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EVALUATION AND SYNTHESIS OF NOVEL ANTIPSYCHOTIC COMPOUNDS FROM LUPEOL

ANKITA WAL[*1], DR. R.S SRIVASTAVA[2] AND DR A.K RAI[1]

[*1]Department of Pharmacy, Pranveer Singh Institute of Technology, Bhauti Road, Kanpur, Uttar Pradesh, India
[2]Professor, Institute of pharmacy ITBHU Varanasi

ABSTRACT

Natural Products traditionally have played an important role in drug discovery and were the basis of most early Medicines. However, despite the promise of these alternative drug discovery methods, there is still a shortage of lead compounds progressing into clinical trials. Plants have been used in treating HIV, TB, antimicrobial and all other diseases. Therefore, the current study was designed to evaluate the antipsychotic potential of lupeol and their semisynthetic derivatives to get a new and potent antipsychotic agent. Lupeol is isolated from bark of crateava nurvala *lupeol.* Lupeol has many activites like antimalarial, anticancer therefore we have planned to make some derivatives of the lupeol, which may further enhance the antipshychotic activity derivatives were prepared The antipsychotic activity of lupeol derivatives of It is established that they have effective antipshychotic action. Therefore, the current study was designed to evaluate the antipsychotic potential of lupeol and its semisynthetic derivatives. *C. nurvala* stem bark was extracted by cold maceration with 95% ethanol and concentrated through rotary vacuum evaporator at 40^0C under reduced pressure. The concentrated ethanolic extract was defatted with petroleum ether and fractionated with chloroform successively. Lupeol derivatives were prepared through a three step reaction with different amines, aliphatic and aromatic moieties. A series of derivatives of lupeol were assayed for antipshychotic activity in actophotometer and compulsive behaviour (Stereotypy) in Plus Maze Model in rats. Few derivatives of lupeol showed more potent activity as compared to the basic molecule, lupeol. The results of the present study clearly indicated that the derivatives and lupeol isolated from *C. nurvala* and synthetic lupeol analogs possess significant antipsychotic activity. It is thus concluded that lupeol skeleton deserves further investigation for the development of more potent and non-toxic new antipshychotic agents for therapeutic applications.

Keywords: lupeol, stereotype behaviour, locomotar, crateava nurvala, antipsychotic.

INTRODUCTION

Mental illness is any disease or condition affecting the brain that influences the way a person thinks, feels, behaves and/or relates to others and to his or her surroundings. Although the symptoms of mental illness can range from mild to severe and are different depending on the type of mental illness, a person with an untreated mental illness often is unable to cope with life's daily routines and demands.[1]Psychosis is the term given to the more severe forms of psychiatric disorder, during which hallucinations or delusions, violence and impaired insight may occur.[2] Psychotic disorders is reflected as significant psychological and social repercussions for everyday living.[3] Around three percent experience psychosis, more frequent than diabetes. Unlike infection, where the cause and effect are clear, most CNS ailments follow a complex biology, and have differing outcomes depending on predisposing factors. Even though the available drugs fulfil the requirements in the segment, there is need for more effective drugs that are better tolerated and cost effective to enhance the long term compliance.[4] In several other indications, such as delusional disorders, borderline psychoses, neurological conditions, or behavioral disturbances, clinical studies have been less comprehensive and

often limited to particular antipsychotics, although results seemed to be positive in terms of remarkable improvement of patients. The use of conventional antipsychotics in the elderly is strongly limited by severe and intolerable side effects.[5] In fact, conventional antipsychotics are D_2 receptor antagonists and inhibit dopaminergic neurotransmission in a dose-related manner, whereas atypical agents cause serotonin and dopamine D_2 receptor antagonism.[6-8] The use of atypical antipsychotic drugs in the elderly has become wider and wider in recent years; in fact, these agents have novel receptor binding profiles, good efficacy regarding negative symptoms, and few adverse effects, particularly in terms of reduced extra pyramidal symptoms (EPS). Psychosis is explained in terms of neurotransmitter dopamine. It is assumed that the dopamine of psychosis has been influential and states that psychosis results from an over activity of dopamine function in the brain, in particular the mesolimbic pathway. There exists a complex relationship in between dopamine and psychosis. The dopamine receptor D2 suppresses and D1 receptor increases adenylate cyclase activity. The blocked dopamine spills over to the D1 receptors during the administration of D2 blocking drugs. The increased adenylate cyclase activity affects genetic expression in the nerve cell; usually this process takes certain time period. Hence, antipsychotic drugs take a week or two to reduce the symptoms of psychosis.[9] Antipsychotics are divided into two categories. The first category is called as typical antipsychotics and also referred to as first generation antipsychotics or classical neuroleptics or major tranquilizers or conventional antipsychotics. Second category drugs, known as atypical antipsychotics or second generation antipsychotics, are used to treat psychiatric conditions. [10]

MATERIALS AND METHODS

Instrumentation

The melting points of compounds were measured in open capillaries in electrical heated melting point apparatus of Jindal, S.M. Scientific Instruments Pvt. Ltd., New Delhi. IR spectra were recorded on PerkinElmer FT-IR Spectrometer Spectrum Two and values are expressed in wave numbers (cm^{-1}). ^{1}H-NMR spectrums were recorded on JEOL, JNM-ECS 400 MHz using Chloroform-D ($CDCl_3$) as solvent. Tetramethylsilane (TMS, δ0.00 ppm) was used as internal standard in ^{1}H NMR. Purity and Mass analysis were recorded on WATERS-Q-TOF Premier-HAB213 mass spectrometer. All the chemicals were procured from Aldrich, Qualikems and Merck Chemicals.

Animals

White Albino Rats of Wister strain weighing 150±5g and Swiss albino mice of both gender weighing 20±5g and studies on them. After observing the usual formalities lay down by IAEC as per provisions made by CPCSEA. All the animals were housed in laboratory cages in animal house maintained at 23±2ºC under standard light/dark cycle. All the animals had free access to standard food pellets and filtered water.

Drugs

Diazepam (Calmtack, Indus Pharma Pvt. Ltd.) this is dissolved in DMSO (2mg/kg body weight), Apomorphine (Zyprima, Cadila Pharmaceuticals Ltd) (2.5 mg/kg body weight, ip), Haloperidol (Haldol, East West Pharma) (2.5 mg/kg body weight, ip) were dissolved in normal saline. Control animals were treated with distilled water in the same period.

Plant material

Stem bark of Crataeva nurvala was authenticate from Chandra Shekhar Azad, University of Agriculture & Technology, Kanpur and its reference is CSA/DOH/ 2015-16/ 31 . The plants were selected on the basis of their folk medicinal value.

Lupeol

Lupeol was isolated from the hexane fraction of the stem bark *Crataeva nurvula* and It was named as compound-1. It displayed a molecular ion peak atm/z 426 for molecular ion [M]+ of lupeol and a molecular formula $C_{30}H_{50}O$. The 1 Hand 13C NMR spectra were found exhibiting characteristic signals for lup-20(29)- en-3-ol6. The structure was confirmed by comparison of spectroscopic data of the compound-1 to those described for lupeol [11] and confirmation of the lupeol was also done by thin layer chromatography

Extraction of Lupeol (L-1)

Coarsely powdered (2 kg) raw material of *C. nurvala* stem bark was extracted by cold maceration with 95% ethanol and concentrated through rotary vacuum evaporator at 40^0C under reduced pressure. The concentrated ethanolic extract was defatted with petroleum ether and fractionated with chloroform successively. The

chloroform fraction was concentrated under reduced pressure to afford chloroform soluble light brownish residue (30 g).The compounds were isolated from chloroform fraction through column chromatography using gradient elution technique. The progress of separation was monitored by TLC (silica gel G 60 F254 plates, Merck).Fractions eluted with n-hexane and EtOAc (8:2) resulted an amorphous yellowish white residue which after crystallization with methanol provides colorless crystalline substance termed as Lupeol (L-1) (16 g). M.P is 214-216 ºC, FT-IR spectra hydroxyl group (3302.6 cm-1), Vinyldiene group 3068.1,1636.8,880 cm^{-1}). ^{1}H-NMR (400 MHz, $CDCl_3$, δ, ppm) 4.68 and 4.56 (s, 3H). MS (m/z) 426.

Synthetic Scheme

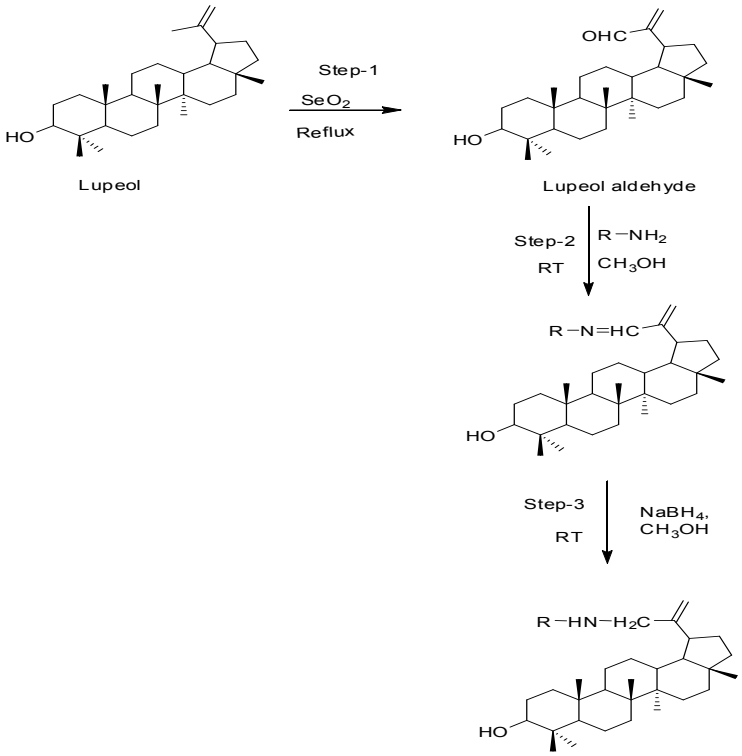
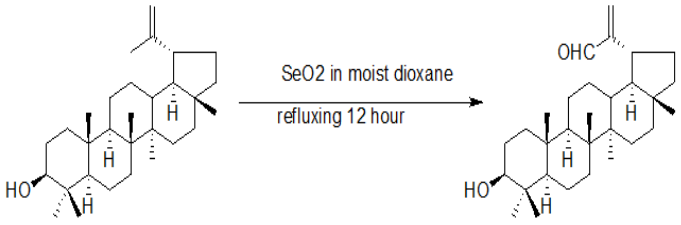


Figure 1
Synthetic Scheme

Chemical Modifications
Step-1
Chemical modification at C-30

Lupeol (1 gm, 2.35 mm) was refluxed with selenium dioxide in dioxane with 3-4 drops of distilled water, for 10-12 hours. After consumption of all of lupeol, reaction mixture was treated with 2.5% aq. KOH, and 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 and was eluted with n- hexane* /ethyl acetate with (9:1) (8:2) (7:3) gave the required pure lupeol aldehyde (LA-1). M.P is 222-226 ºC. FT-IR spectra showed 3283 (OH), 2938 (CH), 1680 (CHO),1463 (-C-H) Bending. ^{1}H-NMR (400MHz, $CDCl_3$, δ, ppm) δ9.48 (s, 1H-CHO), 6.25 and 5.87 (2s, 1H each, H29), 3.13 (m, 1H,H3), 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). MS (m/z) 441.



Lupeol (L-1) **Lupeol Aldehyde (LA-1)**

Formation of **(LA-1)** 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.

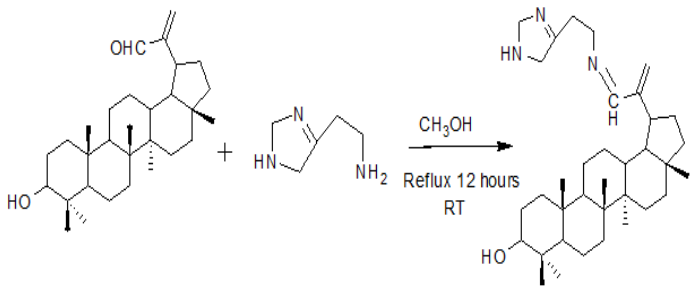
Step -2

Chemical modification at C- 30 aldehyde

In order to this, various changes are done in the isopropenyl side chain of the lupeol.

Reaction with Histamine

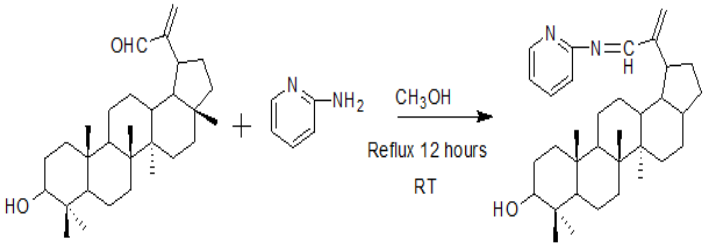
Lupeol Aldehyde (**LA-1**) (1 mole) was refluxed with Histamine (1 mole) in methanol for 12 hours. After that the reaction product was separated by ethyl acetate and water. The organic layer was taken and added sodium sulfate and then filtered it to give the product **LAH-2**. M.P is 217-218 ºC. FT-IR spectra showed 3400 (OH), 2952 (CH), 2379 (C=N), 1655 (CHO), 1559 (NH Bending), 880 (=CH). ^{1}H-NMR (400 MHz, $CDCl_3$, δ, ppm) δ4.8(d,1H,J=16.8,OH), δ2.0 (s,1H,NH), δ4.8(d,1H,J=10.8,OH), δ2.8(m,1H), δ2.1(m,2H,H-2), δ1.65(bunch 24H), δ1.01(s,3H,Me), δ0.92(s,3H,CH_3), δ0.81(s,6H), δ0.75(s,3H,CH_3).



LA-1 ***LAH-2***

Reaction with 2- Aminopyridne

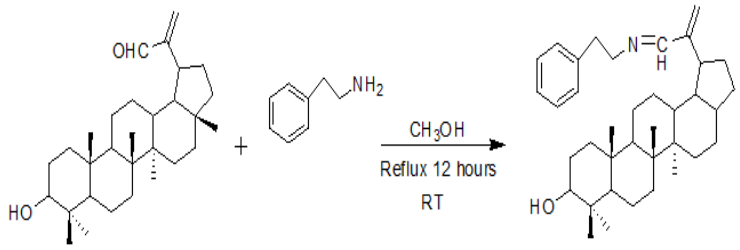
Lupeol Aldehyde (**LA-1**) (1 mole) was refluxed with 2- Aminopyridne (1 mole) in methanol for 12 hours. After that the reaction product was separated by ethyl acetate and water. The organic layer was taken and added sodium sulfate and then filtered it to give the product **LAP-2**. M.P is 232 ºC. FT-IR spectra showed 3443 (OH), 2933 (CH), 2318 (C=N),1712 (CHO), 1645 (NH-Bending). ^{1}H-NMR (400 MHz, $CDCl_3$, δ, ppm) δ7.29(s.1H,NH), δ4.92(s,1H, CN) δ4.91(d,2H,J=14.68,CH_2), δ4.8(d,1H,J=12.8,OH), δ2.8(m,1H), δ2.1(m,2H,H-2),δ1.65(bunch 24H), δ1.01(s,3H,Me), δ0.92(s,3H,CH_3), δ0.81(s,6H), δ0.75(s,3H,CH_3)



LA-1 **LAP-2**

Reaction with Phenyl Ethyl Amine

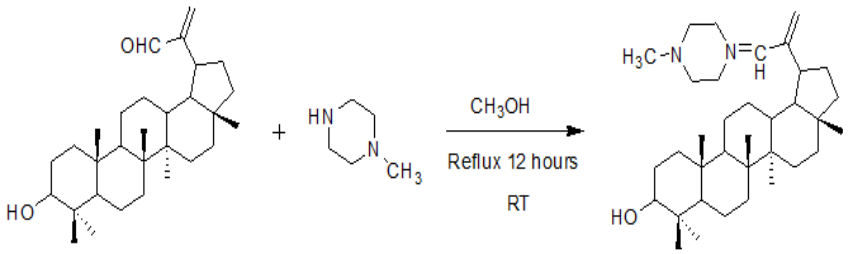
Lupeol Aldehyde (**LA-1**) (1 mole) was refluxed with Phenyl Ethyl Amine (1 mole) in methanol for 12 hours. After that the reaction product was separated by ethyl acetate and water. The organic layer was taken and added sodium sulfate and then filtered it to give the product **LAPEA-2**. M.P is 219 ºC. FT-IR spectra showed 3316 (OH), 2933 (=CH), 2883 (CH), 1693 (NH-Bending), 1453 (C=C), 1378 (C-N). ^{1}H-NMR (400 MHz, $CDCl_3$, δ, ppm) δ5.22(m,1H,CN), δ5.25(s,1H,CN), δ4.8(d,1H,J=10.8,OH), δ3.4(m,2H,CH_2), δ2.8(m,1H), δ2.1(m,2H,H-2), δ1.65(bunch-24H), δ1.01(s,3H,Me), δ0.92(s,3H,CH_3), δ0.81(s,6H), δ0.75(s,3H,CH_3).



LA-1 ***LAPEA-2***

Reaction with Methyl Piperazine

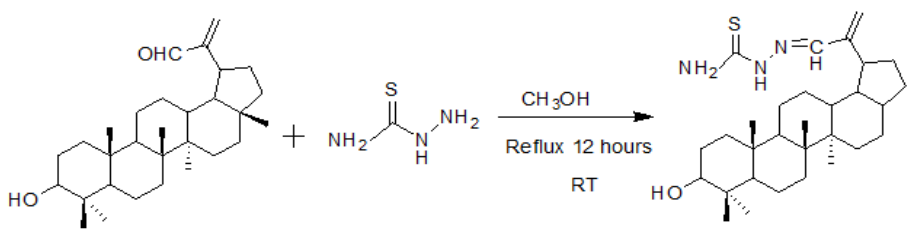
Lupeol Aldehyde (**LA-1**) (1 mole) was refluxed with Methyl Piperazine (1 mole) in methanol for 12 hours. After that the reaction product was separated by ethyl acetate and water. The organic layer was taken and added sodium sulfate and then filtered it to give the product **LAMP-2**. M.P is 224 ºC. FT-IR spectra 3420 (OH), 2924 (=CH), 2853 (CH), 2359 (C=N), 1693 (NH-Bending), 1383 (C-N). ^{1}H-NMR (400 MHz, $CDCl_3$, δ, ppm) δ6.27(s,1H,CN), δ5.89(s,1H,CN), δ3.13(d,2H,J=5.04,CH_2), δ4.8(d,1H,J=12.8,OH), δ2.8(m,1H), δ2.1(m,2H,H-2), δ1.65(bunch-24H), δ1.01(s,3H,Me), δ0.92(s,3H,CH_3), δ0.81(s,6H), δ0.75(s,3H,CH_3).



LA-1 ***LAMP-2***

Reaction with Thiosemicarbazide

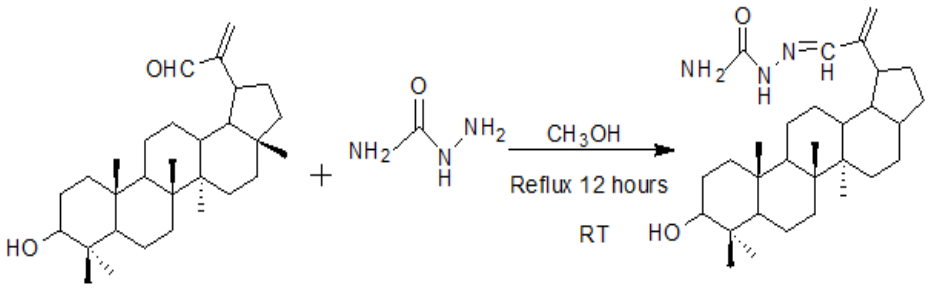
Lupeol Aldehyde (**LA-1**) (1 mole) was refluxed with Thiosemicarbazide (1 mole) in methanol for 12 hours. After that the reaction product was separated by ethyl acetate and water. The organic layer was taken and added sodium sulfate and then filtered it to give the product **LATS-2**. M.P is 220 ºC. FT-IR spectra showed 3431 (OH), 2990 (=CH), 2879 (CH), 2348 (C=N), 1662 (NH-Bending), 1260 (C-N). ^{1}H-NMR (400 MHz, $CDCl_3$, δ, ppm) δ7.34(s,2H,NH_2), δ5.42(s,1H,CN), δ5.18(s,2H,CH_2), δ4.8(d,1H,J=9.8,OH), δ2.2(m,1H,NH), δ2.8(m,1H),δ2.1(m,2H,H-2), δ1.65(bunch-24H), δ1.01(s,3H,Me), δ0.92(s,3H,CH_3), δ0.81(s,6H), δ0.75(s,3H,CH_3).



LA-1 ***LATS-2***

Reaction with Semicarbazide

Lupeol Aldehyde (**LA-1**) (1 mole) was refluxed with Semicarbazide (1 mole) in methanol for 12 hours. After that the reaction product was separated by ethyl acetate and water. The organic layer was taken and added sodium sulfate and then filtered it to give the product **LAS-2**. M.P is 232 ºC. FT-IR spectra showed 3356 (OH), 3010 (=CH), 2881 (CH), 2346 (C=N), 1650 (NH-Bending), 1230 (C-N). ^{1}H-NMR (400 MHz, $CDCl_3$, δ, ppm) δ 7.49(s,2H,NH_2), δ6.45(s,1H,NH), δ4.8(d,1H,J=13.8,OH), δ5.55(s,1H,H) δ5.35(s,1H,H), δ2.8(m,1H), δ2.1(m,2H,H-2), δ1.65(bunch-24H), δ1.01(s,3H,Me), δ0.92(s,3H,CH_3), δ0.81(s,6H), δ0.75(s,3H,CH_3).

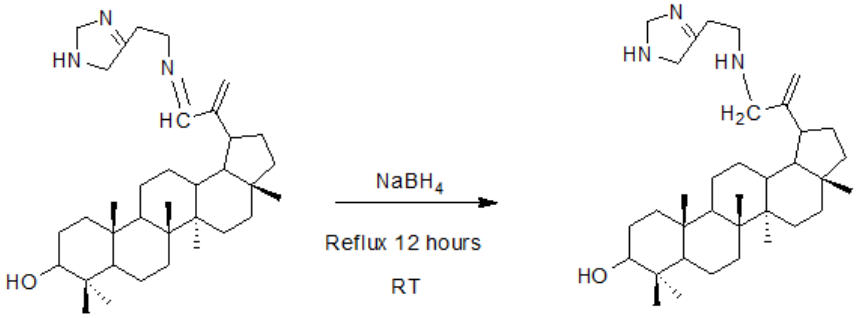


LA-1 ***LAS-2***

Step - 3

Reaction with Histamine

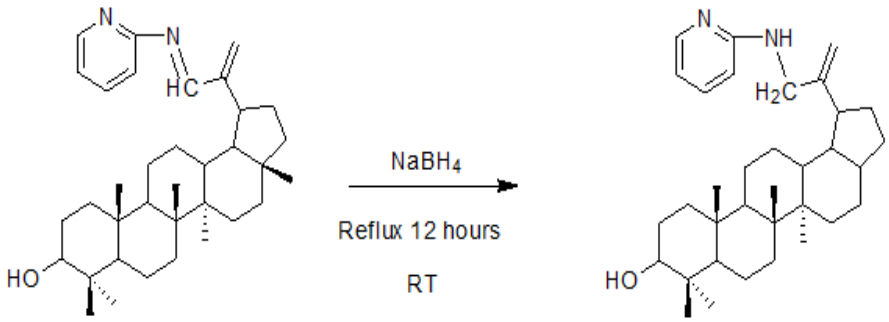
Lupeol Aldehyde Histamine (**LAH-2**) (1 mole) was refluxed with Sodium Borohydride (1.5 mole) in methanol for 12 hours. After that the reaction product was separated by ethyl acetate and water. The organic layer was taken and added sodium sulfate and then filtered it to give the product **LAH-3**. M.P is 226 ºC. FT-IR spectra showed 3454 (OH), 2946 (=CH), 2870 (CH), 2374 (C=N), 1609 (NH-Bending), 1361 (C-N). ^{1}H-NMR (400 MHz, $CDCl_3$, δ, ppm) δ4.8(d,1H,J=16.8,OH), δ2.0(s,1H,NH), δ4.8(d,1H,J=16,OH),δ2.8(m,1H),δ2.1(m,2H,H2),δ1.65(bunch24H),δ1.01(s,3H,Me), δ0.92(s,3H,CH_3), δ0.81(s,6H), δ0.75(s,3H,CH_3).



LAH-2 ***LAH-3***

Reaction with 2- Amino pyridine

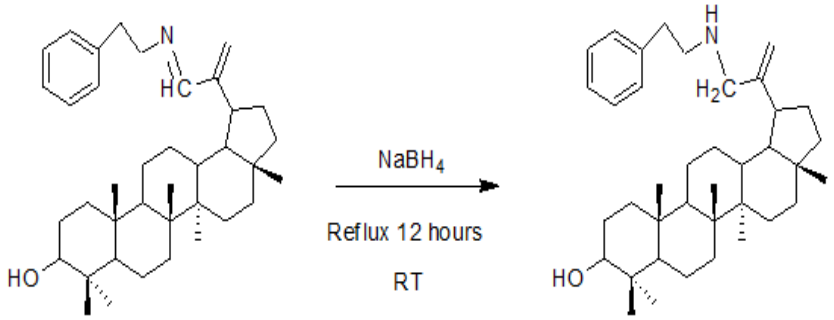
Lupeol Aldehyde aminopyridine (**LAP-2**) (1 mole) was refluxed with Sodium Borohydride (1.5 mole) in methanol for 12 hours. After that the reaction product was separated by ethyl acetate and water. The organic layer was taken and added sodium sulfate and then filtered it to give the product **LAP-3**. M.P is 228 ºC. FT-IR spectra showed 3310 (OH), 2924 (=CH), 2853 (CH), 2340 (C=N), 1707 (NH-Bending), 1452 (-C-H- Bending), 1232 (C-N). ^{1}H-NMR (400 MHz, $CDCl_3$, δ, ppm) δ7.29(s.1H,NH), δ4.92(s,1H,CN),δ4.91(d,2H,J=14.68,CH_2),δ4.8(d,1H,J=12.4,OH), δ2.8(m,1H), δ2.1(m,2H,H-2), δ1.65(bunch-24H), δ1.01(s,3H,Me), δ0.92(s,3H,CH_3), δ0.81(s,6H), δ0.75(s,3H,CH_3).



LAP-2 ***LAP-3***

Reaction with Phenyl Ethyl Amine

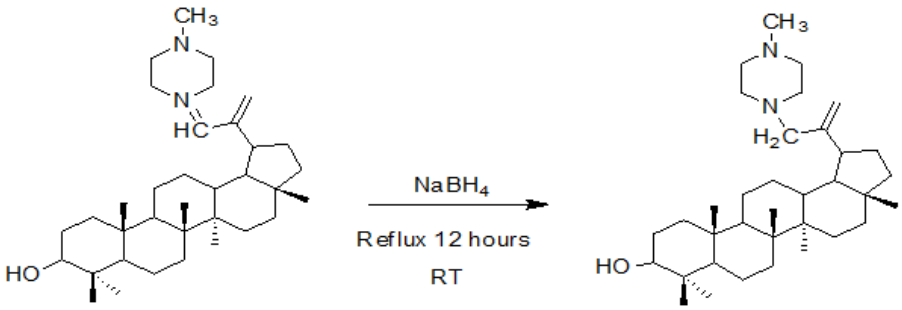
Lupeol Aldehyde Phenyl Ethyl Amine (**LAPEA-2**) (1 mole) was refluxed with Sodium Borohydride (1.5 mole) in methanol for 12 hours. After that the reaction product was separated by ethyl acetate and water. The organic layer was taken and added sodium sulfate and then filtered it to give the product **LAPEA-3**. M.P is 223 ºC. FT-IR spectra showed 3516 (OH), 2922 (=CH), 2854 (CH), 2343 (C=N), 1720 (NH-Bending), 1359 (-C-H Bending). ^{1}H-NMR (400 MHz, $CDCl_3$, δ, ppm) δ5.22 (m,1H,CN), δ6.2(s,1H,CN), δ4.8(d,1H,J=12.8,OH),δ3.4(m,2H,CH_2),δ2.8(m,1H), δ2.1(m,2H,H-2), δ1.65(bunch-24H), δ1.01(s,3H,Me), δ0.92(s,3H,CH_3), δ0.81(s,6H), δ0.75(s,3H,CH_3).



LAPEA-2 **LAPEA-3**

Reaction with Methyl Piperazine

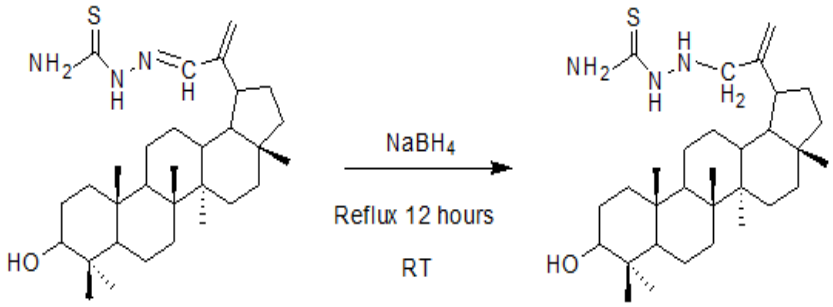
Lupeol Aldehyde Methyl Piperazine (**LAMA-2**) (1 mole) was refluxed with Sodium Borohydride (1.5 mole) in methanol for 12 hours. After that the reaction product was separated by ethyl acetate and water. The organic layer was taken and added sodium sulfate and then filtered it to give the product **LAMA-3**. M.P is 230 ºC. FT-IR spectra showed 3310 (OH), 2914 (=CH), 2844 (CH), 1561 (-C-H Bending). ^{1}H-NMR (400 MHz, $CDCl_3$, δ, ppm) δ6.27(s,1H,CN), δ5.89(s,1H,CN), δ3.13 (d,2H,J=5.04,CH_2), δ4.8 (d,1H,J=17.2,OH), δ2.8(m,1H), δ2.1(m,2H,H-2), δ1.65 (bunch-24H), δ1.01(s,3H,Me), δ0.92(s,3H,CH_3), δ0.81 (s,6H), δ0.75(s,3H,CH_3).



LAMP-2 ***LAMP-3***

Reaction with Thiosemicarbazide

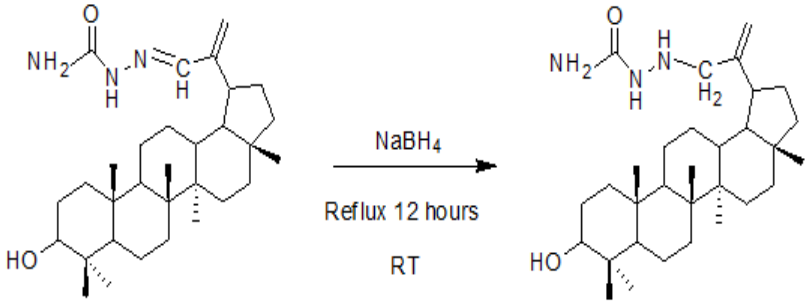
Lupeol Aldehyde Thiosemicarbazide (**LATS-2**) (1 mole) was refluxed with Sodium Borohydride (1.5 mole) in methanol for 12 hours. After that the reaction product was separated by ethyl acetate and water. The organic layer was taken and added sodium sulfate and then filtered it to give the product **LATS-3**. M.P is 231 ºC. FT-IR spectra showed 3367 (OH), 2990 (=CH), 2881 (CH), 2340 (C=N), 1612 (NH-Bending), 1549 (-C-H Bending). ^{1}H-NMR (400 MHz, $CDCl_3$, δ, ppm) δ7.34(s,1H,NH_2), δ5.42 (s,1H,CN), δ5.18(s,2H,CH_2), δ2.2(m,1H,NH), δ4.8(d,1H,J=10.1,OH), δ2.8(m,1H), δ2.1 (m,2H,H-2), δ1.65(bunch-24H), δ1.01(s,3H,Me), δ0.92(s,3H,CH_3), δ0.81(s,6H), δ0.75(s,3H,CH_3).



LATS-2 ***LATS-3***

Reaction with Semicarbazide

Lupeol Aldehyde Semicarbazide (**LAS-2**) (1 mole) was refluxed with Sodium Borohydride (1.5 mole) in methanol for 12 hours. After that the reaction product was separated by ethyl acetate and water. The organic layer was taken and added sodium sulfate and then filtered it to give the product **LAS-3**. M.P is 227 ºC. FT-IR spectra showed 3420 (OH), 2914 (=CH), 2844 (CH), 2266 (C=N), 1561 (-C-H Bending). ^{1}H-NMR (400 MHz, $CDCl_3$, δ, ppm) δ 7.49(s,2H,NH_2), δ6.45(s,1H,NH), δ5.55(s,1H,H) δ5.35(s,1H,H),δ2.8(m,1H), δ4.8(d,1H,J=13.4,OH),δ2.1(m,2H,H-2), δ1.65(bunch-24H),δ1.01(s,3H,Me),δ0.92(s,3H,CH_3),δ0.81(s,6H), δ0.75(s,3H,CH_3).



LAS-2 ***LAS-3***

Acute Toxicity Study

The acute oral toxicity of the Lupeol was evaluated in rats using the procedures described by Organization for Economic Co-operation and Development 423 guidelines.[12] A total of 16 female animals were divided into four dosage groups with 4 animals per dose. The control group was given 10 ml/kg of normal saline. The second, third, fourth and fifth groups were given with a single dose of 250, 500, 1000 mg/kg and 2000 mg/kg of Lupeol, respectively. The albino mice of either sex (body weight20-25) were used. Animals were fasted approximately 12 hours prior to dosing. Following administration of a single dose of herbal preparation, the animals were observed for behavioral changes and general toxicity signs. Results were recorded for the first 30 minutes and at hourly intervals for the next 24 hours and thereafter for a total of 14 days. At the end of the experiment, all of the animals were sacrificed for gross necropsy findings. Animals were observed individually after administration at least once during the first 30 minutes, periodically during the first 24 hours. The acute toxicity study showed that

animals fed by oral gavages tolerated the limit dose of 2000 mg/kg body weight of lupeol .There was no visible signs of acute toxicity during the 14 days of observation. The results of acute toxicity study showed no clinical signs of toxicity and mortality in the lupeol drug treated animals even after administration of 250,500 mg/kg dose. After Sacrifice the animal shows infection in intestine, stomach, liver, ulcer occur in intestinal parts at the 2000 mg/kg of drug. Hence, as per OECD guidelines lethal dose was assigned to be more than 2000 mg/kg.

S.No.	Compound	Conc. (mg/kg, oral)
1	L-1, no toxic	> 250
2	L-1, no toxic	<500
3	L-1,no toxic	<1000
4	L-1, toxic	<2000

Table 1
Acute Toxicity Study

Anti-Psychotic Activity (Actophotometer)

Experimental study protocol have been approved by the Institutional Animal Ethics Committee (IAEC) constituted as per the guidelines laid by the committee for the purpose of control and supervision of experiments on Animals (CPCSEA).The no of CPCSEA is 1273/AC/09. Actophotometer was performed by administering Diazepam (2mg/kg body weight) and synthesized molecules (LAH-3, LAP-3, LAPEA-3, LAMP-3, LATS-3, LAS-3) (n=3) into different groups of White Albino Rats of Wister strain (150-170 g). Rats were maintained on laboratory stock diet. They were fasted for 24 hours before starting the experiment. The rats were numbered and weighted. The Actophotometer was switched on and the rats were placed individually in the activity case for 10 min. The basal locomotor activity score of all rats were noted. Standard, Test, Control were injected on each rats of proposed groups and after 30 min. each animals were retested for 10 min. They were divided into three groups each comprised of three rats. The groups are:- Control group - three rats received 0.8 ml of DMSO, Standard group- three rats received oral dose of Diazepam 2mg/kg body weight, Test group- divided into first to six groups, each containing three rats, received 250 mg of test drug per kg body weight dissolved in 1ml of DMSO. The synthesized compounds LAH-3, LAP-3, LAPEA-3, LAMP-3, LATS-3, LAS-3, were selected for evaluated for their Anti-Psychotic Activity using Actophotometer.

Statistical analysis

All values shown in the figures and tables represent themeans ± S.E.M. values with 95% confidence limits were estimated using Maximum Likelihord Iterative Procedure .[13]Statistical analysis was performed with SPSS software using one-way analysis of variance (ANOVA) followed by Dunnett.s multiple comparison test. $P<0.05$ was considered to be statistically significant.

Compulsive behaviour (Stereotypy) in Plus Maze Model

Compulsive behaviour (Stereotypy) in Plus Maze Model was performed by administering Apomorphine (2.5 mg/kg body weight, ip), Haloperidol (2.5 mg/kg body weight, ip), and synthesized molecules (LAH-3, LAP-3, LAPEA-3, LAMP-3, LATS-3, LAS-3) (n=3) into different groups of Swiss albino mice (20-25 g). Animals were weighed, numbered and divided them into different groups such as control, standard, test. After it trained the animals in elevated plus maze. Prepared the control, standard, test solutions and injected into different groups. The groups are:- Control group - three mice received 0.65 ml of Apomorphine (ip), Standard group- three mice received of Haloperidol (ip) 2.5 mg/kg body weight, Test group- divided into first to six groups, each containing three mice, received 250 mg of test drug per kg body weight. Noted the onset and intensity of rearing, sniffing, and licking behaviour at 0 times, 15, 30, 45, 60 min after the Apomorphine, haloperidol and test drugs were injected. The severity of the response can be scored as + = Presence , - = Absent.

Statistical analysis

Data were analyzed by analysis of variance test followed by turkey's test. All the results were expressed as mean ± SEM. $P < 0.05$ was considered significant. Percent reduction in activity score and fall off time were calculated with reference to respective basal recordings.

RESULT & DISCUSSION

Anti-Psychotic Activity (Actophotometer)

Effect of lupeol derivatives on animal behavior. The compound **LAH-3** and **LAPEA-3** obtained, which shows there is significance difference occurs. $P < 0.05$. Diazepam (2.5 mg/kg, oral) and drug derivatives (250 mg/kg, oral) treated groups showed significant locometer activity when compared with control; however; this psychosis was less with drug derivatives treated group than diazepam-treated group.

Anova is followed by tukkey's Test

Table 2
Anova table

ANOVA Table	SS	df	MS	Table Analysis	Data 1
Treatment (between columns)	3947	7	563.8	One-way analysis of variance	
Residual (within columns)	820.3	16	51.27	P value	P<0.0001
Total	4767	23		P value summary	***
				Are means significant different? (P < 0.05)	Yes
				Number of groups	8
				F	11.00
				R squared	0.8279

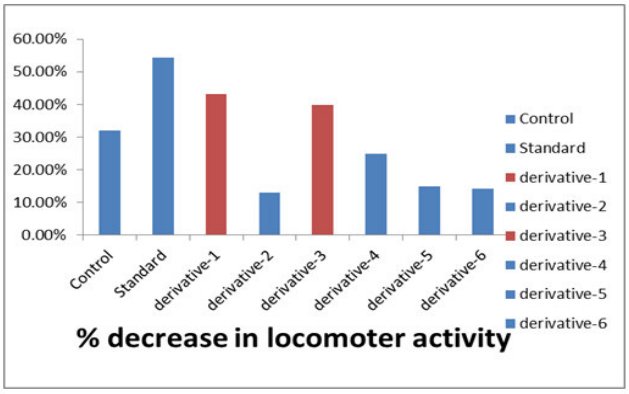


Figure 2
Percentage decrease in locometer activity

Compulsive behaviour (Stereotypy) in Plus Maze Model

All the drug derivatives compounds were subjected to pharmacological evaluation to determine their behaviour symptoms, inhibition of apomorphine induced Rearing, Sniffing, Licking. Swiss albino mice (three mice in each group) of either sex (20-25 g) were used and housed per cage in standard laboratory conditions (12 h light/ dark cycle, 22±2 ºC room temperature). Food and water were available ad libitum. All experiments were approved by institutional ethical Committee. All synthesized compounds were suspended in 1% solution of apomorphine in distilled water and administered by the intraperitoneal (i.p.) route.

Behaviour symptoms

Swiss albino mice (three mice in each group) of either sex (20-25g) were used and kept in plastic cage. All derivatives at their respective doses were given to animals. Each cage contained three animals. The changes in the behavior symptoms were noted down for an interval of 30 minutes for 3 hours and then after 24 hours, the cages were inspected for any mortality of the animals.Haloperidol (2 mg/kg i.p.) and drug derivatives (250 mg/kg, oral) significantly ($P < 0.001$) exhibited psychosis; as evident from decreased rearing, sniffing, and licking behaviour as compared with control.

Observation

Table 3
Severity of response

S.No.	Treatment		Severity of response														
			Rearing					Sniffing					Licking				
			0	15	30	45	60	0	15	30	45	60	0	15	30	45	60
1	Control	M-1	+	+	+	-	+	+	+	-	+	+	-	+	-	+	-
		M-2	+	-	+	+	+	-	+	+	-	+	+	+	+	-	+
		M-3	-	+	+	-	+	+	-	+	+	+	+	-	+	+	+
2	Standard	M-1	+	-	-	-	-	+	-	-	+	-	-	-	+	-	-
		M-2	-	-	-	+	-	-	+	-	-	+	-	-	+	-	-
		M-3	+	-	-	+	-	-	-	-	+	-	-	-	+	-	-
3	LAH-3	M-1	+	-	+	-	+	-	-	+	+	+	-	+	-	-	+
		M-2	+	-	+	-	+	+	-	+	-	-	+	+	+	-	+
		M-3	-	-	+	+	+	-	+	-	-	+	-	-	+	+	+
4	LAP-3	M-1	+	+	+	-	+	+	-	+	+	+	+	+	+	-	+
		M-2	+	+	+	+	+	+	-	-	-	+	+	-	+	-	+
		M-3	+	-	+	-	+	+	-	+	+	+	+	+	+	+	+
5	LAPEA-3	M-1	+	+	+	-	-	-	+	-	+	-	-	-	+	-	+
		M-2	-	+	-	-	+	+	-	+	+	-	+	-	-	+	-
		M-3	-	+	-	+	-	-	+	-	-	+	+	+	+	-	-
6	LAMP-3	M-1	+	-	+	-	+	+	+	+	-	+	+	-	+	-	+
		M-2	+	+	+	+	+	+	-	+	-	+	+	-	+	-	+
		M-3	+	-	+	-	+	+	-	+	-	+	+	+	+	+	+
7	LATS-3	M-1	+	-	+	+	+	+	-	+	-	+	+	-	+	+	+
		M-2	+	-	+	-	+	+	-	+	-	+	+	+	+	-	+
		M-3	+	+	-	-	+	+	+	+	-	+	+	-	+	-	+
8	LAS-3	M-1	+	-	+	-	+	+	+	+	-	+	+	-	+	-	+
		M-2	+	-	+	+	+	+	-	+	+	-	+	-	+	+	+
		M-3	+	-	+	-	+	+	-	+	-	+	+	-	+	-	+

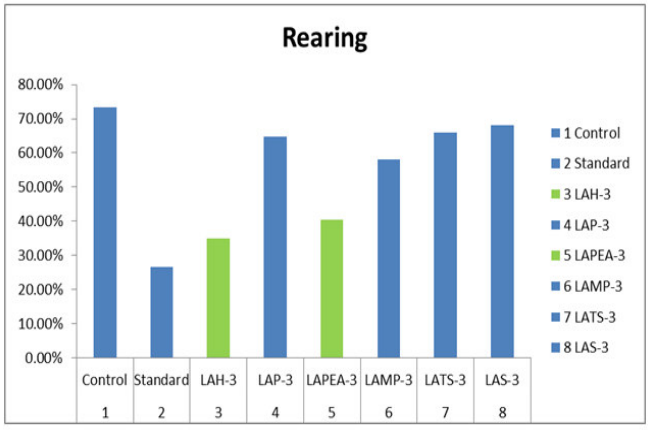


Figure 3
Rearing behaviour of animal

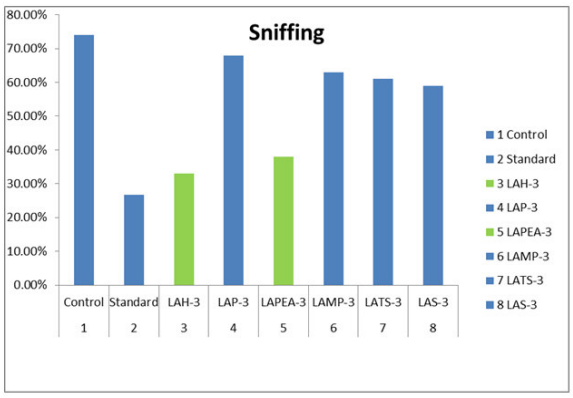


Figure 4
Sniffing Behaviour of animal

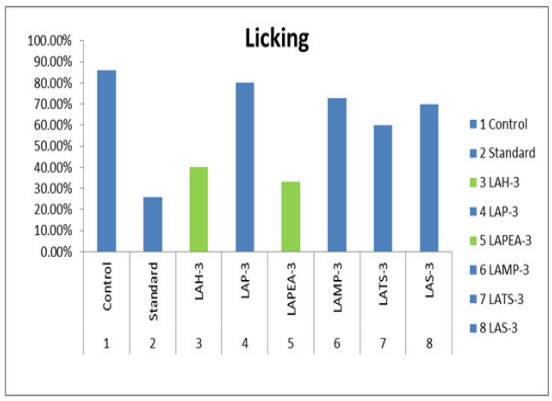


Figure 5
Licking Behaviour of animal

SUMMARY AND CONCLUSION

A series of compounds were prepared using the pathway shown in synthetic schemes (**3.5**). The target compounds were prepared by three steps procedure. In series A, in the first step Lupeol (L-1) was reacted with selenium dioxide and few drops of water to give the product Lupeol aldehyde (LA-1). In the second step, LA-1 was reacted with the different amines to give the final products. At in the last step, the reduction double bond at C-30.All the target compounds were subjected to pharmacological evaluation for behavior symptoms**,** **likes** standing (rearing), continuous sniffing (touching the nose), and licking the body inhibition of apomorphine induced in elevated plus maze model .The semisynthetic derivatives were also subjected to locomotor activity and result shown that compound LAH-3 and LAPEA-3 was found to be active. The acute toxicity of the potent compound in a series was also performed. The pharmacological results suggested that the presence of heterocyclic amines in the ring increased the antipsychotic activity. This is clearly demonstrated that 5 membered Histamine and aromatic amine such as phenyl ethyl amine increases the activity while other substituent has no effect on activity as compared to lupeol. Although inclusion of pyridine, piperazine, semicarbazide and thiosemicarbazide does not increase any effect. Only imidazoline and aromatic amine like phenyl ethyl amine shows the positive response. It is thus concluded that lupeol skeleton deserve further investigation for the development of more potent and non-toxic new agents for therapeutic use.

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