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Convergent synthesis of Biologically significant compounds through Eco-Friendly methods

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ABSTRACT

Pyrazoline and thiazole derivatives are associated with broad spectrum of biological activities. In literature, several conventional and non conventional methods have been reported for the synthesis of thiazolo-pyrazolines, which are associated with many drawbacks like multistep synthetic route, longer reaction time with drastic conditions, difficult workup, low yield and use of expensive & hazardous chemicals. However, convergent one-pot multicomponent synthesis through microwave enhanced chemical reactions, in general & especially on solid support has attracted attention recently. In view of this, different thiazolo-pyrazoline derivatives were synthesized through principles of green chemistry. Some of the compounds were screened for antibacterial and antifungal activities. Compounds show good antimicrobial activity.

Keywords: Thiazolo-pyrazolines, Convergent synthesis, Multicomponent reactions, Microwave enhanced chemical reaction, Green chemistry, Antimicrobial activity.

INTRODUCTION

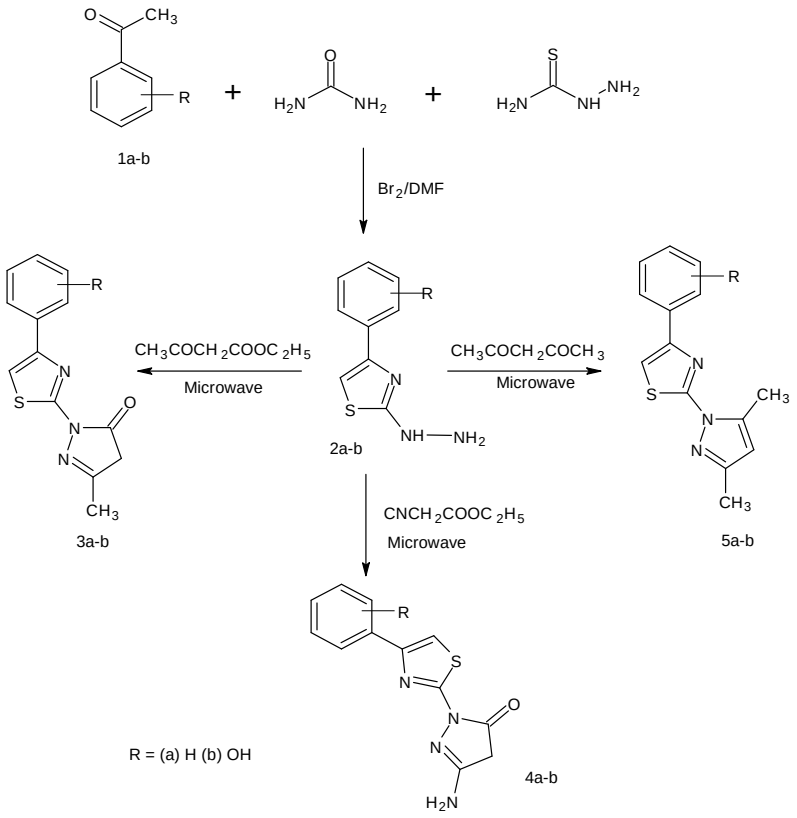
Besides a remarkable progress in the prevention, control and even eradication of infectious diseases with improved hygiene and development of antimicrobials and vaccines, there have been threats of new diseases during the past three decades due to the development of antimicrobial resistance[1-2]. This emerging resistance has resulted in the development of a wide variety of antibiotics. As, sulphur and/or nitrogen heterocycles having pharmaceutical activities are widely occur in nature in the form of alkaloids, vitamins, pigments and as constituents of plant and animal cells, the present work involves the synthesis of thiazolo-pyrazoline derivatives. Literature study reveals[3-15] that these moieties individually or in combination can act as antiinflammatory, antimicrobial, antihypertensive, antituberculosis and anti-HIV activities. In recent years, to minimize the amount of harmful organic solvents used in chemical processes, much attention has been devoted to the use of alternative reaction media. The possibility of performing chemical processes in the absence of solvent (solvent free), has been receiving more attention. The reported examples demonstrate that no-solvent [14-16] reaction are generally faster, gives higher selectivity and yields, and usually require easier work up procedures and simpler equipments. Working on these principles of green chemistry, thiazolo-pyrazoline derivatives were synthesized. The synthesized compounds were tested for their antimicrobial activities.

MATERIALS AND METHODS

In view of the recent emphasis aimed at developing environmentally friendly methodologies for the preparation of organic compounds, herein we report the synthesis compounds **2a-b** by cyclocondensation of urea, substituted acetophenone and thiosemicarbazide. Further, compounds **2a-b** were treated with different reagents with or without solid support using microwave irradiations to form 2-(3,5-dimethyl-1*H*-pyrazol-1-yl)-4- substituted phenyl-1,3-thiazole; 5-methyl-2-(4-substituted phenyl-1,3-thiazol-2-yl)-2,4-dihydro-3*H*-pyrazol-3-one and 5-amino-2-(4-substituted phenyl-1,3-thiazol-2-yl)-2,4-dihydro-3*H*-pyrazol-3-one derivatives (Scheme 1, Table 1).

Table 1: Characterization data of Products (3-5)

Compound	R	Time (min)	M.pt (^{0}C)	Yield%
3a	H	9.0	238-240	81
3b	OH	2.15	229-232	76
4a	H	2.30	220-223	63
4b	OH	2.0	223-226	61
5a	H	2.40	197-200	66
5b	OH	2.30	229-232	76



Scheme 1

Experimental Methods: Melting and boiling points were taken in sulphuric acid bath and are uncorrected. Infrared spectra were recorded in KBr disc on Perkin Elmer FTIR-spectrometer. Proton magnetic resonance spectra were recorded on BRUKER ADVANCE 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-6000 mass spectrometer. For all the reactions, chemicals of BDH standard were used. All solvents were distilled before use.

Synthesis of 4-phenyl-2- hydrazinyl -1,3-thiazole (2a): To the mixture of acetophenone (0.01 mol, 1.2 ml) and urea (0.03 mol, 1.8g) dissolved in DMF, bromine (0.01mol, 0.51ml) was added. After completion of bromination, thiosemicarbazide (0.01 mol, 0.91g) was added and the reaction mixture was stirred for 24 hours. It was kept on water bath for 2 hours, treated with saturated solution of potassium carbonate. The crude product obtained was filtered, washed with distilled water and recrystallized from mixture of chloroform and methanol as yellowish-brown solid.

Synthesis of 4-(2- hydrazinyl -1,3-thiazol-4-yl)phenol (2b): To the mixture of o-hydroxyacetophenone (0.01 mol, 1.36g) and urea (0.03 mol, 1.8g) dissolved in DMF, bromine (0.01mol, 0.51ml) was added. After completion of bromination, thiosemicarbazide (0.01 mol, 0.91g) was added and the reaction mixture was stirred for 24 hours. It was kept on water bath for 2 hours, treated with saturated solution of potassium carbonate. Then neutralized with dil. HCl. The sticky solid obtained was treated with ethyl acetate. The crude product obtained was filtered, washed with distilled water and recrystallized from mixture of chloroform and methanol.

Synthesis of 5-methyl-2-(4-phenyl-1,3-thiazol-2-yl)-2,4-dihydro-3*H*-pyrazol-3-one (3a): A mixture of 4-phenyl-2- hydrazinyl-1,3-thiazole (2a, 1g), ethyl acetoacetate (0.68 ml), 3-4 drops of glacial acetic acid were grinded with silica gel using pestle and mortar. The reaction mixture was subjected to microwave pulse at 320W, for 9 minutes. The reaction mixture was kept overnight. The product was extracted with help of alcohol. The reaction was followed by TLC and conditions were standardized. The crude product was filtered, washed with alcohol and recrystallized from mixture of methanol and benzene as brown solid.

Synthesis of 2-[4-(4-hydroxyphenyl)-1,3-thiazol-2-yl]-5-methyl-2,4-dihydro-3*H*-pyrazol-3-one (3b): A mixture of 4-(2- hydrazinyl-1,3-thiazol-4-yl)phenol (2b, 1g), ethyl acetoacetate (0.70 ml), 3-4 drops of glacial acetic acid was subjected to microwave pulse at 320W, for 2.15 minutes. The reaction mixture was kept overnight. The product was extracted with help of alcohol. The reaction was followed by TLC and conditions were standardized. The crude product was filtered, washed with alcohol and recrystallized from mixture of methanol and benzene.

Synthesis of 5-amino-2-(4-phenyl-1,3-thiazol-2-yl)-2,4-dihydro-3*H*-pyrazol-3-one (4a) : A mixture of 4-phenyl-2- hydrazinyl -1,3-thiazole (2a, 1g), ethyl cyanoacetate (0.56 ml), 3-4 drops of glacial acetic acid were grinded with silica gel using pestle and mortar. The reaction mixture was subjected to microwave pulse at 320W for 2-5 minutes. The mixture was kept overnight. The product was extracted with help of alcohol. The reaction was followed by TLC and conditions were standardized. The crude product was filtered, washed with alcohol and recrystallized from mixture of methanol and benzene, as brown solid.

Synthesis of 5-amino-2-[4-(4-hydroxyphenyl)-1,3-thiazol-2-yl]-2,4-dihydro-3*H*-pyrazol-3-one (4b) : A mixture of 4-(2- hydrazinyl-1,3-thiazol-4-yl)phenol (2b, 1g), ethyl cyanoacetate (0.51 ml), 3-4 drops of glacial acetic acid was subjected to microwave pulse at 320W for 2 minutes. The mixture was kept overnight. The product was extracted with help of alcohol. The reaction was followed by TLC and conditions were standardized. The crude product was filtered, washed with alcohol and recrystallized from mixture of methanol and benzene.

Synthesis of 2-(3,5-dimethyl-1*H*-pyrazol-1-yl)-4-phenyl-1,3-thiazole (5a) : A mixture of 4-phenyl-2-hydrazinyl-1,3-thiazole (2a, 1g), acetylacetone (0.52 ml), 3-4 drops of glacial acetic acid were grinded with silica gel using pestle and mortar. The reaction mixture was subjected to microwave pulse at 320W for 6 minutes. The mixture was kept overnight. The product was extracted with the help of alcohol. The reaction was followed by TLC and conditions were standardized. The crude was filtered, washed with alcohol and recrystallized from mixture of methanol and benzene as brown solid.

Synthesis of 4-[2-(3,5-dimethyl-1*H*-pyrazol-1-yl)-1,3-thiazol-4-yl]phenol (5b) : A mixture of 4-(2-hydrazinyl-1,3-thiazol-4-yl)phenol (2b, 1g), acetylacetone (0.48 ml), 3-4 drops of glacial acetic acid was subjected to microwave pulse at 320W for 2.30 minutes. The mixture was kept overnight. The product was extracted with the help of alcohol. The reaction was followed by TLC and conditions were standardized. The crude was filtered, washed with alcohol and recrystallized from mixture of methanol and benzene.

RESULTS AND DISCUSSION

The results of synthesised compounds obtained are given below.

Compound **2a:** M.p: 164-166^{0}C, Yield: 63%, IR (KBr) cm^{-1}: 3468 & 3324 (NH), 3054 (Ar-H), 1645 (C=N), ^{1}H NMR (δ, ppm) : 5.42 (s, 2H, NH_2), 6.75 (s, 2H, N-H & C_5-H), 7.86 (d, 2H, Ar-H), 7.40 (d,3H,Ar-H) Also, NH protons got exchanged with D_2O.

Compound **2b:** M.p: 207-209^{0}C, Yield: 66%, IR (KBr) cm^{-1}: 3553 (O-H), 3439 & 3313 (NH,) 1600 (C=N), ^{1}H NMR (δ , ppm) : 10.35 (s, 1H, OH), 7.98 (s, Ar-H), 6.98 (s, 1H, NH), 6.84 (s, 1H, C_5-H), 3.92 (s, 2H, NH_2).

Compound **3a:** IR (KBr) cm^{-1}: 1672 (C=O), 3054 (Ar-H), 1620 (C=N) , ^{1}H NMR (δ,ppm) : 1.24 (s, 3H, CH_3), 2.55 (s, 2H, CH_2), 6.50 (s, 1H, C_5-H), 7.38 (m, Ar-H), Mass(m/z): 280, 258, 248, 243, 226, 215,160.

Compound **3b:** IR (KBr) cm^{-1}: 3468 (O-H), 3051 (Ar-H), 1649 (C=O), ^{1}H NMR (δ, ppm) : 9.98 (s, 1H, OH), 7.84 (m, Ar-H), 6.83 (s, 1H, C_5-H), 2.82 (s, 2H, CH_2), 1.95 (s, 3H, CH_3)

Compound **4a**: IR (KBr) cm^{-1}: 3196 (NH_2), 3091 (Ar-H), 1680 (C=O), 1600 (C=N), Mass (m/z): 258, 244,216,200,174,160.

Compound **4b**: IR (KBr) cm^{-1}: 3472 (O-H), 3450 (NH_2), 1615 (C=O), 1594 (C=N), ^{1}H NMR (δ, ppm): 9.96 (s, 1H, OH), 7.82 (m, Ar-H), 6.80 (s, 3H, C_5-H), 2.83 (s, 2H, CH_2).

Compound **5a:** IR (KBr) cm^{-1}: 3080 (Ar-H), 1600 (C=N) , ^{1}H NMR (δ, ppm): 1.25 (s, 3H, CH3a), 2.13 (s, 3H, CH3b), 7.39 (m, Ar-H).

Compound **5b:** IR (KBr) cm^{-1}: 3222 (O-H), 3020 (Ar-H,) 1630 (C=N), ^{1}H NMR (δ, ppm): 10.02 (s, 1H, OH), 7.91 (m, Ar-H), 2.12 (s, 3H, CH_3b), 1.94 (s, 3H, CH_3a)

Antimicrobial Activities: Antimicrobial activities were checked by Agar diffusion method[17-18] and Serial tube dilution technique.

Agar diffusion method: Initially, the stock cultures of bacteria were revived by inoculating in broth media and grown at 37^{0}C for 18 hrs. The agar plates of the above media were prepared and wells were made in the plate. Each plate was inoculated with 18h old cultures (100 ml, 10^{-4} cfu) and spread evenly on the plate. After 20 min, the wells were filled with of compound at different concentrations. The control wells

with Gentamycin were also prepared. All the plates were incubated at 37^0C for 24h and the diameter of inhibition zone were noted.
Solvent Used: DMSO; Concentrations screened: 0.0625, 0.125, 0.25, 0.5, 1.0, 2.0 mg
Sample preparation: 20 mg sample was dissolved in 1 ml of solvent
Stock sample Concentration: 20 mg ml^{-1}
Bacteria analyzed: Entero toxin producing Escherichia coli (ETEC), Staphylococcus aureus, Methicillin resistant Staphylococcus aureus (MRSA)
Media used: Peptone-10g, NaCl-10g and Yeast extract 5g, Agar 20 g in 1000 ml of distilled water.

Table 2: Diameter of inhibition zones of different compounds

Sample No.	Conc. mg	*E.coli*	*S.aureus*	*S. aureus (MRSA)*
3a	0.625	0	0	0
	0.125	0	0	0
	0.25	0.3	0	0
	0.5	0.4	0	0.3
	1.0	0.4	0	0.5
	2.0	0.5	0.2	0.6
4a	0.625	0	0	0
	0.125	0	0	0
	0.25	0	0	0
	0.5	0	0	0
	1.0	0.4	0.3	0.3
	2.0	0.6	0.4	0.7
5a	0.625	0	0	0
	0.125	0	0	0
	0.25	0	0	0
	0.5	0.2	0	0
	1.0	0.4	0	0.5
	2.0	0.6	0.3	0.6

Table 3: Diameter of inhibition zones of Gentamycin

Conc. µg	Zone of inhibition (cm)		
	E.coli	*S.aureus*	*S.aureus (MRSA)*
25	1.8	1.3	0.7
50	2	1.8	1.5
100	2.3	2.1	2
200	2.6	2.5	2.4
400	2.8	2.7	2.5
800	3.1	3.4	2.7
MIC	25	<25	<25

Serial Tube Dilution Technique: Minimum inhibitory concentration (MIC) is the lowest concentration of an antimicrobial that will inhibit the visible growth of a microorganism after overnight incubation. In the present study, MIC was determined[19-20] 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 the concentration of compound is below the inhibitory level and the culture will become turbid (cloudy). The tube in which growth will not occur above the inhibitory level, will remain clear. Therefore, MIC was determined by choosing the lowest concentration in which no growth occurs (table 4).

Preparation of the inoculums: The test bacteria grown at 37^0C in nutrient agar medium was diluted in sterile nutrient broth medium in such a manner that the suspension contain about 10^7 cells ml^{-1}. This suspension was used as the inoculums.

Procedure: Six test tubes were taken for antibacterial activities.1 ml of nutrient broth medium was poured to each of the tube. These test tubes were cotton plugged and sterilized in an autoclave for 15 lbs sq. inch $^{-1}$ pressure. After cooling, 1 ml of the sample solution (made from tested compound and DMSO) was added to the 1st tube and mixed well and then 1 ml of this content was transferred to the 2nd test tube. The content of the second test tube was mixed well and again 1 ml of this mixture was transferred to the 3rd test tube. This process of serial dilution was continued up to the 6th tube. The tubes were inoculated by 0.1 ml of the bacterial suspension and then mixed well. All the test tubes were incubated at 37^0C for 24^0C. The highest dilution without growth is the minimal inhibitory concentration (MIC).The whole process was repeated for antifungal activities also.

Table 4: Results of MIC of some of the compounds (μg ml^{-1})

S. No.	**Compd. No.**	**E.coli** Gram -ve	**S.A.** Gram +ve	**K.P.** Gram -ve	**P.A.** Gram -ve.	**S.P.** Gram +ve	**B.S** Gram. +ve	**P.F** Gram-ve.	**A.J.**	**P.G.**
1.	3a	12.5	25	25	25	12.5	12.5	25	25	25
2.	3b	12.5	25	12.5	25	12.5	12.5	12.5	25	12.5
3.	4b	12.5	25	25	12.5	12.5	12.5	12.5	12.5	25
4.	4a	50	25	25	12.5	12.5	25	12.5	25	12.5
5.	5a	12.5	25	50	12.5	12.5	12.5	12.5	25	12.5
6.	5b	12.5	50	50	12.5	12.5	25	25	25	12.5
7.	Amoxicilln	3.12	3.12	3.12	3.12	3.12	3.12	3.12	------	------
8.	Fluconazole	-----	------	-------	-------	------	----	----	6.25	6.25

E.coli = Escherichia coli, S.A. = Staphylococcus aureus, K.P. = Klebsiella pneumoniae, P.A. = Pseudomonas aeruginosa, S.P. = Streptococcus pyogenes, B.S. = Bacillus subtilis, P.F. = Pseudomonas fluorescens, A.J. = Aspergillus janus, P.G.= Pencillium glabrum.

APPLICATIONS

The biologically significant compounds have been synthesized through the ecofriendly methods. These reactions reduce the amount of toxic wastes and by products in chemical transformations.

CONCLUSIONS

The present method provides a high speed, efficient, environmentally benign modification of classical Biginelli reaction without using an expensive reagent. Furthermore, biologically significant compounds have been synthesized, through the ecofriendly methods. The solid support used in the reactions reduce the amount of toxic wastes and by products in chemical transformations. Through this modification, precious

solvents can be saved, reaction time can be reduced and overall yield has been improved by reducing the no. of steps. Thus, it is a step towards green chemistry.

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