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Design and Synthesis of Carbamazepine-Alkyne Conjugate as Antidiabetic Agent: Study of Chemical Descriptors (log P and π)

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In this work, the carbamazepine-alkyne derivative [**4**] was synthesized, using the three components system (carbamazepine [**1**], benzaldehyde [**2**] and 1-hexyne [**3**]) in presence of cupric chloride such as catalyst. Additionally, the antidiabetic activity of **1** and **4** compounds was evaluated *in vivo* in a diabetic animal model. The structure of **4** was confirmed by spectroscopy and spectrometry data. The ^{1}H NMR spectrum showed, up field shifts at 1.23 ppm corresponding to methyl and 2.10 ppm for methylene involved in the alkyne-fragment. Another signals at 6.72 ppm for proton of azepine-ring and several signals (7.25-7.78 ppm) corresponding to the protons of phenyls groups was found. Another results showed that compound **4** induce antidiabetic activity in comparison with compound **1**. To delineate the structural chemical requirements of both compounds **1** and **4** on the antidiabetic activity another parameters such as, the descriptors log P and π were calculated. The results showed an increase in both log P and π values of compound **4** with respect to compound **1**. In conclusion, these results indicate that the antidiabetic activity of compound **4** depend on their chemical structure and of the lipophilic contributions of the parent molecule and its substituent.

Key Words: Carbamazepine-alkyne, Benzaldehyde, 1-Hexyne, Antidiabetic activity.

INTRODUCTION

Several decades ago has witnessed a growing interest for combinatorial chemistry on development of new therapeutics agents for treatment of diseases diverse[1-3]. In this sense, new drugs have been developed for control of diabetes[4,5]. There are several reports which show the synthesis of various alkyne-derivatives for control of diabetes, for example, a series of (5-substituted pyrrolidinyl-2-carbonyl)-2-cyanopyrrolidine (C5-Pro-Pro) analogues was discovered as dipeptidyl peptidase

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IV (DPPIV) inhibitors as a potential treatment of diabetes and obesity[6]. Additionally, the discoveries of a series of 4-phenethynyldihydrocinnamic acid agonists were synthesized for the treatment of type II diabetes[7]. Another studies showed the preparation of 2-[4-{2-(2S,5R)-2-cyano-5-ethynyl-1-pyrrolidinyl]-2-oxoethyl]-amino]-4-methyl-1-piperidinyl]-4-yridinecarboxylic acid (ABT-279) for the treatment of diabetes[8]. In addition, the 2-alkynyl-8-aryl-9-methyladenine derivative was synthesized and this compound showed hypoglycemic activity in a diabetic animal model[9]. Nevertheless, it is important to mention here that despite its wide scope, the former protocols to synthesize drugs for diabetes treatment suffer from several drawbacks *e.g.*, some reagents have a limited stability and its preparation can be dangerous. Therefore in this work, our initial design included a facile synthesis of carbamazepine-alkyne derivative that contains in the azepine-ring of carbamazepine-alkyne conjugate nucleus a spacer arm with both phenyl and alkyne functional groups. The route involve preparation of carbamazepine-alkyne derivative [**4**] using first the three components system (carbamazepine [**1**], benzaldehyde [**2**] and 1-hexyne [**3**]) in presence of cupric chloride such as catalyst. Additionally, the hypoglycemic activity of carbamazepine-alkyne conjugate was evaluated on an animal model of diabetes.

EXPERIMENTAL

Carbamazepine and the other compounds evaluated in this study were purchased from Sigma-Aldrich Co., Ltd. The melting points for the different compounds were determined on 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. ^{1}H 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.

Synthesis of N-hex-1-ynyl-N-phenyl-5*H*-dibenzo[b,f]azepine-5-carboxamide (4): A solution of carbamazepine 100 mg (0.42 mmol), 1-hexyne 48 µL (0.42 mmol), benzaldehyde 43 µL (0.42 mmol) in ethanol 10 mL was stirring by 10 min at room temperature. After cupric chloride anhydrous 113 mg (0.84 mmol) was added and the mixture was stirring by 48 h at room temperature. The reaction mixture was evaporated to a smaller volume, 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 70 % of product, m.p. 107 ºC; UV (MeOH) λ_{max} (log ε) 215 (1.04), 284 (0.34) nm; IR (V_{max}, cm^{-1}): 3482, 2361, 1736; ^{1}H NMR (300 MHz, $CDCl_3$) δ_H; 1.23 (3H, s, CH_3, t, J = 6.3 Hz), 1.65-1.81 (m, 4H), 2.1 (m, 2H, T, J = 6.7 Hz), 6.72 (s, 2H, azepine-ring), 7.25-7.78 (m, 13H). ^{13}C NMR (74.5 MHz, $CDCl_3$) δ_C; 13.63 (C-CH_3), 21.98, 22.40, 31.07, 51.07, 64.33 (C≡C-C), 84.40 (N-C≡C) , 120.70, 123.88, 123.89, 125.51, 126.46,

128.01, 129.14, 129.16, 129.36, 129.38, 134.25, 137.65, 138.08, 142.19 (C=C-N, azepine-ring), 142.90 (C-C-N, phenyl) 166.58 (O=C-N). EIMS (30 ev) m/z (rel. int.), 392.20 (12, M^+), 322 (64), 303 (30), 255 (27), 213 (58), 209 (27), 161 (28), 147 (46), 105 (66), 91 (100). Anal. $C_{27}H_{24}N_2O$: C, 82.68; H, 6.12; N, 7.10. Calcd. (%) for C, 82.62; H, 6.16; N, 7.14.

QSAR: To estimate the logarithmic octanol-water partition coefficient (log P) and π of carbamazepine and carbamazepine-alkyne conjugate, the log Kow method (atom/fragment contribution), introduced by Mannhold and Waterbeemd[10], available as the KOWWIN software was used.

Biological method: All experimental procedures and protocols used in this investigation were reviewed and apprdoved by the Animal Care and Use Committee of Universidad Autonoma de Campeche (UAC) and were in accordance with the Guide for the Care and Use of Laboratory Animals (Washington, DC: National Academy Press, 1996). Male rats (Wisstar; weighing 200-250 g) were obtained from UAC.

Experimental induction of diabetes in rats: The rats were injected with alloxan monohydrate dissolved in sterile normal saline at a dose of 150 mg/kg body wt. intraperitoneally[11]. After 2 weeks, rats with moderate diabetes having glycosuria (indicated by Benedict's qualitative test) and hyperglycaemia (*i.e.*, with a blood glucose ≥ 200 mg/dL) were used for the experiment.

Experimental design and treatment: In the experiment a total of 36 rats were used. Diabetes was induced in rats 2 weeks before starting the experiment. The rats were divided into six groups after the induction of diabetes. In the experiment six rats were used in each group (30 diabetic surviving rats, six normal rats) as followed.

Group 1: Normal rats given with 2 mL of normal saline. **Group 2:** Diabetic control rats given with 2 mL of normal saline; **Group 3:** Diabetic rats given aqueous solution of glibenclamide (600 mg/kg body wt.) daily were using an intragastric tube for 30 days. **Group 4:** Diabetic rats given aqueous solution of metformin (350 mg/kg body wt.) daily were using an intragastric tube for 30 days. **Group 5:** Diabetic rats given aqueous solution of carbamazepine-alkyne conjugate (20 mg/kg body wt.) daily were using an intragastric tube for 30 days. **Group 6:** Diabetic rats given aqueous solution of carbamazepine (20 mg/kg body wt.) daily were using an intragastric tube for 30 days.

Biochemical assays

Measured in acute form: Blood glucose was determined from tail blood with a rapid glucose analyzer (Accutrend Sensor Comfort; Roche, U.S.A) every 48 h.

Statistical analysis: All the experimental data were statistically evaluated and the significance of various treatments was calculated using Student's *t*-test. All the results were expressed as mean ± SD.

RESULTS AND DISCUSSION

It is important to mention here that many procedures use the three components system in order to synthesize several compounds. The most widely practiced method employs boric acid[12], silica sulfuric acid[13], poly(4-vinylpyridine-codivynylbenzene)-Cu(II) complex[14], H_2SO_4[15], silica triflate[16] and phosphorus pentoxide[17]. Nevertheless, despite its wide scope, the former protocols suffer from several drawbacks *e.g.*, some reagents have a limited stability and its preparation can be dangerous. Analyze these data and the reports which indicate that copper(I) reagent has been found to be an efficient catalyst for an enantioselective one-pot three-component synthesis between aldehydes, amines and alkynes[18,19]. In this work we report a straightforward route for synthesis of carbamazepine-alkyne derivative (**4**) using first the three components system (carbamazepine [**1**], benzaldheide [**2**] and 1-hexyne [**3**]) in presence of cupric chloride such as catalyst (Fig. 1).

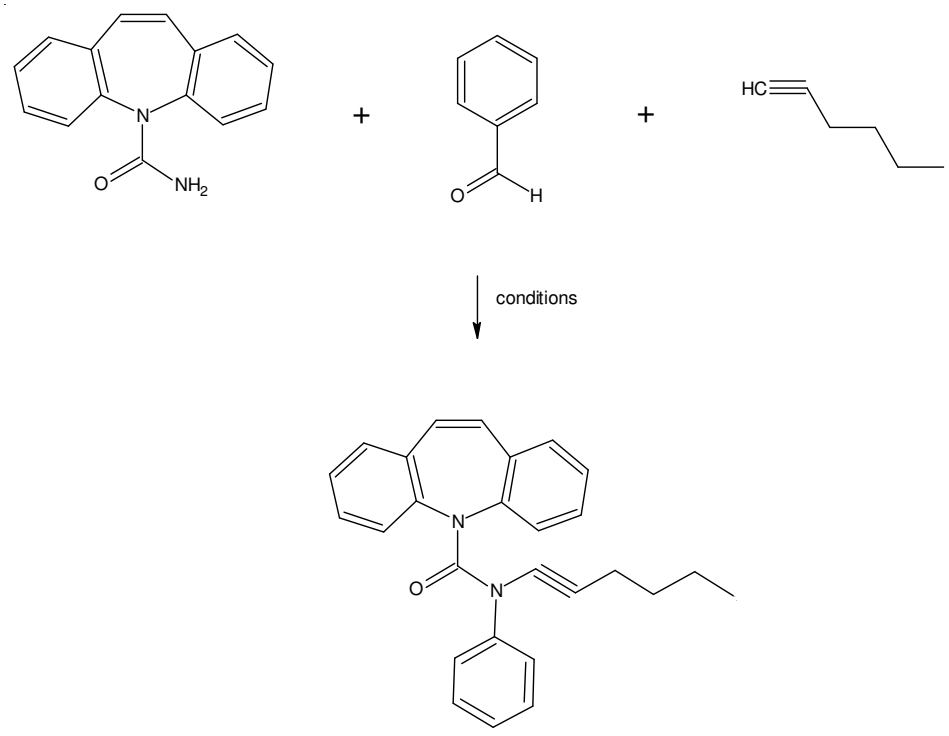

Fig. 1. Synthesis of carbamazepine-alkyne derivative [**4**] using the three components system (carbamazepine [**1**], benzaldheide [**2**] and 1-hexyne [**3**]). Conditions: cupric chloride anhydrous/ethanol

The results indicate that ^{1}H NMR spectrum of carbamazepine-alkyne conjugate showed a signal at 1.23 ppm corresponding to methyl present in the alkyne-fragment. In addition, other signals at 6.72 ppm for proton of azepine-ring and 2.10 ppm for

methylene involved in the alkyne-fragment were found. Finally, several signals (7.25-7.78 ppm) corresponding to the protons of phenyls groups was found. The ^{13}C NMR spectra displays chemical shifts at 13.63 ppm for the carbon of methyl group present in the alkyne-fragment. The chemical shift of the methylenes involved in the alkyne-fragment is found out at 21.98-31.07 ppm. Additionally, two signals characteristics at 64.33 (C≡C-C) and 84.40 ppm (N-C≡C) for carbons corresponding to alkyne-fragment. In addition, two chemical shifts at 142.19 (C=C-N, azepine-ring) and 142.90 (C-C-N, phenyl-ring) were found.

At down field there are several signals (120.70-138.08 ppm) corresponding to carbons involved in the heterocyclic rings. Finally, a signal at 166.58 ppm for the carbon involved in the fragment O=C-N was found. Additionally, the presence of the carbamazepine-alkyne derivative was further confirmed from mass spectrum which showed a molecular ion at m/z 392.20.

On the other hand, the antidiabetic activity of carbamazepine-alkyne was evaluated. In this sense, changes of plasma glucose levels were determinate (Fig. 2) after oral administration of carbamazepine-alkyne conjugate in diabetic rats, using metformin, glibenclamide as control. It is important to mention, that diabetes was induced with alloxan. There are reports that alloxan causes massive reduction in insulin release, through the destruction of β-cells of the islets of Langerhans[20].

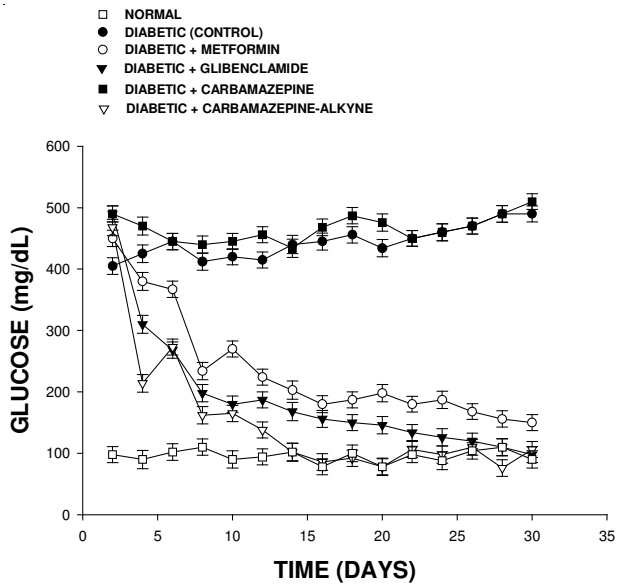

Fig. 2. Antidiabetic activity induced by carbamazepine-alkyne conjugate and control (metformin, glibenclamide and carbamazepine) in diabetic rat. The results showed that glibenclamide significantly reduced ($p = 0.05$) the blood glucose levels. Additionally, another results showed that carbamazepine-alkyne conjugate significantly decrease the blood glucose levels ($p = 0.06$) in comparison with metformin and carbamazepine. The effects are expressed as mean ± SD

The results showed that glibenclamide significantly reduced (490-98 mg/dL; p = 0.05) the blood glucose levels (450-150 mg/dL). Additionally, another results showed that carbamazepine-alkyne conjugate significantly decrease the blood glucose levels (468-106 mg/dL; p = 0.06) in comparison with metformin (450-150 mg/dL; p = 0.05). These results indicate that the antidiabetic activity of carbamazepine-alkyne, could depend of their chemical structure. To evaluate this hypothesis, the biological activity of carbamazepine fragment involved in chemical structure of carbamazepine-alkyne conjugate was evaluated. The results showed that carbamazepine (Fig. 2) did not induce antidiabetic effects (490-510 mg/dL). These experimental data suggest that changes in the chemical structure of carbamazepine to form carbamazepine-alkyne conjugate (Fig. 1) induce antidiabetic activity.

To delineate the structural chemical requirements of both carbamazepine and carbamazepine-alkyne conjugate in the antidiabetic activity. We calculate other parameters such as the descriptors log P and π[21]. 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[22]. The difference between the substituted and unsubstituted log P values is conditioned by the π value for the particular substituent. Hammett showed that p values measure the free energy change caused by particular substituent to relate to biological activity[23,24]. The log P and π parameters was calculated by the method proposed by Mannhold and Waterbeemd *et al.*[10]. The results (Fig. 3, Table-1) showed an increase in both

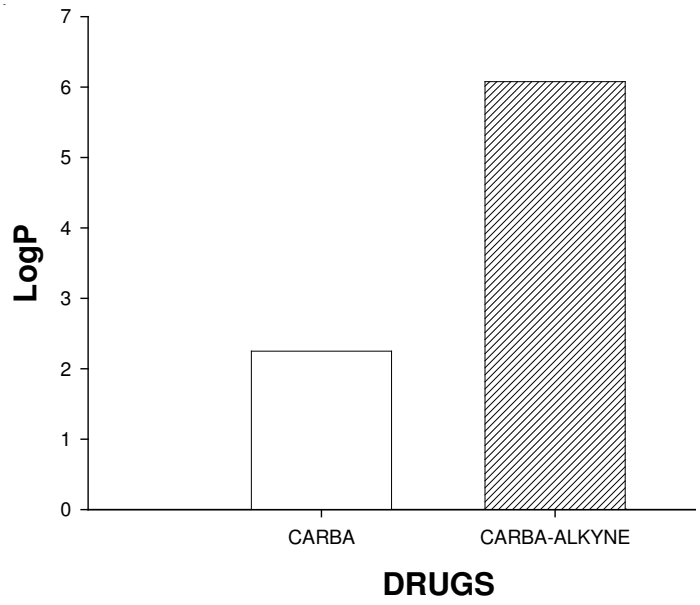

Fig. 3. log P of carbamazepine and carbamazepine-alkyne conjugate

TABLE-1
log P OF CARBAMAZEPINE AND CARBAMAZEPINE-ALKYNE CONJUGATE

Compounds	Fragment	Contribution
Carbamazepine	=CH- or =C< [olefinc carbon]	0.7672
	$-NH_2$ [aliphatic attach]	-1.4148
	Aromatic carbon	3.5280
	-NC (=O)N- [urea]	1.0453
	-N- [aliphatic N, two aromatic attach]	-0.4657
	Di-N urea/acetamide aromatic correction	-1.4406
	Equation constant	0.2290
	log Kow	2.2484
	π	1.8101
Carbamazepine-alkyne	$-CH_3$ [aliphatic carbon]	0.5473
	$-CH_2-$ [aliphatic carbon]	1.4733
	=CH- or =C< [olefinic carbon]	0.7672
	≡C [acetylenic carbon]	0.2668
	Aromatic carbon	5.2920
	-N [aliphatic N, one aromatic attach]	-0.9170
	-NC (=O)N- [urea]	1.0453
	-N- [aliphatic N, two aromatic attach]	-0.4657
	Di-N urea/acetamide aromatic correction	-21609
	Equation constant	0.2290
	log Kow	6.0773
	π	3.8289

log P and π values of carbamazepine-alkyne conjugate with respect to carbamazepine. This phenomenon is conditioned mainly, by the contribution of all substituent atoms involved in the chemical structure of carbamazepine-alkyne, as is showed in the Table-1. The results showed that aliphatic carbons ($-CH_3$ and $-CH_2$), acetylenic carbons (C≡C) and aromatic carbons involved in the chemical structure of carbamazepine-alkyne contribute to high lipophilicity in comparison with carbamazepine. This result is supported by the QSAR studies on structurally for others compounds as oxlyl aryl amino benzoic acid derivatives which indicate that substituent involved in their chemical structure induced changes in both the lipophilicity and antidiabetic activity[25].

Conclusion

The results obtained indicate that the antidiabetic activity of compound **4** depends on its chemical structure and of the lipophilic contributions of the parent molecule and its substituent.

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