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Synthesis of novel *N*-(2-hydroxy-2-*p*-tolylethyl)-amide and *N*-(2-oxo-2-*p*-tolylethyl)-amide derivatives and their antidyslipidemic and antioxidant activity ☆

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ABSTRACT

In continuation of our drug discovery program on metabolic diseases, we identified an alkaloidal amide, that is, Aegeline (**V**) from the plant *Aegle marmelos* leaves as a dual acting agent (antihyperlipidemic and antihyperglycemic). We therefore synthesized a series of alkaloidal amides [*N*-(2-hydroxy-2-*p*-tolylethyl)-amides and *N*-(2-oxo-2-*p*-tolylethyl)-amide derivatives] related to Aegeline and screened for their in vivo antihyperlipidemic activity in Triton induced hyperlipidemia model. The synthetic compounds **4**, **17** and **20** showed equipotent activity to the natural product, that is, Aegeline (**V**). These compounds also showed strong antioxidant activity, which support their antihyperlipidemic activity. Compound **12** showed better antihyperlipidemic and antioxidant profile than the natural product **V**.

When carbohydrates are in low supply or their breakdown is incomplete, fats become the preferred source of energy in diabetic patients. As a result, the fatty acids are mobilized into the general circulation leading to secondary triglyceridemia in which total serum lipids in particular triglycerides as well as the levels of cholesterol and phospholipids increase. 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. An increase in plasma lipids, particularly cholesterol, is a common feature of atherosclerosis, a condition involving arterial damage, which may lead to ischemic heart disease, myocardial infarction, and cerebrovascular accidents. These conditions are responsible for one-third of deaths in industrialized nations.[1]

The discovery of new drugs from traditional medicine is not a new phenomenon (Fig. 1). Christophe et al.[2] discovered glucose induced insulin secretion in vitro and ex vivo of 4-hydroxyisoleucine. Furthermore, in type 2 diabetic rat model the compound was active and partly corrected hyperglycemia and glucose tolerance. Recently we have reported the lipid lowering activity of 4-hydroxyisoleucine in high fat diet fed hamster model.[3] Metformin (**I**) is currently used as antidiabetic agent in the treatment of type 2 diabetes. Metformin 1 and its analogues[4] were synthesized on the basis of a natural product lead, that is, galegine (**II**).[5] The synthetic cholesterol lowering statins such as fluvastatin (**III**),[6] cerivastatin[7] were synthesized on the basis of natural product lead, that is, mevastatin (**IV**).[8] The plant derived saponin derivative, pamaqueside (CP-148623),[9] has been reported for cholesterol absorption up to 35–40% and the fish oils, which contain fatty acids such as eicosapentaenoic acid and docosahexaenoic acids, have been reported for their lowering activity on triglycerides and cholesterol.[10] The plant *Aegle marmelos* is commonly known as 'bael' in India,[11] belongs to the family of Rutaceae, widely of diabetes mellitus. Our bioactivity-guided fractionation and isolation work led to discovery of Aegeline (**V**) as an antihyperglycemic and antidyslipidemic principle.[12] Chatterjee and Bose[13] isolated the Aegeline (**V**) for the first time in 1952 and its chemical structure and stereochemistry has been elucidated by synthesis.[14] Various methods for the synthesis of optically active Aegeline (**V**) have also been appeared in the literature.[15] As a part of our drug discovery program on antidyslipidemic agents, we synthesized several analogue compounds of the natural product **V** (Aegeline) and studied their antidyslipidemic activity in Triton induced hyperlipidemia model (Fig. 2).

The synthesis of alkaloidal amide derivatives started with a reaction between *p*-methyl phenacyl bromide (**1**) and NaN_3 in MeOH to provide *p*-methylphenacylazide (**2**). The phenacyl azide (**2**) was reduced to give common synthon, *p*-methyl phenylacetamide (**3**) and the resultant *p*-methyl phenylacetamide was reacted with various acid/acid chlorides such as substituted cinnamic acids, substituted benzoic acid, phenylacetic acids and

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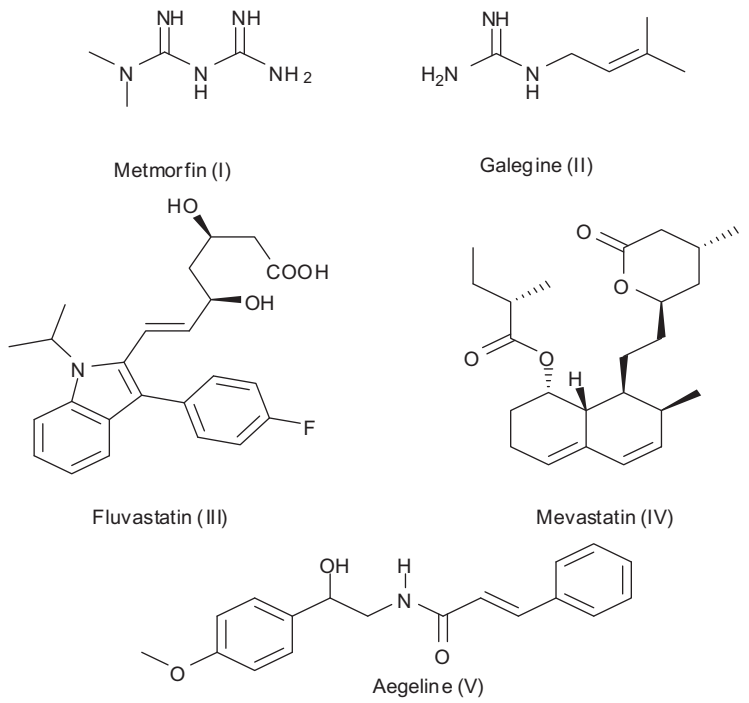


Figure 1. Naturally occurring and synthetic antidiabetic and antidyslipidemic agents.

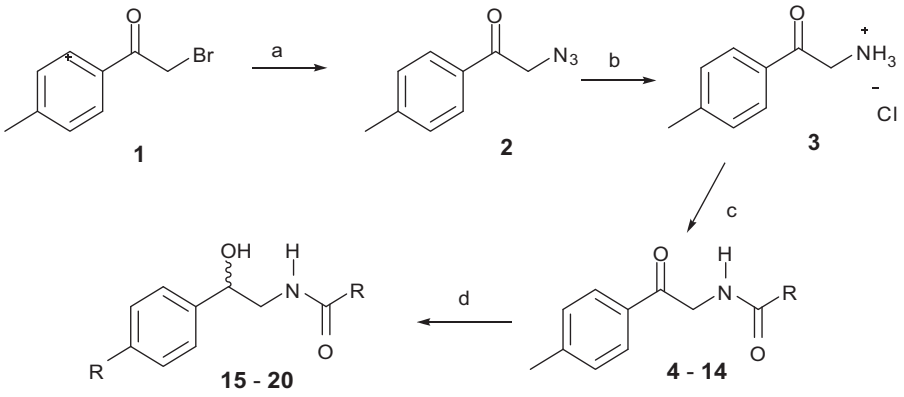


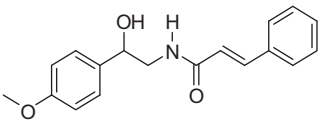
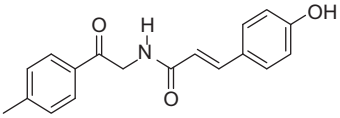
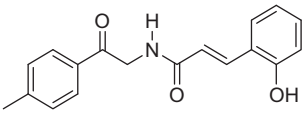
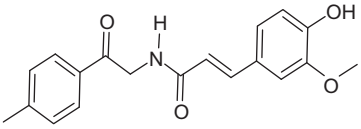
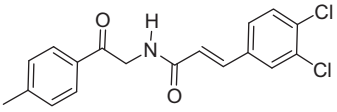
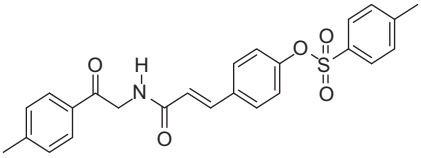
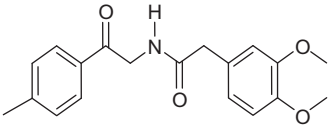
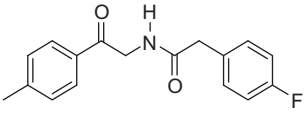
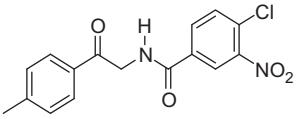
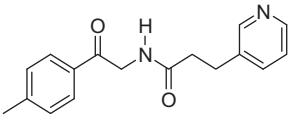
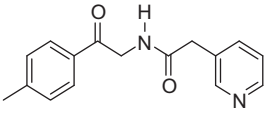
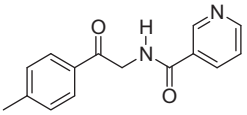
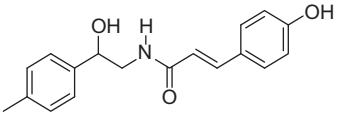
Figure 2. Synthesis of Aegeline analogues. Reagents: (a) NaN_3, MeOH and H_2O. (b) 10% Pd/C, concd HCl and dry MeOH. (c) acid, DEA, DIC, DCM and 0 °C to rt. (d) $NaBH_4$ and MeOH.

heterocyclic acids to provide the various amides **4**–**14** (Table 1) and subsequent reduction of ketone with $NaBH_4$ in MeOH at room temperature resulted in the synthesis of *p*-methyl phenylethanolamine derivatives **15**–**20** (Table 1). The double bond geometry has been determined as trans for compounds **4**–**8** and **15**–**18** by their coupling constant values in ^{1}H NMR spectral data. The optical rotation values of **15**–**20** indicated that these compounds appear to be mixture of enantiomers.[16]

Male rats of Charles forest strain (100–150 g) were divided into four groups [control, triton-induced, triton+compound (**4**–**20**), and Gemfibrozil (100 mg/kg) treated] containing six rats in each group. In this experiment of 18 h, hyperlipidemia was developed by administration of triton WR-1339 (Sigma chemical company, St. Louis, MO, USA) at a dose of 400 mg/kg. b.w. intraperitoneally to animals of all the groups except the control. These derivatives were macerated with gum acacia (0.2% w/v), suspended in water and fed simultaneously with triton with a dose of 100 mg/kg po to the animals of treated group and the diet being withdrawn. Animals of control and triton group without treatment with compounds **4**–**20** were given same amount of gum acacia suspension (vehicle). After 18 h of treatment the animals were anaesthetized with thiopentone solution (50 mg/kg b.w.) prepared in normal saline and then 1.0 ml blood was withdrawn from retro-orbital sinus using glass capillary in EDTA coated Eppendorf tube (3.0 mg/ml blood). The blood was centrifuged at 4 °C for 10 min and plasma was separated. Plasma was diluted with normal saline (ratio of 1:3) and used for analysis of total cholesterol (TC), triglycerides (TG) and phospholipids (PL) by standard enzymatic methods[17,18] and post-heparin lipolytic activity (PHLA) were assayed using spectrophotometer and Beckmann auto-analyzer and standard kits purchased from Beckmann Coulter International, USA.[19]

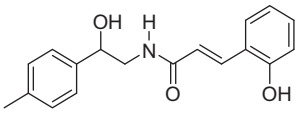
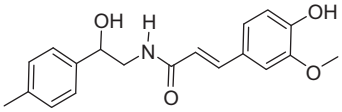
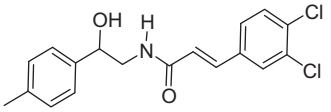
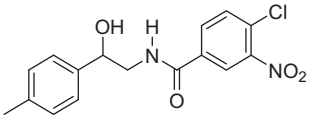
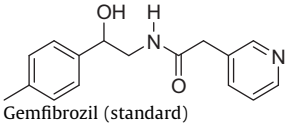
The naturally occurring Aegeline (**V**) lowered the TC by 24%, TG by 22% and PL by 23%, protein by 24% and also reactivated the post-heparin lipolytic activity (PHLA) by 15% as compared to control at a dose of 100 mg/kg.[12,20] All the synthesized compounds were also screened in the same model at a dose of 100 mg/kg. In the

Table 1
Antihyperlipidemic activity of keto-amide (**4**–**14**) and amino-alcohol (**15**–**20**) series compounds in Triton induced hyperlipidemia model at a dose of 100 mg/kg

S. No	Compound	Total cholesterol[a]	Triglyceride[a]	Phospholipid[a]	Protein[b]	PHLA[c]
V	 AEGELINE	−24***	−22**	−23***	−24***	+14*
04		−22**	−21**	−23***	20**	+19*
05		−11*	−09	−10*	−08 NS	+10*
06		−05	−10*	−06	−10*	+13*
07		−12*	−12*	−12*	−13*	+06
08		−07	−04	−09	−06	+07
09		−10*	−12*	−14*	−14*	+12*
10		−14*	−11*	−15*	−19*	+06
11		−04	−09	−05	−09	+08
12		−24***	−22**	−24***	−21**	+20**
13		−07	−05	−07	−07	+08
14		−04	−02	−06	−04	+06
15		−07	−09	−09	−08	+07

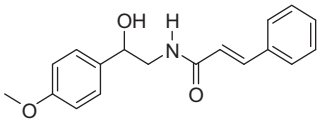
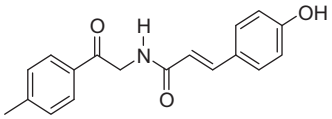
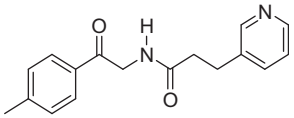
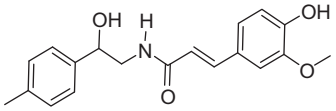
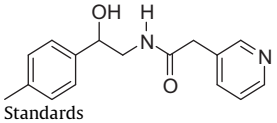
(*continued on next page*)

Table 1 (*continued*)

S. No	Compound	Total cholesterol[a]	Triglyceride[a]	Phospholipid[a]	Protein[b]	PHLA[c]
16		−08	−09	−07	−08	+05
17		−24***	−23***	−22**	−21**	+21**
18		−16*	−14*	−13*	−15*	+14*
19		−05	−11*	−07	−11*	+08
20		−23***	−25***	−22**	−23***	+19*
	Gemfibrozil (standard)	−33***	−31***	−36***	−31***	+23***

Units of keto-amide series compounds: a: mg/dl; b: g/dl; c: n mol of free fatty acids formed/h/ml of plasma; d: Triton treated group compared with control and Triton plus drug treated group compared with Triton group: Values are mean ± SD of six rats, ***p <0.001; **p <0.01; *p <0.05; values without star = Non significant.

Table 2
Antioxidant activity of Aegeline and synthetic compounds

S. No	Compound	Dose (μg/ml)	Super oxide anions (O_2^-)[a]	Hydroxyl radicals (·OH)[b]	Microsomal lipid peroxidation[b]
V		100 200	−19* −25***	−18* −22**	−12* −31***
	Aegeline				
4		100 200	−17* −31***	−17* −29***	−22** −29***
12		100 200	−24*** −31***	−19* −33***	−19* −29***
17		100 200	−21** −32***	−18* −27***	−19* −23***
20		100 200	−17* −31***	−17* −29***	−22** −29***
	Standards	200	−69*** (Allopurinol)	−45*** (Mannitol)	−63*** (α-tocoferol)

Units: a: n mole formazone formed/minute. b: n mole MDA formed/h. Values are mean ± SD of six rats, ***p <0.001; **p <0.01; *p <0.05.

keto-amide series (**4**–**14**) compounds **4** and **12** showed good lipid lowering activity similar to the natural products. Compound **4** lowered the TC by 22%, TG by 21% PL by 23%, Protein by 20% and PHLA reactivation by 19%, where as **12** lowered the TC by 24%, TG by 22%, PL by 24%, Protein by 21% and reactivation of PHLA by 20%. In the hydroxyl amine series (**15**–**20**) compound **17** and **20** showed good lipid lowering activity. Compound **17** lowered the TC by 24%, TG by 23% and PL by 22%, Protein by 21% and PHLA reactivation by 21%. The amino-alcohol derivative with heterocyclic acid **20** lowered the TC by 23%, TG by 25% and PL by 22%, Protein by 23% and PHLA reactivation by 19%.

Recent studies have demonstrated that the generation of large quantities of reactive oxygen species can cause activation of lipid peroxidation, protein modification, which leads to cardiovascular diseases (CVD).[21] Therefore we have screened Aegeline (**V**) and synthetic compounds (**4**–**20**) for their antioxidant activity (Table

2). Superoxide anions were generated enzymatically[22] by xanthine (160 mM), xanthine oxidase (0.04 U) and nitroblue tetrazolium (320 mM) in the absence or presence of compounds (100 mg/ml) in 100 mM phosphate buffer (pH 8.2). Fractions were sonicated well in phosphate buffer before use. The reaction mixtures were incubated at 37 °C and after 30 min the reaction was stopped by adding 0.5 ml glacial acetic acid. The amount of formazone formed was measured at 560 nm on a spectrophotometer. Percentage inhibition was calculated taking absorption coefficient of formazone as 7.2×10^3 M/cm. In another set of experiment, an effect of compounds on generation of hydroxyl radicals (OH^-) was also studied by non-enzymic reactants.[23] Briefly OH^- was generated in a non-enzymic system comprised of deoxyribose (2.8 mM), $FeSO_4{\cdot}7H_2O$ (2 mM), sodium ascorbate (2.0 mM) and H_2O_2 (2.8 mM) in 50 mM KH_2PO_4 buffer, pH 7.4 to a final volume of 2.5 ml. The above reaction mixtures in the absence or presence of compounds (100 mg/ml) were incubated at 37 °C for 90 min. Reference samples and reagent blanks were also run simultaneously. Malondialdehyde (MDA) content in both experimental and reference samples were estimated spectrophotometrically by thiobarbituric acid method as mentioned above.[18,24]

The natural products **V** exhibited moderate antioxidant activity at 200 μg/ml concentration. The compounds **4**, **12**, **17** and **20**, which are active in hyperlipidemia studies exhibited better antioxidant activity than the natural products at the same concentration (Table 2).

In conclusion a series of alkaloidal amides (**4**–**20**) related to natural product Aegeline (**V**) have been synthesized in our laboratory and screened for their antihyperlipidemic and antioxidant activity. Some of the compounds of this series (**4**, **17** and **20**) are equipotent to the natural product **V** in antihyperlipidemia studies and better antioxidants than the natural product **V**. Compound **12** showed better antihyperlipidemic and antioxidant profile than the natural product **V**. Our preliminary structure activity relationship data indicated that *p*-methylphenylacetamide derivatives (**4** and **12**) are as potent as *p*-methyl phenylethanolamine derivatives (**17** and **20**). It appears that stereochemistry is not so important in the activity, however further studies are required to confirm the importance of stereochemistry. Further work is in progress to synthesize large number of compounds to confirm the structure activity relationship and also to evaluate the antihyperglycemic activity of the compounds presented in this manuscript to develop dual acting synthetic agent..

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Supplementary data

Supplementary data (spectroscopic characterization of all new compounds along with their IR, ^1H, ^{13}C spectra, Mass and biological screening) associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2011.08.099.

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16. Please see the Supplementary data for procedures for the synthesis of compounds, spectral data, stereochemistry, optical rotation and melting points of synthetic compounds.
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