

HEALTH SCIENCE

Manuscript info:

Received October 12, 2019., Accepted November 17, 2019., Published December 20, 2019.

DESIGN, SYNTHESIS AND BIOLOGICAL ACTIVITIES OF NEW ALKYLATED ISATIN-DERIVATIVES

Ruchi Bhatnagar*, Dr Jaya Pandey, Dr Deepa Panhekar*****

**Department of Chemistry, Amity University,
Lucknow Campus, Lucknow-226028, India

***Department of Chemistry, Dr. Ambedkar College,
Nagpur-440008, India



<http://dx.doi.org/10.26739/2573-5616-2019-12-6>

Abstract: 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

Recommended citation: Ruchi Bhatnagar, Dr Jaya Pandey, Dr Deepa Panhekar. DESIGN, SYNTHESIS AND BIOLOGICAL ACTIVITIES OF NEW ALKYLATED ISATIN-DERIVATIVES. 11-12. American Journal of Research P. 54-60 (2019).

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, hydroxy 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 position 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 piperidine 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.

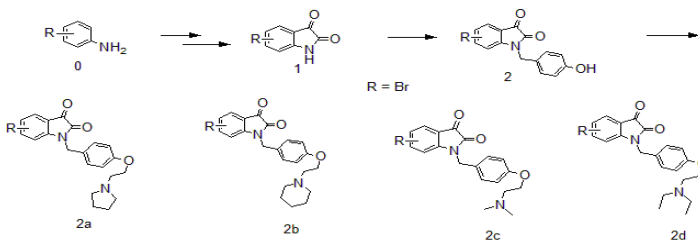
Experimental

Procedure for synthesizing the compounds for each step is described. The melting points were measured in open capillaries "Toshniwal melting point apparatus". The Bruker 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%, MP702K; ¹H NMR (200 MHz, CDCl₃): □ 6.60-6.61 (d, 2H, Ar-H, phenyl), □ 6.60-6.89 (d, 2H, Ar-H, phenyl), □ 4.00-4.22(s, 2H, CH₂), □ 7.50 -7.72 (s, 1H, Ar-H, 5-bromo indole- 2,3-dione), □ 7.30 -7.69 (s, 1H, Ar-H, 5-bromo indole-2,3-dione), □ 7.40 -7.96 (s, 1H, Ar-H, 5-bromo indole- 2,3-dione); FAB-MS m/z 330, Anal Calcd for C₁₅H₁₀BrNO₃ (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): □ 3.0 -4.04 (s, 2H, CH₂), □ 2.50 -2.78 (s, 2H, CH₂), □ 1.45 -1.59 (d, 4H, pyrrolidyl), □ 2.00 -2.25 (d, 4H, pyrrolidyl), □ 6.60-6.65(d, 2H, Ar-H, phenyl), □ 6.90-6.95(d, 2H, Ar-H, phenyl), □ 4.00-4.22(s, 2H, CH₂), □ 7.50 -7.72 (s, 1H, Ar-H, 5-bromo indole- 2,3-dione), □ 7.30 -7.69 (s, 1H, Ar-H, 5-bromo indole- 2,3-dione), □ 7.40 -7.96 (s, 1H, Ar-H, 5-bromo indole- 2,3-dione); FAB-MS m/z 428, Anal Calcd for C₂₁H₂₁BrN₂O₃ (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): □ 3.0 -4.04 (s, 2H, CH₂), □ 2.50 -2.78 (s, 2H, CH₂), □ 2.20-2.24 (d, 4H, piperidyl), □ 1.30 -1.50 (t, 6H, piperidyl), □ 6.60- 6.65(d, 2H, Ar-H, phenyl), □ 6.85- 6.95(d, 2H, Ar-H, phenyl), □ 4.00-4.22(s, 2H, CH₂), □ 7.50 -7.72 (s, 1H, Ar-H, 5-bromo indole- 2,3-dione), □ 7.30 -7.69 (s, 1H, Ar-H, 5-bromo indole- 2,3-dione), □ 7.40 -7.96 (s, 1H, Ar-H, 5-bromo indole- 2,3-dione); FAB-MS m/z 442, Anal Calcd for C₂₂H₂₃BrN₂O₃ (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): □ 3.0 -4.04 (s, 2H, CH₂), □ 2.50 -2.78 (s, 2H, CH₂), □ 2.50-2.27 (d, 6H dimethyl amino.), □ 6.65(d,2H, Ar-H, phenyl), □ 6.95(d,2H, Ar-H, phenyl), □ 4.00-4.22(s, 2H, CH₂), □ 7.50 -7.72 (s, 1H, Ar-H, 5-bromo indole- 2,3-dione), □ 7.30 -7.69 (s, 1H, Ar-H, 5-bromo indole- 2,3-dione), □ 7.40 -7.96 (s, 1H, Ar-H, 5-bromo indole- 2,3-dione); FAB-MS m/z 402, Anal Calcd for C₁₉H₁₉BrN₂O₃ (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): □ 3.0 -4.04 (s, 2H, CH₂), □ 2.50 -2.78 (s, 2H, CH₂), □ 2.50-2.40 (d, 4H, diethyl amino), □ 1.00 -1.00 (t, 6H, diethyl amino), □ 6.65(d,2H, Ar-H, phenyl), □ 6.95(d,2H, Ar-H, phenyl), □ 4.00-4.22(s, 2H, CH₂) □ 4.00-4.22(s, 2H, CH₂), □ 7.50 -7.72 (s, 1H, Ar-H, 5-bromo indole- 2,3-dione), □ 7.30 -7.69 (s, 1H, Ar-H, 5-bromo indole- 2,3-dione), □ 7.40 -7.96 (s, 1H, Ar-H, 5-bromo indole- 2,3-dione); FAB-MS m/z 430, Anal Calcd for C₂₁H₂₃BrN₂O₃ (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	
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

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

Acknowledgement

The author wish to thank Dr Jaya Pandey (Amity University) and Dr. Deepa Pahnekar (Ambedkar College, Nagpur) for providing their continuous

support in the process of synthesizing compounds and their evaluation. I also thanks Department of Science and Technology for funding to Amity University for this work.

Reference

1. G. Chen, XJ. Hao, Natural Product Research and Development, 22,356-360,2010.
2. G. Chen, XJ. Hao, QY. Sun , J. Ding ,Rapid eco-friendly synthesis andbioactivities of 3-nitromethylene-1,3-dihydro-indol-2-one analogues.Chemical Papers,64(5), 673-377,2010
3. G. Chen, XJ. Hao, QY .Sun , J. Ding,Synthesis and antitumor activityevaluation of regioselective spiro [pyrrolidine-2, 3'-oxindole] compounds.Heterocyclic Communication, 15(5),355-361,2009
4. G.Chen, J.Yang, S. Gao, HP. He, SL. Li, YT.Di, Y.Chang, Y.Lu ,XJ.Hao, Spiro[pyrrolidine-2,3'-oxindole] derivatives synthesized by novelregionsselective 1,3-di polar cycloadditions, Mol Divers,16,151-156,2012
5. G.Chen, J.Zhang, Y.Tag, XJ.Hao, SZ.Mu, icheal addition between Isatin and acrylate derivatives.Letters in Organic Chemistry,8(9),614-617,2011
6. JFM .DaSilva, SJ.Garden, A.Zda C Pinto ,The chemistry of isatins: a reviewfrom 1975 to 1999. The Journal of the Brazilian Chemical Society,12,273-324,2001
7. S.N Pandeya, S. Smitha, S. Stables, J. P. "Anticonvulsant and sedative-hypnotic activities of N-substituted isatin semicarbazones" Arch. Pharm. Med. Chem. ,4, 129-134,2002.
8. Pandeya, S. N, D ,Sriram.,; P.Yogeeswari,S.Ananthan, "Antituberculous activity of norfloxacin mannich bases with isatin derivatives" Chemotherapy, 47, 266-269,2001.
9. T.D.Brewerton, ,J.Zealberg, J.Lydiard, R. B, Glover, V. Sandler, M,Ballenger, J. C. "CSF isatin is elevated in bulimia nervosa" Biol. Psychiatry, 37, 481-483,1995.
- 10.K.L Vine, J.Locke, J. M, Ranson, M, Benkendorff, K, Pyne, S. G. Bremner, J. B. In vitro cytotoxicity evaluation of some substituted isatin derivatives. Bioorg. Med. Chem., 15, 931-938, 2007.
- 11.K. LVine.,; Lidia Matesic; Locke, J. M.; Danielle Skropeta: Recent highlights in the development of Isatin based anticancer agents University of Wollongong, Research Online 2013
- 12.J.T.Nguyen, J.A. Wells, ,Direct activation of the apoptosis machinery as a mechanism to target cancer cells. Proc. Natl. Acad. Sci. U S A, 100, 7533-7538,2003
- 13.Y.Liu, H.Lashuel,. A. Choi, S. Xing, X. Case, A. Ni, J. Yeh, L. A. Cuny, G. D. Stein, R. L. Lansbury, P. T., Jr. Discovery of inhibitors that elucidate the role of UCH-L1 activity in the H1299 lung cancer cell line. Chem. Biol., 10, 837-846,2003
- 14.G.Bacher, B.Nickel, ,P.Emig, V.anhoefer, U,Seeber, S, Shandra, A, Klenner, T,Beckers, T. D-24851, a novel synthetic microtubule inhibitor, exerts curative antitumoral activity in vivo, shows efficacy toward multidrug-resistant tumor cells, and lacks neurotoxicity. Cancer Res., 61, 392-399,2001.
- 15.K.Vine., L. Locke, J. M. Ranson, M. Pyne, S.G. Bremner, J. B. An investigation into the cytotoxicity and mode of action of some novel N-alkyl-substituted isatins. J. Med. Chem., 50, 5109-5117,2007.

16. K. Vine, L.; Matesic, L.; Locke, J. M.; Ranson, M.; Skropeta, D. Cytotoxic and anticancer activities of isatin and its derivatives: a comprehensive review from 2000-2008. *Anticancer Agents Med Chem*, 9, 397-414, 2009.

17. L. Matesic, J. Locke, M. Bremner, J. B. Pyne, S. G. Skropeta, D. Ranson, M. K. L. Vine, N-phenethyl and N-naphthylmethyl isatins and analogues as in vitro cytotoxic agents. *Bioorg. Med. Chem.*, 16, 3118-3124, 2008

18. N. R. Modi, R. J. Shah, M. J. Patel, M. Suthar, B. Chauhan, F. Patel, L. J. Design, synthesis, and QSAR study of novel 2-(2,3-dioxo-2,3-dihydro-1H-indol-1-yl)-N-phenylacetamide derivatives as cytotoxic agents. *Medicinal Chemistry Research*, 20, 615-625, 2011.

19. P. Singh, S. Kaur, V. Kumar, P. Bedi, M. Mahajan, P. Sehar, I. Pal, H. C. Saxena, A. K. Synthesis and in vitro cytotoxic evaluation of N-alkylbromo and N-alkylphthalimido-isatins. *Bioorg Med Chem Lett.*, 21, 3017-3020, 2011