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Iodine-catalyzed condensation of isatin with indoles: A facile synthesis of di(indolyl)indolin-2-ones and evaluation of their cytotoxicity

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ABSTRACT

Isatin reacts smoothly with indoles in the presence of a catalytic amount of molecular iodine under mild conditions to afford a novel class of di(indolyl)indolin-2-one derivatives in good yields. These molecules are found to possess a promising cytotoxicity against cancer cells only but not on normal cells.

Indoles and its derivatives are found abundantly in Nature and are known to exhibit potent physiological properties.[1] In particular, 3,3-diaryloxindole is frequently found in clinical drugs and biologically active compounds and are known to possess anti-proliferative, antibacterial, anti-protozoal and anti-inflammatory activities.[2] They have also been used as laxatives[3] and some lead molecules are known to facilitate Ca^{2+} depletion mediated inhibition of translation initiation.[4] Furthermore, a large number of bis(indolyl)methanes have been isolated from natural sources.[5] Some of these natural products for example, vibrindole A have shown promising biological activity.[6] Generally, 3,3-di(indolyl)indolin-2-ones are prepared by coupling of isatin with indoles under acidic conditions.[7,8] However, only a few methodologies has been reported for the synthesis of di(indolyl)indolin-2-ones.[9]

Therefore, the development of simple and efficient methods is desirable to generate structurally varied di(indolyl)indolin-2-ones with a variety of substituents.

Recently, molecular iodine has received considerable attention in organic synthesis because of its high tolerance to air and moisture, low-cost, nontoxic nature and ready availability, affording the corresponding products in excellent yields with high selectivity. The mild Lewis acidity associated with iodine has led to its use in organic synthesis using catalytic to stoichiometric amounts.[10]

In continuation of our interest on catalytic use of molecular iodine for various transformations,[11] we herein report for the first time, a direct and metal-free condensation of isatin with indoles using a catalytic amount of molecular iodine. Accordingly, we first attempted the coupling of 1 equiv of isatin (**1**) with 2 equiv of indole (**2**) in the presence of 10 mol % of molecular iodine. The reaction proceeds well in dichloromethane at room temperature and the corresponding di(indolyl)indolin-2-one **3a** was obtained in 82% yield (Scheme 1).

Next we examined the scope of this method with respect to various indoles. Interestingly, substituted indoles such as 5-cyano-, 2-methyl-5-nitro-, methyl 5-carboxylate, 2-methyl-, 5-nitro-, 2-phenyl-, 5-bromo-, 5-chloro-, and 1-methyl derivatives underwent a smooth coupling with isatin to furnish the corresponding di(indolyl)indolin-2-one derivatives in good yields (Table 1, entries b–j). Notably, electron-deficient indoles such as 2-methyl-5-nitro-, 5-cyano-, methyl 5-carboxylate and 5-nitro-, derivatives also participated well coupling with isatin under similar conditions (Table 1, entries b, c, d and f). Furthermore, *N*-methylindole also gave the desired product in 85% yield (Table 1, entry j). This method works well with both electron-rich as well as electron-deficient indoles. The products were characterized and confirmed by NMR, IR, and mass spectroscopy. In all cases, the reactions proceeded well at room temperature affording the corresponding di(indolyl)indolin-2-ones in good yields. However, in the absence of iodine, the reaction failed to give the desired

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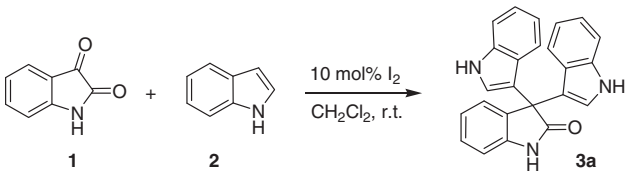


Scheme 1. Synthesis of di(indolyl)indolin-2-one **3a**.

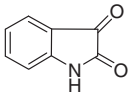
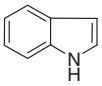
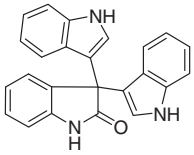
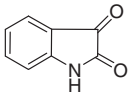
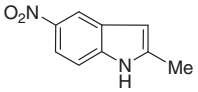
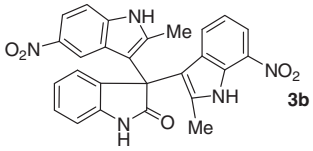
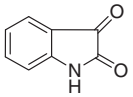
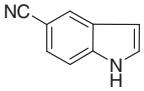
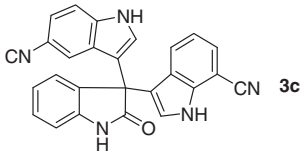
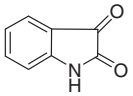
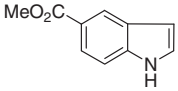
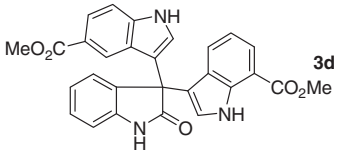
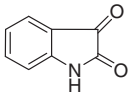
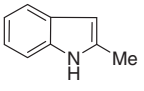
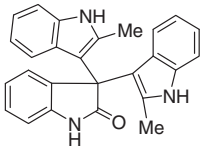
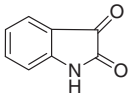
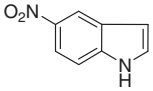
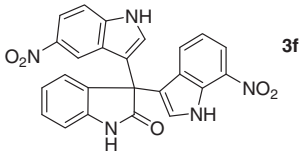
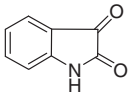
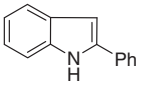
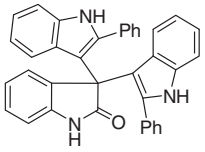
products. This clearly indicates that molecular iodine is essential to facilitate the reaction. The scope and generality of this process is illustrated with respect to various indoles and the results are presented in Table 1.[12] The salient features of this method are good yields, mild reaction conditions and low-cost of the catalyst.

Mechanistically, we assume that the reaction proceeds by the activation of isatin by molecular iodine. This is followed by a nucleophilic attack of indole on isatin led to the formation of 3-hydroxy-3,3'-biindolin-2-one. A subsequent dehydration of 3-hydroxy-3,3'-biindolin-2-one (**A**) and addition of another equivalent of indole on a conjugated 3*H*-indol-3-ylidene (**B**) would give the desired di(indolyl)indolin-2-one (Scheme 2).

Cytotoxicity: Cellular viability in the presence of test compounds was determined by MTT-microcultured tetrazolium assay following the reported protocol.[13] Human lung cancer cell line, A549; human breast cancer cell line, MDA-MB-231; (estrogen receptor-negative); and a human neuroblastoma cell line, SK-N-SH along with a MRC5, human normal lung cell line are employed in the current study.

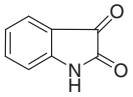
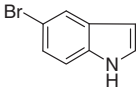
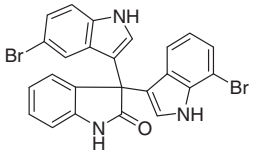
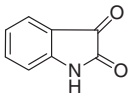
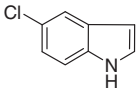
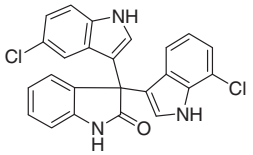
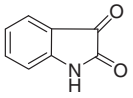
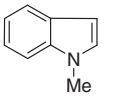
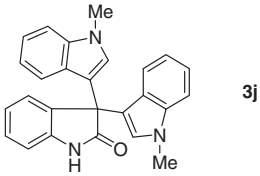
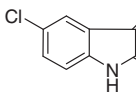
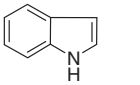
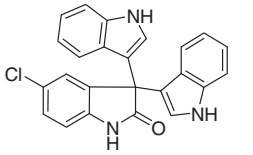
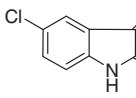
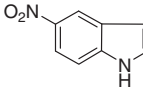
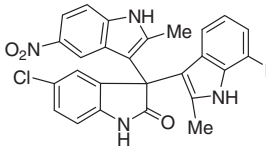
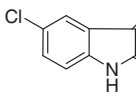
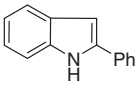
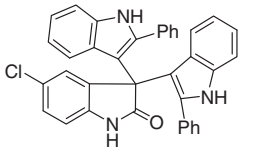
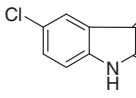
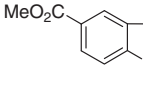
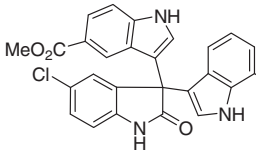
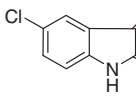
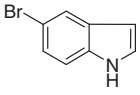
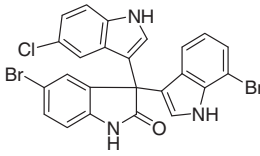
All the four types of cell lines were seeded to flat bottom 96 (10,000 cells/100 μl) well plate and cultured in the medium containing 10% serum and incubated for 24 h in a 5% CO_2 humid chamber so that the cells can adhere to the surface. 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) was dissolved in PBS at 5 mg/mL and sterile filtered. Different concentrations of the compounds were added to the adhered cells. After 48 h, MTT solution (10 μl per well) was added to the culture plate. Cells were further incubated in the CO_2 chamber for 2 h. Following this, media was removed and 100 μl of DMSO was added. Absorbance was measured at 562 nm in a multimode microplate

Table 1
Synthesis of di(indolyl)indolin-2-one derivatives from isatin and indoles

Entry	Isatin (**1**)	Indole (**2**)	Product (**3**)[a]	Time (h)	Yield[b] (%)
a			 **3a**	14	82
b			 **3b**	16	78
c			 **3c**	18	85
d			 **3d**	15	80
e			 **3e**	16	75
f			 **3f**	18	80
g			 **3g**	20	82

(*continued on next page*)

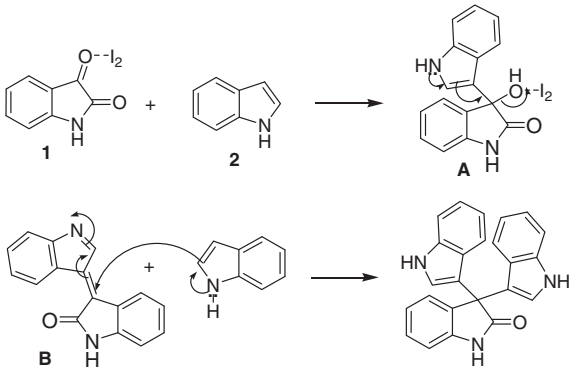
Table 1 (*continued*)

Entry	Isatin (**1**)	Indole (**2**)	Product (**3**)[a]	Time (h)	Yield[b] (%)
h			 **3h**	18	85
i			 **3i**	22	70
j			 **3j**	19	85
k			 **3k**	12	68
l			 **3l**	14	70
m			 **3m**	13	75
n			 **3n**	14	74
o			 **3o**	15	80

[a] The products were characterized by NMR, IR and mass spectroscopy.
[b] Yield refers to pure products after chromatography.

reader (Tecan GENios). All the experiments were carried out in triplicates.

The IC_{50} values are calculated from the percentage of cytotoxicity and are presented in Table 2. Except compound **3m** (Table 2, entry 13), all compounds in the present study display systematic cytotoxicity at near μM concentration on the neuroblasoma cell lines (SK-N-SH). More than 50% of the compounds inhibit the cell growth in the A549 cell lines near one μM concentration. Only five compounds affect the breast cancer cell lines (MDA-MB 231) in the sub-micromolar range. Surprisingly, none of the compounds (Table 2) possess any cytotoxicity on the non-cancerous breast cells (MRC5). Biochemical studies are underway to understand



Scheme 2. A plausible reaction pathway.

Table 2
Cytotoxic study of products **3a**–**o**

S. No.	Product (3)	MDA-MB 231 (μM)	SK-N-SH (μM)	A549 (μM)	MRC5 (μM)
1	**3a**	>100	0.90 ± 0.06	1.23 ± 0.030	>100
2	**3b**	99.42 ± 7.5	0.94 ± 0.04	0.89 ± 0.037	>100
3	**3c**	99.14 ± 5.5	0.94 ± 0.06	0.97 ± 0.127	>100
4	**3d**	>100	0.98 ± 0.04	0.75 ± 0.04	>100
5	**3e**	>100	0.98 ± 0.03	0.92 ± 0.06	>100
6	**3f**	9.98 ± 0.31	0.99 ± 0.03	7.90 ± 0.26	>100
7	**3g**	>100	9.6 ± 0.72	8.91 ± 0.17	>100
8	**3h**	9.95 ± 0.32	0.93 ± 0.10	0.96 ± 0.4	>100
9	**3i**	10.00 ± 0.18	1.31 ± 0.08	>100	>100
10	**3j**	>100	1.32 ± 0.24	>100	>100
11	**3k**	9.99 ± 0.20	1.28 ± 0.01	76.3 ± 0.7	>100
12	**3l**	10.05 ± 0.34	1.23 ± 0.11	69.4 ± 0.264	>100
13	**3m**	>100	>100	>100	>100
14	**3n**	10.00 ± 0.13	9.9 ± 0.24	71.5 ± 0.751	>100
15	**3o**	>100	1.04 ± 0.14	>100	>100
16	Isatin	>100	9.84 ± 0.16	13.5 ± 0.3	>100
17	Indole	>100	1.78 ± 0.42	>100	15.58 ± 0.35
18	Standard (Doxorubicin)	8.14 ± 0.14	0.97 ± 0.03	15.07 ± 0.13	14.84 ± 0.25

the biological pathways that are affected by these compounds specifically in cancer cells and not in normal cell lines.

In summary, we have developed a novel method for the synthesis of di(indolyl)indolin-2-one derivatives by means of a coupling of isatin with indoles using a catalytic amount of molecular iodine under mild conditions. This method is simple and convenient to prepare a wide range of di(indolyl)indolin-2-one derivatives which are found to possess strong cytotoxicity against only cancer cell lines and not normal ones. This provides a platform for further development of these compounds into promising anticancer agents.

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12. *General procedure*: To a stirred solution of isatin (1 mmol) and indole (2 mmol) in dry dichloromethane (4 mL) was added molecular iodine (26 mg, 10 mol %) at room temperature. The resulting mixture was stirred at room temperature for the appropriate time (Table 1). After complete conversion, as indicated by TLC, the reaction mixture was quenched by saturated sodium thiosulphate solution (4 mL) and extracted with dichloromethane (3 × 10 mL). The combined organic layers were dried over anhydrous Na_2SO_4, concentrated in vacuo and purified by column chromatography on silica gel (Merck, 60–120 mesh, ethyl acetate–hexane, 3:7) to afford the pure di(indolyl)indolin-2-one. Spectral data for the selected products: 2′-Methyl-3-(2-methyl-5-nitro-1*H*-indol-3-yl)-7′-nitro-3,3′-biindolin-2-one (**3b**, Table 1): White solid, mp 340–342 °C; ^{1}H NMR (500 MHz, DMSO): δ 11.26 (d, 2H, *J* = 7.3 Hz), 10.5–10.6 (br s, 1H), 7.82 (d, 2H, *J* = 9.4 Hz), 7.75 (s, 1H), 7.61 (s, 1H) 7.29 (d, 2H, *J* = 7.3 Hz), 7.26 (t, 1H, *J* = 7.3 Hz), 7.15 (d, 1H, *J* = 7.3 Hz), 7.03 (d, 1H, *J* = 7.3 Hz), 6.91 (t, 1H, *J* = 7.3 Hz), 2.10 (s, 3H), 2.01 (s, 3H); ^{13}C NMR (300 MHz, $CDCl_3$ + DMSO): δ 178.23, 141.0, 140.0, 135.1, 137.1, 136.6, 133.4, 128.1, 126.2, 126.0, 125.1, 121.4, 116.3, 116.1, 115.3, 115.2, 111.9, 111.5, 110.3, 109.8, 12.95, 12.9; IR (KBr): *v* 3325, 3238, 1690, 1472, 1328 cm^{-1}; ESI (MS): *m*/*z*: 499 (M+NH_4); 504(M+Na); HRMS calcd for $C_{26}H_{19}N_3O_5Na$: 504.1260, found, 504.1283. Methyl 3′-(5-(methoxycarbonyl)-1*H*-indol-3-yl)-2′-oxo-3,3′-biindoline-7-carboxylate (**3d**, Table 1): White solid, mp 255–257′°C; ^{1}H NMR (500 MHz, DMSO): δ 10.92–10.96 (br s, 2H), 10.11–10.55 (br s, 1H), 8.08 (s, 2H), 7.69 (d, 2H, *J* = 8.5 Hz), 7.35 (d, 2H, *J* = 8.5 Hz), 7.19 (t, 2H, *J* = 8.5 Hz), 7.02 (d, 1H, *J* = 7.6 Hz), 6.94 (d, 2H, *J* = 1.9 Hz), 6.91 (t, 1H, *J* = 7.6 Hz), 3.78 (s, 6H); ^{13}C NMR (300 MHz, $CDCl_3$ + DMSO): δ 178.3, 167.1, 141.2, 139.6, 133.7, 128.3, 126.2, 125.0, 124.7, 123.5, 122.1, 121.7, 119.9, 115.6, 111.7, 109.8, 51.6, 52.3; IR (KBr): *v* 3335, 3236, 1700, 1615, 1270, 1122 cm^{-1}; ESI (MS): *m*/*z*: 497 (M+NH_4); 502(M+Na); HRMS calcd for $C_{28}H_{21}N_3O_5Na$: 502.1358, found: 502.1378. 7′-Nitro-3-(5-nitro-1*H*-indol-3-yl)-3,3′-biindolin-2-one (**3f**, Table 1): White solid, mp 350–352 °C; ^{1}H NMR (500 MHz, DMSO): δ 11.27–11.39 (br s, 2H), 10.5–10.7 (br s, 1H), 8.31 (s, 2H), 7.93 (d, 2H, *J* = 8.3 Hz), 7.43 (d, 2H, *J* = 8.3 Hz), 7.23 (d, 2H, *J* = 7.3 Hz), 7.10 (s, 2H), 7.06 (d, 2H, *J* = 7.3 Hz), 6.96 (t, 1H, *J* = 7.3 Hz); ^{13}C NMR (300 MHz, $CDCl_3$ + DMSO): δ 177.7, 140.8, 139.9, 132.6, 128.3, 127.9, 124.6, 124.3, 121.8, 117.2, 116.4, 112.1, 109.8, 59.4, 51.7, 20.4, 13.7; IR (KBr): *v* 3325, 3238, 1690, 1245, 1127; ESI(MS): *m*/*z*: 471(M+NH_4); 476 (M+Na); HRMS calcd for $C_{24}H_{15}N_5O_5Na$: 476.099, found: 476.097.
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