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The Inhibitory Effects of Squalene-Derived Triterpenes on Protein Phosphatase PP2A

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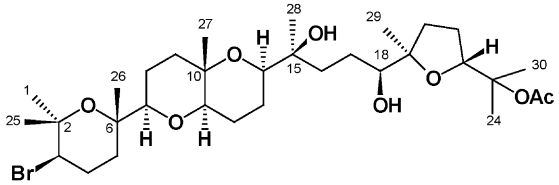
Abstract—This paper reports testing 15 polyether triterpenes with a squalene carbon skeleton for inhibitory effects on type 2A protein phosphatase. Two compounds, 16-hydroxydehydrothyrsiferol **10** and thyrsenol B **14**, exhibited significant inhibitory action at a concentration of 10 μM. Comparison with thyrsiferyl-23-acetate **1** showed that a similar spatial disposition for the hydroxy group around C-15 or C-16 was the structural feature shared by these metabolites.

Protein phosphorylation is now recognised as a most important means of regulating protein function in eukariotic cells, switching cellular activity from one state to another, and appears to have two main functions. It is the major mechanism for cells to respond to extracellular signals such as hormones and growth factors and is also responsible for the timing of events which occur at set stages in the cell cycle, such as DNA synthesis or mitosis. These processes are catalysed by protein kinases (PKs) and reversed by protein phosphatases (PPs).[1] The quickest route to identify the particular protein kinase or phosphatase involved in a system is to use pharmacological inhibitors or activators in intact cells. In the case of protein kinases, such compounds have been available for some time, but only in the last decade has a variety of very potent inhibitors been discovered for protein phosphatases, and the impact has been considerable.[2]

Okadaic acid (OA), a cytotoxic marine polyether was reported to have a potent inhibitory effect on serine/threonine protein phosphatases PP1 and PP2A, [3,4] generating a new okadaic class of inhibitor that has been widely used as powerful tool in the study of biological processes mediated by protein phosphorylation. This group of inhibitors is structurally diverse and includes cyclic peptides such as microcystins and nodularins; other polyketides such as tautomycin (TM) and calyculin A (CL-A), and terpenoids such as cantharidin and thyrsiferyl-23-acetate (TA), **1**.[5] The choice of inhibitor depends on its efficacy and selectivity. These substances show a strong inhibitory effect on both PP1 and PP2A, while some also inhibit PP2B to a lesser extent which makes it difficult to study the specific function of each molecular species on protein phosphatase.

Thyrsiferyl-23-acetate (TA), **1**, was isolated in 1985 from the red alga *Laurencia obtusa*,[6] and discovered to be a PPs inhibitor in 1994. It has been shown to inhibit PP2A potently and specifically, depending on the enzyme concentration, but has no effect on PP1, PP2B, PP2C or PTP.[7]

We have recently concluded studies of secondary metabolites isolated from the red alga *Laurencia viridis*, an indigenous Canary Islands species. Dehydrothyrsiferol (DHT) **2** was isolated from this alga together with several new polyethers, **3**–**17**, which possess strong cytotoxic activity.[8] As they are structurally related to thyrsiferyl-23-acetate (TA) **1**,[9–13] it was decided to study their inhibitory activity.



1 Thyrsiferyl-23-acetate (TA)

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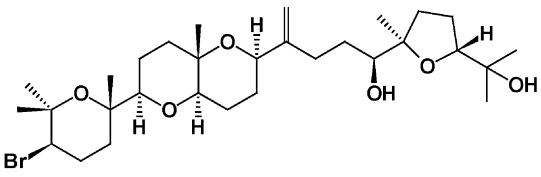
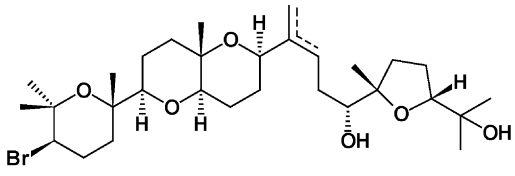
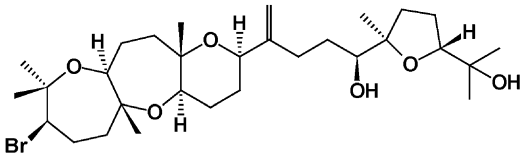


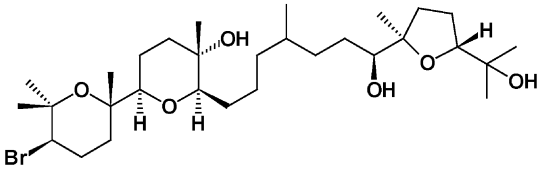
2 Dehydrothyrsiferol (DHT)



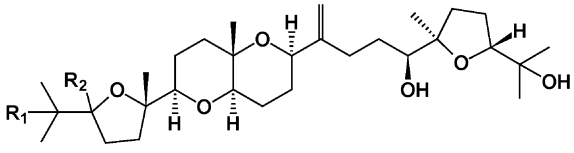
3 Dehydrovenustatriol $\Delta^{15,28}$
17 15,16-Dehydrovenustatriol $Z\,\Delta^{15,16}$



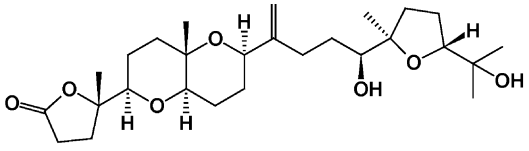
4 Dioxepandehydrothyrsiferol



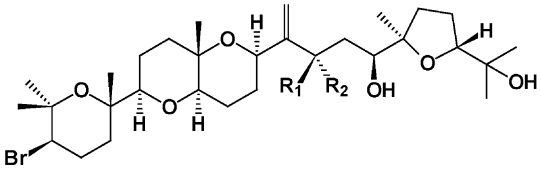
5 Clavidol



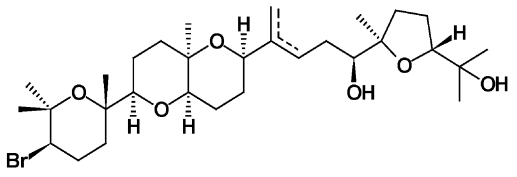
6 Pseudodehydrothyrsiferol R_1=OH; R_2=αH
7 R_1=H; R_2=βOCH$_3$
8 R_1=OCH$_3$; R_2=αH



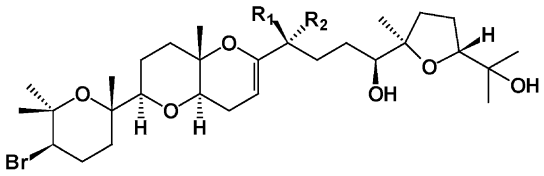
9 Lactodehydrothyrsiferol



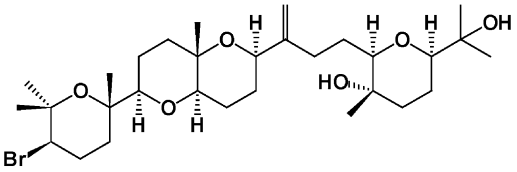
10 16-Hydroxydehydrothyrsiferol R_1=H; R_2=OH
11 16-*epi*-Hydroxydehydrothyrsiferol R_1=OH; R_2=H



12 10-*epi*-15,16-Dehydrothyrsiferol $E\,\Delta^{15,16}$
16 10-*epi*-Dehydrothyrsiferol $\Delta^{15,28}$



13 Thyrsenol A R_1=OH; R_2=CH$_2$OH
14 Thyrsenol B R_1=CH$_2$OH; R_2=OH



15 Isodehydrothyrsiferol

To determine the effect of these metabolites on PP2A, in vitro assays with pure compounds were undertaken. Inhibitory effects were examined in a fluorescent enzyme inhibition assay using fluorescein diphosphate as substrate.[14] Inhibition assays were performed in a microtiter plate, and PP2A was inhibited by adding solutions of pure compound of 1, 10, 100 and 250 μM for **2**–**15** and 100, 125, 150, 200 and 250 μM for **16**–**17**. The resulting fluorescence enhancement derived from the enzymatic hydrolysis of the substrate was quantified on a fluorescence plate reader.[15] The results of this assay are set out in Tables 1 and 2.

Table 1 and Figure 1 show the percentages of PP2A inhibition for substances in which the activity is within the range 1–250 μM, and Table 2 and Figure 2 give the

Table 1. Inhibition percentage on PP2A in the range 1–250 μM

Compd	Concentrations			
	1 μM	10 μM	100 μM	250 μM
2	8.2	12.2	25.2	37.7
3	7.5	23.7	66.8	95.3
4	18.9	20.4	59.7	66.5
5	43.6	45.9	57.8	74.5
6	0	0	40.4	55.4
7	0	2.7	43.1	55.4
8	0	4.7	65.1	57.2
9	33.7	40.1	49.4	63.2
10	41.1	93.4	100	100
11	42.2	45.6	65.1	79.3
12	17.9	41.9	99.5	100
13	15.2	31.8	89.6	97.8
14	52.8	93.2	99.1	100
15	0	6.2	74.7	81.3

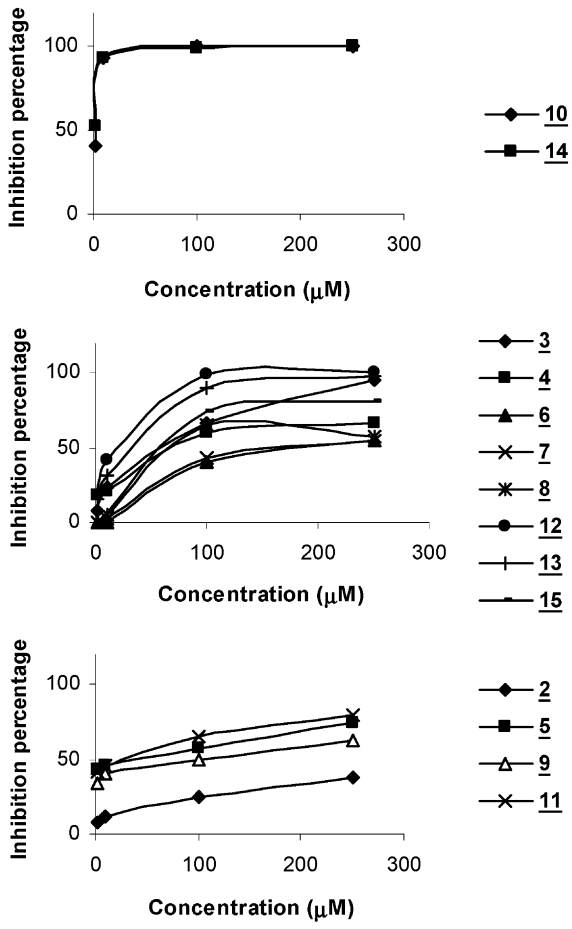


Figure 1. Inhibition percentage curves corresponding to the compounds listed in Table 1 with a range 1–250 μM.

Table 2. Inhibition percentage on PP2A in the range 100–250 μM

Compd	Concentrations				
	100 μM	125 μM	150 μM	200 μM	250 μM
16	11.4	24.0	26.1	47.8	66.4
17	6.5	18.8	32.7	35.8	59.8

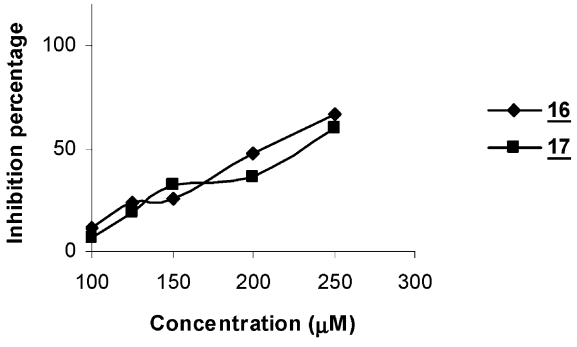


Figure 2. Inhibition percentage curves for compounds with a range between 100 and 250 μM.

values for compounds **16** and **17** with a range of 100 to 250 μM. These results showed that, qualitatively, all compounds possess the inhibitory effects of TA **1** on PP2A. Between them, the activity shown by compounds **10** and **14** was significantly higher than for the others, 93.4 and 93.2% inhibition, respectively, of protein phosphatase type 2A at a concentration of 10 μM.

A comparative analysis of the PP2A inhibitory effects and the chemical structures of our compounds showed that neither modification of size and stereochemistry at rings A (compounds **4**, **6** and **9**), B (compound **4**) and D (compound **15**) nor the lack of ring C (compound **5**), alter the inhibitory activity shown by the lead compound in this series, dehydrothyrsiferol **2**. The most potent activity was observed for 16- hydroxy-dehydrothyrsiferol **10** and thyrsenol B **14**, which induce inhibitory effects with values of IC_{50} <10 μM, similar to those published for **1**.[16,17] Compounds **10**, **14** and **1** are characterized by the presence of a hydroxy group at carbon C-15 or C-16 with a similar spatial disposition. This suggests that this moiety may be one of the fundamental factors related to intrinsic activity. In conclusion, the structure–activity relationships and inhibitory effects of polyether triterpenoids as presented in this communication should provide a rational basis for the future design of new ether drugs containing a fragment where the orientation of the hydroxy moiety at C-15 and C-16 is a crucial factor.

Acknowledgements

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