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Synthesis and in vitro cytotoxic evaluation of *N*-alkylbromo and *N*-alkylphthalimido-isatins

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ABSTRACT

The manuscript pertains to the synthesis and in vitro cytotoxic evaluation of a series of *N*-alkylbromo and *N*-alkylphthalimido-isatins against four different human cancer cell lines namely Colon: HCT-15; Liver: Hep-2; Lung: A-549 and Leukemia: THP-1 at 10 and 100 μM concentrations. The active compounds based on preliminary studies were evaluated for their IC_{50} value against six cell lines viz. Colo-205, HCT-15 (Colon), THP-1 (Leukemia), A-549 (Lung), PC-3 (Prostate) and HeLa (Cervix). The active analogue **IS-4** exhibited IC_{50} values of 4.57, 10.90, 11.75, 12.40 and 54.20 μM against HeLa, PC-3, HCT-15, THP-1 and Colo-205, respectively.

Cancer is known to be one of the major causes of mortality and the development of newer and potent anticancer agents is considered to be of great significance because of the problems like severe toxicity as well as resistance with the existing drugs. Anticancer agents exert their biological effect usually by targeting various intracellular targets; therefore, the current research in the area mainly focuses on therapeutic targets involved in cell proliferation. Inhibition of the cancer cell proliferation is one of the most effective principles in the treatment of cancer using chemotherapy. Many compounds presently used in cancer treatment affect proliferation and/or lead to cancer cell death via apoptosis, necrosis, or alike. Indole derivatives have been found to exhibit anticancer activity by interacting with different intracellular targets. Naturally occurring and synthetic indolocarbazoles indicated inhibitory activity against cyclindependent kinases (CDKs) and antiproliferative activities in a variety of cell lines.[1,2]

Isatin (1*H*-indole-2,3-dione) and its derivatives demonstrate a diverse array of biological and pharmacological activities including anticonvulsant, antibacterial, antifungal, antiviral and anticancer properties.[3] This broad spectrum of biochemical targets has been facilitated by the synthetic versatility of isatin, which has allowed the generation of a large number of structurally diverse derivatives including analogues derived from substitution of the aryl ring, and/or derivatisation of the isatin nitrogen and C2/C3 carbonyl moieties.[4] *N*-Alkylated indoles are well known to exhibit anticancer activity.[5] However, until recently, little has been reported on the antineoplastic activity of *N*-alkyl- and *N*-aryl-isatins. Recent revelations by Nguyen and Wells showed the apoptosis behaviour of *N*-alkyl isatins in a panel of human cancer cell lines.[6] The approach has been further extended by Vine et al. through introduction of various hydrophobic substituents like *N*-phenethyl, *N*-benzyl and *N*-2-naphthylmethyl on the parent nucleus that significantly increased their cytotoxicity towards lymphoma cells and against a wide range of human cancer cell lines, including MDA-MB-231 metastatic breast adenocarcinoma cells and U937 human monocyte-like histiocytic lymphoma cells.[7]

Thus in continuation of our interest towards the synthesis of novel heterocyclic compounds with diverse medicinal potential[8] and the lack of extensive literary reports on the cytotoxicity studies of *N*-alkylisatins, we report herein the synthesis and cytotoxic evaluation of novel *N*-alkylbromo and *N*-alkylphthalimido-isatins against four different human cancer cell lines namely Colon: HCT-15; Liver: Hep-2; Lung: A-549 and Leukemia: THP-1 at 10 and 100 μM concentrations. The alkyl chain length has been varied to ascertain the quantitative structure–activity relationship of the resulting scaffolds against a panel of human cancer cell lines. The replacement of bromo with phthalimido substituent has been done on the basis of literature rationale[7] as the anticancer potential increases with introduction of aryl substituent on *N*-alkylated isatins.

The procedure for the synthesis of the *N*-bromoalkyl derivatives was based on a combination of the literature methods.[9]

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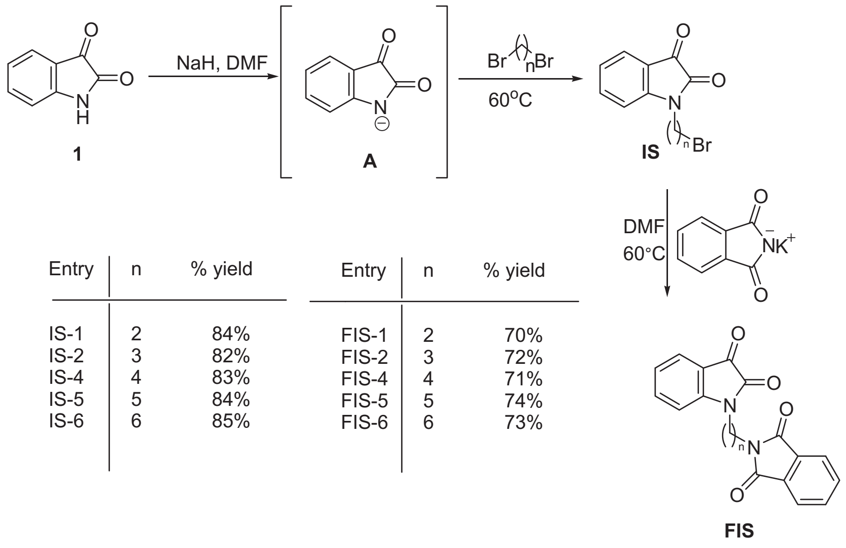
N-bromoalkyl isatin derivatives **IS** were synthesised by treating a solution of isatin in DMF with sodium hydride (NaH) resulting in the formation of intense purple coloured anion that was subsequently reacted with appropriate dibromoalkane (Scheme 1). The reaction mixture was then heated to 60 °C until the completion of reaction as evidenced by tlc. This upon quenching with water, extraction with ethyl acetate and subsequent concentration under reduced pressure resulted in the isolation of *N*-alkylbromo isatins (**IS 1**-**6**).

The anionic intermediate **A**, being an ambident anion could undergo *N*- or *O*-alkylation, however spectral evidences viz. IR, ^{1}H and ^{13}C spectroscopic studies exhibited exclusive *N*-alkylation.[12] The compound **IS**-**4**, for example, analyzed for $C_{12}H_{12}BrNO_2$ showed a molecular ion peak at 283. Its IR spectrum showed a sharp absorptions at 1607 and 1738 cm^{-1} corresponding to the two carbonyls of the isatin ring. Its ^{1}H NMR showed a four proton multiplet at δ 1.95 corresponding to two –CH$_2$, a multiplet at δ 3.45 corresponding to CH$_2$Br and another multiplet at δ 4.12 (J = 7.6 Hz) for –NCH$_2$ apart from the aromatic protons. The presence of requisite number of carbons in ^{13}C NMR further corroborates the assigned structure. Further, the compound **IS** upon stirring with potassium phthalimide in DMF at 60 °C resulted in the isolation of *N*-alkylphthlamido **FIS** in good yields (Scheme 1). The structures of **FIS** were established on the basis of analytical evidences and spectral data, the details of which are provided in the experimental section while salient features are mentioned here.[13] The compound **FIS**-**6**, for example, analyzed for $C_{22}H_{20}N_2O_4$ showed a molecular ion peak at 378. Its IR spectrum showed sharp absorptions at 1637, 1709, 1743 and 1768 cm^{-1} corresponding to the carbonyls. Its ^{1}H NMR spectrum showed a multiplet at δ 1.55 corresponding to –CH$_2$–CH$_2$–CH$_2$–CH$_2$, a triplet at δ 3.65 (J = 7.2 Hz) and another triplet at δ 4.06 (J = 7.5 Hz) apart from the aromatic protons. ^{13}C NMR further showed the presence of isatin amide carbonyl at 157.9, phthalimido carbonyls at 168.3 and an isatin keto-carbonyl at 181.7 along with the required carbons confirming the assigned structure.

The human cancer cell lines were procured from National Cancer Institute, Frederick, PO Box B, Frederick, MD 21702-1201, U.S.A. Cells were grown in tissue culture flasks in complete growth medium (RPMI-1640 medium with 2 mM glutamine, pH 7.4, supplemented with 10% fetal calf serum, 100 µg/ml streptomycin and 100 units/ml penicillin) in a carbon dioxide incubator (37 °C, 5% CO$_2$, 90% relative humidity). The cells at subconfluent stage were harvested from the flask by treatment with trypsin [0.05% in PBS (pH 7.4) containing 0.02% EDTA]. Cells with viability of more than 98% as determined by trypan blue exclusion, were used for determination of cytotoxicity. The cell suspension of 1×10^5 cells/ml was prepared in complete growth medium. Stock solutions (2×10^{-2} M) of compounds were prepared in DMSO. The stock solutions were serially diluted with complete growth medium containing 50 µg/ml of gentamycin to obtain working test solutions of required concentrations. The final DMSO concentration was 0.5% and 0.05% when the test material was used at 100 and 10 µM, respectively.

In vitro cytotoxicity against human cancer cell lines of different tissues was determined[10,11] using 96-well tissue culture plates. The 100 µL of cell suspension was added to each well of the 96-well tissue culture plate. The cells were allowed to grow in carbon dioxide incubator (37 °C, 5% CO$_2$, 90% RH) for 24 h. Test materials in complete growth medium (100 µL) were added after 24 h of incubation to the wells containing cell suspension. The plates were further incubated for 48 h in a carbon dioxide incubator. The cell growth was stopped by gently layering trichloroacetic acid (50%, 50 µL) on top of the medium in all the wells. The plates were incubated at 4 °C for 1 h to fix the cells attached to the bottom of the wells. The liquid of all the wells was gently pipetted out and discarded. The plates were washed five times with distilled water to remove trichloroacetic acid, growth medium low molecular weight metabolites, serum proteins, etc., and air-dried. The plates were stained with sulforhodamine B dye (0.4% in 1% acetic acid, 100 µL) for 30 min. The plates were washed five times with 1% acetic acid and then air-dried.[11] The adsorbed dye was dissolved in Tris–HCl Buffer (100 µL, 0.01 M, pH 10.4) and plates were gently stirred for 10 min on a mechanical stirrer. The optical density (OD) was recorded on ELISA reader at 540 nm. The cell growth was determined by subtracting mean OD value of respective blank from the mean OD value of experimental set. Percent growth in presence of test material was calculated considering the growth in absence of any test material as 100% and in turn percent growth inhibition in presence of test material was calculated.

The newly synthesized *N*-alkylbromo and *N*-alkylphthalimido derivatives were initially tested for their cytotoxicity against a panel of four human cancer cell lines including HCT-15, Hep-2, A-549 and THP-1 using Sulforhodamine B assay. The concentration dependent cytotoxicity of these compounds against human cancer cell lines is shown in Table 1. Adriamycin has been used as standard in case of A-549 and THP-1 while 5-fluorouracil and



Entry	n	% yield
IS-1	2	84%
IS-2	3	82%
IS-4	4	83%
IS-5	5	84%
IS-6	6	85%

Entry	n	% yield
FIS-1	2	70%
FIS-2	3	72%
FIS-4	4	71%
FIS-5	5	74%
FIS-6	6	73%

Scheme 1.

Table 1
In vitro cytotoxicity against human cancer cell lines

Serial No.	Compound	Concd (μM)	Tissue			
			Colon	Liver	Lung	Leukemia
			Cell lines			
			HCT-15	Hep-2	A-549	THP-1
			% Growth inhibition			
1	**IS-1** ($n = 2$)	10	4	14	38	14
		100	89	71	44	96
2	**IS-2** ($n = 3$)	10	59	36	0	80
		100	93	93	37	95
3	**IS-4** ($n = 4$)	10	62	33	30	90
		100	88	68	49	90
4	**IS-5** ($n = 5$)	10	62	40	0	85
		100	91	81	40	91
5	**IS-6** ($n = 6$)	10	67	48	0	94
		100	95	86	53	93
6	**FIS-1** ($n = 2$)	10	3	10	18	9
		100	86	68	43	96
7	**FIS-2** ($n = 3$)	10	1	11	0	8
		100	58	30	1	96
8	**FIS-4** ($n = 4$)	10	4	15	0	44
		100	89	73	36	92
9	**FIS-5** ($n = 5$)	10	9	10	4	60
		100	97	85	36	93
10	**FIS-6** ($n = 6$)	10	49	39	0	96
		100	91	90	37	95
	Adriamycin	1	–	–	55	96
	5-Fluorouracil	1	31	–	–	–
	Mitomycin-c	1	–	31	–	–

Table 2
IC_{50} determination of cytotoxicity of compounds against human cancer cell lines

S. No.	Compound	Tissue					
		Colon		Leukemia	Lung	Prostate	Cervix
		Cell lines					
		Colo-205	HCT-15	THP-1	A-549	PC-3	HeLa
		IC_{50} (μM)					
1	**IS-2**	>100	13.84	>100	>100	15.26	7.77
2	**IS-4**	54.20	11.75	12.40	>100	10.90	4.57
3	**IS-5**	>100	8.46	42.30	>100	11.45	5.49
4	**IS-6**	>100	12.38	26.50	>100	11.72	11.97
5	**FIS-6**	>100	9.73	>100	>100	12.77	8.82

mitomycin-C have been used as standards in case of HCT-15 and Hep-2 cancer cell lines, respectively, for evaluating the cytotoxic profiles of the newly synthesized scaffolds. Although, the test compounds showed comparatively poor cytotoxic profiles compared to the standard agents, however, they exhibited an interesting structure–activity relationship and substituent effect with considerable% growth inhibition at 10 μM. The results clearly revealed that the *N*-alkylbromo compounds showed concentration dependent cytotoxicity in case of HCT-15, Hep-2 and THP-1 with substantial increase in growth inhibition as the concentration increases from 10 to 100 μM. Further, the cytotoxicity increases by increasing carbon linkers between nitrogen and bromo group as evidenced by compounds **IS-2**, **IS-4**, **IS-5** and **IS-6** which showed maximum growth inhibition in case of HCT-15, Hep-2 and THP-1 while the compound **IS-1** showed poor growth inhibition. The compound **IS-1** and **IS-4** showed moderate growth inhibition against A-549 while the other *N*-alkyl bromo derivatives showed practically no cytotoxicity against the same cell line. The *N*-alkylphthalimido-isatins have shown considerably poor cytotoxicity at 10 μM when compared with *N*-alkyl bromo derivatives; however considerable cytotoxicity was noticed as the concentration is increased from 10 to 100 μM. Further, the cytotoxicity of *N*-alkylphthalimido-isatins also increased with the increase in alkyl chain length as evidenced by the comparable cytotoxic profiles of the compound **FIS-6** with that of *N*-alkylbromo derivatives viz. **IS-2**, **IS-4**, **IS-5** and **IS-6**. Compound **FIS-1**, **FIS-2**, **FIS-4** and **FIS-5** showed poor cytotoxicity against HCT-15, Hep-2, A-549 cancer cell lines while the compound **FIS-6** showed moderate cytotoxicity against liver and lung cancer cell lines. At 100 μM compound, **FIS-1**, **FIS-2**, **FIS-4**, **FIS-5** showed moderate to good cytotoxicity against HCT-15, Hep-2, THP-1 but poor cytotoxicity against A-549 cancer cell lines.

Among the ten compounds evaluated for their cytotoxicity, **IS-2**, **IS-4**, **IS-5**, **IS-6** and **FIS-6** showed maximum cell growth inhibition at 10 μM concentration against the THP-1 cell line and were further evaluated for their IC_{50} values, which is the concentration required to inhibit 50% of cell viability by the test compounds after exposure to cells, as summarized in Table 2. The cytotoxic effect of these compounds was evaluated against six cell lines, Colo-205, HCT-15, THP-1, A-549, PC-3 and HeLa. The effect was more pronounced against HeLa followed by PC-3 cell line. The most active analogue in the *N*-bromoalkyl series was **IS-4** which exhibited IC_{50} values of 4.57, 10.90, 11.75, 12.40 and 54.20 μM against HeLa, PC-3, HCT-15, THP-1 and Colo-205, respectively, as compared to other compounds of the series. However, all the compounds were devoid of any cytotoxic activity against A-549.

The present communication describes the synthesis and cytotoxic evaluation of a number of *N*-alkylbromo and *N*-alkylphthaimido-Isatins against a range of human cancer cell lines. During initial studies, all compounds of *N*-alkylbromo series viz. **IS-1**, **IS-2**, **IS-4**, **IS-5**, **IS-6** and one of the *N*-alkylphthalimido derivatives **FIS-6**, showed good cytotoxicity against HCT-15, Hep-2, THP-1 cancer cell lines with effects more pronounced in case of THP-1. These preliminary results indicate a marked dependence of cytotoxicity on the alkyl chain length as well as a preference for bromo substitution. The potent compounds were further evaluated for their IC_{50} values against six human cancer cell lines and all the compounds have shown good cytotoxic profiles in case of HeLa. **IS-4** and **IS-5** were the two most active compounds against all the cell lines tested except A-549 and Colo-205 and have IC_{50} values of 4.57 and 5.49 μM against HeLa cells, respectively. The results described in the present manuscript indicate that these compounds could serve as the basis for the development of a new group of cancer chemotherapeutics.

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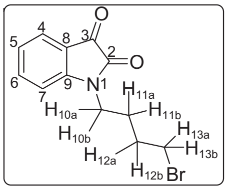
12. *General procedure and analytical data for N-alkyl bromo isatin* ***IS:*** To a stirred suspension of Sodium hydride (1.5 mmol) in dry DMF (10 ml) was added isatin (1 mmol) and the resulting solution was stirred at room temperature for 20 min before the addition of dibromoalkane (1 mmol). The reaction mixture was heated to 60 °C with constant stirring for about 2 h. After the completion of reaction, as evidenced by tlc, it was quenched by drop wise addition of water (20 ml) and extracted with ethyl acetate (3 × 30 ml). The combined organic layers were washed with brine solution, dried over anhydrous Na_2SO_4 and concentrated in vacuo. Purification of the reaction mixture by column chromatography using hexane: ethyl acetate (8:2) furnished the desired *N*-alkylbromo derivatives in good yields. *1-(4-Bromo-butyl)-1H-indole-2,3-dione* (**IS-4**): yellow solid, mp 100–102 °C.
   ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.95 (m, 4H, $H_{11a,11b}$, $H_{12a,12b}$), δ 3.45 (m, 2H, $H_{13a,13b}$), δ 4.12(m, 2H, $H_{10a,10b}$), δ 7.54 (m,4H, Ar-H). ^{13}C NMR: δ 28.09, 29.52, 36.60, 41.04, 117.75, 120.77, 124.21, 129.95, 139.31, 144.62, 157.96, 181.46. ν_{max} (KBr)/cm^{-1} 1607, 1738 MS (EI) *m*/*z* 283 (M^+). Calcd for $C_{12}H_{12}BrNO_2$: C, 51.09; H, 4.29; N, 4.96. Found: C, 51.32, H, 4.62, N, 4.70.



13. *Procedure and analytical data for N-alkylphthalimido* ***FIS***: To a stirred solution of *N*-bromoisatin (**IS**) (1 mmol) in anhydrous DMF was added potassium phthalimide (2 mmol) and the reaction mixture was heated to 60 °C for 2 h. On completion as monitored by tlc, water (20 ml) was added to the reaction mixture and extracted with ethyl acetate (3 × 30 ml). Combined organic layers were washed with brine solution, dried over anhydrous Na_2SO_4 and concentrated under reduced pressure to yield crude yellow solid which was then recrystallized by using hexane:ethyl acetate (5:5).
   1-[6-(1,3-Dioxo-1,3-dihydro-isoindol-2-yl)-hexyl]-1H-indole-2,3-dione (**FIS-6**): yellow solid, mp 80–83 °C.
   ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.55 (m, 8H, $H_{11a,11b}$, $H_{12a,12b}$,$H_{13a,13b}$,$H_{14a,14b}$), δ 3.65 (t, 7.2 Hz, 2H, $H_{15a,15b}$), δ 4.06 (t, 7.5 Hz, 2H, $H_{10a,10b}$), δ 7.75(m, 8H, ArH). ^{13}C NMR: δ 25.94, 26.32, 28.28, 29.20, 37.65, 41.80, 117.73, 120.75, 123.20, 123.48, 124.06, 129.67, 132.03, 133.84, 134.20, 139.21, 140.38, 144.83, 157.90, 168.35, 181.71. ν_{max} (KBr)/cm^{-1} 1637, 1709, 1743, 1768 MS (EI) *m*/*z* 378 (M^+). Calcd for $C_{22}H_{20}N_2O_4$: C, 70.20; H, 5.36; N, 7.44. Found: C, 70.56, H, 5.72, N, 7.21.