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Synthesis and pharmacological evaluation of novel potential ultrashort-acting β-blockers

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The basic relationship between chemical structure and pharmacological activity of eight newly developed potential ultrashort-acting β-adrenergic blockers was evaluated. The compounds studied are derivatives of arylcarbonyloxyaminopropanols and were prepared by four-step synthesis. All the compounds evaluated showed weak antiisoprenaline (β-adrenergic receptor blocking) activity and antiarrhythmic (antiouabain) activity.

1. Introduction

β-Adrenergic receptor blocking agents are essential drugs used mainly in the treatment of cardiovascular diseases [1–3]. However, patients depending on their long-term administration may exhibit some serious side effects such as bradycardia, hypotension, aggravation of heart failure or bronchospasm. These side effects are more common after intravenous than oral administration of β-blockers and can persist for several hours [4]. Therefore, ultrashort-acting β-blockers have been developed for emergent intravenous treatment of atrial fibrillation or when control of heart rate is desirable [5]. The drugs esmolol [6], flestolol [7] and landiolol [8] are the examples used clinically. A short duration of action can be achieved by incorporation of a metabolically unstable ester group on the nitrogen [9] or the aryl function [10] which makes the molecule more susceptible to rapid hydrolysis in plasma or by erythrocyte esterases.

On the basis of our previous studies of structure-activity relationship the *para*-carbonyl substitution of the phenoxyaminopropanol ring was found to be the most pharmacologically active and selective for myocardial tissue [11, 12]. In order to prepare short acting β-blockers we have synthesized eight new derivatives of β-adrenergic receptor blocking phenoxyaminopropanoles. The structure of the pattern molecule was modified with the ester group inserted between the aromatic and amine moiety of the connecting chain. Two series of 3-alkylamino-1-(4-carbalkoxyaminobenzoylpropan-2-ols were prepared and their basic presupposed pharmacological activities were evaluated.

2. Investigations, results and discussion

2.1. Chemistry

Synthesis of the novel derivatives of arylcarbonyloxyaminopropanols is shown in the Scheme and was carried out according to the general method first reported by Kam et al. [13]. The corresponding 4-carbalkoxyaminobenzoic acids **1a–d** were prepared by the reaction of 4-aminobenzoic acid with appropriate alkylchloroformiates. These acids were reacted with thionyl chloride to obtain the acid chlorides **2a–d**. Glycidyl esters **3a–d** were prepared by the reaction of the acid chloride with glycidol. Physicochemical data for compounds **3a–d** are given in Table 1. In the last step of the synthesis, glycidyl esters were re-

Scheme

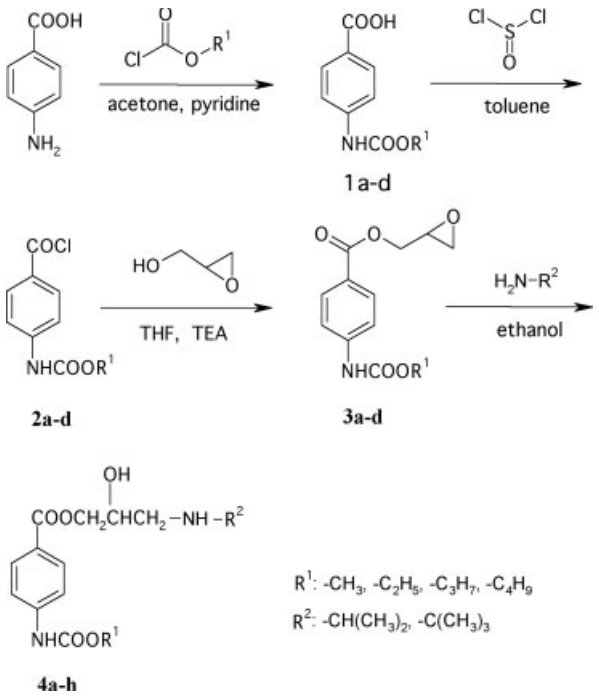
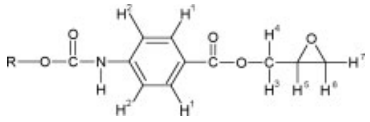


Table 1: Experimental and physico-chemical data of 2,3-epoxypropyl-4-carbalkoxy-aminobenzoates



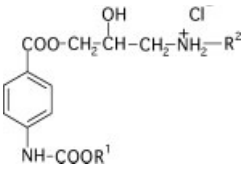
Compd.	R^1	Formula Mr	M.p. (°C) Solvent	Yield (%)	R_F*	^{1}H NMR δ (ppm), J (Hz)
3a	CH_3	$C_{12}H_{13}NO_5$ 251.24	125–127 P	80	0.57	δ = 8.01 (d, J = 7, ArH^1, 2 H), 7.46 (d, J = 7, ArH^2, 2 H), 6.94 (s, NH, 1 H), 4.65 (dd, $J_1 = 2.9$, $J_2 = 12.5$, H^3, 1 H), 4.15 (dd, $J_1 = 6.2$, $J_2 = 12.5$, H^4, 1 H), 3.80 (s, CH_3, 3 H), 3.38–3.32 (m, H^5, 1 H), 2.93–2.88 (m, H^6, 1 H), 2.76–2.72 (m, H^7, 1 H)
3b	C_2H_5	$C_{13}H_{15}NO_5$ 265.27	104–106 P	73	0.63	δ = 8.01 (d, J = 8.4, ArH^1, 2 H), 7.46 (d, J = 8.4, ArH^2, 2 H), 6.99 (s, NH, 1 H), 4.65 (dd, $J_1 = 2.9$, $J_2 = 12.5$, H^3, 1 H), 4.30–4.10 (m, $H^4 + CH_2$, 3 H), 3.36–3.34 (m, H^5, 1 H), 2.93–2.89 (m, H^6, 1 H), 2.76–2.72 (m, H^7, 1 H), 1.32 (t, J = 7.1, CH_3, 3 H)
3c	C_3H_7	$C_{14}H_{17}NO_4$ 279.29	109–112 P	66	0.67	δ = 8.02 (d, J = 8,4, ArH^1, 2 H), 7.48 (d, J = 8.4, ArH^2, 2 H), 7.00 (s, NH, 1 H), 4.65 (dd, $J_1 = 2.9$, $J_2 = 12.8$, H^3, 1 H), 4.19–4.10 (m, $\underline{H}^4 + C\underline{H}_2CH_2CH_3$, 3 H), 3.37–3.35 (m, H^5, 1 H), 2.93–2.89 (m, H^6, 1 H), 2.76–2.72 (m, H^7, 1 H), 1.76–1.65 (m, $CH_2C\underline{H}_2CH_3$, 2 H), 0.98 (t, J = 7.5, CH_3, 3 H)
3d	C_4H_9	$C_{15}H_{19}NO_4$ 293.32	98–101 P	67	0.71	δ = 8.00 (d, J = 8.8, ArH^1, 2 H), 7.48 (d, J = 8.8, ArH^2, 2 H), 6.93 (s, NH, 1 H), 4.64 (dd, $J_1 = 2.9$, $J_2 = 12.3$, H^3, 1 H), 4.22–4.10 (m, $\underline{H}^4 + C\underline{H}_2CH_2CH_2CH_3$, 3 H), 3.35–3.33 (m, H^5, 1 H), 2.93–2.88 (m, H^6, 1 H), 2.75–2.72 (m, H^7, 1 H), 1.70–1.60 (m, $CH_2C\underline{H}_2CH_2CH_3$, 2 H), 1.45–1.36 (m, $CH_2CH_2C\underline{H}_2CH_3$, 2 H), 0.95 (t, J = 7.1, $C\underline{H}_3$, 3 H)

P-propan-2-ol; *-acetone: petroleum ether (1 : 1)

acted with appropriate amines to obtain the final compounds **4a–h**.

The structures of all the final compounds were confirmed by elemental analysis as well as FTIR, ^{1}H and ^{13}C NMR spectra. The proposed structures, melting points, and reaction yields are shown in Table 2. Selected spectral data for the prepared compounds are presented in Table 3.

Table 2: Experimental and physico-chemical data of 3-alkylamino-1-(4-carbalkoxy-aminobenzoyl)propan-2-ols



Compd.	R^1	R^2	Formula Mr	M.p. (°C) Solvent	Yield (%)	R_F*
4a	CH_3	$CH(CH_3)_2$	$C_{15}H_{23}N_2O_5Cl$ 346.81	182–184 P	62	0.34
4b	C_2H_5	$CH(CH_3)_2$	$C_{16}H_{25}N_2O_5Cl$ 360.84	186–188 P	67	0.42
4c	C_3H_7	$CH(CH_3)_2$	$C_{17}H_{27}N_2O_5Cl$ 374.87	191–193 P	68	0.47
4d	C_4H_9	$CH(CH_3)_2$	$C_{18}H_{29}N_2O_5Cl$ 388.90	174–179 P	74	0.56
4e	CH_3	$C(CH_3)_3$	$C_{16}H_{25}N_2O_5Cl$ 360.84	192–195 M/E	65	0.44
4f	C_2H_5	$C(CH_3)_3$	$C_{17}H_{27}N_2O_5Cl$ 374.87	200–204 M/E	71	0.52
4g	C_3H_7	$C(CH_3)_3$	$C_{18}H_{29}N_2O_5Cl$ 388.90	196–199 M/E	73	0.55
4h	C_4H_9	$C(CH_3)_3$	$C_{19}H_{31}N_2O_5Cl$ 402.92	189–191 M/E	78	0.58

P – propan-2-ol, M – methanol, E – diethyl ether, * ethyl acetate: diethylamine (8 : 1)

2.2. Pharmacological activity

2.2.1. Antiisoprenaline activity

The potential β-adrenolytic efficiency of the compounds was evaluated as the ability of the compounds to antagonise the effect of isoprenaline induced positive chronotropic responses on isolated guinea pig atria. The specific antiisoprenaline activity of the compounds was expressed as pA_2 values shown in Table 4. All the compounds tested showed moderate antiisoprenaline activity where pA_2 values varied from 5.82 ± 0.26 to 6.66 ± 0.17. The lowest pA_2 values were found with the methyl derivatives ($pA_2 = 6.09 \pm 0.28$ and 5.82 ± 0.26) and the values increased with the length of the side chain substitution on the carbonyl function. The highest antiisoprenaline activity was observed in the butyl derivatives ($pA_2 = 6.50 \pm 0.15$ and 6.66 ± 0.17). However, in comparison with our previous studies, the insertion of the ester group into the connecting oxyaminopropanol chain decreased the antiisoprenaline activity of the original phenoxyaminopropanols more than 10 times [11, 12].

Likewise, the tested compounds were found less effective in antiisoprenaline activity experiments on tracheal tissue (β_2) ($pA_2 = 5.29 \pm 0.10$ to 6.01 ± 0.07). Thus, the cardioselectivity, expressed as β_1/β_2 antagonist potency ratio, was low for methyl- and ethylcarbonyl derivatives but increased significantly for propyl- and butylcarbonyl derivatives. The most cardioselective compound **4c** showed 16.2 times higher affinity to atrial than to tracheal tissue. The results obtained correlate well with the studies performed earlier [12].

2.2.2. Antiarrhythmic activity

The influence of the compounds on heart rate was measured firstly in *in vitro* conditions. All tested compounds at conc. 1×10^{-6} mol · l^{-1} significantly ($p < 0.05$) influenced the spontaneous rate of isolated guinea pig atria

Table 3: Spectral data of 3-alkylamino-1-(4-carbalkoxyaminobenzoyl)propan-2-ols

Compd.	IR (cm^{-1})	^{1}H NMR-δ (ppm), J (Hz)				
		−NH−	$-N^+H_2-$	ArH	OH	alkyl
4a	3309 (O−H) 2959 (N−H) 1748 (C=O) 1701 (C_{NH}=O) 1231 (C_{AR}−O)	s 10.10	bs 9.12 bs 8.64	d 7.94 (J = 8.8) d 7.63 (J = 8.8)	s 5.94	s 3.79 CH_3 m 1.28–1.22 $CH(C\underline{H}_3)_2$
4b	3328 (O−H) 2983 (N−H) 1723 (C=O) 1697 (C_{NH}=O) 1228 (C_{AR}−O)	s 10.11	bs 9.12 bs 8.65	d 7.95 (J = 8.8) d 7.61 (J = 8.8)	s 5.95	m 1.28–1.17 $CH(C\underline{H}_3)_2 + CH_2C\underline{H}_3$
4c	3317 (O−H) 2967 (N−H) 1729 (C=O) 1697 (C_{NH}=O) 1230 (C_{AR}−O)	s 10.10	bs 9.11 bs 8.64	d 7.94 (J = 8.8) d 7.62 (J = 8.8)	s 4.94	t 4.06 (J = 6.8) $C\underline{H}_2CH_2CH_3$ m 1.70–1.56 $CH_2C\underline{H}_2CH_3$ m 1.29–1.24 $CH(C\underline{H}_3)_2$ t 0.93 (J = 7.1) $CH_2CH_2C\underline{H}_3$
4d	3317 (O−H) 2967 (N−H) 1732 (C=O) 1701 (C_{NH}=O) 1227 (C_{AR}−O)	s 10.10	bs 9.10 bs 8.64	d 7.94 (J = 8.8) d 7.62 (J = 8.8)	s 4.94	t 4.10 (J = 6.4) $C\underline{H}_2(CH_2)_2CH_3$ m 1.64–1.57 $CH_2C\underline{H}_2CH_2CH_3$ m 1.43–1.32 $CH_2CH_2C\underline{H}_2CH_3$ m 1.28–1.23 $CH(C\underline{H}_3)_2$ t 0.94 (J = 7.3) $CH_2(CH_2)_2C\underline{H}_3$
4e	3321 (O−H) 2977 (N−H) 1735 (C=O) 1692 (C_{NH}=O) 1235 (C_{AR}−O)	s 10.16	bs 9.12 bs 8.47	d 7.96 (J = 8.8) d 7.62 (J = 8.8)	s 4.94	s 3.67 CH_3, s 1.31 $C(CH_3)_3$
4f	3325 (O−H) 2981 (N−H) 1739 (C=O) 1712 (C_{NH}=O) 1231 (C_{AR}−O)	s 10.10	bs 9.17 bs 8.48	d 7.94 (J = 6.7) d 7.61 (J = 6.7)	s 4.92	m 1.31–1.21 $C(CH_3)_3 + CH_2C\underline{H}_3$
4g	3320 (O−H) 2973 (N−H) 1720 (C=O) 1698 (C_{NH}=O) 1229 (C_{AR}−O)	s 10.12	bs 9.19 bs 8.48	d 7.94 (J = 8.8) d 7.62 (J = 8.4)	s 4.94	t 4.06 (J = 6.8) $C\underline{H}_2CH_2CH_3$ m 1.69–1.55 $CH_2C\underline{H}_2CH_3$ s 1.30 $C(CH_3)_3$ t 0.93 (J = 7.3) $CH_2CH_2C\underline{H}_3$
4h	3321 (O−H) 2962 (N−H) 1719 (C=O) 1699 (C_{NH}=O) 1227 (C_{AR}−O)	s 10.10	bs 9.12 bs 8.47	d 7.94 (J = 8.8) d 7.62 (J8.8)	s 4.94	t 4.10 (J6.4) $C\underline{H}_2(CH_2)_2CH_3$ m 1.64–1.31 $CH_2C\underline{H}_2CH_2CH_3 + C(CH_3)_3$ t 0.91 (J = 7.3) $CH_2(CH_2)_2C\underline{H}_3$

(Table 4). The heart rate, measured over 20 min after addition the compound in solution to the system (at 1, 5, 10, 15 and 20 min after addition), decreased in the range of 1.5% (**4e**) to 14.6% (**4h**). The maximal negative chronotropic effect was observed, and is given in the Table, at the longest recorded interval, i.e. at 20 min after drug administration. These findings did not correspond with our primary assumption of a very short biological half-life.

Table 4: Negative chronotropic activity in isolated guinea pig spontaneously beating atria of evaluated compounds at 10^{-6} mol · l^{-1}, their pA_2 values determined in atria and tracheal muscle of guinea pigs and β_1/β_2 selectivity ratios calculated as antilog ($pA_2\beta_1 - pA_2\beta_2$)

Compd.	Heart rate (%)	pA_2 values		β_1/β_2 ratio
		Atria (β_1)	Trachea (β_2)	
4a	89.8 ± 2.2*	6.09 ± 0.28	4.97 ± 0.13	1.3
4b	91.2 ± 1.9*	6.34 ± 0.17	6.01 ± 0.07	2.8
4c	91.3 ± 2.3*	6.41 ± 0.24	4.44 ± 0.14	9.3
4d	87.2 ± 2.9*	6.40 ± 0.14	4.29 ± 0.10	16.2
4e	98.4 ± 6.8	5.82 ± 0.26	4.74 ± 0.04	1.2
4f	90.4 ± 2.4*	6.16 ± 0.11	4.64 ± 0.07	3.2
4g	88.4 ± 1.1*	6.34 ± 0.41	4.80 ± 0.06	3.4
4h	84.4 ± 2.0*	6.66 ± 0.17	4.76 ± 0.07	7.9

Each value represents the mean ± SEM from 4–7 experiments.
* $p < 0{,}05$ against saline-treated group

Results of the studies on antiarrhythmic activity, comparing the increase in ouabain consumption needed to induce heart rhythm disturbances in guinea pigs, are shown in the Fig. Pretreatment of the animals with the compounds at conc. 1×10^{-6} mol · kg^{-1} i.v., delayed ouabaine-induced onset of extrasystoles, fibrillation and cardiac arrest. The ability of *N-tert*. butyl derivatives to deplay the onset of heart disturbances was significantly higher in all the evaluated parameters than that of isopropyl derivatives. Non of the isopropyl derivatives, except compound **4d** (butylcarbonyl derivative), differed significantly from the control. We are conscious that our preliminary assumed experimental approaches about rapid metabolization and elimination half-life have not been taken into consideration. However we suggest that the screening form at of our primary evaluation could give sufficient information about potential protective effects on heart

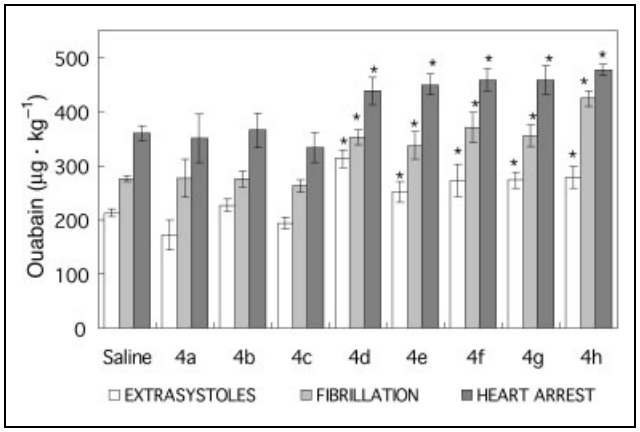


Fig.: Protection provided by evaluated compounds at conc. 1×10^{-6} mol · kg^{-1} against ouabain-induced arrhythmias in anesthetized guinea pigs. Each bar represents the mean ± SEM from 6–7 experiments.
* Statistical significance ($p < 0.05$) was determined against saline-treated group.

functions. Such approaches will indeed be needed for further experimentation.

One may speculate that the increased amino group substitution may have altered some of the membrane physicochemical properties, which would be expected to affect changes in membrane ionic permeability [14]. In addition, changes in the alkylcarbonyl group of the aromatic ring may change some of the β-adrenoreceptor binding properties of the compounds, and hence result in improved antiarrhythmic responses [15]. In conclusion, the present findings indicate that incorporation of the ester group in the connecting chain of phenoxyaminopropanols decreases the β-adrenergic receptor blocking activity of the original compounds. It is possible that other structural modifications of the pattern molecule, particularly in the amino group or lipophilic phenyl ring could positively influence both its pharmacological and biochemical properties. However, other experiments, particularly physico-chemical and pharmacokinetic, remain to be performed to evaluate the possible therapeutic effectiveness of these novel chemical structures.

3. Experimental

3.1. Chemistry

The IR spectra were recorded on a Nicolet Impact 410 FTIR spectrophotometer using the KBr disc technique in the range of 400–4000 cm^{-1}. The NMR spectra were measured with a Varian Gemini-200 spectrometer (^{1}H NMR spectra by 200 MHz and ^{13}C NMR spectra by 50 MHz), and elemental analysis was performed using a CARLO ERBA 1110 Elemental Analyzer. All the results were in an acceptable range. Melting points were determined on the Koffler apparatus and are uncorrected. TLC was carried out using silica gel plates (Silufol® UV 254) and the spots were visualized under UV at 254 nm.

3.1.1. Synthesis of 4-carbalkoxyaminobenzoic acids **1a–d**

To a solution of p-aminobenzoic acid (0.1 mol) and pyridine (0.1 mol) in acetone (100 ml) alkylchloroformiate (0.1 mol) was added dropwise. The mixture was refluxed for 1.5 h, evaporated to dryness and washed with water (0.5 l). The crude acid was recrystallized from ethanol.

3.1.2. Synthesis of 4-carbalkoxyaminobenzoyl chlorides **2a–d**

To a suspension of **1a–d** (0.1 mol) in toluene (120 ml) thionylchloride (0.2 mol) was added. The resulting mixture was refluxed for 2 h and evaporated to dryness. The crude chloride was recrystallized from toluene.

3.1.3. Synthesis of 2,3-epoxypropyl-4-carbalkoxyaminobenzoates **3a–d**

To a three-neck round-bottom flask glycidol (0.25 mol) and THF (50 ml) were added. The solution was cooled to −5 °C and triethylamine (0.25 mol) was added. To the resulting solution was added dropwise a solution of **2a–d** (0.2 mol) in THF (250 ml) at −5 to 5 °C. The mixture was stirred at room temperature for 3 h, filtered and evaporated to dryness. The product was recrystallized from propan-2-ol.

3.1.4. Synthesis of 3-alkylamino-1-(4-carbalkoxyaminobenzoyl)propan-2-ols **4a–h**

A mixture of **3a–d** (0.2 mol) and the appropriate alkylamine (0.25 mol) in ethanol (150 ml) was heated at 65 °C for 4 h. The solvent was evaporated under reduced pressure and the residing oil was dissolved in diethyl ether. The solution of the base was converted to its chloride salt by addition of ethereal HCl. The amine salt was collected by filtration and recrystallized in propan-2-ol to give the white crystals.

3.2. Pharmacology

3.2.1. Antiisoprenaline activity on isolated guinea pig right atria

The isolated guinea pig right heart atria was connected to an isometric transducer in Tyrode solution at 30 °C under a static tension of 1 g and gassed with pneumoxide (5% CO_2). After 30 min stabilisation isoprenaline chloride was added cumulatively (10^{-11} to 10^{-5} mol · l^{-1}) and concentration-response curves were plotted before and 20 min after addition of the compounds at conc. 1×10^{-6} mol · l^{-1}. Changes in the atrial rate beats were recorded 1, 5, 10, 15 and 20 min after drug application. The affinity for isoprenaline was expressed as the EC_{50} value (agonist concentration producing 50% of maximal response). The antagonist potency of the compounds was calculated from the shift in concentration-response curves (CRC) of isoprenaline and expressed as the dissociation constant (pA_2 value) according to the modified method of Van Rossum [16].

3.2.2. Antiisoprenaline activity on guinea pig smooth tracheal muscle

Tracheal strip from guinea pigs was isolated and placed in a thermostated bath containing Krebs solution at 37 °C under resting tension of 2 g and aerated with pneumoxide. After 1 h of stabilisation the muscle was precontracted with histamine (6.7×10^{-7} mol · l^{-1}) and then isoprenaline was applied cumulatively to cause relaxation and concentration-response curves were obtained. The compound being tested was then introduced to the system and allowed to equilibrate for 30 min before the isoprenaline curve was reestablished. The EC_{50} and pA_2 values were then calculated from the shift in CRC of isoprenaline according the method described above.

3.2.3. Ouabain-induced arrhythmias in guinea pigs

Guinea pigs (280–340 g), anesthetized with urethane (1.5 g · kg^{-1}, i.p.), were used as experimental animals. Cardiac rhythm disturbances were induced with ouabain injected continuously into the jugular vein as an infusion (36 µg × 0.33 ml^{-1} · min^{-1}) and were recorded by ECG. The antiarrhythmic activity of the compounds (1×10^{-6} mol · kg^{-1}), given preventively 5 min before ouabain infusion, was determined by comparing the dose of ouabain necessary to induce arrhythmias, fibrillation and heart arrest in animals pretreated with the studied compounds and with the arrhythmogenic dose of ouabain in saline-pretreated animals.

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