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Preliminary communication

5-(4′-Substituted-2′-nitroanilino)-1,2,3-triazoles as new potential potassium channel activators. I

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Abstract – By the hypothesised correlation with the large conductance Ca^{++}-activated potassium channel (BK_{Ca}) openers NS 004 and NS 1619, bearing a benzimidazolone ring, a series of new 5-(4′-substituted-2′-nitroanilino)-1,2,3-triazoles were synthesised and tested on in vitro isolated vascular preparation. The compounds were prepared starting from the appropriately substituted 2-nitro-phenylazides by 1,3-dipolar cycloaddition reaction to cyanoacetamide and following Dimroth isomerisation of the corresponding 1-arylsubstituted-5-amino-1,2,3-triazoles. The analogous 5-(4′-substituted-2′-amino-anilino)-1,2,3-triazoles were also prepared to assess the role of the nitro group in the pharmacophoric model. Almost all the nitro compounds showed a vasorelaxant activity on endothelium-denuded rat aortic rings with a potency comparable to that recorded for the reference compound NS 1619. Such a vasorelaxing activity was significantly reduced by the increase of the level of membrane depolarisation and by the potassium channel blocker 4-aminopyridine with a pharmacodynamic behaviour consistent with a potassium channel activation. © 2000 Éditions scientifiques et médicales Elsevier SAS

1,2,3-triazoles / potassium channel activators

1. Introduction

In previous papers we reported synthesis and structure evidence of new 1-(1,2,3-triazol-4-yl)-benzotriazoles [1] and of analogous 1-(1,2,3-triazol-4-yl)-benzimidazolones [2]. These structures attracted our interest for medicinal chemistry because they may be correlated to NS 004 and NS 1619 [3], which are two of the few actually available openers of the large conductance Ca^{++}-activated potassium channels (BK_{Ca}). Such a pharmacological activity represents an interesting approach in the treatment of several cardiovascular disorders without the typical side-effects of classical vasodilators [4].

The preparation of both series of compounds, triazolyl-benzotriazoles or triazolyl-benzimidazolones, followed a synthetic route based upon common 5-anilino-triazole intermediates, consisting of a structure which could also be correlated to the above mentioned openers of the BK_{Ca} channels (*figure 1*). Therefore some intermediates were preliminarily tested on in vitro isolated endothelium denuded rat aortic preparations, showing strong vasorelaxing properties [5].

2. Chemistry

This paper describes the synthesis and pharmacological evaluation of a series of 5-(4′-substituted-2′-nitroanilino)-1,2,3-triazoles (**2a**–**g**) and of the corresponding aminoderivatives **3a–g** (*figure 2*).

All the azides, excluding the 4-*sec*-butyl-2-nitrophenylazide **1g**, have been described in the literature and were prepared from the appropriate primary aromatic amines via diazonium salt and treatment with sodium azide: 2-nitrophenylazide **1a** [6], 4-methyl-2-nitrophenylazide **1b** [7], 4-methoxy-2-nitrophenylazide **1c** [8], 4-chloro-2-nitrophenylazide **1d** [9], 4-fluoro-2-nitrophenylazide **1e** [10] and 4-trifluoromethyl-2-nitrophenylazide **1f** [10].

The 1,3-dipolar cycloaddition reaction of the appropriate azide **1a**–**g** to the activated methylene compound

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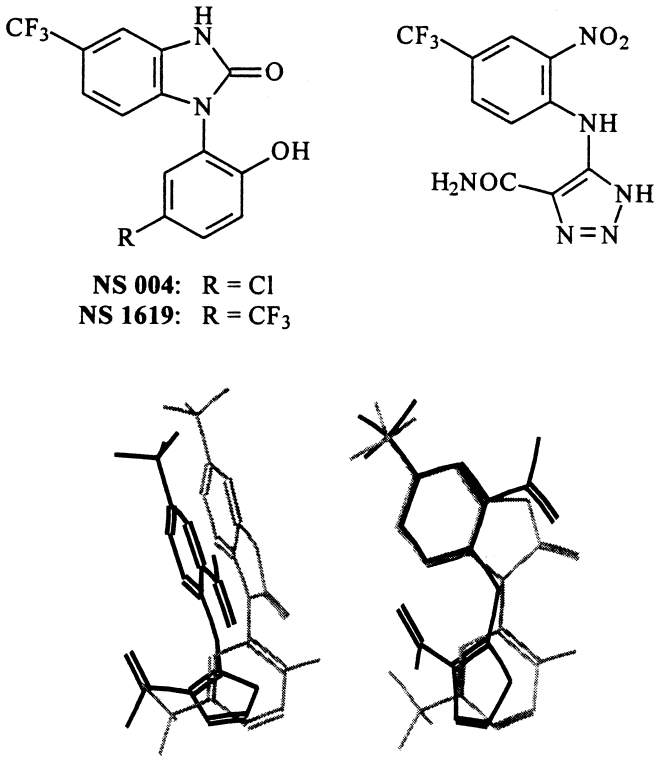


NS 004: R = Cl
NS 1619: R = CF_3

Figure 1. Chemical structures and two orthogonal hypothetical tridimensional overlaps of NS1619 (grey lines) and 5-(4-trifluoromethyl-2-nitroanilino)4-carboxamido-1,2,3-triazole (black lines).

cyanoacetamide was carried out in the usual manner in anhydrous ethanol in the presence of sodium ethoxide at room temperature for 1 night (*figure 2*).

Generally the insoluble 1-(4′-substituted-2′-nitrophenyl)-4-carboxamido-5-amino-1H-1,2,3-triazole intermediate was not isolated, but it was directly converted to the desired Dimroth isomer by addition of a small amount of 10% sodium hydroxide solution and heating of the reaction mixture.

The Dimroth isomers **2a**–**g**, which turned acidic because of the presence of the triazole proton, went in the aqueous alkaline solution and were isolated by acidification after previous washing with an organic solvent (*table I*). The amino derivatives **3a**–**g** (*figure 2*) were obtained by catalytic hydrogenation of the corresponding nitro derivatives **2a**–**g** at room temperature and pressure. As previously described [1], the carboxylic acid **4** and the corresponding decarboxylated compound **5** (*figure 3*) were obtained by alkaline hydrolysis of **1a** and heating of the acid **4** in DMF, respectively.

The structures of all the new compounds were assigned on the basis of the well-known reaction mechanisms, as shown in previous works [1] and [2], and were confirmed by analytical and spectroscopic data.

3. Pharmacology

There is actually strong evidence of a hyperpolarisation of the vascular smooth muscle cells, mediated by drugs activating the BK_{Ca} potassium channels, whose presence has been widely demonstrated (by functional and/or electrophysiological investigations) in many circulatory areas, such as the rabbit pulmonary artery [11], the rat portal vein [12] and the rat aorta [13]. Thus, the functional evaluation of a vasorelaxing activity of the tested compounds was chosen as a preliminary screening method to unmask a possible potassium channel opening effect.

4. Results and discussion

Almost all the compounds bearing the nitro group on the benzene ring, and the carboxamido substituent on the 1,2,3-triazole heterocyclic moiety, showed vasorelaxing properties (*table II*). These effects consisted of a full abolition of the contractile tone induced by the depolarising stimulus (administration of KCl 20 mM), with potency orders of magnitude weakly lower or also comparable to that recorded for the reference compound NS1619. Only the fluorine-substituted compound **2e**, belonging to this series, showed an unusual profile of activity, with a very low efficacy (< 50%) that made the calculation of the potency value impossible. This last experimental observation could be probably explained by the strong electronic influence of the fluorine atom, determining a dramatic negative impact on the effectiveness of the molecule; however, more detailed hypotheses are, actually, premature, because of the few available data. Other preliminary observations of the structure–activity relationships allowed us to observe that the absence of any substituent in position 4 of the benzene group (**2a**) led to a decrease of potency (but not of efficacy), whilst the presence of the *sec*-buthylic substituent could be correlated with the highest value of potency, recorded for the compound **2g**. This preliminary experimental data could also suggest a direct correlation between the potency and the steric hindrance or the lipophilicity in this position; whilst the electronegativity of the substituent would appear to exert a negative influence on the potency.

The replacement of the carboxamido substituent, bound to the triazole ring in the active compound **2a**, by the

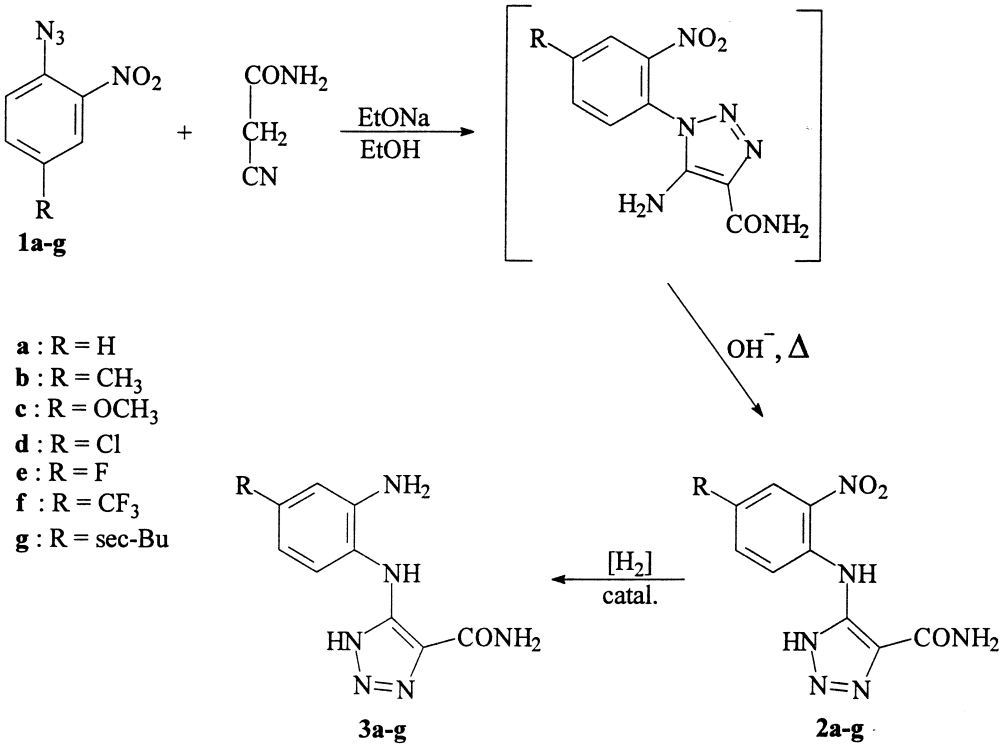


Figure 2. Preparation of compounds **2** and **3**.

carboxylic group (**4**) or by the hydrogen atom (**5**), determined an almost complete fall of the vasorelaxant activity.

To assess the importance of the presence of the oxygen atom(s) of the nitro group, clearly mimicking the position (and the function of an H-bond acceptor?) of the carbonyl group of the benzimidazolone reference compounds, the nitro group was replaced by the corresponding amino group. All the tested compounds possessing this structural characteristic (**3b**, **c**, **f** and **g**) were devoid of any significant vasorelaxing activity.

Finally, a further investigation of the possible potassium channel opening mechanism of action was also performed in the isolated vessels. It is widely reported that drugs, acting through the potassium channel activation, possess the highest degree of 'depolarisation-sensitive' inhibition of the functional responses among the several classes of vasodilators [14]. Therefore, the selected compound **2f** ($IC_{50} = 4.79 \pm 0.089$, $E_{max} = 100$) was also tested on isolated aortae, whose contractile tone was induced by a higher level of membrane depolarisation (due to the administration of KCl 80 mM). In such an experimental condition, significant decreases of potency ($IC_{50} = 4.22 \pm 0.018$) and of efficacy ($E_{max} = 84 \pm 4\%$) could be observed, fitting the profile of response expected for a potassium channel opener. Surprisingly, the reference benzimidazolone compound NS1619 did not show any significant decrease of activity when administered to preparations pre-contracted by KCl 80 mM. This anomalous behaviour could be due to different ancillary mechanisms of actions, whose existence has been already suggested by the literature [13]. Furthermore, the vasorelaxing activity of **2f** was also significantly inhibited ($IC_{50} = 4.25 \pm 0.025$, $E_{max} = 71 \pm 3$) by 4-aminopyridine (3 mM), a widely used non-selective blocker of several potassium channel subtypes.

It is concluded that the 5-(4′-substituted-2′-nitroanilino)-4-carboxamido-1,2,3-triazole derivatives can represent a new chemical class of vasodilators. The first preliminary experimental observation can suggest a potassium channel opening mechanistic hypothesis. An accurate pharmacological characterisation of the target of these drugs is actually in progress by means of functional and electrophysiological tools.

Table I. Chemical and physical properties of derivatives **2** and **3**[1].

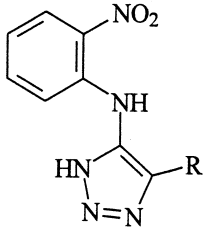
Compound	Yield (%)	Crystall. solvent	M.p. (°C)	Mass. M^+	m/z. base peak	Elemental analysis	Calcd./found		
							C	H	N
2b	56	EtOH	278–282 dec	262	216	$C_{10}H_{10}N_6O_3$	45.80 45.68	3.84 3.51	32.05 32.15
2c	31	AcOH	271–274 dec	278	232	$C_{10}H_{10}N_6O_4$	43.17 43.27	3.62 3.90	30.21 30.51
2d	25	MeOH	303–307 dec	282	236	$C_9H_7N_6O_3Cl$	38.25 38.43	2.50 2.46	29.73 29.79
2e	30	MeOH	285–298 dec	266	44	$C_9H_7N_6O_3F$	40.61 40.57	2.65 2.71	31.57 31.51
2f	28	MeOH	253–257	316	270	$C_{10}H_7N_6O_3F_3$	37.99 38.04	2.23 2.29	26.58 26.71
2g	46	MeOH	174–176	304	44	$C_{13}H_{16}N_6O_3$	51.31 51.12	5.30 5.06	27.62 27.29
3b	83	EtOH	189–191	232	44	$C_{10}H_{12}N_6O$	51.72 51.85	5.21 5.18	36.19 36.10
3c	86	EtOH	191–194	248	133	$C_{10}H_{12}N_6O_2$	48.38 48.36	4.87 4.56	33.85 34.17
3d	91	H_2O	178–182	252	44	$C_9H_9N_6OCl$	42.78 42.89	3.59 3.31	33.26 32.99
3e	84	H_2O	202–205	236	44	$C_9H_9N_6OF$	45.76 45.73	3.84 3.51	35.58 35.69
3f	96	H_2O	168–171	286	286	$C_{10}H_9N_6OF_3$	41.96 42.29	3.17 2.97	29.36 29.03
3g	91	MeOH/ H_2O	165–168	274	44	$C_{13}H_{18}N_6O$	56.92 56.64	6.61 6.84	30.64 30.31

[1] Compounds **2a** and **3a** are reported in the literature [1].

5. Experimental protocols

5.1. Chemistry

Melting points were determined on a Kofler hot-stage and are uncorrected. IR spectra in nujol mulls were recorded on a Mattson Genesis series FTIR spectrometer. ^{1}H-NMR spectra were recorded with a Varian CFT-20 spectrometer in DMSO-d_6 in δ units, using TMS as the internal standard. Mass spectra were performed with a Hewlett Packard MS/System 5988. Elemental analyses (C, H, N) were within ± 0.4% of the theoretical values and were performed on a Carlo Erba Elemental Analyzer Mod. 1106 apparatus. TLC data were obtained with Riedel de Haen, 37360 DC-Karten F_{254}, 0.2 mm, eluting with a 1:3 AcOEt/petroleum ether mixture. Petroleum ether corresponds to the fraction boiling at 40–60 °C.



4 : R = COOH
5 : R = H

Figure 3. Previously prepared compounds **4** and **5**.

Table II. Potency (pIC_{50}) and efficacy (E_{max} %) values of the vasorelaxing compounds **2a**–**g**[1].

Compound	pIC_{50}	E_{max} %
2a	3.98 ± 0.066	100
2b	4.73 ± 0.11	100
2c	4.55 ± 0.11	100
2d	4.61 ± 0.048	100
2e	not calculated	41 ± 1
2f	4.79 ± 0.089	100
2g	5.14 ± 0.049	100
NS 1619	5.30 ± 0.29	100

[1] The tested compounds **3b**, **c**, **f**, **g**, **4** and **5** did not show significant vasorelaxant effects.

Short distillations were performed with a Buchi GKR 50 tubular oven.

Compounds **2a** and **3a** are reported in the literature [1].

5.1.1. 4-sec-*Butyl-2-nitro-acetanilide*

To an ice-cooled (0–5 °C) and stirred solution of 4-*sec*-butyl-acetanilide [15] (4.50 g, 23.5 mmol) in 100 mL of AcOH, 30 mL of fuming HNO_3 (d 1.482) were added dropwise. The ice-bath was removed, the mixture was heated at 50 °C for 6 h and, after cooling, it was poured into crushed ice. The suspension was alkalinised (pH ≅ 10) with solid Na_2CO_3 and extracted with $CHCl_3$. After evaporation of the solvent, the residue was stirred with AcOEt/petroleum ether 1:9 mixture and the insoluble material, consisting of the starting solid, was filtered off. This separation could be achieved by flash-chromatography through a silica gel column, eluting with AcOEt/petroleum ether 1:3 mixture. Evaporation of the solvent gave the title compound as an oil (4.03 g, yield 72%) which could be purified by short distillation at 160–170 °C/2 mm Hg; m.p. 14–16 °C (Lit. [16] 15–17 °C). Anal.: $C_{12}H_{16}N_2O_3$. Found: C 61.05, H 6.62, N 11.52; calculated: C 61.02, H 6.78, N 11.86. IR (cm^{-1}): 1 514 and 1 360 (NO_2). MS (m/z,): 236 [M^+], 43 [100].

5.1.2. 4-sec-*Butyl-2-nitroaniline*

A solution of 4-*sec*-butyl-2-nitro-acetanilide (4.0 g, 17 mmol) in 40 mL of 50% H_2SO_4 was heated under reflux for 1 night. After cooling the mixture was alkalinised (pH ≅ 10) with 10% NaOH and extracted with $CHCl_3$. The solution, dried ($MgSO_4$) and evaporated in vacuo providing the title compound as an oil (3.0 g, yield 92%) which was purified by short distillation at 165–170 °C/2.5 mm Hg). Anal.: C_{10} $H_{14}N_2O_2$. Found: C 62.08, H 7.03, N 14.08; calculated: C 61.86, H 7.22, N 14.43. IR (cm^{-1}): 3 490 and 3 371 (NH_2). MS (m/z): 194 [M^+], 165 [100].

5.1.3. 4-sec-*Butyl-2-nitrophenylazide* **1g**

To a stirred and cooled (0–5 °C) solution of 4-*sec*-butyl-2-nitroaniline (3.0 g, 15.5 mmol) in 100 mL of 18% HCl, 10 mL of aqueous solution of $NaNO_2$ (1.30 g, 18.8 mmol) were added drop by drop. After 15–20 min, a solution of NaN_3 (1.20 g, 18.8 mmol) in 10 mL of H_2O was added dropwise under vigorous stirring. After approximately 1 h the solution was extracted with Et_2O and the organic layer was dried ($MgSO_4$) and evaporated to give the title compound as an oil which was used without purification: 2.60 g, yield 76%; IR (cm^{-1}): 2 130 (N_3).

5.1.4. 4-Carboxamido-5-(4-substituted-2-nitroanilino)-1,2,3-triazoles **2b–g**

To a stirred solution of sodium ethoxide (0.41 g, 18 mmol of Na) in 25 mL of absolute ethanol, 1.50 g (18 mmol) of cyanoacetamide were added. After 15–20 min the suspension was cooled in an ice-bath (–10 °C) and 15 mmoles of the appropriate 4-substituted-2-nitrophenylazide (**1b**, **1c**, **1d**, **1e**, **1f** or **1g**) in 25 mL of absolute ethanol were added drop by drop keeping the temperature < 0–5 °C. After 1 h the ice-bath was removed and stirring was continued at room temperature for 1 night. The solvent was evaporated under reduced pressure, H_2O and 3–5 mL of 10% NaOH were added and the mixture was heated under reflux for 15–20 min. After cooling, the reaction mixture was paper filtered from the formed nitroaniline and by acidification (pH 1–2) of the filtrate the title compounds precipitated and were collected by filtration (*table I*).

5.1.5. 4-Carboxamido-5-(4-substituted-2-aminoanilino)-1,2,3-triazoles **3b–g**

To a solution or suspension of the appropriate nitro-derivative **2b–g** (5.0 mmol) in ≈ 200 mL of MeOH, 10% Pd/C (0.100 g) was added and the mixture was hydrogenated at room temperature and pressure. The catalyst was filtered off, washed with hot MeOH and the filtrate was evaporated in vacuo to give the title compounds (*table I*).

5.2. Pharmacology

All the procedures, performed on experimental animals, were carried out following the guidelines of the European Community Council Directive 86-609.

To determine a possible vasodilator mechanism of action, the compounds were tested on isolated thoracic aortae of male normotensive Wistar rats (250–350 g). The rats were killed by cervical dislocation under light ether anaesthesia and bled. The aortae were immediately excised, freed of extraneous tissues and the endothelium was removed by gently rubbing the intimal surface of the vessels. Aortic rings were suspended, under a preload of 2 g, in 10 mL organ baths containing Tyrode solution (composition of saline in mM: NaCl 136.8; KCl 2.95; $CaCl_2$ 1.80; $MgSO_4 \cdot 7H_2O$ 1.05; NaH_2PO_4 0.41; $NaHCO_3$ 11.9; glucose 5.5), thermostated at 37 °C and continuously bubbled with a mixture of O_2 (95%) and CO_2 (5%). Changes in tension were recorded by means of an isometric transducer (Basile mod. 7005), connected with a unirecord microdynamometer (Basile mod. 7050).

After an equilibration period of 60 min the endothelial integrity was confirmed by acetylcholine (ACh) (55 μM)-induced relaxation of norepinephrine (NE, 1 μM)-precontracted tissues. A relaxation < 20% of the NE-

induced contraction was considered representative of an acceptable lack of the endothelial layer, while the organs showing a relaxation ≥ 20% (i.e. significant presence of the endothelium), were not used in the experimental procedures. 30–40 min after the confirmation of the endothelium removal, the aortic preparations were contracted by treatment with a single concentration of KCl (20 mM) and when the contraction reached a stable plateau, 3-fold increasing concentrations of the tested compounds and of the reference compound NS 1619 (10 nM to 1 mM) were added cumulatively. In parallel sets of experiments, to investigate the influence of a higher level of depolarisation on the responses evoked by the tested compound, the aortic preparations were contracted by KCl 80 mM. Then, 3-fold increasing concentrations of the compounds (10 nM to 1 mM) were added cumulatively. Furthermore, the vasorelaxing activity was also evaluated on KCl 20 mM-contracted vessels, in the presence of the potassium channel blocker 4-aminopyridine (3 mM).

Preliminary experiments showed that both the KCl (20 and 80 mM)-induced contractions remained constant in a stable tonic state for at least 40 min. Norepinephrine hydrochloride (Sigma), acetylcholine chloride (Sigma), 4-aminopyridine and KCl were dissolved in bi-distilled water. All the synthesised 1,2,3-triazole derivatives and the reference compound NS 1619 (RBI) were dissolved (10 mM) in NaOH 0.1 N. All the further dilutions were performed in bi-distilled water. All the solutions were prepared immediately before the pharmacological experimental procedures. Previous experiments showed a complete ineffectiveness of the administration of the vehicle.

5.3. Data analysis

The efficacy of the vasorelaxing responses was expressed as maximal relaxant effect (E_{max}), calculated as a % of the contractile tone developed by the smooth muscle preparation, after the depolarising stimulus induced by KCl 20 or 80 mM. Previous experiments demonstrated an almost complete quantitative equivalence between the contractile responses evoked by the two different concentrations of KCl, since the concentration 20 mM could substantially induce a maximal effect in endothelium denuded aortic rings.

The parameter of potency of the vasorelaxing effects was expressed as pIC_{50}, representing the negative logarithm of the vasodilator molar concentration determining a half reduction of the contractile tone induced by the contractile agent. The above parameter was calculated by means of a non-linear regression analysis of the sigmoidal concentration–response curves (computer program: GraphPad Prism).

All the results are expressed as mean SEM of 4–6 experiments. The statistical comparison of experimental data was performed by two-tailed Student *t*-test and Anova. A value of $P < 0.05$ was considered as representative of significant differences.

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