Lupane triterpenoids, selective butyrylcholinesterase inhibitors

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Abstract

Alzheimer´s disease (AD) is a progressive neurodegenerative disorder associated with memory impairment and cognitive deficit. It is characterized by 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 used to treat AD. In the healthy brain, another enzyme, namely butyrylcholinesterase (BChE), is involved in the metabolic degradation of ACh. 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.

The chemistry of lupane-type triterpenoids has been actively explored due to their biological and pharmacological properties. Abundant in many plants, these metabolites are valuable natural raw materials to perform chemical modifications. In the present work, we aimed to evaluate natural and semisynthetic lupanes as potential *in vitro* cholinesterase inhibitors. Lupeol (**1**) (lup-20(29)-en-3β-ol) and calenduladiol (**2**) (lup-20(29)-en-3β,16β-diol) have been isolated from *Chuquiraga erinacea* subsp. *erinacea* (Asteraceae), an endemic species growing wildly in our region. Semisynthetic lupanes **3**-**17** have been prepared from them. All of them failed to inhibit AChE, but we found that most of calenduladiol derivatives exhibited BChE inhibition. The best BChE inhibitors were 3β-hydroxylup-20(29)-en-16-one (**5**) and 3,16-dioxolup-20(29)-en-30-al (**15**) with IC_{50} values of 28.9 and 21.5 μM, respectively, an interesting result considering that the role of BChE is more relevant as the disease progresses.

Keywords

Cholinesterase inhibitors, triterpenoids, semisynthetic lupanes.

Introduction

Neurodegenerative diseases gain more and more of importance in ageing societies. Especially, the number of patients suffering from Alzheimer´s disease (AD) increased continuously during the last decades.

One characteristic feature of AD is a decreased level of the neurotransmitter acetylcholine (ACh). This results in a decline in memory and recognition. Usually, the level of ACh is controlled by the enzyme acetylcholinesterase (AChE), which cleaves the neurotransmitter in the postsynaptic area. Another enzyme, butyrylcholinesterase (BChE) is also able to hydrolyze ACh, although it doesn´t possess the same affinity for the neurotransmitter as AChE does. Drugs currently used for the treatment of AD inhibit the two ACh controlling enzymes, AChE as well as BChE. However the AChE inhibitors retard the progress of AD only in a very early stage; during the progress of AD, the loss of AChE-activity is compensated by BChE.[1] Therefore, the searching of new selective BChE inhibitors should provide additional benefits in the treatment of AD.

Triterpenoids are naturally occurring compounds with ubiquitous distribution and a wide range of biological activities.[2-4] Pentacyclic triterpenoids provide privileged structures for further modifications and structure activity relationship (SAR) studies.[5-7] Lupanes in particular, have attracted attention due to the broad range of biological and pharmacological properties that they exhibit such as anticancer, antitumor, anti-inflammatory, anti-HIV, anticholinesterase, insecticidal and antimalarial activities.[3,4,8-15]

Our interest in bioactive triterpenes, prompted us to synthesize a series of derivatives from natural lupeol (**1**) and calenduladiol (**2**), isolated from *Chuquiraga erinacea* D. Don. subsp. *erinacea* (Asteraceae).[14-16] Lupeol (**1**) and calenduladiol (**2**) are pentacyclic triterpenes belonging to the lupane family. In a previous work, we observed the enhancement of the inhibitory activity against BChE of **2** by the introduction of keto groups at C-3, C-16 and C-30.[15] In this paper we report the preparation of 15 lupane derivatives from compound **1** and **2**, and their ability as potential cholinesterase inhibitors.

Experimental Section

General Experimental Procedures

All reactions were monitored by thin layer chromatography (TLC) (Silicagel 60 F254 sheets, 0.2 mm 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** and **2** were isolated from *C. erinacea* as previously described.[14] Compounds **6**, **13** and **15** were obtained by oxidation or sequential oxidations of **2**, as described in our previous work.[15] Compounds **10** and **11**, and compounds **16** and **17** were prepared from **6** and **15**, respectively, by reaction with hydroxylamine hydrochloride.

*Synthesis of compounds **3-5, 7-9, 12, 14***

Lup-20(29)-en-3-one (3). Jones reagent was added slowly to a solution of **1** (50.0 mg, 0.12 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 (90:10) to yield 47.4 mg (0.11 mmol, 95.3%) of **3** as a white solid. ^{13}C NMR (100 MHz, $CDCl_3$) δ 218.0 (C-3), 150.8 (C-20), 109.5 (C-29), 55.0 (C-5), 49.9 (C-9), 48.3 (C-18), 48.0 (C-19), 47.4 (C-4), 43.1 (C-17), 43.1 (C-14), 40.9 (C-8), 40.1 (C-22), 39.7 (C-13), 38.2 (C-1), 36.9 (C-10), 35.7 (C-16), 34.2 (C-2), 33.7 (C-7), 29.9 (C-21), 27.5 (C-15), 26.8 (C-23), 25.2 (C-12), 21.6 (C-24), 21.1 (C-11), 19.8 (C-30), 19.8 (C-6), 18.1 (C-28), 16.0 (C-25), 16.2 (C-26), 15.9 (C-27).

16β-hydroxylup-20(29)-en-3-one (4) and **3β-hydroxylup-20(29)-en-16-one (5)**. Following the same procedure described for **3**, 50.0 mg (0.11 mmol) of **2** were oxidized to obtain 10.0 mg (0.02 mmol) (9.9%) of **4** and 12.8 mg (0.03 mmol, 12.7%) of **5**. ^{13}C NMR-**4** (100 MHz, $CDCl_3$) δ 218.2 (C-3), 150.0 (C-20), 110.0 (C-29), 77.2 (C-16), 55.1 (C-5), 49.5 (C-9), 48.8 (C-17), 47.8 (C-18), 47.7 (C-19), 47.5 (C-4), 44.3 (C-14), 41.0 (C-8), 39.8 (C-1), 37.8 (C-22), 37.5 (C-13), 37.0 (C-10), 37.0 (C-15), 34.2 (C-2), 33.7 (C-7), 30.0 (C-21), 26.8 (C-23), 24.9 (C-12), 21.5 (C-11), 21.2 (C-24), 19.8 (C-6), 19.5 (C-30), 16.2 (C-25), 16.1 (C-27), 15.9 (C-26), 11.8 (C-28).
^{13}C NMR-**5** (100 MHz, $CDCl_3$) δ 216.1 (C-16), 148.9 (C-20), 110.8 (C-29), 79.0 (C-3), 56.7 (C-5), 55.3 (C-17), 50.1 (C-9), 49.6 (C-18), 48.2 (C-14), 47.5 (C-19), 45.0 (C-15), 41.2 (C-8), 39.0 (C-1), 38.9 (C-4), 37.6 (C-13), 37.3 (C-10), 34.3 (C-7), 31.3 (C-22), 28.7 (C-21), 28.1 (C-23), 27.5 (C-2), 24.8 (C-12), 20.8 (C-11), 19.1 (C-30), 18.4 (C-6), 18.1 (C-28), 16.6 (C-26), 16.1 (C-25), 15.5 (C-24), 15.5 (C-27).

16β-hydroxy-3-oxolup-1,20(29)-dien-30-al (7) A solution of **4** (8.0 mg, 0.02 mmol) in EtOH (5 mL) was treated with SeO_2 (5.9 mg, 0.05 mmol). The reaction mixture was heated under reflux until the disappearance of the starting material was confirmed by TLC (30 h). Then, the reaction mixture was cooled and EtOH was removed under reduced pressure. The crude was treated with water (10 mL) and extracted with CH_2Cl_2 (3 x 15 mL). The combined organic extracts were dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The residue was purified by column

flash chromatography on silica gel with hexane/AcOEt (83:17) to afford 1.8 mg (0.004 mmol, 21.8%) of **7**. ^{13}C NMR (75 MHz, $CDCl_3$) δ 205.5 (C-3), 195.0 (C-30), 160.0 (C-2), 156.2 (C-20), 132.4 (C-29), 125.4 (C-1), 76.9 (C-16), 53.6 (C-5), 49.0 (C-17, C-18), 44.8 (C-4), 44.2 (C-9), 44.1 (C-14), 41.9 (C-8), 39.6 (C-10), 37.9 (C-22), 37.3 (C-13, C-19), 36.9 (C-15), 33.9 (C-7), 31.7 (C-21), 27.9 (C-24), 27.1 (C-12), 21.5 (C-23), 21.3 (C-11), 19.3 (C-25), 19.1 (C-6), 16.6 (C-26), 14.3 (C-27), 11.8 (C-28).

3β-hydroxy-16-oxolup-20(29)-en-30-al (8). Ketone **5** (14.0 mg, 0.03 mmol) was converted in compound **8** (6.8 mg, 0.01 mmol, 47.2%) under the same conditions described for compound **7**. ^{13}C NMR (75 MHz, $CDCl_3$) δ 215.5 (C-16), 194.7 (C-30), 156.5 (C-20), 132.42 (C-29), 79.0 (C-3), 56.9 (C-5), 55.3 (C-17), 49.9 (C-9, C-18), 47.8 (C-14), 45.0 (C-15), 41.1 (C-8), 39.0 (C-1), 38.8 (C-4), 37.3 (C-13), 37.2 (C-10, C-19), 34.3 (C-7), 31.3 (C-22), 29.8 (C-21), 28.1 (C-23), 27.5 (C-2), 27.1 (C-12), 20.8 (C-11), 18.3 (C-6), 18.0 (C-28), 16.5 (C-26), 16.1 (C-25), 15.5 (C-24), 15.4 (C-27).

Lup-20(29)-en-3-oxime (9). Three equiv of hydroxylamine hydrochloride (18.8 mg) and a solution of 11.6 mg of sodium acetate (2 equiv) in 5 mL of H_2O were added to a solution of 40.0 mg (0.09 mmol) of compound **3** in 2 mL of EtOH. The reaction mixture was stirred at room temperature over 30 h, and then the EtOH was evaporated. The residue was treated with H_2O and extracted with dichloromethane (3 × 15 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 (95:5) to yield 12.9 mg (0.03 mmol, 31.1%) of **9**. ^{13}C NMR (75 MHz, $CDCl_3$) δ 167.4 (C-3), 151.0 (C-20), 109.5 (C-29), 55.6 (C-5), 50.2 (C-9), 48.4 (C-18), 48.1 (C-19), 43.1 (C-17), 43.1 (C-14), 41.0 (C-8), 40.4 (C-22), 38.9 (C-1, C-4), 38.3 (C-13), 37.4 (C-10), 35.7 (C-16), 34.5 (C-7), 30.0 (C-21), 27.6 (C-23), 27.6 (C-2, C-15), 25.3 (C-12), 23.0 (C-24), 21.7 (C-11), 19.8 (C-30), 18.2 (C-6, C-28), 16.1 (C-26), 16.1 (C-25), 14.6 (C-27).

3β-hydroxylup-20(29)-en-30-al (12). Following the same procedure described for compound **7**, 40.0 mg (0.09 mmol) of **1** were oxidized to obtained 18.0 mg (0.04 mmol, 43.6%) of **12**. ^{13}C NMR (75 MHz, $CDCl_3$) δ 195.2 (C-30), 157.4 (C-20), 133.3 (C-29), 79.1 (C-3), 55.4 (C-5), 50.4 (C-9, C-18), 43.4 (C-17), 42.8 (C-14), 40.9 (C-8), 40.1 (C-22), 39.0 (C-4), 38.9 (C-1), 37.9 (C-13), 37.3 (C-10, C-19), 35.5 (C-16), 34.4 (C-7), 28.1 (C-21, C-23), 27.5 (C-2, C-12), 27.5 (C-15), 21.1 (C-11), 18.5 (C-6), 17.9 (C-28), 16.2 (C-25), 16.1 (C-26), 15.5 (C-24), 14.6 (C-27).

3-oxolup-20(29)-en-30-al (14). Compound **12** (15.0 mg, 0.03 mmol) was converted in **14** (7.9 mg, 0.02 mmol, 53.0%) under the same conditions described for derivative **3**. ^{13}C NMR (75 MHz, MeOD) δ 218.2 (C-3), 195.2 (C-30), 157.3 (C-20), 133.3 (C-29), 55.1 (C-5), 49.8 (C-9, C-18), 47.5 (C-4), 43.4 (C-14), 42.9 (C-17), 40.9 (C-8), 40.1 (C-22), 39.8 (C-1), 38.0 (C-13, C-19), 37.0 (C-10), 35.5 (C-16), 34.3 (C-2), 33.7 (C-7), 27.5 (C-12, C-15), 26.7 (C-21, C-23), 21.6 (C-11), 21.2 (C-24), 19.8 (C-6), 17.9 (C-28), 16.0 (C-25), 15.9 (C-26), 14.5 (C-27).

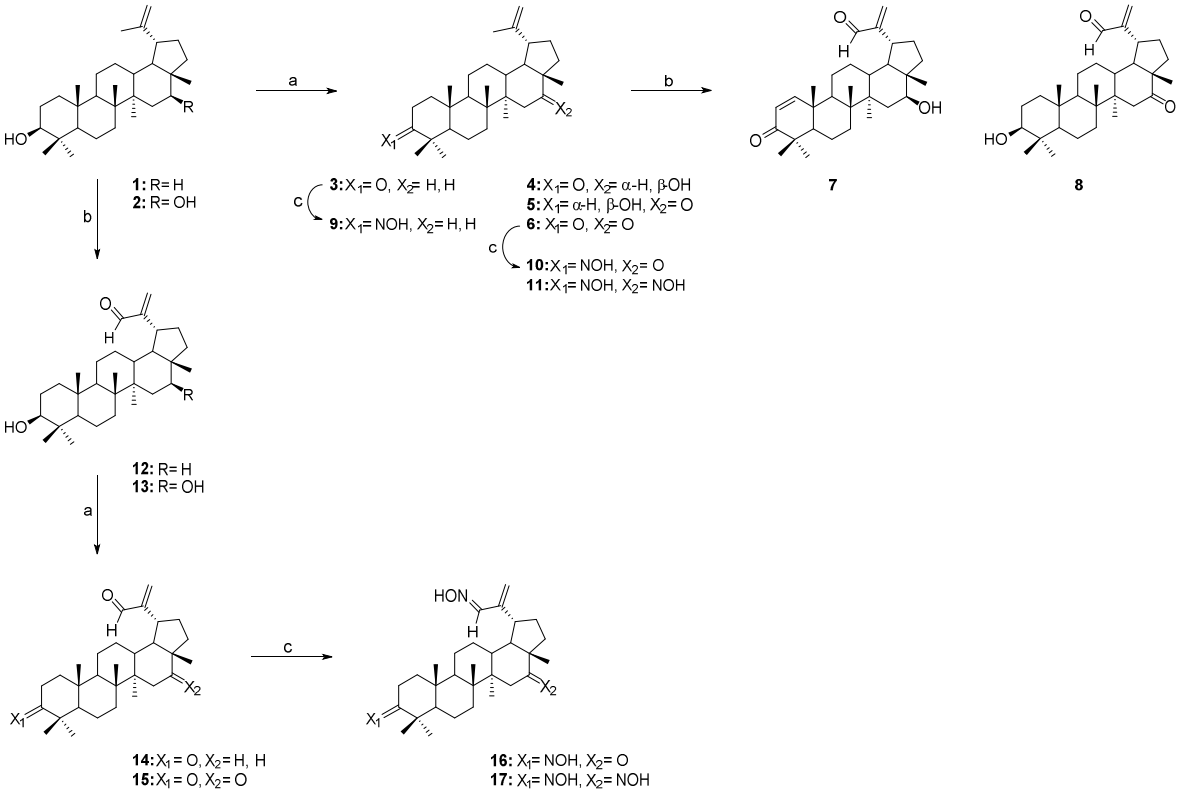
Cholinesterase inhibition assay: Electric eel (*Torpedo californica*) AChE and horse serum BChE were used as source of both the cholinesterases. AChE and BChE inhibitory activities were measured *in vitro* by the spectrophotometric method developed by Ellman, with eserine and tacrine as reference compounds.[17] The results for AChE and BChE inhibition values are summarized in Table 1.

Results and Discussion

We report here the synthesis of 15 lupanes (**3**-**17**). These compounds have been obtained by oxidation (**3**-**6**, **12**, **13**) or sequential oxidations (**7**, **8**, **14**, **15**) and by reaction with hydroxylamine

hydrochloride (**9-11**, **16**, **17)** (Figure 1). Their structures were characterized by ^{1}H and ^{13}C NMR spectroscopy.

Figure 1. Synthesis of derivatives **3-17**.



a) Jones reagent; b) SeO_2, EtOH, reflux; c) $NH_2OH.HCl$, NaOAc, EtOH/H_2O.

As a part of our continuing efforts directed toward the synthesis of new cholinesterase inhibitors, we became interested in evaluate the role of the keto group at C-16. Therefore we decided to carry out the synthesis of 11 derivatives of calenduladiol, 6 of which have a keto group at C-16, compounds **5**, **6**, **8**, **10**, **15** and **16**. They were compared with the same derivatives without modifying the C-16 (**5** vs **2**, **6** vs **4**, **8** vs **13**, **10** vs **11**, **15** vs **7, 16** vs **17**). We found that all failed to inhibit AChE, but 16-oxo derivatives of calenduladiol exhibited BChE inhibition. The best BChE inhibitors were 3β-hydroxylup-20(29)-en-16-one (**5**) and 3,16-dioxolup-20(29)-en-30-al (**15**) with IC_{50} values of 28.9 and 21.5 μM respectively (Table 1).

In order to confirm the importance of the keto group at C-16 on the antiBChE activity, we compared the same derivatives of calenduladiol (**2**) unfunctionalized at C-16 obtained from lupeol (**1**) (**5** vs **1**, **6** vs **3**, **8** vs **12**, **10** vs **9** and **15** vs **14**). None of the lupeol derivatives (**3**, **9**, **12**, **14**) inhibited the enzyme AChE or BChE (Table 1).

These results suggest that the keto group at C-16 may be responsible for the antiBChE activity for this type of molecules.

Table 1. Inhibition of AChE and BChE activity and selectivity index.

Compounds	AChE [a]		BChE [b]		Selectivity index [c]
	% inhibition at 200 µM	IC_{50} (µM)	% inhibition at 200 µM	IC_{50} (µM)	
1	21.3 ± 2.7	> 200	31.0 ± 2.2	> 200	
2	8.1 ± 0.2	> 200	42.0 ± 0.8	> 200	
3	5.7 ± 0.4	-	3.2 ± 1.0	-	
4	12.6 ± 1.5	> 200	43.5 ± 0.9	> 200	
5	40.2 ± 2.1	> 200	> 100	28.9 ± 0.1	> 6.92
6	6.4 ± 0.3	-	61.4 ± 0.5	154.6 ± 2.3	
7	n.i.[d]	-	44.6 ± 0.6	> 200	
8	29.7 ± 0.8	> 200	> 100	76.8 ± 0.3	> 2.60
9	5.3 ± 1.0	-	43.7 ± 0.4	> 200	
10	7.6 ± 1.2	-	82.8 ± 0.9	83.7 ± 0.1	
11	2.4 ± 1.0	-	47.4 ± 0.7	> 200	
12	8.8 ± 1.2	-	10.2 ± 1.4	-	
13	43.5 ± 1.1	> 200	42.0 ± 4.4	-	
14	n.i.[d]	-	28.9 ± 3.1	-	
15	21.7 ± 1.2	> 200	86.5 ± 2.7	21.5 ± 1.2	> 9.30
16	n.i.[d]	-	55.1 ± 1.2	174.2 ± 0.1	
17	4.3 ± 1.5	-	29.2 ± 1.0	-	
eserine	-	0.011 ± 0.001	-	0.014 ± 0.001	
tacrine	-	0.029 ± 0.002	-	0.004 ± 0.001	

[a] AChE from *electric eel*; [b] BChE from horse serum; [c] Selectivity Index = IC_{50} (AChE)/IC_{50} (BChE); [d] n.i. no inhibition.

Conclusions

We have obtained a series of natural and semi-synthesized lupane-type triterpenes, by oxidation or sequential oxidations, and by reaction with hydroxylamine hydrochloride. Our results on BChE inhibition of calenduladiol analogs which have been oxidized at the C-16 position indicate

that they could be promising leader compounds to develop a strategy for the enhancement of pharmacological properties of this type of BChE inhibitors.

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