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DESIGN, SYNTHESIS, SPECTRAL ANALYSIS DFT, ANTIMICROBIAL AND DOCKING ACTIVITIES OF SOME NOVEL BENZENETHIOL BASED DERIVATIVES OF BENZIMIDAZOLE

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ABSTRACT

A series of novel benzimidazole derivatives 4a-c were obtained by two-step synthesis from various substituted benzenethiol with N-((1H-benzo[d]imidazol-2-yl) methyl)-4-chloroaniline. All the Synthesized compounds were characterized by IR, ^{1}H and ^{13}C NMR. The Synthesized compounds were further studied using density functional theory (DFT) calculations at the Becke3 Lee Yang Par level with 6-311G (d, p) basis set. The frontier molecular orbitals (HOMO and LUMO) were computed to determine the distribution of charge density and possible site for electrophilic and nucleophilic reactions. The newly synthesized compounds were screened for their anti-fungal and antibacterial activities using serial dilution and agar diffusion methods respectively. A molecular docking study has supported the antimicrobial activity of the synthesized compounds.

KEYWORDS

2-(chloromethyl)-1H-benzo[d] imidazole, IR, NMR, DFT and Docking.

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INTRODUCTON

Benz imidazole and benzimidazole derivatives belong to the family of heterocyclic compounds which are widely used in medicinal chemistry because of their significant properties as therapeutics in clinical applications. Benzimidazole is a versatile pharmacophore producing a diverse range of biological activities including anticancer[1], antihypertensive[2], anthelminthic[3-5], antiprotozoal[6,7], antimicrobial[8-13], analgesic[14], anti-inflammatory[15,16] and anti-hepatitis-B-virus[17]etc. The most prominent

benzimidazole compound in nature is N-ribosyl-dimethylbenzimidazole, which serve as an axial ligand for cobalt in vitamin B_{12}[18] Optimization of substituent around the benzimidazole nucleus has resulted in many drugs like albendazole, mebendazole, thiabendazole as anthelmintic, omeprazole, lansoprazole, pantoprazole as proton pump inhibiters[19]. Though all seven positions in the benzimidazole nucleus can be substituted with a variety of chemical entities, but most of the biologically active benzimidazole base compounds been functional groups at 1, 2 and (or) 5 (or) 6 positions. Based on these findings the aim of this study was to synthesize a novel series of benzimidazole derivatives derived from 2-chloromethyl-1H-benzimidazole and screened for antimicrobial activities. The synthesized compounds were characterized by IR, 1DNMR (^{1}H, ^{13C}) and CHN analysis.

EXPERIMENTAL

MATERIAL AND METHODS

Melting points (mp) were reordered on open capillary melting point apparatus and are uncorrected. IR spectra were recorded in AVATAR 330 FT-IR Thermo Nicholet spectrophotometer (range 4000-400 cm^{-1}) as KBr pellets. ^{1}H NMR spectra were recorded with a Bruker AMX-400 spectrometer operating at 400.23 MHz spectrometer at room temperature, using TMS as internal standard. Splitting patterns are indicated as follows: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; br; broad peak. ^{13}C NMR spectra were recorded on the same instrument at 100.63 MHz and are referenced using the central line of the solvent signal (DMSO-d_6 septet at δ = 39.5 ppm). Thin layer chromatography was carried out on (Fluka) Silica Gel. All the chromatographic purifications were performed with silica gel 60 (100-200 or 200-400 mesh), whereas all TLC (Silica gel) was performed on silica gel coated (Merk Kiesel 60 GF-254, 0.2 mm thickness) sheets. All reagents and solvents commercially obtained (Sigma-Aldrich®, Himedia®) were used directly and without further purification

General procedure

Synthesis of (1H-Benzimidazole-2-ylmethyl)-(4-chloro-phenyl)-amine[20]

2-Chloromethyl-1H-benzimidazole (0.01 mol) and K_2CO_3 (0.02mol) were stirred at room temperature in dimethylformamide (DMF, 20 ml) for half an hour and pinch of KI was added and *p*-chloroaniline (0.01mol) was added. The reaction was refluxed for 12 hrs until TLC showed completion of reaction. The reaction mixture was poured into water (20ml) and the mixture was extracted with ethyl acetate (3X20 ml). The organic extracts were washed with water, dried over anhydrous sodium sulphate and concentrated to obtain crude product. The residue was recrystallized from diethyl ether to give pure compound.

Computational analysis

The synthesized compounds have been computed using Gaussian 09W [9] software program package. The diffuse basis set used in the package is 6-311G (d, p) which are additionally included by'd' polarization function on main-group elements and 'p' on only hydrogen atoms. These polarization functions were used for detailed description of polar bonds of molecules [10, 11]. It should be noted that 'p' polarization function gives importance to hydrogen atoms because they are useful for reproducing the out-of-plane vibrations and also for a better description of the vibrational modes and molecular geometry of the molecule. In the Gaussian 09W [9] package at the DFT/Becke3 Lee Yang Par/6-311G (d, p) method.

RESULTS AND DISCUSSION

Chemistry

The synthesis of various benzimidazole derivatives were prepared by condensing N-((1H-benzo[d]imidazol-2-yl) methyl)-4-chloroaniline with several substituted benzene thiols (benzene thiol, chlorobenzene thiol, p- chlorobenzene thiol,) by refluxing in ethanol for 12hrs (Scheme No.1). The crude products were further recrystallised from methanol. The structural confirmation of the benzimidazole derivatives was done by various spectroscopic techniques including NMR, IR. Thin layer chromatography (TLC) was employed to

monitor reaction progress and to determine the purity of the products. The structures of the obtained compounds were identified and characterized by elemental analysis, FT-IR, and by 1 H NMR and 13C NMR spectroscopy. The presence of benzimidazole derivatives was confirmed by FTIR measurement: All the compounds showed absorption bands of the N-H stretching frequency of the imidazole moiety) at 3409-3475 cm^{-1}. The benzimidazole derivatives display a strong intensity band in the IR spectra, at 1614-1619 cm^{-1} which is attributed to CN provides a strong evidence for the -C-N- linkage formed between benzimidazole ring and aromatic amines moieties confirm the formation of compounds 4a-d. A prominent band appeared at ~747 cm^{-1} is due to C-Cl deformation vibration. Several medium intensity bands appeared in the 3040-3056 cm^{-1} region of the spectra is due to the stretching of CHvibrations.

In the 1 H NMR spectra of benzimidazole derivatives, the integral intensities of each signal are found to agree with the number of different types of protons present. In the spectra of the compounds, a singlet signal is appeared for NH proton (1H) in the range of 7.78. This supports the formation of benzimidazole derivatives by the condensation of benzimidazole with amines.

In ^{1}HNMR spectrum of compound 4a-4c show broad singlet in the region of 12.2ppm which is due to free NH group present. A singlet at 4.4ppm (4a-4c) shows the presence of methylene group in 2-chloromethyl-1H-benzimidazole derivatives. Aromatic protons appeared as multiplet at 7.56-6.76ppm show the presence of 2-(2-chlorophenyl)-1H-benzo[d] imidazole derivatives. On focusing the ^{13}CNMR spectral assignments, the signals at 148 is due to C-N of amine compounds (4a-4d).The aromatic carbon signal is appeared at 123.2-124.5ppm (4a-4c) and 44.24ppm show the presence of methylene carbon of 2-chloromethyl-1H-benzimidazolemoiety(4a-4c).

Analytical data

Synthesis of (1H-Benzimidazole-2-ylmethyl)-(4-chloro-phenyl)-amine

A mixture of (1H-Benzimidazole-2-ylmethyl)-(4-chloro-phenyl)-amine (0.01mol) and benzene thiol (0.01mol) and KI (0.01 mol) in 50 ml of ethanol was heated under reflux for 12 h, KOH (0.01mol in5mL of water) was added with continuous stirring for 2 h. Finally the reaction mixture was left aside at room temperature and then poured into crushed ice. The solid product that precipitated was filtered off, recrystallized from ethanol and dried in vacuum desiccators. The synthetic route for the target compounds 4a–4c is shown in Scheme No.1.

N-((1H-benzo[d]imidazol-2-yl) methyl)-4-(phenylthio) aniline (4a)

Yellow crystal; m.p. 187–192^{o}C; Yield 65%; IR (KBr): ν_{max} in cm^{-1}: 3475(-NH),3066(aromatic–CH), 1604(-CN), 3015(aliphatic–CH), ^{1}H NMR (400 MHz, DMSO-d6): δ (ppm) 12.03 (s, 1H, benzimidazolic NH), 6.56-7.48 (13H, m, aromatic protons), 8.46 (s,NH), 4.46(2H, s, protons of CH2 linkage). ^{13}C NMR (100.63 MHz, $CDCl_3$): δ (ppm) 148.1, 142.2, 141.4, 135.3, 131.5, 130.2, 129.4, 128.2, 126.4 125.3, 123.4, 117.1, 115.2, 44.3:Anal. calcd for $C_{20}H_{17}N_3S$ (331): C, 73.46; H, 5.51; N, 12.88; S, 9.72.

N-((1H-benzo[d]imidazol-2-yl) methyl)-4-(2-chlorophenylthio) aniline (4b)

Pale yellow crystal; m.p. 167–178^{o}C; Yield 58%; IR (KBr): ν_{max} in cm^{-1}: 3408(-NH), 3035(aromatic–CH), 1614(-CN), 3012(aliphatic–CH) 747(C-Cl), ^{1}H NMR (400 MHz, DMSO-d6): δ (ppm) 12.21 (s, 1H, benzimidazolic NH), 6.52-7.56 (12H,m,aromatic protons),7.85 (s,NH),4.41(2H,s,protons of CH2 linkage). ^{13}C NMR (100.63 MHz, $CDCl_3$): δ (ppm) 147.8, 141.2, 140.2, 134.3, 132.5, 130.5, 129.4, 127.2, 126.3 125.3, 123.4, 117.1, 115.244.3:Anal. calcd for C20H16ClN3S (365): C, 65.81; H, 4.21; Cl, 9.80; N, 11.24; S, 8.45.

N-((1H-benzo[d]imidazol-2-yl) methyl)-4-(4-chlorophenylthio) aniline (4c)

yellow crystal; m.p. 172–179^{o}C; Yield 68%; IR (KBr): ν_{max} in cm^{-1}: 3402(-NH), 3045(aromatic–CH), 1619(-CN), 3018(aliphatic–CH) 749(C-Cl), ^{1}H NMR (400 MHz, DMSO-d6): δ (ppm) 12.18 (s, 1H,

benzimidazolic NH), 6.62-7.58 (12H, m, aromatic protons), 7.78 (s,NH), 4.42(2H, s, protons of CH2 linkage). ^{13}C NMR (100.63 MHz, $CDCl_3$): δ (ppm) 148.2, 141.6, 140.8, 135.3, 133.2. 132.5, 130.1, 129.3, 127.2 125.5, 123.1, 117.6, and 115.4 44.2:Anal. calcd for $C_{20}H_{16}ClN_3S$ (365): C, 66.61; H, 4.52; Cl, 9.65; N, 11.31; S, 8.36.

DFT studies

Geometry optimisation

The optimized ground state structure with atomic numbering of 4a-4c has been given in the Figure No.1. The optimized parameters of the synthesized molecules calculated by B3LPY/6-31G (d, p) are listed in Table No.1. As a result of partial protonation of both nitrogen atoms C4-N5 and C3-N2 bond lengths in benzimidazole moiety are approximately equal for all compounds 4a-4c. This result was confirmed by the fact that the bond lengths C4-N5 and C3-N2 in benzimidazole moiety are 1.381 and 1.381 Å in 4a, 1.432 and 1.420 Å in 4b, 1.418 and 1.434 Å in 4c.This result was supported by the fact that the computed dihedral angles for N5-C1-C10-C15, N5-C1-C10-C11, N17-C18-N21-C11, N19-C18-N21-C11, and N17-N16-C13-N21 are 0° or 180°.

HOMO and LUMO analysis

The frontier molecular orbitals can offer a reasonable qualitative prediction of the excitation properties and the ability of electron support. The highest molecular orbital (HOMO) is outermost higher energy orbital containing electrons so it act as an electron donor. The lowest unoccupied molecular orbital is the lowest energy orbital that has the room to accept electrons so it acts as an electron acceptor. The HOMO and LUMO are also very popular quantum chemical parameters which determine the molecular reactivity. The energies of the HOMO and LUMO orbitals of the title compound are calculated using B3LYP/6-311G (d, p) levels and shown in Figure No.2.

In frontier energy molecular orbital diagram (Figure No.2), the HOMO is distributed on aromatic rings (especially electron donating like methyl group substituents) and phenyl ring in second postion of benzimidazole for all compounds 4a-c. The lower value of HOMO–LUMO energy gaps of the compound 4a-c reflects increased charge transfer within the molecule. HOMO-LUMO energy gap values for the compounds 4a-c values are listed in Figure No.2

Procedure for Antibacterial studies[16,18,21]

The antibacterial activities of the synthesized compounds against different pathogens were determined by Agar Well diffusion method. Using sterile inoculation loop, 20 pure colonies of the test organism are transferred to 5ml of sterile nutrient broth and incubated at 37^oC overnight for 18hrs. The modified agar well diffusion method of Perez *et al*[21]. Was employed. Each selective medium was inoculated with the microorganism suspended in sterile water. Once the agar was solidified, it was punched with a six millimeters diameter wells and filled with 50µg/ml of the sample and blanks (ethanol and antibiotic). The test was carried out by triplicate. The plates were incubated at 35 ± 2°C for 24 h.

The results of antibacterial activity of the synthesized compounds against the pathogenic strains viz., *Klebsiella pneumonia* ATCC-15499(K. Pneumonia), *Salmonella typhi* ATCC-24930 (S.typhi), *staphylococcus aureus* ATCC-25833(S.aureus), *Bacillus Subtilis* ATCC-461(B.Subtilis), *Pseudomonas. Auruginosa* ATCC-27853(P. Auruginosa), Escherichia coli ATCC-25840 (E. coli) by agar well diffusion method. And their MIC's were compared with ciprofloxacin standard drug. MIC values in Table No.1 revealed that compound 4b exhibited excellent activity against E. coli at MIC 5µg/ml than other derivatives.

Molecular Docking

We have performed a molecular modeling study to investigate the possible binding conformation for the benzimidazole based heterocyclic amines compounds by inhibiting E. coli enzyme (biotin carboxylase) binding site which may be give an idea about the carboxylase activity and mechanism of action. The crystal structure (PDB code: 3JZI) was downloaded directly from the Protein Data Bank (www.rcsb.org). Docking simulations were performed with simple and fast molecule 1-click docking server. The interaction between the protein

and ligands were viewed through using using Accelrys Discovery Studio Visualizer software[22]. The docking results showed that the best scored confirmation showed a very similar fashion compared to the standard.

Among the docking results Compound 4b N-((1H-benzo[d]imidazol-2-yl) methyl)-4-(2-chlorophenylthio) aniline has given the top docking score. The docking score and protein interactions of the compounds were tabulated in Table No.2. The 2D and 3D interactions of the compound 4b were shown in Figure No.3. LYS-238 is on the top centre of the cavity which is involved in π-electron interaction with 4b. GLU-276 forms a hydrogen bond with NH group of Benzimidazole moiety.

Table No.1: Antibacterial activities of compounds 4a-4c, for bacterial strains in MIC (µg/ml)

S.No	Bacterial strains (MIC)	COMPOUNDS			
		Ciprofloxacin	4a	4b	4c
1	K. pneumoniae	15	40	50	50
2	S. Typhic	10	45	50	50
3	S. Aureus	5	50	45	45
4	B.Subtilis	5	30	35	35
5	P. auruginosa	5	30	40	30
6	E.coli	5	10	5	15

Table No.2: Docking Results

S.No	Compound	Docking Score kcal/mol	Interacting Residues
1	4a	-8.7	HIS 236, LYS-238
2	4b	-9.0	HIS 238, TYR-203, LYS-238
3	4c	-8.2	HIS 236, TYR-203

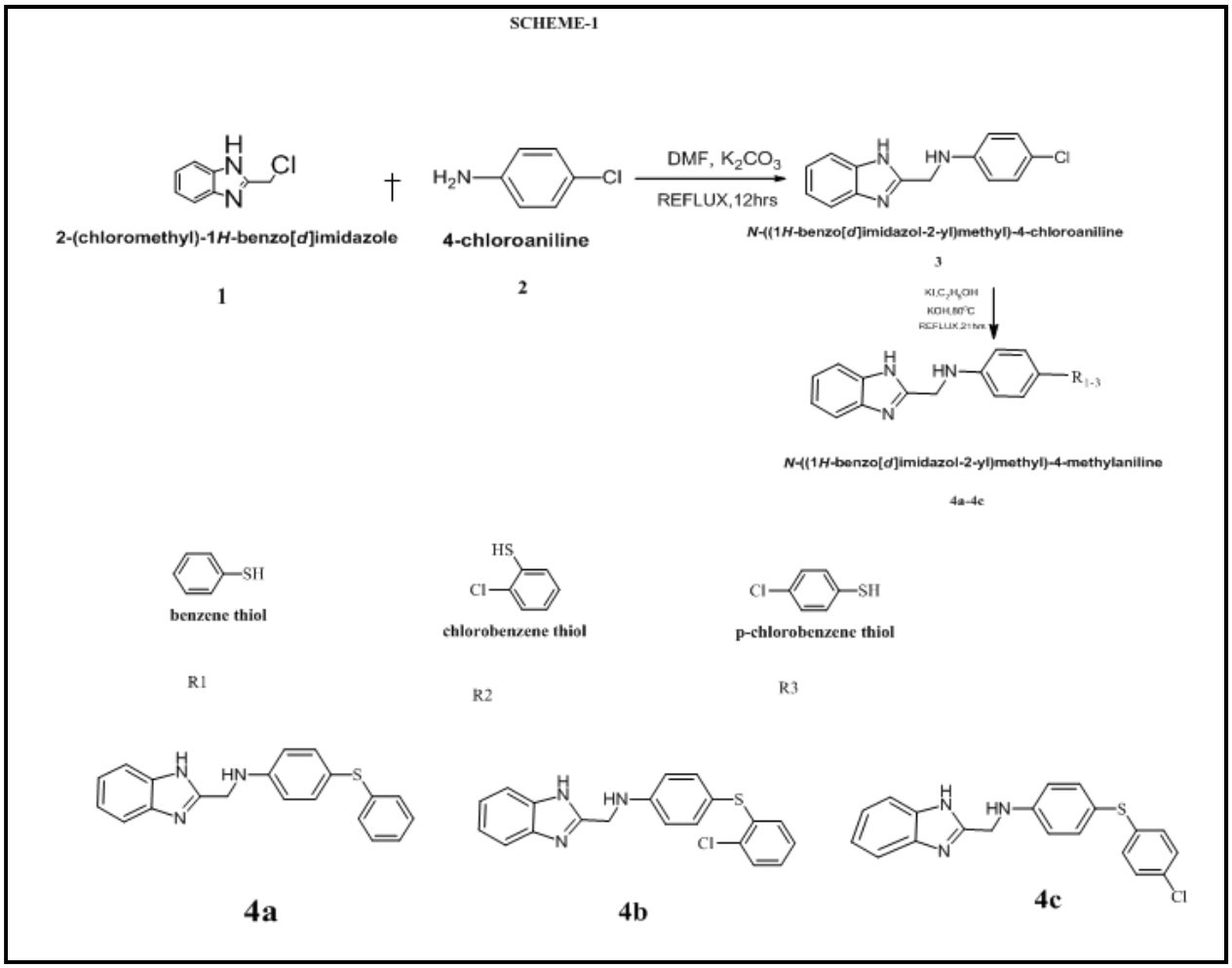


Figure No.1: Scheme of synthesis

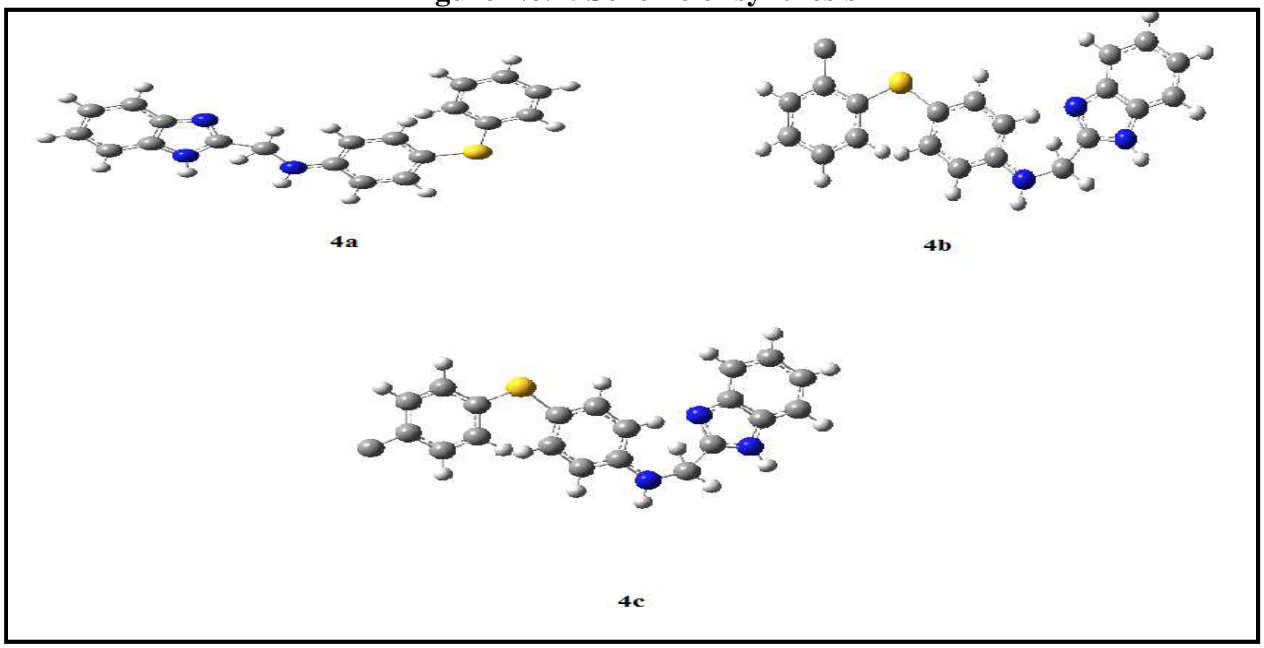
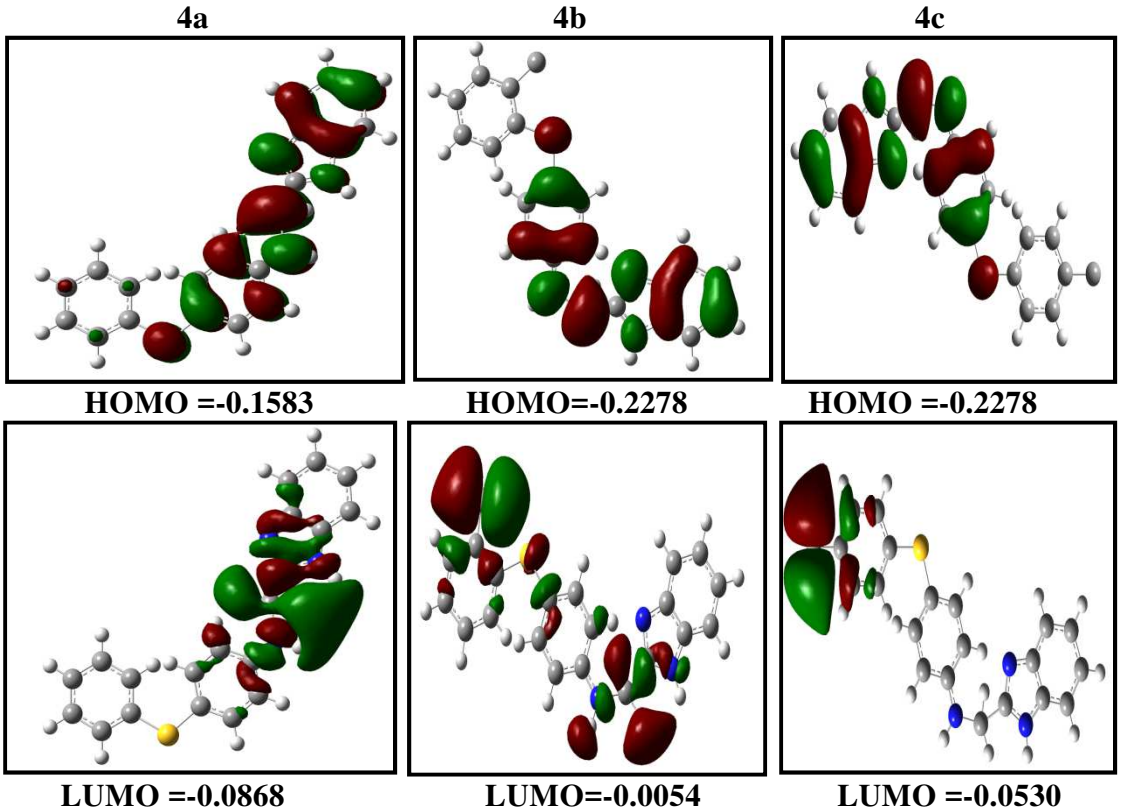
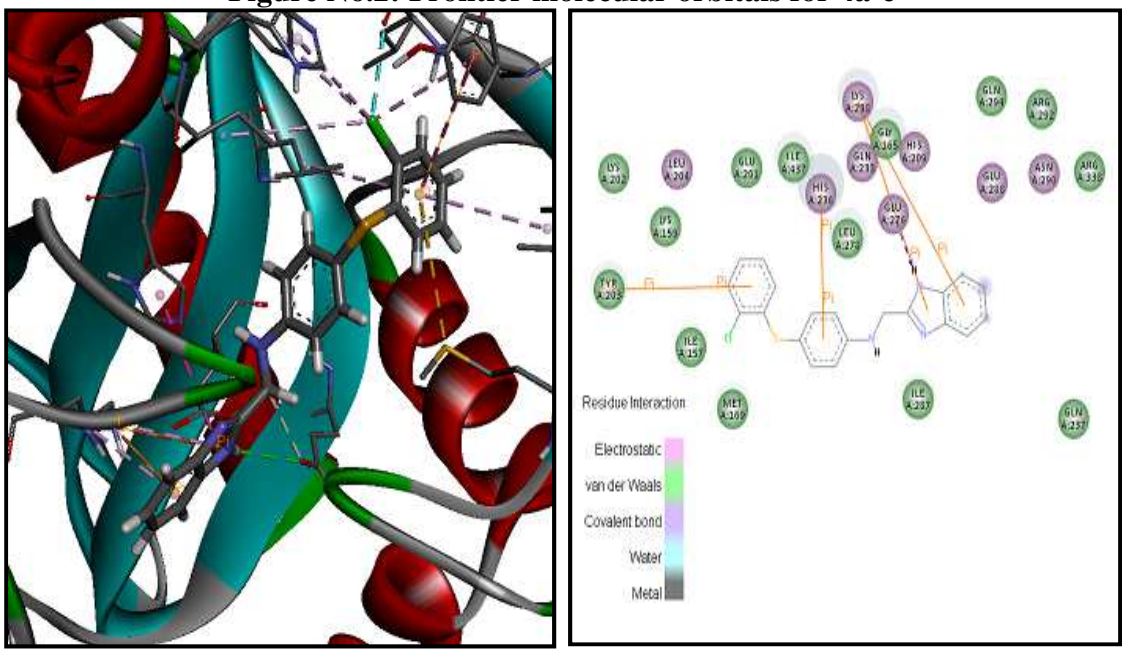


Figure No.1: Optimized geometries of 4a-c



$E_{LUMO} - E_{HOMO}$ (ΔE)= 0.0715 $E_{LUMO} - E_{HOMO}$ (ΔE)= 0.1723 $E_{LUMO} - E_{HOMO}$ (ΔE)= 0.1747

Figure No.2: Frontier molecular orbitals for 4a-c



3D view **2D view**

Docking scores =-9.0kcal/mol

Figure No.3: Molecular docking of 4b

CONCLUSION

In the present work we have synthesised 2-Chloromethyl-1H-benzimidazole derivatives from benzthiols. We have calculated the equilibrium geometries, frontier orbitals, MESP of 4a-c at DFT level employing the 6-31G (d,p) basis set. The lower frontier orbital energy gap, the higher dipole moment and polarisability values make 4a the more reactive and more polar as compared to the 4b-c. They were characterized by IR, ^{1}H, ^{13}C NMR. All the newly synthesized compounds were tested for antibacterial and antifungal activity by agar well diffusion and serial dilution method. Among the screened samples, compound 4bexhibited as most active against bacterial strain E. coli compared to other synthesized compounds.

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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