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Preliminary communication

Synthesis of 1-(2,6-dichlorophenyl)-3-methylene-1,3-dihydro-indol-2-one derivatives and *in vitro* anticancer evaluation against SW620 colon cancer cell line

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Abstract

A small library of 2-indolinone derivatives with the 2,6-dichlorophenyl ring at the N_1 position and with varying substitutions including aryl groups at the 3-position were synthesized, and their structures were confirmed by spectral analysis. All molecules were screened for their *in vitro* cytotoxic activity on SW620 colon cancer cell lines. Among the designed series compounds **4c**, **4f** and **4j** were found to be active at concentrations of 2–15 μg/ml. Some 3D-QSAR models were also built to understand the structure–activity relationship.

Keywords: 2-Indolinones; SW620 cell line; Colon cancer; Anticancer activity; 3D-QSAR

1. Introduction

2-Indolinone derivatives have recently been established as anticancer compounds [1] and more specifically as tyrosine kinase inhibitors (SU5416, SU5614, SU6668, SU6597, SU6663 and SU6561) that block kit activation and growth of small cell in lung cancer [2]. Targeting receptor kinase by novel indolinone derivatives like BIBF1000 in multiple myeloma abrogration of stroma derived interleukin-6 secretion and induction of apoptosis in cytogenetically with definite subgroups have been now identified [3]. Various other analogs have been evaluated as kinase inhibitors, anticancer and antiangiogenic agents with encouraging results [4].

After the first report of fibroblast growth factor receptor (FGFr-1) co-crystallization with SU4984 and SU5402, the crystal structure of a receptor tyrosine kinase (RTK) bound to a competitive inhibitor of ATP was identified [5]. These compounds are also inhibitors of both the vascular endothelial growth factor receptor (TK flk-1) and vascular endothelial growth factor (VEGF). SU4984 and SU5402 inhibit the autophosphorylation of FGFr with IC_{50} values of 10–40 μM [6].

SU11248 is designed to bind in particular, VEGF receptor, PDGF receptors α and β, Flt3 and C-KIT tyrosine kinases [7,8]. The other derivatives studied are SU6577 and SU6663.

All molecules of SU series are indolines, unsubstituted at the nitrogen of the indole ring, while the molecules developed

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by Boehringer Ingelheim Pharma, Ingelheim (DE) are oncolytic and various tyrosine kinase receptor inhibitors and similar to the Sugen molecules, but substituted in the 6-position of the indolinone nucleus [9].

More recently Andreani et al. [10] extensively studied several *E* isomers of 3-(3,4,5-trimethoxybenzylidene)-1,3-dihydroindol-2-ones as anticancer agents. Moreover, (2-chloroindolyl)methylene-2-indolinone derivatives were studied as CDK1/cyclinB inhibitors by the same author [11]. The closely structurally related compounds 3-(((4-phenyl)-piperazine-1-yl)-alkyl)-3-alkyl-1,3-dihydro-2*H*-indol-2-one derivatives and related compounds have shown as CNS antagonists which bind 5-HT_2C and a1 receptors [12].

The present study was initiated with the aim of identifying the structural requirement of the unsubstituted nitrogen of 2-indolinone towards anticancer activity. Though several such molecules have entered Phase I–IV studies, none of the molecules being studied have an *N*-substitution. In many tyrosine kinase inhibitors, the 2,6-dichlorophenyl fragment has proven to be very potent [13], and therefore using the fragment based approach, the 2,6-dichlorophenyl moiety has been appended to the N_1 ring nitrogen of the 2-indolinone scaffold.

2. Experimental

2.1. Chemistry

2.1.1. Materials and methods

^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker Avance II 300 MHz NMR spectrometer using TMS as the internal reference. IR spectra were recorded on a Shimadzu FR-IR-8400 spectrometer using the KBr pellet method. Thin layer chromatography was performed on silica gel (Merck 60 F_{254}). Mass spectra were recorded on a JEOL SX 102/DA-6000 FAB spectrometer.

2.1.2. Synthesis of 2-chloro-N-(2,6-dichlorophenyl)-N-phenyl-acetamide (**2**)

Compound **2** was prepared according to cited literature [14–17].

2.1.3. Synthesis of 1-(2,6-dichlorophenyl)-2-indolinone (**3**)

Compound **3** is also prepared according to cited literature [14–17].

2.1.4. General procedure for the synthesis of 1-(2,6-dichlorophenyl)-3-(un/substituted phenyl)methylene-1,3-dihydro-indol-2-ones (**4a–t**)

1-(2,6-Dichlorophenyl)-2-indolinone (0.01 M) with the corresponding aldehyde (0.01 M) was refluxed together in ethanol using piperidine as a catalyst. The reaction was monitored by TLC. After the completion of the reaction, the reaction mixture was allowed to cool. The solid product was separated by filtration, washed with diethyl ether and dried *in vacuo*. Finally, the compounds of the designed series purified by crystallization with methanol leave the pure products.

2.1.5. Spectral data of the compounds

*2.1.5.1. 1-(2,6-Dichlorophenyl)-1,3-dihydro-indol-2-one (**4a**).* IR (KBr, cm^{-1}): 1707 (C=O str.), 1157 (C–N str.), 1628, 1589 (C=C str., aromatic), 812 (OOP bending), 758 (C–Cl str.); ^{1}H NMR ($CDCl_3$) $\delta = 3.22$ (s, 3H), 6.94 (t, 1H, $J = 9.18$ Hz), 7.11 (t, 1H, $J = 8.34$ Hz), 7.42 (m, 4H), 7.83 (d, 1H, $J = 8.66$); ^{13}C NMR ($CDCl_3$) $\delta = 206.5$, 144.2, 137.4, 134.8, 129.9, 129.5, 127.9, 127.7, 127.3, 126.8, 42.0, 39.9; FAB-mass (*m/z*): 278 (M^+).

*2.1.5.2. 1-(2,6-Dichlorophenyl)-3-((phenyl)methylene)-1,3-dihydro-indol-2-one (**4b**).* IR (KBr, cm^{-1}): 1712 (C=O str.), 1186 (C–N str.), 1624 (C=C str., alkene), 1606, 1562 (C=C str., aromatic), 694, 744 (OOP bending), 778 (C–Cl str.); ^{1}H NMR ($CDCl_3$) $\delta = 6.39$ (d, 1H, $J = 7.81$ Hz), 6.93 (t, 1H, $J = 8.13$ Hz), 7.19 (t, 1H, $J = 8.05$ Hz), 7.36 (m, 1H), 7.50 (m, 5H), 7.72 (m, 3H), 7.97 (s, 1H); ^{13}C NMR ($CDCl_3$) $\delta = 197.1$, 144.2, 140.2, 139.5, 138.1, 135.7, 135.3, 135.0, 129.3, 128.7, 128.4, 128.2, 127.9, 127.5, 127.0, 126.7, 126.1, 37.8; FAB-mass (*m/z*): 365 (M^+), 367 ($M + 2$).

*2.1.5.3. 1-(2,6-Dichlorophenyl)-3-((2-chlorophenyl)methylene)-1,3-dihydro-indol-2-one (**4c**).* IR (KBr, cm^{-1}): 1716 (C=O str.), 1176 (C–N str.), 1639 (C=C str., alkene), 1606, 1589 (C=C str., aromatic), 825 (OOP bending), 790 (C–Cl str.); ^{1}H NMR ($CDCl_3$) $\delta = 6.40$ (d, 1H, $J = 7.89$ Hz), 6.95 (t, 1H, $J = 7.30$ Hz), 7.21 (tt, 1H, $J = 7.13$ Hz), 7.38 (dd, 1H, $J = 6.90$), 7.46–7.54 (m, 4H), 7.66 (d, 1H, $J = 7.02$ Hz), 7.68 (d, 2H, $J = 5.55$ Hz), 7.88 (s, 1H); ^{13}C NMR ($CDCl_3$) $\delta = 196.9$, 144.2, 141.3, 139.4, 138.7, 135.0, 134.7, 133.3, 131.6, 129.7, 129.7, 128.5, 128.3, 128.0, 127.8, 127.4, 126.9, 126.5, 126.2, 37.8; FAB-mass (*m/z*): 400 (M^+).

*2.1.5.4. 1-(2,6-Dichlorophenyl)-3((3-chlorophenyl)methylene)-1,3-dihydro-indol-2-one (**4d**).* IR (KBr, cm^{-1}): 1703 (C=O str.), 1208 (C–N str.), 1668 (C=C str., alkene), 1618, 1570 (C=C str., aromatic), 679, 760 (OOP bending), 811 (C–Cl str.); ^{1}H NMR ($CDCl_3$) $\delta = 6.73$ (t, 1H, $J = 9.29$ Hz), 6.94 (t, 1H, $J = 8.74$ Hz), 7.15 (d, 1H, $J = 8.82$ Hz), 7.43 (d, 2H, $J = 7.90$ Hz), 7.58 (t, 1H, $J = 9.76$), 7.71 (m, 4H), 7.83 (d, 1H, $J = 7.81$ Hz), 7.96 (s, 1H); ^{13}C NMR ($CDCl_3$) $\delta = 197.0$, 144.3, 140.9, 139.5, 138.8, 136.4, 135.4, 135.0, 134.6, 130.5, 129.7, 128.4, 128.6, 128.0, 127.4, 127.0, 126.4 126.1, 124.5, 37.8; FAB-mass (*m/z*): 400 (M^+).

*2.1.5.5. 1-(2,6-Dichlorophenyl)-3-((4-chlorophenyl)methylene)-1,3-dihydro-indol-2-one (**4e**).* IR (KBr, cm^{-1}): 1713 (C=O str.), 1164 (C–N str.), 1680 (C=C str., alkene), 1622, 1545 (C=C str., aromatic), 679, 734 (OOP bending), 757 (C–Cl str.); ^{1}H NMR ($CDCl_3$) $\delta = 6.72$ (t, 1H, $J = 8.79$ Hz), 7.08 (m, 4H), 7.24 (d, 2H, $J = 9.11$ Hz), 7.41 (m, 3H), 7.62 (d, 1H, $J = 8.42$ Hz), 7.73 (s, 1H); ^{13}C NMR ($CDCl_3$) $\delta = 197.0$, 144.0, 141.0, 139.6, 138.9, 135.7, 134.9, 133.6, 133.8, 129.4,

128.9, 128.6, 128.2, 127.6, 127.8, 127.0, 126.6, 37.7; FAB-mass (*m/z*): 400 (M^+).

*2.1.5.6. 1-(2,6-Dichlorophenyl)-3-((2,4-dichlorophenyl)methylene)-1,3-dihydro-indol-2-one (**4f**).* IR (KBr, cm^{-1}): 1712 (C=O str.), 1182 (C–N str.), 1635 (C=C str., alkene), 1606, 1577 (C=C str., aromatic), 817, 864 (OOP bending), 778 (C–Cl str.); ^1H NMR ($CDCl_3$) $\delta = 6.40$ (d, 1H, $J =$ 7.60 Hz), 6.92 (tt, 1H, $J = 7.49$, 1.20 Hz), 7.21 (tt, 1H, $J = 7.49$, 1.18 Hz), 7.39 (m, 3H), 7.52 (m, 2H), 7.76 (d, 2H, $J = 8.34$ Hz), 7.89 (s, 1H); ^{13}C NMR ($CDCl_3$) $\delta = 196.9$, 144.2, 140.9, 135.7, 135.3, 139.7, 138.9, 135.3, 132.8, 131.7, 130.7, 129.6, 129.4, 128.9, 128.3, 127.7, 127.0, 126.7, 37.7; FAB-mass (*m/z*): 435 (M^+).

*2.1.5.7. 1-(2,6-Dichlorophenyl)-3-((2,6-dichlorophenyl)methylene)-1,3-dihydro-indol-2-one (**4g**).* IR (KBr, cm^{-1}): 1694 (C=O str.), 1142 (C–N str.), 1667 (C=C str., alkene), 1652, 1605 (C=C str., aromatic), 656, 772 (OOP bending), 812 (C–Cl str.); ^1H NMR ($CDCl_3$) $\delta = 6.93$ (m, 2H), 7.12 (d, 1H, $J = 7.89$ Hz), 7.27 (d, 2H, $J = 8.83$ Hz), 7.39 (d, 2H, $J = 8.77$ Hz), 7.48 (m, 1H), 7.79 (d, 1H, $J = 8.14$ Hz), 7.87 (d, 1H, $J = 8.91$ Hz), 8.04 (s, 1H); ^{13}C NMR ($CDCl_3$) $\delta = 197.1$, 144.0, 141.2, 139.8, 138.9, 136.0, 135.7, 135.0, 132.9, 131.3, 129.5, 128.8, 128.0, 127.5, 127.0, 126.5, 37.7; FAB-mass (*m/z*): 435 (M^+).

*2.1.5.8. 1-(2,6-Dichlorophenyl)-3-((4-methoxyphenyl)methylene)-1,3-dihydro-indol-2-one (**4h**).* IR (KBr, cm^{-1}): 1718 (C=O str.), 1216 (C–N str.), 1688 (C=C str., alkene), 1610, 1536 (C=C str., aromatic), 723 (OOP bending), 766 (C–Cl str.); ^1H NMR ($CDCl_3$) $\delta = 3.86$ (s, 3H), 6.68 (d, 2H, $J = 9.82$ Hz), 6.82 (t, 1H, $J = 7.86$ Hz), 6.97 (t, 1H, $J =$ 8.33 Hz), 7.10 (d, 2H, $J = 9.12$ Hz), 7.23 (d, 1H, $J = 8.56$ Hz), 7.47 (m, 3H), 7.74 (d, 1H, $J = 8.71$ Hz), 7.91 (s, 1H); ^{13}C NMR ($CDCl_3$) $\delta = 197.2$, 160.0, 144.2, 141.4, 138.9, 139.5, 135.2, 134.8, 129.5, 128.3, 128.0, 127.7, 127.4, 127.0, 126.2, 114.6, 66.6, 37.5; FAB-mass (*m/z*): 395 (M^+).

*2.1.5.9. 1-(2,6-Dichlorophenyl)-3-((2-nitrophenyl)methylene)-1,3-dihydro-indol-2-one (**4i**).* IR (KBr, cm^{-1}): 1720 (C=O str.), 1174 (C–N str.), 1645 (C=C str., alkene), 1608, 1568 (C=C str., aromatic), 1523 (NO_2 str.), 742 (OOP bending), 792 (C–Cl str.); ^1H NMR ($CDCl_3$) $\delta = 6.40$ (d, 1H, $J = 7.89$ Hz), 6.83 (t, 1H, $J = 7.04$ Hz), 6.94 (d, 1H, $J =$ 7.32 Hz), 7.18 (tt, 1H, $J = 7.90$ Hz), 7.39 (dd, 1H, $J =$ 7.31 Hz), 7.53 (d, 2H, $J = 7.59$ Hz), 7.67 (tt, 1H, $J = 7.82$, 1.45 Hz), 7.76 (tt, 1H, $J = 6.00$, 1.17 Hz), 7.83 (d, 1H, $J = 6.76$ Hz), 8.19 (s, 1H), 8.34 (dd, 1H, $J = 8.10$, 1.21 Hz); ^{13}C NMR ($CDCl_3$) $\delta = 197.3$, 146.5, 144.2, 140.9, 139.5, 138.3, 135.2, 134.9, 130.5, 129.5, 129.0, 128.5, 128.2, 127.9, 127.5, 126.8, 126.4, 120.7, 37.9; FAB-mass (*m/z*): 410 (M^+).

*2.1.5.10. 1-(2,6-Dichlorophenyl)-3-((4-fluorophenyl)methylene)-1,3-dihydro-indol-2-one (**4j**).* IR (KBr, cm^{-1}): 1720 (C=O str.), 1157 (C–N str.), 1639 (C=C str., alkene), 1598, 1564 (C=C str., aromatic), 1234 (C–F str.), 817 (OOP bending), 786 (C–Cl str.); ^1H NMR ($CDCl_3$) $\delta = 6.42$ (d, 1H, $J = 7.80$ Hz), 6.95 (m, 1H), 7.15–7.25 (m, 2H), 7.39 (dd, 1H, $J = 7.30$, 1.47 Hz), 7.52 (d, 2H, $J = 7.63$ Hz), 7.68–7.75 (m, 4H), 7.91 (s, 1H); ^{13}C NMR ($CDCl_3$) $\delta = 196.7$, 162.5, 144.2, 140.4, 139.6, 138.9, 135.5, 135.0, 131.4, 129.6, 128.4, 128.2, 127.8, 127.3, 126.9, 126.4, 115.7, 37.8; FAB-mass (*m/z*): 383 (M^+).

*2.1.5.11. 1-(2,6-Dichlorophenyl)-3-((3-bromobenzylidene)methylene)-1,3-dihydro-indol-2-one (**4k**).* IR (KBr, cm^{-1}): 1702 (C=O str.), 1214 (C–N str.), 1650 (C=C str., alkene), 1613, 1577 (C=C str., aromatic), 617, 743 (OOP bending), 718 (C–Cl str.); ^1H NMR ($CDCl_3$) $\delta = 6.87$ (t, 1H, $J = 9.29$ Hz), 7.04 (t, 1H, $J = 7.81$ Hz), 7.13 (t, 1H, $J = 8.07$ Hz), 7.24 (m, 3H), 7.41 (t, 1H, $J = 8.51$ Hz), 7.47 (d, 1H, $J = 8.86$ Hz), 7.58 (m, 3H), 7.66 (s, 1H); ^{13}C NMR ($CDCl_3$) $\delta = 197.2$, 144.1, 141.2, 139.5, 138.4, 137.6, 135.4, 134.9, 131.3, 130.0, 129.4, 128.7, 128.2, 127.6, 126.7, 126.3, 125.5, 123.2, 37.8; FAB-mass (*m/z*): 444 (M^+).

*2.1.5.12. 1-(2,6-Dichlorophenyl)-3-((3-phenoxyphenyl)methylene)-1,3-dihydro-indol-2-one (**4l**).* IR (KBr, cm^{-1}): 1722 (C=O str.), 1138 (C–N str.), 1690 (C=C str., alkene), 1625, 1545 (C=C str., aromatic), 667, 759 (OOP bending), 810 (C–Cl str.); ^1H NMR ($CDCl_3$) $\delta = 6.59$ (t, 1H, $J = 9.36$ Hz), 6.74 (t, 1H, $J = 7.78$ Hz), 6.91 (dd, 2H, $J = 8.41$ Hz), 7.07 (m, 5H), 7.19 (d, 2H, $J = 8.06$ Hz), 7.23 (t, 1H, $J = 8.92$ Hz), 7.39 (d, 1H, $J = 9.14$ Hz), 7.46 (d, 2H, $J = 8.17$ Hz), 7.58 (d, 1H, $J = 7.93$ Hz), 7.66 (s, 1H); ^{13}C NMR ($CDCl_3$) $\delta = 197.3$, 144.3, 157.5, 157.1, 140.9, 139.7, 139.0, 135.4, 135.3, 135.2, 129.9, 128.7, 128.6, 128.6, 127.8, 126.7, 122.4, 119.9, 117.8, 116.9, 114.2, 37.2; FAB-mass (*m/z*): 457 (M^+).

*2.1.5.13. 1-(2,6-Dichlorophenyl)-3-((4-hydroxyphenyl)methylene)-1,3-dihydro-indol-2-one (**4m**).* IR (KBr, cm^{-1}): 3418 (O–H str.), 1688 (C=O str.), 1107 (C–N str.), 1678 (C=C str., alkene), 1610, 1579 (C=C str., aromatic), 656, 741 (OOP bending), 717 (C–Cl str.); ^1H NMR ($CDCl_3$) $\delta = 6.92$ (d, 2H, $J = 7.15$ Hz), 6.98 (s, 1H), 7.18 (t, 1H, $J = 8.20$ Hz), 7.22 (t, 1H, $J = 7.11$ Hz), 7.26 (t, 1H, $J = 8.35$ Hz), 7.31 (d, 2H, $J = 7.68$ Hz), 7.38 (d, 2H, $J = 8.66$ Hz), 7.41 (d, 1H, $J = 7.81$ Hz), 7.44 (d, 1H, $J = 8.14$ Hz), 7.61 (s, 1H); ^{13}C NMR ($CDCl_3$) $\delta = 197.2$, 157.9, 144.2, 141.2, 139.8, 138.9, 135.3, 134.9, 129.6, 128.2, 128.0, 128.2, 127.3, 126.9, 126.4, 116.2, 37.9; FAB-mass (*m/z*): 381 (M^+).

*2.1.5.14. 1-(2,6-Dichlorophenyl)-3-((3,4-dimethoxyphenyl)methylene)-1,3-dihydro-indol-2-one (**4n**).* IR (KBr, cm^{-1}): 2977, 2845 (C–H str.), 1693 (C=O str.), 1147 (C–N str.), 1612 (C=C str., alkene), 1600, 1566 (C=C str., aromatic), 885, 775 (OOP bending), 1024 (C–O–C str.); ^1H NMR ($CDCl_3$) $\delta = 3.94$ (s, 3H), 3.96 (s, 3H), 6.39 (d, 1H, $J = 7.65$ Hz), 6.91 (d, 1H, $J = 8.34$ Hz), 7.11 (tt, 1H, $J = 7.92$ Hz), 7.19 (tt, 1H, $J = 7.28$ Hz), 7.37 (dd, 1H), 7.53 (d, 2H, $J = 9.00$ Hz), 7.61

(s, 1H), 7.65 (m, 2H), 8.67 (d, 1H, $J = 1.68$ Hz); ^{13}C NMR ($CDCl_3$) $\delta = 197.3$, 150.0, 149.0, 144.3, 140.9, 139.4, 138.9, 135.4, 134.9, 129.5, 128.2, 128.0, 127.1, 127.2, 126.5, 119.9, 115.7, 111.9, 56.9, 37.8; FAB-mass (*m/z*): 425 (M^+).

*2.1.5.15. 1-(2,6-Dichlorophenyl)-3-((4-(dimethylamino)phenyl)-methylene)-1,3-dihydro-indol-2-one (**4o**).* IR (KBr, cm^{-1}): 2912, 2856 (C–H str.), 1710 (C=O str.), 1222 (C–N str.), 1620 (C=C str., alkene), 1589, 1536 (C=C str., aromatic), 678, 785 (OOP bending), 780 (C–Cl str.); ^{1}H NMR ($CDCl_3$) $\delta = 3.11$ (s, 6H), 6.83 (d, 2H, $J = 8.10$ Hz), 6.88 (t, 1H, $J = 9.32$ Hz), 6.98 (t, 1H, $J = 7.18$ Hz), 7.06 (t, 1H, $J = 7.83$ Hz), 7.18 (dd, 2H, $J = 9.03$ Hz), 7.31 (d, 1H, $J = 8.47$ Hz), 7.38 (d, 1H, $J = 8.35$ Hz), 7.44 (m, 2H), 7.61 (s, 1H); ^{13}C NMR ($CDCl_3$) $\delta = 197.2$, 149.0, 144.3, 141.2, 139.7, 139.2, 135.4, 134.0, 129.5, 128.8, 128.2, 127.8, 127.2, 126.9, 126.4, 124.8, 114.7, 40.8, 37.7; FAB-mass (*m/z*): 408 (M^+).

*2.1.5.16. 1-(2,6-Dichlorophenyl)-3-((naphthalen-1-yl)methylene)-1,3-dihydro-indol-2-one (**4p**).* IR (KBr, cm^{-1}): 1712 (C=O str.), 1177 (C–N str.), 1688 (C=C str., alkene), 1627, 1576 (C=C str., aromatic), 669, 728 (OOP bending), 807 (C–Cl str.); ^{1}H NMR ($CDCl_3$) $\delta = 6.87$ (t, 1H, $J = 8.83$ Hz), 6.95 (d, 1H, $J = 8.03$ Hz), 7.04 (t, 1H, $J = 9.56$ Hz), 7.13 (d, 2H, $J = 7.41$ Hz), 7.26 (d, 2H, $J = 8.12$ Hz), 7.33 (m, 3H), 7.41 (t, 1H, $J = 7.16$ Hz), 7.46 (d, 1H, $J = 7.79$ Hz), 7.55 (s, 1H), 7.68 (d, 2H, $J = 9.12$ Hz); ^{13}C NMR ($CDCl_3$) $\delta = 197.2$, 144.2, 141.3, 139.6, 139.0, 135.8, 135.0, 134.6, 133.4, 131.3, 129.4, 128.5, 128.4, 128.0, 127.8, 127.5, 127.0, 126.7, 126.1, 125.8, 125.5, 124.4, 123.4, 37.7; FAB-mass (*m/z*): 415 (M^+).

*2.1.5.17. 1-(2,6-Dichlorophenyl)-3-((furan-2-yl)methylene)-1,3-dihydro-indol-2-one (**4q**).* IR (KBr, cm^{-1}): 1708 (C=O str.), 1186 (C–N str.), 1629 (C=C str., alkene), 1602, 1562 (C=C str., aromatic), 779 (C–Cl str.); ^{1}H NMR ($CDCl_3$) $\delta = 6.41$ (d, 1H, $J = 7.47$ Hz), 6.50 (dd, 1H), 6.96 (d, 1H, $J = 3.45$ Hz), 7.14 (tt, 1H, $J = 6.00$, 1.30 Hz), 7.24 (tt, 1H, $J = 6.00$, 1.20 Hz), 7.37 (dd, 1H, $J = 9.00$ Hz), 7.51 (d, 2H, $J = 8.10$ Hz), 7.58 (s, 1H), 7.80 (d, 1H, $J = 1.72$ Hz), 8.58 (d, 1H, $J = 6.86$ Hz); ^{13}C NMR ($CDCl_3$) $\delta = 197.1$, 152.0, 146.3, 145.5, 144.2, 140.9, 135.4, 134.9, 129.7, 129.4, 128.9, 128.2, 127.4, 127.0, 126.4, 112.9, 111.6, 36.9; FAB-mass (*m/z*): 355 (M^+).

*2.1.5.18. 1-(2,6-Dichlorophenyl)-3-((2-chloroquinolin-3-yl)methylene)-1,3-dihydro-indol-2-one (**4r**).* IR (KBr, cm^{-1}): 1694 (C=O str.), 1109 (C–N str.), 1668 (C=C str., alkene), 1605, 1535 (C=C str., aromatic), 656, 738 (OOP bending), 764 (C–Cl str.); ^{1}H NMR ($CDCl_3$) $\delta = 6.94$ (dd, 1H, $J = 9.17$ Hz), 7.11 (d, 1H, $J = 8.34$ Hz), 7.19 (t, 1H, $J = 8.84$ Hz), 7.26 (d, 2H, $J = 8.06$ Hz), 7.38 (m, 3H), 7.53 (dd, 1H, $J = 8.79$ Hz), 7.59 (t, 1H, $J = 7.83$ Hz), 7.72 (s, 1H), 7.86 (d, 1H, $J = 7.18$ Hz), 7.94 (d, 1H, $J = 8.17$ Hz); ^{13}C NMR ($CDCl_3$) $\delta = 197.1$, 149.9, 147.5, 145.2, 144.2, 141.1, 139.0, 136.1, 135.5, 135.3, 131.5, 130.5, 129.5, 128.7, 128.2, 127.9, 127.5, 127.1, 126.8, 126.6, 126.4, 37.7; FAB-mass (*m/z*): 451 (M^+).

*2.1.5.19. 1-(2,6-Dichlorophenyl)-3-((2-chloro-6-methylquinolin-3-yl)methylene)-1,3-dihydro-indol-2-one (**4s**).* IR (KBr, cm^{-1}): 2966 (C–H str.), 1708 (C=O str.), 1181 (C–N str.), 1677 (C=C str., alkene), 1610, 1560 (C=C str., aromatic), 687, 749 (OOP bending), 782 (C–Cl str.); ^{1}H NMR ($CDCl_3$) $\delta = 2.14$ (s, 3H), 6.74 (d, 1H, $J = 8.80$ Hz), 6.87 (d, 1H, $J = 8.17$ Hz), 6.98 (t, 1H, $J = 7.44$ Hz), 7.11 (d, 2H, $J = 9.82$ Hz), 7.28 (t, 1H, $J = 7.18$ Hz), 7.44 (dd, 2H, $J = 8.18$ Hz), 7.57 (s, 1H), 7.72 (m, 3H); ^{13}C NMR ($CDCl_3$) $\delta = 197.1$, 148.8, 145.6, 145.5, 144.4, 140.9, 139.2, 137.1, 135.0, 134.7, 132.5, 130.8, 129.0, 128.5, 128.2, 127.6, 126.9, 126.7, 126.1, 126.0, 125.9, 37.8, 24.5; FAB-mass (*m/z*): 465 (M^+).

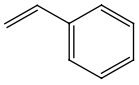
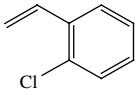
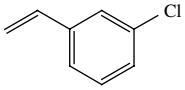
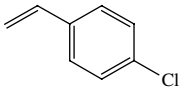
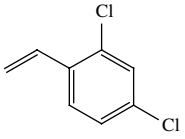
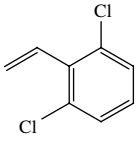
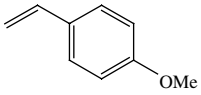
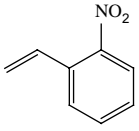
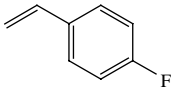
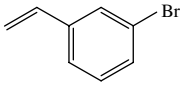
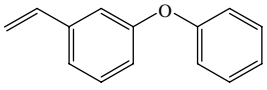
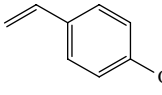
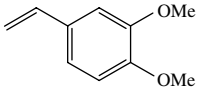
*2.1.5.20. 1-(2,6-Dichlorophenyl)-3-((2-chloro-7,8-dimethylquinolin-3-yl)methylene)-1,3-dihydro-indol-2-one (**4t**).* IR (KBr, cm^{-1}): 2980, 2867 (C–H str.), 1721 (C=O str.), 1220 (C–N str.), 1634 (C=C str., alkene), 1602, 1527 (C=C str., aromatic), 650, 731 (OOP bending), 770 (C–Cl str.); ^{1}H NMR ($CDCl_3$) $\delta = 2.16$ (s, 3H), 2.21 (s, 3H), 7.08 (d, 1H, $J = 8.12$ Hz), 7.11 (t, 1H, $J = 7.19$ Hz), 7.28 (d, 2H, $J = 8.52$ Hz), 7.40 (d, 1H, $J = 7.18$ Hz), 7.54 (s, 1H), 7.68 (m, 2H), 7.73 (m, 3H); ^{13}C NMR ($CDCl_3$) $\delta = 196.9$, 155.2, 148.9, 145.6, 144.2, 140.7, 138.5, 138.9, 135.9, 135.3, 134.5, 129.9, 129.1, 128.6, 128.1, 127.3, 127.0, 126.8, 126.3, 125.8, 124.6, 37.8, 17.5, 13.2; FAB-mass (*m/z*): 479 (M^+).

2.2. Biology

2.2.1. Cytotoxicity assay

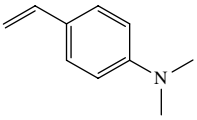
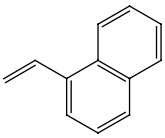
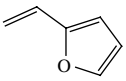
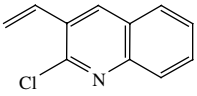
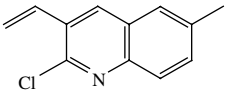
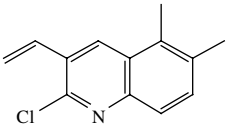
Derivatives of 1-(2,6-dichlorophenyl)-3-methylene-1,3-dihydro-indol-2-one (**4a–t**, Table 1) were screened for cytotoxic activity at 1–100 μM concentration in a human colon cancer cell line (SW620) [13]. Briefly, a three-day MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) *in vitro* cytotoxicity assay was performed. This test is based on the principle of uptake of MTT, a tetrazolium salt, by the metabolically active cells where it is metabolized by active mitochondria into a blue colored formazan product that is read spectrophotometrically. MTT was dissolved in phosphate buffered saline at a pH of 7.4 to obtain an MTT concentration of 5 mg/ml; the resulting mixture was filtered through a 0.22-μm filter to sterilize and remove a small amount of insoluble residue. A total of 10,000 cells were seeded in a 96-well culture plate and incubated with various concentrations of 1-(2,6-dichlorophenyl)-3-methylene-1,3-dihydro-indol-2-one derivatives (**4a–t**) in a CO_2 incubator for 72 h. Control cells not treated with 1-(2,6-dichlorophenyl)-3-methylene-1,3-dihydro-indol-2-one derivatives (**4a–t**) were similarly incubated. The assay was terminated after 72 h by adding 125 μg (25 μL) MTT to each well, followed by incubation for 3 h, and finally added 50 μL of 10% SDS–0.01 N HCl to each well to lyse the cells and dissolve formazan. After incubating for 1 h, the plate was read spectrophotometrically at 540 nm and the

Table 1
Anticancer activity of 1-(2,6-dichlorophenyl)-3-methylene-1,3-dihydro-indol-2-one derivatives against SW620 colon cell line

Compounds	Substitution (R)	Yield[b] (%)	Mp (°C)	R_f[a]	ED_{50} (μg/ml)
4a	H	62	128	0.52	>100
4b		58	150	0.66	100
4c		52	178	0.54	15
4d		55	144	0.64	50
4e		60	164	0.73	100
4f		64	184–185	0.75	2
4g		53	190	0.51	>100
4h		57	175	0.44	>100
4i		48	160	0.35	90
4j		56	152	0.47	5
4k		52	149–151	0.67	50
4l		59	148	0.65	50
4m		60	218	0.61	50
4n		52	174	0.38	50

(*continued on next page*)

Table 1 (*continued*)

Compounds	Substitution (R)	Yield[b] (%)	Mp (°C)	R_f[a]	ED_{50} (μg/ml)
4o		46	220–222	0.42	>100
4p		55	178	0.72	>100
4q		54	184	0.41	>100
4r		57	256–258	0.58	25
4s		58	278	0.67	40
4t		51	260	0.68	>100

[a] Solvent system: ethyl acetate/toluene, 0.5:9.5.
[b] Yield (%) for the transformation from compound **3** to **4**.

cytotoxicity percentage calculated using the formula: cytotoxicity percentage $= (1 - X/R_1) \times 100$, where $X =$ (absorbance of treated sample at 540 nm) − (absorbance of blank at 540 nm) and $R_1 =$ absorbance of control sample at 540 nm.

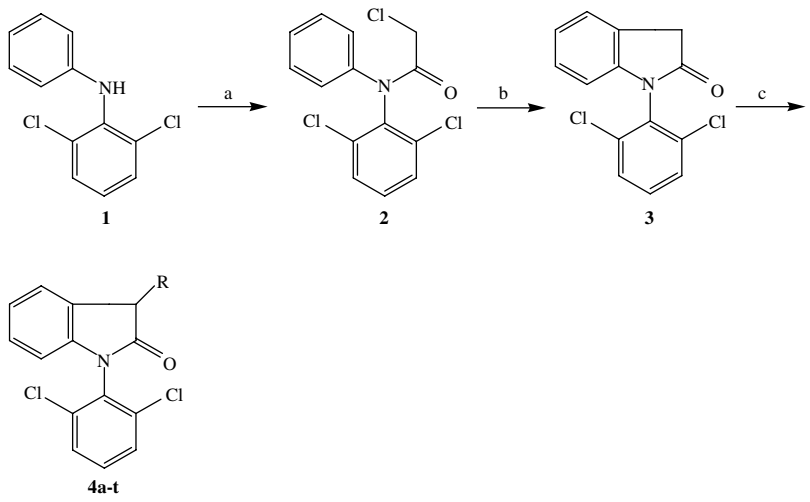
2.3. QSAR studies

The 3D-QSAR techniques, comparative molecular field analysis [18] and comparative molecular similarity indices analysis (CoMSIA) [19,20] were carried out with Sybyl v.7.1 [21] running on a Pentium IV computer under the Linux Red Hat Enterprise WS4.0. The 3D-QSAR models were built using both atom fit and field fit alignment techniques with default settings of the parameters for 3D-QSAR *viz*. 3D cubic lattice, grid spacing, probe groups and energy cut-off. The QSAR models were based on a training set of 13 diverse molecules and were externally validated on a test set of seven molecules. Further, statistical parameters like r^2, q^2, PRESS, r^2_{pred}, standard error (SDEP) and F value were calculated to determine the robustness of the 3D-QSAR models.

3. Results and discussion

2-Indolinone derivatives with 2,6-dichlorophenyl ring on the N_1 position were synthesized as displayed in reaction of Scheme 1. *N*-Phenyl-2,6-dichloroaniline (**1**) was refluxed with chloroacetyl chloride to give compound **2**. Compound **2** was heated with anhydrous aluminum chloride at 160 °C to give compound **3**. The arylidene was synthesized by refluxing compound **3** with corresponding aldehydes in ethanol using piperidine as the catalyst for an appropriate time to give 1-(2,6-dichlorophenyl)-3-methylene-1,3-dihydro-indol-2-one derivatives (**4a–t**). Different aromatic aldehydes were chosen to obtain compounds with halogen substitutions at various positions of the aromatic ring on the benzylidine side chain. Compounds with bulky group at the 3-position (*e.g.* *m*-phenoxyphenyl, 3,4-dimethoxyphenyl, 4-dimethyl amino phenyl, and 1-naphthyl) were also synthesized using the appropriate aldehyde. Furfuraldehyde and substituted quinoline aldehydes were also used to get compounds substituted with heterocycles at the 3-position of the 2-indolinone nucleus. Thus, a series of 20 diverse 2-indolinone derivatives with varying substitutions at the 3-position were synthesized. The crystal structure of compound **4b** was obtained by XRD [22] to establish the structure and conformation, in addition to ^{1}H NMR, ^{13}C NMR, mass and IR spectral studies.

The synthesized derivatives were tested for *in vitro* cytotoxic activity on SW620 colon cancer cell line. Compound **4f** (Table 1) was found to be the most potent compound which has two chloro groups at the C_2' and C_4' positions of the benzylidine ring. Compound **4j** with a fluoro substituent at the C_4' position also shows good potency with a low ED_{50} value.



Scheme 1. Synthesis of 1-(2,6-dichlorophenyl)-3-methylene-1,3-dihydro-indol-2-one derivatives. *Reagents and conditions*: (a) $ClCH_2COCl$, reflux 5 h; (b) anhydrous $AlCl_3$, 160 °C, 2 h; (c) aldehydes, EtOH, piperidine, reflux 5–7 h.

Compound **4c** with a chloro substituent at the *ortho* position also shows good potency. Thus, it can be observed that halo substitutions either at the *ortho* or *para* positions of the benzylidine ring enhance the anticancer activity considerably. On the other hand, a halogen at the *meta* position causes a loss in the potency. Thus, compounds **4d** and **4k** having a chlorine and bromine atom, respectively, at the *meta* position, are nearly inactive.

The QSAR studies also suggest that bulky substituents at the *ortho* position of the phenyl ring at the 3-position should increase the activity. Similarly electropositive groups at the *meta* position of the phenyl ring at the 3-position of the indolinone ring and electron-withdrawing groups in the 2,4-positions could enhance the activity. The hydrophobic groups at the 3-position of the phenyl ring may add to the activity, but if placed at the 4-position of the phenyl ring or at the 8-position of the quinoline ring will diminish the anticancer activity.

4. Conclusions

In this preliminary communication, we show that compounds with 2,6-dichlorophenyl at the N_1 position of 2-indolinone ring have potent anticancer activity which can be modulated by groups at the 3-position. Some of the compounds show excellent anticancer activity (2 μM). To arrive at the structure with optimal activity a 3D-QSAR study was carried out and based on the QSAR results more compounds are being designed and work is in progress.

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