# Semi-synthetic analogs of 17-hydroxycativic acid and their biological evaluation

Natalia Paola Alza[1] and Ana Paula Murray[1]

[1] INQUISUR-CONICET, Departamento de Química, Universidad Nacional del Sur, Avenida Alem 1253, Bahía Blanca, 8000, Argentina. natalia.alza@uns.edu.ar

## Abstract

A new series of analogs of the naturally occurring 17-hydroxycativic acid (**1**) were synthesized and evaluated as cholinesterase inhibitors. All derivatives have been fully characterized by mono- and bidimensional NMR spectroscopy. The butyrylcholinesterase (BChE) inhibitory activity of the analogs was of the same order of magnitude as that of compound **1** ($IC_{50}$ = 171.1 μM), while the acetylcholinesterase (AChE) inhibition was not improved by these derivatizations, being **1** the most potent AChE inhibitor of the series ($IC_{50}$ =21.1 μM). These results suggest that both carboxylic group (C15) and allylic alcohol (C17) could be important for AChE inhibitory activity.

## Keywords

*Grindelia ventanensis*, cholinesterase inhibition, 17-hydroxycativic acid, semisynthesis.

## Introduction

Alzheimer´s disease (AD) is a progressive neurodegenerative disorder associated with memory impairment and cognitive deficit. Although many factors have been implicated in AD, its etiology and pathogenesis remains unclear. The cholinergic hypothesis postulates that at least some of the cognitive decline results from the low levels of the neurotransmitter acetylcholine (ACh) in the brain of AD patients. The inhibition of acetylcholinesterase (AChE), the enzyme that catalyzes ACh hydrolysis, is the main therapeutic strategy followed to treat AD. Recent reports suggest that drug development would be targeted not only to increasing AChE inhibition but also selectivity for AChE over butyrylcholinesterase (BuChE) [1], which activity decreases in synapses of AD patients.

Labdane diterpenes have demonstrated several biological activities, such as antiproliferative against different cancer cell lines and gastroprotective [2-6]. Structural modifications of these diterpenes had led derivatives with improved bioactivities [3-6].

In the course of our ongoing studies of natural products from our regional flora with AChE inhibitory activity, a bioassay-guided fractionation of the ethanolic extract of *Grindelia ventanesis* Bartola & Tortosa (Asteraceae) resulted in the isolation of a labdane diterpene of the normal series identified as 17-hydroxycativic acid (**1**). Taking into account that **1** showed a significant inhibition of AChE ($IC_{50}$ = 21.1 μM), selectivity over BuChE ($IC_{50}$ = 171.1 μM) and that it was easily isolated from the plant extract in a very good yield (150 mg/ 100 g of aerial parts), we decided to obtain semisynthetic analogs of this natural diterpene through simple structural modifications of two of the three hot spots of this molecule: the carboxylic group (C15) and the primary allylic alcohol (C17) (Figure 1).

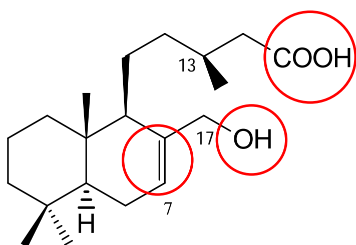



**Figure 1** Hot spots of compound **1**.

## Experimental

**General.** NMR measurements were carried out at 22 °C from DMSO-$d_6$ or $CDCl_3$ solutions, on a Bruker ARX300 spectrometer operated at 300 and 75 MHz for hydrogen and carbon, respectively. Chemical shifts are given in ppm ($\delta$) with TMS as an internal standard. Silica gel 60 (Merck, 200 - 425 mesh) was used for column chromatography. Analytical TLC was performed on Silicagel 60 $F_{254}$ sheets (0.2 mm thickness, Merck). The *p*-anisaldehyde-acetic acid spray reagent was used for detection. UV spectra were recorded on a JASCO V-630BIO spectrophotometer. Microwave assisted reactions were carried out in a CEM Discover reactor. Acetylcholinesterase from *electric eel* (type VI-S), 5,5′-Dithiobis(2-nitrobenzoic acid) (DTNB), acetylthiocholine iodide (ATCI), butyrylthiocholine iodide (BTCI) and tacrine were purchased from Sigma. Butyrylcholinesterase (horse serum) was purchased from MP Biomedicals.

**Synthesis of 2.** $(CH_3)_3N.SO_3$ (0.4 mmol) was added to a solution of **1** (0.1 mmol) in dry DMF (3 ml). The reaction mixture was irradiated and stirred at 150°C for 5 min in a sealed tube in a microwave reactor. After evaporation to dryness, the residue was eluted through Amberlite CG-120 (sodium form) with methanol (50 ml), evaporated under reduced pressure and purified by column chromatography on silicagel using $CH_2Cl_2$/MeOH. Fractions eluted with $CH_2Cl_2$/MeOH (87.5:12.5) afforded pure **2** (23.7 mg, 55 %). For $^1$H-NMR and $^{13}$C-NMR data see tables 1 and 2.

**Synthesis of 3.** To a suspension of $LiAlH_4$ (1.0 mmol) in dry THF (2 ml) was added dropwise **1** (0.1 mmol) in THF (0.5 ml) at 0°C. This mixture was refluxed for 6 h and left overnight without heating. Acidulated water was added (20 ml) and extracted with $CH_2Cl_2$ (3×10 ml). The organic phase was dried over $CaCl_2$ and evaporated under reduced pressure. The residue was chromatographed on silicagel and elution with $CH_2Cl_2$/MeOH 99:1 afforded diol **3** (16.1 mg, 55.3 %). For $^1$H-NMR and $^{13}$C-NMR data see tables 1 and 2.

**Synthesis of 4.** Compound **1** (0.1 mmol) was added to a mixture of $Ac_2O$ (31.7 mmol) and pyridine (37.7 mmol) and left at room temperature for 24 h. The reaction mixture was poured onto distilled water (20 ml) and partitioned with $CH_2Cl_2$ (3×8 mL). The organic layer was washed with water, dried over $CaCl_2$ and purified via silicagel column chromatography with $CH_2Cl_2$/MeOH to obtain by elution with 99:1 compound **4** (19.0 mg, 45.3 %). For $^1$H-NMR and $^{13}$C-NMR data see tables 1 and 2.

**Synthesis of 5** Compound **1** (0.1 mmol) and $K_2CO_3$ (0.2 mmol) were added to DMF (5 ml) and stirred at room temperature for 10 min, after which $ICH_3$ (0.4 mmol) was added. After being stirred for another 24 h, the reaction mixture was poured onto water (20 ml) and partitioned with $CH_2Cl_2$ (3×8 ml) and AcOEt (3×10 ml). Both organic layers together were washed with saturated NaCl, dried over $CaCl_2$ and chromatographed over silicagel with hexane/AcOEt. Elution with

hexane/AcOEt 85:15 afforded methyl ester **5** (16.4 mg, 53.2 %). For $^{1}$H-NMR and $^{13}$C-NMR data see tables 1 and 2.

**NMR data for semisynthetic derivatives.**

Table 1. *$^{1}$H-NMR spectroscopic data for compouns **1-5**, δ in ppm, J in Hz.*

| Position | | | | | |
|---|---|---|---|---|---|
| | **1**[a] | **2**[a] | **3**[b] | **4**[a] | **5**[b] |
| **1** | 0.91 m<br>1.79 os | 0.92<br>1.80 os | 0.98 dd<br>(4.2, 12.9)<br>1.87 m | 0.94<br>1.81 os | 0.97 os<br>1.85 m |
| **2** | 1.40 m | 1.41 m | 1.47 m | 1.42 m | 1.50 os |
| **3** | 1.11 os<br>1.36 os | 1.12 os<br>1.37 os | 1.16 os<br>1.41 os | 1.13 os<br>1.37 os | 1.15 os<br>1.40 m |
| **5** | 1.13 os | 1.12 os | 1.21 m | 1.14 os | 1.20 m |
| **6** | 1.84 m<br>1.97 os | 1.83<br>1.98 | 1.91 m<br>2.05 m | 1.87 d<br>2.01 d | 1.90 os<br>2.05 m |
| **7** | 5.62 br s | 5.74 br s | 5.75 m | 5.74 br s | 5.75 t (2.7) |
| **9** | 1.64 br s | 1.63 br s | 1.78 br s | 1.67 br s | 1.77 br s |
| **11** | 1.09 os<br>1.39 os | 1.10 os<br>1.38 os | 1.14 os<br>1.52 m | 1.12 os<br>1.39 os | 1.16 os<br>1.51 os |
| **12** | 1.09 os<br>1.52 m | 1.10 os<br>1.60 os | 1.14 os<br>1.66 m | 1.11 os<br>1.39 os | 1.16 os<br>1.63 m |
| **13** | 1.79 os | 1.77 os | 1.57 os | 1.77 os | 1.93 os |
| **14** | 1.93 os<br>2.21 dd<br>(5.4, 15.0) | 1.91 os<br>2.30 dd<br>(4.5, 14.7) | 1.43 os<br>1.57 os | 1.94 os<br>2.19 dd<br>(5.7, 14.7) | 2.16 dd (7.5)<br>2.33 dd<br>(6.6, 14.7) |
| **15** | | | 3.69 m | | |
| **16** | 0.87 d (6.5) | 0.87 d (6.3) | 0.93 d (6.3) | 0.87 d (6.6) | 0.96 d (6.6) |
| **17** | 3.78 d<br>3.85 d | 4.02 d (11.1)<br>4.17 d (11.1) | 3.97 d (12.3.)<br>4.13 d (12.3) | 4.33 d (12.3)<br>4.48 d (12.3) | 3.96 d (12.3)<br>4.16 d (12.3) |
| **18** | 0.82 s | 0.83 s | 0.86 s | 0.83 s | 0.85 s |
| **19** | 0.84 s | 0.85 s | 0.89 s | 0.84 s | 0.88 s |
| **20** | 0.69 s | 0.70 s | 0.75 s | 0.71 s | 0.74 s |
| | | | | 1.99 s<br>($COCH_3$) | 3.66 s<br>($COOCH_3$) |

[a] Registered in DMSO-$d_6$, [b] Registered in $CDCl_3$

Table 2. *$^{13}$C-NMR spectroscopic data for compouns **1-5**, δ in ppm.*

| Position | | | | | |
|---|---|---|---|---|---|
| | **1**[a] | **2**[a] | **3**[b] | **4**[a] | **5**[b] |
| **1** | 38.8 t | 38.7 t | 39.3 t | 38.4 t | 39.2 t |
| **2** | 18.6 t | 18.4 t | 18.9 t | 18.3 t | 18.9 t |
| **3** | 42.1 t | 41.8 t | 42.4 t | 41.7 t | 42.4 t |

| 4 | 32.8 s | 32.6 s | 33.1 s | 32.7 s | 33.1 s |
|---|---|---|---|---|---|
| 5 | 49.9 d | 49.5 d | 50.1 d | 49.3 d | 50.0 d |
| 6 | 23.2 t | 23.2 t | 23.9 t | 23.2 t | 23.8 t |
| 7 | 121.7 d | 125.9 d | 125.7 d | 127.8 d | 125.7 d |
| 8 | 139.5 s | 135.4 s | 139.7 s | 134.0 s | 139.4 s |
| 9 | 52.6 d | 52.1 d | 52.9 d | 52.1 d | 52.6 d |
| 10 | 36.6 s | 36.4 s | 36.9 s | 36.4 s | 36.9 s |
| 11 | 23.8 t | 23.4 t | 24.4 t | 23.3 t | 24.4 t |
| 12 | 38.6 t | 37.7 t | 38.9 t | 38.0 t | 38.6 t |
| 13 | 30.8 d | 30.6 d | 30.8 d | 30.4 d | 31.5 d |
| 14 | 41.3 t | 40.9 t | 39.7 t | 41.2 t | 41.4 t |
| 15 | 174.4 s | 174.3 s | 61.3 t | 174.1 s | 174.1 s |
| 16 | 20.0 c | 19.7 c | 20.0 c | 19.8 c | 20.1 c |
| 17 | 63.4 t | 68.5 t | 66.2 t | 66.9 t | 66.0 t |
| 18 | 33.2 c | 33.0 c | 33.3 c | 32.9 c | 33.3 c |
| 19 | 21.9 c | 21.8 c | 22.0 c | 21.7 c | 22.0 c |
| 20 | 13.7 c | 13.6 c | 13.8 c | 13.5 c | 13.7 c |
| | | | | 170.2 s<br>20.8 c ( $COCH_3$) | 51.6 c<br>($COOCH_3$) |

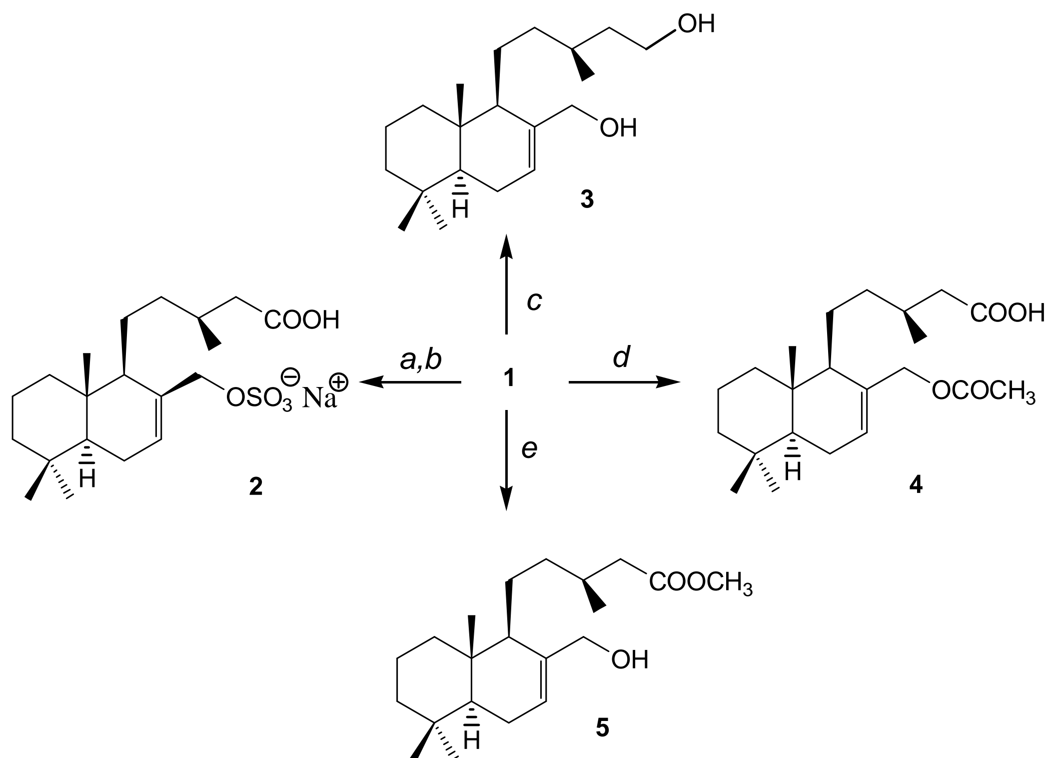
[a] Registered in DMSO-$d_6$, [b] Registered in $CDCl_3$

**Cholinesterase inhibition assay**

Electric eel (*Torpedo californica*) AChE and horse serum BuChE were used as source of both the cholinesterases. AChE and BuChE inhibitory activities were measured *in vitro* by the spectrophotometric method developed by Ellman with slight modification[7]. The lyophilized enzyme, 500U AChE/300U BuChE, was prepared in buffer A (8 mM $K_2HPO_4$, 2.3 mM $NaH_2PO_4$) to obtain 5/3 U/mL stock solution. Further enzyme dilution was carried out with buffer B (8 mM $K_2HPO_4$, 2.3 mM $NaH_2PO_4$, 0.15 M NaCl, 0.05% Tween 20, pH 7.6) to produce 0.126/0.06 U/mL enzyme solution. Samples were dissolved in buffer B using 2.5% of MeOH as cosolvent. Enzyme solution (300 $\mu$L) and 300 $\mu$L of sample solution were mixed in a test tube and incubated for 60/120 min at room temperature. The reaction was started by adding 600 $\mu$L of the substrate solution (0.5 mM DTNB, 0.6 mM ATCI/BTCI, 0.1 M $Na_2HPO_4$, pH 7.5). The absorbance was read at 405 nm for 180 s at 27°C. Enzyme activity was calculated by comparing reaction rates for the sample to the blank. All the reactions were performed in triplicate. $IC_{50}$ values were determined with GraphPad Prism 5. Tacrine (99%) was used as the reference AChE/BuChE inhibitor.

**Results and discussion**

In the present work four derivatives of 17-hydroxycativic acid isolated from *G. ventanensis* were prepared as depicted in Scheme. Compound **2** was obtained by sulfation with trimethylamine-sulfur trioxide complex of the hydroxyl group; reduction of the carboxylic acid to alcohol using $LiAlH_4$ afforded diol **3**; acetylation with $Ac_2O$/pyridine of the hydroxyl group gave **4**; using $K_2CO_3$ and $CH_3I$, compound **5** was synthesized. These derivatives are new diterpenes and have been fully characterized by mono- and bidimensional NMR spectroscopy (Tables 1 and 2).




Scheme. (a) $(CH_3)_3N.SO_3$, DMF, 5 min, 150°C, microwave; (b) Amberlite CG-120 (MeOH); (c) LiAlH4, THF; (d) $Ac_2O$, Py; (e) $ICH_3$, $K_2CO_3$, DMF.

The AChE and BuChE inhibitory activity of the semisynthetic compounds **2-5** were evaluated and compared to that of the natural compound **1**. Even though **1** has a significant inhibition of AChE ($IC_{50}$ = 21.1 μM), derivatives **2-5** showed no AChE inhibitory activity. On the other hand, the BuChE inhibition of **3-5** resulted to be of the same order of magnitude of **1** ($IC_{50}$ = 171.1 μM) with $IC_{50}$ values 373.2, 219.6 and 143.7 μM respectively, showing weak BuChE inhibitory activity. Compound **2** did not inhibit this enzyme.

**Conclusions**

These preliminary results suggest that both carboxylic group (C15) and allylic alcohol (C17) could be important functional groups for AChE inhibitory activity of this type of labdane diterpenes. Structural modifications achieved until now have not enhanced AChE inhibition, while compounds **3-5** showed selectivity for BuChE over AChE.

**Acknowledgments** - This work was financially supported by the National Research Council of Argentina (CONICET), Universidad Nacional del Sur (Argentina) and ANPCYT. A.P.M. is a Research Member of CONICET. N.P.A. is grateful to CONICET for her doctoral fellowship.

**References:**

1- Wang, B.; Mai, Y.-C.; Li, Y.; Hou, J.-Q.; Huang, S.-L-.; Ou, T.-M.; Tan, J.-H.; An, L.-K.; Li, D.; Gu, L.-Q.; Huang, Z.-S. *Eur. J. Med. Chem.* **2010**, *45*, 1415.

2- Chinou, I. *Curr. Med. Chem.* **2005**, *12 (11)*, 1295.

3- Pertino, M.W.; Theoduloz, C.; Palenzuela, J.A.; Afonso, M.M.; Yesilada, E.; Monsalve, F.; González, P.; Droguett, P.; Schmeda-Hirschmann, G. *Molecules* **2011**, *16(10)*, 8614.

4- Pertino, M.W.; Theoduloz, C.; Bastías, M.; Schmeda-Hirschmann, G. *Molecules* **2013**, *18*, 5936.

5- Schmeda-Hirschmann, G; Pertino, M.W.; Rodríguez, J.A.; Monsalve, F.; Droguett, D.; Theoduloz, C. *Molecules* **2010**, *15*, 7378.

6- Schmeda-Hirschmann, G.; Rodríguez, J.A.; Theoduloz, C.; Valderrama, J.A. *Planta Med.* **2011**, *77*, 340.

7- Di Giovanni, S.; Borloz, A.; Urbain, A.; Marston, A.; Hostettmann, K.; Carrupt, P.A.; Reist, M. *Eur. J. Pharm. Sci.* **2008**, *33*, 109.