Isoellipticine: targeting cell proliferation by a structured approach

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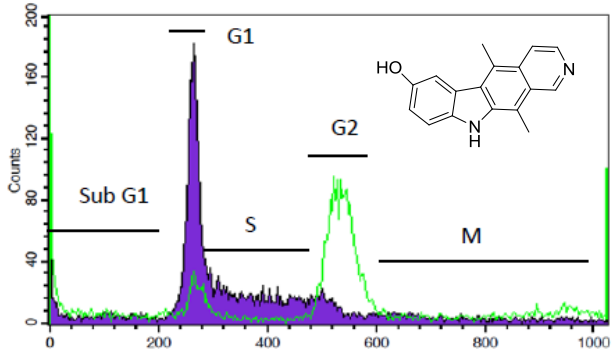
- http://publish.ucc.ie/researchprofiles/D004/fmccarthy



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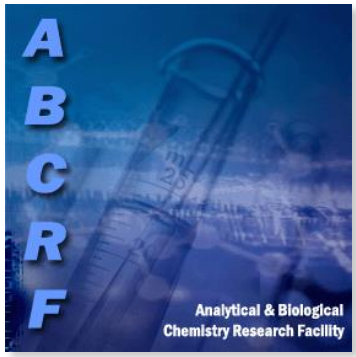

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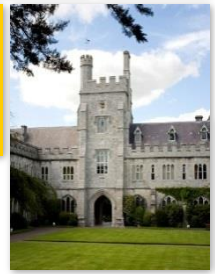
UNIVERSITY COLLEGE CORK

- ★ **UCC** was founded in 1845
- ★ **UCC** is an international university in the heart of Ireland's Energy, Food, Pharmaceutical and Software ICT industries
  - Eight of the world's top 10 pharmaceutical companies and Ireland's top 4 food companies are located in Munster
  - 25% of national energy needs are produced in Cork
- ★ **UCC** is host to Ireland's largest ICT institute – The Tyndall Institute
- ★ **UCC** supports regional development
- ★ **UCC** works closely with Irish and International agencies to help industry to access its world class resources



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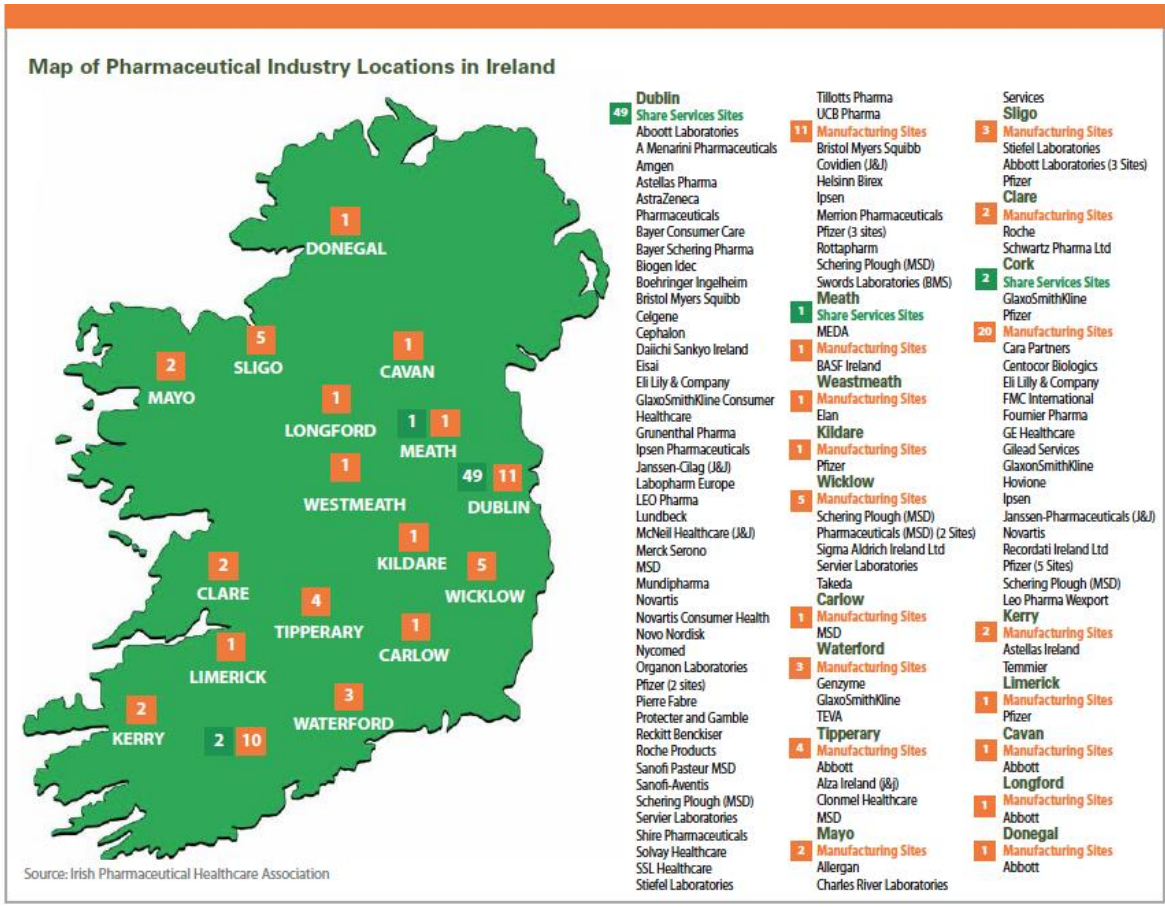
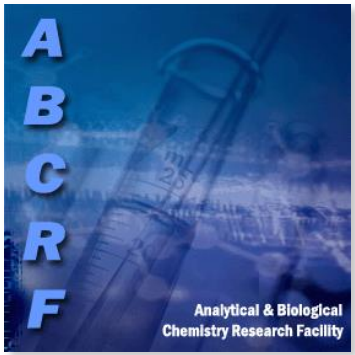
Pharma Industry – Irish context

- >120 overseas companies
- 9 of the 10 largest pharma
- Largest net exporter of pharmaceuticals in the EU – accounts for >50% of our exports

- Diversifying the nature of investment
  - Away from original bulk active plants
  - Towards higher value activities

- Maintaining a culture of innovation
- Drug Discovery





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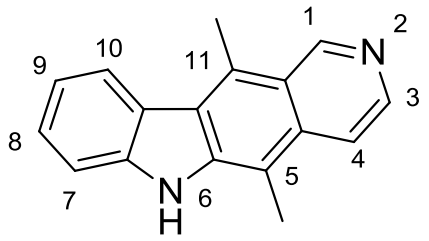
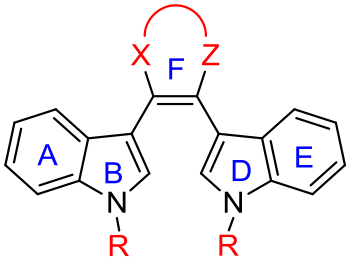
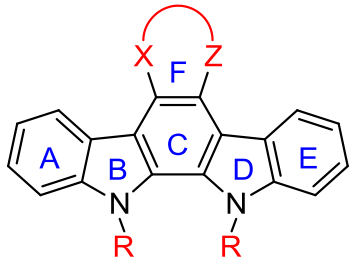
MuTaLig Specific Research Areas

- Ellipticine & Isoellipticine

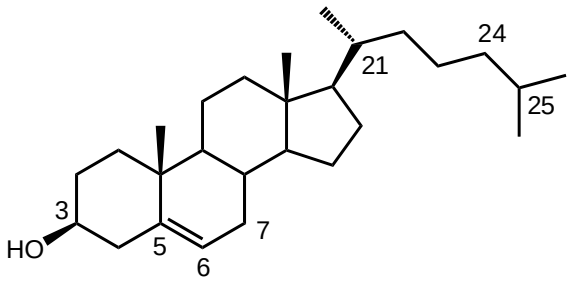


- Bisindolylmaleimide & Indolocarbazole
  - Novel heterocycles
  - Topo I inhibition
  - Excellent antiproliferative activity
  - 10nM kinase inhibition

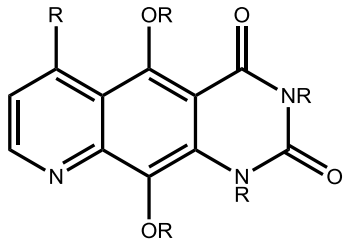




- Cholesterol & Phytosterols
  - Oxidation products
  - Food industry

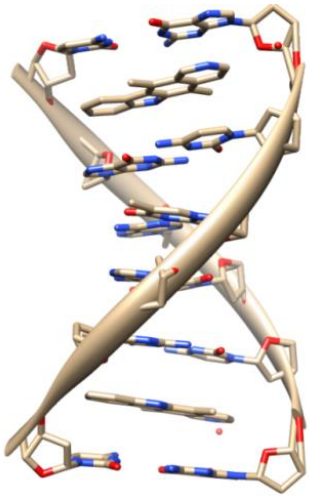


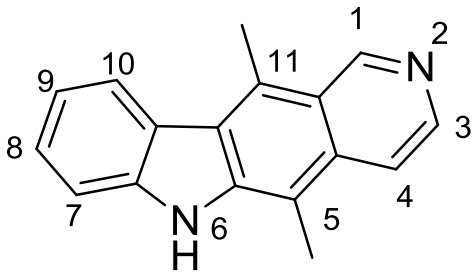
- Quinazolines and Quinolones
  - SK1/HIV integrase

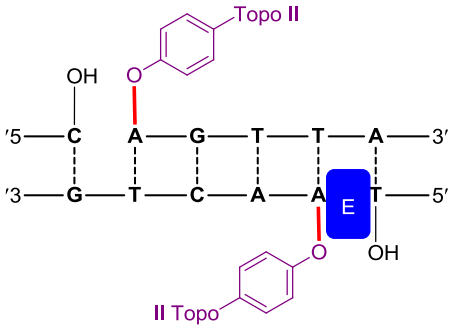


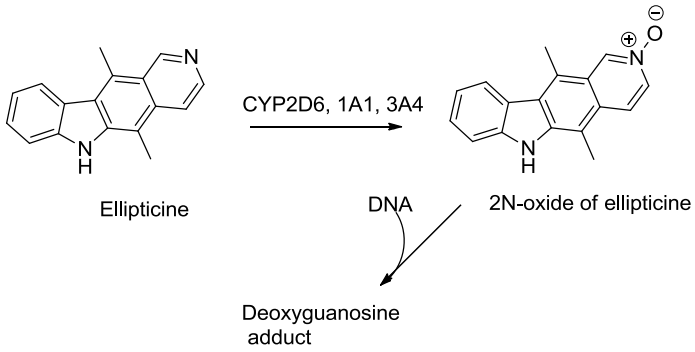
Ellipticine

- Isolated from leaves of *Oschrosia Elliptica* Labill in 1959
- Potent anti-tumour effects









- First established modes of action were intercalation, topoisomerase II inhibition and formation of cytotoxic adducts with DNA

Goodwin, S.; Smith, A.F.; Horning E.C. *Journal of the American Chemical Society* **1959,** *81,* (8)1903-1908
O'Sullivan, E.C.; Miller, C.M.; Deane, F.M.; McCarthy F.O. *Studies in Natural Products Chemistry* **2013**, *39*, (6) 189-232

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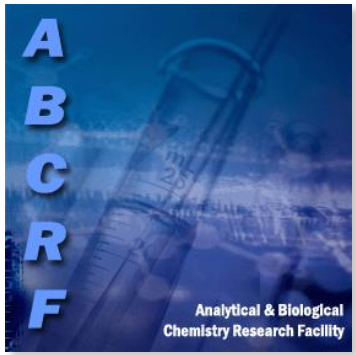

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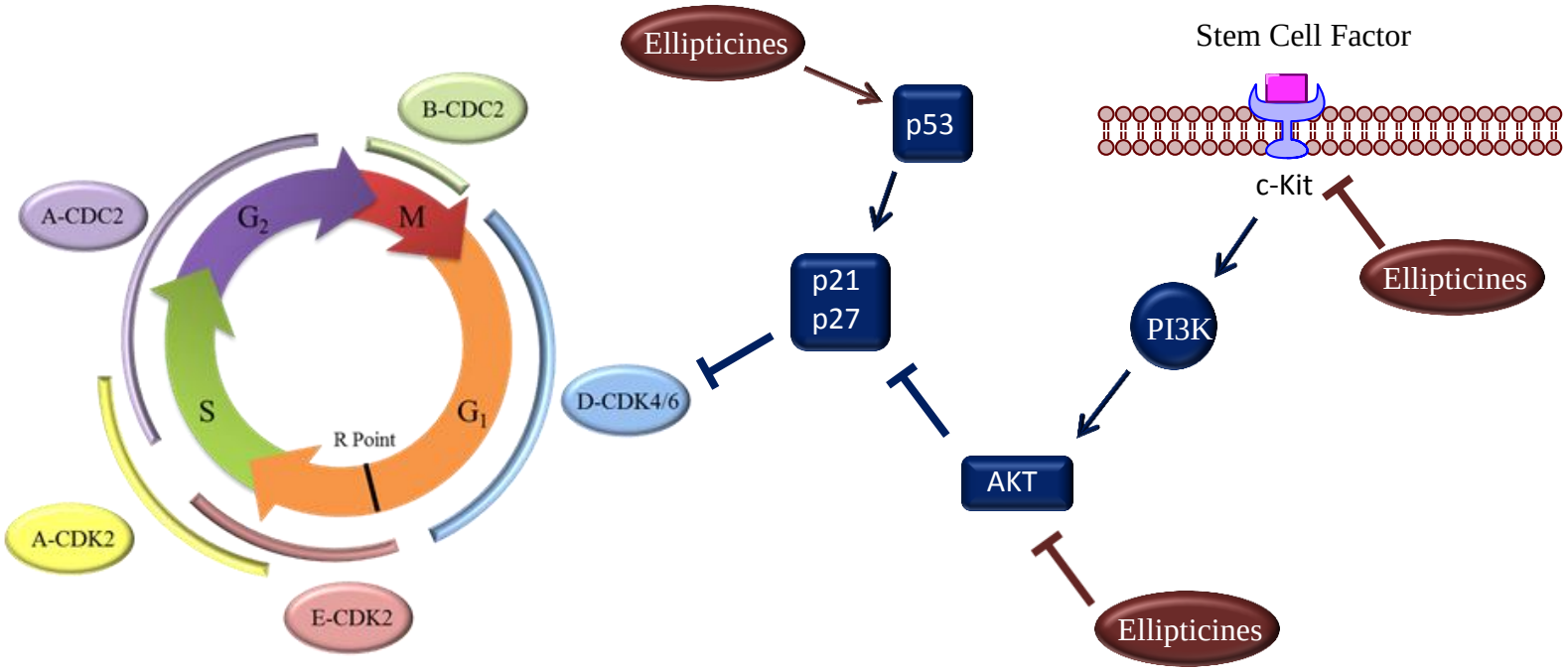
Ellipticine 1990 – present: Old drug, new targets



- Recently, Ellipticines shown to affect the regulation of the cell cycle, including restoration of function to mutant p53 tumour suppressor protein.
- Inhibition AKT kinase and wild-type and mutant c-Kit kinase is reported
- Latterly, reported mechanisms of action include uncoupling of oxidative phosphorylation, activation of the mitochondrial pro-apoptotic pathway and inhibition of RNA polymerase 1

O'Sullivan, E.C.; Miller, C.M.; Deane, F.M.; McCarthy F.O. *Studies in Natural Products Chemistry* **2013**, *39*, (6) 189-232.
Andrews, W.J.; Panova, T.; Normand, C.; Gadal, O.; Tikhonova, I.G.; Panov, K.I. *Journal of Biological Chemistry* **2013**, (288) 4567-4582

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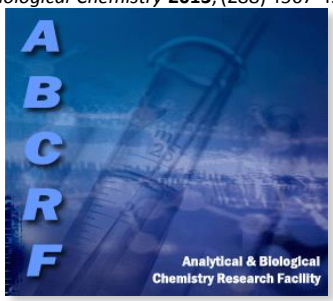

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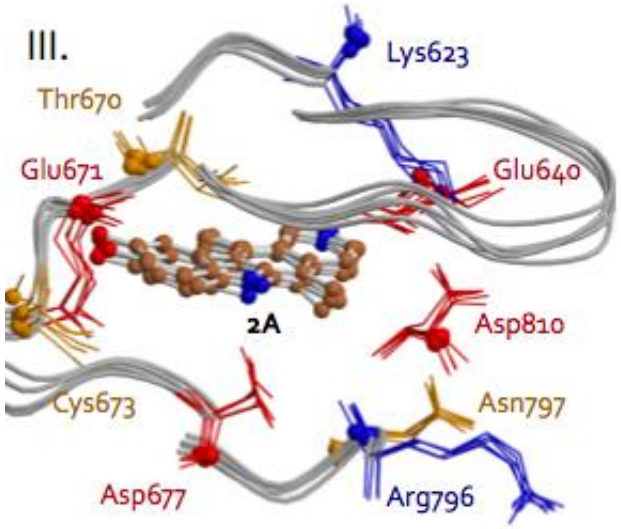
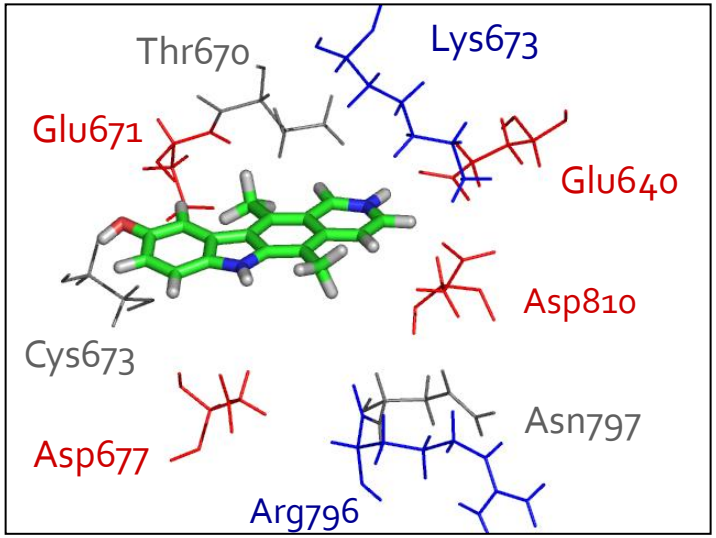
Molecular Modelling – creating a rationale

Molecular Dynamics simulations of the binding mode of 9-hydroxyellipticine in the c-Kit kinase active site.

- Five different orientations of 9-hydroxyellipticine were investigated.
- Two nanoseconds of molecular dynamics were performed for each complex.
- New binding mode proposed.



Five snapshots of the c-Kit active site at 20 ps intervals from the last 80 ps of the MD trajectory.



Major hydrogen bonding interactions:

Protonated N-2:Glu640 9-Hydroxy group:Glu671

D. Thompson, C. Miller, F. O. McCarthy, *Biochemistry,* **2008**, 47, 10333-10344

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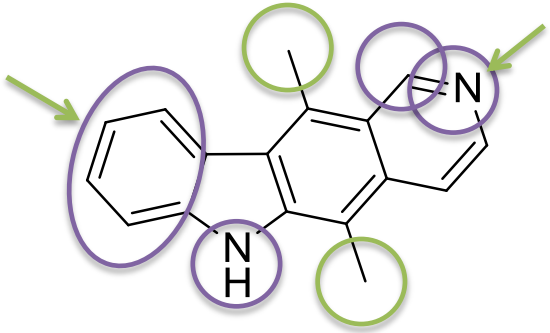
Novel Potential Drugs?

- Isoellipticine is a synthetic isomer of ellipticine which displays promising anti-cancer activity but has been subject to little investigation.
- Aim to increase potency and selectivity by substituent modification on the isoellipticine template, specifically at position 2, 7 and 10.

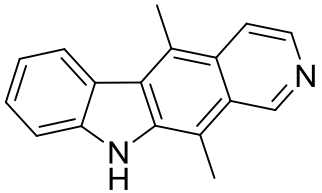


Previous work focused on:

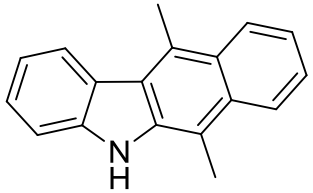
- A-ring substitution – particularly C-9
- *N*-2 Ellipticinium salts
- *N*-6 Substitution
- C-1 Substitution

New areas for investigation:

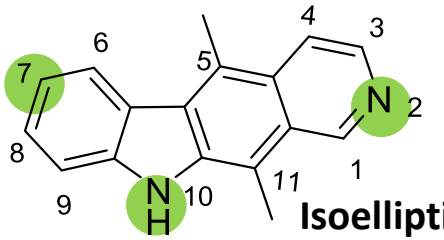
- Novel A/D-rings
- C-11 Substitution
- C-5 Substitution
- Ellipticine Analogues



Isoellipticine



Deazaellipticine



Isoellipticine

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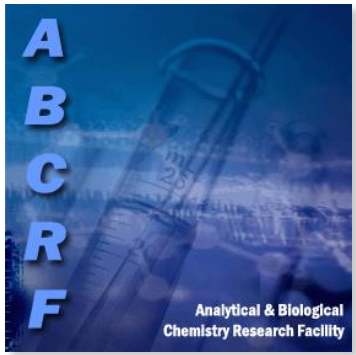

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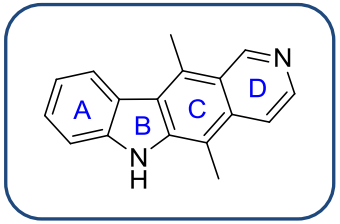
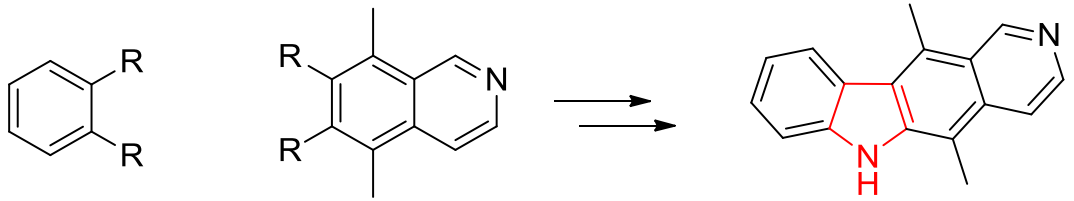
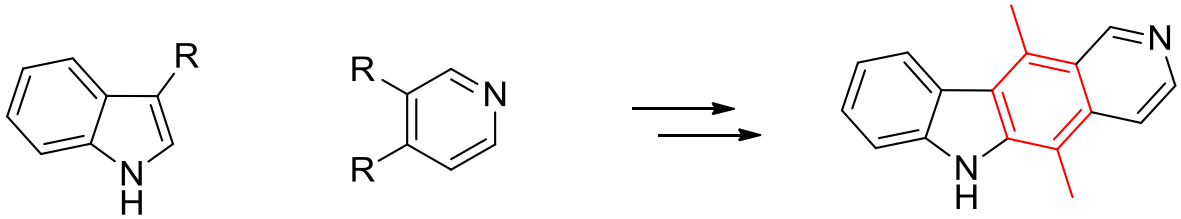
Synthesis of Ellipticines



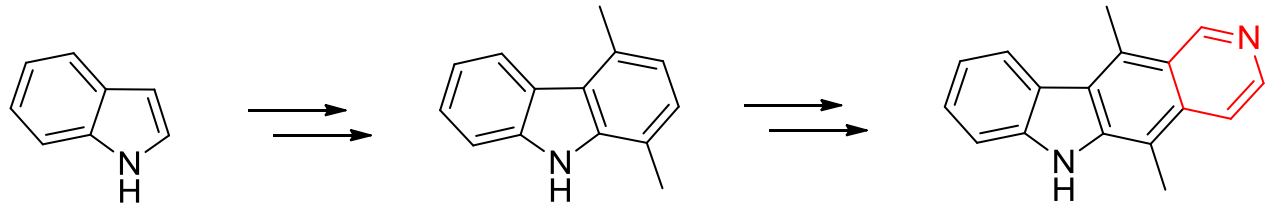
B-Type



C-Type



D-Type



C. M. Miller and F. O. McCarthy. RSC Adv. 2012, **2**, 8883-8918
F.M. Deane, E.C. O'Sullivan, A.R. Maguire, J. Gilbert, J.A. Sakoff, A. McCluskey, F.O. McCarthy. *Org. Biomol. Chem*., 2013,**11**, 1334-1344
F.M. Deane, C.M. Miller, A.R. Maguire, F.O. McCarthy. *J. Het Chem.* 2011, **48 (4)**, 814–823
C.M. Miller, E.C. O'Sullivan, K.J. Devine and F.O. McCarthy. *Org. Biomol. Chem.,* 2012,**10**, 7912-7921

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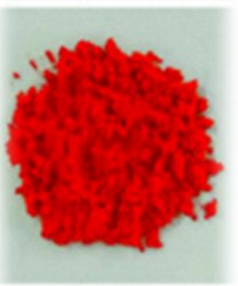
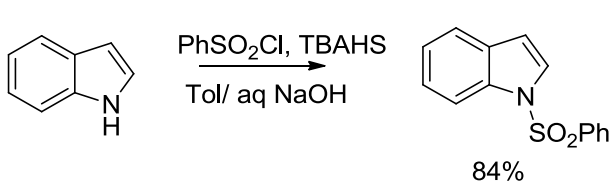
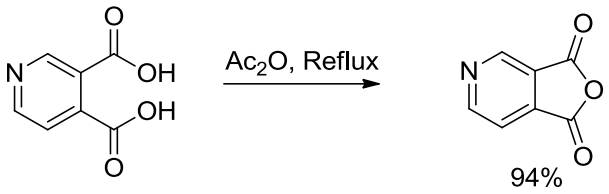
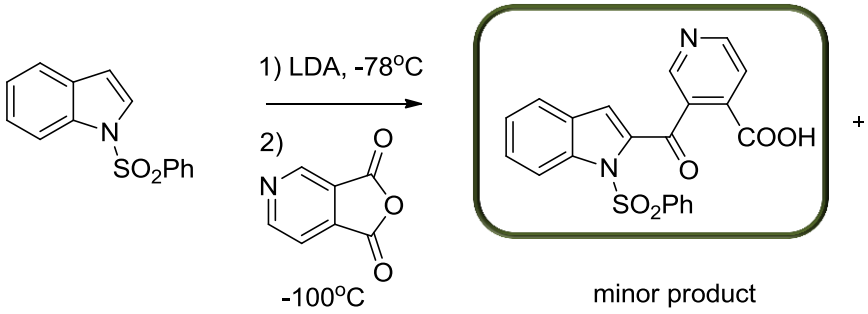
Isoellipticine synthesis

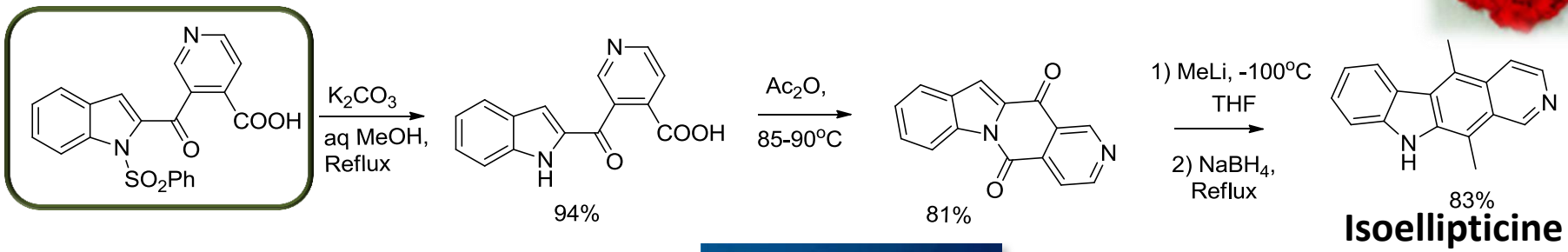






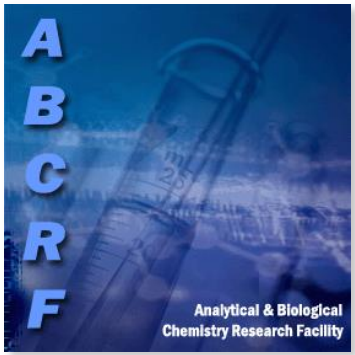


Total yield 79% (4:1 ratio major to minor isomer)



Ellipticine

Isoellipticine



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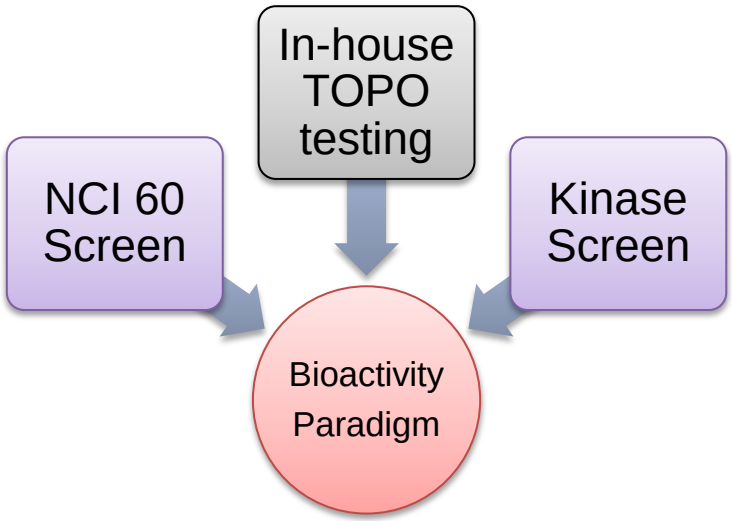
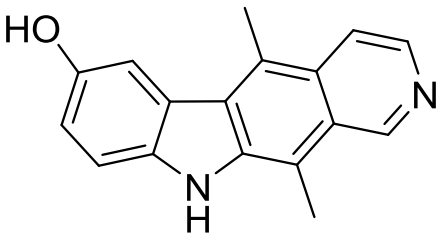
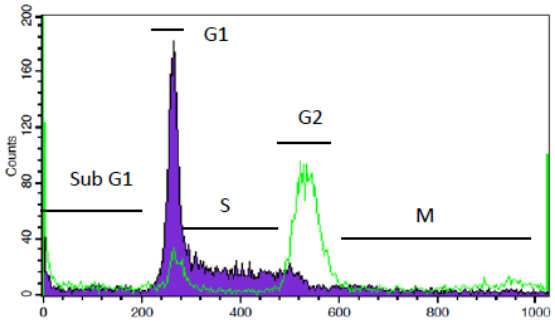
Biological evaluation of novel isoellipticines

- Biological evaluation follows a predetermined programme beginning with cellular antiproliferative activity as measured at the NCI 60 cell line screen.
- Active compounds are profiled for Topoisomerase I and II inhibition.
- Active compounds are profiled for kinase inhibition in collaboration.







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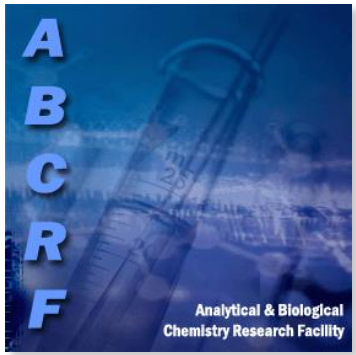

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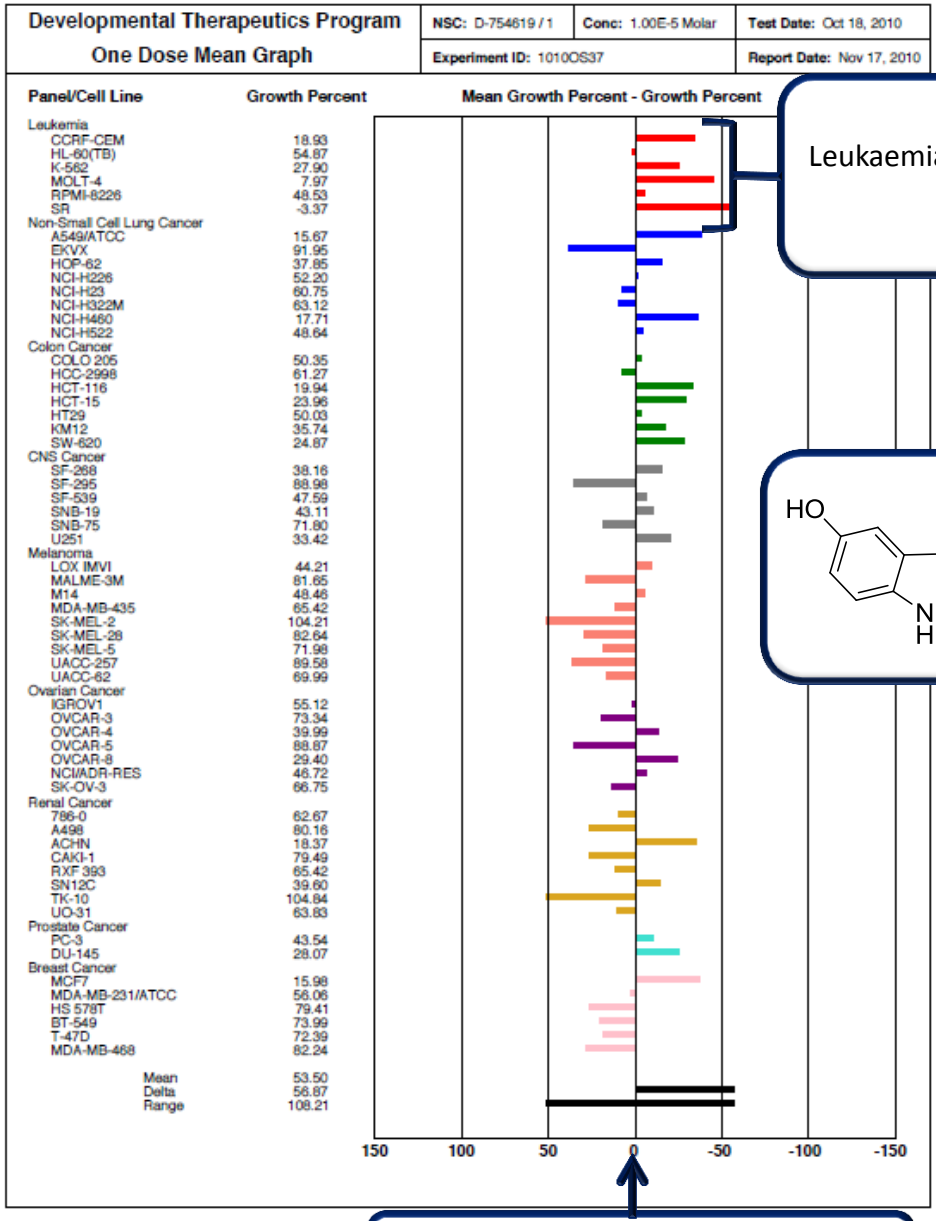
NCI screening – One dose

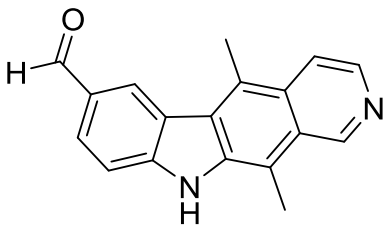
- Initial screening comprises testing *in vitro* against a panel of 60 cancer cell lines at a concentration of 10µM.
- Must satisfy inhibition threshold in a minimum number of cell lines to progress to 5 dose.
- Over 50 of our ellipticine derivatives have been brought forward for five-dose screen and tested against the cell line panel at concentrations ranging from 100 µM to 10 nM.
- Dose-response curves are generated for each cell line.Three characteristic *in vitro* parameters, GI_{50}, TGI and LC_{50}, are calculated for each cell line in response to the presence of the different drug candidates.



NCI 60-Cell Line Screen

1st Generation Compounds

- Single dose (10 μM)



7-Formylisoellipticine

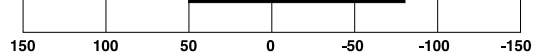
Mean Growth % = 51.47

Developmental Therapeutics Program — One Dose Mean Graph

NSC: D-754620 / 1 | Conc: 1.00E-5 Molar | Test Date: Oct 18, 2010

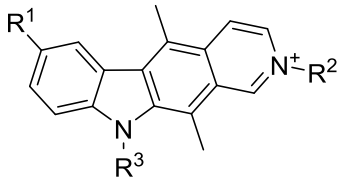
Experiment ID: 1010OS37 | Report Date: Nov 17, 2010

Panel/Cell Line	Growth Percent
Leukemia	
CCRF-CEM	11.07
HL-60(TB)	23.54
K-562	54.44
MOLT-4	-2.46
RPMI-8226	11.10
SR	-28.84
Non-Small Cell Lung Cancer	
A549/ATCC	81.69
EKVX	101.55
HOP-62	48.47
NCI-H226	58.38
NCI-H23	38.33
NCI-H322M	90.32
NCI-H460	45.56
NCI-H522	66.97
Colon Cancer	
COLO 205	80.78
HCC-2998	81.45
HCT-116	15.62
HCT-15	25.75
HT29	49.71
KM12	84.56
SW-620	31.91
CNS Cancer	
SF-268	35.01
SF-295	88.82
SF-539	45.83
SNB-19	32.07
SNB-75	66.00
U251	23.79
Melanoma	
LOX IMVI	10.80
MALME-3M	92.11
M14	39.85
MDA-MB-435	52.83
SK-MEL-2	64.50
SK-MEL-28	76.22
SK-MEL-5	58.08
UACC-257	96.84
UACC-62	34.89
Ovarian Cancer	
IGROV1	59.31
OVCAR-3	62.98
OVCAR-5	95.08
OVCAR-8	24.23
NCI/ADR-RES	44.95
SK-OV-3	61.00
Renal Cancer	
786-0	67.90
A498	62.12
ACHN	39.00
CAKI-1	92.70
RXF 393	44.71
SN12C	31.53
TK-10	83.95
UO-31	58.59
Prostate Cancer	
PC-3	39.66
DU-145	36.83
Breast Cancer	
MCF7	6.80
MDA-MB-231/ATCC	66.95
HS 578T	51.05
BT-549	64.37
T-47D	61.58
MDA-MB-468	42.73
Mean	51.47
Delta	80.31
Range	130.39



2nd Generation Compounds

- Single dose (10 μM)
- Not a simple relationship….

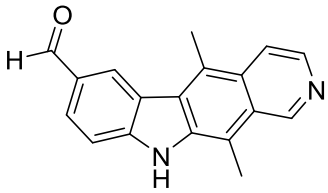


CNS Cancer	
SF-268	16.79
SF-295	13.44
SF-539	28.51
SNB-19	19.85
SNB-75	36.84
U251	11.15

C.M. Miller, E.C. O'Sullivan, K.J. Devine and F.O. McCarthy. *Org. Biomol. Chem.*, 2012,**10**, 7912-7921

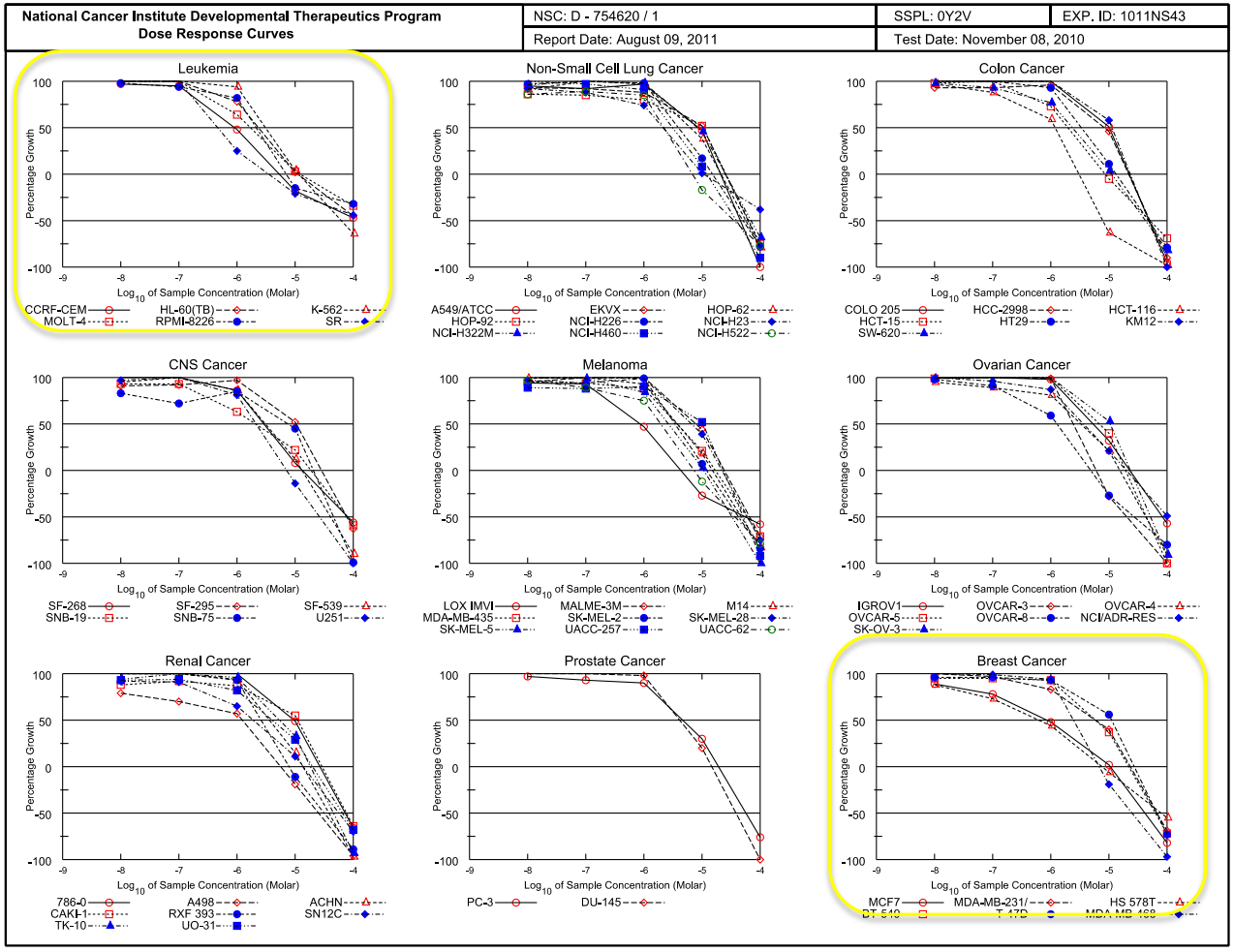
NCI 60-Cell Line Screen – Five dose

- Five dose results



7-Formylisoellipticine

Type	Line	GI_{50} (μM)
Leukaemia	CCRF-CEM	0.89
	SR	0.49
Breast	MCF-7	0.88
	HS 578T	0.24

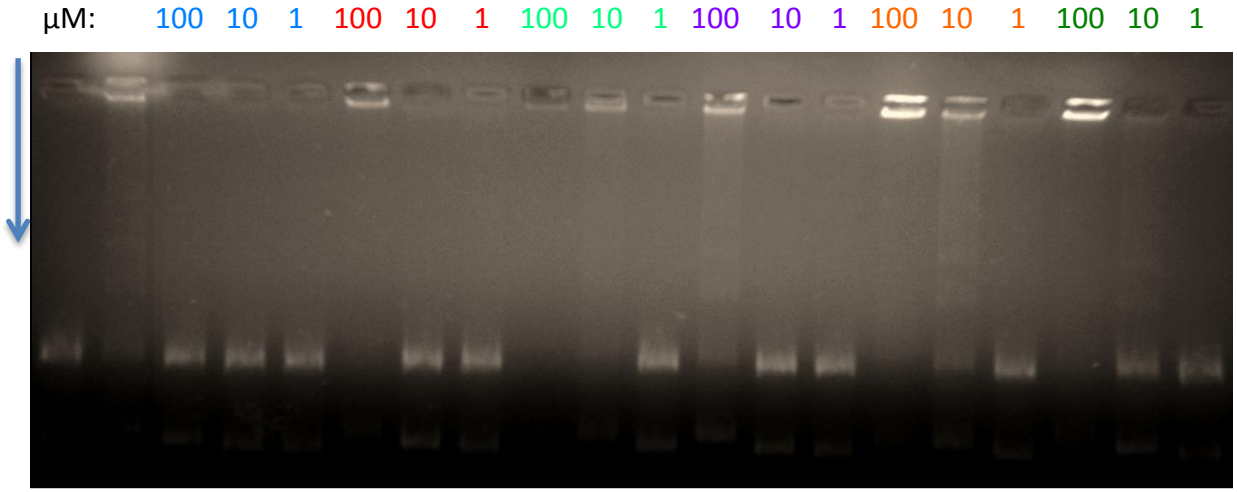


Five dose assay (10 nM, 100nM, 1 μM, 10 μM, 100 μM)

C.M. Miller, E.C. O'Sullivan, K.J. Devine and F.O. McCarthy. *Org. Biomol. Chem.*, 2012,**10**, 7912-7921

Topoisomerase II Decatenation Assay

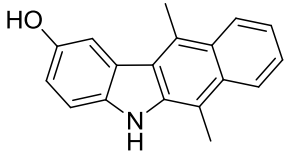
A-Ring Substituted Ellipticines and Analogues – 3-fold dilution



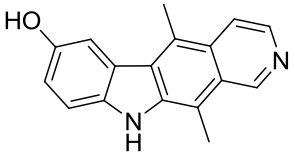
Lane	A	B	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
Activity	-	+	-	-	-	+	-	-	+	+	-	+	-	-	+	+	-	+	-	-

A, positive control = kDNA +ATP + Topo II
B, negative control = kDNA +ATP + Topo II + 100 µM ellipticine
C = dilution solvent / blank
Inhibitory activity: (-) = not active against topo II; (+) = topo II inhibition.

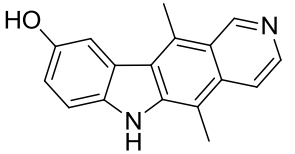
1 – 3



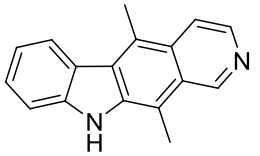
4 – 6



7 – 9



10 – 12



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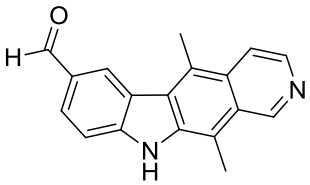
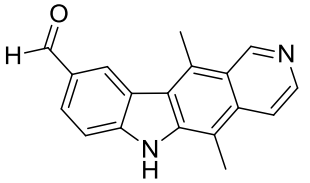


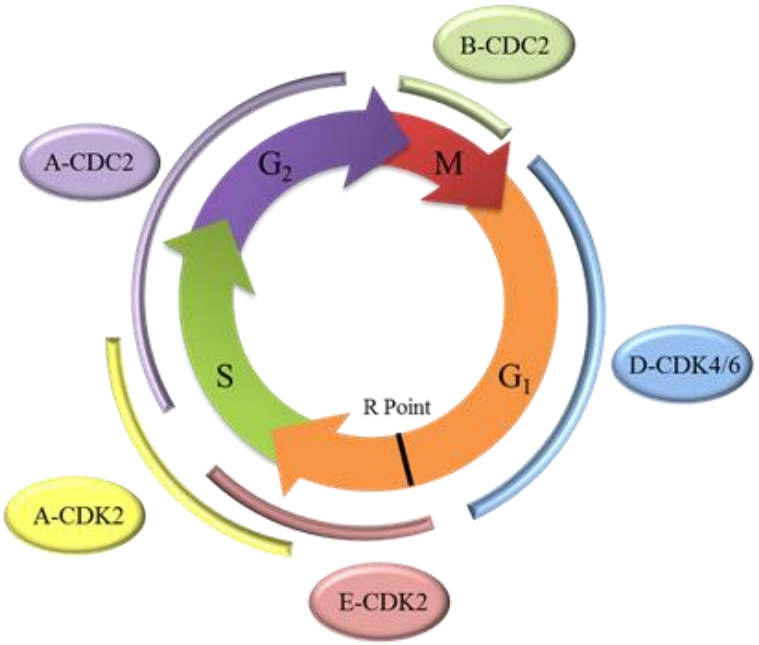
16 - 18



C.M. Miller, E.C. O'Sullivan, K.J. Devine and F.O. McCarthy. *Org. Biomol. Chem.,* 2012,**10**, 7912-7921

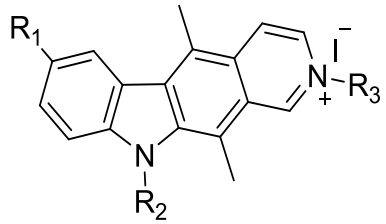
Structures for cell cycle study

- Acute myeloid leukaemia cell line MV4-11
- Flow cytometry as starting point
- Enables determination of the proportion of cells in each stage of the cell cycle after exposure to the sample compound



- 7-Formyl and 7-hydroxyl derivatives chosen as parent compounds showed selectivity for leukaemia cell lines in NCI testing.

A

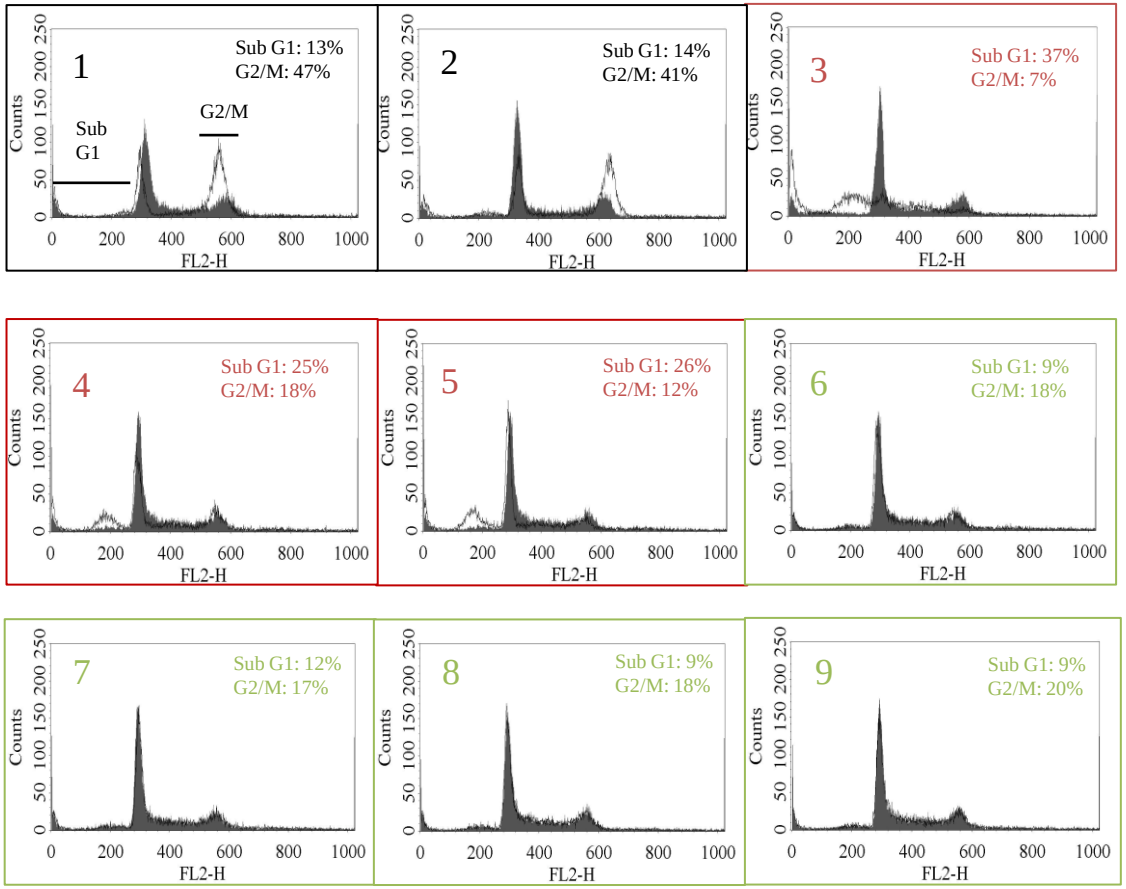


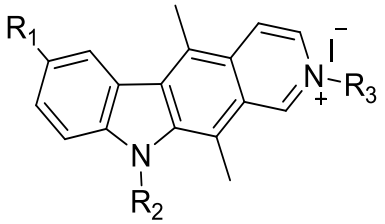
Compound	R_1	R_2	R_3
1	OH	H	-
2	OH	CH_3	-
3	CHO	CH_3	-
4	CHO	CH_2CH_3	-
5	CHO	$CH(CH_3)_2$	-
6	CHO	H	CH_2CH_3
7	OH	H	CH_3
8	OH	H	CH_2CH_3
9	CHO	H	CH_3

Structure of isoellipticine derivatives

E. G. Russell, E. C. O'Sullivan, C. M. Miller, J. Stanicka, F. O. McCarthy, T. G. Cotter. *Inv. New Drugs* (2014) **32 (6)**, 1113-1122

Flow cytometry for compounds 1-9





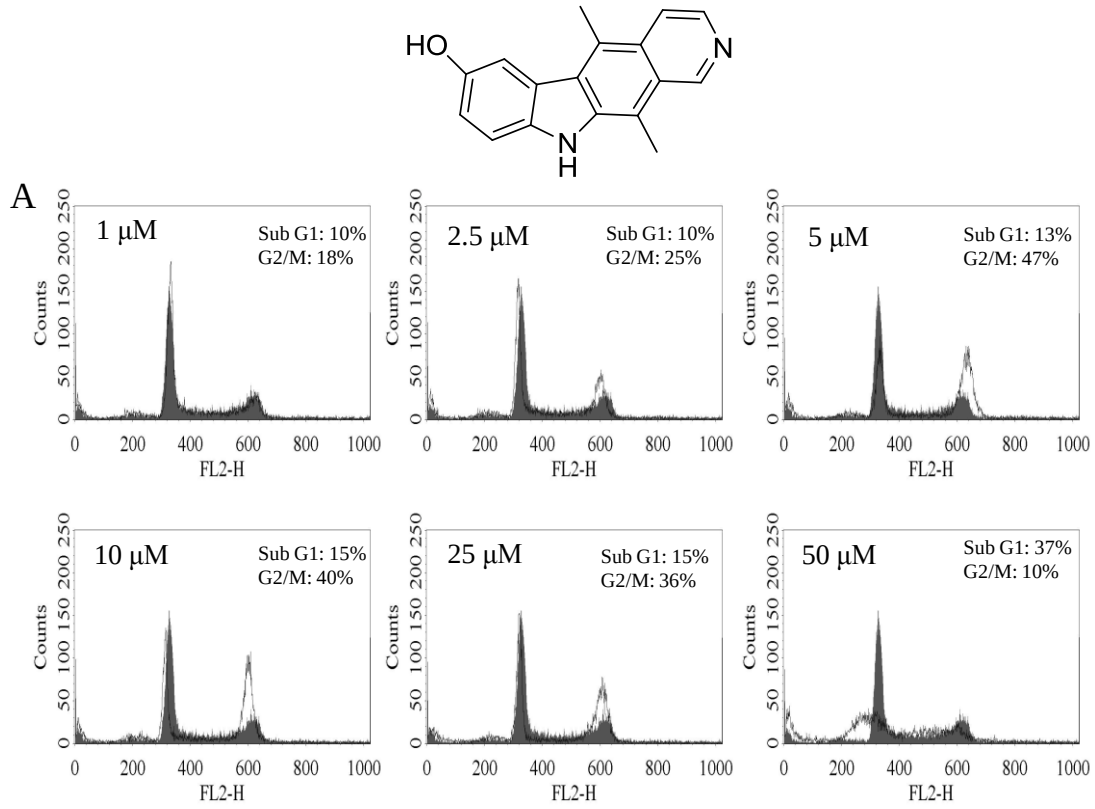
Compound	R_1	R_2	R_3
1	OH	H	-
2	OH	CH_3	-
3	CHO	CH_3	-
4	CHO	CH_2CH_3	-
5	CHO	$CH(CH_3)_2$	-
6	CHO	H	CH_2CH_3
7	OH	H	CH_3
8	OH	H	CH_2CH_3
9	CHO	H	CH_3

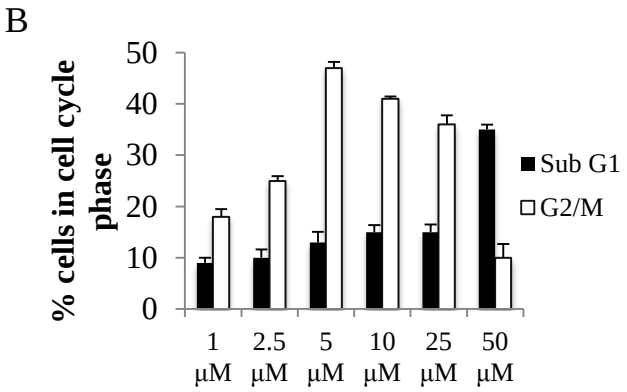
The panel of isoellipticine derivatives had contrasting effects on MV4-11 cell cycle

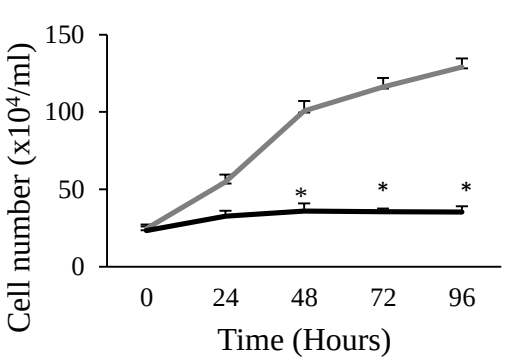
The cell cycle of MV4-11 cells incubated with 5 µM of each derivative for 24 hours was analyzed by propidium iodide staining. A representative profile is shown. Black line = 5 µM isoellipticine derivative, grey = 0.5 % DMSO control. Values represent the percentage (%) of cells in each cell cycle phase of the cells treated with isoellipticine.

E. G. Russell, E. C. O'Sullivan, C. M. Miller, J. Stanicka, F. O. McCarthy, T. G. Cotter. *Inv. New Drugs* (2014) **32 (6)**, 1113-1122

First IsoE lead: Flow cytometry of 7OH-IsoE at different concentrations



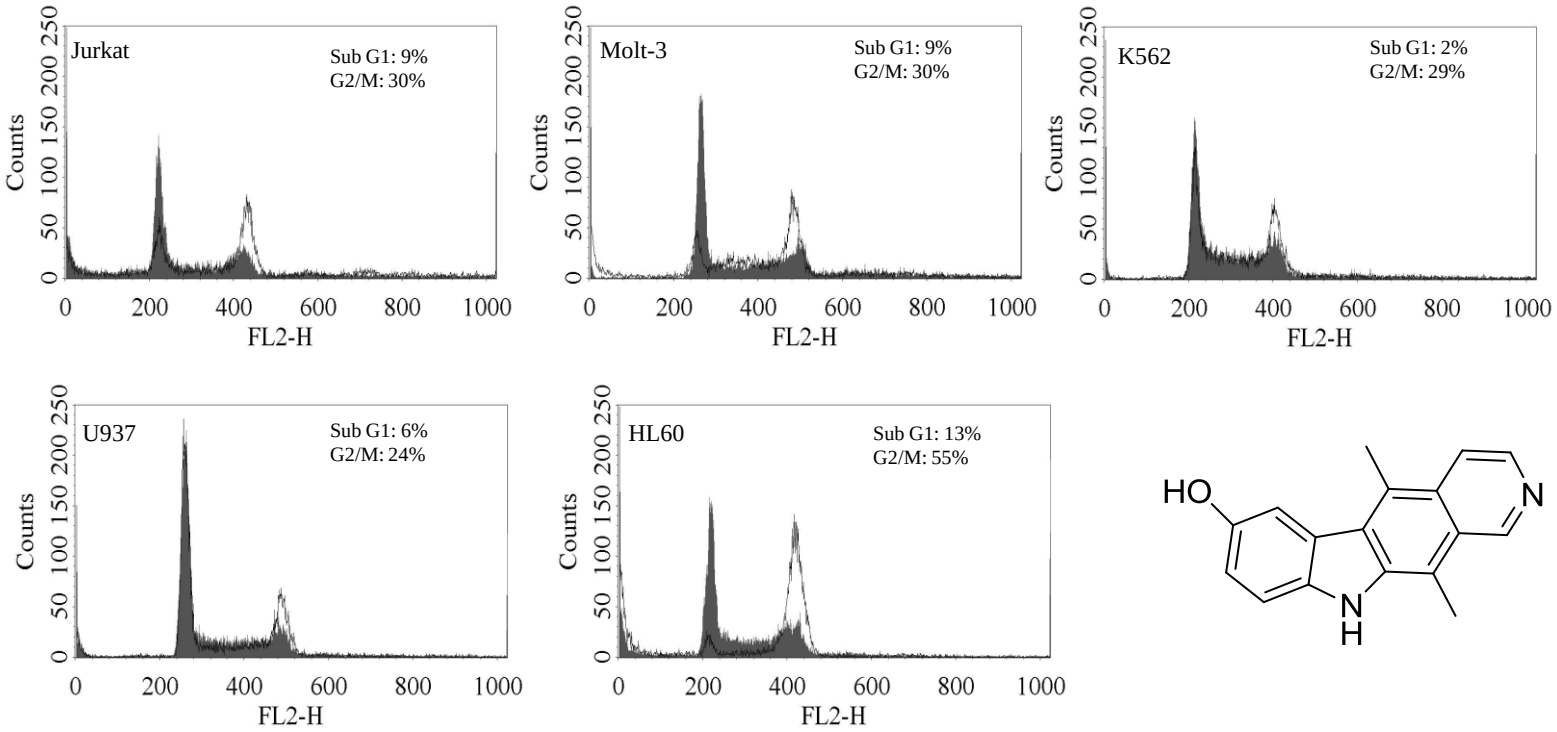




5 µM 7-hydroxyisoelliptine causes G2/M cell cycle arrest in MV4-11 cells

A. The cell cycle of MV4-11 cells incubated with different doses of 7-hydroxyisoelliptine for 24 hours was analyzed by propidium iodide staining. A representative profile is shown. Values represent the percentage (%) of cells in each cell cycle phase of the cells treated with 7-hydroxyisoellipticine. **B.** Quantification of cell cycle analysis. **C.** The effects of 5 µM 7-hydroxyisoellipticine on cell number measured by trypan blue exclusion. Black line = 5 µM 7-hydroxyisoellipticine, grey = 0.5 % DMSO control. * = p-value < 0.01. The error bars represent ±SD.

Flow cytometry against other leukaemia cell lines

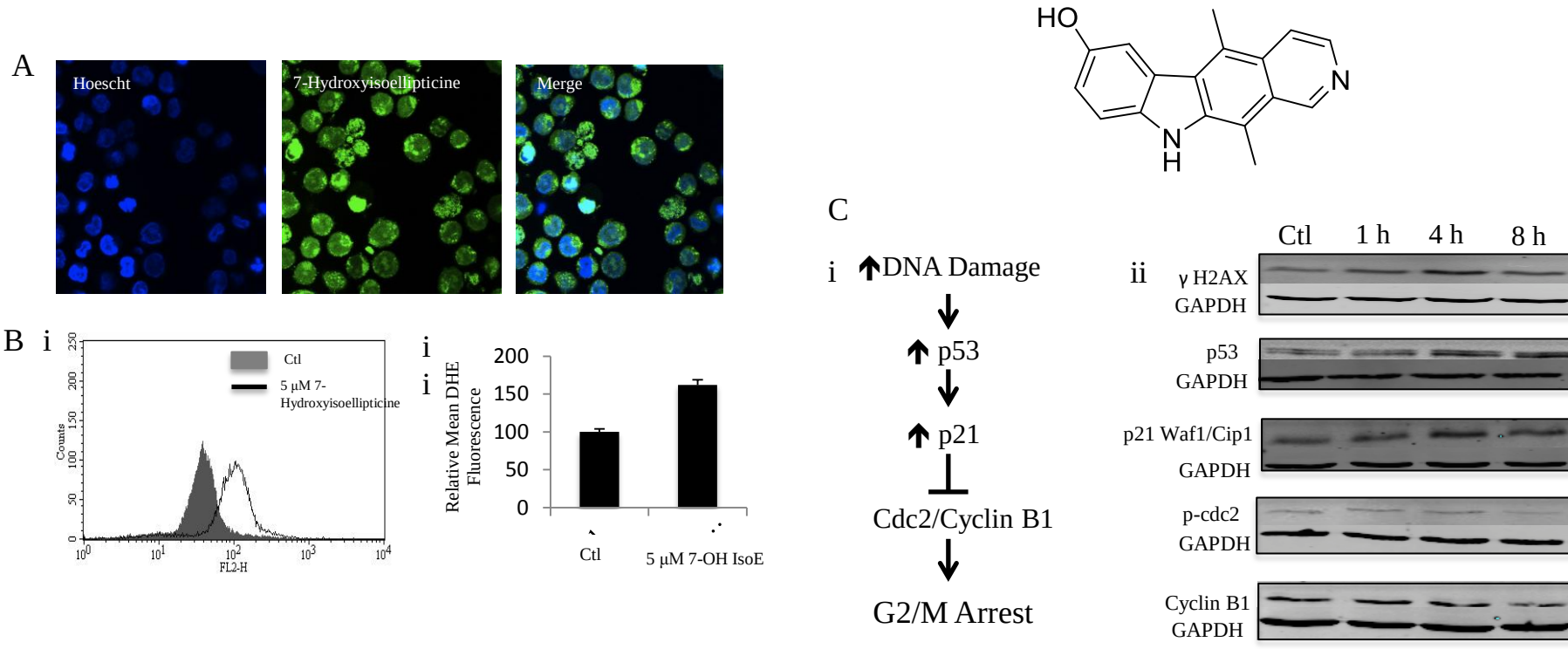


5 µM 7-hydroxyisoellipticine causes a G2/M cell cycle arrest in a number of leukaemia cell lines

The cell cycle of leukaemia cells incubated with 5 µM 7-hydroxyisoellipticine or 0.5% DMSO control for 24 hours was analyzed by propidium iodide staining of nuclei and flow cytometry. Values represent the percentage (%) of cells in each cell cycle phase of the cells treated with 7-hydroxyisoellipticine. Black line = 5 µM 7-hydroxyisoellipticine, grey = 0.5 % DMSO control.

E. G. Russell, E. C. O'Sullivan, C. M. Miller, J. Stanicka, F. O. McCarthy, T. G. Cotter. *Inv. New Drugs* (2014) **32 (6)**, 1113-1122

Distribution and mechanism of action studies



7-Hydroxyisoellipticine activates the p53 pathway and increases ROS levels

A. Cells were treated with 5 µM 7-hydroxyisoellipticine for 4 hours and imaged using Olympus FluoView FV1000-ASW confocal microscope. **B. i.** ROS levels were measured by flow cytometry using dihydroethidium. **ii.** Quantification of ROS levels. Grey = Control, black = 5 µM 7-hydroxyisoellipticine. The error bars represent ±SD. **C. i.** Schematic of the DNA damage pathway. **ii.** Western blot of γH2AX, p53, p21 Waf1/Cip1, phospho-cdc2 and cyclin B1 protein expression in MV4-11 cell treated with 0.5% DMSO control (Ctl) and with 5 µM 7-hydroxyisoellipticine at 1, 4, 8 and 24 hours respectively using GAPDH as a loading control.

Summary: G2/M cell cycle arrest of 7-OH IsoE

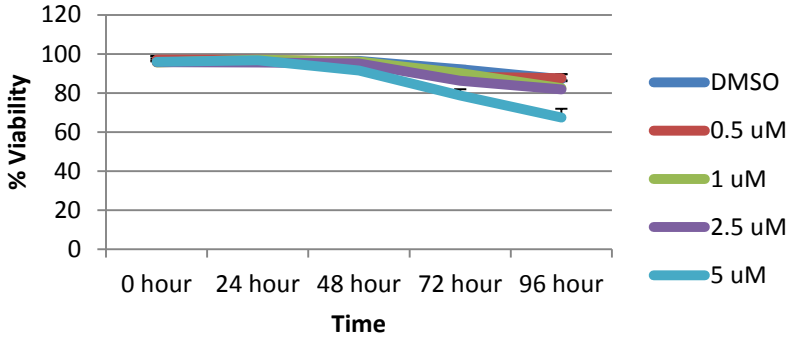


Figure 1: The effect of 7-hydroxyisoellipticine on MV4-11 viability.

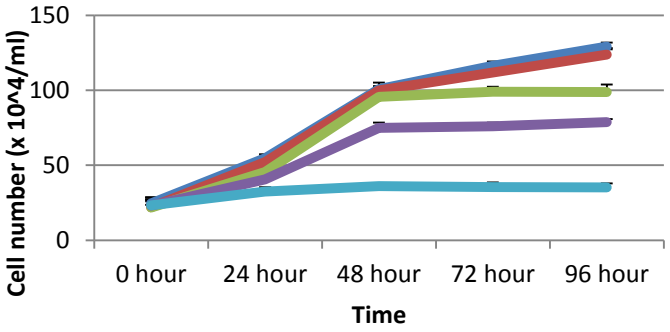
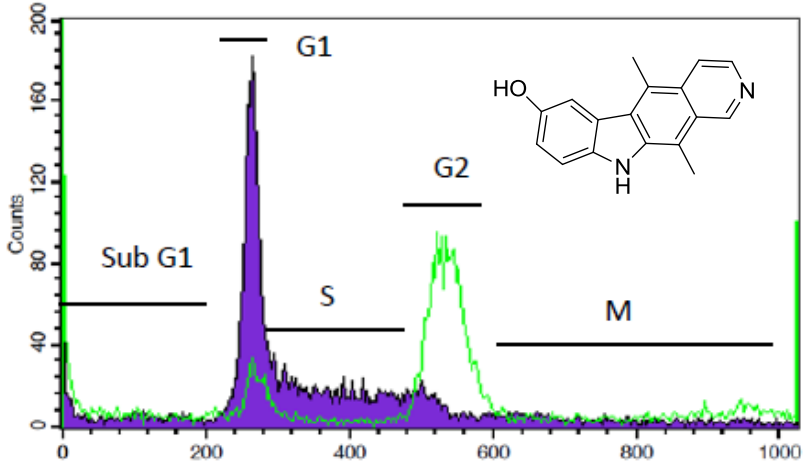


Figure 2: The effect of 7-hydroxyisoellipticine on MV4-11 proliferation.

- After 96 hours 70% of cells are still viable after treatment with 5 µM 7-hydroxyisoellipticine however cell numbers are not increasing.
- G2/M arrest also evident in five other leukaemia cell lines tested



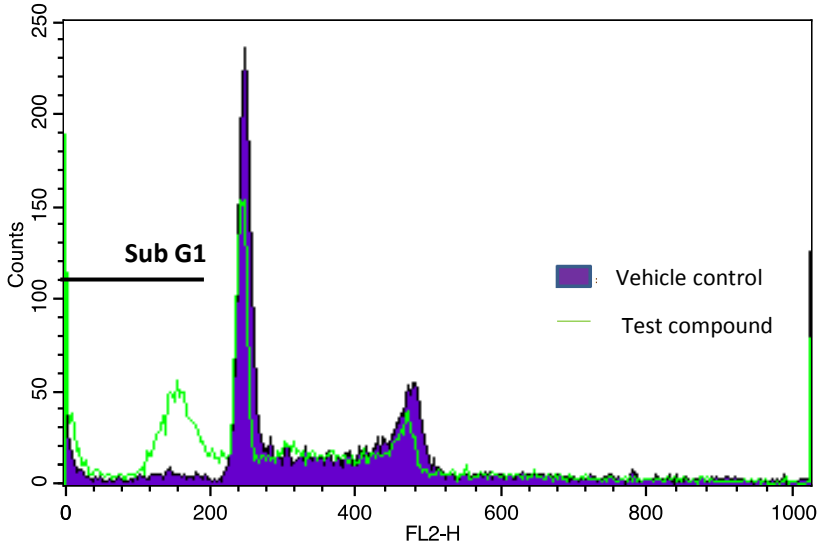
Vehicle control

7-Hydroxyisoellipticine

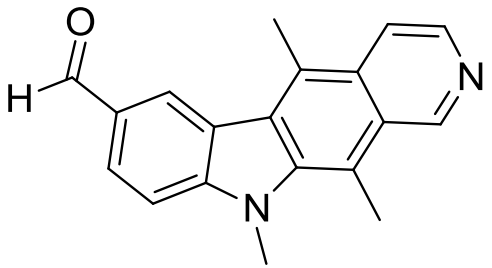
- 7-Hydroxyisoellipticine causes G2/M arrest
- Indication that it acts as a cytostatic rather than cytotoxic agent
- Cytostatic compounds possess attractive features for inclusion in chemotherapy

Sub G1/Cytotoxic effect of other of IsoE's

- Isoellipticine derivatives also synthesised to explore whether this cytostatic effect could be replicated with increased potency
- Sub G1 peak observed when cells treated with 7-formyl derivatives and N2/N10 7-hydroxyl derivatives indicating they act as cytotoxic agents

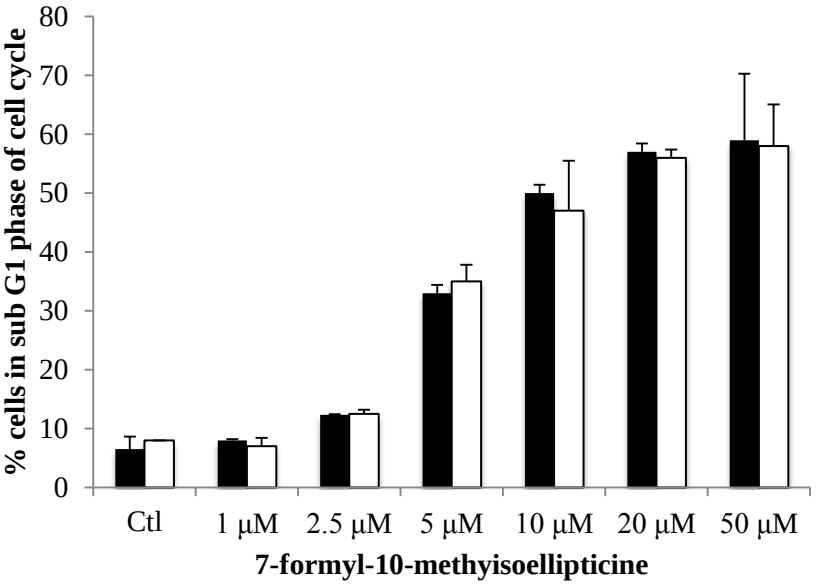


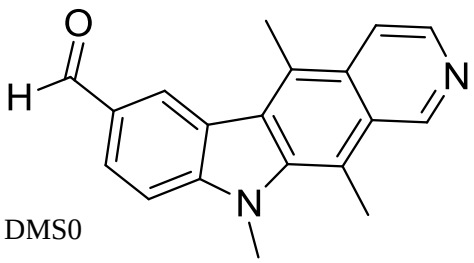
- Adaptability of template allows for additional cytotoxic activity



- Led us to consider the potential *in vivo* effects of this cytotoxic action using FM-IE (above)
- Embarked on a screen of the suitability of this agent in cancer treatment

Effect of different delivery methods of FM-IE on cells





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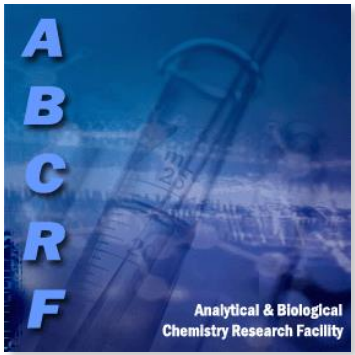

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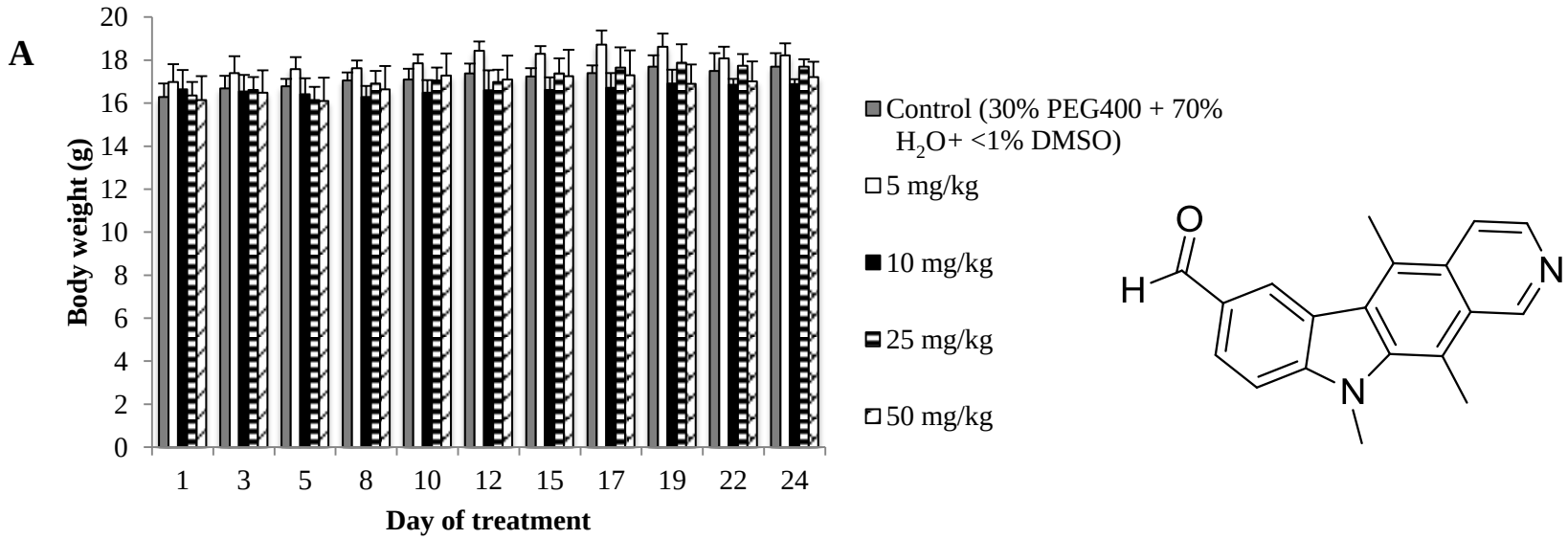
Effect of different dosages of FM-IE on body weight

- *In vivo* toxicity was assessed using female BALB/c mice.
- Animals (5 mice/group) injected i.p. with FM-IE at doses of 5, 10, 25 and 50 mg per kg body weight in a 0.2 ml injection volume at the indicated times.
- Sacrificed on day 25, major organs were stained using haematoxylin and eosin, and serum was collected to monitor ALT and AST levels



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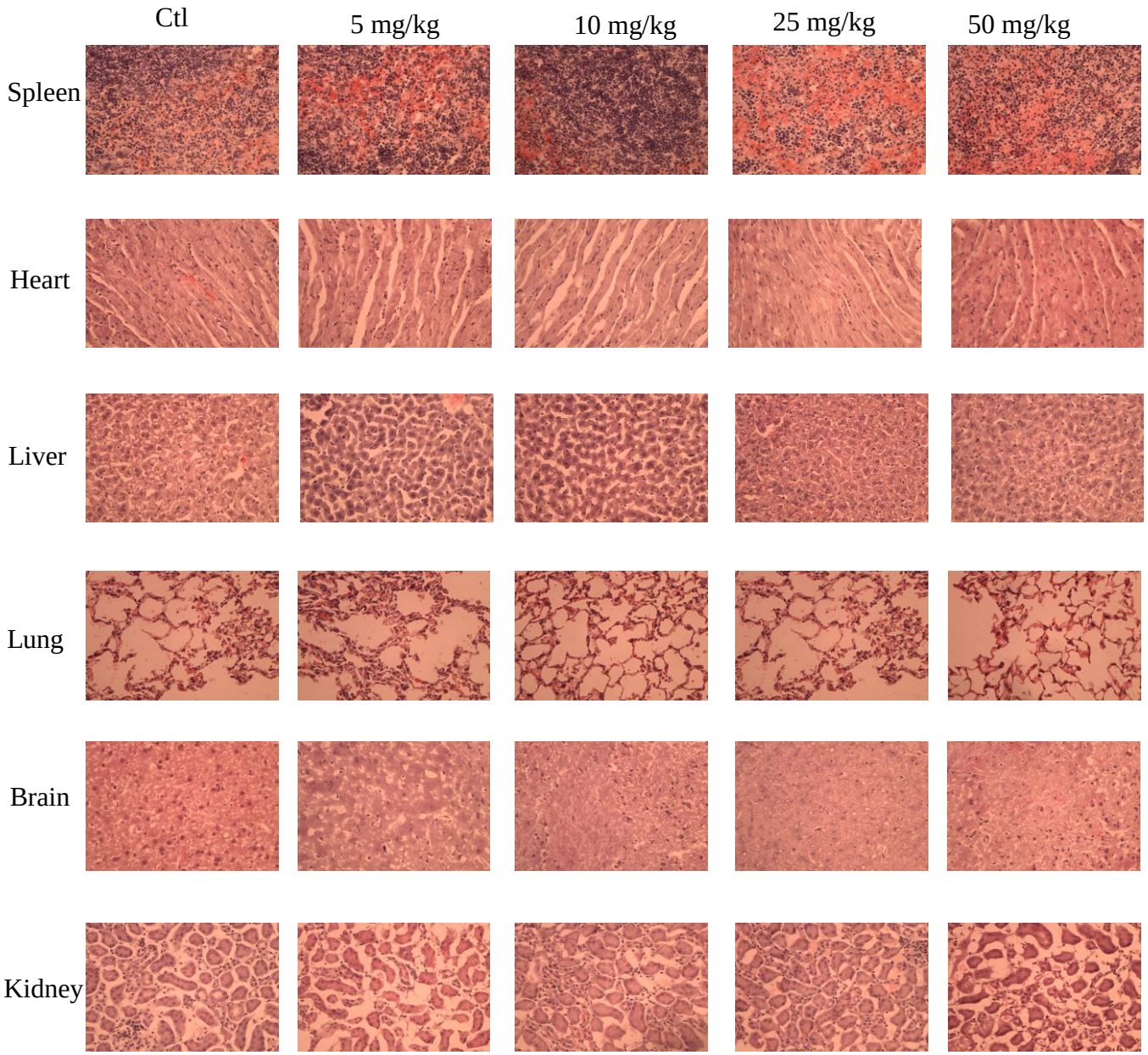
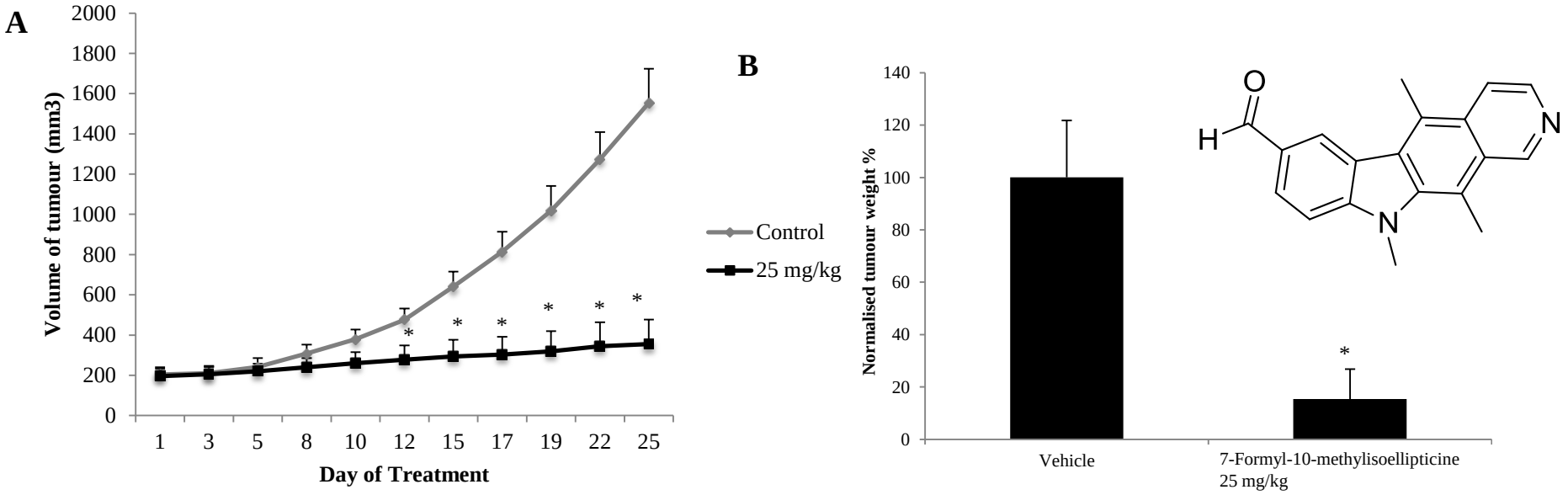
Effect of FM-IE on normal tissues/organs



FM-IE

- Female CB17 (SCID) mice were used for the *in vivo* anti-tumour study and the model was established by subcutaneous injection of MV4-11 cells [1 x 10^7 into the flank].
- Once average tumour volume reached approximately 200 mm^3 animals (8 mice/group) were i.p. injected with FM-IE at a dose of 25 mg per kg body weight in a 0.2 ml volume.



Russell EG[1], Guo J[2], O'Sullivan EC[3], O'Driscoll CM[2], McCarthy FO[3], Cotter TG[4]. Invest New Drugs. 2016 Feb;34(1):15-23.

7-Formyl-10-methylisoellipticine, a novel ellipticine derivative, induces mitochondrial reactive oxygen species (ROS) and shows anti-leukaemic activity in mice.

cost
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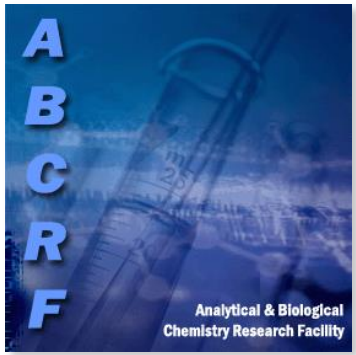

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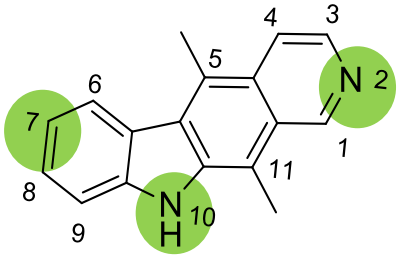
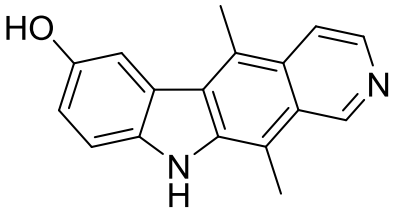
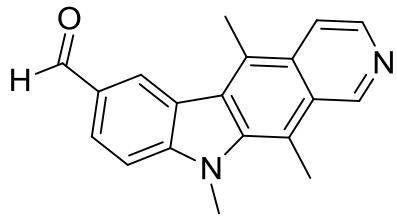
Conclusions

- Over 100 novel isoellipticines have been generated to date with >40 taken forward to 5 dose testing at the NCI
  - Mean growth inhibitions as low as -20% observed
  - Bulkier substitutions at N10 were found to be generally less active but could result in enhanced selectivity
  - COMPARE analysis has been performed to identify intracellular targets and led to flow cytometry studies





- 7-Hydroxyisoellipticine (7H IE) induces G2/M cell cycle arrest by inducing ROS and activating the DNA damage pathway.



- 7-Formyl-10-methyl isoellipticine (FM-IE) induces significant G1/S arrest and cytotoxicity in leukaemia cell cultures and is apparently safe for administration in the short term.

- FM-IE shrinks tumour growth by >70% in the leukaemia model and further *in vivo* studies are planned.

- Multiple targets can be accessed from the isoellipticine template.

Acknowledgments

- Funding
  - Irish Research Council
  - PRTLI
- Ellipticine team
  - Fiona Deane PhD, Charlotte Miller PhD
  - Elaine O'Sullivan PhD, Mary McKee
  - Dr. Eileen Russell, Prof. Thomas Cotter
  - Dr. Jianfeng Guo, Prof. Caitriona O'Driscoll
  - Dr. Ken Devine
  - National Cancer Institute