International Journal of Science and Research (IJSR)
ISSN (Online): 2319-7064
Index Copernicus Value (2013): 6.14 | Impact Factor (2013): 4.438

Synthesis of Novel N^1-(4-Fluorobenzoyl)-1-methyl-3-phenyl-4(N-4- sulfamoylphenylazo)-1, 2-diazole

Sumit Bhatt*, J. S. Jangwan

*Department of Chemistry HNB Garhwal University, SRT Campus Badshahi Thaul, Tehri garhwal 249199 (Uttarakhand) India

*Author to whom correspondence should be addressed. E-mail: sumit.inorganic@gmail.com Tel.: +91-999-728-7568

Abstract: ***Novel N^1-(4-amino benzoyl)-1-methyl-3-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 diazonium salt solution of Diethyl sulphanilamide. Which with 4-amino benzoic acid hydrazide to form the final in compound. The synthesized compound was subjected to anti inflammatory activity and it exhibited moderate anti inflammatory activity at the end of 180 mins. (p is less than 0.01 We have synthesized the sulfanilamide derivatives. Then all sulfanilamide derivatives [1a-1e] diazotization with $NaNo_2$ and HCl by 0.5°C. Then the newly synthesized compound N^1-(4-amino benzoyl)-1-methyl-3-phenyl-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. The urine was collected up to 5 h after administration and subsequently up to 24 h after administration. The acidity and urine volume were measured immediately. The urinary sodium and potassium levels were determined using a flame photometer, and the chloride level was determined by argentometric titration. The diuretic index and diuretic activity were calculated mathematically.***

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

1. 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 six membered (Pyron) 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 bio-organic and medicinal chemistry with application in drug history[24]. 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 [2-7].

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[5].

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[4].

2. Material and Method

All melting points were determined in open capillary and are uncorrected. The IR spectra were recorded on Perkin-Elmer 157 and Shimadzu spectrometer. ^{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 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.

2.1 General

Melting points of the N^1-(4-Fluorobenzoyl)-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-5-phenyllpropane-1, 3-dione

An ice cooled solution of 1-methyl-5-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. 2].

On analysis, it was found to be N^1-4-sulphamoylphenyl hydrazono-1-methyl-3- phenylpropane-1, 3-dione [Fig. 2].

N^1-4-sulphamoylphenyl hydrazono-1-methyl-5-phenylpropane-1, 3-dione

A yellow crystalline powder, mp 198-200 ^{o}C, Yield 82.36%, molecular formula $C_{22}H_{18}O_4N_3S$, anal. Calcd for $C_{22}H_{18}O_4N_3S$ (348.78): C, 55.10; H, 4.34; O, 18.35; N, 12.04; S, 10.17. Found: C, 54.93; H, 4.56; O, 18.17; N, 12.49; 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, 2.82 (s, 3H CH_3), 6.75-7.69 (m, 9H, Ar- H), 6.93 (s, 2H NH_2), 10.43 (s, 1H NH).

Step-II

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

N^1-(4-Fluorobenzoyl)-1-methyl-3-phenyl-4(N-4-sulfamoylphenylazo)-1, 2-diazole

A yellow crystalline powder, mp 226-229 ^{o}C, 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, 18.13; S, 7.64. Found: C, 58.99; H, 4.64; O, 10.29; N, 18.37; S, 7.71. IR (KBr) in cm^{-1} 740 (C-C), 1240 (C-N), 1535 (C=C of aromatic ring), 1588 (C=N), 1460 (N=N), 3055 (aromatic C-H), 3135 (NH), 1709 (C=O), 3082 (NH_2), 3283 (SO_2NH_2). ^{1}HNMR ($CDCl_3$) δ in ppm, 2.78 (s, 3H CH_3), 6.65-7.58 (m, 13, Ar-H), 7.10 (m, 4H NH_2).

2.4 Animals

Healthy adult Male Wistar albino rats of either sex weighing (200-250 gm) of inbred colony strains were used in the study[Table 2]. 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 four treatment groups of four animals each and kept in polypropylene cages covered and housed under standard conditions of temperature [Table 6], environmentally, humidity, dark light cycle (12h-12h) and diet also [Table 4]. All the experimental animals were housed at a temperature of 25 ± 2°C and 40–50% humidity in a 12:12 ± 1 h light–dark cycle. The rats were fed with standard rat pellets Reddy Research Laboratory, Hyderabad and water ad libitum [Table 7]. The study protocol was approved by the National Institute of Animal Biotechnology (NIAB) Miyapur, Hyderabad and all the animal experiments were carried out in accordance with the guidelines of the National Institute of Communicable Diseases, New Delhi, India.

2.5 Experimental Design and Procedure:

Rats were assigned into four groups of 4 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 mins the injection of synthesized compound. Dose volume not exceeding 0.10 ml/100gm 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 mins. The % inhibition of edema was calculated at the end of 180 mins by using the formula

% inhibition = 100 x (1-Vt /Vc)
Vt/Vc = edema volume in the rat treated with test drug and control respectively.

2.6 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 p.o. The activity observed Fig. 1 (a) & 1 (b) was compared with the standard drug diclofenac sodium. All the compounds have exhibited anti-inflammatory activity after 60 mins and 180 mins. After 60 mins, activity of all the test compounds were found comparable to that of standard drug diclofenac sodium. But after 180 mins, the activity of test compounds was found to be less than standard drug.

Of the compounds screened, Synthesized compounds in Scheme-2 were found to show significant anti-inflammatory activity at the end of 180 mins ($p<0.001$) comparable with the standard Diclofenac sodium. The Synthesized compound exhibited moderate anti-inflammatory activity at the end of 180 mins ($p<0.01$).

(a) (b)

Figure 1: Action of anti-inflammatory activity on rats

2.7 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 [10-14]. 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-Fluorobenzoyl)-1-methyl-3-phenyl-4(N-4-sulfamoylphenylazo)-1, 2-diazole at a dose of 25 mg/kg, 50 mg/kg, 100 mg/kg and 200 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-Fluorobenzoyl)-1-methyl-3-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.

2.8 Experimental Design
Animals were deprived of food and water 18 hours before the experiment. The volume of the dose was administered 10 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[10].

2.9 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.

3. 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-Fluorobenzoyl)-1-methyl-3-phenyl-4(N-4-sulfamoylphenylazo)-1, 2-diazole. The Diuretic activity of the N^1-(4-Fluorobenzoyl)- 1-methyl-3-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].

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.

The present study demonstrates that, the synthesized N^1-(4-Fluorobenzoyl)-1-methyl-3-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-Fluorobenzoyl)-1-methyl-3-phenyl-4(N-4-sulfamoylphenylazo)-1, 2-diazole was found to be superior to that of standard drugs.

4. Conclusion

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[16].

The cycloaddition reaction of 1, 2-diones with hydrzides to obtain 1, 2-disubstituted pyrazole derivatives was attempted by employing various reagents and reaction conditions in Scheme-2. 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 [18].

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 [19].

The antifungal activity, of the synthesized 1, 2-disubstituted pyrazole derivatives revealed that the compound showed good activity against tested fungi [22], [23].

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 but the effect declined at higher dose [15].

Scheme-I

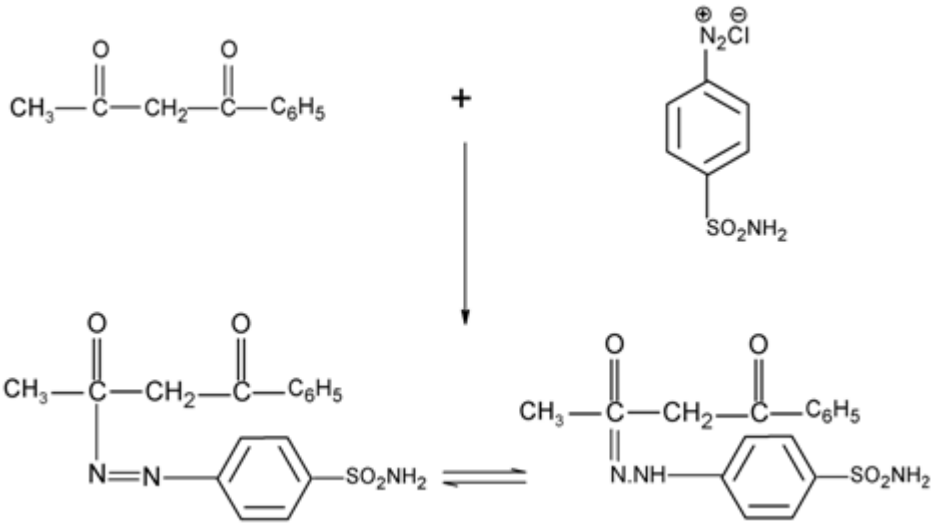

Figure 2: Synthesis of N1-4-sulphamoylphenyl hydrazono-1-methyl-3-phenylpropane-1, 3-dione

Scheme-II

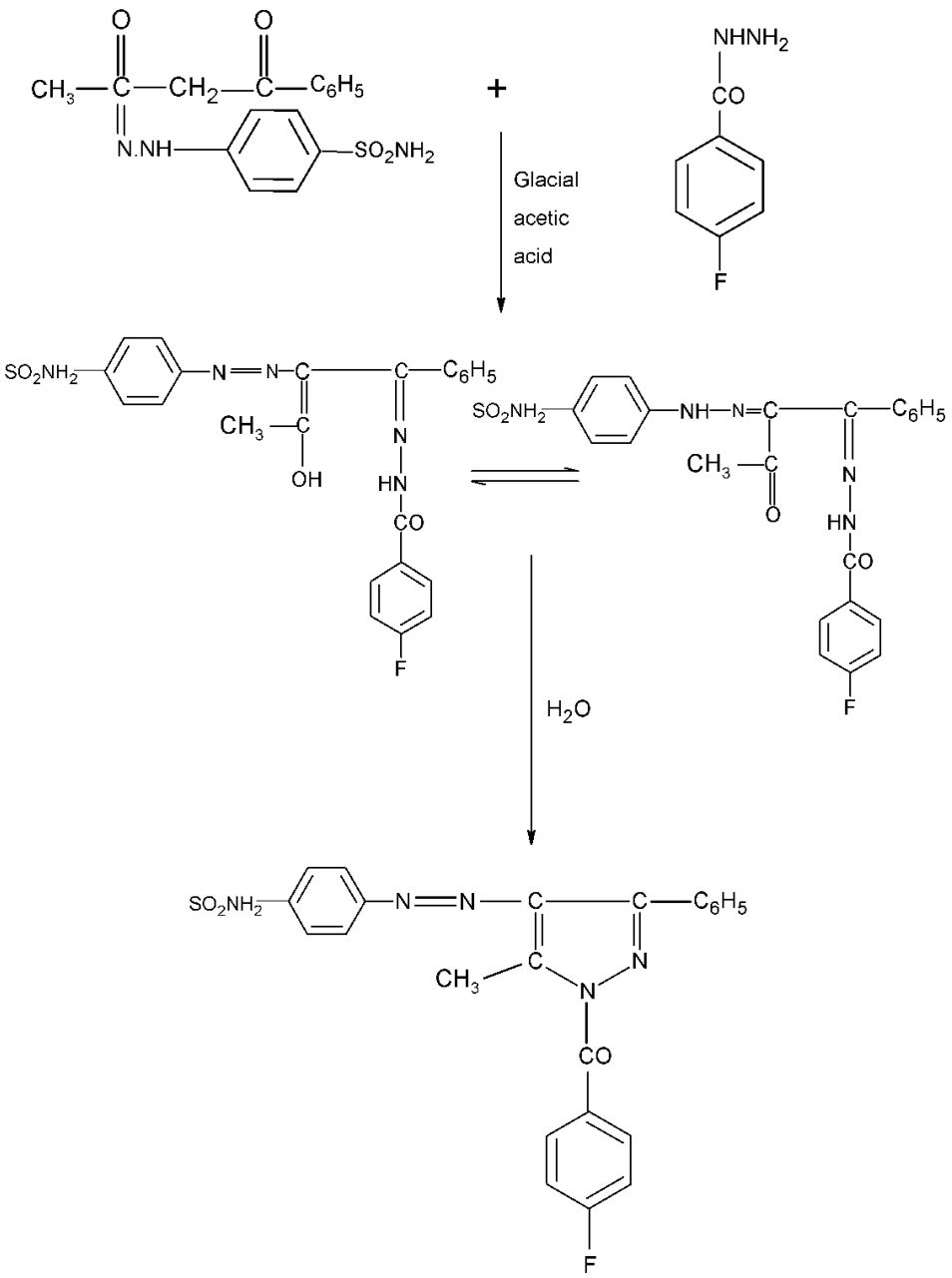

Figure 3: Synthesis of N^1-(4-fluoro benzoyl)-1-methyl-3-phenyl-4(N-4-sulfamoylphenylazo)-1, 2-diazole

Table 1: Effect of N1-(4-Fluorobenzoyl)-1-methyl-3-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/100 g)			Na^+/Cl Ratio	Diuretic index
				Na^+	K^+	Cl^-		
I	Normal saline	35 ml/kg	5.88±0.08	83.6±0.18	74.48±0.12	79.70±0.15	1.18	----
II	Furosemide	35 mg/k	11.15±0.09*	183.56±0.27*	162.50±0.16*	172.44±0.19*	1.16	1.95
III	N'-(4-Fluorobenzoyl)-1-methyl-5-phenyl-4(N-4-sulfamoylphenylazo)-1,2- diazole	50 mg/kg	7.75±0.09*	93.10±0.18***	88.40±0.27***	90.40±0.25***	1.09	1.36
IV		100 mg/kg	7.70±0.09*	95.28±0.13*	88.48±0.07*	91.22±0.09*	1.08	1.36
V		198 mg/kg	6.68±0.09*	87.89±0.15*	68.50±0.12*	72.50±0.14*	1.29	1.18
VI		390 mg/kg	7.16±0.18**	90.89±0.09*	72.64±0.07**	82.62±.08**	1.27	1.28

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

Table 2: Physiological Norms of Commonly Used Laboratory Animals

	Mouse	**Rat**
Weight at Birth(grams)	1-2	4-5
Age at Weaning (weeks)	3	3
Wt. At Weaning (grams)	9-12	40-50
Age at Maturity W=weeks Y=Years	6-8w	10-12w
Wt. at Maturity	18-22g	150-200g
Adult Weight	25-30g	200-300g
Rectal Temp0C(Average)	37.4	37.5
Respiratory Rate per minute	90-180	18-150
Pulse Rate per minute (Average)	600	300
Life span (Years)	1.5-2.0	2.5-3.0
Diploid Chromosome number 2n=	40	42

Table 3: Reproductive Date of Commonly Used Laboratory Animals

	Mouse	**Rat**
Oestrus Cycle(days)	4-5	13-15h
Duration of Oestrus	2-3 h after Est. (spont.)	8-10 h (spont.)
Time of Ovulation h=hour da=day	21	21
Gestation Period (days) average	6-10	8-12
Litter size	6-10	8-12
Oestrus after parturition	Post partum	Post partum
Reproductive Life span (years)	1	1
Mating System M:F ratio	Pair/Trio/Harem	Pair/ Harem
Max. number Of females per male	5	5
Mammary Glands (T.A.P.) no. Of pairs	3,1,1 Five	3,1,2 Six

A=Abdominal, P=Pelvic, T=Thoracic, h=hours, d=days = Menstrual Cycle

Table 4: Housing and Environmental Requirements for commonly used Laboratory Animals

	Mouse	Rat
Avg. Adult weight	25-30g	200-300g
Type of Housing	Cage	Cage
Floor Area per Animal (sq.cm)	65-100	100-150
Cage height Minimum(cm)	12	14
Room TempºC	22-24	22-24
Relative Humidity(%)	45-60	50-60
Suitable Bedding Material	Paddy husk saw dust	Paddy husk saw dusk
Nesting Material	Paper cutting	Paper cutting
Ventilation Air changes per hour	10-12	10-12
Light Intensity (LUX)	300-400	300-400
Photocycle (Light:Dark)	12:12	12:12

Table 5: Nutritional Requirements of Common Laboratory Animals

Nutritional Requirements	Mouse	Rat
Protein(%)	18.00	12.00
Fat(%)	5.00	-
Linoleic acid(%)	0.30	0.60
Fiber(%)	5.00	-
Digestible energy (Kcal)	3000	3800
Vitamins		
A(1U/kg) 15000	500	4000
D(1U/kg)	150	1000(e)
E (1U/kg)	20	30(f)
K1(1U/kg)	3000	50(g)
C(mg/kg)	t	t
Biotin(mg/kg)	0.20	t
Choline(mg/kg)	600	1000
Folic acid(mg/kg)	0.50	1.00
Niacin(mg/kg)	10.00	20.00
Pantothenic acid	10.00	8.00
Riboflavin	7.00	3.00
Thaimine	5.00	4.00
VitaminB6	1.00	6.00
VitaminB12	10.00	50.00
Minerals (mg/kg)		
Calcium(%)	0.40	0.50
Chloride(%)	t	0.50
Magnesium(%)	0.05	0.04

Table 6: Requirements for Transport of Laboratory Animals by Road, Rail and Air Nutritional Requirements of Common Laboratory Animals

Requirements	Mouse	Rat
Maximum No. of Animals per Cage	25	25
Material used In Transport	Metal Cardboard Synthetic	Metal Cardboard Synthetic
Space per Animal(sq.)	20-25	80-100
Minimum height Of Box(cm)	12	14

Table 7: Commonly used Anaesthetic Drugs for Laboratory Animals

Drugs (mg/kg)	Mouse	Rat
Ketamine Hcl	22-24i/m	22-24i/m
Pentobarbitone	35i/v	25i/v
Sodium	50i/p	50i/p
Thiopentone	25i/v 50i/p	20i/v 40i/p
Urethane	-	0.75i/p

Atropine : Dose 0.02-0.05mg/kg for all species by s/c or i/m or i/v routes used to reduce salivary and bronchial secretions and protect heart from vagal inhibition, given prior to anaesthesia I/m= intramuscular, i/v = ntravenous, i/p = intraperitoneal, s/c = subcutaneous

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