PYRIDINE AND BENZOISOTHIAZOLE BASED PYRAZOLINES: SYNTHESIS, CHARACTERIZATION, BIOLOGICAL ACTIVITY, MOLECULAR DOCKING AND ADMET STUDY

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Synthesis, characterization, antioxidant and antimicrobial activities of novel pyrazolines and phenylpyrazoline containing substituted pyridine and piperazine benzoisothiazole moieties have been reported. When these synthesized compounds were exposed for antioxidant screening, some among them exhibited prominent DPPH radical scavenging activity and superoxide radical (SOR) scavenging activity where ascorbic acid used as standard. During the antimicrobial screening compounds, some derivatives were found to be very active against *Cryptococcus neoformans*, which was supported on the basis of higher free binding energies with methionyl-tRNA synthetase.

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INTRODUCTION

Pyrazolines, a class of five-membered heterocycles, is recognized for never-ending research and development in terms of therapeutic potential. As a result, pyrazolines have become an obvious core of numerous drugs having diverse activities.[1] The broad spectrum of activities shown by the pyrazolines encourages searchers to modify their structures by building with some biologically important heterocyclic compounds like alkyl, aromatic, heterocyclic rings and other groups at different positions on the ring which helps to create a novel class of desired compounds.

Pyrazoline and its analogs act as significant pharmacies and synthons in the field of organic chemistry and drug designing. They are well-acknowledged for pharmacologically interesting heterocyclic systems through recent literature survey.[2] Pyrazolines possess a wide range of biological properties such as anticancer,[3-5] anti-inflammatory,[6] antibacterial,[7] anti-depressive and anticonvulsant,[8] antimicrobial,[9] antinociceptives[10] and enzyme inhibitors.[11] Thus pyrazolines are well recognized for various biological activities.[12,13] Hence, synthesis of new heterocycles bearing pyrazolines remains the core area of research. Numerous methods were developed for the synthesis of pyrazoline and their synthetic counterparts, among which the classical method involves cyclization of the Michael acceptor unit (chalcone) with hydrazine hydrate or phenylhydrazine in the presence of cyclizing agents like acetic acid or simple heating procedure.[14]

Thus, considering the biological significance of pyrazolines and in continuation of our efforts in the development of biologically active entities;[15-17] in the present work, we report the synthesis, characterization, antimicrobial and antioxidant activities of some novel pyrazolines bearing pyridine and benzoisothiazole containing piperazine moiety.

The results of biological activities were supported by molecular docking studies. ADMET study was performed to know the drug-likeness and toxicity profile of the synthesized compounds.

MATERIALS AND METHODS

Melting points were recorded in an electrothermal melting point apparatus and were uncorrected. The consumption of starting material and formation of the products was monitored on silica precoated thin layer chromatography plate using 40 % ethyl acetate : n-hexane as the mobile phase. FTIR spectra were recorded using KBr pellets (100 mg) on Shimadzu FT-IR spectrophotometer. ^{1}H and ^{13}C NMR spectra were recorded on Bruker 400 MHz spectrometer and chemical shifts were expressed in δ ppm with reference to tetramethylsilane (TMS) as the internal standard.

In the antimicrobial activity, the zones of inhibition and the antioxidant activity performed spectrophotometrically were expressed as mean ± SD of three replicates.

General procedure for the synthesis of pyrazolines

A mixture of chalcone (0.7g, 0.16 mol) and hydrazine hydrate (1.0 mL) or phenylhydrazine in ethanol (1.0 mL) was stirred for 2 h at 25-35 °C. Sometimes heating at 45-50 °C was required for dehydration. The precipitated solid was filtered off to get a crude product which was crystallized with hot ethanol to get pure white color material.

2-((4-(4,5-Dihydro-3-phenyl-1*H*-pyrazol-5-yl)-2-methoxyphenoxy)methyl)-3,4-dimethoxypyridine (1)

^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 2.89 (dd, *J* = 10.4 & 10.8 Hz, 1H), 3.39 (dd, *J* = 10.4 & 10.8 Hz, 1H), 3.72 (3H, s), 3.77 (3H, s), 3.90 (3H, s), 4.78 (t, 1H), 5.08 (s, 2H), 6.85 (d, *J* = 8.4 Hz, 1H), 7.00 (s, 1H), 7.04 (d, *J* = 8.4 Hz, 1H), 7.14 (d, *J* = 5.6 Hz, 1H), 7.31 (d, *J* = 6.8 Hz, 1H), 7.37 (t, *J* = 7.2 Hz, 2H) , 7.50 (s, 1H, -NH), 7.61 (d, *J* = 7.6 Hz, 2H), 8.20 (d, *J* = 5.6 Hz, 1H); ^{13}C NMR (101.3 MHz, $CDCl_3$) δ ppm 41.2, 54.9, 55.2, 61.42, 62.58, 68.11, 107.65, 109.61, 114.65, 116.4(2), 118.51, 118.94, 127.44, 130.21, 134.70, 144.66, 145.79, 148.07, 149.85, 149.95, 154.16, 157.61, 158.75; ESIMS m/z 420.4 $[M + H]^+$.

2-(5-(4-((3,4-Dimethoxypyridin-2-yl)methoxy)-3-methoxyphenyl)-4,5-dihydro-1*H*-pyrazol-3-yl)phenol (2*)

^{1}H-NMR (400 MHz, DMSO-d_6) δ ppm 3.07 (dd, *J* =10.8 Hz & 11.2 Hz, 1H); 3.56 (dd, *J* = 10.4 Hz & 11.2 Hz, 1H); 3.72 (s, 3H); 3.77 (s, 3H); 3.90 (s, 3H); 4.8 (t, 1H); 5.03 (s, 2H); 6.87-6.91 (m, 2H); 7.03 (s, 1H); 7.07 (d, *J* = 8.4 Hz, 1H); 7.15 (d, *J* = 5.6 Hz, 1H); 7.22 (t, *J* = 8.0 Hz, 2H); 7.29 (d, *J* =7.6 Hz, 1H); 8.21 (d, *J* = 5.6 Hz, 1H); 10.19 (s, 1H); 11.18 (s, 1H); ^{13}C NMR (101.3 MHz, $CDCl_3$) δ ppm 41.51; 55.59; 55.7; 61.4; 62.5; 68.1; 107.6; 109.6; 114.6; 116.4(2); 118.5; 118.9; 127.4; 130.2; 134.7; 144.6; 145.7; 148.0; 149.8; 149.9;154.1; 157.6; and 158.7;): FT-IR (KBr) cm^{-1} 761, 825, 1163, 1224, 1352, 1496, 1591 & 1516; ESIMS m/z 436.4 $[M + H]^+$.

2-(5-(4-((3,4-Dimethoxypyridin-2-yl)methoxy)-3-methoxyphenyl)-4,5-dihydro-1*H*-pyrazol-3-yl)-4-methylphenol (3)

^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 2.23 (s, 3H), 3.05 (dd, *J* = 10.8 & 11.2, 1H), 3.31 (dd, *J* = 10.4 & 10.6, 1H), 3.75 (s, 3H), 3.78 (s, 3H), 3.90 (s, 3H), 4.99 (t, 1H), 5.06 (s, 2H), 6.45 (bs, 2H), 6.77 (d, *J* = 8.4 Hz, 1H), 6.95 (d, *J* = 7.2 Hz, 1H), 7.07 (d, *J* = 7.6 Hz, 1H), 7.11-7.16 (m, 3H), 7.59 (s, 1H), 8.22 (d, *J* = 5.6 Hz, 1H); ^{13}C NMR (101.3 MHz, $CDCl_3$) δ ppm 20.69, 31.19, 55.69, 55.91, 61.55, 68.19, 77.34, 107.74, 109.73, 113.95, 117.42, 118.54, 120.77, 123.64, 130.70, 131.03, 133.18, 142.67, 144.82, 145.89, 148.53, 149.87, 150.09, 154.14, 158.8; ESIMS m/z 450.4 $[M+H]^+$.

2-((4-(4,5-Dihydro-3-(2-methoxyphenyl)-1*H*-pyrazol-5-yl)-2-methoxyphenoxy)methyl)-3,4-dimethoxypyridine (4)

^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 2.91 (dd, *J* = 10.8 & 11.2 Hz, 1H), 3.38 (dd, *J* = 10.4 & 10.8 Hz, 1H), 3.71 (s, 3H), 3.74 (s, 3H), 3.75 (s, 3H), 3.87 (s, 3H), 4.69 (t, 1H), 5.00 (s, 2H), 6.85 (d, *J* = 7.2 Hz, 1H), 6.92 (t, 2H), 6.97 (s, 1H), 7.02 (dd, *J* = 5.2 & 4.4 Hz, 1H), 7.12 (d, *J* = 5.6 Hz, 1H), 7.27 (d, *J* = 7.6 Hz, 1H), 7.30 (d, *J* =2.8 Hz, 1H), 7.62 (d, *J* = 7.6 Hz, 1H), 8.19 (d, *J* = 5.6 Hz, 1H); ESIMS m/z 450.4 $[M + H]^+$.

2-((4-(3-(4-Fluorophenyl)-4,5-dihydro-1$_H$-pyrazol-5-yl)-2-methoxyphenoxy)methyl)-3,4-dimethoxy pyridine (5)

^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 2.84 (dd, *J* = 10.8 & 10.8 Hz, 1H), 3.39 (dd, *J* = 9.6.4 & 10.4 Hz, 1H), 3.71 (s, 3H), 3.74 (s, 3H), 3.77 (s, 3H), 3.90 (s, 3H), 4.66 (t, 1H), 5.08 (s, 2H), 6.88 (d, *J* = 7.2 Hz, 1H), 6.92 (t, 2H), 6.97 (s, 1H), 7.02 (dd, *J* = 5.2 & 4.4 Hz, 1H), 7.11 (t, *J* = 8.0 Hz, 2H), 7.15 (d, *J* = 5.6 Hz, 1H), 7.55 (dd, *J* = 5.6 & 4.8 Hz, 2H), 8.23 (d, *J* = 5.6 Hz, 1H); 41.32, 55.76, 59.64, 64.17, 71.99, 105.8, 109.51, 114.27, 115.29, 115.42, 118.44, 126.15, 126.54, (127.48, 127.58 F-C) , 129.03, 129.06, 135.56, 147.51, 148.75, 149.86, 150.11, 154.18, 161.55 (F-C), 164.0 (F-C), 164.19; ESIMS m/z 438.3 $[M + H]^+$.

6-(5-(4-((3,4-Dimethoxypyridin-2-yl)methoxy)-3-methoxyphenyl)-4,5-dihydro-1*H*-pyrazol-3-yl)-2*H*-benzo[*b*][1,4]oxazin-3(4*H*)-one (6)

^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 2.79 (dd, *J* = 9.2 & 9.6 Hz, 1H), 3.71 (s, 3H), 3.76 (s, 3H), 3.79 (dd, *J* = 10.8 & 10.8 Hz, 1H), 3.90 (s, 3H), 4.59 (s, 2H), 4.75 (s, 2H) 5.02 (s, 2H), 6.85 (d, *J* = 7.2 Hz, 1H), 6.94 (d, *J* = 8.4 Hz, 1H), 6.95 (s, 1H), 7.05 (d, *J* = 8.4 Hz, 1H), 7.09 (d, *J* = 8.4 Hz, 1H), 7.14 (d, *J* = 5.6 Hz, 1H), 7.25 (s, 1H), 7.41 (s, 1H), 8.22 (d, *J* = 5.6 Hz, 1H), 10.21 (s, 1H), 10.77 (s, 1H), ^{13}C NMR (101.3 MHz, $CDCl_3$) δ ppm 41.55, 55.65, 55.78, 61.48, 63.99, 67.16, 67.93, 107.70, 109.69, 113.04, 114.04, 116.57, 118.49, 122.17, 126.57, 127.60, 135.39, 144.18, 144.67, 145.77, 147.88, 149.79, 149.99, 150.73, 158.87, 165.57; FT-IR (KBr) cm^{-1} 1597, 1521, 1498, 1354, 1228, 1166, 828, 767; ESIMS m/z 491.4 $[M + H]^+$.

2-((4-(4,5-Dihydro-3-phenyl-1*H*-pyrazol-5-yl)-2-methoxyphenoxy)methyl)-4-methoxy-3,5-dimethylpyridine (7)

^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 2.22 (s, 3H), 2.25 (s, 3H), 2.89 (dd, *J* = 10.4 & 10.8 Hz, 1H), 3.39 (dd, *J* = 10.4 & 10.8 Hz, 1H), 7.04 (d, *J* = 8.4 Hz, 1H), 3.73 (s, 6H), 4.78 (t, 1H), 5.08 (s, 2H), 6.85 (d, *J* = 8.4 Hz, 1H), 7.00 (s, 1H), 7.04 (d, 1H), 7.31 (d, *J* = 6.8 Hz, 1H), 7.37 (t, *J* = 7.2 Hz, 2H), 7.50 (s, 1H, -NH), 7.61 (d, *J* = 7.6 Hz, 2H), 8.20 (s, 1H); ^{13}C NMR 101.3 MHz, $CDCl_3$) δ ppm 10.7, 13.2, 41.3, 55.8, 59.7, 64.1, 72.1, 109.3, 114.3, 118.5, 125.8(2), 126.2, 126.6, 128.4(2), 128.6, 132.7, 135.7, 147.6, 148.8, 149.9, 151.2, 154.2, 164.2; ESIMS m/z 418.3$[M + H]^+$.

2-(5-(4-((4-Methoxy-3,5-dimethyl pyridin-2-yl)methoxy)-3-methoxy phenyl) -4,5-dihydro-1*H*-pyrazol-3-yl)phenol (8)

^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 2.20 (s, 3H), 2.24 (s, 3H), 2.98 (dd, *J* = 12.8 & 11.2 Hz, 1H), 3.54 (dd, *J* = 10.4 & 10.4 Hz, 1H), 3.72 (s, 6H), 4.77(t, *J* = 10.0 Hz, 1H), 5.07(s, 2H), 6.85 - 6.89 (m, 3H), 7.02 (s, 1H), 7.05 (d, *J* = 8.0 Hz, 1H), 7.20 (t, *J* = 8.0 Hz, 1H), 7.27 (d, *J* = 8.0 Hz, 1H) , 7.75 (s, 1H, -NH), 8.19 (s, 1H), 11.15 (s, 1H, -OH); ^{13}C NMR (101.3 MHz, $CDCl_3$) δ ppm 10.7, 13.21, 41.4, 55.8, 59.7, 62.5, 71.9, 109.6, 114.3, 116.3(2), 118.5, 118.9, 126.2, 126.6, 127.4, 130.1, 134.8, 147.7, 148.7, 149.9, 154.0, 154.1, 157.5, 164.1; ESIMS m/z 434.3$[M + H]^+$.

2-(5-(4-((4-Methoxy-3,5-dimethylpyridin-2-yl)methoxy)-3-methoxyphenyl)-4,5-dihydro-1*H*-pyrazol-3-yl)-4-methylphenol (9)

^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 2.20 (s, 3H), 2.24 (s, 3H), 2.36 (s, 3H), 2.99 (dd, *J* = 12.8 & 11.2 Hz, 1H), 3.54 (dd, *J* = 10.4 & 10.4 Hz, 1H), 3.72 (s, 6H), 4.77 (t, 1H), 5.08 (s, 2H), 6.85-6.89 (m, 2H), 7.02 (s, 1H), 7.05 (d, *J* = 8.0 Hz, 1H), 7.20 (t, *J* = 7.6 Hz, 1H), 7.27 (d, *J* = 8.0 Hz, 1H), 7.75 (s, 1H, -NH), 8.19 (s, 1H), 11.15 (s, 1H, -OH); ^{13}C NMR (101.3 MHz, $CDCl_3$) δ ppm 10.74, 13.21, 31.12, 41.49, 55.81, 59.72, 62.5, 71.9, 109.6, 114.3, 116.3, 118.5, 118.9, 126.2, 126.6, 127.4, 130.1(2), 134.8, 147.7, 148.7, 149.9, 154.0, 154.1, 157.5, 164.1; ESIMS m/z 448.4 $[M + H]^+$.

2-((4-(3-(4-Fluorophenyl)-4,5-dihydro-1*H*-pyrazol-5-yl)-2-methoxyphenoxy)methyl)-3,4-dimethoxypyridine (10)

^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 2.22 (3H, s), 2.25 (3H, s), 2.84 (1H, dd, *J* = 10.8 & 10.8 Hz), 3.37 (1H, dd, *J* = 10.8 &10.8 Hz), 3.73 (6H, s), 4.78 (1H, t, *J* = 10.4 Hz), 5.08 (2H, s), 6.85 (1H, d, *J* = 8.4 Hz), 7.00 (1H, s), 7.05 (1H, d, *J* = 8.4 Hz), 7.22 (1H, t, *J* = 8.0 Hz), 7.49 (1H, s), 7.65 (1H, t, *J* = 6.8 Hz), 8.20 (1H, s); ^{13}C NMR (101.3 MHz) in $CDCl_3$) δ ppm 10.7, 13.1, 41.3, 55.7, 59.6, 64.1, 71.9, 109.5, 114.2, 115.2, 115.4, 118.4, 126.1, 126.5, (127.4,127.5), (129.03, 129.06), 135.5,147.5, 148.7, 149.8, 150.1, 154.1, 161.5 (F-C), 164.0 (F-C), 164.1; ESIMS m/z 436.3 $[M + H]^+$.

6-(5-(4-((4-Methoxy-3,5-dimethylpyridin-2-yl)methoxy)-3-methoxyphenyl)-4,5-dihydro-1*H*-pyrazol-3-yl)-2*H*-benzo[*b*][1,4]oxazin-3(4*H*)-one (11)

^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 2.22 (s, 3H), 2.25 (s, 3H), 2.78 (dd, *J* = 11.2 & 11.2 Hz, 1H), 3.34 (dd, *J* = 12.8 Hz & 12.8 Hz, 1H), 3.73 (s, 3H), 3.74 (s, 3H), 4.59 (s, 2H), 4.75 (t, *J* = 10.8 Hz, 1H), 5.08 (s, 2H), 6.85 (d, *J* = 8.0 Hz, 1H), 6.93 (d, *J* = 8.4 Hz, 1H), 7.0 (s, 1H), 7.05 (d, *J* = 8.0 Hz, 1H), 7.09 (d, *J* = 8.8 Hz, 1H), 7.25 (s, 1H), 7.40 (s, 1H, -NH), 8.2 (s, 1H), 10.79 (bs, 1H); ^{13}C NMR (101.3 MHz, $CDCl_3$) δ ppm 10.7, 13.2, 41.5, 55.7,59.7, 63.8, 67.0, 71.7, 109.7, 113.0, 114.2, 116.4, 118.5, 122.0, 126.2, 126.5, 126.6, 127.4, 135.5, 144.1, 147.5, 148.6, 149.8, 150.6, 154.0, 164.2, 165.6; ESIMS m/z 490.3 $[M + H]^+$.

3-(4-(4-(4-(4,5-Dihydro-3-phenyl-1*H*-pyrazol-5-yl)-2-methoxyphenoxy)butyl)piperazin-1-yl)benzo[*d*]isothiazole (12)

^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 1.61 (m, 2H), 1.75 (m, 2H), 2.45 (t, 2H), 2.59 (bpeak, 4H), 2.98 (dd, *J* = 11.2 Hz & 11.2 Hz, 1H), 3.63 (dd, *J* = 10.4 Hz & 11.2 Hz, 1H), 3.43 (bpeak, 4H), 3.75 (s, 3H), 3.97 (t, 2H), 4.78 (t, 1H), 6.86 (d, *J* = 2.4Hz, 1H), 6.93 (d, *J* = 8.4 Hz, 1H), 7.01 (t, *J* = 2.4 Hz, 1H), 7.31 (t, *J* = 7.2 Hz, 1H), 7.43 (t, *J* = 6.8 Hz, 2H), 7.52 (d, *J* = 3.6 Hz, 1H, -NH), 7.56 (t, *J* = 5.4 Hz, 1H), 7.62 (d, *J* = 7.2 Hz, 2H), 8.05 (d, *J* = 8 Hz, 2H); ESIMS m/z 542.4 $[M + H]^+$.

2-(5-(4-(4-(4-(Benzo[*d*]isothiazol-3-yl)piperazin-1-yl)butoxy)-3-methoxyphenyl)-4,5-dihydro-1*H*-pyrazol-3-yl)phenol (13)

^{1}H-NMR (400 MHz, $CDCl_3$) δ ppm 1.61 (m, 2H), 1.75 (m, 2H) H_2, 2.42 (t, 2H), 2.59 (m, 4H), 2.98 (dd, *J* = 11.6 & 11.2 Hz, 1H), 3.43 (bpeak, 4H), 3.56 (dd, *J* = 10.4 & 10.4 Hz, 1H), 3.76 (s, 3H), 3.97 (t, 2H), 4.78 (t, 1H), 6.86-6.95 (m, 4H), 7.03 (d, *J* = 2.4 Hz, 1H), 7.23 (t, *J* = 8.0 Hz, 1H), 7.29 (dd, *J* = 1.2 & 8.4 Hz, 1H), 7.43 (t, *J* = 8.0 Hz, 1H), 7.56 (t, *J* = 8.0 Hz, 1H), 7.78 (d, *J* = 8.0 Hz, 1H), 8.04 (d, *J* = 8.4 Hz, 2H), 11.18 (s, 1H), 8.17 (d, *J* = 8.0 Hz , 2H); ^{13}C NMR (101.3 MHz, $CDCl_3$) δ ppm 23.2, 27, 41.6, 49.9(2), 52.9(2), 55.9, 58.1, 62.6, 68.7, 109.7, 113.0, 116.4, 118.6, 118.9, 120.4, 127.37, 127.60, 123.7, 123.8, 127.4, 127.4, 127.9, 130.2, 134.3, 148.1, 149.6, 152.6, 154.2, 157.6, 163.8; FT-IR (KBr) cm^{-1} 758, 838, 1137, 1236, 1253, 1375, 1484, 1592 & 1514; ESIMS m/z 558.4 $[M + H]^+$.

3-(4-(4-(4-(3-(4-Fluorophenyl)-4,5-dihydro-1*H*-pyrazol-5-yl)-2-methoxyphenoxy)butyl)piperazin-1-yl) benzo[*d*]isothiazole (14)

^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 1.74 (m, 2H), 1.90 (m, 2H), 2.51 (t, 2H), 2.66 (m, 4H), 3.00 (dd, *J* = 9.2 Hz & 9.2 Hz, 1H), 3.42 (dd, *J* = 10.8 Hz & 10.8 Hz, 1H), 3.56 (t, 4H), 3.85 (s, 3H), 4.06 (t, 1H), 4.88 (t, 2H), 6.87 (d, *J* = 8.0 Hz, 1H), 6.93 (d, *J* = 8.4 Hz, 1H), 6.99 (d, *J* = 2.4 Hz, 1H), 7.21 (t, 1H), 7.43 (t, 1H), 7.51 (d, *J* = 3.6 Hz, 2H), 7.65 (dd, *J* = 5.2 Hz & 5.6 H_Z, 2H), 8.05 (d, *J* = 8.4 Hz, 2H); ^{13}C NMR (101.3 MHz, $CDCl_3$) δ ppm 23.2, 27.0, 41.5, 49.95(2), 52.9(2), 55.9, 58.1, 64.2, 68.7, 109.7, 112.7, 115.3, 115.5, 118.5, 120.4, 123.8(2), 127.4, 127.6, 127.7, 127.9, 129.1, 135.0, 148.0, 149.6, 150.4, 152.6, 162.0 (F-C), 163.8, 164.0(F-C); ESIMS m/z 560.4 $[M + H]^+$.

6-((R)-5-(4-(4-(4-(Benzo[*d*]isothiazol-3-yl)piperazin-1-yl)butoxy)-3-methoxyphenyl)-4,5-dihydro-1*H*-pyrazol-3-yl)-2*H*-benzo[*b*][1,4]oxazin-3(4*H*)-one (15)

^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 1.61 (m, 2H), 1.74 (m, 2H), 2.42 (t, 2H), 2.59 (bpeak, 4H), 2.77 (dd, *J* = 10.8 Hz & 10.8 Hz, 1H), 3.31 (dd, *J* = 10.4 & 10.2 Hz, 1H), 3.33 (bpeak, 4H), 3.75 (t, 3H), 3.96 (t, 2H), 4.59 (s, 2H), 4.69 (t, 1H), 6.84 (d, *J* = 8.4 Hz, 2H), 6.926 (d, *J* = 7.6 Hz, 2H), 6.98 (d, *J* = 1.2 Hz, 1H), 7.08 (d, *J* = 8.0 Hz, 1H), 7.263 (d, *J* = 1.6 Hz, 1H), 7.41-7.45 (m, 2H), 7.55 (t, 1H), 8.05 (d, *J* = 8.0 Hz, 2H), ^{13}C NMR (101.3 MHz, $CDCl_3$) δ ppm 23.2, 27.0, 41.6, 49.9(2), 52.9(2), 55.9, 58.1, 63.9, 67.1, 68.7, 109.8, 112.9, 116.5, 118.5, 120.4(2), 122.2, 123.7, 123.8, 126.5, 127.4, 127.5, 127.9, 134.9, 144.2, 147.9, 149.5, 150.7, 152.6, 163.8, 165.6; ESIMS m/z 613.4 $[M + H]^+$.

3-(4-(4-(4-(3-(3-(4-Fluorophenyl)-1-isopropyl-1*H*-indol-6-yl)-4,5-dihydro-1*H*-pyrazol-5-yl)-2-methoxyphenoxy)butyl)-piperazine-1-yl)benzo[*d*]isothiazole (16)

^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 1.51 (d, 6H), 1.62 (m, 2H), 1.74 (m, 2H), 2.42 (m, 2H), 2.59 (bpeak, 4H), 2.95 (dd, *J* = 11.2 Hz & 10.8 Hz, 1H), 3.43 (bpeak, 4H), 3.50 (dd, *J* = 10.4 & 10.4 Hz, 1H), 3.76 (s, 3H), 3.97 (d, 2H), 4.78 (t, 1H), 4.81 (m, 1H), 6.89 (m, 3H), 7.03 (s, 1H), 7.24-7.32 (m, 3H), 7.43 (t, 1H), 7.56 (t, 1H), 7.62 (t, 2H), 7.71 (dd, *J* = 5.6 Hz & 5.6 Hz, 2H), 7.85 (s, 1H), 7.95 (s, 1H), 8.05 (d, *J* = 7.2 Hz, 2H); ^{13}C NMR (101.3 MHz, $CDCl_3$) δ ppm 22.7,

23.2, 27.1, 42.0, 47.3, 49.9(2), 52.9(2), 55.9, 58.2, 63.9, 68.8, 109.7, 110.0, 112.9, 115.4, 115.6, 116.6, 117.9, 118.1, 118.6, 120.1, 120.4, 121.8, 123.7, 123.8, 125.1, 126.0, 127.4, 127.9(2), 128.7, 128.8, 131.4, 135.6, 136.3, 147.9, 149.6, 152.6, 152.9, 163.8; ESIMS m/z 717.6 $[M + H]^+$.

3-(4-(4-(4-(4,5-Dihydro-1,3-diphenyl-1H-pyrazol-5-yl)-2-methoxyphenoxy)butyl)piperazin-1-yl)benzo[d]isothiazole (*17*)

^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 1.72 (m, 2H), 1.88 (m, 2H), 2.47 (t, d, *J* = 7.2 Hz, 2H), 2.67 (bpeak, 4H), 3.14 (dd, *J* = 7.6 Hz & 7.6 Hz, 1H), 3.56 (bpeak, 4H), 3.77-3.84 (m, 4H), 4.04 (t, *J* = 6.8 Hz, 2H), 5.18 (dd, *J* = 8.0 Hz & 8.0 Hz, 1H), 6.78 (t, *J* = 7.6 Hz, 1H), 6.82-6.87 (m, 3H), 7.09 (d, *J* = 8.0 Hz 2H), 7.17 (t, *J* = 8.0 Hz, 2H), 7.32-7.40 (m, 4H), 7.46 (t, *J* = 7.6 Hz, 1H), 7.73 (d, *J* = 7.2 Hz, 2H), 7.80 (d, *J* = 8.0 Hz, 1H), 7.90 (d, *J* = 8.0 Hz, 1H); ^{13}C NMR ($CDCl_3$) δ ppm 23.3, 27.1, 43.6, 50.0(2), 52.9(2), 55.9, 58.2, 64.6, 68.7, 109.0, 113.1, 113.4(2), 118.0, 119.1, 120.5, 123.7, 123.8, 125.6(2), 127.4, 127.9, 128.4(2), 128.7(2), 132.7, 135.2, 145.1, 146.8, 147.7, 149.9, 152.6, 163.8; ESIMS m/z 618.5 $[M + H]^+$.

3-(4-(4-(4-(3-(4-Fluorophenyl)-4,5-dihydro-1-phenyl-1H-pyrazol-5-yl)-2-methoxyphenoxy) butyl)piperazin-1-yl)benzo[*d*]isothiazole (18)

^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 1.73 (m, 2H), 1.89 (m, 2H), 2.50 (t, 2H), 2.69 (bpeak, 4H), 3.11 (dd, *J* = 7.6 Hz & 7.2 Hz, 1H), 3.56 (bpeak, 4H), 3.75-3.88 (m, 4H), 4.04 (t, *J* = 7.2 Hz, 2H), 5.19 (dd, *J* = 8.0 Hz, 8.0 Hz, 1H), 6.73 (t, *J* = 7.6 Hz, 1H), 6.84 (d, *J* = 8.8 Hz, 2H), 7.08 (d, *J* = 4.4 Hz, 2H), 7.32-7.36 (m, 2H), 7.42-7.47 (m, 2H), 7.55-7.58 (m, 2H), 7.80 (d, *J* = 8.0 Hz, 1H), 7.18 (t, *J* = 7.6 Hz, 2H), 7.36 (t, *J* = 7.2 Hz, 2H), 7.47 (t, *J* = 7.6 Hz, 1H), 7.71 (t, *J* = 7.6 Hz, 2H), 7.81(d, *J* = 8.4 Hz, 1H), 7.90 (d, *J* = 8.0 Hz, 1H); ESIMS m/z 636.5 $[M + H]^+$.

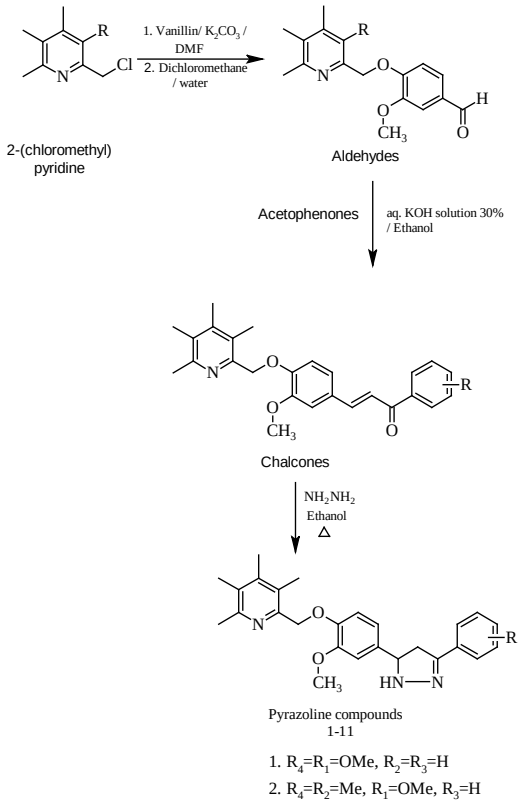
3-(4-(4-(4-(3-(3-(4-Fluorophenyl)-1-isopropyl-1*H*-indol-6-yl)-4,5-dihydro-1-phenyl-1*H*-pyrazol-5-yl)-2-methoxyphenoxy)-butyl)piperazin-1-yl)benzo[*d*]isothiazole (19)

^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 1.58 (m, 6H), 1.72 (m, 2H), 1.89 (m, 2H), 2.49 (t, 2H), 2.67 (bpeak, 4H), 3.23 (dd, *J* = 10.8 Hz & 11.2 Hz, 1H), 3.56 (bpeak, 4H), 3.79 (s, 3H), 3.90 (dd, *J* = 10.2 Hz & 10.8 Hz, 1H), 4.03 (t, 2H), 4.73 (m, 1H), 5.15 (dd, *J* = 9.6 Hz & 9.6 Hz, 1H), 6.74-6.90 (m, 5H), 7.09-7.20 (m, 5H), 7.32-7.36 (m, 2H), 7.42-7.47 (m, 2H), 7.55-7.58 (m, 2H), 7.80 (d, *J* = 8.0 Hz, 1H), 7.91 (dd, *J* = 8.4 Hz & 5.6 Hz, 2H), 7.94 (s, 1H); ^{13}C NMR (101.3 MHz, $CDCl_3$) δ ppm 22.8(2), 27.1, 44.2, 47.3, 50.0(2), 52.9(2), 55.98, 58.2, 64.6, 68.7, 109.1, 110.1, 113.1, 113.3(2), 115.5, 115.7, 116.7, 117.6, 118.0, 118.7, 120.0, 120.5(2), 121.8, 123.8, 123.8, 125.0, 126.0, 127.4(2), 128.0, 128.7, 128.8, 131.4, 131.4, 135.7, 136.3, 145.6, 147.7, 148.4, 150.0, 152.7, 160.1, 162.6, 163.9; ESIMS m/z 793.7 $[M+H]^+$.

RESULT AND DISCUSSIONS

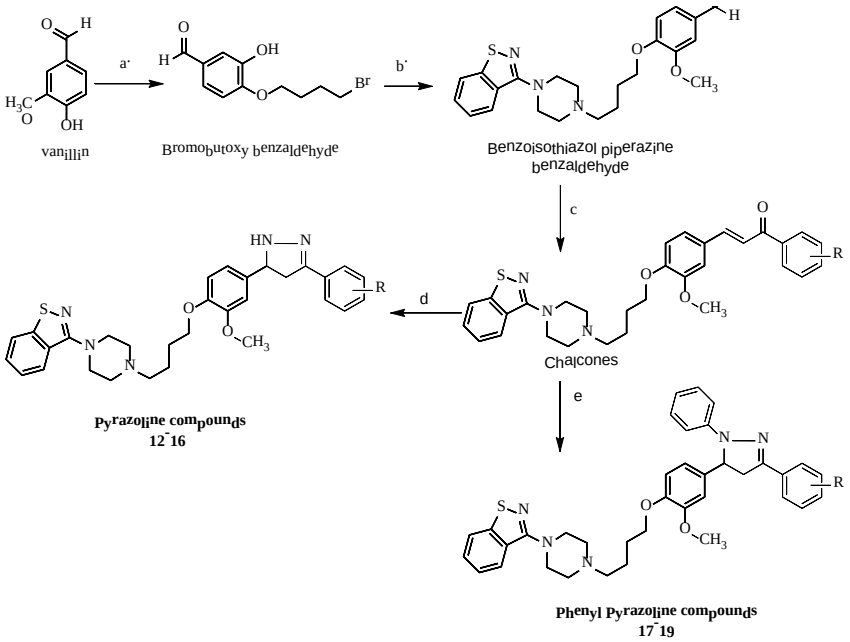
In this communication, two new series of pyrazoline containing substituted chloromethyl pyridine and alkoxy linkage side chain containing benzoisothiazole represented as in Schemes 1 and 2. The essentials of synthesized compounds were confirmed by their physical properties and spectroscopic techniques like ^{1}H NMR, ^{13}C NMR, Mass spectroscopy and FT-IR.

Substituted chloromethylpyridines were treated with vanillin at room temperature in the presence of potassium carbonate and DMF used as a solvent to get aldehydes. These aldehydes were reacted further with different acetophenones, as shown in Table 1, in the presence of 30 % aq. KOH solution where ethanol used as a solvent in the reaction to form the respective chalcones. Pyrazolines were synthesized by refluxing chalcones with hydrazine hydrate in the presence of ethanol as the solvent.



Scheme 1. Synthesis of pyrazoline compounds (**1-11**).

Spectroscopic data confirmed the assigned structure for 1-19, where its ^{1}H NMR, ^{13}C NMR and mass spectroscopy where disappearance of the C=C bond two trans proton coupling having *J* value ~16.0 Hz of chalcones and formation of three peaks displayed in pyrazoline (δ ~2.8, 3.4 and a triplet at ~4.7 ppm (D_2O-exchangeable, 1H, NH)). ^{13}C-NMR shows signals corresponding to respective types of carbon in different chemical shifts of pyrazoline compounds details of the chemical shift were captured in the characterization part.



Scheme 2. Synthesis of pyrazoline and phenyl pyrazoline compounds (**12-19**): Reaction conditions a) K_2CO_3/DMF/1,4-dibromobutane, dichloromethane and water. b) K_2CO_3/DMF/3-(piperazin-1-yl)benzo[*d*]isothiazole, isopropyl alcohol. c) 30% aq. solution of KOH. d) NH_2NH_2/ethanol/ $\triangle$ e) $PhNHNH_2$, EtOH, $\triangle$

Mass spectroscopy gives corresponding [M+1] molecular ion peak at their corresponding molecular weight, as shown in Table 1. The IR spectrum of compounds showed a broad band at ~3446 cm^{-1} recognized for -OH group and the characteristic N=N band was assigned at 1591 cm^{-1} two strong bands at ~1650 cm^{-1} and ~1607 cm^{-1} referring to carbonyl and carbon-carbon double bond disappearance confirmed the formation of pyrazolines compounds.

The synthesized compounds were assigned on the basis of ^1H NMR, ^{13}C NMR, mass and IR spectral analytical studies to confirm the proposed structures. Physical constant data were tabulated in Table 1.

Molecular docking

To study binding conformations of the synthesized compounds, the Autodock Vina program was used for docking pyrazoline derivatives into the active sites of methionyl-tRNA synthetase. The AutoDock Tools 1.5.4 (ADT) was used to prepare the input files for docking.[18] The crystal structure of *Thermus thermophilus* methionyl-tRNA synthetase reveals two RNA-binding modules. All water molecules and ions were removed from the protein crystallographic structures; polar hydrogens were added and partial atomic charges were assigned by Kollman united charges method.[19-21]

Sidechains of lysine, arginine, and histidine residues were protonated while the carboxylic groups of glutamic and aspartic acid were deprotonated. For each ligand, nonpolar hydrogens were merged, Gasteiger charges were assigned and rotatable bonds were set up. The structures were then saved in the corresponding pdbqt file required for the Autodock.

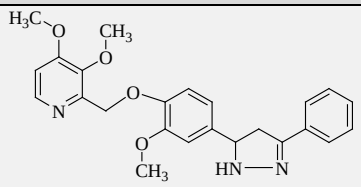
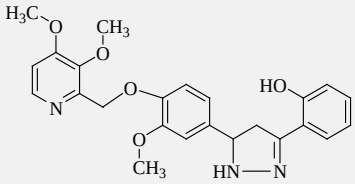
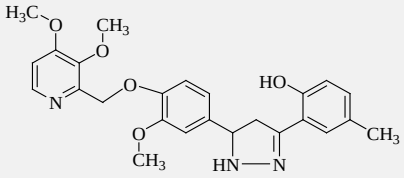
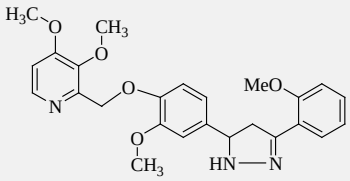
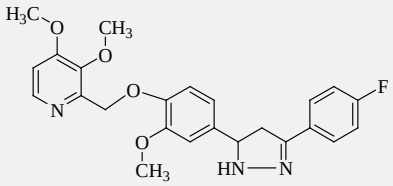
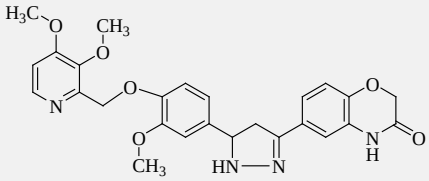
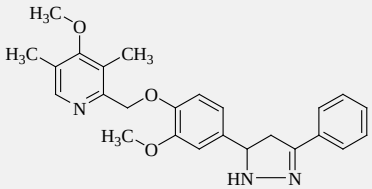
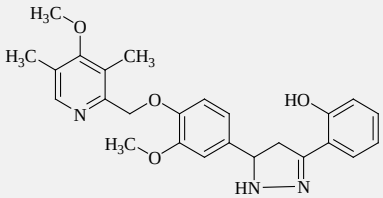
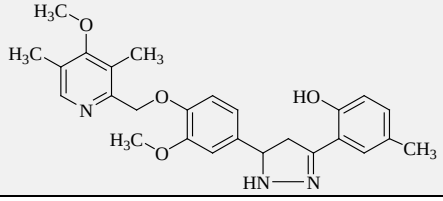
Antioxidant activity

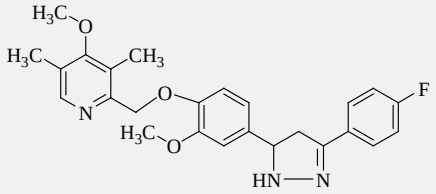
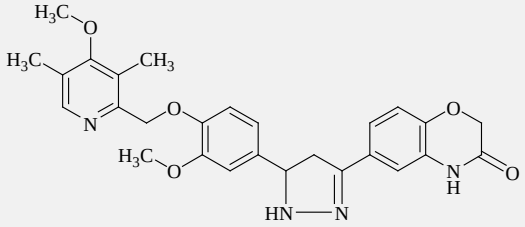
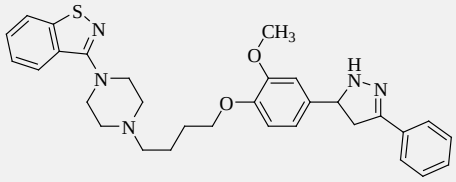
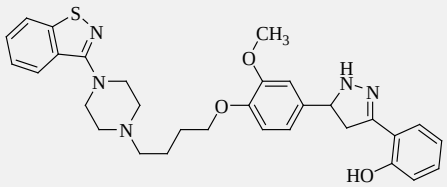
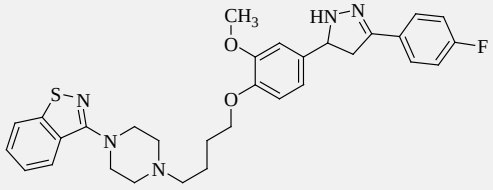
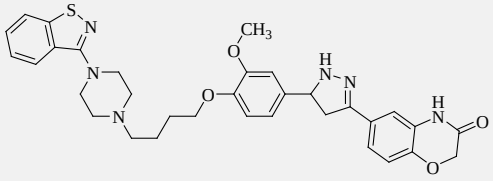
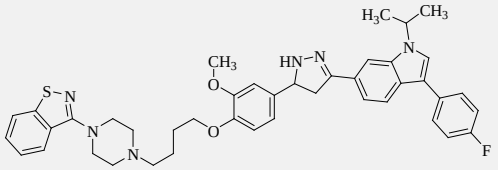
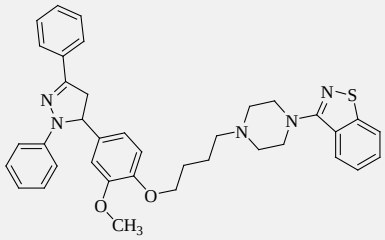
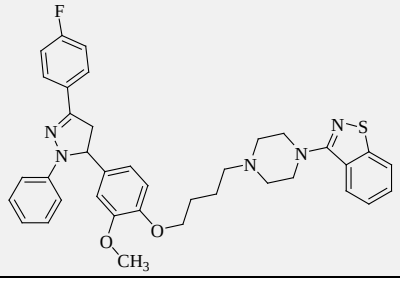
Antioxidant potential of the synthesized compounds was measured in terms of DPPH, OH and superoxide radical (SOR) scavenging activity carried out using reported methods.[22] following the procedure as discussed in our previous publications.[16] The results of antioxidant activity are summarized in Table 2. The detailed procedure for the antioxidant activity is provided in the supplementary material.

Antimicrobial screening

To confirm the inhibitory activity, the hit compounds were re-tested against the strains in a dose-response assay to determine the minimum inhibitory concentration (MIC) of the compounds. Samples were prepared in DMSO and water to a final testing concentration of 32 µg mL^{-1} or 20 µM in 384-well, non-binding surface plate (NBS) for each bacterial/fungal strain, in duplicate (n=2), and keeping the final DMSO concentration to a maximum of 1 % DMSO. All the sample-preparation was done using liquid handling robots. All bacteria were cultured in cation-adjusted Mueller Hinton broth (CAMHB) at 37 °C overnight. A sample of each culture was then diluted 40-fold in fresh broth and incubated at 37 °C for 1.5-3 h. The resultant mid-log phase cultures were diluted (CFU mL^{-1} measured by OD600), added to each well of the compound containing plates giving a cell density of 5×105 CFU mL^{-1} and a total volume of 50 µL. All the plates were covered and incubated at 37 °C for 18 h without shaking.

Table 1. Physical constant of the synthesized compounds.

Code	Structure of compounds	Molecular formula	Molecular wt.	M. P., °C	Yield, %
1		$C_{24}H_{25}N_3O_4$	419.47	171	92
2		$C_{24}H_{25}N_3O_5$	435.47	176	87
3		$C_{25}H_{27}N_3O_5$	449.5	175	83
4		$C_{25}H_{27}N_3O_5$	449.5	173	85
5		$C_{24}H_{24}FN_3O_4$	437.46	169	88
6		$C_{26}H_{26}N_4O_6$	490.51	207	93
7		$C_{25}H_{27}N_3O_3$	417.5	161	70
8		$C_{25}H_{27}N_3O_4$	433.5	163	68
9		$C_{26}H_{29}N_3O_4$	447.53	165	69

10		$C_{25}H_{26}FN_3O_3$	435.49	166	70
11		$C_{27}H_{28}N_4O_5$	488.54	225	72
12		$C_{31}H_{35}N_5O_2S$	541.71	163	69.8
13		$C_{31}H_{35}N_5O_3S$	557.71	171	78
14		$C_{31}H_{34}FN_5O_2S$	559.7	160	79
15		$C_{33}H_{36}N_6O_4S$	612.74	171	78
16		$C_{42}H_{45}FN_6O_2S$	716.91	172	80
17		$C_{37}H_{39}N_5O_2S$	617.8	168	71
18		$C_{37}H_{38}FN_5O_2S$	635.79	164	69

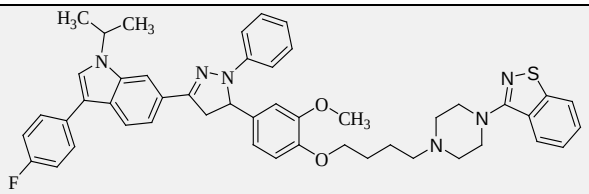
19		$C_{48}H_{49}FN_6O_2S$	793.01	174	70

Table 2. Radical scavenging profile of the synthesized compounds

Compound	% Radical scavenging activity		
	DPPH	OH	SOR
1	NR	44.76 ± 1.03	32.74 ± 1.27
2	40.76 ± 1.76	26.01 ± 1.09	60.52 ± 1.03
3	20.76 ± 1.63	11.59 ± 1.36	32.41 ± 0.89
4	23.62 ± 1.61	11.99 ± 0.72	41.36 ± 0.56
5	78.52 ± 1.51	07.82 ± 0.64	52.16 ± 2.14
6	37.09 ± 1.49	31.95 ± 0.76	53.14 ± 1.29
7	39.16 ± 0.13	27.03 ± 0.14	32.63 ± 1.34
8	56.93 ± 1.93	12.04 ± 1.20	21.52 ± 1.87
9	41.15 ± 2.14	12.70 ± 1.91	43.87 ± 1.64
10	52.71 ± 1.02	12.48 ± 1.85	36.46 ± 1.35
11	20.28 ± 0.21	34.61 ± 1.43	35.82 ± 0.87
12	33.11 ± 0.52	10.49 ± 1.84	37.42 ± 1.49
13	47.53 ± 0.63	31.95 ± 0.12	53.45 ± 1.26
14	64.02 ± 0.84	15.43 ± 0.46	45.61 ± 1.47
15	59.88 ± 2.14	17.31 ± 1.74	37.46 ± 1.67
16	37.81 ± 1.84	21.22 ± 1.61	44.21 ± 1.34
17	68.88 ± 0.83	07.82 ± 1.76	55.41 ± 0.57
18	78.88 ± 0.73	31.95 ± 0.73	65.43 ± 0.39
19	75.02 ± 2.59	31.98 ± 2.46	37.63 ± 0.16
*AA	91.25 ± 1.91	89.11 ± 1.67	79.86 ± 1.24

*AA: ascorbic acid. Results represented here are the mean of *n*=3±SD. NR: No reaction under the experimental conditions.

Fungi strains were cultured for 3 days on yeast extract-peptone dextrose (YPD) agar at 30 °C. A yeast suspension of 1 x 106 to 5 x 106 CFU mL^{-1} (as determined by OD530) was prepared from five colonies. The suspension was subsequently diluted and added to each well of the compound containing plates giving a final cell density of fungi suspension of 2.5 ×103 CFU mL^{-1} and a total volume of 50 µL. All plates were covered and incubated at 35 °C for 24 h without shaking.

Colistin and Vancomycin were used as positive bacterial inhibitor standard for gram-negative and gram-positive bacteria, respectively. Fluconazole was used as a positive fungal inhibitor standard for *C. Albicans* and *C. neoformans.* The antibiotics were provided in 4 concentrations, with 2 above and 2 below its MIC value, and plated into the first 8 wells of column 23 of the 384-well NBS plates. The quality control (QC) of the assays was determined by the antimicrobial controls and the *Z*'-factor (using positive and negative controls). Each plate was deemed to fulfill the quality criteria (pass QC) if the *Z*'-factor was above 0.4, and the antimicrobial standards showed the full range of activity, with full growth inhibition at their highest concentration, and no growth inhibition at their lowest concentration.

The inhibition of bacterial growth was determined by measuring absorbance at 600 nm (OD600) using a Tecan M1000 Pro monochromator plate reader. The percentage of growth inhibition was calculated for each well using the negative control (media only) and positive control (bacteria without inhibitors) on the same plate as references. The significance of the inhibition values was determined by modifying *Z*-scores, calculated using the median of the samples (no controls) on the same plate. Samples with inhibition values above 80 % and *Z*-score above 2.5 for either replicate (*n*=2 on different plates) were classed as actives, whereas samples with inhibition values between 50 – 80 % and *Z*-score below 2.5 for either replicate (*n*=2 on different plates) were classed as partially active.

Table 3. Material assays for antimicrobial activity

Material	Code	Brand	Catalogue No.
Compound preparation	Plate PP	Corning	3364
Assay plates	NBS 384w	Corning	3640
Growth media-bacteria	CAMHB	Bacto Laboratories	212322
Culture agar-fungi	YPD	Becton Dickinson	242720
Growth media-fungi	YNB	Becton Dickinson	233520
Resazurin		Sigma-Aldrich	R7017

Percentage growth inhibition of individual samples was calculated based on negative controls (media only) and positive controls (bacterial media without inhibitors). The percentage of growth inhibition was calculated for each well using the negative control (media only) and positive control (without inhibitors) on the same plate. The growth rates for all bacteria and fungi have a variation of -/+ 10 %, which lies is within the expected normal distribution of microbial growth. □

Molecular docking

The synthesized compounds were docked against the active site of Thermus thermophilus methionyl-tRNA synthetase using Autodock vina docking tool whose results were expressed in terms of docking scores (Table 8). Analysis of docking interaction reveals that the pyrazoles bearing benzoisothiazole ring (**12**, **13** and **15**) were the most active compounds against methionyl-tRNA synthetase. Results of observed antimicrobial activities are in good agreement for these tilted compounds in terms of higher negative values of *Z*-scores.

The benzisothiazole linked pyrazolines **12, 13** and **15** bind efficiently into the active site residues like TYR13, GLU54, ARG132, ASP50, ASP260, GLY21, HIS22, LEU290, ILE261, VAL226, and LYS297.

Table 4. Standards for antimicrobial activity

Sample	Sample ID	Full MW	Stcok conc. μg mL^{-1}	Solvent	Source
Colistin	Sulfate MCC_000094:02	1400.63	10.0	DMSO	Sigma; C4461
Vancomycin	HCL MCC_000095:02	1485.71	10.0	DMSO	Sigma; 861987
Fluconazole	MCC_008383:01	306.27	2.56	DMSO	Sigma; F8929

Table 5. Bacterial and fungal species used along with abbreviations

ID	Batch	Microbe	Strain	Description
GN_001	02	*Escherichia coli*	ATCC 25922	FDA control strain
GN_003	02	*Klebsiella pneumoniae*	ATCC 700603	MDR
GN_034	02	*Acinetobacter baumannii*	ATCC 19606	Type strain
GN_042	02	*Pseudomonas aeruginosa*	ATCC 27853	Quality control strain
GP_020	02	*Staphylococcus aureus*	ATCC 43300	MRSA
FG_001	01	*Candida albicans*	ATCC 90028	CLSI reference
FG_002	01	*Cryptococcus neoformans*	ATCC 208821	Type strain

All antibiotic controls displayed inhibitory values within the expected range.

Table 6. Brief results of antimicrobial activity

Strain ID	Microba	Antibiotic	Pass / Fail
GN_001:02	*E. coli*	Colistin	Pass
GN_003:02	*K. pneumoniae*	Colistin	Pass
GN_034:02	*A. baumannii*	Colistin	Pass
GN_042:02	*P. aeruginosa*	Colistin	Pass
GP_020:02	*S. aureus (MRSA)*	Vancomycin	Pass
FG_001:01	*C. albicans*	Fluconazole	Pass
FG_002:01	*C. neoformans (H99)*	Fluconazole	Pass

Table 7. Different bacteria, fungi and abbreviations used for antimicrobial activity

Abbreviation	Name	Description	Strain	Organism	Type
Sa	*Staphylococcus aureus*	MRSA	ATCC 43300	Bacteria	G+ve
Ec	*Escherichia coli*	FDA control	ATCC 25922	Bacteria	G-ve
Kp	*Klebsiella pneumoniae*	MDR	ATCC 700603	Bacteria	G-ve
Ab	*Acinetobacter baumannii*	Type strain	ATCC 19606	Bacteria	G-ve
Pa	*Pseudomonas aeruginosa*	Type strain	ATCC 27853	Bacteria	G-ve
Ca	*Candida albicans*	CLSI reference	ATCC 90028	Fungi	Yeast
Cn	*Cryptococcus neoformans var. grubii*	Type strain	H99; ATCC 208821	Fungi	Yeast

The 2*H*-1,4-benzoxazin-3(4*H*)-one derivatives **15** (-8.5243) interacts with the polar and charged amino acid residues THR55 and GLU54 with a carbonyl oxygen atom and an oxygen atom of oxazine at the distance of 1.90, 1.93 and 1.93 Å to form conventional hydrogen bond interactions. The charged amino acid ASP50 and hydrophobic amino acid TYR13 form conventional hydrogen bond interactions with pyrazole nitrogen and methoxyl oxygen atom with a distance of 1.87 and 1.90 Å, respectively. The polar amino acid HIS22 and ASP260 amino acid interact with a methoxyl hydrogen atom and the hydrogen atom of the piperazine ring to form a C-H bond and conventional hydrogen bond interactions.

The aliphatic amino acids VAL226, ILE261, GLY288, LEU290, and charged amino acid LYS297 interact with π electron cloud to form π-donor hydrogen and π-alkyl interactions (Figure 1).

The benzoisothiazole derivative **12** (-7.9567) interacts with the polar and charged amino acid of the active site. The charged amino acid ASP260 interacts with hydrogens of the piperazine ring to form conventional hydrogen bond interactions and C-H bond at a distance of 1.96, 2.53, 2.59 and 2.54 Å, respectively.

Table 8. In vitro activity and molecular docking of synthesized compounds

Compound code	Z-Score							Free binding energy, kcal mol^{-1}
	Sa	Ec	Kp	Pa	Ab	Ca	Cn	
1	1.76	1.29	0.73	0.6	1.12	-4.54	1.95	-6.4069
2	0.96	-0.13	-1.09	1.33	-0.42	-2.63	1.52	-5.6208
3	1.31	-0.52	0.96	-1.56	0.14	0.05	-0.21	-6.9635
4	1.13	1.24	0.97	-1.04	0.21	0.49	0.05	-6.0319
5	0.29	-0.2	0.73	-0.6	0.34	-2.25	-0.13	-7.2436
6	0.13	0	0.7	-1.23	0.08	0.1	2.19	-6.584
7	0.99	-1.17	83	-0.62	0.32	0.55	-0.12	-4.5155
8	0.95	1	1.39	0.2	1.24	0.63	0.74	-6.3774
9	1.4	-1.66	0.7	-1.53	0.54	-0.83	-0.13	-5.5377
10	-0.11	0.04	-0.57	-1.35	-0.86	-0.89	0.22	-5.2221
11	-0.74	-1.24	-0.72	-0.8	-1.16	-0.48	2.11	-6.1736
12	0.72	1.38	0.26	0.65	1	0.47	*-2.78*	-7.9567
13	0.49	-0.49	0.52	-1.46	-0.19	-0.11	*-2.77*	-7.6732
14	0.24	0.83	0.44	-1.64	0.2	0.55	-0.81	-6.6984
15	0.39	-0.08	0.28	0.76	-0.04	0.67	*-2.91*	-8.5248
16	1.34	1.13	1.76	1.19	-1.62	-1.11	-0.41	-6.9736
17	3.31	3.77	3.9	0.9	2.53	-5.85	2.49	-6.351
18	0.42	0.52	0.78	-0.43	0.24	0.12	1.17	-5.5277
19	2.38	1.94	3.41	-0.23	3.39	-0.2	1.91	-5.112

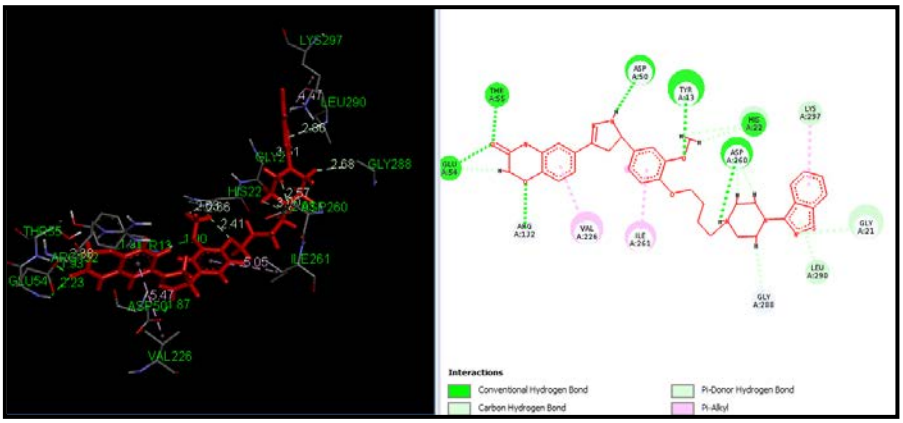


Figure 2. Binding pose and molecular interactions of **15** in the active site of methionyl-tRNA synthetase.

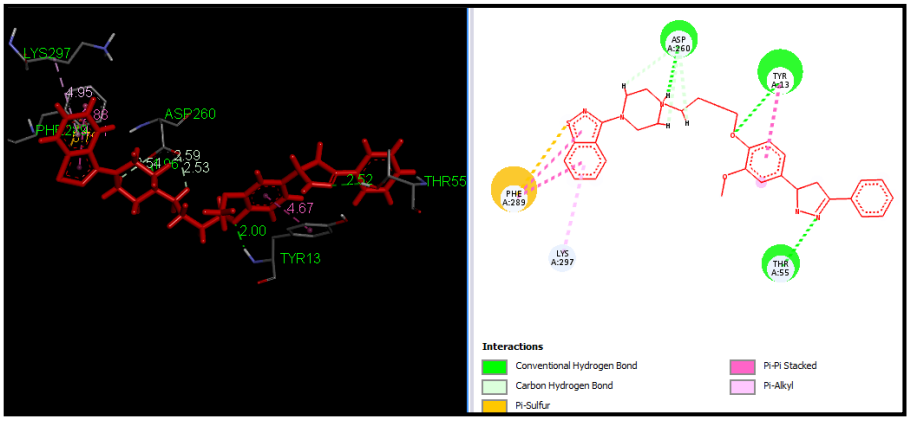


Figure 3. Binding pose and molecular interactions of **12** in the active site of methionyl-tRNA synthetase.

The polar amino acid THR55 and hydrophobic amino acid TYR13 interacts with pyrazole nitrogen atoms and phenoxy oxygen atom to form conventional hydrogen bond interaction with a distance of 2.00 and 2.52 Å respectively. The π-electron clouds of benzoisothiazole and phenoxy rings create π-sulfur, π-π stacked and π-alkyl interactions with various distances (Figure 2).

CONCLUSION

In summary, the present article reports a novel series of pyrazolines from chalcones and hydrazine hydrate or phenylhydrazine in the presence of ethanol as the alcoholic solvent. All structures of the newly synthesized molecules were characterized by spectroscopic data - ^{1}H NMR, ^{13}C NMR, Mass, and IR. Further, these newly synthesized compounds were evaluated for antioxidant and antimicrobial activity. Compounds **5**, **18** and **19** were found to be potentially active antioxidants in terms of the DPPH radical scavenging assay, whereas **2** and **18** compounds showed better activity by superoxide radical (SOR) scavenging assay method in but less as compared with the standard ascorbic acid. Compounds **12, 13 and 15** were partially active against *Cryptococcus neoformans var. grubii* fungi. The molecular docking study and *in-vitro* antibacterial and antifungal activity suggested that **12, 13** and **15** are the most active among all synthesized derivatives and will serve as excellent leads in the antimicrobial and antioxidant drug discovery process.

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