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RESEARCH ARTICLE

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* $H_{37}Rv$.

Keywords: Piperazine, 2-Chloromethyl-3-(*N*-isonicotinamide-yl)-4*H*quinazolinone, 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 heterocycles[1], among them quinazolinone have remained always a major source for therapeutic drugs[2]. Also they are the building block for more than 150 naturally occurring pharmacologically active alkaloids and commercial drugs[3,4].

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 activity[5]. On the other hand, piperazine and substituted piperazine are important pharmacophore that can be found in many marketed drugs and drugs under clinical trials[6,7]. Also, piperazines were explored with several biological activity[8-11]. Actually the polarity of nitrogen atoms of piperazine ring enhances favorable interaction with biomacromolecules and thus confers the biological activity[12,13].

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[14-17] with promising antimicrobial activity, herein, we report the synthesis and evaluation of *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 (ν_{max} in cm^{-1}) were recorded on a Perkin-Elmer 1720 FT-IR spectrometer (KBr pellets). ^{1}H-NMR and ^{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.

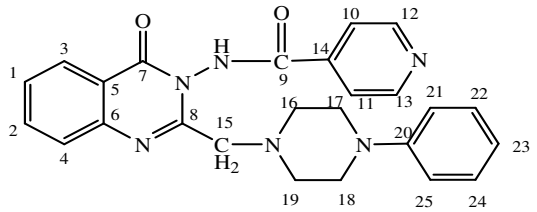


Figure 1. Numbering system for 13C-NMR of compounds **4a, 4c-g** and **4i-1**

*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 ºC; 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^{-1}; ^{1}H NMR (400MHz, DMSO-d_6) δ ppm: 1.82 (s, 2H, CH_2), 2.48-2.65 (m, 8H, 4×CH_2), 6.73-8.28 (m, 13H, Ar-H), 8.54 (s, 1H, NH-CO); ^{13}C NMR (100MHz, DMSO-d_6) δ 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 $C_{25}H_{24}N_6O_2$: 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 ºC; 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^{-1}; ^{1}H NMR (400MHz, DMSO-d_6) δ ppm: 1.74 (s, 2H, CH_2), 2.34-2.46

(m,8H, 4×CH_2), 2.60 (s, 2H, CH_2), 7.32-8.12 (m, 13H, Ar-H), 8.67 (s, 1H, NH-CO); ^{13}C NMR (100MHz, DMSO-*d_6*) δ ppm: 51.28 (C_{15}), 55.48 (C_{16}, C_{19}), 57.32 (C_{17}, C_{18}), 60.23 (CH_2), 122.32-136.27 (C_1-C_5, C_{10}-C_{11},C_{20}-C_{25}), 142.20 (C_{14}), 147.63 (C_6), 150.39 (C_{12}-C_{13}), 164.14 (C_8), 167.23 (C_7), 168.40 (C_9); MS: *m/z* $[454.21]^+$; Analysis calculated for $C_{26}H_{26}N_6O_2$: 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 ºC; FTIR (KBr) *v*: 3358 (NH), 3053 (C-H Aromatic), 2947 (C-H aliphatic), 1673 (C=O of amide), 1631 (C=O of quinazolinone), 1367 (C=N), 1278 (C-N), 1224 (N-N) cm^{-1}; ^{1}H NMR (400MHz, DMSO-*d_6*) δ ppm: 1.81 (s, 2H, CH_2), 2.32 (s, 3H, CH_3), 2.49-2.67 (m, 8H, 4×CH_2), 7.27-8.28 (m, 8H, Ar-H), 8.70 (s, 1H, NH-CO); ^{13}C NMR (100MHz, DMSO-*d_6*) δ ppm: 38.62 (CH_3), 51.35 (C_{15}), 55.18 (C_{16}, C_{19}), 57.24 (C_{17}, C_{18}), 122.24-133.38 (C_1-C_5, C_{10}-C_{11}), 142.34 (C_{14}), 147.58 (C_6), 150.43 (C_{12}-C_{13}), 164.21 (C_8), 167.30 (C_7), 168.44 (C_9); MS: *m/z* $[378.18]^+$; Analysis calculated for $C_{20}H_{22}N_6O_2$: 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 ºC; FTIR (KBr) *v*: 3362 (NH), 3049 (C-H Aromatic), 2956 (C-H aliphatic), 1680 (C=O of amide), 1638 (C=O of quinazolinone), 1372 (C=N), 1294 (C-N), 1218 (N-N) cm^{-1}; ^{1}H NMR (400MHz, DMSO-*d_6*) δ ppm: 1.92 (s, 2H, CH_2), 2.36-2.58 (m, 8H, 4×CH_2), 3.88 (s, 2H, OCH_3), 6.57-8.06 (m, 12H, Ar-H), 8.73 (s, 1H, NH-CO); ^{13}C NMR (100MHz, DMSO-*d_6*) δ ppm: 51.29 (C_{15}), 55.19 (C_{16}, C_{19}), 56.27 (OCH_3), 57.83 (C_{17}, C_{18}), 115.89-133.13 (C_1-C_5, C_{10}-C_{11},C_{20}-C_{23},C_{25}), 142.26 (C_{14}), 146.52 (C_{24}), 147.75 (C_6), 150.23 (C_{12}-C_{13}), 164.20 (C_8), 167.25 (C_7), 168.49 (C_9); MS: *m/z* $[470.21]^+$; Analysis calculated for $C_{26}H_{26}N_6O_3$: C, 66.37; H, 5.57; N, 17.86. Found: C, 66.48; H, 5.42; N, 17.93%.

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

Yield, 67%; m.p. 236-238 ºC; FTIR (KBr) *v*: 3357 (NH), 3061 (C-H Aromatic), 2948 (C-H aliphatic), 1674 (C=O of amide), 1623 (C=O of quinazolinone), 1365 (C=N), 1305 (C-N), 1226 (N-N) cm^{-1}; ^{1}H NMR (400MHz, DMSO-*d_6*) δ ppm: 1.84 (s, 2H, CH_2), 2.40-2.64 (m, 8H, 4×CH_2), 3.90 (s, 2H, OCH_3), 6.71-8.13 (m, 12H, Ar-H), 8.79 (s, 1H, NH-CO); ^{13}C NMR (100MHz, DMSO-*d_6*) δ ppm: 51.38 (C_{15}), 55.30 (C_{16}, C_{19}), 56.15 (OCH_3), 57.78 (C_{17}, C_{18}), 115.94-136.13 (C_1-C_5, C_{10}-C_{11}, C_{20}-C_{21}, C_{23}-C_{25}), 142.35 (C_{14}), 147.82 (C_6), 150.18 (C_{12}-C_{13}), 151.43 (C_{22}), 164.16 (C_8), 167.30 (C_7), 168.53 (C_9); MS: *m/z* $[470.21]^+$; Analysis calculated for $C_{26}H_{26}N_6O_3$: 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 ºC; FTIR (KBr) ν: 3364 (NH), 3047 (C-H Aromatic), 2953 (C-H aliphatic), 1682 (C=O of amide), 1630 (C=O of quinazolinone), 1357 (C=N), 1291 (C-N), 1230 (N-N) cm^{-1}; ^{1}H NMR (400MHz, DMSO-*d_6*) δ ppm: 1.98 (s, 2H, CH_2), 2.39-2.68 (m, 8H, 4×CH_2), 6.57-8.10 (m, 11H, Ar-H), 8.84 (s, 1H, NH-CO); ^{13}C NMR (100MHz, DMSO-*d_6*) δ ppm: 51.48 (C_{15}), 55.36 (C_{16}, C_{19}), 57.25 (C_{17}, C_{18}), 116.79-135.81 (C_1-C_5, C_{10}-C_{11}, C_{20}-C_{24}), 142.23 (C_{14}), 146.48 (C_{25}), 147.78 (C_6), 150.22 (C_{12}-C_{13}), 164.21 (C_8), 167.28 (C_7), 168.45 (C_9); MS: *m/z* $[508.12]^+$; Analysis calculated for $C_{25}H_{22}Cl_2N_6O_2$: 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 quinazolinone), 1360 (C=N), 1290 (C-N), 1231 (N-N), 552 (C-I) cm^{-1}; ^1H NMR (400MHz, DMSO-d_6) δ ppm: 1.75 (s, 2H, CH_2), 2.50-2.76 (m, 8H, 4×CH_2), 6.51-8.28 (m, 12H, Ar-H), 8.63 (s, 1H, NH-CO); ^{13}C NMR (100MHz, DMSO-d_6) δ ppm: 51.17 (C_{15}), 55.31 (C_{16}, C_{19}), 57.52 (C_{17}, C_{18}), 95.80 (C_1), 122.51-137.38 (C_3-C_5, C_{10}-C_{11},C_{20}-C_{24}), 142.17 (C_2), 142.54 (C_{14}), 144.40 (C_{25}), 147.49 (C_6), 150.42 (C_{12}-C_{13}), 164.19 (C_8), 167.21 (C_7), 168.46 (C_9); MS: *m/z* $[566.09]^+$; Analysis calculated for $C_{25}H_{23}IN_6O_2$: 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) ν: 3367 (NH), 3072 (C-H Aromatic), 2946 (C-H aliphatic), 1688 (C=O of amide), 1628 (C=O of quinazolinone), 1364 (C=N), 1284 (C-N), 1220 (N-N), 542 (C-I) cm^{-1}; ^1H NMR (400MHz, DMSO-d_6) δ ppm: 1.85 (s, 2H, CH_2), 2.35-2.68 (m, 8H, 4×CH_2), 3.58 (s, 2H, CH_2), 7.34-8.19 (m, 12H, Ar-H), 8.59 (s, 1H, NH-CO); ^{13}C-NMR (100MHz, DMSO-d_6) δ ppm: 51.17 (C_{15}), 55.53 (C_{16}, C_{19}), 57.24 (C_{17}, C_{18}), 60.12 (CH_2), 95.74 (C_1), 122.26-136.30 (C_3-C_5, C_{10}-C_{11},C_{20}-C_{25}), 142.11 (C_2), 142.48 (C_{14}), 147.51 (C_6), 150.33 (C_{12}-C_{13}), 164.16 (C_8), 167.25 (C_7), 168.35 (C_9); MS: *m/z* $[580.11]^+$; Analysis calculated for $C_{26}H_{25}IN_6O_2$: 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 quinazolinone), 1358 (C=N), 1271 (C-N), 1226 (N-N), 538 (C-I) cm^{-1}; ^1H NMR (400MHz, DMSO-d_6) δ ppm: 1.76 (s, 2H, CH_2), 2.18 (s, 3H, CH_3), 2.46-2.70 (m, 8H, 4×CH_2), 7.23-8.30 (m, 7H, Ar-H), 8.74 (s, 1H, NH-CO); ^{13}C-NMR (100MHz, DMSO-d_6) δ ppm: 38.36 (CH_3), 51.43 (C_{15}), 55.26 (C_{16}, C_{19}), 57.38 (C_{17}, C_{18}), 95.67 (C_1), 122.13-137.13 (C_3-C_5, C_{10}-C_{11}), 142.14 (C_2), 142.52 (C_{14}), 147.60 (C_6), 150.38 (C_{12}-C_{13}), 164.15 (C_8), 167.37 (C_7), 168.51 (C_9); MS: *m/z* $[504.08]^+$; Analysis calculated for $C_{20}H_{21}IN_6O_2$: 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 quinazolinone), 1324 (C=N), 1283 (C-N), 1247 (N-N), 542 (C-I) cm^{-1}; ^1H NMR (400MHz, DMSO-d_6) δ ppm: 1.86 (s, 2H, CH_2), 2.36-2.63 (m, 8H, 4×CH_2), 3.79 (s, 2H, OCH_3), 6.52-8.16 (m, 11H, Ar-H), 8.68 (s, 1H, NH-CO); ^{13}C NMR (100MHz, DMSO-d_6) δ ppm: 51.34 (C_{15}), 55.30 (C_{16}, C_{19}), 56.15 (OCH_3), 57.80 (C_{17}, C_{18}), 95.53 (C_1), 122.18-137.23 (C_3-C_5, C_{10}-C_{11},C_{20}-C_{23},C_{25}), 142.17 (C_2), 142.48 (C_{14}), 146.59 (C_{24}), 147.64 (C_6), 150.14 (C_{12}-C_{13}), 164.18 (C_8), 167.31 (C_7), 168.53 (C_9); MS: *m/z* $[596.10]^+$; Analysis calculated for $C_{26}H_{25}IN_6O_3$: 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 quinazolinone), 1316 (C=N), 1276 (C-N),

1253 (N-N), 531 (C-I) cm^{-1}; ^1H 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_3), 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_3), 57.70 (C_{17}, C_{18}), 95.44 (C_1), 122.23-137.34 (C_3-C_5, C_{10}-C_{11},C_{20}-C_{21},C_{23}-C_{25}), 142.23 (C_2), 142.56 (C_{14}), 147.87 (C_6), 150.20 (C_{12}-C_{13}), 151.52 (C_{22}), 164.13 (C_8), 167.26 (C_7), 168.45 (C_9); MS: *m/z* $[596.10]^+$; Analysis calculated for $C_{26}H_{25}IN_6O_3$: 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 ***(4l)***

Yield, 69%; m.p. 232-234 ºC; FTIR (KBr) *v*: 3351 (NH), 3048 (C-H Aromatic), 2943 (C-H aliphatic), 1678 (C=O of amide), 1649 (C=O of quinazolinone), 1314 (C=N), 1282 (C-N), 1260 (N-N), 526 (C-I) cm^{-1}; ^1H 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_{15}), 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_6), 150.16 (C_{12}-C_{13}), 164.23 (C_8), 167.30 (C_7), 168.37 (C_9); MS: *m/z* $[634.01]^+$; Analysis calculated for $C_{25}H_{21}Cl_2IN_6O_2$: 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[18]. 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, 50, 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.

Table 1. *In vitro* antibacterial activity (MIC µg/mL) of compounds **4a-l**

Entry	R	R'	*E.c.*	*P.a.*	*Kl.p*	*S.t.*	*S.a.*	*S.p.*	*B.s.*
4a	-H	C_6H_5	250	250	500	250	200	500	250
4b	-H	$C_6H_5CH_2$	200	250	200	200	200	250	150
4c	-H	CH_3	500	250	500	500	500	250	250
4d	-H	2-OCH_3 C_6H_5	125	150	150	200	200	150	200
4e	-H	4-OCH_3 C_6H_5	250	250	200	250	100	62.5	100
4f	-H	2,3-diCl C_6H_5	62.5	100	62.5	62.5	100	125	100
4g	-I	C_6H_5	500	250	250	500	250	250	500
4h	-I	$C_6H_5CH_2$	200	100	200	200	250	250	200
4i	-I	CH_3	500	500	250	500	250	500	250
4j	-I	2-OCH_3 C_6H_5	150	200	150	250	250	150	200
4k	-I	4-OCH_3 C_6H_5	150	125	150	100	62.5	100	62.5
4l	-I	2,3-diCl C_6H_5	125	62.5	150	150	200	125	150
Gentamycin	-	-	0.05	1	0.05	1	0.25	0.5	-
Ampicilin	-	-	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= Kl. 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 method[19,20] 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 ºC for 24 h followed by streaking of *mycobacterium tuberculosis $H_{37}Rv$* ($5x10^4$ bacilli per tube). These tubes were then incubated at 37 ºC. 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**

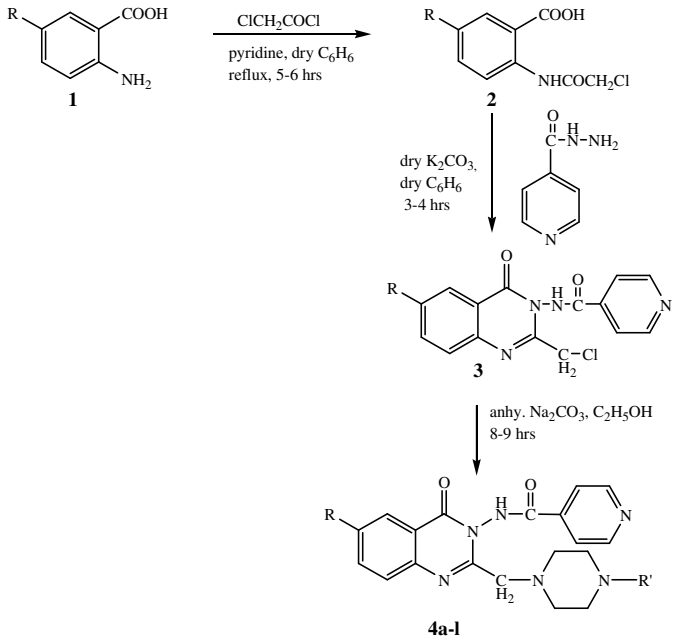
Entry	*C.a.*	*A.n.*	*A.c.*
4a	1000	1000	1000
4b	1000	500	1000
4c	500	1000	500
4d	500	250	500
4e	250	200	200
4f	500	500	500
4g	250	>1000	>1000
4h	1000	>1000	1000
4i	1000	500	1000
4j	500	500	250
4k	250	200	250
4l	500	500	1000
Nystatin	100	100	100
Greseofulvin	500	100	100

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 chloroacetylchloride using dry benzene as solvent[21]. Further cyclisation[21] of **2a/2b** with isonicotinic acid hydrazide in the presence of dry K_2CO_3, 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, (^{1}H & ^{13}C)-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). In

the ^{1}H-NMR spectra of compounds **4a-l**, NH peaks were observed as singlet at about δ 8.54-8.84 ppm region. In addition, protons of piperazine were observed as multiplate at about δ 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 δ 51.17-51.48 ppm and carbon of piperazine ring was observed in the region of about δ 55.18-55.48 and δ 57.24-57.83 ppm.



Scheme 1. Synthesis of piperazinyl-quinazoline-1-one (**4a-I**)

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 > 4-subsituted > 2- substituted is the order for better activity. Therefore, it can be inferred that presence of polar substituent imparts much towards antimicrobial potency[12,13,22].

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 µg/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[12,13,22].

Table 3. *In vitro* antitubercular activity of compounds **4a-l**

Entry	*M.Tuberculosis* $H_{37}Rv$ (MIC µg/mL) *(MTCC 200)*	% Inhibition	Clogp*
4a	250	85	0.61
4b	100	95	1.47
4c	250	88	-0.24
4d	250	90	0.63
4e	150	91	0.63
4f	50	96	2.15
4g	250	89	1.80
4h	62.5	96	2.65
4i	250	34	0.93
4j	250	87	1.82
4k	200	90	1.82
4l	62.5	95	3.33
Rifampicin	40	98	6.04
Isoniazid	0.20	99	-0.60

**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 antituberculosis 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.

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