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Utrasound Mediated Synthesis Of Novel Pyrazoline Derivatives As Antimicrobial Agents

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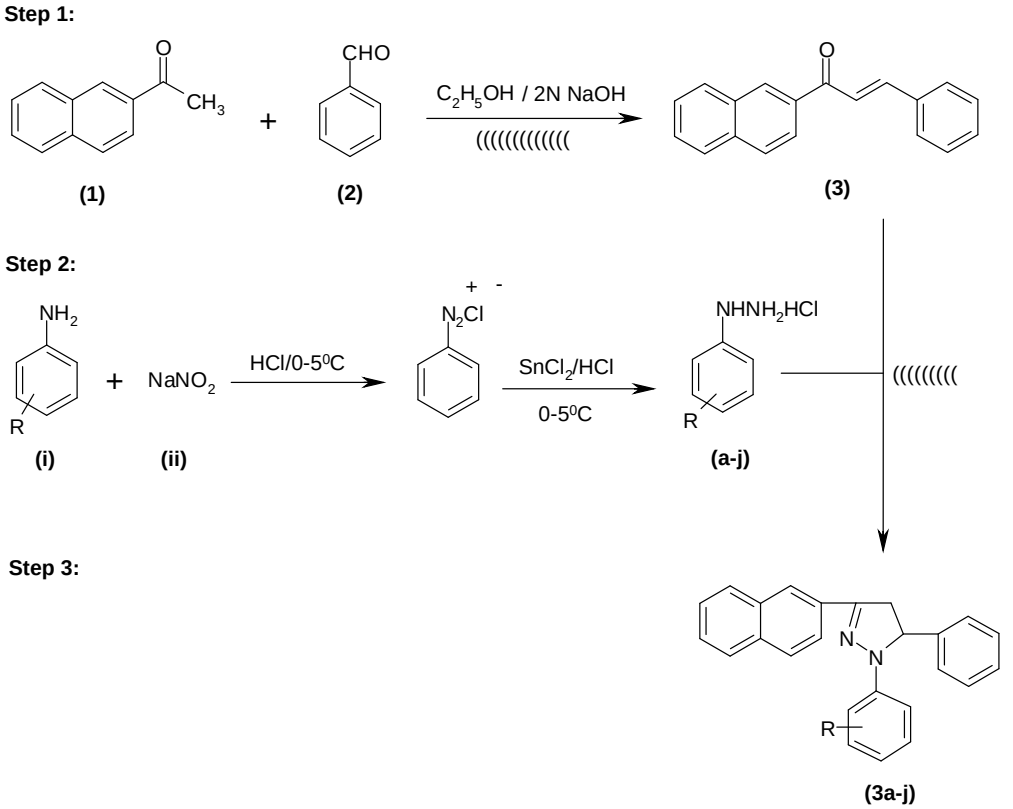
Abstract: The synthesis and antimicrobial activity of some novel pyrazoline derivatives **3(a-j)** are synthesized by Claisen-Schmidt condensation of 2-acetylnaphthalene and benzaldehyde to give respective chalcone and further cyclised with substituted phenylhydrazine in glacial acetic acid using ultrasonic irradiation in lesser time with higher yields. All the compounds were characterized by physical and spectral data. The compounds were screened for anti-microbial activities. Compounds **3i & 3d** were found to possess significant anti-bacterial activity against both gram positive and gram negative bacteria at the tested concentrations when compared with that of standard drug ampicillin. In anti-fungal study, compounds **3e, 3h** and **3j** have exhibited significant anti fungal activity when compared with standard drug fluconazole. These compounds can be further exploited to get the potent lead compound.
Key words: 2-Pyrazolines, Sonochemistry, Antimicrobial activity.

INTRODUCTION

The quest for safe and potent drug is always fascinating and challenging for medicinal chemist. Large number of drugs containing simple heterocyclic or a combination of different heterocyclic moieties being used as therapeutic agents and these compounds are also essential for the human life. Increasing evidence suggest that pyrazolines are well known important nitrogen containing five membered heterocyclic compounds. They possess a broad range of biological activities such as antibacterial [1-3], antifungal [4], antitubercular[5-6], antimalarial [7], antiamoebic [8], anti-HIV [9-10], anti-inflammatory [11-13], antioxidant [14-15], antipyretic [16], antidiabetic [17], antidepressant [18-19], anticancer [20-21] and also useful synthons in organic synthesis [22]. The traditional synthesis of 2-pyrazolines involves the base catalyzed Claisen-Schmidt condensation of aryl methyl ketones and aldehydes to give chalcones, which undergo a subsequent cyclization with hydrazines to yield respective 2-pyrazolines [23]. The recent interest in ultrasound (sonochemistry) has increasingly been used in organic synthesis and become an exciting field of research. The chemical effects of ultrasound are diverse and include substantial improvements in both stoichiometric and catalytic chemical reactions when compared with traditional methods. Ultrasonic irradiation accelerates reactivity, reduces reaction time and also improves the yield. As a part of our research interest in the development of multifunctional libraries of pyrazolines, therefore it was felt worthwhile to study these reactions under ultrasonic irradiation with the aim of decreasing the reaction time and increasing the yield (Scheme 1) and also evaluate the antimicrobial activity of synthesized compounds.

GENERAL SCHEME:



EXPERIMENTAL

All the chemicals and solvents used were of synthetic grade obtained from Sd Fine chemicals and AVRA labs. Completion of the reactions was monitored time to time by analytical thin layer chromatography (TLC) using E-Merck 0.25mm silica gel plates. Visualization was accomplished with UV light (256nm) and iodine chamber. The purity of compounds was checked by a single spot in TLC and solvent system for TLC was determined on trial & error basis. All the melting points were determined in open capillaries, using Boitus melting point apparatus, expressed in ºC and are uncorrected. All the IR spectra were recorded on SCHIMADZU FT-IR SPECTROPHOTOMETER by using 1 % potassium bromide discs. The Electronic Spray Ionization mass spectra were recorded on Agilent 1100 series. The ^{1}H NMR spectra of the compounds were recorded on Bruker AMX 400 MHz NMR spectrophotometer using TMS as an internal standard and the values are expressed in δ ppm.

General Procedure for Synthesis of 1-(naphthalen-6-yl)-3-phenylprop-2-en-1-one (1):

To a mixture of 2-acetylnaphthalene (**1**) (0.01 mol) and benzaldehyde (**2**) (0.01 mol) were dissolved in ethanol (50 mL). To this reaction mixture aqueous sodium hydroxide (70 %, 5 mL) was added drop wise with constant stirring. The reaction mixture was irradiated by an ultrasonic generator (Citizen Make) in a water-bath at 30-35ºC for 3 min. The solid product so formed was diluted with water and neutralized with 2*N* HCl. Then it was filtered, washed well with cold water and recrystallized from ethanol to afford yellow solid product (**3**).

Synthesis of substituted phenyl hydrazines (a-j):

Substituted aniline (i) (0.05 mol) and water (20 ml) were placed in conical flask and the mixture was stirred while conc. HCl (20 ml) was added. The reaction flask was cooled rapidly to 0ºC by using ice-bath. The temperature was maintained to 0ºC while the solution of sodium nitrite **(ii)** (0.053 mol, 3.6 gms) in 9 ml of water was added to flask. The diazotization required about 15 min.Then the reaction mixture was poured with stirring into solution of stannous chloride dehydrate 23 gms, 0.13 mol) in HCl (25 ml) at 0ºC.The resulting mixture was stored at 0ºC overnight, the separated product was filtered, washed with HCl and then with ether. It was recrystallized from water. Purity of the compounds was established by a single spot in TLC and was further confirmed by melting point.

Synthesis of 1-(substituted phenyl)-4,5-dihydro-3-(naphthalen-6-yl)-5-phenyl-1*H*-pyrazole (3a-j):

To a mixture of 1-(naphthalen-6-yl)-3-phenylprop-2-en-1-one **(1)** (0.01 mol), substituted phenyl hydrazine hydrochloride **(a-j)** (0.01 mol) and glacial acetic acid (30mL) were taken into a 100 mL conical flask. This reaction flask was suspended at the center of the ultrasonic cleaning-bath to get the maximum ultrasound energy and sonicated until crystals appeared or starting chalcone disappeared. The reaction mixture was poured into crushed ice and left overnight. The precipitate was separated by filtration, washed well with water, dried and recrystallized from suitable solvent to afford pure crystals **(3a-j)**. The purity of the compound was checked by TLC using a mixture of hexane and ethyl acetate as a mobile phase.

Antimicrobial activity:

All the synthesized compounds **3a-j** were screened for their antibacterial activity against *Staphylococcus aureus* (NCIM-2079), *Bacillus subtilis* (NCIM-2063), *Escherichia coli* (NCIM-2068) and *Proteus vulgaris* (NCIM-2027) by serial tube dilution technique [24-25] using ampicillin as reference standard, and antifungal activity against *Aspergillus* niger (ATCC-6275) and *Candida tropicalis* (ATCC-1369) by using fluconazole as reference standard. The observed minimum inhibitory concentrations (MIC) values for all the synthesized compounds are presented in Table 4.

RESULTS AND DISCUSSION

i) *Chemistry*

Synthesis of 1-(naphthalen-6-yl)-3-phenylprop-2-en-1-one (1):

The synthesis involves condensation of 2-acetylnaphthalene with benzaldehyde in presence of 2*N* NaOH in absolute ethanol by employing ultrasound irradiation to give 1-(naphthalen-6-yl)-3-phenylprop-2-en-1-one **(1)**. Homogeneity of the compound was checked by TLC. Solvent system used was ethylacetate:n-hexane (2:8). Compound **1** analyzed for $C_{19}H_{14}O$ and it showing sharp melting point at 108-110^oC. The compound further confirmed by IR spectrum in which characteristic bands observed at 1661 (C=O), 1573 (C=C of Ar), 1468 (CH=CH). The ^1H NMR (δ ppm) spectrum of compound **1** showed characteristic signals of CO-CH= and =CH-Ar at 7.25 and 7.80 as doublets respectively, a multiplet in between 7.25–8.70 integrating for the twelve aromatic protons.

Synthesis of Phenyl hydrazine hydrochloride (1a):

Aniline was reacted with sodium nitrite in presence of HCl to give respective diazonium salt, followed by reduction in presence of stannous chloride dihydrate in HCl to give phenylhydrazine hydrochloride **(1a)**. Homogeneity of the compound was checked by a single spot in TLC and was further confirmed by melting point given in the literature. The Physical data of compounds **(b-j)** given in table 1.

Synthesis of 4,5-dihydro-3-(naphthalen-6-yl)-5-phenyl-1-*p*-tolyl-1*H*-pyrazole:

To a mixture of 1-(naphthalen-6-yl)-3-phenylprop-2-en-1-one **(1)** (0.01 mol), 4-methyl phenylhydrazine hydrochloride **(b)** (0.01 mol) and glacial acetic acid (30mL) were taken into a 100 mL conical flask. This reaction flask was suspended at the center of the ultrasonic cleaning-bath to get the maximum ultrasound energy and sonicated until crystals appeared or starting chalcone disappeared. The reaction mixture was poured into crushed ice and left overnight. The precipitate was separated by filtration, washed well with water, dried and recrystallized from ethanol to afford pure crystals **(3b)**. The purity of the compound was checked by TLC using a mixture of hexane and ethyl acetate (30:70) as a mobile phase. Further confirmation was done by spectral data. Compound **3b** analyzed for $C_{26}H_{22}N_2$, m.p 168-170^oC, well supported by its $[M+H]^+$ ion at *m/z* 363.22 in its positive mode electrospray ionization mass spectrum. The IR (cm^{-1}) spectrum showed the characteristic intense bands at 1596 (C=N), 1379 (C-N) and 1497 (C=C of Ar). The ^1H NMR spectrum (300 MHz, $CDCl_3$) showed the characteristic H_A, H_B, H_X protons at δ 3.22, 3.90 and 5.35 respectively as doublet of doublets (dd) with J_{AB}=16.98 Hz, J_{AX}=7.50 Hz and J_{BX}=9.35 Hz. The spectrum also showed a singlet at 2.31 (3H, s, Ar-CH3) and also accounted for all other 16 aromatic protons which appeared in between δ 6.80-8.20. Based on the above spectral data the structure of the compound **3b** was confirmed as 4,5-dihydro-3-(naphthalen-6-yl)-5-phenyl-1-*p*-tolyl-1*H*-pyrazole. By adopting the above synthetic procedure, compounds (**3a** to **3j**) were also synthesized. All these compounds are new and their physical and spectral data were presented separately in the table 2 and 3.

Table 1: Physical data of phenylhydrazines (1a-j)

Compound	Ar	Formula	Melting Point (^{0}C)	Yield (%)
1a	Phenyl	$C_6H_9N_2Cl$	250-252	72%
1b	4-tolyl	$C_7H_{11}N_2Cl$	155-158	68%
1c	4-chlorophenyl	$C_6H_8N_2Cl_2$	216-218	78%
1d	2,4-dichlorophenyl	$C_6H_7N_2Cl_3$	206-208	64%
1e	4-dimethylaminophenyl	$C_8H_{14}N_3Cl$	215-217	74%
1f	2-chlorophenyl	$C_6H_8N_2Cl_2$	200-203	65%
1g	2-tolyl	$C_7H_{11}N_2Cl$	195-196	63%
1h	2,4-dimethylphenyl	$C_8H_{13}N_2Cl$	182-184	66%
1i	4-fluorophenyl	$C_6H_8N_2ClF$	>300	69%
1j	4-nitrophenyl	$C_6H_8N_3ClO_2$	194-196	72%

Table 2: Physical data of 2-pyrazoline derivatives (3a-j)

Compound	Ar	Formula	Melting Point(^{0}C)	Yield %)
3a	Phenyl	$C_{25}H_{20}N_2$	192-198	76%
3b	4-tolyl	$C_{26}H_{22}N_2$	200-205	77%
3c	4-chlorophenyl	$C_{25}H_{19}N_2Cl$	170-179	70%
3d	2,4-dichlorophenyl	$C_{25}H_{18}N_2Cl_2$	189-195	72%
3e	4-dimethylaminophenyl	$C_{27}H_{25}N_3$	190-198	69%
3f	2-chlorophenyl	$C_{25}H_{19}N_2Cl$	198-204	74%
3g	2-tolyl	$C_{26}H_{27}N_2$	185-191	75%
3h	2, 4-dimethylphenyl	$C_{27}H_{24}N_2$	179-182	74%
3i	4-fluorophenyl	$C_{25}H_{19}N_2F$	172-180	76%
3j	4-nitrophenyl	$C_{25}H_{19}N_3O_2$	197-202	72%

Table 3: ^{1}H NMR of 2-pyrazoline derivatives (3b-e)

Compound	Chemical shift (δ) in ppm
3b	3.22 (1H, dd, HA), 3.90 (1H, dd, HB), 5.35 (1H, dd, Hx), 2.31 (3 H,s, Ar-CH3) and 6.80-8.20 (16 H, Ar-H)
3c	3.20 (1H, dd, HA), 3.91 (1H, dd, HB), 5.28 (1H, dd, Hx), 6.80-8.20 (16 H, Ar-H)
3d	3.15 (1H, dd, HA), 4.10 (1H, dd, HB), 5.60 (1H, dd, Hx), 6.80-8.20 (15 H, Ar-H)
3e	3.21 (1H, dd, HA), 3.84 (1H, dd, HB), 5.22 (1H, dd, Hx), 2.90 (6H, s, -N-(CH3)2, 6.67-8.20 (16 H, Ar-H)

Table 4. Antimicrobial activity of 2-pyrazoline derivatives (3a-j). (Expressed as MIC in µg/mL)

	Antimicrobial activity					
Compound	***B.subtilis***	***S.aureus***	***E.coli***	***P.vulgaris***	***A.* niger**	***C. tropicalis***
3a	256	-	256	128	256	256
3b	256	-	-	-	128	128
3c	128	256	256	128	256	256
3d	64	128	64	128	256	128
3e	256	256	128	256	16	64
3f	256	256	256	128	256	128
3g	256	256	-	-	256	256
3h	256	256	256	128	64	128
3i	32	64	64	64	256	128
3j	128	128	128	256	128	128
Ampicillin	<1	<1	<1	<1	-	-
Fluconazole	-	-	-	-	<2	<2

(-) indicates MIC > 256

ii) Antibacterial activity:

All synthesized pyrazoles (3a-j) have been evaluated for their antibacterial activity against *E. coli*, *P. vulgaris* (Gram-negative) and *S. aureus* and *B. subtilis* (Gram-positive) using serial tube dilution method. The results of this evaluation compared with ampicillin as reference standard. From the above results it is evident that all the pyrazoles showed antibacterial activity with different MIC values against the tested organisms, but not comparable with that of the standard. Among the tested compounds 3i (4-fluorophenyl) and 3d (2,4-dichlorophenyl) was found to be potent against *B. subtilis* with a MIC value of 32 µg/mL and 64 µg/mL respectively. The compound 3c (2-chlorophenyl) and 3j (4-nitrophenyl) was found to be moderately potent against *B. subtilis* with a MIC value of 128 µg/mL. The compound 3f was active against *E. coli* with a MIC value of 64 µg/mL. The compounds 3d & 3j were active against *P. vulgaris* with a MIC value of 64 µg/mL. The other compounds also exhibited activity with a MIC values ranging from 128-256 µg/mL.

Among all the compounds tested, compounds 3i and 3d possessed maximum activity which may be due to electron withdrawing substituents such as 4-fluorophenyl and 2,4-dichlorophenyl moieties at N-1 position of pyrazoline and thus reveals the importance of such groups for favorable antibacterial activity. This also suggested that pyrazoline having more number of these substituents at different positions of the aromatic or heteroaromatic rings when synthesized may demonstrate promising antibacterial activity. Infact, it was observed in the present study that N-1 substituted phenyl ring contributed favourably to the antibacterial activity.

iii) Antifungal activity:

The antifungal activity of pyrazolines (3a-j) have been evaluated against *A.niger* and *C.tropicalis* and fluconazole employed as reference standard by using serial tube dilution method. A close examination of the antifungal data of pyrazolines revealed that some of the compounds in this series have been found effective against both fungi at 16 µg/mL concentration level when compared with reference standard fluconazole. Among the compounds tested for antifungal activity, compounds 3e (4-dimethylaminophenyl) and 3h (2,4-dimethylphenyl) and 3j (4-nitrophenyl) found to be potent against *A. niger* with a MIC value of 64 and 128 µg/mL. All the other compounds showed activity with a MIC values ranging from 128-256 µg/mL which was less when compared to the activity of other compound tested. Compounds 3e, 3h, 3j and 3b possessed maximum activity which may be due to the presence of 4-dimethylaminophenyl, 2,4-dimethylphenyl, 4-nitrophenyl and 4-methylphenyl pharmacophore at N-1 position of pyrazoline structure. This reveals the importance of the electronic effects of the substituents present on the aromatic ring in enhancing the antifungal activity. Moreover it has been found that compounds **3a**, **3c**, **3d** and **3i** also exhibited weak activity against both the fungi.

CONCLUSION

In the present study we have demonstrated a simple, efficient and cleaner strategy for the synthesis of 2-pyrazolines by reacting of different phenylhydrazines with chalcone in glacial acetic acid under ultrasound irradiation conditions. With encouraging antimicrobial activity results, all the synthesized compounds need to be evaluated in terms of active concentration and also examine the mechanism of compounds responsible for antimicrobial activity. All the synthesized compounds can be further explored for structural modifications and studies concerning the structure-activity relationships are in progress in our laboratory.

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