

Cytotoxicity study of some indophenines and isatin derivatives

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Abstract

Eight indophenines were synthesized for the interest of studying biological activity especially for cytotoxicity. The cytotoxicity of some indophenines and some isatin derivatives was studied by the brine shrimp lethality bioassay. It was observed that all the indophenines from thiophene, thiazol and isatin derivatives showed potential cytotoxicity against brine shrimp nauplii and the structure activity relationships (SAR) of these compounds have been reported.

Introduction

Isatin (1H-indole-2,3-dione) and its derivatives possessing an indole nucleus have attracted great attention in recent years due to their wide variety of biological activities and pharmacological studies as insecticides (Bahmaria and Deliwala, 1968), fungicides (Chen and Rhodes, 1996), anticancer (Holla et al., 2000) and anti-inflammatory. Isatins are very important compounds due to their antifungal properties (Islam et al., 1998). Moreover, isatins are the synthetic precursors of some biologically important compounds such as quinoline 1, 2, 3-thiadiazoline (Ram et al., 1980). Besides that, the triazines [5, 6-b] indole-3-thione derivatives have attracted considerable attention in the field of medicine due to their antifungal, antimalarial and antiparasitic properties (Pal et al., 1991). Therefore, the research interest on isatins has expanded day by day. Because of the

continuing interest about isatins and its derivatives (Saha et al., 1992), the present study aims at the synthesis of some indophenines from isatins, thiophene, and thiazol. This work has been carried out from the synthetic point of view along with their biological (Islam et al., 1990) property especially cytotoxicity by brine shrimp lethality bioassay (Meyer et al., 1982; Solis et al., 1993).

Materials and Methods

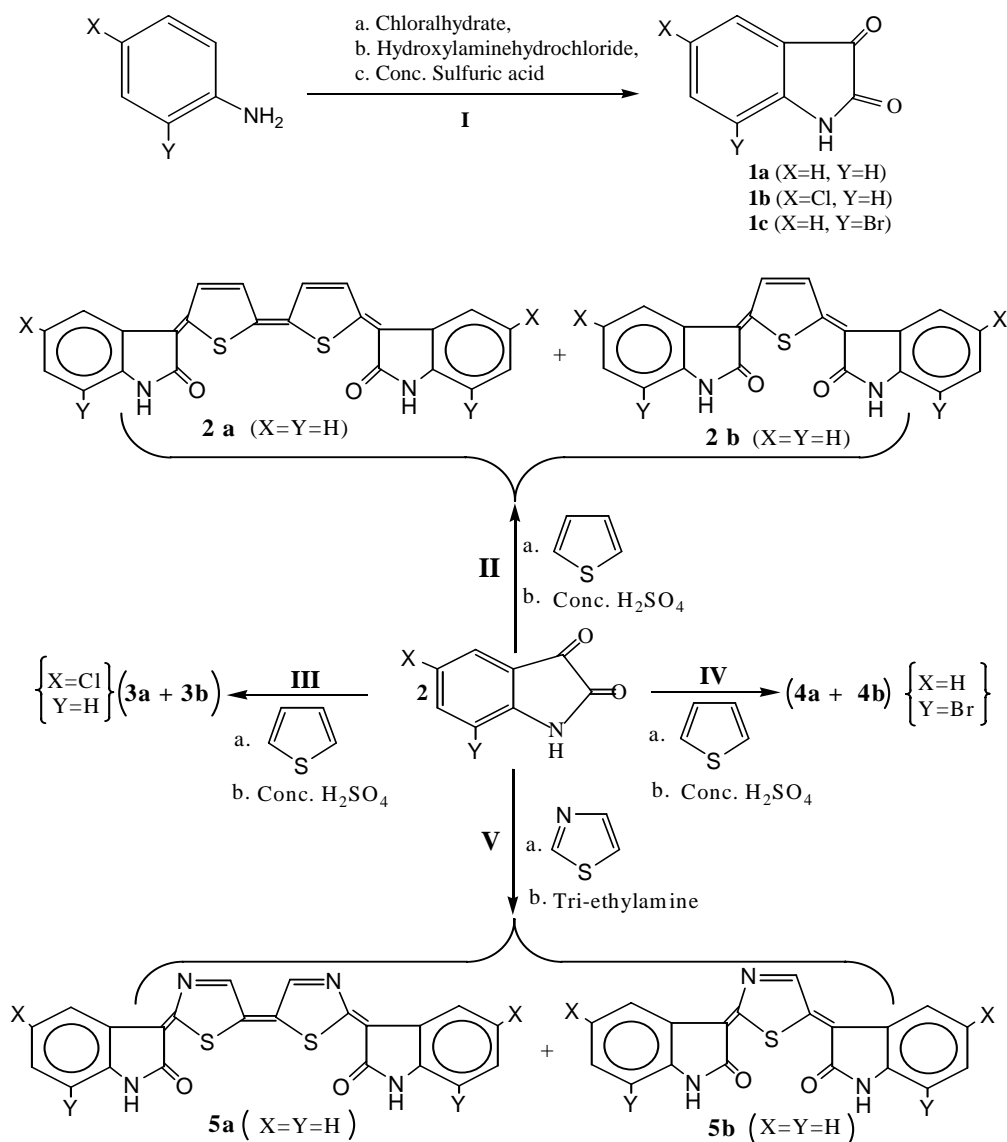
Physical and spectral data of the compounds were recorded on a Fischer Johns electrothermal melting point apparatus by thin disc method, on a DR-8001 Shimadzu FT-IR spectrophotometer, on a WP-400 NMR spectrophotometer using DMSO-d₆ as the solvent with tetramethylsilane as internal standard and on a Kratos MS-25 using DH-88 data system.



Isatin and substituted isatins **1a**, **1b** and **1c** (Sandmeyer, 1919) were synthesized from the condensation of corresponding aniline with chloralhydrate and hydroxylaminehydrochloride followed by the cyclization with concentrated sulfuric acid. Again, some indophenines (Gregory et al., 1993) were synthesized from thiophene and isatin by the standard procedure. Indophenines **2a** and **2b** were obtained when isatin reacts with thiophene under refluxing condition in the molar ratio of 2:1 respectively in the presence of concentrated sulfuric acid. Indophenines **3a**, **3b**, **4a**

and **4b** were prepared under identical conditions (as **2a** and **2b**) from 5-chloroisatin and 7-bromoisatin respectively. Again, indophenines **5a** and **5b** were prepared by the reaction between isatin and thiazol in the molar ratio of 2:1 in presence of triethylamine under intensive stirring at 80°C (**Scheme 1**). The cytotoxicity of the synthesized compounds was studied by brine shrimp lethality bioassay (Solis et al., 1993).

Test animal: Brine shrimps (*Artemia Salina*) were used as test animal for the investigation of cytotoxicity.



Scheme 1

Hatching and maintenance of brine shrimp: The preferred condition for brine shrimp (temperature 28-30°C, salinity 30-35 ppt, pH 8-9, and strong aeration) was established by mixing sodium chloride salts in water. After obtaining the desired condition, about one teaspoon of brine shrimp eggs was added to the beaker. After 12 hour hatching aggregated brine shrimp nauplii were collected in another beaker and rinse with fresh water and applied for testing.

Preparation of test sample: For the cytotoxicity study, dimethylsulphoxide (DMSO) was used as a solvent and the mortality of brine shrimp nauplii in this DMSO solution was almost zero. Different concentrations (150, 100 and 50 µg/ml) of each test samples were prepared with DMSO. Then 10-12 brine shrimp nauplii were transferred to each test tube using micropipette.

Counting of nauplii: The numbers of survived nauplii in each test tube were counted. The percentage of mortality of brine shrimp was calculated for each sample that gives different mortality for different concentrations. An approximate linear correlation was observed when logarithm of concentration was plotted against percentage of mortality and the values of LC₅₀ were calculated for each sample. The LC₅₀ represents the concentration of a compound, which will kill, or inactive 50 percent of the test animal. LC₅₀ is inversely proportional to the toxicity of a compound, i.e. the lower the LC₅₀ the higher the toxicity.

Results

Reaction I: After recrystallization, the products **1a**, **1b** and **1c** were obtained as brick-red solids with the yield of 90% and m. p. ranging from 188°-195°C. The structures of the compounds are consistent with the physical constants and spectral data. The IR spectra of the compounds **1a-1c** show a sharp band at 1772 cm⁻¹ corresponding to the vC=O keto group and another sharp band at 1740 cm⁻¹ indicates the vC=O lactam. Again, the ¹H-NMR signal for >NH is also observed in the respective region as a singlet and a signal for aromatic ring as multiplet.

Reaction II: The major product from crude mass afforded **2a** in 60% yield, (deep blue, λ_{max} 625 nm) after purification over silica gel column chromatography. **IR:** ν_{max} nujul (cm⁻¹) 3450 cm⁻¹ (m, sh, vN-H, lactam), 3100 cm⁻¹ (sh, vC-H, aromatic), 1749 cm⁻¹ (sh, vC=O), 1684 cm⁻¹ (sh, vC=C) and 1605, 1508 cm⁻¹ (sh, vC=C, aromatic); **¹H-NMR:** δ 10.5 (s, 1H, N-H), 7.3-7.5 (m, 4H, alkene) and 6.9-7.0 (m, 8H, aromatic); **mass spectrum:** m/z 426 (**M**⁺, 25), 213 (33), 185 (20), 153 (100), 132 (38), 89 (20) and 77 (66). And **2b** was afforded as minor product (λ_{max} 620 nm) in 15% yields which was brown in color with the melting point 260°- 265°C and also more polar comparing to **2a**. **IR:** 3400 cm⁻¹ (s, sh, vN-H), 3050 cm⁻¹ (sh, vC-H aromatic), 1749 cm⁻¹ (sh, vC=O) and 1670 cm⁻¹ (sh, vC=C); **¹H-NMR:** δ 10.4 (s, 1H, N-H), 8.5 (d, 2H, alkene), 7.2-7.5 (m, 8H, aromatic); **mass spectrum:** m/z 344 (**M**⁺, 40), 213 (67), 185 (45), 153 (100), 132 (56), 89 (69), 77 (80).

Reaction III: The major product **3a** was obtained as a blue solid having a yield of 45% (λ_{max} 638 nm) with m.p. >300°C after purification over silica gel column chromatography. The IR spectra of compound **3a** show a band at 1670 cm⁻¹ corresponding to the C=C in the conjugated system and another strong band at 1749 cm⁻¹ is observed for C=O. **¹H-NMR:** δ 10 (s, 1H, N-H), 6.7-6.9 (m, 6H, aromatic); **mass spectrum:** m/z 494 (**M**⁺, 20), 247 (39), 187 (100), 166 (40), 89 (20) and 77 (60). Again, the minor product **3b** was obtained as a brownish solid having a yield of 20%. **IR:** ν_{max} nujul (cm⁻¹) 3300 cm⁻¹ (s, sh, vN-H), 3100 cm⁻¹ (sh, vC-H, aromatic), 1747 cm⁻¹ (sh, vC=O), 1682 cm⁻¹ (sh, vC=C); **¹H-NMR:** δ 10.3 (s, 1H, N-H), 7.2-7.4 (m, 6H, aromatic); **mass spectrum:** m/z 412 (**M**⁺, 25), 247 (30), 219 (35), 187 (100), 89 (59) and 77 (60).

Reaction IV: The major product **4a** was obtained as a blue solid in 55% yield (λ_{max} 650 nm) after purification over silica gel column chromatography. **IR:** ν_{max} nujul (cm⁻¹) 3312 cm⁻¹ (s, sh, vN-H), 3100 cm⁻¹ (sh, vC-H, aromatic), 1716 cm⁻¹ (sh, vC=O), 1653 cm⁻¹ (sh, vC=C); **¹H-NMR:** δ 11.4 (s, 1H, N-H), 6.9-7.1 (m, 6H, aromatic); **mass spectrum:** m/z 582 (**M**⁺, 15), 263 (30), 231 (100), 89 (50) and 77 (70). Second product **4b** was obtained as brown solid, λ_{max} 620 nm and m. p. 220°-225°C. **IR:** ν_{max} nujul (cm⁻¹) 3450-3500 cm⁻¹ (m,

sh, ν N-H), 3100 cm^{-1} (sh, ν C-H, aromatic), 1716 cm^{-1} (sh, ν C=O), 1693 cm^{-1} (sh, ν C=C); $^1\text{H-NMR}$: δ 9.3 (s, 1H, N-H), 8.1-8.2 (d, 2H, alkene), 7.2-7.4 (m, 6H, aromatic); **mass spectrum**: m/z 500 (M^+ , 40), 291 (54), 263 (40), 231 (100) and 155 (55).

Reaction V: The crude was chromatographed on silica gel column to separate the mixture which gives the major product **5a** as a blue solid in 50% yield, m. p. 290°-295°C. **IR**: ν_{max} nujol (cm^{-1}) 3300 cm^{-1} (m, sh, ν N-H, lactam), 3080 cm^{-1} (sh, ν C-H, aromatic), 1716 cm^{-1} (sh, ν C=O), 1684 cm^{-1} (sh, ν C=N), 1645 (sh, ν C=C); $^1\text{H-NMR}$: δ 10.0 (s, 1H, N-H, lactam), 8.0 (s, 2H, alkene), 6.9-7.0 (m, aromatic); **mass spectrum**: m/z 428 (M^+ , 15), 214

(72), 186 (22), 154 (100), 132 (58), 89 (20) and 77 (21). Another product **5b** was obtained as brownish solid having a yield of 20%, m. p. 175°-180°C. **IR**: ν_{max} nujol (cm^{-1}) 3450 cm^{-1} (m, sh, ν N-H), 3063 cm^{-1} (sh, ν C-H aromatic), 1740 cm^{-1} (sh, ν C=O), 1682 cm^{-1} (sh, ν C=N), 1670 cm^{-1} (sh, ν C=C); $^1\text{H-NMR}$: δ 10.5 (s, 1H, N-H), 9.0 (s, 1H, a, N=CH), 7.1-7.3 (m, 8H, aromatic); **mass spectrum**: m/z 345 (M^+ , 35), 214 (46), 186 (40), 154 (100), 89 (15) and 77 (35).

Cytotoxicity: The cytotoxicity study of the synthesized compounds was investigated on brine shrimp as a test organism and the LC_{50} values for the synthesized compounds are shown in Figure 1.

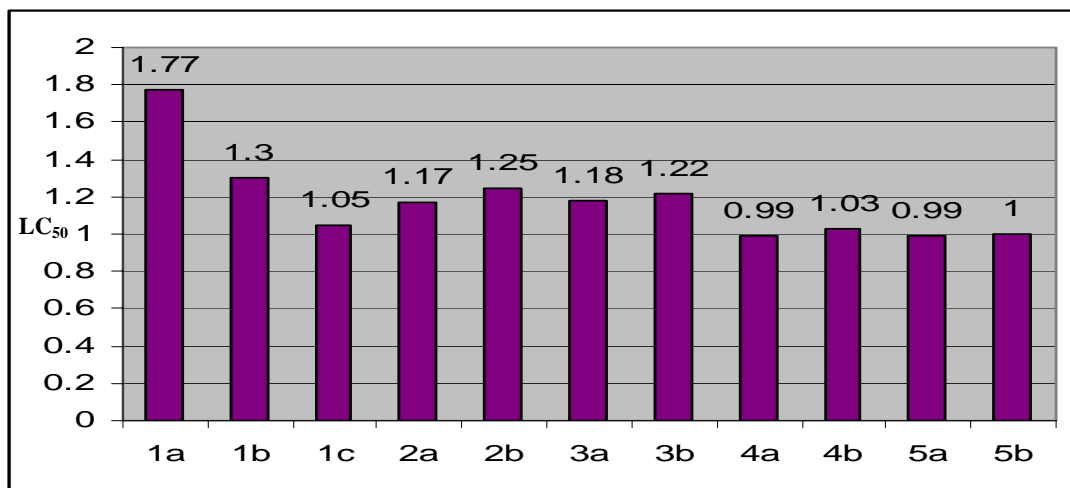
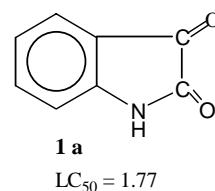
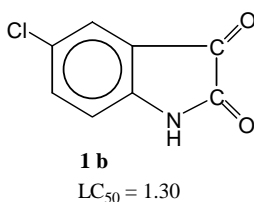
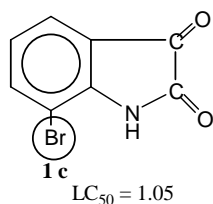


Figure 1: LC_{50} values of the synthesized compounds **1a** - **5b**.

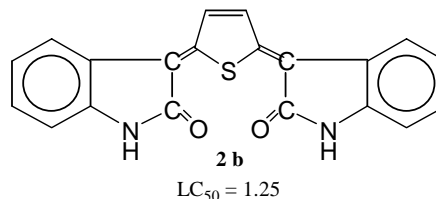
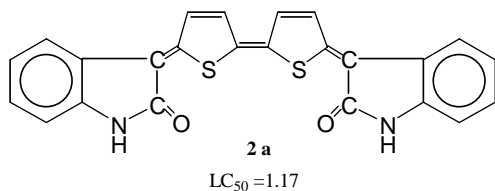
Structure Activity Relationship (SAR): 1) From the Figure 1, it is found that compound **1c** shows the pronounced cytotoxicity property than that of **1b**.

On the other hand, **1b** had higher cytotoxicity property than **1a**, the non-substituted isatin. Thus the order of cytotoxicity activity was $\text{Br} > \text{Cl} > \text{H}$.

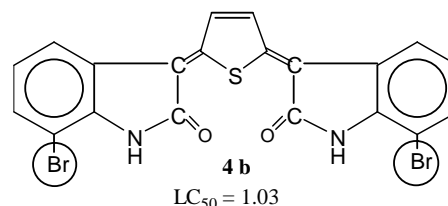
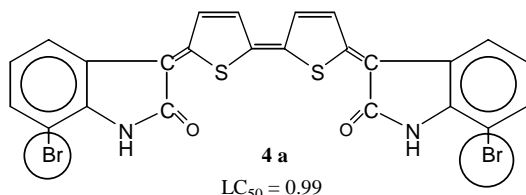


2) The indophenines **2b** with the insertion of one thiophene moiety showed enhanced cytotoxicity

than **1a**. The compound **2a** with two thiophene moieties had still higher activity than **2b**.

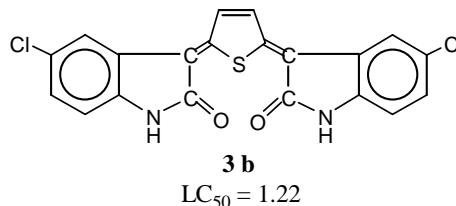
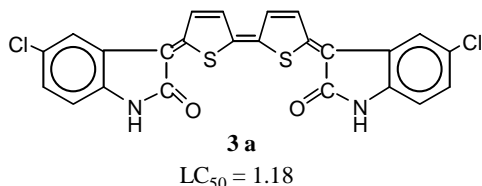


3) The indophenines **4a** and **4b** with Br atom in C - 7 showed very high cytotoxicity that was similar to above trend.



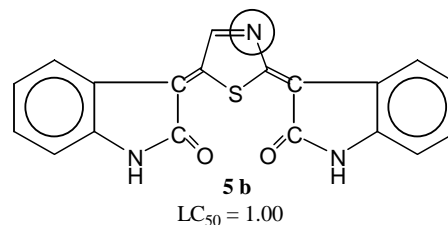
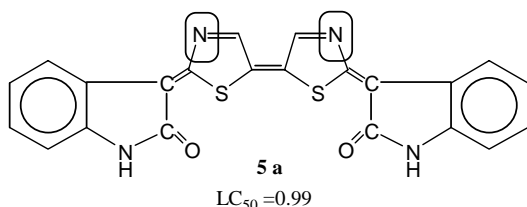
4) Again the indophenines **3a** and **3b** with Cl atom in C - 5 showed different cytotoxicity against brine shrimp

nauplii, **3a** showed greater cytotoxicity than **3b** again the similar trend was observed.



5) The indophenines **5a** and **5b** also showed very high cytotoxicity. The cytotoxicity was enhanced

due to the insertion of hetero atom N in the thiazol ring.



6) It is to be mention here that compounds (2a-5b) with other heterocyclic systems like isatin-1,3,4-thiadiazoline (Hossain et al., 2007) it was found that indophenines with thiophene moiety enhanced the cytotoxicity 24 times than isatin-1,3,4-thiadiazoline.

Discussion

The indophenines (**2a-2b**, **3a-3b**, **4a-4b** and **5a-5b**) have been synthesized from isatins, thiophene and thiazol in moderate yields. The products have been characterized with the help of spectral analysis from Japan (Tokushima University) and German (Ruhr University).

The SAR study of the synthesized compounds showed different cytotoxicity against brine shrimp. The chemical structure of a compound is very important as the relatively minor modification in the compound may results in major change in biological properties. The isatin **1a** is almost inactive but the introduction of a chlorine atom in 5- position of the benzene ring (**1b**) enhanced the cytotoxicity. Again, insertion of a bromine atom in 7-position of the benzene ring (**1c**) further enhanced the cytotoxicity as **1c**>**1b**>**1a**, although the position of bromine and chlorine atoms are not identical. Indophenines **2a** and **2b** show higher cytotoxicity than that of isatins **1a**, **1b** and **1c** due to the presence of thiophene moieties. Again, the indophenine **2a** with two thiophene moieties

shows still higher cytotoxicity than that of **2b** with one thiophene moiety. On the other hand, the indophenines **4a** and **4b** show still greater cytotoxicity against brine shrimp nauplii than that of **3a** and **3b**, due to the presence of bromine atom in the indophenines. Also the indophenines **5a** and **5b** show very high cytotoxicity property against brine shrimp nauplii. The cytotoxicity enhancement is due to the presence of hetero atom N and S in the thiazol ring. Thus the compounds **1c**, **4a**, **4b**, **5a** and **5b** are very active and the compounds **2a**, **2b**, **3a** and **3b** are moderately active.

The present study shows that the cytotoxicity increases by the introduction of Br atom in the benzene ring than that of Cl atom and the greater is the numbers of heterocycles adjacent to the isatin ring, higher is the cytotoxicity. Especially the bulky molecules that contain two thiophene moieties have better cytotoxicity, which means, it hinders the replication of DNA molecule.

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