Novel Analogues of Salicylic Acid as Uricosuric and Analgesic Agents

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2-Substituted benzoic acid derivatives are known to possess wide variety of chemotherapeutic activity. Among these 2-hydroxy-benzoic acid derivatives are found to be very useful uricosuric agents and have powerful analgesic activity. In the present research work, two series of 2-substituted benzoic acid derivatives were synthesized and structure elucidation was done by spectral analysis using IR and pNMR spectroscopy. They were then tested for uricosuric and analgesic activities.

2-Substituted-5-(sulfonamide) salicylic acids have been reported for their powerful analgesic activity. In spite of availability and extensive use of colchicine, steroids, NSAIDs, uricosuric agents and xanthine oxidase inhibitors in the treatment of gout, this disease has still remained incurable and the research for therapeutically more effective molecule has been continued unabated. Therefore it is advisable to have a drug having uricosuric as well as analgesic activity. The extent of uricosuric activity along with the analgesic activity has to be further studied in detail. Literature survey revealed that probenecid is a potent uricosuric agent and salicylic acid is known to possess analgesic activity. Therefore an attempt has been made to synthesize compounds, which will possess uricosuric as well as analgesic activities.

Melting points were determined in open capillary tubes and uncorrected. Purity of the compounds was checked by thin layer chromatography using precoated silica gel G plates. IR spectra were recorded on IR-Jasco-8400 and IR-Bomem-MB series (FTIR) and pNMR spectra were recorded on Bruker-200MHz and Amex-500 Bruker-500MHz

2-substituted-5-(dialkyl sulfamoyl) benzoic acids were synthesised following a general procedure that was previously reported[1,2]. The procedure followed was briefly, o-iodobenzoic acid (0.004 mol) and *o*-hydroxybenzoic acid (0.007 mol) were added slowly and steadily with continuous stirring to excess of chlorosulfonic acid in a 250 ml round bottom flask. The flask was continuously swirled in an ice-bath between 0-5° for about an hour. The reaction mixtures were then heated using a calcium chloride guard tube maintaining the required temperature and time so as to obtain 2-iodo-5-(chloro sulfonyl) benzoic acid (mp: 155°) and 2-hydroxy-5-(chloro sulfonyl) benzoic acid (reported mp:169-171° and obtained mp:169°), respectively.

Following methods were attempted in this step, the acetone method, in which 50 ml of dry acetone was taken in a 250 ml beaker and the beaker was placed on a magnetic stirrer. 2-Substituted-5-(chloro sulfonyl) benzoic acids and secondary amines were added to the dry acetone in (1:3) molar ratio. The stirring was continued for one hour and then the mixture was concentrated to 1/3 of its original volume in a water bath, the reaction mixture was further treated with the cold water. Dilute hydrochloric acid was then added drop wise with continuous stirring to precipitate the crude product. These crude products were filtered and dilute sodium bi carbonate was added followed by addition of dilute hydrochloric acid to give corresponding acids, which were further recrystallized with benzene and methanol. No impurity was observed.

In the pyridine method, equal moles of 2-substituted-5-(chloro sulfonyl) benzoic acids and secondary amines were added to 20 ml of pyridine solution placed in a 250 ml beaker which was kept aside for 24 h. After 24 h the beaker was placed in a boiling water bath with stirring for 20 min. Ice-cold dilute hydrochloric acid was added to the reaction mixture and the precipitate of crude products obtained were

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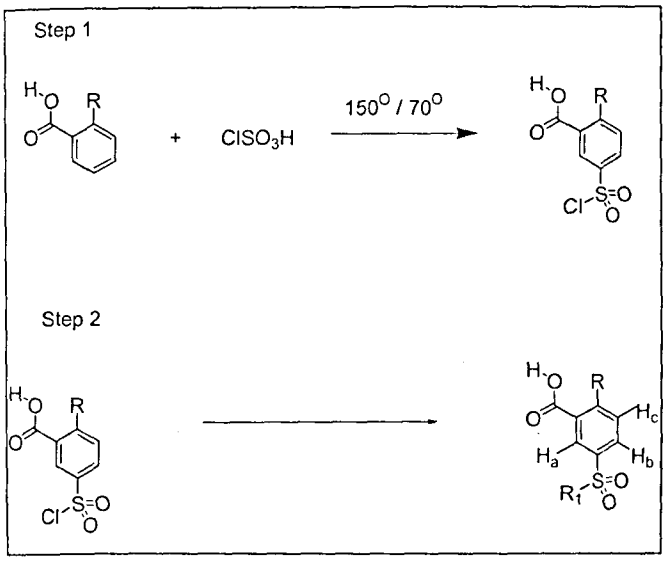

Fig. 1: Scheme of synthesis

R – hydroxy or iodo group, R_1 - different secondary amines, step 1 chlorosulfonation of 2-substituted benzoic acids, step 2 synthesis of 2-substituted-5-(N-dialkyl sulfamoyl) benzoic acids step 2 synthesis of 2-substituted-5-(N-dialkyl sulfamoyl) benzoic acids

filtered, drained and washed with water, which were further recrystallized with benzene. Impurity was observed.

Following the above two procedures 2-substituted-5-(dialkyl sulfamoyl) benzoic acids were obtained as crystalline products. Six compounds (Table 1) were synthesized and characterized by pNMR, and five out of these compounds were further characterized by IR. pNMR spectra were recorded using TMS as internal standard and chemical shifts have been expressed in δ ppm. IR and pNMR spectral data[3,4] of the synthesized compounds are as follows; 2-iodo [5-(N-dipropyl sulfonyl) amino] benzoic acid, IR (KBr): ortho-substitution phenyl C-H bonding–748 cm^{-1}, carboxylic acids, hydroxyl stretching–2510 cm^{-1}, carboxylate anion stretching-1579 cm^{-1}, carbonyl stretching-1680 cm^{-1}, C-O stretching and O-H deformation-1412, 1256 cm^{-1}, asymmetric SO_2- stretching–1299 cm^{-1}, symmetric SO_2-stretching-1162 cm^{-1}, C-I stretching-581 cm^{-1}, p-NMR: CH_2–CH_2-*CH_3*(triplet, J-7.3, 7.3 Hz, P-6 and δ-0.89); -CH_2 –*CH_2*–CH_3 (sextet, J- 7.5, 7.4, 7.5, 7.4, 7.2 Hz, P–4 and δ-1.57); -N-*CH_2*– CH_2-CH_3 (Triplet, J-7.6, 7.7 Hz, P-4, and δ-3.12); $CDCl_3$ δ-7.26; {aromatic proton-H_a-singlet, P–1 and δ-8.37; H_b– doublet, J-8.3 Hz, P–1 and δ -8.19; H_c– doublet, J-8.2 Hz, P–1 and δ-7.59}.

2-Hydroxy-[5-(N-diethyl sulfonyl) amino] benzoic acid, p NMR: -CH_2-*CH_3*(triplet, J-7.1, 7.1, Hz, P-6 and δ-1.15); -N-*CH_2*–CH_3 (quartet, J-7.1, 7.1, 7.1 Hz, P–4 and δ-3.24); aromatic –OH (singlet, P–1 and δ-4.46); $CDCl_3$ δ-7.27; {aromatic proton - H_a - singlet, P–1 and d-8.39; H_b - doublet J-8.8 Hz , P–1 and δ-7.89; H_c - doublet J-8.8 Hz, P–1 and δ-7.12}. 2-Hydroxy-[5-(N-dipropyl sulfonyl) amino] benzoic acid, IR (KBr): ortho-substitution phenyl C-H bonding–737

TABLE 1: 2-IODO-5-(DIALKYL SULFAMOYL) BENZOIC ACIDS AND 2-HYDROXY-5-(DIALKYL SULFAMOYL) BENZOIC ACIDS

C. No	R	R_1	Method	M.P (°)	Yield (%)	Molecular Formula
1	I*	-N-(CH_2-CH_3)$_2$	Pyridine	218-20	22.7	$C_{11}H_{14}INO_4S$
2	I*ϕ	-N-(CH_2-CH_2-CH_3)$_2$	Acetone	90-91	21.22	$C_{13}H_{18}INO_4S$
3	I*	-N-(CH-{CH_3}$_2$)$_2$	Pyridine	209-11	21.22	$C_{13}H_{18}INO_4S$
4	OH*ϕ	-N-(CH_2-CH_3)$_2$	Acetone	85-86	34.78	$C_{11}H_{15}NO_5S$
5	OH*ϕ	-N-(CH_2-CH_2-CH_3)$_2$	Acetone	123–24	39.37	$C_{13}H_{19}NO_5S$
6	OH*ϕ	-N-(CH-{CH_3}$_2$)$_2$	Acetone	191-92	35.43	$C_{13}H_{19}NO_5S$
7	OH*ϕ	-N-(CH_2-CH_2-CH_2-CH_3)	Acetone	85-86	25.9	$C_{15}H_{23}NO_5S$
8	OH*ϕ	CH_3-N-C_6H_5	Acetone	155 –56	42.0	$C_{14}H_{13}NO_5S$
8A	OH*ϕ	CH_3-N-C_6H_5	Pyridine	156-158	39.0	$C_{14}H_{13}NO_5S$

*IR spectra were recorded and ϕ pNMR were taken

cm^{-1}, carboxylic acids, hydroxyl stretching–2533 cm^{-1}, carboxylate anion stretching-1582 cm^{-1}, carbonyl stretching-1669 cm^{-1}, asymmetric SO_2- stretching–1307 cm^{-1}, symmetric SO_2- stretching-1164 cm^{-1}, p NMR: -CH_2– CH_2-*CH_3* (triplet, J-7.3, 7.3 Hz, P-6 and δ -0.89); -CH_2 –*CH_2*–CH_3 (sextet, J-7.3, 7.6, 7.3, 7.6, 7.1 Hz, P–4 and δ-1.56); -N-*CH_2*– CH_2-CH_3 (triplet, J-7.3, 7.8 Hz, P-4, and δ-3.11); $CDCl_3$ δ-7.27; {aromatic proton - H_a - singlet, P–1 and δ -8.42; H_b - doublet J-8.8 Hz, P–1 and δ-7.9; H_c - doublet J-8.8 Hz, P–1 and δ-7.14}.

2-Hydroxy-[5-(N-diisopropyl sulfonyl) amino] benzoic acid, IR (KBr): ortho-substitution phenyl C-H bonding–732 cm-1, carboxylic acids, hydroxyl stretching–2514 cm^{-1}, carboxylate anion stretching-1586 cm^{-1}, carbonyl stretching-1691 cm^{-1}, C-O stretching and O-H deformation-1408, 1320 cm^{-1}, asymmetric SO_2- stretching–1287 cm^{-1}, p NMR: -CH-*$(CH_3)_2$* (doublet, J–6.5 Hz, P-12 and δ-1.30); -N-*CH*-$(CH_3)_2$ (quintet, J-6.8, 6.6, 6.8, 6.8 Hz, P–2 and δ-3.69); $CDCl_3$ δ-7.27; {aromatic proton - H_a - singlet, P–1 and δ-8.46; H_b – doublet, J–6.8, P–1 and δ-7.95; H_c – doublet, J-8.8 Hz, P–1 and δ-7.11}. 2-Hydroxy-[5-(N-dibutyl sulfonyl) amino] benzoic acid , IR (KBr): carboxylic acids, hydroxyl stretching–2470 cm^{-1}, carboxylate anion stretching-1583 cm^{-1}, carbonyl stretching-1681 cm^{-1}, p NMR: -CH_2-CH_2-CH_2-*CH_3* (triplet, J-7.3, 7.1 Hz, P- 6 and δ-0.9); -CH_2-CH_2-*CH_2*-CH_3 (quintet, J-7.3, 7.3, 7.3, 6.8 Hz, P–4 and δ-1.31); -CH_2-*CH_2*-CH_2-CH_3 (quartet, J–7.8, 6.8, 8 Hz, P–4 and δ-1.51); -N-*CH_2*-CH_2-CH_2-CH_3 (triplet, J-6.8, 8 Hz, P–4 and δ-3.13); $CDCl_3$ d-7.27; {aromatic proton - H_a - singlet, P–1 and δ-8.38; H_b – doublet, J–8.8 Hz, P–1 and δ-7.87; H_c – doublet, J-8.8 Hz, P–1 and δ-7.11}.

2-Hydroxy-[5-(N-methyl phenyl sulfonyl) amino] benzoic acid, IR (KBr): ortho-substitution phenyl C-H bonding–717 cm^{-1}, carboxylic acids, hydroxyl stretching–2524 cm^{-1}, carboxylate anion stretching-1581 cm^{-1}, carbonyl stretching-1670 cm^{-1} , asymmetric SO_2- stretching–1294 cm^{-1}, symmetric SO_2- stretching-1142 cm^{-1}, p NMR: -N-*CH_3* (singlet, P-3, and δ-3.2); $CDCl_3$ δ-7.26; {benzoic acid ring proton - H_a –doublet, J–1.5 Hz , P–1 and δ-8.17; H_b – [doublet, doublet - doublet, J-2 Hz, and δ-7.59, doublet, J–2 Hz, P–1 and, δ-7.57]; H_c – doublet, J–9 Hz, P–1 and δ-7.03}, {benzene ring proton - H_{a1b1} –quintet, J-7, 7.5, 7.5, 7 Hz, P–4 and δ-7.32; H_{c1} –doublet, J–8 Hz, P–1 and δ-7.13}, aromatic–COOH (singlet, P-1, and δ-11.01), where, P – No of protons and J – Joules constant.

The animal experiments were performed as per the protocol approved by Institutional Animal Ethics Commit-

TABLE 2: EXPERIMENTAL FINDING OF PRELIMINARY SCREENING FOR URICOSURIC ACTIVITY IN MALE WISTAR RATS

Time Interval (min)		Average concentration of dye in µg/100ul of plasma diluted to 10 ml at the given interval of time, in six animals (Mean±SE)							
		S_d	C_d	D_d					
				2	4	5	6	7	8
00 (t_1)		0	0	0	0	0	0	0	0
30 (t_2)		44±.28	53±.43	45±.37	40±.34	40±.36	40±.36	48±0.34	49±.57
60 (t_3)		38±.46	29±.3	37±.37	34±.29	34±.24	33±.29	40±0.34	42±.29
90 (t_4)		32±.43	14±.26	31±.24	25±.18	27±.26	24±.26	30±0.29	31±.18
$T_2 - t_3$		6	24	8	6	6	7	8	7
$T_3 - t_4$		6	15	8	9	7	9	10	11
% RA ♣	t_2–t_3	75	-	72.41	75	75	75	66.6	70.8
	t_3-t_4	60	-	42.85	40	53.33	40.0	33.3	26.6

♣ -Percentage retention activity at a particular time interval (t_2–t_3) and (t_3-t_4) in µg of the respective drug in male Wistar rats. Each point represents the mean±SEM of 6 animals per group the two-tailed P<0.0001. SE - standard error, S_d stands for the standard drug (probenecid), C_d -control, D_d -(2, 4, 5, 6, 7 and 8) title compound synthesized in the laboratory.

TABLE 3: PRELIMINARY SCREENING OF ANALGESIC ACTIVITY IN MALE WISTAR RATS (TAIL FLICK METHOD)

Comp/ concn (mg/kg)	BRT (s)	Analgesic activity at different interval after drug treatments response time (s) min						
		10	20	30	40	60	80	90
Std /100	3.0±.03	4.8±.04	6.0±.04	5.4±.03	4.8±.03	4.2±.04	3.9±.01	3.0±.02
4/100	2.5 ± .05	4.0±0.4	5.0±.04	4.0±.03	3.0±.03	2.5±.01		
5/100	2.8± .02	5.0±.04	5.6±.04	5.6±.03	4.8±.04	3.8±.03	2.8±.02	
6/100	2.7± .03	4.9±.04	5.4±.04	4.9±.03	4.3±.04	3.5±.03	2.7±.02	
7/50	3.0± .04	6.0±.05	6.0±.03	6.0±.04	5.4±.03	4.8±.03	3.9±.03	3.0±.03
8/50	2.5± .02	5.0±.05	5.0±.02	5.0±.02	4.5±.02	3.8±.03	3.0±.03	2.5±.02

Concn –concentration, BRT–basal response time, Comp–compound, M-mean, Std-standard drug (acetyl salicylic acid) and each point represents the mean±SEM of 6 animals per group the two-tailed P<0.0001

tee (No.25/1999/CPCSEA). Group of six male Wistar rats weighing between 200-270 g were treated intraperitoneally with the test compounds (32 mg/kg) or the standard (32 mg/kg), before 10 min of intraperitoneal injection with 2.5 ml/kg of a 3% aqueous solution of phenolsulfonphthalein., blood samples were withdrawn using the retro-orbital puncture method after 30, 60 and 90 min. The blood was centrifuged and plasma (0.1 ml) was diluted with 1 ml of sodium bicarbonate solution and 8.9 ml of saline was added. Using colorimeter extinction at 546 nm was determined[5-11].

Standard curve of concentration (20-100 μg/ml) was plotted against absorbance. Percent retention activity (% RA) of the drug $=[(C)_d-(D)_d]\times100\div(C)_d$, where, $(D)_d$ is the average concentration of dye in μg/100 μl of plasma diluted to 10.0 ml at the given interval of time, in six male Wistar rats, when the respective drugs, $(S)_d$, $(2)_d$ to $(8)_d$ were administered [$(3)_d$ was not administered], $(C)_d$ is the average concentration of dye in μg/100 μl of plasma diluted to 10.0 ml at the given interval of time, in six male Wistar rats, when no drug was administered (control group) and $(S)_d$ represents the average concentration of dye in μg/100 μl of plasma diluted to 10.0 ml at the given interval of time, in six male Wistar rats when the standard drug was administered. It can be automated for objective measurement. This produces reliable measurements of thresholds, and has become one of the standard tests for determining the analgesic properties of drugs or behavioural treatments. New baselines are established with each new testing session. Increase in the latency for the rat to flick its tail is indicative of analgesia.

The tail of the male Wistar rat was placed in the analgesiometer. The coil temperature was raised by the passage of electric current and a fix current was given to a particular rat throughout the experiment, which was previously adjusted to ensure a tail withdrawal reflex within 2.5-4.0 s prior to the drug treatment. Tail flick latencies were measured at 10 min intervals until a baseline was obtained over three consecutive trials. Only rats showing a stable baseline after three trials were used. For cut-off time, (stable baselinex2) s was established to minimize the probability of skin damage. Group of six male Wistar rats weighing between 100-170 g were treated intraperitoneally with the test compounds.

The basic aim of the project was to have compounds which possess weak to moderate uricosuric and strong analgesic activities. The weak to moderate uricosuric activity is desired because uricosuric agents like probenecid and sulphinpyrazone cannot be given during the gout attack. If the uricosuric agent is given than it causes rebound attack due to the increase in uric acid excretion. Hence, we were interested in having compounds, which possess weak/moderate uricosuric and srong analgesic activities, so that it can reduce the pain (normally during gout attack any NSAID can be given). All this factor prompted us to make series, which could possess strong analgesic and weak or moderate uricosuric activities. Salicylic acid substituted with sulfonamide group is known to possess strong analgesic activity. Hence, we were keen to have compounds, which were analogues of probenecid and salicylic acid. The purpose of having a weak to moderate uricosuric agent can be obtained by replacing different secondary amines. The

uricosuric activities of the derivatives were compared with probenecid and they have significant activity in comparison to probenecid.

Uricosuric activities of novel analogues in experimental model animal are tabulated in Table 2. The possibility of uricosuric activity of various title compounds synthesized has been observed from experimental findings in terms of plasma dye retention time. Significant uricosuric activity was observed in all the compounds in comparison to probenecid. The salicylic acid and probenecid are known to have antiuricosuric activity, when they are less in concentration and the same paradoxical effect was observed with these derivatives. The analgesic activity of benzoic acid derivatives are tabulated in Table 3 evaluated by analgesiometer (INCO) using tail flick method. Five compounds were tested and were compared with acetyl salicylic acid as standard. Of these, compounds 7 and 8 showed better analgesic activity in terms of onset as well as duration of action as compared to acetyl salicylic acid. The combination therapy of uricosuric and analgesic agent during acute attack could cause precipitation. It would be very interesting to see the dual activity of a drug possessing weak to moderate uricosuric as well as strong analgesic effect in person suffering from acute attack of gout.

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REFERENCES

1. Smiles, S. and Stewart, J., **J. Chem. Soc.**, 1921, 119, 1792.
2. Stewart, J., **J. Chem. Soc.**, 1922, 121, 2555.
3. Dyer, J.R., Eds., In; Application of Absorption Spectroscopy of Organic Compounds, Prentice-Hall, New Jersey, 1994, 33.
4. Abdullah, A.A., Eds., In; Florey, K., Analytical Profiles of Drug Substances, Academic press, New York, 1984, 10, 639.
5. Vogel, H.G., Eds., In; Drug Discovery and Evaluation, Springer-Verlag Berlin Heidelberg, Germany 1997, 178.
6. Scarborough, H.C. and McKinney, G.R., **J. Med. Chem.**, 1962, 5, 175.
7. Turner, R.A., Eds., In; Screening Methods in Pharmacology Vol. 1, Academic Press, New York, 1965, 262.
8. Turner, R.A., Eds., In; Screening Methods in Pharmacology, Vol. 2, Academic Press, New York, 1971, 228.
9. Oliveira, M.A. and Prado, W.A., **Braz. J. Med. Biol. Res.**, 1998, 31, 681.
10. D'Amour, F. E. and Smith, D. L., **J. Pharmacol. Exp. Ther.**, 1941 72, 74.
11. Gracely, R. H. Eds., In; Advances in Behavioral Medicine, Volume 1, JAI Press, New York, 1985, 211.