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RESEARCH ARTICLE

One-pot synthesis and molecular docking study of pyrazoline derivatives as an anticancer agent

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Abstract

Background: Pyrazoline is a series of heterocyclics with an N–N bond linkage, which is the determining factor in their biological activities, including anticancer. **Objectives:** This research aims to synthesise pyrazoline derivative compounds with anticancer potential. **Method:** 4-Metoxyacetophenon, halogen-substituted benzaldehyde, and phenylhydrazine were used to prepare pyrazoline derivatives (**4a, b**) under basic conditions using a microwave-assisted, one-pot, three-component reaction method. UV, FTIR, ^{1}H-NMR, and HRMS spectrometers were used to confirm the molecular structure of pyrazolines (**4a, b**) using their UV, FTIR, ^{1}H-NMR, and HRMS spectra. The anticancer activity of the compounds (**4a, b**) was evaluated using molecular docking studies to observe the receptor-ligand interaction of the compounds with the Estrogen Receptor Erα. **Result:** The pyrazoline (4a, b) produced positive results, with approximately 40% yield. It can be used as an anticancer agent due to its binding free energy values of -9.74 and -9.29 respectively, and a receptor-ligan interaction. **Conclusion:** New pyrazolines (4a, b) have been successfully synthesised with good yields through the one-pot three-component reaction and have potential as anti-breast cancer agents because of their good affinity for the ERα evidenced by the negative binding free energy values and receptor-ligand interactions that are similar to natural ligand, 4-OHT.

Introduction

Pyrazolines are a unique structural class of five-membered rings with two nitrogen atoms and a double bond (Kumar *et al*., 2020). Pyrazolines are members of the azole group, which also includes several other members that can be differentiated from one another based on the types of heteroatoms found in the five rings. Pyrazoline has been shown to have a number o biological effects, including anticancer, antioxidant, antibacterial, antifungal, antidepressant, and anti-inflammatory properties. (Sharma *et al*., 2014)

Breast cancer is the subtype of cancer that ranks as the second leading cause of death in women (Lumachi, 2015; Sun *et al*., 2017). Oestrogen is known to play a role in the development of breast cancer. because more than 70% of breast cancer cases are ER-positive, the primary target of chemotherapy is oestrogen receptors. Tamoxifen is the most effective drug for the treatment of breast cancer, but it has also been linked to many negative side effects, including the development of osteoporosis in patients who take it for an extended period (Ali *et al.*, 2016). As a result, there is a pressing need for the continued research and development of new anticancer therapeutic agents that are more efficient and have less of a negative impact on the body. (Constantinescu & Lungu, 2021).

Pyrazoline is an alkaloid compound that is found in nature only in extremely small amounts (Sakthinathan *et al*., 2012). This is because it is difficult for living organisms to form the N-N bond that is found in the pyrazoline ring (Ahmad *et al.*, 2016). The process of synthesis is an effective method for obtaining pyrazoline compounds with a variety of structural configurations. A one-pot reaction involving aldehydes, ketones, and hydrazine was used to produce pyrazoline compounds in this investigation. The reaction took place in a single step and involved all three components occurring simultaneously in the same vessel. Green

chemistry makes use of this synthesis method because it can reduce the amount of time, solvents, and energy consumed while simultaneously increasing the amount of product that is produced. Several different researchers have successfully carried out the one-pot synthesis of pyrazoline derivatives in the past. Hawaiz and researchers performed a one-pot synthesis of azo-pyrazoline compounds from azo-acetophenone, benzaldehyde and phenylhydrazine (Hawaiz *et al*., 2014).

Method

Synthesis

4-Methoxyacetophenone (3 mmol) and halogen-substituted benzaldehyde (2a, b; 3 mmol) were dissolved in 30 mL of ethanol to initiate the synthesis of compounds 4a, b. KOH 3N (10 mL) and phenylhydrazine (7-9 mmol) were added to the mixture. The mixture compound was exposed to microwave irradiation at a power level of 180 Watts for three to six minutes. TLC was utilised to monitor the reaction. The precipitate was filtered off and washed with aqua DM and methanol upon completion of the reaction. Using n-hexane, 4a and 4b were obtained through recrystallisation. Figure 1 depicts a schema reaction.

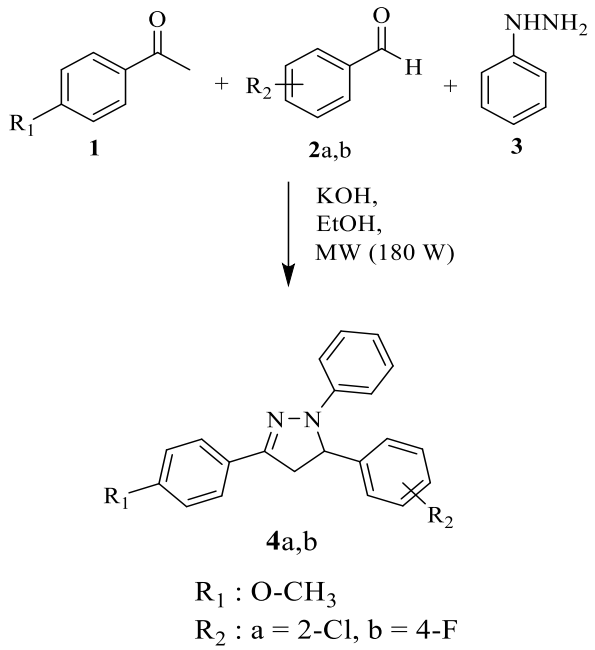

Figure 1: Schematic one-pot synthesis of pyrazolines

5-(2-chlorophenyl)-3-(4-methoxyphenyl)-1-phenyl-4,5-dihydro-1H-pyrazole (4a)

Molecular formula: $C_{22}H_{19}ClN_2O$, white solid, yield 47.2 %, mp: 110–111°C. UV (EtOH) λmax (nm): 210, 248 and 353. FTIR (KBr) ῡ (cm^{-1}): 3068, 2937, 1601, 1499, 1253, 1138, and 747. ^{1}H NMR ($CDCl_3$) δ (ppm): 7.68-7.69 (d, *J* = 8 Hz, 2H, Ar-H), 7.46 (dd, *Ja* = 7.5 Hz and *Jb* = 1 Hz, 1H, Ar-H), 7.20 (m, 5H, Ar-H), 7.01 (d, *J* = 2 Hz, 2H, Ar-H), 6.92-6.93 (d, *J*=2 Hz, 2H, Ar-H), 6.80 (t, *Ja* and *Jb*=7 Hz, 1H, Ar-H), 5.61 (dd, *JXB*=12 Hz and *JAX*=7 Hz, 1H, Hx), 3.96 (dd, *JBX*=12 Hz and *JBA*=17 Hz, 1H, HB), 3.85 (s, 3H, -OCH^3), 3.04 (dd, *JAX*=7 Hz dan *JAB*=17 Hz, 1H, HA). HRMS (*m/z*): $[M+H]^+$ calc: 363,1264; found: 363,1266.

5-(4-fluorophenyl)-3-(4-methoxyphenyl)-1-phenyl-4,5-dihydro-1H-pyrazole (4b)

Molecular formula: $C_{22}H_{19}FN_2O$, white solid, yield 46.8 %, mp: 129–130°C. UV (EtOH) λmax (nm): 247 and 353. FTIR (KBr) ῡ (cm^{-1}): 3071, 2993, 1607, 1506, 1321, 1306, 1217. ^{1}H NMR ($CDCl_3$) δ (ppm): 7.68 (d, *J* = 8.5 Hz, 2H, Ar-H); 7.31 (dd, *J* = 8.5 Hz and 5 Hz, 2H, Ar-H); 7.19 (dd, *J* = 8.5 Hz and 7 Hz, 2H, Ar-H); 7.04 (m, 4H, Ar-H); 6.93 (d, *J* = 8.5 Hz, 2H, Ar-H); 6.79 (t, *J* = 7 Hz, 1H, Ar-H); 3.85 (s, 3H, O-CH_3); 5.23 (dd, *JXB* = 12 Hz and *JXA* = 7 Hz, 1H, Hx); 3.81 (dd, *JBX*=12 Hz and *JAB*=17 Hz, 1H, HB); 3.09 (dd, *JAB*=17 Hz and *JAX*=7 Hz, 1H, HA). HRMS (*m/z*): $[M+H]^+$ calc: 347,1560; found: 347,1554.

Molecular docking procedure

Using the ChemDraw Professional 16.0.0.82 programme, the structures of the synthesised pyrazoline and the natural ligand 4-hydroxytamoxifen (4-OHT) for comparison were drawn (PerkinElmer). To find the most stable conformation, the energy was minimised. All ligands were made by combining a Gasteiger charge with hydrogen. Energy usage was reduced. The human oestrogen receptor (ERα) crystal structure was obtained from the Protein Data Bank (ID:3ERT). To prepare the receptors, hydrogen and Kollman charges were added.

Using AutoDock 4.2.6, docking studies were conducted on the prepared ligand and receptor molecule. By re-docking the natural ligand (4-OHT) within the active site, the docking protocol was validated. After the method has been validated, synthesised ligands can be docked using the same receptor, site, and parameter settings as their natural counterparts. After docking is complete, the conformation with the lowest binding energy is chosen, and the receptor-ligand complex is visualised using the software BIOVIA Discovery Studio Visualizer 2021.

Results

5-(2-chlorophenyl)-3-(4-methoxyphenyl)-1-phenyl4,5-dihydro-1H-pyrazole (4a)

Molecular formula: $C_{22}H_{19}ClN_2O$, white solid, yield 47.2 %, mp: 110–111°C. UV (EtOH) λmax (nm): 210, 248 and 353. FTIR (KBr) ῡ (cm−1): 3068, 2937, 1601, 1499, 1253, 1138, and 747. 1H NMR (CDCl3) δ (ppm): 7.68-7.69 (d, J = 8 Hz, 2H, Ar-H), 7.46 (dd, Ja = 7.5 Hz and Jb = 1 Hz, 1H, Ar-H), 7.20 (m, 5H, Ar-H), 7.01 (d, J = 2 Hz, 2H, Ar-H), 6.92-6.93 (d, J = 2 Hz, 2H, Ar-H), 6.80 (t, Ja and Jb = 7 Hz, 1H, Ar-H), 5.61 (dd, JXB = 12 Hz and JAX = 7 Hz, 1H, Hx), 3.96 (dd, JBX = 12 Hz and JBA = 17 Hz, 1H, HB), 3.85 (s, 3H, -OCH3), 3.04 (dd, JAX = 7 Hz dan JAB = 17 Hz, 1H, HA). HRMS (m/z): [M+H]+ calc: 363,1264; found: 363,1266.

5-(4-fluorophenyl)-3-(4-methoxyphenyl)-1-phenyl4,5-dihydro-1H-pyrazole (4b)

Molecular formula: $C_{22}H_{19}FN_2O$, white solid, yield 46.8 %, mp: 129–130°C. UV (EtOH) λmax (nm): 247 and 353. FTIR (KBr) ῡ (cm−1): 3071, 2993, 1607, 1506, 1321, 1306, 1217. 1H NMR (CDCl3) δ (ppm): 7.68 (d, J = 8.5 Hz, 2H, Ar-H); 7.31 (dd, J = 8.5 Hz and 5 Hz, 2H, Ar-H); 7.19 (dd, J = 8.5 Hz and 7 Hz, 2H, Ar-H); 7.04 (m, 4H, Ar-H); 6.93 (d, J = 8.5 Hz, 2H, Ar-H); 6.79 (t, J = 7 Hz, 1H, Ar-H); 3.85 (s, 3H, O-CH3); 5.23 (dd, JXB = 12 Hz and JXA = 7 Hz, 1H, Hx); 3.81 (dd, JBX = 12 Hz and JAB = 17 Hz, 1H, HB); 3.09 (dd, JAB = 17 Hz and JAX = 7 Hz, 1H, HA). HRMS (m/z): [M+H]+ calc: 347,1560; found: 347,1554.

Molecular Docking

The docking results obtained binding free energy value (S) and the ligand-receptor interaction which can be seen in Table I. The docking results showed that pyrazoline 4a has a lower binding free energy value compared to pyrazoline 4b but not lower than the natural ligand 4-hydroxytamoxifen.

Table I: Molecular docking results of synthesised compounds against ERα

Compounds	Binding Free Energy (Kcal/mole)	Interaction with residue		
		Hydrogen Bond	Van der Waals	Hydrophobic
4a	-9.74	-	Glu353, Arg394, Thr347, Phe404, Leu428, Ile 424, Trp383	Leu346, Leu525, Met343, Leu349, Leu387, Ala350, Leu391, Leu384, Met388, Met421, Met528
4b	-9.29	Arg394	Thr347, Leu384, His524, Gly420, Ile424, Met388, Trp383, Met421, Leu349	Leu346, Leu525, Met343, Leu387, Ala350, Leu391, Gly521, Glu353
4-OHT	-11.69	Arg394, Glu353	Thr347, Met343, His524, Glu419, Gly521, Gly420, Leu428, Met388, Leu349, Trp383, Leu354, Asp351, Ile424, Leu384	Ala350, Leu387, Phe404, Met421, Leu525, Leu346, Leu391

Discussion

Chemistry

Pyrazoline derivatives (4a, b) were successfully synthesised using a one-pot, one-step reaction. As illustrated in Figure 1, pyrazoline compounds (4a, b) were produced via a three-component reaction involving 4-methoxyacetophenone, halogen substituted benzaldehyde (2a, b), and phenylhydrazine. Pyrazoline synthesis can be performed under acid and basic conditions (Farooq & Ngaini, 2019). This study was carried out under basic condition using KOH as catalyst.

The one-pot synthesis of pyrazoline (4a, b) was conducted using 180 W of microwave irradiation. The power source was selected based on the findings (Zamri *et al*., 2016). In the course of the reaction, acetophenone and benzaldehyde will form a chalcone intermediate compound. Without undergoing a separation step, the chalcone intermediate reacts directly with phenylhydrazine to produce the desired molecule. Condensation is the mechanism for the formation of chalcone intermediates. Furthermore, the chalcone will undergo a cyclisation reaction with phenylhydrazine to produce pyrazolines. Figure 2 depicts the reaction mechanism for the formation of the compounds (4a, b).

Based on the reaction mechanism for the formation of pyrazoline compounds, it is possible to explain how the strong base catalyst KOH aids in the formation of enolate ions from ketones by binding to the acidic H present in ketones. The enolate ion will attack the

electrophilic aldehyde, forming a chalcone intermediate compound and releasing water. In addition, the chalcone will react with phenylhydrazine in the presence of H^+ and OH^- ions from water to produce the desired pyrazoline compound.

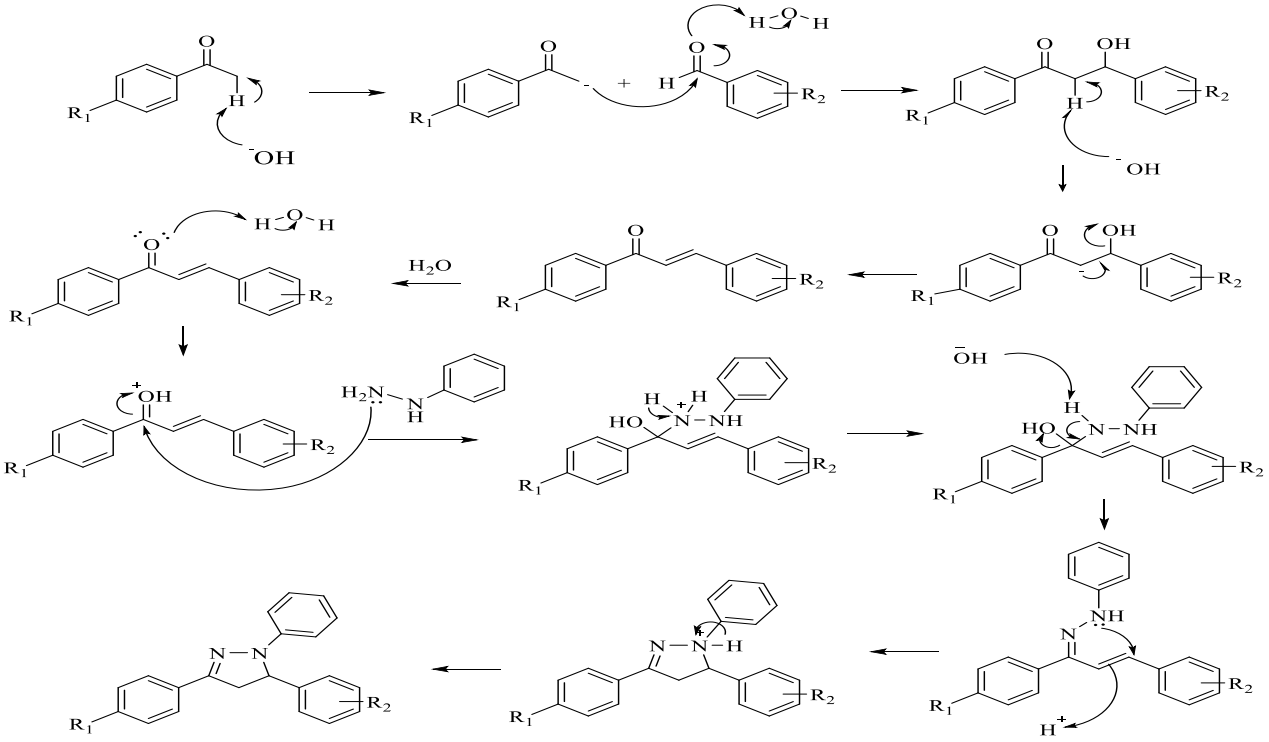

Figure 2: Reaction mechanism synthesis of pyrazoline derivative

By elucidating FTIR, ^{1}H-NMR, and HRMS spectra, the structures of the pyrazoline compounds were confirmed. The compounds 4a and 4b showed IR absorption bands of pyrazoline ring system. IR absorptions at 2937 and 2993 cm^{-1} indicate aliphatic C-H bond vibration, at 1600 cm^{-1} indicate C=N bond vibration and at 1253 and 1321 cm^{-1} indicate C-N bond vibration. In ^{1}H-NMR spectra, the pyrazoline ring is represented by the peaks of proton types HA, HB, and HX. The HA and HB protons are attached to the same carbon atom (C4), so they have a geminal coupling interaction. The vicinal coupling also exists between the protons of HA and HB. Protons HA and HB appeared as doublets of doublets at approximately three to four ppm, and proton Hx appeared at approximately five ppm. The methoxy protons (O-CH_3) appeared as singlets at 3.85 ppm, whereas aromatic protons (Ar-H) appeared between six to eight ppm.

Molecular Docking Study

In this study, the docking process involved several ligands, including pyrazolines synthesised (4a,b) and 4-hydroxytamoxifen (4OHT) as a positive control. 4-Hydroxy tamoxifen (4OHT) is a breast cancer drug still in use today. The receptor used in this molecular docking study is estrogen receptor alpha (ERα). Estrogen receptors are the main target for breast cancer treatment because more than 70% of breast cancer patients are ER-positive breast cancer (Sun *et al*., 2017). ERα receptors play an important role in the differentiation and proliferation of breast cancer cells as they are involved in regulating epithelial cell division (Abdel-Hafiz, 2017). 3ERT downloaded from the Protein Data Bank bound to the natural ligand 4OHT with a structural resolution of < 2 Å (3ERT:1.9 Å).

A Molecular Docking study was conducted to gain a deeper understanding of the compounds' binding modes, binding free energies, and binding interactions. The affinity of the ligand for the receptor is seen from the value of the binding free energy. A negative binding free energy (S) indicates that the ligand spontaneously binds to the receptor. The lower the binding free energy value, the greater the propensity to form a ligand-receptor bond (Pantsar & Poso, 2018). With dock scores of -9.74 and -9.29, respectively, the docking analysis revealed that compounds 4a and 4b exhibit favourable binding modes. Nonetheless, with a docking score of -11.69, it is inferior to the natural ligand 4OHT. The bond modes of compounds 4a, 4b, and the natural ligand 4OHT with the amino acid residues of the active site are illustrated in Figure 3. In compounds 4a and 4b, the pyrazoline ring interacts with Leu346 via a pi-amide

stacking interaction. Compound 4a interacts with Met343, Leu525, Ala350, Leu349, and Met421 through hydrophobic interactions. In compound 4b, the phenoxy oxygen forms a hydrogen bond with Arg394, whereas the fluoro group forms a halogen bond with Gly521. Compound 4b interacts hydrophobically with Met343, Leu525, Ala350, Leu346 and Leu387. Compounds 4a and 4b have interactions with Thr347, Glu353, and Arg394 that are crucial to the antagonistic activity of 4OHT against ERα.

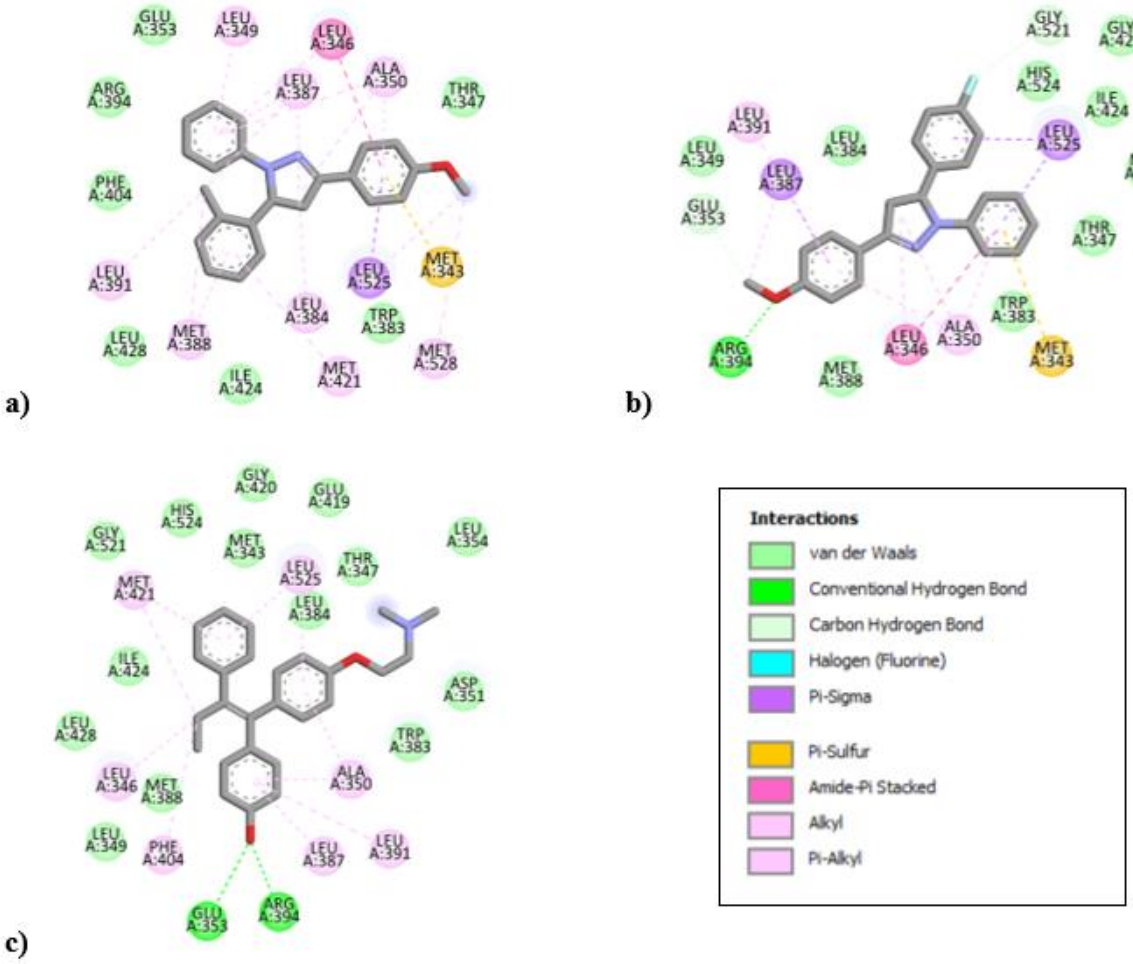

Figure 3: interactions of ligands with ERα receptor; a) 4a, b) 4b, and c) natural ligand 4OHT

Conclusion

In conclusion, pyrazoline derivatives have been synthesised as a novel class of anticancer agents. As evidenced by the negative docking score and the presence of ligand-receptor interactions, the anticancer activity of pyrazoline compounds 4a and 4b on ERα receptors was confirmed by molecular docking analyses. Therefore, additional tests of the anti-cancer activity of the synthesised compounds are required.

Acknowledgement

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