0.172	0.093	0.004	-0.351	0.035	-0.176	3.80	0.031	9.563	-9.5632	-1.1239
10	20	6.28	0.062	23.22	252.27	311.41	721.12	-0.182	0.067	0.012	-0.351	-0.007	-0.143	3.98	-1.725	9.619	-9.6194	-1.0997
11	78	5.93	0.079	24.15	241.25	303.47	682.17	-0.182	0.069	0.035	-0.351	-0.008	-0.120	3.05	-2.533	9.030	-9.0304	-1.0429

[a] Calculated by HyperChem 3.0 program
[b] Calculated by MOLCAD program
[c] Calculated by SYBYL 6.5 program using Gasteiger Hückel method
[d] Calculated by MOPAC 6.0-PM3 program

Table 3. The correlation matrix of all variables used in QSAR studies.

	% IR*	Hydrophobic		Steric				Electronic										
		logP	LP	MR	MW	MS	MV	$N1_{GH}$	$C1_{GH}$	$C2_{GH}$	$N1_{PM3}$	$C1_{PM3}$	$C2_{PM3}$	Dipole	ES	Ioniz	HOMO	LUMO
%IR*	1.000	-0.311	-0.331	-0.007	-0.207	0.123	-0.366	0.129	0.092	-0.304	0.051	0.022	0.047	0.141	-0.014	0.241	-0.241	-0.056
logP		1.000	0.795	-0.485	0.327	0.063	0.261	-0.121	-0.083	-0.129	-0.559	-0.045	-0.266	0.080	-0.532	-0.370	0.370	-0.043
LP			1.000	-0.527	0.063	-0.213	-0.088	-0.053	0.205	-0.077	-0.803	0.071	-0.037	0.078	-0.504	-0.598	0.598	-0.039
MR				1.000	0.443	0.134	0.484	-0.642	-0.491	0.064	0.599	-0.680	0.134	-0.656	0.207	-0.224	0.224	0.807
MW					1.000	0.367	0.933	-0.579	-0.670	-0.354	0.102	-0.547	-0.388	-0.383	-0.287	-0.557	0.557	0.466
MS						1.000	0.592	0.274	-0.128	-0.312	0.333	0.239	-0.276	-0.119	0.101	0.040	-0.040	-0.008
MV							1.000	-0.497	-0.694	-0.269	0.212	-0.50	-0.496	-0.415	-0.092	-0.352	0.352	0.479
$N1_{GH}$								1.000	0.732	-0.012	-0.042	0.983	0.114	0.526	0.267	0.518	-0.518	-0.860
$C1_{GH}$									1.000	0.322	-0.19	0.803	0.548	0.221	0.103	0.186	-0.186	-0.576
$C2_{GH}$										1.000	0.104	-0.015	0.143	-0.261	0.369	0.346	-0.346	0.101
$N1_{PM3}$											1.000	-0.148	0.343	-0.472	0.170	0.300	-0.300	0.280
$C1_{PM3}$												1.000	0.153	0.517	0.170	0.392	-0.392	-0.864
$C2_{PM3}$													1.000	-0.263	-0.224	-0.156	0.156	0.111
Dipole														1.000	0.053	0.369	-0.369	-0.774
Es															1.000	0.632	-0.632	-0.110
Ioniz																1.000	-1.000	-0.511
HOMO																	1.000	0.511
LUMO																		1.000

* % IR = % inhibition

This model indicates the contribution of electronic descriptors (ie, $C2_{GH}$, dipole and HOMO energy), hydrophobic descriptor (logP), and steric descriptor (MV) to the enzyme inhibitory activity of these phthalimide compounds. The negative value of the coefficient for the $C2_{GH}$ variable suggests that high electron density around C2 position (Figure 2) will increase biological activity. Therefore, the presence of a strong electron donating substituent at this position will be preferred. The negative value of the coefficient for the HOMO, implies that a low energy of HOMO within the molecule correlates with increased activity. Dipole moment, another electronic descriptor, is a good indicator of the overall polarity within the molecule. The addition of this parameter to the MLR analysis obtained the higher q^2 in every

model studied. This indicates that the dipole moment could be essential for increasing the quality of the QSAR model. The positive coefficient of the logP descriptor which relates to the hydrophobicity of the molecule suggested that increasing the lipophilicity might increase the activity. This corresponds to the presence of the hydrophobic binding site in HIV-1 RT (Pro 95, Leu 100, Val 106, Tyr 181, Tyr 188, Gly 190, Trp 229, Leu 234, and PRO 236). The negative coefficient of the molecular volume descriptor, one of the steric properties, means that a molecule could be too large in size, and then it would not fit into such a hydrophobic pocket in the enzyme non-substrate binding site.

The experimental and calculated (predicted) percent inhibition of compounds in the training set based on the equation above are shown in Table 4 and the residual plot of both values is given in Figure 4. The results indicate a good linear regression of correlation between experimental and calculated percent inhibition. Three phthalimide compounds, ie, compound 12, 13 and 14 which were not included in the training set, were used to test the predictive power of the derived QSAR equation. As seen from the result in Table 5, this QSAR equation gave good predictive ability with the predicted activity corresponding well to the experimental activity. Moreover, a three-dimensional QSAR technique, comparative molecular field analysis (CoMFA) was also applied to the same training set. The CoMFA model was obtained with cross-validated r^2 (q^2) = 0.646 (S_{PRESS} = 28.474 and number of the optimum components = 3) including the lowest unoccupied molecular orbital (LUMO) energies in addition to CoMFA fields (manuscript in preparation). Table 6 summarizes

Table 4. The experimental and calculated percent inhibition of phthalimide derivatives.

Cpd	% inhibition		Residual
	Experimental	Calculated	
1	62	62.2	-0.2
2	84	84.2	-0.2
3	21	28.4	-7.4
4	32	35.3	-3.3
5	76	76.9	-0.9
6	3	3.4	-0.4
7	11	13.2	-2.2
8	8	2.1	5.9
9	41	26.7	14.3
10	20	31.3	-11.3
11	78	72.3	5.7

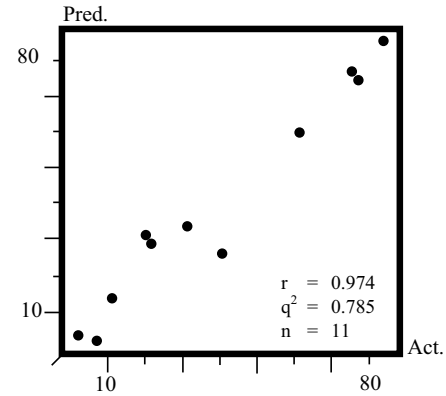
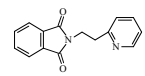
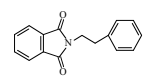
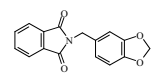


Fig 4. Plot of experimental (actual) versus predicted (calculated based on QSAR equation) percent inhibition of phthalimide compounds in the training set.

Table 5. The predicted activity of compounds not included in training set using the QSAR model.

Cpd	Structures	% inhibition		Residual
		Experiment[a]	Predicted	
12		52 ± 10.3	53.0	-1.0
13		67 ± 4.3	69.2	-2.2
14		94 ± 0.8	108.9	-14.9

* compound was omitted from QSAR table in CoMFA study.

Table 6. The experimental and predicted percent inhibitions calculated from the classical QSAR equation and CoMFA model.

		% Inhibition		
	Cpd		Classical QSAR	CoMFA
	1	62	62.2	63.0
	2	84	84.2	86.3
	3	21	28.4	22.5
	4	32	35.3	*
Training	5	76	76.9	75.6
set	6	3	3.4	2.3
	7	11	13.2	16.1
	8	8	2.1	8.1
	9	41	26.7	34.9
	10	20	31.3	18.5
	11	78	72.3	76.5
	12	52	53.0	53.3
Test set	13	67	69.2	66.9
	14	94	108.9	89.1

* compound 4 was omitted from QSAR table in CoMFA study

the calculated activity of compounds in the training set and test set using QSAR equation and CoMFA model. Both classical and CoMFA models gave comparable correlation between experimental and calculated values.

Conclusion

This QSAR study suggested the structural features of compounds in the phthalimide series which possess promising high HIV-1 reverse transcriptase inhibitory activity. This study also provided predictive utility for untested compounds, and also, it was able to predict activity of new compounds prior to synthesis.

Acknowledgements

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