Synthesis and Characterization of Two Danazol Derivatives

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Received 16 March 2010; Revised 25 May 2010; Accepted 20 June 2010

Abstract: Two danazol derivatives were synthesized and characterized by spectral analyses. In order to characterize the structural and chemical requirements of danazol derivatives, several parameters such as $\log P$, π , R_m and V_m were evaluated. The results showed an increase in the values of $\log P$ for the compound **6** in comparison with **3**. The compound **3** showed an increase in the values of π , R_m and V_m with respect to **6**. These data indicate a high degree of lipophilicity and a low steric impediment for compound **6** in comparison with **3**.

Keywords: Danazol derivatives, Lipophilicity, Steric impediment, Synthesis.

Introduction

The studies for the establishment of parameters relationship between biological activity of steroids and its molecular structure (QSAR), plays an important role in the development of new steroid derivatives¹⁻². For example the QSAR analysis of a series of steroids binding to globulin was made using the electro topological state index for each atom in the molecule³. Additionally, other studies showed a quantitative structure-activity relationship among some steroids with several parameters such as molecular volume, Van der Waals volume and including difference and square steric terms.^{4,5} The relationship of dihydrotestosterone–ciprofloxacin conjugates with the descriptors such as $\log P$, π , R_m and V_m .⁶ was evaluated recently. In this work our initial design included the synthesis of two danazol derivatives and its relationship with the $\log P$, π , R_m and V_m .

Experimental

Hemisuccinate of danazol (**1**) was prepared accord to the method reported by Figueroa and co-workers⁷. Danazol (**4**) and the other compounds evaluated in this study were purchased from Sigma-Aldrich Co., Ltd. The melting points of the different compounds were determined using an Electrothermal (900 model). Ultraviolet spectroscopy (UV) was carried out in dry methanol on a Perkin-Elmer model 552 spectrophotometer and infrared spectra (IR) was recorded using KBr pellets on a Perkin Elmer Lambda 40 spectrometer. ¹H and ¹³C NMR spectra were recorded on a Varian VXR-300/5 FT NMR spectrometer at 300 and 75.4 MHz in CDCl₃ using TMS as internal standard. EIMS spectra were obtained with a Finnigan Trace GCPolaris Q. spectrometer. Elementary analysis data were acquired from a Perkin Elmer Ser. II CHNS/O 2400 elemental analyzer.

Succinic acid mono-[6-[(2-amino-ethylamino)-methyl]-1-ethynyl-10a,12a-dimethyl-2,3,3a,3b,4,5,10,10a,10b,11,12,12a-dodecahydro-1H-7-oxa-8-aza-dicyclopenta[a,h]-phenanthren-1-yl]ester (3)

200 Mg (0.46 mmol) of compound **1** was added to solution of ethylenediamine hydrochloride (**2**), 100 mg (0.75 mmol) in 10 mL formaldehyde was gently refluxed for 48 h and then cooled to room temperature. After the solvent was removed under vacuum and the crude product was purified by crystallization from methanol:hexane:water (3:2:1) mixture. The yield of the product was 75% and m.p 184 °C.

UV (MeOH) $\lambda_{\max}$: (log ϵ) 210 (2.54), 282 (3.36) nm; IR $\nu_{\max}$: 3330, 1680 cm⁻¹; ¹H NMR (300 MHz, CDCl₃) δ_{H} : 0.84 (m, 1H), 0.92 (m, 1H), 0.96 (s, 3H), 1.01 (s, 3H), 1.53-1.70 (m, 4H), 1.71-1.82 (m, 4H), 1.87-1.89 (m, 2H), 1.95-2.03 (m, 3H), 2.30-2.34 (m, 2H), 2.57 (m, 2H), 2.67 (m, 2H), 2.74-2.80 (m, 4H), 2.96 (s, 1H), 3.79 (broad, 4H), 3.98 (s, 2H), 8.03 (s, 1H) ppm. ¹³C NMR (75.4 MHz, CDCl₃) δ_{C} : 14.00 (C-22), 19.81 (C-21), 20.83 (C-19), 22.00 (C-17), 22.90 (C-13), 29.45 (C-34), 30.60 (C-33), 30.98 (C-18), 32.17 (C-20), 33.93 (C-12), 36.60 (C-8), 41.17 (C-31), 41.42 (C-6), 46.19 (C-28), 46.40 (C-10), 51.91 (C-30), 53.10 (C-9), 56.60 (C-7), 75.44 (C-27), 84.00 (C-11), 84.70 (C-26), 107.34 (C-4), 120.30 (C-15), 145.00 (C-16), 148.66 (C-3), 162.02 (C-14), 168.40 (C-40), 173.60 (C-35) ppm. MS (70 eV): m/z = 509.20 ([M+ 10]⁺), 432.20, 285.13, 215.28. Anal. Calcd. to C₂₉H₃₉N₃O₅: C, 68.34; H, 7.71; N, 8.25, O, 15.70. Found. C, 68.30; H, 7.67; N, 8.19.

6-[(1-Ethynyl-1-hydroxy-10a,12a-dimethyl-2,3,3a,3b,4,5,10,10a,11,12,12a,dodecahydro-1H-7-oxa-8-aza-dicyclopenta[a,h]phenanthren-6-yl-methyl)-amino]hexanoic acid (6)

A solution of compound **4** (200 mg, 0.59 mmol) and 6-aminohexanoic acid (**5**) (155 mg, 1.18 mmol) in 10 mL of formaldehyde was gently refluxed for 48 h and then cooled to room temperature. After the solvent was removed under vacuum and the crude product was purified by crystallization from methanol:hexane:water (3:2:1) mixture. The product yield was 65% and m.p 168-170 °C.

UV (MeOH) $\lambda_{\max}$: (log ϵ) 208 (1.96), 284 (1.61) nm; IR $\nu_{\max}$ 3300, 1730 cm⁻¹; ¹H NMR (300 MHz, CDCl₃) δ_{H} : 0.78 (s, 3H), 0.79-0.82 (m, 2H), 1.01 (s, 3H), 1.22 (d, 2H), 1.29 (m, 1H), 1.40-1.61 (m, 7H), 1.71-1.81 (m, 6H), 1.94-2.02 (m, 3H), 2.03 (m, 1H), 2.34 (m, 1H), 2.40-2.43 (d, 2H), 3.08 (s, 1H), 3.76 (s, 2H), 5.60 (broad, 3H), 8.03 (1H) ppm. ¹³C NMR (75.4 MHz, CDCl₃) δ_{C} : 14.04 (C-22), 19.10 (C-21), 20.97 (C-19), 22.54 (C-17), 22.94 (C-13), 25.30 (C-30), 25.48 (C-31), 28.95 (C-29), 30.98 (C-18), 31.75 (C-20), 33.57 (C-32), 33.93 (C-5), 37.00 (C-8), 37.10 (C-12), 38.00 (C-6), 46.28 (C-26), 46.70 (C-10), 48.60 (C-28),

52.88 (C-9), 54.41 (C-7), 75.14 (C-25), 85.85 (C-11), 90.03 (C-24), 109.84 (C-1), 116.84 (C-15), 141.96 (C-16), 148.16 (C-3), 159.79 (C-14), 175.30 (C-33) ppm; MS (70 eV): m/z = 480.60 ($[M + 18]^+$), 3.19.44, 275.28. Anal. Calcd. to $C_{29}H_{40}N_2O_4$: C, 72.47; H, 8.39; N, 5.83, O, 13.32. Found. C, 72.38; H, 8.36; N, 5.80.

QSAR

ACD Log P and KOWWIN programs^{8,9} were used to estimate the parameters $\log P$, π , R_m , V_m of the compounds studied.

Results and Discussion

It is important to mention that several steroid derivatives has been developed using the Mannich reaction. The structural chemistry of these compounds involves an activated methyl group in ring A and B¹⁰, or the aliphatic side chain attached to C₁₇. Several androgen derivatives¹¹, have been synthesized in this way. The reactivity of hydrogen atom involved in the ring A of compound **1** was studied in this work by means of Mannich reaction. The first step was achieved by reacting the compound **1** with ethylenediamine (**2**) and formaldehyde to form compound **3** (Figure 1). ¹H NMR spectrum of compound **3** showed signals at 0.84 and 0.96 ppm corresponding to methyl group presents in the steroid nucleus. Additionally, several signals at 2.67, 2.74 and 3.98 for methylenes bound to A-ring of compound **3**; at 2.57 and 2.80 ppm for methylenes bound to carboxyl group were found. Finally, two signals at 2.96 ppm for the proton of alkyne group and at 8.03 ppm corresponding to the acidic hydrogen of C(=O)-OH were found. ¹³C NMR spectra display chemical shifts at 14.00 and 19.81 ppm for the carbons of methyl presents in the steroid nucleus. The chemical shift of the methylenes bound to carboxyl group is found at 29.45, 29.48 and 168.40 ppm. Other signals at 41.17-51.91 ppm for the methylenes bound to amino group were found. There are several signals (20.83-162.02 ppm) corresponding to carbons involved in the steroid nucleus; at 168.40 ppm for ester group and at 173.60 ppm for free carboxyl group. The presence of **3** was further confirmed from mass spectrum which showed a molecular ion at m/z 509.60.

The second step was achieved by the reaction of compound **4** with aminocaproic acid (**5**) in presence of formaldehyde to form compound **6** (Figure 2). ¹H NMR spectra of **6** showed chemical shifts at 0.78 and 1.01 ppm for methyl group present in the steroid nucleus. Additionally, several signals at 1.22, 2.40-2.43 and 3.76 ppm were found for methylenes involved in arm bound to steroid. Finally, other signals at 3.06 ppm for the proton of alkyne group at 5.60 ppm corresponding to the acidic hydrogen of C(=O)-OH were found. ¹³C NMR spectra displays chemical shifts at 14.04 and 19.10 ppm for the carbons of methyl group presents in the steroid nucleus of compound **6**. Another chemical shifts at 25.30-28.95 and 46.28-48.60 ppm for carbons of methylenes involved in arm bound to the steroid nucleus were exhibited. In addition, two signals at 75.14 and 90.03 ppm for carbons corresponding to alkyne group were exhibited. Another chemical shifts at 175.30 for free carboxyl group. The presence of compound **6** was further confirmed from mass spectrum which showed a molecular ion at m/z 480.60.

In order to characterize the structural and chemical requirements of the compounds **3** and **6**, the descriptors¹² such as $\log P$ and π were calculated. $\log P$ describes the logarithmic octanol-water partition coefficient; therefore, it represents the lipophilic effects of a molecule that includes the sum of the lipophilic contributions of the parent molecule and its substituents¹³. The difference between the substituted and unsubstituted $\log P$ values is conditioned by the π value for a particular substituent¹⁴. In this work, the $\log P$ and π parameters were calculated by the method reported by Leo¹⁵. The results (Table 1 and 2, see)

showed an increase in $\log P$ and a decrease in the π values of compound **6** with respect to **3**. These results showed that aliphatic carbons ($-\text{CH}_2-$) in compound **6** contribute to the high lipophilicity in comparison with **3**.

Table 1. Physicochemical parameters LogKow and π of hemisuccinate of danazol-ethylenediamine (**3**)

Compound	LogKow Fragment	Contributions
Hemisuccinate of danazol-ethylen-diamine	-CH3 [aliphatic carbon]	1.0946
	-CH2- [aliphatic carbon]	5.8932
	-CH [aliphatic carbon]	1.0842
	=CH- or =C< [olefinic carbon]	0.7672
	#C [acetylenic carbon]	0.2668
	-NH2 [aliphatic attach]	-1.4148
	-NH- [aliphatic attach]	-1.4962
	Aromatic Carbon	0.8820
	-COOH [acid, aliphatic attach]	-0.6895
	-C(=O)O [ester, aliphatic attach]	-0.9505
	Aromatic Oxygen	-0.0423
	Aromatic Nitrogen [5-member ring]	-0.5262
	-tert Carbon [3 or more carbon attach]	0.8028
	Fused aliphatic ring unit correction	-2.0526
	Oxazole ring (non-fused) correction	-0.3279
	Amino acid (non- α carbon type) cor.	-3.2000
	Equation Constant	0.2290
	Log Kow	0.3198
	π	3.8869

Table 2. Physicochemical parameters LogKow and π of danazol-aminocaproic acid (**6**)

	LogKow Fragment	Contributions
Danazol-aminocaproic acid	-CH3 [aliphatic carbon]	1.0946
	-CH2- [aliphatic carbon]	6.3843
	-CH [aliphatic carbon]	1.0842
	=CH- or =C< [olefinic carbon]	0.7672
	[acetylenic carbon]	0.2668
	-OH [hydroxy, aliphatic attach]	-1.4086
	-NH- [aliphatic attach]	-1.4962
	Aromatic Carbon	0.8820
	-COOH [acid, aliphatic attach]	-0.6895
	Aromatic Oxygen	-0.0423
	Aromatic Nitrogen [5-member ring]	-0.5262
	-tert Carbon [3 or more carbon attach]	0.8028
	Fused aliphatic ring unit correction	-2.0526
	Oxazole ring (non-fused) correction	-0.3279
	Amino acid (non- α carbon type) cor.	-3.2000
	Equation Constant	0.2290
	Log Kow	1.7676
	π	2.4391

On the other hand, other steric constants such as the molar volume (V_m) and molar refractivity (R_m) were calculated (Table 3). These options are a useful tool for the correlation of different properties that depend on characteristics of substituents attached to a constant reaction center. The results showed an increase in both R_m and V_m values for **3** in comparison

with **6**. These data indicate a high steric impediment and different conformational preferences and internal rotation of **3** in comparison with **6**. These data are supported by studies of Bryantsev and coworkers¹⁶, who showed that conformational differences between several chemical functional groups have important consequences.

Table 3. Physicochemical parameters R_m and V_m of compounds **3** and **6**

Compound	R_m , cm ³	V_m , cm ³
3	137.26	401.90
6	133.31	393.00

Conclusion

The results indicate a high degree of lipophilicity and a low steric impediment of compound **6** in comparison with **3**.

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