

Note

Synthesis of new substituted sulfonylhydrazinecarboxamides and sulfonylhydrazinecarbothioamides having antifungal and antibacterial activities

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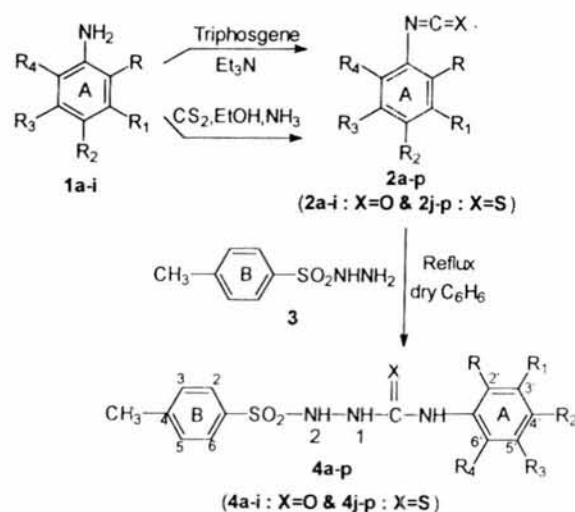
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p-Toluenesulfonylhydrazide **3** on condensation with substituted phenyl isocyanates **2a-i** and substituted phenyl isothiocyanates **2j-p** gives *N*,2-disubstituted sulfonylhydrazinecarboxamides **4a-i** and *N*,2-disubstituted sulfonylhydrazinecarbothioamides **4j-p** respectively. Compounds **4a-p** have been found to be bactericidal against *S.aureus* and *S.typhi* while compound **4n** shows fungicidal activity against *T.mentagrophytes* and *T.rubrum*.

N,2-Disubstituted sulfonylhydrazinecarboxamides and *N*,2-disubstituted sulfonylhydrazinecarbothioamides are reported in literature to possess blood sugar lowering activity^{1,2}, antibacterial activity³, and are also used as blowing agents⁴ in foamed plastics. In view of these reports we have synthesized some new sulfonylhydrazinecarboxamides **4a-i** and sulfonylhydrazinecarbothioamides **4j-p** (Scheme I; cf. Table I). *p*-Toluenesulfonyl hydrazide **3** was prepared by reacting *p*-toluenesulfonyl chloride with hydrazine hydrate⁵. Phenyl isocyanates **2a-i** were prepared by condensation of amines with triphosgene⁶⁻⁸ (Table II) and phenyl isothiocyanates **2j-p** by reaction of amines with carbon disulphide and ammonia as described in literature⁹. Sulfonylhydrazinecarboxamides **4a-i** and sulfonylhydrazinecarbothioamides **4j-p** were prepared by condensation of *p*-toluenesulfonylhydrazide **3** with isocyanates **2a-i** and isothiocyanates **2j-p** respectively. Compounds **4a-p** were purified by column chromatography and further by crystallization. Structures of all the compounds were supported by elemental analyses (Table I), UV, IR and ¹H NMR spectral data.

Antibacterial activity: The bactericidal activity of compounds **4a-p** was determined against *S.aureus* and *S.typhi*. The tests were carried out in DMF and all the compounds **4a-p** showed activity against *S.aureus* and *S.typhi* at different concentrations (Table III).

Methodology: The test compounds were subjected



1a,2a & 4a	: R = F; R ₁ = R ₂ = R ₃ = R ₄ = H
1b,2b & 4b	: R = R ₁ = R ₃ = R ₄ = H; R ₂ = F
1c,2c & 4c	: R = R ₂ = R ₃ = R ₄ = H; R ₁ = F
1d,2d & 4d	: R = Cl; R ₁ = R ₂ = R ₃ = R ₄ = H
1e,2e & 4e	: R = R ₂ = R ₃ = R ₄ = H; R ₁ = Cl
1f,2f & 4f	: R = R ₁ = R ₃ = R ₄ = H; R ₂ = Cl
1g,2g & 4g	: R = R ₂ = R ₃ = R ₄ = H; R ₁ = CF ₃
1h,2h & 4h	: R = R ₁ = Cl; R ₂ = R ₃ = R ₄ = H
1i,2i & 4i	: cyclohexyl
2j & 4j	: R = F; R ₁ = R ₂ = R ₃ = R ₄ = H
2k & 4k	: R = R ₂ = R ₃ = R ₄ = H; R ₁ = F
2l & 4l	: R = R ₁ = R ₃ = R ₄ = H; R ₂ = F
2m & 4m	: R = R ₂ = R ₃ = R ₄ = H; R ₁ = Cl
2n & 4n	: R = R ₁ = R ₃ = R ₄ = H; R ₂ = Cl
2o & 4o	: R = R ₂ = R ₃ = R ₄ = H; R ₁ = CF ₃
2p & 4p	: cyclohexyl

Scheme I

to screening *in vitro* by the tube dilution technique¹⁰ against Gram positive and Gram negative bacteria *Staphylococcus aureus* and *Salmonella typhi* using Mueller Hinton broth as the culture medium.

Muellar Hinton broth (sterilized) was dispensed in each borosilicate test tube (150 × 20 mm). The drug solution was added in order to attain final drug concentration of 200, 100, 40, 25, 20, 10, 5, 2, 1, 0.5, 0.25 etc µg/mL.

At the end, innoculum of standard suspension 0.1 mL of the desired bacterial strain (*S.aureus* or *S.typhi*) containing 10⁶ bacilli/mL was added. The tubes were incubated at 37°C for 48 hr and then examined for the presence or absence of growth of the test organism. The lowest concentration which showed no visible growth was taken as MIC (Minimum Inhibiting Concentration).

Table I—Characterization data of compounds 4a-p.

Compd	m.p. (°C) (solvent)*	<i>R_f</i> Value (Eluent) [†]	Yield (%)	Mol. formula	Calcd (Found)%				
					C	H	N	S	Cl
4a	199-200 (1)	0.463	60	C ₁₄ H ₁₄ FN ₃ O ₃ S	52.01 (52.5)	4.33 4.5	13.00 13.4	9.91 9.6	— (—)
4b	239 (2)	0.32	83	C ₁₄ H ₁₄ FN ₃ O ₃ S	52.01 (52.3)	4.33 4.4	13.00 13.1	9.91 9.5	- (—)
4c	215 (3)	0.192	67	C ₁₄ H ₁₄ FN ₃ O ₃ S	52.01 (52.4)	4.33 4.9	13.00 13.3	9.91 9.6	- (—)
4d	172 (3)	0.514	59	C ₁₄ H ₁₄ ClN ₃ O ₃ S	49.48 (49.3)	4.12 4.6	12.37 12.0	9.42 8.9	10.45 10.4
4e	191-2 (3)	0.474	83	C ₁₄ H ₁₄ ClN ₃ O ₃ S	49.48 (49.0)	4.12 4.5	12.37 12.0	9.42 9.6	10.45 10.0
4f	244-5 (2)	0.56	59	C ₁₄ H ₁₄ ClN ₃ O ₃ S	49.48 (49.0)	4.12 4.6	12.37 12.0	9.42 9.7	10.45 10.8
4g	204-5 (3)	0.521	72	C ₁₅ H ₁₄ F ₃ N ₃ O ₃ S	48.25 (48.4)	3.75 3.9	11.26 11.7	8.58 8.1	- (—)
4h	202 (3)	0.666	72	C ₁₄ H ₁₃ Cl ₂ N ₃ O ₃ S	44.92 (45.3)	3.47 3.5	11.23 10.8	8.55 8.6	18.98 18.8
4i	211 (3)	0.327	80	C ₁₄ H ₁₂ N ₃ O ₃ S	54.02 (53.8)	6.75 7.2	13.50 13.0	10.29 10.6	- (—)
4j	201-2 (3)	0.35	67	C ₁₄ H ₁₄ FN ₃ O ₂ S ₂	49.56 (49.8)	4.13 4.4	12.39 12.7	18.88 18.3	- (—)
4k	195 (3)	0.633	78	C ₁₄ H ₁₄ FN ₃ O ₂ S ₂	49.56 (49.5)	4.13 4.4	12.39 12.7	18.88 18.4	- (—)
4l	203-4 (2)	0.571	57	C ₁₄ H ₁₄ FN ₃ O ₂ S ₂	49.56 (49.1)	4.13 4.6	12.39 12.7	18.88 19.0	- (—)
4m	180 (1)	0.18	76	C ₁₄ H ₁₄ ClN ₃ O ₂ S ₂	47.25 (47.3)	3.94 4.5	11.81 11.7	18.00 18.8	9.98 10.5
4n	208 (1)	0.79	80	C ₁₄ H ₁₄ ClN ₃ O ₂ S ₂	47.25 (46.7)	3.94 4.4	11.81 11.5	18.00 18.5	9.98 10.2
4o	195-7 (3)	0.6	51	C ₁₅ H ₁₄ F ₃ N ₃ O ₂ S ₂	46.27 (46.8)	3.59 3.3	10.79 10.4	16.45 16.1	— (—)
4p	200-1 (3)	0.227	67	C ₁₄ H ₁₂ N ₃ O ₂ S ₂	51.37 (51.0)	6.42 6.9	12.84 12.6	19.57 19.6	- (—)

All compounds 4a-p crystallized as needles.

* (1) Ethyl acetate-petroleum ether (60-80)°(3:1), (2) methanol, (3) ethyl acetate.

Eluent for column chromatography : Ethyl acetate for 4a, 4b, 4c, 4d, 4e, 4f, 4g, 4i; ethyl acetate-acetone (6:4) for 4h; (8:2) for 4n; chloroform-ethyl acetate (2:8) for 4j, 4k, 4l, 4m; and (6:4) for 4o, 4p.

† Eluent for TLC : Chloroform-ethyl acetate (8:2) for 4a, 4d, 4e, 4g, 4m, 4o; (6:4) for 4b, 4l; (2:8) for 4f; (7:3) for 4i, 4k; (9:1) for 4j; chloroform for 4c, 4p and ethyl acetate for 4h, 4n.

Ampicillin and Trimethoprim were used as standard drugs against Gram positive and Gram negative bacteria, respectively and were subjected to similar screening programs. MIC of Ampicillin was 0.01 µg/mL against *S. aureus*, and that of Trimethoprim was 1 µg/ml against *S. typhi*.

Antifungal activity : The antifungal activity of compounds 4a-p was determined against *Candida*

albicans, *Trichophyton mentagrophytes* and *Trichophyton rubrum*. The tests were carried out in DMF and only 4n showed activity against *T. mentagrophytes* and *T. rubrum* (Table IV).

Methodology : The organism employed for the test were *Candida albicans*, *Trichophyton mentagrophytes* and *Trichophyton rubrum*.

These organisms were maintained on Sabouraud's

agar slants by regular subculturing. *C. albicans* was cultured every week and the rest of the organisms at the interval of 12 days.

Schraufstatter¹¹ medium was employed for testing and pH medium was adjusted to 5.2. The medium (sterilized) was dispensed in each borosilicate tube. The drug solution was added in order to attain final drug concentration as 200, 100, 40, 25, 10, 5, 2, 1, 0.5 µg/mL. The tubes were incubated at room temperature (28-30°C) in a dark place. Incubation period of *C. albicans* was 7 days where as that of *T. mentagrophytes* and *T. rubrum* was 14 days. The

presence and absence of growth was noted visually. The lowest concentration which showed no visible growth was taken as end point (MIC).

Miconazole was used as standard drug and was subjected to same screening pattern. MIC of miconazole was 6.25 µg/mL for *C. albicans* and 1.56 µg/mL for *T. mentagrophyte* and *T. rubrum*.

Experimental Section

The general procedures adopted for the preparation of different compounds are described below:

Preparation of *p*-chlorophenyl isocyanate 2f. To triphosgene (3.56 g, 0.012 mole) and triethylamine (2.62 g, 0.024 mole) in dry benzene (30 mL), was added *p*-chloroaniline (3.0 g, 0.024 mole) in dry benzene (20 mL) over a period of 20 min. During addition the temperature of the reaction mixture was raised to reflux. After refluxing for 3 hr, the reaction mixture was cooled and filtered. From the filtrate, benzene was removed by distillation and the residual liquid distilled under reduced pressure to give **2f**, b.p. 48-55/1mm, yield 1.3 g, (36% theory); IR (neat): 2240 (N = C = O) cm⁻¹.

Preparation of *N*-(*p*-chlorophenyl)-2-(*p*-toluenesulfonyl)hydrazinecarboxamide 4f. To **3** (0.558 g, 0.003 mole) in sodium dried benzene (15 mL) was added **2f** (0.46 g, 0.003 mole) in dry benzene (15 mL) and the reaction mixture refluxed for 3 hr, cooled

Table II—Characterization data of isocyanates **2a-i** by triphosgene method

Compd.	b.p. °C/1mm	Yield (%)	IR (neat) (cm ⁻¹)	Reported bp °C/1mm by other methods
2a	41-44	32	2250	65/18mm
2b	42-47	38	2250	68/15mm
2c	42-45	29	2250	69/19mm
2d	53-55	54	2250	200-3
2e	50-54	69	2250	58-62/2.2mm
2f	48-55	36	2250	87/8.5mm
2g	43-45	42	2250	54.5/11mm
2h	58-66	56	2250	not reported
2i	60-65	62	2250	63-5/1mm

Table III—Antibacterial activity of compounds **4a-p**

Minimum Inhibitory Concentration (MIC) (µg/mL) against

Compd	<i>S. aureas</i>				<i>S. typhi</i>			
	12.5	25	50	100	12.5	25	50	100
4a	—	—	—	+	—	—	—	+
4b	—	—	—	+	—	—	—	+
4c	—	—	—	+	—	—	—	+
4d	—	—	+	—	—	—	+	—
4e	—	+	—	—	—	+	—	—
4f	—	—	—	+	—	—	—	+
4g	—	—	—	+	—	—	—	+
4h	—	—	—	+	—	—	—	+
4i	—	—	—	+	—	+	—	—
4j	—	+	—	—	—	+	—	—
4k	—	+	—	—	—	+	—	—
4l	—	—	—	+	—	—	—	+
4m	—	+	—	—	—	+	—	—
4n	—	—	+	—	+	—	—	—
4o	—	+	—	—	—	+	—	—
4p	—	+	—	—	—	+	—	—
Ampicillin	Active at 0.01 µg/mL				Trimethoprim std	Active at 1.0 µg/mL		

Table IV — Antifungal activity of compounds **4a-p**
Minimum Inhibiting Concentrations (MIC) ($\mu\text{g/mL}$) against

Compd	<i>C. albicans</i>		<i>T. mentagrophytes</i>		<i>T. rubrum</i>	
	25	200	25	200	25	200
4a-m	—	—	—	—	—	—
4n	—	—	+	—	—	+
4o-p	—	—	—	—	—	—
Miconazole	Active at 6.25 $\mu\text{g/mL}$		Active at 1.56 $\mu\text{g/mL}$		Active at 1.56 $\mu\text{g/mL}$	

filtered and dried, mp of the crude product was 244°C. The compound was purified by column chromatography over silica gel (60-100 mesh) using solvents with increasing polarity. Elution with ethyl acetate gave a white solid, which on crystallization from methanol gave **4f**, mp 244-45°C, yield 0.6 g (59% theory). Purity of the compound was determined by TLC on silica gel (100-120 mesh); R_f 0.56 [eluent, chloroform-ethyl acetate (2:8)]; UV (MeOH) : 206 (log ϵ 3.9880), 239.5 (log ϵ 3.0434) nm; IR (KBr) : 3370, 3310, 3150 (NH), 3050 (=CH phenyl), 2970, 2930 (CH-str), 1690 (C=O), 1600, 1560, 1500 (NH def), 1430, 1400 (CH def), 1340, 1135(SO₂), 820(substituted benzene), 755(C-Cl) cm^{-1} ; ¹H NMR (DMSO-*d*₆) : δ 2.36 (CH₃ attached to B ring), 2.48 (DMSO protons), 3.32 (nH₂O present in DMSO-*d*₆), 7.26 (2H, d, C₃-H and C₅-H of B ring, J = 8.9 Hz), 7.38 (2H, d, C₃-H and C₅-H of A ring, J = 7.8 Hz), 7.40 (2H, d, C₂-H and C₆-H of A ring, J = 8.7 Hz), 7.73 (2H, d, C₂-H and C₆-H of B ring, J = 8.2 Hz), 8.39 (1H, s, NH attached to A ring), 8.68 (1H, s, N-NH-C=O), 9.58 (1H, s, NH attached to SO₂).

Preparation of *p*-chlorophenyl isothiocyanate **2n.** *p*-Chlorophenyl isothiocyanate was prepared by the method described in literature, bp 61-66°C/1mm, mp 47°C, yield 1.6 g (40% theory) IR (KBr): 2100 (N=C=S) cm^{-1} .

Preparation of *N*-(*p*-chlorophenyl)-2-(*p*-toluenesulfonyl)hydrazinecarbothioamide **4n.** To **3** (0.554g, 0.003mole) in dry benzene (15mL) was added **2n** (0.5g, 0.003mole) in dry benzene (15mL) and the reaction mixture refluxed for 3 hr, cooled, filtered and dried, mp of the crude product 204-5°C. The compound was purified by column chromatography over silica gel (60-100 mesh) using solvents of increasing polarity. Elution with ethyl

acetate-acetone (8:2) gave a white solid which was crystallised from ethyl acetate-petroleum ether (60-80°) (3:1), mp 208°C, yield 0.8g (80% theory). Purity of the compound was determined by TLC on silica gel (100-120 mesh); R_f 0.79 [eluent : ethyl acetate]; UV (MeOH) : 207 (log ϵ 4.0049, 226.5 (log ϵ 4.0567) nm; IR (KBr): 3320, 3180, 3060 (NH), 3010 (=CH phenyl), 2950 (CH str), 1600, 1540, 1520 (NH def), 1490, 1420, 1400 (CH def), 1350, 1140(SO₂), 1220 (C-S), 830(NH out of plane), 810 (substituted benzene), 710 (C-Cl) cm^{-1} ; ¹H NMR (DMSO-*d*₆) : δ 2.39 (CH₃ attached to B ring), 2.5 (DMSO protons), 3.31 (nH₂O present in DMSO-*d*₆), 7.35 (4H, s, C₃-H and C₅-H of A ring, C₃-H and C₅-H of B ring), 7.41 (2H, d, C₂-H and C₆-H of A ring, J = 8.05 Hz), 7.75 (2H, d, C₂-H and C₆-H of B ring, J = 8.42 Hz), 9.74 (1H, s, NH attached to A ring), 9.84 (1H, s, -N-NH-C=O), 9.98 (1H, s, NH attached to SO₂).

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