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Synthesis of 3,3-diindolyl oxyindoles efficiently catalysed by $FeCl_3$ and their in vitro evaluation for anticancer activity

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ABSTRACT

A simple and highly efficient method has been developed for the synthesis of 3,3-diindolyl oxyindoles by the reaction of indoles with isatin or 5-fluoro isatin using a catalytic amount (5 mol %) of $FeCl_3$ at room temperature in a short reaction time in high yields. All these compounds were evaluated against a panel of five human cancer lines and most of them showed potent cytotoxicity. Compound **4b** showed IC_{50} of 4.7 and 5 μM against SK-N-SH and DU-145 cell lines, respectively, whereas **4c**, **4d**, **4f** and **4k** showed IC_{50} of 2.2, 1.2, 3.6 and 3.6 μM, respectively, against DU-145 cell line. Interestingly, some of the compounds are selectively potent in prostate cancer (DU-145) with IC_{50} values of 1.2–19.6 μM.

Cancer has become the second cause of mortality in the world and the development of potent and specific anticancer agents is urgently needed 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, current research mainly focuses on therapeutic targets involved in cell proliferation. However, identification of the exact target for particular class of compounds for their anticancer activity is one of the challenges to improve efficacy. Indole derivatives have been found to exhibit anticancer activity by interacting with different intracellular targets. Naturally occurring and synthetic indolocarbazoles indicated inhibitory activity against cyclin-dependent kinases (CDKs) and antiproliferative activities in a variety of cell lines.[1,2] Recently, bis(indolyl)maleimides (**1**, Fig. 1) have been identified as NAD^+-dependent histone deacetylases (HDAC) inhibitors.[3] Most recently, 3-arylidineindoline-2-one analogues (**2**), containing an oxyindole scaffold exhibited potential HDAC inhibitory activity.[4] Furthermore, oxyindole hybrids (**3**) showed micromolar activity against lung cancer cells by partial depletion of intracellular Ca^{2+} stores and phosphorylation in a growth inhibition assay.[5] In addition, bis-indoles and a number of indole derivatives are reported to display potent anticancer activity.[6a–g]

On the basis of previous studies[3–6] on oxyindoles and bisindolyl maleimides it was speculated that combining the structural characteristics of both these moieties could produce considerable enhancement in the anticancer activity of such compounds. This prompted us to design molecules wherein the two indolyl moieties are linked to the same carbon atom of an indoline-2-one. Keeping this objective in mind bis-indole derivatives, namely 3,3-diindolyl oxyindoles have been prepared and evaluated for anticancer activity. Further in continuation to our previous studies in search of potent molecules that exhibited anticancer activity,[7] we have

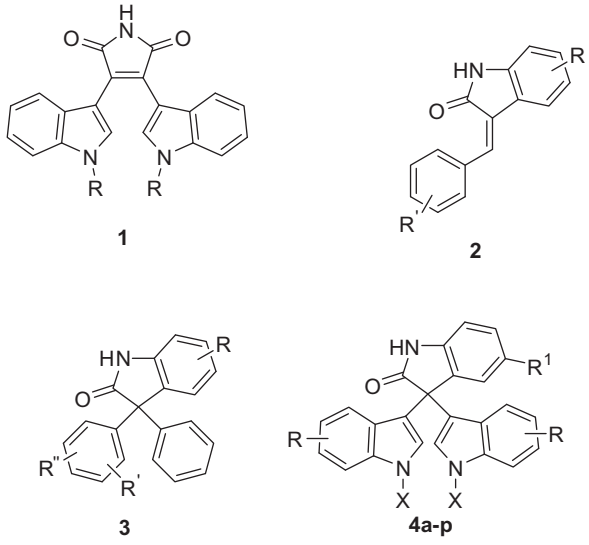

Figure 1. Chemical structures of anticancer indole derivatives.

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synthesized 3,3-diindolyl oxyindoles employing $FeCl_3$ as the catalyst. Various synthetic methods for the preparation of 3,3-diindolyl oxyindoles have been reported in the literature.[8a–f] However, the present method improved the yields using a new catalyst and the procedure is simple as well as convenient. In recent years iron(III) chloride is found to be a powerful Lewis acid catalyst in various organic transformations[9a–i] under mild reaction conditions, moreover iron salts are cheaper with low toxicity, eco-friendly and hence this is considered as a practical catalyst in a number of organic reactions. As a part of our research programme on evaluation of biological activity for different heterocyclic scaffolds, we herein report a new and efficient method to prepare oxyindoles[10] under mild conditions and evaluated them for their anticancer potential.

We first attempted the reaction of isatin with indole and reaction was carried out using 5 mol % anhydrous $FeCl_3$ in acetonitrile for <1 h at room temperature. Later considering the encouraging results we expanded our attention towards a various indoles and isatins to produce 3,3-diindolyl oxyindole derivatives in high yields. The reaction pathway probably involves the activation of carbonyl group of isatin moiety as well as indole ring by Fe(III), and this is similar to that has been reported earlier.[8c] Both electron donating and electron withdrawing substituents on indoles, reacted effectively with isatin under the same reaction conditions (Scheme 1, Table 1). Whereas, electron deficient substituents on indole moiety (**4g** and **4o**) required longer reaction times to afford corresponding oxyindoles with lower yields than those of their electron-rich counterparts (**4b–d**, **4j** and **4k**). However, halogen substituents on indole and isatin did not show much effect on the reaction times and yields of the desired products. Further, *N*-methyl indole also reacted with isatin and 5-fluoroisatin efficiently to furnish corresponding 3,3-diindolyl oxyindoles (**4h** and **4p**) in high yields.

All the synthesized compounds were evaluated for in vitro cytotoxicity against a panel of five human cancer cell lines including lung (A-549), CNS (SK-N-SH), breast (MCF-7), liver (Hep-2) and prostate (DU-145) by using MTT assay.[11] IC_{50} values (in μM), which is the concentration required to inhibit 50% of cell viability by the test compounds after exposure to cells, have been determined. Results in Table 2 indicate that most of the compounds displayed good anticancer potency against the cell lines tested in the assay. Compounds **4c, 4d, 4f** and **4k** showed excellent anticancer potency with IC_{50} values of 2.2, 1.2, 3.6 and 3.6 μM, respectively, against prostate cancer cell line DU-145 cell line in comparison to structurally related analogues, **3** (GI_{50} = 1.08–12.51 μM) against DU-145 cell line.[5] In most cases, methoxy substituents in the 4-, 5- and 6-position of the indole ring led, for **4b–d**, to significant increase in potency compared to other analogues. Compounds possessing methoxy group in the 5- and 6-position (**4c** and **4d**) showed higher potency in DU-145 with IC_{50} values of 2.2 and 1.2 μM, respectively, compared to **4b** having methoxy group in the 4-position which exhibited IC_{50} value of 5 μM in the same cell line. On the other hand, introduction of a fluoro substituent in the 5-position of the oxyindole ring led, for **4j–l**, to reduction in potency to some extent particularly against liver cancer cell line (Hep G-2) with IC_{50} values in the range of 16.0–36.6 μM. Compounds with nitro substituents at indole ring (**4g** and **4o**) also exhibited significant activity against DU-145 cell line with IC_{50} values of 8.0 and 7.5 μM, but found to be inactive against breast cancer cell line MCF-7. Compound **4n** with bromo substituent at indole ring selectively more active in SK-N-SH cell line with

Table 1
$FeCl_3$ catalysed synthesis of 3,3-diindolyl oxyindoles[a] (**4a–p**)

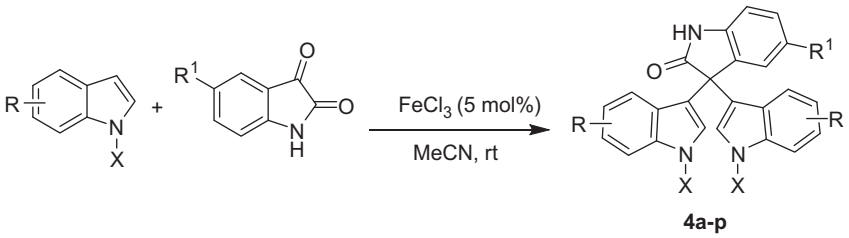
Compd	R	R^1	X	Time (min)	Yield[b] (%)
4a	H	H	H	15	93
4b	4-OCH_3	H	H	10	82
4c	5-OCH_3	H	H	10	95
4d	6-OCH_3	H	H	10	92
4e	5-Cl	H	H	30	93
4f	5-Br	H	H	15	89
4g	5-NO_2	H	H	15	81
4h	H	H	CH_3	20	90
4i	H	F	H	10	82
4j	4-OCH_3	F	H	10	80
4k	5-OCH_3	F	H	10	78
4l	6-OCH_3	F	H	30	85
4m	5-Cl	F	H	10	94
4n	5-Br	F	H	50	92
4o	5-NO_2	F	H	60	84
4p	H	F	CH_3	20	92

[a] All the reactions were performed using 5 mol % catalyst.
[b] Yields refer to isolated products.

Table 2
Anticancer potency of compounds **4a–p** in selected human cancer cell lines

Compd	IC_{50} (μM)[a]				
	A549[b]	SK-N-SH[c]	MCF-7[d]	Hep G-2[e]	DU-145[f]
4a	8.6	11.3	49.8	20.4	8.7
4b	15.3	4.7	14.1	9.0	5.0
4c	11.2	3.9	33.6	30.2	2.2
4d	7.8	13.6	45.4	12.0	1.2
4e	8.3	9.4	—[g]	14.6	8.1
4f	8.0	13.2	46.8	15.8	3.6
4g	10.3	11.8	—[g]	23.4	8.0
4h	10.1	5.1	—[g]	12.0	5.6
4i	12.7	67.3	23.6	6.6	12.8
4j	7.8	12.1	7.5	18.0	9.4
4k	10.6	7.0	—[g]	36.6	3.6
4l	14.4	4.1	12.0	16.0	13.1
4m	12.1	8.5	24.8	16.6	19.6
4n	10.3	2.8	12.8	12.8	16.3
4o	7.7	6.8	—[g]	23.8	7.5
4p	17.5	6.5	9.2	13	11

[a] Mean values from three separated experiments.
[b] Lung cancer.
[c] CNS cancer.
[d] Breast cancer.
[e] Liver cancer.
[f] Prostate cancer.
[g] Inactive up to concentration 100 μM.



Scheme 1. Synthesis of 3,3-diindolyl oxyindoles (**4a–p**).

an IC_{50} value of 2.8 μM, where as fluorine substituent did not alter the potency of the compounds. Incorporation of a methyl group in 1-position of indole moiety (**4h** and **4p**) also displayed considerable cytotoxicity in all most all cell lines tested.

Notably, some of the compounds displayed marked potency selectively against prostate cancer cell line and CNS cancer cell line with IC_{50} value up to 1.2 and 2.8 μM, respectively. It is expected that these compounds which resemble with indole derivatives (**1–3**), could exhibit anticancer activity by acting as HDAC or CDK inhibitors. However, further studies by structural modifications in both isatin and indole moieties to improve the anticancer efficacy is likely to provide an insight in to the mechanism of action of diindolyl oxyindoles.

In conclusion, we have developed a simple and highly efficient method for the conversion of 3,3-diindolyl oxyindoles from indole and isatin using 5 mol % of $FeCl_3$ in high yields. The advantages of this method over previous reports include its simplicity, clean reactions, high yields, shorter reaction times and use of inexpensive catalyst. These compounds were screened for their anticancer potency and compounds **4b**, **4c**, **4f** and **4k** have exhibited potential anticancer potency thereby suggesting that these scaffolds could be further developed as possible anticancer agents by the structural modification in both indole and oxyindole moieties for improving the anticancer efficacy.

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10. *General*: To a mixture of isatin (1 mmol) and indole (2 mmol), $FeCl_3$ (5 mol %) was added. The reaction mixture was stirred at room temperature for the appropriate time (Table 1). After completion of the reaction as monitored by TLC the solvent was removed under vacuum and quenching with a saturated solution of $NaHCO_3$ and the products were extracted into ethyl acetate (3 × 30 mL). The combined organic layers were washed with water, dried over anhydrous Na_2SO_4 and concentrated in vacuo. Purification by column chromatography using hexane/ethyl acetate (9:1) furnished the corresponding 3,3-diindolyl oxyindoles. **4d**: White solid, mp 242–243 °C; ^1H NMR (300 MHz, DMSO-d_6): δ 10.7 (br s, 2H, NH), 10.5 (br s, 1H, NH), 7.25 (d, *J* = 8.3 Hz, 3H, Ar–H), 7.20 (d, *J* = 7.6 Hz, 1H, Ar–H), 7.01–6.89 (m, 2H, Ar–H), 6.84 (d, *J* = 3.0 Hz, 2H, Ar–H), 6.73–6.64 (m, 4H, Ar–H), 3.52 (s, 6H, OCH_3). ^{13}C NMR (75 MHz, DMSO-d_6): 178.3, 152.4, 141.4, 134.6, 132.2, 127.8, 126.4, 125.2, 124.9, 121.5, 113.6, 112.1, 110.4, 109.4, 103.4, 55.1, 52.6; LRMS (ESI, *m*/*z*) 424 $(M+1)^+$; IR (KBr) (ν_{max}/cm^{-1}): 3382 (NH), 1686 (C=O), 1481, 1213 (C–O), 804, 755.
4o: Pale yellow solid, mp 333–334 °C; ^1H NMR (300 MHz, DMSO-d_6): δ 11.8 (br s, 2H, NH), 11.0 (br s, 1H, NH), 8.23 (s, 2H, Ar–H), 7.97 (d, *J* = 9.8 Hz, 2H, Ar–H), 7.58 (d, *J* = 8.9 Hz, 2H, Ar–H), 7.25 (s, 2H, Ar–H), 7.20–7.00 (m, 3H, Ar–H). ^{13}C NMR (75 MHz, DMSO-d_6):178.0, 159.7, 156.6, 140.3, 137.4, 134.6, 128.5, 124.5, 117.4, 116.8, 116.1, 115.1, 112.8, 112.5, 111.1, 52.6; LRMS (ESI, *m*/*z*) 472 $(M+1)^+$; IR (KBr) (ν_{max}/cm^{-1}): 3324 (NH), 1677 (C=O), 1519 (NO_2 asym), 1479, 1342 (NO_2 sym).
11. *Cell proliferation assay using MTT*:
This assay is a quantitative colorimetric method for determination of cell survival and proliferation.[12] The assessed parameter is the metabolic activity of viable cells. Metabolically active cells reduce pale yellow tetrazolium salt (MTT) to a dark blue water-insoluble formazan, which can be directly quantified after solubilisation with DMSO. The absorbance of the formazan directly correlates with the number of viable cells. The cells were plated in 96-well plates at a density of 2.0×10^4 in 200 μL of medium per well of 96-well plate. Cultures were incubated with different concentrations of test material and incubated for 48 h. The medium was replaced with fresh medium containing 100 μg/mL of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) for 2–3 h. The supernatant was aspirated and MTT-formazon crystals dissolved in 100 μL DMSO; OD measured at λ 540 nm (reference wavelength, λ 620 nm) on ELISA reader cell viability% was calculated by comparing the absorbance of treated versus untreated cells.
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