

SYNTHESIS AND ANTIMICROBIAL ACTIVITY OF SOME NEWER BENZIMIDAZOLE DERIVATIVES

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ABSTRACT

Objective: Benzimidazoles are an important group of heterocyclic compounds which are biologically active. The main objective to synthesized a new series of *N*-1 as well as 2-substituted benzimidazoles *N*-{(2-Methyl/Mercapto-1*H*-benzo[*d*]imidazol-1-yl)methyl} substituted benzenamine (**1a-1c**) & (**3a-3c**), *N*-{(2-Methyl/Mercapto-1*H*-benzo[*d*]imidazol-1-yl)(substituted phenyl)methyl} substituted benzenamine (**2a-2d**) & (**4a-4d**) were synthesized.

Methods: All the newly synthesized compounds have been characterized on the basis of analytical and spectral data. The compounds were evaluated for *in-vitro* antibacterial and antifungal activity using disc diffusion methods on nutrient agar medium.

Results: All the synthesized compounds were confirmed by physical parameters (Solubility, melting point), chromatographic methods (TLC) and spectroscopic methods (IR, ¹H NMR, Mass spectroscopy and elemental analysis). The newly synthesized compounds were screened for antibacterial and antifungal activity by disc diffusion methods on nutrient agar medium. The results revealed that newly synthesized compounds **2a**, **2c**, **4a** and **4c** were found to be potent against *E. coli*; **1a**, **2b**, **3a** and **4b** against *B. subtilis*; **1b** and **3b** against *P. aeruginosa*; **1c**, **2d**, **3c** and **4d** against *S. aureus* and antifungal activity **2b**, **2d**, **4b** and **4d** against *C. albicans* while **1a**, **1c**, **3a** and **3c** were found to be potent against *A. niger*.

Conclusion: Modifications on benzimidazole moiety displayed valuable biological activities. Substituted benzimidazole exhibited significant to moderate antibacterial and antifungal activities.

KEYWORDS: Benzimidazole, Antibacterial activity, Antifungal activity, Disc diffusion method.

INTRODUCTION

Benzimidazole is a bicyclic ring system in which benzene ring fused with 4- and 5- position of the imidazole ring, imidazole ring contain two nitrogen atoms at nonadjacent position. Benzimidazole is a very important pharmacophore in drug discovery, and its derivatives are used as an important class of bioactive molecules in the field of new drug development.^[1] Benzimidazole nucleus has capability to inhibit the growth of various bacteria, yeast, fungi, protozoa and helminthes.^[2] Benzimidazole are the versatile pharmacophore having various biological activities like antibacterial^[3], antifungal^[4], Anthelmintic^[5], antiprotozoal^[6], anticoagulant^[7], analgesic, anti-inflammatory^[8], anticancer^[9], anti-HIV^[10], antiulcer^[11], antiviral^[12], antihistaminic^[13], antioxidant^[14], anticonvulsant^[15], hypolipidemic activities.^[16]

There are various methods for synthesis of these nitrogen containing compounds. My new research work study various *N*-1 as well as 2-substituted benzimidazole derivatives were synthesised and were evaluated for antimicrobial activity. The synthesized compounds were characterized by IR, ¹H NMR, Mass spectroscopy and Elemental analysis.

MATERIALS AND METHODS

Melting points were determined in open capillary tube and are uncorrected. Thin layer chromatography was performed to monitor the reactions and to determine the purity of the products on silica gel G (E. Merck) plates using 10% methanol in chloroform as a mobile phase. IR spectra were recorded on Perkin-Elmer FTIR-8400S spectrometer (SHIMADZU, Japan) by KBr pellet technique. ¹H NMR spectra were recorded on Bruker DRX-300 (300 MHz) spectrophotometer in DMSO-d₆ using TMS as internal standard (Chemical shift in δ ppm). Mass spectrum was obtained using ESI-MS (Schimadzu-2010AT) under Electro Spray Ionization (ESI) technique. Microanalyses for C, H, N were performed in Carlo Erba EA 1108 elemental analyzer. All the chemicals used were procured from Qualigens[®] Fine chemicals, Mumbai and Central Drug House (P.) Ltd., New Delhi.

Procedure for synthesis of 2-Methyl-1*H*-benzo[d]imidazole

o-Phenylenediamine dihydrochloride (10 mmol), water 5 ml and acetic acid (30 mmol) were added to the flask and the reaction mixture was refluxed for 1 hr. The flask was then removed, cooled at room temperature and conc. ammonia solution was added slowly with constant stirring until the reaction mixture become alkaline. The product was precipitated out, washed with ice cold water, filtered, dried and recrystallized from aq. Ethanol.

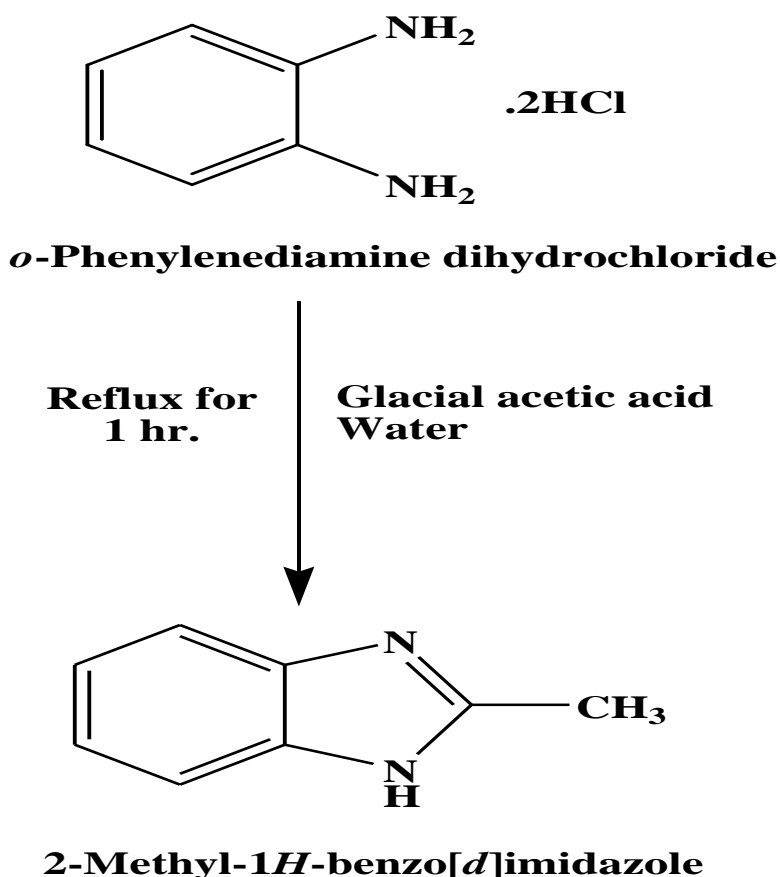
Procedure for Synthesis of *N*-{(2-Methyl/Mercapto-1*H*-benzo[*d*]imidazol-1-yl)methyl}substituted benzenamine (1a-1c) & (3a-3c), *N*-{(2-Methyl/Mercapto-1*H*-benzo[*d*]imidazol-1-yl)(substituted phenyl)methyl}substituted benzenamine (2a-2d) & (4a-4d)

Eqimolar quantities of compound 2-Methyl/Mercapto benzimidazole (10 mmol), Substituted aryl amine (10 mmol) and formaldehyde/Substituted benzaldehyde (10 mmol) were taken in 15 ml. of ethanol and refluxed for 10-24 hrs. On cooling, the product formed was filtered, dried and purified by recrystallization with 40% aq. Ethanol.

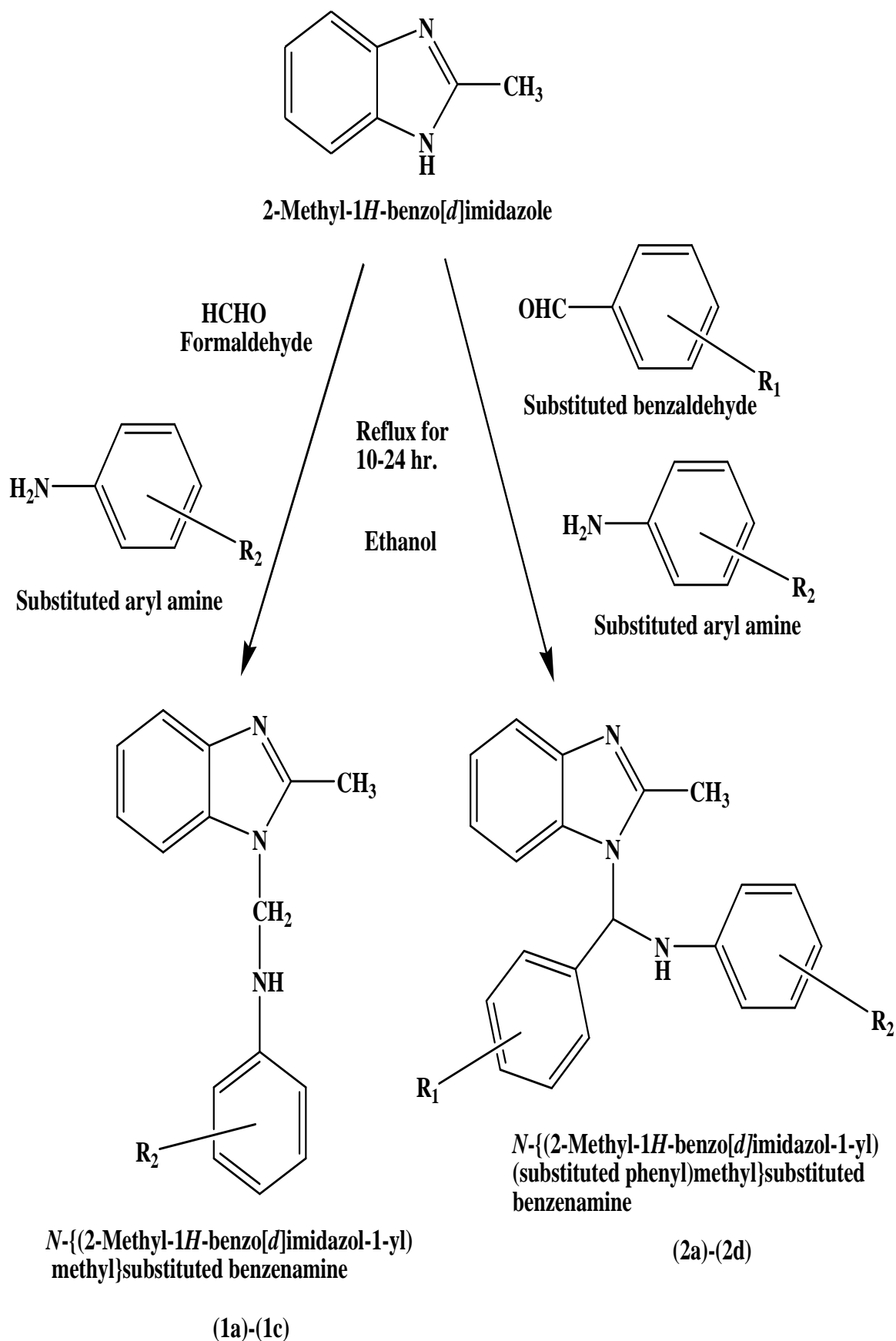
SCHEME

Scheme-1:

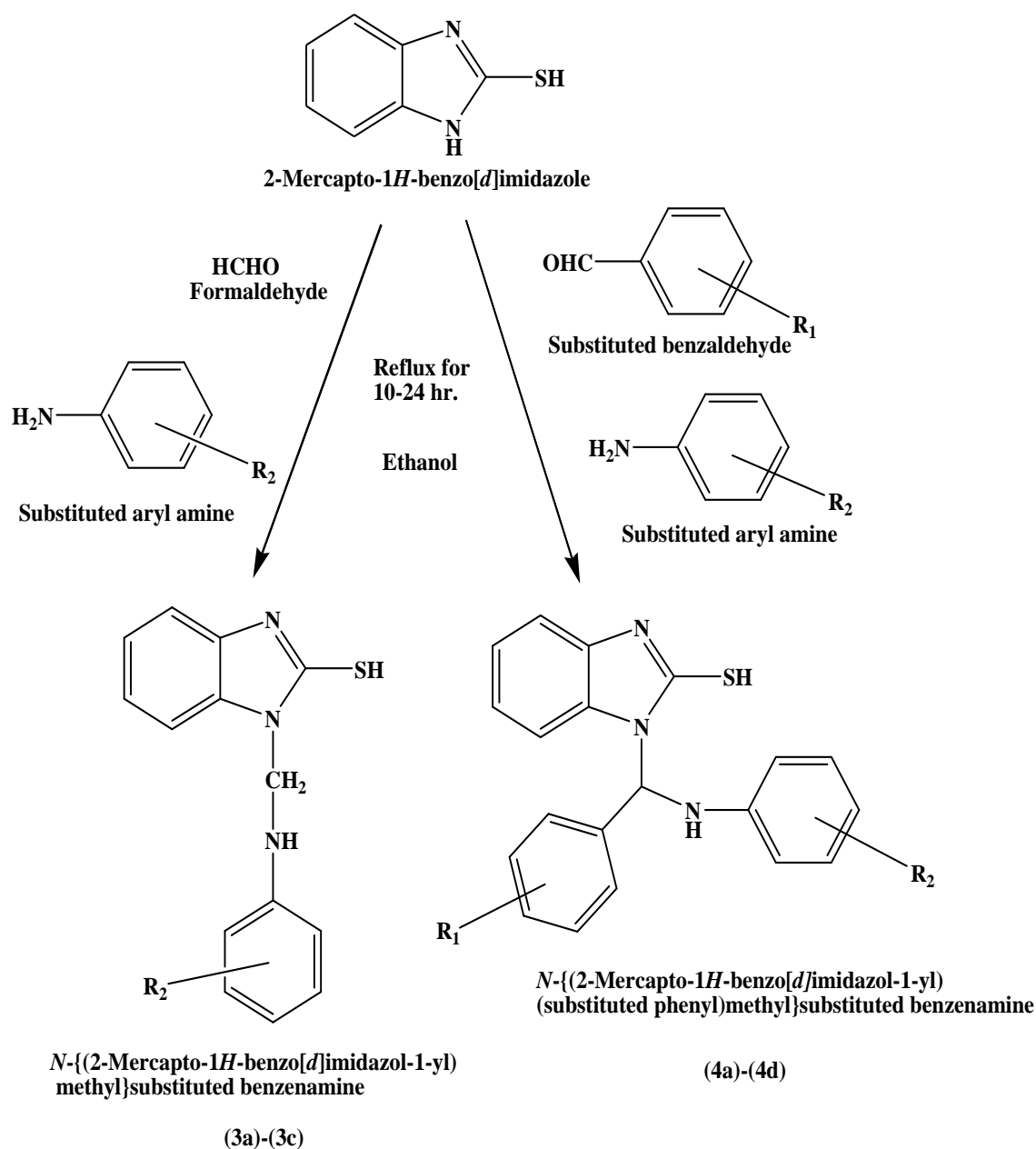
Step-1:



Step-2:



Scheme-2:



COMPOUND DETAIL

N-{(2'-Methyl-1H-benzo[d]imidazol-1-yl)methyl}-4-nitrobenzenamine (1a)

Yield: 81.41 %, m.p. 144-146°C; Elemental analysis Calcd for C₁₅H₁₄N₄O₂: C, 63.82; H, 5.00; N, 19.85. Found: C, 63.80; H, 5.01; N, 19.81 %; FTIR (KBr, ν_{\max} , cm⁻¹): 3365 (N-H str. (2° amine)), 3051 (Aromatic C-H str.), 2935 (Aliphatic C-H str.), 1677 (C=N str.), 1630 (Aromatic C=C str.), 1585 (Aromatic C-C str.), 1419 (N-O str.), 1315 (Aromatic C-N str.), 1174 (Aliphatic C-N str.), 813 (C-H *p*-disubstituted benzene (def.)); ¹H NMR (DMSO-d₆) δ (ppm): 2.70 (s, 3H, CH₃), 4.10 (s, 1H, N-H, D₂O exchangeable), 4.32 (s, 2H, CH₂), 7.203-

7.249 (t, 2H, Ar-H), 7.410-7.425 (d, 2H, Ar-H), 7.501-7.527 (d, 2H, Ar-H), 7.854-7.895 (d, 2H, Ar-H); MS (ESI) m/z [% rel. abundance]: 282 (100) $[M]^+$, 283 (16) $[M+1]^+$.

3-Methoxy-*N*-{(2'-methyl-1*H*-benzo[*d*]imidazol-1-yl)methyl}benzenamine (1b)

Yield: 75.7 %, m.p. 164-166°C; Elemental analysis Calcd for $C_{16}H_{17}N_3O$: C, 71.89; H, 6.41; N, 15.72. Found: C, 71.87; H, 6.40; N, 15.69 %; FTIR (KBr, ν_{\max} , cm^{-1}): 3350 (N-H str. (2°amine)), 3056 (Aromatic C-H str.), 2921 (Aliphatic C-H str.), 1687 (C=N Str.), 1620 (Aromatic C=C str.), 1271 (Aromatic C-N str.), 1209 (Aliphatic C-N str.), 1084 (C-O-C str.), 693 (C-H *m*-disubstituted benzene (def.)); 1H NMR (DMSO- d_6) δ (ppm): 2.320 (s, 3H, CH_3), 3.734 (s, 3H, OCH_3), 4.122 (s, 1H, N-H, D_2O exchangeable), 4.601 (s, 2H, CH_2), 6.080-6.093 (d, 1H, Ar-H), 6.408 (s, 1H, Ar-H), 6.700-6.777 (t, 1H, Ar-H), 6.940-6.957 (d, 1H, Ar-H), 7.260-7.283 (t, 2H, Ar-H), 7.509-7.525 (d, 2H, Ar-H); MS (ESI) m/z [% rel. abundance]: 267 (100) $[M]^+$, 268 (18) $[M+1]^+$.

4-Chloro-*N*-{(2'-methyl-1*H*-benzo[*d*]imidazol-1-yl) methyl} benzenamine (1c)

Yield: 63.71 %, m.p. 180-182°C; Elemental analysis Calcd for $C_{15}H_{14}N_3Cl$: C, 66.30; H, 5.19; N, 15.12; Cl, 13.05. Found: C, 66.27; H, 5.18; N, 15.09; Cl, 13.01 %; FTIR (KBr, ν_{\max} , cm^{-1}): 3356 (N-H str. (2°amine)), 3037 (Aromatic C-H str.), 2935 (Aliphatic C-H str.), 1664 (C=N str.), 1623 (C=C ring str.), 1282 (Aromatic C-N str.), 1164 (Aliphatic C-N str.), 1092 cm^{-1} (Aromatic C-Cl str.), 829 (C-H *p*-disubstituted benzene (def.)); 1H NMR (DMSO- d_6) δ (ppm): 2.102 (s, 3H, CH_3), 4.00 (s, 1H, N-H, D_2O exchangeable), 4.410 (s, 2H, CH_2), 7.053-7.109 (t, 2H, Ar-H), 7.210-7.235 (d, 2H, Ar-H), 7.421-7.439 (d, 2H, Ar-H), 7.654-7.690 (d, 2H, Ar-H); MS (ESI) m/z [% rel. abundance]: 271 (100) $[M]^+$, 272 (16) $[M+1]^+$, 273 (27) $[M+2]^+$.

4-{(4'-Nitrophenylamino)(2''-methyl-1*H*-benzo[*d*]imidazol-1-yl)methyl}phenol (2a)

Yield: 78.2 %, m.p. 178-180°C; Elemental analysis Calcd for $C_{21}H_{18}N_4O_3$: C, 67.37; H, 4.85; N, 14.96. Found: C, 67.35; H, 4.84; N, 14.93 %; FTIR (KBr, ν_{\max} , cm^{-1}): 3640 (O-H str.), 3361 (N-H str. (2°amine)), 3072 (Aromatic C-H str.), 2896 (Aliphatic C-H str.), 1675 (C=N Str.), 1625 (Aromatic C=C str.), 1483 (N-O Str.), 1313 (Aromatic C-N str.), 1186 (Aliphatic C-N str.), 840 (C-H *p*-disubstituted benzene (def.)); 1H NMR (DMSO- d_6) δ (ppm): 2.803 (s, 3H, CH_3), 4.502 (s, 1H, N-H, D_2O exchangeable), 5.20 (s, 1H, OH, D_2O exchangeable), 6.106 (s, 1H, CH), 6.603-6.625 (d, 2H, Ar-H), 6.804-6.829 (d, 2H, Ar-H), 6.904-6.983 (t,

2H, Ar-H), 6.259-6.285 (d, 2H, Ar-H), 7.310-7.329 (d, 2H, Ar-H), 7.901-7.925 (d, 2H, Ar-H); MS (ESI) m/z [% rel. abundance]: 374 (100) $[M]^+$, 375 (16) $[M+1]^+$.

***N*-{(4'-Fluorophenyl)(2''-methyl-1*H*-benzo[*d*]imidazol-1-yl)methyl}-4-nitrobenzenamine (2b)**

Yield: 85.3 %, m.p. 134-136°C; Elemental analysis Calcd for $C_{21}H_{17}FN_4O_2$: C, 67.01; H, 4.55; F, 5.05; N, 14.89. Found: C, 67.02; H, 4.52; F, 5.02; N, 14.86 %; FTIR (KBr, ν_{\max} , cm^{-1}): 3357 (N-H str. (2°amine)), 3055 (Aromatic C-H str.), 2889 (Aliphatic C-H str.), 1680 (C=N str.), 1616 (Aromatic C=C str.), 1431 (N-O Str.), 1313 (Aromatic C-N str.), 1205 (C-F Str.), 1103 (Aliphatic C-N str.), 820 (C-H *p*-disubstituted benzene (def.)); 1H NMR (DMSO- d_6) δ (ppm): 2.401 (s, 3H, CH_3), 4.00 (s, 1H, N-H, D_2O exchangeable), 6.10 (s, 1H, CH), 6.920-6.957 (d, 2H, Ar-H), 7.240-7.248 (d, 2H, Ar-H), 7.303-7.399 (t, 2H, Ar-H), 7.512-7.528 (d, 2H, Ar-H), 7.802-7.815 (d, 2H, Ar-H), 7.902-7.957 (d, 2H, Ar-H); MS (ESI) m/z [% rel. abundance]: 376 (100) $[M]^+$, 377 (12) $[M+1]^+$, 378 (24) $[M+2]^+$.

***N*-{(3-Methoxyphenyl)(2''-methyl-1*H*-benzo[*d*]imidazol-1-yl)methyl}-4-nitrobenzenamine (2c)**

Yield: 83.5 %, m.p. 152-154°C; Elemental analysis Calcd for $C_{22}H_{20}N_4O_3$: C, 68.03; H, 5.19; N, 14.42. Found: C, 68.01; H, 5.16; N, 14.41 %; FTIR (KBr, ν_{\max} , cm^{-1}): 3361 (N-H str. (2°amine)), 3062 (Aromatic C-H str.), 2898 (Aliphatic C-H str.), 1687 (C=N str.), 1610 (Aromatic C=C str.), 1476 (N-O str.), 1313 (Aromatic C-N str.), 1190 (C-O-C str.), 1125 (Aliphatic C-N str.), 812 (C-H *p*-disubstituted benzene (def.)), 705 (C-H *m*-disubstituted benzene (def.)); 1H NMR (DMSO- d_6) δ (ppm): 2.203 (s, 3H, CH_3), 3.734 (s, 3H, OCH_3), 4.312 (s, 1H, N-H, D_2O exchangeable), 6.280 (s, 1H, CH), 6.093-6.548 (d, 1H, Ar-H), 6.600 (s, 1H, Ar-H), 6.738-6.840 (t, 1H, Ar-H), 6.957-7.060 (d, 1H, Ar-H), 7.269-7.299 (t, 2H, Ar-H), 7.305-7.359 (d, 2H, Ar-H), 7.505-7.529 (d, 2H, Ar-H), 7.925-7.945 (d, 2H, Ar-H); MS (ESI) m/z [% rel. abundance]: 388 (100) $[M]^+$, 389 (16) $[M+1]^+$.

***N*-{(3'-Nitrophenyl)(2''-methyl-1*H*-benzo[*d*]imidazol-1-yl)methyl}-4-chlorobenzenamine (2d)**

Yield: 68.97 %, m.p. 218-220°C; Elemental analysis Calcd for $C_{21}H_{17}N_4O_2Cl$: C, 64.21; H, 4.36; N, 14.26; Cl, 9.02. Found: C, 64.19; H, 4.35; N, 14.23; Cl, 9.01%; FTIR (KBr, ν_{\max} , cm^{-1}): 3380 (N-H str. (2°amine)), 3049 (Aromatic C-H str.), 2910 (Aliphatic C-H str.), 1664 (C=N str.), 1610 (Aromatic C=C str.), 1446 (N-O str.), 1332 (Aromatic C-N str.), 1226

(Aliphatic C-N str.), 1092 (C-Cl str.), 834 (C-H *p*-Disubstituted benzene (def.)), 691 (C-H *m*-disubstituted benzene (def.)); ^1H NMR (DMSO- d_6) $\delta(\text{ppm})$: 2.300 (s, 3H, CH_3), 4.207 (s, 1H, N-H, D_2O exchangeable), 6.103 (s, 1H, CH), 7.103-7.179 (t, 2H, Ar-H), 7.210-7.243 (d, 2H, Ar-H), 7.302-7.349 (d, 2H, Ar-H), 7.410-7.435 (d, 1H, Ar-H), 7.501-7.584 (t, 1H, Ar-H), 7.605-7.641 (d, 2H, Ar-H), 7.907 (s, 1H, Ar-H), 7.914-8.025 (d, 1H, Ar-H); MS (ESI) m/z [% rel. abundance]: 392 (100) $[\text{M}]^+$, 393 (12) $[\text{M}+1]^+$, 394 (23) $[\text{M}+2]^+$.

1-((4'-Nitrophenylamino) methyl)-1*H*-benzo[d]imidazole-2-thiol (3a)

Yield: 87.7 %, m.p. 236-238°C; Elemental analysis Calcd for $\text{C}_{14}\text{H}_{12}\text{N}_4\text{O}_2\text{S}$: C, 55.99; H, 4.03; N, 18.65; S, 10.68. Found: C, 55.97; H, 4.01; N, 18.63; S, 10.62 %; FTIR (KBr, ν_{max} , cm^{-1}): 3370 (N-H str. (2°amine)), 3075 (Aromatic C-H str.), 2925 (Aliphatic C-H str.), 2559 (S-H Str.), 1635 (Aromatic C=C Str.), 1623 (C=N Str.), 1593 (Aromatic C-C str.), 1454 (N-O Str.), 1325 (Aromatic C-N str.), 1180 (Aliphatic C-N str.), 806 (C-H *p*-disubstituted benzene (def.)); ^1H NMR (DMSO- d_6) $\delta(\text{ppm})$: 3.007 (s, 1H, SH, D_2O exchangeable), 4.000 (s, 1H, N-H, D_2O exchangeable), 4.502 (s, 2H, CH_2), 7.215-7.269 (t, 2H, Ar-H), 7.304-7.325 (d, 2H, Ar-H), 7.610-7.630 (d, 2H, Ar-H), 7.904-7.923 (d, 2H, Ar-H); MS (ESI) m/z [% rel. abundance]: 300 (100) $[\text{M}]^+$, 301 (15) $[\text{M}+1]^+$.

1-((3'-Methoxyphenylamino) methyl)-1*H*-benzo[d]imidazole-2-thiol (3b)

Yield: 83.8 %, m.p. 220-222°C; Elemental analysis Calcd for $\text{C}_{15}\text{H}_{15}\text{N}_3\text{OS}$: C, 63.13; H, 5.30; N, 14.73; S, 11.24. Found: C, 63.11; H, 5.28; N, 14.71; S, 11.22 %; FTIR (KBr, ν_{max} , cm^{-1}): 3364 (N-H str. (2°amine)), 3072 (Aromatic C-H str.), 2879 (Aliphatic C-H str.), 2572 (S-H Str.), 1682 (C=N str.), 1614 (Aromatic C=C str.), 1274 (Aromatic C-N str.), 1190 (Aliphatic C-N Str.), 1107 (C-O-C Str.), 710 (C-H *m*-Disubstituted benzene (def.)); ^1H NMR (DMSO- d_6) $\delta(\text{ppm})$: 3.220 (s, 1H, SH, D_2O exchangeable), 3.730 (s, 3H, OCH_3), 4.000 (s, 1H, N-H, D_2O exchangeable), 4.302 (s, 2H, CH_2), 6.110-6.133 (d, 1H, Ar-H), 6.408 (s, 1H, Ar-H), 6.701-6.767 (t, 1H, Ar-H), 6.940-6.953 (d, 1H, Ar-H), 7.310-7.364 (t, 2H, Ar-H), 7.733-7.765 (d, 2H, Ar-H); MS (ESI) m/z [% rel. abundance]: 285 (100) $[\text{M}]^+$, 286 (15) $[\text{M}+1]^+$.

1-((4'-Chlorophenylamino) methyl)-1*H*-benzo[d]imidazole-2-thiol (3c)

Yield: 83.4 %, m.p. 252-254°C; Elemental analysis Calcd for $\text{C}_{14}\text{H}_{12}\text{N}_3\text{SCl}$: C, 58.03; H, 4.17; N, 14.50; S, 11.07; Cl, 12.23. Found: C, 58.01; H, 4.15; N, 14.47; S, 11.02; Cl, 12.21 %; FTIR (KBr, ν_{max} , cm^{-1}): 3362 (N-H str. (2°amine)), 3071 (Aromatic C-H str.), 2952 (Aliphatic C-H str.), 2571 (S-H str.), 1683 (C=N str.), 1602 (C=C ring str.), 1317 (Aromatic

C-N str.), 1182 (Aliphatic C-N str.), 1095 cm^{-1} (Aromatic -Cl str.), 835 (C-H *p*-disubstituted (def.)); ^1H NMR (DMSO- d_6) $\delta(\text{ppm})$: 3.102 (s, 1H, SH, D_2O exchangeable), 4.010 (s, 1H, N-H, D_2O exchangeable), 4.402 (s, 2H, CH_2), 7.043-7.103 (t, 2H, Ar-H), 7.216-7.243 (d, 2H, Ar-H), 7.401-7.435 (d, 2H, Ar-H), 7.509-7.524 (d, 2H, Ar-H); MS (ESI) m/z [% rel. abundance]: 289 (100) $[\text{M}]^+$, 290 (15) $[\text{M}+1]^+$, 291 (26) $[\text{M}+2]^+$.

4-((4'-Nitrophenylamino)(2''-mercapto-1*H*-benzo[*d*]imidazol-1-yl)methyl)phenol (4a)

Yield: 69.6 %, m.p. 210-212°C; Elemental analysis Calcd for $\text{C}_{20}\text{H}_{16}\text{N}_4\text{O}_3\text{S}$: C, 61.21; H, 4.11; N, 14.28; S, 8.17. Found: C, 61.18; H, 4.09; N, 14.25; S, 8.14 %; FTIR (KBr, ν_{max} , cm^{-1}): 3620 (O-H str.), 3359 (N-H str. (2°amine)), 3063 (Aromatic C-H str.), 2893 (Aliphatic C-H str.), 2570 (S-H Str.), 1661 (C=N str.), 1631 (Aromatic C=C str.), 1469 (N-O Str.), 1303 (Aromatic C-N str.), 1176 (Aliphatic C-N str.), 823 (C-H *p*-disubstituted benzene (def.)); ^1H NMR (DMSO- d_6) $\delta(\text{ppm})$: 3.401 (s, 1H, SH, D_2O exchangeable), 4.300 (s, 1H, N-H, D_2O exchangeable), 5.000 (s, 1H, OH, D_2O exchangeable), 6.206 (s, 1H, CH), 6.603-6.625 (d, 2H, Ar-H), 6.807-6.823 (d, 2H, Ar-H), 7.156-7.181 (d, 2H, Ar-H), 7.309-7.385 (t, 2H, Ar-H), 7.479-7.505 (d, 2H, Ar-H), 7.810-7.844 (d, 2H, Ar-H); MS (ESI) m/z [% rel. abundance]: 392 (100) $[\text{M}]^+$, 393 (12) $[\text{M}+1]^+$.

1-((4'-Nitrophenylamino)(4''-fluorophenyl)methyl)-1*H*-benzo[*d*]imidazole-2-thiol (4b):

Yield: 84.76 %, m.p. 240-242°C; Elemental analysis Calcd for $\text{C}_{20}\text{H}_{14}\text{FN}_3\text{O}_2\text{S}$: C, 63.31; H, 3.72; F, 5.03; N, 11.08; S, 8.45. Found: C, 63.16; H, 3.53; F, 5.01; N, 11.02; S, 8.20 %; FTIR (KBr, ν_{max} , cm^{-1}): 3386 (N-H str. (2°amine)), 3089 (Aromatic C-H str.), 2887 (Aliphatic C-H str.), 2557 (S-H Str.), 1685 (C=N str.), 1620 (Aromatic C=C str.), 1413 (N-O str.), 1299 (Aromatic C-N str.), 1208 (C-F str.), 1145 (Aliphatic C-N str.), 820 (C-H *p*-disubstituted benzene (def.)); ^1H NMR (DMSO- d_6) $\delta(\text{ppm})$: 3.100 (s, 1H, SH, D_2O exchangeable), 4.010 (s, 1H, N-H, D_2O exchangeable), 6.109 (s, 1H, CH), 6.932-6.951 (d, 2H, Ar-H), 7.210-7.283 (t, 2H, Ar-H), 7.311-7.334 (d, 2H, Ar-H), 7.602-7.629 (d, 2H, Ar-H), 7.832-7.853 (d, 2H, Ar-H), 7.941-7.959 (d, 2H, Ar-H); MS (ESI) m/z [% rel. abundance]: 394 (100) $[\text{M}]^+$, 395 (12) $[\text{M}+1]^+$, 396 (25) $[\text{M}+2]^+$.

1-((4'-Nitrophenylamino)(3''-methoxyphenyl)methyl)-1*H*-benzo[*d*]imidazole-2-thiol (4c)

Yield: 80.7 %, m.p. 226-228°C; Elemental analysis Calcd for $\text{C}_{21}\text{H}_{17}\text{N}_3\text{O}_3\text{S}$: C, 64.43; H, 4.38; N, 10.73; S, 8.19. Found: C, 64.41; H, 4.36; N, 10.71; S, 8.16 %; FTIR (KBr, ν_{max} , cm^{-1}): 3361 (N-H str. (2°amine)), 3040 (Aromatic C-H str.), 2881 (Aliphatic C-H str.), 2578 (S-H

str.), 1675 (C=N str.), 1618 (Aromatic C=C str.), 1467 (N-O str.), 1313 (Aromatic C-N str.), 1186 (C-O-C str.), 1110 (Aliphatic C-N str.), 820 (C-H *p*-disubstituted benzene (def.)), 708 (C-H *m*-disubstituted benzene (def.)); ^1H NMR (DMSO- d_6) δ (ppm): 3.200 (s, 1H, SH, D_2O exchangeable), 3.704 (s, 3H, OCH_3), 4.212 (s, 1H, N-H, D_2O exchangeable), 6.120 (s, 1H, CH), 6.193-6.528 (d, 1H, Ar-H), 6.600 (s, 1H, Ar-H), 6.728-6.900 (t, 1H, Ar-H), 6.927-7.030 (d, 1H, Ar-H), 7.209-7.289 (t, 2H, Ar-H), 7.305-7.399 (d, 2H, Ar-H), 7.605-7.629 (d, 2H, Ar-H), 7.813-7.845 (d, 2H, Ar-H); MS (ESI) m/z [% rel. abundance]: 406 (100) $[\text{M}]^+$, 407 (12) $[\text{M}+1]^+$.

1-{(4'-Chlorophenylamino)(3''-nitrophenyl)methyl}-1*H*-benzo[*d*]imidazole-2-thiol (4d)

Yield: 73.05 %, m.p. 190-192°C; Elemental analysis Calcd for $\text{C}_{20}\text{H}_{15}\text{N}_4\text{O}_2\text{SCl}$: C, 58.46; H, 3.68; N, 13.64; S, 7.80; Cl, 8.63. Found: C, 58.45; H, 3.63; N, 13.62; S, 7.76; Cl, 8.61 %; FTIR (KBr, ν_{max} , cm^{-1}): 3380 (N-H str. (2° amine)), 3080 (Aromatic C-H str.), 2958 (Aliphatic C-H str.), 2559 (S-H Str.), 1685 (C=N str.), 1602 (Aromatic C=C str.), 1421 (N-O str.), 1326 (Aromatic C-N str.), 1180 (Aliphatic C-N str.), 1090 (C-Cl str.), 812 (C-H *p*-disubstituted benzene (def.)), 703 (C-H *m*-disubstituted benzene (def.)); ^1H NMR (DMSO- d_6) δ (ppm): 3.102 (s, 1H, SH, D_2O exchangeable), 4.102 (s, 1H, N-H, D_2O exchangeable), 6.107 (s, 1H, CH), 7.103-7.189 (t, 2H, Ar-H), 7.212-7.240 (d, 2H, Ar-H), 7.303-7.342 (d, 1H, Ar-H), 7.401-7.437 (d, 2H, Ar-H), 7.510-7.574 (t, 1H, Ar-H), 7.705-7.731 (d, 2H, Ar-H), 7.907 (s, 1H, Ar-H), 7.915-8.035 (d, 1H, Ar-H); MS (ESI) m/z [% rel. abundance]: 410 (100) $[\text{M}]^+$, 411 (12) $[\text{M}+1]^+$, 412 (25) $[\text{M}+2]^+$.

Antibacterial activity

The antibacterial activity of newly synthesized compounds were tested by disc diffusion method on nutrient agar medium against following bacterial strains i.e. *S. aureus*, *B. subtilis* (Gram positive) and *E. coli*, *P. aeruginosa* (Gram negative). In the disc diffusion method, paper disc impregnated with compounds dissolved in DMSO at concentration 25, 50 and 100 $\mu\text{g ml}^{-1}$ were used. Disc impregnated with DMSO were used as solvent control for antibacterial activity because of free solubility of test compounds. The microorganism culture was spread over nutrient agar media in petri dishes, and then the disc impregnated with the solution was placed on the surface of the media inoculated with the bacterial strain. The plates were incubated at 35°C for 24 hrs for bacterial cultures. After incubation, the zone of inhibition around the disc was observed. The zone of inhibition indicates that the compounds inhibit growth of microorganism. Each testing is done in triplicate. Ciprofloxacin at conc.

50 $\mu\text{g ml}^{-1}$ were used as standard drug for antibacterial activity. Results were interpreted in terms of diameter (mm) of zone of inhibition. The results of antibacterial studies are presented in table- 1

Antifungal activity

The antifungal activities of newly synthesized compounds were tested by disc diffusion method on nutrient agar medium against following fungal strains i.e. *A. niger* and *C. albicans*. In the disc diffusion method, paper disc impregnated with compounds dissolved in DMF at concentration 25, 50 and 100 $\mu\text{g ml}^{-1}$ were used. Disc impregnated with DMF were used as solvent control for antifungal activity because of free solubility of test compounds. The microorganism culture was spread over nutrient agar media in petri dishes, and then the disc impregnated with the solution was placed on the surface of the media inoculated with the fungal strain. The plates were incubated at 25°C for 48 hrs for fungal strains. After incubation, the zone of inhibition around the disc was observed. The zone of inhibition indicates that the compounds inhibit growth of microorganism. Each testing is done in triplicate. Fluconazole at concentration 50 $\mu\text{g ml}^{-1}$ was used as standard drug for antifungal activity. Results were interpreted in terms of diameter (mm) of zone of inhibition. The results of antifungal studies are presented in table- 1

Table 1: Antibacterial and antifungal activity data of the synthesized compounds

Compound No.	Diameter of zone of inhibition in mm (MIC 50 $\mu\text{g/ml}$)					
	<i>E. coli</i>	<i>P. aeureginosa</i>	<i>B. subtilis</i>	<i>S. aureus</i>	<i>C. albicans</i>	<i>A. niger</i>
1a	10.70	10.56	13.16	11.43	12.53	16.25
1b	11.46	13.31	10.76	10.17	10.71	15.37
1c	10.42	12.19	11.53	13.05	12.23	16.13
2a	13.30	12.10	10.53	10.27	15.56	12.11
2b	10.56	11.32	13.26	11.21	17.36	13.63
2c	13.20	12.51	10.47	10.53	15.23	13.61
2d	10.12	11.28	11.26	13.26	16.12	12.40
3a	10.33	11.15	13.06	11.35	12.54	16.09
3b	11.33	13.28	10.52	10.09	11.06	15.22
3c	10.09	11.53	11.17	12.85	11.21	16.17
4a	13.15	12.15	10.61	10.40	13.43	11.40
4b	10.25	12.30	13.10	11.15	17.28	13.12
4c	13.10	12.23	10.51	11.30	14.20	11.23
4d	10.11	11.42	11.21	13.17	16.19	13.53
Ciprofloxacin	13.62	13.47	13.32	13.51	-	-
Fluconazole	-	-	-	-	17.92	17.38
Controle	-	-	-	-	-	-

Note: - Average zone diameter in mm of triplicates

RESULTS AND DISCUSSION

Antibacterial activity

The newly synthesized compounds were screened for *in-vitro* antibacterial activity using disc diffusion method. The antibacterial activities of all the synthesized compounds were carried out against the pathogenic bacterial strains *S. aureus*, *B. subtilis* (gram positive) and *E. coli*, *P. aeruginosa* (gram negative). The zone of inhibition was measured by antibiotic zone reader. The results revealed that the newly synthesized compounds 2a, 2c, 4a and 4c showed good antibacterial activity with 13.30, 13.20, 13.15 and 13.10 mm zone of inhibition respectively against *E. coli* when given at concentration $50\mu\text{g ml}^{-1}$ whereas under identical conditions standard drug ciprofloxacin showed 13.62mm zone of inhibition. Compounds 1a, 1b, 1c, 2b, 2d, 3a, 3b, 3c, 4b and 4d showed moderate antibacterial activity with 10.70, 11.46, 10.42, 10.56, 10.12, 10.33, 11.33, 10.09, 10.25 and 10.11mm zone of inhibition respectively against *E. coli*. Compounds 1a, 2b, 3a and 4b showed good antibacterial activity with 13.16, 13.26, 13.06 and 13.10mm zone of inhibition against *B. subtilis* when given at concentration $50\mu\text{g ml}^{-1}$ where as under identical conditions standard drug ciprofloxacin showed 13.32mm zone of inhibition. Compounds 1b, 1c, 2a, 2c, 2d, 3b, 3c, 4a, 4c and 4d showed moderate antibacterial activity with 10.76, 11.53, 10.53, 10.47, 11.26, 10.52, 11.17, 10.61, 10.51 and 11.21mm zone of inhibition respectively against *B. subtilis*. Compound 1b and 3b showed good antibacterial activity with 13.31 and 13.28mm zone of inhibition against *P. aeruginosa* when given at concentration $50\mu\text{g ml}^{-1}$ where as under identical conditions standard drug ciprofloxacin showed 13.47mm zone of inhibition. Compounds 1a, 1c, 2a, 2b, 2c, 2d, 3a, 3c, 4a, 4b, 4c and 4d showed moderate antibacterial activity with 10.56, 12.19, 12.10, 11.32, 12.51, 11.28, 11.15, 11.53, 12.15, 12.30, 12.23 and 11.42mm zone of inhibition respectively against *P. aeruginosa*. Compounds 1c, 2d, 3c and 4d showed good antibacterial activity with 13.05, 13.26, 12.85 and 13.17mm zone of inhibition against *S. aureus* when given at concentration $50\mu\text{g ml}^{-1}$ where as under identical conditions standard drug ciprofloxacin showed 13.51mm zone of inhibition. Compounds 1a, 1b, 2a, 2b, 2c, 3a, 3b, 4a, 4b and 4c showed moderate antibacterial activity with 11.43, 10.17, 10.27, 11.21, 10.53, 11.35, 10.09, 10.40, 11.15 and 11.30mm zone of inhibition respectively against *S. aureus*.

Antifungal activity

The newly synthesized compounds were screened for *in-vitro* antifungal activity using disc diffusion method. The following fungal strains were used: *C. albicans* and *A. niger*. The results revealed that the newly synthesized compounds 2b, 2d, 4b and 4d showed good

antifungal activity with 17.36, 16.12, 17.28 and 16.19mm zone of inhibition respectively against *C. albicans* when given at concentration $50\mu\text{gml}^{-1}$ whereas under identical conditions standard drug fluconazole showed 17.92mm zone of inhibition. Compounds 1a, 1b, 1c, 2a, 2c, 3a, 3b, 3c, 4a and 4c showed moderate antifungal activity with 12.53, 10.71, 12.23, 15.56, 15.23, 12.54, 11.06, 11.21, 13.43 and 14.20mm zone of inhibition respectively against *C. albicans*. Compounds 1a, 1c, 3a and 3c showed good antifungal activity with 16.25, 16.13, 16.09 and 16.17mm zone of inhibition respectively against *A. niger* when given at concentration $50\mu\text{g ml}^{-1}$ where as under identical conditions standard drug fluconazole showed 17.38mm zone of inhibition. Compounds 1b, 2a, 2b, 2c, 2d, 3b, 4a, 4b, 4c and 4d showed moderate antifungal activity with 15.37, 12.11, 13.63, 13.61, 12.40, 15.22, 11.40, 13.12, 11.23 and 13.53mm zone of inhibition respectively against *A. niger*.

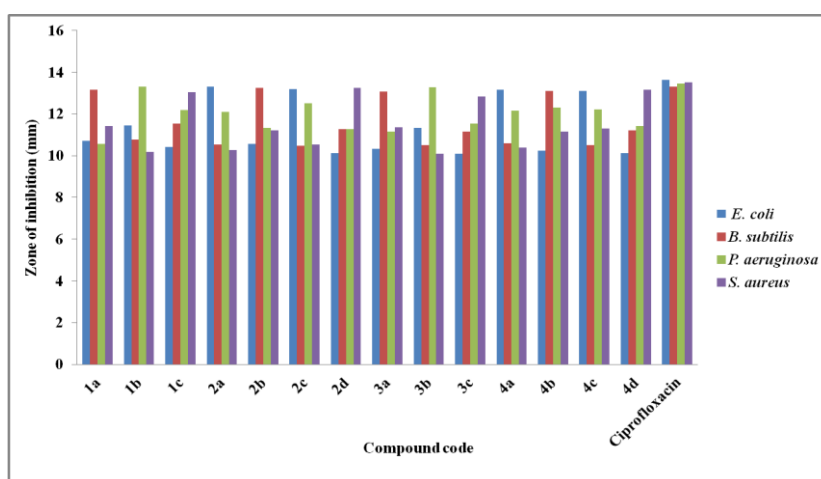


Fig. 1: Antibacterial activity against bacterial strains *S. aureus*, *B. subtilis* (Gram positive) and *E. coli*, *P. aeruginosa* (Gram negative).

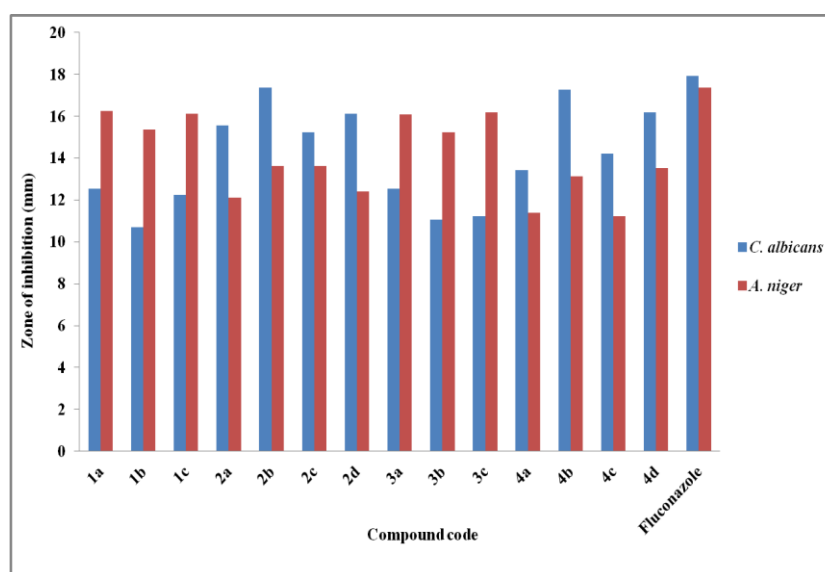


Fig. 2: Antifungal activity against following fungal strains *A. niger* and *C. albicans*.

CONCLUSION

All the synthesized compounds were confirmed by physical parameters (Solubility, melting point), chromatographic methods (TLC) and spectroscopic methods (IR, ^1H NMR, Mass spectroscopy and elemental analysis). The newly synthesized compounds were screened for antibacterial and antifungal activity by disc diffusion methods on nutrient agar medium. The antibacterial and antifungal activity presented in Figure 1 and 2. The results revealed that newly synthesized compounds **2a**, **2c**, **4a** and **4c** were found to be potent against *E. coli* (MIC 50 $\mu\text{g/ml}$); **1a**, **2b**, **3a** and **4b** against *B. subtilis* (MIC 50 $\mu\text{g/ml}$); **1b** and **3b** against *P. aeruginosa* (MIC 50 $\mu\text{g/ml}$); **1c**, **2d**, **3c** and **4d** against *S. aureus* (MIC 50 $\mu\text{g/ml}$) and antifungal activity **2b**, **2d**, **4b** and **4d** against *C. albicans* (MIC 50 $\mu\text{g/ml}$) while **1a**, **1c**, **3a** and **3c** were found to be potent against *A. niger* (MIC 50 $\mu\text{g/ml}$).

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