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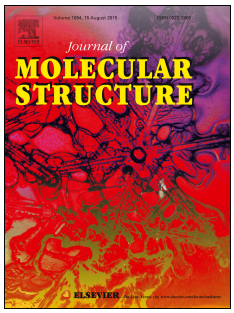


Density functional theory molecular modeling, chemical synthesis, and antimicrobial behaviour of selected benzimidazole derivatives

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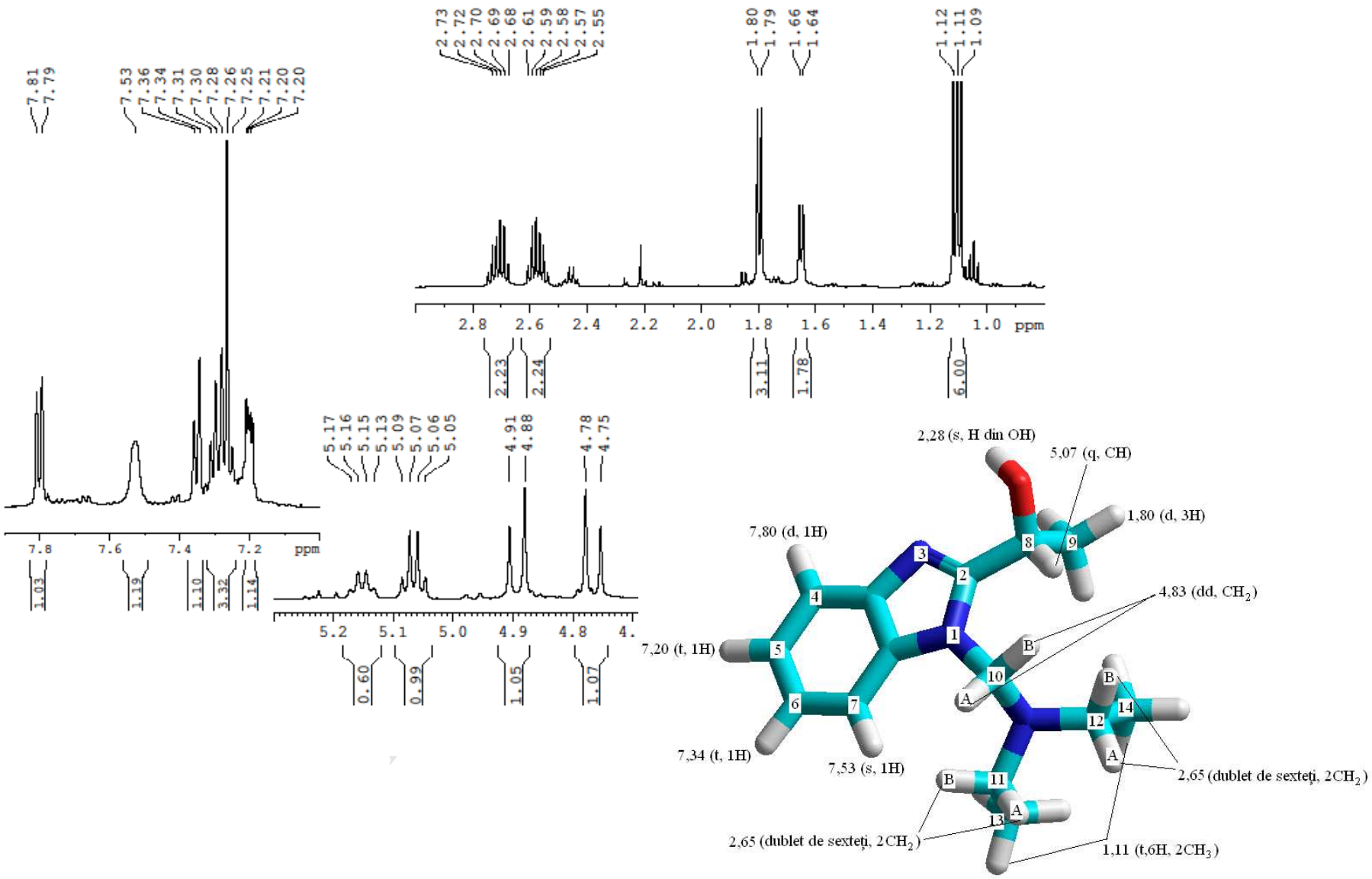
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5,07 (q, CH)
1,80 (d, 3H)
7,80 (d, 1H)
4,83 (dd, CH2)
7,20 (t, 1H)
7,34 (t, 1H)
7,53 (s, 1H)
2,65 (dublet de sexteți, 2CH2)
2,65 (dublet de sexteți, 2CH2)
1,11 (t,6H, 2CH3)

# *Density functional theory molecular modeling, chemical synthesis, and antimicrobial behaviour of selected benzimidazole derivatives*

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## *Abstract*

Eco-friendly, one-pot, solvent-free synthesis of biologically active 2-substituted benzimidazoles is presented and discussed herein. Novel N-*Mannich* bases are synthesized from benzimidazoles, secondary amines and formaldehyde, and their structures are confirmed by $^{1}$H nuclear magnetic resonance (NMR), Fourier transform infrared (FTIR), and elemental analysis. All benzimidazole derivatives are evaluated by qualitative and quantitative methods against 9 bacterial strains. The largest microbicide and anti-biofilm effect is observed for the 2-(1-hydroxyethyl)-compounds. Density functional theory (DFT) modeling of the molecular structure and frontier molecular orbitals, *i.e.* highest occupied molecular orbital and lowest unoccupied molecular orbital (HOMO / LUMO), is accomplished by using the *GAMESS 2012* software. Antimicrobial activity is correlated with the electronic parameters (chemical hardness, electronic chemical potential, global electrophilicity index), *Mullikan* atomic charges and geometric parameters of the benzimidazole compounds. The planarity of the compound, symmetry of the molecule, and the presence of a nucleophilic group, are advantages for a high antimicrobial activity. Finally, we briefly show that further accurate processing of such compounds into thin films and hybrid structures, *e.g.* by laser ablation matrix-assisted pulsed laser evaporation and/or laser-induced forward transfer, may indeed provide simple and environmental friendly, state-of-the-art solutions for antimicrobial coatings.

***Keywords***: *2-substituted benzimidazoles; antimicrobial activity; spectral analysis; disc diffusion, minimal inhibitory concentration (MIC), biofilm, laser processing.*

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## 1. Introduction

With the occurrence of multi-drug, extended-drug, and pan-drug resistance phenotypes, bacterial resistance to antibiotics is one of the most important global health problem, justifying the necessity of identifying innovative, radical antimicrobial agents [1]. The antibiotic resistance problem is amplified by the ability of bacteria to grow in a sessile, adherent state, developing microbial communities called biofilms, that exhibit a phenotypic resistance, *i.e.* tolerance, rendering them to 1,000-fold more resistant to antibiotic treatments with respect to their planktonic counterparts [2, 3].

Benzimidazole is a bi-cyclic aromatic organic compound, consisting of the fusion of benzene and imidazole. Together with its derivatives, they represent an important class of bioactive molecules of biological and pharmaceutical interest [4], mainly used as ligands for transition metal complexes. To better exemplify, the most prominent natural benzimidazole compound is N-ribosyl-dimethylbenzimidazole, which serves as an axial ligand for cobalt in vitamin $B_{12}$. They are important not only as structural units in natural compounds and synthetic pharmaceuticals, but have also elicited considerable theoretical interest. In recent years, reports on compounds which contain the benzimidazole moiety have increased. Various pharmaceutical applications of such compounds include anticancer [5-10], antihypertensive [11, 12], anti-inflammatory [13, 14], antimicrobial [15-17], antiviral [18, 19] and lipid modulating [20, 21] activities. Some benzimidazole compounds (*e.g.* astemizole) have demonstrated anti-prion activity, with a view to possible treatment for *Creutzfeldt–Jakob* disease, while other compounds (*e.g.* albendazole) are currently used as medication for the treatment of a variety of parasitic worm infestations. Some benzimidazole compounds also exhibit fungicidal properties, mainly due to their binding to the fungal microtubules and stopping hyphal growth. They are valuable agents in *Alzheimer* disease [22], and are also used as psychoactive drugs [23]. Furthermore, benzimidazoles are mentioned as anticoagulants [24] and antidiabetic agents [25].

In this work, we present and discuss results on the eco-friendly synthesis of 2-substituted benzimidazoles and new *Mannich* bases (*Figure 1*), density functional theory (DFT) study for these structures, antimicrobial activity screening, followed by a brief discussion on their potential use as antimicrobial coatings accomplished by thin film laser processing. The antimicrobial activity of the compounds was assessed on Gram-negative (*Escherichia coli* ATCC 8739, *Klebsiella pneumoniae* 40, *Enterobacter cloacae* 56, *Pseudomonas aeruginosa* ATCC 27853, *Acinetobacter baumannii complex* 230) and Gram-positive (*Staphylococcus aureus* ATCC 6538, *Stapylococus saprophyticus* ATCC 15305, *Enterococcus faecium* 17, *Enterococus faecalis* ATCC 29212) bacterial strains.

## 2. Experimental section

### 2.1 *Materials and devices*

All chemicals and reagents (provided by *Sigma-Aldrich*) were used without any further purification. Elemental analysis was performed with a "multi EA 4000" device from "Analytik Jena". Fourier transform infrared (FTIR) spectra have been acquired by using a "Vertex 70-Bruker" spectrophotometer, in KBr pellets. The $^1$H nuclear magnetic resonance (NMR) spectra have been recorded on a "Varian Inova-400" (400 MHz, 2 RF channels) in deuterated dimethyl sulfoxide (DMSO-$d_6$). The chemical shifts (δ, ppm, TMS internal standard) by $^1$H NMR and the FTIR spectra have all been recorded at room temperature.

### 2.2 *Synthesis of benzimidazole compounds*

A mixture of organic acid (40 mmol) and *o*-phenylenediamine (40 mmol), thoroughly grounded with a pestle in a mortar at room temperature until liquefied, was subsequently heated at 140°C for 1.5 - 2 h. The progress of the reaction was monitored by thin-layer chromatography (TLC). After cooling, the resulting mass was washed with water, filtered, and the final product was re-crystallized. The synthesized 2-benzimidazoles were then characterized and confirmed by comparing their physical data with those of literature known compounds, as follows.

#### 2.2a *Synthesis 2-substituted benzimidazoles*

(**1A**) *(S)-1-(1H-Benzo[d]imidazole-2-yl)ethanol*, colourless solid. Yield 80%; m.p. 177-178°C, similar to [26] (m.p. 176-178°C) IR (cm$^{-1}$, KBr): 3356 (aromatic –NH bending), 3364 (OH stretching), 3070 (aromatic C-H stretching), 1505 (C-N stretching), 1458 (-C=C stretching), 1315 (-C-N stretching); $^1$H-RMN: δ 7,58 (d, 2H), 7,26 (t, 2H), 1,72 (d, 3H, $CH_3$), 5,21 (q, 1H), 2.3 (brs, 1H, NH), 2,28 (s, 1H, OH). Elemental analysis (%) found for $C_9H_{10}N_2O$: C, 66.57; H, 6.25; N, 17.29; O, 9.89. calcd.: C, 66.65; H, 6.21; N, 17.27; O, 9.86%.

(**2B**) *2-Phenyl-1H-benzo[d]imidazole*, white solid. Yield 75%; m.p. 291-293°C, similar to [27] (m.p. 291-293°C); IR (cm$^{-1}$, KBr): 3352 (aromatic –NH bending), 3044 (aromatic C-H stretching), 1622 (alkenyl C=C stretch), 1587, 1537 (aromatic ring stretch) 1458 (-C=C stretch), 1410 (vinyl C-H in-plane bend), 1312, 1290 (vinylidene C-H in-plane bend); $^1$H-RMN: δ 12.91 (brs, 1H, NH), 8.13 (d, 2H, J = 7.0 Hz), 7.51–7.43 (m, 5H), 7.19 (brs, 2 H). Elemental analysis (%) found for $C_{13}H_{10}N_2$: C, 80.35; H, 5.22; N, 14.41. Calcd: C, 80.41; H, 5.17; N, 14.42%).

(**3B**) *2-o-Tolil-1H-benzo[d]imidazole*, colourless solid. Yield 72%; m.p. 220-221°C, similar to [28] (m.p. 220-222°C); IR ($cm^{-1}$, KBr): 3355 (aromatic –NH bending), 3087, 3062 (aromatic C-H stretching), 2925 (alkyl stretch), 1620 (alkenyl C=C stretch), 1614, 1505 (aromatic ring C-C stretch) 1458 (-C=C stretch), 1410 (vinyl C-H in-plane bend), 1086, 1035 (in plane C-H bending), 738 (C-H bending); $^1$H-NMR: δ 13.08 (br s, 1H), 7.82 - 7.75 (m, 3H), 7.60 - 7.58 (m, 1H), 7.55 - 7.43 (m, 4H), 2.56 (s, 3H). Elemental analysis (%) found for $C_{14}H_{12}N_2$: C, 80.72; H, 5.72; N, 13.55. Calcd: C, 80.77; H, 5.76; N, 13.46%).

(**4B**) *1-(1H-benzo[d]imidazole-2-yl)ethanone*, synthesized according to the procedure described in [29]. Brown crystals, Yield (90%). IR ($cm^{-1}$, KBr): 3356 (aromatic –NH bending), 3070 (aromatic C-H stretching), 1705 (C=O stretch), 1612, 1505 (aromatic ring C-C stretch), 1458 (-C=C stretching), 1315 (-C-N stretching); $^1$H-RMN: δ 2,83 (s, 3H), 7,58 (t, 2H), 7,91 (s, 1H), 7,54 (s, 1H), 7,26 (s, 2H), 10,25 (s, 1H, NH). Elemental analysis (%) found for $C_9H_8N_2O$: C, 67.41; H, 5.03; N, 17.52; O, 10.04. Calcd: C, 67.50; H, 5.00; N, 17.50; O, 10.00%).

2.2b *Synthesis of the Mannich bases*

A *Mannich* base is a beta-amino-ketone, formed by the reaction of an amine, formaldehyde (or another aldehyde) and a carboxilic acid. Here, the *Mannich* bases were prepared in a solution of 2-substituted benzimidazole (0.005 mol) in 10 ml ethanol, 0.005 mol of secondary amine and 0.005 mol of formaldehyde. The reaction mixtures were stirred at room temperature for 2h and then refluxed for an additional 3h. After cooling, the products were filtered, dried, and re-crystallized from dimethylformamide (DMF).

(**1M**) *Dimethyl(2-o-tolyl-1H-benzo[d]imidazole-1-yl)methanamine*. Pale yellow (crystals). TLC: $R_f$ (ethyl acetate : methanol : chloroform 4:3:3 v/v): 0.65; Yield 65%; m.p. 175-176°C. IR ($cm^{-1}$, KBr): 2990, 1450, 1439 methyl $CH_{asym}$; 1485 $CH_2$ bending; 1210 aromatic tertiary amine; 1028m skeletal C-C vibrations; 1583 vs C=C aromatic stretch; 2876 methylene CH asym/sym stretch; 766 *orto*-phenylene. $^1$H-RMN: δ 7,65 (dd, 2H, J = 2, 7Hz), 7,35 (d, 1H, J = 8Hz), 7,23 (m, 2H), 7,11 (m, 3H), 4,80 (s, 2H, $CH_2$), 2,27 (s, 6H, $CH_3$). Elemental analysis (%) found for $C_{17}H_{19}N_3$: C, 76.91; H, 7.22; N, 15.86. Calcd for $C_{13}H_{10}N_2$: C, 76.95; H, 7.21; N, 15.83%).

(**2M**) *1-(1-((Diethylamino)methyl)-1H-benzo[d]imidazol-2-yl)ethanol*. Pale yellow (semisolid). TLC: $R_f$ (ethyl acetate : methanol : chloroform 4:3:3 v/v): 0.72; Yield 75%; IR ($cm^{-1}$, KBr): 3200-3400 normal ‘‘polymeric’’ OH stretch, 3080 (aromatic C-H stretch), 2904, 2822 methyne C-H stretch, 1457

vC=C aromatic stretch, 1352, 1341 methyne C-H bend, 1314, 1223 aromatic tertiary amine, 1271 primary OH in plan bend, 743 primary OH out-of-plane bend. $^{1}$H-RMN: δ 7,80 (d, 1H, J = 2 Hz), 7,53 (s, 1H), 7.34 (t, 1H, J = 3 Hz), 7.20 (t, 1H, J = 3Hz), 5.07 (q, CH, J = 2Hz), 4.83 (dd, $CH_2$, J = 3, 14 Hz), 2.65 (doublet sextet, 2 $CH_2$, J = 1, 12 Hz), 2.20 (s, 1H from OH), 1.80 (d, 3H, $CH_3$, J = 2Hz), 1.11 (t, 6H, 2 $CH_3$, J = 2Hz). Elemental analysis (%) found for $C_{14}H_{12}N_2O$: C, 72.05; H, 9.03; N, 12.04; O, 6.88, calcd.: C, 72.10; H, 9.01; N, 12.01; O, 6.87%.

2.3 *Antimicrobial activity assays*

2.3a *Qualitative screening of the antimicrobial activity*

Standardized bacterial suspensions with a density of 1.5 -3 x $10^8$ CFU $mL^{-1}$ (corresponding to the 0.5 *McFarland* nephelometric standard) were obtained from 15-18h fresh bacterial cultures developed on solid media. The compounds were suspended in DMSO to prepare a stock solution of 10 mg /$mL^{-1}$ concentration. The antimicrobial activity was tested on *Mueller–Hinton Agar* (MHA) medium. The qualitative screening was performed by an adapted disc diffusion method [30].

2.3b *Quantitative assay of the antimicrobial activity*

The quantitative assay of the antimicrobial activity was performed by liquid medium microdilution method in 96 multi-well plates. Two-fold serial dilutions of the compounds solutions (ranging between 1000 µg and 4 µg $mL^{-1}$) were performed in a 200 µL volume of broth, and each was well seeded with 50 µL microbial inoculum. Bacterial culture positive controls (wells containing culture medium seeded with the microbial inoculum) as well negative sterility controls (containing only culture medium) were used. The influence of the DMSO solvent was also quantified in a series of wells containing DMSO, diluted accordingly with the dilution scheme used for the tested compounds. The multi-well plates were incubated for 24h at 37$^{o}$C, and the minimal inhibitory concentration (MIC) values were considered as the lowest concentration of the tested compound that inhibited the visible growth of the microbial overnight cultures, as compared to the positive control, correlated with a decreased value of the absorbance read at 600 nm (by using an “Apollo LB 911” ELISA reader).

2.3c *Quantitative assay of the anti-biofilm activity*

In order to evaluate the influence of the obtained compounds upon the colonization ability of microbial strains to the inert substratum, a microtiter method was employed. The multi-well plates

used for the MIC assay were emptied and washed three times with phosphate buffered saline. The biofilm formed on the plastic wells wall. After it was fixed for 5 min with cold methanol and coloured for 15 min by violet crystal solution, it was re-suspended in a 33% acetic acid solution. The minimal biofilm eradication concentration (MBEC) values were considered at the lowest concentration of the tested compound that inhibited the development of biofilm on the plate wells, as revealed by the decreased values of the optical density of the coloured solution at 490 nm, and as compared to that of the positive control [31].

2.4 *Computational and modeling details*

A quantum mechanical modeling method was implemented for each benzimidazole compound, using the *GAMESS 2012* software [32], in order to assess their structural parameters. The modeling was performed on a computer cluster consisting of 12 nodes and 96 cores running on Linux CeontOS. The results were visualized using *wxMacMolPlt* [33]. The molecular geometries of the benzimidazoles were optimized by using DFT at *M11/ktzvp* level of theory. *Tuhlar's M11* [34] is a modern range-separated hybrid functional that provides better results compared to the traditional *B3LYP* functional class of approximations to the exchange–correlation energy in DFT. Also, the basis set that we used is a more recent one (*Karlsruhe* valence triple zeta basis with a set of single polarization), introduced by *Prof. Ahlrichs* [35, 36]. The parameters used for geometry optimization are the default ones used in *GAMESS*, and the geometry used during the calculations is described by "natural internal coordinates" generated by the software.

**3. Results and discussion**

3.1 *Synthesis of benzimidazoles and benzimidazole Mannich bases*

Three 2-substituted-benzimidazoles have been synthesized using an eco-friendly, one-pot, solvent-free reaction, from 1,2-phenylenediamine and the corresponding carboxylic acid: (S)-lactic, benzoic, and *o*-toluic acid, respectively. For the lactic acid (liquid), the reactants were mixed thoroughly for a given period of time, instead of grinding. It should be noted that, by the eco-friendly method presented herein, better yields have been obtained in comparison to those reported in literature by other classical methods [37]. *1-(1H-Benzo[d]imidazole-2-yl)ethanone* (**4B**) was obtained by classic oxidation of *(S)-1-(1H-benzo[d]imidazole-2-yl)ethanol* with potassium dichromate and acetic acid. As it was previously mentioned, the new *Mannich* bases were synthesized by stirring of the 2-

substituted benzimidazoles, the secondary amine, and the formaldehyde, in a molar ratio of 1:1:3. The two methylene protons from the -$CH_2$- group in the second *Mannich* base (**2M)** are diastereotopic and, as a consequence, they appear as a doublet in doublets at 4.83 ppm. Also, the protons from -$CH_2$- ethylene groups are diastereotopic, and a doublet of sextets at 2.65 ppm can be observed. Antimicrobial properties of all benzimidazole derivatives have been screened, as they are presented and discussed in the following sections.

3.2 *Antimicrobial activity*

The antimicrobial activity of these compounds have been assessed against a range of planktonic and adherent bacterial strains, including all *"ESKAPE"* pathogens, which are exhibiting epidemiologically important resistance mechanisms, namely *Escherichia coli (E. coli), Staphylococcus aureus (S. aureus), Klebsiella pneumoniae (K. pneumoniae), Acinetobacter baumannii (A. baumannii), Pseudomonas aeruginosa (P. aeruginosa)* and *Enterococcus faecalis (E. faecalis)* [38]. In the qualitative assay, we have quantified the growth inhibition zones, induced after the deposition of 10 µl of the DMSO stock solution (10 mg/ml concentration) over the microbial culture (*Table 1*). From the tested compounds, the most active proved to be **3B**, active against five of the 9 tested strains, mostly Gram-positive ones, followed by **2B** and **1B**, each of them active against four tested microbial strains. Compound **2B** exhibited the largest spectrum of activity, including Gram positive cocci (*E. faecium*), *Pseudomonadaceae* (*P. aeruginosa, A. baumannii*) and *Enterobacteriaceae* (*E. coli*), while compound **1B** antimicrobial spectrum included only Gram-negative strains. Compound **2M** was active against the two *Enterococcus sp.* strains and the *E. cloacae* strain, while compounds **4B**, **1M** exhibited a much lower and narrower antimicrobial activity, against two or one of the tested Gram positive cocci strains. The quantitative assay of the microbicidal activity for the tested compounds confirmed the results obtained in the qualitative assay only for the compound **2B**, for which the same spectrum of activity has been recorded, with MIC values ranging from 0.5 to 0.125 mg/ml (*Table 2*). Compound **3B** exhibited moderate antimicrobial activity (MIC of 0.25-0.5 mg/ml) against the members of *Pseudomonadaceae* family and very good MIC (0.031 mg/ml) against *E. coli.* Compound **1B** exhibited very low MIC values against *S. saprophyticus* (0.002 mg/ml)*, P. aeruginosa* (0.008 mg/ml) and *K. pneumoniae* (0.031 mg/ml). Compound **2M** was active against the two *Enterococcus sp.* strains, with MIC values ranging from 0.008 tp 0.031 mg/ml and the *K. pneumoniae* strain (0.031 mg/ml). The investigation of the anti-biofilm activity of the obtained compounds revealed a different behaviour as

compared to their microbicidal properties (*Table 3*). Compound **1B** proved to be the most effective anti-biofilm agent, inhibiting the biofilm development of strains belonging to all three bacterial groups, *i.e.* Gram positive cocci, *Pseudomonadaceae* and *Enterobacteriaceae,* the lowest MBEC value (0. 008 mg/ml) being recorded against *E. faecium*. The second most active anti-biofilm compound was **2M**, also exhibiting low MBEC against the ability to form biofilms by three strains belonging to all three tested bacterial groups. Compound **2B** exhibited anti-biofilm activity against *E. faecium* and *Pseudomonadaceae* strains, with very good anti-*P. aeruginosa* activity. Compound **4B** exhibited very good activity against *E. faecium* with MBEC of 0.008 mg/mL. It is to be noticed that two of the most feared, biofilm forming opportunistic and nosocomial pathogens, *i.e. E. faecium* and *P. aeruginosa*, proved to be very susceptible to at least 4 of the tested compounds, proving their utility for the development of novel anti-biofilm agents.

*3.3 Electronic and structural properties*

The molecular geometries of the benzimidazole compounds were optimized using DFT at *M11/ktzvp* level [34-36]. The parameters of the optimized geometries of the compounds were subsequently determined. Relatively small molecules belonging to the group of symmetry C1, with lengths between 8.935 Å and 10.775 Å can be seen in *Table 4*. The smallest molecule is **1B**, which has the best anti-biofilm activity. *Mannich* base **1M** has a total energy of -823.309 Hartree (Ha), thus it is the most stable of all compounds, which correlates very well with its poor antimicrobial activity.

3.3a *Electronic parameters of the benzimidazole compounds*

The following parameters of the benzimidazole compounds have been obtained by using DFT at *M11/ktzvp* level of theory, *i.e.* $E_{HOMO}$ (energy of high occupied molecular orbital), $E_{LUMO}$ (energy of low unnoccupied molecular orbital), $E_{gap}$ ($E_{HOMO}$- $E_{LUMO}$), $\eta$ (chemical hardness), $\mu$ (electronic chemical potential), $\omega$ (global electrophilicity index), are shown in *Table 5*. These were used to appreciate chemical reactivity indices of benzimidazole compounds.

Chattaraj *et al.* [39] noted that the larger the HOMO–LUMO energy gap is, the harder and more stable/less reactive the molecule reveals to be. This means that the order of the decreasing the reactivity from the $E_{gap}$ value is:

**2B > 3B > 4B > 1M > 1B > 2M**.

It can be seen that for compounds **1B** and **2M**, the data correlate poorly. The stability and the reactivity of an organic compound are correlated with the chemical hardness, which may be calculated by using the following equation [40]:

$$\eta = \frac{E_{LUMO} - E_{HOMO}}{2}$$

From the calculated values of the chemical hardness (η) of the studied benzimidazoles and *Mannich* bases, one can see that compound **2B** should be the most reactive, because it is less hard of all, followed by **3B**, **4B**, **1M**, and **1B**, **2M** should be the least reactive. Again, one could say that for compound **1B** data do not fit all. *Parr* defined the negative of the electronegativity as the electronic chemical potential of a molecule, determined by the equation:

$$\mu = \frac{E_{HOMO} + E_{LUMO}}{2}$$

It is known that the greater the electronic chemical potential (μ) is, the less stable or more reactive the compound reveals to be. In *Table 4*, one can see that the decreasing order of the compounds reactivity is as follows:

**1B > 1M > 2M > 3B > 2B > 4B**.

Compound **1M** correlates the worst in this case, but for the first time we find a parameter that correlates the activity of compound **1B**. *Parr* introduced the global electrophilicity index using the electronic chemical potential and chemical hardness [41]:

$$\omega = \frac{\mu^2}{2\eta}$$

This index measures the stability of one molecule after it accepts an electronic charge from the surrounding environment. In *Table 5*, one can notice a decreasing order for the ω parameter, which means an increasing order for the stability of the molecules:

**1B > 2M > 1M> 3B > 2B > 4B**.

Compound **1B** is the strongest nucleophile, while **4B** is the strongest electrophile. We observe a good correlation for the antimicrobial activity for all compounds, except for the **1M** compound. An explanation for their poor activity with respect to the geometrical parameters will be provided.

3.3b *Atomic charges and geometric parameters of the benzimidazole compounds*

The nucleophilicity and electrophilicity concepts are related with the atomic charges on the atoms. The *Mulliken* charges on the atoms were calculated using DFT at *M11/ktzvp* level of theory (*Table 6*). The numbering of the atoms in the compounds discussed herein is shown in *Figure 2*. The calculated geometric parameters for all compounds are summarized in *Table 7* and *Table 8*. To the best of our knowledge, there is no experimental data on the geometric structure of these benzimidazoles to be found in literature. The following observations can be made relative to atomic charges and the antimicrobial activity of the discussed benzimidazoles.

Charges of -0.435 and -0.454 can be observed on the oxygen atom in **1B** and the **2M** molecule, respectively, which suggests that this is the most reactive atom for a substitution reaction. However, the base **2M** is less reactive than the benzimidazole **1B**. A possible explanation for this behaviour is the stereochemistry of the **1B**, which can be seen from the data for the bond lengths, bond and dihedral angles (*Table 7* and *Table 8*). Therefore, **1B** has a slight deviation from co-planarity due to the -OH and -$CH_3$ groups. However, these are almost symmetric and are therefore compensated. About 5° deviation from co-planarity, meaning (α-β)/2, is finally determined in the **1B** compound. In the case of **2M**, the same angle is 30.5°, a big deviation from co-planarity due to the presence of nitrogen substituents. In addition, the substituents to nitrogen are unsymmetrically located, *i.e.* the angles γ and δ, and a deviation of about 44 degrees was determined, (γ-δ)/2.

Compound **4B** has a charge of -0,354 on oxygen atom (hybridized $sp^2$), not available to be involved in substitution. It also exhibits minimum nucleophilicity and, as a consequence, reduced reactivity. However, in this case the stereochemistry of the compound is very advantageous, because the entire molecule is planar, as (α-β)/2 is 0,05 degrees. These facts explain the reactivity of the **4B** against about 22% of strains.

Benzimidazole **2B** is perfectly planar. This fact explains their good reactivity against ~44% of the strains, although a chemical substituent with high charge is not present in this molecule.

Benzimidazole **3B** has a tolyl ring rotated with 28.761°, a deviation from co-planarity of the entire molecule; however, the -$CH_3$ substituent has a high charge, *i.e.* -0.685, which possibly functions as a strong nucleophile, as it can be seen from the large spectra of strains in this case.

The *Mannich* base compound **1B** has a tolyl ring rotated with 66.348°, which is double compared with benzimidazole **3B**, and also a big deviation from the co-planarity of the N-substituents,

*i.e.* (θ-τ)/2, meaning 73.14°. Such a stereochemical structure doesn't favour antimicrobial activity of the **1M**, in concordance with the qualitative and quantitative determinations.

3.3c *Chemical shifts*

Finally, in order to find and compare the chemical shifts of the protons in our new compounds, we used data from literature [42, 43]. In NMR spectroscopy, the chemical shift is the resonant frequency of a nucleus relative to a standard in a magnetic field, while the position and number of chemical shifts are diagnostic of the structure of a molecule. Two scenarios have been employed. For the hydrogen atoms bound to $sp^3$ carbon atoms, the following equation was used:

$$\delta_{XYZC\text{-}H} = (0.23 + \sigma_X + \sigma_Y + \sigma_Z)\ \text{ppm},$$

where: $\sigma_X$, $\sigma_Y$, $\sigma_Z$ are the increments for the substituents of the calculated hydrogen atom. Likewise, for the aromatic hydrogen atoms bound to $sp^2$ carbon atoms, the following equation was used:

$$\delta_{Ar\text{-}H} = (7.27 + \sigma_{ortho} + \sigma_{meta} + \sigma_{para})\ \text{ppm},$$

where: $\sigma_{ortho}$, $\sigma_{meta}$, $\sigma_{para}$ are the increments for the substituents in the *ortho*, *meta*, and *para* positions, respectively. For the *Mannich* bases (**1M** and **2M)**, the determined chemical shifts (in ppm) are presented in black colour while the calculated ones (also in ppm) are in blue colour (*Figure 3*). In both cases, one can observe a reasonable agreement between the determined and the calculated chemical shifts, hence confirming our approach, except for the circumstances in which the values for the special increment are not available and a difference can be observed between the two values (*e.g.* the case of the -$CH_3$ group).

To summarize, we show that the electronic parameters of the benzimidazole compounds are in accordance with their antimicrobial activity. Such results fit with previously reported data for the coumarin compounds [44]. Further accurate processing of such compounds into thin films and hybrid structures, *e.g.* by laser ablation matrix-assisted pulsed laser evaporation (MAPLE) and/or laser-induced forward transfer (LIFT) [45-52] could provide simple and environmental friendly, state-of-the-art solutions for antimicrobial applications. It was shown that the antimicrobial activity of lysozyme [46, 49-52] and lactoferrin [53-55] is preserved after laser processing, as thin films. Therefore,

embedded and/or multi-layered structures [55], patterned coatings and blends [56-59] can be further imagined, in which novel structures can be designed and produced to further enhance, adjust and control such microbicidal / anti-biofilm properties.

**4. Conclusion**

Six benzimidazole compounds, including new *Mannich* bases, have been synthesized, characterized, presented and discussed herein. Density functional theory analysis of the molecular structure, together with the HOMO-LUMO orbitals, has been employed by using the *GAMESS 2012* software. It has been found that the electronic and structural parameters of the benzimidazole compounds are well correlated to their antimicrobial activity. The tested compounds exhibited different microbicidal and anti-biofilm features, being active against a wide spectrum of Gram positive and Gram negative bacterial strains both in planktonic and adherent state, endorsing their potential in developing novel anti-microbial and anti-biofilm agents. The microbicidal activity of the compounds is linked to the presence of the nucleophilic groups within the molecule, such as -OH or -$CH_3$. Therefore, the largest microbicidal and anti-biofilm effect is noticed for the OH-compounds, in both types, *i.e.* benzimidazole and *Mannich* bases. The planarity and the symmetry of the molecule also represent strong support in their antimicrobial activity. Finally, we briefly show that further accurate processing of such compound into thin films and hybrid structures, *e.g.* by laser ablation matrix-assisted pulsed laser evaporation and/or laser-induced forward transfer, and may provide simple and environmental friendly, state-of-the-art solutions for antimicrobial coating applications.

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***List of tables and figures (table and figure captions):***

***Table 1.*** The antimicrobial activity of the tested compounds expressed semi-quantitatively, as the absence of the microbial growth (-), slight decrease of the microbial growth (+/-), total inhibition of microbial growth (+, ++).

***Table 2.*** The MIC (μg $mL^{-1}$) values of the tested compounds against the tested microbial strains.

***Table 3.*** The MBEC (μg $mL^{-1}$) values of the tested compounds against the tested microbial strains.

***Table 4.*** The symmetry group, length of molecule and total energy of the benzimidazoles.

***Table 5.*** Chemical reactivity indices for compounds **1B**-**4B** and **1M**-**2M**.

***Table 6.*** *Mulliken* atomic charges for compounds **1B**-**4B** and **1M**-**2M**.

***Table 7.*** Bond lengths values of the discussed benzimidazoles.

***Table 8.*** Bond and dihedral angles of the discussed benzimidazoles.

***Figure 1.*** The optimized structures of benzimidazole compounds **1B-4B**, **1M**, **2M** (black-C; grey-H; red-O; blue-N).

***Figure 2.*** Atom numbering, exemplified on Mannich bases **1M** (like in **2B**, **3B**) and **2M** (like in **1B**, **4B**).

***Figure 3.*** The determined (in black) and calculated (in blue) chemical shifts for the *Mannich* base **1M** and **2M**.

| Compound/Tested strain | | 1B | 2B | 3B | 4B | 1M | 2M |
|---|---|---|---|---|---|---|---|
| Gram positive cocci | *S. aureus* | +/- | +/- | + | - | +/- | +/- |
| | *S. saprophyticus* | + | +/- | ++ | + | +/- | +/- |
| | *E. faecium* | +/- | + | + | + | + | + |
| | *E. faecalis* | +/- | +/- | ++ | - | +/- | ++ |
| Gram negative bacilli (*Pseudomonadaceae* family) | *P. aeruginosa* | + | + | ++ | - | +/- | +/- |
| | *A. baumannii* | ++ | + | +/- | - | +/- | +/- |
| Gram negative baciili (*Enterobacteriaceae* family) | *K. pneumoniae* | + | +/- | +/- | - | +/- | +/- |
| | *E. cloacae* | +/- | +/- | +/- | - | +/- | ++ |
| | *E. coli* | + | + | +/- | - | +/- | +/- |

***Table 1*.** The antimicrobial activity of the tested compounds expressed semiquantitatively, as the absence of the microbial growth (-), slight decrese of the microbial growth (+/-), total inhibition of microbial growth (+, ++).

| Compound/Tested strain | | 1B | 2B | 3B | 4B | 1M | 2M |
|---|---|---|---|---|---|---|---|
| Gram positive cocci | *S. aureus* | >1 | >1 | >1 | >1 | >1 | >1 |
| | *S. saprophyticus* | 0.002 | >1 | >1 | >1 | >1 | >1 |
| | *E. faecium* | >1 | 0.125 | >1 | >1 | >1 | 0.008 |
| | *E. faecalis* | >1 | >1 | >1 | >1 | >1 | 0.03125 |
| Gram negative baciili (*Pseudomonadaceae* family) | *P. aeruginosa* | 0.008 | 0.25 | 0.25 | >1 | >1 | >1 |
| | *A. baumanii* | >1 | 0.5 | 0.5 | >1 | >1 | >1 |
| Gram negative baciili (*Enterobacteriaceae* family) | *K. pneumoniae* | 0.03125 | >1 | >1 | >1 | >1 | 0.03125 |
| | *E. cloacae* | >1 | >1 | >1 | >1 | >1 | >1 |
| | *E. coli* | >1 | 0.5 | 0.03125 | >1 | >1 | >1 |

***Table 2.*** The MIC (μg $mL^{-1}$) values of the tested compounds against the tested microbial strains.

| Compound/Tested strain | | 1B | 2B | 3B | 4B | 1M | 2M |
|---|---|---|---|---|---|---|---|
| Gram positive cocci | *S. aureus* | >1 | >1 | >1 | >1 | >1 | >1 |
| | *S. saprophyticus* | 0.002 | >1 | >1 | 0.03125 | >1 | >1 |
| | *E. faecium* | 0.002 | 0.125 | >1 | 0.008 | 0.25 | 0.008 |
| | *E. faecalis* | >1 | >1 | >1 | >1 | 1 | 0.25 |
| Gram negative baciili (*Pseudomonadaceae* family) | *P. aeruginosa* | 0.01562 | 0.3125 | 0.3125 | >1 | >1 | 0.0625 |
| | *A. baumanii* | >1 | 0.5 | 0.25 | >1 | >1 | >1 |
| Gram negative baciili (*Enterobacteriaceae* family) | *K. pneumoniae* | 0.03125 | >1 | >1 | >1 | >1 | 0.03125 |
| | *E. coli* | >1 | >1 | >1 | >1 | >1 | >1 |

***Table 3.*** The MBEC (μg $mL^{-1}$) values of the tested compounds against the tested microbial strains.

| Compound | Symmetry | Length (Å) | $E_{tot}$ (Ha) |
|---|---|---|---|
| **1B** | C1 | 8.935 | -533.628 |
| **2B** | C1 | 10.823 | -610.780 |
| **3B** | C1 | 10.733 | -650.080 |
| **4B** | C1 | 8.974 | -532.427 |
| **1M** | C1 | 10.775 | *-823.309* |
| **2M** | C1 | 10.322 | -785.457 |

***Table 4*.** The symmetry group, length of molecule and total energy of the benzimidazoles.

| Energy (Hartree) | | *Benzimidazoles* | | | | *Mannich bases* | |
|---|---|---|---|---|---|---|---|
| | | **1B** | **2B** | **3B** | **4B** | **1M** | **2M** |
| | $E_{HOMO}$ | -0.322 | -0.310 | -0.311 | -0.337 | -0.315 | -0.325 |
| | $E_{LUMO}$ | 0.048 | 0.016 | 0.023 | -0.001 | 0.039 | 0.046 |
| | $E_{gap}$ | -0.370 | -0.326 | -0.334 | -0.336 | -0.354 | -0.371 |
| | $\eta$ | 0.185 | 0.163 | 0.167 | 0.168 | 0.177 | 0.1855 |
| | $\mu$ | -0.137 | -0.152 | -0.144 | -0.169 | -0.138 | -0.1395 |
| | $\omega$ | 0.050 | 0.070 | 0.062 | 0.085 | 0.053 | 0.052 |
| Antimicrobial activity | Qualitative | 44.44% | 44.44% | 55.55% | 22.22% | 11.11% | 22.22% |
| | Quantitative (MIC) | 33.33% | 44.44% | 33.33% | - | - | 33.33% |
| | Quantitative (MBEC) | 44.44% | 33.33% | 22.22% | 22.22% | 11.11% | 44.44% |

***Table 5.*** Chemical reactivity indices for compounds **1B**-**4B** and **1M**-**2M**.

| Atom | *Mulliken charge* | | | | | |
|---|---|---|---|---|---|---|
| | **1B** | **2B** | **3B** | **4B** | **1M** | **2M** |
| C-1 (Benzimidazole) | -0.132 | -0.128 | -0.135 | -0.127 | -0.131 | 0.132 |
| C-2 (Benzimidazole) | -0.189 | -0.190 | -0.185 | -0.178 | -0.185 | -0.186 |
| C-3 (Benzimidazole) | -0.089 | -0.072 | -0.092 | -0.095 | -0.022 | -0.077 |
| C-4 (Benzimidazole) | 0.013 | -0.019 | -0.007 | 0.000 | -0.066 | -0.039 |
| C-5(Benzimidazole) | 0.010 | -0.02 | 0.006 | 0.013 | -0.035 | 0.023 |
| C-6 (Benzimidazole) | -0.143 | -0.140 | -0.136 | -0.140 | -0.138 | -0.135 |
| N-7 (Benzimidazole) | -0.389 | -0.146 | -0.406 | -0.354 | -0.295 | -0.268 |
| C-8 (Benzimidazole) | 0.167 | 0.389 | 0.195 | 0.218 | -0.002 | 0.267 |
| N-9 (Benzimidazole) | -0.312 | -0.333 | -0.325 | -0.307 | -0.271 | -0.394 |
| C-10 ($CH^{B1,M2}$, $Ph^{B2,B3,M1}$, $CO^{B4}$) | -0.020 | -0.341 | -0.106 | 0.301 | -0.046 | -0.106 |
| O-11 ($OH^{B1,M2}$, $CO^{B4}$) | ***-0.435*** | - | - | *-0.374* | - | ***-0.454*** |
| C-11 (Ph) | - | -0.154 | -0.039 | - | -0.085 | - |
| C-12 ($CH_3^{B1,B4,M2}$, $Ph^{B2,B3,M1}$) | -0.349 | -0.130 | -0.156 | -0.552 | -0.134 | -0.421 |
| C-13 (Ph) | - | -0.155 | -0.161 | - | -0.168 | - |
| C-14' ($Ph^{B2,B3,M1}$) | - | -0.183 | -0.117 | - | -0.067 | - |
| C-14 ($CH_2^{M1,M2)}$ | - | - | - | - | -0.296 | -0.408 |
| N-15 | - | - | - | - | *-0.213* | -0.203 |
| C-15 (Ph) | - | -0.021 | 0.063 | - | -0.060 | - |
| C-16 ($CH_3$-$tol^{B3,M1}$, $CH_2^{M2}$) | - | - | ***-0.685*** | - | **-0.543** | -0.240 |
| C-17 ($CH_3$) | - | - | - | - | -0.378 | -0.408 |
| C-18 ($CH_2$) | - | - | - | - | - | -0.250 |
| C-19 ($CH_3$) | - | - | - | - | -0.426 | -0.369 |

Ph: Phenyl; Tol: Tolyl

***Table 6.*** Mulliken atomic charges for compounds **1B**-**4B** and **1M**-**2M**.

| Chemical bond | *Length (Å)* | | | |
|---|---|---|---|---|
| | **1B** | **4B** | **1M** | **2M** |
| $C^{8}$-$C^{10}$ | 1.507 | 1.489 | - | 1.505 |
| $C^{10}$-$C^{12}$ | 1.518 | 1.498 | - | 1.531 |
| $C^{10}$-$O^{11}$ | 1.427 | 1.209 | - | 1.411 |
| $O^{11}$-$H^{19}$ | 0.961 | - | - | 1.097 |
| $N^{7}$-$C^{14}$ | - | - | 1.444 | 1.449 |
| $C^{14}$-$N^{15}$ | - | - | 1.450 | 1.448 |
| $N^{15}$-$C^{17}$ | - | - | 1.452 | - |
| $N^{15}$-$C^{16}$ | - | - | - | 1.464 |
| $N^{15}$-$C^{19}$ | - | - | 1.455 | - |
| $N^{15}$-$C^{18}$ | - | - | - | 1.464 |
| $C^{16}$-$C^{17}$ | - | - | - | 1.525 |
| $C^{18}$-$C^{19}$ | - | - | - | 1.523 |

***Table 7.*** Bond lengths values of the discussed benzimidazoles.

| Parameter | *Angle value* | | | | | |
|---|---|---|---|---|---|---|
| | **1B** | **2B** | **3B** | **4B** | **1M** | **2M** |
| $C^{8}C^{10}C^{12}$ | 111.228 | - | - | 116.581 | - | 110.467 |
| $C^{8}C^{10}O^{11}$ | 109.564 | - | - | 118.760 | - | 109.028 |
| $N^{7}C^{14}C^{15}$ | - | - | - | - | 111.186 | 112.093 |
| $N^{7}C^{8}C^{10}C^{12}$ | 148.807 | - | - | 0,390 | - | 77.533 |
| $N^{9}C^{8}C^{10}C^{12}$ (α) | 33.184 | - | - | 0.435 | - | 99.982 |
| $N^{7}C^{8}C^{10}O^{11}$ (β) | 23.312 | - | - | 0.335 | - | 161.069 |
| $N^{7}C^{8}C^{10}C^{15}$ | - | 180.000 | *28.761* | - | *66.348* | - |
| $C^{8}C^{10}C^{15}C^{16}$ | - | - | 1.832 | - | 1.673 | - |
| $N^{7}C^{14}N^{15}C^{17}$ (θ) | - | - | - | - | 174.328 | - |
| $N^{7}C^{14}N^{15}C^{16}$ (γ) | - | - | - | - | - | 160.232 |
| $N^{7}C^{14}N^{15}C^{19}$ (τ) | - | - | - | - | 28.047 | - |
| $N^{7}C^{14}N^{15}C^{18}$ (δ) | - | - | - | - | - | 72.499 |
| $C^{14}N^{15}C^{16}C^{17}$ | - | - | - | - | - | 79.587 |
| $C^{14}N^{15}C^{18}C^{19}$ | - | - | - | - | - | 160.545 |

***Table 8.*** Bond and dihedral angles of the discussed benzimidazoles.

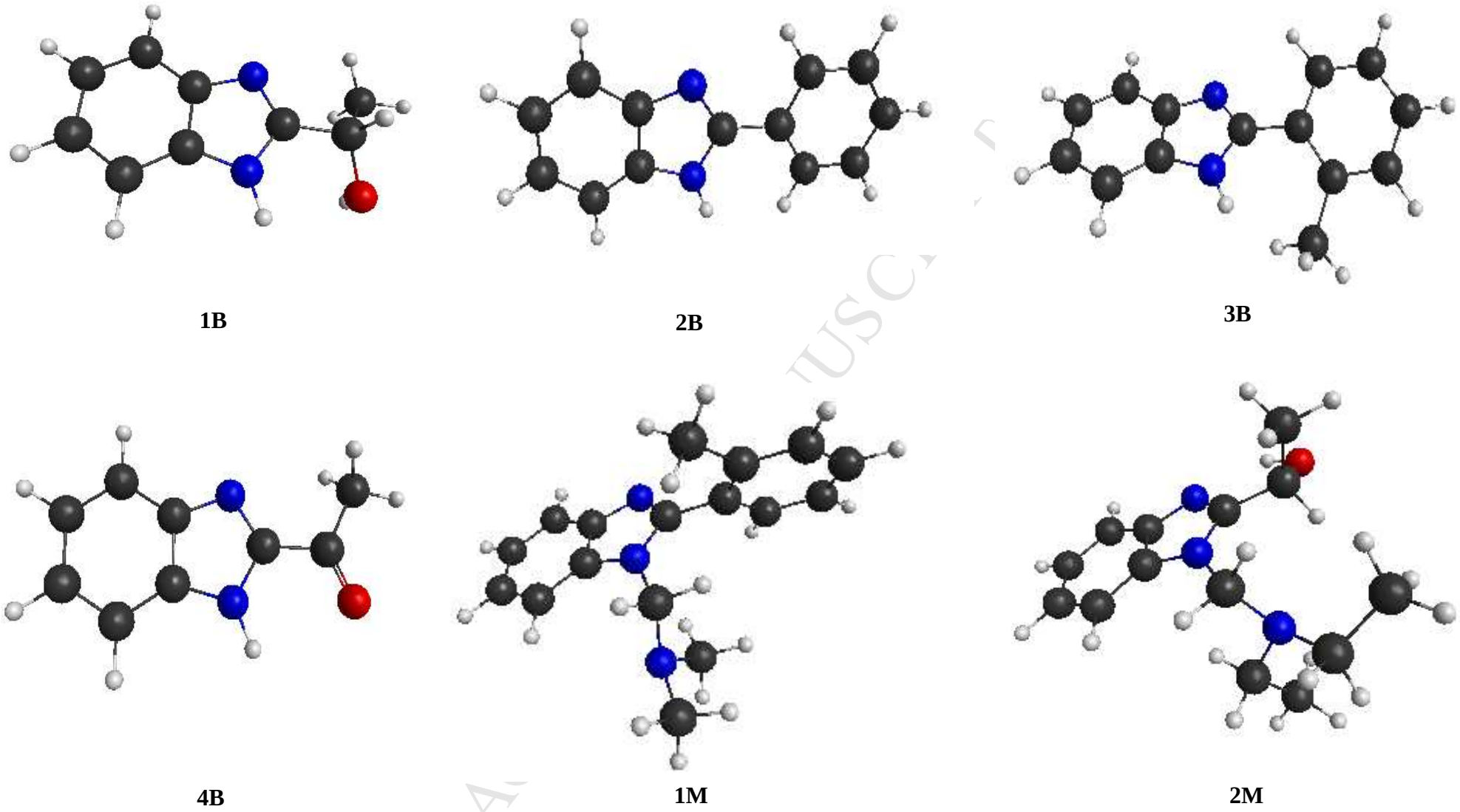
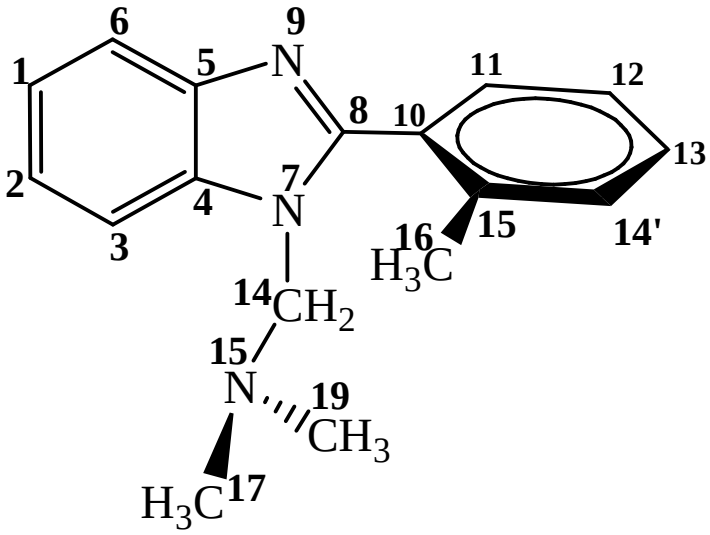
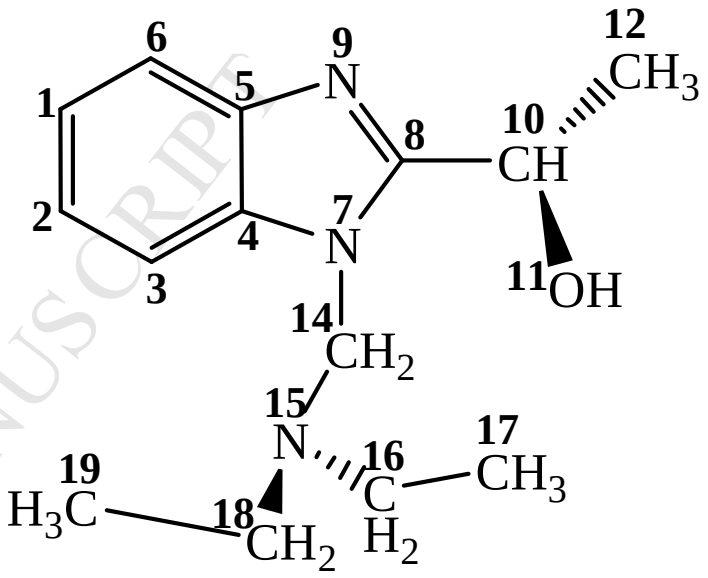



***Figure 1*.** The optimized structures of benzimidazole compounds **1B-4B**, **1M**, **2M** (black-C; gray-H; red-O; blue-N).



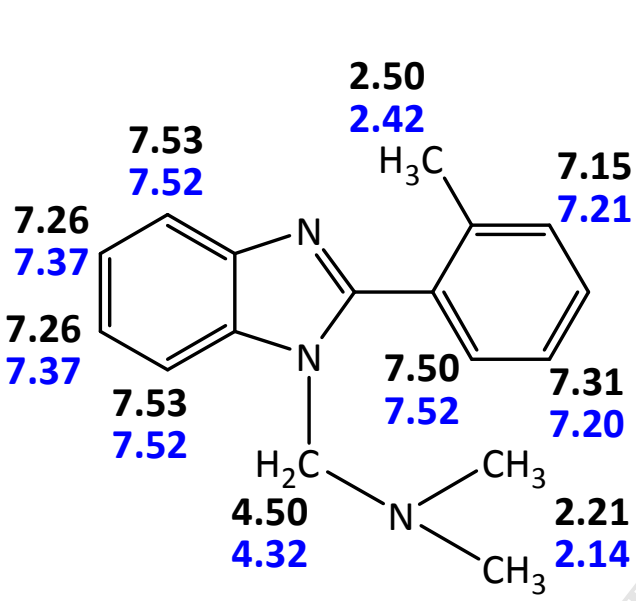

**1M**



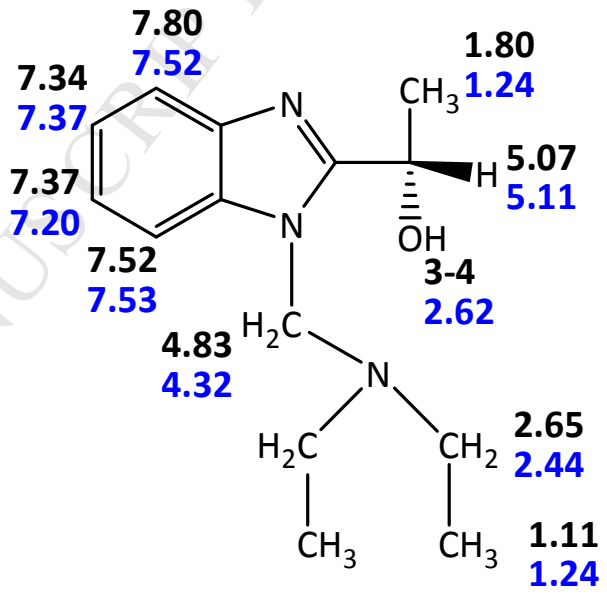

**2M**



***Figure 2*.** Atom numbering exemplified on *Mannich* bases **1M** (as in **2B**, **3B**) and **2M** (as in **1B**, **4B**).




1M




2M

***Figure 3.*** The determined (in black) and calculated (in blue) chemical shifts for the *Mannich* base **1M** and **2M**.

## Highlights

- Eco-friendly one-pot solvent-free synthesis of bioactive 2-substituted benzimidazoles
- N-*Mannich* bases synthesized from benzimidazoles, secondary amines and formaldehyde
- Compounds evaluated by qualitative & quantitative methods against 9 bacterial strains
- Density functional theory (DFT) modeling is accomplished with *GAMESS 2012* software
- Discussion on the laser-processing of such compounds for antimicrobial coatings