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Thiosemicarbazone and Benzimidazole Hybrid Molecules: The Privileged Scaffolds for Anti-Bacterial Activity

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Abstract:
Ten thiosemicarbazones and Benzimidazole Hybrid molecules were synthesized and purified to investigate antibacterial activity. MIC values of the compounds were determined by the Cup borer method against *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus mirabilis*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *streptococcus pneumonia.* 2MOQ is an important target for antibacterial drugs. Despite the development of resistance against 2MOQ inhibitors drugs, there is still significant potential for designing new chemical entity with affordable, safe and efficacious antibacterials. In present study thiosemicarbazone-benzimidazole hybrids were designed and interaction of these conjugate hybrids was investigated by docking studies in the binding site of PDB ID: 2MOQ using Glide v 5.6. Among the series of designed compounds five compounds with good potential were synthesized. Structural confirmation of these compounds was done by FT-IR, 1H-NMR and Mass spectroscopy. The activity of compound AD2, AD3, AD7, AD8 and AD10 was found to be good against various strains. The above study could be very useful for further design and development of new antibacterials.

Keywords: 2MOQ, thiosemicarbazone hybrid molecules, docking, antibiacterials.

1. INTRODUCTION

Infectious disease is generally caused by microbes called pathogen, which enter into the body and affect the body to function properly. Human beings are been in constant exposure to pathogen like bacteria, fungi, viruses and protozoa from many decades. WHO report estimated that 26 percent of annual deaths occur around the world because of microbial diseases [1]. The incidence of microbial infection has increased on frightening level over the world as a result of development of antimicrobial resistance to currently available antimicrobial drugs, so there is urgent need for development of new antimicrobial agents which have a broad spectrum of activity against the resistant micro-organism. The most recent studies and reported literature revealed that heterocycles containing an azole ring system are found to exhibit a wide spectrum of biological activities like antimicrobial, anti-inflammatory, anticonvulsant, antituberculosis, anticancer and antihyperlipidemic activities. Several marketed drugs such as Acetazolamide, Methazolamide (antiglaucoma), and sulfamethizole, Cefazedone, Cefazolin, Ceftezole (antibacterial), [2],[4]. In addition of benzimidazole this benzimidazole nucleus and various biological compounds such as purine base of the DNA are having same structure.It combined with thiosemicarbazone due to its iron-chelating properties overcome rapid development of anti-bacterial resistance problems. Encouraged by these results and research towards the synthesis of novel antibacterial agents. New series of thiosemicarbazone- benzimidazole hybrids were designed to develop structurally diverse series of compounds in order to gain structural insight for improved antibacterial activity [3]. Thus in the present study, the synthesis and antibacterial activity of a new series of thiosemicarbazone-benzimidazole hybrids is reported Also, the interaction of these hybrids in the binding site of 2MOQ using Glide docking studies was also studied.

2. MATERIALS AND METHODS

2.1 Computational studies

2.1.1 Molecular Docking

Docking studies were carried out using Glide v5.6, Schrödinger LLC, NewYork (http:// www. Schrodinger.com). The computational process of searching for a ligand that is able to fit both geometrically and energetically into the binding site of protein is called molecular docking. All the compounds were docked in active site of protein [PDB ID: 2MOQ]. Molecular docking studies involved ligand preparation, protein preparation, receptor grid generation, docking studies and further analysis of docking results. The steps involved in docking studies are as follows:

2.1.2 Chem Draw Ultra 2D 8.0

The 2Dstructure of thiosemicarbazone and benzimidazole hybrid derivative was drawn by using Chem Draw Ultra 8.0 developed by Cambridge Pvt. Ltd and the structure of each compound was analyzed for correction error in bond order and converted to 3D structure with the help of 3D optimization tool was saved in .mol file compatible with maestro format.

2.1.3 Ligand preparation

All the built compound structures with their 3D .mol file were imported in maestro v9.1. By using the LigPrep version 2.4 (2010), the drawn ligand was geometry optimized and partial atomic charges were computed by using the Optimized Potentials for Liquid Simulations 2005 (OPLS 2005) force field. Various possible ionization states were generated at pH 7 ± 2.0 to generate single low energy 3-D structures for each of the input structure and the rest of the parameter values by defaults[7].

2.1.4 Protein preparation

The three dimensional structure of [PDB ID: 2MOQ] was obtained from the RCSB protein data bank (http:// www.rcsb.org/pdb), the best proteins were selected by

analyzing the protein with Ramachandran plot and regions. After selection, Protein preparation wizard (2010) of Schrodinger suite has been used to prepare protein. The proteins were preprocessed, the bond orders were assigned to residues of proteins, hydrogen atoms were added and tautomeric states at their normal pH (7.0) were generated. Impref minimization was carried out using the OPLS 2005 molecular mechanics force field with cut off RMSD of 0.3 Å[8].

2.1.5 Receptor grid generation

Minimized protein was used for grid generation which involves selected ligand as the reference as it signifies the binding sites of drug with respect to the target. The active site is generally represented as an enclosing box at the centroid of workspace ligand. All ligands were docked into this grid structure [6].

2.1.6 Site Map studies

The Site Map studies were carried out using the flip no flip model of target protein (PDB: 2MOQ) by employing Site Map v2.4 program (Schrodinger Inc.). Site Map generates information on the character of binding sites using novel search and analysis facilities, and provides information to Maestro for visualization of the sites. A Site Map calculation begins with an initial search stage that determines one or more regions on or near the protein surface, called sites, which may be suitable for binding of a ligand to the receptor. The search uses a grid of points, called site points, to locate the sites. In the second stage, contour maps are generated, producing hydrophobic and hydrophilic maps. The hydrophilic maps are further divided into donor, acceptor, and metal-binding regions. The most important property generated by Site Map is an overall Site Score, which has proven to be effective at identifying known binding sites in co-crystallized complexes. The evaluation stage, which concludes the calculation, assesses each site by calculating various properties like size, volume, exposure, enclosure, contact, hydrophobicity, hydrophilicity and donor/acceptor ratio. It provides a fast and effective means for identifying potential binding sites and predicting their drug ability with a high degree of confidence. It generates hydrophobic & hydrophilic map, hydrogen bond acceptor & hydrogen bond donor map, metal-binding map, surface map(Fig.1)

The default appearances of the six types of map are as follows:

- Hydrophobic map—yellow mesh
- Hydrophilic map—green mesh
- Hydrogen-bond donor map—blue mesh
- Hydrogen-bond acceptor map—red mesh
- Metal-binding map—pink mesh
- Surface map—gray surface, 50% transparent.

2.1.7 Docking studies

Ligand docking was done by using Glide, v 5.6. The prepared ligands and the file obtained from receptor grid generation panel were selected and all the designed hybrids of thiosemicarbazone and benzimidazole derivatives were docked within the binding site of 2MOQ. Flexible docking was done by employing Extra Precision (XP) mode of Glide. Glide score of compounds was obtained and various interaction of ligand with protein was studied. The final energy evaluation was done with the GlideScore and a single best pose was generated as output for a particular ligand with the help of following equation.

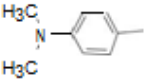
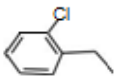
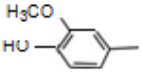
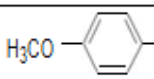
GScore = a*vdW+ b*Coul + Lipo + H bond + Metal + Bury P+ RotB + Site

Where vdW = Vander Waal energy, Coul = Coulomb energy, Lipo = Lipophilic contact term, HBond = Hydrogen-bonding term, Metal = Metal-binding term, BuryP = Penalty for buried polar group, RotB = Penalty for freezing rotable bonds, Site = Polar interaction at active site, and the coefficient of vdW and Coul are a = 0.065, b = 0.0130. The best pose for a given ligand was determined by the Emodel score, while different compounds were ranked using Glide score [6].

2.1.8 ADMET predictions by QikProp

The above prepared ligands were then neutralized and checked for their ADMET properties using QikProp v3.3, 2010. QikProp helps in analyzing pharmacokinetics and pharmacodynamics of the ligand by accessing the drug like properties. It also provides significant range of values for comparing these molecular properties with those of 95% of already known pharmaceutical drugs. The selected properties are known to influence metabolism, cell permeation and bioavailability. Most of drug failures have been reported in early and late pipeline stage due to undesired pharmacokinetics and toxicity problems. If these issues can be addressed early, it would be extremely advantageous for the drug discovery process. The use of insilico methods to predict ADMET properties is projected as a first step in this direction to analyze the novel chemical entities to prevent wasting time on lead candidates that would be toxic or metabolized by the body into an inactive form and unable to cross membranes, and the results of such analysis are herein reported in Table 22. All designed compounds showed ADMET properties in acceptable range along with the Lipnski rule of five[13].

Table .1.compound code, substitutions & iupac name of tiosemicarbazide-benzimidazole hybrids. (AD2, AD3, AD7, AD8 AND AD10)

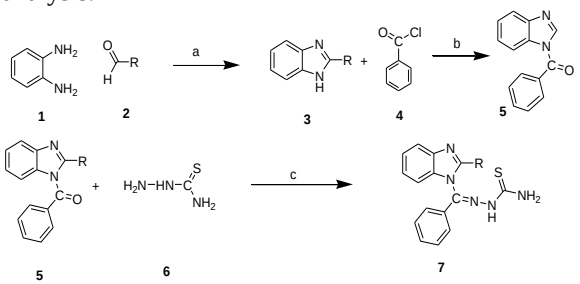
COM. CODE	R	IUPAC NAME
AD2		(E)-1-((2-(4-(dimethylamino)phenyl)-1H-benzo[d]imidazol-1-yl)(phenyl)methylene)thiosemicarbazide
AD3		(E)-1-((2-(2-chlorobenzyl)-1H-benzo[d]imidazol-1-yl)(phenyl)methylene)thiosemicarbazide
AD7		(E)-1-((2-(4-hydroxy-3-methoxyphenyl)-1H-benzo[d]imidazol-1-yl)(phenyl)methylene)thiosemicarbazide
AD8	-CH3	(E)-1-((2-methyl-1H-benzo[d]imidazol-1 syl)(phenyl)methylene)thiosemicarbazide
AD10		(E)-1-((2-(4-methoxyphenyl)-1H-benzo[d]imidazol-1-yl)(phenyl)methylene)thiosemicarbazide

2.2 Synthesis

2.2.1 Chemistry

A series of ten 2-substituted-1H-benzo[d]imidazol-1-yl(phenyl) methylene thiosemicarbazide AD2, AD3, AD7, AD8 and AD10 derivatives were synthesized according to the synthetic route presented in Scheme 1. One of the key intermediates, 2-substituted-1*H*-benzo[d]imidazole (3) was synthesized by reacting substituted aldehydes with *o*-phenylenediamine in the presence of ammonium chloride and ethanol. The 2-substituted -1*H*-benzo[d]imidazole-1-yl)methanone(5) were synthesized by the reaction between 2-substituted-1*H*-benzo[d]imidazole (3) and benzoyl chloride (4) in the presence

of sodium hydrogen carbonate and dilute HCl. 2- substituted-1H-benzo [d]imidazol-1-yl(phenyl) methylene thiosemi carbazide AD2, AD3, AD7, AD8 and AD10 was synthesized by reacting with second intermediate 2-substituted-1*H*-benzo [d]imidazole-1-yl) methanone (5)and thiosemicarbazide in the presence of Glacial acetic acid and methanol. The formation of compounds was confirmed by IR, 1H NMR and mass spectral analysis.



Scheme.1. Synthetic scheme of Thiosemicarbazone derivatives.

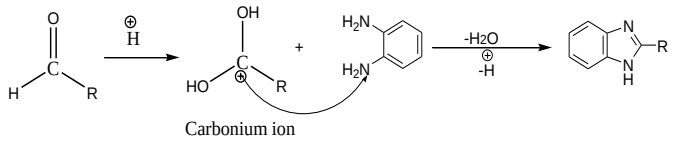
REAGENTS AND CONDITIONS:-

1. NH_4Cl, ethanol, 80-90 ^oC, 2 hr stirring
2. $NaHCO_3$, dilute HCl
3. Methanol, Glacial Acetic acid 70-80 ^oC, 4 hr reflux

2.2.2 Mechanism of Reaction

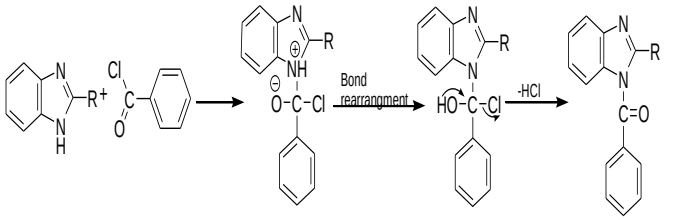
Step-1 Mechanism of synthesis of 2-substituted-1*H*-benzo[d]imidazole(3)

The role of ammonium chloride was to activate carboxyl group by addition of proton to carbonium ion. The reaction mechanism involved carbonium ion intermediate and eliminated H_2O molecule.



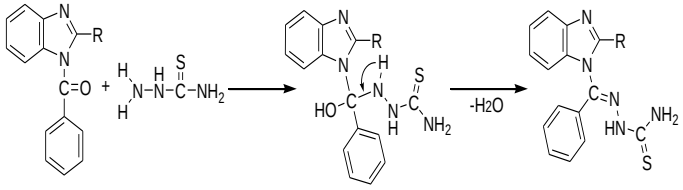
Step-2 Mechanism of synthesis of 2-(substituted-1*H*-benzo[d]imidazole-1-yl)methanone(5)

Nucleophilic attack of nitrogen on electrophilic carbon of benzoyl chloride and eliminated HCl after bond rearrangement.



Step-3Mechanism of synthesis of 2-(substituted-1*H*-benzo [d]imidazole-1-yl) (Phenyl) (methylene) thiosemicarbazide (7)

Nucleophilic attack of Nitrogen on electrophilic carbonyl carbon. After that it formed hemiacetal and hemiketal.Than it is attacked by a second amine to form a compound with a carbon bound to two amine groups.



Nitrogen was deprotonated & electrons from this N-H bond push the oxygen off of the carbon leaving C=N double bond (an imine or Schiff base) & displaced water molecule.

2.2.3 General procedure for synthesis of thiosemicarbazone and benzimidazole hybrids (AD2, AD3. AD7, AD8 and AD10)

A mixture of o-phenylenediamine (0.92 mmol) and substituted aldehydes and acids (0.92 mmol) in 4 ml of ethanol was added NH_4Cl (30 mol%). The resulting mixture was stirred for 2 hr at 80^{o}C. The completion of the reaction was confirmed by TLC ethylacetate: hexane, (2:1 v/v).The reaction mixture placed into ice cold water and the product was precipitated as pale yellow solid and it was dried and purified by recrystallization from ethanol to give pure product(First Intermediate). 0.025 mole of the above product was dissolved in 15ml of 10% $NaHCO_3$ solution, 0.04 Mole (1.5ml) of benzoyl chloride was added, and the reaction mixture was shaken vigorously in an FBF the stopper was removed from time to time since CO_2 evolved. mThan dilute HCl was added and the precipitate obtained was recrystallized from ethanol. Thiosemicarbazide (0.01mol) and above product (0.01mol) were taken into the 100ml RBF. The reaction was carried out in methanol with catalytic amount of glacial acetic acid at refluxed temperature for 4 hr. After completion of the reaction, obtained solid product was filtered and purified by recrystallization from ethanol. Thin layer chromatography was used to monitor the progress of the reactions. Infrared spectra were recorded on SHIMADZU FT/IR spectrophotometer using KBr pellets at S.G.S.I.T.S, Indore, and values were expressed in cm^{-1}. 1HNMR spectra were recorded using a Bruker ADVANCE II 400 NMR spectrophotometer at IIT Bombay and values were reported in ppm downfield from TMS (Tetramethylsilane) as an internal standard. The NMR spectra were obtained in Acetone. The molecular mass of synthesized hybrid was determined by mass spectroscopy. Mass spectra were recorded using CIF Mass Facility IISER Bhopal and results were reported in terms of their m/z values. Melting points were determined by open capillary method.

(E)-1-((2-(4-(dimethylamino)phenyl)-1H-benzo[d] imidazol-1-yl)(phenyl)methylene)thiosemicarbazide (AD2):

Brown ; Yield 75%; m.p. 242-246^{o}C; IR (KBr, cm^{-1}): 3259.84 (N-H, stretch), 3103.6 (C-H,stretch, aromatic), 1644.39 (C=N), 1287.54 (C=S),1164.09 (C-N); 1H-NMR(Acetone): δ ^{1}H NMR (Acetone-d_6) δ ppm): 7.850 (1H, d, Benzimidazol), 2.037 (2H, s, Amine), 3.160(2H, s, CH_2-N), 7.510 (1H, s, H-N, aromatic), 1.917 (3H, s, -CH_3); the molecular ion peak was not observed but m/z at 406.1 (M-8) and 351.1 fragment peak that confirmed the structure of compound.

(E)-1-((2-(2-chlorobenzyl)-1H-benzo[d]imidazol-1-yl) (phenyl)methylene) thiosemicarbazide(AD3):

Brown;Yield 72%;m.p.196-198^{o}C; IR(KBr,cm-1): 3369.79 (N-H, stretch), 2972.43 (C-H,stretch, aromatic), 1643.42 (C=N), 1525.76 (N-H,bend),1166.02(C-N), 1287.54 (C=s), 684.76 (C-H, bend, aromatic), 604.71 (C-Cl); 1H-NMR(DMSO): δ ^{1}H NMR (Acetone-d_6) δ ppm): 7.545 (1H, d, Benzimidazol), 2.044 (2H, s, Amine), 7.510 (1H, s, H-N, aromatic), 1.977 (1H, t, -CH); the molecular ion peak was not observed but m/z at 416.9 (M-2).

(E)-1-((2-(4-hydroxy-3-methoxyphenyl)-1H-benzo[d] imidazol-1-yl)(phenyl)methylene)thiosemicarbazide (AD7):

Yellow; Yield 70%; m.p. 190-194^{o}C; IR (KBr, cm^{-1}): 3170.70(N-H, stretch), 2870.30 (C-H,stretch, aromatic), 1645.35 (C=N), 1525.76 (N-H, bend), 1445.71 (C=C, aromatic), 1165.05(C-N), 684.76 (C-H, bend, aromatic), 1002.06(O-H); ^{1}H NMR (Acetone) δ ppm: 7.536 (1H, d,

Benzimidazol), 2.011 (2H, s, Amine), 7.311 (1H, s, H-N, aromatic), 1.944 (3H, s,); the molecular ion peak was not observed but m/z at 378.1 (M-39) and 351.1 fragment peak that confirmed the structure of compound.

(E)-1-((2-methyl-1H-benzo[d]imidazol-1yl)(phenyl) methylene) thiosemicarbazide(AD8):
Yellow ; Yield 72%; m.p. 220-224^{o}C; IR (KBr, cm^{-1}): 3371.17 (N-H, stretch), 3065.02 (C-H,stretch, aromatic), 1642.42 (C=N), 1445.71 (C=C, aromatic), 1149.9(C-N), 684.76 (C-H, bend, aromatic); ^{1}H NMR (Acetone-d_6) δ ppm): 7.743 (1H, d, Benzimidazol), 2.400(2H, s, Amine), 7.763 (1H, s, H-N, aromatic), 3.415 (2H, d, CH_2-X); the molecular ion peak was not observed but m/z at 304(M+5) confirmed the structure of compound.

(E)-1-((2-(4-methoxyphenyl)-1H-benzo[d]imidazol-1-yl) (phenyl) methylene) thiosemicarbazide(AD10):
Yellow; Yield 78%; m.p. 186-190^{o}C; IR (KBr, cm-1): 3264.66 (N-H, stretch), 2981.4 (C-H,stretch, aromatic), 1619.41 (C=N), 1525.76 (N-H, bend), 1445.71 (C=C, aromatic), 1162.16(C-N), 1285.61(C=S), 684.76 (C-H, bend, aromatic), 2778.5.13 (-OCH_3); ^{1}H NMR (Acetone-d_6) δ ppm): 7.675 (1H, d, Benzimidazol), 2.297 (2H, s, Amine),4.398(1H,s, C=C-H) 7.367 (1H, s, H-N, aromatic), 1.548(3H,t,CH_3); the molecular ion peak was not observed but m/z at 354.3(M-46)and 345.1 that confirmed the structure of compound.

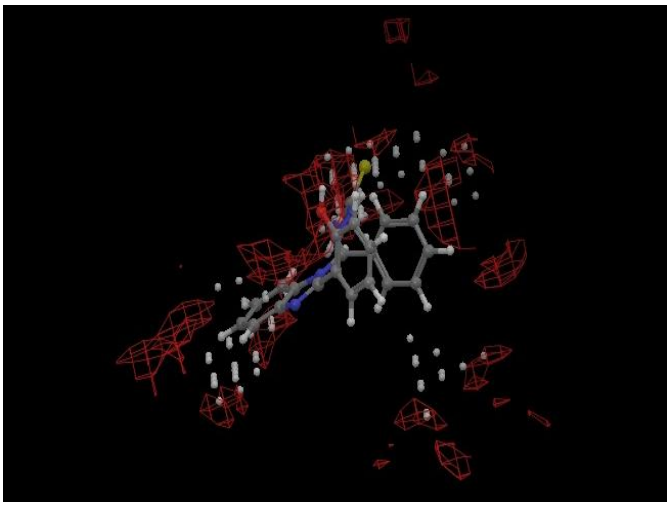
2.3 *In vitro* Anti bacterial activity:
All the synthesized compounds were screened for *in-vitro* antibacterial activity. The *in-vitro* antibacterial assay was performed by Cup borer method. The pure strain of bacteria was obtained from American Type Culture Collection Centre, USA.

Cup Borer Method-
Nutritional agar plate was divided into 4 quadrant section. These sections were labeled with the compound code and name of the bacteria. Turbidity of the nutrient broth was matched with the standards. The surface on the nutrient agar plate was inoculated with the help of sterile cotton swab after expressing the culture from the swab by pressing on a rotating tube above the culture level. The surface of nutrient agar plate was covered evenly by swabbing in three directions. A final sweep was made on the agar rim with the swab. Three to five minutes were allowed for the agar to dry. The well was prepared with the help of sterile cup borer having diameter of 6mm and at equal distance from wall of petri-dish the well was labeled with cone extractor and was poured in to the brim. Plates were incubated at 37^{0}C for 24 hours. After incubation period the antimicrobial activity of synthesized compound was measured in terms of area of zone of inhibition appearance around the well. Each well was filled with 10mg of sample and considered as 100% growth of bacteria throughout the plate. Area of plate was divided into four quadrants. The zone of inhibition was then measured in terms of area in mm^2 and compared with the area of whole quadrant.

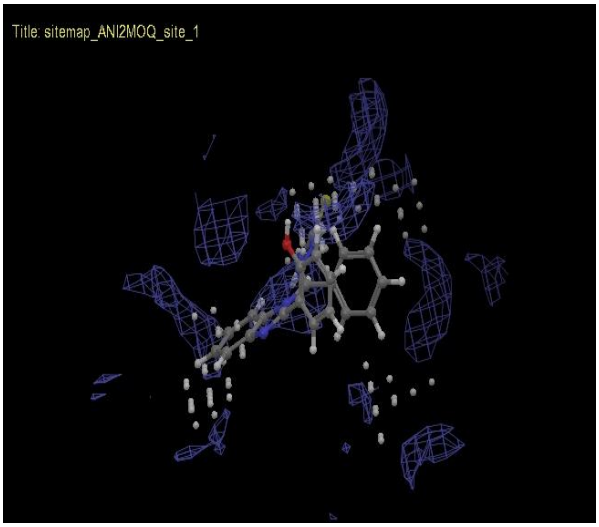
3. RESULTS AND DISCUSSION
3.1 Docking studies:
The result of the extra precision docking experiments of all the designed compounds along with standard drug is summarized in table 2. The X-ray crystallographic structure of PDB ID: 2MOQ were obtained from protein data bank through internet. Inspection of Thiosemicarbazone-benzimidazole hybrids in the active site of enzyme revealed hydrogen bonding and π-π interaction with the residues ALA 319, SER 317, ALA217 and ASN 218.All the designed compounds revealed molecular interaction into the active site of enzyme. The observed interaction of compound AD7 into the active site of 2MOQ enzyme are shown in Fig 1 Sitemaps of 2MOQ with co-crystallized ligand with inside pocket. The good binding interaction of compound with enzyme explains highest antibacterial activity.

Table.2. Compound Code, Glide Score, Emodel Energy, Rmsd, Amino Acid Interactions and IC_{50} Values of Compounds and Standad with 2MOQ.

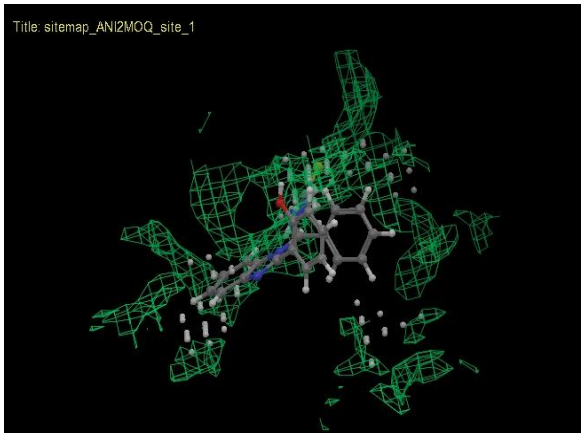
COM. CODE	DOCKING SCORE	GLIDE EMODEL ENERGY	RMSD	INTERACTING AMINO ACID	AVG. IC50 VALUE
AD1	-5.067	-64.315	0.003	ALA 219, SER 319 ,	24.2
AD2	-6.509	-55.381	0.005	ASP 107, Gly 110	27
AD3	-6.648	-43.921	0.004	SER 216, SER 317	27.33
AD4	-7.082	-59.923	0.023	ASN 216, ALA 217	16.5
AD5	-8.010	-47.280	0.015	ASN 218,LEU 216	25.33
AD6	-7.834	-69.656	0.005	SER317, ARG 318	28.2
AD7	-8.572	-54.976	0.008	SER 215, LEU 216	22
AD8	-6.776	-64.286	0.046	ASN 218, ALA 217	28
AD9	-6.507	-64.103	0.049	ALA 319, SER 317,ALA320	27.4
AD10	-7.637	-65.558	0.007	ALA 217, ASN 218	25.66
AMP	5.208	-62.456	0.043	ASP 32	-



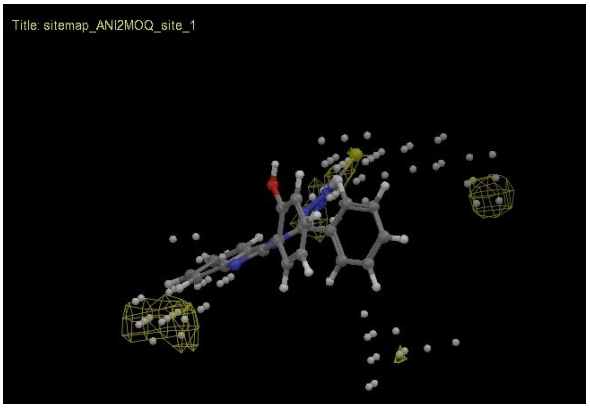
(a) AD7 Acceptor



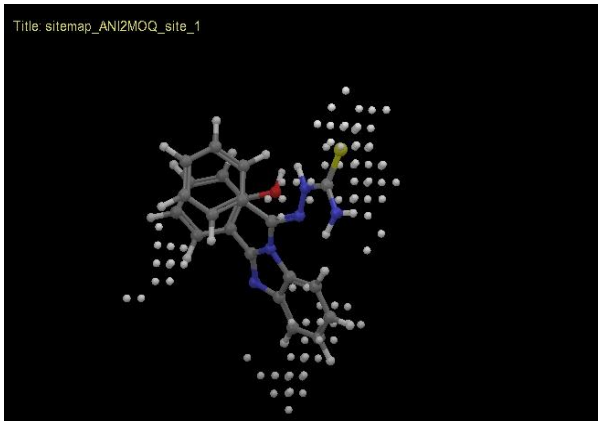
(b) AD7 Donor



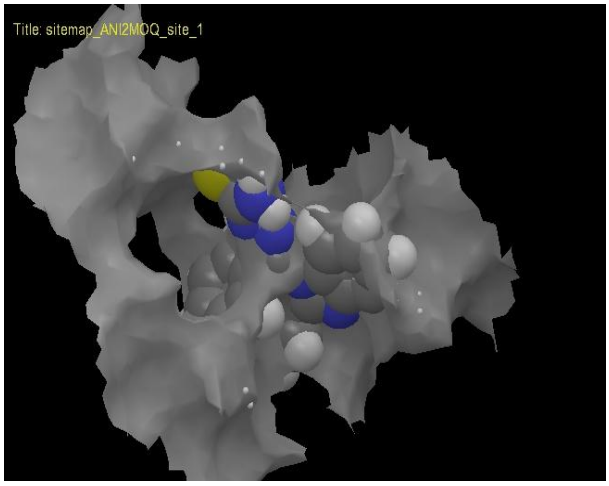
(c) AD7 Hydrophilic



(d) AD7 Hydrophobic



(e) AD7 Metal binding site



(f) AD7 Surface filing

Figure.1. Sitemaps of 2MOQ with co-crystallized ligand with inside pocket

3.2 ADMET Profile

The drug likeness of all the synthesized compounds was determined by the help of Qikprop v3.0 tool of schrodinger software. Results are shown TABLE 3:

Table.3. Qikprop pharmacokinetic analysis of designed compound

Com. code	MW	QP Log $P_{o/w}$	QP LogS	QP Log BBB	% Human abs (%)	#rtv FG Descri-ptors	Rule Of Five
AD1	405	4.74	-6.01	-0.07	100	0	0
AD2	405	4.73	-5.90	-0.05	100	0	0
AD3	405	4.39	-5.55	-0.03	100	0	0
AD4	416	3.43	-5.32	-1.42	87.09	0	0
AD5	416	3.58	-5.14	-1.13	92.27	0	0
AD6	431	4.32	-5.55	-0.59	100	0	0
AD7	414	4.47	-5.80	-0.54	100	0	0
AD8	419	5.05	-6.40	-0.44	100	0	1
AD9	433	3.27	-3.95	-0.91	71.42	0	0
AD10	417	3.58	-4.99	-0.90	100	0	0

1. **Molecular weight(MW):** MW of the molecule (accepted range:130.0 - 725.0.
2. **Log $P_{o/w}$:** Octanol / Water partition coefficient (Log $P_{o/w}$) (-2 - 6.5).
3. **QPLog BBB:** Predicted brain/blood partition coefficient. (-3.0 - 1.2).
4. **QPLogS:** Predicted aqueous solubility, log S. S in mol dm^{-3} is the concentration of the solute in a saturated solution that is in equilibrium with the crystalline solid.(-6.5 - 0.5).
5. **Human absorption:** Predicted human oral absorption on 0 to 100% scale. The prediction is based on a quantitative multiple linear regression model. This property usually correlates well with human oral absorption, as both measure the same property (>80% is high; <25% is poor).
6. **#rtvFG Descriptors:** Number of reactive functional groups; the specific groups like silicon present(toxic), aluminium present (toxic),acyl halide, alkyl halide etc. The presence of these groups can lead to decomposition, reactivity, or toxicity problems *in vivo*.(0-2).
7. **Rule of Five:** Numbers of violations of Lipinski's rule of five. The rules are: mol MW < 500, QPLog$P_{o/w}$ < 5,donorHB ≤ 5, and accptHB ≤ 10. Compounds that satisfy these rules are considered drug like. (The "five" refers to the limits, which are multiples of 5.) (Maximum is 4).

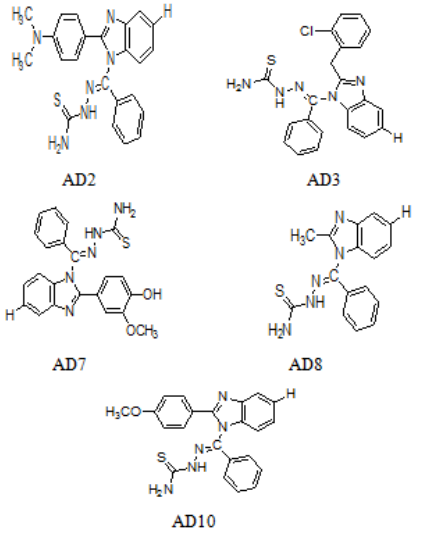
3.3 Synthesis:

Synthesis of thiosemicarbazone -benzimidazole hybrids were carried out as outlined in reaction scheme 1. Thiosemicarbazone -benzimidazole hybrid derivatives AD2, AD3, AD7, AD8 and AD10 were synthesized by reaction of *o*-phenylene diamine with substituted aldehydes and acids in the presence of ethanol and NH_4Cl (30 mol%). The resulting mixture was stirred for 2 hr at 80°C. The reaction mixture placed into ice cold water and the product was precipitated as pale yellow solid and it was dried and purified by recrystallization from ethanol to give pure product (3). Above product was dissolved in 10% $NaHCO_3$ solution and benzoyl

chloride was added and the reaction mixture was shaken vigorously in an FBF the stopper was removed from time to time since CO_2 evolved. Than dilute HCl was added and the precipitate obtained were recrystallized from ethanol (5). Thiosemicarbazide and above product were taken into the 100ml RBF. The reaction was carried out in methanol with catalytic amount of glacial acetic acid and refluxed for 4hr. After completion of the reaction, obtained solid product (7) was filtered and purified by recrystallization from ethanol. The completion of the reaction was confirmed by TLC, ethylacetate: hexane, (2:1 v/v).

3.3 *In vitro* Antibacterial activity:

All the five synthesized hybrids were evaluated for their antibacterial activity against Escherichia *coli*, *Klebsiella pneumoniae*, *Proteus mirabilis*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *streptococcus pneumonia.* In order to get structural insight, two point variations were made in the thiosemicarbazone–benzimidazole hybrids (AD2, AD3, AD7, AD8 and AD10). In the structural motive of these hybrids, the Thiosemicarbazone were kept common and variation was made in benzimidazole ring. Among these hybrids, compound AD2, AD3, AD7 was found to be active against *Staphylococcus aureus*, *streptococcus pneumonia* and AD7, AD8, AD10 was found to be active against *Escherichia coli*, *Klebsiella pneumoniae*, (Table 4).



Figur.2. Structure of synthesized compounds

Table.4. IC50 Values Of Synthesized Compound:

COM. CODE	*S. aureus* Gm(+) mg/ml	*S. pneumoniae* Gm(+) mg/ml	*Proteous* Gm (-) mg/ml	*Kleb. Pheu-moniae* Gm (-) mg/ml	*P. aerugi-nosa* Gm(-) mg/ml	*E.coliG m(-) mg/ml*
AD2	224.9	74.9	20.84	33.55	-	-
AD3	32.9	31.24	-	33.1	-	25.29
AD5	19.2	37.8	33.55	33.1	29.98	29.98
AD7	279.9	40.3	-	-	-	-
AD8	80.3	34.9	39.98	29.79	26.79	-
AD9	41.24	39.29	-	27.40	-	-
AD10	64.9	80.31	34.65	17.40	71.18	29.29
AMP	20	35.05	32.45	-	-	24.28

4. CONCLUSION

In the present study, we have reported docking, synthesis and antibacterial activity of series of thiosemicarbazone-benzimidazole hybrids. XP Glide docking scores and docking poses of designed compounds and standard suggest that these compounds adopt similar binding mode with active site residue of 2MOQ as hydrogen bond, hydrophobic and π-π stacking interactions, which help in the stabilization of drug in active site. The *in-vitro* evaluation of synthesized hybrids against *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus mirabilis*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *streptococcus pneumonia.* Sensitive strain. The compound AD2, AD3 and AD7 having good *in-vitro* antibacterial activity and described in present study reveals that in near future, they could be developed as lead for antibacterial compounds.

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6. REFERENCES

[1]. Y. Kotaiah, et al, (2014), Synthesis, docking and evaluation of antioxidant and antimicrobial activities of novel 1,2,4-triazolo [3,4-b] [1,3,4] thiadiazol-6-yl), selenopheno[2,3-d] pyrimidines. European Journal of Medicinal Chemistry. 75 195-202.

[2]. B. Chandrakantha, et al, (2014), Synthesis and biological evaluation of novel substituted 1,3,4-thiadiazole and 2,6-di aryl substituted imidazo [2,1-b] [1,3,4] thiadiazole derivatives. European Journal of Medicinal Chemistry. 71 316-323.

[3]. Patel, H., et al, (2016), an efficient synthesis of novel carbohydrate and thiosemicarbazone hybrid benzimidazole derivatives and their antimicrobial evaluation, Indian journal of Chemistry Vol.55B, pp.604-612

[4]. Tuncay Karakurt, et al., (2016) Novel aldehyde and thiosemicarbazone derivatives: Synthesis, spectroscopic characterization, structural studies and molecular docking studies, Journal of Molecular Structure

[5]. Sashidhara KV, Kumar M, Modukuri RK, Srivastava RK and Puri SK. Antiplasmodial activity of novel ketoenamine chalcone-chloroquine based hybrid pharmacophores. Bioorganic and Medicinal Chemistry. 2012; 20:2971-2981.

[6]. Glide 5.6 (2010) Schrodinger, LLC, New York.

[7]. Ligprep 2.4 (2010) Schrodinger, LLC, New York.

[8]. Protein Preparation Wizard (2010) Schrodinger, LLC, New York.

[9]. Maestro 9.1 (2010) Schrodinger, LLC, New York.

[10]. Kouznetsov VV and Gomez-Barrio(2009). A.Recent developments in the design and synthesis of hybrid molecules based on aminoquinoline ring and their antiplasmodial evaluation. European Journal of Medicinal Chemistry.;44: 3091-3113.

[11]. Kumar D, Khan SI, Tekwani BL, Ponnan P and Rawat DS. (2015) 4-Aminoquinoline-Pyrimidine hybrids:Synthesis, antimalarial activity, heme binding and docking studies. European Journal of Medicinal Chemistry. 89: 490-502.

[12]. G. Swastika and D. Biplab, (2014) Molecular Docking Studies and ADME Prediction of Novel Isatin Analogs with Potent Anti-EGFR Activity. Med Chem Lett. 558-568.

[13]. L. Zhang, et al, (2012) Screening of Potent Inhibitor of H1N1 Influenza NS1 CPSF30 Binding Pocket by Molecular Docking. Advances in Infectious Diseases. 292-96.

[14]. Kubinyi, H. Folkers, G. & Martin Y. C., (1998). *3D-QSAR in Drug Design*. [Online] Available at: https:// books. google.co.in/books id [Accessed5Jan.2016].