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# SYNTHESIS AND BIOLOGICAL EVALUATION OF SOME NOVEL TRIAZOLE DERIVATIVES

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## ABSTRACT:

A series of eighteen novel 2-[(4-amino-5- pyridin-3-yl-4*H*-1,2,4-triazol-3-yl)thio]-*N*-(substituted phenyl) propanamides 3(a-i) and 2-[(4-amino-5-phenyl-4*H*-1,2,4-triazol-3-yl) thio]-*N*-(substituted phenyl) propanamides 5(a-i) were synthesized by reacting 4-amino-5-pyridin-3-yl-4*H*-1,2,4-triazole-3-thiol (2) and 4-amino-5-phenyl-4*H*-1,2,4-triazole-3-thiol (4) with the respective 2-chloro-*N*-(substituted phenyl) propanamides 1(a-i) in acetonitrile and triethylamine. The formation of the compounds was established considering the output obtained from spectral analysis. All the synthesized compounds were evaluated for their antimicrobial activities as well as antioxidant property. Most of the compounds have shown comparable antifungal activity against *Candida spp* with respect to the standard antifungal agent fluconazole, whereas almost all the compounds were found to be weakly active against pathogenic bacteria taken for the study. In addition to its prominent antifungal potency, most of the compounds exhibited significant free radical scavenging activity as well. Docking studies with chimeric enzyme of *Candida* $P450_{DM}$ modeled from the structure of *Mycobacterium* $P450_{DM}$ (PDB entry code 1EA1) was also performed for the highest active compound 3c in order to investigate the binding pattern of the same.

**Keywords:** 1,2,4-triazole, Propanamides, Fluconazole, Ciprofloxacin, Nitricoxide

## [I] INTRODUCTION

In the last few decades, the chemistry of 1, 2, 4-triazoles and their fused heterocyclic derivatives have received considerable attention owing to their synthetic and effective biological importance. 1, 2, 4-triazole moiety has been incorporated into a wide variety of therapeutically interesting drug candidates including antifungal [1,2], antineoplastic [3,4,5], antiviral [6,7,8], sedatives, anxiolytics [9], anti-convulsants [10], anti-migraine [11], anti-histaminics [12], anti-tubercular [13,14], and anti-HIV [15] agents. Triazole units have attracted considerable attention in fields, such as medicinal and agrochemical research as well as in the material sciences due to their unique structure and properties [16]. Considering the advancement made in triazole chemistry and biology, an endeavor has been made to synthesis and evaluate some novel trisubstituted

1,2,4-triazole derivatives as 3(a-i) and 5(a-i) for antimicrobial and antioxidant activities. Molecular modelling studies were carried out of the highest active compound 3c in the active site of chimeric 1EA1 to analyze the binding mode.

## [II] MATERIALS AND METHODS

### 2.1. Synthetic study [17, 18]

The reagents were procured from Spectrochem., Merck India Pvt. Ltd., Bengal Chemicals, Kolkata and Otto Chemicals India and were of analytical grade. 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). The melting points of the synthesized compounds were measured by capillary method and are reported uncorrected. The FT-IR pellets were prepared by using Perkin-Elmer punches with KBr.The FT-IR Spectra were measured in SPECTRUM Px FT-IR Spectrometer PERKIN ELMER (serial no: 78625). $^{1}$HNMR spectra were recorded on AMX-400 NMR spectrophotometer at 400 MHz using DMSO-$d_6$ as the solvent and tetramethylsilane as an internal standard. Mass spectra (m/z) of the compounds were recorded on a QTOF Micro YAZ63 spectrometer using electron ionization technique.

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

The respective aromatic amine (0.033 mol) was dissolved in 15 ml of glacial acetic acid. 2-chlorpropionyl chloride (0.037 mol) was added dropwise to this solution while cooling in ice bath. During the addition the reaction mixture was vigorously stirred to drive the reaction to completion. A precipitate was obtained which was filtered and washed with ether. The crude product was dried and subsequently recrystallized from dehydrated ethanol to yield 2-chloro-*N*-(substituted phenyl) propanamide.

2-chloro-*N*-(2-nitrophenyl) propanamide **(1a)**
Yellow crystalline solid (5.0 g, 94%); mp: 80-82$^{o}$C; IR (KBr, cm$^{-1}$) 3355, 1694, 1542;
$^{1}$H-NMR (DMSO-$d_6$, $\delta$ ppm): 8.40 (s; 1H; N$\underline{H}$), 7.46-7.16 (m; 4H; Ar-$\underline{H}$), 4.28(q; 1H; C$\underline{H}$-CO)
MS m/z: 242 [M$^{+}$]

2-chloro-*N*-(4-nitrophenyl) propanamide **(1b)**
Yellow crystalline solid (4.8 g, 91%); mp: 138-140$^{o}$C; IR (KBr, cm$^{-1}$) 3365, 1690, 1532

2-chloro-*N*-(2-bromophenyl) propanamide **(1c)**
White crystalline solid (4.4 g, 90%); mp: 218-220$^{o}$C; IR (KBr, cm$^{-1}$) 3335, 1680, 1539

2-chloro-*N*-(4-bromophenyl) propanamide **(1d)**
White crystalline solid (4.2 g, 86%); mp: 112-114$^{o}$C; IR (KBr, cm$^{-1}$) 3419, 3052, 1700

2-chloro-*N*-(2-methoxyphenyl) propanamide **(1e)**
White crystalline solid (5.0 g, 90%); mp: 220-222$^{o}$C; IR (KBr, cm$^{-1}$) 3310, 1684, 1538

2-chloro-*N*-(4-methoxyphenyl) propanamide **(1f)**
White crystalline solid (5.1 g, 92%); mp: 108-110$^{o}$C; IR (KBr, cm$^{-1}$) 3253, 1659, 1111, 1548
2-chloro-*N*-(4-ethoxyphenyl) propanamide **(1g)**
White crystalline solid (4.7 g, 89%); mp: 230-232$^{o}$C; IR (KBr, cm$^{-1}$) 3260, 1658, 1547, 1118

*N*-benzyl-2-chloro propanamide **(1h)**
White crystalline solid (4.9 g, 88%); mp: 258-260$^{o}$C; IR (KBr, cm$^{-1}$) 3270, 3032, 2973, 1653

2-chloro-*N*-(3-chlorophenyl) propanamide **(1i)**
White crystalline solid (4.9 g, 89%); mp: 58-60$^{o}$C; IR (KBr, cm$^{-1}$) 3249, 3049, 2982, 1667

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

To a solution of 4-amino-5-pyridin-3-yl-4*H*-1,2,4-triazole-3-thiol (0.00052 mol) in 15 ml of

acetonitrile was added the respective 2-chloro-*N*-(substituted phenyl) propanamide (0.00052 mol) and triethylamine (0.00104) dropwise. The reaction mixture was refluxed for 4 h. Later, 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, dried and was recrystallized from dehydrated ethanol to yield the product 2-[(4-amino-5-pyridin-3-yl-4*H*-1,2,4-triazol-3-yl) thio] - *N*- (substituted phenyl) propanamide 3(a-i).

2-[(4-amino-5-pyridin-3-yl-4*H*-1,2,4-triazol-3-yl)thio]-*N*-(2-nitrophenyl)propanamide **(3a)**
White crystalline solid (0.150 g, 75%); mp: 190-192°C; IR (KBr, $cm^{-1}$) 3233, 2976, 1694, 1476; $^1$H-NMR (DMSO-$d_6$, $\delta$ ppm): 10.41 (s; 1H; NH), 9.16 (d; 4H; Pyridyl H), 7.46-7.16 (m; 4H; Ar-H), 4.29(q; 1H; CH), 2.75 (s; 2H, $NH_2$), 1.74 (s; 3H; $CH_3$)
MS m/z: 385 [$M^+$]; Anal. found: C, 49.82; H, 3.89; N, 25.40 %; Calcd for $C_{16}H_{15}N_7O_3S$, C, 49.86; H, 3.92; N, 25.44 %.

2-[(4-amino-5-pyridin-3-yl-4*H*-1,2,4-triazol-3-yl)thio]-*N*-(4-nitrophenyl)propanamide **(3b)**
White crystalline solid (0.144 g, 72%); mp: 210-212°C; IR (KBr, $cm^{-1}$) 3233, 1648, 1562, 1487; $^1$H-NMR (DMSO-$d_6$, $\delta$ ppm): 10.46 (s; 1H; NH), 9.16 (d; 4H; Pyridyl H), 7.47-6.85 (m; 4H; Ar-H), 4.27(q; 1H; CH), 2.38 (s; 2H, $NH_2$), 1.92 (s; 3H; $CH_3$)
MS m/z: 385 [$M^+$]; Anal. found: C,49.81; H, 3.90; N, 25.41%; Calcd for $C_{16}H_{15}N_7O_3S$, C, 49.86; H, 3.92; N, 25.44 %.

2-[(4-amino-5-pyridin-3-yl-4*H*-1,2,4-triazol-3-yl)thio]-*N*-(2-bromophenyl)propanamide **(3c)**
White crystalline solid (0.160 g, 73%); mp: 174-176°C; IR (KBr, $cm^{-1}$) 3233, 2976, 1651, 1566, 1476; $^1$H-NMR (DMSO-$d_6$, $\delta$ ppm): 10.22 (s; 1H; NH), 9.16 (d; 4H; Pyridyl H), 8.10-7.50 (m; 4H; Ar-H), 1.86 (s; 3H; $CH_3$)
MS m/z: 419 [$M^+$]; Anal. found: C, 45.79; H, 3.56; N, 20.00 %; Calcd for $C_{16}H_{15}BrN_6OS$, C, 45.83; H, 3.61; N, 20.04 %.

2-[(4-amino-5-pyridin-3-yl-4*H*-1,2,4-triazol-3-yl)thio]-*N*-(4-bromophenyl)propanamide **(3d)**
White crystalline solid (0.165 g, 76%); mp: 182-184°C; IR (KBr, $cm^{-1}$) 3233, 1651, 1476; $^1$H-NMR (DMSO-$d_6$, $\delta$ ppm): 10.57 (s; 1H; NH), 8.03-8.00 (m; 4H; Pyridyl H), 7.53-7.52 (m; 4H; Ar-H), 3.067 (q; 1H; CH), 2.89 (s; 2H, $NH_2$), 1.92 (s; 3H; $CH_3$)
MS m/z: 419 [$M^+$]; Anal. found: C, 45.78; H, 3.59; N, 20.01%; Calcd for $C_{16}H_{15}BrN_6OS$, C, 45.83; H, 3.61; N, 20.04 %.

2-[(4-amino-5-pyridin-3-yl-4*H*-1,2,4-triazol-3-yl)thio]-*N*-(2-methoxyphenyl)propanamide **(3e)**
White crystalline solid (0.162 g, 84%); mp: 144-146°C; IR (KBr, $cm^{-1}$) 3233, 2976, 1640, 1475, 1130; $^1$H-NMR (DMSO-$d_6$, $\delta$ ppm): 10.24 (s; 1H; NH), 8.70-8.07 (d; 4H; Pyridyl H), 7.47-7.12 (m; 4H; Ar-H), 4.29(q; 1H; CH), 3.85(s; 3H; $OCH_3$), 3.55 (s; 2H, $NH_2$), 1.83 (s; 3H; $CH_3$)
MS m/z: 370 [$M^+$]; Anal. found: C, 55.10; H, 4.88; N, 22.66%; Calcd for $C_{17}H_{18}N_6O_2S$, C, 55.12; H, 4.90; N, 22.69%.

2-[(4-amino-5-pyridin-3-yl-4*H*-1,2,4-triazol-3-yl)thio]-*N*-(4-methoxyphenyl)propanamide **(3f)**
White crystalline solid (0.152 g, 79%); mp: 192-194°C; IR (KBr, $cm^{-1}$) 3234, 2975, 1651, 1566, 1475; $^1$H-NMR (DMSO-$d_6$, $\delta$ ppm): 10.55 (s; 1H; NH), 9.16-8.29 (d; 4H; Pyridyl H), 7.46-6.86 (m; 4H; Ar-H), 4.25 (q; 1H; CH), 3.80 (s; 3H; $OCH_3$), 2.88 (s; 2H, $NH_2$), 1.93 (s; 3H; $CH_3$)
MS m/z: 370 [$M^+$]; Anal. found: C, 55.08; H, 4.86; N, 22.65%; Calcd for $C_{17}H_{18}N_6O_2S$, C, 55.12; H, 4.90; N, 22.69 %.

2-[(4-amino-5-pyridin-3-yl-4*H*-1,2,4-triazol-3-yl)thio]-*N*-(4-ethoxyphenyl)propanamide**(3g)**
White crystalline solid (0.140 g, 70%); mp: 200-202°C; IR (KBr, $cm^{-1}$) 3233, 2976, 1640, 1567, 1476, 1130; $^1$H-NMR (DMSO-$d_6$, $\delta$ ppm): 10.48 (s; 1H; N<u>H</u>), 9.16-8.70 (d; 4H; Pyridyl <u>H</u>), 7.48-6.84 (m; 4H; Ar-<u>H</u>), 4.17(q; 1H; C<u>H</u>), 4.07 (q; 2H; $OC\underline{H}_2C\underline{H}_3$), 3.57 (s; 2H, $N\underline{H}_2$), 1.83 (s; 3H; $C\underline{H}_3$), 1.35 (t; 3H; $OCH_2C\underline{H}_3$)
MS m/z: 386 [$M^+$]; Anal. found: C, 56.21; H, 5.20; N, 21.83%; Calcd for $C_{18}H_{20}N_6O_2S$, C, 56.23; H, 5.24; N, 21.86%.

2-[(4-amino-5-pyridin-3-yl-4*H*-1,2,4-triazol-3-yl)thio]-*N*-benzylpropanamide **(3h)**
White crystalline solid (0.135 g, 73%); mp: 152-154°C; IR (KBr, $cm^{-1}$) 3232, 2976, 1694, 1597, 1477; $^1$H-NMR (DMSO-$d_6$, $\delta$ ppm): 10.32 (s; 1H; N<u>H</u>), 9.16-8.29 (d; 4H; Pyridyl <u>H</u>), 7.47-7.31 (m; 5H; Ar-<u>H</u>), 4.10 (q; 1H; C<u>H</u>), 1.67 (s; 3H; $C\underline{H}_3$)
MS m/z: 354 [$M^+$]; Anal. found: C, 57.57; H, 5.08; N, 23.69%; Calcd for $C_{17}H_{18}N_6OS$, C, 57.61; H, 5.12; N, 23.71%.

2-[(4-amino-5-pyridin-3-yl-4*H*-1,2,4-triazol-3-yl)thio]-*N*-(3-chlorophenyl)propanamide **(3i)**
White crystalline solid (0.148 g, 76%); mp: 214-216°C; IR (KBr, $cm^{-1}$) 3321, 2937, 1695, 1599, 1475; $^1$H-NMR (DMSO-$d_6$, $\delta$ ppm): 10.46 (s; 1H; N<u>H</u>), 9.16 (d; 4H; Pyridyl <u>H</u>), 7.75-7.16 (m; 4H; Ar-<u>H</u>), 4.38 (q; 1H; C<u>H</u>), 3.39 (s; 2H, $N\underline{H}_2$), 1.78 (s; 3H; $C\underline{H}_3$)
MS m/z: 375 [$M^+$]; Anal. found: C, 51.23; H, 3.99; N, 22.39%; Calcd for $C_{16}H_{15}ClN_6OS$, C, 51.27; H, 4.03; N, 22.42%.

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

To a solution of 4-amino-5-phenyl-4*H*-1,2,4-triazole-3-thiol (0.00052 mol) in 15 ml of acetonitrile was added the respective 2-chloro-*N*-(substituted phenyl) propanamide (0.00052 mol) and triethylamine (0.00104) dropwise. The reaction mixture was refluxed for 4 h. Later, the reaction mixture was cooled and to it was added 10 ml of water, 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, dried and was recrystallized from dehydrated ethanol to yield the product 2-[(4-amino-5-phenyl-4*H*-1,2,4-triazol-3-yl)thio]-*N*-(substituted phenyl) propanamide 5(a-i).

2-[(4-amino-5-phenyl-4*H*-1,2,4-triazol-3-yl)thio]-*N*-(2-nitrophenyl)propanamide **(5a)**
White crystalline solid (0.152 g, 76%); mp: 187-189°C; IR (KBr, $cm^{-1}$) 3233, 2976, 1620, 1476; $^1$H-NMR (DMSO-$d_6$, $\delta$ ppm): 10.60 (s; 1H; N<u>H</u>), 8.05-7.50 (m; 5H; Ar-<u>H</u>), 7.72-6.85 (d; 4H; Ar-<u>H</u>), 4.24 (q; 1H; C<u>H</u>), 3.72 (s; 2H, $N\underline{H}_2$), 1.80 (s; 3H; $C\underline{H}_3$)
MS m/z: 384 [$M^+$]; Anal. found: C, 53.08; H, 4.17; N, 21.82 %; Calcd for $C_{17}H_{16}N_6O_3S$, C, 53.12; H, 4.20; N, 21.86 %.

2-[(4-amino-5-phenyl-4*H*-1,2,4-triazol-3-yl)thio]-*N*-(4-nitrophenyl)propanamide **(5b)**
White crystalline solid (0.142 g, 71%); mp: 199-201°C; IR (KBr, $cm^{-1}$) 3233, 1648, 1562, 1487; $^1$H-NMR (DMSO-$d_6$, $\delta$ ppm): 10.23 (s; 1H; N<u>H</u>), 8.10-7.50 (m; 5H; Ar-<u>H</u>), 7.39-6.94 (m; 4H; Ar-<u>H</u>), 4.38 (q; 1H; C<u>H</u>), 3.13 (s; 2H, $N\underline{H}_2$), 1.93 (s; 3H; $C\underline{H}_3$)
MS m/z: 384 [$M^+$]; Anal. found: C, 53.10; H, 4.19; N, 21.81%; Calcd for $C_{17}H_{16}N_6O_3S$, C, 53.12; H, 4.20; N, 21.86 %.

2-[(4-amino-5-phenyl-4*H*-1,2,4-triazol-3-yl)thio]-*N*-(2-bromophenyl)propanamide **(5c)**
White crystalline solid (0.152 g, 70%); mp: 188-190°C; IR (KBr, $cm^{-1}$) 3233, 2976, 1476;
$^1$H-NMR (DMSO-$d_6$, $\delta$ ppm): 10.32 (s; 1H; N<u>H</u>), 8.06-7.51 (m; 5H; Ar-<u>H</u>), 8.06-7.27 (m; 4H; Ar-<u>H</u>), 4.76 (q; 1H; C<u>H</u>), 3.46 (s; 2H, $N\underline{H}_2$), 1.80 (s; 3H; $C\underline{H}_3$)

MS m/z: 418 [$M^+$]; Anal. found: C, 48.79; H, 3.83; N, 16.71%; Calcd for $C_{17}H_{16}BrN_5OS$, C, 48.81; H, 3.86; N, 16.74 %.

2-[(4-amino-5-phenyl-4*H*-1,2,4-triazol-3-yl)thio]-*N*-(4-bromophenyl)propanamide **(5d)**
White crystalline solid (0.100 g, 70%); mp: 220-222ºC; IR (KBr, $cm^{-1}$) 3233, 2976, 1631, 1476; $^1$H-NMR (DMSO-$d_6$, $\delta$ ppm): 10.31 (s; 1H; N$\underline{H}$), 8.05-7.50 (m; 5H; Ar-$\underline{H}$), 7.50 (m; 4H; Ar-$\underline{H}$), 4.34 (q; 1H; C$\underline{H}$), 3.21 (s; 2H, N$\underline{H}_2$), 1.74 (s; 3H; C$\underline{H}_3$)
MS m/z: 418 [$M^+$]; Anal. found: C, 48.78; H, 3.82; N, 16.70 %; Calcd for $C_{17}H_{16}BrN_5OS$, C, 48.81; H, 3.86; N, 16.74%.

2-[(4-amino-5-phenyl-4*H*-1,2,4-triazol-3-yl)thio]-*N*-(2-methoxyphenyl)propanamide **(5e)**
White crystalline solid (0.146 g, 76%); mp: 230-232ºC; IR (KBr, $cm^{-1}$) 3405, 2975, 1633, 1476, 1076; $^1$H-NMR (DMSO-$d_6$, $\delta$ ppm): 10.40 (s; 1H; N$\underline{H}$), 8.06-7.50 (m; 5H; Ar-$\underline{H}$), 8.06-7.12 (m; 4H; Ar-$\underline{H}$), 4.38 (q; 1H; C$\underline{H}$), 3.85 (s; 3H; OC$\underline{H}_3$), 2.99 (s; 2H, N$\underline{H}_2$), 1.75 (s; 3H; C$\underline{H}_3$)
MS m/z: 369 [$M^+$]; Anal. found: C, 58.50; H, 5.15; N, 18.92%; Calcd for $C_{18}H_{19}N_5O_2S$, C, 58.52; H, 5.18; N, 18.96%.

2-[(4-amino-5-phenyl-4*H*-1,2,4-triazol-3-yl)thio]-*N*-(4-methoxyphenyl)propanamide **(5f)**
White crystalline solid (0.168 g, 87%); mp: 226-228ºC; IR (KBr, $cm^{-1}$) 3310, 2971, 1610, 1476, 1070; $^1$H-NMR (DMSO-$d_6$, $\delta$ ppm): 10.22 (s; 1H; N$\underline{H}$), 8.05-7.48 (m; 5H; Ar-$\underline{H}$), 7.48-6.86 (m; 4H; Ar-$\underline{H}$), 4.19 (q; 1H; C$\underline{H}$), 3.80 (s; 3H; OC$\underline{H}_3$), 3.39 (s; 2H, N$\underline{H}_2$), 1.84 (s; 3H; C$\underline{H}_3$)
MS m/z: 369 [$M^+$]; Anal. found: C, 58.49; H, 5.15; N, 18.94 %; Calcd for $C_{18}H_{19}N_5O_2S$, C, 58.52; H, 5.18; N, 18.96%.

2-[(4-amino-5-phenyl-4*H*-1,2,4-triazol-3-yl)thio]-*N*-(4-ethoxyphenyl)propanamide **(5g)**
White crystalline solid (0.162 g, 81%); mp: 196-198ºC; IR (KBr, $cm^{-1}$) 3233, 2976, 1651, 1476, 1130; $^1$H-NMR (DMSO-$d_6$, $\delta$ ppm): 10.28 (s; 1H; N$\underline{H}$), 8.05-7.48 (m; 5H; Ar-$\underline{H}$), 7.48-6.83 (m; 4H; Ar-$\underline{H}$), 4.05 (q; 2H; OC$\underline{H}_2$C$\underline{H}_3$), 3.46 (s; 2H, N$\underline{H}_2$), 1.85 (s; 3H; C$\underline{H}_3$), 1.34 (t; 3H; OCH$_2$C$\underline{H}_3$)
MS m/z: 383 [$M^+$]; Anal. found: C, 59.48; H, 5.50; N, 18.23%; Calcd for $C_{19}H_{21}N_5O_2S$, C, 59.51; H, 5.52; N, 18.26%.

2-[(4-amino-5-phenyl-4*H*-1,2,4-triazol-3-yl)thio]-*N*-benzylpropanamide (5h)
White crystalline solid (0.138 g, 75%); mp: 190-192ºC; IR (KBr, $cm^{-1}$) 3230, 2978, 1640, 1597, 1477; $^1$H-NMR (DMSO-$d_6$, $\delta$ ppm): 10.35 (s; 1H; N$\underline{H}$), 8.05-7.50 (m; 5H; Ar-$\underline{H}$), 7.30-7.23 (m; 5H; benzyl Ar-$\underline{H}$), 4.51 (d; 2H; C$\underline{H}_2$), 4.23 (q; 1H; C$\underline{H}$), 2.60 (s; 2H, N$\underline{H}_2$), 1.72 (s; 3H; C$\underline{H}_3$)
MS m/z: 353 [$M^+$]; Anal. found: C, 61.15; H, 5.40; N, 19.78 %; Calcd for $C_{18}H_{19}N_5OS$, C, 61.17; H, 5.42; N, 19.81%.

2-[(4-amino-5-phenyl-4*H*-1,2,4-triazol-3-yl)thio]-*N*-(3-chlorophenyl)propanamide **(5i)**
White crystalline solid (0.142 g, 73%); mp: 244-246ºC; IR (KBr, $cm^{-1}$) 3233, 2976, 1630, 1476; $^1$H-NMR (DMSO-$d_6$, $\delta$ ppm): 10.38 (s; 1H; N$\underline{H}$), 8.05-7.42 (m; 5H; Ar-$\underline{H}$), 7.75-7.16 (m; 4H; Ar-$\underline{H}$), 4.38 (q; 1H; C$\underline{H}$), 3.39 (s; 2H, N$\underline{H}_2$), 1.78 (s; 3H; C$\underline{H}_3$)
MS m/z: 373 [$M^+$]; Anal. found: C, 54.58; H, 4.27; N, 18.69%; Calcd for $C_{17}H_{16}ClN_5OS$, C, 54.61; H, 4.31; N, 18.73%.

### 2.2. Antimicrobial study

#### 2.2.1. 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.

**2.2.2. 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), *C. dubliniensis* (SSKM), *C. tropicalis* (ATCC-750) and *C. urusial* (SSKM). Two different concentrations of sample solutions were prepared using double distilled water at the strength of 10 and 50 µg/ml. The test micro organisms 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 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 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 sterilised 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. Thus 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.

**2.3. Antioxidant study**

**Nitric Oxide Scavenging Activity Test [22-26]**

A serial solution of different strengths was prepared in DMSO (50, 100, 200, 300, 400 and 500 µg/ml). To these different solutions, equal volume of 5 mM sodium nitroprusside solution in Phosphate buffer pH 7.4 was added and kept for incubation for 2.5 h to 3 h in dark, at 25-29 $^{o}$C. Then, the incubated solutions were treated again with equal volumes of Griess reagent (mixture of sulphanilic acid and naphthylethylenediamine dihydrochloride) and kept for another 10 mins. The absorbance for the nitric oxide scavenging activity was measured by by UV Vis spectrophotometric method using UV-Vis

Spectrophotometer (UV1-THERMO INSIGHT, UK).Thus prepared final solutions were analysed and their respective absorbance at 540nm were recorded. The absorbance of each solution was then compared with standard nitrite solution and percentage inhibition of NO was calculated using the formula given below:

$$\% \text{ inhibition}=[(A_o - A_t)/A_o] \times 100$$

Where, $A_o$ = absorbance of the standard nitrite solution, $A_t$ = absorbance of the sample solution

The standard nitrite solution used was prepared with sodium nitrite solution in deionised water.

**2.4. Molecular modeling**

All computational studies were carried out using Glide v 5.0 [27] software package installed in a single machine running on a 3.4 GHz Pentium 4 processor with 1GB RAM and 160 GB Hard Disk with Red Hat Linux Enterprise version 5.0 as the Operating System.

**Ligand Structure Preparation**

Ligand structure with highest antifungal activity, like 3c was drawn and optimized using PRODRG online server [28] and saved in PDB format. By using the Ligprep utility of Glide, this structure was geometry optimized by using the Optimized Potentials for Liquid Simulations-2005 (OPLS-2005) force field [29, 30] with the steepest descent followed by truncated Newton conjugate gradient protocol. Partial atomic charges were computed using the OPLS-2005 force field.

**Protein Structure Preparation**

It was observed from the literature that high homology exists between the *Mycobacterium* $P450_{DM}$ and *Candida* $P450_{DM}$. A chimeric enzyme has been built from the crystallographic structure of the complex 1EA1 [31] in which the residues that are arranged in a range of 7Å from Fluconazole (TPF) were substituted with those of *Candida* $P450_{DM}$. The chimeric enzyme for the *Candida albicans* (CYP51) was constructed from that of mycobacterium $P\text{-}450_{DM}$ CYP51 extracted from the protein data bank (PDB entry code 1EA1) as per the reported method [32].Overall 12 residues that were arranged in a range of 7 Å from fluconazole were substituted with those of *Candida* $P\text{-}450_{DM}$. Substitutions were made by replacement of the residues Pro77, Phe78, Met79, Arg96, Met99, Leu100, Phe255, Ala256, His258, Ile322, Ile323 and Leu324 by Lys77, His78, Leu79, Leu96, Lys99, Phe100, Met255, Gly256, Gln258, His322, Ser323 and Ile324, which were thought to be necessary for the ligand-receptor interaction. Water molecules of crystallization were removed from the complex, and the protein was optimized for docking using the protein preparation wizard provided by Schrodinger Inc. Partial atomic charges were assigned according to the OPLS-AA force field.

**Validation of Docking Protocol**

The most suitable method of evaluating the accuracy of a docking procedure is to determine how closely the lowest energy pose predicted by the scoring function resembles an experimental binding mode as determined by X-ray crystallography. In the present study, Extra Precision Glide docking procedure was validated by removing TPF from the binding site and re-docking it to the binding pocket of chimeric 1EA1. We found a very good agreement between the localization of the inhibitors upon docking. The root mean square deviations (RMSD) between the predicted conformation and the observed X-ray crystallographic conformation of compound was 1.602 Å (<3 Å).

**Docking**

The most active compound 3c was docked into the coordinates of the crystal structure of chimeric 1EA1. All docking calculations were performed using the “Extra Precision” (XP) mode of Glide Program v5.0. A grid was prepared with the center defined by the co-crystallized ligand TPF for 1EA1. During the docking process, Glide initially

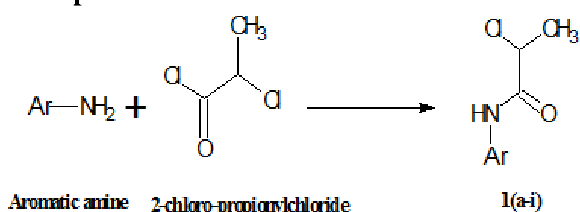
performed a complete systematic search of the conformational, orientational and positional space of the docked ligand and eliminating unwanted conformations using scoring and followed by energy optimization. Finally the conformations were further refined *via* Monte Carlo sampling of pose conformation. Predicting the binding affinity and rank-ordering ligands in database screens was implemented by modified and expanded version of the Glide Score scoring function.

## [III] RESULTS

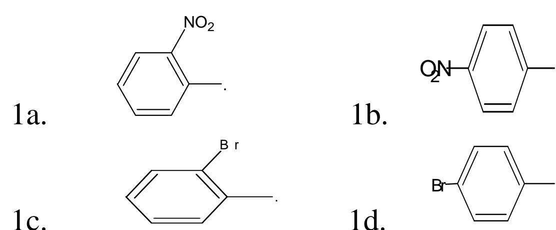
### 3.1. Synthetic study

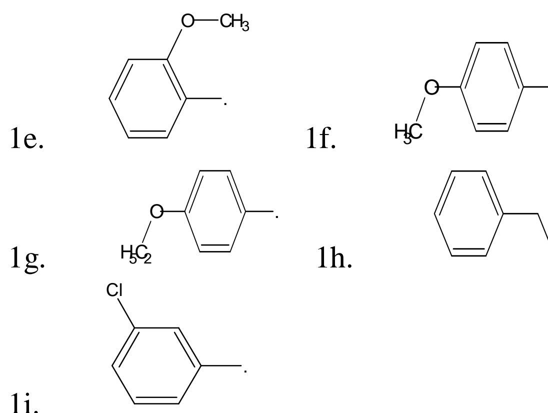
In this present study 2-chloro-*N*-(substituted phenyl) propanamides 1(a-i) were synthesized by reported method [17]. Compounds 4-amino-5-(pyridin-3-yl)-4*H*-1,2,4-triazole-3-thiols (2) and 4-amino-5-phenyl-4*H*-1,2,4-triazole-3-thiols (4) were synthesized by the method reported for triazoles [18]. Compounds 1(a-i) were next refluxed with compounds 2 and 4 in presence of triethylamine in acetonitrile yielding eighteen 2-[(4-amino-5- pyridin-3-yl-4*H*-1,2,4-triazol-3-yl)thio]-*N*-(substituted phenyl)propanamides 3(a-i) and 2-[(4-amino-5- phenyl-4*H*-1,2,4-triazol-3-yl)thio]-*N*-(substituted phenyl)propanamides and 5(a-i) respectively.

**Step1:**

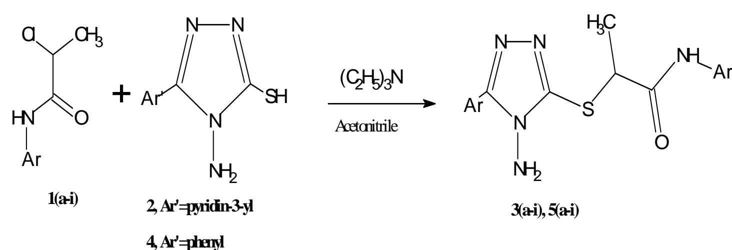



Where Ar=




**Step 2:**




**Scheme 1.** Synthetic route of compounds; 2-[(4-amino-5- pyridin-3-yl/ phenyl-4*H* 1,2,4-triazol-3-yl)thio]-*N*-(substituted phenyl)propanamide 3(a-i), 5(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 3419-3230 $cm^{-1}$ indicating the presence of N-H stretch of aromatic secondary amide. A strong band in the range of 1694-1630 $cm^{-1}$ 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. A strong band in the range of 1130-1076 $cm^{-1}$ indicates the presence of C-O-C stretching of the compounds holding methoxy group in the compounds 1e, 1f, 3e, 3f, 5e, 5f and ethoxy group in the compounds 1g, 3g and 5g.

$^{1}$H NMR spectrum of the compounds 3a-i and 5a-i showed a singlet of one proton at $\delta$ 10.57-9.48, assigned to the secondary N-H of amide. A multiplet of four protons at $\delta$ 8.10-6.85 was assigned to the disubstituted aromatic ring attached to the aliphatic end by means of an amide linkage. In case of compound 3h and 5h a multiplet of four protons at $\delta$ 7.43-7.23 and at $\delta$ 7.30-7.23 respectively was observed. A quartet of one proton at $\delta$ 4.10-3.067 was attributed to the secondary carbon holding the methyl group. A singlet of three protons at $\delta$ 3.85-3.80 was observed for four compounds, namely 3e, 3f, 5e and 5f and was assigned to the methyl protons of the methoxy group. On the other hand, for ethoxy compounds 3g and 5g at $\delta$ 4.05-4.07 a quartet of two protons was observed for the methylene group and a triplet of three protons between $\delta$ 1.35-1.34 was seen for the terminal methyl group. Finally, at $\delta$ 1.93-1.67 a singlet of three protons was assigned to the branched methyl protons of the linker group $CHCH_3CONH$ of propanamide.

The results obtained from mass analysis were very much 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.

## 3.2. Antimicrobial study

### 3.2.1. Antibacterial study [19]

All the compounds 3(a-i) and 5(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 all the compounds 3(a-i) and 5(a-i) were found to be weakly active compared to the reference standard ciprofloxacin against all pathogenic bacterial strains considered for the study. The results are summarized in Table 1.

**[Table-1]**.

**Table: 1.** Minimum Inhibitory concentration (MIC) of Test Compounds 3 (a-i) and 5 (a-i) against *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*

| **Compounds** | **MIC (µg/ml)** | | |
|---|---|---|---|
| | ***Staphylococcus aureus*** | ***Bacillus subtilis*** | ***Escherichia coli*** |
| 3a | 400 | 400 | 500 |
| 3b | 400 | 500 | 500 |
| 3c | 300 | 300 | 400 |
| 3d | 200 | 200 | 300 |
| 3e | 500 | 500 | 500 |
| 3f | 400 | 500 | 500 |
| 3g | 400 | 400 | 400 |
| 3h | 500 | 500 | 500 |
| 3i | 200 | 300 | 300 |
| 5a | 300 | 300 | 400 |
| 5b | 400 | 400 | 500 |
| 5c | 200 | 200 | 200 |
| 5d | 200 | 300 | 300 |
| 5e | 400 | 400 | 400 |
| 5f | 500 | 500 | 500 |
| 5g | 500 | 500 | 500 |
| 5h | 500 | 500 | 500 |
| 5i | 300 | 200 | 200 |
| Ciprofloxacin | 0.78 | 0.78 | 0.78 |

### 3.2.2. Antifungal study [19-21]

All the compounds 3(a-i) and 5(a-i) were screened for their *in vitro antifungal activity against* the fungal strains namely *Candida albicans, Candida tropicalis*, *Candida dubliniensis* and *Candida urusial.* All the compounds were tested at different concentrations, 15, 20, 25, 50 and 100 µg/ml. Compound 3c exhibited highest activity against all the strains of *Candida* compared to the standard fluconazole. Compounds 3d, 3i and 5c also exhibited highest activity against *Candida albicans* and *Candida tropicalis* while exhibiting weak activity against the other two strains of

*Candida.* The superiority can be attributed to the halogen substituents as either bromo at *ortho* and *para* position or chloro at *meta* position were present in the stated compounds. Compounds 3a and 5d with nitro and bromo substituents at *ortho* and *para* position also exhibited good activity against both *Candida albicans and Canduda tropicalis* compared to the reference standard. Moderate activity was observed for all the other compounds. The results are summarized in Table 2.

**[Table-2].**

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

**Table: 2.** Minimum Inhibitory Concentration (MIC) of Test Compounds 3 (a-i) and 5 (a-i) against *Candida urusial*, *Candida tropicalis*, *Candida dubliniensis*, *Candida albicans.*

In case of *Candida tropicalis* highest activity was observed in compounds 3a with a nitro group at *meta* position, 3c with a bromo substituent at *ortho* position and 3d in which a *para* position was occupied with bromo group, whereas compounds 3b, 5a, 5b, 5c, 5d and 5i were moderate in their activities. All the other compounds were found to be weakly active while acting against the fungi, viz.; *Candida urusial* and *Candida dubliniensis.*

### 3.3. Antioxidant study [22-26]

All the compounds (3a-i) and (5a-i) were evaluated for their *in vitro* antioxidant properties. Nitric oxide (NO) was generated by sodium nitropruside in solution. In the presence of an antioxidant or nitric oxide scavenger the amount of NO generated was less. The excess NO was then estimated by Griess reagent which is a mixture of sulphanilic acid and naphthylethylenediamine dihydrochloride. The nitric oxide generated pink colour complex estimated at 540 nm. Nitric oxide (NO) was generated from sodium nitroprusside (SNP) and was measured by the Griess reagent. The results are summarized in Table 3.

**[Table-3].**

| **Compounds** | **PERCENTAGE INHIBITION** | | | | | |
|---|---|---|---|---|---|---|
| | **50 µg/ml** | **100 µg/ml** | **200 µg/ml** | **300 µg/ml** | **400 µg/ml** | **500 µg/ml** |
| 3a | 08 | 20 | 60 | 70 | 80 | 82 |
| 3b | 14 | 34 | 58 | 63 | 79 | 81 |
| 3c | 07 | 13 | 43 | 59 | 70 | 79 |
| 3d | 31 | 42 | 60 | 63 | 69 | 74 |
| 3e | 28 | 40 | 58 | 62 | 72 | 78 |
| 3f | 16 | 38 | 60 | 70 | 76 | 79 |
| 3g | 30 | 41 | 59 | 65 | 75 | 80 |
| 3h | 33 | 40 | 48 | 60 | 71 | 80 |
| 3i | 11 | 27 | 40 | 58 | 79 | 83 |
| 5a | 11 | 21 | 43 | 53 | 65 | 85 |
| 5b | 17 | 24 | 38 | 49 | 57 | 74 |
| 5c | 21 | 31 | 51 | 65 | 75 | 86 |
| 5d | 16 | 27 | 36 | 45 | 58 | 74 |
| 5e | 18 | 26 | 35 | 47 | 59 | 79 |
| 5f | 13 | 17 | 27 | 38 | 57 | 73 |
| 5g | 08 | 14 | 28 | 40 | 57 | 70 |
| 5h | 11 | 20 | 30 | 41 | 53 | 63 |
| 5i | 10 | 21 | 32 | 44 | 57 | 80 |

**Table: 3**. Results for NO scavenging activity

SNP in aqueous solution at physiological pH spontaneously generates NO, which interacts with oxygen to produce nitrite ions that can be estimated by the use of Griess Reagent. Scavengers of NO compete with oxygen leading to

reduced production of NO-SNP. The synthesized compounds showed a remarkable nitric oxide (NO) scavenging property. 80% and above inhibition at a concentration of 500µg/ml was observed for the compounds 3a, 3b, 3h, 3i, 5a, 5c and 5i. The rest of the compounds, except compound 5h, at similar concentration exhibited 70% and above inhibitory capacity. Information procured from the above study clearly indicates a good account of antioxidant property of the compounds under investigation.

### 3.3. Molecular modeling

*In silico* docking studies were carried out to investigate the interaction of the 3c with the chimeric 1EA1 in figure 2 using the Extra Precision (XP) mode of Glide software. To validate the Glide software, we first modelled the interaction between fluconazole (TPF) and chimeric 1EA1. Superimposition of the experimental bound (co-crystallized) conformation of TPF and that predicted by Glide are shown in figure 1.

**Fig: 1**

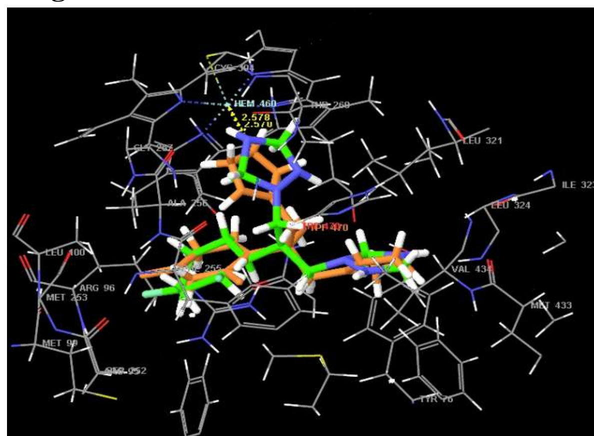



**Fig: 1.** Validation of docking protocol. Superimposition of experimental bound (co-crystallized) conformation of TPF (orange) and that predicted by Glide colored as carbon: green, hydrogen: cyan, nitrogen: blue, and oxygen: red. Active site amino acid residues are represented by lines. Hydrogen bond interaction is represented by yellow dotted lines.

**Fig: 2**

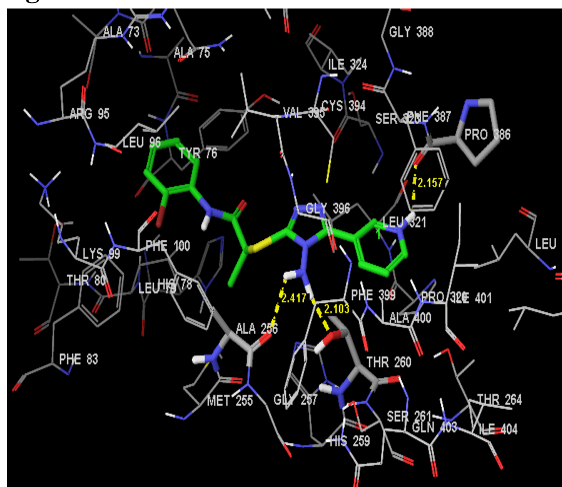



**Fig: 2.** Molecular model of compound 3c in the binding pocket of chimeric 1EA1. Active site amino acid residue and the inhibitor are represented as tubes with the atoms of inhibitor colored as carbon: green, hydrogen: cyan, nitrogen: blue, and oxygen: red. Hydrogen bond interactions are represented by dotted lines.

Glide successfully reproduced the experimental binding conformations of TPF in the binding pocket of chimeric 1EA1 with an acceptable root-mean-square deviation (RMSD) of 1.602 Å (<3Å). In our model of compound **3c** with the enzyme binding pocket in Figure 2, the pyridyl nitrogen forms a hydrogen bond with PRO386 ($NH_{pyridyl}\cdots OH_{PRO386}$ = 2.157Å). The primary amino group attached to triazole ring forms two hydrogen bond with ALA256 and GLY257 respectively (primary $NH_{triazole}\cdots CO_{ALA256}$ = 2.417Å and primary $NH_{triazole}\cdots CO_{GLY257}$ = 2.103Å). The substituted phenyl ring also makes favorable interaction with the hydrophobic pocket formed by the residues like, PHE100, TYR76, LEU96. The 1,2,4 triazole nucleus exhibits favorable interactions with the another hydrophobic cavity comprised of GLY396, and VAL395 of the binding pocket of chimeric 1EA1. Glide score obtained for the compound 3c was -5.89, whereas for TPF (fluconazole) was found to be -6.05. The closeness

of the score, as well as the interaction pattern with the active site residues clearly suggests the high potency of the synthesized compound 3c as an antifungal agent.

## [IV] CONCLUSION

A series of eighteen novel 2-[(4-amino-5-pyridin-3-yl-4*H*-1,2,4-triazol-3-yl)thio]-*N*-(substituted phenyl) propanamides 3(a-i) and 2-[(4-amino-5-phenyl-4*H*-1,2,4-triazol-3-yl)thio]-*N*-(substituted phenyl) propanamides 5(a-i) were synthesized by reacting 4-amino-5-pyridin-3-yl-4*H*-1,2,4-triazole-3-thiols (2) and 4-amino-5-phenyl-4*H*-1,2,4-triazole-3-thiols (4) with the respective 2-chloro-*N*-(substituted phenyl) propanamides 1(a-i). 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 antimicrobial potential and antioxidant property. Despite their weak antibacterial activity, the antifungal property was worth mentioning. This property may be attributed to the halogen substituents, as most of the *ortho* as well as *para* halogenated compounds were found to be superior in their pathogenic resistance potential. Alkoxy and benzylated compounds were the weakest link in this study as most of them were weak in their behavioral pattern. Most of the compounds also exhibited significant antioxidant property. Thus, it is concluded that the triazole moiety with the $CHCH_3CONH$ linker attached to a phenyl ring substituted with electron withdrawing groups may provide a suitable scaffold from which potential antifungal agents may be developed. Therefore, further optimization of the centroid as 1,2,4-triazole with several other substituents must be needed to ensure the immense potentiality of this type of structural skeleton.

## FINANCIAL DISCLOSURE

All the experiments were carried out exclusively in the different Laboratories at Gupta College of Technological Sciences, Asansol. The total expenditure during the process of this study was provided by the College.

## ACKNOWLEDGEMENT

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