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Benzoflavones as cholesterol esterase inhibitors: Synthesis, biological evaluation and docking studies

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ABSTRACT

A library of forty 7,8-benzoflavone derivatives was synthesized and evaluated for their inhibitory potential against cholesterol esterase (CEase). Among all the synthesized compounds seven benzoflavone derivatives (**A-7**, **A-8**, **A-10**, **A-11**, **A-12**, **A-13**, **A-15**) exhibited significant inhibition against CEase in *in vitro* enzymatic assay. Compound **A-12** showed the most promising activity with IC_{50} value of 0.78 nM against cholesterol esterase. Enzyme kinetic studies carried out for **A-12**, revealed its mixed-type inhibition approach. Molecular protein–ligand docking studies were also performed to figure out the key binding interactions of **A-12** with the amino acid residues of the enzyme's active site. The **A-12** fits well at the catalytic site and is stabilized by hydrophobic interactions. It completely blocks the catalytic assembly of CEase and prevents it to participate in ester hydrolysis mechanism. The favorable binding conformation of **A-12** suggests its prevailing role as CEase inhibitor.

Cholesterol is a vital component of cell membrane and possesses many physiological functions. The greatest percentage of cholesterol is used in cytoplasm for bile acid synthesis.[1] Hypercholesterolemia produced either by cholesterol feeding or by cholesterol-free, purified diets ("endogenous" Hypercholesterolemia) results in the accumulation of cholesterol in adipose tissue. As there is a well-established link between plasma cholesterol level and coronary artery disease, the reduction of cholesterol level in plasma, particularly in low density lipoprotein (LDL) lowers the risk of cardiovascular events.[2] The contribution of elevated plasma cholesterol specifically, LDL-cholesterol, to other diseases including cancer, obesity, and diabetes has made control of plasma cholesterol a major health aim.[3]

Pancreatic cholesterol esterase is the member of α/β hydrolase family of proteins which catalyses the hydrolysis of dietary cholesterol ester into free cholesterol in the lumen of small intestine.[4] It is also thought that the transport of cholesterol micelles to enterocytes is performed by this enzyme.[5] As the combined role of CEase in the absorption and transport of cholesterol, its inhibition is important by the development of novel moieties which helps in treating hypercholesterolemia and associated diseases such as coronary heart disease.[6]

From the last decade, there are several classes of potent CEase inhibitors have been developed,[7] so far, including 6-chloro-2-pyrones,[8] thieno[1,3]-oxazin-4-ones,[9] carbamates,[10] aryl phosphates and phosphonates,[11] chloroisocoumarins,[12] phosphaisocoumarins,[13] thiazolidinediones,[5] phosphorylated flavonoids,[14] 2-(1H-Indol-3-yl)-4-phenylquinolines[15] and 3-phenyl substituted 1,3,4-oxadiazol-2(3H)-ones[16] (Fig. 1). However, most of these inhibitors are not highly selective and they could also inhibit other serine hydrolases, such as acetylcholinesterase (AChE), butyrylcholinesterase (BChE), Pseudomonas species lipase (PSL), chymotrypsin (CT) and trypsin.[14]

Flavonoids, including flavones, flavonols, isoflavones and flavanones, are a large class of polyphenolic compounds widely distributed in herbs and foods of plant origin, and exhibit diversified biological activities, such as antioxidant, anti-proliferative, anti-tumor, anti-microbial, estrogenic, acetyl cholinesterase, anti-inflammatory activities and are also used in cancer, cardiovascular disease, neurodegenerative disorders and enzyme inhibition.[17] Among them, the flavones have been considerably explored due to their ability to modulate several enzyme systems involved in a number of diseases.[14]

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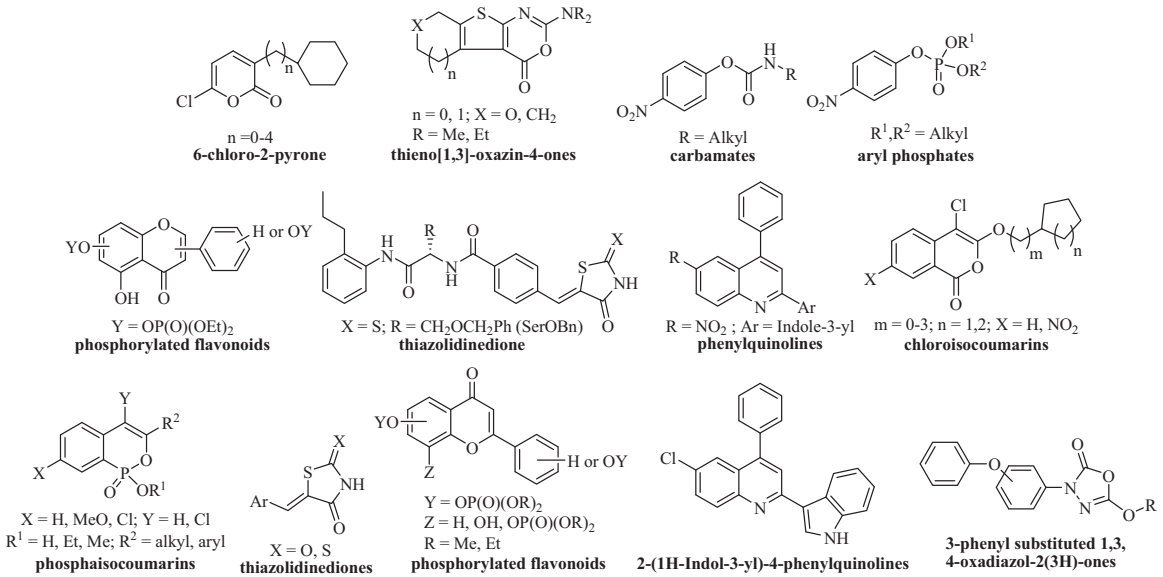
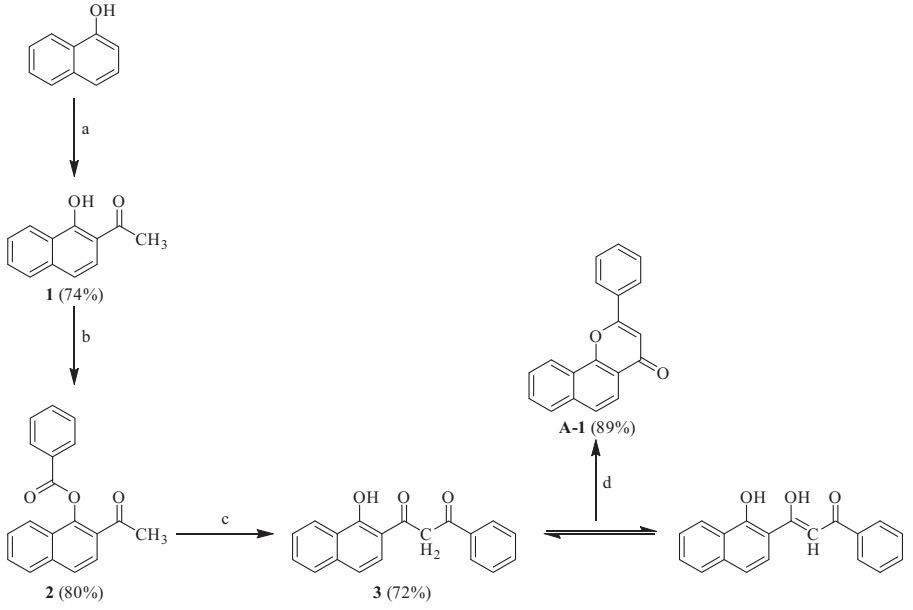


Fig. 1. Reported CEase inhibitors.

In view of various medicinal attributes of flavones, we here report for the first time, benzoflavone derivatives as potent CEase inhibitors. Thus, in the present study, various benzoflavone analogs were synthesized and evaluated for their inhibitory potential against CEase enzyme using spectrophotometric assay. The type of inhibition and the interactions of the most potent inhibitor with CEase enzyme had also been figured out.

Benzoflavones were synthesized *via* Scheme 1. α-Naphthol was subjected to fries rearrangement and the product (**1**) was benzoylated using various substituted benzoylchlorides to obtain **2**. Product **2** was then subjected to Baker Venkataraman rearrangement. The Baker Venkataraman rearranged product (**3**) existed in enol form (confirmed by the appearance of singlets for two D_2O exchangeable protons at 15.2 and 13.68 ppm along with the vinylic proton to carbonyl which appeared as a merged signal in a multiplet at 7.26–7.36 ppm). Compound **3** was then cyclized by treatment with sulphuric acid to yield the desired benzoflavone (**A-1**).[18] All the reactions proceeded smoothly with diverse benzoylchlorides (Table 1) and products were obtained in good yields. No Retro-Diels fragmentation was observed for benzoflavones in the mass spectrum. The structures of the synthesized compounds were elucidated by ^{1}H NMR, ^{13}C NMR, HRMS and Elemental Analysis. All spectral data were in accordance with assumed structures (Supplementary Material).

All the synthesized benzoflavones were evaluated for their inhibitory potential against CEase enzyme using spectrophotometric assay as described in the literature.[15] Compounds with CEase enzyme inhibition of more than 60% at 50 nM were further evaluated at concentrations of 1, 5, 10 and 25 nM in order to calculate their IC_{50} values (Table 1).



Scheme 1. Synthesis of benzoflavone derivative. Reagents and conditions: (a) MW, $ZnCl_2$, CH_3COOH, 20 min; (b) benzoyl chloride, pyridine, stirring rt, 1 h; (c) KOH, pyridine, warm, 30 min; and (d) a drop of conc. H_2SO_4, CH_3COOH, reflux, 1 h.