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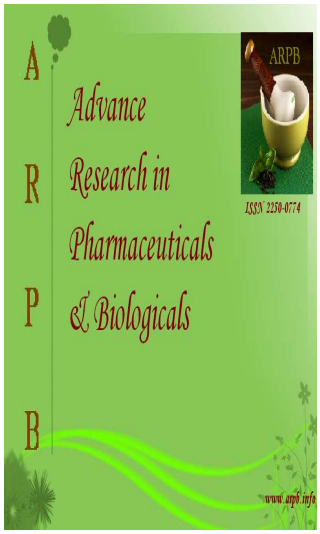

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QSAR ANALYSIS OF A SERIES OF 1-(2-(2,2,2-TRIFLUOROETHOXY) ETHYL-1 H-PYRAZOLO[4,3-D]PYRIMIDINES: AS POTENT PHOSPHODIESTERASE 5 (PDE5) INHIBITORS

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ABSTRACT:

A series of recently synthesized 1-(2-(2,2,2-Trifluoroethoxy) ethyl-1 H-pyrazolo[4,3-d]pyrimidines as Potent Phosphodiesterase 5 (PDE5) inhibitor was subjected to Quantitative Structure Activity Relationship (QSAR) Analysis . QSAR investigation based on semiemperical AM1 calculations reveals that thermodynamic, electronic & topology parameters are responsible for PDE5 inhibitory activity. Attempt in correlating the derived physiochemical properties with the PDE5 inhibitory activity resulted in some statistically significant QSAR models with good predictive ability. Our study explored some interesting findings for the design of potent new class of PDE5 inhibitors.

KEYWORDS: QSAR, PDE 5 inhibitors, Sildenafil, Erectile Dysfunction.

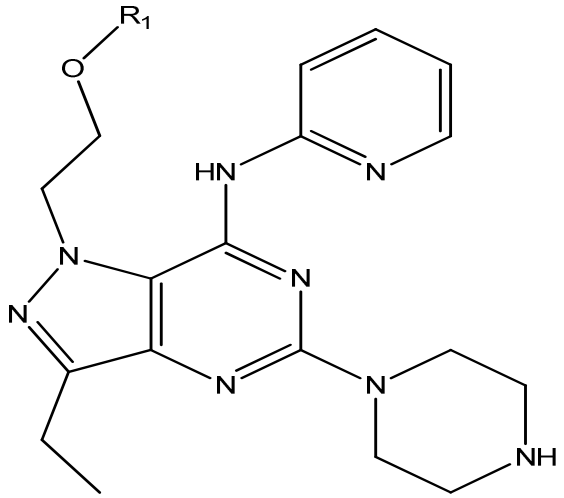
INTRODUCTION

Sildenafil, sold as Viagra and Revatio, is successfully used in the treatment of erectile dysfunction (ED) and pulmonary arterial hypertension (PAH). Sildenafil acts by competitive inhibition of phosphodiesterase 5 (PDE5)[1]. PDE5 regulates guanosine cyclic 3',5'-monophosphate (cGMP) levels by converting cGMP to GMP. cGMP interacts with protein kinase G which leads to the reduction of intracellular Ca+ levels and vasodilation[2]. By inhibiting PDE5, cGMP levels rise resulting in vasodilation of the vascular endothelium allowing for the treatment of ED and PAH. Greater deal of attention has been paid to the pyrazolo pyrimidine heterocyclic analogues as PDE5 inhibitors ever since the discovery of Sildenafil, tadalafil & Verdenafil. Recently Michael B. T., Brad A. A. et. al. have reported a novel series of 1-(2(2,2,2-trifluoroethoxy)ethyl)1H-pyrazolo-[4,3-d] pyrimidines as PDE 5 inhibitor[3]. In spite of this spectacular & unprecedented rate of progress in this therapeutic area, no molecular modelling-QSAR studies have been reported for this new lead structure. Owing to our special interest & in continuation of our research effort towards QSAR analysis, we attempt to subject this new series of molecule for an effective QSAR analysis.

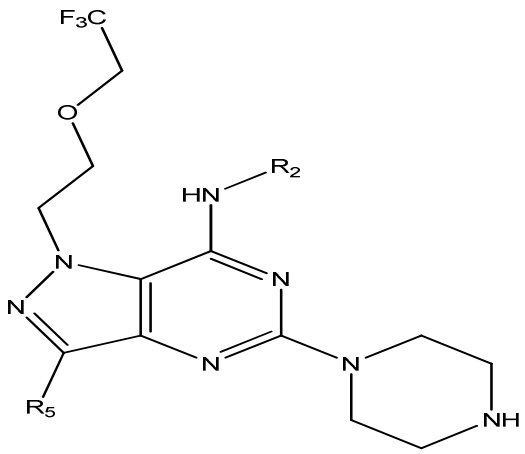
MATERIALS & METHODS

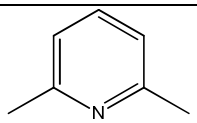
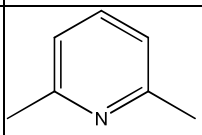
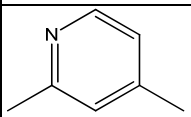
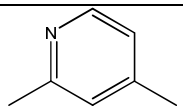
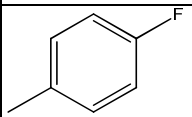
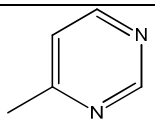
The PDE5 inhibitory activities of 1-(2-(2,2,2-Trifluoroethoxy) ethyl-1 H-pyrazolo[4,3-d]pyrimidines are listed in Table No 1.

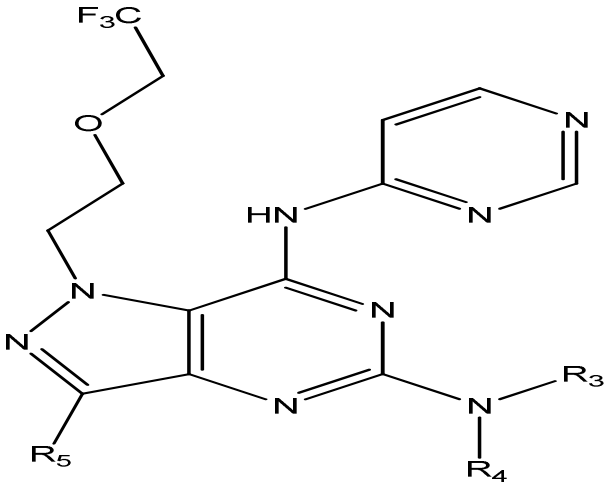
Table No 1: The PDE5 inhibitory activities of 1-(2-(2,2,2-Trifluoroethoxy) ethyl-1 H-pyrazolo[4,3-d]pyrimidines

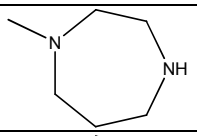
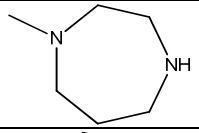
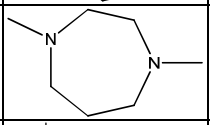
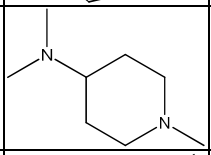
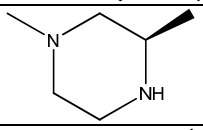
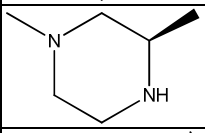
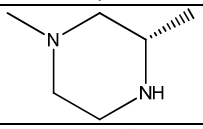
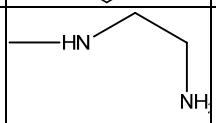
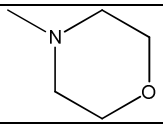
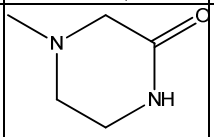


Comp	R^1	PDE5 IC_{50} (µM)	PDE5 PIC_{50} (µM)
1	Et	0.0004	-0.294296
2	n-Pr	0.0003	-0.2838588
3	i-Pr	0.00086	-0.3262109
4	CH_2CF_3	0.00065	-0.3137662



Comp	R^2	R^5	PDE5 IC_{50} (µM)	PDE5 PIC_{50} (µM)
1		Me	0.00078	-0.3217601
2		Et	0.00101	-0.3338142
3		Me	0.00184	-0.3656064
4		Et	0.00328	-0.402556
5		Et	0.00009	-0.2471725
6		Et	0.00015	-0.2615125



Comp	NR^3R^4	R^5	PDE5 IC_{50} (µM)	PDE5 PIC_{50} (µM)
1		Me	0.00979	-0.4977062
2		Et	0.00315	-0.3997299
3		Et	0.00648	-0.4569496
4		Et	0.00035	-0.2893575
5		Me	0.00014	-0.2594793
6		Et	0.00007	-0.2406796
7		Me	0.00094	-0.330374
8		Et	0.00952	-0.4947157
9		Et	0.00392	-0.4155043
10		Et	0.00296	-0.3954588

A dataset of 20 molecules have been taken from literature[3]. PDE5 inhibitory activity data (IC50 value in µM) were converted to negative logarithm (pIC50) to get the linear relationship in the QSAR equation. The sketched 2D structures were converted to 3D structures and were subjected to energy minimization using

semiemperical quantum mechanics module implemented on molecular orbital package (MOPAC) version, fixing maximum iteration limit to 1000, root mean square (RMS) gradient to 0.001 kcal/mol and applying the theory of AM1 Hamiltonian using closed shell restricted wave function. In order to cover wide range of physiochemical properties to describe the observed activity in a better way, thermodynamic, electronic, steric & topology parameters were calculated for the energy minimized and geometrically optimized structures[4] from MM2 server. The 20 derivatives selected for the study were divided into training set of 15 compounds and test set of 05 compounds by random selection method. Quantitative model building was accomplished through sequential multiple regression analysis using the method of least square in val_stat software.

The statistical quality of the models was gauged by parameters like correlation coefficient (r) or coefficient of determination (r^2), standard error of estimate (s), fisher's F-value. To ascertain the predictivity of the model, internal validation using Leave One Out (LOO) cross validation process, bootstrapping technique and randomization test were performed. Sum of squared prediction errors called predictive residual sum squares (PRESS) are calculated as the sum of squares of the differences between predicted and observed values of the activity.

The significance of individual descriptor is gauged from its standard error, as it is considerably smaller than its regression coefficient. The correlation matrix among the various predictor variables was examined regularly in order to avoid simple colinearity problem. The parameters having intercorrelation above 0.5 and those are not significant at 99.9 % confidence interval were not considered whilst deriving QSAR models.

RESULT & DISCUSSION

Several models were developed but for the sake of brevity only two statically significant models were discussed here.

Model No 1:

BA = [-1184.39(± 1242.17)] + E_{HOMO}[1] [-143.093(± 154.422)] + BEND [2] [1.12438(± 1.90969)] + NON1,4VDW [3] [-8.24666e+008(± 1.5089e+009)]

n=15, r=0.561543, r^2=0.315331, variance=3305.19, Std=5.74908, F=1.68872

Optimized model no. 1a:

BA = [32.9279(± 300.869)] + E_{HOMO} [-0.161132(± 36.7718)] + BEND [-0.101618(± 0.419622)] + NON1,4VDW [-1.36273e+008(± 3.1801e+008)]

n=14,r=0.355653,r^2=0.126489,variance=129.788,std=1.13925,F=0.482685

[1] E_{HOMO} = Energy of highest occupied molecular orbital

[2] BEND = Bend energy

[3] NON1,4VDW = Non 1,4 Vander Walls Force

Optimized model no. 1b:

BA = [57.2794(± 56.9482)] + E_{HOMO} [6.9732(± 7.01682)] + BEND [-0.0514258(± 0.0795875)] + NON1,4VDW [-1.23787e+008(± 6.01133e+007)]

n=13,r=0.925693,r^2=0.856907,variance=4.45869,std=0.211156,F=17.9654

The derived model explains 85.69 % of variance in observed activity. The tri parametric equation of Model 1 describes the PDE5 inhibitory activity of 15 analogs as a function of their electronic (E_{HOMO}) & thermodynamic (BEND, NON 1,4 VDW) Parameters. E_{HOMO} is contributing positively while BEND & NON 1,4 VDW are negatively contributing to PDE5 inhibitory activity.

The satisfactory value of internal validation, crossvalidated correlation coefficient (Q^2), standard deviation of prediction (Spress), standard error of prediction (SDEP), bootstrapping squared correlation coefficient (r^2 bsp) and chance correlation <0.01 in the randomized biological activity test revealed that the results are not based on chance correlation. The models Q^2 value > 0.4 supported the predictive ability and significance of the model.

To validate the derived models externally, biological activity of the test set molecules were predicted using models derived from training set.

Model no. 2: BA = [-983.25(± 1201.16)] +E_{HOMO} [-79.6512(± 146.5)] + CSEV[4] [11.8886(± 20.1678)] + TVC[5] [-42.6595(± 79.0268)]

n=15, r=0.49686, r^2=0.24687, variance=3654.31, std=6.04509, F=1.2019

Optimized model no. 2a:

BA = [81.2378 (± 271.054)] + E_{HOMO} [4.30142 (± 30.3706)] + CSEV [-2.00053 (± 4.30255)] + TVC [0.581114(± 16.2864)]

n=14,r=0.349548,r^2=0.122184,variance=132.118,std=1.14943,F=0.463969

Optimized model no. 2b:

BA = [145.323 (± 72.0213)] + E_{HOMO} [16.1305 (± 8.27124)] + CSEV [-1.41075 (± 1.13197)] + TVC [3.28796 (± 4.29501)]

n=13,r=0.877477,r^2=0.769966,variance=8.71024,std=0.295131,F=10.0416

Optimized model no. 2c:

BA = [115.881 (± 38.0269)] + E_{HOMO} [12.0322 (± 4.47946)] + CSEV [-1.22358 (± 0.569245)] + TVC [2.93784 (± 2.14345)]

n=12,r=0.941443,r^2=0.886315,variance=2.05226,std=0.143257,F=20.79

The derived model explains 88.63 % of variance in observed activity. The tri parametric equation of Model 2 describes the PDE5 inhibitory activity of 15 analogs as a function of their electronic (E_{HOMO}) and steric (CSEV) & topology (TVC) properties. The presence of outlier was further confirmed by

[4] CSEV = Connoly Solvent excluded volume

[5] TVC = Total valence connectivity

higher Z-score value. Compound 6 and 17 were omitted stepwise upon deriving model 1 & compound 3, 6, & 17 in Model 2. Retention of these compounds in model lowers the stastical significance hence QSAR analysis was performed without it. Deletion of outlier showed improved r^2 value and cross-validated Q^2 value of the model. Validation parameters of both the models are shown in Table No 2.

Table 2: Validation parameters for Model 1 & Model 2.

Model	r^2 boot	S boot	chance	Q	Spress	Sdep
1	0.841106	0.213477	<0.001	0.648675	0.330864	0.275295
2	0.911585	0.0714711	<0.001	0.719436	0.225051	0.183753

Both the models are statically significant in terms of r, r^2, F-value, r^2_{bs} and Q^2. Model 2 shows better r, r^2, F-value, r^2_{bs} and Q^2 values, hence model 2 is better and significant for designing new analogues. Cross correlations of the descriptors are lower than 0.5 (shown in Table No 3).

Table 3: Correlation Matrix among parameters for Model 1 & 2.

Model 1			
	E_{HOMO}	CSEV	TVC
E_{HOMO}	1.000000		
CSEV	0.438175	1.000000	
TVC	0.534400	0.169920	1.000000
Model 2			
	E_{HOMO}	BEND	NON 1,4 VDW
E_{HOMO}	1.000000		
BEND	0.076848	1.000000	
NON 1,4 VDW	0.504505	0.320002	1.000000

Predicted, calculated activity, Z- value and descriptors of training and test set molecules for Model 2 is shown in Table No 4.

The correlation between observed, predicted and calculated activity for training molecules of Model 2 is given in figure 1-2.

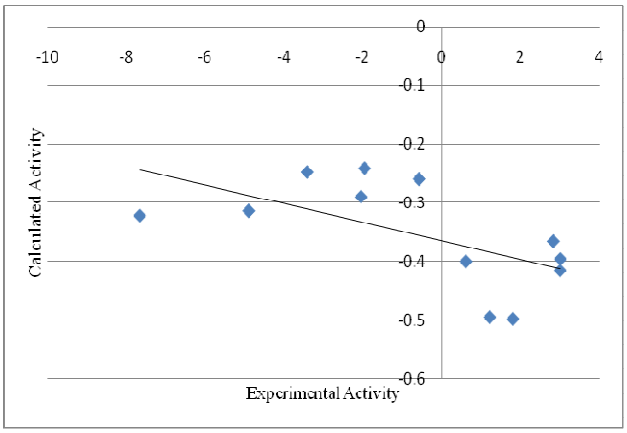


Fig. 1: Experimental versus Calculated activity for Training set molecules of Model 2

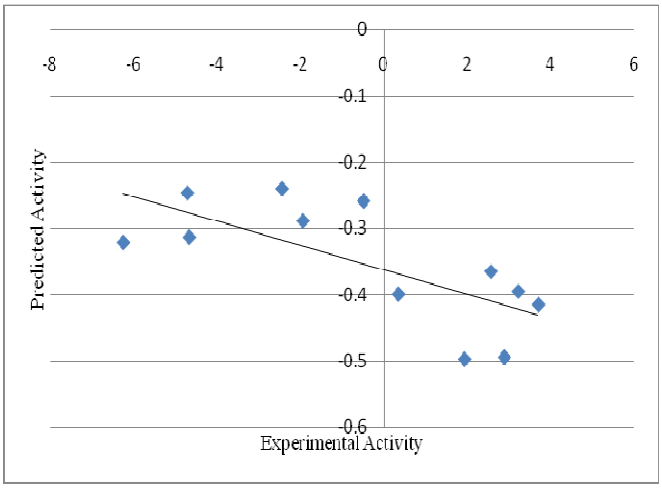


Fig. 2: Experimental versus Predicted activity for Training set molecules of Model 2

The Positive contribution of E_{HOMO} suggests that electrophilicity is important for PDE 5 inhibitory activity. The electron withdrawing groups increases the HOMO energy and in turn increases the electrophilicity of the ligands. This in turn would increase the PDE5 inhibitory activity.

Table 4: Observed, predicted, calculated activity and descriptors of training and test set molecules for Model 2.

Comp	Calculated Activity	Z- Value	Loo Predicted Activity	Descriptor		
				E_{HOMO}	BEND	NON 1,4 VDW
1	-	-	-2.77003	-6.023	29.7602	-3.3246
2	-	-	-3.90265	-6.024	29.8131	-3.684
4	-4.89783	-0.366105	-4.67163	-6.026	29.9837	-3.9435
5	-7.65759	-1.3176	-6.25216	-6.02	31.502	-4.2751
7	2.81326	0.788111	2.56119	-5.49	27.7493	-4.4446
8	-	-	1.58903	-5.425	28.4082	-4.8531
9	-3.41762	2.01475	-4.7159	-5.97	29.9165	-3.697
10	-	-	3.42343	-5.415	27.9202	-4.4729
11	1.79301	-0.641489	1.92534	-5.437	28.9384	-4.5137
12	0.603731	1.14837	0.341789	-5.373	29.6547	-4.8823
13	-	-	-3.988	-5.363	32.7122	-5.2128
14	-2.05504	-0.113172	-1.94631	-5.382	30.099	-5.5654
15	-0.585275	-0.479516	-0.491367	-5.459	30.2732	-4.6772
16	-1.96307	0.898146	-2.44806	-5.395	31.0324	-5.0921
18	1.20597	-0.150746	2.88251	-5.734	24.2267	-5.4595
19	2.98772	-1.06591	3.70023	-5.419	29.364	-4.0035
20	2.99513	-0.714845	3.21875	-5.424	28.1851	-4.4715

Fig. 3 shows the HOMO orbital of the active molecule of the series (com-16).

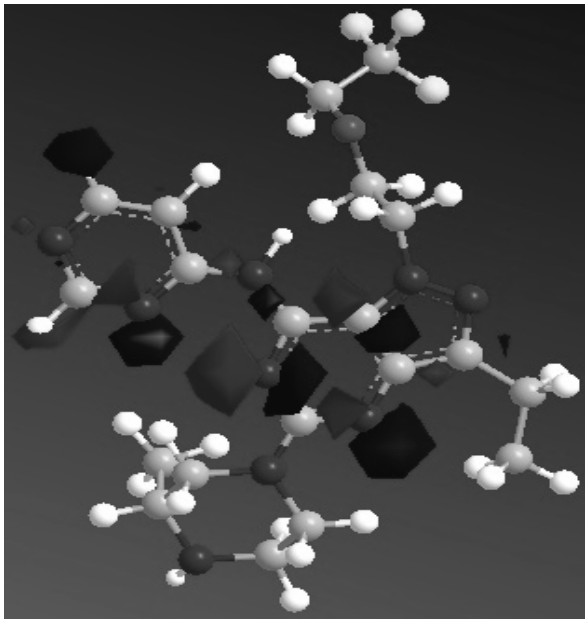


Fig. 3: HOMO molecular orbital of most active molecule of series (com-16)

Connolly's solvent Excluded Volume, a steric descriptor, represents the surface area that is not in contact with the solvent. The descriptor bears negative coefficient in the model, suggesting increase in the bulkiness of the substituents and molecular solvent excluded surface area is not conducive to the activity.

The TVC is topology descriptor, contributing positively to activity. Molecular topology is a very useful tool for describing molecular structures and has been used for efficient analysis of QSAR data[5]. The molecule-topology index is calculated based only on the framework of a molecule, although some non-bond forces are important in QSAR or QSPR[6]. Topological descriptor helps to differentiate the molecules according mostly to their size, degree of branching, flexibility and overall shape. Positive coefficient in the model, suggest that increase in molecular branching &

flexibility increases the activity, although the negative coefficient of CSEV suggest that too much bulkier substituents may appear to be detrimental to the PDE 5 inhibitory activity.

CONCLUSION

QSAR studies provide deeper insight into the mechanism of action of compounds that ultimately becomes of great importance in modification of the structure of compounds. The finding of the study will be helpful in the design of potent PDE 5 inhibitors.

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