SYNTHESIS, ANTI-INFLAMMATORY AND ANTIMICROBIAL ACTIVITY OF 2-(ISOXAZOL-5-YL)-PHENYL-4-METHYLBENZENE SULFONATE DERIVATIVES

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A B S T R A C T

A new series of 2-(isoxazol-5-yl) phenyl 4-methylbenzene sulfonate derivatives (5a-e) were prepared by the cyclo-condensation reaction of 2-[3-(dimethylamino) acryloyl]-phenyl 4-methylbenzene sulfonates (4a-e) and hydroxylamine hydrochloride in presence of acetic acid. The synthesized compounds were screened for their antimicrobial and anti-inflammatory activities. They were found active against microorganisms and showed significant anti-inflammatory activity.

Keywords: **Arylsulfonate; COX-2 inhibitors; Enaminone; Anti-inflammatory activity; Antimicrobial activity**

1. Introduction

The history of heterocyclic compounds described the prominent role of isoxazole derivatives as medicinally active compounds.[1] A wide variety of biologically active compounds are known to possess isoxazole nucleus in their chemical structure. On the other hand, the aryl sulphonate group is a common functionality present in many molecules and has found broad application in medicines, drugs and agricultural chemistry.[2] The compounds containing aryl sulfonate moiety have dominated the surfactant industry and received considerable attention during last two decades as they are endowed with variety of biological activities like papillomavirus microbicidal[3] and anti-neoplastic.[4] Isoxazole derivatives have diverse biological properties such as a typical antipsychotic,[5] antibacterial,[6] anti-inflammatory,[7] antitumor,[8] insecticidal[9] and antioxidant.[10]

Some heterocyclic compounds containing isoxazole moiety were discovered as non-steroidal anti-inflammatory drug (NSAIDs). The COX-1 and COX-2 are the iso-forms of non-steroidal anti-inflammatory drugs differ in their functions.[11] Prostaglandins whose biosynthesis involves the cyclooxygenase-1 enzyme is responsible for the physiological housekeeping functions such as platelet aggregation and gastric cytoprotection, while COX-2 is inducible which creates problems like inflammation and pain.[12]

In the course of search for COX-2 inhibitors, it was observed that the structure of several heterocyclic compounds containing isoxazole nucleus were discovered as COX-2 inhibitors. The selective inhibition of COX-2 prevents the gastrointestinal irritation and gives relief from pain and inflammation. The over expression of COX-2 enzyme has been shown to promote angiogenesis, [13] cell proliferation[14] and inhibit apoptosis.[15] In association with these applications, we report herein the selective synthesis of 2-(isoxazol-5-yl) phenyl 4-methylbenzene sulfonate derivatives (5a-e).

2. Experimental

General procedure for the synthesis of enaminones (3a-e): To a mixture of o-hydroxy acetophenone (0.05mol) and N, N-dimethyl formamide dimethyl acetal (0.05mol), dry toluene (85ml) was added and reaction mixture was refluxed for 6-7 hrs on water bath. After completion of reaction as indicated by TLC the reaction mixture was filtered and concentrated under vacuum. The crude solid obtained was washed with cold toluene (3 x 10ml) to enhance the purity of product and crystallized with ethyl alcohol.

General procedure for the synthesis of 2-[3-(dimethylamino)-acryloyl]-phenyl-4-methylbenzene sulfonate (4a-e): To a mixture of compound 3a-e (0.01mol), p-toluene sulfonyl chloride (0.01mol) and anhydrous K_2CO_3 (0.015 mol) was grinded well in mortar for 7-8

minutes. The reaction mixture was then allowed to leave for 1h at room temperature; a pasty solid obtained was poured into cold water and stirred well for 10 minute. A solid separated out was filtered, washed with cold dilute sodium hydroxide solution and finally by cold water. The crude product obtained was crystallized from ethyl alcohol.

General procedure for the synthesis of 2-(isoxazol-5-yl) phenyl-4-methylbenzene sulfonates (5a-e): To a mixture of compound 4a-e (0.01mol) and hydroxylamine hydrochloride (0.01mol) in glacial acetic acid (20 ml) was refluxed on water bath for 3-4 hours. The reaction mixture was brought to room temperature and diluted with 20 ml cold water and then neutralized with sodium bicarbonate. The solid obtained was filtered, washed with cold water, dried and crystallized from ethyl alcohol to give products 5a-e.

Compound **5a**: 2-(Isoxazol-5-yl) phenyl-4-methylbenzene sulfonate: Yield: 83 %; M. p: 158-160 °C; ^{1}H-NMR (DMSO-d6) δ: 2.41(S, 3H), 6.71(d, 1H), 6.89(d, 1H), 6.91(t, 1H), 7.04(t, 1H), 7.25(d, 1H), 7.36(d, 2H), 7.61(d, 1H), 7.87(d, 2H). ^{13}C-NMR ($CDCl_3$): 21.41, 103.16, 116.29, 119.42, 122.60, 127.09, 127.24, 128.10, 129.46, 130.74, 131.60, 145.73, 150.66, 154.41, 163.57, 165.83.

Compound **5b**: 2, 4-Diiodo-6-(isoxazol-5-yl) phenyl-4-methylbenzene sulfonate: Yield: 87 %; M. p: 177-180 °C; ^{1}H-NMR (DMSO-d6) δ: 2.45 (S, 3H), 6.52 (d, 1H), 7.30 (d, 2H), 7.67 (S, 1H), 8.07 (d, 2H), 8.08 (d, 1H), 8.10 (S, 1H). ^{13}C-NMR ($CDCl_3$): 21.69, 92.59, 104.03, 125.22, 128.63, 129.96, 133.54, 138.01, 146.21, 147.08, 149.27, 150.41, 162.89, 164.23.

Compound **5c**: 2, 4-Dibromo-6-(isoxazol-5-yl) phenyl-4-methylbenzene sulfonate: Yield: 88 %; M. p: 142-145 °C; ^{1}H-NMR (DMSO-d6) δ: 2.45 (S, 3H), 6.58 (d, 1H), 7.30 (d, 2H), 7.65 (S, 1H), 7.80 (S, 1H), 7.92 (d, 1H), 8.12 (d, 2H). ^{13}C-NMR ($CDCl_3$): 21.64, 104.25, 119.99, 121.02, 125.65, 128.40, 129.87, 131.00, 137.46, 143.24, 146. 14, 150.52, 162.66

Compound **5d**: 2-Iodo-6-(isoxazol-5-yl)-3,4-dimethylphenyl-4-methylbenzene sulfonate: Yield: 82 %; M. p: 112-115 °C; ^{1}H-NMR (DMSO-d6) δ: 2.43 (S, 3H), 2.47 (S, 3H), 2.53 (S, 3H), 6.37 (d, 1H), 6.82 (S, 1H), 7.26 (d, 2H), 7.65 (d, 1H), 8.01 (d, 2H). ^{13}CNMR ($CDCl_3$): 21.47, 26.48, 29.54, 92.58, 102.91, 125.47, 128.39, 129.65, 133.89, 145.59, 150.17, 155.27 and 164.55.

Compound **5e**: 4-Chloro-2-(isoxazol-5-yl)phenyl-4-methylbenzene sulfonate: Yield: 90%; M. p: 126-128 °C; ^{1}H-NMR (DMSO-d6) δ: 2.41 (s, 3H), 6.71 (d, 1H), 7.25 (d, 1H), 7.36 (d, 2H), 7.57 (s, 1H), 7.61 (d, 2H), 7.87 (d, 2H). ^{13}CNMR ($CDCl_3$): 21.65, 92.55, 104.02, 117.98, 125.74, 125.78, 127.21, 129.87, 130.02, 130.24, 138.03, 141.51, 149.31, 150.49, 162.89, 164.24.

3. Results & Discussion

Anti-inflammatory activity

The normal control, Indomethacin and test compounds were administered to the rats 30 minutes before the injection of 0.1ml of 1% Carrageenan suspension in normal saline. The test drugs 50 mg/kg and the standard drug 10 mg/kg were dosed to the animals. The animals were divided into eight groups containing six animals in each group. Male and female adult Wistar albino rats marked H, B, and T having weight 25-50 gm were used for the study. The animals were kept overnight on fasting. The anti-inflammatory activity study was carried by using Winter et al. method.[16] The experimental procedures were carried out under the guidelines of Institutional Animal Ethics Committee (IAEC) at National Toxicology Centre, Pune. A no. 26 gauge needle was used to inject the Carrageenan suspension into the sub planar region of the right hind paw. Immediately thereafter the oedema volume of the injected paws were measured plethysmographically by water displacement method. For the comparison purpose volume of oedema at various prefixed time intervals 1h, 2h, 4h and 6h was measured. The difference between paw volumes of the treated animals was measured and the mean oedema volume was calculated. Percentage reduction in oedema volume was calculated by using the formula, % reduction = 100 x V0-Vt/V0. Where, V0 = Volume of the paw of control at time 't'. Vt = Volume of the paw of drug treated at time 't'. From the obtained data, the mean oedema volume and percentage reduction in oedema was calculated. The results are presented in Table-1. The SD and SEM were calculated by using ANOVA, Dunnet's't' test. The compound no. 5b, 5c and 5d showed significant anti-inflammatory activity in comparison with standard drug Indomethacin.

Antimicrobial activity

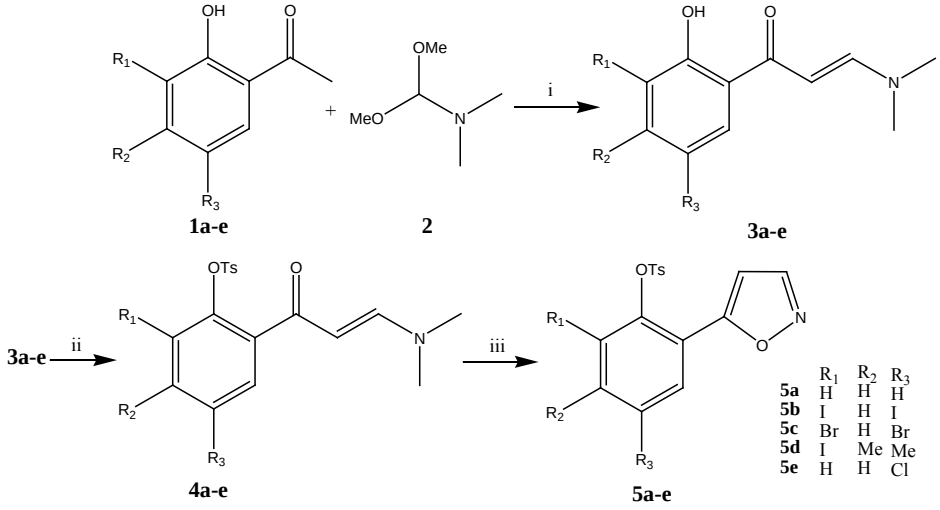
The antibacterial activity of the test samples 5a-e was determined by agar cup plate method[17] using ampicillin (100µg/ml) as standard drug and four pathogens such as *Bacillus subtilis, Staphylococcus aureus, Escherichia coli and Pseudomonas aeruginosa*. This method was based on diffusion of antibacterial component from reservoir bore to the surrounding inoculated nutrient agar medium so that the growth of microorganisms was inhibited as circular zone around the bore. The concentration of test compounds was 100µg/ml and was prepared in dimethyl Sulfoxide (DMSO). The test samples and standard drug were placed in a bore made in Petri dishes, which contains different pathogens and were incubated at 37 °C for 24 hours. The zone of inhibitions around the bore was measured after 24 hours. The antibacterial activity was classified as standards (>22mm), highly active (15-22 mm), moderately active (10-15 mm), least active (7-10 mm) and less than 7 mm was taken as inactive. The antifungal activity of synthesized compounds was determined by using *Aspergillus niger, Aspergillus flavus* and *Fusarium oxysporium* pathogens. Dimethyl sulphoxide was used as control and dextrose agar as culture medium for antifungal activity. Norcadine (100µg/ml) was used as standard drug for the comparison and determination of their antifungal activities. The data are recorded in Table-2.

The minimum inhibitory concentration (MIC) against the organisms was determined by the method of serial dilutions.[18] Stock solutions of standard compound and synthesized compound having concentration 250µg /ml was prepared by dissolving 25 mg of synthesized compound in 2ml of DMSO and was made 100 ml with sterile distilled water. From this stock solution, the solutions of different concentrations such as 50µg/ml, 25µg/ml, 12.5µg/ml, 6.25µg/ml and 3.12µg/ml were prepared. The results are presented in Table-3.

4. Conclusion

In conclusion, a simple, efficient and cost-effective procedure was developed for the synthesis of 2-(isoxazol-5-yl) phenyl 4-methylbenzene sulfonate derivatives (5a-e) by using inexpensive and commercially available K_2CO_3 as catalyst and acetic acid as solvent medium. The compound 5a and its derivatives were investigated for their anti-inflammatory and antimicrobial activity. The synthesized compounds (5b-d) showed significant anti-inflammatory activity when compared with standard Indomethacin. The antimicrobial activity of compound 5b, 5c, 5d and 5e were found to be more active compared with standard drugs. The minimum inhibitory concentration data also showed the lowest concentration of an antimicrobial (5b, 5c and 5e) that would inhibit the visible growth of a microorganism after overnight incubation.

1.1. Structure



Scheme-1: Reaction conditions: (i) Toluene, 7-8 h; (ii) p-Toluene sulfonyl chloride, K_2CO_3, 7-8 min.; (iii) Acetic acid, $NH_2OH.HCl$, 3-4 h.

1.2. Table 1: Anti-inflammatory activity of isoxazole derivatives (5a-e)

Group (n)	Substance	Dose mg/kg	Difference in paw Oedema volume after							
			1 h		2 h		4 h		6 h	
			Mean ± SEM	% ROV	Mean ± SEM	% ROV	Mean ± SEM	% ROV	Mean ± SEM	% ROV
1.	Control	0.1 ml	4.94 0.219	-	4.63[a] 0.210	-	4.93 0.446	-	4.73 0.262	-
2.	Standard	10	4.56[a] 0.256	7.69	4.16 0.171	10.15	4.29 0.231	12.98	3.96[a] 0.182	16.27
3.	5a	50	4.90 0.021	0.81	4.50 0.087	2.80	4.71[b] 0.315	4.46	4.41[a] 0.142	6.76
4.	5b	50	4.65 0.158	5.87	4.29[b] 0.072	7.34	4.41 0.132	10.54	4.07 0.245	13.95
5.	5c	50	4.74[b] 0.317	4.04	4.46[b] 0.218	3.67	4.59[b] 0.415	6.89	4.27[a] 0.231	9.72
6.	5d	50	4.70[b] 0.210	4.85	4.40[a] 0.212	4.96	4.46[c] 0.415	9.53	4.23[b] 0.236	10.57
7.	5e	50	4.88[b] 0.203	1.21	4.47[b] 0.124	3.45	4.67[c] 0.455	5.27	4.36[b] 0.156	7.82

n: Six albino rats in each group; ROV: Reduction in Oedema volume; ± SEM: The standard error of the mean; Standard: Indomethacin drug; Significance level: [a]$p<0.05$, [b]$p<0.01$, [c]$p< 0.001$ compared with respective control

Table 2: Antibacterial and antifungal activity of synthesized compounds (5a-e)

Compound	bacteria (zone of inhibition in mm)			fungi (zone of inhibition in mm)			
	A	B	C	D	E	F	G
5a	08	10	12	09	-	-	10
5b	14	16	19	14	13	19	08
5c	12	18	15	13	10	17	14
5d	13	15	10	12	08	-	13
5e	18	21	20	16	22	18	15
Std.	20	21	22	24	25	20	26

A = *Bacillus subtilis*; B = *Staphylococcus aureus*; C = *Pseudomonas aeruginosa*; D = *Escherichia coli*; E= *Aspergillus niger*; F= *Aspergillus flavus*; G= *Fusarium oxysporium*; Concentration of the test compounds (5a-e) and standard drugs (Ampicillin & Norcadine): 100 µg/ml, Solvent Control: DMSO.

Table 3: Minimum inhibitory concentration (MIC µg /ml) of isoxazole derivatives

Compound	A	B	C	D	E	F	G
5a	25.0	25.0	25.0	50.0	50.0	50.0	25.0
5b	12.5	12.5	6.25	25.0	20.0	6.25	20.0
5c	6.25	3.12	3.12	12.5	6.25	3.12	6.25
5d	6.25	12.5	6.25	25.0	12.5	50.0	25.0
5e	3.12	3.12	3.12	6.25	3.15	12.5	12.5
Std.	3.12	3.12	3.12	6.25	3.12	6.25	3.12

A= Bacillus subtilis; B= Staphylococcus aureus; C= Pseudomonas aeruginosa; D= Escherichia coli; E= Aspergillus niger; F= Aspergillus flavus; G= Fusarium oxysporium; Concentration of the test compounds (5a-e) and standard drug.

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