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Development of 5-(Aryl)-3-phenyl-1*H*-pyrazole Derivatives as Potent Antimicrobial Compounds

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A series of 16 chalcone compounds were synthesized by Claisen-Schmidt condensation of various aldehydes with acetophenone using KOH as a base in ethanol. The reaction affords the desired products in good yields. Then all the 16 compounds were converted into pyrazoles by treating with hydrazine hydrate in ethanol under reflux condition. Both chalcones and pyrazoles were screened for their *in vitro* antibacterial (*Escherichia coli*, *Staphylococcus aureus* and *Pseudomonas aeruginosa*) and antifungal (*Aspergillus flavus*, *Chrysosporium keratinophilum* and *Candida albicans*) activity. Biological activities of these compounds were compared with those of commercially available antibiotic ampicillin and antifungal agent miconazole. Pyrazoles were found to be most active and effective than corresponding chalcones for antimicrobial activity. Out of the 7 pyrazole compounds tested for antibacterial and antifungal activity, 5 compounds, **4h**, **4j**, **4l**, **4m** and **4n** are turned out to be potent antimicrobial agents. Therefore these derivatives could serve as a highly promising molecules for further development.

Keywords: Antibacterial, Antifungal, Heterocyclic and Substituted pyrazoles, Chalcone.

INTRODUCTION

Chalcones (**1**) and pyrazoles (**2**) are the most important classes of flavonoids and alkaloids respectively present across the whole plant kingdom [1,2]. Both have interesting bioactive features associated with several biological activities. Chalcones and their derivatives demonstrate wide range of biological activities such as antidiabetic, antineoplastic, antihypertensive, antiretroviral, anti-inflammatory [3], antiparasitic [4], anti-histaminic [5], antimalarial [6], antioxidant [7], antifungal [8], antiobesity [9], antiplatelet [10], antitubercular [11], immuno-suppressant [12] *etc*. Also, pyrazole nucleus has found considerable attention due to its potent medicinal scaffolds and exhibits full spectrum of biological activities. Many pyrazole derivatives have already found their application as antimicrobial [13-16], antifungal [17], anticancer [18-20], anti-inflammatory [13,21-26], antiviral [14], antioxidant [27], *etc*.

Chalcone is an acyclic compound with substituted aromatic ring at both side of the double bond which allows the molecule to adopt many conformations due to weak π-π interactions which may affect the biological activities of these compounds [28]. We envisage that, introducing cyclic structure to chalcone compound (**3**) restricts the conformational freedom of the aromatic rings, thereby improving the bioactive potency of the resulting compounds.

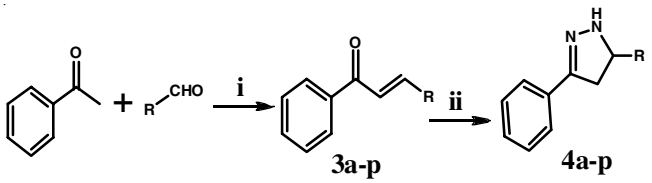
Owing to the interesting applications of pyrazoles in the field of medicinal chemistry, the combination of pyrazole nucleus with the additional substituents at 2 and 4 positions of pyrazole ring, will increase the diversity and becomes fruitful area of study for their biological activity. It was thought that, a pyrazole ring substituted with different aryl groups at 5 and 3 positions might possess enhanced biologically activity. In view of these facts, here we report the synthesis of chalcones and derivatizing them in to pyrazoles by reacting with hydrazine with the aim of comparing and exploring their antimicrobial activity.

EXPERIMENTAL

All chemicals were acquired from Sigma-Aldrich Chemical Co (Sigma-Aldrich, Bengalore, India). The infrared

spectra (KBr) were recorded using Shimadzu 8201PC instrument operating on 4000-400 cm^{-1}. The proton NMR and Carbon NMR were recorded using Agilent V NMRS-400 instrument with $CDCl_3$ and the chemical shifts are expressed in ppm. The mass spectra (EI) were recorded using Jeol JMS D-300 spectrometer.

The chalcones **3(a-p)** were synthesized by base catalyzed Claisen-Schmidt condensation reaction of acetophenone with different substituted aromatic aldehydes using known literature method. The chalcones so obtained were refluxed with hydrazine hydrate solution in ethanol yield 4,5-dihydro,3,5-disubstituted pyrazoles **4(a-p)** (**Scheme-I**).



Scheme-I: Reagent and conditions: (i) KOH, ethanol, reflux; (ii) NH_2-NH_2; ethanol, reflux

General procedure for chalcone synthesis 3(a-p): A mixture of acetophenone (6 mmol) and arylaldehyde (6 mmol) were dissolved in ethanol (30 mL), then 40 % solution of NaOH (6 mL) was added drop-wise, while the temperature was kept below 10 °C. The reaction mixture was stirred under this condition for 1 h. The stirring was continued at room temperature for 4 h. Thereafter the reaction mixture was poured into ice-water. The precipitated solid was filtered off and recrystallized from aqueous ethanol.

(E)-1-Phenyl-3-(3,4,5-trimethoxyphenyl)prop-2-en-1-one (3a): ^{1}H NMR: ($CDCl_3$) δ 8.06 (s, 1H); 7.89 (s, 1H); 7.64 (m, 2H); 7.59 (s, 1H); 3.83 (s, 9H). ^{13}C NMR ($CDCl_3$) δ 189.7, 153.0, 145.1, 138.4, 137.9, 134.5, 129.2, 128.5, 126.4, 121.3, 103.8, 60.8, 56.1. Calculated mass for ($C_{18}H_{18}O_4$) 298.33; Observed 298.42. IR (nujal) cm^{-1}: 1656, 1597, 1541.

(E)-1-Phenyl-3-(3,5-dimethoxyphenyl)prop-2-en-1-one (3b): ^{1}H NMR: ($CDCl_3$) δ 8.06 (d,1H); 7.90 (d, 1H), 7.64 (m, 2H). ^{13}C NMR ($CDCl_3$) δ 189.7, 161.5, 145.1, 137.9, 134.5, 134.1, 129.2 128.5, 105.5, 99.6, 55.8. Calculated mass for ($C_{17}H_{16}O_3$) 268.11; Observed 268. 23. IR (nujal) cm^1: 1657, 1597, 1544.

(E)-1-Phenyl-3-(3,4-dimethoxyphenyl)prop-2-en-1-one (3c): ^{1}H NMR: ($CDCl_3$) δ 8.06 (s, 1H); 7.89 (m, 2H); 7.73 (s, 1H); 7.64 (m, 2H), 7.18 (m, 1H);6.94(d, 1H); 3.38 (s, 6H). ^{13}C NMR ($CDCl_3$) δ 189.7, 149.7, 145.1, 137.9, 134.5 129.2, 128.5, 127.3, 122.5, 111.7, 111.5. Calculated mass for ($C_{17}H_{16}O_3$) 268.31, observed 269.15. IR (nujal) cm^1: 1649, 1598, 1541.

(E)-3-(2-Hydroxy-3-methoxyphenyl)-1-phenylprop-2-en-1-one (3d): ^{1}H NMR: ($CDCl_3$) δ 8.33(d, 1H); 7.89 (m, 2H); 7.73 (m, 2H); 7.64 (m, 2H); 7.42 (d, 1H); 7.15(m, 4H); 3.83 (s, 3H). ^{13}C NMR ($CDCl_3$) δ 189.7; 141.0, 137.9, 134.5, 129.2, 128.5, 121.2, 117.6. Calculated mass for. ($C_{16}H_{14}O_3$) 254.09; Observed 255.21. IR (nujal) cm^1: 1658, 1593, 1539.

(E)-3-(2-Hydroxyphenyl)-1-phenylprop-2-en-1-one (3e): ^{1}H NMR: ($CDCl_3$) δ 8.31 (d, 1H); 7.87 (m, 2H); 7.73 (m, 1H); 7.64 (m, 2H); 7. 42 (d, 1H); 6.72 (m, 2H). ^{13}C NMR ($CDCl_3$) δ 189.7, 157.1, 141.0, 137.9, 134.5, 129.2, 128.9, 128.5, 121.2, 117.6. Calculated mass for ($C_{15}H_{14}O_2$) 224.08; Observed 225.12. IR (nujal) cm^1: 1655, 1597, 1544.

(E)-3-(2-Bromo-3-hydroxyphenyl)-1-phenylprop-2-en-1-one (3f): ^{1}H NMR: ($CDCl_3$) δ 8.23 (d, 1H); 7.89 (m, 3H); 7.73 (m, 2H); 7.64 (m, 2H); 7.47 (m, 2H); 7.42 (d, 1H); 6.83 (m, 2H). ^{13}C NMR ($CDCl_3$) δ 189.3, 137.9, 145.1, 121.3, 128.5, 129.2, 134.5, 113.9, 156.5, 129.0, 115.5. Calculated mass for ($C_{15}H_{11}O_2Br$) 301.99; Observed 303.09. IR (nujal) cm^1: 1658, 1592, 1543.

(E)-3-(2,4-Dibromo-6-hydroxyphenyl)-1-phenylprop-2-en-1-one (3g): ^{1}H NMR: ($CDCl_3$) δ 8.13(d, 1H); 7.42 (d, 1H); 7.87 (m, 2H); 7.64 (m, 2H); 7.59 (m. 1H); 7.53(s, 1H); 7.09 (s, 1H). ^{13}C NMR ($CDCl_3$) δ 188.7, 162.4, 146.7, 140.5, 137.9, 129.0, 128.5, 128.6, 116.3. Calculated mass for ($C_{15}H_{10}O_2Br_2$) 379.90; Observed 381.03. IR (nujal) cm^1: 1651, 1593, 1539.

(E)-3-(4-Fluorophenyl)-1-phenylprop-2-en-1-one (3h): ^{1}H NMR: ($CDCl_3$) δ 8.06(d, 1H); 7.89 (d, 1H); 7.64 (m, 2H); 7.73 (m. 1H); 7.59 (m, 2H); 7.51(s, 1H); 7.09 (s, 1H). ^{13}C NMR ($CDCl_3$) δ 188.4, 162.3, 145.7, 142.5, 138.9, 137.4, 129.05, 128.1, 125.6, 116.3. Calculated mass for ($C_{15}H_{11}OF$) 226.25; Observed 226. 23. IR (nujal) cm^1: 1651, 1597, 1541. IR (nujal) cm^1: 1659, 1601, 1545.

(E)-3-(3-Nitrophenyl)-1-phenylprop-2-en-1-one (3i): ^{1}H NMR: ($CDCl_3$) δ 8.16(d, 1H); 7.82 (d, 1H); 7.74 (m, 2H); 7.69 (m. 1H); 7.63 (m, 2H); 7.55(s, 1H); 7.29 (s, 1H). ^{13}C NMR ($CDCl_3$) δ 189.9, 162.3, 147.7, 141.5, 137.9, 137.47, 134.6, 129.52, 128.5, 123.1. Calculated mass for ($C_{15}H_{11}NO_3$) 253.25; Observed 256. 25. IR (nujal) cm^1: 1654, 1589, 1539.

(E)-3-(4-Chlorophenyl)-1-phenylprop-2-en-1-one (3j) ^{1}H NMR: ($CDCl_3$) δ 8.01(d, 1H); 7.81 (d, 1H); 7.66 (m, 2H); 7.73 (m. 1H); 7.61 (m, 2H); 7.55(s, 1H); 7.19 (s, 1H). ^{13}C NMR ($CDCl_3$) δ 189.7, 164.2, 148.7, 144.5, 138.1, 134.5, 128.15, 126.1, 123.6, 114.3. Calculated mass for ($C_{15}H_{11}OCl$) 242.70; Observed 243.83. IR (nujal) cm^1: 1653, 1599, 1544.

(E)-3-(4-*t*-Butylphenyl)-1-phenylprop-2-en-1-one (3k): ^{1}H NMR: ($CDCl_3$) δ 8.06(d, 1H); 7.89 (d, 1H); 7.64 (m, 2H); 7.61 (m. 1H); 7.57 (m, 2H); 7.22(s, 1H); 1.35 (s, 9H). ^{13}C NMR ($CDCl_3$) δ 186.1, 150.5, 146.2, 138.7, 134.5, 128.1, 124.5, 128.15, 126.1, 34.3, 31.6. Calculated mass for ($C_{19}H_{20}O$) 264.36; Observed 264.43. IR (nujal) cm^1: 165,0 1599, 1548.

(E)-1-Phenyl-3-(pyridine-2-yl)prop-2-en-1-one (3l): ^{1}H NMR: ($CDCl_3$) δ 8.84 (m, 2H); 8.02(d, 1H); 7.98 (m, 1H); 7.59(d, 1H); 7.64 (m. 1H); 7.57 (m, 2H). ^{13}C NMR ($CDCl_3$) δ 189.7, 154.7, 148.6, 143.7, 137.9, 134.5, 129.1, 128.5, 124.3, 122.7,122.1. Calculated mass for ($C_{14}H_{11}NO$) 209.08; Observed 210.03. IR (nujal) cm^1: 1655, 1594, 1538.

(E)-1-Phenyl-3-(pyridine-3-yl)prop-2-en-1-one (3m): ^{1}H NMR: ($CDCl_3$) δ 8.84 (m, 2H); 8.02(d, 1H); 7.98 (m, 1H); 7.59(d, 1H); 7.64 (m. 1H); 7.57 (m, 2H). ^{13}C NMR ($CDCl_3$) δ 189.7, 154.7, 148.6, 143.7, 137.9, 134.5, 129.1, 128.5, 124.3, 122.7,122.1. Calculated mass for ($C_{14}H_{11}NO$) 209.08; Observed 210.03. IR (nujal) cm^1: 1662, 1607, 1541.

(E)-1-Phenyl-3-(pyridine-4-yl)prop-2-en-1-one (3n): ^{1}H NMR: ($CDCl_3$) δ 8.74 (m, 2H); 8.06(d, 1H); 7.88 (m, 1H); 7.69(d, 1H); 7.62 (m. 1H); 7.54 (m, 2H). ^{13}C NMR ($CDCl_3$) δ 189.9, 155.7, 148.9, 145.7, 138.9, 135.5, 129.7, 128.9, 125.3, 121.7,120.2. Calculated mass for ($C_{14}H_{11}NO$) 209.08; Observed 210.05. IR (nujal) cm^1: 1655, 1593, 1544.

(E)-1-(Phenyl)-1-phenylprop-2-en-1-one (3p): ^{1}H NMR: ($CDCl_3$) δ 8.06 (d, 1H); 7.06 (m, 5H); 7.59 (d, 1H); 7.73 (m, 5H). ^{13}C NMR ($CDCl_3$) δ 189.3, 145.1, 137.9, 135.2, 134.5, 129.2, 128.5, 127.9. Calculated mass for ($C_{15}H_{12}O_2$) 208.8; Observed 209.5. IR (nujal) cm^1: 1646, 1588, 1536.

(E)-1-(Furan-2-yl)-1-phenylprop-2-en-1-one (3o): ^{1}H NMR: ($CDCl_3$) δ 8.17 (d, 1H); 7.9(d, 1H); 7.89 (m, 3H); 7.73 (m, 1H); 7.65 (d, 1H); 6.87 (m, 2H). ^{13}C NMR ($CDCl_3$) δ 189.7, 151.7, 143.7, 137.9, 134.5, 129.2, 128.5, 127.3, 113.8, 112.7. Calculated mass for ($C_{13}H_{10}O_2$) 198.08; Observed 199.05. IR (nujal) cm^1: 1657, 1597, 1551.

General procedure for synthesis of 3,4-dihydro-3,5-disubstituted pyrazoles 4(a-p): The suspension of the corresponding chalcone **3** (440 mg, 2 mmol) and hydrazine hydrate (1.5 mL, 10 mmol) in ethanol (10 mL) was refluxed for 3 h and left overnight at room temperature. The solid precipitate formed was filtered off. The resulting compound obtained was recrystallized from hot ethanol to afford compound **4** as buff crystals.

3-Phenyl-5(3,4,5-trimethoxyphenyl)-4,5-dihydro-1*H*-pyrazole (4a): ^{1}H NMR: ($CDCl_3$) δ 7.67 (m, 5H); 7.0 (s, 1H); 6.52 (m, 2H); 3.92 (d, 2H); 3.83 (s, 9H); 3.31 (t, 1H). ^{13}C NMR ($CDCl_3$) δ 152.6, 151.7, 137.2, 136.4, 128.8, 102.2, 56.1, 51.7, 42.4. Calculated mass for ($C_{18}H_{20}N_2O_3$) 312.15; Observed 313.25. IR (KBr, cm^{-1}): 3391, 3322, 1632, 1603.

5-(3,5,-Dimethoxyphenyl)-3-phenyl-4,5-dihydro-1*H*-pyrazole (4b): ^{1}H NMR: ($CDCl_3$) δ 7.52 (m, 5H); 6.96 (s, 1H); 6.52 (m, 32H); 3.94 (d, 2H); 3.81 (s, 6H); 3.43 (t, 1H). ^{13}C NMR ($CDCl_3$) δ 151.7, 147.8, 136.8, 136.2, 131.1, 128.8, 121.2, 118.9, 56.3, 51.4, 42.8. Calculated mass for ($C_{17}H_{18}N_2O_2$) 282.14; Observed 283.21. IR (KBr, cm^{-1}): 3391, 3322, 1632, 1603.

5-(3,4-Dimethoxyphenyl)-3-phenyl-4,5-dihydro-1*H*-pyrazole (4c): ^{1}H NMR: ($CDCl_3$) δ 7.52 (m, 5H); 7.1 (s, 1H); 6.63 (m, 3H); 3.94 (d, 2H); 3.81 (s, 6H); 3.33 (t, 1H). ^{13}C NMR ($CDCl_3$) δ 161.4, 151.5, 145.5, 137.2, 136.4, 128.8, 128.2, 104.3, 55.8, 51.3, 42.6. Calculated mass for ($C_{17}H_{18}N_2O_2$) 282.14; Observed 283.18. IR (KBr, cm^{-1}): 3398, 3332, 1635, 1609.

2-Methoxy-6-(3-phenyl-4,5-dihydro-1*H*-pyrazol-5-yl)phenol (4d): ^{1}H NMR: ($CDCl_3$) δ 7.67 (m, 5H); 7.0 (s, 1H); 6.68 (m, 3H); 5.35 (s, 1H); 3.93 (d, 2H); 3.81 (s, 3H); 3.43 (t, 1H). ^{13}C NMR ($CDCl_3$) δ 151.7, 147.8, 143.5, 139.2, 131.1, 128.8, 118.2, 51.5, 42.9. Calculated mass for ($C_{16}H_{16}N_2O_2$) 268.15; Observed 269.19. IR (KBr, cm^{-1}): 3392, 3323, 1636, 1606.

2-(3-Phenyl-4,5-dihydro-1*H*-pyrazol-5-yl)phenol (4e): ^{1}H NMR: ($CDCl_3$) δ 7.52 (m, 5H); 7.12 (m, 4H); 6.98 (s, 1H); 5.37 (s, 1H); 3.95 (d, 2H); 3.38 (t, 1H). ^{13}C NMR ($CDCl_3$) δ 154.1, 151.3, 137.4, 131.2, 130.9, 128.6, 126.2, 121.1, 115.7, 44.5, 42.9. Calculated mass for ($C_{15}H_{14}N_2O$) 238.11; Observed 239.16. IR (KBr, cm^{-1}): 3399, 3328, 1642, 1613.

2-Bromo-3-(3-phenyl-4,5-dihydro-1*H*-pyrazol-5-yl)phenol (4f): ^{1}H NMR: ($CDCl_3$) δ 7.67 (m, 5H); 7.08 (s, 1H); 6.72 (m, 3H); 5.35 (s, 1H); 3.94 (d, 2H); 3.91 (t, 1H). ^{13}C NMR ($CDCl_3$) δ 156.4, 151.7, 147.1, 136.4, 131.1, 128.9, 128.2, 121.7, 114.3, 47.6, 41.9. Calculated mass for ($C_{15}H_{13}N_2OBr$) 316.02; Observed 317.14. IR (KBr, cm^{-1}): 3393, 3326, 1633, 1605.

3,5-Dibromo-2-(3-phenyl-4,5-dihydro-1*H*-pyrazol-5-yl)phenol (4g): ^{1}H NMR: ($CDCl_3$) δ 7.67 (s, 1H); 7.52 (m, 5H); 7.02 (s, 1H); 6.67 (m, 1H); 5.55 (s, 1H); 3.91 (d, 2H); 3.88 (t, 1H). ^{13}C NMR ($CDCl_3$) δ 158.4, 157.4, 151.7, 139.1, 136.4, 131.3, 128.8, 128.1, 125.7, 118.3, 42.6, 41.1. Calculated mass for ($C_{15}H_{13}N_2OBr$) 393.93; Observed 395.04. IR (KBr, cm^{-1}): 3397, 3322, 1634, 1613.

5-(4-Fluorophenyl)-3-phenyl-4,5-dihydro-1*H*-pyrazole (4h): ^{1}H NMR: ($CDCl_3$) δ 7.54 (m, 5H); 7.27 (m, 4H); 7.03 (s, 1H); 3.87 (d, 2H); 3.45 (t, 1H). ^{13}C NMR ($CDCl_3$) δ 160.9, 151.7, 139.2, 136.7, 131.08, 128.5, 128.2, 114.8, 51.1, 42.6. Calculated mass for ($C_{17}H_{13}N_2F$) 240.11; Observed 241.25. IR (KBr, cm^{-1}): 3391, 3322, 1637, 1602.

5-(3-Nitrophenyl)-3-phenyl-4,5-dihydro-1*H*-pyrazole (4i): ^{1}H NMR: ($CDCl_3$) δ 8.18 (m, 2H); 7.68 (m, 2H); 7.52 (m, 5H); 3.87 (d, 2H); 3.45 (t, 1H). ^{13}C NMR ($CDCl_3$) δ, 151.17, 147.4, 144.4, 136.4, 133.8, 131.02, 129.4, 128.2, 1124.8, 50.9, 42.4. Calculated mass for ($C_{15}H_{13}N_3FO_2$) 267.28; Observed 268.12. IR (KBr, cm^{-1}): 3398, 3325, 1637, 1602.

5-(4-Chlorophenyl)-3-phenyl-4,5-dihydro-1*H*-pyrazole (4j): ^{1}H NMR: ($CDCl_3$) δ 7.54 (m, 5H); 7.27 (m, 4H); 7.03 (s, 1H); 3.87 (d, 2H); 3.45 (t, 1H). ^{13}C NMR ($CDCl_3$) δ 151.7, 136.4, 132.3, 131.12, 128.6, 127.4, 114.8, 51.4, 41.9. Calculated mass for ($C_{15}H_{13}N_2Cl$) 256.73; Observed 256.81. IR (KBr, cm^{-1}): 3396, 3323, 1639, 1605.

5-(4-*tert*-Butylphenyl)-3-phenyl-4,5-dihydro-1*H*-pyrazole (4k): ^{1}H NMR: ($CDCl_3$) δ 7.52 (m, 5H); 7.21 (m, 4H); 7.03 (s, 1H); 3.94 (d, 2H); 3.63 (t, 1H) 1.35 (s, 9H). ^{13}C NMR ($CDCl_3$) δ 151.7, 149.3, 140.4, 136.4, 131.3, 131.12, 128.6, 125.4, 51.5, 43.1, 34.2, 31.3. Calculated mass for ($C_{19}H_{22}N_2$) 278.18; Observed 279.22. IR (KBr, cm^{-1}): 3399, 3322, 1638, 1606.

2-(3-Phenyl-4,5-dihydro-1*H*-pyrazol-5yl)-pyridine (4l): ^{1}H NMR: ($CDCl_3$) δ 8.46 (m, 1H); 7.55 (m, 5H); 7.49 (m, 3H); 7.05 (s, 1H); 3.92 (d, 2H); 3.73 (t, 1H). ^{13}C NMR ($CDCl_3$) δ 152.2, 151.7, 144.4, 136.4, 131.0, 128.8, 127.9, 121.0, 51.2, 42.6. Calculated mass for ($C_{14}H_{13}N_3$) 223.11; Observed 224.18. IR (KBr, cm^{-1}): 3392, 3324, 1631, 1604.

3-(3-Phenyl-4,5-dihydro-1*H*-pyrazol-5yl)-pyridine (4m): ^{1}H NMR: ($CDCl_3$) δ 7.66 (m, 1H); 7.52 (m, 5H); 7.43 (m, 3H); 7.15 (s, 1H); 3.88 (d, 2H); 4.13 (t, 1H). ^{13}C NMR ($CDCl_3$) δ 151.7, 143.2, 136.5, 131.3, 127.8, 126.9, 121.5, 52.2, 41.9. Calculated mass for ($C_{14}H_{13}N_3$) 223.11; Observed 224.15. IR (KBr, cm^{-1}): 3394, 3328, 1637, 1601.

4-(3-Phenyl-4,5-dihydro-1*H*-pyrazol-5yl)-pyridine (4n): ^{1}H NMR: ($CDCl_3$) δ 8.15 (d, 2H); 7.56 (m, 5H); 7.35 (d, 2H); 7.05 (s, 1H); 3.94 (d, 2H); 3.83 (t, 1H). ^{13}C NMR ($CDCl_3$) δ 152.6, 149.7, 149.4, 136.6, 128.3, 128.1, 123.9, 123.0, 51.1, 42.8. Calculated mass for ($C_{14}H_{13}N_3$) 223.11; Observed 224.14. IR (KBr, cm^{-1}): 3396, 3323, 1634, 1605.

5-(Furan-2-yl)3-phenyl-4,5-dihydro-1*H*-pyrazole (4o): ^{1}H NMR: ($CDCl_3$) δ 7.52 (m, 5H); 6.43 (m, 3H); 7.15 (s, 1H); 3.94 (d, 2H); 4.1 (t, 1H). ^{13}C NMR ($CDCl_3$) δ 152.4, 151.4, 141.5, 136.4, 131.0, 128.8, 128.2, 111.0, 109.2, 50.8, 43.7. Calculated mass for ($C_{13}H_{12}N_2O$) 212.19; Observed 213.27. IR (KBr, cm^{-1}): 3396, 3324, 16323, 1601.

3,5-Diphenyl-4,5-dihydro-1*H*-pyrazole (4p): ^{1}H NMR: ($CDCl_3$) δ 7.52 (m, 5H); 7.29 (m, 5H); 7.03 (s, 1H); 3.91 (d,

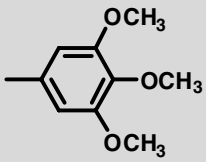
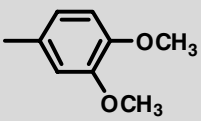
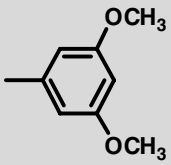
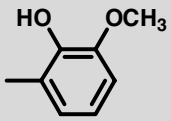
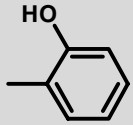
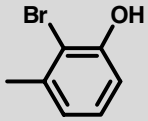
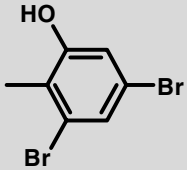
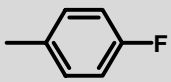
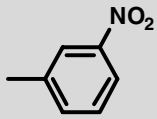
2H); 3.1 (t, 1H). ^{13}C NMR ($CDCl_3$) δ 151.7, 143.5, 136.4, 131.2, 128.5, 128.2, 126.9, 126.0, 51.8, 42.4. Calculated mass for ($C_{15}H_{14}N_2$) 222.12; Observed 223.29. IR (KBr, cm^{-1}): 3398, 3327, 1636, 1605.

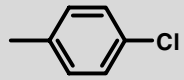
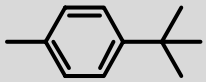
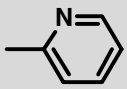
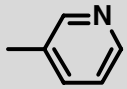
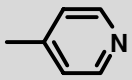
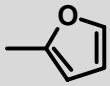
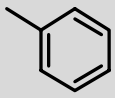
RESULTS AND DISCUSSION

Initially, we screened all the synthesized chalcones **3(a-p)** and pyrazoles **4(a-p)** to investigate their antibacterial activity against *Staphylococcus aureus*, using ampicilin as a standard and antifungal activity against *Candida albicans* using miconazole as a standard by the disc diffusion method. The compounds were dissolved in propylene glycol (PG) at concentrations of 0.2-0.22 mg/mL. Propylene glycol did not show any inhibitory effect on the growth of the test organisms. The results of the antibacterial and antifungal activities are given in Table-1. Pyrazole derivatives have higher inhibition zones than chalcones derivatives, which represent their better activity. Activity data in the Table-1 reveals that the compounds with electron withdrawing substituent on aromatic ring like Br, F, Cl, NO_2 (entry 7-10) are more active than the compounds with electron releasing substituent on aromatic ring (entry 1-6). It reveals that the small structural differences can have greater influences on the antimicrobial activity (entry 2-4). Similarly, the same substitution pattern on different positions (entry 12-14) leads to a different role in the inhibition of microorganisms.

For our next study, we selected the pyrazole derivatives, which showed a better inhibition zone by the disc diffusion method to determine their MIC values for different bacterial and fungal species.

TABLE-1
in vitro ANTIBACTERIAL AND ANTIFUNGAL ACTIVITY RESULTS OF **3a-p** AND **4a-p** WHICH WERE OBTAINED BY THE DISC DIFFUSION METHOD

Entry	R =	Compound	*S. aureus*	*C. albicans*	Compound	*S. aureus*	*C. albicans*
1		**3a**	7	16	**4a**	10	19
2		**3b**	7	18	**4b**	12	21
3		**3c**	9	18	**4c**	13	22
4		**3d**	11	20	**4d**	15	23
5		**3e**	12	21	**4e**	12	25
6		**3f**	12	25	**4f**	12	20
7		**3g**	14	29	**4g**	17	18
8		**3h**	16	45	**4h**	18	34
9		**3i**	20	42	**4i**	19	36

10		**3j**	18	32	**4j**	17	24
11		**3k**	12	24	**4k**	12	18
12		**3l**	14	30	**4l**	15	28
13		**3m**	18	37	**4m**	19	35
14		**3n**	21	40	**4n**	21	39
15		**3o**	16	28	**4o**	17	28
16		**3p**	10	15	**4p**	12	18
17	Ampicilin	–	20	–	–	–	–
18	Miconazole	–	–	10	–	–	–

Antibacterial activity: The antibacterial activity was determined by well plate method in Mueller-Hinton Agar. The compounds were tested against a panel of pathogenic microorganisms, including *Escherichia coli, Staphylococcus aureus* and *Pseudomonas aeruginosa*. Microorganism strains were maintained on nutrient agar medium at 37 °C. The cultures were inoculated in fresh 10 mL Nutrient Broth to yield an initial suspension of approximately 10^{100} cfu/mL. All broths were then incubated statically at the aforementioned temperatures for microorganisms for 18-24 h so that all cells were in the stationary phase. Susceptibility of the test organism to the compounds was determined by employing the well plate technique. The bacterial suspensions were diluted tenfold in distilled water and 0.1 mL from the appropriate dilution was spread plated on nutrient agar in order to obtain a population of approximately 10^6 cfu/plate. The wells were dug in each Petri plate using a sterilized cork borer. The test compounds were dissolved in DMSO and appropriate dilutions were made (5 and 0.5 µg/mL). The same procedure was repeated for other microorganisms. Each experiment was carried out in triplicate. After the inoculation of organism and compound, the Petri plates were incubated for 24 h at 37 °C. After the incubation, the inhibition zone was measured and the values for dimethyl sulphoxide (DMSO) were subtracted to get the actual values.

Antifungal activity: The fungal strains used in this study were *Aspergillus flavus, Chrysosporium keratinophilum* and *Candida albicans*. The required amounts of each fungal strain were removed from the stock and suspended in 5 mL of distilled water with 2 drops of Tween-80. This suspension was uniformly spread on Petri plates containing potato dextrose agar media using sterile swabs. After applying the samples into the wells formed by using the same technique for tests on bacteria, the plates were incubated at 25 °C for 3 days. The plates were then examined for the presence of zones of inhibition and the results were recorded. Miconazole was used as a standard drug. The MIC values of the compounds were determined by the two-fold serial dilution technique.

All the compounds exhibited variable antibacterial and antifungal activity against the tested bacterial and fungal strains and the results indicated that among the tested compounds, **4h**, **4j**, **4l**, **4m** and **4n** all are having electron withdrawing group Cl, F, NO_2 and N at different position of the phenyl ring showed good antibacterial and antifungal activity towards all bacterial strains at concentrations of 3.25 to 1.25 µg/mL and all fungal strains at concentrations of 1.75 to 0.25 µg/mL, respectively compared to standard drugs. Rest of the compounds showed fair or moderate activity. Results of antibacterial and antifungal studies are presented in Table-2.

Fluorine substituted compound **4h** (MIC: 2.75 µg/mL) exhibited high activity against gram negative *E. coli* compared to other halogen substituted compound. Also, antifungal activity of compound **4h** exhibited equipotent activity (MIC: 1.75 µg/mL) against both *Candida albicans* and *Chrysosporium keratinophilum.*

The antifungal activity of pyrazole compound increased 2 to 5 times by replacing the substituted phenyl ring with pyridine. Compound **4m** (MIC: 1.5 µg/mL) shows 3 fold more active against gram negative bacteria of *E. coli* and *Staphylococcus aureus*. Its antifungal activity also improves by 10 fold (MIC 0.5 µg/mL) against *Chrysosporium keratinophilum*, *Candida albicans* and was considered as an effective antifungal agent. Inspired by this finding, we further synthesized pyridine ring substituted at 3- (compound **4m**) and 4- (compound **4n**) positions to test these modifications for biological activity. The results of antimicrobial and antifungal studies of these compounds reveals that, compound **4n** display 10 fold more potency than standard compounds and turn out to be highly potent antibacterial (MIC 1.25 µg/mL) and antifungal (MIC 0.25 µg/mL) agent.

This study reveals that the presence of substituents on the phenyl ring at 4-position plays important role in antimicrobial

TABLE-2
in vitro ANTIMICROBIAL AND ANTIFUNGAL ACTIVITIES OF PYRAZOLE DERIVATIVES (MIC)

Entry	Compound	Bacterial strain (μg/mL)			Fungal strain		
		E. coli	*S. aureus*	*P. aeruginosa*	*A. flavus*	*C. keratinophilum*	*C. albicans*
1	**4g**	5.0	4.5	15.0	6.75	4.75	4.0
2	**4h**	2.75	2.75	7.5	3.0	1.75	1.75
3	**4i**	4.25	3.75	9.5	5.5	3.0	2.5
4	**4j**	3.0	2.50	9.5	5.25	2.75	2.0
5	**4l**	2.5	2.0	5.0	3.25	0.75	0.75
6	**4m**	1.50	2.0	3.5	1.5	0.5	0.5
7	**4n**	1.25	1.25	2.0	0.5	0.25	0.25
8	Ampicillin	2.25	2.0	3.0	–	–	–
9	Miconazole	–	–	–	0.2	0.45	0.45

activity. The enhanced activity of **4n**, **4m**, **4l**, **4j** and **4h** is due to the presence of electron withdrawing groups attached at 3, or 4-position of phenyl rings. However, in general, compounds containing heteroatom as a part of the ring showed better antibacterial and antifungal activity than the compounds with ring substituents. The absence of such pharmacophore on phenyl ring fails to exhibit both antibacterial as well as antifungal activity. From the antimicrobial results it is cleared that the pyrazole compounds are potential antifungal agents than antibacterial agents.

Conclusion

In conclusion, chalcone and pyrazole compounds with similar substituents were tested and compare them with antimicrobial screening. Among the tested compounds, pyrazoles were found to be the most active against Gram-negative bacterial strain *E. coli* compared with standard ampicillin. A combination of one heterocyclic systems namely pyridine has enhanced the pharmacological effect and hence they are ideally suited for further modifications to obtain more efficacious antibacterial and antifungal compounds.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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