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Letter

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Discovery of Natural Product Derived Labdane Appended Triazoles as Potent Pancreatic Lipase Inhibitors

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KEYWORDS. Zingiberaceae, Curcuma amada, Triazoles, Pancreatic lipase inhibition, Obesity related disorders.

ABSTRACT: Obesity contributes to the genesis of many metabolic disorders including dyslipidemia, coronary heart disease (CHD), nonalcoholic fatty liver, type 2 diabetes etc. Pancreatic lipase plays a vital role in food fat digestion and absorption. Therefore to control obesity, inhibition of pancreatic lipase is the active therapy. Thus, a novel natural product derived labdane appended triazoles with pancreatic lipase inhibition potential was designed and synthesized. Among these hybrids, **6b** and **6f** exhibited excellent inhibitory activity (IC_{50} 0.75 ± 0.02 μM and 0.77 ± 0.01 μM), slightly better than that of the positive control Orlistat (IC_{50} 0.8 ± 0.03 μM). Compounds **6c**, **6e**, and **6g-j**inhibited the PL comparable to that of positive control. Interestingly none of the compounds showed cytotoxicity (Hep G2) in the concentration ranging from 0.5-100 μM. Overall results reveal potential of labdane appended triazoles as anti-obesity agents.

Obesity is a medical condition with excess body fat accumulation to the extent which leads to serious health consequences. Obesity contributes to the genesis of many metabolic disorders including dyslipidemia,[1] coronary heart disease (CHD),[2] nonalcoholic fatty liver,[3] type 2 diabetes[4] etc. In 2017 WHO reported that the global obesity has almost tripled since 1975.[5] As per literature more than 340 million children and adolescents between 5 and 19 aged are overweight or obese.[5] As a result, physicians are prompted to take aggressive treatments in lifestyle changes, pharmacological interventions, and surgical therapies before a severe consequence become clinically apparent. Among these various treatments, pharmacotherapy is the most commonly used one.[6-7]

Pancreatic lipase plays a vital role in food fat digestion and absorption.[8] Therefore to control obesity, inhibition of pancreatic lipase enzyme is the active therapy. Among the existing medicines, Orlistat is one such precise drug used for the inhibition of pancreatic lipase. In recent times, natural/herbal products also considered as an alternative medicine for the treatment of obesity and related disorders.[9-10] Many plant-based extracts reported for the treatment of obesity and associated diseases. For instance, ethanolic extracts of *Terminalia paniculata* showed very good anti-lipase and anti-obesity activities.[11] Likewise, some terpenoid saponins from the leaves of *Acantho panaxsenticosus*,[12] the phenolic acids from fermented oats,[13] apple polyphenols,[14] and flavan-3-ol digalate esters of oolong tea are found to have substantial pancreatic lipase inhibitory property.[15]

Based on the traditional applications and its large medicinal properties in a long history of Ayurvedic medicine, we have selected *Curcuma amada Roxb*. for the exploration of its inhibition properties against the pancreatic lipase. *C. amada*, an edible ginger is one of the rhizomatous species in the Zingiberaceae family. The rhizomes of *C. amada* are a rich source of essential oils, and more than 130 phytochemicals are isolated,[16] which possesses various biological properties *viz*. antimicrobial, antioxidant, anti-inflammatory, anticancer, cardiovascular and gastrointestinal disorders etc.[16-19]

Therefore, in continuation of our focused on going research interest on natural products and natural product based lead molecule[20-22] identification for therapeutic applications, in the present work, we have isolated the major compound (*E*)-Labda-8(17),12-diene-15,16-dial. This was synthetically transformed to rationally designed triazole appended analogues and

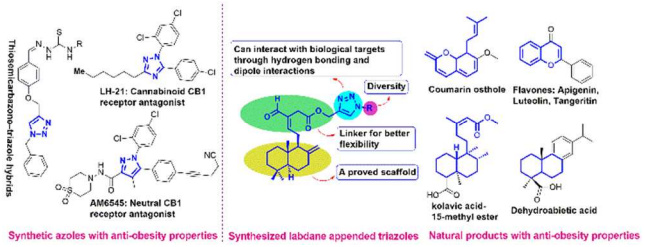
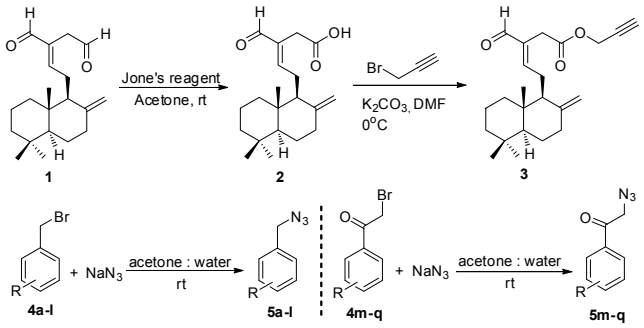


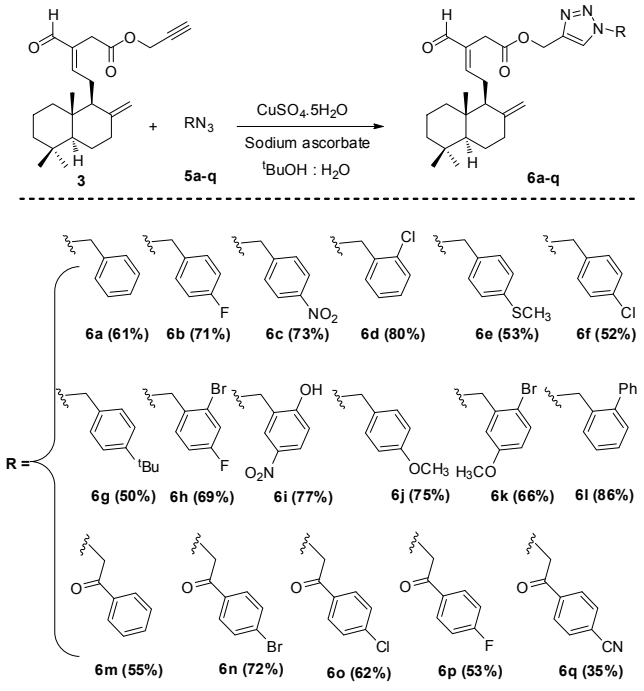
Figure 1. Rationale for the synthesis of labdane appended triazoles

- evaluated for their pancreatic lipase inhibitory potential. The rationale for the targeted synthesis of triazole appended analogues is based upon its broad spectrum of biological properties.[23-24] Triazole and pyrazole based synthetic drugs are also well-known for the treatment of obesity and related disorders (**Figure 1**).[25-27] To begin with, the fresh rhizomes of *C.amada* were collected from CTCRI, Thiruvananthapuram India in February 2017. By following standard protocol (**supporting information**) compound **1** (*E*)-labda 8(17), 12-diene -15, 16-dial was isolated from the chloroform extract as a colorless solid and confirmed by using various spectroscopic data IR, ^{1}H NMR, ^{13}C NMR, HRMS, etc.[19,28] A series of variously substituted triazole appended labdane derivatives were prepared by a three-step protocol starting from compound **1**. Compound 1, when subjected to Jone's oxidation, one of the aldehydes of compound 1 is selectively oxidized into acid derivative, Zerumin A (2). The alkyne intermediate (**3**) of Zerumin A, prepared in excellent yields by treating **2** with propargyl bromide (**Scheme 1**). The propargylated labdane undergoes the click reaction with various substituted benzyl -

Scheme 1. Synthesis of propargylated labdane (3) and substituted benzyl and phenacylazides (5a-q).

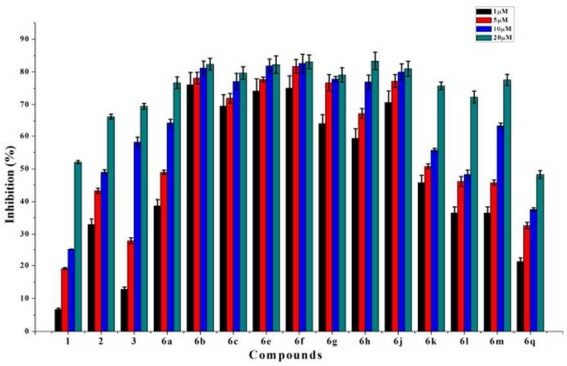


Scheme 2. Synthesis of labdane appended triazoles by Click chemistry (6a-q)



- and phenacyl azides (**5**) at room temperature to provide 1,2,3- triazole appended labdane derivatives **(6a-q)** in good to excellent yields (**Scheme 2**). All the synthesized derivatives are fully characterized by IR, NMR and HRMS spectral data (**supporting information**).

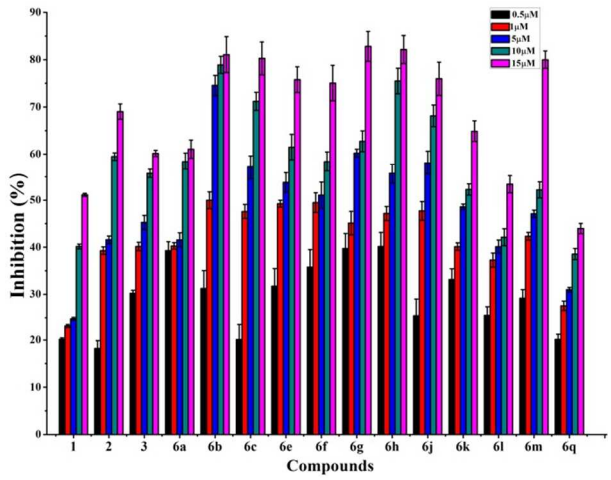
The isolated *E*-labda 8(17), 12-diene -15, 16-dial **(1)**, semi-synthetic intermediates and targeted triazole appendages were evaluated for the inhibitory activity against pancreatic lipase (PL) (Method A)[29] (**supporting information**). The parent molecule labdane dial exhibited moderate activity. Among the tested, majority of the triazole hybrids showed strong PL inhibitory activity in the concentration ranging from 0.75-14.63 μM. Initially, the percentage of enzyme inhibition of compounds at various concentrations (1, 5, 10, and 20 μM) was performed. At lower concentration of 1 μM, compounds **6b**, and **6f** showed inhibition of 75 to 80 %. At 20 μM concentration the derivatives **6b, 6c, 6e, 6f, 6g, 6h** and **6j** exhibited maximum inhibition percentage of 80 to 85 (**Figure 2**). The other compounds (**6a, 6k, 6l, 6m** and **6q**) also showed better tendency to inhibit the PL. In contrast, few derivatives (**6d, 6i, 6n, 6o,** and **6p**) failed to show any significant effect on pancreatic lipase. Overall, the labdane-triazole hybrids inhibited the PL through the concentration dependent manner.



Isolates and synthetic derivatives are tested at concentrations 1-20 μM. Each experiment was independently performed four times in duplicates and expressed as mean ± SD (n=4).

Figure 2. The percentage inhibition of isolates and synthetic derivatives against pancreatic lipase in various concentrations.

In an attempt to validate the potential of the compounds, the PL inhibitory activity was repeated using Human Pancreatic lipase (Method B)[30] (**supporting information**). As expected, the compounds followed the similar trend in exhibiting the inhibitory potential with IC_{50} values in the range of 1.10-14.48 μM concentration. The derivatives **6b, 6c, 6g,** and **6h** presented maximum PL inhibition percentage of 80 to 85 at 15 μM concentration (**Figure 3**). Similarly, triazole analogues **6d, 6i, 6n, 6o,** and **6p** have failed to inhibit the Human Pancreatic lipase. The IC_{50} values of active compounds with maximum inhibitory potential are calculated and, summarized in **Table 1**. The IC_{50} results revealed (Method A) that the labdane traizole appendages **6b** and **6f** exhibited an excellent inhibitory activity with 0.75 ± 0.02 μM and 0.77 ± 0.01 μM respectively, which is slightly better than that of positive control Orlistat (IC_{50} 0.8 ± 0.03 μM). Compounds **6c, 6e,** and **6g-j** inhibited the pancreatic lipase comparable to that of positive control in the range IC_{50} 0.8 to 0.9 μM. Whereas, the parent molecule **1**, -



Isolates and synthetic derivatives are tested at concentrations 0.5-15 μM. Each experiment was independently performed four times in duplicates and expressed as means ± SD (n=4).

Figure 3. The percentage of isolates and synthetic derivatives against Human pancreatic lipase in various concentrations.

Table 1. 50% inhibitory concentration (IC_{50}) evaluation of labdane appended triazoles.

	IC_{50} (μM)[a]	
Compound	**Method A**	**Method B**
1	14.63 ± 0.11	14.48 ± 0.51
2	10.30 ± 2.71	11.75 ± 0.64
3	8.64 ± 3.13	7.38 ± 0.77
6a	5.35 ± 1.20	9.15 ± 0.27
6b	0.75 ± 0.02	1.1 ± 0.53
6c	0.85 ± 0.03	2.01± 0.73
6e	0.80 ± 0.05	1.65 ± 0.93
6f	0.77 ± 0.01	1.25 ± 0.78
6g	0.91 ± 0.02	2.28 ± 0.88
6h	0.95 ± 0.02	2.31± 0.62
6j	0.84 ± 0.07	1.87± 0.14
6k	4.43 ± 1.02	6.90 ± 0.73
6l	10.36 ± 2.20	13.48 ± 0.53
6m	6.22 ± 2.04	7.82 ± 0.48
Orlistat	0.80 ± 0.03	0.19 ± 0.62

[a]The IC_{50} values of PL inhibitory activity for selected compounds and the positive control Orlistat. Values are mean ± SD of four independent experiments performed in duplicates.

- semisynthetic intermediates **2** and **3**, and labdane triazole derivatives **6a, 6k, 6l** and **6m** showed moderate potency in the range of IC_{50} 5.35 - 14.63 μM. However, against the Human pancreatic lipase (Method B), labdane triazole appendages **6b** and **6f** being the more potent analogues exhibited the inhibitory activities with IC_{50} values 1.1 ± 0.53 μM and 1.25 ± 0.78 μM respectively. Compounds **6c**, **6e**, and **6g-j** also showed promising ability in inhibiting the PL with IC_{50} values in the range of 1.65 to 2.31μM. The parent molecule and other derivatives showed moderate activity. The preliminary studies also revealed that the compounds are bound to the active site of PL and probably exhibited a covalent interaction with PL. Nevertheless, extended kinetics and crystallographic studies to be conducted for further authentication of a mode of interaction. We believe this is the first report on the pancreatic lipase inhibitory activity of labdane dial and its semi-synthetic triazole appendages.

In a structure-activity relationship point of view, we observed that the labdane-triazole hybrids incorporating benzyl azides **(6a-l)** were more active than the phenacyl azides **(6m-q)**. Most of the analogues synthesized from benzyl azides exhibited excellent inhibition property slightly better than or equal to that of Orlistat. In contrast, the triazole analogues synthesized from phenacyl azides **(6m-q)** did not show any significant inhibition potential except for the compound **6m**. In precise, among the triazoles incorporated from the variously substituted benzyl azides, all the para-substituted analogues showed the lowest IC_{50}. However, the unsubstituted **(6a)**, ortho- and meta-substituted benzyl azide incorporated triazole appendages showed moderate activity. Interestingly, there was no clear trend followed by the nature of the para substitution, i.e., among the halogen, electron donating and electron withdrawing groups. Overall, among the various substituted hybrids **6b** and **6f** with *p*-F and *p*-Cl substituted benzyl azide incorporated triazole appendages were found to be the most potent candidates of the series.

The molecular docking studies have also performed[31] in the present study to understand the basic knowledge on how these compounds have blocked human pancreatic lipase activity. There were twenty compounds docked on human pancreatic lipase to understand their possible binding site with respect to the binding energies of structure conformations of compounds (**Figure 4**; **Figure S**, **supporting information**). The top ranked stable structure conformations on protein have shown least Gibb's free binding energies. The docking results have shown that all the triazole derivatives are able to interact with lipase at possible predicted site with different binding energies (**Table S; supporting information**). However, further experimental studies (*ie,* site directed mutagenesis) are much needed to understand the precise mechanism of action of these compounds. This data provided us a vital clue about the interaction sites, which helps in our future in-depth studies towards lipase inhibition.

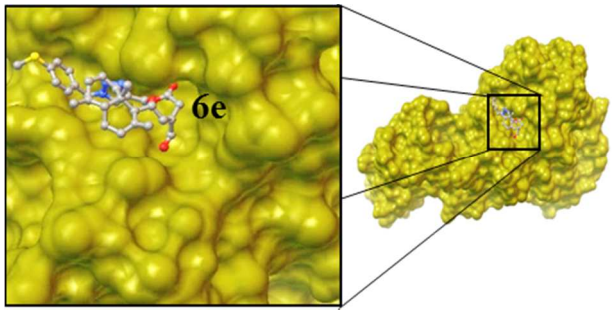
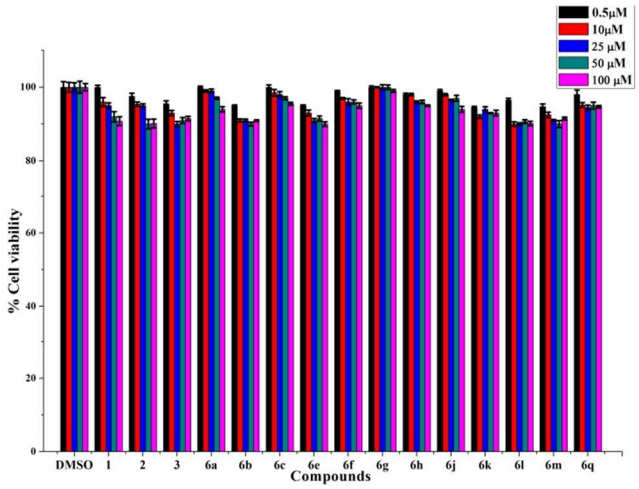


Figure 4. The representative Molecular docking studies of compound **6e.**

Further, in vitro toxicity of isolated and semi-synthetic derivatives is carried out to analyze the effect of compounds on human liver cell lines. This method is primarily used to identify potentially hazardous compounds and their toxic effect in

the early stage of development of therapeutic drugs. The cytotoxicity of compounds on Hep G2 human liver carcinoma-derived cell lines was measured by MTT assay.[32] The cytotoxic effect of each compound was estimated by calculating the percentage of cell viability in a dose-dependent manner ranging from 0.5 μM to 100 μM (**Figure 5**). Based on the percentage of cell viability, compound **6a, 6c, 6f, 6g, 6h, 6j, 6k** and **6q** were found to be the least toxic on Hep G2 human liver cell lines, and none of the compounds showed any signs of toxicity at all the tested concentrations.



Cytotoxic effect of each compound is expressed as percentage of cell viability in dose dependent manner. Values are mean ± SD of four independent experiments performed in duplicates.

Figure 5. Cytotoxic study of isolates and selected semi synthetic derivatives by MTT assay.

In summary, a new series of natural product derived labdane-triazole hybrids were designed, synthesized and evaluated for pancreatic lipase inhibitory potential. Among the semi-synthetic derivatives, **6b** and **6f** are most active candidates of the series with excellent PL inhibitory activity slightly higher than that of the positive control Orlistat. Hybrids **6c, 6e,** and **6g-j** inhibited the PL comparable to that of positive control. The structure-activity relationship studies suggested that the *p*-substitution on benzyl azides seems to be vital for improved PL inhibition. Cytotoxicity of the compounds on Hep G2 human liver carcinoma-derived cell line was measured by MTT assay. Based on the percentage of cell viability, none of the compounds showed any signs of toxicity at all the tested concentrations. This is the first report on the PL inhibitory activity of labdane dial and its semi synthetic triazole appendages. Our findings will provide useful insights for the design and synthesis of novel PL inhibitors. Presently, a detailed study to elucidate the molecular mechanistic action of an anti-obesity effect of the compounds **6b** and **6f** by using in vitro and in vivo experimental models and structural optimization are in progress in our laboratory.

ASSOCIATED CONTENT

Supporting Information.

The Supporting Information is available free of charge on the ACS Publications website.

Experimental details of synthesis, pancreatic lipase activity assay MTT assay, Molecular docking studies, characterization data and copies of ^{1}H, ^{13}C NMR spectra of all the newly synthesized compounds (PDF).

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Author Contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

Notes

The authors declare no competing financial interest.

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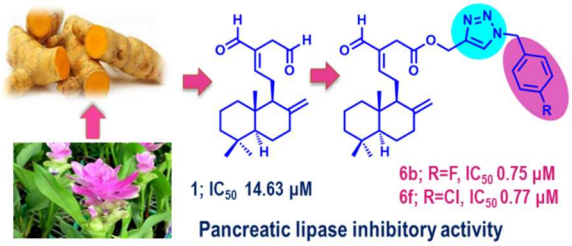
1; IC50 14.63 µM
6b; R=F, IC50 0.75 µM
6f; R=Cl, IC50 0.77 µM
Pancreatic lipase inhibitory activity

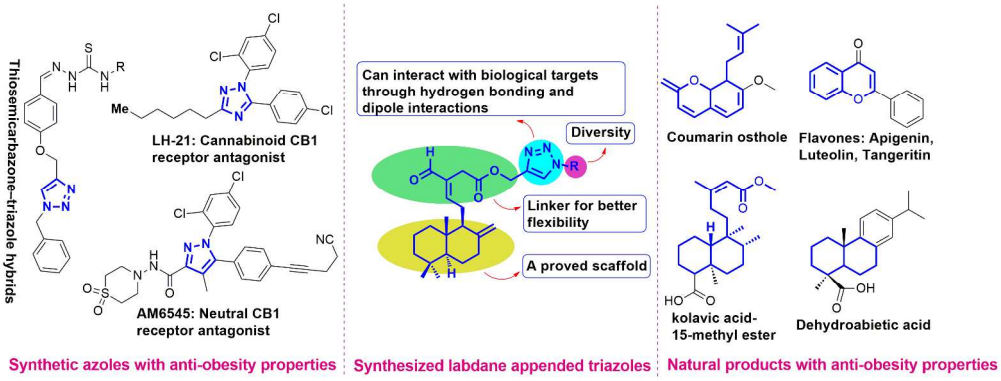

Figure 1. Rationale for the synthesis of labdane appended triazoles

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