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Synthesis, characterization, electronic structure, vibrational assignment, HOMO-LUMO, Mulliken charge analysis, docking, antioxidant and anticancer studies of (1-methyl)-2-(2-arylaminothiazol-4-yl)benzimidazole

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The compound (1-methyl)-2-(2-arylaminothiazol-4-yl)benzimidazole has been characterized by IR spectra. The theoretical calculations of the mentioned compound have been carried out using the more popular density functional theory method, Beck's-3-parameter-Lee Yang Parr (B3LYP) in 6-31G basic set. Optimized geometries of the molecule have been described and collated with the experimental values. The experimental atomic charges demonstrate adequate concurrence with the theoretical prediction from DFT. Theoretical spectral values have been interpreted and compared with FT-IR spectra. The IR spectra are obtained and assigned by vibrational analysis. The Mulliken population analysis on atomic charges has been computed using DFT calculation. A correlation between the theoretical and the experimental spectra has been achieved. The calculated HOMO and LUMO energy gaps also confirm that charge transfer occurs within the molecule. The compound C2 has highest activity against Hep-C cell line. Five membered heterocyclic moieties have been proved to posses strong biological activities. Hence a series of noval benzimidazolyl thiazoles have been synthesized by multi-step reaction from 2-acetyl benzimidazole and hydroxylamine hydrochloride as starting materials and structure characterized by NMR and mass spectroscopy. The antioxidant activities of the derivatives have been determined by DPPH scavenging assay. The synthesized compounds show remarkable antioxidant activity. They show excellent anticancer activity.

Keywords: Gaussian, DFT, B3LYP, Mulliken charges, HOMO, LUMO, DPPH, Docking, Anti-oxidant, Anticancer

Benzimidazole is an aromatic heterocyclic organic compound. The synthesis of benzimidazole based poly heterocycles draw the attention of pharmacist from the last few decades as it functions as an important pharmacophore in medicinal chemistry and pharmacology[1]. The biological activities of benzimidazole and thiazole are such as antimicrobial, antifungal, anti-inflammatory antiviral, anticancer and antiulcerative, *etc*.[2] The potency of these clinically useful drugs in treatment of microbial infections and other activities encouraged the development of some more potent and significant compounds[3]. Benzimidazole are remarkably effective compounds, extensive biochemical and pharmacological studies have confirmed that these molecules are effective against various strains of microorganism[4]. In the present work to study the optimized geometry of the molecule were obtained by means of density functional theory using standard B3LYP /6-31G basic sets with Gaussian 09 software packages. Mulliken atomic charges, Bond length, Bond Order, HOMO, LUMO properties were also obtained theoretically[5,6]. Electronic properties like Frontier molecular orbital energy, ionization potential, hardness, electronegativity, softness and dipole moment were calculated[7,8]. Hence it is worthwhile to exploit these simple molecules with different functionalities. Owing to their versatile chemotherapeutic importance. It appeared of interest to synthesis novel benzimidazolyl thiazole derivatives to find their antioxidant property and to characterize structure of the most active compound by DFT.

Computational Details

In the present work, the density functional method (DFT) has been employed using beck's three parameter hybrid exchange functional with the Lee-Yang-Parr Correlation functional to optimize the structure of the molecule to calculate the electronic structure of the title molecule. The entire calculations were performed at the DFT(B3LYP) method 6-31G(d.p) basic set by using Gaussian 09W program package, geometry optimization[9,10]. The optimized molecular structure parameters were used in the harmonic vibrational frequency. From the optimized structure of the compounds has been used to calculate the molecular electrostatic potential, highest occupied orbital, lowest unoccupied molecular orbital energy by using Gaussian 09 software[11,12]. The optimized structure parameters were used in the vibrational frequency calculations at the HF and DFT levels to characterize all stationary points as minima. The electron correlations is taken into account in DFT *via* the exchange correlation term E_{xc}, which includes the exchange energy arising from the antisymmetric of the quantum mechanical wave function and the dynamic correlation, in the motion of individual electrons: it makes DFT dominant over the conventional HF procedure[13,14]. The DFT hybrid B3LYP functional also tends to overestimate the fundamental modes in comparison to the other DFT methods; therefore scaling factors have to be used to obtain considerably theoretical data. The calculated geometric parameters represent a good approximation, and are the basis for the calculation of other parameters such as polarizability, vibrational frequencies and thermodynamic properties[15,16].

Materials and Methods

All the reagents used were of analytical grade purchased From E. Merck and were purified prior to use. Melting points were determined on Digital Program Rate Melting points apparatus and were uncorrected. Proton NMR measurements were performed on a Bruker Advance Ш, 400 MHz NMR spectrometer and chemical shifts were reported in δ(ppm). Mass spectral studies were performed on (ESI-MS)

Experimental Section

An aqueous solution of ammonium thiocyanate (3.8g, 0.05 mol in 5 mL water) was prepared and diluted to 20 mL. The solution is heated at 90°C. Aryl amine (0.05 mol) was added drop-wise with vigorous stirring. It is then boiled until it is reduced to a syrupy liquid and further solidified to quite thick yellow mass. This was added rapidly to crushed ice and stirred well. The solid formed was filtered, dried and recrystallized from ethanol water (1:2) to obtain white crystals of N aryl thioureas. Yellow color crystals obtained.

Molecular Docking

Molecular docking was accomplished by using Auto Dock Vina. Auto Dock tools (ADT) were used to assigned polar hydrogen atoms. Each and every compound (C1-C4) were found to have minimum binding energy ranging from –7.1 to –7.8 kJ/mol.

Spectral characterization of the synthesized compounds

(1-Methyl)-2-(2-phenylaminothiazol-4yl)benzimidazole

^{1}H NMR (400 MHz, DMSO-d_6): δ 7.37 -7.48 (m, 4H, ArH), 4.1 (s, 3H, N-CH_3), 7.21- 7.29 (m,4H, ArH), 7.05 (t,8.4Hz,1H, ArH),7.8 (s,1H, H-5 of thiazole), 8.54 (s,1H.NH); MS: *m/z* 307(M^+).

(1-Methyl)-2-(2-chlorophenylaminothiazol-4yl) benzimidazole

^{1}H NMR (400 MHz, DMSO-d_6): δ 7.33 -7.45 (m, 4H, ArH), 4.11 (s, 3H, N-CH_3), 7.26- 7.3 (m, 4H, ArH), 7.8 (s, 1H, H-5 of thiazole), 8.89 (s, 1H, NH); MS: *m/z* 414(M^+).

(1-Methyl)-2-(2-methoxyphenylaminothiazol-4yl) benzimidazole

^{1}H NMR (400 MHz, DMSO-d_6): δ 7.30 -7.48 (m, 4H, ArH), 4.0 (s, 3H, N-CH_3), 7.22- 7.29 (m, 4H, ArH), 7.9 (s, 1H, H-5 of thiazole), 8.05 (s, 1H, NH), 3.75 (s, 3H); MS: *m/z* 397(M^+).

(1-Methyl)-2-(2-ethoxyphenylaminothiazol-4yl)benzimidazole

^{1}H NMR (400 MHz, DMSO-d_6): δ 7.52 -7.48 (m, 4H, ArH), 4.35 (s, 3H, N-CH_3), 7.34- 7.39 (m, 4H, ArH), 1.47 (t, 7 Hz, 3H, CH_3), (s, 1H, H-5 of thiazole), 8.05 (s, 1H, NH); MS: *m/z* 428(M^+).

Results and Discussion

Optimized geometry

The optimized structure of the compounds can be calculated using DFT theory at B3LYP/ 6-31Gbasic set level[17]. From the most optimized geometries are performed by B3LYP of DFT molecule with atoms numbering shown in (Fig. 1). And the difference of

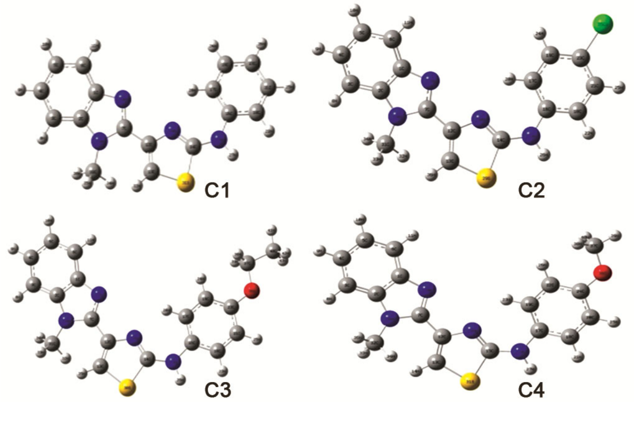


Fig. 1 — Optimized structure of (1-methyl)-2-(2-arylaminothiazol-4yl)benzimidazole

atoms arrangement will lead to a difference in the computational results involves significant modifications on the geometrical parameters are shown in the (Table 1). The bond length is the average distance between the nuclei of two atoms bonded together in a molecules[18,19]. Bonds with higher bond order values have short bond length and *vice versa*[20]. The investigation of bond order may envisage that the weakest bonds may be cleaved better and they have a relatively low pi bond character. Bond between S_1 and C_2 possess higher bond length 1.874 inB3LYP method

Mulliken Atomic Charge

The Mulliken population analysis is probably the best known of all models for predicating individual atomic charges which is computationally very popular due to its simplicity. Mulliken charges were shown to be highly basic set dependent and unpredictable with marked fluctuations in partial charges (Table 2), (Fig. 2). The solvent effect of the atomic charge distributions of derived compounds, based on the B3LYP/6-31G model[21]. Mulliken atomic charge values posses an important part in the implementation of quantum chemical calculation since atomic charges effect dipole moment, electronic structure, molecular polarizability and more a lot of properties of molecular system. The most of the carbon and nitrogen atoms have negative charges while the sulphur and hydrogen atoms have positive charges. But H_{31} was gained maximum positive charge than the other hydrogen atoms, due to the presence of electronegative atom N_{22}. They act as a acceptor atoms. Besides all oxygen atoms of the optimized compounds were shown to have negative charge, which act as donor atoms. By determining the electron population of each atom in the defined basic function, the Mulliken charges were calculated.

Electronic properties

Frontier molecular orbitals are those in which chemical reaction actually occur. The orbital HOMO (High occupied molecular orbital) measures the electron donor, and the LUMO (low-unoccupied molecular orbital) measure the electron acceptor. From these definition it should be observed that the higher the energy of the HOMO greater electron donating ability, and the lower the energy of the LUMO is less resistance to accept electron Table 3. The molecular chemical stability can be characterized by the energy gap between the HOMO and the LUMO orbitals (Fig. 3). During molecular interaction, the LUMO accept electrons and its energy corresponds to the electron affinity (EA), while the HOMO represents electron donors and its energy is associated with the ionization potential (IP). The ionization energy and the electron affinity can be expressed through HOMO and LUMO orbital energies as $IE = E_{HOMO}$ and $EA = E_{LUMO}$. The hardness corresponds to the gap between HOMO and LUMO of the energies. Hardness (ŋ) has been defined as the second unoriginal of the E measures the stability and the molecular reactivity,

$$ŋ(\text{Hardness}) = (IE-EA)/2$$

$$x(\text{Electronegativity}) = (IE+EA)/2$$

The electron affinity can be used in the combination with ionization energy to give electronic chemical potential, $\mu = ½(E_{HOMO+}E_{LUMO})$. The hardness corresponds to the gap between homo, lumo orbital energies[22]. The larger the homo-lumo energy gap the harder the molecule. The global hardness $ŋ=½(E_{LUMO\text{-}}E_{HOMO})$. The global softness parameter used to measure the molecular stability and reactivity $S(\text{softness})={}^1/ŋ$.

Docking Study

Molecular docking has become an increasingly important tool for drug discovery. Molecular docking is the study of how two or more molecular structures, for example, the drug and catalyst or macromolecular receptor, fit into a perfect fit (Fig. 4). The molecular docking technique is used for the interaction between a small molecule and protein at the atomic level. The PDB format of target protein was acquired from Protein Data Bank (PDB). The synthesized compounds act as ligands and the structure was optimized by Gaussian 09 software with B3LYP/631G level and the ligand was converted into PDB format with the help of Openbabel GUI (C) 2006. The binding energy and the proper conformation of the ligand in certain groove of the target protein were obtained by PyRx-Python prescription 0.8. The hydrogen bonding and hydrophobic interaction between thiazole and protein molecule was identified by Pymol software.

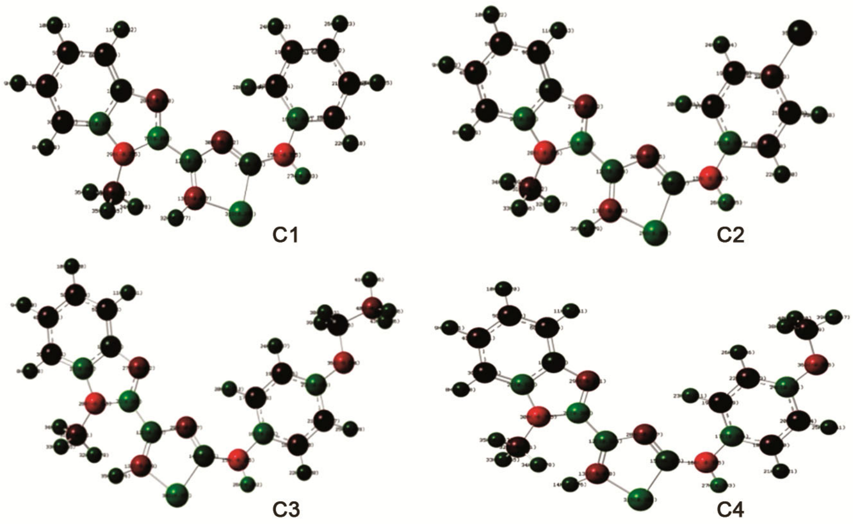
Drug likeness is a complex balance of structural features and a molecular property which was determined whether the molecule has pharmacological activity which is similar to drugs. The Lipinski rule of five describes the molecular properties of a drug's pharmacokinetics in human

Table 1 — Geometrical parameters of (1-methyl) -2-(2-arylaminothiazol-4yl)benzimidazole

Atom	Bond length data of (1-methyl)-2-(2-arylaminothiazol-4yl)benzimidazole				
	Bond Length (Å)				
$S_1 – C_2$	1.874	1.842	1.842	1.843	1.843
$C_2 – N_3$	1.310	1.309	1.309	1.310	1.310
$N_3 – C_4$	1.400	1.400	1.409	1.400	1.400
$C_4 – C_5$	1.366	1.366	1.357	1.365	1.365
$C_5 – H_{23}$	1.076	1.076	1.079	1.076	1.076
$C_4 – C_6$	1.457	1.457	1.450	1.457	1.457
$C_6 – N_7$	1.335	1.335	1.363	1.335	1.335
$N_7– C_{14}$	1.394	1.394	1.472	1.394	1.394
$C_{14} – C_8$	1.401	1.401	1.347	1.401	1.401
$C_8 – H_{24}$	1.084	1.083	1.069	1.084	1.084
$C_8– C_9$	1.393	1.393	1.392	1.393	1.393
$C_9 – H_{25}$	1.085	1.085	1.085	1.085	1.085
$C_9– C_{10}$	1.414	1.414	1.417	1.414	1.414
$C_{10}– H_{26}$	1.085	1.085	1.086	1.085	1.085
$C_{10} – C_{11}$	1.396	1.396	1.396	1.396	1.396
$C_{11} – H_{27}$	1.083	1.083	1.083	1.083	1.083
$C_{11} – C_{13}$	1.399	1.399	1.345	1.399	1.399
$C_{13}– C_{14}$	1.419	1.419	1.405	1.397	1.397
$C_{13} – N_{12}$	1.397	1.397	1.397	1.397	1.396
$N_{12}– C_{15}$	1.470	1.470	1.470	1.470	1.470
$C_{15} – H_{29}$	1.091	1.091	1.090	1.091	1.091
C_{15} - H_{28}	1.096	1.096	1.096	1.096	1.096
C_{15} -C_{16}	1.520	1.520	1.540	1.520	1.520
C_{16} - C_{17}	1.404	1.404	1.401	1.404	1.404
$C_{17}– H_{30}$	1.085	1.085	1.085	1.085	1.085
c17 –C_{18}	1.398	1.398	1.401	1.398	1.398
c18 – H_{31}	1.085	1.085	1.085	1.085	1.085
C_{18} - C_{19}	1.400	1.400	1.401	1.400	1.400
C_{19} - H_{32}	1.085	1.085	1.070	1.085	1.085
C_{19} -C_{20}	1.398	1.398	1.402	1.392	1.398
C_{20} - H_{33}	1.085	1.085	1.085	1.085	1.085
C_{20} -C_{21}	1.399	1.399	1.401	1.399	1.399
C_{21}- H_{34}	1.087	1.087	1.070	1.087	1.085
C_{21}- C_{16}	1.403	1.403	1.401	1.403	1.403
C_2-N_{22}	1.368	1.370	1.469	1.367	1.365
C_{38} -C_{39}	1.405	1.409	1.401	1.403	1.401
C_{39} - H_{44}	1.082	1.081	1.087	1.086	1.085
C_{39}- C_{40}	1.398	1.398	1.401	1.398	1.400
C_{40}-H_{45}	1.085	1.081	1.081	1.086	1.086
C_{40}-C_{35}	1.392	1.398	1.392	1.406	1.400
C_{35}-H_{41}	1.084	1.082	1.085	1.086	1.083
C_{35}-C_{36}	1.400	1.393	1.401	1.406	1.404
C_{36}-H_{42}	1.085	1.085	1.072	1.086	1.085
C_{36}-C_{37}	1.394	1.394	1.395	1.393	1.389
C_{37}-H_{43}	1.086	1.085	1.082	1.082	1.082
C_{35}-Cl_{46}	–	1.092	–	–	–
C_{35}-O_{46}	–	–	1.430	–	1.389
O_{46}-C_{47}	–	–	1.433	–	1.462
C_{47}-H_{48}	–	–	1.070	1.090	–
C_{47}-H_{49}	–	–	1.070	1.094	1.098
C_{40}-H_{50}	–	–	1.071	–	1.098

Table 2 — Mulliken charge analysis of (1-methyl)-2-(2-arylaminothiazol-4yl)benzimidazole

Atom	Mulliken atomic charges in arylthiazole			
	1A	1B	1C	1D
S1	0.436	0.439	0.427	0.433
C2	0.184	0.183	0.178	0.184
N3	–0.450	–0.447	–0.449	–0.451
C4	0.303	0.302	0.303	0.303
C5	–0.495	–0.494	–0.495	–0.496
C6	0.415	0.416	0.414	0.415
N7	–0.455	–0.456	–0.457	–0.457
C8	–0.139	–0.106	–0.107	–0.107
C9	–0.139	–0.139	–0.139	–0.139
C10	–0.153	0.153	–0.153	–0.153
C11	–0.080	–0.079	–0.080	–0.080
N12	–0.741	–0.741	–0.741	–0.741
C13	0.325	0.325	0.325	0.325
C14	0.089	0.089	0.089	0.089
C15	–0.187	–0.187	–0.187	–0.187
C16	0.112	0.112	0.112	0.112
C17	–0.134	–0.135	–0.134	–0.134
C18	–0.130	–0.130	–0.130	–0.130
C19	–0.121	–0.121	–0.121	–0.121
C20	–0.126	–0.126	–0.126	–0.126
C21	–0.167	–0.157	–0.167	–0.167
N22	–0.782	–0.784	–0.769	–0.780
H23	0.207	0.210	0.205	0.206
H24	0.137	0.138	0.139	0.137
H25	0.119	0.120	0.118	0.118
H26	0.119	0.121	0.119	0.119
H27	0.138	0.138	0.137	0.137
H28	0.152	0.153	0.151	0.151
H29	0.210	0.208	0.211	0.210
H30	0.141	0.141	0.142	0.142
H31	0.129	0.129	0.128	0.128
H32	0.125	0.127	0.125	0.126
H33	0.125	0.127	0.125	0.126
H34	0.122	0.123	0.121	0.122
C35	–0.113	–0.228	0.292	0.125
C36	–0.134	–0.106	–0.142	–0.164
C37	–0.154	–0.151	–0.157	–0.151
C38	0.313	0.321	0.291	0.307
C39	–0.115	–0.109	–0.113	–0.112
C40	0.157	–0.121	–0145	–0.180
H41	0.130	0.164	0.136	0.128
H42	0.133	0.141	0.134	0.129
H43	0.130	0.167	0.160	0.157
H44	0.157	0.166	0.151	0.129
H45	0.135	0.141	0.343	0.344
H46	0.345	–	–	–
O46	–	–	–0.560	–
C47	–	–	–0.172	–
H48	–	–	0.149	0.159
H49	–	–	0.173	0.147
H50	–	–	0.151	–
C46	–	–	–	–0.481
H47	–	–	–	0.149
C48	–	–	–	–
H51	–	–	–	
H52	–	–	–	
H53	–	–	–	



C1-(1-methyl)-2-(2-phenylaminothiazol-4yl)benzimidazole
C2-(1-methyl)-2-(2-chlorophenylaminothiazol-4yl)benzimidazole
C3-(1-methyl)-2-(2-ethoxyphenylaminothiazol-4yl)benzimidazole
C4-(1-methyl)-2-(2-methoxyphenylaminothiazol-4yl)benzimidazole

Fig. 2 — Mulliken charge of (1-methyl)-2-(2-arylaminothiazol-4yl)benzimidazole

Table 3 — Calculated electronic parameters

Parameters	C_1	C_2	C_3	C_4
HOMO	–0.03278	–0.03933	–0.03089	–0.03089
LUMO	–0.23546	–0.23844	–0.19417	–0.23463
ΔE	0.20268	0.19911	0.16328	0.20374
Ionization potential(I)	–0.03278	–0.03933	–0.03089	–0.03089
Electron Affinity(A)	–0.23546	–0.23844	–0.19417	–0.23463
Electronegativity(x)	0.13416	0.13888	0.11253	0.13276
Hardness(ŋ)	–0.10134	–0.09955	–0.08164	–0.10187
Softness(s)	0.05067	0.04977	0.04082	0.05093

body as well as their absorption, distribution, metabolism and excretion (ADME)[23].

The docking results concluded that hydrogen bonding interactions contributed significantly for its ligand binding and minimum binding affinity values (Table 4). In this article, all the benzimidazole derivative ligands shows highest binding efficiency with suitable protein hence it is very potential for anticancer drug. From these evidence further understanding the structures, their authenticity and biomolecular activity.

Vibrational Assignments

The vibrational assignment in the present work are based on B3LYP /6-31G frequencies. The detailed vibrational assignments of the fundamental modes of (1-methyl)-2-(2-arylaminothiazol-4yl)benzimidazole are given below (Fig. 5 and Fig. 6).

C-H Vibration

The aromatic structure shows the presence of C-H stretching vibrations in the region 3300-3100 cm^{-1}, which has been characterized region for the ready identification of C-H stretching vibrations. The B3LYP/3-21G level at 6-31G basic set give the same frequency at 3239 cm^{-1}, 3227 cm^{-1}, 3218 cm^{-1} and 3195 cm^{-1}. The title compounds has both out of plane and in plane aromatic C-H bending vibrations are observed in the region of 1650-1000 cm^{-1}. From the theoretical calculation indicates the scaled frequency value at 1450 cm^{-1} assigned with C-H plane bending vibration. The out of plane bending mode of C-H vibrations are

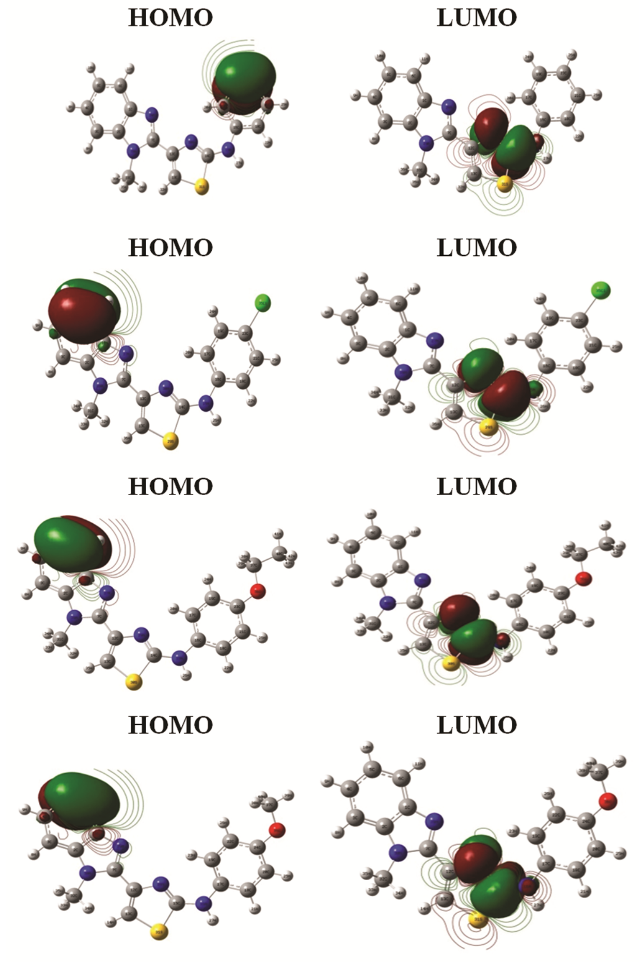


Fig. 3 — HOMO and LUMO of (1-methyl)-2-(2-arylaminothiazol-4yl)benzimidazole

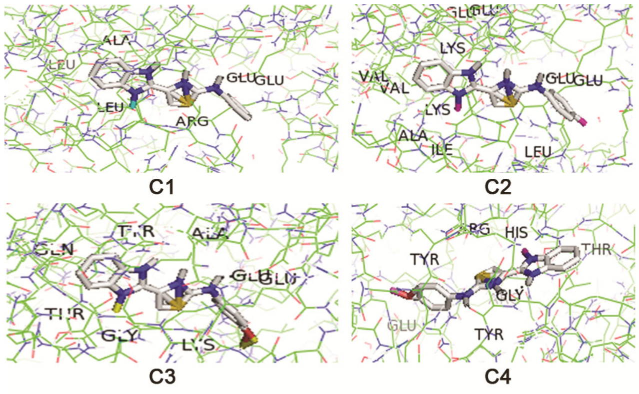


Fig. 4 — Docking images

assigned even through a good agreement with literature observation.

C=C Vibration

Conjugation of the C=C bond with a double bond or an aromatic rings decrease the frequency of the bond. In our present compound have three C=C symmetric vibrations. The position of the modes is determined not so much by the nature of the substituent by the form of substitution around the ring. They are calculated as 1591 cm^{-1} and 1567 cm^{-1} for B3LYP method.

Table 4 — Binding affinity and residues of (1-methyl)-2-(2-arylaminothiazol- 4yl)benzimidazole

Compd	Docking score	Residue involved in hydrogen bonding
C_1	–7.1	LEU – 248,GLU- 196,ARG- 251
C_2	–7.8	GLU -247, LYS -196
C_3	–7.3	TYR-247,GLU-245,LYS-255
C_4	–7.2	HIS – 247,TYR -189, THR- 196

C=O Vibration

The C=O stretching vibration observed in a region 1642 cm^{-1} with B3LYP/6-31G basic set. This is agreement with the very strong frequencies of 1631 cm^{-1} in FT-IR spectra. The FT-IR spectra shows a band at 1632 cm^{-1} confirms the presence of C=0 stretching vibrations. The stretching vibrations calculated with HF level shows slightly higher range of the frequencies than the theoretical results. The out of plane C=O bending vibration mode with the frequency 150 cm^{-1} found to be in excellent agreement with the literature value.

C-C Vibration

The C-C aromatic stretching vibrations give rise to characteristic band observed in IR spectra from the range of 1600-1400 cm^{-1}. The IR bands are 1592, 1566, 1516 and 1494 cm^{-1}. The C-C in plane bending frequencies are observed in the region 1500 cm^{-1}. The theoretically calculated C-C out of plane bending vibration has been found to be consisted in the region 753, 621, 441, 427 cm^{-1}.

C-N Vibration

A C-N Group attached to the ring, a band of good intensity has been absorbed in the region of 1500-1200 cm^{-1} and its being attributed of C-N stretching modes appears in our calculation at 1572, 1481, 1330, 1210 cm^{-1}.

N-H vibration

The NH stretching vibration observed generally at 3300-3500 cm^{-1}. The NH vibration of the compounds, theoretically at 3600, 3272, 3290, 3205 and 3350 cm^{-1}.

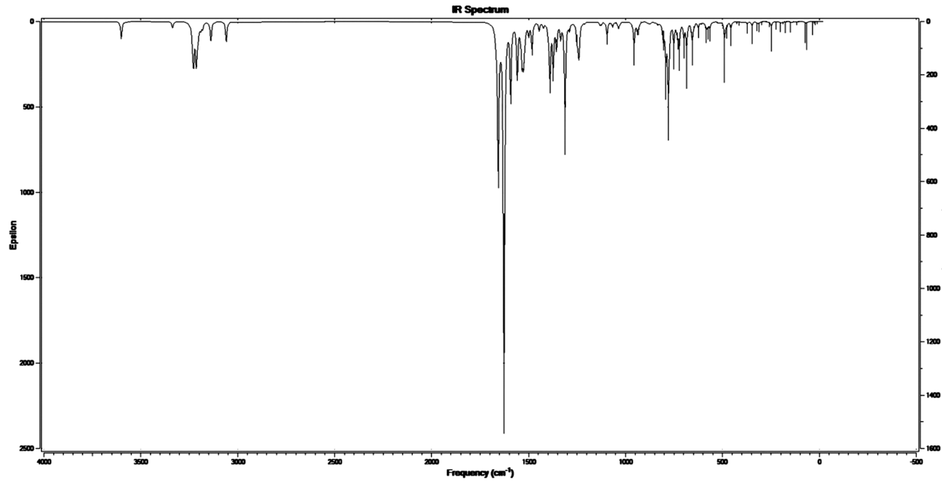


Fig. 5 — Theoretical IR spectra of (1-methyl) -2-(2-phenylaminothiazol-4yl)benzimidazole

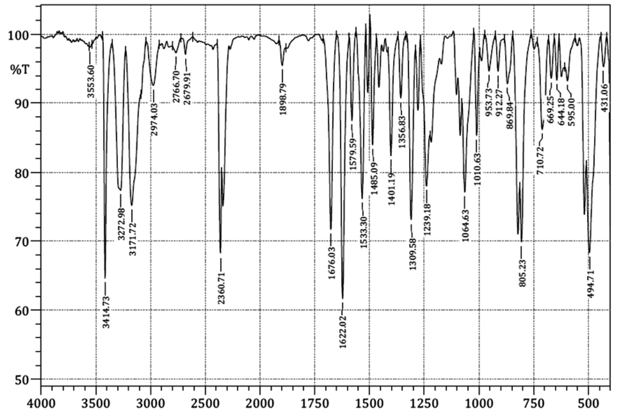


Fig. 6 — Experimental IR spectra of (1-methyl)-2-(2-phenylaminothiazol- 4yl)benzimidazole

Methyl group vibration

The compounds which contain a CH_3 group in the substitution chain. The stretching vibrations are generally observed in the region 3450 cm^{-1}. There are nine fundamentals one can expect to a CH_3 group, which namely the symmetrical stretching in CH_3 (CH_3 sym. str) and asymmetric stretching (in plane hydrogen stretching mode). The theoretically computed values of 3153 cm^{-1} for CH_3 in symmetric stretching 3112 cm^{-1}, 3170 cm^{-1} for CH_3 asymmetric stretching have shown excellent values. The first band is assigned to C_{36}-H(mode 96) stretching vibrations. The C_{22}-H andC_{23}-H (mode 98) symmetric stretching vibrations in the bond is located in the region of 2940 cm^{-1} to 3200 cm^{-1}. And the mode (89)C_{37}-H, mode (97)C_{38}-H asymmetric stretching vibrations were found in 2957 cm^{-1} to1457 cm^{-1}. The theoretical values shows excellent agreement with literature of symmetric and asymmetric stretching vibration of the CH_3 group respectively.

Ring vibration

The C-C stretching vibrations in an aromatic compound from the band in the region 1640 cm^{-1} - 1440 cm^{-1}. In the present study the scaled frequency values at 1516 cm^{-1}, 1490 cm^{-1}, 1481 cm^{-1}. In the present study the scaled frequency values at 1516 cm^{-1}, 1490 cm^{-1}, 1481 cm^{-1} are assigned to C-C stretching vibrations. From the ring C-C stretching vibration of the aromatic bands appear at 1659, 1572, 1311, 1182, 1062 and 837 cm^{-1}. Many ring modes are affected by the substitution in the aromatic ring.

DPPH Scavenging assay

The samples were made up with methanol to different concentrations (50, 100, 250, 500 and 750 µm). 2 mL of each sample was allowed to react with 2 mL of stable free radical, 1,1,-diphenyl-2- picryl hydrazyl radical (DDPH) for 0.5 h at 37°C. The deep purple colour of the DPPH solution turns yellow in the presence of antioxidants. The concentrations of DDPH was kept as 10^{-15} mol. After incubation the absorbance of all the solutions was measured at 517 nm. *tert*-Butyl-4-hydroxyanisole was used as a standard[24]. Percent antioxidant activity of the samples was determined in comparison with a methanol treated control group by using the following formula.

% Antioxidant activity = OD test compound – OD control/ OD control × 100

Where OD is the optical Activity.

Table 5 — Antioxidant activities of (1-methyl)-2-(2-arylaminothiazol-4-yl)benzimidazole

Compd	IC_{50} value (µM)
C_1	330
C_2	52
C_3	132
C_4	427

Table 7 — Anti-cancer activities of (1-methyl)-2-(2-arylaminothiazol-4yl)benzimidazole

Sample Concentration (µg/mL)	Percentage Viability	
	$\mathbf{C_2}$	$\mathbf{C_3}$
Control	100	100
6.25	67.7774	82.55096
12.5	51.93333	62.222
25	44.63527	48.26991
50	32.46375	40.58186

Table 6 — Anti-tubercular activities of(1-methyl)-2-(2-arylaminothiazol-4yl)benzimidazole

Compd	Diameter of inhibition zone (mm)				
	25 µg/disc	50 µg/disc	100 µg/disc	150 µg/disc	Standard 100 µg/disc
$\mathbf{C_2}$	–	–	7	8	10
$\mathbf{C_3}$	–	–	6	9	10

Two replicates of the analysis were undertaken and results averaged. A linear regression plot with absccia and ordinate as concentration of samples and % antioxidant activity respectively was plotted to determine the IC_{50} (effective concentrations for scavenging 50% of the initial DPPH) values (Table 5).

The antioxidant data shows that the compound C2-(1-benzyl)-2-(2-chlorophenylaminothiazol-4yl)-benzimidazole has IC_{50} value 52 µM and possesses excellent antioxidant activity.

Anti-tubercular Activity

Tuberculosis is a major health issue and its treatment is so complicated that emergence of new and efficient drugs that shorten the time of treatment is necessary.

Method used

The bacterial inoculums was uniformly spread using sterile cotton swab on a sterile Petri dish MH agar and wells were made in the plate. The wells were filled with samples of different concentrations. The systems were incubated for 24 h at 36ºC ± 1ºC, under aerobic conditions. After incubation, inhibition of the bacterial growth (Clear zone) was measured in mm.

Anti-tubercular activities of (1-methyl)-2-(2-arylaminothiazol-4yl)benzimidazole

The compounds has(1-methyl)-2-(2-chlorophenylaminothiazol-4yl)benzimidazole the highest docking score**.** Hence *in vitro* anti-tubercular assay is performed for both the compounds (Table 6). The anti-tubercular activities of is presented in (1-methyl)-2-(2-arylaminothiazol-4yl)benzimidazole Table 3.22.

The result reveals that the compoundC_3 **(**1-methyl)-2-(2-ethoxyphenylaminothiazol-4yl)benzimidazole and (1-methyl)-2-(2-chlorophenylaminothiazol-4yl) benzimidazole posses remarkable anti-tubercular activity.

Anticancer activities of (1-methyl)-2-(2-methoxy phenylaminothiazol-4yl)benzimidazole and (1-methyl)-2-(2-chlorophenylaminothiazol-4yl) benzimidazole

The compounds which have highest antioxidant activities $\mathbf{C_2}$ and $\mathbf{C_3}$ were screened for their anticancer activity against HT-29 (human colon adenocarcinoma) cell line by MTT method (Table 7). From the MTT test, cytotoxic effects of the compounds were examined and percentage viability was calculated.

Conclusion

Some interesting benzimidazole thiazole derivatives were designed and synthesized. Their structures were confirmed using NMR and mass spectral analysis. The antioxidant study of the synthesized derivatives showed among the five compounds, (1-methyl)-2-(2-chlorophenylaminothiazol-4yl)benzimidazole showed best antioxidant activity. The molecular geometry, vibrational frequencies, infrared intensities of the molecule has been aided by using hybrid density functional method (B3LYP) with 6-31G basic set. The analysis of HOMO-LUMO energies suggests that the charge transfer is taking place within the molecule. The feasibility of the hydrogen bonding was explained by mulliken charge analysis. From the FT-IR spectral analysis and the theoretical estimation show minor variation of the vibrational pattern. The complete molecular structure parameters and thermodynamic properties of the optimized geometry of the compounds have been obtained from DFT calculations. Docking study of these benzimidazole derivative shows as good oral drug for candidates. It is seen from these properties that the molecule donates electrons easily, which is essential for efficient antioxidant activity. The compound (1-methyl)-2-(2-chlorophenylaminothi azol-4yl) benzimidazole is highly active against Mycobacterium Tuberculosis. It exhibited the highest anticancer activity

Supplementary Information

Supplementary information is available in the website http://nopr.niscpr.res.in/handle/123456789/58776.

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