

SYNTHESIS OF PYRAZOLINES AND ISOXAZOLES AS POTENTIAL ANTIMICROBIAL AGENTS.

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ABSTRACT : **m,m'-Bis-(p-acetophenylaminosulphonyl)-benzophenone 1** on condensation with various araldehydes yielded chalcones **2a-p** which on cyclization with phenyl hydrazine and hydroxylamine hydrochloride furnished the corresponding **m,m'-bis- (5-aryl-1-phenyl-pyrazolin-3-yl-p-phenylaminosulphonyl)-benzophenones 3a-p** and **m,m'-bis (5-arylisoxazol-3-yl-p-phenylaminosulphonyl)-benzophenones 4a-p**. The structure of the compounds have been confirmed from elemental analyses, IR, ¹H PMR and Mass spectral data. All the products have been evaluated for their *in vitro* growth inhibitory activity against different microbes.

INTRODUCTION

Pyrazolines and isoxazoles play a vital role owing to their wide range of biological activities (1-6). Furthermore benzophenone moiety has been found to enhance the wide variety of pharmacological activities (7-9). In the light of these interesting biological activities, it appeared of interest to synthesise some novel derivatives bearing benzophenone moiety and study their biopotential behavior.

The starting compound benzophenone-m,m'-bis sulphonyl chloride on reaction with 4-amino acetophenone yielded **m,m'-bis-(p-acetophenylaminosulphonyl)-benzophenones 1** which on condensation with different araldehydes yielded **m,m'-bis-(substituted benzal-p-acetophenylamino sulphonyl)-benzophenones 2a-p**. Compounds **2a-p** on treatment with phenyl hydrazine and glacial acetic acid furnished **m,m'-bis(5-aryl-1-phenyl pyrazolin-3-yl-p-phenylaminosulphonyl) -benzophenones 3a-p**. Moreover the compounds **2a-p** on cyclization with hydroxylamine hydrochloride in ethanol in presence of sodium acetate afforded **m,m'-bis-(5-aryl isoxazol-6-yl-p-phenylaminosulphonyl)-benzophenones 4a-p** (cf. Scheme.).

The structures of the synthesised compounds were assigned on the basis of elemental analyses, IR, ¹H-PMR and Mass spectral data. The compounds were evaluated for their antimicrobial and antimycobacterial activity.

RESULTS AND DISCUSSION

All the compounds reported in Table-2 were tested *in vitro* for their antimicrobial activity against various microbes. Under identical conditions, the standard antibiotics showed zone of inhibition like Ampicillin 16-28 mm, Chloramphenicol 21 mm, Norfloxacin 20 mm against bacterial strains and Griseofulvin 25 mm against fungi *A. niger* and antitubercular activity against *Mycobacterium tuberculosis H37 Rv* at a concentration of 12.5 μ g/ml.

Looking to the structure activity relationship, 1-phenyl-pyrazoline and isoxazole derivatives were found to be remarkably active against *B. megaterium*. Promising activity was also observed in the case of *B. subtilis*. Chalcone derivatives bearing 4-chloro and 4-hydroxy-3-methoxy substituents **2d,2k** showed significant activity against *B. subtilis*, *E. coli* and fungi *A. awamori*. Compounds **2d,2i,2k,2m,3f,3g,3l,3n,4a,4f,4h,4k,4l,4n** and **4o** were highly active against *B. megaterium*. Similarly, compounds **2j,2k,2d,2f,4d,4h,4m** were showed significant activity against *E. coli*. The compounds **3a,3d,3k,3m,3n,4g,4l,4m** and **4p** were highly active against *B. subtilis*. Moreover **2i** and **2k** compounds showed considerable potency against *P. fluorescens*. The compounds **2d** was highly active against *A. awamori*, however **3g,3j,3o** and **4g** compounds showed significant activity against *A. niger*. While the compound **3i** showed remarkable activity against *Mycobacterium tuberculosis H37 Rv*.

IN VITRO EVALUATION OF PHARMACOLOGICAL STUDIES

The antimicrobial activity was assayed by using the cup-plate agar diffusion method (10) by measuring the inhibition zone in mm. All the compounds were screened in vitro for their antimicrobial activity against a variety of bacterial strains such as *Bacillus megaterium*, *Escherichia coli*, *Bacillus subtilis* and fungi *Aspergillus niger* & *Aspergillus awamori* at a 50 μ g concentration. Known antibiotics like Chloramphenicol, Ampicillin, Norfloxacin and Griseofulvin were used for comparison purpose.

The antitubercular evaluation of the compounds was carried out at " Tuberculosis Antimicrobial Acquisition and Coordinating Facility " (TAACF) USA. Primary screening of the compounds for antitubercular activity have been conducted at 12.5 μ g/ml against *Mycobacterium tuberculosis H37 Rv*, in BACTEC 12B medium using BACTEC 460 radiometric system. Antitubercular activity data were compared with standard drug Rifampin at 0.125 μ g/ml concentration which showed 94% inhibition.

EXPERIMENTAL

All the melting points are uncorrected. Infrared Spectra (KBr) were recorded on a Specord 75-IR Spectrophotometer, ^1H -PMR Spectra on Bruker Spectrometer (300 MHz) using CDCl_3 + DMSO-d_6 as solvent and Mass Spectra on Jeol JMS D - 300 Spectrophotometer.

Preparation of $\text{m},\text{m}'\text{-Bis-(p-acetophenylaminosulphonyl)-benzophenones 1.}$ (11)

Preparation of $\text{m},\text{m}'\text{-Bis-(substituted benzal-p-acetophenyl amino sulphonyl)-benzophenones 2a-p}$

4-Methoxy benzaldehyde (0.02 mole, 2.26 ml) in dioxan (20 ml) was added to the mixture of $\text{m},\text{m}'\text{-bis-(p-acetophenylaminosulphonyl)-benzophenones (1, 0.01 mol,)}$ in dioxan (30 ml) and aqueous sodium hydroxide (40%, 5 ml) stirred at 20-30°C for 24hrs. The contents were poured into crushed ice and isolated by acidification and crystallised from dioxan to give **2m**, yield 86%, m.p. 121°. Calcd. for $\text{C}_{45}\text{H}_{36}\text{N}_2\text{O}_9\text{S}_2$: C 66.50, H 4.43, N 3.40 %. Found : C 66.40, H 4.40, N 2.90 ; IR_{max} (KBr) : 3419 (N-H str.) 1668 (C=O str.), 1603 (C=O str.), 1339 (S=O str.), 1303 (C-N str.), 1161 (S=O str.) cm^{-1} ; ^1H -PMR (CDCl_3) : δ 3.85 [s, 6H, $(\text{Ar}-\text{O}-\text{CH}_3)_2$], 6.92(d, 2H, $\text{CH}=\text{CH}$), 7.23-8.27 (m, 24H, Ar-H), 7.92 (d, 2H, $\text{CH}=\text{CH}$), 10.50 [s, 2H, $(\text{NH})_2$]; FAB MS : m/z 813 (M), 695, 497, 364, 289, 252, 161, 415, 154, 107, 79 (B. P.).

Similarly, other members of **2a-p** were prepared.

Preparation of $\text{m},\text{m}'\text{-Bis-(5-aryl-1-phenyl-pyrazolin-3-yl-p-phenyl aminosulphonyl)-benzophenones 3a-p .}$

A mixture of chalcones (**2l**, 0.01 mol) in 25 ml acetic acid and phenyl hydrazine (0.02 mol) was refluxed for 12 hrs. The resulting mixture was poured into ice and acidified with acetic acid. The product was isolated and crystallised from dioxan to give **3l**, yield 51%, m.p. 153°. Calcd. for $\text{C}_{57}\text{H}_{48}\text{N}_6\text{O}_7\text{S}_2$: C 68.95, H 4.84, N 8.47 %. Found C 68.91, H 4.75, N 8.44%. IR_{max} (KBr) : 3200 (N-H str.), 1600 (C=O str.), 1570 (C=N str.), 1392 (C-N str.) 1336 (S=O str.), 1158 (S=O str.) cm^{-1} . **3p** ^1H -PMR δ ($\text{CDCl}_3+\text{DMSO-d}_6$) : 3.00 [dd, 2H, $(\text{CH}_A)_2$], 3.1 [dd, 2H, $(\text{CH}_B)_2$], 3.71 [s, 6H, $(\text{OCH}_3)_2$], 3.77 [s, 12H, $(\text{OCH}_3)_2$], 5.48 [dd, 2H, $(\text{CH}_x)_2$], 7.15-8.2 (m, 32H, Ar-H).

Similarly, other members of **3a-p** were prepared. The physical constants are recorded in Table No. I.

Preparation of *m,m'*-Bis-(5-aryl(isoxazol-3-yl)-p-phenyl aminosulphonyl)-benzophenones **4a-p.**

Anhydrous sodium acetate dissolved in a minimum amount of hot acetic acid was added to a solution of hydroxylamine hydrochloride (0.02 mol) in ethanol (20 ml). This solution was added to a solution of chalcones (**2I**, 0.01 mol) in ethanol. The mixture was heated under reflux on an oil-bath for 8 hrs., concentrated and neutralised with sodium hydroxide. The product was isolated and crystallised from ethanol to give **4I**, yield 58 %, m.p. 145°C. Calcd. for $C_{45}H_{34}N_4O_9S_2$: C 64.44, H 4.06, N 6.68 %. Found C 64.40, H 4.00, N 6.60 %. IR_{max} (KBr): 3428 (N-H str.), 1610 (C=O str.), 1584 (C=C str.), 1462 (C=N str.), 1334 (S=O str.), 1301 (C-N str.), cm^{-1} . ^1H -PMR δ (CDCl_3) : 3.73 [s, 6H, $(\text{OCH}_3)_2$], 6.63-8.04 (m, 22H, Ar-H), 8.16 [s, 2H, $(\text{NH})_2$]; **4p** FAB MS : m/z 958(M), 946, 782, 651, 622, 460, 424, 307, 273, 232, 154, 137(B.P.), 107, 79

Similarly, other members of **4a-p** were prepared. The physical constants are given in Table I.

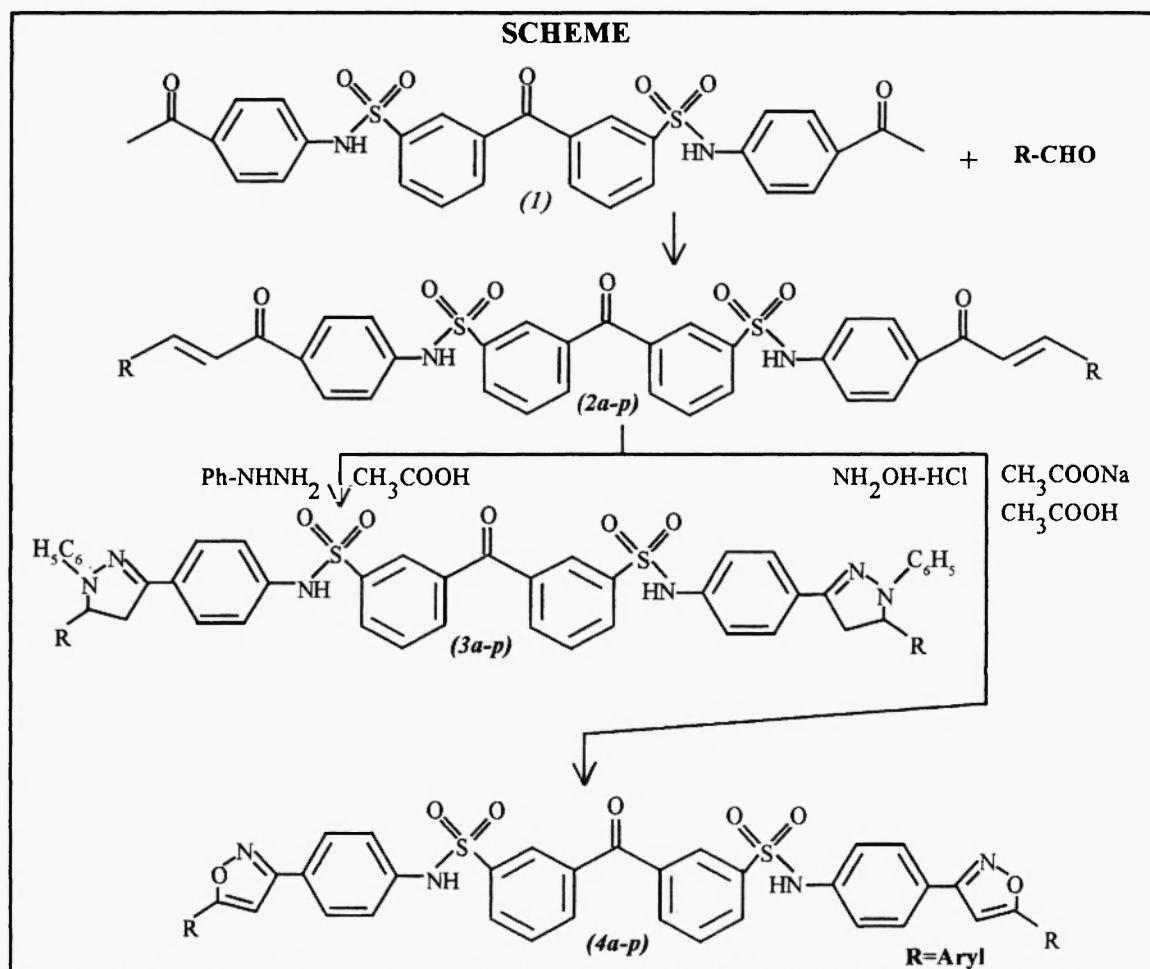


TABLE 1 : Physical constant of the compounds 3a-p and 4a-p.

Compd.	R	Molecular Formula	M.P. (°C)	Yield (%)	% of N	
					Found	Calcd.
3a	C ₆ H ₅ -	C ₅₅ H ₄₄ N ₆ O ₅ S ₂	141	59	9.00	9.01
3b	3-NH ₂ -C ₆ H ₄ -	C ₅₅ H ₄₆ N ₈ O ₅ S ₂	182	66	11.60	11.64
3c	3-Br-C ₆ H ₄ -	C ₅₅ H ₄₂ N ₆ O ₅ S ₂ Br ₂	159	63	7.63	7.71
3d	4-Cl-C ₆ H ₄ -	C ₅₅ H ₄₂ N ₆ O ₅ S ₂ Cl ₂	207	49	8.34	8.39
3e	2,4-Cl ₂ -C ₆ H ₃ -	C ₅₅ H ₄₀ N ₆ O ₅ S ₂ Cl ₄	154	55	7.80	7.85
3f	2,6-Cl ₂ -C ₆ H ₃	C ₅₅ H ₄₀ N ₆ O ₅ S ₂ Cl ₄	157	71	7.77	7.85
3g	4-(CH ₃) ₂ -C ₆ H ₄ -	C ₅₉ H ₅₄ N ₈ O ₅ S ₂	161	63	10.13	11.00
3h	O-C ₄ H ₃ -	C ₅₁ H ₄₀ N ₆ O ₇ S ₂	154	65	9.18	9.20
3i	2-OH-C ₆ H ₄ -	C ₅₅ H ₄₄ N ₆ O ₇ S ₂	167	58	8.71	8.72
3j	4-OH-C ₆ H ₄ -	C ₅₅ H ₄₄ N ₆ O ₇ S ₂	185	74	8.68	8.72
3k	4-OH-3-OCH ₃ -C ₆ H ₃ -	C ₅₇ H ₄₈ N ₆ O ₉ S ₂	167	68	8.71	8.20
3l	3-OCH ₃ -C ₆ H ₄ -	C ₅₇ H ₄₈ N ₆ O ₇ S ₂	153	51	8.44	8.47
3m	4-OCH ₃ -C ₆ H ₄ -	C ₅₇ H ₄₈ N ₆ O ₇ S ₂	163	50	8.40	8.47
3n	3-NO ₂ -C ₆ H ₄ -	C ₅₅ H ₄₂ N ₈ O ₉ S ₂	191	60	10.92	10.96
3o	S-C ₄ H ₃ -	C ₅₁ H ₄₀ N ₆ O ₅ S ₄	149	55	8.80	8.90
3p	3,4,5-(OCH ₃) ₃ -C ₆ H ₂ -	C ₆₁ H ₅₆ N ₆ O ₁₁ S ₂	190	58	7.50	7.55
4a	C ₆ H ₅ -	C ₄₃ H ₃₀ N ₄ O ₇ S ₂	130	69	7.13	7.20
4b	3-NH ₂ -C ₆ H ₄ -	C ₄₃ H ₃₂ N ₆ O ₇ S ₂	190	59	10.34	10.39
4c	3-Br-C ₆ H ₄ -	C ₄₃ H ₂₈ N ₄ O ₇ S ₂ Br ₂	160	67	5.97	6.00
4d	4-Cl-C ₆ H ₄ -	C ₄₃ H ₂₈ N ₄ O ₇ S ₂ Cl ₂	135	54	6.60	6.61
4e	2,4-Cl ₂ -C ₆ H ₃ -	C ₄₃ H ₂₆ N ₄ O ₇ S ₂ Cl ₄	155	48	6.10	6.11
4f	2,6-Cl ₂ -C ₆ H ₃	C ₄₃ H ₂₆ N ₄ O ₇ S ₂ Cl ₄	172	67	6.08	6.11
4g	4-(CH ₃) ₂ -C ₆ H ₄ -	C ₄₇ H ₄₀ N ₆ O ₇ S ₂	144	63	9.65	9.72
4h	O-C ₄ H ₃ -	C ₃₉ H ₂₆ N ₄ O ₉ S ₂	142	72	7.30	7.39
4i	2-OH-C ₆ H ₄ -	C ₄₃ H ₃₀ N ₄ O ₉ S ₂	145	75	6.84	6.91
4j	4-OH-C ₆ H ₄ -	C ₄₃ H ₃₀ N ₄ O ₉ S ₂	132	60	6.87	6.91
4k	4-OH-3-OCH ₃ -C ₆ H ₃ -	C ₄₄ H ₃₆ N ₄ O ₁₁ S ₂	210	55	6.46	6.51
4l	3-OCH ₃ -C ₆ H ₄ -	C ₄₅ H ₃₄ N ₄ O ₉ S ₂	145	58	6.60	6.68
4m	4-OCH ₃ -C ₆ H ₄ -	C ₄₅ H ₃₄ N ₄ O ₉ S ₂	158	50	6.62	6.68
4n	3-NO ₂ -C ₆ H ₄ -	C ₄₃ H ₂₈ N ₆ O ₁₁ S ₂	125	51	9.64	9.67
4o	S-C ₄ H ₃ -	C ₃₉ H ₂₆ N ₄ O ₇ S ₄	131	65	7.03	7.09
4p	3,4,5-(OCH ₃) ₃ -C ₆ H ₂ -	C ₄₉ H ₄₂ N ₄ O ₁₃ S ₂	165	67	5.80	5.84

TABLE 2 : Antimicrobial data (inhibition zone =17-24 mm at 50 μ g concentration) and Antitubercular data (% inhibition < 50%) of those compounds which exhibited highest activity

B.mega	B.subtilis	E.coli	A.niger	A.awamori	M. tuberculosis H37 Rv
3a,3d,3e,3k, 3m,3n,4g,4l, 4m,4p	2m,2i,2d,2k , 3f,3g,3l,3n, 4a,4f,4h,4k, 4l,4n,4o	2j,2k,2d,2f, 4d,4h,4m	3g,3j,3o,4g	2d	2d,3e,3o,3i,3k, 3i* (* % inhi >90 %)
Ampicillin (16-28 mm)	Norfloxacin (20 mm)	Chloramphenicol (21 mm)	Griseofulvin (25 mm)	Griseofulvin (20 mm)	Rifampin (94 % inhi.)

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