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Isatin derivatives with activity against apoptosis-resistant cancer cells[†]

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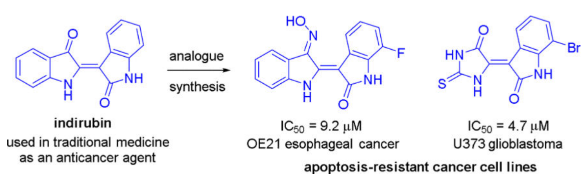
Abstract

In a search of small molecules active against apoptosis-resistant cancer cells, a series of isatin-based heterocyclic compounds were synthesized and found to inhibit proliferation of cancer cell lines resistant to apoptosis. The synthesis of these compounds involved a condensation of commercially available, active methylene heterocycles with isatin proceeding in moderate to excellent yields. The heterocyclic scaffolds prepared in the current investigation appear to be a useful starting point for the development of agents to fight cancers with apoptosis resistance, and thus, associated with dismal prognoses.

Graphical abstract



Keywords

Anticancer activity; Apoptosis resistance; Indirubin; Isatin; Cytotoxic compounds

Cancers with intrinsic resistance to apoptosis are generally poorly responsive to current chemotherapeutic agents, most of which work through the induction of apoptosis. These cancers, which include tumors of the lung, liver, stomach, esophagus, pancreas, as well as melanoma and glioblastoma, represent a major challenge in the clinic in terms of treatment

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strategies.[1] For example, patients afflicted by glioblastoma multiforme,[2,3] have a median survival expectancy of less than 14 months when treated with the best available protocol that involves chemotherapy with temozolomide.[4] Indeed, due to their high resistance to apoptosis, glioblastoma cells respond poorly to conventional chemotherapy with proapoptotic agents.[3] In addition, the majority of cancer patients die from tumor metastases. Metastatic cells have acquired resistance to cell death known as anoikis, which is normally triggered due to the lost of contact with the extracellular matrix or neighboring cells.[5] The ability to survive anoikis renders metastatic cells resistant to the large majority of proapoptotic agents as well.[3,6] Therefore, a search for novel anticancer agents that can overcome cancer cell resistance to apoptosis is an important pursuit.

In this connection, natural products represent a valuable source of not only cytotoxic compounds, but also those molecules capable of overcoming the intrinsic resistance of cancer cells to apoptosis.[7–10] Our recent studies have focused on the class on natural products known as indirubins (Figure 1A).[11] The 2,3`-bis-indoles have been reported to modulate glycogen synthase kinase (GSK)-3,[11,12] 5-lipoxygenase (5-LO),[13] tyrosine kinases,[14] and aryl hydrocarbon (Ah) receptor.[15] Indirubin derivatives that attracted our interest were indirubin-3`-oximes based on a report from Meijer’s group, showing that 7-bromoindirubine-3`-oxime (7-BIO, Figure 1B) triggers necrotic, caspase-independent cancer cell death,[16] indicating the promise of this type of compounds in overcoming apoptosis resistance.[17] This discovery prompted us to synthesize a series of indirubin derivatives, as well as related isatin-based heterocyclic scaffolds (Figure 1C), and evaluate them against apoptosis-resistant cancer cells.

For the synthesis of indirubin analogs (**4a–g**), most of which bear a halogen atom in the ring (as in 7-BIO), we employed a base-catalyzed condensation of substituted isatins (**1a–e**) with indoxyl acetate (**2**) and 1-indanone (**3a**). 3-Coumaranone (**3b**) failed to react with **1a** neatly due to a rapid self-condensation, but a switch from basic to acidic conditions facilitated a clean reaction and we were able to obtain a high yield of **4h**. Compounds **4a–f** were further reacted with an excess of hydroxylamine hydrochloride in pyridine providing 3’-oximes **5a–f** (Scheme 1).

To extend the series beyond the indirubin scaffold, we synthesized compounds resulting from the condensation of isatin with other active methylene heterocycles (Scheme 2). Our selection of suitable heterocycles was based on their previously described biological and therapeutic relevance. Thus, hydantoin (**6**) derivatives are represented by approved drugs (Phenytoin, Fosphenytoin), pesticides (Imiprothrin, Iprodione), or inhibitors of matrix metalloproteinases (MMPs).[18] Thiohydantoin (**7**) derivatives have been identified as antimicrobials[19] and antitumor[20] agents with topoisomerase I inhibitory activities.[21] Rhodanine (**8**) derivatives are well known beta-lactamase inhibitors.[22,23] Analogues of thiazolidinedione (**9**) are peroxisome proliferator-activated receptor (PPAR) agonists and have been used as antidiabetic drugs.[24] Condensation of isatins with the above mentioned heterocycles was found to work best with sodium acetate in acetic acid, providing the desired isatin derivatives **10a–f** in satisfactory yields (Scheme 2).

Having synthesized the library, we proceeded with the *in vitro* evaluation of their growth inhibitory activity against the panel of nine cancer cell lines (Table 1). The panel included cell lines with demonstrated resistance towards the induction of apoptosis, such as U373 glioblastoma,[25] A549 non-small cell lung cancer,[26] SKMEL-28 melanoma,[27] OE21 esophageal cancer[28] along with apoptosis sensitive cells, incuding HS683 glioma, B16F10 murine melanoma, MCF7 breast cancer and PC-3 prostate cancer (the apoptosis sensitivity of the LoVo colon cancer cell line is unavailable in the literature). As a reference, we used beta-lapachone, shown to be active against a variety of genetically distinct cancers and capable of inducing caspase-independent apoptosis, necrosis and autophagy.[29] The analysis of the antiproliferative activities reveals single digit micromolar potencies of Meijer's compound **5e** and its regioisomer **5b**. The other synthesized compounds all display growth inhibitory properties in single- to double-digit micromolar range depending on the cell line. In a manner similar to **5e** and beta-lapachone, for the majority of the synthesized compounds there was no difference between the activities against apoptosis-sensitive and resistant cell lines, indicating that they are capable of overcoming apoptosis resistance. For example, thiohydantoin isatin derivative **10c** displays single digit micromolar potencies against apoptosis-resistant glioblastoma (GBM) and non-small lung cancer (NSCLC), as well as apoptosis sensitive murine melanoma (B16F10) cells. Similar observations were made for indirubin-3`-oximes **5a** and **5c**, capable of displaying single digit micromolar potencies against representative cell lines of both types. On the other hand, 3(2*H*)-benzofuranone isatin derivative **4h** appears to be much more active against the apoptosis-sensitive subpanel, even displaying nanomolar activity against B16F10 cells.

In conclusion, preparation and anticancer evaluation of indirubin derivatives and related isatin heterocycles revealed their single to double digit micromolar activity against a panel of cancer cell lines consisting of apoptosis-sensitive, as well as those with demonstrated apoptosis resistant properties. The results indicate that the majority of synthesized compounds show equal effectiveness against apoptosis resistant and sensitive cells, indicating their potential to overcome apoptosis resistance. Thus, this type of compounds could be used as a starting point for the development of agents active against cancers associated with dismal clinical outcomes. The progress on this work will be reported in due course.

Acknowledgments

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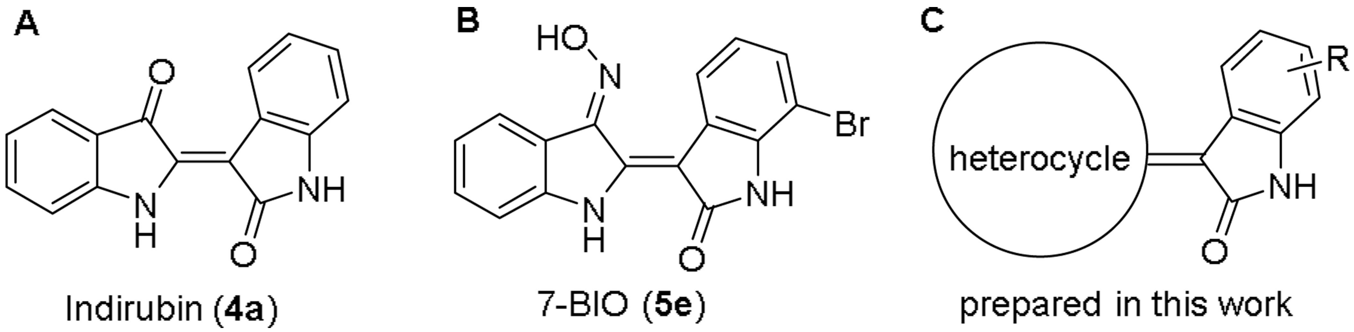

Figure 1.
Structures of natural and synthetic isatin derivatives relevant to the current study

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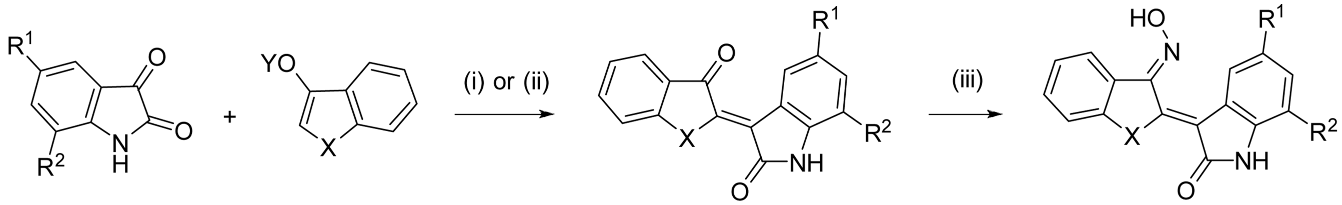


1a: R^1, R^2 = H
1b: R^1 = Br, R^2 = H
1c: R^1 = H, R^2 = F
1d: R^1 = H, R^2 = Cl
1e: R^1 = H, R^2 = Br

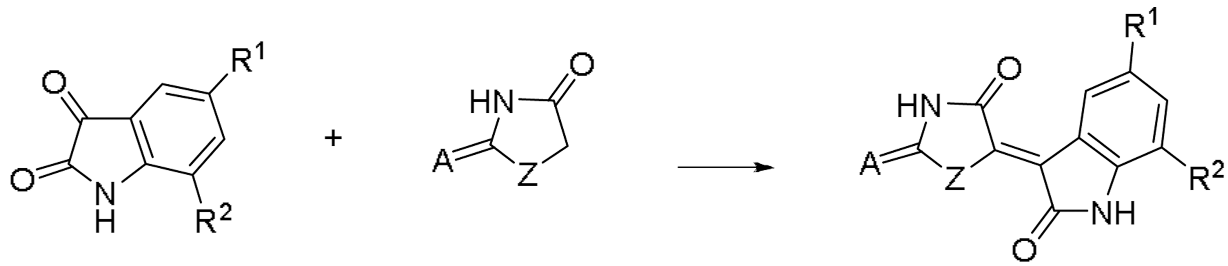
2: X = NH, Y = Ac
3a: X = CH_2, Y = H
3b: X = O, Y = H

4a: R^1, R^2 = H, X = NH
4b: R^1 = Br, R^2 = H, X = NH
4c: R^1 = H, R^2 = F, X = NH
4d: R^1 = H, R^2 = Cl, X = NH
4e: R^1 = H, R^2 = Br, X = NH
4f: R^1 = H, R^2 = H, X = CH_2
4g: R^1 = Br, R^2 = H, X = CH_2
4h: R^1, R^2 = H, X = O

5a: R^1, R^2 = H, X = NH
5b: R^1 = Br, R^2 = H, X = NH
5c: R^1 = H, R^2 = F, X = NH
5d: R^1 = H, R^2 = Cl, X = NH
5e: R^1 = H, R^2 = Br, X = NH
5f: R^1 = H, R^2 = H, X = CH_2

Scheme 1.

Synthesis of isatin derivatives **4a–h** and their 3'-oximes **5a–f**. (i) Na_2CO_3, MeOH, rt, 6–24 h (73% (**4a**), 81% (**4b**), 93% (**4c**), 88% (**4d**), 91% (**4e**), 86% (**4f**), 59% (**4g**)); (ii) AcONa, AcOH, 110 °C, 24 h (54% (**4h**)); (iii) NH_2OH•HCl, pyridine, 120 °C, 2 h (71% (**5a**), 59% (**5b**), 65% (**5c**), 73% (**5d**), 69% (**5e**), 36% (**5f**)).



1a: R^1, R^2 = H
1b: R^1 = Br, R^2 = H
1e: R^1 = H, R^2 = Br

6: A = O, Z = NH
7: A = S, Z = NH
8: A = S, Z = S
9: A = O, Z = S

10a: R^1, R^2 = H, A = O, Z = NH
10b: R^1, R^2 = H, A = S, Z = NH
10c: R^1 = H, R^2 = Br, A = S, Z = NH
10d: R^1, R^2 = H, A = S, Z = S
10e: R^1 = Br, R^2 = H, A = S, Z = S
10f: R^1, R^2 = H, A = O, Z = S

Scheme 2.
Synthesis of compounds **10a–f**. AcONa, AcOH, 110 °C, 24 h. (65% (**10a**), 52% (**10b**), 64% (**10c**), 58% (**10d**), 85% (**10e**), 66% (**10f**)).

Table 1

In vitro antiproliferative activities of the synthesized compounds

Compound	IC_{50} in vitro values, μM[a]								
	resistant to apoptosis				sensitive to apoptosis				unknown
	U373 (GBM)	A549 (NSCLC)	OE21 (esophageal cancer)	SKMEL-28 (melanoma)	HS683 (Glioma)	B16F10 (melanoma, mouse)	PC-3 (prostate cancer)	MCF-7 (breast cancer)	LoVo (colon cancer)
4a	^[b]	^	30.5	^	^	0.2	^	^	^
4b	8.6	^	3.8	^	14.1	3.8	^	37.3	12.0
4c	45.8	^	29	^	38.7	16.3	^	44.8	42.0
4d	^	^	35.3	^	55.2	15.7	^	^	^
4e	8.1	^	8.7	^	6.9	3.2	^	52.8	32.3
4f	^	^	25.1	^	^	^	^	70.2	52.6
4g	41.4	^	56.0	^	47.3	69.1	^	65.2	52.8
4h	27.7	23.3	25.9	23.8	25.7	<0.01	36.1	0.3	4.1
5a	18.0	27.0	8.1	26.6	6.0	5.4	24.3	9.2	8.9
5b	8.0	7.2	3.6	17.3	8.4	0.5	6.8	5.8	6.6
5c	19.7	33.3	9.2	44.6	9.3	7.8	26.0	26.2	9.0
5d	31.5	45.2	19.6	40.1	25.3	12.9	29.7	27.5	10.2
5e	6.6	13.9	3.9	9.8	8.1	5.3	7.8	4.9	5.6
5f	28.0	28.7	27.8	33.7	22.9	32.6	33.1	38.5	18.7
10a	^	66.5	52.3	^	84.7	73.3	95.5	94.6	76.1
10b	51.9	45.8	38.7	62.9	52.9	41.6	75.5	87.8	60.2
10c	4.7	3.8	20.2	11.7	40.8	5.0	22.8	51.0	37.0
10d	85.3	99.0	47.2	96.6	93.3	73.5	^	^	69.3
10e	41.7	36.2	62.5	53.5	93.6	0.1	44.3	82.9	52.1
10f	86.4	60.7	38.0	^	89.7	2.8	67.5	^	5.2
β-Lapachone	0.4	0.8	0.6	0.6	0.4	0.3	<0.01	0.5	0.3

[a] IC_{50} is the compound concentration that reduces by 50% the growth of a given cell line (as compared to the control value) after having cultured the cells for 72h with the compound of interest, as determined with the MTT assay. Each experiment was carried out in sextuplicates, numbers in table are arithmetic mean values.

[b] ^ - more than 100 μM.