

# Synthesis of hydantoin and thiohydantoin related compounds from benzil and study of their cytotoxicity

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## Abstract

Condensation of benzil (**1**) with urea, monophenyl urea and diphenyl urea in the presence of absolute ethanol using 30% aqueous NaOH gave the products **1a**, **1b** and **1c** respectively and also with thiourea, monomethyl thiourea, dimethyl thiourea and diethyl thiourea the products **2a**, **2b**, **2c** and **2d** were obtained. Methylation of the product, **2a** in the presence of dimethyl formamide (DMF) using K<sub>2</sub>CO<sub>3</sub> formed **2**. The compounds **1b**, **1c**, **2b**, **2c** and **2** showed highly cytotoxic activity and the compounds **1a**, **2a**, **2d** showed relatively low cytotoxic activity against brine shrimp lethality bioassay.

## Introduction

Several types of substituted isatin heterocyclic derivatives were synthesized and found cytotoxic activity of these compounds by screening tests (Islam et al., 2001a; Islam et al., 2001b; Lingcon et al., 2001). Methyl and bromine groups in the benzene ring of isatin,  $\Delta^2$ -1,3,5-thiadiazolines show more cytotoxic activity. For this interest, the title compounds have been synthesized for screening tests whether they show reasonable lethal effect on brine shrimp or not. We report to see herein the results of the synthesis of the mentioned compounds (Scheme 1), spectral characterization and their cytotoxic effects by brine shrimp lethality bioassay (Anderson et al., 1999).

## Materials and Methods

Melting points are not corrected. IR spectra recorded on a Shimadzu DR 8001 FT-IR spectrometer, NMR spectra on a WP 200 spectrometer using TMS as internal

standard and mass spectra on an MS Kratos mass spectrometer.

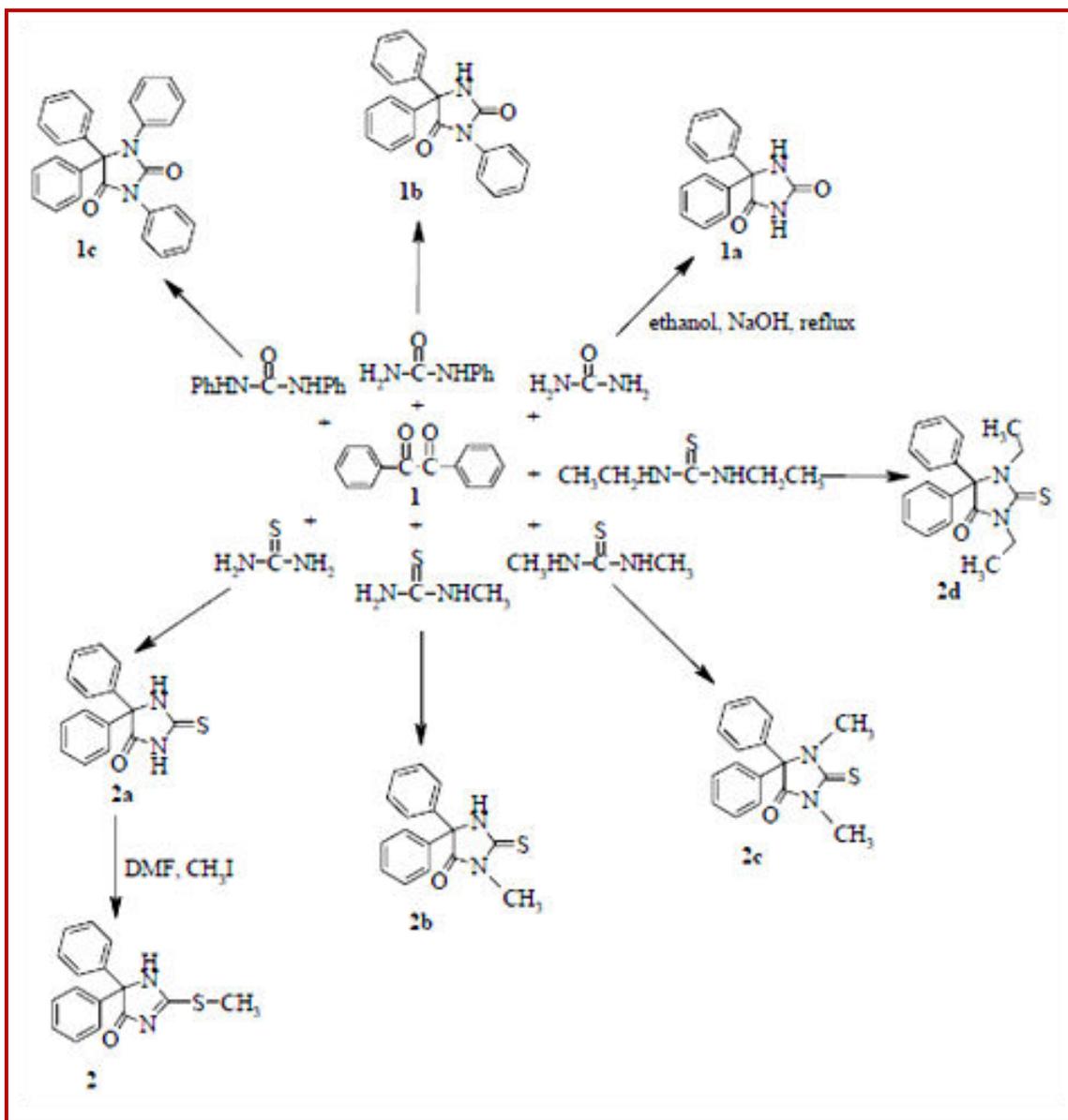
### Preparation of mono- and diphenyl urea

Mono-phenyl urea and diphenyl urea were prepared from aniline hydrochloride and urea following the reported procedure (Furniss et al., 1998).

### Preparation of 5,5-diphenylhydantoin (**1a**) (Muccioli et al., 2003)

Benzil, **1** (0.8 g, 3.8 mmol/L) was placed in a 100 mL round-bottomed flask with urea, (0.4 g, 6.7 mmol/L) in the molar ratio of ca 1:2. Absolute ethanol (12 mL) and 30% aqueous sodium hydroxide (2.5 mL) were added to these reactants. Boiling chips were also added to this solution and a condenser was attached after wrapping the ground glass joint with Teflon tape, and was heated (110-120°C) the mixture under reflux (2 hours). The reaction mixture was cooled before adding 15 mL of water. The solution was not clear, so the suspended solids were removed by filtration. Then the clear solu-





Scheme 1

tion was cautiously acidified with concentrated hydrochloric acid and the product was collected by vacuum filtration and washed thoroughly with water.

Then the product, **1a** was recrystallized by ethanol, dried, the m.p. 294-295°C and yields 0.750 g (80%). **IR:**  $\nu_{\text{Nujol}}(\text{cm}^{-1})$  3271 (s,  $\nu\text{N-H}$  amide, CO-NH-CO); 3204 (s,  $\nu\text{N-H}$  amide, CO-NH-CPh<sub>2</sub>); 3055 (m,  $\nu\text{C-H}$  aromatic); 1772 (s,  $\nu\text{C=O}$  amide, NH-CO-CPh<sub>2</sub>); 1741 (s,  $\nu\text{C=O}$  amide, NH-CO-NH); 1597, 1541, 1508 (s,  $\nu\text{C=C}$ , aromatic). **<sup>1</sup>H-NMR** (DMSO):  $\delta$  10.10 (s, 1H, NH, CO-NH-CO);  $\delta$  9.10 (s, 1H, NH, CO-NH-CPh<sub>2</sub>);  $\delta$  7.80-7.00 (m, 10H, C-H, aromatic). **<sup>13</sup>C-NMR** (DMSO):  $\delta$  175.19 (C-

-4);  $\delta$  156.36 (C-2);  $\delta$  140.27 (C-6);  $\delta$  128.87 (C-7);  $\delta$  128.39 (C-8);  $\delta$  126.93 (C-9);  $\delta$  70.56 (C-5). **Mass:** m/z (% of relative intensity) 252 (M<sup>+</sup>-3%), 176 (30%), 154 (100%), 128 (3%), 77 (15%), 51 (8%), and 39 (10%). The molecular ion peak appears at m/z 252 due to C<sub>15</sub>H<sub>12</sub>N<sub>2</sub>O<sub>2</sub>.

#### Preparation of 3,5,5-triphenyl hydantoin (**1b**)

Benzil, **1**; (0.148 g, 0.70 mmol/L) and the monophenylurea; (0.298 g, 1.40 mmol/L) in the molar ratio of ca 1:2 were refluxed in the absolute ethanol and the procedure for **1b** was similar to that of the compound, **1a**. The product, **1-a1** was colorless solid; m.p. 172-173°C having

yields 0.077 g, 50%. **IR:**  $\nu$  Nujol (cm<sup>-1</sup>) 3220 (s,  $\nu$ N-H amide); 3055 (m,  $\nu$ C-H aromatic); 1716 (s,  $\nu$ C=O amide, NPh-CO-C-Ph<sub>2</sub>); 1637 (s,  $\nu$ C=O amide, NH-CO-NPh); 1595, 1541, 1508 ( $\nu$ C=C, aromatic). **<sup>1</sup>H-NMR** (DMSO):  $\delta$  9.40 (s, 1H, NH, amide);  $\delta$  7.80-7.40 (m, 15H, C-H, aromatic). **<sup>13</sup>C-NMR** (DMSO):  $\delta$  173.06 (C-4);  $\delta$  154.32 (C-2);  $\delta$  141.99 (C-10);  $\delta$  140.60 (C-6);  $\delta$  129.05 (C-7)  $\delta$  128.30 (C-8);  $\delta$  127.97 (C-9);  $\delta$  127.33 (C-12);  $\delta$  121.53 (C-13);  $\delta$  117.70 (C-11); 68.70 (C-5). **Mass:** m/z (% of relative intensity) 328 (M<sup>+</sup>3%), 176 (25%), 154 (100%), 136 (68%), 120 (12%), 55 (27%), 43 (26%). The molecular ion peak appears at m/z 328 due to C<sub>21</sub>H<sub>16</sub>N<sub>2</sub>O<sub>2</sub>.

#### Preparation of 1,3,5,5-Tetraphenyl hydantoin (1c)

Benzil **1**; (0.148 g, 0.70 mmol/L) and the above 1,3-diphenylurea, a3; (0.298 g, 1.40 mmol/L) in the molar ratio of ca 1:2 were refluxed in the absolute ethanol and the procedure of **1c** was similar to that of the compound, **1a**. The product, **1c** was off white powder. It was recrystallized in methanol, dried and m.p. 163-165°C and yields were 0.07 g, 42%. **IR:**  $\nu$  Nujol (cm<sup>-1</sup>) 3110 (w,  $\nu$ C-H aromatic); 1716 (s,  $\nu$ C=O amide, NPh-CO-CPh<sub>2</sub>); 1635 (s,  $\nu$ C=O amide, NPh-CO-NPh); 1595, 1541, 1508 ( $\nu$ C=C, aromatic). **<sup>1</sup>H-NMR** (DMSO):  $\delta$  7.20-6.70 (m, 20H, C-H, aromatic). **<sup>13</sup>C-NMR** (DMSO):  $\delta$  173.10 (C-4);  $\delta$  154.25 (C-2);  $\delta$  141.88 (C-10);  $\delta$  140.60 (C-6);  $\delta$  139.88 (C-14)  $\delta$  129.33 (C-7)  $\delta$  128.71 (C-8);  $\delta$  127.99 (C-9);  $\delta$  127.38 (C-12);  $\delta$  127.26 (C-13);  $\delta$  127.12 (C-16)  $\delta$  121.60 (C-17);  $\delta$  117.73 (C-11);  $\delta$  109.48 (C-15)  $\delta$  69.50 (C-5). **Mass:** m/z (% of relative intensity) 404 (M<sup>+</sup>3%), 369 (75%), 347(68%), 302 (15%), 259 (7%), 233 (12%), 211 (100%), 182 (92%), 136 (29%), 93 (41%). The molecular ion peak appears at m/z 404 due to C<sub>27</sub>H<sub>20</sub>N<sub>2</sub>O<sub>2</sub>.

#### Preparation of 5,5-diphenyl-2-thiohydantoin (2a)

The compound, **2a** was prepared from Benzil, **1**; (0.80 g, 3.81 mmol/L) and thiourea; (0.58 g, 7.63 mmol/L) following the procedure of **1a**. The product was recrystallized in ethanol, m.p. 234-235°C yielded 0.595 g, 94%. **IR:**  $\nu$  Nujol (cm<sup>-1</sup>) 3255 (s, b,  $\nu$ N-H amide, CO-NH-CS); 3135 (b,  $\nu$ N-H, amide, CPh<sub>2</sub>-NH-CS); 3010 (s, C-H aromatic); 1749 (s,  $\nu$ C=O amide); 1558, 1541, 1508 ( $\nu$ C=C, benzene); 1215 (s,  $\nu$ C=S). **<sup>1</sup>H-NMR** (DMSO):  $\delta$  7.80-7.60 (m, 10H, C-H, aromatic),  $\delta$  1.10-1.12 (1H, SH). **<sup>13</sup>C-NMR** (DMSO):  $\delta$  181.65 (C-4);  $\delta$  175.54 (C-2);  $\delta$  138.70 (C-6);  $\delta$  129.13 (C-7);  $\delta$  128.80 (C-8);  $\delta$  126.92 (C-9); 73.92 (C-5). **Mass:** m/z (% of relative intensity) 268 (M<sup>+</sup>12%), 182 (18%), 154 (100%), 136 (59%), 120 (12%), 107 (19%), 77 (17%), 57 (27%), 43 (23%). The molecular ion peak appears at m/z 268 due to C<sub>15</sub>H<sub>12</sub>N<sub>2</sub>OS.

#### Preparation of 3-methyl-5, 5-diphenyl-2-thiohydantoin (2b)

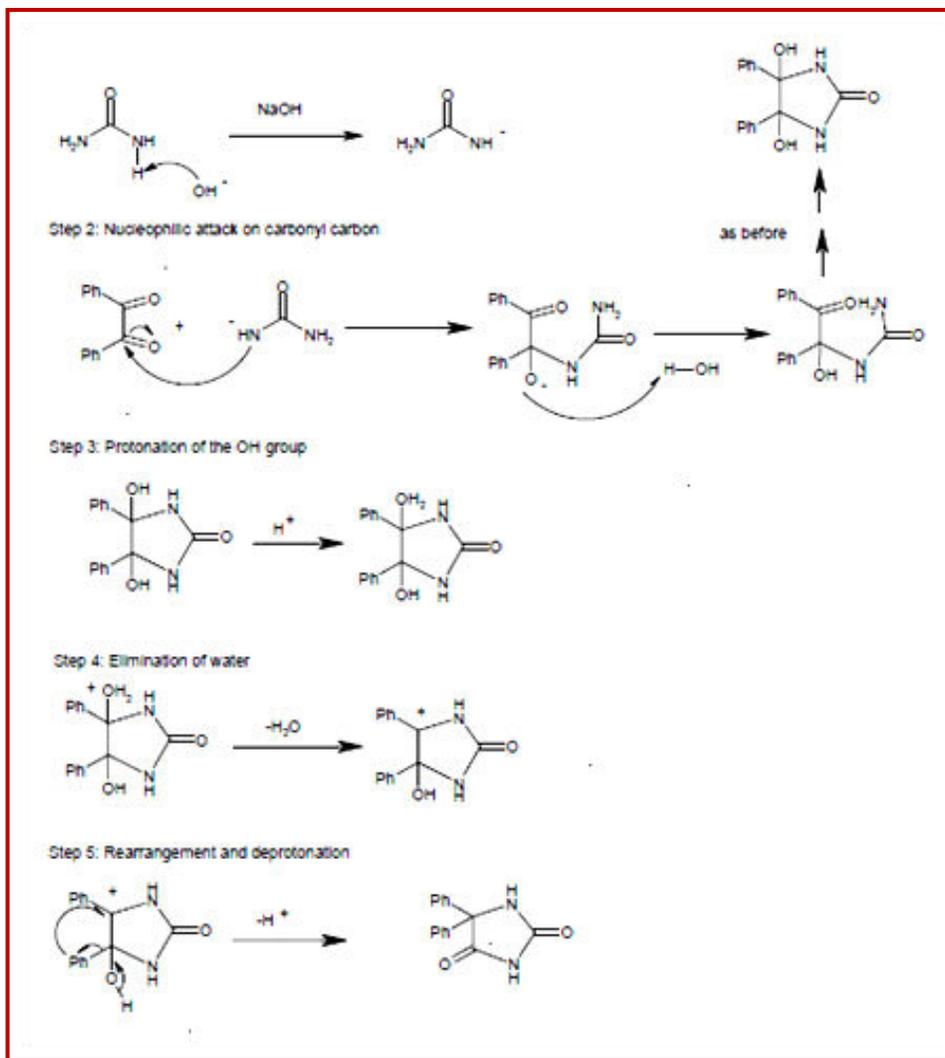
Benzil, **1**; (0.84 g, 4.00 mmol/L) with methylthiourea (0.72 g, 8.00 mmol/L) in the molar ratio of ca 1:2 were refluxed in ethanol and the procedure for the compound, **2b** is similar to that of the compound, **1a**. The product was recrystallized in ethanol; m.p. 182-183°C having yields 1.0 g, 94%. **IR:**  $\nu$  Nujol (cm<sup>-1</sup>) 3250 (b,  $\nu$ N-H thiamide); 3110 (s, w,  $\nu$ C-H aromatic); 2950, 2900 (s,  $\nu$ C-H, aliphatic), 1716 (s,  $\nu$ C=O amide); 1595, 1541, 1508 ( $\nu$ C=C, aromatic); 1215 (s,  $\nu$ C=S). **<sup>1</sup>H-NMR** (DMSO):  $\delta$  7.20-6.70 (m, 10H, C-H, aromatic),  $\delta$  3.50 (s, 3H, C-H, aliphatic),  $\delta$  1.30 (s, 1H, SH). **<sup>13</sup>C-NMR** (DMSO):  $\delta$  181.94 (C-2);  $\delta$  173.93 (C-4);  $\delta$  138.55 (C-6);  $\delta$  129.13 (C-7)  $\delta$  128.89 (C-8);  $\delta$  127.44 (C-9);  $\delta$  71.76 (C-5);  $\delta$  27.79 (C-10). **Mass:** m/z (% of relative intensity) 282 (M<sup>+</sup>100%), 205 (22%), 180(68%), 165 (50%), 121 (7%), 104 (72%), 77 (49%), 59 (6%), 51 (17%), 39 (3%). The molecular ion peak appears at m/z 282 due to C<sub>16</sub>H<sub>14</sub>N<sub>2</sub>OS.

#### Preparation of 1,3-dimethyl-5, 5-diphenyl-2-thiohydantoin (2c)

Compound, **2c** was obtained from benzil, **1**; (1.00 g, 4.76 mmol/L) and 1,3-dimethylthiourea; (1.00 g, 9.61 mmol/L) in ethanol following the procedure of **1a**. Compound **2c** was obtained as white crystalline solids, m.p. 154-156°C having 0.950 g, yielded 85%. **IR:**  $\nu$  Nujol (cm<sup>-1</sup>) 3030 (s,  $\nu$ C-H aromatic); 2930, 2858 (s,  $\nu$ C-H, aliphatic); 1643 (s,  $\nu$ C=O amide); 1470, 1448 (s,  $\nu$ C=C, aromatic); 1296 (s,  $\nu$ C-N); 1126 (s,  $\nu$ C=S stretching vibration). **<sup>1</sup>H-NMR** (DMSO):  $\delta$  7.00-6.60 (m, 10H, C-H, aromatic),  $\delta$  3.30 (bs, 6H, CH<sub>3</sub>). **<sup>13</sup>C-NMR** (DMSO):  $\delta$  184.08 (C-2, C-4);  $\delta$  137.90 (C-6);  $\delta$  127.97 (C-7)  $\delta$  127.74 (C-8);  $\delta$  127.12 (C-9);  $\delta$  95.39 (C-5);  $\delta$  33.41 (C-10);  $\delta$  30.54 (C-11). **Mass:** m/z (% of relative intensity) 296 (M<sup>+</sup>100%), 224 (23%), 209(13%), 176 (10%), 154 (31%), 136 (45%), 105 (56%), 91 (13%), 77 (14%), 43 (12%). The molecular ion peak appears at m/z 296 due to C<sub>17</sub>H<sub>16</sub>N<sub>2</sub>OS.

#### Preparation of 1,3-diethyl-5, 5-diphenyl-2-thiohydantoin (2d)

Refluxing of benzil, **1**; (0.84 g, 4.00 mmol/L) and 1,3-diethylthiourea; (1.06 g, 8.00 mmol/L) and the following procedure of **1a** gave the product **2d**. The product was recrystallized in ethanol, m.p.116-117°C having yielded 0.297 g, 45%. **IR:**  $\nu$  Nujol (cm<sup>-1</sup>) 3110 (w,  $\nu$ C-H aromatic); 2990, 2830 (s,  $\nu$ C-H, aliphatic); 1716 (s,  $\nu$ C=O amide, NPh-CO-C-Ph<sub>2</sub>); 1635 (s,  $\nu$ C=O amide, NPh-CO-NPh); 1595, 1541, 1508( $\nu$ C=C, aromatic).



Scheme 2

**<sup>1</sup>H-NMR** (DMSO):  $\delta$  7.00-6.80 (m, 10H, C-H, aromatic),  $\delta$  3.60 (q, 2H, <sup>11</sup>CH<sub>2</sub>),  $\delta$  3.30 (q, 2H, <sup>13</sup>CH<sub>2</sub>),  $\delta$  1.20 (t, 6H, CH<sub>3</sub>). **<sup>13</sup>C-NMR** (DMSO):  $\delta$  183.31 (C-2);  $\delta$  183.21 (C-4);  $\delta$  140.07 (C-6);  $\delta$  128.17 (C-7);  $\delta$  127.97 (C-8);  $\delta$  127.83 (C-9);  $\delta$  99.62 (C-5);  $\delta$  96.54 (C-11);  $\delta$  96.28 (C-13);  $\delta$  14.85 (C-12);  $\delta$  14.43 (C-14). **Mass**: m/z (% of relative intensity) 324 (M<sup>+</sup>+12%), 194 (28%), 165 (19%), 150 (55%), 121 (12%), 105 (100%), 86 (9%), 77 (50%), 51 (5%), 29 (21%). The molecular ion peak appears at m/z 324 due to C<sub>19</sub>H<sub>20</sub>N<sub>2</sub>O<sub>5</sub>.

**Preparation of S-methyl-5, 5-Diphenyl-2-thiohydantoin (2)** (Muccioli et al., 2003)

The compound, **2a** (0.200 g, 0.71 mmol/L) was dissolved in anhydrous DMF (0.70 mL) and K<sub>2</sub>CO<sub>3</sub> (0.41 g, 0.31 mmol/L) then iodomethane (0.70 mL, 0.71 mmol/L) were added to this mixture and stirred

overnight at room temperature. The mixture was poured into distilled water. The resulting precipitate was collected, dried and recrystallized from ethanol (If some of K<sub>2</sub>CO<sub>3</sub> are non-reacted then hot ethanol solution of product is filtered to separate K<sub>2</sub>CO<sub>3</sub> from the product) the product, **2**. The yields of the product, **2** were 0.75 g, 38% having m.p. 172-174°C. **IR**:  $\nu$  Nujol (cm<sup>-1</sup>) 3240 (w,  $\nu$ N-H, amine); 3010 (s,  $\nu$ C-H, aromatic); 2900, 2855 (s,  $\nu$ C-H, aliphatic); 1725 (s,  $\nu$ C=O); 1684 (s,  $\nu$ C=N); 1585, 1506, 1489 ( $\nu$ C=C, aromatic). **<sup>1</sup>H-NMR** (DMSO):  $\delta$  7.40-7.20 (m, 10H, C-H, aromatic),  $\delta$  3.20 (s, 1H, NH),  $\delta$  2.70 (s, 3H, C-H, aliphatic). **<sup>13</sup>C-NMR** (DMSO):  $\delta$  180.36 (C-2);  $\delta$  162.42 (C-4);  $\delta$  140.80 (C-6);  $\delta$  128.72 (C-7);  $\delta$  127.97 (C-8);  $\delta$  127.14 (C-9);  $\delta$  77.94 (C-5);  $\delta$  26.87 (C-10). **Mass**: m/z (% of relative intensity) 282 (M<sup>+</sup>+2%) 176 (27%), 165 (12%), 154 (100%), 136 (70%), 120 (13%), 107 (19%), 88 (18%), 77 (15%), 51 (5%), 43 (8%).

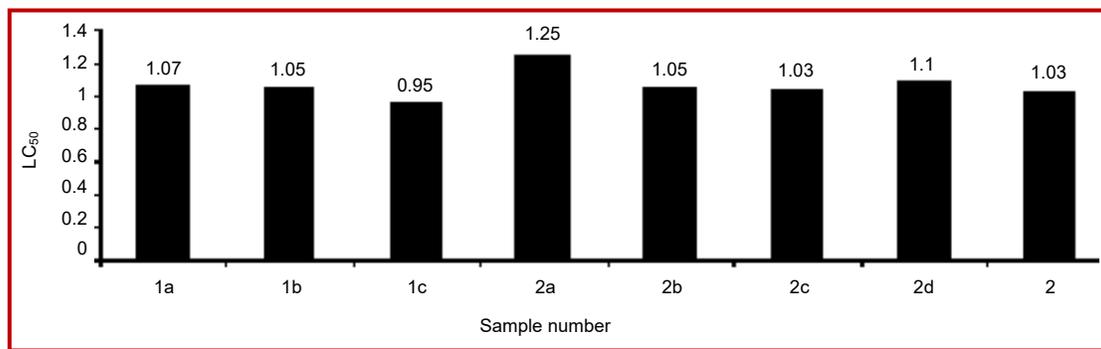


Figure 1: Comparative graphical representation of the LC<sub>50</sub> of toxicity for the synthesized compounds against brine shrimp lethality test

The molecular ion peak appears at  $m/z$  282 due to  $C_{16}H_{14}N_2OS$ .

## Results and Discussion

Simple base catalyzed condensation of benzil, **1** with urea, monophenyl urea and diphenyl urea in absolute ethanol furnished **1a**, **1b**, **1c** whereas with thiourea, methyl thiourea, dimethyl thiourea and diethyl thiourea gave **2a**, **2b**, **2c** and **2d**. The formation of these products follows the pinacole-pinacolone type (Shukla and Trivedi, 1997) rearrangement that is shown in the Scheme 2 (e.g., compound, **1a**). The IR spectrum of the compound, **1a** at  $3271\text{ cm}^{-1}$  indicates the presence of NH group of amide moiety. The other band at  $3204\text{ cm}^{-1}$  also indicates the presence of NH in  $\text{Ph}_2\text{C-NH-CO}$ . The clear band at  $3055\text{ cm}^{-1}$  shows the presence of C-H stretching vibration of phenyl ring. The lower absorption at  $1772\text{ cm}^{-1}$  assigns C=O group of amide in  $\text{Ph}_2\text{-CO-NH-}$ , on the other hand the band at  $1741\text{ cm}^{-1}$  corresponds to other C=O group in  $\text{NH-CO-NH}$ . The bands at  $1597\text{ cm}^{-1}$ ,  $1541\text{ cm}^{-1}$ ,  $1508\text{ cm}^{-1}$  give the strong support of C=C stretching vibration of phenyl ring.  $^1\text{H-NMR}$  spectrum shows two singlets at  $\delta$  10.1 and  $\delta$  9.1, which would be assigned for NH proton of amide. The aromatic protons appear as multiplet at  $\delta$  7.8-7.0.  $^{13}\text{C-NMR}$  spectrum at  $\delta$  175.2 (C-4) and  $\delta$  156.4 (C-2) is for the carbonyl groups of amide. The aromatic carbons are designated by the following values  $\delta$  140.3 (C-6),  $\delta$  128.9 (C-7),  $\delta$  128.4 (C-8),  $\delta$  126.9 (C-9) and at  $\delta$  70.6 (C-5). In the MS spectrum of the compound, **1a** the molecular ion peak ( $M^+3\%$ ) appears at  $m/z$  252 that corresponding to the molecular formula  $C_{15}H_{12}N_2O_2$ . In this spectrum the base peak is formed at  $m/z$  154.

*Methylation of 2a with  $\text{CH}_3\text{I}$  in the presence of  $\text{K}_2\text{CO}_3$  in dry DMF gave the product (2)*

In the IR spectrum of the compound, **2** the sharp band at  $3240\text{ cm}^{-1}$  indicates the presence of NH group of amide. The signal at  $3010\text{ cm}^{-1}$  locates the presence of C-H stretching vibration in aromatic. The bands at  $2900\text{ cm}^{-1}$  and  $2855\text{ cm}^{-1}$  indicate the presence of C-H group for  $\text{CH}_3$ . The clear and sharp band at  $1725\text{ cm}^{-1}$  is assigned for C=O group. The band at  $1684\text{ cm}^{-1}$  indicates the presence of C=N group. The bands at 1585, 1506 and 1489 give the strong support for the presence of C=C stretching vibration in the aromatic ring. In  $^1\text{H-NMR}$  spectrum the aromatic protons appear as multiplet at  $\delta$  7.4-7.2. The singlet at  $\delta$  3.2 indicates the presence of NH group and the singlet at  $\delta$  2.7 is observed for  $\text{CH}_3$  group in  $\text{S-CH}_3$ . In  $^{13}\text{C-NMR}$  spectrum the signals at  $\delta$  180.4 (C-2) and  $\delta$  162.4 (C-4) appears for both C=S and C=O groups respectively. The aromatic carbons are designated by the following values)  $\delta$  140.8 (C-6),  $\delta$  128.7 (C-7),  $\delta$  128.0 (C-8),  $\delta$  127.1 (C-9). The value at 77.9 (C-5) appears for the aliphatic carbon with various substituted groups. The signal at  $\delta$  26.9 (C-10) is concerned for simple aliphatic carbon  $\text{CH}_3$ . In the MS spectrum the molecular ion peak ( $M^+2\%$ ) appears at  $m/z$  282 that corresponding to the molecular formula  $C_{16}H_{14}N_2OS$ . In this spectrum the base peak is formed at  $m/z$  154.

## Cytotoxicity

Cytotoxicity of all the compounds was measured by brine shrimp lethality bioassay method. Cisplatin, a recognized anti-cancer drug was used as reference to compare the efficacy of the synthesized compounds. Compounds **1a**, **1b**, **1c**, **2a**, **2b**, **2c**, **2d** and **2** showed potential cytotoxicity but compounds **1b**, **1c**, **2b**, **2c** and **2** that showed highly cytotoxic activity and compounds **1a**, **2a**, **2d** showed relatively low cytotoxic activity. The LC<sub>50</sub> values of the titled compounds are represented in the Figure 1.

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