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Synthesis and cytotoxic evaluation of four new 6*E*-hydroximinosteroids

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ABSTRACT

Four new 6*E*-hydroximinosteroids (**1**, **2a**, **3** and **4**) have been synthesized from the corresponding ketones, 2β,3β-dihydroxy-5α-cholestan-6-one (**5**), 2α,3α-dihydroxy-5α-cholestan-6-one (**6**), 2β,3α-dihydroxy-5α-cholestan-6-one (**7**) and 2β,3α-dihydroxy-5α-cholestan-6-one-disulfate (**8**). The cytotoxic activity of the steroidal oximes was evaluated against two prostate carcinoma cell lines (PC-3 and LNCaP) and compared with that of five polyhydroxylated sulfated analogs (**8**–**12**). Oxime **3** and trisulfated analog **11** were the most active compounds with IC_{50} values of 10.8 μM (PC-3) and 7.9 μM (LNCaP), respectively.

1. Introduction

Marine organisms have become an important source of steroids with unusual and interesting structures. Among these secondary metabolites, sulfated polyhydroxysterols have been described from Porifera and Ophiuroidea (Echinodermata) [1–3], and some rare steroidal oximes have been isolated from *Cinachyrella* sponges [4,5]. These compounds have attracted considerable interest due to their broad spectrum of biological activities, such as ligands of human nuclear receptors [6], inhibition of protein tyrosine kinases [7,8], antiviral [9], cytotoxic [10,11], and antifouling activities [12]. In recent years, a variety of marine steroidal analogs have been synthesized in order to develop new bioactive compounds. In previous works [13,14], we have synthesized new di- and trisulfated steroids with acetylcholinesterase inhibitory activity and established that the activity was dependent on the location and the configurations of the sulfate groups on rings A and B. In 2009, Cui et al. [15] reported the synthesis of 3β,6β-dihydroxysterol disulfates and determined the importance of the cholesterol-type side chain for the cytotoxic activity.

Recently, several 6-hydroximinosteroids have been synthesized and evaluated for their cytotoxic activity against human tumor cell lines in vitro [10,16–20]. These studies have revealed the importance of oxygenated positions in ring A and a cholesterol-type side chain for an increase in cytotoxic activity.

These results prompted us to synthesize three new 6*E*-hydroximinosteroids with hydroxy groups at C-2 and C-3 (α/β configurations) (**1**, **2a**, and **3**) and one new 2β,3α-disulfated analog (**4**) (Scheme 1), and evaluate their anti-tumor activity against two human prostate carcinoma cell lines (PC-3 and LNCaP). In order to establish structure/activity relationships, the cytotoxic activity of the four 6*E*-hydroximinosteroids was compared with that of five sulfated analogs (**8**–**12**) (Fig. 1) previously synthesized by our group [13,14,21].

2. Experimental

2.1. General methods

Melting points (m.p.) were determined on a Fisher Johns apparatus and are uncorrected. ^{1}H NMR, ^{13}C NMR, HSQC-DEPT, HMBC, COSY and NOESY spectra were recorded on a Bruker AM 500 spectrometer. Chemical shifts (δ) are given in ppm downfield from TMS as the internal standard. 2D NMR spectra were obtained using standard Bruker software. High resolution mass spectra were determined on a Bruker micrOTOF-Q II mass spectrometer with ESI as ionization source. IR spectra were acquired on a FT-IR Nicolet Magna 550 spectrometer. Analytical thin layer chromatography (TLC) was performed on pre-coated silica plates (Merck F_{254}, 0.2 mm thickness); TLC of the sulfated steroids was performed on silica gel F_{254} (*n*-BuOH/AcOH/H_2O (12:3:5)) and detected by spraying with sulfuric acid (10% H_2O). Solid phase extraction tubes of silica gel (55 μm) were purchased from Phenomenex.

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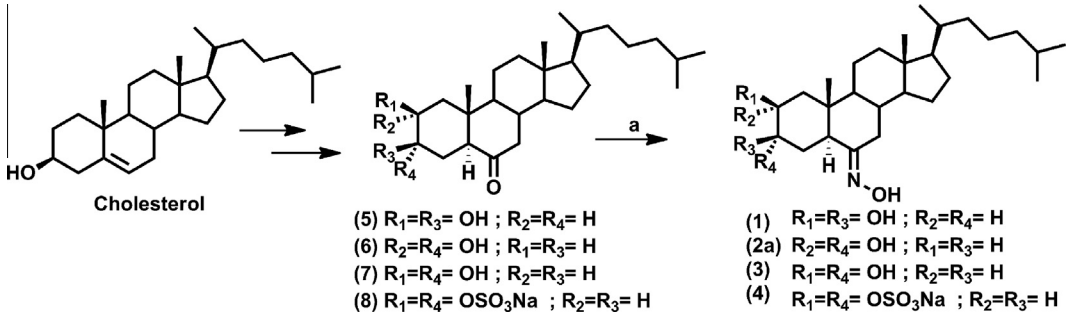

Scheme 1. Conditions: (a) $NH_2OH{\cdot}HCl$, $NaAcO{\cdot}3H_2O$, ethanol, room temperature.

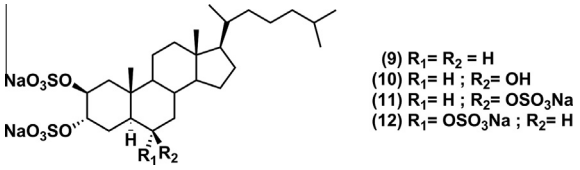

Fig. 1. Chemical structures of sulfated polyhydroxysteroids (**9**–**13**).

All chemicals and solvents were analytical grade and solvents were purified by general methods before being used.

2β,3β-Dihydroxy-5α-cholestan-6-one (**5**), 2α,3α-dihydroxy-5α-cholestan-6-one (**6**), 2β,3α-dihydroxy-5α-cholestan-6-one (**7**) and 2β,3α-dihydroxy-5α-cholestan-6-one-disulfate (**8**) were used as starting materials for the synthesis of compounds **1**, **2a**, **3** and **4**. Compounds **5**–**8** were obtained from cholesterol following the procedure described in our previous works [13,14].

2.2. General procedure for the synthesis of 6E-hydroximinosteroids

$NaAcO{\cdot}3H_2O$ (16.5 mg, 0.12 mmol) and $NH_2OH{\cdot}HCl$ (19.5 mg, 0.16 mmol) were added to a solution of 6-keto steroid (0.10 mmol) in 95% ethanol (4.95 mL). The mixture was stirred at room temperature until no 6-keto steroid was observed by TLC. The reaction product obtained by evaporation of the solvent under reduced pressure was purified over silica gel (55 μm) using cyclohexane enriched with EtOAc.

2.2.1. 2β,3β-Dihydroxy-6E-hydroximino-5α-cholestane (*1*)

The reaction of 2β,3β-dihydroxy-5α-cholestan-6-one (**5**) (4.3 mg, 0.010 mmol) in 95% ethanol with $NaAcO{\cdot}3H_2O$ and $NH_2OH{\cdot}HCl$ as described in 2.2 led to 4.3 mg of compound **1** (96%), m.p. 190 °C (decomp) (acetone). ^{1}H NMR δ (DMSO-d_6): 0.62 (s, 3H, H-18), 0.82 (s, 3H, H-19), 0.84 (d, J = 6.6 Hz, 6H, H-26, H-27), 0.89 (d, J = 6.3 Hz, 3H, H-21), 3.13 (dd, J = 13.5, 4.6 Hz, 1-H, H-7β), 3.33 (m, 1H, H-3α), 3.77 (m, 1H, H-2α), 10.29 (s, 1H, NOH). ^{13}C NMR (DMSO-d_6): 42.4 (C-1), 68.7 (C-2), 71.3 (C-3), 26.0 (C-4), 49.5 (C-5), 157.1 (C-6), 28.9 (C-7), 34.6 (C-8), 54.2 (C-9), 38.0 (C-10), 21.0 (C-11), 39.5 (C-12), 42.2 (C-13), 55.9 (C-14), 23.7 (C-15), 27.8 (C-16), 55.6 (C-17), 11.8 (C-18), 14.5 (C-19), 35.1 (C-20), 18.5 (C-21), 35.6 (C-22), 23.2 (C-23), 38.9 (C-24), 27.4 (C-25), 22.6 (C-26), 22.4 (C-27). HREIMS (ESI+), calculated for $C_{27}H_{48}NO_3$ $[M+H]^+$: 434.3629, found m/z = 434.3626. FT-IR (NaBr, film, cm^{-1}) 3376 (ν O–H), 1463 (δ_{as} CH_3), 1374 (δ_s CH_3), 1651 (ν C=N).

2.2.2. 2α,3α-Dihydroxy-6E-hydroximino-5α-cholestane (*2a*)

The reaction of 2α,3α-dihydroxy-5α-cholestan-6-one (**6**) (5.2 mg, 0.012 mmol) in 95% ethanol with $NaAcO{\cdot}3H_2O$ and $NH_2OH{\cdot}HCl$ as described in 2.2 led to 5.1 mg of a mixture of isomers **2a** and **2b** (98%). Recrystallization of the mixture afforded the *E*-isomer **2a**, m.p. 200 °C (decomp) (acetone–H_2O). ^{1}H NMR δ (DMSO-d_6): 0.61 (s, 3H, H-18), 0.63 (s, 3H, H-19), 0.84 (d, J = 6.5 Hz, 6H, H-26, H-27), 0.89 (d, J = 6.3 Hz, 3H, H-21), 3.15 (dd, J = 13.5, 4.3 Hz, 1H, H-7β), 3.48 (m, 1H, H-2β), 3.76 (bs, 1H, H-3β), 10.25 (s, 1H, NOH). ^{13}C NMR (DMSO-d_6): 39.9 (C-1), 67.4 (C-2), 67.9 (C-3), 28.5 (C-4), 42.9 (C-5), 157.5 (C-6), 29.1 (C-7), 35.2 (C-8), 53.7 (C-9), 34.9 (C-10), 20.7 (C-11), 39.2 (C-12), 42.4 (C-13), 55.9 (C-14), 23.7 (C-15), 27.8 (C-16), 55.5 (C-17), 11.8 (C-18), 12.8 (C-19), 35.6 (C-20), 18.5 (C-21), 35.6 (C-22), 23.2 (C-23), 38.9 (C-24), 27.4 (C-25), 22.4 (C-26), 22.7 (C-27). HREIMS (ESI+), calculated for $C_{27}H_{48}NO_3$ $[M+H]^+$: 434.3629, found m/z = 434.3627. FT-IR (NaBr, film, cm^{-1}) 3322 (ν O–H), 1454 (δ_{as} CH_3), 1374 (δ_s CH_3), 1657 (ν C=N).

2.2.3. 2β,3α-Dihydroxy-6E-hydroximino-5α-cholestane (*3*)

The reaction of 2β,3α-dihydroxy-5α-cholestan-6-one (**7**) (11 mg, 0.026 mmol) in 95% ethanol with $NaAcO{\cdot}3H_2O$ and $NH_2OH{\cdot}HCl$ as described in 2.2 led to 10.3 mg of compound **3** (90.5%), m.p. 230 °C (decomp) (acetone–H_2O). ^{1}H NMR δ (CD_3OD): 0.70 (s, 3H, H-18), 0.88 (d, J = 6.6 Hz, 6H, H-26, H-27), 0.94 (d, J = 6.5 Hz, 3H, H-21), 0.92 (s, 3H, H-19), 3.33 (dd, J = 13.5, 4.7 Hz, 1H, H-7β), 3.81 (m, 1H, H-2α), 3.84 (m, 1H, H-3β), 9.99 (bs, 1H, NOH). ^{13}C NMR (CD_3OD): 40.0 (C-1), 71.3 (C-2), 70.5 (C-3), 25.5 (C-4), 45.2 (C-5), 161.8 (C-6), 30.6 (C-7), 36.6 (C-8), 56.5 (C-9), 40.0 (C-10), 22.2 (C-11), 41.1 (C-12), 44.1 (C-13), 57.9 (C-14), 25.1 (C-15), 29.2 (C-16), 57.5 (C-17), 12.5 (C-18), 15.0 (C-19), 37.1 (C-20), 19.2 (C-21), 37.3 (C-22), 24.9 (C-23), 40.7 (C-24), 29.1 (C-25), 22.9 (C-26), 23.2 (C-27). HREIMS (ESI+), calculated for $C_{27}H_{48}NO_3$ $[M+H]^+$: 434.3629, found m/z = 434.3608. FT-IR (NaBr, film, cm^{-1}) 3364 (ν O–H), 1455 (δ_{as} CH_3), 1374 (δ_s CH_3), 1658 (ν C=N).

2.2.4. 2β,3α-Dihydroxy-6E-hydroximino-5α-cholestane-2,3-disulfate (*4*)

The reaction of 2β,3α-dihydroxy-5α-cholestan-6-one (**8**) (28.6 mg, 0.046 mmol) in 95% ethanol with $NaAcO{\cdot}3H_2O$ and $NH_2OH{\cdot}HCl$ as described in 2.2 led to 28.7 mg of compound **4** (98%), m.p. 210 °C (decomp) (MeOH). ^{1}H NMR δ (CD_3OD): 0.69 (s, 3H, H-18), 0.88 (d, J = 6.7 Hz, 6H, H-26, H-27), 0.91 (s, 3H, H-19), 0.94 (d, J = 6.5 Hz, 3H, H-21), 3.32* (1H, H-7β), 4.75 (m, 1H, H-2α), 4.77 (m, 1-H, H-3β)), 10.01 (bs, 1H, NOH). ^{13}C NMR (CD_3OD): 38.3 (C-1), 75.51 (C-2), 75.51 (C-3), 24.1 (C-4), 45.2 (C-5), 160.1 (C-6), 30.5 (C-7), 36.6 (C-8), 56.4 (C-9), 39.6 (C-10), 22.2 (C-11), 41.1 (C-12), 44.1 (C-13), 57.9 (C-14), 25.1 (C-15), 29.2 (C-16), 57.5 (C-17), 12.5 (C-18), 14.6 (C-19), 37.1 (C-20), 19.2 (C-21), 37.3 (C-22), 24.9 (C-23), 40.7 (C-24), 29.1 (C-25), 22.9 (C-26), 23.2 (C-27) *under CD_3OD signal. HREIMS (ESI-), calculated for $C_{27}H_{45}NNaO_9S_2$ $[M-Na]^-$: 614.2439, found m/z = 614.2421. FT-IR (KBr, cm^{-1}) 3448 (ν O–H), 1402 (δ_s CH_3), 1652 (ν C=N), 1227 (δ_s S=O).

2.3. Cytotoxic activity

2.3.1. Cell culture

Prostate cancer cell lines LNCaP and PC-3 cells were obtained from the American Type Culture Collection (Manassas, VA, USA) and were maintained at 37 °C in a humidified incubator with a 5% CO_2/95% air atmosphere in RPMI 1640 supplemented with 10% FCS.

2.3.2. Treatment of cancer cells

Optimum seeding density for 96-well plates was determined for both cell lines. LNCaP cells (10^4 cells/100 μL) and PC-3 cells (5×10^3 cells/100 μL) were seeded into each well of a 96-well microtiter plate. After incubation for 24 h, the media were aspirated and replaced with 100 μL of serum free medium containing different concentration of each compound. Wells with serum free medium and wells with an equal amount of DMSO were used as negative controls. Triplicate wells were prepared for each individual dose.

2.3.3. Assay for cell viability

MTS cell viability analysis was performed 24 h post treatment using the Cell Titer 96 AQueous One Solution Proliferation Assay System (Promega), in which viable cells convert MTS tetrazolium into a formazan-colored product (OD490 nm). Following the manufacturer's instructions, 20 μL of MTS reagent were added to each well and cells were incubated at 37 °C for 1 h. Absorbance was detected at 490 nm on a Thermo Scientific Multiskan FC plate reader. The IC_{50} value was defined as the concentration of compound yielding 50% cell survival.

3. Results and discussion

3.1. Chemistry

The steroidal oximes **1**–**4**, with a hydroximino group at C-6 on ring B, were synthesized from the corresponding ketones (**5**–**8**), which were obtained from cholesterol, as described previously [13,14] (Scheme 1). Synthesis of hydroximinosteroids **1**, **3** and **4** afforded only the *E*-isomer, but oximination of **6** gave a mixture of oximes **2a** (*E*) and **2b** (*Z*) in a 98.7:1.3 ratio, as established by ^{1}H NMR. The ^{1}H NMR spectrum of the mixture showed two singlets at 10.29 (*Z*) and 10.25 (*E*) ppm for the NOH group, and duplicate signals for C-6 (157.52(*E*)/157.45(*Z*)), C-2 (67.36(*E*)/67.24(*Z*)), and C-3 (67.85(*E*)/67.74(*Z*)) were observed in the ^{13}C NMR spectrum. Assignments of the NMR signals corresponding to the *Z*-isomer **2b** were established on comparison with those of the pure *E*-isomer **2a** obtained by recrystallization of the mixture.

The structures of the 6*E*-hydroximinosteroids were confirmed by analysis of IR, proton and carbon NMR chemical shifts at C-6 and C-7. In the IR spectra, the absorptions of 1696, 1710, 1690 and 1699 cm^{-1} for the original carbonyl group in compounds **5**, **6** [14], **7** and **8** [13], were absent and replaced by a new absorption at 1651, 1657, 1658, and 1652 cm^{-1} (C=N) for oximes **1**, **2a**, **3** and **4**, respectively [19]. Resonances showing H-7β as a double doublet at 3.1–3.3 ppm were shifted downfield due to the deshielding effect of the hydroxyl oxygen of the oxime, which were indicative of the *E*-configuration of 6-hydroximino in steroids **1**, **2a**, **3** and **4** [19]. This assignment was confirmed by the correlation between H-7β and C-6 at ≈160 ppm in the HMBC spectra of the compounds. The ^{1}H NMR spectra also showed the presence of a singlet at 9.99–10.29 ppm for the NOH group.

Compound 2β,3α-dihydroxy-6*E*-hydroximine-5α-cholestane-2,3-disulfate (**4**) was synthesized in order to determine the influence of the sulfate groups on the cytotoxicity and compare it to the corresponding diol **3**. It is worth mentioning that sulfation of the oxime **3** was not feasible because the 6-hydroximino group was converted into the former carbonyl group at the sulfation reaction conditions [20]. For this reason, oximation of 2β,3α-disulfated ketone **8** was necessary.

3.2. Evaluation of the cytotoxic activity

To determine the effect of the hydroxyl groups at C-2 and C-3 as well as their configurations on the biological function of 6*E*-hydroximinosteroids, we evaluated the cytotoxicity of compounds **1**–**3** in vitro on two human prostate carcinoma cell lines, PC-3 (androgen independent) and LNCaP (androgen dependent). From the data shown in Table 1, *cis* hydroximinosteroids **1** and **2a** were less active against PC-3 than isomer **3**, suggesting the importance of the hydroxyl configurations at C-2 and C-3 on the inhibitory activity of this tumor cell line. The introduction of hydroxyl groups at C-2 (β) and C-3 (α) resulted in a remarkable increase in the cytotoxic activity against PC-3 in comparison with 6*E*-hydroximinosteroids with a cholesterol-type side chain, a 4,5-double bond and a hydroxyl or keto group at C-3 (IC_{50} 78.3–84.8 μM) [17,18]. Sulfation of hydroxyl groups in **4** does not seem to have an influence on the level of cytotoxic activity against PC-3 because **3** and **4** showed similar IC_{50} values (10.8 μM and 12.9 μM, respectively) but an enhancement was observed for disulfated compound **4** against LNCaP. These results are in accordance with the cytotoxic activities displayed by sulfated hydroximinosteroids against some tumor cell lines [20]. Recently, the synthesis of sulfated marine sterols has attracted the attention of organic chemists and pharmacologists due to their broad spectrum of biological activities. Cui et al. [15] have synthesized three disodium 3β,6β-dihydroxysterol disulfates with different side chains and determined that 3β,6β-dihydroxy-5α-cholestane disulfate exhibited antitumor activity against PC-3, among other human carcinoma cell lines. These results prompted us to compare the cytotoxic activity against PC-3 and LNCaP cell lines of 6*E*-hydroximinosteroid **4** with that of five related 2β,3α-disulfated polyhydroxysteroids (**8**–**12**) with different functional groups at C-6, previously synthesized by our group [13,14]. Compounds **8**, **9**, **11** and **12** were active against both prostate carcinoma cell lines, while **10** with a hydroxyl group at C-6 (β) was inactive against PC-3 (IC_{50} > 100 μM) and moderately active against LNCaP (IC_{50} = 35.1 μM). Compound **11** containing three sulfate groups at C-2 (β), C-3 (α) and C-6 (β) remarkably increased its cytotoxic activity against LNCaP cells (IC_{50} 7.9 μM) in comparison with analogs **4**, **8**–**10** and **12**. In contrast, the cytotoxic activity against PC-3 cells was not significantly different between analogs **4**, **8**, **9**, **11** and **12**. These results indicate that the presence of *trans* diaxial sulfate groups at C-2 and C-3 together with an *E*-hydroximino or a sulfate group at C-6 (β) increase the cytotoxic activity against LN-CaP cells. Our findings provide new evidence of the cytotoxic activity of 6*E*-hydroximinosteroids and sulfated analogs with different functional groups at C-6.

Table 1
Summary of the in vitro cytotoxic activities.

Compound	IC_{50} (μM)	
	PC-3	LNCaP
1	39.3 ± 2.6	>100
2a	31.0 ± 2.4	26.2 ± 2.4
3	10.8 ± 2.6	44.8 ± 2.6
4	12.9 ± 1.7	13.9 ± 1.8
8	16.7 ± 1.7	20.3 ± 1.8
9	21.2 ± 1.7	20.7 ± 1.8
10	>100	35.1 ± 1.7
11	15.7 ± 1.4	7.9 ± 1.6
12	20.3 ± 1.4	21.8 ± 1.5

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References

[1] Stonik VA. Marine polar steroids. Russ Chem Rev 2001;70:673–715.
[2] ÍAuria MV, Minale L, Riccio R. Polyoxygenated steroids of marine origin. Chem Rev 1993;93:1839–95.
[3] Sarma NS, Krishna MSR, Rao SR. Sterol ring system oxidation pattern in marine sponges. Marine Drugs 2005;3:84–111.
[4] Rodríguez J, Nuñez L, Peiyinho S, Jiménez C. Isolation of the first natural 6-hydroximino-4-en-3-one steroids from the sponge *Cynachyrella* spp. Tetrahedron Lett 1997;38:1833–6.
[5] Xiao DJ, Peng XD, Deng SZ, Ma WJ, Wu HM. Structure elucidation of (3*E*)-cholest-4-en-3,6-dione-3-oxime in marine sponge *Cynachyrella australiensis* from the south China sea. Chin J Org Chem 2005;25:1606–9.
[6] Fiorucci S, Distrutti É, Bifulco G, ÍAuria MV, Zampella A. Marine sponge steroids as nuclear ligands. Trends Pharmacol Sci 2012;33:591–601.
[7] Fu X, Schmitz FJ, Lee RH, Papkoff JS, Slate DL. Inhibition of protein tyrosine kinase pp60v-src: sterol sulfates from the brittle star *Ophiarachna incrassata*. J Nat Prod 1994;57:1591–4.
[8] Whitson EL, Bugni TS, Chockalingam PS, Concepcion GP, Harper MK, He M, Hooper JNA, Mangalindan GC, Ritacco F, Ireland CM. Spheciosterol sulfates, PKC_inhibitors from a philippine sponge *Spheciospongia* sp.. J Nat Prod 2008;71:1213–7.
[9] Comin MJ, Maier MS, Roccatagliata AJ, Pujol CA, Damonte EB. Evaluation of the antiviral activity of natural sulfated polyhydroxysteroids and their synthetic derivatives and analogs. Steroids 1999;64:335–40.
[10] Deive N, Rodríguez J, Jiménez C. Synthesis of cytotoxic 6*E*.hydroximino-4-ene steroids: structure/activity studies. J Med Chem 2001;44:2612–8.
[11] Marinho PR, Simas NK, Kuster RM, Duarte RS, Fracalanzza SEL, Ferreira DF, Romanos MTV, Muricy G, Giambiagi-Demarval M, Laport MS. Antibacterial activity and cytotoxicity analysis of halistanol sulfate from marine sponge *Petromia citrina*. J Antimicrob Chemother 2012;67:2396–400.
[12] Nguyen XC, Longeon A, Pham VC, Urvois F, Bressy C, Trinh TTV, Nguyen HN, Phan VK, Chau VM, Briand JF, Bourguet-Kondracki ML. Antifouling 26,27-cyclosterols from the Vietnamese marine sponge *Xestospongia testudinaria*. J Nat Prod 2013;76:1313–8.
[13] Richmond V, Garrido Santos G, Murray AP, Maier MS. Synthesis and acetylcholinesterase inhibitory activity of 2β,3α-disulfoxy-5α-cholestan-6-one. Steroids 2011;76:1160–5.
[14] Richmond V, Murray AP, Maier MS. Synthesis and acetylcholinesterase inhibitory activity of polyhydroxylated sulfated steroids: structure/activity studies. Steroids 2013;78:1141–7.
[15] Cui J, Wang H, Huang Y, Xin Y, Zhou A. Synthesis and cytotoxic analysis of some disodium 3β.6β-dihydroxysterol disulfates. Steroids 2009;74:1057–60.
[16] Poza J, Rega M, Paz V, Alonso B, Rodriguez J, Salvador N, et al. Synthesis and evaluation of new 6-hydroximinosteroid analogs as cytotoxic agents. Bioorg Med Chem 2007;15:4722–40.
[17] Cui J, Huang L, Fan L, Zhou A. A facile and efficient synthesis of some (6*E*)-hydroximino-4-en-3-one steroids, steroidal oximes from *Cinachyrella* spp. Sponges. Steroids 2008;73:252–6.
[18] Cui JG, Fan L, Huang LL, Liu AH, Zhou AM. Synthesis and evaluation of some steroidal oximes as cytotoxic agents: structure activity studies (I). Steroids 2009;74:62–72.
[19] Cui J, Fan L, Huang Y, Xin Y, Zhou A. Synthesis and evaluation of some steroidal oximes as cytotoxic agents: structure activity studies (II). Steroids 2009;74:989–95.
[20] Huang Y, Cui J, Li Y, Fan L, Jiao Y, Su S. Synthesis and antiproliferative activity of some sulfated hydroximinosterols. Med Chem Res 2013;22:409–14.
[21] Garrido Santos GA, Murray AP, Pujol CA, Damonte EB, Maier MS. Synthesis and antiviral activity of sulfated and acetylated derivatives of 2β,3α-dihydroxy-5α-cholestane. Steroids 2006;68:125–32.