Synthesis of 3,16,30-trioxolup-20(29)-ene, a selective butyrylcholinesterase inhibitor, from a natural triterpene

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Abstract

Six new lupanes type triterpenoids derivatives have been synthesized using lup-20(29)-en-3β,16β-diol (**1**) and 30-oxolup-20(29)-en-3β,16β-diol (**2**) as starting material. Ketones **3** (lup-20(29)-en-3,16-dione) and **4** (3,16,30-trioxolup-20(29)-ene) were obtained by Jone´s oxidation of compounds **1** and **2**, respectively. Further reaction of **3** and **4** with hydroxylamine hydrochloride provided compounds **5** (lup-20(29)-en-16-one-3-oxime), **6** (lup-20(29)-en-3,16-dioxime), **7** (lup-20(29)-en-16-one-3,30-dioxime) and **8** (lup-20(29)-en-3,16,30-trioxime). Derivatives **3-8** were obtained in moderate - good yields. Their structures were confirmed by analysis of their ^{1}H and ^{13}C NMR and ESI-MS spectra. The complete assignation of the signals was achieved with the aid of 2D NMR experiments (COSY, HSQC, HMBC, NOESY).

All of the new derivatives were evaluated as potential *in vitro* butyrylcholinesterase (BChE) inhibitors by the Ellman´s colorimetric method. Their anti acetylcholinesterase (AChE) activity was evaluated in order to determine the BChE/AChE selectivity of compounds **3**-**8**. All of them failed to inhibit AChE, but we found that the best BChE inhibition was observed for 3,16,30-trioxolup-20(29)-ene (**4**) with an IC_{50} value of 21.5 μM, which elicited a selective inhibitor profile.

Keywords

Butyrylcholinesterase activity, 3,16,30-trioxolup-20(29)-ene, pentacyclic triterpenes, semisynthesis.

Introduction

The chemistry of lupane-type triterpenoids has been actively explored due to and their biological and pharmacological properties which include anti-inflammatory[1], anti-oedematous[2], antitumor[3] and anti-HIV activities[4]. Abundant in many plants, these metabolites are valuable natural raw materials to perform chemical modifications and obtain semisynthetic analogs for structure-activity relationship studies.

According to the cholinergic hypothesis, the inhibition of cholinesterase increases the levels of acetylcholine in the brain, thus improving cholinergic functions in Alzheimer´s Disease (AD) patients. Butyrylcholinesterase (BChE) is one of the enzymes involved in the metabolic degradation of acetylcholine, together with acetylcholinesterase (AChE). BChE activity increases as AD progresses, which suggests that BChE may play an important role at the latter stages of AD. Therefore, selective BChE inhibitors attract interest nowadays.

In our continuing search of new anti-cholinesterase agents, we found that calenduladiol (**1**), obtained from ethanolic extract of *Chuquiraga erinacea* (Asteraceae) in good yields, showed a moderate cholinesterase inhibition[5]. That result encouraged us synthesize derivatives of this natural compound. Calenduladiol (**1**) (lup-20(29)-en-3β,16β-diol) is a pentacyclic lupane-type triterpene with the interesting feature of being hydroxylated at C-16. Previous reports indicate that this position is essential for a biological activity. In the present work we report the synthesis of several new derivatives from natural calenduladiol and their evaluation as potential cholinesterase inhibitors.

Experimental Section

General Experimental Procedures.

All reactions were monitored by thin layer chromatography (TLC) (Silicagel 60 F254 sheets, 0.2mm thickness; Merck). The *p*-anisaldehyde-acetic acid spray reagent, and UV light (254 and 366 nm) were used for detection. Silicagel 60 (70-230 mesh; Fluka) and Silicagel flash (200-425 mesh; Fluka) were used for column chromatography. NMR spectra were recorded in $CDCl_3$ or MeOD with Bruker ARX300 and AMX400 instruments. BChE, AChE, butyrylthiocholine iodide (BTChI), acetylthiocholine iodide (ATChI), 5,50-dithiobis (2-nitrobenzoic acid) (DTNB) and tacrine (99% pure) were purchased from Sigma. Compound **1** was isolated from *C. erinacea* as previously described[5]. Compound **2** was obtained by allylic oxidation of **1**, as previously reported[6].

*Synthesis of compounds **3-8***

Lup-20(29)-en-3,16-dione (3). Jones' reagent was added slowly to a solution of **1** (100.0 mg, 0.23 mmol) in 3 mL of acetone, at 0 °C. The reaction mixture was stirred for 30 min, quenched with 2-propanol, filtered through Florisil, and concentrated under vacuum. The residue was purified by column chromatography on silicagel using hexane/EtOAc (95:5) to yield 41.4 mg (0.09 mmol) (42%) of **3** as a white solid. ^{13}C NMR (100 MHz, $CDCl_3$) δ 217.80 (C-3), 215.76 (C-16), 148.80 (C-20), 110.81 (C-29), 56.69 (C-17), 54.80 (C-5), 49.44 (C-18), 49.40 (C-9), 48.11 (C-14), 47.39 (C-4, C-19), 44.92 (C-15), 41.04 (C-8), 39.62 (C-1), 37.67 (C-13), 36.93 (C-10), 34.14 (C-7), 33.58 (C-2), 31.20 (C-22), 28.63 (C-21), 26.83 (C-23), 24.80 (C-12), 21.27 (C-11), 21.14 (C-24), 19.68 (C-6), 19.03 (C-30), 18.13 (C-28), 16.30 (C-26), 15.97 (C-25), 15.39 (C-27).

Lup-20(29)-en-3,16,30-trione (4). Following the same procedure described for **3**, 100 mg (0.22 mmol) of **2** were oxidated to obtain 25.8 mg (0.06 mmol) (26%) of **4**. ^{13}C NMR (100 MHz, $CDCl_3$) δ 217.86 (C-3), 215.37 (C-16), 194.78 (C-30), 156.22 (C-20), 133.32 (C-29), 56.91 (C-17), 54.83 (C-5), 49.16 (C-9, C-18), 47.72 (C-14), 47.43 (C-4), 44.91 (C-15), 40.98 (C-8), 39.61 (C-1), 37.33 (C-13,C-19), 36.91 (C-10), 34.17 (C-7), 33.57 (C-2,C-22), 31.28 (C-12, C-21), 26.78 (C-23), 21.25 (C-11), 21.20 (C-24), 19.65 (C-6), 18.06 (C-28), 16.30 (C-26), 15.93 (C-25), 15.38 (C-27).

Lup-20(29)-en-16-one-3-oxime (5). Four equiv of hydroxylamine hydrochloride (29.7 mg) and a solution of 18.7 mg of sodium acetate (2 equiv) in 5 mL of H_2O were added to a solution of 50.0 mg (0.11 mmol) of compound **3** in 2 mL of EtOH. The reaction mixture was stirred at room temperature over 36 h, and then the EtOH was evaporated. The residue was treated with H_2O and extracted with dichloromethane (3 × 5 mL). The combined organic extracts were dried over $MgSO_4$, filtered, and concentrated. The residue was purified by column chromatography on silicagel using hexane/EtOAc (87:13) to yield 12.9 mg (0.03 mmol) (25%) of **5**. ^{13}C NMR (100 MHz, $CDCl_3$) δ 215.97 (C-16), 167.05 (C-3), 148.89 (C-20), 110.81 (C-29), 56.74 (C-17), 55.43 (C-5), 49.69 (C-18), 49.50 (C-9), 48.16 (C-14) , 47.45 (C-19) , 44.97 (C-15) , 41.18 (C-8), 40.36 (C-4), 38.83 (C-1), 37.66 (C-13), 37.31 (C-10), 33.92 (C-7), 31.25 (C-22), 28.67 (C-21), 27.53 (C-23), 24.84 (C-12), 23.06 (C-24), 21.08 (C-11), 19.11 (C-2), 19.06 (C-30), 18.16 (C-28), 17.18 (C-6), 16.49 (C-26), 15.84 (C-25), 15.40 (C-27).

Lup-20(29)-en-3,16-dioxime (6). Six equiv of hydroxylamine hydrochloride (39.8 mg) and a solution of 33.8 mg of sodium acetate (4 equiv) in 5 mL of H_2O were added to a solution of 45.0 mg (0.10 mmol) of compound **3** in 2 mL of EtOH. The reaction mixture was refluxed for 72 h, and then the EtOH was evaporated. The residue was treated with H_2O and extracted with dichloromethane (3 × 5 mL). The combined organic extracts were dried over $MgSO_4$, filtered, and concentrated. The residue was purified by column chromatography on silicagel using hexane/EtOAc (85:15) to yield 16.8 mg (0.03 mmol) (35%) of **6**. ^{13}C NMR (75 MHz, $CDCl_3$) δ 167.19 (C-3), 165.25 (C-16), 149.63 (C-20), 110.51 (C-29), 56.75 (C-5), 50.14 (C-18), 50.01 (C-9), 48.84 (C-17), 47.38 (C-19), 46.11 (C-14), 41.12 (C-8), 41.06 (C-4), 39.66 (C-1), 37.91 (C-13), 37.52 (C-10), 34.51 (C-7), 33.28

(C-22), 29.57 (C-21), 27.38 (C-15), 26.44 (C-23), 24.78 (C-12), 22.56 (C-24), 20.88 (C-11), 19.17 (C-30), 18.93 (C-2), 18.89 (C-28), 17.18 (C-6), 16.58 (C-26), 15.76 (C-25), 15.13 (C-27).

Lup-20(29)-en-16-one-3,30-dioxime (7). Following the same procedure described for compound **5**, 5 mg (0.01 mmol) (23%) of **7** were obtained from 20 mg (0.04 mmol) of ketone **4**. ^{13}C NMR (75 MHz, $CDCl_3$) δ 215.89 (C-16), 167.32 (C-3), 150.69 (C-20), 119.32 (C-29), 56.79 (C-17), 55.42 (C-5), 49.54 (C-18), 47.93 (C-14), 45.02 (C-15), 41.13 (C-8), 40.31 (C-4), 38.80 (C-1), 37.55 (C-13), 37.27 (C-10), 33.91 (C-7), 31.15 (C-22), 29.85 (C-12), 27.53 (C-23), 26.55 (C-21), 23.07 (C-24), 21.13 (C-11), 19.10 (C-2), 18.04 (C-28), 17.08 (C-6), 16.49 (C-26), 15.82 (C-25), 15.45 (C-27).

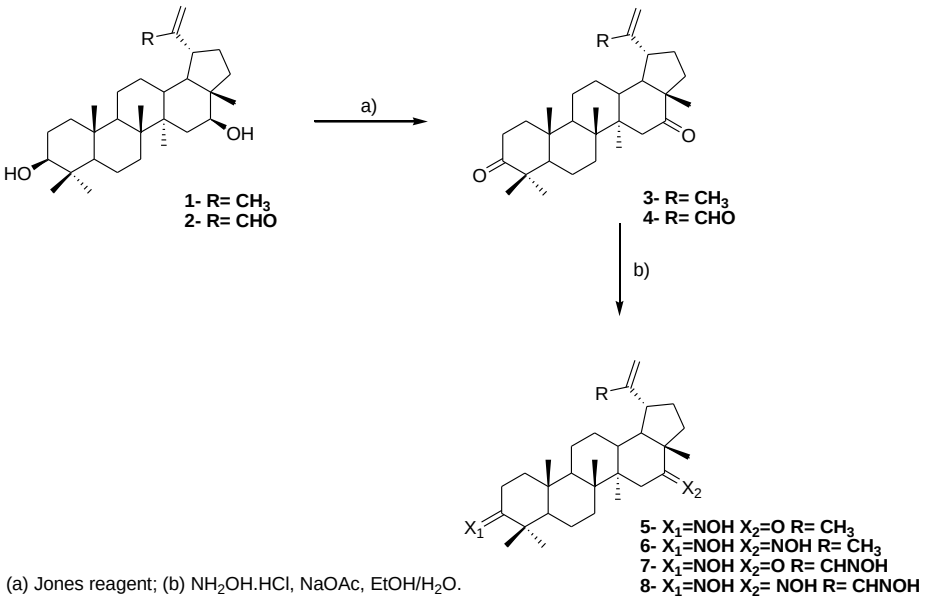
Lup-20(29)-en-3,16,30-trioxime (8). Ketone **4** (20 mg, 0.04 mmol) was converted in compound **8** (6.7 mg, 0.01 mmol, 31%) under the same conditions described for compound **6**. ^{13}C NMR (75 MHz, MeOD) δ 167.16 (C-3), 166.43 (C-16), 152.82 (C-20), 150.69 (C-29), 56.64 (C-5), 51.09 (C-18), 49.85 (C-9), 49.57 (C-19), 46.53 (C-8), 42.12 (C-14), 40.98 (C-4), 39.72 (C-1), 39.24 (C-13), 38.25 (C-10), 35.11 (C-7), 34.22 (C-22), 30.75 (C-15), 28.16 (C-12), 27.67 (C-21), 23.49 (C-24), 22.16 (C-2), 20.20 (C-11), 19.33 (C-28), 17.88 (C-6), 16.79 (C-26), 16.16 (C-25), 15.27 (C-27).

Cholinesterase inhibition assay: Electric eel (*Torpedo californica*) AChE and horse serum BChE were used as source of both the cholinesterases. AChE and BChE inhibitory activities were measured *in vitro* by the spectrophotometric method developed by Ellman with slight modification, as previously described[7, 8].

Results and Discussion

We report here the synthesis of six new lupanes (**3**-**8**). These compounds have been obtained by sequential oxidations and reaction with NH_2OH of the starting compounds **1** and **2** (Figure 1). Their structures were characterized by ^{1}H and ^{13}C NMR spectroscopy.

Figure 1- Synthesis of derivatives **3**-**8**.



(a) Jones reagent; (b) $NH_2OH.HCl$, NaOAc, $EtOH/H_2O$.

Compounds **1**-**8** were screened for AChE and BChE inhibition using the Ellman's assay[7]. Enzyme activity was calculated by comparing reaction rates for the samples to the blank as previously reported[9]. None of the new derivatives (**3**-**8**) inhibited the enzyme AChE. On the other

hand, BChE inhibition was observed for compounds **1-8** (Table 1). These results suggest that these type of compounds have selectivity towards BChE. 3,16,30-trioxolup-20(29)-ene (**4**) resulted to be the most potent BChE inhibitor with an IC value of 21,5 μM.

Table 1- *In vitro* BChE inhibitory activity. [a]

Compound	Inhibition ± S.D.[b](%)	IC_{50} ± S.D.(μM)
1	42,0 ± 1,6	>200
2	42,0 ± 0,3	>200
3	33,4 ± 0,5	>200
4	86,5 ± 2,7	21,5 ± 2,3
5	82,8 ± 1,5	83,7 ± 3,0
6	22,6 ± 0,3	>200
7	52,7 ± 2,3	174,2 ± 2,5
8	29,2 ± 0,8	>200
tacrine[c]	99,0 ± 0,3	0,003 ± 0,001

[a]Samples were dissolved in buffer with MeOH as cosolvent (final conc. 2,5%).Values represent the mean of three replicates ± standard deviation. [b] Sample concentration 200 μM. [c] Positive control.

Conclusions

We have obtained a series of natural and semi-synthesized lupane-type triterpenes, by oxidation at C-3, C-16 and C-30, followed by treatment with NH_2OH in order to obtain the corresponding oximes. Our results on BChE inhibition of these analogs indicate that 3,16,30-trioxolup-20(29)-ene (**4**) could be a promising leader compound to develop a strategy for the enhancement of pharmacological properties of this type of BChE inhibitor.

Acknowledgements

This work was supported by Universidad Nacional del Sur, Instituto Universitario de Bio-Orgánica (Universidad de La Laguna), CIC and CONICET. A.P. Murray is Research Member of the National Research Council of Argentina (CONICET) and M.B. Faraoni is Research Member of Scientific Research Commission (CIC).

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