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3D-QSAR Study of 2-Morpholinochromones as Selective Phosphodiesterase-2 Inhibitors

PRADEEP K. GOEL, RAKESH K. JATAV and N. KAWATHEKAR*
Department of Pharmacy, Shri G.S. Institute of Technology and Science
23, Park Road, Indore-452 003, India
E-mail: neha_kawathekar@yahoo.co.in

Three-dimensional quantitative structure activity relationship study on series of 2-morpholinochromones with phosphodiesterase-2 inhibitory activity was performed using a combination of various thermodynamic, electronic and spatial descriptors. Several statistical regression expressions were obtained using sequential multiple linear regression analysis. The best QSAR model ($r > 0.80$, Fischer test value = 8.45, $S < 0.37$, change correlation < 0.001) have acceptable statistical quality and predictive potential as indicated by the value of cross-validated squared correlation coefficient ($Q^2 > 0.43$). Thermodynamic parameters (standard Gibb's free energy, stretch energy) and electronic parameters (dipole moment, HOMO energy) were found to have significant correlation with biological activity.

Key Words: 3D-QSAR, Phosphodiesterase-2 2-Morpholinochromones.

INTRODUCTION

Phosphodiesterase (PDE) is a key enzyme involved in cellular functions such as cell division, cell differentiation, ion channel, ion transport and energy metabolism. The inhibition of PDE activity increase cellular levels of the key second messengers, cyclic adenosine 5′-monophosphate (cAMP) and cyclic guanosine 5′-monophosphate (cGMP), thereby activating specific protein phosphorylation cascades that elicit a variety of functional responses[1,2]. At the present time there are eleven known PDE isoenzyme famiiies[3] (PDE 1-11), which share the property of hydrolyzing cyclic nucleotides to their 5-monophosphate counterparts. For a number of reasons, interest in the potential therapeutic utility of selective PDE inhibitors has largely been focussed on drugs capable of inhibiting phosphodiesterase-2 (PDE2), which is widely expressed in human tissues and hydrolyses both cAMP and cGMP. This activity may be significant as PDE2 has contrasting enzyme kinetics to other PDEs and is also allosterically activated by cGMP[4]. The understanding of phophodiesterases has been accelerated in recent years by the advances of molecular cloning and concomitant development of isoform selective inhibitors[5].

Recently, a number of PDE2 inhibitors [such as sulindac sulfone and erythro-9 (2-hydroxy-3-nonyl)adenine analogues][6] have shown enormous application in diseases such as cancer[7], scleroderma and cognitive disorders. Considering the recent interest in the development of PDE2 inhibitors, the authors decided to emphasize their attention on 3D-QSAR[8, 9] of 2-morpholinochromones to identify and optimize molecular properties for better inhibition of PDE2.

EXPERIMENTAL

The PDE2 inhibitory data of 2-morpholinochromones (Tables 1 and 2, Fig. 1) were taken from the reported work of Abbott *et al.*[6] All the biological activity

TABLE-1
COMPARISON OF OBSERVED AND LEAVE ONE OUT (LOO) PREDICTED pIC_{50} VALUES OF COMPOUNDS USED IN TRAINING SET

Compd. No.	R_6	R_7	R_8	IC_{50}[a] (μM)	Observed pIC_{50}	Predicted (LOO)[b] pIC_{50}
1	H	H	Ph	54	–0.0696	0.1511
2	H	H	(4-F)Ph	43	0.1224	–0.2873
3	H	H	(2-CH_3)Ph	47	0.0521	0.1099
4	H	H	(2-CH_3O)Ph	47	0.0521	–0.0135
5	H	H	(2-Cl)Ph	62	–0.2126	0.1113
6	H	H	(4-PhO)Ph	75	–0.4771	–0.8517
7	Me	H	Ph	60	–0.1760	0.0199
8	OMe	H	Ph	75	–0.4771	0.0046
9	OMe	H	(2-Cl)Ph	78	–0.5496	–0.1562
10	OMe	H	(4-Cl)Ph	67	–0.3075	–0.4539
11	OH	H	Ph	69	–0.3474	0.1505
12	OMe	H	H	38	0.2126	0.1634
13	H	H	OH	19	0.6297	1.0046
14	H	H	OCH_2Ph	40	0.1760	0.1344
15	H	H	OCH_2 (3-pyridyl)	35	0.2688	0.7884
16	H	H	$(CH_2)_2Ph$	43	0.1224	0.0471
17	H	OCH_2Ph	Me	38	0.2126	0.0743
18	H	$(CH_2)_2$(4-Me-1-piperazinyl)	Me	–2	–1.7077	–1.1155
19	H	Ph	Me	31	0.3474	0.0222
20	H	C≡CPh	Me	44	0.1047	–0.4344
21	H	OCH_2Ph	H	12	0.8653	0.0047
22	H	OCH_2(3-pyridyl)	H	22	0.5496	–0.1563
23	H	C≡CPh	H	92	–1.0606	–0.5965

[a]Inhibitory activity of 2-morpholinochromones evaluated *in-vitro* as inhibitors of isolated human platelets phosphodiesterases (% base).
[b]Leave-one-out (LOO) method.

data (% base at 50 μM or IC_{50}) were converted to pIC_{50} (negative logarithm of IC_{50}) to get a linear relationship in the biological activity. IC_{50} refers to the micromolar concentration of the compounds required for 50% inhibition of PDE2 activities. 30 compounds selected for study were divided into training set of 23 compounds and test set of 7 compounds by random selection method[10]. For molecular modelling and calculation of various descriptors, we have used different modules provided in the software[11] (C.S. ChemOffice) have been used. The 2D structures were converted to 3D structures in the Chem-3D Ultra 6.0 of ChemOffice 6.0. The energy of the molecule was minimized in molecular mechanics 2 (MM2) by fixing root mean square (RMS) gradient to 0.1 kcal/mol Å which is followed by MOPAC by fixing RMS gradient to 0.0001 kcal/mol Å by applying the Austin model-1 (AM1) Hamiltonian approximation method[12]. Various descriptors[13-16] calculated for the energy minimized and geometrically optimized structures, are: bend energy (Eb), connolly accessible surface area (AS), connolly molecular surface area (MS), connolly solvent excluded volume (SEV), dipole moment (DPL), dipole energy (ED), electronic energy (EleE), exact mass (Mass), highest occupied molecular orbital energy (HOMO), lowest unoccupied molecular orbital energy (LUMO), heat of formation (HF), Henry's law constant (H), molar refractivity (MR), molecular weight (MW), non-1,4 van der Waals' energy (EV), ovality, partition coefficient or PC (octanol/water), principal moment of inertia along X, Y and Z axis (PMIX, PMIY and PMIZ respectively), repulsion energy (RE), log P, stretch energy (ES), torsion energy (ET), total energy (E), van der Waals' 1,4 energy (E14), stretch bend energy (Esb) and standard Gibb's free energy (G).

TABLE-2
COMPARISON OF OBSERVED AND PREDICTED pIC_{50} VALUES OF COMPOUNDS USED IN TEST SET

Compd No.	R_6	R_7	R_8	IC_{50}^{a} (μM)	Observed pIC_{50}	Predicted (LOO)[b] pIC_{50}
Test 1	H	H	(2-CF_3)Ph	43	0.3638	0.5726
Test 2	Et	H	Ph	47	–0.2122	–0.5693
Test 3	OMe	H	(3-Cl)Ph	87	–0.2633	–0.0274
Test 4	H	H	OMe	20	0.4497	0.6922
Test 5	H	H	C≡CPh	93	–1.2176	–1.2457
Test 6	H	OCH_2 (3-pyridyl)	Me	35	–0.0930	–0.2219
Test 7	H	Ph	H	9	0.2192	0.4485

[a]Inhibitory activity of 2-morpholinochromones evaluated *in-vitro* as inhibitors of isolated human platelets phosphodiesterases (% base).
[b]Leave-one-out (LOO) method.

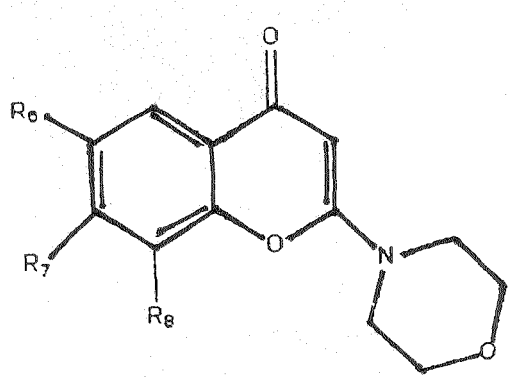

Fig. 1. 2-Morpholinochromones used in present study

Sequential multiple linear regression (MLR)[17, 18] analysis was carried out taking calculated descriptors as independent variables and biological activity as dependent variable using VALSTAT software.

RESULTS AND DISCUSSION

The results of sequential multiple regression analysis are given in Table-3. Among several QSAR models, some models were selected on the basis of

TABLE-3
STATISTICALLY SIGNIFICANT QSAR MODELS DEVELOPED BY MULTIPLE LINEAR REGRESSION (MLR) ANALYSIS

Model No.	Models
1	BA = [–20.1739 (±20.6157)] + G[–0.0018 (±0.0014)] + DPL [0.174 (±0.2065)] + HOMO [–2.3945 (±2.3184)] + ES [–0.4288 (±0.2132)]. n = 23, r = 0.81, S = 0.36, F = 8.45, Q^2 = 0.44
2	BA = [1.5756 (±0.8483)] + G [–0.0024 (±0.0013)] + PMIX [–0.0004 (±0.0004)] + ES [–0.3042 (±0.1979)] + PC [0.1573 (±0.1922)]. n = 23, r = 0.80, S = 0.37, F = 8.07, Q^2 = 0.37
3	BA = [–16.2471 (±20.3482)] + G [–0.0013 (±0.0014)] + HOMO [–2.0583 (±2.3061)] + LUMO [0.8381 (±1.1122)] + ES [–0.4334 (±0.2166)]. n = 23, r = 0.80, S = 0.37, F = 8.06, Q^2 = 0.26
4	BA = [1.5711 (±0.8600)] + LOGP [0.1846 (±0.2371)] + G [–0.0026 (±0.0014)] + PMIX [–0.0004 (±0.0003)] + ES [–0.3106 (±0.1989)]. n = 23, r = 0.79, S = 0.37, F = 7.89, Q^2 = 0.37
5	BA = [–21.9898 (±22.3298)] + G [–0.0021 (±0.0016)] + HOMO [–2.6050 (±2.4967)] + ES [–0.4254 (±0.2189)] + E14 [0.0292 (±0.0429)]. n = 23, r = 0.79, S = 0.37, F = 7.78, Q^2 = 0.39
6	BA = [–13.1986 (±21.2209)] + G [–0.0016 ± 0.00143)] + PMIX [–0.0002 (±0.0003)] + HOMO [–1.6939 (±2.3953)] + ES [–0.3913 (±0.2269)]. n = 23, r = 0.79, S = 0.37, F = 7.61, Q^2 = 0.30
7	BA = [–17.0177 (±21.0724)] + G [–0.0015 (±0.0015)] + HOMO [–2.1175 (±2.3895)] + Ev [0.0447 (±0.0838)] + ES [–0.4599 (±0.2323)]. n = 23, r = 0.79, S = 0.38, F = 7.29, Q^2 = 0.33

n = no. of compounds, r = coefficient of correlation, F-ratio = sequential Fischer test value, S = standard error, Q^2 = cross validated coefficient of determination, BA = biological activity (–log IC_{50})

correlation coefficient (r), parameters that have least inter-correlation with each other (Table-5), leave-one-out cross validated squared correlation coefficient (Q^2) and intercept of best fit line. The best model, *i.e.*, model 1, explains more than 65% of variance in biological activity. The inter-correlation between the descriptors in the selected tetraparametric model is < 0.44. The model explains the contribution of dipole moment, stretch energy, HOMO energy and standard Gibb's free energy towards inhibition of PDE2 and can be analyzed in terms of no. of compounds (n), correlation coefficient (r), sequential Fischer test (F-ratio) and standard deviation (S) as shown in Table-3. The results show that electronic and thermodynamic parameters govern the activity of 2-morpholinochromones as PDE2 inhibitors. The model has better statistical significance > 99.9% as the calculated Fischer test value (F-value) exceeds the tabulated F-value ($F_{4, 25} = 8.25$). The model was tested for outlier by Z-score method, no compound was found to be outlier. To ascertain the predictivity of the model; internal validation using leave-one-out cross validation process, bootstrapping technique and randomization test were performed. The satisfactory values of internal validation, cross validated squared correlation coefficient (Q^2), predicted residual sum of squares (S_{press}), standard error of prediction (SDEP), bootstrapping squared correlation coefficient (r^2_{bsp}) and chance correlation in the randomized biological activity test revealed that the results were not based on chance correlation (Table-4). The model's $Q^2 > 0.43$ supported the predictive ability and significance of the model (Table-1 and Fig. 2). The r^2_{bsp} was at par with r^2, supported the robustness of the model, as well as indicated that no single

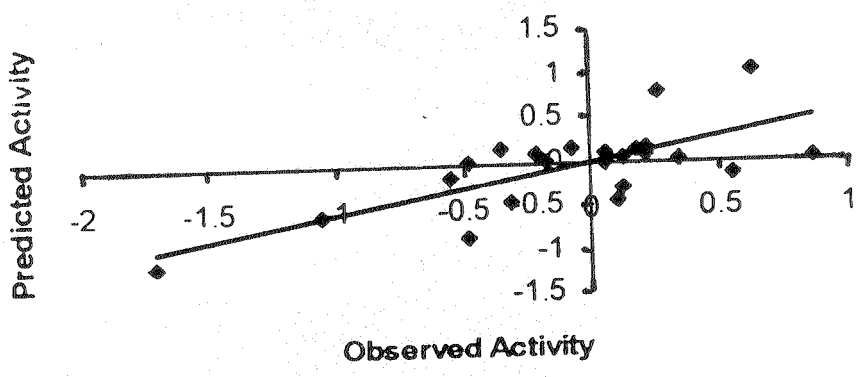

Fig. 2. A plot of observed pIC_{50} *vs.* predicted (leave one out) pIC_{50} for training set

compound of the series contributed much more to the model. The validity of this model was further confirmed by external validation. The external validation of the model has been done by predicting the biological activity of seven test compounds (Tests 1–7) of the series that have not been used in deriving the model. The predicted coefficient of determination (r^2_{pred}) > 0.47, as determined between observed and predicted activities (Table-2) for the test compounds. is well above the statistical limit of significance (> 0.3). The graph between observed and predicted activity (Fig. 3) shows high correlation between them.

TABLE-4
STATISTICAL PARAMETERS OF INTERNAL AND EXTERNAL VALIDATION FOR MODEL 1

n	r^2	Q^2	r^2_{bsp}	S_{press}	SDEP	r^2_{pred}	Chance
23	0.65	0.44	0.63	0.46	0.41	0.47	< 0.001

n = no. of compounds, r^2 = coefficient of determination, Q^2 = cross validated r^2, r^2_{bsp} = bootstrapping r^2, S_{press} = predicted residual sum of squares,
SDEP = standard error of prediction, r^2_{pred} = coefficient of determination of prediction.

TABLE-5
INTERCORRELATION MATRIX OF DESCRIPTORS USED IN MODELS

	LOGP	G	PMIX	DPL	HOMO	LUMO	Ev	ES	E14	PC
LOGP	1.000									
G	0.413	1.000								
PMIX	0.496	0.026	1.000							
DPL	0.271	0.334	0.284	1.000						
HOMO	0.309	0.436	0.104	0.332	1.000					
LUMO	0.137	0.230	0.202	0.319	0.086	1.000				
Ev	0.119	0.111	0.242	0.242	0.078	0.155	1.000			
ES	0.039	0.031	0.168	0.086	0.408	0.041	0.264	1.000		
E14	0.312	0.574	0.042	0.264	0.539	0.214	0.260	0.160	1.000	
PC	0.968	0.259	0.546	0.139	0.233	0.185	0.134	0.022	0.022	1.000

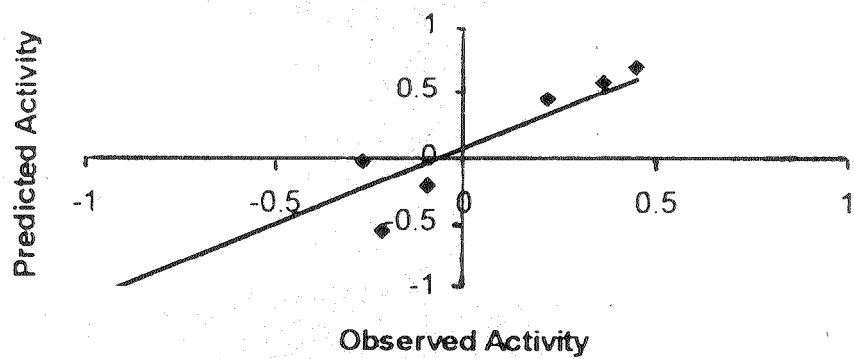

Fig. 3. A plot of observed pIC_{50} *vs.* predicted pIC_{50} for test set

The model shows that dipole moment has positive contribution while HOMO energy, stretch energy and standard Gibb's free energy (G) have negative contribution towards PDE2 inhibitory activity. The dipole moment is representative of interaction of the polar group suggesting that the substitution of moiety which is more polar in nature is favourable for determining the behaviour of the molecule in the vicinity of the receptor. The HOMO energy is representative of the nucleophilic nature of the molecule that suggests that the substitution of electron withdrawing group is favourable for the activity and the molecule may interact with electron-rich area of the receptor. Stretch energy signifies the importance of conformational rigidity of the molecule for its inhibitory activity. The standard Gibb's free energy (thermodynamic descriptor) is representative of binding of molecule with enzyme, the negative coefficient of which in the selected model reveals that substitution with

the functional moieties which impart high standard Gibb's free energy to the molecule will be detrimental for the activity.

Conclusion

The above study reveals the importance of polar substitution and electron withdrawing groups for the PDE2 inhibition. Substituents of high polarity and those with high electrophilic nature will have positive influence on biological activity. Moreover, the results shows that substitution with functional groups of high standard Gibb's free energy will decrease the activity as suggested by its negative coefficient. These results may help in designing a new analogue with more potent PDE2 inhibitory activity.

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