SYNTHESIS AND ANTIMICROBIAL EVOLUTION OF SOME THIAZOLE BASED ISOXAZOLINE MOIETIES AS POTENTIAL ANTITUBERCULAR AGENTS

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ABSTRACT

Since the versatile nature of the isoxazoline derivatives in the form of biological activity, it was a thought of interest to prepare a new class of isoxazolines viz., N-(4-(4-nitrophenyl)thiazol-2-yl)-5-phenyl-4,5-dihydroisoxazol-3-amine moieties have been synthesized. Further these entities were screened for their in-vitro antitubercular activity against *M.Tuberculosis H37Rv* and also antimicrobial activity against various (Gram +ve), (Gram -ve) bacterial strains and various fungal strains. The results revealed that most of the compounds shown promising activity against all the strains employed compared with standard drugs. The synthesized compounds were characterized by IR, ^{1}H NMR, ^{13}C NMR spectral data and elemental analysis studies.

KEYWORDS: Isoxazoline, Antimicrobial, Antitubercular, Thiazole

INTRODUCTION

Small ring heterocycles containing nitrogen, sulfur and oxygen have been under investigation for a long time because of their most precious medicinal properties. In past several decades the isoxazoline and its derivatives have also taken a considerable pharmacological importance (Ozden *et al.*, 2005). A large number of isoxazoline derivatives have been reported to possess various types of important biological activities, such as antitubercular (Tangallapally *et al.*, 2007), anti-inflammatory (Dravyakar *et al.*, 2008), antimicrobial (Varma, 2003; Sharma *et al.*, 2009; Gaonkar *et al.*, 2007; Nagar and Shah, 2003; Abdel-Rahman *et al.*, 2007; Badadhe *et al.*, 2011; Shah and Desai, 2007), antiviral (Sokolov *et al.*, 1981), anti HIV (Ali *et al.*, 2011) and anticonvulsant (Uno *et al.*, 1979) activities.

On the other hand since the discovery of heterocyclic nucleus the chemistry of thiazole and its derivatives continue to draw attention of organic chemists due to their important role as a widely exploited pharmacophore in medicinal chemistry having varied biological activity such as anti-inflammatory (Holla *et al.*, 2003), antibacterial (Sadek *et al.*, 2011), antifungal (Pandeya *et al.*, 1999), antimycobacterial (Andreani *et al.*, 2001; Pattan *et al.*, 2006) have been found to be associated with thiazolidinone derivatives. It has been noticed that introduction of an additional ring to the thiazole core tends to exert profound influence in conferring novel biological activities in those molecules. In view of these facts and in connection with our studies we turn our attention to synthesize some new isoxazoline derivatives of thiazole (AI-Saadi *et al.*, 2008) as a biological agent. These two rings are connected with the bridge of secondary amine which one exhibit prominent biological activity. In the present discussion we are focusing on the antitubercular activity of synthesized moieties to prevent TB disease by which bacterium one third of the globes population infects.

In view of these above findings it was contemplated to design and synthesize some new isoxazoline derivatives incorporating with thiazole moiety. These moieties were further screened for their antimycobacterial and antimicrobial activity.

EXPERIMENTAL

MATERIALS AND METHODS

All the melting points were determined in open capillary method and are uncorrected. IR spectra were recorded for KBr disc on Shimadzu-8400 FT-IR spectrophotometer. ^{1}H and ^{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 ppm and TMS as an internal standard. Purity of the compounds checked by TLC on silica gel 'G' plates. Reactions were monitored by thin layer chromatography (TLC) on silica gel, plates were visualizing with ultraviolet light (or) iodine.

Reaction conditions:a- Iodine, Ethanol; b- Dry benzene; c-NaOH, DMF; d- Hydroxylamine-hydrochloride (NH4OH.HCl), KOH, DMF

General procedure for the preparation of 4-(4-Nitro-phenyl)-4,5-dihydro-thiazol-2-ylamine (1)

An equimolar mixture of p-nitro acetophenone (0.01mol) and iodine (0.01) and 2-equvalents of thiourea in a round bottomed flask, with a 20ml of ethanol were heated with continuous stirring upto 8 Hours. After completion of the reaction reaction mixture was cooled and then filtered it the solid product obtained and washed with diethylether, solution of sodium thiosulphate, and water respectively. Finally the product was dried and recrystallized. The progress of the reaction was checked by TLC.

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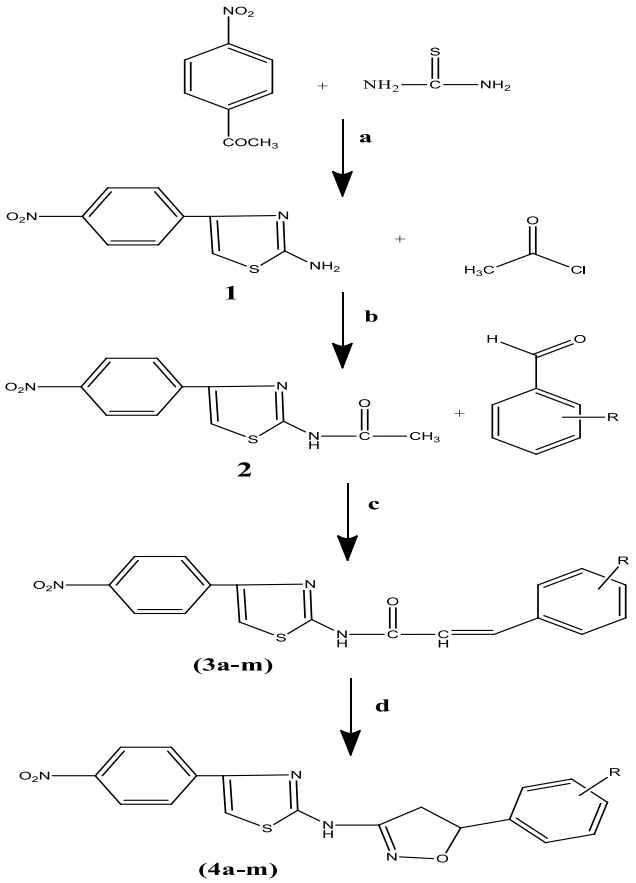

Scheme 1: Synthesis of Isoxazoline Derivatives

General procedure for the preparation of N-(4-(4-nitrophenyl)thiazol-2-yl)acetamide (2)

(0.01mol) of 4-(4-Nitro-phenyl)-4,5-dihydro-thiazol-2-ylamine was taken in 20ml of dry benzene and acetylchloride (0.01mol) was added in drop wise with continuous stirring up to 7-8 hours, finally the excess solvent was distilled off and separated the solid part from the reaction and recrystallized. The progress of the reaction was checked by TLC. IR (KBr) cm^{-1}: 3055(Ar ring), 650(C-S), 1595.61 (C=N).

General procedure for the preparation of (Z)-N-(4-(4-nitrophenyl)thiazol-2-yl)-3-phenyl acryl amide (3)

A solution of (0.01mol) of N-(4-(4-nitrophenyl)thiazol-2-yl)acetamide in DMF(25ml) and (0.01mol) of different aromatic aldehydes were stirred for few minutes and added 2% NaOH solution and refluxed it with continuous stirring up to 6-7 hours the progress of the reaction was checked by TLC. After completion of the reaction the reaction mixture was cooled and then poured it in to crushed ice finally the solid obtained was filtered and recrystallized. IR (KBr) cm^{-1}: 3050(Ar ring), 679(C-S), 1578.28 (C=N), 3238(Ar-H), 1550 (N-H).

General procedure for the preparation of N-(4-(4-nitrophenyl)thiazol-2-yl)-5-phenyl-4,5-dihydroisoxazol-3-amine 4(a-m):

A mixture of (Z)-N-(4-(4-nitrophenyl)thiazol-2-yl)-3-phenylacrylamide (0.01mol) and (0.01mol) of hydroxyl amine hydrochloride taken in 15ml of DMF then added (0.01mol) of KOH, refluxed it with continuous stirring up to 5-6 hours the progress of the reaction was checked by TLC. After completion of the reaction the reaction mixture was cooled and then poured it in to crushed ice finally the solid obtained was filtered and recrystallized. In this way all the isoxazoline derivatives 4(a-m) have been synthesized. Details regarding the structure determination of various synthesized isoxazoline derivatives 4(a-m) on the basis of IR, NMR spectral data studies as given below. The physical data of the synthesized compounds represented in Table 1

N-(4-(4-nitrophenyl)thiazol-2-yl)-5-phenyl-4,5 dihydroisoxazole-3-amine (4a)

IR (KBr) cm^{-1}: 3367.19 (N-H in ring), 3016.19 (Ar-H), 3053 (C-H str in ring), 2869(CH_2), 1581.28 (C=N), 1279.68 (C-N). 1539.03 (N-O), 981(O-CH), 689(C-S); ^{1}H NMR (400MHz, DMSO-d_6) δ ppm: 6.9-7.31 (m, 9H, Ar-H), 4.51 (t, 1H, O-CH), 2.6(d,2H, CH_2),

8.01(s, 1H, N-H); ^{13}C NMR (100MHz, DMSO-d_6) δ ppm: 149.8, 134.1, 129.4, 123.2, 128.8, 118.2(aromatics) , 151.3, 141.0,101.2(thiazole), 120.7,59.1, 53.1(isoxazoline).

5-(3-nitrophenyl)-n-(4-(4-nitrophenyl)thiazol-2-yl)-4,5-dihydroisoxazole-3-amine (4b)

IR (KBr) cm^{-1}: 3364.39 (N-H in ring), 3021.57 (Ar-H), 3057.16 (C-H str in ring), 2871.66 (CH_2), 1581.28 (C=N), 1279.68 (C-N). 1543.72 (N-O), 977(O-CH), 689.18(C-S); ^{1}H NMR (400MHz, DMSO-d_6) δ ppm: 7.05-7.31 (m, 8H, Ar-H), 5.27 (t, 1H, O-CH), 3.98(d,2H, CH_2), 7.98 (s, 1H, N-H); ^{13}C NMR (100MHz, DMSO-d_6) δ ppm: 149.8, 145.2, 134.3, 129.4, 123.2, (aromatics) , 152.1, 141.8, 108.3 (thiazole), 119.3,60.3, 56.7. (isoxazoline).

5-(4-(dimethylamino)phenyl)-N-(4-(4-nitrophenyl)thiazol-2-yl)-4,5-dihydro isoxazole-3-amine (4c)

IR (KBr) cm^{-1}: 3367.15 (N-H in ring), 3017.23 (Ar-H), 3064.98 (C-H str in ring), 2869.18 (CH_2), 1581.16 (C=N), 1279.01 (C-N). 1568.17 (N-O), 993(O-CH), 701.26(C-S), 2951(CH_3); ^{1}H NMR (400MHz, DMSO-d_6) δ ppm: 6.81-7.42 (m, 8H, Ar-H), 4.99 (t, 1H, O-CH), 3.37(d,2H, CH_2), 8.08 (s, 1H, N-H),2.90(S, 6H, $N(CH_3)_2$. ; ^{13}C NMR (100MHz, DMSO-d_6) δ ppm: 150.3, 149.8, 133.7, 129.4, 117.78, 113.65, (aromatics) , 151.8, 142.1, 108.5, (thiazole), 120.1,60.1, 55.8 (isoxazoline), 40.1(C in CH_3).

5-(4-methoxyphenyl)-N-(4-(4-nitrophenyl)thiazol-2-yl)-4,5-dihydroisoxazole-3-amine (4d)

IR (KBr) cm^{-1}: 3351.19 (N-H in ring), 3028.63 (Ar-H), 3057.16 (C-H str in ring), 2904.43 (CH_2), 1596.74 (C=N), 1283.26 (C-N). 1518.72 (N-O), 965.29(O-CH), 701.58(C-S),1225.83(C-O-C), 2946.11(CH_3); ^{1}H NMR (400MHz, DMSO-d_6) δ ppm: 7.1-7.42(m, 8H, Ar-H), 4.51 (t, 1H, O-CH), 3.9(d,2H, CH_2), 8.01 (s, 1H, N-H),3.1(S, 3H,OCH_3); ^{13}C NMR (100MHz, DMSO-d_6) δ ppm: 159.7, 148.7, 133.9, 129.7, 121.6, 113.9, (aromatics) , 150.91, 141.56, 98.3 (thiazole), 119.1, 63.1, 59.8. (isoxazoline).55.4(C in CH_3).

2-methoxy-6-(3-((4-(4-nitrophenyl)thiazol-2-yl)amino)-4,5-dihydroisoxazol-5-yl)phenol (4e)

IR (KBr) cm^{-1}: 3361.08 (N-H in ring), 3034.91 (Ar-H), 3063.45 (C-H str in ring), 2916.07 (CH_2), 1581.16 (C=N), 1279.59 (C-N). 1471.53 (N-O), 981.26(O-CH), 693.53(C-S),1216.47(C-O-C), 2943.65(CH_3), 3416.93(O-H); ^{1}H NMR (400MHz, DMSO-d_6) δ ppm: 7.0-7.59(m,7H, Ar-H), 4.98 (t, 1H, O-CH), 4.0(d,2H, CH_2), 8.03 (s, 1H, N-H),3.85(S, 3H,OCH_3). 5.31(S, 1H, OH); ^{13}C NMR (100MHz, DMSO-d_6) δ ppm:154.1, 148.7, 134.2, 130.6, 123.1, 129.7, 121.0, (aromatics), 151.5, 142.7, 101.8 (thiazole), 120.1, 59.1, 57.8 (isoxazoline).52.7(C in CH_3).

5-(4-nitrophenyl)-N-(4-(4-nitrophenyl)thiazol-2-yl)-4,5-dihydroisoxazole-3-amine (4f):

IR (KBr) cm^{-1}: 3364.39 (N-H in ring), 3021.57 (Ar-H), 3057.16 (C-H str in ring), 2871.66 (CH_2), 1581.28 (C=N), 1279.68 (C-N). 1548.26 (N-O), 977(O-CH), 689.18(C-S); ^{1}H NMR (400MHz, DMSO-d_6) δ ppm: 6.8-7.35 (m, 8H, Ar-H), 5.27 (t, 1H, O-CH), 3.98(d,2H, CH_2), 7.98 (s, 1H, N-H); ^{13}C NMR (100MHz, DMSO-d_6) δ ppm: 148.9, 145.2, 134.3, 129.4, 123.2, (aromatics) , 152.1, 141.8, 108.3 (thiazole), 119.3,60.3, 56.7. (isoxazoline).

N-(4-(4-nitrophenyl)thiazol-2-yl)-5-(3,4,5-trimethoxyphenyl)-4,5-dihydro isoxazole-3-amine (4g)

IR (KBr) cm^{-1}: 3351.19 (N-H in ring), 3028.63 (Ar-H), 3057.16 (C-H str in ring), 2904.43 (CH_2), 1596.74 (C=N), 1283.26 (C-N). 1518.72 (N-O), 965.29(O-CH), 701.58(C-S),1243.83(C-O-C), 2946.11(CH_3); ^{1}H NMR (400MHz, DMSO-d_6) δ ppm: 7.03-7.60(m, 6H, Ar-H), 4.92 (t, 1H, O-CH), 3.9(d,2H, CH_2), 8.02 (s, 1H, N-H),3.71(S, 9H,OCH_3); ^{13}C NMR (100MHz, DMSO-d_6) δ ppm: 161.3, 160.7, 149.1, 133.9, 129.7, 121.6, 113.9 (aromatics) , 151.18, 142.1, 101.79 (thiazole), 119.3,60.1, 58.3 (isoxazoline), 56.1,53.6(C in OCH_3).

4-(3-((4-(4-nitrophenyl)thiazol-2-yl)amino)-4,5-dihydroisoxazol-5-yl)phenol (4h)

IR (KBr) cm^{-1}: 3358.41 (N-H in ring), 3027.57 (Ar-H), 3059.08 (C-H str in ring), 2916.45 (CH_2), 1583.59 (C=N), 1301.68 (C-N). 1428.23 (N-O), 965.29(O-CH), 701.29(C-S), 3416.78(O-H); ^{1}H NMR (400MHz, DMSO-d_6) δ ppm: 7.1-7.65(m,8H, Ar-H), 4.78 (t, 1H, O-CH), 3.98(d,2H, CH_2), 7.98 (s, 1H, N-H), 5.27(S, 1H, OH); ^{13}C NMR (100MHz, DMSO-d_6) δ ppm:153.4, 130.1, 148.2, 134.5, 130.9, 129.1, 123.2, 120.8 (aromatics) , 151.5, 140, 109.7 (thiazole), 120.6, 61.3, 58.8 (isoxazoline).

5-(2-nitrophenyl)-N-(4-(4-nitrophenyl)thiazol-2-yl)-4,5-dihydroisoxazole-3-amine (4i)

IR (KBr) cm^{-1}: 3364.39 (N-H in ring), 3021.57 (Ar-H), 3057.16 (C-H str in ring), 2871.66 (CH_2), 1581.28 (C=N), 1279.68 (C-N). 1545.11 (N-O), 977(O-CH), 689.18(C-S); ^{1}H NMR (400MHz, DMSO-d_6) δ ppm: 7.07-7.56 (m, 8H, Ar-H), 5.21 (t, 1H, O-CH), 4.01(d,2H, CH_2), 8.0 (s, 1H, N-H); ^{13}C NMR (100MHz, DMSO-d_6) δ ppm: 149.8, 145.2, 134.3, 129.4, 123.2, (aromatics) , 152.1, 141.8, 108.3 (thiazole), 119.3,60.3, 56.7. (isoxazoline).

5-(4-bromophenyl)-N-(4-(4-nitrophenyl)thiazol-2-yl)-4,5-dihydroisoxazole-3-amine (4j)

IR (KBr) cm^{-1}: 3363.21 (N-H in ring), 3028.20 (Ar-H), 3051.43 (C-H str in ring), 2828.91 (CH_2), 1581.27 (C=N), 1300.63 (C-N). 1511.49 (N-O), 965(O-CH), 695.84(C-S),1128.07(C-Br); ^{1}H NMR (400MHz, DMSO-d_6) δ ppm: 7.1-7.42 (m, 8H, Ar-H), 4.98 (t, 1H, O-CH), 4.1(d,2H, CH_2), 8.09 (s, 1H, N-H); ^{13}C NMR (100MHz, DMSO-d_6) δ ppm: 148.2, 133.7, 136.4, 129.5, 128.1, 126.8, 123.9 (aromatics) , 151.3, 141.7, 100.9 (thiazole), 120.1, 60.8, 58.1. (isoxazoline).

5-(4-chlorophenyl)-N-(4-(4-nitrophenyl)thiazol-2-yl)-4,5-dihydroisoxazole-3-amine (4k)

IR (KBr) cm^{-1}: 3363.21 (N-H in ring), 3028.20 (Ar-H), 3051.43 (C-H str in ring), 2828.91 (CH_2), 1581.27 (C=N), 1300.63 (C-N). 1511.49 (N-O), 965(O-CH), 695.84(C-S),1081.68(C-Cl); ^{1}H NMR (400MHz, DMSO-d_6) δ ppm: 7.3-7.71 (m, 8H, Ar-H), 4.76 (t, 1H, O-CH), 3.98(d,2H, CH_2), 8.12 (s, 1H, N-H); ^{13}C NMR (100MHz, DMSO-d_6) δ ppm: 149.0, 135.6, 130.6, 129.5, 127.9, 126.1, 124.7, (aromatics) , 153.6, 142.1, 102.3 (thiazole), 120.1, 64.1, 59.3. (isoxazoline).

2-(3-((4-(4-nitrophenyl)thiazol-2-yl)amino)-4,5-dihydroisoxazol-5-yl)phenol (4l):

IR (KBr) cm^{-1}: 3358.41 (N-H in ring), 3027.57 (Ar-H), 3059.08 (C-H str in ring), 2916.45 (CH_2), 1583.59 (C=N), 1301.68 (C-N). 1428.23 (N-O), 965.29(O-CH), 701.29(C-S), 3428.03(O-H); ^{1}H NMR (400MHz, DMSO-d_6) δ ppm: 6.65-7.48(m,8H, Ar-H), 4.81 (t, 1H, O-CH), 3.91(d,2H, CH_2), 8.1 (s, 1H, N-H), 5.15(S, 1H, OH); ^{13}C NMR (100MHz, DMSO-d_6) δ ppm:153.4, 130.1, 148.2, 134.5, 130.9, 129.1, 123.2, 120.8 (aromatics) , 151.5, 140, 109.7 (thiazole), 120.6, 61.3, 58.8 (isoxazoline).

2-methoxy-5-(3-((4-(4-nitrophenyl)thiazol-2-yl)amino)-4,5-dihydroisoxazol-5-yl)phenol (4m):

IR (KBr) cm^{-1}: 3361.08 (N-H in ring), 3039.15 (Ar-H), 3063.45 (C-H str in ring), 2916.07 (CH_2), 1581.16 (C=N), 1279.59 (C-N). 1471.53 (N-O), 981.26(O-CH), 691.22(C-S),1225.14(C-O-C), 2943.65(CH_3), 3418.16(O-H); ^{1}H NMR (400MHz, DMSO-d_6) δ ppm: 6.94-7.48(m,7H, Ar-H), 4.86 (t, 1H, O-CH), 3.92 (d,2H, CH_2), 8.01 (s, 1H, N-H),3.64(S, 3H,OCH_3). 5.45(S, 1H, OH); ^{13}C NMR (100MHz, DMSO-d_6) δ ppm:155.6, 149.2, 134.2, 130.6, 123.1, 129.7, 121.0,(aromatics), 150.1, 142.7, 101.8 (thiazole), 120.1, 59.1, 57.8 (isoxazoline). 54.2(C IN CH_3).

Table 1: Physical and Analytical Data of The Synthesized Isoxazoline (4a-m) Derivatives

Comp.code	Formula	M.P(°c)	Yield(%)	Elemental Analysis (calc. / found) (%)					
				C		H		N	
4a	$C_{18}H_{14}N_4O_3S$	125	53.4	59.01	58.84	3.85	3.71	15.29	15.18
4b	$C_{18}H_{13}N_5O_5S$	164	73.8	52.55	52.43	3.19	3.10	17.02	16.94
4c	$C_{20}H_{19}N_5O_3S$	129	67.2	58.67	58.58	4.68	4.54	17.10	17.02
4d	$C_{19}H_{16}N_4O_4S$	138	64.5	57.57	57.41	4.07	3.94	14.13	14.02
4e	$C_{19}H_{16}N_4O_5S$	132	61.7	55.33	55.28	3.91	3.86	13.58	13.51
4f	$C_{18}H_{13}N_5O_5S$	172	69.2	52.55	52.41	3.19	3.04	17.02	16.95
4g	$C_{21}H_{20}N_4O_6S$	136	63.6	55.26	55.14	4.42	4.31	12.27	12.15
4h	$C_{18}H_{14}N_4O_4S$	124	59.5	56.54	56.41	3.69	3.57	14.65	14.52
4i	$C_{18}H_{13}N_5O_5S$	170	76.8	52.55	52.43	3.19	3.03	17.02	16.87
4j	$C_{18}H_{13}BrN_4O_3S$	146	68.1	48.55	48.41	2.94	2.81	12.58	12.43
4k	$C_{18}H_{13}ClN_4O_3S$	150	71.4	53.94	53.88	3.27	3.20	13.98	13.91
4l	$C_{18}H_{14}N_4O_4S$	128	55.7	56.54	56.41	3.69	3.49	14.65	14.53
4m	$C_{19}H_{16}N_4O_5S$	138	58.3	55.33	55.21	3.91	3.83	13.58	13.47

Antimycobacterial Activity Procedure

All the synthesized compounds of series 4a-m were evaluated for their anti tubercular activity (Seelam and Shrivastava, 2011). 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 37ºC for 24hours followed by streaking of M. tuberculosis H37Rv (5 × 104 bacilli pertube). These tubes were then incubated at 37 °C. 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. Isoniazid was used as standard drug. The MIC levels of compounds 4a-m against these organisms were given in Table 3.

RESULTS AND DISCUSSION

To synthesize new heterocyclic lead we approached a versatile route for the preparation of isoxazoline derivative incorporating with thiazole moiety. After the formation of thiazole moiety (1) it was going to acetylation with acetylchloride in the presence of dry benzene it is afforded to N-(4-(4-nitrophenyl)thiazol-2-yl)acetamide (2). This compound (2) was further reacted with various aromatic aldehydes to give chalcones (3a-m). Then these chalcone derivatives were involved in the cyclisation process by the reaction of hydroxyl amine hydrochloride in presence of alkaline medium which gives the title compounds (4a-m), the route of reaction was represented by Scheme 1. These title compounds were confirmed by IR spectral data with the absorption bands at 3367.19 (N-H in ring), 3016.19 (Ar-H), 3053 (C-H str in ring), 2869(CH_2), 1581.28 (C=N), 1279.68 (C-N). 1539.03 (N-O), 981(O-CH), 689(C-S) by this observations the respected functional groups were confirmed along with NMR data is also supporting respected compound 6.9-7.31 (m, 9H, Ar-H), 4.51 (t, 1H, O-CH), 2.6(d,2H, CH_2), 8.01(s, 1H, N-H); ^{13}C NMR (100MHz, DMSO-d_6) δ ppm: 149.8, 134.1, 129.4, 123.2, 128.8, 118.2(aromatics) , 151.3, 141.0,101.2 (thiazole), 120.7,59.1, 53.1(isoxazoline). The resultant final products were screened for their antimicrobial activity against various types of organisms employed. The selected strains for anti-bacterial activity were collected from the microbial type collection (MTCC) and gene bank (Institute of microbial technology, Chandigarh, India). For this study we used various microorganisms. For antibacterial activity the following bacterial strains were used *E. coli* (MTCC 443), *B. subtilis* (MTCC 441), *B. thuringiensis* (MTCC No: 4714), *P. aeruginosa* (MTCC 1688), and the following fungal strains *F. oxysporum* (MTCC No: 7392), *C. albicans* (MTCC 227), *A. fumigatus* (MTCC 3008).

Table 2: Antibacterial And Antifungal Activities of Synthesized compounds (4a-m).

			Bacteria				Fungi	
Comp.code	**R**	**B.subt**	**B.thur**	**E.coli**	**P.aeru**	**A.fumig**	**F.oxys**	**C.albica**
4a	H	-	-	25	-	25	25	12.5
4b	3-NO_2	3.125	6.25	3.125	3.125	-	>50	-
4c	$N(CH3)_2$	25	50	-	>50	6.25	6.25	6.25
4d	4-OCH_3	12.5	25	-	25	3.125	3.125	3.125
4e	2-OH-3-OCH_3	50	>50	-	-	12.5	6.25	6.25
4f	4-NO_2	3.125	3.125	<3.125	3.125	25	-	-
4g	3,4,5-Trimethoxy	>50	-	>50	-	>3.125	6.25	6.25
4h	4-OH	50	-	-	>50	3.125	6.25	>3.125
4i	2-NO_2	<3.125	3.125	3.125	3.125	-	-	50
4j	4-Br	3.125	6.25	6.25	6.25	25	-	-
4k	4-Cl	3.125	6.25	6.25	3.125	-	50	-
4l	2-OH	-	50	50	-	6.25	3.125	3.125
4m	4-OH-3-OCH_3	50	-	>50	-	12.5	6.25	6.25
Streptomycin	-	3.125	6.25	6.25	6.25	-	-	-
Treflucan	-	-	-	-	-	3.125	3.125	3.125

PHARMACOLOGY

Antimicrobial Studies

The results depicted in Table–2 revealed that most of the tested compounds displayed variable inhibitory effects on the growth of tested bacterial and fungal strains. The synthesized isoxazoline derivatives had shown potential antibacterial and antifungal activity on comparison with the standards, the compounds 4b, 4f, 4i, 4k have shown good activity against *Bacillus Subtilis* , compounds 4f, 4i, 4k have shown promising activity on *Bacillus Thuringiensis*, the compounds 4b, 4f, 4i, 4k are potent against both *E.Coli.* and, *Pseudomonus Aeruginosa*. in general comparison of the results the derivatives 4b, 4f, 4i effective against selected bacteria. On overall assessment of the antimicrobial data, the nitro substituted derivative '4i' was highly potent against all bacterial strains on rest of all other derivatives. Antifungal activity the compounds 4d, 4h, 4l have been shown fine antifungal activity against *candida albicans*. the compound '4d' (4-methoxy) is highly active against all the antifungal strains, the compounds 4c, 4d, 4g, 4h are active against *Aspergillus fumigates* and the compounds 4d, 4l showedgood activity against *F.*

oxysporum the results have been showed in Table 2. The remaining compounds have also shown moderate to good antimicrobial activity aginst all the strains employed. Keeping this anti microbial information, the biologically active synthesized heterocyclic derivatives, we further proceed to anti-tubercular activity.

Anti tubercular Activity

Anti tubercular activity of the synthesized isoxazoline derivatives were screened for their in vitro using *Mycobacterium Tuberculosis* H37Rv, and Isoniazid as a control. Due to the presence of bridge at secondary amine in between isoxazoline and thiazole groups showed good anti tubercular activity, and the compounds 4b, 4f, 4i, 4k have shown good activity against the test organisms. The results were revealed in the Table 3.

Table 3: Antitubercular Activity of Compounds (4a-m)

Comp.code	MIC(µg/ml)	Comp.code	MIC(µg/ml)
4a	>25	4h	25
4b	>3.125	4i	3.125
4c	12.50	4j	12.50
4d	25	4k	3.125
4e	>25	4l	>25
4f	>3.125	4m	>25
4g	>25	Isoniazid	4

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