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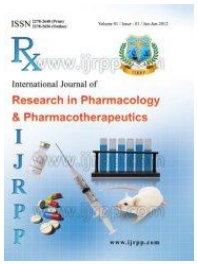

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A Study on anti-Inflammatory activity of Thiazolo-Thiourea Sydnones in albino rats

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ABSTRACT

Sydnones are proved to be important members of the meso-ionic system and are studied extensively for their pharmacological activities. In the present study several newly synthesized sydnones have been investigated for their anti-inflammatory effect. In the present study experiments for both acute and chronic anti-inflammatory activity were carried out viz., Carrageenan induced rat paw edema inhibition test to study the acute anti-inflammatory activity and cotton pallet granuloma inhibition test to study the chronic anti-inflammatory activity. These meso-ionic compounds-sydnones have been shown to possess anti-inflammatory. Among the various substituted thiazolyl thiourea sydnones 10 compounds were tried initially employing Carrageenan induced rat paw edema inhibition test for picking up an agent with anti-inflammatory activity. Compounds 3, 6, 8 and 9 which have shown considerable acute anti-inflammatory activity

INTRODUCTION

A compound may be appropriately called meso-ionic if it is a five membered heterocycle[1], which cannot be represented satisfactorily by any one covalent or polar structure and possesses a sextet of electrons in association with the five atoms comprising the ring[2] The term meso-ionic was first suggested by Baker etal3 to describe the type of molecules which defied covalent representation. Many biological activities have been claimed for meso-ionic systems in general and sydnones in particular.[4] Sydnones are proved to be important members of the meso-ionic system and are studied extensively for their pharmacological activities.[5] The reason being amino acids are precursors of sydnones.[6] Sydnone ring when it is intact it is active when hydrolyzed becomes inactive.[7] Inflammation is a protective response intended to eliminate the cause of cell injury. Several types of cells are involved in inflammatory process to eliminate the cause of cell injury. Leucocytes are activated by injurious agent by locally produced mediators and they help to eliminate the cause of cell injury.[8] These processes that eliminate the cause of cell injury may also cause harm by affecting the normal cells which are not injured. It can cause more damage to the body and the injury will become more severe because of inflammation.[9] If inflammation is not stopped it can cause injury even to normal host cells that are not injured. In inflammation there will be activated vascular permeability with fluid and

protein leakage from blood vessels.[9,10] Vasodilatation is induced by chemical mediators such as histamine; increased vascular permeability is induced by histamine, kinnins and other mediators.[10] There is leukocyte recruitment to the site of injury where infectious pathogens or damaged tissues may be located and they are activated to perform their functions. Leucocyte recruitment consists of loose attachment and rolling on endothelium.[8,10] Various cells are involved in inflammation, initially neutrophills predominate and they are later replaced by macrophages. Leucocytes can eliminate microbes and dead cells by phagocytosis, followed by their destruction in phagolysosomes.[11] 11Anti-inflammatory drugs are drugs which decrease inflammation they are as follows. NSAIDS12: nonselective COX inhibitors,-preferential COX 2 inhibitors, selective COX 2 inhibitors,-analgesic antipyretics with poor anti-inflammatory action

NSAIDS[12]

Nonselective COX inhibitors,-preferential COX 2 inhibitors, selective COX 2 inhibitors,-analgesic antipyretics with poor anti-inflammatory action Corticosteroids: hydrocortisone, prednisolone, methylprednisolone, triamcinolone, dexamethasone, and betamethasone. NSAIDS inhibit COX enzyme which inhibits prostaglandin production which inhibit process of inflammation.[13] Corticosteroids inhibit phospholipase – decrease production of PG s, LT s, and PAF, they decrease production of interleukins, chemo taxis is interfered.[14] Adhesion and localization of leucocytes is interfered. Inflammatory process is suppressed and they prevent tissue damage.[12, 15]

ANIMAL MODELS OF INFLAMMATION

UVB induced erythema in guinea pigs; animals are exposed to UV light which causes erythema.

CARRAGEEN INDUCED RAT PAW EDEMA

Tissue injury caused by this irritant initiates a cascade of events leading to formation of exudates.

PLEURAL EXUDATION METHOD

Various irritants injected into pleural cavity of rats and guinea pigs have been used to cause inflammation.

COTTON PELLET INDUCED GRANULOMA

Sterile cotton pellet are inserted subcutaneously in the skin, in the back of rats and mice .Cotton pellets cause granuloma formation.

ADJUVANT ARTHRITIS

Arthritis is induced by S/C injection of freunds complete adjuvant, sub plantar injection results in a primary no immune localized inflammatory response in the paw followed by a secondary immune systemic disease.

MATERIALS AND METHODS

MATERIALS USED

CHEMICALS

Synthetic thiazolo thiourea substituted sydnones, carrageenan, carboxymethyl cellulose, gumacacia, aspirin, and phenylbutazone.

EQUIPMENTS

Plathysmograph, ryles tube, dissection set, wooden mouth gag, syringes, skin suturing set, magnifying lens, mortar and pestle, sterile cotton wool pellets. Of all the methods available for screening the compounds for the anti-inflammatory activities, the following methods were adopted with all the limitations of availability of test compounds, animals and equipments. Carrageenan induced rat paw edema inhibition method to study the effects in acute phase of inflammatory process[16]. Cotton wool pallet granuloma inhibition method to study the efficacy of the test compounds in chronic inflammation[19]

ANTI-INFLAMMATORY TESTS

Study of anti-inflammatory activity using rat paw edema method: the method was mainly that of winter etal[17]. The measurement of edema volume was by plathysmographic method where mercury displacement was the index. This plathysmographic method was based on Waldheim and Domanzoz (1951) standardized by U.K.Seth and Dadkar (1972). The apparatus consists of a vertically placed cylindrical glass tube A. this tube is 8 cms in length and 14 mm inside diameter. Tube A is attached to another vertical glass tube B by means of a horizontal glass tube C. The inside diameter of B and C tube is 3mm.further A tube is connected to a graduated pipette D of 5ml capacity with 100 equal divisions

per ml. by means of glass tube E. pipette D has a stop cock G and is connected to another vertical glass tube F which is connected to another stop cock I and a 20 ml glass syringe by means of a pressure rubber tubings. Glass marking pencils were used to mark lines around A and B. the mercury was filled up to the zero mark in the tube D and the resulted mercury levels in A & B tubes were marked by glass marking pencils. The procedure requires two operators, one for dipping the rat paw in the mercury of the cylinder A and other for measuring and recording the paw volume simultaneously. The hind paw edema was produced by injecting 0.1 ml of 1% Carrageenan into the sub plantar region of the left hind paw of the rat. A line was marked at the level of the malleolus by skin marking pencil so that every time the foot was dipped to the same mark. The hind foot of the animal was dipped into the mercury up to the mark of the malleolus. This resulted in rise of mercury column above the marking lines on the glass tubes. The rat foot was held firmly for few seconds by the first operator till the second operator brought the level of the mercury in the tube back to the line marked i.e., the original level of mercury in the tube B before dipping of the hind foot of the animal by withdrawing air with the syringe after closing the stop cock G and opening the stop cock I. This maneuver resulted in the rise of mercury column in the glass tube D which was read off. To facilitate reading as soon as the level was brought back to dipping level, the stopcock I was closed and the animal foot was allowed to be removed from the cylinder. After the reading was complete both the stopcock were opened, the piston of the syringe was pushed back completely. The mercury levels now returned to the original levels and the apparatus was once again ready for the next recording. The hind paw volume immediately after Carrageenan injection was noted .this was compared with the same paw volume 4th hour after Carrageenan injection and increase in volume was the amount of edema formed.[17] The amount of edema formed in the control animals was designated as Vc. the amount of edema formed in the animals treated with drugs was designated as Vt, and percentage inhibition was calculated as follows[17,18] Percentage inhibition = 100x1-Vt/Vc. For this study 72 albino rats of either sex weighing between 100 to 150 gm were divided into 12 groups of six each. The animals were not given food for 18 hrs but water was allowed adlibit. Then first ten groups received the ten test compounds in the dose of 100mg/kg body weight as fine suspension in 1 ml of 4% gum acacia orally. The eleventh group received phenylbutazone in the dose of 100mg/kg body weight as suspension in 1 ml of 4% gum acacia orally and twelth group served as control receiving 1 ml of 4% gum acacia orally. Food and water were not allowed during experimentation. One hour later 0.1ml of 1% Carrageenan in 0.5% carboxy methyl cellulose was injected into the sub plantar region of the left hind paw of the rat. A mark was put on the leg at the maleolus, to facilitate the dipping of the leg to the same level every time. This animal was now taken to the apparatus and its hind foot was extended and held firmly by the 1st operator and was dipped in the mercury in the tube A up to the mark on the malleolus. The second operator adjusted the apparatus to read off the volume of the paw.[18] The procedure was repeated till it covered all the animals including the control group. All the animals were once more subjected to the plathysmographic reading to note the paw volume 3 hrs after Carrageenan injection. The procedure was repeated for all group rats every ½ hrly till five hours after Carrageenan injection. Rats were observed for 8 hours after drug administration. The control group showed maximum edema around 4th hour of Carrageenan injection. The difference between maximum edema ie.4th hour reading and initial reading provided the actual edema volume. The edema produced in the control group animals were tabulated and the mean was calculated to give the value of Vc the edema produced in the groups of animals treated with various compounds was also similarly tabulated and the mean was calculated to give various Vt values .the percentage inhibition was calculated by using the formula. The results of this series of experiments are given in the table1.we were interested to perform all the tests with all the compounds. But we were restrained by the amount s of individual compounds that were available to use. Hence the further tests were performed with only compounds no 3, 6, 8 and 9. Study of the anti-inflammatory activity by using cotton pallet method[19]: The method of Meir,Sculler and Desaulles[20] as described by Finney and Somera[21],with slight modifications was adopted was adopted 36 albino rats of either sex were divided into

six groups of six each. The first five groups received the compounds 3, 6, 8 and 9 and phenylbutazone respectively in the dose of 100mg/kg body weight in 1ml of 4% gum acacia, the sixth group received 1 ml of 4% gum acacia only as in the previous experiments .the area over the flanks of the rats was shaved and cleaned with alcohol. Under light ether anesthesia with aseptic precautions a small incision was made on the left flank and two subcutaneous pockets were created by separating the skin from underlying tissues. Two sterilized cotton pallets of 10 gm each were now pushed into the pockets .the wound was closed by using silk thread. The same procedure was followed for the right flank of the same animal, thus implanting a total of four cotton pallets in the animal. The procedure was repeated for all the animals of all the above groups. The animals were kept in clean sterilized cages. Food and water was withheld for 4 hours after the above procedure and there afterwards was allowed freely. Next day i.e., the second day the animals were administered the compounds no 3, 6, 8, and 9, phenylbutazone and gum acacia to their respective groups in the same dose and in the similar manner. The time of administration of the drugs was same on all days i.e. 10 to 11 AM .the animals were dosed for 7 days. On the 8^{th} day the animals were sacrificed and the implanted cotton pallets were removed. Each pellet was cleaned of extraneous tissue. They were separated and dried overnight in a hot air oven at 60^{o}c.the drying was continued till the weight of the pellets remained constant. The weights of the individual pellets were noted and the subtraction of 10 mg gave the amount of edema formed in each pallet. The edema formation in the control group and the groups treated with compound 3, 6, 8 and 9 and phenyl butazone was tabulated and the percentage inhibition was calculated as in previous experiments. The results are given in table 2.

RESULTS

TABLE 1: Showing the Results of Carrageenan Induced Rat Paw Edema Inhibition Test (edema volume in ml in rats treated with)

Rat no	Control	Phenyl	Compounds									
		butazone	1	2	3	4	5	6	7	8	9	10
1	0.56	0.10	0.30	0.38	0.42	0.38	0.46	0.18	0.42	0.26	0.44	0.60
2	0.62	0.08	0.38	0.40	0.38	0.48	0.30	0.30	0.38	0.22	0.28	0.34
3	0.66	0.12	0.42	0.38	0.34	0.40	0.40	0.42	0.38	0.32	0.36	0.38
4	0.58	0.20	0.36	0.44	0.32	0.38	0.40	0.50	0.46	0.26	0.14	0.36
5	0.60	0.16	0.44	0.30	0.34	0.36	0.46	0.38	0.28	0.26	0.16	0.24
6	0.60	0.16	0.40	0.38	0.32	0.22	0.48	0.24	0.30	0.24	0.42	0.30
Mean	0.60	0.13	0.38	0.38	0.35	0.37	0.41	0.34	0.37	0.26	0.30	0.37
S.D.	0.03	0.04	0.04	0.04	0.04	0.08	0.06	0.12	0.06	0.03	0.12	0.12
't'	-	23.5	10.78	10.78	12.25	6.59	6.94	5.15	8.40	19.63	5.94	4.55
% inh	-	78.33	36.66	36.66	41.66	38.33	31.66	43.33	38.33	60.00	50.0	38.33

"T" test all are significant at $P<0.05$ at DF=10. Table showing the amount of edema formed in the hind paw of the rat .four hours after the sub plantar injection of 0.1 ml of 1% Carrageenan in control group and groups treated with phenylbutazone and compound 1 to 10.the drugs were administered an hour before the Carrageenan injection in the dose of 100mg/kg body weight. Percent inhibition was calculated using the formula %inhibition = 100/1-Vt/Vc. Where Vc represents the edema volume in

control group and Vt represents the edema volume in groups treated with test compounds.

TABLE -II : Showing Cotton Pallet Granuloma Inhibition Test Results in Rats

	Control		Phenylbutazone		Compound 3		Compound 6		Compound 8		Compound 9	
Rat no	**Gain in wt (mgs)**	**Mean**	**Gain in wt (mgs)**	**Mean**	**Gain in wt (mgs)**	**Mean**	**Gain in wt (mgs)**	**Mean**	**Gain in wt (mgs)**	**Mean**	**Gain in wt (mgs)**	**Mean**
1	52,59,47,57	70	18,14,14,18	16	33,34,33,34	33.5	33,35,35,33	34.0	32,34,34,32	33.0	40,30,28,28	29.5
2	50,62,56,48	54	22,24,22,20	22	40,30,28,28	29.5	36,36,33,36	35.2	36,36,33,36	35.2	28,24,32,26	27.5
3	52,52,70,56	57.5	20,26,26,28	25	30,40,26,26	30.5	30,40,26,24	30.0	34,36,34,34	34.5	24,38,26,26	28.7
4	64,44,46,52	51.5	28,30,27,22	26.7	36,36,33,36	35.2	34,34,28,32	32.0	34,34,28,32	32.0	30,40,26,26	30.5
5	56,56,44,50	51.5	25,22,29,28	26	35,36,34,38	35.8	28,32,34,34	32.0	32,36,34,32	33.5	24,24,28,28	26.0
6	49,56,56,54	53.7	18,25,24,26	23.2	36,36,33,35	35.0	30,41,28,24	30.8	36,36,26,32	32.5	26,22,38,32	31.5
T.Mean		53.67		23.17		33.58		32.33		33.45		28.91
S.D.		6.25		4.53		3.87		4.41		2.51		5.24
C.V.		11.64		19.55		11.52		13.64		7.50		18.12
%-inh		--		57		38.00		36.60		40.80		30.60

Table showing the gain in weight of the individual cotton pallets, their mean and their total mean and the percent inhibition achieved for the groups of rats (6 each) treated with phenylbutazone and test compounds 3, 6, 8 and 9 compared with control group. Percent inhibition was calculated using the formula: % inhibition=100/1-Wt/Wc. Where Wc represents the gain in weight in the control groups and Wt represents the gain in weight in group treated with test compounds and phenylbutazone. Coefficient of variation is calculated as follows. C.V=S.D/A.M x100 where C.V. is coefficient of variation; S.D. is standard deviation; A.M is arithmetic mean

DISCUSSION

Dr Upadhya etal synthesized a series of thiazolyl substituted sydnones and a number of compounds have shown considerable anti-inflammatory activities[22] there are reports that a thiourea moiety in an imidazolyl ring potentiates the anti-inflammatory activity of compounds. In the present study experiments for both acute and chronic anti-inflammatory activity were carried out viz., Carrageenan induced rat paw edema inhibition test to study the acute anti-inflammatory activity and cotton pallet granuloma inhibition test to study the chronic anti-inflammatory activity. Among the various substituted thiazolyl thiourea sydnones 10 compounds were tried initially employing Carrageenan induced rat paw edema inhibition test for picking up an agent with anti-inflammatory activity. Compounds 3, 6, 8 and 9 which have shown considerable acute anti-inflammatory activity were subjected to further study so as to obtain chronic anti-inflammatory activity using cotton pallet granuloma inhibition method. Among the 10 compounds screened for anti-inflammatory activity by using rat paw edema inhibition test, compound 8 have chloro substitution on the phenyl group at position 3 of sydnone ring in the phenyl group of thiourea exhibits a maximum activity (60%).all other combinations like bromophenyl (compounds 3, 9) or benzoyl (compounds 4, 7) or allyl (compound 10) or nil substitutions in place of P-chlorophenyl on thiourea moiety reduces the activity. Also substitutions by methyl (compounds 5,6) on phenyl or by only phenyl at position 3 of sydnone ring(compound 1,2,3 and 4) reduces the acute anti-inflammatory activity.

From the above results, substitution of chlorine on phenyl at position 3 of sydnone ring and on phenyl at

thiourea moiety appears to enhance the acute anti-inflammatory activity. It will be interesting to check the acute anti-inflammatory activity of compounds having bromine at both the positions viz., on phenyl ring at position 3 of the sydnone ring and on phenyl ring attached to thiourea moiety. Compounds 3, 6, 8 and 9 were subjected to test chronic anti-inflammatory activity of cotton pallet implantation method. As contrasted with analgesic activity .P-bromophenyl substitution on the thiourea moiety does not favor chronic anti-inflammatory activity. Substitution of the chlorine in place of bromine does not seem to alter the chronic anti-inflammatory activity much. P-chlorophenyl substitution at position 3 of sydnone ring does not seem to confer marked increased over tolyl or phenyl substitutions. All the four compounds have shown considerable chronic anti-inflammatory activity, compared to that of phenylbutazone. It would be interesting to check of this same group of compounds varying the said chloro, bromo, tolyl substitutions or other substitutions for chronic anti-inflammatory activity. Observations regarding increase or decrease of either acute or chronic anti-inflammatory activity or analgesic activity will be more worthy only after checking the other substitutions in the different positions already referred to.

SUMMARY AND CONCLUSIONS

The present study was undertaken to find out whether newly synthesized meso-ionic compounds substituted thiazolyl thiourea sydnones possessed anti-inflammatory activity. Initially 10 compounds were screened for anti-inflammatory activity on Carrageenan induced rat paw edema test. Four compounds namely 3, 6, 8 and 9 which showed higher anti-inflammatory activity than others were subjected to further tests to ascertain chronic anti-inflammatory activity and analgesic activity. Cotton pallet induced granuloma test gave the chronic anti-inflammatory activity of the compounds. Compounds 3, 6, 8 and 9 have shown significant anti-inflammatory activity in both acute and chronic models. Of all compounds compound no 8 seems most promising in our study at the dose administered .we were limited by the merge availability of these new compounds. The compounds have not proved toxic acutely up to the dose of 400mg/kg body weight and 100mg/kg body weight every day for seven days. With the greater availability of these compounds the possibility of higher dose yielding much better anti-inflammatory and analgesic effects is there. A significant degree of anti-inflammatory activity has been demonstrated with these substituted thiazolyl thiourea sydnones at the dose of 100mg/kg body weight by oral route. The possibility of making further minor changes on the molecule with the yield of higher anti-inflammatory activity and analgesic is an exciting possibility to be explored.

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