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Synthesis and antimicrobial activity of 5-amino-2,7-diaryl-6-cyano- 3-isonicotinamido thiazolo[4,5-*b*]-2,3,4,7-tetrahydropyridines, 2,7-diaryl-6-cyano-3-isonicotinamido thiazolo[4,5-*b*]-2,3,4,5,6,7-hexahydropyrid-5-ones, 2,7-diaryl-5-amino-3-isonicotinamido thiazolo[4,5-*d*][1,3-]thiazines and 2,6-diaryl-3-isonicotinamido thiazolo[4,5-*c*] pyrazolines

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5-Amino-2,7-diaryl-6-cyano-3-isonicotinamido thiazolo[4,5-*b*]-2,3,4,7-tetrahydropyridines **3** and 2,7-diaryl-6-cyano-3-isonicotinamido thiazolo[4,5-*b*]-2,3,4,5,6,7-hexahydropyrid-5-ones **4** have been prepared by Michael addition between α,β-unsaturated ketones 2 and malononitrile/ethyl cyanoacetate in the presence of excess ammonium acetate. 2,7-Diaryl-5-amino-3-isonicotinamido thiazolo[4,5-*d*][1,3]thiazines **5** and 2,6-diaryl-3-isonicotinamido thiazolo[4,5-*c*]pyrazolines **6** have been prepared by cyclocondensation of α,β-unsaturated ketones **2** with thiourea/hydrazine hydrate, respectively. The antimicrobial activity of the representative samples are assayed against bacteria *E. coli, B. subtilis, S. aureus* and fungi *A. niger, P. oryzae* and *F. oxysporum* at 100 μg/mL concentration.

Keywords: Thiazolo[4,5-*b*]pyridines, thiazolo[4,5-*b*]pyridones, thiazolo- [4,5-*d*][1,3]thiazines, thiazolo[4,5-*c*]pyrazolines, bactericidal, fungicial activity

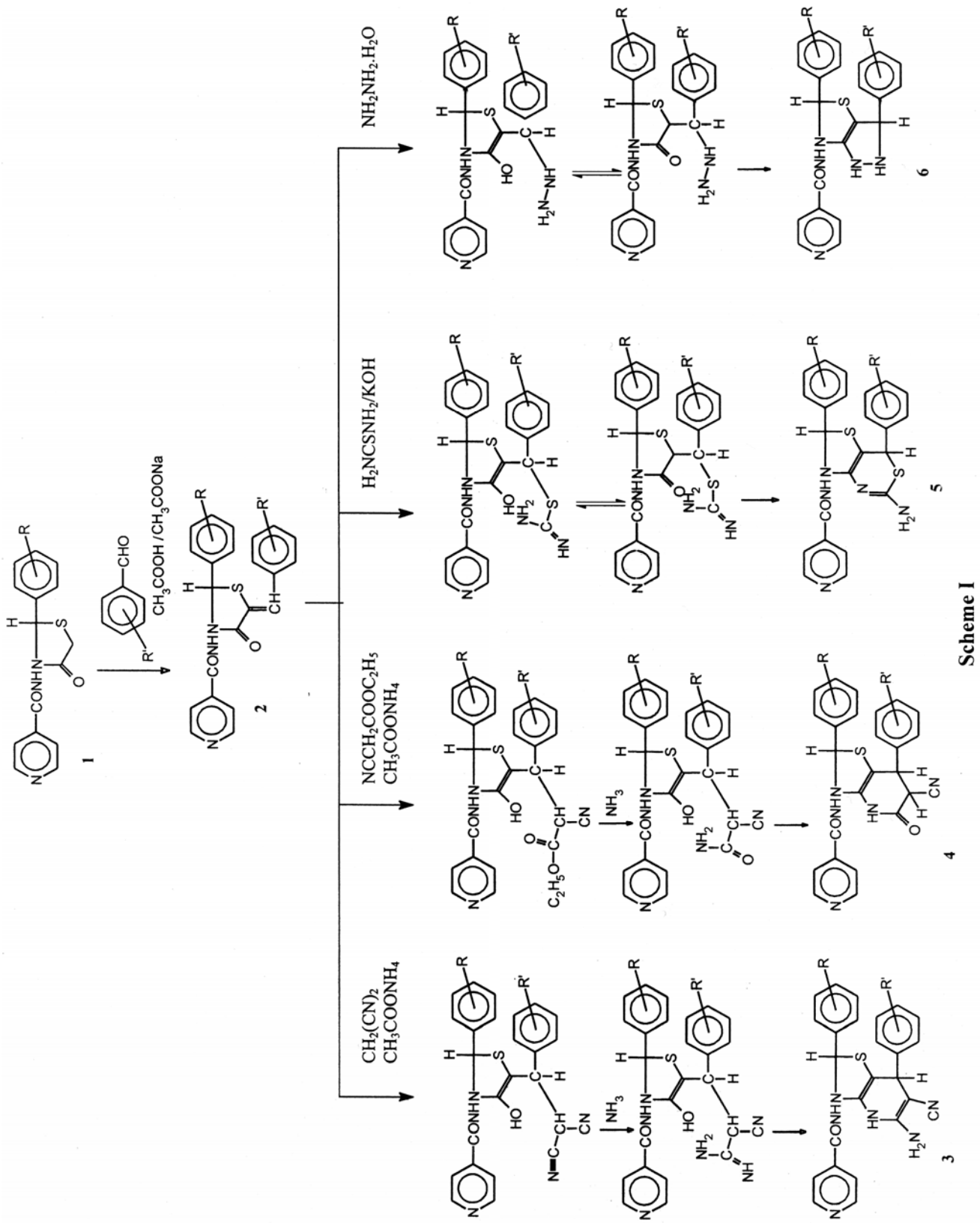
IPC: Int.Cl.[8] C07D

Thiazolidinone derivatives are well known for their diverse biological activities[1-3] such as fungicidal, bactericidal, insecticidal, herbicidal, virucidal, anti-inflammatory, anti-tubercular, anthelmintic, anti-thyroidal, cardiovascular, potential to pentobarbital-induced sleeping potentials etc. Similarly pyridine[4-6], thiazole[7-9], thiazine[10] and pyrazoline[11,12] heterocyclic nuclei exhibit numerous biocidal activities and antitubercular activity of isoniazide is well documented[13,14]. Keeping these facts in mind and in continuation of our earlier work on fused heterocycles[15-17] of pesticidal interest and guided by the observations that sometimes the fusion of two or more heterocyclic nuclei enhances the biological profile many fold than its parent nuclei. The title compounds having thiazole nuclei fused with cyanopyridine, cyanopyridone, thiazine or pyrazoline nuclei in a single molecule having isonicotinamide group as a side chain have been synthesised.

The required thiazolidinones **1** have been synthesised by the reaction between isonicotinic acid hydrazide, aromatic aldehydes and thioglycollic acid in ethanol. Condensation of these thiazolidinones **1** with aromatic aldehydes under Knoevenagal conditions furnished the corresponding arylideno thiazolidinones **2** having α,β-unsaturated carbonyl function which have been used as a component of Michael addition. The arylideno thiazolidinones **2** when condensed with an equimolar quantity of malononitrile or ethyl cyanoacetate in the presence of excess ammonium acetate afforded the title compounds **3** and **4**, respectively. The reaction involves first, the nucleophilic attack by the methylene groups of malononitrile or ethyl cyanoacetate at the β-carbon atom of the α,β-unsaturated carbonyl function leading to the Michael type adducts which undergo cyclodehydration to give thiazolo- [4,5-*b*]pyridines **3** and thiazolo[4,5-*b*]pyridones **4**. Cyclocondensation of arylideno thiazolidinones **2** with thiourea in ethanolic KOH solution through Michael type adduct formation or with hydrazine hydrate, furnished the title compounds thiazolo[4,5-*d*][1,3]thiazines **5** and thiazolo [4,5-*c*] pyrazolines **6**, respectively (**Scheme I, Table I**).

Antimicrobial Activity

Bactericidal Activity. The bactericidal activity of the synthesised compounds have been assayed against *Escherichia coli, Bacillus subtilies* and *Streptococcus aureus* by using cup plate agar diffusion method of


CH2(CN)2
CH3COONH4
NCCH2COOC2H5
CH3COONH4
H2NCSNH2/KOH
NH2NH2.H2O
CH3COOH /CH3COONa
CHO
NH3
1
2
3
4
5
6

Scheme I

Table I — Physical characterisation of compound **1-5** and **6**

Compd	R	R'	m.p. °C	Yield (%)	Mol. formula	Found % (Calcd)		
						C	H	N
1a	H	-	144	85	$C_{15}H_{13}N_3O_2S$	60.12	4.29	14.11
						(60.20	4.35	14.05)
1b	4-Cl	-	201	96	$C_{15}H_{12}N_3O_2SCl$	53.88	3.52	12.67
						(53.97	3.60	12.59)
1c	4-CH_3	-	173-74	86	$C_{16}H_{15}N_3O_2S$	61.23	4.75	13.50
						(61.34	4.79	13.42)
1d	4-OCH_3	-	133	88	$C_{16}H_{15}N_3O_3S$	58.43	4.49	12.85
						(58.36	4.56	12.76)
2a	H	4-Cl	167	78	$C_{22}H_{16}N_3O_2SCl$	62.51	3.95	10.05
						(62.63	3.80	9.96)
2b	4-Cl	4-OCH_3	170	80	$C_{23}H_{18}N_3O_3SCl$	61.25	4.10	9.25
						(61.13	3.99	9.30)
2c	4-CH_3	4-Cl	156-57	75	$C_{23}H_{18}N_3O_2SCl$	63.52	4.25	9.53
						(63.38	4.13	9.64)
2d	4-OCH_3	4-Cl	155	83	$C_{23}H_{18}N_3O_3SCl$	61.00	3.86	9.45
						(61.13	3.99	9.30)
2e	4-OCH_3	4-OCH_3	147-48	83	$C_{24}H_{21}N_3O_4S$	64.51	4.75	9.29
						(64.43	4.70	9.40)
2f	4-Cl	H	174	81	$C_{22}H_{16}N_3O_2SCl$	62.77	3.91	9.87
						(62.63	3.80	9.96)
3a	H	4-Cl	159-60	65	$C_{25}H_{19}N_6OSCl$	61.73	4.11	17.42
						(61.66)	3.91	17.27)
3b	4-Cl	4-OCH_3	167-68	60	$C_{26}H_{21}N_6O_2SCl$	60.52	4.17	16.15
						(60.41	4.07	16.26)
3c	4-CH_3	4-Cl	140	70	$C_{26}H_{21}N_6OSCl$	62.19	4.32	16.90
						(62.34	4.20	16.78)
3d	4-OCH_3	4-Cl	144-45	70	$C_{26}H_{21}N_6O_2SCl$	60.54	4.16	16.39
						(60.41	4.07	16.26)
3e	4-OCH_3	4-OCH_3	153	65	$C_{27}H_{24}N_6O_3S$	63.35	4.83	16.27
						(63.28	4.69	16.41)
4a	H	4-Cl	171	68	$C_{25}H_{18}N_5O_2SCl$	61.70	3.75	14.19
						(61.54	3.69	14.36)
4b	4-Cl	4-OCH_3	177	67	$C_{26}H_{20}N_5O_3SCl$	60.17	3.93	13.45
						(60.29	3.86	13.53)
4c	4-CH_3	4-Cl	185	65	$C_{26}H_{20}N_5O_2SCl$	62.34	4.15	14.07
						(62.21	3.99	13.96)
4d	4-OCH_3	4-Cl	149	70	$C_{26}H_{20}N_5O_3SCl$	60.41	3.95	13.70
						(60.29	3.86	13.53)
4e	4-OCH_3	4-OCH_3	158	72	$C_{27}H_{23}N_5O_4S$	63.24	4.61	13.81
						(63.16	4.48	13.65)
5a	4-Cl	H	179	65	$C_{23}H_{18}N_5OS_2Cl$	57.69	3.59	14.64
						(57.56	3.75	14.60)
5b	4-Cl	4-OCH_3	160-62	68	$C_{24}H_{20}N_5O_2S_2Cl$	56.70	4.03	13.65
						(56.53	3.93	13.74)
5c	4-CH_3	4-Cl	140	64	$C_{24}H_{20}N_5OS_2Cl$	58.39	4.16	14.27
						(58.36	4.05	14.18)
5d	4-OCH_3	4-Cl	150-51	60	$C_{24}H_{20}N_5O_2S_2Cl$	56.49	3.81	13.86
						(56.53	3.93	13.74)
5e	4-OCH_3	4-OCH_3	165-66	65	$C_{25}H_{23}N_5O_3S_2$	59.33	4.39	13.97
						(59.41	4.55	13.86)
6a	4-Cl	H	164	66	$C_{22}H_{18}N_5OSCl$	60.69	4.17	16.21
						(60.62	4.13	16.07)
6b	4-Cl	4-OCH_3	145	69	$C_{23}H_{20}N_5O_2SCl$	59.15	4.21	15.16
						(59.29	4.30	15.04)
6c	4-CH_3	4-Cl	136	70	$C_{23}H_{20}N_5OSCl$	61.50	4.37	15.49
						(61.40	4.45	15.57)
6d	4-OCH_3	4-Cl	153	72	$C_{23}H_{20}N_5O_2SCl$	59.18	4.39	15.11
						(59.29	4.30	15.04)
6e	4-OCH_3	4-OCH_3	150	64	$C_{24}H_{23}N_5O_3S$	62.60	5.11	15.07
						(62.47	4.99	15.18)

Table II — Antimicrobial activity of compounds **3a-c – 6a-c** at 100 μ/mL concentration

Compd	Bactericidal activity			Fungicidal activity		
	E. coli	*B. subtilis*	*S. aureus*	*A. niger*	*P. oryzae*	*F. oxysporum*
3a	20	22	18	4	5	4
3b	23	24	19	4	6	5
3c	18	18	19	4	5	5
4a	20	21	15	3	5	4
4b	22	19	17	4	6	5
4c	19	18	15	4	5	5
5a	20	19	17	5	6	5
5b	21	18	19	6	6	5
5c	20	19	19	6	6	5
6a	22	17	16	5	5	4
6b	20	18	17	5	6	4
6c	20	16	15	5	6	5
Norfloxacin	29	26	27	-	-	-
Ciprofloxacin	27	25	25	-	-	-

A L Barry[18]. Bacteria were cultured in nutrient agar medium and the solution of the compounds were made in DMSO at 100 μg/mL concentration. The bacteria were precultured overnight in nutrient broth at 37±1 °C. Filter paper disc (5 mm in diameter) containing the compounds were placed at 37 °C for 24 hr. After incubation period, the inhibition zone were measured in mm. Known antibiotics like norfloxacin and ciprofloxacin were used for comparison at same concentration. The results are recorded in **Table II**.

Fungicidal Activity. The fungicidal activity of the compounds were evaluated against *Aspergillus niger*, *Pyricularia oryzae* and *Fusarium oxysporum* by filter paper disc method[19] at 100 μg/mL concentration using standard malt agar culture medium. The filter paper disc (5 mm in diameter, Whatman filter paper no. 1) impregnated with 1 mL of test solution of the toxicant, was dried and aseptically transferred to a petriplate (80 mm diameter) containing 10 mL of culture medium. Only mycelial disc of test fungus was inoculated at 28±1 °C for seven days. The experiment was repeated in triplicate. After seven days, inhibition in fungal growth was determined as a difference in growth between control plates and those treated with test compound. The percentage inhibition of mycelial growth was calculated by following equation[20].

$$\text{Growth Inhibition} = \frac{C - T}{C} \times 100$$

where C is the average diameter of the fungal colony (in mm) in control plates and T is the average diameter of the fungal colony (in mm) in the treated plates. The fungal activity being rated at **1**(0-30 %), **2**(31-50 %), **3**(51-65 %), **4**(66-80 %), **5**(81-90 %), **6**(91-95 %) and **7**(96-100 %) and are recorded in **Table II**.

The antimicrobial activity data of the tested compounds revealed that compounds **3b, 4b, 6a** were comparatively more active against *E. coli*; compounds **3a, 3b, 4a** were more active against *B. subtilis*, compounds **3b**, **3c**, **5a**, **5b** were more active against *S. aureus*; compounds **5b**, **5c** were active against *A. niger* and compounds **3b**, **4b**, **5a**, **5c**, **6b** and **6c** were active against *P. oryzae* at 100 μg/mL concentration.

Experimental Section

Melting points are taken in open capillaries and are uncorrected. IR spectra are recorded in KBr on PE 781 IR spectrophotometer (cm^{-1}), ^1H NMR spectra in DMSO-d_6 on a Varian EM 390-CW spectrometer at 90 MHz using TMS as internal reference (chemical shifts in δ, ppm) and mass spectra on a Finnigan MAT 8230 mass spectrometer.

3-Isonicotinamido-2-phenyl-4-thiazolidinone 1a-d. A mixture of isoni-cotinamide (0.01 mole) and benzaldehyde (0.01 mole) in ethanol was refluxed for 1 hr. Mercaptoacetic acid (0.011 mole) was added to the reaction mixture and refluxed for 3 hr. It was cooled, poured into water and excess of mercapto acetic acid was neutralised by adding sodium bicarbonate solution. The resulting solid mass was filtered, washed with water, dried and recrystallised from aq. ethanol to give the thiazolidinones **1a-d** which are recorded in **Table I**.

5-(4-Chlorobenzylidene)-3-isonicotinamido-2-phenyl-4-thiazolidinone 2a.

A mixture of 3-isonicotinamido-2-phenyl-4-thiazolidinone **1a** (0.01 mole), 4-chlorobenzaldehyde (0.01 mole) and anh. sodium acetate (0.015 mole) in gl. acetic acid was refluxed for 3 hr. The reaction mixture was poured into beaker and left for sometime and ice-cold water was added to it. The compound thus separated out was filtered, washed with water, dried and recrystallised from aq. ethanol to give **2a**, m.p. 167 °C, yield 78%. IR (KBr, cm^{-1}): 3180 (NH), 3035 (C-H aromatic), 1676 (CONH), 1665 (O=C-C=C-), 1585, 1565, 1510 (aromatic ring); ^{1}H NMR (DMSO-d_6): δ 6.8-8.2 (m, 15H, ArH, O=C-C=CH- & SCH), 12 (b, 1H, NH).

The compounds **2b-f** were prepared from **1b-d** using similar procedure (**Table I**).

5-Amino-7-(4-chlorophenyl)-6-cyano-3-isonicotinamido-2-phenyl thiazolo [4,5-*b*]-2,3,4,7-tetrahydropyridines 3a. A mixture of **2a** (4.21g, 0.01 mole), malononitrile (0.66 g, 0.01 mole) and ammonium acetate (6.16 g, 0.08 mole) was fused for 2 hr, dioxane (10 mL) was then added and refluxed for 4 hr. The reaction mixture was cooled and poured into ice-cold water. The resulting solid was filtered, washed with water, dried and recrystallised from aq. ethanol to give **3a**, m.p. 159-60 °C, yield 65%. IR (KBr, cm^{-1}): 3460, 3235, 3160, 3080 (NH_2, NH & CONH), 2250 (C≡N), 1680 (CONH), 1620, 1585, 1535, 1510 (aromatic ring); ^{1}H NMR (DMSO-d_6): δ 6.8-8.9 (m, 17H, ArH, NH_2 & NH), 12.1 (b, 1H, NH); MS: m/z 486, 488 (M^+).

The compounds **3b-e** were prepared from **2b-e** using similar procedure (**Table I**).

6-Cyano-7-(4-chlorophenyl)-3-isonicotinamido-2-phenyl thiazolo[4,5-*b*]-2,3,4,5,6,7-hexahydropyrid-5-one 4a. A mixture of **2a** (4.21 g, 0.01 mole), ethyl cyanoacetate (1.13 g, 0.01 mole) and ammonium acetate (6.16 g, 0.08 mole) was fused for 2 hr, dioxane (10 mL) was then added and refluxed for 4 hr. The reaction mixture was cooled and poured into ice-cold water. The resulting mass was filtered, washed with water, dried and recrystallised from aq. ethanol to give **4a**, m.p. 171 °C, yield 68%. IR (KBr, cm^{-1}): 3180, 3140 (NH & CONH), 2250 (C≡N), 1670, 1660 (CONH), 1620, 1590, 1560, 1510 (aromatic ring); ^{1}H NMR (DMSO-d_6): δ 7.1-8.3 (m, 16H, ArH & 2H, H-6 & H-7), 9.6 (b, 1H, NH), 11.8 (b, 1H, NH); MS: m/z 487, 489 (M^+).

Other compounds **4b-e** were prepared from **2b-e** using similar procedure and are recorded in **Table I**.

5-Amino-2-chlorophenyl-3-isonicotinamido-7-(4-methoxyphenyl) thiazolo [4,5-*d*][1,3] thiazines 5b. A mixture of **2b** (4.5 g, 0.01 mole), thiourea (0.91 g, 0.012 mole) and KOH (0.73 g, 0.013 mole) in ethanol was refluxed for 4 hr. The resulting mixture was poured into cold water. The product thus obtained was filtered, dried and recrystallised from aq. ethanol to give **5b**, m.p. 160-61 °C, yield 62%. IR (KBr, cm^{-1}): 3465, 3250, 3080 (NH_2 & NH), 3030 (C-H aromatic), 2960, 2880 (C-H aliphatic), 1680 (CONH), 1640 (C=NH), 1620 (C=C), 1585, 1565, 1530, 1505 (aromatic ring), 1320 (C-N-), 1275 (C-S-C); ^{1}H NMR (DMSO-d_6): δ 3.8 (s, 3H, OCH_3), 6.8-8.2 (15H, ArH & 2H, 2 × NH), 11.6 (b, 1H, NH); MS: m/z 509, 511 (M^+).

The compounds **5a** and **5c-e** were prepared from **2a** and **2c-e** using similar procedure (**Table I**).

2-Chlorophenyl-3-isonicotinamido thiazolo[4,5-*c*]pyrazolines 6b. A mixture of **2b** (4.5 g, 0.01 mole) and hydrazine hydrate (0.015 mole) in ethanol was refluxed for 4 hr. The resulting mixture was poured into cold water. The product thus obtained was filtered, dried ands recrystallised from aq. ethanol to give **6b**, m.p. 145 °C, yield 68%. IR (KBr, cm^{-1}): 3250, 3080 (NH), 1680 (CONH), 1620 (C=C); ^{1}H NMR (DMSO-d_6): δ 3.8 (s, 3H, OCH_3), 5.3 (d, 1H, NH), 6.8-8.2 (m, 15H, ArH & NH), 11.6 (b, 1H, NH); MS: m/z 465, 467 (M^+).

The compounds **6a** and **6c-e** were prepared from **2a** and **2c-e** using similar procedure (**Table I**).

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