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Design, Molecular Docking, DFT and Antimicrobial Studies of Novel Benzimdazole Derivatives

A. Adhilakshmi[1], S. Darlin Quine[2]

[1]Department of Chemistry, Krishnasamy College of Science, Arts and Management for Women, Cuddalore-607109, Tamilnadu, India

[2]Post Graduate and Research Department of Chemistry, Government Arts College, C. Mutlur, Chidambaram-608001, Tamilnadu, India
email: *kapilesh071978[at]gmail.com*

Abstract: ***In the study, we have successfully synthesized N-((1H-benzo [d]imidazol-2-yl) methyl)-4-chloroaniline by the reaction of 4-chloroaniline with 2-(chloromethyl)-1H-benzo [d]imidazole. Thereafter novel derivative would be subjected to synthesize (1a&1b) by N-((1H-benzo [d]imidazol-2-yl) methyl)-4-methylaniline with 1, 2, 4-triazole and N-methyl-1H-1, 2, 4-triazol-3-amine These synthesized compounds (1a&1b) were characterized by FT-IR, ^{1}H NMR and ^{13}C spectroscopy, and the theoretical investigation of MEPs, HOMO, LUMO, and the energy gap of HOMO-LUMO were calculated by B3LYP/6-311G (d, p) method. The Synthesized compounds were evaluated for their antibacterial activities using agar diffusion methods respectively. A molecular docking study has supported the antimicrobial activity of the synthesized compounds.***

Keywords: 4-chloroaniline, 2-(chloromethyl)-1H-benzo [d]imidazole, Molecular docking, DFT

1. Introduction

Benzimidazole and their derivatives are an important class of heterocycles with a wide range of pharmacological and biological activities. Benzimidazole ring displays an important heterocyclic pharmacophor in drug discovery. The pharmacological application of benzimidazole analogs found potent inhibitors of various enzymes involved and therapeutic uses including as anti-diabetic, anti-cancer, anti-microbial, anti-parasitic, analgesics, anti-viral, anti-histamine and also neurological, endocrinological, and ophthalmological drugs [1, 2, 3, 4, 5, 6, 7, 8, 9]. Looking at the antimicrobial importance of benzimidazole and triazole scaffolds, and regarding our previous studies on benzimidazoles [10, 11, 12, 13, 14, 15, 16], we planned to synthesize a hybrid molecules that involve two different pharmacophores. Thus, in this study, two novel N-((1H-benzo [d] imidazol-2-yl) methyl)-4-(1H-1, 2, 4-triazol-1-yl) aniline and N1-((1H-benzo [d]imidazol-2-yl) methyl)-N4-(1H-1, 2, 4-triazol-3-yl) benzene-1, 4-diamine (**1a and 1b**) were synthesized and investigated for antimicrobial activities. A molecular docking study has supported the antimicrobial activity of the synthesized compounds.

2. Experimental

Material and Methods

All reagents and solvents commercially obtained (Sigma-Aldrich®, Himedia®) were used directly and without further purification. . Melting point ranges of solid compounds were reordered on open capillary tubes using a hot stage apparatus and are uncorrected. IR spectra were obtained by Jasco FTS 3000MX (KBr pellets).1H NMR spectra were recorded with a Bruker AVANCE III 500 & 400 MHz spectrometer at room temperature, using TMS as internal standard.1^{3}C NMR spectra were recorded on the same instrument at 125.76 & 100.00 MHz and are referenced using the central line of the solvent signal (DMSO-d_6 septet at δ = 39.5 ppm Elemental analyses (C, H and N) were performed with a Perkin Elmer 2400 Series II CHN Analyzer. 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.

3. General Procedure

Synthesis of (1H-Benzimidazole-2-ylmethyl)-(4-chloro-phenyl)-amine [17]

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 (0.01 mol) was added. The reaction was refluxed for 12 hrs until TLC showed completion of reaction. The reaction mixture was poured into water (20 ml) 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

Density functional theory (DFT) has become very popular in recent years. The molecular geometry optimizations were performed by density functional theory [18] with Becke's three parameters and the Lee-Yang-Parr functional (B3LYP) [19-20] with the standard 6-311++G (d, p) basis set with Gaussian 09 software package. No symmetry restriction was applied during geometry optimization.

4. Results and Discussion

Chemistry

Triazole based benzimidazole derivatives were synthesized by condensing N-((1H-benzo [d]imidazol-2-yl) methyl)-4-chloroaniline with 1, 2, 4-Triazole and 1, 2, 4-triazol-3-amine by refluxing in ethanol for 20-30hrs

(Scheme 1). The progress of the reaction was monitored by TLC. Upon completion of the reaction, the mixture was allowed to cool to room temperature. The resulting residue was crystallized from methanol to give pure compound at room temperature. All the synthesized molecules were characterized by IR, ^{1}H and ^{13}C NMR spectroscopy. Moreover absorption bands ranging from 1290-1303 cm^{-1} correspond with the-C-N-linkage formed between benzimidazole ring and heteroaromatic amines moieties confirm the formation of compounds **1a and 1b.** The band observed at 3022-3010 cm^{-1} is ascribed to aromatic CH stretching. The 400MHz NMR spectrum of **1b** reveals two singlets at 8.03 and 12.01ppm are assigned to N3H and N1H of triazole moiety. This supports the formation of benzimidazole derivatives by the condensation of benzimidazole with 1, 2, 4-triazol-3-amine.

In ^{13}C NMR spectroscopy, the signals in the region 115.6-123.2 ppm in all the compounds are characteristic of C4, C5, C6 and C7 carbons in benzimidazole moiety. The C8 and C9 carbons of benzimidazole ring are observed at 139.4 and 141.2 ppm respectively. The signal observed at 152.4 ppm should be due to C2 carbon of the benzimidazole ring. In compound **1b**, signals at 148.9 and 146.2 ppm are assigned to the two carbons of C17 and C20 of triazole moiety.

Spectral data

Synthesis of (1H-Benzimidazole-2-ylmethyl)-(4-chloro-phenyl)-amine
A mixture of (1H-Benzimidazole-2-ylmethyl)-(4-chloro-phenyl)-amine (0.01 mol) and benzene thiol (0.01 mol) and KI (0.01 mol) in 50 ml of ethanol was heated under reflux for 12 h, KOH (0.01 mol in 5 mL 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 **1a and 1b** is shown in fig.1.

N-((1H-benzo [d]imidazol-2-yl) methyl)-4-(1H-1, 2, 4-triazol-1-yl) aniline (1a).
Pale yellow crystal; m. p.352^{0}C; Yield 69%; IR (KBr): ν_{max} in cm^{-1}: 3488 (-NH), 3051 (aromatic–CH), 1615 (-CN), 3022 (aliphatic–CH), ^{1}H NMR (400 MHz, DMSO-*d6*): δ (ppm) 13.02 (s, 1H, benzimidazolic NH), 6.72-7.56 (8H, m, aromatic protons), 7.03 (s, NH), 8.92-9.01 (s, 2CH Triazole), 12.01 (s, NH triazole), 4.40 (2H, s, protons of CH2 linkage).^{13}C NMR (100.63 MHz, $CDCl_3$): δ (ppm) 151.4, 149.1, 143.2, 141.5, 139.2, 130.5, 123.5, 118.4, 115.6, 44.23: Anal. calcd for $C_{16}H_{14}N_6$ (290.13): C, 66.19; H, 4.86; N, 28.95.

N1-((1H-benzo [d]imidazol-2-yl) methyl)-N4-(1H-1, 2, 4-triazol-3-yl) benzene-1, 4-diamine (1b).
Begie yellow solid; m. p.167–178^{0}C; Yield 52%; IR (KBr): ν_{max} in cm^{-1}: 3377 (-NH), 3044 (aromatic–CH), 1614 (-CN), 3010 (aliphatic–CH), ^{1}H NMR (400 MHz, DMSO-*d6*): δ (ppm) 13.05 (s, 1H, benzimidazolic NH), 6.58-7.52 (8H, m, aromatic protons), 7.03 (s, NH), 8.03 and 13.01 (s, 2NH protons of triazole), 6.92 (s, CH triazole), 4.42 (2H, s, protons of CH2 linkage).^{13}C NMR (100.63 MHz, $CDCl_3$): δ (ppm) 152.4, 148.9, 146.2, 140.2, 136.5, 127.2, 123.3, 118.1, 115.2 44.2Anal. calcd for: $C_{16}H_{15}N_7$ (305.14): C, 62.94; H, 4.95; N, 32.11.

DFT studies

Geometry optimization
The optimized geometry of the synthesized molecules calculated by B3LPY/6-31G (d, p) are shown in Fig 2. . 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 1a and 1b. This result was confirmed by the fact that the bond lengths C4-N5 and C3-N2 in benzimidazole moiety are 1.387&1.398 Å in 1a, 1.444 & 1.411 Å in 1b. The molecular structure of 1a and 1b were non-planar, as shown by the dihedral angles of 26.37 to-40.46°, 11.14 to-12.75° between the planes of central benzimidazole ring and triazole moiety.

HOMO and LUMO analysis
Investigation of molecular orbitals and spatial distribution of other molecular properties are useful for many purposes. Molecular orbitals can provide important insight into bonding and other chemical properties. The analysis of the wave function indicates that the electron absorption corresponds to the transition from the ground state to the first excited sate and is mainly described by one-electron excitation from highest occupied molecular orbital (HOMO) to the lowest unoccupied orbital (LUMO). Highest occupied molecular orbital and the lowest unoccupied molecular orbital are very important parameters for quantum chemistry. The HOMO represents ability to donate an electron and LUMO represents the ability to obtain an electron. The HOMO-LUMO energy gap for the compounds 1a and 1b were calculated by B3LYP/6-311G (d, p) level of theory. The frontier orbital gap helps to characterize the chemical reactivity and kinetic stability of the molecule. A molecule with a small frontier orbital gap is more polarizable and is generally associated with high chemical reactivity and is also termed as soft molecule. The Eigen values of HOMO and LUMO and their energy gap reflect the chemical activity of the molecule shown in fig 3.

Procedure for Antibacterial studies [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^{o}C overnight for 18hrs. The modified agar well diffusion method of Perez *et al.*, 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-1 revealed that compound 1a exhibited excellent activity against E. Coli at MIC 10 µg/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 **1a** N1-((1H-benzo [d]imidazol-2-yl) methyl)-N4-(1H-1, 2, 4-triazol-3-yl) benzene-1, 4-diamine has given the top docking score shown in fig 4. The docking score and protein interactions of the compounds were tabulated in Table 2.

5. Conclusion

Novel triazole based benzimidazole derivatives were synthesized in reasonably good yields. They were characterized by IR, ^{1}H, ^{13}C NMR spectroscopy. All the newly synthesized compounds were tested for antimicrobial activity by agar well diffusion method. Among the screened samples compound 1b exhibited as most active against E. Coli compared to other synthesized compounds.

6. Acknowledgements

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Table 1: Antibacterial activities of compounds 1a and 1b, for bacterial strains in MIC (µg/ml)

Bacterial strains (MIC)	Compounds		
	Ciprofloxacin	1a	1b
K. pneumoniae	15	45	40
S. Typhic	10	50	45
S. Aureus	5	40	40
B. Subtilis	5	35	40
P. auruginosa	5	40	35
E. coli	5	10	15

Table 2: Docking Results

Compound	Docking Score kcal/mol	Interacting Residues
1a	-6.7	PHE 241
1b	-5.5	LYS 353, ARG 356

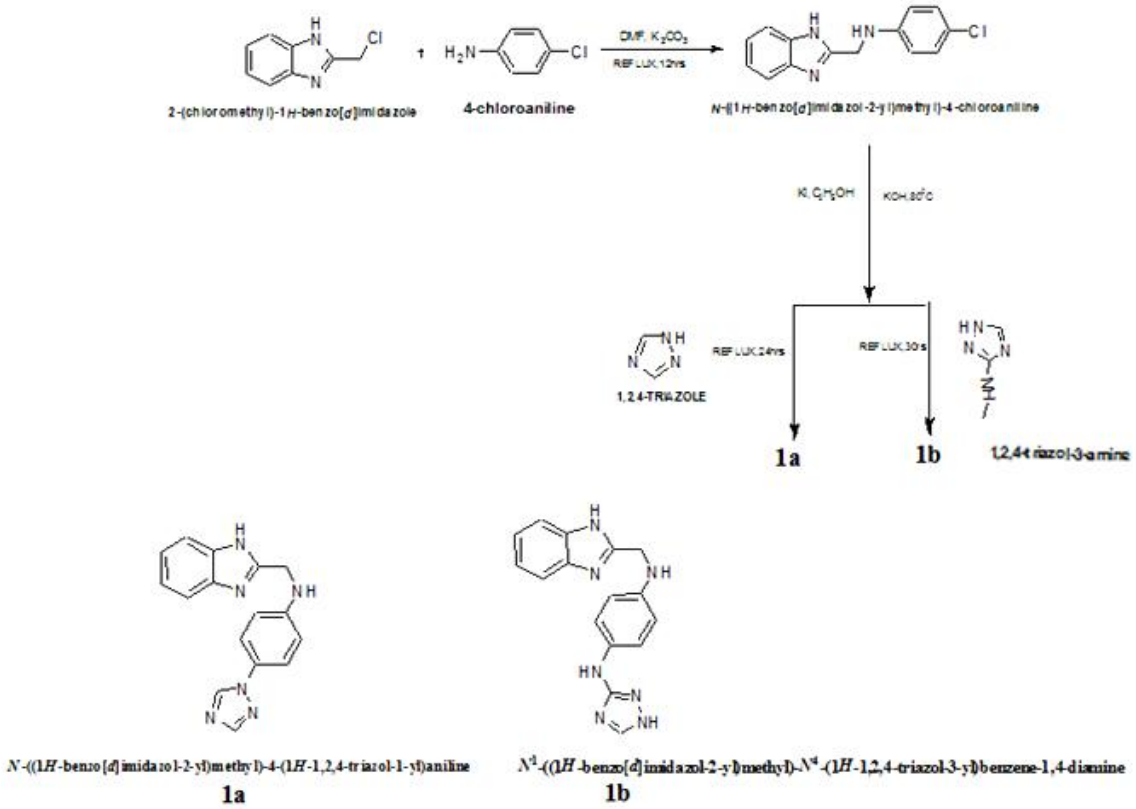
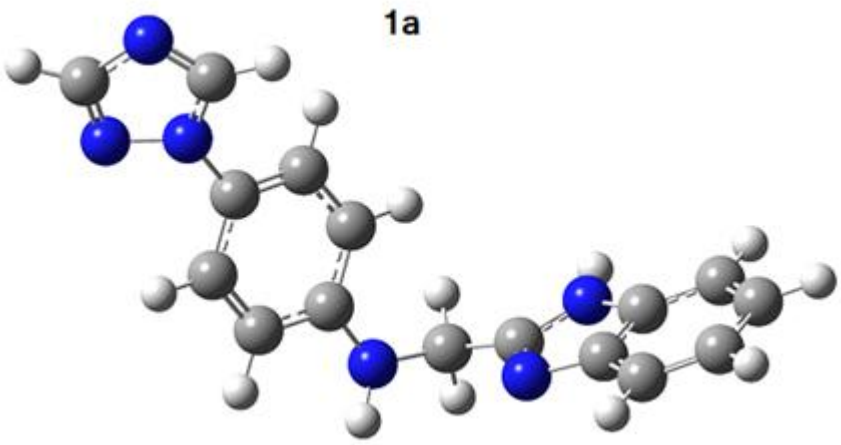


Figure 1: Scheme of synthesis



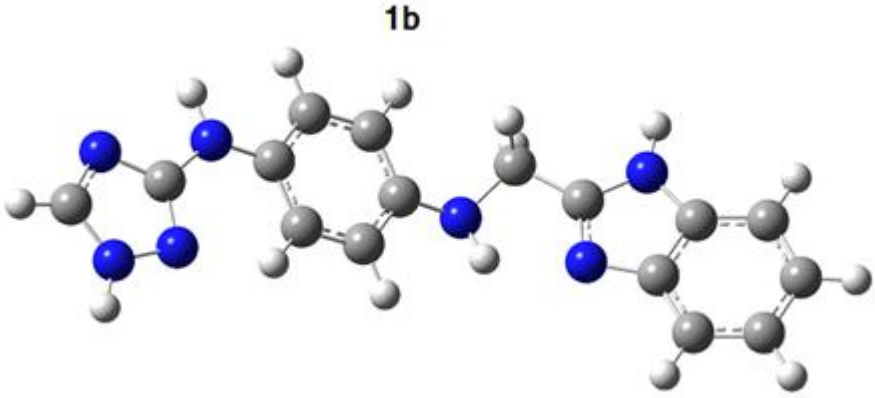


Figure 2: Optimized geometries of 1a and 1b

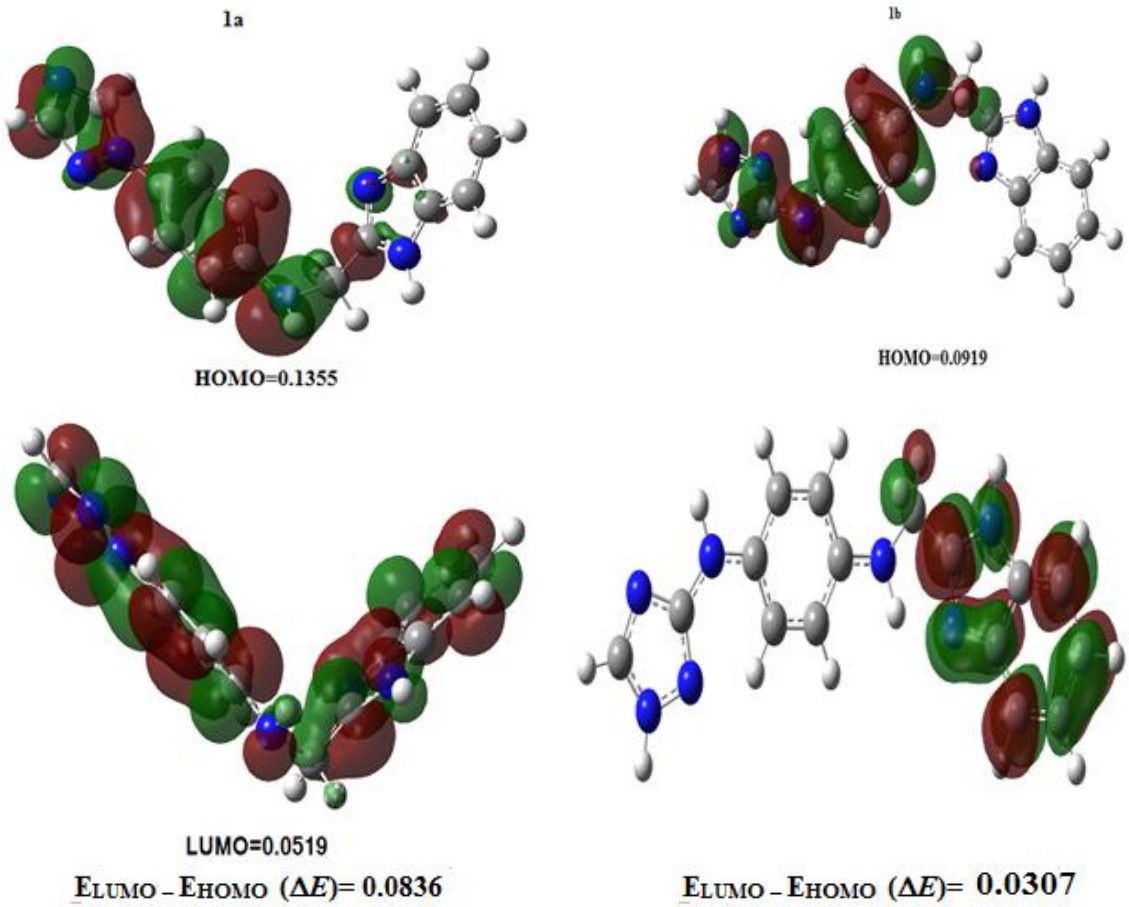
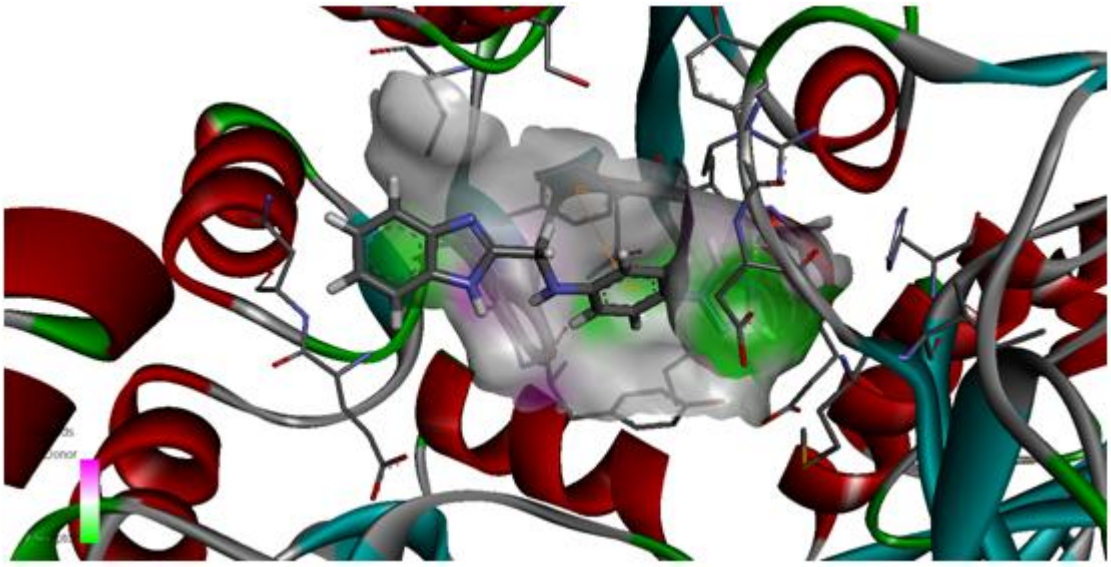
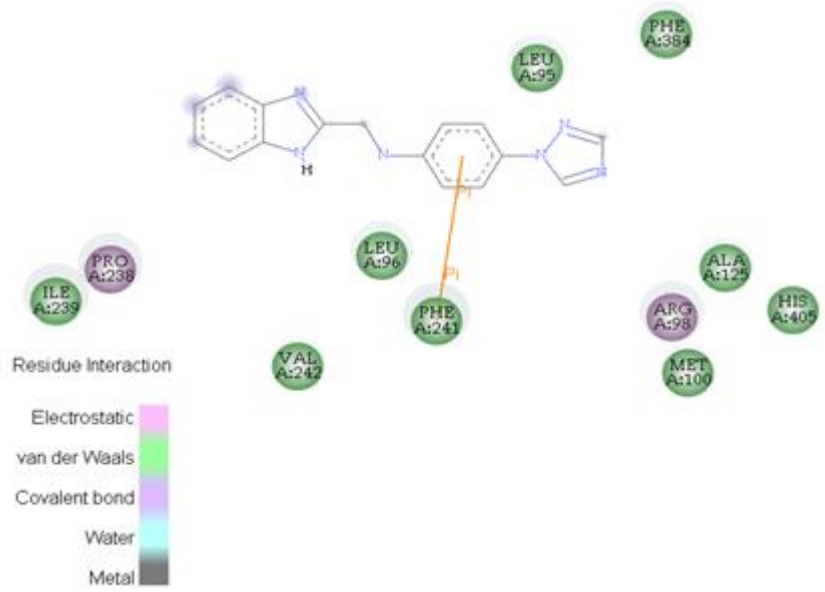


Figure 3: Frontier molecular orbitals for 1a and 1b



3D VIEW



2D VIEW

Docking scores =-6.7kcal/mol

Figure 4: Molecular docking of 1a