Synthesis and Pharmacological Activities of Some New 5-Substituted-2-mercapto-1,3,4-oxadiazoles

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Desai, *et al.*: New 5-Substituted-2-mercapto- 1,3,4-oxadiazoles: Synthesis and Evaluation

In this study, various 5-β-[(N-benzenesulphony/tosyl)-4-(un) substituted anilino]ethyl-2-mercapto-1,3,4-oxadiazole (4a-f), with sulphonamide moiety at the side chain have been synthesised. The structures of the newly synthesised compounds have been established on the basis of their spectral data and elemental analysis. All the compounds were screened for antimicrobial activities against *Escherichia coli*, *Bacillus cirroflagellosus*, *Aspergillus niger*. *Colletotrichum capsici* and antituberclosis activity against *Mycobacterium tuberculosis H37Rv* strain. Only two compounds 4b (73%) and 4e (54%), have shown moderate antituberculosis activity. All the compounds have shown moderate antiinflamatory activity and least ulcerogenecity. Most of the compounds have shown significant analgesic activity (64.20-120.72%) in comparison with the standard, Aspirin (49.39%) In the MES method, however only compound 4a, exhibited a protection of 33.33%, and others failed to protect.

Key words: Antimicrobial, analgesic, antiinflamatory, antitubercular, anticonvulsant, 1,3,4-oxadiazole

Various 5-substituted-2-mercapto-1,3,4-oxadiazoles posses significant antimicrobial[1-3], antiinflammatory[4-6], analgesic[4-6], anticonvulsant[7,8], antitubercular[9,10] and hypoglycemic[11] activities. In view of the established pharmacological activities of 1,3,4-oxadiazoles and in continuation[12-14] of our quest for 1,3,4-oxadiazoles with better pharmacological activities, we report herein the synthesis, pharmacological and antimicrobial activities of some new 5-β-[(N-benzenesulphonyl/tosyl)-4-(un) substituted anilino] ethyl-2-mercapto-1,3,4-oxadiazoles (4a-f) with "sulphonamide isostere" a pharmacologically active moiety at the 5th position of 1,3,4-oxadiazole nucleus. All the compounds were screened for antituberculosis activity against *Mycobacterium tuberculosis H37Rv* strain and antimicrobial activities against *Escherichia coli*, *Bacillus cirroflagellosus*, *Aspergillus niger* and *Colletotrichum capsici*. Same compounds were also screened for pharmacological activities such as antiinflammatory, analgesic, anticonvulsant, change in adrenal gland weight and ulcerogenic activity. Synthetic route is, depicted as Scheme 1.

Melting points were determined in open capillaries and are uncorrected. IR spectra in KBr, were recorded on a Perkin Elmer Spectrophotometer and 1H-NMR spectra on a Varian 300 MHz NMR spectrometer using TMS as an internal standard (chemical shifts in δ ppm). Mass spectra were recorded on Finnigan Mat 8230 spectrophotometer.

Methyl-β-[(4-H/CH_3/Cl)anilino]propionates, (1a-f), were prepared according to the literature methods[15-17]. Esters were sulphonylated/tosylated with benzenesulphonyl/tosyl chloride to methyl-β-[(N-benzenesulphonyl/tosyl)anilino]propionates) (2a-f)[18]. β-[(N-Benzenesulphonyl/tosyl)-4-(un)substituted anilino]propionic acid hydrazides (3a-f)[19], were prepared by refluxing a mixture of methyl-β-[(N-benzenesulphonyl/tosyl)anilino]propionates (2a-f) in ethanol with hydrazine hydrate. Hydrazides (3a-f), when refluxed with an alcoholic solution of potassium hydroxide and carbon disulphide yielded the title compounds. Title compounds were characterised by IR, ^{1}H-NMR and mass spectral studies.

General procedure for preparation of 5-β-[(N-benzene sulphonyl/tosyl)-4-un(substituted anilino]ethyl-2-mercapto-1,3,4-oxadiazoles (4a-f), involved the addition of carbon disulphide (2.43g, 0.02 mole) dropwise to a clear solution of potassium hydroxide (1.12 g, 0.02 mol) in water (10 ml) and β-[(N-benzenesulphonyl/tosyl)-4-(un)substituted anilino] propionic acid hydrazide (3a-f) (0.02 mol) in 15 ml ethanol with stirring and cooling in ice. Reaction mixture was refluxed on water bath till the evolution

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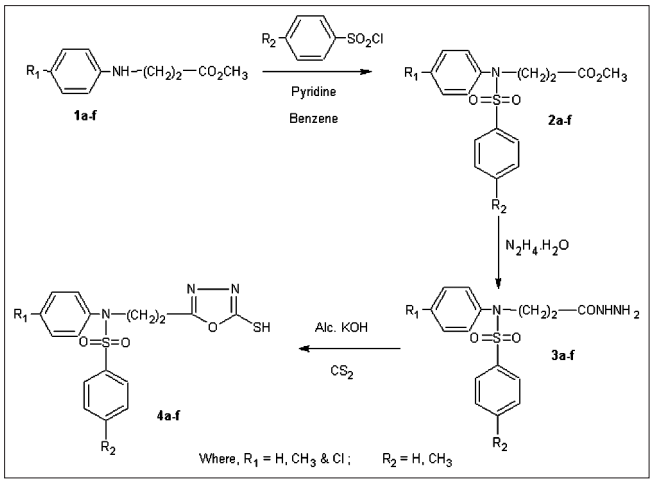

Scheme 1: Synthesis of 5-β-[(N-benzene sulphonyl/tosyl)-4-(un) substituted anilino]ethyl-2-mercapto-1,3,4-oxadiazoles (4a-f)

of hydrogen sulphide gas ceased (8-10 h). The reaction mixture was concentrated in vacuo, residual mass was poured on crushed ice and neutralised with acetic acid. The precipitated oxadiazole was separated and crystallised from ethyl alcohol. 4d: IR (KBr) υ cm^{-1}: 3500(b) (NH), 3000(s) (CH_2), 1680(s), 1540 (m) (>C=C< and >C=N-), 1190 (s) (-C-O-C-), 1420 (m) (>C-N-), 1200(s), 1150 (m) (>C=S), 1380 (s) (SO_2). 4d: ^{1}H-NMR (DMSO-d6**):** δ 2.34 (s, 3H, CH_3), 2.40 (s, 3H, CH_3), 2.91 (t, 2H, -CH_2-), 3.89 (t, 2H, -N-CH_2), 6.89-7.63 (m, 8H, ArH), 10.68 (br.s. 1H, -NH-C=S, D_2O-exchangeable). 4d: MS; (m/z, Rel.Abund): 274 (99), 155 (99), 119 (78), 105 (24), 76 (10), 66 (16). 4e: IR (KBr) υ cm^{-1}:3400(b) (NH), 3000(s) (CH_2), 1660(s), 1540 (m) (>C=C< and >C=N-), 1190 (s) (-C-O-C-), 1430 (m) (>C-N), 1220(s), 1120(m) (>C=S), 1370 (s) (SO_2). 4e: ^{1}H-NMR (DMSO-d6**)**: δ, 2.90 (t, 2H, CH_2), 3.90 (t, 2H, -N-CH_2), 7.01-7.81 (m, 9H, ArH) 13.98 (br.s., 1H, -NH-C=S – D_2O exchangeable). Physicochemical properties of title oxadiazoles are enumerated in Table 1**.**

After establishing the physicochemical and spectral properties, the newly synthesised oxadiazoles were tested for their antituberculosis activity against *M. Tuberculosis H37Rv* by Bactec 460 radiometric system at Southern Research Institute, Frederic Research Centre, Frederic MD. All the compounds were tested at a concentration of 12.5 µg/ml. Rifampicin was used as a standard (97% at 12.5 µg/ml).

Newly synthesised oxadiazoles, (4a-f) were first subjected to acute toxicity study by Miller and Tainter[20] method, in order to determine their LD_{50}. The graphically calculated LD_{50} values of the newly synthesised compounds were found to be 2291 (4e), 1405±17.04 (4d and 4a), 1086±17.04 (4b and 4c), 816±13.08 (4f).

Antiinflammatory activity of 1,3,4-oxadiazoles (4a-f) was studied by carrageenan induced rat paw edema method (acute) due to Wilhelmi *et al*[21], cotton pallet induced granulation tissue formation method (chronic) due to Meir *et al.*[22] and adrenal weight change methods. An arbitrary scoring system[23] was followed to determine the severity of the ulcers. Analgesic activity of newly synthesised oxadiazoles (4a-f) was carried out by radiant heat induced rat tail flick method[24] using aspirin as a standard. Anticonvulsant activity was studied by MES method[25,26]. The percentage protections were then assessed by Fischer's exact test[27].

All the compounds were screened for antimicrobial activities against Gram negative bacterium *Escherichia coli*, Gram positive bacterium *Bacillus cirroflagellosus* and fungi *Aspergillus niger* and *Colletotrichum capsici* by cup plate method[28]. Antimicrobial activity is calculated as relative percent inhibition, RI with reference to the standard. DMF was used as solvent control. Cotrimoxazole (trimethoprim 500 mg and sulphamethoxazole 800 mg) and Diflucan (fluconazole) are used as standards for antibacterial and antifungal respectively.

In the carrageenan induced rat paw edema method, all the compounds exhibited moderate to minimum antiinflammatory activity, 4a (19.36% , 43.35%, 40.15%), 4b (--, 39.26%, 59.11%), 4c (--, 43.39%, 57.61%), 4d (--, 43.31%, 72.31%), 4e (51.59%, 47.37%, 60.10%) 4f (19.36%, 79.76%, 75.07%), in comparison with the standard aspirin (54.84%, 55.05%, 73.81%) after 1, 3, 5 h respectively. However the same compounds exhibited better antiinflammatory activity 4a (57.84%), 4b (53.72%), 4c (46.14%), 4d (61.57%), 4e (50.97%), 4f (58.69%)**,** in comparison to aspirin (41.886%) in the cotton pellet method. This has been further substantiated by the suppression of adrenal gland weight of the compounds (58.27-45.09) in comparison with that of the standard aspirin (50.13%) except for compounds 4c and 4d**.** All the synthetic 1,3,4-oxadiazoles have shown lesser degree (20.00-40.00) of ulcerogenecity, as compared to standard aspirin (43.33) Table 2**.**

TABLE 1: PHYSICOCHEMICAL PROPERTIES, ANTIMICROBIAL AND ANTI-TUBERCULAR ACTIVITIES OF 1,3,4-OXADIAZOLES (4a-f)

Compd* No	R_1	R_2	M.P, (°)	Yield (%)	Antimicrobial activity , (RI)				Antituberculosis activity** *M. tuberculosis* (Percent inhibition)
					E. coli	*B. cirro*	*A. niger*	*C. capsici*	
4a	H	H	133-35	45	20.68	------	38.22	100.00	30
4b	H	CH_3	80-81	40	20.68	------	20.72	64.65	73
4c	CH_3	H	130-31	46	----	36.99	20.72	126.93	34
4d	CH_3	CH_3	140-41	47	46.06	------	78.76	223.90	36
4e	Cl	H	100-01	45	20.68	4.34	48.52	126.93	54
4f	Cl	CH_3	90-91	46	-----	72.37	48.51	206.06	----

*All the compounds were analysed for C, H and N. The experimental values were within ± 0.04% of the calculated value. ** - Rifampicin as standard (97%, at 12.5 µg/ml), Concentration (antituberculosis activity) - MIC: 12.5 µg/ml (Bactec 460 radiometric system). Concentration (antimicrobial activity)- 100 µg/ml (Cup plate method). DMF as solvent control; Cotrimoxazole (Trimethoprim 500 mg and Sulphamethoxazole 800 mg) and Flucanazole as standards for antibacterial and antifungal respectively. Relative percent inhibition, RI = [100 x (X-Y)/(Z-Y)]; where X , Y and Z are total area of inhibitions in test, solvent (DMF) and standard respectively; Area = πr^2; where r = Radius of zone of inhibition.

TABLE 2: PHARMACOLOGICAL ACTIVITIES OF 5-β-[(N-BENZENESULPHONYL/TOSYL)-4-(UN)SUBSTITUTED ANILINO] ETHYL-2-MERCAPTO-1,3,4-OXADIAZOLES (4a-f)

Comp no	Dose (mg/Kg b.w.,)	Ulcer index	Antiinflammatory activity[a] Percentage inhibition					Analgesic activity[a] percent protection (*P*<0.05)	Anti convulsant[b] activity
			Carrageenan method			Cotton pellet method	Adrenal weight method	Rat tail flicK method	MES protection
			1 h	3 h	5 h				
Std (a/b)	200	43.33 <0.02	54.84 <0.01	55.05 <0.001	73.81 <0.001	41.89 <0.01	50.13 <0.001	49.39	100 >1(s)
4a	100	33.33 >0.1	19.36 >0.5	43.35>0.02	40.15 <0.001	57.84 <0.001	48.28 <0.001	68.24	33.33 <0.03 (ns)
4b	100	36.66 <0.05	----	39.26 >0.1	59.11 <0.01	53.72 <0.001	45.09 <0.001	83.04	0 --- (ns)
4c	100	20.00 >0.5	----	43.39 <0.1	57.61 <0.01	46.14 <0.001	28.61 <0.001	64.20	0 ---(ns)
4d	100	40.00 <0.02	----	43.31 <0.01	72.31 <0.001	61.57 <0.001	30.43 <0.001	76.31	0 ---(ns)
4e	200	40.00 <0.02	51.59 >0.1	47.37 <0.02	60.10 <0.001	50.97 <0.001	58.27 <0.001	120.72	0 ---(ns)
4f	75	36.66 <0.05	19.36 >0.5	79.76 <0.001	75.07 <0.001	58.69 <0.001	50.31 <0.001	109.95	0 ---(ns)

Std (Standard) a- Aspirin and b- Phenytoin sodium. LD50 ± D (Miller and Tainter method): 2291 (4e), 1405±17.04 (4d), 1405±12.23 (4a), 1086±17.04 (4b,4c), 816±13.08 (4f); a-standard used for antiinflamatory and analgesic activities; b-standard used for anticonvulsant activity; statistical differences between the treatment and the control group of animals were evaluated by student's t test for evaluation of the analgesic and antiinflammatory activity; s- Significant, ns-Non significant Dose for anticonvulsant activity; Std Phenytoin sodium(18mg/kg b.w), 4a-4d (200 mg/kg b.w), 4e and 4f (150mg/kg b.w)

In the radiant heat induced rat tail flick method, all the compounds exhibited most significant analgesic activity in comparison with aspirin (49.39%, < 0.01). The activity is in the order 4e (120.72%), 4f (109.95%), 4b (83.04%), 4d (76.31%), 4a (68.24%), 4c (64.20%) (Table 2).

In the maximum electroshock seizure (MES) method only compound 4a has shown a protection of (33.33%) in comparison with the standard Phenytoin (100%) (Table 2). All the compounds have shown greater antifungal activity against *Colletotrichum capsici*. The activity expressed as RI, enumerated in the decreasing order is 4f (206.06%), 4d (223.90%), 4c and 4e (126.93%), 4a (100.00%) and 4b (64.65%). However the same compounds exhibited minimum antifungal activity (20.72-48.52%) against *Aspergillus niger*, except compound 4d (78.76%). The compounds have shown moderate to minimum antibacterial activity, 4a, 4b, 4e (20.68%), 4d (46.06) against *Escherichia coli* and 4c (36.99%), 4e (4.34%) and 4f (72.37%) against *Bacillus cirroflagellosus* respectively (Table 1). Only compound 4b (73%) has shown moderate antituberculosis activity in comparison with the standard Rifampicin which has 97% at 12.5 µg/ml, other compounds exhibited lesser activity (30-54%) against, *Mycobacterium tuberculosis H37Rv* (Table 1).

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