https://doi.org/10.35845/kmuj.2025.23603

ORIGINAL ARTICLE

Potential therapeutic compounds against hypercholesterolemia: an in-silico analysis

Sara Tahir [1], Madeeha Khan [2], Azam Shareef [2] Hizbullah Khan [3], Ishrat Jabeen [4], Ubair Aziz [4], Quratul Ain [1], Mohammad Iqbal Khan [1], Fouzia Sadiq [1]✉

1: Shifa Tameer-e-Millat University, Islamabad, Pakistan
2: Department of Biochemistry, Abdul Wali Khan University Mardan, Mardan, Pakistan
3: The Center for Microbes, Development and Health, Institute Pasteur of Shanghai, Chinese Academy of Sciences, University of Chinese Academy of Sciences, Shanghai, China
4: School of Interdisciplinary Engineering and Sciences, National University of Sciences and Technology, Islamabad, Pakistan

Email✉ : director.research@stmu.edu.pk
Contact #: +92-51-8493038

Date Submitted: February 13, 2024
Date Revised: May 15, 2025
Date Accepted: May 31, 2025

ABSTRACT

Objective: To identify and evaluate potential alternative compounds targeting HMG-CoA reductase through in-silico methods for the treatment of hypercholesterolemia, aiming for improved safety and pharmacokinetic profiles compared to existing statins.

Methods: We employed in-silico analysis to identify molecules that can bind to drug target of statins; 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA reductase) and could be used as potential drugs against hypercholesterolemia and with fewer side effects. Molecular docking analysis of the eight compounds [25-Hydroxycholesterol (25HC), TAK-715, Flufenamic acid, Piceatannol, Retinol, Nortriptyline hydrochloride, Losmapimod and AG555 (tyrphostinb46)] was performed against HMG-CoA to probe the ligand protein interaction profile. Subsequently, molecular dynamics simulation was performed by Maestro in Schrodinger Suite to study the dynamic behavior of each compound. Molinspiration server was utilized for the in-silico drug-likeness and bioactivity while the SwissADME and admetSAR were used for ADMET analysis.

Results: All eight repurposed compounds showed favorable binding interactions with HMG-CoA reductase and demonstrated good ADMET properties, including blood-brain barrier permeability, CYP2D6 binding, intestinal absorption, and Caco-2 permeability. TAK-715 and Tyrphostin b46 exhibited the lowest docking scores and stable molecular dynamics, indicating strong binding affinity. Piceatannol showed the most stable ligand-protein complex in RMSF analysis. Drug-likeness and toxicity assessments confirmed the acceptable pharmacokinetic profiles. Overall, TAK-715, Tyrphostin b46, Losmapimod, and Flufenamic acid emerged as the most promising candidates for further exploration in hypercholesterolemia therapy.

Conclusion: This study identifies repurposed compounds, particularly TAK-715 and Tyrphostin b46, as promising HMG-CoA reductase inhibitors. Their favorable profiles warrant further experimental validation for hypercholesterolemia treatment.

Keywords: Hypercholesterolemia (MeSH); Statins (MeSH); Hydroxymethylglutaryl-CoA Reductase Inhibitors (MeSH); HMG-CoA reductase (MeSH); In-silico (MeSH); Computer Simulation (MeSH); Molecular Docking Simulation (MeSH).

THIS ARTICLE MAY BE CITED AS: Tahir S, Khan M, Shareef A, Khan H, Jabeen I, Aziz U, et al. Potential therapeutic compounds against hypercholesterolemia: an in-silico analysis. Khyber Med Univ J 2025;17(2):213-22. https://doi.org/10.35845/kmuj.2025.23603

INTRODUCTION

Hypercholesterolemia refers to dyslipidemia with increased levels of circulating cholesterol that can accumulate within the lining of the vascular walls, interrupting its structure and function, leading to cardiovascular, cerebrovascular, and peripheral vascular diseases.[1] Primary hypercholesterolemia can be due to common hypercholesterolemia, familial hypercholesterolemia, combined hyperlipidemia, and severe hypertriglyceridemia.[1] Secondary causes of hypercholesterolemia can either be caused by existing disorders (type 2 diabetes mellitus, kidney disorders, etc.) or by certain drugs or by excessive intake of dietary cholesterol and smoking.[1] Hypercholesterolemia has been identified as a causal risk factor for atherosclerotic cardiovascular disease (ASCVD), which remains a leading cause of morbidity and mortality around the world, with 523.2 million cases and 18.6 million deaths in 2019 worldwide.[2]

Generally, lifestyle modifications and dietary interventions are recommended as a primary treatment option for managing hypercholesterolemia. Statins and other drugs are a secondary treatment option for the reduction of LDL-C levels and prevention of ASCVD.[3] Statins reduce the total cholesterol and LDL-C in blood by inhibiting the rate-limiting enzyme in cholesterol biosynthesis, HMG-CoA reductase, which converts HMG-CoA to mevalonate that further leads to the production of cholesterol and isoprenoid through a series of biochemical reactions.[4]

Generally, statins can result in approximately 20% to 55% reduction in LDL-C level.[5] Despite the ability of the statins to lower cholesterol, statin withdrawal and non-adherence are constant problems. Statin-associated muscle symptoms (SAMSs), accounting for 72% of all the adverse side effects of statins, are the major reason for discontinuation.[5] Moreover, the efficiency of lipid-lowering activity of statins could also be influenced by genetic polymorphism in the solute carrier organic transporter 1B1 (SLCO1B1) gene, which is expressed at the basolateral membrane of hepatocytes and helps internalize statins.[6] Two most common polymorphisms, SLCO1B1 521T>C and 388A>G, identified in ethnically

diverse populations, play a major role in interindividual differences in statin disposition and response and can lead to reduced hepatic uptake and clearance of statins and increased risk of statin-induced adverse impacts.[6] These two genotypes have been identified among various ethnic groups in the Pakistani population, which could potentially impact the pharmacokinetics and pharmacodynamics of statins.[6]

Ezetimibe is a second-line therapy along with statins.[3] Other drugs such as proprotein convertase subtilisin/kexin type 9 inhibitors (PCSK9 inhibitors), mipomersen, and bempedoic acid are promising therapies for managing dyslipidemias[3,7] While these agents are effective in reducing cholesterol levels, their use is often restricted due to issues of tolerability, cost, and limited global accessibility.[7] Moreover, interindividual variability in response, partly attributed to genetic polymorphisms such as those in the SLCO1B1 gene[6] further emphasizes the need for alternative therapeutic strategies. Drug repurposing presents a cost-effective and time-efficient approach for identifying promising candidates. Accordingly, the present study was undertaken to identify and evaluate alternative compounds targeting HMG-CoA reductase through in-silico methods, with the aim of discovering safer and pharmacokinetically favorable agents as potential substitutes for current statin therapy.

METHODS

Selection of the compounds: Datasets2tools repository retrieved compounds for the queried disease.[8] A total of 100 compounds including FDA-approved drugs that reverse hypercholesterolemia were retrieved from Datasets2tools. The ligands with a poor drug-like properties were discarded by employing drug-likeness and absorption, distribution, metabolism, excretion, and toxicity (ADMET) analysis. The major parameters for drug-likeness and ADMET-related properties including Lipinski's rule of five, pharmacokinetic properties, and toxicity were considered for further screening of compounds analysis. Eight of the compounds were short listed for further analyses. Tak-715, Flufenamic acid, Piceatannol, Retinol, Nortriptyline hydrochloride and AG555 were finalized for further analysis, as they passed the major parameters criteria for drug selection (drug likeliness and ADMET analysis) whereas 25-hydroxycholesterol was selected as it binds with HMG-CoA reductase which is the drug target for statins,[9] while Losmapimod was selected owing to its anti-atherosclerotic properties. The canonical SMILES of the compounds were taken from PubChem database (https://pubchem.ncbi.nlm.nih.gov/). Open Babel tool was utilized for PDB-format 3D structure of compounds (https://openbabel.org/).

Protein preparation: The protein structure was retrieved from the protein data bank (PDB) (https://www.rcsb.org/). The 3D structure of the enzyme HMG-CoA reductase (PDB ID: 1dQ9) was downloaded in the PDB format. The energy minimization was done by the MOEserver(https://www.chemcomp.com/Products.htm). The online software Datasets2Tools [8] was used for the prediction of compounds that could help in reversing hypercholesterolemia.

Molecular docking: The molecular docking of the compounds was done by MOE software version 19 (https://www.chemcomp.com/Products.htm), which uses docking algorithms such as Dock, Triangle Matcher, and London dG for protein-ligand binding studies. The binding pocket selected for the docking analysis was based on the spatial conformation of the ligand naturally present in HMG-CoA reductase. The three-dimensional pose evaluation was performed using PyMOL (https://pymol.org/2/) .

Molecular dynamics simulation: All molecular dynamics simulation were performed using Maestro in Schrodinger Suite (https://www.schrodinger.com) (V.12.8.117). The protein complexes were then solvated with TIP3P water models in a cubical box with distance of at least 10 Å from each side of the box with protein-ligand complex. The system was neutralized by adding Sodium (Na+) and Chloride (Cl−), maintaining a concentration of 0.15 M. Finally, 5 ns Molecular Dynamics Simulation was carried out using Maestro. The stability of each protein-ligand complex was assessed by evaluating the CαRMSD and CαRMSF, analysis with respect to the starting structures.

In silico drug-likeliness and bioactivity prediction: Molinspiration server(https://www.molinspiration.com) was used to analyze the drug-likeliness and bioactivity of the selected compounds. There are 2 important features in the drug-likeliness analysis by the Molinspiration server that is: lipophilicity level (log P) and polar surface area (PSA) directly linked with the pharmacokinetic properties (PK) of the compounds. The ideal value for lipophilicity is logP < 5 whereas for TPSA<100.

Lipinski's rule of five helps to describe the molecular properties of drug compounds that are essential for assessment of vital pharmacokinetic features like absorption, distribution, metabolism, and excretion.

During bioactivity analysis, the bioactivity score of the compounds concerning G protein-coupled receptors (GPCR), ion channel modulators, kinase inhibitors, nuclear receptor ligands, protease inhibitors, and other enzyme targets was calculated by Bayesian statistics.

In silico ADMET analysis: The pharmacokinetic features ADMET of the drugs were predicted by the SwissADME server (http://www.swissadme.ch/) and admetSAR server (http://lmmd.ecust.edu.cn/admetsar2/) The absorption (A) of excellent drugs is determined by the parameters such as membrane permeability (determined by the Caco-2 colon cancer cell line), human intestinal absorption (HIA), and the presence of either a P-glycoprotein substrate or inhibitor. The ability to pass the blood-brain barrier is crucial for drug distribution (D) (BBB) whereas the CYP, MATE1, and OATP1B1- OATP1B3 models are used to calculate drug metabolism (M) while the drug's excretion (E) is calculated using the renal OCT substrate. The drug's toxicity (T) is then projected based on the suppression of human Ether-a-go-go-related genes, carcinogenicity, and

acute oral toxicity.

RESULTS

Docking analysis: To identify more drugs against hypercholesterolemia, the selected compounds (25-hydroxycholesterol, Flufenamic acid, piceatannol, AG555, nortriptyline hydrochloride, retinol, Losmapimod and tak-715) were docked with the active site of HMG-CoA reductase.

All the compounds displayed effective interaction with HMG-CoA reductase (Figure 1).

However, after simulation the compound Flufenamic acid forms 1 hydrogen and ionic bond with Lys^{722} and 01 ionic bond with Arg^{568} of the enzyme

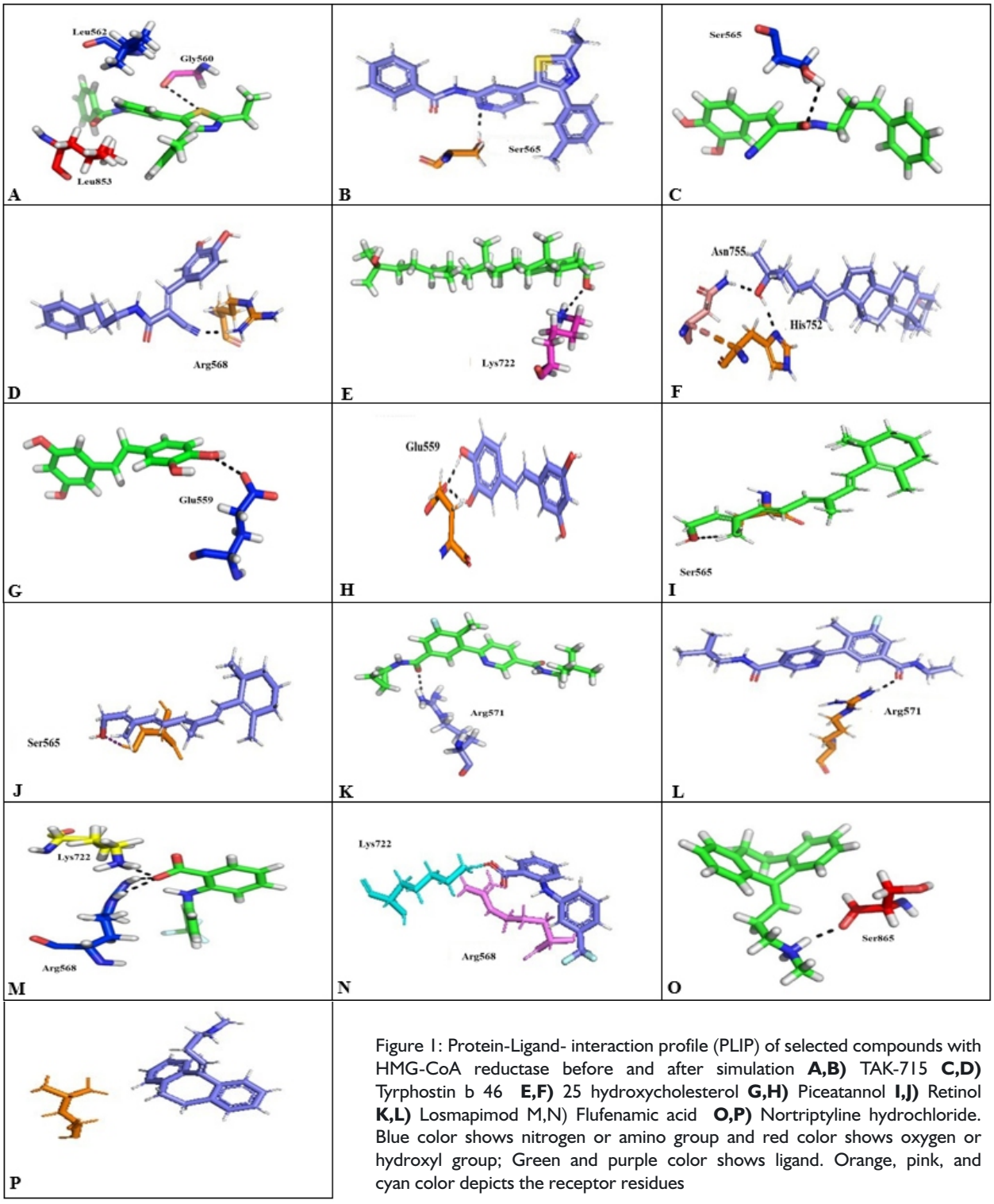

Figure 1: Protein-Ligand- interaction profile (PLIP) of selected compounds with HMG-CoA reductase before and after simulation **A,B)** TAK-715 **C,D)** Tyrphostin b 46 **E,F)** 25 hydroxycholesterol **G,H)** Piceatannol **I,J)** Retinol **K,L)** Losmapimod M,N) Flufenamic acid **O,P)** Nortriptyline hydrochloride. Blue color shows nitrogen or amino group and red color shows oxygen or hydroxyl group; Green and purple color shows ligand. Orange, pink, and cyan color depicts the receptor residues

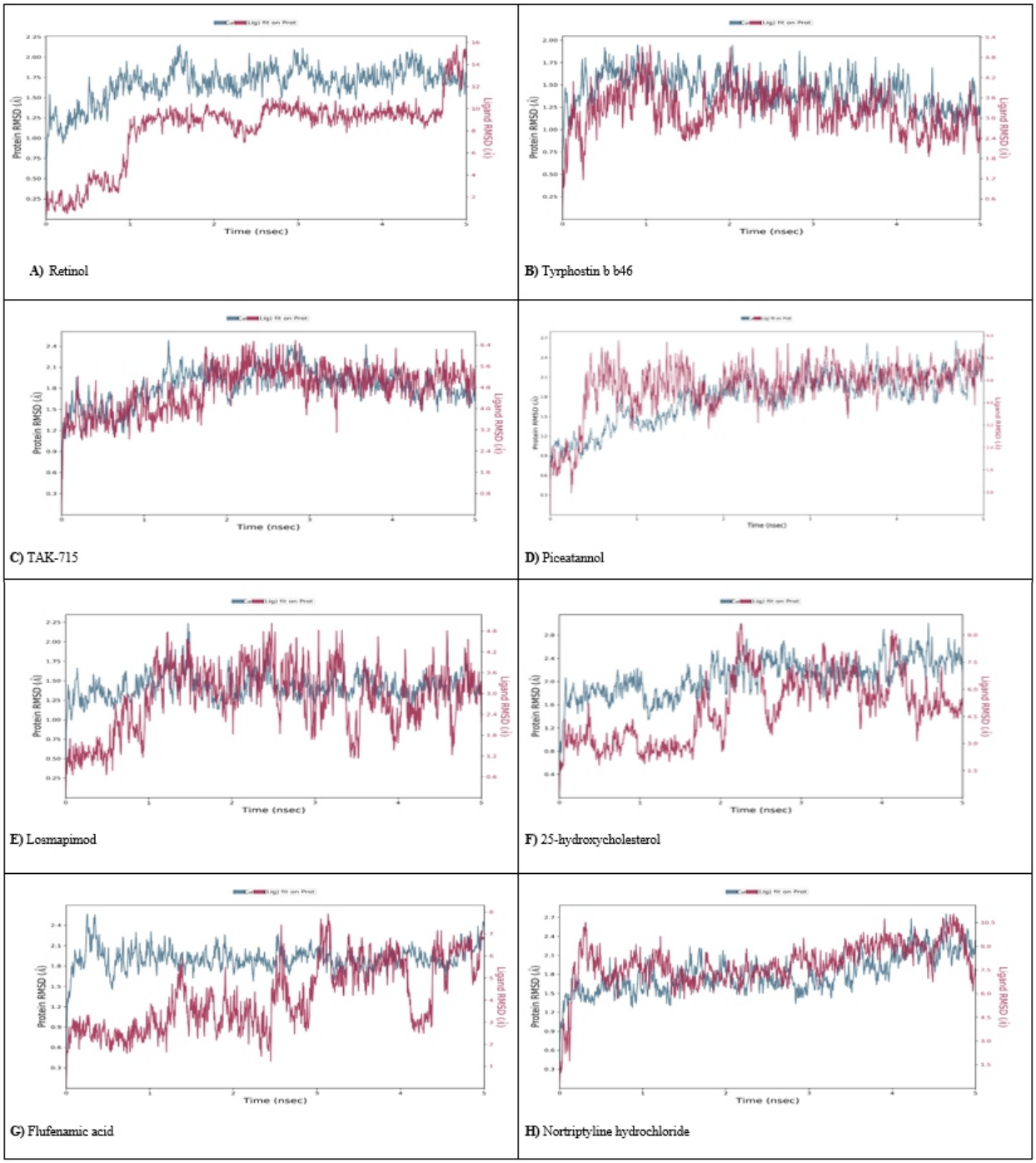

Figure 2: RMSD graph of compounds with HMG-CoA reductase **A)** Retinol **B)** Tyrphostin b46 **C)** TAK-715 **D)** Piceatannol **E)** Losmapimod **F)** 25-hydroxycholesterol **G)** Flufenamic acid **H)** Nortriptyline hydrochloride; Blue color shows protein and dark pink color shows ligand.

while Piceatannol forms 2 hydrogen bonds with active site residue Glu[559]. The interaction of Losmapimod remains same before and after simulation as it forms 1 hydrogen bond with residue Arg[571] of the enzyme. 25-hydroxycholesterol forms 2 hydrogen bonds with Asn755 and His[752] and has 01 $\pi-H$ bonding with His[861] of the enzyme. Tyrphostinb46 forms a single interaction with Arg568 of the enzyme. TAK-715 and retinol formed 1 hydrogen bond with residue Ser[565] while nortriptyline seemed to be non-reactive as it showed no interaction. After visualizing the ligand and receptor interactions, we identified a ligand showing the minimum S score among all the compounds. TAK-715 and Tyrphostin b46 showed minimum S score towards HMG-CoA reductase (Supplemental Table S4).

Table I: Molinspiration bioactivity analysis of compounds under study

Compound name	GPCR	Ion channel modulator	Kinase inhibitor	Nuclear receptor ligand	Protease inhibitor	Enzyme inhibitor
25-hydroxych-olesterol	0.20	0.06	0.44	0.92	0.07	0.59
Tyrphostinb46	-0.15	-0.15	-0.15	-0.12	-0.06	-0.08
Flufenamic acid	-0.02	0.06	0.18	0.16	-0.21	0.06
Nortriptyline hydrochloride	0.68	0.48	0.16	0.35	0.23	0.69
Retinol	-0.01	0.32	-0.25	1.02	-0.16	0.06
Piceatannol	-0.12	0.05	-0.11	0.08	-0.34	0.07
Tak-715	0.12	-0.31	0.50	0.11	-0.09	0.10
Losmapimod	0.12	-0.03	0.36	-0.18	0.14	0.06

GPCR: G protein-coupled receptors

Table II: Molinspiration Drug likeliness analysis of compounds under study

Compound name	Lipinski's parameters				
	Molecular weight ≤ 500DA	miLogP ≤ 5	H-bond donor ≤ 5	H bond acceptor ≤ 10	Violations <4
25-hydroxych-olesterol	402.66	6.42	2	2	1
Ag555	322.36	2.85	3	5	0
Flufenamic acid	281.23	4.84	2	3	0
Nortriptyline hydrochloride	263.38	3.94	1	1	0
Piceatannol	244.25	2.50	4	4	0
Retinol	286.46	5.92	1	1	1
Tak-715	399.52	5.12	1	4	1
Losmapimod	383.47	3.52	2	5	0

Losmapimod and Flufenamic acid exhibited better binding affinity than the other four studied compounds i.e., retinol, 25-hydroxycholesterol, piceatannol and nortriptyline hydrochloride. However, the lowest score shows the overall binding affinity for a target protein. The best docking energy is illustrated by lowest value of S score. On a comparative analysis of minimum S score, TAK-715 displayed highest binding affinity towards HMG-CoA reductase followed by Tyrphostin-b46 and nortriptyline.

Molecular dynamics simulation: Molecular dynamic (MD) simulations were carried out for all 8 compounds having good interactions, best docking scores, as well as better ADMET properties. The stability of all the compounds with the ligand was studied, considering the RMSD parameters of the whole complex. Simulations were run for a total of 5 ns to analyze the comparative stability of the compounds with reference structure. The reference structure of the protein displayed constant stability throughout the entire simulation period. The stability of the main structure of the protein measured in terms of the RMSD indicates that the protein's core structure remained mainly unchanged despite being introduced with diverse ligands in the binding pocket. The ligands displayed different levels of structural deviations from their initial binding poses. Three compounds (Retinol, Tyrphostin b46, and TAK-715) showed lower RMSD and thus indicate relatively stable binding conformation throughout the 5ns simulation (Figure 2).

An average RMSD change of only 1.2 angstroms indicating negligible deviations was demonstrated by the protein structure. During the simulation, the RMSD values for the protein structure remained within a relatively narrow range, changing between 1.0 Å and 2.2 Å. The consistent stability illustrates the flexibility of the protein's core conformation within the complex. Simultaneously, the compound retinol demonstrated a relatively stable conformation from 2.5 ns to 4.8 ns, with an RMSD difference of 3.0 Å. The RMSD values for the ligand consistently fell within the range of 8.0 Å to 11.0 Å during this period, suggestive of a distinct and dynamically favored binding pose (Figure 2a). This stable ligand behavior features the significance of the Retinol-HMG interaction and proposes the presence of a vigorous ligand-protein binding mode.

In Tyrphostinb46-HMG Complex, the protein structure remained stable throughout the 5ns simulation, with an RMSD change of 0.9Å (Angstroms), changing within the range of 1.0Å to 1.9Å. The ligand also maintained relative stability, with an RMSD change of 2.6Å, ranging from 2.4Å to 5.0Å (Figure 2b). Similar to the Tyrphostinb46-HMG complex, in the TAK-715-HMG complex the protein structure displayed stability, with an RMSD change of 1.3Å within the range of 1.1Å to 2.4Å. The ligand also reserved a stable conformation, with a lower RMSD of 3.0Å, upholding this stability from 1ns to the end of the simulation (Figure 2c).

The protein structure in the Piceatannol-HMG complex, attained stability after 1.5ns of simulation, with an RMSD change of 0.9Å, inconsistent between 1.7Å and 2.6Å. However, the ligand showed less stability, with a variable RMSD ranging from 3.2Å to

6.2Å, initially at 0.5ns (Figure 2d). The protein structure remained relatively stable throughout the 5ns simulation, with an RMSD change of 1.2Å within the range of 1.0Å to 2.2Å in the Losmapimod-HMG complex. However, the ligand exhibited instability after 0.6ns, with an RMSD change of 3.6Å, varying between 1.2Å and 4.8Å (Figure 2e). The protein structure sustained stability with an RMSD change of 2.0Å, shifting from 0.8Å to 2.8Å in the 25-hydroxycholesterol-HMG complex. The ligand showed stability from 1ns to 1.8ns, with an RMSD difference of 1.6Å, ranging from 1.8Å to 3.4Å (Figure 2f). The protein structure in the Flufenamic Acid-HMG complex remained relatively stable after 0.7ns, with an RMSD change of 0.5Å within the range of 1.7Å to 2.2Å. However, due to half of the ligand structure being anchored and the other half freely hanging, structural changes caused higher oscillations in the RMSD graph, with a change of 6.0Å ranging from 2.0Å to 8.0Å (Figure 2g).

But, in the Nortriptyline Hydrochloride-HMG complex, the protein structure retained stability throughout the simulation, with an RMSD change of 1.2Å ranging from 1.2Å to 2.4Å. Yet, the ligand displayed higher RMSD values, with variations from 6.5Å to 10.5Å and displayed instability (Figure 2h). However, the RMSF results revealed that the residues binding with each compound had a completely different pattern of fluctuations (Supplemental Figure S1).

TAK-715 had higher peaks for residues 19-21 (2.2 Å - 3.9 Å), indicating that the ligand was more flexible in this region. However, the other residues to which the ligand was bound were more stable. Tyrphostin b46 also had higher peaks for residues 1-3 (1.9 Å-2.9 Å), 12-16 (1.2 Å -2.1 Å), and 21-23 (1.9Å-2.5Å), demonstrating that the ligand was more flexible in these regions. However, considering other parameters such as RMSD and docking score, this compound could still be considered a good candidate in term of binding stability. Retinol had fluctuations for residues 2-20 (2.9 Å -5.8 Å), showing that the ligand was more flexible in this region. The peaks with higher RMSF showed that the complex was less stable. 25-hydroxycholesterol had higher peaks for ligand-bound residues 4-23 (2.6 Å-4.5Å), signifying that the ligand was more flexible in this region. The higher RMSF indicated the less stability of this compound. Piceatannol had very less fluctuations for residues 2-3 (1.5Å -2 Å), showing that the ligand was very stable in this region. A higher peak was observed in Losmapimod for residues 6-11 (1Å-3.9Å), but the rest of the residues to which the ligand was attached showed very less fluctuations, designating that the complex was stable overall. Flufenamic acid had fluctuations for residues 1-3 (4.1Å) and 17-19 (3.2Å -4.2Å), but the stability of the complex was less clear. Nortriptyline hydrochloride had greater fluctuations for residues 2-19 (2.2Å -3.8Å), showing that the ligand was more flexible in this region. The stability of the complex was unclear.

Overall, the RMSF plots showed that Piceatannol had the most stable ligand-protein complex, while TAK-715, Tyrphostin b46, Retinol, and Nortriptyline hydrochloride had less stable complexes than piceatannol.

In silico prediction of drug-likeness property and bioactivity score: Molinspiration evaluates the significant molecular properties of Octanol-water partition coefficient logP (milogP), Topological polar surface area (TPSA), number of hydrogen acceptor and donor of the compounds. The results indicate that the milogP value of AG555 (2.85), Flufenamic acid (4.84), nortriptyline hydrochloride (3.94), Losmapimod (3.52) and piceatannol (2.14) had ideal lipophilicity (logP < 5); while 25-hydroxycholesterol (6.42), tak-715 (5.12) and retinol (5.92) were predicted to have poor lipophilicity (logP > 5).

The TPSA of 25-hydroxycholesterol (40.46), Flufenamic acid (49.33), retinol (20.23), nortriptyline hydrochloride (12.03), tak-715 (54.88), AG555 (93.35), piceatannol (80.92) and Losmapimod (71.09) were ≤ 140 Å, which shows that the compounds have good oral absorption or membrane permeability. Most of the targets of existing drugs belong to one of the mentioned protein families: G protein-coupled receptors (GPCR), ion channels, kinases, nuclear hormone receptors, proteases, and other enzymes (Table I). The molecule having bioactivity score greater than 0.00 is expected to have significant biological activity and values -0.50 to 0.00 are likely to be moderately active. The molecule with a value less than -0.50, is assumed to be inactive.

All compounds followed the Lipinski rule 'rule of five' (lipophilicity, molecular weight, hydrogen bonding, charge, and polar surface area) (Table II). According to this rule, the molecules must have a molecular weight ≤ 500DA, hydrogen bond donors ≤ 5 (expressed as the sum of OHs and NHs) and acceptors ≤ 10 (expressed as the sum of Ns and Os), calculated octanol-water and partition coefficient log P≤ 5 and < 4 number of violations. Three of the compounds, 25-hydroxycholesterol, nortriptyline hydrochloride and retinol showed one violation of the Lipinski rule.

Bioavailability Radar demonstrates the quick evaluation of drug-likeness. The compounds under study had most of the properties stated in the bioavailability radar (Supplemental Table SI).

In silico ADMET analysis: The absorption (A) analysis shows that all the compounds except Losmapimod had high Caco-2 permeability suggesting their absorption by the human intestine. Only nortriptyline hydrochloride, Losmapimod and TAK-715 are substrates for P-glycoprotein.

The Distribution (D) analysis showed that only Flufenamic acid, Retinol, Losmapimod and nortriptyline hydrochloride can cross the blood brain barrier easily. BOILED-Egg for ADMET/Drug-likeness is a precise analytical model used to evaluate numerous points of drug discovery. This model works by evaluating the lipophilicity and polarity of small molecules. The boiled egg model consists of the yolk (i.e., the physicochemical area for very plausible BBB permeation) and the white (i.e., the physicochemical area for very plausible HIA absorption).[39] (Supplemental Table S2). The two most important cytochrome P450s (CYP) subtypes are CYP2D6 and CYP3A4, that are enzyme-systems vital for metabolizing drug in the liver. The metabolism (M) analysis showed that 25-hydroxycholesterol was a non-inhibitor

to CYP2D6 and CYP3A4 whereas tyrphostinb46, retinol, Losmapimod and TAK-715 were inhibitors for both CYP2D6 and CYP3A4. Flufenamic acid and nortriptyline hydrochloride were inhibitors for CYP2D6 and a non-inhibitor of CYP3A4. Piceatannol was an inhibitor for CYP3A4 whereas it was a non-inhibitor to CYP2D6. The results revealed that 25-hydroxycholesterol, piceatannol, Flufenamic acid, nortriptyline hydrochloride, TAK-715, Losmapimod, and tyrphostinb46 non-inhibitors for OATP2B1 and inhibitors for OATP1B1 and OATP1B3, whereas retinoic acid was non-inhibitor for OATP2B1, OATP1B1, and OATP1B3.

Excretion (E) of drugs is linked to their hydrophilicity and molecular weight. In the kidney, organic cation transporters (OCTs) and multi-drug and toxin extrusion proteins (MATE) are the important carriers for the clearance of cationic drugs in the urine. Flufenamic acid, 25-hydroxycholestrol, piceatannol, TAK-715, Losmapimod, retinol and tyrphostinb46 were non-inhibitors to MATE-1 and OCT-2 whereas nortriptyline hydrochloride was non-inhibitor to MATE-1 and inhibitor to OCT-2.

The toxicity (T) analysis revealed that all compounds are non-carcinogenic, non-eye corrosive, and weak inhibitors of Human ether -a -go-go- related gene inhibition. In acute oral toxicity, Flufenamic acid belongs to category II which shows that it is moderately toxic and irritating whereas the remaining compounds fit in category III which might indicate slight toxicity and irritation potential of these compounds (Supplemental Table S3).

DISCUSSION

The present study gives the analysis of eight compounds that have the potential to combat different diseases like rheumatoid arthritis, facioscapulohumeral dystrophy (FSHD), cancer, inflammation, skin conditions, nerve pain, and depression were studied for their probable inhibitory activity against HMG-CoA reductase.

25-hydroxycholesterol: 25-hydroxycholesterol (25HC) is a cholesterol metabolite that binds to 1) a variety of receptors including nuclear receptors LXRs (Liver X receptors), retinoic acid receptor- (RAR-) related orphan receptors (ROR) and the estrogen receptor α (ERα)[9], as well as membrane receptor G protein-coupled receptor 183,[9] 2) a few oxysterols binding proteins, such as the insulin-induced gene protein (INSIG), Niemann-Pick protein (NPC), and 3) steroidogenic acute regulatory-related lipid transfer (START) domain proteins.[9] The lipid-lowering activity of 25HC has been observed in both in vivo and in vitro studies, where 25HC resulted in the reduction in total cholesterol and LDL-C levels, along with the suppression of HMG CoA reductase level.[10,11] Furthermore, a significant increase in the formation of oxysterol sulfate by the overexpression of sulfotransferase 2B1b (SULT2B1b) was observed in mice fed with a high-fat diet, which led to the inhibition of LXRα/SREBP-1c, thus reducing circulating and hepatic lipid levels. However, this phenomenon happens only when there is enough 25HC.[12] The present study revealed that 25 HC effectively binds with HMG CoA reductase and is therefore expected to inhibit the production of cholesterol. Moreover, the physicochemical and ADMET properties also strongly support this compound.

Piceatannol: Piceatannol is a naturally occurring polyhydroxylated compound present in a variety of fruits, and possesses beneficial effects against hyperlipidemia, atherosclerosis, and cancer.[13] It has been shown to significantly reduce LDL-C, TC, and TG levels and then LDL-C/HDL-C ratio in vivo.[14] Our docking analyses showed that piceatannol formed interactions through active site residue Glu^{559} and exhibited strong binding with HMG CoA reductase. Due to the formation of probable interaction and good physical and chemical properties, this study suggests that piceatannol might be a strong candidate for the treatment of hypercholesterolemia.

Flufenamic acid: Flufenamic acid (FFA) is an FDA-approved drug that belongs to the class of nonsteroidal anti-inflammatory drugs (NSAIDs)[15] and is a member of anthranilic acid derivatives. FFA can increase LDL binding, cell association, and degradation in HepG2 cells, by increasing the expression of mRNA of LDLR protein.[16] Our results indicate that FFA is capable of binding with HMG CoA reductase with a significant docking score of (-6.1119), presented good interactions with CoA binding pocket residues, and showed putative ADMET properties. Therefore, this study suggests that Flufenamic acid could be a potential candidate for lowering lipid levels.

Retinol: Retinol is a derivative of vitamin A, that possesses anti-aging and anti-inflammatory activity, however, it does not exert significant biological action on tissues, rather it needs to be converted into more active metabolites particularly retinoic acid to function.[17] Retinoic acid treatment of apoE-deficient mice decreased total and LDL cholesterol and increased HDL cholesterol.[18] In another study, retinoic acid decreased serum cholesterol and triglyceride levels in mice.[19] Because of its antagonistic behavior towards lipid levels and its ability to attain stable conformation with HMG-CoA reductase during molecular dynamic simulation with a lower RMSD points towards the potential of retinol as a promising candidate against hypercholesterolemia.

Nortriptyline hydrochloride: Nortriptyline hydrochloride is a tricyclic antidepressant that inhibits the reuptake of neurotransmitters serotonin and norepinephrine at the presynaptic neuronal membrane. Moreover, it can also inhibit the activity of histamine, 5-hydroxytryptamine, and acetylcholine.[20] Our results revealed that Nortriptyline hydrochloride can bind with HMG CoA reductase with a low docking score (-5.2947). Moreover, the ADMET results revealed that this compound is a non-carcinogenic, non-eye corrosive, weak inhibitor of human ether-a-go-go related genes, has caco2 permeability, and could be absorbed in the human intestine which suggests it is an efficient drug candidate for repurposing against hypercholesterolemia.

Ag555: AG555 is an epidermal growth factor receptor (EGFR) tyrosine kinase inhibitor.[21] The inhibition of EGFR results in reduced protein expression associated with lipogenesis and

cholesterol synthesis.[21] The current study found that Tyrphostinb46 binds HMG-CoA reductase with a low docking score (-7.0207) and interacts with ARG[568] in the CoA binding pocket residue. Furthermore, the physicochemical and ADMET properties of this compound are favorably suggesting tyrphostinb46 to be effective for the management of hypercholesterolemia.

TAK 715 and losapimod: TAK 715 is an inhibitor of p38 mitogen-activated protein kinases (MAPK) and is used as an anti-arthritis agent[22] whereas Losmapimod is a new, orally administered inhibitor of p38 mitogen-activated protein kinase (MAPK) α and β.[3] Data from randomized controlled trials show that Losmapimod treatment resulted in reduced vascular inflammation along with the reduction of inflammatory biomarkers.[24,25] TAK-715 displayed the lowest docking score in this study and maintained a stable conformation through the simulation period with a lesser RMSD whereas Losmapimod showed good ADMET properties. We indicate that the high affinity for binding between HMG-CoA reductase and these two compounds may contribute to the inhibition of cholesterol synthesis.

CONCLUSION

This in-silico study identified various repurposed compounds, 25-hydroxycholesterol, TAK-715, retinol, tyrphostin b46, flufenamic acid, piceatannol, losmapimod, and nortriptyline hydrochloride, as potential inhibitors of HMG-CoA reductase, the key enzyme in cholesterol biosynthesis. Among these, TAK-715 and tyrphostin b46 demonstrated the strongest binding affinity, stable molecular dynamics, and favorable ADMET profiles, highlighting their promise as alternative lipid-lowering agents. While most compounds adhered to drug-likeness criteria and showed low predicted toxicity, the diverse attributes observed across the group suggest varying degrees of efficacy.

These findings support drug repurposing as a cost-effective strategy for identifying novel therapeutic candidates against hypercholesterolemia. Further in-vitro and in-vivo studies are warranted to explore and harness the therapeutic implications of these compounds in biological systems against hypercholesterolemia.

ACKNOWLEDGEMENT

The authors are grateful to Dr Ian Matthews (ChemOvation Ltd) for his intellectual input during conception of the study.

REFERENCES

1. Ahangari N, Mobarhan MG, Sahebkar A, Pasdar A. Molecular aspects of hypercholesterolemia treatment: current perspectives and hopes. Ann Med 2018;50(4):303-11. https://doi.org/10.1080/07853890.2018.1457795
2. Nordestgaard BG, Chapman MJ, Humphries SE, Ginsberg HN, Masana L, Descamps OS, et al. Familial hypercholesterolaemia is underdiagnosed and undertreated in the general population: guidance for clinicians to prevent coronary heart disease. Eur Heart J 2013;34(45):3478-90. https://doi.org/10.1093/eurheartj/eht273
3. Mach F, Baigent C, Catapano AL,Koskinas KC, Casula M, Badimon L, et al. 2019 ESC/EAS guidelines for the management of dyslipidaemias: lipid modification to reduce cardiovascular risk. Eur Heart J 2020;14(1):111-88. https://doi.org/10.1093/eurheartj/ehz455
4. Beltowski J, Wojcicka G, Jamroz-Wisniewska A. Adverse effects of statins - mechanisms and consequences. Curr Drug Saf 2009;4(3):209-28. https://doi.org/10.2174/157488609789006949
5. Ward NC, Watts GF, Eckel RH. Statin toxicity: mechanistic insights and clinical implications. Circ Res 2019;124(2):328-50. https://doi.org/10.1161/CIRCRESAHA.118.312782
6. Rajput TA, Naveed AK, Khan S, Farooqi ZUR. Frequencies of two functionally significant SNPs and their haplotypes of organic anion transporting polypeptide 1B1 SLCO1B1 gene in six ethnic groups of Pakistani population. Iran J Basic Med Sci 2014;17(6):441-7.
7. Tromp TR, Hartgers ML, Hovingh GK, Vallejo-Vaz AJ, Ray KK, Soran H, et al. Worldwide experience of homozygous familial hypercholesterolaemia: retrospective cohort study. Lancet 2022;399(10326):719-28. https://doi.org/10.1016/S0140-6736(21)02001-8
8. Torre D, Krawczuk P, Jagodnik KM, Lachmann A, Wang Z, Wang L, et al. Datasets2Tools, repository and search engine for bioinformatics datasets, tools and canned analyses. Sci Data 2018;5(Dcic):1-10.https://doi.org/10.1038/sdata.2018.23
9. Cao Q, Liu Z, Xiong Y, Zhong Z, Ye Q. Multiple roles of 25-hydroxycholesterol in lipid metabolism, antivirus process, inflammatory response, and cell survival. Oxid Med Cell Longev 2020;2020:8893305. https://doi.org/10.1155/2020/8893305
10. Wu C, Zhao J, Li R, Feng F, He Y, Li Y, et al. Modulation of antiviral immunity and therapeutic efficacy by 25-hydroxycholesterol in chronically SIV-infected, ART-treated rhesus macaques. Virol Sin 2021;36(5):1197-1209. https://doi.org/10.1007/s12250-021-00407-6
11. Liu Y, Wei Z, Zhang Y, Ma X, Chen Y, Yu M, et al. Activation of liver X receptor plays a central role in antiviral actions of 25-hydroxycholesterol. J Lipid Res 2018;59(12):2287-96. https://doi.org/10.1194/jlr.M084558
12. Wang Y, Li X, Ren S. Cholesterol metabolites 25-hydroxycholesterol and 25-hydroxycholesterol 3-sulfate are

potent paired regulators: from discovery to clinical usage. Metabolites 2021;11(1):1-14. https://doi.org/10.3390/metabo11010009

13. Eid BG, Abdel-Naim AB. Piceatannol attenuates testosterone-induced benign prostatic hyperplasia in rats by modulation of Nrf2/HO-1/NFκB axis. Front Pharmacol 2020;11(December):1-11.https://doi.org/10.3389/fphar.2020.614897

14. Tung YC, Lin YH, Chen HJ, Chou S-C, Cheng AC, Kalyanam N, et al. Piceatannol exerts anti-obesity effects in C57BL/6 mice through modulating adipogenic proteins and gut microbiota. Molecules 2016;21(11):1419. https://doi.org/10.3390/molecules21111419

15. Chi Y, Li K, Yan Q, Koizumi S, Shi L, Takahashi S, et al. Nonsteroidal anti-inflammatory drug flufenamic acid is a potent activator of AMP-activated protein kinase. J Pharmacol Exp Ther 2011;339(1):257-66. https://doi.org/10.1124/jpet.111.183020

16. Al Rayyes O, Ahren B, Floren CH. Enhancement of low density lipoprotein catabolism by non-steroidal anti-inflammatory drugs in cultured HepG2 cells. Eur J Pharmacol 1999;372(3):311-8. https://doi.org/10.1016/S0014-2999(99)00246-0

17. Zasada M, Budzisz E. Retinoids: active molecules influencing skin structure formation in cosmetic and dermatological treatments. Postep Dermatologii i Alergol 2019;36(4):392-7. https://doi.org/10.5114/ada.2019.87443

18. Zhou W, Lin J, Chen H, Wang J, Liu Y, Xia M. Retinoic acid induces macrophage cholesterol efflux and inhibits atherosclerotic plaque formation in apoE-deficient mice. Br J Nutr 2015;114(4):509-18. https://doi.org/10.1017/S0007114515002159

19. He Y, Gong L, Fang Y, Zhan Q, Liu HX, Lu Y, et al. The role of retinoic acid in hepatic lipid homeostasis defined by genomic binding and transcriptome profiling. BMC Genomics 2013;14(1):1-11. https://doi.org/10.1186/1471-2164-14-575

20. Merwar G, Gibbons JR, Hosseini SA, Saadabadi A. Nortriptyline. 2023 Jun 5. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing. Availble from URL: https://www.ncbi.nlm.nih.gov/books/NBK482214/

21. Choung S, Kim JM, Joung KH, Lee ES, Kim HJ, Ku BJ. Epidermal growth factor receptor inhibition attenuates non-alcoholic fatty liver disease in diet-induced obese mice. PLoS One 2019;14(2):1-12. https://doi.org/10.1371/journal.pone.0210828

22. Miwatashi S, Arikawa Y, Kotani E, Miyamoto M, Naruo K, Kimura H, et al. Novel inhibitor of p38 MAP kinase as an anti-TNF-α drug: Discovery of N-[4-[2-ethyl-4-(3-methylphenyl)-1,3-thiazol-5-yl]-2-pyridyl]benzamide (TAK-715) as a potent and orally active anti-rheumatoid arthritis agent. J Med Chem 2005;48(19):5966-79. https://doi.org/10.1021/jm050165o

23. Willette RN, Eybye ME, Olzinski AR, Behm DJ, Aiyar N, Maniscalco K, et al. Differential effects of p38 mitogen-activated protein kinase and cyclooxygenase 2 inhibitors in a model of cardiovascular disease. J Pharmacol Exp Ther 2009;330(3):964-70. https://doi.org/10.1124/jpet.109.154443

24. O O'Donoghue ML, Glaser R, C-ave-nder MA, Aylward PE, Bonaca MP, Budaj A, et al. Effect of losmapimod on cardiovascular outcomes in patients hospitalized with acute myocardial infarction: a randomized clinical trial. JAMA 2016;315(15):1591-9. https://doi.org/10.1001/jama.2016.3609

25. Elkhawad M, Rudd JH, Sarov-Blat L, Cai G, Wells R, Davies LC, et al. Effects of p38 mitogen-activated protein kinase inhibition on vascular and systemic inflammation in patients with atherosclerosis. JACC Cardiovasc Imaging 2012;5(9):911-22.https://doi.org/10.1016/j.jcmg.2012.02.016

SUPPLEMENTARY INFORMATION

Supplementary information accompanies this paper at
http://www.kmuj.kmu.edu.pk/article/view/23603

AUTHORS' CONTRIBUTION

The following authors have made substantial contributions to the manuscript as under:

ST, MK, AS, HK & UA: Analysis and interpretation of data, drafting the manuscript, approval of the final version to be published

IJ & QA: Analysis and interpretation of data, critical review, approval of the final version to be published

MIK: Conception, critical review, approval of the final version to be published

FS: Conception and study design, acquisition, analysis and interpretation of data, critical review, approval of the final version to be published

Authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

CONFLICT OF INTEREST

Authors declared no conflict of interest, whether financial or otherwise, that could influence the integrity, objectivity, or validity of their research work.

GRANT SUPPORT AND FINANCIAL DISCLOSURE

Authors declared no specific grant for this research from any funding agency in the public, commercial or non-profit sectors

DATA SHARING STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request

KMUJ web address: www.kmuj.kmu.edu.pk
Email address: kmuj@kmu.edu.pk