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5,6-Benzoflavones as cholesterol esterase inhibitors: Synthesis, biological evaluation and docking studies

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Abstract: In continuous efforts to develop potent cholesterol esterase (CEase) inhibitors, a series of 5,6-benzoflavone derivatives was rationally designed and synthesized by changing the position of benzene ring attached to flavone skeleton in previously reported (7,8-benzoflavones). All the synthesized compounds were checked for their inhibitory potential against cholesterol esterase (CEase) by using spectrophotometric assay. Among the series of forty compounds, seven derivatives (**B-10** to **B-16**) exhibited above 90 percent inhibition against CEase in *in-vitro* enzymatic assay. Compound **B-16** showed the most promising activity with IC_{50} value of 0.73 nM against cholesterol esterase. To determine the type of inhibition, enzyme kinetic studies were carried out for **B-16**, which revealed its mixed-type inhibition approach. Moreover, to figure out the key binding interactions of **B-16** with the amino acid residues of the enzyme's active site, molecular protein–ligand docking studies were also performed. The **B-16** completely blocks the catalytic assembly of CEase and prevents it to participate in ester hydrolysis mechanism. The favorable binding conformation of **B-16** suggests its prevailing role as CEase inhibitor. The overall study stated that the *cis*-orientation of Ring A with respect to carbonyl group of Ring C is responsible for potent CEase inhibitory activity of newly synthesized compounds.

Keywords: 5,6-benzoflavones, Baker Venkataraman rearrangement, cholesterol esterase inhibition, enzyme kinetics, docking studies.

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Introduction:

Cholesterol is a vital component of cell membrane which possesses many physiological functions. Plasma cholesterol level is linked to many diseases such as coronary artery disease, cancer, obesity, and diabetes, which is a major health aim worldwide.[1] In this respect, control of the cholesterol level has gained much attention.

Pancreatic cholesterol esterase (CEase) is an important serine hydrolase that plays imperative role in the absorption of dietary cholesterol. The transport of cholesterol micelles to enterocytes is also performed by this enzyme.[2] As its dual role in absorption and transportation, the inhibition of CEase is very important and thus a potential approach to treat hypercholesterolemia and atherosclerosis.[3] In the recent past, several classes of potent CEase inhibitors have been developed including aryl phosphates and phosphonates,[4] carbamates,[5] chloroisocoumarins,[6] 6-chloro-2-pyrones,[7] 2-(1H-Indol-3-yl)-4-phenylquinolines,[8] 3-phenyl substituted 1,3,4-oxadiazol-2(3H)-ones,[9] phosphaisocoumarins,[10] phosphorylated flavonoids,[11] thiazolidinediones,[2] and thieno[1,3]-oxazin-4-ones[12] (**Figure 1**). However, most of the reported inhibitors are not highly selective for CEase and could also inhibit other serine hydrolases, such as acetylcholinesterase (AChE), butylcholinesterase (BuChE), Pseudomonas species lipase, chymotrypsin and trypsin.[3,13-16] One of the major reasons behind their in-selectivity is that all serine enzymes share the similar catalytic triad of Ser-His-Asp (Glu) and mechanism of acylatione-deacylation.[17]

Inspired from various biological attributes of flavones, we have recently reported a series of 7,8-benzoflavone derivatives as potential CEase inhibitors.[18] Among this whole series of compounds, twenty seven molecules were found to exhibit above 60% inhibition against CEase enzyme with the IC_{50} values ranging from 0.78 to 47.80 nM. Compound **A** was the most promising molecule among the series of reported molecules (**Figure 2**). Prompted from these significant *in-vitro* results, a new series of the compounds have been designed by simply changing the orientation of Ring A, from *trans*- to *cis*-configuration with respect to carbonyl group of Ring C (**Figure 2**). Docking study demonstrated that, Ring A gets positioned in a well-defined cavity formed by Gly106, Gly107, GLu193, Ser194 and Ala19 within the active site of CEase. While, Ring A of 7,8-benzoflavone derivatives only surrounded by Ile323 and His435 amino acid residues. We concluded from these particular findings that the *cis*-orientation of Ring A with respect to carbonyl group of Ring C might be responsible for good CEase inhibitory

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activity of newly designed compounds. In present study, the designed compounds were synthesized in order to evaluate the inhibitory potential against CEase enzyme by using *in-vitro* spectrophotometric assay. The type of inhibition and the various types of interactions of the most potent inhibitor with CEase had also been figured out.

Results and Discussion:

5,6-benzoflavone derivatives were synthesized *via* Scheme **1**. β-naphthol was subjected to fries rearrangement and the product (**1**) was benzoylated using benzoylchloride 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 singlet for two D_2O exchangeable protons at 11.35 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 5,6-benzoflavone (**B-1)**. Some of the synthesized molecules are previously reported by various research groups (mentioned below) but most of the molecules reported herein are novel to the best of our knowledge. All the reactions proceeded smoothly with diverse benzoylchlorides (**Table 1**) and products were obtained in good yields. No Retro-Diels fragmentation was observed for derivatives in the mass spectrum. The structures of the synthesized compounds were elucidated by ^{1}H NMR, ^{13}C NMR and MASS spectrum. All spectral data were in accordance with assumed structures.

***In vitro* screening:**

CEase inhibition assay of all the synthetics was performed by using spectrophotometric assay as described in the literature[8] and the results were compared with potent cholesterol esterase inhibitor (**PF**) reported by Wei Y *et al*.[11]

In-vitro results showed that among series of forty compounds, nine compounds exhibited significant percentage inhibition against CEase enzyme (above 90 % inhibition). Compound **B-16** was found to be endowed with most potent percentage inhibition against CEase with 100% inhibition. Careful examination of **Table 1** revealed an interesting structure activity relationship similar to the reported benzoflavone derivatives (7,8-benzoflavones) as CEase inhibitors. Any substitution on Ring D [phenyl (at 2nd position of 5,6-benzoflavone)] significantly influences the cholesterol esterase inhibitory activity. Placement of halogen atoms on this phenyl ring

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considerably increases the potency against cholesterol esterase enzyme. It has also been cleared that as the size of halogen atom increases, inhibitory potency significantly decreases. Ring D (phenyl) with deactivating groups (nitro, cholomethyl, triflоromethyl and acetoxy) favors the inhibitory activity whereas, Ring D with activating groups (dimethylamino, methoxy, methyl and triflоromethoxy), disfavors the activity against CEase inhibition. Thus, the overall preference order of the substituent on phenyl ring at 2nd position of 5,6-benzoflavone nucleus for the inhibition of cholesterol esterase enzyme is as follows: -Cl > -F > -Br > -I > -NO_2 > -CH_2Cl > -CF_3 > -$OCOCH_3$ > -H > -OCF_3 > -CH_3 > -OCH_3 > -$N(CH_3)_2$ (**Figure 3**). Compounds with CEase enzyme inhibition of more than 60% at 50 nM were further evaluated at four different concentrations (1, 5, 10 and 25 nM) in order to calculate their IC_{50} values. Exceptionally, the IC_{50} value of unsubstituted compound **B-1** was also calculated to better describe the structure activity relationship. The IC_{50} value of most potent compound **B-16** (0.73 nM) was found comparable to that of literature value of phosphorylated flavonoid (**PF**, IC_{50} = 0.72 nM)[11] (**Table 1**). Most excitingly, the whole series was found to be more active as compare to the previous series of compounds (7,8-benzoflavones) with the IC_{50} values ranging from 0.72-31.35. Moreover, the experimentation for the evaluation to check the specificity of most potent compound towards the cholesterol esterase enzyme is also under progress.

Enzyme kinetics study:

The most potent compound among the series (**B-16**) was further investigated for the type of inhibition by performing enzyme kinetic studies.[19] The Lineweaver-Burk plot (**Figure 4**) revealed that the compound **B-16** was a mixed-type CEase inhibitor. The pattern of graph shows that it is a form of mixed inhibition scenario. The K_m, V_{max} and slope are all affected by the inhibitor. The inhibitor has increased the K_m and slope (K_m/V_{max}) while decreasing the V_{max}. Moreover carefully observing the **Figure 4** it was found that intersecting lines on the graph converge to the left of the *y*-axis and above the *x*-axis which indicates that the value of α (a constant that defines the degree to which inhibitor binding affects the affinity of the enzyme for substrate) is greater than 1. This confirms that the inhibitor preferentially binds to the free enzyme and not to the enzyme substrate complex.

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Docking studies:

Various types of the binding interactions of most potent compound **B-16** within the active site of *human* cholesterolesterase enzyme (*h*CEase), were also streamlined by using molecular modeling studies. The active site apparatus of *h*CEase consist of a catalytic triad and an oxyanion hole.[20] The catalytic triad is made of Ser194, Asp320, and His435 residues and serves as general acid-base and nucleophilic catalytic entity along with an oxyanion hole consisting of Gly107, Ala108, and Ala195 residues.[20,21] The hydroxyl group of Ser194 acts as nucleophile and is necessary for the hydrolytic reaction. The serine lipases and serine proteases also possess the Ser-Asp-His catalytic triad and share the catalytic mechanism with *h*CEase. In the present docking study, the *h*CEase residues within the radius of 10 Å around the hydroxyl function of Ser194 were defined to form the active site of the enzyme.[21]

The **B-16** fits well at the catalytic site and stabilized by H-bonds, polar and van der Walls interactions (**Figure 5** and **6**). Interestingly, the Gly107, Ala108, and Ala195 residues of oxyanion hole were involved in H-bond interactions with the carbonyl oxygen of Ring C (H-bond acceptor; d = 2.01 to 2.19 Å). The three H-bonds showed their significance in the tight binding of **B-16** with *h*CEase. Rings A, B and C were stabilized by van der Waals interaction with Ser194, His435 and Ala436. In addition to this, the Ring C of **B-16** is placed in a well-defined cavity formed by Gly106, Gly107, Glu193, Ser194 and Ala195 and suggested to be stabilized by dispersion interactions. Ring D (dichlorophenyl) gets positioned in a hydrophobic cavity created by Trp227, Phe324, Leu392 and Phe393 residues and involved in face-to-face pai-pai stacking interaction with Trp227 and Phe324. The study showed that the **B-16** completely blocks the catalytic assembly of *h*CEase. Its binding with the oxyanion hole prevents it to participate in ester hydrolysis mechanism. The favorable binding conformation of **B-16** suggests its prevailing role as *h*CEase inhibitor.

While comparing the docking conformation of **B-16** and **A** (lead compound), the **B-16** showed more strong binding with *h*CEase as shown by higher Gold score than **A** (Gold score = 54.35 and 46.06 for **B-16** and **A**, respectively). Both compounds shared common pharmacophoric features except the position of Ring A (**Figure 2**). In contrary to **B-16**, the Ring A of "compound **A"** is in *trans* conformation to the carbonyl function at Ring C and surrounded by Ile323 and His435 only. Therefore, this can be suggested that *cis*-conformation of Ring A and carbonyl function at

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Ring C is more favorable for activity than *trans*-conformation. This pattern of docking is in full agreement with the *in-vitro* results. (**Figure 7**).

***In silico* studies**:

Furthermore, physico-chemical properties like Absorption, Distribution, Metabolism and Excretion (ADME) of the synthesized compounds were determined *in silico* by using the web-based applications MarvinSketch (http://www.chemaxon.com/) and PreADMET (http://preadmet.bmdrc.org/). The Caco-2 cell, MDCK cell, Blood Brain Barrier (BBB) & Skin permeabilities, human intestinal absorption and plasma protein binding affinities were predicted and summarized in **Table 2**. Results indicated that the compounds are predicted to have lower blood brain barrier permeation which are less likely to cause neurotoxicity. In other words we can say that the synthesized compounds cannot alter the normal activity of the neuronal cells. Basicity and lipophilicity of the synthesized compounds were determined with ChemAxon software MarvinSketch and the results are shown in **Table 3** which describes the compliance of all the synthesized compounds with the Lipinski rule of five. Tabular values indicated that a) all the molecules have molecular weight in the range of 286-408 which lies in the limit of 180-500 b) compounds have no H-bond donating property c) compounds followed the H-bond acceptor criteria (< 10) d) molar refractivity found very consistence in the range of 83.64-98.07 which lies well in the accepted value range 40-130 and e) a log P of all the compounds found lower than 5.6 indicating that the compounds are not very lipophilic. These results suggest that all the compounds follow the Lipinski rule of five and ADME properties which makes them pharmacologically efficient for clinical use in future.

Conclusion:

A series of 5,6-benzoflavone derivatives rationally designed, synthesized and characterized by using ^{1}H NMR, ^{13}C NMR, MASS and Elemental Analysis. All the synthetics were evaluated for *in-vitro* cholesterol esterase inhibitory activity. Among all the derivatives, **B-16** was found to be endowed with most potent enzyme inhibition with IC_{50} value of 0.73 nM. Enzyme kinetic study confirmed that the inhibitor **B-16** preferentially binds to the free enzyme and not to the enzyme substrate complex (mixed type inhibition). Docking study suggested that the compound **B-16** fits well in the active site of cholesterol esterase enzyme and completely blocks its catalytic

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assembly. The study also concluded that *cis*-conformation of Ring A and carbonyl function at Ring C is well tolerable for CEase inhibition. In silico parameters revealed that the compounds with improved CEase inhibitory potential could be act as a hit lead molecules for further development of pharmacologically active CEase inhibitory framework.

Experimental:

Materials and measurements

The reagents were purchased from Sigma Aldrich, Loba and CDH, India and used without further purification. The porcine cholesterol esterase enzyme was also procured from Sigma Aldrich. All yields refer to isolated products after purification. Products were characterized by comparison with authentic samples and by spectroscopic data (^{1}H, ^{13}C NMR and MASS). ^{1}H NMR and ^{13}C NMR Spectra were recorded on JEOL AL 300 NMR Spectrometer. The spectra were measured in $CDCl_3$ relative to TMS (0.00 ppm). In ^{1}H NMR chemical shifts were reported in δ values using tetramethylsilane as internal standard with number of protons, multiplicities (s-singlet, d-doublet, t-triplet, q-quartet, m-multiplet, dd-double doublet) and coupling constants (*J*) in Hz (Hertz) in the solvent indicated. HRMS was recorded on micrOTOF-QII Bruker Daltonik LC–MS/MS High Resolution Mass Spectrometer. Melting points were determined in open capillaries and were uncorrected.

Procedure for Synthesis of 1-(2-hydroxynaphthalen-1-yl)ethanone (1)

β-naphthol (1mmol) was treated with glacial acetic acid (1.2 mmol) in the presence of $Zncl_2$ (0.41 mmol) under microwave irradiation for 20 mins at 200°C. The crude mixture was dissolved in methanol and adsorbed on silica (60-120 #). The desired product was purified by column chromatography with increasing percentage of ethyl acetate in hexane as eluting solvent. The characterization data for the 1-(2-hydroxynaphthalen-1-yl)ethanone is as follows:

Yield: 60 %, mp: 62-68ºC. ^{1}H NMR ($CDCl_3$, 300 MHz, δ, TMS = 0): 13.48 (1H, s, OH), 8.09 (1H, d, *J* = 8.4 Hz, ArH), 7.89 (1H, d, *J* = 9.0 Hz, ArH), 7.78 (1H, d, *J* = 8.1 Hz, ArH), 7.55-7.60 (1H, m, ArH), 7.37-7.42 (1H, m, ArH), 7.14 (1H, d, *J* = 9.0 Hz, ArH), 2.87 (3H, s, $COCH_3$). ^{13}C NMR ($CDCl_3$, 75 MHz, δ, TMS = 0): 35.22, 117.55, 122.59, 126.49, 127.06, 130.85, 131.28,

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132.31, 134.63, 140.25, 166.81, 207.44. Anal. Calcd. For $C_{12}H_{10}O_2$: C, 77.40; H, 5.41; O, 17.18; Found: C, 77.32; H, 5.55.

Procedure for Synthesis of 1-acetylnaphthalen-2-yl benzoate (2)

To a solution of 1-(2-hydroxynaphthalen-1-yl)ethanone (0.01 mol) in pyridine **(5 ml),** benzoylchloride (1 eq) was added and stirred for 1 hr at room temperature. The mixture was poured on ice and precipitated solid was collected and dried.

Yield: 84 %, mp: 49-53ºC. ^{1}H NMR ($CDCl_3$, 300 MHz, δ, TMS = 0): 8.19-8.21 (2H, m, ArH), 7.81-7.96 (3H, m, ArH), 7.64-7.67 (1H, m, ArH), 7.53-7.55 (4H, m, ArH), 7.36-7.40 (1H, m, ArH), 2.62 (3H, s, $COCH_3$); ^{13}C NMR ($CDCl_3$, 75 MHz, δ, TMS = 0): 35.01, 124.08, 127.11, 128.84, 130.34, 131.05, 131.33, 131.44, 132.44, 132.95, 133.65, 134.25, 136.76, 147.57, 167.52, 205.70. Anal. Calcd. for $C_{19}H_{14}O_3$: C, 78.61; H, 4.86; Found: C, 78.93; H, 4.61.

Procedure for Synthesis of 1-(2-hydroxynaphthalen-1-yl)-3-phenylpropane-1,3-dione (3)

The mixture of 1-acetylnaphthalen-2-yl benzoate (0.01 mmol), KOH (1 mmol) and pyridine (2 ml) was warmed on a water bath for 15 mins. Acetic acid solution (10%, 1.3 ml) was added to the cooled mixture. The crude mixture was dissolved in ethyl acetate and adsorbed on silica (60-120 #). The desired product was purified by column chromatography with increasing percentage of ethyl acetate in hexane as eluting solvent. The characterization data for the 1-(2-hydroxynaphthalen-1-yl)-3-phenylpropane-1,3-dione is as follows:

Yield: 65%; ^{1}H NMR ($CDCl_3$, 300 MHz, δ, TMS = 0): 11.35 (1H, s, D_2O exchangeable proton), 8.27-8.30 (1H, d, J = 8.4 Hz, ArH), 7.90 (1H, s, ArH), 7.67-7.70 (1H, m, ArH), 7.39-7.42 (1H, m, ArH), 7.15-7.26 (5H, m, ArH), 6.66 (1H, s, ArH), 1.29 (2H, s, -CH_2-). Anal. Calcd. for $C_{19}H_{14}O_3$: C, 78.61; H, 4.86; O, 16.53; Found: C, 78.72; H, 4.73.

Procedure for Synthesis of 5,6-benzoflavones (B-1)

To a solution of the 1-(2-hydroxynaphthalen-1-yl)-3-phenylpropane-1,3-dione (1 mmol) in acetic acid (5 ml) was added a drop of concentrated sulfuric acid and the mixture was refluxed for 1

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hour. The cooled mixture was poured into ice and the product was collected by simple filtration. The characterization data for the synthesized derivatives is given below:

3-phenyl-1H-benzo[*f*]chromen-1-one **(B-1)**[22-24]**:** Yield 91%, mp 157-162°C. ^{1}H NMR ($CDCl_3$, 300 MHz, δ, TMS = 0): 9.78 (1H, d, *J* = 9.0 Hz, ArH), 7.77-7.79 (1H, m, ArH), 7.73-7.74 (1H, m, ArH), 7.56-7.61 (3H, m, ArH), 7.36-7.47 (5H, m, ArH), 6.93 (1H, s, -CH-). ^{13}C NMR ($CDCl_3$, 75 MHz, δ, TMS = 0): 108.64, 119.15, 120.43, 122.56, 124.45, 125.32, 126.45, 127.54, 128.32, 129.34, 129.21, 131,65, 131.34, 136.65, 153.34, 162.23, 178.34. MS: m/z: 273 (M^++1). Anal. Calcd for $C_{19}H_{12}O_2$: C, 83.81; H, 4.44; Found: C, 83.59; H, 4.54.

All the 5,6-benzoflavone derivatives were synthesized with above given procedure and their characterization data is given below:

3-(4-methoxyphenyl)-1H-benzo[*f*]chromen-1-one (B-2)[22,25]**:** Yield 88%, mp 72-78°C. ^{1}H NMR ($CDCl_3$, 300 MHz, δ, TMS = 0): 10.11 (1H, m, ArH), 8.13-8.17 (3H, m, ArH), 8.07 (1H, m, ArH), 7.97 (1H, m, ArH), 7.41 (2H, d, *J* = 8.5 Hz, ArH), 7.01-7.03 (2H, m, ArH), 6.95 (1H, s, -CH-), 3.96 (3H, s, OCH_3). ^{13}C NMR ($CDCl_3$, 75 MHz, δ, TMS = 0): 56.93, 108.27, 114.16, 118.45, 119.54, 121.66, 123.45, 123.99, 126.75, 127.16, 128.34, 130.12, 130.67, 135.76, 153.34, 162.56, 177.76. MS: m/z: 303 (M^++1). Anal. Calcd for $C_{20}H_{14}O_3$: C, 79.46; H, 4.67; Found: C, 79.57; H, 4.42.

3-(3,4-dimethoxyphenyl)-1H-benzo[*f*]chromen-1-one (B-3)[22]**:** Yield 84%, mp 99-105°C. ^{1}H NMR ($CDCl_3$, 300 MHz, δ, TMS = 0): 10.07 (1H, d, *J* = 8.9 Hz, ArH), 8.12-8.19 (3H, m, ArH), 7.93-7.99 (2H, m, ArH), 7.72-7.78 (3H, m, ArH), 6.94 (1H, s, -CH-), 3.92 (3H, s, OCH_3), 3.96 (3H, s, OCH_3). ^{13}C NMR ($CDCl_3$, 75 MHz, δ, TMS = 0): 55.83, 56.35, 101.43, 102.99, 1105.54, 107.34, 110.76, 119.45, 122.76, 123.34, 123.76, 123.45, 128.76, 128.34, 129.65, 130.56, 135.34, 148.76, 153.34, 162.54, 177.34. MS: m/z: 322 (M^+ + 1). Anal. Calcd for $C_{21}H_{16}O_4$: C, 75.89; H, 4.85; Found: C, 75.91; H, 4.71.

3-(2,4-dimethoxyphenyl)-1H-benzo[*f*]chromen-1-one (B-4)[25]**:** Yield 89%, mp 105-113°C. ^{1}H NMR ($CDCl_3$, 300 MHz, δ, TMS = 0): 10.11 (1H, m, ArH), 8.12-8.14 (3H, m, ArH), 8.06-8.09 (2H, m, ArH), 7.76 (2H, m, ArH), 7.22-7.24 (1H, m, ArH), 7.18 (1H, s, -CH-), 3.94 (3H, s, OCH_3), 3.98 (3H, s, OCH_3). ^{13}C NMR ($CDCl_3$, 75 MHz, δ, TMS = 0): 55.95, 56.40, 101.34, 102.35, 105.43, 107.22, 119.97, 122.60, 123.74, 123.91, 128.10, 128.44, 128.76, 129.45, 130.66,

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135.88, 153.68, 158.75, 160.88, 162.20, 177.33. MS: m/z: 322 (M^+ + 1). Anal. Calcd for $C_{21}H_{16}O_4$: C, 75.89; H, 4.85; Found: C, 75.66; H, 4.91.

3-(3-trifluoromethoxyphenyl)-1H-benzo[*f*]chromen-1-one (B-5): Yield 83%, mp: 96-101°C, ^{1}H NMR ($CDCl_3$, 300 MHz, δ, TMS = 0): 10.08 (1H, d, *J* = 8.5 Hz, ArH), 8.14-8.18 (3H, m, ArH), 8.02-8.04 (2H, m, ArH), 7.21-7.26 (2H, m, ArH), 7.09-7.16 (2H, m, ArH), 6.95 (1H, s, -CH-): ^{13}C NMR ($CDCl_3$, 75 MHz, δ, TMS = 0): 110.55, 111.42, 113.67, 118.34, 120.21, 120.65, 122.76, 123.45, 123.65, 128.67, 128.99, 129.54, 129.99, 130.43, 131.78, 135.45, 153.76, 159.34, 162.55, 177.23. MS: m/z: 357 (M^+ + 1). Anal. Calcd for $C_{20}H_{11}F_3O_3$: C, 67.42; H, 3.11; F, 16.00; Found C, 67.55; H, 2.99; F, 16.09.

3-(4-trifluoromethoxyphenyl)-1H-benzo[*f*]chromen-1-one (B-6): Yield 87%, mp: 115-120°C. ^{1}H NMR ($CDCl_3$, 300 MHz, δ, TMS = 0): 10.09 (1H, d, *J* = 8.6 Hz, ArH), 8.14-8.19 (3H, m, ArH), 8.06-8.09 (2H, m, ArH), 7.88-7.91 (2H, m, ArH), 7.23 (2H, d, *J* = 8.5 Hz, ArH), 6.98 (1H, s, -CH-). ^{13}C NMR ($CDCl_3$, 75 MHz, δ, TMS = 0): 110.76, 115.34, 118.95, 120.76, 120.34, 122.23, 123.21, 123.56, 125.11, 128.17, 128.34, 129.76, 130.45, 135.34, 153.87, 162.45, 177.45. MS: m/z: 357 (M^+ + 1). Anal. Calcd for $C_{20}H_{11}F_3O_3$; C, 67.42; H, 3.11; F, 16.00; Found: C, 67.36; H, 3.24; F, 16.01.

3-(2-fluorophenyl)-1H-benzo[*f*]chromen-1-one (B-7)[23,24]: Yield 76%, mp 105-109°C. ^{1}H NMR ($CDCl_3$, 300 MHz, δ, TMS = 0): 10.06 (1H, d, *J* = 8.4 Hz, ArH), 8.13 (1H, d, *J* = 9.0 Hz, ArH), 7.91-7.99 (2H, m, ArH), 7.75-7.81 (1H, m, ArH), 7.35-7.66 (5H, m, ArH), 7.13 (1H, s, -CH-). ^{13}C NMR ($CDCl_3$, 75 MHz, δ, TMS = 0): 115.16, 115.26, 116.90, 117.08, 117.52, 123.78, 124.36, 124.66, 126.71, 127.20, 128.17, 128.90, 129.33, 129.56, 130.41, 130.62, 132.70, 132.77, 135.65, 137.47, 156.40, 157.53, 180.28. MS: m/z: 291 (M^+ + 1). Anal. calcd. for $C_{19}H_{11}FO_2$: C, 78.61; H, 3.82; F, 6.54; Found: C, 78.55; H, 3.91; F, 6.44.

3-(3-fluorophenyl)-1H-benzo[*f*]chromen-1-one (B-8): Yield 78%, mp 123-128°C. ^{1}H NMR ($CDCl_3$, 300 MHz, δ, TMS = 0): 10.03 (1H, d, *J* = 8.6 Hz, ArH), 8.12-8.14 (1H, m, ArH), 7.92-7.95 (2H, m, ArH), 7.75-7.79 (1H, m, ArH), 7.62-7.66 (3H, m, ArH), 7.34-7.36 (2H, m, ArH), 7.14 (1H, s, -CH-). ^{13}C NMR ($CDCl_3$, 75 MHz, δ, TMS = 0): 114.13, 115.45, 116.96, 117.56, 117.56, 123.74, 124.33, 124.68, 126.75, 127.27, 128.13, 128.96, 129.34, 129.87, 130.45, 130.34,

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132.77, 132.45, 135.67, 137.44, 156.45, 157.56, 180.45. MS: m/z: 291 (M^+ + 1). Anal. calcd. for $C_{19}H_{11}FO_2$: C, 78.61; H, 3.82; F, 6.54; Found: C, 78.74; H, 3.75; F, 6.55.

3-(4-fluorophenyl)-1H-benzo[*f*]chromen-1-one (B-9): Yield 77%, mp 130-136°C. ^{1}H NMR ($CDCl_3$, 300 MHz, δ, TMS = 0): 10.04 (1H, d, *J* = 8.5 Hz, ArH), 8.13-8.15 (1H, m, ArH), 7.91-7.94 (2H, m, ArH), 7.75-7.79 (2H, m, ArH), 7.62-7.66 (2H, m, ArH), 7.22 (2H, d, *J* = 8.9 Hz, ArH), 7.13 (1H, s, -CH-). ^{13}C NMR ($CDCl_3$, 75 MHz, δ, TMS = 0): 114.16, 115.43, 116.94, 117.57, 117.54, 123.75, 124.38, 124.64, 126.76, 127.23, 128.16, 128.94, 129.33, 129.84, 130.47, 130.33, 132.72, 132.44, 135.63, 137.45, 156.42, 157.53, 180.42. MS: m/z: 291 (M^+ + 1). Anal. calcd. for $C_{19}H_{11}FO_2$: C, 78.61; H, 3.82; F, 6.54; Found: C, 78.72; H, 3.64; F, 6.59.

3-(2,6-difluorophenyl)-1H-benzo[*f*]chromen-1-one (B-10)[23,24]: Yield 73%, mp 89-93°C. ^{1}H NMR ($CDCl_3$, 300 MHz, δ, TMS = 0): 10.05 (1H, d, *J* = 8.6 Hz, ArH), 8.13-8.16 (3H, m, ArH), 8.05-8.09 (2H, m, ArH), 7.55-7.58 (1H, m, ArH), 7.23-7.29 (2H, m, ArH), 6.93 (1H, s, -CH-). ^{13}C NMR ($CDCl_3$, 75 MHz, δ, TMS = 0): 114.22, 115.46, 116.93, 117.57, 117.53, 123.74, 128.12, 128.64, 129.96, 130.46, 135.34, 153.05, 158.44, 162.26, 179.99. MS: m/z: 309 (M^+ + 1). Anal. calcd. for $C_{19}H_{10}F_2O_2$: C, 74.03; H, 3.27; F, 12.33; Found: C, 74.22; H, 3.17; F, 12.44.

3-(2,4-difluorophenyl)-1H-benzo[*f*]chromen-1-one (B-11): Yield 76%, mp 103-108°C. ^{1}H NMR ($CDCl_3$, 300 MHz, δ, TMS = 0): 10.07 (1H, d, *J* = 8.4 Hz, ArH), 8.10-8.15 (3H, m, ArH), 7.99-8.03 (2H, m, ArH), 7.64-7.69 (1H, m, ArH), 7.21-7.25 (2H, m, ArH), 6.91 (1H, s, -CH-). ^{13}C NMR ($CDCl_3$, 75 MHz, δ, TMS = 0): 115.21, 115.88, 116.97, 117.53, 117.56, 123.73, 128.17, 128.63, 129.93, 130.47, 135.39, 153.03, 158.47, 162.24, 180.34. MS: m/z: 309 (M^+ + 1). Anal. calcd. for $C_{19}H_{10}F_2O_2$: C, 74.03; H, 3.27; F, 12.33; Found: C, 74.22; H, 3.36; F, 12.23.

3-(3,5-difluorophenyl)-1H-benzo[*f*]chromen-1-one (B-12): Yield 78%, mp 124-139°C. ^{1}H NMR ($CDCl_3$, 300 MHz, δ, TMS = 0): 9.80 (1H, d, *J* = 8.4 Hz, ArH), 7.97 (1H, d, *J* = 9.0 Hz, ArH), 7.75 (1H, d, *J* = 8.1 Hz, ArH), 7.42-7.58 (4H, m, ArH), 7.29-7.33 (2H, m, ArH), 6.76-6.85 (1H, m, -CH-). ^{13}C NMR ($CDCl_3$, 75 MHz, δ, TMS = 0): 114.56, 115.41, 116.93, 117.54, 117.56, 123.77, 128.18, 128.69, 129.94, 130.43, 135.32, 153.01, 158.42, 162.23, 179.94. MS: m/z: 309 (M^+ + 1). Anal. calcd. for $C_{19}H_{10}F_2O_2$: C, 74.03; H, 3.27; F, 12.33; Found: C, 73.99; H, 3.34; F, 12.15.

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3-(2,5-difluorophenyl)-1H-benzo[*f*]chromen-1-one (B-13): Yield 81%, mp 131-136°C. ^{1}H NMR ($CDCl_3$, 300 MHz, δ, TMS = 0): 10.09 (1H, m, ArH), 8.15-8.19 (3H, m, ArH), 8.02-8.05 (2H, m, ArH), 7.33-7.42 (3H, m, ArH), 6.85 (1H, s, -CH-). ^{13}C NMR ($CDCl_3$, 75 MHz, δ, TMS = 0): 106.64, 109.09, 109.31, 111.43, 117.36, 126.90, 127.11, 128.24, 129.49, 130.33, 130.71, 135.96, 137.01, 157.27, 162.43, 164.31, 179.84. MS: m/z: 309 (M^+ + 1). Anal. calcd. for $C_{19}H_{10}F_2O_2$: C, 74.03; H, 3.27; F, 12.33; Found: C, 74.21; H, 3.08; F, 12.66.

3-(3,4-difluorophenyl)-1H-benzo[*f*]chromen-1-one (B-14): Yield 79%, mp 104-107°C. ^{1}H NMR ($CDCl_3$, 300 MHz, δ, TMS = 0): 10.06 (1H, m, ArH), 8.12-8.17 (3H, m, ArH), 8.02-8.05 (2H, m, ArH), 7.42-7.45 (1H, m, ArH), 7.38 (1H, s, ArH), 7.29-7.32 (1H, m, ArH), 6.94 (1H, s, -CH-). ^{13}C NMR ($CDCl_3$, 75 MHz, δ, TMS = 0): 115.23, 115.65, 116.96, 117.53, 117.55, 123.71, 128.16, 128.67, 129.93, 130.42, 135.37, 153.08, 158.41, 162.23, 179.95. MS: m/z: 309 (M^+ + 1). Anal. calcd. for $C_{19}H_{10}F_2O_2$: C, 74.03; H, 3.27; F, 12.33; Found: C, 74.32; H, 3.45; F, 12.10.

3-(3-chlorophenyl)-1H-benzo[*f*]chromen-1-one (B-15): Yield 78%, mp 160-166°C. ^{1}H NMR ($CDCl_3$, 300 MHz, δ, TMS = 0): 10.07 (1H, m, ArH), 8.11-8.17 (3H, m, ArH), 8.00-8.04 (2H, m, ArH), 7.66 (1H, s, ArH), 7.23-7.30 (3H, m, ArH), 6.96 (1H, s, -CH-). ^{13}C NMR ($CDCl_3$, 75 MHz, δ, TMS = 0): 115.45, 117.45, 117.65, 126.26, 127.64, 128.36, 129.08, 130.33, 131.64, 132.37, 133.35, 134.34, 135.43, 136.55, 153.54, 161.18, 180.05. MS: m/z: 307 (M^+ + 1). Anal. calcd. for $C_{19}H_{11}ClO_2$: C, 74.40; H, 3.61; Cl, 11.56; Found: C, 74.50; H, 3.59; Cl, 11.60.

3-(2,3-dichlorophenyl)-1H-benzo[*f*]chromen-1-one (B-16): Yield 84%, mp 160-165°C. ^{1}H NMR ($CDCl_3$, 300 MHz, δ, TMS = 0): 10.08 (1H, m, ArH), 8.15-8.19 (3H, m, ArH), 7.98-8.03 (2H, m, ArH), 7.32-7.36 (3H, m, ArH), 6.81 (1H, s, -CH-). ^{13}C NMR ($CDCl_3$, 75 MHz, δ, TMS = 0): 115.97, 117.26, 117.55, 126.80, 127.16, 127.64, 128.21, 129.02, 129.40, 130.41, 130.69, 131.67, 132.51, 133.71, 134.61, 135.83, 157.79, 159.70, 179.79. MS: m/z: 341 (M^+ + 1). Anal. calcd. for $C_{19}H_{10}Cl_2O_2$: C, 66.89; H, 2.95; Cl, 20.78; Found: C, 66.99; H, 2.85; Cl, 20.91.

3-(2-bromophenyl)-1H-benzo[*f*]chromen-1-one (B-17): Yield 75%, mp 153-159°C. ^{1}H NMR ($CDCl_3$, 300 MHz, δ, TMS = 0): 10.08 (1H, d, *J* = 8.7 Hz, ArH), 8.13 (1H, d, *J* = 9.0 Hz, ArH), 7.94 (1H, *d, J* = 7.5 Hz, ArH), 7.40-7.80 (7H, m, ArH), 6.78 (1H, s, -CH-). ^{13}C NMR ($CDCl_3$, 75 MHz, δ, TMS = 0): 115.12, 116.89, 117.07, 117.11, 117.51, 119.90, 124.64, 124.66, 126.71, 127.19, 128.18, 128.90, 129.33, 130.61, 132.71, 132.79, 135.68, 157.54, 180.32. MS: m/z: 350

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(M^+ + 1). Anal. calcd. for $C_{19}H_{11}BrO_2$: C, 64.98; H, 3.16; Br, 22.75; Found: C, 65.05; H, 3.03; Br, 22.88.

3-(3-bromophenyl)-1H-benzo[*f*]chromen-1-one (B-18): Yield 78%, mp 168-171°C. ^{1}H NMR ($CDCl_3$, 300 MHz, δ, TMS = 0): 10.04 (1H, d), 8.15 (1H, d, *J* = 9.0 Hz, ArH), 7.95 (1H, m, ArH), 7.77-7.83 (3H, m, ArH), 7.63 (1H, s, ArH), 7.23-7.33 (3H, m ArH), 6.98 (1H, s, -CH-). ^{13}C NMR ($CDCl_3$, 75 MHz, δ, TMS = 0): 110.53, 117.23, 117.43, 126.08, 126.79, 127.18, 127.47, 128.16, 129.35, 130.34, 130.42, 130.67, 132.35, 135.66, 157.33, 159.72, 179.97. MS: m/z: 350 (M^+ + 1). Anal. calcd. for $C_{19}H_{11}BrO_2$: C, 64.98; H, 3.16; Br, 22.75; Found: C, 65.02; H, 3.09; Br, 22.79.

3-(4-bromophenyl)-1H-benzo[*f*]chromen-1-one (B-19): Yield 80%, mp 220-225°C. ^{1}H NMR ($CDCl_3$, 300 MHz, δ, TMS = 0): 10.06 (1H, d, *J* = 8.7 Hz, ArH), 8.14 (1H, d, *J* = 9.0 Hz, ArH), 7.93 (1H, d, J = 8.1 Hz, ArH), 7.78-7.85 (3H, m, ArH), 7.61-7.70 (4H, m, ArH), 6.97 (1H, s, -CH-). ^{13}C NMR ($CDCl_3$, 75 MHz, δ, TMS = 0): 110.57, 117.28, 117.46, 126.05, 126.74, 127.16, 127.49, 128.18, 129.34, 130.34, 130.43, 130.65, 132.37, 135.65, 157.32, 159.76, 180.07. MS: m/z: 350 (M^++1). Anal. calcd. for $C_{19}H_{11}BrO_2$: C, 64.98; H, 3.16; Br, 22.75; Found: C, 64.78; H, 3.00; Br, 22.65.

3-(2-iodophenyl)-1H-benzo[*f*]chromen-1-one (B-20): Yield 69%, mp 141-146°C. ^{1}H NMR ($CDCl_3$, 300 MHz, δ, TMS = 0): 10.05 (1H, m, ArH), 8.19-8.25 (3H, m, ArH), 8.11-8.13 (2H, m, ArH), 7.82-7.84 (1H, m, ArH), 7.31-7.36 (3H, m, ArH), 6.96 (1H, s, -CH-). ^{13}C NMR ($CDCl_3$, 75 MHz, δ, TMS = 0): 110.32, 113.65, 119.25, 120.65, 122.82, 124.16, 125.62, 127.26, 128.12, 128.56, 129.47, 130.81, 131.94, 136.03, 138.07, 140.53, 154.02, 165.25, 180.12. MS: m/z: 398 (M^+ + 1). Anal. calcd. for $C_{19}H_{11}IO_2$: C, 57.31; H, 2.78; I, 31.87; Found: C, 57.42; H, 2.88; I, 31.93.

3-(4-iodophenyl)-1H-benzo[*f*]chromen-1-one (B-21): Yield 72%, mp 133-139°C. ^{1}H NMR ($CDCl_3$, 300 MHz, δ, TMS = 0): 10.08 (1H, m, ArH), 8.19-8.25 (3H, m, ArH), 8.11-8.13 (2H, m, ArH), 7.88 (2H, d, *J* = 8.9 Hz, ArH), 7.25-7.29 (2H, m, ArH), 6.95 (1H, s, -CH-). ^{13}C NMR ($CDCl_3$, 75 MHz, δ, TMS = 0): 110.55, 113.69, 119.28, 120.62, 122.87, 124.13, 125.67, 127.22, 128.16, 128.53, 129.42, 130.87, 131.92, 136.34, 138.09, 140.51, 154.07, 165.29, 180.18. MS:

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m/z: 398 (M^+ + 1). Anal. calcd. for $C_{19}H_{11}IO_2$: C, 57.31; H, 2.78; I, 31.87; Found: C, 57.28; H, 2.79; I, 31.77.

3-(3-nitrophenyl)-1H-benzo[*f*]chromen-1-one (B-22): Yield 79%, mp 139-141°C. ^{1}H NMR ($CDCl_3$, 300 MHz, δ, TMS = 0): 10.15 (1H, m, ArH), 8.42 (1H, m, ArH), 8.11-8.14 (3H, m, ArH), 7.78-7.85 (2H, m, ArH), 7.62-7.65 (2H, m, ArH), 7.07 (1H, s, -CH-). ^{13}C NMR ($CDCl_3$, 75 MHz, δ, TMS = 0): 112.48, 115.93, 117.35, 124.25, 124.30, 126.77, 126.99, 127.14, 128.20, 128.31, 129.31, 129.62, 136.14, 136.16, 137.45, 157.45, 158.11, 179.74. MS: m/z: 318 (M^+ + 1). Anal. calcd. for $C_{19}H_{11}NO_4$: C, 71.92; H, 3.49; N, 4.41; Found: C, 72.00; H, 3.33; N, 4.34.

3-(4-nitrophenyl)-1H-benzo[*f*]chromen-1-one (B-23): Yield 76%, mp: 102-106°C. ^{1}H NMR ($CDCl_3$, 300 MHz, δ, TMS = 0): 10.14 (1H, d, *J* = 8.4 Hz, ArH), 8.41 (1H, d, *J* = 8.7 Hz, ArH), 8.14-8.19 (3H, m, ArH), 7.95 (1H, d, *J* = 8.4 Hz, ArH), 7.78-7.80 (1H, m, ArH), 7.65-7.68 (2H, m, ArH), 7.08 (1H, s, -CH-). ^{13}C NMR ($CDCl_3$, 75 MHz, δ, TMS = 0): 112.48, 115.93, 117.35, 124.25, 124.30, 126.77, 126.99, 127.14, 128.20, 128.31, 129.31, 129.62, 136.14, 136.16, 137.45, 157.45, 158.11, 179.74. MS: m/z: 318 (M^+ + 1). Anal. calcd. for $C_{19}H_{11}NO_4$: C, 71.92; H, 3.49; N, 4.41; Found: C, 71.85; H, 3.35; N, 4.65.

3-(3,5-dinitrophenyl)-1H-benzo[*f*]chromen-1-one (B-24): Yield 81%, mp 101-106°C. ^{1}H NMR ($CDCl_3$, 300 MHz, δ, TMS = 0): 10.15 (1H, s, ArH), 9.08 (1H, s, ArH), 8.65-8.70 (2H, m, ArH), 8.11 (1H, m, ArH), 7.97-7.99 (1H, m, ArH), 7.77-7.84 (3H, m, ArH), 7.07 (1H, s, -CH-). ^{13}C NMR ($CDCl_3$, 75 MHz, δ, TMS = 0): 112.43, 115.97, 117.39, 124.22, 124.36, 126.73, 126.93, 127.17, 128.24, 128.36, 129.37, 129.67, 136.18, 136.13, 137.46, 157.47, 158.18, 180.75 MS: m/z: 363 (M^++1). Anal. calcd. for $C_{19}H_{10}N_2O_6$: C, 62.76; H, 2.93; N, 7.35; Found: C, 62.66; H, 3.03; N, 7.30.

3-*p*-tolyl-1H-benzo[*f*]chromen-1-one (B-25)[24]: Yield 85%, mp: 96-102°C. ^{1}H NMR ($CDCl_3$, 300 MHz, δ, TMS = 0): 10.04 (1H, m, ArH), 8.12-8.18 (3H, m, ArH), 8.01-8.06 (2H, m, ArH), 7.33 (2H, d, *J* = 8.4 Hz, ArH), 7.11-7.15 (2H, m, ArH), 7.01 (1H, s, -CH-), 2.45 (3H, s, $-CH_3$). ^{13}C NMR ($CDCl_3$, 75 MHz, δ, TMS = 0): 26.45, 110.26, 119.93, 122.65, 123.77, 123.95, 126.33, 127.46, 128.18, 128.63, 129.07, 129.93, 130.45, 135.32, 138.55, 153.04, 162.25, 180.24. MS: m/z: 287 (M^++1). Anal. calcd. for $C_{20}H_{14}O_2$: C, 83.90; H, 4.93; Found: C, 83.80; H, 4.98.

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3-(4-(trifluoromethyl)phenyl)-1H-benzo[*f*]chromen-1-one (B-26): Yield 85%, mp: 115-119°C, ^{1}H NMR ($CDCl_3$, 300 MHz, δ, TMS = 0): 10.04 (1H, m, ArH), 8.14-8.19 (3H, m, ArH), 8.01-8.05 (2H, m, ArH), 7.88 (2H, d, *J* = 8.9 Hz, ArH), 7.41-7.45 (2H, m, ArH) 6.97 (1H, s, -CH-). ^{13}C NMR ($CDCl_3$, 75 MHz, δ, TMS = 0): 110.221, 119.91, 122.62, 123.73, 123.95, 126.52, 128.12, 128.64, 129.93, 130.25, 130.46, 133.57, 135.31, 153.03, 162.22, 177.25. MS: m/z: 341 (M^++1). Anal. calcd. for $C_{20}H_{11}F_3O_2$: C, 70.59; H, 3.26; F, 16.75; Found: C, 70.68; H, 3.06; F, 16.82.

3-(3-(trifluoromethyl)phenyl)-1H-benzo[*f*]chromen-1-one (B-27): Yield 74%, mp 106-110°C. ^{1}H NMR ($CDCl_3$, 300 MHz, δ, TMS = 0): 10.07 (1H, m, ArH), 8.13-8.17 (3H, m ArH), 8.00-8.04 (2H, m, ArH), 7.88 (1H, s, ArH), 7.21-7.33 (3H, m, ArH), 6.97 (1H, s, -CH-). ^{13}C NMR ($CDCl_3$, 75 MHz, δ, TMS = 0): 111.45, 119.95, 122.66, 122.83, 123.76, 123.93, 124.05, 124.67, 128.17, 128.67, 129.74, 129.93, 130.45, 130.76, 130.92, 135.33, 153.05, 162.26, 180.28. MS: m/z: 341 (M^++1). Anal. calcd. for $C_{20}H_{11}F_3O_2$: C, 70.59; H, 3.26; F, 16.75; Found: C, 70.49; H, 3.35; F, 16.65.

3-(2-(trifluoromethyl)phenyl)-1H-benzo[*f*]chromen-1-one (B-28): Yield: 81%; mp 105-108°C. ^{1}H NMR ($CDCl_3$, 300 MHz, δ, TMS = 0): 10.06 (1H, m, ArH), 8.11-8.14 (3H, m, ArH), 8.00-8.04 (2H, m, ArH), 7.88-7.91 (1H, m, ArH), 7.21-7.33 (3H, m, ArH), 6.97 (1H, s, -CH-). ^{13}C NMR ($CDCl_3$, 75 MHz, δ, TMS = 0): 110.45, 119.94, 122.63, 122.85, 123.76, 123.96, 124.05, 124.64, 128.13, 128.62, 129.71, 129.92, 130.43, 130.74, 130.95, 135.36, 153.07, 162.28, 180.29. MS: m/z: 341 (M^++1). Anal. calcd. for $C_{20}H_{11}F_3O_2$: C, 70.59; H, 3.26; F, 16.75; Found: C, 70.48; H, 3.35; F, 16.38.

3-(3-(trifluoromethyl)phenyl)-1H-benzo[*f*]chromen-1-one (B-29): Yield 80%, mp 96-101°C. ^{1}H NMR ($CDCl_3$, 300 MHz, δ, TMS = 0): 10.09 (1H, m, ArH), 8.13-8.18 (3H, m, ArH), 7.97-8.02 (2H, m, ArH), 7.84 (1H, s, ArH), 7.41-7.53 (3H, m, ArH), 6.93 (1H, s, -CH-). ^{13}C NMR ($CDCl_3$, 75 MHz, δ, TMS = 0): 110.48, 119.98, 122.67, 122.86, 123.75, 123.94, 124.03, 124.62, 128.12, 128.61, 129.74, 129.95, 130.46, 130.77, 130.94, 135.33, 153.06, 162.23, 180.25. MS: m/z: 409 (M^++1). Anal. calcd. for $C_{21}H_{10}F_6O_2$: C, 61.78; H, 2.47; F, 27.92; Found: C, 61.68; H, 2.55; F, 27.83.

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3-(4-fluoro-2-(trifluoromethyl)phenyl)-1H-benzo[*f*]chromen-1-one (B-30): Yield 81%, mp 155-159°C. ^{1}H NMR ($CDCl_3$, 300 MHz, δ, TMS = 0): 10.06 (1H, d, *J* = 8.6 Hz, ArH), 8.13-8.16 (3H, m, ArH), 8.06 (1H, m, ArH), 8.01 (1H, m, ArH), 7.64-7.66 (1H, m, ArH), 7.44-7.47 (1H, m, ArH), 7.23 (1H, m, ArH), 6.86 (1H, s, -CH-). ^{13}C NMR ($CDCl_3$, 75 MHz, δ, TMS = 0): 110.65, 110.99, 114.75, 117.47, 119.99, 121.45, 122.69, 123.74, 123.96, 125.94, 126.63, 128.17, 128.67, 129.94, 130.47, 135.34, 153.05, 162.25, 164.55, 180.42. MS: m/z: 359 (M^++1). Anal. calcd. for $C_{20}H_{10}F_4O_2$: C, 67.05; H, 2.81; F, 21.21; Found: C, 67.11; H, 2.79; F, 21.33.

3-(2-fluoro-4-(trifluoromethyl)phenyl)-1H-benzo[*f*]chromen-1-one (B-31): Yield 82%, mp 151-155°C. ^{1}H NMR ($CDCl_3$, 300 MHz, δ, TMS = 0): 10.08 (1H, d, *J* = 8.6 Hz, ArH), 8.14-8.17 (3H, m, ArH), 8.03-8.06 (2H, m, ArH), 7.22-7.43 (3H, m, ArH), 6.85 (1H, s, -CH-). ^{13}C NMR ($CDCl_3$, 75 MHz, δ, TMS = 0): 110.23, 110.67, 114.74, 117.45, 119.67, 121.65, 122.76, 123.34, 123.78, 125.45, 126.67, 128.45, 128.89, 129.45, 130.67, 135.67, 153.65, 162.67, 164.56, 180.56. MS: m/z: 359 (M^+ +1). Anal. calcd. for $C_{20}H_{10}F_4O_2$: C, 67.05; H, 2.81; F, 21.21; Found: C, 67.12; H, 2.76; F, 21.34.

3-(2-fluoro-6-(trifluoromethyl)phenyl)-1H-benzo[*f*]chromen-1-one (B-32): Yield 73%, mp 122-127°C. ^{1}H NMR ($CDCl_3$, 300 MHz, δ, TMS = 0): 10.07 (1H, d, *J* = 8.6 Hz, ArH), 8.12-8.14 (3H, m, ArH), 8.02-8.06 (2H, m, ArH), 7.19-7.28 (3H, m, ArH), 6.86 (1H, s, -CH-). ^{13}C NMR ($CDCl_3$, 75 MHz, δ, TMS = 0): 110.64, 110.93, 114.74, 117.45, 119.93, 121.45, 122.66, 123.74, 123.93, 125.92, 126.65, 128.14, 128.64, 129.93, 130.44, 135.36, 153.04, 162.27, 164.54, 180.47. MS: m/z: 359 (M^++1). Anal. calcd. for $C_{20}H_{10}F_4O_2$: C, 67.05; H, 2.81; F, 21.21; Found: C, 67.22; H, 2.76; F, 21.32.

3-(5-fluoro-2-(trifluoromethyl)phenyl)-1H-benzo[*f*]chromen-1-one (B-33): Yield 74%, mp 144-147°C. ^{1}H NMR ($CDCl_3$, 300 MHz, δ, TMS = 0): 10.05 (1H, m, ArH), 8.10-8.13 (3H, m, ArH), 8.02-8.05 (2H, m, ArH), 7.74-7.78 (1H, m, ArH), 7.11-7.23 (2H, m, ArH), 6.85 (1H, s, -CH-). ^{13}C NMR ($CDCl_3$, 75 MHz, δ, TMS = 0): 110.62, 110.92, 114.73, 117.49, 119.98, 121.48, 122.69, 123.78, 123.97, 125.96, 126.68, 128.16, 128.67, 129.95, 130.46, 135.34, 153.07, 162.25, 164.56, 180.43. MS: m/z: 359 (M^++1). Anal. calcd. for $C_{20}H_{10}F_4O_2$: C, 67.05; H, 2.81; F, 21.21; Found: C, 67.25; H, 2.65; F, 21.54.

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3-(3-fluoro-5-(trifluoromethyl)phenyl)-1H-benzo[*f*]chromen-1-one (B-34): Yield 69%, mp 110-115°C. ^{1}H NMR ($CDCl_3$, 300 MHz, δ, TMS = 0): 10.11 (1H, m, ArH), 8.14-8.19 (3H, m, ArH), 8.02-8.00 (2H, m, ArH), 7.66 (1H, s, ArH), 7.21 (2H, m, ArH), 6.94 (1H, s, -CH-). ^{13}C NMR ($CDCl_3$, 75 MHz, δ, TMS = 0): 110.67, 110.95, 114.45, 117.78, 119.45, 121.98, 122.45, 123.67, 123.76, 125.54, 126.67, 128.54, 128.89, 129.43, 130.54, 135.55, 153.43, 162.54, 164.34, 180.23. MS: m/z: 359 (M^++1). Anal. calcd. for $C_{20}H_{10}F_4O_2$: C, 67.05; H, 2.81; F, 21.21; Found: C, 67.14; H, 2.74; F, 21.45.

3-(4-fluoro-3-(trifluoromethyl)phenyl)-1H-benzo[*f*]chromen-1-one (B-35): Yield 81%, mp 158-164°C. ^{1}H NMR ($CDCl_3$, 300 MHz, δ, TMS = 0): 10.10 (1H, m, ArH), 8.16-8.20 (3H, m, ArH), 8.04-8.08 (2H, m, ArH), 7.69 (1H, s, ArH), 7.44 (1H, d, *J* = 8.9 Hz, ArH), 7.23-7.25 (1H, m, ArH), 6.96 (1H, s, -CH-). ^{13}C NMR ($CDCl_3$, 75 MHz, δ, TMS = 0): 110.64, 110.93, 114.74, 117.45, 119.93, 121.45, 122.66, 123.74, 123.93, 125.92, 126.65, 128.14, 128.64, 129.93, 130.44, 135.36, 153.04, 162.27, 164.54, 180.47. MS: m/z: 359 (M^++1). Anal. calcd. for $C_{20}H_{10}F_4O_2$: C, 67.05; H, 2.81; F, 21.21; Found: C, 67.21; H, 2.66; F, 21.29.

3-(4-(chloromethyl)phenyl)-1H-benzo[*f*]chromen-1-one (B-36): Yield 74%, mp 116-120°C. ^{1}H NMR ($CDCl_3$, 300 MHz, δ, TMS = 0): 10.05 (1H, m, ArH), 8.11-8.15 (3H, m, ArH), 8.05-8.07 (2H, m, ArH), 7.66 (2H, d, *J* = 8.6 Hz, ArH), 7.29 (2H, d, *J* = 8.6 Hz, ArH), 6.98 (1H, s, -CH-), 4.64 (2H, s, CH_2Cl). ^{13}C NMR ($CDCl_3$, 75 MHz, δ, TMS = 0): 48.54, 110.56, 119.87, 122.45, 123.34, 123.78, 125.56, 128.45, 128.76, 128.99, 129.45, 130.43, 130.55, 135.67, 137.34, 153.56, 162.56, 180.34. MS: m/z: 321 (M^++1). Anal. calcd. for $C_{20}H_{13}ClO_2$: C, 74.89; H, 4.08; Cl, 11.05; Found: C, 74.79; H, 4.18; Cl, 11.25.

3-(3-(chloromethyl)phenyl)-1H-benzo[*f*]chromen-1-one (B-37): Yield 77%, mp 101-105°C. ^{1}H NMR ($CDCl_3$, 300 MHz, δ, TMS = 0): 10.11 (1H, m, ArH), 8.14-8.17 (3H, m, ArH), 8.06-8.09 (2H, m, ArH), 7.33-7.49 (3H, m, ArH), 6.98 (1H, s, -CH-), 4.69 (2H, s, CH_2Cl). ^{13}C NMR ($CDCl_3$, 75 MHz, δ, TMS = 0): 48.23, 110.23, 119.45, 122.45, 123.65, 123.45, 125.65, 128.54, 128.34, 128.34, 129.45, 130.56, 130.45, 135.23, 137.56, 153.34, 162.23, 180.45. MS: m/z: 321 (M^++1). Anal. calcd. for $C_{20}H_{13}ClO_2$: C, 74.89; H, 4.08; Cl, 11.05; Found: C, 74.78; H, 4.24; Cl, 10.96.

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3-(4-(dimethylamino)phenyl)-1H-benzo[*f*]chromen-1-one (B-38): Yield 74%, mp 100-105°C. ^{1}H NMR ($CDCl_3$, 300 MHz, δ, TMS = 0): 10.04 (1H, m, ArH), 8.14-8.17 (3H, m, ArH), 8.05-8.07 (2H, m, ArH), 7.41 (2H, d, *J* = 8.7 Hz, ArH), 7.22-7.25 (2H, m, ArH), 6.99 (1H, s, -CH-), 2.89 (3H, s, $N(CH_3)_2$), 2.83 (3H, s, $N(CH_3)_2$). ^{13}C NMR ($CDCl_3$, 75 MHz, δ, TMS = 0): 42.56, 42.78, 110.56, 114.34, 119.54, 119.89, 122.65, 123.45, 123.67, 127.45, 128.34, 128.69, 129.56, 130.45, 135.67, 147.54, 153.56, 162.24, 180.56. MS: m/z: 316 (M^++1). Anal. calcd. for $C_{21}H_{17}NO_2$: C, 79.98; H, 5.43; N, 4.44; Found: C, 79.86; H, 5.66; N, 4.34.

3-(3-(acetoxy)phenyl)-1H-benzo[*f*]chromen-1-one (B-39): Yield 78%, mp 160-165°C. ^{1}H NMR ($CDCl_3$, 300 MHz, δ, TMS = 0): 10.11 (1H, m, ArH), 8.14-8.18 (3H, m, ArH), 8.03-8.05 (2H, m, ArH), 7.77-7.82 (2H, m, ArH), 7.33-7.35 (2H, m, ArH), 6.94 (1H, s, -CH-), 2.16 (3H, s, $OCOCH_3$). ^{13}C NMR ($CDCl_3$, 75 MHz, δ, TMS = 0): 23.34, 110.65, 119.45, 121.46, 121.84, 122.65, 123.74, 123.92, 126.76, 127.56, 128.16, 128.66, 129.97, 130.47, 135.37, 153.08, 162.28, 168.75, 180.32. MS: m/z: 331 (M^++1). Anal. calcd. for $C_{21}H_{14}O_4$: C, 76.35; H, 4.27; Found: C, 76.29; H, 4.34.

3-(4-(acetoxy)phenyl)-1H-benzo[*f*]chromen-1-one (B-40): Yield 81%, mp 159-163°C. ^{1}H NMR ($CDCl_3$, 300 MHz, δ, TMS = 0): 10.10 (1H, m, ArH), 8.14-8.18 (3H, m, ArH), 8.02-8.04 (2H, m, ArH), 7.66 (2H, d, *J* = 8.5 Hz, ArH), 7.13-7.15 (2H, m, ArH), 6.96 (1H, s, -CH-), 2.14 (3H, s, $OCOCH_3$). ^{13}C NMR ($CDCl_3$, 75 MHz, δ, TMS = 0): 23.54, 110.25, 119.96, 121.48, 121.86, 122.67, 123.74, 123.95, 126.73, 127.66, 128.14, 128.62, 129.93, 130.42, 135.35, 153.02, 162.22, 168.75, 179.99. MS: m/z: 331 (M^++1). Anal. calcd. for $C_{21}H_{14}O_4$: C, 76.35; H, 4.27; Found: C, 76.42 H, 4.18.

In vitro cholesterol esterase assay

Porcine cholesterol esterase inhibition was assayed spectrophotometrically at 405 nm at 25°C. Assay buffer was 100 mM sodium phosphate, 100 mM NaCl, pH 7.0. A stock solution of CEase (200 µg/mL) was prepared in 100 mM sodium phosphate buffer, pH 7.0 and kept at 0°C. A 1:200 dilution was done with the same buffer immediately before starting the measurement. Sodium taurocholate (12 mM) was dissolved in assay buffer and kept at 25°C. A stock solution of para-nitrophenyl butyrate (20 mM) was prepared in acetonitrile. The final concentration of acetonitrile was 3%, of the substrate para-nitrophenyl butyrate 20 µM, and of sodium taurocholate 6 mM. Assays were performed with a final concentration of 10 ng/mL of CEase.

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Into a cuvette containing 430 µL assay buffer, 500 µL of the sodium taurocholate solution, 20 µL acetonitrile, 10 µL of the para-nitrophenyl butyrate solution, and 30 µL of an inhibitor solution in DMSO were added and thoroughly mixed. After incubation for 5 min at 25°C, the reaction was initiated by adding 10 µL of the enzyme solution 1µg/mL. All the experiments were performed in triplicate and values were expressed as means of three experiments.[8]

Enzyme kinetics study

Synthesized compounds were further investigated for the type of inhibition and enzyme kinetics study were carried out. The Lineweaver-Burk plot will be established from which we can calculate the K_m, V_{max} of the slope of inhibitor and the value of α (a constant that defines the degree to which inhibitor binding affects the affinity of the enzyme for substrate).[19]

Docking study

To study the binding mode of the synthesized compounds, we have docked the most potent compound **B-16** at the catalytic site of lipase. The X-ray coordinates of catalytic domain of human bile salt activated lipase (*h*BAL, *h*CEase) was obtained from protein data bank (PDB entry: 1F6W; Resolution 2.3Å).[20] The docking study was carried out using GOLD v5.3 software.[26] Gold performs genetic algorithm based ligand docking to optimize the conformation of ligand at the receptor binding site. The Goldscore fitness function was used to evaluate the various conformations of ligand at the binding site. Goldscore comprises of four components: protein–ligand hydrogen bond energy, protein–ligand van der Waals (vdw) energy, ligand internal vdw energy and ligand torsional strain energy.[26] **B-16** was docked ten times and conformation associated with highest scoring value was considered to analyze various drug-receptor interactions. The Structure of **B-16** was drawn in ChemDraw Ultra (2010) and subjected to energy minimization using the MM2 force field as implemented in Chem 3D Ultra software.

Conflict of interest

The authors declare no competing interests.

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Captions

Figure 1. Reported CEase inhibitors[18]

Scheme 1. Synthesis of 5,6-benzoflavone. Reagents and conditions: (a) MW, $ZnCl_2$, CH_3COOH, 20 min; (b) benzoyl chloride, pyridine, stirring rt, 1 h; (c) KOH, pyridine, warm, 15 min; (d) a drop of conc. H_2SO_4, CH_3COOH, reflux, 30 min.

Figure 2. Design strategy for target compounds

Figure 3. Structure Activity Relationship

Figure 4. Lineweaver-Burk plot of **B-16**

Figure 5. Schematic 2D representations of *h*Cease-**B-16** complex showing H-bond and van der Walls interactions (Figure generated by LIGPLOT[27])

Figure 6. Docked conformation of **B-16** at the catalytic site of *h*CEase (**B-16**: carbon atoms are shown in green; only hydrogens which are involved in H-bond interactions are shown in white color)

Figure 7. Docked conformation of **B-16** and **A** (lead compound) at the catalytic site of *h*CEase (**B-16**: shown in green; **A**: shown in purple)

Table 1. Various substituted 5,6-Benzoflavone derivatives and their CEase inhibitory activity

Table 2. In silico ADME properties of 5,6-Benzoflavone derivatives

Table 3. Physicochemical parameters of 5,6-Benzoflavone derivatives

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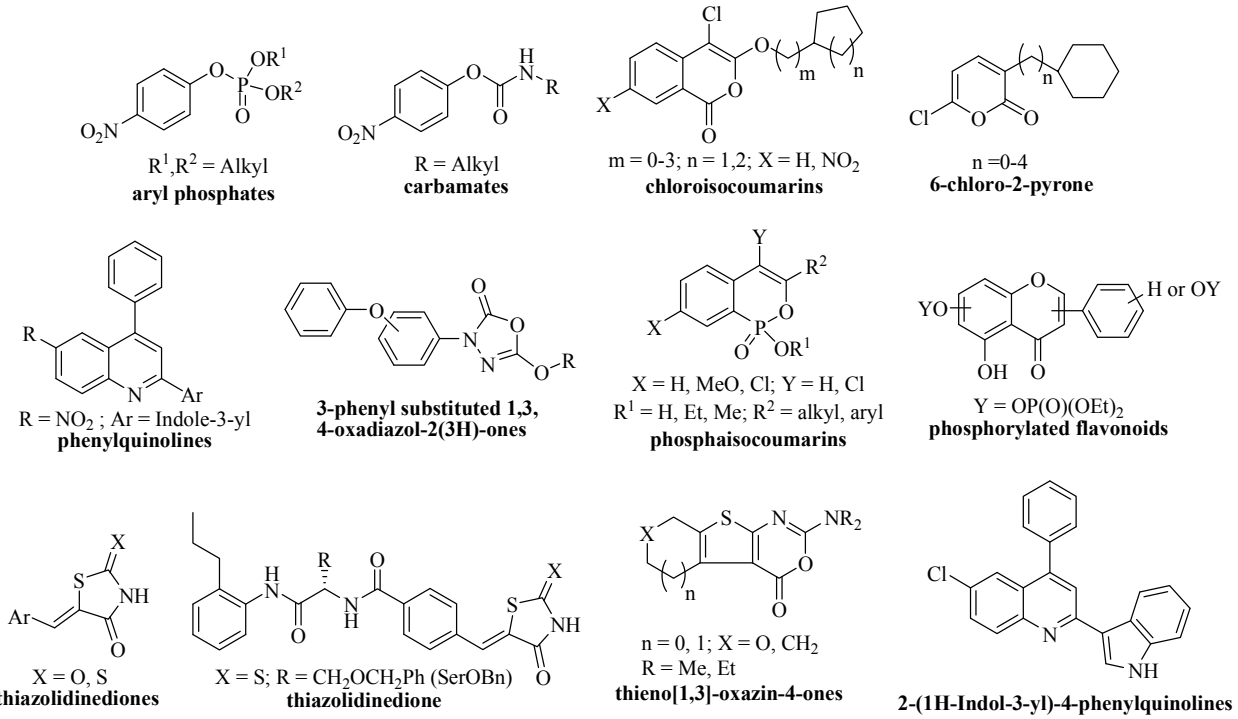

Figure 1. Reported CEase inhibitors[18]

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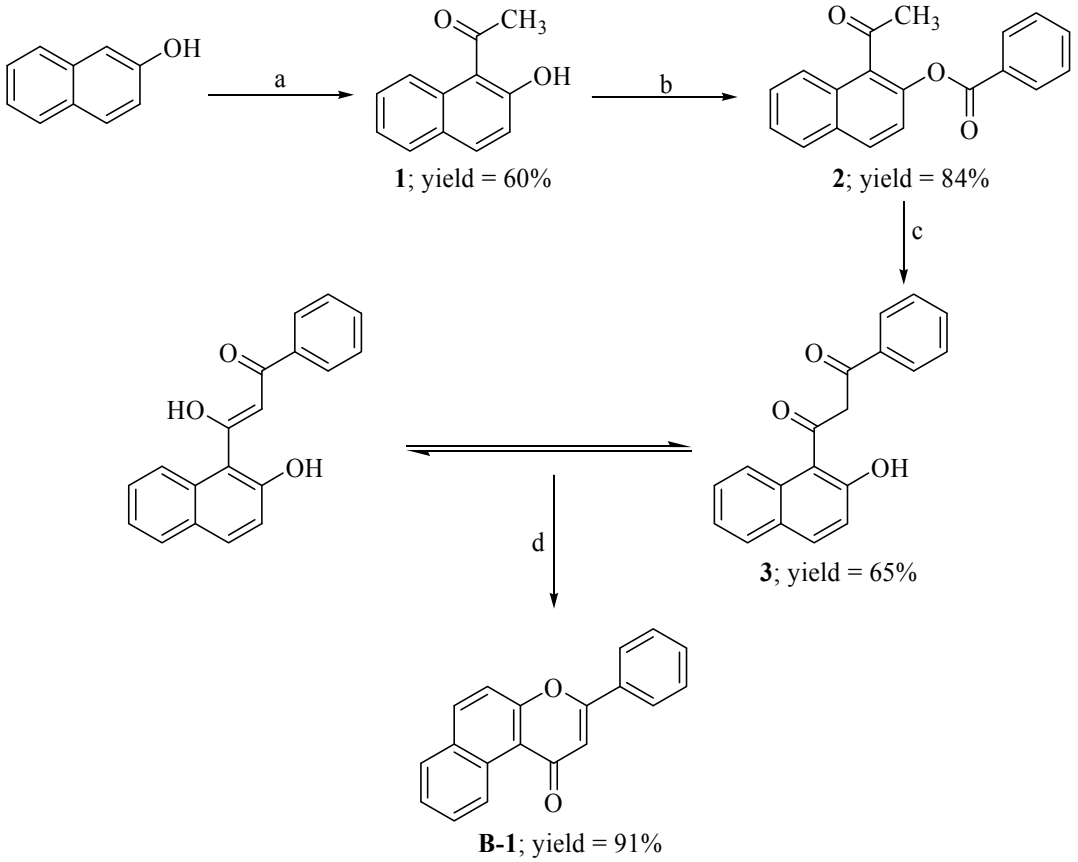

Scheme 1. Synthesis of 5,6-benzoflavones. Reagents and conditions: (a) MW, $ZnCl_2$, CH_3COOH, 20 min; (b) benzoyl chloride, pyridine, stirring rt, 1 h; (c) KOH, pyridine, warm, 15 min; (d) a drop of conc. H_2SO_4, CH_3COOH, reflux, 30 min.

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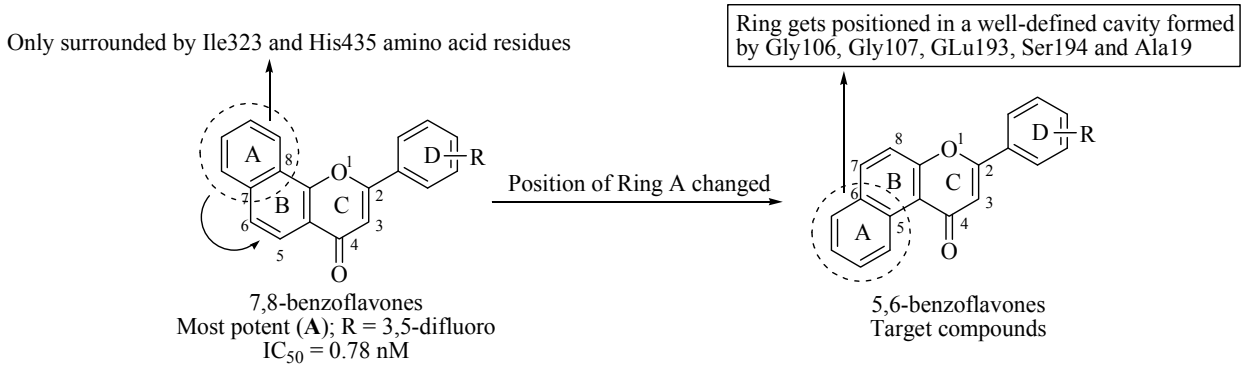

Figure 2. Design strategy for target compounds

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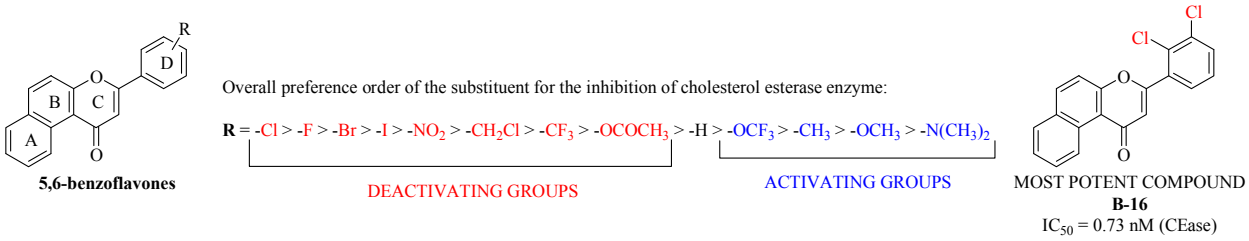

Figure 3. Structure Activity Relationship

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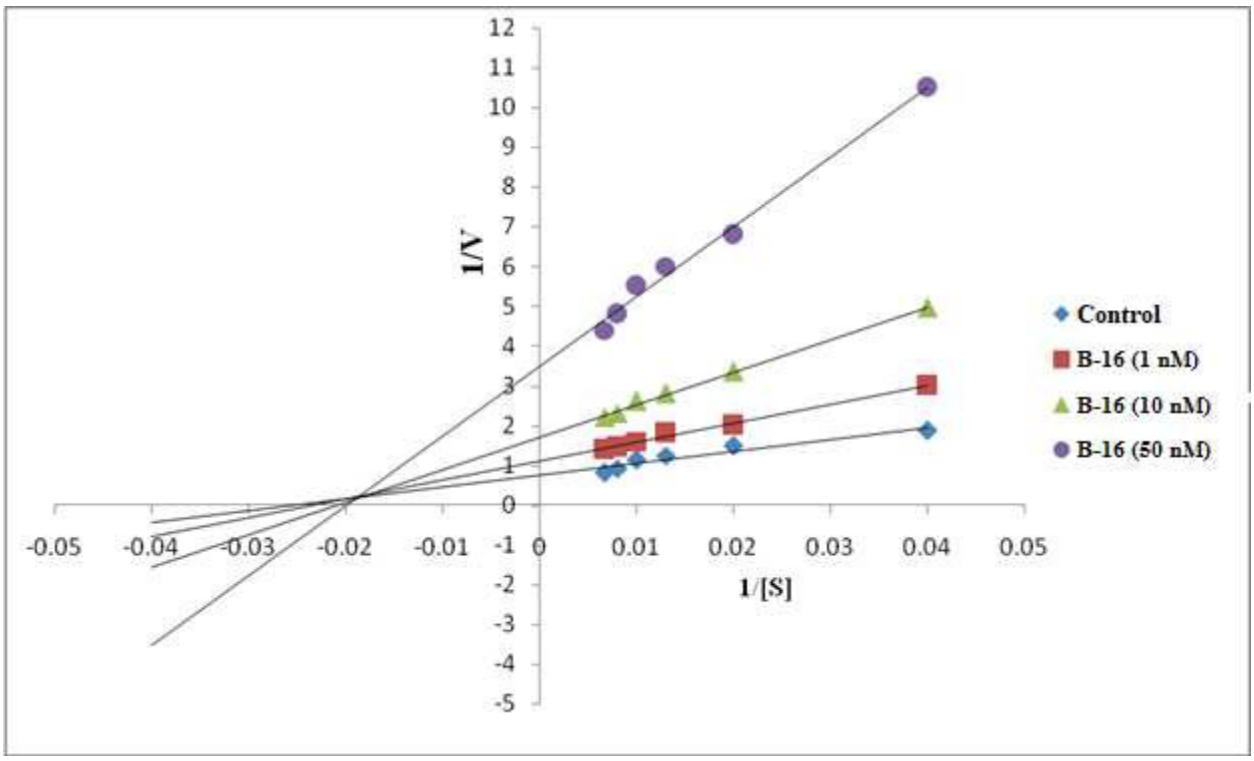

Figure 4. Lineweaver-Burk plot of **B-16**

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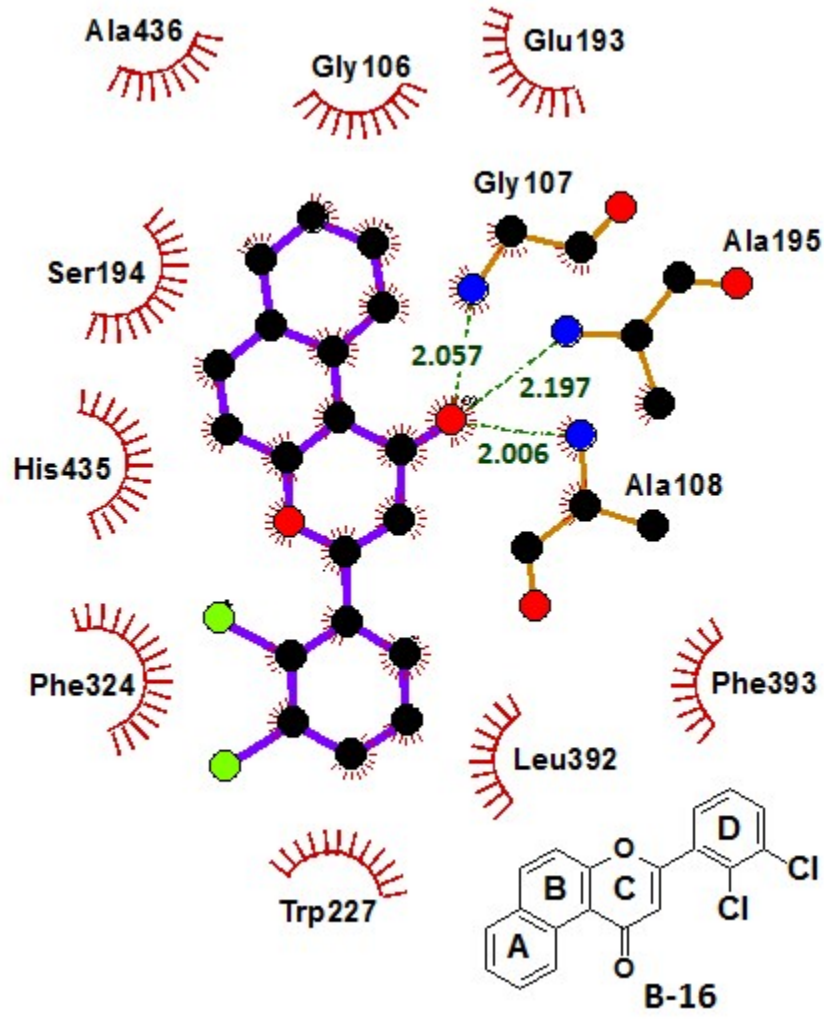

Figure 5. Schematic 2D representations of *h*CEase-**B-16** complex showing H-bond and van der Waals interactions (Figure generated by LIGPLOT[27])

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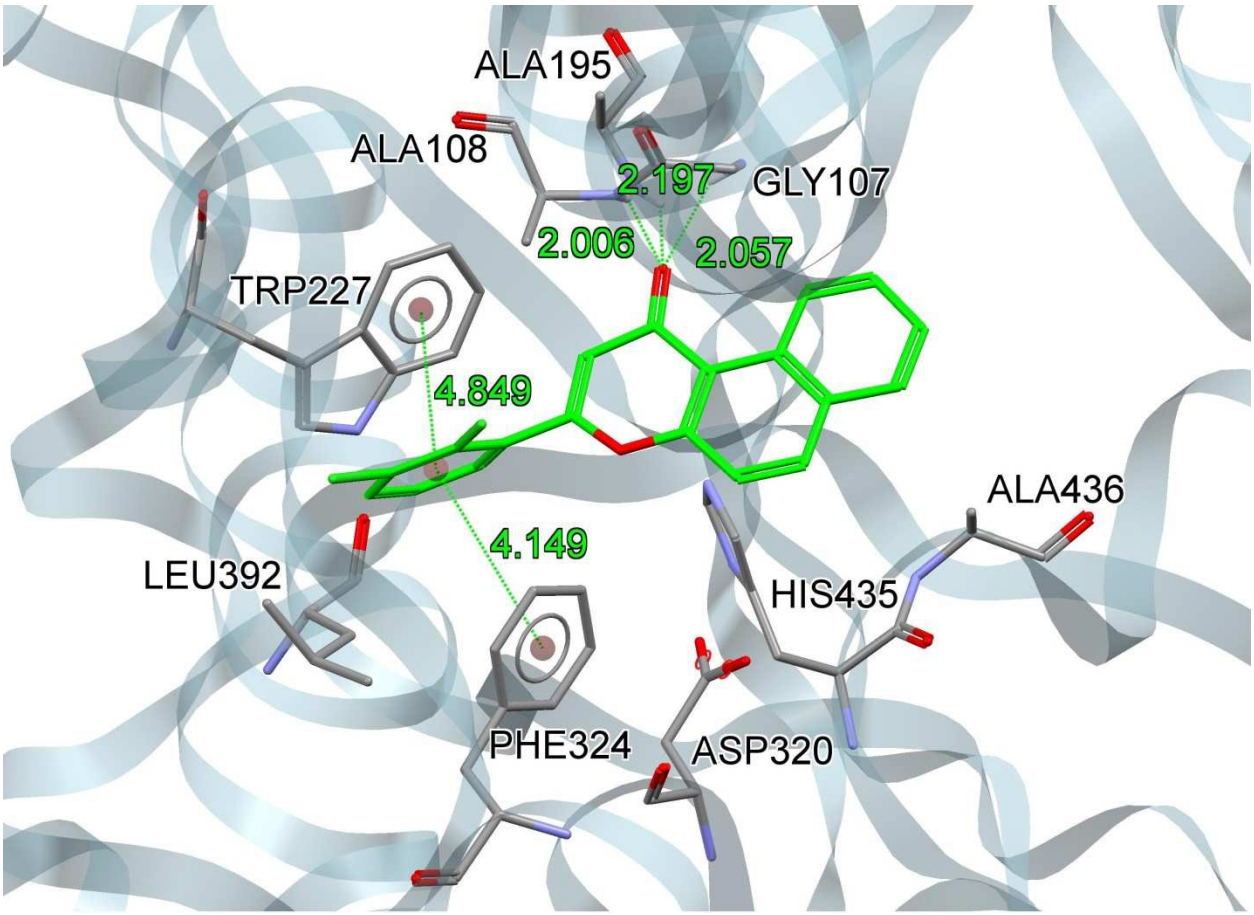

Figure 6. Docked conformation of **B-16** at the catalytic site of *h*CEase (**B-16**: carbon atoms are shown in green; only hydrogens which are involved in H-bond interactions are shown)

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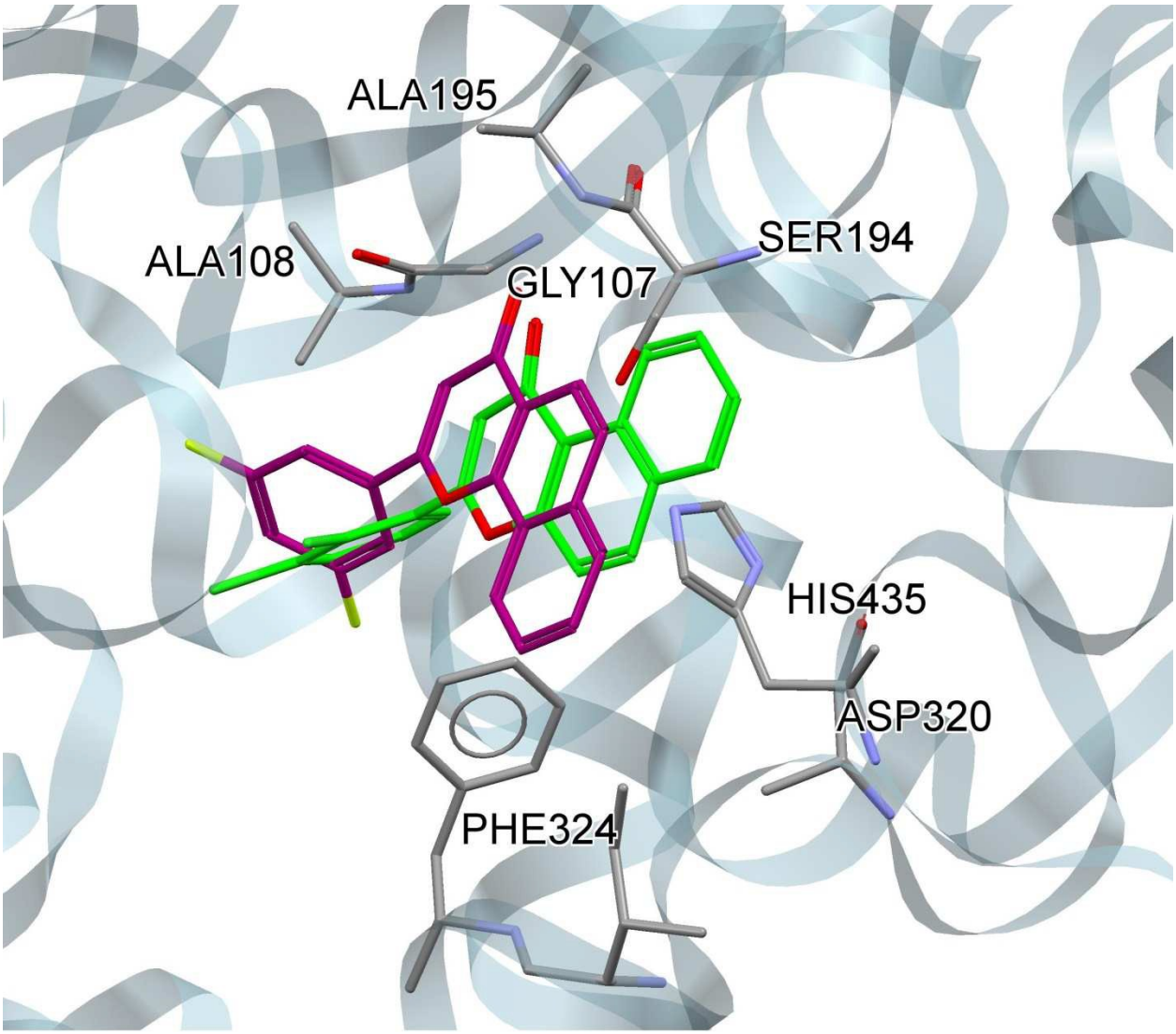
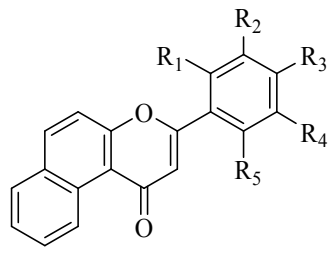


Figure 7. Docked conformation of **B-16** and **A** (lead compound) at the catalytic site of *h*CEase (**B-16**: shown in green; **A**: shown in purple)

Table 1. Various substituted 5,6-Benzoflavone derivatives and their CEase inhibitory activity



Code	R_1	R_2	R_3	R_4	R_5	IC_{50} (nM ± SD)
B-1	H	H	H	H	H	51.06 ± 2.33
B-2	H	H	OCH_3	H	H	ND
B -3	H	OCH_3	OCH_3	H	H	ND
B -4	OCH_3	H	OCH_3	H	H	ND
B -5	H	OCF_3	H	H	H	ND
B -6	H	H	OCF_3	H	H	ND
B-7	F	H	H	H	H	2.59 ± 0.33
B-8	H	F	H	H	H	3.90 ± 0.43
B-9	H	H	F	H	H	4.50 ± 0.54
B-10	F	H	H	H	F	1.19 ± 0.24
B-11	F	H	F	H	H	1.46 ± 0.34
B-12	H	F	H	F	H	0.99 ± 0.17
B-13	F	H	H	F	H	1.08 ± 0.19
B-14	H	F	F	H	H	1.15 ± 0.23
B-15	H	Cl	H	H	H	0.83 ± 0.14
B-16	Cl	Cl	H	H	H	0.73 ± 0.09
B-17	Br	H	H	H	H	4.69 ± 0.76
B-18	H	Br	H	H	H	6.01 ± 0.54
B-19	H	H	Br	H	H	8.15 ± 0.56
B-20	I	H	H	H	H	9.55 ± 0.67
B-21	H	H	I	H	H	13.11 ± 0.56
B-22	H	NO_2	H	H	H	16.44 ± 0.76
B-23	H	H	NO_2	H	H	18.01 ± 0.65
B-24	H	NO_2	H	NO_2	H	9.80 ± 0.53
B-25	H	H	CH_3	H	H	ND
B-26	H	H	CF_3	H	H	ND
B-27	H	CF_3	H	H	H	ND
B-28	CF_3	H	H	H	H	31.35 ± 0.99
B-29	H	CF_3	H	CF_3	H	ND
B-30	CF_3	H	F	H	H	ND
B-31	F	H	CF_3	H	H	23.11 ± 0.75
B-32	F	H	H	H	CF_3	29.17 ± 0.66
B-33	CF_3	H	H	F	H	27.79 ± 0.88
B-34	H	F	H	CF_3	H	29.11 ± 0.34
B-35	H	CF_3	F	H	H	ND
B-36	H	H	CH_2Cl	H	H	22.23 ± 0.85
B-37	H	CH_2Cl	H	H	H	20.05 ± 0.94
B-38	H	H	$N(CH_3)_2$	H	H	ND
B-39	H	$OCOCH_3$	H	H	H	ND
B-40	H	H	$OCOCH_3$	H	H	ND
PF						0.72 ± 0.06

SD-Standard Deviation, NA- Not Determined, *PF-Phosphorelated Flavonoid[11]

Table 2. In silico ADME properties of 5,6-Benzoflavone derivatives

	Absorption			Distribution		
Compound	Human intestinal absorption (HIA)%	*In vitro* Caco-2 cell permeability (nm/sec)	*In vitro* MDCK cell permeability (nm/sec)	*In vitro* skin permeability (logKp) cm/hr	*In vitro* plasma protein binding (%)	*In vivo* blood brain barrier penetration (C.brain/C.blood)
B-1	100.00	56.73	50.02	-2.65	95.97	2.75
B-2	98.80	57.28	2.31	-2.80	95.48	0.08
B-3	97.64	56.64	4.46	-2.93	93.63	0.04
B-4	97.64	56.64	4.46	-2.93	93.03	0.04
B-5	98.80	27.98	7.46	-1.73	96.49	1.70
B-6	98.80	27.90	0.13	-1.73	99.05	1.36
B-7	100.00	54.71	20.11	-2.91	98.40	2.17
B-8	100.00	54.75	27.06	-2.94	100.00	0.28
B-9	100.00	55.39	3.48	-2.94	100.00	0.24
B-10	100.00	53.72	6,62	-3.06	99.69	1.82
B-11	100.00	54.73	0.87	-3.09	100.00	0.30
B-12	100.00	73.77	10.53	-3.12	100.00	0.30
B-13	100.00	53.76	8.28	-3.09	100.00	0.39
B-14	100.00	54.70	23.36	-3.09	100.00	0.38
B-15	100.00	47.91	26.61	-2.70	96.47	0.42
B-16	100.00	50.14	20.56	-2.62	100.00	0.80
B-17	100.00	46.44	0.16	-2.57	100.00	1.27
B-18	100.00	46.57	0.12	-2.58	100.00	0.45
B-19	100.00	46.46	0.01	-2.58	100.00	0.46
B-20	100.00	46.45	0.23	-2.62	100.00	1.07
B-21	100.00	46.43	0.18	-2.64	100.00	0.36
B-22	98.49	21.43	0.42	-2.86	95.25	0.01
B-23	98.49	15.17	0.33	-2.86	96.00	0.01
B-24	96.03	20.38	0.06	-2.83	94.55	0.04
B-25	100.00	56.22	5.38	-2.56	95.75	0.36
B-26	100.00	41.23	0.93	-1.89	99.68	0.95
B-27	100.00	41.36	0.07	-1.89	98.73	1.11
B-28	100.00	41.61	3.42	-1.89	97.32	0.58
B-29	100.00	42.14	0.04	-1.47	95.99	3.89
B-30	100.00	48.21	0.15	-1.96	99.97	0.85
B-31	100.00	47.41	0.07	-1.96	100.00	1.20
B-32	100.00	47.73	1.05	-1.95	100.00	0.72
B-33	100.00	47.72	1.22	-1.96	98.13	0.77
B-34	100.00	48.21	0.06	-1.96	100.00	1.49
B-35	100.00	48.63	0.04	-1.96	100.00	1.61
B-36	100.00	25.60	2.79	-2.60	100.00	0.39
B-37	100.00	25.58	18.06	-2.60	100.00	0.49
B-38	100.00	57.87	0.37	-2.81	92.67	0.16
B-39	97.37	45.13	2.63	-2.79	94.14	0.03
B-40	97.37	48.99	0.22	-2.79	93.78	0.02

Table 3. Physicochemical parameters of 5,6-Benzoflavone derivatives

Compound	Molecular weight	No. of H-bond donors	No. of H-bond acceptors	Molar Refractivity	Log P	No. of Lipinski Violation
B-1	272	0	2	83.42	3.90	0
B-2	302	0	3	89.88	3.80	0
B-3	332	0	4	96.35	3.64	0
B-4	332	0	4	96.35	3.64	0
B-5	356	0	2	89.40	4.83	0
B-6	356	0	2	89.40	4.83	0
B-7	290	0	2	83.64	4.10	0
B-8	290	0	2	83.64	4.10	0
B-9	290	0	2	83.64	4.10	0
B-10	308	0	2	83.85	4.24	0
B-11	308	0	2	83.85	4.24	0
B-12	308	0	2	83.85	4.24	0
B-13	308	0	2	83.85	4.24	0
B-14	308	0	2	83.85	4.24	0
B-15	306	0	2	88.23	4.56	0
B-16	341	0	2	93.03	5.16	0
B-17	351	0	2	91.04	4.73	0
B-18	351	0	2	91.04	4.73	0
B-19	351	0	2	91.04	4.73	0
B-20	398	0	2	96.78	4.89	0
B-21	398	0	2	96.78	4.89	0
B-22	317	0	4	90.75	3.90	0
B-23	317	0	4	90.75	3.90	0
B-24	362	0	6	98.07	3.84	0
B-25	286	0	2	88.46	4.47	0
B-26	340	0	2	89.40	4.83	0
B-27	340	0	2	89.40	4.83	0
B-28	340	0	2	89.40	4.83	0
B-29	408	0	2	95.37	5.71	0
B-30	358	0	2	89.61	4.98	0
B-31	358	0	2	89.61	4.98	0
B-32	358	0	2	89.61	4.98	0
B-33	358	0	2	89.61	4.98	0
B-34	358	0	2	89.61	4.98	0
B-35	358	0	2	89.61	4.98	0
B-36	320	0	2	93.29	4.54	0
B-37	320	0	2	93.29	4.54	0
B-38	315	0	3	97.85	4.06	0
B-39	330	0	3	94.55	3.56	0
B-40	330	0	3	94.55	3.56	0

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GRAPHICAL ABSTRACT

5,6-Benzoflavones as cholesterol esterase inhibitors: Synthesis, biological evaluation and docking studies

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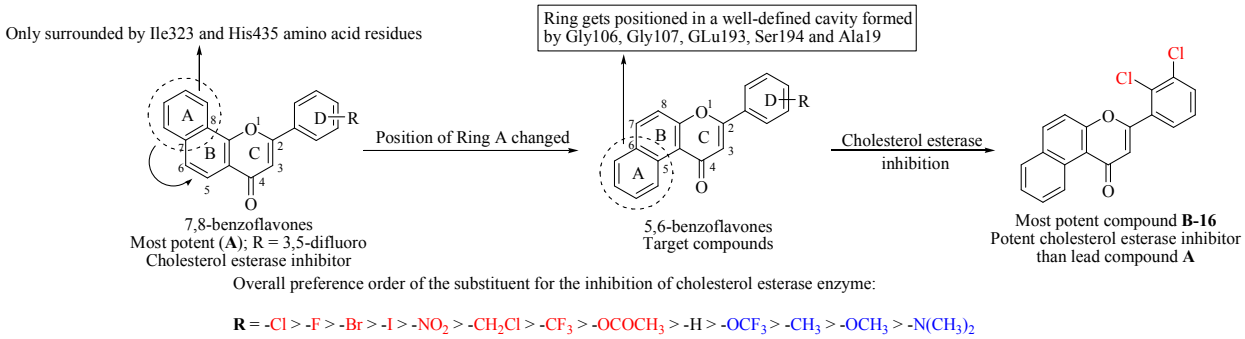

A library of forty 5,6-Benzoflavone derivatives was synthesized and evaluated for their inhibitory potential against cholesterol esterase (CEase) enzyme.