Microwave Assisted Synthesis of Some Novel 2-(3-Chlorobenzyl)-1H-Benzimidazole Derivatives and Determination of Their Antimicrobial Activity and pKa Values

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In the present research, synthesis of new 2-(3-chlorobenzyl)-1H-benzimidazole derivatives bearing N-benzylidene, 1,2,4-triazole and N-acylhydrazone moiety is investigated. These novel compounds have been tested for their antimicrobial effects against two of Gram-positive bacterial strains, namely Bacillus subtilis and Staphylococcus epidermidis, and four Gram-negative bacterial strains which were Enterobactercloaceae, Klebsiellapneumonie, Proteus vulgaris, and Salmonella typhymirium. According to their MIC values, which reflect their activity on the bacteria, were classified in three grops: best, good and moderate. So, the best MIC values were obtained ***6a*** *and* ***6b****. On the other hand, the acidity values of pKa for* ***6a-d*** *derivatives were calculated with MOPAC 2012 computer program using physico-chemical semi-empirical methods (AM1, MNDO, MNDOD, PM3, PM6, PM6-DH2, PM7 and RM1) in water, ethyl alcohol, methyl alcohol, n-propyl alcohol, isopropyl akcohol, n-butyl alcohol, tert-butyl alcohol, ethylene glycol, N,N-dimethyl formamide, N,N-dimetyl sulfoxide and acetonitrile at 25°C.*

Keywords:benzimidazoles, hydrazinecarbothioamides, 1,2,4-triazoles, N-acylhydrazones, antimicrobial activity, pKa and MOPAC 2012 computer program

For biological and pharmaceutical reasons, synthesis of novel benzimidazole derivatives and investigation of their chemical and biological behavior has gained great interest in recent decades [1]. Substituted benzimidazole derivatives possess broad spectrum of biological activity in both medicinal and pharmaceutical, such as, anticonvulsants [2], antitubercular [3], analgesics [4], anticancer [5], antiviral [6], antihelmintic [7], antioxidant [8] and antimicrobial [9]. Benzimidazoles are widely used as drugs like lansoprazole, pantoprazole, omeprazole as proton pump inhibitors [10]; albendazole, mebendazole, thiabendazole as antihelmintics [11]; astemizole as antihistaminic [12] and pimobendan, ionodilator and rifaximin as anticancer [13-14]. In addition, 1,2,4-triazole derivatives are used quite often in pharmacological, medicinal, and agricultural applications [15]. Various compounds containing 1,2,4-triazole ring are well known drugs like vorozole, letrozole and anastrozole (anti cancer drugs)[16], fluconazole, itraconazole, ravuconazole, voriconazole and posaconazole (antifungal drugs) [17-18], rizatriptan (antimigraine drug) [19] and ribavirin (antiviral drug) [20], On the other hand, benzimidazoles containing 1,2,4-triazole ring have also been reported to exhibit potential pharmacological properties [21-23].

Acid dissociation constants, pKa values, are important parameters which reflect the degree of ionization of a molecule in solution at different pH values [24]. Dissociation constants of a substance can be determined by different methods. Potentiometric, chromatographic and electrophoretic methods have been used widely. Further, sophisticated computer programs have also been used for determination of pKa. This aproach has been employed in different fields such as stereochemical and conformational structure determinations.

As an extention of our studies on benzimidazole derivatives [22, 23, 25-27], it was contemplated to synthesize a series of new 2-(3-chlorobenzyl)-1H-benzimidazole derivatives bearing hydrazinecarbothioamide, 1,2,4-triazole and N-benzylidene moity and screen them for their antimicrobial activity. Moreover, the pKa values of compounds **6a-d** were also calculated employing MOPAC 2012 computer programme to evaluate the structure-activity relationship.

Experimental part

Chemistry

All the chemicals were supplied from Merck, Aldrich and Fluka. Melting points were determined on capillary tubes on a Buchi oil heating melting point apparatus and uncorrected. ^{1}H-NMR was performed on Varian- Mercury 400 MHz spectrometer in DMSO-d_6 using TMS as internal. The IR spectra were recorded on a Perkin- Elmer 100 FTIR spectrophotometer as KBr pellets. The elemental compositions were determined on a Carlo Erba1106 CHN analyzer; the experimental values were in agreement (±0.4 %) with the calculated ones. Mass spectra were recorded on Thermo Scientific Quantum Access max LC-MS spectrometer. A mono- mode CEM- Discover microwave was used to carry out microwave reactions in 30 mL microwave process vials with temperature control by infrared detection temperature sensor. All reactions were monitored by TLC using precoated aluminum sheets (silica gel 60 F 2.54 0.2 mm thickness).

*Synthesis of ethyl 2-(2-(3-chlorobenzyl)-1H-benzimidazol-1-yl)acetate (**3**)*

A solution of compound **2** (0.01 mol) in acetone (10 mL) was taken in a closed vessel, dry K_2CO_3 (0.03 mol) and ethyl bromoacetate (0.01mol) were added. The mixture was irradiated in microwave at 90°C, 10 min (hold time) at 300 Watt maximum power. After the reaction was completed, monitored by TLC (EtAc: Hexane, 4:1), the

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mixture was cooled, taken in a beaker and the product was precipitated by addition of water and was filtered, dried and recrystallized from ethanol-water (1:1) to afford the desired product **3**.

Yield 94%; m.p. 149-150 °C; IR (KBr): ν= 1731 (C=O), 1596 (C=N), 1214 (C-O) cm^{-1};^{1}H NMR (400 MHz, DMSO-d_6): δ= 1.15 (3H, t, CH_3), 3.96 (2H, q, OCH_2), 4.37 (2H, s, CH_2), 5.32 (2H, s, NCH_2), 7.24-7.83 (8H, m, Ar-H); Anal. Calcd. for. $C_{18}H_{17}ClN_2O_2$: C, 65.75; H, 5.21; N, 8.52 Found: C, 65.79; H, 5.24; N, 8.56 %. LC-MS: m/z: 329.92 $[M+H]^+$.

*Synthesis of 2-(2-(3-chlorobenzyl)-1H-benzimidazol-1-yl)acetohydrazide (**4**)*

A mixture of compound **3** (0.01 mol) and hydrazine hydrate (0.025 mol) in absolute ethanol (10 mL) was irradiated in closed vessels with the pressure control at 120°C for 7 min (hold time) at 300 W maximum power. After the completion of the reaction, (monitored by TLC, ethylacetate:Hexane, 3:1), the mixture was cooled to room temperature. The precipitate was filtered off and recrystallized from ethanol.

Yield 83 %;m.p. 187-188 °C; IR (KBr): ν = 3323 (NH_2NH), 1650 (C=O), 1604 (C=N) cm^{-1};^{1}H NMR (400 MHz, DMSO-d_6): δ= 4.34 (2H, s, CH_2), 4.38 (2H, s, NH_2, exch. D_2O), 5.25 (2H, s, NCH_2), 7.44-7.15 (8H, m, Ar-H), 9.58 (1H, s, NH, exch. D_2O); Anal. Calcd. for. $C_{16}H_{15}ClN_4O$: C, 61.05; H, 4.80; N, 17.80 Found: C, 61.10; H, 4.86; N, 17.75 %.LC-MS: m/z: 315.80$[M+H]^+$.

*Synthesis of Compounds **5a-d***

A mixture of compound **4** (0.01 mol) and corresponding isothiocyanate (0.01 mol) in ethanol (20 mL) was irradiated in microwave at 76-78 °C and 13 min. at 200 Watt maximum power (monitored by TLC, ethyl acetate/hexane= 3:1). Then, the mixture was cooled to room temperature and the crude product was observed by addition of water, filtered off and recrystallized from ethanol-water (1:2) to afford the desired products.

*1-(2-(2-(3-Chlorobenzyl)-1H-benzimidazol-1-yl)acetyl)-4-methylthiosemicarbazide (**5a**)*

Yield 91 %;m.p. 200-201 °C; IR (KBr): ν = 3285, 3250 (NH), 1679 (C=O), 1550 (C=N), 1236 (C=S) cm^{-1}; ^{1}H-NMR (DMSO-d_6, 200 MHz)δ (ppm): 2.93 (3H, s, CH_3), 4.25 (2H, s, CH_2), 4.97 (2H, s, N-CH_2), 7.19-7.59 (8H, m, Ar-H), 8.10, 9.39, 10.30 (3H, s, NH, exch. D_2O); Anal. Calcd. For $C_{18}H_{18}ClN_5OS$: C, 55.74; H, 4.68; N, 18.06; S, 8.27. Found: C, 55.39; H, 4.71; N, 18.13; S, 8.24. LC-MS: m/z: 388.91$[M+H]^+$.

*1-(2-(2-(3-Chlorobenzyl)-1H-benzimidazol-1-yl)acetyl)-4-ethylthiosemicarbazide (**5b**)*

Yield 90 %;m.p. 217-218 °C; IR (KBr): ν = 3382, 3292 (NH), 1684 (C=O), 1595 (C=N), 1307 (C=S) cm^{-1}; ^{1}H-NMR (DMSO-d_6, 400 MHz)δ (ppm): 1.09 (3H, t, *J*= 4.2 Hz, 3H, CH_3), 3.12 (2H, m, CH_2), 3.91 (2H, s, CH_2), 4.70 (2H, s, N-CH_2), 6.90-7.29 (8H, m, Ar-H), 7.89, 9.05, 10.09 (3H, s, NH, exch. D_2O); Anal. Calcd. For $C_{19}H_{20}ClN_5OS$: C, 56.78; H, 5.02; N, 17.43; S, 7.98. Found: C, 56.83; H, 5.05; N, 17.45; S, 7.94. LC-MS: m/z: 402.94$[M+H]^+$.

*1-(2-(2-(3-Chlorobenzyl)-1H-benzimidazol-1-yl)acetyl)-4-phenylthiosemicarbazide (**5c**)*

Yield 89 %;m.p. 250-252 °C; IR (KBr): ν = 3380, 3288 (NH), 1690 (C=O), 1593 (C=N), 1305 (C=S) cm^{-1}; ^{1}H-NMR (400 MHz, DMSO-d_6): δ= 4.40 (2H, s, CH_2), 5.29 (2H, s, NCH_2), 6.85-7.57 (13H, m, Ar-H), 9.43, 10.11, 10.38 (3H, s, NH, exch. D_2O); Anal. Calcd. for. $C_{23}H_{20}ClN_5OS$: C, 61.39; H, 4.48; N, 15.56, S, 7.13. Found: C, 61.43; H, 4.51; N, 15.62, S, 7.09. %.LC-MS: m/z: 450.87$[M+H]^+$.

*1-(2-(2-(3-Chlorobenzyl)-1H-benzimidazol-1-yl)acetyl)-4-benzylthiosemicarbazide (**5d**)*

Yield 85 %;m.p. 254-255 °C; IR (KBr): ν = 3285 (NH), 1666 (C=O), 1598 (C=N), 1297 (C=S) cm^{-1}; ^{1}H-NMR (DMSO-d_6, 200 MHz)δ (ppm): 4.22 (2H, s, CH_2), 4.31(2H, s, CH_2), 5.05 (2H, s, N-CH_2), 7.17-7.58 (13H, m, Ar-H), 8.06, 9.67, 10.25 (3H, s, NH, exch. D_2O); Anal. Calcd. For $C_{24}H_{22}ClN_5OS$: C, 62.13; H, 4.78; N, 15.09; S, 6.91. Found: C, 62.17; H, 4.82; N, 15.02; S, 6.88. LC-MS: m/z: 465.00$[M+H]^+$.

*Synthesis of Compounds **6a-d***

A solution of corresponding carbothioamide **5a-d** (0.01 mol) in ethanol/water (1:1) was irradiated in microwave at 120 °C and 10 min. at 200 Watt maximum power in the presence of 2N NaOH. Then, the resulting solution was cooled to room temperature and acidified to pH 5-6 with 37% HCl. The crude product was filtered off, washed with water and recrystallized from ethanol to afford compound **6a-d**.

*5-((2-(3-Chlorobenzyl)-1H-benzimidazol-1-yl)methyl)-4-methyl-4H-1,2,4-triazole-3-thiol (**6a**)*

Yield 84 %;m.p. 232-233 °C; IR (KBr): ν = 2540 (SH), 1599, 1576 (C=N), 1328 (C=S) cm^{-1};^{1}H-NMR (DMSO-d_6, 200 MHz)δ (ppm): 3.45 (3H, s, NCH_3), 4.28 (2H, s, CH_2), 5.65 (2H, s, N-CH_2), 7.17-7.59 (8H, m, Ar-H), 13.46 (1H, s, -SH); Anal. Calcd. For $C_{18}H_{16}ClN_5S$: C, 58.45; H, 4.36; N, 18.93; S, 8.67. Found: C, 58.49; H, 4.32; N, 18.97; S, 8.60. LC-MS: m/z: 370.91$[M+H]^+$.

*5-((2-(3-Chlorobenzyl)-1H-benzimidazol-1-yl)methyl)-4-ethyl-4H-1,2,4-triazole-3-thiol (**6b**)*

Yield 81 %;m.p. 238-239 °C; IR (KBr): ν = 2620 (SH), 1599, 1575 (C=N), 1352 (C=S) cm^{-1}; ^{1}H-NMR (DMSO-d_6, 200 MHz)δ (ppm): 1.07 (3H, t, J= 8 Hz, CH_3), 3.99 (2H, q, J= 8 Hz, NCH_2), 4.31 (2H, s, CH_2), 5.69 (2H, s, N-CH_2), 7.16-7.60 (8H, m, Ar-H), 13.50 (1H, s, SH); Anal. Calcd. for. $C_{19}H_{18}ClN_5S$: C, 59.44; H, 4.73; N, 18.24; S, 8.35. Found: C, 59.49; H, 4.77; N, 18.26; S, 8.30. %. LC-MS: m/z: 384.93$[M+H]^+$.

*5-((2-(3-Chlorobenzyl)-1H-benzimidazol-1-yl)methyl)-4-phenyl-4H-1,2,4-triazole-3-thiol (**6c**)*

Yield 83 %;m.p. 269-270 °C; IR (KBr): ν = 2653 (SH), 1591, 1576 (C=N), 1303 (C=S) cm^{-1}; ^{1}H-NMR (400 MHz, DMSO-d_6): δ= 4.07 (2H, s, CH_2), 5.52 (2H, s, NCH_2), 6.98-7.73 (13H, m, Ar-H), 13.97 (1H, s, SH); Anal. Calcd. for. $C_{23}H_{18}ClN_5S$: C, 63.95; H, 4.20; N, 16.21; S, 7.42. Found: C, 63.90; H, 4.23; N, 16.24; S, 7.38. %. LC-MS: m/z: 432.97$[M+H]^+$.

*5-((2-(3-Chlorobenzyl)-1H-benzimidazol-1-yl)methyl)-4-benzyl-4H-1,2,4-triazole-3-thiol (**6d**)*

Yield 77 %;m.p. 260-261°C; IR (KBr): ν = 2659 (SH), 1597, 1548 (C=N), 1297 (C=S) cm^{-1}; ^{1}H-NMR (DMSO-d_6, 200 MHz)δ (ppm): 4.23 (2H, s, CH_2), 4.33 (2H, s, CH_2), 5.64 (2H, s, N-CH_2), 7.167-7.59 (13H, m, Ar-H), 13.53 (1H, s, SH); Anal. Calcd. For $C_{24}H_{20}ClN_5S$: C, 64.64; H, 4.52; N, 15.70; S, 7.19. Found: C, 64.69; H, 4.55; N, 15.73; S, 7.15. LC-MS: m/z: 447.02$[M+H]^+$.

*Synthesis of Compounds **7a-c***

To a solution of compound 4 (0.01mol) in dry ethanol (20mL) (containing 0.5mL glacial aceticacid), corresponding aromatic aldehyde (0.01mol) was added, and the mixture was refluxed for 4-6h (monitored by TLC, ethylacetate : hexane 3:1). After cooling the mixture to room temperature, a white solid appeared. This crude product was filtrated and washed with ethanol to obtain the desired product.

*2-(2-(3-Chlorobenzyl)-1H-benzimidazol-1-yl)-N'-(4-chlorobenzylidene)acetohydrazide (**7a**)*

Yield 95 %;m.p. 253-254 °C; IR (KBr): 3123 (NH), 1697 (C=N), 1608, 1596 (CP%N) cm^{-1}; ^{1}H-NMR (DMSO-d6, 200 MHz): 4.28 (2H, s, CH_2), 5.50 and 5.04 (2H, s, N-CH_2, *trans* and *cis* conformer), 7.14-7.80 (12H, m, Ar-H), 8.03 and 8.23 (1H, s, NP%CH, *trans* and *cis* conformer), 11.78 and 11.88 (1H, s, NH, *trans* and *cis* conformer 78/22, exch. D_2O); The ratio of *trans* and *cis* conformers: 78/22. Anal. Calcd for $C_{23}H_{18}Cl_2N_4O$: C, 63.17; H, 4.15; N, 12.81. Found: C, 63.22; H, 4.18; N, 12.77. LC-MS: m/z: 438.47 $[M+H]^+$.

*2-(2-(3-Chlorobenzyl)-1H-benzimidazol-1-yl)-N'-(4-(dimethylamino)benzylidene)acetohydrazide(**7b**)*

Yield 93 %;m.p. 237-238 °C; IR (KBr): 3218 (NH), 1672 (C=N), 1598, 1577 (CP%N) cm^{-1}; ^{1}H-NMR (DMSO-d6, 200 MHz): 2.95 (6H, s, $2NCH_3$), 4.26 (2H, s, CH_2), 5.43 and 4.99 (2H, s, N-CH_2, *trans* and *cis* conformer), 6.71-7.57 (12H, m, Ar-H), 7.90 and 8.07 (1H, s, NP%CH, E *trans* and *cis* conformer), 11.44, 11.52 (1H, s, NH, *trans* and *cis* conformer, exch. D_2O); The ratio of *trans* and *cis* conformers: 77/23. Anal. Calcdfor $C_{25}H_{24}ClN_5O$: C, 67.33; H, 5.42; N, 15.70. Found: C, 67.38; H, 5.45; N, 15.66. LC-MS: m/z: 446.99$[M+H]^+$.

*2-(2-(3-Chlorobenzyl)-1H-benzimidazol-1-yl)-N'-(3-bromo-4-fluorobenzylidene) acetohydrazide (**7c**)*

Yield 90 %;m.p. 260-261 °C; IR (KBr): 3198 (NH), 1686 (C=N), 1610, 1598 (CP%N) cm^{-1}; ^{1}H-NMR (DMSO-d6, 200 MHz): 4.26 (2H, s, CH_2), 5.53 and 5.05 (2H, s, N-CH_2, *trans* and *cis* conformer), 7.14-8.00 (11H, m, Ar-H), 8.16 and 8.20 (1H, s, NP%CH, *trans* and *cis* conformer), 11.81, 11.95 (1H, s, NH, *trans* and *cis* conformer, exch. D_2O); The ratio of *trans* and *cis* conformers: 77/23. Anal. Calcd for $C_{23}H_{17}BrClFN_4O$: C, 55.28; H, 3.43; N, 11.21. Found: C, 55.33; H, 3.47; N, 11.17. LC-MS: m/z: 500.83. $[M+H]^+$.

Antimicrobial Aactivity

The Minimum Inhibition Concentration (MIC) Tests

The qualitative screening of the susceptibility spectra of different microbial strains to the complexes was performed by the quantitative assay of minimal inhibitory concentration (MIC, g/mL) based on liquid medium serial microdilution [28, 29]. The MIC assays were performed in LB medium at pH 7.2. The stock solutions of the newly synthesized compounds were prepared in dimethyl sulfoxide (DMSO). The dilution series of the chemical compounds to be tested were prepared from 1500 to 11.72 μg/mL concentrations in 100 μL medium. The well plates were incubated at 37.0 ± 1 °C for 18–24 h. Dimethy-sulphoxide, LB medium with or without antibiotic, ampicillin, were used as solvent control, positive, and negative controls, respectively. The MIC was taken to be the last well in the dilution series that did not exhibit growth as determined on the basis of turbidity.

The determination of MIC [29] was done with two of Gram-positive bacterial strains, namely *Bacillus subtilis* (ATCC 66333) and *Staphylococcus epidermidis*, and four Gram-negative bacterial strains which were *Enterobactercloaceae* (ATCC 13047), *Klebsiella pneumonie* (ATCC 13883), *Proteus vulgaris* (ATCC 13315), and *Salmonella typhymirium* (ATCC 14028). These were inoculated into Luria broth medium containing 1% tryptone, 0.5% yeast extract, 0.5% sodium chloride. The *p*H of the medium was adjusted to 7.2 and incubated at 37°C for 18-24 h. The optical density of the bacteria from mid-log phase of growth was measured at 600 nm and diluted in fresh medium so as to get an optical density of 0.004 (corresponding to 5 x 10^5 colony forming units/mL).

Acidity

Theoretical calculations were carried out using AM1, MNDO, MNDOD, PM3, PM6, PM6-DH2, PM7 and RM1 semi empirical methods in the MOPAC 2012 computer program. Compound **6a-d** were optimized to a gradient form in water, ethyl alcohol, methyl alcohol, n-propyl alcohol, isopropyl akcohol, n-butyl alcohol, tert-butyl alcohol, ethylene glycol, N,N-dimethyl formamide, N,N-dimetyl sulfoxide and acetonitrile at 25°C.

The primary approximates of the geometry of all the structures were obtained by Augmented MM3 and MMFF molecular mechanic methods, followed by full optimization of all geometrical variables (bond lengths, bond angles and dihedral angles), without any symmetry constraint, using the semi-empirical AM1, MNDO, MNDOD, PM3, PM6, PM6-DH2, PM7 and RM1 quantum chemical methods [30]. One of the usually used methods to search for the effect of substituent on an equilibrium proces is the practice of the Hammett equation [31].

The semi empirical calculations that were used in the present work are based on the following reaction.

$$AH + S \rightleftharpoons SH^+ + A^-$$

In this reaction AH is the weak acid and SH^+ is the protonated acid. S is using solvent and A^- is conjugated base of weak acids. Semi empirical calculations were carried out using the following reactions.

$$\Delta G_{Reaction} = [\Delta G_{(SH+)} + \Delta G_{(A-)}] - [\Delta G_{(AH)} + \Delta G_{(S)}]$$

$$pKa = \Delta G_{Reaction} / 2.303RT$$

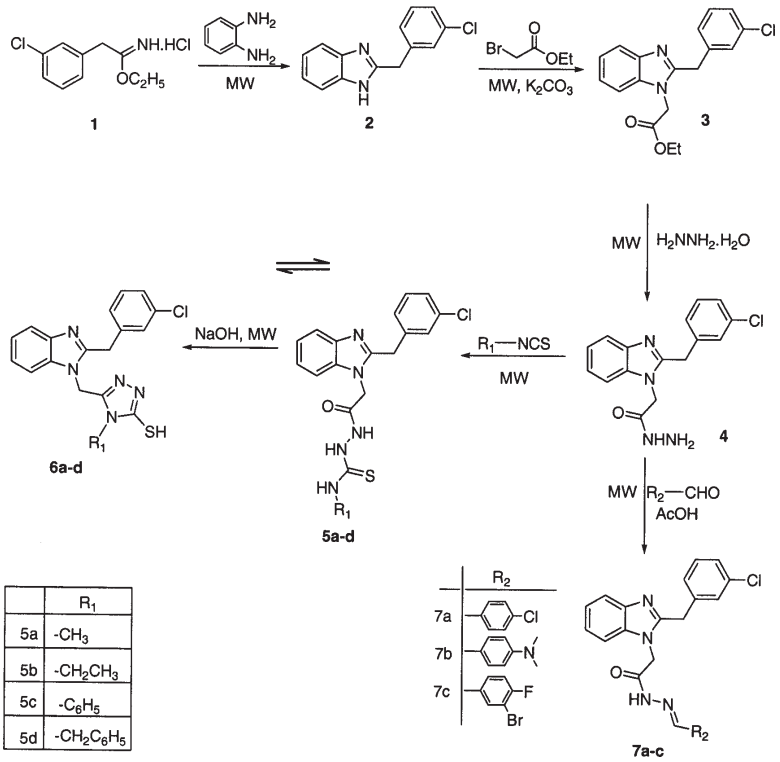
$\Delta H_{Formation}$ and ΔS are calculated for each species involved in the reaction. In this study, $\Delta G_{Reaction}$ thermodynamic values were calculated from determined $\Delta H_{Formation}$ and ΔS using the semi-empirical methods.

Results and discussion

Chemistry

In this study, a shorter procedure for the synthesis of compound **2** was used. Firstly, compound **1** was prepared according to the literature [32]. To synthesize benzimidazole compound (**2**) [33], compound **1** was reacted with 1,2-phenylenediamine derivatives under microwave irradiation with high yields (scheme 1) [23].

Treatment of compound **2** with ethyl bromoacetate in acetone by microwave irradiation gave the ethyl 2-(2-(3-chlorobenzyl)-1H-benzimidazol-1-yl)acetate (**3**), which converted by hydrazine hydrate to afford 2-(2-(3-chlorobenzyl)-1H-benzimidazol-1-yl)acetohydrazide (**4**). Hydrazinecarbothioamide derivatives (**5a-d**) were synthesized via condensation of corresponding acid hydrazide (**4**) and alkyl/aryl isothiocyanates in ethanol by microwave irradiation. The cyclization of compounds **5a-d** in the presence of sodium hydroxide by microwave



	R_1
5a	$-CH_3$
5b	$-CH_2CH_3$
5c	$-C_6H_5$
5d	$-CH_2C_6H_5$

Scheme 1. Synthetic pathway for the preparation of compounds **2-7**

irradiation resulted in the formation of **6a-d**. Reaction of compound **4** with equimolar amount of various aromatic aldehydes in ethanol by microwave irradiation afforded the corresponding arylidenhydrazide derivatives (**7a–c**) at good yields. The structures of synthesized novel compounds were confirmed by IR, ^{1}H NMR, elemental analyses and mass spectra. In the IR spectra of compound **3** the C=O absorption bands were observed at 1731 cm^{-1} regions. In the ^{1}H NMR spectra of this compounds were seen as a triplet at δ 1.15 ppm (3H, OCH_2CH_3), as a quartet δ 3.96 ppm (2H, OCH_2) and as a singlet δ 5.32 ppm (2H,N-CH_2). The IR spectrum of **4** showed absorption bands at 3323 and 1650 cm^{-1}due to NH_2+NH and C=O groups, respectively. In the ^{1}H NMR spectrum of this compound the signals due to NH_2 and NH protons were observed at 4.38 and 9.58 ppm, respectively. The IR spectrum of compounds **5a-d** displayed absorption bands at around 3250-3385 cm^{-1}due to 3 NH functional groups. The three protons of NH groups resonated as three singlet at between 7.89-9.43, 9.05-10.11 and 10.09-10.38 ppm (exchangeable with D_2O) in the^{1}H NMR spectrum of these compounds. The IR spectra of compounds **6a-d** showed absorption bands between 2540-2653 cm^{-1}due to SH stretching. In the^{1}H NMR spectra of these compounds the SH protons were seen around 13.46-13.97 ppm. The IR spectra of **7a-c** showed the N-H and C=O bands at about 3193-3218 and 1672-1697 cm^{-1}. In the ^{1}H NMR spectra of compounds **7a-c** N-CH_2, N=CH and NH proton signals were recorded as double singlets. According to the literature, N-benzylidene derivatives may exist as *E*/*Z* geometrical isomers about C=N double bonds and *cis*/*trans* amide conformers [34-36]. It is known that the N=CH double bond restricts the rotation and increase the formation of *E* and *Z* isomers that the *E* isomer is dominate [34-36]. It has been reported that when N-benzylidene derivatives are dissolved in polar solvents such as DMSO-d_6, the geometrical *E* isomers of compouns **7a-c** undergo a rapid *cis*and *trans* amide equilibrium, in which the *trans* conformer peredominates [34-36]. The *E* isomers and the *cis* and *trans* conformer ratios can easily be determined by

Compounds		Gram Positive		Gram Negative			
		B. subtilis	*S. epidermidis*	*E. cloacae*	*K. pneumoniae*	*P. vulgaris*	*S. typhimirium*
1	**3**	750	750	750	750	750	750
2	**4**	750	750	750	750	750	750
3	**5a**	750	750	750	750	750	750
4	**5b**	750	750	750	750	750	750
5	**5d**	750	750	750	750	750	750
6	**6a**	750	187.5	375	187.5	187.5	750
7	**6b**	750	375	750	375	375	750
8	**6c**	750	750	750	750	750	750
9	**6d**	750	750	750	750	750	750
10	**7a**	750	750	750	750	750	750
11	**7b**	750	750	750	750	750	750
DMSO		+	+	+	+	+	+
LB broth		+	+	+	+	+	+
LB broth + amp		-	-	-	-	-	-

DMSO: dimethyl sulfoxide, LB broth: This medium without chemical component and antibiotic as positive control, amp: ampicilline,
"+": not effected forgrowing, "-"; no growth.

Table 1
IN-VITRO ANTIBACTERIAL ACTIVITY DATA (MIC VALUE μg/mL) OF COMPOUNDS **3-7**

Solvent	MODEL	ΔH(S) (kcal/mol)	ΔH(SH^+) (kcal/mol)	ΔS(S) (cal/Kmol)	ΔS(SH^+) (cal/Kmol)	ΔG(S) (kcal/mol)	ΔG(SH^+) (kcal/mol)
Methyl Alcohol	AM1	-5.7053	13.8259	5.6929	5.7958	-7.4018	12.0988
	MNDO	-5.7379	13.5332	5.7225	5.9576	-7.4432	11.7578
	MNDOD	-5.7354	13.4874	5.7083	5.8420	-7.4365	11.7465
	PM3	-5.1898	21.9471	5.7122	5.8687	-6.8920	20.1982
	PM6	-4.8346	13.7694	5.7450	5.8077	-6.5466	12.0387
	PM6-DH2	-4.8356	13.7665	5.7449	5.8033	-6.5476	12.0371
	PM7	-4.8938	14.4233	5.7402	5.9224	-6.6044	12.6584
	RM1	-5.0127	14.4897	5.6924	5.8744	-6.7090	12.7391

Table 2
AM1, MNDO, MNDOD, PM3, PM6, PM6-DH2, PM7 AND RM1CALCULATED THERMODYNAMIC DATA OF THE STUDIED METHYL ALCOHOLAT 25°C

Comp.	MODEL	ΔH(AH) (kcal/mol)	ΔH(A^-) (kcal/mol)	ΔS(AH) (cal/Kmol)	ΔS(A^-) (cal/Kmol)	ΔG(AH) (kcal/mol)	ΔG(A^-) (kcal/mol)
6a	AM1	16.9074	9.3789	15.8278	16.0346	13.7418	6.1720
	MNDO	10.2097	6.3038	16.0606	16.8153	6.8370	2.7726
	MNDOD	11.0221	7.1297	16.1483	16.2562	7.4695	3.5533
	PM3	11.6880	6.4836	16.4260	16.6407	7.9100	2.6562
	PM6	11.3946	5.5619	16.4341	16.2108	7.4504	1.6713
	PM6-DH2	10.1515	4.5802	16.2511	16.2049	6.0887	0.5290
	PM7	11.1201	5.4495	16.1900	16.2442	6.9107	1.2260
	RM1	11.1343	8.2766	16.2197	16.8787	6.7550	3.7194

Table 3
AM1, MNDO, MNDOD, PM3, PM6, PM6-DH2, PM7 AND RM1CALCULATED THERMODYNAMIC DATA OF THE STUDIED COMPOUND **6A** AT 25°C

^{1}H NMR integration. In this study, two sets of signals each belonging to the NCH_2, N=CH and NH group of trans and cis conformers were observed 5.53-4.99, 7.90-8.20, 11.44-11.95 ppm, respectively.

Antibacterial Activities

All the synthesized compounds were tested against three *Gram-positive* and *negative* bacteria in accordance with published protocols. The results were compared with the standard drug, ampicillin (table 1). As can be seen from the table, all compounds showed anti-bacterial effect, ranging from good to moderate, with a minimum inhibitory concentration of 11.72-1500 μg/mL in dimethyl sulfoxide. The minimum inhibition concentrations were determined in triplicates against the test bacteria and the results are shown in table 1.

First of all, we see that the highest effect was observed by compound **6a** with a MIC value of 187.5μg/mL against *S.epidermidis*, *K.pneumonie* (ATCC 13883), and *P. vulgaris* (ATCC 13315). Compound **6b** also has the high activity against *S.aureus* (ATCC 25923), *K.pneumonie* (ATCC 13883), and *P. vulgaris* (ATCC 13315) with a MIC value of 375 μg/mL. Compounds **6a** and **6b** have the same effect against *B.subtilis* (ATCC 66333) and *E.cloaceae* (ATCC 13047) with a MIC value of 750 μg/mL. It is interesting that the other compounds have the same effect on all the bacteria that exhibited the same MIC value of 750 μg/mL (table 1).

Acidity

The acidity of a compound depends on several factors. The two most important factors are the solvent effect and molecular structure. $\Delta H_{Formation}$ and ΔS is calculated for each

Table 4
AM1, MNDO, MNDOD, PM3, PM6, PM6-DH2, PM7 AND RM1CALCULATED PKA VALUES FOR THE INVESTIGATED MOLECULES IN WATER, ETHYL ALCOHOL, METHYL ALCOHOL, N-PROPYL ALCOHOL, ISOPROPYL AKCOHOL, N-BUTYL ALCOHOL, *TERT*-BUTYL ALCOHOL, ETHYLENE GLYCOL,*N,N*-DIMETHYL FORMAMIDE, *N,N*-DIMETYL SULFOXIDE AND ACETONITRILE AT 25°C

Comp.	Solvent	pKa							
		MODEL							
		AM1	MNDO	MNDOD	PM3	PM6	PM6-DH2	PM7	RM1
6a	Water	13.8420	16.0366	15.3908	15.1687	12.4896	12.1817	12.2650	13.5654
	Ethyl alcohol	12.1990	15.4978	14.9176	14.0627	10.8423	10.7152	10.5695	12.6433
	Methyl alcohol	13.0361	15.7513	15.1647	20.7473	11.6605	11.3853	11.4123	13.2837
	n-Propyl alcohol	12.2564	15.4000	14.8476	14.0803	10.8677	10.6201	10.6379	12.6451
	Isopropyl akcohol	11.6808	15.2231	14.6787	13.4796	10.1933	9.9688	9.9641	12.2161
	n-Butyl alcohol	12.2209	15.3782	14.8266	14.0450	10.7976	10.5520	10.5420	12.6101
	tert-Butyl alcohol	11.2367	14.9944	14.4602	13.0768	9.6176	9.4096	8.8351	11.9809
	Ethylene glycol	12.7646	15.6335	15.0705	14.3229	11.6535	11.3772	11.2874	12.8901
	N,N-Dimethyl formamide	11.2276	15.4511	14.5425	12.7531	11.2664	11.1115	9.7307	9.8209
	N,N-Dimetyl sulfoxide	7.8379	10.2233	11.8420	9.3483	7.9533	7.8605	7.6429	9.7665
	Acetonitrile	11.0817	15.4174	14.8645	11.6293	10.2772	10.0533	9.6575	11.1429
6b	Water	13.4627	16.1204	15.2445	15.0821	12.4874	11.8550	12.2762	13.7922
	Ethyl alcohol	11.8197	15.5817	14.7712	13.9760	10.8400	10.3885	10.5806	12.8702
	Methyl alcohol	12.6567	15.8352	15.0184	20.6606	11.6582	11.0586	11.4235	13.5106
	n-Propyl alcohol	11.8770	15.4839	14.7013	13.9936	10.8654	10.2933	10.6491	12.8720
	Isopropyl akcohol	11.3014	15.3070	14.5323	13.3930	10.1910	9.6421	9.9753	12.4429
	n-Butyl alcohol	11.8415	15.4620	14.6803	13.9584	10.7953	10.2252	10.5532	12.8370

	tert-Butyl alcohol	10.8574	15.0783	14.3139	12.9902	9.6153	9.0828	8.8463	12.2078
	Ethylene glycol	12.3852	15.7174	14.9242	14.2363	11.6512	11.0505	11.2986	13.1170
	N,N-Dimethyl formamide	10.8482	15.5350	14.3962	12.6665	11.2641	10.7847	9.7419	10.0478
	N,N-Dimetyl sulfoxide	7.4585	10.3072	11.6956	9.2616	7.9510	7.5337	7.6540	9.9934
	Acetonitrile	10.7023	15.5013	14.7182	11.5427	10.2749	9.7265	9.6687	11.3698
6c	Water	18.3175	15.8880	14.9581	15.2191	12.4941	11.4469	12.1038	13.3064
	Ethyl alcohol	16.6745	15.3492	14.4849	14.1130	10.8468	9.9804	10.4083	12.3843
	Methyl alcohol	17.5116	15.6027	14.7320	20.7976	11.6650	10.6505	11.2511	13.0247
	n-Propyl alcohol	16.7319	15.2514	14.4149	14.1306	10.8722	9.8852	10.4767	12.3861
	Isopropyl akcohol	16.1563	15.0745	14.2460	13.5300	10.1977	9.2340	9.8029	11.9571
	n-Butyl alcohol	16.6963	15.2296	14.3939	14.0954	10.8020	9.8171	10.3808	12.3512
	tert-Butyl alcohol	15.7122	14.8458	14.0275	13.1272	9.6221	8.6747	8.6739	11.7220
	Ethylene glycol	17.2400	15.4849	14.6378	14.3733	11.6580	10.6423	11.1262	12.6311
	N,N-Dimethyl formamide	15.7030	15.3025	14.1098	12.8035	11.2709	10.3766	9.5695	9.5619
	N,N-Dimetyl sulfoxide	12.3133	10.0747	11.4093	9.3986	7.9577	7.1256	7.4817	9.5075
	Acetonitrile	15.5572	15.2688	14.4318	11.6797	10.2817	9.3184	9.4963	10.8839
6d	Water	17.9110	16.0572	14.9403	15.3437	12.5596	11.8738	12.8236	13.0174
	Ethyl alcohol	16.2680	15.5184	14.4671	14.2377	10.9122	10.4073	11.1281	12.0954
	Methyl alcohol	17.1051	15.7719	14.7142	20.9223	11.7304	11.0774	11.9709	12.7358
	n-Propyl alcohol	16.3254	15.4206	14.3972	14.2553	10.9376	10.3121	11.1965	12.0972
	Isopropyl akcohol	15.7498	15.2437	14.2282	13.6546	10.2632	9.6609	10.5227	11.6681
	n-Butyl alcohol	16.2899	15.3988	14.3761	14.2200	10.8675	10.2440	11.1006	12.0622
	tert-Butyl alcohol	15.3057	15.0150	14.0098	13.2519	9.6876	9.1016	9.3937	11.4330
	Ethylene glycol	16.8336	15.6541	14.6201	14.4979	11.7234	11.0693	11.8460	12.3422
	N,N-Dimethyl formamide	15.2966	15.4717	14.0920	12.9281	11.3364	10.8035	10.2893	9.2730
	N,N-Dimetyl sulfoxide	11.9069	10.2439	11.3915	9.5233	8.0232	7.5525	8.2015	9.2186
	Acetonitrile	15.1507	15.4380	14.4140	11.8043	10.3471	9.7453	10.2161	10.5950

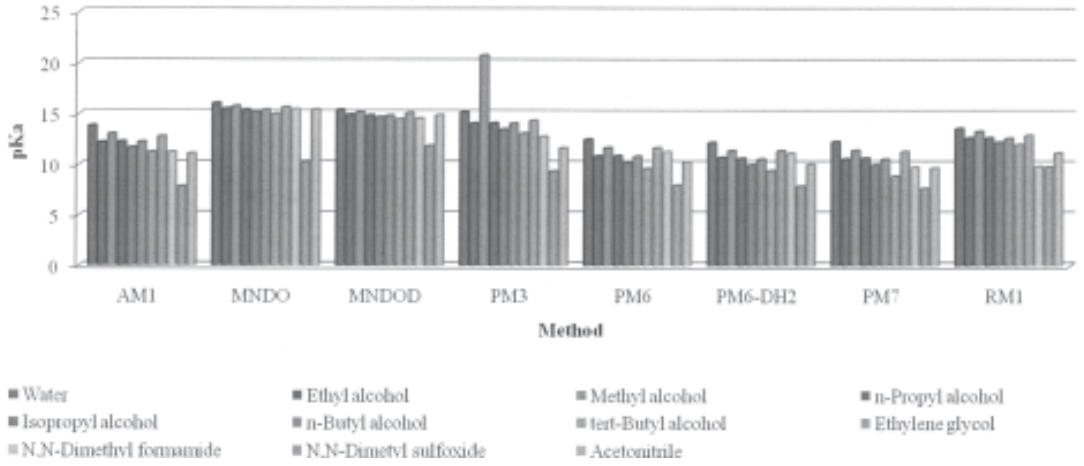


Fig. 1. AM1, MNDO, MNDOD, PM3, PM6, PM6-DH2, PM7 and RM1 calculated pKa values of graphical representation of Compound 6a in water, ethyl alcohol, methyl alcohol, n-propyl alcohol, isopropyl alcohol, n-butyl alcohol, tert-butyl alcohol, ethylene glycol,*N,N*-dimethyl formamide, *N,N*-dimetyl sulfoxide and acetonitrile at 25°C

species, solvents and compounds that involved in the reaction. Calculations for methyl alcohol were given as example in table 2. In this study, $\Delta G_{Reaction}$ thermodynamic values were calculated from determined $\Delta H_{Formation}$ and ΔS using the semi-empirical methods. Thermodynamic data were also introduced into the charts. Compound **6a** was given as example in table 3. Depending on these data pKa values were calculated and given in table 4. When the dielectric permittivity of solvents is taken into consideration, the acidic arrangement can be expected as follows: water (ε:78)>N,N-dimetyl sulfoxide (ε:46) >ethylene glycol (ε:38)>N,N-dimethyl formamide (ε:37)>acetonitrile (ε:36)>methyl alcohol (ε:33)>ethyl alcohol (ε:24)>n-propyl alcohol (ε:20.3)>isopropyl alcohol (ε:19.4)>n-butyl alcohol (ε:17.5)>tert-butyl alcohol (ε:12). As seen in table 4, the acidic order for compounds; **6a** (pKa:7.8379) and **6b** (pKa:7.4585) in N,N-dimethyl sulfoxide by AM1, **6c** (pKa:7.1256) and **6d** (pKa:7.5525) in N,N-dimethyl sulfoxide by PM6-DH2 showed high acidic properties. Compound **6a** (pKa:20.7473), **6b** (pKa:20.6606), **6c** (pKa:20.6606) and **6d** (pKa:20.9223) in methyl alcohol by PM3 showed low acidic properties. All pKa values calculated in different solvents found for compound **6a** are given in figure 1 as an example of studied compounds.

Conclusions

In conclusion, a series of 2-(3-chlorobenzyl)-1H-benzimidazole derivatives bearing hydrazine-carbothioamide, 1,2,4-triazole and N-benzylidene moiety were synthesized by microwave technique and were screened for their antimicrobial activity. It was clear that the bacterial growth was inhibited by compounds **6a** and **6b**. Other compounds have the same effect against all bacteria strain tested in this study at the same concentration. The

compounds **6a-d** were tested for their protons pull and protons release potential for acidity. The acidity changed in the order of 6b> 6a> 6c> 6d that was obtained in PM3 and PM6 methods.

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