Special Issue on "Medicinal Chemistry"

J. Indian Chem. Soc.,
Vol. 97, August 2020, pp. 1265-1271

Design, synthesis and biological evaluation of *O*-alkyl umbelliferone derivatives as pancreatic lipase inhibitors

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Manuscript received online 13 July 2020, accepted 29 July 2020

A series of coumarin derivatives were synthesised through *O*-alkylation of umbelliferone. These derivatives were screened for their pancreatic lipase (PL) inhibitory potential. The PL inhibitory effect of compounds with various benzyl and long chain alkyl substituents on umbelliferone were analysed. The compound **1g,** having the geranyl substituent was found to have better PL inhibitory potential with an IC_{50} of 21.64 μM. The compounds **1a-j** were subjected to molecular docking into the crystal structure of human PL. The molecular docking results are in correlation with the *in vitro* PL inhibition activity, wherein compound **1g** showed a higher MolDock score of –122.12 kcal/mol. The long chain alkyl groups were found to have PL inhibition due to additional interactions with lid domain amino acids (Phe215, Tyr114, Phe77), as revealed by molecular docking study.

Keywords: Umbelliferone, pancreatic lipase, molecular docking, *O*-alkylation.

Introduction

Obesity is one among the most significant public health challenges of the 21st century. The World Health Organization (WHO) defines overweight and obesity as abnormal or excessive fat accumulation that poses a risk to health[1]. Earlier obesity used to be considered as a metabolic disorder; however, recently it has been declared as a chronic, relapsing progressive disease[2]. In addition, obesity is associated with various comorbidities, including diabetes and cardiovascular diseases etc. Among the various targets that have been explored for the treatment/management of obesity[3,4], the pancreatic lipase (PL), a key enzyme for lipid absorption, is considered as a valid target for obesity. The human PL is a key enzyme responsible for the digestion of dietary triglycerides into monoglycerides[5] and hence its inhibition leads to the proper management of obesity.

Orlistat is the one of the clinically approved drugs for long term treatment/management and of obesity and it acts through the inhibition of PL[6]. It is a β-lactone containing semi-synthetic analogue of lipstatin that exerts its activity by covalently blocking the Ser152 of the lipase active site[7]. However, orlistat is associated with a greater incidence of gastric side effects such as frequent bowel movements, oily stools and steatorrhea etc. Further, the long-term usage of orlistat is associated with severe adverse effects such as nephrotoxicity, acute pancreatitis, gall stones, and hepatotoxicity etc.[8]. The current pharmacotherapy possesses various problems such as fewer number of drugs acting through pancreatic lipase, adverse effects of the available PL inhibitor etc. These clearly highlight the urgent need of safe and efficacious PL inhibitor for the treatment/management of obesity.

Numerous plants derived secondary metabolites are reported to possess potential anti-obesity effects. Among the various classes, coumarin containing compounds have been reported to show promising anti-obesity effects via various mechanism. Coumarins represent an important class of natural and synthetic analogues of oxygen-containing heterocycles. They have a typical benzo-[α]-pyrone structure with high electron density and good charge transport properties. Coumarin derivatives such as osthole (*Cnidium monnieri)* exhibited its anti-obesity effects by positive modulation of Peroxisome Proliferator-Activated Receptor (PPAR)-α/γ mediated target genes while, pteryxin (*Peucedanum*

japonicum) exhibited its activity by inhibiting the transcriptions factors for lipid synthesis. However, the PL inhibitory potential of coumarins are under explored[9,10]. The furo[3,2-*c*]coumarin derivatives exhibited PL inhibition with IC_{50} value of 9.37 μg/mL, while cleomiscosin B and C (Fraxinus rhynchophylla) exhibited moderate PL inhibition (IC_{50} > 100 μM)[11,12].

Various pharmacophoric features that are required for the PL inhibition have been reported by numerous groups[5,13,14]. Effects of alkyl chain substituted compounds have been explored previously for the potential PL inhibitory activity. Morusalnol A containing geranyl substitution exhibited an activity of 0.71 μM, while cudraflavanone A with prenyl substitution resulted in 6.5 μM of IC_{50} values[15] (Fig. 1). The long alkyl chains are reported to possess the lid-domain interaction in the active site of the PL.

By considering the anti-obesity effects of coumarin moieties and potential effects of alkylation on PL inhibitions, herein we plan to explore the effects of *O*-alkylation on the umbelliferone (7-Hydroxy coumarin) in PL inhibitory effects.

Experimental

Chemistry:

Synthetic route for the preparation of 7-alkoxy umbelliferone derivatives is summarized in Scheme 1. The 7-alkoxy umbelliferone derivatives were synthesized by the reaction of commercially available umbelliferone **1** with various alkyl bromides in the presence of an hydrous potassium carbonate in dry acetone. Structures of the synthesized derivatives **1a-j** were determined on the basis of physical and spectral data (^1H and ^{13}C NMR, LC-MS).

All solvents used were of analytical grade. Silica gel of 60–120 mesh (CDH Laboratory Reagents, India) was used for column chromatography. The reactions were monitored

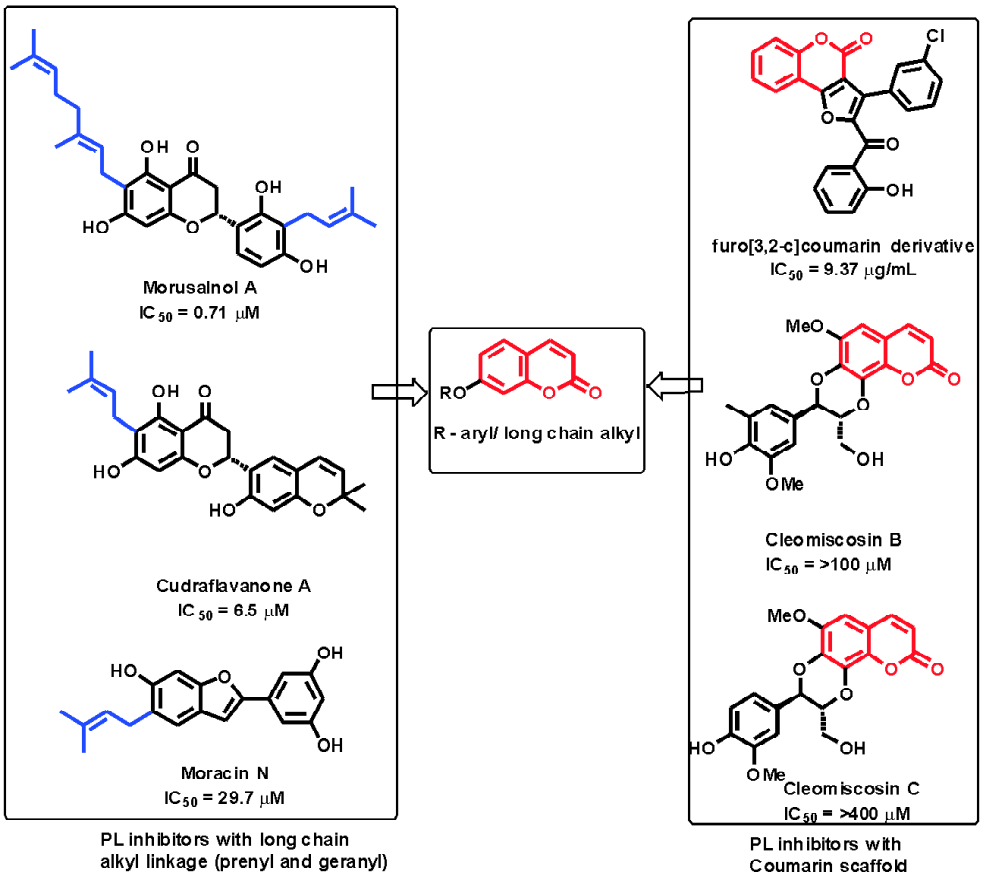
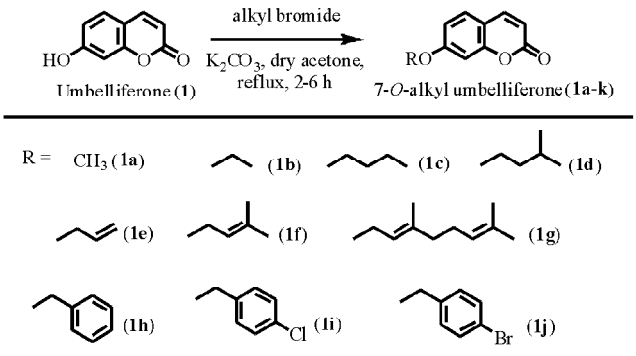


Fig. 1. Rationale for designing umbelliferone derivatives as PL inhibitors.



Scheme 1. Schematic representation involved in the synthesis of umbelliferone derivatives (**1a-j**).

by TLC plates of silica gel GF_{254} (Merck, Germany). Melting points were recorded using capillary melting point apparatus (Scientific, India) and are uncorrected. Mass data of the compounds were recorded on LC-MS (Waters, USA). The ^1H/^{13}C NMR were recorded on Bruker AVANCE 300/75 and 400/100 MHz using $CDCl_3$ as a solvent. Chemical shifts are reported in δ scale from an internal standard of solvent (tetramethylsilane).

*General procedure for the synthesis of 7-O-alkyl umbelliferone (**1a-j**):*

In a 50 mL two necked round-bottomed flask equipped with a magnetic stirrer, a condenser, and a nitrogen inlet, umbelliferone (1 equiv.), K_2CO_3 (3 equiv.) and 10 mL of anhydrous acetone were added. The mixture was heated under reflux for 15 min under nitrogen atmosphere and cooled to room temperature before the dropwise addition of alkyl bromide (1.5 equiv.). The resulting mixture was heated under reflux for another 2–6 h. The reaction was quenched with water (10 mL), followed by extraction with EtOAc (2×50 mL) and washed with brine solution. After drying (Na_2SO_4) and removal of the solvent, the residue was purified by column chromatography using hexane/EtOAc as mobile phase (gradient elution), to afford the corresponding compounds **1a-j**[14]. All the derivatives were characterized by ^1H, ^{13}C NMR and mass data.

*7-O-Methyl umbelliferone **1a:*** White solid; yield 86%; m.p. 120–122°C; ^1H NMR ($CDCl_3$, 300 MHz): δ 3.87 (s, 3H), 6.23 (d, 1H, *J* 9.6 Hz), 6.82 (d,1H, *J* 1.8 Hz), 6.83 (dd, 1H, *J* 8.7, 1.8 Hz), 7.36 (d, 1H, *J* 8.7 Hz), 7.62 (d, 1H, *J* 9.6 Hz); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 56.3, 101.4, 113.1, 113.6, 113.6, 129.3, 143.9, 156.4, 161.7, 163.4; APCI-MS: *m/z* 177 $[M+1]^+$.

*7-O-Ethyl umbelliferone **1b**:* Yellow liquid; yield 92%; ^1H NMR ($CDCl_3$, 400 MHz): δ 2.76 (t, 3H, *J* 7.2 Hz), 3.58 (q, 2H, *J* 6.8 Hz), 6.21 (d, 1H, *J* 9.6 Hz, H-3), 6.78 (d,1H, *J* 2.4 Hz, H-8), 6.81 (dd, 1H, *J* 8.4, 2.4 Hz, H-6), 7.35 (d, 1H, *J* 8.8 Hz, H-5), 7.62 (d, 1H, *J* 9.2 Hz, H-4); ^{13}C NMR ($CDCl_3$,100 MHz): δ 31.3, 36.4, 101.3, 112.4, 112.9, 113.5, 128.7, 143.4, 155.9, 162.2, 162.5.

*7-O-Butyl umbelliferone **1c**:* White solid; yield 67%; m.p. 40–42°C; ^1H NMR ($CDCl_3$, 400 MHz): δ 0.98 (t, 3H, *J* 7.2 Hz), 1.52 (m, 2H), 1.80 (m, 2H), 4.01 (t, 2H, *J* 6.8 Hz), 6.21 (d, 1H, *J* 9.6 Hz), 6.77 (d, 1H, *J* 2.4 Hz), 6.81 (dd, 1H, *J* 8.8, 2.4 Hz), 7.34 (d, 1H, *J* 8.8 Hz), 7.62 (d, 1H, *J* 9.6 Hz); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 13.7, 19.1, 30.7, 68.1, 101.2, 112.3, 112.9, 113.2, 128.7, 143.4, 155.8, 161.2, 162.4; ESI-MS: *m/z* 219 $[M+1]^+$.

*7-O-Isoamyl umbelliferone **1d**:* White solid; yield 61%; m.p. 45–47°C; ^1H NMR ($CDCl_3$, 400 MHz): δ 0.96 (d, 6H, *J* 6.4 Hz), 1.68 (q, 2H, *J* 6.8 Hz), 1.82 (m, 1H), 4.04 (t, 2H, *J* 6.8 Hz), 6.22 (d, 1H, *J* 9.6 Hz), 6.79 (d, 1H, *J* 2.0 Hz), 6.81 (dd, 1H, *J* 8.8, 2.0 Hz), 7.34 (d, 1H, *J* 8.8 Hz), 7.62 (d, 1H, *J* 9.6 Hz); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 22.5, 22.6, 24.9, 37.6, 67.0, 101.3, 112.3, 112.8, 112.9, 128.7, 143.4, 155.8, 161.2, 162.3; APCI-MS: *m/z* 233 $[M+1]^+$.

*7-O-Allyl umbelliferone **1e**:* White solid; yield 90%; m.p. 78–81°C; ^1H NMR ($CDCl_3$, 300 MHz): δ 4.59 (d, 2H, *J* 5.1 Hz), 5.32 (dd, 1H, *J* 10.5, 0.9 Hz), 5.41 (dd, 1H, *J* 17.1, 1.2 Hz), 5.98 (m, 1H), 6.23 (d, 1H, *J* 9.3 Hz), 6.82 (d, 1H, *J* 2.1 Hz), 6.84 (dd, 1H, *J* 8.4, 2.1 Hz), 7.35 (d, 1H, *J* 8.4 Hz), 7.62 (d, 1H, *J* 9.3 Hz); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 69.8, 102.2, 113.1, 113.6, 113.7, 119.0, 129.2, 132.6, 143.9, 156.3, 161.7, 162.3; ESI-MS: *m/z* 203 $[M+1]^+$.

*7-O-Prenyl umbelliferone **1f**:* White solid; yield 90%; m.p. 74–75°C; ^1H NMR ($CDCl_3$, 300 MHz): δ 1.77 (s, 3H), 1.81 (s, 3H), 4.56 (d, 2H, *J* 6.6 Hz), 5.45 (t, 1H, *J* 6.0 Hz), 6.22 (d, 1H, *J* 9.4 Hz), 6.82 (d, 1H, *J* 2.4 Hz), 6.82 (dd, 1H, *J* 8.7, 2.4 Hz), 7.34 (d, 1H, *J* 8.7 Hz), 7.61 (d, 1H, *J* 9.4 Hz); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 18.8, 26.3, 65.9, 102.1, 112.9, 113.5, 113.7, 119.1, 129.2, 139.8, 144.0, 156.4, 161.8, 162.6; ESI-MS: *m/z* 231 $[M+1]^+$.

7-O-Geranyl umbelliferone ***1g****:* White solid; yield 90%; m.p. 74–75°C; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 1.65 (s, 3H), 1.72 (s, 3H), 1.81 (s, 3H), 2.26 (m, 4H), 4.65 (d, 2H, *J* 6.5 Hz), 5.13 (m, 1H), 5.52 (dt, 1H, *J* 6.5, 1.0 Hz), 6.29 (d, 1H, *J* 9.5 Hz), 6.87 (d, 1H, *J* 2.3 Hz), 6.90 (dd, 1H, *J* 8.5, 2.4 Hz), 7.41 (d, 1H, *J* 8.5 Hz), 7.69 (d, 1H, *J* 9.5 Hz); ^{13}C NMR ($CDCl_3$): δ 17.2, 18.1, 29.7, 30.1, 39.9, 65.9, 102.0, 112.8, 113.3, 113.6, 118.8, 124.0, 129.1, 132.3, 142.7, 143.9, 152.2, 161.7, 162.5

7-O-Benzyl umbelliferone ***1h****:* White solid; yield 91%; m.p. 152–154°C; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 5.13 (s, 2H), 6.23 (d, 1H, *J* 9.6 Hz), 6.89 (d, 1H, *J* 2.1 Hz), 6.89 (dd, 1H, *J* 8.4, 2.1 Hz), 7.34 (d, 1H, *J* 8.4 Hz), 7.42 (s, 5H), 7.61 (d, 1H, *J* 9.6 Hz); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 71.0, 102.4, 113.2, 113.8, 113.8, 128.0, 128.9, 129.3, 136.3, 143.9, 156.3, 161.7, 162.4; APCI-MS: *m/z* 253 $[M+1]^+$.

7-O-p-Chlorobenzyl umbelliferone ***1i****:* White solid; yield 91%; m.p. 100–106°C; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 5.11 (s, 2H), 6.28 (d, 1H, *J* 9.6 Hz), 6.88 (d, 1H, *J* 2.4 Hz), 6.92 (dd, 1H, *J* 8.4, 2.4 Hz), 7.41–7.40 (m, 5H), 7.65 (d, 1H, *J* 9.6 Hz); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 69.7, 101.9, 112.9, 113.2, 113.4, 128.8, 128.9, 129.0, 134.3, 134.3, 143.3, 158.8, 161.1, 161.6.

7-O-p-Bromobenzyl umbelliferone ***1j****:* White solid; yield 91%; m.p.134–138°C; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 5.09 (s, 2H), 6.33 (d, 1H, *J* 9.6 Hz), 6.87 (d, 1H, *J* 2.0 Hz), 6.92 (dd, 1H, *J* 8.8, 2.4 Hz), 7.33 (d, 2H, *J* 8.4 Hz), 7.40 (d, 1H, *J* 8.4 Hz), 7.55 (d, 2H, *J* 8.4 Hz), 7.65 (d, 1H, *J* 9.6 Hz); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 69.7, 101.9, 112.9, 113.1, 113.4, 122.4, 128.9, 129.1, 131.9, 134.8, 143.3, 155.8, 161.0, 161.6

Pancreatic lipase inhibition assay:

Porcine PL (Type II), *p*-nitrophenyl butyrate and orlistat were procured from Sigma-Aldrich (St. Louis, MO, USA). Sodium chloride (molecular biology grade) and Tris buffer were procured from Sisco Research Laboratories (MH, India). All other chemicals and solvents were of analytical grade. The PL inhibition assay was performed as per the standardized protocol[5,16]. The enzyme solutions were prepared immediately before use.

Briefly, crude porcine PL (5 mg/mL) was suspended in Tris-HCl buffer (pH 7.4). The mixture is subjected to vigorous shaking, followed by centrifugation (3000 g, 10 min) and the supernatant was collected. Stock solutions of the orlistat and the synthesized coumarin derivatives were prepared in DMSO. A linear concentration (0.78–2000 μg/mL) were used as stock solutions. The 875 μL of Tris-HCl buffer and 100 μL of PL solution were pre-incubated (5 min) with 20 μL various concentrations of test solutions at 37°C. Further, 5 μL of the substrate (4-nitrophenyl butyrate, 10 mM in acetonitrile) were added and measured the amount of released *p*-nitrophenol at 405 nm [BioTek EPOCH microplate spectrophotometer (VT, USA)]. The percentage inhibition was calculated using the formula

$$\% \text{ Inhibition} = [1 - (A_T/A_E)]\times 100$$

where A_E is the absorbance of enzyme control (without inhibitor), and A_T is the difference between the absorbance of the test sample, with and without substrate. The IC_{50} values were calculated from the slope of linear regression curve.

Molecular docking studies:

The molecular docking study of the synthesized coumarin derivatives was performed using Molegro Virtual Docker 6.0 (CLC bio). The structures of all the coumarin derivatives were drawnin ChemDraw and its energy minimized structure were obtained from 3D module of Chem BioOffice v12 (Perkin-Elmer, USA). The energy minimized molecules were docked into the active sites of human PL (PDB ID: 1LPB), that was obtained from protein data bank[17,18].

Results and discussion

Chemistry:

The 7-*O*-alkyl umbelliferone derivatives (**1a-j)** were synthesised as per the Scheme 1. Initially, the hydroxyl proton of **1** was abstracted by anhydrous K_2CO_3 and addition of various alkyl halides resulted in *O*-alkylated derivatives of umbelliferone (**1a-j**). The structures of all the synthesised molecules were characterised using IR, NMR (^{1}H and ^{13}C) and HRMS analysis. The appearance of alkyl chains in the NMR spectra at a range of 1.65 to 5.52 ppm in ^{1}H NMR and 20–40 ppm in ^{13}C NMR further confirmed the attachment of alkyl groups to the coumarin moieties.

Pancreatic lipase inhibition assay:

The PL inhibitory activity of the synthesised 7-*O*-alkyl

Table 1. PL inhibitory activity (*In vitro*) of 7-*O*-alkyl umbelliferone derivatives (**1a-j**)

Sl. No.	Compound	R	IC_{50} (μM)[a]
1.	**1**	H	160.19±3.25
2.	**1a**	Methyl	121.96±11.40
3.	**1b**	Ethyl	59.06±3.76
4.	**1c**	Butyl	23.70±1.61
5.	**1d**	Isoamyl	74.23±4.80
6.	**1e**	Allyl	54.19±4.63
7.	**1f**	Prenyl	40.59±2.59
8.	**1g**	Geranyl	21.6 ±1.15
9.	**1h**	Benzyl	114.89±7.51
10.	**1i**	4-Chlorobenzyl	98.53±5.70
11.	**1j**	4-Bromobenzyl	46.66±3.65
Orlistat			1.07±0.11

[a]All experiments were performed in triplicate and the values are expressed in mean±SEM.

umbelliferone derivatives (**1a**-**j**) was evaluated using porcine PL (Type II) and the results are summarised in Table 1. Orlistat (reference) exhibited a potent PL inhibitory activity (IC_{50} 1.07 μM). The synthesised analogues exhibited an IC_{50} in the ranges of 21.64 to 160.19 μM. Umbelliferone exhibited an activity of 160.19 μM, while the compound **1g** with a geranyl linkage was found to be highly active in the series (IC_{50} of 21.64 μM). Alkylation of the umbelliferone with aromatic substituents resulted in **1h**-**1j**, that exhibited a moderately inhibitory potential towards PL. The extent in the degree of alkylation resulted in the increment of PL inhibitory activity. Further, the synthesised alkylated analogues exhibited a greater potential than the parent compound (umbelliferone), that clearly highlighted the importance of alkylation for potential PL inhibition.

Molecular modelling:

The three-dimensional structure of the human PL is comprised of 449 amino acids, with a highly restricted catalytic triad of His263-Asp176-Ser =152, that are enclosed in a hydrophobic lid domain (Gly76-Lys80, Leu213-Met217). During the fat digestion process, alkyl chain of triglycerides interacts with the lid domain via various hydrophobic interactions that successively leads to the opening of the catalytic site. Further, carbonyl carbon of the ester linkage present in the triglycerides undergo nucleophilic attack by the Ser152 (1LPB active site)[5,19].

In order to understand the interaction of 7-*O*-alkyl umbelliferone derivatives (**1a-j**) at the active site of human PL (PDB ID: 1LPB), molecular docking study was performed. The obtained results are summarized in Table 2. The redocked pose co-crystallized ligand (methoxyundecyl phosphinic acid) deviated from native pose by a RMSD of 1.7 Å validated the grid parameters. The obtained results of molecular docking are in agreement with the *in vitro* PL activity, wherein the carbonyl carbon of the coumarin analogues existed in proximity to the Ser152 with a distance range of 3.20–3.51 Å. The most active analogue **1g** (IC_{50} 21.62 μM) with the geranyl attachment resulted in greater MolDock Score of –122.12 kcal/mol and exhibited additional interac-

Table 2. MolDock score and interaction summary of **1a-j** at 1LPB active site

Compound	MolDock score (kcal/mol)	H-Bond	π-Alkyl interaction	π-Cation interaction
1	–71.64	Gly76, His151, Ser152	Phe77	His263
1a	–79.13	Gly76, His151, Ser152	Phe77	His263
1b	–92.36	Gly76, His151, Ser152	Tyr114	His263
1c	–101.45	Gly76, His151, Ser152	Phe215	His263
1d	–94.26	Gly76, Ser152	Tyr114	His151, His263
1e	–93.74	Gly76, Phe77, His151, Ser152	Phe77, Tyr114	His263
1f	–98.58	Gly76, His151, Ser152	Phe77, Tyr114, Phe215	His263
1g	–122.12	Gly76, His151	Phe77, Tyr114, Pro180, Phe215	Asp79, His151, His263
1h	–83.15	Gly76, Ser152	Ala178, His263	Asp79, His151, His263
1i	–88.38	Gly76, His151, Ser152	Phe77, Phe215	His263
1j	–95.26	Gly76, His151, Ser152	Phe77, Phe215	His263
Orlistat	–152.41	Gly76, Phe77, Ser152, His263	Phe215	His263

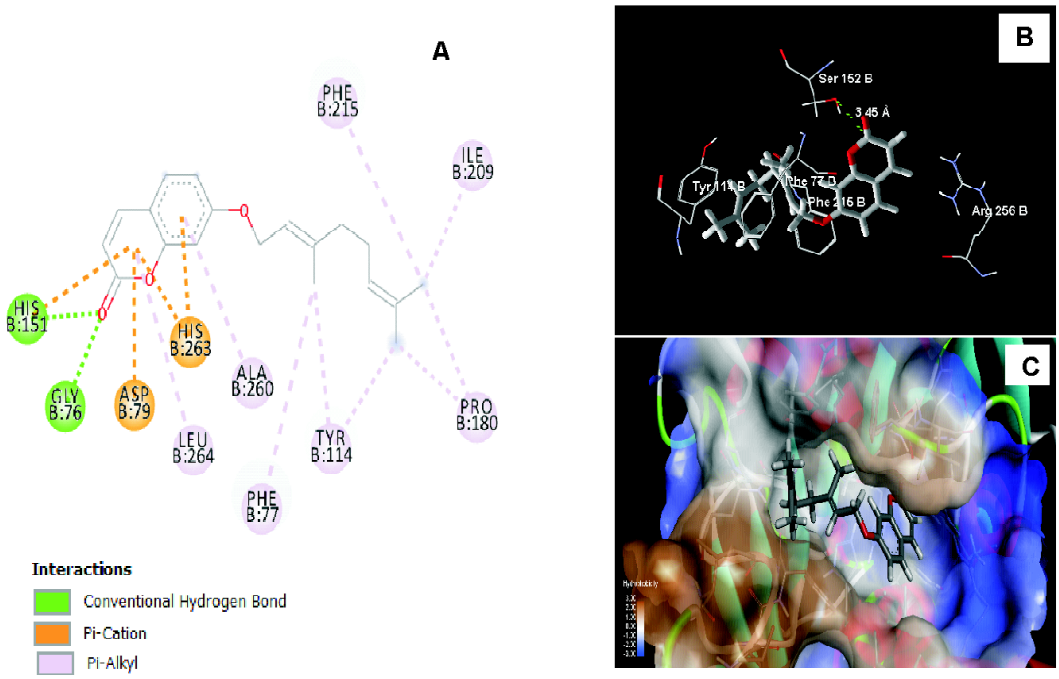


Fig. 2. (A) 2-D Interactions diagram of **1g** with 1LPB; (B) 3-D Interactions diagram of **1g** with 1 LPB highlighting the lid domain interactions and reactive carbonyl position with reference to Ser152; (C) Representation of **1g** in the binding pocket of 1LPB.

tions with lid domain amino acids (Phe215, Tyr114, Phe77, Pro180) (Fig. 2). The butyl substituted analogue exhibited a higher potential than the prenyl substitution (MolDock Score of –101.45 and –98.58, respectively).

Conclusions

In conclusion, a series of *O*-alkyl substituted umbelliferone derivatives were synthesised and evaluated for their *in vitro* PL inhibitory potential. Amongst the synthesised compounds, compound **1g** with a geranyl group was found to be more active with an IC_{50} of 21.64 μM as compared with umbelliferone (IC_{50} = 160.19 μM). The molecular modelling studies were in correlation with *in vitro* PL inhibitory activities. Although, the alkyl substitution to the umbelliferone resulted in candidates with PL inhibitory potential, further structural refinements are required for the development of potent PL inhibitors. This study demonstrates the scope for development of potent drug candidates from the natural products by the necessary structural modifications.

Acknowledgements

The authors acknowledge Birla Institute of Technology and Science, Pilani (BITS Pilani), Pilani Campus, Rajasthan, India for providing infrastructure. Ms. Nisha Yadav acknowledge DST for INSPIRE fellowship (#IF 180833). Mr. Ginson George acknowledges the fellowship received from the Council for Scientific and Industrial Research (CSIR), New Delhi, India (File No: 09/719(0100)/2019-EMR-I).

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