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Research Article

Synthesis of novel 5-substituted isatin derivatives via Suzuki-Miyaura coupling reaction: Cytotoxic evaluation and in-silico studies

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ABSTRACT

A number of 5-substituted isatin derivatives (**2–6**) were synthesized from *N*-benzylisatin (**1**) by multistep organic reaction. Bromination of isatin followed by Suzuki-Miyaura coupling reaction with commercially available organoboron reagents afforded the compounds **2–6** with moderate yield. All the compounds were characterized using different spectroscopic techniques and their cytotoxicity was studied by brine shrimp lethality bioassay. Among these compounds, **2, 3, 5**, and **6** showed moderate cytotoxicity with LC_{50} values 54.0, 76.5, 58.3, and 76.3 μg/mL, respectively, whereas compound **4** showed significant cytotoxicity with an LC_{50} value of 33.2 μg/mL. Structural modifications significantly affected electronic energy, dipole moment, and charge distribution. Compound **6** showed the highest dipole moment, suggesting greater polarity and potential intermolecular interaction capability. Frontier molecular orbital analysis identified compound 4 as the most reactive analogue, characterized by the lowest HOMO-LUMO energy gap, lowest hardness, and highest softness.

Introduction

Isatin, a naturally occurring heterocyclic compound, is found in many biologically active molecules and pharmaceutical agents. In the last two decades, research based on isatin has attracted increasing interest for a broad spectrum of activities of isatin and its derivatives, such as anticancer (Pavia et al., 2021; Yu et al., 2016), antidepressant (Pakravan et al., 2013), anticonvulsant (Shahid et al., 2016), antifungal (Pandeya et al., 2002), anti-HIV (Selvam et al., 2008), and anti-inflammatory (Socca et al., 2014), etc.

Moreover, it’s also used as a precursor for the synthesis of various drug molecules (Alshams et al., 2025; Singh et al., 2017; Varun et al., 2019).

Vine et al. (2007) have reported that *N*-benzyl isatin derivatives have potential cytotoxicity against some lymphoma cells as well as a series of human cancer cell lines, including human leukemic (K562, U937, and Jurkat), liver (HepG2), breast (MDA-MB-231 and MCF-7), prostate (PC-3), and colorectal (HCT-116) cell lines. In addition, Chinnasamy's group found that some *N*-1 and C-5 disubstituted molecules, such as 1- (substitutedbenzylidene)-3-[1-(morpholino /piperidinomethyl)-2,3-dioxoindolin-5-yl] urea derivatives exhibited antiepileptic activity and neurotoxicity (Prakash and Raja, 2011). Later on, Zhang et al reported that *N*-1 benzyl and C-5 phenyl substituted isatin derivatives exhibited excellent cancer inhibitory activity in vitro against K562 cell lines and HepG2 cell lines (Zhang et al, 2018). The hydrophobic moiety of these molecules may contribute to their biological activity by effective interactions with the binding sites of the receptors as well as enzyme through hydrophobic interactions. Therefore, increasing the hydrophobic moiety is a

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promising strategy to enhance the cytotoxicity of the existing bioactive molecules.

Moreover, increasing the number of isatin moieties in same molecule may provide the scope for strong DNA intercalation and effective binding with cancer-related enzymes. In this article, we have mentioned the synthesis of *N*-benzyl isatin derivatives, particularly those substituted at the C-5 position as well as phenyl-bridged isatin dimer by Suzuki-Miyaura coupling reaction and their cytotoxic properties.

Materials and Methods

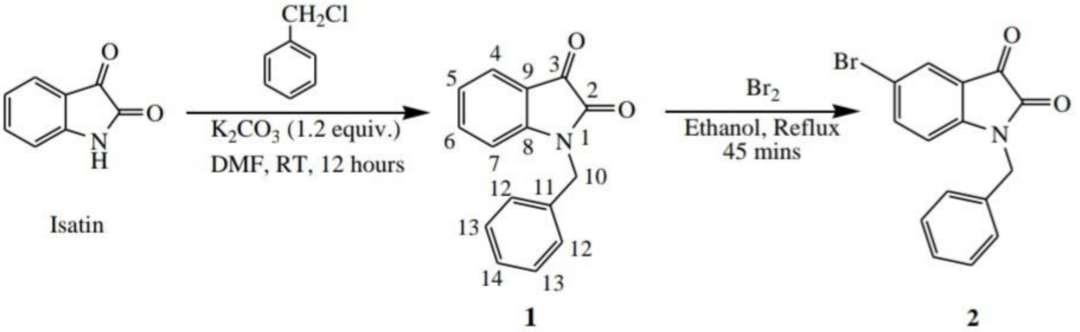
General

Fisher-John's electrothermal melting point apparatus was used for recording the melting points of all the synthesized compounds. The thin layer chromatography (TLC) was performed on silica gel, 60 GF_{254} (Merck), to monitor the progress of the reaction and to check the purity of the synthesized products. Solvents that were used during present investigation were purified by distillation at the boiling point of the respective solvents.

Evaporation of solvents was carried by a vacuum rotary evaporator under reduced pressure at a temperature below 60 °C. The compounds of a mixture were separated by column chromatography. Infrared spectra were recorded on a Shimadzu FTIR-8300 Spectrophotometer in KBr. ^1H NMR spectra were recorded by Bruker 400 MHz instrument. UV-visible spectra were recorded by Specord 205 PC UV-Vis Spectrophotometer.

Preparation of *N*-benzylisatin (1) from isatin

In a 500 ml round-bottom flask, 500 mg (3.398 mmole, 1 eq.) of isatin was dissolved in 12 ml of DMF. Then 563.54 mg (4.0776 mmole, 1.2 eq.) of K_2CO_3 and 25 drops (473.13 mg, 3.74 mmole, 1 eq.) of benzyl chloride were added into the solution. The reaction was allowed to proceed for 24 hours at room temperature. The crude product was extracted by toluene. After recrystallization from *n*-hexane, yielding orange-red transparent crystal, (421mg, 84.2%); melting point 130-132 °C and R_f 0.60 (*n*-hexane: ethyl acetate = 2:1). ^1H NMR (400 MHz, $CDCl_3$) δ 4.96 (s, 2H, H_{10}), 6.80 (d, 1H, J=7.8 Hz, H_7), 7.12 (t, 1H, J = 7.8 Hz, H_5), 7.31-7.40 (m, 5H, H_{12-14}), 7.51 (td, 1H, J = 7.8, 1.2 Hz, H_6), 7.64 (d, 1H, J =7.6 Hz, H_4). FT-IR (KBr) υ_{max} 3086 (C-H str., aromatic), 2916, 2856 (C-H str., alkane), 1747 (C=O, ketone), 1732 (C=O, amide), 1610 (C=C str., aromatic), 1469 (CH_2 bend), 1456 (C-H bend, aliphatic) 1357.89 (C-N str.), cm^{-1}. UV-Visible: λ_{max} = 249 nm; ε =5.08x10^6 M^{-1} cm^{-1}.



Scheme1. Preparation of compound 1-2.

Preparation of 5-bromo-*N*-benzylisatin (2)

In a 250 mL round-bottom flask, 50 mg (0.21 mmole, 1 eq.) of *N*-benzylisatin was dissolved in 5 mL of ethanol. Subsequently, 1 mL of bromine (Br_2) was carefully added dropwise to the solution. The reaction mixture was refluxed in an oil bath for 45 minutes. Upon completion of reaction, solvent was evaporated under reduced pressure. The crude mass was purified by silica gel column chromatography using a solvent *n*-hexane and ethyl acetate (7:1). The product was recrystallized from *n*-hexane and obtained as an orange solid (35 mg, 70%); melting point 127-129 °C, and R_f = 0.30 (*n*-hexane: ethyl acetate = 7:1). ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.95 (s, 2H, H_{10}), 6.69 (d, 1H, J = 8.4 Hz, H_7), 7.61(dd, 1H, J = 8.4, 2.0 Hz, H_6), 7.75 (d, 1H, J = 1.6 Hz, H_4), 7.3-7.44 (m, 5H, H_{12-14}). FTIR (KBr) υ_{max} 3099 (C-H str., aromatic), 2920 and 2850 (C-H str., alkane), 1749 (C=O, ketone), 1730 (C=O, amide), 1606 (C=C str.), 1467, 1414 (CH_2 bend, aliphatic), 1321 (C-N str.), 821 and 696 (C-H str. aromatic), 630 (C-Br str) cm^{-1}. UV-visible: λ_{max} = 254 nm, ε =3.44x10^6 M^{-1} cm^{-1}.

Preparation of compound 3-6

A two-necked round-bottom flask was charged with 5-bromo-*N*-benzylisatin (1.0 eq.) and organo boron reagents (1.5 eq. or 0.75 eq) in DMF and purged with nitrogen (N_2). Subsequently, $Pd(PPh_3)_4$ (0.1 eq.) and potassium carbonate (4.0 eq.) were added. The mixture was stirred at 70-80 °C for 24 hours under an inert atmosphere. After a usual workup by toluene, the products were purified by silica gel column chromatography (*n*-hexane and ethyl acetate = 5:1) followed by the recrystallization from *n*-hexane.

Compound 3

Orange solid (56 mg, 56%); melting point 98-102 °C and R_f = 0.50 (*n*-hexane: ethyl acetate = 5:1). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.30 (s, 9H, H_{20}), 4.94 (s, 2H, H_{10}), 6.76 (d, 2H, J = 8.4 Hz, H_{17}), 6.82 (d, 1H, J = 8.4 Hz, H_7), 7.23 (d, 2H, J = 8.8 Hz, H_{16}), 7.35-7.44 (m, 5H, H_{12-14}), 7.68 (d, 1H, J = 8.4 Hz, H_6), 7.82 (s, 1H, H_4). ^{13}C NMR (100.6 MHz, $CDCl_3$) δ 30.80, 34.85, 43.7, 118.15, 123.71, 126.04, 126.21, 126.41, 127.51, 128.23, 129.16, 134.55, 135.99, 136.59, 137.37, 149.54, 151.12, 153.30, 158.50. DEPT-135 (400 MHz, $CDCl_3$), Positive signals: 30.8, 126.04, 126.21, 126.41, 127.51, 128.23, 129.16, 135.99, Negative signal: 43.7. FT-IR (KBr) υ_{max} 3028 (C-H str., aromatic), 2959 2852 (C-H str., alkane), 1762-1691(br. C=O, ketone and amide), 1618 (C=C str., aromatic), 1331 (C-N str.), 1481, 1448 (CH_2 bend) cm^{-1}, UV-visible: λ_{max}= 270 nm, ε = 4.05x10^6 M^{-1} cm^{-1}.

Compound 4

Orange solid (20 mg, 20%); melting point 102-105 °C and R_f = 0.50 (*n*-hexane: ethyl acetate = 5:1). ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 1.62(s, 9H, H_{22}), 4.99 (s, 2H, H_{10}), 6.92 (d, 1H, J=8.0 Hz, H_7), 7.35-7.42 (m, 5H, H_{12-14}), 7.63 (s, 2H, H_{19}), 7.94 (d, 2H, J = 8.8 Hz, H_{18}), 8.05 (d, 2H, J = 8.8 Hz, H_{17}), 8.214 (s, 2H, H_{16}), 8.26 (d, 1H, J = 8.0 Hz, H_6), 8.68 (s, 1H, H_4). FT-IR (KBr) υ_{max} 3028 (C-H str., aromatic), 2959, 2868 (C-H str., alkane), 1761-1687 (C=O, ketone and amide) 1618 (C=C str., aromatic), 1448 (CH_2 bend, aliphatic), 1330 (C-N str.), 823, 696 (C-H bend), cm^{-1}. UV-Visible: λ_{max}= 281 nm, ε =3.77x10^6 M^{-1} cm^{-1}.

Compound 5

Orange solid (50 mg, 50%); melting point 111-115 °C and R_f = 0.40 (*n*-hexane: ethyl acetate = 5:2). ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.98 (s, 4H, H_{10}), 6.90 (d, 2H, J = 8.0 Hz, H_7), 7.03 (t, 1H, J = 8.0 Hz, H_5), 7.51 (d, 2H, J = 7.2 Hz, H_6), 7.77 (d, 2H, J = 8.4 Hz, $H_{4', 6'}$), 7.88 (s, 2H, H_4), 7.30-7.40 (m, 10H, H_{12-16}), 7.92 (s, 1H, $H_{2'}$). ^{13}C NMR (100.6 MHz, $CDCl_3$) δ 44.24, 111.37, 118.19, 123.85, 124.87, 125.82, 126.79, 127.51, 128.27, 129.10, 134.5, 136.90, 137.20, 139.88, 142.67, 150.03, 158.5. DEPT-135 (^{13}C 100.6 MHz, $CDCl_3$): δ Positive signals: 118.19, 123.85, 124.87, 125.82, 126.79, 127.51, 128.27, 129.10, 136.90. Negative signal: 44.24. FTIR (KBr) υ_{max} 3059 (C-H str., aromatic), 2928, 2853 (C-H str., alkane), 1780-1670 (br. C=O, ketone and amide), 1618 (C=C str., aromatic), 1473, 1435 (CH_2 bend, alkane), 1344 (C-N str.), 823, 698 (C-H bend, aromatic) cm^{-1}. UV-Visible: λ_{max}= 272 nm; ε =2.90x10^6 M^{-1} cm^{-1}.

Compound 6

Orange solid (20 mg, 20%); melting point 115-120°C and R_f = 0.40 (*n*-hexane: ethyl acetate = 5:2). ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.98 (s, 4H, H_{10}), 7.42-7.51 (m, 10H, H_{12-16}), 7.56 (d, 2H, J = 8.0 Hz, H_7), 7.68 (d, 2H, J = 7.8 Hz, H_6), 7.69 (s, 4H, $H_{2', 3'}$), 7.72 (s 2H, H_4).

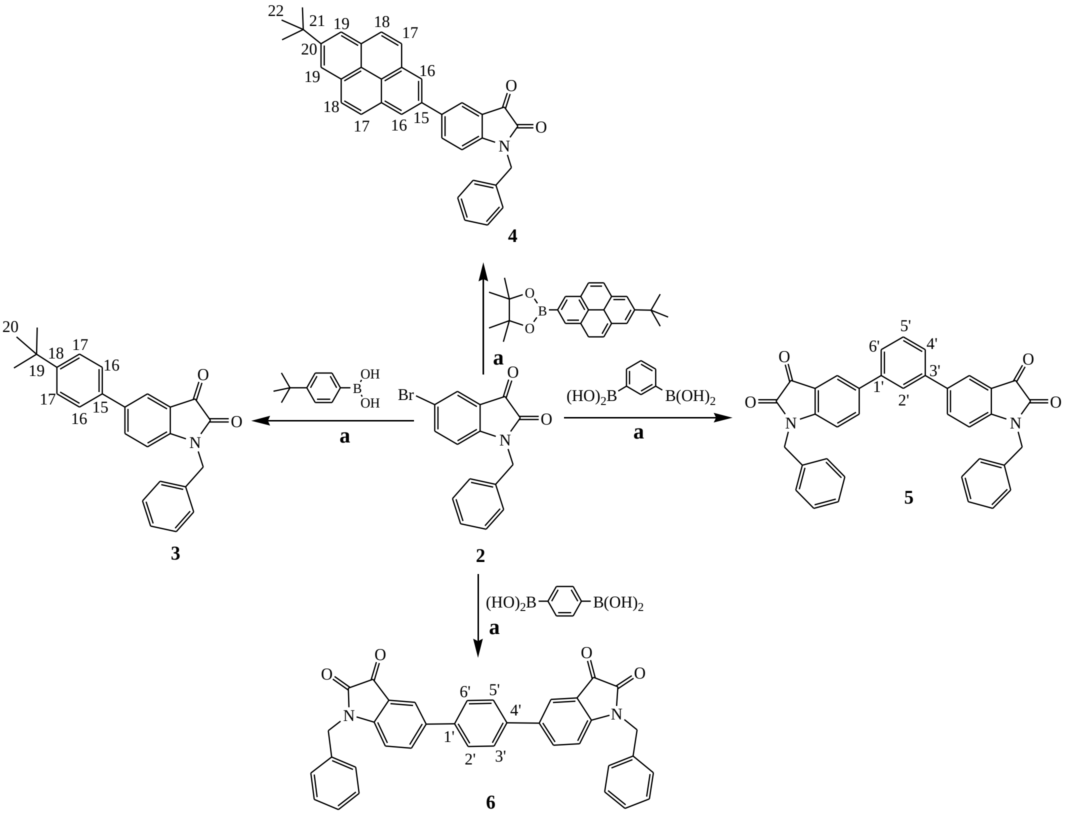
FTIR (KBr) υ_{max} 3058 (C-H str., aromatic), 2926, 2854 (C-H str., alkane), 1781–1674 (C=O, ketone and amide), 1617 (C=C str., aromatic), 1473, 1436 (CH_2 bend), 1346 (C-N str.), 824, 696 (C-H bend) cm^{-1}, UV-visible: λ_{max} = 269 nm; $\varepsilon = 2.89 \times 10^6$ M^{-1} cm^{-1}.

Results and Discussion

Isatin is poorly soluble in most of the organic solvents. To increase the solubility, *N*-benzyl isatin **1** was prepared by the reaction of isatin with benzyl chloride in the presence of K_2CO_3 in DMF with good yield. The treatment of *N*-benzyl isatin **1** with bromine solution afforded the compound 5-bromo *N*-Benzyl isatin, **2**, which was used as a precursor for the synthesis of desired molecules.

The compound **1** is a known compound (Huda et al., 2020). Introduction of bromine at the 5-position of isatin made the ^1H NMR spectrum simpler. The signal for H_4 proton appeared as a doublet at 7.75 ppm by *meta*-coupling with H_6 (J = 1.6 Hz). The H_6 proton was found as a doublet of doublet (dd) at 7.61 ppm by *ortho*- and *meta*-coupling (J = 8.4 Hz and J = 2.0 Hz) with H_7 and H_4, respectively, whereas signal for H_7 proton appeared as a sharp doublet at 6.69 ppm. The absorption band at 630 cm^{-1} may be due to the C-Br bond. The Suzuki Miyaura coupling reaction of compound **2** with *tert*-butyl phenyl boronic acid produced compound **3**, whereas the reaction with pyrene boronic acid pinacol ester afforded compound **4**. The Suzuki Miyaura coupling reaction of compound **2** with 1,3-diboronic acid and 1,4-diboronic acid afforded compounds **5** and **6** respectively having two isatin moieties.

The structure of the compound 3 was confirmed by the presence of the signals for two protons, H_{16} and H_{17}. These protons appeared as doublets at 7.23 and 6.76 ppm. The structure of this compound is also supported by ^{13}C NMR, DEPT-135 and IR data.



Scheme 2. Preparation of compounds 3, 4, 5, and 6 (a; $Pd(PPh_3)_4$, K_2CO_3, DMF, 90 °C, 24 h)

The pyrene protons (H_{17} and H_{18}) of compounds **4** appeared asdoublets at 8.05 and 7.93 ppm. Other two pyrene protons (H_{16} and H_{19}) were observed as singlets at 8.21 and 7.63 ppm. In compounds **5** and **6**, protons of isatin moieties showed similar splitting pattern except for the bridging benzene. The four phenyl protons of compound **6** showed a sharp singlet, whereas four protons of phenyl ring in compound **5** showed a singlet at 7.92 ppm a doublet at 7.77 ppm, and a triplet at 7.03 ppm for $H_{2'}$, $H_{4',6'}$ and $H_{5'}$, respectively.

Brine shrimp lethality test

The cytotoxicity of the compounds was studied by Brine shrimp lethality bioassay (Perry et al.; 2008). In this method, the test solutions of different concentrations, such as 200, 100, 50, 25, 12.5, and 6.25 µg/mL were prepared by the combination of DMSO and brine. Then, 10 nauplii in 5 mL of brine were introduced to 5 mL of the solution of the synthesized compounds. The process also included a control to eliminate any other effect except the test molecule. After 24 hours, the test tubes were examined using a magnifying glass against a dark background, and the number of surviving nauplii in each tube was counted. This information was used to compute the proportion of brine shrimp nauplii for each concentration. The formula was used to determine the percentage of mortality.

$$\%\ \text{Mortality} = \frac{\text{No.of nauplii taken} - \text{No.of nauplii alive}}{\text{No.of nauplii taken}} \times 100$$

The median lethal concentration (LC_{50}) is typically used to express the effectiveness of the concentration-mortality relationship of synthesized compounds. It is calculated using the linear regression approach by plotting the percentage of deaths against the corresponding log(concentration). The LC_{50} value is obtained by drawing a vertical line to the horizontal axis from 50% of the mortality of the test sample.

The cytotoxicity studies of the synthesized compounds demonstrated that compounds **1, 2, 3, 5** and **6** exhibited moderate cytotoxic activity whereas compound **4** showed significant cytotoxicity relative to the standard vincristine sulfate. These results imply that the presence of hydrophobic moiety at 5-position of isatin is useful for cytotoxicity. Increasing size hydrophobic moiety like phenyl to pyrene may provide the scope of greater interaction with the binding site through Vander Waals force which was revealed by compounds **4**. The LC_{50} values of the synthesized compounds is depicted in following bar chart.

In-Silico studies

Optimization and DFT calculation of the synthesized compounds

On the BIOVIA Drawer, the molecules were sketched. The next step was to create three-dimensional structures by DFT complete optimization using Becke's exchange functional that included the LYP correlation functional with Lee, Yang, and Parr's (LYP) (Becke, 1988; Lee et al., 1988; Parr and Zhou, 1993). The compounds created were all fine-tuned using the 3-21G, basis set (Bergner et al., 1993). Following optimization, the stationary points were verified to correspond to minima on the potential energy surface by performing additional vibrational frequency calculations. We looked at every chemical's electronic energy, enthalpy, dipole moment, and partial charge. For hardness and softness of all compounds were calculated using the energies (ε) of their frontier HOMOs and LUMOs. Taking into account the Parr and Pearson interpretation of DFT (Geerlings and De Proft, 2008) and Koopmans' theorem (Pearson, 1986), the following equation was used to compute the hardness (η) and softness (S) of the medicines.

$$\text{Hardness}\ (\eta) = \frac{\varepsilon_{HOMO} - \varepsilon_{LUMO}}{2}, \quad \text{Softness}\ (S) = \frac{1}{\eta}$$

Table 1. Data for brine shrimp lethality bioassay for vincristine sulfate.

Comp. No	Conc. (μg/ml)	Log conc.	No. of nauplii taken (N_0)	No. of nauplii dead	No. of nauplii alive (N_1)	Mortality, $M=\frac{N0-N1}{N0}*100$	logLC$_{50}$ (μg/ml)	LC$_{50}$ (μg/ml)
Stand.	0.313	-0.505	10	10	10	0	0.544	3.50
	0.625	-0.204	10	10	10	0		
	1.25	0.097	13	2	11	15		
	2.5	0.398	10	5	5	50		
	5	0.699	13	7	6	54		
	10	1.00	10	8	2	80		
1	12.5	1.10	10	2	8	20	1.94	87.5
	25	1.40	10	3	7	30		
	50	1.70	10	4	6	40		
	100	2.00	10	5	5	50		
	200	2.30	10	7	3	70		
	400	2.60	10	7	3	70		
2	12.5	1.10	10	2	8	20	1.73	54.0
	25	1.40	10	4	6	40		
	50	1.70	10	5	5	50		
	100	2.00	10	6	4	60		
	200	2.30	10	7	3	70		
	400	2.60	10	9	1	90		
3	12.5	1.10	10	1	9	10	1.88	76.5
	25	1.40	10	2	8	20		
	50	1.70	10	5	5	50		
	100	2.00	10	6	4	60		
	200	2.30	10	7	3	70		
	400	2.60	10	8	2	80		
4	12.5	1.10	10	1	9	10	1.52	33.2
	25	1.40	10	2	8	20		
	50	1.70	10	4	6	40		
	100	2.00	10	6	4	60		
	200	2.30	10	8	2	80		
	400	2.60	10	10	0	100		
5	12.5	1.10	10	2	8	20	1.77	58.3
	25	1.40	10	3	7	30		
	50	1.70	10	6	4	60		
	100	2.00	10	6	4	60		
	200	2.30	10	7	3	70		
	400	2.60	10	8	2	80		
6	12.5	1.10	10	1	9	10	1.88	76.3
	25	1.40	10	3	7	30		
	50	1.70	10	4	6	40		
	100	2.00	10	5	5	50		
	200	2.30	10	7	3	70		
	400	2.60	10	9	1	90		

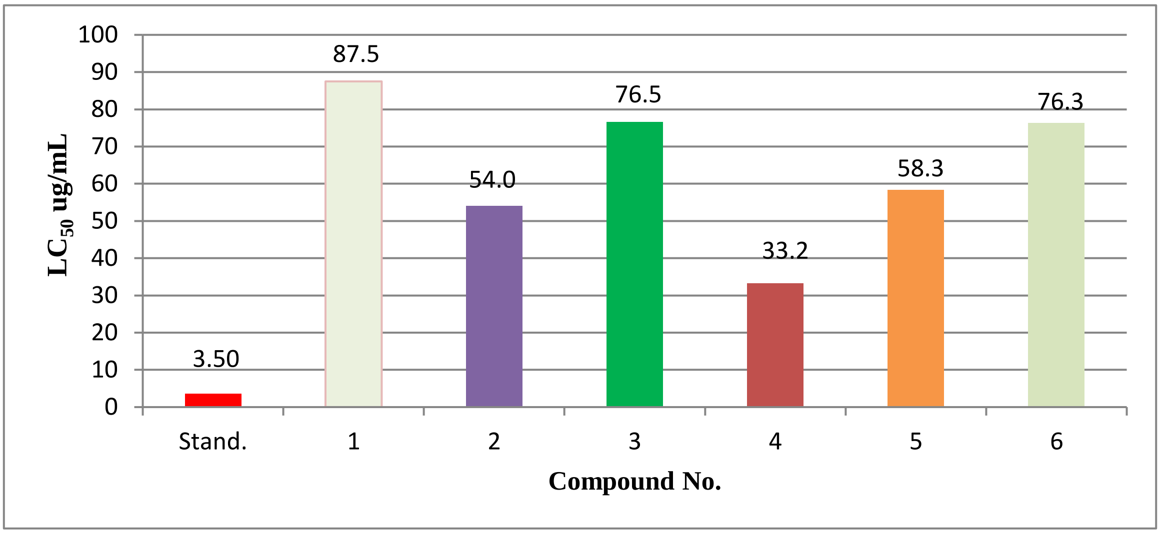


Fig. 1. The LC_{50} values of the synthesized molecules.

Strategies and optimization of analogues

As conformational features of a molecule critically influence its physical and chemical properties, all compounds were subjected to full geometry optimization using DFT. Table 2 illustrates the stoichiometry, electronic energy, predicted energy change (in Hartree), and dipole moment (Debye) of the synthesized analogues and the optimized structures are depicted in Fig. 2. According to Table 2, it is clear that modifications significantly influenced the structural properties of the compound in terms of energy, partial charge distribution, and dipole moment. The highest energy was observed for 1, while 6 showed the highest dipole moment of 7.779877 Debye, representing high polarity in nature and compound **5** exhibit lowest dipole moment of 0.002800 Debye. It is important to note that incorporation of the heteroatom significantly reduced the improved dipole moment.

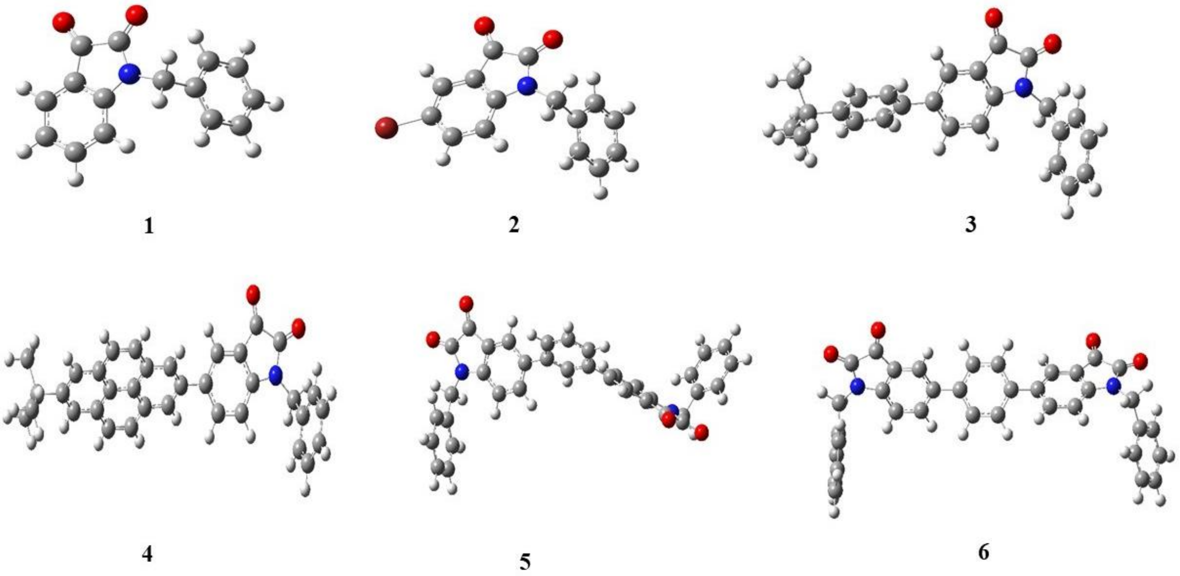

1
2
3
4
5
6

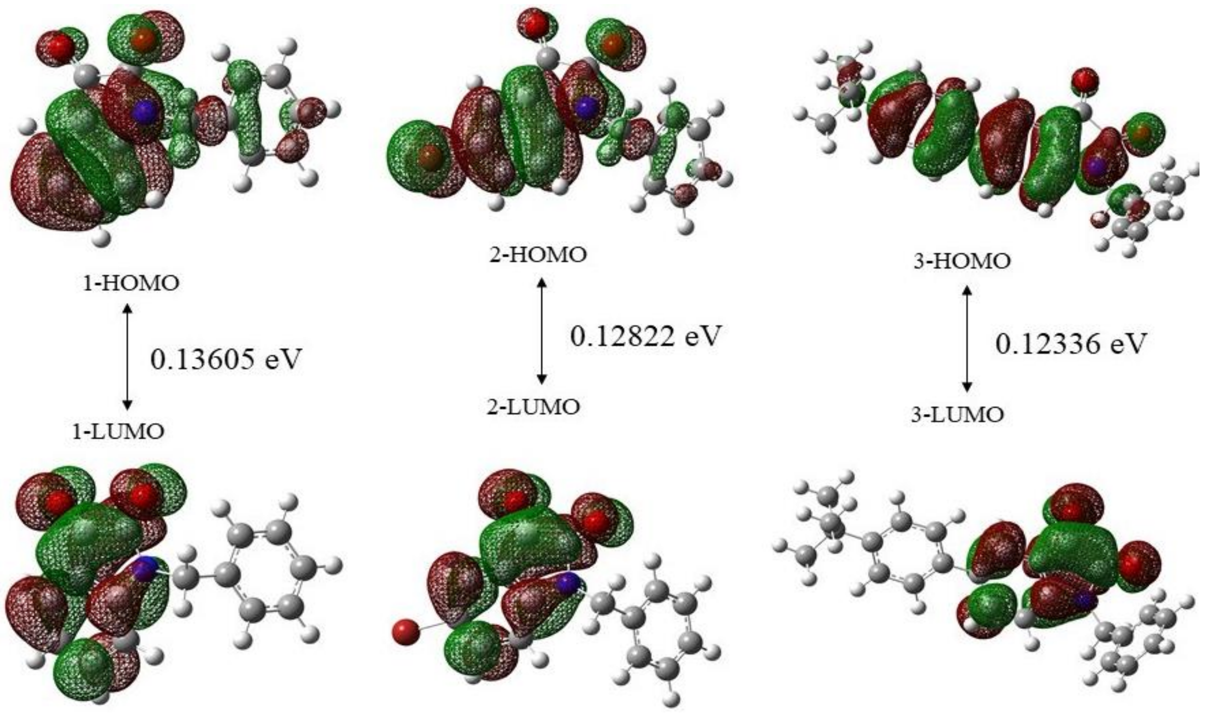
Fig. 2. Most stable optimized structures of all analogues. All analogues were optimized in the gas phase at the B3LYP level in Gaussian 09.

Table 2. The stoichiometry, electronic energy, predicted energy change (in Hartree) and dipole moment (Debye) of the synthesis analogues.

Sample ID	Stoichiometry	Electronic Energy	Dipole Moment (Debye)	Free energy	Predicted energy change
1	$C_{15}H_{11}NO_2$	–779.119	5.317	0.1854	–3.52e-08
2	$C_{15}H_{10}NO_2Br$	–3340.32	4.881	0.1725	–1.03e-08
3	$C_{25}H_{23}NO_2$	–1165.31	5.631	0.3665	–4.72e-09
4	$C_{35}H_{27}NO_2$	–1546.72	5.838	0.4661	–6.42e-10
5	$C_{30}H_{24}N_2O_4$	–1786.83	4.208	0.4450	–1.34e-08
6	$C_{30}H_{24}N_2O_4$	–1786.83	7.780	0.4445	–1.83e-08

Analysis of frontier molecular orbitals

The frontier molecular orbitals are the most important in a molecule and are considered to characterize the chemical reactivity and kinetic stability. These frontier molecular orbitals are the highest occupied molecular orbital (HOMO) and the lowest unoccupied molecular orbital (LUMO). Fig. 3 and Table 3 show the values of orbital energies, along with the two global chemical descriptors, hardness and softness, which are also calculated for all compounds. The highest softness was observed for compound **4** and it also showed the lowest HOMO-LUMO gap and hardness, indicating that the molecule is more reactive than other compounds, according to (Pearson, 1986).



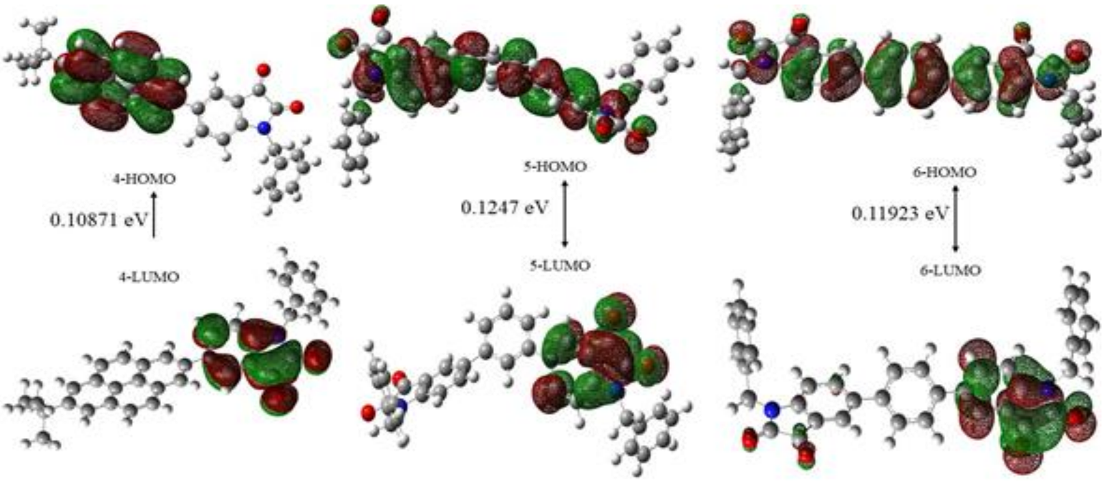


Fig. 3. Synthesis analogues HOMO and LUMO configurations.

Table 3. Results of frontier molecular properties.

Sample ID	HOMO	LUMO	Gap	Hardens	Softness
1	–0.23145	–0.09540	0.13605	0.06803	14.70048
2	–0.23171	–0.10349	0.12822	0.06411	15.59819
3	–0.21774	–0.09438	0.12336	0.06168	16.21271
4	–0.20434	–0.09563	0.10871	0.05436	18.39757
5	–0.22434	–0.09964	0.1247	0.06235	16.03849
6	–0.21816	–0.09893	0.11923	0.05962	16.7743

Conclusion

A number of 5-substituted *N*-benzyl isatins were successfully synthesized by Suzuki- Miyaura coupling reaction and studied their cytotoxicity by Brine Shrimp Lethality Bioassay. Compounds **2, 3, 5,** and **6** showed moderate cytotoxicity (LC_{50} = 54.0-76.5 μg/mL), while compound 4 exhibited significantly higher activity with an LC_{50} of 33.2 μg/mL. DFT calculations at the B3LYP/3-21G level confirmed the optimized geometries and stability of all synthesized analogues. The results demonstrate that structural modifications significantly affect electronic energy, dipole moment, and charge distribution, indicating strong structure-property relationships. Among the compounds, compound 6 exhibited the highest dipole moment, suggesting greater polarity Frontier molecular orbital analysis identified compound **4** as the most reactive analogue, characterized by the lowest HOMO-LUMO energy gap, lowest hardness, and highest softness. Overall, these findings highlight compound **4** as a promising candidate for further biological investigation and emphasize the effectiveness of DFT-based approaches in predicting molecular reactivity and guiding rational design.

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Authors contribution

Md Awlad Hossain: Conceptualization, Supervision, Data analysis and manuscript writing; Monisha Akter: Laboratory experiments and Data collection; Md. Farid Uddin: Laboratory experiments and In silico studies; Ananta Kumar Das: In silico studies; Kaushik Saha: Data analysis and manuscript editing.

Conflict of interest

The authors declare no conflict of interest regarding the publication of this paper.

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