Exploring Natural Product Chemistry and Biology with Multicomponent Reactions. 5. Discovery of a Novel Tubulin-Targeting Scaffold Derived from the Rigidin Family of Marine Alkaloids

1

Liliya V. Frolova, Igor V. Magedov, Anntherese E. Romero, Menuka Karki, Isaiah Otero, Kathryn Hayden, Nikolai M. Evdokimov, Laetitia Moreno Y. Banuls, Shiva K. Rastogi, W. Ross Smith, Shi-Long Lu, Robert Kiss, Charles B. Shuster, Ernest Hamel, Tania Betancourt, Snezna Rogelj and Alexander Kornienko

J. Med. Chem., 2013, **56**, 6886-6900

(Texas State Uni, San Marcos, Texas)

A. Manos-Turvey,

Wipf Group Current Literature

October 12th, 2013

Active Natural Products as Inspiration for MCR Scaffolds

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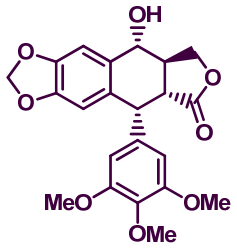
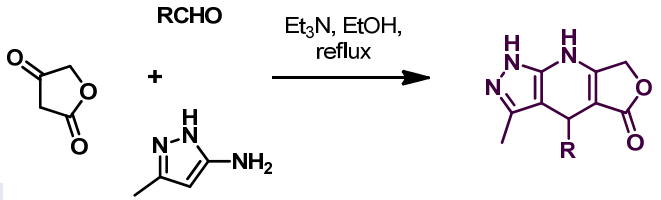


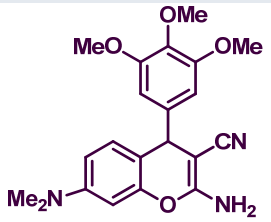
podophyllotoxin

Structural simplification of bioactive natural products with multicomponent synthesis: Dihydropyridopyrazole analogues of podophyllotoxin

Igor V. Magedov,[a,b,*] Madhuri Manpadi,[c] Elena Rozhkova,[a] Nikolai M. Przheval'skii,[a] Snezna Rogelj,[d] Scott T. Shors,[d] Wim F. A. Steelant,[c] Severine Van slambrouck[c] and Alexander Kornienko[c,*]



J. Med. Chem. **2008**, *51*, 2561–2570 256

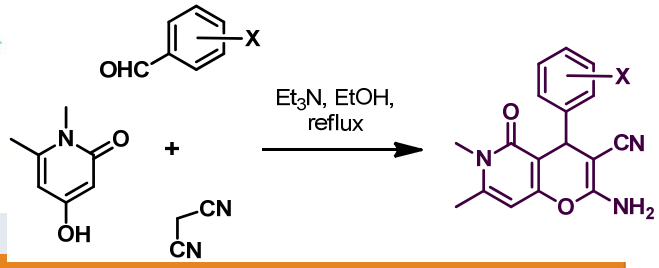


chromene derivative

Structural Simplification of Bioactive Natural Products with Multicomponent Synthesis. 2. Antiproliferative and Antitubulin Activities of Pyrano[3,2-*c*]pyridones and Pyrano[3,2-*c*]quinolones

Igor V. Magedov,*[,†,‡] Madhuri Manpadi,[†] Marcia A. Ogasawara,[†,§] Adriana S. Dhawan,[§] Snezna Rogelj,[§] Severine Van slambrouck,[†] Wim F. A. Steelant,[†] Nikolai M. Evdokimov,[‡] Pavel Y. Uglinskii,[‡] Eerik M. Elias,[†] Erica J. Knee,[†] Paul Tongwa,[||] Mikhail Yu. Antipin,[||,⊥] and Alexander Kornienko*[,†]

Department of Chemistry, New Mexico Institute of Mining and Technology, Socorro, New Mexico 87801, Department of Organic Chemistry, Timiryazev Agriculture Academy, Moscow 127550, Russia, Department of Biology, New Mexico Institute of Mining and Technology, Socorro, New Mexico 87801, Department of Natural Sciences, New Mexico Highlands University, Las Vegas, New Mexico 87701, Institute of Organoelement Compounds, Russian Academy of Sciences, Moscow, Russia



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Journal of Medicinal Chemistry

J. Med. Chem. 2011, 54, 2012–2021

ARTICLE

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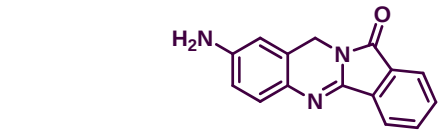
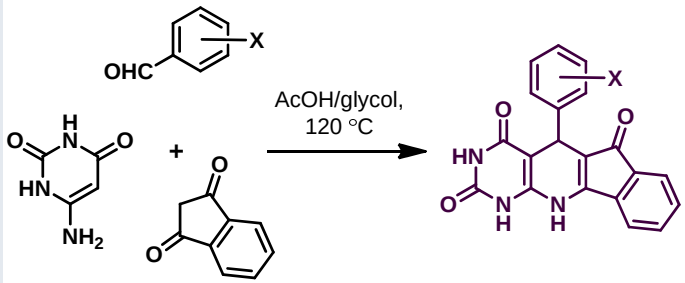
Structural Simplification of Bioactive Natural Products with Multicomponent Synthesis. 3. Fused Uracil-Containing Heterocycles as Novel Topoisomerase-Targeting Agents

Nikolai M. Evdokimov,[†] Severine Van slambrouck,[†] Petra Heffeter,[‡] Lee Tu,[†] Benjamin Le Calvé,[§] Delphine Lamoral-Theys,[§] Carla J. Hooten,[†] Pavel Y. Uglinskii,[∥] Snezna Rogelj,[⊥] Robert Kiss,[§] Wim F. A. Steelant,[†] Walter Berger,[‡] Jeremy J. Yang,[#] Cristian G. Bologa,[#] Alexander Kornienko,[*,†] and Igor V. Magedov[*,†]





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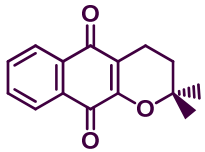
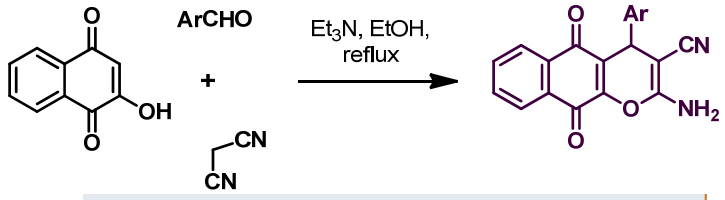


Structural simplification of bioactive natural products with multicomponent synthesis. 4. 4*H*-Pyrano-[2,3-*b*]naphthoquinones with anticancer activity

Igor V. Magedov[a,*], Artem S. Kireev[a], Aaron R. Jenkins[a], Nikolai M. Evdokimov[a], Dustin T. Lima[a], Paul Tongwa[b], Jeff Altig[a], Wim F. A. Steelant[a,c], Severine Van slambrouck[a], Mikhail Yu. Antipin[b], Alexander Kornienko[a,*]



Active Natural Products as Inspiration for MCR Scaffolds

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Exploring Natural Product Chemistry and Biology with Multicomponent Reactions. 5. Discovery of a Novel Tubulin-Targeting Scaffold Derived from the Rigidin Family of Marine Alkaloids

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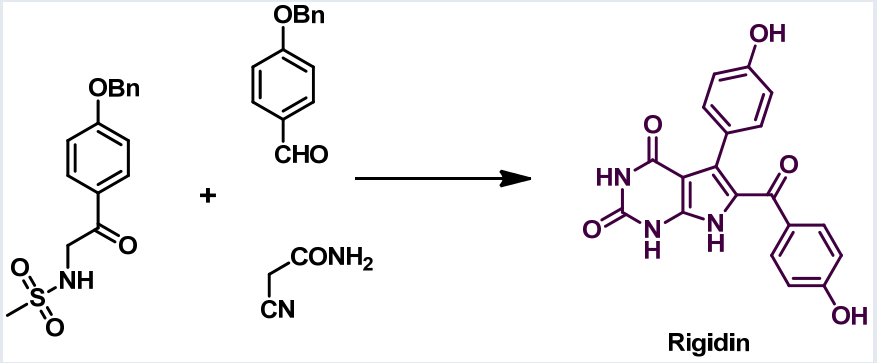
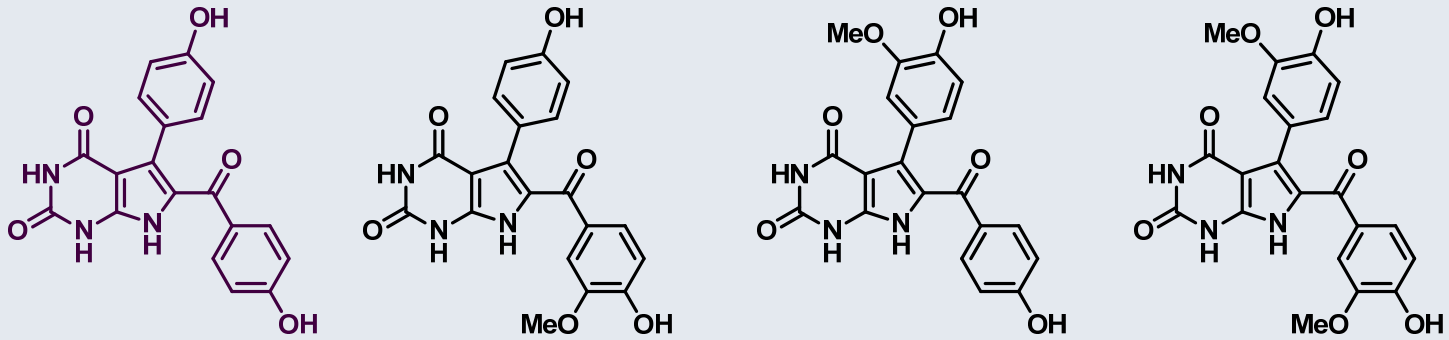


Rigidin: Pyrrolopyrimidine Alkaloids

- Rigidin A was first isolated in 1990 from *Eudistoma* cf. *rigida* in Okinawan, Japan.
- Rigidin B-D were then isolated from the same region in *Cystodytes* sp. in 2002.



Rigidin A
(0.0015% wet weight
12 mg, 0.00075%)

Rigidin B
(4.9 mg, 0.000031% wet weight)

Rigidin C
(1.3 mg, 0.00008%)

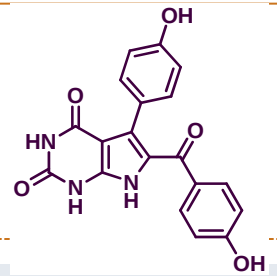
Rigidin D
(0.6 mg, 0.00004%)

- Initial interest lay in the bioactivity of these compounds
  - Rigidin A is a proven calmodulin antagonist, while B, C and D were reported to show inhibition of murine leukemia

J. Kobayashi, J.-f. Cheng, Y. Kikuchi, M. Ishibashi, S. Yamamura, Y. Ohizumi, T. Ohta, S. Nozoe, Tett. Lett., **1990**, *31*, 4617-4620

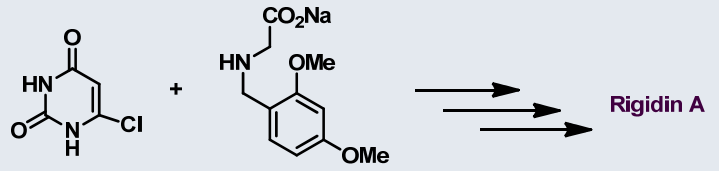
M. Tsuda, K. Nozawa, K. Shimbo, J. Kobayashi, J. Nat. Prod., **2003**, *66*, 292-294

Prior Syntheses of Rigidins A-D



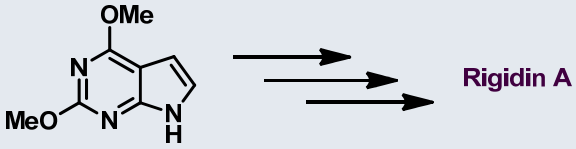
6

- 1993, Edstrom and Wei
  - 68 mg, 9 steps, 26%



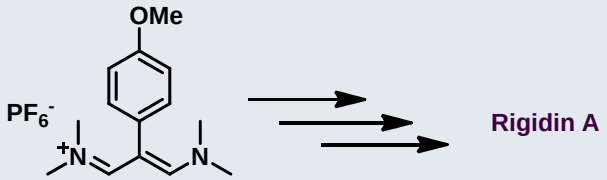
E.D. Edstrom, Y. Wei, J. Org. Chem., **1993**, *58*, 403-407

- 1994 and 1996, Yamanaka and co-workers
  - 6 steps, 9%; 30 mg, 5 steps, 11%



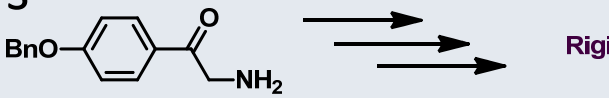
T. Sakamoto, Y. Kondo, S. Sato, H. Yamanaka, Tett. Lett., **1994**, *35*, 2919-2920;
T. Sakamoto, Y. Kondo, S. Sato, H. Yamanaka , J. Chem. Soc. Perkin Trans. I, **1996**, 459-464

- 2006, Sikorski and co-workers
  - 30 mg, 9 steps, 15%



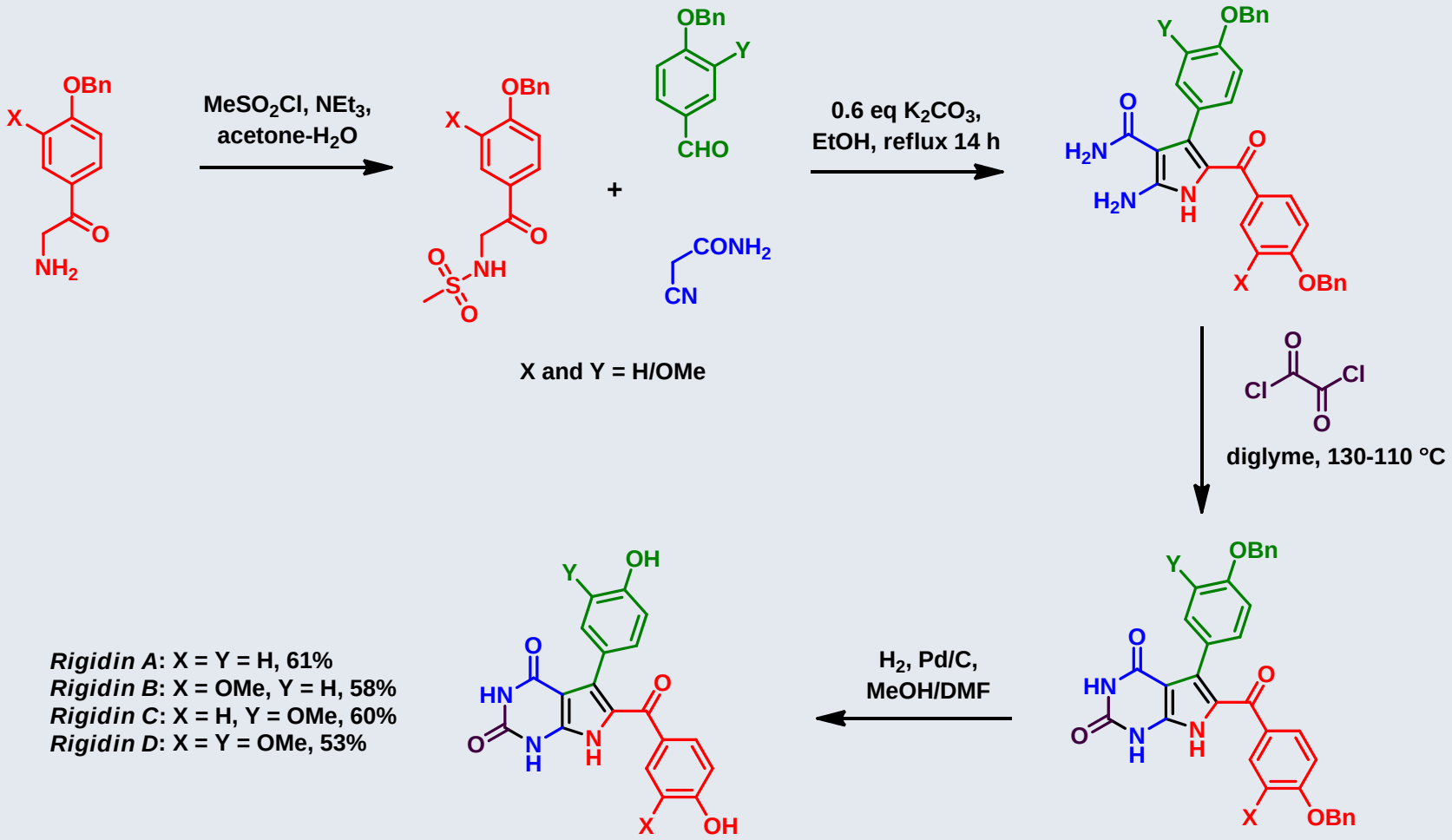
J.T. Gupton,....., J.A. Sikorski, Tetrahedron, 2006, *62*, 8243-8255

- 2011, Kornienko, Magedov and co-workers
  - 25 mg, 4 steps, 61%



L.V. Frolova, N.M. Evdokimov, K. Hayden, I. Malik, S. Rogelj, A. Kornienko, I.V. Magedov, Org. Lett., **2011**, *13*, 1118-1121

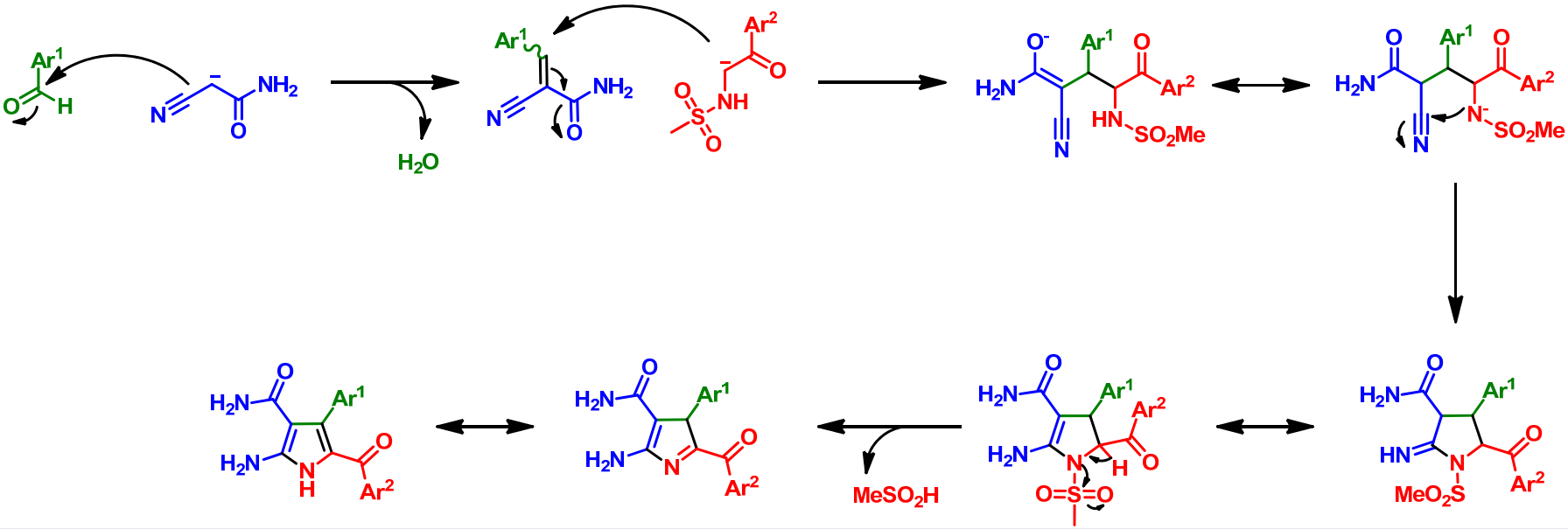
The Kornienko-Magedov Synthesis



Rigidin A: X = Y = H, 61%
Rigidin B: X = OMe, Y = H, 58%
Rigidin C: X = H, Y = OMe, 60%
Rigidin D: X = Y = OMe, 53%

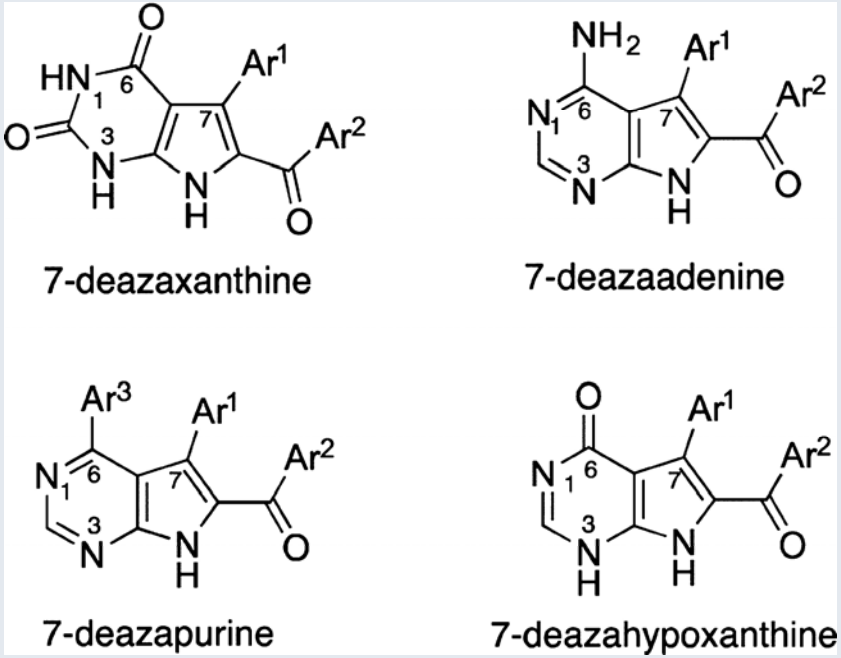
L.V. Frolova, N.M. Evdokimov, K. Hayden, I. Malik, S. Rogelj, A. Kornienko, I.V. Magedov, Org. Lett., **2011**, *13*, 1118-1121

MCR Mechanism

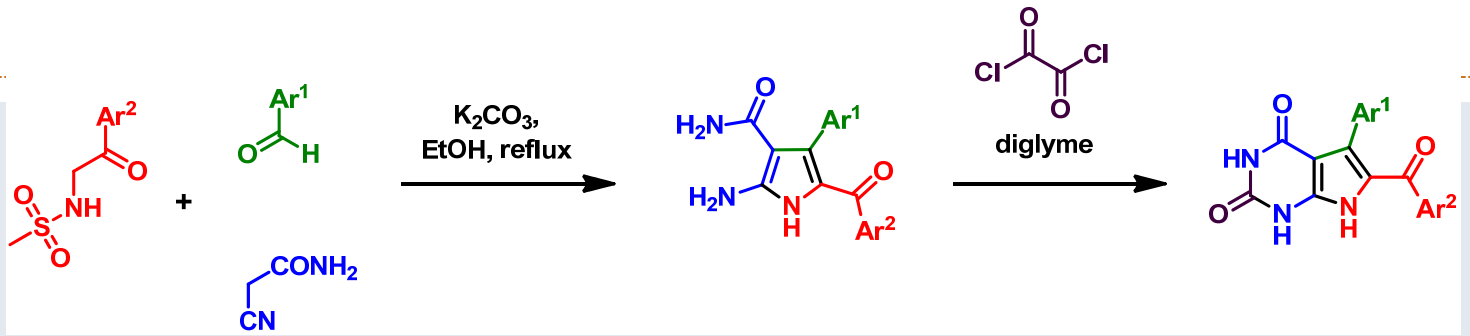


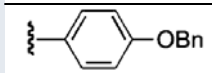
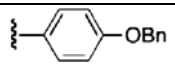
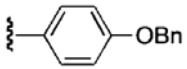
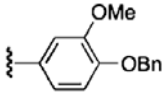
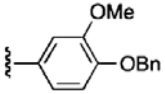
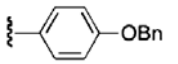
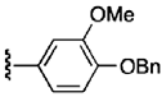
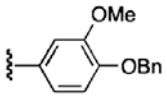
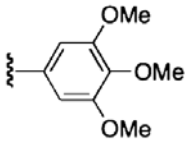
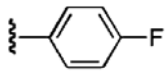
Aim: To Exploit the Rigidin Scaffold MCR

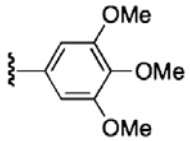
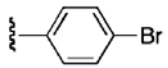
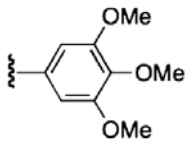
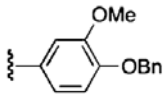
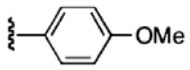
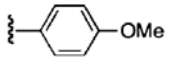
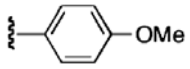
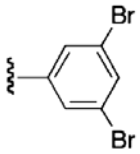
- synthesise analogues of Rigidin A-D and test for bioactivity
- investigate bioactivity of further MCR scaffolds obtained through adaptations of the original Rigidin forming reactants:



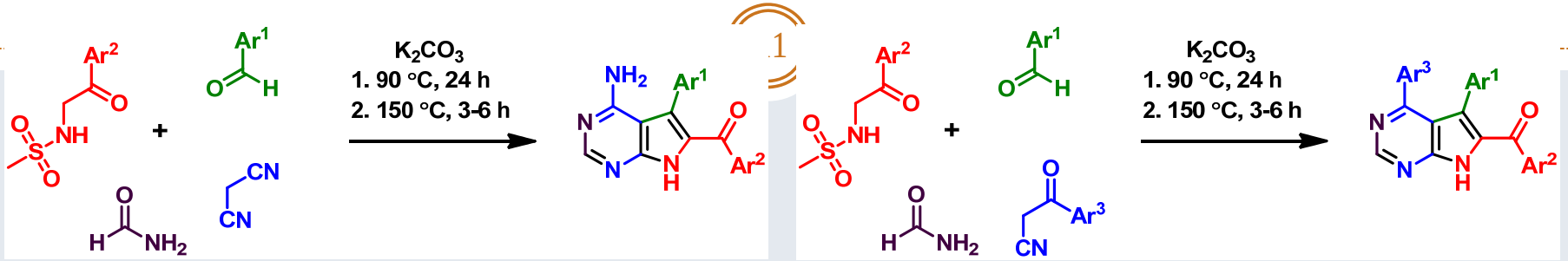
7-Deazaxanthine Synthetic Analogues

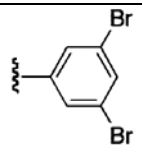
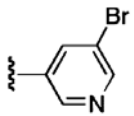


Ar1	Ar2	overall yield %
		64
		62
		62
		57
		74

Ar1	Ar2	overall yield %
		64
		59
		57
	Ph	62
	Ph	43

4 Component Reaction Discoveries: 7-Deazaadenine and 7-Deazapurine Analogues



Ar1	Ar2	overall yield %
	Ph	58
	Ph	66
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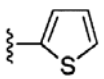
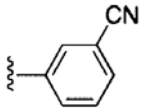
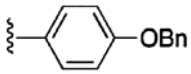
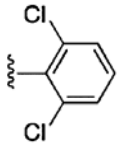
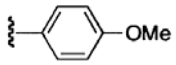
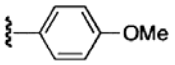
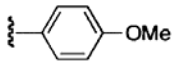
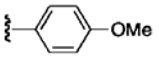
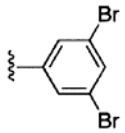
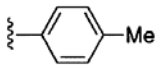
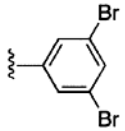
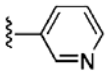
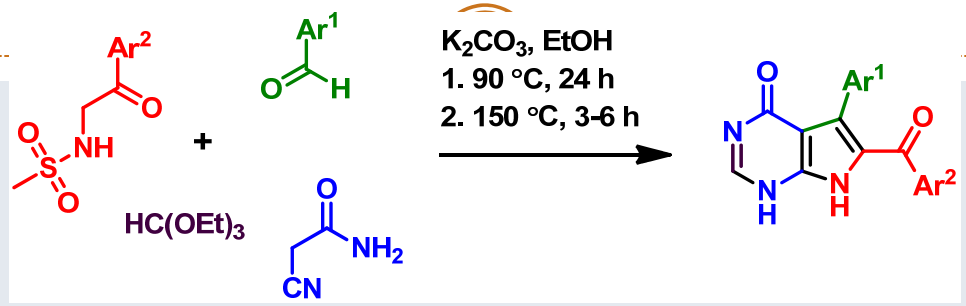
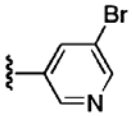
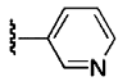
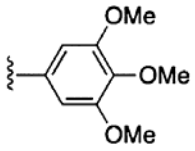
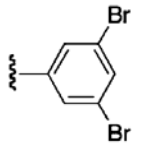
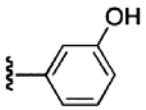
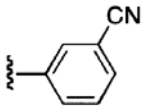
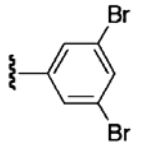
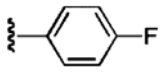
	Ph	85
		54
	Ph	72

Ar1	Ar2	Ar3	overall yield %
			63
	Ph	Ph	30
	Ph		33
	Ph	Ph	39
	Ph	Ph	22

4 Component Reaction Discoveries: 7-Deazahypoxanthine Analogues

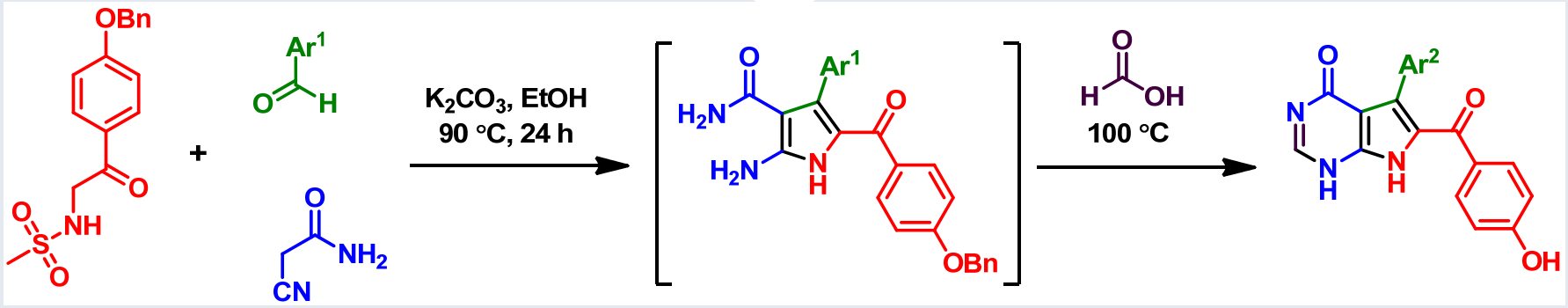


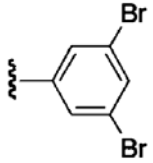
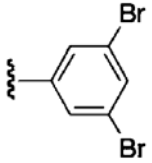
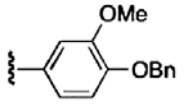
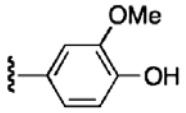
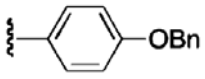
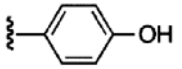
Ar1	Ar2	overall yield %
Ph	Ph	71
	Ph	88
	Ph	61
	Ph	64

Ar1	Ar2	overall yield %
	Ph	77
	Ph	26
	Ph	47
		56

(22 compounds in total)

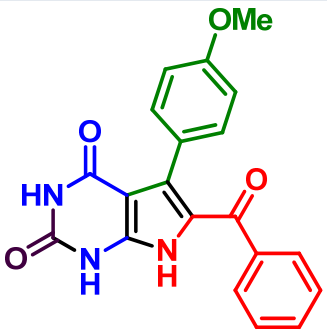
4 Component Reaction Discoveries: 7-Deazahypoxanthine Analogues



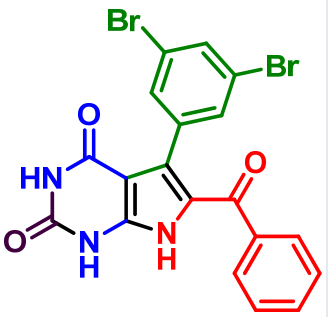
Ar[1]	Ar[2]	overall yield %
Ph	Ph	88
		70
		64
		71

Antiproliferative Activity

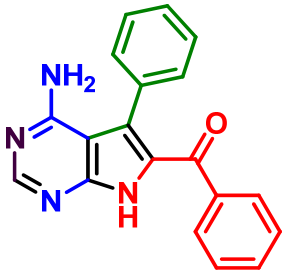
- compounds were tested against HeLa cells and MCF-7 cells
  - Rigidin A-D and related 7-deazaxanthine analogues were inactive (GI_{50} > 100 μM) or only weakly active
  - the 7-deazaadenine and 7-deazapurine analogues (4-CR) showed higher potency in select cases



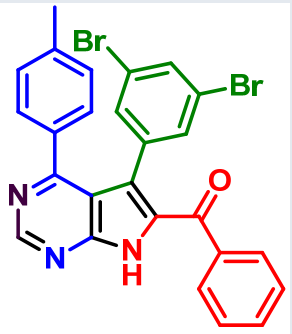
HeLa GI_{50} = 80 μM
MCF-7 GI_{50} = 14 μM



HeLa GI_{50} = 71 μM
MCF-7 GI_{50} = 47 μM



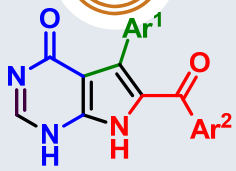
HeLa GI_{50} = 4.4 μM
MCF-7 GI_{50} = 3.9 μM

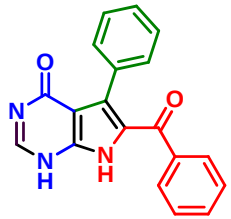


HeLa GI_{50} = 9.0 μM
MCF-7 GI_{50} = 7.0 μM

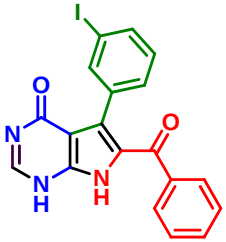
Antiproliferative Activity of the 7-Deazahypoxanthines

15

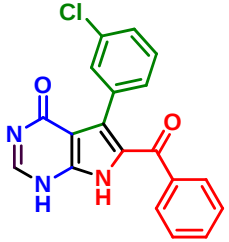




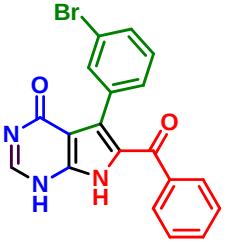
HeLa GI_{50} = 0.035 μM
MCF-7 GI_{50} = 0.040 μM



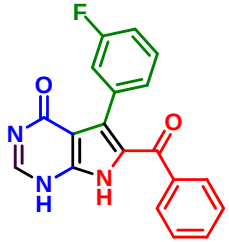
HeLa GI_{50} = 0.2 μM
MCF-7 GI_{50} = 0.15 μM



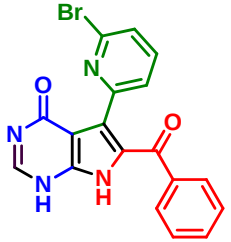
HeLa GI_{50} = 0.1 μM
MCF-7 GI_{50} = 0.06 μM



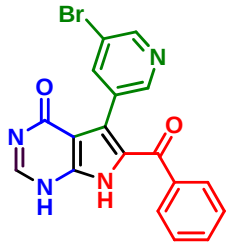
HeLa GI_{50} = 0.13 μM
MCF-7 GI_{50} = 0.06 μM



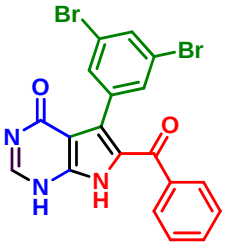
HeLa GI_{50} = 0.065 μM
MCF-7 GI_{50} = 0.070 μM



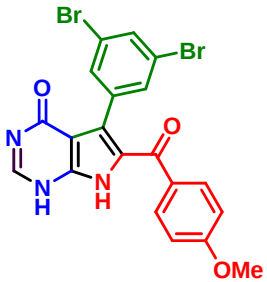
HeLa GI_{50} = 0.15 μM
MCF-7 GI_{50} = 0.135 μM



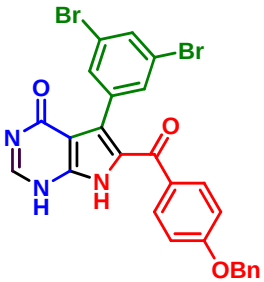
HeLa GI_{50} = 0.080 μM
MCF-7 GI_{50} = 0.087 μM



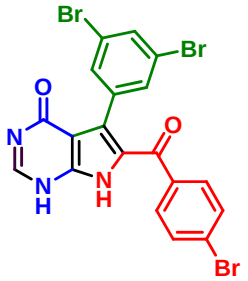
HeLa GI_{50} = 0.095 μM
MCF-7 GI_{50} = 0.095 μM



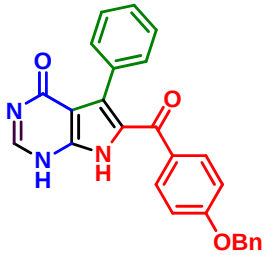
HeLa GI_{50} = 0.30 μM
MCF-7 GI_{50} = 0.11 μM



HeLa GI_{50} = 0.40 μM
MCF-7 GI_{50} = 0.31 μM



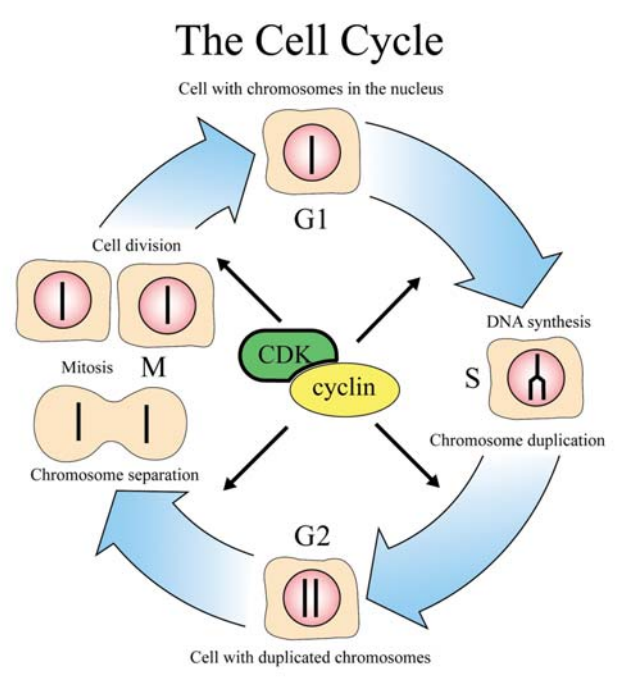
HeLa GI_{50} = 2.2 μM
MCF-7 GI_{50} = 0.6 μM

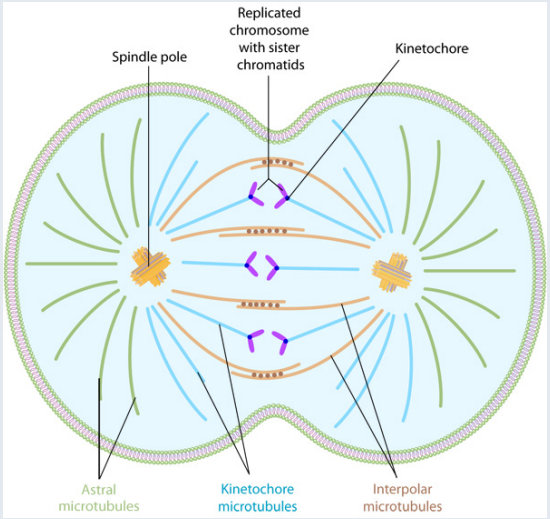


HeLa GI_{50} = 0.045 μM
MCF-7 GI_{50} = 0.053 μM

Mode of Action: Tubulin Dynamics?

- the 7-deazahypoxanthines appeared to induce cellular morphology changes attributed to tubulin dynamic inhibition
  - tubulin polymers form microtubules, involved in cell division and mitotic spindle formation (cell division , which may account for antiproliferative activity



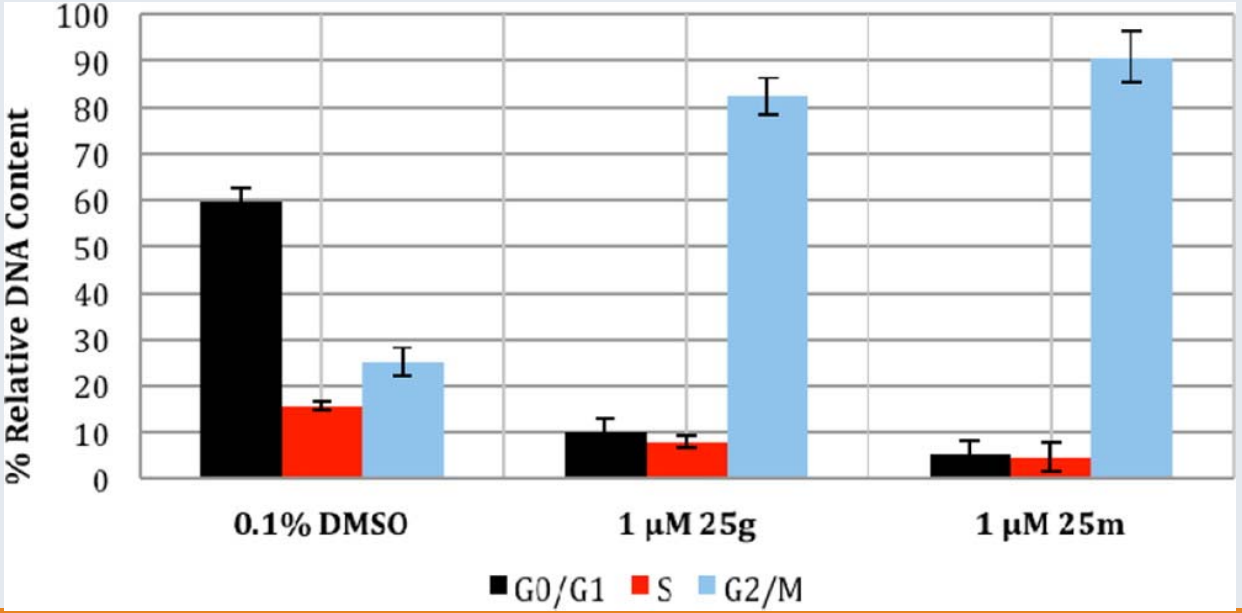


http://fhs-bio-wiki.pbworks.com/w/page/12145745/Cell%20Cycle

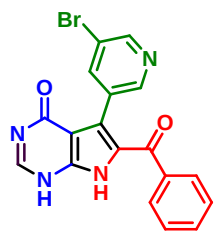
http://www.nature.com/scitable/content/types-of-microtubules-involved-in-mitosis-14752887

Mode of Action: Tubulin Dynamics?

- the 7-deazahypoxanthines appeared to induce cellular morphology changes attributed to tubulin dynamic inhibition
  - cell cycle analyses were run in HeLa cells using a dye which measures DNA mass per cell
  - cells are arresting in the G2/M phase, indicative of microtubule assembly disruption

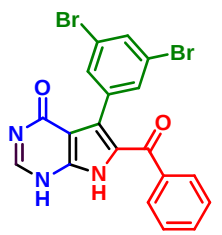


25g



HeLa GI_{50} = 0.080 μM
MCF-7 GI_{50} = 0.087 μM

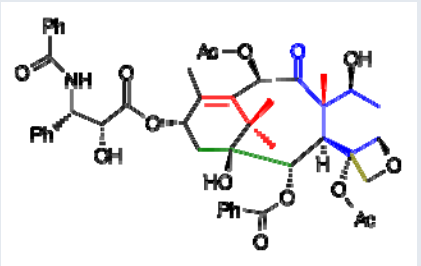
25m

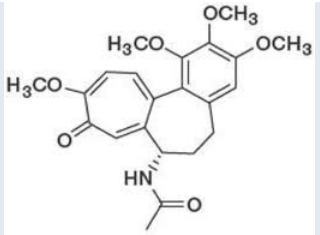


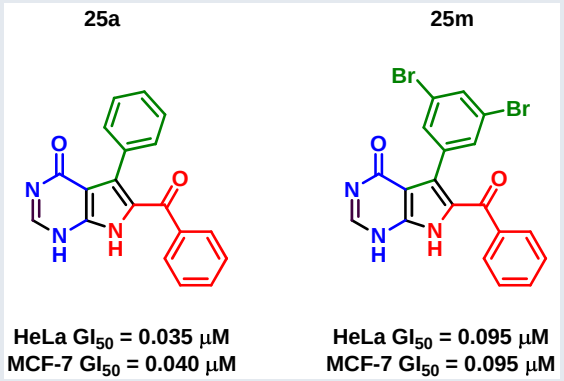
HeLa GI_{50} = 0.095 μM
MCF-7 GI_{50} = 0.095 μM

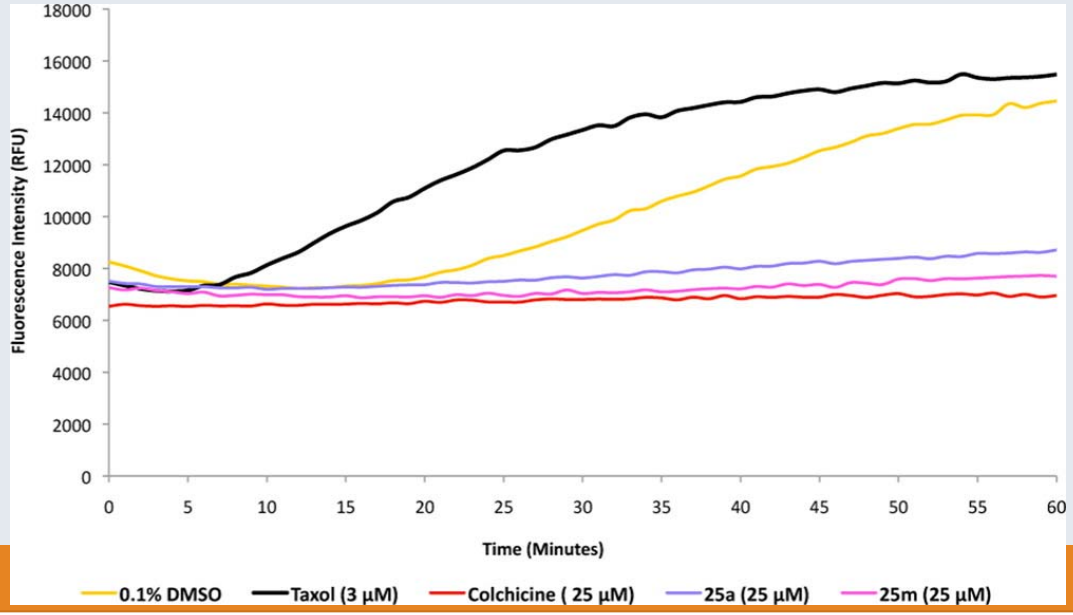
Effect Upon Tubulin Assembly

- the 7-deazahypoxanthine analogues were tested for tubulin polymerisation interactions
  - measured polymerisation by fluorescent courtesy of a fluorescent reporter into the microtubules
  - tested alongside taxol (microtubule stabiliser) and colchicine (microtubule destabiliser)
  - two potent inhibitors of microtubule formation were identified



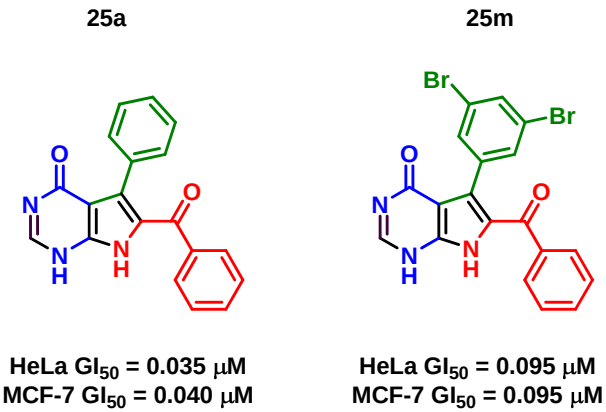






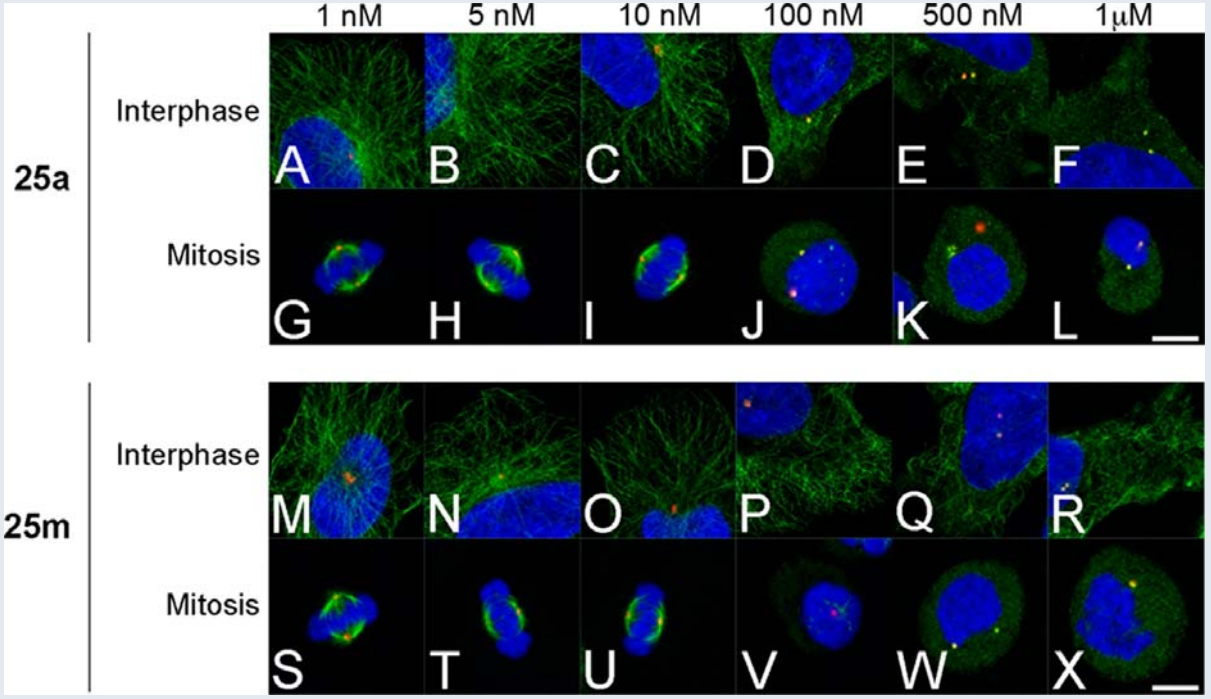
Microtubule Organisation

- 25a and 25m were tested in HeLa cells (3 h) and microtubule morphology was measured
  - probed for microtubules (green), centrosome marker pericentrin (red) and DNA (blue)
  - Bar = 10 µm



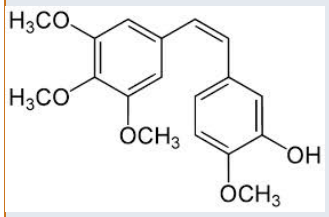
HeLa GI_{50} = 0.035 µM
MCF-7 GI_{50} = 0.040 µM

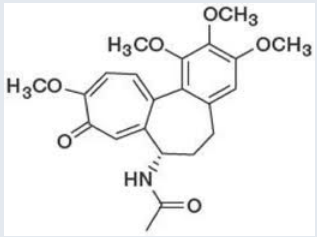
HeLa GI_{50} = 0.095 µM
MCF-7 GI_{50} = 0.095 µM



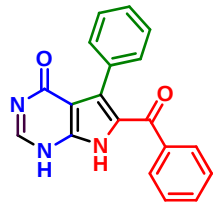
Source of Tubulin Assembly Inhibition

- tested 25a and 25m for tubulin polymerisation inhibition alongside combretastin A-4 and for the ability to displace colchicine
  - carried out using a quantitative turbidimetric assay (assembly measured at 350 nm)



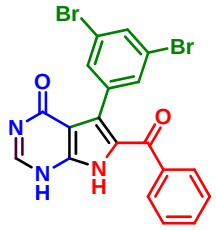


25a



HeLa GI_{50} = 0.035 μM
MCF-7 GI_{50} = 0.040 μM

25m



HeLa GI_{50} = 0.095 μM
MCF-7 GI_{50} = 0.095 μM

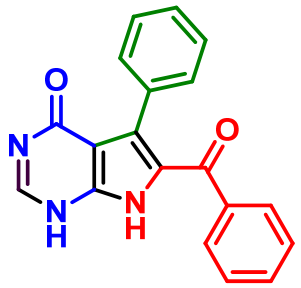
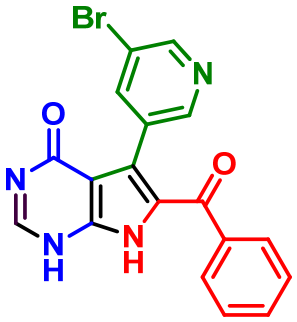
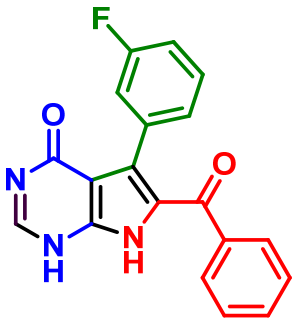
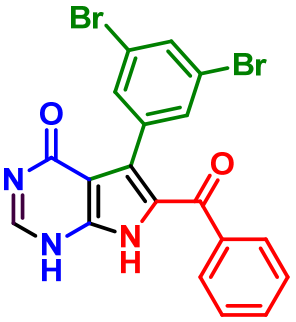
compound	inhibition of tubulin assembly[b] IC$_{50}$ (μM) ± SD	inhibition of colchicine binding[c] % inhibition ± SD 5 μM inhibitor	1 μM inhibitor
CA-4[a]	0.96 ± 0.07	99 ± 0.4	89 ± 0.6
25a	1.2 ± 0.1	88 ± 0.7	58 ± 4
25m	1.6 ± 0.04	71 ± 0.5	ND

[a]CA-4 = combretastatin A-4. [b]Inhibition of tubulin polymerization by selected compounds. Tubulin was at 10 μM. [c]% Inhibition of [^{3}H]colchicine (5 μM) binding to tubulin (1 μM) by selected compounds.

E. Hamel, Cell Biochem. Biophys., **2003**, *38*, 1-22

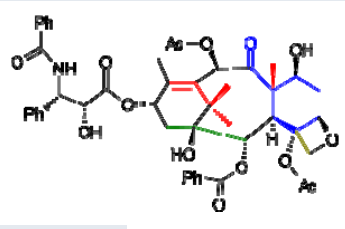
Colchicine Tubulin Binding Site

- no FDA approved colchicine tubulin binding pocket inhibitors however these agents have key characteristics:
  - good oral bioavailability
  - MDR tumour cell growth inhibitors
  - low neurotoxicity

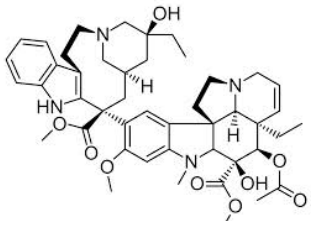
25a	25g	25e	25m
			
Solubility: 10 μM	**Solubility: 30 μM**	**Solubility: 65 μM**	**Solubility: 40 μM**
HeLa GI_{50} = 0.035 μM MCF-7 GI_{50} = 0.040 μM	HeLa GI_{50} = 0.080 μM MCF-7 GI_{50} = 0.087 μM	HeLa GI_{50} = 0.065 μM MCF-7 GI_{50} = 0.070 μM	HeLa GI_{50} = 0.095 μM MCF-7 GI_{50} = 0.095 μM

MDR Cell Antiproliferative Activity

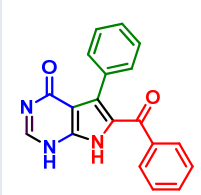
- colchicine site agents are usually insensitive to P-glycoprotein
  - sensitivity of 25a and 25m was measured against MES-SA (parent uterine sarcoma cell line) and MDR resistant MES-SA/Dx3 (resistant to a number of P-gp substrates



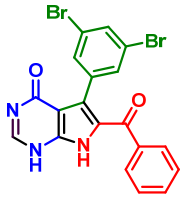
25a



25m



HeLa GI_{50} = 0.035 μM
MCF-7 GI_{50} = 0.040 μM



HeLa GI_{50} = 0.095 μM
MCF-7 GI_{50} = 0.095 μM

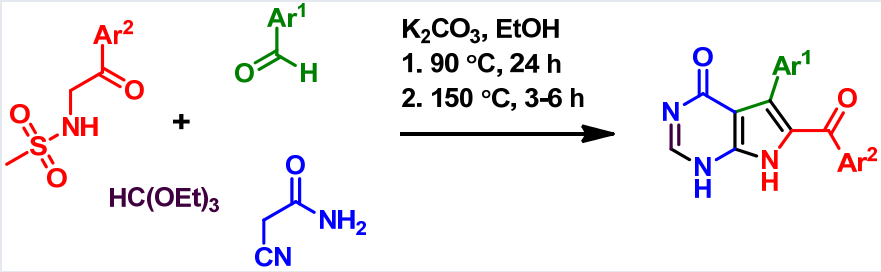
	GI_{50} in vitro values (nM)[a]	
	MES-SA	MES-SA/Dx5
taxol	7 ± 1	9800 ± 283
vinblastine	6 ± 1	5000 ± 1414
25m	81 ± 6	394 ± 10
25a	30 ± 4	70 ± 4

[a]Concentration required to reduce the viability of cells by 50% after a 48 h treatment with the indicated compounds relative to a DMSO control ± SD from two independent experiments, each performed in four replicates, as determined by the MTT assay.

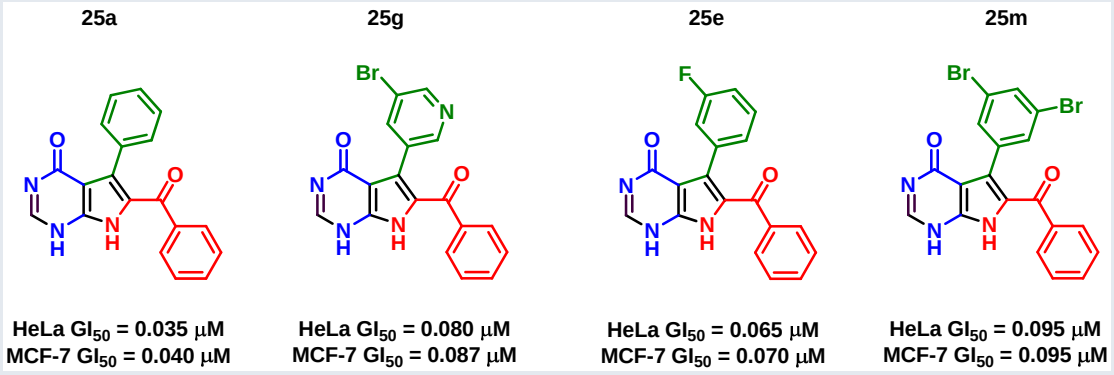
  - Furthermore: 25a, 25g and 25m all maintained nM antiproliferative activity against a selection of dismal prognoses cancer lines and tumour mestases cell lines

Conclusions

- MCRs were used to synthesise a range of analogues with structures of biological interest



- The 7-deazahypoxanthine compounds showed greatest activity



  - the action of these compounds is attributed to tubulin polymerisation inhibition, thereby impeding microtubule dynamics