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# Synthesis of novel $N^1$-(4-amino benzoyl)-3-methyl-5-phenyl-4(N-4-sulfamoylphenylazo)-1, 2-diazole

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***Abstract—Novel $N^1$-(4-amino benzoyl)-3-methyl-5-phenyl-4(N-4- sulfamoylphenylazo)-1,2-diazole has been synthesized by two step processes. Synthesis of $N^1$-4-sulphamoylphenyl hydrazono-1-methyl-3-phenylpropane-1, 3-dione by the interaction of 1-methyl-3-phenylpropane-1,3-dione and sulphanilamide. Which interacting with 4-amino benzoic acid hydrazide to form the final compound. The newly synthesized compound 4-amino benzoyl)-3-methyl-5-phenyl-4(N-4- sulfamoylphenylazo)-1, 2-diazoles were screened for diuretic activity.***

***Keywords—Synthesis, Diuretic activity, Sulphanilamide, 1,3-diketone, 4-amino benzoic acid hydrazide***.

## I. INTRODUCTION

Nitrogen based heterocyclic compounds are very important in the field of medicinal chemistry. 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.

## II. MATERIAL AND METHOD

The 1,3-diketones, sulphanilamide, 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. Chem. TLC was performed on pre-coated plastic sheets of silica gel G/UV-254 of 0.2 mm thickness (Macherey-Nagel, Germany).

***General***

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.

***Step-I***

***Synthesis of $N^1$-4-sulphamoylphenyl hydrazono-1-methyl-3-phenylpropane-1,3-dione***

An ice cooled solution of 1-methyl-3-phenylpropane-1,3-dione (0.02 mole) in ethanol containing sodium acetate (6 grams) a diazotized solution of sulphanilamide (0.05 mole) were gradually added with stirring and cooling. The reaction mixture was further stirring for 20 minutes, the coloured hydrazono compounds precipitated by addition of ice cold water. It was filtered off, washed with water, dried and recrystallised 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. 1].

***$N^1$-4-sulphamoylphenyl hydrazono-1-methyl-3-phenylpropane-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 C<u>H</u>$_3$), 6.75-7.68 (m, 9H, Ar-H), 6.92 (s, 2H N<u>H</u>$_2$), 10.43 (s, 1H N<u>H</u>).

***Step-II***

***Synthesis of $N^1$-(4-amino benzoyl)-3-methyl-5-phenyl-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 mole) in glacial acetic acid was added to 4-amino benzoic acid hydrazide (0.05 mole) refluxed on water bath for 8 hours and left overnight. On cooling, shining coloured 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. 2].

***$N^1$-(4-amino benzoyl)-3-methyl-5-phenyl-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 C<u>H</u>$_3$), 6.65-7.58 (m, 13, Ar-<u>H</u>), 7.10 (m, 4H N<u>H</u>$_2$).

***Animals***

Adult's male Wistar albino rats, each in the weight range of 180-200gm were used for this experiment. They were procured from National Vertinary Research centre, Bareilly, India. 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.

***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 by body weight; (III), (IV), (V) and (VI) was received $N^1$-(4-amino benzoyl)-3-methyl-5-phenyl-4(N-4-sulfamoylphenylazo)-1,2-diazole at a dose of 50 mg/kg , 100 mg/kg, 200 mg/kg and 400 mg/kg 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)-3-methyl-5-phenyl-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 hours before the experiment. The volume of the dose was administered 5 ml/kg 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 hours 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.

## III. RESULT 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)-3-methyl-5-phenyl-4(N-4-sulfamoylphenylazo)-1,2-diazole. The Diuretic activity of the $N^1$-(4-amino benzoyl)-3-methyl-5-phenyl-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].

In present study, the synthesized $N^1$-(4-amino benzoyl)-3-methyl-5-phenyl-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)-3-methyl-5-phenyl-4(N-4-sulfamoylphenylazo)-1,2-diazole was found to be superior to that of standard drugs.

## IV. CONCLUSION

The present study revealed that, synthesized compound $N^1$-(4-amino benzoyl)-3-methyl-5-phenyl-4(N-4-sulfamoylphenylazo)-1,2-diazole possess significant diuretic activity at 100 and 200 mg/kg but the effect declined at higher dose.

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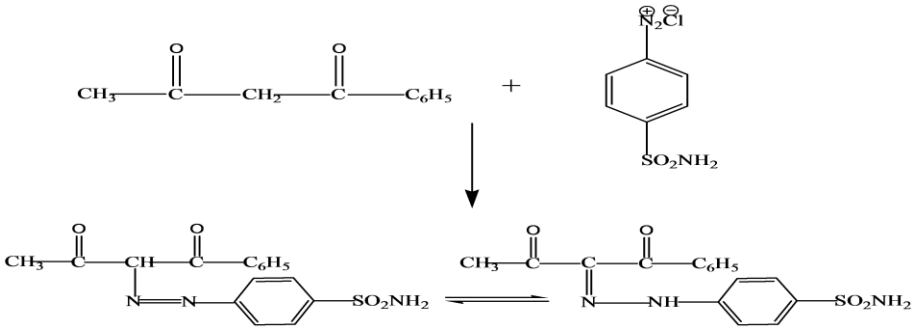

**Fig. 1 Synthesis of N[1]-4-sulphamoylphenyl hydrazono-1-methyl-3-phenylpropane-1, 3-dione**

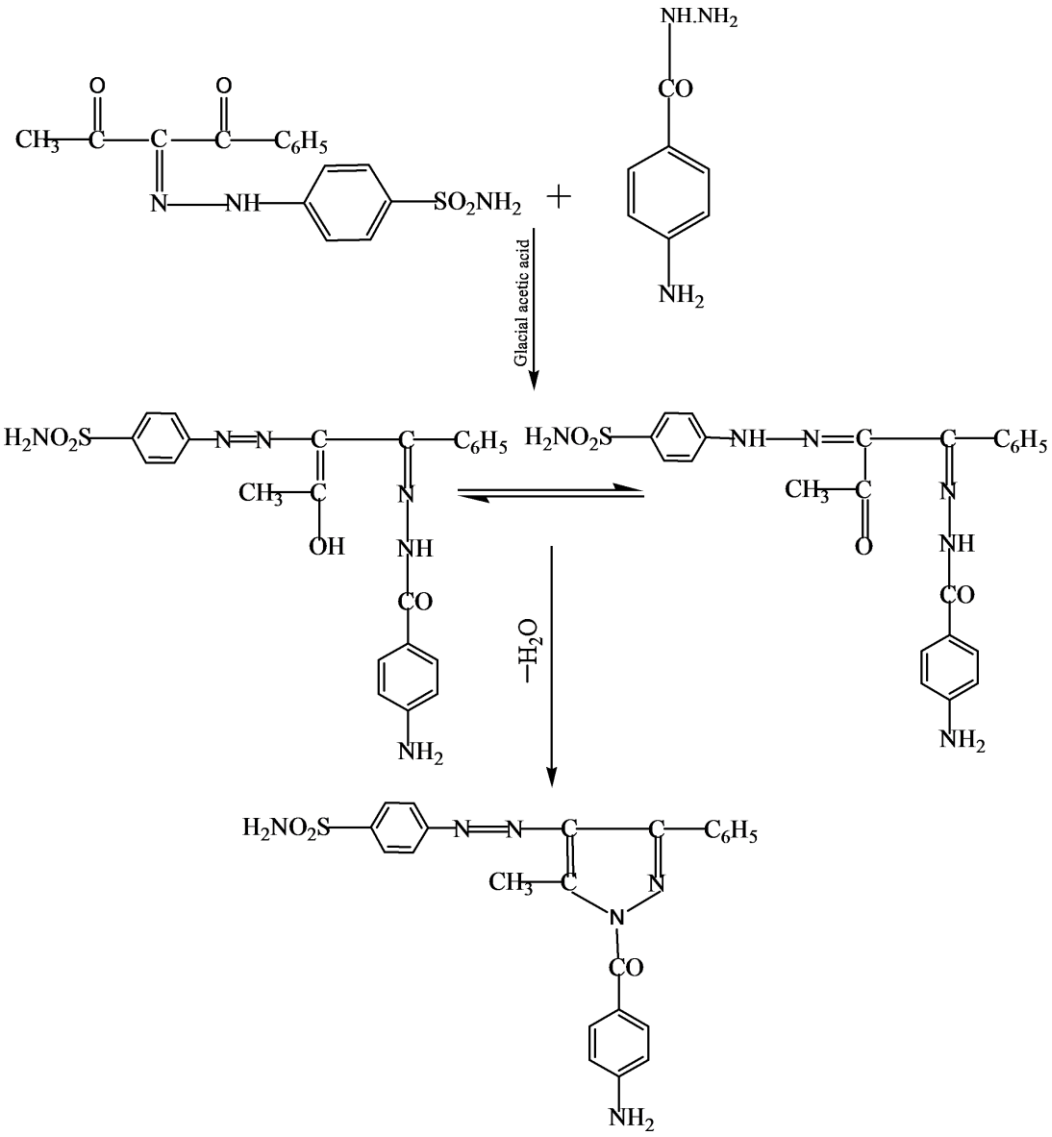


**Fig. 2. Synthesis of N[1]-(4-amino benzoyl)-3-methyl-5-phenyl-4(N-4-sulfamoylphenylazo)-1, 2-diazole .**

**Table 1. Effect of $N^1$-(4-amino benzoyl)-3-methyl-5-phenyl-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/kg | 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)-3-methyl-5-phenyl-4(N-4-sulfamoylphenylazo)-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**.