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SYNTHESIS, CHARACTERIZATION AND ANTIBACTERIALBIOASSAY AND MOLECULAR MODELING STUDIES OF NOVEL(E)-N-(5-CHLORO-2-(2-OXOAZETIDIN-1-YL)BENZYLIDENE)BENZOHYDRAZIDE DERIVATIVES

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ABSTRACT:
A series of novel (E)-N'-(5-chloro-2-(2-oxoazetidin-1-yl)benzylidene)benzohydrazide derivatives(4a-j) were prepared by the reaction of 5-chloro-2-(2-oxoazetidin-1-yl)benzaldehyde with different benzohydrazides. The synthesized compounds were characterized by IR, Mass, and ^{1}H NMR and screened for their antibacterial activities. The compounds 4d, 4h and 4i bearing -NO_2, –OH, and-SO_2Me, moieties exhibited excellent antibacterial activity, compounds 4a, 4b and 4c with halo group's substitution, displayed moderate antibacterial activity. To understand the ligand–protein interactions in terms of the binding affinity, docking studies were performed using Molegro Virtual Docker (MVD-2013, 6.0) for the compounds **4(a-j)**with DNA gyrase protein. It was observed that the in silico docking analysis is in agreement with the biological activity.

KEY WORDS: Azetidinones; Hydrazones; Antibacterial Activity; Molecular Modeling

INTRODUCTION:

The chemistry of β-lactams plays an important role in organic chemistry since the discovery of Penicillin by Sir Alexander Fleming in 1928. Later Cephalosporins were discovered and both were used as successful antibiotics. Because of development of bacterial resistance to widely used antibiotics of this type a need for new active functionalized β-lactam series motivated a research in this area. The β –ring is a common structural feature of a number of broad spectrum β-lactam antibiotics including penicillins I, cephalosporins II, carbapenems III, nocardicin A IV and monobactams which have been widely used as chemotherapeutic agents to treat bacterial infection and microbial diseases[i]. These β-lactams (Azetidinones) are very important class of compounds possessing wide range of biological activities such as antibacterial,anti-inflammatory,antihyperlipidemic,CNS activity,tryptase inhibitory, human leukocyte elastase inhibitory,antihyperglycemic,vasopressinV1a antagonist, and anticancer activity,

antimicrobial,antitumor,antitubercular,cytotoxic,enzyme inhibitors, elastase inhibitors and cholesterol absorption inhibitors[ii-xvii], in recent past these derivatives are also found to be moderately active against several types of cancers. The biological activity of the β-lactams skeleton is generally believed to be associated with the chemical reactivity of their β-lactam ring and on the substituents especially at nitrogen of the 2-azetidinone ring[xviii].
Hydrazones, related to aldehydes and ketones belong to a class of organic compounds with the structure, R^1 R^2 C=NNH[xix], which contain C = N bond, conjugated with a lone pair of electrons of the functional nitrogen atom[xx]. These compounds possess diverse biological and pharmacological properties such as antimicrobial,anti-inflammatory, analgesic, antifungal, anti-tubercular, antiviral, anticancer, antiplatelet, antimalarial, anticonvulsant, cardio protective, antihelmintic, antiprotozoal[xxi], anti-trypanosomal[xxii], antischistosomiasisetc[xxiii].
The combination of hydrazones with other functional groups may lead to compounds with unique physical, chemical and biological behavior[xxiv].

This prompted us to take up the present work of synthesis of new azitidinone analogs by linking β-lactams with hydrazone motifs. The biological activity of the synthesized compounds may be augmented. The molecular modeling studies would help us to know the suitable chemical environment for high efficacy.

MATERIALS AND METHODS:

Chemicals and solvents used were purchased from Merck. All the reagents were of analytical grade. Thin-layer chromatography (TLC) was performed on E.Merck AL silica gel 60 F254 plates and visualized under UV light. IR spectra were recorded as KBr pellet with a Perkin-Elmer spectrum FTIR instrument and only diagnostic and/or intense peaks are reported. 1 H NMR spectra were recorded in DMSO- d6 with a Varian Mercury plus 400 MHz instrument. Signals due to the residual protonated solvent (1 H NMR) served as the internal standard. All the chemical shifts were reported in δ (ppm) using TMS as an internal standard. The 1 H NMR chemical shifts and coupling constants were determined assuming first-order behavior. Multiplicity is indicated by one or more of the following: s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), br (broad); the list of coupling constants (J) corresponds to the order of multiplicity assignment. Mass spectra were recorded with a PE Sciex model API 3000 instrument. The 2-bromo-5-chlorobenzaldehyde 1 and all the benzoic acids used for the preparation of 3a-j were purchased from commercial sources.

Experimental procedure for synthesis of 5-chloro-2-(2-oxoazetidin-1-yl) benzaldehyde2:
A mixture of CuI (0.69 mmol), 2-Bromo-5-chloro-benzaldehyde (8.43 mmol), 2-azetidinone (7.03 mmol), K_2CO_3 (14.03 mmol), N,N'-dimethylethylenediamine (1.4 mmol) and toluene (5.0 mL) was charged in a sealed tube under argon. The reaction mixture was stirred at 110°C for 6 h on a preheated oil bath. The resulting suspension was allowed to reach room temperature and diluted with EtOAc followed by water and filtered through celite bed. The organic layer was separated and washed with water, brine solution and dried over Na_2SO_4, filtered and concentrated. The residue was purified by flash chromatography on silica gel (100-200 mesh) using EtOAc: Pet-ether as eluant to afford pure compound 2. Pale yellow solid; Yield: 82%; M.p: 114-117°C; 1H NMR (400 MHz, $CDCl_3$) δ 10.19 (s, 1H), 7.85 (d, 1H, J = 2.4 Hz), 7.51 (dd, 1H, J = 2.4, 8.8 Hz), 7.35 (d, 1H, J = 8.4 Hz), 3.86 (t, 2H, J = 4.8 Hz), 3.22 (t, 2H, J = 4.4 Hz).

General procedure for synthesis of Benzohydrazide derivatives 3 (a- j): To a stirred solution of benzoic acids (6.42 mmol) in ethanol (3 mL) was added catalytic quantity of conc.H_2SO_4 and heated to reflux for 6 – 10 h. The reaction mixture was diluted with ethyl acetate followed by water. The organic layer was washed with saturated $NaHCO_3$ followed by water and brine

solution. The organic layer was dried over sodium sulphate, filtered and evaporated to obtain respective ethyl benzoates derivatives.

To a stirred solution of ethyl benzoates (3 mmol) derivatives in ethanol was added hydrazine-hydrate (5.44 mmol) and refluxed for 6 – 12 h. The reaction mixture was diluted with ethylacetate followed by water. The organic layer was dried over sodium sulphate, filtered and evaporated to obtain respective benzohydrazide derivatives 3 (a-j). The yields of the products varied from 75 – 88%.

Experimental Procedure for the Synthesis of Hydrazone derivatives 4(a-j): To a stirred solution of compound 2 (100 mg, 1.0 mmol) in ethanol was added corresponding benzohydrazides3(a-j) (1.0 mmol) and refluxed for 1 h. The reaction medium was poured into water and extracted with ethylacetate. The organic layer was washed with water followed by brine solution, dried over anhydrous sodium sulphate, filtered and concentrated under reduced pressure, to obtain the pure compounds. Yields of the products varied between 76 and 87%.

(E)-N'-(5-chloro-2-(2-oxoazetidin-1-yl)benzylidene)-2-iodobenzohydrazide (4a): Pale yellow solid; Yield: 82%; M.p: 122-123°C; IR (KBr): υmax 3442.3, 3219.6, 3048.9, 1746.2, 1724.0, 1680.7, 1661.7, 1538.9, 1483.0, 1417.4, 1407.8, 1364.4, 1285.3, 1256.4, 1150.3, 1015.3, 930.5, 883.2, 819.6, 744.0 cm-1;1H NMR (400 MHz, DMSO-d6): δ 12.07 (s, 1H), 8.48 (*8.26 , s, 1H), 7.96-7.94 (m, 2H), 7.54-7.50 (m, 4H), 7.35-7.10 (m, 1H), 3.83-3.80 (m, 2H), 3.11-3.08 (m, 2H); ESI-MS: m/z, 454 (M+H)+.

(E)-N'-(5-chloro-2-(2-oxoazetidin-1-yl)benzylidene)-2-bromobenzohydrazide (4b): Pale yellow solid; Yield: 76%; M.p: 112-113°C; 1H NMR (400 MHz, DMSO-d6): δ 12.10 (*12.08, s, 1H), 8.47 (*8.25 , s, 1H), 7.91 (m, 2H), 7.73-7.69 (m, 1H), 7.53-74 (m, 7H), 7.27 (s, 1H), 3.81 (* 3.76, s,1H), 3.09 (d, J = 4.0Hz, 2H); ESI-MS: m/z, 407.9 (M+H)+.

(E)-N'-(5-chloro-2-(2-oxoazetidin-1-yl)benzylidene)-3-chlorobenzohydrazide (4c): White solid; Yield: 80%; M.p: 116-117°C; IR (KBr): υmax 3440, 2249.6, 2123.2, 1665.2, 1659.4, 1642.1, 1631.5, 1055.8, 1027.9, 1008.6, 822.5, 759.8, 668.2, 624.8 cm-1;1H NMR (400 MHz, DMSO-d6): δ 12.16 (s, 1H), 8.61 (s, 1H), 7.95 (s, 1H), 7.89 (d, J = 9.2 Hz, 2H), 7.70 (d, J = 8.4 Hz, 1H), 7.60-7.52 (m, 3H), 3.86 (t, J = 4.8 Hz, 2H), 3.16 (t, J = 4.8 Hz, 2H); ESI-MS: m/z, 362.0 (M+H)+.

(E)-N'-(5-chloro-2-(2-oxoazetidin-1-yl)benzylidene)-3-nitrobenzohydrazide (4d): Yellow solid; Yield: 85%; M.p: 144-145°C; IR (KBr): υmax 3433.5, 1753.9, 1657.5, 1649.8, 1526.4, 1482.0, 1408.7, 1365.4, 1348.0, 1298.8, 1271.8, 1150.3, 723.2 cm-1; 1H NMR (400 MHz, DMSO-d6): δ 12.38 (s, 1H), 8.74 (s, 1H), 8.64 (s, 1H), 8.47 (d, J = 8.0 Hz, 1H), 8.38 (d, J = 8.0 Hz, 1H),7.91-7.88 (m, 2H), 7.57-7.51(m, 2H), 3.87 (t, J = 4.4 Hz, 2H), 3.16 (t, J = 4.4 Hz, 2H); ESI-MS: m/z, 373.0 (M+H)+.

(E)-N'-(5-chloro-2-(2-oxoazetidin-1-yl)benzylidene)-4-bromobenzohydrazide (4e):yellow solid; Yield: 78%; M.p: 118-119°C; 1H NMR (400 MHz, DMSO-d6): δ 12.11 (s, 1H), 8.61 (s, 1H), 7.88 (d, J = 5.6 Hz, 2H), 7.86 (s, 1H), 7.76 (d, J = 5.2 Hz, 2H), 7.53-7.50 (m, 2H), 3.84 (s, 2H), 3.14 (s, 2H); ESI-MS: m/z, 406.0 (M+H)+.

(E)-N'-(5-chloro-2-(2-oxoazetidin-1-yl)benzylidene)-4-chlorobenzohydrazide (4f): Pale yellow solid; Yield: 87%; M.p: 132-133°C; 1H NMR (400 MHz, DMSO-d6): δ 12.11 (s, 1H), 8.61 (s, 1H), 7.95 (d, J = 6.8 Hz, 2H), 7.89 (s, 1H), 7.63 (d, J = 8.0 Hz, 2H), 7.53 (s, 2H), 3.84 (s, 2H), 3.14 (s, 2H); ESI-MS: m/z, 362.3 (M+H)+.

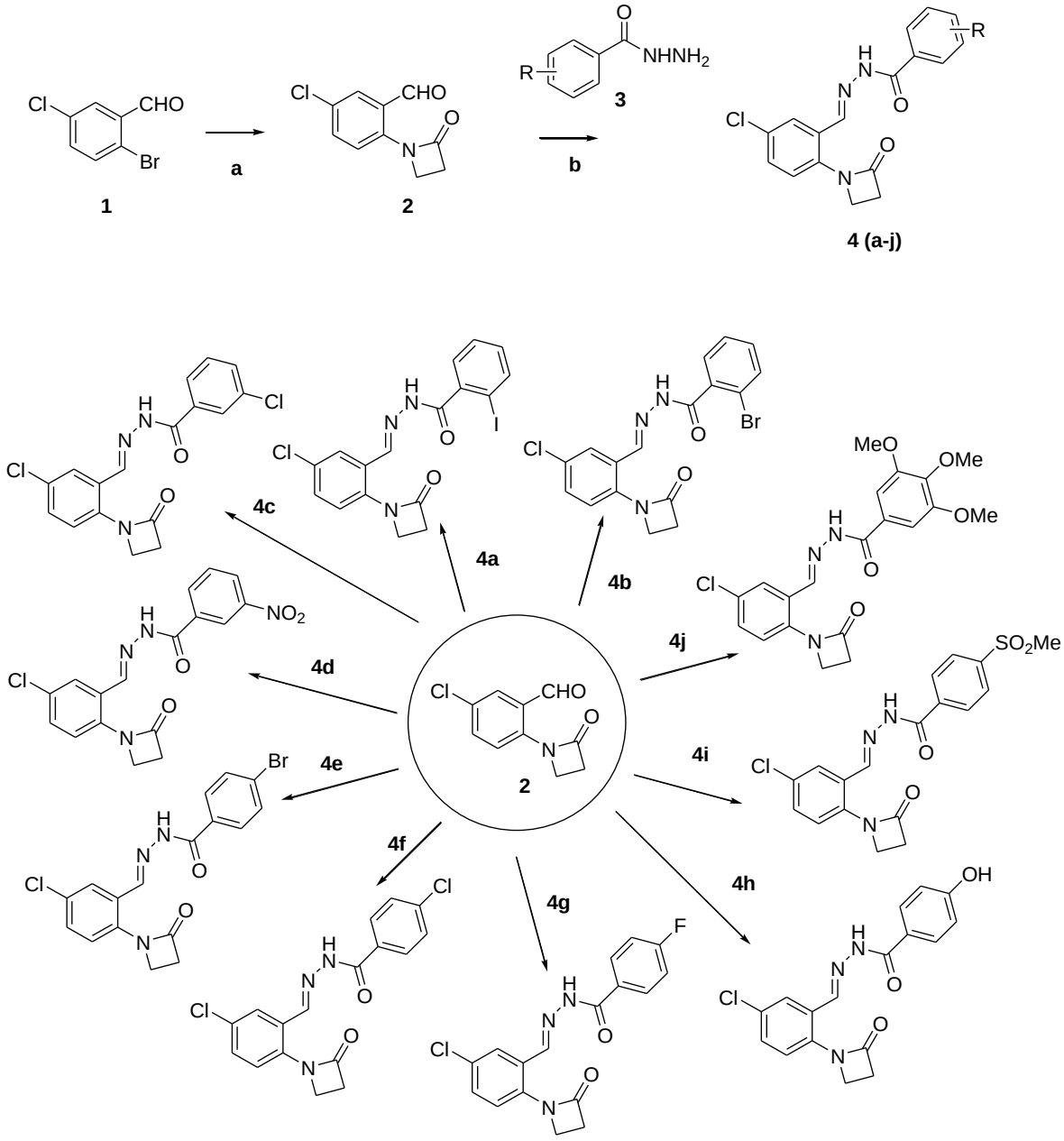
2.4.7.**(E)-N'-(5-chloro-2-(2-oxoazetidin-1-yl)benzylidene)-4-fluorobenzohydrazide**

(4g): Yellow solid; Yield: 80%; M.p: 122-123°C; IR (KBr): υmax 3654.4, 3434.6, 2250.2, 2124.2, 1729.8, 1659.4, 1650.8, 1556.3, 1538.9, 1485.9, 1372.1, 1283.4, 1055.8, 1027.9, 1008.6, 823.5,760.8 cm-1; 1H NMR (400 MHz, DMSO-d6): δ 12.09 (s, 1H), 8.61 (s, 1H), 8.01 (t, J = 5.6 Hz, 2H), 7.89 (s, 1H), 7.53 (s, 2H), 7.41 (t, J = 8.8 Hz, 2H), 3.86 (t, J = 4.4 Hz, 2H), 3.15 (t, J = 4.4 Hz, 2H); ESI-MS: m/z, 346.0 (M+H)+.

(E)-N'-(5-chloro-2-(2-oxoazetidin-1-yl)benzylidene)-4-hydroxybenzohydrazide (4h): White solid; Yield: 75%; M.p: 146-147°C; IR (KBr): υmax 3433.2, 3284.2, 1739.5, 1728.9, 1648.8, 1603.5, 1510.10, 1478.2, 1376.0, 1282.4, 1175.4, 853.3, 763.7 cm-1; 1H NMR (400 MHz, DMSO-d6): δ 11.86 (s, 1H), 10.16 9s, 1H), 8.58 (s, 1H), 7.83 (brs, 1H), 7.81 (d, J = 8.8 Hz, 2H), 7.53-7.50 (m, 2H), 6.88 (d, J = 8.8 Hz, 2H), 3.85 (t, J = 4.4 Hz, 2H), 3.15 (t, J = 4.4 Hz, 2H); ESI-MS: m/z, 344 (M+H)+.

(E)-N'-(5-chloro-2-(2-oxoazetidin-1-yl)benzylidene)-4-(methyl)sulfonyl)bromobenzohydrazide (4i): Yellow solid; Yield: 77%; M.p: 133-134°C; IR (KBr): υmax 3443.3, 3182.0, 3054.7, 2923.6, 2833.9, 1763.6, 1748.2, 1659.4, 1562.1, 1551.5, 1405.9, 1376.0, 1349.0, 1319.1, 1299.8, 1157.1, 1145.5, 1133.0, 1087.7, 963.3, 939.2, 782.0, 754.0 cm-1; 1H NMR (400 MHz, DMSO-d6): δ 12.29 (s, 1H), 8.63 (s, 1H), 8.15-8.09 (m, 4H), 7.91 (s, 1H), 7.55-7.48 (m, 2H), 3.88 (t, J = 6.8 Hz, 2H), 3.33 (s, 3H), 3.17 (t, J = 7.6 Hz, 2H); ESI-MS: m/z, 406 (M+H)+.

(E)-N'-(5-chloro-2-(2-oxoazetidin-1-yl)benzylidene)-3,4,5-trimethoxybenzohydrazide (4j): Pale yellow solid; Yield: 79%; M.p: 125-126°C; IR (KBr): υmax 3443.3, 3229.2, 3063.4, 2836.8, 1759.7, 1643.1, 1584.2, 1551.5, 1504.2, 1483.0, 1415.5, 1364.4, 1351.9, 1332.6, 1237.1, 1128.2, 1074.2, 856.2, 771.4 cm-1; 1H NMR (400 MHz, DMSO-d6): δ 11.96 (s, 1H), 8.59 (s, 1H), 7.9 (s, 1H), 7.56-7.52 (m, 2H), 7.21 (s, 2H), 3.86 (s, 6H), 3.84 (d, J = 4.4 Hz, 2H), 3.73 (s, 3H), 3.15 (t, J = 4.4 Hz, 2H); ESI-MS: m/z, 418 (M+H)+.



Scheme.1 Synthesis of Hydrazone derivatives 5-chloro-2-(2-oxoazetidin-1-yl) benzaldehyde **(4a–j)**

Experimental Conditions: a) 2-Azetidinone, CuI, K_2CO_3, DMF, Toluene, 110°C, 6 h;
b) Benzohydrazides**3(a-j)**, ethanol, reflux, 1 h.

ANTIBACTERIAL ASSAY:

The synthesized novel (E)-N-(5-chloro-2-(2-oxoazetidin-1-yl)benzylidene)benzohydrazide derivatives 4a-j, were dissolved in dimethylsulphoxide at 25 µg/mL concentration and screened against Gram negative strains: i) Escherichia coli (MTCC 443), (ii) Pseudomonas aeruginosa(MTCC 424) and Gram positive strains: (iii) Staphylococcus aureus(MTCC 96) strains iv) Streptococcus pyogenes(MTCC 442) using agar well diffusion method according to the literature protocol[xxv-xxvii]. The composition of nutrient agar medium was Bactotryptone (10 g), Yeast extract (5 g), NaCl (10 g), final pH 7.4.

After 18 h the exponentially growing cultures of the four bacteria in nutrient broth at 37°C were diluted in sterile broth. From each of these diluted cultures, 1mL was added to 100 mL sterilized

and cooled nutrient agar media to give a final bacterial count of 1 x 106 cell/ml. The plates were set at room temperature and later dried at 37°C for 20h. Paper discs (6mm, punched from whatmann no 41 paper) were ultraviolet sterilized and used for the assays. Discs were soaked in different concentration of the test solution and placed on the inoculated agar media at regular intervals of 6-7 cm, care was taken to ensure that excess solution was not on the discs. The plates were incubated at 37°C in an inverted fashion. Activity was determined by zones showing complete inhibition (mm). Growth inhibition was calculated with reference to positive control. All the samples were taken in triplicates.

MOLECULAR MODELING STUDIES:

Model building: The current study is performed using the following programs. Molegro Virtual Docker ((MVD-2013, 6.0) which performs flexible ligand docking. The structure of hydrazone derivatives was drawn in ChemSketch and the molecule was saved in MolDock format. Molegro module works in five steps:

Step 1: Start with crystal coordinates of target receptor

Step2: Generate molecular surface for receptor

Step 3: Generate spheres to fill the active site of the receptor: the spheres become potential locations for ligand atoms

Step 4: Matching: Sphere centers are then matched to the ligand atoms, to determine possible orientations for the ligand

Step 5: Scoring: Find the top scoring orientation

RESULTS AND DISCUSSION:

The synthesized hydrazone derivatives were represented in **Scheme-1**. Initial compound 5-chloro-2-(2-oxoazetidin-1-yl) benzaldehyde **2**, was synthesized in good yield by condensation of 2-bromo-5-chloro-benzaldehyde and 2-azetidinone in presence of CuI, K_2CO_3 in DMF as solvent. Condensation of compound **2** with various benzohydrazide derivative **3(a-j)** under reflux condition in ethanol, furnished corresponding hydrazones **4(a-j).**The structures of all the newly synthesized compounds were confirmed by ^{1}H NMR, mass and IR spectroscopic techniques. Formation of 5-chloro-2-(2-oxoazetidin-1-yl) benzaldehyde **2**wasconfirmed by^{1}H NMR analysis. The proton signal due to –CHO was observed at 10.19 ppm and the two methylene (-CH_2)protons of the azetidinone is centered at δ values 3.86 and 3.22 ppm as triplets, with two proton integration. The structures of the synthesized compounds **4(a-j**) were confirmed on the basis of absence of the aldehydic proton, and the appearance of a new signal due to the azomethine (-CH=N) group appeared at *δ* values between 8.26 - 8.64 ppm in all the compounds. The –C=O-**NH**- protons appearing as singlets resonated at δ values between 11.86 and 12.38 ppm. Furthermore, the protons of compounds **4a** and **4b** –(-C=O-NH and -CH=N-) exhibited as two separate signals in ^{1}HNMR spectra in between 12.07 – 12.10 ppm and 8.25 - 8.48 ppm respectively due to the nitrogen inversion.

In the IR spectra, all derivatives **4(a-j**) exhibited a strong characteristic band in the region 1763-1650 cm^{-1} due to the C=O stretching vibration. The N-H stretching vibration of compounds **4(a-j**) gave rise to a band at 3654.4- 3219.6 cm^{-1}.The stretching bands for C=C and C=N groups were observed at 1642.1 - 1483.0 cm^{-1}. The mass spectra of the compounds showed (M+1) peaks, and are in agreement with their molecular formula.

ANTIBACTERIAL ACTIVITY:

The outcome of the antibacterial activity information of the newly synthesized (*E*)-N-(5-chloro-2-(2-oxoazetidin-1-yl)benzylidene)benzohydrazide derivatives **4(a-j)**is presented in **Table.1** The synthesized compounds have shown broad spectrum of activity against gram

negative and gram positive bacterialstrains.Within theseries of hydrazone derivatives **4(a-j)**, it is observed that compounds **4d, 4h** and **4i** exhibited elevated antibacterial activity against gram negative bacterial strain E.Coli MTCC443, 4h and 4i have shown excellent antibacterial activity against all the testedbacterial strains both gram negative and gram positivecompared to standard drug, while the compounds **4g** and **4j** displayed equipotent activity while the compounds **4a-4c, 4e** and **4f** displayed moderate antibacterial activity against all the tested bacterial strains *viz.*,*Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*and *Streptococcus pyogenes*. The significant antibacterial activity of compounds **4d, 4h and 4i** is attributed to the presence of -NO_2, -OH and -SO_2Me substituents, while the compounds **4g** and **4j** having the substituent's 4-F and 3,4,5-OMe displayed equipotent antibacterial activity and all the remaining compounds **4a-4c, 4e and 4f** displayed moderate activity. From the above antibacterial data it may be suggested that, by further hit and trials on modification of R in the (*E*)-N-(5-chloro-2-(2-oxoazetidin-1-yl)benzylidene)benzohydrazide derivatives **4a-j,** thederivatives may lead to a promising antibacterial agent.

MOLECULAR DOCKING STUDIES:

The *in vitro* antibacterial bioassay results were promising and to further validate our experimental results, molecular docking studies for the selected compounds **4(a-j)** were carried out to predict the most probable mode of binding of the hydrazone derivatives with topoisomerase ATPase enzyme using Molegro virtual docker (MVD-2013, 6.0)[xxviii], module of receptor-ligand interaction section available under Discovery studio client 3.5. The Hyperchemsoftware[xxix] calculation has been used to get energy minimized, geometry optimized structures of the compounds. The optimized structures are used as ligands during docking studies in the binding site of protein (pdbId :3TTZ) cavity. The docking studies reveal crucial information regarding interaction mode of ligand with enzyme inside binding pockets. **Figure 2** shows the native crystal structure of 2-[(3S,4R)-4-{[(3,4-dichloro-5-methyl-1H-pyrrol-2-yl)carbonyl]amino}-3-fluoropiperidin-1-yl]-1,3-thiazole-5-carboxylic acid bound to the active site of topoisomerase ATPase enzyme obtained from protein data bank[xxx], with the PDB ID: 3TTZ). Docking data of the synthesized compounds are given in Table 2. Interaction energy and antibacterial activity have shown good correlation. The most favored binding modes of hydrazone derivatives **4(a-j)** and standard drug Norfloxacin with topoisomerase ATPase are shown below. The amino acid residues actively involved in the active site of our interest are Arg198,leu202,leu205,Val165,Lys163,His150,Tyr141,Glu164,Val48,Trp49,Glu50,Val52,Asp53,His45,His46,Arg42,Ile148,Ile56,Leu202,His16,Lys163,Glu41 etc.

For the compounds **4(a-j)**, the MolDock score ranged from-113.159 Kcal/mol to -128.499 Kcal/mol**(Table 2)** while the MolDock score for standard drug Norfloxacin was -75.9403 Kcal/mol. The best poses of the docked compound are shown in both Molegro Virtual docker (MVD-2013, 6.0) and Discovery studio client 3.5.

The compounds have shown hydrogen bonding interactions and aromatic π interactions with the active site amino acids, the common amino acids involved interaction with the compounds are Arg42, Try49, Arg198, His45, 46, Asn54, Ser55,Lys163. The nitrogen and oxygen of the hydrazide group of compound **4h** shows hydrogen bond interactions with Asn54, ser55, ser129 and aromatic π interactions with amino acids Val131, Ser129, Ile86. The sulphonyl oxygen of compound **4i** shows hydrogen bonding interactions with His46, aromatic π interactions with Try49, Arg42, Arg198, His45, 46. The nitrogen atom of hydrazide group and azitidine ring in **4d** compound shows hydrogen bond interaction with Arg42. Oxygen atom of -NO_2 group on aromatic ring shows hydrogen bond interactions with Lys163 and aromatic π interactions with amino acids His45, 46, Trp49.

Table.1 Antibacterial activity of compounds **4(a-j) zone of inhibition (mm)**

Compound No.	R	Gram negative		Gram positive	
		E.coli MTCC443	P.aeruginosa MTCC424	S.aureus MTCC96	S.pyogenes MTCC 442
		Zone of inhibition		Zone of inhibition	
4a	2-iodo	16	14	15	14
4b	2-Bromo	14	15	14	12
4c	3-Chloro	16	12	16	12
4d	3-Nitro	27	22	27	22
4e	4-Bromo	14	12	15	14
4f	4-Chloro	15	12	14	12
4g	4-Fluoro	25	19	25	19
4h	4-Hydroxy	28	24	27	21
4i	4-Methylsulfonyl	27	23	26	23
4j	3,4,5-Trimethoxy	25	19	25	19
Standard Drug	Norfloxacin	25	19	25	19

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Figure.1 In vitro antibacterial activities of 4(a-j).

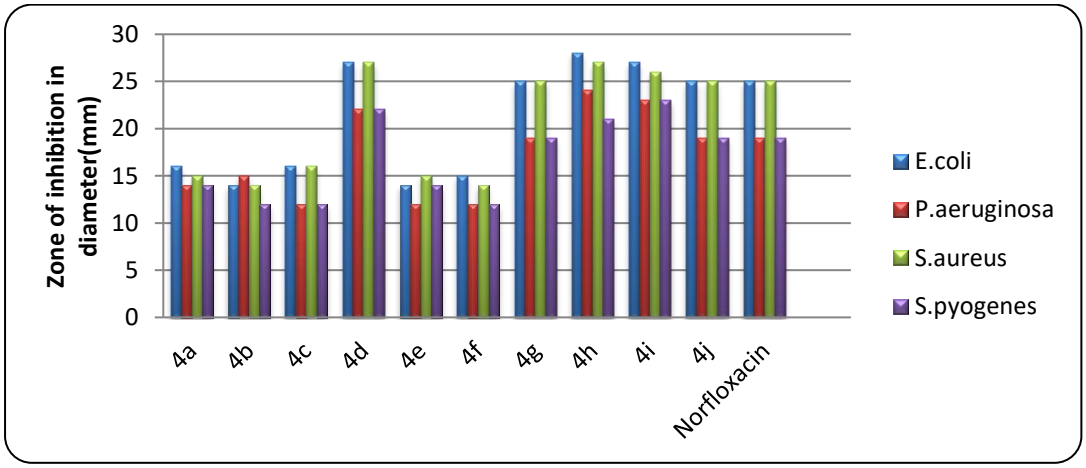


Table.2

S.No	Ligand	Moldock score[Grid]	Moldock score	Rerank score	RMSD	Torsions
1	**4a**	-113.159	-112.095	-79.1404	22.525	4
2	**4b**	-113.297	-108.432	-80.5497	23.2037	4
3	**4c**	-125.193	-119.303	-84.2329	23.0558	4
4	**4d**	-122.258	-118.48	-96.7951	27.2207	5

5	**4e**	-117.176	-106.175	-85.8318	29.6359	4
6	**4f**	-114.962	-111.498	-72.0984	33.4782	4
7	**4g**	-116.493	-110.526	-76.9324	20.2802	4
8	**4h**	-128.499	-125.773	-81.1847	25.1247	4
9	**4i**	-127.553	-124.139	-77.014	28.1245	5
10	**4j**	-128.093	-126.185	-86.7551	27.9916	5
11	**Norfloxacin**	-75.9403	-64.2549	-49.2883	29.5459	2

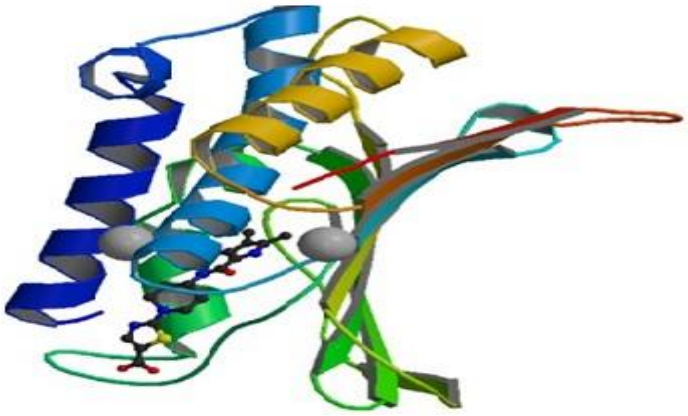
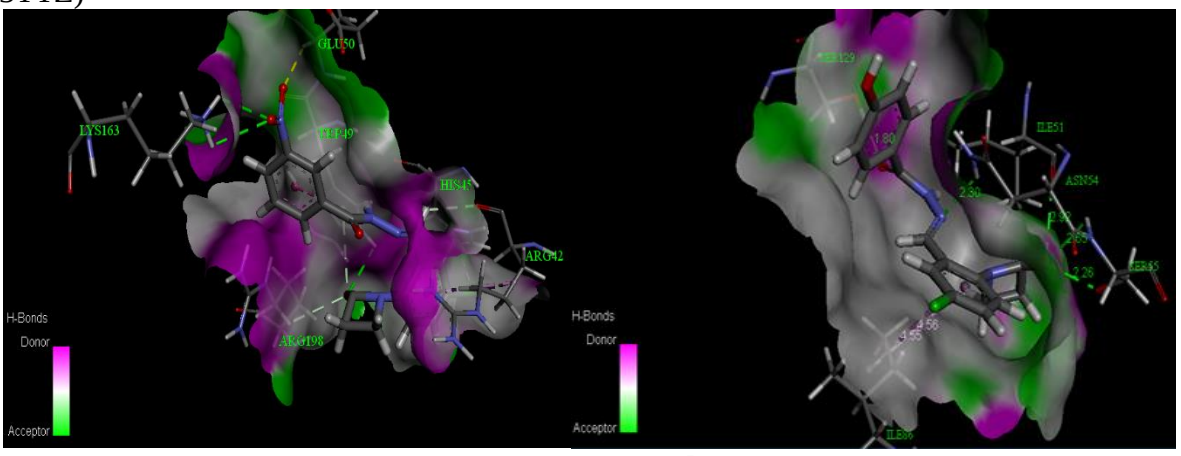
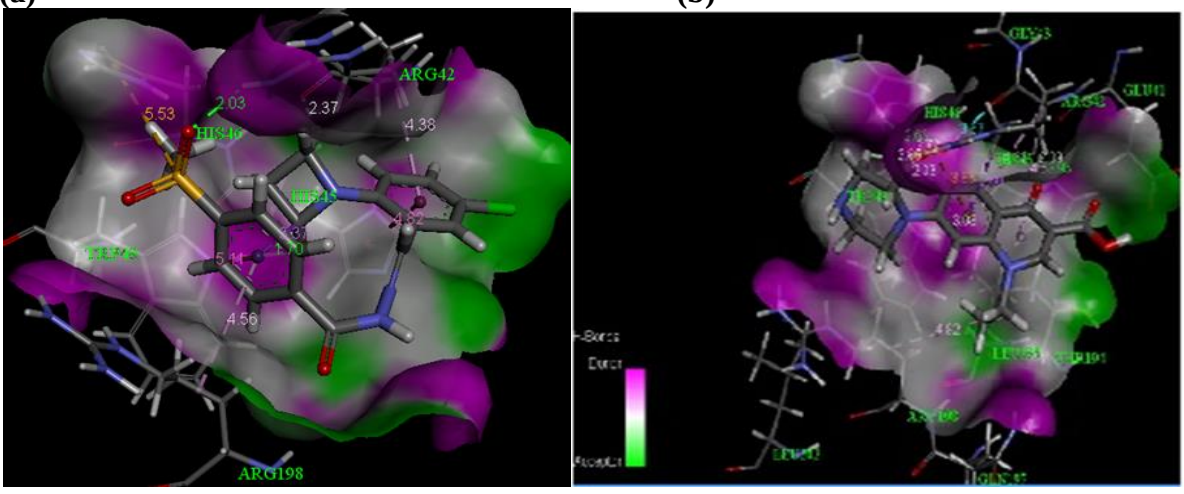


Figure.2 Secondary structure (complete protein) of topoisomerase ATPase enzyme (PDB ID 3TTZ)



(a) **(b)**



(c) **(d)**

Figure 3: Showing the compounds docked in best of its confirmation into the binding site of 3TTZ with Discovery studio client 3.5**(a)**molecule **4d** in the active site of3TTZ (topoisomerase ATPase enzyme)showing hydrogen bond interactions between N of hydrazide and azitidine ring and Arg42, O of -NO_2 with Lys163 and aromatic π interactions with amino acids His45, 46, Trp49. **(b)**molecule**4h**in the active site of3TTZ (DNA gyrase protein) showing hydrogen bond interactions between Asn54, ser55, ser129 and aromatic π interactions with amino acids Val131, Ser129, Ile86**(c)**molecule **4i** in the active site of 3TTZ(DNA gyrase protein) showing hydrogen bond interactions between His46 (2.03) aromatic π interactions with Try49, Arg42, Arg198, His45, 46**(d)** Binding mode of **Norfloxacin** with 3TTZ.

CONCLUSION:

All the synthesized azetidinone derivatives have shown good activity against both gram negative and gram positive bacterial strains.The compounds 4d, 4h and 4i with Nitro,Hydroxy and Methyl sulphonyl group have shown good activity, while compounds 4g and 4j containing Flouro and methoxy group exhibited equipotent activity, 4a-4c, 4e and 4f containing halo groups displayed moderate activity, the in silico studies reveal the compounds 4d, 4h and 4i with good dock score and best binding energy values are found to be best fitting ligands with in the active site of topoisomerase ATPase enzyme. The molecular modeling studies results, correlated well with the experimental biological activity. The hydrogen bonding and aromatic π interactions and electron withdrawing groups are found to be responsible for high efficacy of hydrazone derivatives.

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