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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

In vitro cytotoxicity evaluation of diversely substituted *N*-aryl-2-oxindoles

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Abstract In continuation with our previous work, structurally diverse 2-indolinones bearing 2,6-dichloroaryl fragment at N^1 and (hetero)aryl benzylidene at C^3 were evaluated for their antitumor activity on a panel of cancer cell lines such as MCF-7 (breast), MiapaCa2 (pancreas), KB (oral), HuTu80 (stomach), L132 (lung), B16F10 (melanoma), and Molt4 (leukemia) from various human organs. Among the screened compound library, molecules **4e, 4k,** and **4r** have shown excellent cytotoxicity on a stomach cancer cell line. Moreover, a significant number of compounds have also shown promising cytotoxicity on pancreas and oral cancer cell lines.

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Introduction

Indolinone is ubiquitous heterocycle, which can serve as unique and versatile scaffold for the discovery of new antitumor and antiangiogenic agents (Andreani *et al.*, 2010; Andreani *et al.*, 2007, Zang and Go, 2009). Various kinase inhibitors (containing 2-indolinone motif) have been intensively studied for the inhibition of vascular endothelial growth factor receptor (VEGFR), c-Kit (the protein product of the proto-oncogen Kit), Flt 3 (FMS-like tyrosine kinase), PDGFR-α/β (platelet derived growth factor receptor), and CSF-1-R (colony stimulating factor 1 receptor) (Krug and Hilgeroth, 2008) (Fig. 1). SU6668 (TSU-68, Orantanib) is proved as selective inhibitor of Flk-1/KDR, PDGFR (Sun *et al.*, 1999), and epidermal growth factor receptor (FGFR) (Laird *et al.*, 2002). Moreover, SU11274 was also identified as a prototype ATP-competitive small molecule inhibitor of the catalytic activity of Met enzyme versus other tyrosine kinases (Sattler *et al.*, 2003). Receptor tyrosine kinases (RTKs) have proved to be attractive targets for therapeutic intervention, and significant progress has been made in the recent years in developing selective inhibitors (Beenken and Mohammadi, 2009; Guagnano *et al.*, 2011). Notably, SU11652 is also considered as PDGFR, Flk-1/KDR, and FGFR-1 inhibitors (Heryanto *et al.*, 2003). Sunitinib (Sutent), a multi-targeted receptor tyrosine kinase inhibitor, interfering with tumor blood vessel formation, is approved by FDA in 2006 for the treatment of advanced renal cell carcinoma (RCC) and gastrointestinal stromal tumors (GIST) (Atkins *et al.*, 2006). Sunitinib is the first anticancer drug which received

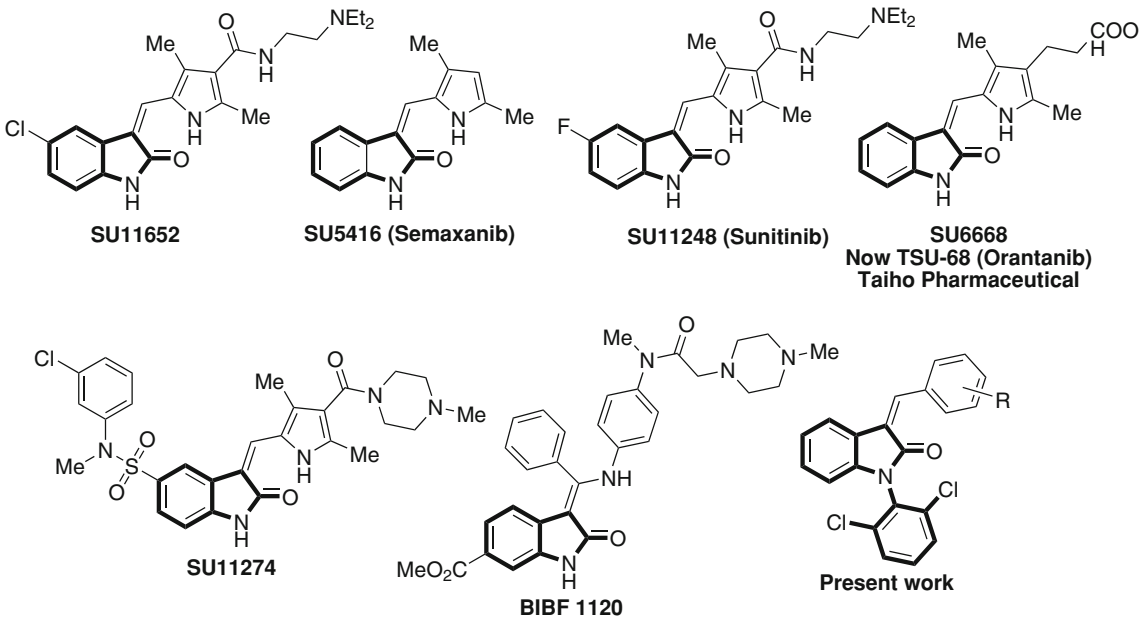


Fig. 1 Various indolinone motif at different clinical stages

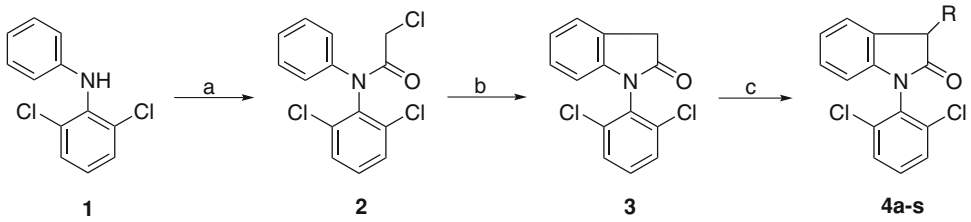
simultaneous FDA approval for two different indications. Semaxanib is a potent and selective Flk-1/KDR VEGFR tyrosine kinase inhibitor (Sun *et al.*, 1998). Unfortunately, Semaxanib and SU6668 failed in phase II clinical trial conducted with advanced soft tissue sarcoma. Importantly, BIBF1120 (Vergatef), a triple angio kinase inhibitor discovered by Boehringer, is currently in phase III clinical trials in non-small cell lung cancer (Roth *et al.*, 2009).

There is a plethora of literature on diversely substituted oxindoles as potent anticancers. A series of 3-benzylideneindolin-2-ones were evaluated for induction of NQO1 in murine Hepa1c1c7 cells as well as for antiproliferative activity (Zang and Go, 2009). Moreover, a series of pyrrolo-fused six or seven membered alicyclic ring were found as potent VEGFR, PDGFR, and c-Kit inhibitors. In continuation, pyrrolo-fused cyclohexanone in the 2-indolinone derivative possessing phenylsulfonamido group was also screened as aurora kinase inhibitors (Chiang *et al.*, 2010; Cho *et al.*, 2010). Remarkable contribution has been made by Andreani and co-workers in this research area so far. Recently, *E*-3-(3-indolylmethylene)-1,3-dihydroindol-2-ones tested on MCF-7 breast cancer cell lines, gave rise to a block in cell cycle progression with cell arrest in the G2/M phase (Andreani *et al.*, 2010). The effect on growth of the antitumor activity of 3-(5-imidazo[2,1-*b*]thiazolyl/thiadiazolyl methylene)-2-indolinones was elegantly addressed by the same group. In order to investigate the mechanism of action of the most potent antitumor agent, the effect on growth of HT-29 colon carcinoma cells was studied (Andreani *et al.*, 2008). The antitumor activity of indolinone bearing a 3,4,5-trimethoxyphenyl (Andreani *et al.*, 2006), since it is present in well-known antitumor agents such as colchicine, combretastatin, and podophyllotoxin were also successfully achieved. Though there is a huge progress on oxindole as a potent anticancer, the exploration of N^1 functionalized oxindoles are still in its infancy. Consequently, 1-substituted 3-pyridinyl/quinolyl methylidenylindolin-2-ones were evaluated for differentiation-inducing activity toward HL-60 cells. Among the designed molecules, quinolyl analog was proved to be the most potent (Hung *et al.*, 2008).

Results and discussion

In the current communication, we aimed to probe the structural and biochemical requirement for cytotoxicity study of N^1 arylated 2-oxindoles. Though several lead molecules are in various clinical stages, none of the molecule being studied has N^1 substitution. Numerous tyrosine kinase inhibitors having 2,6-dichloroaryl fragment have proven to be very potent and hence using the fragment based approach, the 2,6-dichlorophenyl ring is appended at N^1 in the core structure. In our previous paper we have revealed the syntheses, characterization, and anticancer evaluation on SW620 (colon) cancer cell line of *N*-arylated oxindoles (Virsodia *et al.*, 2009) (Scheme 1). Encouraged by the promising starting point, we drew our attention toward the extension of the work (Fig. 2). Herein we have evaluated the in vitro cytotoxic activity on a panel of additional cell lines, such as MCF-7 (breast), MiapaCa2 (pancreas), KB (oral), HuTu80 (stomach), L132 (lung), B16F10 (melanoma), and Molt4 (leukemia) from

Scheme 1 Synthesis of 2-indolinone derivatives. *Reagents and conditions: a* $ClCH_2COCl$, reflux, 5 h, *b* Anhydrous $AlCl_3$, 160 °C, 2 h, *c* RCHO, ethanol, piperidine (10 mol%), 5–7 h



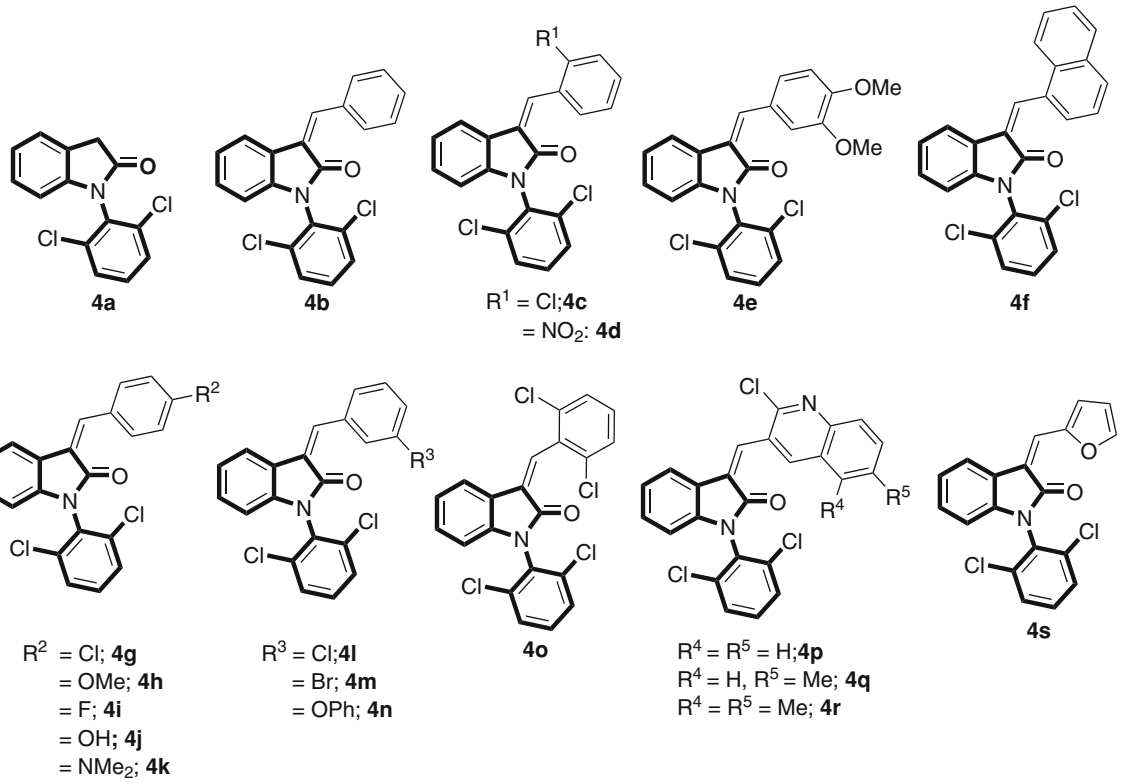


Fig. 2 2-Indolinone building blocks employed in the in vitro cytotoxicity study

various human organs by MTT method (Alley *et al.*, 1988). The target compounds encompasses versatile donor and acceptor groups in the benzylidene ring designed to mimic the *N*-phenyl ring of the 4-anilino quinazoline skeleton of Erlotinib and Gefitinib (Table 1). Of the designed molecules, the parent *N*-aryl-2-indolinone, compound **4a** was found to be inactive on all cell lines tested. From this observation we may hypothesize that *N*-arylation may not be indispensible structural motif for anticancer activity but benzylidene ring might be playing a role for the biological activity. The cytotoxic activity of unsubstituted benzylidene analog **4b** was found to be highly active on Molt4, HuTu80, and B16F10; while no promising cytotoxicity was observed on other cell lines. Hence, it is interesting to note that the cytotoxic activity depends on the presence of neither benzylidene ring nor 2,6-dichlorophenyl fragments, but it might depend on electronic requirement of the corresponding rings. On examination of activity profile of compound **4d,** encompasses strong electron withdrawing *ortho* nitro group in the benzylidene ring, exerted excellent cytotoxic activity on KB cell line and was observed significantly active. Likewise, it was also found noteworthy on MiapaCa2 and Mol4 cell lines. Sterically congested naphthalene derivative not having any hydrophilic or lipophilic groups, compound **4f**, was found to be inactive on all cell lines, except HuTu80. Interestingly, strong electron donor dimethoxy analog, **4e** has exhibited excellent cytotoxic activity on HuTu80 and found to be the most potent in the designed series. The same compound was also notorious on MCF-7, MiapaCa2, and KB cell lines, while found to be inactive on L132 and B16F10 cell lines. The results were quite surprising for the molecule **4h** having mono methoxy benzylidene ring. The cytotoxicity of **4h** was dramatically reduced on all cell lines. On the contrary, sterically demanding phenoxy benzylidene, **4n** was found to be significantly potent on KB and Molt4 cell lines while moderately active on HuTu80 and inactive on MCF-7, MiapaCa2, and B16F10 cell lines. In addition, *N,N*-dimethyl benzylidene analog **4k** showed remarkable cytotoxic activity on HuTu80 cell line, moderately active on KB, and inactive on rest of

Table 1 In vitro cytotoxicity evaluation on various cancer cell lines

Molecules	MCF7 (Breast)	MiapaCa2 (Pancreas)	KB (Oral)	HuTu80 (Stomach)	L132 (Lung)	B16F10 (Melanoma)	Molt4 (Leukemia)
4a	>100	>100	100	>100	>100	>100	ND
4b	10	>100	10	8	>100	9	7
4c	9	>100	5	>100	>100	39	7
4d	ND	5	1	10	10	>100	6
4e	10	5	10	<1	>100	>100	ND
4f	>100	>100	>100	10	>100	>100	ND
4g	5	5	2	6	9	>100	ND
4h	>100	>100	>100	>100	>100	>100	ND
4i	9	5	5	8	13	38	ND
4j	20	2	2	25	30	33	ND
4k	>100	>100	15	<1	>100	>100	ND
4l	ND	5	2	7	16	8	6
4m	5	5	2	7	19	8	7
4n	100	>100	5	10	24	>100	6
4o	>100	>100	50	100	>100	>100	ND
4p	>100	5	10	ND	ND	ND	ND
4q	>100	ND	100	ND	ND	ND	ND
4r	>100	ND	10	<1	98	>100	ND
4s	>100	10	5	18	>100	>100	ND
Gefitinib	0.89	0.51	0.33	0.77	>1	0.91	>1

ED_{50} (μg/mL)

ND not determined

the cell lines tested. The molecule having donor *para* hydroxy group in the benzylidene fragment, compound **4j** showed excellent cytotoxic activity on MiapaCa2 and KB cell lines. No significant cytotoxic activity was observed on other cell lines. The moderate electron withdrawing halogenated derivative, compound **4c** has exhibited good cytotoxic activity on KB and Molt4 cell lines, while found to be moderately active on MCF-7 and inactive on MiapaCa2, HuTu80, and L132 cell lines. The *para* chloro analog, compound **4g** showed excellent cytotoxic activity on KB cell lines and later it was found potent on MCF-7, MiapaCa2, HuTu80, and L132 cell lines. The incorporation of fluorine group into organic molecules can serve to dramatically alter many of the physical properties of such compounds, including lipophilicity, metabolic stability, and conformational behavior. For these reasons, we have rationally introduced electron poor fluoro atom in compound **4i**, which was found to be noteworthy on all cell lines. Additionally, the *meta* halogenated derivatives such as *m*-chloro and *m*-bromo substituted analogs, **4l** and **4m** have also shown significant activity on KB and MiapaCa2 cell lines. The compound **4m** was also noteworthy on rest of the cell lines under study. The tetra-chloro substituted indolinone derivative, compound **4o** was found to be the least cytotoxic; it is thought that steric interaction may prevent the flexibility of the ring. After the incorporation of bulky heterocycle at C^3 of 2-indolinone, 2-Cl quinolyl benzylidene analogs, **4p**, **4q**, and **4r**, the cytotoxicity of compound **4p** on MiapaCa2 is quite noteworthy. Compound **4q** was not a molecule of interest for cytotoxicity evaluation and was found to be inactive on cell lines tested. The only analog **4r**, having two donor methyl groups in the quinoline ring has exhibited excellent cytotoxicity on HuTu80 cell lines. Furan substituted 2-oxindole, compound **4s** has also shown good cytotoxic activity on KB cell line, while found to be moderately active on MiapaCa2 cell line and inactive on other cell lines. Overall, most of the 2-oxindoles were found to be inactive on B16F10 and L132 cell lines. The SAR analysis clearly revealed that the compounds having donor groups on the benzylidene fragment were far active than corresponding acceptor analogs.

Experimental

Chemistry

General remarks

Unless otherwise stated, all reactions were carried out in pre-dried glasswares under N_2 atmosphere. Chemicals and solvents were purchased commercially. Yields refer to isolated compounds, estimated to be >95 % pure as determined by ^{1}H-NMR. ^{1}H-NMR and ^{13}C-NMR spectra were recorded on a Bruker Avance II 300 MHz NMR spectrometer using TMS as an internal reference. IR

spectra were recorded on a Shimadzu FTIR-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.

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

Compound **2** was prepared according to the literature described procedure (Moser *et al.*, 1990).

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

Compound **3** was prepared according to the literature described procedure (Moser *et al.*, 1990).

Representative 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 (10 mmol) and corresponding aldehyde (10 mmol) were refluxed in ethanol using piperidine (10 mol%) as a catalyst for 5–7 h. The reaction was monitored by TLC (EtOAc/PhMe 5/9.5). 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*. The products were purified by recrystallization in methanol to leave the pure products.

Spectral data of the compounds

1-(2,6-Dichlorophenyl)-1,3-dihydro-indol-2-one ***(4a)*** Yield: 62 %, m.p. (°C): 128. $R_f = 0.52$. IR (KBr, cm^{-1}): 1,707 (C=O str.), 1,628, 1,589 (C=C str., aromatic), 1,157 (C–N str.), 812 (OOP bending), 758 (C–Cl str.). ^{1}H-NMR ($CDCl_3$): δ 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, $J = 8.66$ Hz). ^{13}C-NMR ($CDCl_3$): δ 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^+).

1-{[(2,6-Dichlorophenyl)-3-phenyl]methylene}-1,3-dihydro-indol-2-one ***(4b)*** Yield: 58 %, m.p. (°C): 150. $R_f = 0.66$. IR (KBr, cm^{-1}): 1,712 (C=O str.), 1,624 (C=C str., alkene), 1,606, 1,562 (C=C str., aromatic), 1,186 (C–N str.), 7,78 (C–Cl str.), 744 (OOP bending), 694. ^{1}H-NMR ($CDCl_3$): δ 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 ($CDCl_3$): δ 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).

1-{[(2,6-Dichlorophenyl)-3-(2-chlorophenyl)]methylene}-1,3-dihydro-indol-2-one ***(4c)*** Yield: 52 %, m.p. (°C): 178. $R_f = 0.54$. IR (KBr, cm^{-1}): 1,716 (C=O str.), 1,639 (C=C str., alkene), 1,606, 1,589 (C=C str., aromatic), 1,176 (C–N str.), 825 (OOP bending), 790 (C–Cl str.). ^{1}H-NMR ($CDCl_3$): δ 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, $J = 6.90$ Hz), 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 ($CDCl_3$): δ 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^+).

1-{[(2,6-Dichlorophenyl)-3-(2-nitrophenyl)]methylene}-1,3-dihydro-indol-2-one ***(4d)*** Yield: 48 %, m.p. (°C): 160. $R_f = 0.35$. IR (KBr, cm^{-1}): 1,720 (C=O str.), 1,645 (C=C str., alkene), 1,608, 1,568, (C=C str., aromatic), 1,523 (NO_2 str.), 1174 (C–N str.), 792 (C–Cl str.). 742 (OOP bending). ^{1}H-NMR ($CDCl_3$): δ 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 ($CDCl_3$): δ 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^+).

1-{[(2,6-Dichlorophenyl)-3-(3,4-dimethoxyphenyl)]methylene}-1,3-dihydro-indol-2-one ***(4e)*** Yield: 52 %, m.p. (°C): 174. $R_f = 0.38$. IR (KBr, cm^{-1}): 2,977, 2,845 (C–H str.), 1,693 (C=O str.), 1,612 (C=C str., alkene), 1,600, 1,566 (C=C str., aromatic), 1,147 (C–N str.), 1,024 (C–O–C str.) 885, 775 (OOP bending). ^{1}H-NMR ($CDCl_3$): δ 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, $J = 9$ Hz), 7.61 (s, 1H), 7.65 (m, 2H), 8.67 (d, 1H, $J = 1.68$ Hz). ^{13}C-NMR ($CDCl_3$): δ 197.3, 150.0, 149.0, 144.3, 140.9, 139.4, 138.9, 135.4, 134.9, 129.5, 128.2, 128.0, 127.2, 127.1, 126.5, 119.9, 115.7, 111.9, 56.9, 37.8. FAB-mass (*m/z*): 425 (M^+).

1-{[(2,6-Dichlorophenyl)-3-(naphthalen-1-yl)]methylene}-1,3-dihydro-indol-2-one ***(4f)*** Yield: 55 %, m.p. (°C): 178. $R_f = 0.72$. IR (KBr, cm^{-1}); 1,712 (C=O str.), 1,688 (C=C str., alkene), 1,627, 1,576 (C=C str., aromatic), 1,177 (C–N str.), 807 (C–Cl str.), 728 (OOP bending), 669. ^{1}H NMR

($CDCl_3$): δ 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 ($CDCl_3$): δ 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^+).

1-{[(2,6-Dichlorophenyl)-3-(4-chlorophenyl)]methylene}-1,3-dihydro-indol-2-one ***(4g)*** Yield: 60 %, m.p. (°C): 164. $R_f = 0.73$. IR (KBr, cm^{-1}): 1,713 (C=O str.), 1,680 (C=C str., alkene), 1,622, 1,545 (C=C str., aromatic), 1,164 (C–N str.), 757 (C–Cl str.), 734 (OOP bending), 679. ^{1}H-NMR ($CDCl_3$): δ 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 ($CDCl_3$): δ 197.0, 144.0, 141.0, 139.6, 138.9, 135.7, 134.9, 133.8, 133.6, 129.4, 128.9, 128.6, 128.2, 127.8, 127.6, 127.0, 126.6, 37.7; FAB-mass (*m/z*): 400 (M^+).

1-{[(2,6-Dichlorophenyl)-3-(4-methoxyphenyl)]methylene}-1,3-dihydro-indol-2-one ***(4h)*** Yield: 57 %, m.p. (°C): 175. $R_f = 0.44$. IR (KBr, cm^{-1}): 1,718 (C=O str.), 1,688 (C=C str., alkene), 1,610, 1,536 (C=C str., aromatic), 1,216 (C–N str.), 766 (C–Cl str.), 723 (OOP bending). ^{1}H-NMR ($CDCl_3$): δ 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 ($CDCl_3$): δ 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^+).

1-{[(2,6-Dichlorophenyl)-3-(4-fluorophenyl)]methylene}-1,3-dihydro-indol-2-one ***(4i)*** Yield: 56 %, m.p. (°C): 152. $R_f = 0.47$. IR (KBr, cm^{-1}): 1720 (C=O str.), 1,639 (C=C str., alkene), 1,598, 1,564 (C=C str., aromatic), 1,234 (C–F str.), 1,157 (C–N str.), 817 (OOP bending), 786 (C–Cl str.). ^{1}H-NMR ($CDCl_3$): δ 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 ($CDCl_3$): δ 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^+).

1-{[(2,6-Dichlorophenyl)-3-(4-hydroxyphenyl)]methylene}-1,3-dihydro-indol-2-one ***(4j)*** Yield: 60 %, m.p. (°C): 218. $R_f = 0.61$. IR (KBr, cm^{-1}): 3,418, (O–H str.), 1,688 (C=O str.), 1,678 (C=C str., alkene), 1,610, 1,579 (C=C str., aromatic), 1,107 (C–N str.), 741 (OOP bending), 717 (C–Cl str.), 656. ^{1}H-NMR ($CDCl_3$): δ 6.92 (d, 2H, $J = 7.15$ Hz), 6.98 (s, 1H), 7.18 (t, 1H, $J = 8.2$ 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 ($CDCl_3$): δ 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^+).

1-{[(2,6-Dichlorophenyl)-3-(4-(dimethylamino)phenyl)]-methylene}-1,3-dihydro-indol-2-one ***(4k)*** Yield: 46 %, m.p. (°C): 220–222. $R_f = 0.42$. IR (KBr, cm^{-1}): 2,912, 2,856 (C–H str.), 1710 (C=O str.), 1,620 (C=C str., alkene), 1,589, 1,536 (C=C str., aromatic), 1,222 (C–N str.), 785 (OOP bending), 780 (C–Cl str.) 678. ^{1}H-NMR ($CDCl_3$): δ 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 ($CDCl_3$): δ 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^+).

1-{[(2,6-Dichlorophenyl)-3-(3-chlorophenyl)]methylene}-1,3-dihydro-indol-2-one ***(4l)*** Yield: 55 %, m.p. (°C): 144. $R_f = 0.64$. IR (KBr, cm^{-1}): 1,703 (C=O str.), 1,668 (C=C str., alkene), 1,618, 1,570 (C= str., aromatic), 1,208 (C–N str.), 811 (C–Cl str.), 760 (OOP bending), 679. ^{1}H-NMR ($CDCl_3$): δ 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, $J = 9.76$ Hz), 7.71 (m, 4H), 7.83 (d, 1H, $J = 7.81$ Hz), 7.96 (s, 1H). ^{13}C-NMR ($CDCl_3$): δ 197.0, 144.3, 140.9, 139.5, 138.8, 136.4, 135.4, 135.0, 134.6, 130.5, 129.7, 128.6, 128.4, 128.0, 127.4, 127.0, 126.4 126.1, 124.5, 37.8; FAB-mass (*m/z*): 400 (M^+).

1-{[(2,6-Dichlorophenyl)-3-(3-bromobenzylidene)]methylene}-1,3-dihydro-indol-2-one ***(4m)*** Yield: 52 %, m.p. (°C): 149–151. $R_f = 0.67$. IR (KBr, cm^{-1}): 1,702 (C=O str.), 1,650 (C=C str., alkene), 1,613, 1,577 (C=C str., aromatic), 1,214 (C–N str.), 743 (OOP bending), 718 (C–Cl str.). 617. ^{1}H-NMR ($CDCl_3$): δ 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 ($CDCl_3$): δ 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^+).

1-{[(2,6-Dichlorophenyl)-3-(3-phenoxyphenyl)]methylene}-1,3-dihydro-indol-2-one ***(4n)*** Yield: 59 %, m.p. (°C): 148. $R_f = 0.65$. IR (KBr, cm^{-1}): 1,722 (C=O str.), 1,690 (C=C str., alkene), 1,625, 1,545 (C=C str., aromatic), 1,138 (C–N str.), 810 (C–Cl str.), 759 (OOP bending), 667. ^{1}H-NMR ($CDCl_3$): δ 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 ($CDCl_3$): δ 197.3, 157.5, 157.1, 144.3, 140.9, 139.7, 139.0, 135.4, 135.3, 135.2, 129.9, 128.9, 128.5, 128.2, 127.8, 126.7, 122.4, 119.9, 117.8, 116.9, 114.2, 37.2; FAB-mass (*m/z*): 457 (M^+).

1-{[(2,6-Dichlorophenyl)-3-(4-methoxyphenyl)]methylene}-1,3-dihydro-indol-2-one ***(4o)*** Yield: 53 %, m.p. (°C): 190. $R_f = 0.44$. IR (KBr, cm^{-1}): 1,718 (C=O str.), 1,688 (C=C str., alkene), 1,610, 1,536 (C=C str., aromatic), 1,216 (C–N str.), 766 (C–Cl str.). 723 (OOP bending). ^{1}H-NMR ($CDCl_3$): δ 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 ($CDCl_3$): δ 197.2, 160.0, 144.2, 141.4, 139.5, 138.9, 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^+).

1-{[(2,6-Dichlorophenyl)-3-(2-chloroquinolin-3-yl)]methylene}-1,3-dihydro-indol-2-one ***(4p)*** Yield: 57 %, m.p. (°C): 256–258. $R_f = 0.58$. IR (KBr, cm^{-1}): 1,694 (C=O str.), 1,668 (C=C str., alkene), 1,605, 1,535 (C=C str., aromatic), 1,109 (C–N str.), 738 (OOP bending), 764 (C–Cl str.), 656.^{1}H-NMR ($CDCl_3$): δ 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 ($CDCl_3$): δ 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^+).

1-{[(2,6-Dichlorophenyl)-3-(2-chloro-6-methylquinolin-3-yl)]methylene}-1,3-dihydro-indol-2-one ***(4q)*** Yield: 58 %, m.p. (°C): 278 (dec.). $R_f = 0.67$. IR (KBr, cm^{-1}): 2,966 (C–H str.), 1,708 (C=O str.), 1,677 (C=C str., alkene), 1,610, 1,560 (C=C str., aromatic), 1,181 (C–N str.), 782 (C–Cl str.), 749 (OOP bending), 687. ^{1}H-NMR ($CDCl_3$): δ 2.14 (s, 3H), 6.74 (d, 1H, $J = 8.8$ 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 ($CDCl_3$): δ 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^+).

1-{[(2,6-Dichlorophenyl)-3-(2-chloro-7,8-dimethylquinolin-3-yl)]methylene}-1,3-dihydro-indol-2-one ***(4r)*** Yield: 51 %, m.p. (°C): 260. $R_f = 0.68$. IR (KBr, cm^{-1}): 2,980, 2,867 (C–H str.), 1,721 (C=O str.), 1,634 (C=C str., alkene), 1,602, 1,527 (C=C str., aromatic), 1,220 (C–N str.), 770 (C–Cl str.), 731 (OOP bending), 650. ^{1}H-NMR ($CDCl_3$): δ 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 ($CDCl_3$): δ 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^+).

1-{[(2,6-Dichlorophenyl)-3-(furan-2-yl)]methylene}-1,3-dihydro-indol-2-one ***(4s)*** Yield: 54 %, m.p. (°C): 184. $R_f = 0.41$. IR (KBr, cm^{-1}): 1,708 (C=O str.), 1,629 (C=C str., alkene), 1,602, 1,562 (C=C str., aromatic), 1,186 (C–N str.), 779 (C–Cl str.). ^{1}H-NMR ($CDCl_3$): δ 6.41 (d, 1H, $J = 7.47$ Hz), 6.50 (dd, 1H), 6.96 (d, 1H, $J = 3.45$ Hz), 7.14 (tt, 1H, $J = 6.0$, 1.3 Hz), 7.24 (tt, 1H, $J = 6.0$, 1.2 Hz), 7.37 (dd, 1H, $J = 9.0$ Hz), 7.51 (d, 2H, $J = 8.1$ Hz), 7.58 (s, 1H), 7.80 (d, 1H, $J = 1.72$ Hz), 8.58 (d, 1H, $J = 6.86$ Hz). ^{13}C-NMR ($CDCl_3$): δ 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^+).

Cytotoxicity assay

The cytotoxicity study performed is a three-day in vitro MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) based assay. This test is based on the principle of uptake of MTT by the metabolically active cells. MTT is metabolized by active mitochondria into a blue-colored formazan product which can be read spectrophotometrically. Cells were plated at a density of 10,000 cells per well in 96-well culture plates and incubated with various concentrations of compounds **4a–s** at 37 °C, 5 % CO_2, and 95 % humidity for 72 h. Control cells were not treated with the target molecules **(4a–s)**. The assay was terminated after 72 h by addition of 25 µL of filter sterilized MTT (5 mg/mL) to each well. After 3 h, supernatant was removed from each well and the formazan complex formed in cells was resuspended in 150 µL of DMSO. For suspension cell lines, after 3 h of MTT addition, 50 µL of 10 % SDS—0.01 N HCl was added to each well to lyse the cells and dissolve the formazan complex. After incubating

for 1 h, the absorbance of each well was measured spectrophotometrically at 540 nm. The cytotoxicity percentage was calculated using the formula Cytotoxicity percentage = $(1 - X/R_1) * 100$, where X = absorbance of treated sample at 540 nm, R_1 = absorbance of control sample at 540 nm. The results from the assay are depicted in Table 1.

Conclusion

In summary, molecules **4e, 4k,** and **4r** emerged as potential cytotoxic agents on stomach cancer cell lines. A significant number of compounds have also shown promising cytotoxicity on pancreas and oral cancer cell lines. The extended study of the 2-oxindoles on a series of cell lines has shown potent anticancer activity revealing that modulation by groups at C_3 varies the cytotoxicity. The synthesis of more focused library, especially with 2,6-difluoro aryl fragment and its anticancer evaluation is in progress. The results will be disseminated in due course.

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Conflicts of interest The author declares that they have no conflicts of interest.

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