Section A-Research paper

Novel triazole derivatives' design, synthesis, characterisation, molecular docking, and in vitro antimicrobial activity of N-(4-(3-oxomorpholino) phenyl)-2-(4-((phenylamino)methyl)-1h-1,2,3-triazol-1-yl) acetamide.

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ABSTRACT:

The novel collection of triazole derivatives has been synthesized, characterized through spectroscopic methods (Infra-Red, Proton NMR, and Mass Spectroscopy). The biological activity and MIC (Minimal Inhibitory Concentration) of this as synthesized compound N-(4-(3-oxomorpholino)phenyl)-2-(4-((phenylamino)methyl)-1H-1,2,3-triazol-1-yl)acetamide **(HP1-HP10)** have been screened against gram-negative pathogens (2) and fungi (3). HP03 and HP07 have potential action against gram-negative bacteria as well as fungus strains. And molecular docking studies site specific of all the ten tetrazole moieties done through PyRx shown the compound HP03 and HP07 have good binding affinity against PDB: 1KZN,1UKC,1IYL,4JVI,5C5G.

KEYWORDS: *triazole derivative, click-chemistry, acetamide, molecular-docking & antimicrobial pursuit.*

Specifications Table

Subject area	*Organic Chemistry, Computational Chemistry, Bio Chemistry*
Compounds	*Triazole*
Data category	*Spectral, synthesized, computational simulations, molecular docking, etc.*
Data acquisition format	*NMR, IR, Mass spectra*
Data type	*Analyzed*
Procedure	*Copper sulphate catalyzed synthesis triazole derivatives by click*

	chemistry
Data accessibility	*Manuscript and supplementary data enclosed with this article*

1. Introduction

Triazoles fall under the heterocyclic compound group and have a 5-membered ring made up of 3 nitrogen atoms and 4 carbon atoms. In the region where hydrogen and nitrogen connect, the 1,2,3-triazole and 1,2,4-triazole tautomeric bureaucracy protrudes. Triazole homes have been used in a variety of ways for years. Plant safety products include epoxiconazole, propiconazole, and derivatives of the triazole family [1].

Plant growth inhibitors include paclobutrazol and uniconazole [2]. A few triazole compounds, including benzotriazole and naphthotriazole, have been used to prevent copper from corroding [3]. Triazole activity has also been used in biochemistry and medicine. A brassinosteroid biosynthesis inhibitor is brassinazole [4]. Triazole compounds have a very significant organic impact, as evidenced by studies. They have antibacterial [5], fungicidal [6,7], antiviral [8], antiproliferative [9,10], cytotoxic and antitumor [11], anti-leishmanial [12], anti-tubercular [13], anti-inflammatory and analgesic [14], anticonvulsant [15], anti-obesity [16], and antimalarial [17] activity. Studies on triazoles as Alzheimer's disease inhibitors have also produced positive results [18]. Fluconazole, Itraconazole, Voriconazole, and Posaconazole are indexed substances that are frequently found in antifungal medications among the triazole derivatives [19]. People with lowered immunity, malnutrition, the elderly, and alcohol addicts are particularly susceptible to this condition.

The extraordinary infectiousness of tuberculosis is a significant factor in its treatment, which is why triazole derivatives' anti-tuberculosis properties are currently the subject of in-depth research. Mycobacterium tuberculosis, Mycobacterium bovis, and Mycobacterium africanum—commonly cause the tuberculosis definitive process. The triazole compounds have unique anti-tuberculosis activity, it turns out [20,21]. Our lab has conducted additional triazole-related research [22, 23]. Additional structural alterations to those derivatives may also lead to a surge in anti-tuberculosis medications.

On the basis of all of this knowledge, attempt to begin designing a novel triazole moiety of biologically effective. This research discusses the synthesis of these new triazoles, their molecular docking investigations, and their in vitro anti-bacterial and anti-fungal capabilities.

Experimental

1.1 Material and Characterizations

Regarding air or moisture-touchy compounds, all chemicals were procured from Spectro-chem (Mumbai), Sigma-Aldrich (Mumbai), Loba-Chemie, and Merck-India. Syringes were used to changeover solutions, and oven-dried glassware was used throughout. Reaction progress was confirmed by TLC (thin-layer chromatography) using 20x20 cm aluminum plates coated in silica gel 60 F254 procured from Merck. Melting factors had been measured using an open capillary approach and the melting factor equipment (uncorrected). Excess solvents were evaporated by using BUCHI rotary evaporator. IR spectrum were captured using the DRS prob., which is represented in ν, on the FTIR-8400 spectrometer (/cm). GCMS-QP-2010 Shimadzu make was used to obtain the products' mass fragmentation. NMR was recorded by digesting the sample in CDCl3/DMSO-d6 (in a 3/1 ratio) or DMSO-d6 with the help of Bruker AVANCE II 400 MHz. Chemical shifts (ppm) were expressed downfield from tetramethyl silane (TMS), which was utilised as an inner reference, in ppm (components per million). Unique splitting style were observed: singlet (s), doublet (d), triplet (t), quadruplet (q), multiplats (m), extensive singlet (brs), and double doublet (dd).

2.1.1. Preparation of *2-chloro-N-(4-(3-oxomorpholino) phenyl) acetamide* (INT-2).

A dropwise addition of 1 eq of chloroacetyl chloride to a solution of 1 eq of substituted amine in acetone was made, and the resulting mixture was stirred at room temperature for 15 minutes. In order to separate the reaction mixture into a stable intermediate product that has been filtered and washed with water, the reaction liquid was then put onto crushed ice. Dry it without purification and utilise it in the subsequent step.

2.1.2. Preparation of *2-azido-N-(4-(3-oxomorpholino) phenyl) acetamide* (INT-3).

A 0.1 mmol. solution of INT-2 in DMF was mixed with sodium azide (NaN3) (0.3 mmol). The ensuing combination was stirred at room temperature for twenty-four hours after the complete reaction mixture had been combined before being immediately poured over crushed ice. With a filter, products dried and separated.

2.1.3. Preparation of *N-(prop-2-yn-1-yl) aniline* (INT-4).

150ml of acetone were replaced with 50mmol of aniline, and 100mmol of anhydrous K2CO3 were added while stirring. Propargyl bromide (55mmol) began to gently charge

after five to ten minutes. Reflux after the addition for three hours. Monitored on TLC was the new reaction. The reaction mass was put into the crushed ice after the reaction's conclusion, which was verified by TLC. The separated product should be filtered, water-rinsed, and dried.

2.1.4. Preparation of substitute *N-(4-(3-oxomorpholino) phenyl)-2-(4-((phenylamino) methyl)-1H-1,2,3-triazol-1-yl) acetamide (**HP1-HP10**)*

The addition of a catalytic amount of sodium ascorbate and copper sulphate pentahydrate transformed INT-3 and INT-4 into brought at RT in the RBF containing DMF: H2O: n-butanol (1:1:1), DMF: H2O: INT-3 (1 eq), and INT-4 (1 eq). After the reaction has finished, aggregate was poured into the overburdened ace to clear out the separated product. Stir the resulting solution for RT for twenty-four hours. Was with diluted ammonia and once more cleared the product.

2.1.5. *2-(4-(((4-chlorophenyl) amino) methyl)-1H-1,2,3-triazol-1-yl)-N-(4-(3-oxomorpholino) phenyl) acetamide (HP-1)*

Yellow Solid, Rf value 0.40 (Ethyl acetate 8: Hexane 2) IR (KBr pallet) in cm^{-1}: 3279, 3076, 2864, 1712, 1650, 1551, 1483, 1372, 1256, 1154, 1061, 853, 781, 692. ^{1}H NMR (DMSO) in δ ppm: 6.30 to 7.57 (Complex, 9H), 10.51 (Singlet, 1H), 7.97-7.98 (Singlet, 1H), 3.69 (Singlet, 2H), 3.94 (Singlet, 2H), 4.18 (Singlet, 2H), 4.30 (Singlet, 2H), 5.29 (Singlet, 2H). ^{13}C NMR (DEPT):(75 MHz, $CDCl_3$); 38.39, 49.00, 52.11, 63.48, 67.71, 113.69, 119.23, 119.50, 124.43, 126.00, 128.54, 136.54, 137.17, 147.30, 164.30, 165.96. Mass (m/z): 440 (M^+), Ana. Calculated for Molecular formula $C_{21}H_{21}ClN_6O_3$ is C; 57.21%, H; 4.80%, N; 19.06% Found C; 57.25%, H; 4.85%, N; 19.09%.

2.1.6. *2-(4-(((4-bromophenyl) amino) methyl)-1H-1,2,3-triazol-1-yl)-N-(4-(3-oxomorpholino) phenyl) acetamide (HP-2)*

White Solid, Rf value 0.43 (Ethyl acetate 8: Hexane 2), IR (KBr pallet) in cm^{-1}: 3399, 3258, 2910,1692, 1599, 1484, 1367, 1238, 1159, 1121, 875, 820, 751. ^{1}H NMR (DMSO) in δ ppm: 6.35 to 7.59 (Complex, 9H), 10.51 (Singlet, 1H), 7.97 (Singlet, 1H), 3.69 (Singlet, 2H), 3.94-3.95 (Singlet, 2H), 4.18 (Singlet, 2H), 4.29-4.31 (Singlet, 2H), 5.29 (Singlet, 2H). ^{13}C NMR (DEPT):(75 MHz, $CDCl_3$); 38.29, 48.99, 52.10, 63.47, 67.71, 106.61, 114.26, 119.48, 124.46, 125.98, 131.34, 136.54, 137.16, 147.64, 164.29,

165.93. Mass (m/z): 485 (M^+), Ana. Calculated for Molecular formula $C_{21}H_{21}BrN_6O_3$ is C; 51.97%, H; 4.36%, N; 17.32% Found C; 51.95%, H; 4.30%, N; 17.31%

2.1.7. *2-(4-(((4-nitrophenyl) amino) methyl)-1H-1,2,3-triazol-1-yl)-N-(4-(3-oxomorpholino) phenyl) acetamide (HP-3)*

White Solid, Rf value 0.42 (Ethyl acetate 8: Hexane 2), IR (KBr pallet) in cm^{-1}: 3398, 3257, 2911,1692, 1598, 1485, 1367, 1237, 1151, 1122, 876, 820, 752. ^{1}H NMR (DMSO) in δ ppm: 6.30 to 7.58 (Complex, 9H), 10.50 (Singlet, 1H), 7.96 (Singlet, 1H), 3.68 (Singlet, 2H), 3.94-3.96 (Singlet, 2H), 4.17 (Singlet, 2H), 4.29-4.30 (Singlet, 2H), 5.28 (Singlet, 2H). ^{13}C NMR (DEPT):(75 MHz, $CDCl_3$); 38.41, 48.96, 52.31, 63.57, 67.75, 114.36, 119.23, 119.55, 124.43, 126.10, 128.55, 136.23, 137.27, 147.35, 164.40, 165.86. Mass (m/z): 451 (M^+), Ana. Calculated for Molecular formula $C_{21}H_{21}BN_7O_5$ is C; 55.87%, H; 4.79%, N; 21.72% Found C; 55.86%, H; 4.78%, N; 21.70%

2.1.8. *N-(4-(3-oxomorpholino) phenyl)-2-(4-((phenylamino)methyl)-1H-1,2,3-triazol-1-yl) acetamide (HP-4)*

White Solid, Rf value 0.40 (Ethyl acetate 8: Hexane 2), IR (KBr pallet) in cm^{-1}: 3397, 3157, 2811, 1691, 1597, 1484, 1366, 1236, 1115, 1120.26, 876, 821, 750. ^{1}H NMR (DMSO) in δ ppm: 6.20 to 7.52 (Complex, 10H), 10.51 (Singlet, 1H), 7.95 (Singlet, 1H), 3.68 (Singlet, 2H), 3.94-3.95 (Singlet, 2H), 4.18 (Singlet, 2H), 4.29-4.31 (Singlet, 2H), 5.28 (Singlet, 2H). ^{13}C NMR (DEPT):(75 MHz, $CDCl_3$); 38.29, 48.99, 52.10, 63.47, 67.71, 106.61, 114.26, 119.48, 124.46, 125.98, 131.44, 136.75, 137.16, 147.74, 164.31, 165.60. Mass (m/z): 406 (M^+), Ana. Calculated for Molecular formula $C_{21}H_{22}N_6O_3$ is C; 62.06%, H; 5.46%, N; 20.68% Found C; 62.02%, H; 5.45%, N; 20.60% 2.1.9. *N-(4-(3-oxomorpholino) phenyl)-2-(4-((p-tolylamino) methyl)-1H-1,2,3-triazol-1-yl) acetamide (HP-5)*

White Solid, Rf value 0.41 (Ethyl acetate 8: Hexane 2), IR (KBr pallet) in cm^{-1}: 3396, 3155, 2711, 1690, 1598, 1485, 1367, 1236, 1115, 1120, 876, 821, 750. ^{1}H NMR (DMSO) in δ ppm: 6.21 to 7.52 (Complex, 9H), 10.51 (Singlet, 1H), 7.95 (Singlet, 1H), 3.68 (Singlet, 2H), 3.94-3.95 (Singlet, 2H), 4.18 (Singlet, 2H), 4.29-4.31 (Singlet, 2H), 5.28 (Singlet, 2H), 1.22 (Singlet, 3H). ^{13}C NMR (DEPT):(75 MHz, $CDCl_3$); 19.51, 37.45, 48.04, 52.15, 63.51, 67.51, 112.87, 119.51, 119.64, 123.54, 127.01, 127.98, 137.21, 137.45, 147.80, 164.03, 165.99. Mass (m/z): 420 (M^+), Ana. Calculated for

Molecular formula $C_{22}H_{24}N_6O_3$ is C; 62.84%, H; 5.75%, N; 19.99% Found C; 62.82%, H; 5.72%, N; 19.98%

2.1.10. *2-(4-(((4-methoxyphenyl)amino)methyl)-1H-1,2,3-triazol-1-yl)-N-(4-(3-oxomorpholino)phenyl)acetamide (HP-6)*

White Solid, Rf value 0.43 (Ethyl acetate 8: Hexane 2) IR (KBr pallet) in cm^{-1}: 3397, 3154, 2722, 1688, 1597, 1486, 1367, 1236, 1115, 1120, 876, 822, 751. ^1H NMR (DMSO) in δ ppm: 6.22 to 7.52 (complex, 9H), 10.50 (Singlet, 1H), 7.90 (Singlet, 1H), 3.68 (Singlet, 2H), 3.94-3.92 (Singlet, 2H), 4.16 (Singlet, 2H), 4.28-4.31 (Singlet, 2H), 5.27 (Singlet, 2H), 3.22 (Singlet, 3H). ^{13}C NMR (DEPT):(75 MHz, $CDCl_3$); 16.54, 38.31, 48.85, 52.50, 63.45, 67.64, 106.56, 114.52, 119.45, 124.32, 125.78, 131.31, 136.81, 137.15, 147.51, 164.52, 165.89. Mass (m/z): 436 (M^+), Ana. Calculated for Molecular formula $C_{22}H_{24}N_6O_4$ is C; 60.54%, H; 5.54%, N; 19.25% Found C; 60.50%, H; 5.52%, N; 19.22%

2.1.11.*4-(((1-(2-oxo-2-((4-(3-oxomorpholino)phenyl)amino)ethyl)-1H-1,2,3-triazol-4-yl)methyl)amino)benzoic acid (HP-7)*

White Solid, Rf value 0.40 (Ethyl acetate 8: Hexane 2), IR (KBr pallet) in cm^{-1}: 3397, 3155, 2722, 1689, 1598, 1486, 1366, 1236.59, 1151, 1121, 877, 822, 751. ^1H NMR (DMSO) in δ ppm: 6.20 to 7.52 (complex, 9H), 10.02 (Singlet, 1H), 10.52 (Singlet, 1H), 7.91 (Singlet, 1H), 3.67 (Singlet, 2H), 3.94-3.92 (Singlet, 2H), 4.14 (Singlet, 2H), 4.26-4.31 (Singlet, 2H), 5.27 (Singlet, 2H). ^{13}C NMR (DEPT):(75 MHz, $CDCl_3$); 38.41, 49.05, 51.18, 62.45, 67.17, 111.54, 119.12, 119.41, 124.42, 126.57, 127.54, 135.54, 137.15, 146.34, 163.35, 166.96, 178.69. Mass (m/z): 450 (M^+), Ana. Calculated for Molecular formula $C_{22}H_{22}N_6O_5$ is C; 58.66%, H; 4.92%, N; 18.66% Found C; 58.60%, H; 4.90%, N; 18.64%

2.1.12. *methyl4-(((1-(2-oxo-2-((4-(3-oxomorpholino)phenyl)amino)ethyl)-1H-1,2,3-triazol-4-yl)methyl)amino)benzoate (HP-8)*

White Solid, Rf value 0.42 (Ethyl acetate 8: Hexane 2), IR (KBr pallet) in cm^{-1}: 3396, 3155, 2722, 1700, 1597, 1486, 1366, 1235, 1150, 1121, 877, 821, 752. ^1H NMR (DMSO) in δ ppm: 6.22 to 7.52 (complex, 9H), 10.51 (Singlet, 1H), 7.92 (Singlet, 1H), 3.65 (Singlet, 2H), 3.94-3.92 (Singlet, 2H), 4.14 (Singlet, 2H), 4.26-4.32 (Singlet, 2H), 5.26 (Singlet, 2H), 2.92 (Singlet, 3H). Mass (m/z): 464 (M^+), ^{13}C NMR (DEPT):(75 MHz, $CDCl_3$); 17.54, 38.35, 48.74, 52.54, 63.45, 67.12, 106.35, 114.47, 119.48, 124.78,

125.52, 131.41, 136.47, 137.10, 147.01, 164.41, 165.42, 176.78. Ana. Calculated for Molecular formula $C_{23}H_{24}N_6O_5$ is C; 59.48%, H; 5.21%, N; 18.09% Found C; 59.49%, H; 5.22%, N; 18.10%

2.1.13. *2-(4-(((4-hydroxyphenyl)amino)methyl)-1H-1,2,3-triazol-1-yl)-N-(4-(3-oxomorpholino) phenyl)acetamide (HP-9)*

White Solid, Rf value 0.43 (Ethyl acetate 8: Hexane 2) IR (KBr pallet) in cm^{-1}: 3196, 3154, 2720, 1600, 1597, 1486, 1366, 1235, 1150, 1122, 877, 821, 752. ^{1}H NMR (DMSO) in δ ppm: 6.20 to 7.52 (complex, 9H), 10.51 (Singlet, 1H), 7.92 (Singlet, 1H), 3.65 (Singlet, 2H), 3.94-3.90 (Singlet, 2H), 4.15 (Singlet, 2H), 4.26-4.31 (Singlet, 2H), 5.25 (Singlet, 2H), 5.20 (Singlet, 1H), ^{13}C NMR (DEPT):(75 MHz, $CDCl_3$); 39.10, 48.50, 52.33, 63.47, 67.54, 112.79, 118.22, 118.54, 124.47, 125.41, 126.21, 134.47, 138.10, 148.32, 166.12, 167.96. Mass (m/z): 422 (M^+), Ana. Calculated for Molecular formula $C_{21}H_{22}N_6O_4$ is C; 59.71%, H; 5.25%, N; 19.89% Found C; 59.72%, H; 5.26%, N; 19.88%

2.1.14. *2-(4-(((4-acetamidophenyl)amino)methyl)-1H-1,2,3-triazol-1-yl)-N-(4-(3-oxomorpholino) phenyl)acetamide (HP-10)*

White Solid, Rf value 0.42 (Ethyl acetate 8: Hexane 2) IR (KBr pallet) in cm^{-1}: 3296, 3154, 2720, 1650, 1596, 1486, 1366, 1235, 1150, 1121, 876, 821, 750. ^{1}H NMR (DMSO) in δ ppm: 6.20 to 7.55 (complex, 9H), 10.52 (Singlet, 1H), 7.90 (Singlet, 1H), 3.60 (Singlet, 2H), 3.94-3.90 (Singlet, 2H), 4.15 (Singlet, 2H), 4.25-4.31 (Singlet, 2H), 5.26 (Singlet, 2H), 2.98 (Singlet, 3H), 5.56 (Singlet, 1H). ^{13}C NMR (DEPT):(75 MHz, $CDCl_3$); 22.52, 38.31, 48.01, 52.54, 63.10, 67.87, 105.64, 113.22, 118.41, 124.46, 125.74, 130.14, 135.51, 136.17, 148.62, 164.41, 165.54. Mass (m/z): 463 (M^+), Ana. Calculated for Molecular formula $C_{23}H_{25}N_7O_4$ is C; 59.60%, H; 5.54%, N; 21.15% Found C; 59.65%, H; 5.55%, N; 21.19%

2.2 *In silico* molecular docking studies

2.2.1 Ligand preparation.

ligands in 2D. cdx format with the appropriate compounds' structures (**HP1–HP10)** drawn using ChemDraw. All of the ligand's in. sdf format were transformed into.pdb format using Avogadro. UFF functioned as a forcefield to reduce the ligand's energy utilization.

Following energy minimization, all of the ligand molecules were converted. pdb format utilizing Open Babel.

2.2.2. Macromolecule preparation.

Crystal Structure of Candida albicans Myristoyl-CoA: protein N-myristoyl transferase (Nmt) (PDB: 1IYL) with Resolution: 3.20 Å, Aspergillus niger protein EstA (PDB:1UKC) with Resolution: 2.10 Å, Crystal Structure of Aspergillus clavatus Sph3(PDB:5C5G) with Resolution: 1.25 Å, Crystal Structure of E. coli DNA gyrase protein (PDB:1KZN)with Resolution: 2.30 Å, Pseudomonas aeruginosa quorum sensing LysR-type transcriptional regulator (LTTR) protein PqsR (MvfR)(PDB: 4JVI) with Resolution: 2.90 Å were obtained from the (https://www.rcsb.org) and water molecules, ligands, and heteroatoms present in the complex with proteins were removed and collected from the Research Collaboratory for Structural Bioinformatics using Discovery Studio, 2021. The proteins were also stored in .pdb format after the database was deleted.

2.2.3. Active Site Prediction

The online program Computed Atlas for Surface Topography (CASTp) of Proteins was used to determine the active site for each protein.

2.2.4. Ligand-protein docking.

Molecular Docking was performed between ligand (**HP1-HP10**) and protein molecules using PyRx having Auto Dock vina Plugin. The grid box center values for the 1IYL receptor were kept as Site-specific Docking research X: 12.9642887415, Y: 47.7911458235, Z: -0.516956407189. The grid box center values for the 1UKC receptor were kept as Site-specific Docking research X: 11.6594164098, Y: 53.6473705922, Z: -6.36819134173. The grid box center values for the 5C5G receptor were kept as Site-specific Docking research X: 33.533816, Y: 40.877303, Z: 59.161684. The grid box center values for the 1KZN receptor were kept as Site-specific Docking research X: 18.849850, Y: 23.905305, Z: 36.008089. The grid box center values for the receptor were kept as Site-specific Docking research X: -30.449480, Y: 58.642526, Z: 10.595528.

Nine different conformational molecule orientations were achieved by doing dockings while keeping the exhaustiveness parameter at eight. The ligand posture provided the lowest binding energy when docking was complete, and the value of this binding energy in kcal/mol was then chosen for Discovery Studio 2021 to see the interaction between the ligand and protein.

2.3 Antibacterial and Antifungal activity

Two bacterial strains and three fungus strains were used to test the antibacterial activity of synthetic compounds (HP1-HP10) [23,24]. Aspergillus niger (MTCC 282), Candida albicans (MTCC 227), Aspergillus niger (MTCC 1323) and Aspergillus clavatus (MTCC 1323) were used to test the antifungal activity of the same compounds. Ciprofloxacin and norfloxacin (against bacterial strains) and nystatin (against fungal strains) were used as standard reference drugs.

2. Results and Discussion

2.1 Reaction Scheme

The compounds **HP1-HP10** were synthesized as brief in scheme-1

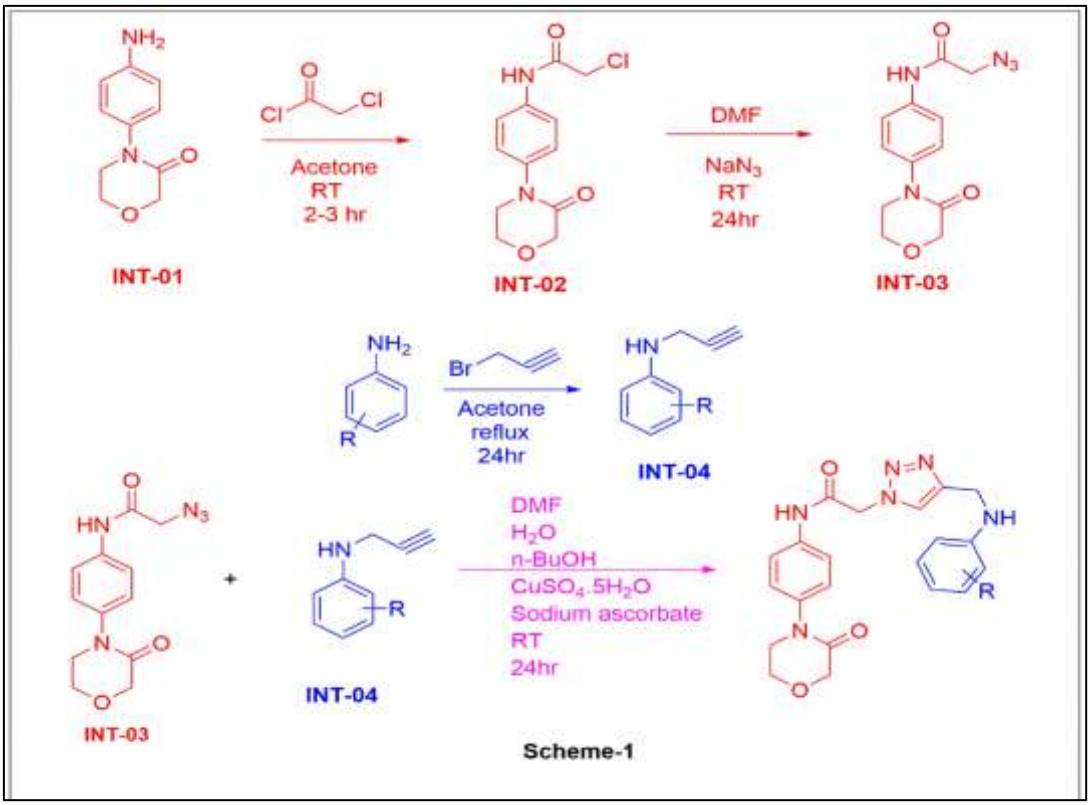


Figure 2: Reaction Scheme

Table.1 Physical constant of synthesized compound (**HP1-HP10**).

Code	Molecular formula	R	Molecular Weight	M.P. ^{0}C	Percentage of Yield
HP-1	$C_{21}H_{21}ClN_6O_3$	-Cl	440	150-152	64
HP-2	$C_{21}H_{21}BrN_6O_3$	-Br	485	178-180	68
HP-3	$C_{21}H_{21}N_7O_5$	-NO_2	451	144-146	54
HP-4	$C_{21}H_{22}N_6O_3$	-H	406	164-166	58
HP-5	$C_{22}H_{24}N_6O_3$	-Me	420	168-170	62
HP-6	$C_{22}H_{24}N_6O_4$	-OMe	436	154-156	54
HP-7	$C_{22}H_{22}N_6O_5$	-COOH	450	182-184	56
HP-8	$C_{23}H_{24}N_6O_5$	-COOR	464	146-148	70
HP-9	$C_{21}H_{22}N_6O_4$	-OH	422	182-184	56
HP-10	$C_{23}H_{25}N_7O_4$	-NHCOMe	463	186-188	58

3.2 Anti-microbial activity

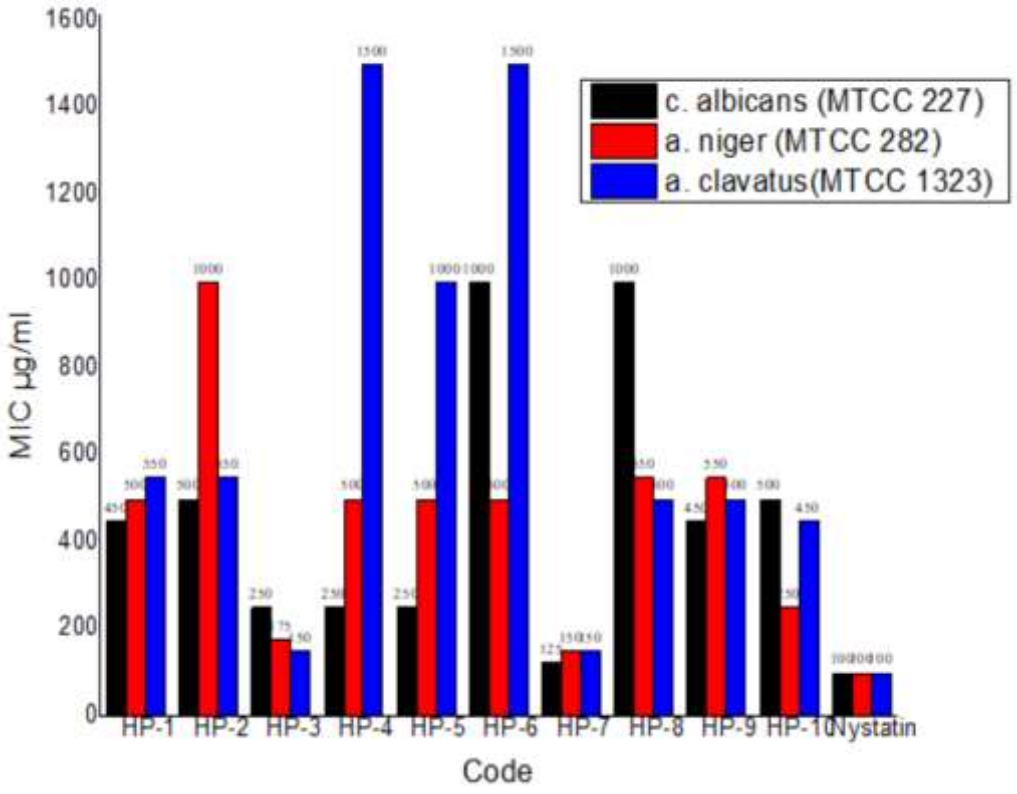


Figure 3.1: Graphical representation of Anti-fungal activities of the compounds (**HP1-HP10**)

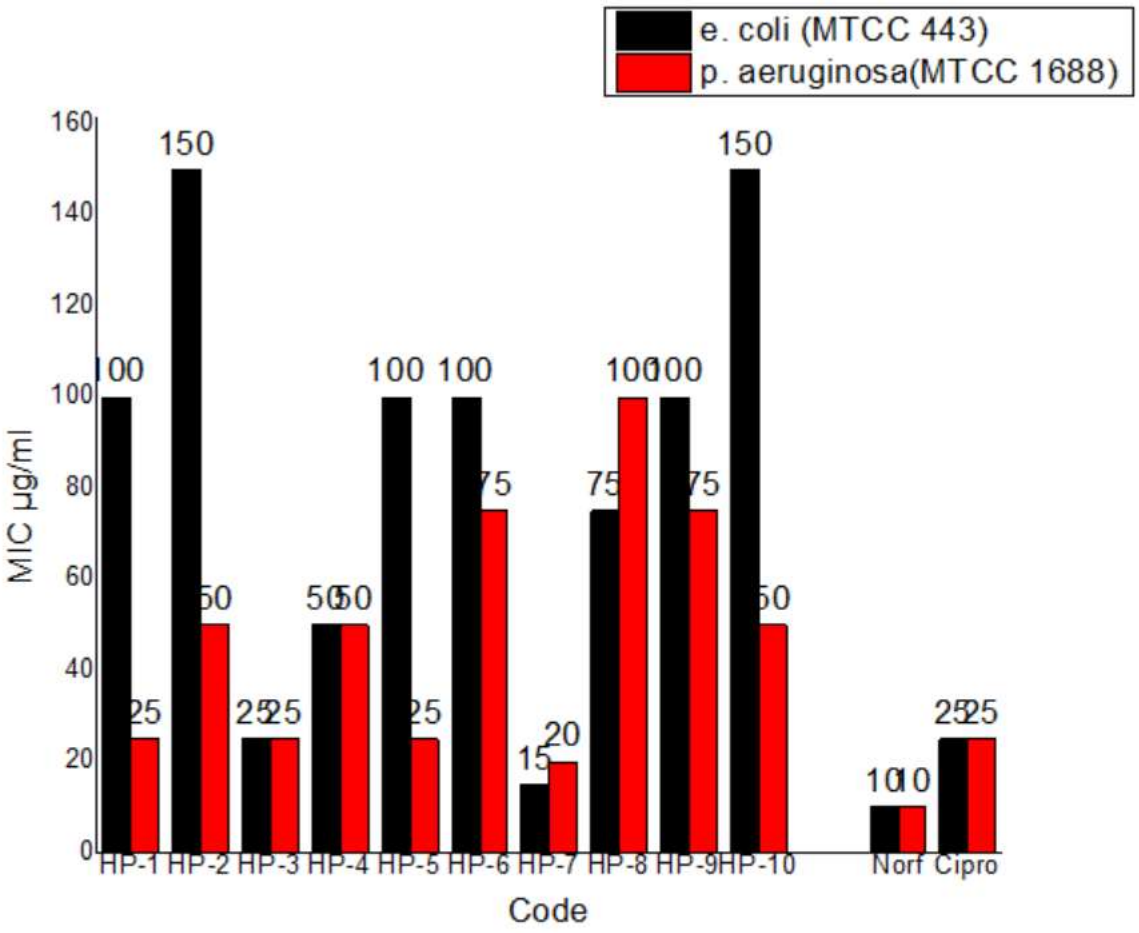


Figure 3.2: Graphical representation of Anti-fungal activities of the compounds (**HP1-HP10**)

Table. 2 Anti-fungal and Anti-bacterial activities of the compound (**HP1-HP10**) as MIC values (μg/ml).

Code	c. albicans (MTCC 227) μg/ml	a. niger (MTCC 282) μg/ml	a. clavatus (MTCC 1323) μg/ml	e. coli (MTCC 443) μg/ml	p. aeruginosa (MTCC 1688) μg/ml
HP-1	450	500	550	100	25
HP-2	500	1000	550	150	50
HP-3	250	175	150	25	25
HP-4	250	500	1500	50	50
HP-5	250	500	1000	100	25
HP-6	1000	500	1500	100	75
HP-7	125	150	150	15	20
HP-8	1000	550	500	75	100
HP-9	450	550	500	100	75
HP-10	500	250	450	150	50
Nystatin	100	100	100	-	-
Norfloxacin	-	-	-	10	10
Ciprofloxacin	-	-	-	25	25

3.3 in-silico study.

Table 3: Binding Energy (Site-specific Docking) in Kcal/mol

Compound code	Protein Pdb:1KZN Binding Energy (Site-specific Docking) Kcal/mol	Protein Pdb:4JVI Binding Energy (Site-specific Docking) Kcal/mol	Protein Pdb:5C5G Binding Energy (Site-specific Docking) Kcal/mol	Protein Pdb:1IYL Binding Energy (Site-specific Docking) Kcal/mol	Protein Pdb:1UKC Binding Energy (Site-specific Docking) Kcal/mol
HP01	-7.7	-8.6	-7.2	-9.7	-7.2
HP02	-7.5	-8.5	-7.0	-9.2	-6.9
HP03	-8.5	-8.8	-7.7	-9.7	-7.9
HP04	-8.0	-8.4	-6.8	-9.8	-7.2
HP05	-7.8	-8.8	-7.1	-9.8	-7.2
HP06	-7.7	-8.2	-6.8	-9.1	-7.4
HP07	-8.6	-8.8	-7.4	-10.1	-7.9
HP08	-7.5	-8.2	-7.0	-9.1	-7.3
HP09	-8.2	-8.5	-7.2	-9.6	-7.2
HP10	-8.1	-8.6	-7.4	-9.4	-7.7

Attributes Compounds against 1KZN

Table 4 : Binding Energy (Site-specific Docking) in Kcal/mol against 1KZN

code	Protein Pdb:1KZN Binding Energy (Site-specific Docking) Kcal/mol	Hydrogen Bonding	Interactions residues	Bond distance (A^0)
HP01	-7.7	3	GLY77, GLU50, ASN46	2.53,3.08,2.29
HP02	-7.5	1	ASN46	2.20
HP03	-8.5	6	ALA96, HIS95, SER121, VAL120, ASN46, ARG76	2.73,2.74,3.31,2.56, 2.85,2.79
HP04	-8.0	2	ASP73, SER121	2.66,2.31
HP05	-7.8	3	GLY77, ASN46	2.25,2.14
HP06	-7.7	2	GLU50, GLY77	2.73,2.81
HP07	-8.6	3	VAL120, SER121, VAL93	2.71,3.36,2.39
HP08	-7.5	3	VAL120, ASP49, ARG136	2.60,2.59,2.33
HP09	-8.2	3	ASN46, VAL93, SER121	2.93,2.21,2.58
HP10	-8.1	4	ASP49, ASN46, ARG136, VAL120	2.63,2.10,6.75,1.90

On the target protein 1KZN, site-specific docking study (X: - 18.849850, Y: 23.905305, Z: 36.008089) discovered that all drugs investigated had negative binding energies ranging from -7.5 to -8.6 kcal/mol. With -8.6 kcal/mol, the substance HP07 had the highest binding energy. Strong hydrogen bonds were established between HP07 and 1KZN at positions 2.71, 3.36, and 2.39 A0 for VAL120, SER121, and VAL93, respectively.

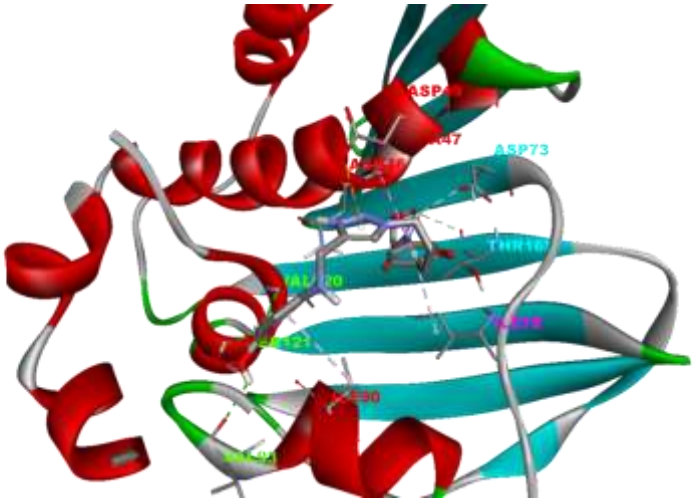


Fig. 3.3: 3D Interaction of HP07 with 1KZN.

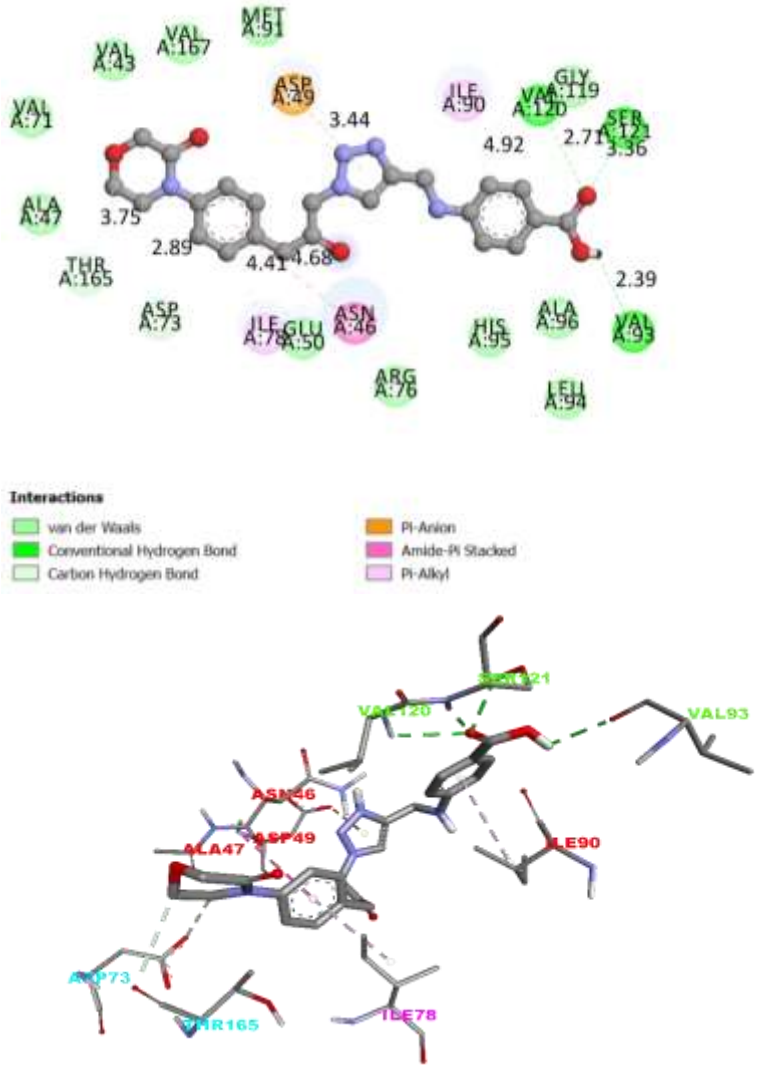


Fig. 3.4: 2D Interaction of HP07 with 1KZN.

Compounds against 4JVI

code	Protein Pdb:4JVI Binding Energy (Site-specific Docking) Kcal/mol	Hydrogen bonding	Interactions residues	Bond distance (A^0)
HP01	-8.6	-	TYR258, ILE186, AL170, ILE236,ALA168, LEU189	5.03,4.10,3.89,3.50, 4.66,5.38
HP02	-8.5	1	LEU197	2.89
HP03	-8.8	2	LEU197, GLN194	2.00, 5.39,
HP04	-8.4	1	ILE236	2.19
HP05	-8.8	1	TYR258	2.09
HP06	-8.2	-	ALA168, VAL170, ILE186, LEU189, VAL211, TYR258, ILE236	4.83,4.20,4.23,5.31, 5.33,5.22,3.55
HP07	-8.8	2	GLN194, LEU208	2.72, 2.53
HP08	-8.2	2	TYR268, LEU153	2.24,2.67
HP09	-8.5	2	ILE236, LEU197	1.88,2.65
HP10	-8.6	2	ARG209, LEU208	2.43,3.02

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 ranging between -8.2 kcal/mol with site-specific docking. The compounds HP03, HP05, and HP07 had the highest binding energy of -8.8 kcal/mol. HP03 formed strong bonds with 4JVI were Hydrogen bonds LEU197, GLN194 at hydrogen bonding at 2.00 and 5.39 Å, HP05 formed strong bonds with 4JVI were hydrogen bonding TYR258 at 2.09 Å and HP07 formed strong bonds with 4JVI were hydrogen bonding GLN194, LEU208 at 2.72, 2.53 A^o.

HP03

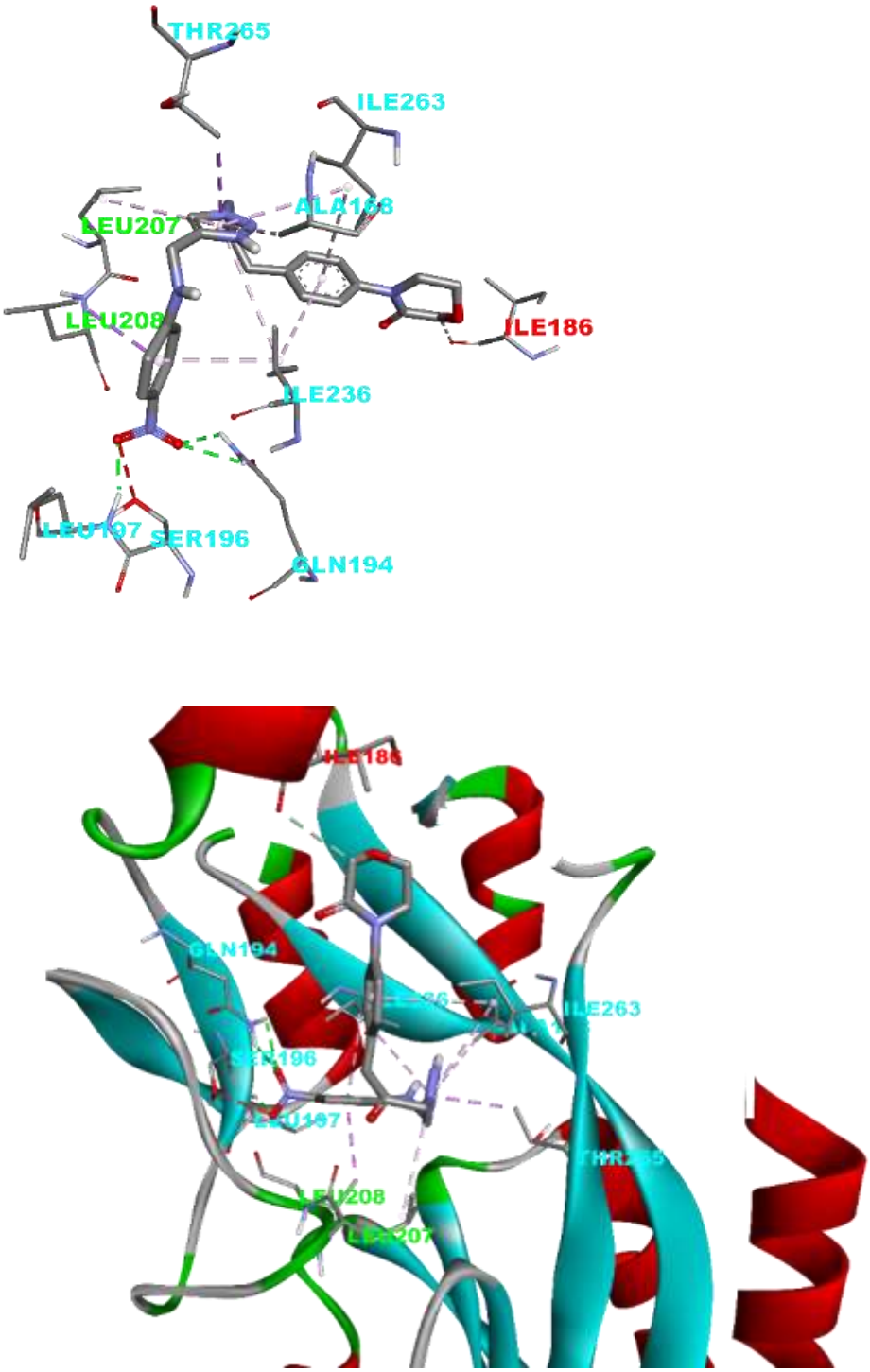


Fig. 3.5: 3D Interaction of HP03 with 4JVI.

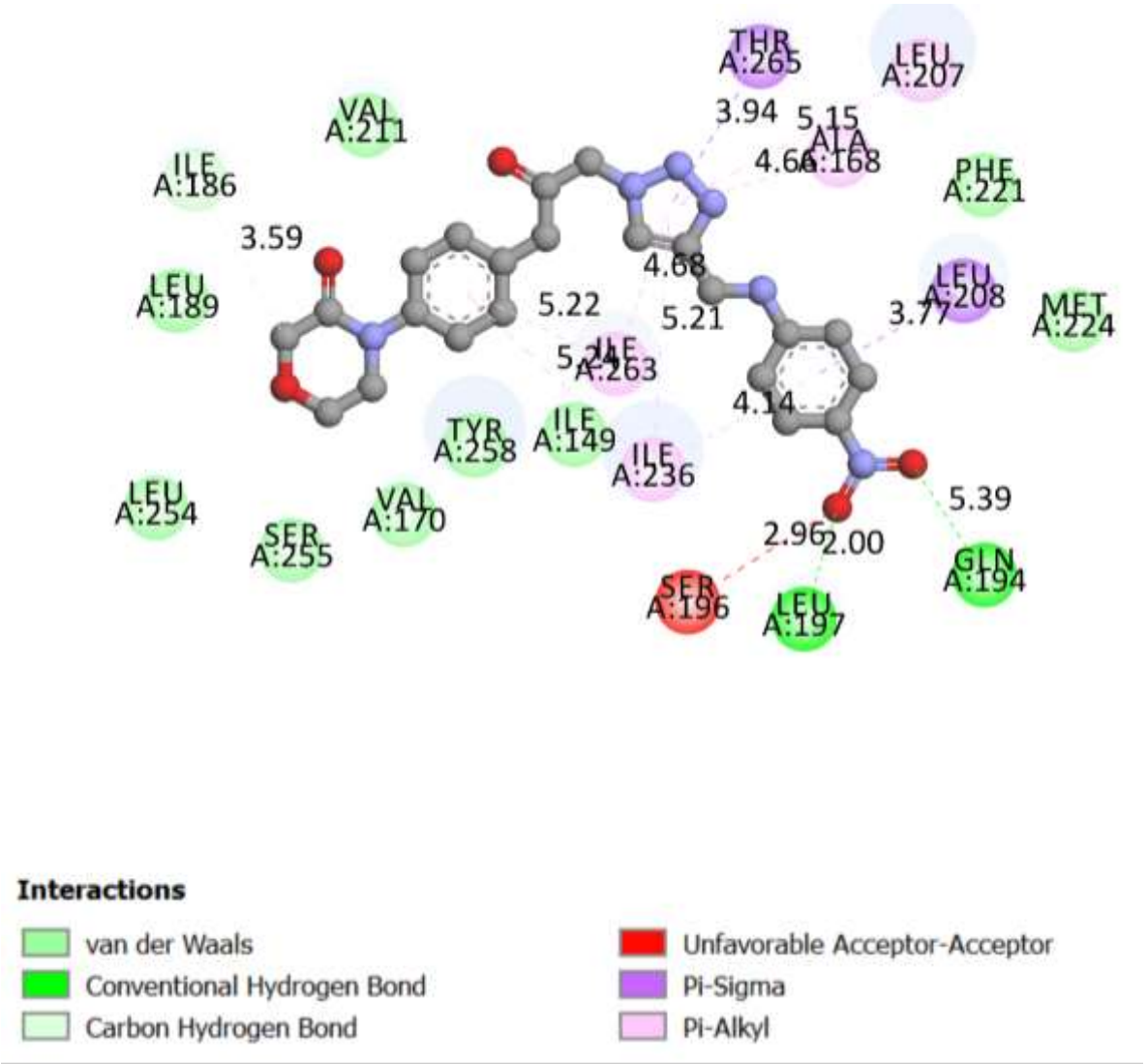
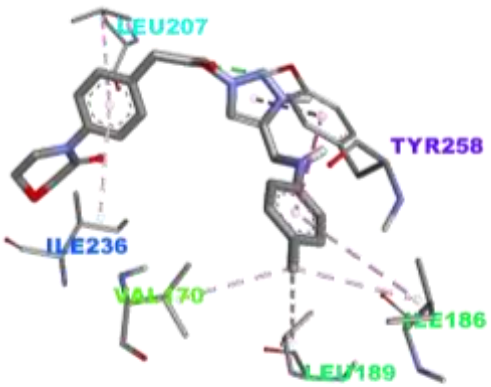


Fig. 3.6: 2D Interaction of HP03 with 4JVI.

HP05



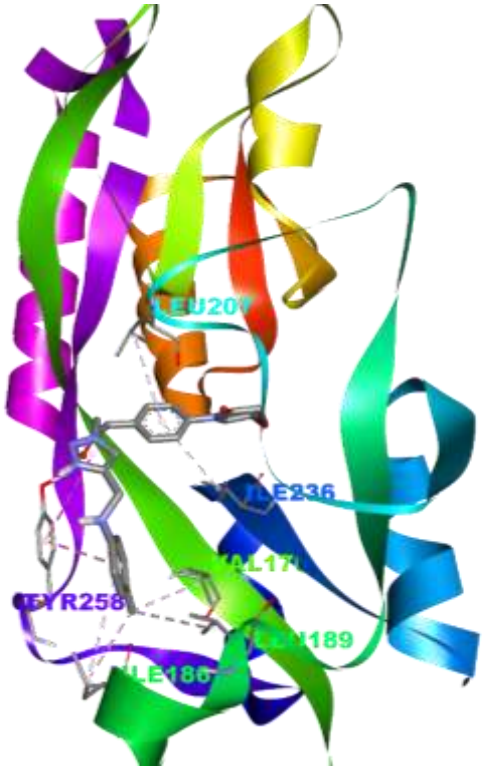


Fig. 3.7: 3D Interaction of HP05 with 4JVI.

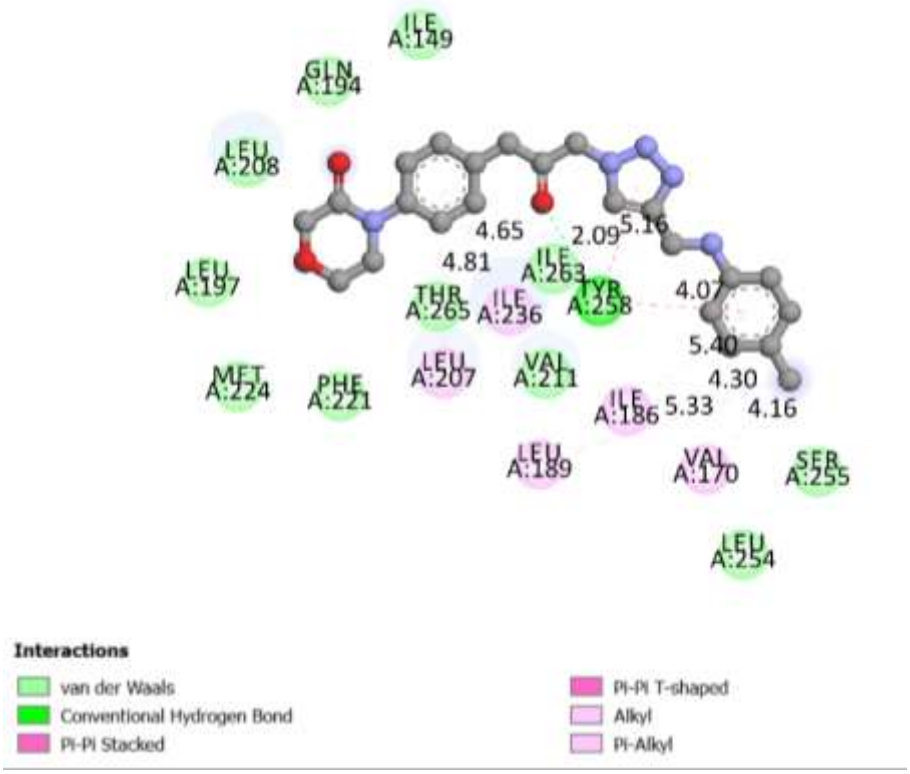
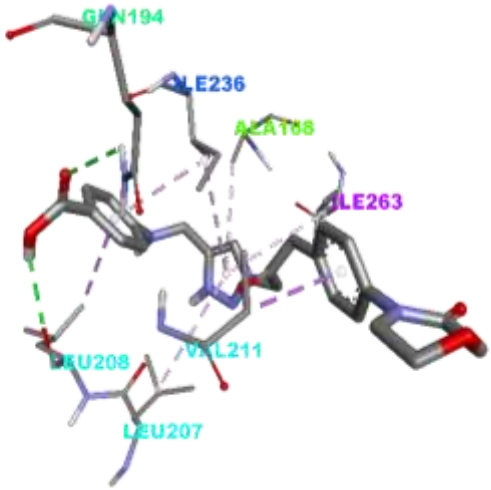


Fig. 3.8: 2D Interaction of HP05 with 4JVI.



HP07

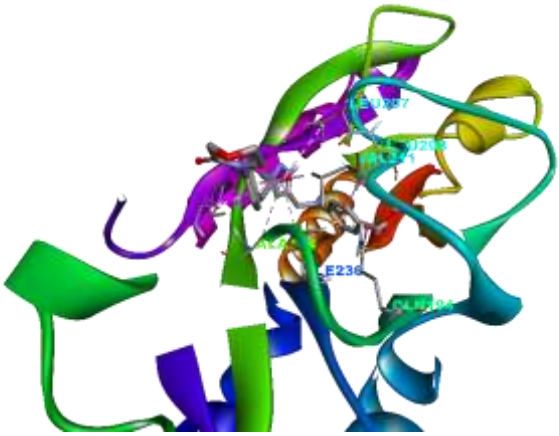


Fig. 3.9: 3D Interaction of HP07 with 4JVI.

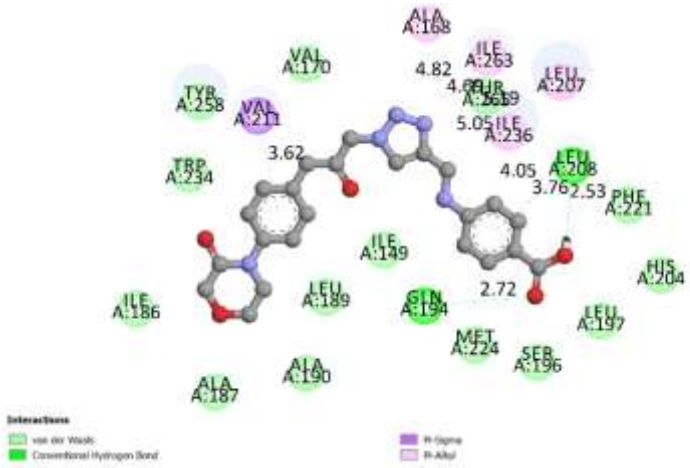


Fig. 3.10: 2D Interaction of HP07 with 4JVI.

Compounds against 5C5G.

code	Protein Pdb:5C5G Binding Energy (Site-specific Docking) Kcal/mol	Hydrogen bonding	Interactions residues	Bond distance (A^0)
HP01	-7.2	1	ASN93	3.05
HP02	-7.0	2	SER96, ASN93,	2.17,2.97
HP03	-7.7	6	SER96, ASN93, TYR288, TYR287	2.19,2.34,2.13,2.09,2.71,3.16
HP04	-6.8	4	SER96, GLU222, TYR287, ASP286	2.63,2.17,2.51,2.61
HP05	-7.1	1	ASN93	3.06
HP06	-6.8	2	SER96, TYR126	2.21,2.71
HP07	-7.4	4	SER96.GLU222, TYR287, TYR288	2.31,2.87,2.62,2.23
HP08	-7.0	5	ASN93, TYR65, ASN202, TYR287, ASP286	2.91,1.99,2.98,2.29,2.71
HP09	-7.2	2	TYR287, TYR288	2.03,2.84
HP10	-7.4	3	TYR287, TYR65, GLU222	2.61,1.88,1.96

According to site-specific docking studies (X: - 33.533816, Y: 40.877303, Z: 59.161684), all drugs tested displayed negative binding energies on the target protein 5C5G. With site-specific docking, the molecule HP03 had the maximum binding energy of -7.7kcal/mol. Hydrogen bonds SER96, ASN93, TYR287, and TYR288 were generated by HP03 and 5C5G at concentrations of 2.19, 2.34, 2.13, 2.09, 2.71, and 3.16, respectively.

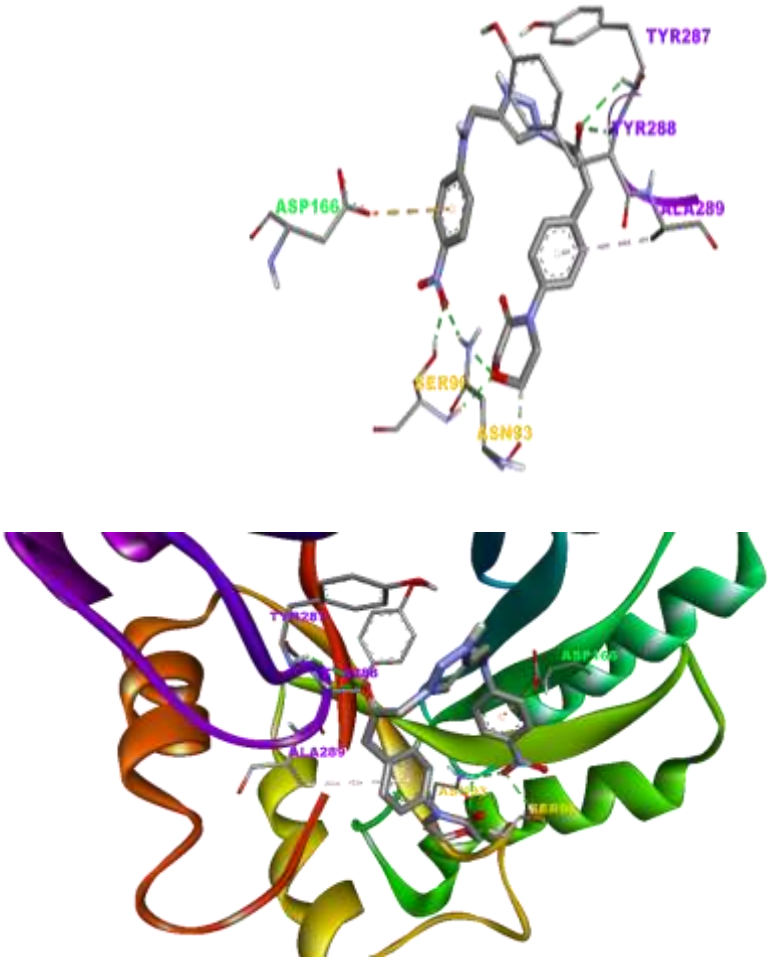


Fig. 3.11: 3D Interaction of HP03 with 5C5G.

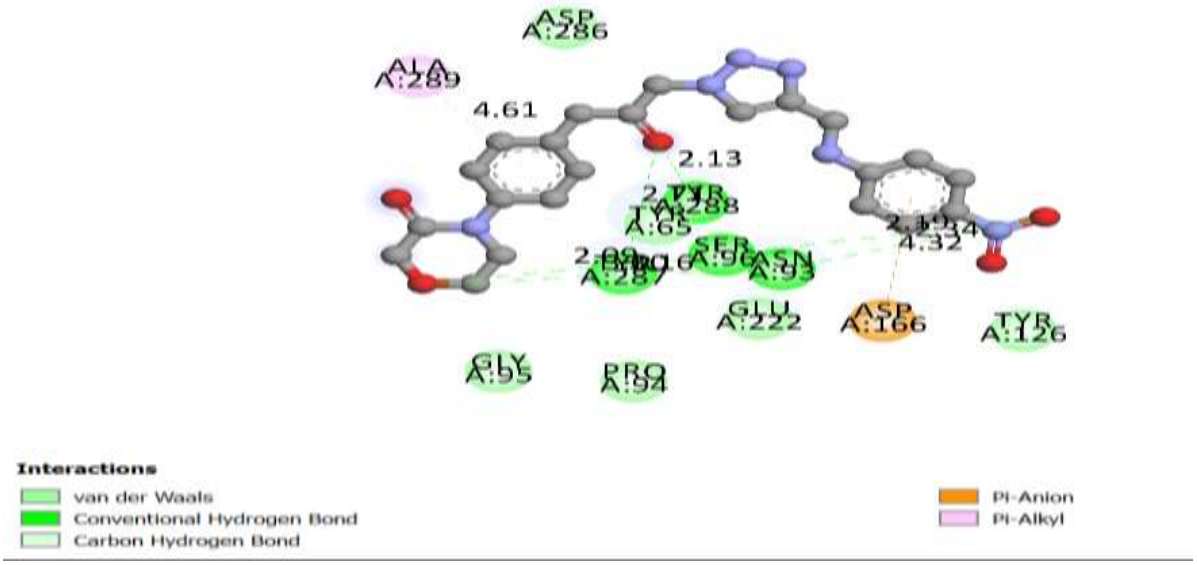


Fig. 3.12: 2D Interaction of HP03 with 5C5G.

Compounds against 1IYL

code	Protein Pdb:1IYL Binding Energy (Site-specific Docking) Kcal/mol	Hydrogen bonding	Interactions residues	Bond distance (A^0)
HP01	-9.7	1	ILE111	2.25
HP02	-9.2	-	LEU415, LEU394, TYR225, CYS393	5.20,5.39,4.94,3.70,3.43
HP03	-9.7	3	ASN392, TYR225, GLU109	2.96,2.72,2.59
HP04	-9.8	2	ASN392, ASN175	2.16,2.60
HP05	-9.8	1	HIS227	3.02
HP06	-9.1	1	CYS393	2.91
HP07	-10.1	2	ASN392, CYS393	2.92,1.96
HP08	-9.1	4	TYR335, ASN392,THR211, TYR107	3.03,2.77,2.44,3.20
HP09	-9.6	3	ASN392,CYS393, LEU451	3.02,2.93,2.71
HP10	-9.4	5	HIS227, ASN392,TYR225, LEU451,TYR107	2.85,2.17,2.85,2.23,2.54

According to site-specific docking research, all drugs tested displayed negative binding energies on the target protein 1IYL (X: - 12.9642887415, Y: 47.7911458235, Z: -0.516956407189). With site-specific docking, the molecule HP07 had the lowest binding energy at -10.1 kcal/mol. Strong hydrogen bonds ASN392, CYS393 at 2.92 and 1.96 were generated by HP07 and 1IYL.

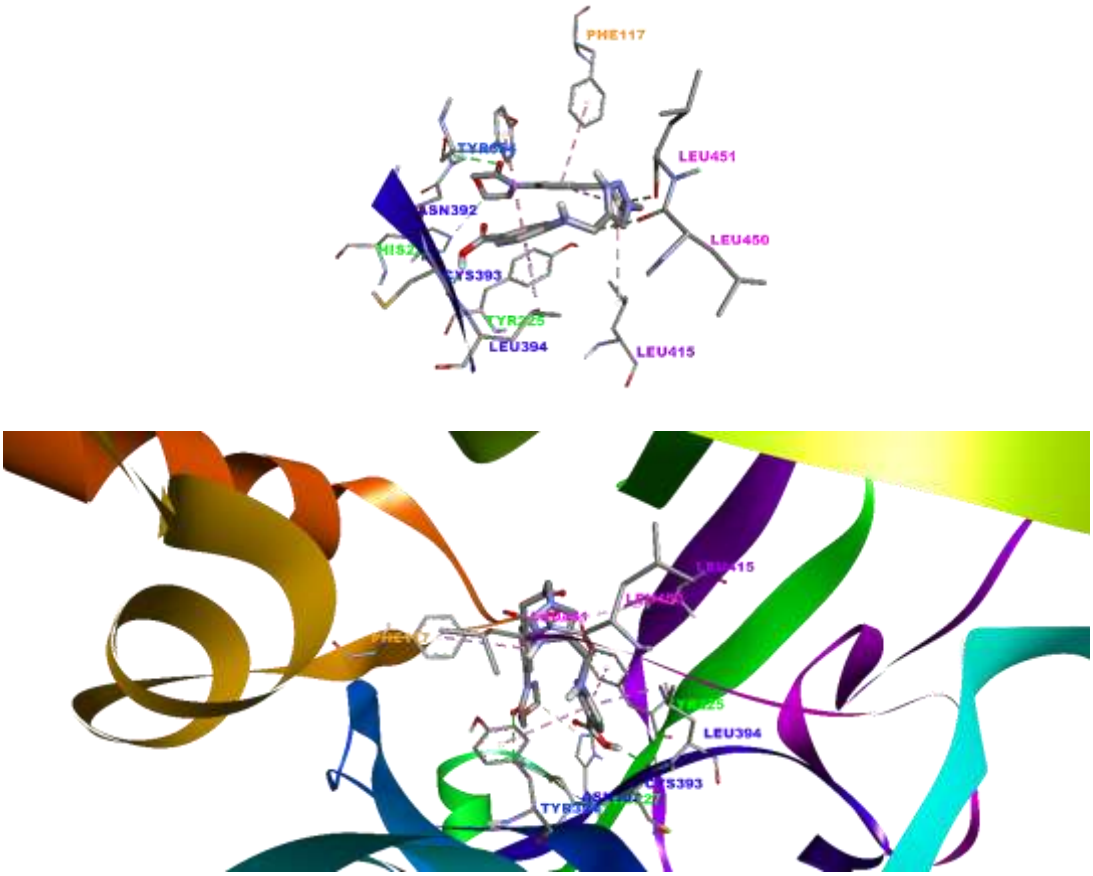


Fig. 3.13: 3D Interaction of HP07 with 1IYL.

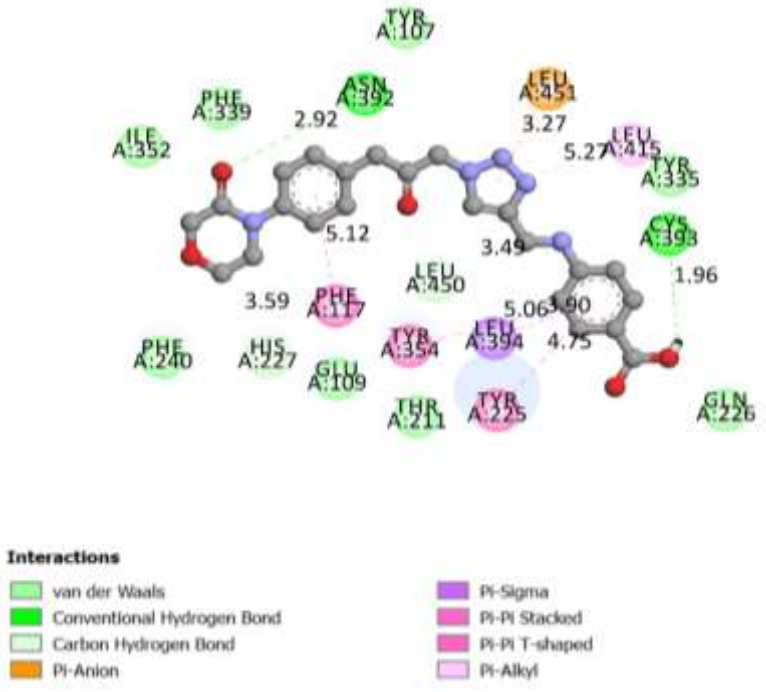


Fig. 3.14: 2D Interaction of HP07 with 1IYL.

Compounds against 1UKC

code	Protein Pdb:1UKC Binding Energy (Site-specific Docking) Kcal/mol	Hydrogen bonding	Interactions residues	Bond distance (A^0)
HP01	-7.2	1	ARG505	2.25
HP02	-6.9	-	ASP397,ASP525,ALA393, SER392,ASP337	5.52,4.41,3.76,4.14,3.48
HP03	-7.9	4	GLU338,GLY339, SER522, ARG505	2.70, 1.90,2.45,2.58
HP04	-7.2	2	SER522, ASP337	2.19,2.41
HP05	-7.2	1	SER522	2.31
HP06	-7.4	3	SER522,ASP337, GLY339	2.29,2.96,2.70
HP07	-7.9	5	GLU338,GLY339, SER522,ALA393, ASP397	2.61,1.86,2.22,2.66,2.52
HP08	-7.3	3	SER522,GLY339, SER392	2.24,1.75,2.88
HP09	-7.2	1	ASP337	2.68
HP10	-7.7	4	LYS379,ALA393, ASP397, ASP525	2.65,3.06,2.08,2.63,2.38

According to site-specific docking study, all of the chemicals tested displayed negative binding energies on the target protein 1UKC (X: - 11.6594164098, Y: 53.6473705922, Z: -6.36819134173). With site-specific docking, the molecules HP03 and HP07 had the maximum binding energy of -7.9 kcal/mol. Hydrogen bonds GLU338, GLY339, SER522, and ARG505 produced strong bonds between HP03 and 1UKC at 2.70, 1.90, and 2.45, respectively. Hydrogen bonds GLU338, GLY339, SER522, and ALA393 formed strong bonds between HP07 and 1UKC at 2.61,1.86, and 2.22, and 2.66, and 2.52 respectively.

HP03

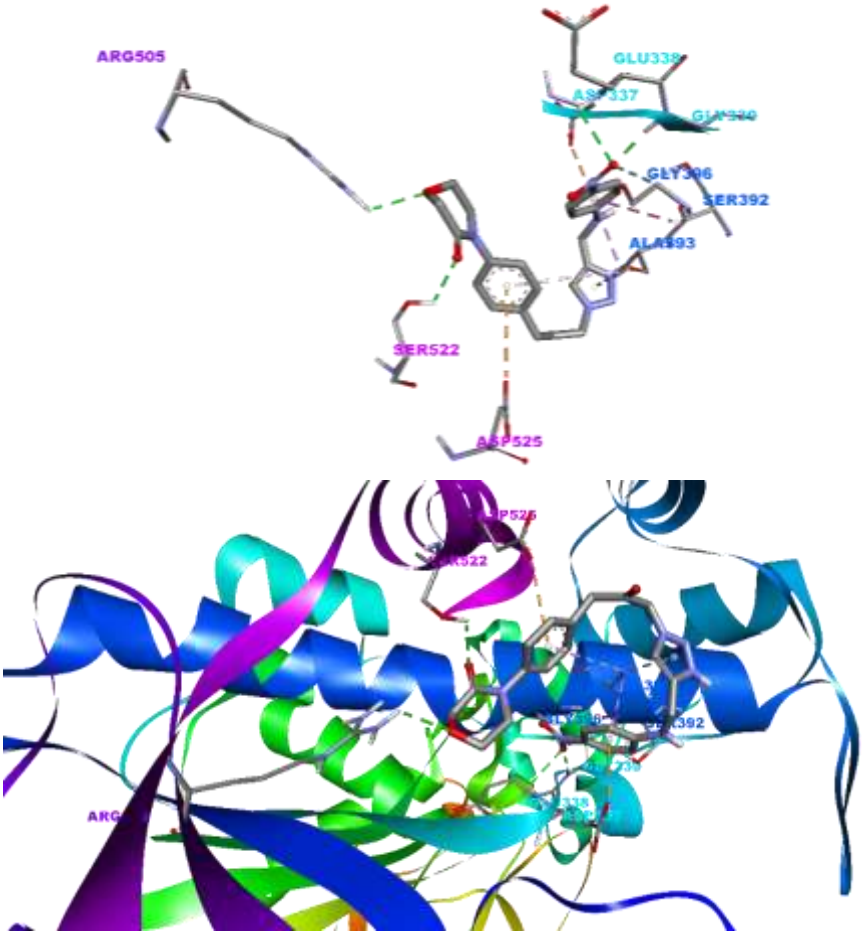


Fig. 3.15: 3D Interaction of HP03 with 1UKC.

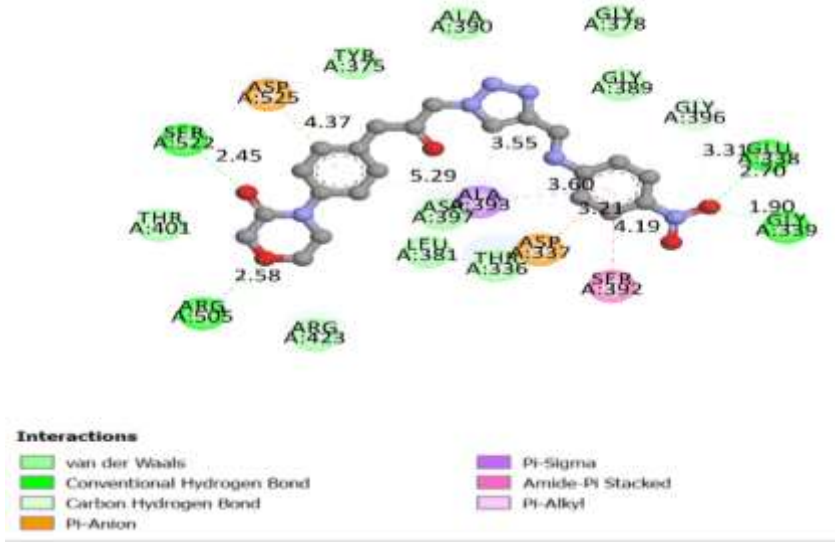


Fig. 3.16: 2D Interaction of HP03 with 1UKC.

HP07

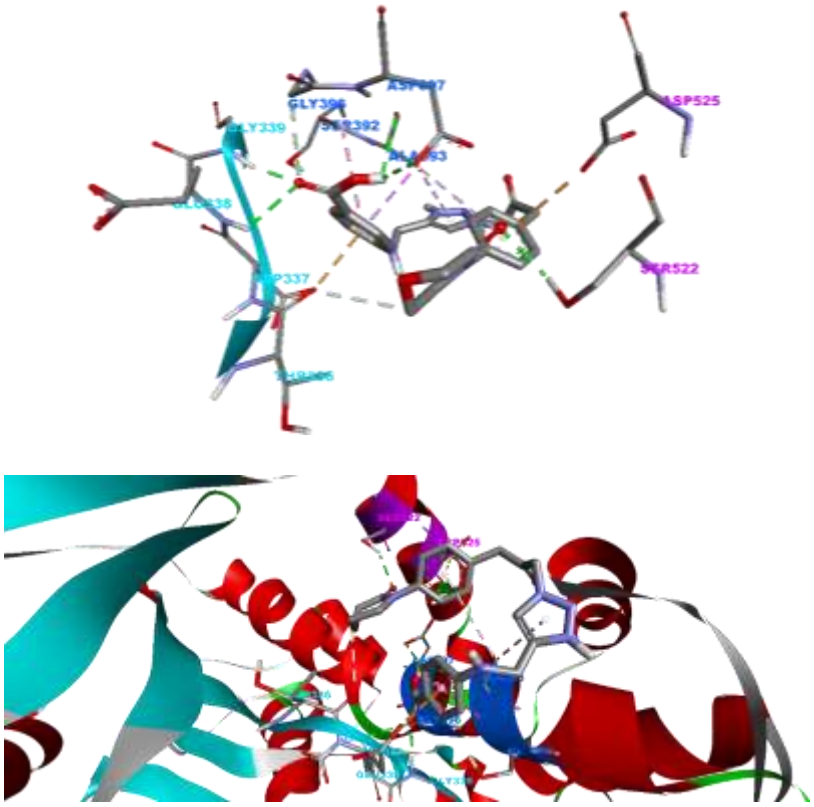


Fig. 3.17: 3D Interaction of HP03 with 1UKC.

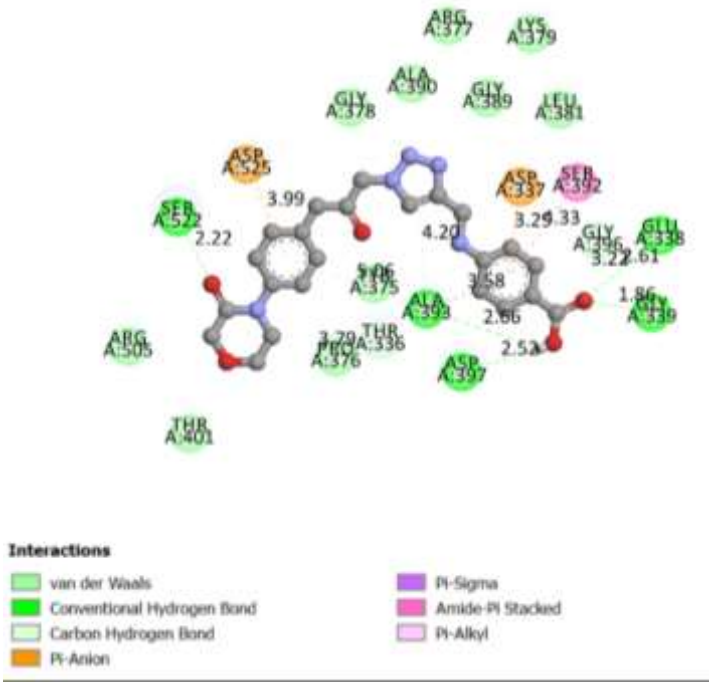


Fig. 3.18: 2D Interaction of HP03 with 1UKC.

3. Conclusion

A new class of N-(4-(3-oxomorpholino)phenyl)-2-(4-((phenylamino) methyl) compounds have been synthesised, characterised, and shown to have antibacterial and antifungal action in vivo and in silico. -1H-1,2,3-triazol-1-yl)acetamide(HP1-HP10). For the successful development of the triazole framework, the click chemistry reaction served as a critical reaction. For each, the minimum inhibitory concentration (MIC) was established. Both HP03 and HP07 may be effective against fungal strains and gram-negative bacteria. Additionally, site-specific molecular docking investigations of all ten tetrazole moieties conducted by PyRx revealed that the compounds HP03 and HP07 exhibit strong binding affinities for PDB: 1KZN, 1UKC, 1IYL, 4JVI, and 5C5G. Such substances might one day prove effective as strong drugs. Purification was typically not necessary because the synthetic procedures were effective and clean because no other byproducts were produced throughout the reaction. The current work is crucial for synthesizing a variety of novel triazole-ringed heterocyclic compounds.

4. Declaration of competing interest

The authors affirm that they have no known financial or interpersonal conflicts that would have appeared to have an impact on the research presented in this study.

5. Data Availability

Click chemistry's copper sulfate-catalyzed triazole derivative synthesis as potential antibacterial drugs linked to molecular docking (Original data).

6. Acknowledgements

The Department of Chemistry at Shree D K V Arts & Science College in Jamnagar provided a lab for the authors' synthetic study, and they are very appreciative.

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