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DYSIDIOLIDE AND RELATED γ-HYDROXY BUTENOLIDE COMPOUNDS AS INHIBITORS OF THE PROTEIN TYROSINE PHOSPHATASE, CDC25

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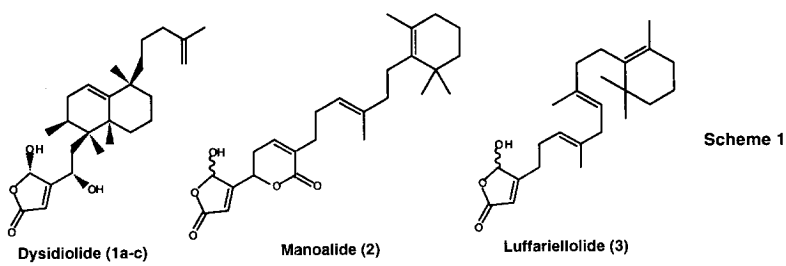
Abstract: Synthetic dysidiolide, as well as several related compounds containing a γ-hydroxybutenolide moiety, were tested in in vitro Cdc25 assays against both synthetic and natural substrates. Contrary to literature values which are in the low micromolar range, we observe only millimolar inhibitory activity for these compounds versus Cdc25 phosphatase.

The dual specificity protein-tyrosine phosphatase (dsPTPase), Cdc25, plays a pivotal role in the regulation of the cell cycle. The three human isoforms—Cdc25A, Cdc25B, and Cdc25C—appear to control specific cell cycle transition points by activating key cyclin-dependent kinases (Cdk). For example, Cdc25C phosphatase is required for entry into mitotsis, and activates the Cdk1/CycB enzyme by dephosphorylating Thr14 and Tyr15 residues on Cdk1.[1] Cdc25A and Cdc25B are known to be oncogenic and are overexpressed in a number of tumor cell lines.[2] Consequently, inhibitors of Cdc25 may exhibit anti-proliferative properties.

We have been interested in the identification of molecules[3] that would inhibit Cdc25 and thereby demonstrate anticancer properties. Recently dysidiolide (**1**) was reported to be a selective and potent inhibitor of Cdc25.[4] This report sparked a great deal of interest in the synthetic community, as noted by three recent syntheses of this material.[5] Of particular note is the presence of a γ-hydroxybutenolide, which has been proposed to serve as a phosphate surrogate in the interaction with Cdc25. We have been intrigued by this proposal since a neutral phosphate mimic would be of significant importance in this area. Herein, we report that we have tested several γ-hydroxybutenolide containing compounds, including synthetically derived dysidiolide, to determine if they are indeed Cdc25 inhibitors in vitro, and to examine the potential of this functional group as a phosphate surrogate.



Dysidiolide (1a-c) Manoalide (2) Luffariellolide (3)

Scheme 1

The IC_{50} data for synthetic and natural isolates of dysidiolide, two closely related natural products, manoalide (**2**) and luffariellolide (**3**), as well as two simpler congeners are shown in Table 1. In addition to the crude extract, **1a**,[4] we also tested the natural enantiomer, **1b**,[5a] and the racemate, **1c**,[5b] for inhibitory activity. Although the different samples of dysidiolide were essentially inactive, using the synthetic substrate mFP,[6] we

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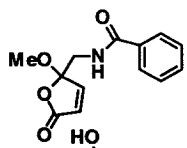
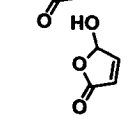
find that **2** and **3** are weak inhibitors of Cdc25. Synthetic dysidiolide also exhibits little, if any, inhibitory activity in a natural substrate-based assay using phosphorylated Cdk2/CycA (unpublished results). Finally, two known competitive inhibitors of dsPTPases, phosphate and tungstate, were tested under our standard assay conditions and were shown to inhibit Cdc25 phosphatase activity in vitro. Of general interest is our observation that the γ-hydroxy butenolide moiety serves as a weak phosphate replacement, and the inhibitory activity reported for dysidiolide may be due to an unidentified component in the crude extract.

Table 1 Inhibition of Cdc25A by γ-hydroxy butenolides

	IC_{50} (μM)
1a (extract)	>50,[a] 9.4 [4]
1b ((-) ent)	>2,000 [b,c]
1c (racemic)	> 2,000 [b,d]
2	1,000 [b]
3	990 [b]
	21,000 [b]
	12,000 [b]
Na_2PO_4	10,000 [b]
Na_2WO_4	30 [b,c,d]

(a) Assay conditions: 56 nM GST-Cdc25A, 60 μM mFP; Buffer:1 mM EDTA, 1 mM DTT, 50 mM Triethanolamine, pH 7.0, 50 mM NaCl, 25 °C. (b) Assay conditions: 5 nM Cdc25A catalytic domain,7 5 μM mFP; Buffer: 1 mM EDTA, 1 mM DTT, 0.01% Tween, 100 mM Imidazole:OAc, 100 mM KOAc, pH 7.2, 25 °C. (c) Tested against 5 nM Cdc25B and also 5 nM Cdc25C catalytic domains; 5 μM mFP; Buffer: 1 mM EDTA, 1 mM DTT, 0.01% Tween, 100 mM Imidazole:OAc, 100 mM KOAc, pH 7.2, 25 °C. (d) Tested against Cdc25A catalytic domain using phosphorylated Cdk2/CycA substrate; Buffer: 1 mM EDTA, 1 mM DTT, 100 mM Imidazole:OAc, 100 mM KOAc, pH 7.2, 25 °C.

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