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Design, synthesis and biological evaluation of novel indolin-2-ones as potent anticancer compounds

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Abstract

The indolin-2-one core is a privileged structure for antitumor agents, especially kinase inhibitors. Twenty-three novel indolin-2-ones were designed by molecular dissection of the anticancer drug indirubin. Seventeen of them exhibited significant inhibition against the tested cell lines, and two of them (**1c** and **1h**) showed IC_{50} values at the submicromolar level against HCT-116 cells. Compounds **1c** and **2c** were also potent inhibitors of the triple-negative breast cancer (TNBC) cell line MDA-MB-231. Flow cytometry was utilized to explore the antitumor mechanism of **1c** and **2c** with MDA-MB-231 cells, and distinct effects were observed on **2c**. Furthermore, immunocytochemical examination of **1c** suggested a destabilization of microtubules, which was significantly different from the effect of **IM**, an indirubin derivative.

Keywords

Indolin-2-one; Privileged structure; Cytotoxicity; Molecular mechanism

The indolin-2-one core is regarded as a privileged structure for antitumor agents, in particular, kinase inhibitors, and numerous indolin-2-one derivatives have been reported as potent kinase inhibitors.[1] Among the examples shown in Fig. 1, Sunitinib[2] and Toceranib

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A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2017.06.019.

phosphate[3] have been approved for cancer therapy by the US FDA, and SU5416,[4] SU6668,[5] SU5614, SU14813, SU9516, and SU4984[1] have been investigated in either clinical trials or preclinical development.

Indirubin (Fig. 2) is a natural product with the bis-indole scaffold. As the active ingredient of the traditional Chinese prescription Danggui Longhui Wan, indirubin is used to treat chronic myeloid leukemia in China.[6,7] It is also reported to possess various biological activities, including anti-HIV,[8] anti-angiogenesis[9] and antiinflammatory[10,11] effects. However, the therapeutic application of indirubin and its derivatives is often hampered by their poor solubility, which is at least in part attributed to the bis-indole scaffold.

We have previously explored the SAR of a series of indirubin-3'-monoxime (**IM**, Fig. 2) derivatives.[12] As a follow-up study and also to expand the SAR of this compound class, two series of indolin-2-one derivatives were designed by molecular dissection of indirubin (Fig. 3). Series **1** was designed by dividing between N1' and C2' to provide a 2-oxo-2-phenylethylidene side chain. From previous SAR studies, fluoro-substitution on ring B might be favored for antitumor activity.[12] Thus, fluorine was maintained at R^1 for most series **1** compounds and substituted phenyl and other aromatic rings were introduced in R^2. In compound **1a**, an isosteric OH group replaced the residual N1' amino. In contrast, series **2** was obtained by breaking the covalent bond between C2' and C3' to leave a (phenylamino)-methylene side chain. To enhance water-solubility, 3-(p-methylpiperazinyl) phenyl was incorporated as R^4 in series **2** compounds, whereas various aromatic and aliphatic substituents were introduced as R^3.

The series **1** and **2** compounds were prepared following previously published protocols[13,14] as presented in Schemes 1 and 2. Briefly, nucleophilic addition of indoline-2,3-dione **3** (Scheme 1) and various ethanones **4** provided the key intermediate **5**. Elimination of H_2O from 5 produced series **1** compounds. The preparation of series **2** compounds began from 5-nitroindolin-2-one (**6**, Scheme 2). After treating **6** with ethylorthoformate and acetic anhydride, the enol ether intermediate **7** was obtained, and **7** was subsequently converted into the enamine **8**. Hydrolysis of the acetyl and reduction of the nitro group in **8** afforded intermediate **10**, which was reacted with different acids to give series **2** compounds.

All compounds in both series **1** and **2** were obtained as a single stereoisomer. The downfield chemical shifts of H-4 and H-2' (Fig. 3) suggested an (*E*)-configuration in series **1** compounds,[13,15] which was further confirmed by the lack of correlation between H-4 and H-2' in the NOESY spectra of compound **1c** (Supporting Data). Similarly, the stereochemistry of compounds in series **2** was assigned as (*Z*)-configuration based on the downfield chemical shifts of H-4 and the apparent correlation between H-4 and H-2' in compound **2c** (Supporting Data).

To explore their potential as antitumor agents, compounds in series **1** and **2** were tested in an MTT assay for their inhibitory activity against HCT-116, HepG2, BGC-823, NCI-H1650 and A2780 tumor cell lines. The results are shown in Tables 1 and 2. With regard to series **1** in Table 1, ten compounds showed significant inhibitory activity against all the tested tumor cell lines, and compounds **1c** and **1h** exhibited submicromolar IC_{50} values against HCT-116

cells. According to the data given in Table 1, it appears that R^1 can accommodate a range of different substituents and might have little effect on the tumor inhibitory activity of this compound class (**1e** *vs* **1f**, **1g** *vs* **1h**). In contrast, the R^2 substituents have a significant impact on the tumor inhibitory activity, although no obvious trends could be deduced from the currently available data. Five compounds in series **2** also displayed significant inhibitory activities against the tested tumor cell lines (Table 2). As observed in series **1**, many various substituents were well-tolerated at R^5 in series **2**. However, aliphatic substituents and heteroaromatic rings might be unfavorable for the cytotoxicity of this compound class (**2a**-**2d** *vs* **2e**-**2g**). Surprisingly, the indirubin derivative **IM** was tested in parallel and was inactive against all the tested cell lines.

Compounds **1c** and **2c**, the most active compound from each series, were selected as representative compounds for further evaluation against MDA-MB-231, MCF-7, A594, KB and KB-vin cells in a sulforhodamine B (SRB) assay, and **IM** was tested in parallel (Table 3). Notably, all three compounds were potent against MDA-MB-231, which are triple-negative breast cancer (TNBC) cells, a clinically aggressive form of breast cancer and generally unresponsive to chemotherapies.

Since **IM** is an effective inhibitor of CDK2 and CDK9,[12] compounds in series **1** and **2** were initially evaluated against CDK2/ Cyclin E1 and CDK9/Cyclin T1 at a concentration of 10 μM. However, neither compound inhibited either CDK system. This observation implied that the molecular dissection of the bis-indole scaffold in **IM** to the indolin-2-one core in **1c** and **2c** resulted in significantly altered pharmacological profiles and an implicit shift in molecular mechanism.

To shed light on the underlying antitumor mechanism of compounds **1c** and **2c**, their effects on cell cycle progression were evaluated in the TNBC cell line MDA-MB-231 by employing flow cytometry.[16] As depicted in Fig. 4, the ratio of G2/M was slightly increased in cells treated with compounds **1c** and **IM** at a concentration five-fold of the corresponding IC_{50} value. The sub-G1 phase was dramatically increased in cells treated with compound **2c**, suggesting that **2c** might induce apoptosis quickly in a target-independent manner. Furthermore, cells treated with **1c** and **IM** were observed with confocal fluorescence microscopy to examine their effects on microtubules (Fig. 5). The microtubule network in interphase cells (unstained by antibody to phosphorylated histone H3) was clearly observed in control cells (DMSO), while unclear in cells treated with **1c**, suggesting that **1c** destabilized microtubules. In contrast, pseudopodia elongation was detected when the cells were treated with **IM**, suggesting no significant effect on microtubule destabilization. These observations implied different mechanisms of action for the two compounds.

In summary, twenty-three novel indolin-2-one compounds were designed, synthesized and evaluated for their cytotoxic activity. Most compounds showed significant antiproliferative activity against the tested cell lines, and notably, two compounds showed potent inhibition against the TNBC cell line MDA-MB-231. To explore the antitumor mechanism of this compound class, flow cytometry and confocal microscopy were utilized and molecular mechanisms different from that of **IM** were implied. The results suggest that the privileged

structure indolin2-one could be a good chemical phenotype for both structural diversity and mechanistic novelty.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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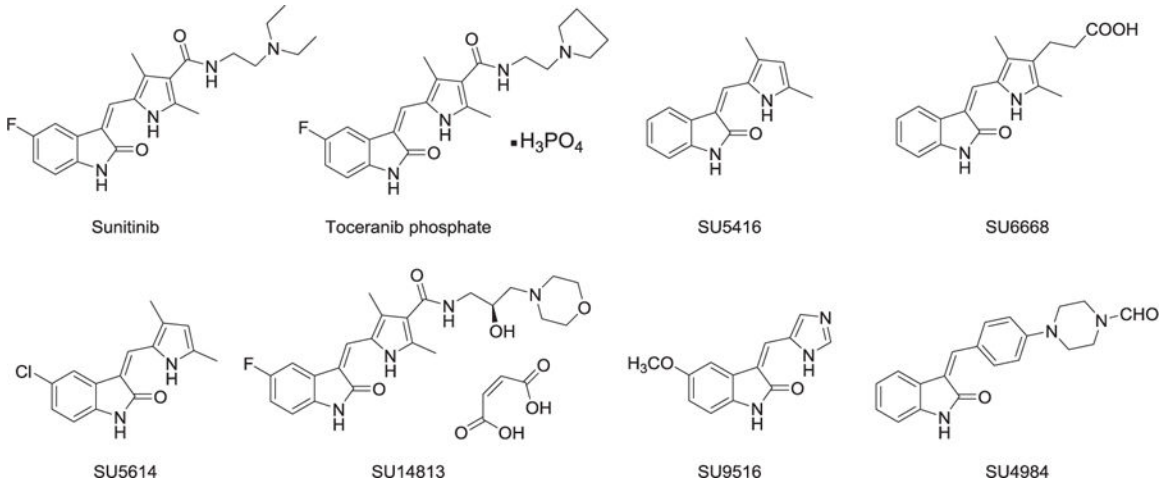
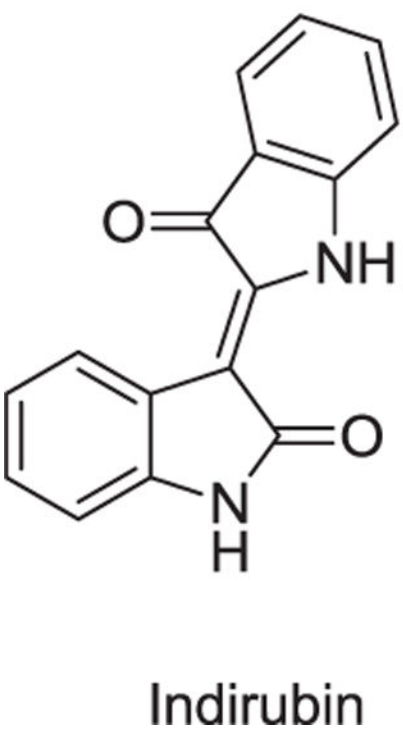
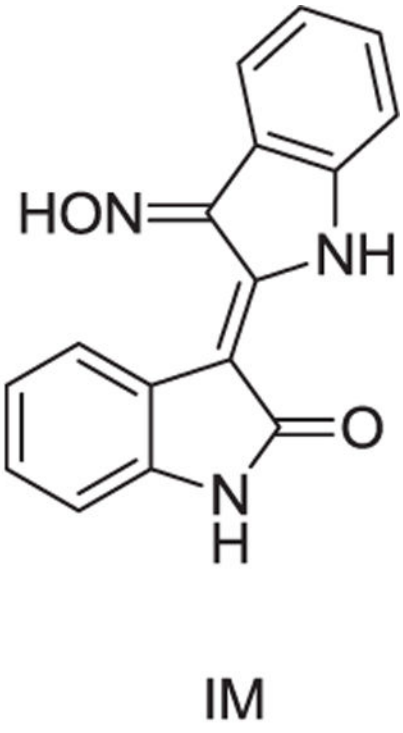


Fig. 1.
Representative indolin-2-ones as kinase inhibitors



Indirubin



IM

Fig. 2.
Structure of indirubin and **IM**

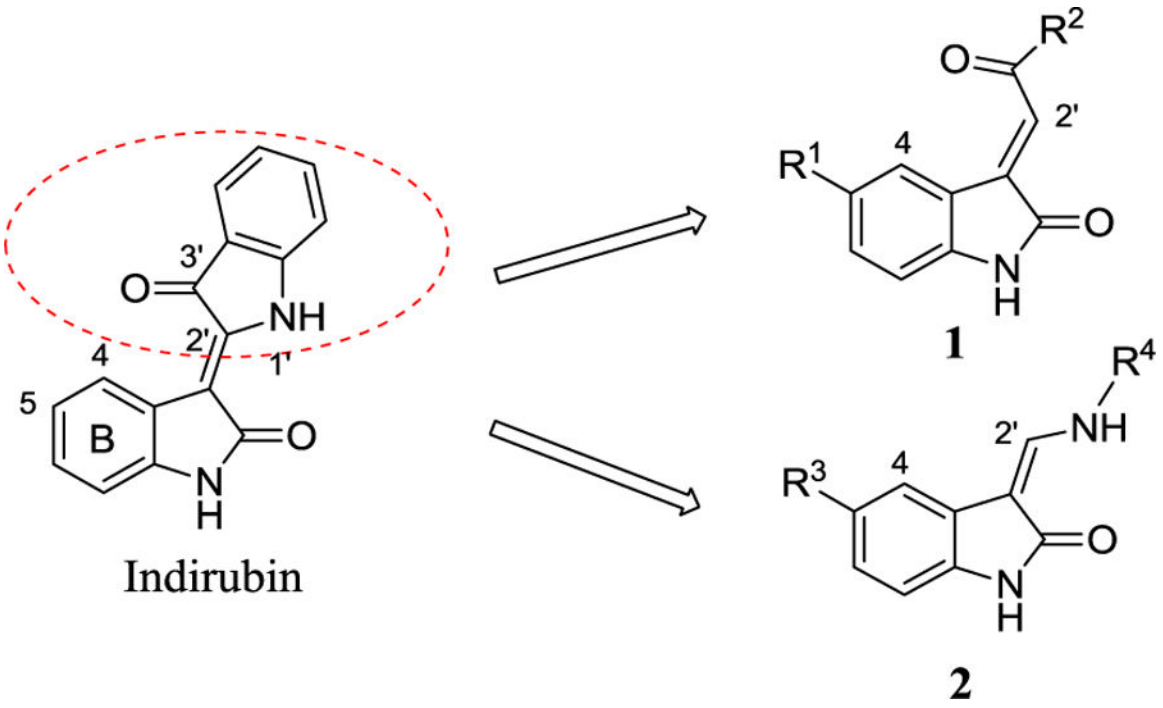


Fig. 3.
Molecular design of the target compounds based on indirubin

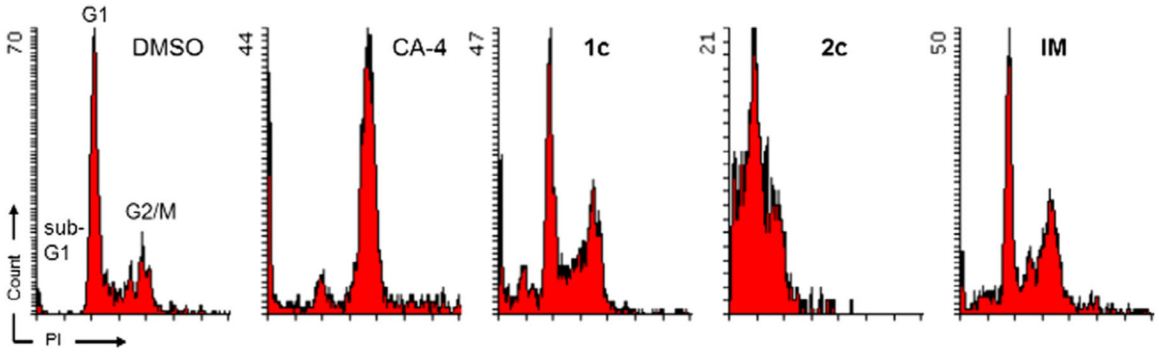


Fig. 4.

Effects of compounds **1c, 2c** and **IM** on cell cycle progression. TNBC MDA-MB-231 cells were treated for 24 h with compounds at a concentration of five-fold the IC_{50} value. Combretastatin A-4 (CA-4) was used at 200 nM as a reference for induction of cell cycle arrest in G2/M. Cells were fixed and stained with propidium iodide (PI) followed by analysis of cell cycle using a flow cytometer.

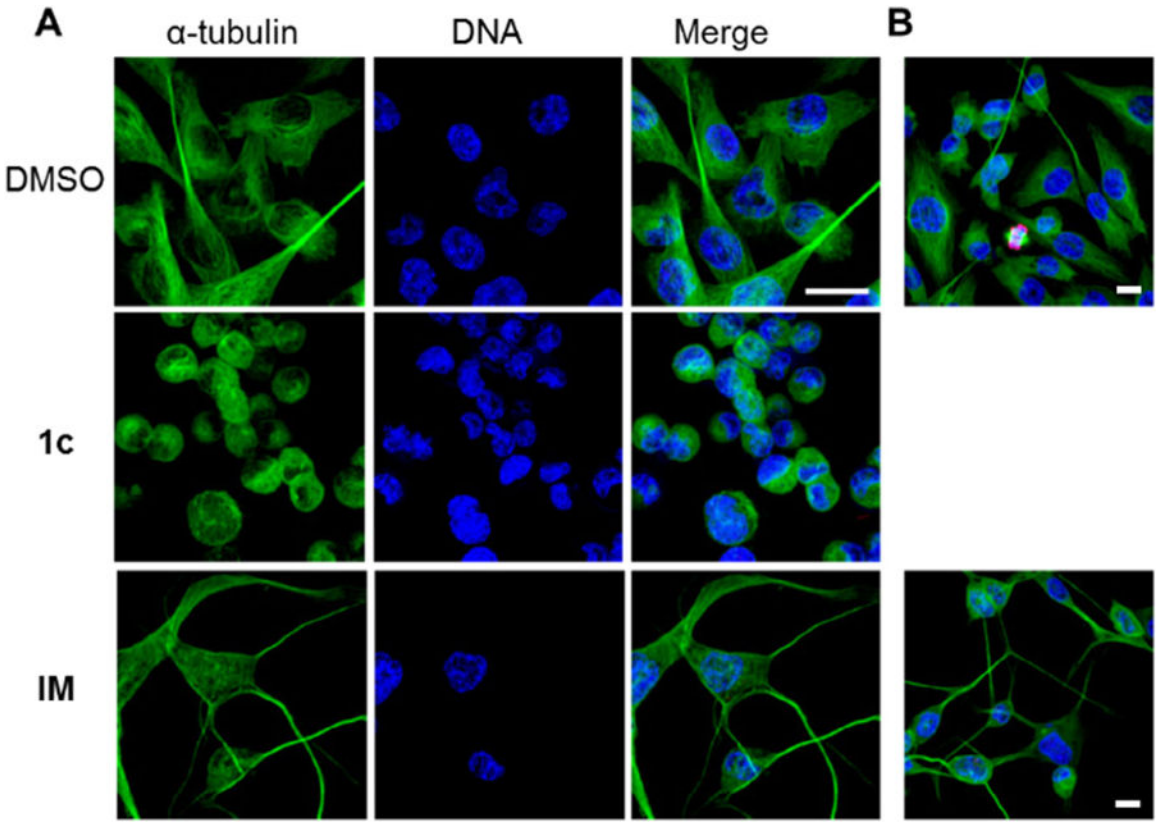
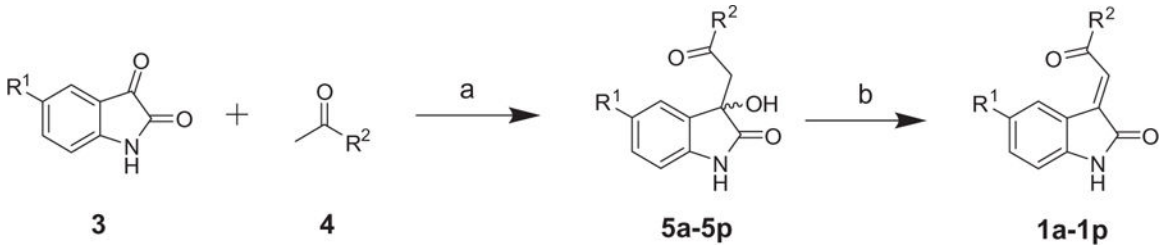


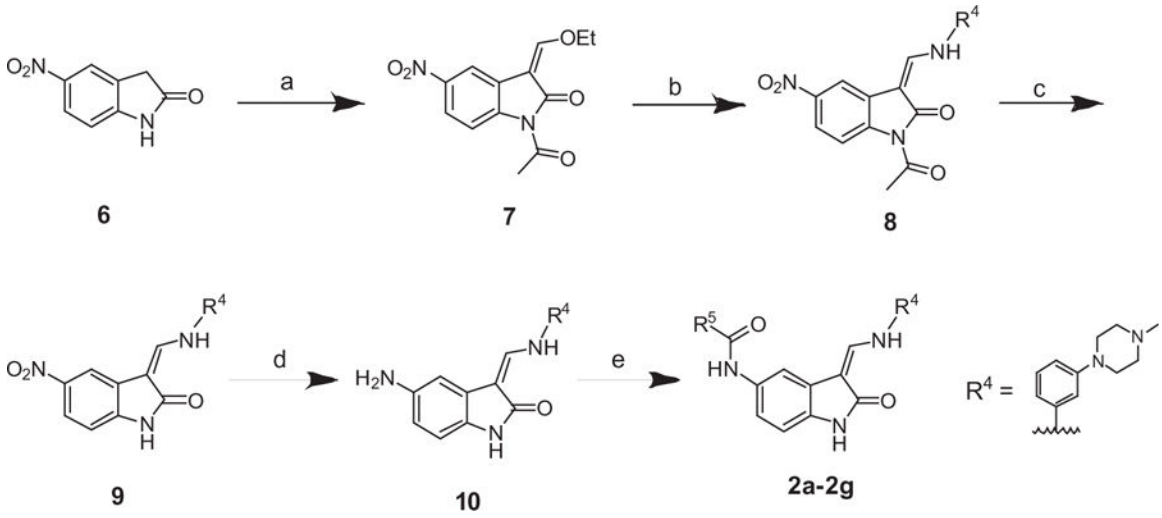
Fig. 5.

Immunocytochemical examination of cells treated with compound **1c** and **IM**. TNBC MDA-MB-231 cells were treated for 24 h with compound at a concentration five-fold the IC_{50} value. Cells were fixed and stained with antibodies to α-tubulin (green) as a marker for the chromosome condensation, and DAPI for DNA (blue). Phosphorylated histone H3 was undetectable in interphase cells. Images were captured by Zeiss LSM700 confocal fluorescence microscope using ×63 (A) or ×40 (B) objective lens. Bar, 25 μm.



Scheme 1.

Synthesis of compounds **1a-1p**. Reagents and conditions: (a) Et_2NH, C_2H_5OH, r.t.; (b) hydrochloric acid, C_2H_5OH, 60 °C, 2–24 h.

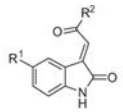


Scheme 2.

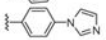
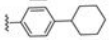
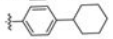
Synthesis of compounds **2a-2g**. Reagents and conditions: (a) $CH(OEt)_3$, acetic anhydride, 150 °C, 1 h; (b) R_2NH_2, dry DMF, 110 °C, 10 h; (c) 1 M NaOH (aq), CH_3OH, r.t., 3–4 h; (d) Pd/C, H_2, CH_3OH/CH_2Cl_2, r.t.; (e) R^5COOH, HATU, triethylamine, DMSO, r.t.

Table 1

Inhibitory activity of compounds **1a–1p** against selected tumor cell lines by the MTT assay.



1a-1p

Compd.	R1	R2	IC_{50} (μM)				
			HCT-116[#]	HepG-2	BGC-823	NCI-H1650	A2780
Taxol	–	–	0.03	0.02	0.001	0.07	0.03
IM	–	–	NA[*]	NA	NA	NA	NA
1a	F		NA	NA	NA	NA	NA
1b	F		1.07	3.44	6.12	3.46	1.58
1c	F		0.76	2.28	4.09	1.22	1.12
1d	F		NA	NA	NA	NA	NA
1e	F		2.52	2.84	4.45	2.73	2.31
1f	NO_2		2.32	3.99	5.90	3.86	2.09
1g	F		3.01	1.86	8.99	6.58	2.44
1h	H		0.86	2.11	9.44	1.27	2.08
1i	F		NA	NA	NA	NA	NA
1j	F		1.38	3.12	6.42	2.84	2.17
1k	F		NA	NA	NA	NA	NA
1l	F		1.84	8.78	7.75	3.22	2.75
1m	F		2.45	3.10	2.66	3.56	2.14
1n	F		NA	NA	NA	NA	NA
1o	F		NA	NA	NA	NA	NA
1p	F		1.41	3.09	8.02	2.73	2.37

[*] NA: not active. No significant inhibition against tumor cell growth was observed at the concentration of 10 μM.

[#] HCT-116 (human colon cancer), HepG2 (liver hepatocellular cells), BGC-823 (human gastric cancer cell line), NCI-H1650 (non-small-cell carcinoma), A2780 (human ovarian cancer).

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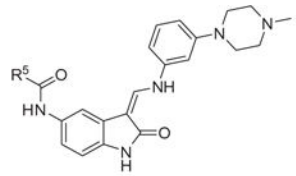
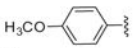
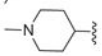
Table 2

Inhibitory activity of compounds **2a-2g** against selected tumor cell lines by the MTT assay.



2a-2g

Compd.	R5	IC_{50} (μM)				
		HCT-116[#]	HepG-2	BGC-823	NCI-H1650	A2780
Taxol	–	0.03	0.02	0.001	0.07	0.03
IM	–	NA[*]	NA	NA	NA	NA
2a		2.01	1.78	3.17	1.87	2.05
2b		2.74	6.72	8.50	4.28	2.91
2c		2.15	3.03	2.67	1.63	2.03
2d		2.28	2.37	2.24	2.17	2.14
2e		NA	5.37	NA	7.56	4.46
2f		9.41	5.07	6.08	5.87	3.37
2g		4.67	NA	NA	NA	NA

[*] NA: not active. No significant inhibition against tumor cell growth was observed at the concentration of 10 μM.

[#] HCT-116 (human colon cancer), HepG2 (liver hepatocellular cells), BGC-823 (human gastric cancer cell line), NCI-H1650 (non-small-cell carcinoma), A2780 (human ovarian cancer).

Table 3

Inhibitory activity of compounds **1c, 2c** and **IM** against selected tumor cell lines by the SRB assay.

Compd.	IC_{50} (μM)				
	MDA-MB-231[#]	MCF-7	A549	KB	KB-VIN
IM	7.59 ± 0.71	6.81 ± 0.21	5.82 ± 0.05	5.34 ± 0.17	5.17 ± 0.07
1c	5.31 ± 0.22	>10	>10	>10	>10
2c	4.43 ± 0.19	4.44 ± 0.10	4.75 ± 0.04	4.40 ± 0.14	7.86 ± 0.08

[#]MDA-MB-231(human breast carcinoma cell line), MCF (Michigan Cancer Foundation-7), A549 (lung cancer), KB (nasopharyngeal carcinoma) and KBvin (vincristine-resistant KB subline).