c. Report of the work done:

i. **BRIEF OBJECTIVE OF THER PROJECT:**

- Application of automated molecular docking and 3D QSAR studies of different classes of NNRTIs for development of a robust pharmacophoric model for design of newer azoles.

- On the basis of the model developed and the docking scores, to synthesize and characterize newer azoles and their analogs.

- To evaluate the synthesized newer azoles and their analogs for HIV-1- RT inhibitory activity and cytotoxicity.

- To carry out molecular modeling and virtual ADME studies to understand the binding mode analyses of the synthesized analogs on the NNIBP of HIV-1-RT for further design and modification of the synthesized analogs.

- To identify and optimize the lead molecule for obtaining a suitable drug candidate which could suppress HIV replication and also inhibit the both wild type and resistant strains of HIV.

ii. Work done so far and results achieved and publications, if any, resulting from the work (Give details of the papers and names of the journals in which it has been published or accepted for publication):

Part A. **Design Studies**:

(i) 3D QSAR studies on a series of NAIMs analogues synthesized by Lagoja *et al* [1] (Model 1) combined with the DAMNI series synthesized by Silvestri *et al* [2,3] (Model 2)using Sybyl 7.1 was carried out by us. The CoMSIA studies (Model 3) of the combined models of both NAIMs and DAMNI analogs revealed that following groups as shown in Fig.1 are required for HIV-1-RT inhibitory activity.

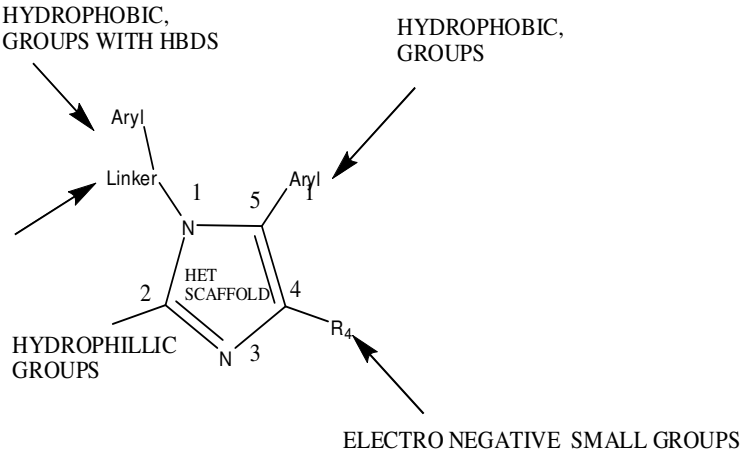
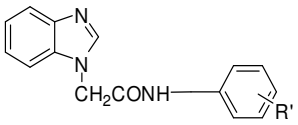


Fig.1: Groups essential for HIV-1RT inhibitory activity.

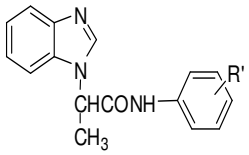
Results of the CoMSIA EHD Model 3: $r^2_{pred} = 0.80$, $r^2_{cv} = 0.532$, $r^2_{ncv} = 0.930$

Based on the 3D QSAR studies and keeping in mind the pharmacophoric requirements of the NNRTIs, various classes of azoles and their analogs were designed as follows:

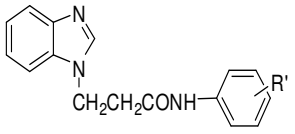
1. **Benzimidazoles [A, B.C]**



[A]



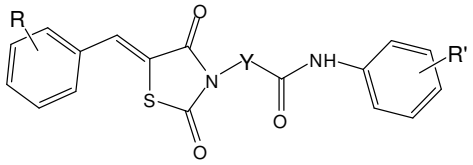
[B]



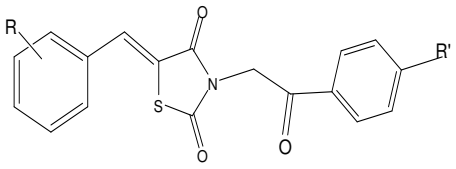
[C]

1. *J. Med. Chem. 2003, 46, 1546-1553*
2. *Bioorg. Med. Chem. Lett. 2000,10 ,253- 256*
3. *J. Med. Chem. 2002, 45, 1567-1576*

2. Thiazolidines **[B, C, D]**



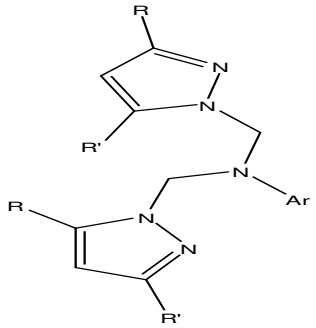
[**B, C**]



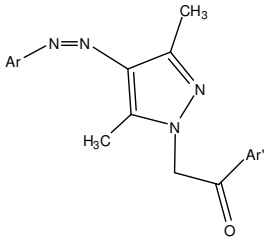
[**D**]

Y=$CH(CH_3)$, CH_2CH_2

3. **Pyrazoles [A, B]**



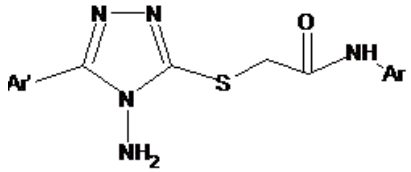
[**A**]



[**B**]

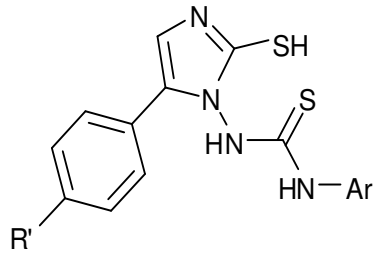
A= R=R'=CH_3 Ar=Aryl Ar=Ar'= Aryl

4. **Triazoles** [A]



. [A] Ar'= phenyl/pyridyl Ar=Aryl

4. **Imidazoles [A]**



[**A**]

R': Br, Cl, OCH_3, CH_3, NO_2, phenyl Ar= Aryl

(ii) Docking studies of the all the designed compounds of benzimidazoles , thiazolidines, triazoles, pyrazoles , imidazoles has been carried out by various software like Glide 5.0 /

Autodock 4.0/Scigress Explorer. After comparative studies, the best docked pose of the highest active molecule in each series of benzimidazoles, thiazolidines, pyrazoles and imidazoles in Glide while the best docked pose of the highest active molecule in triazole series in Autodock has been shown in Figures 3 , 4 , 5, 6 ,7 and 8.

Validation of docking protocol is depicted in Fig. 2.

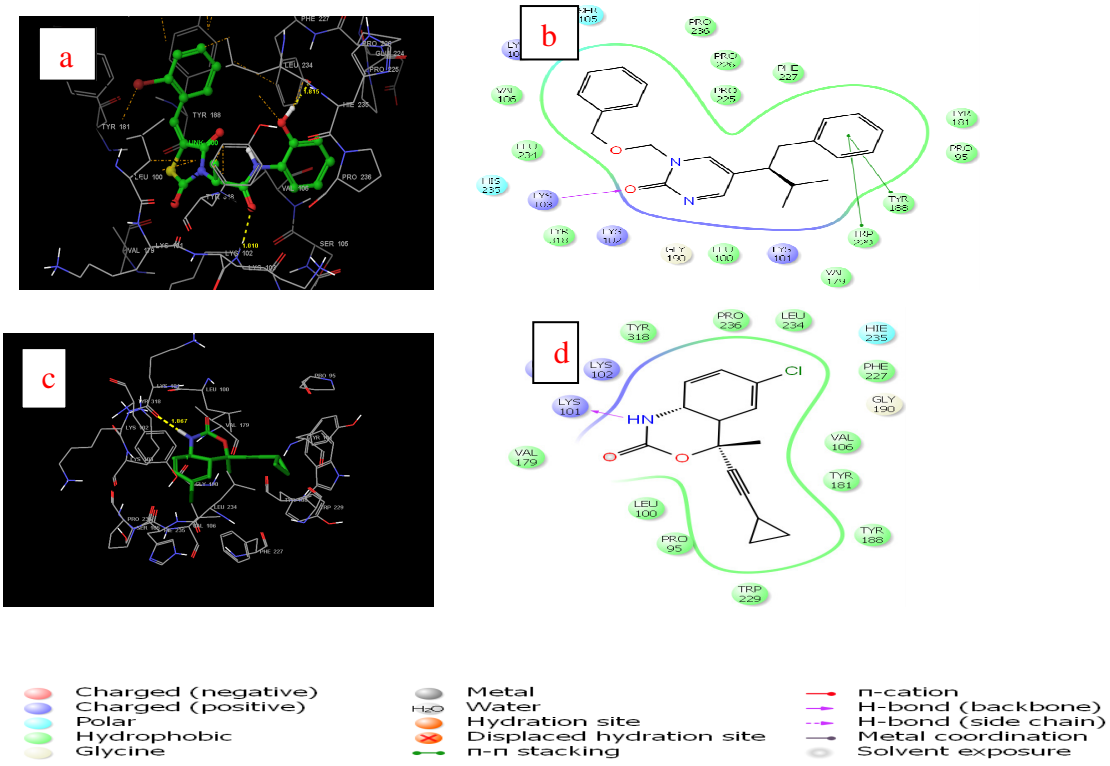


Fig.2.a.Redocked mode of **TNK 651** with the co-crystallized ligand in the NNIBP of HIV-1 RT (PDB code 1RT2). Active site amino acid residues are represented as tubes while the inhibitor is shown as ball and stick model. Hydrogen bond interactions are represented by *yellow* dotted lines. (RMSD) of 0.37 Å. **Glide XP score = -13.29** b. Schematic (2D) representation of interactions of **TNK 651** in the NNIBP of IRT2 .c. Docked pose of **efavirenz** in the NNIBP of 1RT2**.** Active site amino acid residues are represented by lines. Hydrogen bond interactions are represented by *yellow* dotted lines**. Glide XP score =11.30** d**.** Schematic (2D) representation of interactions of **efavirenz** in the NNIBP of IRT2

Docked pose of the most active molecule in the benzimidazole series is given as follows in Fig .3:

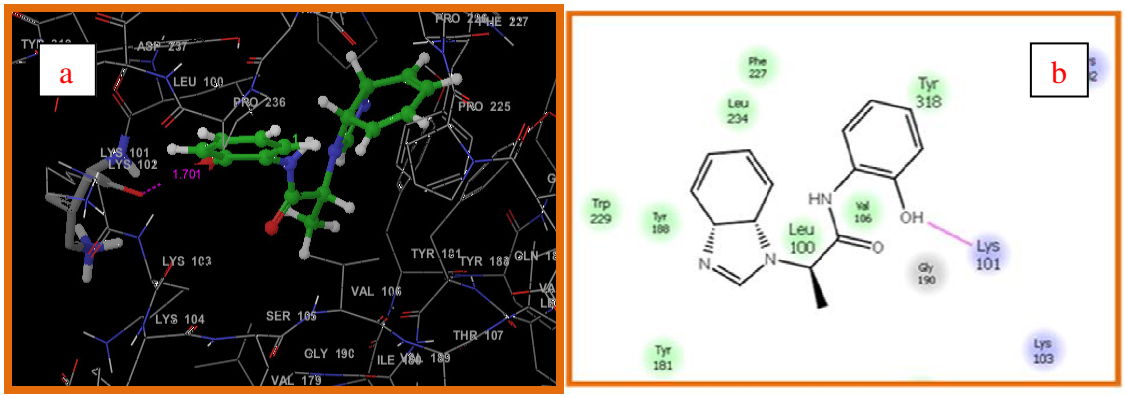
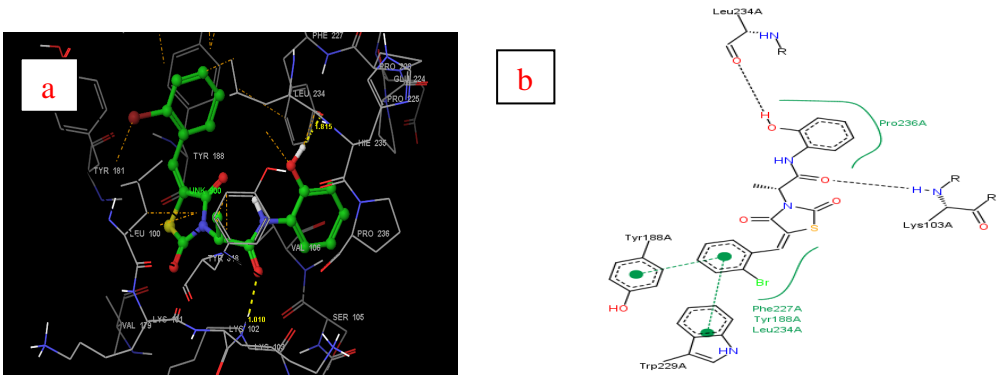


Fig.3.a. Molecular model of most active molecule in the **benzimidazole series** (**B35**)(**R**) in the NNIBP of HIV-1 RT (PDB code 1RT2). (OH $_{\text{PHENYL RING}}$ ___CO $_{\text{LYS101}}$ = 1.701 A°). (**Glide XP Score-10.46).**Active site amino acid residues are represented as tubes, while the inhibitor is shown as ball and stick model with the atoms colored as carbon: *green*, hydrogen: *cyan*, nitrogen: *blue*, oxygen: *red*. Hydrogen bond interaction is represented by *pink* dotted lines .**b**. Schematic (2D) representation of interactions of compound **B35** in the binding pocket of the protein

Docked pose of the most active molecules of the thiazolidine series are given as follows in Fig.4 and 5.



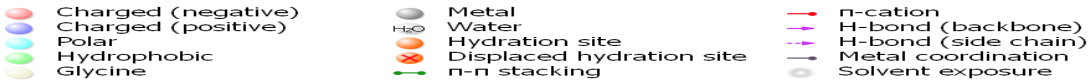


Fig. 4.a Molecular model of most active molecule in the **thiazolidine B and C series** (**B232**)(**R**) in the NNIBP of HIV-1 RT (PDB code 1RT2). ($CO_{CH2CONH}$____NH_{Lys10}3 = 1.01 A °), (OH $_{phenyl}$……CO_{Leu234} = 1.815 A °). **(Glide XP Score-12.47**) . Active site amino acid residues are represented as tubes while the inhibitor is shown as ball and stick model with the atoms colored as carbon: *green*, hydrogen: *cyan*, nitrogen: *blue*, and oxygen: *red*. Hydrogen bond interactions are represented by *yellow* dotted lines. **b.** Schematic (2D) representation of interactions of compound **B232**in in the NNIBP of 1RT2

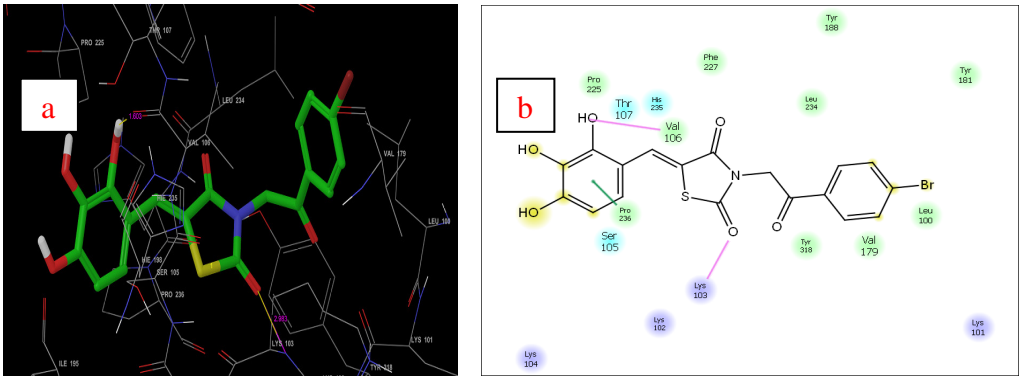


Fig.5.a : Molecular model of most active molecule in the **thiazolidine D series** (**D329)** in the NNIBP of HIV-1 RT (PDB code 1RT2). :($CO_{thiazolidinedione}$____NH Lys103 = 2.98 A°), ($OH_{hydroxybenzylidin}$e ___CO Val106 = 1.60 A °) (**Glide XP Score-11.76).**Active site amino acid residues are represented as tubes while the inhibitor is shown as ball and stick model with the atoms colored as carbon: *green*, hydrogen: *cyan*, nitrogen: *blue*, and oxygen: *red*. Hydrogen bond interactions are represented by *yellow* dotted lines **b.** Schematic (2D) representation of interactions of compound **D 329** in the in the NNIBP of 1RT2.

Docked pose of the most active molecules of the triazole series is given as follows in

Fig.6.

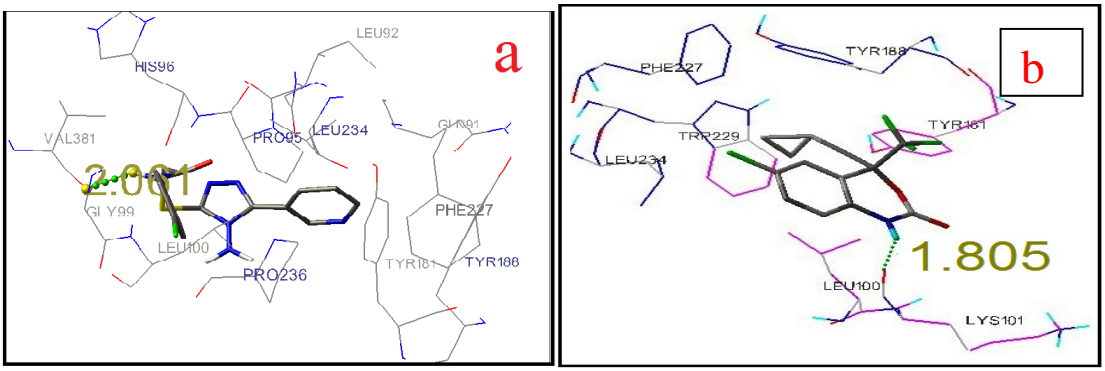


Fig. 6. a,.Molecular model of most active molecule in the **triazole A series** (**A 27**) in the NNIBP of HIV-1 RT (1RT2).b. Molecular model of **efavirenz** in the NNIBP of HIV-1 RT (1RT2 Ligand is shown as stick model and the amino acid residues interacting with the ligands are shown as line model. Hydrogen bond interaction ($NH_{CH2\text{-}C(=O)NH} \cdots CO_{GLY99}$ = 2.061Å).with VAL 381 amino acid residue of reverse transcriptase is shown as dotted spheres.**Autiodock score=10.11**(comparable to dock score of efavirenz=**10.49**).

Docked pose of the most active molecules of the pyrazole series are given as follows in Fig.7.

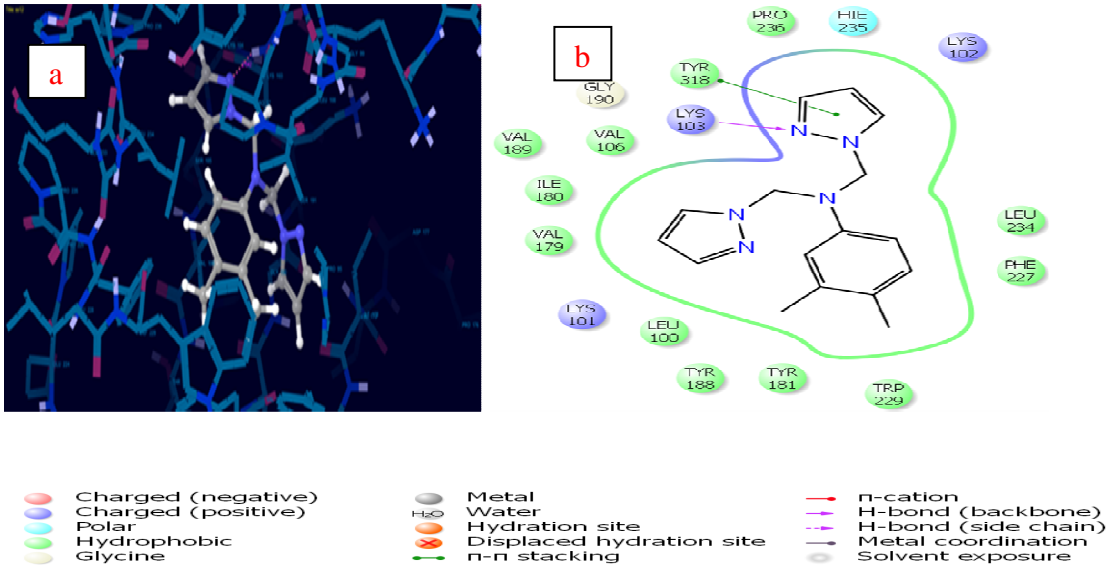


Fig.7.a : Molecular model of most active molecule in the **pyrazole A and B series** (**A12)** in the NNIBP of HIV-1 RT (PDB code 1RT2). (NH_{pyrazole} .....$C{=}O_{\text{Lys 103}}$ = 2.042 A°) (**Glide XP Score=10.08).**Active site amino acid residues are represented as tubes while the inhibitor is shown as ball and stick model with the atoms colored as carbon: *green*, hydrogen: *cyan*, nitrogen: *blue*, and oxygen: *red*. Hydrogen bond interactions are represented by *yellow* dotted lines. **b.** Schematic (2D) representation of interactions of compound **A12** in the NNIBP of 1RT2.

Docked pose of the most active molecule of the imidazole series are given as follows in Fig.7.

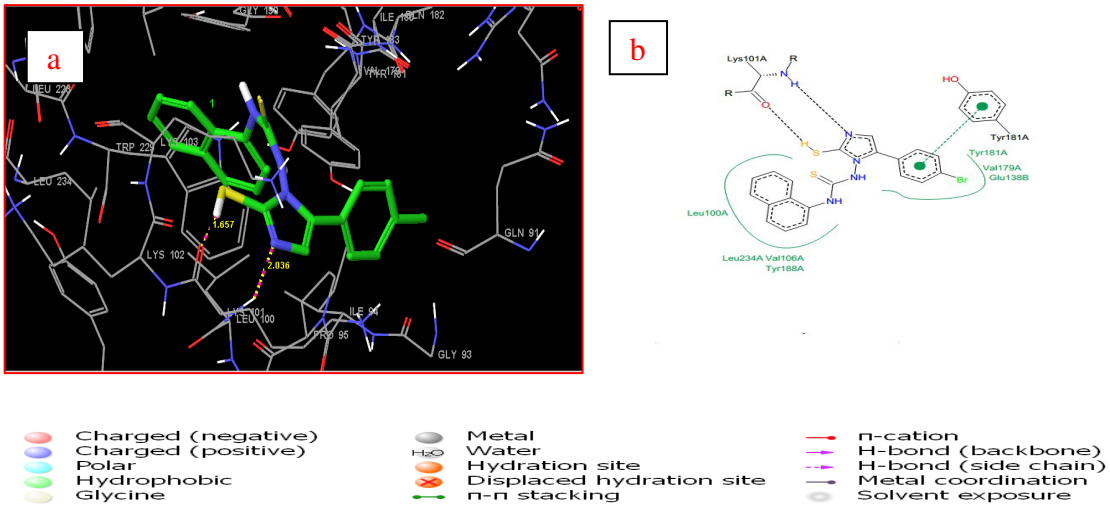
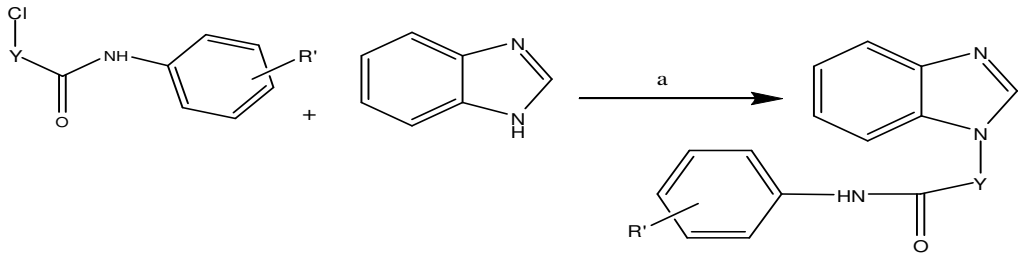


Fig.8.a : Molecular model of most active molecule in the **imidazole A series** (**A73)** in the NNRTI-BP of HIV-1 RT (PDB code 1RT2). ($SH_{imidazole}$.....C=O $_{Lys\ 101}$=1.657 A°, $N3_{imidazole}$.....NH $_{Lys\ 101}$=2.036 A°) (**Glide XP Score-12.14).** Active site amino acid residues are represented as lines while the inhibitor is shown as ball and stick model with the atoms colored as carbon: *green*, hydrogen: *cyan*, nitrogen: *blue*, and oxygen: *red*. Hydrogen bond interactions are represented by *pink* dotted lines. **b.** Schematic (2D) representation of interactions of compound **A73** in the NNIBP of 1RT2.

Part B. Based on the docking scores, compounds with the best scores (comparable to the docking score of standard efavirenz) of the benzimidazole series , thiazolidine series ,

triazole, pyrazole and imidazole series have been synthesized by the following schemes as given below:

i. Scheme 1 for benzimidazoles is given below:

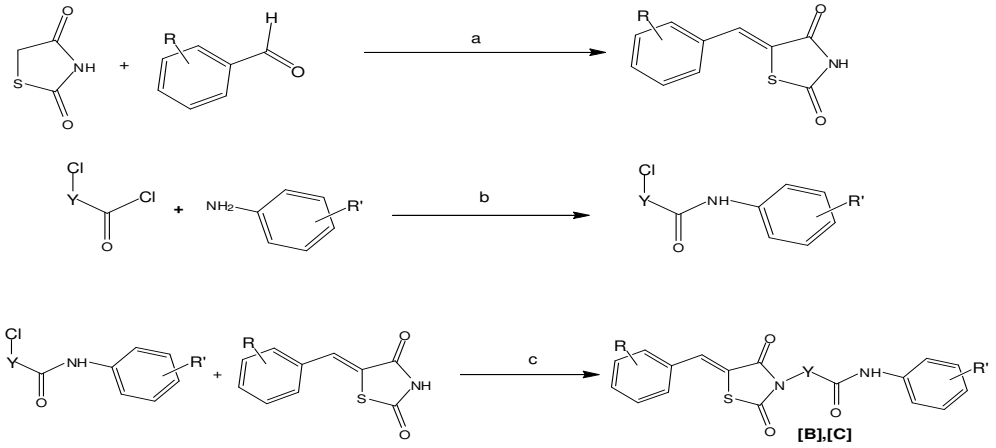


Reagents and conditions:(a)reflux 48 h, DMF [A,B,C]

where Y=- CH_2, $CH(CH_3)$, CH_2CH_2

Scheme 1

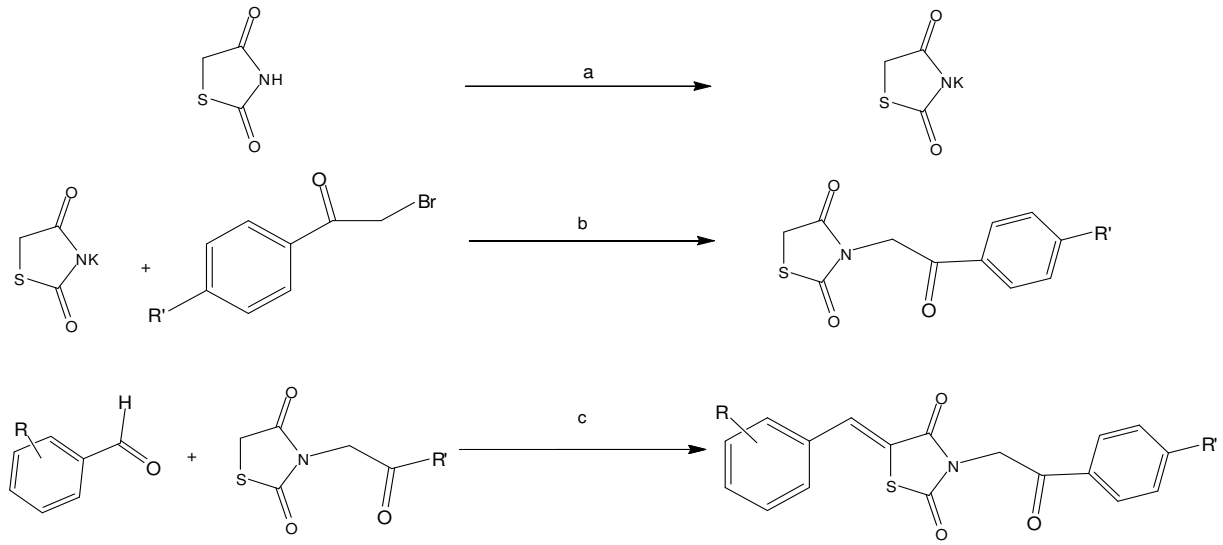
ii. Scheme 2 and Scheme 3 for thiazolidines is given below:



Reagents and conditions: (a) ethanol, piperidine, reflux, 4 h (b) GAA, 0-5^{0}C ,0.5 h, stirring 4 h rt, (c) CH_3CN, triethylamine, reflux 12 h.

where Y= $CH(CH_3)$, CH_2CH_2

Scheme 2

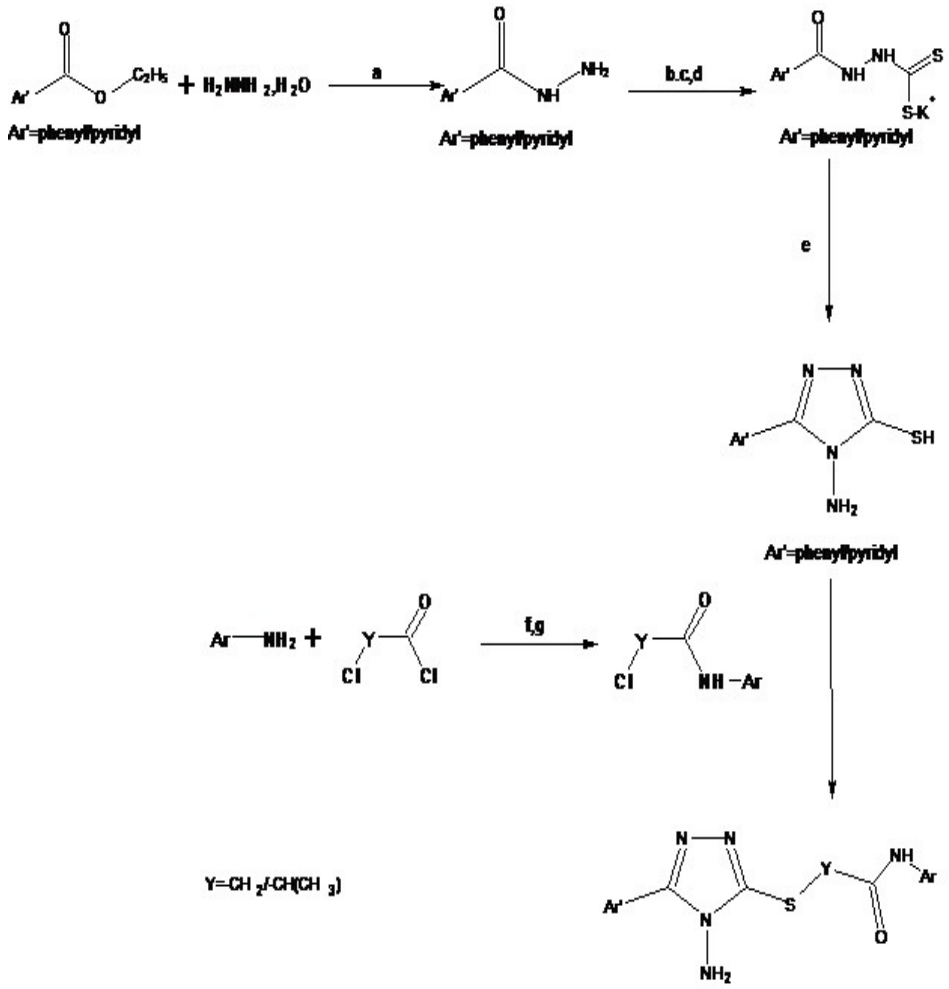


[D]

Reagents and conditions: (a) methanol, KOH, stirring 10 min, (b) methanol, reflux 6 h (c) ethanol, piperidine, reflux 4 h

Scheme 3

iii. Scheme 4 for triazoles is given below

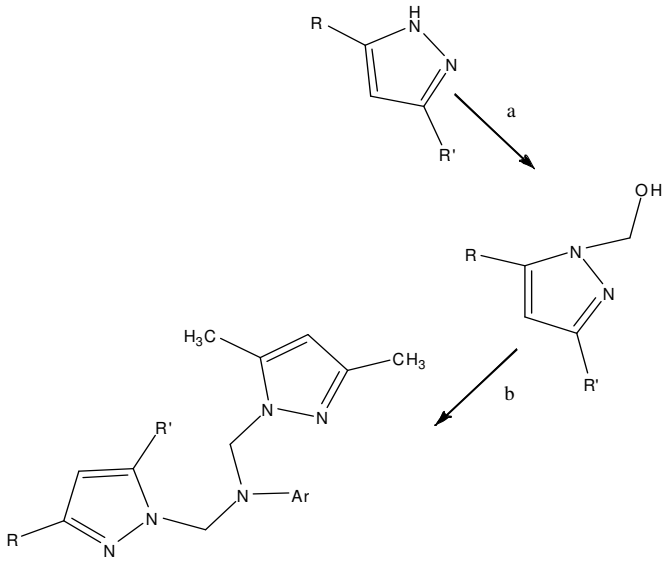


[A]

Reagents and conditions: a; ethanol, reflux, 4 h, b; CS_2, c; KOH, d; ethanol, e; H_2NNH_2, H_2O, reflux, 7 h, f; glacial acetic acid, g; saturated sodium acetate solution

Scheme 4

iv. Scheme 5 and Scheme 6 for pyrazoles is given below

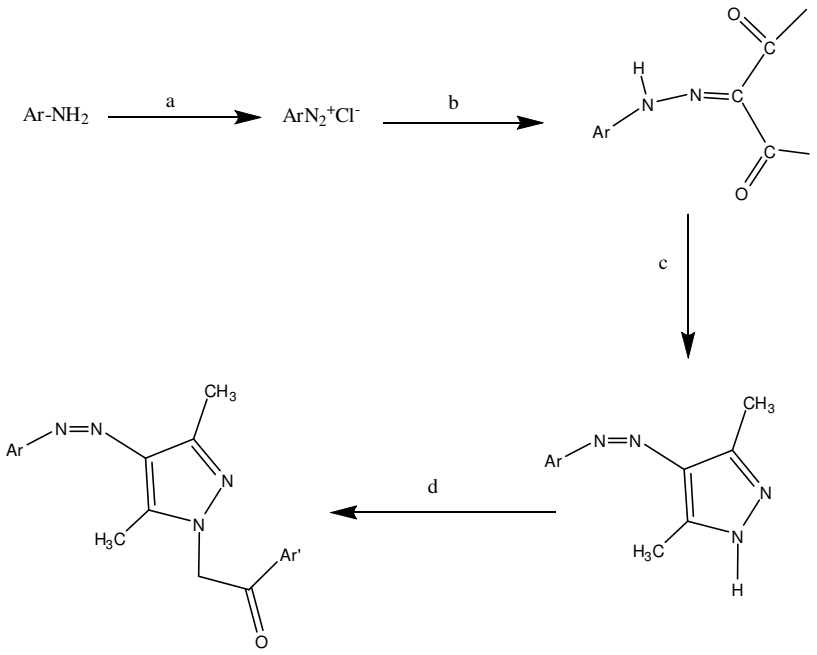


[A]

Where R=R'= CH_3

Reagents and conditions: (a) HCHO, ethanol, 30^0C, 40 h stirring (b)CH_3CN, 70 ^{0}C, aromatic amines , 4 h stirring

Scheme 5

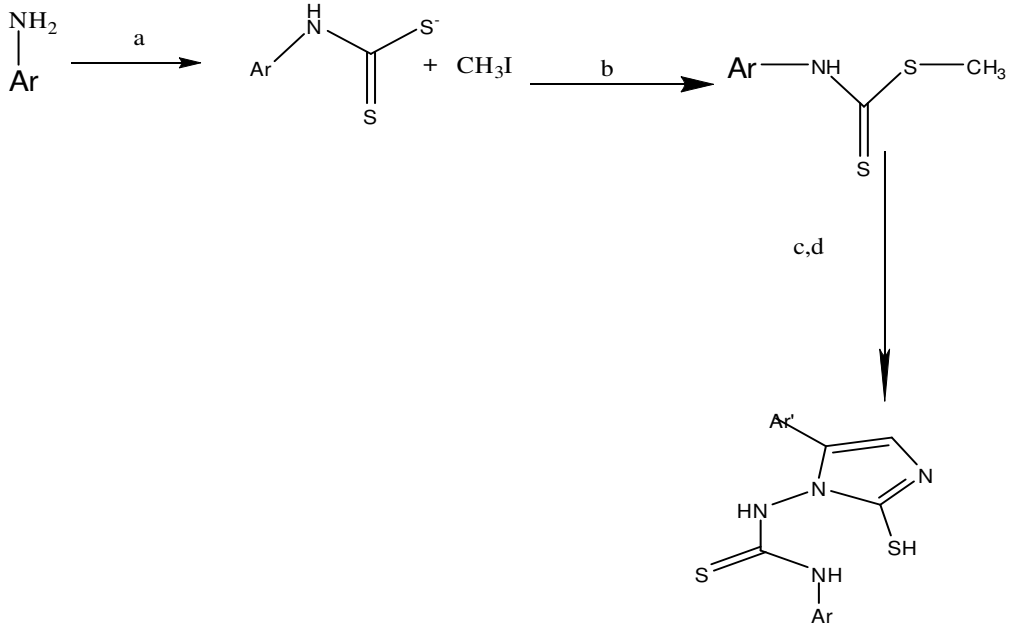


[B]

Reagents and conditions: a) $NaNO_2$, HCl, 0-5^{0}C ,stirring b) Acetylacetone,CH_3COONa,stirring, rt, 2-4 h c) NH_2NH_2,AcOH, reflux,26 h d) appropriate phenacyl bromide, DMF, stirring, 0-5^{0}C

Scheme 6

v. Scheme 7 for imidazoles is given below



[A]

Reagents and conditions : (a)CS_2/NH_4OH,stirring, 0-4^oC,6 h (b)stirring, 0-4^oC, 3 h, methanol (c) NH_2NH_2, stirring, 60^oC, 2 h, methanol , (d)(i)appropriate phenacyl bromide, KCNS, GAA, stirring,60^oC, 1 h (ii) reflux,6 h

Scheme 7

General procedures for synthesis from Scheme 1 to Scheme 7:

Chloranilides(Chloracetamides/propionamides)

Appropriate amine (3.01 mL, 0.033 mol) was dissolved in 12.5 mL glacial acetic acid and appropriate acid chloride (3.70 mL, 0.037 mol) was added dropwise to this solution while cooling in ice-bath. The reaction mixture was stirred in ice-bath for 30 min and then stirred for 4h at room temperature. The mixture was poured into saturated sodium acetate solution. The precipitate was filtered, washed with cold water and dried to give a residue which was recrystallized from methanol to yield white crystals.

Benzimidazoles

3-(1H-benzo[d]imiazol-1-yl)-N-aryl acetamides/propionamides

To a solution of benzimidazole (0.012 M) and appropriate chloranilide (0.012 M) in 20 ml of DMF, K_2CO_3 (0.024 M) was added with stirring. The mixture was refluxed for 10-12 h. Next, the mixture was cooled, poured into crushed ice to yield a precipitate which was filtered, washed with water (3x100 ml) and finally with methanol. The solid residue obtained was recrystallized from ethanol to yield the title compounds.

Thiazolidines

Thiazolidine-2,4-dione

Equimolar amounts of chloroacetic acid (56.4g, 0.6 mol) and thiourea (45.6g, 0.6 mol) was dissolved in 60 mL of water. The mixture was stirred for 15 minutes. To the contents of the flask was added slowly 60 mL concentrated hydrochloric and refluxed for 12 hr at 100-110^0C. Next, the reaction mixture was allowed to cool to yield white crystals which was washed with water and dried. The crude product was recrystallized from ethanol to yield white crystals.

1st scheme

5-(arylidene) thiazolidine-2,4-diones

To a mixture appropriate aldehyde (2.04 mL, 0.02 mol) and thiazolidine-2,4-dione (2.34g, 0.02 mol) was added 20 mL of ethanol. Next, 0.2 mL piperidine was added to this mixture and the resulting solution was refluxed for 4h. The reaction mixture was then cooled and filtered. The precipitate obtained was dried at 50°C and recrystallized from ethanol to yield white crystals.

2-(5-arylidene-2,4-dioxothiazolidin-3-yl)-N-(3-aryl)propanamides or 3(5-arylidene-2,4-dioxothiazolidin-3-yl)-N-(3-aryl)propanamides

The appropriate chloranilide (2g, 0.01mol) and 5-arylidene-thiazolidine-2,4-dione (230)(2.05g, 0.01 mol) was dissolved in 20 mL of acetonitrile. Triethylamine (2.02 mL, 0.02 mol) was added dropwise to this solution with stirring. Next, the reaction mixture was refluxed for 12h, evaporated in vacuo, cooled, poured into crushed ice and then basified with solid potassium carbonate. The resulting precipitate was filtered, washed with water and further washed with n-hexane (3x20 mL). The residue obtained was recrystallized from methanol to yield off white crystals.

2nd scheme:

3-(2-(4-aryl)-2-oxoethyl)thiazolidine-2,4-diones

Thiazolidine -2,4-dione (1.17g, 0.01 mol) was dissolved in 10 mL of methanol. To this mixture was added dropwise a solution of potassium hydroxide (0.56g, 0.01mol) in 3 mL methanol. After completion of addition, the reaction mixture was stirred for 10 min. The appropriate phenacyl bromides (2.34g, 0.01mol) was then added to the reaction mixture. Next, the reaction mixture was stirred for 10 min, and then refluxed for 6h. The resulting product was removed by filtration, washed with water and diethylether. Finally, the residue was recrystallized with ethanol to yield white crystals.

3-(2-(4-chlorophenyl)-2-oxoethyl)- 5-benzylidene thiazolidine-2,4-dione

A mixture of appropriate aldehyde (0.9mL, 0.01mol) and 3-(2-(4-aryl)-2-oxoethyl) thiazolidine-2,4-dione (300) (2.34g, 0.01 mol) was added in 20 mL of ethanol. To this mixture was added 0.2mL piperidine. The resulting solution was refluxed for 4 hr. The reaction mixture was cooled and poured on crushed ice with stirring. The solid product

was separated and dried, washed with toluene and was recrystallized from ethanol to yield white crystals.

Triazoles

Hydrazides

The respective benzoate (0.083 mol, 11.85 ml) was disolved in 30 ml of ethanol and hydrazine hydrate (0.11 mol, 4 ml) was added dropwise in the mixture with continuous stirring. The resulting mixture was refluxed for 6 h. The solvent was removed by distillation and the residue was cooled to room temperature. The precipítate formed was filtered and washed subsequently with wáter, dried and recrystallized from dehydrated ethanol to yield white crystals.

Potassium dithiocarbazinates

Potassium hydroxide (0.0375 mol, 2.10 g) was disolved in 75 ml of absolute alcohol and appropriate substuituted hydrazide (210) (0.025 mol, 3.40 g) was added to the above solution. The mixture was then cooled in an ice-bath. To this mixture carbondisulphide was added (0.0375 mol, 3.6 ml) in small portions with continuous stirring. The reaction mixture was continuously agitated for 15 h. Finally, the mixture was diluted with 100 ml of anhydrous ether. The residue was filtered, washed with ether (3×25 ml), dried and used as such for the next reaction.

4-amino-5-aryl-4H-1,2,4-triazol-3-thiols

A suspensión of appropriate potassium dithiocarbazinate (0.011 mol, 2.11 g) in 10 ml of wáter and hydrazine hydrate (0.033 mol, 1.65 ml) was refluxed for 6 h with occasional shaking. Next, the hot mixture was cooled to room temperature and diluted with 100 ml of wáter. Concentrated hydrochloric acid was added dropwise to make the reaction mixture strongly acidic. The precipitate obtained was filtered, washed throughly with cold wáter and dried. The dried product was further recrytallized from dehydrated ethanol to get white crystals.

2-[(4-amino-5-aryl-4*H*-1,2,4-triazol-3-yl)thio]-*N*-aryl acetamides/propionamides

To a solution of appropriate 4-amino-5-aryl-4*H*-1, 2, 4-triazole-3-thiol (0.0005 mol, 0.10 g) in 15 ml of acetonitrile was added appropriate chloranilides (0.0005 mol, 0.09 g) and triethylamine (0.0010 mol, 0.14 ml) dropwise. The reaction mixture was refluxed for 4 h. Next, the reaction mixture was cooled and 10 ml of water was added to it followed by extraction with chloroform (3 × 10 ml). The aqueous layer was evaporated to yield a solid residue. The residue was then washed with acetone (1×20 ml), dried and was recrystallized from dehydrated ethanol to yield white crystals.

Pyrazoles

1st scheme:

3, 5 dimethyl pyrazole

Hydrazine sulphate(0.5 mole) was dissolved in 10 % sodium hydroxide in a round bottom flask. The flask was immersed in an ice bath and cooled to 15 ^{0}C.Acetyl acetone(0. 5 mole) was added into the mixture dropwise with stirring for 30 mins and the stirring was then continued for 1 hr at 15 ^{0} C. T he contents of the flask were diluted with water to dissolve the organic precipitate which was then extracted with ether. The solvent was then removed by distillation and the residue was crystallized from ethanol to yield the product.

1-hydroxymethyl 3, 5 dimethylpyrazole

To a solution of 3, 5 dimethyl pyrazole(0.034 mole) dissolved in 5ml of ethanol, 3 ml of 35% formalin was added. After stirring at 30^0 C for 40hrs, the mixture was extracted with chloroform to yield the precipitate.

N,N –bis (3, 5 dimethyl pyrazole-1-yl) methyl) aryl amines

The appropriate aryl amine (0.0075mol)was added to a mixture of 1-hydroxymethyl 3, 5 dimethylpyrazole (0.015mol) in acetonitrile.The reaction mixture was stirred at 70^0 C for 4 hrs. Then residue was washed with dichloromethane and water. The organic solvent was removed in vacuo and the residue was recrystallized from dichloromethane /ether to yield the product.

2nd scheme:

3-(2-arylhydrazono)pentane-2,4-diones

The appropriate amine (0.9ml)was dissolved in a mixture of conc. HCl (8ml) and water(10 ml) and cooled to 0 ^{0}C on ice bath. A cold aqueous solution of sodium nitrite (0.02mole) was added to this mixture .The cold diazonium salt solution was filtered and to the filtrate,a cooled solution of acetyl acetone (2.1ml)was added in the presence of sodium nitrite (0.01mol)and sodium acetate (0.05mole)in ethanol and stirred for 2-4hrs at room temperature. Finally pH (8-10) was adjusted with sodium hydroxide and the reaction mixture was kept in ice cold condition , filtered and recrystallized from ethanol to yield the product.

3,5-dimethyl-4-(aryldiazenyl)-1H-pyrazoles

A mixture of the appropriate 3-(2-arylhydrazono)pentane-2,4-dione (0.003 mole) and hydrazine hydrate (0.003mole) was heated under reflux in glacial acetic acid for 26 hrs. The reaction mixture was cooled and the precipitate obtained were filtered and recrystallized from methanol to give the final product.

2. 1-(4-aryl)-2-(3,5-dimethyl-4-(aryldiazenyl)-1H-pyrazol-1-yl)ethanones

To a solution of appropriate 3,5-dimethyl-4-(aryldiazenyl)-1H-pyrazole (0.0125mol) in DMF was added appropriate phenacyl bromide with cooling and mixture was stirred for 24 hrs at 0-5 degree C. The solution was then added to ice cold water and resulting solid was filtered and recrystallized to yield final product.

Imidazoles

Ammonium Aryl Carbamodithioate

To concentrated ammonia (9 ml,0.52 mol) was added carbon disulphide (4.3 ml, 0.071 mol) dropwise with stirring such that the temperature did not rise above 4^{0}C .To the stirred reaction mixture appropriate aromatic amine was added dropwise with vigorous stirring at 0-4^{0}C for 60 min. A milky homogenous mixture was obtained initially which was cooled in ice bath for 1 hr to yield a solid precipitate. It was filtered, washed with ether (2×5 ml) and dried. The product was obtained as white amorphous powder and was used as such for further reaction.

Methyl aryl dithiocarbamate

The appropriate ammonium (aryl) dithiocarbamate (69a) (1.82 g, 0.01 mol) was dissolved in 10 ml methanol with cooling in ice bath. Methyl iodide (0.64 ml, 0.01 mol) was added to the solution and stirred vigorously at a temperature of 0-4^{0}C for 3 hrs. A precipitate was obtained as white powder which was washed with water (5×5 ml) and dried. This product was used as such for further reaction.

***N*-aryl hydrazinecarbothioamides**

To a solution of appropriate methyl (aryl) dithiocarbamate (1.97 g, 0.01 mol) in 15 ml of methanol at 60°C was added 1 ml (0.025 mol)of 85% hydrazine hydrate and then stirred continuously for 2 hrs. A precipitate was cooled at room temperature to yield white crystalline mass which was filtered, washed with cold methanol (3×5 ml) and dried.

1-(2 mercapto-5-(4-aryl)-2- -1*H*-imidazol-1-yl)-3-(substituted phenyl) thioureas

To a solution of the appropriate phenacyl bromide (0.5g, 0.002mol) in glacial acetic acid (10 ml), potassium thiocyanate (0.02 g, 0.0002 mol) was added with continuous stirring at a temperature of 60°C.The reaction mixture was then stirred for 30 mins at the same temperature. N-(2-aryl) hydrazinecarbothioamide (71a) (0.5 g, 0.0002 mol) was added to the reaction mixture and stirring was continued at 60^{0}C for 1 hr. The reaction mixture was then refluxed for 6 hrs. After refluxing was complete, 30ml of water was added to the reaction mixture which yielded a gummy mass .It was washed with ether (25×10 ml). The product precipitated out as an amorphous powder and was recrystallized from ethanol-water mixture.

Part C: Characterization of all the synthesized compounds has been carried out by FTIR, ^{1}H NMR, Mass spectral data and elemental analyses.

Part D:All the synthesized compounds were subjected to HIV-1 -RT assay for *in vitro* activity by our following collaborators: Department of Biochemistry, Faculty of Science, Kasetsart University, 50 Pahon -Yothin Road,Chatuchak, Bangkok-10900 and Department of Pharmaceutical Sciences,Birla Institute of Technology and Sciences, Pilani, Rajasthan.

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Evaluation of *in-vitro* HIV-1 Reverse Transcriptase inhibition activity

In-vitro HIV-RT inhibitory potency of the synthesized analogues was evaluated using colorimetric assay kit procured from Roche diagnostics. All the reagents required to perform RT inhibitory assay were supplied in the kit and the ELSIA procedures for RT inhibition assay was performed as described in the kit protocol (Chen et al. 2013). Standard drug efvarienz was used for comparison purpose.

Briefly, the reaction mixture containing HIV-1 reverse transcriptase (RT) enzyme, viral nucleotides (digoxigenin (DIG)-dUTP, biotin-dUTP and dTTP) and reconstituted template in the incubation buffer with or without inhibitor was incubated at 37 ºC for 1 h in reaction tubes. The reaction mixture was transferred to streptavidine coated microtitre plate (MTP) and incubated for another 1 h at 37 ºC. The biotin-labeled viral nucleotides incorporated in nascent cDNA synthesized was catalyzed by RT were bound to streptavidine. The unbound and un-reacted reaction mixture was removed by gentle washing with washing buffer. Then anti-DIG-POD working solution was added into the MTPs and incubated for 1 h at 37 ºC. The DIG-labeled dNTPs incorporated in cDNA were bound to the anti-DIG-POD antibody. The unbound anti-DIG-PODs were removed by washing and working peroxide substrate (ABST) solution was added to the MTPs. Finally the reaction mixture was incubated at 25 ºC for 15-20 min, until the sufficient green color was developed for detection. The absorbance of the sample was determined at O.D. 405 nm using microtiter plate ELISA reader (Stat Fax 2100). The percentage inhibition of RT activity was calculated by formula as given below:

$$\%\ inhibition\ = 100 - [\frac{OD\ value\ with\ inhibitor - Blank}{OD\ value\ without\ inhibitor - Blank} \times 100]$$

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Some thiazolidines of the [**C**] series have shown significant percentage inhibition of RT in *wt* strain of HIV, upto 73% which is comparable to efavirenz. Some benzimidazoles of the [**C**] series have shown moderate (50-52%) to weak (10-30%) activity. Some imidazoles [A] series also showed some significant activity. Triazoles and pyrazoles showed moderate to mild HIV-1-RT inhibitory activity.

Part E: Molecular modelling studies of the most active compounds for binding mode analysis and virtual ADME studies:

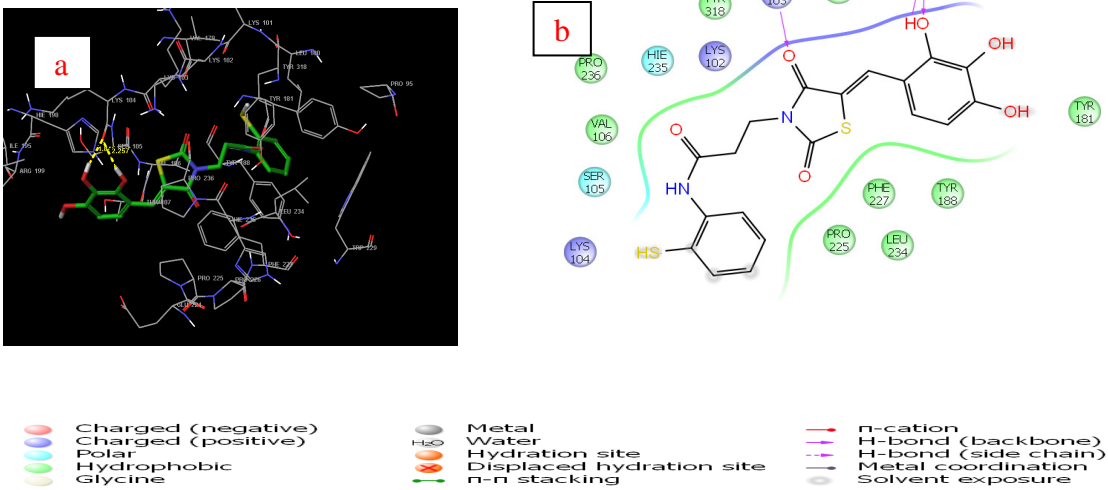

Fig. 8.a.Molecular model of **C292** in the NNIBP of HIV-1 RT (PDB code 1RT2).(C=O $_{\text{thiazolidine dione}}$.....NH $_{\text{Lys 103}}$ =...1.85 Å), (OH $_{\text{phenyl}}$.....C=O $_{\text{Lys 101}=}$ 2.25 Å)(**Glide XP Score-12.92**). Active site amino acid residues are represented as tubes while the inhibitor is shown as ball and stick model with the atoms colored as carbon: green, hydrogen: cyan, nitrogen: blue, and oxygen: red. Hydrogen bond interactions are represented by yellow dotted lines. **b.** Schematic (2D) representation of interactions of compound **C292** in in the NNIBP of 1RT2

With the aim of rationalizing the biological data obtained and considering the best obtained *in vitro* results for the test compound 51(**C292) of thiazolidine diones,** a molecular modeling study was carried out in order to investigate the possible interaction of the highest active test compound 51 in the NNIBP of RT. Ligand structures were drawn within maestro by using build module of Schrodinger Suite 2008 and was

geometry optimized by using the Optimized Potentials for Liquid Simulations-2005 (OPLS_2005) force field. Visual inspection was then performed on the resulting docking solutions of the test compound 51 to analyze the binding modes and key protein ligand interactions and was compared with that of the experimentally determined binding mode and interactions of the bound ligand TNK-651 and that of efavirenz. The key interactions were mainly hydrogen bonding interactions with Lys103 and Lys101 respectively. The carbonyl oxygen in the thiazolidine-2,4-dione moiety forms a strong H-bond interaction with the NH terminal group of Lys103($C{=}O_{\text{thiazolidine dione}}$.....$NH_{\text{Lys 103}}$ =...1.85 Å). Another strong H-bond interaction of the hydrogen atom of the hydroxyl group at the ortho position of the second phenyl ring with one of carbonyl oxygen atoms of Lys101 was observed(OH_{phenyl}……$C{=}O_{\text{Lys 101}}$= 2.25 Å). The phenyl ring in the 2, 3, 4-hydroxybenzaldehyde moiety attached to position 5 of the thiazolidine-2,4-dione ring was favorably oriented in the bigger hydrophobic pocket formed by Tyr181, Tyr188, Leu100 and Val179. Thiazolidine-2,4-dione ring was oriented in the milder hydrophobic pocket formed by Phe227, Pro225 and Leu234. The CH_2CH_2CONH linker attached to the thiazolidine-2,4-dione moiety showed favorable interaction with the amino acid residues Tyr318, Pro236 and Val106 The docking score of test compound 51 was -12.92 comparable and more than that of standard efavirenz (Glide XP score-11.33)(Fig.8).

Similarly the binding mode analysis for imidazoles (test compounds 5, 56 and 60) against HIV-1-RT was investigated by docking studies. Ligand structures were drawn in Chemdraw Professional 15.0. Autodock 4.0.1. program was used to dock the test compound 5 into the non nucleoside inhibitor binding pocket (HIV-1 RT NNIBP) of HIV-1 RT (PDB ID: 1RT2) while test compound 5, 56 and 60 were docked in the active site of glucosamine-6-phosphate synthase (PDB ID: 2VF5)to understand the binding mode analysis regarding antibacterial and antifungal activities.

The NNIBP was prepared by assigning geometrical coordinates of co-crystallized protein of HIV-1 reverse transcriptase with TNK-651 downloaded from Protein Brookehaven Database [108] (PDB ID: 1RT2).

In initial stage of docking, TNK 651 in the NNIBP of 1RT2 was carried out to check the correctness of all docking calculations, reliability and reproducibility of the docking parameters of our study (Fig. 2a and 2b). Autodock was able to calculate binding mode analysis and their conformations of TNK 651 with minimum RMSD of 0.56 ($\geq$ 3 A^0).

The estimated binding free energy for standard TNK 651 was measured as -11.69 kcal/mol while for the test compound 5 was -10.3. An examination of the binding mode of test compound 5 (Fig. 8b) disclosed that the centeroid imidazole ring was oriented towards the bigger hydrophobic pocket of amino acid residues PHE 227, LYS103, TYR 188, LYS101, TYR318 while the linker group attached with phenyl ring C=S-NH was surrounded by amino acids LEU 100 and TYR318. Hydrogen bonding interaction of SH group of test compound 5 and PRO236 was measured as $2.001A^0$ ($SH_{imidazole}$_PRO236 = $2.001A^0$). There were two π-π interactions between phenyl ring of test compound 5 and amino acid TYR181 and TYR188.

Virtual ADME studies viz., molecular weight (MW), (log Po/w), aqueous solubility (QPlogS) ,apparent MDCK cell permeability (QPPMDCK) , brain/blood partition coefficient (QPlogBB) and percent human oral absorption)for all the synthesized compounds has been carried out. All the compounds are lead like in nature

13. ACHIEVEMENTS FROM THE PROJECT:

a) A 3 D QSAR model was developed which was robust enough to predict thr activity of unknown compounds.

b)Basted on 3D QSAR studies and molecular modelling studies a number of azoles were synthesized and characterized.

c)Some of the azoles have shown very interesting activity against the Non nucleoside Inhibitory binding pocket of HIV-1 RT.

Publications:

1	Rohit Singh and Swastika Ganguly, " Design, Synthesis and Evaluation of some novel1-phenyl-3-(5-phenyl-1H-imidazol-1-yl)thiourea derivatives as antiHIV agents "Indian Journal of Pharmaceutical Education and Research, Oct-Dec 2018; Vol. 52(4): 655-65.
2	Rohit Singh and Swastika Ganguly, Synthesis ,Antimicrobial Evaluation and structure activity relationship (SAR)studies of some 1-phenyl-3-(5-phenyl-1H-imidazol-1-yl)thiourea derivatives .Antiinfective Agents. 2017; 15, 1.

3	Rohit Singh and Swastika Ganguly, " Molecular Docking Studies Of Novel Imidazole Analogs as HIV-1-RT Inhibitors "International Journal of Pharmaceutical Sciences and Research, 2017; Vol. 8(9): 1000-07.
4	Rohit Singh and Swastika Ganguly, " Synthesis and antimicrobial evaluation of some 1-phenyl-3-(5-phenyl-1H-imidazol-1-yl)thiourea derivatives "Indian Journal of Heterocyclic Chemistry, Apr-Jun 2018; Vol. 28(3): 361-366.
5	Radhe Shyam Bahare and **Swastika Ganguly**, Comparative molecular docking studies of novel 3, 5-disubstituted thiazolidinedione analogs as HIV-1-RT inhibitors, Medicinal Chemistry Research , (2014) 23(3):1300–1308.

Contributions to the Society: The molecules so developed may be subjected to preclinical trials and if they show possibility they can be further subjected to clinical trials and further be used as novel drug candidates. This would be of great help to the common man who has been suffering from HIV infection.

iii. Has the progress been according to original plan of work and towards achieving the objective. if not, state reasons : The progress is satisfactory and according to the original plan of work.

iv. Please indicate the difficulties, if any, experienced in implementing the Project : During the tenure of the project we only received the first installment of the sanctioned amount of Rs. **13.838 lacs** that is we received only Rs. **9.398 lacs** .

The second installment of **4.44 lacs** was **never released in spite of successful midterm presentation (copy attached)and repeated reminders personally with the utilization certificate dated 17th DECEMBER 2015.**

As such the following difficulties were faced:

1. The research scholar Rohit Singh could not get his fellowship amount per month because of which during the project he struggled with monetary problems.

2. Further chemicals could not be bought for further synthesis which hampered our research work repeatedly. We had to carry out our research work with whatever chemicals we were able to buy from the first installment.
3.Required field work was also not possible because of the non release of the second installment.
On verbal discussion with UGC officials later, it was communicated that only after submission of the completion documents, the accounts may be finalized with the release of the remaining grants.

Collaboration, if any (with Department, University, Industry etc,): Collaborations with the following Institutes**:**

- Department of Biochemistry, Faculty of Science, Kasetsart University, Bangkean, Bangkok 10900, Thailand
- Department of Pharmacy, Birla Institute of Technology & Science, Vidya Vihar, Pilani-333031,Rajasthan. India

v. If project has not been completed, please indicate the approximate time by which it is likely to be completed.
In spite of the non release of the second instalment the project was completed satisfactorily by making good utilization of the first instalment throughout the period of project and even beyond that.

vi. If the project has been completed, please enclose a summary of the findings of the study. Two bound copies of the final report of work done may also be sent to the Commission:
(Two bound copies of Annexure I has been sent with a electronic copy in the CD): For summary please see Annexure II

vii . **Any other information which would help in evaluation of work done on the project. At the completion of the project, the first report should indicate the output, such as (a) Manpower trained (b) Ph. D. awarded (c) Publication of results (d) other impact, if any**

b)**Ph. D Enrolled and Awarded:** Mr. Rohit Singh (PHD/PH/10052/2013) enrolled for Ph.D. programme under this UGC MRP project in the Dept. of Pharm. Sciences and Technology, BIT, Mesra on 17th July 2014.

Ph.D Awarded: . Rohit Singh (PHD/PH/10052/2013)in July 2019

c. Publications: Copy of first page of each publication attached.

1	Rohit Singh and Swastika Ganguly, " Design, Synthesis and Evaluation of some novel1-phenyl-3-(5-phenyl-1H-imidazol-1-yl)thiourea derivatives as antiHIV agents "Indian Journal of Pharmaceutical Education and Research, Oct-Dec 2018; Vol. 52(4): 655-65.
2	Rohit Singh and Swastika Ganguly, Synthesis ,Antimicrobial Evaluation and structure activity relationship (SAR)studies of some 1-phenyl-3-(5-phenyl-1H-imidazol-1-yl)thiourea derivatives .Antiinfective Agents. 2017; 15, 1.
3	Rohit Singh and Swastika Ganguly, " Molecular Docking Studies Of Novel Imidazole Analogs as HIV-1-RT Inhibitors "International Journal of Pharmaceutical Sciences and Research, 2017; Vol. 8(9): 1000-07.
4	Rohit Singh and Swastika Ganguly, " Synthesis and antimicrobial evaluation of some 1-phenyl-3-(5-phenyl-1H-imidazol-1-yl)thiourea derivatives "Indian Journal of Heterocyclic Chemistry, Apr-Jun 2018; Vol. 28(3): 361-366.
5	Radhe Shyam Bahare and **Swastika Ganguly**, Comparative molecular docking studies of novel 3, 5-disubstituted thiazolidinedione analogs as HIV-1-RT inhibitors, Medicinal Chemistry Research , (2014) 23(3):1300–1308.