In-Silico Analysis of Coumarins for Neurodegenerative, Infectious and Metabolic Diseases

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The present study evaluated the binding affinities for a set of coumarins against five key targets which are relevant for anti-Alzheimer's, antimicrobial, anticancer, anti-tuberculosis, and anti-adipogenic activities. Docking score analysis revealed that 3''-Demethylchartreusin **71,** Chartreusin **69** and Fabiatrin **70** and coumarin pyrazole hybrid **298** exhibited significant binding interactions with the targets, making them as the promising lead compounds. Key stabilizing interactions, including H-bonding, π-π, π-σ, π-anion/cation, and π-alkyl interactions, have been analyzed. In-silico drug-likeness analysis was performed on all the coumarins, and another nine coumarins were identified as promising molecules with acceptable pharmacokinetic properties, making them potential multitargeted drug candidates.

Introduction

Coumarins and their derivatives as pharmacophores have sparked much attention because of their ability to engage in non-covalent interactions (hydrophobic, π-π, and electrostatic interactions, hydrogen bonding, metal coordination, Vander Waals forces, etc.) with various active sites in biomolecules [1] and thus display a wide range of biological activities such as antimicrobial, antioxidant, anti-inflammatory, anticancer, anti-HIV, antituberculosis, anti-influenza, anti-Alzheimer, antiviral, antihyperlipidemic, antihypertensive, anticonvulsant, antiadipogenic, and Cytochrome P450 inhibiting, neuroprotective, analgesic, antimalarial, anticancer, antipsychotic and anti-diabetic. So far, some coumarin derivatives for e.g., warfarin (anti-coagulant), armillarisin A (anti-biotic), hymecromone (choleretic and anti-spasmodic), carbochromen (treat coronary disease), phenprocoumon (anti-coagulant), novobiocin (anti-biotic), difenacoum (anti-coagulant), acenocoumarol (anti-coagulant), auraptene (chemopreventive agent), ensaculin (NMDA antagonist and a 5HT1A agonist) and brodifacoum (anti-coagulant) have made their way to clinics [2]. Acetylcholine (ACh), a neurotransmitter essential for cognitive

processes, is hydrolysed by AChE and BChE. Elevated BChE activity exacerbates the cholinergic deficit, contributing to cognitive decline in Alzheimer patients. BChE inhibitors may help delay disease progression and improve quality of life. Coumarin-containing hybrids, including coumermycin A1, clorobiocin, and novobiocin, have been clinically used to treat bacterial infections indicating their potential as templates for new antibiotic development [3]. The GlcN-6-P synthase enzyme is important for the production of N-acetylglucosamine, which is required for cell wall construction in microorganisms. Even a momentary inactivation of the GlcN-6-P synthase is lethal to microorganisms. Some coumarin derivatives, were studied in estrogen-dependent breast (MCF-7) and ovarian cancer (OVCAR) cell lines [4]. Breast cancer therapy is distinguished from other cancer treatments by an emphasis on receptor activation, such as the usage of mammalian target of rapamycin (mTOR). The mTOR is a serine-threonine protein kinase that regulates several important physiological processes, including proliferation, metabolism, protein synthesis, cell growth, and autophagy. In recent years, numerous coumarin scaffolds have been shown to exhibit significant anti-TB properties. For instance, naturally occurring Calanolide A and 3-phenyl-4-syrylcoumarin demonstrated strong anti-*Mtb* activity [5]. The fatty acid elongation system FAS-II is involved in the production of mycolic acids, which are significant and specialized long-chain fatty acids found in the cell membrane of Mycobacterium TB. The protein MabA, also known as FabG1, was recently shown to be a component of FAS-II would catalyse the NADPH specific reduction of long chain beta-ketoacyl derivatives [6]. Coumarin inhibited the HFD-induced up-regulation of fatty acid synthase (FAS) activity. Scoparone and its derivatives decrease cholesterol by inhibiting HMG-CoA reductase activity [7]. Obesity is associated with greater levels of α-amylase. Inhibiting α-amylase and α-glucosidase has been shown to aid weight reduction and glycaemic management [8]. The vast range of functions of coumarin molecules has piqued our interest to investigate natural coumarins and their synthesized derivatives for significant medicinal properties. The present molecular docking study will emphasize a comparative anti-AD, antimicrobial, anticancer, anti-TB and antiadipogenic activity of around 298 natural/synthetic coumarins by determining its binding potency with the respective enzymes. The current investigation highlights molecular docking, interaction analysis, and drug-likeness screening, providing a computational framework for other researchers to refine and validate. The data created can serve as a benchmark for future studies investigating coumarin-based therapeutics and serves as a blueprint for future computational drug discovery projects.

Computational details

Chem 3D program employing the MM2 method was used to optimize the geometrical structures of the coumarins and selective drugs. The crystal structure of Human butyrylcholinesterase (BChE) (PDB: 4TPK), Glucosamine-6-phosphate synthase (GlcN-6-P-synthase) (PDB: 2VF5), mammalian target of rapamycin (mTOR) (PDB: 4DRH), β-ketoacyl-ACP reductase (Mab A) (PDB: 1UZN) and Pancreatic α-amylase (α-amylase) (PDB: 2QV4) were retrieved from the protein data bank. The ligands, co-factors, and solvent molecules, were removed from the protein prior to the docking experiment. Autodock tools were utilized to carry out docking studies [9]. The UCSF Chimera [10] and Biovia software are used to depict the docked conformations of the top hits as well as the stabilizing interactions that exist between them and the amino acids of the target protein. The physicochemical properties, lipophilicity, water solubility, pharmacokinetics, drug likeness and medicinal chemistry friendliness of coumarins, including commercial medicines was predicted by using Swiss ADME online Web tool [11]. Furthermore, the BOILED-Egg model was used to determine the capability for gastrointestinal absorption and the permeability of the blood-brain penetration barrier. Plasma protein binding (PPB), Human Intestinal Absorption (HIA), permeability for Caco-2 cells and hERG was predicted by using PreADMET.

Results and discussion

Molecular Docking Studies

Simple coumarins of around 98 naturals that belongs to different plant sources and 200 synthetics (collected from the literature) were selected and its ability to bind with five proteins–Human butyrylcholinesterase, Glucosamine-6-phosphate synthase, mammalian target of rapamycin, β-ketoacyl-ACP reductase and Pancreatic α-amylase corresponding to five important medicinal properties–anti-Alzheimer, antimicrobial, anticancer, anti-tuberculosis and anti-Adipogenic was evaluated computationally. The docks score of all the coumarins are compared and the top hits are identified. The docking score are also compared with the common drugs which are docked with the same target proteins. The optimized geometry of the coumarins and drugs are docked in the active site of the protein in ten possible orientations. The binding orientation that shows the prominent binding energy was chosen and the appropriate binding energy was noted and various stabilizing interactions were analysed. For the top hits, the best docked orientation was selected, visualized and analysed using Autodock tools, Chimera and Biovia software. The compound number, chemical name and chemical structure are presented in the supplementary **Table S1**. The binding energy of coumarins and drugs with the protein targets is presented in supplementary **Table S2-S6**. Binding mode and interactions of some representative top leads within the active site of the proteins are presented in (**Figure 1**).

The coumarins considered under study showed binding modes within the active sites better than that of the reference drugs.

Docking against BChE

The binding energy varies from -5 to -15 kcal/mol for natural and -5 to -14 kcal/mol for synthetic coumarins. Among the five FDA approved AD drugs, the three cholinesterase inhibitors – donepezil **D1**, rivastigmine **D2** and galantamine **D3** was docked with the enzyme, and their results were used to judge the docking results of the coumarins. Donepezil **D1** had a binding free energy of -9.59 kcal/mol. It possesses π-anion interaction with Asp70, π-σ interaction with Tyr332, π-π T shaped interactions with Trp82, Tyr332, π-alkyl interactions with His438, Ala328, Trp82 amino acid residues. Rivastigmine **D2** had a docking score of -6.83 kcal/mol and establish hydrogen bonds with Ala199, Gly117, Gly116, π-anion & π-cation interactions with Glu197, His438, π-σ interactions with Trp82, Trp231 and π-alkyl interactions with Trp231, His438, Phe329, Ala328. With -8.14 kcal/mol binding free energy, galantamine **D3** creates hydrogen bonds with Ser198, His438 & Gly115. It has π-cation interaction with His438 and π-alkyl interactions with Trp82, His438, Phe398, Phe329. Among natural coumarins 5 compounds that exhibit -12 to -15 are selected as top hits (apiosylskimmin **68**, chartreusin **69**, fabiatrin **70**, 3"-demethylchartreusin **71** & clorobiocin **74**). Chartreusin **69** [12] and 3"-demethylchartreusin **71** are found to have incomparable BE -15 kcal/mol and considered to have higher binding affinity towards the BChE. Chartreusin forms hydrogen bonds with Ser198, His438, Asp70, Pro285 and Thr120 amino acid residues of the protein target. It possesses π-σ interaction with Ala328; π-π T shaped interaction with Tyr332 & π-alkyl interactions with Met437, Tyr440, Trp430, Ala328, Trp82; π-lone pair interaction with Trp82 amino acid (**Figure 1**). 3"-demethylchartreusin interacts with residues Pro285, Trp82, Gly121 by forming four hydrogen bonds. It has π-donor hydrogen bond interactions with Thr120, Gly121, Trp82 and π-alkyl interactions with Trp430, His438, Trp82. Fabiatrin **70** gave exceptional BE -12.67 kcal/mol and forms hydrogen bonds with Tyr332 and Asp70. It has π-donor hydrogen bond interactions with Pro84, Ile69, Thr120, Tyr332 and π-σ interaction with Thr120. Clorobiocin **74** exhibit -12.73 kcal/mol binding affinity and creates hydrogen bond with Glu197 residue, π-cation interaction with His438; π-donor hydrogen bonds with Trp82, Thr120, Phe329, Tyr332; π-π T shaped interactions through coumarin moiety with Phe329, Tyr332 and π-alkyl interactions with Ala328, Phe329, Tyr332. Apiosylskimmin **68** had a docking score of -12.88 kcal/mol and it forms five hydrogen bonds with Pro285, Trp82, Tyr440 and Trp430 residues, π-σ, π-π T shaped, π-alkyl interactions through coumarin moiety with Ala328; Phe329; Met437 and π-donor hydrogen bond interactions

with Gly116, Trp82, Pro285. Daphnetin **7**, artanin **54**, ensaculin **87**, 2'-*O*-ethyl murrangatin **92**, murranganone **93**, paniculatin **94** which experimentally exhibit anti-Alzheimer activity shows binding energy -7 to -11 kcal/mol with the target protein BChE and is supported by the ADMET studies in which **54**, **87**, **92** and **94** exhibit BBB penetration in **Table S9**. Antioxidants such as isomeranzin **21**, isofraxidin **37** shows BE around -6; peceudanol **14**, grandvitinol **15**, auraptene **82** shows around -8 to -9; fraxoside **61**, fraxin **66** shows around -10 to -11 kcal/mol with the target protein. Toddanone **28**; fraxinol **33**; artanin **54** that experimentally exhibited mild to moderate, AChE [13] shows BE -7.4; -5.89; -7.02 kcal/mol respectively with the target protein BChE and BBB penetration. Umbelliferone **2**, peucedanol **14**, grandvitinol **15** and suberosin **56** that experimentally showed significant inhibition against BuChE and appreciable inhibition against AChE [14] exhibit BE -5.82, -9.02, -9.36 and -7.31 kcal/mol and BBB penetration. Aesculetin **6** and daphnetin **7** that experimentally showed potent inhibitory activity against AChE/BChE [3] shows the docking score of -6.84 and -7.17 kcal/mol respectively indicating that the presence of OH and catechol group plays a crucial role in BChE inhibition. The docking score of paniculatin **94,** murranganone **93** and 2'-*O*- ethyl murrangatin **92** are -7.87, -7.44 and -7.64 kcal/mol, among which **92** & **94** exhibit BBB penetration in our study and it is in consistent with the experimental evidence [15]. Similar to the experimental evidences [16], in our investigation, ensaculin **87** exhibited an excellent binding affinity of -11.19 kcal/mol and BBB penetration. Among synthetic coumarins, 4 compounds that display commendable BE -13 to -14 are chosen as top hits (**159, 160, 171** & **298**). 3-carbonyl and 3-carboxyhydrazido coumarin derivative **100, 101** & **102** which displayed *in vitro* inhibitory activity against hMAO-A and hMAO-B [17] shows BE -6.16, -7.52 & -6.53 kcal/mol respectively. **100** and **101** display BBB penetration. 7-ethoxy-4-methyl coumarin **106** which had an agonist effect against the dopamine D2 receptor [3] exhibit BE -6.44 kcal/mol and BBB penetration. 3-Carbamylcoumarins **113** & **114** with hMAO–A/B inhibitory potency [17] exhibit appreciable BE -8.86 & -8.96 kcal/mol and BBB penetration. Three hydroxy-4-methyl coumarins **117-119** shows BE -5.95, -6.10 & -7.58 kcal/mol and BBB penetration. The two coumarins **118** & **119** have an experimental proof of showing radical scavenging activity than Trolox [14]. Their binding energy differences may rely on the differences in the relative positions of OH groups. Ethyl thiosemicarbazone **140**, thiazoline derivatives **141-145** and the thiazolidinone derivatives **146-147** that showed anti-inflammatory activities [14] display noticeable BE -7 to -10 kcal/mol. The compounds with merged 1,2,4-triazole and the coumarin moiety **157-160** characterized by wide range of biological activities and low toxicity profiles display excellent BE -11.71 to -13.15

kcal/mol. **159** shows BE -13.09 kcal/mol and it is stabilized by π-cation interactions with Asp70, Trp82; π-π T shaped interactions with Tyr332, Ala328, Phe329, Trp231, Trp82, Pro285 and π-alkyl interactions through methyl, phenyl groups with Val288, His438, Trp82, Ala328. **160** display outstanding -13.15 kcal/mol binding affinity and develop hydrogen bonds with His438 and Ser198. It owns π-σ interaction with Trp82; π-π T shaped interactions through phenyl & triazole rings with Tyr332, Trp82 and π-alkyl interactions through methyl, phenyl ring of the compound with Phe329, Pro285, Ala328, Tyr128, Leu125 & Tyr114. Coumarins conjugated with salicylic acid **161** &**162** that showed high coagulant activity 1.5 times more active than warfarin [14] exhibit admirable BE -11.97 & -12.56 kcal/mol respectively. A set of four anti-convulsant coumarin derivatives **163-166** exhibit admirable BE -9 to -11.9 kcal/mol. A set of anti-convulsant [14] coumarin-1,2,4-oxadiazole derivatives **167-171** exhibit significant BE -7.77 to -14.08 kcal/mol. The binding affinity increases as substituent varies from Me-ph< Cl-ph< Br-ph< Cl-ph & 4-Me. The compound **171** that possess two oxadiazole substituents exhibit good BE -13.11 kcal/mol. The compounds **172** & **173** which has experimental evidence for their promising inhibitory activity against hMAO-B, eeAChE & eqBuChE [14] shows BE -8.43 & -11.41 kcal/mol and **172** exhibit BBB penetration. 4-methyl-7-aminocoumarin derivatives **175, 176** & **177** which possess oxygen radical scavenging activity [18] shows BE -7.82, -7.85 & -7.72 kcal/mol. Coumarin-1,2,3-triazole-acetamide hybrids **183-192** exhibit excellent BE around -10 to -11 kcal/mol. Coumarin-semicarbazone **211**; thiosemicarbazone **212** & their corresponding pyrazole-coumarin hybrids **213, 214** that showed antioxidant property better than that of ascorbic acid [14] exhibit excellent docking score of -9 to -11 kcal/mol. Novel coumarin derivatives **254** & **255** exhibit substantial BE -11.98 & -12.44 kcal/mol. The most promising anti-inflammatory and analgesic, 7-substituted coumarins **264** & **265** [14] exhibit good BE -10.61 & -11.16 kcal/mol. Anti-inflammatory 7-substituted coumarin **266** exhibit -9.17 kcal/mol. 1,2,3-triazole-chromenone carboxamide derivatives **267** bearing 3,4-dimethyl benzyl connected to 1,2,3-triazole moiety & **268** bearing 3-morpholinopropyl and 2-bromobenzyl moieties that possess AChE and BChE inhibitory potential with reference to donepezil [14] exhibit excellent BE -11.33 & -11.73 kcal/mol. 6,7-dimethoxy-3-substituted coumarin derivative with 3-hydroxy-N, N-dimethylanilino moiety **270** that showed high AChE/BChE selectivity [17] exhibit BE -8.91 kcal/mol and BBB penetration. A potent eeAChE inhibitor coumarin-pyridinium congener **271** [17] exhibit -8.03 kcal/mol and BBB penetration. Donepezil-like coumarin **272** exhibit excellent BE -11.92 kcal/mol. A dual ChE/MAO inhibitor **273** [17] exhibit an outstanding BE -12.73 kcal/mol and BBB

penetration. The anticoagulant acenocoumarol **275**, phenprocoumon **276** and MAO inhibitor pyridizine coumarin hybrid **277** exhibits BE -7 to -8 kcal/mol. A set of geiparvarins **290-292** exhibit appreciable BE -9 to -11 kcal/mol and BBB penetration. Vitamin K antagonist, tecarfarin **295** exhibit BE -7.69 kcal/mol. N-propargylamine derivatives **296** & **297** are P-gp non substrates and display BE -6.94 & -7.24 kcal/mol respectively and BBB penetration. Literature survey revealed that the compound bearing a propargylamine moiety at position 3 **297** displayed the highest and moderate *in vitro* inhibitory activities against MAO-B and AChE respectively [19]. With extraordinary -14.08 kcal/mol binding free energy, coumarin-pyrazole hybrid **298** interacts with the protein target by forming hydrogen bond with Asp70. Methyl group of the compound constructs π-σ interaction with Trp82; phenyl ring forms π-sulfur interaction with Met437 and π-π T shaped interactions with Trp231, Trp82 and phenyl/pyrazole ring forms π-alkyl interactions with Leu286, Val288, His438, Ala328.

Docking against GlcN-6-P-synthase

The binding energy fluctuates from -5 to -13 kcal/mol for natural and -5 to -12 kcal/mol for synthetic coumarins. The most common antibiotics ampicillin **D4**, ciprofloxacinin **D5** and antifungal drug fluconazole **D6** was also docked. Ampicillin **D4** had a binding free energy of -8.88 kcal/mol and reveal the presence of five hydrogen bonds with Thr302, Ser349, Glu488 and Gln348 amino acid residues of the protein target Glms; π-σ interaction with Leu601. Ciprofloxacinin **D5** had a docking score of -7.49 kcal/mol and develop hydrogen bonds with Gln348, Cys300, Ser303 and Glu488; alkyl interaction with Leu601. With -6.89 kcal/mol binding free energy, fluconazole **D6** contributes five hydrogen bonds with Cys300, Gln348, Thr302 & Ser303 residues of the receptor. Its triazole moiety forms π-sulfur interaction with Cys300, π-alkyl interactions with Ala299 & Ala404 and F interacted with Val399, Glu488. It also forms π-donor hydrogen bond interactions with Ala400, Ser401, Leu346, Cys300 & Ser303. Among natural coumarins, 3 compounds that exhibit BE to -13 are selected as top hits (apiosylskimmin **68**, fabiatrin **70** & 3"-demethylchartreusin **71**). 3"-demethylchartreusin **71** found to have BE -13.57 kcal/mol and considered to have higher binding affinity towards the GlcN-6-P-synthase and it is stabilized by four H-bonds with Glu488, Ala602, Val399. Its chartarin moiety forms π-σ; π-sulfur; π-alkyl interactions with Val605; Cys300; Val605, Cys300 (**Figure 1**). Fabiatrin **70** exhibit BE -12.78 kcal/mol and establish four H-bonds with Thr302, Val399, Asp354, π-σ interaction with Val605; π-sulfur interaction with Cys300 and π-alkyl interactions with Val605, Cys300. Apiosylskimmin **68** exhibit BE -12.49 kcal/mol and has shown seven H-bonds interactions with the amino acids Ser347, Ser303, Thr352, Lys603, Gln348, Ala602 and π-alkyl interaction

with Ala404. Similar to its experimental studies that showed modest antibacterial and antifungal activities [20], umbelliferone **2** exhibit BE -6.04 kcal/mol. Pavietin **13** that experimentally showed antifungal effects against *Guignardia aesculi*, [21] exhibit BE -8.26 kcal/mol. Crenulatin **24** and suberosin **56** that showed antifungal activity with 625 mg/L MIC against *Candida albicans* [14] display BE -6.53 & -6.44 kcal/mol respectively. (E)-Suberenol **39** that possess a potent antifungal activity [22], shows a docking score of -7.36 kcal/mol. Osthole **53** was found to have an antifungal effect [12] exhibit BE -6.89 kcal/mol. Ammoresinol **57** and osthruthin **58** that showed antibacterial activity [3] exhibit notable BE -9.24 & -8.16 kcal/mol. Chartreusin **69,** an antibiotic [12] shows appreciable BE -11.36 kcal/mol. 5-Geranyloxy-7-methoxycoumarin **85** and citropten **26** that significantly inhibited mycelial growth [14] show BE -8.15 & -5.67 kcal/mol respectively. Among synthetic coumarins, 4 compounds that display commendable BE -12 are chosen as top hits (**171, 265, 267** & **298**). Two coumarins with sulfonyl urea substituents **115** & **116** shows noticeable BE -8.03 & -8.80 kcal/mol respectively in accordance with Tu *et al.* [18]. **171** with binding free energy -12.01 kcal/mol forms H-bond with Val605 amino acid of the protein target. Coumarin-semicarbazone **211**; thiosemicarbazone **212** that showed a good antibacterial and antifungal activity [14] and their corresponding pyrazole-coumarin hybrids **213, 214** exhibit excellent docking score of -8 to -9 kcal/mol. Coumarin dimers scaffolds **235-238** which are highly active and more potent than ciprofloxacin [14] exhibit appreciable BE around -7 to -8 kcal/mol. Coumarin-pyrazole carboxamide derivatives **239-241** endowed with promising antimicrobial activities [14] exhibit BE -8 kcal/mol. Coumarin-1,2,3-triazole hybrids **242-246** exhibit noticeable BE -10 kcal/mol. Among **242-246**, experimentally the compound **245** was found to be equipotent to fluconazole [14]. Imidazole based coumarin derivatives **247-250** were more efficient against several *Candida* strains [14]. Thiazolyl-coumarin hybrids **260-263** endowed with both antibacterial and antiviral activities [14] exhibit BE -6 to -8 kcal/mol. 7-substituted coumarins **264** & **265** exhibit exceptional BE -11.32 & -12.17 kcal/mol and **265** (R=-OMe) possesses five H-bonds with Ala602 and Ser303; π-anion interaction with Glu488; π-alkyl interactions with Cys300, Ala299, Val605. 1,2,3-triazole-chromenone carboxamide derivatives **267** & **268** exhibit excellent BE -12.96 & -10.12 kcal/mol. **267** forms H-bonds with Gln348 and Thr302; π-alkyl interactions with Leu601, Val605, Cys300. Coumarin-pyrazole hybrid **298** that showed significant antibacterial activity compared to ciprofloxacin [14] exhibit extraordinary BE -12.18 kcal/mol bearing H-bonds with Thr302 and Ala602; π-anion interaction with Glu488; π-alkyl interactions through halogen, phenyl & pyrazole

rings with Val605, Cys300, Leu484, Leu601 and Ala602.

Docking against mTOR

The binding energy differs from -5 to -14 kcal/mol for natural and -5 to -12 kcal/mol for synthetic coumarins. The most common anticancer drugs artemisinin **D7**, doxorubicin **D8** and erlotinib **D9** was also docked. Artemisinin **D7** had a binding free energy of -8.46 kcal/mol and has π-alkyl interactions with Leu2031, Phe2108, Trp2101, Tyr2105, Phe2039 of the target protein mTOR. Doxorubicin **D8** had a docking score of -10.34 kcal/mol and develop hydrogen bonds with Glu2041, Glu2067, Ala112, Phe2039, π-anion & π-cation interactions with Arg2036, Glu2022; π-lone pair interaction with Gly84; π-alkyl interaction with Ala116. With -7.30 kcal/mol binding free energy, erlotinib **D9** shows H-bonds with Ser2035, Lys83 and Gln85 residues. It has π-π & π-anion interactions with Tyr2105, Glu2032 amino acids; π-alkyl interactions with Trp2101, Tyr2105, Leu2031; π-donor hydrogen bonding interactions with Phe2108, Glu2032, Gln85, Tyr2105. Among natural coumarins, 2 compounds that exhibit BE -12 to -14 kcal/mol are selected as top hits (chartreusin **69** & 3"-demethylchartreusin **71**). Chartreusin **69** that showed antitumor properties [12] exhibit BE -12.36 kcal/mol, establishing two H-bonds with Ser2035 and H-bond with Glu2022; π-π T shaped interactions with Phe2039, Trp2101, Tyr2105; π-donor hydrogen bonds with Ser2035, Phe2039 and π-alkyl interaction with Phe2039. 3"-demethylchartreusin **71** is a novel antitumor antibiotic [12], found to have exceptional BE -14.16 kcal/mol and considered to have highest binding affinity towards the mTOR by forming two hydrogen bonds with Glu2032 and one with Ser2035, Tyr2105. Furthermore, π-π; π-donor hydrogen bond; π-alkyl interactions with Phe2108, Tyr2105; Ser2035, Glu2022, Phe2039, Tyr2105; Phe2039, Val86, Phe77 are also present in it (**Figure 1**). Aesuletin **6** that possess many biological activities like antioxidant and apoptotic effects [23] exhibit BE -6.41 kcal/mol. Daphnetin **7** that showed anticancer activity [24] display BE -6.41 kcal/mol. Fraxetin **35**, endowed with inhibitory effects on cancer cell lines [25] display BE -6.66 kcal/mol. Isofraxidin **37** that possess anti-cancer effect shows BE -6.14 kcal/mol. Meranzin hydrate **40** which is used to treat scabies, acne, leprosy [26] exhibit noticeable BE -8.36 kcal/mol. Osthenol **51** that showed the viability of colon cancer [27] display considerable BE -7.1 kcal/mol. Earlier studies showed that osthole **53** was effective in inhibiting the cancer cell lines [12] displays a notable docking score of -7.20 kcal/mol. It exhibits BE -6.36 kcal/mol. Scopolin **63** that showed anti-hepatocellular carcinoma [28] display extraordinary BE -10.04 kcal/mol. Aculeatin **76** that found to be active against human breast cancer [29] exhibit significant BE -7.37 kcal/mol. Phebalosin **79** that prevented the growth of tumours [30] display BE -6.89

kcal/mol. Umbelliprenin **81** endowed with antitumor potential [31] exhibit noteworthy BE -8.55 kcal/mol. A bioactive monoterpene coumarin Auraptene **82** that had chemo-preventive effects [32] exhibit notable BE -8.09 kcal/mol. Farnesiferol C **86** an anti-colon cancer agent [33] exhibits remarkable BE -9.72 kcal/mol. Among synthetic coumarins, 5 compounds that display commendable BE -11 to -12 are chosen as top hits (**158, 159, 171, 273** & **298**). A potent antineoplastic agent **99** show BE -6.09 kcal/mol. Earlier studies showed that 7,8-Dihydroxy-4-methylcoumarin **119** would to induce apoptosis tested in A549 lung cancer cell lines [3]. The hybrid **158** (R_1=OCH_3, R_2 & R_3=H) with binding free energy of -11.49 kcal/mol and stabilized by H-bonding with Gln85; π-donor H-bondings with Ser2035, Glu2032, Gln85; π-π interactions through pyrazole & coumarin moiety with Phe2108, Phe2039 and π-alkyl interactions with Tyr2105, Trp2101, Phe2039. **159** (R_1 & R_3=H, R_2=NEt_2) that possess BE -11.27 kcal/mol forms H-bond with Gln85. Also, it possesses π-cation interaction with Arg2036; π-alkyl interactions through methyl & halogen with Phe2039, Trp2101, Leu2031, Phe2108; π-π interactions through coumarin moiety and phenyl rings with Phe2039, Phe2108; π-donor hydrogen bond interactions with Ser2035, Phe2039, Arg2036, Gln85. *O*-prenylated coumarins **216-219** that showed cytostatic effect [3,14] exhibit appreciable BE -7.92 to -8.50 kcal/mol. Thiazolidinylidene derivative **220** containing a melono nitrile fragment that showed the best cytotoxic activity on human cervix carcinoma cell line KB-3-1 [14] exhibit considerable BE -8.9 kcal/mol. 7-styrylcoumarin derivative **221** that showed the highest activity in inducing apoptosis in human colon adenocarcinoma cells [14] exhibit appreciable BE -8.99 kcal/mol. Coumarin-benzo[b]thiophene 1,1-dioxide conjugate **222** that showed high potency in inducing cancer cell apoptosis [14] display outstanding BE -10.26 kcal/mol. **273** exhibit an outstanding BE -11.30 kcal/mol and forms H-bonding with Glu2032; π-π interactions through benzene ring and coumarin moiety with Tyr2105, Phe2039 and π-alkyl interactions with Trp2101, Tyr2105. Diethylene glycol tethered isatin-1,2,3-triazole-coumarin derivatives **278-289** that showed weak to moderate *in vitro* activity against human cancer cells [14] exhibit excellent BE -9 to -10 kcal/mol. Coumarin-pyrazole hybrid **298** exhibit extraordinary BE -12.71 kcal/mol and are stabilized by π-σ interaction with Trp2101; π-π T shaped interactions through pyrazole and coumarin moiety with Phe2039, Phe2108, Tyr2105; π-alkyl interaction with Arg2036.

Docking against Mab A

The binding energy deviates from -4 to -10 kcal/mol for natural and -4 to -9 kcal/mol for synthetic coumarins. The common anti-TB drugs pyrazinamide **D10**, ethambutol **D11** and isoniazid **D12** was also docked. Pyrazinamide **D10** had a binding free energy of -3.82 kcal/mol and forms H-bonds with Val60 and Val62. It also

constructs π-cation; π-alkyl; π-σ interactions with Arg47; Ala89, Ala111; Val62 residues respectively. Ethambutol **D11** had a docking score of -4.57 kcal/mol and develop three H-bonds with Gly184 & Pro183 amino acid residues of FabG. It possesses π-alkyl interactions with Tyr185, Ile27 and Pro183. With -4.31 kcal/mol binding free energy, isoniazid **D12** forms hydrogen bonds with Ile186, Gly184 and π -alkyl interaction with Pro183. Among natural coumarins 5 compounds that exhibit BE -9 to -10 are selected as top hits (fraxoside **61**, apiosylskimmin **68**, chartreusin **69**, fabiatrin **70** & 3"-demethylchartreusin **71**). Fraxoside **61** exhibit BE -9.06 kcal/mol and acclaim five H-bonds with Ile27, Ile186 and Asn88 amino acid residues of target protein FabG and π-σ interaction with Ile27; π-alkyl interactions with Pro183, Ile27. Apiosylskimmin **68** exhibit -9.96 binding affinity and has hydrogen bonds through carbonyl of coumarin moiety with Ser140 and Gln150; π-σ; π-π T shaped interactions with Met190; Tyr185 respectively. Chartreusin **69** exhibit the docking score of -9 kcal/mol and shows hydrogen bonds with Ser92 and Arg25. Additionally, it has π-σ; π-π interactions through chartarin moiety of phenyl rings with Met190; Tyr153, π-donor hydrogen bonding; alkyl interactions with Asn88, Tyr153, Gly90, Arg25, Met190, Ser92; Met190 respectively. Fabiatrin **70** exhibit BE -9.21 kcal/mol and is stabilized by forming H bondings with Gly22, Asn88, Thr191, Ile186, π-sulfur interaction with Met190 and π-π T shaped interactions with Tyr153. 3"-demethylchartreusin **71** exhibit outstanding BE -10.96 kcal/mol and makes H-bonds with Gly90, Arg25, Ile186 and Ser92 also has π-σ; π-π interactions through chartarin moiety with Met190; Tyr153 & π-donor hydrogen bonds with Arg25, Ser92, Met190, Ile27, Tyr153 and alkyl interaction with Met190. Scopoletin **30** with MeO at C-6, umbelliferone **2** (with H at C-6) and phelodenol **8** (with $HO(CH_2)^2$ at C-6) display BE -5.2, -5.06 and -6.96 kcal/mol respectively. Among synthetic coumarins, 4 compounds exhibit -9.47 to -9.61 are chosen as top hits (**154**, **159**, **171** & **235**). **154** exhibits BE -9.61 kcal/mol and reveal three H-bonds with Ile27 and Gly28. Its coumarin moiety has π-cation interaction with Arg25 and π-alkyl interactions with Ile138, Pro183, Arg47, Ile27. The compounds with merged 1,2,4-triazole and the coumarin moiety **157-160** display commendable BE -8.72 to -9.57 kcal/mol. **159** (R_1 & R_3=H, R_2=NEt_2) shows binding free energy of -9.57 kcal/mol and is stabilized by forming five H-bonds with Ile27, Gly90, Gly28, π-σ interactions with Tyr153, Leu91, π-cation interaction with Arg25, π-alkyl interactions with Met190, Ala89, Pro183 and π-donor hydrogen bonds with Tyr153, Met190, Ile27, Gly90, Arg25 (**Figure 1**). **171** exhibit binding free energy of -9.51 kcal/mol by forming three H-bonds with Ser92 and Tyr153. **235** (R_1=6-Me) with BE -9.47 kcal/mol creates six H-bonds with Lys157, Ile27, Gly90, Tyr153 and Asn88, π-cation interaction with Arg25; π-π

interaction with Tyr153 and π-alkyl interaction with Ile27. **261** & **263** that exhibited experimental anti-TB potential [35] shows BE -6.60 and -8.34 kcal/mol.

Docking against α-amylase

The binding energy diverges from -4 to -13 kcal/mol for natural and -4 to -12 kcal/mol for synthetic coumarins. The most common antiadipogenic drugs benzphetamine **D13**, diethylpropion **D14** and phentermine **D15** were also docked. Benzphetamine **D13** had a binding free energy of -7.48 kcal/mol and possesses π-anion interaction with Asp300; π-σ interactions with Leu165, Leu162 residues of the enzyme. Diethylpropion **D14** had a docking score of -6.72 kcal/mol and forms hydrogen bonding through acyl group with His299, π-lone pair interaction with Tyr62. It also stabilized by creating π-alkyl interactions with Tyr62, Ala198, His101.With -6.75 kcal/mol binding free energy, phentermine **D15** forms H-bonds with Glu233, Asp197. Its phenyl ring exhibit π-anion & π-cation; π-σ; π-alkyl interactions with His201; Glu233; Ile235, Ala198, Lys200, Leu162 respectively. Among natural coumarins, 3 compounds that exhibit BE -12 to -13 kcal/mol are selected as top hits (chartreusin **69**, fabiatrin **70** & 3"-demethylchartreusin **71**). Chartreusin **69** exhibit considerable BE -12.39 kcal/mol and is stabilized by interactions such as three H-bonding with Gln63 and Asp197, π-σ interactions with Thr163, Leu165, Tyr62 and π-alkyl interactions with Ile235, His299, Trp58. Fabiatrin **70** exhibit substantial BE -12.72 kcal/mol exhibit five H-bonds with Trp59, Asp300, Gln63 and Arg195. Through coumarin moiety it also exhibits π-σ interaction with Thr163; π-alkyl interactions with Ile235, Leu162. 3"-demethylchartreusin **71** exhibit outstanding BE -13.49 and is designed by nine H-bonds with Trp59, Gln63, Asp300, Arg195, His305, Glu233 and Asp197, π-σ interactions with Tyr62, Leu165; π-π T shaped interaction with Trp59; π-alkyl interaction with Leu162 (**Figure 1**). Esculetin **6**, which has two hydroxyl moieties at C-6, 7 positions, that significantly blocked the induction of PPAR protein expression and inhibited adipocyte differentiation induced by a PPAR agonist troglitazone [36], exhibit BE -6.42 kcal/mol. Fraxetin **35**, which has two hydroxyl moieties at C-7, 8 positions and a methoxyl moiety at C-6 position, that showed significant inhibitory activity [36] shows BE -6.42 kcal/mol. Interestingly, scopoletin **30**, which has an identical structure to esculetin **6** except an additional methoxyl group at C-6 instead of hydroxyl moiety, that showed weak activity [36] exhibit BE -5.2 kcal/mol. Moreover, addition of hydroxyl moiety to scopoletin **30** significantly increased the activity as shown in fraxetin **35**, whereas addition of methoxyl moiety to fraxetin **35** significantly decreased the activity as shown in fraxidin **34** (BE -5.33 kcal/mol). Furthermore, scopolin **63** that inhibited adipocyte differentiation and lipid accumulation in the preadipocyte cells [37] and that possess five OH

groups display excellent BE -9.06 kcal/mol. These results suggest that a free hydroxyl group of simple coumarin skeleton might be important for exerting anti-adipogenic activity. Marmin **84** (*Citrus maxima* is used in a traditional digestive tea in China and considered to have lipid-lowering effect) [38] exhibit outstanding BE -8.59 kcal/mol. Among synthetic coumarins, 4 compounds that display commendable BE -12 are chosen as top hits (**160, 171, 273** & **298**). The compounds with merged 1,2,4-triazole and the coumarin moiety **157-160** exhibit outstanding BE -10.32 to -12.06 kcal/mol. **160** possess stabilizing interactions such as π-anion through triazole and phenyl ring with Asp197, Glu233, Asp300, π-lone pair through coumarin moiety with Thr163, π-π T shaped and halogen interaction with Trp58, Trp59, π-alkyl with Lys200, His201, Ile235, Ala198, Leu162, Leu165, His305 and Trp59. A set of coumarin-1,2,4-oxadiazole derivatives **167-171** exhibit significant BE -8.40 to -12.26 kcal/mol. **171** is stabilized by H-bonding interaction with Ala106. **273** exhibit an outstanding BE -12.72 kcal/mol and demonstrates hydrogen bonding through amide group with Asp300, π-π interactions with Trp59, Tyr62; alkyl interaction with Leu165. Coumarin-pyrazole hybrid **298** exhibit extraordinary BE -12.27 kcal/mol and shows π-σ interactions through methyl, phenyl & pyrazole rings with Leu165, Leu162, Tyr62; π-cation/π-π T shaped interactions through phenyl rings with His201 & Trp59; π-alkyl interactions with His101, Ala198, His299, Tyr62, Leu165.

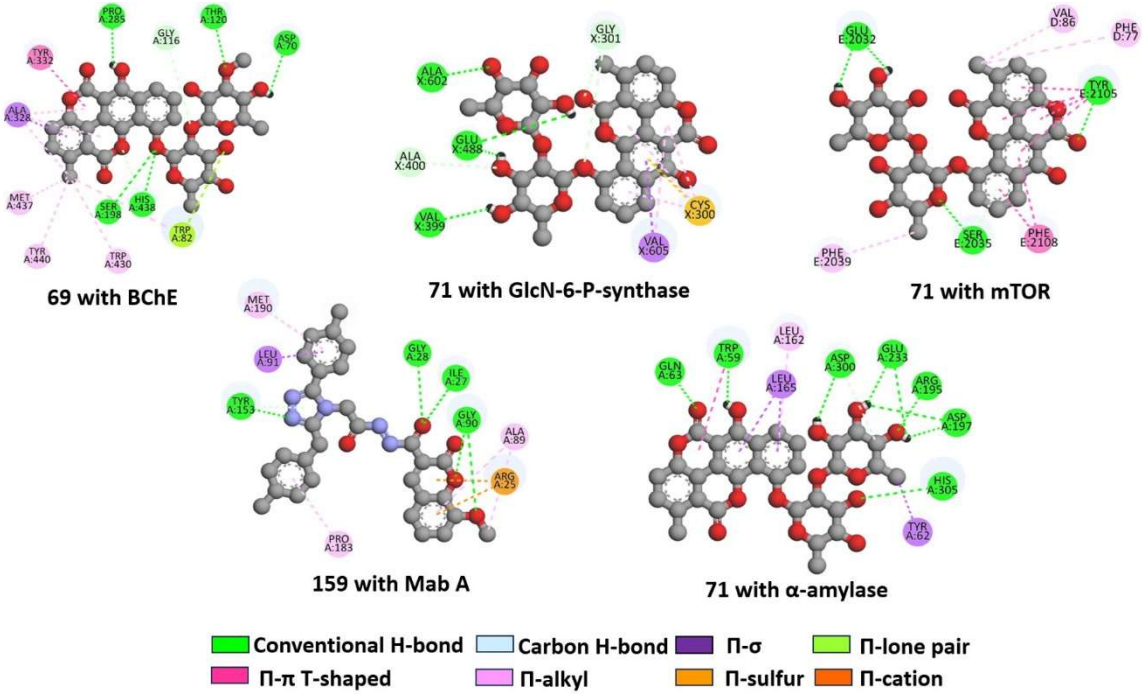

Figure 1. Interactions of some representative top leads within the active site of the proteins.

Based on the docking results, it is resolved that 3”-demethylchartreusin **71** with significant binding affinity with BChE, GlcN-6-P-synthase, mTOR, MabA and α-amylase and hence act as a potential lead compound. Chartreusin **69** and Fabiatrin **70** stand out as the

top contenders for BChE/mTOR and GlcN-6-P-synthase/α-amylase, respectively. Among the synthetic coumarins, pyrazole hybrid **298** functions as the lead that exhibit best molecular docking traits with BChE/mTOR/GlcN-6-P-synthase/α-amylase. The carbohydrazide-triazole-coumarin hybrids **158/159/160** display a leading binding affinity with mTOR/BChE/β-keto acyl reductase respectively and hence considered as potential leads.

Exploring the drug-likeness potential of bioactive coumarins [11]

The predicted data, such as physicochemical properties, lipophilicity, water solubility, pharmacokinetics, drug likeness and medicinal chemistry friendliness of coumarins and drugs under study are deliberated in **Table S7-S10**. 66% (198) of coumarins obey all the five criteria for drug likeliness. 88, 78, 92, 89 and 78% of the coumarins perceive the Lipinski rule of five, Ghose's filter, Veber's rule, Egan's rule and Mugge's filter [39-43] respectively. The drugs **D1-D3**, **D5-D7**, **D9**, **D11** and **D14** follow all the five criteria. 73 and 84% of coumarin observe Pfizer and Golden triangle rules respectively in (**Figure 2**).

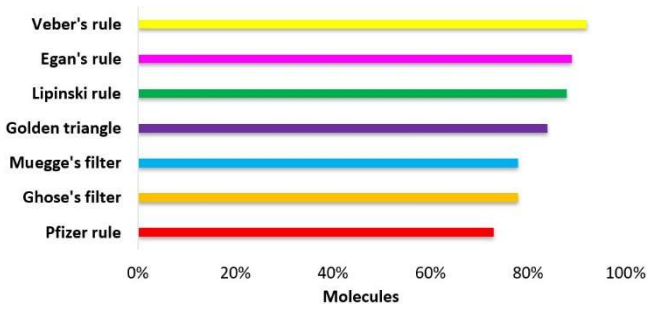

Figure 2. Drug likeness of coumarins 1-298 and drugs D1-D15.

Around 77% of NC and 53% of SC manifest ≤ log P 3 and are found to be lipophilic and have effective absorption. TPSA values of the coumarins and the drugs under study are found to possess characteristic absorption in **Table S7** and (**Figure 3**).

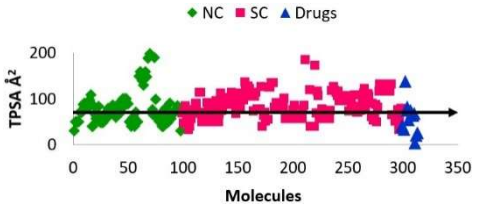

Figure 3. TPSA of the coumarins 1-298 and the drugs D1-D15.

89% of natural coumarins **1-98,** 98% of synthetic coumarins **99-298** have TPSA value less than 140Å^2 indicating its good penetrating ability. Except doxorubicin **D8**, all other drugs under study shows TPSA value less than 140Å^2. 109 (NC-48; SC-61) coumarins and drugs **D1-D3**, **D7**, **D13-D15** that evince TPSA <69Å^2 exactly correlates well with its BBB penetrating ability. Regarding aqueous solubility, 157:122:19 coumarins manifest good(S): moderate (MS): poor (PS) solubility factor under Esol class. 127:125:45 coumarins exhibit good (S): moderate (MS): poor (PS) solubility factor in Ali class. Considering the drugs taken for

comparative purpose, only donepezil **D1**, erlotinib **D9**, benzphetamine **D13** display moderate solubility and all other drugs express solubility under Esol class and Ali class in **Table S8** and (**Figure 4**).

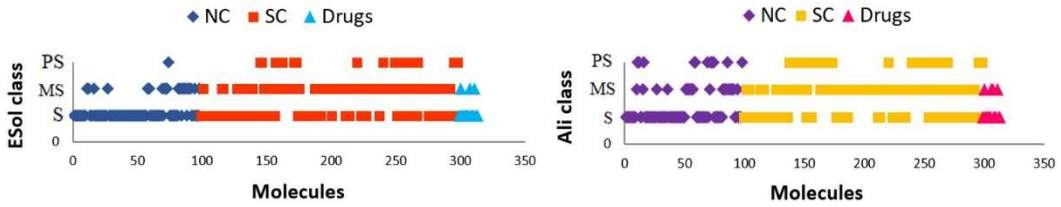

Figure 4. Aqueous Solubility of the coumarins 1-298 and the drugs D1-D15.

86 (NC) and 185 (SC) indicate high GI absorption. Except ampicillin **D4** and doxorubicin **D8** all other drugs show high GI absorption. 54:244 coumarins, 5:10 drugs are found to be P-gp substrates: non-substrates respectively. The same data is also expressed in the form of Boiled Egg model (**Figure 5**).

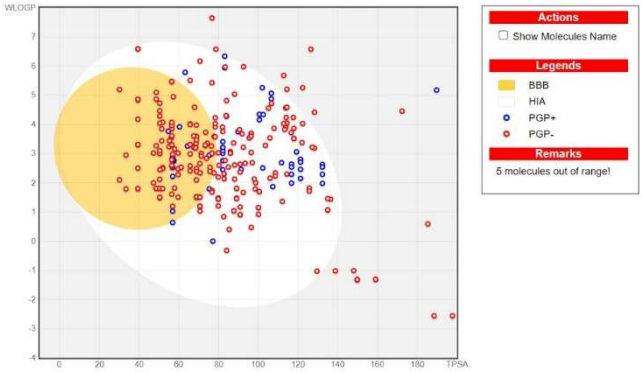

Figure 5. Boiled Egg model for coumarins 1-298.

203 coumarins and 10 drugs possess HIA% ≥95; 81 coumarins and ampicillin **D4**, pyrazinamide **D10** & isoniazid **D12** drugs show ≥75. 285 coumarins and 13 drugs exhibit medium permeability (Caco-2 nm/s). 13 coumarins and ampicillin **D4**, ethambutol **D1** show low permeability. 127 coumarins and artemisinin **D7**, erlotinib **D9** bound strongly with plasma protein. From the pharmacokinetics analysis of the coumarins, it is recognized that 79%, 68%, 48%, 45% and 41% of coumarins, 8 drugs of rivastagmine **D2**, ampicillin **D4**, ciprofloxacinin **D5**, doxorubicin **D8**, pyrazinamide **D10**, ethambutol **D11**, isoniazid **D12** and diethylpropion **D14** are found to be non-inhibitors of CYP2D6, CYP3A4, CYP2C19, CYP2C9 and CYP1A2 enzymes respectively in **Table S9** and (**Figure 6**).

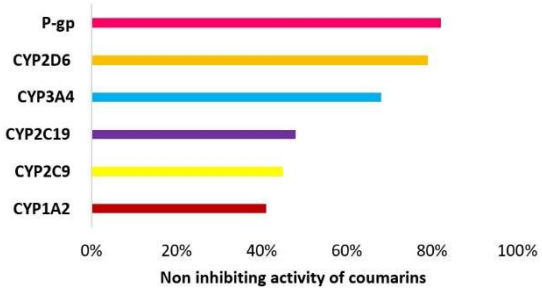

Figure 6. Non inhibiting capacity of the coumarins 1-298 against specific enzymes.

Log Kp ranges from -3 to -10 cm/s for coumarins and -4 to -9 cm/s for drugs **D1-D15** in **Table S9**. The bioavailability score for 94% coumarins was found to be 0.55. 90% of coumarins does not reveal PAINS alert. Regarding hERG parameter, 77 coumarins: 6 drugs (rivastagmine **D2**, galantamine **D3**, ampicillin **D4**, ciprofloxacinin **D5**, artemisinin **D7**, pyrazinamide **D10**) and 212 coumarins: 8 drugs (donepezil **D1**, fluconazole **D6**, erlotinib **D9**, ethambutol **D11**, isoniazid **D12**, benzphetamine **D13**, diethylpropion **D14**,

phentermine **D15**) unveil low and medium risk respectively. 41 natural coumarins and 48 synthetic coumarins shows lead likeness. Synthetic accessibility of 97% coumarins ranges from 1 to 4 indicating that it is easy to synthesize (**Table S10**). In the current study, the drug likeness score for all of the coumarins under review was calculated using MolSoft (https://www.molsoft.com/mprop/) and it is revealed that the majority of the coumarins fall into the category of drugs. Among the 298 coumarins, 172 exhibit binding energy (BE) ≥-7 kcal/mol with all the five target proteins. These 172 coumarins were further examined for their drug-likeness parameters, among which 33 were identified as non-inhibitors of CYPs. Of these 33, 24 comply with Lipinski's rule. Among the 24, 14 shows drug-likeness according to the Ghose, Veber, Egan, Muegge, Pfizer and Golden triangle rules in **Table S11-S14**. Finally, out of these 14, only 9 possess positive responses across all parameters and are therefore considered as the best drug templates (**Table S15)**. In our study, the nine coumarins–Pavietin **13**, Peceudanol **14**, Grandvitinol **15**, Meranzin Hydrate **40**, Minumicrolin **42**, Fatouain C **47**, Fatouain D **48**, Marmin **84** and Clauselenin B **88** possess strong interactions with various amino acid residues of the target proteins (**Figure S1**) and hence stand out with strong binding affinity towards all five targets, passing all drug-likeness criteria to emerge as exceptional multitargeted drug candidates.

Conclusions

In this study, the theoretical evaluation of binding affinities (kcal/mol) of some coumarins with five protein targets was carried out in order to validate their potency and to identify a possible lead molecule. From the docking result, it is found that 3”-demethylchartreusin **71**, Chartreusin **69,** Fabiatrin **70**, carbohydrazide-triazole-coumarin hybrids **158/159/160** and pyrazole hybrid **298** stand out as the top contenders. In-silico drug-likeness analysis helps to identify nine promising coumarins with the acceptable range of pharmacokinetic properties that could strongly interact with target proteins and can be emerge as exceptional multitargeted drug candidates. This broad-spectrum therapeutic approach aligns with the growing trend in polypharmacology, making the findings highly relevant to researchers looking for versatile drug candidates.

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