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Synthesis, anti microbial screening and cytotoxic studies of some novel pyrazole analogs

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ABSTRACT

The objective of the study was to synthesize novel pyrazole analogs and evaluate their antimicrobial, anthelmintic and cytotoxic activity. Novel (Z)-1-(4-chlorophenyl)-2-(3,5-dimethyl-4-(substituited phenyldiazenyl)-1H-pyrazol-1-yl)ethanone derivatives were synthesized by conventional method using potassium carbonate in DMF, under reflux condition. The antibacterial study was carried out against gram positive and gram negative bacteria using ciprofloxacin and norfloxacin as standard drugs. Antifungal activity was compared against fluconazole and evaluated using *Candida albicans* (MTCC 227) and *Aspergillus niger* (NCIM 1056). Anthelmentic activity assay was carried out on Indian earthworm, *Pheretima posthuma*. *In vitro* cytotoxicity studies were also carried out using Dalton's lymphoma ascites cells (DLA) and Ehrlich ascites carcinoma cells (EAC). Results of antibacterial study indicated that the compound 22 showed most promising activity against both Gram-positive and Gram-negative organisms. Compound 21 and 24, exhibited relatively good inhibitory profile against Gram negative organism. In case of compounds 25,27-30 exhibited equally potent antifungal activity as that of the standard drug fluconazole. Moreover, compound 21-24,26 showed excellent activity against *C. albicans* and *A.niger*. The *in vitro* cytotoxicity results and anthelmintic reports indicated compound 22 exhibited promising activity among the tested analogs. These newly synthesized pyrazole analogs, especially 21, 22 and 24 are better scaffolds to develop as broad spectrum chemotherapeutic agents.

INTRODUCTION

Pyrazoles with several sites of alteration offer the flexibility to build up various structural analogs of biomedical concern. Compounds incorporating the pyrazolyl structural unit have shown significant biological activity, which include anti-inflammatory and antipyretic (Rostom *et al*., 2009), antimicrobial (Javed and Hassan, 2013; Shukla *et al*., 2013), anthelmintic activity (Bruce, 2005 ; Wada *et al*., 2001) , antiviral (Rashad *et al*., 2008 ; Mowbray *et al*., 2009) anticancer (Zhang *et al*., 2011), anticonvulsant (Farghaly *et al*., 2014), hypoglycemic (Das *et al*., 2008) carbonic anhydrase inhibitory (Balseven *et al*., 2013), and MAO inhibitory (Kumar *et al*., 2013) activity. It has been found that, 4-arylpyrazole derivatives exhibited unique role due to its antibacterial (Raimondi *et al*., 2012), analgesic (Oru *et al*., 2006), anticancer and DNA photocleavage activities (Kumar *et al*., 2014). A recent report showed that novel (E)-1-aryl-2-(3, 5-dimethyl-4-(aryldiazenyl)-1H-pyrazol-1-yl) ethanones exhibited antimicrobial, antioxidant and UV-mediated DNA damage protective activity (Kaur *et al*., 2015). Moreover, pyrazole analogs exhibiting promising anticancer activities, especially on Ehrlich ascitic carcinoma cells (EAC) (Sunil *et al*., 2013) and on Dalton lymphoma acities cell lines (Ravula *et al*., 2016). Prompted from the above facts, we have synthesized some novel aryl azo pyrazoles using conventional methods using potassium carbonate in DMF under reflux condition . In order to explore biological potential of these novel pyrazole analogs, antibacterial, antifungal, anthelmintic and cytotoxicity activity of the synthesized compounds were carried out.

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MATERIAL AND METHODS

All the reagents used in this study were either of GR grade or of AR grade. They were purchased from commercial suppliers (Sigma Aldrich Company USA, Merck India Ltd,Himedia chemicals). The purity of the final compounds was checked by the TLC method. It was performed on Merck silica gel 60 F254 aluminium sheets using ethyl acetate: hexane (2:8) as eluents.

Iodine chamber and UV chamber used for the visualization of TLC spots. Melting points of compounds were determined on an optimelt automatic apparatus and were uncorrected.FT-IR spectra were recorded on SHIMADZU FT/IR 8400 and were reported in cm^{-1}.^{1}H-NMR spectra were recorded on Bruker advance digital spectrometer 400MHz.Chemical shifts are expressed in δ values (ppm) relative to TMS as an internal standard, using $CDCl_3$ solvent.

The mass spectra were recorded on a Jeol SX-106 instrument. Different steps involved in the synthesis of the final compounds are enumerated in Figure 1. Structures of the final compounds were confirmed using FT-IR, ^{1}H NMR and Mass,elemental analysis.

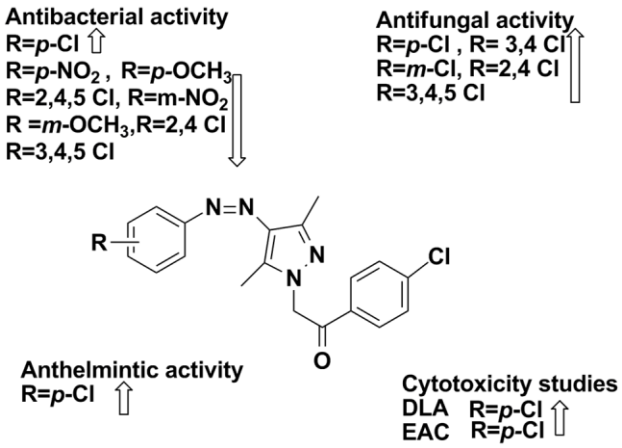


Fig. 1:

EXPERIMENT

Method of synthesis

General procedure for the synthesis of 3-(2-(substituited phenyl)-hydrazono)-pentane-2,4-dione (1- 10)

Intermediates are prepared by the reported procedures (Kaymakçıoğlu *et al*., 2005).Appropriate substituited anilines (0.01 mol) was dissolved in a mixture of conc.HCl (1:2) and water and cooled to 0 ^{0}C on ice bath.A cold aqueous solution of sodium nitrite (0.02mol,1.38) was added.The cold diazonium salt solution was filterd into a cooled solution of acetyl acetone (2.1 ml) in presence of sodium nitrite (0.01mol),0.69) and sodium acetate (0.05mol) in ethanol/methanol (20ml) and stirred room temperature for 2hrs and resulting solid was filtered, dried and purified by recrystallization from ethanol to afford the compound.

General procedure for the synthesis of (Z)-3,5-dimethyl-4-(substituited phenyldiazenyl)-1H-pyrazole (11-20)

A mixture of 3-(2-(substituited phenyl)-hydrazono)-pentane-2,4-dione (1g,0.003mole) and hydrazine hydrate (0.003mole) was heated under reflux in ethanol for 26hr.The reactions was moniterd by TLC using (chloroform: methanol, 8:2).The reaction mixture was cooled and the solids obtained were filterd and washed with water.The compounds were recrystallized from ethanol.

General procedure for the synthesis of (Z)-1-(4-chlorophenyl)-2-(3,5-dimethyl-4-(substituited phenyldiazenyl)-1H-pyrazol-1-yl)ethanone (21-30)

A mixture of (Z)-3,5-dimethyl-4-(substituited phenyldiazenyl)-1H-pyrazole (0.004mol) , 4- chloro phenacyl bromide(0.004mol) and anhydrous potassium carbonate (50mg) was heated under reflux in DMF for 20hrs.After completing the reaction, mixture was poured into ice cold water with stirring to yield the precipitate, which was further recrystallized from ethanol.

Synthesis of (Z)-1-(4-chlorophenyl)-2-(4-((3-chlorophenyl) diazenyl)-3,5-dimethyl-1H-pyrazol-1-yl)ethanone (21)

Yield: 80%, m.p : 172°C ,IR (KBr) (cm^{-1}): 1690 (C=O str), 1553 (C=C str), 1592 (C=N str), ^{1}H NMR ($CDCl_3$,400MHz) 7.29-7.99 (m,8H,Ar-H), 5.52 (s,2H,CH_2), 2.53-2.59 (m,6H,3,5 CH_3) EI-MS : 387.20(M+1) Anal. Calcd for C_{19} H_{16} Cl_2 N_4O : C,58.93; H,4.16; N, 14.47.Found C,58.01; H,4.14; N, 14.07.

Synthesis of ((Z)-1-(4-chlorophenyl)-2-(4-((4-chlorophenyl) diazenyl)-3,5-dimethyl-1H-pyrazol-1-yl)ethanone (22)

Yield:82%, m.p : 178 °C ,IR (KBr) (cm^{-1}): 1690 (C=O str), 1550 (C=C str), 1604 (C=N str), ^{1}H NMR ($CDCl_3$,400MHz) 7.28-7.99 (m,8H,Ar-H), 5.52 (s,2H,CH_2), 2.52-2.59 (m,6H,3,5 CH_3) EI-MS : 387.26(M+1) Anal. Calcd for C_{19} H_{16} Cl_2 N_4O : C,58.93; H,4.16; N, 14.47.Found C,58.11; H,4.12; N, 14.17.

Synthesis of (Z)-1-(4-chlorophenyl)-2-(4-((2,4-dichlorophenyl) diazenyl)-3,5-dimethyl-1H-pyrazol-1-yl)ethanone (23)

Yield: 75 %, m.p : 186 °C ,IR (KBr) (cm^{-1}): 1690 (C=O str), 1552 (C=C str), 1582 (C=N str), ^{1}H NMR ($CDCl_3$,400MHz) 7.32-8.03 (m,7H,Ar-H), 4.90 (s,2H,CH_2), 3.04-3.07 (m,6H,3,5 CH_3)EI-MS : 421.10(M+1) Anal. Calcd for C_{19} $H_{15}Cl_3$ N_4O : C,54.11; H,3.59; N, 13.29.Found C,54.41; H,3.24; N, 13.27

Synthesis of (Z)-1-(4-chlorophenyl)-2-(4-((3,4-dichlorophenyl) diazenyl)-3,5-dimethyl-1H-pyrazol-1-yl)ethanone (24)

Yield:70%, m.p: 180 °C ,IR (KBr) (cm^{-1}): 1690 (C=O str), 1550 (C=C str), 1599 (C=N str), ^{1}H NMR ($CDCl_3$,400MHz) 7.33-8.03 (m,7H,Ar-H), 4.91 (s,2H,CH_2), 3.05-3.07 (m,6H,3,5 CH_3) EI-MS : 421.70(M +1) Anal. Calcd for C_{19} $H_{15}Cl_3$ N_4O : C,54.11; H,3.59; N, 13.29.Found C,54.01; H,3.14; N, 13.07

Synthesis of (Z)-1-(4-chlorophenyl)-2-(3,5-dimethyl-4-((2,4,5-trichlorophenyl)diazenyl)-1H-pyrazol-1-yl)ethanone (25)

Yield:76%, m.p : 170 °C ,IR (KBr) (cm^{-1}): 1688 (C=O str), 1552 (C=C str), 1594 (C=N str), ^{1}H NMR ($CDCl_3$,400MHz) 7.28-7.63 (m,6H,Ar-H), 4.90 (s,2H,CH_2), 2.37-2.77 (m,6H,3,5 CH_3) EI-MS : 456.10(M+2) Anal. Calcd for $C_{19}H_{14}Cl_4N_4O$: C,50.03; H,3.09; N, 12.28.Found C,50.01; H,3.14; N, 12.07

Synthesis of (Z)-1-(4-chlorophenyl)-2-(3,5-dimethyl-4-((3,4,5-trichlorophenyl)diazenyl)-1H-pyrazol-1-yl)ethanone (26)

Yield:70%, m.p : 174 °C ,IR (KBr) (cm^{-1}): 1686 (C=O str), 1552 (C=C str), 1554 (C=N str), ^{1}H NMR ($CDCl_3$,400MHz) 7.30-7.63 (m,6H,Ar-H), 4.99 (s,2H,CH_2), 2.34-2.77 (m,6H,3,5 CH_3) EI-MS : 456.20(M+2) Anal. Calcd for $C_{19}H_{14}Cl_4N_4O$: C,50.03; H,3.09; N, 12.28.Found C,50.41; H,3.24; N, 12.17

Synthesis of (Z)-1-(4-chlorophenyl)-2-(3,5-dimethyl-4-((3-nitrophenyl)diazenyl)-1H-pyrazol-1-yl)ethanone (27)

Yield:76%, m.p : 188°C ,IR (KBr) (cm^{-1}): 1688 (C=O str), 1553 (C=C str), 1594 (C=N str), ^{1}H NMR ($CDCl_3$,400MHz) 7.51-8.33 (m,8H,Ar-H), 5.52 (s,2H,CH_2), 2.54-2.59 (m,6H,3,5 CH_3) EI-MS : 397.21(M^+) Anal. Calcd for $C_{19}H_{16}ClN_5O_3$: C,57.36; H,4.05; N, 17.60.Found C,57.41; H,4.11; N, 17.27

Synthesis of (Z)-1-(4-chlorophenyl)-2-(3,5-dimethyl-4-((4-nitrophenyl)diazenyl)-1H-pyrazol-1-yl)ethanone(28)

Yield:70%, m.p 180 °C ,IR (KBr) (cm^{-1}): 1690 (C=O str), 1552 (C=C str), 1603 (C=N str), ^{1}H NMR ($CDCl_3$,400MHz) 7.51-8.33 (m,8H,Ar-H), 5.52 (s,2H,CH_2), 2.53-2.59 (m,6H,3,5 CH_3) EI-MS : 397.81(M^+) Anal. Calcd for $C_{19}H_{16}ClN_5O_3$: C,57.36; H,4.05; N, 17.60.Found C,57.01; H,4.14; N, 17.07

Synthesis of (Z)-1-(4-chlorophenyl)-2-(4-((3-methoxyphenyl) diazenyl)-3,5-dimethyl-1H-pyrazol-1-yl)ethanone (29)

Yield:78%, m.p : 186 °C ,IR (KBr) (cm^{-1}): 1690 (C=O str), 1552 (C=C str), 1592 (C=N str), ^{1}H NMR ($CDCl_3$,400MHz) 6.93-7.95 (m,8H,Ar-H), 5.27 (s,2H,CH_2), 2.49-2.55 (m,6H,3,5 CH_3) ,3.72-3.87(d,3H,O-CH_3) EI-MS : 382.54(M^+) Anal. Calcd for $C_{20}H_{19}ClN_4O_2$: C,62.74; H,5.00; N, 14.63.Found C,62.61; H,5.01; N, 14.56

Synthesis of (Z)-1-(4-chlorophenyl)-2-(4-((4-methoxyphenyl) diazenyl)-3,5-dimethyl-1H-pyrazol-1-yl)ethanone (30)

Yield:70%, m.p : 180°C, IR (KBr) (cm^{-1}): 1690 (C=O str), 1554 (C=C str), 1580 (C=N str), ^{1}H NMR ($CDCl_3$,400MHz) 6.96-7.95 (m,8H,Ar-H), 5.47 (s,2H,CH_2), 2.50-2.55 (m,6H,3,5 CH_3) ,3.73-3.87(d,3H,O-CH_3) EI-MS : 382.84(M^+) Anal. Calcd for $C_{20}H_{19}ClN_4O_2$: C,62.74; H,5.00; N, 14.63.Found C,62.41; H,5.11; N, 14.66

MICROBIOLOGICAL SCREENING

Antibacterial screening

All the synthesized compounds (21-30) were evaluated for their *in vitro* antimicrobial activity against six bacterial strains : *Escherichia coli* (MTCC 118),*Pseudomonas aeruginosa* (MTCC 647),*Salmonella typhi* (NCIM250), *Klebsiella pneumonia* (MTCC 3384),*Bacillus subtilis* (MTCC 121),*Staphylococcus aureus* (NCIM 2122) and fungal strains : *Candida albicans* (MTCC 227) and *Aspergillus niger* (NCIM 1056) which were procured from Institute of Microbial Technology, Chandigarh, India. The screening methods followed were as per National Committee for Clinical Laboratory Standards (NCCLS) protocol using Mueller Hinton Broth(Becton Dickinson, USA) (NCCL, 1993; NCCL,1992; Desai *et al*., 2013).The compounds which showed better activity while primary screening against microorganism at different concentrations of 1000,500,250 µg/mL was taken in to secondary screening.The second set of screening were performed with dilutions 100µg/mL,50µg/mL, 25µg/mL, 12.5µg/mL, 6.25µg/mL, 3.125µg/mL. The minimum inhibitory concentration (MIC) of the synthesized compounds was determined by two dilution methods. The stock solution (1000µg/mL) of the analogs were prepared in dimethyl sulfoxide (DMSO). The test tube with no compound but with equal volume of solvent DMSO (2 %) served as solvent control. One test tube with no compound and no vehicle but only with nutrient medium served as a negative control to ensure the growth property of the medium. Final concentration of bacterial strain was adjusted 10^4 CFU/mL (Colony Forming Unit per milliliter) . Nutrient medium , Mueller Hinton Broth was used to grow and dilute the test analogs suspension for bacteria. 2% DMSO was taken as a vehicle to maintain the desired concentration of test analogs and standard drugs to perform the screening against microbial strains. The MIC values were measured after incubation at 35°C for a period of 24hrs. The lowest concentration of the test compound that has completely inhibited the growth was reported as MIC.MIC values were also determined for standard drug Ciprofloxacin and Norfloxacin.

Anti fungal screening

The same compounds (21-30) were also screened for antifungal activity as primary screening against *C. albicans* and *A.niger* at various concentrations of 1000,500,250 µg/mL.The compounds found to be active were taken into secondary screening with various concentrations of 100µg/mL,50µg/mL, 25µg/mL,12.5µg/mL,6.25µg/mL,3.125µg/mL. In case of antifungal screening, Sabouroud dextrose broth used as medium. The MIC values were recorded after incubation at 28±2 °C for 72hrs.MIC values also determined for standard drug fluconazole.

Anthelmintic activity studies

The assay was executed on adult Indian earthworm, *Pheretima posthuma* due to its anatomical and physiological similarity with intestinal roundworm parasite of human beings (Vigar, 1984). Anthelmintic assay was performed as per the

method given in literature (Vidyarthi,1967) with minor modifications. All the earthworms were collected from Divyayan Krishi Vigyan Kendra, Jharkhand, India .The earthworms, *P. posthuma* washed with normal saline (0.5%) for about 30 s to remove all faecal matter, were used for anthelmintic study. Earthworms of 2–4 cm in length were used in this experiment and were divided into three groups of six each. All the compounds were dissolved in minimum quantity of 2% v/v Tween 80. Before starting the experiments, all the compounds and standard drug solution were freshly prepared. All the synthesized compounds were subjected to study anthelmintic activity against earthworms at 5, 10 and 20mg/mL concentrations. The paralyzing and death times were noted and their mean was calculated for triplicate sets. Death time was recorded by placing earthworms in warm water and observed for stimulated movement, if the worm was alive. The synthesized compounds (21-30) were evaluated for their *in vitro* anthelmintic activity against *Pheretima posthuma* . Albendazole was used as a standard drug at a dose of 20 mg/mL.The dose used for the newly synthesized compounds were 5, 10 and 20 mg/mL. The results are depicted in Table 2.

Anticancer studies

The test compounds were studied for short term *in vitro* cytotoxicity using Dalton's lymphoma ascites cells(DLA) or Ehrlich Ascites Carcinoma (EAC) cells . The studies were carried out in Amala Cancer Research Centre,Amala Nagar, Thrissur, Kerala.

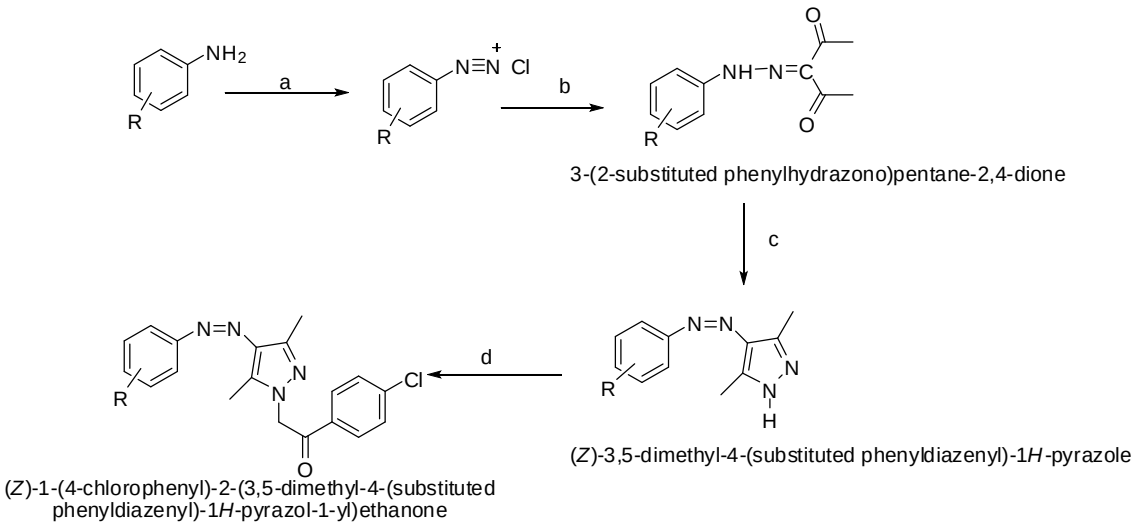
Trypan blue exclusion method:

The tumour cells aspirated from the peritoneal cavity of tumour bearing mice were washed thrice with PBS or normal saline. Cell viability was determined by trypan blue exclusion method (Kuttan *et al*., 1985 ; Babu *et al*., 1995) Viable cell suspension (1×10^6 cells in 0.1 mL) was added to tubes containing various concentrations of the test compounds and the volume was made up to 1ml using phosphate buffered saline (PBS).Control tube contained only cell suspension. These assay mixture were incubated for 3 hour at 37 °C .Further cell suspension was mixed with 0.1 ml of 1% trypan blue and kept for 2-3 minutes and loaded on a haemocytometer. Dead cells take up the blue colour of trypan blue while live cells do not take up the dye. The number of stained and unstained cells were counted separately.

$$\text{Percentage Cytotoxicity} = \frac{\text{No. of Dead cells}}{\text{No. of viable cells} + \text{No. of dead cells}} \text{X100}$$

RESULTS AND DISCUSSION

Various (Z)-1-(4-chlorophenyl)-2-(3,5-dimethyl-4 (substituited phenyldiazenyl)-1H-pyrazol-1-yl) ethanones were synthesized and characterized by analytical, FT-IR, ^{1}H NMR, Mass spectra techniques. The synthetic scheme of thesecompounds is represented in scheme 1. As per literature, Intermediates were prepared by the reported procedures (Kaymakçıoğlu *et al*., 2005). In addition, Kaur *et al.* 2015 have reported few antimicrobial, antioxidant and UV-mediated DNA damage protective photocleavage , anticancer activities of some novel (E)-1-aryl-2-(3, 5-dimethyl-4-(aryldiazenyl)-1H-pyrazol-1-yl)ethanones under solvent-free conditions. To synthesize target analogs, we decided to attach (Z)-3,5-dimethyl-4-(substituited phenyldiazenyl)-1H-pyrazole with 4-chloro phenacyl bromide by conventional method, using potassium carbonate in DMF under reflux condition.The products formed were (Z)-1-(4-chlorophenyl)-2-(4-((3-chlorophenyl)diazenyl)-3,5-dimethyl -1H-pyrazol-1-yl)ethanone (21), ((Z)-1-(4-chlorophenyl) -2-(4-((4-chlorophenyl)diazenyl)-3,5-dimethyl-1H-pyrazol-1-yl) ethanone (22), (Z)-1-(4-chlorophenyl)-2-(4-((2,4-dichlorophenyl)diazenyl)-3,5-dimethyl-1H-pyrazol-1-yl)ethanone (23), (Z)-1-(4-chlorophenyl)-2-(4-((3,4-dichlorophenyl)diazenyl)-3,5-dimethyl-1H-pyrazol-1-yl)ethanone (24), (Z)-1-(4-chlorophenyl)-2-(3,5-dimethyl-4-((2,4,5-trichlorophenyl) diazenyl) -1H-pyrazol-1-yl) ethanone (25), (Z)-1-(4-chlorophenyl)-2-(3,5-dimethyl-4-((3,4,5-trichlorophenyl)diazenyl)-1H-pyrazol-1-yl)ethanone (26). (Z)-1-(4-chlorophenyl)-2-(3,5-dimethyl-4-((3-nitrophenyl)diazenyl)-1H-pyrazol-1-yl) ethanone (27), (Z)-1-(4-chlorophenyl)-2-(3,5-dimethyl-4-((4-nitrophenyl)diazenyl)-1H-pyrazol-1-yl) ethanone (28), (Z)-1-(4-chlorophenyl)-2-(4-((3-methoxyphenyl)diazenyl)-3,5-dimethyl-1H-pyrazol-1-yl)ethanone (29), (Z)-1-(4-chlorophenyl)-2-(4-((4-methoxyphenyl)diazenyl) -3,5-dimethyl-1H-pyrazol-1-yl)ethanone (30).The anti bacterial activity of synthesized compounds was assessed by comparison with ciprofloxacin and norfloxacin against some Gram-positive (*S.aureus* , *B.subtilis*) bacteria and Gram-negative (*E.coli*, *P.aeruginosa* , *S.typhi* , *K.pneumoniae*) bacteria using serial dilution method and results were summarized in Table 1. The anti bacterial results indicated that compounds 22 showed most promising activity against both Gram-positive and Gram-negative organism among the tested compounds. The compounds 21 and 24 showed relatively good inhibitory profile against Gram negative organism. In case of antifungal activities , compounds 21-24,26 showed excellent activity against *C. albicans* and *A.niger* .Moreover, compounds 25, 27-30 exhibited equally potent activity as standard drug fluconazole. Anthelmintic study results showed that, compound 22 exhibited better results among the screened series. The synthesized compounds were selected for the in vitro cell line studies using Dalton's lymphoma ascites cells(DLA) or Ehrlich Ascites Carcinoma (EAC) cells .The percentage cell death was calculated and were compared with the results of standard drug cyclophosphamide. The DLA cytotoxicity studies results depicted that 200µg of the compound 22 showed excellent cytotoxic activity (69% cell death) than the standard drug of the same concentration (45% cell death) (Table 3). The EAC cytotoxicity studies also showed that 200 µg of the compound 22 exibited promising cytotoxic activity (70% cell death) than the standard drug cyclophosphamide of the same concentration (48% cell death), whereas 100 µg and 50 µg of the same compound (22) showed better EAC cytotoxic effect(46% cell death and 30% cell death) than the standard drug (37% cell death and 28% cell death) of the same concentration (Table 4).



Reagents and conditions: a) $NaNO_2$,HCl,0-5^{0}C,stirring b)acetyl acetone,sodium acetate c)hydrazine hydrate,ethanol,reflux d)4-chlorophenacylbromide,potassium carbonate,DMF,reflux

21) *m*-Cl , 22) R=*p*-Cl , 23) R=2,4-Cl , 24) R=3,4-Cl , 25) R=2,4,5-Cl , 26) 3,4,5-Cl, 27) R=*m*-NO_2 , 28) *p*-NO2, 29) R=*m*-OCH_3 ,30) R=*p*-OCH_3

Scheme (1): Synthesis of (Z)-1-(4-chlorophenyl)-2-(3,5-dimethyl-4-(substituited phenyldiazenyl)-1H-pyrazol-1-yl)ethanone (21-30)

Table 1: Minimum Inhibitory concentration (MIC) of test compounds (21-30) against bacterial strains and fungal strains.

Compounds	Microorganisms							
	Gram +^{ve} bacteria			Gram –^{ve} bacteria			Fungal strains	
	S.aureus	**B.subtilis**	***E.coli***	***P.aeruginosa***	***S.typhi***	***K.pneumoniae***	***C.albicans***	***A.niger***
21	6.25	6.25	6.25	6.25	3.12	3.12	3.12	3.12
22	3.12	3.12	3.12	3.12	3.12	3.12	3.12	3.12
23	100	50	50	50	50	-	3.12	3.12
24	6.25	12.5	6.25	12.5	3.12	3.12	3.12	3.12
25	-	100	50	100	12.5	100	12.5	12.5
26	50	100	-	-	-	100	3.12	3.12
27	100	50	-	-	100	100	12.5	12.5
28	12.5	100	100	50	-	-	12.5	12.5
29	50	12.5	50	25	50	50	12.5	12.5
30	12.5	-	-	50	50	-	12.5	12.5
Ciprofloxacin	0.78	1.56	0.39	0.78	0.78	0.78		
Norfloxacin	1.56	0.78	0.78	0.39	1.56	0.78		
Fluconazole							12.5	12.5

Table 2: Anthelmintic activity of test compounds (21 – 30) against *Pheretima Posthuma*.

Compounds	Mean time taken for paralysis and death of organism					
	Paralysis time (Min)			Death time (Min)		
	5mg	**10mg**	**20mg**	**5mg**	**10mg**	**20mg**
21	16.34±1.10	13.45±0.48	08.32±0.52	20.24±1.15	16.14±0.35	10.02±0.57
22	14.20±1.15	10.11±0.61	07.45±0.85	16.02±1.22	13.11±0.79	08.10±0.61
23	15.33±0.78	10.12±0.64	08.35±0.67	18.22±0.63	13.24±0.59	10.21±0.59
24	26.12±1.16	16.10±0.78	10.34±0.77	29.23±1.22	20.35±0.72	11.20±0.64
25	28.10±1.18	18.12±0.56	09.21±0.82	32.04±1.32	22.31±0.71	11.22±0.65
26	17.40±0.59	14.32±0.48	09.24±0.48	20.10±0.44	16.32±0.68	12.29±0.47
27	18.11±0.87	10.35±0.58	08.34±0.87	22.24±1.11	14.20±0.46	10.12±0.79
28	18.80±0.48	9.45±0.54	08.23±0.33	20.23±0.57	11.20±0.46	10.25±0.42
29	25.08±1.30	13.25±0.44	08.19±0.32	28.40±1.21	18.20±0.57	10.30±0.69
30	28.22±1.23	14.20±0.62	08.10±0.67	30.21±1.24	18.20±0.59	10.24±0.49
Albendazole			05.32±0.72			08.35±0.54

Table 3: Percentage cell death –Tested analogs vs .Standard drug (compounds 21-30).

	Percentage cell death -DLA (%)										Standard cyclophosphamide (%)
Conc. µg/mL	21	22	23	24	25	26	27	28	29	30	
200 µg	25	69	10	2	24	39	6	29	45	27	45
100 µg	10	30	5	-	17	22	0	16	30	19	40
50 µg	2	24	-	-	6	8	-	3	18	11	26
20 µg	-	10	-	-	-	-	-	-	6	7	15
10µg		2	2	-	2	-	-	2	-	-	13

Table 4: Percentage cell death –Tested analogs vs .Standard drug (21-30).

	Percentage cell death-EAC (%)										Standard cyclophosphamide
Conc. µg/mL	**21**	**22**	**23**	**24**	**25**	**26**	**27**	**28**	**29**	**30**	**(%)**
200 µg	30	70	10	10	26	39	6	30	45	30	48
100 µg	18	46	5	5	18	22	-	15	30	15	37
50 µg	8	30	-	-	6	8	-	8	18	7	28
20 µg	4	10	-	-	4	-	-	4	6	5	20
10µg	-	5	-	-	2	-	-	2	2	2	13

Structure Activity Relationship (SAR)studies

From these preliminary screening studies, it was found that the presence of halogens in different positions of the aromatic ring of these novel pyrazole analogs assured their activity. The overall effects of substitution pattern on the different biological activities are summarized in Fig 1.

It was interesting to notice that test compound 22 with chloro substitution in para position led to a significant increase in antibacterial activity and anthelmintic and cytotoxic activities.However, presence of halogen substituted in the different positions in the aromatic ring played a substantial increase in the antifungal activities.This indicates that the electron withdrawing groups played a considerable role in a substantial increase in the *in vitro* screening results.

CONCLUSION

In conclusion, a series of (Z)-1-(4-chlorophenyl)-2-(3,5-dimethyl-4- (substituited phenyldiazenyl)-1H-pyrazol-1-yl) ethanones (21-30) has been synthesized using conventional method using potassium carbonate in DMF, under reflux condition and their structures were established by IR,NMR ,Mass and elemental analysis. The results of antibacterial results revealed that compounds 22 showed most promising activity against both Gram-positive and Gram-negative organism among the tested compounds. The compounds 21 and 24 showed relatively good inhibitory profile against Gram negative organism compared to the standard drug ciprofloxacin and Norfloxacin.In case of antifungal activities , compounds 21-24,26 showed excellent activity against *C. albicans* and *A.niger*. Moreover, compounds 25,27-30 exhibited equally potent activity as standard drug fluconazole. However, anthelmintic study results showed that, compound 22 exhibited better results among the screened series. Furthermore, *in vitro* DLA cytotoxicity studies results depicted that 200µg of the compound 22 showed excellent cytotoxic activity (69% cell death) than the standard drug of the same concentration (45% cell death) and EAC cytotoxicity studies also showed that 200 µg of the compound 22 exibited promising cytotoxic activity (70% cell death) than the standard drug cyclophosphamide of the same concentration (48% cell death),whereas 100 µg and 50 µg of the same compound (22) showed better EACcytotoxic effect(46% cell death and 30% cell death) than the standard drug (37% cell death and 28% cell death) of the same concentration . Findings from this study revealed that, these newly synthesized pyrazole analogs especially 21, 22, 24 could serve as better scaffolds to develop as broad spectrum chemotherapeutic agents.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

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