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Original article

Synthesis and evaluation of *in vitro* anti-microbial and anti-tubercular activity of 2-styryl benzimidazoles

Ramya V. Shingalapur, Kallappa M. Hosamani*, Rangappa S. Keri

P. G. Department of Studies in Chemistry, Karnataka University, Pavate Nagar, Dharwad 580 003, India

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ABSTRACT

A new series of novel 5-(nitro/bromo)-styryl-2-benzimidazoles (**1**–**12**) has been synthesized by simple, mild and efficient synthetic protocol by attempted condensation of 5-(nitro/bromo)-*o*-phenylenediamine with *trans*-cinnamic acids in ethylene glycol. Screening for *in vitro* anti-tubercular activity against *Mycobacterium tuberculosis* H_{37} Rv, anti-bacterial activity against *Staphylococcus aureus*, *Escherichia coli*, *Enterococcus faecalis*, *Klebsiella pneumoniae* bacterial strains and anti-fungal activity against *Candida albicans* and *Asperigillus fumigatus* fungal strains were carried out. Compounds **5**, **7**, **8**, **9**, **11** showed higher anti-tubercular activity and compounds **7**, **8**, **10**, **11**, **12** have proved to be effective with MIC (μg/ml) and emerged as lead molecules showing excellent activities against a panel of microorganisms. All synthesized compounds were characterized using IR, ^{1}H, ^{13}C NMR, GC–MS and elemental analysis.

1. Introduction

Tuberculosis (TB), a contagious infection caused by *Mycobacterium tuberculosis* (MTB), still remains the leading cause of the worldwide death among the infectious disease [1,2]. The WHO has estimated that every year about eight million new cases of tuberculosis occur, and up to three million individuals die due to this disease (one person dies every 10 s) [3]. It is also estimated that between 2002 and 2020, approximately a billion people will be newly infected, more than 150 million people will get sick, and 36 million will die of TB. This is also a leading cause of death amongst people who are HIV-positive (13% of AIDS deaths worldwide) [4]. The synergy between tuberculosis and the AIDS epidemic as well as the surge of multidrug-resistant isolates of *M. tuberculosis* has reaffirmed tuberculosis as a primary public health threat. The current threat in TB treatment lies in the emergence of strains resistant to two of the best anti-tubercular drugs, isoniazid (INH) and rifampicin (RIF). The current TB treatment comprises of 3–4 drugs for a period of 6–9 months. Novel drugs are urgently required which can shorten this long-treatment period and target multidrug-resistant strains of TB.

Infectious microbial disease remains a pressing problem worldwide, because microbes have resisted prophylaxis or therapy longer than any other form of life. In recent decades, problems of multidrug-resistant microorganisms have reached an alarming level in many countries around the world. Resistance to a number of anti-microbial agents (β-lactam antibiotics, macrolides, quinolones, and vancomycin) among variety of clinically significant species of bacteria is becoming increasingly important global problem. Also a number of recent clinical reports describe the increasing occurrence of Methicillin Resistant Staphylococcus Aureus (MRSA) which is most disturbing cause of nosocomial infections in developed countries [5,6]. Infections caused by these microorganisms pose a serious challenge to the medicinal community and the need for an effective therapy has led to search for novel anti-microbial agents. In particular, increasing drug resistance among gram-positive bacteria such as *Staphylococci*, *Enterococci* and *Streptococci* is a significant health matter [7]. This has stimulated the scientists for the development of novel molecules to combat these illnesses.

Benzimidazole and its derivatives are reported to be physiologically and pharmacologically active and find applications in the treatment of several diseases like epilepsy, diabetes, anti-fertility [8,9]. It is an important pharmacophore and privileged structure in medicinal chemistry [10,11] encompassing a diverse range of biological activities including anti-bacterial [12], anti-inflammatory, analgesic [13,14], anti-histamine [15], anti-ulcerative [16], anti-oxidant [17], anti-proliferative [18,19], anti-allergic [20,21], anti-tumour [22,23], anti-kinase [24,25], anti-cancer activities [26–30], cytotoxicity [31] and anti-HIV-1 [32,33]. Owing to the importance and in continuation of our ongoing project benzimidazole [34,35], now we wish to describe simple, novel and environmentally benign

* Corresponding author. Tel.: +91 836 2215286; fax: +91 836 2747884,2771275.
E-mail address: dr_hosamani@yahoo.com (K.M. Hosamani).

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approach towards the synthesis of 2-styryl benzimidazole derivatives and *in vitro* screening results of anti-microbial and anti-tubercular activities.

2. Chemistry

The general synthetic strategy employed to obtain the title compounds in excellent yield is depicted in Scheme 1. The cyclocondensation of 5-(nitro/bromo)-substituted-*o*-phenylenediamine with various cinnamic acids in ethylene glycol for 6 h at around 200 °C led to the formation of 5-(nitro/bromo) substituted-2-styryl benzimidazoles. This eco-friendly method for the preparation of styryl benzimidazoles is particularly attractive because it specifically generates crystalline products. All the newly synthesized compounds were characterized using IR, ^{1}H NMR, ^{13}C NMR, GC–MS and elemental analysis.

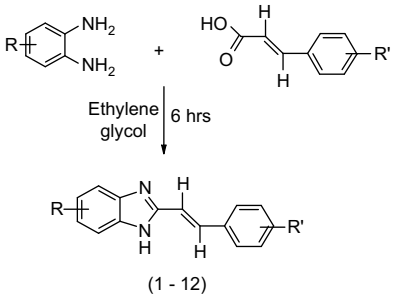
3. Bioevaluations

All the compounds prepared herein were screened for their potential biological activities such as anti-bacterial, anti-fungal and anti-tubercular activities. Anti-tubercular activity was carried out against *M. tuberculosis* H_{37} Rv (ATCC-27294) by Alamar Blue Assay. Bacterial strains *Staphylococcus aureus* [ATCC-25923] and *Enterococcus faecalis* [ATCC-29212] as gram positive, *Klebsiella pneumoniae* [ATCC-13883] and *Escherichia coli* [ATCC-25922] as gram negative and *Candida albicans* [ATCC-10145], *Asperigillus fumigatus* as fungal strains are used for the *in vitro* study. The *in vitro* anti-microbial activity of the compounds was tested by the tube dilution technique [36]. Each of the test compounds and standards ciprofloxacin and fluconazole were dissolved in DMSO initially at 250 μg/ml and then were serially diluted in culture medium as follows: 125, 62.5, 31.250, 16, 8, 4, 2, 1 μg/ml concentrations. The minimum inhibitory concentrations (MICs) were defined as the lowest concentrations of the compounds that prevented visible growth. It was determined that the solvent had no anti-microbial activity against any of the test microorganisms.

4. Results and discussion

4.1. Chemistry

The synthetic strategy involves the reaction of 5-(nitro/bromo)-substituted-*o*-phenylenediamine with variously substituted cinnamic acids. The cyclocondendensation of the reactants in ethylene glycol for 6 h at around 200 °C leads to the formation of 5-(nitro/bromo)-2-styryl benzimidazoles. Maintenance of a high temperature was very essential as it led to a gelatinous substance due to the reduced temperature. An appreciable yield of 75–82% is obtained. Postulated structures of the newly synthesized compounds are in agreement with their IR, ^{1}H NMR, ^{13}C NMR, GC–MS and elemental analysis data. In FT-IR of compound (**1**), a broad peak at 3421.3 cm^{-1} was due to –NH of the benzimidazole moiety. The ^{1}H NMR shows a broad peak at 12.2 ppm due to –NH proton which is also D_2O exchangeable. Sharp doublet was found for vicinal protons at 8.03 ppm with a coupling constant of 16.84 Hz. In ^{13}C NMR the peaks at 114.4, 117.8, 128.54, and 157.4 ppm confirm the formation of benzimidazole moiety. The mass spectrum of compound (**1**) shows the molecular ion peak at *m*/*z* 265 (70%) and [M + 1] peak at *m*/*z* 266 (6%).



R, a = -NO_2, b = -Br

R', a = H , b = 3,4 (OCH_3) , c = 4- CH_3 , d = 3,4- $(CH_2)O_2$, e = 2,4-(Cl) , f = 3 - OH

Scheme 1. Synthesis of 5-(nitro/bromo)-2-styryl benzimidazoles from α, β-unsaturated acids.

4.2. Anti-bacterial activity assay

MIC values for the *in vitro* anti-bacterial studies of the compounds (**1**–**12**) and the standard are represented in Table 1. The anti-bacterial activity of all the compounds against *S. aureus* and *E. faecalis* as Gram (+), *K. pneumoniae* and *E. coli* as Gram (−) bacteria showed good potencies compared to control drug ciprofloxacin. From the results it is apparent that among the synthesized compounds **1**, **7**, **8**, **9**, **11**, **12** showed MIC value of 16–1 μg/ml. Compound **12** showed excellent activity with an MIC of 1 μg/ml and 4 μg/ml against Gram (+) and Gram (−), respectively, which are comparable with ciprofloxacin. Compound **8** exhibited MIC of 2 μg/ml against Gram (+) and 8 μg/ml against *K. pneumoniae*. Compound **7** was also comparable with standard against *S. aureus* with an MIC of 2 μg/ml.

4.3. Anti-fungal activity assay

MIC values for the *in vitro* anti-fungal studies of the compounds (**1**–**12**) and the standard are represented in Table 1. Among test compounds, most interesting activities were found for compounds **7**, **8**, **9** and **12** against *C. albicans* and *A. fumigatus* compared to control fluconazole. Compound **8** was promising with MIC values of 1 and 2 μg/ml, respectively, against both the fungi. Anti-fungal activity indicated that some of the derivatives possessed a broad spectrum of activity against tested fungi; however, compound **8** showed a better spectrum of activity than the reference drug.

Table 1
Anti-microbial activity of synthesized compounds (**1**–**12**).

MIC (μg/ml)						
Comp.	*S. aureus* (25923)[a]	*E. faecalis* (29212)	*K. pneumonia* (13883)	*E. coli* (25922)	*C. albicans* (10145)[a]	*A. fumigatus*
1	8.0	31.250	4.0	31.250	16.125	16.125
2	31.250	31.250	16.125	31.250	16.125	31.250
3	4.0	16.125	16.125	62.5	31.250	31.250
4	31.250	31.250	62.5	31.250	16.125	16.125
5	31.250	31.250	62.5	62.5	16.125	31.250
6	4.0	31.250	31.250	31.250	31.250	62.5
7	2.0	16.125	31.250	16.125	8.0	8.0
8	2.0	2.0	8.0	31.250	1.0	2.0
9	31.250	31.250	8.0	16	4.0	8.0
10	8.0	31.250	16.125	31.250	31.250	31.250
11	16.125	8.0	2.0	31.250	31.250	16.125
12	1.0	1.0	4.0	4.0	4.0	8.0
Ciprofloxacin	0.78	0.70	0.19	0.19	–	–
Fluconazole	–	–	–	–	2.0	2.0

[a] ATCC number.

4.4. Anti-tubercular activity assay

MIC values for the *in vitro* anti-tubercular studies of the compounds (**1**–**12**) and the standard are represented in Table 2. The data of the anti-tubercular activity screening reveals that the compounds **7**, **8**, **9** and **11** showed good activity against *M. tuberculosis* strain, whereas compound **5** showed moderate activity. Encouraging activity was found for the bromo derivatives; however, the nitro derivatives did not show any considerable activity.

5. Conclusion

The preliminary *in vitro* anti-bacterial, anti-fungal and anti-tubercular screening results of novel 5-(nitro/bromo) substituted-2-styryl benzimidazole derivatives reported here have emerged as highly potent anti-bacterial, anti-fungal and anti-tubercular agents. The possible improvements in the activity can be further achieved by slight modifications in the substituents on the basic benzimidazole nucleus. The presence of the bromo group on the aromatic ring has highly increased the activity of the compounds compared to nitro substituent compounds. In the view of the above findings and to identify new candidates that may value in designing new, selective, less toxic anti-microbial and anti-tubercular agents who might serve as new templates, we report the development of potent therapeutics having MIC values $\geq$1 μg/ml. Our findings will now have a very good impact on chemists and biochemists for further investigations in this field of benzimidazoles selectively being bromo substituted.

6. Experimental protocols

6.1. Chemistry

Melting points of the synthesized compounds were determined in open capillaries and are uncorrected. Infrared spectra were recorded using KBr pellets on Nicolet 5700 FT-IR instrument. The ^{1}H NMR and ^{13}C NMR spectra were recorded on Brucker Avance-300 (300 MHz) model spectrophotometer in $CDCl_3$ and DMSO as solvent and TMSi as internal standard with ^{1}H resonant frequency of 300 MHz and ^{13}C resonant frequency of 75 MHz. D_2O exchange was applied to confirm the assignment of the signals of NH protons. The chemical shifts were measured in δ ppm downfield from internal standard TMSi at $\delta = 0$. The TLC was performed on alumina silica gel 60 F_{254} (Merck). The mobile phase was ethyl acetate and *n*-hexane (1:1) and detection was made using UV light and iodine. The resulting compounds were purified by column chromatography. For column chromatography Merck silica gel (0.040–0.063 mm) was used. All the compounds gave C, H and N analysis within ±0.5% of the theoretical values.

Table 2
Anti-tubercular activity of synthesized compounds (**1**–**12**).

Comp	R	R^1	MIC (μg/ml)
1	$-NO_2$	H	>7.25
2	$-NO_2$	3,4-OCH_3	>7.25
3	$-NO_2$	4-CH_3	>7.25
4	$-NO_2$	3,4-O_2CH_2	>7.25
5	**$-NO_2$**	**2,4-Cl**	**>7.25 (45)**
6	$-NO_2$	3-OH	>7.25
7	**–Br**	**–H**	**>7.25 (83)**
8	**–Br**	**3,4-OCH_3**	**>7.25 (54)**
9	**–Br**	**4-CH_3**	**>7.25 (63)**
10	–Br	3,4-O_2CH_2	>7.25
11	**–Br**	**2,4-Cl**	**>7.25 (76)**
12	–Br	3-OH	>7.25

Standard: streptomycin (100% inhibition).
All compounds tested at concentration of 7.25 μg/ml.
The active compounds are marked in bold letters.

6.1.1. General method for the synthesis of 5-(nitro/bromo)-2-styryl benzimidazole

A mixture of 5-substituted-*o*-phenylenediamine (0.01 mol) and cinnamic acid (0.01 mol) in ethylene glycol was stirred for 1 h and then the mixture was refluxed at a temperature of 200 °C for nearly 6 h. The completion of the reaction was monitored through TLC technique. After cooling, the reaction mixture was poured in crushed ice with stirring. The product separated out was filtered, washed with water and dried to obtain 5-(nitro/bromo)-2-styryl-benzimidazole. The compound obtained was chromatographed on silica gel column using $CHCl_3$. The remaining compounds are synthesized in the similar manner.

*6.1.1.1. 5-Nitro-2-styryl-1H-benzimidazole (**1**).* Brown crystals, yield: 79.21%; m.p. 217–219 °C; IR (KBr): υ_{max} in cm^{-1}: 3421.3 (NH), 2921.7 (=C–H), 1629.6 (C=N), 1592.2 (C=C), {1339.8 (Sym), 1518.4 (Asymm)}(NO_2); ^{1}H NMR (DMSO-d_6, 300 MHz) δ ppm: 7.5–8.3 (m, 5H, Ar–H), 8.03 (d, 2H, Vinylic, $^3J_{H-H} = 16.84$ Hz), 12.2 (b, 1H, NH-benzimidazole); ^{13}C NMR (DMSO-d_6, 75 MHz) δ ppm: 39.7, 39.4, 111.9, 114.4, 117.8, 126.28, 128.54, 142.87, 157.4 (6 aryl carbons, 6 phenyl carbons, 2 vinylic carbons and 1 imidazole quaternary carbon); MS (EI) *m*/*z* (%) 170 (100), 265 (M^+, 70), 266 (M + 1, 6), 188 (55), 163 (10), 143 (19), 110 (25), 108 (30), 77 (5), 46 (15); Anal. Calcd. for $C_{15}H_{22}N_3O_2$: C, 67.92; H, 4.18; N, 15.84%. Found: C, 67.81; H, 4.09; N, 15.69%.

*6.1.1.2. 2-[2-(3,4-Dimethoxy-phenyl)-vinyl]-5-nitro-1H-benzimidazole (**2**).* Brown crystals, yield: 82.32%; m.p. 184–186 °C; IR (KBr): υ_{max} in cm^{-1}: 3281.1 (NH), 3000.3 (=C–H), 1712.5 (C=N),1628 (C=C), 1340.2, 1512.8 (NO_2); ^{1}H NMR ($CDCl_3$, 300 MHz) δ ppm: 6.4–8.4 (m, 3 aryl, 3 phenyl, 2 vinylic protons, $^3J_{H-H} = 16.86$ Hz), 2.71 (s, 6H, 2-OCH_3), 10.9 (b, 1H, NH-benzimidazole); ^{13}C NMR ($CDCl_3$, 75 MHz) δ ppm: 21.98, 24.11, 40.92, 108.4, 120.8, 121.85, 152.82, 154.8 (6 aryl carbons, 6 phenyl carbons, 2 vinylic carbons, 1 imidazole quaternary carbon and 2 methoxy carbons); MS (EI) *m*/*z* (%) 174 (100), 325 (M^+, 79), 326 (M + 1, 10), 188 (60), 163 (10), 138 (8), 143 (10), 77 (15), 28 (2); Anal. Calcd. for $C_{18}H_{19}N_3O_4$: C, 63.33; H, 5.61; N, 12.31%. Found: C, 63.35; H, 5.45; N, 12.40%.

*6.1.1.3. 5-Nitro-2-(2-p-tolyl-vinyl)-1H-benzimidazole (**3**).* Light brown crystals, yield: 76.25%; m.p. 192–194 °C; IR (KBr): υ_{max} in cm^{-1}: 3561.7 (NH), 2994.7 (=C–H), 1679.5 (C=N), 1626.6 (C=C), 1515.4, 1338.8 (NO_2); ^{1}H NMR ($CDCl_3$, 300 MHz) δ ppm: 6.9–8.2 (m, 3 aryl, 4 phenyl, 2 vinylic protons, $^3J_{H-H} = 16.25$ Hz), 3.4 (s, 3H, CH_3), 10.5 (b, 1H, NH-benzimidazole); ^{13}C NMR ($CDCl_3$, 75 MHz) δ ppm: 24.15, 55.8, 110.25, 112.8, 119.5, 154.59, 150.20 (1 methane carbon, 6 aryl carbons, 6 phenyl carbons, 2 vinylic carbons and 1 imidazole quaternary carbon); MS (EI) *m*/*z* (%) 165 (100), 279 (M^+, 77), 280 (M + 1, 10), 188 (59), 118 (20), 110 (18), 92 (55), 77(12), 28 (5); Anal. Calcd. for $C_{16}H_{13}N_3O_2$: C, 68.81; H, 4.69; N, 15.05%. Found: C, 68.85; H, 4.55; N, 15.00%.

*6.1.1.4. 2-(2-Benzo [1, 3] dioxol-5-yl-vinyl)-5-nitro-1H-benzimidazole (**4**).* Light brown crystals, yield: 75.32%; m.p. 214–216 °C; IR (KBr): υ_{max} in cm^{-1}: 3411.3 (NH), 2961.0 (=C–H), 1679.3 (C=N), 1618.9 (C=C), 1397.9, 1542.4 (NO_2); ^{1}H NMR ($CDCl_3$, 300 MHz) δ ppm: 6.7–7.8 (m, 3 aryl, 3 phenyl, 2 vinylic protons, $^3J_{H-H} = 16.41$ Hz), 2.6 (s, 2H, CH_2), 11.1 (b, 1H, NH-benzimidazole); ^{13}C NMR ($CDCl_3$, 75 MHz) δ ppm: 15.29, 115.85, 116.02, 117.91, 126.02, 137.53, 140.5, 152.26 (1 dioxy methylene carbon, 6 aryl carbons, 6 phenyl carbons, 2 vinylic carbons and 1 imidazole quaternary carbon); MS (EI) *m*/*z* (%) 169 (100), 309 (M^+, 77), 310 (M + 1, 7), 187 (52), 163 (11), 147 (28), 122

(42), 77 (8), 28 (5); Anal. Calcd. for $C_{18}H_{17}N_3O_4$: C, 63.71; H, 5.05; N, 12.38%. Found: C, 64.00; H, 5.10; N, 12.22%.

*6.1.1.5. 2-[2-(2, 4-Dichloro-phenyl)-vinyl]-5-nitro-1H-benzimidazole (**5**).* Brown crystals, yield: 70.12%; m.p. 212–214 °C; IR (KBr): υ_{max} in cm^{-1}: 3430.4 (NH), 2922.7 (=C–H), 1631.8 (C=N), 1590.6 (C=C), 1340.2, 1545.0 (NO_2); ^1H NMR (DMSO-d_6, 300 MHz) δ ppm: 7.2–8.5 (m, 3 aryl, 3 phenyl, 2 vinylic protons, $^3J_{H-H} = 16.28$ Hz), 11.9 (b, 1H, NH-benzimidazole); ^{13}C NMR (DMSO-d_6, 75 MHz) δ ppm: 30.42, 51.82, 55.4, 111.4, 118.25, 119.7, 121.2, 152.71 (6 aryl carbons, 6 phenyl carbons, 2 vinylic carbons and 1 imidazole quaternary carbon); MS (EI) *m/z* (%) 174 (100), 333 (M^+, 85), 334 (M + 1, 2), 335 (M + 2, 4), 190 (21), 174 (16), 164 (9), 145 (28), 30 (7); Anal. Calcd. for $C_{16}H_{15}N_3O_3$: C, 53.92; H, 2.71; N, 12.58%. Found: C, 53.95; H, 2.80; N, 12.57%.

*6.1.1.6. 3-[2-(5-Nitro-1H-benzimidazole-2-yl)-vinyl]-phenol (**6**).* Brown crystals, yield: 82.24%; m.p. 228–230 °C; IR (KBr): υ_{max} in cm^{-1}: 3515.90 (OH), 3464.2 (NH), 2923.0 (=C–H), 1674.3 (C=N), 1628.1 (C=C), 1339.4, 1517.4 (NO_2); ^1H NMR (DMSO-d_6, 300 MHz) δ ppm: 7.18 - 8.54 (m, 3 aryl, 4 phenyl, 2 vinylic protons, $^3J_{H-H} = 16.88$ Hz), 12.8 (s, 1H, OH) 11.85 (b, 1H, NH-benzimidazole); ^{13}C NMR (DMSO-d_6, 75 MHz) δ ppm: 50.6, 110.27, 118.21, 120.85, 121.7, 151.94 (6 aryl carbons, 6 phenyl carbons, 2 vinylic carbons and 1 imidazole quaternary carbon); MS (EI) *m/z* (%) 174 (100), 281 (M^+, 82), 282 (M + 1, 7), 185 (26), 120 (32), 95 (26), 77 (17), 29 (15); Anal. Calcd. for $C_{16}H_{15}N_3O_3$: C, 64.64; H, 5.09; N, 14.13%. Found: C, 63.52; H, 5.10; N, 14.25%.

*6.1.1.7. 5-Bromo-2-styryl-1H-benzimidazole (**7**).* Pale yellow crystals, yield: 82.12%; m.p. 198–200 °C; IR (KBr): υ_{max} in cm^{-1}: 3430.0 (NH), 2924.5 (=C–H), 1680.9 (C=N), 1620.3 (C=C); ^1H NMR ($CDCl_3$, 300 MHz) δ ppm: 7.32–7.42 (m, 3 aryl, 5 phenyl, 2 vinylic protons, $^3J_{H-H} = 16.82$ Hz), 12.18 (b, 1H, NH-benzimidazole); ^{13}C NMR ($CDCl_3$, 75 MHz) δ ppm: 76.99, 115.72, 125.90, 128.62, 143.8, 152.7 (6 aryl carbons, 6 phenyl carbons, 1 imidazole quaternary carbon and 2 vinylic carbons); MS (EI) *m/z* (%) 155 (100), 298 (M^+, 83), 299 (M + 1, 11), 300 (M + 2, 2), 220 (41), 195 (28), 142 (19), 104 (22), 76 (25); Anal. Calcd. for $C_{15}H_{11}BrN_3$: C, 60.22; H, 3.71; N, 9.36%. Found: C, 60.00; H, 3.85; N, 9.25%.

*6.1.1.8. 5-Bromo-2-[2-(3, 4-dimethoxy-phenyl)-vinyl]-1H-benzimidazole (**8**).* Yellow crystals, yield: 79.35%; m.p. 87–89 °C; IR (KBr): υ_{max} in cm^{-1}: 3406.4 (NH), 2925.1 (=C–H), 1627.7 (C=N), 1544.1 (C=C); ^1H NMR ($CDCl_3$, 300 MHz) δ ppm: 6.7–8.4 (m, 3 aryl, 3 phenyl, 2 vinylic protons, $^3J_{H-H} = 16.85$ Hz), 2.69 (s, 6H, 2-OCH_3), 10.82 (b, 1H, NH-benzimidazole); ^{13}C NMR (CDCl3, 75 MHz) δ ppm: 15.58, 41.85, 110.84, 120.2, 121.5, 153.11, 154.01 (2 methoxy carbons, 6 aryl carbons, 6 phenyl carbons, 2 vinylic carbons and 1 imidazole quaternary carbon); MS (EI) *m/z* (%) 128 (100), 358 (M^+, 75), 359 (M + 1, 4), 360 (M + 2, 6), 220 (40), 195 (20), 163 (28), 139 (22), 45 (12); Anal. Calcd. for $C_{18}H_{19}BrN_2O_2$: C, 57.61; H, 5.10; N, 7.47%. Found: C, 57.60; H, 5.55; N, 7.50%.

*6.1.1.9. 5-Bromo-2-(2-p-tolyl-vinyl)-1H-benzimidazole (**9**).* Pale yellow crystals, yield: 71.32%; m.p. 184–186 °C; IR (KBr): υ_{max} in cm^{-1}: 3430.0 (NH), 2924.9 (=C–H), 1686.1 (C=N), 1621.4 (C=C); ^1H NMR (DMSO-d_6, 300 MHz) δ ppm: 6.2–7.6 (m, 3 aryl, 4 phenyl, 2 vinylic protons, $^3J_{H-H} = 16.20$ Hz), 3.42 (s, 3H, CH_3), 10.28 (b, 1H, NH-benzimidazole); ^{13}C NMR (DMSO-d_6, 75 MHz) δ ppm: 15.27, 55.85, 110.92, 111.99, 120.74, 129.45, 147.28, 154.04 (1 methane carbon, 6 aryl carbons, 6 phenyl carbons, 2 vinylic carbons and 1 imidazole quaternary carbon); MS (EI) *m/z* (%) 174 (100), 313 (M^+, 90), 314 (M + 1, 11), 315 (M + 2, 2), 225 (51), 194 (21), 118 (45), 104 (16), 92 (54), 20 (4); Anal. Calcd. for $C_{16}H_{13}BrN_2$: C, 61.36; H, 4.18; N, 8.94%. Found: C, 61.25; H, 4.52; N, 8.85%.

*6.1.1.10. 2-(2-Benzo [1, 3] dioxol-5-yl-vinyl)-5bromo-1H-benzimidazole (**10**).* Light brown crystals, yield: 81.32%; m.p. 210–212 °C; IR (KBr): υ_{max} in cm^{-1}: 3424.2 (NH), 2924.2 (=C–H), 1664.8 (C=N), 1627.5 (C=C); ^1H NMR ($CDCl_3$, 300 MHz) δ ppm: 6.45–7.55 (m, 4 aryl, 3 phenyl, 2 vinylic protons, $^3J_{H-H} = 16.35$ Hz), 2.52 (s, 2H, CH_2), 11.02 (b, 1H, NH-benzimidazole); ^{13}C NMR (CDCl3, 75 MHz) δ ppm: 15.05, 115.4, 115.9, 117.00, 125.08, 128.24, 140.85, 152.09, 152.7 (1 dioxy methylene carbon, 6 aryl carbons, 6 phenyl carbons, 2 vinylic carbons and 1 imidazole quaternary carbon); MS (EI) m/z (%) 158 (100), 342 (M^+, 77), 343 (M + 1, 12), 344 (M + 2, 5), 222 (45), 147 (26), 124 (32), 110 (20); Anal. Calcd. for $C_{16}H_{11}BrN_2O_2$: C, 56.00; H, 3.23; N, 8.16%. Found: C, 55.99; H, 3.55; N, 8.25%.

*6.1.1.11. 2-[2-(2, 4-Dichloro-phenyl)-vinyl]-5-bromo-1H-benzimidazole (**11**).* Pale yellow crystals, yield: 72.23%; m.p. 204–206 °C; IR (KBr): υ_{max} in cm^{-1}: 3479.9 (NH), 2924.3 (=C–H), 1638.2 (C=N), 1590.6 (C=C); ^1H NMR (DMSO-d_6, 300 MHz) δ ppm: 7.00–7.6 (m, 3 aryl, 3 phenyl, 2 vinylic protons, $^3J_{H-H} = 16.19$ Hz), 11.9 (b, 1H, NH-benzimidazole); ^{13}C NMR (DMSO-d_6, 75 MHz) δ ppm: 51.55, 55.20, 111.89, 115.28, 118.24, 119.00 (6 aryl carbons, 6 phenyl carbons, 2 vinylic carbons and 1 imidazole quaternary carbon); MS (EI) *m/z* (%) 154 (100), 265 (M^+, 82), 266 (M + 1, 9), 267 (M + 2, 5), 192 (31), 169 (24), 148 (62), 118 (20), 104 (22), 77 (15); Anal. Calcd. for $C_{15}H_9BrCl_2N_2$: C, 48.95; H, 2.46; N, 7.61%. Found: C, 48.55; H, 2.37; N, 7.55%.

*6.1.1.12. 3-[2-(5-Bromo-1H-benzimidazole-2-yl)-vinyl]-phenol (**12**).* Light brown crystals, yield: 75.32%; m.p. 99–101 °C; IR (KBr): υ_{max} in cm^{-1}: 3510.40 (OH), 3423.03 (NH), 2925.3 (=C–H), 1635.6 (C=N), 1581.0 (C=C); ^1H NMR (DMSO-d_6, 300 MHz) δ ppm: 6.5–7.85 (m, 3 aryl, 4 phenyl, 2 vinylic protons, $^3J_{H-H} = 16.81$ Hz), 12.43 (b, 1H, OH), 10.40 (b, 1H, NH-benzimidazole); ^{13}C NMR (DMSO-d_6, 75 MHz) δ ppm: 39.7, 56.90, 113.9, 114.14, 115.32, 115.9, 118.17, 122.09, 124.71, 153.70, 158.58 (6 aryl carbons, 6 phenyl carbons, 2 vinylic carbons and 1 imidazole quaternary carbon); MS (EI) *m/z* (%) 128 (100), 315 (M^+, 90), 396 (M + 1, 9), 397 (M + 2, 4), 220 (14), 192 (18), 95 (27), 77 (12), 29 (14), 20 (5); Anal. Calcd. for $C_{15}H_{11}BrN_2O$: C, 57.16; H, 3.52; N, 8.89%. Found: C, 57.25; H, 3.47; N, 8.95%.

6.2. Bioassays

6.2.1. Anti-bacterial assay

The cultures were obtained in Mueller–Hinton Broth (Difco) for all the bacteria after 18–24 hrs of incubation at 37 ± 1 °C. Testing was carried out in Mueller–Hinton Broth at pH 7.4 and twofold dilution technique was applied. A set of tubes containing only inoculated broth was kept as controls. After incubation for 18–24 h at 37 ± 1 °C, the last tube with no growth of microorganism was recorded to represent MIC expressed in μg/ml. Ciprofloxacin was used as standard drug.

6.2.2. Anti-fungal assay

The yeasts were maintained in Sabouraud Dextrose Broth (Difco) after incubation for 48 h at 25 ± 1 °C. Testing was performed in Sabouraud Dextrose Broth at pH 7.4 and the twofold dilution technique was applied. A set of tubes containing only inoculated broth was kept as controls. After incubation for 48 h at 25 ± 1 °C, the last tube with no growth of yeast was recorded to represent MIC expressed in μg/ml. Fluconazole was used as standard drug.

6.2.3. Anti-tubercular assay

Primary screening was conducted at 7.25 μg/ml against *M. tuberculosis* $H_{37}Rv$ (ATCC-27294) in BACTEC 12B [37,38] medium using a broth micro dilution assay [39,40] the microplate Alamar blue assay (MABA) [41]. Compounds exhibiting <90% inhibition in the primary screen were not evaluated further. Compounds

demonstrating at least 90% inhibition were tested at lower concentrations by serial dilution against *M. tuberculosis* H_{37} Rv to determine the minimum inhibitory concentration (MIC) using MABA. Streptomycin was used as a reference drug.

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