International Journal of PharmTech Research
CODEN (USA): IJPRIF, ISSN: 0974-4304
Vol.8, No.9, pp 127-134, 2015

Synthesis, characterization and in-vitro anti TB studies of Isoxazole analogues

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Abstract: A few findings of failure of antibiotics against microorganisms are still unearthed. In this process it is an attempt to digout a few facts regarding anti TB therapy. So therefore a novel class of anti-TB agents viz., isoxazolo-thiazole derivatives incorporating with 4-(4-Nitro phenyl)-thiazole moiety, have been prepared by new synthetic approach, which were characterized by IR, ^{1}H NMR, ^{13}C NMR and MS spectral data and screened for their antimycobacterial activity against *M. tuberculosis H37Rv.* The preliminary results revealed that some of the compounds exhibited promising antimicrobial and remarkable anti-TB activity.

Key words: γ-ferrite; Isoxazole; Thiazole; anti-TB activity.

Introduction:

Since the discovery of heterocyclic nucleus, the chemistry of azoles and their fused heterocyclic derivatives continuously draw the attention of organic chemists due to their various biological activities. The recent literature survey revealed that thiazoles are familiar class of heterocyclic moieties possessing a wide variety of biological activities and their utility in medicine is very much established [1]. Several biological activities of various thiazole and its derivatives have proved the efficiency in combating various diseases and is noticed to have good antibacterial, antifungal activities[2-3], antitubercular activity[4-6] and anti HIV agents [7]. Consequently isoxazole derivatives have also been reported to possess interesting biological activities such as antifungal [8], anti-viral [9], anti-inflammatory and hypoglycemic agents [10].

In view of these above findings, it was contemplated to design and synthesize a new combination of heterocyclic entities in which thiazole and isoxazole derivatives in a single molecular frame work and evaluate their anti-TB activity with remarkable results.

Experimental:

All melting points were measured on open capillary method. IR spectra were recorded for KBr disc on Schimadzu-8400 FTIR spectrophotometer. ^{1}H NMR, ^{13}C NMR spectra were measured on a Bruker Avance II 400 spectrometer, operating at 400, 100.6 MHz respectively. Chemical shifts (δ) are reported in parts per million and TMS as an internal standard. Molecular weights were determined with TOF MS ES Mass spectra. Reactions were monitored by thin layer chromatography (TLC) on silica gel, plates were visualizing with ultraviolet light or iodine. Column chromatography was performed on silica gel 60(0.043-0.06mm) Merck.

4-(4-Nitrophenyl)-thiazol-2-ylamine (1):

A mixture of iodine (0.01 mol) and thiourea (0.02 mol) were mixed and poured in to round bottom flask containing 4-nitro acetophenone (0.01 mol) in ethanol (25 mL), was refluxed for 8h. After the completion of reaction, cool the reaction mixture at room temperature. Filter the solid and wash with diethyl ether to remove the unreacted acetophenone derivative. After that it is washed with sodium thiosulphate solution to remove unreacted iodine. Finally wash with water and dried, recrystallized from hot water yield 78%. IR (KBr) cm^{-1}: 3328.93(N-H), 3041.18 (CH in Ar), 3058.47(C-H in ring), 1549.18 (N=O), 1601.35 (C=N); ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 7.98 (d, J=9.1 Hz, 2H, Ar-H near NO_2), 7.53 (d, J= 8.6 Hz, 2H, Ar-H), 6.87 (s, 1H, C-H in Thiazole ring), 5.61 (s, 2H, NH_2). ^{13}C NMR (100MHz, DMSO-d_6) δ ppm: 169.1, 153.27, 148.13, 143.5, 131.7, 128.31, 109.8.

4-(4-Nitrophenyl)-2'-phenyl-[2, 3']bithiazolyl-4'one (2):

To a mixture of compound **1** (0.01 mol), benzaldehyde (0.01mol), anhydrous γ-ferrite (Fe_2O_3; 0.02 mol) was added and the reaction mixture was refluxed with constant stirring in dry 1,4-dioxane (40mL) for 1/2 h, followed by the addition of thioglycolic acid (0.01 mol). The refluxing and stirring were continued for another 10h. The reaction was monitored by TLC. After the completion of the reaction, a reddish brown amorphous solid $Fe_2O_3.2H_2O/FeO(OH)$ was removed by filtration, filtrate was concentrated to dryness under reduced pressure to achieve compound **2**. Yield 71.5%, m.p.198-200 °C, IR (KBr) cm^{-1}: 3029.42 (CH in Ar), 2911.71 (CH in CH_2), 1648.06 (C=O in ring), 1601.35 (C=N); ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 7.61 (d, J=8.6 Hz, 2H, Ar-H near NO_2), 6.93-7.5 (m, 7H, Ar-H), 5.13 (s, 1H, N-CH-Ar), 3.51 (s, 2H, S-CH_2). ^{13}C NMR (100MHz, DMSO-d_6) δ ppm: 172.9, 168.3, 150.1, 149.8, 146.3, 138.1, 130.1, 128.5, 125.4, 123.7, 123.2, 119.3, 109.8, 61.4, 41.8.

5'-(substituted benzylidene)-4-(4-nitrophenyl)-2'-phenyl-[2,3']bithiazolyl-4'one (3a-h):

A mixture of compound **2** (0.01mol), respective aldehyde (0.01 mol) and anhy- drous CH_3COONa (0.01 mol) in anhydrous glacial acetic acid (50mL) was refluxed for 5 hours. The reaction mixture was concentrated and then poured into ice cold water the solid thus separated was filtered, washed with water and recrystallized from glacial acetic acid to obtain the desired compound as light yellow needles, yield 54.1%.

The other compounds **3a-h** was prepared similarly by treating with correspond- ing aldehydes. And the characterization data of synthesized compounds have been given below

5'-(Benzylidene)-4-(4-nitrophenyl)-2'-phenyl-[2,3']bithiazolyl-4'one 3(a): Yield: 71.3%; m.p.198-200 °C; IR (KBr) cm^{-1}: 3068.23 (C-H in ring), 3013.41 (C-H in Ar), 1695.28 (C=O), 1589.51 (C=N), 1538.16 (N-O), 693.18 (C-S). ^{1}H NMR (400MHz, DMSO-d_6) δ ppm: 7.9 (s, 1H, CH), 7.09-7.41 (m, 14H, Ar-H), 6.07 (s, 1H, CH in ring), 5.21 (s, 1H, N-CH-Ar). ^{13}C NMR (100MHz, DMSO-d_6) δ ppm: 168.7, 162.1, 147.8, 142.3, 134.8, 130.1, 128.5, 127.4, 101.83, 116.8, 63.1.

5'-(4-Nitro benzylidene)-4-(4-nitrophenyl)-2'-phenyl-[2,3']bithiazolyl-4'one 3(b): Yield: 68.9%; m.p.211-213 °C; IR (KBr) cm^{-1}: 3070.03 (C-H in ring), 3027.18 (C-H in Ar), 1693.64 (C=O), 1591.18 (C=N), 1542.18 (N-O), 693.18 (C-S). ^{1}H NMR (400MHz, DMSO-d_6) δ ppm: 7.87 (s, 1H, CH), 7.8 (d, J=9.1 Hz, 4H, Ar-H near NO_2), 6.87-7.39 (m, 9H, Ar-H), 6.07 (s, 1H, CH in ring), 5.24 (s, 1H, N-CH-Ar). ^{13}C NMR (100MHz, DMSO-d_6) δ ppm: 170.1, 164.3, 147.1, 138.6, 129.5, 128.6, 127.4, 119.7, 116.3, 104.5, 68.7.

5'-(4-Chloro benzylidene)-4-(4-nitrophenyl)-2'-phenyl-[2,3']bithiazolyl-4'one 3(c): Yield: 70.4%; m.p.212-214 °C; IR (KBr) cm^{-1}: 3069.01 (C-H in ring), 3018.09 (C-H in Ar), 1695.14 (C=O), 1592.05 (C=N), 1543.27 (N-O), 1093.74 (C-Cl), 693.18 (C-S). ^{1}H NMR (400MHz, DMSO-d_6) δ ppm: 7.91 (s, 1H, CH), 7.81 (d, J=8.6 Hz, 2H, Ar-H near NO_2), 6.96-7.63 (m, 11H, Ar-H), 6.21 (s, 1H, CH in ring), 5.27 (s, 1H, N-CH-Ar). ^{13}C NMR (100MHz, DMSO-d_6) δ ppm: 172.3, 162.1, 154.3, 138.1, 132.3, 130.9, 128.4, 127.4, 101.83, 116.8, 63.1.

5'-(4-Methoxy benzylidene)-4-(4-nitrophenyl)-2'-phenyl-[2,3']bithiazolyl-4'one 3(d): Yield: 63.4%; m.p.225-227 °C; IR (KBr) cm^{-1}: 3068.23 (C-H in ring), 3027.24 (C-H in Ar), 1691.28 (C=O), 1598.55 (C=N), 1538.16 (N-O), 1238.58 (C-O-C), 701.27 (C-S). ^{1}H NMR (400MHz, DMSO-d_6) δ ppm: 7.88 (s, 1H, CH), 7.73

(d, J=8.6 Hz, 2H, Ar-H near NO_2), 6.58 (d, J=9.1 Hz, 2H, Ar-H near OCH_3), 6.9-7.42 (m, 9H, Ar-H), 6.03 (s, 1H, CH in ring), 5.24 (s, 1H, N-CH-Ar), 3.51 (s, 3H, OCH_3). ^{13}C NMR (100MHz, DMSO-d_6) δ ppm: 169.3, 162.1, 155.3, 140.3, 136.18, 129.1, 127.5, 121.87, 115.9, 101.83, 63.1, 58.27.

5'-(4-Dimethyl amine benzylidene)-4-(4-nitrophenyl)-2'-phenyl-[2,3']bithiazolyl-4'one 3(e): Yield: 65.4%; m.p.175-178 °C; IR (KBr) cm^{-1}: 3059.16 (C-H in ring), 3023.51 (C-H in Ar), 2951.28 (CH_3), 1694.03 (C=O), 1596.18 (C=N), 1516.23 (N-O), 699.64 (C-S). ^{1}H NMR (400MHz, DMSO-d_6) δ ppm: 7.91 (s, 1H, =CH), 7.83 (d, J=8.6 Hz, 2H, Ar-H near NO_2), 6.81 (d, J=9.1 Hz, 2H, Ar-H near $N(CH_3)_2$, 7.08-7.61 (m, 9H, Ar-H), 6.11 (s, 1H, CH in ring), 5.38 (s, 1H, N-CH-Ar), 2.81 (s, 6H, CH_3). ^{13}C NMR (100MHz, DMSO-d_6) δ ppm: 168.1, 162.7, 146.23, 135.12, 131.98, 129.3, 130.1, 128.5, 116.8, 101.57, 69.5.

5'-(4-Bromo benzylidene)-4-(4-nitrophenyl)-2'-phenyl-[2,3']bithiazolyl-4'one 3(f): Yield: 59.7%; m.p.203-205 °C; IR (KBr) cm^{-1}: 3069.01 (C-H in ring), 3018.09 (C-H in Ar), 1695.14 (C=O), 1592.05 (C=N), 1543.27 (N-O), 693.18 (C-S). ^{1}H NMR (400MHz, DMSO-d_6) δ ppm: 7.86 (s, 1H, CH), 7.81 (d, J=8.6 Hz, 2H, Ar-H near NO_2), 6.96-7.63 (m, 11H, Ar-H), 6.21 (s, 1H, CH in ring), 5.27 (s, 1H, N-CH-Ar). ^{13}C NMR (100MHz, DMSO-d_6) δ ppm: 172.3, 162.1, 154.3, 138.1, 132.3, 130.9, 128.4, 127.4, 101.83, 116.8, 63.1.

5'-(4-Methyl benzylidene)-4-(4-nitrophenyl)-2'-phenyl-[2,3']bithiazolyl-4'one 3(g): Yield: 67.4%; m.p.208-210 °C; IR (KBr) cm^{-1}: 3061.43 (C-H in ring), 3029.91 (C-H in Ar), 2927.72 (CH_3), 1695.18 (C=O), 1596.17 (C=N), 1538.18 (N-O), 698.16 (C-S). ^{1}H NMR (400MHz, DMSO-d_6) δ ppm: 7.93 (s, 1H, =CH), 7.81 (d, J=8.6 Hz, 2H, Ar-H near NO_2), 6.93 (d, J=9.1 Hz, 2H, Ar-H near CH_3, 7.01-7.48 (m, 9H, Ar-H), 5.99 (s, 1H, CH in ring), 5.38 (s, 1H, N-CH-Ar), 2.54 (s, 3H, CH_3). ^{13}C NMR (100MHz, DMSO-d_6) δ ppm: 169.3, 162.1, 149.78, 139.86, 136.28, 129.85, 126.52, 118.13, 102.83, 68.4.

5'-(2-Methoxy benzylidene)-4-(4-nitrophenyl)-2'-phenyl-[2,3']bithiazolyl-4'one 3(h): Yield: 65.6%; m.p.217-219 °C; IR (KBr) cm^{-1}: 3068.23 (C-H in ring), 3027.24 (C-H in Ar), 1691.28 (C=O), 1598.55 (C=N), 1538.16 (N-O), 1238.58 (C-O-C), 701.27 (C-S). ^{1}H NMR (400MHz, DMSO-d_6) δ ppm: 7.88 (s, 1H, CH), 7.75 (d, J=8.6 Hz, 2H, Ar-H near NO_2), 6.61 (d, J=9.1 Hz, 2H, Ar-H near OCH_3), 6.9-7.42 (m, 9H, Ar-H), 6.03 (s, 1H, CH in ring), 5.24 (s, 1H, N-CH-Ar), 3.51(s, 3H, OCH_3). ^{13}C NMR (100MHz, DMSO-d_6) δ ppm: 169.3, 162.1, 155.3, 140.3, 136.18, 129.1, 127.5, 121.87, 115.9, 101.83, 63.1, 59.14.

6-(4-(4-Nitrophenyl) thiazol-2-yl)-3,5-diphenyl-3,3a,5,6-tetrahydro thiazolo[4,5-c] isoxazole (4a):

A mixture of compound **3a** (0.005 mol), hydroxylamine hydrochloride (0.01 mol) and sodium acetate (0.005mol) in anhydrous glacial acetic acid (20mL), was refluxed for 8h. The reaction mixture was concentrated and then poured into ice cold water, the solid thus separated, was filtered, washed with water and recrystallized from ethanol to afford pure 6a, (61% yield) as reddish brown solid: m.p.217-219 °C.

IR (KBr) cm^{-1} : 3063.11 (C-H in ring), 3049.61 (C-H in Ar), 1507.29 (N-O in Nitro), 913.43 (N-O in ring), 1512.69 (C=N), 1294.31 (C-N); ^{1}H NMR (400MHz,DMSO-d_6) δ ppm: 6.93-7.48 (m, 12H, Ar-H), 7.68 (d, 2H, Ar-H near NO_2), 5.24(s, 1H, N-CH-Ar), 5.57(d, J=2.1 Hz, 1H, CH-O), 4.95(d, J=2.3 Hz, 1H, S-CH) ^{13}C NMR (DMSO-d_6)δ ppm: 51.5, 52.8, 68.1, 102.6, 129.1, 132.7, 160.2, 176.5. MS: *m/z* 487.54[*M*+1].

The other compounds **4b-h** were also prepared similarly by treating with corresponding compounds. **3b-h**. Physical and analytical were given in **Table-I.**

Table-I: Physical and analytical data of compounds 4a-h:

Comp'd	R	Yield (%)	Mol. formula(M. wt)	C calcd (found)	H Calcd (found)	N calcd (found)
4a	H	62	$C_{25}H_{18}N_4O_3S_2$(486.57)	61.71 (61.68)	3.73 (3.77)	11.51 (11.54)
4b	4-NO_2	62.5	$C_{25}H_{17}N_5O_5S_2$(531.56)	56.49 (56.53)	3.22 (3.26)	13.18 (13.22)
4c	4-Cl	59	$C_{25}H_{17}ClN_4O_3S_2$(521.01)	57.63	3.29	10.75

				(57.67)	(3.25)	(10.79)
4d	4-OCH_3	63	$C_{26}H_{20}N_4O_4S_2$(516.59)	60.45 (60.49)	3.90 (3.94)	10.85 (10.89)
4e	4-$N(CH_3)_2$	61.7	$C_{27}H_{23}N_5O_3S_2$(529.63)	61.23 (61.27)	4.38 (4.42)	13.22 (13.18)
4f	4-Br	63	$C_{25}H_{17}BrN_4O_3S_2$(565.46)	53.10 (53.05)	3.03 (3.07)	9.91 (9.95)
4g	4-CH_3	63.5	$C_{26}H_{20}N_4O_3S_2$(500.59)	62.38 (62.42)	4.03 (3.99)	11.19 (11.15)
4h	2-OCH_3	61	$C_{26}H_{20}N_4O_4S_2$(516.59)	60.45 (60.49)	3.90 (3.94)	10.85 (10.81)

3-(4-Nitrophenyl)-6-(4-(4-nitrophenyl) thiazol-2-yl)-5-phenyl-3, 3a, 5, 6-tetrahydro thiazolo[4,5-c] isoxazole (4b):

Yield-62.5%; m.p.225-227 °C; IR (KBr) cm^{-1}: 3049.61 (C-H in Ar), 1516, 1508.13 (N-O in nitro), 926 (N-O in ring), 1512.69 (C=N), 1294.39 (C-N), 681.93(C-S). ^{1}H NMR (400MHz, DMSO-d_6) δ ppm: 7.71 (d, J=8.3 Hz, 4H, Ar-H near NO_2), 6.85- 7.53 (m, 9H, Ar-H), 5.51 (d, J=2.1 Hz, 1H, CH-O), 5.17 (s, 1H, N-CH-Ar), 4.85 (d, J=2.3 Hz, 1H, S-CH). ^{13}C NMR (DMSO-d_6)δ ppm: 51.5, 52.3, 68.1, 102.6, 119.2, 126.2, 128.6, 147.5, 160.2, 176.2; MS: *m*/*z* 532.48[*M*+1].

3-(4-Chlorophenyl)-6-(4-(4-nitrophenyl) thiazol-2-yl)-5-phenyl-3, 3a, 5, 6-tetrahydro thiazolo[4,5-c] isoxazole (4c):

Yield 59%; m.p.231-233 °C; IR (KBr) cm^{-1}: 3063.11 (C-H in ring), 3049.61 (C-H in Ar), 1521.19 (N-O in Nitro), 1512.69 (C=N), 1294.39 (C=N), 926.01 (N-O in ring). ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 7.73 (d, J=8.3 Hz, 2H, Ar-H near NO_2), 7.2-7.51 (m, 9H, Ar-H), 7.12 (d, J=9.1 Hz, 2H, Ar-H near Cl), 5.69 (d, J=2.1 Hz, 1H, CH-O), 5.13 (s, 1H, N-CH-Ar), 4.82 (d, J=2.1 Hz, 1H, S-CH), ^{13}C NMR (DMSO-d_6) δ ppm: 51.5, 52.3, 68.1, 102.6, 128.2, 130.7, 131.5, 138.6, 157.1, 160.2, 176.2; MS: *m*/*z* 523.09[*M*+2], MS: *m*/*z* 522.13[*M*+1].

3-(4-Methoxyphenyl)-6-(4-(4-nitrophenyl)-thiazol-2-yl)-5-phenyl-3, 3a, 5, 6-tetra hydro thiazolo[4,5-c] isoxazole (4d):

Yield-63%; m.p.224-226 °C; IR (KBr) cm^{-1}: 3069.38 (C-H in ring), 3049.23 (C-H in Ar), 1521.19 (N-O in Nitro), 1512.69 (C=N), 1294.39 (C-N), 1269.17 (C-O-C), 918.73 (N-O in ring). ^{1}H NMR (400MHz, DMSO-d_6) δ ppm: 7.68 (d, J=8.6 Hz, 2H, Ar-H near NO_2), 7.01–7.54 (m, 9H, Ar -H), 6.73 (d, J=9.1 Hz, 2H, Ar-H near OCH_3), 5.62 (d, J=2.3 Hz, 1H, O-CH), 5.17 (s, 1H, N-CH-Ar), 4.93 (d, J=2.3 Hz, 1H, S-CH), 3.18 (s, 3H, OCH_3); ^{13}C NMR (DMSO-d_6) δ ppm: 50.9, 51.5, 52.3, 68.1, 102.6, 117.3, 126.8, 136.1, 155.6, 160.2, 176.2; MS: *m*/*z* 517.62[*M*+1].

N-N-dimethyl-4-(6-(4-(4-nitrophenyl) thiazol-2-yl)-5-phenyl-3, 3a, 5, 6-tetrahydro thiazolo[4,5-c] isoxazole –3-yl)aniline (4e):

Yield: 61.7%; m.p.241-243 °C; IR (KBr) cm^{-1}: 3066.31 (C-H in ring), 3049. 18 (C-H in Ar), 2942.51 (C-H in CH_3), 1521.19 (N-O in nitro), 1512.69 (C=N) 923.48 (N-O in ring). ^{1}H NMR (400MHz, DMSO-d_6) δ ppm: 7.79 (d, J=8.6 Hz, 2H, Ar-H near NO_2), 7.08-7.63 (m, 9H, Ar-H), 6.90 (d, J=9.1 Hz, 2H, Ar-H near NMe_2), 5.57 (d, J=2.3 Hz, 1H, CH-O), 5.21 (s, 1H, N-CH-Ar), 4.82 (d, J=2.3 Hz, 1H, CH-S), 2.93 (s, 6H, CH_3); ^{13}C NMR (DMSO-d_6) δ ppm: 29.3, 51.5, 52.3, 68.1, 102.6, 128.6, 132.7, 135.3, 146.2, 157.8, 162.5, 176.2; MS: *m*/*z* 530.55[*M*+1].

3-(4-Bromophenyl)-6-(4-(4-nitrophenyl) thiazol-2-yl)-5-phenyl-3, 3a, 5, 6-tetrahydro thiazolo[4,5-c] isoxazole (4f):

Yield 63%; m.p.238-240 °C; IR (KBr) cm^{-1} ; 3066.31 (C-H in ring), 3049.18 (C-H in Ar), 1521.19 (N-O intro), 1512.69 (C=N), 923.48 (N-O in ring); ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 7.73 (d, J=8.3 Hz, 2H, Ar-H near NO_2), 7.01-7.6 (m, 11H, Ar-H), 5.56 (d, J=2.1 Hz, 1H, CH-O), 5.21 (s, 1H, N-CH-Ar), 4.87 (d, J=2.1 Hz, 1H, CH-S); ^{13}C NMR (DMSO-d_6) δ ppm : 51.2, 51.9, 68.1, 102.6, 123.5, 130.1, 131.5, 162.5, 175; MS: *m*/*z* 567.29[*M*+2], MS: *m*/*z* 566.46[*M*+1].

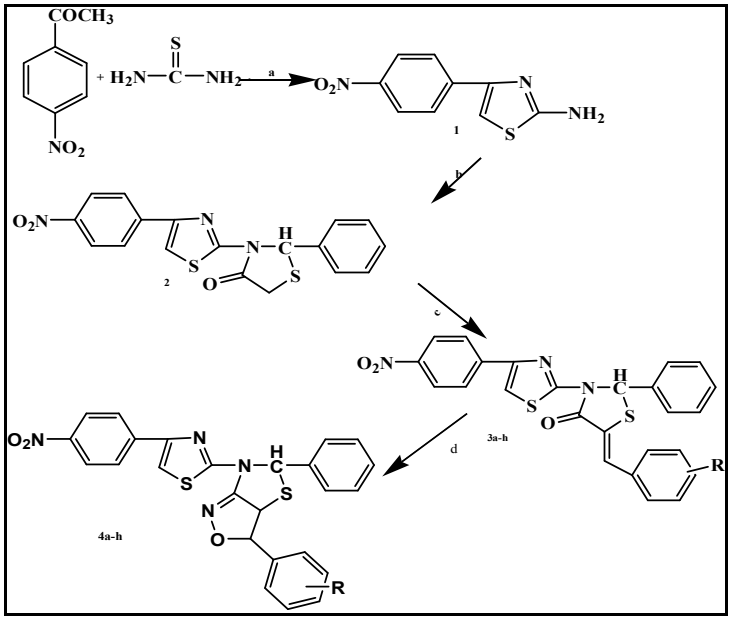
6-(4-(4-Nitrophenyl) thiazol-2-yl)-5-phenyl-3-(p-tolyl)-3, 3a, 5, 6-tetrahydro thiazolo[4,5-c] isoxazole (4g):

Yield 63.5% ; m.p. 243-245 °C; IR (KBr) cm^{-1}; 3059.13 (C-H in ring), 3049.18 (C-H in Ar), 2941.67 (C-H in CH_3), 1512.19 (N-O in nitro), 1513.03 (C=N), 916.77 (N-O in ring); ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 7.73 (d, J=8.3 Hz, 2H, Ar-H near NO_2), 6.85-7.45 (m, 11H, Ar-H), 5.56 (d, J=2.3 Hz, 1H, CH-O), 5.21 (s, 1H, N-CH-Ar), 4.91(d, J=2.3 Hz, 1H, CH-S), 2.78 (s, 3H, OCH_3); ^{13}C NMR (DMSO-d_6) δ ppm : 28.3, 51.2, 51.9, 68.1, 102.6, 124.3, 128.1, 139.5, 157.1, 162.5, 176.2; MS: *m*/*z* 501.68[*M*+1].

3-(2-Methoxyphenyl)-6-(4-(4-nitrophenyl) thiazol-2-yl)-5-phenyl-3, 3a, 5, 6-tetrahydro thiazolo[4,5-c] isoxazole (4h):

Yield- 61% ; m.p.227-229 °C ; IR (KBr) cm^{-1} ; 3063.47 (C-H in ring), 3049.18 (C-H in Ar), 1512.19 (N-O in nitro), 1512.69 (C=N), 1271.84 (C-O-C), 918.15 (N-O in ring); ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 7.68 (d, J=8.3 Hz, 2H, Ar-H near NO_2), 7.05-7.51 (m, 9H, Ar-H), 6.81(d, J=9.1 Hz, 2H, Ar-H near OCH_3), 5.56 (d, J=2.1 Hz, 1H, CH-O), 5.17(s, 1H, N-CH-Ar), 4.91(d, J=2.3 Hz, 1H, CH-S), 3.36 (s, 3H, OCH_3); ^{13}C NMR (DMSO-d_6) δ ppm : 50.8, 51.3, 52.1, 68.1, 102.6, 117.3, 126.8, 129.7, 137.3, 155.1, 157.8, 162.5, 176.2; MS: *m*/*z* 517.51[*M*+1].

Scheme-1: Synthesis of compounds 4a-h.



R=H, 4-NO_2, 4-Cl, 4-OCH_3, 4-$N(CH_3)_2$, 4-Br, 4-CH_3, 2-OCH_3

Reaction conditions: a) I_2, Ethanol, 8h; b) γ-ferrite, Benzaldehyde, $SHCH_2COOH$, 1, 4-dioxane; c) aromatic aldehydes, anhydrous.CH_3COONa, glacial.AcOH. **d**) $NH_2NH_2.H_2O$; anhydrous. CH_3COONa, glacial AcOH;

Biological Activity:

Antimycobacterial activity:

All the synthesized compounds of series (**4a-h**) were evaluated for their antitubercular activity. Drug susceptibility and determination of MIC of the test compounds against *M. tuberculosis H37Rv* were performed by agar micro dilution method, where two fold dilutions of each test compound were added into 7H10 agars supplemented with OADC and organism. A culture of used microorganism *M. tuberculosis H37Rv* growing on L-J medium was harvested in 0.85% saline with 0.05% Tween-80. A suspension of compounds was prepared in DMSO. This suspension was added to (in tubes) 7H10 middle brook's medium (containing 1.7 ml medium and 0.2 ml OADC supplement) at different concentrations of compound keeping the volume constant, that is, 0.1 ml medium was allowed to cool keeping the tubes in slanting position. These tubes were then incubated at 37°C for 24h followed by streaking of *M. tuberculosis H37Rv* (5×104 bacilli per tube). Growth of bacilli was seen after 30 days of incubation. Tubes having the compounds were controlled with control tubes where medium alone was incubated with H37Rv. The concentration at which complete inhibition of colonies occurred was taken as active concentration of test compound. Streptomycin was used as standard drug. The MIC levels of some active compounds (**4a-h**) against these organisms were given in **Table II.**

Table-II: Antituberculosis activity of compounds 4a-h.

Compound	MIC (μg/ml) $H_{37}Rv$
4a	6.25
4b	3.25
4c	6.25
4d	12.5
4e	>6.25
4f	3.125
4g	>12.5
4h	>25
Streptomycin	3.125

Results and Discussions:

Chemistry:

The synthesis of 3-(substituted-phenyl)-6-(4-(4-nitrophenyl) thiazol-2-yl)-5-phenyl-3,3a,5,6-tetrahydro thiazolo[4,5-c] isoxazole (**4a-h**) compounds were prepared by the key intermediate **2**. The key intermediate **2** was prepared by the one pot three component condensation reaction involving 4-(4-Nitrophenyl)-thiazol-2-ylamine (**1**), benzaldehyde, and mercapto acetic acid in the presence of γ-ferrite as a catalyst[11]. Further this compound was reacted with various aromatic aldehydes in the presence of anhydrous CH_3COONa through Knoevenegal reaction which gave 5'-(substituted benzylidene)-4-(4-nitrophenyl)-2'-phenyl-[2, 3']bithiazolyl-4'one (**3a-h**). Thus resulted compounds 3a-h were then converted into title compounds **4a-h** by condensation with hydroxylamine hydrochloride. The structures of newly synthesized compounds were confirmed by their IR, ^{1}H NMR,^{13}C NMR, MS spectral data.

In the present work, a series of new compounds were synthesized. **Scheme-1** illustrates the path used for the preparation of target compounds by the use of p-nitro acetophenone and thiourea as preliminary materials. The structures of target compounds were confirmed by IR, (^{1}H&^{13}C) NMR and MS spectral data. In the IR spectra of compounds **4a-h** disappearance of amide carbonyl (C=O) absorption band at ~1695 cm^{-1}, which was present in compound **3**, confirmed the cyclization or involvement of α, β- unsaturated carbonyl(C=O) system. The N-O band of isoxazole moiety was observed at about 920 cm^{-1} respectively.

In the ^{1}H NMR spectra of compounds **4a-h** were recorded in DMSO-d_6. The signal due to CH-O protons of isoxazole moiety appeared at about 5.5 ppm as a doublet. These signals demonstrate that the

cyclization step had occurred as isoxazole. All the other aromatic protons of **4a-h** were observed at the expected regions.

In the ^{13}C NMR spectrum of compounds **4a-h** were recovered in DMSO-d_6.The signals corresponding to the carbons of isoxazolo-thiazole compounds observed nearly at about 51, 52, 68, 104, 126, 128, 147, 157, 162, 176 ppm are proof of further evidence of their structures. Mass spectra of all the synthesized compounds showed M^+/M^++1 peaks in agreement with their molecular formula.

Anti-TB activity:

The results depicted in **Table-II** revealed that most of the tested compounds displayed variable inhibitory effects on the growth of the tested *M. tuberculosis H37Rv* strains.

Generally compounds possessing mesomerically electron withdrawing and inductively electron donating groups showed good antitubercular activity. In this view (**4b** and **4f**) were equipotent to Streptomycin activity against *M. tuberculosis H37Rv* (MIC 3.125 µg/ml). Besides this the compounds **4a, 4c** and **4e** were 50% less potent than Streptomycin against *M. tuberculosis H37Rv* (MIC 6.25 µg/ml).

Conclusion:

In the present communication our aim has been verified by the synthesis and biological evaluation of 3-(subtituted-phenyl)-6-(4-(4-nitrophenyl) thiazol-2-yl)-5-phenyl-3,3a,5,6-tetrahydro thiazolo[4,5-c] isoxazole (**4a-h**) derivatives, in which 4-aryl- thizole moiety incorporating with thiazolo-isoxazole moieties in a same molecular framework. The obtained results revealed that most of the tested compounds exhibited good antitubercular activities comparable with standard commercial drugs, and they become lead molecules for further synthetic and biological evaluation. Finally it can be concluded that this class of molecules certainly hold great promise towards pursuit of discovering novel class of anti-TB agents.

Acknowledgement:

The authors are grateful to The Head, Department of Chemistry, K L University for providing necessary facilities to carry out this work. We are grateful to The Director, SAIF, Panjab University for providing spectral data. We are grateful to The Director Microcare Laboratory Surat for providing antimycobacterial data.

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