INTERNATIONAL JOURNAL OF RESEARCH IN PHARMACY AND CHEMISTRY

Available online at www.ijrpc.com

Research Article

SYNTHESIS AND ANTIMICROBIAL ACTIVITY OF SOME NEW 1,3,5-TRISUBSTITUTED-2-PYRAZOLINES

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ABSTRACT

A variety of 1, 3, 5-trisubstituted-2-pyrazolines (3a-k) were synthesized by reacting various chalcones with phenyl hydrazine hydrochloride. The required chalcones were prepared by condensation of 3-acetylthiophene with various substituted aromatic / hetero-aromatic aldehydes in the presence of alkali. All these compounds were characterized by IR, ^{1}H NMR and elemental analyses. The newly synthesized compounds were evaluated for their antimicrobial activity and some of them have shown significant activity when compared with the standard.

Keywords: 1,3,5-trisubstituted-2-pyrazolines, Antimicrobial activity.

INTRODUCTION

Considerable interest has been focused on the pyrazoline structure, which has been known to possess a broad spectrum of biological activities such as tranquilizing, muscle relaxant, psycho-analeptic, anticonvulsant, antihypertensive and antidepressant activities[1-6]. The discovery of this class of drugs provides an outstanding case history of modern drug development and also points out the unpredictability of biological activity from structural modification of a prototype drug molecule. Prodrug-based monoamine oxidase (MAO) inhibitors having hydrazide, hydrazine and amine moiety such as isocarboxazide[7], phenelzine[8], meclobemide[9,10] show prominent antidepressant activity in laboratory animals and humans. Additionally, tranylcypromine like MAO inhibitors mechanism-based inactivators and they are metabolized by MAO with one electron of the nitrogen pair to generate an imine, the other residing on a methylene carbon. The structures of synthesized compounds are very similar to that of isocarboxazide. Earlier studies by Parmar et al.[3] and soni et al.[4] demonstrated monoamine oxidase inhibitory activities of some 1, 3, 5-triphenyl-2-pyrazolines, 1-thiocarbamoyl-3,5-diphenyl-2-pyrazolines and bicyclic pyrazolines in behavioural despair test.[11-14] These observations prompted the authors to carryout the synthesis of some new 1, 3, 5-trisubstituted-2-pyrazolines (3a-k) which were also evaluated for their antimicrobial activity.

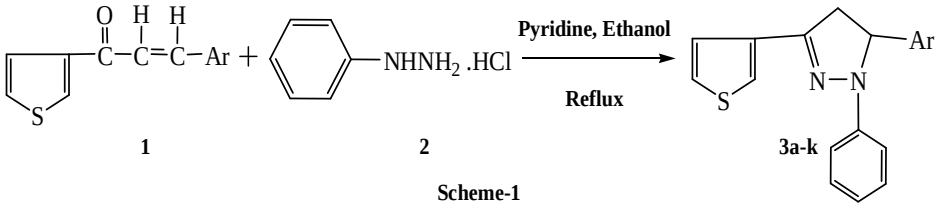
EXPERIMENTAL

Chemicals and solvents were of reagent grade and used without further purification. Melting points were determined on a capillary melting point apparatus and are uncorrected.^{1}H NMR spectra were recorded in the indicated solvent on Bruker WM 400 MHz spectrometer with TMS as internal standard. IR spectra were recorded in KBr on Perkin-Elmer AC-1 spectrophotometer. Microanalyses were performed on Carlo Erba EA-1108 element analyzer and were within the ± 0.4% of the theoretical values. Column chromatography was performed on silica gel (Merck, 60-120 mesh).

GENERAL PROCEDURE FOR THE PREPARATION OF PYRIMIDINES

A mixture of chalcones (3a-k) of 3-acetylthiophene (1 mmole), phenylhydrazine hydrochloride (1 mmole) in absolute ethanol (20 mL) and pyridine (0.3 mL) was added drop wise at room temperature. After that the mixture was refluxed for 5-6 h and the solvent was evaporated completely. The reaction

mixture was poured into ice -cold water and the solid mass that separated out was filtered, dried and purified by column chromatography with ethylacetate/ hexane and recrystallized from chloroform to give 1, 3, 5-trisubstituted-2-pyrazolines (3a-k) (Scheme 1). The chemical and spectral data of the compounds (3a-k) are given in Tables 1 and 2.



Scheme-1

Where **Ar**

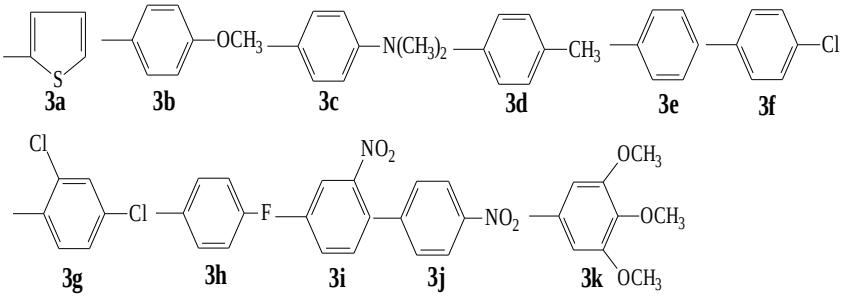


Table 1: Physical characterization data of the compounds (3a-k)

Compound	Ar	Molecular formula	M.p (°C)	Yield (%)
3a	2"-thienyl	$C_{17}H_{14}N_2S_2$ (C,H,N) [a]	210	71
3b	4"-methoxyphenyl	$C_{20}H_{15}N_2OS$ (C,H,N) [a]	214	68
3c	4"-dimethylaminophenyl	$C_{21}H_{21}N_3S$ (C,H,N) [a]	137	74
3d	4"-methylphenyl	$C_{20}H_{18}N_2S$ (C,H,N) [a]	172	68
3e	Phenyl	$C_{19}H_{16}N_2S$ (C,H,N) [a]	212	76
3f	4"-chlorophenyl	$C_{19}H_{15}ClN_2S$ (C,H,N) [a]	239	74
3g	2",4"-dichlorophenyl	$C_{19}H_{14}Cl_2N_2S$ (C,H,N) [a]	246	73
3h	4"-fluorophenyl	$C_{19}H_{15}FN_2S$ (C,H,N) [a]	227	68
3i	3"-nitrophenyl	$C_{19}H_{15}N_3O_2S$ (C,H,N) [a]	205	71
3j	4"- nitrophenyl	$C_{19}H_{15}N_3O_2S$ (C,H,N) [a]	231	72
3k	3",4"5"-trimethoxyphenyl	$C_{22}H_{22}N_2O_3S$ (C,H,N) [a]	237	71

[a] Elemental analyses for C, H,N are within ± 0.4% of the theoretical values

Table 2: Spectral data of the compounds (3a-k)

Compound	IR (KBr, cm^{-1})	^1H NMR ($CDCl_3$, ppm)*
3a	1594 (C=N), 1112 (C-N) and 706 (C-S)	3.25 (1H, dd, H_A), 3.80 (1H, dd, H_B), 5.50 (1H, dd, H_x) and 6.80-7.50 (11H, Ar-H)
3b	1642 (C=N), 1355 (C-N), 670 (C-S) and 1160 (-O-CH_3)	3.13 (1H, dd, H_A), 3.77 (1H, dd, H_B), 5.25 (1H, dd, H_x), 3.85 (3H, s, -OCH_3) and 6.70-7.62 (12H, Ar-H)
3c	1520 (C=N), 1080 (C-N) and 690 (C-S)	3.10 (6H, s, N $(CH_3)_2$), 3.21 (1H, dd, H_A), 3.80 (1H, dd, H_B), 5.50 (1H, dd, H_x) and 6.70-8.10 (12H, Ar-H)
3d	1648 (C=N), 1352 (C-N), 660 (C-S) and 1160 (-O-CH_3)	2.35 (3H, s, Ar-CH_3), 3.15 (1H, dd, H_A), 3.83 (1H, dd, H_B), 5.28 (1H, dd, H_x) and 6.80-7.50 (12H, Ar-H)
3e	1525 (C=N), 1078 (C-N), 698 (C-S) and 1160 (-O-CH_3)	3.16 (1H, dd, H_A), 3.88 (1H, dd, H_B), 5.29 (1H, dd, H_x) and 6.75-7.50 (13H, Ar-H)
3f	1595 (C=N), 1097(C-N), 689 (C-S) and 822(C-Cl)	3.18 (1H, dd, H_A), 3.85 (1H, dd, H_B), 5.30 (1H, dd, H_x) and 6.40-7.90 (12H, Ar-H)
3g	1645 (C=N), 1082 (C-N), 855 (C-Cl) and 716 (C-S)	3.05 (1H, dd, H_A), 3.91(1H, dd, H_B), 5.6 (1H, dd, H_x) and 6.70-7.60 (11H, Ar-H)
3h	1640 (C=N), 1080 (C-N), 870 (C-F) and 680 (C-S)	3.18 (1H, dd, H_A), 3.88 (1H, dd, H_B), 5.28 (1H, dd, H_x) and 6.80-7.45 (12H, Ar-H)
3i	1648 (C=N), 1080 (C-N), 1510 (N=O, asymmetric), 1335 (N=O, symmetric) and 660 (C-S)	3.15 (1H, dd, H_A), 3.86 (1H, dd, H_B), 5.35 (1H, dd, Hx) and 6.45-7.80 (12H, Ar-H)
3j	1594 (C=N), 1542 (N=O, asymmetric), 1336 (N=O, symmetric), 1105(C-N) and 693 (C-S)	3.15 (1H, dd, H_A), 3.85 (1H, dd, H_B), 5.25 (1H, dd, Hx) and 6.50-7.60 (12H, Ar-H)
3k	1640 (C=N), 1068 (C-N), 655 (C-S) and 1180 (-O-CH_3)	3.5 (1H, dd, H_A), 3.78 (1H, dd, H_B), 5.20 (1H, dd, Hx) and 6.45-7.60 (10H, Ar-H)
* s, singlet; dd, double doublet; d, doublet; m, multiplet		

ANTIMICROBIAL ACTIVITY

Cup plate method [15,16] using Mueller – Hinton agar medium was employed to study the preliminary antibacterial activity of (3a-k) against *B. subtilis, B. pumilis*, *E. coli* and *P. vulgaris*. The agar medium was purchased from Hi media Laboratories Ltd., Mumbai, India. Preparation of nutrient broth, subculture, base layer medium, agar medium and peptone waster was done as per the standard procedure. Each test compound (5 mg) was dissolved in 5 mL of dimethyl sulfoxide (1000 µg/mL). Volumes of 0.05 mL and 0.1 mL of each compound were used for testing.

Same cup plate method using PDA medium was employed to study the preliminary antifungal activity of (3a-k) against *A. niger* and *P. crysogenium*. The PDA medium was purchased from Hi media Laboratories Ltd., Mumbai, India. Preparation of nutrient broth, subculture, base layer medium and PDA medium was done as per the standard procedure. Each test compound (5 mg) was dissolved in 5 mL of dimethyl sulfoxide (1000 µg/mL). Volumes of 0.05 mL (50 µg) and 0.1 mL (100 µg) of each compound were used for testing. The cups each of 9 mm diameter were made by scooping out the medium with a sterilized cork borer in a petri dish, which was streaked with the organisms. The solutions of each test compound (0.05 mL and 0.1 mL) were added separately in the cups and petri dishes were subsequently incubated. Sparfloxacin and Fluconazole were used as standard (reference) drugs and dimethyl sulfoxide as a control which did not reveal any inhibition. Zone of inhibition produced by each compound was measured in mm and the results are presented in Tables 3 and 4.

Table 3: Antibacterial Activity of Pyrimidine Derivatives (3a-k)

Compound		Zone of inhibition (in mm)			
		B.subtilis	*B.pumilis*	*E.coli*	*P.vulgaris*
3a	A	8	9	7	9
	B	12	12	10	13
3b	A	10	9	8	9
	B	12	14	11	13
3c	A	11	12	11	13
	B	13	14	9	11
3d	A	10	9	8	9
	B	14	13	11	13
3e	A	11	10	9	9
	B	15	14	12	14
3f	A	13	10	13	10
	B	17	15	16	16
3g	A	14	12	12	11
	B	20	18	15	17
3h	A	15	14	14	12
	B	21	18	18	18
3i	A	10	9	8	9
	B	14	13	11	13
3j	A	12	11	13	11
	B	18	17	16	16
3k	A	11	12	10	13
	B	13	13	9	11
C		-	-	-	-
S (0.1 mL)	A	22	26	19	24
	B	25	28	24	26

A: 0.05 mL (50µg); B: 0.1 mL (100µg);
C: Control (DMSO); S: Standard (Sparfloxacin)

Table 4: Antifungal Activity of Pyrimidine Derivatives (3a-k)

Compound		Zone of inhibition (in mm)	
		A.niger	*P.crysogenium*
3a	A	8	8
	B	11	12
3b	A	9	9
	B	13	14
3c	A	11	13
	B	14	12
3d	A	11	8
	B	13	12
3e	A	10	10
	B	14	14
3f	A	8	9
	B	14	14
3g	A	11	10
	B	14	14
3h	A	9	8
	B	14	14
3i	A	9	9
	B	13	13
3j	A	8	10
	B	14	14
3k	A	12	13
	B	13	11
C		-	-
S (0.1 mL)	A	23	24
	B	28	27

A: 0.05 mL (50µg); B: 0.1 mL (100µg);
C: Control (DMSO); S: Standard (Fluconazole)

RESULTS AND DISCUSSION

Among the compounds tested for antibacterial activity, compounds **3h** and **3g** showed the highest inhibition zones, but not comparable to that of the standard drug at the dose levels tested. **3f**, **3j** also showed zones of inhibition, but less than those obtained for **3h** and **3g**.

From the results it is evident that a fluorine substituent present at *para* position on the phenyl ring (**3h**) enhanced the antibacterial activity and this was also observed in our study in case of substituted pyrimidines having this group. Hence pyrazolines with a phenyl group having number of fluorine substituents can be synthesized as such compounds likely to possess significant activity. Similarly compounds having fluorine substituent on the thiophene ring can also be synthesized and tested for its antibacterial activity. The study also reveals pyrazolines having the phenyl group substituted with other halogens can also be synthesized to have potent compounds.

Among the compounds tested for antifungal activity, **3h** exhibited the highest activity followed by **3g** and **3f**. In general, many of these compounds have not showed much antifungal activity when compared to fluconazole used as the standard. From the results it is evident that the compounds having halogens like chlorine (**3f**) and fluorine (**3h**) found to be more potent and hence compounds having number of these substituents on different positions of the phenyl ring can be synthesized in order to improve the activity further. Since, no studies in the present case were carried out with bromine as a substituent, such compounds having bromine at one or more positions of the phenyl ring can be synthesized to enhance the antifungal activity. Introduction of nitro group also contributed favorably to the antifungal activity (**3i**) and hence compounds with more nitro groups at different positions of the phenyl ring, if synthesized, may exhibit significant antifungal activity. Attempts can also be made to have the above substituents on the thiophene ring also in order to have a cumulative positive effect on the antifungal activity of the pyrazolines.

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