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RESEARCH ARTICLE

SOLID SUPPORT SYNTHESIS OF THIAZOLO-PYRAZOLINE DERIVATIVES AND EVALUATION OF THEIR ANTIMICROBIAL ACTIVITIES

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ABSTRACT

The microwave-assisted synthesis of thiazolo-pyrazoline derivatives was carried out, using silica gel as solid support i.e. through an eco-friendly technique. Keeping in view of the wider therapeutic applications of these derivatives, they are screened for antibacterial activities against *E.coli, Klebsiella pneumonia, Pseudomonas aeruginosa, S.aureus and B.subtilis* strains and for antifungal activities against *Aspergillus janus* and *Pencilliun glabrum* strains using amoxicillin and fluconazole as standard drugs respectively.

INTRODUCTION

Heterocyclic compounds include many of the biochemical materials essential for life; e.g. vitamins, hormones, nucleic acids, that carry genetic information and control inheritance. Modern society is dependent on synthetic heterocycles for many uses such as drugs, pesticides, plastics, dyes, cosmetics, information storage, solvents, antioxidants, vulcanization accelerators (Li *et al.*, 2011; Martins *et al.*, 2004). Literature survey reveals that the organic moiety having nitrogen and sulphur atom results higher efficiency against various diseases (Chitamber, *et al.*, 1997). Keeping this in view, we planned to synthesize a system like thiazolyl pyrazole that contains both the thiazole ring and pyrazole ring and to study the combined effect of these rings on the biological properties. To synthesize these compounds, an attempt was made to use greener methods of synthesis. i.e microwave-assisted solvent-free organic synthesis (MASFOS) has been developed as an environmental friendly process. Microwave chemistry is rapid, give higher yields, high purity of products, uniform heating and greater reproducibility of chemical reactions.

EXPERIMENTAL

Melting points were taken in sulphuric acid bath and are uncorrected. Infrared spectra were recorded in KBr disc on Perkin Elmer FTIR-spectrometer and Proton Magnetic Resonance spectra were recorded on BRUKER AVANCE II 400 NMR spectrometer with auto sampler and with tetramethylsilane (TMS) as internal standard. Mass spectra were recorded by Regional Sophisticated instrumentation centre on JEOL 5x102/DA-600 MASS spectrometer of Punjab University, Chandigarh. For all the reactions, chemicals of SD fine standard were used. All solvents were distilled before use.

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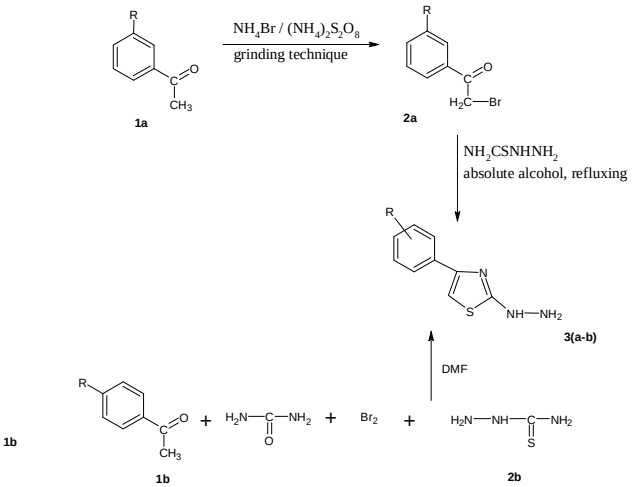
SYNTHESIS OF THIAZOLO PYRAZOLINE DERIVATIVES

Scheme 1

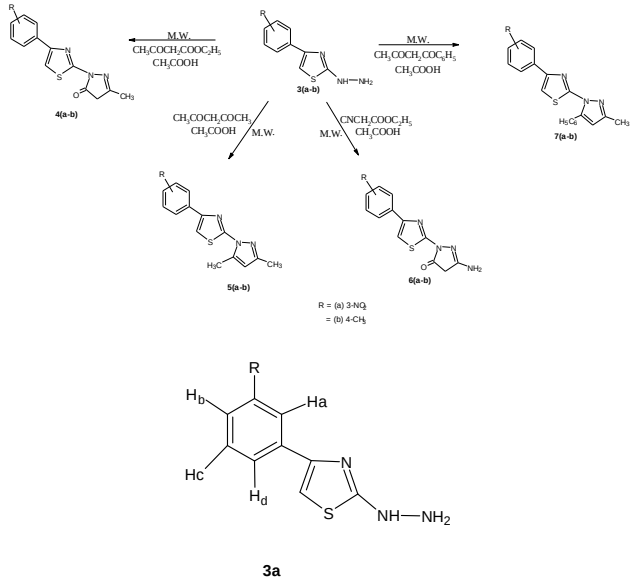


A typical procedure is given below

2a. Synthesis of 2-bromo-1-(3-nitrophenyl)ethanone

A mixture of 3-nitroacetophenone (1a) (0.01mol), ammonium bromide (0.02mol) and ammonium persulphate (0.025mol) moist with 6-10 drops of water was grinded using mortar and pestle at room temperature for 25 minutes. The reaction mixture was kept at room temperature overnight. The completion of reaction was checked by Thin Layer Chromatography. The reaction mixture was diluted with ice cold water. A solid precipitated upon dilution. The solid was

Scheme 2



collected by the vacuum filteration and recrystallised from ethanol as creamish solid. M.P. 87-89^{0}C, Lit.M.P. 92-94^{0}C, Expt. Yield-66%.

I.R. (KBr) cm^{-1}: 3089(aromatic C-H), 2924 & 2857(aliphatic C-H), 1692(C=O), 1526(NO_2 antisymmetric) and 1349(NO_2 symmetric). ^{1}H N.M.R. (DMSO- d_6), δ (ppm): 4.33(s, 2H, CH_2), 8.59(t, Ar-Ha, *J*=2.0Hz), 8.35(dd, Ar-Hb,Hd, $J_{m,o}$=1.1Hz, 6.8Hz), 8.20(d(dd), Ar-Hc, $J_{p,o}$=0.6, 6.9, 7.3Hz).

3a. Synthesis of 2-hydrazinyl-4-(3-nitrophenyl)-1,3-thiazole

The mixture of 2a (0.004mol), thiosemicarbazide (0.004mol) and absolute alcohol (25 mL) were added in 100mL round bottom flask. The contents were allowed to reflux for 26-27 hrs. The completion of reaction was checked by Thin Layer Chromatography. On completion of reaction, the solid was separated at the bottom. The solid was filtered and it was allowed to dry and recrystallised from mixture of methanol and chloroform as creamish white shinning solid. M.P.-221-223^{0}C, Expt. Yield-85%.

I.R. (KBr) cm^{-1}: 3371(NH_2), 3138(NH), 3055(aromatic C-H), 1606(C=N), 1523(NO_2 antisymmetric), 1348(NO_2 symmetric). ^{1}H N.M.R. (DMSO- d_6), δ (ppm): 8.59(t, Ar-Ha, *J*=2.0Hz), 8.35(dd, Ar-Hb,Hd $J_{m,o}$=1.1, 6.8Hz), 8.20(d(dd), Ar-Hc, $J_{p,o}$=0.6, 6.9, 7.3Hz), 7.99(s, 1H, C-5 of thiazole ring), 7.63(t, 1H, NH), 5.05(s, 2H, NH_2).

4a. Synthesis of 5-methyl-2-[4-(3-nitrophenyl)-1,3-thiazol-2-yl]-2,4-dihydro-3H-pyrazol-3-one

A mixture of 3a (0.004mol), ethylacetoacetate (0.004mol) and 3-4 drops of glacial acetic acid were grinded with pre-conditioned silica-gel using pestle and mortar. The reaction mixture was subjected to microwave pulse at 320W, for 15 minutes. The reaction mixture was kept overnight. The product was extracted with the help of alcohol. The reaction was followed by Thin Layer Chromatography and the conditions were standardized. The crude product was filtered, washed with alcohol and recrystallised from mixture of methanol and benzene as orange solid. M.P.-195^{0}C, Expt. Yield-40%.

I.R. (KBr) cm^{-1}: 3055(aromatic C-H), 2922(aliphatic C-H), 1680(C=O), 1607(C=N), 1523(NO_2 antisymmetric), 1348(NO_2 symmetric). ^{1}H N.M.R. (DMSO- d_6), δ (ppm): 8.59(t, Ar-Ha, *J*=2.0Hz), 8.35(dd, Ar-Hb,Hd $J_{m,o}$=1.1, 6.8Hz), 8.20(d(dd), Ar-Hc, $J_{p,o}$=0.6, 6.9, 7.3Hz), 7.99(s, 1H, C-5), 7.63(s, 1H, C-H), 2.55 (s, 2H,CH_2), 2.19(s, 3H, CH_3). Mass spectrum (EI, m/z): 295,277, 273, 270, 238, 204, 203, 160.

5a. Synthesis of 2-(3,5-dimethyl-1H-pyrazol-1-yl)-4-(3-nitrophenyl)-1,3-thiazole

A mixture of 3a (0.004mol), acetyl acetone (0.004mol) and 3-4 drops of glacial acetic acid were grinded with pre-conditioned silica-gel using pestle and mortar. The reaction mixture was subjected to microwave pulse at 320W, for 15 minutes. The reaction mixture was kept overnight. The product was extracted with the help of alcohol. The reaction was followed by Thin Layer Chromatography and the conditions were standardized. The crude product was filtered, washed with alcohol and recrystallised from mixture of methanol and benzene as orange solid. M.P.-217-219^{0}C, Expt. Yield-40%.

I.R. (KBr) cm^{-1}: 3054(aromatic C-H), 2921(aliphatic C-H), 1607(C=N), 1508((NO_2 antisymmetric), 1348(NO_2 symmetric). ^{1}H N.M.R. (DMSO- d_6), δ (ppm): 8.59(t, Ar-Ha, *J*=2.0Hz), 8.35(dd, Ar-Hb,Hd $J_{m,o}$=1.1, 6.8Hz), 8.20(d(dd), Ar-Hc, $J_{p,o}$=0.6, 6.9, 7.3Hz), 7.99(s, 1H, C-5), 7.63(s, 1H, C-H), 2.39(s, 3Ha',CH_3), 1.00(s,3Hb' CH_3).

Mass spectrum (EI, m/z): 217, 205, 203(100%), 178, 160.

6a. 5-amino-2-[4-(3-nitrophenyl)-1,3-thiazol-2-yl]-2,4-dihydro-3H-pyrazol-3-one

A mixture of 3a (0.004mol), ethylcyanoacetate (0.004mol) and 3-4 drops of glacial acetic acid were grinded with pre-conditioned silica-gel using pestle and mortar. The reaction mixture was subjected to microwave pulse at 320W, for 15 minutes. The reaction mixture was kept overnight. The product was extracted with the help of alcohol. The reaction was followed by Thin Layer Chromatography and the conditions were standardized. The crude product was filtered, washed with alcohol and recrystallised from mixture of methanol and benzene as light brown solid. M.P.-213-215^{0}C, Expt. Yield-60%.

I.R. (KBr) cm^{-1}: 3193(NH), 3055(aromatic C-H), 2922(aliphatic C-H), 1687(C=O), 1606(C=N), 1508((NO_2 antisymmetric), 1348(NO_2 symmetric).

^{1}H N.M.R. (DMSO- d_6), δ (ppm): 8.59(t, Ar-Ha, *J*=2.0Hz), 8.35(dd, Ar-Hb, Hd, $J_{m,o}$=1.1Hz, 6.8Hz), 8.20(d(dd), Ar-Hc, $J_{p,o}$=0.6, 6.9, 7.3Hz), 7.63(brs, 3H, NH_2 & C-5), 2.39(s, 2H, CH_2).

Mass spectrum (EI, m/z): 289(100%), 287, 270, 261, 257, 245, 219.

7a. Synthesis of 2-(3-methyl-5-phenyl-1H-pyrazol-1-yl)-4-(3-nitrophenyl)-1,3-thiazole

A mixture of 3a (0.004mol), benzoyl acetone (0.004mol) and 3-4 drops of glacial acetic acid were grinded with pre-conditioned silica-gel using pestle and mortar. The reaction mixture was subjected to microwave pulse at 320W, for 21minutes and 10seconds. The reaction mixture was kept overnight. The product was extracted with the help of alcohol. The reaction was followed by Thin Layer Chromatography and the conditions were standardized. The crude product was filtered, washed with alcohol and recrystallised from mixture of methanol and benzene as dark orange solid. M.P. 208-210^{0}C, Expt. Yield-62-63%.

I.R. (KBr) cm^{-1}: 3055(aromatic C-H), 2922(aliphatic C-H), 1607(C=C), 1600(C=N), 1528((NO_2 antisymmetric), 1348(NO_2 symmetric).

^{1}H N.M.R. (DMSO-d_6), δ (ppm): 8.59(t, Ar-Ha, *J*=2.0Hz), 8.35(dd, Ar-Hb,Hd $J_{m,o}$=1.1, 6.8Hz), 8.20(d(dd), Ar-Hc, $J_{p,o}$=0.6, 6.9, 7.3Hz), 7.99(s, 1H, C-5), 7.63(s, 5H, C_6H_5), 6.39(s, 1H, C-H), 2.54(s, 3H, CH_3).

Mass spectrum (EI, m/z): 360, 338(100%), 317, 285, 208.

3b. Synthesis of 2-hydrazinyl-4-(4-methylphenyl)-1,3-thiazole

In a mixture of 4-methylacetophenone (1b) (0.01mol) and urea (0.03mol), dissolved in D.M.F., bromine was added (0.01mol) maintaining the temperature near 0^0C by keeping in ice-bath. After completion of bromination (when the solution became colorless), thiosemicarbazide (2b) (0.01mol) was added and reaction mixture was stirred for 13-14hours at 15-20^0C. Then it was kept on water-bath for about 2 hours and treated with saturated solution of potassium carbonate. A pasty brown solid was obtained and it was dissolved using acetone. Then it was concentrated and was poured into crushed ice. Yellowish solid was obtained, which was then filtered, washed with distilled water and recrystallised from chloroform as yellowish solid.M.P.62-64^0C, Expt. Yield-60%.

I.R. (KBr) cm^{-1}: 3413(NH), 3027(aromatic C-H), 2919(CH_3), 1612(C=N), 1554(C=C).

^{1}H N.M.R. ($CDCl_3$), δ (ppm): 7.68(dd, Ar-Hb, Hd, $J_{m,o}$=1.8,6.1), 7.20(m, Ar-Ha, Hc, C-5), 4.89(s, 2H, NH_2), 2.36(m, NH, CH_3).

4b. Synthesis of 5-methyl-2-[4-(4-methylphenyl)-1,3-thiazol-2-yl]-2,4-dihydro-3H-pyrazol-3-one

A mixture of 3b (0.004mol), ethylacetoacetate (0.004mol) and 3-4 drops of glacial acetic acid were grinded with pre-conditioned silica-gel using pestle and mortar. The reaction mixture was subjected to microwave pulse at 320W, for 8minutes and 10seconds. The reaction mixture was kept overnight. The product was extracted with the help of alcohol. The reaction was followed by Thin Layer Chromatography and the conditions were standardized. The crude product was filtered, washed with alcohol and recrystallised from mixture of methanol and benzene as brown solid. M.P. 173-175^0C, Expt. Yield-60%.

I.R. (KBr) cm^{-1}: 3027(aromatic C-H), 2917(CH_3), 1718(C=O), 1608(C=N), 1557(C=C).

^{1}H N.M.R. ($CDCl_3$), δ (ppm): 7.69(d, Ar-Ha, Ar-Hb), 7.26(m, Ar-Hc, Ar-Hd, C-5), 4.3(q, 2H, CH_2), 2.37(m, two CH_3).

5b.Synthesis of 2-(3,5-dimethyl-1H-pyrazol-1-yl)-4-methylphenyl-1,3-thiazole:-

A mixture of 3b (0.004mol), acetyl acetone (0.004mol) and 3-4 drops of glacial acetic acid were grinded with pre-conditioned silica-gel using pestle and mortar. The reaction mixture was subjected to microwave pulse at 320W, for 30minutes and 10seconds. The reaction mixture was kept overnight. The product was extracted with the help of alcohol. The reaction was followed by Thin Layer Chromatography and the conditions were standardized. The crude product was filtered, washed with alcohol and recrystallised from mixture of methanol and benzene as dark brown solid. M.P.108-110^0C, Expt. Yield-40%.

^{1}H N.M.R. ($CDCl_3$), δ (ppm): 7.70(d, Ar-Hb), 7.69(d, Ar-Hc), 7.65(brs,Ar-Hd, Ha), 6.7(s, 1H attached to C-5 0f thiazole ring), 2.77(s, 1H, C-H), 2.39(s, 3H, CH_3 attached to benzene moiety), 1.80(brs, 3Hb', CH_3), 1.25(brs, 3Ha', CH_3).

6b. Synthesis of 5-amino-2-(4-methylphenyl-1,3-thiazole-2-yl)-2,4-dihydro-3H-pyrazol-3-one

A mixture of 3b (0.004mol), ethylcyanoacetate (0.004mol) and 3-4 drops of glacial acetic acid were grinded with pre-conditioned silica-gel using pestle and mortar. The reaction mixture was subjected to microwave pulse at 320W, for 26minutes and 10seconds. The reaction mixture was kept overnight. The product was extracted with the help of alcohol. The reaction was followed by Thin Layer Chromatography and the conditions were standardized. The crude product was filtered, washed with alcohol and recrystallised from mixture of methanol and benzene as dark brown solid. M.P.92-94^0C, Expt. Yield-40%.

^{1}H N.M.R. ($CDCl_3$), δ (ppm): 7.71(d, Ar-Ha), 7.69(d, Ar-Hd), 7.26(brs, Ar-Hc, Hb and C-5), 3.45(s, 2H, NH_2), 2.40(brs, 5H, CH_3 & NH_2).

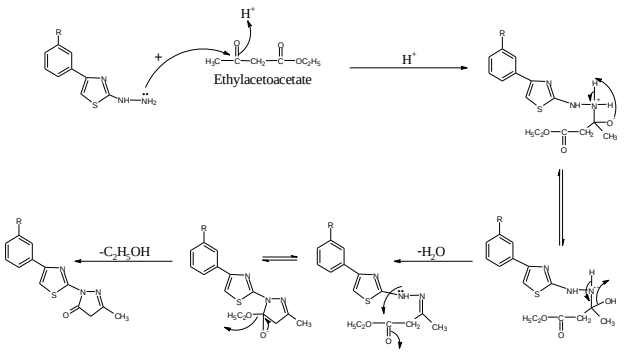
7b. Synthesis of 2-(3-methyl-5-phenyl-1H-pyrazol-1-yl)-4-methylphenyl-1,3-thiazole

A mixture of 3b (0.004mol), benzoyl acetone (0.004mol) and 3-4 drops of glacial acetic acid were grinded with pre-conditioned silica-gel using pestle and mortar. The reaction mixture was subjected to microwave pulse at 320W, for 5minutes and 43seconds. The reaction mixture was kept overnight. The product was extracted with the help of alcohol. The reaction was followed by Thin Layer Chromatography and the conditions were standardized. The crude product was filtered, washed with alcohol and recrystallised from mixture of methanol and benzene as light brown solid. M.P.-93-95^0C, Expt. Yield-60%.

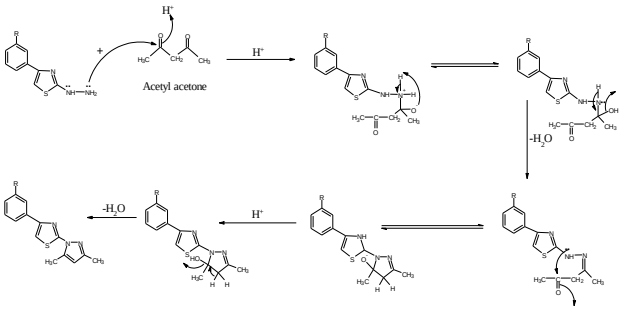
^{1}H N.M.R. ($CDCl_3$), δ (ppm): 7.26(m, aromatic protons), 2.58(s,1H, methine group of pyrazole ring), 1.66(brs, 6H, CH_3 attached to benzene ring and pyrazole moiety).

REACTION MECHANISM

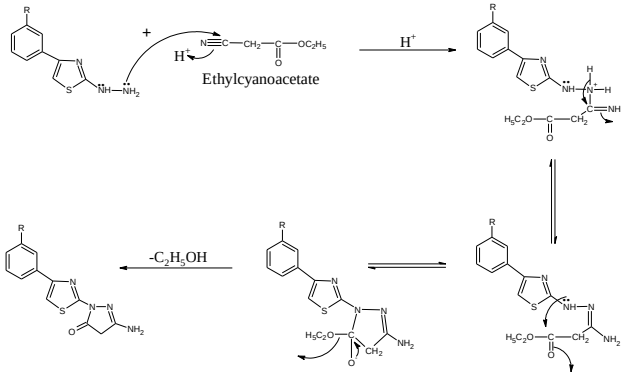
1) Reaction with ethylacetoacetate is in accordance with the following mechanism



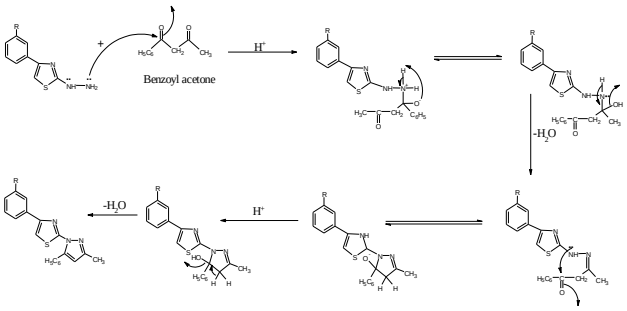
2) Reaction with acetyl acetone is in accordance with the following mechanism



3) Reaction with ethylcyanoacetate is in accordance with the following mechanism



4) Reaction with benzoyl acetone is in accordance with the following mechanism



will remain clear. Therefore, M.I.C. was determined by choosing lowest concentration in which no growth occurs (Table 1).

Preparation of the inoculums

The test bacteria grown at 37^0C in nutrient agar medium were diluted in sterile nutrient broth medium in such a manner that the suspension contains about 10^7 cells/mL. This suspension was used as the inoculums.

Procedure

1) 5 test tubes were taken for anti-bacterial activities.
2) 1mL of nutrient broth medium was poured to each of the tube.
3) These test tubes were cotton plugged and sterilized in an autoclave for 15lbs/sq. inch pressure.
4) After cool, 1mL of sample solution (made from tested compound and DMSO) was added to 1st tube and mixed well and then 1mL of this content was transferred to 2nd tube.
5) The content of the 2nd test tube was mixed well and again 1mL of this mixture was transferred to 3rd test tube. This process of serial dilution was continued up to 5th tube.
6) 1mL of the contents were removed from tube 5th and discarded.
7) The tubes were inoculated by 0.1mL of the bacterial suspension and then mixed well.
8) All the test tubes were inoculated at 37^0C for 24 hours.
9) The highest dilution without growth is the M.I.C.
10) All the samples were tested at the concentration of 128, 64, 32, 16, 8, 4, 2, and 1.
11) The whole process was repeated for anti-fungal activities.

Table 1. In vitro antimicrobial MIC (µg/mL) of prepared compounds

Compound No.	Gram-negative bacteria			Gram-positive bacteria		Fungi	
	Escherichia coli	*Klebsiella pneumoniae*	*Pseudomonas aeruginosa*	*Staphylococcus aureus*	*Bacillus subtilis*	*Aspergillus janus*	*Pencillium glabrum*
4a	32	32	16	16	16	16	16
5a	16	16	16	16	16	16	32
6a	8	16	32	8	16	16	16
7a	16	16	32	16	16	8	16
4b	16	16	8	4	8	32	16
5b	16	8	16	16	16	32	16
6b	16	16	8	16	16	32	32
7b	16	16	16	16	8	16	16
Amoxicillin	4	4	4	4	4	--	--
Fluconazole	--	--	--	--	--	2	2

EVALUATION OF BIOLOGICAL ACTIVITY

The prepared compounds were screened for antimicrobial activities by "Serial Tube Dilution Technique". The microbial strains used in our study were obtained from Microbial Type Culture Collection & Gene Bank (MTCC)-Chandigarh, India. The strains used in the present study were *Escherichia coli* MTCC (443), *Staphylococcus aureus* MTCC (96), *Klebsiella pneumoniae* MTCC (3384), *Bacillus subtilis* MTCC (441), *Pseudomonas aeruginosa* MTCC (424), *Aspergillus janus* MTCC (2751), *Penicillium glabrum* MTCC (4951).

Serial Tube Dilution Technique

Minimum Inhibitory Concentration (M.I.C.) is the lowest concentration of an antimicrobial that will inhibit the visible growth of a micro-organism after overnight incubation. In the present study, M.I.C. was determined (Delima, *et al*., 1992; Labouta, *et al.*, 1987) using "Serial Tube Dilution Technique". In this technique, the tubes of broth medium, containing graded doses of compounds are inoculated with the test organisms. After suitable incubation, growth will occur in those tubes where concentration of compounds is below the inhibitory level and the culture will become turbid (cloudy). The tube, in which growth will not occur above the inhibitory level,

CONCLUSION

Thiazoles and Pyrazolines individually or in combination are involved in a wide variety of biological activities. The wider applications of thiazolo-pyrazoline derivatives prompted us to synthesize these classes of compounds. The synthesis was carried out through microwave irradiations using silica gel as solid support. Thus, reaction time was reduced, precious solvents were saved and the overall yield was improved by reducing the number of steps. Thus, it's a step towards Greener Chemistry. Also from antimicrobial evaluation, it may be concluded that out of the nitro-substituted compounds, compound 6a was found to be most active against the bacterial strain *S.aureus* and *E. coli* at M.I.C. of 8µg/mL while compound 7a exhibited significant activity against the fungal strain *A. janus* at the M.I.C. of 8µg/mL . And out the methyl substituted compounds, the compound 4b was found to be the most active against *S. aureus* at M.I.C. 4µg/mL among the studied compounds. While compound 4b also inhibited the growth of strain *B. subtilis* and *P. aeruginosa* at M.I.C. of 8µg/mL. The compound 6b and 7b also showed significant activity at M.I.C. of 8µg/mL against the micro-organisms *P. aeruginosa* and *B. subtilis*. And the rest of the compounds showed moderate activity against tested microbial strains at M.I.C. of 32-16µg/mL.

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