[c0003]

Cantharidin: The Next Generation. Towards Selective Inhibitors of Protein Phosphatase 1 and 2A

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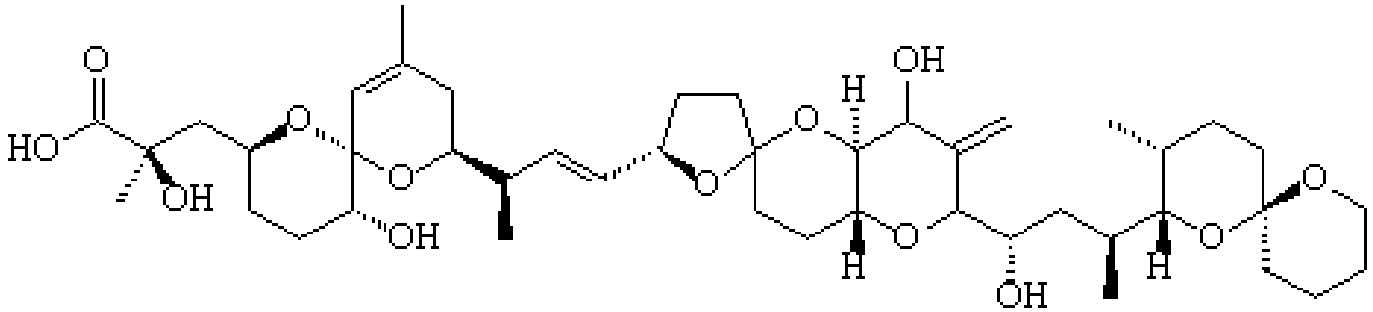
Introduction

Reversible protein phosphorylation plays a pivotal role in cellular signal transduction, moderating such diverse functions as neurotransmission, muscle contraction, glycogen synthesis, T-cell activation and cell proliferation.[1-5]

Serin/threonine phosphatases, which are responsible for protein dephosphorylation. Of the serine/threonine phosphatases, protein phosphatases 1 and 2A (PP1 and PP2A, respectively) share sequence identity between both enzyme subunits (50% for residues 23-292; 43% overall), are present in all eukarytoic cells and are together responsible for 90% of all cellular dephosphorylation.

An interesting link between PP1 and PP2A is their shared sensitivity towards a structurally diverse family of natural products: the okadaic acid (**1**) class of compounds.



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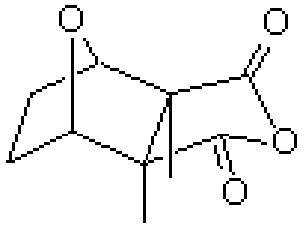
However, with the exceptions of (**1**), cantharidin (**2**) and thyrisferyl 23-acetate (**3**) (being PP2A selective) they exhibit poor selectivity.



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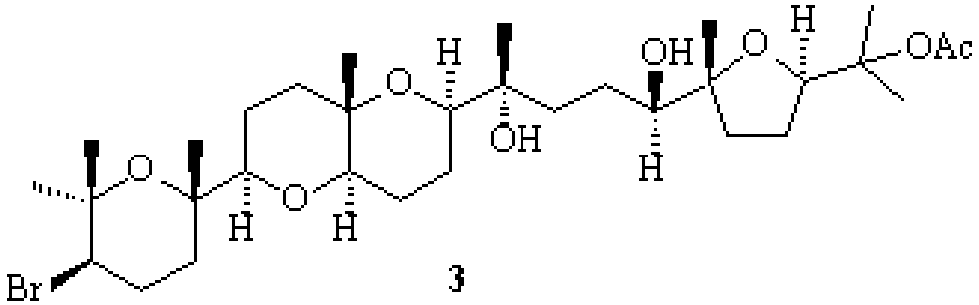

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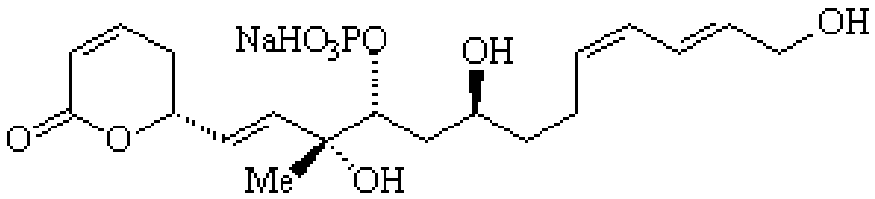
The current challenge remains the development of an inhibitor with either absolute specificity or high enough selectivity, which renders the inhibitor effectively specific in vivo.

Phosphatase Inhibitors: Friend or Foe?



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Fostrecin (**4**) exhibits >40 000 fold selectivity for PP2A (IC_{50} 3.4nM) over PP1. As with other protein phosphatase inhibitors (eg okadaic acid, calyculin A), fostrecin also abrogates the G_2 checkpoint of the cell cycle.[6]

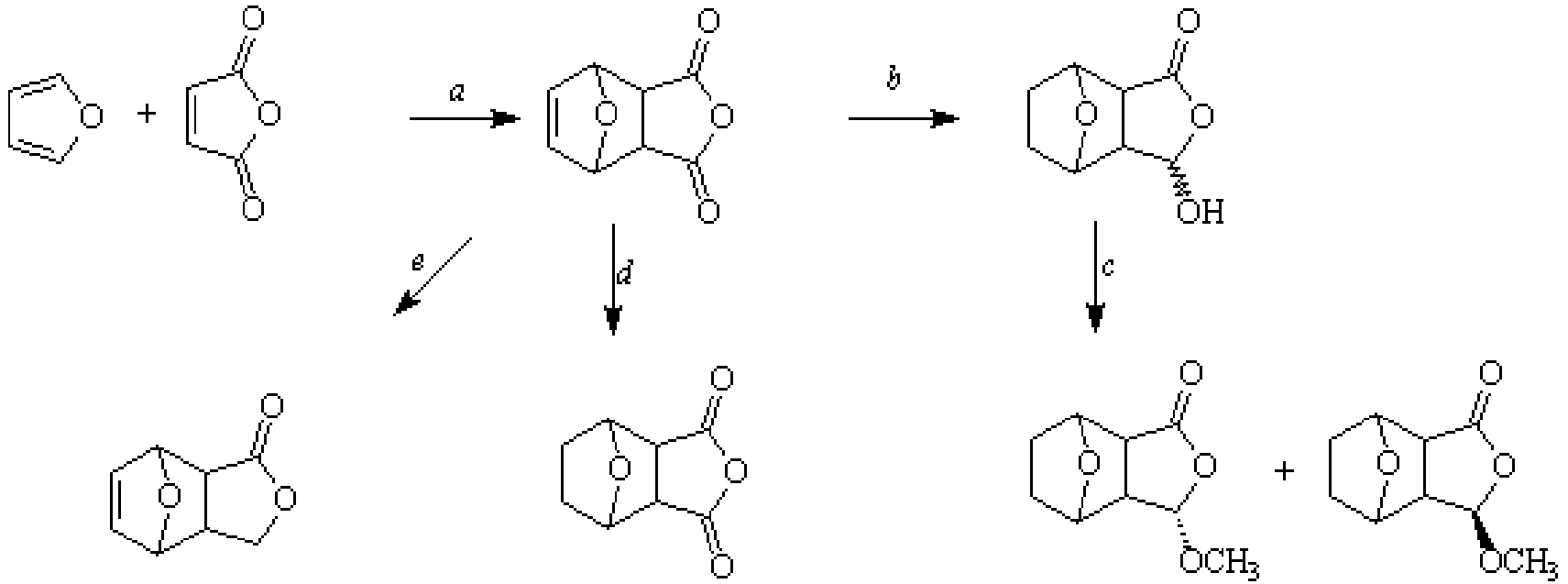
Abrogation of the G_2 checkpoint forces the cells prematurely into mitosis with inadequate spindle formation and unreplicated DNA.[2] In contrast to okadaic acid, fostrecin is cytotoxic as a single agent and is currently undergoing phase 1 clinical trials at NCI as an anti-cancer agent (active against leukemia (L1210, IC_{50} 0.46 M), lung, breast, and ovarian cancer cells and displays efficacious in vivo anti-tumour activity against L1210 leukemia in mice). ***This is perhaps a surprising result, given that okadaic acid (and other members of this class of compounds) is hepatotoxic and known tumour promotors.***[7]

Fostriecin, however is sensitive to oxidation, and loses 50% of its ability to promote entry into mitosis during the first 30 Min of incubation in cell culture medium.

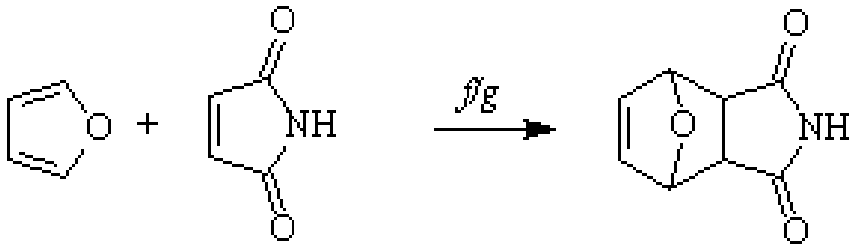
It is becoming increasingly apparent that the abrogation of the G_2 checkpoint in the cell cycle via protein phosphatase inhibition clearly offers new avenues for anti-cancer research.

Our recent studies have revolved around the bioisostereic replacement of the heteroatom in cantharidin, here were report our recent findings in light of the anti-cancer activity of fostrecin.

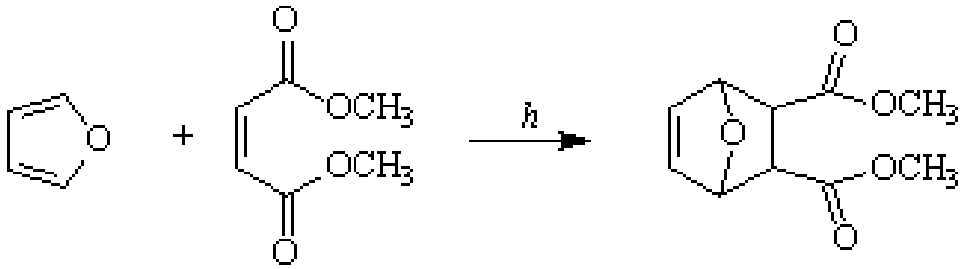
Chemistry



Scheme 1. *a*. Furan:maleic anhydride (5:1), diethylether, 2d, RT, 96%; *b*. H_2 / 10% Pd-C/ EtOH; *c*. *p*-TosOH, MeOH, chromatography; *d*. H_2 / 10% Pd-C/ Acetone; *e*. $NaBH_4$, then HCl.



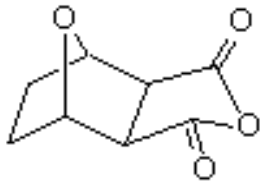
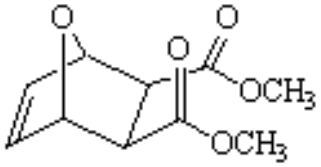
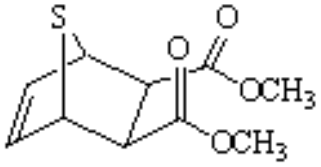
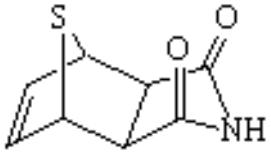
Scheme 2. Reagents and Conditions: *f*. Furan:maleimide (5:1), diethyl ether, 7d, in dark, 75%, exo product; *g*. Furan:Maleimide (5:1), diethylether, sealed tube 12h, 90ºC, 66%, endo product.



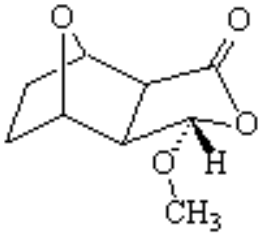
Scheme 3. Reagents and Conditions: *h*. Furan: dimethylmaleate (2:1), CH_2Cl_2, 17 Kbar, 40ºC, 61 h, 56%.

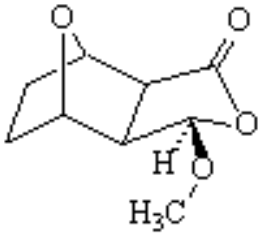
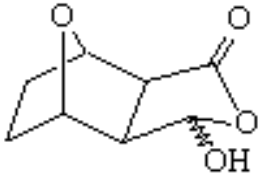
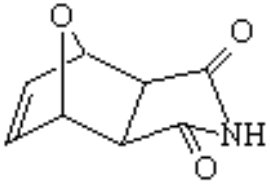
Protein Phosphatase Inhibition

Anhydride Modified Cantharidin Analogues

Compound	Inhibition of PP1 (%)	Inhibition of PP2A (%)	Selectivity PP2A/PP1
	90 IC_{50} 2.4 M	97 IC_{50} 2.1 M	0.875
	46 IC_{50} 50 M	6 IC_{50}>10, 000 M	>200
	3	3	Not determined
	15	69*	Not determined

*IC_{50} determination in progress

Compound	Inhibition of PP1 (%)	Inhibition of PP2A (%)	Selectivity PP2A/PP1
	13	11	Not determined
	15	8	Not determined

			
	9	11	Not determined
	-	15	Not determined

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6. Roberge M; Tudan C; Hung SMF; Harder K W; Jericho F R; Anderson H; *Cancer Research* 1994, *54* 6115.

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Comments

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