Scientific paper

New Mono, Bis-2,2-(arylidineaminophenyl)benzimidazoles: Synthesis and Antimicrobial Investigation

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Abstract

Some new mono and bis-2,2-(arylidineaminophenyl)benzimidazole derivatives have been synthesized and characterized via IR, ^{1}H NMR, ^{13}C NMR, MS and elemental analysis. All the products were screened for antibacterial activity against *Staphylococcus aureus*, *Bacillus subtilis*, *Streptococcus pyogenes, Salmonella typhimurium, Escherichia coli* and *Klebsiella pneumonia* and antifungal activity against *Aspergillus niger*, *Candida albicans* and *Trichoderma viridae*. Compounds **5**, **6** exhibited potent in vitro antibacterial activity against *S. aureus*, *B. subtilis*, *S. pyogenes* whereas compounds **13**–**16** exerted superior activity against all tested bacterial and fungal strains even than standard drugs Ampicillin and Ketoconazole.

Keywords: 2,2-(arylidineaminophenyl)benzimidazole, synthesis, antibacterial activity, antifungal activity

1. Introduction

Heterocycles make up an exceedingly important class of compounds due to their expansive range of applications. They are predominant among all types of pharmaceuticals, agrochemicals and veterinary products.[1–8] This comes as no surprise, since the most potent natural compounds, the alkaloids, are heterocyles. Manipulations of heterocyclic compounds are easy to perform, very subtle, and nevertheless have a huge impact on their reactivity. This is why heterocycles are so widely used. Nitrogen heterocycles in particular exhibit diverse biological and pharmacological activities[9–11] due in part to the similarities with many natural and synthetic molecules with known biological significance.[12] Specifically, benzimidazole nucleus is a constituent of several natural and non-natural products such as Vitamin B_{12},[13] marine alkaloid kealiiquinone,[14] benzimidazole nucleosides[15] etc. Some of their derivatives are marketed as anti-fungal agents such as Carbendazim[16] and anti-helmintic agents such as Mebendazole and Thiabendazole.[17] The benzimidazole has been an important pharmacophore and privileged structure in medicinal chemistry, encompassing a plethora of useful biological activities such as antimicrobial,[18–20] anti cancer,[21–23] anti HIV,[24–26] etc. Benzimidazoles are fundamental structural units not only in the pharmaceutical industry but also in several other fields such as agricultural, electronic, and polymer chemistry.[27–30] Owing to the immense importance and varied bioactivities exhibited by benzimidazoles, efforts have been made from time to time to generate libraries of these compounds and screen them for potential biological activities.[31] These observations have encouraged us to synthesize some new products containing the benzimidazole moiety hoping to obtain new compounds with potential biological activity. In the present study, we performed the synthesis and biological evaluation of some new mono and bis-2,2-(arylideneaminophenyl)benzimidazole.

2. Results and Discussion

The synthesis of mono and bis-2,2-(arylidineaminophenyl)benzimidazoles is summarized in Scheme 1. In the present investigation, the starting compound, 2-(1*H*-benzimidazol-2-yl)aniline (**A**) (lit. mp 214 °C) was prepared by reacting 1,2-benzenediamine (OPD) with 2-amino-

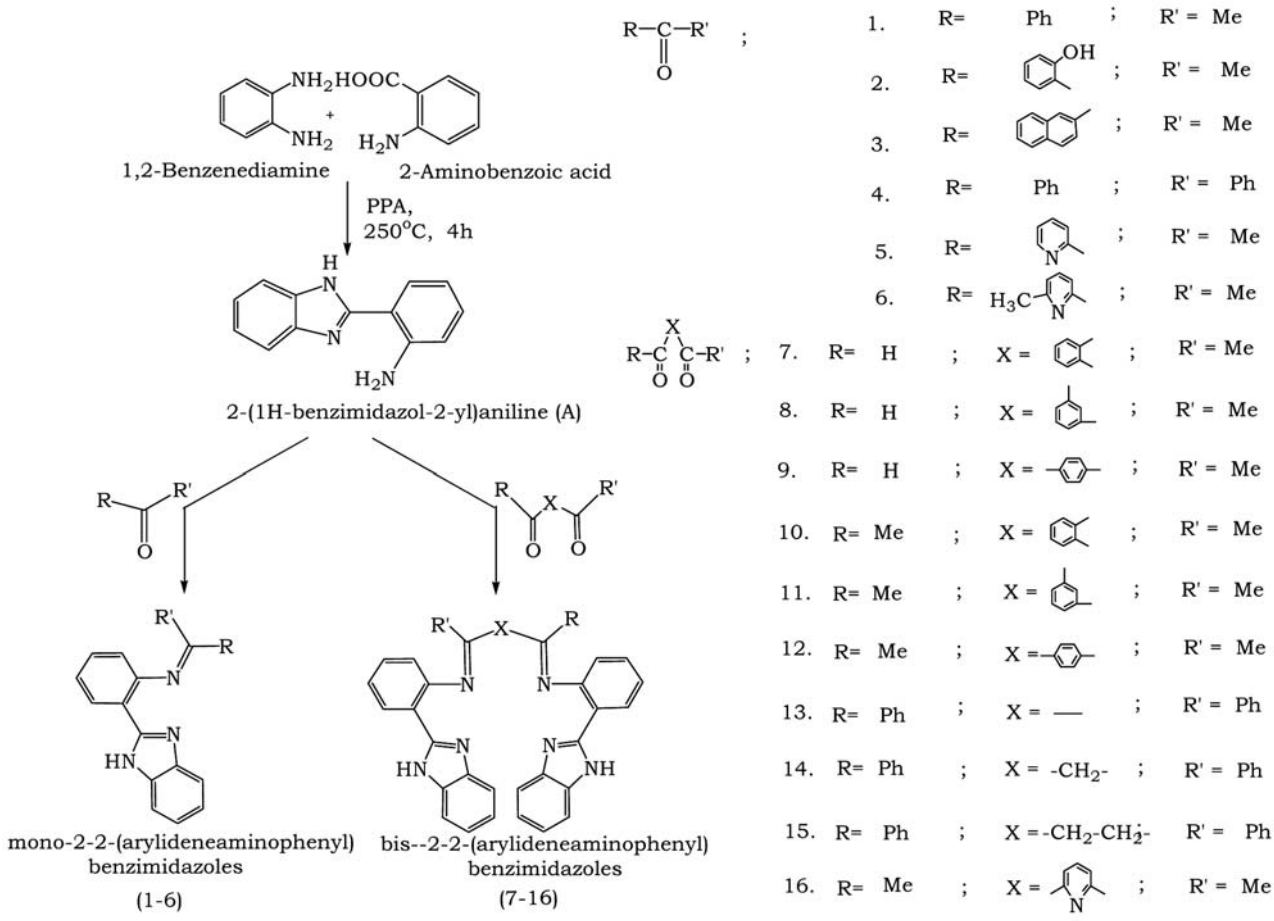
benzoic acid in freshly prepared polyphosphoric acid (PPA). The reaction was carried out according to the procedure described by Hein et al.[32] The target compounds **1–16** were synthesized by condensation of the amino group of 2-(1*H*-benzimidazol-2-yl)aniline; with various mono (R–CO–R^1) and dicarbonyl (R–CO–X–CO–R^1) compounds.

IR spectra of the compound **A** showed absorption band at 3350 cm^{-1} assigned to aminophenyl ring and in the case of 2-(1*H*-benzimidazol-2-yl)aniline (**A**) and 2,2-(arylidineaminophenyl)benzimidazoles **1–16**, two absorption bands in the region 3260–3300 cm^{-1} and 1420 cm^{-1} were assigned to NH stretching and bending vibrations of imidazolyl ring, respectively.[33,34] The absence of aminophenyl ring absorption at 3350 cm^{-1} and appearance of a strong intensity band in the IR spectra of compounds **1–16** in the range of 1615–1635cm^{-1} attributable to C=N vibrations and provides a strong evidence for the condensation and also confirms the formation of the azomethines.[35]

The ^{1}H NMR spectra of the compound **A** as well as its derivatives **1–16** have been recorded in $CDCl_3$ using TMS as internal standard. In the spectra of 2-(1*H*-benzimidazol-2-yl)aniline (**A**), signals at δ 6.4 and 8.25 ppm appeared corresponding to free amino and imidazolyl protons, respectively.[33,35] The aromatic protons of various environments present in all compounds appeared as multiplets in the range of 6.71–7.96 ppm.[35,36] However, in the spectra of target compounds the disappearance of signals at 6.4 which is due to NH_2 protons of compound **A** supports the involvement of amino group in the condensation and confirms the formation of azomethines**.**

^{13}C NMR spectra of all the compounds contain signals in the range of 156.3–169.2 ppm confirming the presence of carbon, which is doubly bonded to nitrogen. The aromatic carbons of various environments present in all the compounds appeared as signals in the range of 113.1–149.4 ppm.[36] The ^{1}H and ^{13}C NMR spectra of compound **1** are presented in Figures 1 and 2 respectively (see support information).

All the compounds showed a single peak in ESI–MS suggesting the purity of the azomethines. The FAB mass spectrum of compound **1** shows a parent peak at *m/z* 311 (M^+, 74%) corresponding to the molecular formula $C_{21}H_{17}N_3$. The mass spectrum shows major fragments at *m/z* values of 234 (100%), 233 (39%), 208 (35%), 207 (56%), 195 (85%), 193 (45%), 118 (65%), 117 (58%),



Scheme 1. Synthetic route of mono, bis-2,2-(arylidineaminophenyl)benzimidazoles

Table 1. Zone of inhibition of newly synthesized compounds against different bacteria.

Compound (1000 µg/mL)	Zone of inhibition (mm)					
	Gram-positive bacteria			Gram-negative bacteria		
	S. aureus	*B. subtilis*	*S. pyogenes*	*S. typhimurium*	*E. coli*	*K. pneumonia*
1	15	12	11	12	18	6
2	10	10	9	12	20	15
3	18	14	13	13	11	16
4	20	16	15	11	12	10
5	50	42	49	40	35	40
6	51	45	50	42	30	40
7	20	19	21	25	20	19
8	19	21	20	21	18	20
9	21	18	20	18	19	18
10	20	21	25	20	21	20
11	21	18	24	22	19	18
12	25	20	25	20	21	25
13	50	45	51	45	48	48
14	50	50	50	48	45	50
15	52	45	52	50	48	52
16	55	50	50	52	55	50
Ampicillin	48	39	35	45	40	45

Table 2. Zone of inhibition of newly synthesized compounds against different fungi.

Compound (1000 µg/mL)	Zone of inhibition (mm)		
	A. niger	*C. albicans*	*T. viridae*
1	**10**	**10**	**9**
2	16	13	13
3	13	11	6
4	9	10	12
5	35	36	40
6	40	35	38
7	20	18	19
8	19	21	20
9	18	16	18
10	21	20	19
11	25	15	21
12	21	20	25
13	50	42	45
14	50	46	48
15	55	50	50
16	52	52	51
Ketoconazole	45	40	41

104 (55%), 91 (25%), and 76 (16%). These indicate the fragmentation pattern and their intensities give an idea about the abundance and stability of the fragments.

3. Antimicrobial Activity

The *in vitro* antimicrobial activity was performed by cup-plate method.[37,38] All the synthesized benzimidazole derivatives were screened for antibacterial activity against *Staphylococcus aureus*, *Bacillus subtilis*, *Streptococcus pyogenes* (Gram positive) and *Salmonella typhimurium*, *Escherichia coli*, *Klebsiella pneumonia* (Gram negative) bacterial strains using Ampicillin as the standard. The antifungal activity was investigated against *Aspergillus niger*, *Candida albicans*, *Trichoderma viridae* fungal strains using Ketoconazole as the standard. Preliminary screening for sixteen benzimidazole derivatives was performed at fixed concentrations of 1000 µg/mL. Screening results are summarized in Tables 1 and 2. The antimicrobial screening revealed that all the tested compounds **1**–**6** and **7**–**16** showed moderate to good inhibition towards all tested strains. The maximum inhibition was observed for **5** and **6** against three Gram-positive bacterial strains (*S. aureus, S. pyogenes*). Furthermore, the most potent activity was observed in four bis-2,2-(arylidineaminophenyl)benzimidazole derivatives **13**–**16** against all bacterial and fungal strains when compared to respective standard drugs Ampicillin and Ketoconazole. The minimum inhibitory concentration[39] of these benzimidazole derivatives was also verified by the liquid dilution method in which the effectiveness was observed at lower concentrations.

3. 1. Determination of Minimum Inhibitory Concentration (MIC)

The minimum inhibitory concentration of the compounds **5**, **6** and **7**–**16** against *S. aureus*, *B. subtilis*, *S. pyogenes*, *S. typhimurium*, *E. coli*, *K. pneumonia* (bacterial strains), *A. niger*, *C. albicans*, *T. viridae* (fungal strains) were determined by liquid dilution method. Stock solutions of test samples with 2.5, 5, 10, 15, 20, 25, 30, 35, 40, 45 and 50 µg/mL concentrations were prepared with the appropriate solvent. The solutions of standard drugs, Ampicillin and Ketoconazole were prepared in the same concentrations. Inoculums of the bacterial and fungal culture

were also prepared. To a series of tubes each containing 1 mL of the benzimidazole derivative solution with different concentrations, 0.2 mL of the inoculum was added. Further 3.8 mL of the sterile water was added to each of the test tubes. These test tubes were incubated for 24 h and observed for the presence of turbidity. This method was repeated by changing the benzimidazole derivatives with the standard drug Ampicillin (in the case of bacteria) and with Ketoconazole (in the case of fungi) for comparison. The minimum inhibitory concentration at which no growth was observed was taken as the MIC value.

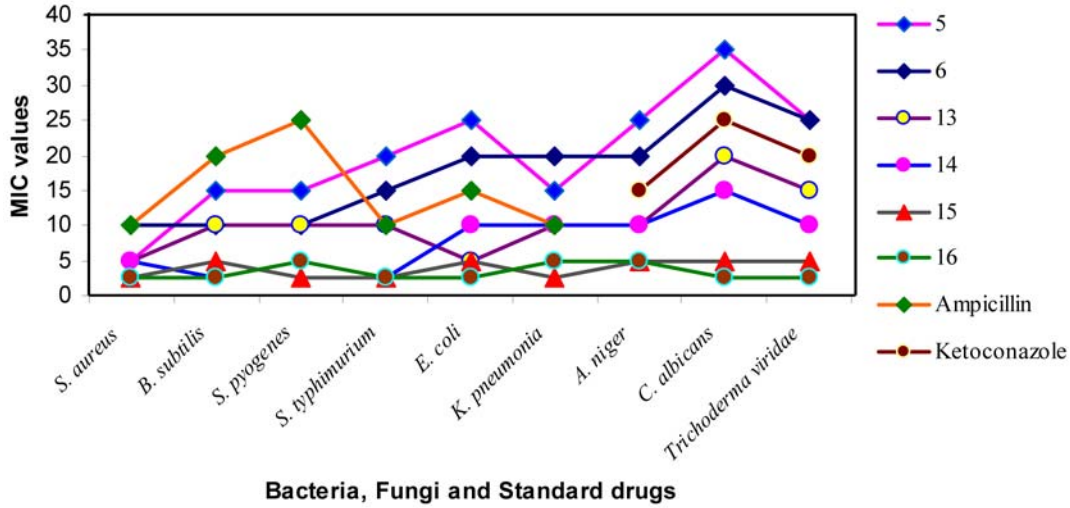


Fig. 1. Comparison of MIC values (in μg/mL) of mono, bis-2,2-(arylidineaminophenyl)benzimidazoles and standard drugs against different bacteria and fungi.

Comparison of MIC values (in μg/mL) of the effective benzimidazole derivatives and standard drugs against different bacteria and fungi is presented in Figure 1. From this study it is clear that compounds **5** and **6** are showing enhanced activity when compared to Ampicillin towards inhibiting three Gram-positve bacterial strains (*S. aureus*, *B. subtilis*, *S. pyogenes*). Furthermore, it has been found that four bis-2,2-(arylidineaminophenyl)benzimidazole derivatives **13**–**16** are antimicrobially active and show superior activity than standard drugs Ampicillin and Ketoconazole.

4. Experimental

Melting points were determined in open capillary tubes and are uncorrected. Analar grade reagents and freshly distilled solvents were used throughout the investigations. Purity of the compounds was checked by TLC using Merck 60 F254 silica gel plates. Micro analytical (C, N, H) data was obtained by using a Perkin–Elmer 2400 CHN elemental analyzer. The IR spectra were recorded in KBr pellets on Perkin–Elmer 283 spectrophotometer. ^{1}H-NMR spectra were acquired at 400 MHz, and ^{13}C-NMR at 67.93 MHz on a Bruker NMR spectrometer (Bruker Bioscience, USA). FAB mass spectra were recorded on a Finnigan-MAT 1020 instrument (Thermo Electron Corporation, Bremen, Germany). An ion trap mass spectrometer (Agilent Series LC/MSD Trap SL) equipped with an electrospray ionization (ESI) source was used for MS analyses (Agilent, Palo Alto, CA, USA). Hot air oven (Instrument and equipment Pvt. Ltd., Mumbai), incubator (Instrument and equipment Pvt. Ltd., Mumbai), laminar airflow unit (Clas laminar technologies Pvt. Ltd. Secunderabad), autoclave (Medica instrument Mfg. Co., Mumbai) were used. Organisms *S. aureus*, *B. subtilis*, *S. pyogenes* (Gram positive), *S. typhimurium*, *E. coli*, *K. pneumonia* (Gram negative) bacteria and *A. niger*, *C. albicans*, *T. viridae* fungi were used.

General procedure for the preparation of 2-(1*H*-benzimidazol-2-yl)aniline (A): 2-(1*H*-benzimidazol-2-yl)aniline (**A**) was prepared by reacting 1,2-benzenediamine (OPD) with 2-aminobenzoic acid in freshly prepared polyphosphoric acid (PPA) at 250 °C for 4 h.

Preparation of mono and bis-2,2-(arylidineaminophenyl)benzimidazoles 1–6 and 7–16: To a hot stirred solution of 2-(1*H*-benzimidazol-2-yl)aniline (**A**) (2 mmol) in ethanol (20 mL) the appropriate ketone **1**–**6** (2 mmol) and 2–3 drops of acetic acid were added. In the case of bis-2,2-(arylidineaminophenyl)benzimidazole derivatives **7**–**16** 4 mmol of **A** was taken. The resulting mixture was refluxed for 2 h. The product was filtered and recrystalized from the mixture of dichloromethane and methanol.

***N*-[2-(1*H*-benzo[d]imidazol-2-yl)phenyl]-*N*-[(*E*)-1-phenylethylidene]amine (1).**

Yield 79%; solid, mp 218–220 °C; IR (KBr) ν 3260, 2860, 1625, 1590, 1575, 1130 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 2.30 (s, 3H, CH_3), 6.83 (t, 1H, Ar, *J* = 7.4 Hz), 7.05–7.18 (m, 4H, Ar), 7.29–7.40 (m, 6H, Ar), 7.63 (d, 1H, Ar, *J* = 7.3 Hz), 7.95 (d, 1H, Ar, *J* = 7.3 Hz), 8.89 (s, 1H, imidazolyl NH); ^{13}C NMR (67.93 MHz, $CDCl_3$) δ 19.4, 114.2, 118.2, 121.4, 123.2, 125.2, 130.5, 131.2, 132.2, 136.5, 141.2, 142,6, 143.8, 146.3, 165.7 (21C, Ar-C); Anal. Found: C, 81.08; H, 5.57; N, 14.01. Calcd for $C_{21}H_{17}N_3$: C, 81.00; H, 5.50; N, 13.49. MS: *m/z* 311 $[M]^+$.

(*E*)-2-(1-(2-(1*H*-benzo[d]imidazol-2-yl)phenylimino)ethyl)phenol (2). Yield 81%; solid, mp 225–227 °C; IR (KBr) ν 3420, 3300, 2865, 1630, 1590, 1580, 1128 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 2.33 (s, 3H, CH_3), 6.84–7.18 (m, 6H, Ar), 7.25–7.38 (m, 4H, Ar), 7.63 (d, 1H, Ar, *J* = 7.3 Hz), 7.94 (d, 1H, Ar, *J* = 7.3 Hz), 8.91 (s, 1H, imidazolyl NH) 10.22 (s, 1H, OH); ^{13}CNMR (67.93 MHz, $CDCl_3$) δ 19.5, 114.2, 117.1, 118.4, 123.8, 126.2, 130.5, 132.2, 133.6, 136.1, 141.2, 142,6, 143.1, 145.4, 153.7, 167.5 (21C, Ar-C); Anal. Found: C, 77.65; H, 5.81; N, 12.85. Calcd for $C_{21}H_{17}N_3O$: C, 77.04; H, 5.23; N, 12.84. MS: *m/z* 327 $[M]^+$.

***N*-[2-(1*H*-benzo[d]imidazol-2-yl)phenyl]-*N*-[(*E*)-1-(1-naphthyl)ethylidene] amine (3).** Yield 75%; solid, mp 220–222 °C; IR (KBr) ν 3290, 2868, 1635, 1590, 1578, 1125 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 2.32 (s, 3H, CH_3), 6.83 (t, 1H, Ar, *J* = 7.4 Hz), 7.06–7.18 (m, 6H, Ar,), 7.22–7.63 (m, 7H, Ar), 7.95 (d, 1H, Ar, *J* = 7.3 Hz), 8.89 (s, 1H, imidazolyl NH); ^{13}C NMR (67.93 MHz, $CDCl_3$) δ 19.6, 114.1, 118.2, 123.1, 123.5, 126.2, 130.5, 130.8, 131.2, 132.5, 136.3, 141.1, 142,5, 143.8, 146.3, 166.7 (25C, Ar-C); Anal. Found: C, 83.26; H, 5.31; N, 12.01. Calcd for $C_{25}H_{19}N_3$: C, 83.08; H, 5.30; N, 11.63. MS: *m/z* 361 $[M]^+$.

***N*-[2-(1*H*-benzo[d]imidazol-2-yl)phenyl]-*N*-diphenylmethyleneamine (4).** Yield 79%; solid, mp 219–221 °C; IR (KBr) ν 3300, 2870, 1635, 1592, 1576, 1130 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 2.30 (s, 3H, CH_3), 6.83 (t, 1H, Ar, *J* = 7.4 Hz), 7.05–7.31 (m, 8H, Ar), 7.58–7.64 (m, 5H, Ar), 7.95 (d, 1H, Ar, *J* = 7.3 Hz), 8.89 (s, 1H, imidazolyl NH); ^{13}C NMR (67.93 MHz, $CDCl_3$) δ 19.4, 113.9, 117.2, 123.0, 124.1, 126.5, 130.5, 131.8, 132.2, 136.9, 141.0, 142,2, 145.1, 150.8, 165.7 (26C, Ar-C); Anal. Found: C, 83.68; H, 5.47; N, 11.31. Calcd for $C_{26}H_{19}N_3$: C, 83.62; H, 5.13; N, 11.25. MS: *m/z* 373 $[M]^+$.

***N*-[2-(1*H*-benzo[d]imidazol-2-yl)phenyl]-*N*-[(*E*)-1-(2-pyridyl)ethylidene] amine (5).** Yield 82%; solid, mp 225–227 °C; IR (KBr) ν 3295, 2865, 1630, 1610, 1580, 1100 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 2.30 (s, 3H, CH_3), 6.83–7.10 (m, 3H, Ar), 7.15–7.30 (m, 4H, Ar), 7.62–7.85 (m, 3H, Ar), 7.94 (d, 1H, Ar, *J* = 7.3 Hz), 8.72 (d, 1H, *J* = 5.25 Hz), 8.89 (s, 1H, imidazolyl NH); ^{13}C NMR (67.93 MHz, $CDCl_3$) δ 19.8, 113.4, 114.0, 119.4, 123.6, 125.1, 131.6, 137.2, 140.8, 142,5, 143.6, 147.2, 160.4, 167.2 (20C, Ar-C), Anal. Found: C, 76.92; H, 5.10; N, 17.95. Calcd for $C_{20}H_{16}N_4$: C, 76.90; H, 5.16; N, 17.94. MS: *m/z* 312 $[M]^+$.

***N*-[2-(1*H*-benzo[d]imidazol-2-yl)phenyl]-*N*-[(*E*)-1-(6-methyl-2-pyridyl)ethylidene]amine (6).** Yield 76%; solid, mp 231–233 °C; IR (KBr) ν 3275, 2860, 1635, 1610, 1582, 1115 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 2.28 (s, 3H, CH_3), 2.32 (s, 3H, CH_3), 6.71–7.18 (m, 5H, Ar), 7.29–7.30 (m, 2H, Ar); 7.62 (d, 1H, Ar, *J* = 7.3 Hz), 7.75–7.82 (m, 2H, Ar), 7.94 (d, 1H, Ar, *J* = 7.3 Hz), 8.89 (s, 1H, imidazolyl NH); ^{13}C NMR (67.93 MHz, $CDCl_3$) δ 20.1, 113.1, 114.5, 118.2, 123.5, 126.2, 130.5, 130.8, 131.2, 132.7, 136.5, 140.2, 142,8, 143.6, 146.3, 149.4, 161.4, 166.8 (21C, Ar-C), Anal. Found: C, 77.22; H, 5.57; N, 17.80. Calcd for $C_{21}H_{18}N_4$: C, 77.28; H, 5.56; N, 17.17. MS: *m/z* 326 $[M]^+$.

***N*-[2-(1*H*benzimidazol-2-yl)phenyl]-*N*-[(2-{*N*-[2-(1*H*-benzimidazol-2-yl)phen yl]ethanimidoyl}phenyl)methylidene]amine (7).** Yield 75%; solid, mp 248–250 °C; IR (KBr) ν 3300, 2865, 1630, 1605, 1585, 1120 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 2.26 (s, 3H, CH_3), 6.81–6.83 (m, 2H, Ar), 7.08–7.19 (m, 8H, Ar), 7.28–7.44 (m, 8H, Ar), 7.95 (d, 2H, Ar, *J* = 7.3 Hz), 8.31 (s, 1H, CH=N), 8.89 (s, 2H, NH); ^{13}C NMR (67.93 MHz, $CDCl_3$) δ 19.6, 113.5, 114.8, 118.2, 123.1, 125.2, 128.3, 130.5, 132.2, 133.5, 138.3, 140.1, 142,6, 144.2, 146.6, 158.2, 169.2 (35C, Ar-C); Anal. Found: C, 80.08; H, 5.02; N, 15.82. Calcd for $C_{35}H_{26}N_6$: C, 79.22; H, 4.94; N, 15.84. MS: *m/z* 530 $[M]^+$.

***N*-[2-(1*H*-benzimidazol-2-yl)phenyl]-*N*-[(3-{*N*-[2-(1*H*-benzimidazol-2-yl) phenyl]ethanimidoyl}phenyl)methylidene]amine (8).** Yield 72%; solid, mp 242–244 °C; IR (KBr) ν 3290, 2870, 1625, 1605, 1575, 1130 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 2.20 (s, 3H, CH_3), 6.82–6.85 (m, 4H, Ar), 7.05–7.18 (m, 8H, Ar), 7.35–7.42 (m, 2H, Ar), 7.62 (t, 2H, Ar, *J* = 6.4 Hz), 7.89–7.96 (m, 4H, Ar), 8.28 (s, 1H, CH=N), 8.89 (s, 2H, NH); ^{13}C NMR (67.93 MHz, $CDCl_3$) δ 20.1, 113.3, 114.4, 118.4, 122.1, 124.2, 126.4, 128.3, 130.9, 132.2, 133.5, 135.3, 141.1, 144.6, 146.5, 156.8, 168.9 (35C, Ar-C); Anal. Found: C, 80.02; H, 5.01; N, 15.95. Calcd for $C_{35}H_{26}N_6$: C, 79.22; H, 4.94; N, 15.84. MS: *m/z* 530 $[M]^+$.

***N*-((*E*)-4-((*E*)-1-(2-(1*H*-benzo[d]imidazol-2-yl)phenylimino)ethyl)benzylidene) -2-(1*H*-benzo[d]imidazol-2-yl) benzenamine (9).** Yield 75%; solid, mp 255–257°C; IR (KBr) ν 3298, 2865, 1621, 1600, 1580, 1130 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 2.25 (s, 3H, CH_3), 6.80–6.83 (m, 4H, Ar), 7.05–7.09 (m, 4H, Ar), 7.13–7.18 (m, 4H, Ar), 7.39–7.42 (m, 6H, Ar), 7.95 (d, 2H, Ar, *J* = 7.3 Hz), 8.23 (s, 1H, CH=N), 8.87 (s, 2H, NH); ^{13}C NMR (67.93

MHz, $CDCl_3$) δ 20.2, 113.6, 114.2, 119.1, 123.5, 125.2, 127.3, 128.3, 130.5, 131.2, 137.3, 141.1, 142,6, 144.2, 156.6, 168.3 (35C, Ar-C); Anal. Found: C, 80.01; H, 5.07; N, 15.84. Calcd for $C_{35}H_{26}N_6$: C, 79.22; H, 4.94; N, 15.84. MS: *m/z* 530 $[M]^+$.

(*E*)-*N*-(1-(2-((*Z*)-1-(2-(1*H*-benzo[d]imidazol-2-yl) phenylimino)ethyl)phenyl)ethylidene)-2-(1*H*-benzo[d] imidazol-2-yl)benzenamine (10). Yield 78%; solid, mp 250–252ºC; IR (KBr) ν 3295, 2865, 1630, 1605, 1585, 1125 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 2.26 (s, 6H, CH_3), 6.82–7.19 (m, 10H, Ar), 7.28–7.44 (m, 8H, Ar), 7.95 (d, 2H, Ar, *J* = 7.3 Hz), 8.90 (s, 2H, NH); ^{13}C NMR (67.93 MHz, $CDCl_3$) δ 20.6, 113.2, 114.7, 119.2, 123.1, 124.5, 125.2, 127.3, 128.3, 130.5, 131.2, 133.9, 136.3, 141.1, 142,4, 144.8, 146.6, 165.2, 169.2 (36C, Ar-C); Anal. Found: C, 79.48; H, 5.22; N, 15.80. Calcd for $C_{36}H_{28}N_6$: C, 79.39; H, 5.18; N, 15.43. MS: *m/z* 544 $[M]^+$.

(*E*)-*N*-(1-(3-((*Z*)-1-(2-(1*H*-benzo[d]imidazol-2-yl) phenylimino)ethyl)phenyl)ethylidene)-2-(1*H*-benzo[d] imidazol-2-yl)benzenamine (11). Yield 76%; solid, mp 249–251ºC; IR (KBr) ν 3300, 2875, 1625, 1608, 1575, 1128 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 2.22 (s, 6H, CH_3), 6.82–6.89 (m, 4H, Ar), 7.06–7.42 (m, 10H, Ar), 7.62–7.95 (m, 6H, Ar), 8.91 (s, 2H, NH); ^{13}C NMR (67.93 MHz, $CDCl_3$) δ 21.0, 113.3, 114.2, 122.1, 124.1, 126.7, 128.2, 130.9, 132.1, 133.9, 136.1, 140.2, 142,8, 145.5, 146.1, 165.8, 168.2 (36C, Ar-C); Anal. Found: C, 80.01; H, 5.21; N, 15.95. Calcd for $C_{36}H_{28}N_6$: C, 79.39; H, 5.18; N, 15.43. MS: *m/z* 544 $[M]^+$.

(*E*)-*N*-(1-(4-((*Z*)-1-(2-(1*H*-benzo[d]imidazol-2-yl) phenylimino)ethyl)phenyl)ethylidene)-2-(1*H*-benzo[d] imidazol-2-yl)benzenamine (12). Yield 82%; solid, mp 241–243 °C; IR (KBr) ν 3290, 2860, 1625, 1605, 1575, 1130 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 2.21 (s, 6H, CH_3), 6.80–6.84 (m, 4H, Ar), 7.06–7.16 (m, 6H, Ar), 7.20–7.42 (m, 8H, Ar), 7.95 (d, 2H, Ar, *J* = 7.3 Hz), 8.87 (s, 2H, NH); ^{13}C NMR (67.93 MHz, $CDCl_3$) δ 20.5, 113.5, 114.4, 123.5, 125.2, 127.3, 128.3,130.5, 131.2, 132.2, 137.3, 142,6, 144.2, 162.6, 168.3 (36C, Ar-C); Anal. Found: C, 79.48; H, 5.27; N, 15.80. Calcd for $C_{36}H_{28}N_6$: C, 79.39; H, 5.18; N, 15.43. MS: *m/z* 544 $[M]^+$.

2-(1*H*-benzimidazol-2-yl)-*N*-(2-{[2-(1*H*-benzimidazol-2-yl)phenyl]imino}-1,2-diphenylethylidene)aniline (13). Yield 76%; solid, mp 238–240 °C; IR (KBr) ν 3300, 2870, 1615, 1605, 1575, 1125 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 6.80–7.15 (m, 6H, Ar), 7.22–7.32 (m, 10H, Ar), 7.45–7.65 (m, 8H, Ar), 7.95 (d, 2H, Ar, *J* = 7.3 Hz), 8.90 (s, 2H, imidazolyl NH); ^{13}C NMR (67.93 MHz, $CDCl_3$) δ 114.1, 118.2, 123.1, 123.4, 126.2, 130.6, 130.9, 131.2, 132.8, 136.6, 140.2, 142,6, 143.9, 162.3, 165.7 (40C, Ar-C); Anal. Found: C, 81.05; H, 4.87; N, 14.25. Calcd for $C_{40}H_{28}N_6$: C, 81.06; H, 4.76; N, 14.18. MS: *m/z* 592 $[M]^+$.

(*E*)-*N*-((*Z*)-3-(2-(1*H*-benzo[d]imidazol-2-yl)phenylimino)-1,3-diphenylpropyl idene)-2-(1*H*-benzo[d]imidazol-2-yl)benzenamine (14). Yield 81%; solid, mp 228–230 °C; IR (KBr) ν 3285, 2885, 1620, 1608, 1585, 1120 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 2.35 (s, 2H, CH_2), 6.83 (t, 2H, Ar, *J* = 7.4 Hz), 7.09–7.15 (m, 4H, Ar), 7.25–7.32 (m, 10H, Ar), 7.38–7.79 (m, 8H, Ar), 7.95 (d, 2H, Ar, *J* = 7.3 Hz), 8.91 (s, 2H, imidazolyl NH); ^{13}C NMR (67.93 MHz, $CDCl_3$) δ 35.2, 113.6, 114.5, 118.7, 122.5, 125.2, 130.5, 132.2, 133.5, 136.5, 141.9, 142,2, 143.8, 160.3, 165.7 (41C, Ar-C); Anal. Found: C, 81.26; H, 5.02; N, 14.02. Calcd for $C_{41}H_{30}N_6$: C, 81.17; H, 4.98; N, 13.85. MS: *m/z* 606 $[M]^+$.

(*Z*)-*N*-((*E*)-4-(2-(1*H*-benzo[d]imidazol-2-yl)phenylimino)-1,4-diphenylbutylidene)-2-(1*H*-benzo[d]imidazol-2-yl)benzenamine (15). Yield 75%; solid, mp 215–217 °C; IR (KBr) ν 3289, 2868, 1632, 1610, 1580, 1126 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 2.30 (s, 4H, CH_2), 6.82 (t, 2H, Ar, *J* = 7.4 Hz), 7.06–7.12 (m, 5H, Ar), 7.22–7.35 (m, 9H, Ar), 7.35–7.64 (m, 8H, Ar), 7.94 (d, 2H, Ar, *J* = 7.3 Hz), 8.94 (s, 2H, imidazolyl NH); ^{13}C NMR (67.93 MHz, $CDCl_3$) δ 31.4, 113.8, 114.9, 118.2, 123.8, 124.1, 126.7, 130.4, 132.7, 136.1, 141.8, 142,6, 143.2, 156.3, 165.7 (42C, Ar-C); Anal. Found: C, 81.30; H, 5.22; N, 13.65. Calcd for $C_{42}H_{32}N_6$: C, 81.27; H, 5.20; N, 13.54. MS: *m/z* 620 $[M]^+$.

(*E*)-*N*-(1-(6-((*Z*)-1-(2-(1*H*-benzo[d]imidazol-2-yl) phenylimino)ethyl)pyridin-2-yl)ethylidene)-2-(1*H*-benzo[d]imidazol-2-yl)benzenamine (16). Yield 79%; solid, mp 244–246 °C; IR (KBr) ν 3295, 2862, 1635, 1608, 1585, 1125 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 2.19 (s, 6H, CH_3), 6.81–7.12 (m, 5H, Ar), 7.20–7.31 (m, 6H, Ar), 7.39–7.42 (m, 4H, Ar), 7.89–7.95 (m, 4H, Ar), 8.87 (s, 2H, NH); ^{13}CNMR (67.93 MHz, $CDCl_3$) δ 20.1, 113.3, 114.4, 121.6, 123.2, 124.6, 127.1, 128.4, 130.8, 132.6, 140.8, 142,6, 144.2, 146.6, 152.8, 164.2, 168.4 (35C, Ar-C); Anal. Found: C, 77.55; H, 5.02; N, 17.98. Calcd for $C_{35}H_{27}N_7$: C, 77.04; H, 4.99; N, 17.97. MS: *m/z* 545 $[M]^+$.

5. Conclusion

Benzimidazole derivatives have been widely used in the pharmaceutical industry and medicine because of their antimicrobial, antipyretic and anticancer properties. We have described the synthesis, characterization and antimicrobial studies of new mono, bis-2,2-(arylidineaminophenyl)benzimidazoles. We have chosen six bacterial and three fungal strains for microbial studies of these benzimidazole derivatives. From this study it is evident that compounds **5** and **6** are showing better activity when compared to Ampicillin towards inhibiting three gram positive bacterial strains. Furthermore, the most potent activity

was observed in some of the bis-2,2-(arylidineaminophenyl)benzimidazoles **13–16** against all tested strains when compared to respective standard drugs Ampicillin and Ketoconazole. A possible explanation for this result is that the antibacterial and antifungal activity of compounds may depend on the basic skeleton of molecule as well as on the nature of substituents such as pyridyl groups.

6. References

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Povzetek

Sintetizirali smo nekatere nove mono in bis-2-o-(arilidineaminofenil)benzimidazolne derivate in jih opredelili z IR, ^{1}H NMR, ^{13}C NMR, MS in elementno analizo. Vse produkte smo preverili na antibakterijsko aktivnost proti *Staphylococcus aureus*, *Bacillus subtilis*, *Streptococcus pyogenes, Salmonella typhimurium, Escherichia coli* in *Klebsiella pneumonia* in protiglivno aktivnost proti *Aspergillus niger, Candida albicans* in *Trichoderma viridae*. Spojine **5, 6** so izkazale učinkovito *in vitro* antibakterijsko aktivnost proti *S. aureus*, *B. subtilis*, *S. pyogenes*. Spojine **13-16** pa so pokazale najboljšo aktivnost proti vsem testiranim bakterijskim in glivnim sevom, boljšo kot standardna zdravila ampicilin in ketokonazol.