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Research Article

Novel class of semi synthetic natural products derived from lupeol as anti tubercular agents

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Abstract

Novel lupeol pyrazoline and pyrimidines derivatives were prepared. Triterpene lupeol isolated from plant *Euphorbia tirucalli* was chemically modified and derivatives 4a, 4b and 5 were evaluated anti tubercular activity invitro.

Keywords: Lupeol, *Euphorbia tirucalli*, Anti tubercular

INTRODUCTION

Natural products remain a prolific source for the discovery of new drugs and drug leads even from vedic period (Harvey, 2008). Many human illnesses are caused by infections with microbes like viruses or bacteria or fungi (Mojahidul et al., 2008). Tuberculosis is endemic in India and its prevalence is reported to be increasing in patients with human immunedeficiency virus (HIV) infection (Hira and Dupont, 1998). Tuberculosis (TB) is a chronic necrotizing bacterial infection with wide variety of manifestations caused by Mycobacterium tuberculosis, which has been a scourge of humanity for thousands of years and remains one of the prevalent health tribulations in the world. Tuberculosis (TB) is contagious and airborne disease. It is a disease of poverty affecting mostly young adults in their most productive years. 95% of TB deaths are in the developing world. TB is among the three greatest causes of death among women aged 15-44, 320,000 women died from TB in 2010. The WHO estimated that 17% of the 9.2 million new cases of active TB had some form of drug-resistant TB (DR-TB); of these, 3.1% or 440000 individuals had multidrug- resistant (MDR- TB) (Mulla and Khan, 2011). Tuberculosis (TB) is one of the oldest and most pervasive diseases in history (Okunade et al., 2004; Yves, 2007). As much as one-third of the world population is currently infected and more than five thousand (5000) people die from TB everyday (Imramovsky et al.,2007). 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 (Corbett et al., 2003).

Mycobacterium tuberculosis, causing tuberculosis is a single infectious agent killing roughly two million people annually throughout the world as per WHO report about one third of the world population is infected with this bacterium (Tripathi and Tiwari, 2005)

Current TB chemotherapy is based on the combination of four anti-TB drugs which inhibit the bacterial metabolism, particularly the cell wall synthesis (Casenghi, 2012). During the therapy, the goal of this drug combination strategy is to prevent effectively the mutational events (Cantón and Ruiz-garbajosa, 2011). According to their action mode, first and second line anti-TB drugs are grouped into cell wall inhibitors (INH, EMB, ethionamide (ETH), and cycloserine (DCS)), protein synthesis inhibitors (RIF, fluoroquinolones, STR, kanamycin (KAN)), and membrane energy metabolism inhibitors (PZA). Current chemotherapy principally inhibits cell processes such as cell wall biosynthesis and DNA replication, and they only turn to be active regarding bacteria in active growth (Cantón and Ruiz-garbajosa, 2012).

RIF and PZA have a partial sterilizing activity and they play an important role in the decrease of therapy from 18 to 6 months, even though there is a persistent population surviving these two agents. Consequently the current therapy ensures a clinical cure but fails to obtain a bacteriological cure (Casenghi, 2012).

Need for new drug regime

There are several major problems associated with currently available treatment of TB they are:

- The duration and complexity often resulting in non adherence and leading to emergence of resistance and continuous spread of the disease.
- Adverse events in response to anti tubercular drugs.
- Some drugs for drug-resistant TB are not available everywhere and are less effective, more toxic, and have longer use.
- Co-infection of TB and HIV, where their combined treatment involves a high pill count with associated adherence problems, overlapping toxicity profiles, drug interaction and risk of immune reconstitution syndrome (Orme, 2000). Search for new drugs for treatment of Tuberculosis. Antimicrobial agents and chemotherapy. 2000; 45: pp. 1943-1946.)

MATERIALS AND METHOD

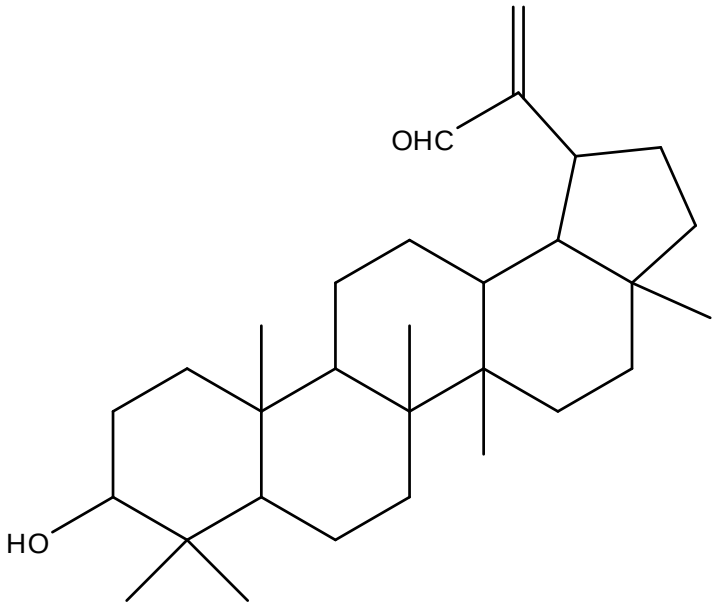
Isolation of lupeol (1) Lupeol (1), isolated from the whole plant of Euphorbia tirucalli with chloroform through maceration for seven to ten days. The extract was concentrated by evaporation under reduced pressure at 40ºC using rotavapour. Further amount of lupeol was isolated by column chromatography of mother liquor which on repeated crystallization gave pure lupeol.

Chemical modifications

In this article, we describe the work on the isopropenyl Side chain. Functionalisation of Lupeol (1) has been carried out by its allylic oxidation by SeO2 to lupeol-aldehyde (2). Aldehyde (2) was selected for hetrocyclic rings formation due to bifunctional reactive centre in the molecule. For this phenyl keto substituted derivative of lupeol (3) was synthesized by the action of o-hydroxy acetophenone with ethanol and thionyl chloride. The compound (3) was converted to pyrazoline analogues. This was done by reaction of compound (3) with hydrazine derivatives in the solvent ethanol. These reactions led to the formation of compounds (4a) and (4b). Formation of compounds (4a) and (4b) were confirmed by melting point, I.R. spectroscopy, NMR spectroscopy and MASS spectroscopy. Lupeol aldehyde (2) was reacted with 4-dimethylaminopyridine in presence of triethyl amine and tetra hydrofuran to obtain pyridine derivative of lupeol (5).

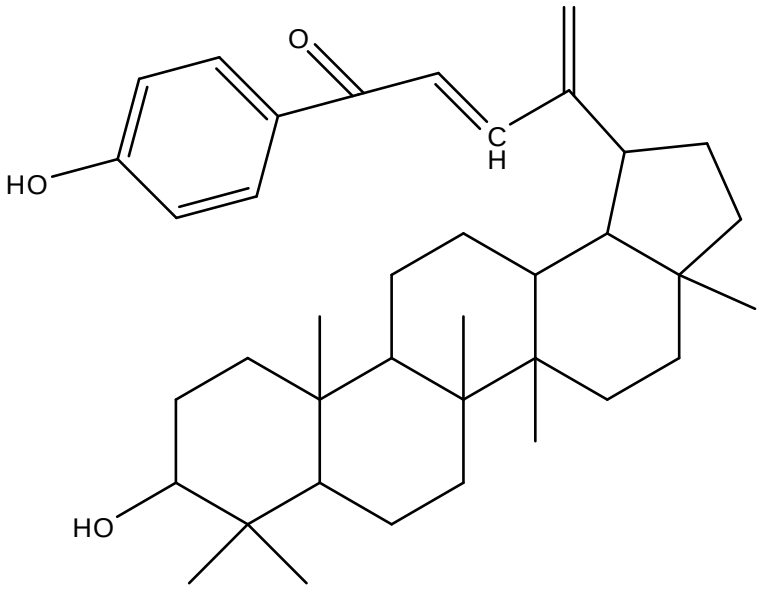
Experimental

Lupeol aldehyde (2) Lupeol (2 gm) was refluxed with selenium dioxide in dioxane with 10-12 drops of distilled water, for 24 - 48 hr. Completion of reaction was detected on TLC plates. When all the lupeol was consumed, reaction mixture was passed through column of silica. Then it is treated with 2.5% aq. KOH, and was extracted with chloroform. Organic layer was washed with distilled water till it become neutral, dried over sodium sulphate, and was evaporated in vacuum. Then the reaction mixture was chromatographed over silica gel column, packed in hexane and was eluted with 5, 10, 20% ethyl acetate/ hexane to obtain lupeol aldehyde (2) in yield 50%.



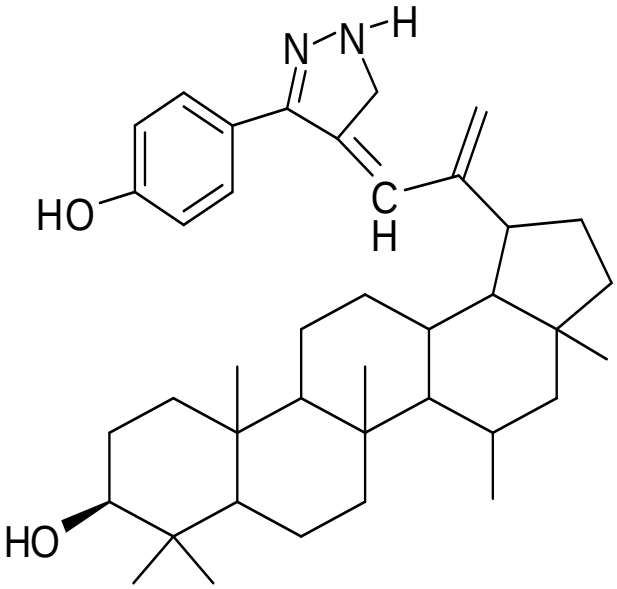
IUPAC:2-(9-hydroxy-3a,5a,5b,8,8,11a-hexamethylicosahydro1Hcyclopenta[a]chrysene-1-yl) acrylaldehyde M.P: 225ºC; Mass (ESI): m/z 441 (M+1); IR (KBR, cm^{-1}): 3326, 2945.9, 2855, 2325, 1743.1, 1641.98, 1454.4, 1380.51, 1261.34, 1109, 1015.34, 948.1, 757.19; ^{1}H-NMR (500 MHz, $CHCl_3$): δ 9.48 (s, 1H-CHO), 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).

Place lupeol aldehyde (0.01 Mole) and O- hydroxy acetophenone (0.01 Mole) in 5 ml absolute ethanol and 0.05 ml of thionyl chloride in conical flask provided with mechanical stirrer. Stirring was continued for 2 hrs at room temperature. After complete stirring and kept the solution for 12 hrs at room temperature. Precipitation was done by cold water. Lastly filtered the reaction mixture using whattman filter paper and washed with cold water to obtain the desired product.



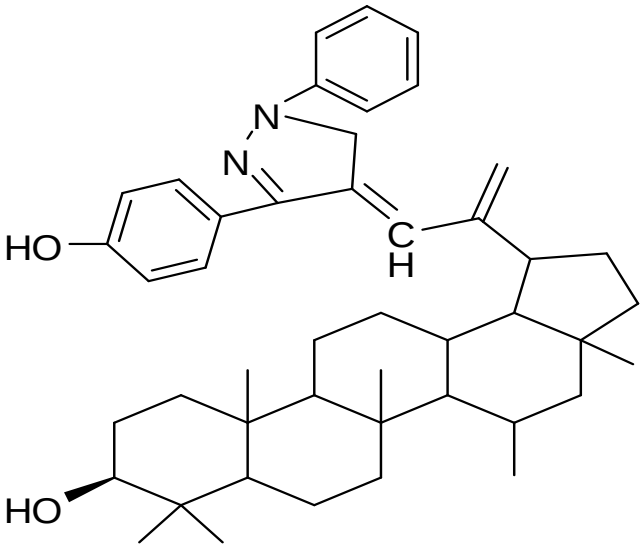
IUPAC:(E)-4-(9-hydroxy-3a,5a,5b,8,8,11a-hexamethylicosahydro-1H-cyclopenta[a]chrysen-1-yl)-1-(4-hydroxyphenyl)penta-2,4-dien-1-one

Place a mixture of (3) in round bottom flask with ethanol and then 6-8 drops of Hydrazine hydrate was added drop wise in the reaction mixture. The reaction mixture was heated under reflux for 24 hrs at 25ºC. 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 (4a).



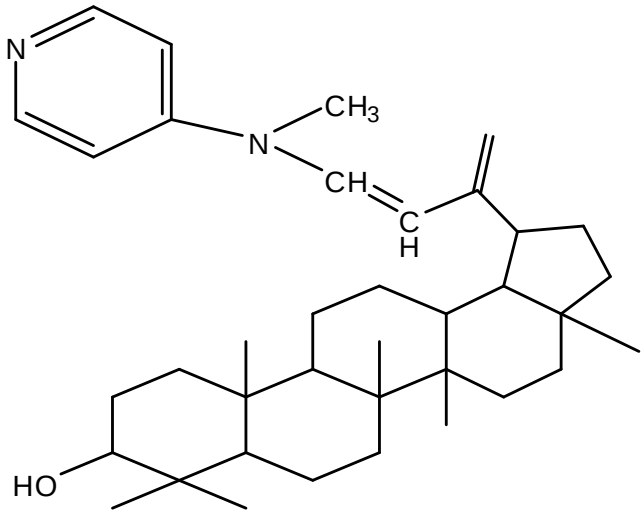
IUPAC:(9S)-1-((E)-3-(3-(4-hydroxyphenyl)-1H-pyrazol-4(5H)-ylidene)prop-1-en-2-yl)-3a,5,5b,8,8,11a-hexamethylicosahydro-1H-cyclopenta[a]chrysene-9-ol. M.P: 250ºC; Mass (ESI): m/z (227.1183) (M+1); IR (KBR, cm^{-1}): 3760.7, 3404.4, 2920.9, 2855.2, 1634.05, 1588.4, 1494.5, 1452.3, 1414.8, 1312.5, 1297.5, 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).

Place a mixture of (3) in round bottom flask with ethanol and then 6-8 drops of Phenyl hydrazine was added drop wise in the reaction mixture. The reaction mixture was heated under reflux for 24 hrs at 25ºC. 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 (4b).



IUPAC:(9S)-1-((E)-3-(3-(4-hydroxyphenyl)-1-phenyl-1H-pyrazol-4(5H)-ylidene)prop-1-en-2-yl)-3a,5,5b,8,8,11a-hexamethylicosahydro-1H-cyclopenta[a]chrysene-9-ol. 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).

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.



IUPAC:3a,5a,5b,8,8,11a-hexamethyl-1-(4-(methyl(pyridine-4-yl)amino)buta-1,3-dien-2-yl)icosahydro-1H-cyclopenta[a]chrysene-9-ol
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).

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.	Anti tubercular activity
1	4a	8.50 (µg/ ml)
2	4b	10.0 (µg/ ml)
3	5	>25.0 (µg/ ml)

DISCUSSION

Tuberculosis has been a major health problem for developing countries including India. Due to increase in MDR and XDR strains of *M. tuberculosis,* there is an urgent need of finding newer anti-mycobacterial agents to combat this problem.

Natural products and/or their semi-synthetic derivatives can lead to novel anti mycobacterial drugs and may have important role in the chemotherapy of tuberculosis. The literature reports the antimycobacterial activity of many classes of natural products such as alkanes, phenolics, acetogenic quinones, flavonoids, and triterpenes.

Detailed analysis of results (Table 1) showed that formation of lupeol derivatives show antitubercular activity.

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 4a, 4b. 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.

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