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Synthesis of Glibenclamide-Pregnenolone Conjugate and Its Relationship with Physico-chemical Descriptors log P, π, R_m, V_m, P_c and S_t

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In this study, a glibenclamide-pregnenolone conjugate was synthesized. The route involved a reaction of substitution of chloride atom involved in the chemical structure of glibenclamide to form the compound **2** using sodium hydroxide. Additionally, the compound **2** was bound to pregnenolone succinate to form the glibenclamide-pregnenolone conjugate (**4**) in the presence of dicyclohexylcarbodiimide and *p*-toluene sulfonic acid. To delineate the structural chemical requirements of the compounds **2** and **4**, some parameters such as the physico-chemical descriptors log P, π, R_m, V_m, P_c and S_t were calculated. The results showed an increase in log P, π, R_m, V_m, P_c values for **4** in comparison with compound **2**. These data indicate that steric impediment, conformational preferences and internal rotation of compound **4** could influence the degree of lipophilicity of this compound.

Key Words: Physicochemical descriptors, Glibenclamide, Steroid nucleus, Lipophilicity.

INTRODUCTION

Since several decades ago have been synthesized some urea and sulfonylurea derivatives with a wide spectrum of biological activity[1,2]. For example, there are several reports which show the synthesis of symmetric urea derivatives with carbon dioxide in ionic liquids[3]. In addition, other reports showed the preparation of N,N,N′,N′-unsymmetrical tetrasubstituted ureas using N,N′-carbonyldibenzotriazole[4]. Other studies showed the preparation of a series of N-(*o*-fluorophenoxyacetyl)thioureas by the reaction between *o*-fluorophenoxyacetyl isothiocyanate and 4,6-disubstituted-2-aminopyrimidines[5]. There are report[6] on the synthesis of N-(4-isopropylphenyl)-N-(coumarin-4-sulfonyl)urea by the reaction of 4-coumarinsulfonamide with 4-isopropylphenyl isocyanate using $SnCl_4$ as catalyst. Additionally, others studies showed the preparation of 2-thiazolyl- and 2-furfuryl-ureas using 2-aminothiazole or 2-furfurylamine in presence of isocyanate[7].

On the other hand, there are reports on the synthesis of sulfonylurea conjugates, for example the coupling of glibenclamide (1-[4-[2-(2-aminobenzamido)ethyl]-phenylsulfonyl]-3-cyclohexylurea) with bovine serum albumin (BSA)[8]. In addition, other study shows the preparation of a glibenclamide-glucose conjugate[9]. All these experimental results show several procedures are available for synthesis of urea and sulfonylurea derivatives and conjugates, nevertheless expensive reagents and special conditions are required. Therefore, in this work our initial design included a facile synthesis of a glibenclamide-pregnenolone conjugate that contains a spacer arm with di-ester groups between the glibenclamide fragment and steroid nucleus. In addition to delineate the structural chemical requirements of glibenclamide -pregnenolone derivative with the lipophilicity degree, several physicochemical parameters such as log P, π, R_m, V_m, P_c and S_t were evaluated.

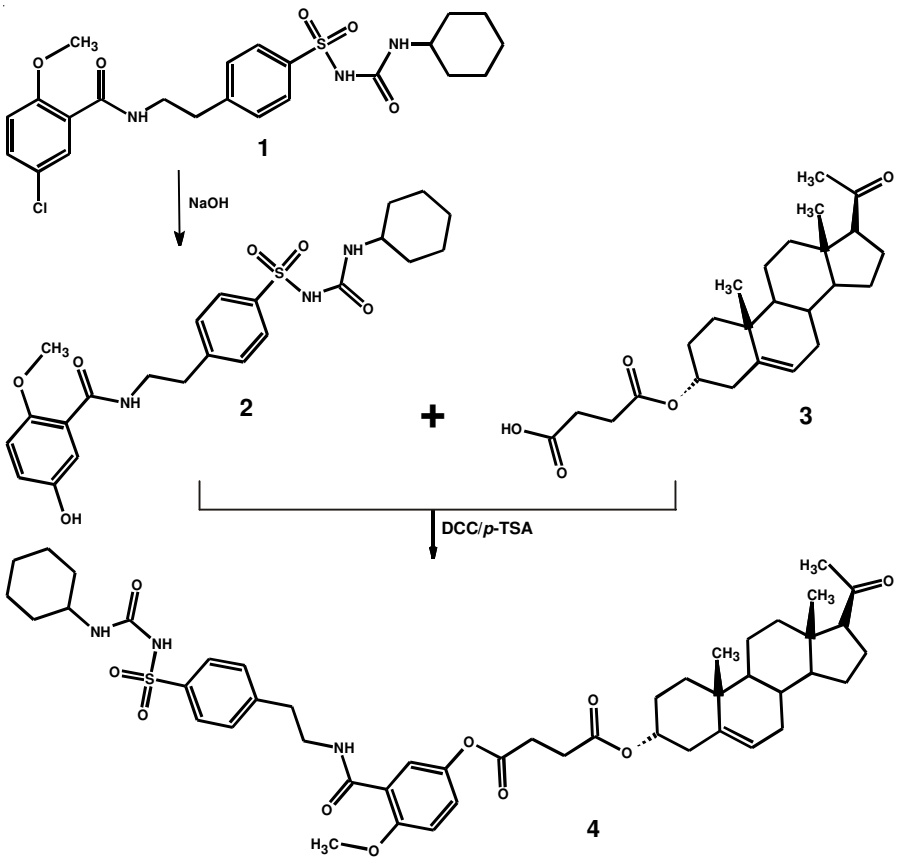
EXPERIMENTAL

Hemisuccinate of pregnenolone was prepared according to a previously reported method by several investigators[10,11]. Glibenclamide and the other compounds were purchased from Sigma-Aldrich Co., Ltd. The melting points for the different compounds were determined on an Electrothermal (900 model). Infrared spectra (IR) was recorded using KBr pellets on a Perkin-Elmer Lambda 40 spectrometer. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Varian VXR-300/5 FT NMR spectrometer at 300 and 75.4 MHz in $CDCl_3$ 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/0 2400 elemental analyzer.

N-[2-(4-[N-(cyclohexylcarbamoyl)sulfamoyl]-phenyl)-ethyl]-5-hydroxy-2-methoxybenzamide (2): A solution of glibenclamide (100 mg, 0.20 mmol) and sodium hydroxide (16 mg, 0.40 mmol) in ethanol 10 mL was stirring by 24 h at room temperature. The reaction mixture was evaporated to a smaller volume. After the mixture was diluted with water and extracted with chloroform. The organic phase was evaporated to dryness under reduced pressure, the residue was purified by crystallization from methanol:water (3:1) yielding 60 % of product, m.p. 112-115 ºC; IR (ν_{max}, cm^{-1}): 3402, 2130, 1655, 1530; ^{1}H NMR (300 MHz, DMSO-d_6) δ_H: 1.45 (m, 2H), 1.50 (m, 3H), 1.83 (m, 2H), 2.01 (m,2H), 2.84 (t, 2 H, J = 7 Hz), 3.48 (t, 2H, J = 7 Hz), 3.76 (m, 1 H), 3.95 (s, 3H), 6.72 (d, 1 H), 6.92 (m, 1H), 6.94 (m, 1H), 7.47 (d, 2H), 7.84 (d, 2H), 8.26 (broad, 4H) ppm. ^{13}C NMR (75.4 MHz, DMSO-d_6) δ_C: 24.59 (C-3, C-5), 25.47 (C-4), 33.56 (C-2, C-6), 36.06 (C-20), 40.30 (C-21), 47.28 (C-1), 56.03 (C-32), 113.30 (C-27), 113.62 (C-30), 120.47 (C-25), 122.99 (C-28), 128.98 (C-19, C-15), 129.54 (C-18, C-16), 136.17 (C-14), 141.43 (C-17), 147.64 (C-29), 147.89 (C-26), 154.75 (C-8), 163.57 (C-23), ppm. MS (70 eV): m/z = 475.00 [M^+], 334, 295.4, 151, 108, 83. Anal. calcd. for $C_{23}H_{29}N_3O_6S$: C, 58.09; H, 6.15; N, 8.84; S, 6.74. Found: C, 58.05; H, 6.19.

Succinic acid 17-acetyl-10,13-dimethyl-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1*H*-cyclopenta[a]-phenanthren-3-yl-ester-4-methoxy-3-[2-[4-(N-(cyclohexyl-carbamoyl)]sulfamoyl-phenyl]ethylcarbamoyl]phenyl ester (4): A solution of compound **3** (100 mg, 0.21 mmol) and hemisuccinate of pregnenolone (87 mg, 0.21 mmol), 1,3-dicyclohexylcarbodiimide (0.87 mg, 0.42 mmol) and *p*-toluene sulfonic acid (76 mg, 0.40 mmol) in 10 mL ethanol was stirring by 24 h at room temperature. The reaction mixture was evaporated to a smaller volume. After the mixture was diluted with water and extracted with chloroform (**Scheme-I**). The organic phase was evaporated to dryness under reduced pressure, the residue was purified by crystallization from methanol: water (3:1) yielding 70 % of product, m.p. 178-180 ºC; IR (ν_{max}, cm^{-1}): 2128, 1720, 1536; ^{1}H NMR (300 MHz, $CDCl_3$) δ_H: 0.64 (s, 3H), 1.01 (s, 3H), 1.06-1.38 (m, 5H), 1.45 (m, 2H), 146-1.49 (m, 2H), 1.53-1.60 (m, 4H), 1.61-1.70 (m, 5H), 1.83 (m, 2H), 1.88-1.92 (m, 2H), 2.03 (m, 2H), 2.07-2.08 (m, 2H), 2.13 (s, 3H), 2.17-2.51 (m, 4H), 2.64 (m, 2H), 2.84 (m, 2H), 2.86 (m, 2H), 3.46 (t, 2H, J = 6.7 Hz), 3.77 (m, 1H), 3.86 (s, 3H), 4.39-5.45 (m, 2H), 7.08-7.13 (m, 3H), 7.41 (m, 2H), 7.71 (m, 2H), 8.06 (s, 3H) ppm. ^{13}C NMR (75.4 MHz, $CDCl_3$) δ_C: 13.17 (C-59), 19.48 (C-58), 21.92 (C-50), 22.90 (C-55), 23.61 (C-62), 24.59 (C-3, C-5), 25.47 (C-4), 27.80 (C-42), 30.00 (C-37), 30.71 (C-36), 31.40 (C-56), 31.80 (C-46, C-54), 33.56 (C-2, C-6) 36.56 (C-20), 36.95 (C-43), 37.60 (C-44), 38.21 (C-51), 38.80 (C-49), 40.00 (C-21), 43.91 (C-48), 47.28 (C-1), 49.90 (C-45), 54.81 (C-32), 56.78 (C-47), 63.91 (C-57), 73.95 (C-41), 113.27 (C-27), 120.80 (C-25), 122.23 (C-30), 122.59 (C-53), 127.84 (C-19), 127.88 (C-15), 128.53 (C-18, C-16), 130.23 (C-28), 135.81 (C-14), 139.60 (C-52), 142.11 (C-17), 146.02 (C-26), 147.00 (C-29), 154.75 (C-8),



Scheme-I: Synthesis of glibenclamide-pregnenolone conjugate. Reaction of **2** with pregnenolone succinate (**3**) in presence of 1,3-dycyclohexylcarbodiimide (DCC) and *p*-toluensulfonic acid (*p*-TSA)

168.82 (C-23), 171.88 (C-34), 173.00 (C-38), 209.20 (C-60) ppm. MS (70 eV): m/z = 873.38 [M^+], 233.10 (100), 198.20 (65). Anal. calcd. for $C_{48}H_{63}N_3O_{10}S$: C, 65.96; H, 7.26; N, 4.81; S, 3.67. Found: C, 65.44; H, 7.23.

RESULTS AND DISCUSSION

In this study, a straightforward route is reported for the synthesis of glibenclamide-pregnenolone derivative. The first step was achieved by the synthesis of the compound **2** which contains in the phenyl ring a hydroxyl group by the substitution of chloride. It is important to mention that there are several reports of substitution reaction in the literature[12-14], nevertheless in this study was used sodium hydroxide such nucleophilic agent to form the compound **2**. ^{1}H NMR spectrum of **2** shows signals at 1.45-2.01 and 3.76 ppm for methylenes present in the hexane ring; at 2.48 and 3.48 for methylenes bound to phenyl group. In addition, others signals at 3.95 ppm for protons of methoxy group; at 6.72-7.84 ppm for phenyl groups were found. Finally, a signal at 8.26 ppm for both amide and hydroxyl groups was found. The ^{13}C NMR spectrum contains peaks at chemical shifts of 24.59-33.56 and 47.28 ppm for the carbons of the methyl groups present in the cyclohexane ring. The chemical shifts of methylenes coupling to the phenyl ring are at 36.06 and 40.30 ppm. In addition, other signal at 56.03 ppm for methoxy group was found. At downfield there are several signals (113.30-147.89 ppm) corresponding to the carbons of the aromatic rings and at 154.75 ppm for amide group and at 163.57 ppm for urea group. Finally, in the mass spectrum the molecular ion is atm/z = 475.00 ([M + H]$^+$), which confirms the structure of compound **2**.

The second step involves esterification of the hydroxyl group of the compound **2** by the reaction with pregnenolone succinate (**3**) to form glibencalmide-pregnenolone derivative (**4**). It is important to mention that diverse reagents are available for producing ester derivatives[15,16]; nevertheless, most conventional methods have found only limited use for this purpose. During recent years, carbodiimides and especially dicyclohexylcarbodiimide (DCC) have attracted increasing attention as condensing agents in ester synthesis[17,18]. Nevertheless, it is important to mention here that when dicyclohexylcarbodiimide is used as condensing agent in esters synthesis, yields of the esters are often unsatisfactory because of formation of the N-acylurea derivative as by-product. Some reports reveal that addition of a catalytic amount of a strong acid to the esterification reaction in the presence of dicyclohexyl-carbodiimide considerably increases the yield of esters and reduces the formation of the N-acylurea compound[19]. For this reason, esterification of the hydroxyl group of compounds **2** with **3** in the presence of dicyclohexylcarbodiimide and *p*-toluene sulfonic acid (**Scheme-I**) was used to increase the yield of glibenclamide-pregnenolone derivative (**4**). ^{1}H NMR spectrum of compound **4** shows signals at 0.64, 1.01 and 2.13 ppm for methyl groups present in the heterocyclic ring and at 1.06-1.49, 1.61-1.92, 2.07-2.51 and 4.39-5.45 ppm for the protons involved in the steroid nucleus. Other signals at 1.45-3.77 for cyclohexane ring; at 2.64 and 2.84 for methylene groups involved in the spacer arm between the glibenclamide and steroid nucleus; at 2.86 and 3.46 for methylenes of arm bound to phenyl group. In addition, other signals at 3.86 ppm for proton corresponding to methoxy group; at 7.08-7.71 ppm for methylenes of phenyl groups. Finally, the spectrum contains a signal with a chemical shift of 8.06 ppm for both amide and urea groups.

^{13}C NMR spectrum of compound **4** contains peaks at chemical shifts of 13.17-19.48 and 23.61 ppm for the carbons of the methyl groups present in the heterocyclic ring. Other signals at 21.92-22.90, 27.80, 31.40-31.80, 36.95-38.80, 43.91, 49.90, 56.78-73.95, 122.59 and 139.60 ppm for steroid nucleus were found. The chemical shifts of methylenes involved in cyclohexane ring are at 24.59-25.47, 33.56 and 47.28 ppm. In addition, other signals at 30.00-30.71 ppm for spacer arm involved between the glibenclamide fragment and steroid nucleus; at 36.56 and 40.00 ppm for methylenes joined to phenyl group; at 56.78, 113.27-122.23, 127.84-135.81 and 142.60-147.00 ppm for carbons of phenyl groups. Several chemical shifts at 154.75 for urea group; at 168.82 ppm for amide group; at 171.88-173.00 ppm for ester groups and 209.20 for ketone group are also found. Finally, in the mass spectrum the molecular ion is at m/z = 873.38 ([M + H]$^+$), which confirms the structure of **4**.

On the other hand, to delineate the structural chemical requirements of both compounds **2** and **4**, other parameters such as the descriptors log P and π[20] were calculated. It is important to mention that log P estimates the logarithmic octanol-water partition coefficient. Therefore the log P represents the lipophilic effects of a molecule which includes the sum of the lipophilic contributions of the parent molecule and its substituent[21]. The difference between the substituted and unsubstituted log P values is conditioned by the π value for the particular substituent. Hammett showed that π values measure the free energy change caused by particular substituent to relate to biological activity[22,23]. The log P and π parameters were calculated by the method proposed by Mannhold and Waterbeemd *et al.*[24]. It is important to mention that also the compound **2** was evaluated with the purpose to know if there are differences in its lipophilicity degree with respect to compound **4**. The results (Tables 1 and 2) showed an increase in both log P and π values of compound **4** with respect to compound **2** and glibenclamide. This phenomenon is conditioned mainly, by the contribution of all substituent atoms involved in the chemical structure of compound **4**, as is showed in the Tables 1 and 2. These results showed that aliphatic carbons (-CH_3, -CH_2 and -CH) in compound **4** contribute to increase the lipophilicity in comparison with compound **2**. These data indicate that a change in the degree of lipophilicity depend of structural chemistry characteristic of compounds studied. Nevertheless, it is important to mention that there are studies which suggest that log P is relationship with some steric constants such as the molar volume (V_m) and molar refractivity (R_m)[24], these physicochemical parameters are a useful tool for the correlation of different properties that depend on characteristics of substituents attached to a constant reaction center. Therefore in study, both V_m and R_m descriptors were evaluated using the ACDLabs program[25,26]. The results showed an increase in both R_m and V_m and values for compound **4** in comparison with compound **2** (Table-3). These data indicate that steric

TABLE-1
PHYSICO-CHEMICAL PARAMETERS log P
OF COMPOUNDS **2** AND **4**

Program	Compounds	
	2	**4**
ALOGPs	2.99	5.84
AC log P	3.08	7.16
AB/log P	2.57	8.18
miLogP	3.61	7.84
ALOGP	3.21	7.16
MLOGP	1.57	4.56
KOWWIN	3.67	8.30
XLOGP2	3.65	7.60
XLOGP3	3.83	8.07
Average log P	3.13 (± 0.71)	7.19(±1.24)

TABLE-2
PHYSICO-CHEMICAL PARAMETERS
log Kow AND π OF COMPOUNDS **2** AND **4**

log Kow fragment	Contributions
Compound **2**	
$-CH_3$ [aliphatic carbon]	0.5473
$-CH_2-$ [aliphatic carbon]	3.4377
-CH [aliphatic carbon]	0.3614
-NH- [aliphatic attach]	-4.4886
Aromatic carbon	3.5280
-OH [hydroxy, aromatic attach]	-0.4802
-O- [oxygen, one aromatic attach]	-0.4664
-C(=O)N [aromatic attach]	0.1599
$-SO_2-N$ [aromatic attach]	-0.2079
-NC(=O)N- [urea]	1.0453
Equation Constant	0.2290
log Kow	3.6655
π	-1.125
Compound **4**	
$-CH_3$ [aliphatic carbon]	2.1892
$-CH_2-$ [aliphatic carbon]	8.3487
-CH [aliphatic carbon]	2.1684
=CH- or =C< [olefinc carbon]	0.7672
-NH- [aliphatic attach]	-4.4886
Aromatic carbon	3.5280
-O- [oxygen, one aromatic attach]	-0.4664
-C(=O)- [carbonyl, aliphatic attach]	-1.5586
-C(=O)O [ester, aliphatic attach]	-1.9010
-C(=O)N [aromatic attach]	0.1599
$-SO_2-N$ [aromatic attach]	-0.2079
-NC(=O)N- [urea]	1.0453
-*tert*-Carbon [3 or more carbon attach]	0.5352
Fused aliphatic ring unit correction	-2.0526
Equation Constant	0.2290
log Kow	8.2958
π	4.6303

TABLE-3
PHYSICO-CHEMICAL PARAMETERS log Kow
AND π OF COMPOUNDS **2** AND **4**

Compd.	R_m (cm^3)	V_m (cm^3)	P_c (cm^3)	S_t (dyne/cm)
2	123.60 ± 0.4	348.90 ± 5.0	993.40 ± 6.0	65.70 ± 5.0
4	233.08 ± 0.4	676.00 ± 5.0	882.70 ± 6.0	60.10 ± 5.0

impediment, conformational preferences and internal rotation of compound **4** could influence the degree of lipophilicity of this compound.

On the other hand, it is important to mention that there are reports which suggest that V_m is directly related to parachor (P_c) and surface tension (S_t) which are cumulative effects of the different intra- and inter-molecular forces involved in the structural chemistry of some compounds[27,28]. The results indicate that values of P_c for compound **4** were high in comparison with **2** (Table-2) nevertheless, S_t was low in compound **4** with respect to compound **2**. These data indicate that this physico-chemical parameters can also conditioned the degree of lipophilicity of compounds **2** and **4**. This premise could be supported by other studies[29] which indicate that R_m, V_m, P_c and S_t can condition by the degree of lipophilicity of some steroid derivatives and consequently affect its biological activity.

Conclusion

In this study, a facile synthesis of a glibenclamide-pregnenolone conjugate was development and several physico-chemical descriptors were evaluated. The results showed an increase in the values of log P, π, R_m, V_m and P_c in compound **4** with respect to compound **2**. These data suggest that physico-chemical parameters can be relationship with the degree of lipophilicity of compound **4**.

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