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Novel Synthesis, Characterization and Biological Evaluation of Substituted Pyrazolidine-3, 5-Dione

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Abstract

An efficient lead molecule Pyrazolidine-3, 5-dione derivative has been developed as anti-inflammatory and antibacterial agents. In the present investigation, the compounds **(RS-1 to RS-10)** were synthesized according to Scheme 1 using 4-substituted benzoic acid as starting material. This acid is converted into the respective ester **(R_1 to R_5)** and then ester is converted into hydrazide **(R_2A_1 to R_2A_5 & R_2B_1 to R_2B_5)**, and finally into the pyrazolidine-3,5-diones **(tested compound)**. The compounds were evaluated for their anti-inflammatory activity by the carrageen an-induced paw edema method and anti-bacterial activity using zone of inhibition method. Out of these compounds **RS-6, RS-9, RS-10,** and **RS-2** were found to be the most active synthesized compounds, with significant anti-inflammatory activity. The Structure Activity Relationship (SAR) of these synthesized compounds showed that substitution with Nitro and Chloro group at para position of phenyl ring is produce optimum activity, as among all the synthesized compounds, the most active ones were **RS-6, RS-9,** and **RS-10**. Another structural feature of all the synthesized compounds was that phenyl substitution over Nitrogen of Pyarazolidine-3,5-dione also produce appreciable activity. Therefore these synthesized molecules can be considered as lead molecules for future investigations.

ABBREVIATIONS

FAB: Fast Atom Bombardment; TLC: Thin Layer Chromatography; IR: Infra Red; OECD: Organization for Economic Cooperation and Development

INTRODUCTION

In the last two decades, the chemistry of pyrazolidine-3,5-dione and their fused heterocyclic derivatives have received considerable attention owing to their synthetic and effective biological importance. In spite of a large number of antibiotics and chemotherapeutics available for medicinal use, the treatment of infectious diseases still remains an important and challenging problem because of a combination of factors including emerging infectious diseases and the increasing number of multi-drug resistant microbial pathogens, so it revealed a substantial need for new classes of antimicrobial agents. There is really perceived need for the discovery of new compounds endowed with different activity. Through the various molecules designed and synthesized for this aim, in recent years, active research has been initiated on heterocycles and the chemistry of Pyrazolidine-3,5-dione has received considerable attention owing to their synthetic and effective biological importance. Pyrazolidine-3,5-dione moiety has been incorporated into a wide variety of therapeutically interesting drug candidates including anti-inflammatory and antimicrobial [1]. There are many marketed drugs containing the pyrazolidine-3,5-diones group e.g., Feprazone [2], Sulfinpyrazone [3], Phenyl butazone, Kebuzone, Mofebutazone [4] etc. From the literatures, it may be predicted that Pyrazolidine-3,5-dione moiety represents important pharmacophore and plays a vital role in medicinal agents. A degree of respectability has been bestowed upon Pyrazolidine-3,5-dione derivatives due to their wide range of biological activities such as antibacterial [5], antifungal [6], anti-inflammatory [7], analgesic [8], and hypoglycemic properties [9]. In the design of new drugs, the combination of different pharmacophores frame may lead to compounds with interesting biological profiles. As alkyl, benzyl, phenyl incorporated Pyrazolidine-3,5-dione displayed varied pharmacological properties. Prompted by these investigations we synthesized compounds containing Pyrazolidine-3,5-dione

with attached benzoyl group and evaluated them for their anti-inflammatory and antimicrobial activity [10].

MATERIALS AND METHODS

Chemicals

All the solvents were of LR grade and were obtained from Merck, Rankem, Fluka, and S.D. Fine Chemicals. The Melting points were recorded in open capillary tubes and are uncorrected. The purity of the synthesized compounds was confirmed by TLC using silica gel G. Visualization was done using iodine vapours or sulfuric acid (30% v/v).

IR spectra were recorded in KBr discs in Nicolet-6700 spectrophotometer. Chemical shifts were expressed in parts per million (δ) units. ^{13}C NMR spectra were recorded on Bruker Avance II 400 MHz apparatus using DMSO-d_6 as the solvent, TMS as an internal standard. Mass spectra were recorded on Jeol Sx 102/DA-600 mass spectrometer/Data system using FAB technique and nitrogen analysis was done on elemental analyzer Elementar Vario EL III Carlo Erba 1108.

Anti-inflammatory and analgesic activities were performed in the animal house of the R.K.D.F College of Pharmacy (M.P.), and the experimental protocol was approved by the Animal Ethical Committee of R.K.D.F College of Pharmacy (M.P.)-India. (780/05/ab/CPCSEA).

Animals and microbial cultures

Wister albino rats (120-200 g) and swiss albino mice (20-30 g) of either sex were used. The animals housed under standard laboratory conditions maintained at 25±1˚C and fewer than 12 / 12 h light /dark cycle and fed with standard pellet diet (Gold Mohur brand, Lipton India Ltd.) and water ad libitum. Animal experiments were approved by the Institutional Animal Ethical Committee for the purpose of Control and Supervision of Experiments on Animals (CPCSEA-780), constituted under the directives of Ministry of Social Justice and Empowerment, Government of India. The microbial cultures of bacteria *Proteus mirabilis* (MTCC 425), *Pseudomonas aeruginosa* (MTCC 424), *Bacillus subtilis* (MTCC 619) and *Staphylococus aureus* (MTCC 96) were used for the evaluation of anti-bacterial activity.

Chemistry

The preparation of different substituted pyrazolidine-3,5-dione by the initial reagent i.e, from substituted benzoic acid consist of three steps [11,12]. Synthesis of the Pyrazolidine-3,5-dione derivatives is shown in (figure 2).

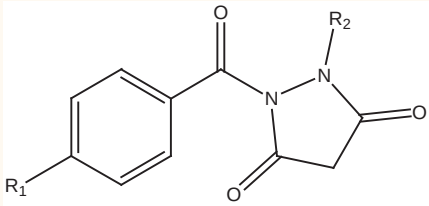

Figure 1 Basic Molecule.

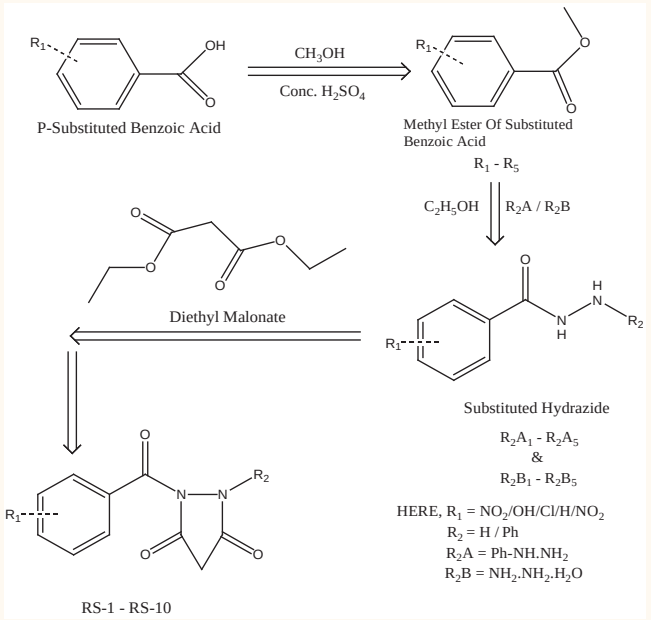

Figure 2 Scheme of Synthesis of Title Compound.

(Step-1) Synthesis of Different Para Substituted Benzoic Acid Methyl Esters (R_1 to R_5)

One mole of substituted benzoic acid was dissolved in 70-80 mL methanol and taken in a round bottom flask. After it 1.5-2.0ml sulphuric acid was added from side and some porecelain chips were putted to avoid bumping. The whole reaction mixture was subjected to reflux for 6hrs. After refluxing the mixture was cooled to room temperature and equal quantity of water has been added to it. This makes ester and water layer separated but the two layer was not distinctive one, so for this ester is separated with the help of carbon tetrachloride from separating funnel and washed with 0.6M sodium bicarbonate. After shaking and venting the funnel, removed the sodium bicarbonate layer and tested to see if it was basic. Separated the organic layer and dried it with anhydrous magnesium sulphate & removed the $MgSO_4$ by gravity filtration. Carbon tetrachloride was evaporated and respective ester was obtained which is again recrystallized with ethanol. Yield is 89%.

(Step - 2) Synthesis of Different substituted Hydrazides of Substituted Methyl Benzoates (R_2A_1 to R_2A_5 and R_2B_1 to R_2B_5)

0.1mole of different Substituted methyl benzoates previously dissolved in ethanol was taken in round bottom flask and mixed with 9-10ml of hydrazine hydrate or phenyl hydrazine, 1-2 drops of acetic acid was also added along with some porcelain chips. The whole system is subjected to reflux for 8-9 hrs. After refluxing product (respective hydrazide) was obtained which is again recrystallize with absolute ethanol? Yield is 73%.

(Step-3) Synthesis of Different Substituted 1-benzoyl-pyrazolidine-3,5-dione (RS-1 to RS-10)

After the successful completion of the above two steps the last step concludes the reaction with cyclisation, the formation

of different substituted pyrazolidine-3,5-dione molecule was carried out. For this equimolar quantity of the different substituted hydrazide was reacted with diethyl malonate to obtain the final product. Yield is 41%.

Biological Evaluation

In biological evaluation antibacterial activity and anti-inflammatory activity was performed.

Anti-Bacterial Activity

The newly synthesized compounds were screened for their antibacterial activity against bacterial strains by disc diffusion method [13] at 70 µg/mL, 50 µg/mL and 30 µg/mL concentrations, respectively. Standard inoculums (1-2×10^7 c.f.u. /mL 0.5 McFarland standards) was introduced onto the surface of sterile agar plates and a sterile glass spreader was used for even distribution of the inoculums [14]. The discs measuring 6 mm in diameter were prepared from what man no. 1 filter paper and sterilized by dry heat at 140 °C for 1 h. The sterile discs previously soaked in known concentrations of the test compounds were placed in nutrient agar medium [15]. The plates were inverted and incubated for 24 hr at 37 °C. Vancomycin and amikacin were used as a standard drug. The inhibition zones (in mm) were measured and compared with the controls. All the tests were performed in triplicate and average reading was taken [16].

Acute Toxicity Study

This involves the estimation of the median lethal dose (LD_{50}), which is the dose that will kill 50% of the animal population within 24 hours post treatment with the test substance. All animal experiments were conducted under the conditions of the Animal Scientific Procedures and as per the OECD-423 Guideline [17]. The experimental protocol was approved by the Animal Ethical Committee of R.K.D.F College of Pharmacy (M.P.)-India. (780/05/ab/CPCSEA). Groups of Swiss albino mice consisted of three animals and were maintained in colony cages at 25 ± 2ºC, relative humidity of 50 – 60%, under a 12 hour light and dark cycle; they were fed the standard animal feed and water ad libitum.

When there is no information on a substance to be tested, for animal welfare reasons, it is recommended to use the starting dose of 300 mg/kg body weight. The animals were given the lowest dose of 300 mg/kg of the compounds at the first instance. Then the animals were observed for three days. They were treated orally with different doses of tested compounds (200, 400, 600, 800, 1000, 2000, 2500 mg/kg^{-1}). The animals were then observed for 24 hours for any behavioral effects such as nervousness, excitement, dullness, in-coordination or even death.

Anti-inflammatory Activity

The anti-inflammatory activity was evaluated by the carrageen an induced, paw edema method. Albino rats of Wistar strains, weighing 100 – 200 g, of either sex, were divided into ten groups of six animals each. The animals were maintained under normal environmental conditions. They were fed the standard feed, with water ad libitum. To each group of six animals, with the exception of the control group, the tested compounds (100 mg/kg of body weight) were administered orally (p.o.). The control group received an equivalent amount of CMC. To one group the standard drug Indomethacin (10 mg/kg) was administered. After one hour, carrageen an (0.1 mL, 1% w/v solution in sterile saline) was injected into the sub plantar tissue of the left paw of all the animals. The right paw served as the reference non-inflamed paw for comparison [18]. The initial paw volume was measured using a plethysmograph within 30 seconds of the injection. After three hours, the final paw volume of each animal was measured. The percentage of reduction in the paw volume was calculated by subtracting the difference between the right and left hind paw volumes in the treated group from the difference in the control group and dividing it by the difference in the control group. The anti-inflammatory activity of the tested compounds and the standard reference drug was determined by using the formula, anti-inflammatory activity (%) = (1-Vt/Vc) × 100, where Vt represented the mean increase in paw volume of rats treated with test compounds and Vc represented the mean increase in paw volume in the control group of rats [19].

RESULTS AND DISCUSSION

In the present investigation, the compounds **(RS-1 to RS-10)** were synthesized according to Scheme 1 using 4-substituted benzoic acid as starting material. This acid is converted into the respective ester **(R_1 to R_5)** and then ester is converted into hydrazide **(R_2A_1 to R_2A_5 & R_2B_1 to R_2B_5)**, and finally into the pyrazolidine-3,5-diones **(tested compound)**. The compounds were evaluated for their anti-inflammatory activity by the carrageen an-induced paw edema method and anti-bacterial activity using zone of inhibition method.

Chemistry

Syntheses of the designed compounds have been performed as showed in scheme. After completion of synthesis, physicochemical characterization of the synthesized compounds has done. The result of solubility studies shows that all

Table 1: List of Synthesized Compound.

Compound Code	R_1	R_2	YIELD (%)
RS-1	NO_2	H	62
RS-2	NO_2		90
RS-3	Cl	H	74
RS-4	Cl		80
RS-5	OH	H	68
RS-6	OH		73
RS-7	H	H	78
RS-8	H		55
RS-9	NH_2	H	66
RS-10	NH_2		73

synthesized compound are more soluble in a non polar solvent rather than polar solvent. All synthesized compound posses a maximum solubility in methanol which confer a hydrophobicity of the synthesized compounds. The synthesized compounds were also characterized by elemental analysis, IR, ^{13}C NMR, and mass spectra. The IR spectra of each synthesized compound showed a characteristic absorption in accordance to their structural functional group, IR spectra peaks 3062 (Ar, C-H), 2943 (alkanes), 1646 (amide, C=O), 3250, 3100 (>N-H), 1705, 1700 (>C=O, Pyrazolidine-3,5-dione), 1540 (>C-N), 1618, 1460 (>C=C). This clearly tells us about different functional groups present in the synthesized compounds.

In ^{13}C- NMR spectra, Carbon of the Pyrazolidine-3,5-dione nucleus were observed in the range of δ 165.5 – 170.3 ppm. Aromatic carbons of phenyl were found between δ 128.3 to 140.8 and carbons of amide were found to be in between δ 165.8 and 168.3 ppm, respectively. A solvent peak of DMSO-d_6 was observed at 46.9 ppm. Analytical and spectral data were in good agreement with the composition of the synthesized compounds and the data are given in (Table 4). The physicochemical properties of the titled compounds are presented in (Table 3).

Anti Bacterial Activity

The compounds were evaluated for antimicrobial activity using disc diffusion method. A few of the compounds showed good activity compared to standard drug. The data given in the (Table 5) include the size of filter paper disc (6 mm). The results of antibacterial activity showed that some appreciable inhibitions were shown by compounds **RS-1**, **RS-4** and **RS-10**. The most active compound was **RS-4**, which contain Chloro group attached to phenyl ring at para position and phenyl ring at nitrogen atom of Pyrazolidine3, 5dione ring. Its zone of inhibition at 70 µg/mL concentration against *Staphylococcus aureus* (11 mm), *Bacillus subtilis* (11 mm), *Pseudomonas aeruginosa* (12 mm) and *Proteus mirabilis* (10 mm) were comparable to the standard drugs i.e., vancomycin (30 µg/mL) for Gram positive and amikacin (30 µg/mL) for Gram negative organisms. Among the bacterial strains taken for antibacterial activity, these three active compounds provided good response against *P. Aeruginosa*.

Acute Toxicity Study

As per the OECD Guidelines-423 (Acute toxic classic method) LD_{50} was calculated for all the synthesized compounds and the

Table 2: Solubility profile of the synthesized substituted Pyrazolidine-3,5-dione.

S. No.	Compound Code	Solvents				
		Water	DMSO	Methanol	Absolute Ethanol	Ether
1	RS-1	—	+ + +	+ + +	+ +	+ +
2	RS-2	—	+ + +	+ + +	+ + +	+ +
3	RS-3	—	+ + +	+ + +	+ +	+ +
4	RS-4	—	+ + +	+ + +	+ +	+ +
5	RS-5	—	+ + +	+ + +	+ +	+ +
6	RS-6	—	+ + +	+ + +	+ +	+ +
7	RS-7	—	+ + +	+ + +	+ +	+ +
8	RS-8	—	+ + +	+ + +	+ +	+ +
9	RS-9	—	+ + +	+ + +	+ +	+
10	RS-10	—	+ + +	+ + +	+	+

Abbreviations: — insoluble, ++ slightly soluble, +++ soluble

Table 3: Physico-chemical Properties of the Synthesized Compounds.

Comp. Code	Mol. Formula	Mol. Wt.	% Nitrogen		% yield of synthesized compound	M.P. found of synthesized compound
			Calculated	Found		
RS-1	$C_{10}H_7O_5N_3$	249.18	16.86	16.86	62	230-235
RS-2	$C_{16}H_{11}O_5N_3$	325.28	12.92	13.13	90	224-228
RS-3	$C_{10}H_7O_3N_2Cl$	238.63	11.72	11.99	74	137-140
RS-4	$C_{16}H_{11}O_3N_2Cl$	314.72	8.90	8.77	80	144-146
RS-5	$C_{10}H_8O_4N_2$	220.18	12.72	12.96	68	186-188
RS-6	$C_{16}H_{12}O_4N_2$	296.28	9.46	9.65	73	176-178
RS-7	$C_{10}H_8O_3N_2$	204.18	13.72	13.78	78	152-154
RS-8	$C_{16}H_{12}O_3N_2$	280.08	9.99	10.01	55	164-166
RS-9	$C_{10}H_9O_3N_3$	219.20	19.17	19.17	61	172-174
RS-10	$C_{16}H_{13}O_3N_3$	295.29	14.23	14.25	52	189-191

Abbreviations: M.P: Molecular Weight

Table 4: IR, ^{13}C-NMR & MASS spectrum of synthesized compound.

S.NO.	COMPOUND	IR(KBR) : V_{max}/cm^{-1}	^{13}C NMR δ ppm(DMSO-d_6/TMS)	FAB MASS
1.	RS-1	3062 (Ar, C-H), 2943 (alkanes), 1646 (amide, C=O), 3250, 3100 (>N-H), 1705, 1700 (>C=O, Pyrazolidine-3,5-dione), 1540 (>C-N), 1618, 1460 (>C=C)	170.3(C, amide), 46.9(CH_2, aliphatic), 168.3(C, amide), 165.3(C, amide), 139.6(C, benzene), 128.2(CH, benzene), 123.7(CH, benzene), 151.8(C, benzene), 123.7(CH, benzene), 128.2(CH, benzene)	249 $(M+H)^+$
2.	RS-2	3056, 3062 (Ar, C-H), 2945 (alkanes), 1646 (amide, C=O), 3232, 3110 (>N-H), 1701, 1720 (>C=O, Pyrazolidine-3,5-dione), 1540 (>C-N), 1625, 1465 (>C=C)	165.8(C, amide), 44.3(CH_2, aliphatic), 168.3(C, amide), 165.3(C, amide), 139.6(C, benzene), 128.2(CH, benzene), 123.7(CH, benzene),151.8(C, benzene), 123.7(CH,benzene),128.2(CH, benzene), 140.8(C,benzene),120.4,128.7,124.1,128.7,120.4 (CH, benzene)	325 $(M+H)^+$
3.	RS-3	3065 (Ar, C-H), 2941 (alkanes), 1646 (amide, C=O), 3240, 3110 (>N-H), 1710, 1700 (>C=O, Pyrazolidine-3,5-dione), 1542 (>C-N), 1620, 1462 (>C=C)	170.3(C, amide), 46.9(CH_2, aliphatic), 168.3(C, amide), 165.3(C, amide), 131.6(C, benzene), 128.7(CH, benzene), 129.0(CH, benzene), 137.2(C, benzene), 129.0(CH, benzene), 128.7(CH, benzene)	238 $(M+H)^+$
4.	RS-4	3061, 3051 (Ar, C-H), 2943 (alkanes), 1636 (amide, C=O), 3255, 3104 (>N-H), 1708, 1710 (>C=O, Pyrazolidine-3,5-dione), 1541 (>C-N), 1623, 1463 (>C=C)	165.8(C, amide), 44.3(CH_2, aliphatic), 168.3(C, amide), 165.3(C, amide), 131.6(C, benzene), 128.7(CH, benzene), 129(CH, benzene), 137.2(C, benzene), 129.0(CH, benzene), 128.2(CH, benzene), 140.8(C,benzene), 120.4,128.7,124.1,128.7,120.4 (CH, benzene)	314 $(M+H)^+$
5.	RS-5	3059 (Ar, C-H), 2942 (alkanes), 1647 (amide, C=O), 3253, 3105 (>N-H), 1705, 1698 (>C=O, Pyrazolidine-3,5-dione), 1543 (>C-N), 1615, 1462 (>C=C)	170.3(C, amide), 46.9(CH_2, aliphatic), 168.3(C, amide), 165.3(C, amide), 126.1(C, benzene), 128.7(CH, benzene), 115.8(CH, benzene), 160.7(C, benzene), 115.8(CH, benzene), 128.7(CH, benzene)	220 $(M+H)^+$
6.	RS-6	3057, 3052 (Ar, C-H), 2939 (alkanes), 1646 (amide, C=O), 3247, 3100 (>N-H), 1705, 1700 (>C=O, Pyrazolidine-3,5-dione), 1530 (>C-N), 1618, 1460 (>C=C)	165.8(C, amide), 44.3(CH_2, aliphatic), 168.3(C, amide), 165.3(C, amide), 130.5(C, benzene), 127.2, 129.3(CH, benzene) 141.1(C, benzene), 129.3, 127.2(CH, benzene), 140.8(C, benzene), 120.4,128.7,124.1,128.7,120.4 (CH, benzene)	296 $(M+H)^+$
7.	RS-7	3062 (Ar, C-H), 2943 (alkanes), 1646 (amide, C=O), 3250, 3100 (>N-H), 1705, 1700 (>C=O, Pyrazolidine-3,5-dione), 1540 (>C-N), 1618, 1460 (>C=C)	170.3(C, amide), 46.9(CH_2, aliphatic), 168.3(C, amide), 165.3(C, amide), 133.5(C, benzene), 127.3, 128.6, 131.9, 128.6, 127.3(CH, benzene)	204 $(M+H)^+$
8.	RS-8	3077, 3062 (Ar, C-H), 2943 (alkanes), 1646 (amide, C=O), 3250, 3100 (>N-H), 1708, 1710 (>C=O, Pyrazolidine-3,5-dione), 1544 (>C-N), 1618, 1458 (>C=C)	165.8(C, amide), 44.3(CH_2, aliphatic), 168.3(C, amide), 165.3(C, amide), 133.5(C, benzene), 127.3, 128.6, 131.9, 128.6, 127.3(CH, benzene), 140.8(C, benzene), 120.4,128.7,124.1,128.7,120.4 (CH, benzene)	280 $(M+H)^+$
9.	RS-9	3061 (Ar, C-H), 2943 (alkanes), 1646 (amide, C=O), 3250, 3100 (>N-H), 1705, 1700 (>C=O, Pyrazolidine-3,5-dione), 1540 (>C-N), 1615, 1462 (>C=C)	172.3(C, amide), 46.9(CH_2, aliphatic), 170.3(C, amide), 165.3(C, amide), 139.6(C, benzene), 128.2(CH, benzene), 115.2(CH, benzene), 151.8(C, benzene), 123.7(CH, benzene), 128.2(CH, benzene)	219 $(M+H)^+$
10.	RS-10	3053, 3061 (Ar, C-H), 2945 (alkanes), 1646 (amide, C=O), 3232, 3110 (>N-H), 1701, 1720 (>C=O, Pyrazolidine-3,5-dione), 1540 (>C-N), 1624, 1463 (>C=C)	172.7(C, amide), 44.3(CH_2, aliphatic), 168.3(C, amide), 165.3(C, amide), 139.6(C, benzene), 128.2(CH, benzene), 123.7(CH, benzene),150.1(C, benzene), 115.2(CH, benzene), 128.2(CH, benzene), 140.8(C,benzene), 120.4,128.7,124.1,128.7,120.4 (CH, benzene)	295 $(M+H)^+$

Abbreviations: IR: Infra Red; NMR: Nuclear Magnetic Resonance; FAB: Fast Atom Bombardment; TMS: Tri Methyl Silane; DMSO: Di Methyl Sulphoxide

cut-off dose was found to be between 1000 and 1500 mg/kg body weight.

Anti-inflammatory Activity

The pharmacological evaluation of the tested compounds **(RS-1 to RS-10)** was carried out as per the protocol specified. The anti-inflammatory activity of the synthesized compounds was carried out using the carrageen an-induced rat paw edema method. The anti-inflammatory activity data for the compounds is given in (Table 6). At the dose level of 100 mg/kg, **RS-6, RS-**

Table 5: Antibacterial activity of synthesized compounds.

Compound	Zone of Inhibition (in mm)											
	Staphylococus aureus (MTCC 96)			Bacillus subtilis (MTCC 619)			Pseudomonas aeruginosa (MTCC 424)			Proteus mirabilis (MTCC 425)		
	70 (µg/mL)	50 (µg/mL)	30 (µg/mL)	70 (µg/mL)	50 (µg/mL)	30 (µg/mL)	70 (µg/mL)	50 (µg/mL)	30 (µg/mL)	70 (µg/mL)	50 (µg/mL)	30 (µg/mL)
RS-1	7.46 ± 0.51	7.23 ± 0.58	6.90 ± 0.70	7.26 ± 0.74	6.20 ± 0.46	5.96 ± 0.80	7.96 ± 0.56	7.13 ± 0.61	6.10 ± 0.46	8.93 ± 0.80	7.13 ± 0.61	6.83 ± 1.00
RS-2	09.11 ± 0.80	9.56 ± 0.55	8.20 ± 0.60	9.93 ± 0.61	8.96 ± 0.70	7.03 ± 0.64	10.16 ± 0.55	08.96 ± 0.70	8.16 ± 0.57	9.13 ± 0.77	8.13 ± 0.66	7.06 ± 0.71
RS-3	7.90 ± 0.40	7.20 ± 0.46	6.23 ± 0.42	8.20 ± 0.62	09.96 ± 0.47	8.30 ± 0.56	8.40 ± 0.30	7.23 ± 0.56	6.16 + 0.56	8.20 ± 0.46	7.40 ± 0.30	7.30 ± 0.46
RS-4	11.23 ± 0.55	10.23 ± 0.42	9.23 ± 0.55	11.33 ± 0.26	7.23 ± 0.56	6.16 ± 0.56	12.13 ± 0.85	09.91 ± 0.47	9.36 ± 0.75	10.23 ± 0.55	9.26 ± 0.55	8.36 ± 0.75
RS-5	7.90 ± 0.70	7.30 ± 0.56	6.56 ± 0.40	8.16 ± 0.56	7.23 ± 0.55	6.10 ± 0.10	9.30 ± 0.56	8.20 ± 0.53	7.11 ± 0.56	7.26 ± 0.56	6.61 ± 0.46	6.33 ± 0.21
RS-6	9.03 ± 0.56	8.23 ± 0.56	7.26 ± 0.50	7.36 ± 0.35	6.30 ± 0.51	7.16 ± 0.68	7.13 ± 0.54	6.30 ± 0.36	6.13 ± 0.51	7.23 + 0.51	6.43 ± 0.60	6.23 ± 0.65
RS-7	6.193 ± 0.61	6.23 ± 0.51	6.20 ± 0.53	8.13 ± 0.56	7.16 ± 0.47	6.13 ± 0.61	8.26 ± 1.36	7.40 ± 0.30	6.23 ± 0.58	8.13 ± 0.56	6.90 ± 0.56	7.03 ± 0.85
RS-8	6.96 ± 0.70	6.93 ± 0.61	6.13 ± 0.61	9.30 ± 0.56	7.99 ± 0.36	3.13 ± 0.56	9.13 ± 0.56	8.86 ± 0.56	7.23 ± 0.58	8.23 ± 0.61	7.06 ± 0.70	6.40 ± 0.80
RS-9	7.23 ± 0.56	7.16 ± 0.75	6.30 ± 0.56	7.13 ± 0.55	6.10 ± 0.56	6.10 ± 0.55	7.90 ± 0.75	7.10 ± 0.56	6.26 ± 0.50	9.40 ± 0.56	8.60 ± 0.56	7.06 ± 0.56
RS-10	7.10 ± 0.70	6.13 ± 0.42	5.95 ± 0.10	6.11 ± 0.56	6.19 ± 0.46	6.05 ± 0.10	5.99 ± 0.77	5.17 ± 0.56	6.10 + 0.10	7.01 ± 0.61	6.23 ± 0.21	6.06 ± 0.70
VAN.			18.20 ± 0.20			17.43 ± 0.25						
AMIK.									22.10 ± 0.26			21.27 ± 0.46

Abbreviations: Van: Vancomycin; Amik: Amikacin

Table 6: Anti-inflammatory effect of synthesized drug on carrageen an induced paw edema in rats.

COMPOUND	CARAGEENAN-INDUCED RAT PAW EDEMA	
	After 3 hours Paw Volume ml ± SEM	Percentage Inhibition (After 3 hours)
RS-1	0.59 ± 0.004**	15.57
RS-2	0.50 ± 0.008**	28.17
RS-3	0.57 ± 0.005**	18.34
RS-4	0.46 ± 0.005**	32.23
RS-5	0.49 ± 0.010**	29.46
RS-6	0.44 ± 0.007**	36.40
RS-7	0.51 ± 0.011**	26.68
RS-8	0.65 ± 0.013**	7.93
RS-9	0.43 ± 0.007**	37.79
RS-10	0.42 ± 0.007**	39.38
Indomethacin Control	0.26 ± 0.011**	61.60

The results were statistically significant *($P < 0.05$) and ** ($P < 0.01$) during the observation. Significance was calculated by using the one-way ANOVA with Dunnett's t- test. Versus control. -- showed no inhibition in the selected model dose of Indomethacin taken at 10 mg/kg.

9, RS-10, and **RS-2** exhibited appreciable inhibition of edema, especially **RS-10**, which exhibited a percentage of edema inhibition of 40.28%, which was comparable to that of the standard drug indomethacin (62.50% at 10 mg/kg dose). Among the compounds tested, compounds **RS-6, RS-9, RS-10,** and **RS-2** exhibited mild anti-inflammatory activity. SAR study of the synthesized compounds showed that Nitro and Chloro group at para position of phenyl ring is good for optimum activity.

CONCLUSION

In the present study, synthesis of a new series of substituted pyrazolidine-3,5-diones **(RS-1 to RS-10)** has been described. Out of these compounds **RS-6, RS-9, RS-10,** and **RS-2** were found to be the most active synthesized compounds, with significant anti-inflammatory activity. The Structure Activity Relationship (SAR) of these synthesized compounds showed that substitution with

Nitro and Chloro group at para position of phenyl ring is produce optimum activity, as among all the synthesized compounds, the most active ones were **RS-6, RS-9,** and **RS-10**. Another structural feature of all the synthesized compounds was that phenyl substitution over Nitrogen of Pyarazolidine-3,5-dione also produce appreciable activity.

Out of all these synthesized compounds, the most promising compound was **RS-2**, which showed not only appreciable anti-inflammatory activity, but also antibacterial activity. It can be concluded that substituted pyrazolidine-3,5-diones moiety displays not only good anti-inflammatory activity but also provides good antibacterial activity as well. Nitro substitution is better than other group on pyrazolidine-3,5-diones moiety. These compounds can be considered as lead molecules for future investigations.

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