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Research Article

# DESIGN, SYNTHESIS, QSAR STUDIES AND *IN VITRO* EVALUATION OF NOVEL TRIAZOLOPIPERAZINE BASED B-AMINO AMIDES AS DIPEPTIDYL PEPTIDASE-IV (DPP-IV) INHIBITORS: PART-I

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**ABSTRACT**

Objective: A dataset of triazolopiperazine based β-amino amide derivatives were tested for their inhibitory activities against the Dipeptidyl peptidase-IV [DPP-IV], the enzyme responsible for peripheral degradation of glucagon like peptide-1 [GLP-1]; thereby acting as antihyperglycaemic agents. Quantitative structure-activity relationship [QSAR] study of some recently studied series of DPP-IV inhibitors will provide the rationale to structure modification for the better efficacy and potency to DPP-IV inhibition. The main objective of the present work is to find out the minimum structural requirements for DPP-IV inhibitors with better $IC_{50}$ values containing triazolopiperazines as scaffold using QSAR studies.

Methods: QSAR analysis was applied to 12 candidates of the triazolopiperazine based β-amino amide derivatives using a combination of various physicochemical, steric, electronic, structural, molecular, constitutional descriptors and lipophilic parameters. Descriptors such as Ghose-Crippen $LogK_{o/w}$ [ALogP], Dipole length [DL] and number of double bond [nDB] were calculated. QSAR models were derived and validated to judge the reliability of models. Statistical significance of the generated best QSAR model was analyzed by cross validation. Molecules were synthesized and characterized by TLC and $^1$H-NMR.

Results: Present study offered good, predictive and statistically significant models. Among all the derived models, model-4 which correlates ALogP, nDB and DL descriptors with DPP-IV inhibition ($R^2$ = 0.8131) was found to be best model.

Conclusion: The best model obtained shows that ALogP, nDB and DL properties of the molecules are the important parameters that need to be considered while designing new potent DPP-IV inhibitors.

**Keywords:** QSAR, DPP-IV inhibitors, triazolopiperazine derivatives.

## INTRODUCTION

Computational methods aid rapid generation of new hypothesis-driven experiments for predicting activities of new compounds. The QSAR has vital role in current drug design. Nowadays, QSAR is widely applied for the activity prediction of diverse series of biological and/or chemical compounds. QSAR enumerate the relationship between a biological activity of molecules and permit the prediction of properties from structural and electronic parameters and/or descriptors.[1,2,3]

### DPP-IV enzyme and DPP-IV inhibitors

DPP-IV is an ubiquitous enzyme[4,5,6] acts as key regulator of incretin hormones[7] which belongs to the serine protease family and responsible for the degradation of incretin hormones[8,9,5]. The most important substrates for DPP-IV are incretins: GLP-1 having very short half-lives because of rapid degradation into its inactive form by DPP-IV. Therefore, inhibiting DPP-IV prolongs the half life of GLP-1 and promote the desirable effects of GLP-1 and GIP.[8,10] DPP-IV inhibitors inhibit the enzyme DPP-IV. Currently available oral therapeutic medications [sulphonylureas, meglitinide] having side effects like weight gain and hypoglycaemia. DPP-IV inhibitors are devoid of hypoglycemia or body-weight gain.[11,12,8] A large number of DPP-IV inhibitors have been reported in the literature including Sitagliptin (MK-0431), Vildagliptin [LAF237], Saxagliptin [BMS-47711] and Aloglipti[SYR-322].[13,14,15]

Keeping this in mind, in order to find highly potent DPP-IV inhibitors with minimal side effects, a QSAR studies on some reported series of molecules, will provide the rationale to structural modification for identification of the better potent DPP-IV inhibitors in order to promote the activity of incretin hormones. Considering the wealth of previously reported DPP-IV inhibitors, the fused heterocycle moiety of sitagliptin is recognized as a key pharmacophore that contributes to its good potency and selectivity. A new series of triazolopiperazines in [Figure 1] with better $IC_{50}$ values have been reported by the replacement of nitrogen atom from the 2 to 3 position in sitagliptin molecule.[16]

Thus, DPP-IV inhibitor seems to represent an efficient and well-tolerated new class of oral normoglycemic agents with a potential beneficial effect on pancreatic function.

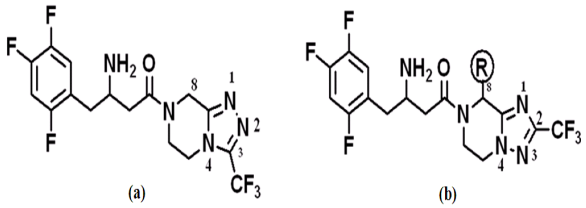



**Fig. 1: It shows General structures of sitagliptin $IC_{50}$= 18nM (a) and triazolopiperazine (b) derivative**

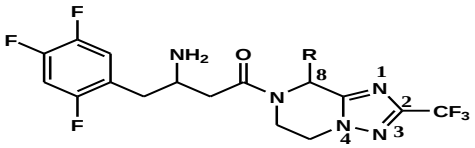
## MATERIALS AND METHODS

DPP-IV inhibitory activity data of 12 compounds was taken from the published work[16]. The experimental $IC_{50}$ values were evaluated. Table 1 represents the inhibitory properties of fused heterocycle derived DPP-IV inhibitors with varied 8- position substituents[16].

### *In silico Screening*

Chemdraw 8.0 was used to convert 2D ChemSketch files into 3D Mol Files. Further these were uploaded into the server to obtain the dipole length of the compound/s, ALogP values and nDB. Predictions are done with the help of Marvin bean- firstly generated the smile file and these smiles are then uploaded in to E-Dragon software an online server can be brought in computation of descriptors[17]. Calculation of descriptors has been achieved using E-dragon. A set of triazolopiperazine derivative were used for multiple linear regression [MLR] model generation. Table 2 represents the values of descriptors chosen for MLR model. Compounds having only definite activities were considered for the QSAR study. Compounds with insignificant activities were excluded from the dataset.

**Table 1: It shows inhibitory properties of fused heterocycle-derived DPP-IV inhibitors with varied 8- position substituents [16]**



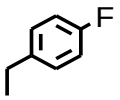
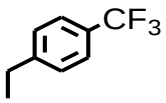
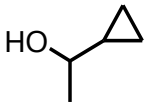
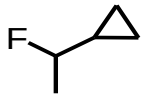
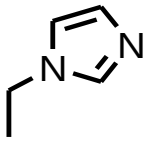
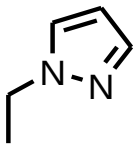
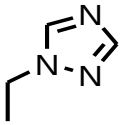
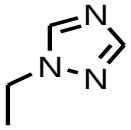
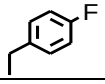
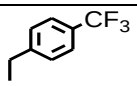
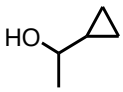
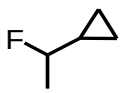
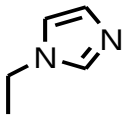
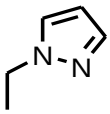
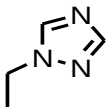
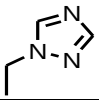

| Compound | -R group | DPP-IV $IC_{50}$ (nM) | QPP $IC_{50}$ (nM) | DPP-VIII $IC_{50}$ (nM) | DPP-IX $IC_{50}$ (nM) |
|---|---|---|---|---|---|
| **1** | -H | 59 | 54,000 | 62,000 | >100,000 |
| **2** | -$CH_3$(*S*) | 274 | 72,000 | >100,000 | >100,000 |
| **3** | - $CH_3$(*R*) | 25 | 25,000 | >100,000 | >100,000 |
| **4** |  | 4 | 60,000 | 25,000 | 27,000 |
| **5** |  | 4 | 15,000 | 16,000 | 27,000 |
| **6** |  | 7 | 7400 | 71,000 | 54,000 |
| **7** |  | 12 | 33,000 | 86,000 | >100,000 |
| **8** |  | 18 | 5767 | >100,000 | 77,000 |
| **9** |  | 2 | 26,000 | >100,000 | 27,000 |
| **10** |  | 4 | 9600 | >100,000 | >100,000 |
| **11** |  | 5 | 49,000 | >100,000 | >100,000 |
| **12** |  | 2 | 22,000 | >100,000 | >100,000 |

**Table 2: It shows values of molecular descriptors used in the regression analysis.**

| Compound | -R | Biological Activity; DPP-IV (Log 1/C of $IC_{50}$ nM) | ALogP | nDB | Dipole length |
|---|---|---|---|---|---|
| 1 | -H | 0.5647 | 2.23 | 0.089 | 4.41999 |
| 2 | -$CH_3$ (S) | 0.4102 | 2.61 | 0.083 | 4.42486 |
| 3 | - $CH_3$ (R) | 0.7153 | 2.61 | 0.083 | 4.32732 |
| 4 |  | 1.6611 | 4.37 | 0.102 | 6.75171 |

| | | | | | |
|---|---|---|---|---|---|
| 5 |  | 1.6611 | 5.11 | 0.097 | 7.65634 |
| 6 |  | 1.1834 | 3.47 | 0.107 | 4.6858 |
| 7 |  | 0.9266 | 2.57 | 0.105 | 4.44169 |
| 8 |  | 0.7986 | 3.51 | 0.107 | 5.07307 |
| 9 |  | 3.3222 | 2.01 | 0.107 | 7.21201 |
| 10 |  | 1.6611 | 2.56 | 0.107 | 5.02329 |
| 11 |  | 3.3222 | 1.88 | 0.109 | 6.36066 |
| 12 |  | 1.4308 | 1.88 | 0.109 | 5.66571 |

Graph Pad Instat 3.06 was used to design linear models. In this, descriptors were added and deleted stepwise to obtain linear model. The reference drugs were not included in model generation as they belong to a different structural series. Inhibitory activity data determined as $IC_{50}$ [nM] were first transformed to the negative logarithms of molar $IC_{50}$ [log1/C], [Table 2] which was used as a dependent variable in the QSAR study. Here, descriptor acts as input for the software Graph Pad Instat 3.06 and then the sequential addition method was applied for selecting the descriptors for DPP-IV inhibitory effect of triazolopiperazine based β-amino amides derivatives based on lower values of interdependency. When the number of descriptors are 5 or 6 times lesser than the number of molecules, then only MLR can be used. Therefore in this case, only 3 descriptors [ALogP, nDB and DL] are used to build a good QSAR model in order to avoid a high chance of spurious correlations.

Initially got the numbers of equations which are best fit in the QSAR rules for MLR analysis but due to possible nonlinearity of the best fit, some of the equations were rejected. The rejected equations are as follows:

[A:BA] = 28.155-0.2198*[B:MW]+0.1797*[C:ALogP]+0.0008625*[D:RE] + 3.371E-06*[E:BI] +38.139*[F:Ms]+0.1879*[G:ARR]……..**Model 1**

n = 12, $R^2$ = 0.8557, P value = 0.0502, F value = 4.9403

The above model 1 though the $R^2$ was significant [i.e. it should be > 0.8], this model was rejected because F [Fisher index] value was less which determines a 99% significant level of the generated equations[18].

[A:BA] = 69.875-0.3373*[B:MW]+0.5144*[C:ALogP] + 0.001256*[D:RE] + 4.291E-06*[E:BI] - 14.671*[F:Ms]+0.6248*[G:ARR]+0.9349*[H:Sv]……..**Model 2**

n = 12, $R^2$ = 0.8791, P value = 0.0931, F value = 4.1558

The above model 2 though the $R^2$ was significant, this model was rejected because F value was less and P value [it should be < 0.05] was greater than 0.05[18].

[A:BA]=780.89-9.424*[B:MW]-17410*[C:nDB]-419.44*[D:x1]-0.4109*[E:TE]+346.51* [F:ARR]-14.142*[G:HOMO]-11.611*[H:ALogP]-0.009542*[I:MTI]-170.82*

[J:SCBO]……..**Model 3**

n = 12, $R^2$ = 0.99, P value = 0.0444, F value = 21.898

The above model 3 though the $R^2$, F value, and P value was significant, this model was rejected because it included many descriptors which are from same descriptor family and these are dependent [nDB, ARR, SCBO]. If descriptors are from the same descriptor family then $R^2$ get ultimately high i.e. significant and QSAR should be based upon independent parameters. Because of this reason, the above model was rejected. Another reason to reject this model is, it contains 9 descriptors but according to n/5 rule model should contain 2-3 descriptors.

After rejecting all the above equations, the following equation is accepted having three main parameters which possess lowest value of ALogP, highest value of nDB and highest value of DL.

[A:BA] = – 3.628 – 0.4063*[B:ALogP] + 34.313*[C:nDB] + 0.5288*[D:DL]……..**Model 4**

n = 12, $R^2$ = 0.8131, P value = 0.0028, F value = 11.6041

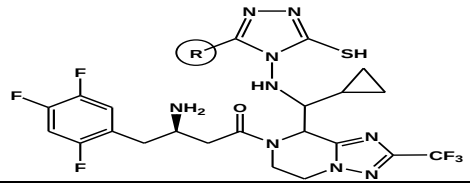
The above model 4 is selected because it satisfies all the requirements of the QSAR. The values like $R^2$, P value, F value are within the limit. The correlation matrix for three descriptors with biological activities is shown in Table 3. This suggests that there is no or less interdependency among the descriptors used in the study.

**Table 3: It shows correlation matrix of best QSAR model [Model 4]**

| Particulars | ALogP | nDB | DL | BA |
|---|---|---|---|---|
| **B:ALogP** | 1.0000 | -0.0610 | 0.3397 | -0.1952 |
| **C:nDB** | -0.0610 | 1.0000 | 0.2704 | 0.5819 |
| **D:DL** | 0.3397 | 0.2704 | 1.0000 | 0.6994 |

Based on above set of QSAR data selected, the substitutions shown in Table 4 at 8- position of compound having lowest value of ALogP, highest value of nDB, highest value of DL.

**Table 4: It shows substitution of proposed functional groups in triazolopiperazine ring**



| S. No. | -R | Predicted $IC_{50}$ (nM) |
|---|---|---|
| **$XXIR_1$** | -H | 4.35 |
| **$XXIR_2$** | $-CH_3$ | 3.41 |

**DPP-IV Enzyme Inhibition Assay**

DPP-IV inhibition was determined through *in-vitro* assay by measuring the rate of hydrolysis of a surrogate substrate, H-Gly-Pro-7-amino-4-methylcoumarin [H-Gly-Pro-AMC].

The graph of predicted $IC_{50}$ [nM] and reported $IC_{50}$ [nM] triazolopiperazine based β-amino amides was plotted in Figure 2 and the $R^2$ is 0.8334 which indicates that the generated QSAR model shows linear relationship between predicted and observed $IC_{50}$ and Table 5 shows results of DPP-IV enzyme inhibition assay [observed $IC_{50}$ [nM]

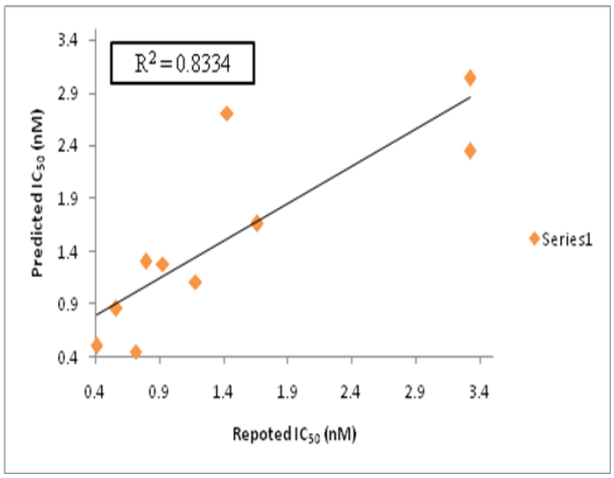



**Fig. 2: It shows graph of predicted $IC_{50}$ [nM] vs. reported $IC_{50}$[nM] values of triazolopiperazine based β-amino amides**

**Table 5: It shows results of DPP-IV Enzyme Inhibition Assay**

| S. No. | Predicted $IC_{50}$ [nM] | Observed $IC_{50}$[nM] |
|---|---|---|
| **$XXIR_1$** | 4.35 | 1.2 |
| **$XXIR_2$** | 3.41 | 0.8 |

A QSAR study has been carried out with respect to three major components that is development of QSAR model, its validation and utility of developed models. The fundamental aspect of any QSAR analysis is validation[19]. The following cross validation parameters were calculated to test the validation of developed models; PRESS, SSY, SPRESS, $r^2$cv and $r^2$adj Table 6. The following equations are used to calculate above parameters[20].

$$PRESS = \sum (Yobs - Ycal)^2 \quad (1)$$

$$SSY = \sum (Yobs - Ymean)^2 \quad (2)$$

$$SPRESS = \sqrt{PRESS \backslash n} \quad (3)$$

$$r^2cv \text{ or } Q2 = 1 - PRESS \quad (4)$$

SSY

$$r^2adj = 1 - (r^2)[n-1/n-p-1] \quad (5)$$

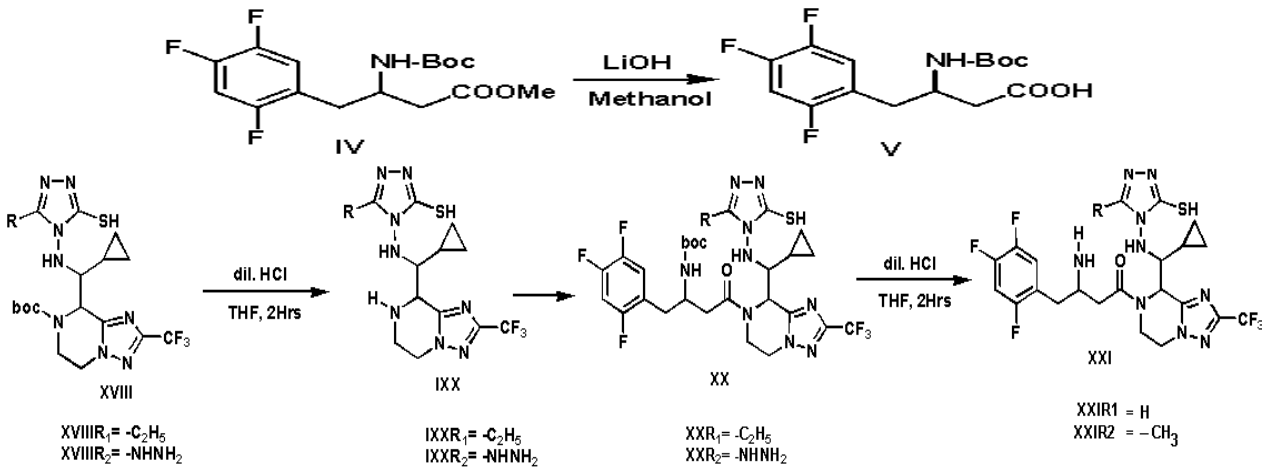
**Table 6: It shows cross validation of best QSAR model [Model 4]**

| S. No. | Statistical Parameters | Standard Limits | Calculated Limits |
|---|---|---|---|
| **1** | PRESS | << SSY | 3.2241 |
| **2** | SSY | - | 10.3100 |
| **3** | $S_{PRESS}$ | Low | 0.5183 |
| **4** | $r^2_{cv}$ or $Q^2$ | > 0.9 | 0.6872 |
| **5** | $r^2_{adj}$ | ≥ 0.6 | 0.6840 |
| **6** | PREES/SSY | < 0.4 | 0.3100 |

where, *Yobs*, *Ycalc* and *Ymean* are observed, calculated and mean values; *n* is number of compounds; *p* is number of independent parameters.

PRESS [PRediction Error Sum of Squares] is used for predicting sum of squares. To validate MLR model with respect to predictability, PRESS is utilized. The variation between *Yobs* and *Ycal* is called the prediction error. The sum of the squared prediction errors is known as PRESS value, provided which observations are independent. If PRESS value is smaller than SSY [squares of the response values] then predictability of model is better than chance and it is statistically significant. The values of PRESS and SSY were found 3.2241 and 10.3100 respectively. Here, PRESS is smaller than SSY which indicates model is statistically significant.

The ratio of PRESS/SSY indicates approximate confidence intervals of predictions of new observations and should be smaller than 0.4. The ratio of PRESS/SSY was found to be 0.31. Thus, it proves that the data obtained by QSAR is significant. With the help of PRESS value, $r^2$cv statistic or $r^2$ cross validated can be calculated which shows the prediction ability of the model.




**Scheme**

Many a times, $r^2cv$ and $r^2adj$ are considered as a proof of the high predictability of QSAR models. If the $r^2cv$ or $Q^2 > 0.9$ then the QSAR model has high predictive power, but the recent studies shows reverse of it. The $r^2cv$ or $Q^2$ value for generated QSAR was found to be 0.6872 i.e. it determines predictive ability of QSAR models[21,20,22].

**Synthesis**

Melting points were determined in open capillary method on Veego [India] electronic apparatus and are uncorrected. The Ultraviolet absorption spectra are determined in methanol on JASCO V-530, UV-Visible double beam spectrophotometer. The IR spectra of synthesized compounds were recorded on Shimadzu IR Affinity-1 spectrophotometer using KBr disc. The $^1H$ [400MHz] NMR spectra were recorded on a Bruker ACF 200 spectrometer fitted with an Aspect 3000 computer in $CDCl_3$ and DMSO. The chemical shifts were expressed in parts per million [δ ppm], downfield from tetramethylsilane [TMS] as an internal standard. Column chromatography was performed for purification of compounds on Spectrochem silica gel [60-120 mesh]. TLCs were carried out on pre-coated silica gel GF254 aluminium sheets [Merck 5554].

**Synthesis of methyl-3-(*tert*-butoxycarbonylamino)-4-(2,4,5-trifluorophenyl)-butanoate (IV)**

(*tert*-butyl-4-diazo-3-oxo-1-(2,4,5-trifluorophenyl)-butan-2-yl diazoketone in methanol, DIPEA [1.2eq.] and silver benzoate [1.1eq.] were added and the mixture was stirred at 70ºC for 15Hrs. The reaction mixture was concentrated to remove the MeOH. The product was extracted from the aqueous layer with ethyl acetate and was purified by column chromatography to obtain white solid.

**Synthesis of 3-(*tert*-butoxycarbonylamino)-4-(2,4,5-trifluorophenyl)-butanoic acid (V)**

Methanolic solution of methyl 3-(*tert*-butoxycarbonylamino)-4-(2,4,5-trifluorophenyl)-butanoate [1eq.] and aqueous solution of LiOH [2M] were added and stirred at rt for 6Hrs. The precipitated solid that separated was removed by filtration and the residual filtrate was evaporated to obtain white solid.

**Synthesis of *tert*-butyl-8-(cyclopropyl(3-mercapto-4H-1,2,4-triazol-4-ylamino)methyl)**

**-2-(trifluoromethyl)-5,6-dihydro-[1,2,4]-triazolo-[1,5-a]-pyrazine-7-(8H)-carboxylate (XVIIIR$_1$ and XXIIIR$_2$)[16,23]**

(*tert*-butyl-8-(cyclopropyl(tosyloxy)methyl)-2-(trifluoromethyl)-5,6-dihydro-[1,2,4]-triazolo[1,5-α]-pyrazine-7(8H)-carboxylate), palladium [4.25g, 0.04 mol, 4eq.] acetic acid [1.2ml, 0.02mol, 2eq.], potassium carbonate [5.52g, 0.04mol, 4eq.], and 50ml of methanol were added in to the round bottom flask stirred for 30min. After stirring 8-(Chloro(cyclopropyl)methyl)-2-(trifluoromethyl)-5,6,7,8-tetrahydro-[1,2,4]-triazole-[1,5α]-pyrazine [2.80g, 0.01mol, 1eq.] was added, stirred mixture at 60ºC for 2Hrs, concentrated the mixture under reduced pressure.

**Synthesis of 4-(cyclopropyl(2-(trifluoromethyl)-5,6,7,8-tetrahydro-[1,2,4]-triazolo-[1,5-a]-pyrazin-8-yl)methylamino)-4H-1,2,4-triazole-3-thiol (IXXR$_1$ and IXXR$_1$)[16,23]**

To a solution of (*tert*-butyl-8-(cyclopropyl-(3-mercapto-4H-1,2,4-triazol-4-yl-amino)-methyl)-2-(trifluoromethyl)-5,6-dihydro-[1,2,4]-triazolo-[1,5-a]-pyrazine-7-(8H)-carboxylate [1.05g, 0.00181mol, 1eq.] in methanol, a saturated methanolic hydrogen chloride [0.13g, 0.0036 mol, 2eq.] solution at 0°C was added. After being stirred at rt for 1Hr, the solution was concentrated to give a white foamy solid.

**Synthesis of *tert*-butyl-4-(8-(cyclopropyl(3-mercapto-4H-1,2,4-triazol-4-ylamino)methyl)-2-(trifluoromethyl)-5,6-dihydro-[1,2,4]-triazolo-[1,5-a] pyrazin-7-(8H)-yl)-4-oxo-1-(2,4,5-trifluorophenyl)-butan-2-ylcarbamate (XXR$_1$ and XXR$_2$)[16]**

The substituted triazolopiperazine [0.045mol, 1eq.] and a Boc-protected β-amino acid moiety [25.89g, 0.045mol, 1eq.] in DMF were added into round bottom flask. Further, HOBt [0.0495mol, 1.1eq.] and followed by EDC [0.0495mol, 1.1eq] was added. After being stirred at rt for 15Hrs, the reaction mixture was then acidified with 6N HCl. DMF was evaporated tore give a viscous residue, which was partitioned between EtOAc and saturated aqueous $NaHCO_3$ solution. The aqueous layer was extracted three times with ethyl acetate and was dried over anhydrous sodium sulfate, EtOAc was removed on rotary evaporator. Then, reaction mixture was recrystallized from ethyl acetate and petroleum ether to obtain the compound.

**Synthesis of Synthesis of 3-amino-1-(8-(cyclopropyl(3-mercapto-4H-1,2,4-triazol-4-ylamino)methyl)-2-(trifluoromethyl)-5,6-dihydro-[1,2,4]-triazolo-[1,5-a]-pyrazin-7-(8H)-yl)-4-(2,4,5-trifluorophenyl)-butan-1-one (XXIR$_1$ and XXIR$_2$)[16]**

To a methanolic solution of *tert*-butyl-4-(8-(cyclopropyl(3-mercapto-4H-1,2,4-triazol-4-ylamino)methyl)-2-(trifluoromethyl) -5,6-di-hydro-[1,2,4]-triazolo-[1,5-a]pyrazin-7(8H)-yl)-4-oxo-1-(2,4,5-trifluoro-phenyl)-butan-2-yl-carbamate [0.00181mol, 1eq.], aqueous solution of HCl [0.0036mol, 2eq.] was added at 0°C. After being stirred at rt for 2Hr, the solution was concentrated to give a white foamy solid. The physiochemical characteristics of **XXIR$_1$: M. pt:** 158ºC, **TLC:** Pet. Ether : EtOAc [75 : 25], Yield: 34%, **R$_f$ :** 0.48, **$^1$H-NMR [shift in δ ppm]:** 3.8 (s, 1H, SH), 1.2 (d, 1H, CH), 2 (d, 1H CH), 2.9 (t, 4H,$NCH_2CH_2N$), 2.6 (d, 2H, $COCH_2$), 6.7 (s, 1H, Ar- H), 6.8 (s, 1H, Ar- H), 5 (d, 1H, NH), 4 (t, 2H, $NH_2$), 2.3 (m, 1H, $CHNH_2$), 1.2 (d, 2H, $CH_2CHNH_2$), 1 (q, 1H, CH), 0.3 (m, 1H, CH), 0.1 (m, 2H, $CH_2$), **XXIR$_2$: M. pt:** 212ºC, **TLC:** Pet. Ether : EtOAc (75 : 25), Yield: 26%, **R$_f$ :** 0.35, **$^1$H-NMR [shift in δ ppm]:** 3.8 (s, 1H, SH), 2 (d, 1H, CH), 2.2 (q, 3H $CH_3$), 2.85 (t, 4H,$NCH_2CH_2N$), 2.7 (d, 2H, $COCH_2$), 6.7 (s, 1H, Ar-H), 6.8 (s, 1H, Ar- H), 5 (d, 1H, NH), 4 (t, 2H, $NH_2$), 2.1 (m, 1H, $CHNH_2$), 1.2 (d, 2H, $CH_2CHNH_2$), 0.6 (q, 1H, CH), 0.3 (m, 1H, CH), 0.1 (m, 2H, $CH_2$)

**RESULTS AND DISCUSSION**

***In silico* Screening**

From the library of designed molecules, two molecules [**XXIR$_1$**& **XXIR$_2$**] were prioritized by using descriptors prediction from E-Dragon online server. Graph Pad Instat 3.06 was used to design linear models.

**Synthesis & Characterization**

Two molecules [**XXIR$_1$** & **XXIR$_2$**] were synthesized by the proposed scheme. By removal of protecting group- Boc using dil HCl, present scheme was successfully yielded 34% and 26% as compound $XXIR_1$ and its hydrazine derivative $XXIR_2$. The overall yield of final compound/s $XXIR_1$ and its hydrazine derivative $XXIR_2$ were found to be 45.18% and 44.72% respectively. All the reactions were monitored by TLC with suitable solvent system. Final test compounds were characterized with $^1$H-NMR for characteristic peaks. Uncorrected melting points were reported which were sharp.

**CONCLUSION**

QSAR studies of reported triazolopiperazine class of DPP-IV inhibitors have led to the discovery of **XXIR$_1$** and **XXIR$_2$** as selective and potent inhibitors of DPP-IV. The design, synthesis, QSAR studies and biological evaluation of novel triazolopiperazine based β-amino amides as DPP-IV inhibitors confirms the utility of QSAR techniques in optimizing the lead molecules in process of drug discovery. The results of the study indicate that DPP-IV inhibitory activities of triazolopiperazine based inhibitors can be successfully explained in terms of physicochemical parameters of the molecule. The obtained correlation suggest that increase in the number of carbon atoms along with sulfur and more number of nitrogen/s will augment inhibitory activity of these molecules against DPP-IV probably by virtue of carbon rotation in the active site of the enzymes. From these studies, it was revealed that the prime electrotopological requirements for better inhibition of DPP-IV should have at least two heterocyclic rings either fused or connected with one single bond. QSAR studies of set of 12 reported compounds helped us in predicting further potent DPP-IV inhibitors i.e. **XXIR$_1$** and **XXIR$_2$** with higher predicted $IC_{50}$ values, 4.35nM and 3.41nM respectively. *In-vitro* DPP-IV inhibition assay was carried out for synthesized compounds **XXIR$_1$** and **XXIR$_2$**, which showed better inhibition with $IC_{50}$ values 1.2nM

and 0.8nM respectively. These $IC_{50}$ values were found to be 3 fold and 4 fold potent than predicted $IC_{50}$ values of **$XXIR_1$** and **$XXIR_2$**.

**REFERENCES**

1. MH Bohari, HK Srivastava, GN Sastry. Analogue-based approaches in anti-cancer compound modelling: the relevance of QSAR models. Org. Medi Chem Lett. 2011; 1: 1-12.
2. S Mbarki, K Dguigui, ME Hallaoui. Construction of 3D-QSAR models to predict antiamoebic activities of pyrazoline and dioxazoles derivatives. J. Mater. Environ. Sci. 2011; 2: 61-70.
3. MS Khan, ZH Khan. Molecular Modeling for Generation of Structural and Molecular Electronic Descriptors for QSAR Using Quantum Mechanical Semiempirical and ab initio Methods. Genome Inform. 2003; 14: 486-487.
4. NA Thornberry, B Gallwitz. Mechanism of action of inhibitors of dipeptidyl-peptidase-4 (DPP-4). Best Practice Res Clin Endocrino Metabol. 2009; 23: 479-486.
5. BD Green, VA Gault, PR Flatt et al. Comparative effects of GLP-1 and GIP on cAMP production, insulin secretion, and in-vivo antidiabetic actions following substitution of $Ala^8$/$Ala^2$ with 2-aminobutyric acid. Archives of Biochem and Biophy. 2004; 428: 136–143.
6. M Kirby, MT Yu Denise, SP O'connor, MD Gorrell, Inhibitor selectivity in the clinical application of dipeptidyl peptidase-4 inhibition. Clinical Sci. 2010; 118: 31-41.
7. GR Lankas, B Leiting, RS Roy, GJ Eiermann et al. Dipeptidyl Peptidase IV Inhibition for the Treatment of Type 2 Diabetes, Potential Importance of Selectivity Over Dipeptidyl Peptidases 8 and 9. Diabetes. 2005; 54: 2988-2994.
8. N Mulakayala, CHU Reddy, Iqbal, JM Pal. Synthesis of dipeptidyl peptidase-4 inhibitors: a brief overview. Tetrahedron. 2010; 66: 4919-4938.
9. HJ Kim, WY Kwak, JP Min, JY Lee, TH Yoon, HD Kim et al. Discovery of DA-1229: A potent, long acting dipeptidyl peptidase-4 inhibitor for the treatment of type 2 diabetes. Bioorg Medi Chem Lett. 2011; 21: 3809–3812.
10. H Duez, B Cariou, B Staels. DPP-4 inhibitors in the treatment of type 2 diabetes. Biochem Pharmaco. 2012,;83: 823–832.
11. M Nauck, U Smith. Incretin-based therapy: how do incretin mimetics and DPP-4 inhibitors fit into treatment algorithms for type 2 diabetic patients? Best Practice Res Clin Endocrino & Metabol. 2009; 23: 513-523.
12. T Biftu, G Scapin, S Singh, D Feng, JW Becker, G Eiermann et al. Rational design of a novel, potent, and orally bioavailable cyclohexylamine DPP-4 inhibitor by application of molecular modeling and X-ray crystallography of sitagliptin. Bioorg Medi Chem Lett. 2007; 17: 3384–3387.
13. A Nitta, H Fujii, S Sakami, Y Nishimura, T Ohyama, M Satoh et al. (3R)- 3-Amino-4-(2,4,5-trifluorophenyl)-N-{4-[6-(2-methoxyethoxy)-benzothiazol-2-yl]-tetrahydropyran-4-yl}-butanamide as a potent dipeptidyl peptidase IV inhibitor for the treatment of type 2 diabetes. Bioorg Medi Chem Lett. 2008; 18: 5435–5438.
14. H Xie, L Zeng, S Zeng, X Lu, G Zhang, X Zhao et al. Novel pyrrolopyrimidine analogues as potent dipeptidyl peptidase IV inhibitors based on pharmacokinetic property-driven optimization. Eu J Medi Chem. 2012; 52: 205-212.
15. D Kim, JE Kowalchick, SD Edmondson et al. Triazolopiperazine-amides as dipeptidyl peptidase IV inhibitors: Close analogs of JANUVIA[™] (sitagliptin phosphate). Bioorg Medi Chem Lett. 2007; 17: 3373–3377.
16. JE Kowalchick, B Leiting, KD Pryor, F Marsilio, JK Wu, H He et al. Design, synthesis, and biological evaluation of triazolopiperazine-based β-amino amides as potent, orally active dipeptidyl peptidase IV (DPP-4) inhibitors. Bioorg Medi Chemi Lett. 2007; 17: 5934–5939.
17. IV Tetko. Computing chemistry on the web. Drug Discov. Today. 2005; 10: 1497-1500.
18. SP Gupta. QSAR and Molecular Modeling. Anamaya Publishers, New Delhi. 2011.
19. R Veerasamy, H Rajak, A Jain, S Sivadasan, CP Varghese, RK Agrawal. Validation of QSAR Models - Strategies and Importance. Int J Drug Design and Discov. 2011; 2: 511-519.
20. SO Podunavac-Kuzmanovic, DD Cvetkovic, DJ Barna. QSAR Analysis of 2-Amino or 2-Methyl-1-Substituted Benzimidazoles Against Pseudomonas aeruginosa. Int. J. Mol. Sci.. 2009; 10: 1670-1682.
21. S Wold. Validation of QSAR's. Quant. Struct.-Act. Relat. 1991; 10: 191-193.
22. M Tichy, M Rucki. Validation of QSAR models for legislative purposes. Interdisc Toxicol. 2009; 2: 184-186.
23. S Jubie, P Sikdar, S Antony1, R Kalirajan et al. Synthesis And Biological Evaluation Of Some Schiff Bases of [4-(Amino) -5-Phenyl- 4h-1, 2, 4-Triazole- 3- Thiol. Pak. J. Pharm. Sci. 2011; 24:109-112.