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Synthesis and biological evaluation of novel ellipticine salt derivatives as anticancer agents

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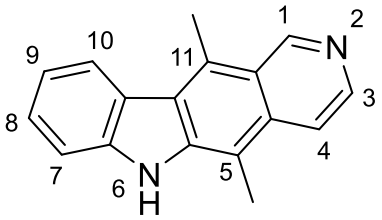
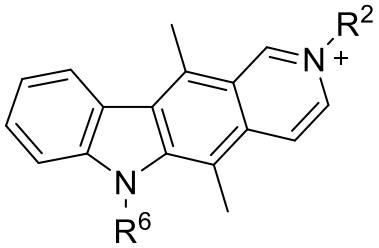
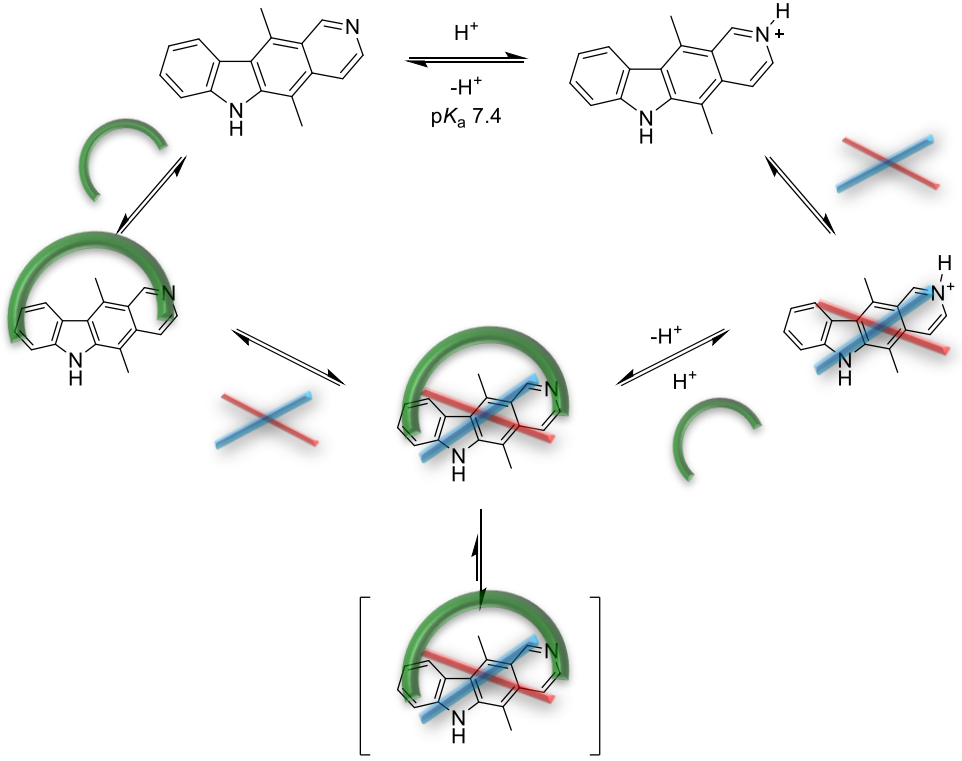
Coláiste na hOllscoile Corcaigh

ABCRF Analytical & Biological Chemistry Research Facility

Synthesis and biological evaluation of novel ellipticine salt derivatives as anticancer agents







Abstract:

Cancer is the second leading cause of death worldwide, killing an estimated 1 in 6 people. Ellipticine is a natural product which has potent anticancer activity and has been subject to extensive study since its discovery, in 1959, with the key aim of identifying derivatives with clinical application.
Functionalisation of the ellipticine pharmacophore is key to developing potent and selective analogues. For example, generation of quaternary ellipticine salts, helps to overcome issues surrounding solubility and can improve selectivity whereas the most potent anticancer ellipticine derivatives have a hydroxyl or methoxy substituent at the 9-position. This work outlines the synthesis of quaternary ellipticine salts and their subsequent biological evaluation. Alkyl groups were introduced at the 6-position, as well as formyl or hydroxy groups at the 9-position, as these substituents have been previously shown to improve activity.
Biological evaluation encompassed measurement of growth inhibition against twelve cancer cell lines and submission to the NCI 60 Cell Lines Screen. Substitution at the 9-position greatly improved activity, while increasing substituent size at the 6-position led to lower potency. A number of potent derivatives have been identified following biological evaluation, with long chain alkyl salts displaying sub-micromolar average GI_{50} values.

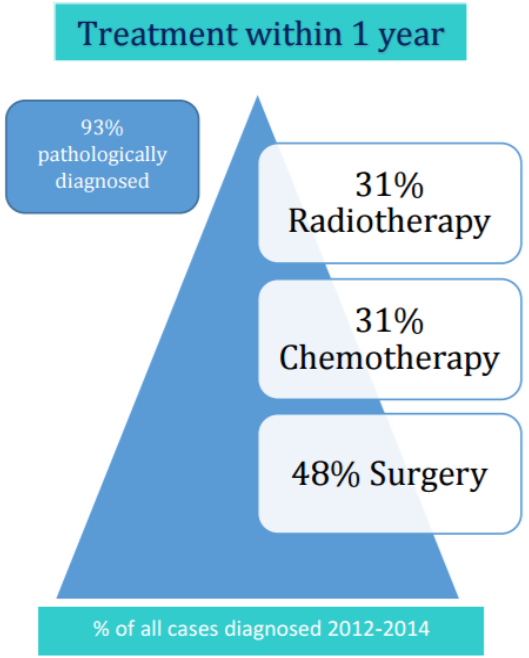
Keywords
Ellipticine; cancer; ellipticinium salts; NCI

Cancer

- Cancer is responsible for 1 in 6 deaths worldwide
- The cumulative lifetime risk, of developing an invasive cancer is approximately 1 in 4 for women and 1 in 3 for men

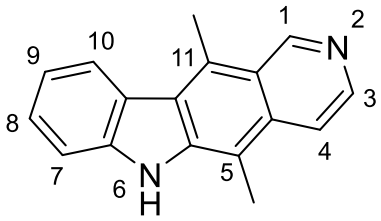
- The NCR of Ireland predicts that by 2020, 1 in 2 Irish people will develop cancer

- New and effective chemotherapeutic treatments are essential

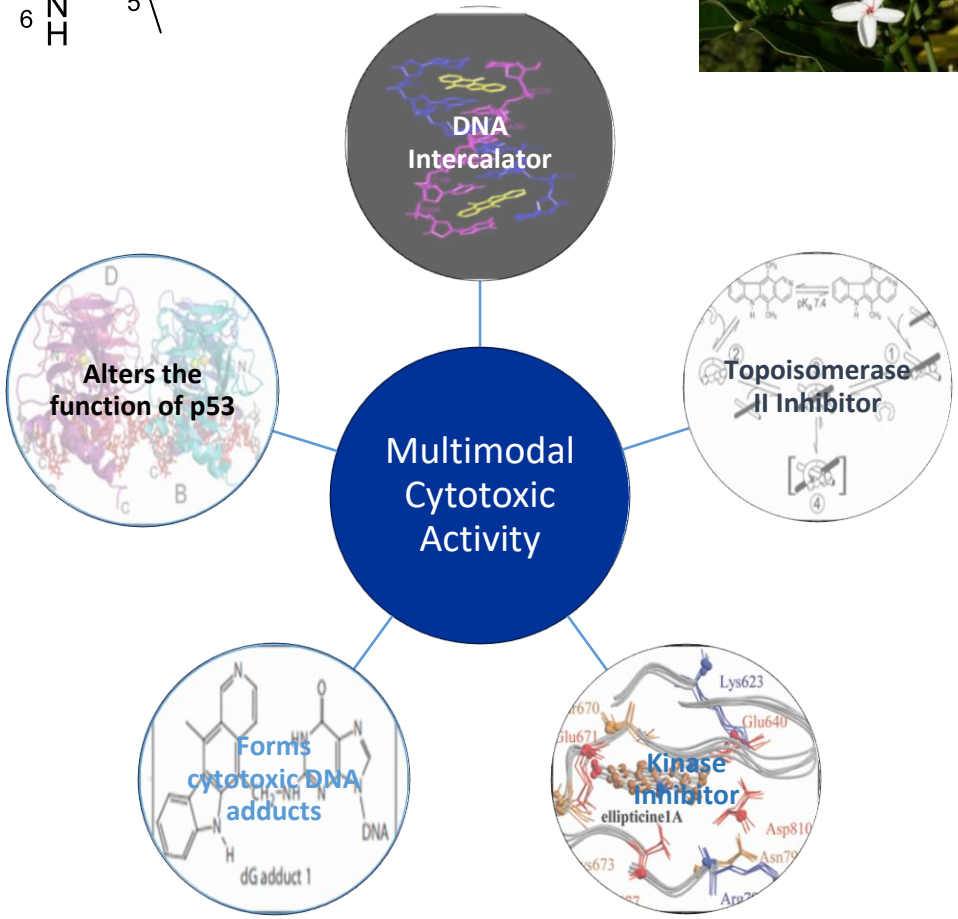


World Health Organisation: http://www.who.int/news-room/fact-sheets/detail/cancer, 2018.
Data sourced from the Central Statistics Office, Ireland
National Cancer Registry (2016) Cancer in Ireland 1994-2014: Annual Report of the National Cancer Registry. NCR, Cork, Ireland.
Image sourced from the National Cancer Registry Factsheet (Overview and Most Common Cancers)

Ellipticine



- Ellipticine is a naturally occurring alkaloid first isolated in 1959
- Found to have extensive anticancer properties but limited by side effects and poor solubility
- Derivatives have progressed to phase II of clinical trials

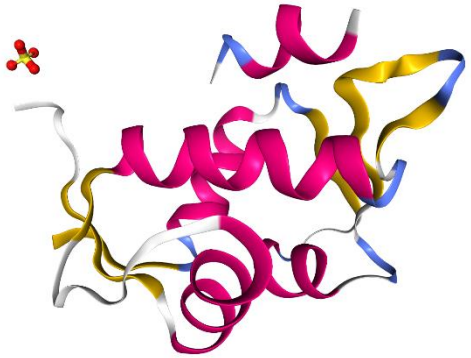


Goodwin, S. *et al., Journal of the American Chemical Society* **1959**, *81*, 1903
Dalton, L.; *et al*., *Australian Journal of Chemistry* **1967**, *20*, 2715.
Auclair, C. *Archives of Biochemistry and Biophysics* **1987**, *259*, 1.

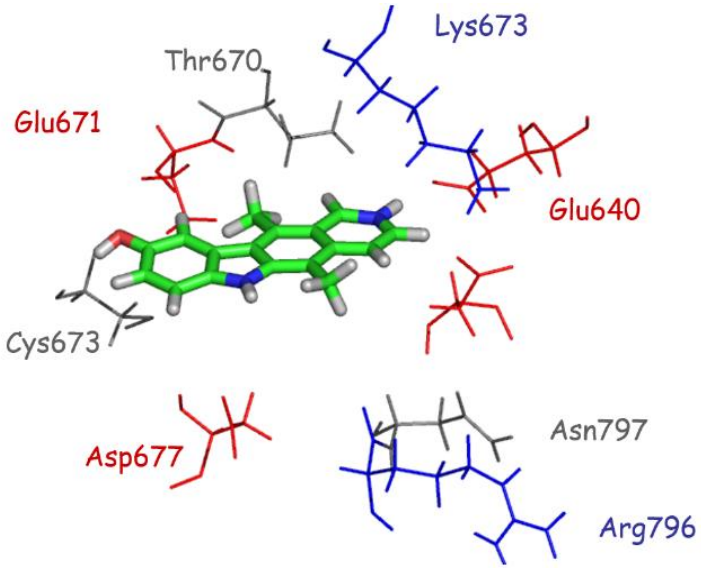
Ellipticine: Old drug with new targets?

Recent work on the ellipticine pharmacophore has shown a significant impact on cell cycle regulation.

- Ellipticine has been shown to restore the function of mutant p53. p53 is referred to as the guardian of the genome and is associated with over 50% of cancers
- It has been shown to impact kinases, including AKT, helping to restore apoptotic signalling in cancer cells. Molecular modelling has been used to examine the binding of 9-hydroxyellipticine to c-Kit kinase



p53 crystal structure



Ellipticine bound in the active site of c-Kit

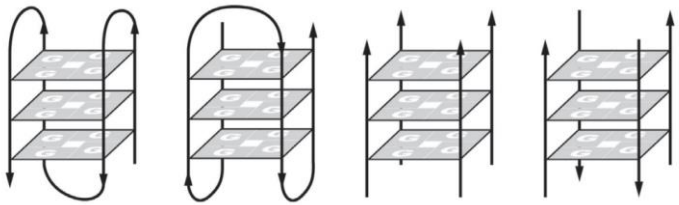
Peng, Y.*; et al*, J. *Oncogene* **2003**, *22*, 4478.
D. Thompson, *et al*, *Biochemistry*, 2008, 47, 10333-10344
O'Sullivan, E. C.; *et al*. In *Studies in Natural Products Chemistry*; Atta ur, R., Ed.; Elsevier: 2013; Vol. Volume 39, p 189.

Ellipticine: Old drug with new targets?

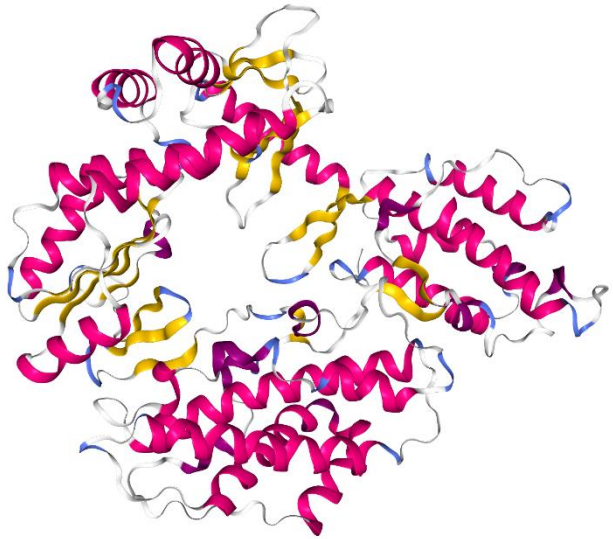
- Interactions of 9-substituted ellipticine derivatives with G-quadruplexes, can inhibit telomerase induced cell immortality, which is closely associated with cancer

- Interactions with chromatin, histone octamers and chromosomal DNA have posed another potential mechanism of action

- 9-Hydroxyellipticine has been shown to disrupt the activity of RNA polymerase I, an enzyme which is fundamental to protein synthesis and linked to cancers which are challenging to treat



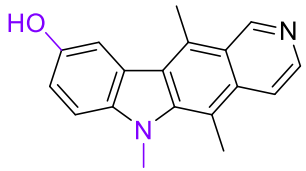
Representation of G-quad structures

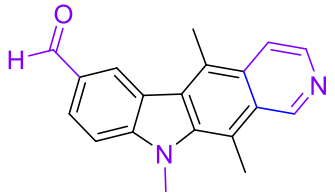


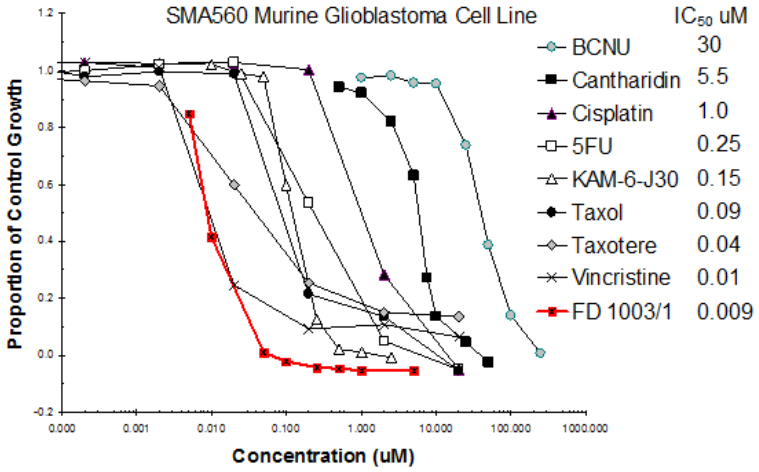
RNA Pol I crystal structure

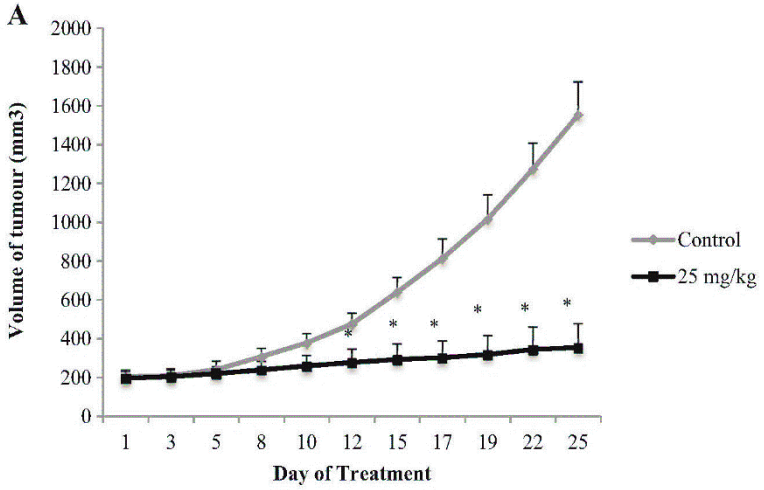
Brown, R. V.; *et al*. *Journal of the American Chemical Society* **2017**, *139*, 7456.
Andrews, W. J.; *et al*. *Journal of Biological Chemistry* **2013**, *288*, 4567.

Introduction: Recent developments within the McCarthy group









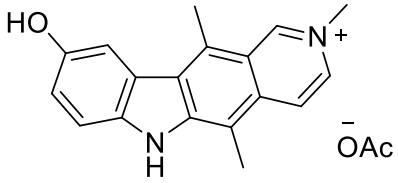
9-Hydroxy-6-methylellipticine has been shown to better activity than known anticancer agents, including 5-fluorouracil, against a murine glioblastoma cell line

7-Formyl-10-methylisoellipticine was employed in *in vivo* testing resulting in a seven-fold reduction in tumour growth in an acute myeloid leukaemia xenograft mouse model when compared to a control

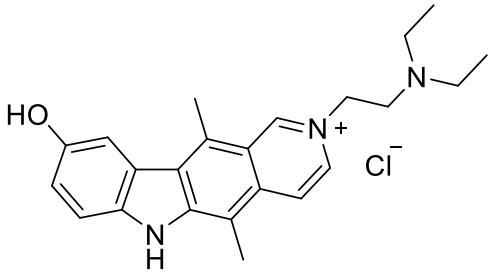
Deane, F. M. *PhD Thesis* University College Cork, 2009
Russell, E. G.*et al. Invest New Drugs* **2016**, *34*, 15.

Quaternary ellipticine salts and clinical trials

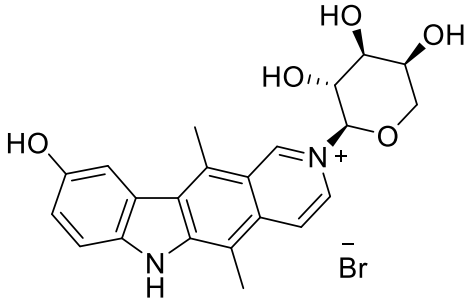
- Ellipticine derivatives which are substituted at the 2-position have greatly improved aqueous solubility and as a result improved bioavailability
- Ellipticine derivatives which have progressed to clinical trials are often quaternised, including Celiptium, Datelliptine and Elliprabin
- Celiptium has been shown to intercalate, to affect TOP2, to form cytotoxic DNA adducts and to have potent activity against a number of cancerous cell lines



Celiptium



Datellipine



Elliprabin

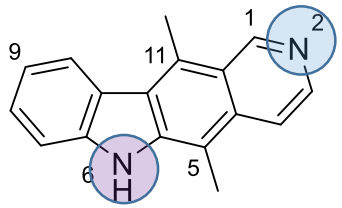
Activity by Design?

Modification of the ellipticine template has been shown to improve selectivity and increase potency. This project aimed to develop and biologically evaluate a panel of quaternary ellipticine derivatives

Previous work focused on:

- A-ring substitution, especially *C*-9
- *C*-1 substitution
- *C*-5 and *C*-11 modification

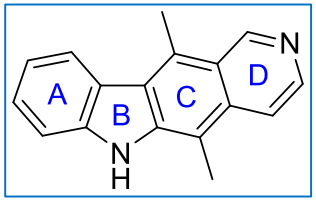
New investigation focused on:



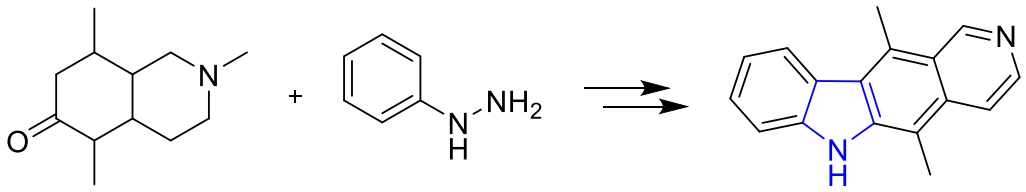
Quaternisation of the 2-position to improve solubility and probe binding interactions of DNA and topoisomerases

Substitution at the 6-position to probe the effect on bioactivity and eliminate potential bio-oxidation products in cellular assays

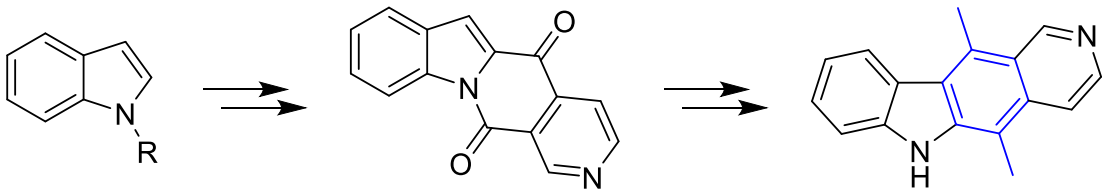
Routes to ellipticine



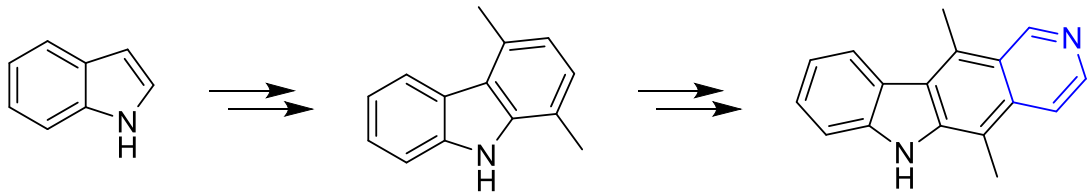
B-type



C-type

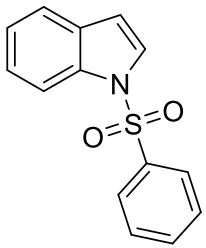


D-type



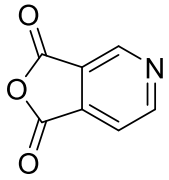
Miller, C. M.; O'Sullivan, E. C.; Devine, K. J.; McCarthy, F. O. *Organic & Biomolecular Chemistry* **2012**, *10*, 7912.

The Gribble Synthesis of Ellipticine

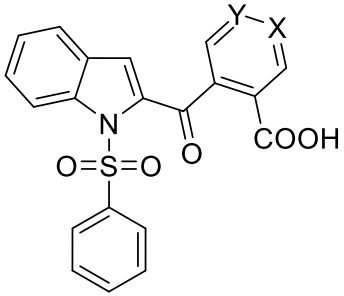


93% from indole

(i) LDA, THF
–78 °C
(ii) THF, –100 °C

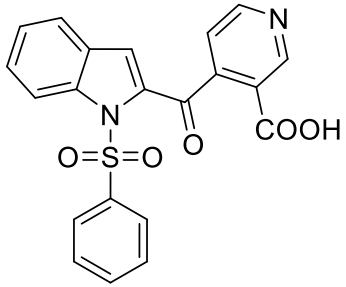


79% from pyridine-3,4-dicarboxylic acid

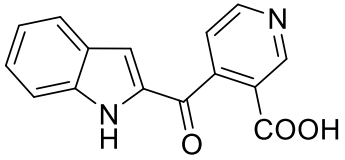


After fractional recrystallisation
major: X=N, Y=CH, 63%
minor: X=CH, Y=N, 14%

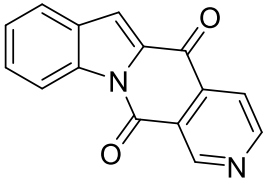
Fractional recrystallisation



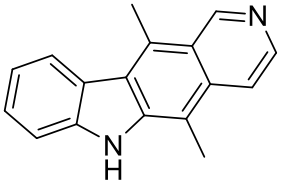
K_2CO_3
$MeOH, H_2O$
reflux
96%



Ac_2O
85-90 °C
69%

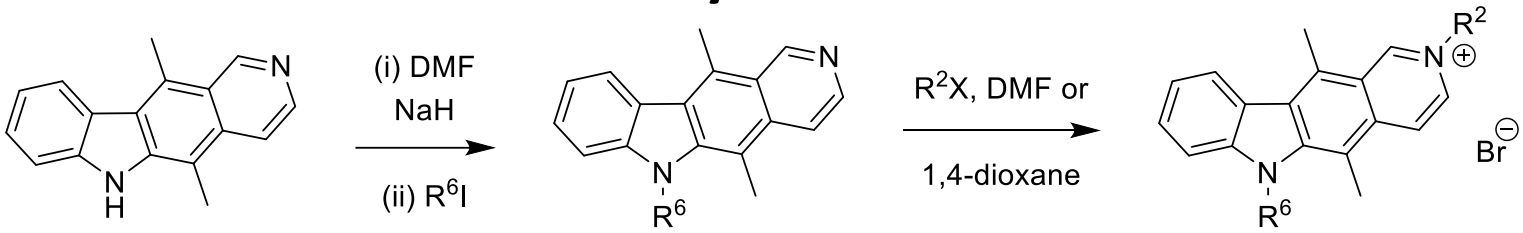


(i) MeLi, THF
–100 °C
(ii) $NaBH_4$, EtOH
reflux
71%



Saulnier, M. G.; Gribble, G. W. *The Journal of Organic Chemistry* **1983**, *48*, 2690.

Derivatisation and Quaternary Salt Formation



R^6 = Me 92%, Et 63%, *i*Pr 35%

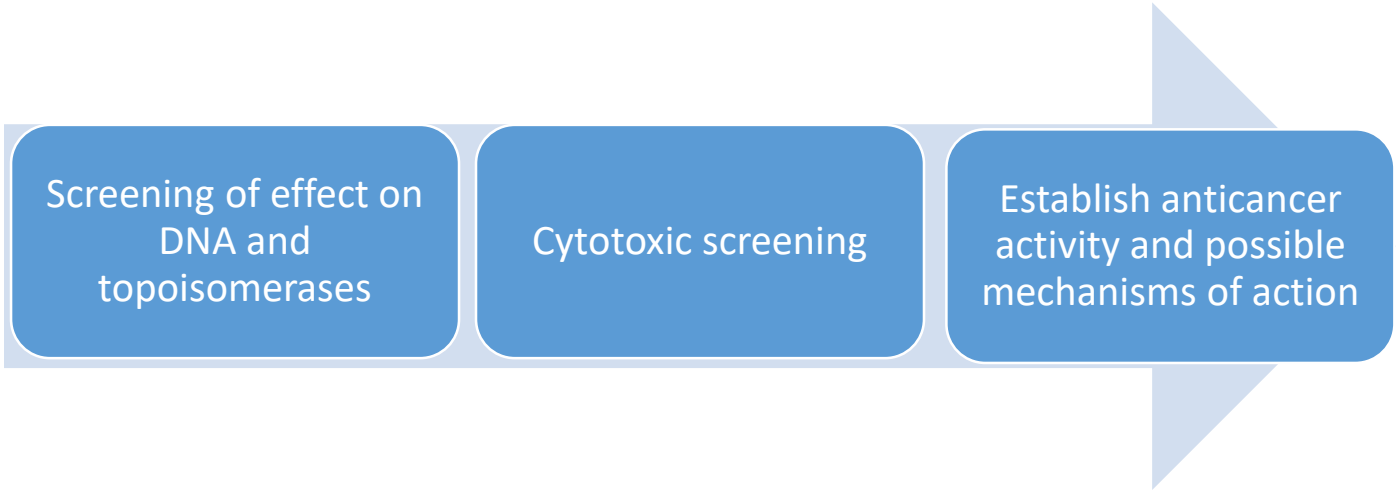
Entry	R^6	R^2	Yield (%)	Entry	R^6	R^2	Yield (%)
1	H	$(CH_2)_2CO_2H$	48*	7	CH_3	$(CH_2)_2CO_2H$	57*
2	H	$(CH_2)_4CO_2H$	17	8	CH_3	$(CH_2)_4CO_2H$	75
3	H	$(CH_2)_5CO_2H$	60	9	CH_3	$(CH_2)_5CO_2H$	74
4	H	$(CH_2)_5CONH_2$	66	10	CH_3	$(CH_2)_5CONH_2$	77
5	H	$(CH_2)_5CN$	59	11	CH_3	$(CH_2)_5CN$	62
6	H	$(CH_2)_5CONHSO_2CH_3$	52	12	CH_3	$(CH_2)_5CONHSO_2CH_3$	64

* Contains a trace amount of starting elliptiine

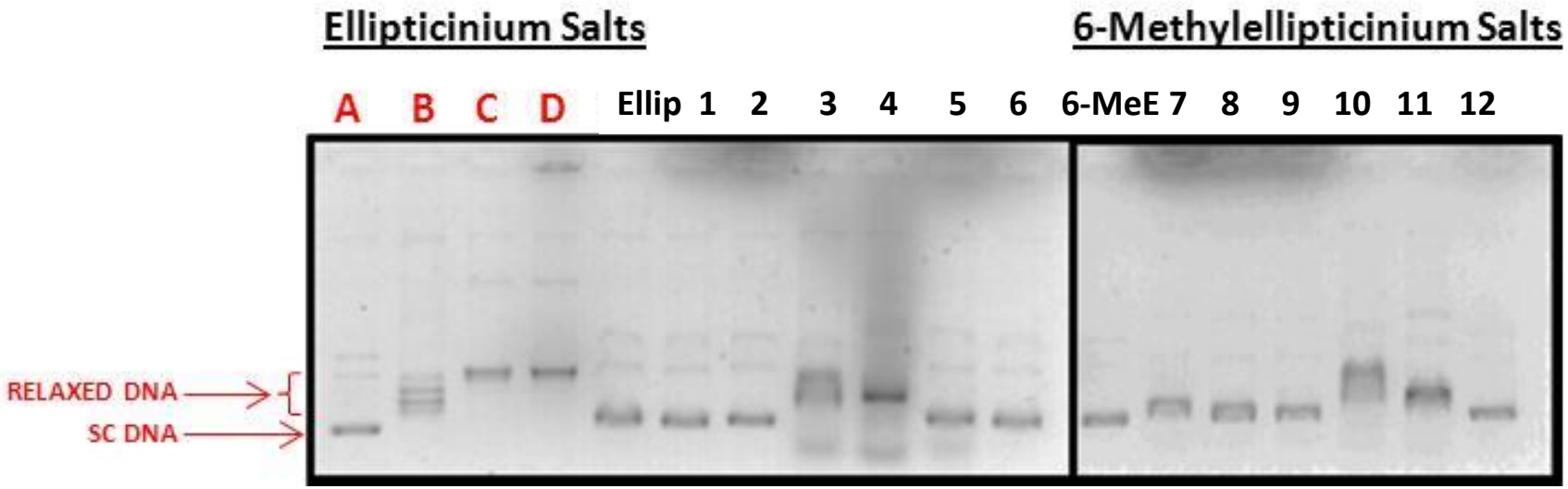
Deane, F. M.; O'Sullivan, E. C.; Maguire, A. R.; Gilbert, J.; Sakoff, J. A.; McCluskey, A.; McCarthy, F. O. *Organic & Biomolecular Chemistry* **2013**, *11*, 1334.
Miller, C. M.; O'Sullivan, E. C.; Devine, K. J.; McCarthy, F. O. *Organic & Biomolecular Chemistry* **2012**, *10*, 7912.

Establishing biological activity

- Biological activity was assessed in a number of ways to develop a better understanding of the mechanism of action of these novel derivatives
- Complementary techniques were employed to identify the most potent derivatives

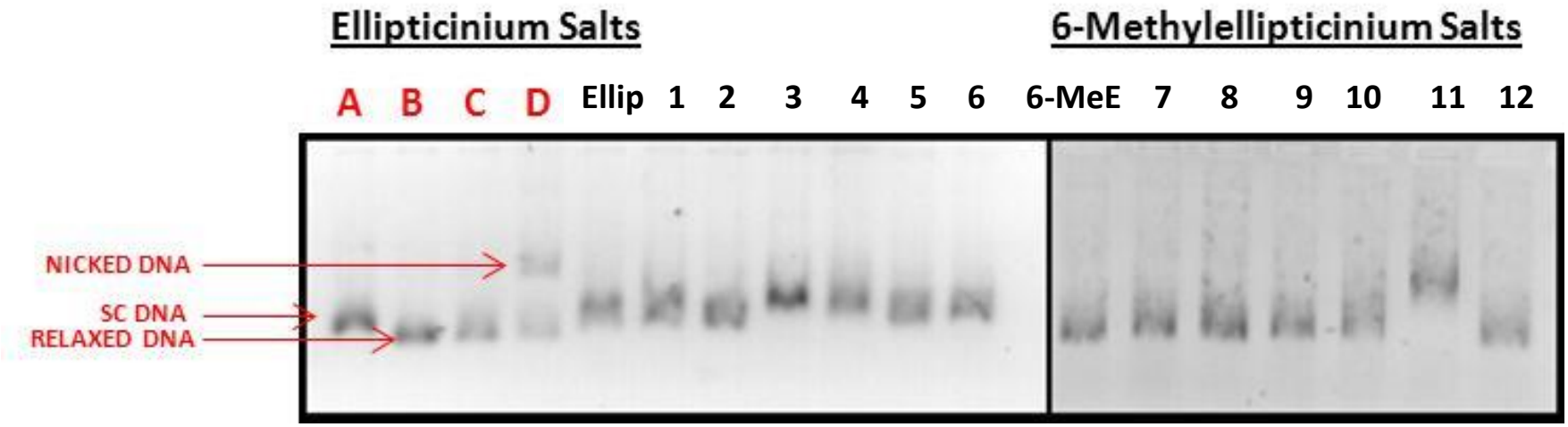


Topoisomerase Assays: Unwinding Assay



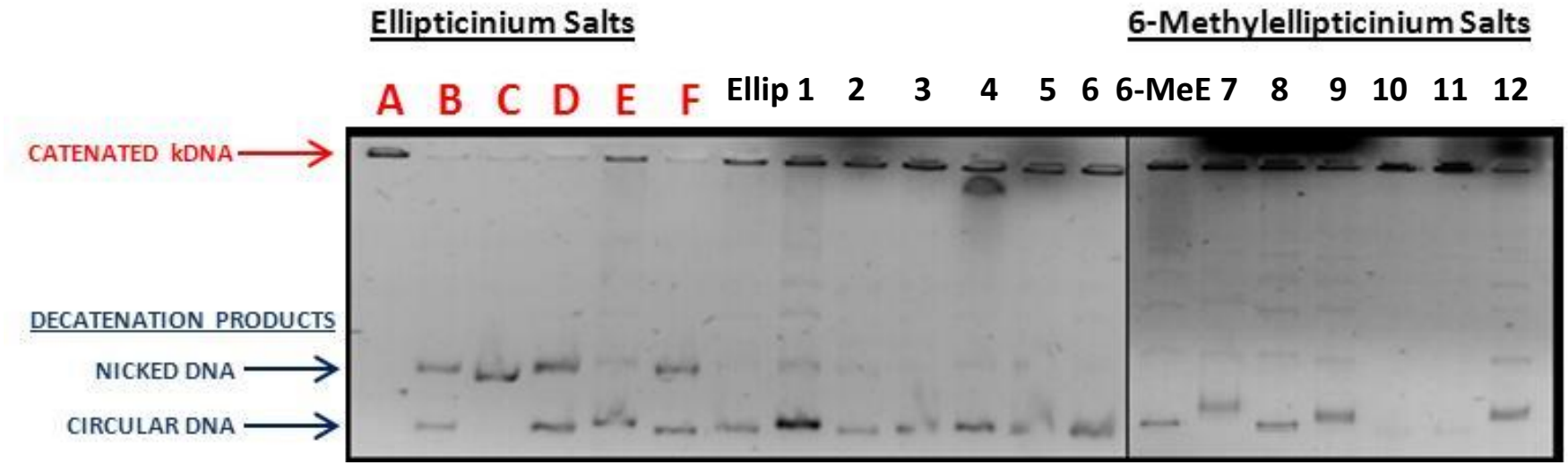
Influence of ellipticine (Ellip), 6-methylellipticine (6-Me E) and ellipticinium salts **1**–1**2** on the relaxation of plasmid DNA by topoisomerase (topo) I. Supercoiled DNA (SC DNA) was incubated without or with human topo I in the absence and presence of the test compounds which were all analysed at a final concentration of 100 µM. Control inhibitor camptothecin (CPT) was used at a final concentration of 200 µM. DNA samples were separated on agarose gel without ethidium bromide. Lanes **Ellip, 1-6** and **6-Me E**, **7-12** contain selected compounds **Ellip, 1-6** and **6-Me E**, **7-12** respectively, SC DNA and topo I. **A** = SC DNA; **B** = Relaxed DNA; **C** = SC DNA and topo I; **D** = SC DNA, topo I and CPT.

Topoisomerase Assays: Topo I Cleavage Assay



Influence of ellipticine (**Ellip**), ellipticinium salts **1**–**6**; 6-methylellipticine (**6-Me E**), and 6-methylellipticinium salts **7-12** on the relaxation of plasmid DNA by topoisomerase (topo) I. Conditions were identical to the topo I unwinding assay with the exception that DNA samples were separated on a gel containing ethidium bromide (0.5 µg/mL). Supercoiled DNA (SC DNA) was incubated without or with human topo I in the absence and presence of the test compounds which were all analysed at a final concentration of 100 µM. Control inhibitor camptothecin (CPT) was used at a final concentration of 200 µM. Gel lanes are identified by the compound number at the top of each gel. Gel flow was from top to bottom. **A** = SC DNA; **B** = Relaxed DNA; **C** = SC DNA and topo I; **D** = SC DNA, topo I and CPT.

Topoisomerase Assays: Topo II Decatenation Assay



Influence of ellipticine (**Ellip**), ellipticinium salts **1**–**6**; 6-methylellipticine (**6-Me E**), and 6-methylellipticinium salts **7-12** on DNA decatenation. All of the compounds were tested at a 100 µM final concentration. ATP was added to all reactions containing topo II and DNA samples were separated on a 1% agarose gel containing ethidium bromide (0.5 µg/mL). Gel lanes are identified by the compound number at the top of each gel. Gel flow was from top to bottom. **A** = Catenated kDNA (Cat DNA); **B** = Decatenated kDNA (Decat DNA); **C** = Linearized kDNA (Lin DNA); **D =** Cat DNA, ATP and topo II; **E =** Cat DNA, ATP, topo II and ellipticine; **F =** Cat DNA, ATP, topo II and VP-16.

Results and discussion

- The topo I unwinding assay primarily examines the DNA binding affinities of the derivatives. Ellipticine behaved as a typical intercalator and showed non-specific inhibition of topo I, producing a band similar to SC DNA. Derivatives **1**, **2**, **5** and **6** displayed the same DNA band formation suggesting they too intercalate with DNA

- Topo I poisons such as camptothecin stabilise the DNA-topo I intermediate resulting in an increase in the nicked DNA product. When tested at 100 µM drug concentration none of the analogues formed a second DNA band which corresponded to nicked DNA. This suggests that the derivatives are acting as non-specific topo I inhibitors and not topo I poisons

- The ability to inhibit topo II was examined using a decatenation assay. Ellipticine only modestly promotes the formation of more cleavage intermediates but it is able to strongly inhibit the decatenation reaction while intercalated to DNA. The derivatives, when compared to ellipticine, retained their potency and at least partly were able to inhibit the decatenation reaction. Compounds **10** and **11** were found to fully inhibit the reaction and also strongly bound to DNA in the topo I relaxation assay

- These findings suggest that ellipticine salts do not act as a topoisomerase poisons but are capable of interacting with DNA, with certain derivatives displaying complete inhibition of cleavage

Cytotoxic Assay

- The panel were then subjected to a cytotoxic screen, assessing activity against the following cell lines, which are used routinely in antiproliferative screening: HT29, SW480 (colon); MCF-7 (breast); A2780 (ovarian); H460 (lung); A431 (skin); DU145 (prostate); BE2-C (neuroblastoma); SJ-G2, U87 (glioblastoma); MIA (pancreas) and SMA (murine glioblastoma).

- Analogues were initially screened at a 25 µM drug concentration and those that inhibited growth by ≥ 90% in 12 cell lines or had specific activity progressed to a full dose response screening and GI_{50} determination.

- GI_{50} values were established by examining the difference between the optical density values on day one and those at the end of the drug exposure period.

- Ellipticine did not progress to the second stage of screening and as a result the values from the first stage of screening are included as percentage growth inhibition.

- Ellipticine derivatives **2**, **5**, **8**, **10**, **11** and **12** progressed to the second stage of testing, as well as known anticancer agents irinotecan and etoposide.

Cytotoxic screening results

Entry	HT29[a]	SW480[a]	MCF-7[b]	A2780[c]	H460[d]	A431[e]	DU145[f]	BE2-C[g]	SJ-G2[h]	MIA[i]	SMA[j]	U87[h]
CPT-11	9.3±0.4	18±2.6	5.0± 0.0	1.0±0.0	3.3±0.9	3.2±0.4	1.5±0.2	1.5±0.1	1.5±0.0	9.2±0.4	2.9±0.4	15±3
VP-16	4.4±0.9	2.7±0.8	2.9±0.4	0.04±0.00	0.16±0.00	0.64±0.20	0.4±0.03	0.79±0.06	0.43±0.10	0.7±0.2	0.18±0.0	5.2±1.1
Ellip	*88±1*	*60±2*	*44 ± 7*	*69±3*	*>100*	*40±4*	*6±7*	*50±3*	*45±3*	*13±6*	*60±8*	*41±12*
2	2.0±0.1	2.1±0.1	2.4±0.3	1.6±0.1	1.6±0.03	2.3±0.5	2.5±0.4	2.1±0.2	2.2±0.3	2.4±0.3	0.67±0.12	2.3±0.3
5	3.7±0.3	6.5±0.7	1.4±0.4	2.4±0.2	4.5±0.3	6.2±0.9	4.4±1.0	>50	13±2.2	2.9±0.1	14±2.5	8.5±1.0
6-Me Ellip	0.51±0.02	0.57±0.03	0.90±0.000	0.43±0.04	0.50±0.03	0.62±0.02	0.78±0.03	0.54±0.05	0.69±0.10	0.64±0.03	0.47±0.05	0.58±0.08
8	8.3±1.4	8.3±1.1	9.3±1.4	6.9±0.5	7.4±0.9	10±0.7	9.4±0.9	7.4±0.4	10±0.9	9.5±0.8	7.6±0.8	9.2±0.6
10	7.6±0.7	12±1.2	4.1±0.0	4.8±0.2	4.8±0.6	13±4.1	6.4±1.2	33±0.0	16±4.3	4.0±0.03	28±3.3	11±0.3
11	2.2±0.3	4.3±0.2	1.3±0.1	1.4±0.1	1.7±0.2	3.9±0.1	3.5±0.2	16±2.3	11±2.1	2.3±0.03	10±2.3	3.8±0.2
12	11± 0.3	12±0.2	11±0.6	10±0.4	11±0.7	17±0.7	12±0.3	14±1.3	21±2.9	13±0.7	17±0.7	15±1.2

The effect of ellipticine (**Ellip**), 6-methylellipticinine **(6-Me Ellip)**, ellipticinium salts **2** and **5** and 6-methylellipticinium salts **8** and **10-12** on the growth inhibition of a panel of cancer cell lines. Growth inhibition values are in µM (GI_{50}) and for **Ellip** only the percentage growth inhibition at 25 µM drug concentration is displayed in *italics.* Irinotecan (CPT-11) and etoposide (VP-16).

Human Cancer cell types: [a] Colon; [b] breast; [c] ovarian; [d] lung; [e] skin; [f] prostate; [g] neuroblastoma; [h] glioblastoma; [i] pancreas; and [j] murine glioblastoma.

Cytotoxic screening results

Entry	HT29[a]	SW480[a]	MCF-7[b]	A2780[c]	H460[d]	A431[e]	DU145[f]	BE2-C[g]	SJ-G2[h]	MIA[i]	SMA[j]	U87[h]
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VP-16	4.4±0.9	2.7±0.8	2.9±0.4	0.04±0.00	0.16±0.00	0.64±0.20	0.4±0.03	0.79±0.06	0.43±0.10	0.7±0.2	0.18±0.0	5.2±1.1
Ellip	88±1	60±2	44 ± 7	69±3	>100	40±4	6±7	50±3	45±3	13±6	60±8	41±12
2	**2.0±0.1**	**2.1±0.1**	**2.4±0.3**	**1.6±0.1**	**1.6±0.03**	**2.3±0.5**	**2.5±0.4**	**2.1±0.2**	**2.2±0.3**	**2.4±0.3**	**0.67±0.12**	**2.3±0.3**
5	3.7±0.3	6.5±0.7	1.4±0.4	2.4±0.2	4.5±0.3	6.2±0.9	4.4±1.0	>50	13±2.2	2.9±0.1	14±2.5	8.5±1.0
6-Me Ellip	**0.51±0.1**	**0.57±0.1**	**0.90±0.00**	**0.43±0.1**	**0.50±0.1**	**0.62±0.1**	**0.78±0.1**	**0.54±0.1**	**0.69±0.1**	**0.64±0.1**	**0.47±0.1**	**0.58±0.1**
8	8.3±1.4	8.3±1.1	9.3±1.4	6.9±0.5	7.4±0.9	10±0.7	9.4±0.9	7.4±0.4	10±0.9	9.5±0.8	7.6±0.8	9.2±0.6
10	7.6±0.7	12±1.2	4.1±0.0	4.8±0.2	4.8±0.6	13±4.1	6.4±1.2	33±0.0	16±4.3	4.0±0.03	28±3.3	11±0.3
11	**2.2±0.3**	**4.3±0.2**	**1.3±0.1**	**1.4±0.1**	**1.7±0.2**	**3.9±0.1**	**3.5±0.2**	**16±2.3**	**11±2.1**	**2.3±0.03**	**10±2.3**	**3.8±0.2**
12	11± 0.3	12±0.2	11±0.6	10±0.4	11±0.7	17±0.7	12±0.3	14±1.3	21±2.9	13±0.7	17±0.7	15±1.2

Ellipticinium salt **2**, 6-methylellipticinium salt **11** and 6-methylellipticine had the most potent GI_{50} values of any of the derivatives tested, with activity that is comparable to known anticancer agents irinotecan (CPT-11) and etoposide (VP-16).

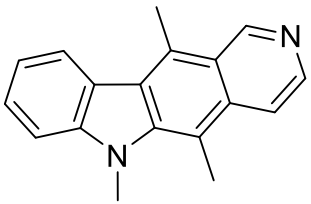
Human Cancer cell types: [a] Colon; [b] breast; [c] ovarian; [d] lung; [e] skin; [f] prostate; [g] neuroblastoma; [h] glioblastoma; [i] pancreas; and [j] murine glioblastoma.

Cytotoxic screening results

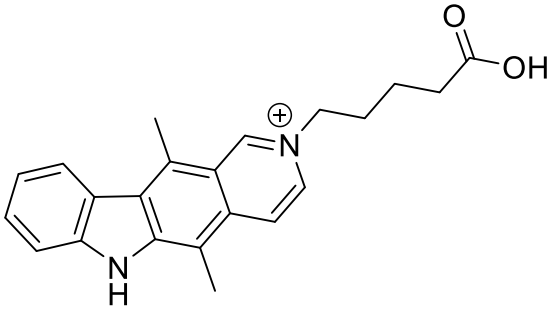
- Ellipticine did not progress to the second phase of testing, while known antitumour agents irinotecan and etoposide did. However, a number of functionalised ellipticine derivatives displayed better activity than these known compounds in the screen.

- 6-Methylellipticine was identified as the most potent derivative screened, with GI_{50} values of 0.43 µM for A2780 and 0.47 µM for SMA.

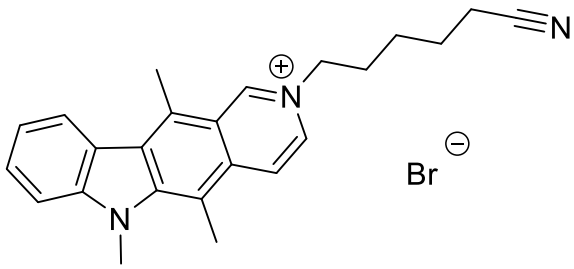
- A number of other long chain derivatives displayed activity at 1 micromolar concentration, highlighting the activity of the panel.



6-Methylellipticine



2



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Conclusions

- A panel of novel ellipticinium and 6-methylellipticinium salts were generated, with side chains at the 2-position
- Derivatives with strong DNA binding affinities were identified and the relationships of the derivatives and topo I and II was examined
- Results from cytotoxic screening identified 6-methylellipticine as the most potent derivative, but a number of the salt derivatives showed micromolar activity
- Future work will see the expansion of substitution at the 2- and 6-position, to further explore the effect of functionalisation
- The introduction of substituents at the 9-position of the pharmacophore will be trialled with the aim of further enhancing biological efficacy

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