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Research Article

SYNTHESIS AND ANTIMICROBIAL ACTIVITY OF 4, 5-DIHYDROPYRAZOLINE DERIVATIVES

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ABSTRACT

In search of new potential antimicrobial agents, series of trisubstituted 4.5-dihydropyrazoline derivatives have been synthesized starting from chalcones. A mixture of 4-phenylbut-3-en-2-one (**1,** 0.001mmol), phenyl hydrazine hydrochloride (**2a-g** 0.001mmol) and sodium acetate (0.002mmol) in ethyl alcohol was stirred at room temperature for 1 hr to get trisubstituted 4,5-dihydropyrazoles (**3a-g**) in good yield. Spectral, X-ray diffraction and elemental analysis confirmed the structures of the products. The synthesised compounds have been evaluated in vitro for their antimicrobial activity. The results revealed that some compounds particularly with chloro substituents act as potential antimicrobial agents.

Keywords: Antifungal, Antibacterial, Crystallographic, Cyclisation, MIC.

INTRODUCTION

The synthesis ofpyrazoline and its analogues has been a subject of consistent interest because of the wide range of applications in the pharmaceutical and agrochemical industries.pyrazolines and its derivatives, a class of well known nitrogen containing heterocyclic compounds occupy an important position in medicinal and pesticide chemistry with diverse biological activities such as anti-inflammatory[1], analgesic[2], antagonist[3], antidepressant and anticonvulsant[4], hypoglycemic[5], antitumour[6], antioxidant[7], and antimicrobial[8]. The wide range of biological activities ofpyrazolines has made them popular synthetic targets. Therefore, extensive research efforts are continually directed at the discovery of new heterocycles with appropriate pharmacological effects.

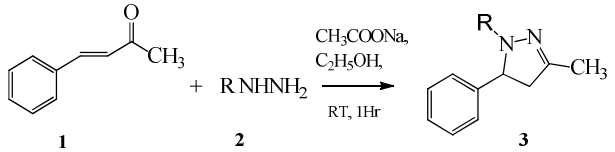
In view of broad spectrum of biological applications; and in continuation of our work onpyrazolines, we herein report the synthesis, characterization and antimicrobial activity of 4, 5-dihydropyrazolines derived from 4-phenylbut-3-en-2-one (Scheme-1).

MATERIALS AND METHODS

The chemicals used were purchased from Aldrich chemicals (India). Melting points were taken in open capillaries using Thomus Hoover melting point apparatus and are uncorrected. The ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker Supercon 400 MHz spectrophotometer in $CDCl_3$; chemical shifts are expressed in δ ppm. The coupling constant (*J*) is expressed in Hz. Mass spectra were obtained on Maspec MSW 9629 spectrophotometer. Elemental analysis was obtained on a Thermo Finnigan Flash EA 1112 CHN analyser. Chromatographic separations were carried out on silica gel (70-230 mesh, Merck) column using benzene: ethyl acetate (8:1 v/v) as eluent.

Synthesis

In a typical procedure, a mixture of 4-phenylbut-3-en-2-one (1, 0.001mmol), phenyl hydrazine hydrochloride (2a-g 0.001mmol) and sodium acetate in ethyl alcohol was stirred at room temperature for 1 h. The progress of the reaction was monitored by TLC. After the completion of the reaction, the mixture was poured into ice cold water. The solid formed was separated and crystallized with acetonitrile to get the title compounds (3a-g) in relatively good yield (Scheme-1).



1 2 3

3 a) R = H; b) R = C_6H_5; c) R = 2,4-(NO_2) C_6H_3; d) R = 4-(OCH_3) C_6H_4; e) R = 4-(Br) C_6H_4; f) R = 4-(Cl) C_6H_4; g) R = 4-(CH_3) C_6H_4.

Scheme-1

Antimicrobial activity

Synthesised compounds (**3a-g**) were evaluated for their antimicrobial activity (MIC) [9] against fungal species *C. albicans, A. niger, A. flavus* and bacteria species *E. coli, S. typhimurium, B. subtilis*. The antibiotics amphotericin B and ciprofloxacin were used as standard drugs against fungi and bacteria species respectively. DMSO is used as control. The experiments were carried out in triplicate; the results were taken as a mean of three determinations.

RESULTS AND DISCUSSION

^{1}H NMR, ^{13}C NMR, elemental analysis and X-ray diffraction crystallographic studies provide the structure proof for the products. The structural assignments were made by NMR analysis by considering compound (**3b**) as the representative compound. In its ^{1}H NMR spectra, Ha, Hb and Hc protons of the pyrazoline ring appeared as a doublet of doublet. The doublets of Ha appeared in the region 2.61-2.67 ppm; doublets of Hb appeared in the region 3.47-3.53 ppm; and that of Hc in the region 5.10-5.14ppm. Doublets of Ha and Hb are due to diastereotopic nature of methylene protons. Among Ha, Hb and Hc protons, Hc is the most deshielded due to its close proximity to benzene ring. Hc couples not only with Ha but also with Hb and appears as doublet of doublet instead of a triplet i.e., the methylene protons of pyrazoline ring (Ha and Hb) exhibited a typical ABX spin system with Hc as a doublet of doublets (Fig-1). Moreover, a collection of signal observed in the aromatic region 6.62-7.37 ppm is due to aromatic protons, C1 and C_5 protons of the pyrazoline ring.

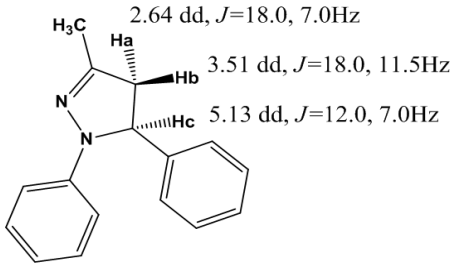


Fig. 1: Proton chemical shifs and couplings of 3b

Further, the structure of (**3b**) was confirmed by single crystal XRD study[10], which was depicted in ORTEP diagram (Fig-2). Analysis of torsion angles, asymmetry parameters and least-square plane calculation shows that it contains two benzene rings (C1-C6 and C8-C13), these two rings are attached to the central pyrazoline ring and the molecules are connected by non classical hydrogen bonds. The dihydropyrazole ring is a shallow envelope, with atom C7 displaced from the other four atoms by 0.298 (2) A. The dihedral angles between the four near coplanar atoms of the central ring and the N- and C-bonded phenyl groups are 13.49 (13) and 82.22 (16) ° respectively.

Fig. 2: ORTEP diagram of 3b with 50% probability ellipsoids

The mass spectrum of all the synthesised compounds showed M+1 molecular ion peak corresponding to its molecular formula which confirmed the formation of these compounds. In ^{13}C NMR spectrum of compound (3b), a signal at 15.99 ppm is assigned to methyl carbon attached to pyrazoline ring at C3. Two signals at 47.74 and 63.75 ppm are assigned to C4 and C5 respectively. One signal at 146.04 ppm is attributed to C3 carbon in the pyrazoline ring. A collection of signals appeared in the region 112.97-149.58 ppm which are ambiguously assigned to aryl carbons. The physical and analytical data; ^{1}H NMR and ^{13}C NMR chemical shifts of synthesised compounds (**3a-g**) are summarized in Table-1, Table-2 and Table-3 respectively.

Table 1: Physical data of Pyrazolines 3a-g

Compound	Molecular formula	Yield (%)	m.p ^0C	Mass	Elemental analysis[a]		
					C (%)	H (%)	N (%)
3a	$C_{10}H_{12}N_2$	58	60-62^{0}C	160.10	74.95	7.52	17.45
3b	$C_{16}H_{16}N_2$	62	90-92^{0}C	236.13	81.36	6.80	11.88
3c	$C_{16}H_{14}N_4O_4$	55	220-222^{0}C	326.10	58.90	4.32	17.15
3d	$C_{17}H_{18}N_2O$	51	138-140^{0}C	266.14	76.70	6.82	10.54
3e	$C_{16}H_{15}BrN_2$	54	98-100^{0}C	314.04	60.97	4.85	8.88
3f	$C_{16}H_{15}ClN_2$	56	101-103^{0}C	270.09	70.91	5.54	10.36
3g	$C_{17}H_{18}N_2$	59	120-122^{0}C	250.15	81.54	7.18	11.18

[a]Observed values; which are within ±0.02% from their theoretical values.

Table 2: Proton chemical shift values of pyrazolines 3a-g (δ ppm)

Compound	Ha	Hb	Hc	$-CH_3/OCH_3$	NH	Aromatic protons
3a	2.62(dd)	3.47 (dd)	5.11(dd)	1.98	6.9	6.61-7.35
3b	2.64 (dd)	3.51(dd)	5.13(dd)	2.01	-	6.62-7.37
3c	2.63 (dd)	3.48 (dd)	5.12(dd)	2.01	-	6.91-8.22
3d	2.56 (dd)	3.46(dd)	5.08(dd)	1.99/3.70	-	6.54-7.21
3e	2.65 (dd)	3.51(dd)	5.13(dd)	2.02	-	6.72-7.39
3f	2.61(dd)	3.50(dd)	5.14(dd)	2.02	-	6.71-7.36
3g	2.63(dd)	3.46(dd)	5.15(dd)	1.99&2.4	-	6.63-7.36

Table 3: ^{13}C NMR chemical shift values of pyrazolines 3a-g (δ ppm)

Compound	C-3	C-4	C-5	$-CH_3/OCH_3$	Aryl carbons
3a	61.62	39.91	46.82	15.81	126.90-142.54
3b	63.75	40.02	47.74	15.99	112.97-149.58
3c	63.81	40.10	48.18	16.20	112.81-150.37
3d	62.90	40.01	47.63	15.91/54.9	118.20-148.63
3e	63.91	41.18	47.90	15.73	112.91-149.64
3f	63.92	41.20	47.89	15.72	112.89-149.69
3g	63.81	40.91	47.72	15.63&21.07	112.54-145.54

The results of minimum inhibitory concentration (MIC) of the synthesised compounds (**3a-g**) against the fungi species *C. albicans*, *A. niger* and *A. flavus* are furnished in Table-4. The investigated compounds showed different degrees of antifungal activity in relation to the tested microbial species. The extent of antifungal activity depended on the microorganism and the type of functional groups present in the molecule. The activity is considerably affected by substituents present at the *para* position of phenyl ring. A close investigation of the *in vitro* antifungal activity profile of the trisubstitutedpyrazolines gives a clear picture of the structure activity correlations among the compounds (**3a-g**) under study. The compounds with halogen, methyl or methoxy function present at C-6 positions of phenyl ring exert varied range of antifungal activity while the compounds with no substituents or $-NO_2$ substituent on the phenyl groups did not exhibit significant antifungal activity. Substitution of halogen at the para position of phenyl groups has promoted the activity against *C. albicans* and *A. flavus*. However, this introduction did not show any improvement against *A. niger*. Similarly, compound which has methoxy group at *para* position only recorded a marked potency.

Table 4: MIC's of the synthesised compounds (3a-g) against fungi species

Compound	Minimum inhibitory concentration (MIC's) in µg/mL*		
	C. albicans	*A. niger*	*A.flavus*
3a	100	100	200
3b	100	**	200
3c	100	**	**
3d	100	100	100
3e	50	50	100
3f	25	100	50
3g	50	100	50
Amphotericin B	25	50	50

*Results are expressed as mean of three determinations (n=3); **No activity observed even at a concentration of 200 µg/mL.

The minimum inhibitory concentration (MIC) results of the synthesised compounds (**3a-g**) against the bacteria species *E. coli, S. typhimurium, B. subtilis* are summarized in Table-5. The test compounds exhibited different degrees of antibacterial activity in relation to the tested microbial species. The activity is considerably affected by substituents present at the *para* position of phenyl ring. The compound (**3a**) having only one aromatic ring substituent showed moderate activity against the organisms tested. The compounds (**3d**) and (**3f**) with halogen, methoxy function present at C-6 positions of phenyl ring exerted very good activity; The compounds (3b) and (3g) with no substitution and –CH3 substituent on aromatic ring exerted moderate activity. however the compound (3c) with –NO2 substituent on the aromatic ring was found inactive even at a higher concentration of 200µg/ml against all the organisms tested.

Table 5: MIC's of the synthesised compounds (3a-g) against bacteria species

Compound	Minimum inhibitory concentration (MIC's) in µg/mL*		
	E. coli	*S. typhimurium*	*B. subtilis*
3a	100	200	100
3b	50	50	100
3c	**	**	**
3d	25	50	25
3e	25	50	25
3f	25	50	25
3g	50	100	50
Ciprofloxacin	25	50	25

*Results are expressed as mean of three determinations (n=3); **No activity observed even at a concentration of 200 µg/mL.

CONCLUSION

In summary, a series of novel trisubstitutedpyrazolines have been synthesized in appreciable yields in an easy accessible method. Results of the antimicrobial activity reveal that some of the compounds particularly with chloro substituents act as potential antimicrobial agents. However, the structure-activity mode of action with the host cell remains of interest.

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