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In vitro antimicrobial, antimycobacterial evalution and synthesis of substituted 1, 2, 4-triazole motifs

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Abstract: In this present study preparation of 2,3,4,5-Tetrafluoro-*N*-(3-(substituted)-5-((quinoxalin-2-yloxy) methyl)-4*H*-1,2,4-triazol-4-yl)benzamides(37-48) condensed with 2-(substituted)-5-((quinoxalin-2-yloxy) methyl)-1,3,4-oxadiazole and 2,3,4,5-tetrafluoro benzo -hydrazide. All synthesized compounds were analyzed by spectral studies and elemental analysis. Antibacterial studies of all the compounds were performed towards *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Staphylococcus pyogenes* and antifungal activities against *Candida albicans*, *Aspergillus niger*, *Aspergillus clavatus*. All compounds were screened for *M. tuberculosis* $H_{37}Rv$. Compounds 39, 40, 41, 45 and 46 exhibited very good antimicrobial activity and among of them compound 39 and 48 exhibited good antituberculosis activity compared with standard drugs rifampicin and isoniazid.

Keywords: 1,2,4-triazol, antimicrobial activity, *M. tuberculosis* $H_{37}Rv$.

Introduction

Tuberculosis is one of the wildly spread dangerous disease, responsible for millions of death. It is an infectious disease caused by Mycobacterium tuberculosis [1]. TB is common among men than women, and affects mainly adults in the most economically productive age groups. According to global tuberculosis report in 2013, an estimated 9.0 million people developed TB and 1.5 million died from this disease. This pathogenic bacteria mainly affects lung (pulmonary TB or PTB), but also affect other sites of the body like brain, bones, kidneys, lymph nodes as the infection can spread via blood from the lung which is called as extra pulmonary tuberculosis (EPT) [2][3]. Tuberculosis control by multidrug resistant is challenge for M. tuberculosis. Multidrug-resistant and extensively drug-resistant tuberculosis (M/XDR-TB) pose major risk. The rapid spread of these forms of TB is a matter of worldwide concern and makes up a serious barrier to TB control [4]. Triazole is

an active nucleus of antifungal heterocyclic compounds [5,6]. Also active as antiviral [7, 8], antibacterial [9] and antituberculosis [10-14]. 1, 2, 3-triazoles and 1, 2, 4-triazoles are very important in pharmaceutical industry. Heterocycles bearing symmetrical triazole is 1, 2, 4-triazole reported to show a broad spectrum of biological activities [15]. Triazoles are familiar class of heterocyclic nucleus containing bio diversity and their utility as medicine is very much-established. 1,2,4-tri substituted triazoles evaluated as potential antitubercular and antimicrobial agents [16-26]. Fungicidal activity of triazole on fungal membrane by inhibition of lanosterol 14α-dimethylase a cytochrome p-450 enzyme. The biological importance of triazole as antifungal and antituberculosis has prompted us to design and synthesize new structural analogues in which substituents could be arranged in a new molecular framework to display possible higher order of antifungal activity. We have synthesized trisubstitured1, 2, 4 triazoles, substitution on 3rd, 4thand 5thposition. In 2, 3, 4, 5-Tetrafluoro-*N*-(3-(substituted)-5-((quinoxalin-2-yloxy) methyl)-4*H*-1, 2, 4-triazol-4-yl) benzamides structure, on 5th position quinazoline nucleus and on 3rd position substituted phenyl ring and substituted quinolone played very important role in biological evolution. Mainly 7-chloro-6-fluoroquinolin-4(4a*H*) one nucleus on 3rd position of 1, 2, 4-triazole highly active compound for antibacterial, antifungal and antituberculosis. When this substitution on 3rd position changed by fluorinated phenyl ring system, it enhance antifungal activity.

Materials and Methods

Melting points of all compounds measured on open capillary method. Reactions were monitored by thin layer chromatography (TLC) on silica gel; plates were visualized with ultraviolet light or iodine. IR absorption spectra were recorded on Perkin-Elmer RX-1 FTIR spectrophotometer using KBr disc. 1 H NMR at 400 MHz and, 13C NMR at 100MHz spectra measured on a Bruker Avance II 400 spectrometer in $CDCl_3$, operating at 400, 100.6 MHz, respectively by using Tetra Methyl Silane(TMS). Mass spectra of selected samples recorded using Waters Q-T of micromass using ESI as ion source (TOF MS ES+) and LCMS 2010 shmiadzu ESI probequadratole detector. Column chromatography performed on silica gel 60 (0.043–0.06 mm) Merck. Elemental analysis performed on Carlo Erba1108 analyser and the result were varying within± 0.04% of the calculated values.

General procedure for the synthesis of Ethyl 2-(quinoxalin-2-yloxy) acetate A:

To the solution of quinoxalin-2-ol (1.46 g, 0.01 mole) in dry DMF (25 mL), anhydrous potassium carbonate (1.38 g, 0.01 mole) and ethyl chloro acetate (1.07 mL, 0.01 mole) added. The resultant mixture stirred at 80 for 15-17 h. The reaction monitored by TLC on silica gel using mobile phase ethyl acetate: toluene (2.5:7.5). After completion of the reaction, cooled and then the reaction mixture added to a large amount of water. The solid separated filtered, washed with excess of water. The crude product purified by crystallization from ethanol and purified by column chromatography.

General procedure for the synthesis of 2-(Quinoxalin-2-yloxy)acetohydrazide B:

To the solution of ethyl 2-(quinoxalin-2-yloxy) acetate A (2.32 g, 0.01 mole) in methanol (25 ml) added hydrazine hydrate (1.65 ml, 0.02 mole). The reaction mixture refluxed on a water bath for 10-12 h. The reaction monitored by TLC on silica gel using ethyl acetate: toluene (2.5:7.5). After completion of the reaction, the solvent evaporated to dryness and the crude solid washed with water and recrystallized from DMF. The residue purified by column chromatography.

General procedure for the synthesis

of 2-(substitutdphenyl)-5-((quinoxalin-2-yloxy) methyl)-1,3,4-oxadiazole C:

A mixture 2-(quinoxalin-2-yloxy) acetohydrazide B (2.18 g, 0.01 mole), and 2,3,4,5,6-penta fluorobenzoic acid (1.94 g, 0.01 mole) in phosphorus oxy chloride (5 mL) refluxed on water bath for 8-9 h. The progress of the reaction monitored by TLC using toluene: ethyl acetate: methanol (70:20:10) as mobile phase. After completion of the reaction, cooled and poured into crushed ice with continuous stirring. The solid mass separated was neutralized with sodium bicarbonate solution (10% w/v). The resulting solid thus obtained collected by filtration, washed well with cold water, dried and crystallized from absolute ethanol. The residue purified by column chromatography. (Yield 85 %, m.p. 218-220^{o}C).IR (cm-1): 2915, 2863 (C-H, asym, sym), 1632 (C=C), 1286, 1135 (C-O-C, asym, sym).1H-NMR (δ ppm) 7.12-8.33 (m, 5H, Ar-H), 5.72 (s, 2H, OCH_2).

Other 2-(substituted)-5-((quinoxalin-2-yloxy) methyl)-1,3,4-oxadiazoles derivatives prepared by the same method.

General procedure for the synthesis of 2,3,4,5-Tetrafluoro-N-(3-(substitutedphenyl)-5-((quinoxalin-2-yloxy) methyl)-4H-1,2,4-triazol-4-yl)benzamide D:

Amixtureof2-(perfluorophenyl)-5-((quinoxalin-2-yloxy)methyl)-1,3,4-oxadiazole(3.76 g, 0.01 mole) and 2,3,4,5-tetrafluorobenzohydrazide (2.08 g,0.01 mole) in dry pyridine (10 mL) refluxed for 18-24 h. The reaction monitored by TLC on silica gel using ethyl acetate: toluene (2.5:7.5). Then cooled and poured on to crush ice. The reaction mass neutralized by dilute hydrochloric acid and resulting solid washed with cold water, dried and crystallized from absolute ethanol. The residue purified by column chromatography. Similarly other 2,3,4,5-Tetrafluoro-*N*-3-(substituted – 5((quinoxalin-2-yloxy)methyl)-4*H*-1,2,4 – triazole-4-yl) benzamides derivatives have beenprepared by the same method.IR (cm^{-1})**:**3433 (NH), 2980, 2846 (C-H, asym, sym), 1673 (C=O),1656 (amide-I), 1643 (C=N), 1517 (amide-II), 1252 (amide-III),1130 (C-F). ^{1}H-NMR (δ ppm):9.85 (*s*, 1H, CONH), 7.10-8.34 (*m*, 6H, Ar-H), 5.75 (*s*, 2H, OCH_2).

Ethyl 2-(quinoxalin-2-yloxy) acetate A: Yield 78 %, m.p. 96-98^{o}C.IR: 2983, 2852, 1733, 1653, 1233, 1107. ^{1}H-NMR (DMSO-*d*6, 600 MHz)**:** 7.10-8.61 (*m*, 5H, Ar-H); 5.02 (*s*, 2H, OCH_2); 4.26-4.29 (*q*, 2H, CH_2 of ester); 1.25-1.29 (*t*, 3H, CH_3 of ester).

2-(Quinoxalin-2-yloxy) acetohydrazide B: Yield 68 %, m. p. 190-194^{o}C. IR: 3420, 3312, 2933, 2852, 1662, 1530, 1220. ^{1}H-NMR (DMSO-*d*6, 600 MHz)**:** 9.49 (*s*, 1H, CONH); 7.27-8.61 (*m*, 5H, Ar-H); 4.94 (*s*, 2H, OCH_2); 3.70 (*s*, 2H, NH_2).

2-(Perfluorophenyl)-5-((quinoxalin-2-yloxy) methyl)-1,3,4-oxadiazole C: Yield 85 %, m. p. 218-220^{o}C. IR: 2915, 2863 (C-H, asym, sym), 1632 (C=C), 1286, 1135 (C-O-C, asym, sym).^{1}H-NMR (δ ppm) 7.12-8.33 (*m*, 5H, Ar-H), 5.72 (*s*, 2H, OCH_2).

2,3,4,5-tetrafluorobenzohydrazide E: IR (KBr, cm^{-1}): 3401, 3345 (-NHNH2), 1653 (amide-I), 1540 (amide-II), 1207(amide-III), 1150 (C-F).

2,3,4,5-Tetrafluoro-N-[3-(4-methoxy-benzyl)-5-(quinoxalin-2-yloxymethyl)-[1,2,4] triazol-4-yl]-benzamide 37. : Yield 57 %, m.p. 141-143^{o}C IR: 3432, 2982, 2843, 1671, 1654, 1644, 1518, 1258, 1138; ^{1}H-NMR (DMSO-*d*6, 600 MHz):9.88 (s, 1H, CONH); 7.13-8.33 (m, 10H, Ar-H); 5.73 (*s*, 2H, OCH_2); 4.34 (*s*, 2H, CH_2); 3.14 (*s*, 3H, OCH_3). ^{13}C-NMR (DMSO-*d*6, 150 MHz)**:** 163.54; 160.58; 156.06; 114.30-146.16; 67.26; 29.07; 55.98; MS: 538 ([M + H]$^{+}$). Anal. calc. for C_{28} H_{20} N_6 F_4O_5 : C 58.00, H

3.37, N 15.61; found: C 57.99, H 3.35, N 15.60.

2,3,4,5-Tetrafluoro-N-[3-(3-methoxy-phenyl)-5-(quinoxalin-2-yloxymethyl)-[1,2,4] triazol-4-yl]-benzamide 38.: Yield 56 %, m.p. 161-163°C IR: 3431, 2981, 2841, 1674, 1656, 1646, 1514, 1254, 1134. ^{1}H-NMR (DMSO-*d*6, 600 MHz): 9.82 (*s*, 1H, CONH); 7.12-8.32 (*m*, 10H, Ar-H); 5.02 (*s*, 2H, OCH_2); 3.13 (*s*, 3H, OCH_3). ^{13}C-NMR (DMSO-*d*6, 150 MHz):163.53; 160.53; 156.03; 114.39-146.18; 67.22; 55.93. MS: 524 ([M + H] $^+$). Anal. calc. for $C_{27}H_{18}N_6F_4O_5$: C 57.24, H 3.08, N 16.03; found: C 57.24, H 3.05, N 16.01.

N-[3-(7-Chloro-6-fluoro-4-oxo-4,4a-dihydro-quinolin-3-yl)-5-(quinoxalin-2-yloxymethyl)-[1,2,4]triazol-4-yl]-2,3,4,5-tetrafluoro-benzamide 39.: Yield 87 %, m.p. 193-195°C IR: 3431, 2980, 2845, 1674, 1666, 1641, 1523, 1251, 1144. ^{1}H-NMR (DMSO-*d*6, 600 MHz): 9.73 (*s*, 1H, CONH); 7.39-8.02 (*m*, 10H, Ar-H); 6.3 (*s*, 2H, CH_2); 5.72 (*s*, 2H, OCH2); 3.10 (*s*, 3H, OCH_3). ^{13}C-NMR (DMSO-*d*6, 150 MHz):163.53; 160.53; 156.03; 119.39-146.18; 67.22; 55.93. MS: 614.07 (M^+ +2), 615.07 (M^+ +1), 613.04 ([M + H] $^+$). Anal. calc. for $C_{29}H_{15}ClN_7F_5O_5$: C 52.83, H 2.13, N 15.97; found: C 52.80, H 2.11, N 15.95.

2,3,4,5-Tetrafluoro-N-[3-(quinoxalin-2-yloxymethyl)-5-(2,3,4,5-tetrafluoro-phenyl)-[1,2,4] triazol-4-yl]-benzamide 40. : Yield 54 %, m.p. 173-175°CIR: 3434, 2984, 2844, 1672, 1652, 1647, 1517, 1257, 1137. ^{1}H-NMR (DMSO-*d*6, 600 MHz): 9.89 (*s*, 1H, CONH); 7.19-8.38 (*m*, 7H, Ar-H); 5.78 (*s*, 2H, OCH_2); ^{13}C-NMR (DMSO-*d*6, 150 MHz): 163.52; 160.52; 156.02; 114.31-146.15; 67.27; MS: 566 ([M + H] $^+$). Anal. calc. for $C_{26}H_{12}N_6F_8O_4$: C 50.90, H 1.78, N 14.84; found: C 50.88, H 1.75, N 14.82.

2,3,4,5-Tetrafluoro-*N*-(3-(perfluorophenyl)-5-((quinoxalin-2-yloxy)methyl)-4*H*-1,2,4-triazol -4-yl)benzamide 41: Yield 65 %, m. p. 152-154°C. IR**:** 3433, 2980, 2846, 1673, 1656, 1643, 1517, 1252, 1130. ^{1}H-NMR (DMSO-*d*6, 600 MHz):9.85 (*s*, 1H, CONH); 7.10-8.34 (*m*, 6H, Ar-H); 5.75 (*s*, 2H, OCH_2).^{13}C-NMR (DMSO-*d*6, 150 MHz): 163.52; 160.55; 156.07; 114.32-146.11; 67.25. MS: 584 ([M + H] $^+$). Anal. calc. for $C_{26}H_{11}N_6F_9O_4$: C 49.33, H 1.55, N 14.38; found: C 49.30, H 1.52, N 14.35.

2,3,4,5-Tetrafluoro-N-[3-pyridin-3-yl-5-(quinoxalin-2-yloxymethyl)-[1,2,4]triazol-4-yl]-benzamide 42.: Yield 55 %, m. p. 169-171°C. IR: 3435, 2985, 2845, 1675, 1655, 1642, 1512, 1253, 1133. ^{1}H-NMR (DMSO-*d*6, 600 MHz): 9.87 (*s*, 1H, CONH); 7.18-8.36 (*m*, 10H, Ar-H); 5.77 (*s*, 2H, OCH_2). ^{13}C-NMR (DMSO-*d*6, 150 MHz): 163.56; 160.57; 156.07; 114.34-146.12; 67.26. MS: 495 ([M + H] $^+$). Anal. calc. for $C_{25}H_{15}N_7F_4O_4$: C 55.76, H 2.65, N 19.79; found: C 55.74, H 2.62, N 19.75.

N-[3-(6-Chloro-pyridin-3-yl)-5-(quinoxalin-2-yloxymethyl)-[1,2,4]triazol-4-yl]-2,3,4,5-tetrafluoro-benzamide 43.: Yield 87 %, m. p. 178-180°C. IR: 3432, 2981, 2841, 1673, 1653, 1648, 1518, 1258, 1138, 752. ^{1}H-NMR (DMSO-*d*6, 600 MHz): 9.82 (*s*, 1H, CONH); 7.12-8.32 (*m*, 9H, Ar-H); 5.72 (*s*, 2H, OCH_2). ^{13}C-NMR (DMSO-*d*6, 150 MHz): 163.55; 160.51; 156.01; 114.37-146.10; 67.28. MS: 529 ([M + H] $^+$), 531 (M^+ +2). Anal. calc. for $C_{25}H_{14}ClN_7F_4O_4$: C 52.14, H 2.28, N 18.51; found: C 52.12, H 2.25, N 18.50.

N-[3-(2-Benzylsulfanyl-pyridin-3-yl)-5-(quinoxalin-2-yloxymethyl)-[1,2,4]triazol-4-yl]-2,3,4,5-tetrafluoro-benzamide 44.: Yield 78 %, m. p. 187-189°C. IR: 3439, 2989, 2849, 1678, 1658, 1645, 1512, 1253, 1133. ^{1}H-NMR (DMSO-*d*6, 600 MHz): 9.83 (*s*, 1H, CONH); 7.13-8.33 (*m*, 14H, Ar-H); 5.73 (*s*, 2H, OCH_2); 4.33 (*s*, 2H, CH_2). ^{13}C-NMR (DMSO-*d*6, 150 MHz): 163.54; 160.54; 156.04; 114.38-146.15; 67.20; 29.07. MS: 617 ([M + H] $^+$). Anal. calc. for $C_{32}H_{21}N_7F_4O_4S$: C 58.34, H 3.10, N 15.88;

found: C 58.31, H 3.07, N 15.85.

2,3,4,5-Tetrafluoro-N-[3-furan-2-yl-5-(quinoxalin-2-yloxymethyl)-[1,2,4]triazol-4-yl]-benzamide 45.: Yield 54 %, m. p. 156-158°C. IR: 3437, 2986, 2846, 1671, 1651, 1644, 1513, 1256, 1235, 1107, 1139. ^{1}H-NMR (DMSO-*d*6, 600 MHz): 9.86 (*s*, 1H, CONH); 7.16-8.39 (*m*, 9H, Ar-H); 5.74 (*s*, 2H, OCH2). ^{13}C-NMR (DMSO-*d*6, 150 MHz): 163.56; 160.58; 156.08; 114.35-146.18, 67.24. MS: 484 ([M + H] $^{+}$). Anal. calc. for $C_{24}H_{14}N_6F_4O_5$: C 54.55, H 2.50, N 17.35; found: C 54.51, H 2.47, N 17.31.

N-[3-(1-Cyclopropyl-6-fluoro-4-oxo-7-piperazin-1-yl-1,4-dihydro-quinolin-3-yl)-5-(quino xalin -2-yloxymethyl)-[1,2,4]triazol-4-yl]-2,3,4,5-tetrafluoro-benzamide 46.: Yield 75 %, m. p. 146-148°C. IR: 3435, 2981, 2846, 1672, 1666, 1641, 1533, 1228. ^{1}H-NMR (DMSO-*d*6, 600 MHz): 9.73 (*s*, 1H, CONH); 7.3-8.6 (*m*, 10H, Ar-H); 6.7 (*s*, 2H, CH_2); 5.76 (*s*, 2H, OCH_2); 3.10 (*s*, 3H, OCH3). ^{13}C-NMR (DMSO-*d*6, 150 MHz):164.53; 160.54; 156.03; 119.39-146.18; 67.22; 57.93. MS: 703.04 ([M + H] $^{+}$). Anal. calc. for $C_{36}H_{28}N_9F_5O_5$: C 58.04, H 3.72, N 17.92; found: C 58.01, H 3.70, N 17.90.

N-[3-(1-Ethyl-6-fluoro-4-oxo-7-piperazin-1-yl-1,4-dihydro-quinolin-3-yl)-5-(quinoxalin-2-yloxymethyl)-[1,2,4]triazol-4-yl]-2,3,4,5-tetrafluoro-benzamide 47.: Yield 65 %, m. p. 196-198°C. IR: 3437, 2987, 2844, 1676, 1660, 1641, 1533, 1228. ^{1}H-NMR (DMSO-*d*6, 600 MHz): 9.73 (*s*, 1H, CONH); 7.3-8.6 (*m*, 10H, Ar-H); 6.3 (*s*, 2H, CH_2); 5.76 (*s*, 2H, OCH_2); 3.10 (*s*, 3H, OCH3). ^{13}C-NMR (DMSO-*d*6, 150 MHz):164.53; 160.54; 156.03; 119.39-145.18; 67.22; 61.93. MS: 691.14 ([M + H] $^{+}$). Anal. calc. for $C_{35}H_{28}N_9F_5O_5$: C 57.31, H 3.79, N 18.23; found: C 57.30, H 3.75, N 18.20.

N-[3-[2-(2,6-Dichloro-phenylamino)-phenyl]-5-(quinoxalin-2-yloxymethyl)-[1,2,4]triazol-4-yl]-2,3,4,5-tetrafluoro-benzamide 48.: Yield 74 %, m. p. 182-184°C. IR: 3438, 3346, 2985, 2845, 1679, 1659, 1649, 1511, 1251, 1131, 751. ^{1}H-NMR (DMSO-*d*6, 600 MHz): 9.84 (*s*, 1H, CONH); 8.54 (*s*, 1H, NH); 7.14-8.36 (*m*, 6H, Ar-H); 5.76 (*s*, 2H, OCH_2), 4.36 (*s*, 2H, CH_2); ^{13}C-NMR (DMSO-*d*6, 150 MHz): 163.59; 160.59; 156.09; 114.39-146.19; 67.29; 29.02. MS: 667 ([M + H]$^{+}$), 669 (M^{+}+1), 671 (M^{+}+2). Anal. calc. for $C_{33}H_{21}Cl_2N_7F_4O_4$: C 55.70, H 2.87, N 14.67; found: C 55.68, H 2.85, N 14.65.

Results and discussion

Chemistry

Synthetic route for all unreported title compounds given in Scheme-1. 1, 2, 4-triazol ring system was prepared by 2-[(5-substituted-1, 3, 4-oxadiazol-2-yl) methoxy] quinoxaline(C). This compound was key intermediate required for the synthesis of title compounds. Quinoxalin-2-ol when react with ethyl chloro acetate in the presence of anhydrous K_2CO_3 yielded ethyl [(quinoxalin-2-yl) oxy] acetate (A). This compound further react with hydrazine hydrate and given2-[(quinoxalin-2-yl)oxy] acetohydrazide (B). To prepare oxadiazole ring, 2-[(quinoxalin-2-yl) oxy] acetohydrazide condensed with substituted aromatic acid in the presence of $POCl_3$, Which yielded 2-[(5-substituted-1,3,4-oxadiazol-2-yl)methoxy]quinoxaline(C). Resulted compound (C) when reacted with 2,3,4,5-tetrafluorobenzohydrazide in the presence of pyridine it yielded final compound 2,3,4,5-Tetrafluoro-*N*-(3-(substituted)-5-((quinoxalin-2-yloxy) methyl)-4*H*-1,2,4-triazol-4-yl) benzamides (D).

Spectral studies of these compounds proved the structure of this compound. IR spectrum of 2,3,4,5-tetrafluorobenzohydrazide show (-$NHNH_2$) absorption band at (3401-3345)cm^{-1}, Which disappeared in 2,3,4,5-Tetrafluoro-*N*-

(3-(perfluorophenyl)-5-((quinoxalin-2-yloxy) methyl)-4*H*-1,2,4-triazol-4-yl)benzamide. IR absorption band of this compound (cm^{-1})**:** 3433 (NH), 2980, 2846 (C-H, asym, sym), 1673 (C=O), 1656 (amide-I), 1643 (C=N), 1517 (amide-II), 1252 (amide-III), 1130 (C-F). ^{1}H NMR spectrum of this compound recorded in DMSO-d6, the signal due to CONH protons appeared at 9.85 ppm as a singlet, the Ar-H protons multiplate at 7.10-8.34 ppm, singlet of 2 protons of OCH_2 at 5.75 ppm. In the ^{13}C NMR spectrum of compounds recovered in DMSO-d6.^{13}C NMR (δ ppm): 163.52 (CO-NH), 160.55 (C5 1,2,4-triazole), 156.07 (C2 1,2,4-triazole), 114.32-146.11 (Ar-C), 67.25 (OCH2). Mass spectra of all the synthesizedcompounds showed M+/M+1 peak, compliance their molecular formula.**Scheme:**

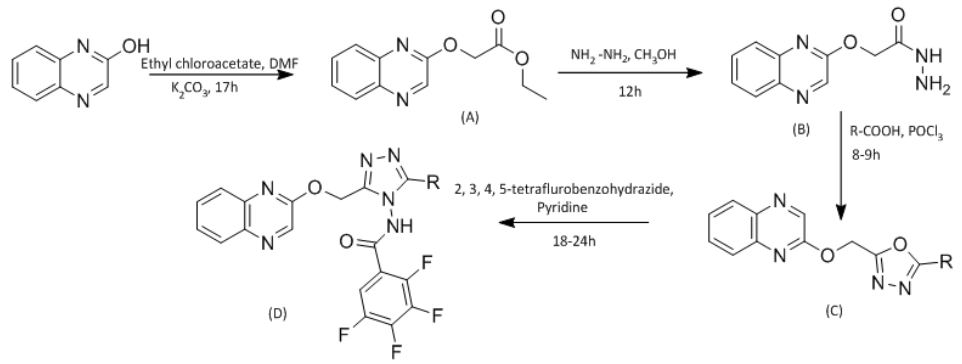
Biological evaluation

In vitro antimicrobial activity

All the newly synthesized compounds 1-12 screened for their antimicrobial activity. This activity determined by the broth micro dilution method according to National Committee for Clinical Laboratory Standard (NCCLS)[27-29]. Standard strains were used for screening of antibacterial and antifungal activities. For antibacterial activity we used *S. aureus* (MTCC 96) and *S. pyogenes*(MTCC 443) as Gram positive, *E. coli*(MTCC 442) and *P. aeruginosa*(MTCC 441) as Gram negative strains by using chloramphenicol and ciprofloxacin as standard antibacterial drug. Antifungal activity screened for three different fungal spices *C. albicans*(MTCC 227), *A. niger*(MTCC 282) and *A. Clavatus*(MTCC 1323). Griseofulvin and nystatine used as a standard antifugal drugs. The strains were procured from Institute of Microbial Technology, Chandigarh.

The results of all compounds against antibacterial and antifungal activity displayed in **Table: 1**. Variable inhibition of all compounds shows against Gram positive and Gram negative bacterial strains. Antibactarial screening of all the newly synthesized compounds showed excellent inhibition against *S. aureus* as compared with standard drug ampicillin. Mainly compound no. 37, 41, 45, and 46 showed outstanding activities against *S. aureus* as compared to ampicillin. Majority of all the tested compounds were sensitive towards gram negative as well as gram positive bacteria.

Scheme: Synthetic pathway for 2,3,4,5-Tetrafluoro-*N*-3-(substituted)-5((quinoxalin-2-yloxy)methyl)-4 *H*-1,2,4-triazol-4-yl)benzamides derivatives D (37-48)



37: R= 1-methoxy-4-ethylbenzene, **38**: R= anisole, **39**: R= 7-chloro-6-fluoroquinolin-4(8*H*)-one, **40**: R= 1,2,3,4-tetrafluorobenzene, **41**: R= 1,2,3,4,5-pentafluorobenzene, **42**: R= pyridine, **43**: R= 2-chloropyridine, **44**: R= 2-(benzylsulfanyl) pyridine, **45**: R= furan, **46**: R= 1-cyclopropyl-6-fluoro-7-(piperazin-1-yl)-8, 8a-dihydroquinolin-4(1*H*)-one, **47**: R= 1-ethyl-6-fluoro-7-(piperazin-1-yl)-8, 8a-dihydroquinolin-4(1H)-one, **48**: R= 2, 6-dichloro-N-(2-ethylphenyl)aniline

Table 1: In vitro antimicrobial activity (MIC, µg/mL) of the synthesized compounds.

Comp. No.	Minimum Inhibitory Concentration (µg/ml)						
	Gram negative bacteria		**Gram positive bacteria**		**Fungal species**		
	E.C. MTCC 443	**P.A. MTCC 741**	**S. A. MTCC 96**	**S. P. MTCC 442**	**C. A. MTCC 227**	**A. N. MTCC 282**	**A. C. MTCC 1323**
37.	250	250	**125**	250	1000	1000	1000
38.	250	250	**250**	250	1000	1000	1000
39.	**100**	**100**	**250**	**100**	1000	**500**	**500**
40.	**100**	200	**200**	**100**	**500**	1000	1000
41.	250	200	**125**	250	**500**	**500**	**500**
42.	250	250	**200**	250	1000	1000	1000
43.	200	200	**250**	200	1000	1000	1000
44.	250	500	**200**	250	1000	1000	1000
45.	200	250	**125**	200	**500**	1000	1000
46.	200	200	**100**	200	**500**	1000	1000
47.	250	500	**250**	250	1000	1000	1000
48.	500	200	**200**	500	1000	1000	1000
A	100	100	250	100	-	-	-
B	50	50	50	50	-	-	-
C	25	25	50	50	-	-	-
D	-	-	-	-	500	100	100
E	-	-	-	-	100	100	100

E. C.:escherichia coli, **P. A.:** pseudomonas aeruginosa, **S.A.:**staphylococcus aureus, **S. P.:** streptococcus pyogenes, **C. A.:**candida albicans, **A. N.:**aspergillus niger,**A. C.:**aspergillus clavatus, **MTCC:** microbial type culture collection**A:** Ampicillin, **B:** Chloramphenicol, **C:** Ciprofloxacin, **D:** Griseofulvin, **E:**Nystatine, **-:** Not tested

Compound no. 39, 40, 46 showed equipotent antibacterial activity as ampicillin. Among of them compound 3 was active against *E. coli*, *P. aeruginosa* &*S. pyogenes*. Whereas compound no. 40 active against *E. coli* and *S. pyogenes.* And compound no. 46 for *S. a*ureus only. No. 37, 41 and 45 also showed good activity towards *S. aureus* as compared to standard drug chloramphenicol and ciprofloxacin. For fungicidal activity the compound No. 40, 41, 45 and 46 displayed very good activity against *C. albicans*(MTCC 227) whereas for *A. niger*(MTCC 282) and *A. clavatus*(MTCC 1323) strain active samples were no. 39 and 41 as compared to standard drug griseofulvin. Quinoxaline- triazole moiety bearing 7-chloro-6-fluoroquinolin-4(4a*H*) one nucleus (comp. 39) respectively showed high degree of activity against all the microorganisms employed. Fluorinated compounds no. 40, 41 and 46 also displayed very good activity against bacteria and fungi as well. No. 41 showed excellent activity against all three species of fungi *C. albicans, A. niger*and *A. Clavatus.* Furan ring containing compound no. 45 also active for gram positive bacteria and fungi especially for *C. Albicans.* Results conclude that all fluorinated synthesized moiety active against both the microorganism.

Anti-tuberculosis activity

All the compounds of this series were tested for anti-tubercular activity. We have used the Minimum Inhibitory Concentrations to evaluate the antituberculosis activity.This classic method yields a quantitative result for the amount of antimicrobial agents that is needed to inhibit growth of specific microorganisms. It is carried out in bottle. Determination of MIC L. J. Slope [30]. All synthesized compounds conducted at 250 µg/mL, 500 µg/mL and 1000 µg/mL.

The bioassay results depicted in **Table 2**revealed that most of the tested compounds moderately inhibitory effects on the growth of the tested M. tuberculosis H_{37}Rv strains. Compound 3 & 12 possessing comparatively good activity among of all these compounds respectively 96% and 99% in 250 µg/mL against *M. tuberculosis H_{37}Rv*. Generally the compounds which containing mesomerically electron withdrawing and inductively electron donating groups shows good anti-tubercular activity. Compound no.3 & 12 containing electron-withdrawing groups (-Cl, -F) have shown promising activity against M. tuberculosis H_{37}Rv. Compounds no. 1 showed 99% inhibition, whereas 2, 4, 6, and 9 showed 95% inhibition and no. 10 at 97% inhibition in 500 µg/mL against M. tuberculosis H_{37}Rv. Above results conclude that compound 3 and 12 may become new class of antituberculosis agent in future.

Table 2: Antitubercular Activity of compounds [37-48].

Compound No.	MIC values (µg/ml) of *M. tuberculosis H37Rv*	%Inhibition
37.	500	99
38.	500	95
39.	**250**	**96**
40.	500	95
41.	1000	98
42.	500	95
43.	1000	99
44.	1000	98
45.	500	95
46.	500	97
47.	1000	98
48.	**250**	**99**
Rifampicin	40	98
Isoniazide	0.20	99

Conclusion

We have synthesized 2,3,4,5-Tetrafluoro-*N*-(3-(substituted)-5-((quinoxalin-2-yloxy) methyl)-4*H*-1,2,4-triazol-4-yl)benzamides (**37-48**). Most of the derivatives were moderately sensitive towards bacteria, fungi and tuberculosis. But

quinoxaline- triazole moiety incorporated with 7-chloro-6-fluoroquinolin-4(4a*H*)one nucleus (Comp.-3) it shown anti-bacterial activity in both Gram positive and Gram negative strain of bacteria, antifungal activity and antituberculosis activity comparable with standard commercial drugs and therefore this compound become lead molecules for further synthetic and biological evaluation. Compound 40, 41, 45 and 46 also active nucleus for antifungal acivity. Whereas compound 39 and 48 were competitively more active for tuberculosis. Finally conclusion for this series is that this class of molecules certainly holds great promise towards the pursuit to discover novel class of antimicrobial and antituberculosis agents.

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