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Synthesis of novel N^1-(4-amino benzoyl)-1,3-dimethyl-4 (N-4- sulfamoylphenylazo)-1, 2-diazole

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ABSTRACT

Novel N^1(4-amino benzoyl)-1,3-dimethyl-4(N-4- sulfamoylphenylazo)-1,2-diazole has been synthesized by two step processes. Synthesis of N^1-4-sulphamoylphenyl hydrazono-1,3-dimethylpropane-1, 3-dione by the interaction of 1,3-dimethypropane-1,3-dione and diazonium salt solution of Diethyl sulphanilamide. Which interacting with 4-amino benzoic acid hydrazide to form the final compound. We have synthesized the sulfamalamide derivatives. Then all sulfamalamide derivatives (1a-1e) diazotization with $NaNo_2$ and HCl ay 0.5°C. Then the newly synthesized compound N^1-(4-amino benzoyl)-1,3-dimethyl-4(N-4- sulfamoylphenylazo)-1, 2-diazoles were screened for diuretic activity. The anti-inflammatory activity for the newely synthesized sulpha/substituted 1,2-diazole compound were carried out by the carrageenan induced rat hind paw edema method by taking Diclofenac sodium as standard.

Keywords: Synthesis, Kofler Hot stage apparatus using determination m.p., Diuretic activity, Sulphanilamide, Diazotization, 4-amino benzoic acid hydrazide.

INTRODUCTION

A wide range of physiological properties are found to be associated with Nitrogen based heterocyclic compounds are very important in the field of medicinal chemistry. Heterocyclic compounds are very widely distributed in nature and essential to the life in various ways. Vitamic C exist in the form of five membered (furan) or sic membered (Pyran) rings containing one oxygen atom. Most member of Vitamin B group possess heterocyclic ring containing nitrogen. One example in Vitamin B_6 (Pyridoxime), which derivative of pyridine essential in amino acid metabolism. Sulpha/Substituted 1,2-diazole is a heterocyclic compound having varied biological activity and still of great scientific interest now a days. They are widely found in bioorganic and medicinal chemistry with application in drug history. The present diazoles were prepared because of its good biological activity. Compounds including a 1,2-diazole nucleus and N-substituted derivatives are known to possess various biological activity[1]. Among these types of molecules have been shown to have various important biological activity such as antibacterial, antifungal, antiviral, diuretic, antituberculostatic, anti-HIV, antihistaminic, anticancer, anticonvulsant, anti-inflammatory and analgesic properties [2-6].

Diuretic compounds that stimulate the excretion of water are potentially useful in many disorders including most of those exhibiting oedema such as congestive heart diseases, nephritis, toxemia of pregnancy, premenstrual tension, hypertension and also play an important role in hypertensive patients and pulmonary congestion [7]. Diuretics like mannitol, thiazides, frusemide, and ethacrinic acid are used in now days. Among these diuretics have some toxic effects. These synthetic diuretics typically inhibit potassium secretion and leads to potassium retention [8]. Sulpha/substituted 1,2-diazoles may serve as the alternative sources for the development of new diuretic agents due to their biological activity. Sulpha/substituted 1,2-diazoles used for the treatment of diuresis in different systems of medicine have shown diuretic activity when tested on animal models. On the basis of the use of diuretics, but no previous pharmacological study was carried out to test the diuretic activity of sulpha/substituted 1,2-diazoles. The main aim of the present investigation was to evaluate the claimed diuretic activity of sulpha/substituted 1,2-diazoles.

The present sulpha/substituted 1,2-diazoles were prepared because of its good biological activity. As sulphonamides have also been reported to exhibit significant antibacterial activity.

MATERIALS AND METHODS

All melting points were determined in open capillary and are uncorrected. The IR spectra were recorded on Perkin-Elmer 157 and Shimadzu spectrometerer. ^{1}H NMR was reported onaveanue-300 MHz instrument using $CDCl_3$ as solvent and TMS as internal standard. The mass spectra were recorded on 7070H spectrometer using ionization energy of 70ev. Elemental analysis were performed on a Carlo Erba 106 Perkin-Elmer model 240 analyzer. 4-amino benzoic acid hydrazide and all reference compound were purchased from Aldrich Chemicals. Ethanol, sodium acetate, glacial acetic acid and all other reagents were purchased from S. D. Fine Chemicals (India). The reactions were monitored on TLC where it is performed on performed on pre-coated plastic sheets of silica gel G/UV-254 of 0.2 mm thickness (Macherey-Nagel, Germany) and the spots were located in iodine chamber. The diazotization of the appropriate sulpha drug and their coupling with reactive methylene compounds was carried out by the method reported in the literature.

Melting points of the N^1(4-amino benzoyl)-3-methyl-5-phenyl-4(N-4-sulfamoylphenylazo)-1,2-diazole was determined using an open-ended capillary tube method and are uncorrected. The purity of the synthesized compound was checked by TLC. A FT-IR spectrum was recorded on a Perkin-Elmer 1605 series. FT-IR in a KBr Disc, ^{1}H NMR spectra was recorded at 300MHz on a Bruker FT-NMR spectrophotometer using TMS as internal standard.

Diketone Synthesis : Some of the most useful reactions of carbonyl involve carbon hydrogen bonds adjacent of the carbonyl group. Such reactions, which can be regarded as the backbone of much synthetic organic chemistry, usually result in the replacement of the hydrogen by some other atom or group, as in the sequence of fig.1

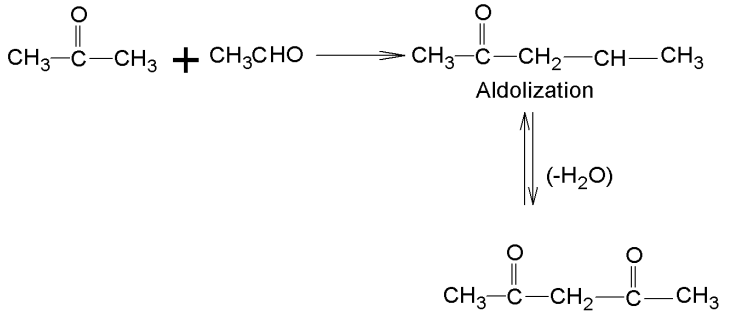


Fig.1

The reaction between a ketone and 2 equivalent of an aliphatic anhydride with BF_3 was found to give the best results when run as rapidly as possible such that despite vigorous external cooling, the temperature reached 80-90^{0}C until the weight increase corresponded to 3 mol of BF_3 per mol of ketone. The intermedinary difluorocarbonyl complexes were isolated by aqueous dilution and purified of crystalline. The work up has been modified to include a titration with KOH (phenolphthalein indicator). So as to minimize retro-claisen decomposition of the product as well as to minimize the precipitation of insoluble solids. The product was then isolated by excration and distillation.

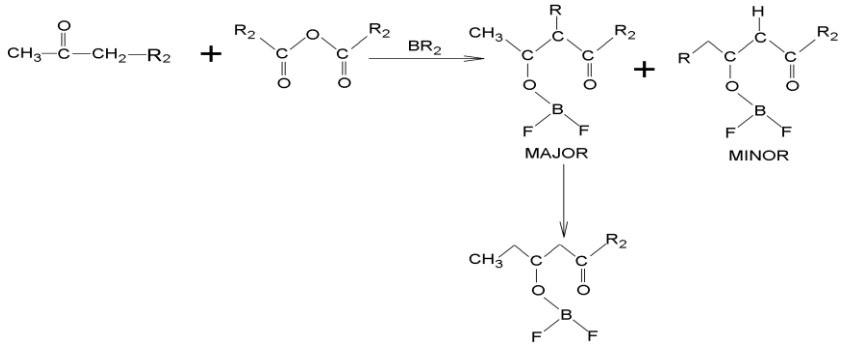


Fig.2

Diazotization: Diazotization of different sulfanilamide deravatives (1a-1e) was dissolved in HCl with stirring and the solution was cooled to 0-5^{0}C in an ice bath. A Solution of sodium nitrite in 5 ml water cooled to 0^{0}C was then added and the reaction mixture was then stirred until the positive test of nitrous acid on starch iodide (e.e. blue colour on starch iodine paper).

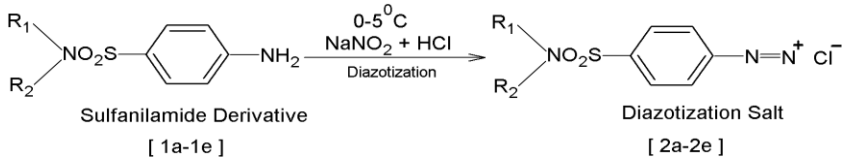


Fig.3

Where

R_1-	R_2
Ethyl	Ethyl
Phenyl	Phenyl
Phenyl	Ethyl
2,4-dichlorophenyl	H
2,6-dichloro-4-nitrophenyl	H

Synthesis of N^1-4-sulphamoylphenyl hydrazono-1,3-dimethylpropane-1,3-dione: An ice cooled solution of 1,3-dimethylpropane-1,3-dione (0.02 mol) in ethanol containing sodium acetate (6 g) a diazotized solution of sulphanilamide (0.05 mol) were gradually added with stirring and cooling. The reaction mixture was further stirring for 20 min, the coloured hydrazono compounds precipitated by addition of ice cold water. It was filtered off, washed with water, dried and recrystallized from

ethanol/acetic acid (Fig. 1). On analysis, it was found to be N^1-4-sulphamoylphenyl hydrazono-1-methyl-3- phenylpropane-1,3-dione (Fig. 4).

N^1-4-sulphamoylphenyl hydrazono-1,3-dimethylpropane-1,3-dione : A yellow crystalline powder, mp 198-200 ^oC, Yield 82.34%, molecular formula $C_{16}H_{15}O_4N_3S$, anal. Calcd for $C_{16}H_{15}O_4N_3S$ (348.76): C, 55.10; H, 4.34; O, 18.35; N, 12.04; S, 10.17. Found: C, 54.92; H, 4.56; O,18.17; N, 12.48; S, 9.87. IR (KBr) in cm^{-1} 1440 (C-C), 1560 (C=N), 1560 (C=C of aromatic ring), 1260 (C-N),1680 (C=O), 3087 (NH), 3275 (SO_2NH_2). ^{1}HNMR ($CDCl_3$) δ in ppm, 2.81 (s, 3H CH_3), 6.75-7.68 (m, 9H, Ar- H), 6.92 (s, 2H NH_2), 10.43 (s, 1H NH).

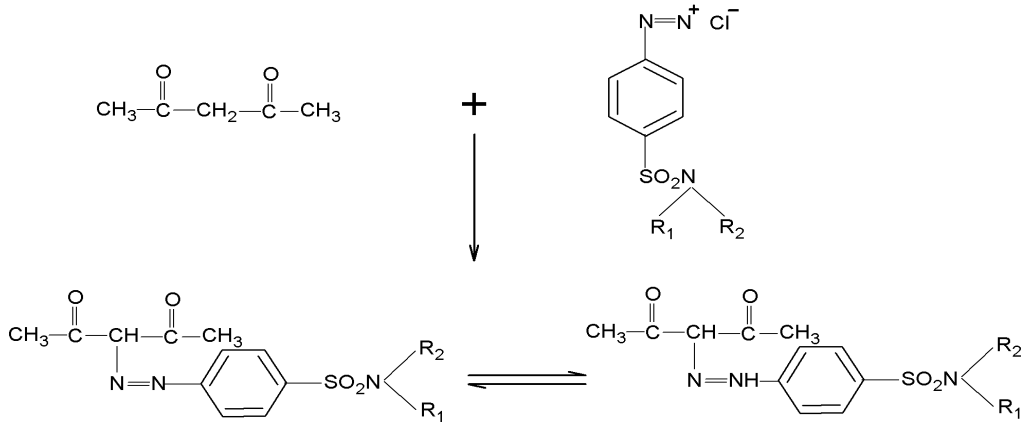


Fig. 4 .Synthesis of N^1-4-sulphamoylphenyl hydrazono-1,3-dimethylpropane-1, 3-dione

Synthesis of N^1-(4-amino benzoyl)-1,3-dimethyl-4(N-4-sulfamoyl phenylazo)-1,2-diazole: A solution of **N^1**-4-sulphamoylphenyl hydrazono-1-methyl-3-phenylpropane-1,3-dione (0.02 mol) in glacial acetic acid was added to 4-amino benzoic acid hydrazide (0.05 mol) refluxed on water bath for 8 hours and left overnight. On cooling, shining colored crystals, separated out which was collected by filtration, washed well with water, dried and recrystallised from glacial acetic acid to give **N^1**-(4-amino benzoyl)-3- methyl-5-phenyl-4(N-4-sulfamoylphenylazo)-1,2-diazole (Fig. 5).

N^1-(4-amino benzoyl)-1,3-dimethyl-4(N-4-sulfamoylphenylazo)-1,2-diazole: A yellow crystalline powder, mp 226-228 ^oC, Yield 72.13%, molecular formula $C_{23}H_{20}O_3N_6S$, anal. Calcd. for $C_{23}H_{20}O_3N_6S$ (463.90): C, 59.55; H, 4.34; O, 10.35; N, 18.12; S, 7.64. Found: C, 58.97; H, 4.64; O, 10.29; N, 18.37; S, 7.73. IR (KBr) in cm^{-1} 740 (C-C), 1240 (C-N), 1535 (C=C of aromatic ring), 1585 (C=N), 1460 (N=N), 3055 (aromatic C-H), 3135 (NH), 1707 (C=O), 3082 (NH_2), 3280 (SO_2NH_2). ^{1}HNMR ($CDCl_3$) δ in ppm, 2.79 (s, 3H CH_3), 6.65-7.58 (m, 13, Ar-H), 7.10 (m, 4H NH_2).

Animals: The adult Wistar albino rats of either sex weighing 200-250 g. They were procured from National Vertinary Research centre, Bareilly, India. They were housed in microloan boxes with standard laboratory diet and water *ad libitum*. The study was conducted after obtaining institutional animal ethical committee clearance. The animals were randomly allocated to six treatment groups of six animals each and kept in polypropylene cages and housed under standard conditions of temperature, humidity, dark light cycle (12h-12h) and diet.

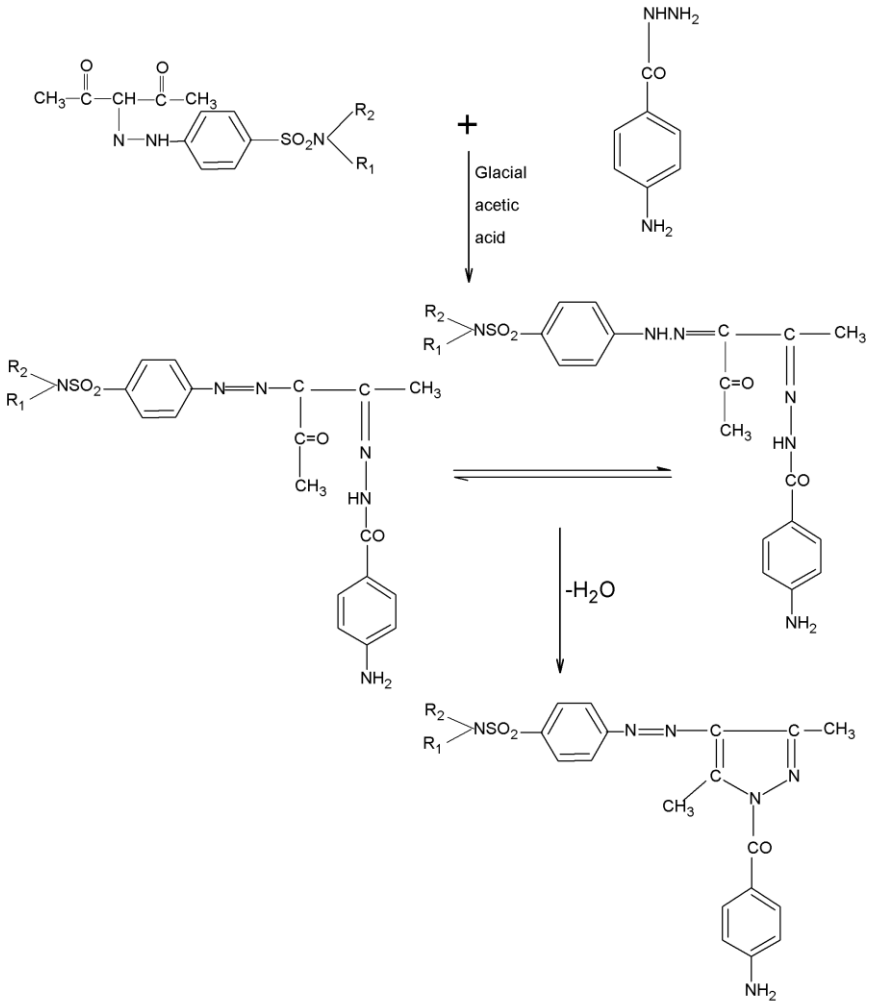


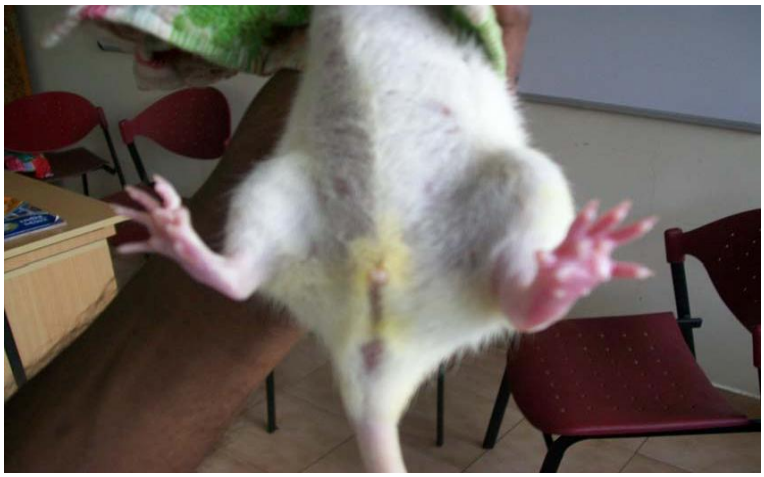
Fig. 5. Synthesis of N^1-(4-amino benzoyl)-1,3-dimethyl-4(N-4-sulfamoylphenylazo)-1, 2-diazole

Experimental design and procedure*:* Rats were assigned into nine groups of 6 animals each. They were marked with picric acid for individual animal identification. The animals were deprived of food overnight (allowed free access to water ad libitium) and the synthetic compounds were administered once before 30 min the injection of synthesized compound. Dose volume not exceeding 0.5 ml $100g^{-1}$ orally was administered. After 30 min. of test compound administration, 0.1 ml of 0.1%w/v of carrageenan in normal saline was injected into the sub plantar region of the left hind paw of the rat. Immediately after the carrageenan injection, the volume of its displacement was measured using plethysmometer. The readings were recorded at 0, 60, 120 & 180 min. The % inhibition of edema was calculated at the end of 180 min by using the formula

% inhibition = 100 x (1-Vt /Vc)

Vt/Vc = edema volume in the rat treated with test drug and control respectively.

Result: Compounds having pyrazole ring were synthesized and screened for anti-inflammatory activity by carageenan induced rat paw edema method at a dose of 50 mg kg^{-1} p.o. The activity observed was compared with the standard drug diclofenac sodium. All the compounds have exhibited anti-inflammatory activity after 1 h and 3 h. After 1 h, activity of all the test compounds were found comparable to that of standard drug diclofenac sodium. But after 3 h, the activity of test compounds was found to be less than standard drug. Of all the compounds screened, derivatives 3, 4, 6 and 7 were found to show significant anti-inflammatory activity at the end of 180 mins ($p<0.001$) comparable with the standard diclofenac sodium. The derivatives 2 and 5 exhibited moderate anti-inflammatory activity at the end of 180 min ($p<0.01$).However, derivative 1 was found to possess least activity.



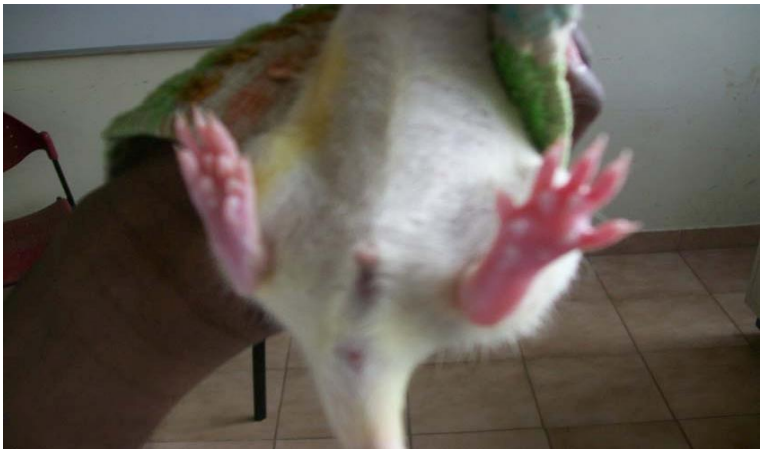


Figure 6: anti-inflammatory activity in rats

Evaluation of diuretic activity: The methods of Lipschitz et al. 1943, Mukherjee et al. 1996 and Murugesan et al. 2000 were followed for the evaluation of diuretic activity [9-13]. The rats were randomly divided into six groups of six animals each as follows: (I) was received only with saline solution. i.e. Normal control; (II) Standard group was received furosemide at a dose of 25 mg kg^{-1} by body weight; (III), (IV), (V) and (VI) was received **N^1**-(4-amino benzoyl)-1,3-dimethyl-4(N-4-sulfamoylphenylazo)-1,2-diazole at a dose of 50 mg kg^{-1} , 100 mg kg^{-1}, 200 mg kg^{-1} and 400 mg kg^{-1} by body weight respectively. Twenty four hours prior to the experiments, the test animals were placed into metabolic cages with withdrawal of food and water. After oral administration of N^1-(4-amino benzoyl)-1,3-dimethyl-4(N^1-4-sulfamoylphenylazo)-1,2-diazole, the urinary output of each group was recorded at

different time intervals from the graduated urine chamber at metabolic cage. Urine samples were analyzed for Na^+ and K^+ concentration by flame photometric method and Cl^- concentration estimated by titrimetrically.

Experimental Design: Animals were deprived of food and water 18 h before the experiment. The volume of the dose was administered 5 ml kg^{-1} by body weight. Immediately after dosing, animals were placed in metabolic cages (2 in one cage), specially designed to separate urine and faces. The urine was collected in measuring cylinder up to 5 h after dosing. During this period, animals were deprived of food and water. The parameters measured were total urine volume, urine concentration of Na^+, K^+ and Cl^-. Concentration of Na^+ and K^+ were determined using flame photometer while Cl^- concentration was estimated titrimetrically using 0.02N $AgNO_3$ with 5% potassium chromate as indicator. Appearance of brick red precipitate was taken as the end point[12].

Statistical analysis: The data were expressed as Mean ± S.E.M. and statistically analyzed using one way ANOVA followed by Dunnet's Test, $P<0.05$ were considered significant.

RESULTS AND DISCUSSION

The best of our knowledge, no previous pharmacological or clinical study has been carried out to best the diuretic activity of N^1-(4-amino benzoyl)-1,3-dimethyl-4(N-4-sulfamoylphenylazo)-1,2-diazole. The Diuretic activity of the N^1-(4-amino benzoyl)-1,3-dimethyl-4(N-4-sulfamoylphenylazo)-1,2-diazole was significant ($P<0.05$) when as compared to control. The graded dose of synthesized drug in normal saline showed a very significant increase in dieresis, natriuresis, GFR (Table. 1).

Table 1. Effect of N^1-(4-amino benzoyl)-1,3-dimethyl-4(N-4-sulfamoylphenylazo)-1, 2-diazole on urine volume and electrolyte concentration.

Group	Treatment	Dose (oral)	Mean urine volume (ml)	Urine electrolyte concentration (m eq/100g)			Na^+/Cl^- Ratio	Diuretic index
				Na^+	K^+	Cl^-		
I	Normal saline	25 ml/kg	5.66±0.08	82.6±0.18	73.44±0.12	78.66±0.15	1.12	----
II	Furosemide	25 mg/k	10.95±0.09 *	183.36±0.27*	162.46±0.16*	172.44±0.19*	1.13	1.93
III	N^1-(4-amino benzoyl)-1, 3-di methyl-4(N-4-sulfamoy lphenyla zo)-1,2- diazole	50 mg/kg	7.65±0.09*	92.86±0.18***	86.32±0.27***	89.46±0.25***	1.08	1.35
IV		100 mg/kg	7.68±0.09*	95.28±0.13*	88.48±0.07*	91.22±0.09*	1.07	1.35
V		200 mg/kg	6.65±0.09*	87.46±0.15*	68.24±0.12*	72.14±0.14*	1.28	1.17
VI		400 mg/kg	7.12±0.18*	90.56±0.09*	72.56±0.07**	82.62±.08**	1.25	1.25

- n = 6 rats per group
- *P<0.01, **P<0.05, ***P<0.001compared to control (normal saline) group using Dennett's 'T' test.

Antibacterial and antifungal activities of the diazoles are most widely studied and some of them are in clinical practice as antimicrobial agents. However, the diazole resistant strains led to develop a new antimicrobial compounds. In particular pyrazole derivatives in recent years are extensively studied for the development of newer antimicrobial agents. Synthesis of pyrazole derivatives requires refluxing of two moieties in alcohol for 5-15 hours depending upon their reactivity's, hence time consuming. Therefore, it is

important to develop a simple technique and procedure to speed up the synthesis of pyrazoles for their biological screening.

In this view, A series of 1,2-disubstituted pyrazole derivatives were synthesized by Cycloaddition reaction of substituted hydrazine's with 1,3-dione (α-β unsaturated ketones) using microwave assisted technique and compared with the conventional method. The synthesized derivatives were characterized on the basis of IR, NMR, and Mass spectral studies. In present study, the synthesized N^1-(4-amino benzoyl)-1,3-dimethyl-4(N-4- sulfamoylphenylazo)-1,2-diazole produced diuretic effect by increasing the excretion of sodium, potassium and chloride. The control of plasma sodium is important in the regulation of blood volume and pressure; the control of plasma potassium is required to maintain proper function of cardiac and skeletal muscles. The regulation of sodium, potassium balance is also intimately related to renal control of acid-base balance. The newly sodium ion is excretion to a greater extent than potassium, which is a very essential quality of a good diuretic with lesser hyperkalaemic effect. The synthesized compound N^1-(4-amino benzoyl)-1,3-dimethyl-4(N-4- sulfamoylphenylazo)-1,2-diazole was found to be superior to that of standard drugs.

APPLICATIONS

The antibacterial activity, of the synthesized 1,2-disubstituted pyrazole derivatives were effective against gram positive and gram negative organisms respectively. The antifungal activity, of the synthesized 1,2-disubstituted pyrazole derivatives showed good activity against tested fungi.

CONCLUSIONS

Substituted 1,2-diazoles are therapeutically important class of heterocyclic compounds. The method used in the present study is one of the best method for introducing substitution at 1,2 positions of the pyrazole ring. The cycloaddition reaction of 1,2-diones with hydrazides to obtain 1,2-disubstituted pyrazole derivatives was attempted by employing various reagents and reaction conditions. However the desired cycloaddition was successful only when the reaction was carried out by using acetic acid as a catalyst and methanol as a solvent. The desired 1,2-disubstituted pyrazole derivatives were obtained in a good yield by microwave assisted method when compared to conventional method. The antibacterial activity, of the synthesized 1,2-disubstituted pyrazole derivatives revealed that the compounds were effective against gram positive and gram negative organisms respectively. The antifungal activity, of the synthesized 1,2-disubstituted pyrazole derivatives revealed that the compound showed good activity against tested fungi.

The present study revealed that, synthesized compound N^1-(4-amino benzoyl)-1,3-dimethyl-4(N-4-sulfamoylphenylazo)-1,2-diazole possess significant diuretic activity at 100 and 200 mg kg^{-1} but the effect declined at higher dose.

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