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IN SILICO MOLECULAR DOCKING AND IN VITRO ANTIBACTERIAL AND ANTIFUNGAL STUDY OF NEWLY SYNTHESIZED QUINOLINE CONTAINING HETEROCYCLIC FRAMWORK

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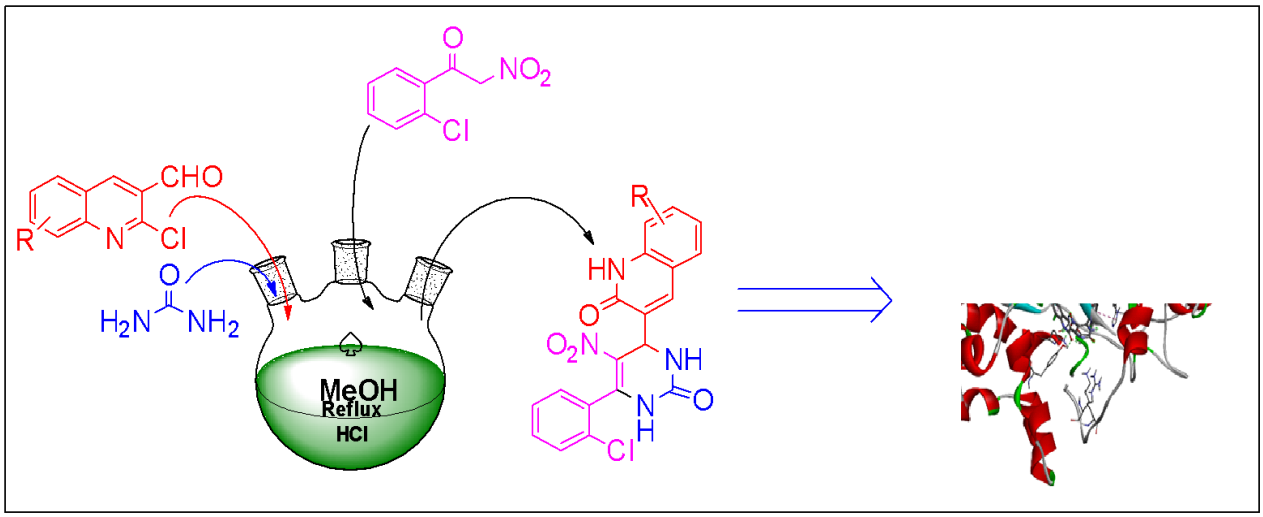
Abstract: In this study novel heterocyclic library of 3-(6-(2-chlorophenyl)-5-nitro-2-oxo-1,2,3,4-tetrahydropyrimidin-4-yl)quinolin-2(1*H*)-one were synthesized and characterize to *in vitro* antimicrobial activity against gram-positive (*S. aureus*, *S. pyogenes*), gram-negative (*E. coli*, *P. aeruginosa*), and fungal strains (*C. albicans*, *A. clavatus*, and *A. niger*) concerning standard drugs. The screened compounds show moderate to good activity against various bacteria and fungi. The compounds were also studied for *in silico* molecular docking with the target protein of bacteria and fungi to investigate potential inhibition properties. The study suggests that compounds some have the potential and after other detailed studies, may be useful for medicinal purposes. The structure of synthesized compounds well characterized by their spectral (IR, ^{1}H NMR and Mass) data. The purity of the synthesized compounds was confirmed by TLC.

KEYWORDS:, Molecular Docking, Pyrimidine, quinoline, methanol & antimicrobial activity.



INTRODUCTION:

The six membered nitrogen containing heterocycles, quinoline (benzazine or 1-azanapthalene or benzo[*b*]pyridine) is one of the very important heterocyclic compounds featuring a nitrogen atom as part of the ring system. The quinoline nucleus constitutes an important core among pharmacologically active synthetic and natural compounds with widespread prevelance[1]. A prominent example is quinine, an alkaloid found in plants. 4-Hydroxy-2-alkylquinolines (HAQs) are involved in antibiotic resistance. Quinoline has slight solubility in cold water, complete solubility in hot water and in most organic solvents. Quinoline exhibits both electrophilic and nucleophilic substitution reactions analogous to those of pyridine and benzene. Its oral absorption and inhalation is not dangerous to human beings. Quinoline has a property to undergo degradation in presence of micro-organisms like *Rhodococcus* species strain Q_1 [2].

Quinolines and related heterocyclic systems represent an important class of alkaloids and are also found as structural frameworks in a large number of biologically active natural products and pharmaceuticals[3]. These nitrogen-containing heterocycles possess broad applications in drug development such as treatment of MCH (Melanin Concentrating Hormone) receptor related disorder[4], cell proliferative disease [5], Melting points were taken in open capillary method and are uncorrected. IR spectra were recorded on FTIR-8400 transmissible spongiform encephalopathies[6], malignant tumor, stomach cancer, brain tumor, and large intestine cancer[7] and bacterial infections in mammals[8]. Substituted quinolines are historically among the most important antimalarial and their immense use in 20th century provided well founded hopes for malaria eradication[9]. Pharmaceutically important quinoline containing molecules such as chloroquine and amidoquine are found as an anti-malarial marketed drug in which a chlorine atom is attached at the C-7 position according to quinoline ring.

In the present work, we report the synthesis of This novel series of Pyrimidine based quinoline derivatives of 3-(6-(2-chlorophenyl)-5-nitro-2-oxo-1,2,3,4-tetrahydropyrimidin-4-yl)quinolin-2(1H)-one and their antimicrobial activity against fungi, gram positive and gram negative bacteria. To study the plausible interaction and affinity of prepared compounds with the target bacterial and fungal proteins, the *in silico* study was performed for the synthesized compounds, which may help understand the interaction mechanism. The main significance of the work is it will provide synthesized and more potent stable molecule for biological response as most of Quinolin derivatives has significant biological activity. As we mentioned above, the significance and biological profile of this class of molecule so our continue efforts towards the synthesis of potential heterocyclic molecules.

2. EXPERIMENTAL:

EXPERIMENTAL MATERIAL AND METHODS:

Anhydrous solvents and all reagents and solvents were purchased from, Spectrochem, Sigma-Aldrich, Lobachemie. And Merck, involving air or moisture-sensitive compounds were performed under a nitrogen atmosphere using oven-dried glassware and syringes to transfer solutions. Thin-layer chromatography (TLC) was conducted by using aluminum plates 20x20 cm coated by silica gel 60 F254 purchased from Merck. Melting points were determined by melting point apparatus (uncorrected) using an open capillary method. Solvents evaporated by the help of a BUCHI rotary evaporator. IR spectra were recorded on FTIR-8400 spectrometer using DRS prob. which expressed in ν (cm-1). Shimadzu GCMS-QP-2010 model was used to achieve Mass spectra of the products. Nuclear magnetic resonance spectra ^{1}HNMR spectra were determined in $CDCl_3$/DMSO-d_6 (in 3/1 ratio) or DMSO-d_6 and were recorded on a Bruker AVANCE II 400 MHz. Chemical shifts (δ scale) were reported in ppm (parts per million) downfield from tetramethylsilane (TMS) used as an internal standard. Splitting patterns are designated as followings: s, singlet; d, doublet; t, triplet; q, quadruplet; m, multiplet; brs, broad singlet; dd, double doublet. Shimadzu GCMS-QP-2010 model was used to achieve Mass spectra of the products.

LIGAND AND MACROMOLECULES PREPARATION

Synthesized structure files created from ChemDraw 20.1.1. Created structures were converted into PDB file using Avogadro 1.2.0.[10]. The bacterial protein structures Crystal structure of esterase A from Streptococcus pyogenes (4ROT.pdb)[11], Crystal structure of S. aureus (1JIJ.pdb)[12], Crystal Structure of Candida albicans N-myristoyltransferase (1IYL.pdb)[13], Crystal Structure of E. coli 24kDa Domain (1KZN.pdb)[14], Crystal structure of PqsR co-inducer binding domain of Pseudomonas aeruginosa (4JVI.pdb)[15], Crystal Structure of Aspergillus niger EstA (1UKC.pdb)[16] and Crystal Structure of Aspergillus clavatus Sph3 (5C5G.pdb)[17] 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).[18]

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.[19] Biovia Discovery Studio 4.5 were used to analyze the docking results.

A. SYNTHESIS OF VARIOUS SUBSTITUTED 2-CHLOROQUINOLINE-3-CARBALDEHYDE (INT-01):

Substituted Aniline (1) (1 mol) was taken in round bottom flask (RBF) in cooling conditions at 0-5 ºC and dropwise addition of acetic anhydride (1.5 mol) was taken place into RBF with continuous stirring. After addition, sulfuric acid (H_2SO_4) was added in catalytic amount to produce acidic medium. Reaction progress was reported by TLC. After the completion of the reaction, the reaction mass was poured in ice-cooled water and produced solid (4) was filtered, washed with cold water and dried. (white solid product yield: 80.02%) In next step, Phosphorus oxychloride ($POCl_3$) (9 mol) was taken in RBF at 0-5 ºC, DMF (3 mol) is added drop wise into $POCl_3$ very slowly by maintaining the temperature with continuous starring. yellow colored salt was observed after

completion of addition and at that stage calculated amount of N-phenylacetamide (3) (1 mol) was added into it & reaction mass was refluxed in oil bath for 16-24 h, by observing the completion of reaction on TLC, reaction mass was poured into crushed ice to get solid precipitate. (Yellow solid product; yield 83.76%).

B. SYNTHESIS OF 1-(2-CHLOROPHENYL)-2-NITROETHANOL (INT-A):

A mixture of 2-chlorobenzaldehyde(0.1mol), nitromethane(0.1 mol) and sodium acetate (0.2 mol) was stirred at RT for 24 h. The reaction was monitored by TLC. After completion of reaction, the solvent was removed under reduced pressure. The residue was poured in water and extracted with ethylacetate. The organic layer was dried and evaporated to afford Int-a in form of viscous oil. This oil was forwarded to next step without further purification.

C. SYNTHESIS OF 1-(2-CHLOROPHENYL)-2-NITROETHANONE (INT-B):

To the suspension of $K_2Cr_2O_7$ (24.8 mmol) in 15 ml water, Int-2 was added drop wise at 0^0C. This mixture was allowed to stir for 30 min and then solution of sulphuric acid (10 ml con H_2SO_4 and 6 ml water) was drop wise added at same temperature. Here the exothermicity was controlled by keeping addition rate very slow. After completion of addition reaction mixture was stirred for 15 min at the same temp. Colour of the reaction mixture turns dark green then it was poured over crushed ice. Separated solid was immediately filtered before temperature rise and was dissolved in saturated $NaHCO_3$ solution. Filtration was again carried out to separate non-dissolved matter. Filtrate was acidified with con HCl. Precipitated solid was filtered and washed with distilled water. Crystallization was carried out from methanol to afford pure **Int-3**.

D. GENERAL SYNTHESIS OF PYRAZOLOPYRIMIDINE:

In 50 ml RBF Int-B (2.5mmol), urea (2.5mmol) and substituted aldehyde (INT-01) were suspended in 20 ml methanol under stirring on magnetic stirrer. 2-3 drops of acid was added into the reaction mixture and reaction mixture was refluxed for 10 to 12 h. The reaction was monitored by TLC. After the completion of the reaction, water was decanted and the solid residue was triturated with methanol to afford pure compound.

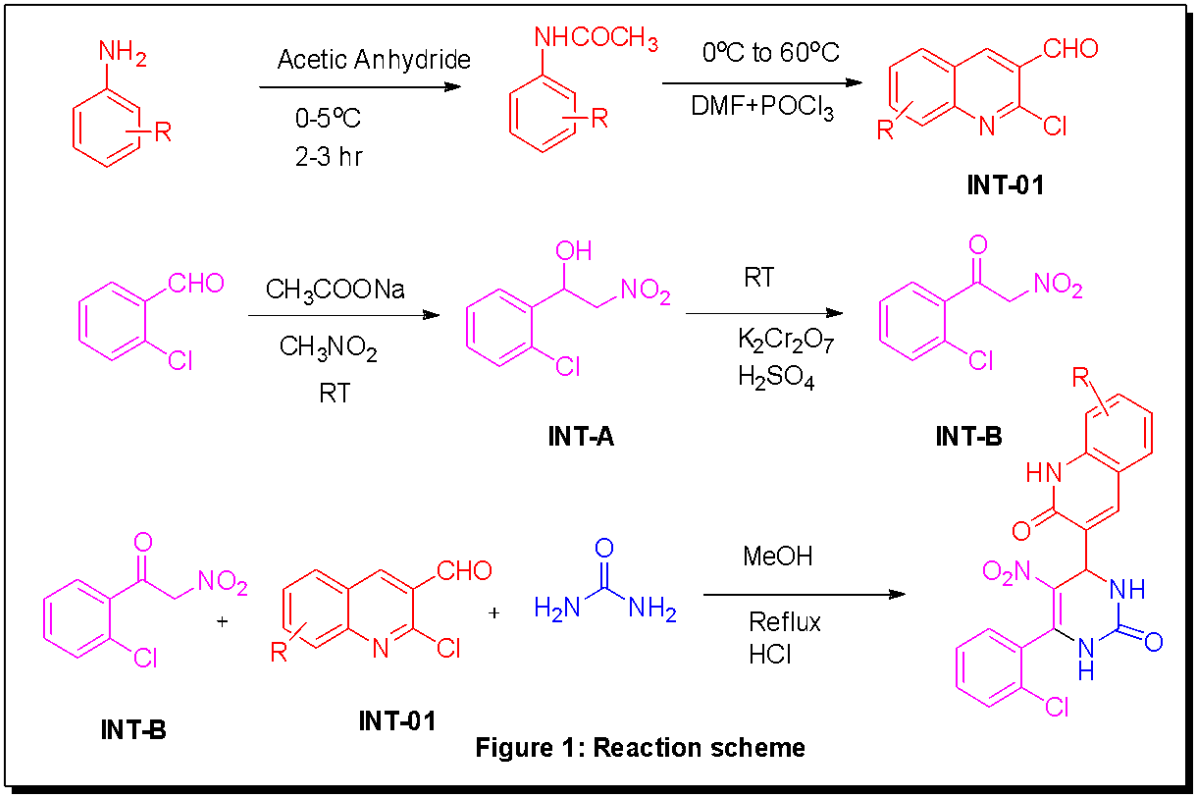


Figure 1: Reaction scheme

TABLE-1 PHYSICAL CONSTANT OF SYNTHESIZED LIBRARY

Code	Molecular formula	Substitution	Molecular Weight	M.P. ^{0}C	Percentage of Yield
MS-10	$C_{19}H_{13}ClN_4O_4$	-H	396	162-164	62
MS-11	$C_{20}H_{15}ClN_4O_4$	6-Me	410	178-180	70
MS-12	$C_{19}H_{12}Cl_2N_4O_4$	6-Cl	430	154-156	68
MS-13	$C_{20}H_{15}ClN_4O_5$	6-Ome	426	158-160	58
MS-14	$C_{19}H_{12}BrClN_4O_4$	6-Br	473	176-178	62
MS-15	$C_{21}H_{17}ClN_4O_4$	6,8- Di Me	424	180-182	54
MS-16	$C_{19}H_{12}ClN_5O_6$	6-NO_2	441	148-150	56
MS-17	$C_{19}H_{12}Cl_2N_4O_4$	8-Cl	430	142-144	72
MS-18	$C_{20}H_{15}ClN_4O_4$	8-Me	410	182-184	56
MS-19	$C_{20}H_{15}ClN_4O_5$	8-Ome	426	186-188	58

SPECTRAL DATA OF SYNTHESIZED COMPOUNDS:

3-[6-(2-chlorophenyl)-5-nitro-2-oxo-1,2,3,4-tetrahydropyrimidin-4-yl]quinolin-2(1*H*)-one(MS-10): Yield 62%, Yellow Solid, mp 162-164 °C. IR spectrum (KBr), *v*, cm^{-1}: 3141, 3022, 2952, 2831, 2769, 1685, 1607, 1519, 1406, 1314, 1188, 1081, 969, 824, 756, 696, 637. ^{1}H NMR spectrum (400 MHz, DMSO-d_6) *δ, ppm*: 10.89 (Singlet, 1H), 8.86 (Singlet, 1H), 7.74-7.45 (Complex, 9H), 7.29 (Singlet, 1H), 6.60-6.59 (Singlet, 1H). Mass (m/z): 396 (M^+), $C_{19}H_{13}ClN_4O_4$, Elemental Analysis Calculated %: C; 57.51, H; 3.30, N; 14.12, Found %: C; 57.50, H; 3.29, N; 14.10.

3-(6-(2-chlorophenyl)-5-nitro-2-oxo-1,2,3,4-tetrahydropyrimidin-4-yl)-6-methylquinolin-2(1*H*)-one (MS-11): Yield 70%, White Solid, mp 178-180°C. IR spectrum (KBr), *v*, cm^{-1}: 3179, 3042, 2827, 2795, 1697, 1510, 1390, 1173, 1025, 913, 847, 745, 699. ^{1}H NMR spectrum (400 MHz, DMSO-d_6) *δ, ppm*: 12.17 (Singlet, 1H), 12.02 (Singlet, 1H), 10.23 (Singlet, 1H), 8.52-7.20 (Complex, 8H), 3.93-3.16 (Singlet, 3H), 1.12 (Singlet, 1H). Mass (m/z): 410 (M^+), $C_{20}H_{15}ClN_4O_4$, Elemental Analysis Calculated %: C; 58.47, H; 3.67, N; 13.64, Found % C; 58.40, H; 3.62, N; 13.60.

6-chloro-3-(6-(2-chlorophenyl)-5-nitro-2-oxo-1,2,3,4-tetrahydropyrimidin-4-yl)quinolin-2(1*H*)-one (MS-12): Yield 68%, White Solid, mp 154-156°C. IR spectrum (KBr), *v*, cm^{-1}: 3140, 3021, 2952, 2831, 2769, 1684, 1607, 1518, 1416, 1314, 1188, 1081, 969, 824, 756, 696, 632. ^{1}H NMR spectrum (400 MHz, DMSO-d_6) *δ, ppm*: 12.12 (Singlet, 1H), 12.05 (Singlet, 1H), 10.29 (Singlet, 1H), 8.50-7.25 (Complex, 8H), 1.15 (Singlet, 1H). Mass (m/z): 430 (M^+), $C_{19}H_{12}Cl_2N_4O_4$, Elemental Analysis Calculated %: C; 52.92, H; 2.80, N; 12.99, Found %: C; 52.90, H; 2.82, N; 12.95.

3-(6-(2-chlorophenyl)-5-nitro-2-oxo-1,2,3,4-tetrahydropyrimidin-4-yl)-6-methoxyquinolin-2(1*H*)-one (MS-13): Yield 58%, White Solid, mp 158-160°C. IR spectrum (KBr), *v*, cm^{-1}: 3140, 3021, 2952, 2831, 2769, 1685, 1606, 1519, 1406, 1314, 1188, 1081, 969, 824, 756, 696, 636. ^{1}H NMR spectrum (400 MHz, DMSO-d_6) *δ, ppm*: 12.33 (Singlet, 1H), 12.04 (Singlet, 1H), 10.25 (Singlet, 1H), 8.50-7.25 (Complex, 8H), 3.98-3.93 (Singlet, 3H), 1.10 (Singlet, 1H). Mass (m/z): 426 (M^+), $C_{20}H_{15}ClN_4O_5$, Elemental Analysis Calculated %: C; 56.28, H; 3.54, N; 13.13, Found %: C; 56.55, H; 3.58, N; 13.25.

6-bromo-3-(6-(2-chlorophenyl)-5-nitro-2-oxo-1,2,3,4-tetrahydropyrimidin-4-yl)quinolin-2(1*H*)-one (MS-14): Yield 62%, White Solid, mp 176-178°C. IR spectrum (KBr), *v*, cm^{-1}: 3140, 3021, 2952, 2831, 2768, 1685, 1606, 1519, 1406, 1314, 1188, 1081, 969, 824, 755, 696, 634. ^{1}H NMR spectrum (400 MHz, DMSO-d_6) *δ, ppm*: 12.18 (Singlet, 1H), 12.05 (Singlet, 1H), 10.28 (Singlet, 1H), 8.52-7.25 (Complex, 8H), 1.10 (Singlet, 1H). Mass (m/z): 473 (M^+), $C_{19}H_{12}BrClN_4O_4$, Elemental Analysis Calculated %: C; 47.97, H; 2.54, N; 11.78, Found %: C; 47.95, H; 2.56, N; 11.72.

3-(6-(2-chlorophenyl)-5-nitro-2-oxo-1,2,3,4-tetrahydropyrimidin-4-yl)-6,8 dimethylquinolin-2(1*H*)-one (MS-15): Yield 54%, White Solid, mp 180-182°C. IR spectrum (KBr), *v*, cm^{-1}: 3140, 3012, 2952, 2830, 2768, 1685, 1606, 1519, 1406, 1314, 1188, 1081, 968, 824, 756, 696, 637. ^{1}H NMR spectrum (400 MHz, DMSO-d_6) *δ, ppm*: 12.26 (Singlet, 1H), 12.00 (Singlet, 1H), 10.22 (Singlet, 1H), 8.50-7.22 (Complex, 7H), 2.28 (Singlet, 6H), 1.12 (Singlet, 1H). Mass (m/z): 424 (M^+), $C_{21}H_{17}ClN_4O_4$, Elemental Analysis Calculated %: C; 59.37, H; 4.03, N; 13.19, Found %: C; 59.35, H; 4.05, N; 13.18.

3-(6-(2-chlorophenyl)-5-nitro-2-oxo-1,2,3,4-tetrahydropyrimidin-4-yl)-6-nitroquinolin-2(1*H*)-one (MS-16): Yield 56%, White Solid, mp 148-150°C. IR spectrum (KBr), *v*, cm^{-1}: 3141, 3021, 2951, 2831, 2768, 1685, 1606, 1519, 1406, 1314, 1188, 1081, 969, 824, 756, 696, 631. ^{1}H NMR spectrum (400 MHz, DMSO-d_6) *δ, ppm*: 12.22 (Singlet, 1H), 12.03 (Singlet, 1H), 10.21 (Singlet, 1H), 8.54-7.25 (Complex, 8H), 1.12 (Singlet, 1H). Mass (m/z): 441 (M^+), $C_{19}H_{12}ClN_5O_6$, Elemental Analysis Calculated %: C; 51.66, H; 2.74, N; 15.85, Found %: C; 51.68, H; 2.76, N; 15.88.

8-chloro-3-(6-(2-chlorophenyl)-5-nitro-2-oxo-1,2,3,4-tetrahydropyrimidin-4-yl)quinolin-2(1*H*)-one (MS-17): Yield 72%, White Solid, mp 142-144°C. IR spectrum (KBr), *v*, cm^{-1}: 3140, 3021, 2952, 2831, 2769, 1684, 1607, 1518, 1416, 1314, 1188, 1081, 969, 824, 756, 696, 632. ^{1}H NMR spectrum (400 MHz, DMSO-d_6) *δ, ppm*: 12.13 (Singlet, 1H), 12.05 (Singlet, 1H), 10.28 (Singlet, 1H), 8.50-7.24 (Complex, 8H), 1.14 (Singlet, 1H). Mass (m/z): 430 (M^+), $C_{19}H_{12}Cl_2N_4O_4$, Elemental Analysis Calculated %: C; 52.92, H; 2.80, N; 12.99, Found %: C; 52.90, H; 2.82, N; 12.95.

3-(6-(2-chlorophenyl)-5-nitro-2-oxo-1,2,3,4-tetrahydropyrimidin-4-yl)-8-methylquinolin-2(1*H*)-one (MS-18): Yield 56%, White Solid, mp 182-184°C. IR spectrum (KBr), *v*, cm^{-1}: 3178, 3042, 2827, 2794, 1697, 1510, 1390, 1173, 1025, 913, 847, 745, 698. ^{1}H NMR spectrum (400 MHz, DMSO-d_6) *δ, ppm*: 12.17 (Singlet, 1H), 12.02 (Singlet, 1H), 10.23 (Singlet, 1H), 8.51-7.20 (Complex, 8H), 3.15-3.92 (Singlet, 3H), 1.11 (Singlet, 1H). Mass (m/z): 410 (M^+), $C_{20}H_{15}ClN_4O_4$, Elemental Analysis Calculated %: C; 58.47, H; 3.67, N; 13.64, Found %: C; 58.40, H; 3.62, N; 13.60.

3-(6-(2-chlorophenyl)-5-nitro-2-oxo-1,2,3,4-tetrahydropyrimidin-4-yl)-8-methoxyquinolin-2(1H)-one (MS-19): Yield 62%, White Solid, mp 162-164 °C. IR spectrum (KBr), *v*, cm^{-1}: 3139, 3021, 2952, 2831, 2769, 1685, 1606, 1519, 1406, 1314, 1188, 1081, 969, 824, 756, 696, 636. ^{1}H NMR spectrum (400 MHz, DMSO-d_6) *δ, ppm*: 3.92-3.98 (Singlet, 3H), 1.10 (Singlet, 1H), 7.25 – 8.50 (Complex, 8H), 10.25 (Singlet, 1H), 12.04 (Singlet, 1H), 12.33 (Singlet, 1H). Mass (m/z): 426 (M^+), Ana. Calculated for Molecular formula $C_{20}H_{15}ClN_4O_5$, Elemental Analysis Calculated %: C; 56.28%, H; 3.54 %, N; 13.13 % Found C; 56.55%, H; 3.58 %, N; 13.25%.

ANTIMICROBIAL ACTIVITY:

Table-2: Antibacterial and antifungal activity of compounds MS-10 to MS-19

Code	Antibacterial activity				Antifungal activity		
	Antibacterial activity (zone in cm), concentration: 1 mg/mL. (0.2 mL)				Antifungal activity (zone in cm), concentration: 1mg/mL (0.2 mL)		
	Gram +*ve* bacteria		Gram –*ve* Bacteria				
	S. aureus	*S. pyogenus*	*P. aeruginosa*	*E. coli.*	*C. albicans*	*A. clavatus*	*A. niger*
MS-10	1.3	2.0	1.1	1.2	1.0	1.0	1.7
MS-11	1.0	1.5	**1.7**	**2.1**	**2.0**	1.5	1.5
MS-12	1.2	1.9	1.6	2.0	1.9	1.7	2.2
MS-13	1.2	2.0	1.3	1.1	0.2	1.3	1.7
MS-14	1.1	1.7	1.2	1.5	1.2	1.6	2.4
MS-15	1.3	1.1	1.3	1.5	1.7	2.3	1.2
MS-16	1.0	**2.1**	1.3	**2.0**	2.0	**2.1**	**3.0**
MS-17	**2.0**	1.8	1.0	1.1	1.2	1.6	2.2
MS-18	1.8	1.5	1.1	1.4	0.2	1.4	-
MS-19	1.0	1.2	1.3	1.7	1.9	1.7	1.7
Streptomycin (200µg/mL)	3.0	2	2	3.2	-	-	-
Ciprofloxacin (200µg/mL)	3.8	4	4	3	-	-	-
Nystatin (200µg/mL)	-	-	-	-	3.2	4	3.5

Antimicrobial activity is the process of killing or inhibiting the pathogenic microbes causing disease.[20] An antimicrobial is an agent that kills microorganisms or stops their growth.[21] Antimicrobials can be anti-bacterial, anti-fungal, or antiviral.[22] Agents that kill microbes are called microbicidal, while those that inhibit their growth are called microbistatic. All agents have different modes of action by which they act against infection. The use of antimicrobial medicines to treat infection is known as antimicrobial chemotherapy. In our current study antibacterial and antifungal activity was tested by the standard agar cup method.[23] All the synthesized compounds were tested for their in vitro antimicrobial activity against Gram +*ve* (*S. aureus, S. pyrogens),* Gram –*ve (E. coli, P. aeruginosa),* and fungal spp. *(C. albicans, A. clavatus,* and *A. niger),* taking streptomycin, ciprofloxacin, and nystatin as standard drugs. Suspension of 24 to 48 h. grown fresh bacterial and fungal culture was prepared in N-broth and potato dextrose broth respectively. All the bacterial and fungal suspension was equally spread onto the sterile Muller Hinton and PDA plates respectively with the help of sterile swabs. Wells were made in the plates (1 cm) with the help of a sterile cork borer. The standard antibiotics were dissolved in sterile distilled water to make the final concentration of 200µg/mL. The synthesized compounds to be tested were dissolved in DMSO up to the final concentration of 1 mg/ml and 0.1 mL of it was loaded in the well. The plate was incubated at 4˚C for 20 minutes for proper diffusion of a compound in agar and then the plates were incubated in the upward position for 24 h at 37˚C for bacterial culture and 48 h at 25˚C for fungal cultures. The control activity against DMSO was also performed. After incubation zone of inhibition was observed and measured.

RESULTS AND DISCUSSION:

The synthesis of various substituted 3-(6-(2-chlorophenyl)-5-nitro-2-oxo-1,2,3,4-tetrahydropyrimidin-4-yl) quinolin-2(1H)-one was carried out successfully. The route includes acetylation, oxidation, cyclization, nitroaldol reactions are performed successfully. **Total ten** compounds were synthesized and well-characterized by various spectroscopic techniques.

IN-VITRO BIOLOGICAL IVOLUATION:

Novel synthesized entities were investigated for their *in-vitro* antibacterial and anti fungal activity. The Bioassay result demonstrated that compounds **MS-10 to MS-19** succeeded to indicate remarkable activity against mentioned microorganism when compared to standard drugs.The result includes that **MS-11** and **MS-16** both compounds displaying notable activity which is shown in Table 1. The reason these compounds are active is because of the presence of groups increasing activity and therefore their activity increases by understanding the structure of two active compounds. **MS-11** and **MS-16** both contain methyl and nitro substitution respectively.

IN-SILICO EVALUATIONS:

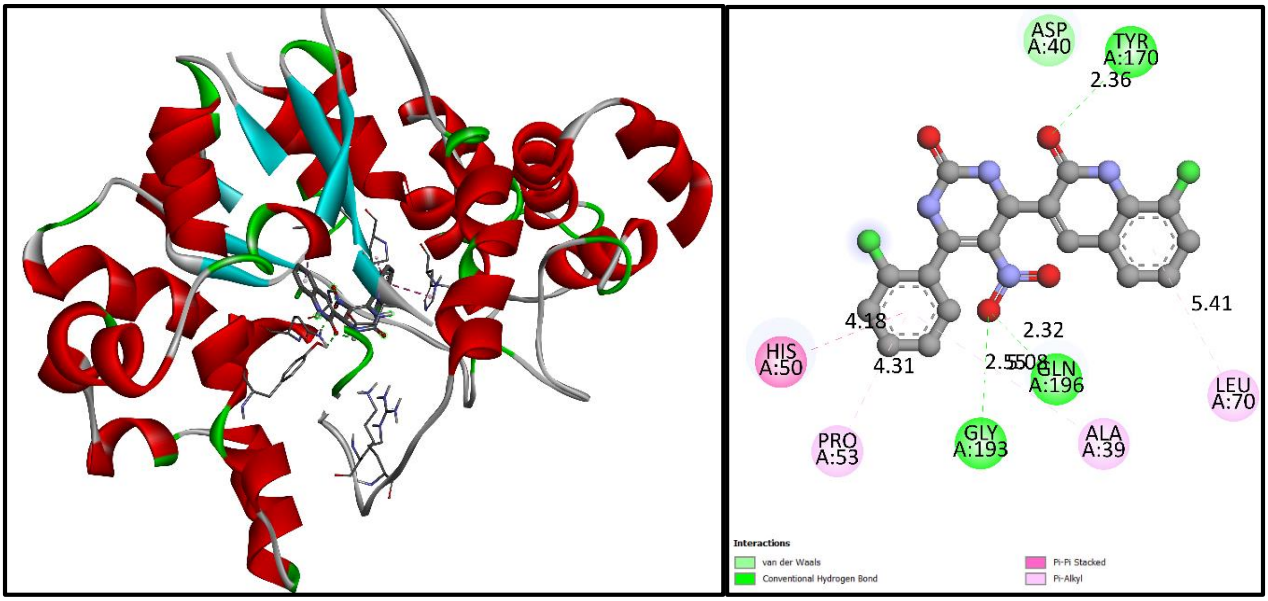
Table 3: Binding Energy (Site-specific Docking) Kcal/mol of compounds **10a** to **10j** to proteins.

	Protein Pdb:1JIJ	Protein Pdb:4ROT	Protein Pdb:4JVI	Protein Pdb:1KZN	Protein Pdb:1IYL	Protein Pdb:5C5G	Protein Pdb:1UKC
MS-10	-9.1	-6.2	-8.9	-8.4	-10.9	-7.8	-7.0
MS-11	-8.7	-6.3	**-9.4**	**-8.5**	**-11.0**	-8.3	-7.3
MS-12	-8.7	-6.3	-9.3	-8.3	-9.8	-8.2	-7.2
MS-13	-8.7	-6.5	-8.9	-7.5	-9.6	-7.9	-7.3
MS-14	-8.6	-6.4	-9.1	-7.8	-9.9	-8.1	-7.2
MS-15	-9.6	-6.1	-8.7	-8.4	-10.7	-8.0	-7.7
MS-16	-9.7	**-6.6**	-9.2	**-8.5**	-9.9	**-8.5**	**-7.8**
MS-17	**-10.5**	-6.3	-8.1	-8.3	-10.9	-7.2	-7.7
MS-18	-10.3	-6.3	-8.0	-8.1	-10.9	-7.1	-7.7
MS-19	-9.2	-6.4	-8.0	-8.0	-10.5	-7.2	-7.5

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 a negative binding energy. The compound **MS-17** had the highest binding energy of **-10.5** kcal/mol with site-specific docking. **MS-17** formed strong bonds with 1JIJ were Hydrogen bonds TYR:170 at 2.36 Å, GLY:193 at 2.55 Å, GLN:196 at 5.08 Å and Pi bonds HIS:50 at 4.18 Å.

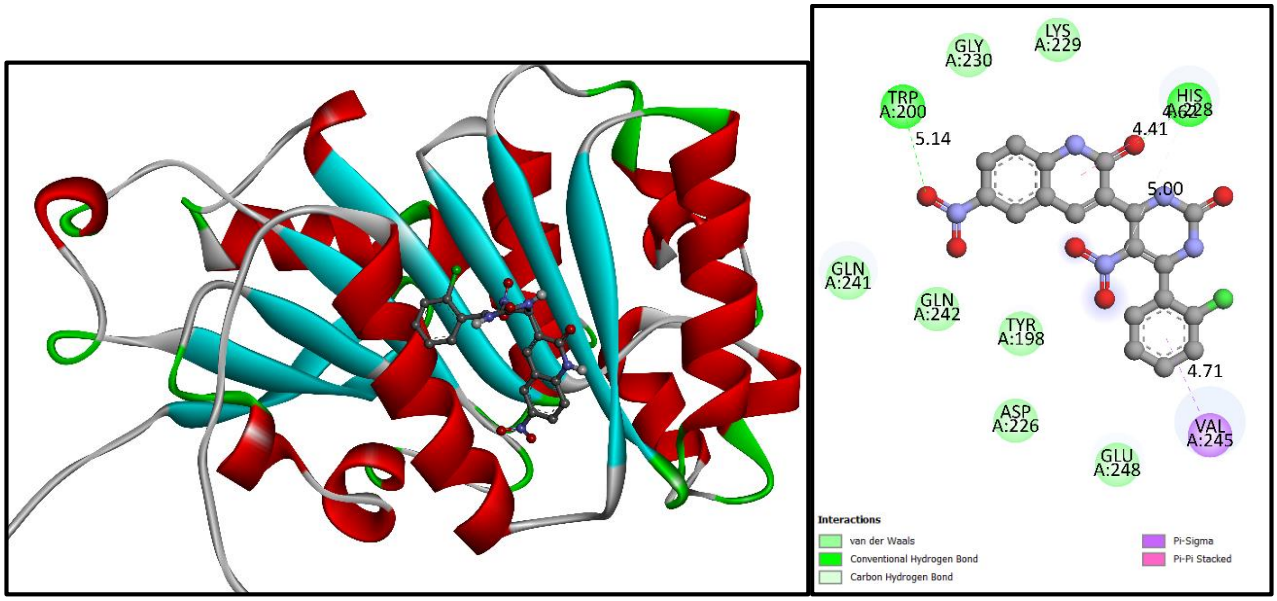
3D and 2D Interaction of **MS-17** with 1JIJ



Compounds against 4ROT

Site-specific Docking research (X: - 4.025250, Y: 31.469568, Z: 8.050351) found that on the target protein 4ROT, all compounds examined had a negative binding energy. The compound **MS-16** had the highest binding energy of **-6.6** kcal/mol with site-specific docking. **MS-16** formed strong bonds with 4ROT were Hydrogen bonds TRP:200 at 5.14 Å, HIS:228 at 4.62 Å, and pi bonds HIS:228 at 4.41 Å, and VAL:245 at 4.71 Å.

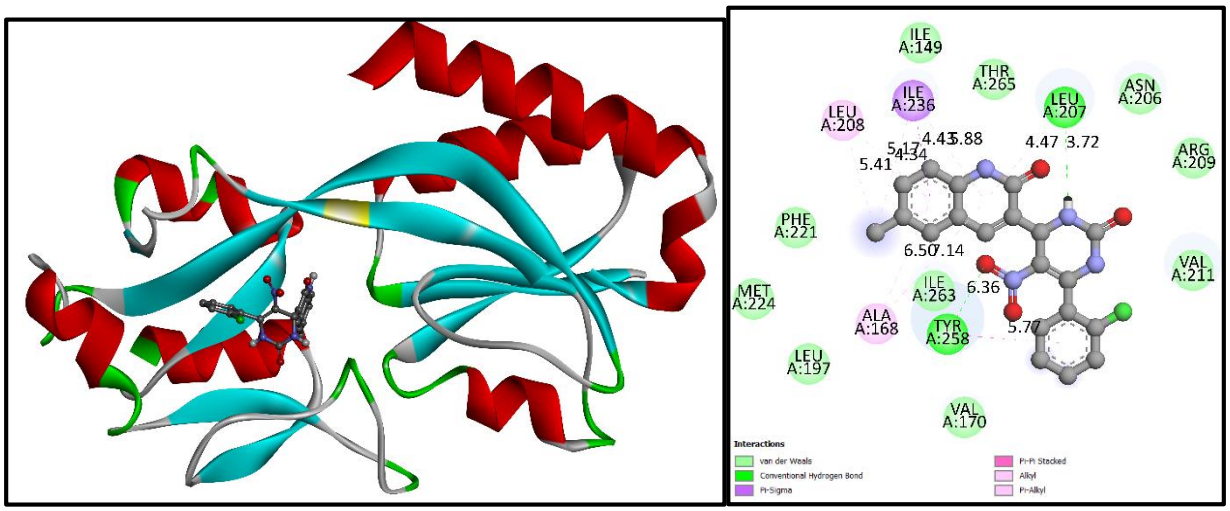
3D and 2D Interaction of **MS-16** with 4ROT



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 a negative binding energy. The compound **MS-11** had the highest binding energy of **-9.4** kcal/mol with site-specific docking. **MS-11** formed strong bonds with 4JVI were Hydrogen bonds LEU:207 at 3.72 Å, TYR:258 at 6.36 Å. and Pi bonds ILE:236 at 4.43 Å, TYR:258 at 5.77 Å.

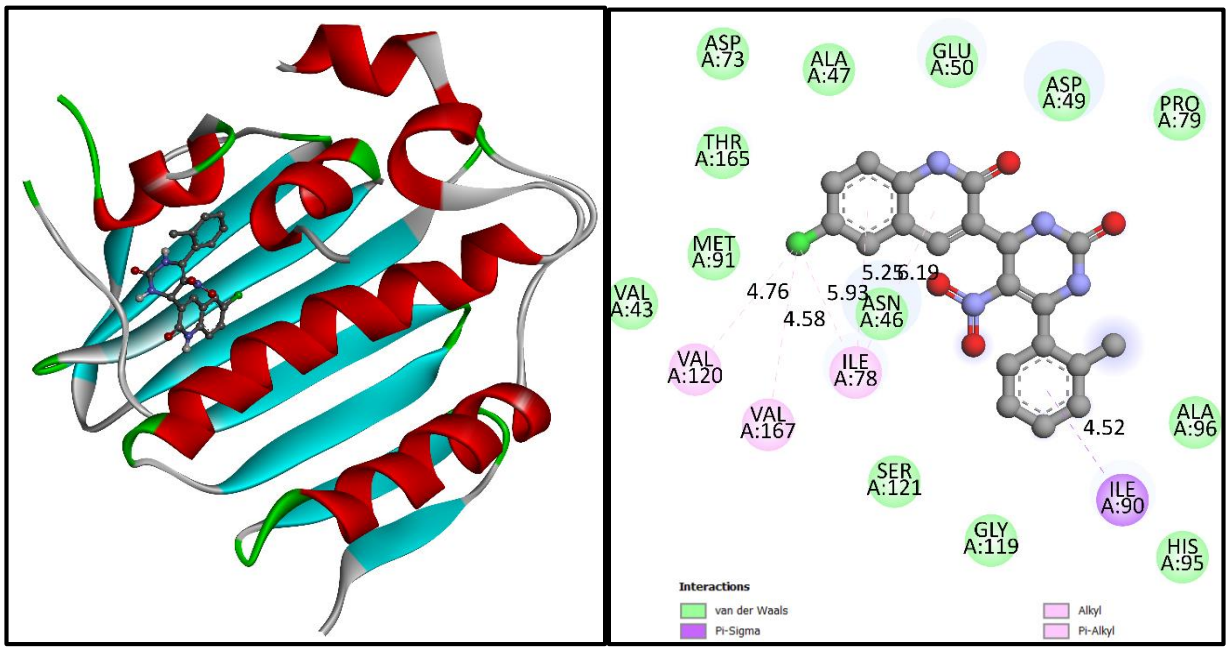
3D and 2D Interaction of **MS-11** with 4JVI.



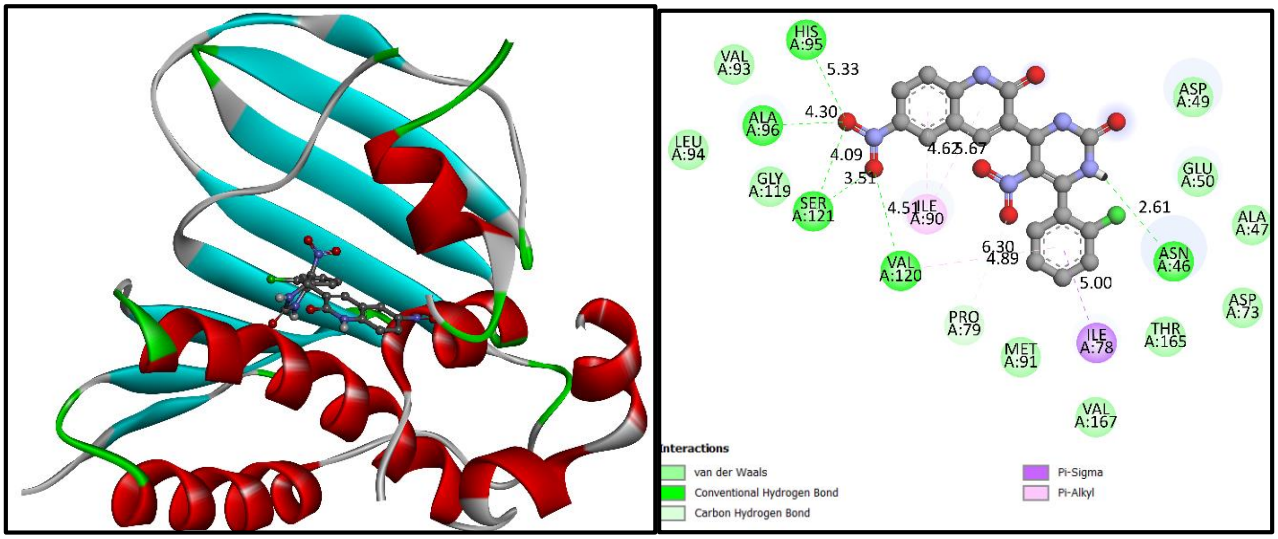
Compounds against 1KZN

Site-specific Docking research (X: 18.849850, Y: 23.905305, Z: 36.008089) found that on the target protein 1KZN, all compounds examined had a negative binding energy. The compound **MS-11** and **MS-16** had the highest binding energy of **-8.5** kcal/mol with site-specific docking. **MS-11** formed strong bonds with 1KZN were Pi bonds ILE:78 at 5.25, 5.93 and 6.19 Å, ILE:90 at 4.52 Å VAL:120 at 4.76 Å and VAL:167 at 4.58 Å and **MS-16** were Hydrogen bonds, ASN:46 at 2.61 Å, HIS:95 at 5.33 Å, ALA:96 at 4.30 Å, VAL:120 at 4.51 Å, SER:121 at 3.51 and 4.09 Å. and Pi bonds ILE:78 at 5.00 Å.

3D and 2D Interaction of **MS-11** with 1KZN.



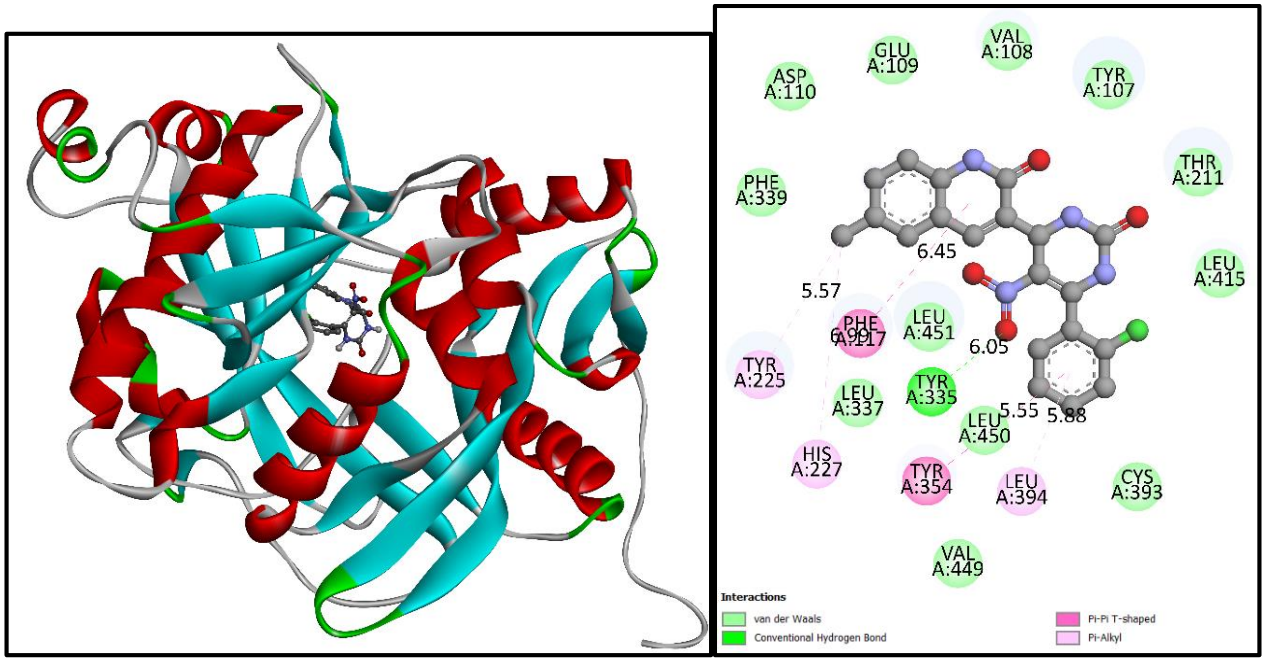
3D and 2D Interaction of **MS-16** with 1KZN.



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 a negative binding energy. The compound **MS-11** had the highest binding energy of **-11.0** kcal/mol with site-specific docking. **MS-11** formed strong bonds with 1IYL were Hydrogen bonds TYR:335 at 6.05 Å, and Pi bonds PHE:117 at 6.45 Å, TYR:354 at 5.55 Å.

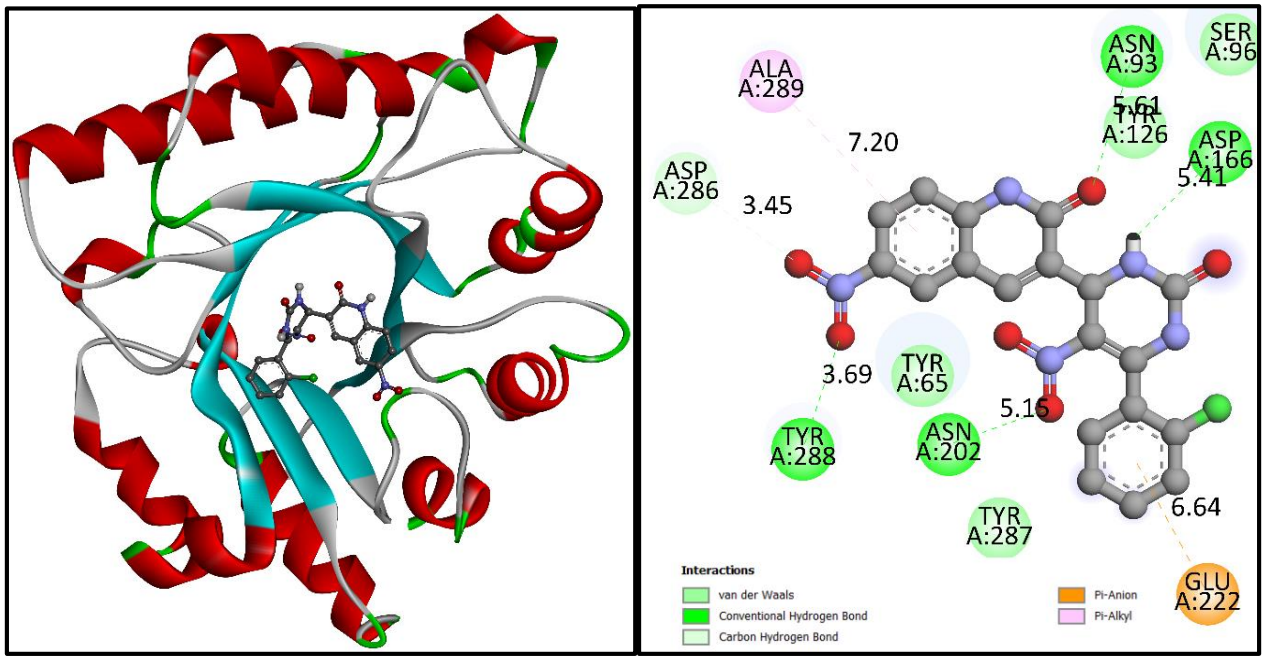
3D and 2D Interaction of **MS-11** with 1IYL.



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 a negative binding energy. The compound **MS-16** had the highest binding energy of **-8.5** kcal/mol with site-specific docking. **MS-16** formed strong bonds with 5C5G were Hydrogen bonds ASN:93 at 5.61 Å, ASP:166 at 5.41 Å, ASN:202 at 5.15 Å, TYR:288 at 3.69 Å, and Pi bonds GLN:222 at 6.64 Å.

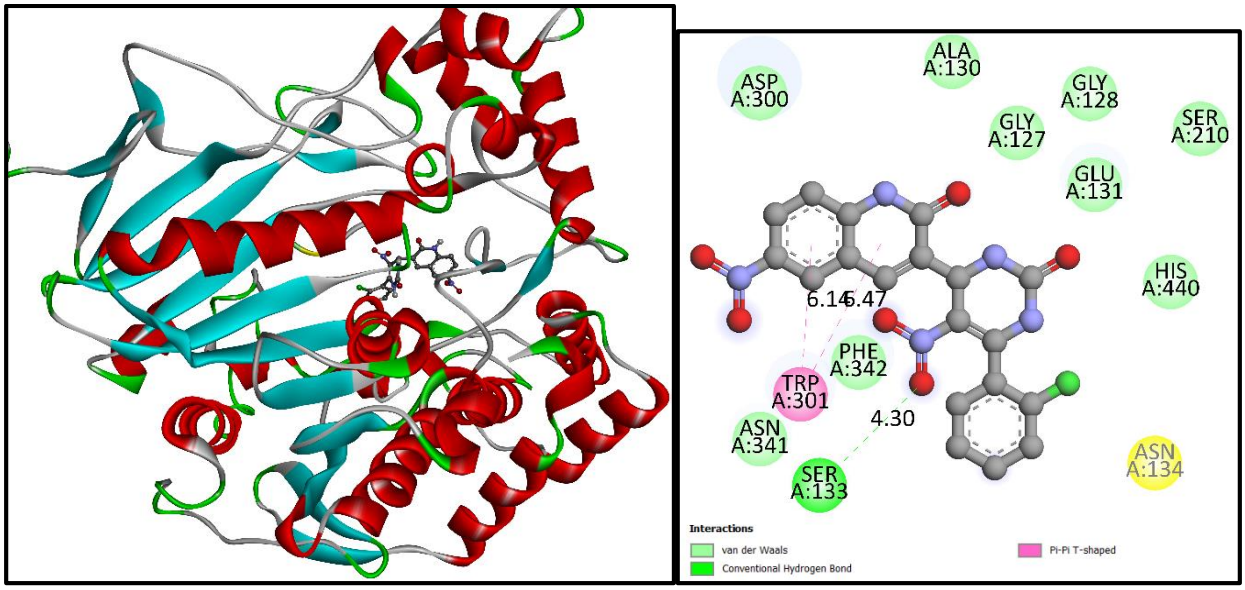
3D and 2D Interaction of **MS-16** with 5C5G.



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 **MS-16** had the highest binding energy of **-7.8** kcal/mol with site-specific docking. **MS-16** formed strong bonds with 1UKC were Hydrogen bonds SER:133 at 4.30 Å, and Pi bonds TRP:301 at 5.47 and 6.14 Å.

3D and 2D Interaction of **MS-16** with 1UKC



CONCLUSION:

The synthesis of various substituted 3-(6-(2-chlorophenyl)-5-nitro-2-oxo-1,2,3,4-tetrahydropyrimidin-4-yl) quinolin-2(1H)-one was carried out successfully. The route includes acetylation, oxidation, cyclization, nitroaldol reactions are performed successfully. **Total ten** compounds were synthesized and well-characterized by various spectroscopic techniques.

in-vivo and *in-silico* antibacterial and antifungal activity of the synthesized compounds reveals that some of the synthesized compounds show excellent drug-like bioactivity. Out of all compounds, **MS-11** and **MS-16** show remarkable antibacterial activity and antifungal activity so these compounds would be of better use in drug development against fungal infection and antibacterial infection.

DECLARATION OF COMPETING INTEREST

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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