Novel Piperazinyl-Quinazoline-4-one Analogs: Design, Synthesis and Evaluation of In Vitro Biological Activity

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Abstract: A series of novel 2-[4-substituted-piperazinyl-methyl]-3-[N-isonicotinamide-yl]-quinazoline-4-one 4a-l were designed, synthesized, characterized and evaluated for in vitro antitubercular, antibacterial and antifungal activity. Compounds 4f, 4h and 4l exhibited excellent antitubercular activity against mycobacterium tuberculosis H37Rv.

Keywords: Piperazine, 2-Chloromethyl-3-(N-isonicotinamide-yl)-4Hquinazolinone, Antitubercular activity

Introduction

Research in heterocyclic chemistry has gained momentum in recent times because more than half of the biologically active molecules belong to various classes of heterocycles1, among them quinazolinone have remained always a major source for therapeutic drugs2. Also they are the building block for more than 150 naturally occurring pharmacologically active alkaloids and commercial drugs3,4.

Structure activity relationship studies of quinazolinone ring system suggest that position 2, 6 and 8 are important for pharmacokinetic property while position 3 attached with different heterocyclic ring system is endowed with better chemotherapeutic activity5. On the other hand, piperazine and substituted piperazine are important pharmacophore that can be found in many marketed drugs and drugs under clinical trials6,7. Also, piperazines were explored with several biological activity8,11. Actually the polarity of nitrogen atoms of piperazine ring enhances favorable interaction with biomacromolecules and thus confers the biological activity12,13.

Since these two heterocyclic moieties constitute two active pharmacophore and are supposed to be highly active, combining these two is expected to have a synergistic effect against pathogens causing infectious diseases. Keeping this in mind and to identify new drug candidates, that may be valued in designing new, potent, selective and less toxic anti-
infective agents and also, our continued interest in the synthesis of novel heterocyclic hybrids\textsuperscript{14-17} with promising antimicrobial activity, herein, we report the synthesis and evaluation of \textit{in vitro} biological activity of novel piperazinyl-quinazolin-4-one analogs. By applying concept molecular hybridization, we have used introduced antitubercular drug isoniazid at position 3, while at position 2, various substituted piperazine derivatives were attached in quinazolin-4-one heterocycles and tried to get promising anti-infective agents.

**Experimental**

The melting points were determined in open glass capillaries and are uncorrected. The purity of all the newly synthesized compounds were routinely checked by TLC (0.5 mm thickness) using silica gel-G coated aluminium plates (Merck) and spots were visualized by exposing the dry plates in iodine vapors. IR spectra ($\nu_{\text{max}}$ in cm\textsuperscript{-1}) were recorded on a Perkin-Elmer 1720 FT-IR spectrometer (KBr pellets). \textsuperscript{1}H-NMR and \textsuperscript{13}C-NMR spectra were recorded on a Bruker AC 400 MHz and 100 MHz spectrometer respectively in DMSO-$d_6$, referenced to TMS. Mass spectra were recorded on Shimadzu LCMS 2010 spectrometer. Elemental analyses (C, H, and N) were conducted using a Carlo Erba analyzer model 1106.

**Figure 1.** Numbering system for 13C-NMR of compounds 4a, 4c-g and 4i-1

**General procedure for the synthesis of 2-[4-substituted-piperazinyl-methyl]-3-[N-isonicotinamide-yl]-quinazoline-4-one (4a-l)**

A mixture of compound 3a/3b (0.5 mmol), appropriate piperazine (0.7 mmol) and anhydrous sodium carbonate (1.50 g) in absolute ethanol was refluxed for 8-9 h (reaction progress was monitored on TLC). After completion, the excess of amine and ethanol was removed by distillation and the residue was treated with 5% NaHCO$_3$ solution to remove acidic impurities, filtered, washed and dried. Final products were crystallized using ethanol to give the title compounds.

**2-[4-Phenyl-piperazinyl-methyl]-3-[N-isonicotinamide-yl]-quinazoline-4-one (4a)**

Yield, 68%; m.p. 179-181 °C; FTIR (KBr) v: 3368 (NH), 3036 (C-H Aromatic), 2954 (C-H aliphatic), 1678 (C=O of amide), 1642 (C=O of quinazolinone), 1352 (C=N), 1290 (C-N), 1226 (N-N) cm\textsuperscript{-1}; \textsuperscript{1}H NMR (400MHz, DMSO-$d_6$) $\delta$ ppm: 1.82 (s, 2H, CH$_2$), 2.48-2.65 (m, 8H, 4 $\times$ CH$_2$), 6.73-8.28 (m, 13H, Ar-H), 8.54 (s, 1H, NH-CO); \textsuperscript{13}C NMR (100MHz, DMSO-$d_6$) $\delta$ ppm: 51.22 (C$_{15}$), 55.30 (C$_{16}$, C$_{19}$), 57.43 (C$_{17}$, C$_{18}$), 122.48-133.27 (C$_1$-$C_5$, C$_{10}$-$C_{11}$, C$_{20}$-$C_{25}$), 142.18 (C$_{14}$), 147.72 (C$_6$), 150.44 (C$_{12}$-$C_{13}$), 164.21 (C$_8$), 167.20 (C$_7$), 168.34 (C$_9$); MS: $m/z$ [440.20]$^+$; Analysis calculated for C$_{25}$H$_{24}$N$_6$O$_2$: C, 68.17; H, 5.49; N, 19.08. Found: C, 68.32; H, 5.66; N, 19.23%.

**2-[4-Benzyl-piperazinyl-methyl]-3-[N-isonicotinamide-yl]-quinazoline-4-one (4b)**

Yield, 72%; m.p. 198-200 °C; FTIR (KBr) v: 3379 (NH), 3048 (C-H Aromatic), 2959 (C-H aliphatic), 1682 (C=O of amide), 1644 (C=O of quinazolinone), 1360 (C=N), 1283 (C-N), 1230 (N-N) cm\textsuperscript{-1}; \textsuperscript{1}H NMR (400MHz, DMSO-$d_6$) $\delta$ ppm: 1.74 (s, 2H, CH$_2$), 2.34-2.65 (m, 8H, 4 $\times$ CH$_2$), 6.73-8.28 (m, 13H, Ar-H), 8.54 (s, 1H, NH-CO); \textsuperscript{13}C NMR (100MHz, DMSO-$d_6$) $\delta$ ppm: 51.22 (C$_{15}$), 55.30 (C$_{16}$, C$_{19}$), 57.43 (C$_{17}$, C$_{18}$), 122.48-133.27 (C$_1$-$C_5$, C$_{10}$-$C_{11}$, C$_{20}$-$C_{25}$), 142.18 (C$_{14}$), 147.72 (C$_6$), 150.44 (C$_{12}$-$C_{13}$), 164.21 (C$_8$), 167.20 (C$_7$), 168.34 (C$_9$); MS: $m/z$ [440.20]$^+$; Analysis calculated for C$_{25}$H$_{24}$N$_6$O$_2$: C, 68.17; H, 5.49; N, 19.08. Found: C, 68.32; H, 5.66; N, 19.23%.

**Figure 1.** Numbering system for 13C-NMR of compounds 4a, 4c-g and 4i-1
(m, 8H, 4×CH₂), 2.60 (s, 2H, CH₂), 7.32-8.12 (m, 13H, Ar-H), 8.67 (s, 1H, NH-CO);
\(^{13}\)C NMR (100MHz, DMSO-d₆) δ ppm: 51.28 (C₁₅), 55.48 (C₁₆, C₁₉), 57.32 (C₁₇, C₁₈), 60.23
(CH₂), 122.32-136.27 (C₁-C₅, C₁₀-C₁₁, C₂₀-C₂₃), 142.20 (C₁₄), 147.63 (C₆), 150.39 (C₁₂-C₁₃),
164.14 (C₇), 167.23 (C₈), 168.40 (C₉); MS: m/z [454.21]; Analysis calculated for
C₃₀H₂₅N₆O₂: C, 68.70; H, 5.77; N, 18.49. Found: C, 68.82; H, 5.59; N, 18.34%.

2-[4-Methyl-piperazinyl-methyl]-3-[N-isonicotinamide-yl]-quinazoline-4-one (4c)

Yield, 70%; m.p. 176-178 °C; FTIR (KBr) ν: 3358 (NH), 3053 (C-H Aromatic), 2947 (C-H aliphatic), 1673 (C=O of amide), 1631 (C=O of quinazoline), 1367 (C=N), 1278 (C-N),
1224 (N-N) cm⁻¹; \(^1\)H NMR (400MHz, DMSO-d₆) δ ppm: 1.81 (s, 2H, CH₂), 2.32 (s, 3H,
CH₃), 2.49-2.67 (m, 8H, 4×CH₂), 7.27-8.28 (m, 8H, Ar-H), 8.70 (s, 1H, NH-CO); \(^{13}\)C NMR
(100MHz, DMSO-d₆) δ ppm: 38.62 (CH₃), 51.35 (C₁₅), 55.18 (C₁₆, C₁₉), 57.24 (C₁₇, C₁₈),
122.4-133.38 (C₁-C₅, C₁₀-C₁₁), 142.34 (C₁₄), 147.58 (C₆), 150.43 (C₁₂-C₁₃), 164.21 (C₈),
167.30 (C₇), 168.44 (C₉); MS: m/z [378.18]; Analysis calculated for C₂₀H₂₂N₆O₂: C, 63.48;
H, 5.86; N, 22.21. Found: C, 63.34; H, 5.75; N, 22.40%.

2-[4-(2-Methoxy-phenyl)-piperazinyl-methyl]-3-[N-isonicotinamide-yl]-quinazoline
-4-one (4d)

Yield, 68%; m.p. 223-225 °C; FTIR (KBr) ν: 3362 (NH), 3049 (C-H Aromatic), 2956 (C-H aliphatic), 1680 (C=O of amide), 1638 (C=O of quinazoline), 1372 (C=N), 1294 (C-N),
1218 (N-N) cm⁻¹; \(^1\)H NMR (400MHz, DMSO-d₆) δ ppm: 1.92 (s, 2H, CH₂), 2.36-2.58 (m, 8H,
4×CH₂), 3.88 (s, 2H, OCH₃), 6.57-8.06 (m, 12H, Ar-H), 8.73 (s, 1H, NH-CO); \(^{13}\)C NMR
(100MHz, DMSO-d₆) δ ppm: 51.29 (C₁₅), 55.19 (C₁₆, C₁₉), 56.27 (OCH₃), 57.83 (C₁₇, C₁₈),
115.89-133.13 (C₁-C₅, C₁₀-C₁₁, C₂₀-C₂₃, C₂₅), 142.26 (C₁₄), 146.52 (C₂₄), 147.75 (C₆), 150.23
(C₁₂-C₁₃), 164.20 (C₈), 167.25 (C₇), 168.49 (C₉); MS: m/z [470.21]; Analysis calculated for
C₂₆H₂₆N₆O₃: C, 66.37; H, 5.57; N, 17.86. Found: C, 66.48; H, 5.42; N, 17.93%.

2-[4-(2-Methoxy-phenyl)-piperazinyl-methyl]-3-[N-isonicotinamide-yl]-quinazoline
-4-one (4e)

Yield, 67%; m.p. 236-238 °C; FTIR (KBr) ν: 3357 (NH), 3061 (C-H Aromatic), 2948 (C-H aliphatic), 1674 (C=O of amide), 1623 (C=O of quinazoline), 1365 (C=N), 1305 (C-N),
1226 (N-N) cm⁻¹; \(^1\)H NMR (400MHz, DMSO-d₆) δ ppm: 1.84 (s, 2H, CH₂), 2.40-2.64 (m, 8H,
4×CH₂), 3.90 (s, 2H, OCH₃), 6.71-8.13 (m, 12H, Ar-H), 8.79 (s, 1H, NH-CO); \(^{13}\)C NMR
(100MHz, DMSO-d₆) δ ppm: 51.38 (C₁₅), 55.30 (C₁₆, C₁₉), 56.15 (OCH₃), 57.78 (C₁₇, C₁₈),
115.94-136.13 (C₁-C₅, C₁₀-C₁₁, C₂₀-C₂₁, C₂₃-C₂₅), 142.35 (C₁₄), 147.82 (C₆), 150.18 (C₁₂-C₁₃),
151.43 (C₂₂), 164.16 (C₈), 167.30 (C₇), 168.53 (C₉); MS: m/z [470.21]; Analysis calculated for
C₂₆H₂₆N₆O₃: C, 66.37; H, 5.57; N, 17.86. Found: C, 66.28; H, 5.76; N, 17.77%.

2-[4-(2,3-Dichloro-phenyl)-piperazinyl-methyl]-3-[N-isonicotinamide-yl]quinazoline
-4-one (4f)

Yield, 73%; m.p. 219-221 °C; FTIR (KBr) ν: 3364 (NH), 3047 (C-H Aromatic), 2953 (C-H aliphatic), 1682 (C=O of amide), 1630 (C=O of quinazoline), 1357 (C=N), 1291 (C-N),
1230 (N-N) cm⁻¹; \(^1\)H NMR (400MHz, DMSO-d₆) δ ppm: 1.98 (s, 2H, CH₂), 2.39-2.68 (m, 8H,
4×CH₂), 6.57-8.10 (m, 11H, Ar-H), 8.84 (s, 1H, NH-CO); \(^{13}\)C NMR (100MHz, DMSO-
δ ppm: 51.48 (C₁₅), 55.36 (C₁₆, C₁₉), 57.25 (C₁₇, C₁₈), 116.79-135.81 (C₁-C₅, C₁₀-C₁₁,
C₂₀-C₂₃), 142.23 (C₁₄), 146.48 (C₂₃), 147.78 (C₆), 150.22 (C₁₂-C₁₃), 164.21 (C₈), 167.28 (C₇),
168.45 (C₉); MS: m/z [508.12]; Analysis calculated for C₂₅H₂₅Cl₂N₇O₂; C, 58.95; H, 4.35;
N, 16.50. Found: C, 58.80; H, 4.56; N, 16.37%.
2-[4-Phenyl-piperazinyl-methyl]-3-[N-isonicotinamide-yl]-6-iodo-quinazoline-4-one (4g)

Yield, 69%; m.p. 217-219 ºC; FTIR (KBr) v: 3350 (NH), 3048 (C-H Aromatic), 2952 (C-H aliphatic), 1684 (C=O of amide), 1623 (C=O of quinazoline), 1360 (C=N), 1290 (C-N), 1231 (N-N), 552 (C-I) cm⁻¹; ¹H NMR (400MHz, DMSO-d₆) δ ppm: 1.75 (s, 2H, CH₂), 2.50-2.76 (m, 8H, 4×CH₂), 6.51-8.28 (m, 12H, Ar-H), 8.63 (s, 1H, NH-CO); ¹³C NMR (100MHz, DMSO-d₆) δ ppm: 51.17 (C₁₅), 55.31 (C₁₆, C₁₉), 57.52 (C₁₇, C₁₈), 95.80 (C₁), 122.51-137.38 (C₃-C₅, C₁₀-C₁₁, C₂₀-C₂₄), 142.17 (C₂), 142.54 (C₁₄), 144.40 (C₂₅), 147.49 (C₆), 150.42 (C₁₂-C₁₃), 164.19 (C₈), 167.21 (C₇), 168.46 (C₉); MS: m/z [566.09]⁺; Analysis calculated for C₂₃H₂₃N₂O₂; C, 53.01; H, 4.09; N, 14.84. Found: C, 53.13; H, 4.20; N, 14.72%.

2-[4-Benzyl-piperazinyl-methyl]-3-[N-isonicotinamide-yl]-6-iodo-quinazoline-4-one (4h)

Yield, 71%; m.p. 209-211 ºC; FTIR (KBr) v: 3367 (NH), 3072 (C-H Aromatic), 2946 (C-H aliphatic), 1688 (C=O of amide), 1628 (C=O of quinazoline), 1364 (C=N), 1284 (C-N), 1220 (N-N), 542 (C-I) cm⁻¹; ¹H NMR (400MHz, DMSO-d₆) δ ppm: 1.85 (s, 2H, CH₂), 2.35-2.68 (m, 8H, 4×CH₂), 3.58 (s, 2H, CH₂), 7.34-8.19 (m, 12H, Ar-H), 8.59 (s, 1H, NH-CO); ¹³C NMR (100MHz, DMSO-d₆) δ ppm: 51.17 (C₁₅), 55.31 (C₁₆, C₁₉), 57.24 (C₁₇, C₁₈), 60.12 (CH₂), 95.74 (C₁), 122.26-136.30 (C₃-C₅, C₁₀-C₁₁, C₂₀-C₂₄), 142.11 (C₂), 142.48 (C₁₄), 147.51 (C₆), 150.33 (C₁₂-C₁₃), 164.16 (C₆), 167.25 (C₇), 168.35 (C₉); MS: m/z [580.11]⁺; Analysis calculated for C₂₅H₂₅N₆O₂; C, 53.80; H, 4.34; N, 14.48. Found: C, 53.87; H, 4.45; N, 14.32%.

2-[4-Methyl-piperazinyl-methyl]-3-[N-isonicotinamide-yl]-6-iodo-quinazoline-4-one (4i)

Yield, 68%; m.p. 189-191 ºC; FTIR (KBr) v: 3354 (NH), 3064 (C-H Aromatic), 2956 (C-H aliphatic), 1677 (C=O of amide), 1635 (C=O of quinazoline), 1358 (C=N), 1271 (C-N), 1226 (N-N), 538 (C-I) cm⁻¹; ¹H NMR (400MHz, DMSO-d₆) δ ppm: 1.76 (s, 2H, CH₂), 2.18 (s, 3H, CH₃), 2.46-2.70 (m, 8H, 4×CH₂), 7.23-8.30 (m, 7H, Ar-H), 8.74 (s, 1H, NH-CO); ¹³C NMR (100MHz, DMSO-d₆) δ ppm: 38.36 (CH₃), 51.43 (C₁₅), 55.26 (C₁₆, C₁₉), 57.38 (C₁₇, C₁₈), 95.67 (C₁), 122.13-137.13 (C₃-C₅, C₁₀-C₁₁), 142.14 (C₂), 142.52 (C₁₄), 147.60 (C₆), 150.38 (C₁₂-C₁₃), 164.15 (C₆), 167.37 (C₇), 168.51 (C₉); MS: m/z [504.08]⁺; Analysis calculated for C₂₅H₂₅N₆O₂; C, 47.63; H, 4.20; N, 16.66. Found: C, 47.56; H, 4.27; N, 16.54%.

2-[4-(2-Methoxy-phenyl)-piperazinyl-methyl]-3-[N-isonicotinamide-yl]-6-iodo-quinazoline-4-one (4j)

Yield, 70%; m.p. 234-236 ºC; FTIR (KBr) v: 3336 (NH), 3065 (C-H Aromatic), 2924 (C-H aliphatic), 1697 (C=O of amide), 1630 (C=O of quinazoline), 1324 (C=N), 1283 (C-N), 1247 (N-N), 542 (C-I) cm⁻¹; ¹H NMR (400MHz, DMSO-d₆) δ ppm: 1.86 (s, 2H, CH₂), 2.36-2.63 (m, 8H, 4×CH₂), 3.79 (s, 2H, OCH₃), 6.52-8.16 (m, 11H, Ar-H), 8.68 (s, 1H, NH-CO); ¹³C NMR (100MHz, DMSO-d₆) δ ppm: 51.34 (C₁₅), 55.30 (C₁₆, C₁₉), 56.15 (OCH₃), 57.80 (C₁₇, C₁₈), 95.53 (C₁), 122.18-137.23 (C₃-C₅, C₁₀-C₁₁, C₂₀-C₂₄), 142.17 (C₂), 142.48 (C₁₄), 146.59 (C₂₅), 147.64 (C₆), 150.14 (C₁₂-C₁₃), 164.18 (C₆), 167.31 (C₇), 168.53 (C₉); MS: m/z [596.10]⁺; Analysis calculated for C₂₆H₂₅N₆O₂; C, 52.36; H, 4.22; N, 14.09. Found: C, 52.45; H, 4.36; N, 14.13%.

2-[4-(4-Methoxy-phenyl)-piperazinyl-methyl]-3-[N-isonicotinamide-yl]-6-iodo-quinazoline-4-one (4k)

Yield, 71%; m.p. 243-245 ºC; FTIR (KBr) v: 3348 (NH), 3054 (C-H Aromatic), 2932 (C-H aliphatic), 1684 (C=O of amide), 1637 (C=O of quinazoline), 1316 (C=N), 1276 (C-N),
1253 (N-N), 531 (C-I) cm$^{-1}$; $^1$H NMR (400MHz, DMSO-$d_6$) δ ppm: 1.78 (s, 2H, CH$_2$), 2.32-2.53 (m, 8H, 4×CH$_2$), 3.86 (s, 2H, OCH$_2$), 6.68-8.16 (m, 11H, Ar-H), 8.64 (s, 1H, NH-CO); $^{13}$C NMR (100MHz, DMSO-$d_6$) δ ppm: 51.43 (C$_{15}$), 55.26 (C$_{16}$, C$_{19}$), 56.21 (OCH$_2$), 57.70 (C$_{17}$, C$_{18}$), 95.44 (C$_1$), 122.23-137.34 (C$_{2}$-C$_{5}$, C$_{16}$-C$_{19}$, C$_{20}$-C$_{21}$, C$_{22}$-C$_{23}$), 142.23 (C$_2$), 142.56 (C$_{13}$), 147.87 (C$_9$), 150.20 (C$_{12}$-C$_{13}$), 151.52 (C$_{22}$), 164.13 (C$_9$), 167.26 (C$_7$), 168.45 (C$_6$); MS: m/z [596.10]$^{+}$; Analysis calculated for C$_{26}$H$_{25}$IN$_2$O$_3$: C, 52.36; H, 4.22; N, 14.09. Found: C, 52.23; H, 4.35; N, 14.17%.

2-[4-(2,3-Dichloro-phenyl)-piperazinyl-methyl]-3-[N-isonicotinamide-yl]-6-iodo-quinazoline-4-one (4I)

Yield, 69%; m.p. 232-234 °C; FTIR (KBr) ν: 3351 (NH), 3048 (C-H Aromatic), 2943 (C-H aliphatic), 1678 (C=O of amide), 1649 (C=O of quinazolinone), 1311 (C=N), 1282 (C-N), 1260 (N-N), 526 (C-I) cm$^{-1}$; $^1$H NMR (400MHz, DMSO-$d_6$) δ ppm: 1.85 (s, 2H, CH$_2$), 2.35-2.57 (m, 8H, 4×CH$_2$), 6.47-8.14 (m, 10H, Ar-H), 8.78 (s, 1H, NH-CO); $^{13}$C NMR (100MHz, DMSO-$d_6$) δ ppm: 51.35 (C$_{2}$), 55.48 (C$_{16}$, C$_{19}$), 57.30 (C$_{17}$, C$_{18}$), 95.40 (C$_1$), 116.12-137.30 (C$_3$-C$_5$, C$_{10}$-C$_{11}$, C$_{20}$-C$_{24}$), 142.18 (C$_2$), 142.64 (C$_{14}$), 146.50 (C$_{25}$), 147.73 (C$_8$), 150.16 (C$_{12}$-C$_{13}$), 164.23 (C$_9$), 167.30 (C$_7$), 168.37 (C$_6$); MS: m/z [634.01]$^{+}$; Analysis calculated for C$_{26}$H$_{22}$Cl$_2$I$_3$N$_2$O$_2$: C, 47.27; H, 3.33; N, 13.23. Found: C, 47.38; H, 3.25; N, 13.34%.

In vitro evaluation of antibacterial and antifungal activities

The MIC (Minimal Inhibition Concentration) of synthesized compounds was carried out by broth dilution method. Serial dilutions were prepared for the purpose of the primary and secondary screening. Each synthesized drug was diluted obtaining 1000 µg/mL concentration, as a stock solution. In the primary screening 500, 250, 150 and 125 µg/mL of the synthesized drugs was taken. The active synthesized drugs found in this primary screening were further tested in a second set of dilution against all microorganisms. The drugs found active in primary screening were similarly diluted to obtain 100, 62.5, 25, 12.5, 6.250, 3.125 and 1.5625 µg/mL concentrations. The highest dilution showing at least 99% inhibition is taken as MIC. The results of antibacterial and antifungal activity are summarized in Table 1.

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<tr>
<td>4h</td>
<td>-I</td>
<td>C$_6$H$_5$CH$_2$</td>
<td>200</td>
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<td>4i</td>
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<td>CH$_3$</td>
<td>500</td>
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<tr>
<td>4j</td>
<td>-I</td>
<td>2-OCH$_3$C$_6$H$_5$</td>
<td>150</td>
<td>200</td>
<td>150</td>
<td>250</td>
<td>150</td>
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<tr>
<td>4k</td>
<td>-I</td>
<td>4-OCH$_3$C$_6$H$_5$</td>
<td>150</td>
<td>125</td>
<td>150</td>
<td>100</td>
<td>62.5</td>
<td>100</td>
<td>62.5</td>
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<tr>
<td>4l</td>
<td>-I</td>
<td>2,3-diClC$_6$H$_5$</td>
<td>125</td>
<td>62.5</td>
<td>150</td>
<td>150</td>
<td>200</td>
<td>125</td>
<td>150</td>
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</table>

Gentamycin - - 0.05 1 0.05 1 0.25 0.5 -
Ampicillin - - 100 100 100 100 250 100 -
Chloramphenicol - - 50 50 50 50 50 50 -
Ciprofloxacin - - 25 25 25 25 50 50 -
Norfloxacin - - 10 10 10 10 10 10 -

E.c = E. coli (MTCC 443); P.a = P. aeruginosa (MTCC 1688); Kl.p = K. pneumoniae (MTCC109); S.t = S. typhi (MTCC98); S.a = S. aureus (MTCC 96); S.p = S. pyogenus (MTCC 442); B.s = B. subtilis (MTCC 441)
In vitro evaluation of antitubercular activity

Drug susceptibility and determination of MIC of the test compounds against *mycobacterium tuberculosis H*₃₇*Rv* were performed by L. J. (Lowenstein and Jensen) MIC method¹⁹,²⁰ for the measurement of MIC. Stock solutions of primary 1000, 500, 250 µg/mL and secondary 200, 150, 100, 62.5, 50, 25, 12.5, 6.25 and 3.25 µg/mL dilutions of each test compound in dimethylsulphoxide (DMSO) were added liquid L. J. Medium and then media were sterilized by inspissations method. A culture of *mycobacterium tuberculosis H*₃₇*Rv* growing on L. J. Medium was harvested in 0.85% saline in bijou bottles. These tubes were then incubated at 37±1 °C for 24 h followed by streaking of *mycobacterium tuberculosis H*₃₇*Rv* (5x10⁴ bacilli per tube). These tubes were then incubated at 37 °C. Growth of bacilli was seen after 12 days, 22 days and finally 28 days of incubation. Tubes having the compounds were compared with control tubes where medium alone was incubated with *mycobacterium tuberculosis H*₃₇*Rv*. The concentration at which no development of colonies occurred or <20 colonies was taken as MIC concentration of test compound. The standard strain *mycobacterium tuberculosis H*₃₇*Rv* was tested with known drug rifampicin and isoniazid.

The antitubercular data are shown in Table 2.

| Table 2. In vitro antifungal activity (MIC µg/mL) of compounds 4a-l |
|---------------------|--------|--------|--------|
| Entry  | C.a.  | A.n.  | A.c.  |
| 4a     | 1000  | 1000  | 1000  |
| 4b     | 1000  | 500   | 1000  |
| 4c     | 500   | 1000  | 500   |
| 4d     | 500   | 250   | 500   |
| 4e     | 250   | 200   | 200   |
| 4f     | 500   | 500   | 500   |
| 4g     | 250   | >1000 | >1000 |
| 4h     | 1000  | >1000 | 1000  |
| 4i     | 1000  | 500   | 1000  |
| 4j     | 500   | 500   | 250   |
| 4k     | 250   | 200   | 250   |
| 4l     | 500   | 500   | 1000  |
| Nystatin | 100   | 100   | 100   |
| Greseofulvin | 500   | 100   | 100   |

C.A= C. albicans (MTCC 227); A.N= A. niger (MTCC 282); A.C= A. clavatus (MTCC 1323).

Results and Discussion

The synthetic protocol used to synthesize the title compounds is outlined in Scheme 1. Compounds 4a-l was synthesized by using commercially available isonicotinic acid hydrazide. First, N-chloroacetyl substituted anthranilic acid 2a/2b was synthesized by the reaction of substituted anthranilic acid 1a/1b with chloroacetylenechloride using dry benzene as solvent²¹. Further cyclisation²¹ of 2a/2b with isonicotinic acid hydrazide in the presence of dry K₂CO₃, yielded 2-chloromethyl-3-[N-isonicotinamide-yl]-substituted-quinazolin-4-one 3a/3b, which on further condensation with various substituted piperazine derivatives in the presence of anhydrous sodium carbonate gives desired compounds 4a-l. The structure of synthesized compounds was established by IR, (¹H & ¹³C)-NMR, elemental analysis and mass spectral analysis. In the IR spectrum of 4a-l, the most characteristic absorption bands observed at 3336-3379 cm⁻¹ (NH), 2924-2959 cm⁻¹ (C-H aliphatic) and 1271-1305 (C-N).
the $^1$H-NMR spectra of compounds 4a-l, NH peaks were observed as singlet at about $\delta$ 8.54-8.84 ppm region. In addition, protons of piperazine were observed as multiplet at about $\delta$ 2.32-2.76 ppm region. All the other aromatic and aliphatic protons were observed at expected regions. From the $^{13}$C-NMR spectra it was observed that aliphatic carbon attached with piperazine ring appear at about $\delta$ 51.17-51.48 ppm and carbon of piperazine ring was observed in the region of about $\delta$ 55.18-55.48 and $\delta$ 57.24-57.83 ppm.

\[
\text{Scheme 1. Synthesis of piperazinyl-quinazoline-1-one (4a-l)}
\]

From in vitro antibacterial and antifungal activity data, it is confirmed that compound 4f and 4k exhibited excellent activity against all tested gram negative strains and gram positive strains respectively while compounds 4d, 4j and 4k displayed comparable activity against gram-negative strains. Other compounds are found to be moderate to good active against all antibacterial strain tested as compared to standard antibiotics. The in vitro antifungal activity data demonstrate that compounds 4e and 4k exhibited excellent antifungal activity against the fungal strain tested.

In general, the order of antibacterial activity of the substituent is 2, 3-dichloro phenyl > 4-methoxy phenyl > 2-methoxy phenyl > benzyl > H > methyl and also 2,3-disubstituted > 2- substituted is the order for better activity. Therefore, it can be inferred that presence of polar substituent imparts much towards antimicrobial potency.

The encouraging results from the antibacterial and antifungal studies impelled us to go for preliminary screening of synthesized compounds against mycobacterium tuberculosis H$_{37}$Rv. Among the newly synthesized compounds, compound 4f, 4h and 4l produced highest efficacy and exhibited $>$95% inhibition at a concentration of 50 and 62.5 $\mu$g/mL against mycobacterium tuberculosis H$_{37}$Rv. Thus, 2,3-dichloro substituent displayed relatively higher inhibitory activity in general. On the other hand with methoxy group 4d, 4e, 4j and 4k showed relatively low inhibitory activity against mycobacterium tuberculosis H$_{37}$Rv. Thus, introduction of electron withdrawing substituent gives excellent antitubercular potency; this may be due to increased lipophilicity or with favorable steric hinderance.

\[
\text{Scheme 1. Synthesis of piperazinyl-quinazoline-1-one (4a-l)}
\]
Table 3. *In vitro* antitubercular activity of compounds 4a-l

<table>
<thead>
<tr>
<th>Entry</th>
<th>M.Tuberculosis (MIC µg/mL)</th>
<th>H₃Rv (MTCC 200)</th>
<th>% Inhibition</th>
<th>Clogp*</th>
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<tr>
<td>4a</td>
<td>250</td>
<td>85</td>
<td>0.61</td>
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<td>4b</td>
<td>100</td>
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<td>4c</td>
<td>250</td>
<td>88</td>
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</tr>
<tr>
<td>4d</td>
<td>250</td>
<td>90</td>
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<tr>
<td>4e</td>
<td>150</td>
<td>91</td>
<td>0.63</td>
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<td>4f</td>
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<td>96</td>
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<td>89</td>
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<tr>
<td>4h</td>
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<td>96</td>
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<tr>
<td>4i</td>
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<td></td>
</tr>
<tr>
<td>4l</td>
<td>62.5</td>
<td>95</td>
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<td>Rifampicin</td>
<td>40</td>
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<tr>
<td>Isoniazid</td>
<td>0.20</td>
<td>99</td>
<td>-0.60</td>
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</table>

*Theoretical values of log P were calculated using commercially available chem draw program

Conclusion

Among all the newly synthesized compounds some compounds are showing good antituberculous effect due to presence of three pharmacologically active nucleus *viz.* quinazolinone, piperazine and isoniazid. The importance of such work lies in the possibility that the new compounds obtained using such molecular hybridization might be a more efficacious against bacteria, mycobacteria and fungal infections, for which a thorough investigation regarding its structure activity relationship, toxicity and *in vivo* biological effects is essential, which is underway in our laboratory.

Acknowledgement

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References


