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Isolation and modification of pseudohybrid plant (Lupeol)

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Abstract
To start with, a brief introduction has been presented on Malaria as a serious endemic disease and the need to develop new structural classes of antimalarial agents with novel and different mechanism of action. Stress has been given to the fast emerging multidrug resistance of P.falciparum to the commonly used drug chloroquine. *Crateva nurvala* (Capparidaceae) is a plant from which Lupeol has been isolated and its reported antimalarial activity prompted to prepare semisynthetic derivatives for development of new antimalarial agent for better activity. In the treatment of malaria, natural produts are unending source of new lead and their role is not new.
The structure of Lupeol is reminiscent of that of cholesterol, and the compound is expected to be able to enter the cellular membranes. Due to the presence of a single hydroxy group and a large, apolar skeleton, and Lupeol acts as an amphiphile. According to the bilayer hypothesis Stomatocytes are generally formed when a lipophilic compound is incorporated into and expands the inner layer of the lipid membrane .Such changes appear to be more prohibiting with respect to parasite growth than incorporation of an amphiphile into the outer layer, as in case of echinocytogenic compounds. The two possible sites for chemical modification present in Lupeol are ring-A, and isopropenyl side chain. Modification on ring-A, ring-A expansion and cleavage followed by introduction of antimalarial pharmacophores and modification at isopropenyl side chain is targeted as represented in **1 a**
Keywords : Antiurolithiatic, Isopropenyl , PCC, Triterpene , Quinoline

Introduction

Crataeva nurvala, three leaved Caper commonly known as Steaved Tree in English and Varuna in Sanskrit, a moderate sized deciduous tree[1-2] . Whole of the plants are used for medicinal purpose [3-5] . Mature bark is generally used for extraction which is 6-15 cm long, 3-10 cm wide with thickness varying from 5-12 mm. The water extract also contains tertiary and quarternery bases including choline. The leaves yield flavonoids including rutin, quercetin and isoquercetin. Long chain Lupeol fatty ester showed inhibitory activity against drug resistant strains of *P. falciparum at a dose of* 1.02-18.53 µg/ml. Lupeol exhibits inhibitory activity on *P. falciparum* growth in vitro but lacks in vivo activity in mice infected with *P. berghei* Lupeol causes membrane shape changes of erythrocytes toward stomatocytic forms observable at concentrations below its IC_{50} (11.8 µg/ml or 27.7 µM). The Lupeol can also be used for fungal infection in humans. It may be administered intravenously or intraperitoneally by infusion or injection. Lupeol derivatives like Lupenone, Lupeol acetate have also been found to show antifungal activity. Lupenone were evaluated for their antiviral activities against Herpes Simplex Virus and African Swine Fever Virus. Lupeol (50mg/kg) and Lupeol Acetate (50 mg/kg) were investigated for there anti- inflammatory activity in comparison with the commonly used non-steroidal anti-inflammatory drug, Indomethacin (3 mg/kg) in rats [6-8] . The presence of anti-inflammatory activity in Lupeol & Lupeol Acetate seems interesting, since they possess hydro-aromatic ring systems more or less similar to that of steroid and devoid of side-effects. Lupeol linoleate anIndomethacin showed a reduction in paw swelling by 39, 58 and 35%, respectively, in adjuvant arthritis [9-14]. Lupeol and its linoleate ester derivative, in ameliorating the lipidemic–oxidative abnormalities in the early stage of hypercholesterolemic atherosclerosis. Antiurolithiatic activity of Lupeol was assessed in rats by observing the weight of the stone, biochemical analysis of serum and urine, and histopathology of bladder and kidney. it is not only prevented the formation of vesical calculi but also reduced the size of the preformed stones. Lupeol acetate decreased the incidence of gastric ulceration induced by pyloric ligation .Thus it appear that this novel abundantly available 6-6-6-6-5-pentacyclic triterpene

having a wide range of biological activities, can be used as template for a particular activity by grafting / crafting different pharmacophores, which can be optimized by chemical modification. i.e. one can generate pseudo hybrid natural products as novel bioactive compounds.

Material and method

Isolation of Lupeol 1

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.

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 spectum .In 1H-NMR spectrum a pair of two singlets at δ 4.68 and 4.56 along with a singlet for 3 protons at δ1.68 is indicating for the presence of isoprenyl side chain present in the molecule. Six singlets for 3 protons each and a muliplet for one proton at δ 3.23 is as according to the six methyl groups and H-3 proton connected with OH, present in Lupeol .The IR spectrum showed hydroxyl group (3302.6 cm-1), Vinyldiene group 3068.1,1636.8,880 cm-1) respectively.

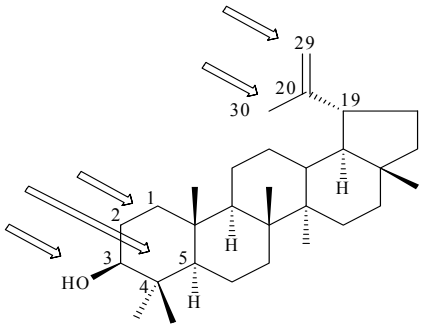


Figure 1a

Chemical Modifications

Chemical modification at C-30

Allylic oxidation of Lupeol with SeO_2 in moist dioxane under refluxing condition gave the aldehyde **2** .

Scheme 1

Formation of **2** generated an additional functional group (Aldehyde) in Lupeol which can be used to build different heterocycles on this bi-functional three carbon unit.In NMR spectrum,a new proton at 9.5 appeared along with olefinic proton.

Chemical modification at bi-functional three carbon unit (C 20-29 double bond and C 30 aldehyde):

In order to diversify the Lupeol at this end, it was started with the synthesis of 3-substituted quinoline by using Skraup synthesis. Lupeol aldehyde **2** on reaction with substituted aniline in freshly distilled toluene, in the presence of Conc. H_2SO_4 as a catalyst. Conc sulphuric acid also acts as a dehydrogenating agent . Shiff base formed as a intermediate in this reaction which furthur undergoes cyclisation.

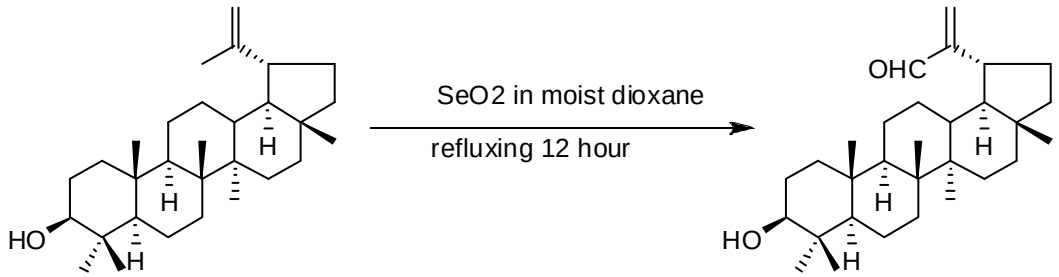
Scheme 3

The above mentioned subsituted quinolines crafted on Lupeol nucleus side chain can be converted to keto analogoues which can be visualized as products derived from lupenone , a another natural product.This was done by oxidation with PCC (Pyridinium chloro chromate).

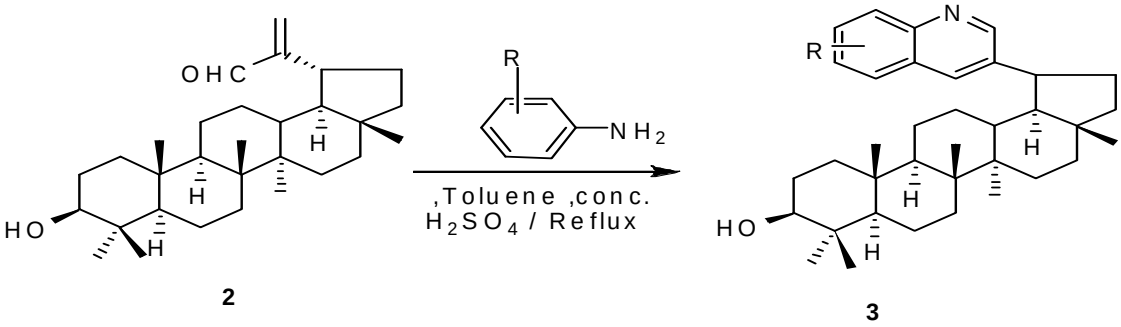
Scheme 4

Lupenone hybrid compound **4a** on treatment with hydroxylamine hydrochloride in pyridine gave its oxime **5a (Scheme 4)** in a very good yield .It displayed $(M+H)^+$ peak at 545, in its FAB mass spectrum. Compound **5a** was subjected to reduction with LAH in THF to give corresponding amine **6a** in good yield. Formation of **6a** was confirmed by decrease of 14 units in its molecular weight. Molecular ion at. m/e at 531 (M+1) in FAB Mass spectrum was observed. In IR spectrum a peak at 1594 was disappeared (C=N-OH) and which confirmed the structure of **6**.

Scheme 1

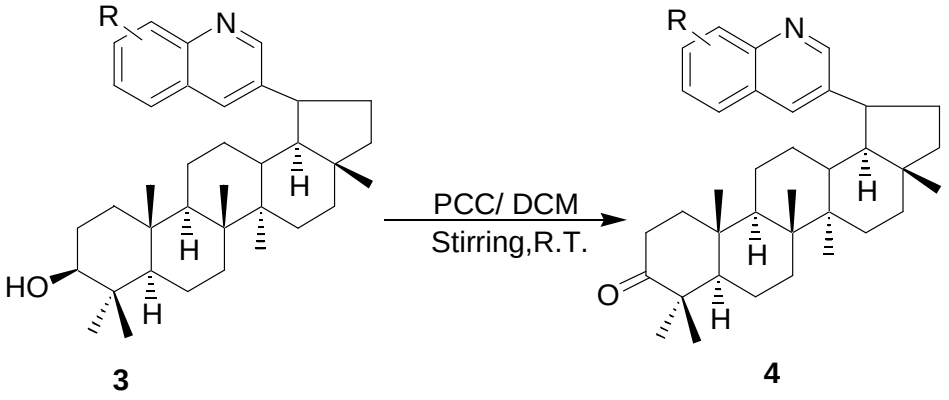


Scheme 2



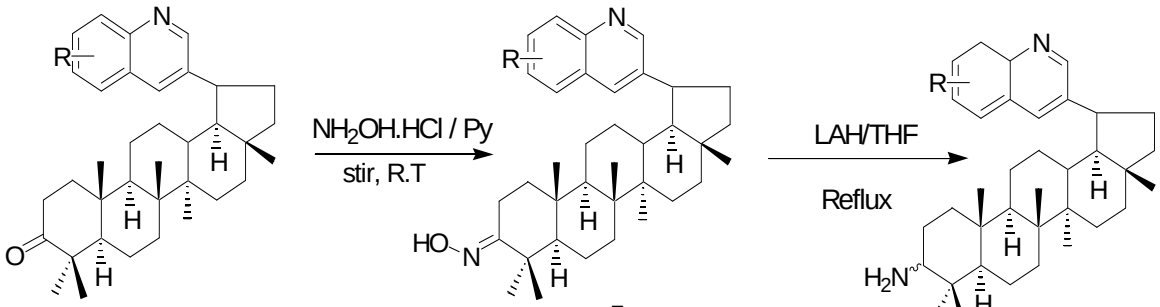
3a R= F, 3b R= 2,3dimethyl,
3c R= 4 CF_3, 3d R= 3 CF_3,
3e R= 2 OCH_3

Scheme 3



4a R= F, 4b R= 2,3dimethyl,
4c R= 4 CF_3, 4d R= 3 CF_3,
4e R= 2 OCH_3

Scheme4



4a **R=F** **5a** **R=F** **6 a** **R=F**

Experimental

Isolation of Lupeol 1a

Stem bark of Crataeva nurvala (80 kg) was grinded and extracted with commercial alcohol (95% Ethanol). Crude Lupeol from concentrated ethanol extract was filtered out and crystallized repeatedly (chloroform/MeOH to give white solid. (201 gm). 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 (820 mg), while elution with 7-10% ethyl acetate in hexane yielded Lupeol (12 gm).

mp: 212-214ºC, **Mass (FAB):** m/z 427 (M+1) **^{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 2a

Lupeol (1 gm, 2.35 mmol) was refluxed with selenium dioxide in dioxane with 3-4 drops of distilled water, for 8 hr. After consumption of all of Lupeol, reaction mixture was passed through ciliate, treated with 2.5% aq. KOH, and was extracted with chloroform. Organic layer was washed with distilled water till it became neutral, dried over sodium sulphate, and was evaporated in vacuum. this reaction mixture was chromatographed over silica gel column, packed in Hexane and was eluted with hexane, 5, 10, and 20 % ethyl acetate /hexane Elution with 20 % ethyl acetate /hexane gave the required product **2** in yield (50%).

mp : 222-225ºC, **Mass (FAB):** m/z 441 (M+1) **^{1}H-NMR (300 MHz, $CDCl_3$):** δ9.48 (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)

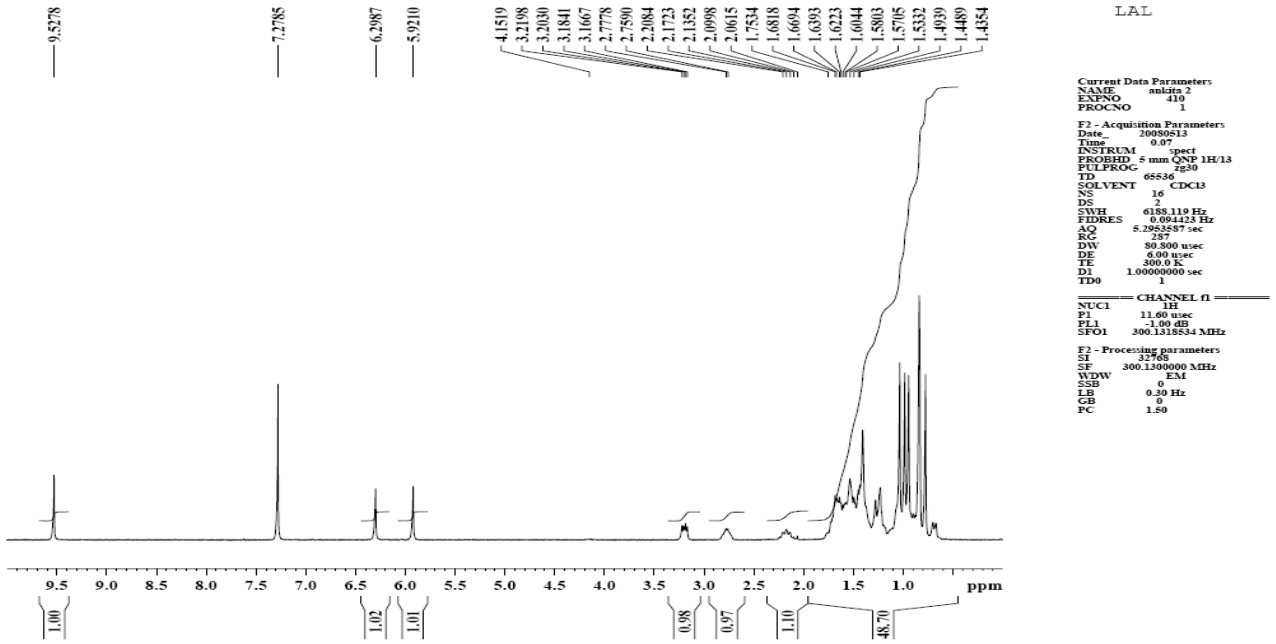
8-fluoro-3-lupenyl quinoline 3a

Lupeol aldehyde **2** (1 g, 2.27mmol) was refluxed with 2 fluoro aniline (.378ml, 3.41 mmole, 1.5equvi) gave crude product, which was chromatographed over silica gel, Column was packed in hexane and was eluted with 5, 10, 15% , ethyl acetate /hexane. Elution with 15 % of ethyl acetate /hexane gave the required white amorphous solid which was dried in vacuum giving pure 3-substituted quinoline. **3a** (450 mg).

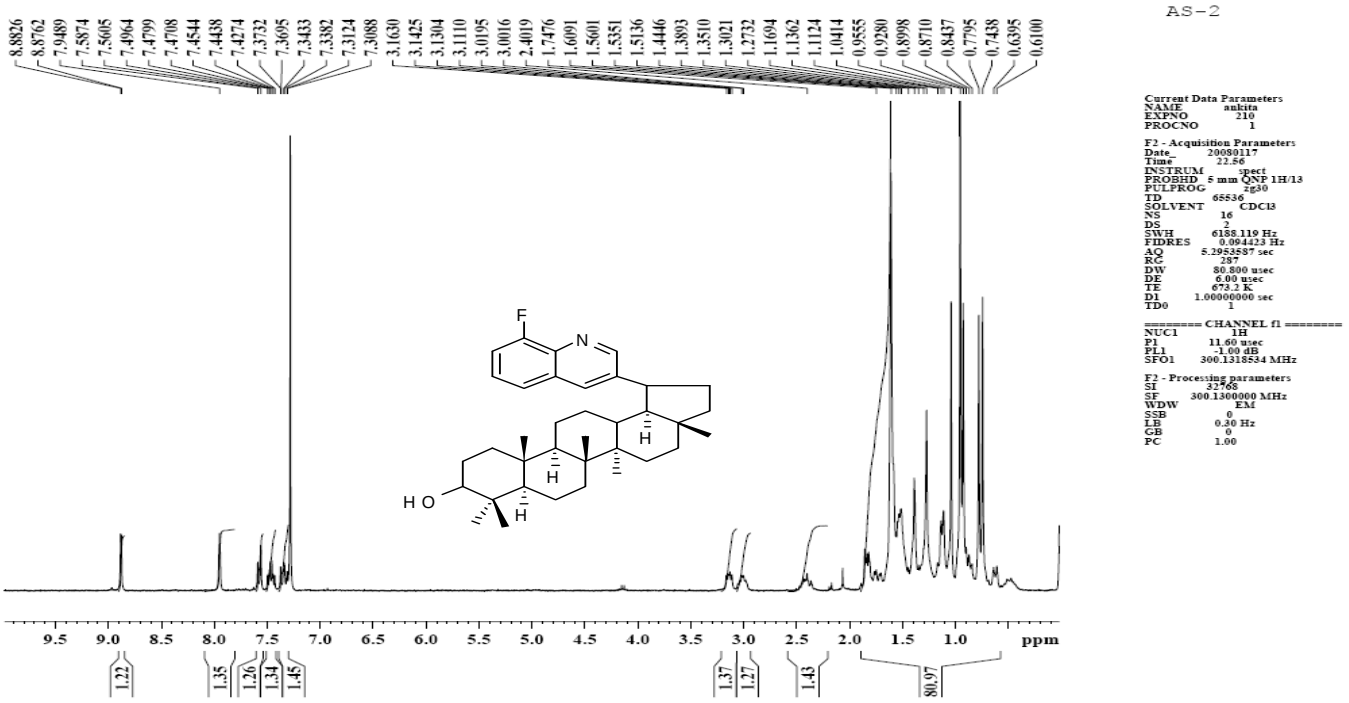
mp: < 240ºC **Mass (ESI):** m/z 532 (M+1) **^{1}H-NMR (200 MHz, $CDCl_3$):** δ8.84 (d, 1H, *J* = 2.1 Hz, Ar-H), 7.81 (d, 1H, *J*=1.8 Hz, Ar-H), 7.60(m, 1H, *J*=7.8 Hz, Ar-H), 7.48 (m, 1H, *J* =6.2 Hz, Ar-H), 7.41 (m, 1H, Ar-H),3.11 (m, 1H, H-3), 2.76 (m, 1H), 2.12 (m, 1H), 1.65-1.27 (bunch, 24 H),1.01 (s, 3H, -Me), 0.96 (s, 3H, -Me), 0.92 (s, 3H, -Me), 0.81 (s, 6H), 0.75 (s, 3H, -Me)

7,8-dimethyl- 3- lupenyl quinoline 3b

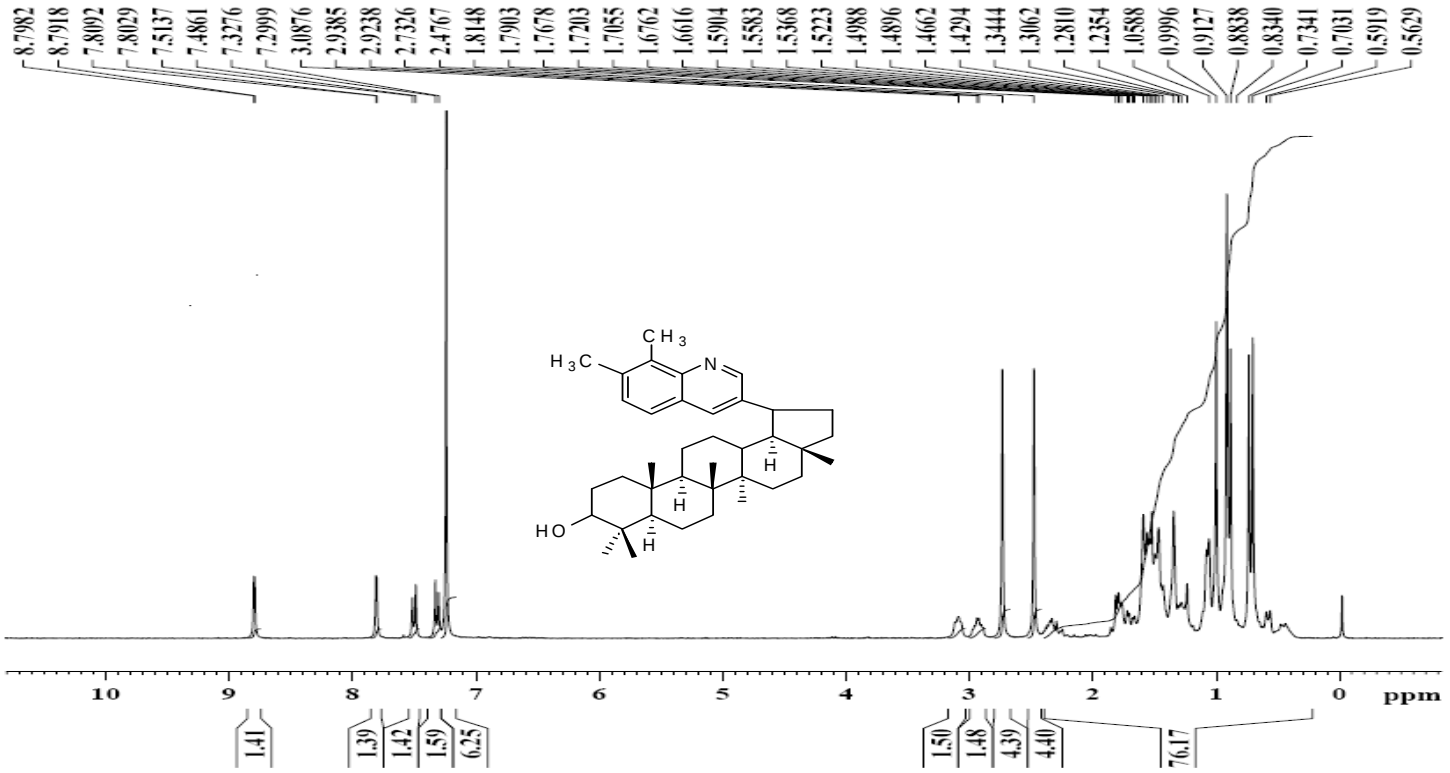
Lupeol aldehyde **2** (1gm, .4545 mmol) was refluxed with 2,3 dimethyl aniline (416mg, 0.6817 mmole,1.5 equvi) as in the above case gave crude product, which was chromatographed over silica gel, Column



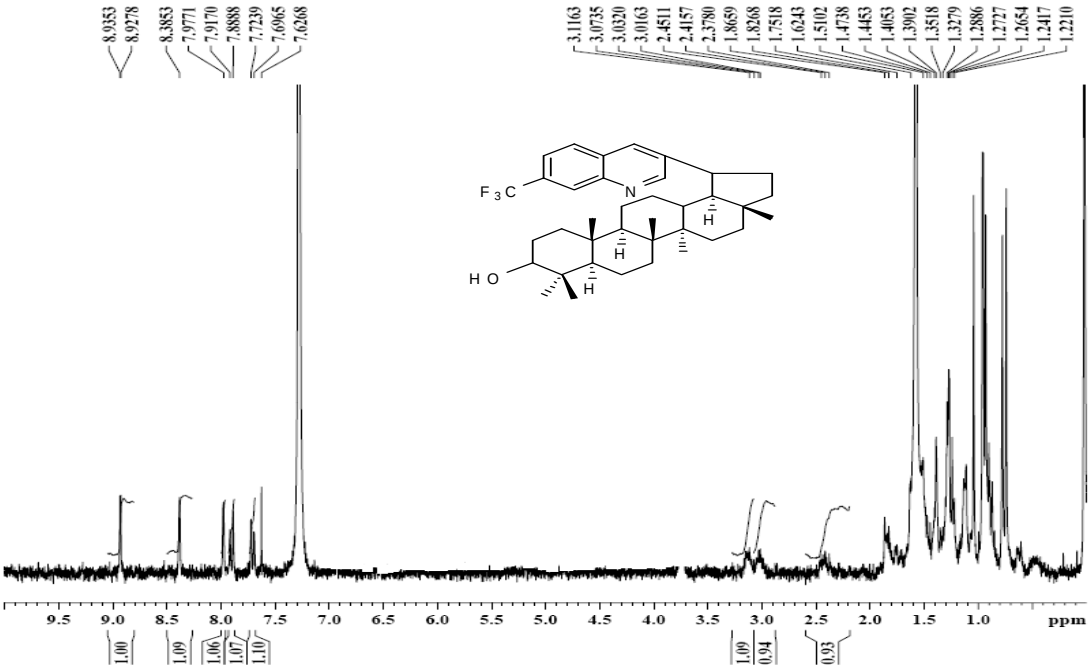
^{1}H NMR (300 MHz ,$CDCl_3$) spectra of Lupeol aldehyde 2a



^{1}H NMR (300 MHz ,$CDCl_3$) spectra of 8-fluoro-3-lupenyl quinoline



^{1}H NMR (300 MHz ,$CDCl_3$) spectra of 6-trifluromethyl-3-lupenyl quinoline



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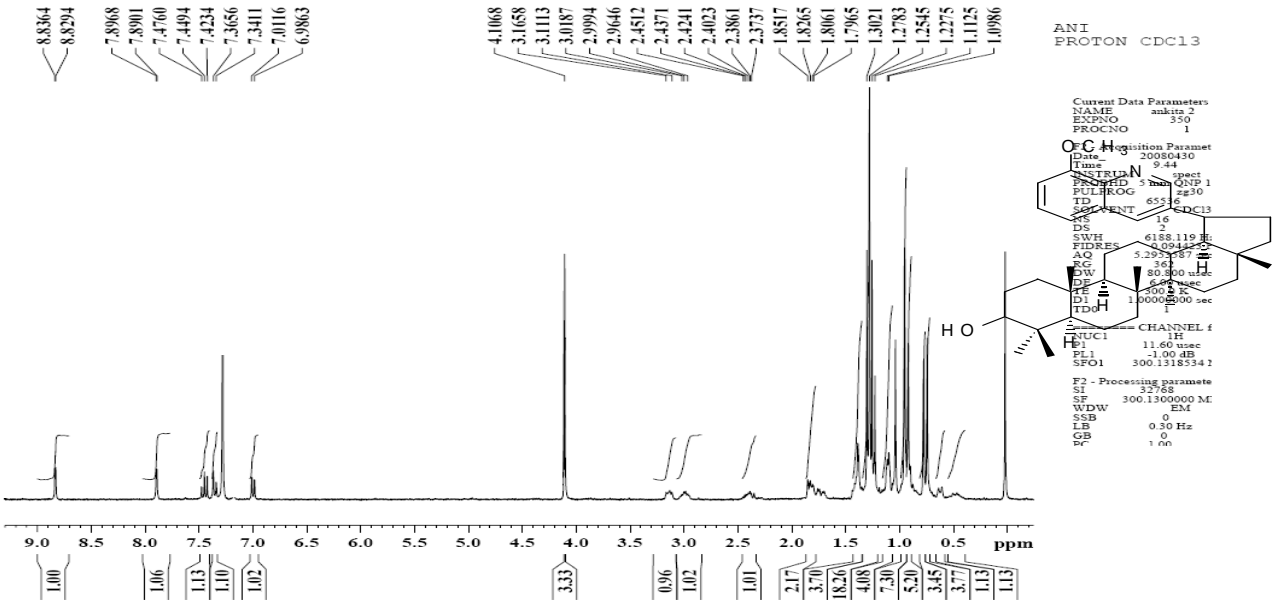
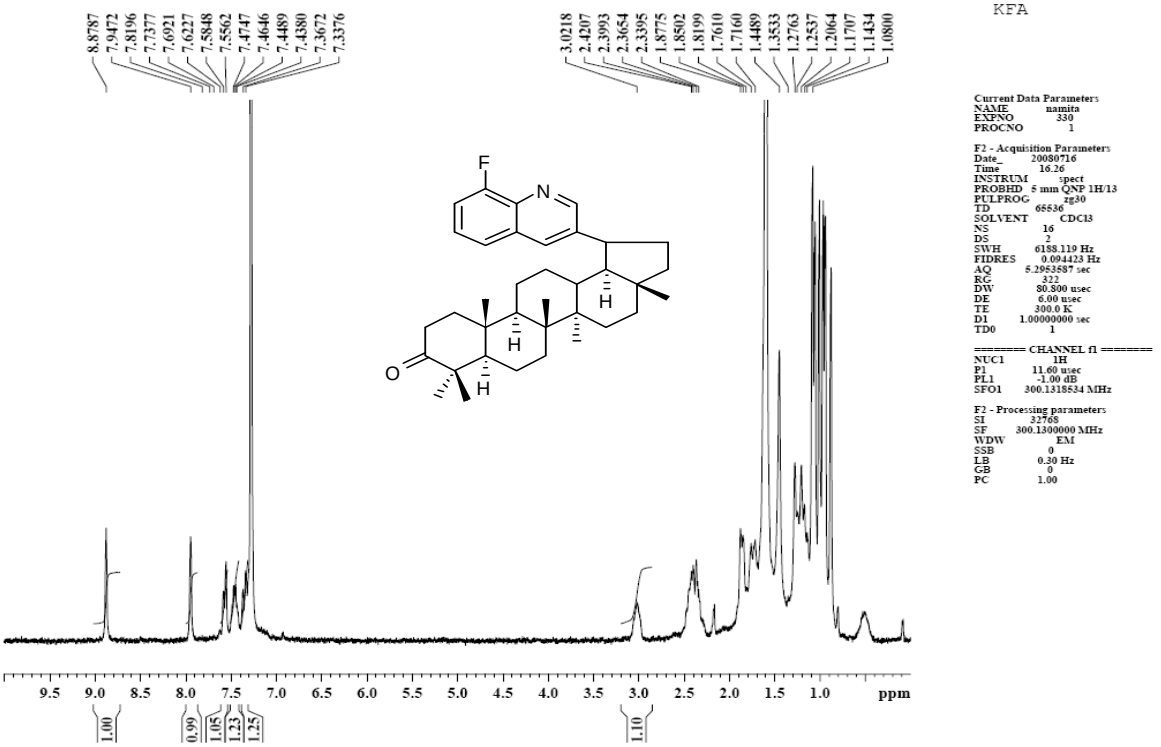
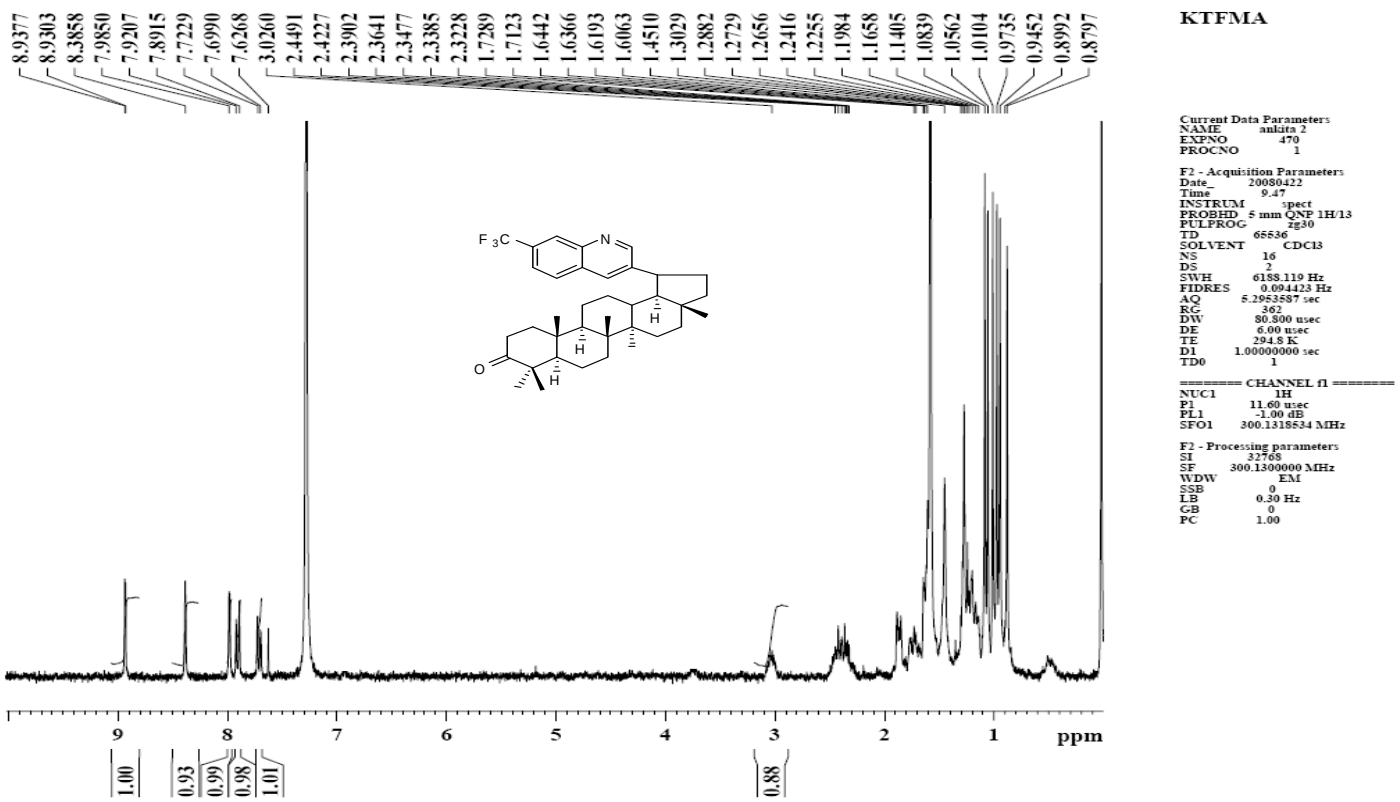


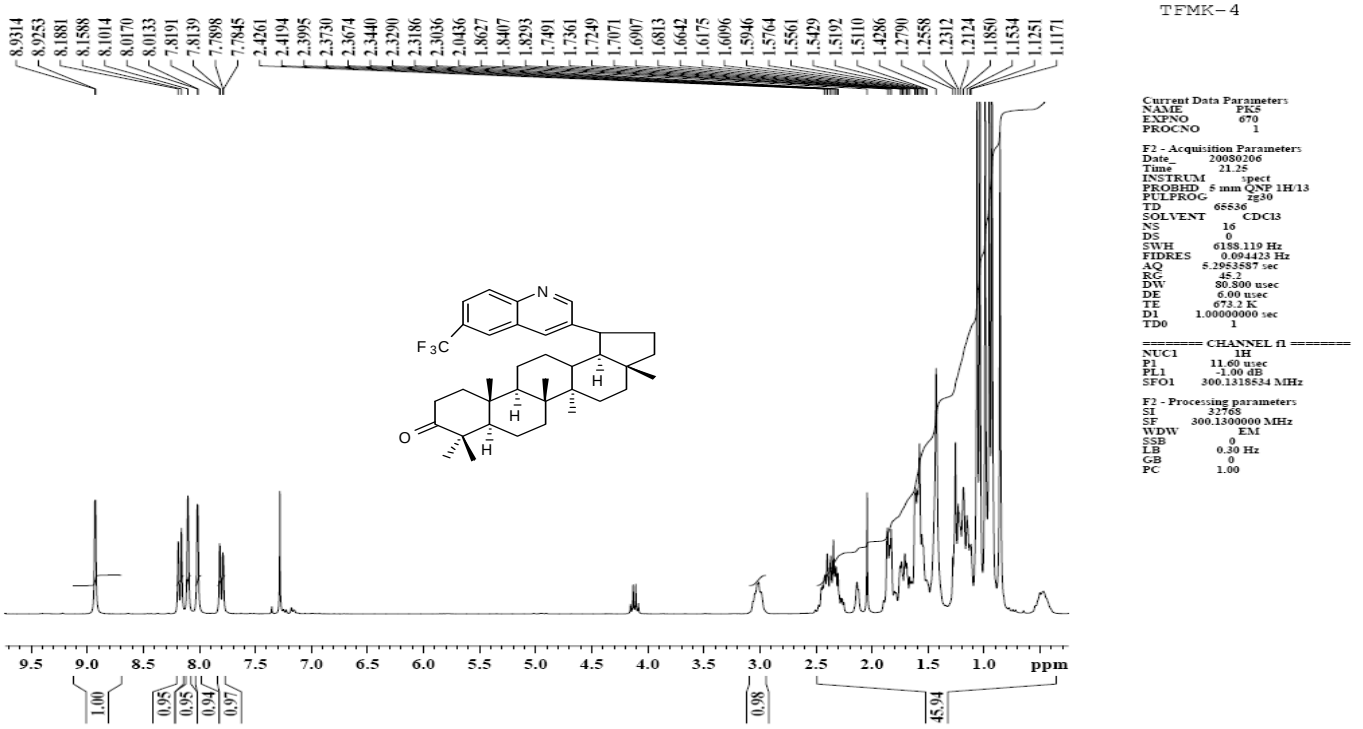
^{1}H NMR (300 MHz ,$CDCl_3$) spectra of 8-methoxy-3-lupenyl quinoline



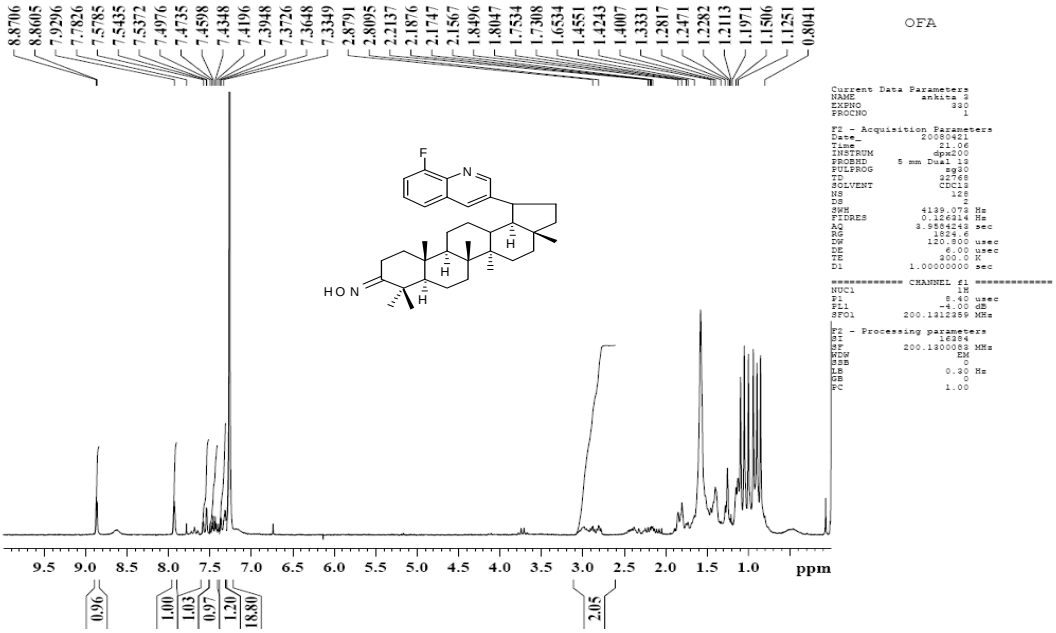
^{1}H NMR (300 MHz ,$CDCl_3$) spectra of 8-fluoro-3-(lupenone) quinoline



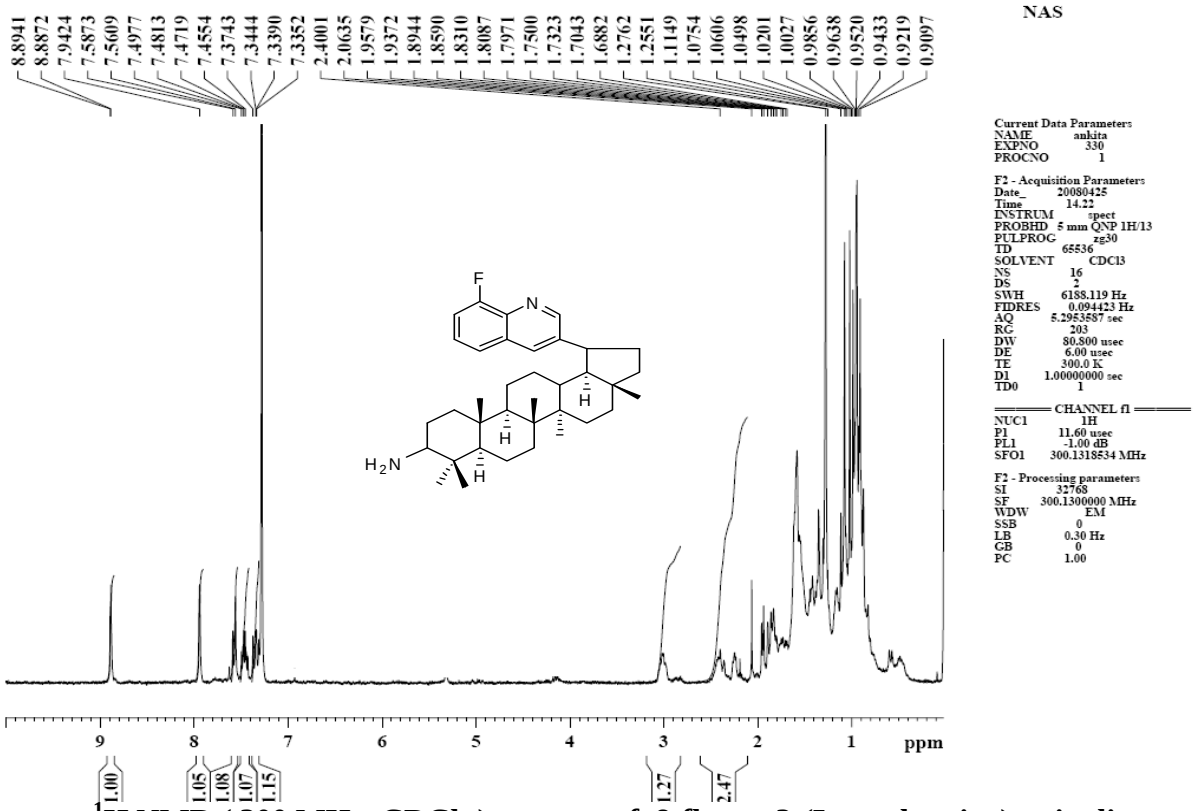
^{1}H NMR (300 MHz ,$CDCl_3$) spectra of 7-trifluromethyl-3-lupenone quinoline



^{1}H NMR (300 MHz ,$CDCl_3$) spectra of 6-trifluromethyl-3-lupenone quinoline



^{1}H NMR (300 MHz ,$CDCl_3$) spectra of 8-fluoro-3-(Lupeol oxime) quinoline



^{1}H NMR (300 MHz ,$CDCl_3$) spectra of 8-fluoro-3-(Lupeol amine) quinoline

Table a:Anti-malarial activity of Lupeol derivatives

MIC: Minimum concentration inhibiting development of ring stage parasites into the schizonts.

Sl no.	Compound No.	Antimalarial activity
	Lupeol	
1	**3a**	10
2	**4a**	10
3	**5a**	10
4	**6a**	>50

was packed in hexane and was eluted by 5%,10%, and 15% ethylacetate /hexane. Elution with 15% of ethylacetate /hexane gave the required white amorphous solid which was dried in vacuum giving pure 3-substituted quinoline **3b** (400 mg)

mp: 270ºC **Mass (ESI):** m/z 542 (M+1), **^{1}H-NMR (300 MHz, CDCl3):** δ 8.82 (d, 1H, *J*=1.9 Hz, Ar-H), 7.83 (d, 2H, *J*=1.8 Hz, Ar-H), 7.51(d, 1H, *J* = 8.2, Ar-H), 7.31 (d, 1H, *J* = 8.2, Ar-H) 3.1 (m, 1H, H-3), 2.9 (m, 1H), 2.7(s, 3H, Ar-CH_3), 2.4 (s, 3H, Ar-CH_3), 2.1(m, 2H, H-2), 1.65-1.27 (bunch, 24 H), 1.01(s, 3H, CH_3), 0.92 (S, 3H, -Me), 0.81 (s, 6H,), 0.75 (s, 3H, -Me).

HRMS: Measured mass: 541.4285

Calculated mass: 541.4284 for C H N O

6-trifluromethyl-3-lupenyl quinoline 3c

Lupeol aldehyde 2 (1 gm) was refluxed with 4-trifluromethylaniline (538.41 mg.,3.41 mmol, 1.5equvi.) dissolved in 100 ml round bottomed flask in 20 ml distilled toluene with catalytic amount of concentrated H_2S0_4 (one drop) for 12 hr. After the completion of reaction (monitored by TLC), toluene was removed by rotavapour and was extracted with chloroform .Organic layer was washed with water dried over sodium sulphate and solvent was evaporated in vacuum giving crude product, which was chromatographed over silica gel, Column was packed in hexane and was eluted by 5,10,15,and 20% ethylacetate /hexane. Elution with 20 % ethylacetate/hexane gave the required white amorphous solid which was dried in vacuum giving pure 4 trifluromethyl substituted quinoline (400mg).

m.p:260ºC **(MassESI):** m/z 582 (M+1) **^{1}H-NMR (300 MHz, $CDCl_3$):** δ8.92 (d, 1H, *J*=1.9 Hz, Ar-H), δ 8.21 (d, 1H, *J*=8.7 Hz, Ar-H), δ 8.1 (d, 1H, *J*=1.6 Hz, Ar-H), δ 8.0 (d, 1 H, *J*=1.9 Hz, Ar-H), δ 7.8 (dd, 1H, J_1=8.8 Hz, J_2 = 1.62 Hz, Ar-H), δ 3.11 (m, 1H, H-3), δ 2.8 (m, 1H,), δ 2.1 (m, 2H, H-2) 1.65-1.27 (bunch, 24 H),1.01 (s, 3H, -Me), 0.96 (s, 3H, -Me), δ 0.92 (s, 3H, -Me), δ 0.81 (s, 6H), δ 0.75 (s, 3H, -Me).

HRMS: Measured mass:581.3798

Calculated mass: 581.3845 for C H N O F

7-trifluromethyl-3-lupenyl quinoline 3d

Lupeol aldehyde 2 (1 gm) was refluxed with 3-trifluromethylaniline (538.41mg.,3.41 mmol,1.5equvi.) dissolved in 50 ml round bottomed flask in 20 ml distilled toluene with catalytic amount of concentrated H_2S0_4 (one drop) for 12 hr. After the completion of reaction (monitored by TLC), toluene removed by rotavapour, and was extracted with chloroform .Organic layer was washed with water dried over sodium sulphate and solvent was evaporated in vacuum giving crude product, which was chromatographed over silica gel, Column was packed in hexane and was eluted by 5,10,15, 20 % chloroform /hexane. Elution with 25 % chloroform /hexane. gave the required white amorphous solid which was dried in vacuum giving pure 4 trifluromethyl substituted quinoline (400mg).

m.p:200ºC **Mass (ESI):**m/z 582 (M+1),**^{1}H-NMR (300 MHz, $CDCl_3$):** δ8.9(d, 1H, J=1.98 Hz, Ar-H), δ 8.2(d, 1H, J=8.76 Hz, Ar-H), δ 8.1(d, 1H, J=1.65 Hz Ar-H), δ 8.0(d, 1H, J=1.94 Hz, Ar-H), δ 7.8 (dd, 1H,J=8.76Hz, Ar-H J=1.62Hz,Ar-H), δ 3.11 (m, 1H, H3), δ 2.8(M, 1H,), δ 2.1 (m, 2H, H-2) 1.65-1.27 (bunch, 24 H),1.01 (s, 3H, -Me), 0.96 (s,

3H, -Me), δ 0.92 (s, 3H, -Me), δ 0.81 (s, 6H), δ 0.75 (s, 3H, -Me).
HRMS: Measured mass:581.3806
Calculated mass: 581.3845 FOR C H N O F

8-methoxy-3-lupenyl quinoline 3e

Lupeol aldehyde **2** (1gm, .4545 mmol) was refluxed with O anisidine (.08ml,0.6817mmole,1.5equvi) dissolved in 100 ml round bottomed flask in 20 ml distilled toluene with catalytic amount of concentrated H_2S0_4 (one drop) for 12 hr. After the completion of reaction (monitored by TLC), toluene removed by rotavapour, and was extracted with chloroform .Organic layer was washed with water dried over sodium sulphate and solvent was evaporated in vacuum giving crude product, which was chromatographed over silica gel, Column was packed in hexane and was eluted by 5,10,15, 20, 25, 30, 40, 50 % ethylacetate /hexane. Elution with 60 % ethylacetate/hexane gave the required white amorphous solid which was dried in vacuum giving pure 3-substituted quinoline **3e** (410 mg).

m.p: 220-22ºC, **Mass (ESI):** m/z 544 (M+1), **^{1}H-NMR (300 MHz, $CDCl_3$):** δ 8.82 (d, 1H, *J* =2.1 Hz, Ar-H), δ 7.91 (d, 1H, *J* =2.2 Hz, Ar-H), δ 7.4 (t , 1H, *J*= 7.9 Hz, Ar-H), δ 7.3 (d, 1H, *J*=8.0 Hz, Ar-H), δ 7.0 (d, 1H, *J*= 7.9 Hz, Ar-H), δ 4.12 (s, 3H, OCH_3), δ 3.11 (m, 1H, H-3), δ 2.8 (m, 1H,), δ 2.1 (m, 2H, H-2) 1.65-1.27 (bunch, 24 H),1.01 (s, 3H, -Me), 0.96 (s, 3H, -Me), δ 0.92 (s, 3H, -Me), δ 0.81 (s, 6H), δ 0.75 (s, 3H, -Me).

8-fluoro-3-(lupenone) quinoline 4a

3-substituted quinoline Lupeol derivative **3a** (200mg, 0.3766 mmol) dissolved in DCM (15 ml) was stirred overnight at room temperature with PCC (121 mg, 0.5649 mmol, 1.5 eqv.). After completion of reaction (TLC), work up as the reaction miture was concentrated upto3/4th .Addition of ether and settled down the reaction mixture.After settling fiter with hyflow& alumina bed then concentrate the reaction mixture gave 180mg of almost pure 3-substituted quinoline lupenone derivative **4a.**

mp: 105-106 ºC , **Mass (ESI):** m/z 530 (M+1), **^{1}H-NMR (200 MHz, $CDCl_3$):** δ8.8 (d, 1H, *J*=2.1 Hz, Ar-H), 7.8 (d, 1H, *J*=1.8 Hz, Ar-H), 7.60(m, 1H, *J*=7.86 Hz Ar-H),7.48 (m, 1H, *J*=6.24 Hz, Ar-H), 7.41 (m, 1H, Ar-H), 2.76 (m, 1H), 2.12 (m, 2H),1.65-1.27 (bunch, 22 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) .

7,8 dimethyl-3-lupenone quinoline 4b

3-substituted quinoline Lupeol derivative **4a** (200mg, 0.3766 mmol) dissolved in DCM (15 ml) was stirred overnight at room temperature with PCC (121 mg, 0.5649 mmol, 1.5 eqv.). After completion of reaction (TLC), work up as in the above case gave 180mg of almost pure 3-substituted quinoline lupenone derivative.

mp: 242ºC , **Mass (ESI):** m/z 540 (M+1), **^{1}H-NMR (200 MHz, CDCl3):** δ8.8 (d, 1H, *J*=1.76 Hz, Ar-H), 7.83 (d, 2H, *J*=1.94 Hz, Ar-H),7.51 (d, 1H, *J*=8.3, Ar-H), 7.4 (d, 1H, *J*=8.32, Ar-H), 2.8 (m, 1H,), 2. 7 (s, 3H, Ar-Me),2.5 (s,3H, Ar-CH3), 2.1 (m, 2H, H-2), 1.65-1.27 (bunch, 24 H), 1.01(s, 3H, CH_3), 0.92 (S, 3H, -Me), 0.81 (s, 6H,), 0.75 (s, 3H, -Me).

6-trifluromethyl-3-lupenone quinoline 4c

4-substituted quinoline Lupeol derivative **3c** (200mg, 0.3766 mmol) dissolved in DCM (15 ml) was stirred overnight at room temperature with PCC (121 mg, 0.5649 mmol, 1.5 eqv.). After completion of reaction (TLC), work up as in the above case gave 180mg of almost pure 3-substituted quinoline lupenone derivative.

m.p: 220ºC **, Mass (ESI):**m/z 580 (M+1) **^{1}H-NMR (300 MHz, $CDCl_3$):** δ 8.9 (d, 1H, *J*=1.98 Hz, Ar-H), δ 8.2 (d, 1H, *J*=8.76 Hz, Ar-H), δ 8.1 (d, 1H, *J*=1.65 Hz, Ar-H), δ 8.0 (d, 1H, *J*=1.94 Hz, Ar-H), δ 7.8 (dd, 1H, J_1=8.76 Hz, J_2=1.62 Hz, Ar-H), δ 2.8 (m, 1H, H-3), , δ 2.1 (m, 2H, H-2) 1.65-1.27 (bunch, 24 H),1.01 (s, 3H, -Me), 0.96 (s, 3H, -Me), δ 0.92 (s, 3H, -Me), δ 0.81 (s, 6H), δ 0.75 (s, 3H, -Me).
HRMS: Measured mass: 579.3689

Calculated mass: 579.3688

7-trifluromethyl-3-lupenone quinoline 4d

3-substituted quinoline Lupeol derivative **3d** (200mg, 0.3766 mmol) dissolved in DCM (15 ml) was stirred overnight at room temperature with PCC (121 mg, 0.5649 mmol, 1.5 eqv.). After completion of reaction (TLC), work up as in the above case gave 170 mg of pure 3-substituted quinoline lupenone derivative.

m.p:110ºC **Mass (ESI):**m/z 582 (M+1)**^{1}H-NMR (300 MHz, $CDCl_3$):** δ8.9(d, 1H, J=1.98 Hz, Ar-H), δ 8.2(d, 1H, J=8.76 Hz, Ar-H), δ 8.1(d, 1H, J=1.65 Hz Ar-H), δ 8.0(d, 1H, J=1.94 Hz, Ar-H), δ 7.8 (dd, 1H,J=8.76Hz, Ar-H J=1.62Hz,Ar-H), δ 2.8 (m, 1H, H3), , δ 2.1 (m, 2H, H-2) 1.65-1.27 (bunch, 24 H),1.01 (s, 3H, -Me), 0.96 (s, 3H, -Me), δ 0.92 (s, 3H, -Me), δ 0.81 (s, 6H), δ 0.75 (s, 3H, -Me).

8 methoxy-3-lupenone quinoline 4e

3-substituted quinoline Lupeol derivative **3e** (1gm) dissolved in DCM (15 ml) was stirred overnight at room temperature with PCC (625 mg, 1.5 eqv.). After completion of reaction (TLC), work up as the reaction miture was concentrated upto3/4th .Addition of ether and settled down the reaction mixture.After settling fiter with hyflow & alumina bed then concentrate the reaction mixture gave 180mg of pure 3-substituted quinoline lupenone derivative **4e.**

m.p:131ºC **Mass (ESI):** m/z 542 (M+1),

^{1}H-NMR (300 MHz, $CDCl_3$): δ 8.82 (d, 1H, *J* =2.0 Hz, Ar-H), δ 7.91 (d, 1H, *J* =2.1 Hz, Ar-H), δ 7.4 (t , 1H, *J*= 8.0 Hz, Ar-H), δ 7.3 (d, 1H, *J*=8.1 Hz, Ar-H), δ 7.0 (d, 1H, *J*= 7.9 Hz, Ar-H), δ 4.12 (s, 3H, OCH_3), δ 2.8 (m, 1H,), δ 2.1 (m, 2H,H-2) 1.65-1.27 (bunch, 24 H),1.01 (s, 3H, -Me), 0.96 (s, 3H, -Me), δ 0.92 (s, 3H, -Me), δ 0.81 (s, 6H), δ 0.75 (s, 3H, -Me).

8-fluoro-3-(Lupeol oxime) quinoline 5a

3-substituted quinoline lupenone derivative **4a** (100mg, 0.1890 mmol) was stirred in 50 ml round bottomed flask with $NH_2OH.HCl$ (32.8mg, 0.4725 mmol, 2.5 eqv.) in pyridine (5 ml) for 12 hr. After the completion of reaction (monitored by TLC), pyridine was removed by vacuum distillation with stirring, as a azeotropic mixture with toluene. Crude solid reaction mixture so obtained was washed with Dilute HCl (5%), water, and a small amount of methanol through sintered funnel. White amorphous solid so obtained was dried in vacuum gave pure quinoline substituted lupenone oxime **5a** (80mg).

mp: 139-140ºC **Mass (ESI):** m/z 545 (M+1)

.**^{1}H-NMR (300 MHz, $CDCl_3$):** 9.20 (bs, 1H, N=O-H),δ8.8(d, 1H, J=2.1 Hz, Ar-H), 7.8(d, 1H, J=1.8 Hz, Ar-H),7.60(m, 1H, J=7.86 Hz Ar-H),7.48(m, 1H, J=6.24 Hz, Ar-H),7.41 (m, 1H, Ar-H), 2.76 (m, 1H), 2.12 (m, 2H),1.65-1.27 (bunch, 22 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)

8-fluoro-3-(Lupeol amine) quinoline 6a

3-substituted quinoline lupenone oxime **5a** (50mg, 0.0919 mmol), dissolved in dry THF was refluxed with LAH (50mg) for 24 hr. Confirming the completion of reaction by TLC, reaction mixture was quenched with cold water in ice-cold condition, and was extracted with ether, washed the organic layer with water, dried over sodium sulphate and was evaporated giving almost pure 3-substituted quinoline Lupeol Amine **6a** (30mg).

mp: 138-140 ºC **Mass (ESI):** m/z 531 (M+1),

^{1}H-NMR (300 MHz, CDCl3: δ8.8 (d, 1H, J=2.1 Hz, Ar-H), 7.9(d, 1H, J=1.8 Hz, Ar-H),7.5(m, 1H, Ar-H),7.4(m, 1H,Ar-H),7.3 (m, 1H, Ar-H),3.1 (m, 1H, H3), 2.37 (m, 2H, NH_2), 2.12 (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) .

Result

MIC of all the Lupeol derivatives tested are given in table-a.

Discussion

Detailed analysis of results (Table-1) showed that formation of 8-fluoro quinoline **3a** increases the activity and

MIC was at 10 μg/ml, C3-OH of ring-A of 8-fluoro quinoline **3a** when oxidized to 4a **,** does not increases the activity further and MIC was still at 10 μg/ml. Formation of oxime **5a** does not increase the activity and MIC was retained at 10 μg/ml, However, replacement of 3-OH of ring-A in Lupeol by NH_2 **6a** did not improved any activity of the parent molecule Lupeol **1.**

Conclusion

Among the Lupeol derivatives tested few have shown MIC at 50 μg/ml, while others showed >50 μg/ml the most active compound 3a,4a,& 5a showed MIC 10 μg/ml. Activity of Lupeol could be increased to a level of 10 μg/ml.This clearly demonsrate that fluorine group introduction in quinoline increases the activity while other substituent in quinoline ring has no effect on activity as compared to Lupeol.

It is thus concluded that Lupeol skeleton deserve further investigation for the development of more potent and non-toxic new agents for therapeutic use. Further optimization of it is needed to have a compound of clinical trial **.**

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