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Research Article

COMPARATIVE STUDY ON CONVENTIONAL, MICROWAVE AND ULTRASOUND-ASSISTED SYNTHESIS AND BIOLOGICAL EVALUATION OF TETRAZOLE DERIVATIVES

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ABSTRACT

In the present study a series of novel 6-(4-nitrophenyl)-4-substituted-3-(1H-tetrazol-5-yl)pyridin-2(1H)-one derivatives(3a-3j) by conventional, microwave and ultrasound assisted synthesis method. Advantages of the ultra sound effect were observed and high yields of the products were obtained after20–45 min ultrasonication. Characterization and structural elucidation of the products was realized based on chemical, analytical and spectral analyses. The results clearly demonstrated a high efficiency of the ultrasonic systems was achieved in the chemical processes. New compound were screened for anti-microbial evaluation. The results revealed that many of the synthesized 6-(4-nitrophenyl)-4-substituted-3-(1H-tetrazol-5-yl)pyridin-2(1H)-one derivatives has good anti-microbial activity.

KEYWORDS: *Chalcones, Tetrazole, Pyridin-2(1H)-one, Anti-microbial activity.*

INTRODUCTION

Heterocyclic chemistry is a very important branch of organic chemistry accounting for nearly one-third of modern publications. In fact two thirds of organic compounds are heterocyclic compounds [1]. A cyclic organic compound containing all carbon atoms in ring formation is referred to as a carbocyclic compound. If at least one atom other than carbon forms a part of the ring system then it is designated as a heterocyclic compound. Nitrogen, oxygen and sulfur are the most common heteroatoms but heterocyclic rings containing other hetero atoms are also widely known [2].

The literature survey reveals that tetrazole derivatives have proved to be good bioactive molecules. They have shown diverse biological activities like anti-bacterial [3], anti-fungal [4] anti-inflammatory [5], anti-tubercular [6], anticancer [7], anticonvulsant [8], antioxidant [9], analgesic [10] and anti proliferative [9] activities etc. Similarly pyridin-2(1*H*)-one are of biological importance having anti-bacterial [3], anti-fungal [11], anti-inflammatory [12], anti-tubercular [13], anticancer [14], anticonvulsant [15] activities.

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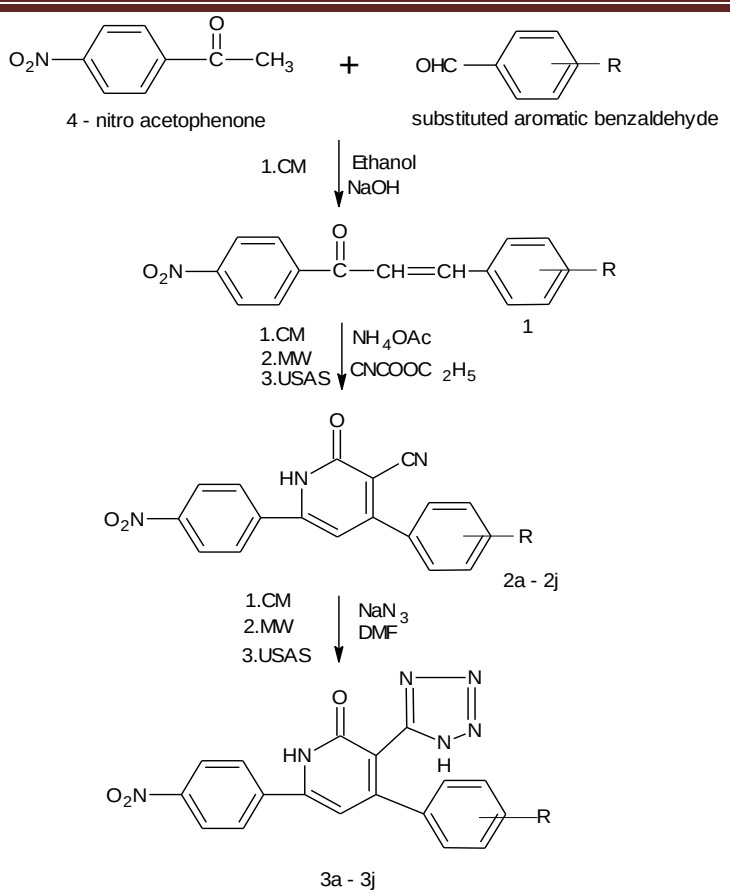
Ultrasound-assisted synthesis has attracted much attention during the past few decades. One advantage of using cavitation as an energy source to promote organic reactions includes shorter reaction times [16]. During the cavitation process, cavitation induces very high local temperatures and pressure inside the bubbles and enhances mass transfer and turbulent flow in the liquid. This method has been considered as a clean and convenient in organic synthesis compared with traditional methods. In continuation of our previous work on synthesis of various azole derivatives, we have decided to explore the efficient, simple, and fast synthesis of their corresponding heterocycles using ultrasound irradiation [17]. Based on the aforementioned considerations and as extension of our search for effective antimicrobial agents, The present study deals with the reaction of the step (1) compound was with 4-nitro acetophenone with aromatic aldehyde in the presence of NaoH and ethanol to give Chalcones. The chalcone which was synthesis in step (1) reacted with ethyl cyanoacetate, ammonium acetate and ethanol to produce compound (2a-2j). The compound (3a-3j) was obtained by treatment of (2a-2j) with ammonium chloride, sodium azide and DMF by conventional, microwave and ultrasound assisted synthesis methods and compared with respect to various parameters like yield and time [18].

We designed and synthesized new tetrazole andpyridin-2(1*H*)-one compounds conventional, microwave and under ultrasound irradiation. The compounds were assigned on the basis of elemental analysis, IR and 1H NMR spectral data. The synthesized compounds were evaluated *in vitro* for their antimicrobial activity.



R=4-Cl, 4-NO2, 4-Br, 4-OH, 3-Cl, 3-NO2, 4-N(CH3)2, 2,4-(OCH3)2, Furoyl, cinnamoyl

1. CM:*Conventional method;* **2. MW:***Microwave assisted synthesis* **3. USAO:** *Ultrasound assisted synthesis*

Scheme1: Scheme of tetrazole derivatives

MATERIAL AND METHODS

All chemicals used were of synthetic grade. Melting points were determined with open capillary method and were uncorrected. FT-IR spectra were recorded on a JASCO-FT-IR model 400 spectrophotometer, ^{1}H-NMR spectra were recorded in deuterated DMSO on a Varian mercury FT-NMR model 'Avance-II(Bruker) instrument, using TMS as internal standard.

Experimental work:

Step 1: Synthesis ofChalcones (1):

A mixture of 4-nitro acetophenone (0.01M), aromatic aldehyde (0.01M) was weighed accurately and dissolved in sufficient amount of ethanol and NaOH solution by shaking it vigorously. Then the reaction mixture was set for stirring on the magnetic stirrer for about 10 hr. After the completion of the reaction, crushed ice was added into reaction mixture to get precipitated product. The precipitated product was filtered, dried and recrystallized from ethanol.

Step 2: Synthesis of 6-(4-nitrophenyl)-2-oxo-4-(substituted phenyl)-1,2-dihydropyridine-3-carbonitrilederivatives(2a-2j)

Method 1: Conventional synthesis:

A mixture of chalcone (0.01M) (synthesis in step 1), ethyl cynoacetate (0.01M), ammonium acetate (0.01M), and ethanol (20ml) was refluxed for 3hrs.after completion of reaction cooled it and poured into ice cold water. The precipitated solid was obtained and it was filtered, dried and re-crystallized from ethanol.

Method 2: Microwave assisted synthesis

Mix 0.01M of chalcone (synthesis in step1), 0.01M of ethyl cyanoacetate (1.13gm), 0.01M of ammonium acetate (1.54gm), and ethanol (20ml) in microwave flask. Irradiated the reaction flask in microwave at level 3 for 3mins.After completion of reaction the flask was removed and kept it into ice bath. Added hydrochloric acid into reaction to occur precipitation of the step 2 product. The solid residue was filtered, dried and recrystallized from ethanol.

Method 3: Ultrasound assisted synthesis

Mix 0.01M of chalcone (synthesis in step1), 0.01M of ethyl cyanoacetate (1.13gm), 0.01M of ammonium acetate (1.54gm), and ethanol (20ml) was placed in ultrasonic bath for the period of 20-22 min. at 20-25^{0}c and Subsequently, the reaction mixture was left overnight and then concentrated under reduced pressure. The solid residue was collected, washed with water and recrystallized from ethanol to afford pure product.

Step -3 Synthesis of6-(4-nitrophenyl)-4-substituted phenyl-3-(1*H*-tetrazol-5-yl)pyridin-2(1*H*)-one derivatives (3a-3j)

Method 1: Conventional synthesis :

A mixture of step 2 compound (0.01M), sodiumazide (0.01M), dimethyl formamide (0.01M), and ammonium chloride

(0.01M) was refluxed in water bath for 7hrs. The solvent was removed and dissolved in 100ml of water and carefully acidify with hydrochloric acid to pH2.The solution was cooled in ice bath. The solid residue was filtered, dried and recrystallized from ethanol.

Method 2: Microwave assisted synthesis:

Mix 0.01M of step 2 compound, sodium azide (0.01M), dimethyl Formamide (0.01M), and ammonium chloride (0.01M) in microwave flask. Irradiated the flask in microwave at level 4 for 3 mins. After completion of reaction the flask was removed and whole mixture dissolved in 100ml of water and acidify with hydrochloric acid upto pH2. After that cooled whole solution in ice bath. The solid residue was filtered, dried and recrystallized from ethanol

Method 3 : Ultrasound assisted synthesis:

Mix 0.01M of step 2 compound, sodium azide (0.01M), dimethyl formamide (0.01M), and ammonium chloride (0.01M) was placed in ultrasonic bath for the period indicated in Table no. 2 at 20-25^{0}c and Subsequently, the reaction mixture was left overnight and then concentrated under reduced pressure. The solid residue was collected, washed with water and recrystallized from ethanol.

Spectral data of compounds [IR (KBr), ν cm–1 and ^{1}H-NMR (DMSO), δ ppm]:

3a:4-(4-chlorophenyl)-6-(4-nitrophenyl)-3-(1H-tetrazol-5-yl)pyridin-2(1H)one:

FT-IR:2967(C-H),1715(C=O),1653(N-H),1363(R-NO2),720(C-Cl).

^{1}H-NMR:7.4-8.6(8H,m,Ar-H),5.1-7.1(2H,s,pyridinone).

3b:4,6-bis(4-nitrophenyl)-3-(1H-tetrazol-5-yl)pyridin-2(1H)-one:

FT-IR:2965(C-H),1683(C=O),1540(N-H),1317 (R-NO2).

^{1}H-NMR:7.9-8.5(8H,m,Ar-H),5.2-7.1(2H,s,pyridinone).

3c:4-(4-bromophenyl)-6-(4-nitrophenyl)-3-(1H-tetrazol-5-yl)pyridin-2(1H)-one:

FT-IR:2968(C-H),1716(C=O),1663(N-H),1558(R-NO2),669(C-Cl).

^{1}H-NMR:7.4-8.6 (8H,m,Ar-H),5.1-7.0 (2H,s,pyridinone).

3d:4-(4-hydroxyphenyl)-6-(4-nitrophenyl)-3-(1H-tetrazol-5-yl)pyridin-2(1H)-one:

FT-IR:3628(R-OH),2965(C-H),1683(C=O),1540(N-H),1317(R-NO2).

^{1}H-NMR:6.9-8.6(8H,m,Ar-H),5.1-7.0(2H,s,pyridinone)5.1-7(1H,m,OH).

3e:4-(3-chlorophenyl)-6-(4-nitrophenyl)-3-(1H-tetrazol-5-yl)pyridin-2(1H)-one:

FT-IR:2967(C-H),1715(C=O),1653(N-H),1363(R-NO2),720(C-Cl).

^{1}H-NMR:7.2-8.6(8H,m,Ar-H),5.0-7.1(2H,s,pyridinone).

3f:4-(3-nitrophenyl)-6-(4-nitrophenyl)-3-(1H-tetrazol-5-yl) pyridin-2(1H)-one:

FT-IR:2965(C-H),1683(C=O),1540(N-H),1317(R-NO2).

^{1}H-NMR:7..4-8.6 (8H,m,Ar-H),5.1-7.4(2H,s,pyridinone).

3g:4-[4-(dimethylamino)phenyl]-6-(4-nitrophenyl)-3-(1H-tetrazol-5-yl)pyridin-2(1H)-one:

FT-IR:1396(C-H),1771 (C=O),3446(N-H),1568 (R-NO2),1318(C-N).

^{1}H-NMR:7.1-8.6(8H,m,Ar-H),4.9-7.0 (2H,s,pyridinone), 2.866(6H,s,N-(CH3)2).

3h:4-(2,4-dimethoxyphenyl)-6-(4-nitrophenyl)-3-(1H-tetrazol-5-yl)pyridin-2(1H)-one:

FT-IR: 2965 (C-H), 1683 (C=O), 1540(N-H), 1317 (R-NO2),1226 (C-O).

^{1}H-NMR:6.7-8.6 (8H,m,Ar-H),4.9-7.0 (2H,s,pyridinone),3.9-3.9 (6H,s,O-CH3).

3i:4-(furan-2-yl)-6-(4-nitrophenyl)-3-(1H-tetrazol-5-yl) pyridin-2(1H)-one

FT-IR: 2966 (C-H), 1715 (C=O), 1652 (N-H), 1558 (R-NO2), 1226 (C-O).

^{1}H-NMR: 6.8-8.6 (8H,m,Ar-H), 4.9-7.0 (2H,s, pyridinone).

3j:6-(4-nitrophenyl)-4-(2-phenylethenyl)-3-(1H-tetrazol-5-yl) pyridin-2(1H)-one:

FT-IR: 1396(C-H), 1771 (C=O), 3446 (N-H), 1568 (R-NO2), 1662 (C=C).

^{1}H-NMR: 7.1-8.6 (8H,m,Ar-H), 5.4-6.6 (2H,s, pyridinone),7.4-7.5(2H,m,CH=CH).

Table No. 1: Physical characteristics of compound(3a-3j)

Comp	'R' Group	Mol. formula	Mol. Wt.	M.P.(^{0}C)	Rf Value	Colour
3a	4-Cl	$C_{18}H_{11}ClN_6O_3$	394	186	0.56	Brownish yellow
3b	4-NO_2	$C_{18}H_{11}N_7O_5$	405	206	0.63	Dark Brown
3c	4-Br	$C_{18}H_{11}BrN_6O_3$	439	176	0.69	Brown
3d	4-OH	$C_{18}H_{12}N_6O_4$	376	170	0.67	Brown
3e	3-Cl	$C_{18}H_{11}ClN_6O_3$	394	190	0.52	Brownish yellow
3f	3-NO_2	$C_{18}H_{11}N_7O_5$	405	204	0.66	Blackish brown
3g	4-$N(CH_3)_2$	$C_{20}H_{17}N7O_3$	376	230	0.45	Brownish yellow
3h	2,4-$(OCH_3)_2$	$C_{20}H_{16}N_6O_5$	420	236	0.62	Yellow
3i	Furyl	$C_{16}H_{10}N_6O_4$	350	232	0.55	Blackish brown
3j	Cinnamoyl	$C_{20}H_{14}N_6O_3$	386	206	0.56	Brown

Table No. 2: Compariosin data between various methods for compound (3a-3j)

Compound	Conventional conditions		Microwave irraditation		Ultrasound irradiation	
	Time (min)	Yield (%)	Time (min)	Yield (%)	Time (min)	Yield (%)
3a	420	63	4	65	35	88
3b	420	65	4	68	40	76
3c	420	70	4	75	40	83
3d	420	68	5	70	30	73
3e	420	71	6	70	30	85
3f	420	58	5	60	35	68
3g	420	72	6	75	25	80
3h	420	66	5	68	25	77
3i	420	64	5	70	30	70
3j	420	74	3	75	30	85

2.3 Antimicrobial activity:

In vitro antibacterial activity of the synthesized compounds was tested against Gram positive bacteria viz. *S. aureus* (NCIM 2079), *B. subtilis* (NCIM 2920) and Gram-negative bacteria *E. coli* (NCIM2065) and *P. aeruginosa* (NCIM 2200).Compounds were diluted in DMSO with 1 µg/mL concentrations for bioassay. Micro-broth dilution method used to determine *in vitro* minimum inhibitory concentrations (MIC) of compounds in 96-well micro titreplates. Test compounds were serially double diluted in growth medium. Plates were incubated at 30°Cfor fungi and 37°C for bacteria for 24 h. Results are presented in Table 3.

RESULT AND DISCUSSION

In the present work, chalcone derivatives 1 were treated with ammonium acetate and ethylcyanoacetate in ethanol to produce the 6-(4-nitrophenyl)-2-oxo-4-(substituted phenyl)-1,2-dihydropyridine-3-carbonitrile derivatives (2a-2j) derivatives . The reaction of compound 2a-2j with sodium azide in presence of ammonium chloride produces 6-(4-nitrophenyl)-4-substituted phenyl-3-(1H-tetrazol-5-yl)pyridin-2(1H)-one derivatives (3a-3j)(Scheme 1).Application of microwave and ultrasound shortened the reaction time of the generation of pyrimidines from 7 h under classical conditions to 25-40 min. In addition, the yieldsof the products were improved by 05–22 % in comparison with those obtained bythe conventional and microwave assisted synthesis method s (Table 2).Conventional heating of the sonicating reaction mixture to the same (bulk)t emperature did not lead to any significant differences in the yields and times. In the view of the interest in green chemistry for the synthesis of organic compounds, an optimized procedure for the preparation of tetrazole derivatives was developed. These reactions were realized under milder and cleaner conditions. While with thermal heating these reactions required 7h at70–80 °C, the new method was performed at room temperature for shorter times.

The IR spectra of synthesized pyridine(1*H*)-one containing tetrazole (3a-3j) were taken the compounds shows the all functional group within the range. The synthesized tetrazole derivatives (3a-3j) are also characterized by ^{1}H-NMR spectroscopy the compound shows chemical shift at 6.86-8.60 due to aromatic protons. The expected signals for different types of protons such as hydroxy, methyl, methoxy group were observed for the derivative within the range. The overall spectral data confirms the structure of synthesized compounds.

The synthesized tetrazole derivatives were screened for antimicrobial activity using DMSO as a solvent against the organisms, Gram positive organisms: *Staphylococcus aureus, B. subtillis* and Gram negative organism,: *Escherichiacoli, P. Aeruginosa* by MIC method on nutrient broth media.

Clotrimazole was used as standard drug for antimicrobial activity. Standard norfloxacin shows potent activity at MIC of 75, 50, 100, 150 against *Staphylococcus aureus, B. subtillis, Escherichia coli* and *P. Aeruginosa.* Phenyl substituted derivatives with 4-Cl, 4-Br, 4-OH, 3-NO_2 (3a, 3c, 3d, 3f) are shows good activity and compounds 3-Cl,2,4-$(OCH_3)_2$, Furan (3e, 3h, 3i) group substituted are shows moderate activity remaining three compounds shows less activity.

Table No. 3: Antimicrobial careening data of compound 3a-3j

Comp. code	MIC (µg/mL) G^{+ve} bacteria		MIC (µg/mL) G^{-ve} bacteria	
	S.aureus	*B.subtillis*	*E.coli*	*Pseudomonas aeruginosa*
3a	75	50	50	75
3b	50	75	100	50
3c	100	75	75	100
3d	150	100	200	150
3e	75	75	100	50
3f	100	50	100	75
3g	200	150	150	100
3h	150	100	75	50
3i	200	100	150	150
3j	150	100	100	75
Clotrimazole	50	25	50	50

CONCLUSION

An optimized procedure for the preparation of tetrazole derivativesunder mild and clean conditions was described by microwave and ultrasound assisted synthetic methods was described. The advantages of ultrasoundin chemical reactions, such as shorter reaction times, higher yields andmilder conditions, could be of use in industrial applications in the pharmaceuticalor fine chemical industries.Thederivatives with electrophilic substitution like 4-Cl, 4-Br, 3-Cl, 3-NO_2 on phenyl ring (Comp. code- 3a, 3c, 3e, 3f) shows promising anti-microbial activity than nucleophilic substitution.

This study shows that ultrasound-assisted synthesis is an important tool of green chemistry as illustrated by the reactions presented here. Moreover, it is a fairly new technique it is now increasingly applied in research such as organic synthesis and development of tetrazoles. The advantages of ultrasound in chemical reactions are milder conditions, shorter reaction times, higher yields and environmental friendly reaction conditions, but the increasing requirement for environmentally clean technology that minimizes the production of waste at source is an important factor.

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