

Cytotoxicity study of dimethylisatin and its heterocyclic derivatives

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Article Info

Received: 2 August 2007

Accepted: 10 September 2007

Available Online: 22 January 2008

DOI: 10.3329/bjp.v2i2.572

Cite this article:

Hossain MM, Islam N, Khan R, Islam MR. Cytotoxicity study of dimethylisatin and its heterocyclic derivatives. Bangladesh J Pharmacol. 2007; 2: 66-70.

Abstract

Isatin derivatives are bioactive molecules. To study the cytotoxicity and eventually the anticancer activities against cancer cell lines, a series of dimethyl-substituted isatin derivatives (**4-8**) starting from isatin thiosemicarbazones (**3**) had been synthesized in high yields. Investigation of the cytotoxicity of these compounds was carried out against brine shrimp by lethality bioassay. The present study shows that compounds **4**, **5**, **6** and **8'** with heterocyclic moiety had pronounced cytotoxicity whereas **7**, **7'** and **8** were moderately active. It is remarkable that the substituent, X = -OCH₃ has greater activity than the bromine atom in this series.

Introduction

Isatin chemically known as 1H-indole-2,3-dione has become a popular topic due to its manifold uses. The chemistry of isatin and its derivatives is particularly interesting because of their potential application in medicinal chemistry. Isatins are very important compounds due to their antifungal properties (Islam et al., 1998). Moreover, isatins are the synthetic precursors of some biologically important heterocyclic compounds such as quinoline 1,3,4-thiadiazolines etc (Ram et al., 1980; Raj et al., 2003). The biological activity of Δ^2 -1,3,4-thiadiazoline heterocycles can be obtained from the corresponding schiff bases of isatins (Islam et al., 2000, 2001, 2003). Besides, 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). Over the last five decades the considerable research effort has been devoted to the field of HIV and AIDS, a fatal disease, to develop or immunosuppressive agents across the world. Surprisingly isatin derivatives show anti-HIV activity too (Silvam et al., 2001). Therefore, the research interest on isatins has expended day to day. Here some important heterocyclic derivatives of isatins

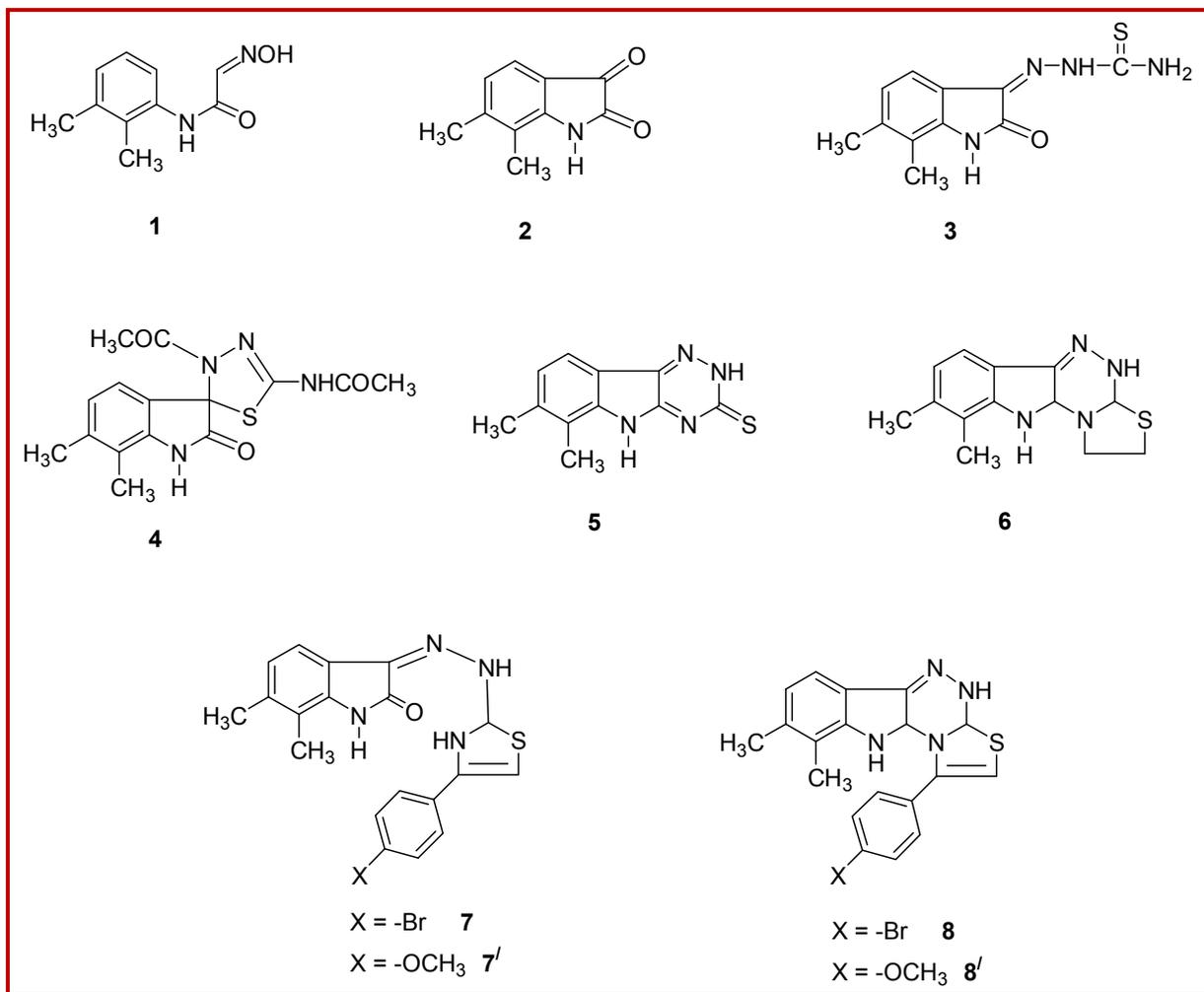
and consequent study of their cytotoxicity are reported.

Materials and Methods

The melting points of the synthesized compounds were recorded by thin disc method on a Fischer Johns electro-thermal melting point apparatus. Infrared spectra were recorded on DR-8001, Shimadzu FT-IR spectrophotometer, ¹H-NMR spectra on a WP-400 NMR spectrophotometer using tetramethylsilane as internal standard. Mass spectra were obtained on a Kratos MS-25 using DH-88 data system.

The tested compound (Scheme 1) **2**, **3**, **4**, **5**, **6**, **7**, **7'**, **8** and **8'** were synthesized by the standard procedure. The characterization of synthesized compounds was carried out by IR, ¹H-NMR and mass spectroscopic methods (Table I). According to standard sandmeyer procedure (Kondrashova and Shvekhgeimer, 2002) oximinoacetanilide **1** was synthesized from 2,6-dimethyl aniline by using chloral hydrate. The resulting pale yellow solid was cyclized with concentrated H₂SO₄ under warm condition to give the corresponding dimethylisatin **2** as a red solid in high yield. Compound **2** was treated with thiosemicarbazide in glacial acetic acid under refluxing





Scheme 1

condition for 3 hours. Cooling followed by neutralization of the reaction mixture gave a crude solid, which was recrystallized from methanol to give 3 as yellow crystals in high yield (Table II). Refluxing of compound 3 with acetic anhydride gave a crude solid mass, which was recrystallized from methanol to give 4 as a yellow solid in high yield. On the other hand, alkali (4% KOH) mediated refluxing of compound 3 for 3 hours gave compound 5 as orange crystals in moderate yield. The ring extended compound 6 was obtained in high yield from 5 with the treatment of 1,2-dibromoethane in DMF. Besides, the treatment of compound 3 with the derivatives of phenacylbromide in a mixture of ethanol and DMF gave the corresponding phenylthiazoles 7 and 7' in very good yields. Finally POCl₃ mediated dehydration of 7 and 7' gave thiazolotriazinoindoles 8 and 8' respectively in excellent yield.

Cytotoxicity studies

The cytotoxicity study of the synthesized compounds was investigated on brine shrimp as a test organism for convenience (Solis et al., 1993). 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, 132 and 165 ppm of each compounds were placed in separate test tubes by microsyringe, 1 mL of extra dimethyl sulfoxide was given in each test tube with 10-12 brine shrimp. 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 the LD₅₀ values were calculated by plotting percentage of mortality against time (Figure 1). LD₅₀ of an agent is the dose, which will kill, or inactive 50% of the test animal. By this method the LD₅₀ values for all the synthesized compounds were obtained (Table III).

Table I

Spectroscopic data of the synthesized compounds

| Compounds | IR (cm ⁻¹) | ¹ H-NMR (δ-value) | Mass (m/z) |
|-----------|---|--|---|
| 1 | 3320 (OH), 3352 (NH), 3060 (Ph-H), 3020 (=CH), 2920,2864 (CH ₃), 1655 (C=O, amide), 1612 (C=N), 1551,1473 (C=C) | 12.09 (s, 1H, OH), 9.53 (s, 1H, amide), 7.64 (s, 1H, N=CH), 6.9-7.3 (m, 3H, Ar-H), 2.24 (s, 3H, CH ₃), 2.06 (s, 3H, CH ₃) | 192 (M ⁺ ; 88), 175 (94), 160 (100),147 (42), 132 (32), 120 (56), 105 (37), 91(38),77 (58) |
| 2 | 3195 (NH), 3090 (Ph-H), 2942, 2880 (CH ₃), 1736 (C=O, lactum), 1618 (C=O, Keto), 1597,1505 (C=C, aromatic) | 11.10 (s, 1H, NHCO), 7.3 (d, 1H, J _o =7.55 Hz, Ar-H), 6.8 (d, 1H, J _o =7.55 Hz, Ar-H), 2.20 (s, 3H, CH ₃), 1.95 (s, 3H, CH ₃) | 175 (M ⁺ ; 96), 147 (46), 119 (100), 118(58), 104 (34), 91 (24), 77 (12), 74 (8), 65 (16) |
| 3 | 3155,3020 (NH ₂), 3067 (Ph-H), 2937,2885 (CH, Saturated), 1695 (C=O, lactum), 1610,1489 (C=C, aromatic) | 12.84 (s, 1H, NHCO), 11.50 (s, 1H, NH-CS), 10.40 (s, 1H, CS-NH ₂), 7.25 (d, 1H, J=7.64 Hz, Ar-H), 6.89 (d, 1H, J=7.64 Hz, Ar-H), 2.20 (s, 3H, CH ₃), 2.02 (s, 3H, CH ₃) | 248 (M ⁺ ;12), 264 (46), 220 (38), 161 (72), 133 (42), 118 (58), 91 (100), 77(80), 65(56), 29 (64) |
| 4 | 3175 (NH), 3090 (Ph-H), 2920, 2875 (CH, Saturated), 1742 (C=O, acetyl), 1693 (C=O, lactum), 1616, 1499 (C=C, aromatic) | 12.02 (s, 1H, NHCO, lactum), 8.50 (s, 1H, NH-CO), 7.20 (d, 1H, J=7.64 Hz, Ar-H), 6.85 (d, 1H, J=7.64 Hz, Ar-H), 2.40 (s, 3H, N-NCOCH ₃), 2.3 0 (s, 3H, C-NHCOCH ₃), 2.06 (s, 3H, CH ₃), 2.02 (s, 3H, CH ₃) | 332 (M ⁺ ; 22), 318 (10), 304 (18), 290 (30), 276 (72), 248 (100), 234 (42), 205 (50), 191 (48), 177 (80), 163 (56), 130 (62), 103 (42), 91 (64) |
| 5 | 3205 (NH), 3060 (CH, aromatic), 2925, 2865 (CH, saturated), 1595, 1489 (C=C, aromatic), 1142 (C=S) | 13.20 (s, 1H, NHCS), 10.50 (s, 1H, NH-N), 7.20 (d, 1H, J=7.60 Hz, Ar-H), 6.90 (d, 1H, J=7.60 Hz, Ar-H), 2.20 (s, 3H, CH ₃), 2.10 (s, 3H, CH ₃) | 230 (M ⁺ ; 60), 201 (48), 186 (100), 173(22), 155 (62), 130 (58), 91 (60), 77 (80) |
| 6 | 3090 (CH, aromatic), 2925, 2855 (CH, saturated), 1616 (C=N), 1689, 1487 (C=C, aromatic) | 7.25 (d, 1H, J=7.55 Hz, Ar-H), 6.85 (d, 1H, J=7.55 Hz, Ar-H), 4.95 (t, 2H, CH ₂ -N), 3.85 (t, 2H, CH ₂ -S), 2.20 (s, 3H, CH ₃), 2.15 (s, 3H, CH ₃) | 256 (M ⁺ ; 10), 242 (56), 210 (100), 197(46), 171 (52), 156 (58), 154 (60), 140 (72), 130 (18), 102 (42) |
| 7 | 3175 (NH), 3060 (CH, aromatic), 2910, 2840 (CH, saturated), 1693 (C=O, lactum), 1616, 1479 (C=C, aromatic) | 13.40 (s, 1H, NHCO), 11.45 (s, 1H, NH-N), 7.70 (d, 2H, J=8.01 Hz, Ar-H), 7.40 (d, 2H, J=8.01 Hz, Ar-H), 7.20 (d, 1H, J=7.45 Hz, Ar-H), 7.10 (d, 1H, J=7.45 Hz, Ar-H), 7.50 (s, 1H, =C-H), 2.18 (s, 3H, CH ₃), 2.10 (s, 3H, CH ₃) | 347 (M ⁺ 81/79; 4), 319 (20), 242 (12), 147 (46), 133(100), 105 (68), 91 (80), 77 (58), 65 (40) |
| 7' | 3175 (NH), 3080 (CH, aromatic), 2920, 2865 (CH, saturated), 1686 (C=O, lactum), 1599, 1508 (C=C, aromatic) | 14.40 (s, 1H, NHCO), 11.30 (s, 1H, NH-N), 7.90 (d, 2H, J=8.51 Hz, Ar-H), 7.25 (d, 1H, J=7.46 Hz, Ar-H), 7.15 (d, 1H, J=7.46 Hz, Ar-H), 7.20 (s, 1H, =C-H), 3.60 (s, 3H, OCH ₃), 2.20 (s, 3H, CH ₃), 2.10 (s, 3H, CH ₃) | 378 (M ⁺ ; 10), 350 (12), 338 (48), 326 (30), 295 (22), 219 (68), 214 (86), 188 (60), 160 (42),140 (100), 109 (58), 91 (40) |
| 8 | 3095 (CH, aromatic), 2920, 2860 (CH, saturated), 1616, 1479 (C=C, aromatic) | 7.70 (d, 2H, J=8.56 Hz, Ar-H), 7.40 (d, 2H, J=8.56 Hz, Ar-H), 7.20 (d, 1H, J=7.58 Hz, Ar-H), 7.10 (d, 1H, J=7.58 Hz, Ar-H), 7.50 (s, 1H, =C-H), 2.18 (s, 3H, CH ₃), 2.10 (s, 3H, CH ₃) | 329 (M ⁺ 81/79; 2), 297 (10), 271 (12), 220 (78), 211 (42), 192 (80), 185 (38), 134(100), 120 (10), 91 (28) |
| 8' | 3060 (CH, aromatic), 2920, 2855 (CH, saturated), 1589, 1487 (C=C, aromatic) | 7.88 (d, 2H, J=8.60 Hz, Ar-H), 6.85 (d, 2H, J=8.60 Hz, Ar-H), 7.20 (d, 1H, J=7.52 Hz, Ar-H), 7.10 (d, 1H, J=7.52 Hz, Ar-H), 7.30 (s, 1H, =C-H), 3.40 (s, 3H, OCH ₃), 2.10 (s, 3H, CH ₃), 2.02 (s, 3H, CH ₃) | 360 (M ⁺ ; 60), 328 (30), 297 (40), 242 (38), 221 (28), 212 (74), 193 (100), 186 (60), 135 (62) |

| Table II | | | |
|--|--------------------------|------------------------------|--------|
| Physical constants and %yield of synthesized compounds (2-8) | | | |
| Compound No. | Melting temperature (°C) | Color | %Yield |
| 2 | 210-211 | Red crystalline solid | 75 |
| 3 | 217-218 | Yellow crystalline solid | 77 |
| 4 | 163-164 | Pale yellow solid | 86 |
| 5 | 275-276 | Orange red crystalline solid | 69 |
| 6 | 256-257 | Orange red crystalline solid | 62 |
| 7 | 274-275 | Yellowish crystalline solid | 77 |
| 7' | 255-256 | Paleyellow crystalline solid | 77 |
| 8 | 261-262 | Dark brown crystalline solid | 85 |
| 8' | 276-278 | Dark brown crystalline solid | 87 |

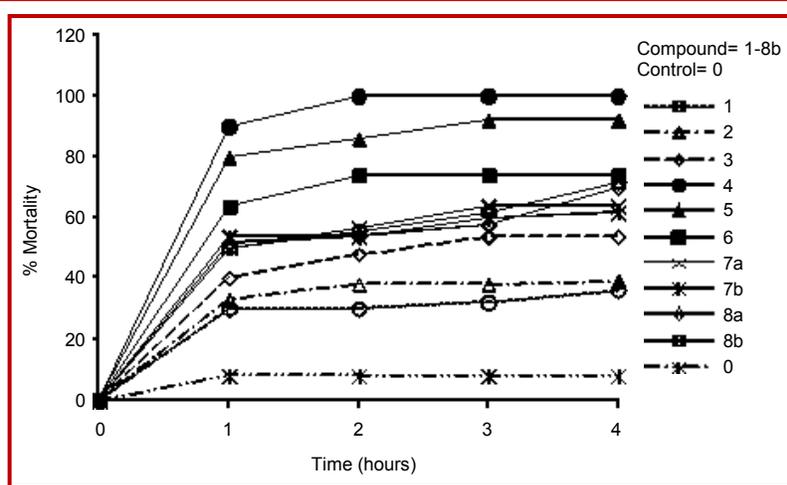


Figure 1: Brine shrimp lethality of different compounds in different incubation time

| Table III | | | | | |
|---|------------------------------|---------------|--------------|------------------------------|-------------------|
| Cytotoxicity of the synthesized compounds | | | | | |
| Compound No. | LD ₅₀ value (ppm) | Remarks | Compound no. | LD ₅₀ value (ppm) | Remarks |
| 1 | 99 | Less active | 6 | 22 | Highly active |
| 2 | 96 | Less active | 7 | 36 | Moderately active |
| 3 | 52 | Weakly active | 7' | 31 | Moderately active |
| 4 | 24 | Highly active | 8 | 29 | Moderately active |
| 5 | 24 | Highly active | 8' | 24 | Highly active |

Results

The structures of the synthesized compounds were proved by analyzing the spectroscopic data. The results of the IR, ¹H-NMR and MS spectra are given in the Table I. From the cytotoxicity study it was seen that compounds **4**, **5**, **6** and **8'** were highly active whereas compounds **7**, **7'** and **8** were moderately active and the compound **3** showed poor activity against brine shrimp. The rest of the compounds **1** and **2** were comparatively inactive.

Discussion

The structure activity relationship study of the synthesized compounds showed that dimethyl isatin along with its precursor was almost inactive. But when it was converted to Schiff base of thiosemicarbazide, it showed moderate activity. Thus sulfur atom played an important role for bioactivity.

Further cyclization of compound **3** showed greater activity. Besides this, a number of compounds containing

several types of ring systems were synthesized from dimethyl isatin for studying the effect of extended cycle on bioactivity. An important finding of the present study showed that the greater the number of cycles adjacent to the isatin ring, the higher was the cytotoxicity (compound **4**, 3 rings, LD₅₀ 24 ppm; compound **5**, 3 rings, LD₅₀ 24 ppm; compound **6**, 4 rings, LD₅₀ 22 ppm). But compounds containing rings far apart from the isatin ring system showed lower activity (compound **7** and **7'**).

Selvam et al., 2006 showed that brominated isatin derivatives had higher activity but this study showed that it was the methoxy group which showed greater activity (compound **7**, LD₅₀ 36 ppm; compound **7'**, LD₅₀ 31 ppm and compound **8**, LD₅₀ 29 ppm; compound **8'**, LD₅₀ 24 ppm).

Acknowledgement

We express our gratitude and thanks to Prof. Helmut Duddeck, Institute of Organic Chemistry, Hannover University, Germany for kindly supplying the MS and NMR spectra of the synthesized compounds.

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