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**Research Article**

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# Synthesis, biological evaluation and docking study of 2-amino-4,6-diaryl-pyrimidines as novel non-nucleoside HIV-1reverse transcriptase inhibitors

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**ABSTRACT**

*A novel series of substituted 2-Amino-4,6-diarylpyrimidines (DAPY's)as Non-nucleoside reverse transcriptase inhibitors (NNRTIs) were designed, synthesized and evaluated for in vitro reverse transcriptase (RT) inhibition activity. Out of the reported compounds,* ***4a, 4i, 4j*** *and* ***4n*** *showed potent anti-HIV activity as compared to standard rilpivirine. The other compounds displayed moderate activity against HIV-1. Binding affinities of the designed NCEs were studied on reverse transcriptase enzyme using docking studies and showed possible horseshoe conformation as required for the DAPY category of RT inhibitors. A correlation was found between the anti-HIV activity and the electrostatic energy interaction with Lys 101 residue.*

**Key words:** NNRTI, DAPY, RT inhibitors, docking

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## INTRODUCTION

Acquired immunodeficiency syndrome (AIDS) is a collection of symptoms and infections resulting from the specific damage to the immune system caused by infection with the human immunodeficiency virus (HIV) [1, 2]. As per the global report of United Nation programme on AIDS 2013, 35.3 million peoples were living with HIV, 2.3 million new HIV infections and 1.6 million AIDS-related deaths in 2012. Thus, AIDS is still one of the leading pandemic diseases worldwide [3]. Highly Active Antiretroviral Therapy (HAART) provided an effective way to treat AIDS patients by dramatically decreasing the morbidity and mortality from the infection of HIV-1. However, a significant proportion of patients did not fully benefit from HAART, as virus showed resistance to the available drugs, as a consequence of monotherapy and/or suboptimal combination therapy regimens [4]. Thus, it is an urgent need to design and develop new anti-HIV drugs with improved potency to halt the spread of HIV [5]. Non-nucleoside reverse transcriptase inhibitors (NNRTIs) of HIV-1 RT are one of the most promising areas of currently available anti-HIV therapies because of their diversity and specificity in targeting this enzyme [6]. However, the efficacy of NNRTIs is seriously hampered by the emergence of mutant viral strains. Therefore, it is imperative to look for new chemical entities (NCEs) having broad-spectrum activity against a variety of clinically relevant mutant RT enzymes with minimal cytotoxicity.

As per the report, [7] it has been observed that all NNRTI bind at the same site in the RT, despite the chemical heterogeneity of NNRTIs. The NNRTIs block the HIV-1 RT reaction through interaction with an allosterically located, non-substrate binding site. When bound into their pocket at the HIV-1 RT, the first generation NNRTIs maintains a very similar conformational 'butterfly-like' shape having hydrophilic centre as a 'body' and two hydrophobic moieties representing the 'wings'. Second generation, very potent and resilient DAPY compounds can

bind in multiple modes within the highly flexible NNRTI binding site of RT with horseshoe pattern. In particular, DAPYs have been regarded as one of the most successful scaffolds for NNRTIs because of their potent antiviral activity against wild-type and mutant strains of the HIV-1. Based on the above observation we thought to design new molecular scaffolds having important core of diarylpyrimidine (DAPY), a hydrophilic centre as a 'body', and two hydrophobic aromatic rings as wings. Thus, we thought logical to synthesis series of 2-(4,6-Diphenylpyrimidin-2-ylamino)-N-phenylacetamide for possible reverse transcriptase inhibitor activity.

## EXPERIMENTAL SECTION

Melting points were determined on Veego VMP-D digital melting point apparatus and were uncorrected. $^{1}$H NMR and $^{13}$C NMR spectra were recorded on a VARIAN MERCURY YH-300 NMR spectrometer (300MHz) with TMS as internal standard and DMSO as a solvent. Mass spectra were recorded on time of flight mass spectrometer. FT-IR spectra were recorded on JASCO FT-IR 4100 using KBr powder. The Labsystem Multiscan Microplate reader was used to determine absorbance of the sample to calculate the % RT inhibitory activity.

**General procedure for synthesis of N-Chloroacetylarylamine (1)**
Chloroacetylchloride (0.03 mol, 2.4 ml) was added to benzene (30ml) and mixture was stirred in water bath. The solution of aryl amine (aniline, 0.02mol) in benzene (30 ml) was added dropwise and refluxed for 2hrs, and then cooled the reaction mixture. The resulting white precipitate was filtered, washed with benzene, and purified by recrystallization from alcohol [9].

**General procedure for synthesis of substituted Benzylideneacetophenones/chalcones (2 a-f)**
The substituted aromatic aldehydes (0.01 mol) and substituted acetophenones (0.01mol) was dissolved in 50 ml of ethanol and maintained at 5-10 $^{0}$C, 5 ml of 70% aqueous NaOH was added dropwise with constant stirring. The reaction mixture was further stirred for 3-4 hrs and left over night and neutralized with conc.HCl, and crystallized from ethanol [10].

**General procedure for synthesis of substituted 2-Amino-4,6-diarylpyrimidines (3 a-f)**
A mixture of chalcone (0.01mol) and guanidine hydrochloride (0.01mol) in30 ml of ethanol was refluxed for 6 hrs, cooled and poured into crushed ice. The 2-Amino-4,6-diarylsubstituted pyrimidine was obtained as a precipitate. The precipitate was thoroughly washed with ice cold ethanol, filtered and dried. The obtained crude product was purified by recrystallization with ethanol. The same procedure was performed by using substituted chalcones with guanidine hydrochloride to obtain designed derivatives [10].

**General procedure for synthesis of substituted 2-(4, 6-Diphenylpyrimidin-2-ylamino)-N-phenylacetamide (4)**
A solution of 2-Amino-4,6-diarylpyrimidine (0.005 mol) and N-Chloroacetylarylamine (0.005 mol) in pyridine (50 ml) was refluxed for 3-7 hr. After completion of reaction, excess of pyridine was distilled off and resulting solid was treated with methanol to yield white crystals of titled compounds. The resulting crystals were filtered and washed with methanol and water. The progress of reaction was monitored by TLC.

The spectral characterizations of synthesized derivatives are given below

**2-[4-(2,4-Dichlorophenyl)-6-phenylpyrimidine-2-ylamino]-N-phenyl-acetamide (4a)**
Brown solid (210 mg, 42%): FT-IR (KBr)(*ν*, cm$^{-1}$) 3122, 2929, 1683, 1630, 1594, 1259, 445; $^{1}$H NMR (DMSO) δ (ppm): 2.04 (s, 1H, NH); 3.48 (d, 2H, $CH_2$); 5.73 (s, 1H, NH); 7.07-9.01 (m, 14H, ArH); $^{13}$C NMR (DMSO-d6) δ ppm: 166 (C-H, amide),138-127 (aromatic), 161-163 (pyrimidine), 60-62 (aliphatic, $CH_2$); MS: m/z449.03 $(M)^{+}$, 450.23 $(M+1)^{+}$.

**2-[4-(4-Bromophenyl)-6-(2,4-dichlorophenyl)pyrimidin-2-ylamino]-N-phenyl-acetamide(4b)**
Yellow solid (310 mg, 62%): FT-IR (KBr) (*ν*, cm$^{-1}$) 3023, 2962, 1685, 1633, 1583, 1355, 1214,4623; $^{1}$H NMR (DMSO)δ (ppm): 2.47 (s, 1H, NH); 3.20 (d, 2H, $CH_2$); 5.76 (s, 1H, NH); 7.07-9.12 (m, 13H, ArH);$^{13}$C NMR (DMSO-d6) δ ppm: 168 (C-H, amide), 138-128 (aromatic), 161-163 (pyrimidine), 60-62 (aliphatic, $CH_2$); MS: m/z528.33 $(M)^{+}$.

**2-[4-(4-Chlorophenyl)-6-(3-methoxyphenyl)pyrimidine-2-ylamino]-N-phenyl-acetamide(4c)**
Light brown solid (310 mg, 62%): FT-IR (KBr) ($\nu$, $cm^{-1}$) 3099, 2904, 1596, 1259, 462; $^1H$ NMR (DMSO)δ (ppm): 2.10 (s, 1H, NH); 3.82 (d, 2H, $CH_2$); 3.35 (s, 3H, $OCH_3$); 5.76 (s, 1H, NH); 7.07-9.06 (m, 14H, ArH); $^{13}C$ NMR (DMSO-d6) δ ppm: 168 (C-H, amide), 138-126 (aromatic), 160-163 (pyrimidine), 61-63 (aliphatic, $CH_2$), 55-56 ($OCH_3$); MS: m/z444.39 $(M)^+$, 445.59 $(M+1)^+$.

**2-[4-(4-Chlorophenyl)-6-(3,4-dimethoxyphenyl)pyrimidin-2-ylamino]-N-phenyl-acetamide (4d)**
Light yellow solid (290 mg, 58%): FT-IR (KBr) ($\nu$, $cm^{-1}$) 3057, 1689, 1651, 1596, 1264, 448; $^1H$ NMR δ (ppm) : 2.10 (s, 1H); 3.30 (d, 2H); 3.90 (s, 6H); 5.77 (s, 1H); 7.07-8.10 (m, 13H); $^{13}C$ NMR (DMSO-d6) δ ppm: 168 (C-H, amide),138-126 (aromatic), 160-163 (pyrimidine), 61-63 (aliphatic, $CH_2$), 55-56 ($OCH_3$); MS: m/z474.64 $(M)^+$, 475.74 $(M+1)^+$.

**2-[4-(2,4-Dichlorophenyl)-6-(3,4-dimethoxyphenyl)pyrimidin-2-ylamino]-N-phenyl-acetamide (4e)**
Light brown solid (310 mg, 62%): FT-IR (KBr) ($\nu$, $cm^{-1}$) 3006, 1650, 1633, 1575, 1263, 447; $^1H$ NMR δ (ppm): 1.30 (s, 1H); 3.20 (d, 2H); 4.00 (s, 6H); 6.10 (s, 1H); 6.94-8.20 (m, 12H); $^{13}C$ NMR (DMSO-d6) δ ppm: 166 (C-H, amide), 138-128 (aromatic), 160-163 (pyrimidine), 61-63 (aliphatic, $CH_2$), 54-56 ($OCH_3$); MS: m/z509.28 $(M)^+$, 511.23 $(M+2)^+$.

**2-[4-(4-Bromophenyl)-6-(2-hydroxyphenyl)pyrimidin-2-ylamino]-N-phenylacetamide (4f)**
Light yellow solid (390 mg, 78%): FT-IR (KBr) ($\nu$, $cm^{-1}$) 3351, 3176, 1683, 1633, 1596, 1259; $^1H$ NMR δ (ppm) : 1.80 (s, 1H); 4.00 (d, 2H); 6.10 (s, 1H); 6.80 (s, 1H); 7.07-8.10 (m, 14H); $^{13}C$ NMR (DMSO-d6) δ ppm: 168 (C-H, amide), 138-119 (aromatic), 161-163 (pyrimidine), 60-62 (aliphatic, $CH_2$), 146 (C-OH); MS: m/z477.39 (M+1).

**2-(4-(4-(trifluoromethyl)phenyl)-6-(2,4-dimethoxyphenyl)pyrimidin-2-ylamino)-N-phenylacetamide (4g)**
Brown solid (310 mg, 78%) : FT-IR (KBr) ($\nu$, $cm^{-1}$) 3361, 3168, 2921, 2852, 1674, 1490, 1100; $^1H$ NMR (DMSO) δ (ppm): 4.00 (s, 1H, NH); 3.82 (d, 2H, $CH_2$); 7.23 (s, 1H, NH); 6.61-7.85 (m,13H, ArH); $^{13}C$ NMR (DMSO-d6) δ ppm : 168 (C-H, amide), 098-161 (aromatic), 101-165 (pyrimidine), 55-56 (aliphatic, $CH_2$); MS: m/z 508.49 $(M)^+$, 509.49 $(M+1)^+$.

**2-(6-(2,4-dimethoxyphenyl)-4-(3,4-dimethoxyphenyl)pyrimidin-2-ylamino)-N-phenylacetamide (4h)**
Brown solid (190 mg, 54%) : FT-IR (KBr) ($\nu$, $cm^{-1}$) 3359, 3168, 2921, 2361, 1664, 1409, 1258; $^1H$ NMR (DMSO) δ (ppm) : 4.00 (s, 1H, NH); 3.82 (d, 2H, $CH_2$); 7.23 (s, 1H, NH); 6.61-7.85 (m, 12H, ArH); $^{13}C$ NMR (DMSO-d6) δ ppm : 168 (C-H, amide), 098-161 (aromatic), 101-165 (pyrimidine), 55-56 (aliphatic, $CH_2$); MS: m/z 500.49 $(M)^+$, 501.49 $(M+1)^+$.

**2-(4-(2-fluorophenyl)-6-(2,4-dimethoxyphenyl)pyrimidin-2-ylamino)-N-phenylacetamide (4i)**
Brown solid (305 mg,75%) : FT-IR (KBr) ($\nu$, $cm^{-1}$) 3398, 3168, 2850, 2156, 1671, 1448; $^1H$ NMR (DMSO) δ (ppm) : 4.00 (s, 1H, NH); 3.82 (d, 2H, $CH_2$); 7.23 (s, 1H, NH); 6.61-7.85 (m, 13H, ArH); $^{13}C$ NMR (DMSO-d6) δ ppm : 168 (C-H, amide), 098-158 (aromatic), 101-165 (pyrimidine), 55-56 (aliphatic, $CH_2$); MS: m/z 458.18 $(M)^+$, 459.18 $(M+1)^+$.

**2-(4-(2,4-dimethoxyphenyl)-6-(4-fluorophenyl)pyrimidin-2-ylamino)-N-phenylacetamide(4j)**
Faint Yellow solid (410 mg, 82%) : FT-IR (KBr) ($\nu$, $cm^{-1}$) 3384, 3168, 2952, 1671, 1467; $^1H$ NMR (DMSO) δ (ppm) : 4.00 (s, 1H, NH); 3.82 (d, 2H, $CH_2$); 7.23 (s, 1H, NH); 6.61-7.85 (m, 13H, ArH); $^{13}C$ NMR (DMSO-d6) δ ppm : 168 (C-H, amide), 098-162 (aromatic), 101-165 (pyrimidine), 55-56 (aliphatic, $CH_2$); MS: m/z 458.18 $(M)^+$, 459.18 $(M+1)^+$.

**2-(4-(4-hydroxyphenyl)-6-(4-(trifluoromethyl)phenyl)pyrimidin-2-ylamino)-N-phenylacetamide(4k)**
Black solid (210 mg, 50%) : FT-IR (KBr) ($\nu$, $cm^{-1}$) 3369, 3168, 2922, 1665, 1556, 1447; $^1H$ NMR (DMSO) δ (ppm) : 4.00 (s, 1H, NH); 3.82 (d, 2H, $CH_2$); 7.23 (s, 1H, NH); 6.86-7.85 (m, 14H, ArH); $^{13}C$ NMR (DMSO-d6) δ ppm : 168 (C-H, amide), 116-158 (aromatic), 101-165 (pyrimidine), 55 (aliphatic, $CH_2$); MS: m/z 464.44 $(M)^+$, 465.44 $(M+1)^+$.

**2-(4-(3,4-dimethoxyphenyl)-6-(4-hydroxyphenyl)pyrimidin-2-ylamino)-N-phenylacetamide (4l)**
Black solid (280 mg, 66%) : FT-IR (KBr) ($\nu$, $cm^{-1}$) 3393, 3168, 2923, 1663, 1490; $^1H$ NMR (DMSO) δ (ppm) : 4.00 (s, 1H, NH); 3.82 (d, 2H, $CH_2$); 7.23 (s, 1H, NH); 6.86-7.85 (m, 13H, ArH); $^{13}C$ NMR (DMSO-d6) δ ppm : 168 (C-

H, amide), 111-150 (aromatic), 101-165 (pyrimidine), 55-56 (aliphatic, $CH_2$); MS: m/z 456.49 $(M)^+$, 457.49 $(M+1)^+$.

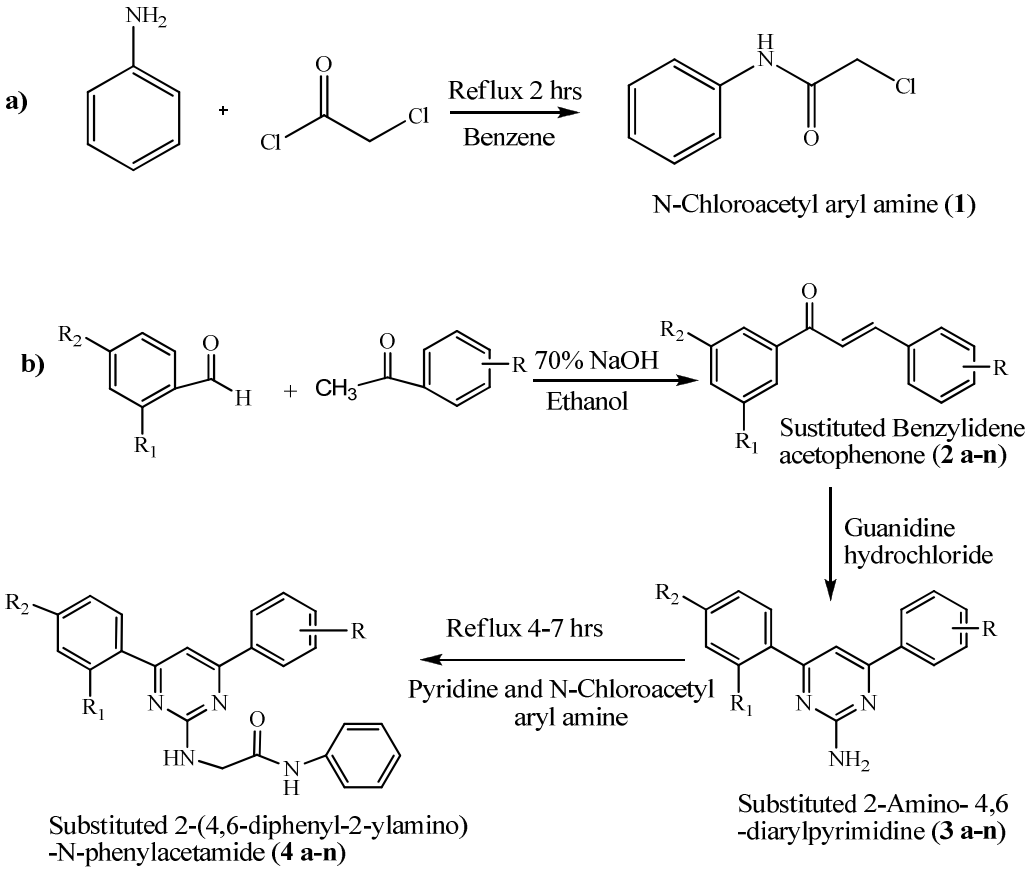
**2-(4-(2-fluorophenyl)-6-(4-hydroxyphenyl)pyrimidin-2-ylamino)-N-phenylacetamide (4m)**
Black solid (180 mg, 62%) : FT-IR (KBr) ($\nu$, $cm^{-1}$) 3354, 3168, 2923, 2166, 1666, 1490; $^1H$ NMR (DMSO) δ (ppm): 4.00 (s, 1H, NH); 3.82 (d, 2H, $CH_2$); 7.23 (s, 1H, NH); 6.86-7.85 (m, 14H, ArH); $^{13}C$ NMR (DMSO-d6) δ ppm : 168 (C-H, amide), 116-158 (aromatic), 101-165 (pyrimidine), 55 (aliphatic, $CH_2$); MS: m/z 414.15 $(M)^+$, 415.15 $(M+1)^+$.

**2-(4-(4-fluorophenyl)-6-(4-hydroxyphenyl)pyrimidin-2-ylamino)-N-phenylacetamide (4n)**
Black solid (310 mg, 70%) : FT-IR (KBr) ($\nu$, $cm^{-1}$) 3366, 3168, 2929, 1669, 1447;$^1H$ NMR (DMSO) δ (ppm): 4.00 (s, 1H, NH); 3.82 (d, 2H, $CH_2$); 7.23 (s, 1H, NH); 6.86-7.85 (m, 14H, ArH); $^{13}C$ NMR (DMSO-d6) δ ppm : 168 (C-H, amide), 116-162 (aromatic), 101-165 (pyrimidine), 55 (aliphatic, $CH_2$); MS: m/z 414.15 $(M)^+$, 415.15 $(M+1)^+$.

***In vitro* RT inhibition assay**
The standard rilpivirine and test compounds (**4a-n**) were powdered finely and suspended in DMSO. Finally 20μg/mL sample was used for *in vitro* assay. The RT inhibition assay was performed by using an RT assay kit (Roche). The procedure for assaying RT inhibition was performed as reported protocol [11]. Briefly, the reaction mixture consists of template primer complex, dNTPs and reverse transcriptase (RT) enzyme in the lysis buffer with or without inhibitors. After 1hr incubation at $37^0$C, the reaction mixture was transferred to streptavidine-coated microtitre plate (MTP). The biotin-labeled dNTPs that are incorporated in the template due to activity of RT were bound to streptavidine. The unbound dNTPs were washed using washing buffer and anti-DIG-POD was added to the MTP. The DIG-labeled dNTPs incorporated in the template were bound to anti-DIG-POD Antibody. The unbound anti-DIG-POD was washed and the peroxide substrate (ABST) was added to the MTP. A colored reaction product was produced during the cleavage of the substrate catalyzed by a peroxide enzyme. The absorbance of the sample was determined at optical density (OD) 405 nm using micro titer plate ELISA reader. The % inhibition was calculated using following formula.

$$\% \text{ Inhibition} = 100 \times \left( \frac{\text{OD at 405nm with inhibitor}}{\text{OD at 405nm without inhibitor}} \right) 100$$

**Docking Methodology**
With the aim to investigate the binding mode of newly synthesized compounds, molecular modeling study was performed by the means of Glide 5.0 (Schrodinger Maestro 8.5). The crystal structure of HIV-1 RT complexed with diarylpyrimidine inhibitor Rilpivirin (PDB ID : 2ZD1) was retrieved from RCSB protein data bank and used as target for molecular modeling studies. First protein preparation procedure was used to obtained satisfactory starting structure for docking studies. This facility is designed to ensure chemical correctness and to optimize the protein structure for further analysis. The process adds hydrogen, neutralize appropriate amino acid chains, delete water molecule. A partial minimization on the co-crystallize structure was done using minimization force field OPLS 2005. The minimization was terminated when energy converged or the RMSD reached a maximum cutoff of 0.30 Å. The prepared protein structure was used to generate receptor grid using option from menu receptor grid generation. No scaling was done for Van der waals (vdw) radii of nonpolar receptor atom. An enclosing box was used as docking space, centered on allosteric site using Rilpivirin crystallographic position as reference the ligand diameter midpoint box was set to default value (10 Å). Structures of the synthesized compounds were processed using Schrodinger Ligprep utility. This generates number of low energy 3D structures. Ligand docking was performed using 0.80 (scaling factor) to scale the vdw radii of the nonpolar ligand atoms with a charge cutoff 0.15. Glide score standard precision used as scoring method.




**Scheme 1** Synthetic scheme designed compounds **(4a-n)**

| Compd. Id | R | $R_1$ | $R_2$ |
|---|---|---|---|
| **2a-4a** | H | Cl | Cl |
| **2b-4b** | Br (p) | Cl | Cl |
| **2c-4c** | $OCH_3$ (m) | H | Cl |
| **2d-4d** | di-$OCH_3$ (m,p) | H | Cl |
| **2e-4e** | di-$OCH_3$ (m,p) | Cl | Cl |
| **2f-4f** | Br (p) | OH | H |
| **2g-4g** | 4-$CF_3$ | $OCH_3$ | $OCH_3$ |
| **2h-4h** | 3-$OCH_3$, 4-$OCH_3$ | $OCH_3$ | $OCH_3$ |
| **2i-4i** | 2-F | $OCH_3$ | $OCH_3$ |
| **2j-4j** | 4-F | $OCH_3$ | $OCH_3$ |
| **2k-4k** | 4-$CF_3$ | - | OH |
| **2l-4l** | 3-$OCH_3$, 4-$OCH_3$ | - | OH |
| **2m-4m** | 2-F | - | OH |
| **2n-4n** | 4-F | - | OH |

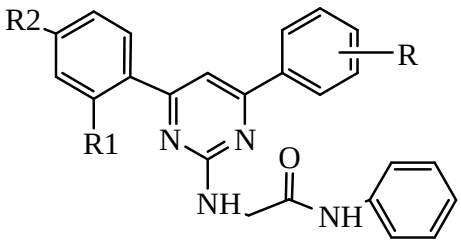



**Table 1 Characterization data for substituted 2-(4,6-Diphenylpyrimidin-2-ylamino)-N-phenyl-acetamide (4 a-n)**

| Compd. Id | Molecular formula | Molecular weight (g) | % Yield | Melting point ($^0$C) |
|---|---|---|---|---|
| **4a** | $C_{24}H_{18}Cl_2N_4O$ | 449.33 | 42 | **225-228** |
| **4b** | $C_{24}H_{17}BrCl_2N_4O$ | 528.23 | 62 | **216-219** |
| **4c** | $C_{25}H_{21}ClN_4O_2$ | 444.91 | 62 | **231-233** |
| **4d** | $C_{26}H_{23}ClN_4O_3$ | 474.94 | 58 | **135-137** |
| **4e** | $C_{26}H_{22}Cl_2N_4O_3$ | 509.38 | 62 | **159-160** |
| **4f** | $C_{24}H_{19}BrN_4O_2$ | 475.34 | 78 | **236-238** |
| **4g** | $C_{27}H_{23}F_3N_4O_3$ | 508.49 | 78 | **230-232** |
| **4h** | $C_{28}H_{28}N_4O_5$ | 500.55 | 54 | **220-223** |
| **4i** | $C_{26}H_{23}FN_4O_3$ | 458.18 | 75 | **210-212** |
| **4j** | $C_{26}H_{23}FN_4O_3$ | 458.18 | 82 | **170-172** |
| **4k** | $C_{25}H_{19}F_3N_4O_2$ | 464.44 | 50 | **193-195** |
| **4l** | $C_{26}H_{24}N_4O_4$ | 456.49 | 66 | **179-181** |
| **4m** | $C_{24}H_{19}FN_4O_2$ | 414.15 | 62 | **189-190** |
| **4n** | $C_{24}H_{19}FN_4O_2$ | 414.15 | 70 | **209-211** |

**Table 2 Percentage inhibition activity of compounds (4 a- n)**

| Compound ID | % Inhibition |
|---|---|
| **Control** | 0.12 |
| **4a** | **90.90** |
| **4b** | 89.88 |
| **4c** | 88.63 |
| **4d** | 87.15 |
| **4e** | 87.38 |
| **4f** | **90.34** |
| **4g** | **86.23** |
| **4h** | **82.11** |
| **4i** | **91.52** |
| **4j** | **92.20** |
| **4k** | **83.12** |
| **4l** | **82.25** |
| **4m** | **90.14** |
| **4n** | **91.45** |
| **Rilpivirine** | 99.92 |

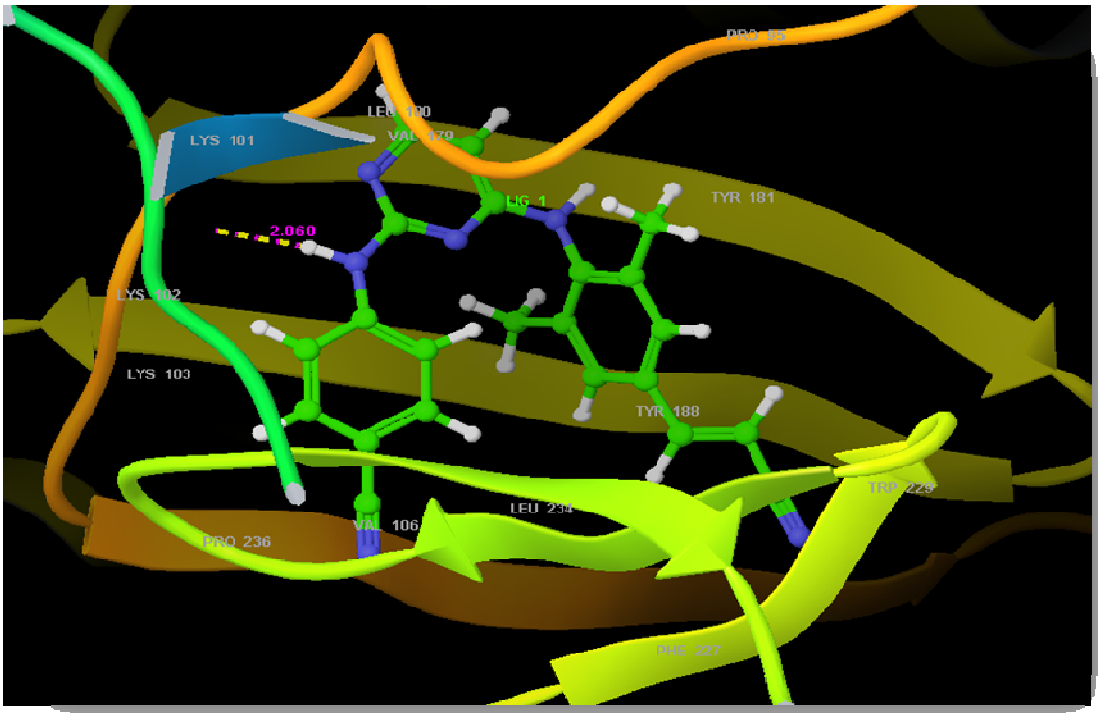



**Fig. 1 Ribbon representation of binding mode of standard rilpivirine showing H-bond with Lys101**

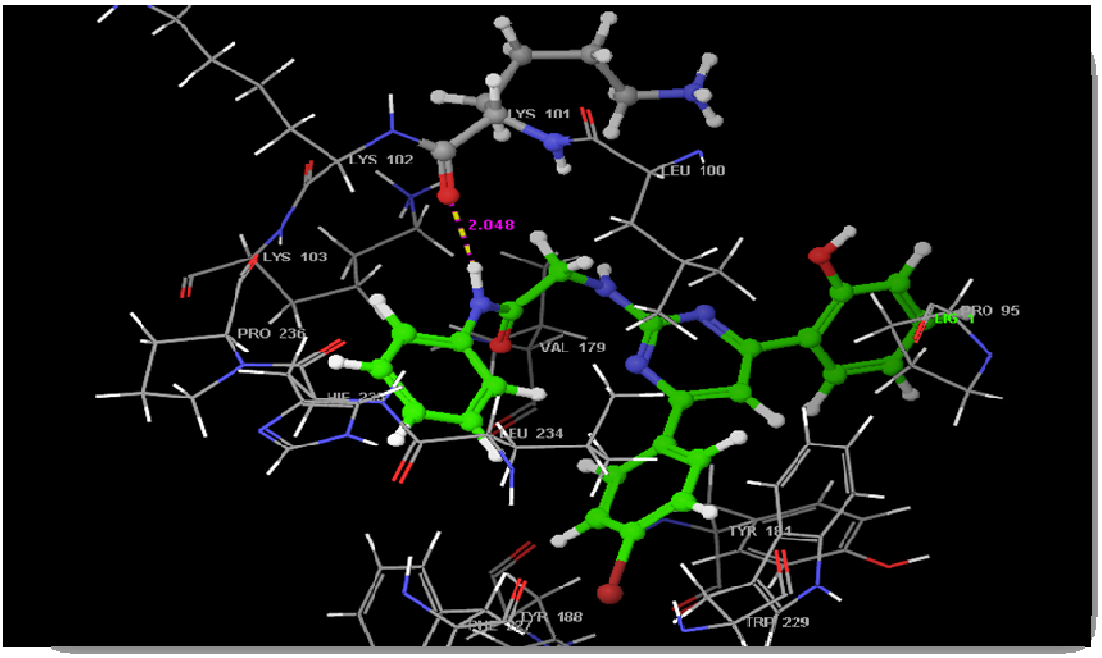
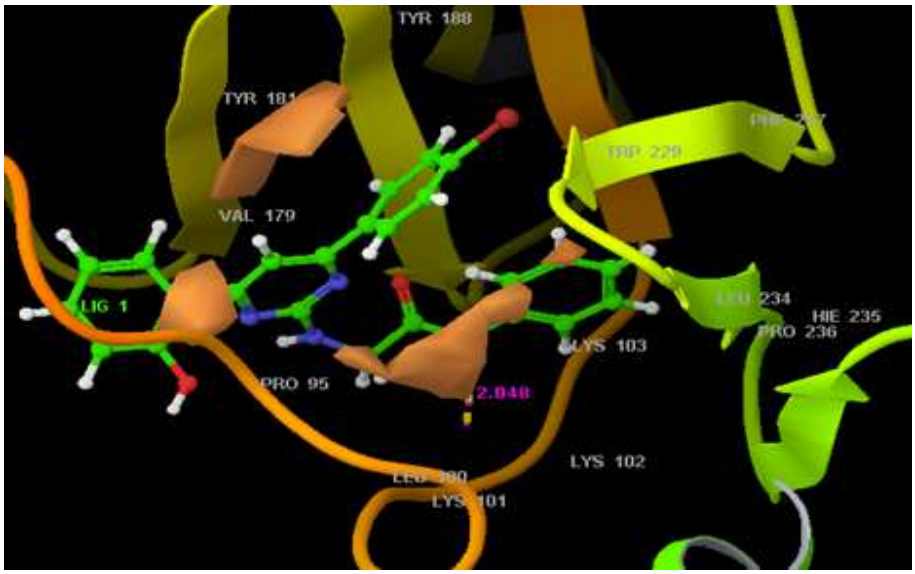



**Fig. 2 Binding mode of 4f with HIV-1 RT showing H-bond with Lys 101**

**a)**




**b)**

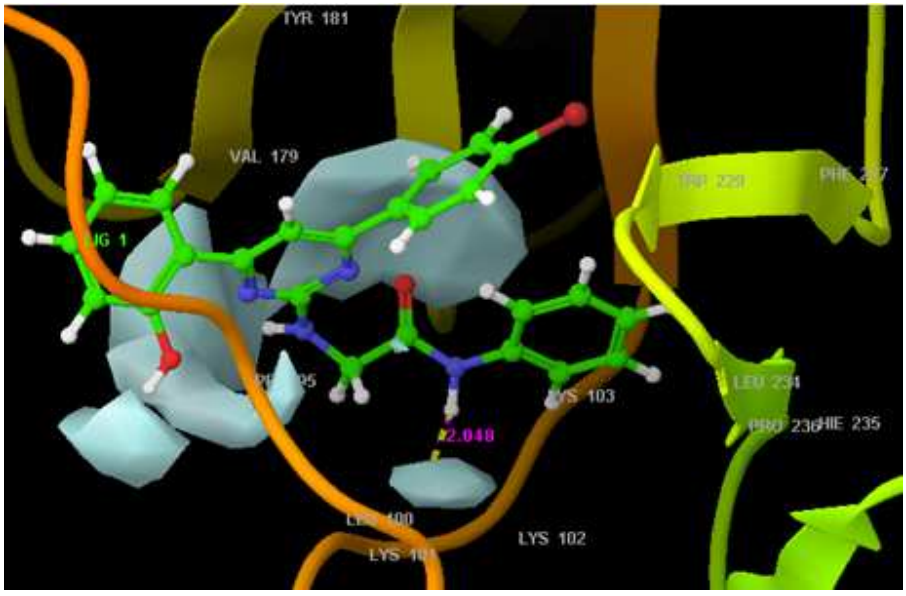



**Fig. 3 Ribbon representation of binding mode of 4f showing a) Hydrophobic region (orange color), b) Hydrophilic region (cyne color)**

## RESULTS AND DISCUSSION

### Chemistry

Synthesis scheme of target compounds **4a–n** is outlined in **scheme 1**. The first step is Claisen-Schmidt condensation reaction, which involved the reaction of substituted benzaldehydes with substituted acetophenones in ethanol and sodium hydroxide to yield a substituted benzylidene acetophenones/chalcones. Synthesized chalcones were subjected to cyclo-condensation reaction with guanidine hydrochloride in presence of ethanol which gave 2-Amino-4,6-diarylpyrimidines. The target compounds, 2-(4,6-Diphenylpyrimidin-2-ylamino)-N-phenylacetamide **(4a-n, Table 1)**, were synthesized by nucleophilic addition, elimination reaction between N-Chloro-acetylarylamine and 2-Amino-4,6-diarylpyrimidines in presence of pyridine.

**Biological activity**

The *invitro* RT inhibition assay was performed by using an RT assay kit (Roche) [8]. The procedure for performing RT inhibition assay was carried out as per the previously reported kit protocol. The entire synthesized compound showed comparable activity as compared with standard rilpivirine. Among the synthesized compound **4a (90.90%), 4b (89.88%) 4f (90.34%), 4j (92.20%), 4i (91.52%), 4m (90.14%), 4n (91.45%)** inhibition showed significant RT inhibitory activity as compare with standard rilpivirine **(99.92%)**. Compounds with–F, -Br, –Cl and –OH substitution at C2 and C4 position on the phenyl ring was found to increases RT inhibitory activity.

Compounds **4c, 4d, 4e, 4g, 4h, 4k** and **4l** showed good RT inhibitory activity as compared to rilpirivine. Compound **4d** showed poor RT inhibitory activity as compared to compounds in the series, may be because of presence of dimethoxy group on aromatic ring in the structure.

**Docking study**

Docking studies of synthesized compounds were carried out to see the interaction of the compounds with active site of RT enzyme. The docking studies showed that compound **4f** ranked higher (G Score -11.69) among the synthesized compounds. The glide score of the compound **4f** is less as compared to standard rilpirivirine (-12.90) (Fig.1). The interaction of compounds showed that amide –NH group of **4f** forms hydrogen bonding with –C=O group of Lys 101 (Fig. 2) and it lies in hydrophilic region. Diaryl wing and side chain aromatic ring fits into the aromatic rich hydrophobic cavity surrounded by Tyr 181, Tyr 188, Pro 277, Trp 229, Leu 234, and Pro 95 (Fig.3a). The phenyl ring at right wing particularly interacts with Tyr 181 giving rise to positive π stacking interaction. Central pyrimidine ring, 2-hydroxy group and hydrogen bond lies in the hydrophilic cavity (Fig. 3b).

## CONCLUSION

Compounds belonging to the series of substituted 2-(4,6-Diphenylpyrimidin-2-ylamino)-N-phenylacetamide have been designed, synthesized and evaluated for RT inhibitory activity. All the reported compounds possess good RT inhibitory activity as compared with standard rilpivirine. The docking studies indicate that central pyrimidine ring is having polar character which is important for RT inhibitory activity. Hydrogen bond between –NH of compound **4f** and carbonyl group of Lys 101 is prime requirement for inhibition and it increases affinity of compound towards HIV-1 RT enzyme. It is further concluded that compound with pyrimidine ring having substitutions of 2-Hydroxyphenyl at C-6 and 4-Bromophenyl at C-4 position showed higher activity. This may form possible horseshoe conformation of 4, 6 substitution on pyrimidine ring as required for the DAPY category of RT inhibitors. π stacking interactions of phenyl ring with the aromatic amino acids in hydrophobic cavity concludes proper positioning of 2 phenyl rings in eastern and western wings of non nucleoside inhibitor binding pocket (NNIBP).

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