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PAPER

Synthesis and biological evaluation of novel isoellipticine derivatives and salts†

Charlotte M. Miller,[a] Elaine C. O'Sullivan,[a] Ken J. Devine[b] and Florence O. McCarthy*[a]

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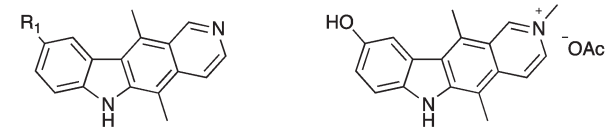
Synthesis of novel 7-substituted isoellipticines and isoellipticinium salts is described, with optimisation of routes, representing a new class of anti-cancer agent. Initial assessment of biological activity using a topoisomerase II decatenation assay and NCI screening highlighted strong anti-cancer activity, further developed in a panel of isoellipticinium salts. Interestingly, low correlation between results of the topoisomerase II decatenation assay and NCI screen throughout the panel suggest that topo II is not the most important biological target with respect to anti-cancer activity in this new class of compounds. Results also suggest that solubility is not the limiting factor in activity of the isoellipticinium salts. Overall, 20 novel ellipticine analogues were prepared and full anti-cancer profiling was completed for 13 isoellipticine derivatives and salts. Two compounds display significant specificity towards CNS cancer cell lines and are lead compounds for future development.

Introduction

The tetracyclic natural product ellipticine **1** (5,11-dimethyl-6*H*-pyrido[4,3-*b*]carbazole, Fig. 1) was isolated from the leaf material of *Ochrosia elliptica* Labill by Goodwin *et al.* in 1959.[1] This small tropical evergreen tree belonging to the Apocynaceae family also contained several other alkaloids, including 9-methoxyellipticine **2**. Ellipticine has since been isolated from several other plants of the Apocynaceae family (*Ochrosia vieillardii, Ochrosia acuminate and Ochrosia moorei*)[2–4] and from *Strychnos dinkagei* of the Loganiaceae family.[5,6]

Ellipticine and its derivatives were found to exhibit potent anticancer activity and have been subject to much study over the last fifty years. In particular, Celiptium (9-hydroxy-*N*-methylellipticinium acetate) **3** and 9-hydroxyellipticine **4** were early lead compounds which progressed to phase II clinical trials.[7–9]

Several key mechanisms of action have been found to contribute to ellipticine anticancer activity. These include DNA intercalation and topoisomerase II inhibition, two closely related mechanisms which have been well established in the literature.[10–12] Biooxidation and adduct formation have also been proposed to play a role in ellipticine cytotoxicity, and have been subject to much study in the last decade.[13–15] More recent investigations have shown that ellipticines induce multifaceted biological responses, including interaction with kinases such as c-Kit and AKT and also p53 tumour suppressor.[16–23]



R¹=H, Ellipticine **1**
R¹=OMe, 9-Methoxyellipticine **2**
R¹=OH, 9-Hydroxyellipticine **4**

Celiptium **3**

Fig. 1 Ellipticine derivatives.

In 1994, collation of NCI screening results for a large panel of ellipticinium salts revealed significant CNS cancer cell line selectivity.[24,25] A range of *N*-methylellipticinium salts with varying counterions (I, OAc, Cl, OSO_2Me) and C-9 substituents (H, OMe, Me, Cl) displayed selectivity for the six CNS cancer cell lines over the other 54 lines in the panel. Interestingly, neither the parent ellipticine compounds nor the lead ellipticinium salt, Celiptium **3**, displayed this selectivity. In general, a decrease in selectivity for CNS cell lines was observed with increasing size of the C-9 substituent.[25]

Isoellipticine **5** (Fig. 2) is a non-natural isomer of ellipticine, first synthesised in 1967, it possesses similar anticancer properties to ellipticine but has not been extensively investigated.[26] Our aim was to further investigate the biological activity of the isoellipticine family of compounds by synthesis of novel 7-substituted isoellipticines and isoellipticinium salts. In particular, if the isoellipticinium salts proved to be as potent and selective as their ellipticine analogues, we proposed further investigation of

[a]*Department of Chemistry, Analytical and Biological Chemistry Research Facility, University College Cork, Western Road, Cork, Ireland. E-mail: f.mccarthy@ucc.ie; Tel: +353 21 4901695*
[b]*School of Pharmacy, University College Cork, Western Road, Cork, Ireland*

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the side chain functionally at the N-2 position. The appeal of this strategy lay in the possibility of increasing both solubility and activity through salt formation.

Results and discussion

Synthesis of isoellipticine and deazaellipticine derivatives

Isoellipticine **5** was prepared *via* Sauliner and Gribble's versatile route.[27] The novel derivatives 7-formylisoellipticine **6** and 7-hydroxyisoellipticine **7** were synthesised using Plug's conditions for the Duff and Baeyer–Villiger reactions.[7] The highly regioselective Duff reaction of isoellipticine **5** with hexamethylenetetramine in trifluoroacetic acid gave 7-formylisoellipticine **6** in 77% yield (Scheme 1).

The Baeyer–Villiger reaction was initially carried out under the conditions reported by Plug *et al.* (Scheme 1, *conditions (i)*), however this procedure gave low yields (15%–34%), so a more reliable preparation was sought. First, the issue of solubility was considered, increasing the amount of sulfuric acid to a 5% v/v solution in methanol resulted in full dissolution of 7-formylisoellipticine and an increased yield of 71%. These reaction conditions were repeated several times and gave consistently high yields between 60 and 70% (Scheme 2).

Fujishiro and Mitamura investigated the Baeyer–Villiger reaction of polycyclic aromatic aldehydes and found that use of formic acid as both acid and reaction solvent gave excellent yields and purity.[28] These conditions were applied to the preparation of 7-hydroxyisoellipticine **7**, however only starting material was recovered after work-up. Finally, a solid phase Dakin reaction was investigated on the basis of work published by Da Silva *et al.*[29] The reaction carried out by carefully grinding 7-formylisoellipticine **6** and *m*-CPBA with a pestle and mortar (for safety precautions, see Experimental section) to give 7-hydroxyisoellipticine **7** in 12% yield. Overall, the best results for the Baeyer–Villiger reaction were obtained with hydrogen peroxide and a 5% v/v solution of sulfuric acid in methanol.

In an alternative preparation of 7-hydroxyisoellipticine **7**, 7-methoxyisoellipticine **8** (synthesised *via* the Gribble route from 5-methoxyindole) was demethylated with pyridine hydrochloride in a yield of 82%.

As an additional comparison with ellipticine and isoellipticine derivatives, the novel 2-hydroxydeazaellipticine **9** (6,11-dimethyl-5*H*-benzo[*b*]carbazol-2-ol), was synthesized in 18% yield over six steps from 5-methoxy-1-(phenylsulfonyl)-1*H*-indole **10**. Lithiation at C-2 of the indole, followed by coupling with phthalic anhydride gave 2-(5-methoxy-1-(phenylsulfonyl)-1*H*-

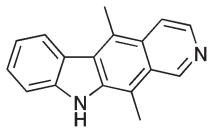
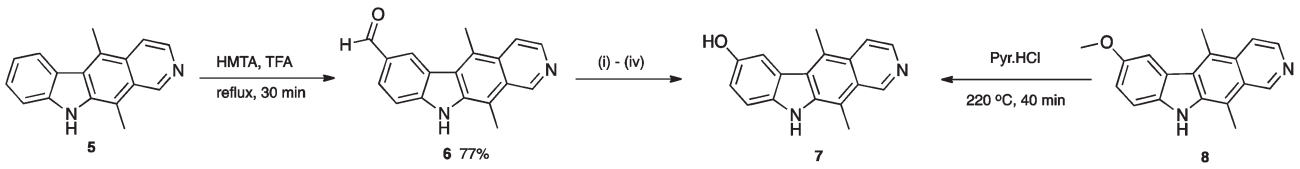
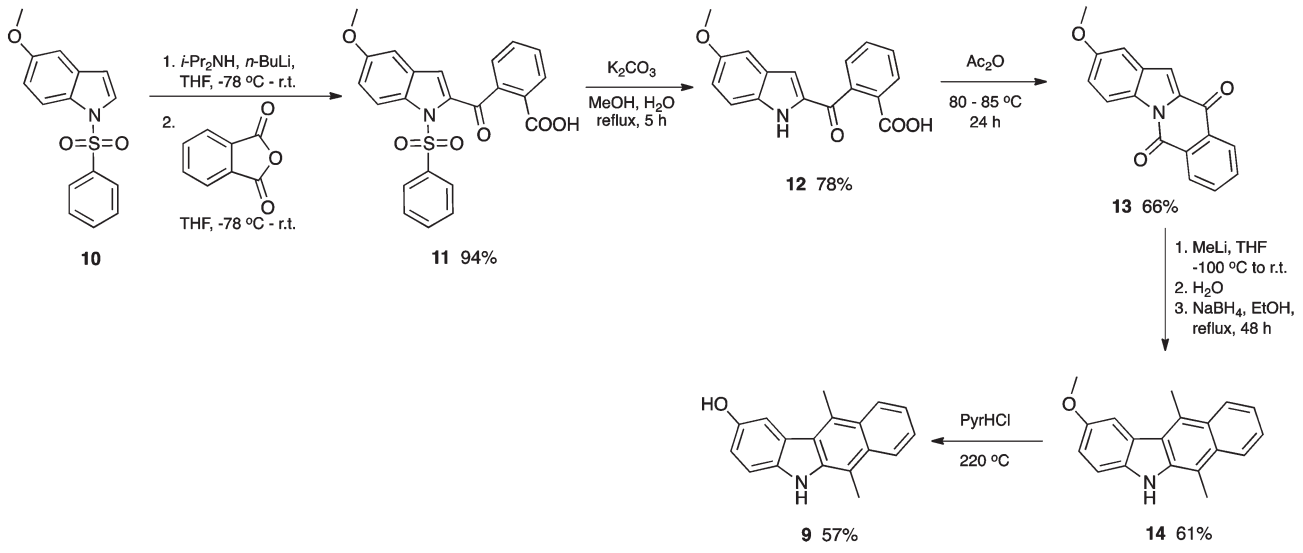


Fig. 2 Isoellipticine **5**.



Scheme 1 Synthesis of 7-substituted isoellipticines. Conditions of Baeyer–Villiger reaction: (i) H_2O_2, H_2SO_4, MeOH; (ii) H_2O_2, H_2SO_4 in MeOH (5% v/v); (iii) H_2O_2, formic acid; (iv) *m*-CPBA, solid phase.



Scheme 2 Synthesis of 2-hydroxydeazaellipticine **9**.

indole-2-carbonyl)benzoic acid **11** in 94% yield. Deprotection to **12** proceeded smoothly (78%), followed by cyclisation to 2-methoxyindolo[1,2-*b*]isoquinoline-6,11-dione **13** in 66% yield. 2-Methoxydeazaellipticine **14** was prepared by alkylation with methyllithium followed by cyclisation with sodium borohydride and was subsequently demethylated with pyridine hydrochloride to give the novel 2-hydroxydeazaellipticine **9** in 57% yield.

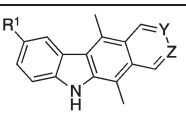
Biological activity of isoellipticines *versus* ellipticines

Inhibition of topoisomerase II is considered to be a key mechanism of action for the ellipticine family of compounds and was evaluated using a decatenation assay. In addition to this, the overall cytotoxicity of the novel derivatives was assessed using the NCI 60-cell line screen. Initially, the effect of A and D-ring variation across ellipticine, isoellipticine and deazaellipticine analogues was investigated (Table 1).

All compounds showed inhibition of topoisomerase II decatenation at 100 μM concentration except 2–hydroxydeazaellipticine **9**, indicating that the pyridine nitrogen is essential for activity. The novel 7-substituted isoellipticines are at least as active as their ellipticine counterparts and, in addition to this, 9-hydroxyellipticine **4** and 7-formylisoellipticine **6** are both active at 10 μM.

In the NCI 60-cell line screen, 7-formylisoellipticine **6** and 7-hydroxyisoellipticine **7** both displayed moderate activity, with mean growth values across the cell line panel of 51.47% and 53.50% respectively at 10 μM concentration. 7-Formylisoellipticine **6** showed selectivity for leukaemia cell lines, with mean growth in most cell lines below 10%. Highest activity was seen against the SR cell line at −28.84% growth after 48 hours. Other lines that responded well to 7-formylisoellipticine (10.80%) and MCF7 (6.80%). 7-Hydroxyisoellipticine **7** also displayed selectivity against leukaemia cell lines, with most lines below 30% growth. It also showed good activity against two non-small cell lung cancer lines and several colon cancer lines. These early results established the promise of the isoellipticine class of compounds as anti-cancer therapeutics.

Table 1 Comparison of topoisomerase II activity in ellipticine analogues



Compound number	R^1	Y	Z	Topo II inhibition
5	H	CH	N	+
1	H	N	CH	+
7	OH	CH	N	+
4	OH	N	CH	++
9	OH	CH	CH	−
6	CHO	CH	N	++
15	CHO	N	CH	+

+ Inhibition observed at 100 μM; ++ inhibition at 10 μM; +++ inhibition at 1 μM; − no activity observed.

Synthesis and biological evaluation of isoellipticinium salts

These favourable initial results prompted investigation of novel salts of 7-formylisoellipticine **6** and 7-hydroxyisoellipticine **7**. A large panel of salts was prepared by heating isoellipticine **5**, 7-formylisoellipticine **6** or 7-hydroxyisoellipticine **7** with an alkyl halide in dimethylformamide and triturating with diethyl ether to give the novel isoellipticinium salts in excellent to modest yields (Scheme 3 and Table 2). The salts of 7-hydroxyisoellipticine were highly hydroscopic, resulting in lower yields.

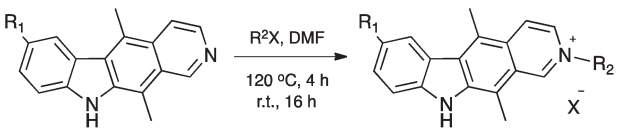
Given the anti-cancer activity of the *N*-methylellipticinium salts, *N*-methylisoellipticinium iodide **16** was predicted to show some activity and indeed it displayed moderate topoisomerase II inhibition and cytotoxicity in the NCI screen. In the single dose screen (10 μM concentration), **16** displayed 65.75% mean growth over 58 cell lines and also displayed the CNS cell line selectivity that had previously been observed in the *N*-methylellipticinium salts, with growth reduced to between 11 and 36% across all CNS lines.[24] This equates to selectivity of 3.36 (mean of 52 non-CNS lines/mean of 6 CNS lines; where 1 = no selectivity). *N*-Methylisoellipticinium iodide **16** also gave excellent activity against the breast cancer cell line MDA-MB-468 with −31.95% growth.

Next, the optimum length of the side chain was investigated using nitrile halides (**17**, **18**, **19**), of these the six carbon chain give moderate topo II inhibition and cytotoxicity. Investigation of acid, amide and sulphonamide functionality resulted in decreased cytotoxicity, while maintaining some topo II inhibition, with the amide, 2-(6′-carboxamidohexyl)isoellipticinium bromide **21** displaying inhibition at 10 μM.

7-Formylisoellipticine **6** had performed well in initial testing and it was therefore surprising that none of its salt derivatives were cytotoxic in the NCI screen, however, 2-(5′-cyanopentyl)-7-formylisoellipticinium bromide **24** showed the highest level of topo II activity in the screen, with inhibition at 10 μM and partial inhibition at 1 μM. Surprisingly, given the activity of Celiptium, none of the 7-hydroxyisoellipticinium salts showed significant topo II inhibition or general cytotoxicity.

Overall, three novel isoellipticines displayed strong inhibition of topo II at 10 μM, two of which are isoellipticinium salts. It appears that both long and short chain salts are tolerated in the DNA–topoisomerase II-drug complex, however a six carbon chain with either nitrile or amide functionality was optimum for topo II inhibition (**21**, **24**). Interestingly, none of the salts with a carboxylic acid side chain were active (**20**, **23** and **27**). A-ring substitution is also favourable for inhibition (Table 1), however the combination of an A-ring substituent and quaternary isoellipticinium salt did not dramatically increase activity, and in fact several such derivatives were less active.

In the NCI screen, the carboxylic acid, nitrile, amide and sulphonamide isoellipticinium salts displayed rather disappointing



Scheme 3 Salt formation.

Table 2 Yields and biological activity of novel isoellipticinium salts

Compound number	R^1	R^2	X	Yield %	Topo II inhibition	NCI mean growth %
16	H	CH_3	I	47	+	65.75
17	H	C_3H_6CN	Cl	79	–	—
18	H	C_4H_8CN	Cl	88	–	—
19	H	$C_5H_{10}CN$	Br	82	+	75.68
20	H	$C_5H_{10}COOH$	Br	77	–	99.78
21	H	$C_5H_{10}CONH_2$	Br	84	++	91.68
22	H	$C_5H_{10}CONHSO_2CH_3$	Br	76	+	—
23	CHO	$C_5H_{10}COOH$	Br	49	–	95.87
24	CHO	$C_5H_{10}CN$	Br	63	++	92.33
25	CHO	$C_5H_{10}CONH_2$	Br	60	–	92.99
26	CHO	$C_5H_{10}CONHSO_2CH_3$	Br	54	–	95.43
27	OH	$C_5H_{10}COOH$	Br	32	–	—
28	OH	$C_5H_{10}CN$	Br	31	+	89.52
29	OH	$C_5H_{10}CONH_2$	Br	46	+	95.78
30	OH	$C_5H_{10}CONHSO_2CH_3$	Br	52	–	84.58

+ Inhibition observed at 100 μM; ++ inhibition at 10 μM; +++ inhibition at 1 μM; – no activity observed.

cytotoxicity overall. The best mean growth was recorded for 2-(5′-cyanopentyl)isoellipticinium bromide **19** (75.68%) and this was also the only other salt to display the CNS selectivity observed in the *N*-methylisoellipticinium salt. CNS cell line growth ranged between 25.19 and 69.30% for this compound and with lower selectivity than *N*-methylisoellipticinium iodide **16** at 1.67 (mean of 52 non-CNS lines/mean of 6 CNS lines; where 1 = no selectivity). Growth of the melanoma cell line, SK-MEL-5 was also significantly inhibited at 14.31%, along with the renal line, SN12C (19.33%).

The biological importance of C-9 substituents in the ellipticine series is well established, and a similar effect was anticipated in the 7-substituted isoellipticine series, however this was not the case, 2-(5′-cyanopentyl)-7-formylisoellipticinium bromide **24** and 2-(5′-cyanopentyl)-7-hydroxyisoellipticinium bromide **28** gave mean growth values of 92.33% and 89.52%. This trend was common to all the 7-formyl and 7-hydroxyisoellipticinium salts.

Table 3 Selected five dose data for 7-formylisoellipticine **6**

Cancer type	Cell line	GI_{50} (μM)	LC_{50} (μM)
Leukaemia	CCRF-CEM	0.894	>100
	SR	0.487	>100
Breast	MCF7	0.879	41.3
	HS 578T	0.620	78.2
Melanoma	LOX IMIV	0.861	56.0

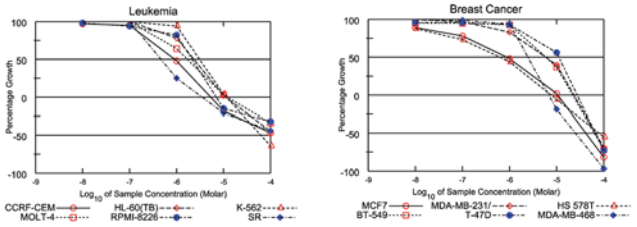


Fig. 3 Dose response curves for 7-formylisoellipticine **6**.

NCI five-dose data for isoellipticine derivatives

Of the novel isoellipticine and isoellipticinium derivatives, four were selected by the NCI for five dose screening, these include 7-formylisoellipticine **6**, 7-hydroxyisoellipticine **7**, *N*-methylisoellipticinium iodide **16** and 2-(5′-cyanopentyl)isoellipticinium bromide **19**.

7-Formylisoellipticine **6** exhibited good GI_{50} values (concentration at which growth is limited to 50%) in leukaemia and breast cancer cell lines, particularly in CCRF-CEM, SR, MCF7, HS578T and also the melanoma line, LOX IMIV (Table 3 and Fig. 3).

These results correlate well with the single dose data discussed above. While the dose response curve for leukaemia cell lines levels off to give high LC_{50} values *i.e.* a cytostatic rather than cytotoxic effect, that of breast cancer and the melanoma line LOX IMIV display moderate LC_{50} values (concentration at which <50% of cells survive after 48 hours).

Each of the compounds selected for five-dose testing was also analysed using the COMPARE program to identify agents with similar activity patterns across the NCI-60 panel. The correlation was carried out using COMPARE analysis of GI_{50} values of each isoellipticine derivative with those of the NCI synthetic compounds database. Surprisingly, in the case of 7-formylisoellipticine **6**, no significant correlation was found with any ellipticine or isoellipticine analogue or indeed with any of the NCI standard agents. This unusual result suggests that 7-formylisoellipticine **6** does not exert its effects *via* the currently known mechanisms of NCI agents and further elucidation of its cellular effects is currently underway.

7-Hydroxyisoellipticine **7** displayed a mean growth of 53.50% in the single dose testing, however five dose testing highlights a decline in activity at lower concentrations, particularly in leukaemia cell lines. Thus, the sub-micromolar GI_{50} values are found in one leukaemia cell line and two NSCLC lines (Table 4 and Fig. 4).

COMPARE analysis of 7-hydroxyisoellipticine **7** give a correlation of 0.705 with another ellipticine isomer, 5,11-dimethyl-10*H*-pyrido[2,3-*b*]carbazol-7-ol **31**, although **7** is the more active of the two (Fig. 5). An additional correlation of 0.785

Table 4 Selected five dose data for 7-hydroxyisoellipticine **7**

Cancer type	Cell line	GI_{50} (μM)	LC_{50} (μM)
Leukaemia	MOLT-4	0.517	>100
NSCLC	A549/ATCC	0.888	34.3
	NCI-H460	0.569	30.6

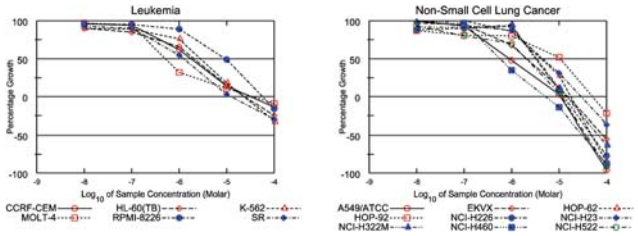
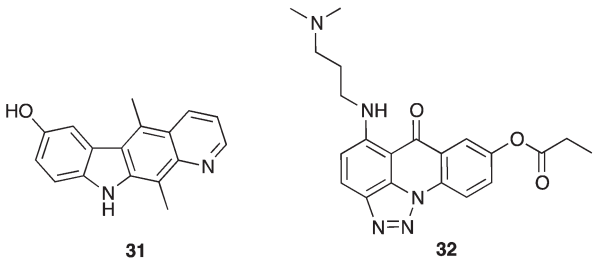


Fig. 4 Dose response curves for 7-hydroxyisoellipticine **7**.



31 **32**

Fig. 5 Compounds with COMPARE analysis correlation to 7-hydroxyisoellipticine **7**.

Table 5 Selected five dose data for *N*-methylisoellipticinium iodide **16**

Cancer type	Cell line	GI_{50} (μM)	LC_{50} (μM)
CNS	SF-268	0.676	40.2
	SNB-19	0.607	>100
	U251	0.276	40.2
Renal	A498	0.683	>100
	SN12C	0.526	>100
Breast	MDA-MB-468	0.442	6.51

was found with the tricyclic compound 5-((3-(dimethylamino)-propyl)amino)-6-oxo-6*H*-[1,2,3]triazolo[4,5,1-*de*]acridin-8-yl propionate **32**, however the mechanism of action of this drug is unknown.

Significant selectivity for CNS cancer cell lines was displayed in the single dose data for *N*-methylisoellipticinium iodide **16** and this translated to excellent GI_{50} values in the five dose assay. In addition, strong activity was seen in two renal cancer and one breast cancer line (Table 5 and Fig. 6).

As expected, COMPARE analysis of *N*-methylisoellipticinium iodide **16** gave a strong correlation to the corresponding ellipticine, *N*-methylellipticinium iodide. The correlation value was 0.775, with the ellipticine isomer displaying marginally stronger activity.

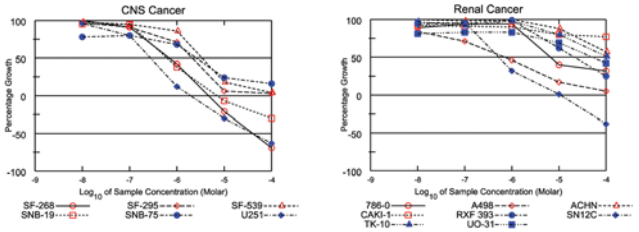


Fig. 6 Dose response curves for *N*–methylisoellipticinium iodide **16**.

Table 6 Selected five dose data for 2-(5′-cyanopentyl)isoellipticinium bromide **19**

Cancer type	Cell line	GI_{50} (μM)	LC_{50} (μM)
CNS	SF-268	4.95	>100
	SF-295	2.47	>100
	SF-539	4.64	>100
	SNB-19	2.40	>100
	U251	2.05	>100
Renal	SN12C	1.50	>100
Breast	MDA-MB-468	1.22	51.6

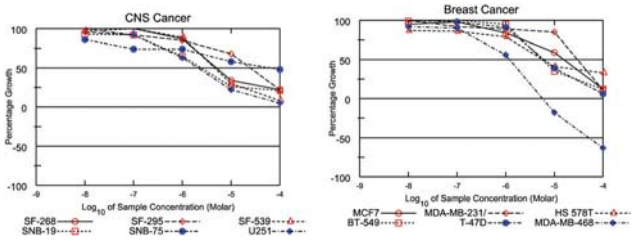


Fig. 7 Dose response curves for 2-(5′-cyanopentyl)isoellipticinium bromide **19**.

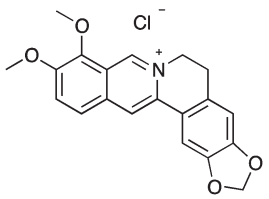


Fig. 8 Berberine **33**.

The nitrile salt, 2-(5′-cyanopentyl)isoellipticinium bromide **19** also displayed CNS specificity in the single dose mean graph, however with a mean growth of 75.68%, this compound was not expected to be particularly active in the five dose assay. Indeed, no sub-micromolar GI_{50} values were recorded for **19** (Table 6 and Fig. 7), although the renal line SN12C and the breast cancer line MDA-MB-468 displayed GI_{50} values of 1.50 μM and 1.22 μM respectively.

2-(5′-Cyanopentyl)isoellipticinium bromide **19** gave a correlation of 0.681 to 2-methylisoellipticinium iodide **16** and a stronger correlation of 0.789 to the natural product berberine **33** (Fig. 8). Interestingly, berberine is known to interact with multidrug resistance protein MDR1 or P-glycoprotein, a mechanism also attributed to the ellipticines.[30]

Conclusions

The 7-substituted isoellipticines represent a new class of anti-cancer agent with a unique NCI activity profile. Interestingly, low correlation between results of the topoisomerase II decatenation assay and NCI screen throughout the panel suggests that topo II is not the most important biological target with respect to anti-cancer activity in this new class of compounds.

In particular, the CNS specific agents *N*-methylisoellipticinium iodide **16** and 2-(5′-cyanopentyl)isoellipticinium bromide **19** are of significant interest in treatment of a disease with very poor prognosis and a lower age profile than most other cancers. Preliminary results show that the longer chain isoellipticinium salts are less active than the simple methyl derivative and further investigation of the structure activity relationship is required. These results also suggest that solubility is not the limiting factor in activity of the isoellipticinium salts, given that all the longer chain salts had improved aqueous solubility over the predecessor **16**.

The mechanism of action of these new compounds is largely unknown and comparison with current data on NCI cell line characteristics has not yielded any significant correlations.[31] In particular, studies which characterised the NCI cell line expression of p53 tumour suppressor, reductase enzyme and topoisomerase I gave no correlation with isoellipticines **6**, **7**, **16** and **19**.[32–34] On COMPARE analysis, compounds **7**, **16** and **19** appear to correlate to some extent with ellipticine congeners, however 7-formylisoellipticine **6** has a unique mode of action and may be a significant lead for future work especially given its poor solubility. Further investigation of the mechanism of action of all four compounds will be pursued.

Overall, 20 novel ellipticine analogues were prepared and full anti-cancer profiling was completed for 13 isoellipticine derivatives and salts. Significant SAR studies will now follow in order to determine where potency and solubility can be improved in this new class of anti-cancer compounds.

Experimental

General procedures

Solvents were distilled prior to use as follows: dichloromethane was distilled from phosphorous pentoxide; ethyl acetate was distilled from potassium carbonate; ethanol and methanol were distilled from magnesium in the presence of iodine; toluene was distilled from sodium and benzophenone; hexane was distilled prior to use; tetrahydrofuran was freshly distilled from sodium and benzophenone. Diethyl ether was obtained pure from Riedel-de Haën. Organic phases were dried using anhydrous magnesium sulphate. All commercial reagents were used without further purification unless otherwise stated. 5-Methoxy-1-(phenylsulfonyl)-1*H*-indole **10** was prepared from 5-methoxyindole in 94% yield.

Infrared spectra were recorded as a thin film on sodium chloride plates for liquids or potassium bromide (KBr) disc for solids on a Perkin Elmer Spectrum 100 FT-IR spectrometer.

^{1}H (300 MHz) and ^{13}C (75 MHz) NMR spectra were recorded on a Bruker Avance 300 NMR spectrometer. ^{1}H (400 MHz) NMR spectra were recorded on a Bruker Avance 400 NMR spectrometer. ^{1}H (600 MHz) and ^{13}C (150.9 MHz) NMR spectra were recorded on a Bruker Avance III 600 MHz NMR spectrometer equipped with a dual CH cryoprobe. All spectra were recorded at room temperature (~20 °C) in deuterated chloroform ($CDCl_3$) with tetramethylsilane (TMS) as an internal standard, or deuterated dimethylsulfoxide (DMSO-d_6). ^{1}H NMR spectra recorded in deuterated dimethylsulfoxide (DMSO-d_6) were assigned using the DMSO-d_6 peak as the reference peak. Chemical shifts (δ_H and δ_C) are expressed in parts per million (ppm) relative to the reference peak. Coupling constants (*J*) are expressed in Hertz (Hz). Splitting patterns in ^{1}H NMR spectra are designated as s (singlet), br s (broad singlet), d (doublet), br d (broad doublet), t (triplet), q (quartet), dd (doublet of doublets), dt (doublet of triplets), ddd (doublet of doublet of doublets), ddt (doublet of doublet of triplets) and m (multiplet).

Low resolution mass spectra were recorded on a Waters Quattro Micro triple quadrupole spectrometer (QAA1202) in electrospray ionisation (ESI) mode using 50% acetonitrile–water containing 0.1% formic acid as eluent. High resolution mass spectra (HRMS) were recorded on a Waters LCT Premier Time of Flight spectrometer in electrospray ionisation (ESI) mode using 50% acetonitrile–water containing 0.1% formic acid as eluent.

Elemental analyses were preformed by the Microanalysis Laboratory, National University of Ireland, Cork, using Perkin Elmer 240 and Exeter Analytical CE440 elemental analysers. Melting points were measured in a uni-melt Thomas Hoover capillary melting point apparatus and are uncorrected. Thin layer chromatography (TLC) was carried out on precoated silica gel plates (Merck 60 PF254) or aluminium oxide TLC plates (Sigma). Visualisation was achieved by UV light detection (254 nm).

Synthesis of 7-substituted isoellipticines

5,11-Dimethyl-10*H*-pyrido[3,4-*b*]carbazole-7-carbaldehyde 6. A solution of 5,11-dimethyl-10*H*-pyrido[3,4-*b*]carbazole **5** (2.433 g, 9.88 mmol) in trifluoroacetic acid (160 mL), was treated with hexamethylenetetramine (13.847 g, 98.78 mmol, 10 eq.) portionwise over 5 minutes. The resulting deep red solution was heated to reflux for 30 minutes. On cooling, the reaction mixture was concentrated to approx. one quarter volume and water (250 mL) was added. The solution was cooled to 0 °C and neutralized with solid sodium bicarbonate while stirring vigorously. Dichloromethane (300 mL) was added and the mixture was stirred for 2 hours. The aqueous layer was extracted with dichloromethane–methanol (90 : 10, 5 × 200 mL). Combined organic extracts were dried and concentrated under reduced pressure to give the product as an orange solid (1.013 g). A precipitate in the aqueous layer, which did not extract into the organic phase, was filtered and dried thoroughly under vacuum (0.1 mm Hg) to give additional pure product (1.064 g, combined yield 2.077 g, 76.7%). m.p. 270–271 °C; ν_{max}/cm^{-1} (KBr): 3153 (NH), 1672 (C═O), 1599 (C═C arom.), 1469 (C═C arom.), 1381, 1285, 1216, 1113, 1014, 810; δ_H (600 MHz, DMSO-d_6): 2.86 [3H, s, C(11)CH_3], 3.05 [3H, s, C(5)CH_3], 7.61 [1H, d, *J* 8.3, C(9)H], 8.01 [1H, d, *J* 8.5, C(8)H], 8.07 [1H, d, *J* 5.9, C(3)H], 8.42 [1H, d, *J* 5.9, C(4)H], 8.74 [1H, s, C(6)H], 9.52 [1H, s, C(1)H],

10.04 [1H, s, C(7)C*H*O], 11.83 [1H, s, N(10)H]; δ_C (150.9 MHz, DMSO-d_6): 11.7 [CH_3, C(11)*C*H_3]; 14.4 [CH_3, C(5)*C*H_3]; 110.9 (CH, aromatic CH), 111.9 (C, aromatic C), 116.9 (CH, aromatic CH), 122.5 (C, aromatic C), 125.1 (C, aromatic C), 125.4 (C, aromatic C), 125.6 (C, aromatic C), 127.7 (CH, aromatic CH), 128.2 (C, aromatic C), 128.4 (CH, aromatic CH), 128.8 (C, aromatic C), 138.4 (C, aromatic C), 138.7 (CH, aromatic CH), 146.9 (C, aromatic C), 148.7 (CH, aromatic CH), 191.8 (C, *C*HO); *m*/*z* (ESI$^+$): 275 [(M + H)$^+$ 30%], 115 (100%), 105 (70%); HRMS (ESI$^+$): Exact mass calculated for $C_{18}H_{15}N_2O^+$ 275.1184. Found 275.1179. Anal. calculated for $C_{18}H_{14}N_2O$: C, 78.81; H, 5.14; N, 10.21. Found: C, 78.96; H, 5.42; N, 9.91.

5,11-Dimethyl-10*H*-pyrido[3,4-*b*]carbazol-7-ol 7

(a) Baeyer–Villiger reaction with hydrogen peroxide and sulfuric acid. 5,11-Dimethyl-10*H*-pyrido[3,4-*b*]carbazole-7-carbaldehyde **6** (201 mg, 0.733 mmol) was added to a solution of sulfuric acid in methanol (150 mL, 5% v/v). The deep red solution was treated with hydrogen peroxide (1 mL, 30% w/v soln.) and heated to reflux for 6 hours. The mixture was cooled to 0 °C and stirred vigorously while adjusting to pH 12 with solid sodium bicarbonate. After 30 minutes stirring at room temperature the solution was neutralised to pH 7 with 20% aqueous HCl and stirred overnight with dichloromethane (70 mL). The layers were separated and the aqueous layer was extracted with dichloromethane–methanol (90 : 10, 6 × 30 mL) while sequentially adjusting the pH (7, 5, 10, 7) with 20% aqueous HCl and 10% aqueous NaOH. Combined organic extracts were dried and concentrated under reduced pressure to give a bright orange solid (137 mg, 71.3%). m.p. >300 °C (without melting); ν_{max}/cm^{-1} (KBr): 3388 (OH), 3241 (NH), 1658 (C═C arom.), 1635 (C═C arom.), 1451, 1410, 1220, 1116 (C–O stretch); δ_H (300 MHz, DMSO-d_6): 2.85 [3H, s, C(11)CH_3], 3.02 [3H, s, C(5)CH_3], 7.00 [1H, dd, *J* 8.6, 2.3, C(8)H], 7.33 [1H, d, *J* 8.6, C(9)H], 7.72 [1H, d, *J* 2.3, C(6)H], 8.01 [1H, dd, *J* 6.0, 0.2, C(3)H], 8.30 [1H, d, *J* 5.8, C(4)H], 9.05 [1H, s, C(7)O*H*, assigned from D_2O exchange experiment], 9.48 [1H, s, C(1)H], 10.96 [1H, s, N(10)H]; δ_C (75.5 MHz, DMSO-d_6): 11.8 [CH_3, C(11)*C*H_3], 14.3 [CH_3, C(5)*C*H_3], 109.3 [CH, C(6)H], 110.3 (C, aromatic C), 111.0 [CH, C(9)H], 116.6 [CH, C(8)H], 116.8 [CH, C(3)H], 123.2 (C, aromatic C), 124.6 (C, aromatic C), 125.1 (C, aromatic C), 125.8 (C, aromatic C), 127.6 (C, aromatic C), 136.9 (C, aromatic C), 138.1 [CH, C(4)H], 139.1 (C, aromatic C), 148.7 [CH, C(1)H], 150.5 (C, aromatic C); *m*/*z* (ESI$^+$): 263 [(M + H)$^+$ 100%]; HRMS (ESI): Exact mass calculated for $C_{17}H_{15}N_2O^+$ 263.1184. Found 263.1181.

(b) Baeyer–Villiger reaction with hydrogen peroxide and formic acid. A stirred solution of 5,11-dimethyl-10*H*-pyrido[3,4-*b*]carbazole-7-carbaldehyde **6** (129 mg, 0.470 mmol) in formic acid (10 mL) was treated with hydrogen peroxide (0.06 mL, 30% w/v soln., 1.2 eq.) and stirred at room temperature for 24 hours. TLC analysis showed no reaction, so additional hydrogen peroxide (0.06 mL) was added, and the reaction was heated to 40 °C for 6 hours. On cooling, water (10 mL) was added and the solution was basified with 10% aqueous NaOH to pH 12. After stirring for 15 minutes, the solution was neutralised with 20% aqueous HCl and extraction was attempted with dichloromethane–methanol (90 : 10, 3 × 20 mL), however this was unsuccessful and no product was obtained after concentrating under reduced pressure. During extraction an precipitate formed in the aqueous layer, this was filtered to give a brown solid, which was found to be unreacted starting material (63 mg, 48.8% recovery).

(c) Solid phase Baeyer–Villiger reaction with m-*CPBA.* 5,11-Dimethyl-10*H*-pyrido[3,4-*b*]carbazole-7-carbaldehyde **6** (413 mg, 1.51 mmol) was ground with a pestle and mortar to give a fine orange powder. *m*-Chloroperbenzoic acid (519 mg, 2.32 mmol, 77%) was added and gently ground.‡ After 5 minutes the mixture melted and bubbled slightly and the reaction was continued for a further 15 minutes. Sodium hydroxide (10% soln., 5 mL) was added and stirred for 10 minutes before transferring to a conical flask and neutralising with 20% aqueous HCl. The solution was concentrated under reduced pressure and purified by column chromatography with dichloromethane–methanol (95 : 5–90 : 10) containing 10 drops of triethylamine per 500 mL of eluent. However further impurity remained and additional column chromatography was required to give the desired product as a red solid (47 mg, 11.9%).

(d) Demethylation of 7-methoxyisoellipticine. 7-Methoxy-5,11-dimethyl-10*H*-pyrido[3,4-*b*]carbazole **8** (69 mg, 0.250 mmol) and pyridine hydrochloride (1.155 g, 10.0 mmol) were heated with a heat gun for 3 minutes until melted and bubbling slightly and then on a sand bath at ~220 °C for 40 minutes. The reaction was cooled to 0 °C, brine (10 mL) was added and stirred for 30 minutes. The mixture was filtered, washed with water (20 mL) and dried. Column chromatography on basic alumina with dichloromethane–methanol (95 : 5) gave the product as a red solid (53 mg, 81.5%). Analysis was identical to that quoted in method A above.

Synthesis of 2-hydroxydeazaellipticine

2-(5-Methoxy-1-(phenylsulfonyl)-1*H*-indole-2-carbonyl)benzoic acid 11. A solution of diisopropylamine (14.7 mL, 0.108 mol) in THF (150 mL) at −78 °C, under N_2, was treated with *n*-BuLi (39.6 mL, 0.099 mol, 2.5 M soln.) *via* cannula and stirred for 20 minutes. 5-Methoxy-1-(phenylsulfonyl)-1*H*-indole **10** (25.850 g, 0.090 mol) in THF (150 mL) was added over 15 minutes, maintaining the temperature below −78 °C. The mixture was allowed to warm to 15 °C over 3 hours. The mixture was recooled to −78 °C and treated with phthalic anhydride (19.991 g, 0.135 mol) in THF (100 mL), maintaining the temperature below −78 °C. The reaction mixture was allowed to warm to room temperature overnight. The solvent was evaporated under reduced pressure and the residue was dissolved in water (600 mL). The solution was cooled to 0 °C and acidified to pH 2 with 20% aqueous HCl. The resulting precipitate was collected by vacuum filtration and washed with water (200 mL) to give a cream solid. The crude product was recrystallised from acetone and collected in two crops as a cream solid (36.634 g, 93.5%). m.p. 205–207 °C (acetone); ν_{max}/cm^{-1} (KBr): 2982 (OH, broad) 1720 (C═O, acid), 1682 (C═O, ketone), 1584

‡*Note*: Due to the explosion and fire hazard of grinding *m*-CPBA, several precautions were taken. These included the use of a blast shield for the duration of the reaction and heavy-duty gloves worn over disposable gloves to protect hands and arms. A sand–gravel mixture was kept in the fumehood in case of fire.

(C═C arom.), 1444, 1371, 1351 (SO_2 asymm. stretch), 1243 (C–O), 1144 (SO_2 symm. stretch); δ_H (300 MHz, DMSO-d_6): 3.75 [3H, s, C(5)OCH_3], 6.99 [1H, s, C(3)H], 7.13 [1H, dd, *J* 9.1, 2.7, C(6)H], 7.21 [1H, d, *J* 2.3, C(4)H], 7.57–7.59 [1H, m, C(3′)H], 7.64–7.79 [5H, m, C(4′)H, C(5′)H, SO_2Ph-C(3)H, SO_2Ph-C(4)H, SO_2Ph-C(5)H], 7.87–7.89 [1H, m, C(6′)H], 8.06 [1H, d, *J* 9.1, C(7)H], 8.13–8.16 [2H, m, SO_2Ph-C(2)H, SO_2Ph-C(6)H], 13.30 (1H, br s, COO*H*); δ_C (75.5 MHz, DMSO-d_6): 55.4 [CH_3, C(5)O*C*H_3], 104.8 [CH, C(4)H], 116.1 [CH, C(7)H], 117.8 [CH, C(6)H], 121.1 [CH, C(3)H], 127.1 [CH, two overlapping aromatic CH, SO_2Ph-C(2)H, SO_2Ph-C(6)H], 128.4 (C, aromatic C), 129.2 [CH, C(3′)H], 129.4 [CH, three overlapping aromatic CH, C(6′)H, SO_2Ph-C(3)H, SO_2Ph-C(5)H], 131.29 (CH, aromatic CH), 131.32 (CH, aromatic CH), 132.4 (C, aromatic C), 133.3 (C, aromatic C), 134.3 (CH, aromatic CH), 138.6 (C, aromatic C), 138.8 (C, aromatic C), 139.3 (C, aromatic C), 156.3 (C, aromatic C), 167.8 [C, C(2′)*C*OOH], 185.2 [C, C(2)C═O]; *m*/*z* (ESI^+): 436 [$(M + H)^+$ 100%]; HRMS (ESI^+): Exact mass calculated for $C_{23}H_{18}NO_6S^+$ 436.0855. Found 436.0862.

2-(5-Methoxy-1*H*-indole-2-carbonyl)benzoic acid 12. A solution of 2-(5-methoxy-1-(phenylsulfonyl)-1*H*-indole-2-carbonyl)-benzoic acid **11** (36.635 g, 0.084 mol) and potassium carbonate (46.438 g, 0.336 mol) in methanol (900 mL) and water (300 mL) was heated to reflux for 5 hours. The mixture was allowed to cool and the methanol was evaporated under reduced pressure. The residue was treated with water (500 mL), cooled to 0 °C and acidified to pH 2 with 20% aqueous HCl, with vigorous stirring. The aqueous layer was extracted with ethyl acetate (3 × 200 mL) and the combined organic extracts were washed with water (1 × 200 mL) and brine (2 × 200 mL), dried and concentrated under reduced pressure to give an orange solid. This was recrystallised from acetone to give a yellow solid in several crops (19.361 g, 78.1%). m.p. 219–221 °C (acetone); ν_{max}/cm^{-1} (KBr): 3212 (OH, broad), 1703 (C═O × 2, broad), 1590 (C═C arom.), 1524 (C═C arom.), 1455, 1397, 1266 (C–O), 1134, 1034; δ_H (400 MHz, DMSO-d_6): 3.73 [3H, s, C(5)OCH_3], 6.49 [1H, dd, *J* 2.2, 0.7, C(3)H], 6.96 [1H, dd, *J* 9.0, 2.5, C(6)H], 7.07 [1H, d, *J* 2.4, C(4)H], 7.38 [1H, d, *J* 9.0, C(7)H], 7.57 [1H, dd, *J* 7.4, 1.3, C(3′)H], 7.67 [1H, td, *J* 7.5, 1.3, C(5′)H], 7.72 [1H, td, *J* 7.4, 1.4, C(4′)H], 7.97 [1H, dd, *J* 7.6, 1.3, C(6′)H], 11.88 [1H, s, N(1)H], 13.12 (1H, br s, COO*H*); δ_C (75.5 MHz, DMSO-d_6): 55.7 [CH_3, C(5)O*C*H_3], 102.4 (CH, aromatic CH), 110.5 (CH, aromatic CH), 113.6 (CH, aromatic CH), 117.3 (CH, aromatic CH), 127.1 (C, aromatic C), 127.9 (CH, aromatic CH), 129.6 (CH, aromatic CH), 129.9 (CH, aromatic CH), 130.8 (C, aromatic C), 131.8 (CH, aromatic CH), 133.5 (C, aromatic C), 136.2 (C, aromatic C), 140.8 (C, aromatic C), 153.9 (C, aromatic C), 167.3 [C, C(2′)*C*OOH], 187.7 [C, C(2)C═O]; *m*/*z* (ESI^-): 294 [$(M - H)^-$ 100%]; HRMS (ESI^+): Exact mass calculated for $C_{17}H_{14}NO_4^+$ 296.0923. Found 296.0933.

2-Methoxyindolo[1,2-*b*]isoquinoline-6,11-dione 13. 2-(5-Methoxy-1*H*-indole-2-carbonyl)benzoic acid **12** (5.857 g, 19.8 mmol) in acetic anhydride (500 mL) was heated to 90 °C under N_2 for 24 hours. The reaction mixture was concentrated to approximately half volume and stored at 4 °C overnight. The resulting precipitate was collected by vacuum filtration, washed with water (500 mL) and dried at 0.1 mbar to give the product as gold needles (2.932 g). The filtrate was completely evaporated under reduced pressure and recrystallised from acetone in two crops to give additional product (0.702 g, combined yield 3.634 g, 66.1%). m.p. 223–225 °C (acetone); ν_{max}/cm^{-1} (KBr): 3070 (CH), 2974 (CH_3 asymm. stretch), 2833 (CH_3 symm. stretch), 1693 (C═O, ketone), 1659 (C═O, lactam), 1599 (C═C arom.), 1547 (C═C arom.), 1477, 1377, 1255 (C–O), 1152; δ_H (300 MHz, DMSO-d_6): 3.84 [3H, s, C(5)OCH_3], 7.24 [1H, dd, *J* 9.1, 2.6, C(3)H], 7.36 [1H, d, *J* 2.5, C(1)H], 7.65 [1H, s, C(12)H], 7.89–7.98 [2H, m, C(8)H, C(9)H], 8.15–8.18 [1H, m, C(7)H or C(10)H], 8.30–8.33 [1H, m, C(7)H or C(10)H], 8.38 [1H, d, *J* 9.1, C(4)H]; δ_C (75.5 MHz, DMSO-d_6): 55.5 [CH_3, C(2)O*C*H_3], 105.3 (CH, aromatic CH), 115.2 (CH, aromatic CH), 117.2 (CH, aromatic CH), 119.0 (CH, aromatic CH), 126.3 (CH, aromatic CH), 128.7 (CH, aromatic CH), 129.5 (C, aromatic C), 130.8 (C, aromatic C), 131.2 (C, aromatic C), 133.0 (C, aromatic C), 134.0 (C, aromatic C), 134.3 (CH, aromatic CH), 134.7 (CH, aromatic CH), 156.9 (C, aromatic C), 158.6 [C, C(6)═O], 175.0 [C, C(11)═O]; *m*/*z* (ESI^+): 278 [$(M + H)^+$ 100%]; HRMS (ESI^+): Exact mass calculated for $C_{17}H_{12}NO_3^+$ 278.0817. Found 278.0806.

2-Methoxy-6,11-dimethyl-5*H*-benzo[*b*]carbazole 14. A solution of 2-methoxyindolo[1,2-*b*]isoquinoline-6,11-dione **13** (0.401 g, 1.45 mmol) in THF (100 mL) at −100 °C under N_2 was treated drop-wise with methyllithium (1.9 mL, 3.05 mmol, 1.6 M soln.). The resulting mixture was maintained below −100 °C for 1 hour and then allowed to warm to room temperature overnight. The reaction was quenched with water (5 mL) and solvent was removed under reduced pressure. The residue was dissolved in absolute ethanol (130 mL), treated with sodium borohydride (0.546 g, 14.4 mmol) and heated to reflux. The reaction was refluxed for a total of 48 hours with a second portion of sodium borohydride (0.546 g, 14.4 mmol) added after 6 hours and a third portion (0.546 g, 14.4 mmol) after 26 hours. On cooling, the solvent was evaporated under reduced pressure and water (120 mL) was added. The aqueous layer was extracted with dichloromethane (3 × 100 mL), adjusted to pH 7 with 20% aqueous HCl and extracted again with dichloromethane (2 × 50 mL). Combined organic extracts were washed with water (1 × 100 mL) and brine (1 × 100 mL), dried and concentrated under reduced pressure. Purification by column chromatography, eluting with hexane–ethyl acetate (70 : 30), gave **14** (0.245 g, 61.4%). m.p. 184–186 °C (Lit. 188 °C);[17] ν_{max}/cm^{-1} (KBr): 3414 (NH), 2930 (CH_3 asymm. stretch), 2849 (CH_3 symm. stretch), 1624 (C═C arom.), 1582 (C═C arom.), 1485, 1223 (C–O), 1132; δ_H (300 MHz, DMSO-d_6): 2.81 [3H, s, C(6)CH_3], 3.17 [3H, s, C(11)CH_3], 3.90 [3H, s, C(2)OCH_3], 7.15 [1H, dd, *J* 8.7, 2.3, C(3)H], 7.39–7.43 [1H, m, C(9)H], overlapping with 7.45 [1H, d, *J* 8.6, C(4)H], 7.48–7.54 [1H, m, C(8)H], 7.87 [1H, d, *J* 2.4, C(1)H], 8.10 [1H, d, *J* 8.5, C(7)H], 8.32 [1H, d, *J* 8.5, C(10)H], 10.84 [1H, s, N(5)H]; δ_C (75.5 MHz, DMSO-d_6): 12.6 [CH_3, C(6)*C*H_3], 15.0 [CH_3, C(11)*C*H_3], 55.8 [CH_3, C(2)O*C*H_3], 107.7 [CH, C(1)H], 108.8 (C, aromatic C), 110.7 [CH, C(4)H], 114.6 [CH, C(3)H], 121.5 [CH, C(9)H], 122.7 (C, aromatic C), 123.1 [CH, C(7)H], 123.7 (C, aromatic C), 124.3 [CH, C(8)H], 124.5 [CH, C(10)H], 125.8 (C, aromatic C), 126.4 (C, aromatic C), 130.6 (C, aromatic C), 137.5 (C, aromatic C), 138.9 (C,

aromatic C), 152.6 (C, aromatic C); *m*/*z* (ESI$^+$): 276 [(M + H)$^+$ 100%]; HRMS (ESI$^+$): Exact mass calculated for $C_{19}H_{18}NO^+$ 276.1388. Found 276.1388.

6,11-Dimethyl-5*H*-benzo[*b*]carbazol-2-ol 9. 2-Methoxy-6,11-dimethyl-5*H*-benzo[*b*]carbazole **14** (145 mg, 0.527 mmol) and pyridine hydrochloride (1.217 g, 10.5 mmol) were heated to 220 °C for 2 hours. The reaction was cooled to 0 °C, water (10 mL) was added and extracted with dichloromethane (2 × 10 mL) and dichloromethane–methanol (90 : 10, 2 × 10 mL). Combined organic extracts were washed with 1 M HCl (2 × 10 mL) and brine (1 × 10 mL), dried and concentrated under reduced pressure. Purification by column chromatography, eluting with hexane–ethyl acetate (85 : 15), gave the product as a yellow solid (78 mg, 56.5%). m.p. 215–217 °C; ν_{max}/cm^{-1} (KBr): 3343 (OH), 2917 (CH_3 asymm. stretch), 2849 (CH_3 symm. stretch), 1622 (C═C arom.), 1565 (C═C arom.), 1477, 1222 (C–O stretch), 1185, 1131; δ_H (300 MHz, DMSO-d$_6$): 2.79 [3H, s, C(6)C*H*$_3$], 3.12 [3H, s, C(11)C*H*$_3$], 6.98 [1H, dd, *J* 8.5, 2.3, C(3)H], 7.33 [1H, d, *J* 8.5, C(4)H], 7.40 [1H, overlapping ddd, *J* 8.3, 6.7, 1.4, C(9)H], 7.50 [1H, overlapping ddd, *J* 8.3, 6.7, 1.4, C(8)H], 7.75 [1H, d, *J* 2.3, C(1)H], 8.09 [1H, d, *J* 8.1, C(7)H], 8.29 [1H, d, *J* 7.9, C(10)H], 8.95 [1H, s, C(2)O*H*], 10.68 [1H, s, N(5)H]; δ_C (75.5 MHz, DMSO-d$_6$): 12.5 [CH_3, C(6)*C*H$_3$], 15.0 [CH_3, C(11)*C*H$_3$], 107.7 (C, aromatic C), 108.8 (CH, aromatic CH), 110.7 (CH, aromatic CH), 114.5 (CH, aromatic CH), 121.5 (CH, aromatic CH), 122.7 (C, aromatic C), 123.0 (CH, aromatic CH), 123.7 (C, aromatic C), 124.2 (CH, aromatic CH), 124.5 (CH, aromatic CH), 125.8 (C, aromatic C), 126.3 (C, aromatic C), 130.6 (C, aromatic C), 137.5 (C, aromatic C), 138.9 (C, aromatic C), 152.6 (C, aromatic C); *m*/*z* (ESI$^+$): 262 [(M + H)$^+$ 40%], 261 (100%); HRMS (ESI$^+$): Exact mass calculated for $C_{18}H_{16}NO^+$ 262.1232. Found 262.1226.

Representative syntheses of isoellipticinium salts

Full experimental data for salts **18–30** may be found in the ESI.†

2-Methylisoellipticinium iodide 16. To a stirred suspension of 5,11-dimethyl-10*H*-pyrido[3,4-*b*]carbazole **5** (72 mg, 0.292 mmol) in dimethylformamide (4 mL), was added iodomethane (0.02 mL, 0.321 mmol, 1.1 eq.). The reaction mixture was stirred at room temperature for 3 hours. The mixture was cooled to 0 °C and cold diethyl ether (3 mL) was added. The resulting precipitate was collected by vacuum filtration and washed with hexane (3 mL) and cold diethyl ether (5 mL) to give the product as an orange solid (53 mg, 46.7%). m.p. >350 °C without melting; ν_{max}/cm^{-1} (KBr): 3400 (NH), 3195 (CH), 3004 (CH), 2862 (symm. CH_3 stretch), 1636 (C═C arom.), 1619 (C═C arom.), 1418, 1316, 1193; δ_H (600 MHz, DMSO-d$_6$): 2.94 [3H, s, C(11)C*H*$_3$], 3.13 [3H, s, C(5)C*H*$_3$], 4.45 [3H, s, N(2)C*H*$_3$], 7.31 [1H, overlapping ddd appearing as a td, *J* 6.9, 0.7, C(7)H], 7.61–7.64 [2H, m, C(8)H, C(9)H], 8.37 [1H, d, *J* 6.9, C(3)H], 8.40 [1H, d, *J* 8.0, C(6)H], 8.69 [1H, d, *J* 7.0, C(4)H], 9.89 [1H, s, C(1)H], 11.90 [1H, s, N(10)H]; δ_C (150.9 MHz, DMSO-d$_6$): 12.3 [CH_3, C(11)*C*H$_3$], 14.8 [CH_3, C(5)*C*H$_3$], 47.2 [CH_3, N(2)*C*H$_3$], 111.3 (CH, aromatic CH), 115.2 (C, aromatic C), 120.1 (CH, aromatic CH), 121.6 (C, aromatic C), 122.1 (CH, aromatic CH), 123.6 (C, aromatic C), 125.1 (CH, aromatic CH), 126.4 (C, aromatic C), 128.0 (C, aromatic C), 129.7 (CH, aromatic CH), 129.8 (CH, aromatic CH), 130.0 (C, aromatic C), 139.8 (C, aromatic C), 143.7 (C, aromatic C), 146.5 (CH, aromatic CH); *m*/*z* (ESI$^+$): 261 [(M)$^+$ 100%]; HRMS (ESI$^+$): Exact mass calculated for $C_{18}H_{17}N_2^+$ 261.1392. Found 261.1385.

2-(3′-Cyanopropyl)isoellipticinium chloride 17. 5,11-Dimethyl-10*H*-pyrido[3,4-*b*]carbazole **5** (198 mg, 0.80 mmol) and 4-chlorobutryonitrile (0.091 mL, 0.96 mmol) in dimethylformamide (5 mL) were heated to 120 °C for 48 hours. The dark pink solution was cooled to 0 °C and excess cold ether added. The resulting dark pink precipitate was filtered and washed with cold ether to yield product as a red powder (222 mg, 79%). m.p. 236–240 °C; ν_{max}/cm^{-1} (KBr): 3384 (NH), 3153 (CH, aromatic), 3091 (CH_3), 2246 (CN, nitrile), 1634 (C═C, aromatic), 1619 (C═C, aromatic), 1417, 1321; δ_H (300 MHz, DMSO-d$_6$): 2.28–2.41 [2H, m, C(2′)H$_2$], 2.69 [2H, t, *J* 7.3, C(3′)C*H*$_2$], 2.91 [3H, s, C(11)CH$_3$], 2.98 [3H, s, C(5)CH$_3$]. 4.80 [2H, t, *J* 7.0, C(1′)H$_2$], 7.18–7.25 [1H, m, C(7)H], 7.53–7.59 [2H, m, C(8)H, C(9)H], 8.25 [1H, d, *J* 8.1, C(6)H], 8.44 [1H, d, *J* 7.1, C(3)H], 8.57 [1H, d, *J* 7.1, C(4)H], 9.97 [1H, s, C(1)H], 12.13 (1H, s, N*H*); δ_C (75.5 MHz; DMSO-d$_6$): 12.55 [CH_3, C(11) *C*H$_3$], 13.64 (CH_2), 14.64 [CH_3, C(5)*C*H$_3$], 26.44 (CH_2), 58.57 [CH_2, C(1′)H$_2$], 111.38 (CH, aromatic), 115.61 (C, aromatic C), 119.68 (C, aromatic C), 119.81 (CH, aromatic), 121.35 (C, *C*N), 122.32 (CH, aromatic), 123.58 (C, aromatic C), 124.82 (CH, aromatic), 126.07 (C, aromatic C), 128.06 (C, aromatic C), 128.56 (CH, aromatic), 129.47 (CH, aromatic), 129.89 (C, aromatic C), 139.65 (C, aromatic C), 143.71 (C, aromatic C), 146.21 (CH, aromatic); *m*/*z* (ESI$^+$): 314 [(M$^+$), 100%]; HRMS (ESI): Exact mass calculated for $C_{21}H_{20}N_3^+$: 314.1657. Found: 314.1664.

Topoisomerase II decatenation assay

The decatenation assay kit was obtained from Inspiralis, Norwich Bioincubator, Norwich Research Park, Colney, Norwich, UK. The kit comprised of the following: assay buffer (supplied as 10× stock) containing 50 mM Tris-HCl (pH 7.5), 125 mM NaCl, 10 mM $MgCl_2$, 5 mM DTT and 100 μg mL^{-1} albumin; dilution buffer containing 50 mM Tris-HCl (pH 7.5), 100 mM NaCl, 1 mM DTT, 0.5 mM EDTA, 50% (v/v) glycerol, 50 μg mL^{-1} albumin; ATP 30 mM; kDNA (100 ng μL^{-1}); 10 U μL^{-1} human topoisomerase II in dilution buffer; 5× stop buffer containing 2.5% SDS, 15% Ficoll-400, 0.05% bromophenol blue, 0.05% xylene cyanol and 25 mM EDTA. Tris-acetate-EDTA buffer (supplied as 10× buffer) and agarose were obtained from Sigma Life Sciences (Dublin, Ireland) and Safe View Stain was supplied by NBS Biologicals, Cambridgeshire, England.

The topo II decatenation assay protocol involved initial incubation of each inhibitor candidate (100 μM) along with a stock solution containing water, ATP, assay buffer, kDNA obtained from the mitochondrial DNA of *Crithidia fasciculate*, and topo II, at 37 °C for 1 hour. Following addition of stop buffer, agarose DNA gel electrophoresis was run at 50 V for 2 hours using a Consort EV243 power pack, to determine the relative amounts of decatenated DNA bands obtained in each compound lane. Positive (water), as well as negative controls (ellipticine) were incorporated in order to validate the results of each run. The resulting

gels were viewed under U.V. light using a DNR Bio-Imaging System and photographed using GelCapture software.

NCI-60 anti-cancer screening

Tested compounds were initially solubilised in DMSO, diluted into RPMI 1640 and 5% fetal bovine serum/L-glutamine, and added to 96-well plates containing cell lines previously cultured for 24 hours. After 48-hour incubation, the media were removed, and the cells were fixed and stained with sulforhodamine B to determine overall percent growth/total protein content. Unbound dye was removed with five washes of 1% acetic acid, and the plates were allowed to air dry. The dye was then resolubilised in Tris buffer, and the colorimetric absorbance was measured (515 nm). Growth inhibition was measured relative to the response generated from proliferating cells cultured under identical conditions for 48 hours. In the five-dose study, serial 5 × 10-fold dilution from an initial DMSO stock solution was performed, prior to incubation at each individual concentration.

Using seven absorbance measurements [time zero (Tz), control growth (C), and test growth in the presence of drug at the five concentration levels (Ti)], the percentage growth is calculated at each of the drug concentrations levels. Percentage growth inhibition is calculated as:

$$[(\mathrm{Ti} - \mathrm{Tz})/(\mathrm{C} - \mathrm{Tz})] \times 100 \text{ for concentrations for which } \mathrm{Ti} \geq \mathrm{Tz}$$

$$[(\mathrm{Ti} - \mathrm{Tz})/\mathrm{Tz}] \times 100 \text{ for concentrations for which } \mathrm{Ti} < \mathrm{Tz}.$$

Three dose response parameters are calculated for each experimental agent. Growth inhibition of 50% (GI50) is calculated from $[(\mathrm{Ti} - \mathrm{Tz})/(\mathrm{C} - \mathrm{Tz})] \times 100 = 50$, which is the drug concentration resulting in a 50% reduction in the net protein increase (as measured by SRB staining) in control cells during the drug incubation. The drug concentration resulting in total growth inhibition (TGI) is calculated from Ti = Tz. The LC50 (concentration of drug resulting in a 50% reduction in the measured protein at the end of the drug treatment as compared to that at the beginning) indicating a net loss of cells following treatment is calculated from $[(\mathrm{Ti} - \mathrm{Tz})/\mathrm{Tz}] \times 100 = -50$. Values are calculated for each of these three parameters if the level of activity is reached; however, if the effect is not reached or is exceeded, the value for that parameter is expressed as greater or less than the maximum or minimum concentration tested.[35]

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