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Synthesis and Multiple Biological Activities of Azomethines and 4-Thiazolidinones

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4-(2-Methoxy-5-methylphenyl)-1-(substituted benzal) thiosemicarbazides IIa-m and 2-(arylidene-hydrazono)-3-(2-methoxy-5-methylphenyl)-4-thiazolidinones IIIa-m have been prepared by the condensation of 2-methoxy-5-methylphenyl thiosemicarbazide I with different aromatic carboxaldehydes, which on cyclocondensation with chloroacetic acid in presence of sodium acetate in glacial acetic acid yielded 4-thiazolidinones IIIa-m. The characterisation of the compounds have been done on the basis of elemental analyses, IR, IH NMR and mass spectral study. All the compounds have been evaluated for their *in vitro* growth inhibiting activity against several microbes and most of the compounds have also been screened for antitubercular activity against *Mycobacterium tuberculosis* $H_{37}Rv$. Some selected compounds have been evaluated for their *in vitro* anticancer screen aimed at identifying agents having cell type specificity using batteries of cell lines derived from human solid tumor at National Cancer Institute, U.S.A.

Considerable interest has been shown in the field of thiazolidinone chemistry due to their wide range of therapeutic activities such as anticancer[1], antimicrobia[2], herbicida[3] and antithyroid[4]. Thiosemicarbazide derivatives have become attractive target in organic synthesis because of their reactivity and biological significance such as anticancer[5], antitumor[6], antimicrobia[7], antiviral[8] and antiiflammatory[9]. Continuous increase in bacterial resistance to existing drugs has been resulted due to wide spread use of antibiotics leading to research on new substances possessing antimicrobial activity.

Awareness of wide spread tuberculosis epidemic and the emergence of MDRTB have stressed the urgent need for new, effective antitubercular drugs. Because of spread of HIV infection, the menace of TB has reached alarming properties world wide[10]. To achieve this goal and on the basis of above considerations, some new 4-thiazolidinone derivatives have been investigated.

The key intermediate 2-methoxy-5-methylphenyl thiosemicarbazide I, ammonia and carbon disulphide with

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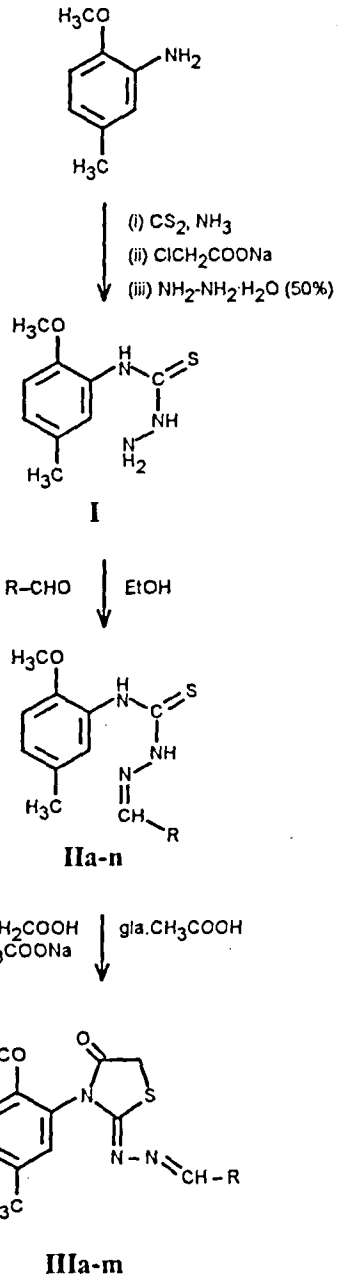
sodium on condesation with different aromatic carboxaldehydes afforded respective 4-(2-methoxy-5-methylphenyD-1-(substituted benzal) thiosemicarbazones II_{a-n} which when subjected to cyclocondensation with chloroacetic acid furnished 2-(arylidene-hydrazono)-3-(2-methoxy-5-methylphenyl)-4-thiazolidinones III_{a-m}. All the compounds have been characterized by elemental analyses, IR, ^{1}HNMR and Mass spectral study. All the compounds have been screened *in vitro* for antimicrobial activity against different strains of bacteria and fungi and most compounds have also been screened for antitubercular activity against Mycobacterium tuberculosis $H_{37}Rv$ strain. Some selected compounds were evaluated for *in vitro* anticancer activity against different cell lines at National Cancer Institute, Bethesda, U.S.A.

All the melting points were determined using open capillary method and are uncorrected. Infrared spectra were recorded on a Shimadzu FTIR-8400 spectrophotometer using KBr pellet and ^{1}H NMR spectra on a Bruker spectrophotometer (300 MHz) using $CDCl_3$ as an internal solvent and Mass spectra on a Jeol D-300 at 70 e.v. Purity of the compounds was routinely checked using thin layer chromatography.

2-Methoxy-5-methylphenyl thiosemicarbazide (I) 2-Methoxy-5-methylaniline(13.7 g, 0.1 mol) was dissolved in ethanol (95%, 50 ml) and ammonia solution (20 ml) was added to it. The reaction mixture was cooled below 30° and carbon disulphide (8 ml) was added slowly within 15 min with shaking. After complete addition of CS_2, the solution was allowed to stand for 1 h. Sodium chloroacetate (9.4 g, 0.1 mol) was added followed by 50% hydrazine hydrate (20 ml). The mixture was warmed gently, filtered, concentrated and kept over night. Next day, the product was filtered, crystallised from ethanol yield 60%, m.p. 125°, (Found: C, 51.10; H, 6.13;N, 19.85; $C_9H_{13}ON_3S$ requires: C, 51.18; H, 6.16; N, 19.90%).

4-(2-Methoxy-5-methylphenyl)-l-(substituted benzal) thiosemicarbazides (IIh) was prepared by heating a mixture of 2-methoxy-5-methylphenyl thiosemicarbazide (2.11 g, 0.01 mol), 4-methoxy benzaldehyde (1.36 g, 0.01 mol) in ethanol (25 ml)) and a few drops of glacial acetic acid for 2 h. The reaction mixture on cooling yielded a product and crystallised from ethanol, yield 62%, m.p. 174°. (Found: C, 61.95; H, 5.70; N, 12.64; $C_{17}H_{19}O_2N_3S$ requires: C, 62.00; H, 5.77; N, 12.76%) IR (KBr) υ max: 3400 (N-H), 2960 (-CH-), 1596 (C=N), 1238 (C-O-C), 1130 cm^{-1} (C-S). ^{1}H NMR ($CDCl_3$): δ 2.35 (s, 3H, $-CH_3$), 3.84 (s, 3H, $-OCH_3$), 3.90 (s, 3H, $-OCH_3$), 6.81-8.58 (m, 8H,Ar-H+ =C-H), 9.84 (s, 1H, -NH), 10.00 (s, 1H, -NH). MS: m/z 329 (m+), 323, 268, 255, 253, 241, 226, 192, 179, 161, 134, 122. Similarly, other members of II were prepared. The physical data are given in Table 1.

2-(Arylidene-hydrazono)-3-(2-methoxy-5-methylphenyl)-4-thiazolidinones (IIIa) was prepared by heating a mixture of 4-methoxy-5-methylphenyl-1-(benzal) thiosemicarbaride (2.99 g, 0.01 mol) and chloroacetic acid



Scheme

TABLE 1: CHARACTERIZATION DATA AND BIOLOGICAL ACTIVITY OF THE IIA-N* AND IIIA-M*.

Comp.	R	Yield (%)	MP°	Molecular Formula	% of Nitrogen Calcd./Found	Zone of inhibition Antibacterial activity					Antitubercular activity % growth mycobacterium tuberculosis $H_{37}Rv$
						E. coli	*P. vulgaris*	*V. mega*	*S. aureus*	*A. niger*	
IIa	Phenyl	58	140	$C_{16}H_{17}ON_3S$	14.04/14.00	18	12	14	14	10	07
IIb	2-Chlorophenyl	60	180	$C_{16}H_{16}ON_3SCl$	12.59/12.48	20	14	15	12	15	03
IIc	4-Chlorophenyl	56	190	$C_{16}H_{16}ON_3SCl$	12.59/12.43	16	18	12	17	13	00
IId	3,4-Dimethoxyphenyl	50	182	$C_{18}H_{21}O_3N_3S$	11.69/11.61	18	13	14	16	18	00
IIe	α-Furyl	68	176(d)	$C_{14}H_{15}O_2N_3S$	14.53/14.41	16	18	15	14	12	00
IIf	2-Hydroxyphenyl	62	210	$C_{16}H_{17}O_2N_3S$	13.33/13.22	20	18	21	15	11	04
IIg	4-Hydroxyphenyl	55	202	$C_{16}H_{17}O_2N_3S$	13.33/13.30	15	17	14	16	10	04
IIh	4-Methoxyphenyl	62	174	$C_{17}H_{19}O_2N_3S$	12.76/12.64	17	14	13	14	16	52
IIi	3-Methoxy-4-Hydroxyphenyl	68	218	$C_{17}H_{19}O_3N_3S$	12.17/12.10	19	18	15	19	12	00
IIj	2-Nitrophenyl	70	146	$C_{16}H_{16}O_3N_4S$	16.27/16.21	19	16	18	17	14	00
IIk	3-Nitrophenyl	56	206	$C_{16}H_{16}O_3N_4S$	16.27/16.18	15	16	14	19	16	00
IIl	4-Mercaptomethylphenyl	63	172	$C_{17}H_{19}O_3N_3S_2$	11.14/11.22	16	15	12	20	11	49
IIm	3,4,5-Trimethoxyphenyl	57	170	$C_{19}H_{23}O_4N_3S$	10.79/10.70	18	19	15	15	18	00
IIn	Cinnamyl	61	196	$C_{18}H_{19}ON_3S$	12.92/12.85	17	16	18	16	12	00
IIIa	Phenyl	48	118	$C_{18}H_{17}O_2N_3S$	12.38/12.30	15	11	17	12	18	-
IIIb	2-Chlorophenyl	52	94	$C_{18}H_{16}O_2N_3SCl$	11.24/11.20	13	14	16	16	15	-
IIIc	4-Chlorophenyl	58	106	$C_{18}H_{16}O_2N_3SCl$	11.24/11.12	14	18	18	15	14	-
IIId	3,4-Dimethoxyphenyl	49	70	$C_{20}H_{21}O_4N_3S$	10.52/10.48	15	18	18	16	14	-
IIIe	α-Furyl	45	150(d)	$C_{16}H_{15}O_3N_3S$	12.76/12.70	18	12	16	20	16	-
IIIf	2-Hydroxyphenyl	52	102	$C_{18}H_{17}O_3N_3S$	11.83/11.75	12	11	20	12	12	-
IIIg	4-Hydroxyphenyl	58	128	$C_{18}H_{17}O_3N_3S$	11.83/11.80	16	14	13	17	20	-
IIIh	4-Methoxyphenyl	50	>280	$C_{19}H_{19}O_3N_3S$	11.38/11.25	14	16	15	12	13	-
IIIi	3-Methoxy-4-Hydroxyphenyl	62	100	$C_{19}H_{19}O_4N_3S$	10.90/10.82	12	15	19	13	17	-
IIIj	2-Nitrophenyl	41	96	$C_{18}H_{16}O_4N_4S$	14.58/14.49	15	13	12	14	13	-
IIIk	3-Nitrophenyl	45	136	$C_{18}H_{16}O_4N_4S$	14 58/14.55	18	15	14	16	16	-
IIIl	3,4,5-Trimethoxyphenyl	60	70	$C_{21}H_{23}O_5N_3S$	9.79/9.72	12	10	11	13	14	-
IIIm	Cinnamyl	40	124	$C_{20}H_{19}O_2N_3S$	11.50/11.43	12	15	18	15	12	-

Results of standard antibiotics: amoxicillin 20-26 mm; erythromycin 22-25 mm; ampicillin 18-20 mm; griseofulvin 26 mm; ciprofloxacin 20-26 mm and rifampin: 98%. *All the compounds gave satifactory C, H and N analysis.

(0.94 g, 0.01 mol) in glacial acetic acid on water bath for 5 h. The reaction product was poured in to ice water, the solid obtained was collected and crystallized from acetic acid, yield 48%, m.p. 118°. (Found: C, 63.68; H, 5.00; N, 12.30; $C_{18}H_{17}O_2N_3S$ requires: C, 63.71; H, 5.01; N, 12.38%). IR (KBr) υ max: 2970 (-C-H), 1728 (C=0), 1581 (C=N), 1410 (-CH_2-CO str.), 1250 (C-O-C str.), 1028 (N-N str.), 692 cm^{-1} (C-S-C str.). ^1H NMR (TFA): δ 2.35 (s, 3H, -CH_3), 4.00 (s, 3H, -OCH_3), 4.42 (s, 2H, -CO-CH_2), 6.80-7.90 (m, 9H, Ar-H + N=CH). MS: m/z 339 (m^{+1}), 324, 294, 266, 178, 162, 147, 136, 122, 119. Similarly, other members of III were prepared. The physical data are given in Table 1.

The synthesised compounds were evaluated for antimicrobial activity against Gram negative bacterial strains such as *Escherichia coli, Proteus vulgaris,* Gram positive bacterial strains such as *Bacillus megaterium, Staphylococcus aureus* and *Aspergillus niger,* the fungal strain, using cup plate agar diffusion method[11]. Known standard antibiotics, ampicillin, amoxicillin, ciprofloxacin, erythromycin and griseofulvin were used for comparison purposes. The concentration of the test compounds/standard in each cup was 40 μg. The zones of inhibition were measured in mm.

The antitubercular evaluation of the compounds was carried out at Tuberculosis Antimicrobial Acquisition and Coordinating Facility (TAACF) U.S.A. Primary screening of some synthesised compounds for *in vitro* tubercular activity was conducted at a concentration of 6.25 μg/ml against *Mycobacterium tuberculosis* $H_{37}Rv$ in BACTEC 12B medium using the BACTEC 460 radiometric system. The antitubercular activity of some of the compounds were compared with the standard drug, rifampin at a 0.25 μg/ml concentration which showed 98% inhibition.

The anticancer screening of some selected compounds was carried out at National Cancer Institute, Department of health and human services, Bethesda, U.S.A. The study is related with *in vitro* anticancer screen aimed at identifying agents having cell type specificity using batteries of cell lines derived from human solid tumors. At the primary anticancer assay, a 3-cell panel consisting of NCF-7 (Breast), NCI-H 460 (Lung) and SF-268 (CNS) has been used. The compounds exhibiting inhibition to 32% or less (negative nos.) indicate active molecule. The results for each test against are reported to the % growth of treated cell when compared to the uncontrolled cells.

Table 1 describes the *in vitro* antimicrobial activity against *B. megaterium, S. aureus, E. coli, P. vulgaris* and *A. niger* and antitubercular activity against *M. tuberculosis* $H_{37}Rv$ of the synthesised compounds II_{a-n} and III_{a-m}, respectively. It can be concluded from the Table 1 that the compounds IIb, IIf. IIi, IIj, IIIe, IIIk displayed maximum activity against *E.coli.* The compounds IIf, IIIf, IIIi showed highest activity against *B. megaterium.* While the compounds IIm, IIIe, IIId and IIi, IIk, III, IIIe exhibited significant activity against *P.vulgaris* and *S. aureus,* respectively. In case of *A. niger* the compounds IId, IIm, IIIa, IIIg showed significant activity.

Looking at the structure activity relationship, the antitubercular activity data showed that compounds bearing substituted methyl or methoxy group in 4-position display mild to moderate antitubercular activity towards *Mycobacterium tuberculosis* $H_{37}Rv$. The antitubercular data were compared with standard drug rifampin at 0.25 μg/ml concentration which showed 98% inhibition. Schiffs bases IIe, IIh and III have been evaluated for anticancer activity. All of them exhibited significant anticancer activity against different batteries of cell lines and were found to be active.

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