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Synthesis of novel triterpenoid (lupeol) derivatives and their in vivo antihyperglycemic and antidyslipidemic activity ☆

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ABSTRACT

The triterpenoid, lupeol (**1**) has been isolated from the leaves extract of *Aegle marmelos*. Few novel derivatives (**2**–**13**) were synthesized from the naturally occurring lupeol (**1**) and screened for their antihyperglycemic activity (**2**–**11**) and antidyslipidemic activity (**2–4** and **12–13**). The derivative **4** lowered the blood glucose levels by 18.2% and 25.0% at 5 h and 24 h, respectively, in sucrose challenged streptozotocin induced diabetic rats (STZ-S) model at the dose of 100 mg/kg body weight. The compound **4** also significantly lowered 40% (P <0.001) in triglycerides, 30% (P <0.05) in glycerol, 24% (P <0.05) in cholesterol quantity and also improved the HDL–cholesterol by 5% in dyslipidemic hamster model at the dose of 50 mg/kg b.wt.

Diabetes mellitus is an independent risk factor for the development of coronary artery diseases, myocardial infarction, hypertension and dyslipidemia. Clinically diabetic patients are characterized by marked increase in blood glucose level followed by mild hyperlipidemia. Non-insulin dependent diabetes mellitus (NIDDM) accounts for approximately 80–90% of all cases[1] and it is the fastest growing global threat to public health. If the current trend continues, it is likely to result in an estimated 215 million sufferers from NIDDM worldwide by the year 2010.[2,3] This number is expected to increase as medical advances extend life expectancy and more widespread access to a calorie rich diet promotes the prevalence of obesity. When carbohydrates are in low supply or their breakdown is incomplete, fats become the preferred source of energy. Fatty acids are mobilized into the general circulation leading to secondary triglyceridemia in which total serum lipids in particular triglycerides as well as in the levels of cholesterol and phospholipids increases.

This rise is proportional to the severity of the diabetes. Uncontrolled diabetes is manifested by a very high rise in triglycerides and fatty acid levels[4] for one third of deaths in industrialized nations.[5] Therefore, a drug, having twofold properties is in great demand. Several research groups are focusing to develop such dual-acting agents (Fig. 1). Despite the remarkable progress in the management of diabetes mellitus by synthetic drugs,[6] there has been a renewed interest in medicinal plants because of the side effects of synthetic drugs. The discovery of new drugs from traditional medicine is not a new phenomenon (Fig. 1). In continuation of our drug discovery program on antidiabetic and antidyslipidemic agents, we discovered antidyslipidemic activity in naturally occurring lupeol (**1**), which was isolated from the leaves of *Aegle marmelos*.[7] Recently Sudhahar et al.[8] also, reported hypercholesterolemia in lupeol and linoleate ester of lupeol. The linoleate ester of lupeol appears to be a good prodrug of lupeol in Sudhahar et al. studies.[9] Prodrugs play a major role in absorption, distribution, metabolism, excretion (ADME) and improves the oral bioavailability. In our own studies the lupeol (**1**) exhibited the dyslipidemic activity. We therefore prepared few novel ester derivatives of lupeol (**1**) and studied their in vivo antihyperglycemic activity in Streptozotocin induced diabetic rats model and antidyslipidemic activity in hamster model.

The syntheses of non-natural ester analogues **2**–**13** of lupeol (**1**) were accomplished by subjecting various aromatic and aliphatic acids with lupeol (Scheme 1) by employing DCC-DMAP esterification protocol in dry CH_2Cl_2.

Ten novel ester derivatives **2**–**11** of lupeol were studied for their antihyperglycemic activity in streptozotocin induced diabetic rats (STZ-S model)[10] at a dose of 100 mg/kg body weight. Out of these 10 derivatives nicotinic acid derivative **4** was the most potent compound (−18.3% at 5 h and −25.0% at 24 h) followed by 2 (pyridin-2-yl) acetic acid **7** (−20.5% at 5 h and −18.2% at 24 h). Extension of the alkyl chain between acid group and pyridine moiety (**6** and **8**) led to decreased activity. Hippuric acid derivative **5** also showed good activity (−13.8% at 5 h and −23.6% at 24 h). *p*-Chlorobenzoic acid derivative **2** and *p*-nitrobenzoic acid derivative

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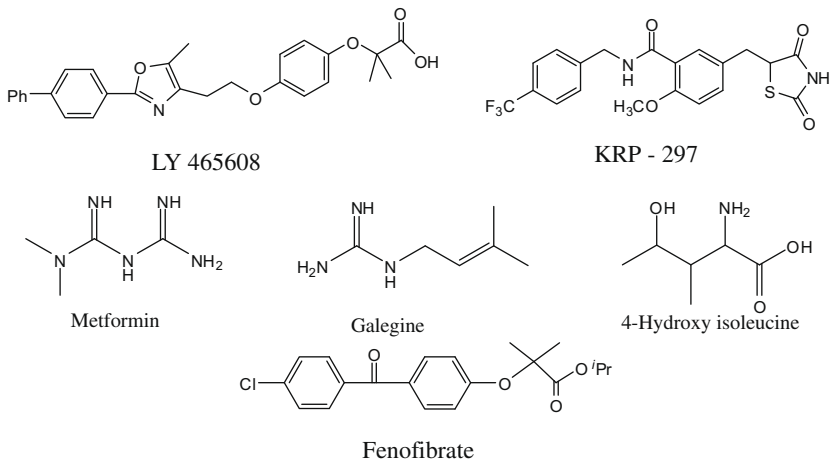
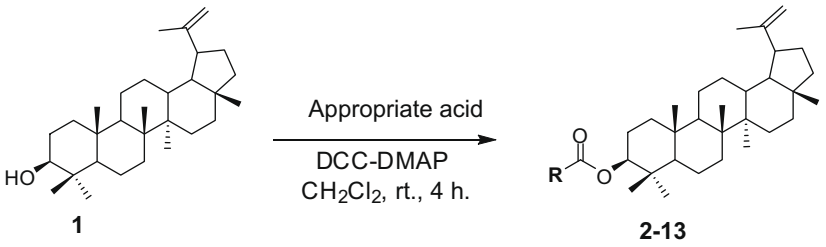
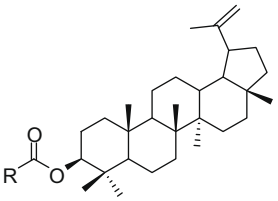


Figure 1. Dual-acting agents in clinical trials (LY 465608 and KRP-297); synthetic antidiabetic agent (Metformin); naturally occurring antidiabetic agents (Galegine and 4-Hydroxy isoleucine) and synthetic triglyceride lowering drug (Fenofibrate).



Scheme 1. Synthesis of novel lupeol esters **2**–**13** using DCC-DMAP esterification protocol in dry CH_2Cl_2.

Table 1
In vivo antihyperglycemic activity of lupeol ester derivatives **2**–**11** in STZ- S model



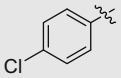
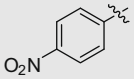
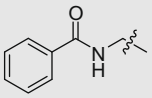
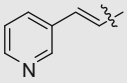
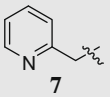
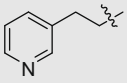
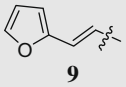
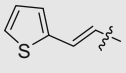
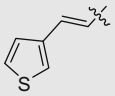
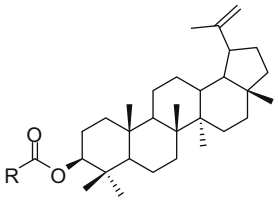
Entry	Compound (R)	Test dose (mg/kg)	% Antihyperglycemic activity in STZS	
			5 h	24 h
1	 **2**	100	−17.8*	−19.6*
2	 **3**	100	−14.8	−21.1*
3	 **4**	100	−18.3*	−25.0**
4	 **5**	100	−13.8	−23.6**

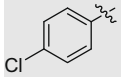
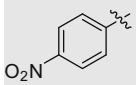
Table 1 (*continued*)

Entry	Compound (R)	Test dose (mg/kg)	% Antihyperglycemic activity in STZS	
			5 h	24 h
5	 **6**	100	−13.4	−15.7*
6	 **7**	100	−20.5*	−18.2*
7	 **8**	100	−8.21	−10.4
8	 **9**	100	−15.8*	−18.5*
9	 **10**	100	−12.5	−14.9
10	 **11**	100	−16.9*	−21.5*
11	Metformin (Fig. 1)	100	−23.5**	−26.5**

*p <0.05 and **p <0.01. Control.

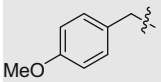
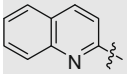
Table 2
Percentage decrease/increase in plasma lipids from baseline with the treatment of lupeol esters **2**–**4** and **12**–**13** in dyslipidemic hamsters (values are mean ± SD of eight hamsters in each group)



Test sample	Food intake (g)	TG (mM)	CHO (mM)	HDL–C (mM)	GLU (mM)	GLY (mM)	H/C
Vehicle	6.79	12.14 ± 2.14	12.51 ± 2.99	2.59 ± 1.06	87.94 ± 5.24	1.35 ± 0.30	0.21
 **2** 50 mg/kg	7.10 +4	10.85 ± 4.19 −11	9.18 ± 4.78 −27	2.23 ± 2.19 −14	93.2 ± 15.21 +5	1.16 ± 0.21 −14	0.24 +14
 **3** 50 mg/kg	7.73 +4	9.76 ± 1.74 −20*	8.27 ± 2.47 −34*	1.81 ± 1.44 −30	93.2 ± 7.45 +6	1.07 ± 0.09 −21	0.22 +4

(*continued on next page*)

Table 2 (*continued*)

Test sample	Food intake (g)	TG (mM)	CHO (mM)	HDL–C (mM)	GLU (mM)	GLY (mM)	H/C
 **4** 50 mg/kg	7.34 +8	7.31 ± 2.19 −40***	8.25 ± 2.9 −24*	2.72 ± 1.64 +5	89.73 ± 23.1 +2	0.94 ± 0.24 −30*	0.33 +57
 **12** 50 mg/kg	6.52 −4	14.66 ± 5.9 +20	9.40 ± 2.59 −25	2.47 ± 1.70 −5	91.52 ± 19.91 +4	1.25 ± 0.21 −8	0.26 +24
 **13** 50 mg/kg	7.51 +11	11.35 ± 6.85 −7	9.10 ± 4.01 −27	1.82 ± 1.38 −30	81.22 ± 15.02 −8	1.08 ± 0.38 −20	0.20 −5
Fenofibrate 108 mg/kg (Fig. 1)	—	−42*	−18	NC	—	−36**	+10

P <0.05 (*), *P* <0.01 (**), *P* <0.001 (***); NC = no change.

3 lowered the blood glucose levels to −17.8%, −14.8% at 5 h and −19.6%, −21.1% at 24 h, respectively. The five-membered furan and thiophene ester derivatives **9**, **10** and **11** were also lowered the blood glucose levels to −15.8%, −12.5%, −16.9% at 5 h and −18.5%, −14.9%, −21.5% at 24 h, respectively. The reference drug metformin lowered the blood glucose levels −23.5% at 5 h and −26.5% at 24 h in the same model (Table 1).

Activity guided fractionation and isolation work on *A. marmelos* led to identify the dyslipidemic activity in lupeol (**1**). Lupeol (**1**) lowered the triglyceride by 26%, cholesterol by 9%, glycerol by 10%, free fatty acids by 23% and increased the HDL–cholesterol by 44% (*P* <0.001) and the HDL/cholesterol ratio was 63% in hamster model at 100 mg/kg body weight.[10] Five novel ester derivatives of lupeol (**2**–**4**, **12** and **13**) were screened for their antidylipidemic activity in the same model at a dose of 50 mg/kg body weight. Nicotinic acid derivative **4** showed better lipid lowering profile than other derivatives. It significantly lowered 40% (*P* <0.001) in triglycerides, 30% (*P* <0.05) in glycerol, 24% (*P* <0.05) in cholesterol quantity. It also improved the HDL–cholesterol by 5%. It is also noteworthy to mention here that **4** also exhibited good antihyperglycemic activity. *p*-Nitrobenzoic acid derivative **3** showed good lowering in triglycerides (20%), cholesterol (34%), glycerol (21%), however **3** also lowered the HDL–cholesterol, which is undesired side effect in dyslipidimia treatment. Compound **2**, **12** and **13** showed good cholesterol lowering property; mild effect in lowering triglycerides, but these derivatives also possesses undesired effect (HDL–C lowering). The reference drug fenofibrate lowered the triglycerides 42%, cholesterol 18% and glycerol 36% at a dose of 108 mg/kg body weight in the same model (Table 2). There is increasing evidence that hepatic insulin resistance is associated with an increased production of free fatty acid and hypertriglyceridemia. The circulating triglycerides and free fatty acid are commonly elevated in obese and diabetic subjects and lower the ability of insulin to suppress hepatic glucose production by activating gluconeogenesis yet inhibiting glycolysis.[11] Reduction in the circulating triglycerides and other fatty acid by ester derivative such as **4** in our studies might be playing a major role to improve the hyperglycaemia and strengthen the insulin response.

In conclusion ester derivatives of abundantly available lupeol (**1**) was prepared and screened for their in vivo antihyperglycemic activity and also antidyslipidemic activity. Nicotinic acid derivative **4** turned out to be dual-acting (antihyperglycemic and antidyslipidemic activity) agent in our studies. Further work is in progress to find out the mechanism of action of **4** in dyslipidemic activity.

Acknowledgments

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Supplementary data

The spectral data (^{1}H, ^{13}C NMR, ES and FAB Mass), experimental procedure of novel synthetic derivatives and experimental procedure of biological activity of lupeol are available. Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2009.05.034.

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