AMERICAN JOURNAL OF PHARMTECH RESEARCH

Journal home page: http://www.ajptr.com/

Design, Synthesis, Characterization and Biological Evaluation of some novel Heterocyclic derivatives as Anti-Tubercular agents

Ayyadurai Jerad Suresh*[1], Parakkot Ramakrishnan Surya[1], Antonyraj Velankani[1]

1. Department of pharmaceutical chemistry, College of Pharmacy, Madras Medical College, Chennai-600003, India

ABSTRACT

Tuberculosis is a serious threat to public health throughout the world. Schiff bases are compounds carrying the imine or azomethine (–C=N–) functional group. Recent studies report that benzimidazole based Schiff bases possess antibacterial, antimicrobial, anti-tubercular, and anti-inflammatory activities. [1,2] Further it has been known that fluoroaniline moiety can have profound and unexpected results in biological activity [19]. A series of benzimidazole and fluroaniline based Schiff bases were designed and docked against crucial mtb enzyme target Glutamine synthetase1. The molecules with good docking-score and multiple interactions were chosen for synthesis. Compounds (BE1, BE2, FA-1, FA-2, and FA-3) were synthesized by reflux condensation reaction with good yield. The newly synthesized compounds were characterized by spectral methods and evaluated for anti- mycobacterial activity against tuberculosis H37RV strain. Anti-tubercular activity was carried out by using Microplate Alamar Blue Assay (MABA) method. The experimental results revealed that Compounds (BE1& BE2) showed promising anti-tubercular activity with an MIC below 0.8 mcg/mL while (FA-1, FA-2, and FA-3) showed moderate anti tubercular activity with an MIC below 6.25 and 12.5 mcg/mL.

Key words: Benzimidazole, Schiff base, Fluoroaniline, Docking, Anti-tubercular.

*Corresponding Author Email: ajsuresh2001@yahoo.co.uk

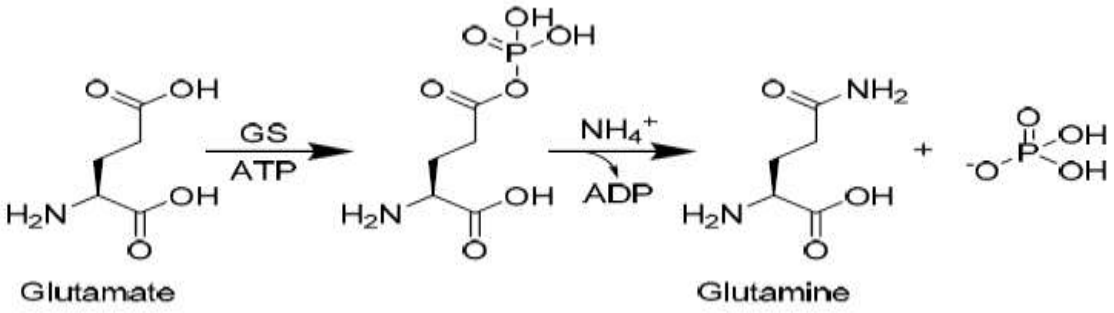
Received 29 October 2015, Accepted 03 November 2015

Please cite this article as: Suresh AJ *et al*., Design, Synthesis, Characterization and Biological Evaluation of some novel Heterocyclic derivatives as Anti-Tubercular agents. American Journal of PharmTech Research 2015.

INTRODUCTION

Tuberculosis (TB), become a major global health concern. Tuberculosis is a common infectious disease caused by the Mycobacterium tuberculosis. TB is second only to HIV/AIDS as the greatest killer worldwide due to a single infectious agent. Tuberculosis is a curable and preventable disease. The emergence of new "multidrug-resistant tuberculosis" (MDR-TB) strains, ''extensively drug-resistant tuberculosis'' (XDR-TB) and "totally drug resistant tuberculosis" (TDR-TB) has complicated the management of tuberculosis disease. This indicates an urgent need to develop new potential anti-tubercular drugs. [3]

Glutamine synthetase is an enzyme that plays an essential role in the metabolism of nitrogen by catalyzing the condensation of glutamate and ammonia to form glutamine. [4]



Schiff bases are condensation products of primary amines with carbonyl compounds. Schiff bases are the compounds carrying imine or azomethine functional group and are found to be a versatile pharmacophore for design and development of various bioactive lead molecules. Schiff bases exhibit useful biological activities such as anti-inflammatory, analgesic, anticonvulsant anticancer, antidepressant, antimicrobial and anti tubercular activities.

Benzimidazole is the heterocyclic compound formed from benzene and imidazole ring. It is regarded as a promising class of bioactive heterocyclic molecules that exhibit various biological activities. The synthesis of novel benzimidazole derivatives remains an impotrant focus on medical research.

MATERIALS AND METHODS

Docking

Docking involves the fitting of a molecule into the target structure in a variety of positions, conformations and orientations to find out the most stable posse. A process of design and discovery of the new chemical entities using an automated docking programs GLIDE, Auto Dock and Argus Lab.[7] It searches molecules (ligands) have maximum favorable interactions with

receptors usually a protein. Docking is done by using Argus Lab software. Argus Lab 4.0 software distributed freely available for windows platforms by plannaria software. [8]

Insilco Screening of Drug Likeness

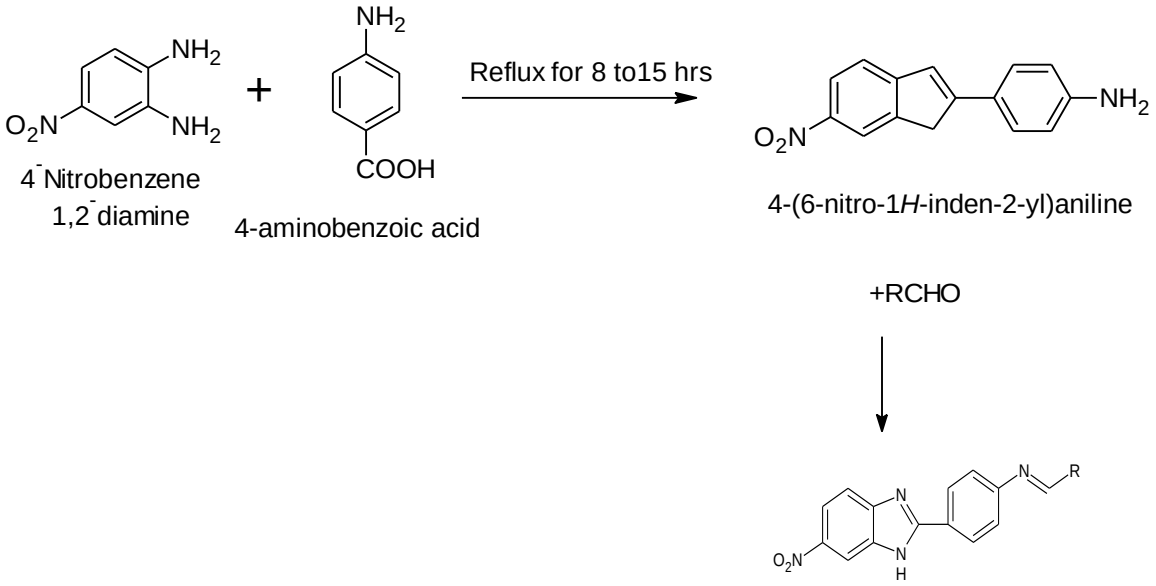
A drug to be pharmacologically active and exert the action it should possess pharmacokinetic properties like absorption, distribution, metabolism and excretion. In the field of drug research and development many drug failures occur due to unfavorable ADME properties. This has to be out earlier in the process of drug discovery. Some computational methods (in silico tools) have been evolved to investigate the most suitable drug molecules before synthesis. It is used to predict whether a molecule is likely to be orally bio-available or to evaluate drug likeness. [9]

Toxicity Risk Assessment

In silico toxicity prediction is done by using OSIRIS Property Explorer. It is free software available for access in the Organic Chemistry Portal. Using this prediction tool, mutagenicity, tumerogenicity, skin irritation and reproductive effects can be calculated. The prediction properties relies on a precompiled set of structure fragment that gives rises to toxicity alerts in case they are encountered in the structure currently drawn.[10]

Experimental section:

Scheme: 1

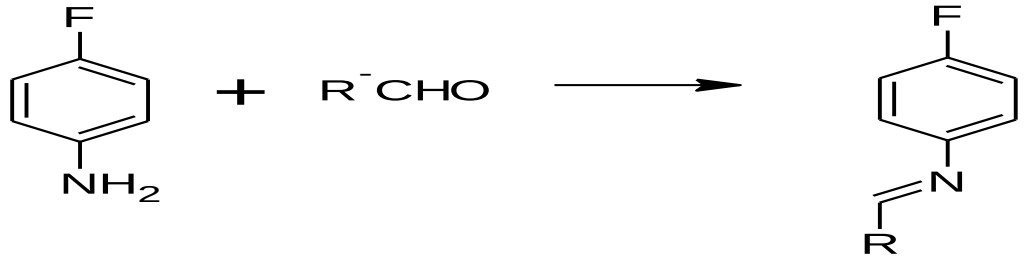


Aldehydes used:

BE1= Benzaldehyde

BE2= 3, 4-dimethoxybenzaldehyde

Scheme: 2



Compounds FA 1, FA2 AND FA3, were synthesized by using 4-fluroraniline as primary amine and 1*H*-pyrrole-2-carbaldehyde, pyridine-4-carbaldehyde and 2-nitrobenzaldehyde as respective aromatic aldehydes.

Procedure:

Synthesis of 4-(6-nitro-1H-inden-2yl) aniline

The mixture of 4-nitro o-phenylenediamine (0.01mol), p-amino benzoic acid (0.01mol) and 4N HCl (20ml) was refluxed for 6 hours. Completion of the reaction was monitored by TLC. After the completion of the reaction, the reaction mixture was poured into crushed ice. Then the solution was neutralized with 1N NaOH. The resulting precipitate was filtered, washed with water and recrystallized using ethanol. [11]

Synthesis of Schiff base

An equimolar quantity of amine and substituted aromatic aldehydes was refluxed for 10-15 hours in 20ml of ethanol. Completion of the reaction was monitored by TLC. After the completion of the reaction mixture was filtered and dried. The product was recrystallized by using ethanol. [12]

S.NO	Mol.Wt	Melting Point(^{0}C)	Molecular Formula	Solubility	Colour	Yield
BE-1	342.35	185	$C_{20}H_{14}N_4O_2$	Methanol, Ethylacetate	Browinish Black	82.6%
BE-2	402.40	165	$C_{22}H_{18}N_4O_4$	Methanol, Ethylacetate	Yelowish Orange	78.9%
FA-1	244.22	182	$C_{13}H_9N_2O_2F$	Methanol, Ethylacetate	Pale yellow	96%
FA-2	188.20	173	$C_{11}H_9N_2F$	Methanol, Ethylacetate.	Buff color	99%
FA-3	200.21	161	$C_{12}H_9FN_2$	Methanol, Ethylacetate	Pale Brown	96%

Spectral Analysis:

4-(6-nitro-1*H*-benzimidazol-2-yl)-*N*-[(*E*)-phenylmethylidene] aniline: IR- using KBR pellet method. Ar-CH str (2982.04 cm^{-1}), Aliphatic CH str (2923.45 cm^{-1}), 2^0 Amine (3232.45 cm^{-1}),

C=N str (1620.08 cm^{-1}). NMR: ^{1}HNMR δPPM (δ3.3 singlet 1H), (δ6.8 singlet 1H), (δ7-8.5 multiblet 6H), Purity 91%, Mass- 342.35 (M^{-1} 341.11).

***N*-[(*E*)-(3, 4-dimethoxyphenyl)methylidene]-4-(6-nitro-1*H*-benzimidazol, 2yl)aniline :** IR- using KBR pellet method. Ar-CH str (3186.14 cm^{-1}), Aliphatic CH str (2936.51 cm^{-1}), 2^{0} Amine (3361.61 cm^{-1}), C=N str (1620.08 cm^{-1}). NMR: ^{1}HNMR δPPM (δ9 singlet 1H), (δ7-8 multiblet), Mass- 402.40 (402.33).

4-fluoro-*N*-[(*E*)-(2-nitrophenyl)methylidene]aniline: IR- using KBR pellet method. Ar-CH str (3109.02 cm^{-1}), Aliphatic CH str (2916.16 cm^{-1}), C-F (1350.07 cm^{-1}), C=N str (1627.80 cm^{-1}). NMR: ^{1}HNMR δPPM (δ9 singlet 1H), (δ7-8 multiblet), Purity 100%, Mass- 244.22

4-fluoro-*N*-[(*E*)-3*H*-pyrrol-2-ylmethylidene] aniline: IR- using KBR pellet method. Ar-CH str (3078.04 cm^{-1}), Aliphatic CH str (2912.21 cm^{-1}), 2^{0} Amine (3433.04 cm^{-1}), C=N str (1686.93 cm^{-1}). NMR: ^{1}HNMR δPPM (δ7-7.6 multiplet), (δ8-8.7 doublet), (δ7-7.7 multiblet), Purity 100%, Mass- 188.20

4-fluoro-*N*-[(*E*)-pyridin-4-ylmethylidene] aniline: IR- using KBR pellet method. Ar-CH str (3070.45 cm^{-1}), Aliphatic CH str (2916.16 cm^{-1}), C-F str (1334.34 cm^{-1}), C=N str (1627.67 cm^{-1}). NMR: ^{1}HNMR δPPM (δ2.6 singlet), (δ7-8.2 multiplet), Purity 100%, Mass- 200.21.

BIOLOGICAL ACTIVITY:

MABA Procedure:

The anti-mycobacterial activity of compounds were assessed against M. tuberculosis using Micro plate Alamar Blue assay (MABA). Briefly, 200µl of sterile deionized water was added to all outer perimeter wells of sterile 96 wells plate to minimized evaporation of medium in the test wells during incubation. The 96 wells plate received 100 µl of the Middlebrook 7H9 broth and serial dilution of compounds were made directly on plate. The final drug concentrations tested were 100 to 0.8 µg/ml. Plates were covered and sealed with parafilm and incubated at 37ºC for five days. After this time, 25µl of freshly prepared 1:1 mixture of Alamar Blue reagent and 10% tween 80 was added to the plate and incubated for 24 hrs. A blue color in the well was interpreted as no bacterial growth, and pink color was scored as growth. The MIC was defined as lowest drug concentration which prevented the color change from blue to pink. [17]

RESULTS AND DISCUSSION

In our present research work, we have synthesized benzimidazole and fluoroaniline based Schiff base derivatives. The Synthesized compounds were purified by column chromatographic techniques. Characterization of the purified compounds was carried out by spectral methods. The

final compounds were screened for anti-tubercular activity.

Docking

The designed molecules were docked against the selected target Glutamine synthetase1. The best docked pose was selected based on the docking score and the multiple interactions.

On comparison, it was found that, interaction of amino acids Lysine (Lys403/250) and Isoleucine (Ile416/31) was most common for compounds BE1,BE2 and Standard drugs INH and Pyrazinamide. Whereas Phenyl Alanine (Phe139/212/232) among FA1, FA 2 AND FA 3.

Docking score for the synthesized compounds BE1: -7.988, BE2: -8.466, FA1: -9.2939, FA2: -8.1748 and FA3: -8.7845 Kcal/mol. Standard drugs INH -6.029 and Pyrazinamide -5.559Kcal/mol.

Figure1 and 2: Compounds Docked Against the Protein 4acf of Glutamine Synthetase 1

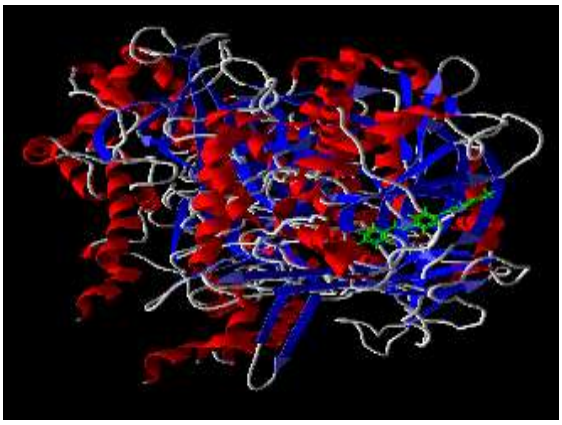


Figure1: BE-1 with 4 ACF

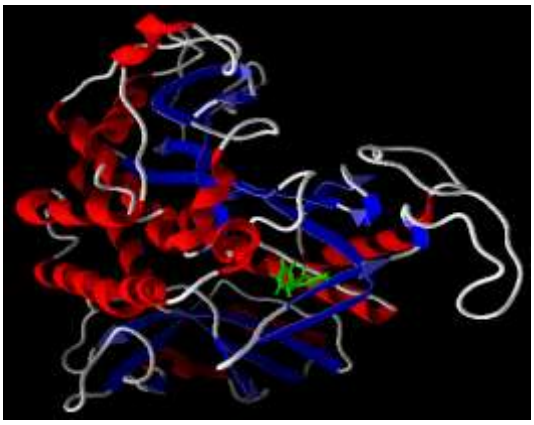


Figure2: FA-1 with 4 ACF

Figure3 and 4: Interactions between the Compounds and the Protein

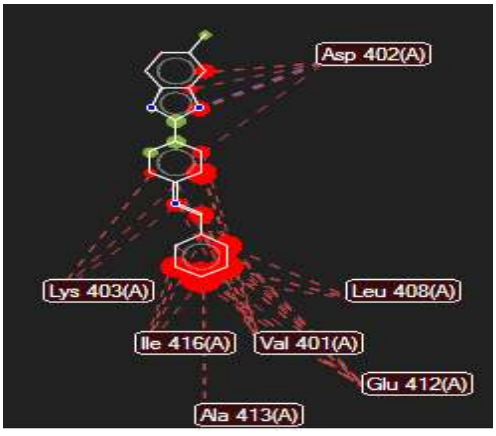


Figure 3: BE-1 with 4ACF

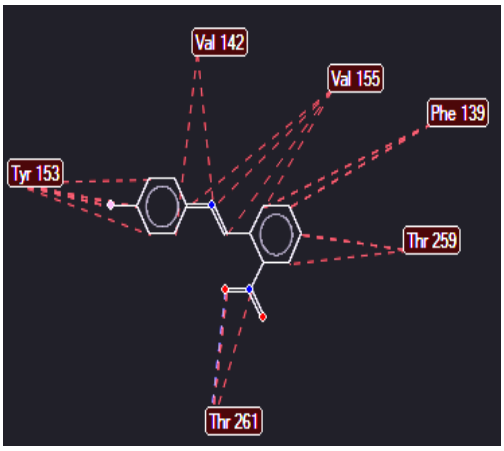


Figure 4: FA-1with 4ACF

Figure 5 and 6: Toxicity Prediction by Osiris:

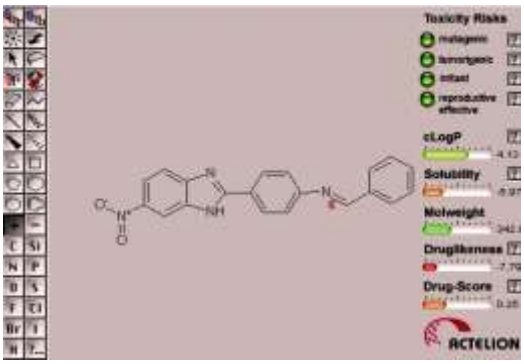


Figure5: BE-1

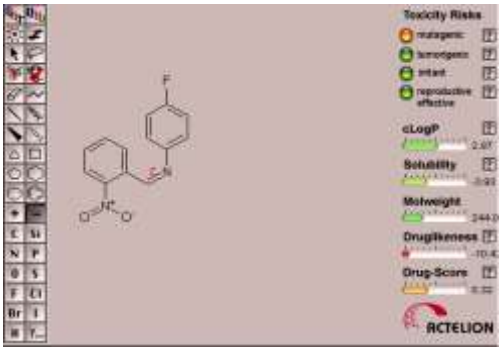


Figure6: FA-1

Table 1: Docking Score of compounds against 4acf

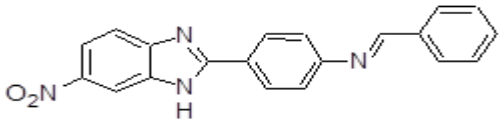
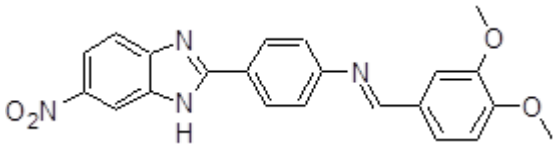
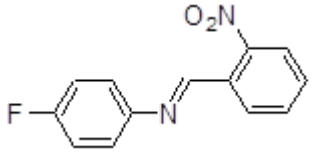
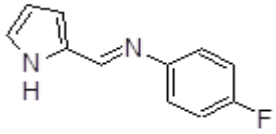
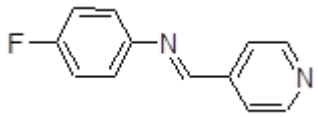
COMPOUND ID	COMPOUND STRUCTURE	SCORE
BE-1		-7.92
BE-2		-8.46
FA-1		-9.29
FA-2		-8.17
FA-3		-8.78

Table 2: Toxicity prediction:

- ✓ OSIRIS Property Explorer
- ✓ Mutagenicity, tumorogenicity, skin irritancy, and reproductive effects can be calculated.

Compound Code	Mutagenicity	Tumorogenicity	Skin Irritancy	Reproductive Effect
BE-1	Green	Green	Green	Green
BE-2	Green	Green	Green	Green
FA-1	Yellow	Green	Green	Green
FA-2	Yellow	Green	Green	Green
FA-3	Green	Green	Green	Green

Green– Nontoxic, **Yellow** – Moderately toxic, **Red** - Severely toxic

Table 3: Anti Mycrobial Screening

All the compounds showed good and moderate activity against mycobacterium tuberculosis. The inhibition of growth of bacteria measured by µg and ng.

MABA REPORTS

S.NO	SAMPLE	100 µg/ml	50 µg/ml	25 µg/ml	12.5 µg/ml	6.25 µg/ml	3.12 µg/ml	1.6 µg/ml	0.8 µg/ml
1.	BE-1	S	S	S	S	S	S	S	S
2.	BE-2	S	S	S	S	S	S	S	S
3.	FA-1	S	S	S	S	R	R	R	R
4.	FA-2	S	S	S	S	S	R	R	R
5.	FA-3	S	S	S	S	R	R	R	R

NOTE: S-sensitive, R-Resistant, Strain Used- M.tuberculosis (H37 RV strain)

Synthesis

The compounds were synthesized as per the scheme. All the synthesized compounds were purified by chromatographic techniques.

Melting point: The melting point of the synthesized compound was determined by one end open capillary tube method. The temperature at which the compound starts losing its crystallinity and changes from solid to liquid form was found recorded.

BE-1: Brownish black solid; MP- 185ºC, BE-2: Yellowish orange solid; MP- 165ºC, Pale yellow solid; MP- 95ºC, FA-2: Buff color solid; MP- 98ºC and FA-3: Pale brown solid; MP- 98ºC.

Yield: Products obtained with a yield of about 78-99%.

Biological activity: The in-vitro anti-tubercular activity of compounds were assessed against M. tuberculosis using Micro plate Alamar Blue assay (MABA). All the compounds showed good activity against mycobacterium tuberculosis, BE1: 0.8, BE2: 0.8, FA1: 12.5, FA2: 6.25 and FA3: 12.5 mcg/ML.

Chemistry

The structures of the final compound were confirmed on the basis of spectral studies. All the newly synthesized compounds were characterized by IR, NMR and MASS Spectroscopy. IR spectra of 4-(6-nitro-1*H*-benzimidazol-2-yl)-*N*-[(*E*)-phenylmethylidene]aniline showed strong absorption band at 3232 cm^{-1}, 1690 cm^{-1} for C=N and 1194 cm^{-1} for C-N. ^{1}HNMR δPPM (δ3.3 singlet, Aliphatic proton), (δ6.8 singlet, N-H proton), (δ6.8 singlet, Aromatic proton).The mass spectra of the compound BE-1 revealed the molecular ion peak at 342.35m/z. m^{-1} at 341.11.

CONCLUSION

All the synthesized compounds showed promising anti tubercular activity. Compounds (BE1&

BE2) showed promising anti-tubercular activity with an MIC below 0.8 mcg/mL while (FA-1, FA-2, and FA-3) showed moderate anti tubercular activity with an MIC below 12.5, 6.25 and 12.5 mcg/mL respectively. This compounds compares with the standard antitubercular agents like Isoniazid, Streptomycin etc. The benzimidazole compounds are far more potent than the fluorinated derivatives. Further, structural modifications of the synthesized compounds will aid in the development of more potential molecules against the pathogen.

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