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Synthesis and antibacterial evaluations of (3,5-dimethyl-1*H*-pyrazol-4-yl)-phenyl-diazenes

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ABSTRACT

A series of (3,5-dimethyl-1H-pyrazol-4-yl)-phenyl-diazenes were prepared by condensing hydrazine hydrate with 3-phenylazo-pentane-2, 4 dione. The synthesized compounds were screened for their antibacterial activity against four types of bacteria. The (3,5-dimethyl-1H-pyrazol-4-yl)-(4-chloro-phenyl)-diazene showed strong activity against E.coli, B.cereus, P.vulgaris selectively.

Keywords: Diazines, anti-bacterial activity, *E.Coli, B. cereus, P. vulgaris, S. aureseus.*

INTRODUCTION

The pronounced synthetic utility of heterocycles in the area of pharmaceuticals [1-3], dyes and pigment[4], technology[5,6] and natural products[7], has always encouraged organic chemist to synthesize novel heterocyclic compounds. Pyrazole is one of the influenced heterocycles and its various derivatives have shown antitubercular[8], antimicrobial[9], antitumor[10] properties. In addition they also act as inhibitor of apoptosis[11] and COX-2 inhibitors[12]. They also possess important co-ordination property[13]. Various strategies employed for the synthesis of pyrazoles include1, 3-Dipolar cycloaddition of diazo compounds onto triple bond[14], cycloaddition of 6-nitroquinoline with aromatic hydrazones[15], biaryl coupling[16] and addition of hydrazines to 1,3-dicarbonyl compounds and α,β-unsaturated compounds[17-19]. Among these, addition of hydrazine to 1,3-dicarbonyl compounds is the most easily accessible route for the synthesis of pyrazoles.

The usage of most of the antimicrobial agents is limited, not only by the rapidly developing drug resistance, but also by the unsatisfactory status of present treatments of bacterial and fungal infections and drug side effects[20-23].This has spewed the scientists to develop the new antibacterial agents having broad antimicrobial spectrum.

In continuation of our research directed towards the development of biodynamic heterocyclic compounds [24], we report herein the two-step high yielding synthesis of Pyrazoles derivatives by condensation of 1,3 dicarbonyl compounds with hydrazine hydrate and their biological activities against various strains of bacteria.

EXPERIMENTAL SECTION

All the reagents were AR grade and used without further purification. The melting points of the compounds were taken in open capillary and are uncorrected. Infrared spectra were recorded on 1310 FT-IR spectrometer with KBr pellets. NMR and C^{13}NMR spectra were recorded on BRUKER AMX 300MHz in $CDCl_3$ using tetramethyl silane as an internal standard. Mass spectra were recorded on SHIMADZU GCQP-2010 and API 4000 by ion trap system.

General procedure for the synthesis of desired compounds (3a-f)
The equimolar mixture of substituted 3-phenylazo-pentane-2, 4 dione (5mmol), hydrazine hydrate (5mmol) in 10mL methanol was warmed on water bath till completion of the reaction as monitored by TLC. After completion of reaction, the mixture was poured over ice-cooled water, the formed precipitate was filtered and further purified by column chromatography using petroleum ether and ethyl acetate (9:1) as an elutant. All the compounds were fully characterized by IR, NMR, C^{13}-NMR and Mass spectroscopic methods.

Spectroscopic data for synthesized compounds:
(3,5-Dimethyl-1H-pyrazol-4-yl)-phenyl-diazene(3a)
IR (KBr) :3373, 3172 cm^{-1}. PMR ($CDCl_3$) δ 2.6(s, 6H, CH_3), 7.4(m, 3H, Ar-H), 7.8(q, 2H, Ar-H). ^{13}C NMR ($CDCl_3$) δ12.16, 100.03, 121.83, 128.91, 129.50, 134.72, 153.53. MS m/z 201.

(3,5-Dimethyl-1H-pyrazol-4-yl)-(4-Chloro-phenyl)-diazene(3b)
IR (KBr) 3209 cm^{-1} PMR ($CDCl_3$) δ 2.5 (s, 6H, CH_3), 7.45 (d, 2H, Ar-H),7.72 (d, 2H, Ar-H) ^{13}C NMR ($CDCl_3$) δ 12.38, 100.27, 123.08, 129.10, 134.68, 135.23, 151.98, MS m/z 235.05, 95.04 75.84.

(3,5-Dimethyl-1H-pyrazol-4-yl)-(4-methoxy-phenyl)-diazene (3c)
IR (KBr) 3382 cm^{-1}. PMR ($CDCl_3$) δ 2.5 (s ,6H, CH_3), 3.8(s, 3H, OCH_3),7.00(d, 2H, Ar-H), 7.8(d, 2H, Ar-H). ^{13}CNMR ($CDCl_3$) δ12.14, 55.52, 100.12, 114.03, 123.40, 147.84, 160.89, MS m/z 230, 123 (base peak), 95, 77, 65.

(3,5-Dimethyl-1H-pyrazol-4-yl)-(2-methoxy-phenyl)-diazene (3d)
IR (KBr) 3380 cm^{-1}. PMR ($CDCl_3$) δ 2.58 (6H, CH_3), 3.9(s, 3H, OCH_3), 7.04 (q, 2H, Ar-H), 3.1.5 (d, 2H, Ar-H) . ^{13}CNMR ($CDCl_3$) 12.03, 56.47, 99.99, 112.93, 116.29, 120.83, 130.75, 156.19, MS m/z 230.34, 97.08, 75.72.

(3,5-Dimethyl-1H-pyrazol-4-yl)-(4-methyl-phenyl)-diazene (3e*)*
IR (KBr) 3373,3172 cm^{-1}. PMR ($CDCl_3$) δ 2.45 (3H, CH_3), 2.61 (6H, CH_3), 7.28 (d, 2H, Ar-H),7.70 (d, 2H, Ar-H) . ^{13}CNMR ($CDCl_3$) δ 12.11, 21.36, 121.77, 129.54, 134.62, 139.74, 141.21, 151.61. MS m/z 214.35, 92.04.

(3,5-Dimethyl-1H-pyrazol-4-yl)-(4-hydroxy-phenyl)-diazene (3f)
IR (KBr) 3419, 3207 cm^{-1}.PMR ($CDCl_3$) δ 2.56 (6H, CH_3), (m,7.03, 2H Ar-H), 7.26 (q,1H, Ar-H), 7.8 (q, 1H, Ar-H), 12.6 (s, 1H, -OH). ^{13}CNMR ($CDCl_3$) δ 12.21, 100.03, 117.87, 119.74, 131.45, 131.56, 152.07. MS m/z 216.36.

RESULTS AND DISCUSSION

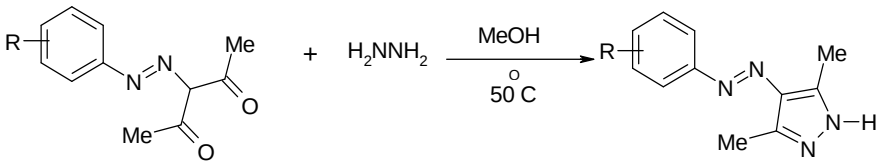
Chemistry

The key synthetic intermediates, 1,3-dicarbonyl compounds, 3-phenylazo-pentane-2, 4-diones used for synthesis of (3,5-Dimethyl-1H-pyrazol-4-yl)-phenyl-diazenes were prepared by the coupling of diazoniun salt with acetyl acetone in an alkaline medium[25] to get yellow to brown coloured compounds.

The diazocoupling reaction takes place between diazoniun salts and 1,3-diketone compounds. The diazonium salts of some weak aromatic amines have been found to be difficult to couple with acetyl acetone. However, aromatic amines possessing electron-donating groups give excellent yields. Hence, we have used amines possessing electron-donating group for the said reaction. It has been observed that the 1,3-diketones derived from coupling of diazonium salt of *para* substituted amines like *p*-chloroaniline, *p*-anisidine, *p*-toluidine have been given good yields. The results have been shown in Table1

The method reported for synthesis of (3,5-dimethyl-1H-pyrazol-4-yl)-phenyl-diazenein involves condensation of 3-phenylazo-pentane-2, 4-dione with hydrazine hydrate in automated micro channel reactor system[27]. Herein, we synthesized said pyrazoles by the condensation of equimolar quantities of substituted diketones (5mmol) with hydrazine hydrate (5mmol) at 50 ^oC in methanol. Progress of the reaction was monitored by TLC. After completion of reaction, the reaction mixture was poured over ice cold water to get the product. It offered (3,5-dimethyl1H-pyrazol-4-yl)-phenyl-diazenein in good yields (Scheme 1). The results have been shown in Table 1 .The reaction also proceeded at room temperature but yield was less as compared to the reaction at higher temperature. The NMR spectrum showed a singlet at 2.5 δ corresponding to pyrazole attached to two symmetrical methyl groups. Further, mass spectrum showed a mass peak at m/z 95 indicating ring closure has been takes place.

Scheme 1



1 **2** **3a-f**

Table 1

Compound	Time (min)	Yield (%)[a]	Mp ^0C	
			Obs.	Lit.
3a	70	84	140	143
3b	60	90	168	172
3c	60	80	175	-
3d	80	85	142	-
3e	60	80	157	157
3f	85	84	156	-
a- Yield refers to isolated products				

Antibacterial activity

The antibacterial activity of all the synthesized compounds were examined against *Escherichia coli* (NCIM2089), *Bacillus cereus* (NCIM2156), *Proteus vulgaris* (NCIM2027), *Staphylococcus aureseus* (NCIM5021) *via* Agar Well Diffusion Method. Initially, the solutions were prepared in DMF for a) 50 ppm, b) 80 ppm, c) 100 ppm, d) 200 ppm concentrations. All the tests were carried out by agar well diffusion method. In this method, the solution of the test compound for above concentrations was added in a cooled solidified agar layer for its antibacterial activity.

Agar Well Diffusion Method:

Well-sterilized molten agar measuring about 30mL was spread into sterilized Petri dish under aseptic condition in laminar flow. The suspension of each bacterium was prepared in sterile saline water and it was inoculated by spread plate technique. Sample-well was made by sterile borer. For each sample, there were four wells for 50, 80, 100, 200 ppm sample solution. With the help of micropipette, 50 µL solution of each concentration was added in the well and negative control was prepared in same solvent employed to dissolve test compounds. Tetracycline was used as the positive control. Plates were incubated at 37^0 C for 24 hr.

The antimicrobial activity of the synthesized compounds showed excellent activities against *Escherichia.coli* (NCIM2089), *Bacillus cereus* (NCIM2156), *Proteus vulgaris* (NCIM2027), *Staphylococcus aureseus* (NCIM5021). Among these, the pyrazole derived from 4-chloro (3-phenylazo-pentane-2, 4-dione) **3b** was found to be highly active against *Escherichia coli, Bacillus cereus, Proteus vulgaris* and they are comparable to standard. The zone of inhibition for the synthesized compound has been mentioned in Table 2.

The minimum inhibitory concentration (MIC) for the compound 5b was estimated by broth dilution assay by preparing concentration of the compound at 10 to 50 µg/mL.A set of tubes with above concentrations were in growth medium (LB broth).These tubes were then inoculated with the microorganisms, incubated for 18-24 h, and observed for growth of bacteria. The broth tubes that appears turbid, indicates bacterial growth while tubes remain transparent indicate no growth. The MIC of **3b** for *Escherichia coli, Bacillus cereus, and Proteus vulgaris* were reported as per result obtained. The MIC results have given in the Table 3.

Table: 2 Zone of inhibition in mm against *Escherichia coli, Bacillus cereus, Proteus vulgaris, Staphylococcus aureseus* for 50, 80, 100, 200 ppm concentrations

Entry	*E.Coli* Conc. in ppm				*P. Vulgaris* Conc. in ppm				*B.Cereus* Conc. in ppm				*S.aureus* Conc. in ppm			
	50	80	100	200	50	80	100	200	50	80	100	200	50	80	100	200
3a	15	15	17	19	-	-	-	8	-	-	-	11	-	-	-	11
3b	20	25	30	35	19	20	23	38	14	17	25	30	-	-	-	10
3c	-	12	19	20	14	18	20	22	12	18	20	22	-	-	-	-
3d	-	-	-	-	-	14	17	25	-	-	-	-	-	-	-	12
3e	-	-	-	-	-	-	-	07	-	-	17	19	-	-	-	-
3f	15	15	18	20	-	-	14	15	-	-	-	-	-	-	-	-
control	-				-				-				-			
Tetracycline	18				11				11				21			

Table: 3 MIC in ppm and its zone of inhibition (in mm) values for compound 3b for *Escherichia coli, Bacillus cereus, Proteus vulgaris* bacteria

Bacteria	MIC µg/ml
E. coli	14
B.cereus	19
P.vulgaris	17

SAR Study

The potential antibacterial activity showed by compound **3b** was highest among the synthesized derivatives. The electronic nature of chlorine is probably responsible for the potential antibacterial activity. On the contrary compounds **3d** and **3f** possessing electron donating methyl and methoxy groups almost resulted in medium activity. Thus electron-withdrawing nature of chlorine is likely to be active part for antimicrobial activity.

CONCLUSION

In this article, we uncover the potential antibacterial activity of above mentioned compounds, some of them are new. Among these (3, 5-Dimethyl1H-pyrazol-4-yl)-(4-Chloro-phenyl)-diazene was found to be effective against *Escherichia coli*, *Bacillus cereus*, *Proteus vulgaris* has been an applicable achievement of this article.

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