RESEARCH ARTICLE

Synthesis, evaluation and binding mode analysis of some novel triazole derivatives as antimicrobials

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ABSTRACT

A series of twenty seven novel *N*-(substituted phenyl)-2-(1*H*-1,2,4-triazol-1-yl)acetamides 3(a-i), 2-[(4-amino-5-phenyl-4*H*-1,2,4-triazol-3-yl) thio]-*N*-(substituted phenyl) acetamides 5(a-i) and 2-[(4-amino-5- pyridin-3-yl-4*H*-1,2,4-triazol-3-yl)thio]-*N*-(substituted phenyl) acetamides 7(a-i) were synthesized by reacting 1*H*-1,2,4-triazole (2), 4-amino-5-pyridin-3-yl-4*H*-1,2,4-triazole-3-thiol (4) and 4-amino-5-phenyl-4*H*-1,2,4-triazole-3-thiol (5) with the respective 2-chloro-*N*-(substituted phenyl) acetamides 1(a-i) in acetonitrile and triethylamine. Structure elucidation of all the synthesized compounds was made on the basis of the data obtained out of various spectral studies. All the synthesized compounds were evaluated for HIV-1 reverse transcriptase (RT) inhibitory activity, antibacterial and antifungal activities. Compound 7i was the most promising among all with 52% HIV inhibition. Most of the compounds showed comparable antifungal activity against *Candida spp* with respect to the standard antifungal agent fluconazole, whereas they exhibited weak inhibitory property against pathogenic bacteria taken for the study. Docking studies with reverse transcriptase enzyme of (PDB entry code 1RT2) was also performed for the highest active compound 7i in order to investigate the binding pattern of the same.

Keywords: 1, 2, 4-triazole, Acetamides, Efavirenz, Fluconazole, Ciprofloxacin, Reverse Transcriptase

INTRODUCTION

Clinically many substituted 1, 2, 4-triazole derivatives are acknowledged to possess a wide range of bioactivities. The various activities associated with this moiety are anti-HIV, anti microbial, analgesic and anti-inflammatory, antineoplastic, sedatives, anxiolytics, anti-convulsants, antimigraine, antiobese and other activities. [1-14] This heterocyclic moiety have gained substantial attention in fields, such as medicinal and agrochemical research as well as in the material sciences due to their unique structure and properties. [15] Considering the development in triazole chemistry and biology, an initiative had been taken to synthesize and evaluate a series of twenty seven novel substituted 1, 2, 4-triazole derivatives as 3(a-i), 5(a-i) and 7(a-i) for anti-HIV, and antimicrobial activities. Binding mode analysis was performed for the highest active compound 7i in the active site of reverse transcriptase with the PDB entry code 1RT2.

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MATERIALS AND METHODS

The experimental work is described under Synthetic Studies, Anti-HIV studies, Antimicrobial Studies, FQlogP determination and Molecular Docking Studies.

Synthetic studies

The melting points reported are uncorrected. The FT-IR spectra of synthesized compounds were taken on SPECTRUM Px FT-IR Spectrometer PERKIN ELMER (serial no: 78625). ^{1}HNMR spectra was recorded on AMX-400 NMR spectrophotometer at 400 MHz using DMSO-d_6 as the solvent and tetramethylsilane as an internal standard. The mass spectra (m/z) of the compounds synthesized were recorded on a QTOF Micro YAZ63 spectrometer using electrospray ionization (ESI). Elemental analysis was performed on Vario EL III M/s Elementor C, H, N, S, O analyzer. The reagents were procured from Spectrochem., Merck India Pvt. Ltd., Bengal Chemicals, Kolkata and Otto Chemicals India and other chemicals used in the studies were either of A.R or G.R quality. Anhydrous sodium sulphate was used as the drying agent. In process monitoring of reaction was done on activated

silica gel coated plates and the solvent system used was benzene: chloroform: ethylacetate (6:3:1).

General Procedure for the preparation of 2-chloro-*N*-(substituted phenyl) acetamides 1(a-i)

To a solution of respective aromatic amine (0.033 mole) in 15 ml of glacial acetic acid was added chloroacetyl chloride (0.037 mole) dropwise while cooling in ice-bath. It was stirred for 30 min in ice-bath. Further, the mixture was stirred for 1 h at room temperature. The mixture was then poured into saturated sodium acetate solution. The precipitate was filtered and washed with cold water. It was dried and recrystallized from dehydrated ethanol to yield white crystals of 2-chloro-*N*-(substituted phenyl) acetamide.

N-benzyl-2-chloroacetamide (1a)
Yield: 5.26 g (87%), mp: 92 °C
Anal:
IR(KBr) (cm^{-1}): 3278.81 (N-H stretching), 3063.04 (aromatic C-H stretching), 2951.53 (aliphatic C-H stretching), 1649.07 (C=O stretching), 1452.81 (aromatic C=C stretching), 745.65 (C-Cl stretching).

2-chloro-N-(2-methoxyphenyl)acetamide (1b)
Yield: 6.19 g (94%), mp: 165 °C
Anal:
IR(KBr) (cm^{-1}): 3295.68 (N-H stretching), 2957.94 (aliphatic C-H stretching), 1665.83 (C=O stretching amide), 1413.71 (aromatic C=C stretching), 1151.85 (C-O-C stretching), 712.09 (C-Cl stretching).

2-chloro-*N*-(4-methoxyphenyl)acetamide (1c)
Yield: 6.05 g (92%), mp: 118 °C
Anal:
IR(KBr) (cm^{-1}): 3295.68 (N-H stretching), 2957.94 (aliphatic C-H stretching), 1665.83 (C=O stretching amide), 1413.71 (aromatic C=C stretching), 1151.85 (C-O-C stretching), 712.09 (C-Cl stretching).

2-chloro-N-(3-chlorophenyl)acetamide (1d)
Yield: 5.92 g (88%), mp: 138 °C
Anal:
IR(KBr) (cm^{-1}): 3268.33 (N-H stretching), 3100.74 (aromatic C-H stretching), 2972.59 (aliphatic C-H stretching), 1675.39 (C=O stretching amide), 691.56 (C-Cl stretching).

2-chloro-N-(4-ethoxyphenyl)acetamide (1e)
Yield: 6.27 g (89%), mp: 148 °C
Anal:
IR(KBr) (cm^{-1}): 3273.83 (N-H stretching), 3003.47 (aromatic C-H stretching), 2976.33 (aliphatic C-H stretching), 1673.72 (C=O stretching amide), 1171.80 (C-O-C stretching), 705.15 (C-Cl stretching).

2-chloro-*N*-(2-nitrophenyl)acetamide (1f)
Yield: 6.37 g (90%), mp: 89 °C
Anal:
IR(KBr) (cm^{-1}): 3307.64(N-H stretching), 3017.18 (aromatic C-H stretching), 2940.54 (aliphatic C-H stretching), 1691.10 (C=O stretching amide), 1514.98 (N-O asymmetric stretching), 1338.98 (N-O symmetric stretching), 1410.43 (aromatic C=C stretching), 715.60 (C-Cl stretching).

2-chloro-N-(4-nitrophenyl)acetamide (1g)
Yield: 6.30 g (89%), mp: 178 °C
Anal:
IR(KBr) (cm^{-1}): 3276.33(N-H stretching), 2940.54 (aliphatic C-H stretching), 1687.12 (C=O stretching amide), 1510.00 (N-O asymmetric stretching), 1339.84 (N-O symmetric stretching), 1406.93 (aromatic C=C stretching), 719.68 (C-Cl stretching).

2-chloro-N-(2-bromophenyl)acetamide (1h)
Yield: 7.62 g (67%), mp: 67 °C
Anal:
IR(KBr) (cm^{-1}): 3251.35 (N-H stretching), 3042.43 (aromatic C-H stretching), 2957.94 (aliphatic C-H stretching), 1665.83 (C=O stretching amide), 1438.19 (aromatic C=C stretching), 752.02 (C-Cl stretching), 571.72 (C-Br stretching).

2-chloro-N-(4-bromophenyl)acetamide (1i)

Yield: 7.38 g (90%), mp: 182 °C

Anal:

IR(KBr) (cm^{-1}): 3263.33 (N-H stretching), 3076.46 (aromatic C-H stretching), 2917.04 (aliphatic C-H stretching), 1671.52 (C=O stretching amide), 1487.75 (aromatic C=C stretching), 772.34 (C-Cl stretching), 499.84 (C-Br stretching).

General Procedure for the preparation of N-(aryl)-2-(1H-1, 2, 4-triazol-1-yl)acetamides (3a-i)

To a solution of 1H-1,2,4-triazole (2) (0.0014 mol, 0.10 g) in 15 ml of acetonitrile was added 2-chloro-*N*-(substituted phenyl) acetamides (1a-i) (0.0014 mole) and triethylamine (0.0028 mole) dropwise. The reaction mixture was refluxed for 4 h. After this, the reaction mixture was cooled to room temperature and poured in water (10 ml). The mixture was then washed with chloroform (3 × 10 ml). The aqueous layer was separated and evaporated to yield a solid residue. The residue was then washed with acetone (1× 20 ml), dried and recrystallized from dehydrated ethanol to yield white crystals.

N-benzyl-2-(1*H*-1,2,4-triazol-1-yl)acetamide (3a)

Yield: 0.27 g (86%), mp: 219 °C

Anal:

IR(KBr) (cm^{-1}): 2975 (aliphatic C-H stretching), 1610 (C=O stretching), 1475 (C=N stretching).

^{1}H-NMR (DMSO-d_6, 400 MHz) δ ppm: 10.37 (s;1H; NH), 7.51 (s; 1H; H-5), 7.41 (s; 1H; H-3), 7.42-7.50 (m; 5H; Ar-H), 4.00 (s; 2H; CH_2-CO), 3.07 (d; 2H; NH-CH_2). MS m/z: 216 [M^+].

Calcd for $C_{11}H_{12}N_4O$, C, 61.10; H, 5.59; N, 25.91 Found: C, 60.98; H, 5.19; N, 25.41.

N-(2-methoxyphenyl)-2-(1*H*-1,2,4-triazol-1-yl)acetamide (3b)

Yield: 0.30 g, (89%), mp: 208 °C

Anal:

IR (KBr) (cm^{-1}): 2976 (aliphatic C-H stretching), 1625 (C=O stretching), 1507 (C=N stretching). ^{1}H-NMR (DMSO-d_6, 400 MHz) δ ppm: 10.69 (s; 1H; NH), 8.03-8.01 (t; 1H; H-5), 7.86 (t; 1H; H-3), 7.53-7.57 (m; 4H; Ar-H), 5.81 (s; 2H; CH_2-CO), 3.42 (s; 3H; OCH_3). MS m/z: 232 [M^+].

Calcd for $C_{11}H_{12}N_4O_2$, C, 56.89; H, 5.20; N, 24.12.Found: C, 56.19; H, 5.19; N, 24.02.

N-(4-methoxyphenyl)-2-(1*H*-1,2,4-triazol-1-yl)acetamide (3c)

Yield: 0.280 g, 83%, mp: 225 °C

Anal:

IR (KBr) (cm^{-1}): 2976 (aliphatic C-H stretching), 1676 (C=O stretching), 1514 (C=N stretching).

^{1}H-NMR (DMSO-d_6, 400 MHz) δ ppm: 10.77 (s; 1H; NH), 9.27 (s; 1H; H-5), 8.47 (s; 1H; H-3), 7.48-7.55 (m; 4H; Ar-H), 4.99 (s; 2H; CH_2-CO), 3.73 (s; 3H; OCH_3). MS m/z: 232 [M^+].

Calcd for $C_{11}H_{12}N_4O_2$, C, 56.89; H, 5.20; N, 24.12.Found: C, 56.19; H, 5.10; N, 23.92.

N-(3-chlorophenyl)-2-(1*H*-1,2,4-triazol-1-yl)acetamide (3d)

Yield: 0.278 g, 81%, mp: 256 °C

Anal:

IR (KBr) (cm^{-1}): 2975 (aliphatic CH stretching), 1620 (C=O stretching), 1475 (C=N stretching).

^{1}H-NMR (DMSO-d_6, 400 MHz) δ ppm: 10.73 (s; 1H; NH), 9.27 (s; 1H; H-5), 8.48 (s; 1H; H-3), 7.49-7.55 (m; 4H; Ar-H), 4.99 (s; 2H; CH_2-CO). MS m/z: 236 [M^+].

Calcd for $C_{10}H_9ClN_4O$, C,50.75; H,3.83; N,23.67; Found: C,50.15; H, 3.63; N,23.41.

N-(4-ethoxyphenyl)-2-(1*H*-1,2,4-triazol-1-yl)acetamide (3e)

Yield: 0.265 g, 74%, mp: 222 °C,

Anal:

IR (KBr) (cm^{-1}): 1671 (C=O stretching), 1545 (C=N stretching).

^{1}H-NMR (DMSO-d_6, 400 MHz) δ ppm: 10.80 (d; 1H; NH), 9.28 (d; 1H; H-5), 8.50 (s; 1H; H-3), 7.50-7.53 (m; 4H; Ar-H), 5.40 (s; 2H; CH_2-CO), 4.01 (q; 2H; OCH_2CH_3), 1.32 (t; 3H; OCH_2CH_3). MS m/z: 245 [M^+].

Calcd for $C_{12}H_{14}N_4O_2$, C,58.53; H,5.73; N,22.75. Found: C,58.13 ; H,5.64 ; N,22.25.

N-(2-nitrophenyl)-2-(1*H*-1,2,4-triazol-1-yl)acetamide (3f)

Yield: 0.257 g, 71%, mp: 242 °C

Anal:

IR (KBr) (cm^{-1}): 2968 (aliphatic CH stretching), 1698 (C=O stretching), 1507 (C=N stretching). ^{1}H-NMR (DMSO-d_6, 400 MHz) δ ppm: 10.70 (s; 1H; NH), 8.01-8.02 (t; 1H; H-5), 7.84-7.86 (t; 1H; H-3),7.53-7.57 (m; 4H; Ar-H), 5.60 (s; 2H, CH_2-CO). MS m/z: 247 [M^+].

Calcd for $C_{10}H_9N_5O_3$, C,48.58 ; H,3.67 ; N,28.33. Found: C, 48.38; H, 3.27; N, 28.13.

N-(4-nitrophenyl)-2-(1*H*-1,2,4-triazol-1-yl)acetamide (3g)

Yield: 0.270 g, 75%, mp: 221 °C

Anal:

IR (KBr, cm^{-1}): 3397 (NH stretching), 2976 (aliphatic CH stretching), 1630 (C=O stretching).

^{1}H-NMR (DMSO-d_6, 400 MHz) δ ppm: 10.26 (s; 1H; NH), 9.27 (s; 1H; H-5), 8.47 (s; 1H; H-3), 7.49-7.55 (m; 4H; Ar-H), 5.50 (s; 2H, CH_2-CO). MS m/z: 247 [M^+].

Calcd for $C_{10}H_9N_5O_3$,C, 48.58; H, 3.67; N, 28.33. Found: C, 48.18; H, 3.14; N, 28.03.

N-(2-bromophenyl)-2-(1*H*-1,2,4-triazol-1-yl)acetamide (3h)

Yield: 0.376 g, 92%, mp: 190 °C

Anal:

IR (KBr) (cm^{-1}): 2974 (aliphatic CH stretching), 1674 (C=O stretching), 1513 (C=N stretching).

^{1}H-NMR (DMSO-d_6, 400 MHz) δ ppm: 10.57 (s; 1H; NH), 8.64 (s; 1H; H-5), 8.31 (s; 1H; H-3), 7.52-7.66 (m; 4H; Ar-H), 5.12 (s; 2H; CH_2-CO). MS m/z: 281 [M^+].

Calcd for $C_{10}H_9BrN_4O$, C, 42.73; H, 3.23; N, 19.93. Found: C, 42.12; H, 3.04; N, 19.74.

N-(4-bromophenyl)-2-(1*H*-1,2,4-triazol-1-yl)acetamide (3i)

Yield: 0.367 g, 90%, mp: 230 °C

Anal:

IR (KBr) (cm^{-1}): 2976 (aliphatic CH stretching), 1681 (C=O stretching), 1550 (C=N stretching).

^{1}H-NMR (DMSO-d_6, 400 MHz) δ ppm:10.67 (s; 1H; NH), 8.64 (s; 1H; H-5), 8.31 (s; 1H; H-3), 7.49-7.55 (m; 4H; Ar-H), 5.12 (s; 2H; CH_2-CO). MS m/z: 281 [M^+].

Calcd for $C_{10}H_9BrN_4$ O, C, 42.73; H, 3.23; N, 19.93. Found: C, 42.33; H, 3.08; N, 19.78.

2-[(4-amino-5-aryl-4H-1,2,4-triazol-3-yl)thio]-N-(aryl)acetamides (4,6)

General procedure for the preparation of arylhydrazide

Arylbenzoate (0.083 mole) was disolved in 30 ml of ethanol and hydrazine hydrate (0.11 mole) 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.

Benzohydrazide

Yield: 3.49 g (51.01%), mp: 107 °C

Anal:

IR (KBr) (cm^{-1}): 3300 (N-H stretching), 3020 (aromatic C-H stretching), 1662 (C=O stretching).

Nicotinohydrazide

Yield: 7.81 g (64.80%), mp: 162 °C

Anal:

IR (KBr) (cm^{-1}): 3270 (N-H stretching), 3050 (aromatic C-H stretching), 1682 (C=O stretching).

General procedure for the preparation of Potassium dithioarylcarbazinate

Potassium hydroxide (0.0375 mole) was dissolved in 75 ml of absolute alcohol and arylhydrazide (0.025 mole) was added to the above solution. The mixture was then cooled in an ice-bath. To this mixture carbondisulphide was added (0.0375 mole) 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.

Potassium dithiobenzocarbazinate

Yield: 3.74 g (60.00%), mp: 292 °C

Anal:

IR (KBr) (cm^{-1}): 3210 (N-H stretching), 3056 (aromatic C-H stretching), 1759 (C=O stretching), 1671 (C-N stretching), 1290 (C=S stretching).

Potassium dithiopyridocarbazinate

Yield: 3.70 g (59.13 %), mp: 210 °C

Anal:

IR (KBr) (cm^{-1}): 3250 (N-H stretching), 3037 (aromatic C-H stretching), 1729 (C=O stretching).

General Procedure for the preparation of 4-amino-5-aryl-4H-1,2,4-triazol-3-thiol

A suspensión of potassium dithioarylcarbazinate (0.011 mole) in 10 ml of wáter and hydrazine hydrate (0.033 mole) 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.

4-amino-5-phenyl-4H-1,2,4-triazol-3-thiol (4)

Yield: 1.53 g (94.64%), mp: 168 °C

Anal:

IR (KBr) (cm^{-1}): 3175 (N-H stretching), 3075 (aromatic C-H stretching), 1625 (C-N stretching), 1225 (S-H stretching).

4-amino-5-pyridyl-4H-1, 2,4-triazol-3-thiol (6)

Yield: 1.61 g (75.86%), mp: 242 °C

Anal:

IR (KBr) (cm^{-1}): 3233 (N-H stretching), 3056 (aromatic C-H stretching), 1464 (aromatic C=C stretching), 1290 (S-H stretching), 1237 (N-N=C stretching).

General procedure for the preparation of 2-[(4-amino-5-aryl-4*H*-1,2,4-triazol-3-yl)thio]-*N*-(substituted phenyl)acetamide (5a-i), (7a-i)

To a solution of 4-amino-5-aryl-4*H*-1,2,4-triazole-3-thiol (4 and 6) (0.0005 mole) in 15 ml of acetonitrile was added 2-chloro-*N*-(substituted phenyl) acetamides (1a-i) (0.0005 mole) and triethylamine (0.0010 mole) drop wise. 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.

2-[(4-amino-5-phenyl-4*H*-1,2,4-triazol-3-yl)thio]-*N*-benzylacetamide (5a)

Yield: 0.148 g (84%), mp: 227 °C

Anal:

IR(KBr) (cm^{-1}): 3435(N-H stretching), 2975(aliphatic CH stretching), 1602(C=O stretching), 1476(C=N stretching of triazole).

^{1}H-NMR (DMSO-d_6, 400 MHz) δ ppm: 10.70 (s; 1H; NH), 8.01-8.03 (m; 5H; Ar´-H), 7.50-7.54 (m; 5H; Ar-H), 5.79 (s; 2H; CH_2CO), 4.32-4.34 (d; 2H; $NHCH_2$), 3.89 (s; 2H, NH_2). MS m/z: 339 [M^+].

Calcd for C_{17} H_{17} N_5 O S, C, 60.16; H, 5.05; N, 20.63. Found: C, 60.08; H, 4.98 ; N, 20.41.

2-[(4-amino-5-phenyl-4H-1,2,4-triazol-3-yl)thio]-N-(2-methoxyphenyl)acetamide (5b)

Yield: 0.149 g (80%), mp: 250 °C

Anal:

IR(KBr) (cm^{-1}): 3322(NH stretching), 2975(aliphatic CH stretching), 1640(C=O stretching).

^{1}H-NMR (DMSO-d_6, 400 MHz) δ ppm: 10.51(s; 1H; NH), 7.83-7.86(m; 5H; Ar´-H), 7.53-7.57(m; 4H; Ar-H), 5.70 (s; 2H; CH_2CO), 3.40(s; 2H, NH_2), 3.02-3.07(m; 3H; OCH_3). MS m/z: 355 [M^+].

Calcd for C_{17} H_{17} N_5 O_2 S, C,57.45 ; H, 4.82; N,19.70. Found: C, 57.12; H,4.62 ; N,19.17.

2-[(4-amino-5-phenyl-4H-1,2,4-triazol-3-yl)thio]-N-(4-methoxyphenyl)acetamide (5c)

Yield: 0.132 g (72%), mp: 216 °C

Anal:

IR(KBr) (cm^{-1}): 3322 (NH stretching), 2975 (aliphatic CH stretching), 1640 (C=O stretching), 1475 (C=N stretching).

^{1}H-NMR (DMSO-d_6, 400 MHz) δ ppm:10.42 (s; 1H; NH), 8.01-8.03 (m; 5H; Ar´-H), 7.52-7.54 (m; 4H; Ar-H), 5.78 (s; 2H; CH_2CO), 3.40 (s; 2H, NH_2), 3.02-3.07(m; 3H; OCH_3). MS m/z:355 [M^+].

Calcd for C_{17} H_{17} N_5 O_2 S, C, 57.45; H, 4.82; N, 19.70. Found: C, 57.25; H, 4.62; N, 19.27.

2-[(4-amino-5-phenyl-4H-1,2,4-triazol-3-yl)thio]-N-(3-chlorophenyl)acetamide (5d)

Yield: 0.149 g (80%), mp: 244 °C

Anal:

IR(KBr) (cm^{-1}): 3323 (NH stretching), 2975 (aliphatic CH stretching), 1602 (C=O stretching), 1475 (C=N stretching of triazole).

^{1}H-NMR (DMSO-d_6, 400 MHz) δ ppm: 10.51-10.54 (d; 1H; NH), 8.01-8.03 (m; 4H; Ar-H), 7.52-7.54 (m; 5H; Ar´-H), 5.80 (s; 2H; CH_2CO), 3.40 (s; 2H, NH_2). MS m/z: 373 [M^+].

Calcd for C_{16} H_{14} Cl N_5 O S, C, 51.36 ; H, 3.92 ; N, 19.46. Found: C, 51.98; H, 3.58; N, 19.06.

2-[(4-amino-5-phenyl-4H-1,2,4-triazol-3-yl)thio]-N-(4-ethoxyphenyl)acetamide (5e)

Yield: 0.172 g (89%), mp: 236°C

Anal:

IR(KBr) (cm^{-1}): 3322 (NH stretching), 2976 (aliphatic CH stretching), 1597 (C=O stretching), 1470(C=N stretching of triazole).

^{1}H-NMR (DMSO-d_6, 400 MHz) δ ppm: 10.23 (s; 1H; NH), 8.00-8.03 (m; 4H; Ar-H), 7.52-7.54 (m; 5H; Ar´-H), 5.80 (s; 2H; CH_2CO), 3.40 (s; 2H, NH_2), 3.02-3.08 (q; 2H; OCH_2CH_3), 1.18-1.22(t; 3H; OCH_2CH_3). MS m/z: 369 [M^+].

Calcd for C_{18} H_{19} N_5 O_2 S, C, 58.52 ; H, 5.18 ; N, 18.96. Found: C, 58.14; H, 5.10; N, 18.56.

2-[(4-amino-5-phenyl-4H-1,2,4-triazol-3-yl)thio]-N-(2-nitrophenyl)acetamide (5f)

Yield: 0.145 g (75%), mp: 248 °C

Anal:

IR(KBr) (cm^{-1}): 3429 (NH stretching), 2976 (aliphatic CH stretching), 1601 (C=O stretching), 1475 (C=N stretching of triazole).

^{1}H-NMR (DMSO-d_6, 400 MHz) δ ppm: 9.95 (s; 1H; NH), 8.71-8.72 (m; 5H; Ar´-H), 7.56-7.59 (m; 4H; Ar-H), 5.79 (s; 2H; CH_2CO), 3.30 (s; 2H, NH_2). MS m/z:370 [M^+].

Calcd for C_{16} H_{14} N_6 O_3 S, C, 51.88; H, 3.81 ; N, 22.69. Found: C, 51.58 ; H, 3.52 ; N, 22.49.

2-[(4-amino-5-phenyl-4H-1,2,4-triazol-3-yl)thio]-N-(4-nitrophenyl)acetamide (5g)

Yield: 0.158 g (82%), mp: 210 °C

Anal:

IR(KBr) (cm^{-1}): 3323(NH stretching), 2976(aliphatic CH stretching), 1475(C=N stretching)

^{1}H-NMR (DMSO-d_6, 400 MHz) δ ppm: 10.38 (s; 1H; NH), 8.37-8.40 (m; 5H; Ar´-H), 7.57-7.60 (m; 4H; Ar-H), 5.81 (s; 2H; CH_2CO), 3.40 (s; 2H, NH_2). MS m/z: 370 [M^+].

Calcd for C_{16} H_{14} N_6 O_3 S, C, 51.88; H, 3.81 ; N, 22.69. Found: C, 51.33 ; H, 3.51; N, 22.12.

2-[(4-amino-5-phenyl-4H-1,2,4-triazol-3-yl)thio]-N-(2-bromophenyl)acetamide (5h)

Yield: 0.179 g (85%), mp: 244 °C

Anal:

IR(KBr) (cm^{-1}): 3427 (NH stretching), 2975 (aliphatic CH stretching), 1602 (C=O stretching), 1475 (C=N stretching).

^{1}H-NMR (DMSO-d_6, 400 MHz) δ ppm: 10.58 (s; 1H; NH), 8.01-8.03 (m; 4H; Ar-H), 7.54-7.57 (m; 5H; Ar´-H), 5.80 (s; 2H; CH_2CO), 3.40 (s; 2H, NH_2). MS m/z:404 [M^+].

Calcd for $C_{16}H_{14}BrN_5O$, C, 47.53 ; H, 3.49 ; N, 17.32. Found: C, 47.13 ; H, 3.19 ; N, 17.41.

2-[(4-amino-5-phenyl-4H-1,2,4-triazol-3-yl)thio]-N-(4-bromophenyl)acetamide (5i)

Yield: 0.163 g (77%), mp: 217 °C

Anal:

IR(KBr) (cm^{-1}): 3323 (N-H stretching), 2976 (aliphatic CH stretching), 1600 (C=O stretching), 1475 (C=N stretching).

^{1}H-NMR (DMSO-d_6, 400 MHz) δ ppm: 10.55 (s; 1H; NH), 8.01-8.03 (m; 5H; Ar´-H), 7.52-7.54 (m; 4H; Ar-H), 5.79 (s; 2H; CH_2CO), 3.40 (s; 2H, NH_2). MS m/z:404 [M^+].

Calcd for $C_{16}H_{14}BrN_5OS$, C, 47.53 ; H, 3.49 ; N, 17.32. Found: C, 47.28 ; H, 3.11 ; N, 17.11.

2-[(4-amino-5-pyridin-3-yl-4*H*-1,2,4-triazol-3-yl)thio]-*N*-benzylacetamide (7a)

Yield: 0.145 g (82%), mp: 170 °C

Anal:

IR(KBr) (cm^{-1}): 3322 (N-H stretching), 2975 (aliphatic C-H stretching), 1601 (C=O stretching), 1475 (C=N stretching).

^{1}H-NMR (DMSO-d_6, 400 MHz) δ ppm: 9.69 (s; 1H; NH), 8.71-8.73 (m; 4H; Ar'-H), 7.25-7.34 (m; 5H; Ar-H), 5.65 (s; 2H; CH_2CO), 4.38-4.39 (d; 2H; $NHCH_2$), 3.40 (s; 2H, NH_2). MS m/z: 340 [M^+].

Calcd for $C_{16}H_{16}N_6OS$, C, 56.45; H, 4.77; N, 24.69. Found: C, 56.08; H, 4.64; N, 24.41.

2-[(4-amino-5-pyridin-3-yl-4*H*-1,2,4-triazol-3-yl)thio]-*N*-(2-methoxyphenyl)acetamide (7b)

Yield: 0.155 g (84%), mp: 176 °C

Anal:

IR(KBr) (cm^{-1}): 3234 (N-H stretching), 2975 (aliphatic C-H stretching), 1601 (C=O stretching), 1475 (C=N stretching).

^{1}H-NMR (DMSO-d_6, 400 MHz) δ ppm: 10.36 (s; 1H; NH), 8.37-8.39 (m; 4H; Ar'-H), 7.56-7.60 (m; 4H; Ar-H), 5.80 (s; 2H; CH_2CO), 3.40 (s; 2H, NH_2), 3.02-3.08 (m; 3H; OCH_3). MS m/z: 356 [M^+].

Calcd for $C_{16}H_{16}N_6O_2S$, C, 53.92; H, 4.52; N, 23.58. Found: C, 53.67; H, 4.19; N, 23.23.

2-[(4-amino-5-pyridin-3-yl-4*H*-1,2,4-triazol-3-yl)thio]-*N*-(4-methoxyphenyl)acetamide (7c)

Yield: 0.159 g (86%), mp: 230 °C

Anal:

IR(KBr) (cm^{-1}): 3322 (N-H stretching), 2975 (aliphatic C-H stretching), 1475 (C=N stretching).

^{1}H-NMR (DMSO-d_6, 400 MHz) δ ppm: 10.36 (s; 1H; NH), 8.37-8.40 (m; 4H; Ar'-H), 7.57-7.60 (m; 4H; Ar-H), 5.81 (s; 2H; CH_2CO), 3.70 (s; 3H; OCH_3), 3.40 (s; 2H, NH_2). MS m/z: 356 [M^+].

Calcd for $C_{16}H_{16}N_6O_2S$, C, 53.92; H, 4.52; N, 23.58. Found: C, 53.98; H, 4.37; N, 23.47.

2-[(4-amino-5-pyridin-3-yl-4*H*-1,2,4-triazol-3-yl)thio]-*N*-(3-chlorophenyl)acetamide (7d)

Yield: 0.157 g (84%), mp: 188 °C

Anal:

IR(KBr) (cm^{-1}): 3426(N-H stretching), 1598(C=O stretching), 1476(C=N stretching)

^{1}H-NMR (DMSO-d_6, 400 MHz) δ ppm: 10.23 (s; 1H; NH), 8.71-8.73 (m; 4H; Ar'-H), 7.56-7.60 (m; 4H; Ar-H), 5.80 (s; 2H; CH_2CO), 3.40 (s; 2H, NH_2). MS m/z: 360 [M^+].

Calcd for $C_{15}H_{13}ClN_6OS$, C, 49.93; H, 3.63; N, 23.29. Found: C, 49.27; H, 3.37; N, 23.36.

2-[(4-amino-5-pyridin-3-yl-4*H*-1,2,4-triazol-3-yl)thio]-*N*-(4-ethoxyphenyl)acetamide (7e)

Yield: 0.149 g (78%), mp: 210 °C

Anal:

IR(KBr) (cm^{-1}): 3322(N-H stretching), 2975(aliphatic C-H stretching), 1601(C=O stretching), 1475(C=N stretching).

^{1}H-NMR (DMSO-d_6, 400 MHz) δ ppm: 10.32 (s; 1H; NH), 8.00-8.02 (d; 4H; Ar'-H), 7.52-7.54 (m; 4H; Ar-H), 5.79 (s; 2H; CH_2CO), 3.42 (s; 2H, NH_2), 3.02-3.08(q; 2H; OCH_2CH_3), 1.18-1.22(t; 2H; OCH_2CH_3).MS m/z: 372 [M^+].

Calcd for $C_{17}H_{18}NO_2S$, C, 55.12; H, 4.90; N, 22.69. Found: C, 55.02; H, 4.77; N, 22.43.

2-[(4-amino-5-pyridin-3-yl-4*H*-1,2,4-triazol-3-yl)thio]-*N*-(2-nitrophenyl)acetamide (7f)

Yield: 0.151 g (78%), mp: 214 °C

Anal:

IR(KBr) (cm^{-1}): 3377 (N-H stretching), 2972 (aliphatic C-H stretching), 1699 (C=O stretching), 1515 (C=N stretching).

^{1}H-NMR (DMSO-d_6, 400 MHz) δ ppm: 10.42 (s; 1H; NH), 8.71-8.74 (m; 4H; Ar'-H), 7.56-7.60 (m; 4H; Ar-H), 5.82 (s; 2H; CH_2CO), 3.30 (s; 2H, NH_2). MS /z: 371 [M^+].

Calcd for $C_{15}H_{13}N_7O_3S$, C, 48.51; H, 3.53; N, 26.40. Found: C, 48.11; H, 3.19; N, 26.22.

2-[(4-amino-5-pyridin-3-yl-4*H*-1,2,4-triazol-3-yl)thio]-*N*-(4-nitrophenyl)acetamide (7g)

Yield: 0.147 g (76%), mp: 220 °C

Anal:

IR(KBr) (cm^{-1}): 3322 (N-H stretching), 2975 (aliphatic C-H stretching), 1596 (C=O stretching).

^{1}H-NMR (DMSO-d_6, 400 MHz) δ ppm: 10.43 (s; 1H; NH), 8.71-8.73 (m; 4H; Ar'-H), 7.56-7.60 (m; 4H; Ar-H), 5.80 (s; 2H; CH_2CO), 3.40 (s; 2H, NH_2). MS m/z: 371 [M^+].

Calcd for $C_{15}H_{13}N_7O_3S$, C, 48.51; H, 3.53; N, 26.40. Found: C, 48.13; H, 3.27; N, 26.09.

2-[(4-amino-5-pyridin-3-yl-4*H*-1,2,4-triazol-3-yl)thio]-*N*-(2-bromophenyl)acetamide (7h)

Yield: 0.168 g (80%), mp: 228 °C

Anal:

IR(KBr) (cm^{-1}): 3322 (N-H stretching), 2976 (aliphatic CH stretching), 1596 (C=O stretching), 1475 (C=N stretching).

^{1}H-NMR (DMSO-d_6, 400 MHz) δ ppm: 10.51 (s; 1H; NH), 8.37-8.40 (m; 4H; Ar'-H), 7.56-7.60 (m; 4H; Ar-H), 5.81 (s; 2H; CH_2CO), 3.40 (s; 2H, NH_2). MS m/z: 405 [M^+].

Calcd for $C_{15}H_{13}BrN_6OS$, C, 44.45; H, 3.23; N, 20.74. Found: C, 44.34; H, 3.03; N, 20.46.

2-[(4-amino-5-pyridin-3-yl-4*H*-1,2,4-triazol-3-yl)thio]-*N*-(4-bromophenyl)acetamide (7i)

Yield: 0.187 g (89%), mp: 200 °C

Anal:

IR(KBr) (cm^{-1}): 3443 (NH stretching), 1690 (C=O stretching), 1541 (C=N stretching).

^{1}H-NMR (DMSO-d_6, 400 MHz) δ ppm: 10.33 (s; 1H; NH), 8.37-8.40 (m; 4H; Ar'-H), 7.56-7.60 (m; 4H; Ar-H), 5.80 (s; 2H; CH_2CO), 3.40 (s; 2H; NH_2).MS m/z: 405 [M^+].

Calcd for $C_{15}H_{13}BrN_6OS$, C, 44.45; H, 3.23; N, 20.74. Found: C, 44.15; H, 3.67; N, 20.44.

Antimicrobial study

Anti-HIV study

Microplate assay of HIV-1 RT activity

All the above synthesized compounds 3a-i, 5a-i and 7a-i were evaluated for HIV-1 reverse transcriptase (RT) inhibitory activity by using microplate assay with efavirenz as the standard. This HIV-1 reverse transcriptase inhibitory activity has been carried out following the reported fluorescence based method. [17] All reagents used were provided in the EnzChek® Reverse Transcriptase Assay Kit (Molecular Probes, USA). A mixture of 5 microlitre (µL) of 1 mg/mL 350 bases-poly(rA) ribonucleotide template and 5 µL of 50 µg/mL oligo $d(T)_{16}$ primer in a nuclease-free microcentrifuge tube were incubated at room temperature (25°C) for 1 h to allow the primer/template annealing. The primer/template was prepared by 200-fold dilution in polymerization buffer. The primer/template was aliquoted and kept at −20°C until used. 5 µL of 8 micromolar (µM) stock purified HIV-1 reverse transcriptase was aliquoted and kept at −80°C until used. The working enzyme was diluted to 400 nanomolar (nM) with 50 mM Tris-HCl, 20% glycerol, 2 mM DTT, pH 7.5. The assays were performed in a total volume of 15 µL of the polymerization reaction. The reaction containing 3 µL

of 400 nM recombinant HIV-1 RT (the final concentration is 80 nM), 2 µL of TE buffer (10 mM Tris–HCl at pH 7.5) were added and gently mixed on ice prior. The polymerization reaction was initiated by the addition of 10 µL of the primer/template and incubated at room temperature for 30 minutes. After the reactions reached the desired incubation time, 5 µL of 0.2 M EDTA was added to stop the polymerization reaction (RTControl). The blank reaction was prepared by mixing 5 µL of 0.2 M EDTA with enzyme before adding primer/template (After termination of the reactions, the plate was gently shaken and incubated at room temperature for 3 min to allow the formation of a stable heteroduplex DNA/RNA complex, followed by addition of 180 µL of PicoGreen reagent diluted 700-fold with TE buffer for each well, making the final volume 200 µL and incubation at room temperature in the dark for 3 min, during which PicoGreen binds to double-stranded DNA and RNA-DNA hybrids and this was followed by measurement of fluorescence with a fluorometer (excitation 485 nm; emission 535 nm).

To test the inhibition efficiency of the test compounds, all the compounds were dissolved in dimethyl sulfoxide (DMSO) to make a 20 mM stock solution. 10 mM working solution of each inhibitor was further diluted by 10 mM Tris–HCl, pH 7.5 containing 50% DMSO. Two µL of each inhibitor and 3 µL of 400 nM recombinant HIV-1 RT were added and gently mixed on ice prior. The reaction was initiated by the addition of 10 µL of the primer/template and incubated at room temperature for 30 minutes. After the reactions reached the desired incubation time, 5 µL of 0.2 M EDTA was added to stop the polymerization reaction. The relative inhibitory effect of HIV-1RT activity was compared by using the percent inhibition, which was calculated via the following eq: [18]

$$\% \text{ Relative Inhibition} = \frac{[(\text{RTControl-RTBlank})-(\text{RTSample-RTBlank})]*100}{[(\text{RTControl-RTBlank})]}$$

where :

RTControl = Fluorescence without sample (normal RT reaction or Positive control)

RTBlank = Fluorescence without enzyme activity (Negative control)

RTSample = Fluorescence with sample

Antibacterial study[19]

The principle composition of nutrient agar medium (media no 1) included peptone (1%), sodium chloride (0.5%), beef extract (0.5%), agar (1.5%) and distilled water (100 ml qs).

pH of the solution was adjusted to 7.2-7.6. Three different bacterial strains were used, namely *Staphylococcus aureus* (ATCC-25293), *Bacillus subtilis* (PZ6633) and *Escherichia coli* (ATCC25922). The sample solutions were prepared using double distilled water at a concentration of 200, 300, 400 and 500 µg/ml. The test micro organisms were maintained in slant tubes as solid culture and in test tube (containing 2.5 ml of media 1). At first, the solid slants were inoculated from the original stock culture and finally the liquid media was inoculated from the solid slants. The microbes were allowed to incubate for 24 h at a temperature of 37°C. 20 ml of liquid agar media was taken in McCartney bottle and sterilised in autoclave at 121°C for 15 mins at 15 psi. The sterilised media was then poured into sterilised petri plates aseptically in a horizontal laminar air flow chamber. The layers of media were uniformly distributed. The media was then allowed to dry in the aseptic chamber itself and followed by inoculating the bacterial strains separately in the petri plates. Anti bacterial activity was determined by cup-plate method. In order to determine the anti bacterial activity, the inoculated petri plates were divided into four quarters and to each quarter a well was made in the media with the help of a sterilised cork borer. The known concentrations of the standard drug as ciprofloxacin and the test compounds were added to the wells so that the volume fills up the well uniformly. Thus applied plates were then kept in refrigerator for 10 mins followed by incubation in BOD incubator for 24 h

at 37°C. After 24 h the minimum inhibitory concentration (MIC) of the compounds was determined.

Antifungal Study [19-21]

The various components used for fungal agar medium (media No-3) were glucose (4%), peptone (1%), agar (2%) and distilled water (100 ml qs). The pH of the media was adjusted to 5.4. Four different fungal strains used in this process were *Candida albicans* (NCIM3471), *Candida dubliniensis* (ATCC 90029), *Candida tropicalis* (ATCC-750) and *Candida urusial* (SSKM). Two different concentrations of sample solutions were prepared using double distilled water at the strength of 10 µg/ml and 50 µg/ml. The test microorganisms were maintained in slant tubes as solid culture and in test tube (containing 2.5 ml of media 3). At first, the solid slants were inoculated from the original stock culture and finally the liquid media were inoculated from the solid slants. The microbes were allowed to incubate for 24 h at a temperature of 28 °C. 20 ml of liquid agar media was taken in McCartney bottle and sterilized in autoclave at 121°C for 15 min at 15 psi. The sterilized media was then poured into sterilized petri plates aseptically in a horizontal laminar air flow chamber. The layers of media were uniformly distributed. The media was then allowed to dry in the aseptic chamber itself and followed by inoculating the fungal strains separately in the petri plates. Antifungal activity was determined by cup-plate method**.** For this purpose the inoculated petri plates were divided into four quarters and to each quarter a well was made in the media with the help of a sterilized cork borer. The known concentrations of the standard drug and the test compounds were added to the wells so that the volume fills up the well uniformly. The applied plates were then kept in refrigerator for 10 mins and followed by incubation in BOD incubator for 72 h at 28 °C. After 72 h the minimum inhibitory concentration (MIC) of the compounds was determined.

FQLog P Determination

Calculation of FQLogP for the synthesized compounds were carried out using ADME Works Model Builder v 4.5, [23] an add on module of Scigress Explorer ultra v 7.7.4.0 [24] installed in a single machine running on a 3.4 GHz Pentium 4 processor with 1GB RAM and 160 GB Hard Disk. Structures of the designed molecules were drawn in the Scigress Explorer work space and saved in the .csf format. Molecules in .csf format were imported to Project Leader and from the chemical property of the cell, LogP was selected and then evaluated for the selected molecules. The project was saved and exported to ADME Works model builder in .sdf format and the FQLogP descriptor was then added from the add descriptor option menu and predicted.

Molecular docking studies

The molecular docking studies were carried out using Autodock 4.0.1 [25-29] installed on a single machine running on a 3.4 GHz Pentium 4 processor with FEDORA-8 (LINUX) as the Operating System. The X-ray co-crystal structure (Protein Data Bank entry code 1RT2) of wt RT with NNRTI (TNK 999) was used as the initial structure for the computational studies. [30] All the residues within 20 Å core from TNK 999 were used to define the NNIBP. The starting conformations for docking studies were obtained using molecular dynamics with simulated annealing by heating at 1000 K for 1 ps and then cooling at 200 K for 1ps as implemented in SYBYL 7.1.[31]

Autodock 4.0.1 [25-29] was used to explore the binding conformation of TNK 999 and for the other test molecules. Initially the ligand and water molecules from the X-ray co-crystal structure of 1RT2 were discarded, polar hydrogen atoms were added to the protein and later Gasteiger charges were assigned. All the non-polar hydrogens were merged. All the residues of the protein were kept rigid except PHE227 and LYS103 which are flexible. The protein was saved in PDBQT format. The AutodockTools package version 1.4.6 was employed to generate the docking input files

and to analyze the docking results. For the docking, a grid spacing of 0.375 Å and 60 x 60 x 60 number of points was used. The grid was centered on the allosteric site, using the TNK999 crystallographic position as reference. The AutoGrid program generated separate grid maps for all atom types of the ligand structures plus one for electrostatic interactions. Autodock generated 50 possible binding conformations, i.e., 50 runs for each docking by using Lamarckian Genetic Algorithm (LGA) searches. A default protocol was applied, with an initial population of 150 randomly placed individuals, a maximum number of 2.5 x 105 energy evaluations, and a maximum number of 2.7 x 104 generations. A mutation rate of 0.02 and a crossover rate of 0.8 were used.

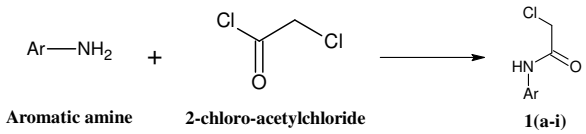
The docking calibration was performed with the reference compound TNK 999 and further compared with cross docking study using standard Efavirenz. The docking study reveals the experimental binding conformations of the reference molecule TNK 999 with acceptable root-mean-square deviation (RMSD) of 0.56 Å. The structures of the newly synthesized compounds were drawn and optimized using PRODRG online server[32] and saved in PDB format. The optimized 3D structures of the ligands (synthesized compounds) were used for the docking studies. The binding mode and interactions in the non-nucleosidal inhibitory binding pocket were analyzed for the significant compounds.

RESULTS AND DISCUSSION

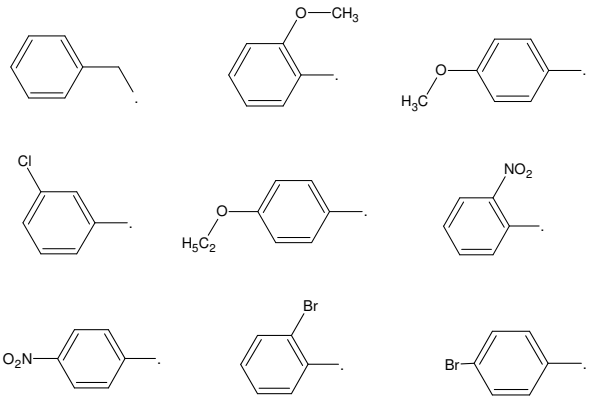
Synthetic study

In this present study 2-chloro-*N*-(substituted phenyl) acetamides 1(a-i) were synthesized by the method described in experimental section. Compounds 4-amino-5-phenyl-4*H*-1,2,4-triazole-3-thiols (4) and 4-amino-5-(pyridin-3-yl)-4*H*-1,2,4-triazole-3-thiols (6) were synthesized by the method reported for triazoles [16]. Compounds 1(a-i) were next refluxed with compounds 2, 4 and 6 in presence of triethylamine in acetonitrile yielding twenty seven N-(aryl)-2-(1H-1, 2, 4-triazol-1-yl)acetamides, 2-[(4-amino-5- pyridin-3-yl-4*H*-1,2,4-triazol-3-yl)thio]-*N*-(substituted phenyl)acetamides 5(a-i) and 2-[(4-amino-5- phenyl-4*H*-1,2,4-triazol-3-yl)thio]-*N*-(substituted phenyl)acetamides 7(a-i) respectively.

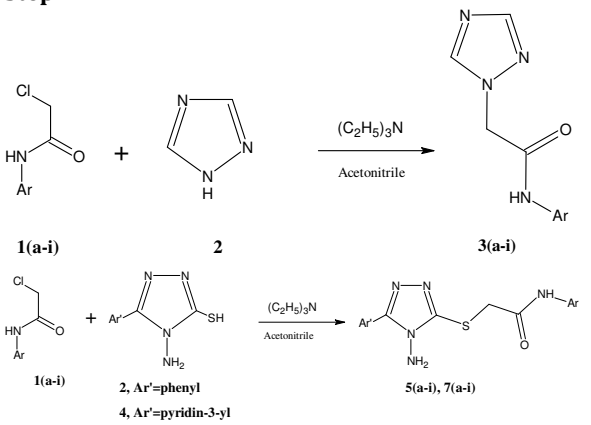
Step1:



Where Ar=



Step 2:



Scheme 1. Synthetic route of compounds; N-(aryl)-2-(1H-1, 2, 4-triazol-1-yl)acetamides 3(a-i), 2-[(4-amino-5- phenyl-4*H* /pyridin-3-yl 1,2,4-triazol-3-yl)thio]-*N*-(substituted phenyl)propanamide 5(a-i), 7(a-i)

IR spectra of all the compounds under study clearly demonstrate the existence of various structural fragments which constitutes the entire molecular framework. A strong intense band appeared in the range of 3443-3322 cm^{-1} indicating the presence of N-H stretch of aromatic secondary amide. A strong band in the range of 1698-1596 cm^{-1} confirms the presence

of C=O stretch in all the compounds under investigation. Appearance of a sharp peak in all the compounds at 1476 cm^{-1} confirm the presence of C=N stretch of the central ring as 1,2,4- triazole.

^1H NMR spectrum of the compounds 3(a-i), 5(a-i) and 7(a-i) showed a singlet of one proton at δ 9.69-10.80, assigned to the secondary N-H of amide. A multiplet of four protons for compounds 7(a-i) at δ 8.00-8.73 was assigned to pyridyl ring attached to the 5th carbon of triazole ring, whereas for compounds 5(a-i) at δ 8.72-7.83 a multiplet of five protons was observed for the phenyl ring attached to the the 5th carbon of triazole ring. A multiplet of four protons at δ 7.49-7.66 was assigned to the disubstituted aromatic ring attached to the aliphatic end by an amide linkage. In case of compound 3a, 5a and 7a a multiplet of five protons at δ 7.25-7.54 was observed. A singlet at 4.00-5.82 was assigned to two protons of CH_2CO spacer. For compounds 3(a-i) a singlet of one proton each H-3 and H-5 of 1,2,4 triazole appeared at δ 7.41-9.28.

A singlet of three protons at δ 3.02-3.73 was observed for six compounds, namely 3b, 3c, 5b, 5c 7b and 7c was assigned to the methyl protons of the methoxy group. On the other hand, for ethoxy compounds 3e, 5e and 7e at δ 3.02-4.01 a quartet of two protons was observed for the methylene group and a triplet of three protons between δ 1.18-1.32 was observed for the terminal methyl group. The results obtained from mass analysis were quite significant as the data related to molecular ion peak of all the compounds is in good agreement with the respective molecular formula. The elemental analysis data of all the compounds were within ±0.5% of the theoretical values. On the basis of all the spectral information furnished, it can be inferred that the molecular framework under study confirmed for the newly compounds.

Antimicrobial study

HIV-1 Reverse Transcriptase inhibitory activity

The test compounds 3(a-i), 5(a-i) and 7(a-i) were studied for their percentage inhibitory reverse transcriptase activity against wild type strain of HIV-1. Efavirenz was used as the standard. Among these compounds, test compound 7i showed highest RT inhibitory activity with 52% inhibition comparable to standard drug efavirenz. Test compounds 3b, 3g and 7c showed HIV-1 RT inhibition more than 30%. The test compounds 3c, 3d, 3h, 5a, 5b, 5d, 5i, 7a, 7b, 7e and 7f showed weak inhibitory activity around 20 to 30%, against HIV-1 Reverse Transcriptase. Compound 3a and 7g were completely inactive. The other test compounds possessed very weak HIV-1 RT inhibitory activity. Therefore, it is evident that test compounds containing the spacer groups CH_2-CONH with the phenyl group substituted with electronically denser groups such as halogens, preferentially bromo group at *para* position, methoxy group at *ortho* position, nitro group at *para* position were able to inhibit HIV-1 RT significantly by acting on the non nucleoside inhibitory binding pocket of RT enzyme.

Table1: Percentage HIV-1 RT inhibitory activity of the test compounds on wild type (*wt*) strain of HIV-1

Test Compound	Percentage of RT inhibitory activity	Test Compound	Percentage of RT inhibitory activity	Test Compound	Percentage of RT inhibitory activity
3a	00	5a	21	7a	23
3b	34	5b	27	7b	23
3c	22	5c	06	7c	34
3d	21	5d	27	7d	15
3e	13	5e	17	7e	29
3f	10	5f	09	7f	28
3g	30	5g	06	7g	00
3h	28	5h	17	7h	17
3i	17	5i	24	7i	52

Standard: Efavirenz; Percentage of RT inhibitory activity: 98

* The concentration used was 1 µM.

Antibacterial study[19]

All the compounds 3(a-i), 5(a-i) and 7(a-i) were screened for their *in vitro* antibacterial activity against standard organisms *Staphylococcus aureus* (ATCC-25293), *Bacillus subtilis* (PZ6633) and *Escherichia coli* (ATCC25922) by cup plate method. Ciprofloxacin was

used as the standard. Minimum Inhibitory Concentration (MIC) of the compounds was determined. The MIC is the lowest concentration of tested compounds that completely inhibited the growth of the test organisms after 24 h of incubation at 37 °C. Antibacterial study reflected that compound 7i, was moderately active against all pathogenic bacterial strains considered for the study compared to the reference standard ciprofloxacin All the other compounds 3(a-i), 5(a-i) and 7(ah), were found to be weakly active against all pathogenic bacterial strains. The results are summarized in Table 2.

Table 2: Minimum Inhibitory concentration (MIC) of Test Compounds 3 (a-i), 5 (a-i) and 7 (a-i) against *Staphylococcus aureus* (ATCC-25293), *Bacillus subtilis* (PZ6633), *Escherichia coli* (ATCC25922)

Test Compound	MIC (µg/ml)		
	Staphylococcus aureus	***Bacillus subtilis***	***Escherichia coli***
3a	500	400	500
3b	500	400	300
3c	500	400	500
3d	500	400	400
3e	400	500	500
3f	400	400	500
3g	300	300	200
3h	500	500	400
3i	500	300	300
5a	500	300	500
5b	500	400	400
5c	400	400	500
5d	300	400	500
5e	300	500	500
5f	300	500	500
5g	300	400	400
5h	400	400	400
5i	100	200	300
7a	400	300	300
7b	500	300	300
7c	500	400	400
7d	500	400	500
7e	400	500	400
7f	200	300	100
7g	400	500	500
7h	300	500	500
7i	50	100	100
Ciprofloxacin	0.78	0.78	0.78

Antifungal study

All the compounds 3(a-i), 5 (a-i) and 7(a-i) were screened for their *in vitro antifungal activity against* the fungal strains namely *Candida albicans* (NCIM3471), *Candida tropicalis* (ATCC-750), *Candida dubliniensis* (ATCC 90029) and *Candida urusial*(SSKM). All the compounds were tested at different concentrations, 15, 20, 25, 50 and 100 µg/ml. Compound 7i exhibited highest activity against all the strains of *Candida* compared to the standard fluconazole. Compounds 3c, 3h, 7a and 7b also exhibited highest activity against *Candida albicans* and *Candida tropicalis* while exhibiting moderate activity against the other two strains of *Candida.* The superiority can be attributed to the halogen and alkoxy substituents as either bromo at *ortho* and *para* position or methoxy at *ortho* and *para* position were present in the stated compounds, the benzyl substituent attached with the general structural framework also exhibited good activity against both *Candida albicans and Candida tropicalis* compared to the reference standard. Moderate activity was observed for all the other compounds. The results are summarized in Table 3.

Table 3. Minimum Inhibitory Concentration (MIC) of Test Compounds 3 (a-i), 5 (a-i) and 7 (a-i) against *Candida albicans* (NCIM3471), *Candida tropicalis* (ATCC-750), *Candida dubliniensis* (ATCC 90029), *Candida urusial* (SSKM)

Test Compound	MIC (µg/ml)			
	Candida albicans	***Candida tropicalis***	***Candida dubliniensis***	***Candida urusial***
3a	50	25	50	100
3b	25	25	50	25
3c	20	20	20	20
3d	25	25	25	25
3e	50	50	25	25
3f	100	50	100	100
3g	25	25	50	25
3h	20	20	20	25
3i	25	25	25	25
5a	50	50	50	50
5b	100	50	50	100
5c	25	25	20	25
5d	50	25	25	50
5e	25	50	50	100
5f	20	50	20	20
5g	20	25	15	25
5h	25	50	25	25
5i	25	25	50	25
7a	20	15	50	20
7b	20	20	20	50
7c	50	50	25	50
7d	50	50	50	25
7e	25	20	25	25
7f	50	20	20	25
7g	25	50	50	50
7h	50	50	50	100
7i	15	20	15	15
Fluconazole	12.5	10	15	15

Most of the compounds were found to be moderate to weakly active while acting against the fungi, viz.; *Candida urusial* and *Candida dubliniensis.*

FQLogP

LogP represents the octanol-water partition coefficient (the ratio of the concentrations of a given chemical in octanol and in water at the equilibrium and at a specified temperature).The FQLogP algorithm divides a chemical structure into fragments (atoms or larger functional groups), and sums up the parameters characteristic to every group to yield the LogP estimate. The substructure search engine is able to recognize the hybridization of atoms in molecules and the aromaticity. [22]

The FQLogP of all the synthesized compounds 3(a-i), 5(a-i) and 7(a-i) were calculated using ADME Works Model Builder v 4.5 [23] and the results are summarized in Table 4. ADME Works Model Builder is a tool dedicated to building mathematical models that can later be used for predicting various chemical and biological properties of compounds. Model Builder can also be used for analyzing non-chemical data imported from a CSV file, but its functionality is then limited to statistical analysis and model building only.

Table 4: FQLogP of synthesized compounds 3(a-i), 5(a-i) and 7(a-i)

Compound	FQLogP	Compound	FQLogP	Compound	FQLogP
3a	1.03	5a	3.78	7a	1.93
3b	0.45	5b	1.49	7b	1.35
3c	0.44	5c	1.47	7c	1.34
3d	1.21	5d	3.96	7d	2.11
3e	0.81	5e	3.57	7e	1.72
3f	0.40	5f	1.45	7f	1.31
3g	0.39	5g	2.34	7g	1.30
3h	1.30	5h	4.05	7h	2.20
3i	1.28	5i	2.32	7i	2.18

Molecular docking study

The binding mode of the highest active test compound 7i exhibiting significant HIV-1 reverse transcriptase inhibitory activity was investigated by docking studies. Ligand structures were drawn and optimized using PRODRG [32] online server and saved in PDB format. In order to understand the binding mode of these compounds, Autodock 4.0.1 [25-29] program was used to dock test compound 7i into the HIV-1 RT non-nucleoside inhibitor binding pocket (HIV-1 RT NNIBP). The non nucleosidal inhibitory binding pocket (NNIBP) was obtained using the coordinates of HIV-1-RT/TNK 999 taken from the Protein Brookehaven Database [33] (PDB entry code 1RT2).

The docking experiments were carried out using the Lamarckian genetic algorithm with local search (GA-LS) hybrid formalism of the docking program Autodock 4.0.1. Initially, the docking of TNK 999 was performed to ensure the validity of docking calculations, reliability and reproducibility of the docking parameters for our study (Fig. 1). It was evident that the docked pose of the redocked ligand (green colored ligand) was almost superimposed with that of the co-crystallized ligand (grey colored ligand). A hydrogen bond interaction (1.905 Å) was observed between the hydrogen atom of uracil ring (NH) of TNK 999 and oxygen atom of LYS 103 (CO) residue. Autodock was able to reproduce the experimental binding conformation of TNK 999 within a minimal root mean square deviation (RMSD= 0.56 Å).

Next, docking experiment was performed using standard molecule Efavirenz with 1RT2 for comparison purpose. It was observed that, it forms hydrogen bond interactions (1.805 Å) with LYS 101 amino acid residue in the NNIBP of Reverse Transcriptase (Fig. 2).

The estimated binding free energy and predicted inhibitory constant values for the active test compound 7i was -9.19 kcal/mol and 183.01 nM. It was interesting to note that the experimental results of the test compound 7i correlated well with the estimated binding free energy and predicted inhibitory constant values. The binding mode of the highest active compound is shown in Fig. 3.

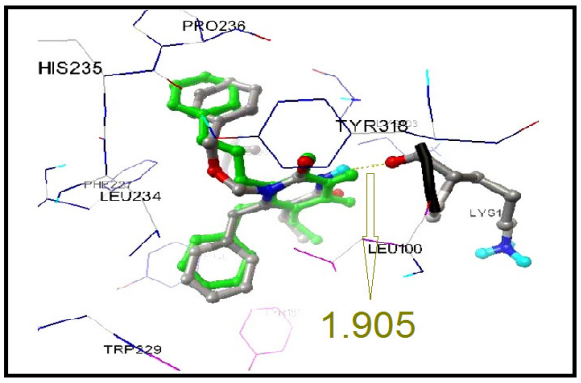


Fig. 1: Redocked mode of co-crystallized ligand TNK 999 in the NNIBP of HIV-1 RT (1RT2). Ligand is shown as stick model and the amino acid residues interacting with the ligands are shown as line model. Hydrogen bond interaction (1.905 Å) with LYS 103 amino acid residue of Reverse Transcriptase is shown as dotted spheres. Rest of the protein is suppressed for clarification purposes.

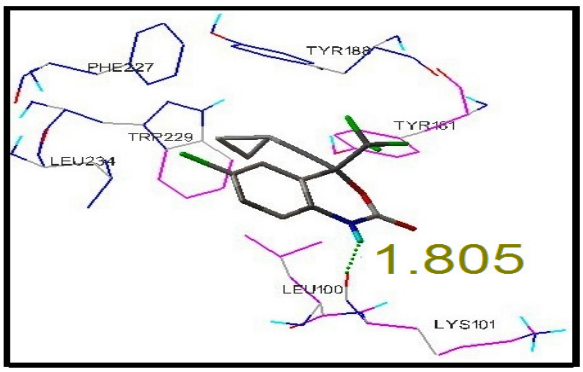


Fig. 2. Binding mode of Efavirenz in the NNIBP of HIV-1 RT (1RT2). Ligand is shown as stick model and the amino acid residues interacting with the ligands are shown as line model. Hydrogen bond interactions (1.805 Å) with LYS 101 amino acid residues of Reverse Transcriptase respectively are shown as dotted spheres. Rest of the protein is suppressed for clarification purposes.

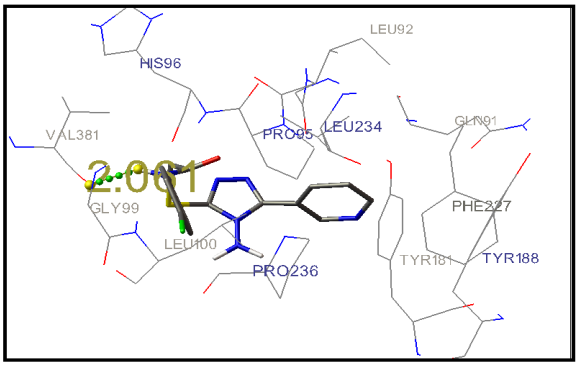


Fig. 3. Binding mode of highest active test compound (7i) in the NNIBP of HIV-1 RT (1RT2). Ligand is shown as stick model and the amino acid residues interacting with the ligands are shown as line model. Hydrogen bond interaction (2.061 Å) with VAL 381 amino acid residue of Reverse Transcriptase is shown as dotted spheres. Rest of the protein is suppressed for clarification purposes.

An investigation of the binding mode of test compound 7i (Fig. 3) revealed that the centroid 1,2,4-triazole ring was surrounded by PRO 95, PRO 236, LEU 100 and HIS 96,whereas the pyridyl portion was oriented towards the hydrophobic pocket formed by the side chains of TYR 181, TYR 188, PRO 236 and PHE 227. The phenyl ring substituted with bromo group at *para* position is surrounded by the residues of LEU 100 and GLY99. The hydrophilic spacer group CH_2-CO-NH is surrounded by the residues of GLY 99, HIS 96 and LYS 103 of HIV-1 RT. A hydrogen bond interaction (2.061 Å) between NH hydrogen atom of spacer CH_2-C(=O)NH group of test compound 7i and oxygen atom of C=O group of GLY99 residue was observed. These interactions may explain the significant inhibitory activities of the compound 7i in the NNIBP of HIV -1- RT.

CONCLUSION

A series of twenty seven novel *N*-(substituted phenyl)-2-(1*H*-1,2,4-triazol-1-yl)acetamides 3(a-i), 2-[(4-amino-5-phenyl-4*H*-1,2,4-triazol-3-yl) thio]-*N*-(substituted phenyl) acetamides 5(a-i) and 2-[(4-amino-5- pyridin-3-yl-4*H*-1,2,4-triazol-3-yl)thio]-*N*-(substituted phenyl) acetamides 7(a-i) were synthesized by reacting 1*H*-1,2,4-triazole (2) 4-amino-5-pyridin-3-yl-4*H*-1,2,4-triazole-3-thiol (4) and 4-amino-5-phenyl-4*H*-1,2,4-triazole-3-thiol (5) with the respective 2-chloro-*N*-(substituted phenyl) acetamides 1(a-i) in acetonitrile and triethylamine. All the final compounds were characterized on the basis of FTIR, ^{1}H NMR and mass spectral data. All the compounds were evaluated for their anti-HIV and antimicrobial potential. Despite their weak antibacterial activity, the antifungal property was very promising. However, few compounds have also showed inhibition towards reverse transcriptase enzyme. This biological property may be conferred to the electron rich substituents, viz.; halogen as bromo, alkoxy as methoxy, nitro were found to be superior in their inhibitory potential. Benzylated compounds were the weakest link in this study as most of them

were weak in their behavioral pattern except compound 7a which exhibited significant antifungal activity. Thus, it is concluded that the triazole moiety with the CH_2CONH spacer attached to aryl moiety substituted with electron withdrawing groups may provide an useful therapeutically effective chemical framework from which potential anti-HIV as well as antifungal agents may be developed. Therefore, further optimization of this prototypical molecular skeleton with lots of diversified molecular fragments may generate a robust therapeutically effective compound.

List of abbreviations

RT: Reverse Transcriptase

HIV: Human Immunodeficiency Virus

NNIBP: Non-nucleoside Inhibitory Binding Pocket

WT: Wild Type

FTIR: Fourier Transform Infrared

NMR: Nucleo Magnetic Resonance

nM: nano mole

GA-LS: Lamarckian genetic algorithm with local search

MIC: Minimum Inhibitory Concentration

LGA: Lamarckian Genetic Algorithm

h: h

MS: Mass Spectrometry

MHz: Mega hertz

ESI: Electrospray Ionization

mp: melting point

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