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SYNTHESIS AND ANTICONVULSANT ACTIVITY OF SOME NOVEL ISATIN SCHIFF'S BASES

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Abstract: A series of isatin schiff's bases **(3a-j)** were synthesized and characterized by their spectral data and screened for anticonvulsant and toxicity screening. Some of investigated compounds showed significant anticonvulsant activity.
Keywords: Isatin, schiff's bases, anticonvulsant activity

Introduction

Isatin (indol-2,3-dione) is a resourceful endogenous heterocyclic molecule identified in human being and rat tissues. Several of its derivatives were reported to exhibit a wide range of promising pharmacodynamic profile like anticonvulsant,[1,2] anti-HIV,[3] cytotoxic,[4] tuberculostatic,[5] anti-microbial.[6] At milimolar concentrations isatin has been found to inhibit different enzymes, an effect that may contribute to its anti infective actions.[7] Isatin has been preferred because during initial screening it has shown activity in the MES test .[8] In view of potent anticonvulsant activity of isatin, we have synthesized a novel series of 3-substituted isatin derivatives by following schiff's reaction and evaluate them for their anticonvulsant activity.

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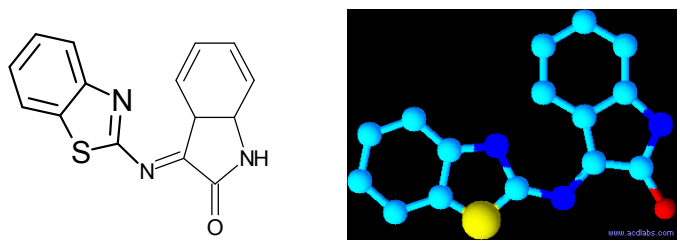
Materials and Methods

Chemistry

All compounds were purified by column chromatography and confirmatory establishment of structure was done by melting point, TLC, UV, IR and ^{1}H NMR. Column chromatography was performed using silica gel (Qualigens, particle size 60-120 mm). TLC was performed on silica gel TLC plates. All melting points were recorded on a DECIBEL digital melting point apparatus. IR spectra were recorded on 8400S SHIMADZU spectrometer. ^{1}H NMR spectra were recorded on a dpx300 spectrometer (analysis laboratory, IIT, New Delhi). Physical properties of the synthesized compounds are listed in **Table** 1 whereas scheme of synthesis is given in **Figure 2** and **3**



(a) **(b)**

Figure 1: (a) Structural formula and (b) 3D-structure of Compund 3a.

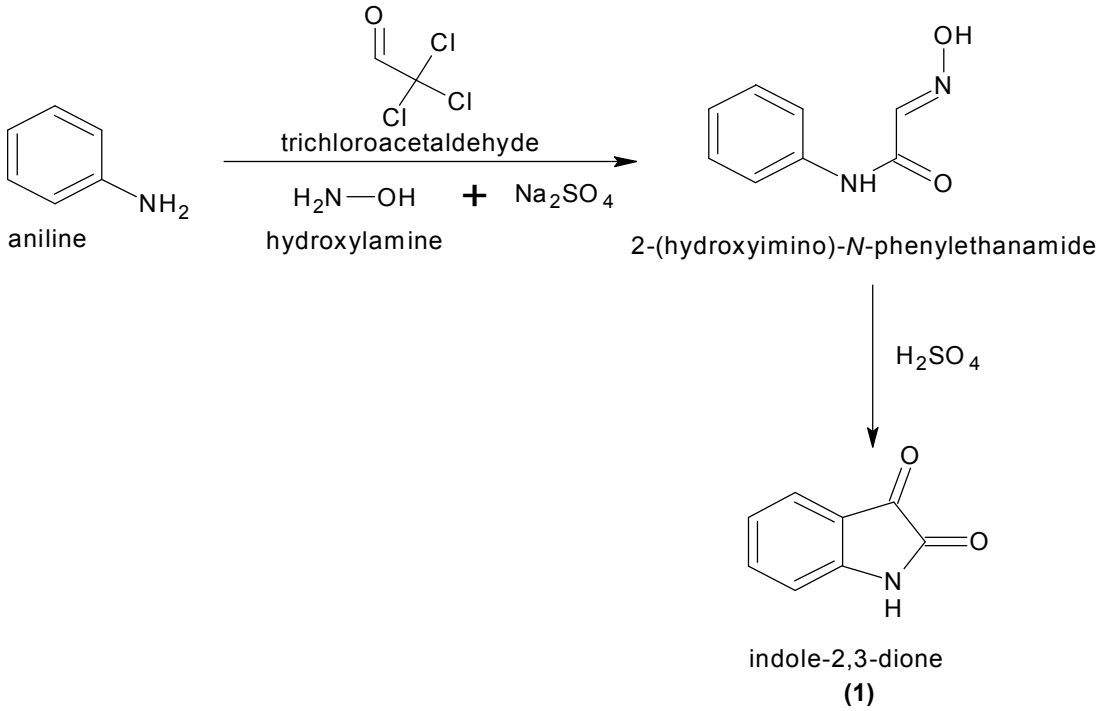


Figure 2: Scheme 1 Synthesis of Indole-2,3 dione (1)

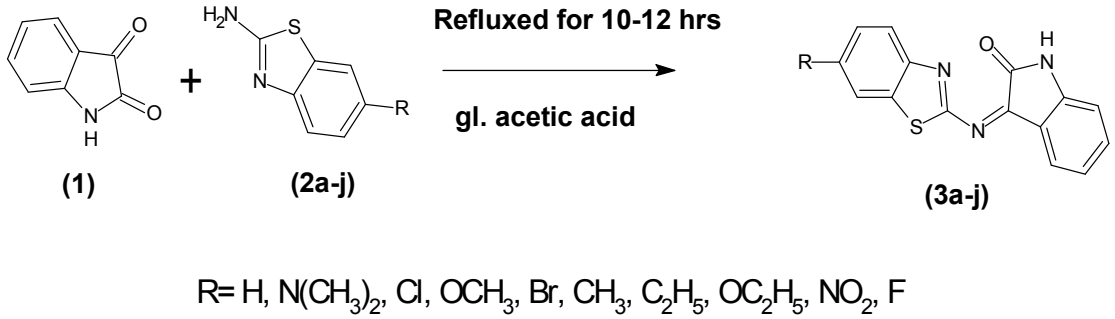


Figure 3: Scheme 2 of synthesis of compounds 3a-3j

General procedure for the synthesis of 6(un)substituted 2-amino benzothiazoles (2a-j)

To glacial acetic acid (20 ml), cooled to 20 °C, was added 0.08 mole of potassium thiocynate and 0.01 mole of (un)substituted anilines. The mixture was placed in a freezing mixture of ice-salt and mechanically stirred. 1.6 ml of Bromine in 6 ml of glacial acetic acid was added from a dropping funnel at such a rate that the temperature never rose beyond 0^0C. After all the bromine was added (105 min), the solution was stirred for 2 hrs below room temperature and at room temperature for 10 hrs. It was then allowed to stand overnight, during which period an orange precipitate settled at the bottom. Water (6 ml) was added quickly and the slurry was heated to 85^0C on steam-bath and filtered while hot. The orange residue was placed in reaction flask and treated with 10 ml of glacial acetic acid, heated again to 85^0C and filtered. The combined filtrate was cooled and the precipitate was collected, re-crystallized from benzene: ethanol.

General procedure for the synthesis of Schiff's Bases (3a-j)

A mixture of equimolar quantities of 6-(un)substituted 1, 3 benzothiazol-2-amine and indole 2, 3dione were dissolved in 20 ml of absolute alcohol, refluxed for 10-12 hrs in presence of few drops of glacial acetic acid. Orange red spangles of the product crystallized out on cooling and were collected, washed with chilled methanol and recrystallized (ethanol: benzene; 1:1) to give chromatographically pure products. In some of cases column chromatography over neutral alumina (hexane: ethyl acetate; 12:1) was necessary prior to crystallization. Following the same procedure, compounds (3b-j) were prepared.

Spectral data

3-(1, 3-benzothiazol-2-ylimino)-1, 3-dihydro-2H-indol-2-one (3a)

IR (KBr, cm^{-1}) 1609 (C=N), 1654 (C=O), 3061 (N-H); ^1H NMR δ 6.6-7.8 (m, 8H, Ar-H), 9.5 (s, 1H, NH), m/z: 279.05 (100.0%), 280.05 (17.2%), 281.04 (4.5%), 281.05 (1.8%), 280.04 (1.1%). Analytical (%) Calculated C (64.50%) H (3.25%) N (15.04%) O (5.73%) S (11.48%), found C (64.54%) H (3.26%) N (15.14%) O (5.70%) S (11.42%)

3-{[6-(dimethylamino)-1,3-benzothiazol-2-yl]imino}-1,3-dihydro-2H-indol-2-one (3b)

IR (KBr, cm^{-1}) 1604 (C=N), 1735 (C=O), 3195 (N-H), 2882, 1438 (C-H), 1342 (C-N); ^1H NMR δ 2.97 (s, 6H, CH), 10.5 (s, 1H, N-H), 6.6-7.8 (m, 7H, Ar-H), 1.1%) m/z: 322.09 (100.0%), 323.09 (20.7%), 324.08 (4.5%), 324.10 (1.6%)Analytical (%) Calculated C (66.66%) H (4.38%) N (17.38%) O (4.96%) S (9.95%), found C (66.63%) H (4.39%) N (5.00%) O (4.98%) S (9.99%)

3-[(6-chloro-1,3-benzothiazol-2-yl)imino]-1,3-dihydro-2H-indol-2-one (3c)

IR (KBr, cm^{-1}) 1612 (C=N), 1735 (C=O), 3195 (N-H), 895 (C-cl); ^1H NMR δ 6.9 – 7.6 (m, 7H, Ar-H), 10.5 (s, 1H, NH); m/z: 313.01 (100.0%), 315.00 (36.5%), 314.01 (17.2%), 316.01 (6.0%), 315.01 (1.7%), 317.00 (1.5%), 314.00 (1.1%) Analytical (%) Calculated C (57.42%) H (2.57%) Cl (11.30%) N (13.39%) O (5.10%) S (10.22%), found C (57.41%) H (2.54%) cl (11.31%) N (13.35%) O (5.09%) S (10.12%)

3-[(6-methoxy-1,3-benzothiazol-2-yl)imino]-1,3-dihydro-2H-indol-2-one (3d)

IR (KBr, cm^{-1}) 1620 (C=N), 1732 (C=O), 3200 (N-H), 2810 (O-CH3); ^1H NMR δ 6.6 – 7.8 (m, 7H, Ar-H), 10.94 (s, 1H, NH), 3.74 (s, 3H, OCH_3); m/z: 309.06 (100.0%), 310.06 (18.3%), 311.05 (4.5%), 311.06 (2.2%), 310.05 (1.1%)Analytical (%) Calculated C (62.12%) H (3.58%) N (13.58%) O (10.34%) S (10.37%), found C (62.02%) H (3.53%) N (13.55%) O (10.35%) S (10.34%)

3-[(6-bromo-1,3-benzothiazol-2-yl)imino]-1,3-dihydro-2H-indol-2-one (3e)

IR (KBr, cm^{-1}) 1652(C=N), 1726 (C=O), 3168 (N-H), 811 (C-Br); ^1H NMR δ 6.7-7.6 (m, 7H, Ar-H), 10.96 (s, 1H, NH); m/z: 356.96 (100.0%), 358.96 (99.0%), 357.96 (17.2%), 359.96 (16.7%), 358.95 (4.5%), 360.95 (4.4%), 359.95 (1.9%), 360.96 (1.8%), 357.95 (1.1%) Analytical (%) Calculated C (50.29%) H (2.25%) Br (22.31%) N (11.73%) O (4.47%) S (8.95%), found C (50.27%) H (2.24%) Br (22.29%) N (11.79%) O (4.44%) S (8.98%)

3-[(6-methyl-1,3-benzothiazol-2-yl)imino]-1,3-dihydro-2H-indol-2-one (3f)

IR (KBr, cm^{-1}) 1620(C=N), 1730 (C=O), 3209 (N-H), 2.32 (s, 3H, CH3); ^1H NMR δ 6.6 – 7.6 (m, 7H, Ar-H), 10.97 (s, 1H, NH); m/z: 293.06 (100.0%), 294.07 (17.5%), 295.06 (4.7%), 294.06 (1.9%), 295.07 (1.8%) Analytical (%) Calculated C (65.51%) H (3.78%) N (14.32%) O (5.45%) S (10.93%), found C (65.50%) H (3.75%) N (14.39%) O (5.46%) S (10.90%)

3-[(6-ethyl-1,3-benzothiazol-2-yl)imino]-1,3-dihydro-2H-indol-2-one (3g)

IR (KBr, cm^{-1}) 1655 (C=N), 1730 (C=O), 3138 (N-H); ^1H NMR δ 6.6 – 7.7 (m, 7H, Ar-H), 10.99 (s, 1H, NH), 4.1-4.4 (q, 2H, CH2 CH3), 1.1-1.4 (t, 2H, CH2 CH_3) m/z: 307.08 (100.0%), 308.08 (19.4%), 309.07 (4.5%), 309.08 (2.2%), 308.07 (1.1%)Analytical (%) Calculated C (66.43%) H (4.26%) N (13.67%) O (5.21%) S (10.43%), found C (66.42%) H (4.27%) N (13.65%) O (5.24%) S (10.43%)

3-[(6-ethoxy-1,3-benzothiazol-2-yl)imino]-1,3-dihydro-2H-indol-2-one (3h)

IR (KBr, cm^{-1}) 1612(C=N), 1726 (C=O), 3195 (N-H) 1280 (Ar-O-C); ^1H NMR δ 6.6 – 7.6 (m, 7H, Ar-H), 10.97 (s, 1H, NH) 1.1-1.4 (q, 2H, CH2CH3), 3.4-4.0 (t, 3H, CH_2CH_3); m/z: 323.07 (100.0%), 324.08 (18.6%), 325.07 (4.7%), 325.08 (2.2%), 324.07 (1.9%) Analytical (%) Calculated C (63.14%) H (4.05%) N (12.99%) O (9.90%) S (9.92%), found C (63.15%) H (4.07%) N (12.89%) O (9.99%) S (9.92%)

3-[(6-nitro-1,3-benzothiazol-2-yl)imino]-1,3-dihydro-2H-indol-2-one (3i)

IR (KBr, cm^{-1}) 1618 (C=N), 1733 (C=O), 3290 (N-H), 1353, 1530 (C-NO_2); ^1H NMR δ 6.6-7.8 (m, 7H, Ar-H), 10.98 (s, 1H, NH), m/z: 324.03 (100.0%), 325.04 (16.4%), 326.03 (4.9%), 325.03 (2.3%), 326.04 (1.9%) Analytical (%) Calculated C (55.55%) H (2.49%) N (17.28%) O (14.80%) S (9.89%), found C (55.57%) H (2.47%) N (17.25%) O (14.83%) S (9.87%)

3-[(6-fluoro-1,3-benzothiazol-2-yl)imino]-1,3-dihydro-2H-indol-2-one (3j)

IR (KBr, cm^{-1}) 1614 (C=N), 1735 (C=O), 3247 (N-H); ^1H NMR δ 6.6-7.7 (m, 7H, Ar-H), 10.95 (s, 1H, NH), m/z: 297.04 (100.0%), 298.04 (17.2%), 299.03 (4.5%), 299.04 (1.8%), 298.03 (1.1%) Analytical (%) Calculated C (60.60%) H (2.71%) F (6.39%) N (14.13%) O (5.38%) S

(10.79%), found C (60.59%) H (2.73%) F (6.40%) N (14.10%) O (5.37%) S (10.77%)

Pharmacology

Anticonvulsant Screening

All the compounds were screened for anticonvulsant properties adopting the anticonvulsant drug development (ADD) program protocol. The mice used were Carworth Farms No. 1, weighing from 19 to 25.5 g (either sex) and 22–33 days old. Accommodation conditions were maintained at 20°C and the number of animals used was 1, 3, 5 and 8 in different experiments. Methyl Cellulose was used for dissolving the test compounds in ScMET and Rotarod Test, while polyethylene glycol was used for MES. The control experiments were performed with solvents alone. Three animals were used in the control test. The test compounds were administered intraperitoneally (0.01 ml g^{-1} body mass) to mice, at doses of 30, 100 and 300 mg kg^{-1} to 1 to 4 mice. The anticonvulsant activity of 3-(1, 3-benzothiazol-2-ylimino)-1, 3-dihydro-2H-indol-2-one and derivatives **(3a-j)** in maximum electroshock (MES) and subcutaneous metrazole (ScMET) test along with their neurotoxicity has been detailed in **Table 2**.

a. Maximal electroshock seizure test (MES)

Maximal seizures were elicited by a 60Hz alternating current of 50mA (5-7 times that is necessary to elicit minimal seizures) intensity delivered for 0.2 seconds via corneal electrodes. A drop of 0.9% w/v sodium chloride instilled in each eye prior to application of electrodes assured adequate electrical contact. Test solutions of all the compounds were prepared in 30% v/v polyethylene glycol 400 (PEG 400) and animals were dosed intraperitoneally 30 min prior to testing. Abolition of the hind limb tonic extension component of the seizure was defined as protection in the MES test.

b. Subcutaneous Metrazole test (scMET)

A Metrazole dose of 85 mg kg^{-1} administered subcutaneously to mice causes seizures in more than 97% of the animals. This is called the convulsive dose 97 (CD97). The test was carried out by giving the metrazole injection approximately 10 minutes before the anticipated time of the peak anticonvulsant drug action. The animals were observed during the following 4 hours for the occurrence of seizures. A threshold convulsion is defined as one episode of clonic spasms which persists for at least 5 seconds. Absence of even a threshold convulsion during the period of observation is taken as the endpoint in this test.

c. Neurotoxicity (NT) screen

Minimal motor impairment was measured in mice by the rotarod test. The mice were trained to stay on an accelerating rotarod that rotates at 6 rpm. The rod diameter was 3.2 cm. Trained animals were given i.p. injection of the test compounds in doses of 30, 100 and 300 mg kg^{-1} body mass**.** Neurotoxicity was indicated by the inability of the animal to maintain equilibrium on the rod for at least 1 min in each of the three trials.

Results and Discussion

In the initial results of these studies, unsubstituted derivative (3a) has shown 100% protection at 300mg of doses administered in MES test and 60% promising protection in ScMET test upto 0.5 hrs however this compound has also shown a 87.5% non-toxic effect. When we introduced various 6-substituted 2-amino benzothiazole in the 3rd position of isatin, in the majority of the cases, there has been no significant change in activity profile of the isatin derivatives; however, introduction of chloro and metoxy derivatives (3c and 3d) has resulted in some potential results. The $N(CH_3)_2$ substituted derivative (3b) has shown 33.33% protection at a dose of 100mg/kg after half an hour, which prolongs after 4 hours without any toxicity but it was found to be toxic at 300mg of doses. Compound 3c has shown activity at a dose of 300mg/kg showing 100% protection after half an hour. In the ScMET test, this compound was found inactive. In the ScMET test, all the compounds were found inactive except 3a. The methoxy substituted compound (3d) has shown only 66.66% protection after half an hour at a dose of 100mg/kg in MES. The toxicity profile of compounds 3a-j was found to be very low excluding compounds 3b and 3d, these compounds were found to be most toxic at a dose of 30 mg/kg. Rest of the compounds was non-toxic at a dose of 30mg/kg. In conclusion compound **3a**, **3b** and **3d** shown potent anticonvulsant activity. Future Goals includes the synthesis of further derivatives of these compounds.

Acknowledgement

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Table: 1 Physical characteristics of compounds 3a-j.

Comp.	R	(Mol. Wt.)	Mol.Formula	R_f	m.p. (^oC)	%yield
3a	H	(279.3164)	$C_{15}H_9N_3OS$	0.315	138	75
3b	$N(CH_3)_2$	(322.38426)	$C_{17}H_{14}N_4OS$	0.391	185	69
3c	Cl	(313.7615)	$C_{15}H_8ClN_3OS$	0.550	172	72
3d	OCH_3	(309.3424)	$C_{16}H_{11}N_3O_2S$	0.219	149	71
3e	Br	(358.3125)	$C_{15}H_8BrN_3OS$	0.277	169	70
3f	CH_3	(293.3430)	$C_{16}H_{11}N_3OS$	0.461	140	63
3g	C_2H_5	(307.3696)	$C_{17}H_{13}N_3OS$	0.361	154	69
3h	OC_2H_5	(308.35438)	$C_{17}H_{12}N_2O_2S$	0.250	125	56
3i	NO_2	(324.3140)	$C_{15}H_8N_4O_3S$	0.400	125	56
3j	F	(297.3069)	$C_{15}H_8FN_3OS$	0.473	130	75

Mol. – Molecular; m.p.-melting point; Wt. – Weight; Comp. – Compound

Table: 2 Anticonvulsant activity (MES & ScMET) & toxicity profile of compounds (3a-j)

Comp. No.	R	Dose	Time in hours					
			MES		ScMET		Tox	
			0.5	4.0	0.5	4.0	0.5	4.0
3a	H	30	0/1	0/1	0/1	0/1	0/4	0/2
		100	0/3	0/3	0/1	0/1	**1/8**	0/4
		300	**1/1**	0/1	**3/5**	0/1	0/4	0/2
3b	$N(CH_3)_2$	30	0/1	0/1	0/1	0/1	0/4	0/2
		100	1/3	**1/3**	0/1	0/1	0/8	0/4
		300	**1/1**	**1/1**	0/1	0/1	**2/4**	0/2
3c	Cl	30	0/1	0/1	0/1	0/1	0/4	0/2
		100	0/3	0/3	0/1	0/1	0/8	0/4
		300	**1/1**	0/1	0/1	0/1	**1/4**	0/2
3d	OCH_3	30	0/1	0/1	0/1	0/1	0/4	0/2
		100	**2/3**	0/3	0/1	0/1	0/8	0/4
		300	**1/1**	**1/1**	0/1	0/1	**2/4**	0/4
3e	Br	30	0/1	0/1	0/1	0/1	0/4	0/2
		100	0/3	0/3	0/1	0/1	0/8	0/4
		300	0/1	0/1	0/1	0/1	0/4	0/2
3f	CH_3	30	0/1	0/1	0/1	0/1	0/4	0/4
		100	2/3	0/3	0/1	0/1	0/8	0/2
		300	1/1	1/1	0/1	0/1	0/4	0/2
3g	C_2H_5	30	0/1	0/1	0/1	0/1	0/4	0/4
		100	0/3	0/3	0/1	0/1	0/8	0/2
		300	0/1	0/1	0/1	0/1	0/4	0/2
3h	OC_2H_5	30	0/1	0/1	0/1	0/1	0/4	0/4
		100	0/3	0/3	0/1	0/1	0/8	0/2
		300	0/1	0/1	0/1	0/1	0/4	0/2
3i	NO_2	30	0/1	0/1	0/1	0/1	0/4	0/4
		100	0/3	0/3	0/1	0/1	0/8	0/2
		300	0/1	0/1	0/1	0/1	0/4	0/2
3j	F	30	0/1	0/1	0/1	0/1	0/4	0/4
		100	0/3	0/3	0/1	0/1	0/8	0/2
		300	0/1	0/1	0/1	0/1	0/4	0/2

Dose: 30, 100 & 300mg/kg in the form of suspension. Values given in that order:
No. of mice protected/No. of mice used.

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