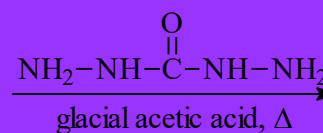
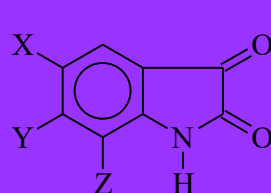
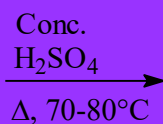


- (i) Cl₃CCH(OH)₂
 (ii) NH₂OH.HCl
 (iii) Conc.HCl
 (iv) Na₂SO₄ solution



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Research Article

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Synthesis of isatin, 5-chloroisatin and their Δ^2 -1, 3, 4 oxadiazoline derivatives for comparative cytotoxicity study on brine shrimp

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Abstract

Isatin (**3a**), isatin 3-carbohydrazone (**4a**), 5-spiro (isatin) 2-(N-acetyl hydrazino) 4-(N-acetyl)- Δ^2 -1,3,4 oxadiazoline (**5a**), and 5-spiro (isatin) 2-hydrazino- Δ^2 -1,3,4 oxadiazoline (**6a**) had been synthesized from the unsubstituted oximinoacetanilide (**2a**). 4-Chlorooximinoacetanilide (**2b**), 5-chloroisatin (**3b**), 5-chloroisatin 3-carbohydrazone (**4b**) and 5-spiro (5'-chloroisatin) 2-(N-acetyl hydrazino) 4-N-acetyl Δ^2 -1,3,4 oxadiazoline (**5b**) compounds had been synthesized from p-chloroaniline. The structures of the products had been characterized from the spectral analysis and comparative cytotoxicity study of them was studied.

Introduction

Isatin, possessing an indole nucleus having both the keto and lactam moiety has aroused tremendous curiosity due to its diverse biological and pharmacological studies. From literature survey it is well known that isatin heterocycles exhibit manifold importance in the field of medicinal chemistry as a potent chemotherapeutic agent. Recently Islam et al., (1992, 2001), in collaboration with National Cancer Institute (NCI) of USA, it was observed that acylated Δ^2 -1,3,4 thiadiazoline derivatives of isatin showed effective anti-cancer activity against a number of cancer cells especially for breast cancer. This eventful observation encouraged us to devotion of further research on isatin heterocycles reacting with carbohydrazone, especially for spiro 1, 3, 4 oxadiazoline derivatives. We report herein the synthesis of compounds shown in the scheme 1 following previous literature (Islam et al., 2001). The synthesized compounds were characterized by spectral analysis of IR, $^1\text{H-NMR}$ and mass spectrometry. The result of the systematic study

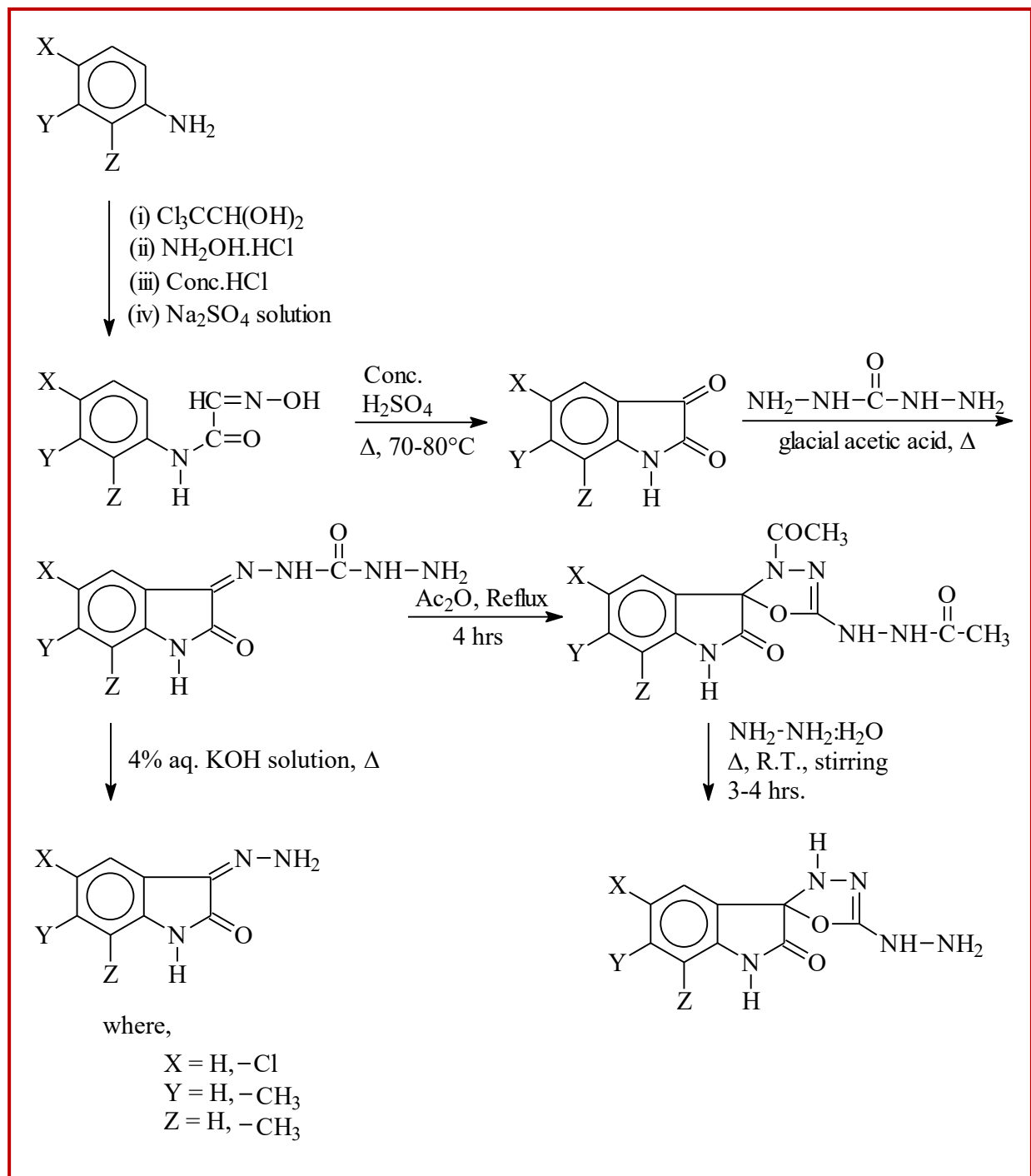
on cytotoxicity of the synthesized compounds on brine shrimp has also been discussed.

Materials and Methods

The melting points of the synthesized compounds were recorded by thin disc method on a FISCHER JOHN'S electro-thermal melting point apparatus. TLC was used for the monitoring the progress of reactions. Infrared spectra were recorded on DR-8001, SHIMADZU FT-IR spectrophotometer as a solid which was finely ground in a small agate mortar in KBr disc. $^1\text{H-NMR}$ spectra were measured by WP 200-NMR spectrometer using TMS (tetramethyl silane) as an internal standard and DMSO- d_6 (dimethyl sulphoxide) as a solvent. Mass spectra were recorded on a high resolution mass spectrometer, KARATAS MS-25 using DH-88 data system.

Synthesis of isatin, 3a: Following the previous literature (Islam et al., 2001) oximinoacetanilide was prepared in 75% yield. After addition of concentrated sulphuric acid





Scheme 1

(14.7 mL) with dry oximinoacetanilide (4 g; 0.024 mole) at 70-80°C the solution was cooled to room temperature and poured upon 10-12 times of its volume of crushed ice. The orange red crude solid product of **3a** was separated from the solution after half an hour which was filtered, washed well with cold water and dried in a vacuum desiccators. The orange red pure product of **3a** was re-crystallized from ethyl acetate having m.p.

180-182°C, yield 2.3 g (65%); R_f 0.4 (PE : EA ; 3 : 2).

$\nu_{\text{max}}^{\text{KBr}} (\text{cm}^{-1})$: 3189 (br, n_{NH} ; amide), 3106 (w, n_{CH} ; aromatic), 1726 (sh, $n_{\text{C=O}}$; keto), 1614 (sh, $n_{\text{C=O}}$; lactam), 1460 (sh, $n_{\text{C-C}}$; aromatic).

$^1\text{H-NMR}$ ($\text{DMSO-}d_6$): δ ppm: 11.02 (s, 1H; NH); 6.90 (d, $J=7.8$ Hz, 1H, $\text{C}_1\text{-H}$); 7.12 (t, 1H, $\text{C}_2\text{-H}$); 7.56 (t, 1H; $\text{C}_3\text{-}$

H); 7.49 (d, J=7.8 Hz, 1H, C₄-H).

MS *m/z* (% of relative intensities): 147 (M⁺, 70), 119 (100), 92 (68), 76 (11), 74 (6) and 64 (32). M⁺ represents the molecular formula of C₈H₅NO₂.

Synthesis of isatin 3-carbohydrazone, 4a: The reaction mixture of carbohydrazide (1 g, 6.8 mmol) in glacial acetic acid (31.6 mL) and a hot solution of isatin (0.6 gm; 6.8 mmol) in glacial acetic acid (33.1 mL) was refluxed for 3-4 hours with vigorous stirring which afforded a crude mass of **4a**. When the reflux was completed, the reaction mixture was cooled, filtered and the crude product was washed well with water. Re-crystallization of the crude **4a** from methanol gave a yellow colored pure compound of **4a** having m.p. >300°C, yield 1.0 g (70%) and R_f 0.3 (PE: EA; 2:1)

IR: $\nu_{\max}^{KBr} (cm^{-1})$: 3395 (w, n_{NH}; 1^o amine; symmetric), 3305 (w, n_{NH}, 1^o amine; asymmetric), 3198 (br, n_{NH}, 2^o amine), 3058 (w, n_{CH}, aromatic), 1730 (sh, n_{C=O}, keto), 1685 (sh, n_{C=O}, lactam), 1624 (sh, n_{C=N}), 1513 (sh, n_{C-C}, aromatic).

¹H-NMR (DMSO-d₆): d ppm: 11.29 (s, 3H; 3'NH); 7.58 (d, J=7.8 Hz, 1H; C₁-H); 7.42 (m, 1H, C₃-H); 7.12 (t, 1H; C₂-H); 7 (d, J=7.8 Hz, 1H, C₄-H).

MS *m/z* (% of relative intensities): 219 (M⁺, 10), 207 (13), 161 (72), 144 (18), 133 (16), 116 (12), 104 (42), 90 (19) and 76 (26). M⁺ represents the molecular formula of C₉H₉N₅O₂.

Synthesis of 5-spiro (isatin) 2(N-acetyl hydrazino)-4-(N-acetyl)-Δ²-1,3,4-oxadiazoline, 5a: The oxidative cyclization of isatin 3-carbohydrazone **4a** (0.4 gm; 2 mmol) with freshly distilled acetic anhydride (55 mL) by refluxing (Kubota et al., 1980) for four hours led to the synthesis of the compound, **5a**. The crude solid mass of **5a** was purified by re-crystallization from ethyl acetate and orange crystalline solid of **5a** was obtained having m.p. 120-122°C, yield 460 mg (80%) and R_f 0.6 (EA : PE ; 1:4).

IR: $\nu_{\max}^{KBr} (cm^{-1})$: 3259 (sh, n_{NH}), 3132 (sh, n_{CH}, aromatic), 2932 (sh, n_{CH}, aliphatic), 1785 (sh, n_{COCH}), 1705 (sh, n_{C=O}, keto), 1660 (sh, n_{C=N}), 1609 (sh, n_{C-C}, aromatic).

¹H-NMR (DMSO-d₆): d ppm: 12.15 (br.s, 3H; 3'NH); 8.17 (d, J=7.8 Hz, 1H, C₁-H); 7.73 (d, 1H; d); 7.51-7.67 (m, 1H; C₃-H); 7.41 (t, 1H; C₂-H); 2.6 (s, 3H, -NCOCH₃); 2.17 (s, 3H; -NHCOCH₃).

MS *m/z* (% of relative intensities): 288 (M⁺-15, 5), 245 (7), 202 (10), 175 (70), 160 (5), 146 (6), 132 (11), 104 (11), 90 (8) and 77 (9)

Synthesis of 5-spiroisatin 2-hydrazino Δ²-1,3,4 oxadiazoline 6a: The reaction of 5-spiro (isatin) 4-(N-acetyl)-2-(N-acetyl amino) D²-1,3,4-oxadiazoline, **5a** (0.2 g, 1 mmol) and hydrazine hydrate (2.5 mL, 98%) with stirring at room temperature for 6 hours afforded precipitate of the crude product of **6a**. The resulting precipitate was filtered, dried in a vacuum desiccators and re-crystallized from water and obtained pale yellow powdered solid of **6a** having m.p. 210-212°C, yield 69.7 mg (40%) and R_f 0.2 (EA : PE ; 1: 4)

IR: $\nu_{\max}^{KBr} (cm^{-1})$: 3358 (sh, n_{NH}), 3158 (br, n_{CH}, aromatic), 1686 (sh, n_{C=O}, lactam), 1657 (sh, n_{C=N}), 1588 (sh, n_{C-C}, aromatic).

¹H-NMR (DMSO-d₆) : d ppm : 11.84 (br.s, 1H, N₁-H, lactam); 11.28 (s, 1H, N₂-H); 10.84 (br.s, 1H, N₃-H); 7.56 (d, J=7.8 Hz., 1H, C₁-H), 7.37 (t, 1H, C₂-H), 7.09-7.16 (m, 1H; C₃-H); 7.00 (d, J=7.8 Hz., 1H, C₄-H).

MS *m/z* (% of relative intensities): 204 (M⁺-15, 10), 149 (14), 138 (8), 119 (6.6), 97 (5.83), 83 (9.58), 55 (40), 43 (96), 28 (100) and 17 (31).

Synthesis of p-chlorooximinoacetanilide, 2b: In accordance with the modified Sandmeyer procedure (Islam et al., 1997) compound **2b** was prepared from p-chloroaniline (8 gm, 0.1 mole), chloral hydrate (10.9 gm, 0.1 mole), saturated aqueous solution of sodium sulfate (157.8 mL), concentrated hydrochloric acid (5.2 mL) and hydroxylamine hydrochloride (13.4 gm, 0.2 mole). The crude product obtained as a pale brown solid which on re-crystallization from ethyl acetate yielded brown colored powdered solid of **2a** having m.p. 170-172°C, R_f 0.5 (PE:EA, 2:1) and yield 1.0 g (80%).

IR: $\nu_{\max}^{KBr} (cm^{-1})$: 3303 (sh, n_{OH}), 3203 (sh, n_{NH}), 3106 (sh, n_{CH}, aromatic), 1663 (sh, n_{CO}, amide), 1623 (sh, n_{C=N}), 1569 (sh, n_{C-C}, aromatic).

¹H-NMR (DMSO-d₆): d ppm: 12.2 (s, 1H, N-OH), 10.2 (s, 1H, NH), 7.62 (s, 1H, CH=N-), 7.71 (d, J=7.6 Hz., 1H, H-3, Ar-H), 7.36 (d, J=7.6 Hz., 1H, H-4, Ar-H).

MS *m/z* (% of relative intensities): 198/200 (M⁺ /M⁺+2, 3:1, ³⁵Cl, 80), 127/129 (³⁵Cl, 100), 181/183 (³⁵Cl, 10), 167/169 (³⁵Cl, 4), 153/155 (³⁵Cl, 26), 144/146 (³⁵Cl, 12), 111/113 (³⁵Cl, 11), 99/101 (³⁵Cl, 24), 90/92 (³⁵Cl, 10) and 75/77 (³⁵Cl, 16).

Synthesis of 5-chloroisatin, 3b: The cyclisation of p-chlorooximinoacetanilide (6 gm, 0.03 mole) according to standard procedure of Sandmeyer treated with concentrated H₂SO₄ (22.0 mL) on controlled temperature led to the synthesis of 5-chloroisatin. The crude product of **3b** was obtained as a pink-red solid which on re-

crystallization from ethyl acetate afforded brick-red powdered solid having m.p. 230-232°C, R_f 0.4 (PE:EA, 1:1) and yield 3.8 g (70%).

IR: $\nu_{\max}^{KBr} (cm^{-1})$: 3097 (w, n_{NH}), 3058 (w, n_{CH}, aromatic), 1704 (sh, n_{C=O}, keto), 1617 (sh, n_{C=O}, lactam), 1470 (sh, n_{C-C}, aromatic).

¹H-NMR (DMSO-*d*₆): δ ppm: 11.12 (s, 1H, NH, lactam), 6.91-6.95 (d, J=7.6 Hz., 1H, H-3, Ar-H), 7.55-7.61 (t, 1H, H-2, Ar-H).

MS *m/z* (% of relative intensities): 181/183 (M⁺ /M⁺+2, 3:1, ³⁵Cl, 48), 153/155 (³⁵Cl, 100), 125/127 (³⁵Cl, 34), 110/112 (³⁵Cl, 7), 98/100 (³⁵Cl, 15), 90 (³⁵Cl, 16), 75 (³⁵Cl, 10), 63 (³⁵Cl, 38).

Synthesis of 5-chloroisatin 3-carbohydrazone, 4b: The refluxing of unimolecular ratio of 5-chloroisatin, **3b** (1.2 gm, 6.6 mmol in 38.0 mL glacial acetic acid) and carbohydrazide (0.6 g, 6.6 mmol in 32.2 mL glacial acetic acid) for four hours afforded precipitate of **4b**. After usual work-up bright yellow solid of **4b** was obtained by re-crystallization from methanol; m.p. >300°C, R_f 0.3 (PE: EA, 1:1) and yield 1.1 gm (70%).

IR: $\nu_{\max}^{KBr} (cm^{-1})$: 3562 (sh, n_{NH}, symmetric), 3415 (sh, n_{NH}, asymmetric), 3345 (w, n_{NH}), 3202 (br, n_{CH}, aromatic), 1786 (sh, n_{C=O}, keto), 1734 (sh, n_{C=O}, lactam), 1620 (sh, n_{C=N}), 1470 (sh, n_{C-C}, aromatic).

¹H-NMR (DMSO-*d*₆): δ ppm: 11.45 (s, 1H, NH, lactam), 8.55 (s, 2H, 2'NH), 7.4 (d, J=7.6 Hz., 1H, H-2, Ar-H), 7 (d, J=7.6 Hz., 1H, H-3, Ar-H).

MS *m/z* (% of relative intensities): No M⁺ / M⁺+2 peak was observed due to loss of thirty mass unit. 223/225 (M⁺ -30/(M⁺+2) -30, 3:1, ³⁵Cl, 12), 207/209 (³⁵Cl, 88), 195/197 (³⁵Cl, 100), 178/180 (³⁵Cl, 10), 151/153 (³⁵Cl, 14), 138/140 (³⁵Cl, 43), 124/126 (³⁵Cl, 15), 112/114 (³⁵Cl, 9), 102 (³⁵Cl, 14), 88 (³⁵Cl, 11), 75 (³⁵Cl, 21).

Synthesis of 5-spiro (5-chloroisatin)-2-(N-acetyl hydrazino)-4-(N-acetyl)-Δ²-1,3,4 oxadiazoline, 5b: The refluxing of 5-chloroisatin 3-carbohy-drazone (0.3 g, 1.1 mmol) with freshly distilled acetic anhydride (84.2 mL) with vigorously stirring for two hours and usual work-up led to a grayish solid of **5b**. The product on re-crystallization from dichloromethane afforded dark grayish solid of **5b** having m.p. 174-175°C, R_f 0.4 (EA: PE, 3:2) and yield 253.7 mg (75%).

IR: $\nu_{\max}^{KBr} (cm^{-1})$: 3255 (sh, n_{NH}), 2933 (w, n_{CH}, aliphatic), 1780 (sh, n_{C=O}, -NHCOCH₃), 1724 (sh, n_{C=O}, lactam), 1616 (sh, n_{C=N}), 1591 (sh, n_{C-C}, aromatic).

¹H-NMR (DMSO-*d*₆): δ ppm: 12.11 (s, 3H, 3'NH), 8.15

(d, J=7.6 Hz., 1H, H-6, Ar-H), 7.82 (s, J=7.6 Hz., 1H, H-4, Ar-H), 7.65 (d, 7.5 Hz, 1H, H-3, Ar-H), 2.61 (s, 3H, terminal -NCOCH₃), 2.19 (s, 3H, -NHCOCH₃).

MS *m/z* (% of relative intensities): No M⁺ / M⁺+2 peak was observed due to loss of fifteen mass unit. 322/324 (M⁺ -15/(M⁺+2) -15, 3:1, ³⁵Cl, 3.2), 321/323 (³⁵Cl, 16), 279/281 (³⁵Cl, 14), 251/253 (³⁵Cl, 13), 236/238 (³⁵Cl, 50), 194 (³⁵Cl, 21), 180 (³⁵Cl, 17), 166 (³⁵Cl, 20), 138 (³⁵Cl, 13), 102 (³⁵Cl, 10).

Demonstration of cytotoxicity: The cytotoxicity study of the synthesized compounds - **3a**, **4a**, **5a**, **6a**, **2b**, **3b**, **4b** and **5b** was investigated on brine shrimp as a test organism for convenience. 1.6 mg of each of the compounds was taken in the corresponding sample vials with 1.6 mL of dimethyl sulfoxide to prepare stock solution. From this stock solution 33, 99 and 132 ppm of each compounds were placed in separate test tubes by micro syringe 1 mL of extra dimethyl sulfoxide was given in each test tube with 10-12 brine shrimp (Microwell, 1997). After 1, 2, 3 and 4 hours the test tubes were observed and the number of survived naupli in each test tube was counted and results were noted. From this the percentage of lethality of brine shrimp naupli was calculated at each concentration for each sample. Then graphs were drawn by plotting percentage of lethality of brine shrimp versus doses (in ppm) of the synthesized compounds which gave rise to the LD₅₀ values of the corresponding compounds.

The LD₅₀ values of the synthesized compounds are given in Table I.

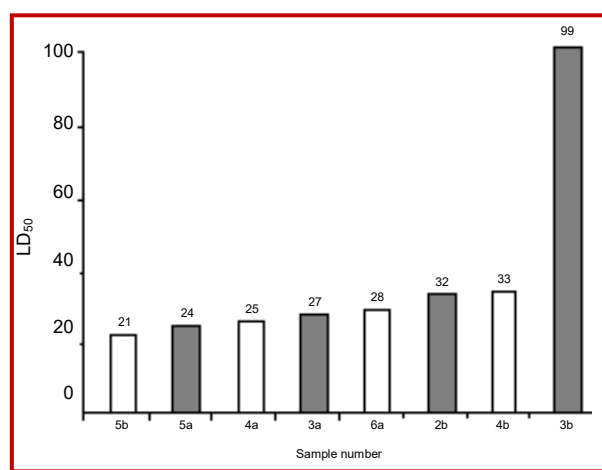
The following graph represents the comparative LD₅₀ values of the synthesized compounds (**3a**, **4a**, **5a**, **6a**, **2b**, **3b**, **4b** and **5b**)

Results and Discussion

The structure of isatin (**3a**) has been confirmed by its IR, ¹H-NMR and mass spectra. The IR bands at 3189 cm⁻¹, 3106 cm⁻¹, 1614 cm⁻¹ and 1460 cm⁻¹ correspond to the presence of broad band of NH, CH (aromatic), C=O (keto), C=O (lactam) and C-C (aromatic) groups respectively. In ¹H-NMR a sharp singlet appearing at δ 11.02 is due to NH proton (lactam). Four aromatic protons of C₁-H, C₂-H, C₃-H, and C₄-H appear at δ 6.90 (d, J=7.8 Hz., 1H, C₄-H), δ 7.12 (t, N, C₂-H), δ 7.56 (t, C₃-H) and δ 7.49 (d, J=7.8 Hz., 1H, C₄-H) respectively. Mass spectrum confirms the structure of **3a** which shows molecular ion peak M⁺ at *m/z* 147.

Reaction of **3a** with carbohydrazide in glacial acetic acid gave **4a**, whose mass spectrum showed molecular ion peak at *m/z* 219 which is consistent with the structure of the compound. Acetylation of **4a** with acetic anhydride afforded **5a**. IR spectrum of **4a** indicates the presences

LD ₅₀ values of the synthesized compounds		
Compound	LD ₅₀	Remarks
5b	21	Highly active
5a	24	
4a	25	
3a	27	Medium active
6a	28	
2b	32	Weakly active
4b	33	
3b	99	Inactive



of acetyl group (-COCH₃) by the band appearing at 1785 cm⁻¹. ¹H-NMR spectrum confirms the presence of two acetyl groups by two singlets at δ 2.2 for NH-COCH₃ and at δ 2.6 due to -NCOCH₃. The expected molecular ion peak (M⁺) was not found due to loss of fifteen mass unit. Two NH protons were not observed in the ¹H-NMR spectrum due to rapid proton-deuteron exchange reaction in deuterated dimethyl sulfoxide solvent.

Reduction of **5a** by hydrazine hydrate stirring at room temperature afforded **6a**. The IR spectrum indicated the absence of any acetyl group which confirmed the reduction of **5a** has taken place. The bands at 3358 cm⁻¹ and 1686 cm⁻¹ indicate the presence of NH and C=O (lactam) groups respectively. In ¹H-NMR a broad singlet appears at δ 11.84 due to NH proton of lactam, whereas a sharp singlet at δ 11.28 and a broad singlet at δ 10.84 indicate the presence of NH proton of N₂-H and NH proton of N₃-H respectively. No signal of two NH₂ protons was observed due to rapid proton-deuteron exchange reaction in deuterated dimethyl sulfoxide solvent. In mass spectrum the molecular ion peak M⁺ was not observed due to loss of fifteen mass unit from **6a** instead the peak appearing at m/z 204 (M⁺-15) is

consistent with the structure of **6a**.

The IR bands of **2b** at 3303 cm⁻¹, 3203 cm⁻¹, 1663 cm⁻¹ and 1623 cm⁻¹ indicates the presence of -OH, -NH, C=O (amide) and C=N groups respectively. The ¹H-NMR shows a doublet at δ 7.7 due to the effect of neighboring electronegative chlorine of aromatic proton H-3 and a doublet at δ 7.4 due to aromatic proton H-4. Two singlets appear at δ 12.2 and at δ 10.2 due to OH and NH proton respectively. In mass spectrum the molecular ion peak at m/z 198/200 (3:1) which is consistent with the isotopic pattern of chlorine and structure of **2b**.

The IR spectrum of **3b** showed bands at 3097 cm⁻¹, 1704 cm⁻¹ and 1617 cm⁻¹ correspond to NH, C=O (keto) and C=O (lactam) groups respectively. The ¹H-NMR shows a broad peak at δ 11.1 due to NH proton. Aromatic proton H-2 undergoes coupling with H-3 and meta coupling with H-1 simultaneously and appears as a triplet at δ 7.55-7.61. A doublet appears at δ 6.91-6.95 due to aromatic proton of H-3. The molecular ion peak at m/z 181/183 (3:1) is characteristic of isotopic pattern of chlorine and consistent with the structure of **3b**.

In IR spectrum of compound **4b**, the bands appear at 3562 cm⁻¹, 1786 cm⁻¹ and 1620 cm⁻¹ indicate the presence of -NH, C=O (keto) and C=N groups respectively. The ¹H-NMR shows a sharp singlet at δ 11.5 due to NH proton of lactam and a singlet at δ 8.5 due to two NH protons but two NH₂ protons were not observed due to rapid proton deuteron exchange reaction in deuterated dimethyl sulfoxide solvent. The mass spectrum did not show the expected molecular ion peak M⁺ due to loss of thirty mass unit and showed the peak at m/z 223/225 (M⁺-30, 3:1) which is characteristic of isotopic pattern of chlorine and consistent with the structure of **4b**.

Acetylation of **4b** with freshly distilled acetic anhydride gave **5b**. The IR spectrum of **4b** shows bands at 1780 cm⁻¹ indicating presence of acetyl group. The ¹H-NMR spectrum of **5b** confirms the presence of two acetyl group groups by two sharp singlets at δ 2.2 and at δ 2.6 due to three protons of -NHCOCH₃ and -NCOCH₃ respectively. A broad singlet appears at δ 12.1 represents three NH protons of H-4, H-5 and H-6. In mass spectrum the molecular ion peak M⁺ was not observed due to loss of fifteen mass unit instead of that a peak appeared at m/z 322/324 (3:1), which is characteristic of isotopic pattern of chlorine and consistent with the structure of **5b**.

On the basis of cytotoxicity study of the synthesized compounds it was found that - carbohydrazido group containing unsubstituted isatin activates the cytotoxic effect of the compound, than that of isatin itself. Spiro 1, 3,4 oxadiazoline derivatives of isatin (**5b**) having chlorine atom showed cytotoxic effect on brine shrimp more significantly than **5a** without chlorine atom.

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