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Arresting cell growth with novel functionalised indolocarbazoles

Florence O. McCarthy[1,*], Hannah J. Winfield[1], Kevin D. O'Shea[1], Michael M. Cahill[1] and Larry T. Pierce[1]

[1] Department of Chemistry and ABCRF, Cavanagh Building, University College Cork, Western Road, Cork, Ireland

- Corresponding author: f.mccarthy@ucc.ie

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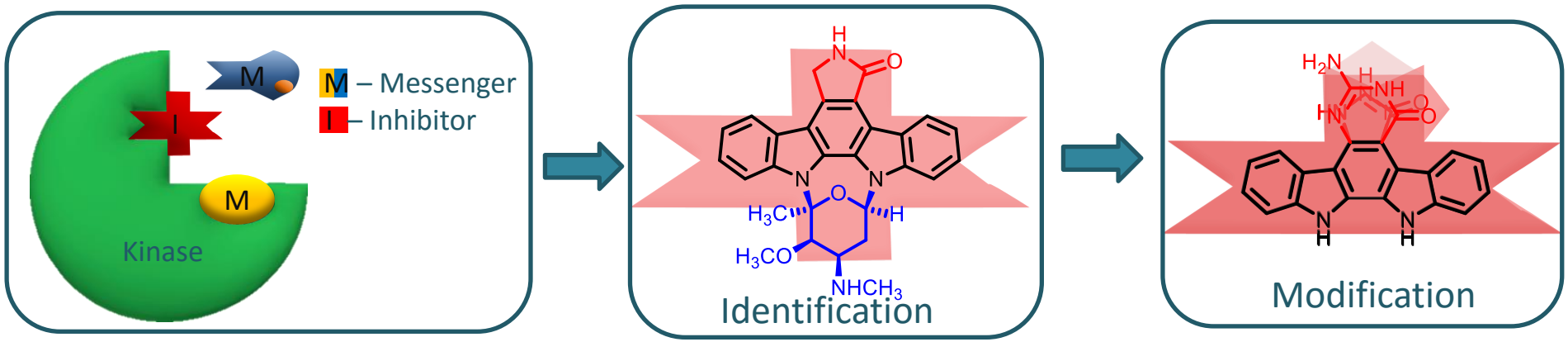
Coláiste na hOllscoile Corcaigh, Éire
University College Cork, Ireland

Arresting cell growth with novel functionalised indolocarbazoles

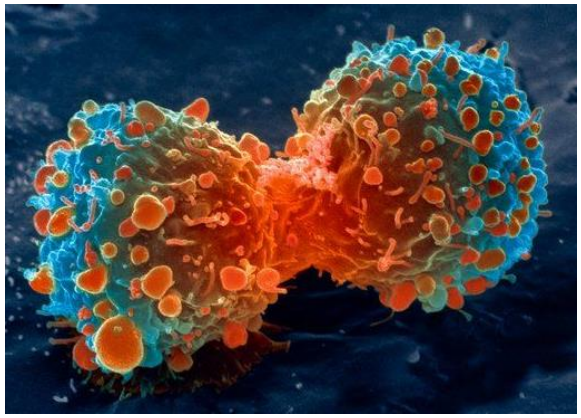
Graphical Abstract

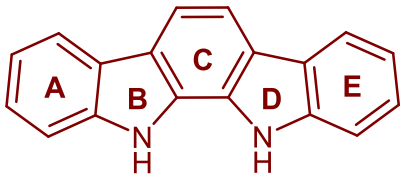


Abstract: Cancer causes about 13% of all human deaths and at least one fifth of all deaths in Europe and North America. Although chemotherapy is increasingly prescribed, it is not without side effects and so new, more selective remedies for cancer sufferers must be found.
Since the discovery of the anticancer properties of the indolocarbazole staurosporine, many analogues have been synthesised in order to obtain compounds that have a higher potency with respect to anticancer mechanisms. The overall objective of this project is to produce selective and highly potent novel anticancer agents through modification of the indolocarbazole structure and a focus of this work is the replacement of the lactam/maleimide heretocycle to form a series of novel indolocarbazole derivatives including the first reported synthesis of a series of novel substituted indolocarbazole uracils.
Biological evaluation via the NCI 60 cell line screen has been completed for a number of these compounds with some showing significant selectivity towards individual leukaemia and melanoma cell lines.

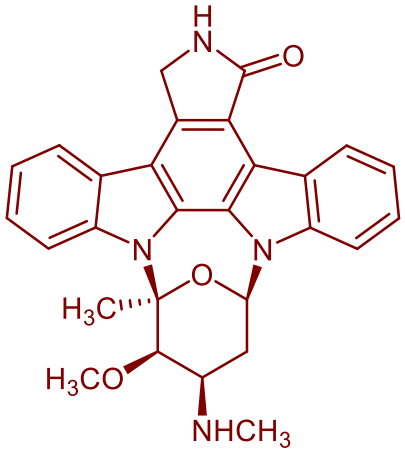
Keywords: indolocarbazole; cancer; kinase; topoisomerase

Cancer and Chemotherapy





Indolo[2,3-*a*]carbazole

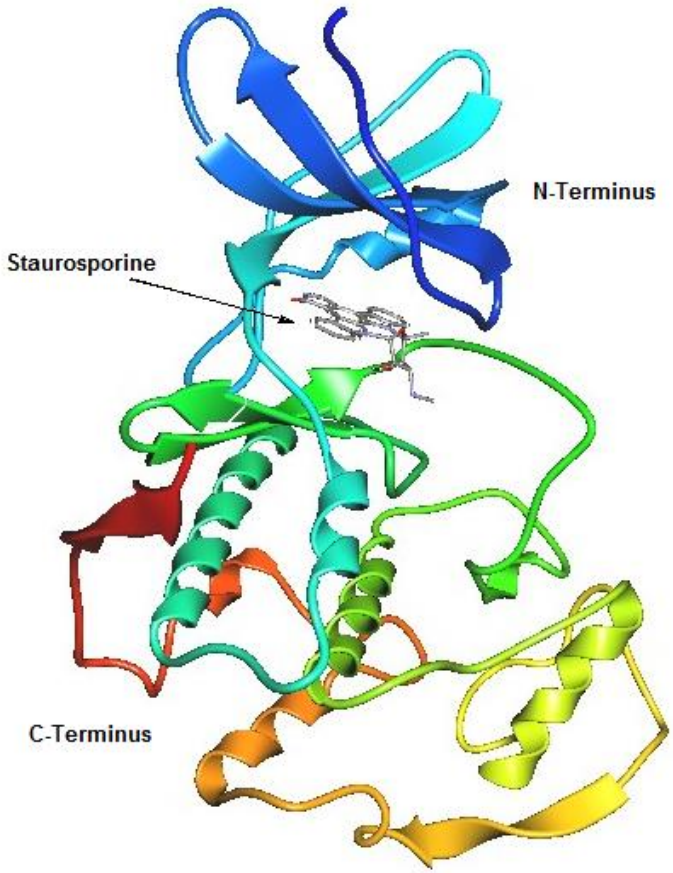


Staurosporine

- Over 3.2 million people in Europe diagnosed with cancer on annual basis.
- Cumulative lifetime risk of invasive cancer in Ireland is approximately 1 in 3 for men and 1 in 4 for women.
- Greater need than ever to pursue targeted cancer therapies via novel drug templates.
- Indolo[2,3-*a*]carbazole (ICZ) pharmacophore has been a major focus to medicinal chemists for over 30 years.
- Staurosporine (STA) first ICZ to be isolated from a natural source; reported by Omura *et al*. in 1977.[1]
- Subsequently shown to be an extraordinarily potent inhibitor of PKC (IC_{50} = 2.7 nM) and strongly cytotoxic against cancer cells.[2]

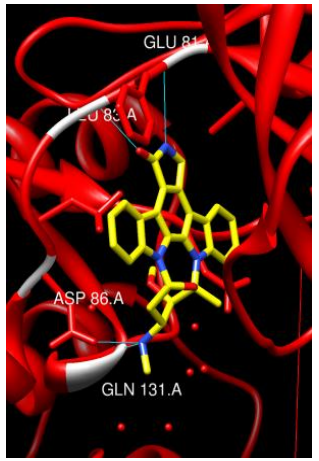
1 Omura, S. et al., *J. Antibiot.*, **1977**, 30, 275
2 Tamaoki, T. et al., *Biochem. Biophys. Res. Commun.*, **1986**, 135, 397

Indolo[2,3-*a*]carbazoles as protein kinase inhibitors



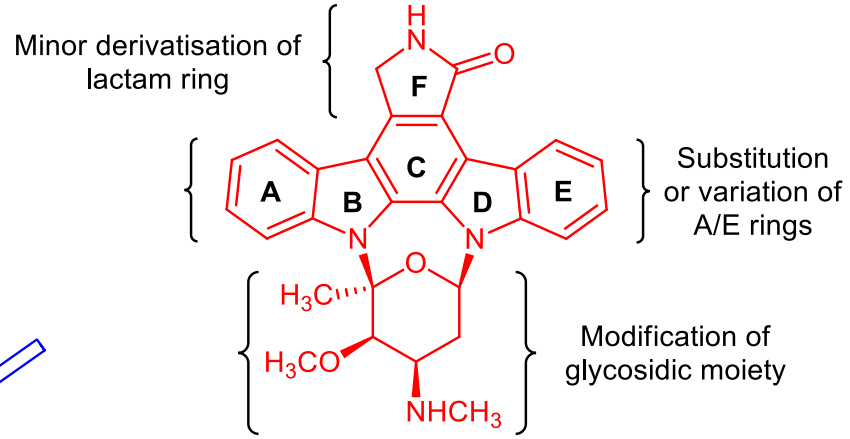
STA in complex with CDK2

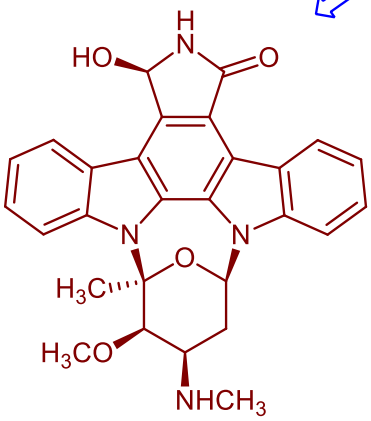
- One of the largest families of proteins in humans, deregulation of protein kinases has been implicated in oncogenesis and the progression of tumours.
- Oncogenic kinases continuously activate signalling pathways that regulate cell cycle progression, proliferation and cell survival.
- STA found to be a nonselective inhibitor of many different kinases, such as PKA (IC_{50} = 15 nM), phosphorylase kinase (IC_{50} = 3 nM) and S6 kinase (IC_{50} = 5 nM).[3]
- Crystal structures resolved for STA in complex with cyclin-dependent kinase 2 (CDK2) and PKA proved inhibition occurs in an ATP-competitive manner.[4,5]
- Although ATP-binding pocket is relatively conserved across pan-kinase domain, exploitation of discreet differences in active site residues and conformations can help to confer selectivity.



3 Meggio, F. et al., *Eur. J. Biochem*, **1995**, 234, 317
4 Lydon, N. et al., *Structure*, **1997**, 5, 1551
5 Engh, R.A. et al., *Structure*, **1997**, 5, 1627

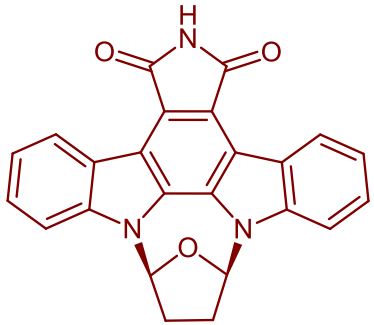
Staurosporine: as a lead for kinase inhibition





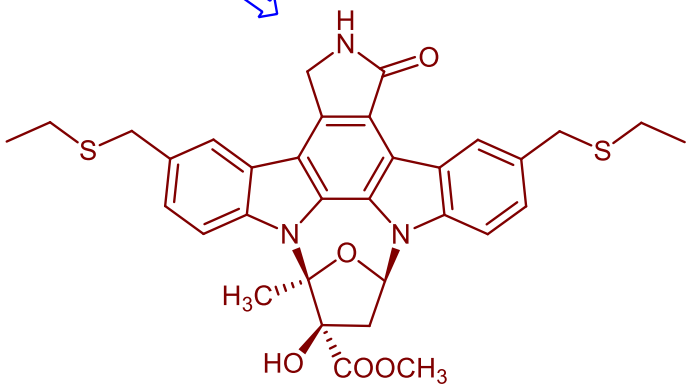
UCN-01

Clinical trials due to highly potent antiproliferative activity.
Inhibitor of: PKC (IC_{50} = 10 nM)
PDK1 (IC_{50} = 5 nM)



SB-218078

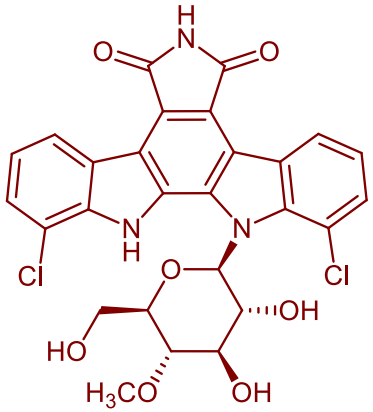
Selective inhibitor of CHK1 (IC_{50} = 15 nM) versus CDK1 (IC_{50} = 250 nM);
PKC (IC_{50} = 1000 nM)



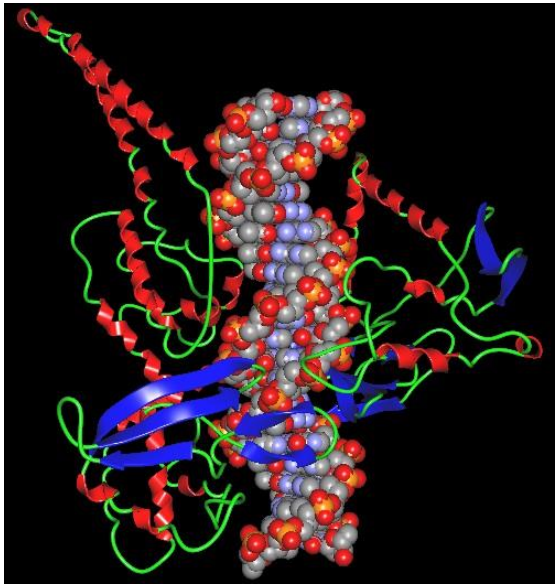
CEP-1347

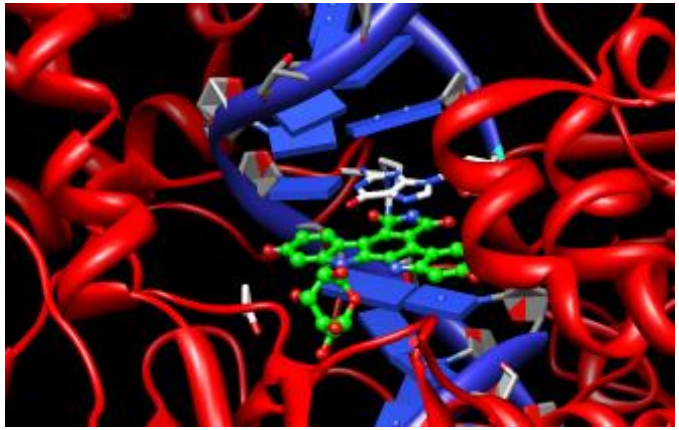
Little or no activity against PKC.
Potent inhibitor of JNK-1 (IC_{50} = 30 nM)

Rebeccamycin: another lead ICZ candidate



Rebeccamycin





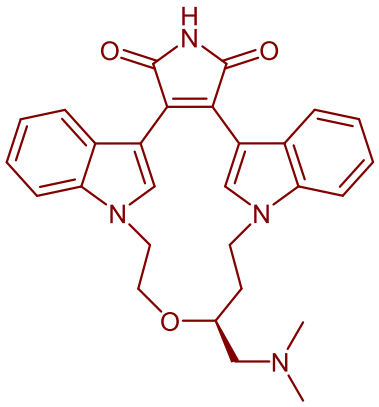
Topo I cleavage complex with human DNA

- Rebeccamycin (REB), an ICZ with one *N*-glycosidic bond, was isolated in 1985 from *Nocardia aerocolonigenes*.[6]
- REB displayed considerable activity against leukemia and melanoma in mice, and inhibited the growth of A549 human lung adenocarcinoma cells, producing single strand breaks in the DNA of these cells.[7]
- Potent anticancer action was linked to its inhibition of topoisomerase I (topo I).

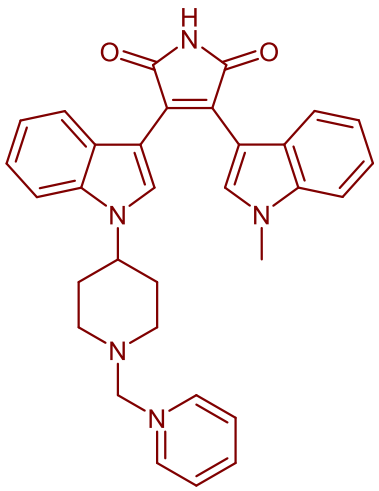
6 Clardy, J. et al., *Tet. Lett.*, **1985**, 26, 4011
7 Tomita, K. et al., *J. Antibiot.*, **1987**, 40, 668

Bisindolylmaleimides: potent ICZ precursors

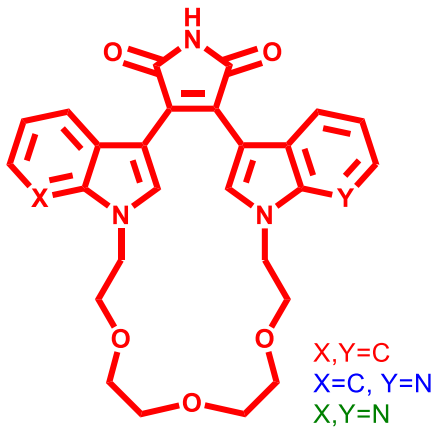
- Bisindolylmaleimides (BIMs) are frequently utilised as synthetic precursors to ICZs, with numerous coupling methods employed to achieve final aromatisation step.
- Also found to possess uniquely potent biological activity, and a number of candidates are under consideration for the treatment of diseases such as non-small cell lung cancer, glioblastoma and diabetic peripheral retinopathy.



Ruboxistaurin
Specific inhibitor of PKC isoforms:
$PKC\beta_1$ (IC_{50} = 4.7 nM)
$PKC\beta_2$ (IC_{50} = 5.9 nM)



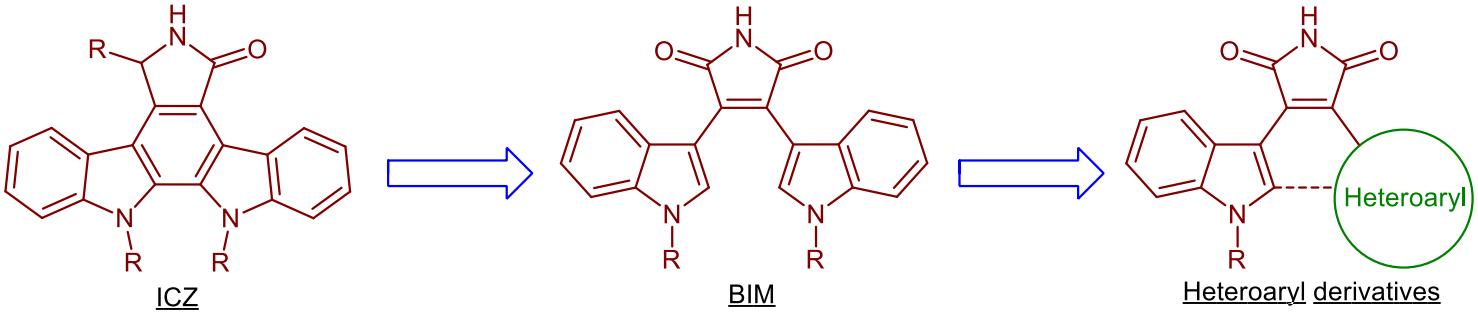
Enzastaurin
Potent inhibitor of PKCβ (IC_{50} = 6 nM)
and AKT/PI3 pathway



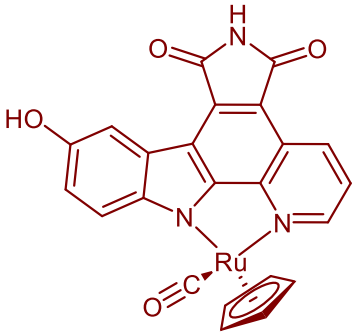
Aza BIMs by Kuo *et al.*[8]
Inclusion of azaindolyl moiety demonstrated remarkable selectivity for GSK-3β

8 Kuo, G.-H. et al., *J. Med. Chem.*, **2003**, 46, 4021

Further diversification of the ICZ pharmacophore

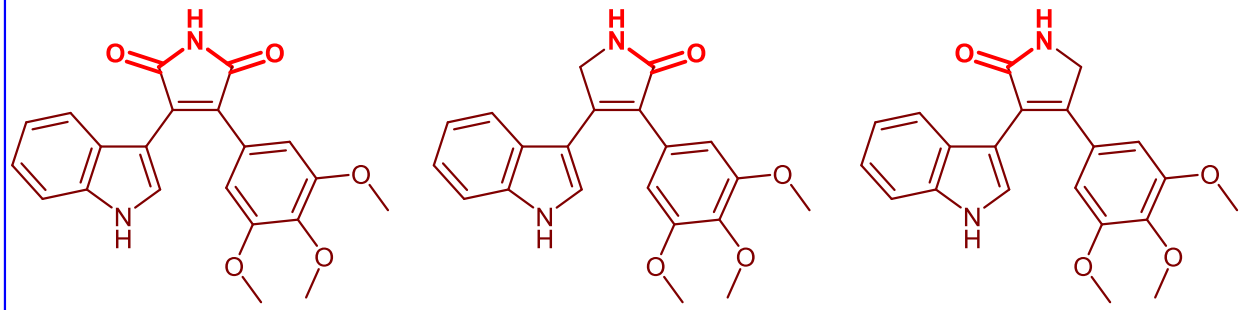


- Appropriation of heteroaryl subunits in place of one indole functionality has been shown to increase kinase inhibition in many instances.



Cyclometalated ICZ analogues by Meggers *et al.*[9]
Indole replaced by pyridine moiety
Inhibits GSK-3α isoform (IC_{50} = 0.3 nM)

VEGF-R inhibitors by Peifer *et al.*[10]
Indole replaced by 3,4,5-trimethoxyphenyl subunit



VEGF-R2 (IC_{50} = 0.0025 μM)
VEGF-R3 (IC_{50} = 0.005 μM)

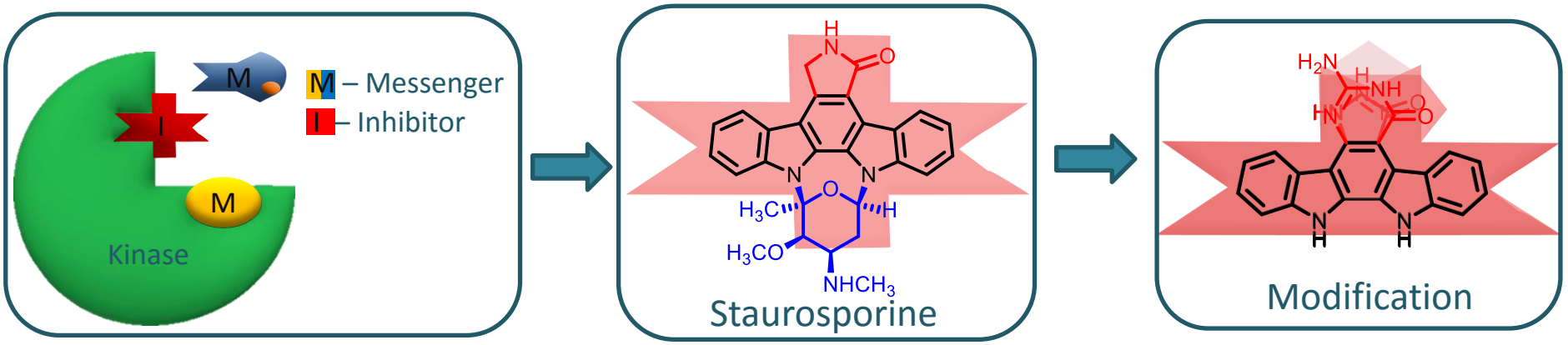
VEGF-R2 (IC_{50} = 0.031 μM)
VEGF-R3 (IC_{50} = 0.037 μM)

VEGF-R2 (IC_{50} = 11 μM)
VEGF-R3 (IC_{50} = 9.4 μM)

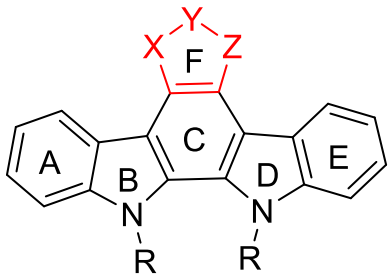
9 Meggers, E. et al., *Synthesis,* **2005**, 9, 1521
10 Peifer, C. et al., *J. Med. Chem.*, **2006**, 49, 7549

Target Indolocarbazoles

- Literature suggests that there is significant scope to modify the indolocarbazole template and maintain biological activity but imbue differentiation of mode of action.
- One area that has been relatively overlooked has been the F-ring and this is the focus of our current work.

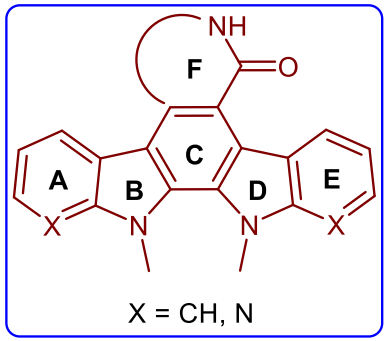


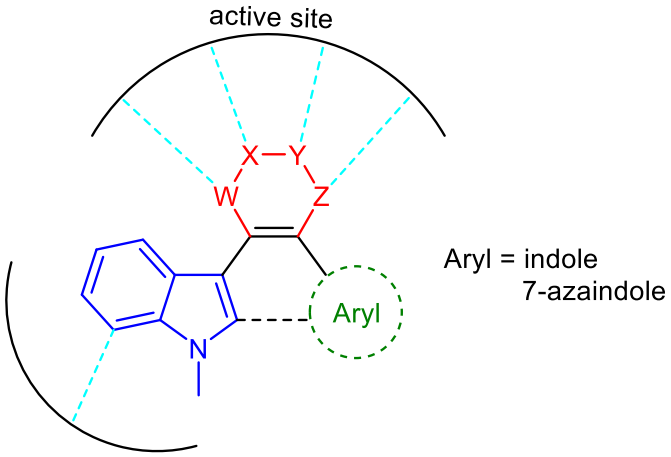
- A common F-ring motif in reported biologically active indolocarbazoles is the lactam/maleimide. We seek to alter the H-bonding framework to isolate new targets.
- Our work to date has focussed on utilising several novel 5- and 6-membered heterocycles (X-Y-Z) to replace this ring with unique biological profiles.



Target Structure

Aims and objectives

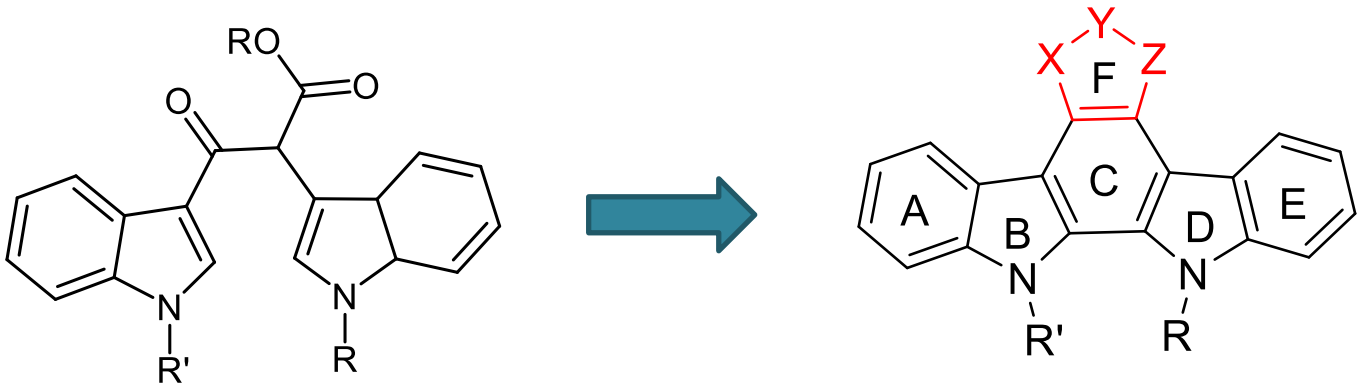




- The primary aim of our program of diversity-oriented synthesis is to explore the paradigm of F-ring modulation in novel indolocarbazoles and azaindolocarbazoles.
- It is envisaged that such modification can help to confer more favourable pharmacological properties and potentially increase bioavailability
- Evaluation undertaken by assessment of cell growth and consequently the influence of these novel templates in the topo I-DNA complex and the exploitation of discrete differences in the kinase active site.
- Initial evaluation of antiproliferative activity is followed by further investigation of discrete biological mechanism of action.

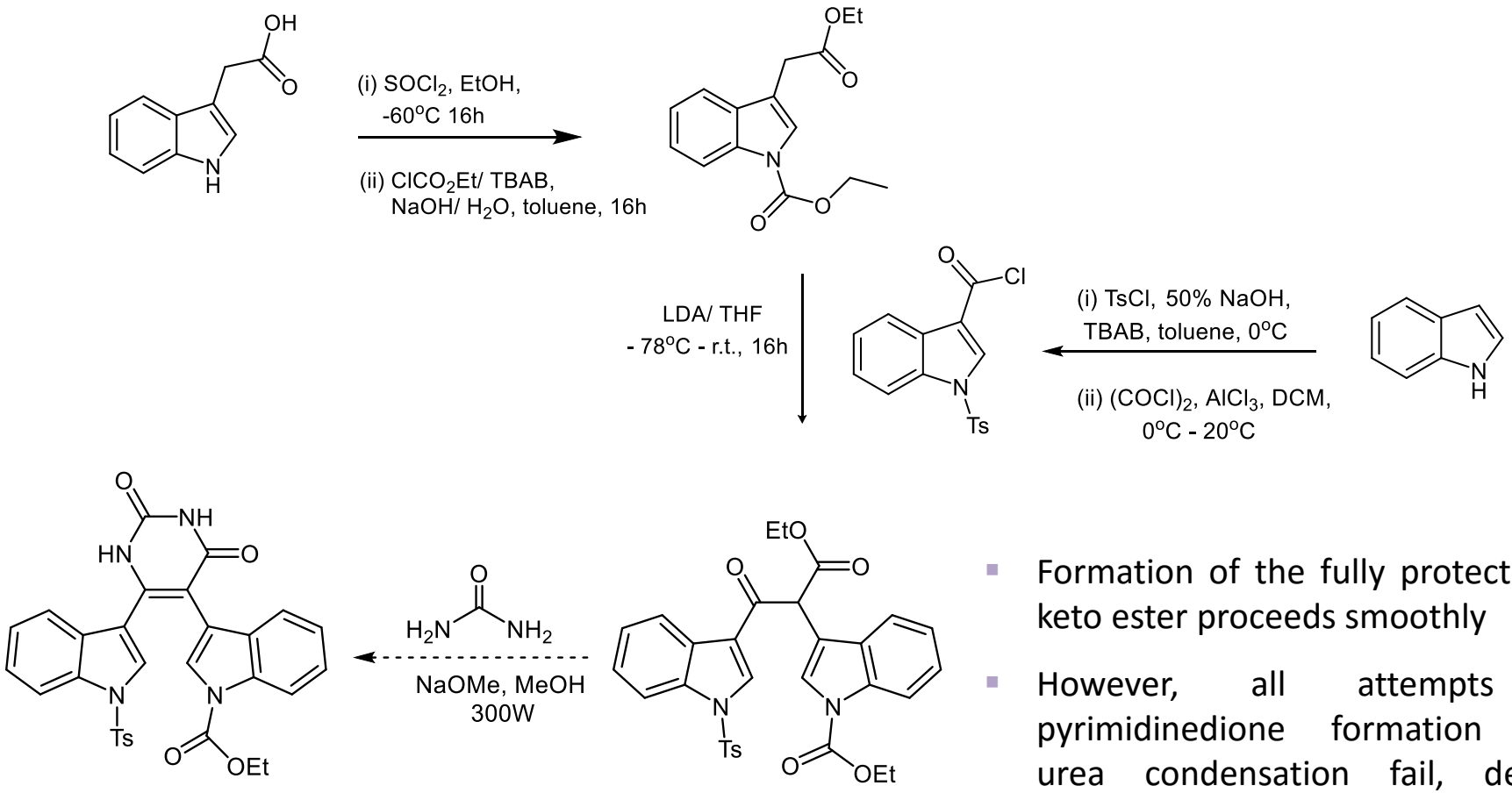
Diversity Orientated Synthesis

- Designed synthesis via a versatile key intermediate
- Bisindolyl β-Keto ester



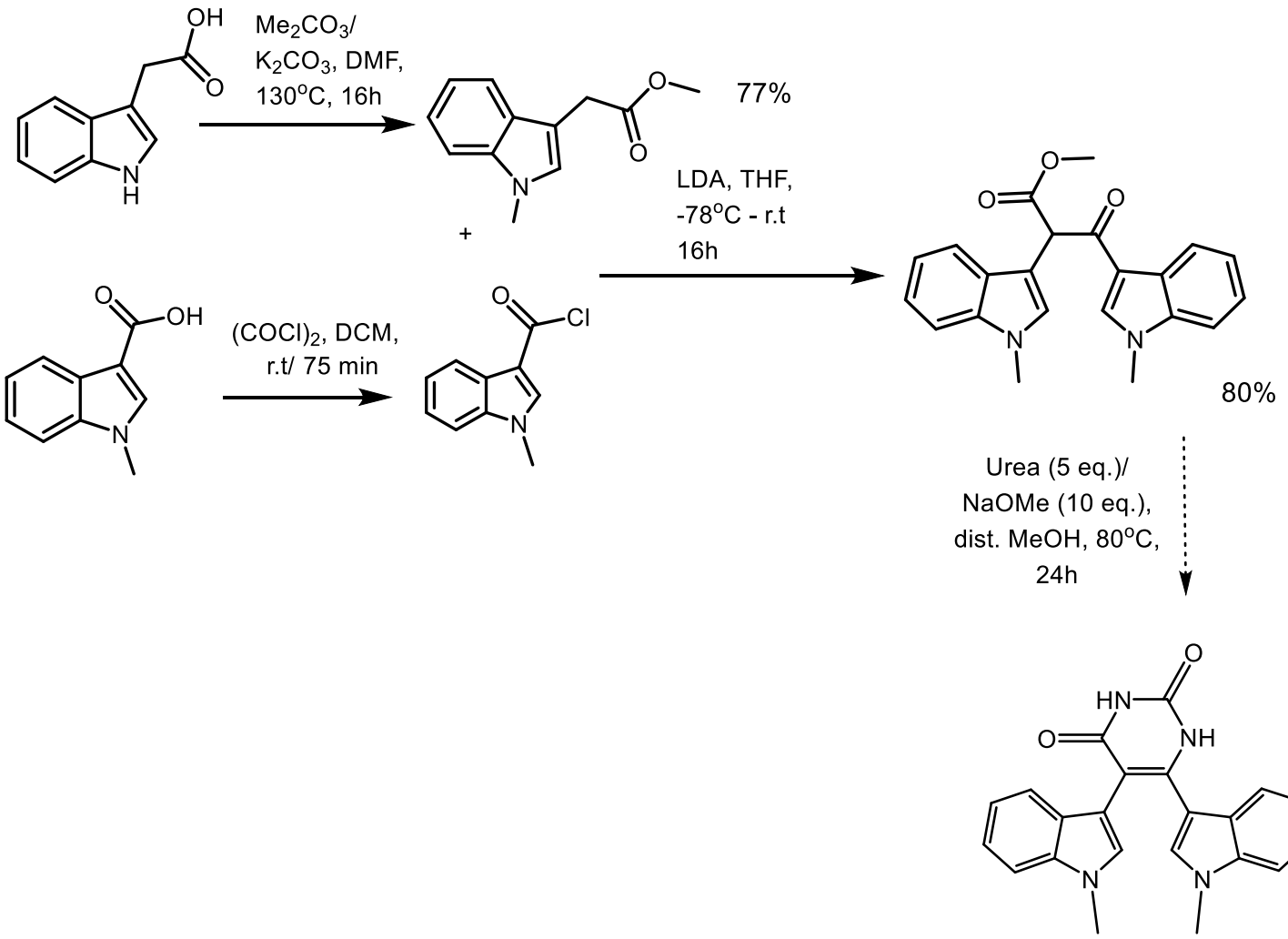
- Subsequent modification to give a series of novel bisindole heterocycles
- Adaptable route provides access to 5- and 6-membered rings
- Cyclisation to final indolocarbazoles reported for the first time
- Starting from indole or 7-azaindole will give rise to indolocarbazoles and azaindolocarbazoles

Initial synthesis of β-keto ester intermediate

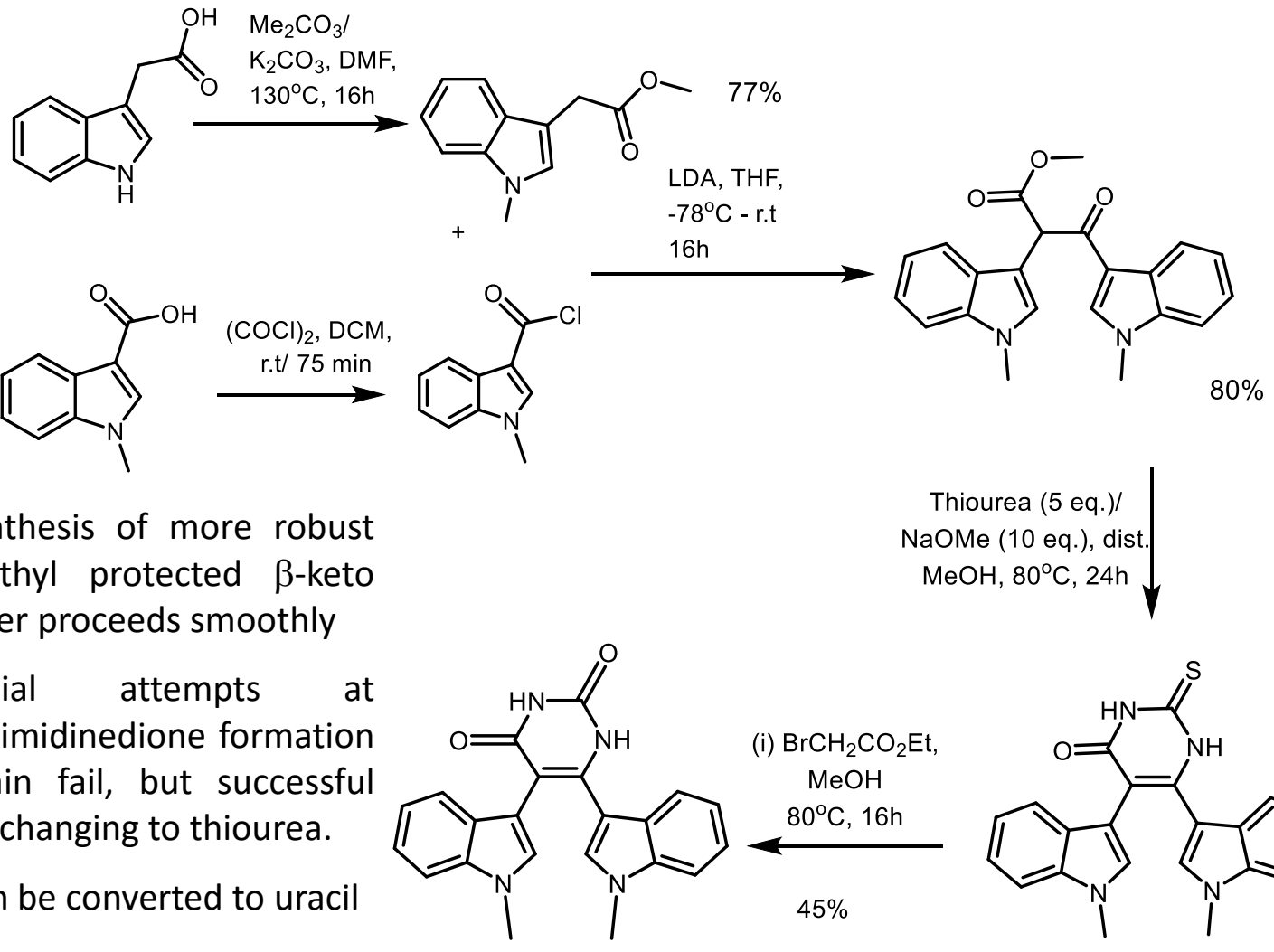


- Formation of the fully protected β-keto ester proceeds smoothly
- However, all attempts at pyrimidinedione formation with urea condensation fail, despite multiple conditions including microwave.

Pyrimidine-2,4-dione synthesis



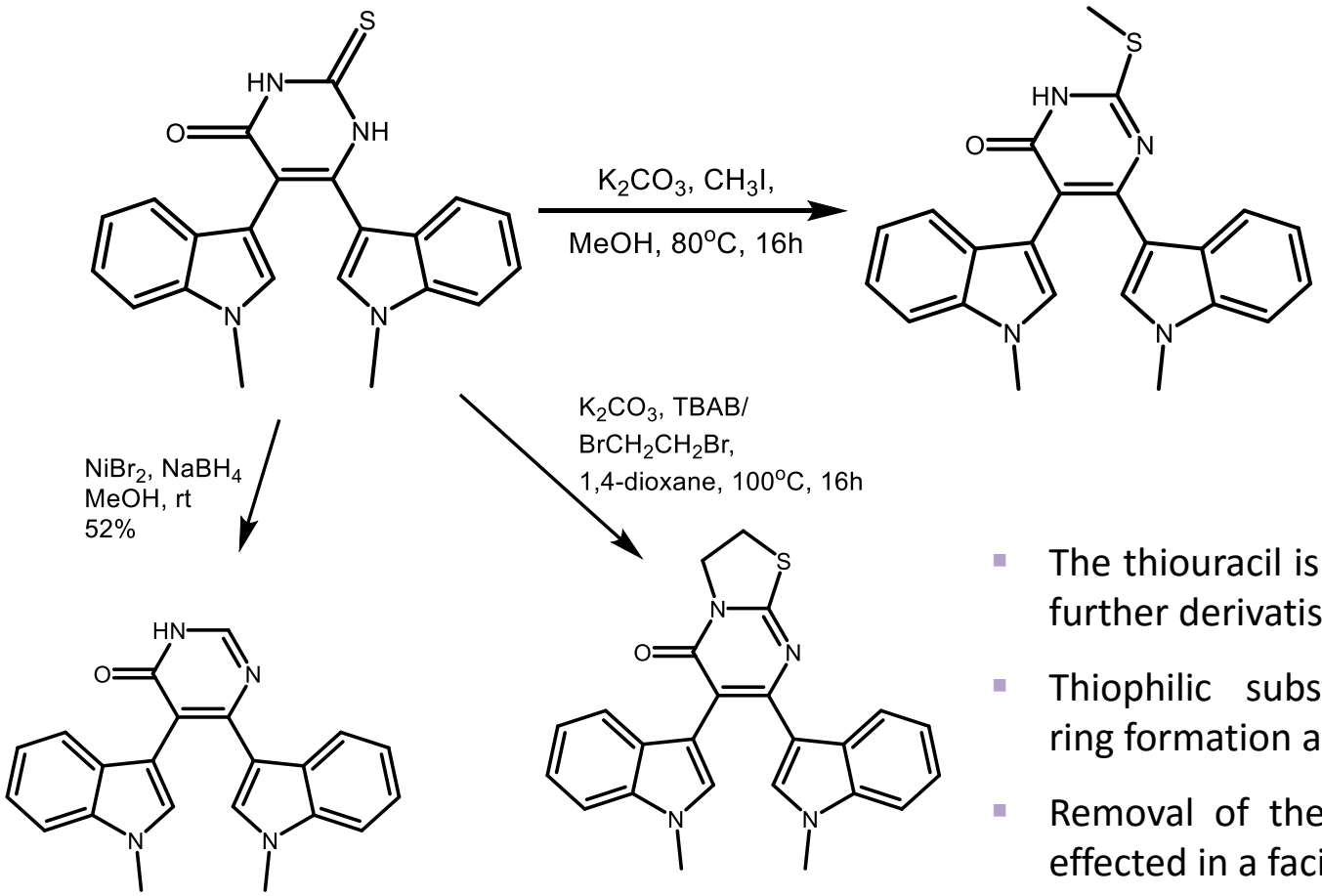
Pyrimidine-2,4-dione synthesis



- Synthesis of more robust methyl protected β-keto ester proceeds smoothly
- Initial attempts at pyrimidinedione formation again fail, but successful on changing to thiourea.
- Can be converted to uracil

11. L.T. Pierce, M.M. Cahill & F. O. McCarthy *Tetrahedron* **2010**, 66(51), 9754-9761

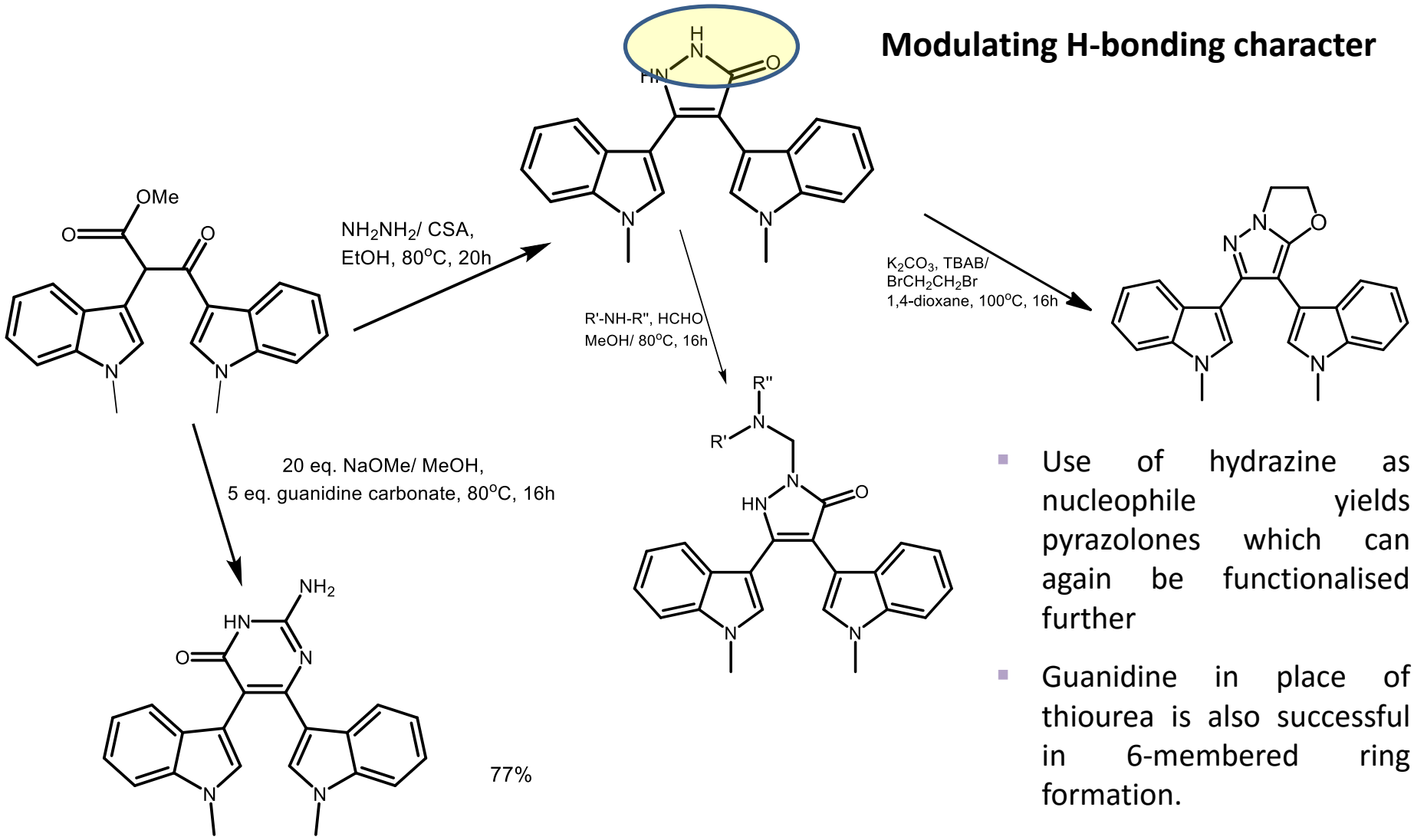
Exploring novel bisindolyl heterocycles



- The thiouracil is a good template for further derivatisation
- Thiophilic substitution and novel ring formation are both possible.
- Removal of the sulfur can also be effected in a facile manner.

11. L.T. Pierce, M.M. Cahill & F. O. McCarthy *Tetrahedron* **2010**, 66(51), 9754-9761

Bisindolyl pyrazolones/aminopyrimidinones



Modulating H-bonding character

- Use of hydrazine as nucleophile yields pyrazolones which can again be functionalised further
- Guanidine in place of thiourea is also successful in 6-membered ring formation.

Bisindolyl pyrimidinone cyclisation study

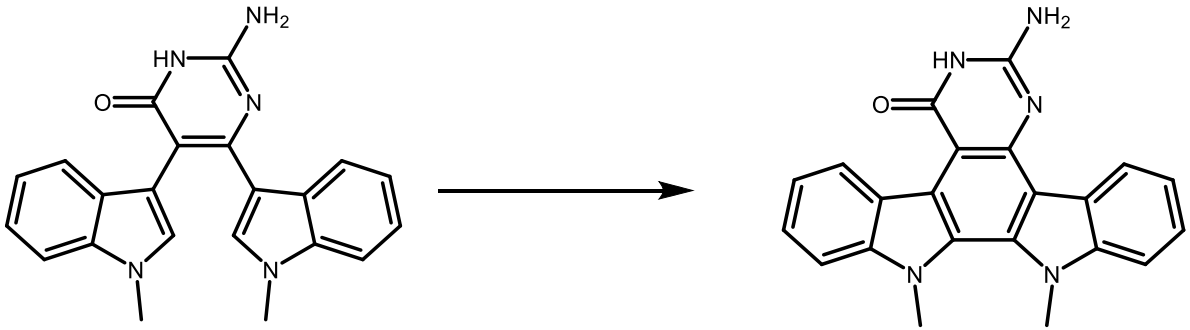


Table 1
Conditions investigated for the oxidative cyclisation of bisindolyl precursor to novel aromatized indolocarbazole[a]

Reagent	Amount	Conditions	Reaction time	Product
$Pd(OAc)_2$	1.0 equiv	DMF, 130°C[b]	20h	-
$Pd(OAc)_2$	5.0 equiv	AcOH, 110°C[c]	24h	-
$Pd(CF_3CO_2)_2$	3.0 equiv	DMF, 100°C[b]	20h	-
$K_3[Fe(CN)_6]$	1.0 equiv	H_2O/ KOH, 100°C[c]	24h	-
$PhI(OAc)_2$	2.5 equiv	DCM, r.t[c]	36h	-
hυ/ I_2	1.0 equiv[d]	toluene, r.t[c]	72h	**SM/Product**
hυ/ I_2	catalytic	CH_3CN/MeOH (3:2)[b]	24h	-
hυ/ I_2	catalytic	CH_3CN/MeOH (3:2)[c,f]	16h	**Product** (53%)
hυ/ I_2	catalytic	CH_3CN/MeOH (3:2)[e,f]	16h	**Product** (55%)

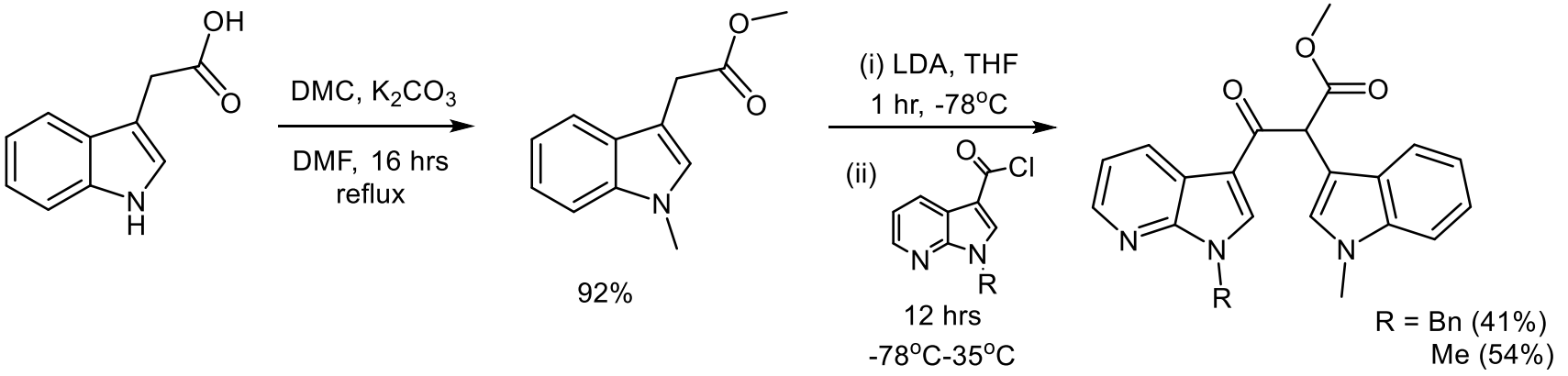
[a]Reactions were performed on 0.27 mmol scale. [b]Inert atmosphere. [c]Open-vessel reaction. [d]Refers to stoichiometry of iodine. [e]Air-bubbling [f]Dilution: 1.0 mg substrate/ 2.5 mL solvent.

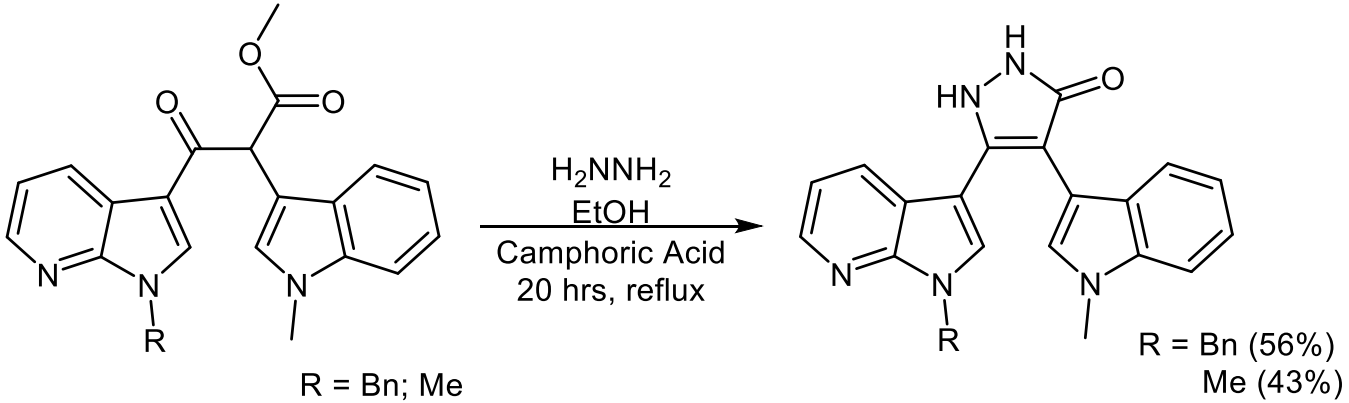
- Indolocarbazole formation from bisindolemaleimide precursors is well described in the literature.
- Specific conditions are required once the maleimide has been converted to another heterocycle.

12. L.T. Pierce, M.M. Cahill, H.J. Winfield & F. O. McCarthy *Eur.J.Med.Chem* **2012**, 56, 292-300

Azaindole β-Keto ester and pyrazolone formation

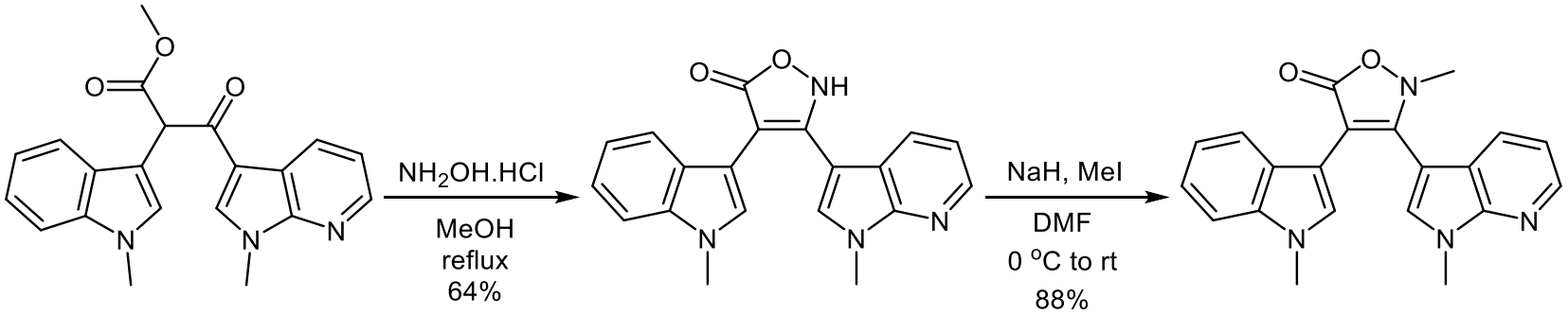
- Temperature control and solubility critical to success

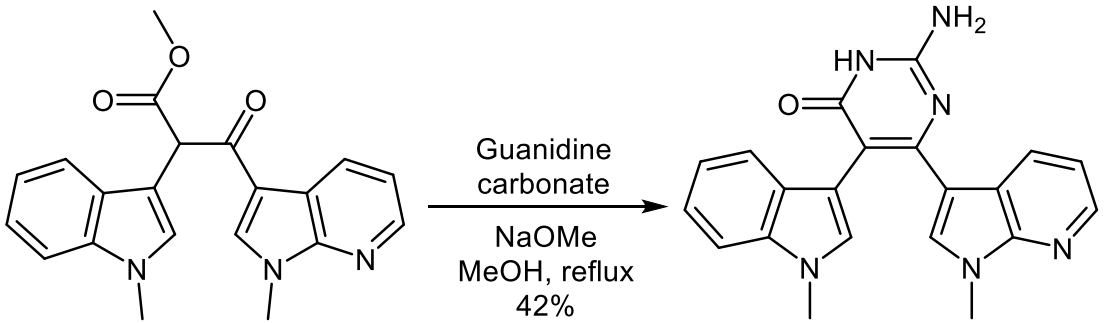




- Azaindole β-keto ester formation is temperature dependant due to solubilty.
- Use of hydrazine forms the pyrazolone in good yield.

Cyclocondensation of β-keto ester to novel F-rings

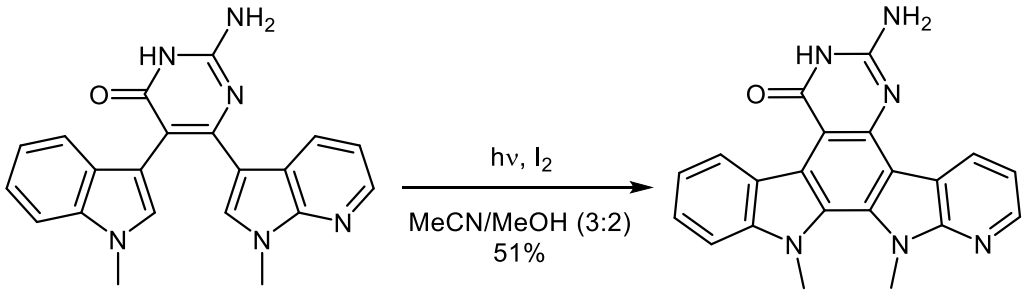


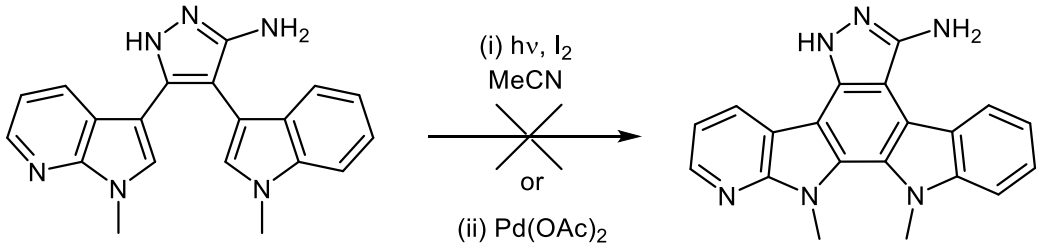


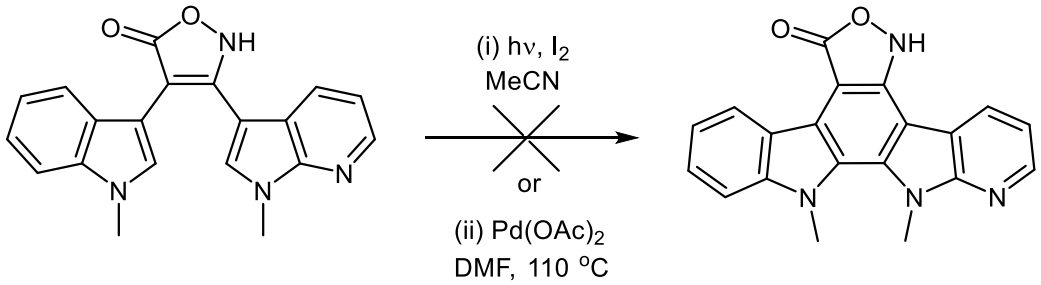
- Use of hydroxylamine as nucleophile yields isoxazolones which can again be functionalised further by simple alkylation.
- Guanidine is again also successful in 6-membered ring formation.

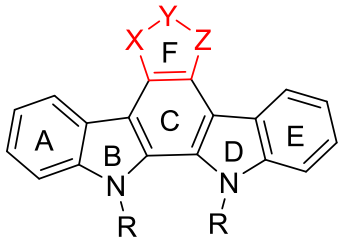
12. L.T. Pierce, M.M. Cahill, H.J. Winfield & F. O. McCarthy *Eur.J.Med.Chem* **2012**, 56, 292-300

Accessing of novel azaindolocarbazoles





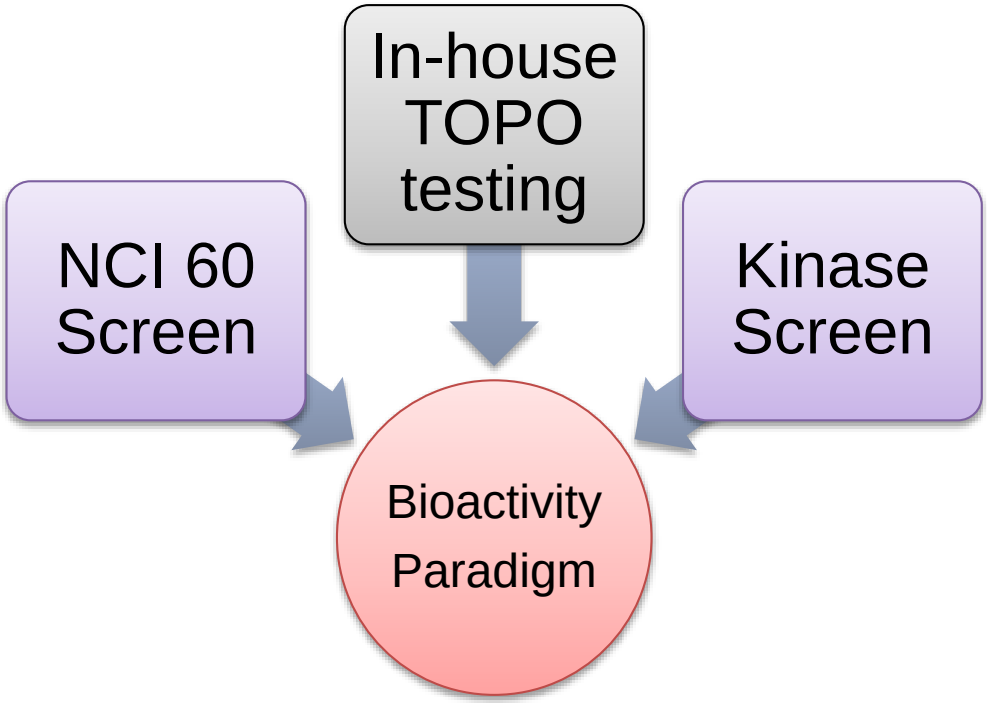




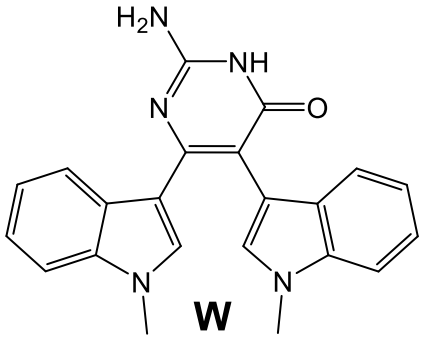
- In order to access the indolocarbazoles, light mediated cyclisation was attempted.
- The isocytosine precursor converts readily to the indolocarbazole
- However, both isoxazolone and aminopyrazole (formed via a different route) fail to cyclise under a variety of conditions.

Biological evaluation of novel indolocarbazoles

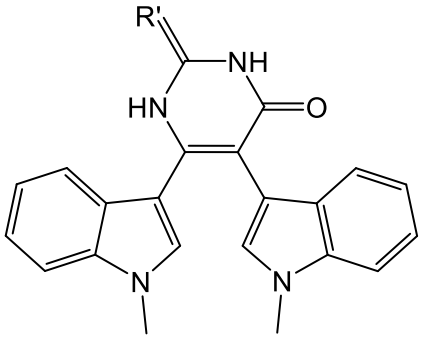
- Biological evaluation follows a predetermined programme beginning with cellular antiproliferative activity as measured at the NCI 60 cell line screen.
- Active compounds are then profiled for Topoisomerase I and II inhibition.
- Active compounds are also profiled for kinase inhibition in collaboration.



Selected NCI *in vitro* cancer cell growth inhibition following incubation with BIMs W-Z

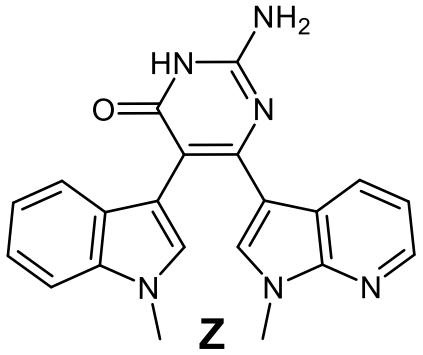


W



X
Y

R'
S
O



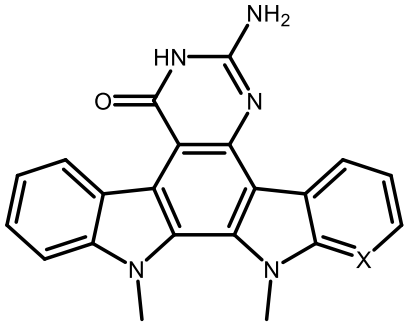
Z

	Cell line† % Growth after 48h (10 µM)§							
	EKVX	SNB-75	U251	*MDA-MB-435*	*IGROV1*	*CAKI-1*	*UO-31*	*MCF7*
W	104.65	103.47	100.33	109.77	68.92	94.60	64.59	77.57
X	74.95	85.66	88.98	77.23	75.70	82.37	75.82	68.22
Y	68.74	70.21	112.97	73.39	62.19	68.59	75.77	60.06
Z	96.34	87.55	77.55	98.77	87.12	89.98	79.03	85.95

†EKVX = non small cell lung cancer; SNB-75,U251 = central nervous system cancer; MDA-MB-435 = melanoma; CAKI-1, UO-31 = renal; IGROV1 = ovarian; MCF7 = breast cancer. §Relative to control cultured in RPMI 1640 medium containing 5% fetal bovine serum/2 mM L-glutamine.

Selected NCI *in vitro* cancer cell growth inhibition following incubation with indolocarbazole A and azaindolocarbazole B

Cell Line†	Compound (% Growth after 48h (10μM))§	
	A	B
CCRF-CEM	35.25	65.33
HL-60 (TB)	34.33	72.81
NCI-H522	46.09	35.05
HCT-116	34.02	63.01
HT29	17.58	78.05
KM12	19.94	59.02
SW-620	31.59	71.95
M14	34.90	68.83
MDA-MB-435	24.36	87.02
SK-MEL-2	31.97	68.46
SK-OV-3	34.92	87.23
ACHN	29.53	54.13
CAKI-1	-14.95	60.13
UO-31	7.16	39.40
MCF7	25.03	45.17



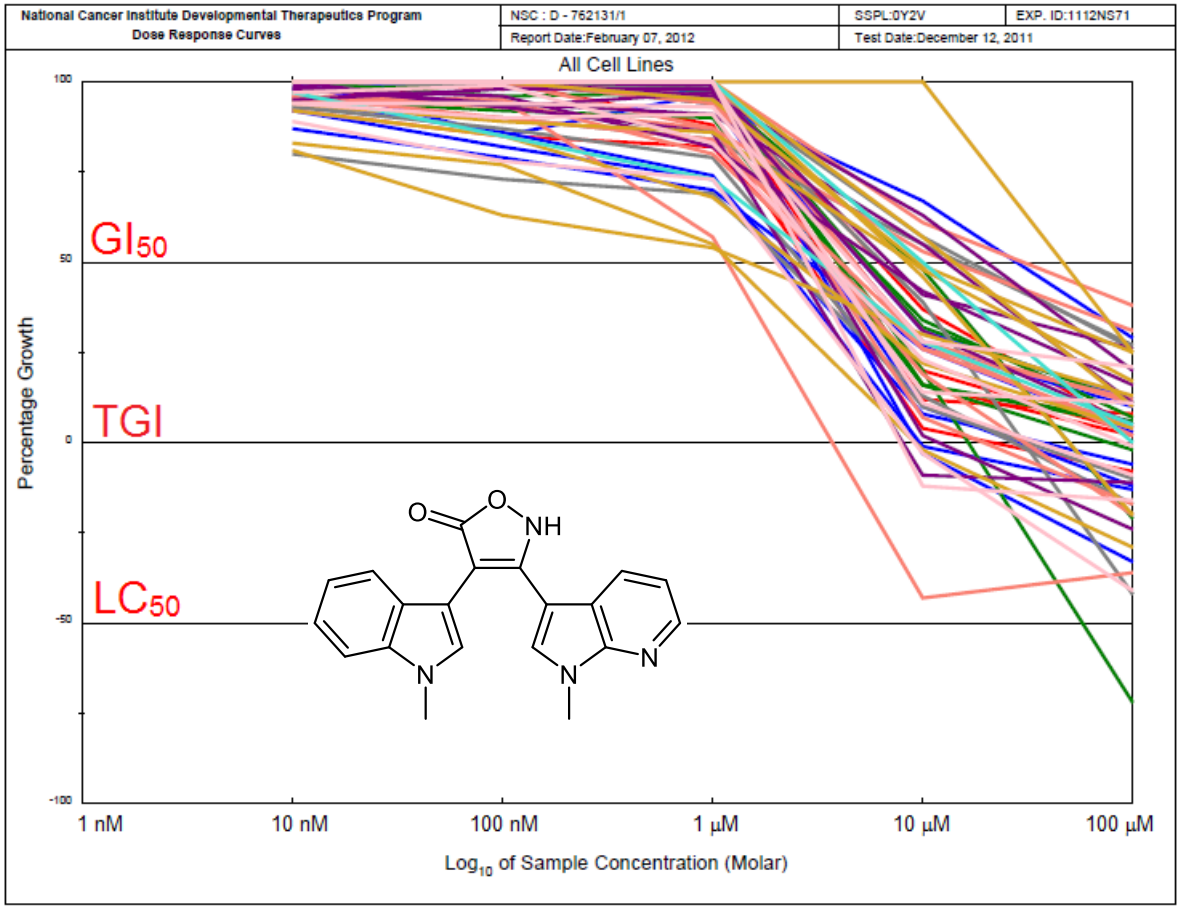
X = CH **A**
X = N **B**

- Conversion to ICZ from BIM results in dramatic increase in potency
- Comparison of the effect of azaindole in place of indole
- Evident that in this case the azaindolocarbazole is less potent
- Not always the case…

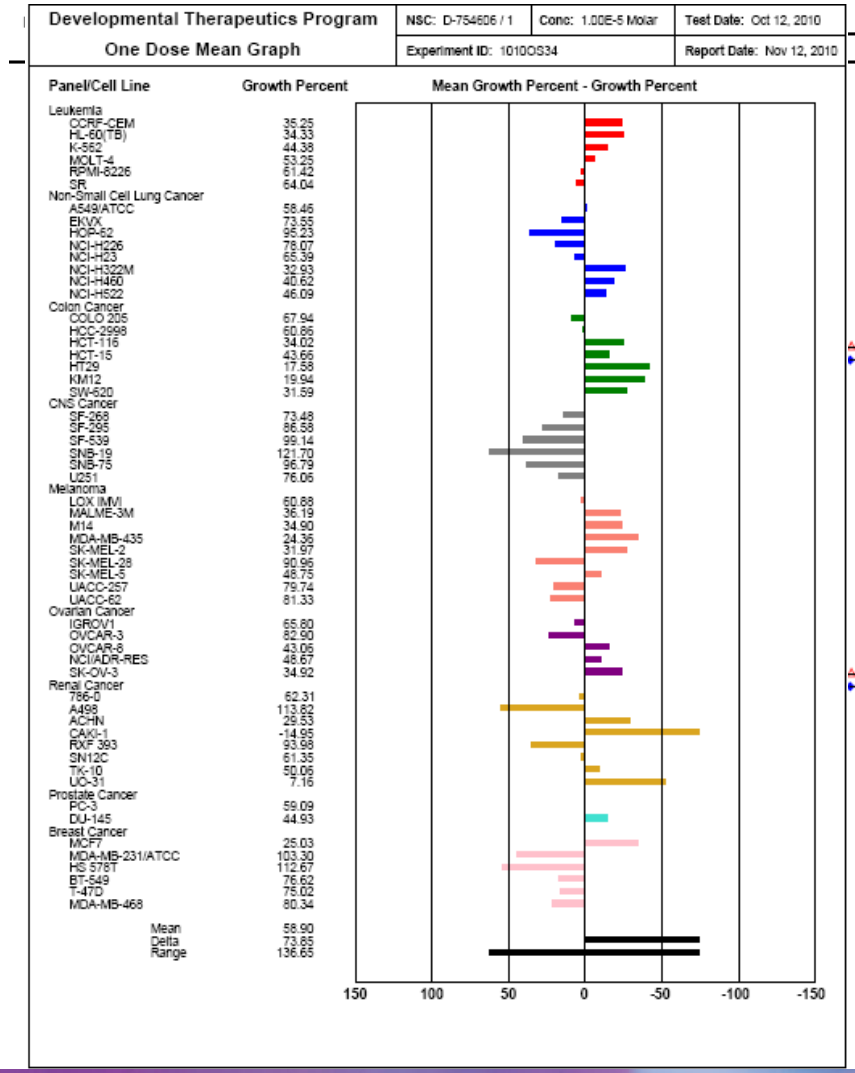
† CCRF-CEM, HL-60 (TB) = Leukaemia; NCI-H522 = non small cell lung cancer; HCT-116, HT29, KM12, SW-620 = colon cancer; M14, MDA-MB-435, SK-MEL-2 = Melanoma; SK-OV-3 = Ovarian cancer; ACHN, CAKI-1, UO-31 = renal; MCF7 = breast cancer. §Relative to control cultured in RPMI 1640 medium containing 5% fetal bovine serum/2 mM L-glutamine.

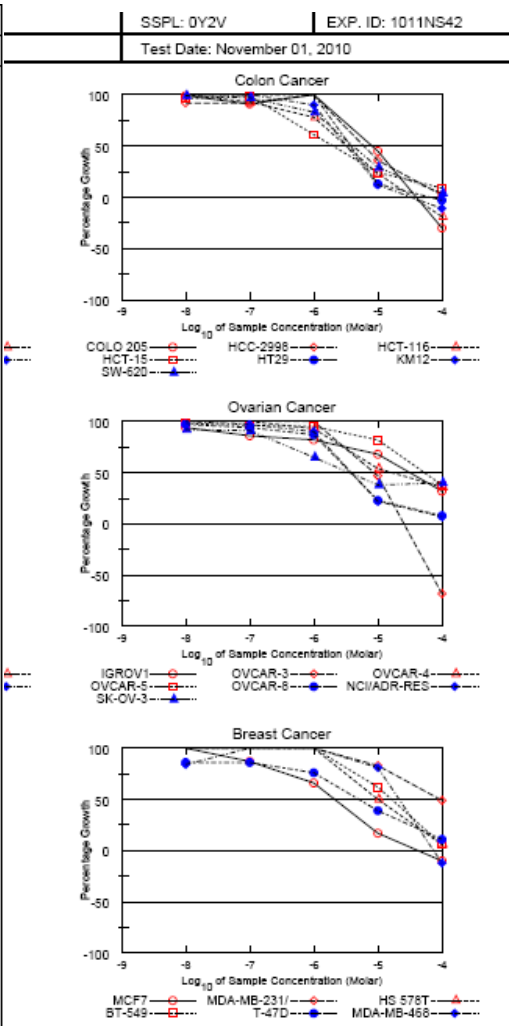
NCI-60 five-dose screen of novel F-rings

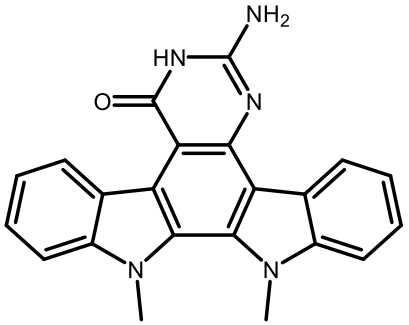
- A number of our BIM and ICZ F-ring derivatives have been brought forward for five-dose screen and tested against the cell line panel at concentrations ranging from 100 µM to 10 nM.
- Dose-response curves are generated for each cell line.
- Three characteristic *in vitro* parameters, GI_{50}, TGI and LC_{50}, were calculated for each cell line in response to the presence of the different drug candidates.
- To date, success has been seen in maleimide, isoxazole, imidazole, pyrazole and pyrazolone 5-membered systems in addition to a number of 6-membered systems.



Full NCI screening profile of Indolocarbazole A



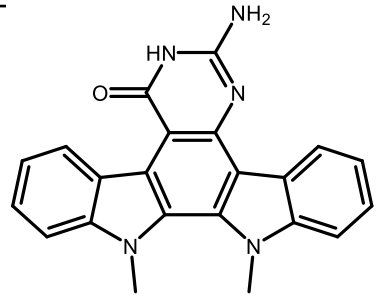




- Not active against Topo II
- Not toxic at highest dose tested
- Remarkable diversity within panels, eg. renal

Full Indolecarbazole A screening data: 5-Dose study

Cell line	Tumour type	% Cell growth after 48h§					GI_{50} µM	TGI µM	LC_{50} µM
		10 nM	100 nM	1 µM	10 µM	100 µM			
SF-295	CNS	85	93	106	7	-57	3.68	12.9	76.7
HCT-15	colon	96	99	61	24	9	1.98	>100	>100
SK-MEL-2	melanoma	103	112	73	-10	-55	1.90	7.59	76.5
SK-MEL-5	melanoma	89	98	87	15	-97	3.25	13.7	37.9
UACC-257	melanoma	93	91	104	64	-62	12.8	32.0	79.7
OVCAR-3	ovarian	112	107	120	47	-68	9.07	25.6	69.8
ACHN	kidney	94	92	51	25	12	**1.11**	>100	**>100**
CAKI-1	kidney	93	85	53	29	3	**1.33**	>100	**>100**
UO-31	kidney	85	72	55	19	17	**1.37**	>100	**>100**



Conclusions

- Successfully synthesised a panel of novel derivatives of the ICZ template.
- Developed two new routes, previously unreported in the literature, to allow access to (aza)indolocarbazoles. A panel of novel bisindole analogues has also been synthesised using this route.
- Explored the theme of F-ring modulation towards the potentiation of inhibitory activity against protein kinases and topoisomerase I.
- Of 45 compounds submitted to date to the National Cancer Institute, 20 have been selected for five-dose screening, and 5 candidates have been brought before the Biological Evaluation Committee.
- Currently undergoing kinase screen in collaboration and a new application to light mediated therapy
- Synthetic efforts have been informed by the results to date and significant improvements in potency are on-stream.

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