



MICROWAVE ASSISTED SYNTHESIS AND ANTIMICROBIAL EVALUATION STUDIES ON PYRAZOLINES

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ABSTRACT

A series of pyrazolines derivatives were synthesized from 2-chloro-3-cyanopyridine under conventional heating and microwave irradiation. The structures of the newly synthesized pyrazolines were established based on IR, ¹H- and ¹³C-NMR and mass spectral data. All the synthesized compounds were screened for their antimicrobial activity. Some of the compounds showed very good activity compared to standard drugs against all pathogenic bacteria and fungi.

KEY WORDS

coumarin; chalcones; Michael addition; microwave irradiation; pyrazolines.

INTRODUCTION

Pyrazolines are well known and important nitrogen containing 5-membered heterocyclic compounds and various methods have been worked out for their synthesis. Numerous pyrazoline derivatives have been found to possess considerable biological activities, which stimulated the research activity in this field.^{1,2}

They have several prominent effects, such as diuretic³, anticonvulsant⁴, herbicidal⁵, bactericidal⁶, anti-allergic⁷, insecticidal⁸, anti-implantation⁹, analgesic¹⁰, antitumor¹¹, anti-inflammatory¹², leukotriene inhibitor¹³, fungicidal¹⁴. They also possess some potent receptor selective biological activity like Nitric oxide synthase (NOS) inhibitor and Cannabinoid CB1 receptor antagonists' activity. 4,5-dihydro-1H-pyrazolines seem to be the most frequently studied pyrazoline type compounds.

Microwave irradiation has gained popularity in the past decade as a powerful tool for the rapid and efficient synthesis of a variety of compounds, resulting from the selective absorption of microwave energy by polar molecules.¹⁵ The application of microwave irradiation

provides enhanced reaction rates and improved product yields in organic synthesis and it is proving quite successful in the formation of a variety of carbon-heteroatom bonds. Recently, considerable efforts have been made in the design and realization of innovative synthetic protocols in organic synthesis, whereby a more eco-sustainable approach was adopted.¹⁶⁻¹⁸

As part of an ongoing research program, the syntheses of new biologically active pyrazoline derivatives, 2-(4-(4,5-dihydro-5-phenyl-1H-pyrazol-3-yl) phenylamino) pyridine-3-carbonitrile, 2-(4-(1-acetyl-4,5-dihydro-5-phenyl-1H-pyrazol-3-yl) phenyl amino) pyridine-3-carbonitrile and 2-(4-(1-phenyl-4,5-dihydro-5-phenyl-1H-pyrazol-3-yl) phenylamino) pyridine-3-carbonitrile under conventional and non-conventional (microwave irradiation) methods, are reported herein.

MATERIAL AND METHODS

Chemicals

Melting points were determined on an electro thermal apparatus using open capillaries and are uncorrected. Thin-layer chromatography was accomplished on 0.2-

mm precoated plates of silica gel G60 F254 (Merck). Visualization was made with UV light (254 and 365nm) or with an iodine vapor. IR spectra were recorded on a Shimadzu-Fourier Transform Infra-Red (FTIR)-8400 Spectrophotometer using KBr disc. ¹H NMR spectra were recorded on a Bruker DPX-400 MHz spectrometer. Chemical shifts are expressed in δ ppm downfield from TMS as an internal standard.

Preparation of 2-(4-acetylphenylamino) pyridine-3-carbonitrile.

Conventional method

A Mixture of 2-chloro-3-cyanopyridine (0.01 M), p-aminoacetophenone (0.01 M) and few drops of Con HCl, taken in RBF with 20 ml Ethanol and was refluxed for 16 hours on water bath. Contain was allowed to cool at room temperature for 30 minutes, the separated solid was collected and washed with cold ethanol, crystallized from hot ethanol.

Microwave irradiation

A Mixture of 2-chloro-3-cyanopyridine (0.01 M), p-aminoacetophenone (0.01 M) and few drops of Con HCl, taken in glass vial equipped with a cap with 20 ml Ethanol and was and then subjected to microwave irradiation at 300 W for 4.2 min. Contain was allowed to cool at room temperature for 30 minutes, the separated solid was collected and washed with cold ethanol, crystallized from hot ethanol.

Preparation of 2-(4-(3-phenylacryloyl) phenylamino) pyridine-3-carbonitrile. (1a/1b/1c)

Conventional method

A Solution of aldehyde (0.01 M) in minimum quantity of DMF (5 ml) was added to the mixture of 2-(4-acetylphenylamino) pyridine-3-carbonitrile in 15ml DMF and 40% KOH was added to make mixture alkaline, the reaction mixture was then stirred for 24 hours at room temperature. The product was isolated by adding mixture in cold water and crystallized it from DMF.

Microwave irradiation

A Solution of aldehyde (0.01 M) in minimum quantity of DMF (5 ml) was added to the mixture of 2-(4-acetylphenylamino) pyridine-3-carbonitrile in 15ml DMF and 40% KOH was added to make mixture alkaline, the reaction mixture was then taken in glass vial equipped with a cap and then subjected to microwave irradiation at 300 W for 4.3 min. The product was isolated by adding mixture in cold water and crystallized it from DMF.

Synthesis of 2-(4-(4, 5-dihydro-5-phenyl-1H-pyrazol-3-yl) phenylamino) pyridine-3-carbonitrile. (2a)

Conventional method

A mixture of 2-(4-(3-phenylacryloyl) phenylamino) pyridine-3-carbonitrile (0.01 M) and hydrazine hydrate (0.05 M) in 25 ml Ethanol was refluxed for 10 hrs. The solution was poured into crushed ice and neutralize with dilute HCl solution. Product was isolated and crystallized from ethanol.

Microwave irradiation

A mixture of 2-(4-(3-phenylacryloyl) phenylamino) pyridine-3-carbonitrile (0.01 M) and hydrazine hydrate (0.05 M) in 25 ml Ethanol was taken in a glass vial equipped with cap and then subjected to microwave irradiation at 300 W for 4.2 min. The solution was poured into crushed ice and neutralize with dilute HCl solution. Product was isolated and crystallized from ethanol.

Synthesis of 2-(4-(1-acetyl-4, 5-dihydro-5-phenyl-1H-pyrazol-3-yl) phenyl amino) pyridine-3-carbonitrile. (2b)

Conventional method

A mixture of 2-(4-(3-phenylacryloyl) phenylamino) pyridine-3-carbonitrile (0.01 M) and hydrazine hydrate (0.05 M) in 15 ml glac acetic acid was refluxed for 8 hrs. The solution was poured into crushed ice and product was isolated and crystallized from ethanol.

Microwave irradiation

A mixture of 2-(4-(3-phenylacryloyl) phenylamino) pyridine-3-carbonitrile (0.01 M) and hydrazine hydrate (0.05 M) in 15 ml glac acetic acid was taken in a glass vial and then subjected to microwave irradiation at 300 W for 4.3 min. The solution was poured into crushed ice and product was isolated and crystallized from ethanol.

Synthesis of 2-(4-(1-phenyl-4, 5-dihydro-5-phenyl-1H-pyrazol-3-yl) phenylamino) pyridine-3-carbonitrile. (2c)

Conventional method

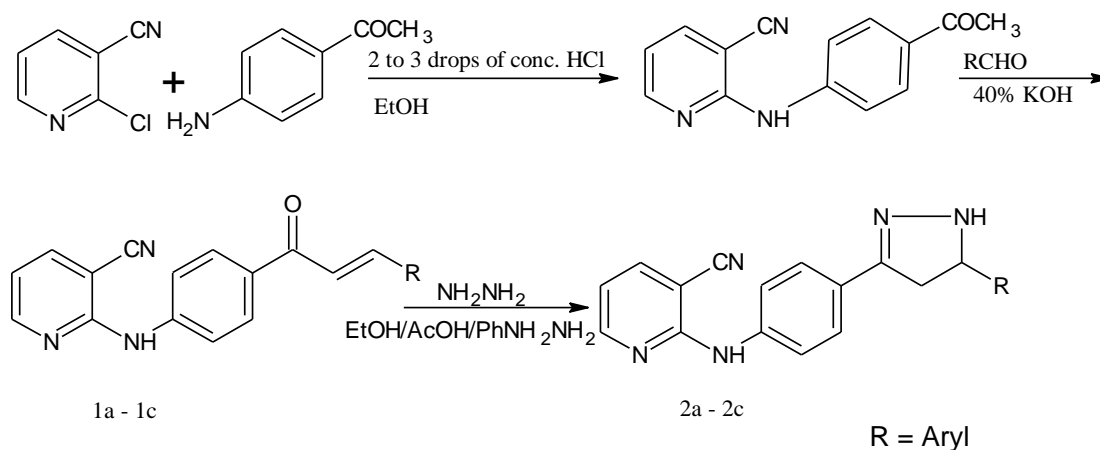
A mixture of 2-(4-(3-phenylacryloyl) phenylamino) pyridine-3-carbonitrile (0.01 M) and Phenyl hydrazine (0.01 M) in 25 ml Ethanol was refluxed for 10 hrs. The solution was poured into crushed ice and neutralize with dilute HCl solution. Product was isolated and crystallized from ethanol.

Microwave irradiation

A mixture of 2-(4-(3-phenylacryloyl) phenylamino) pyridine-3-carbonitrile (0.01 M) and Phenyl hydrazine (0.01 M) in 25 ml Ethanol was taken in a glass vial

equipped with cap and then subjected to microwave irradiation at 300W for 4min. The solution was poured

into crushed ice and neutralize with dilute HCl solution. Product was isolated and crystallized from ethanol



SCHEME – Synthesis of Pyrazoline

Characterization and Spectral Discussion of Synthesized Compounds

2-(4-(4, 5-dihydro-5-phenyl-1H-pyrazol-3-yl) phenylamino) pyridine-3-carbonitrile. (2a)

Yield 59%, m.p. 92–94 C. Rf 0.65.

IR: Alkane: 2958 cm^{-1} (-C-H Str (asym)), 2870 cm^{-1} (-C-H Str (sym)), 1417 cm^{-1} (-C-H def. (asym.)), 1336 cm^{-1} (-C-H def. (sym.)); Aromatic: 3103 cm^{-1} (C-H str), 1550 cm^{-1} (-C=C), 831 cm^{-1} (C-H o.o.p. def); Pyrazoline: C=N str 1519 cm^{-1} , C-N str 1253 cm^{-1} ; Amine: 3302 cm^{-1} N-H str; Nitrile: 2353 cm^{-1} (-C≡N)

^1H NMR Spectral: 2.3 (1H, triplet, -CHx); 2.5-2.6 (2H, double, -CH₂); 6.8 (2H, doublet, -Ar CH); 6.9-7.2 (4H, Multiplet, -Ar CH)

2-(4-(1-acetyl-4, 5-dihydro-5-phenyl-1H-pyrazol-3-yl) phenyl amino) pyridine-3-carbonitrile. (2b)

Yield 63%, m.p. 92 C. Rf 0.66. ^1H NMR (CDCl₃) d = 2.19 (s, 3H, NHCOCH₃), 6.98 (d, 1H, H-3), 7.28–7.46 (m, 5H, phenyl), 7.59 & 8.31 (d, each H, CH,CH), 8.22 (d, 2H, H-4, 6), 9.53 (s, 1H, OH), 11.47 (s, 1H, CONH). MS [EI] m/z 281. Elemental analysis (C₁₇H₁₅N₃O); Calcd. C, 72.58; H, 5.37; N, 4.98; found C, 72.36; H, 5.28; N, 5.10%.

2-(4-(1-phenyl-4, 5-dihydro-5-phenyl-1H-pyrazol-3-yl) phenylamino) pyridine-3-carbonitrile.

Yield 57%, m.p. 112 C. Rf value: 0.63. ^1H NMR (CDCl₃) d = 2.18 (s, 3H, NHCOCH₃), 7.02 (d, 1H, H-3), 7.35–7.49 (m, 4H, H-20, 40, 50, 60), 7.57 & 8.33 (d, each H, CH,CH), 8.25 (d, 2H, H-4,6), 9.67 (s, 1H, OH), 11.39 (s, 1H, CONH). MS [EI] m/z 315. Elemental analysis (C₁₇H₁₄CINO₃); Calcd. C, 64.67; H, 4.47; N, 4.44; found C, 64.58; H, 4.42; N, 4.28%.

Table 1: Physical Data of compound 2a, 2b and 2c

Comp.	R=Aryl	Molecular formula	Molecular weight	Melting point	Yield		Reaction time	
					Conv.	MW	Conv. (hr)	MV
2a	4-OCH ₃ -C ₆ H ₄ -	C ₂₂ H ₁₉ N ₅ O	369	251 – 253	72	85	8	4.2
2b	3, 4-(OCH ₃) ₂ -C ₆ H ₄	C ₂₃ H ₂₁ N ₅ O ₂	399	279 – 281	68	85	8.2	4.3
2c	3, 5-Cl ₂ -2-OH-C ₆ H ₄ -	C ₂₁ H ₁₅ N ₅ OCl ₂	424	160 - 162	65	75	8.4	4

Antibacterial activity

The novel synthesized compounds 2a–c was screened for their antibacterial activity against different types of bacterial strains, i.e., Gram-negative bacterial strains of *Pseudomonas aeruginosa* (9027) and *Escherichia coli* (ATCC-8739), Gram-positive bacterial strains of *Bacillus*

subtilis (ATCC-11778) and *Staphylococcus aureus* (ATCC-9144) at a concentration of 100 $\mu\text{g mL}^{-1}$.

The cultures were diluted with 5 % autoclaved saline and the final volume was adjusted to a concentration of approximately 10⁵–10⁶ CFU mL⁻¹. The synthesized compounds were diluted with acetone for the

antibacterial biological assays. For agar disc diffusion method,³³ the liquid form of the test compound was soaked on to a disc (5 mm) and then allowed to air dry, such that the disc became completely saturated with the test compound. The saturated chemical discs were introduced onto the upper layer of the medium evenly loaded with the bacteria and incubated at 37 °C for 24 to 48 h for better inhibition of the bacteria. The zones of inhibition were measured after 24 to 48 h. All the experiments were performed in triplicate and the results are expressed as zone of inhibition in mm. The zones of inhibition of synthesized compounds 2a–c was compared with the zone of inhibition of the standard antibiotic ampicillin (50 µg mL⁻¹)

RESULTS AND DISCUSSION

The synthesized pyrazoline derivatives were evaluated for their in vitro anti-bacterial activities. The Compounds **2a**, **2b** and **2c** showed the marked zone of inhibition between **24**, **14** and **27mm** against *S. aureus*

while standard drug Ampicillin registered **27mm**. Similarly, compound nos. **2a**, **2b** and **2c** registered zone of inhibition between **21**, **12** and **23mm** against *B. subtilis* while the standard drug exhibited **24mm**. The most promising compounds among all the synthesized compounds showing a maximum zone of inhibition are **2c (27mm)** against *S. aureus* and for *B. subtilis*, the compounds are **2c (23mm)**.

In case of Gram-negative bacteria, compound nos. **2a** and **2c** possess zone of inhibition **25** and **28mm** while the standard drug Ampicillin registered **22mm** against *E. coli*. Compounds **2b** shows **18mm** towards *E. coli*. Similarly, compound **2a** and **2c** showed zone of inhibition between **26 to 31mm**, at the same time the standard drug resulted zone of inhibition of **20mm** against *P.aeruginosa*. Compounds **2b** shows **14 mm** towards *P.aeruginosa*. The most promising compounds against gram-negative bacteria are **2a** and **2c** against *E. coli* and against *P. aeruginosa*.

Table 2: Antibacterial activity data in Zone of Inhibition

S. No.	Compound	Zone of Inhibit			
		<i>S. aureus</i>	<i>B. subtilis</i>	<i>E. coli</i>	<i>P. aeruginosa</i>
1	2a	24	21	25	26
2	2b	14	12	18	14
3	2c	27	23	28	31
5	Solvent	-	-	-	-
6	Ampicillin	27	24	22	20

CONCLUSIONS

Three new compounds 2a–c was synthesized under conventional and microwave irradiation conditions. In microwave irradiation method, the reactions were completed in shorter times with better yields compared to the conventional method. All the new compounds were screened for their antibacterial activities. It was observed that compounds 2a and 2c exhibited more antibacterial activity by comparing with standard ampicillin.

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