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Synthesis, *in vitro* antibacterial, antifungal biological activities along with *in silico* molecular docking studies of substituted 2-[4-(3-oxo-[1,2,4] triazolo [4,3-a]pyridin-2-ylmethyl)-[1,2,3]triazol-1-yl]-N-(4-phenyl-thiazol-2-yl)-acetamide

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A new series of substituted 2-[4-(3-oxo-[1,2,4]-triazolo-[4,3-a]pyridin-2-ylmethyl)-[1,2,3]triazol-1-yl]-N-(4-phenyl-thiazol-2-yl)-acetamide compounds have been synthesized and evaluated for antimicrobial activity. Antibacterial activity has been performed against *S. aureus* and *S. pyogenus* (Gram-positive) and, *E. coli* and *P. aeruginosa* (Gram-negative) bacteria. Antifungal activity was performed against *C. albicans, A. niger* and *A. clavatus*. In the results, selected compounds exhibit significant antibacterial activity in comparison to others. MIC has been compared to currently marketed drugs also.

Keywords: 1,2,4-Triazole, Antimicrobial, Antifungal, MIC, Molecular docking

Antimicrobial resistance is a major issue nowadays. In the last decade, scientists reported many new antimicrobial agents[1-8]. A variety of pharmacological characteristics, including anticancer[9,10], antiviral[11,12], antitubercular[13,14], antifungal[15,16], and antibacterial[17,18] activities, are frequently found in compounds generated from the 1,2,4-triazole (Fig. 1).

Compounds with two or more structural domains that have separate biological characteristics and dual activity are known as hybrid molecules. These substances typically can lower toxicity, overcome drug resistance, and enhance pharmacokinetic characteristics[19,20]. Several hybrid compounds are currently undergoing clinical studies[21-25], revealing their potential to treat a range of disorders. Through the hybridization of 1,2,4-triazole with different antibacterial pharmacophores, numerous novel candidates with outstanding efficacy against both drug-sensitive and drug-resistant microorganisms have been synthesized in recent years.

This work lists the new synthetic techniques for producing functionalized 1,2,4-triazole-containing derivatives and pyridine compounds. These new triazole derivatives are characterized using ^{1}H NMR, FT-IR, elemental analyses, mass spectra, and ^{13}C NMR. With their great potential, these novel synthetic techniques might be employed as antibacterial agents.

Experimental Section

Materials and Method

All of the chemical compounds and solvents used in the synthesized library were obtained from CDH Chemical in Delhi and were used without further purification. Thin-layer chromatography (TLC) was used to monitor reactions on silica gel-G plates (G60 F254 (Merck) with a thickness of 0.5 mm, and ultraviolet light was used for visualization (254 and 365 nm). Melting points were determined using an open capillary technique and were not corrected for temperature. The synthesized compounds' ^{1}H NMR spectra were recorded in DMSO-d_6 solvent with a Bruker-Avance-II (400 MHz). A Shimadzu FTIR-8400 spectrophotometer (Kyoto, Japan) and a DRS probe KBr pallet were used to collect IR spectra. Chemical shifts are measured in parts per million (ppm) downfield from TMS, which serves as an internal standard.

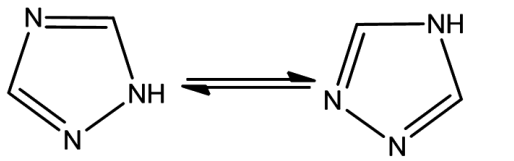


Fig. 1 — Tautomerism of 1,2,4-triazole moiety

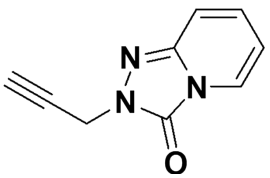
Chemistry

Synthesis of 1,2,4- triazolo4,3-a] pyridine-3-(2*H*)-one, 03 (Step-1)

In 200 mL of 2ethoxyethanol, a mixture of 2-chloropyridine (100 gm, 0.88 mol) and semicarbazide hydrochloride (200 gm, 1.79 mol) was heated to 145-150°C for 12 hours. TLC was used to monitor the reaction's progress. After completion, the reaction mass was cooled to 60°C, and 400 mL of water was added. After cooling to 0°C, the solution was stirred for 0.5 hours. Filtration was used to isolate the precipitated product. 112.0 gm, 94.3% product yield. The above solid was then dissolved in a solution of 30% sodium hydroxide (100 mL) and warmed to 40°C until a clear solution was obtained. The solution was then slowly cooled to 0°C, at which point the product crystallized as sodium salt, yielding a thick slurry. The sodium salt of the product was isolated *via* filtration and washed with chilled water (0°C, 200 mL) before drying for 12 hours at 70°C under reduced pressure (10 mm/Hg). 127.2 gm, 97.0% product yield.

Synthesis of 2-(prop-2-yn-1yl)-[1,2,4] triazolo [4, 3-a] pyiridine-3(2*H*)-one, 04 (Step-2)

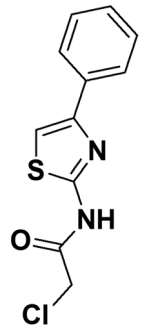
In RBF, dissolve 50 mmol of 1,2,4-triazolo [4,3-a] pyridine-3- (2*H*)-one in 150 mL of acetone and add 100 mmol of anhydrous K_2CO_3 while stirring. After 5 minutes, propargyl bromide (55 mmol) was slowly added. After the addition is complete, continue to stir the reaction mixture for 3-4 hours. TLC was used to monitor the reaction. After the reaction was finished, the reaction mixture was poured into the crushed ice. MDC should be used twice to extract the reaction mixture. Dry the organic layer with sodium sulphate after separating it. Compound 4 is obtained as an oily liquid by evaporating the solvent.



2-(prop-2-yn-1-yl)-[1,2,4]triazolo[4,3-a]pyridine-3(2H)-one

Synthesis of 2-chloro-*N*-(4-phenylthiazol-2-yl) acetamide, 06 (Step-3)

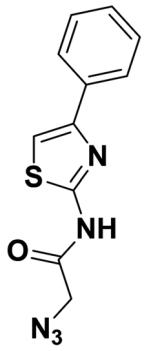
Drop by drop, Chloroacetyl chloride (1 eq.) was added to a solution of various substituted amines (1equi.) in acetone, and the resulting mixture was stirred for 15 minutes at RT. The reaction mixture was then dumped onto crushed ice, and the solid intermediate product was separated and filtered before being washed with water. It is dried and used in the next step without further purification.



2-chloro-*N*-(4-phenylthiazol-2-yl)acetamide

Synthesis of 2-azido-*N*-(4-phenylthiazol-2-yl) acetamide, 07 (Step-4)

Sodium azide (NaN_3) was added to an (1 eq.) solution in DMF (3 eq.)[26]. The resulting mixture was stirred at RT for 24 hours after the reaction mixture was completed; the reaction mixture was poured on crushed ice. Filter and dry the separated product.

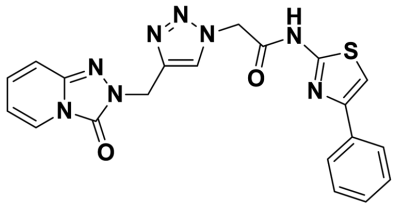


2-azido-*N*-(4-phenylthiazol-2-yl)acetamide

Characterization data of synthesized compounds

Synthesis of 2-[4-(3-oxo-[1,2,4] triazolo[4,3-a]pyridin-2(3*H*)-yl)methyl)-1*H*-[1,2,3] triazol-1-yl]-*N*-(4-phenyl-thiazol-2-yl)-acetamide, 8

An RBF containing DMF:H_2O: *n*-butanol(1:1:1), [04] (1 eq.), and [07] (1 eq.) was added at RT, followed by a catalytic amount of sodium Ascorbate and Copper sulphate pentahydrate. Stir the resulting solution for 24 hours at RT before pouring it into the crushed ace and filtering the separated product. Filter the product again with dilute ammonia.



2-(4-((3-oxo-[1,2,4] triazolo[4,3-*a*]pyridine-2(3*H*)-yl)methyl)-1*H*-1,2,3-triazol-1-yl)-*N*-(4-phenylthiazol-2-yl)acetamide

2-{4-[(3-Oxo-(1,2,4)triazolo(4,3-a)pyridin-2(3*H*)-yl) methyl]-1*H*-1,2,3-triazol-1-yl}*N*-(4-*p*-tolylthiazol-2-yl)acetamide, 8a: Yield 85%. White Solid. m.p.165-167°C. IR (KBr): 646, 686, 749, 778, 819, 926, 964, 992, 1056, 1144, 1173, 1287, 1327, 1368, 1416, 1449, 1486, 1536, 1569, 1608, 1738, 2214, 2813, 2890, 2980, 3040, 3108, 3635, 3730, 3767, 3801, 3888, 3934 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): δ 2.32 s (3H, CH_3), 5.22 s (2H, CH_2), 5.45 s (2H, CH_2), 6.63 s (1H), 7.22 s (4H), 7.57 s (1H), 7.77-7.79 d (2H, J = 6.6 Hz), 7.88-7.89 d (1H, J = 6.6 Hz), 8.16 s (1H), 12.78 brs, (1H, NH). Anal. Calcd for $C_{21}H_{18}N_8O_2S$: C, 56.49; H, 4.06; N, 25.10; S, 7.18. *M* 447. Found: C, 56.52; H, 4.08; N, 25.05; S, 7.16%.

***N*-[4-(4-Chlorophenyl)thiazol-2-yl]-2-{4-[(3-oxo-[1,2,4]triazolo[4,3-a]pyridin-2(3*H*)-yl)methyl]-1*H*-1,2,3-triazol-1-yl}acetamide, 8b**: Yield 82%. White Solid. m.p.180-182°C. IR (KBr): 646, 686, 749, 778, 819, 926, 964, 992, 1056, 1144, 1173, 1287, 1327, 1368, 1416, 1449, 1486, 1536, 1569, 1608, 1738, 2214, 2813, 2890, 3040, 3108, 3635, 3730, 3767, 3801, 3888, 3934 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): δ 5.21 s (2H, CH_2), 5.43 s (2H, CH_2), 6.61-6.64 m (1H),7.22 s (2H), 7.47-7.49 d (2H, J = 8.4 Hz), 7.67 s (1H), 7.87-7.92 m (3H), 8.15 s (1H), 12.78 brs (1H, NH). Anal. Calcd for $C_{20}H_{15}ClN_8O_2S$: C, 51.45; H, 3.24; N, 24.00; S, 6.87. *M* 466. Found: C, 51.52; H, 3.18; N, 24.05; S, 6.86%.

***N*-[4-(4-Bromophenyl)thiazol-2-yl]-2-{4-[(3-oxo-[1,2,4]triazolo[4,3-a]pyridin-2(3*H*)-yl)methyl]-1*H*-1,2,3-triazol-1-yl}acetamide, 8c**: Yield 79%. White Solid. m.p.145-147°C. IR (KBr): 652, 686, 749, 778, 819, 926, 964, 992, 1056, 1144, 1173, 1287, 1327, 1368, 1416, 1449, 1486, 1536, 1569, 1608, 17385, 2214, 2813, 2890, 2980, 3040, 3108, 3635, 3730, 3767, 3801, 3888, 3934 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): δ 5.21 s (2H, CH_2), 5.43 s (2H, CH_2), 6.62-6.65 m (1H),7.22 s (2H),7.46-7.49 d (2H, J = 8.3 Hz),7.66 s (1H), 7.88-7.94 m (3H), 8.16 s (1H), 12.77 brs (1H, NH). Anal. Calcd for $C_{20}H_{15}BrN_8O_2S$: C, 46.98; H, 2.96; N, 21.91; S, 6.27. *M* 510, 512. Found: C, 46.92; H, 2.98; N, 22.05; S, 6.15%.

***N*-[4-(4-Methoxyphenyl)thiazol-2-yl]-2-{4-[(3-oxo-[1,2,4]triazolo[4,3-a]pyridin-2(3*H*)-yl)methyl]-1*H*-1,2,3-triazol-1-yl}acetamide, 8d**: Yield 87%. White Solid. m.p.162-166°C. IR (KBr): 648, 686, 749, 778, 819, 926, 964, 992, 1056, 1144, 1173, 1287, 1327, 1368, 1416, 1449, 1486, 1536, 1569, 1608, 1738, 2214, 2813, 2890, 2980, 3040, 3108, 3635, 3730, 3767, 3801, 3934 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): δ 3.81 s (3H, OCH_3), 5.22 s (2H, CH_2), 5.41 s (2H, CH_2), 6.68-6.65 m (1H), 7.25 s (2H), 7.53-7.50 d (2H, J = 8.2 Hz), 7.69 s (1H), 8.05-7.99 m (3H), 8.15 s (1H), 12.81 brs (1H, NH). Anal. Calcd for $C_{21}H_{18}N_8O_3S$: C, 54.54; H, 3.92; N, 24.23; S, 6.93. *M* 462. Found: C, 54.52; H, 3.91; N, 24.25; S, 6.91%.

2-{4-[(3-Oxo-[1,2,4]triazolo[4,3-a]pyridin-2(3*H*)-yl)methyl]-1*H*-1,2,3-triazol-1-yl}-*N*-(4-phenylthiazol-2-yl)acetamide, 8e: Yield 81%. White Solid. m.p.178-182°C. IR (KBr): 648, 686, 749, 778, 819, 926, 964, 992, 1056, 1144, 1173, 1287, 1327, 1368, 1416, 1449, 1486, 1536, 1569, 1608, 1738, 2214, 2813, 2890, 2980, 3040, 3108, 3635, 3730, 3767, 3801, 3888, 3934 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): δ 5.22 s (2H, CH_2), 5.45 s (2H, CH_2), 6.63 s (1H), 7.22 s (5H), 7.57 s (1H), 7.77-7.75 d (2H, J = 7.9 Hz), 7.87-7.83 d (1H, J = 8.1 Hz), 8.20 s (1H), 12.80 brs (1H, NH). Anal. Calcd for $C_{20}H_{16}N_8O_2S$: C, 55.55; H, 3.73; N, 25.91; S, 7.41. *M* 432. Found: C, 55.57; H, 3.72; N, 25.90; S, 7.40%.

2-{4-[(3-Oxo-[1,2,4]triazolo[4,3-a]pyridin-2(3*H*)-yl]methyl}-1*H*-1,2,3-triazol-1-yl)-*N*-(4-o-tolylthiazol-2-yl)acetamide, 8f: Yield 78%. White Solid. m.p.170-173°C. IR (KBr): 646, 686, 749, 778, 819, 926, 964, 992, 1056, 1144, 1173, 1287, 1327, 1368, 1416, 1449, 1486, 1536, 1569, 1608, 1738, 2214, 2813, 2890, 2980, 3040, 3108, 3635, 3730, 3767, 3801, 3888, 3934 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): δ 2.34 s (3H, CH_3), 5.23 s (2H, CH_2) 5.47 s (2H, CH_2), 6.60 s (1H), 7.24 s (4H), 7.59 s (1H), 7.78-7.77 d (2H, J = 8.4 Hz) 7.87-7.86 d (1H, J = 8.4 Hz), 8.16 s (1H), 12.78 brs (1H, NH). Anal. Calcd for $C_{21}H_{18}N_8O_2S$: C, 56.49; H, 4.06; N, 25.10; S, 7.18. *M* 447. Found: C, 56.52; H, 4.08; N, 25.05; S, 7.16%.

***N*-[4-(2-Chlorophenyl)thiazol-2-yl]-2-{4-[(3-oxo-[1,2,4]triazolo[4,3-a]pyridin-2(3*H*)-yl)methyl]-1*H*-1,2,3-triazol-1-yl}acetamide, 8g**: Yield 62%. White Solid. m.p.149-153°C. IR (KBr): 646, 686, 749, 778,

819, 926, 964, 992, 1056, 1144, 1173, 1287, 1327, 1368, 1416, 1449, 1486, 1536, 1569, 1608, 1738, 2214, 2813, 2890, 2980, 3040, 3108, 3635, 3730, 3767, 3801, 3888, 3934 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): δ 5.65 s (2H, CH_2); 5.70 s (2H, CH_2), 6.64-6.61 m (1H), 7.21 s (2H), 7.49-7.47 d (2H, *J* = 8.2 Hz), 7.69 s (1H), 7.95-7.89 m (3H), 8.16 s (1H), 12.81 brs (1H, NH). Anal. Calcd for $C_{20}H_{15}ClN_8O_2S$: C, 51.45; H, 3.24; N, 24.00; S, 6.87. *M* 466. Found: C, 51.48; H, 3.23; N, 24.01; S, 6.86 %.

***N*-[4-(2-Bromophenyl)thiazol-2-yl]-2-{4-[(3-oxo-[1,2,4]triazolo[4,3-a]pyridin-2(3*H*)-yl)methyl]-1*H*-1,2,3-triazol-1-yl}acetamide, 8h**: Yield 49%. White Solid. m.p.154-157°C. IR (KBr): 652, 686, 749, 778, 819, 926, 964, 992, 1056, 1144, 1173, 1287, 1327, 1368, 1416, 1449, 1486, 1536, 1569, 1608, 1738, 2214, 2813, 2890, 2980, 3040, 3108, 3635, 3730, 3767, 3801, 3888, 3934 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): δ 5.09 s (2H, CH_2), 5.22 s (2H, CH_2), 6.54-6.51 m (1H), 7.21 s (2H), 7.51-7.47 d (2H, *J* = 8.1 Hz), 7.65 s (1H), 7.94-7.87 m (3H), 8.24 s (1H), 12.79 brs (1H, NH). Anal. Calcd for $C_{20}H_{15}BrN_8O_2S$: C, 46.98; H, 2.96; N, 21.91; S, 6.27. *M* 511. Found: C, 46.99; H, 2.98; N, 21.89; S, 6.26%.

***N*-[4-(2-Methoxyphenyl)thiazol-2-yl]-2-{4-[(3-oxo-[1,2,4]triazolo[4,3-a]pyridin-2(3*H*)-yl)methyl]-1*H*-1,2,3-triazol-1-yl}acetamide, 8i**: Yield 87%. White Solid. m.p.138-141°C. IR (KBr): 648, 686, 749, 778, 819, 926, 964, 992, 1056, 1144, 1173, 1287, 1327, 1368, 1416, 1449, 1486, 1536, 1569, 1608, 1738, 2214, 2813, 2890, 2980, 3040, 3108, 3635, 3730, 3767, 3801, 3888, 3934 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): δ 3.83 s (3H, OCH_3), 4.89 s (2H, CH_2), 5.63 s (2H, CH_2), 6.61-6.65 m (1H), 7.25 s (2H), 7.51-7.46 (2H, *J* = 8.3 Hz), 7.65 s (1H), 8.05-7.99 m (3H), 8.15 s (1H), 12.81 brs (1H, NH). Anal. Calcd for $C_{21}H_{18}N_8O_3S$: C, 54.54; H, 3.92; N, 24.23; S, 6.93. *M* 462. Found: C, 54.52; H, 3.94; N, 24.25; S, 7.90%.

***N*-[4-(2-Nitrophenyl)thiazol-2-yl]-2-{4-[(3-oxo-[1,2,4]triazolo[4,3-a]pyridin-2(3*H*)-yl)methyl]-1*H*-1,2,3-triazol-1-yl}acetamide, 8j**: Yield 77%. White Solid. m.p.159-162°C. IR (KBr): 648, 686, 749, 778, 819, 926, 964, 992, 1056, 1144, 1173, 1287, 1327, 1368, 1416, 1449, 1486, 1536, 1569, 1608, 1738, 2214, 2813, 2890, 2980, 3040, 3108, 3635, 3730, 3767, 3801, 3888, 3934 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): δ 4.93 s (2H, CH_2), 5.51 s (2H, CH_2), 6.28 s (1H), 7.21 s (5H), 7.53 s (1H), 7.75-7.71 d (2H), 7.87-7.83 d (1H, *J* = 8.4 Hz), 8.54 s (1H), 12.81 brs (1H, NH). Anal. Calcd for $C_{20}H_{15}N_9O_4S$: C, 50.31; H, 3.17; N, 26.40; S, 6.72. *M* 477. Found: C, 50.32; H, 3.18; N, 26.38; S, 6.69%.

Molecular Docking

Ligand and Macromolecules Preparation

Synthesized structure files created from ChemDraw 20.1.1. Created structures were converted into PDB files using Avogadro 1.2.0.[26] The bacterial protein structures Crystal structure of esterase A from Streptococcus pyogenes (4ROT.pdb)[27], the Crystal structure of S. aureus (1JIJ.pdb)[28], the Crystal Structure of Candida albicans N-myristoyl-transferase (1IYL.pdb)[29], Crystal Structure of E. coli 24kDa Domain (1KZN.pdb)[30], Crystal structure of PqsR co-inducer binding domain of Pseudomonas aeruginosa (4JVI.pdb)[31], Crystal Structure of Aspergillus niger EstA (1UKC.pdb)[32] and Crystal Structure of Aspergillus clavatus Sph3 (5C5G.pdb)[33] were retrieved from RCSB Protein Data Bank (https://www.rcsb.org/).

Active Site Prediction

The COVID proteins' active site was identified using the online tool Computed Atlas for Surface Topography of Proteins (CASTp)[34].

Molecular docking

PyMOL version 2.3.3 (The PyMOL Molecular Graphics System, Version 2.0 Schrödinger, LLC) was used to optimize the ligand and protein. Using Biovia Discovery Studio 4.5 (Biovia, D.S 2019), the water molecule was removed, and polar hydrogens were introduced for protein optimization. PyRx was used to conduct the site-specific docking[35], Biovia Discovery Studio 4.5 was used to analyse the docking results.

In silico evaluations

In silico analysis was accomplished to study the binding affinity and way of interaction of the newly prepared triazoles with the active site of target protein enzymes. In this study, various bacterial (Pdb:1JIJ, Pdb:4ROT, Pdb:1KZN, Pdb:4JVI) and fungal (Pdb:5C5G, Pdb:1IYL, Pdb:1UKC) protein enzyme has been chosen as analysis target for the identification of antibacterial and anti-fungal molecules using docking study. The obtained results for the binding energy are depicted in Table 1.

Table 1 — Binding energy (site-specific docking) in Kcal/mol

Compd	ProteinPdb: 1JIJ	ProteinPdb: 4ROT	Protein Pdb: 1KZN	Protein Pdb: 4JVI	Protein Pdb: 5C5G	Protein Pdb: 1IYL	Protein Pdb: UKC
8a	–10.5	–7.3	–8.9	–10.1	–8.3	–10.1	–7.6
8b	–10.7	–7.2	–8.6	–9.1	–8.2	–10.2	–7.4
8c	–10.8	–7.3	–8.4	–10.0	–8.1	–10.1	–7.4
8d	–10.5	–7.0	–8.4	–9.2	–8.0	–10.1	–7.2
8e	–10.4	–7.1	–8.5	–9.1	–7.8	–10.7	–7.3
8f	–10.5	–7.0	–8.7	–9.5	–8.4	–11.1	–7.4
8g	–10.4	–7.0	–8.6	–9.2	–7.8	–11.0	–7.3
8h	–10.6	–7.2	–8.6	–9.5	–8.1	–11.1	–7.1
8i	–10.4	–6.9	–8.3	–9.2	–7.7	–10.5	–6.9
8j	–10.7	–6.7	–8.5	–9.3	–7.8	–11.2	–7.1

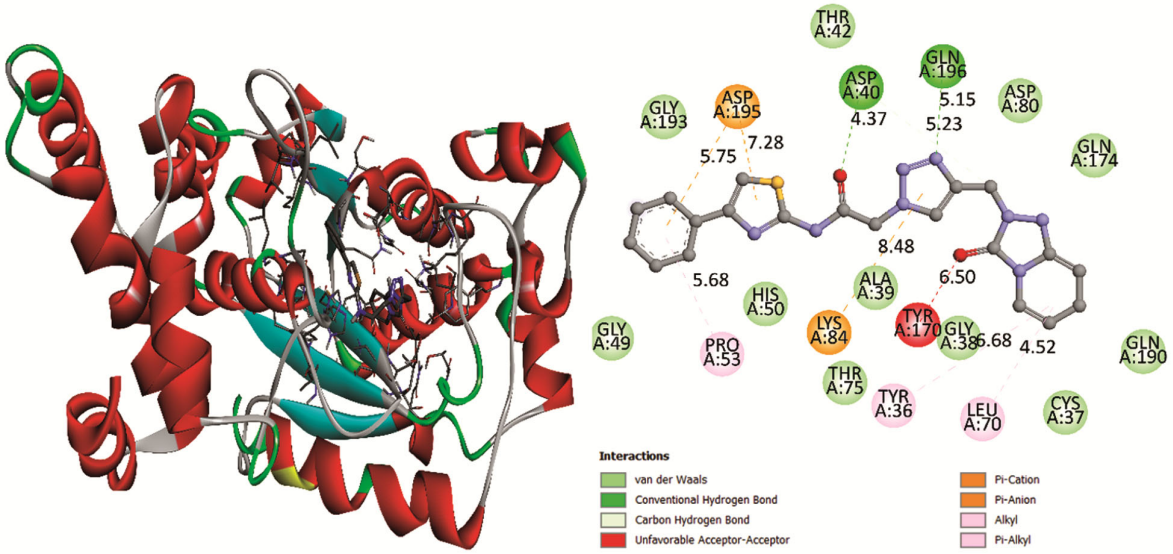


Fig. 2 — 3D and 2D Interaction of **8c** with 1JIJ

Compounds against 1JIJ

Site-specific Docking research (X: -10.949593, Y: 12.537570, Z: 84.757183) found that on the target protein 1JIJ, all compounds examined had negative binding energy. The compound **8c** had the highest binding energy of -10.8 kcal/mol with site-specific docking. **8c** formed strong bonds with 1JIJ were Hydrogen bonds ASP:40 at 4.37 Å, GLN:196 at 5.15 Å. Pi bonds LYS:84 at 8.48 Å, ASP:195 at 5.75 Å and 7.28 Å (Fig. 2).

Compounds against 4ROT

Site-specific Docking research (X: -4.025250, Y: 31.469568, Z: 8.0508a) found that on the target protein 4ROT, all compounds examined had negative binding energy. The compound **8a** and **8c** had the highest binding energy of –7.3 kcal/mol with site-specific docking. **8a** formed strong bonds with 4ROT were Hydrogen bonds TYR:141 at 5.69 Å, TYR:254 at 4.99 Å, Pi bonds GLU:248 at 5.82 Å, PRO:251 at 5.25 Å. and **8c** formed strong bonds with 4ROT were Hydrogen bonds TYR:141 at 5.77 Å, TYR:254 at 4.99 Å, Pi bonds GLU:248 at 5.72 Å, PRO:251 at 5.23 Å (Fig. 3, Fig. 4).

Compounds against 1KZN

Site-specific Docking research (X: 18.849850, Y: 23.905305, Z: 36.008089) found that on the target protein 1KZN, all compounds examined had negative binding energy. The compound **8a** had the highest binding energy of -8.9 kcal/mol with site-specific docking. **8a** formed strong bonds with 1KZN were Hydrogen bonds GLU:50 at 3.35 Å, Pi bonds GLU:50 at 4.24 Å, ASP:49 at 5.85 Å (Fig. 5).

Compounds against 4JVI

Site-specific Docking research (X: -30.449480, Y: 58.642526, Z: 10.595528) found that on the target protein 4JVI, all compounds examined had negative binding energy. The compound **8a** had the highest

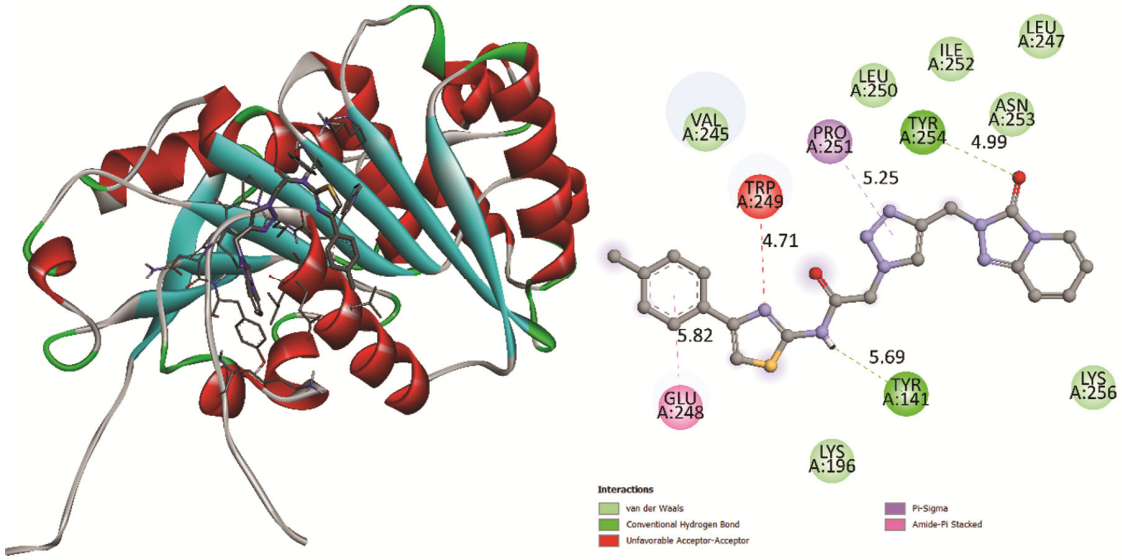


Fig. 3 — 3D and 2D Interaction of **8a** with 4ROT

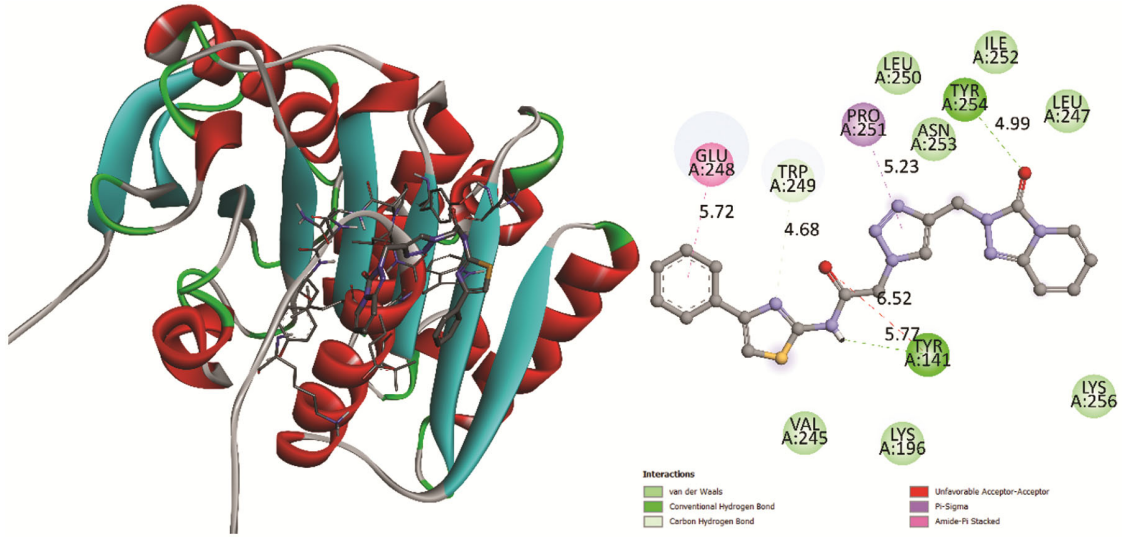


Fig. 4 — 3D and 2D Interaction of **8c** with 4ROT

binding energy of -10.1 kcal/mol with site-specific docking. **8a** formed strong bonds with 4JVI were Hydrogen bonds LEU:208 at 4.33 Å, ARG:209 at 6.35 Å, TYR:258 at 4.58 Å and 4.73 Å, Pi bonds ILE:236 at 4.33 Å, TYR:258 at 4.58 Å and 4.73 Å (Fig. 6).

Compounds against 5C5G

Site-specific Docking research (X: 33.533816, Y: 40.877303, Z: 59.161684) found that on the target protein 5C5G, all compounds examined had negative binding energy. The compound **8f** had the highest binding energy of -8.4 kcal/mol with site-specific docking. **8f** formed strong bonds with 5C5G were Hydrogen bonds ASN:93 at 5.29 Å, SER:96 at 3.35 Å, TYR:126 at 5.87 Å, Pi bonds ASP:166 at 6.58 Å, GLU:222 at 5.79 Å and 6.14 Å, ASP:286 at 6.09 Å (Fig. 7).

Compounds against 1IYL

Site-specific Docking research (X: 12.9642887415, Y: 47.7911458235, Z: -0.516956407189) found that on the target protein 1IYL, all compounds examined had negative binding energy. The compound **8j** had the highest binding energy of -11.2 kcal/mol with site-specific docking. **8j** formed strong bonds with 1IYL were Hydrogen bonds TYR:335 at 5.97 Å, Pi bonds TYR:225 at 5.54 Å, PHE:240 at 7.57 and TYR:354 at 5.55 Å (Fig. 8).

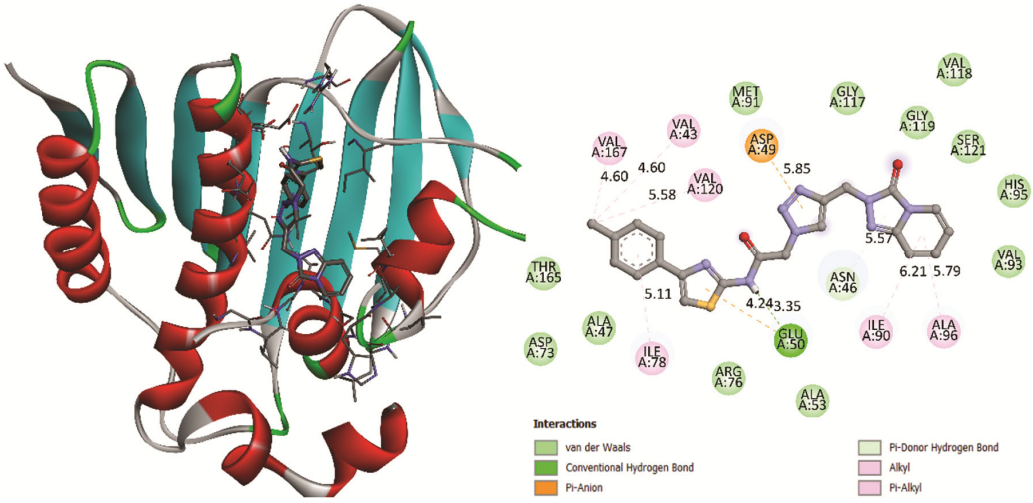


Fig. 5 — 3D and 2D Interaction of **8a** with 1KZN

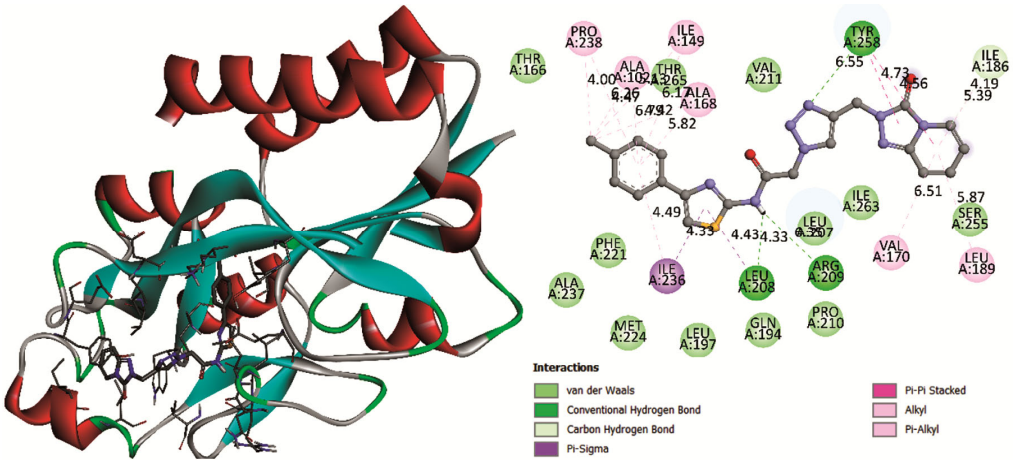


Fig. 6 — 3D and 2D Interaction of **8a** with 4JVI

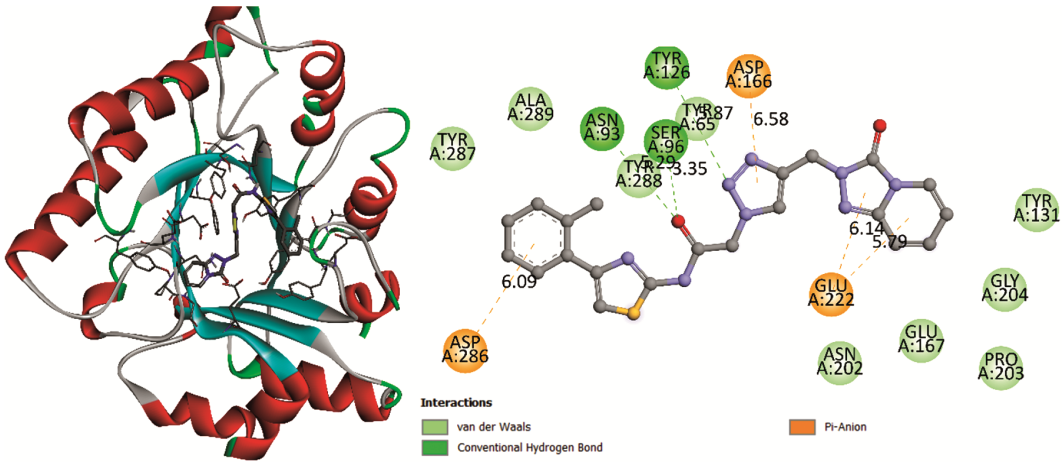


Fig. 7 — 3D and 2D Interaction of **8f** with 5C5G

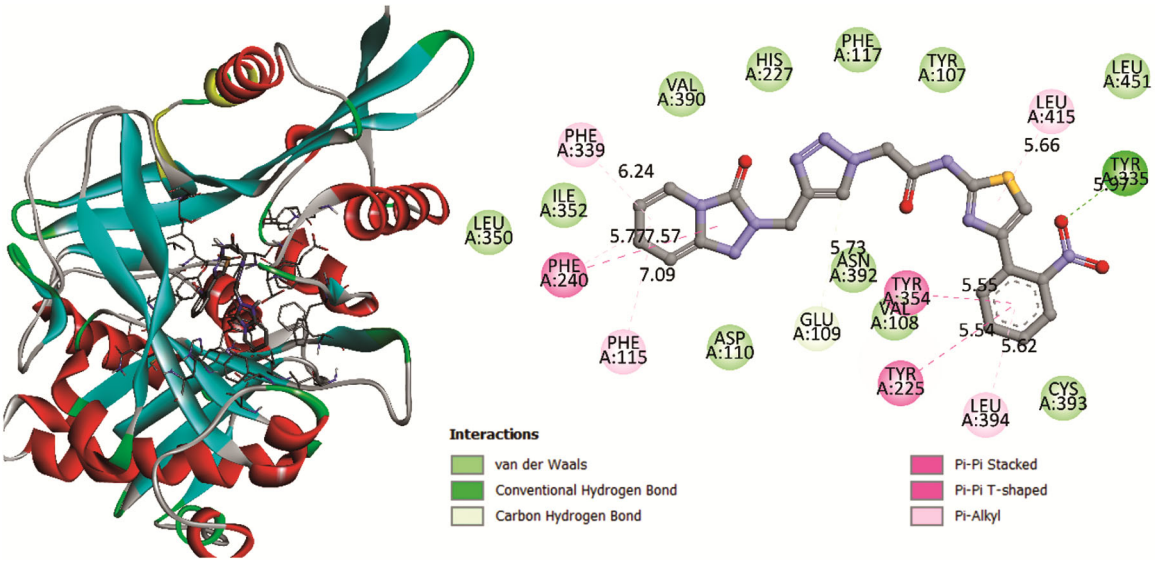


Fig. 8 — 3D and 2D Interaction of **8j** with 1IYL

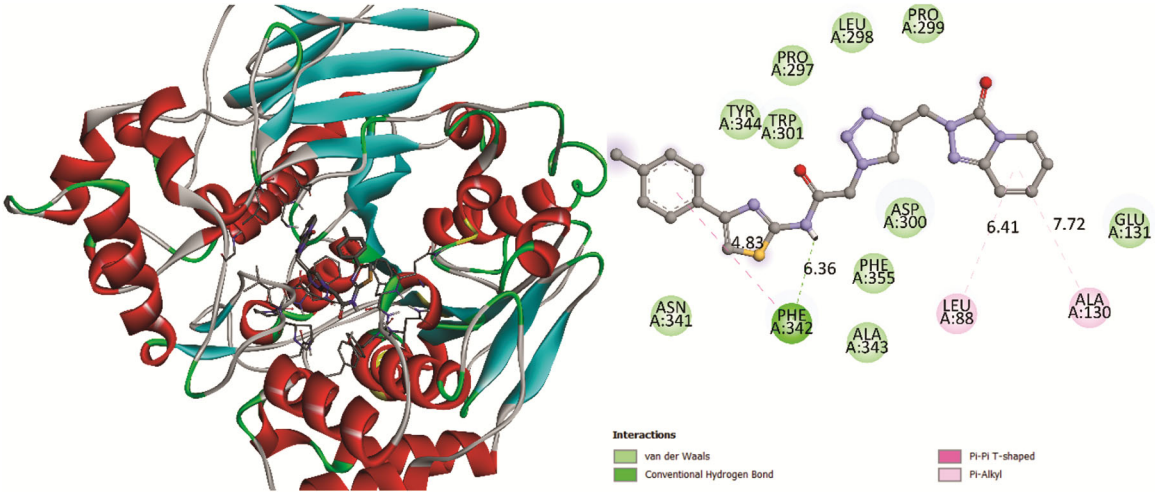


Fig. 9 — 3D and 2D Interaction of **8a** with 1UKC

Compounds against 1UKC

Site-specific Docking research (X: -11.6594164098, Y: 53.6473705922, Z: -6.36819134173) found that on the target protein 1UKC, all compounds examined had a negative binding energy. The compound **8a** had the highest binding energy of -7.6 kcal/mol with site-specific docking. **8a** formed strong bonds with 1UKC were Hydrogen bonds PHE:342 at 6.36 Å, Pi bonds PHE:342 at 4.83 Å, LEU:88 at 6.41 Å, ALA:130 at 7.72 Å (Fig. 9).

Biological activity

Synthesized compounds **8a-j** were evaluated for *in vitro* anti-bacterial and anti-fungal activity against four pathogenic bacteria (*e.g.*, *E. coli, P. aeruginosa, S. aureus,* and *S. pyogenus*, Table 2) and three pathogenic fungi (*e.g.*, *C. albicans, A. niger* and *A. clavatus*, Table 3) using Mueller Hinton Broth and Sabouraud Dextrose Agar as nutritional medium respectively using Broth Dilution Method. To sterilize the growth media, it was autoclaved at 120°C for half an hour and allowed to cool at RT. The medium was mixed with the dimethyl sulphoxide (DMSO) solution of compound **8a-j**. The stain was incubated for 72 hr at 37°C temperature. The inoculum size for test strain was adjusted to 108 CFU (Colony Forming Unit) per mL by comparing turbidity and screened for the activity, Pure DMSO solvent was mixed with media and could serve as blank control. For the anti-bacterial activity, commertially available drugs Gentamycin,

Table 2 — Antibacterial activity

Sr. No.	Compd	Minimal Inhibition Concentration [microgram/mL]			
		E. coli MTCC 443	*P. aeruginosa* MTCC 1688	*S. aureus* MTCC 96	*S. pyogenus* MTCC 442
1	**8a**	50	25	62.5	50
2	**8b**	75	100	62.5	100
3	**8c**	100	25	50	50
4	**8d**	75	100	100	250
5	**8e**	62.5	100	250	250
6	**8f**	62.5	75	250	>250
7	**8g**	75	100	250	100
8	**8h**	100	75	100	100
9	**8i**	100	75	100	>250
10	**8j**	100	100	62.5	>250
11	Gentamycin	0.05	1	0.25	0.5
12	Ampicillin	100	100	250	100
13	Chloramphenicol	50	50	50	50
14	Ciprofloxacin	25	25	50	50

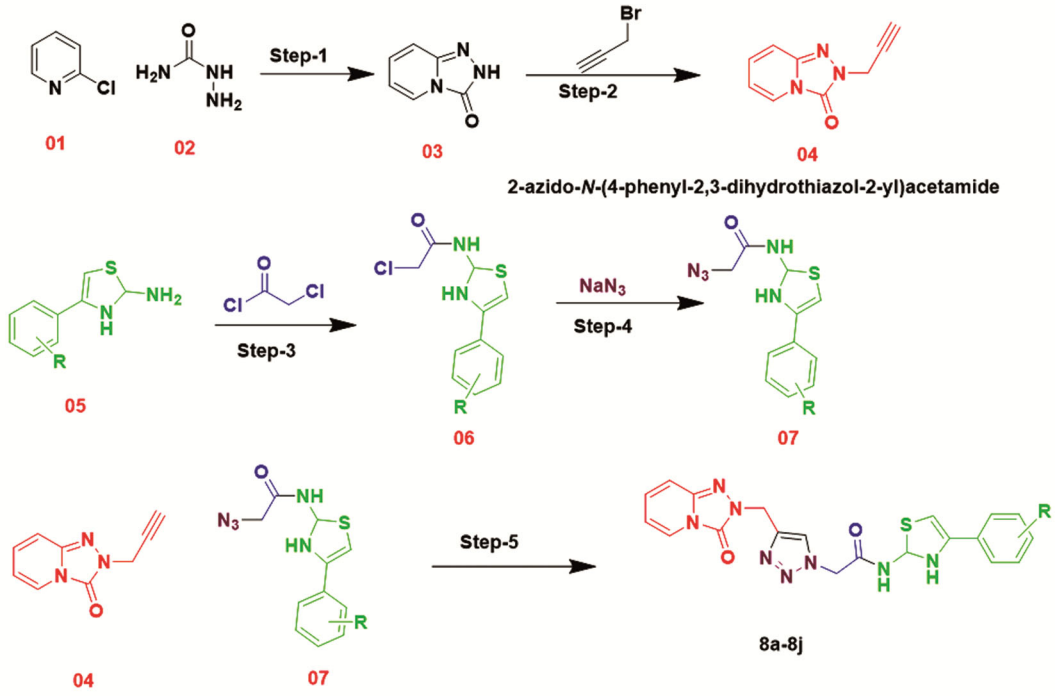
Table 3 — Antifungal activity

Sr. No.	Compd	Minimal fungicidal concentration [microgram/mL]		
		C. abicans MTCC 227	*A. niger* MTCC 282	*A. clavatus* MTCC 1383
1	**8a**	250	1000	1000
2	**8b**	500	1000	1000
3	**8c**	500	1000	>1000
4	**8d**	1000	1000	>1000
5	**8e**	1000	750	>1000
6	**8f**	1000	250	1000
7	**8g**	1000	500	>1000
8	**8h**	750	250	1000
9	**8i**	1000	750	>1000
10	**8j**	1000	250	>1000
11	Nystatin	100	100	100
12	Greseofulvin	500	100	100

Ampicillin, Chloramphenicol, and Ciprofloxacin were used as the positive control. Commercially available drugs Nystatin and Greseofulvin were used as positive control/reference standards for anti-fungal activity. For the activity, two-stage screening was done, primary and secondary screening.

In primary Screening, 1000 µg/mL, 500 µg/mL, and 250 µg/mL concentrations of the synthesized compounds were taken. From these screenings, the compounds found active were further tested in the second set of dilutions against all microorganisms. The secondary screening was carried out with only the compounds found active in primary screening. The stock solutions were similarly diluted to obtain 200 µg /mL 100 µg /mL, 50 µg /mL, 25 µg /mL, 12.5 µg /mL, and 6.25 µg /mL concentrations. The highest dilution, which displayed at least 99% inhibition zone, is considered as a MIC for both activities.

Antimicrobial activity is defined as the act of eradicating or restraining disease-causing pathogenic microbes[36]. Antimicrobial agents are substances that either kill or prevent the growth of microorganisms[37]. Antimicrobial compounds can be antiviral, antifungal, or antibacterial. There are microorganism-killing agents available. Microbicides are microbes that inhibit the growth of other microbes, whereas microbistatic microbes do the opposite[38,39]. To combat infections, the remedies employ various modes of action. The use of antimicrobial medications to treat an infection is referred to as antimicrobial chemotherapy. The agar



Scheme 1 — Synthetic procedure for the compounds **8a-j**

cup method is a widely used method for testing antibacterial and antifungal activity, and it was used in our previous study. The *in vitro* antimicrobial activity of the entire compound synthesized was assessed against Gram +*ve* (*S. Aureus, S. Pyogenus*) and Gram -*ve* (*E. Coli, P. Aeruginosa*), and fungi spp. (*C. Albicans, A. niger, A. Clavatus*) for a period of 24 to 48 hours using streptomycin, ciprofloxacin, and Fresh bacterial and fungal cultures were created and cultivated in N-broth and potato dextrose broth, respectively. All bacterial and fungal suspensions were evenly distributed onto sterile Muller-Hinton and PDA plates using sterile swabs. Small (1 cm) wells were made in the plates with a clean corkscrew. Standard antibiotics were dissolved in sterile distilled water to achieve a final concentration of 200 g/mL. The synthetic compounds were dissolved in DMSO to a final concentration of 1 mg/mL before being added to the well in 0.1 mL. The plate was incubated at 4°C for 20 minutes to ensure adequate compound diffusion on agar. The plates were then incubated for 48 hours at 25°C for fungal cultures and 24 hours at 37°C for bacterial cultures in the upper position. There was also DMSO-control activity. Following incubation, the MIC was examined and measured.

The antibacterial potential of newly synthesized substances was investigated by *Invitro*. When compared to conventional medicines, the Bio-Assay results confirmed that compounds (**8a-j**) were successful in indicating excellent inclination toward well-known microorganisms.

Result and Discussions

Scheme 1 shows the synthesis process for the chosen compounds **(8a to 8j).** 2*H*-[1,2,4] Triazolo[4,3-a] pyridin-3-one (03) was produced in the first phase of the synthesis by the reaction of 2-choloropyridine (01) with semicarbazide (02) in ethoxyethanol solvent. In the subsequent step, (03) reacts with 3-bromopropyne in acetone to create 2-Prop-2-ynyl-2*H*-[1,2,4]triazolo[4,3-a]pyridin-3-one (04). On the other hand, substituted 2-chloro-*N*-(4-phenyl-thiazol-2-yl)-acetamide (06) was prepared by the reaction between substituted-4-Phenyl-thiazol-2-ylamine (05) and chloro-acetyl chloride in presence of acetone. Followed by (06) was reacted with sodium azide in DMF solvent. Which gives rise to the formation of 2-Azido-*N*-(4-phenyl-thiazol-2-yl)-acetamide (07). In the final phase of the process, (04) reacts with previously produced (07) to create the

required heterocyclic molecule (substituted phenyl)-2-[4-(3-oxo-[1,2,4]triazolo[4,3-a]pyridin-2-ylmethyl)-[1,2,3] triazol-1-yl]-*N*-(4-phenyl-thiazol-2-yl)-acetamide. On silica gel-G plates (G60 F254 (Merck) of 0.5 mm thickness, reactions were observed using thin-layer chromatography (TLC), and visualization was completed using ultraviolet light (254 and 365 nm). The uncorrected melting points were determined using an open capillary method.

Conclusion

Demonstration of the synthesis, Characterization, *in vitro* and *in silico* analysis of substituted 2-[4-(3-Oxo-[1,2,4]triazolo[4,3-a]pyridin-2-ylmethyl) [1,2,3] triazol-1-yl]-*N*-(4-phenylthiazol-2-yl)-acetamide has been shown successfully. A total of ten compounds were synthesized and compounds were thoroughly characterized using various spectroscopic techniques. All of the spectroscopic method-based shapes have been completely synthesized and displayed. **8a** and **8c** were found to have potent antibacterial activity against Gram +*ve* (*S. Aureus, S. Pyogenus*) and Gram -*ve* (*E. Coli, P. Aeruginosa*). All synthesized compounds were screened for *in silico* analysis, which also confirmed that **8a** and **8c** were found to have potent antibacterial activity. As a result, similar research could be conducted, MIC could be identified, and such compounds could be similarly investigated and used as a potent drug in the future.

Overall, the response was simple, and the goods were obtained in excellent yields with no additional product improvement or purification required. We synthesized a total of ten compounds, all of which displayed their shape completely using the spectroscopic method. The gift attempt is massive in terms of the synthesis of a wide range of novel entities.

Supplementary Information

Supplementary information is available in the website http://nopr.niscpr.res.in/handle/123456789/58776. The Supplementary Information contains FT-IR, ^{1}H NMR and mass fragmentation profiles.

Conflicts of Interest

There are no conflicts of interest among the authors.

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