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Synthesis and Antitubercular activity of novel lupeol derivatives

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ABSTRACT

Recently, one third of the world's population is infected with Mycobacterium tuberculosis and 8 million new and relapse cases of tuberculosis are reported every year. The emergence of new cases, the increased incidence of multi-drug resistant strains of M. tuberculosis, and the adverse effects of first and second-line antituberculosis drugs have led to renewed research interest in natural products in the hope of discovering new antitubercular leads. Now a days, thousand of Natural and semisynthetic derivatives ,possessing novel, compounds have been reported to positive activity towards M.tuberculosis. In this paper we isolate lupeol first then formation of their pyrazoline derivative .Few derivative of lupeol showed more potent and significant antitubercular activity. In our studies, it was found that the pyrazoline derivatives of lupeol possess better antitubercular potential when compared to lupeol. In this cases natural products such as lupeol with low bioactivity (MICs of 64 µg/ mL), and also those semisynthetic derivatives with remarkable antitubercular activity (MICs of 8.5µg/mL). It is thus concluded that lupeol skeleton deserves further investigation for the development of more potent and non-toxic new antitubercular agents for therapeutic applications.

Keywords: lupeol.antitubercular, HR37 strain

INTRODUCTION

Currently, one third of the world's population is infected with Mycobacterium tuberculosis and 8.9- 9.9 million new and relapse cases of tuberculosis are reported every year [1]. The emergence of new cases, the increased incidence of multi-drug resistant strains of M. tuberculosis, the adverse effects of first- and second-line antituberculosis drugs, and the increased incidence of tuberculosis associated with viral infections (Human Immunodeficiency Virus, HIV) have led to renewed research interest in natural products in the hope of discovering new antitubercular leads [2,3]. During the last two decades, there has been a lot of progress in new techniques to evaluate the antimycobacterial potential of a large number of compounds. Herbal drugs are prescribed widely because of their effectiveness, less side effects and relatively low cost. [4] Therefore, investigation on such agents from traditional medicinal plants has become more important. [5] More than 50 % of the medicines introduced in world are connected with natural compounds to some extent. It can be as native metabolites and synthetically the modified derivative. Natural products and some of their derivatives have been reported to exhibit remarkable growth inhibitory activity towards M. tuberculosis and some of them have been selected as prototype molecules for the development of new antitubercular agents. [6,7] It is estimated that between 2002 and 2020, approximately 1000 million people will be newly infected, over 150 million people will develop diseases and 36 million will die of TB if proper control measures are not established.[8] Lupeol has been reported to be present in diverse species of the plant kingdom. Lupeol is found in edible vegetables and fruits such as white cabbage, pepper, cucumber, tomato, carrot, pea, bitter

root, soy bean, ivy gourd, black tea, figs, strawberries red grapes, mulberries, date palm and guava. Lupeol is also found in abundance in medicinal plants such as, Shea butter plant, licorice, *Tamarindus indica, Celastrus paniculatus, Zanthoxylum riedelianum, Allanblackia monticola, Himatanthus sucuuba, Leptadenia hastata, Crataeva nurvala, Bombax ceiba, Sebastiania adenophora, Aegle marmelos* and *Emblica officinalis*.[9,10] India has a rich history of using various potent herbs and herbal components for treating tuberculosis.

The plant *Crataeva nurvala* Buch.-Ham. belonging to the family Capparidaceae is known as three-leaved caper in English, *neermatalam, nirval* in Malayalam, *Varuna* in Sanskrit and *barun* as well as *barna* in Hindi. The medicinal value of *C. nurvala* has been described against a wide variety of urinary disorders including urolithiasis, in the ancient text "Sushruta Samhita."[11] Among the compounds isolated from the stem bark, lupeol was identified as a major component in association with α- and β-amyrin .[12] Thus, the bark of C. nurvala is contraceptive and cytotoxic and is especially useful in urinary disorders, kidney bladder stones, fever, vomiting and gastric irritation. Root and bark are laxative and lithontripic and increase appetite and biliary secretion13. Leaves are externally rubefacient and used in rheumatism; internally they are given as febrifuge and tonic.[14, 15] In forwarding of our enthusiasm to develop drugs from natural sources, we selected the triterpene lupeol for evaluation of its Antitubercular property. Earlier work has shown that lupeol has great potential as antiarthritic, anti-microbial agent, antiprotozoal agent, anti-diabetic agent, anti-cancerous agent, cardioprotective agent, skin protective agent, hepatoprotective agent, nephroprotective agent.[16] In our studies, lupeol showed Antitubercular activity. Therefore, we planned to prepare derivatives of lupeol for a potent antitubercular agent.. However, to the best of our knowledge, there are no reports about the potential effects of lupeol, and their derivatives on the antitubercular.

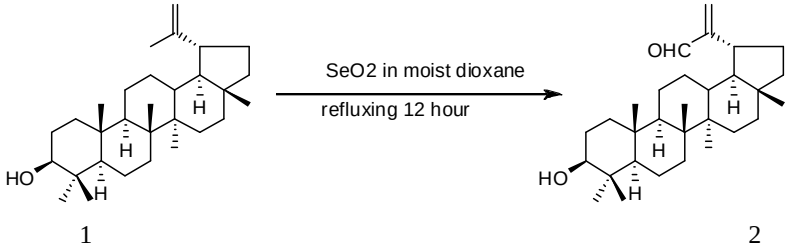
MATERIALS AND METHODS

1H NMR spectra was recorded on a Bruker 300 FT NMR instrument using $CDCl_3$ as solvent and TMS as internal reference (chemical shifts in δ values). IR spectra were measured on a Beckmann Acculab-10 Spectrophotometer. Melting points were determined on a hot stage melting point apparatus .

Collection of the plant material:
Stem bark of *C. nurvula* was purchased from the local market and authenticated by the botanists in Chandra shekhar university azad nagar Kanpur, India

Isolation of lupeol
Lupeol **1**, isolated from the stem bark of *Crataeva nurvala* was extracted from the plant material with 95% ethanol. Extract was concentrated on rotavapour under pressure which on repeated crystallization gave pure lupeol. Further amount of lupeol was isolated by flash column chromatography of mother liquor along with small amount of lupenone. Crude lupeol from concentrated ethanol extract was filtered out and crystallized repeatedly (chloroform/MeOH) to give white solid. Filtrate and collective mother liquor from crystallization was chromatographed over normal silica gel, packed in hexane. 3-5% Ethyl acetate in hexane eluent gave lupenone , while elution with 7-10% ethyl acetate in hexane yielded lupeol . Lupeol gave a +ve Libberman-Burchard test. It showed molecular ion peak at m/z 426 (M^+) in its EI mass spectrum & 409 (M-OH) in FAB mass spectrum. The structure was confirmed by comparison of spectroscopic data of the compound-1 to those described for lupeol [17] and confirmation of the lupeol was also done by TLC with authentic sample of lupeol.

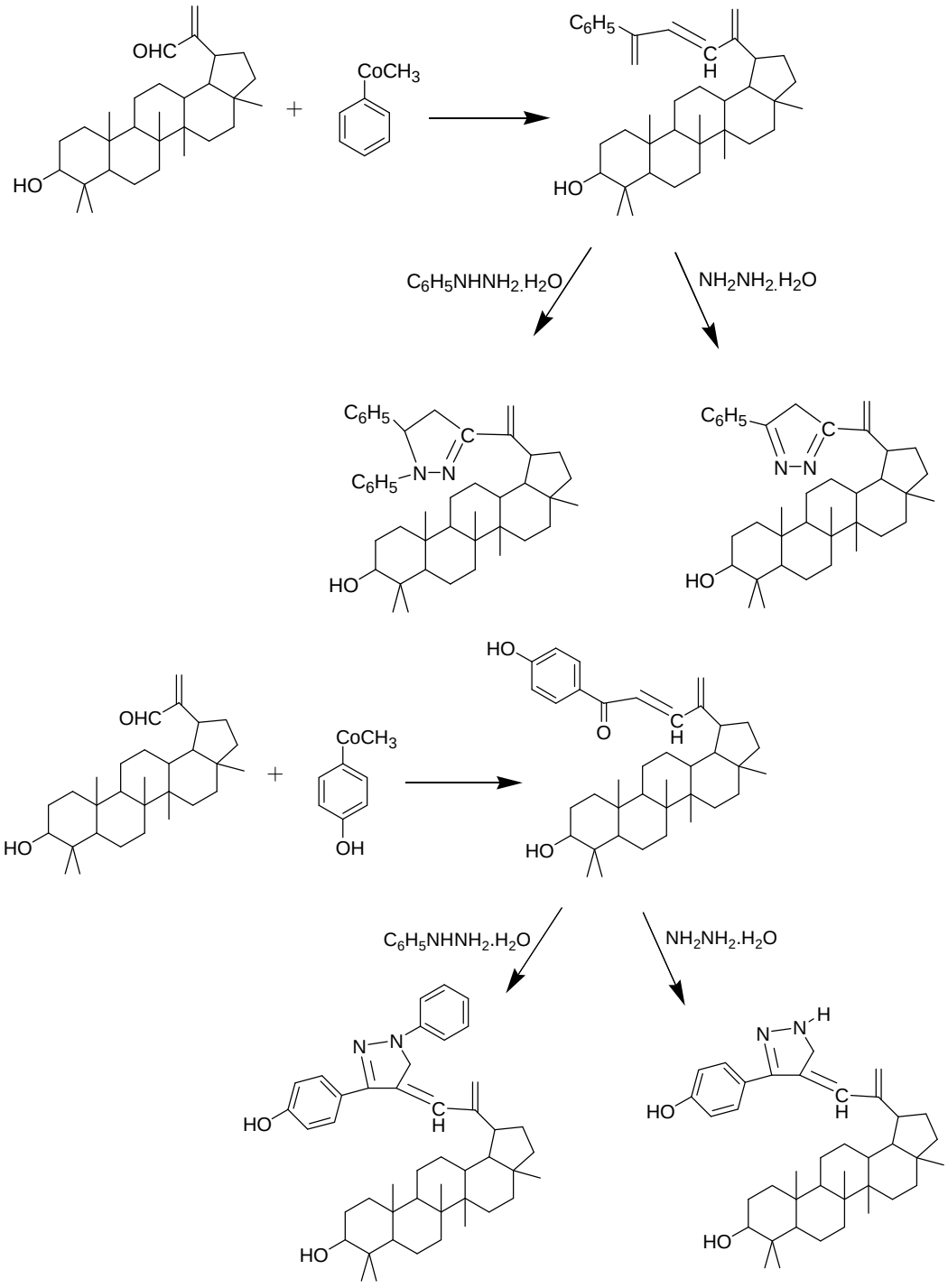
General method for preparation of lupeol aldehyde



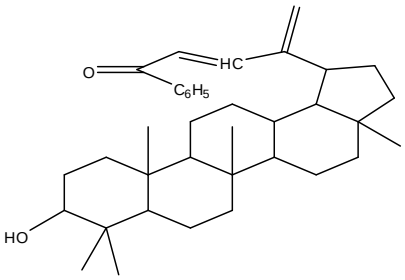
1 2

Scheme 1 Reagents and conditions: SeO_2 Dioxane ,Water 4 drops, 12 hrs, at 120^0 c

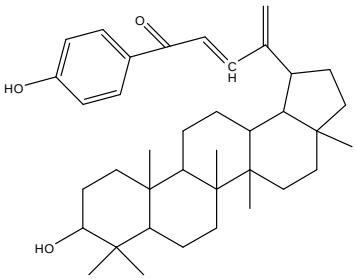
Scheme-2



Place lupeol aldehyde (0.01 mole) reacted with actopheone or O- hydroxy acetophenone (0.01 mole), 5 ml absolute ethanol and 0.05 ml of thionyl chloride. The reaction mixture was refluxed for 2-3 hrs for 2 hrs at room temperature. Precipitation was done by cold water. Lastly filtered the reaction mixture using what man filter paper and washed with cold water to obtain the desired product.(2a,3a)

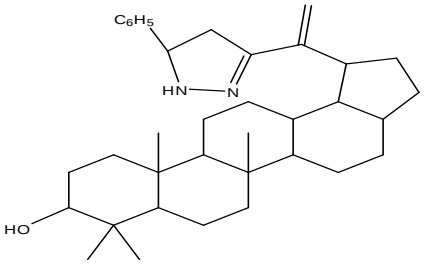


2a

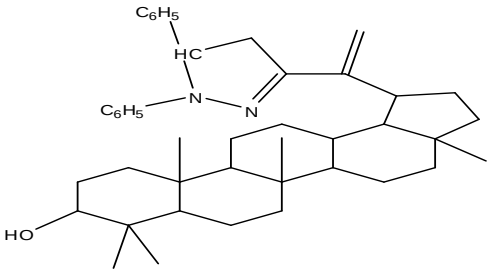


3a

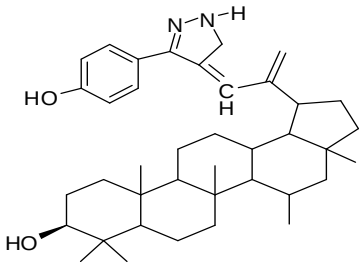
Place a mixture of (2a,3a) in round bottom flask with ethanol and then 6-8 drops of Hydrazine hydrate or phenyl hydrazine was added dropwise in the reaction mixture. The reaction mixture was heated under reflux for 12 hrs at RT. Cooled the reaction mixture and poured into crushed ice with constant stirring. Then filtered the reaction mixture, recrystallized the residue with ethanol and dried the product (2b,2c,3b,3c).



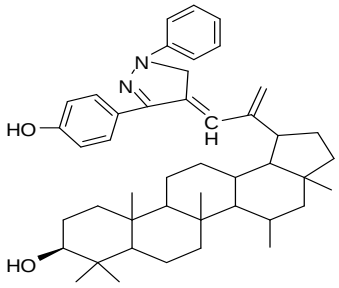
2b



2c



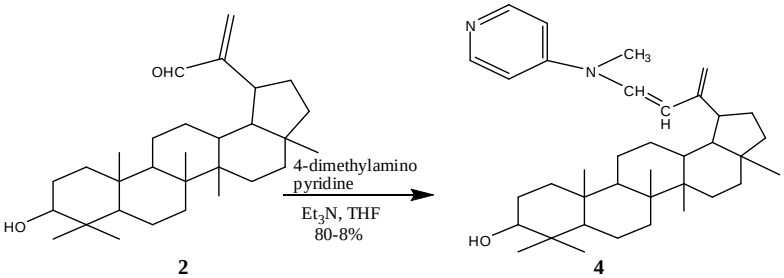
3b



3c

Scheme 3

Place equimolar mixture of lupeol aldehyde (2) and 4- dimethylaminopyridine in round bottom flask with 8-12 drops of triethyl amine and 80-88% THF. The reaction mixture was heated under reflux for 8-12 hrs at 20ºC. Leave the reaction mixture for 24 hrs at room temperature to obtain desired product.



2 **4**

Experimental data

Lupeol 1

M.P: 212-214ºC, Mass (FAB): m/z 427 (M+1), IR (KBr, cm-1): 3302, 3067, 2946, 2667,2363, 1636, 1596, 1460, 1380, 1300, 1190, 1038, 880,669, 545. ^{1}H-NMR (300 MHz, $CDCl_3$): δ 4.68 and 4.56 (2s, 1H each, H-29), 3.23 (m, 1H, H-3), 2.36 (m, 1H, H-2), 1.90 (m, 1H, H-19), 1.68 (s, 3H, H-30), 1.62-1.25 (bunch for 24 H), 1.02 (s, 3H), 0.96 (s, 3H), 0.94 (s, 3H), 0.82 (s, 3H), 0.78 (s, 3H), 0.76 (s, 3H).

Lupeol aldehyde 2

M.P: 225ºC; Mass (ESI): m/z 441 (M+1); IR (KBR, cm^{-1}): 3326, 2945.9, 2855, 2325, 1743.1, 1641.98, 1454.4, 1380.51, 1261.34, 1109, 1015.34, 948.1, 757.19; ^{1}H-NMR (500 MHz, $CHCl_3$): δ 9.48 (s, 1H-CHO), 6.25 and 5.87 (2s, 1H each, H29), 3.13 (m, 1H, H-3), 2.76 (m, 1H), 2.10 (m, 1H), 1.65-1.27 (bunch, 24 H), 1.01 (s, 3H, Me), 0.96 (s, 3H, Me), 0.92 (s, 3H, Me), 0.81 (s, 6H), 0.75 (s, 3H, Me)

Compound 2a

M.P: 217-219ºC, I.R.: ν_{max} (cm^{-1}): 3286.9, 2948.8, 2867.1, 2700, 1744, 1678, 1619.6, 1468, 1378.7, 1188.3, 1106.6, 1040.6, 1013.4, 951.19, 982.28, 873.46, 706.35; Mass (ESI): m/z 543(M+1), 1H-NMR (500 MHz, CDCl3):7.25(s, 1H), 6.59(s, 1H), 4.58(s, 1H), 3.18-3.16(m, 1H), 2.18-2.22(t, 9H), 1.65(s,1H), 1.19-1.2(d,6H), 1.65-1.27 (bunch, 24 H),1.01 (s, 3H, -Me), 0.96 (s, 3H, -Me), 0.92 (s, 3H, -Me), 0.81 (s, 6H), 0.75 (s, 3H, -Me).

Compound 2b

M.P: 200-210 ºC, I.R: ν_{max} (cm-1): 3326.15, 2938.2, 2861, 1641.5, 1556.1, 1457.23, 1489, 1258, 1045, 809, 685; Mass (ESI): m/z 557(M+1), 1H-NMR (500 MHz, $CDCl_3$): 8.21-8.18(m, 2H), 7.96-7.25(m, 7H), 5.6-5.2(m, 1H), 4.28-4.029(m,18H), 3.67 (m, 1H, H-3), 2.155 (m, 1H, H-2), 2.04 (m,1H, H-19), 1.39-1.11 (bunch, 24 H),1.08 (s, 3H, -Me), 1.07 (s, 3H, -Me), 0.87 (s, 3H, -Me), 0.86 (s, 6H), 0.84 (s, 3H, -Me).

Compound 2c

M.P: 190ºC; I.R: ν_{max} (cm^{-1}): 3326.15, 2972.81, 2882.18, 1622, 1453.59, 1380.32, 1326.85, 1273.39, 1087.12, 1045.26, 879.56; Mass (ESI): m/z 633(M+1), 1H-NMR (500 MHz, CDCl3): δ8.08(s, 1H), 7.87 and 7.84(s,1H each),7.7(t,1H), 7.58-7.39(m, 6H), 7.25(s, 2H), 6.54-6.42(m, 1H), 2.3-2.2 (m, 1H, H-3), 1.73 (m, 1H, H-2), 1.41 (m,1H, H-19), 1.28 (d, 2H,), 1.27-1.24 (bunch, 24 H),1.10 (s, 3H, -Me), 0.88 (s, 3H, -Me), 0.87 (s, 3H, -Me), 0.85 (s, 6H), 0.06 (s3H, -Me).

Compound 3b

M.P: 250ºC; Mass (ESI): m/z (227.1183) (M+1); IR (KBR, cm^{-1}): 3760.7, 3404.4, 2920.9, 2855.2, 1634.05, 1588.4, 1494.5, 1452.3, 1414.8, 1312.5, 1297.5, 828.3, 748.53; ^{1}H-NMR (500 MHz, $CHCl_3$): δ 7.64-7.63 (d, CN), 7.25-7.21(m, Ar-H), 4.53-4.51 (m,OH), 2.38 (s, 1H), 1.41-1.12 (bunch, 24H), 1.02 (s, 3H, Me), 1.01 (s, 3H, Me), 0.97 (s, 3H, Me), 0.87 (s, 6H).

Compound 3c

M.P: 290ºC; IR (KBR, cm^{-1}): 3346.7, 3231.6, 2688.1, 1656.6, 1601.1, 1493.4, 1446.5, 1371.5, 1301.3, 1254.4, 1034.2, 748.4, 687.48; ^{1}H-NMR (500 MHz, $CHCl_3$): δ 7.8-7.13 (m, N-H), 4.53-4.51 (m, OH), 2.38 (s, 1H), 1.41-1.12 (bunch, 24H), 1.02 (s, 3H, Me), 1.01 (s, 3H, Me), 0.97 (s, 3H, Me), 0.87 (s, 6H).

Compound 4

M.P: 180ºC; Mass (ESI): m/z (123.0908) (M+1); IR (KBR, cm^{-1}): 2979.9, 2922.4, 2856.8, 1601.1, 1516.8, 1441.8, 1376.5, 1221.6, 1185, 1062.3, 987.34, 940.48, 804.8, 748.4, 659.37; ^{1}H-NMR (500 MHz, $CHCl_3$): δ 8.37-8.35 (m, C-H), 6.78-6.75 (d, C-H), 3.48 (s, C-H, cyclohexane), 1.68 (s, C-H), 0.06 (s, 3H, Me).

Protocol for Screening of compounds for Antimycobacterial Activity

Process Summary:

Stock solution: 10 mM or 10 mg/ml., compound in DMSO.

MIC: IC90 determination for all submitted compounds (using 3.125 µM, 6.25 µM, 12.5 µm, 25.0 µm and 50.0µM conc.) against Mtb H37Rv.

Materials Required: Materials required for MABA assay of the samples are given in the table 1.

Sr No.	Reagent Name (Include all chemicals, enzymes, growth media)
1	Middle brook 7H9 broth (0.05% tween + 0.5% glycerol + 1% casitone + ADC) – incubated for a minimum of 2 days following addition of ADC.
2	ADC
3	DMSO
4	Tween- 80 (10% solution made in MQW)
5	Resazurin (0.02% made in MQW)
6	Milli Q Water
7	7H11 agar plates for CFU plating

Methodology: MABA (Microplate Blue Assay)

Test compounds were suspended in 10 % (v/v) DMSO. Two fold serial dilutions of compounds were made in Middlebrook 7H9 medium supplemented with 10% (v/v) ADC, in 96-well plates (Nunc) in duplicate. An inoculum of 105 CFU/ml was prepared and 200 µL was added per well. Growth controls containing no drug and a sterile control without bacteria were also prepared for each assay.In positive control Rifampicin was added in 2µg/ml was added in the medium containing bacterial culture. The plates were incubated at 37ºC for 5 days before adding 20 µL of sterile 0.01% resazurin to the all wells and incubating for a further 24 h at 37ºC. A change in color from blue (oxidized state) to pink (reduced state) indicated growth of the bacteria. After 24 h, the MIC was determined as the lowest drug concentration that prevented growth and, therefore the occurrence of color change was observed and fluorescence was measured in a microplate fluorometer in bottom-reading mode with excitation at 530 nm and emission at 590 nm.

RESULTS

Hybrid lupeol derivative were evaluated in vitro against Rifampicin sensitive Mtb H37Rv strain by MABA method. Rifampicin was used as standard reference drug. MIC of all the hybrid lupeol derivatives tested is given in table-1.

Table- 1 Anti- tubercular activity of lupeol derivatives

Sr. no.	Compound no.	Antitubercular activity
1	**1**	64 (µg/ ml)
2	2b	>50 (µg/ ml)
3	2c	>50 (µg/ ml)
4	3b	8.50(µg/ ml)
5	3c	10 (µg/ ml)
6	4	>25.0 (µg/ ml)

CONCLUSION

Among the lupeol derivatives tested few have shown MIC at 8.50 µg/ml, 10 µg/ml, while other showed >25 µg/ml. The most active compound 3b, 3c. This clearly demonstrate that pyrazoline moiety in lupeol increases the activity. It is thus concluded that lupeol skeleton deserve further investigation for the development of more potent and nontoxic new agents for therapeutic use. Further optimization is needed to have a compound of clinical trial.

Acknowledgement

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