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Review article:

INHIBITORS OF PANCREATIC LIPASE: STATE OF THE ART AND CLINICAL PERSPECTIVES

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ABSTRACT

Obesity is a disorder of lipid metabolism and continues to be a global problem, ranking fifth for deaths worldwide. It also leads to diabetes, cardiovascular disorders, musculoskeletal disorders and some types of cancer. Obesity is regarded as the output of a long-term imbalance between energy intake and energy expenditure. Digestion and absorption of dietary lipids by pancreatic lipase, a major source of excess calorie intake, can be targeted for development of anti-obesity agents. Being the major factor of concern, food materials and edible plants are most widely studied for the anti-obesity activity, so that they can be incorporated in the routine diet. In this review, an attempt was made to present a current scenario of the bioactive compounds from plant and microbial origin that have been investigated for their pancreatic lipase inhibition. Compounds belonging to various classes of natural products such as alkaloids, carotenoids, glycosides, polyphenols, polysaccharides, saponins and terpenoids are well studied while lipophilic compounds from microbial sources are the most active against the pancreatic lipase. Few studies on the synthetic analogues, structurally similar to the triglycerides have been described in the review. Despite of tremendous research on the finding of potential pancreatic lipase inhibitor, very few compounds have entered the clinical studies and no new molecule after orlistat has been marketed. Along with HTS based screening, detailed structure-activity relationship studies on semi-synthetic and synthetic derivatives might also provide a direction for the development of potential lead(s) or pharmacophore for pancreatic lipase inhibition in order to treat and/or prevent obesity and related disorders.

Keywords: Pancreatic lipase, orlistat, obesity, natural products, clinical perspectives, lipid metabolism

INTRODUCTION

Tremendous health concerns have been raised over a dramatic increase in the prevalence of obesity and related metabolic disorders. Majorly considered as life style disorders of developed countries, obesity is prevailing at alarming speed in developing countries is because of industrialization, fast food intake, decrease in physical activity (Cairns, 2005). According to World Health Organization, 65 % of the world's population live in countries where overweight and obesity kills more people than underweight. More than 1.4 billion adults (age 20 and older) were overweight in 2008. Among them, over 200 million men and nearly 300 million women were obese (WHO, 2014).

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A vast range of health problems co-exist with a weight problem and dysfunction of lipid homeostasis. This interlinked network of metabolic disorders and its co-morbidities involve serious consequences in cardiovascular anomalies (heart failure, hypertension, pulmonary embolism etc.), endocrine imbalance (insulin resistance, glucose intolerance, hypothyroidism etc.), arthritis, urinary incontinence, gastrointestinal complications (gastroesophageal reflux disease, colon cancer, hepatic steatosis etc.). Apart from that obesity and related metabolic disorders disturb life style physically, financially and psychologically. Psychological effect like social discrimination, depression, physical inability etc. separates person from society (Aronne, 2002).

In brief, classification and treatment of the obese patients can be done on the basis of their body weight and height *i.e.* BMI (Kg/m^2). In general population, BMI ranges from 18.5 to 24.9, below and above of which are considered as underweight and overweight respectively. Risk to health starts with a BMI of 25, moderate risk is associated with a BMI of 30 to 34.9 and above which considered as very high risk. BMI above 40 is associated with highest risk of mortality. In terms of anatomy, obesity is classified according to the distribution of body fat deposition. Generally fat deposition occurs in abdomen region and subcutaneous. Visceral fat (gonadal, mesenteric, perirenal, epicardiac) represents a serious risk to health and associated with co-morbidities, whereas subcutaneous fat is not involved in metabolic complications. Some form of weight gain in patients results from drug treatments or certain diseases. It can be classified as secondary or iatrogenic obesity. Contrarily, obesity resulting from an imbalance in fat homeostasis in the body, is classified as primary (Gonzalez-Castejon and Rodriguez-Casado, 2011; Aronne, 2002).

DIFFERENT WAYS TO TREAT OBESITY

Strategic anti-obesity treatments broadly act through peripherally and/or centrally. Current scenario in drug discovery for anti-obesity therapeutics mainly focuses on following mechanisms for energy homeostasis.

1) Centrally acting: by regulation of food intake
2) Peripherally acting: by affecting absorption of dietary fat, affecting storage and metabolism of fat and/or increasing heat generation from dietary fat.

Body weight regulation and energy homeostasis can be viewed as multi-component feedback regulatory mechanisms which provide a vast number of intervening points as targets. In the long term, single point target for body weight management may activate compensatory mechanisms leading to failure of treatment (Barsh, 2000).

CURRENTLY AVAILABLE ANTI-OBESITY REGIME

Sibutramine

Sibutramine (**1**), a centrally acting phenethylamine class of drug currently approved for long-term treatment of obesity in adults, reduces food intake by selective inhibition of reuptake of noradrenaline, serotonin and dopamine and stimulation of sympathetic nervous system, resulting in thermogenesis and lipolysis. Common side effects of sibutramine are due to activation of sympathetic nervous system like dry mouth, insomnia, constipation, headache, anorexia, hypertension and palpitation (Elangbam, 2009) (Figure 1).

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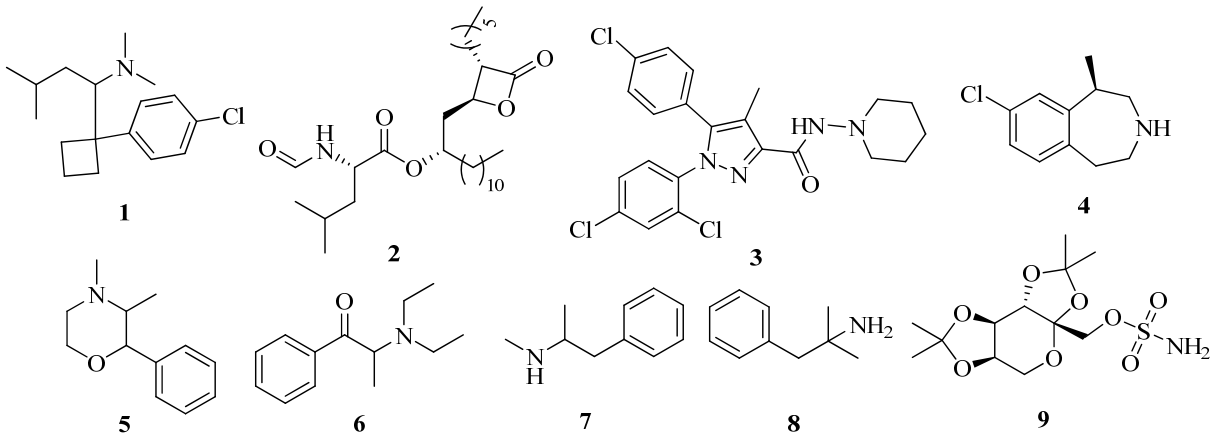

Figure 1: Currently available anti-obesity therapeutics

Orlistat

A potent inhibitor of gastric and pancreatic lipase, orlistat (**2**) is a hydrogenated derivative of lipstatin, produced by *Streptomyces toxytricini* and acts by diminishing the absorption of dietary fat. Orlistat forms a covalent bond with the active serine site of lipases and thus inactivates them to hydrolyze dietary fat. Adverse effects include liquid stools, steatorrhea, abdominal cramping and fat-soluble vitamin deficiencies, fecal urgency, incontinence, flatulence. These unpleasant gastrointestinal side effects are limiting its patient compliance (Kaila and Raman, 2008).

Rimonabant

Appetite regulation poses involvement of cannabinoid-1 (CB_1) receptor which on stimulation increases demand of food. Rimonabant (**3**) reduces food intake by blocking CB_1 receptors and enhances thermogenesis. Side effects include mood changes, nausea and vomiting, diarrhea, headache, dizziness and anxiety (Kaila and Raman, 2008).

Lorcaserin

Lorcaserin (**4**), a selective 5-HT_{2C} receptor agonist developed by Arena pharmaceuticals, has serotonergic properties and acts as an anorectic. 5-HT_{2C} receptors are located in various parts of the brain, including hypothalamus, activation of which leads to proopiomelanocortin production and results in the weight loss through hypophagia (Lam et al., 2008).

Other short term anti-obesity drugs like, phendimetrazine (**5**), diethylpropion (**6**), methamphetamine (**7**), phentermine (**8**) and topiramate (**9**) act centrally but their uses are restricted due to side effects (Elangbam, 2009).

ROLE OF PANCREATIC LIPASE IN LIPID DIGESTION AND ABSORPTION

The deeper understanding of the process of lipid homeostasis *i.e.* absorption, metabolism, transfer, storage, deposition and oxidation, has presented a wide variety of enzymatic targets involved. Dietary fats are mainly regarded as mixed triglycerides, which undergo a complex series of biochemical reactions before absorption in the gastrointestinal tract.

Pancreatic, endothelial, hepatic, lipoprotein lipases are members of the human lipase super family and possess structural similarity. Other tissues like lungs, kidney, skeletal muscles, adipose tissue and placenta also secretes lipase enzymes. Pancreatic acinar cells secrete pancreatic lipase (triacylglycerol acyl hydrolase EC 3.1.1.3), an important enzyme of pancreatic juice responsible for digestion of dietary triglycerides in the small intestine.

Gastric and lingual lipases are responsible for partial hydrolysis of dietary triacyl-

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glycerols into free fatty acids and diacylglycerols. This partial digestion in stomach forms large fat molecule which undergoes emulsification with bile salts to form small droplets of fat. A physical property of emulsion influences the efficiency of digestion. In the emulsion, dietary triglycerides and diglycerides in the center of droplet followed by a mixture of polar lipids, phospholipids, cholesterol, and free fatty acids and later coated with oligosaccharides, denatured proteins, and bile salts. This forms very complex structure. The pancreatic lipase interacts with emulsion droplet which continuously changes its physical properties as products formed, leaves the surface during the process of hydrolysis. Complete hydrolysis process results into free fatty acids, monoacylglycerols, diacylglycerols binds with cholesterol, bile salts, fat soluble vitamins and lysophosphatidic acid to form mixed micelles which can be absorbed by enterocytes. Pancreatic lipase uses a pancreatic protein colipase, as cofactor, to facilitate lipolytic activity. Phosphatidyl choline inhibits lipase-substrate complex. Colipase reverses this inhibition and helps lipase to interact with the scarce surface of the substrate and stabilizes its conformation (Shi and Burn, 2004; Mukherjee, 2003) (Figure 2).

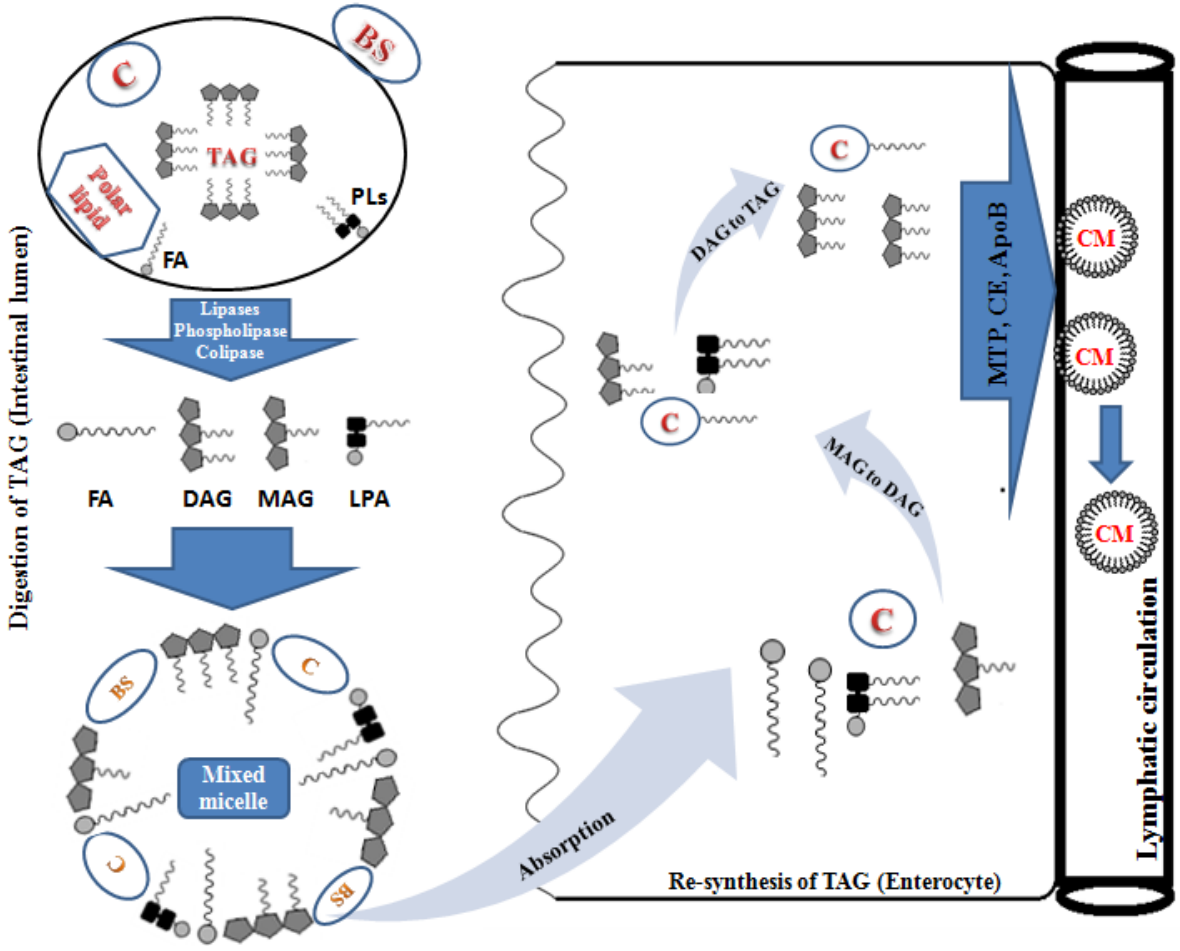

Figure 2: Digestion and absorption of dietary lipids; FA: fatty acids; PLs: phospholipids; C: cholesterol; BS: bile salts; TAG: triacylglycerol; DAG: diacylglycerol; MAG: monoacylglycerol; LPA: lysophosphatidic acid; MTP: microsomal triglyceride transfer protein; CE: cholesterol esters; ApoB: apolipoprotein B; CM: chylomicrons

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APPROACHES TOWARDS PANCREATIC LIPASE INHIBITION

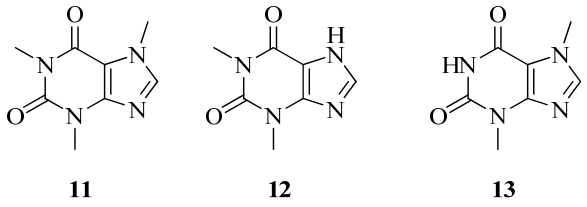
Pancreatic lipase inhibition is the most widely studied mechanism for the identification of potential anti-obesity agents. Only one blockbuster drug, orlistat, approved by FDA and available for the obesity treatment apart from the centrally acting anti-obesity drugs, is acting through the pancreatic lipase inhibition. Discovery of orlistat was done from the naturally occurring molecule lipstatin (**10**, see Figure 9, first scheme**)**. The success of naturally occurring compounds for treatment of obesity has influenced the research for the identification of newer pancreatic lipase inhibitors that lack unpleasant side effects. Till now, many plant extracts and isolated compounds were identified for the pancreatic lipase inhibition. Other than that, many microbial products and isolated compounds, basic protein protamines (Tsujita et al., 1996), ε-polylysine (Tsujita et al., 2006), polysaccharides like chitosan (Sumiyoshi and Kimura, 2006), dietary fibers from wheat bran and cholestyramine (Lairon et al., 1985), soya proteins (Roy and Schneeman, 1981), and synthetic compounds etc. have been studied for inhibitory potential against pancreatic lipase. However, plant and microbial origin isolated molecules were widely studied and reported for the pancreatic lipase inhibition.

PHYTOCHEMICALS AS SOURCE OF PANCREATIC LIPASE INHIBITORS

In the search for biologically active pancreatic lipase inhibitor as anti-obesity agents from natural resources, various plant extracts and their phytochemicals have been screened for their lipase inhibitory activity. Here are some classes of phytochemicals and standardized extracts as follow:

Alkaloids

Caffeine (**11**), theophylline (**12**) and theobromine (**13**) consumed as food components were found to inhibit the hydrolysis of tributyrin and tripalmitate catalyzed by human pancreatic lipase dose dependently. The highest lipase inhibition ratio in tripalmitate and tributyrin hydrolysis were observed as 25.74 % and 79.54 % with caffeine, 29.89 % and 62.79 % with theophylline and 21.08 % and 67.74 % with theobromine, respectively, at the tested dose ranges of 0.015-15 mM (Wikiera et al., 2012) (Figure 3).

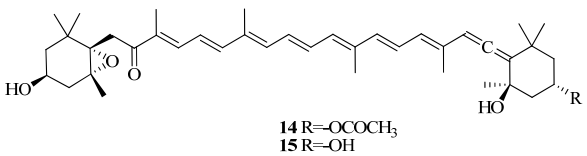


11 **12** **13**

Figure 3: Alkaloids as pancreatic lipase inhibitors

Carotenoids

Fucoxanthin (**14**), a major marine carotenoid found in edible seaweeds, such as *Undaria pinnatifida* and *Sargassum fulvellum*, and its metabolite fucoxanthinol (**15**), have been studied for its inhibitory activity on rat pancreatic lipases. Fucoxanthin is converted into fucoxanthinol in the gastrointestinal tract and released into the lymph. Both marine products have shown inhibition in the hydrolysis of triolein with an IC_{50} of 660 and 764 nM, respectively which is approximately 100-fold higher than the IC_{50} of orlistat (6.8 nM). Fucoxanthin or fucoxanthinol also have shown reduction in lymphatic triglyceride absorption and suppression in the increase in triglyceride concentration in systemic blood (Matsumoto et al., 2010) (Figure 4).



14 R=–$OCOCH_3$
15 R=–OH

Figure 4: Carotenoids as pancreatic lipase inhibitors

Glycosides

Acteoside (**16**), a major active constituent of Chinese tea, *Ligustrum purpurascens* (kudingcha tea) has shown inhibition of pan-

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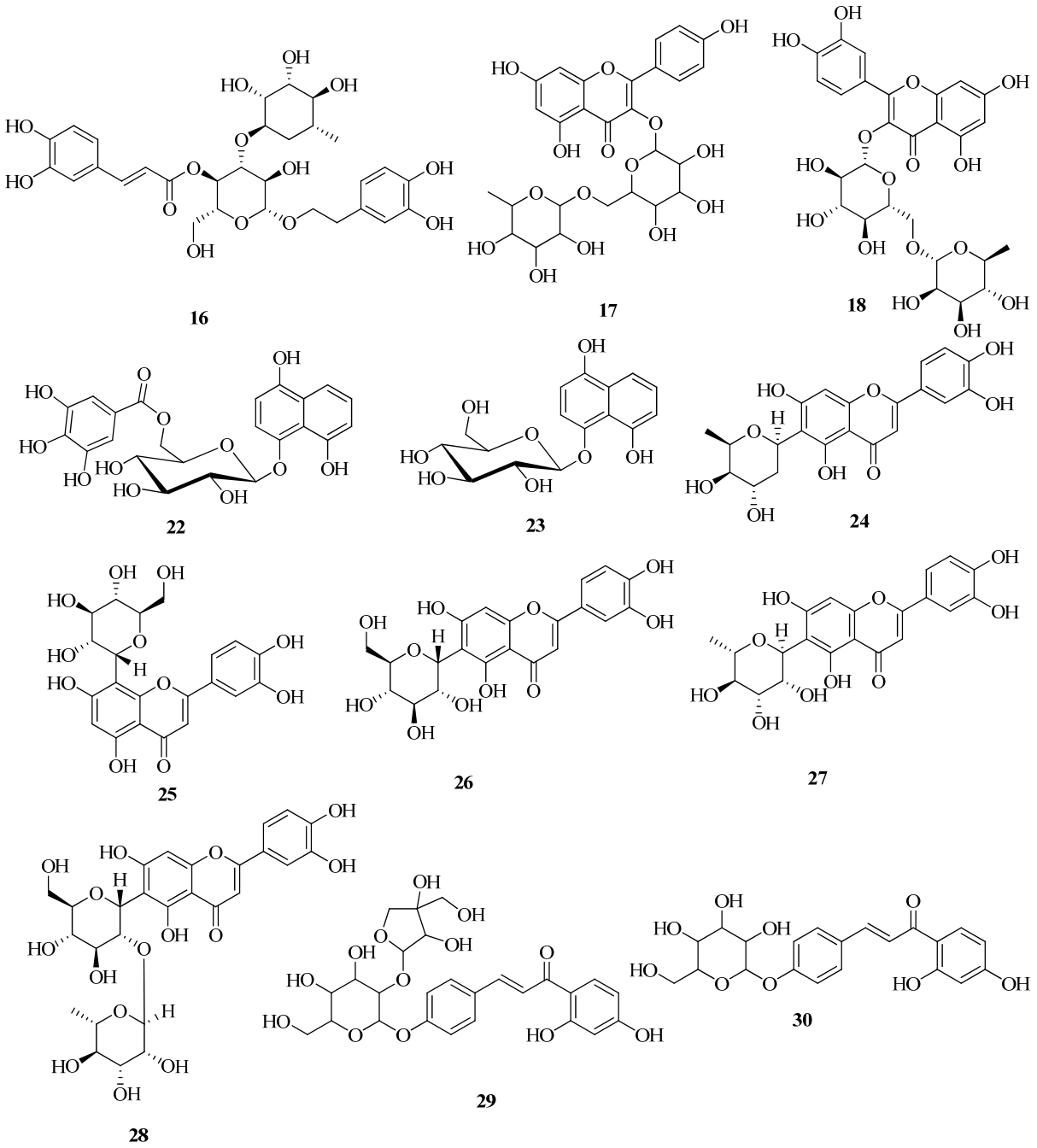

Figure 5: Glycosides as pancreatic lipase inhibitors

creatic lipase with binding to lipase at K_a of 1.88 x 10^4 L/mol. Docking results supported the hydrogen bonding of this molecule with Lys271, Leu272 and Thr68 of lipase which decreases the enzyme catalytic activity (Wu et al., 2014) (Figure 5).

Cassia auriculata (Caesalpiniaceae), a common Asian beverage and medicinal plant, has been traditionally used for diabetes, hyperlipidemia and various other disease conditions. Its crude ethanol extract of the aerial parts has been found to inhibit the pancreatic lipase with the IC_{50} of 6.0µg/mL. Further kaempferol-3-*O*-rutinoside (**17**), rutin (**18**), kaempferol (**19**, Figure 6a), quercetin (**20**, Figure 6a) and luteolin (**21**, Figure 6a), isolated from the *Cassia auriculata*, have been studied for the inhibition of pancreatic lipase. Kaempferol 3-*O*-rutinoside was found to be the most active with the IC_{50} of 2.9 µM while rutin, quercetin and luteolin has been shown weak inhibitory potential

(IC_{50}>100μM), however kaempferol was found almost inactive (IC_{50}>250 μM). Structures of rutin and kaempferol-3-*O*-rutinoside differ by one more hydroxyl group in ring B of the flavonoid skeleton which imparts a significant change in lipase inhibition. Kaempferol-3-*O*-rutinosides has also been present in the *Gingko biloba* and various species of the genus *Ficus*, which are also being reported for the fat-lowering effect *in vivo* (Habtemariam, 2013).

The aqueous extract of fruits of *Juglans mandshurica* possesses inhibitory potential towards pancreatic lipase *in vitro* in a dose dependent manner. The water extract was also found to inhibit increases in the level of plasma triacylglycerol after oral administration of a lipid emulsion in rats. Also, 1,4,8-trihydroxynaphthalene-1-*O*-β-D-[6′-*O*-(3′′,4′′,5′′-trihydroxybenzoyl)]glucopyranoside (**22**), isolated from water extract, showed the strongest inhibitory response of 88 % inhibition while a structurally related compound, α-hydrojuglone-4-glucoside (**23**) inhibited the pancreatic lipase activity by 32 % only at the concentration of 1 mM. However, Gallic acid was found inactive, suggesting the importance of the ester linkage between the galloyl moiety and α-hydrojuglone-4-glucoside (Han et al., 2007) (Figure 5).

Luteolin-6-*C*-β-D-boivinopyranoside (**24**), orientin (**25**), isoorientin (**26**), derhamnosylmaysin (**27**) and isoorientin-2-*O*-α-L-rhamnoside (**28**) from the methanolic extract of the leaves of *Eremochloa ophiuroides* (centipede grass), has reported to inhibit the pancreatic lipase with IC_{50} of 50.5 ± 3.9, 31.6 ± 2.7, 44.6 ± 1.3,25.9 ± 3.7 and 18.5 ± 2.6 μM respectively (Lee et al., 2010). Licuroside (**29**) and isoliquiritoside (**30**) from *Glycyrrhiza glabra* roots showed strong inhibition against pancreatic lipase with IC_{50} of 14.9 and 37.6 μM respectively (Birari et al., 2011) (Figure 5).

Polyphenols

Polyphenols represent the major class for the pancreatic lipase inhibitor. They bind to the enzyme by polyvalent sites present in them. Many fruits and herbal teas have been extensively studied for the pancreatic lipase inhibition due to the presence of polyphenols.

A flavonol, galangin (**31**), isolated from *Alpinia galanga* rhizomes was found to inhibit 50 % pancreatic lipase at 48.20 mg/mL. Further, galangin depicted inhibition of increased body weight, energy intake and parametrial adipose tissue weight induced by cafeteria diet. In addition, galangin was found to produce a significant decrease in serum lipids, liver weight, lipid peroxidation and the accumulation of hepatic triglycerides at the dose of 50 mg/Kg (Kumar and Alagawadi, 2013) (Figure 6a).

Hesperidin (**32**) and neohesperidin (**33**), isolated from the peels of *Citrus unshiu*, depicted reduction in the activity of the porcine pancreatic lipase with the IC_{50} of 32 and 46 μg/mL, respectively, while other flavonoids such as narirutin (**34**) and naringin (**35**) did not show any activity. Further, *in vivo* study on Sprague Dawley rat has shown reduction in plasma triglyceride level in the group fed on 10 % hesperidin as compared to control. However, ingestion of hesperidin has not shown any changes in daily food intake, body weight gain or food efficiency, but the fecal lipid content has increased, suggesting inhibition of pancreatic lipase (Kawaguchi et al., 1997) (Figure 6a).

3-*O*-caffeoyl-4-*O*-galloyl-L-threonic acid (**36**), isolated from *Filipendula kamtschatica* possessing pancreatic lipase's substrate like structure was found to inhibit the enzyme with half maximal concentration of 26 μM (Kato et al., 2012). Methyl chlorogenate (**37**), from the methanolic extract of the leaves of *Eremochloa ophiuroides* (centipede grass), has reported to inhibit pancreatic lipase with IC_{50} values 33.6 ± 2.0 μM (Lee et al., 2010) (Figure 6a).

Licochalcone A (**38**) was reported to inhibit pancreatic lipase with IC_{50} values of 35 μg/mL reversibly and non-competitively with a K_i value of 11.2 μg/mL based on a

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Lineweaver–Burk plot analysis (Won et al., 2007) (Figure 6a).

CT-II, a fraction of the aqueous ethanol extract of fruits of *Cassia mimosoides* L. var. *nomame* Makino (Nomame Herba) has shown *in vitro* porcine pancreatic lipase inhibitory activity with an IC_{50}< 0.1 mg/mL. Chemically, CT-II comprised of proanthocyanidin. The *in vitro* inhibitory activity has been extrapolated to high fat diet rodent model showing fecal fat excretion and suppression of liver stetosis. A dimeric flavan (2S)-3′,4′,7-trihydroxyflavan-(4α→8)-catechin (**39**) from hydromethanolic extract of the fruits showed IC_{50} of 5.5 µM in inhibiting pancreatic lipase (Yamamoto et al., 2000; Hatano et al., 1997) (Figure 6a).

7-Phloroeckol (**40**) has been reported as a significant pancreatic lipase inhibitor *via* bioassay-guided isolation of methanolic extract of brown algae, *Eisenia bicyclis*, with IC_{50} of 12.7 ± 1.0 µM (Eom et al., 2013) (Figure 6a).

Isoliquiritigenin (**41**) and 3,3′,4,4′-tetrahydroxy-2-methoxychalcone (**42**) from *Glycyrrhiza glabra* roots demonstrated strong inhibition against pancreatic lipase with IC_{50} values of 7.3 µM and 35.5 µM, respectively. Further, isoliquiritigenin was found to bind with the key amino acid residues of the pancreatic lipase active site (Birari et al., 2011) (Figure 6a).

Extract of peanut shell of *Arachis hypogaea* depicted inhibition towards lipases such as pancreatic lipase, lipoprotein lipase and hormone sensitive lipase, which may be due to the presence of phenolic constituents *viz*. luteolin, caffeic acid, benzoic acid, ferulic acid and fatty acids (Moreno et al., 2006a).

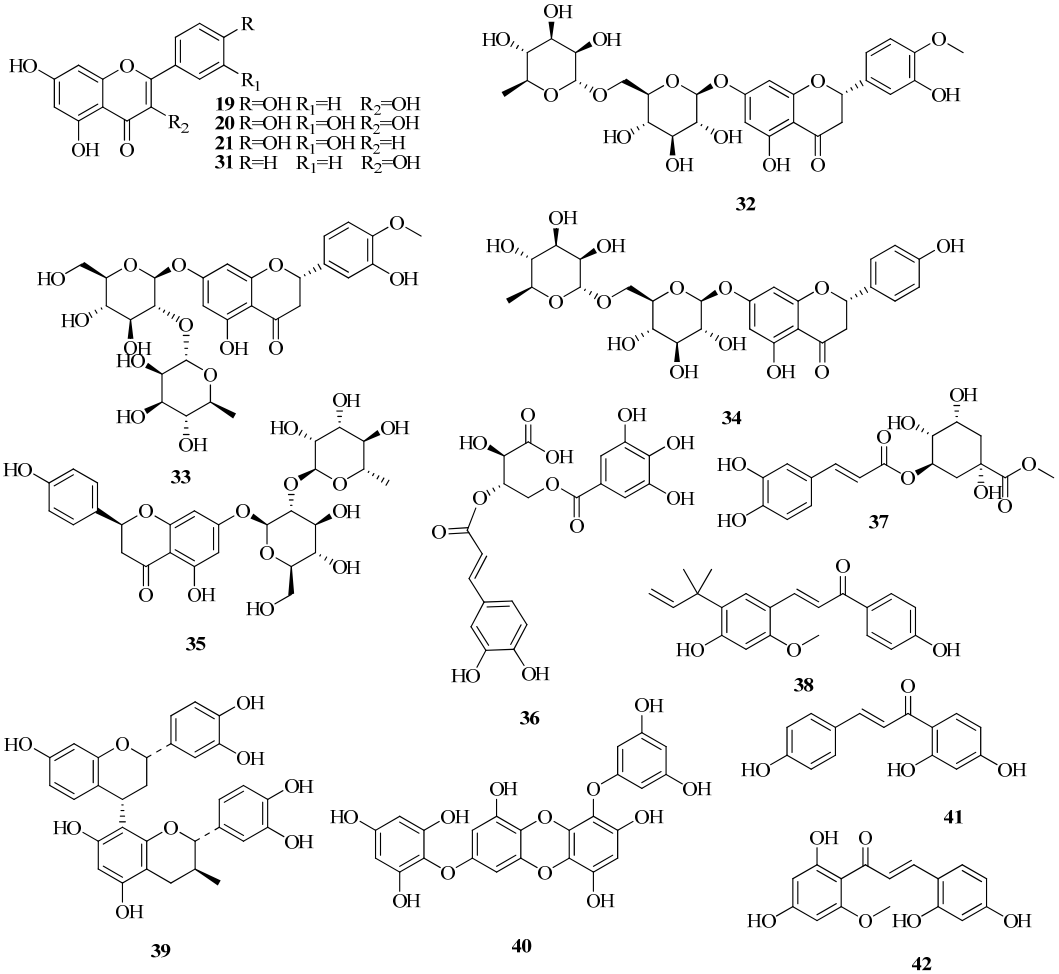

Figure 6a: Polyphenols as pancreatic lipase inhibitors

The EtOAc extract of *Cassia siamea* roots showed 74.3 % enzyme inhibition at 250 µg/mL concentration and bioassay guided fractionation of this extract provided cassiamin A (**43**), a bianthraquinone, as most active compound for pancreatic lipase inhibition with half maximal concentration of 41.8 µM (Kumar et al., 2013) (Figure 6b).

Ethanolic extracts of *Mangifera indica* L. stem bark and leaves exhibited strong inhibition of pancreatic lipase at a concentration of 1 mg/mL. Bark extract also found to reduce the activity of lipoprotein lipase by 75 % at a concentration of 1 mg/mL (Moreno et al., 2006b).

Oolong tea plant is a rich source of polyphenols. An aqueous decoction of the tea plant is widely used as refreshment drink, apart from traditional medicinal use. Flavan-3-ol monogallate esters, (-)-epigallocatechin-3-*O*-gallate (EGCG) (**44)** and flavan-3-ol digallate esters, (-)-epigallocatechin-3,5-digallate (**45**) have been reported to show pancreatic lipase inhibition with an IC_{50} of 0.349 and 0.098 µM respectively. Oppositely, nonesterified flavan-3-ols, such as (+)-catechin (**46**), (-)-epicatechin (**47**), (+)-gallocatechin (**48**), and (-)-epigallocatechin (**49**), were found inactive (IC_{50}> 20 µM). Oolonghomobisflavan A (**50**) and B (**51**, Figure 6b) and oolongtheanin 3′-*O*-gallate (**52**, Figure 6c) possess even more potent inhibition (IC_{50}=0.048, 0.108, and 0.068 µM, respectively) as compared to EGCG while monodesgalloyl (**53**) or didesgalloyl (**54**), oolonghomobisflavans were less active than oolonghomobisflavan A and B. Another interesting parameter found in the structural features of the oolong tea polyphenols is the polymerization as polymerization of flavan-3-ols readily happens by polyphenol oxidase or during the processing of oolong tea. The oolong tea polymerized polyphenols (IC_{50}=0.28 µg/mL), devoid of less-active monomeric flavan-3-ols, was reported to have 5 times stronger inhibition in comparison to the tannase-treated oolong tea polyphenols (IC_{50} =1.38 µg/mL).Based on the structure-activity relationship, it can be concluded that galloyl moiety and/or the polymerization of flavan-3-ols is prerequisite feature for the lipase inhibition. Other active polyphenols identified from oolong tea, prodelphinidin B-2,3,3′-di-*O*-gallate (**55**), assamicain A (**56**), theasinensin D (**57**), oolongtheanin-3′-*O*-gallate (**58**, Figure 6c), theaflavin (**59**, Figure 6d), and theaflavin-3,3′-*O*-gallate (**60**) have been found potent inhibitor against pancreatic lipase with IC_{50} of 0.107, 0.120, 0.098, 0.068, 0.106, and 0.092 µM, respectively (Nakai et al., 2005) (Figure 6d).

In China, *Nelumbo nucifera* leaves have been used to treat obesity. Extracts of leaves have shown lipase and α-amylase inhibition with half maximal concentration of 0.46 and 0.82 mg/mL and thereby inhibit absorption of dietary lipid and carbohydrates. Other than that, it also stimulates β_3 adrenoreceptor mediated lipolysis in 3T3-L1 adipocytes and upregulate UCP3 expression indicating thermogenesis in muscles (Ono et al., 2006).

Apple polyphenol extract (AP) and their procyanidin fractions and other polyphenol fractions, had been reported for inhibition of pancreatic lipase with an IC_{50} of 5.6, 1.4 and 115.9 µg/mL respectively in a dose dependent manner. Interestingly, procyanidin fractions from apple polyphenol extract, according to the degree of polymerization from dimers to nonamers, profoundly inhibited the pancreatic lipase with IC_{50} of >125, 32.9, 6.7, 1.3, 2.3, 0.7, 1.9 and 0.9 µg/mL respectively. Pentamers of procyanidins have more effect as compared to dimers and maximal level of activity can be found in case of pentamer or greater form. Polyphenols contained in the polyphenol fraction, such as (+)-catechin, (-)-epicatechin, phloridzin (**61**), and chlorogenic acid (**62**) and products purified from AP (phloretin-2′-xyloglucoside (**63**) and *p*-coumaroyl quinic acid (**64**)) showed weak inhibitory activity on pancreatic lipase. IC_{50} of phloridzin, phloretin-2′-xyloglucoside, chlorogenic acid, and *p*-coumaroyl quinic acid were 58.7, 44.6, 59.8, and 89.0 µg/mL, respectively (Sugiyama et al., 2007) (Figure 6d).

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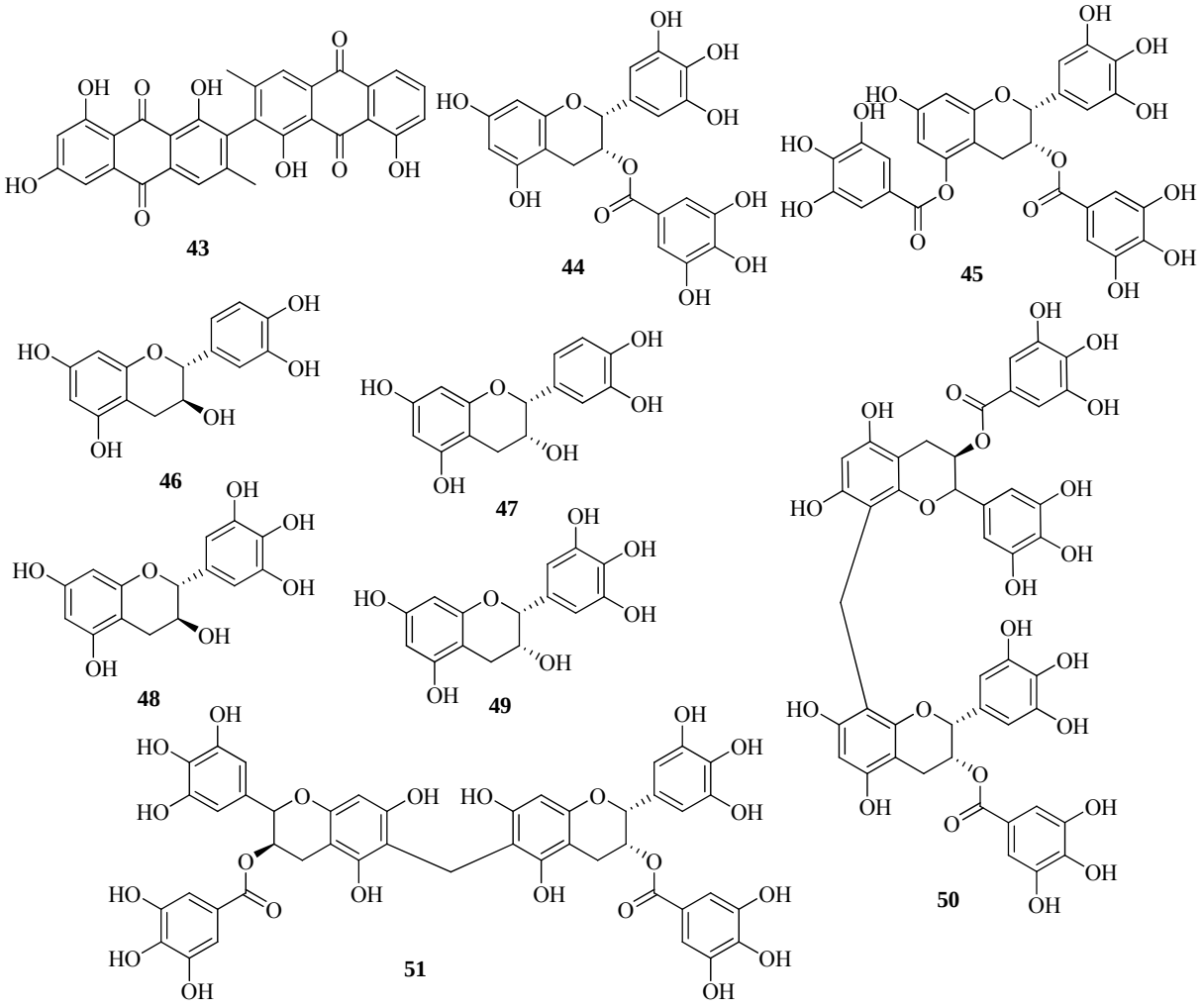

Figure 6b: Polyphenols as pancreatic lipase inhibitors

Grape seed extract has been reported to inhibit various lipases including pancreatic lipase, lipoprotein lipase, and hormone sensitive lipase. It caused a reduction of 80 % and 30 % activity of pancreatic lipase and lipoprotein lipase respectively at a dose of 1 mg/mL. Furthermore, it also suppressed action on hormone sensitive lipase and thereby decreased free fatty acids releasing from adipose tissue (Moreno et al., 2003).

Ethyl acetate fraction of *Alpinia officinarum*, prepared by partitioning of the water extract with organic solvents, has been shown strong inhibition on pancreatic lipase with IC_{50} of 3 mg/mL (triolein as substrate) and 5.6 mg/mL (tributyrin as substrate). Phytochemical investigation of ethyl acetate fraction yielded 3-methyletherglangin (**65**) having IC_{50} of 1.3 mg/mL (triolein as substrate) and 3.3 mg/mL (tributyrin as substrate) towards pancreatic lipase. Water extract (0.1 and 1.0 g/Kg/day), its ethyl acetate fraction (0.1 and 0.5 g/Kg/day) and 3-methyletherganglin (10 and 20 mg/Kg/day) showed significant reduction in the serum triglyceride level in corn oil feeding-induced triglyceridemic mice and triglyceride and cholesterol levels in Triton WR-1339-induced hyperlipidemic mice. It is assumed that 3-methyletherglangin and extract are acting *via* inhibition of pancreatic lipase, which is supported by the study done on the high cholesterol diet induced hyperlipidemic mice (Shin et al., 2003) (Figure 6d).

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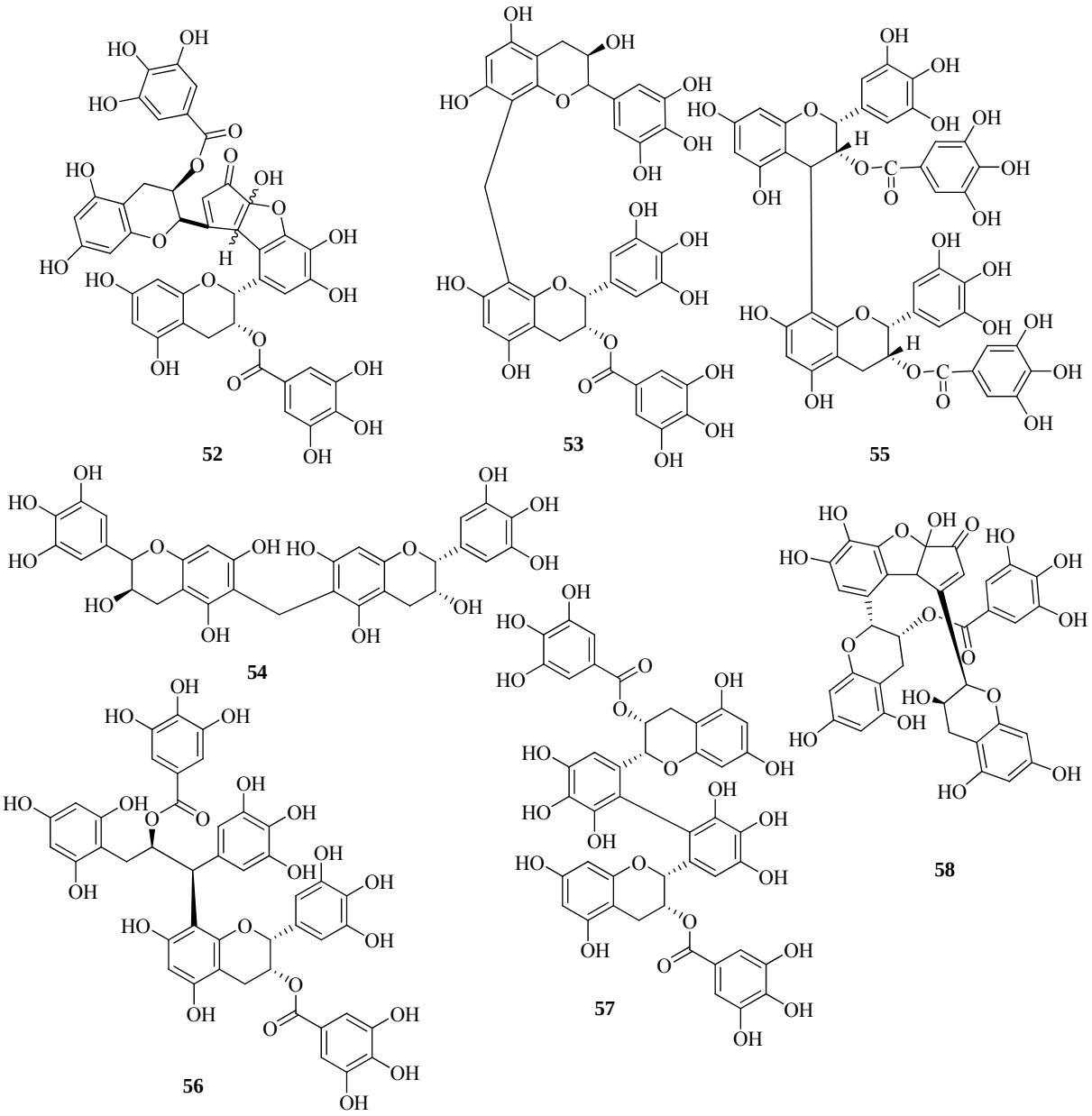

Figure 6c: Polyphenols as pancreatic lipase inhibitors

Yoshikawa et al. (2002) have reported the anti-obesity activity of the hot decoction of the roots of the plant *Salacia reticulata* in female Zucker fatty rats. The extract, majorly rich in polyphenols (24 %), including mangiferin, catechins and condensed tannins, exhibited inhibition of pancreatic lipase (IC_{50}=264 mg/L) and lipoprotein lipase from adipose tissue (IC_{50}=15 mg/L). Furthermore plant extract and its constituents also depicted inhibition of glycerophosphate dehydrogenase and thereby conversion of glucose into triglycerides (Yoshikawa et al., 2002).

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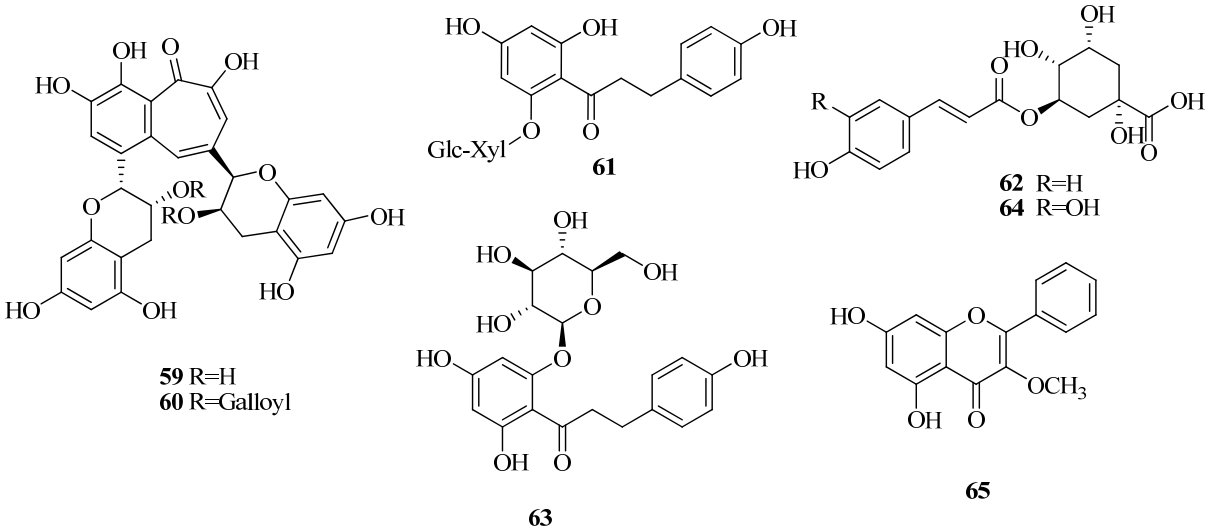

Figure 6d: Polyphenols as pancreatic lipase inhibitors

Polysaccharides

Chitosan, a linear polysaccharide composed of randomly distributed β-(1-4)-linked D-glucosamine (deacetylated unit) and *N*-acetyl-D-glucosamine (acetylated unit), is made by treating chitin from shrimp and other crustacean shells by deacetylation on treatment with alkali at 100 °C. Water soluble chitosan, having a molecular weight of 46 KDa, has demonstrated the inhibitory effect on the pancreatic lipase *in vitro* and reduction in the elevation of plasma triacylglycerol level after the oral lipid tolerance test in mice. At a dose of 300 mg/Kg twice a day, it also found to prevent increases in bodyweight, white adipose tissue weights and liver lipids (cholesterol and triacylglycerol). Furthermore, it also increases the fecal bile acid and fat. Inhibition of pancreatic lipase can be corroborated with an increase in fecal fat excretion and a decrease in the absorption of dietary lipids from the small intestine (Sumiyoshi and Kimura, 2006).

Saponins

Another most important and well studied class of phytochemical targeting pancreatic lipase is saponins. Saponin-rich fraction of the leaves of *Acanthopanax sessiliflorus* yielded sessiloside (**66**) and chiisanoside (**67**) (lupane-type saponins) with IC_{50} values of 0.36 and 0.75 mg/mL, respectively towards *in vitro* lipase inhibition. They also prevented the high fat diet induced weight gain in mice (Yoshizumi et al., 2006). In another reported study, triterpenoid saponins silphioside F (**68**), copteroside B (**69**), hederagenin 3-*O*-β-D-glucuronopyranoside 6′-*O*-methyl ester (**70**) and gypsogenin 3-*O*-β-D-glucuronopyranoside (**71**) from the fruits of *Acanthopanax senticosus*, have been reported for 50 % inhibition of pancreatic lipase at concentration of 0.22, 0.25, 0.26 and 0.29 mM, respectively (Li et al., 2007) (Figure 7a).

The leaves decoction of *Cyclocarya paliurus,* traditionally used as a remedy for prevention of hyperglycemia and diabetes mellitus, inhibited pancreatic lipase with IC_{50} values of 9.1 µg/mL. Further investigation on the leaves provided the structurally dammarane type of triterpene saponins, cyclocarioside A (**72**), II (**73**), and III (**74**) which may have a role in preventing triglyceride absorption (Kurihara et al., 2003) (Figure 7a).

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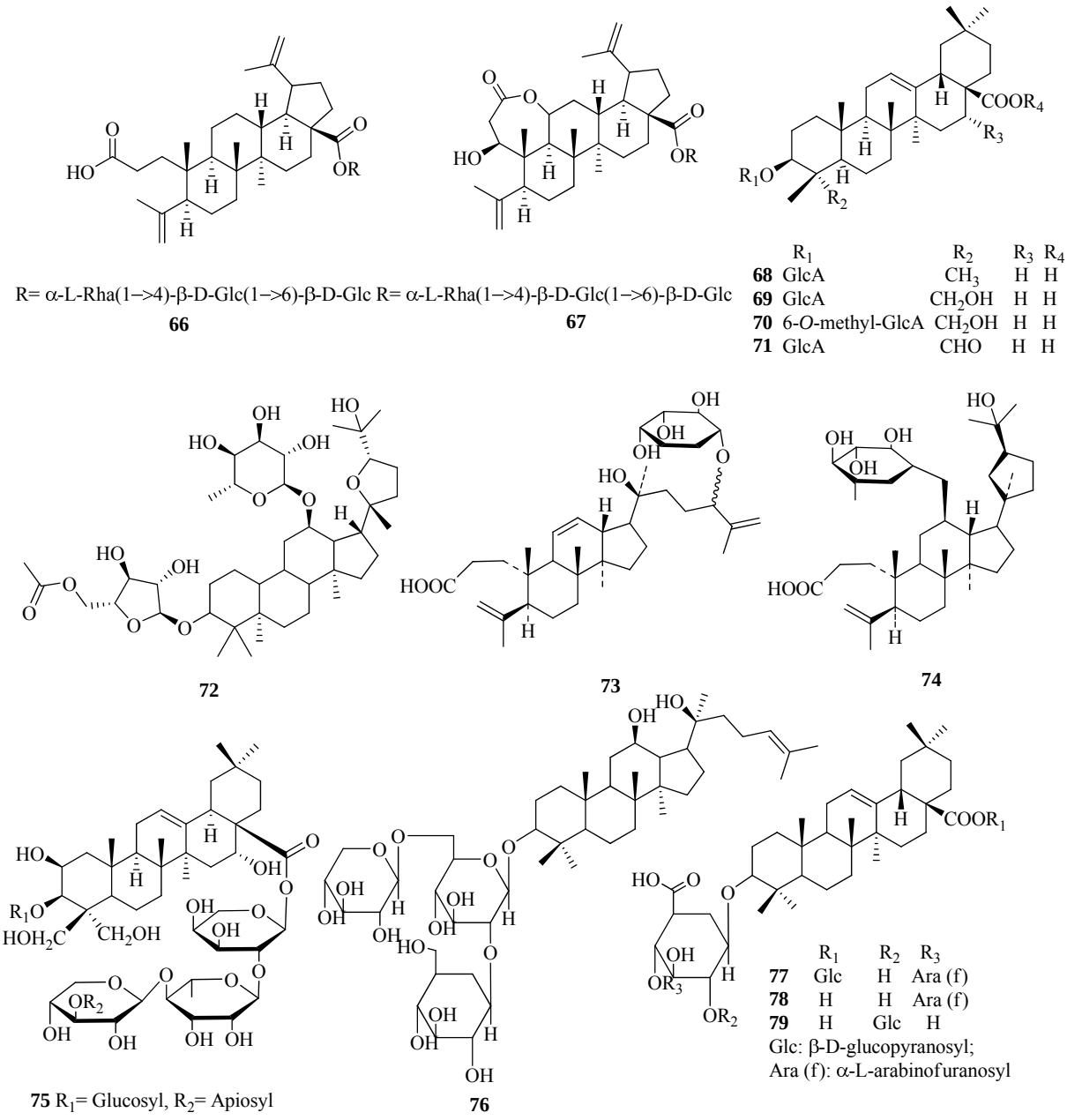

Figure 7a: Saponins as pancreatic lipase inhibitors

Various triterpenoidal saponins have been identified as pancreatic lipase inhibitors from the radix of *Platycodin grandiflorum*. Platycodin D (**75**) has been reported for the inhibition of pancreatic lipase competitively with a K_i of 0.18±0.03 mM. On the plasma triacylglycerol level in rats after the oral administration of lipid emulsion, platycodin D at a dose of 244 mg/Kg has inhibited the elevation. In China and Korea, the roots of this plant have been consumed as food (Han et al., 2000, 2002; Zhao and Kim, 2004; Xu et al., 2005; Zhao et al., 2005) (Figure 7a).

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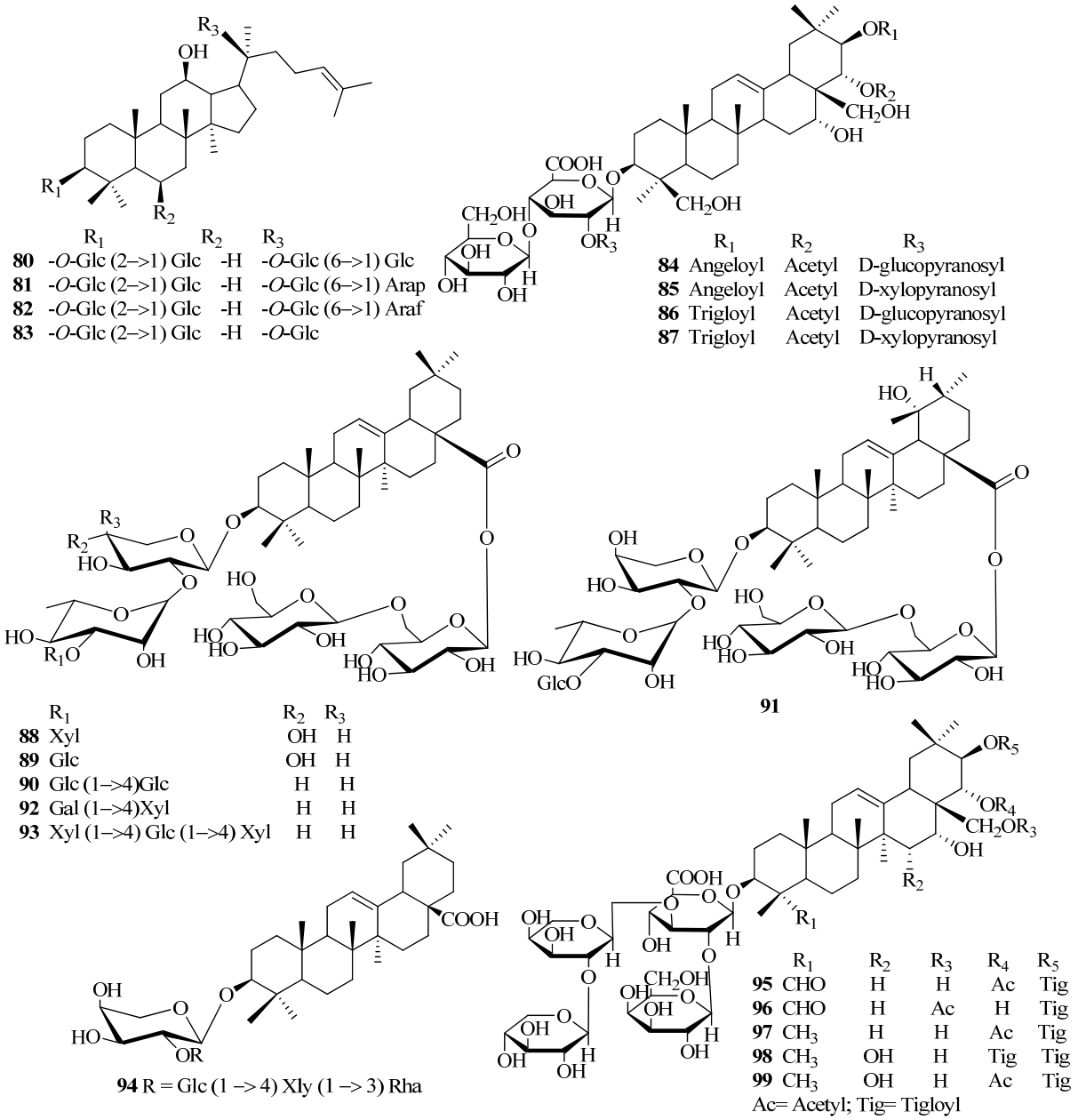

Figure 7b: Saponins as pancreatic lipase inhibitors

From the rhizomes of *Panax japonicas,* total saponin fraction containing chikusetsusaponins inhibited *in vitro* pancreatic lipase and *in vivo* treatment with high fat diet rodent model showed inhibition in weight gain, adipose fat pad weight and increased fat excretion in fecal matter. Total saponin fraction prevented the rise in triglyceride content in plasma in oral lipid emulsion tolerance test. Chikusetsusaponin III (**76**) and IV (**77**), 28-deglucosyl-chikusetsusaponins IV (**78**) and V (**79**) (Figure 7a) isolated from total saponin fraction were also found to inhibit pancreatic lipase (Han et al., 2005). In the similar kind of study, ginsenosides Rb1 (**80**, Figure 7b), Rb2 (**81**), Rc (**82**) and Rd (**83**), isolated from stems and leaves of *Panax quinquefolium* at the concentration of 0.5 mg/mL, inhibited the pancreatic lipase by 78-98 % (Liu et al., 2008) (Figure 7b).

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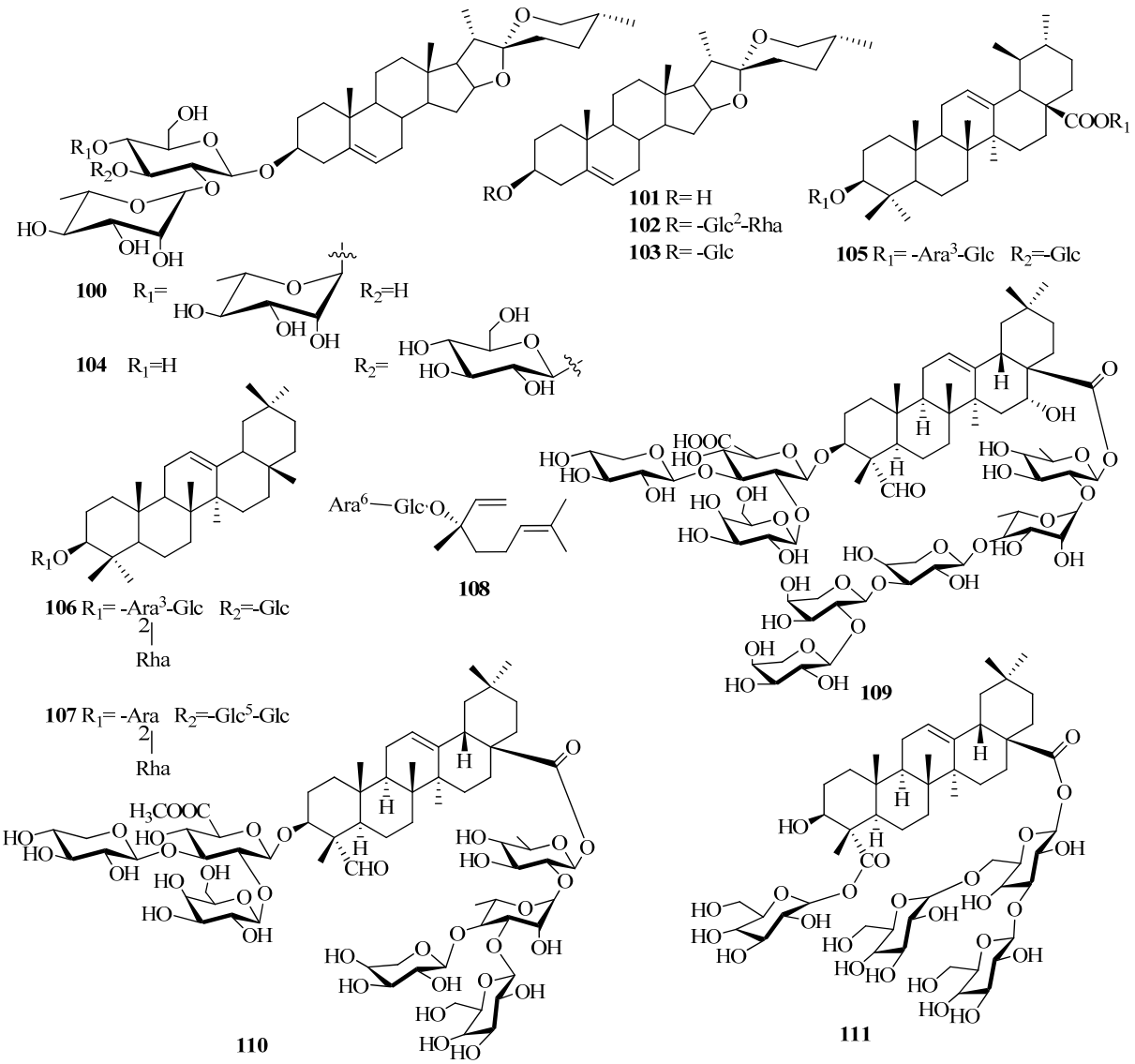

Figure 7c: Saponins as pancreatic lipase inhibitors

Escins, deacetylescins and desacylescins, found in *Aesculus turbinata* have demonstrated the inhibition of pancreatic lipase. Escins were found more active than deacetylescins, followed by desacylescins. Furthermore, angeloyl containing escins Ib (**84**; IC_{50}=24 µg/mL) and IIb (**85**; IC_{50}=14 µg/mL) were found more active than tigloyl containing escins Ia (**86**; IC_{50}=48 µg/mL) and IIa (**87**; IC_{50}=61 µg/mL) (Kimura et al., 2006). Another saponins from *Scabiosa tschiliensis*, scabiosaponins like scabiosaponin E-G (**88-90**), scabiosaponin I (**91**), hookeroside A (**92**) and B (**93**) and prosapogenin 1b (**94**) have been reported for pancreatic lipase inhibition. Prosapogenin 1b at 0.12 mg/mL showed the strongest *in vitro* pancreatic lipase inhibition (Zheng et al., 2004) (Figure 7b).

Similar to polyphenols, saponins from oolong tea possess pancreatic lipase inhibitory potential. Amongst them, teasaponins, composed of acylated oleanene type triterpene oligoglycosides theasaponins E1 (**95**) and E2 (**96**), have shown dose dependent and competitive lipase inhibition with K_m, V_{max} and K_i of 1.42 mg/mL, 476.2 nkat/L and 0.25 mg/mL respectively. In the similar kind of study, three acylated oleanane-type triterpene oligoglycosides, chakasaponins I (**97**),

II (**98**), and III (**99**), isolated from butanol-soluble fraction prepared from the flower buds of Chinese tea plant (*Camellia sinensis* (L.) O.Kuntze; Fujian Province) were reported to have an inhibitory effect against porcine pancreatic lipase with IC_{50} of 0.17, 0.18 and 0.53 mM respectively (Han et al., 1999; Han et al., 2001;Yoshikawa et al., 2009) (Figure 7b).

Dioscin (**100**), diosgenin (**101**), prosapogenin A (**102**) and C (**103**), and gracillin (**104**) from the methanol extract of roots of *Dioscorea nipponica* Makino possessed the inhibitory potential against pancreatic lipase with an IC_{50} of 20, 28, 1.8, 42.2, and 28.9 µg/mL, respectively, along with suppression of increase in plasma triglyceride level (Kwon et al., 2003). Another three triterpene saponins, matesaponin 1 (**105**), nudicaucin C (**106**) and 3-*O*-α-L-rhamnopyranosyl(1→2)-α-L-arabinopyranosyl oleanolic acid 28-*O*-β-D-glucopyranosyl(1→6)-β-D-glucopyranoside (**107**) and one monoterpene oligoglycosides, (R)-linalyl-6-*O*-arabinopyranosyl-β-D-glucopyranoside (**108**), isolated from ethyl acetate and butanol soluble fractions from the leaves of *Ilex paraguariensis* were found to exhibit the potent inhibitory activities at 100 µM concentration with 94, 78, 77 and 83 % respectively (Sugimoto et al., 2009). Three triterpenoidal saponins gypsosaponins A (**109**), B (**110**), and C (**111**) from *Gypsophila oldhamiana* were reported to inhibit the pancreatic lipase enzyme with 58.2 %, 99.2 % and 50.3 % respectively, at the concentration of 1 mg/mL (Zheng et al., 2007) (Figure 7c).

Terpenes

Crocin (**112**) and its metabolite crocetin (**113**) from the fructus of *Gardenia jasminoides* ELLIS water extract, were found to have potent hypotriglyceridemic and hypocholesterolemic effects, along with the pancreatic lipase inhibition with IC_{50} of 2.1 and 2.6 mg/mL respectively. Both compounds reduced the increase of serum triglyceride level in corn oil feeding-induced triglyceridemic mice, and serum triglyceride and total and LDL-cholesterol levels in Triton WR-1339-induced hyperlipidemic mice. They also showed hypolipidemic activity in high cholesterol, high fat or high carbohydrate fed diet induced hyperlipidemic mice. Crocetin has shown more potent activity than crocin (Lee et al., 2005) (Figure 8).

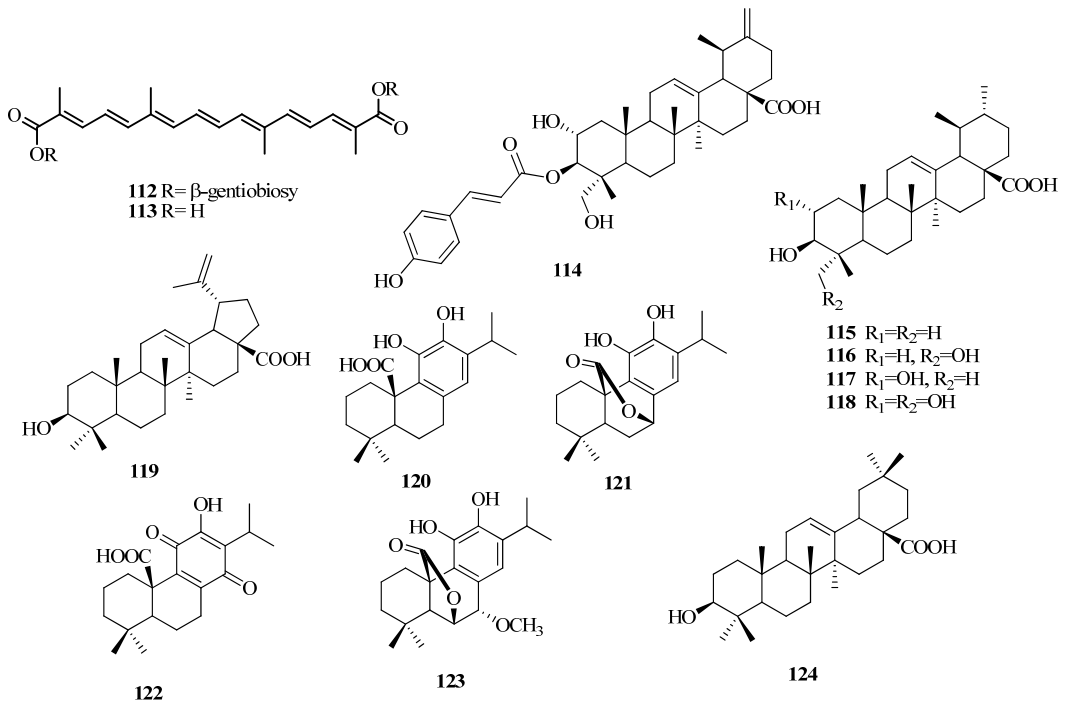

Figure 8: Terpenes as pancreatic lipase inhibitors

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3-*O*-trans-*p*-coumaroyl actinidic acid (**114**), ursolic acid (**115**), 23-hydroxyursolic acid (**116**), corosolic acid (**117**), asiatic acid (**118**) and betulinic acid (**119**), isolated from an ethyl acetate extract of the roots of *Actinidia arguta*, have been reported to possess pancreatic lipase inhibitory activity with IC_{50} of 14.95 ± 0.21, 15.83 ± 1.10, 41.67 ± 0.66, 20.42 ± 0.95, 76.45 ± 0.51 and 21.10 ± 0.55 µM respectively (Jang et al., 2008). Similarly carnosic acid (**120**), carnosol (**121**), roylenoic acid (**122**), 7-methoxyrosmanol (**123**) and oleanolic acid (**124**) from the methanolic extract of *Salvia officinalis* leaves, were reported to inhibit pancreatic lipase with IC_{50} of 12, 4.4, 35, 32 and 83 µg/mL, respectively. Inhibition by carnosic acid was concentration-dependent and competitive with a K_i of 5.4 µg/mL. Furthermore, at oral doses of 5–20 mg/Kg, it also showed suppression of serum triglyceride level increment in olive oil-loaded mice and reduction in body weight and epididymal fat weight in high fat diet fed mice after 14 days (Ninomiya et al., 2004) (Figure 8).

MICROBES AS FLOURISHING SOURCE OF PANCREATIC LIPASE INHIBITORS

Microorganisms are also reported to produce the bioactive molecules in various disease areas. Lipstatin, starting or template molecule for orlistat was the first reported pancreatic lipase inhibitor from the microbial source. Orlistat was the first molecule hit the anti-obesity market after FDA approval. This inspired the researchers throughout the globe to explore the microbial flora in order to discover effective anti-obesity agents.

Lipstatin, from *Streptomyces toxytricini*, irreversibly inhibited the pancreatic lipase with an IC_{50} of 0.14 µm. *In vivo* study also reveals the inhibition of the absorption of dietary triolein in mice while simultaneously administered oleic acid was absorbed which supported the role of pancreatic lipase inhibition. Furthermore, in comparison to the control, it also inhibited 80 % of lipase *ex vivo* as measured in the intestinal fluid of mice, 2 hours after an oral dose of 50 mg/Kg. β-lactone ring cleavage of lipstatin and its derivatives resulted in no inhibition, which suggest the requirement of intact β-lactone ring for the pancreatic lipase inhibitory action (Weibel et al., 1987; Hochuli et al., 1987). Also stereochemistry of the substituent on the C_2 and C_3 of β-lactone ring is equally important for the specificity for being HMG-CoA synthase or lipase inhibitors as (2R, 3R) configuration imparts specificity towards the HMG-CoA synthase while (2S, 3S) configuration imparts specificity towards the pancreatic lipase (Tomoda et al., 2002).

In addition to the lipstatin, β-lactone containing microbial metabolites, valilactone (**125**), percyquinin (**126**), panclicin A-E (**127-131**), ebelactone A (**132**) and B (**133**), vibralactone (**134**) and esterastin (**135**) and non-β-lactone bearing microbial metabolites, (*E*)-4-amino styryl acetate (**136**), ε-polylysine (**137**) and caulerpenyne (**138**) have been identified from microbial source as pancreatic lipase inhibitor (Figure 9).

Valilactone, from the strain MG147-CF2 (closely related to *Streptomyces albolongus*) and esterastin, isolated from *Streptomyces lavendulae* strain MD4-C1, potently inhibited the hog pancreatic lipase (IC_{50} = 0.14 and 0.9 ng/mL respectively) and liver esterase (IC_{50}=29 ng/mL and 50 µg/mL) (Kitahara et al., 1987; Umezawa et al., 1978). Similarly, a β-lactone metabolite percyquinin, from fungal cultures of *Basidiomycete Stereum complicatum*, ST 001837, has been reported for the inhibitory action on pancreatic lipase with an IC_{50} of 2 µM (Hopmann et al., 2003).

Five panclicins A-E, produced by *Streptomyces* sp. NR 0619, have been found as potent pancreatic lipase inhibitors with IC_{50} of 2.9, 2.6, 0.62, 0.66, and 0.89 µM, respectively. Structurally panclicins A and B are the alanine type while panclicins C-E are the glycine type. Latter panclicins are 2-3 fold more potent than orlistat while former panclicins are less potent than the latter ones. Similar to orlistat, inhibition of pancreatic lipase by panclicins was irreversible, but not

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as strong as that of orlistat (Mutoh et al., 1994; Yoshinari et al., 1994).

Ebelactone A and B from the fermentation broth of *Actinomycetes* strain G7-Gl (closely related to *Streptomyces aburaviensis*), have shown inhibition of hog pancreatic lipase (IC_{50}=3 and 0.8 ng/mL, respectively) and liver esterase (IC_{50}=56 and 0.35 ng/ml, respectively) (Umezawa et al., 1980). In the same way, vibralactone, a fused β-lactone type metabolite isolated from the cultures of *Boreostereum vibrans*, was identified as the pancreatic lipase inhibitor with an IC_{50} of 0.4µg/mL using 4-methylumbelliferyl oleate as substrate (Liu et al., 2006).

Chemically belongs to enol acetate of *p*-amino phenyl acetaldehyde class, (*E*)-4-Aminostyryl acetate (**136**), produced by the *Streptomyces* sp. MTCC 5219 which was isolated from the soil sample of cow barnyard in India, was found to inhibit the hydrolysis of trioleate by porcine pancreatic lipase dose dependently with IC_{50} of 7.46 µM (Tokdar et al., 2011) (Figure 9).

ε-polylysine (**137**), a small natural homopolymer of the essential amino acid L-lysine produced by bacterial fermentation *Streptomyces albulus*, is used as a natural preservative in food products. In *in vitro* assay, ε-polylysine exhibited strong inhibition in the hydrolysis of trioleoylglycerol emulsified with phosphatidylcholine and taurocholate by pancreatic lipase with IC_{50} of 0.12 µM. The IC_{50} of ε-polylysine was increased by the addition of emulsifier species such as gum arabic, phosphatidylserine, and phosphatidic acid, by approximately 150, 70, and 230 times, respectively, when compared with phosphatidylcholine emulsion. Mice fed on a high fat diet containing 0.1-0.4 % ε-polylysine has not shown any significant body weight gain and weight of the liver and visceral adipose tissues. Moreover, it also showed decreased plasma triacylglycerol and cholesterol level and liver triacylglycerol content. Also, increment in fecal weights and fecal lipid of mice has suggested that ε-polylysine has an anti-obesity effect by inhibiting intestinal absorption of dietary fat (Tsujita et al., 2006) (Figure 9).

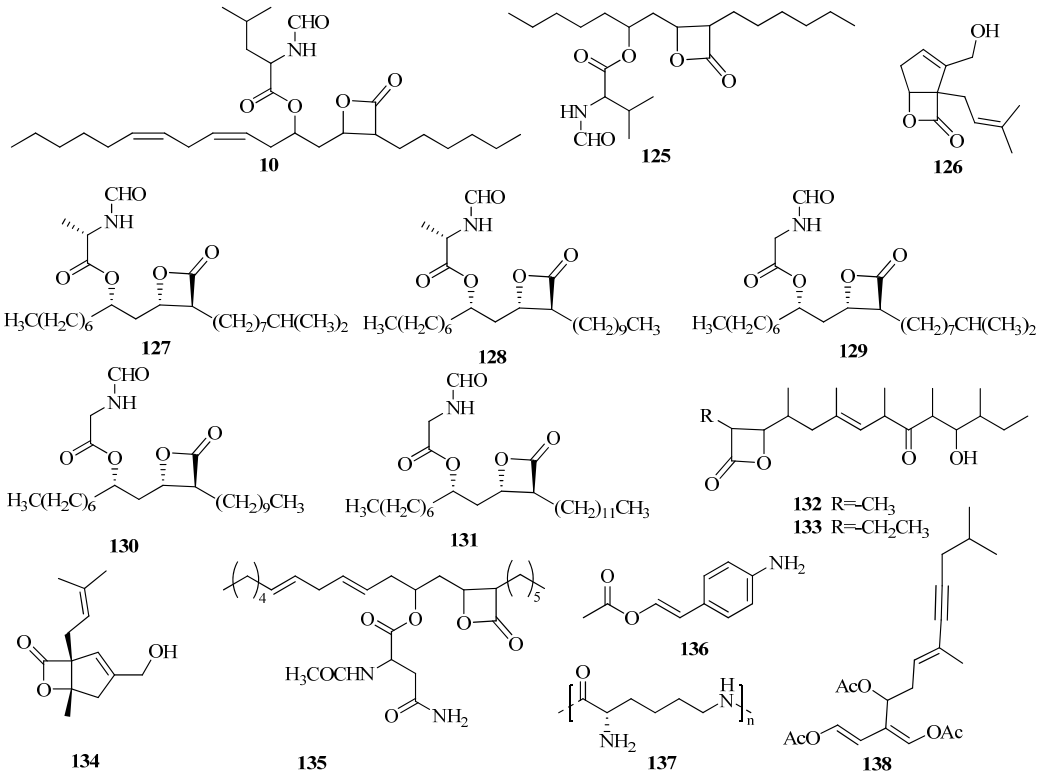

Figure 9: Pancreatic lipase inhibitors from microbial sources

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Caulerpenyne (**138**), from marine algae *Caulerpa taxifolia, was* reported to inhibit lipase activity, competitively with IC_{50} of 2 mM and 13 µM, using emulsified triolein and dispersed 4-methylumbelliferyl oleate as substrates, respectively. *In vivo* study in rats has shown reduction in the plasma triacylglycerol level when caulerpenyne was co-administered with corn oil (Bitou et al., 1999) (Figure 9).

SYNTHETIC PANCREATIC LIPASE INHIBITORS

Commonly employed strategy for analogue synthesis for pancreatic lipase inhibitors is based on the structure of natural substrate of lipase, *i.e*. triglycerides. 1,3-diaminopropan-2-ol, 2-amino alcohol, glycerol and 2-methylglycerol were commonly utilized as backbone to form a triglyceride kind of molecules with modified more stable and/or hindered ester or amide or ether linkages.

Bis-2-oxo amide triacylglycerols, synthesized using 1,3-diaminopropan-2-ol, have been reported as potent human gastric lipase inhibitor. These analogues contain the 2-oxo amide in place of the scissile ester bond at the sn-1 and sn-3 position and ester or ether bond at the sn-2 position. Using the monolayer technique with mixed films of 1,2-dicaprin containing variable proportions of synthesized derivatives, 2-[(2-oxohexadecanoyl)amino]-1-[[(2-oxohexadecanoyl)-amino]methyl]ethyl decanoate (**139**) was found to be the most potent inhibitor, causing a 50 % decrease in human pancreatic and gastric lipase activities at 0.076 and 0.020 surface molar fractions, respectively. Further structure-activity relationship study suggested that the inhibitory potential of synthesized derivatives depends on the nature of the functional group, ester or ether, and the chain length at the sn-2 position (Kotsovolou et al., 2001). Similarly, a lipophilic α-keto amide (**140**), synthesized from 2-amino alcohol showed a 50 % decrease in lipase activity at a 0.14 molar fraction (Chiou et al., 2000). In another similar kind of study, primary and *N*-alkyl α-keto amides prepared from aldehyde and α-keto acids, were studied for inhibitory activity against porcine pancreatic lipase using monolayer technique and methyl 2-[(2-ketododecanoyl)amino] hexadecanoate (**141**) had been identified as potent inhibitor, causing a 50% decrease in lipase activity at a 0.09 molar fraction (Chiou et al., 2001) (Figure 10).

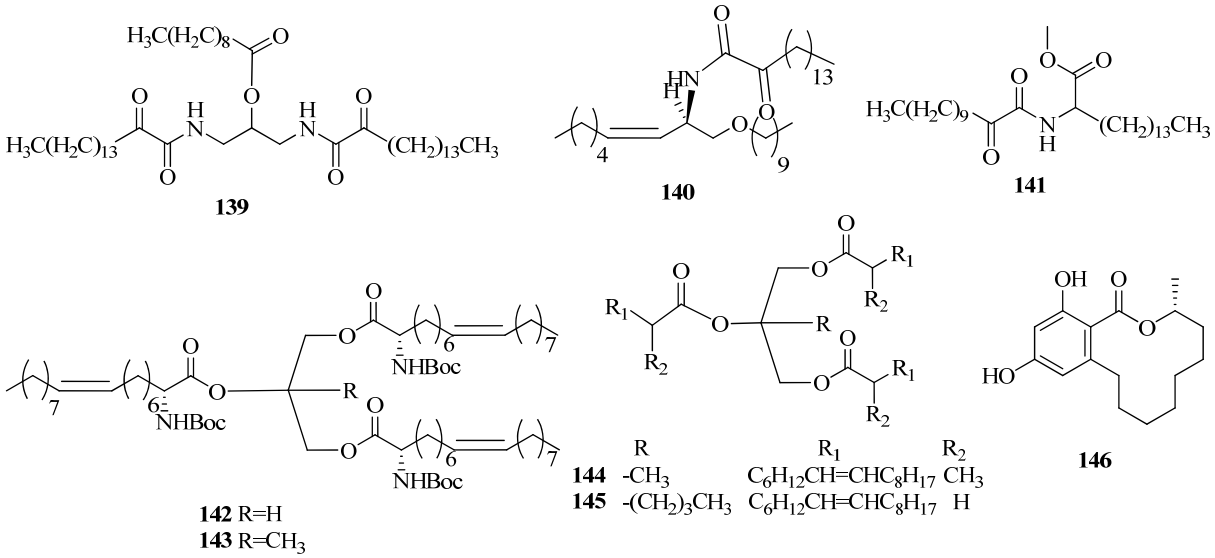

Figure 10: Synthetic pancreatic lipase inhibitors

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Other reported potent human pancreatic lipase inhibitors are triacylglycerol analogues containing 2-(*N*-tert-butoxycarbonylamino) fatty acids. Using the monolayer technique for estimating the lipase inhibition, the triesters of glycerol (**142**) and 2-methylglycerol (**143**) with 2-(*N*-tert-butoxycarbonylamino)oleic acid were found to be potent inhibitors of human pancreatic lipase with 50 % inhibition at 0.003 and 0.002 molar fractions, respectively. They also demonstrated 50 % inhibition against gastric lipase at 0.057 and 0.104 molar fraction, respectively (Magrioti et al., 2004). Similarly, sterically hindered triacylglycerols based on 2-methyl- and 2-butylglycerol, and/or 2-methyl fatty acids have been synthesized and tested for their ability to inhibit human pancreatic and gastric lipases by using the monolayer technique. Triolein analogues that contain a butyl group (**144**) or methyl groups (**145**) at the 2-position of glycerol and the alpha-position of each oleic acid residue have been found as potent inhibitors with a 50 % decrease in human pancreatic lipase activity at 0.003 molar fractions. They have also shown 50% inhibition of gastric lipase at 0.009 and 0.017 molar fraction respectively (Constantinou-Kokotou et al., 2004). Apart from this, a dihydroxy benzomacrolide (**146**) has been reported for the potent inhibitory activity with IC_{50} of 4.73 ± 0.175 μM against pancreatic lipase (Guo et al., 2011) (Figure 10).

CLINICAL STUDIES ON PANCREATIC LIPASE INHIBITORS

In the search of pancreatic lipase inhibitor, a number of plant extracts, isolated phyto-constituents, semi-synthetic and synthetic compounds have been screened for their pancreatic lipase inhibitory activity. Many pancreatic lipase inhibitors are under clinical investigations but only one pancreatic lipase inhibitor, cetilistat (**147**), has completed clinical trials and in the final stage of approval. In October 2012, Alizyme, a biopharmaceutical company in collaboration with Takeda Pharmaceutical has submitted New Drug Application (NDA) for cetilistat to Japan's Ministry of Health, Labour and Welfare for the treatment of obesity, based on the results obtained after phase 3 clinical studies. (Protocol Nos. ATL-962/OCT-001; ATL-962/OCT-002; ATL-962/CCT-002. Takeda Pharmaceutical Company Limited, Osaka, Japan). Cetilistat acts in the same way *via* inhibition of the pancreatic lipase enzyme and thereby inhibiting the breaking down of triglycerides. In human trials, cetilistat was shown to produce similar weight loss to orlistat. However, side effects such as oily, loose stools, fecal incontinence, frequent bowel movements, and flatulence, are similar to orlistat (Yamada et al., 2008; Kopelman et al., 2007) (Figure 11).

Another lipase inhibitor, called GT 389-255, which is under development by Peptimmune, under a license from Genzyme, was a combination of a proprietary pancreatic lipase inhibitor and a fat binding hydrogel polymer for the treatment of obesity. But recent development is unknown since the phase I trial was conducted in 2004. In 2011, Peptimmune filed for Chapter 7 Liquidation (McBride, 2011).

Satiereal (saffron) is recommended as a satiety enhancer and weight loss promoter. In a human clinical trial on humans, it was found to result to lower appetite. One capsule of Satiereal (176.5 mg/day) or an inactive placebo was given to 60 overweight women with no limitation in dietary intake. The saffron extract caused a reduction in snacking and weight loss as compared to the control group after two months treatment (Gout et al., 2010).

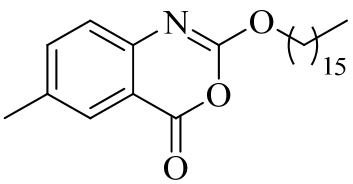

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Figure 11: Pancreatic lipase inhibitor in clinical stage

A double-blinded, randomized, and placebo-controlled clinical study on 32 obese subjects (22.5 g blueberry bioactives, *n*=15 and placebo group, *n*=17 twice daily for six weeks) with daily dietary supplementation with bioactives from blueberries revealed the improvement of insulin sensitivity in obese, nondiabetic, and insulin-resistant participants (Stull et al., 2010).

CONCLUSION

Natural products always are an inspirational source for the development of new types of therapeutics. Despite this scenario, only orlistat is in clinical use. Thus, there is a huge call for newer leads from the natural sources and subsequently to develop them as new anti-obesity therapeutics. Natural compounds and dietary phytomolecules have an advantage of biological friendliness and chemo-diversity. Many reported natural products, particularly the phenolics, terpenes and saponins have already shown profound inhibition of pancreatic lipase. Although, research is continually going on in the development of pancreatic lipase inhibitors from nature, unfortunately none has reached to the clinical use. To increase the number of leads from the natural product libraries for pancreatic lipase inhibition, there is a need to develop a high throughput screening (HTS) protocol. Application of more advanced and recent approach such as structure-activity relationship, *in silico* studies, metabolomics, hyphenated techniques and system biology should be carried out and highly desirable. In addition to this, improvement in the bioavailability of natural products is also necessary for better drug development. Thus, natural product inspired molecules might provide a potential lead or pharmacophore for further development. Detailed structure activity relationship studies on semi-synthetic and synthetic derivatives might also provide a direction for the development of pancreatic lipase inhibitors for the treatment of obesity and related disorders.

Conflict of interest

The authors declare that there is no conflict of interest.

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