ABSTRACT

A series of 5,7-dibromoisatin semicarbazones have been synthesized in good yield, involving aryl urea and aryl semicarbazide formation. The structures of the synthesized compounds were confirmed on the basis of their spectral data. All the compounds were evaluated for anticonvulsant and CNS depressant activities. Anticonvulsant activity was determined after intraperitoneal (i.p.) administration to mice by maximal electroshock (MES) induced seizure method and minimal motor impairment was determined by rotarod test. A computational study was carried out for prediction of pharmacokinetic properties and making them potentially promising agents for the treatment of epilepsy. Compounds (Z)-1-(5,7-dibromo-2-oxoindolin-3-ylidene)-4-(4-chlorophenyl)semicarbazide (DH-05), (Z)-1-(5,7-dibromo-2-oxoindolin-3-ylidene)-4-(3-chloro-4-fluorophenyl)semicarbazide (DH-11) and (Z)-1-(5,7-dibromo-1-methyl-2-oxoindolin-3-ylidene)-4-(3-chloro-4-fluorophenyl)semicarbazide (DH-12) exhibited prominent anticonvulsant effect in the series with little or no neurotoxicity and little CNS depressant effect as compared to standard drug.

Keywords: Dibromoisatin, anticonvulsant, maximal electroshock, antidepressant activity

INTRODUCTION

Epilepsy is a disease of complex nature and of different etiology with huge patient load of varying age groups involving both sexes (Verma et al., 2004). On an average, 0.25 million new cases of epilepsy are reported every year. Mainly two kinds of epilepsy, namely grand mal and petit mal are prevalent (Siddiqui et al., 2007). Available drugs are effective in only 60 – 80 % of epileptic patients.

During the past decade, several new drugs have been approved to be used as antiepileptic drugs (Rufinamide, Retigabine, Pregabiline, Remacemide, etc.) (Yogeeswari et al., 2005). Despite the optimal use of available antiepileptic drugs (AEDs), many patients fail to experience seizure control and others do so only at the expense of significant toxic effects. It is estimated that available medication controls seizures in only 50 % of patients or decrease incidence in only 75 % of patients (Agarwal et al., 2004). Anticonvulsant drugs, showing activity with MES (maximal electroshock) test are generally useful in grand mal (Verma et al., 2004).

Literature survey showed that isatin molecule has positive anticonvulsant effects during its initial screening in the MES test.
(Pandeya et al., 2002; Julian et al., 1952; Popp 1984; Jursic et al., 2002; Hewawasam et al., 2002). Substitution of hydrogen (from –NH in indole nucleus) with methyl group enhanced the lipophilicity of the compounds (Smitha et al., 2008). In recent times, semicarbazones are emerging as novel anticonvulsant drugs. More than 300 compounds have been prepared and tested in animal models. Representative semicarbazones, like 4-bromobenzaldehyde semicarbazone, have shown to have activity comparable with or even exceeding that of phenytoin (Dilantin) in the maximal electroshock (MES)-induced seizure test in mice (Siddiqui et al., 2007). Aryl semicarbazones displaying anticonvulsant activity in the MES screen interact at a specific binding site referred as the hydrogen bonding area and aryl binding site respectively (Dimmock et al., 1995). Terminal amino group of aryl semicarbazones was shown to affect the binding properties at the hydrogen bonding area (Dimmock et al., 1999; Pandeya et al., 1998). However, Pandeya et al. have suggested a new pharmacophore model for semicarbazones displaying anticonvulsant activity (Figure 1). They proposed that terminal amino function of semicarbazones could be substituted with a lipophilic substituted aryl ring (Jain et al., 2010). Proposed pharmacophore model contains four binding sites for interaction with a macromolecular complex in vivo.

1. An aryl hydrophobic binding site (A) with halogen substituent preferably at para position
2. A hydrogen bonding domain (HBD)
3. An electron donor group (D)
4. Another hydrophobic-hydrophilic site controlling the pharmacokinetic properties of the anticonvulsant (C)

These new aspects might be useful for designing prototypic molecules with potential anticonvulsant activity (Figure 2). These observations encouraged us to synthesize a series of 5,7-dibromoisatin semicarbazones which were evaluated their anticonvulsant and toxicological profiles.

CHEMISTRY

The synthesis of 5,7-dibromoisatin semicarbazones DH-01–DH-14 was achieved as depicted in Scheme 1. Substituted aniline was treated with sodium cyanate in the presence of glacial acetic acid according to the known urea preparation method, to yield aryl urea. Then aryl urea on condensation with hydrazine hydrate in ethanol in the presence of sodium hydroxide gave the aryl semicarbazide. The 5,7-dibromoisatin semicarbazones DH-01–DH-14 were prepared by reaction of the appropriate 5,7-dibromoisatin or N-methyl 5,7-dibromoisatin with aryl semicarbazide in presence of anhydrous sodium acetate. The synthesized products showed a single spot on TLC and had IR and 1H-NMR spectra in accordance with their anticipated structures. In general, IR spectra showed the C = N peak at 1610 – 1598 cm–1 and the NH stretching vibrations at 3424 – 3315 cm –1. The 1H-NMR spectrum revealed that the CO–NH proton at 12.19 – 11.25 and the aryl NH proton that showed a singlet at 9.99 – 9.72 were D2O exchangeable. The physical characterization data of the synthesized compounds are given in Table 1.
Figure 2: Structure of proposed general pharmacophore model of the synthesized compound and reported chemical drugs

Table 1: Physico-chemical parameters data of the newly synthesized derivatives

| Code-No | R₁ | R₂ | R₃ | R₄ | Mol. Formula | Mol. Wt | R | % yield | M.P. (°C) | Log P |
|---------|----|----|----|----|--------------|---------|---|---------|----------|-------|
| DH-01   | -  | -  | -  | H  | C₉H₁₈N₂O₂Br₂ | 362     | 0.31| 54     | 253 - 256 | 1.36  |
| DH-02   | -  | -  | -  | CH₃| C₁₀H₁₈N₂O₂Br₂ | 376     | 0.43| 42     | 212 - 214 | 1.59  |
| DH-03   | H  | H  | H  | H  | C₁₁H₂₀N₂O₂Br₂ | 409     | 0.44| 63     | 224 - 226 | 3.25  |
| DH-04   | H  | H  | H  | CH₃| C₁₁H₂₁N₂O₂Br₂ | 423     | 0.60| 66     | 240 - 243 | 3.49  |
| DH-05   | Cl | H  | H  | H  | C₁₁H₂₀N₂O₂Br₂Cl| 443.5  | 0.39| 50     | 177 - 179 | 3.81  |
| DH-06   | Cl | H  | H  | CH₃| C₁₁H₂₁N₂O₂Br₂Cl| 457.5  | 0.45| 44     | 287 - 289 | 4.05  |
| DH-07   | Br | H  | H  | H  | C₁₁H₂₀N₂O₂Br₂ | 488     | 0.36| 63     | 188 - 190 | 4.08  |
| DH-08   | Br | H  | H  | CH₃| C₁₁H₂₁N₂O₂Br₂ | 502     | 0.45| 65     | 273 - 275 | 4.32  |
| Dh-09   | H  | H  | Cl | H  | C₁₁H₂₀N₂O₂Br₂Cl| 443.5  | 0.41| 57     | 180 - 182 | 3.81  |
| DH-10   | Cl | H  | Cl | CH₃| C₁₁H₂₀N₂O₂Br₂Cl| 257.5  | 0.48| 51     | 225 - 227 | 4.05  |
| DH-11   | F  | Cl | H  | H  | C₁₁H₂₀N₂O₂Br₂FCl| 461.5  | 0.39| 41     | 156 - 158 | 3.97  |
| DH-12   | F  | Cl | H  | CH₃| C₁₁H₂₁N₂O₂Br₂FCl| 475.5  | 0.47| 39     | 266 - 268 | 4.21  |
| DH-13   | CH₃| H  | H  | H  | C₁₁H₂₁N₂O₂Br₂ | 423     | 0.55| 45     | 220 - 222 | 3.74  |
| DH-14   | CH₃| H  | H  | CH₃| C₁₁H₂₁N₂O₂Br₂ | 437     | 0.47| 48     | 214 - 216 | 3.98  |
PHARMACOLOGY

**Anticonvulsant screening**

The anticonvulsant evaluation of the synthesized compounds was performed using reported procedure (Krall et al., 1978). Male albino mice (CF-1 strain, 18 – 25 g) were used as experimental animals. The synthesized compounds were suspended in polyethylene glycol (PEG- 400). All experimental protocols were carried out with permission from the Institutional Animal Ethics Committee (IAEC). Animals were obtained from the Central Animal House Facility, DR. K. N. Modi Institute of Pharmaceutical Education and Research, Modinagar, Ghaziabad, Uttar Pradesh, India.

**Electroshock seizure method**

Initial anticonvulsant evaluation of test compounds **DH-01–DH-14** were under taken by maximal electroshock seizure (MES) test following the method of Krall et al. (1978). Maximal seizures were induced by the application of electrical current to the brain via corneal electrodes. For all tests based on MES convulsions, 60 Hz of alternating current (50 mA) was delivered for 0.2 s by corneal electrodes which had been primed with an electrolyte solution containing an anaesthetic agent (0.5 % tetracaine HCl). Abolition of the hind limb tonic extensor spasm was recorded as a measurement of anticonvulsant activity.
Rotarod motor impairment screening

Motor impairment is measured in mice by the rotarod test (Dunham et al., 1957). The mice were trained to stay on an accelerating rotarod (INCO, Ambala, India) that rotated at six revolutions per minute. The rod diameter was 3.2 cm. Motor impairment was indicated by the inability of the animal to maintain equilibrium on the rod for at least 1 min in each of the trials.

CNS depression study

The forced swim method (Porsolt’s swim pool test) (Porsolt et al., 1978) was followed to study the CNS depression. Mice were placed in a chamber (diameter 45 cm, height 20 cm) containing water up to a height of 15 cm at 25 ± 2 °C. Two swim sessions were conducted, an initial 15 min pre-test, followed by a 5 min test session 24 h later. The animals were administered an i.p. injection (100 mg/kg) of the test compounds 30 min before the test session. The period of immobility (passive floating without struggling, making only those movements which are necessary to keep its head above the surface of water) during the 5 min test period were measured.

RESULTS AND DISCUSSION

Anticonvulsant and CNS depressant activities

Synthesized 5,7-dibromoisatin semicarbazone derivatives were screened for their anticonvulsant activity by using standard model MES (maximal electroshock seizure test) for their ability to reduce seizure spread (Table 2). Motor impairment screening of the synthesized compounds was also carried out by rotarod test method and CNS depressant effect of the compounds was determined by porsolt’s force swim pool method. The anticonvulsant activity was tested after 0.5 and 4.0 h time intervals at dose levels of 30, 100, 300 mg/kg body weight. The CNS depressant activity was studied at a dose level of 100 mg/kg body weight. Phenytoin and Carbamazepine were used as standard drugs.

In this series (DH-01–DH-14), compounds DH-03, DH-06 and DH-08 showed protection against induced seizures at maximum dose (300 mg/kg) after 4.0 h. Compound DH-05 showed protection at a dose of 100 mg/kg at 4.0 h and compounds DH-11 and DH-12 showed the activity at the dose of 30 mg/kg at 0.5 h. The compounds DH-02, and DH-04 showed protection at a dose of 100 mg/kg at 4.0 h and compound DH-09 showed protection at a dose of 100 mg/kg at 0.5 h. Compounds DH-11 and DH-12 having R₁ = Flouro, R₂ = Chloro, R₄ = H, and R₁ = Flouro, R₂ = Chloro, R₄ = CH₃ groups respectively were found most active in the series showing activity at 0.5 h at lower dose of 30 mg/kg. In the rotarod motor impairment screening, all compounds except DH-03, DH-05, DH-06 and DH-08, showed motor impairment at the maximum dose of 300 mg/kg.

COMPUTATIONAL STUDY

Calculation of physicochemical parameters

A computational study of synthesized compounds (DH-01–DH-14) was performed for prediction of ADME properties. Polar surface area (TPSA) (Ertl et al., 2000), number of rotatable bonds, molecular volume, number of hydrogen donor and acceptor atoms and violations of Lipinski’s rule of five (Lipinski et al., 2001) were calculated using Molinspiration online property calculation toolkit (Molinspiration Cheminformatics, 2013). Absorption (%ABS) was calculated by: %ABS=109 - (0.345 × TPSA) (Zhao et al., 2002). The theoretical toxicity, druglikeness and drug-score properties were calculated in the Osisris Property Explorer (http://www.organic-chemistry.org).
Table 2: Anticonvulsant and motor impairment screening of synthesized compounds (DH-01-DH-14) using maximal electroshock seizure (MES) Model and Rotarod Model

| Compound Code | MES<sup>a</sup> | Motor Impt.<sup>a</sup> |
|---------------|-----------------|---------------------|
|               | 0.5h 4.0h       | 0.5h 4.0h           |
| DH-01         | - - 300         | - - -              |
| DH-02         | - 100 300       | - - -              |
| DH-03         | - 300 -         | - - -              |
| DH-04         | - 100 -         | - - -              |
| DH-05         | 100 - -         | - - -              |
| DH-06         | 300 - -         | - - -              |
| DH-07         | - - 300         | - - -              |
| DH-08         | - 300 -         | - - -              |
| DH-09         | 100 - -         | - - -              |
| DH-10         | - - 100 -       | - - -              |
| DH-11         | 30 300 -        | - - -              |
| DH-12         | 30 300 -        | - - -              |
| DH-13         | - 100 -         | - - -              |
| DH-14         | - 100 -         | - - -              |
| Control       | - - - 100       | - - -              |
| Phenytoin     | 30 30 100 100   | - - -              |
| Carbamazepine | 30 100 300 -    | - - -              |

<sup>a</sup>Doses of 30, 100, and 300 mg/kg were administered to mice through intraperitoneal route. The figures in the table indicate the minimum dose whereby bioactivity was demonstrated in half or more of mice. The animals were examined 0.5 and 4h after the drug administration. The dash (-) indicates an absence of activity at maximum dose administered (300 mg/kg). Data for Phenytoin and Carbamazepine, used as standard drugs, were obtained referring, Dimmock, J.R., et al 1995 & White, H.S., et al 1995.

Some selected compounds i.e. DH-05, DH-11 and DH-12 having significant anticonvulsant activity were also tested for their CNS depressant effect. These compounds with respect to control, showed 29.05, 51.24 and 32.95 % increase in immobility time respectively. The standard drug carbamazepine showed 58.63 % increase in the immobility time (Table 3). Percentage CNS depression by DH-11 was in the vicinity of carbamazepine while DH-05 and DH-11 fell far behind.

DH-05, with p-chloro in its structure was found to be the most active compound of this study. It was effective in 4.0 h time interval against induced seizure at lower dose of 100 mg/kg, showing no motor impairment effect in comparison to standard drug Carbamazepine. The activity may be due to the presence of chlorine at para position which is well fitted to receptor site though, it showed reduced CNS depressant effect in comparison to standard drug, carbamazepine.

Using the putative binding site theory proposed by Dimmock et al. (2000a, b) subsequently used by others (Pandey et al., 2002; Gibson et al., 2009) in postulating the interaction of anticonvulsant compounds at a specific binding site, the molecule observed to interact with the protein receptor as shown in Figure 3.

![Figure 3: Proposed binding interactions of the title compounds using putative binding site theory (Dimmock et al., 2000a, b; Pandey et al., 2002; Gibson et al., 2009).](image)

Table 3: Data of CNS depressant activity of the selected compounds performed in mice using forced swim test

| Compounds | Duration of Immobility (sec) (mean ± SEM) | % Increased of Immobility |
|-----------|------------------------------------------|---------------------------|
| DH-05     | 68.45 ± 5.28                             | 29.05                     |
| DH-11     | 80.22 ± 2.66                             | 51.24                     |
| DH-12     | 70.52 ± 2.45                             | 32.95                     |
| Carbamazepine | 84.14 ± 1.33               | 58.63                     |
| Control   | 53.04 ± 2.47                             | -                         |

Tested at 100 mg/kg (i.p. in PEG 400); control animals were administered PEG 400 (i.p.). Each value represents the mean ± SEM of six mice. The CNS depressant effect was compared with respect to standard drug. *p < 0.0001. Data was analyzed by unpaired student’s t test.
**Prediction of ADME properties**

A computational study for prediction of ADME properties of synthesized compounds was performed. Topological polar surface area (TPSA), i.e., surface belonging to polar atoms, is a descriptor that was shown to correlate well with passive molecular transport through membranes and, therefore, allows prediction of transport properties of drugs in the intestines and blood-brain barrier crossing (Ertl et al., 2000). The percentage of absorption (%ABS) was calculated using TPSA. From all these parameters, it can be observed that all synthesized compounds exhibited a great %ABS ranging from 74.38 to 82.955% (Table 4). Furthermore, compounds DH-01, DH-07 and DH-08, DH-12 violated only one and two Lipinski’s parameter respectively. None of the other compounds violated Lipinski’s parameters, making them potentially promising agents for epilepsy therapy. Although druglikeness of DH-11 and DH-12 was not comparable to standard drug but DH-05 was shown more druglikeness when compared to the standard drug. Drug scores of DH-11 and DH-12 were low, but for DH-05, it lies in the close proximity with phenatoin. Using oris programe, theoretical toxicity profile (Mutagenic, tumorigenic, irritant and reproductive effective) of the all three compounds DH-05, DH-11 and DH-12 were analysed and found to be equal to that of the standard drug Phenatoin (Figure 4).

**CONCLUSION**

In conclusion, the present study indicated that the synthesized compounds have marked anticonvulsant activity as indicated by the protection against maximal electroshock induced seizures in comparison with standard drug (Phenytoin) with little or no neurotoxicity. Further, the study highlights the importance of the structural features of a compound responsible for the anticonvulsant activity. With semicarbazone structure as a template, many modifications can still be carried out and there is ample scope for further exploration which will lead to a systematic structure-activity relationship.

![Figure 4: Graphical representation of Druglikeness and drugscore values of compounds DH-05, DH-11, DH-12 and Phenytoin](image)
Table 4: Pharmacokinetic parameters important for good oral bioavailability of compounds.

| Compound | % ABS | TPSA (Å²) | n-ROTB | MW  | MV  | n-OHNH donors | n-ON acceptors | Lipinski’s violations |
|----------|-------|-----------|--------|-----|-----|---------------|-----------------|----------------------|
| Rule     | -     | -         | -      | <500| -   | <5            | <10             | <1                   |
| DH-1     | 74.380| 100.347   | 1      | 361.981 | 206.293 | 4             | 6               | 1                    |
| DH-2     | 78.125| 89.49     | 1      | 376.008 | 223.236 | 3             | 6               | 0                    |
| DH-3     | 79.209| 86.351    | 2      | 438.079 | 278.816 | 3             | 6               | 0                    |
| DH-4     | 82.955| 75.494    | 2      | 452.106 | 295.758 | 2             | 6               | 0                    |
| DH-5     | 79.209| 86.351    | 2      | 472.524 | 292.352 | 3             | 6               | 0                    |
| DH-6     | 82.955| 75.494    | 2      | 486.551 | 309.294 | 2             | 6               | 0                    |
| DH-7     | 79.209| 86.351    | 2      | 516.975 | 296.701 | 3             | 6               | 1                    |
| DH-8     | 82.955| 75.494    | 2      | 531.002 | 313.644 | 2             | 6               | 2                    |
| DH-9     | 79.209| 86.351    | 2      | 472.524 | 292.352 | 3             | 6               | 0                    |
| DH-10    | 82.955| 75.494    | 2      | 486.551 | 309.294 | 2             | 6               | 0                    |
| DH-11    | 79.209| 86.351    | 2      | 490.514 | 297.283 | 3             | 6               | 0                    |
| DH-12    | 82.955| 75.494    | 2      | 504.541 | 314.22  | 2             | 6               | 2                    |
| DH-13    | 79.209| 86.351    | 2      | 452.106 | 295.377 | 3             | 6               | 0                    |
| DH-14    | 82.955| 75.494    | 2      | 466.133 | 312.319 | 2             | 6               | 0                    |

%ABS, percentage of absorption; TPSA, topological polar surface area; n-ROTB, number of rotatable bonds; MW, molecular weight; MV, molecular volume; n-OHNH, number of hydrogen bond donors; n-ON, number of hydrogen bond acceptors

EXPERIMENTAL PROTOCOL

Chemistry

Raw materials for the synthesis were purchased from Merck (India), Spectrochem chemicals (India) and S.D. Fine Chemicals (India). The progress of reaction was monitored by thin layer chromatography, performed on Silica gel-GF coated aluminium plate using iodine vapors and UV light as visualizing agents. Evaporation of solvents was performed at reduced pressure, using a Buchi rotary evaporator. Solid products were purified by recrystallization and column chromatography. Melting points of newly synthesized compounds were determined in open glass capillary with the help of digital melting point apparatus and are uncorrected. FT-IR (KBr) spectra were recorded on a Nicolet 5PC FTIR spectrophotometer (λ-max in cm⁻¹). ¹H NMR spectra was recorded on a Brucker Model-300 NMR Spectrometer in DMSO-d⁶ using tetramethylsilane (TMS) as the internal reference (chemical shifts in δ ppm). Chemical shifts are reported in ppm downfield from tetramethylsilane (TMS).

Synthesis of 5,7-dibromoisatin

The synthesis of 5,7-Dibromoisatin was based on the method of Lindwall. Isatin (5.0 g, 34 mmol, 1 equiv) was warmed in ethanol (95 %, 100 mL) with stirring until it dissolved. Bromine (16.3 g, 102 mmol, 5.2 mL, 3.0 equiv) was added dropwise to the stirred isatin solution whilst maintaining the temperature of the reaction mixture between 70 to 75 °C. The solution was cooled to room temperature and placed on ice for 30 min. The resulting precipitate was washed with water and cold ethanol and then recrystallized from ethanol to yield bright orange-red crystals of 5,7-dibromoisatin (60 %), m.p. 251–254 °C (Vine et al., 2007, 248–250 °C).

Synthesis of N-methyl 5,7-dibromoisatin

To a suspension of (0.1 mol) of dibromoisatin, in 200 ml of anhydrous methanol, 100 mL of 10 % methanolic potassium hydroxide solution was added in portions with stirring for 30 min. To this mixture, 15 mL of dimethyl sulfate was added and after 1 h, the solution was filtered to remove potassium methyl sulfate. After removal of about
250 mL of solvent under reduced pressure, 40 mL of warm water was added to the obtained residue. On cooling, orange precipitate occurred which was filtered and dried to obtain N-Methyl 5,7-Dibromoisoatin (Gupta et al., 2010).

**Synthesis of substituted aryl semicarbazides**

Substituted aryl ureas were prepared according to the prescribed procedure (Furniss et al., 1998). The substituted phenyl semicarbazides (H, 2-Cl, 4-Cl, 4-Br, and 4-CH$_3$ etc.) were synthesized according to earlier reported methods.

Phenyl semicarbazide (Pandeya et al., 1999)

Yield: 62 % m. p. 245°C. (lit. 247°C);
N-(2-Chlorophenyl)hydrazinecarboxamide (o-Chlorophenyl semicarbazide) (Pandeya et al., 1999)

Yield : 65 %, m. p. 176°C (lit. 179°C);
N-(4-Chlorophenyl)hydrazinecarboxamide (p-Chlorophenyl semicarbazide) (Pandeya et al., 1999)

Yield : 70 %, m. p. 235°C (lit. 234°C);
N-(4-Bromophenyl)hydrazinecarboxamide (p-Bromophenyl semicarbazide) (Pandeya et al., 2000)

Yield: 40 %, m. p. 267°C (lit. 270°C);
3-Chloro-4-Flourophenyl semicarbazide (Amir et al., 2010)

Yield: 60 %, m. p. 94°C (lit. m.p 90-92°C.)
4-p-tolylsemicarbazide (p-Methylphenyl semicarbazide) (Wheeler et al., 1924.)

Yield: 61 %, m. p. decomposing at 272°C (lit. m.p decomposing at 274°C.)

**General procedure for synthesis of substituted dibromoisoatin semicarbazones**

Dibromoisoatin semicarbazones DH-01 to DH-14 were synthesized according to the procedure specified in literature with some modifications (Amir et al., 2010) as shown in synthetic Scheme 1. To a solution of substituted semicarbazide (0.0025 mole) in 0.5 mL conc. HCl and 12.5 mL water, sodium acetate (0.0025 mole, 0.205g) in 1 mL water g was added to clear the turbidity. This solution mixture was added to an equimolar quantity of the appropriate dibromoisoatin or N-methyl dibromoisoatin in alcohol. The solution was stirred for 15 min. precipitation occurred immediately (if immediate precipitation did not occur, reaction mixture would be allowed to stand for 1-2 h), and the solids were filtered, dried and purified by recrystallization from ethanol. The homogeneity of the compounds was checked by TLC using n-Hexane + ethyl acetate (1:1) as mobile phase.

(Z)-1-(5,7-dibromo-2-oxindolin-3-ylidene)semicarbazide DH-01

IR (KBr): $\nu_{max} = 3267$ (NH), 1720 (C=O), 1610 (C=N), 1021 (C-Br) cm$^{-1}$. 1H NMR (300 MHZ, DMSO-d$_6$) $\delta = 7.33$ (brs, 2 H, =N-NH), 11.56 ppm. Anal. calcd for C$_9$H$_6$Br$_2$N$_4$O$_2$ (359.8858): C 29.88, H 1.86, N 15.62; found C 29.94, H 1.70, N 15.70.

(Z)-1-(5,7-dibromo-1-methyl-2-oxindolin-3-ylidene)semicarbazide DH-02

IR (KBr): $\nu_{max} = 3251$ (NH), 1725 (C=O), 1598 (C=N), 1025 (C-Br) cm$^{-1}$. 1H NMR (300 MHZ, DMSO-d$_6$) $\delta = 2.50$ (s, 3 H, N-CH$_3$), 7.33 (brs, 2 H, CONH), 7.62-7.85 (m, 2H, ArH), 11.56 ppm. Anal. calcd for C$_{10}$H$_8$Br$_2$N$_4$O$_2$ (373.9014): C 31.90, H 2.10, N 14.86; found C 31.94, H 1.70, N 14.98.

(Z)-1-(5,7-dibromo-2-oxindolin-3-ylidene)-4-phenylsemicarbazide DH-03

IR (KBr): $\nu_{max} = 3415$ (NH), 1730 (C=O), 1670 (C=O amide), 1601 (C=N), 1015 (C-Br) cm$^{-1}$. 1H NMR (300 MHZ, DMSO-d$_6$) $\delta = 7.25-7.53$ (m, 5 H, ArH), 7.73-7.84 (m, 2 H, ArH), 9.72 ppm. Anal. calcd for C$_{15}$H$_{10}$Br$_2$N$_4$O$_2$ (435.9171): C 41.10, H 21.90, N 14.86; found C 41.05, H 21.96, N 14.87.
(Z)-1-(5,7-dibromo-1-methyl-2-oxoindolin-3-ylidene)-4-phenylsemicarbazide **DH-04**

IR (KBr): $v_{\text{max}} = 3410$ (NH), 1732 (C=O), 1675 (C=O amide), 1601 (C=N), 1012 (C-Br) cm$^{-1}$.

$^1$H NMR (300 MHz, DMSO-$d_6$) $\delta = 2.58$ (s, 3 H, N-CH$_3$), 7.23-7.63 (m, 5 H, ArH), 7.72-7.81 (m, 2 H, ArH), 9.78 (brs, 1 H, ArNH), 12.10 (s, 1 H, CONH) ppm. Anal. calcd for C$_{16}$H$_{12}$Br$_2$N$_4$O$_2$ (452.1001): C 42.45, H 2.60, N 12.38; found C 42.48, H 2.55, N 12.42.

(Z)-1-(5,7-dibromo-2-oxoindolin-3-ylidene)-4-(4-chlorophenyl)semicarbazide **DH-05**

IR (KBr): $v_{\text{max}} = 3424$ (NH), 1738 (C=O), 1657 (C=O amide), 1607 (C=N), 1018 (C-Br) cm$^{-1}$.

$^1$H NMR (300 MHz, DMSO-$d_6$) $\delta = 7.15$ (d, $J = 8.1$ Hz, 2 H, ArH), 7.75 (d, $J = 8.1$ Hz, 2 H, ArH), 7.76-7.78 (m, 2 H, ArH), 9.76 (brs, 1 H, ArNH), 11.62 (brs, 1 H, NH), 12.03 (s, 1 H, CONH) ppm. Anal. calcd for C$_{15}$H$_{9}$Br$_2$ClN$_4$O$_2$ (469.8781): C 38.16, H 1.90, N 11.80; found C 31.20, H 1.88, N 11.84.

(Z)-1-(5,7-dibromo-1-methyl-2-oxoindolin-3-ylidene)-4-(4-chlorophenyl)semicarbazide **DH-06**

IR (KBr): $v_{\text{max}} = 3343$ (NH), 1730 (C=O), 1652 (C=O amide), 1609 (C=N), 1017 (C-Br) cm$^{-1}$.

$^1$H NMR (300 MHz, DMSO-$d_6$) $\delta = 2.51$ (s, 3 H, N-CH$_3$), 7.40 (d, $J = 6.9$ Hz, 2 H, ArH), 7.80 (d, $J = 14.1$ Hz, 2 H, ArH), 8.06-8.40 (m, 2 H, ArH), 9.75 (brs, 1 H, ArNH), 11.25 (s, 1 H, CONH) ppm. Anal. calcd for C$_{16}$H$_{11}$Br$_2$ClN$_4$O$_2$ (469.8781): C 38.16, H 1.90, N 11.80; found C 31.20, H 1.88, N 11.84.

(Z)-1-(5,7-dibromo-2-oxoindolin-3-ylidene)-4-(4-bromophenyl)semicarbazide **DH-07**

IR (KBr): $v_{\text{max}} = 3324$ (NH), 1740 (C=O), 1648 (C=O amide), 1601 (C=N), 1019 (C-Br) cm$^{-1}$.

$^1$H NMR (300 MHz, DMSO-$d_6$) $\delta = 7.25$ (d, $J = 8.5$ Hz, 2 H, ArH), 7.78 (d, $J = 8.5$ Hz, 2 H, ArH), 7.66-7.79 (m, 2 H, ArH), 9.72 (brs, 1 H, ArNH), 11.70 (brs, 1 H, NH), 12.10 (s, 1 H, CONH) ppm. Anal. calcd for C$_{15}$H$_{11}$Br$_2$N$_4$O$_2$ (513.8276): C 34.80, H 1.70, N 10.80; found C 34.88, H 1.80, N 10.85.

(Z)-1-(5,7-dibromo-1-methyl-2-oxoindolin-3-ylidene)-4-(4-bromophenyl)semicarbazide **DH-08**

IR (KBr): $v_{\text{max}} = 3348$ (NH), 1739 (C=O), 1652 (C=O amide), 1609 (C=N), 1017 (C-Br) cm$^{-1}$.

$^1$H NMR (300 MHz, DMSO-$d_6$) $\delta = 2.56$ (s, 3 H, N-CH$_3$), 7.50 (d, $J = 6.7$ Hz, 2 H, ArH), 7.86 (d, $J = 14.5$ Hz, 2 H, ArH), 8.09-8.42 (m, 2 H, ArH), 9.72 (brs, 1 H, ArNH), 11.65 (s, 1 H, CONH) ppm. Anal. calcd for C$_{15}$H$_{11}$Br$_2$N$_4$O$_2$ (527.8432): C 36.15, H 2.10, N 10.55; found C 36.20, H 2.05, N 10.60.

(Z)-1-(5,7-dibromo-2-oxoindolin-3-ylidene)-4-(2-chlorophenyl)semicarbazide **DH-09**

IR (KBr): $v_{\text{max}} = 3438$ (NH), 1739 (C=O), 1611 (C=N), 1022 (C-Br) cm$^{-1}$.

$^1$H NMR (300 MHz, DMSO-$d_6$) $\delta = 7.51-7.63$ (m, 4 H, ArH), 7.87 (m, 2H, ArH), 9.96 (s, 1 H, ArNH), 11.63 (brs, 1H, NH), 12.09 (s, 1 H, CONH) ppm. Anal. calcd for C$_{15}$H$_{9}$Br$_2$ClN$_4$O$_2$ (469.8781): C 38.10, H 1.90, N 11.80; found C 38.15, H 1.88, N 11.84.

(Z)-1-(5,7-dibromo-1-methyl-2-oxoindolin-3-ylidene)-4-(2-chlorophenyl)semicarbazide **DH-10**

IR (KBr): $v_{\text{max}} = 3416$ (NH), 1729 (C=O), 1601 (C=N), 1021 (C-Br) cm$^{-1}$.

$^1$H NMR (300 MHz, DMSO-$d_6$) $\delta = 2.48$ (s, 3 H, 2.20, N 12.70; found C 41.20, H 2.05, N 12.75.
N-CH₃), 7.31-7.53 (m, 4 H, ArH), 7.76 (m, 2 H, ArH), 9.92 (s, 1 H, ArNH), 12.19 (s, 1 H, CONH) ppm. Anal. calcd for C₁₆H₁₁Br₂ClN₄O₂ (483.8937): C 39.45, H 2.20, N 11.45; found C 39.48, H 2.18, N 11.42.

(Z)-1-(5,7-dibromo-2-oxoindolin-3-ylidene)-4-(3-chloro-4-fluorophenyl)semicarbazide DH-11
IR (KBr): νmax = 3235 (NH), 1728 (C=O), 1607 (C=N), 1021 (C-Br) cm⁻¹. ¹H NMR (300 MHz, DMSO-d₆) δ = 2.51 (s, 3 H, N-CH₃), 7.27-7.45 (m, 3 H, ArH), 7.86 (m, 2 H, ArH), 9.99 (brs, 1 H, ArNH), 12.06 (s, 1 H, CONH) ppm. Anal. calcd for C₁₅H₈Br₂ClFN₄O₂ (487.8687): C 36.70, H 1.60, N 11.40; found C 36.80, H 1.55, N 11.45.

(Z)-1-(5,7-dibromo-1-methyl-2-oxoindolin-3-ylidene)-4-(3-chloro-4-fluorophenyl)semicarbazide DH-12
IR (KBr): νmax = 3233 (NH), 1727 (C=O), 1604 (C=N), 1019 (C-Br) cm⁻¹. ¹H NMR (300 MHz, DMSO-d₆) δ = 2.49 (s, 3 H, N-CH₃), 7.25-7.46 (m, 3 H, ArH), 7.82 (m, 2 H, ArH), 9.95 (brs, 1 H, ArNH), 12.11 (s, 1 H, CONH) ppm. Anal. calcd for C₁₆H₁₀Br₂ClFN₄O₂ (501.8843): C 38.10, H 1.98, N 11.02; found C 38.15, H 1.92, N 11.08.

(Z)-1-(5,7-dibromo-2-oxoindolin-3-ylidene)-4-p-tolylsemicarbazide DH-13
IR (KBr): νmax = 3336 (NH), 1734 (C=O), 1654 (C=O amide), 1598 (C=O amide), 1013 (C-Br) cm⁻¹. ¹H NMR (300 MHz, DMSO-d₆) δ = 2.74 (s, 3 H, ArCH₃), 7.23 (d, J = 7.4 Hz, 2 H, ArH), 7.65 (d, J = 6.4 Hz, 2 H, ArH), 7.73-7.75 (m, 2 H, ArH), 9.85 (brs, 1 H, ArNH), 11.58 (brs, 1 H, NH), 12.14 (s, 1 H, CONH) ppm. Anal. calcd for C₁₆H₁₂Br₂N₄O₂ (449.9327): C 42.45, H 2.60, N 12.30; found C 42.48, H 2.58, N 12.35.

(Z)-1-(5,7-dibromo-1-methyl-2-oxoindolin-3-ylidene)-4-p-tolylsemicarbazide DH-14
IR (KBr): νmax = 3315 (NH), 1737 (C=O), 1656 (C=O amide), 1602 (C=N), 1021 (C-Br) cm⁻¹. ¹H NMR (300 MHz, DMSO-d₆) δ = 2.53 (s, 3 H, N-CH₃), 2.70 (s, 3 H, ArCH₃), 7.45 (d, J = 7.1 Hz, 2 H, ArH), 7.68 (d, J = 6.1 Hz, 2 H, ArH), 7.72-7.76 (m, 2 H, ArH), 9.72 (brs, 1 H, ArNH), 12.08 (s, 1 H, CONH) ppm. Anal. calcd for C₁₇H₁₄Br₂N₄O₂ (463.9484): C 43.75, H 3.00, N 12.00; found C 43.80, H 2.98, N 12.21.

ACKNOWLEDGEMENTS
Authors are grateful to the Director, Dr. K. N. Modi Institute of Pharmaceutical Education and Research, Modinagar, Ghaziabad, Uttar Pradesh, for providing laboratory facilities. Authors are also thankful to Mr. Amar Deep Singh, Dr. K. N. Modi Institute of Pharmaceutical Education and Research, Modinagar, Ghaziabad, Uttar Pradesh, India for their cordial assistance in performing biological activities.

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