Design, Synthesis And Biological Activities Of New Alkylated Isatin–Derivatives

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Abstarct: 5-bromo-Isatin, was opted as the base for synthesizing prospective Isatin derivatives which were synthesized for hypothesized biodynamic activities such as anti-bacterial, anti-fungal and antitumor. The synthesized Isatin derivative were characterized by spectrums and were examined for various biological activities.

Keywords: Isatin, Cancer cell-lines, Anti-tumor activity, Anti-fungal activity, Anti-bacterial activity

INTRODUCTION

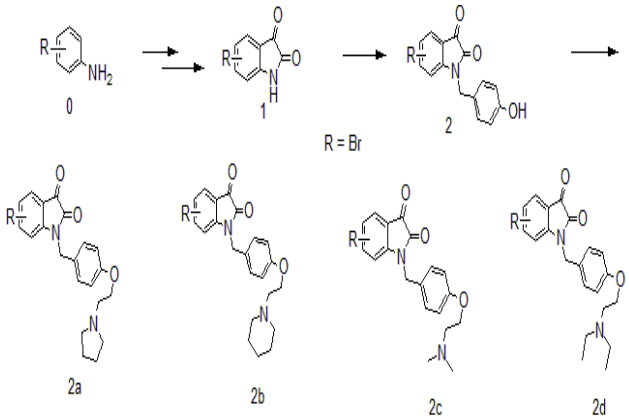
Derivatives of Isatin (1-H-indole-2,3-dione) possess a versatile bioactivity [1] and is used as starting compound for synthesizing a wide range of heterocyclic compounds in drugs preparation [2-6]. The past studies on isatin derivatives are found to possess antitumor, antibacterial, antifungal, anti-HIV, anticonvulsant, antiviral, anti-inflammatory, and many more other biodynamic properties [6]. Drugs containing the isatin skeleton are used to treat diseases such as epilepsy [7], tuberculosis [8], and bulimia [9]. Considering the precedential biological properties of Isatin and its derivatives, there is further scope to create and explore Isatin derivatives for emerging drug-targets. Previous research on isatin derivatives shows that mono-substituted Isatin at aryl ring has greater cytotoxic and other biodynamic properties over un-substituted Isatin. Further, bromo-substituted Isatin was found to possess greater cytotoxic properties over chloro, nitro, hydorxy substituted Isatin. It was found that halogenation substituted Isatin derivatives are the most active compounds, with 5-bromo, 5-iodo, and 5-fluoro isatin being almost 10 times more active than the un-substituted isatin [10]. Moreover, Isatin derivatives with postion 5-substituted were more active over Isatin substituted at other positions and found to possess greater anti-cancer activity [11]. Thus, the starting material for creating prospective isatin derivatives was taken as 5-bromo Isatin. New substances based on proven Isatin scaffolds in combination with other pharmacophoric elements of drugs can be a right approach for the synthesis of new Isatin derivatives for prospective drugs. Study of N-Alkylated Isatin derivatives are found to have anticancer activity [12-14]. It was found that N-methylation greatly improved the cytotoxicity of the Isatin [10-11], SAR studies showed that an aromatic ring with a one or three carbon atom at position 1-N increased the anti-cancer activity [15-19]. Thus, considering the above facts and proven pharmacophoric properties of moiety such as ethyl pyrrolidine, ethyl piperdine di-methyl amino ethane and diethyl amino ethane, intermediate compound N-alkylated 5-bromo Isatin was prepared and used to create new Isatin derivatives with these moieties to investigate for various biodynamic activities such as antitumor, antibacterial and antifungal activities.

EXPERIMENTAL

Procedure for synthesizing the compounds for each step is described. The melting points were measured in open capillaries “Toshniwal melting point apparatus”. The Brucker Avans DRX 300 (300 MHz, FT NMR) spectrometer was used for recording 1H NMR. The values of chemical shift are in ppm scale and coupling constants in Hz. Elemental analysis were done on analyser EA-1108 and were within +3-4% of theoretical values. For checking the purity of the products, pre-coated silica gel 60 F254 was used and the spots were visualized by using iodine vapors.

GENERAL PROCEDURE FOR SYNTHESIS OF COMPOUNDS

The prospective compounds which may have anti-tumor activities were synthesized using base compound 5-bromo Isatin (compound 1) outlined in Scheme-1. The compound 2 “N-(4-hydroxy phenyl methyl) 5-bromo indole-2,3-dione” was prepared after alkylation of compound 1 with p-hydroxy benzyl chloride. The set of compounds (2a -2d) were prepared by etherification of compound 2 with pyrrolidine-ethyl chloride, 2-(piperidyl) ethyl chloride, dimethyl-amino-ethyl chloride, dimethyl-amino-ethyl chloride at para position respectively. All the compounds were prepared as per given in Scheme 1.



***Scheme-1**: Synthesis of compounds (2a-2d)*

Synthesis of N-(4-hydroxy phenyl methyl) 5-bromo indole-2,3-dione (2) Compound 2 was prepared by alkylation of 5-bromo Isatin with 4-hydroxy benzyl chloride. The solution of 5-bromo-isatin (4.42 mmol, 1 g) in acetonitrile (~70 mL), was added alumina/KF (40.7 mmol, 6.48 g) and the mixture was stirred for 5 min until brownish color is obtained. Then 4-hydroxy benzyl chloride (1.5 equiv., 6.6 mmol, 1.49 g) was added to the bottom flask after which the mixture was

refluxed under acetonitrile for 8-10 hours. The mixture was then cooled to room temperature and the suspended alumina/KF was filtered from the solution. Then, The filtrate was evaporated under reduced pressure to afford a solid that was recrystallized from hexanes/chloroform to afford the product. Synthesis of compound N-(4-(2-(pyrrolidyl) ethoxy) phenyl methyl) 5-bromo indole-2, 3-dione (2a – 2d)
For synthesis of compound N-(4-(2-(pyrrolidyl) ethoxy) phenyl methyl) 5-bromo indole-2,3-dione (compound 2a), a mixture of of N-(4-hydroxy phenyl methyl) 5-bromo indole-2,3-dione (3 mmol, 1 g) (compound 2) and 2-(pyrrolidyl) ethyl chloride (9 mmol, 1.2 ml) and KOH (6mmol, 240mg, 2 pellet) was stirred for 16 hours. The other compounds (2b – 2d) were synthesized with the above procedure using the moieties 2-(piperidyl) ethyl chloride, dimethyl-amino-ethyl chloride, dimethyl-amino-ethyl chloride respectively.

Spectral data of selected compounds
N-(4-hydroxy phenyl methyl) 5-bromo indole-2,3-dione (2)
Yield 80%, MP 702K; 1H NMR (200 MHz, CDCl3): δ 6.60-6.61 (d, 2H, Ar-H, phenyl), δ 6.60-6.89 (d, 2H, Ar-H, phenyl), δ 4.00-4.22 (s, 2H, CH2), δ 7.50 -7.72 (s, 1H, Ar-H, 5-bromo indole- 2,3-dione), δ 7.30 -7.69 (s, 1H, Ar-H, 5-bromo indole- 2,3-dione), δ 7.40 -7.96 (s, 1H, Ar-H, 5-bromo indole- 2,3-dione); FAB-MS m/z 330, Anal Calcd for $C_{15}H_{10}BrNO_3$ (Mol Wt.332.15): C,54.24; H,3.03; Br,24.06; N,4.22; O, 14.45 Found: C,54.95; H,3.30; Br,24.67; N,4.78; O, 14.50

N-(4-(2-(pyrrolidyl) ethoxy) phenyl methyl) 5-bromo indole-2,3-dione (2a)
Yield 76%, MP 604 K; 1H NMR (200 MHz, CDCl3): δ 3.0 -4.04 (s, 2H, CH2), δ 2.50 -2.78 (s, 2H, CH2), δ 1.45 -1.59 (d, 4H, pyrrolidyl), δ 2.00 -2.25 (d, 4H, pyrrolidyl), δ 6.60-6.65(d, 2H, Ar-H, phenyl), δ 6.90-6.95(d, 2H, Ar-H, phenyl), δ 4.00-4.22(s, 2H, CH2), δ 7.50 -7.72 (s, 1H, Ar-H, 5-bromo indole-2,3-dione), δ 7.30 -7.69 (s, 1H, Ar-H, 5-bromo indole- 2,3-dione), δ 7.40 -7.96 (s, 1H, Ar-H, 5-bromo indole- 2,3-dione); FAB-MS m/z 428, Anal Calcd for $C_{21}H_{21}BrN_2O_3$ (Mol Wt.429.31): C,58.75; H, 4.93; Br,18.61; N,6.53; O, 11.18 Found: C, 57.95; H, 4.40; Br,18.14; N,6.44; O, 10.95

N-(4-(2-(piperidyl) ethoxy) phenyl methyl) 5-bromo indole-2,3-dione (2b)
Yield 78%, MP 751.85 K; 1H NMR (200 MHz, CDCl3): δ 3.0 -4.04 (s, 2H, CH2), δ 2.50 -2.78 (s, 2H, CH2), δ 2.20-2.24 (d, 4H, piperidyl), δ1.30 -1.50 (t, 6H, piperidyl), δ 6.60-6.65(d, 2H, Ar-H, phenyl), δ 6.85- 6.95(d, 2H, Ar-H, phenyl), δ 4.00-4.22(s, 2H, CH2), δ 7.50 -7.72 (s, 1H, Ar-H, 5-bromo indole- 2,3-dione), δ 7.30 -7.69 (s, 1H, Ar-H, 5-bromo indole- 2,3-dione), δ 7.40 -7.96 (s, 1H, Ar-H, 5-bromo indole- 2,3-dione); FAB-MS m/z 442, Anal Calcd for $C_{22}H_{23}BrN_2O_3$ (Mol Wt.443.33): C,59.60; H,5.23; Br,18.02; N,6.32; O, 10.83 Found: C,53.44; H,5.11; Br,18.01 N,6.21; O, 10.67

N-(4-(2-(dimethyl amino) ethoxy) phenyl methyl) 5-bromo indole-2,3-dione (2c)
Yield 76%, MP 702 K; 1H NMR (200 MHz, CDCl3): δ 3.0 -4.04 (s, 2H, CH2), δ 2.50 -2.78 (s, 2H, CH2), δ 2.50-2.27 (d, 6H dimethyl amino,), δ 6.65(d,2H, Ar-H, phenyl), δ 6.95(d,2H, Ar-H, phenyl), δ 4.00-4.22(s, 2H, CH2), δ 7.50 -7.72 (s, 1H, Ar-H, 5-bromo indole- 2,3-dione), δ 7.30 -7.69 (s, 1H, Ar-H, 5-bromo indole- 2,3-dione), δ 7.40 -7.96 (s, 1H, Ar-H, 5-bromo indole- 2,3-dione); FAB-MS m/z 402, Anal Calcd for $C_{19}H_{19}BrN_2O_3$ (Mol Wt.403.27): C,56.59; H,4.75; Br,19.81; N,6.95; O, 11.90 Found: C,56 44; H,4.22; Br,19.22 N,6.21; O,11.67

N-(4-(2-(diethyl amino) ethoxy) phenyl methyl) 5-bromo indole-2,3-dione (2d)
Yield 79%, MP 725 K; 1H NMR (200 MHz, CDCl3): δ 3.0 -4.04 (s, 2H, CH2), δ 2.50 -2.78 (s, 2H, CH2), δ 2.50-2.40 (d, 4H, diethyl amino), δ1.00 -1.00 (t, 6H, diethyl amino), δ 6.65(d,2H, Ar-H, phenyl), δ 6.95(d,2H, Ar-H, phenyl), δ 4.00-4.22(s, 2H, CH2) δ 4.00-4.22(s, 2H, CH2), δ 7.50 -7.72 (s, 1H, Ar-H, 5-bromo indole- 2,3-dione), δ 7.30 -7.69 (s, 1H, Ar-H, 5-bromo indole- 2,3-dione), δ 7.40 -7.96 (s, 1H, Ar-H, 5-bromo indole- 2,3-dione); FAB-MS m/z 430, Anal Calcd for $C_{21}H_{23}BrN_2O_3$ (Mol Wt.431.32): C,58.48; H,5.37; Br,18.53; N,6.49; O, 11.13 Found: C,58.44; H,5.11; Br,18.33 N,6.21; O, 11.67

RESULT AND DISCUSSION

The objective of the work was to design and synthesize potential Isatin derivatives based on the isatin scaffold in combination with other pharmacophoric moieties of proven drugs. For this, 5-bromo Isatins was used as starting material and further modified by alkylation with p-hydroxy benzyl chloride and then etherification of intermediate compound with pharmacophoric moieties for various biological activities.

Table-1 *below shows the Isatin derivatives (2a-2d) evaluated for anti-tumor activity on cell lines MCF-7 and EVSA-T.*

Compounds	MCF-7 (Cell No. *10^4)	EVSA-T(Cell No. *10^4)	Activity
2a	9.29±0.88	9.89±0.92	Positive
2b	8.95±0.67	8.55±0.62	Positive
2c	8.79±0.52	8.42±0.46	Positive
2d	9.29±0.88	9.89±0.92	Positive

Table 1: *Anti-tumor activity on cell lines MCF-7 and EVSA-T*

Below table shows the results of the Isatin derivatives (2a-2d) evaluated for anti-fungal activity

Anti-fungal activity @ 10 micro-gram/ml concentration				
Samples	A flavus Col. Dia. (mm)	%Inhibition	A niger Col. Dia. (mm)	%Inhibition
2a	0.7 ± 0.005	76.6	0.5 ± 0.003	75
2b	0.5 ± 0.003	83.3	0.4 ± 0.003	80
2c	0.2 ± 0.003	93.3	0.7 ± 0.003	65
2d	0.4 ± 0.004	86.7	0.6 ± 0.004	70

Table 2: *Anti-fungal activity on A flavus and A niger*

Below table shows the results of the Isatin derivatives (2a-2d) evaluated for

Anti-bacterial activity@10 microgram/ml concentration

Compounds	P. aeruginosa Dia. (mm)	S. aureus Dia. (mm)	K. pneumoniae Dia. (mm)
2a	++	+	++
2b	++	++	++
2c	++	++	++
2d	++	++	++

Table 3: *Anti-bacterial activity on P. aeruginosa, S. aureus and K. pneumoniae*

anti-bacterial activity

+ Slightly active (diameter range 6-10 mm)
++ Moderately active (diameter range 10-14 mm)
+++ Highly active (diameter range >14 mm)

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