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Synthesis and Antibacterial Evaluation of some Pyrazoline-Piperazine merged Compounds

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ABSTRACT

Ten new 2-[4-(2, 6-dimethylphenyl) piparazine-1-yl-methyl]-3, 5-substituted phenyl-pyrazoline (3a-j) have been synthesized in 65-85% yield by a condensation of various chalcones with hydrazine hydrate. The structures of the synthesized compounds were confirmed by UV, IR, ^{1}H NMR and Mass spectral data. The Compounds Pyrazoline-Piperazine merged Compounds were evaluated for in vitro antibacterial activity.

Keywords: Pyrazoline, piperazine, synthesis, Spectral analysis, Antibacterial evaluation

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INTRODUCTION

Pyrazole derivatives have a long history of application in agrochemicals and pharmaceutical industry as herbicides and active pharmaceuticals. The recent success of pyrazole COX-2 inhibitor has further highlighted the importance of these heterocycles in medicinal chemistry. The emergence of drug - resistant pathogenic strains in recent years, e.g. Staphylococcus aureus, Entrococcus faecium, Pseudomonas aeruginosa, Stenotrophomonas maltophilia, Salmonella typhi, has been of major concern [1-4].

Number of pyrazoline derivatives has been found to posses considerable biological activities, which stimulated the research activity in this field. 2-Pyrazolines seem to be the most frequently studied pyrazoline type compounds. After the pioneering work of Fischer and Knövenagel in the late nineteenth century, the reaction of α,β-unsaturated aldehydes and ketones with hydrazines became one of the most popular methods for the preparation of 2-pyrazolines.[5-13].

One of the important applications of pyrazoline is the use of pyrazolines as a fluorescent brightening agent. They can absorb light of 300-400 nm and emit blue fluorescence.

Pyrazolines are also acting as whole transporting material in OELD (organic electroluminescent device) because of formation of p-л conjugated system due to one of the nitrogen atom. In the present communication, we report the reaction of different chalcone derivatives with phenyl hydrazine hydrochloride to form pyrozalines. The structures of various synthesized compounds were assigned on the basis of elemental analysis, IR and ^{1}H NMR spectral data. These compounds were also screened for anti bacterial activities.

MATERIAL AND METHODS

All the melting points were determined in open capillary method and are uncorrected. IR spectra were recorded on Perkin-Elmer 237 spectrometer. 1HNMR spectra on a Bruker Avance DPX400 MHz spectrometer with $CDCl_3$ as a solvent and TMS internal standard. The chemical shift values are expressed in part per million (ppm) downfield from the internal standard and signals are quoted as s (singlet), d (doublet), t (triplet) and m (multiplate). Purity of the compounds is checked by TLC plates (Merck) using benzene and ethyl acetate.

Experimental Section:

Preparation of various chalcones (1)

To a well stirred solution of Benzaldehyde (1.06 gm, 0.01 moles) and acetophenone (1.2 gm, 0.01 moles) in ethanol (25 ml), 40% KOH added till the solution become basic. The reaction mixture was stirred for 24 hrs. The contents were poured into ice, acidified, filtered and crystallized from ethanol.

Similarly, other substituted Chalcones have been prepared

Synthesis of pyrazoline derivatives from chalcones (2)

A mixture of Chalcones (2.08 gm, 0.01 moles) in 25 ml of absolute alcohol, add hydrazine hydrate (0.5 gm, 0.01 moles) was refluxed in water bath at temp. 80-90 ºC for 8 hrs. The reaction mixture was poured in to ice. The product was isolated and crystallized from ethanol.

Synthesis of 2-[4-(2, 6 dimethylphenyl) piparazine-1-yl-methyl]-3, 5-substituted phenyl-pyrazoline (3a-j).

A mixture of 3, 5-di phenyl -2H- Pyrazoline (0.01 mole) and formaldehyde (40%, 1.5 ml) in ethanol (20 ml) was stirred at room temp. With a solution of 1-(2, 6-dimethylphenyl) piparazine (0.01 mole) in ethanol (10 ml) for 30 min. The solid product that separated out on standing for a 1 hrs was collected by filtration, washed with ethanol & dried. It was recrystallized from ethanol to yield the compound (3a-j). Which were obtained in 65-85% yield. The analytical and spectral data of compounds (3a-j) are described.

IR spectra of 3, 5-substituted phenyl pyrazolines

Pyrazoline is a heterocyclic compound. The bands due to $-CH_2$ bridge are at nearer to 3100 cm^{-1}. The corresponding N-H in plane and out of plane bending vibrations occurs at 1630 and 699 cm^{-1} respectively. The other band due to aromatic segments at 3 and 5-position are appeared at their respective position. The other unknown bands are due to substitution in aromatic segments.

NMR spectra of 3, 5-substituted phenyl pyrazolines

The NMR spectra of all the compounds show the following common features, while individual ligand having additional signals is due to substitution on aromatic segment.

The signal at 5.7 ppm is responsible for $-CH_2$ bridge of pyrazoline, signal at 11.1 is responsible for N-H proton of pyrazoline, multiple signals between 6.15-7.8 ppm are responsible for aromatic proton. While signal at 2.5 and 3.7-3.9 are due to $-CH_3$ and $-OCH_3$ respectively.

CMR spectra of 3, 5-substituted phenyl pyrazolines

Besides the PMR spectroscopy, the CMR spectroscopy is now more précised method to determine the structure or organic molecules. Considerably greater sensitivity is required for

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^{13}C than for ^{1}H due to low natural abundance of ^{13}C and the lower magnetic moment compared to that of the proton. However, greater resolution is possible with ^{13}C.

The CMR spectra of all the compounds show the following common features, while individual ligand having additional signals is due to substitution on aromatic segment.

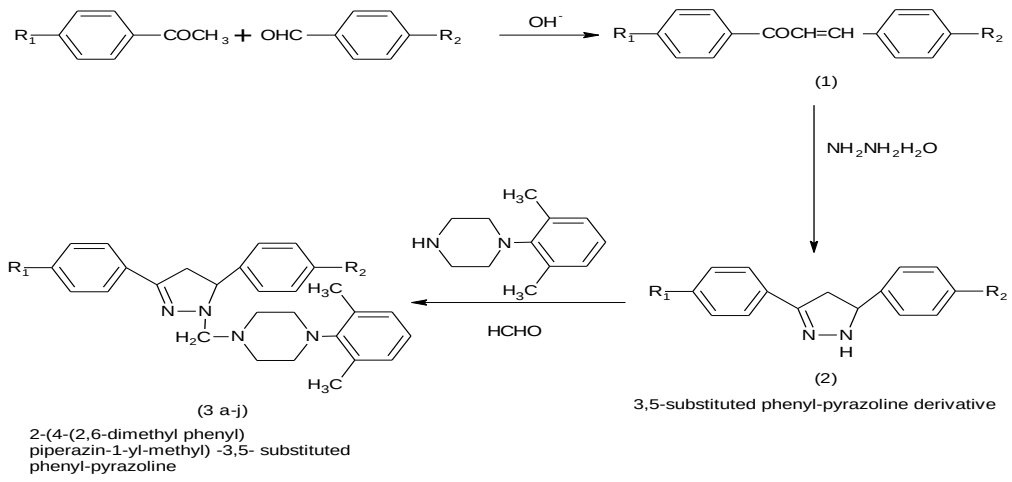
The signals at 135-148 ppm are responsible for $–CH_2$ bridge of pyrazoline, multiple signals between 114-130 ppm are responsible for aromatic segments. While signal at 21 and 56 are due to $–CH_3$ and $–OCH_3$ respectively.

Also LC-MS data of 2j compounds shows molecular ion peak at 303.8 which is consistence with theoretical molecular weight i.e 301.5 g/mole.

Spectral data of 2-[4-(2,6dimethylphenyl) piparazine-1-yl-methyl]-3,5-substituted phenyl-pyrazoline (3a-j).

IR: 3030,1500,1600 (Aromatic C-H stretching), 2850, 2920,1450 (-CH_2- of piperazine ring) **,** **NMR:** 7.1-8.84 (multiplet, aromatic), 3.47 (CH_2 linkage), 3.44-3.52(CH_2 of piperazine+CH_2 bridge), **^{13}CMR:** 136-145 (pyrazoline), 114-130 (benzene), 48 (piperazine)

Figure: Synthetic route of 2-[4-(2,6 dimethylphenyl) piparazine-1-yl-methyl]-3,5- substituted phenyl-pyrazoline



Where,

R_1= -H, -Cl

R_2= -H, -CH_3, -OCH_3, -Cl, -NO_2

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Table 1: Physical Constant of 1-(2,6-dimethylphenyl)piparazine-pyrazolines

Sr. No.	R_1	R_2	Molecular Formula	Molecular Weight gm/mole	M.P. ^{0}C	Yield %	C, H, N & Cl (Calc and Found)		
							C	H	N
3a	-H	-H	$C_{28}H_{31}N_4$	413	233-235	83	75.5	6.3	13.6
							75.4	6.2	13.5
3b	-H	$-CH_3$	$C_{27}H_{27}N_4$	478	260-262	80	67.8	5.6	11.7
							67.8	5.5	11.6
3c	-H	$-OCH_3$	$C_{27}H_{27}N_4O$	494	218-220	78	65.6	5.5	11.3
							65.5	5.4	11.2
3d	-H	-Cl	$C_{26}H_{24}N_4Cl$	498.5	222-224	78	62.6	4.8	11.2
							62.5	4.7	11.0
3e	-H	$-NO_2$	$C_{26}H_{24}N_5O_2Cl$	509	234-236	64	61.3	4.7	13.8
							61.2	4.6	13.6
3f	-Cl	-H	$C_{26}H_{24}N_4Cl$	498.5	227-230	67	62.6	4.8	11.2
							62.5	4.7	11.2
3g	-Cl	$-CH_3$	$C_{27}H_{26}N_4Cl$	512.3	222-224	63	63.2	5.1	10.9
							63.2	5.0	10.8
3h	-Cl	$-OCH_3$	$C_{27}H_{26}N_4OCl$	528.5	225-227	58	61.3	4.9	10.6
							61.3	4.8	10.5
3i	-Cl	-Cl	$C_{26}H_{23}N_4Cl$	533	232-233	63	58.5	4.3	10.5
							58.5	4.3	10.4
3j	-Cl	$-NO_2$	$C_{26}H_{23}N_5O_2Cl$	543.5	234-236	68	57.4	4.2	12.9
							57.2	4.2	12.8

RESULT AND DISCUSSION

Antibacterial activity of 2-[4-(2,6dimethylphenyl) piparazine-1-yl-methyl]-3,5-substituted phenyl-pyrazoline (3 a-j)

The study has been conducted according to the method adopted by Cruickshank et al. Nutrient agar broth was melted in a water bath and cooked to 45^{O}C with gentle shaking to bring about uniform cooling. It was inoculated with 0.5-0.6 ml of 24 hour old culture especially and mixed well by gentle shaking before pouring on the sterilized Petri dish (25 ml each). The poured material was allowed to set (1.5 hour) and there after the “cups” were made by punching into the agar surface with a sterile cork borer and sooping out the punched part of agar. Into this “cups” 0.1 ml of test solution (prepared by dissolving 10gm of sample in 10ml DMF) was added by sterile micropipette. The bacterial data are shown given in Table-3.

Ampicillin, Tetracycline, Gentamycine and Chloramphenicol were used as standard drugs and a solvent control was also run to know the activity of solvent. Activity of standards and inhibition due to DMF are given in Table-2. The results shown by compounds and standards are corrected for DMF.

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Table 2: Antimicrobial activity of Standards and Solvent (DMF)

No.	Name of compound	Zone of inhibition (in mm)			
		Gram positive		Gram negative	
		B.Subtillis	S.Aureus	E.Coli	Ps.Aeruginosa
1	DMF	6	6	6	6
2	Ampicillin	18	15	20	20
3	Tetracycline	21	20	16	24
4	Gentamycin	20	17	18	22
5	Chloramphenicol	18	25	18	23

Table-3 Antimicrobial activity of 2-[4-(2,6-dimethylphenyl) piparazine-1-yl-methyl 3,5-substituted phenyl-pyrazoline. (3 a-j)

Compound	Zone of Inhibition (in mm)			
	Gram positive		Gram negative	
	B.Subtillis	S.Aureus	E.Coli	Ps.Aeruginosa
3a	12	11	10	19
3b	18	14	13	13
3c	19	15	15	11
3d	23	19	17	18
3e	14	16	16	09
3f	15	12	10	11
3g	13	14	09	18
3h	14	16	15	13
3i	22	18	18	16
3j	10	11	09	11

CONCLUSION

The novel pyrazolines were synthesized by condensation of various chalcones with alcohol and hydrazine hydrate in ethanol. After that the compounds were purified by crystallization. The structures of the synthesized compounds were established on the basis of spectral data. Newly synthesized compounds of pyrazolines (3a to 3j) have been tested for their anti bacterial activity against gram positive bacteria B. subtillis, S. aureus, and gram negative bacteria P. aeruginosa and E.coil by the help of borer in agar medium and filled with 0.04ml (40µg) solution of sample in DMF. The compounds 3b, 3c, 3d and 3i were shown significant activities and compound 3a, 3e, 3f, 3g, 3h and 3j have shown moderate activity.

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