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SYNTHESIS CHARACTERIZATION AND BIOLOGICAL EVALUATION OF NOVEL 1-(5-((3,5-DIMETHYL-4-((4-(TRIFLUOROMETHYL)PHENYL)DIAZENYL)-1H-PYRAZOL-1-YL)METHYL)-2-METHYL-2-PHENYL-1,3,4-OXADIAZOL-3(2H)-YL)ETHANONE

***S.Murali Krishna, P.Jagadeeswara Rao, P.Ravisankar Reddy**

[1]*Santhiram College of Engineering & Technology, Nandyal-518501, A.P. India*
[2]*Biological E.Ltd Company, Shameerpet, Hyderabad*
Department of Chemistry

Abstract:
The article is aimed to synthesize, characterize and screening the biological activity of 1-(5-((3,5-dimethyl-4-((4-(trifluoromethyl)phenyl)diazenyl)-1H-pyrazol-1-yl)methyl)-2-methyl-2-phenyl-1,3,4-oxadiazol-3(2H)-yl)ethanone 8(a-f). 1-Chloro-2-phenyldiazene and pentane-2,4-dione were dissolved in DMF. To this reaction mixture anhydrous K_2CO_3 was added and the reaction mixture was stirred at room temperature(35^0C) for 8 hours to afford 3-(phenylamino)pentane-2,4-dione**.** To this reaction mixture hydrazine hydride, chloroethylacetate, acetophenone, EtoH and three drops of acetic acid was added and then heated on a steam bath for 5-6 hrs to obtain 2-(3,5-dimethyl-4-((4-(trifluoromethyl)phenyl)diazenyl)-1H-pyrazol-1-yl)-N'-(1-phenylethylidene)acetohydrazide compound(7). Finally compound 7(a) is treated with acetic anhydride to obtain target molecule 1-(5-((3,5-dimethyl-4-((4-(trifluoromethyl)phenyl)diazenyl)-1H-pyrazol-1-yl)methyl)-2-methyl-2-phenyl-1,3,4-oxadiazol-3(2H)-yl)ethanone. The structure of these newly synthesized compounds were characterised by ^{1}H NMR,^{13}CNMR ,Mass ,IR, and elemental analysis. The antimicriobial activity of the novel compounds was screened by agar disc diffusion method.
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KEYWORDS: Antibacterial activity, Antifungal activity, DMF acetic anhydride,1,3,4 oxadiazole, pyrazole

INDRODUCTION

Hetero cyclic compounds represents an important class of biological molecules.The heterocyclic molecules which ,posses indole,1,3,4 oxadiazole and pyrazole moieties exhibit wide range of biological activities.Pyrazoles are one of the most important alkaloids molecules found extensively in biological systems, which play vital role in many of the biochemical process. Pyrazole ring constitutes an important basic skelton and development of the drug.The classical indole drugs are N-cyclohexyl-5-phenyl-1-(quinolin-2-yl)-1H-pyrazole-4-carboxamideand N,N-diethyl-5-phenyl-1-(quinolin-2-yl)-1H-pyrazole-4-

carboxamide Pyrazole derivetives found to posses highwhichincludes,antibacterial,analgesic,antipyretic,antifungal,antiflamatory,anthelmintic,cardiovascular,anticonvalsant activities [1-5]..

Among the five member heterocyclic compounds, 1,3,4-oxadizoles has become an important synthon for the development new therapeutic agents. Compounds with 1,3,4-oxadiazole core substantiate for broad spectrum of biological activities including antimicrobial[6]., antifungal[7]., antiinflammatory[8]., anticonvulsant[9].,antioxidant, analgesic[10]. and mutagenic acctivity[11].. Compounds containing quinoline moiety are most widely used as antimalarials[12]., antibacterials[13]., antifungals[14]., anticancer agents[15]. and potential HIV-1 integrase inhibitors[16-17].

MATERIALS AND METHODS

Melting points were determined on open capillaries using a cintex melting point apparatus .T.L.C. analysis were performed on precoated silicagel (E-Merck Kieselgel 60 F_{254}) plates and visualisation was done by exposing to iodine vapour .Solvent were purified by standard procedures before use .Column chromatography was conducted by using Silica gel with different solvent systems as elutes. IR Spectra were recorded KBr on perkin –elmer spectrum BX series FTIR spectrometer.H^1-NMR spectrum were recorded on varian zemini 300MHz and 200MHz spectrometers using TMS as internal standard(chemical shifts in & ppm) C^{13}NMR spectra were recorded on a brucker 75MHz spectrometer . Mass spectra were scanned on a varian MATCH -7 and jeol JMSD-300 mass spectrometer at 70 ev.elemental analysis were carried out on carloerba 106 and perkin –analyser . All the chemicals used in the present investigation were purchased from Aldrich chemicals ;U.S.A

EXPERIMENTAL SECTION:

Synthesis of 3-(phenylamino)pentane-2,4-dione(3a):

A mixture of 1-chloro-2-phenyldiazene ,anhydrous K_2CO_3, pentane-2,4-dione and DMF were stirred at room temperature for 8 hours. The reaction mixture was diluted with ice cold water. The separated solid was identified as 3-(phenylamino)pentane-2,4-dione (3a):

Synthesis of 3,5-dimethyl-4-(phenyldiazenyl)-1H-pyrazole(4a):

A solution of 3(a) (0.01mol) and hydrazine hydrate (0.015) in ethanol (20ml) was refluxed for 5 hours.The reaction mixture was cooled and poured in to ice cold water with stirring.The separated solid was filtered, washed with water and recrystalised from ethanol.

ethyl 2-(3,5-dimethyl-4-(phenyldiazenyl)-1H-pyrazol-1-yl)acetate(5a):

An equimolar mixture of ethyl 2-(3,5-dimethyl-4-(phenyldiazenyl)-1H-pyrazol-1-yl)acetate(5a)and chloro ethyl acetate were dissolved in dimethyl formamide solvent and to this reaction mixture anhydrous K_2CO_3 was added and the reaction mixture was stirred at room temperature(35^0C) for 8 hours and the progress of the reaction was monitered by TLC using cyclohexane and ethylacetate solvent mixture (7:3) as eluent the reaction mixture was kept over night. After completion of the reaction the solvent was evaporated on rota-evaporater. The gummy solid was seperated and it was recrystalised from -2-propanol-petrolium ether(80^0c)solvent mixture.The crystaline solid was found to be ethyl 2-(3,5-dimethyl-4-(phenyldiazenyl)-1H-pyrazol-1-yl)acetate(5a) with a yield of 75% and mp 143-145^0C.

2-(3,5-dimethyl-4-(phenyldiazenyl)-1H-pyrazol-1-yl)-N'-(1-phenylethylidene)acetohydrazide(7a):

To the solution of 2-(3,5-dimethyl-4-((4-(trifluoromethyl)phenyl)diazenyl)-1H-pyrazol-1-yl)acetohydrazide 6(a) (0.01mole) in hot methanol (25ml), acetophenone(0.01) and a drop of

glacial aceticacid were added.The solid that seperated on refluxing for 3hours was filtered wash with cold methanol and recrystalised from methanol to give 7(a).M.P.236^{0}C,yield 84%.

The IR(KBr) spectrum of 2-(3,5-dimethyl-4-(phenyldiazenyl)-1H-pyrazol-1-yl)-N'-(1-phenylethylidene)acetohydrazide(7a)was recorded in the range 4000-667cm^{-1} and the absorption signals were found at 3100(-NH),3075(√-Ar-H)**,** 2850 and 2958 (√ aliphatic CH_2 andCH_3), 1725 (√ CO of ester group), 1610(C=N) , and 1155(√ C-O-C of ester group).

^{1}H NMR spectra(300MHZ,$(CD)_2$ SO,TMS): ; 2.53(s,3H,attached to phenyl ring),2.52(s,3H,CH_3 attached to pyrazole ring), 4.90 (s,1 H,-N-NH), 3.98(s,2H,-N-CH_2) 3.55(s,2H,-N-CH_2), 7.15 -8.35 (m,9H due to 9H of two phenyl rings).

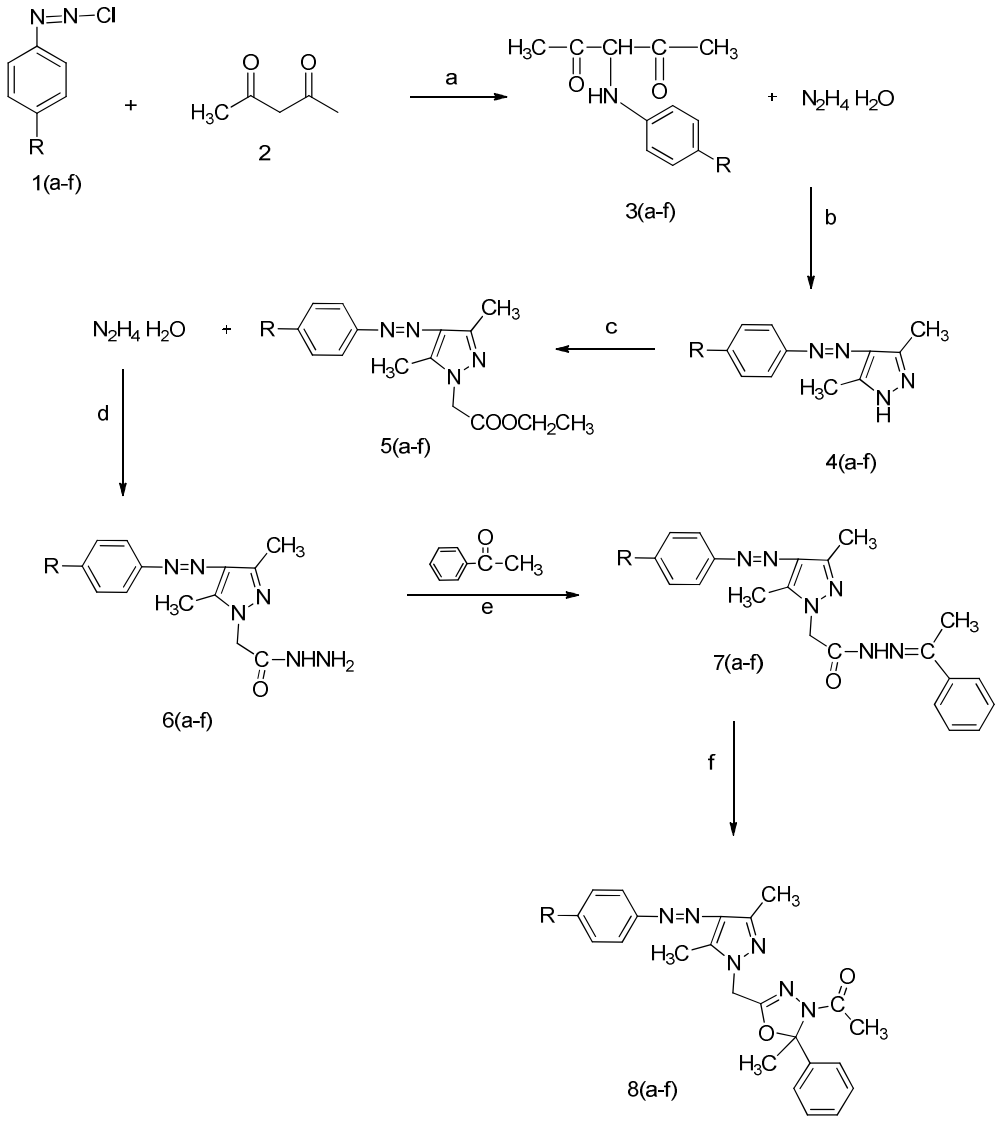
1-(5-((3,5-dimethyl-4-(phenyldiazenyl)-1H-pyrazol-1-yl)methyl)-2-methyl-2-phenyl-1,3,4-oxadiazol-3(2H)-yl)ethanone8(a):

A mixture of 3(a) (0.01mole) and excessive acetic anhydride (10ml) was refluxed for two hours.

The excessive acetic anhydride was distilled off and the residue was poured in to crushed ice. The solid thus obtained was filtered , washed with water and recrystalised from aqueous methanol to furnished obtained compound. M.P.185^{0}C, yield 58 %

The IR(KBr) spectrum of 1-(5-((3,5-dimethyl-4-(phenyldiazenyl)-1H-pyrazol-1-yl)methyl)-2-methyl-2-phenyl-1,3,4-oxadiazol-3(2H)-yl)ethanone8(a)was recorded in the range 4000-667cm^{-1} and the absorption signals were found at 3198(-NH),3045(√-Ar-H)**,** 2975 and 2958 (√ aliphatic CH_2 andCH_3), 1755 (√ CO of ester group), 1640(C=N) , and 1195(√ C-O-C of ester group).

^{1}H NMR spectra(300MHZ,$(CD)_2$ SO,TMS): ; 2.23(s,3H,attached to phenyl ring),2.42(s,3H,CH_3 attached to pyrazole ring), 3.78(s,2H,-N-CH_2) 2.46(s,3H,-CO-CH_3),3.77(s,2H,-N-CH_2), 7.2 -8.5 (m,9H due to 9H of two phenyl rings), 2.55(s,3H,attached to 1,3,4 oxadiazole ring).



	7a	7b	7c	7d	7e	7f
Comp	8a	8b	8c	8d	8e	8f
R	-H	$-CH_3$	$-OCH_3$	-Cl	$-NO_2$	$-CF_3$

Characterization of above compound

COMPOUND	YIELD	M.P.O^{0}C	% of Analysis					
			C		H		N	
			Calcd	FOUND	Calcd	FOUND	Calcd	FOUND
8a	**58%**	**185**	**58.33**	**57.31**	**3.70**	**3.73**	**19.44**	**19.43**
8b	**55%**	**190**	**59.19**	**59.17**	**4.06**	**4.03**	**18.83**	**18.82**
8c	**53%**	**180**	**57.14**	**57.13**	**3.89**	**3.92**	**18.17**	**18.18**
8d	**52%**	**182**	**53.73**	**53.68**	**54.07**	**54.01**	**18.02**	**18.00**
8e	**56%**	**185**	**52.83**	**52.82**	**3.14**	**3.17**	**20.53**	**20.54**
8f	**51%**	**180**	**52.80**	**52.79**	**3.00**	**3.02**	**16.80**	**16.79**

Anti-Bacterial Activity

The anti bacterial activity of synthesized compounds was studied by the disc diffusion method against the following pathogenic organisms. The gram-positive bacteria screened were staphylococcus aureus NCCS 2079 . The gram negative bacteria screened were Escherichia coli NCCS 2065 and pseudomonas aeruginosa NCCS 2200.

The synthesized compounds were used at the concentration of 250 µglml and 500µglml using DMSO as a solvent the Ciprofloxacin 10µglml disc was used as a standard .(Himedia,Laboratories Ltd, Mumbai).

The test results presented in the table -1, suggest that 8b,8d,8e exhibit high activity against the tesed bacteria, the rest of the compounds were found to be moderate active against the tested microorganisms.

Antifungal activity

Compounds were treated at the concentrations of 500µglm and 1000µglml using DMSO as solvent. The standard used was Cyclopiroxolamine 50µglml against both organisms.The test results were presented in the table-2.

TABLE.1- Antibacterial activity by disc diffusion method of pyrazole linked 1,3,4 oxadiazole 8(a.f).

Compound	Zone of inhibition (mm)			
	E.Coli	Staphylococcus	Klebsiella	Pseudomonas aeruginosa
8a	7.5(18)	7.5(20)	7.5(18)	7.5(18)
8b	14(15)	14(15)	14(18)	8(18)
8c	11(10)	-	-	12.5(15)
8d	13(14)	-	7.5(12)	-
8e	14(15)	-	7.5(11)	-
8f	8(18)	8(16)	7.5(18)	-
Ciprofloxacin	6.25(30)	6.25(30)	6.25(27)	6.25(28)

Table-;2 Antifungal activity by disc diffusion method for pyrazole linked 1,3,4 oxadiazole 8(a.f).

Compound	Zone of inhibition (mm)	
	Penicillium	Trichophton
8a	7.5(18)	7.5(18)
8b	13(15)	13(11)
8c	13(10)	-
8d	13(15)	-
8e	13(12)	-
8f	7.5(16)	7.5(18)
Cyclopiroxolamine	7.5(27)	3.12(30)

CONCLUSIONS:

1. ***Further more the substitution with phenyl group having a chloro group at p-position showed better activities.***
2. ***The pyrazole derivatives showed better antibactirial and antifungal activities.***
3. ***1,3,4 oxadiazoles and its derivatives were found to play an important role in medicinalchemistry as herbicidal, fungicidal, bacterial, antiflammatory***.

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