Journal of Chemical Health Risks

www.jchr.org

JCHR (2024) 14(3), 1307-1330 | ISSN:2251-6727

Design, Synthesis, Characterization and Antimicrobial Activity of Schiff Base Containing N, N'-di substituted Benzimidazole-2-Thione Derivatives

Vikas Kumar Sahu*[1], Sarika Sahu[2], Govind Sharma[1], Vinay Sagar Verma[1], Mohit Kumar Sahu[1], Gitanjali Kashyap[1], Akash Chandravanshi[2], Nityapal Singh[3]

[1]Kamla Institute of Pharmaceutical Sciences, Junwani – 490020, Bhilai, Chhattisgarh, India
[2]Rungta Institute of Pharmaceutical Sciences, Kohka - 490024, Bhilai, Chhattisgarh, India
[3]Shri Rawatpura Sarkar Institute of Pharmacy, Kumhari – 490042, Durg, Chhattisgarh, India

Corresponding Author - Sarika Sahu

(Received: 04 February 2024 Revised: 11 March 2024 Accepted: 08 April 2024)

KEYWORDS
Design Schiff base containing N, N'-di substituted Benzimidazole-2-thione; Molecular docking study; Spectral analysis; 1H-benzo[d]imidazole -2(3H)-thione1,3bis-(methyl urea) benzylidine) (3a-3f)

ABSTRACT:
T Medicinal chemistry involves the discovery, development, identification and interpretation of the mode of action of biologically active compounds at the molecular level. The Aim of this research work is to design, synthesis, characterization and antimicrobial activity of Schiff base containing N, N'-di substituted Benzimidazole-2thione derivatives. In the present work, different analogs of Schiff base containing N, N'di substituted Benzimidazole-2-thione derivatives (3a-3f) were designed and subjected for docking study using free available software Argus lab 4.0. Among the designed compound, substituted benzaldehyde containing the functional group like (OH, NO_2, OCH_3 and Cl) and un substituted compound was found to showed good binding energy as compared to another and also the compound substituted with-Cl group shown better binding energy. Furthermore, among the designed compounds which possess good binding energy was selected for synthesis and the new series of different benzaldehyde containing N, N'-di substituted Benzimidazole-2-thione derivatives were prepared by treating intermediate-2 with substituted benzaldehyde by suitable synthetic method. The synthesized new compounds were purified by recrystallization method using suitable solvents and the structure of newly synthesized compound was confirmed by physicochemical and spectral analysis like IR, ^{1}HNMR, ^{13}CNMR and molecular weight of compound was confirmed by GC-MS spectroscopy. On the basis of interpretation of spectral data obtained from spectral analysis, it was concluded that the synthesized compounds were desired compounds i.e. 1H-benzo[d]imidazole-2(3H)-thione-1,3bis(methyl urea) benzylidine) (3a-3f) as expected. Subsequently in the anti-microbial screening, almost all the test compounds exhibited significant antimicrobial activity against both gram positive and gram negative bacteria and synthesized compounds was considered as promising lead candidate for development of new antimicrobial agents.

1. Introduction

Medicinal chemistry involves the discovery, development, identification and interpretation of the mode of action of biologically active compounds at the molecular level. Emphasis is put on drugs, but the significance of medicinal chemistry is also concerned with the study, identification and synthesis of the metabolic products of drugs and related compounds. Medicinal chemistry covers three critical steps. A discovery step, involves the choice of the therapeutic

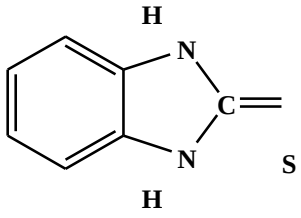
Journal of Chemical Health Risks

www.jchr.org

JCHR (2024) 14(3), 1307-1330 | ISSN:2251-6727

target (receptor, enzyme, transport group, cellular, or in vivo model) and the identification (or discovery) and production of new active substances interacting with the selected target. Such compounds are usually called lead compounds; they can originate from synthetic organic chemistry, from natural sources, or from biotechnological process. Drugs design aims at the development of the drugs with high specificity and therapeutic indeed. An optimization step, which deals with the improvement of the lead structure. [1] The optimization process takes primarily in to account the increase in potency, selectivity and toxicity. Its characteristics are the establishment and analysis of structure activity relationships, in an ideal context to enable the understanding of the molecular mode of action. However, an assessment of the pharmacokinetic parameters such as absorption, distribution, metabolism, excretion and oral bioavailability is almost systematically practiced at an early stage of the development in order to eliminate unsatisfactory candidates. 2 A development step, whose purpose is the continuation of the improvement of the pharmacokinetic properties and the fine tuning of the pharmaceutical properties (chemical formulation) of the active substances in order to render them suitable for clinical use. This chemical formulation process can consist in the preparation of better absorbed compounds, of sustained release formulations, and of water soluble derivatives or in the elimination of properties related to the patient's compliance (causticity, irritation, painful injections and undesirable organoleptic properties). [2] Benzimidazoles are five membered benzoheterocyclic compounds containing two hetero atoms. Both hetero atoms are nitrogen, which are present at non-adjacent position. Benzimidazole derivatives belong to a crucial structural motif that is seen in many pharmaceutically and biologically interesting molecules. Recent publications have been reported to possess a number of significant and diverse biological activities such as fungicide, anti-oxidant, anti-microbial, anthelmintic, anti-cancer, anti-hypertensive, antineoplastic, anti-inflammatory, analgesic, anti-protozoal, and anti-hepatitis B virus activity. Some of their analogues show an array of biological activities, including nonnucleoside HIV-1 reverse transcriptase inhibitors and they selective inhibitors of cyclooxygenase Cox-2.

In view of these activities and synthetic importance, benzimidazoles core and its various derivatives have long been an area of interest and still continue as an active domain for research and industrial field. These versatile biological significance inspired us to synthesize the 1, 3 di-substituted benzimidazoles. [3, 4]



(1)

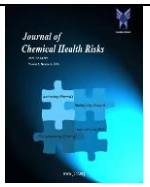
Fig.1. H-benzo[d]imidazole-2(3H)-thione O

Imidazole is the accepted name for the parent compound in the series, the numbering of which follows the accepted pattern for heterocyclic compound. Imidazole or iminazoline is an azapyrrole, the nitrogen atom is separated by one carbon atom. [5] This compound was earlier also called as glyoxalin as it was first prepared in 1958 from glyoxal and ammonia. Although benzimidazole is the commonest name of the parent compound of the series, other names such as benzimidzole and 1, 3-benzodiazole (1) are often used. 1Hbenzimidazole (2) mono acyl derivative of o-phenylenediamine is readily converted into the corresponding benzimidzole by the action of heat alone. [6] These conversions are generally carried out at a temperature somewhat above the melting point of the starting compounds. This is a convenient method for preparing benzimidazoles when monoacyl derivatives are easily obtainable. The procedure may be improved by heating the monoacyl derivative of diamine in an atmosphere of nitrogen to prevent oxidation.The diacyl derivatives of o-phenylenediamines are also converted into benzimidazoles but higher temperatures are required. [7] Urea and thiourea molecule played the central role in the development of organic chemistry since its first documented synthesis in 1828 when the German chemist Friedrich Wohler synthesized first from ammonium cyanate. Urea and thiourea derivatives show a broad spectrum of biological activities such as antioxidant, antibacterial, antiviral, anticancer,

Journal of Chemical Health Risks

www.jchr.org

JCHR (2024) 14(3), 1307-1330 | ISSN:2251-6727



anticonvulsant, analgesic and HDL- elevating properties. Urea structure is shown in fig 2 [8]

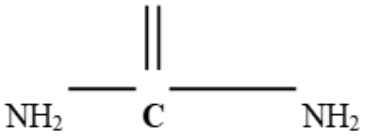


Fig.2. Urea

Molecular docking:

Molecular docking may be defined as an optimization problem, which would describe the "Best-Fit" orientation of a legend that binds to a particular protein of interest and is used to predict the structure of the intermolecular complex formed between two or more molecules. The most interesting case is the protein legend interaction, because of its application in medicines. Ligand is a small molecule, which interacts with proteins binding may occur. These are commonly called binding modes. In modern drug designing, molecular docking is routinely used for understanding drug-receptor interaction. [9]

Types of molecular docking - There are two types of docking

Rigid docking and Flexible docking

Application of Molecular Docking

Application of molecular docking in drug development. Docking is most commonly used in the field of drug design most drugs are small organic molecules and docking may be applied to: Hit identification: docking combined with a scoring function can be used to quickly screen large databases of potential drugs in silico to identify molecules that are likely to bind to protein target of interest. Lead optimization: docking can be used to predict in where and in which relative orientation a ligand binds to a protein (also referred to as the binding mode or pose). [10] This information may in turn be used to design more potent and selective analogs. Bioremediations: protein ligand docking can also be used to predict pollutants that can be degraded by enzyme. Identification of target site, Selection of best drug (based on scoring function), Enzymes and its mechanisms, Protein interactions, Virtual screening of compounds. [11]

Application of molecular modeling in modern drug development

It is used to screening for the side effects that can be caused by the interactions with other proteins, like proteases, cytochrome P450 and others can be done. It is also possible to check the specificity of the potential drug against homologous proteins through docking. Docking is also a widely used tool in predicting protein-protein interactions. Knowledge of the molecular associations aids in understanding a variety of pathways taking place in the living and in revealing of the possible pharmacological targets. [12]

2. Materials and Methods

All the chemicals and reagents were synthetic grade and commercially procured from local vender, Raipur, Chhattisgarh, India (Molychem and Lobachem chemicals). All the synthesized compounds were purified by recrystallization method and characterized by physicochemical properties and spectral analysis. [13]

Molecular docking

Material: Go to Research collaborator for structural bioinformatics (RCSB) website put required name on search box. It provides required data and code of the specific docked compound. Download the search compound on suitable folder on desktop else. Download Argus Lab 4.0.1, Chemdraw 8.0 Make substituent structure with it of design Aniline derivative. Select the standard drug and make structure of compound. [14]

Method:

The structure draw on Chemdraw and file was saved as Chemdraw (*cdx). Then it was copied and pasted in Chemdraw 3D Ultra software. Then the energy minimized and saved in Protein Data Bank (*Pdb). Started Argus Lab went to file and opened, select the save compound which was download from RSCB. Then selected substituent option was minimized and clicked a residue and removes the water molecule and other compound present in it. Clicked on amino acid click on one of them then press Ctl+A to select all then right clicked selected the third option make a group from this ligand. Went to file select open option, selected the prepare structure in the PDB format. [15]

Journal of Chemical Health Risks

www.jchr.org

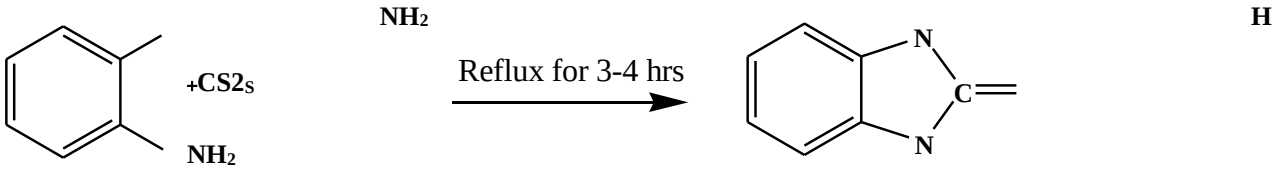
JCHR (2024) 14(3), 1307-1330 | ISSN:2251-6727

Maximized the selected the residue then miscellaneous, then right clicked and selected the second option made a ligand group from this residue. Clicked on group then selected the ligand right click select make a binding site from these groups. Clicked on calculate size to make a grid and stared. Rotamer started its reading then after completion that torsion reading is obtained and recorded. After completing of the reading, we got docked score. Save as in the Argus PDB (*agl). Same procedure was repeated with selected drug. Clicked on folder of target saved in PDB format, then click on residue selected the miscellaneous and right clicked on second i.e. makes a ligand group from this residue. Clicked on group select ligand, went to view the click on hide protein. [16] Clicked on selected ligand then right click select option made a binding site group with this group. Right click select option select render mode and click on ball high cylinder. Selected the same ligand and right clicked select the option which shows hydrogen if hydrogen is present, go to display setting select a rendering style. Noted the number of hydrogen bonds, bond length (A), H-bond with the receptor residue. Made a table through it and compared it to scored best of them and compared with standard drug saved the best image also. [17]

3. Synthesis

General procedure for Synthesis of benzimidazole-2-thione compound

In this work, the synthesis of Benzimidazole-2-thione by O-phenylenediamine and carbon disulfide reaction takes place and the potassium hydroxide is used to enhance the reaction, avoiding the use of high estimation of ammonium salt further shows the effect of various base compounds on the conversion of O-phenylenediamine. It is clear that potassium hydroxide has high reactivity to enhance the reaction of o-phenylenediamine and carbon disulfide .Take in ratio (3:2) (0.0332mol/lit.) O-phenylenediamine (0.3gm) and add equimolar Carbon disulfide (0.23 ml) then add 0.200 gm KOH with 10 ml Ethanol (C_2H_5OH) in round bottom flask. To the above solution add 1.5ml of water with continuous stirring and then reflux for 3 hrs. Then add 10ml warm water to the reaction mixture and cool on ice bath. After cooling add 1ml of CH_3COOH and water with stirring. The product separate as white crystal and the mixture is placed in a refrigerator for 3hrs to complete the crystallization. Then separate out the solid product with the funnel & dry it. [18]



H benzene-1,2-diamine **(1)**

1*H*-benzo[*d*]imidazole-2(3*H*)-thione

Fig.3. Synthesis of compound (1) will be reflux 3-4hrs.

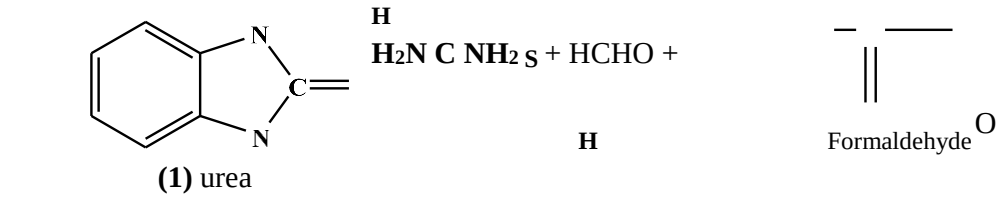
Procedure for synthesis of (1H-benzo[d]imidazole-2(3H)-thione-1,3bis-(methyl urea) compound (2).

Journal of Chemical Health Risks

www.jchr.org

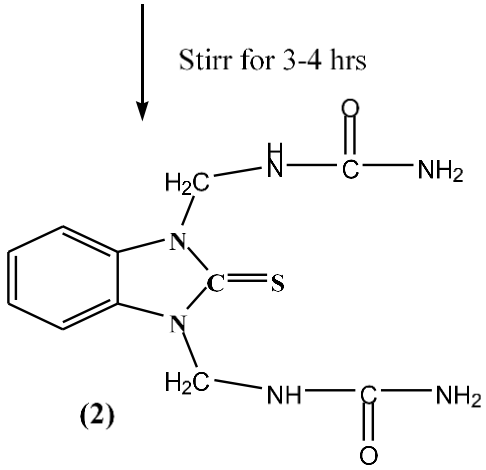
JCHR (2024) 14(3), 1307-1330 | ISSN:2251-6727

Take out the accurate amount of 1st compound (0.01mol) in 250ml beaker and dissolved in 10 ml solvent mixture of ethanol and DMF. Equimolar formaldehyde and urea (0.01mol) was added to the above solution and then stirring for 3-4hours. After stirring completion, the reaction mixture was kept for overnight. Resulting yellowish white precipitate solid was filtered and wash with water and dry. Recrystallization was done with ethanol and DMF. The product compound obtained was weight and calculated the percentage yield.



(1) urea

1*H*-benzo[*d*]imidazole-2(3*H*)-thione



1H-benzo[d]imidazole-2(3H)-thione-1,3bis-(methyl urea)

Fig.4. Synthesis of compound (2) will be magnetic stir 3-4hrs.

Journal of Chemical Health Risks

www.jchr.org

JCHR (2024) 14(3), 1307-1330 | ISSN:2251-6727

4 . Identification and Characterization

Melting point determination

A melting point of any solid compound is a temperature at which it changes state from solid to liquid at atmospheric pressure. It is mostly used physical properties for identification of an organic compound. The determination of melting point of synthesized compounds was done by open capillary tube method using melting point apparatus and data recorded in thermometer. Firstly, a fine capillary of length 5-6cm was taken. Sealed its one end by inserting the end of the capillary tube horizontally into the extreme edge of a small steady Bunsen flame for few seconds, rotating the capillary meanwhile. [19]

Solubility Studies

All the synthesized new compounds were solubility test conducted with polar and nonpolar type of solvents and the procedure was followed as firstly samples was taken and add solvent according to polarity of compound then seen soluble if they are not soluble then slight heating with water bath than check solubility of compound after that note done with room temp or hot temp column of the table. [20]

Determination of Rf-value

The Thin layer chromatography (TLC) of synthesized compound was performed for determination of Rf value. The Rf value is defined as ratio of distance travelled by solute and distance travelled by solvent. For this purpose, silica gel G was used as stationary phase and n-hexane: methanol in the ratio of 3:2 was used as mobile phase and iodine is used as visualizing agent. In TLC plate, we found single spot with different Rf value for each compound. This single spot shows purity of new compound. [21]

Spectral Analysis

UV Spectroscopy for determination of λ max value

The maximum absorbance (λ_{max}) of all the synthesized compounds was determined by using Shimadzu UV-1800 (UV Spectrophotometer). Start the UV spectrum and then correct the base line correction one section then reference sample and then taken the testing sample follow all the procedure to handle the instrument to find the λmax and graph refers to the wavelength in the absorption spectrum where the absorbance is maximum. It acts as a qualitative parameter to compare the absorption range of different molecule. Methanol is used as solvent system. [22]

Infrared spectroscopy

IR spectroscopy is one of most powerful analytical method used for identification of functional group present in organic compound. The IR spectrum of synthesized compound was recorded at Pt. Ravishankar Shukla University, Raipur by SHIMADZU FTIR spectrophotometer using potassium bromide pellet technique.

NMR (Nuclear Magnetic Resonance) Spectroscopy

Nuclear magnetic resonance (NMR) is a spectroscopic technique that detects the energy absorbed by changes in the nuclear spin state. The application of NMR spectroscopy to the study of proteins and nucleic acids has provided unique in- formation on the dynamics and chemical kinetics of these systems. These new synthesized compounds were sending the Indian Institute Of Science and Research Bhopal, (IISERB) India by the used instrument BRUKER (500MHz CDC 13).

Mass Spectroscopy

The mass spectroscopy (GC-MS) is one of the best tools for determination of molecular weight, molecular formula and fragmentation pattern of organic compound by recording mass spectrum. The mass spectrum is a plot of relative abundance against the ratio of mass/charge (m/e). The mass spectra of synthesized compound were recorded at Indian Institute of Education and Science Research (IISER), Bhopal (M.P.) using Agilent 7890A GC with 5975C GC-MS system. [23]

5. Biological Activity

Evaluation of Antimicrobial Activity:

The antimicrobial screening of newly synthesized compounds is done by using microbiological assay. This involves the demonstration of therapeutic efficacy of antimicrobial agents by determining inhibition of

Journal of Chemical Health Risks

www.jchr.org

JCHR (2024) 14(3), 1307-1330 | ISSN:2251-6727

microbial growth under standard conditions. The microbiological assay of antimicrobial agents is performed by comparing the zone of inhibition formed by microorganisms to a specific concentration of antibiotics having a known activity. There are two general methods used for evaluation of antibiotics. [24]

1. Agar diffusion/ cup plate/ cylinder plate method
2. Turbid metric and tube assay method

Agar diffusion method:

This method is based on diffusion of antibiotic containing cup or cylinder into the agar layer containing microorganisms. The zone is formed around the cups. The zone of inhibition is measured in terms of diameter and compared with standard drug. This method gives extent of growth of microorganism.

Turbid metric method:

This method is based on the inhibition of growth of a microbial culture in a uniform solution of the antibiotic in a fluid medium that is favorable to its rapid growth in the absence of the antibiotic. All the newly synthesized compounds were screened for antimicrobial activity by using agar diffusion method at different concentrations against gram positive bacteria *Staphylococcus aureus, Bacillus coagulants, Streptococcus mutants*, gram negative bacteria *Escherichia coli.*

Collection of test microorganisms:

All experimental bacteria and fungi were obtained from the microbial type culture collection and gene bank (MTCC) CSIR- Institute of microbial technology, Chandigarh. All the test microorganisms were maintained in slant and stored in refrigerator. [25]

Table 1: Microbial Cultures

S. No.	Name of microorganisms	Microbial type	MTCC No.
1.	*Staphylococcus mutans*	Gram positive bacteria	MTCC890
2.	*Bacillus coagulants*	Gram positive bacteria	MTCC492
3.	*Escherichia coli*	Gram negative bacteria	MTCC42

Culture Media:

Two culture media were used for the antimicrobial study. Nutrient broth media was used for inoculums preparations of bacteria and nutrient agar media was used for antimicrobial screening of newly synthesized compounds.

Preparation of nutrient broth media:

Table 2: Formula for preparation of nutrient broth media

S. No.	Ingredient	Quantity
1.	Beef extract	10g
2.	Peptone	10g
3.	Sodium chloride	5g
4.	Distilled water	1000ml (q.s.)
5.	pH	7.2-7.4

Procedure:

Accurately weighed all ingredients and dissolved in distilled water. Then media is heated on water bath to dissolve all ingredients till to get clear yellow colored liquid. Plug the broth containing conical flask with cotton swab and placed in autoclave for sterilization of media. Culture media is sterilized at temperature of 121°C at 15 lbs. pressure for 20 minute**.** [26]

Table 3: Formula for preparation of nutrient agar media

S. No.	Ingredient	Quantity
1.	Beef extract	10g
2.	Peptone	10g
3.	Sodium chloride	5g
4.	Distilled water	1000ml(q.s.)
5.	Agar	20g
6.	pH	7.2-7.4

Sub culturing or aseptic transfer of microorganism

Journal of Chemical Health Risks

www.jchr.org

JCHR (2024) 14(3), 1307-1330 | ISSN:2251-6727

All the glassware's were sterilized during the same time of media preparation. The working platform cleaned with disinfectant and flame the burner. The culture media and all glassware's were placed to this sterilized aseptic area. Test tube used for subculture was labeled with name of microorganism and date. The inoculating loop was sterilized by incineration. Hold the test tube in left hand, separate the test tube to form V shape and right hand was used for handling inoculating loop. Remove the plug of test tube near the burner and flame the neck of test tube. Then inserted the inoculating loop into stock culture to pick up small number of microbes. This loop dipped into broth cultured tube and shaken to dislodge the microorganisms. Re-flame the neck of test tube and reseal it with cotton plug. Also re-flame the inoculating loop before keeping it aside. All the subculture test tubes were incubated at 37 for 24 to 48 hours for growth of a pure culture. [28]

Preparation of drug dilutions

The dilutions of test compounds were prepared in Dimethyl sulfoxide (DMSO) and standard drug in distilled water was used for their antimicrobial screening. Preparation of stock solution: Stock solution of test compounds having concentrationg/ml prepared by dissolving 10mg of synthesized compound in up to 10ml/1000 DMSO. [29] Preparation of working solution: From above stock solution of synthesized compoundg/g/ml and 100g/ml, 80g/ml, 60g/ml, 40g/ml different concentration such as 20µg/ml was used as standard antibiotic were prepared and Ciplox (ciprofloxacin) 20 for comparison and it was prepared by using sterile water. [30]

Antimicrobial screening by cup plate/ Agar diffusion method

All the Petri plates were washed thoroughly and sterilized in hot air oven at 160 for one hour. Nutrient agar media was prepared and sterilized in autoclave at 121 and 15 lbs. pressure for 20 minute and temperature maintained at 50-55. Petri plates were prepared by pouring 30 ml of above agar media into Petri plate and allow the medium to solidify for few minutes. The test microorganisms seeded on the surface of Petri plates by spread plate technique using sterile cotton swabs. By using flame sterilized cork borer four to five cups in each plate keeping adequate distance from each other was prepared g/ml and 100g/ml, 80g/ml, 60g/ml, 40g/ml. [31] The different dilutions such as 20µg/ml was used as were prepared using DMSO and Ciplox (ciprofloxacin) 20µg/ml standard antibiotic for comparison and it was prepared by using distilled water. Mark the each cups or cavity as per dilutions. Then standard and test antibiotic dilutions were added in respective labeled cavity of plates. All the Petri plates were transferred in incubator and Incubated at 37 for 48 hours. The size of the zone inhibition recorded against standard dilution and test dilution and the size measured in mm with the help of scale or using antibiotic zone reader. [32]

6. Result and Discussion

In the present project work, a series of Schiff base containing N, N'-disubstituted Benzimidazole-2-thione derivatives (3a-3f) respectively were synthesized by reaction of **(**1H-benzo[d]imidazole-2(3H)-thione-1,3bis-(methyl urea) (2) with substituted benzaldehyde using conventional method. The newly synthesized compounds were purified by method using suitable solvent and characterized by physicochemical and spectral analysis (UV, IR, ^{1}HNMR, ^{13}CNMR, Mass spectroscopy and elemental analysis).

Table 4: Physical properties of synthesized compound (3a-3f)

S. No.	Comp. code	R	Molecular Formula	M.W.	Meltin g Point	% Yield	λmax	Rf value
1.	3a	H	C25H22N6O2S	470.55	264	69%	478nm	0.56cm
2.	3b	4-OH	C25H22N6O4S	502.54	272	64%	462nm	0.63cm
3.	3c	4-NO_2	C25H20N8O6S	560.54	257	71%	476nm	0.61cm

Journal of Chemical Health Risks

www.jchr.org

JCHR (2024) 14(3), 1307-1330 | ISSN:2251-6727

4.	3d	4-Cl	C25H20N6O2SCl2	539.44	273	57%	469nm	0.64cm
5.	3e	4-OCH_3	C27H26N6O4S	530.6	276	66%	473nm	0.54cm
6.	3f	2,4-Cl	C25H18N6O2S Cl4	608.33	281	69%	468nm	0.67cm

The solubility studies of target molecules were done by using various polar and non-polar solvents. The results of solubility studies indicated that all synthesized compounds were soluble in organic solvent. This confirmed the lipophilic nature of the synthesized compounds.

Table 5: Solubility profile of synthesized compound (3a-3f)

S. No.	Solvent	3a	3b	3c	3d	3e	3f
1.	Water	-	-	-	-	-	+
2.	Methanol	++	++	++	++	++	++
3.	Ethanol	+	+	+	+	+	+
4.	Chloroform	+	-	-	-	-	-
5.	Acetone	++	++	++	++	++	+
6.	DMF	+	+	+	+	+	+
7.	n-Hexane	-	-	-	-	-	-
8.	Ethyl acetate	++	++	+	+	+	-
9.	Isopropyl alcohol	++	-	++	++	++	-
10.	Toluene	-	-	-	-	-	-

Where**-:** Soluble (++), slightly soluble (+), Insoluble (-).

FTIR spectrophotometer

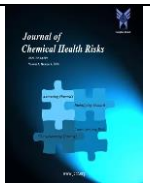
The FTIR spectra of synthesized compounds were obtained by SHIMADZU FTIR Spectrophotometer in Pt. Ravishankar Shukla University, Raipur. The FTIR spectrum of each of the synthesized compounds shows characteristic absorption in accordance to their structural functional groups. The results showed the presence of derivatives which were predicted in the reaction scheme.

FTIR bands of components compounds (1), (2), (3a-3f)

Journal of Chemical Health Risks

www.jchr.org

JCHR (2024) 14(3), 1307-1330 | ISSN:2251-6727



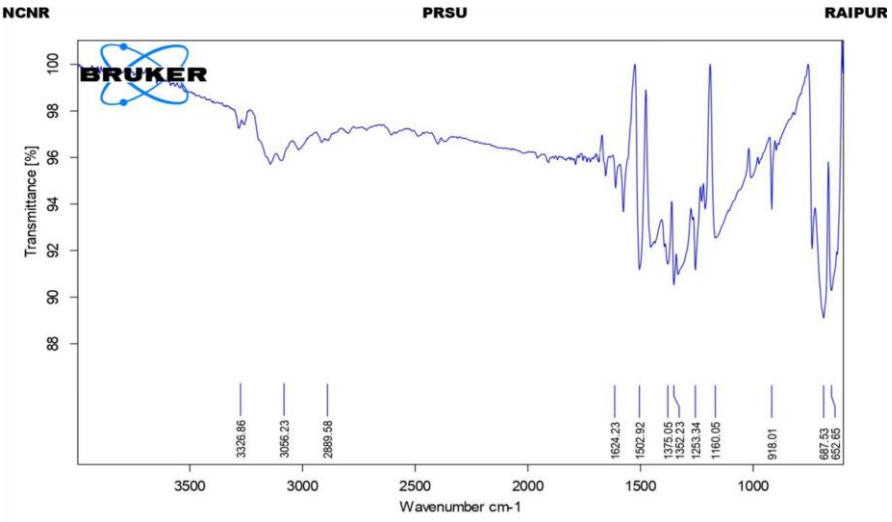


Fig.5. FTIR spectrum for compound (1)

Table 6: FTIR data for compound (1)

S. N.	Wave number (cm^{-1})	Interpretation of functional groups
1.	1624.23	Ar C=C
2.	3056.23	Ar C-H
3.	3326.86	N-H
4.	687.53	C=S

The IR data of compound (1) show presence of N-H stretching frequency at 3326.86cm^{1},
Ar (C-H) stretching frequency at 3056.23cm^{-1}, Ar (C=C) stretching frequency at 1624.23cm^{-1} revealed that formation of benzimidazole-2-thione(1) by reaction of ophenylenediamine with carbon disulfide.

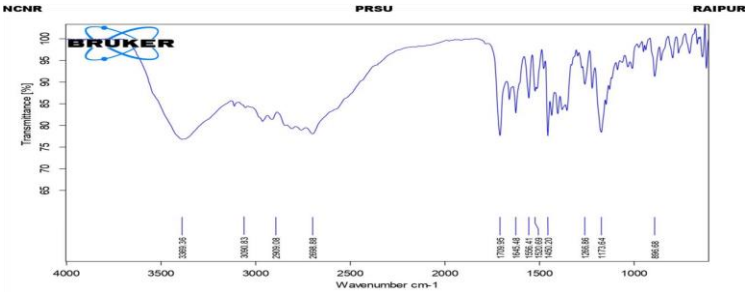


Fig.6. FTIR spectrum for compound (2)

Journal of Chemical Health Risks
www.jchr.org
JCHR (2024) 14(3), 1307-1330 | ISSN:2251-6727

Table 7: FTIR data for compound (2)

S. N.	Wave number(cm^{-1})	Interpretation of functional groups
1.	2909.08	Alkane C-H
2.	1556.41	Ar C=C
3.	3090.83	Ar C-H
4.	3369.36	N-H
5.	1709.95	C=O
6.	896.68	C=S

The above IR data of 1H-benzo[d]imidazole-2(3H)-thione-1,3bis-(methyl urea) (2) reported the presence of C-H (Alkane) stretching band at 2909.08cm^{-1}, N-H stretching band at 3369.36 cm^{-1}, C=O stretching band at 1709.95cm^{-1}, C=S stretching band at 741.30 cm^{1}. All this IR data confirmed that cyclization of compound (1) into compound (2).

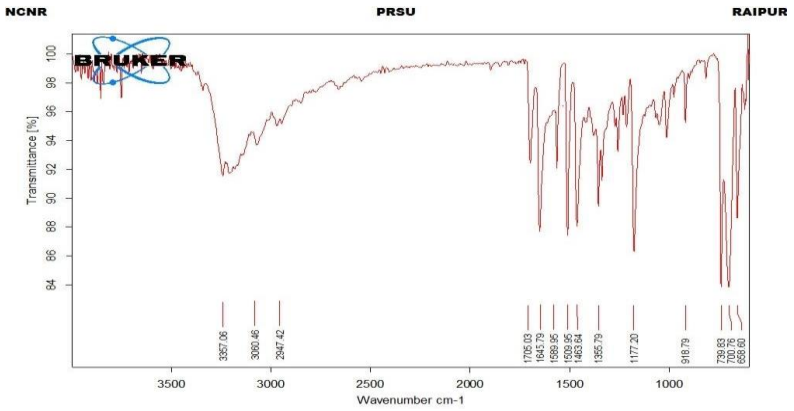


Fig.7. FTIR spectrum for compound (3)

Table 8: FTIR data for compound (3a)

S. N.	Wave number(cm^{-1})	Interpretation of functional groups
1.	2947.42	Alkane C-H
2.	1589.95	Ar C=C
3.	3060.46	Ar C-H

Journal of Chemical Health Risks

www.jchr.org

JCHR (2024) 14(3), 1307-1330 | ISSN:2251-6727

4.	3357.06	N-H
5.	1645.79	C=N
6.	1355.79	C-N
7.	1705.03	C=O
8.	1509.95	N-H
9.	700.76	C=S

IR spectrum of compound (3a) represent broad spectrum of band. The presence of Ar C=C stretching at 1589.95cm^{-1}, Ar C-H stretching at 3060.46 cm^{-1}, C=O stretching at 1705.03cm^{-1}, C-N stretching at 1355.79cm^{-1} and N-H stretching at 3357.06 cm^{-1} revealed that formation of compound (3).

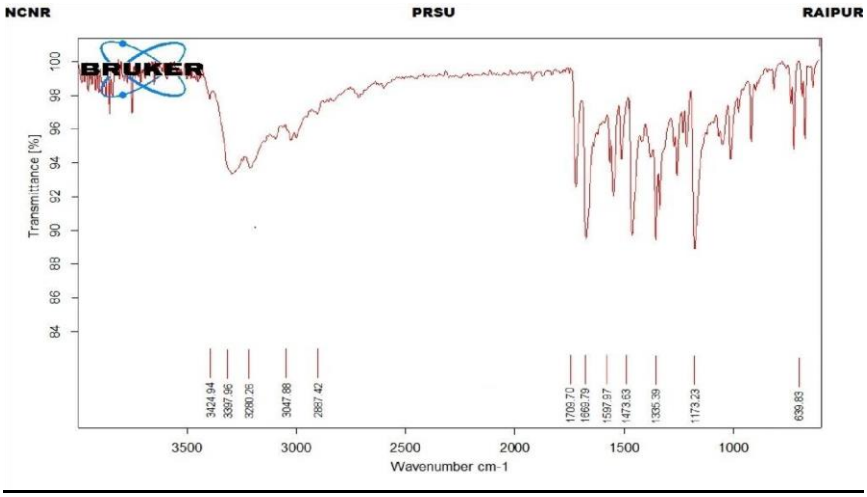


Fig.8. FTIR spectrum for compound (3a)

Table 9: FTIR data for compound (3b)

S. No.	Wave number(cm$^{-1)}$	Interpretation of functional groups
1.	2887.42	Alkane C-H
2.	1597.97	Ar C=C
3.	3047.88	Ar C-H
4.	3280.26-3397.96	Ar-OH
5.	3424.94	N-H
6.	1335.39-1473.63	C-N

Journal of Chemical Health Risks

www.jchr.org

JCHR (2024) 14(3), 1307-1330 | ISSN:2251-6727

7.	1709.70	C=O
8.	1669.79	C=N
8.	639.83	C=S

IR spectrum of compound (3b) represent broad spectrum of band. The presence of Ar C=C stretching at 1597.97cm^{-1}, Ar C-H stretching at 3047.88 cm^{-1}, C-N stretching at 1335.391473.63cm^{-1} and OH stretching at 3280.26-3397.96cm^{-1} revealed that reaction of 4Hydroxybenzaldehyde with compound (3) and confirmed the formation of compound (3b).

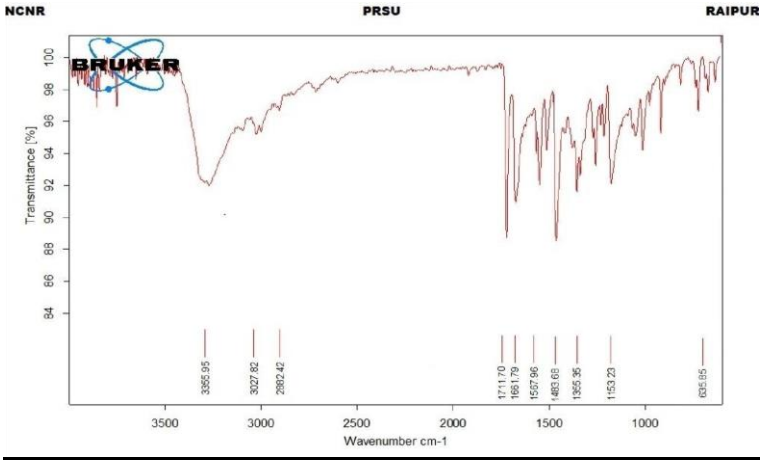


Fig.9. FTIR spectrum for compound (3b)

Table 10: FTIR data for compound (3c)

S. No.	Wave number(cm$^{-1)}$	Interpretation of functional groups
1.	2882.42	Alkane C-H
2.	1567.96	Ar C=C
3.	3027.82	Ar C-H
4.	3355.95	N-H
5.	1355.35	C-N
6.	1661.79	C=N
7.	1483.68	NO_2
8.	1711.70	C=O
9.	635.85	C=S

Journal of Chemical Health Risks

www.jchr.org

JCHR (2024) 14(3), 1307-1330 | ISSN:2251-6727

IR spectrum of compound (3c) represent broad spectrum of band. The presence of Ar C-H stretching at 3027.82 cm^{-1}, N-H stretching at 3355.95cm^{-1}, Ar C-N stretching at 1355.35 cm^{-1}, Ar-NO_2 stretching at 1483.68 cm^{-1} confirmed that formation of compound (3c).

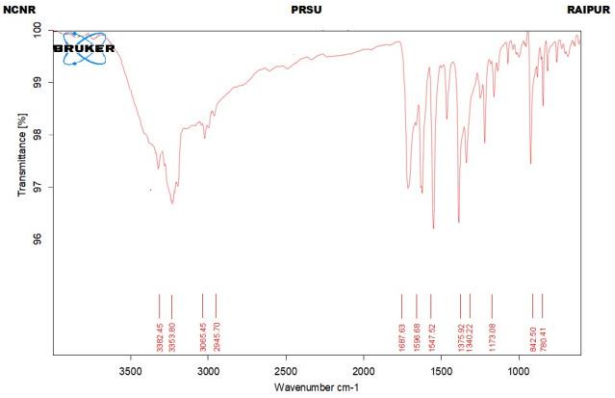


Fig.10. FTIR spectrum for compound (3c)

Table 11: FTIR data for compound (3d)

S.NO.	Wave number(cm^{-1})	Interpretation offunctional groups
1.	2945.70	Alkane C-H
2.	1547.52	Ar C=C
3.	3065.45	Ar C-H
4.	3353.80-3382.45	N-H
5.	1340.22-1375.92	C-N
6.	1596.68	C=N
7.	1687.63	C=O
8.	780.41	C=S
9.	842.50	C-Cl

IR spectrum of compound (3d) represent broad spectrum of band. The presence of Ar C-H stretching at 3065.45 cm^{-1}, N-H stretching at 3353.80-3382.45 cm^{-1}, Ar C-N stretching at 1340.22-1375.92cm^{-1}, Ar C=N stretching at 1596.68 cm^{-1} confirmed that formation of compound (3d).

Journal of Chemical Health Risks

www.jchr.org

JCHR (2024) 14(3), 1307-1330 | ISSN:2251-6727

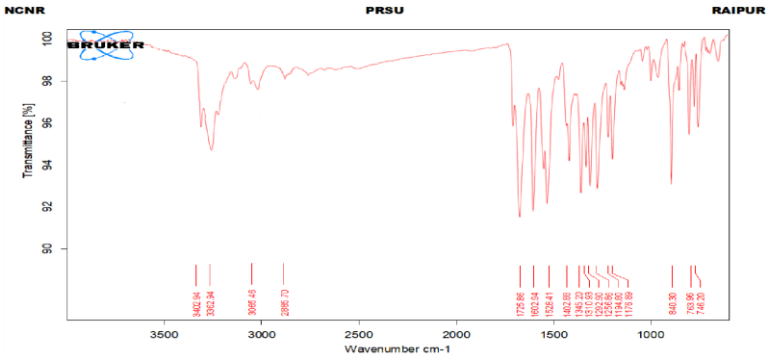


Fig.11. FTIR spectrum for compound (3d)

Table 12: FTIR data for compound (3e)

S.NO.	Wave number(cm^{-1})	Interpretation of functional group
1.	2885.70	Alkane C-H
2.	1602.94	Ar C=C
3.	3065.46	Ar C-H
4.	3362.94-3402.94	N-H
5.	1725.86	C=O
6.	1528.41	C=N
7.	1345.20-1402.88	C-N
8.	1176.89-1310.93	Ar C-O-C
9.	746.20-763.96	C=S

IR spectrum of compound (3e) represent broad spectrum of band. The presence of Ar C-H stretching at 3065.46 cm^{-1}, Ar C-N stretching at 1345.20-1402.88 cm^{-1}, Ar-C=N stretching at 1528.41 cm^{-1} and Ar C-O-C at 1176.89-1310.93cm^{-1} confirmed that formation of compound (3e).

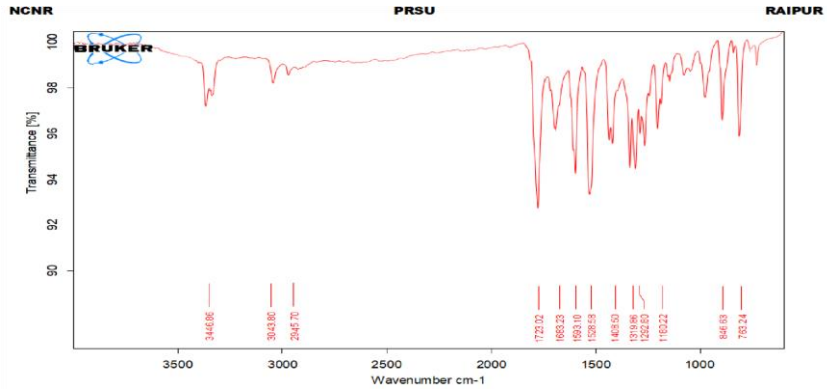


Fig.12. FTIR spectrum for compound (3e)

Journal of Chemical Health Risks

www.jchr.org

JCHR (2024) 14(3), 1307-1330 | ISSN:2251-6727

Table 13: FTIR data for compound (3f)

S. No.	Wave number(cm^{-1})	Interpretation of functional groups
1.	2945.70	Alkane C-H
2.	1593.10	Ar C=C
3.	3043.80	Ar C-H
4.	3446.86	N-H
5.	1292.80-1408.50	C-N
6.	1528.58	C=N
7.	1723.02	C=O
8.	846.63	C-Cl
9.	763.24	C=S

IR spectrum of compound (3f) represent broad spectrum of band. The presence of Ar C-H stretching at 3043.80cm^{-1}, Ar C=C stretching at 1593.10cm^{-1}, Ar C-N stretching at 1292.80-1408.50cm^{-1}, Ar- N-H stretching at 3446.86cm^{-1} confirmed that formation of compound (3f).

NMR (Nuclear Magnetic Resonance) Spectroscopy:
NMR spectrum was recorded at Indian Institute of Education and Science Research (IISER) Bhopal (M.P) India by using instrument BRUKER 500MHZ CDC13 spectrometer And the solvent taken was chloroform as standard. Where the compound (3a) taken. These new synthesized compound (3a) were done under 1HNMR & 13CNMR.

1. ^{1}HNMR (Proton nuclear magnetic resonance).

2.^{13}CNMR (Carbon nuclear magnetic resonance).

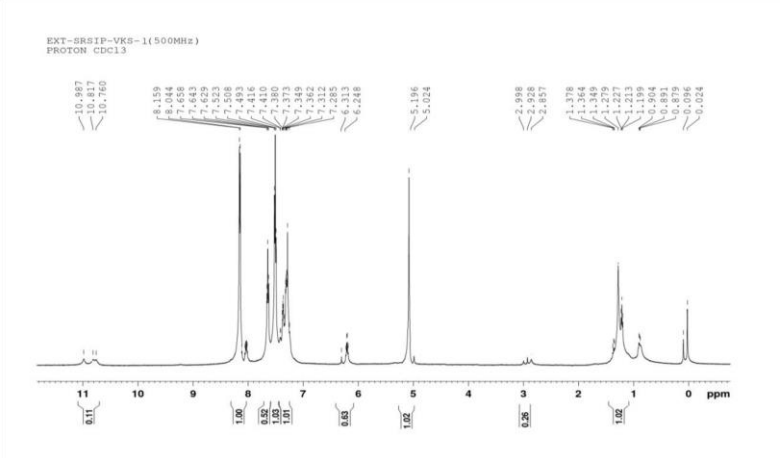


Fig.13. ^{1}HNMR spectrum of compound (3a)

Journal of Chemical Health Risks

www.jchr.org

JCHR (2024) 14(3), 1307-1330 | ISSN:2251-6727

Table 14: [1]HNMR spectrum data of compound (3a)

S. No.	Interpretation of functional groups	Bond	Ppm value
1.	Aromatic C-H	m,10H	7.28-7.65
2.	Benzimidazole-2-thione	m,8H	6.24-6.31
3.	-CH_2	s,4H	5.19
4.	C-H (C=N)	m,2H	8.15
5.	N-H	s,2H	8.04

The[1]HNMR data of compound (3a) shows sharp chemical shift and data reported confirmed the structure of synthesized compound (3a).

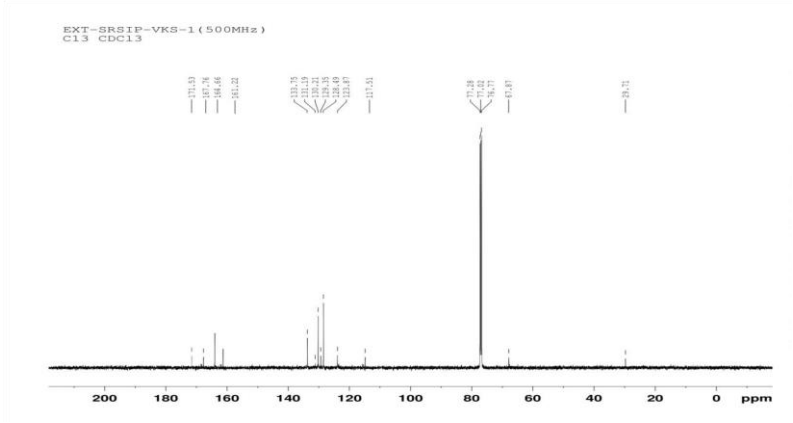


Fig.14. CNMR spectrum of (3a)

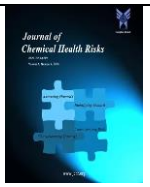
Table 15: [13]CNMR spectrum data of compound (3a)

S. No.	Interpretation of functional groups	Bond	Ppm value
1.	Aromatic C-C (Benzene)	18C	117.51-133.75,
3.	Benzimidazole-2-thione (C=S)	1C	171.53
4.	-CH2	2C	67.87-77.28
5.	C-N (Amide)	2C	167.76
6.	C=N (Imine)	2C	161.22-164.66

Journal of Chemical Health Risks

www.jchr.org

JCHR (2024) 14(3), 1307-1330 | ISSN:2251-6727



The above ^{13}CNMR data of compound (3a) represent shows sharp chemical shift and data reported confirmed the structure of synthesized compound (3a).

Mass Spectroscopy

The mass spectrum of synthesized compound was recorded at IISERB-CIF-Mass Facility Indian Institute of Education and Science Research (IISER) Bhopal (M.P) using Agilent Tech. MS system. Where the compound (3a) taken for the mass spectrum.

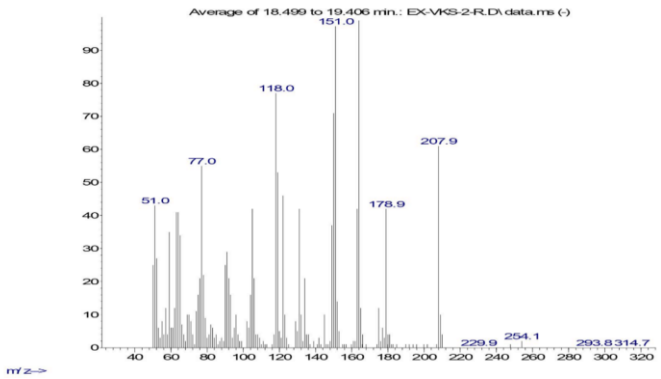


Fig.15. Mass spectrum of compound (3a)

The above mass spectrum of compound (3a) reported for molecular formula $C_{25}H_{22}N_6O_2S$ which illustrates molecular ion peak at (118.0 + 207.9 m/z).

Biological Activity

Antimicrobial Activity:

In-vitro anti- microbial was evaluated by agar disc diffusion (cup plate) method for all the newly synthesized compound (3a-3f) against two gram positive (+Ve) organism (*S mutants, B. coagulants)*, one gram negative (-Ve) organism *(E.coil*). The entire synthesized compound was found to be active against all the microbial strain. Compound (1Hbenzo[d]imidazole-2(3H)-thione-1,3bis-(2,4-dichlorobenzylidine)methyl urea) (3f) showed higher antimicrobial activity than the standard drug at the concentration of 100µg/ml in gram positive strains i.e. *S mutants.* Compound (3d, 3f) at concentration of 100µg/ml showed equal antimicrobial activity compare to standard drug against *S mutants, E.coil.* Compound 3b, 3d & 3f at a concentration of 100µg/ml shows comparable antimicrobial activity against *S mutants, B coagulants, and E.coil* strains compare to standard drug Ciplox (ciprofloxacin) were used as reference standard for antimicrobial activity respectively. Antimicrobial activity result given table number 17.

Table 16: Antimicrobial activity of synthesized compounds (3a-3f)

S. No.	Compound Code	Concentration (µg/ml)	Zone of inhibitions (cm)		
			S. Mutants	***B. coagulants***	***E. coil***
1.	3a	20	07	08	06
		40	09	08	09

		60	11	10	10
		80	11	12	11
		100	13	13	12
2.	3b	20	09	08	08
		40	10	08	10
		60	12	10	12
		80	14	12	14
		100	**16**	**14**	**15**
3.	3c	20	06	07	--
		40	08	08	07
		60	10	10	09
		80	11	11	10
		100	11	12	12
4.	3d	20	10	09	10
		40	11	10	11
		60	13	12	12
		80	15	14	14
		100	**18**	**16**	**16**
5.	3e	20	--	--	06
		40	07	06	08
		60	08	09	09

Journal of Chemical Health Risks
www.jchr.org
JCHR (2024) 14(3), 1307-1330 | ISSN:2251-6727

		80	10	11	10
		100	12	12	11
6.	3f	20	11	10	11
		40	12	11	11
		60	14	12	12
		80	16	13	14
		100	**19**	**16**	**16**
7.	Control (DMSO)	20	00	00	00
8.	Ciprofloxacin	20	18	17	16

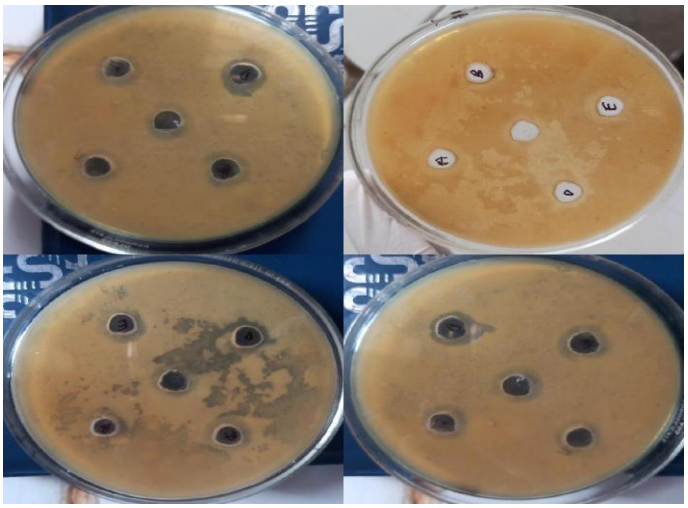


Fig.16. Zone inhibition culture of test compound against gram (+ve) and gram (-ve) bacteria.

Table 17: Comparative anti-microbial study of synthesized compounds (3a-3f)

S. No.	**Compound code**	**Concentration (µg/ml)**	**Zone inhibitions in (mm)**		
			S. mutants	***B. Coagulants***	***E. Coil***
1.	3a	100	13	13	12

Journal of Chemical Health Risks

www.jchr.org

JCHR (2024) 14(3), 1307-1330 | ISSN:2251-6727

2.	3b	100	16	14	15
3.	3c	100	11	12	12
4.	3d	100	**18**	**16**	**16**
5	3e	100	12	12	11
6.	3f	100	19	16	16
7.	Ciprofloxacin	100	18	17	16

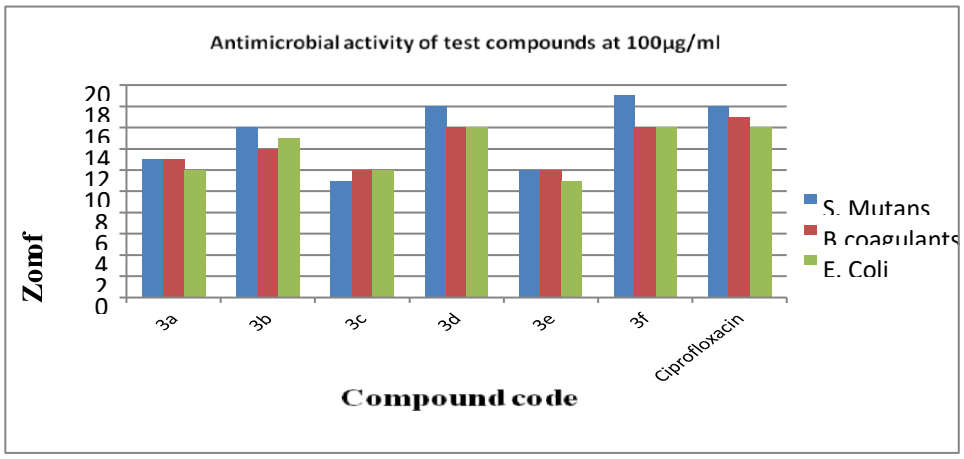


Fig.17. Bar diagram for antimicrobial activity

Molecular docking study

Docking study of all designed compounds was carried out with Argus lab 4.0 to determine the binding energies of predicted ligand- receptor interaction. It has been performed the docking study of a new set of Schiff base containing N, N'-di substituted Benzimidazole2-thione derivatives by binding with active site of DNA gyrase crystal structure (PDB ID: 3U2D) from S. aureus and interacted through various bonds like hydrogen, van der waals bond, carbon-hydrogen bond. From the residue of DNA gyrase 7 amino acids are responsible for formation of bonds with standard drug Ciprofloxacin such as 384ARG (alpha helix), 150 HIS (beta strand), 141TYR (beta strand), 415TYR (coil), 141TYR (beta strand), 264ASP (alpha helix), 387GLU (alpha helix). The test compounds shows different mode of interaction with amino acids located in active site of DNA gyrase to facilitate binding of test compound with active site of DNA gyrase. The oxygen atom and nitrogen atom was forming hydrogen bond with amino groups of protein with specific bond distance. The oxygen atom of amide and hydroxyl group and nitrogen atom of compound (**3b)** make a five hydrogen bond with amino group of Histidine, tyrosine and Arginine (2.864675A^o 150HIS, 2.916301A^o 141TYR, 2.881603 & 2.999295A^o 384ARG, 2.603282A^o 415TYR). The docking study revealed that all test compounds showed significant docking score as compared with standard ciprofloxacin (8. 0755 kcal/mol) and having good binding energy between -9.75982 to -12.8323kcal/mol. Among the entire designed compound hydrogen and Chlorine group substituted compound (3a, 3d, 3f) represent the promising binding energy at -12.8323, -12.2968, -12.284 kcal/mol respectively. The compound substituted with electron donating group exhibited better binding energy than electron withdrawing group

Journal of Chemical Health Risks

www.jchr.org

JCHR (2024) 14(3), 1307-1330 | ISSN:2251-6727

substituted at same position. The docking score of all test compounds were shown in Table-19**.**

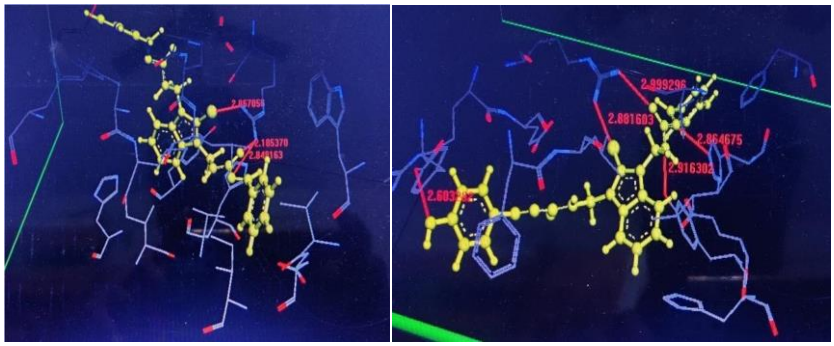


Fig.18. H-bonding score of compound (3a and 3b)

7. Conclusion

In the present work, different analogs of Schiff base containing N, N'-disubstituted Benzimidazole-2-thione derivatives (3a-3f) were designed and subjected for docking study using free available software Argus lab 4.0. Among the designed compound, substituted benzaldehyde containing the functional group like (OH, NO_2, OCH_3 and Cl) and unsubstituted compound was found to showed good binding energy as compared to another and also the compound substituted with -Cl group shown better binding energy. Furthermore, among the designed compounds which possess good binding energy was selected for synthesis and the new series of different benzaldehyde containing N, N'disubstituted Benzimidazole-2-thione derivatives were prepared by treating intermediate2 with substituted benzaldehyde by suitable synthetic method. The synthesized new compounds were purified by recrystallization method using suitable solvents and the structure of newly synthesized compound was confirmed by physicochemical and spectral analysis like IR, ^{1}HNMR, ^{13}CNMR and molecular weight of compound was confirmed by GC-MS spectroscopy. On the basis of interpretation of spectral data obtained from spectral analysis, it was concluded that the synthesized compounds were desired compounds i.e. 1H-benzo[d]imidazole-2(3H)-thione-1,3bis-(methyl urea benzylidine) (3a-3f) as expected. Subsequently in the anti-microbial screening, almost all the test compounds exhibited significant antimicrobial activity against both gram positive and gram negative bacteria and synthesized compounds was considered as promising lead candidate for development of new antimicrobial agents. Further *in-vivo* studies are needed to determine the toxicological effect of these compounds in living bodies. It is still not safe to assume that they are good therapeutic agents and further testing and *in-vivo* experiments are needed to fully assess its pharmacological applicabilities.

References

1. Choudhary Kapill; Bhavsar Rupali; Chaturvedi Prerna; Synthesis and Characterization of Benzimidazole Derivatives for Antimicrobial PropertyInt. Journal of Pharmaceutical Sciences and Medicine (IJPSM), Vol.6 Issue. 8, August-2021, pg. 134-143.
2. Hassan A, Khan N. Novel acyl hydrazones Schiff's bases of benzimidazole-2-thiol. J Anal Pharm Res, 2021; 10(4):151-155.
3. Özgeriş Fatma, Kaci Necmiye, OzgerisBunyamin and GörmezArzu. The Synthesis of Unsymmetrical Urea from Substituted Phenethylamine and the Investigation of its Antibacterial, Anticancer, and Antioxidant Properties. Biointerface Research in Applied Chemistry. (2021);12:7052-7063.
4. Rabia, A. Y. U. B., Anham ZAFAR, Sumaira ABBAS, and AL-SHAKBAN Mundher.

"Synthesis, antimicrobial activity, urease inhibition and molecular docking studies of new proline linked thiourea derivatives." Rev. Roum. Chim. (2020);65(9): 783-788.

5. Naz Sumaira, Zahoor Muhammad, Umar Muhammad, Naveed Alghamdi, Saad Sahibzada, Muhammad Umar Khayam and UlBari Wasim. "Synthesis, characterization, and pharmacological evaluation of thiourea derivatives" Open Chemistry. (2020);18 (1): 764-777.
6. Mohapatra R K, Das P. K, Pradhan M K, El-Ajaily M M, Das D, Salem H F, -E-Zahan M K. Recent advances in urea-and thiourea-based metal complexes: biological, sensor, optical, and corrosion inhibition studies. Comments on Inorganic Chemistry, (2019); 39(3): 127-187.
7. Kodomari Ren,Das P. K, Pradhan M K,Mohapatra R K, El-Ajaily M M, Das D, Salem H F, -E-Zahan M K. Recent advances in urea-and thiourea-based metal complexes: biological, sensor, optical, and corrosion inhibition studies. Comments on Inorganic Chemistry, (2019); 39(3): 127-187.
8. Jha, Das P. K, Pradhan M K,Mohapatra R K, El-Ajaily M M, Das D, Salem H F, -EZahan M K. Recent advances in urea-and thiourea-based metal complexes: biological, sensor, optical, and corrosion inhibition studies. Comments on Inorganic Chemistry, (2019); 39(3): 127-187.
9. El-Ayaan,El-Ajaily M M, Das D, Salem H F, -E-Zahan M K,Jha, Das P. K, Pradhan M K,Mohapatra R K. Recent advances in urea-and thiourea-based metal complexes: biological, sensor, optical, and corrosion inhibition studies. Comments on Inorganic Chemistry, (2019); 39(3): 127-187.
10. Sanna G, Madeddu S, Giliberti G, Piras S, Struga M, Wrzosek M, Szulczyk D. Synthesis and biological evaluation of novel indole-derived thioureas. Molecules. (2018); 23(10): 2554.
11. Mohan, Chandra Kumar, Vinod, Kumari Sarla.Synthesis, characterization, and antimicrobial activity of Schiff bases derived from thiosemicarbazide, 2-acetyle thiophene and thiophene-2 aldehyde. International Research Journal of Pharmacy. (2018); 9. 153-158. 10.7897/2230-8407.097141.
12. : B. S. Balaji, Neha Dalal. An expedient and rapid green chemical synthesis of Nchloro-acetanilide and amides using acid chlorides under metal free neutral conditions, Green Chemistry Letters and Reviews,(2018);11:4, 552-558.
13. Ghorab MM, Alsaid MS, El-Gaby MSA, Elaasser MM, Nissan YM. Antimicrobial and anticancer activity of some novel fluorinated thiourea derivatives carrying sulfonamide moieties: synthesis, biological evaluation and molecular docking. Chem. Cent J. (2017); Apr 7:11(1):32.
14. Divyani G, Priyanka K, Shikha A.Synthetic aspects and Biological Studies of some Heterocycles.Chemistry & Biology Interface. 2017; 7:79-101.
15. SatbirM,Nagoria S,,Suchita S, Singh V. Synthesis and biological activities of 1,4benzothiazine derivatives: An overview.Chemistry& Biology Interface.2017;7:1-18.
16. VioletaI , Perez N . Editorial (Thematic Issue: Towards the Integration of Quantitative and Systems Pharmacology into Drug Discovery: a Systems Level Understanding of Therapeutic and Toxic Effects of Drugs). Current Pharmaceutical Design.2016; 22:6881-6884.
17. Alaqeel SI, Rithe, Rushi. Synthetic approaches to Benzimidazole from ophenylenediamine: A literature review. Journal of Saudi Chemical Society. (2016); 21(2): 229-237.
18. Rushi,Alaqeel SI, Rithe,. Synthetic approaches to Benzimidazole from ophenylenediamine: A literature review. Journal of Saudi Chemical Society. (2016); 21(2): 229-237.
19. Saini Sarika, Neerupma Dhiman, Aman Mittal and Gaurav Kumar. "Synthesis and antioxidant activity of the 2-methyl Benzimidazole." Journal of Drug Delivery andTherapeutics. (2016); 6 (3): 100-102.
20. El-Kihel A, Zouitina S, Guesmi S, Ahbala M, Bauchat P, and Bier sack B. Improved Method of S-Alkylation of 2-Mercaptobenzimidazole Derivativeswith Trialkylphosphite. Asian Journal of Chemistry. (2016); 28(6): 1267–1269.

Journal of Chemical Health Risks

www.jchr.org

JCHR (2024) 14(3), 1307-1330 | ISSN:2251-6727

21. El Sayed H. El Ashry,Yeldez El Kilany, Nariman M. Nahas, Assem Barakat, Nadia Al-Qurashi, Hazem A. Ghabbour and Hoong-Kun Fun, Synthesis and Crystal Structures of Benzimidazole-2-thione Derivatives by Alkylation Reactions, Molecules 2016, 21, 12.
22. Shakeel, A., Altaf, A. A., Qureshi, A. M., & Bad shah, A. Thiourea derivatives in drug design and medicinal chemistry: A short review. J. Drug Des. Med. Chem. (2016); 2(1).
23. Stefanska J, Nowicka G, Struga M,Szulczyk D, Koziol A. E,Augustynowicz-Kopec E. &Sanna G. Antimicrobial and anti-biofilm activity of thiourea derivatives incorporating a 2-aminothiazole scaffold. Chemical and Pharmaceutical Bulletin. (2015); 63(3): 225-236.
24. Sharma Praveen, Dar John, Singh Simranjeet. Synthesis and antimicrobial activities of substituted phenylthioureas. (2015); 133-139.
25. El Ashryel SH, El Kilany Y, Nahas NM, Barakat A, Al-Qurashi N, Ghabbour HA, Fun HK. Synthesis and Crystal Structures of Benzimidazole-2-thione Derivatives by Alkylation Reactions. Molecules. (2015); Dec 22:21(1):E12.
26. Nath Mahendra Mitta and K.Madhavachetty.Synthesis, Spectral Characterization and Anti Bacterial Activity of Novel Thiourea Analogues.International Journal Current Microbial Applied Science. (2015); 4(5): 900-909.
27. Bhaskara Reddy M. Vijaya, Srinivasulu D, Peddanna K, Apparao Ch and Ramesh P. Synthesis and Antioxidant Activity of New Thiazole Analogues Possessing Urea, Thiourea, and Selenourea Functionality. Synthetic Communications.(2015);1532-2432 31.
28. SudhamaniHasti, Basha Thaslim, Venkateswarlu Dr, Vijaya Tartte and Raju C. Synthesis and Characterization of New Thiourea and Urea Derivatives of 6-Fluoro-3(piperidin-4-yl)benzo[d] Isoxazole: In vitro Antimicrobial and Antioxidant Activity. Journal of Chemical Sciences. (2015);127:10:1007/s12039-015-0935-6.
29. Maw-Ling Wang, Biing-Lang Liu. Synthesis of 2-mercaptobenzimidazole from the reaction of o-phenylene diamine and carbon disulfide in the presence of potassium hydroxide. (2012); 38(2): 161–167.
30. SarıbıyıkOY, Serin S Synthesis, Characterization and Catalytic Investigation of Novel Polymeric Thiourea, Designed Monomers and Polymers (2011); 14:2 133-142.
31. Singhal Manmohan, Arindam Paul, Hemendra Pratap Singh, Sushil Kumar Dubey, and Rajendra K. Songara. “Synthesis And Evaluation of Antioxidant Activity of Semicarbazone Derivatives”. International Journal of Pharmaceutical Sciences and Drug Research. (2011); 3 (2): 150-54.
32. Arslan H, Duran N, Borekci G, Koray Ozer C, Akbay C. Antimicrobial Activity of Some Thiourea Derivatives and Their Nickel and Copper Complexes. Molecules. 2009; 14(1):519-527.