

CONCISE ARTICLE

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Cite this: *Med. Chem. Commun.*, 2013, 4, 1452

Received 16th July 2013
 Accepted 16th September 2013
 DOI: 10.1039/c3md00200d
www.rsc.org/medchemcomm

Design, synthesis and antiproliferative activity of indole analogues of indanocine[†]

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The design and synthesis of a novel series of indole-analogues of indanocine is reported, together with their antiproliferative activity in the NCI's panel of cancer cell lines. Indanocine displays potent activity against a wide range of cancer cell lines (mean $GI_{50} < 20$ nM), including drug-resistant cancer cell lines, and also inhibits the migration of metastatic cancer cells. A number of the described indole-analogues display a similar activity profile to indanocine, exhibiting potent antiproliferative activities in several cancer cell lines, and offer new leads for further development.

Introduction

Continuing our interest in compounds that interact with tubulin,^{1,2} we wished to investigate novel analogues of indanocine **1** (Fig. 1). Indanocine **1** is a substituted indanone that displays potent antiproliferative activity against tumour cell lines (mean $GI_{50} < 20$ nM, the mean concentration to inhibit 50% cell growth against the National Cancer Institute's (NCI) panel of cancer cell lines), and is also active against multidrug resistant cell lines.^{3–5} Drug resistance can be due to several factors, including overexpression of the P-glycoprotein drug efflux pump,⁶ and studies have shown that although tumour cells which overexpress the 170 kD P-glycoprotein are resistant to paclitaxel, they remain sensitive to indanocine **1**.⁴

Indanocine **1** is a potent microtubule-destabilizing agent and binds to the colchicine binding site of tubulin.^{7–9} Recent reports have shown that indanocine reduces dynamic instability of microtubules at low concentrations and inhibits migration of metastatic cancer cells.¹⁰ Therefore indanocine and associated analogues are of great interest as they not only have promise as potential chemotherapeutic agents,¹¹ but could also be used to prevent the spread of cancer through metastasis.

Various indanocine analogues have been previously reported,^{5,12–16} however, we wished to apply an approach previously reported by Hudlicky *et al.* in the design of pancratistatin analogue **3** (Fig. 1).¹⁷ Isolated by Pettit *et al.*,¹⁸ pancratistatin **2** is a natural product which exhibits potent anticancer activity,¹⁹ and shows excellent promise as a therapeutic drug, particularly in brain cancers.²⁰ Hudlicky *et al.* proposed that the indole

motif has similar steric and electronic properties to the 4-hydroxy-benzo[1,3]dioxole group present in pancratistatin **2** (highlighted in bold, Fig. 1).¹⁷ In addition, the indole analogue **3** also retains the hydrogen-bonding pattern of the natural product **2**, believed to be important for potency.

Following this work,¹⁷ and the success of other indole-based inhibitors of tubulin polymerisation,²¹ we wished to investigate if the indole motif would also be a suitable bioisostere for the dimethoxyaniline group present in indanocine **1** (highlighted in bold, Fig. 2). We now describe the synthesis and antiproliferative activity of indole-analogues of indanocine (**4** and **5**) outlined in Fig. 3. We wished to investigate both the 5-membered ring cyclic ketone (**4**, as found in indanocine **1**) and the corresponding 6-membered ring core (**5**), since indanones⁵ and tetralones^{14,15,22} of similar compounds were both found to be the most active. In addition, the effects of the N-H hydrogen

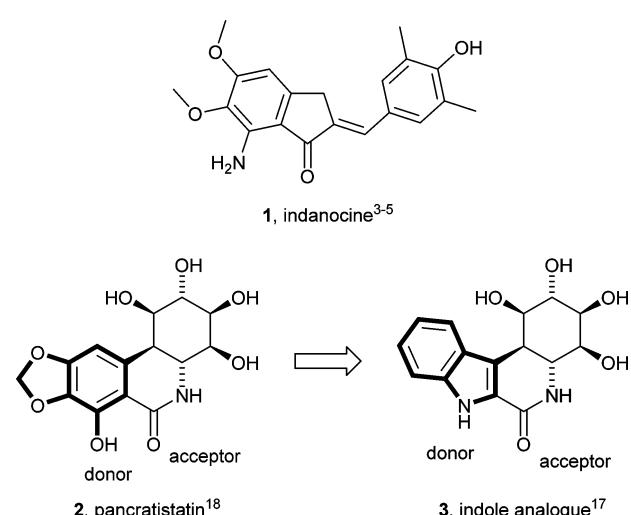


Fig. 1 Structures of indanocine **1**, pancratistatin **2** and an indole-analogue of pancratistatin **3**.

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† Electronic supplementary information (ESI) available: Experimental procedures, characterisation data, ¹H and ¹³C NMR spectra, and COMPARE analyses of compounds **4c**, **5c** and **5g**. See DOI: [10.1039/c3md00200d](https://doi.org/10.1039/c3md00200d)



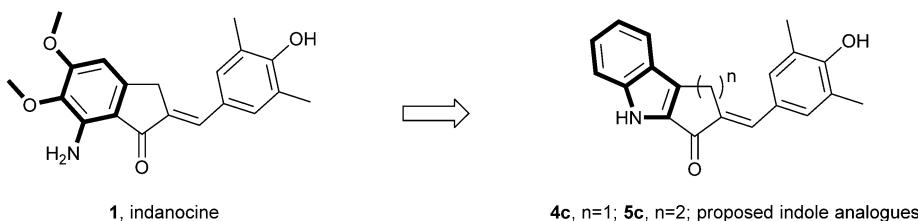
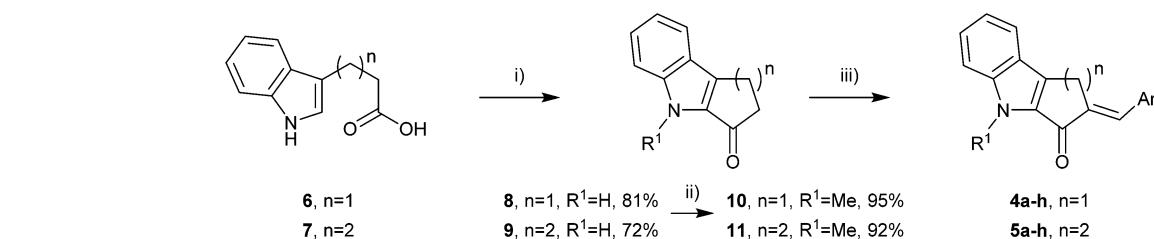


Fig. 2 Proposed indole-analogues of indanocine.



Scheme 1 Synthesis of the indole-analogues 4a-h and 5a-h. (i) PPA (6) or TFAA (7). (ii) KOH/MeI, acetone (iii) See Table 1.

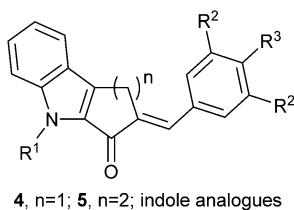


Fig. 3 Indole analogues of indanocine 4 and 5.

bonding interaction ($R^1 = H$, **a-d** and $R^1 = Me$, **e-h**) and various substituted aryl rings ($R^2 = Me$ or OMe and $R^3 = H$ or OH) were also investigated (see Table 1).

Table 1 Yields and NSC numbers of indole analogues 4 and 5 (Fig. 3)

Comp.	R ¹	R ²	R ³	n	Cond. ^a	Yield (%)	NSC ^b
4a	H	Me	H	1	A	72	ns
4b	H	OMe	H	1	A	73	ns
4c	H	Me	OH	1	B	61	756 592
4d	H	OMe	OH	1	B	54	756 591
4e	Me	Me	H	1	A	45	ns
4f	Me	OMe	H	1	A	12	—
4g	Me	Me	OH	1	B	3	ns
4h	Me	OMe	OH	1	B	49	ns
5a	H	Me	H	2	C	29	756 584
5b	H	OMe	H	2	C	47	756 585
5c	H	Me	OH	2	D	21	756 586
5d	H	OMe	OH	2	D	33	756 587
5e	Me	Me	H	2	C	55	756 588
5f	Me	OMe	H	2	C	26	756 589
5g	Me	Me	OH	2	D	11	756 590
5h	Me	OMe	OH	2	D	—	—

^a Reaction conditions for Step (iii) in Scheme 1: A = KOH/EtOH, rt;^{25,26} B = KOH/methoxyethanol, 120 °C; C = LiOH/H₂O/EtOH;²⁷ D = BF₃·OEt₂, dioxane;²⁸ ^b NCI's National Service Center (NSC) number; ns = not selected by the NCI.

Results and discussion

The synthesis of the analogues is shown in Scheme 1 and the yields and conditions in Table 1. Commercially available 3-indolepropionic acid (**6**) or 3-indolebutyric acid (**7**) were treated with PPA²³ or TFAA, respectively, to afford the corresponding cyclic ketones **8** (81%) or **9** (72%, Step i, Scheme 1).

Following previously reported procedures,^{23,24} methylation of the indole nitrogen was readily achieved using KOH/MeI, affording the N-Me derivatives **10** (95%) and **11** (92%, Step ii). Finally, the cyclic ketones **8–11** underwent Claisen–Schmidt condensation with the required aldehyde in the presence of base or Lewis acid to give the indole-analogues **4a–h** and **5a–g**, as shown in Scheme 1 (Step iii) and Table 1. Owing to capricious results, several reaction conditions were used in this transformation,^{25–28} which are described in the ESI.†

Only the *E*-isomers were obtained from the Claisen–Schmidt condensation reaction, as identified by the characteristic downfield shift of the COC=CH proton, due to a deshielding effect caused by diamagnetic anisotropy by the C=O which is *cis* to this proton, as previously noted.²⁹ This assignment was confirmed by key NOESY interactions (see ESI† compounds **5c** and **5g**) and is consistent with observations made with this reaction in similar substrates which also gave the *E*-isomers.^{13–15,22,30}

4-Hydroxy-3,5-dimethylbenzaldehyde and the corresponding 3,5-dimethoxy derivative, syringaldehyde, were investigated as although the former aldehyde gave the substitution pattern observed in indanocine ($R^2 = Me$, Fig. 3), the latter is readily available and inexpensive.

Nine compounds were selected for one-dose preliminary *in vitro* evaluation at 10 μM by the NCI (those with NSC numbers in Table 1). Five of these compounds (**4c**, **5a–c** and

Table 2 Growth inhibition of indanocine **1** and selected analogues **4c**, **5c** and **5g** in the NCI 60 panel of cancer cell lines

Panel	Cell line	GI ₅₀ (μM) ^a			
		1	4c	5c	5g
<i>Leukemia</i>	CCRF-CEM	0.01	2.18	0.36	0.74
	HL-60(TB)	0.01	0.34 ^b	0.21 ^b	0.34 ^b
	K-562	0.01	0.33 ^b	0.32 ^b	0.33 ^b
	MOLT-4	0.01	6.01	0.56	0.80
	RPMI-8226	0.01	0.67	0.47	0.76
	SR	0.01	0.39	0.22	0.34
<i>Non-Small Cell Lung</i>	A549/ATCC	0.01	1.58 ^b	0.55 ^b	0.74 ^b
	EKVK	0.01	2.59	3.38	4.84
	HOP-62	0.02	0.65	0.65	0.72
	HOP-92	100	7.47	0.78	6.56
	NCI-H226	3.98	9.60	3.29	4.38
	NCI-H23	0.01	1.79 ^b	0.65 ^b	2.53 ^b
<i>Colon</i>	NCI-H322M	0.01	10.4	0.53	2.73
	NCI-H460	0.01	0.42	0.41	0.39
	NCI-H522	0.01	0.09	0.13	0.14
	COLO 205	5.01	19.5	14.2	22.9
	HCC-2998	2.00	7.49	2.21	3.61
	HCT-116	0.01	0.50	0.44	0.39
<i>CNS</i>	HCT-15	0.01	0.56	0.42	0.51
	HT29	2.51	15.8	4.32	4.18
	KM12	0.01	0.46	0.39	0.45
	SW-620	0.01	0.41	0.41	0.43
	SF-268	0.01	0.86	0.73	0.72
	SF-295	0.06	2.78	1.88	1.48
<i>Melanoma</i>	SF-539	0.01	0.55	0.32	0.70
	SNB-19	0.01	0.71	0.58	0.63
	SNB-75	—	0.29	0.29	0.41
	U251	0.02	1.12	0.43	0.54
	LOX IMVI	0.01	0.69	0.76	0.70
	MALME-3M	—	0.31 ^b	0.38	0.45
<i>Ovarian</i>	M14	0.01	0.41	0.34	0.39
	MDA-MB-435	0.01	0.16	0.12	0.16
	SK-MEL-2	1.00	0.40	0.32	0.33
	SK-MEL-28	1.00	0.73 ^b	0.74	0.64
	SK-MEL-5	0.01	0.39	0.33	0.41
	UACC-257	100	26.1	0.37	5.83
<i>Renal</i>	UACC-62	0.01	4.57	0.42	0.41
	IGROV1	0.04	3.54	0.74	1.59
	OVCAR-3	0.01	0.51	0.33	0.35
	OVCAR-4	7.94	10.2	1.35	3.87
	OVCAR-5	0.63	11.1	2.35	5.10
	OVCAR-8	0.01	0.99	0.53	0.92
<i>Prostate</i>	NCI/ADR-RES	0.01	0.35	0.31	0.34
	SK-OV-3	0.01	0.73	0.47	1.52
	786-0	0.01	5.96	0.88	3.25
	A498	7.94	4.86	1.83	3.39
	ACHN	0.06	—	0.77	0.83
	CAKI-1	3.98	1.40	0.61	0.84
<i>Breast</i>	RXF 393	0.03	1.43	0.52	1.08
	SN12C	0.01	0.62	0.62	0.72
	TK-10	79.4	6.27 ^b	0.54 ^b	1.08 ^b
	UO-31	0.08	0.32	0.45	0.73
	PC-3	0.01	3.57	1.06	2.49
	DU-145	0.01	2.05	0.54	1.85
	MCF7	0.01	0.70	0.35	0.37
	MDA-MB-231/ATCC	0.01	3.19	1.06	1.06

Table 2 (Contd.)

Panel	Cell line	GI ₅₀ (μM) ^a			
		1	4c	5c	5g
<i>Leukemia</i>	HS 578T	—	0.55	0.38	0.73
	BT-549	10	—	0.53	2.84
	T-47D	100	12.6	0.74	2.06
	MDA-MB-468	—	2.43	0.29	0.31

^a GI₅₀ is concentration required to inhibit growth by 50% as defined by the National Cancer Institute (NCI). Values for **4c**, **5c** and **5g** are the average of two runs, except ^b one run.

5g) were selected for further examination at five-doses. Three of these five analogues were selected for a repeat screen (**4c**, **5c** and **5g**) and the results obtained are shown in Table 2, as the average of these two runs, together with indanocine **1** for comparison.

Indanocine **1** is extremely potent cell growth inhibitor, displaying 10 nM activity against many of the cell lines examined by the NCI. Although our analogues display decreased activities, they retain useful levels of potency in the low sub-micromolar range.

From Table 2, it is apparent that certain cell types appear particularly sensitive to the indole-analogues, such as *Non-Small Cell Lung* NCI-H522 cell line (GI₅₀ = 93 nM, **4c**) and *Melanoma* MDA-MB-435 cells (GI₅₀ = 120 nM, **5c**). Of particular note is that all three analogues display sub-micromolar growth inhibition against the multi-drug resistant NCI/ADR-RES cell line. Other cell lines, however, appear more resilient to the analogues which are also reflected in indanocine **1**, such as the *Melanoma* cell line UACC-257 (GI₅₀ = 100 μM, **1**, and 26.1 μM, **4c**). Interestingly, the cyclohexanone N-H derivative **5c** still retains activity in this cell line (GI₅₀ = 370 nM). Likewise similar discrepancies with compound **5c** are also observed in *Renal* TK-10 and *Breast* T-47D cell lines.

Of the compounds synthesized and examined in this study, the most active antiproliferative analogues are those most similar in structure to indanocine, as predicted in Fig. 2. They all possess a benzylidene with a hydroxyl group (R³), flanked either side with methyl groups (R²); methoxy groups at R² were detrimental to activity. These observations are consistent with previously established SAR around the benzylidene ring⁵ and calculations used to identify important pharmacophoric points for indanocine.³¹

Methylation of the indole nitrogen (R¹) was tolerated in the 6-membered ring series (**5**), as both the N-H (**5c**) and N-Me (**5g**) analogues displayed good activities (Table 2). Although the 5-membered ring N-H analogue **4c** was active, the corresponding N-Me derivative **4g** was not selected by the NCI, so a comparison cannot be made. These results suggest that although changes to the aryl group are not tolerated, indole N-substitution is and demonstrate that further modifications around the indole ring, as observed in many indole-based tubulin inhibitors,²¹ should be investigated in future.

From the final three analogues, the cyclopentanone derivative **4c** was selected by the NCI for consideration by the Biological Evaluation Committee and has been classified as a tubulin binder. COMPARE analysis^{32,33} of the lead compound **4c** using the standard algorithm with Synthetic Compounds gave the strongest correlation (0.795) with centaureidin (NSC 106969), a natural product isolated from the tropical plant *Polymnia fruticosa* which exhibits antiproliferative activity through the inhibition of tubulin polymerisation.^{34,35} Other compounds identified by the COMPARE algorithm included several combretastatin derivatives (see ESI†), which are also tubulin-binding agents and, like indanocine **1**, similarly interact with the colchicine binding site and inhibits tubulin polymerisation.³⁶ Of particular note is that the lead compound **4c** also displayed good correlation (0.646, rank 26) with indanocine **1** (NSC 698666). Of interest is that although combretastatin derivatives were also observed in the COMPARE analyses of both compounds **5c** and **5g**, only the cyclohexanone N-Me analogue **5g** showed any significant correlation with indanocine **1** (0.593, rank 108) as the cyclohexanone N-H derivative **5c** displayed poor correlation (0.426, rank 707).

Conclusions

In summary, we report that the indole group is an effective bioisostere for the dimethoxyaniline motif present in indanocine **1** and describe the synthesis of several indole-based analogues, in either two or three steps from commercially available materials. Several of these compounds were selected for evaluation by the National Cancer Institute (NCI) and analysis using the COMPARE algorithm show that our strategy was successful and that we have identified a novel class of compounds which, like indanocine **1**, interact with tubulin and display potent antiproliferative activity against various cancer cell lines including drug-resistant cells.

Although “indolocene” **4c** is less potent than indanocine **1**, the indole group offers a versatile platform to generate further derivatives with more desirable physicochemical properties and target enhanced potency and selectivity. Such compounds could find potential as novel chemotherapeutic agents to combat cancer or prevent its spread by inhibiting the migration of metastatic cancer cells and will be investigated and reported in due course.

Acknowledgements

We wish to thank Dr Timothy J. Woodman and Dr Anneke Lubben (University of Bath) for their assistance with the NMR and mass spectra, respectively. We also wish to thank Dr Pauline J. Wood (University of Bath) for preliminary cell work. We are extremely grateful to the National Cancer Institute (NCI) for conducting the *in vitro* testing and the University of Bath for providing a studentship for GAT. We also wish to acknowledge RCUK and the University of Bath for the fellowship to LC.

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