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Synthesis, Anticonvulsant and Antimicrobial Activities of Novel Mannich Bases of Isatin Derivatives

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Some novel mannich base isatin derivatives were synthesized by reacting 1-(5-chloro-2-oxoindolin-3-ylidene)-4-(pyridine-2-yl)thiosemicarbazide with formaldehyde and several secondary amines. Their chemical structures were elucidated by means of spectral (FT-IR, ^{1}H NMR and mass) analysis. Investigation of *in vitro* antibacterial and antifungal activity of synthesized compounds was done by disc diffusion method against *B. subtilis, S. aureus, E. coli, P. aeruginosa, C. albicans* and *A. niger*. All the synthesized compounds were screened for anticonvulsant activity by MES method using phenytoin as standard drug. All the compound exhibited moderate to good antibacterial and antifungal activity. All the compounds of the series exhibited significant anticonvulsant activity at 100 mg/kg dose level.

Key Words: Isatin, Mannich base, Antimicrobial, Anticonvulsant.

INTRODUCTION

Current drug therapy for epilepsy suffers from a number of disadvantages including the fact that the convulsions of *ca.* 25 % of the epileptics are inadequately controlled by medication[1]. In recent years antiepileptic drug development is one of the most prominent research areas with the in-depth understanding of the pathophysiology of epilepsy. Isatin was reported to possess proconvulsant and anticonvulsant activities[2] apart from other pharmacological properties like antibacterial[3-5] antifungal[6-8], antiviral[9-11], anti-HIV[12-14], antiprotozoal[15,16] and antihelminthic[17,18] activities. In addition pyridines are associated with diverse biological activities[19,20]. Therefore, it was envisaged that a new series of isatin derivatives with pyridine would possess high antimicrobial and anticonvulsant activity. The chemical structures of the synthesized compounds were confirmed by means of their IR, ^{1}H NMR and mass spectral analysis.

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EXPERIMENTAL

Melting points were determined in open capillary tubes and are uncorrected. The purity of the synthesized compounds was routinely checked by TLC on silica gel G. ^{1}H NMR spectra were recoded on Jeol GSX 400 spectrometer using TMS as an internal standard (chemical shifts in δ, ppm), IR spectra on a Shimadzu FT 8300 infrared spectrophotometer (ν_{max} cm^{-1}) and mass spectra on a Jeol MSMATE spectrometer. The physical and spectral data of the title compounds are given in Table-1.

TABLE-1
PHYSICAL DATA OF COMPOUNDS **4a-4j**

Compd.	R	m.f.	m.p. (ºC) (±2 ºC)	Yield (%)	Elemental analysis %: Found		
					C	H	N
4a	Dimethyl amino	$C_{17}H_{17}N_6OSCl$	156	82	52.48	4.39	21.57
4b	Diethyl amino	$C_{19}H_{21}N_6OSCl$	169	76	54.71	5.06	20.60
4c	Diphenyl amino	$C_{27}H_{21}N_6OSCl$	178	78	63.17	4.11	16.36
4d	Pyrrolidino	$C_{19}H_{19}N_6OSCl$	161	81	54.96	4.61	20.23
4e	Piperidino	$C_{20}H_{21}N_6OSCl$	145	75	55.97	4.91	19.54
4f	Ethyl methyl amino	$C_{18}H_{19}N_6OSCl$	163	80	53.65	4.73	20.85
4g	Methyl propyl amino	$C_{19}H_{21}N_6OSCl$	151	72	54.72	5.06	20.15
4h	Ethyl propyl amino	$C_{20}H_{23}N_6OSCl$	164	83	55.73	5.35	19.46
4i	Aziridino	$C_{17}H_{15}N_6OSCl$	152	78	52.75	3.87	21.70
4j	Azetidino	$C_{18}H_{17}N_6OSCl$	166	71	53.91	4.24	20.96

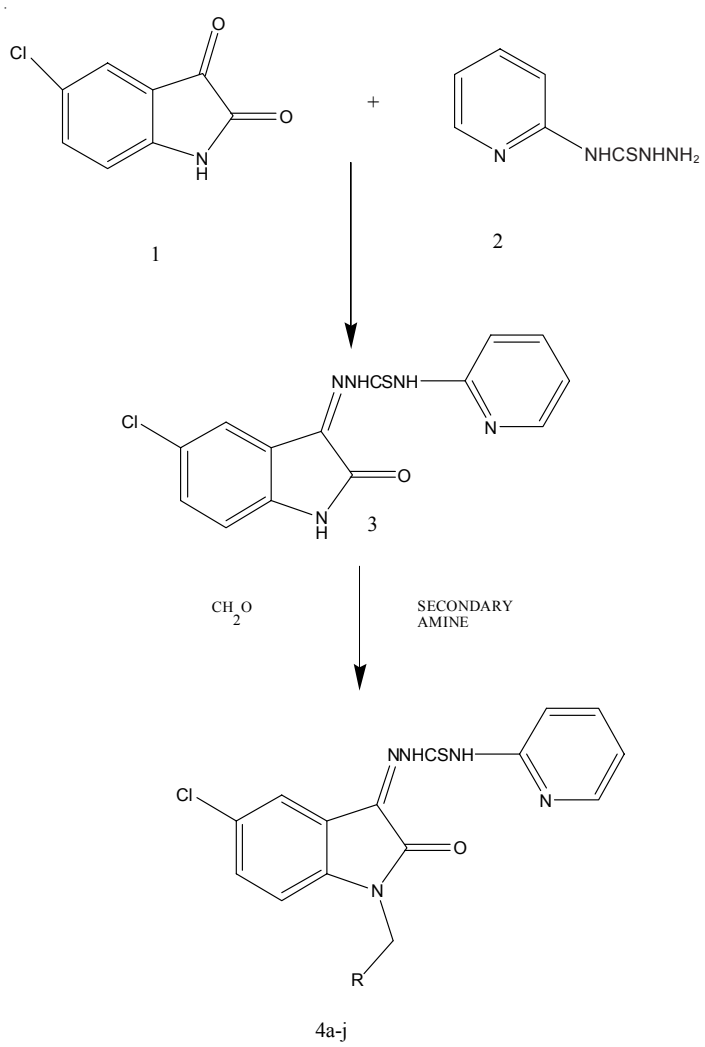
Synthesis of 5-chloro isatin (1): To 55 mL of concentrated sulphuric acid at 50 ºC was added 15 g of dry isonitrosoacetanilide derivative. The solution was heated to 80 °C and was kept at this temperature for about 10 min. It was then cooled to room temperature and poured upon cracked ice. After 1.5 h, the 5-chloro isatin was filtered, washed several times with cold water to remove sulphuric acid and then dried in air.

Synthesis of 4-(pyridine-2-yl) thio semicarbazide (2): To a solution of pyridine (0.001 mol) in DMF was added sodium hydroxide (0.001 mol) and carbon disulphide. The mixture was stirred for 1 h, to the stirred mixture was added hydrazine hydrate (0.01 mol) and stirring continued at 45 °C for 1 h. On adding water a pale yellow solid separated out which is recrystallized from DMF-ethanol.

Synthesis of 1-(5-chloro-2-oxoindolin-3-ylidene)-4-(pyridine-2-yl) thiosemicarbazide (3): Equimolar quantities of 5-chloro isatin and (pyridine-2-yl) thiosemicarbazide were dissolved in warm ethanol containing 1 mL of glacial acetic acid. The reaction mixture was refluxed for 10 h and

set aside. The resultant solid was washed with dilute ethanol dried and recrystallized from ethanol and chloroform mixture.

Synthesis of (4a-4j): A slurry consisting of 1-(5 chloro-2-oxoindolin-3-ylidene)-4-(pyridine-2-yl) thiosemicarbazide (0.002 mol), THF 3 mL and 37 % formalin was made. To this add amine (0.002 mol) drop wise with cooling and shaking. The reaction mixture was allowed to stand at room temperature for 1 h with occasional shaking after, which it was warmed on a steam bath for 15 m. At the end of the period the contents were cooled and the product obtained was recrystallized from chloroform and petroleum ether.



Scheme

1-(5-Chloro-1-[(dimethylamino)methyl)-2-oxoindolin-3-ylidene]-4-(pyridine-2-yl)thiosemicarbazide (4a): The sample was recrystallized using chloroform and petroleum ether. IR (KBr, ν_{max}, cm^{-1}): 3392 (CH, Ar), 1790 (C=O), 1462 (C=N, Ar), 1155 (C=S) and 742 (C-Cl); ^{1}H NMR (DMSO d_6): δ, 7.3-7.6 (m, 4H, Ar-H), 4.03 (t, H, -CH_2-), 7.0 (s, H), 4.0 (s, H, N-H), 8.11 (d, H), 6.60-6.70 (d, 3H, -CH_2-of pyridine), 2.27 (s, 2H, CH_3). Mass: m/z value: 388.09.

1-(5-Chloro-1-[(diethylamino)methyl)-2-oxoindolin-3-ylidene]-4-(pyridine-2-yl)thiosemicarbazide (4b): The sample was recrystallized using chloroform and petroleum ether. IR (KBr, ν_{max}, cm^{-1}): 3351 (CH, Ar), 1740 (C=O), 1437 (C=N, Ar), 1176 (C=S) and 778 (C-Cl); ^{1}H NMR (DMSO d_6): δ, 7.32-7.6 (m, 4H, Ar-H), 4.03 (t, H, -CH_2-), 7.01 (s, H), 4.0 (s, H, N-H), 8.13 (d, H), 6.61-6.72 (d, 3H, -CH_2- of pyridine), 2.40 (m, 2H, -CH_2-), 1.0 (t, 2H, -CH_3). Mass: m/z value: 416.12.

1-(5-Chloro-1-[(diphenylamino)methyl)-2-oxoindolin-3-ylidene]-4-(pyridine-2-yl)thiosemicarbazide (4c): The sample was recrystallized using chloroform and petroleum ether. IR (KBr, ν_{max}, cm^{-1}): 3352 (CH, Ar), 1743 (C=O), 1493 (C=N, Ar), 1085 (C=S) and 680 (C-Cl); ^{1}H NMR (DMSO d_6): d, 7.33-7.62 (m, 4H, Ar-H), 4.73 (d, H, -CH_2-) 7.02 (s, H), 4.01 (s, H, N-H), 8.11 (d, H), 6.63-6.74 (d, 3H, -CH_2- of pyridine), 6.43-7.04 (d, 10H, Ar-H). Mass: m/z value: 512.12.

1-(5-Chloro-2-oxo-1-(pyrrolidin-1-ylmethyl)indolin-3-ylidene)-4-(pyridine-2-yl)thiosemicarbazide (4d): The sample was recrystallized using chloroform and petroleum ether. IR (KBr, ν_{max}, cm^{-1}): 3391 (CH, Ar), 1744 (C=O), 1443 (C=N, Ar), 1055 (C=S) and 788 (C-Cl); ^{1}H NMR (DMSO d_6): δ, 7.34-7.65 (m, 4H, Ar-H), 4.05 (t, H, -CH_2-), 7.04 (s, H), 4.0 (s, H, N-H), 8.14 (d, H), 6.62-6.75 (t, 3H, -CH_2- of pyridine), 1.59-2.25 (m, 4H, pyrrolidine). Mass: m/z value: 414.1.

1-(5-Chloro-2-oxo-1-(piperidin-1-ylmethyl)indolin-3-ylidene)-4-(pyridine-2-yl)thiosemicarbazide (4e): The sample was recrystallized using chloroform and petroleum ether. IR (KBr, ν_{max}, cm^{-1}): 3393 (CH, Ar), 1742 (C=O), 1446 (C=N, Ar), 1058 (C=S) and 676 (C-Cl); ^{1}H NMR (DMSO d_6): δ, 7.34-7.62 (m, 4H, Ar-H), 4.02 (t, H, -CH_2-), 7.04 (s, H), 4.0 (s, H, N-H), 8.14 (d, H), 6.60-6.70 (t, 3H, -CH_2- of pyridine), 1.50-2.24 (s, 5H, piperidine). Mass: m/z value: 428.12.

1-(5-Chloro-1-[(ethyl(methyl)amino)methyl)-2-oxoindolin-3-ylidene]-4-(pyridine-2-yl)thiosemicarbazide (4f): The sample was recrystallized using chloroform and petroleum ether. IR (KBr, ν_{max}, cm^{-1}): 3396 (CH, Ar), 1747 (C=O), 1441 (C=N, Ar), 1058 (C=S) and 698 (C-Cl); ^{1}H NMR (DMSO d_6): δ, 7.30-7.61 (m, 4H, Ar-H), 4.04 (t, H, -CH_2-), 7.02 (s, H), 4.03 (s, H, N-H), 8.11 (d, H), 6.62-6.74 (t, 3H, -CH_2- of pyridine), 1.00-2.40 (d, 3H, -CH_2-CH_3). Mass: m/z value: 402.9.

1-(5-Chloro-1-[(methyl(propyl)amino)methyl]-2-oxoindolin-3-ylidene)-4-(pyridin-2-yl)thiosemicarbazide (4g): The sample was recrystallized using chloroform and petroleum ether. IR (KBr, ν_{max}, cm^{-1}): 3399 (CH, Ar), 1748 (C=O), 1445 (C=N, Ar), 1148 (C=S) and 712 (C-Cl); ^{1}H NMR (DMSO d_6): δ, 7.35-7.63 (m, 4H, Ar-H), 4.02 (t, H, -CH_2-), 7.01 (s, H), 4.02 (s, H, N-H), 8.14 (d, H), 6.61-6.70 (t, 3H, -CH_2- of pyridine), 0.94-2.35 (t, 4H, -CH_2-CH_3). Mass: m/z value: 416.93.

1-(5-Chloro-1-[(ethyl(propyl)amino)methyl]-2-oxoindolin-3-ylidene)-4-(pyridin-2-yl)thiosemicarbazide (4h): The sample was recrystallized using chloroform and petroleum ether. IR (KBr, ν_{max}, cm^{-1}): 3394 (CH, Ar), 1749 (C=O), 1457 (C=N, Ar), 1124 (C=S) and 756 (C-Cl); ^{1}H NMR (DMSO d_6): δ, 7.34-7.65 (m, 4H, Ar-H), 4.01 (t, H, -CH_2-), 7.03 (s, H), 4.04 (s, H, N-H), 8.10 (d, H), 6.66-6.78 (t, 3H, -CH_2- of pyridine), 0.96-2.42 (d, 5H, -CH_2-CH_3). Mass: m/z value: 430.95.

1-(5-Chloro-1-(aziridino)methyl)-2-oxoindolin-3-ylidene)-4-(pyridine-2-yl)thiosemicarbazide (4i): The sample was recrystallized using chloroform and petroleum ether. IR (KBr, ν_{max}, cm^{-1}): 3398 (CH, Ar), 1741 (C=O), 1438 (C=N, Ar), 1098 (C=S) and 617 (C-Cl); ^{1}H NMR (DMSO d_6): δ, 7.34-7.63 (m, 4H, Ar-H), 4.02 (t, H, -CH_2-), 7.02 (s, H), 4.01 (s, H, N-H), 8.15 (d, H), 6.61-6.70 (t, 3H, - CH_2- of pyridine), 1.61(d, 2H, aziridine). Mass: m/z value: 386.07.

1-(5-Chloro-1-(azetidino)methyl-2-oxoindolin-3-ylidene)-4-(pyridin-2-yl)thiosemicarbazide (4j): The sample was recrystallized using chloroform and petroleum ether. IR (KBr, ν_{max}, cm^{-1}): 3389 (CH, Ar), 1750 (C=O), 1479 (C=N, Ar), 1123 (C=S) and 625 (C-Cl); ^{1}H NMR (DMSO d_6): δ, 7.36-7.64 (m, 4H, Ar-H), 4.02 (t, H, -CH_2-), 7.02 (s, H), 4.0 (s, H, N-H), 8.12 (t, H), 6.61-6.72 (t, 3H, -CH_2- of pyridine), 2.23-3.29 (m, 3H, azetidine). Mass: m/z value: 400.09.

Antibacterial activity: The compounds **4a-j** was screened *in vitro* for their antibacterial activity against pathogenic organisms *B. subtilis, S. aureus, E. coli* and *P. aeruginosa* using disc diffusion method at a concentration of 250 µg/mL with DMF as the solvent. After 24 h of incubation at 37 ºC the zones of inhibition formed were measured in mm with standard drug ampicillin and are shown in Table-2.

Antifungal activity: The synthesized compounds were screened for their antifungal activity against *Candida albicans* and *Aspergillus niger* at a concentration of 50 µg/mL with incubation for 72 h at 37 ºC. Standard drug used was griseofulvin. Similar procedure as for antibacterial activity was followed. The activity data are given in Table-2.

Anticonvulsant activity: The anticonvulsant activity of the synthesized compounds were tested against maximal electroshock induced convulsions[21] in albino mice of either sex (weighing 20-25 g). The compounds were

administered at a dose level of 100 mg/kg i.p using phenytoin sodium (25 mg/kg) as standard drug. After 1 h all the groups of mice were subjected to a shock of 50 mA by electro-convulsiometer *via* ear electrodes for 0.2 s and the presence or absence of extensor response was noted. Animals in which extensor response was abolished were taken as protected rats. The data are given in Table-2.

TABLE-2
BIOLOGICAL DATA

Compd.	Antibacterial activity*				Antifungal activity		Anticonvulsant activity**
	SA	BS	EC	PA	CA	AN	
4a	16	19	21	17	12	11	70
4b	20	25	23	22	14	18	40
4c	07	10	11	12	08	07	80
4d	13	08	08	09	11	10	20
4e	18	20	16	21	14	13	30
4f	12	13	13	12	12	14	60
4g	15	18	21	16	07	17	30
4h	21	22	15	20	16	15	50
4i	14	09	07	10	13	09	40
4j	11	14	17	15	11	12	50

SA = *S. aureus*; BS = *B. subtilis*; EC = *E. coli*; PA = *P. aeruginosa*;
CA = *C. albicans*; AN = *A. niger*.
*Zone of inhibition in mm; **Percentage protection.

RESULTS AND DISCUSSION

The synthesized compounds were evaluated for their antimicrobial and anticonvulsant activities. The antimicrobial activity of title compounds revealed that **4b**, **4e** and **4h** exhibited highest activity against *B. subtilis, S. aureus, E. coli, P. aeruginosa, C. albicans* and *A. niger*. But these activities were less than their respective standards. The anticonvulsant activity revealed that three compounds **4a**, **4c** and **4f** exhibited significant activity followed by **4h** and **4j**. However, these compounds showed lesser antimicrobial and anticonvulsant activity as compared to their respective standards.

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