COMPUTATIONAL SCREENING, SYNTHESIS, CHARACTERIZATION AND BIOLOGICAL EVALUATION OF NEW BENZOPYRAN DERIVATIVES AS ANTIHYPERLIPIDIMIC MEDIATORS

Bhartendu Sharma[1], Ranjit Singh[1]

1. *Research Scholar, Adarsh Vijendra Institute of Pharmaceutical Sciences (AVIPS), Shobhit University Gangoh (UP)*
2. *Vice Chancellor, Shobhit University Gangoh (UP)*

ABSTRACT

The design, synthesis, and assessment of a unique series of benzopyran derivatives are covered in this study, which is important in a variety of research fields. The crystal structures of PPRP- (PDB ID: 2ZNN) were included in the protein docking investigation for its antihyperlipidemic action.The compounds E2, E3, E5, E6, E14, E15, E17, and E19 have anti-hyperlipidemic effectiveness, according to significant docking scores.3,4-diaminobenzoic acid was employed as the starting material to produce novel benzopyran-3-carbonyl derivatives. 1H-benzotriazole-5-(1,3,4-oxadiazole)-1-[2-oxo-6-(piperidin-1-yl) and -2-thiol(E1-E20) antihyperlipidemic activity proves that Fenofibrate produced the greatest triglyceride and VLDL level reduction at 6 hours. The triglyceride- and VLDL-lowering effects of benzopyran-3-carbonyl derivatives 200 mg/kg at 24 and 48 hours were significantly reduced, and they were comparable to those of fenofibrate and Simvastatin, fenofibrate, and other derivatives. When compared to control, 200 mg/kg resulted in a substantial (P 0.01) rise in blood HDL levels at 6, 24, and 48 hours.The majority of the molecules synthesized in this study may indeed be promising drug candidates with interesting pharmacological profiles, and most of these derivatives could be useful for further development of better antihyperlipidemic agents according to the docking study, ADME report, and in-vivo activity results.

Keywords: In-vivo anti-hyperlipidemic action, Benzopyran, molecular docking study.

INTRODUCTION

The term "Privileged Scaffold in Drug Discovery" has been used to describe benzopyran, which has received substantial recent evaluation. Benzopyran is one of the naturally occurring chemicals with an oxygen moiety. Different biological effects are demonstrated depending on its replacement pattern. Many synthetic and natural compounds, including genistein, hesperidin, and warfarin, include the benzopyran ring system. It exhibits pharmacological characteristics.The biological activity and behaviors of benzopyran derivatives are very diverse. The benzopyran derivatives simultaneously support their use as therapeutic agents for a variety of disorders. The degree of the biological activity appears to be determined by their structural features that are connected with physicochemical aspects.

According to the literature, we focused on the therapeutic activity of benzopyran derivatives which shows broad biological activities, including anti-cancer, antibacterial, anti-diabetic and anti-inflammatory, antitumor, antimicrobial, anticoagulant, antioxidant, anti-spasmolytic, antifungal, antiviral, anti-helminthic, antitubercular, and anticonvulsant.[1-3]

DOCKING STUDY

MATERIALS AND METHODS:

Docking studies were carried out to analyze the different types of biomolecular interactions and ligand receptor binding affinities. The docking studies were carried out by means of Autodock vina, Biovia Discovery Studio 2020, PyRX, and PyMOL. The docking study was performed on protein namely crystal structures of PPRP-α (PDB ID: 2ZNN) for antihyperlipidemic activity The computational work was performed on a HP 15s-eq0132au Laptop running on AMD Ryzen 7 3700U processor.

Protein preparation

The Crystal structures of PPRP-α (PDB ID: 2ZNN) for antihyperlipidemic activity protein was retrieved from the RCSB Protein Data Bank, All the proteins were prepared by removing the other ligands using Swiss PDB viewer, the prepared proteins were saved in PDB format.

Ligand preparation

The 3-D structures of the ligands were drawn using chemsketch and uploaded in BIOVIA Discovery Studio Visualizer-2020. Ligand minimization was done and using small molecule wizard in 'SMALL MOLECULE' wizard in BIOVIA Discovery Studio Visualizer-2023 and was saved as a cluster sdf file.

Docking studies

To reduce false positives and to identify the perfect orientation of ligand within the active site of protein, docking study acquires its importance. Docking was done using PyRx-Virtual Screening Tool. All ligands were converted to pdbqt in PyRx-Virtual Screening Tool and ligands were selected those as ligands in Vina wizard. The prepared proteins were loaded into the PyRx-Virtual Screening Tool and selected it as macro molecule. An amino acid implicated in binding was calculated together with the interaction energy (interaction between ligand and receptor).

Drug Likeliness Studies

The selected phytochemicals were loaded into DruLiTO in sdf format and carried out the test for drug likeliness.

ADME/T Studies

The SMILES of the selected phytochemicals were loaded into Swiss ADME/T and recorded the ADME/T properties of the same. Results are tabulated as below

Compound Code	Binding Affinity
E1	-8.9
E2	-10.5
E3	-10.6
E4	-9.1
E5	-11.5
E6	-9.6
E7	-10.9
E8	-11.7
E9	-10.5
E10	-8.2
E11	-7.6
E12	-10.8
E13	-11.1
E14	-12.9
E15	-9.8
E16	-7.4
E17	-8.3
E18	-8.9
E19	-11.8
E20	-9.8
Atorvastatin	-6.6

Table No 1: Molecular Docking Scores of Selected Compounds with Protein PPRP-α (PDB ID: 2ZNN)

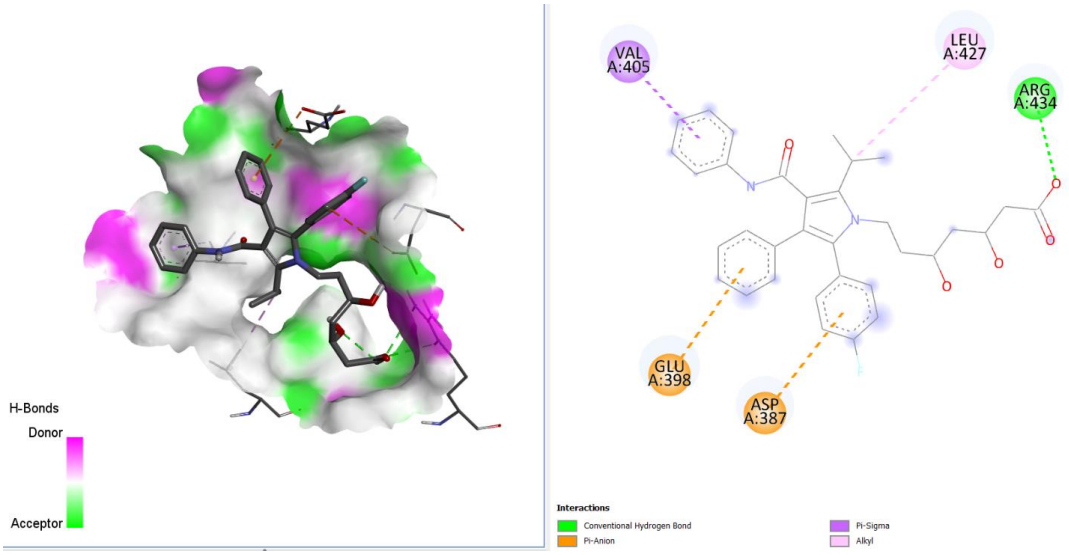


Figure No 1: 3D & 2D interactions of Atorvastatin with 2ZNN

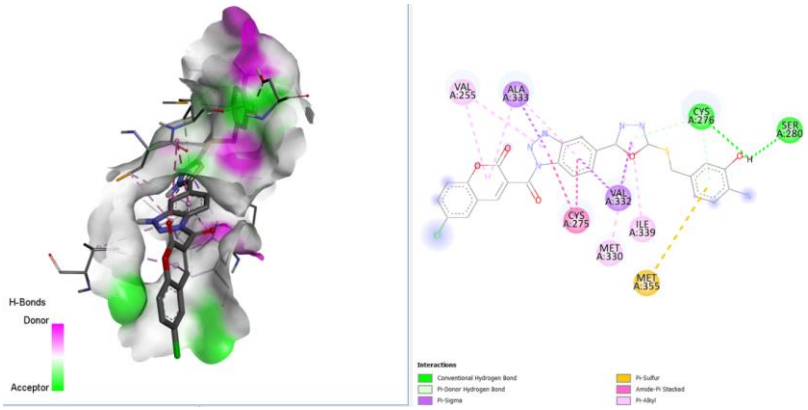


Figure No 2: 3D & 2D interactions of E14 with 2ZNN

Compound Code	Mol.Wt	LogP	Rotatable Bonds	Acceptors	Donors	Lipinki's Violations
E1	517.954	4.5743	5	10	0	1
E2	531.981	4.88272	5	10	0	1
E3	547.98	4.58832	5	11	1	2
E4	566.426	5.53612	5	10	0	2
E5	549.971	5.02182	5	10	0	2
E6	610.877	5.64522	5	10	0	2
E7	612.849	5.0424	5	11	1	2
E8	549.952	3.9855	5	12	2	2
E9	551.943	4.419	5	11	1	2
E10	575.99	4.4825	6	12	1	3
E11	574.018	5.08532	6	11	0	4
E12	559.991	4.7769	6	11	0	3
E13	575.006	4.3591	6	12	1	3
E14	561.023	4.7189	6	11	1	3
E15	546.996	4.46492	5	11	1	2
E16	548.968	3.8621	5	12	2	2
E17	532.969	4.1565	5	11	1	2
E18	598.018	3.821	6	12	1	3
E19	562.951	4.4825	6	12	0	3
E20	576.978	4.79092	6	12	0	3
Atorvastatin	558.65	6.3136	12	5	4	3

Table No 2: reveals the drug likeliness studies have proved that all the synthesised compounds are within the limit and are druggable.

Compound code	Water solubility	$Caco_2$ permeability	Intestinal absorption	Skin Permeability	P-glycoprotein substrate	P-glycoprotein I inhibitor	P-glycoprotein II inhibitor	VDss (human)	Fraction unbound	BBB permeability	CNS permeability
E1	-4.537	0.777	100	-2.736	No	Yes	Yes	0.051	0.101	-1.668	-3.12
E2	-4.625	0.689	100	-2.736	No	Yes	Yes	0.1	0.123	-1.677	-3.06
E3	-4.234	0.764	100	-2.736	No	Yes	Yes	0.171	0.082	-1.749	-3.285
E4	-4.73	0.625	100	-2.736	No	Yes	Yes	0.195	0.125	-1.844	-2.998
E5	-4.275	0.727	100	-2.736	No	Yes	Yes	0.072	0.143	-1.877	-3.186
E6	-4.753	0.627	100	-2.736	No	Yes	Yes	0.214	0.124	-1.852	-2.984
E7	-4.177	0.625	100	-2.736	No	Yes	Yes	0.168	0.14	-1.978	-3.254
E8	-3.89	0.172	94.696	-2.735	Yes	Yes	Yes	0.001	0.076	-1.602	-3.612
E9	-3.889	0.675	100	-2.735	No	Yes	Yes	-0.014	0.159	-1.942	-3.445
E10	-4.147	0.201	99.315	-2.735	No	Yes	Yes	0.018	0.127	-1.955	-3.469
E11	-4.549	0.561	100	-2.736	No	Yes	Yes	0.086	0.112	-1.89	-3.21
E12	-4.483	0.639	100	-2.736	No	Yes	Yes	0.04	0.105	-1.897	-3.271
E13	-4.366	0.202	100	-2.735	No	Yes	Yes	0.043	0.096	-1.907	-3.387
E14	-4.644	0.181	100	-2.736	Yes	Yes	Yes	0.14	0.106	-1.74	-3.147
E15	-4.339	0.239	100	-2.736	Yes	Yes	Yes	0.184	0.116	-1.746	-3.187
E16	-3.979	0.178	95.867	-2.735	Yes	Yes	Yes	0.031	0.042	-1.554	-3.529
E17	-4.249	0.212	100	-2.736	Yes	Yes	Yes	0.062	0.089	-1.754	-3.236
E18	-3.211	-0.146	67.351	-2.735	No	No	Yes	-0.767	0.114	-2.406	-3.72
E19	-4.599	0.035	100	-2.735	No	Yes	Yes	-0.201	0.108	-2.181	-3.252
E20	-4.655	0.094	100	-2.735	No	Yes	Yes	-0.1	0.121	-2.171	-3.202
Atorvastatin	-3.065	0.597	64.049	-2.735	Yes	No	No	-1.698	0.149	-1.468	-2.716

Table No. 3: In-Silico Absorption Studies

Compound code	VDss (human)	Fraction unbound (human)	BBB permeability	CNS permeability
E1	0.051	0.101	-1.668	-3.12
E2	0.1	0.123	-1.677	-3.06
E3	0.171	0.082	-1.749	-3.285
E4	0.195	0.125	-1.844	-2.998
E5	0.072	0.143	-1.877	-3.186
E6	0.214	0.124	-1.852	-2.984
E7	0.168	0.14	-1.978	-3.254
E8	0.001	0.076	-1.602	-3.612
E9	-0.014	0.159	-1.942	-3.445
E10	0.018	0.127	-1.955	-3.469
E11	0.086	0.112	-1.89	-3.21
E12	0.04	0.105	-1.897	-3.271
E13	0.043	0.096	-1.907	-3.387
E14	0.14	0.106	-1.74	-3.147
E15	0.184	0.116	-1.746	-3.187
E16	0.031	0.042	-1.554	-3.529
E17	0.062	0.089	-1.754	-3.236
E18	-0.767	0.114	-2.406	-3.72
E19	-0.201	0.108	-2.181	-3.252
E20	-0.1	0.121	-2.171	-3.202
Atorvastatin	-1.698	0.149	-1.468	-2.716

Table No.4: In-Silico Distribution Studies

Compound code	CYP2D6 substrate	CYP3A4 substrate	CYP1A2 inhibitor	CYP2C9 inhibitor	CYP2C9 inhibitor	CYP2D6 inhibitor	CYP3A4 inhibitor
E1	No	Yes	No	No	Yes	No	Yes
E2	No	Yes	No	No	Yes	No	Yes
E3	No	Yes	No	No	Yes	No	Yes
E4	No	Yes	No	No	Yes	No	No
E5	No	Yes	No	No	Yes	No	Yes
E6	No	Yes	No	No	Yes	No	No
E7	No	Yes	No	Yes	Yes	No	Yes
E8	No	Yes	No	No	Yes	No	Yes

E9	No	Yes	No	No	Yes	No	Yes
E10	No	Yes	No	No	Yes	No	Yes
E11	No	Yes	No	No	Yes	No	Yes
E12	No	Yes	No	No	Yes	No	Yes
E13	No	Yes	No	No	Yes	No	Yes
E14	No	Yes	No	No	Yes	No	Yes
E15	No	Yes	No	No	Yes	No	Yes
E16	No	Yes	No	No	Yes	No	Yes
E17	No	Yes	No	No	Yes	No	Yes
E18	No	Yes	No	No	No	No	No
E19	No	Yes	No	No	Yes	No	Yes
E20	No	Yes	No	No	Yes	No	Yes
Atorvastatin	Yes	Yes	No	No	Yes	No	No

Table No 5: In-Silico Metabolism Studies

Compound code	Total Clearance	Renal OCT2 substrate
E1	-0.152	No
E2	-0.209	No
E3	-0.308	No
E4	-0.328	No
E5	-0.34	No
E6	-0.349	No
E7	-0.388	No
E8	-0.276	No
E9	-0.307	No
E10	-0.256	No
E11	-0.385	No
E12	-0.279	No
E13	-0.452	No
E14	-0.407	No
E15	-0.425	No
E16	-0.296	No
E17	-0.384	No
E18	-0.257	No

E19	-0.133	No
E20	-0.237	No
Atorvastatin	0.247	No

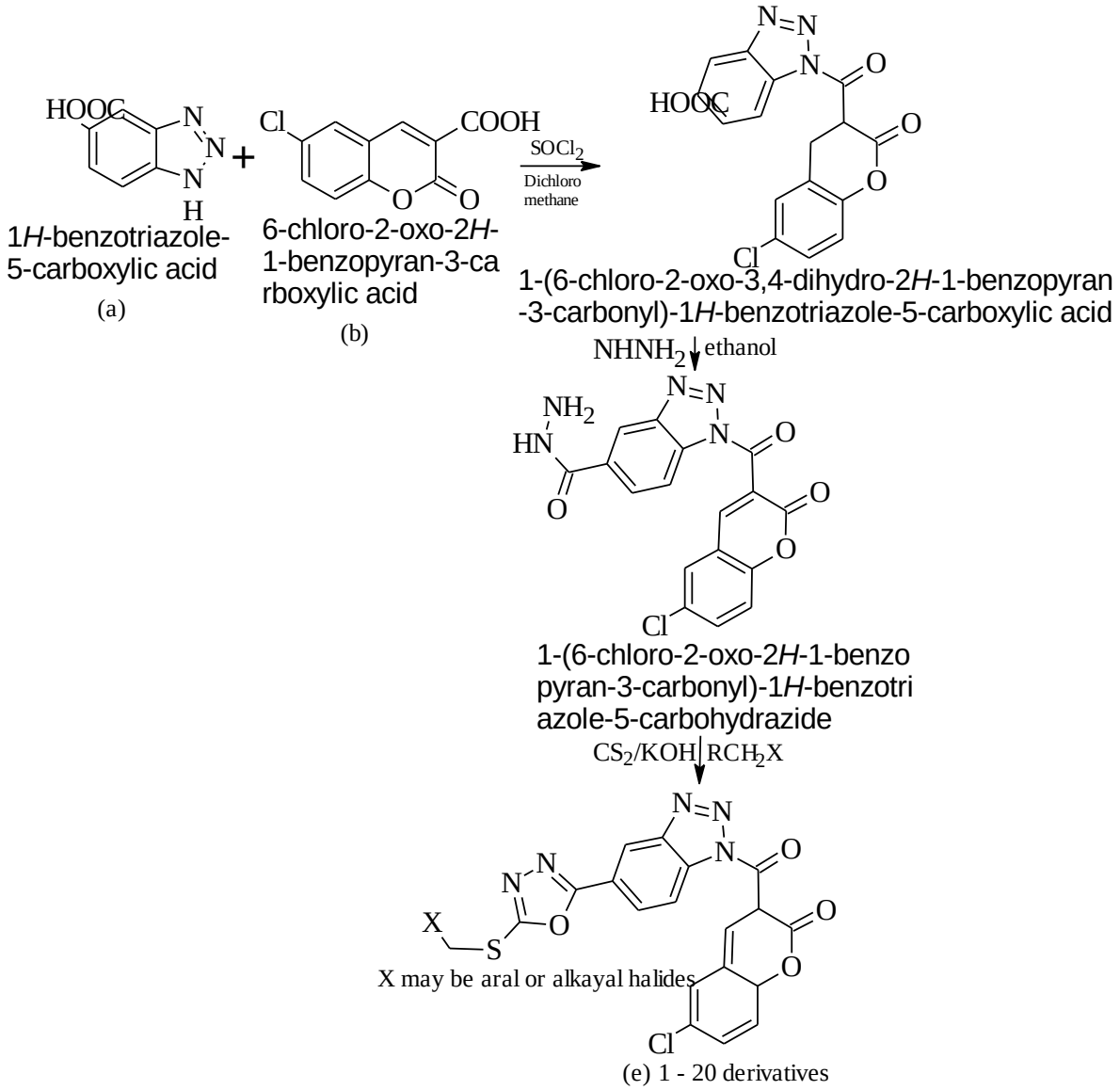
Table No. 6: In-Silico Excretion Studies

Compound code	AMES toxicity	Max. tolerated dose (human)	hERG I inhibitor	hERG II inhibitor	Oral Rat Acute Toxicity (LD50)	Oral Rat Chronic	Hepatotoxicity	Skin T.Pyriformis toxicity	Minnow toxicity
E1	No	0.503	No	Yes	2.551	0.581	Yes	No	0.285	-2.012
E2	No	0.407	No	Yes	2.611	0.622	Yes	No	0.285	-2.014
E3	No	0.267	No	Yes	2.731	0.651	Yes	No	0.285	-1.802
E4	No	0.362	No	Yes	2.681	0.424	Yes	No	0.285	-2.811
E5	No	0.453	No	Yes	2.527	0.564	Yes	No	0.285	-2.176
E6	No	0.364	No	Yes	2.685	0.413	Yes	No	0.285	-2.957
E7	No	0.539	No	Yes	2.664	0.43	No	No	0.285	-2.112
E8	No	0.437	No	Yes	2.574	2.732	Yes	No	0.285	-1.141
E9	No	0.578	No	Yes	2.465	0.661	Yes	No	0.285	-1.515
E10	No	0.536	No	Yes	2.602	0.629	Yes	No	0.285	-1.467
E11	No	0.374	No	Yes	2.634	0.627	Yes	No	0.285	-2.411
E12	No	0.435	No	Yes	2.576	0.583	Yes	No	0.285	-2.409
E13	No	0.445	No	Yes	2.622	0.534	Yes	No	0.285	-1.612
E14	No	0.46	No	Yes	2.67	0.541	Yes	N	0.285	-1.993

								o		
E15	No	0.445	No	Yes	2.668	0.562	Yes	No	0.285	-1.795
E16	No	0.384	No	Yes	2.673	2.563	Yes	No	0.285	-1.003
E17	No	0.321	No	Yes	2.692	0.533	Yes	No	0.285	-1.215
E18	No	0.96	No	No	2.572	0.463	Yes	No	0.285	-1.896
E19	Yes	0.324	No	Yes	2.494	0.564	Yes	No	0.285	-3.036
E20	No	0.243	No	Yes	2.512	0.606	Yes	No	0.285	-3.652
Atorvastatin	No	0.412	No	No	2.495	3.353	Yes	No	0.285	-2.975

Table No. 7: In-Silico Toxicity Studies

SCHEME



MATERIALS AND METHODS

All the chemicals used were procured from Sigma Aldrich, Merck and CDH laboratory chemical suppliers and purity of starting materials used for reactions was confirmed by checking their melting point or boiling point and by thin layer chromatography (TLC).

Step-I Preparation of 1*H*-benzotriazole-5-carboxylic acid (a)

Dissolve 1.3g of 3,4-diaminobenzoic acid in a mixture of 1.5ml of hydrochloric acid and 5ml water in a beaker. Stir until the solid dissolves, warm gently if necessary. Cool the solution to 15˚C. Stir well simultaneously add a solution of 2g sodium nitrite in 2ml water. Reaction mixture become warm within 2-3 minutes and reaches a temperature of about 85˚C and then begins to cool. Colour changes from deep red to pale brown. Continue stirring for 15 minutes till the temperature falls between 35-40˚C. Let the mixture to chill in ice bath for 30 minutes. Filter the product and wash it with cold water

it gives 1*H*-benzotriazole-5-carboxylic acid (a). The crude product was recrystallized from aqueous ethanol. The purity of the product was confirmed by a single spot-on TLC.

Step-II Preparation of 6-chloro-2-oxo-2*H*-1-benzopyran-3-carboxylic acid (b)

A mixture of 5-chloro-2-hydroxybenzaldehyde (1 mmol), 2,2-dimethyl-1,3-dioxane-4,6-dione (1 mmol), and 25ml ethanol and pyridine (20 mol%) was stirred at 110°C for a 15 min. The progress of the reaction was monitored by using TLC. After completion of the reaction gives 6-chloro-2-oxo-2*H*-1-benzopyran-3-carboxylic acid (b), the solid was washed thoroughly with water, and finally purified by recrystallizing in ethanol. The purity of the product was confirmed by a single spot-on TLC.

Step-III Preparation of 1-(6-chloro-2-oxo-3,4-dihydro-2*H*-1-benzopyran-3-carbonyl)-1*H*-benzotriazole-5-carboxylic acid (c)

Reflux the mixture of 1*H*-benzotriazole-5-carboxylic acid (a) (0.01mol) with 6-chloro-2-oxo-2*H*-1-benzopyran-3-carboxylic acid (b) (0.01mol) with mixture of 6ml of thionyl chloride and 6ml of dichloro methane for 8 hours. The progress of the reaction was monitored by using TLC. After completion of the reaction, gives 1-(6-chloro-2-oxo-3, 4-dihydro-2*H*-1-benzopyran-3-carbonyl)-1*H*-benzotriazole-5-carboxylic acid (c), the crude product was recrystallized with aqueous ethanol. The purity of the product was confirmed by a single spot-on TLC.

Step-IV Preparation of Derivatives of compound 1-(6-chloro-2-oxo-2*H*-1-benzopyran-3-carbonyl)-1*H*-benzotriazole-5-carbohydrazide (d)

To the solution of 1-(6-chloro-2-oxo-3,4-dihydro-2*H*-1-benzopyran-3-carbonyl)-1*H*-benzotriazole-5-carboxylic acid (c) (6gm, 0.01mol) in 15ml of ethanol, 99% hydrazine hydrate (1.94ml, 0.03mol) was added and the reaction mixture was refluxed on water bath for 4hrs. After cooling, the precipitate was filtered off, washed with water and dried under vacuum (60^{o} C) to this a mixture of 0.01mole aromatic aldehyde was added and few drops of glacial acetic acid in 30 ml ethanol was further added refluxed for 5 hours, the residue was stirred with 50 ml ice cold water and filtered off, and dried under vacuum to obtain derivatives of compound 1-(6-chloro-2-oxo-2*H*-1-benzopyran-3-carbonyl)-1*H*-benzotriazole-5-carbohydrazide (d). The purity of the product was confirmed by a single spot-on TLC.

Step-V Preparation of Derivatives of compound 1-(6-chloro-2-oxo-2*H*-1-benzopyran-3-carbonyl)-1*H*-benzotriazole-5-(1,3,4-oxadiazole)-2-thiol (E1-E20)

A mixture of 1-(6-chloro-2-oxo-2*H*-1-benzopyran-3-carbonyl)-1*H*-benzotriazole-5-carbohydrazide (0.01mol)10ml and 0.6 ml carbondisulphide was added in a solution of KOH 0.56 gm in 50 ml water then 50 ml ethanol was refluxed on water bath for about 3-4 hours then the reaction mixture was acidified with conc. HCL the solid product was filtered and washedwith water and dried under vacuum (50° C) to this substituted aromatic or aliphatic halides with few drops of glacial acetic acid was added and refluxed for 4-5hrs to obtain derivatives of compound 1-(6-chloro-2-oxo-2*H*-1-benzopyran-3-carbonyl)-1*H*-benzotriazole-5-(1,3,4-oxadiazole)-2-thiol (E1-E20). The crude product

was recrystallized using 50% aqueous ethanol. The purityof the product was confirmed by a single spot-on TLC plate.

IR Spectra of compound E6 is 3150 cm^{-1} N-H Stretch Of 1^0 amine, 3000-3150 cm^{-1} N-H stretch 2° or 3° amine, 2900-2950 cm^{-1} Aromatic C-H Stretch, 2400 cm^{-1} Aliphatic -C-H Stretch, 1500, 1570, 1615 cm^{-1} C = O Stretch, 700-750 cm^{-1} -CH, CH_3, CH_2 Stretch 950 cm^{-1} -C-S stretch and 850 cm^{-1} -X stretch. ^{1}HNMR Spectra in δ is 1.161, 1.501, 1.518, 2.442, 2.952-$(CH3)_2$, CH(7H), 6.5-8.1 Ar-H(multiplate). ^{13}CNMR 1C –$CH_2$28.96, 1C $CH_3$54.27 and 22C from Aromatic ring 120-155 and2C from C=O 166.80, 167.42 and M^+ Peaks (Mass Peak)at m/z 541.4 and Base Peak is 610.2

BIOLOGICAL EVALUATION

Pharmacology

The animals used in the examination were sheltered in analogy of the School of Pharmacy and Emerging Sciences, Baddi University of Emerging Sciences & Technology, Solan , Himachal Pradesh, which follows the guidelines and regulation set by the Committee for the Control and Administration of Experiments on Animals (CPCSEA), Ministry of Social Justice and Empowerment, Government of India. The studies were attempted with previous approval from the Institutional Animal Ethics committee (IAEC) and ultimate care was taken to establish that the animals were handling in the most kind and satisfactory manner. Albino male/female Wistar rats of either sex weighing between 200 - 220gm were used.

IAECPermission

The permission of Institutional Animal Ethics Committee (IAEC), duly constituted as per CPCSEA guidelines was obtained from School of Pharmacy and Emerging Sciences, Baddi University of Emerging Sciences & Technology, Solan, Himachal Pradesh for the study. The permission letter is enclosed.

Acutetoxicity studies

Acute oral toxicity studies of benzopyran-3-carbonyl derivatives were carried out in male Albino Wistar rats using the limit test or main test (Up and down procedure) as per OECD guideline (425).Derivatives were dissolved in DMSO and a single oral dose of 200 mg/kg was used for limit test in the overnight fasted animals. The animals were observed continuously for first 6 hrs after dosing and thereafter for 14 days for toxicity signs, morbidity and mortality. The dose for main test was selected from the default progression factor on the basis of onset, duration and severity of toxic sign, morbidity and time of death in limit test. When the main test was performed, the high dose at which animal showed mortality and low dose at which animal survived were used to calculate LD_{50} by using AOT software.

No signs of lethality or morbidity were detected in the rats given different doses up to 5000 mg/kg of benzopyran-3-carbonyl derivatives for two weeks. Therefore; the median lethal dose (LD_{50}) of derivatives was higher than 5000mg/kg for all benzopyran-3-carbonyl derivatives.

Anti-hyperlipdemic activity

Chemicals

Triton WR-1339, AGAPPE diagnostic kits, phosphotungstate and magnesium acetate reagent.

Method

Albino male Wistar rats weighing between 150gm to 200gm were assigned to various groups of six animals each. Animals were fasted for 16 h prior to the experiment with water *ad libitum*. The various benzopyran-3-carbonyl derivatives each at doses of 200 mg/kg body weight, simvastatin at 4 mg/kg and fenofibrate at 20 mg/kg, were administered p.o. to groups II to VI, respectively. Group I served as control. On the day of the experiment, the animals of the groups II-IV received the respective drugs by oral route. Simultaneously, all the animals received Triton WR-1339 at 100 mg/kg body weight by intraperitoneal route. The control animals were given only Triton WR-1339 at 100 mg/kg body weight. Serum cholesterol, triglyceride, and high density lipoproteins were estimated at 6, 24, and 48 h using AGAPPE diagnostic kits. Blood samples were withdrawn by retro-orbital puncture. Total cholesterol was estimated by CHOD-PAP methodology, Triglycerides by GPO-PAP methodology, and HDL by the precipitation method using phosphotungstate magnesium acetate reagent.

VLDL was calculated using the formula,

$$VLDL = \frac{Triglycerides}{5}$$

LDL cholesterol was calculated as,

$$LDL = Total\ cholestrol - HDL - \frac{Triglycrides}{5}$$

***Table No 8: effects of* benzopyran-3-carbonyl derivativeson total cholesterol and low-density lipids levels in triton-induced hyperlipidemic rats**

Compounds	*6hrs*		24hrs		48hrs	
	Serum cholesterol(mg/dl)	*Serum LDL(mg/dl)*	*Serum cholesterol(mg/dl)*	*Serum LDL(mg/dl)*	*Serum cholesterol(mg/dl)*	*Serum LDL(mg/dl)*
Control	*110.26±0.25*	*101.45±0.36*	*92.45±1.02*	*76.41±0.34*	*84.12±0.10*	*65.25±0.28*
E2	**90.40±0.62**	**52.71±0.95**	**74.56±0.52**	**51.39±1.16**	**67.85±1.78**	**47.58±0.27**
E3	**91.20±0.67**	**55.42±0.46**	**69.74±0.63**	**52.79±1.25**	**60.38±1.68**	**50.36±0.98**
E5	**84.36±0.78**	**58.36±1.55**	**66.35±0.45**	**55.63±0.34**	**58.96±1.34**	**52.75±0.43**
E6	**80.55±0.92**	**59.12±1.35**	**71.32±1.24**	**57.86±0.85**	**64.23±0.98**	**55.63±1.02**
E14	**72.53±1.25**	**48.64±2.12**	**65.94±1.34**	**36.52±0.75**	**56.64±0.78**	**35.64±1.34**
E15	**88.41±1.45**	**45.69±1.75**	**69.28±1.62**	**44.89±0.36**	**62.58±0.45**	**40.25±1.78**
E17	**94.13±0.22**	**46.95±1.68**	**71.64±1.74**	**45.28±0.45**	**61.37±0.76**	**42.36±1.85**
E19	**90.34±1.37**	**64.13±1.75**	**77.85±1.29**	**55.78±0.75**	**48.56±0.97**	**54.78±0.75**
Simvastatin	**34.74±0.60**	**38.40±1.64**	**70.28±1.45**	**35.46±1.25**	**45.46±0.70**	**34.52±0.96**
Fenofibrate	**36.85±0.45**	**39.47±1.37**	**66.85±1.60**	**38.72±1.46**	**45.12±0.68**	**42.95±0.85**

Values are expressed as mean ± S.D. (n = 6). Cholesterol and LDL concentrations are estimated by the standard method and the values are expressed as mg/dl serum. $P < 0.05$, $P < 0.01$, $P < 0.001$, when compared with the control group; Simvastatin; Fenofibrate

Table No 9: Effects ofbenzopyran-3-carbonyl derivatives on total triglyceride and very low-density lipids levels in triton-induced hyperlipidemic rats

Compounds	*6hrs*		24hrs		48hrs	
	Serum Triglyceride(mg/dl)	***Serum VLDL(mg/dl)***	***Serum Triglyceride(mg/dl))***	***Serum VLDL(mg/dl)***	***Serum Triglyceride(mg/dl)***	***Serum VLDL(mg/dl)***
Control	***72.15±1.34***	***14.12±0.42***	***68.75±0.55***	***13.58±0.10***	***64.72±0.35***	***11.46±0.32***
E3	**63.50±0.67**	**16.75±0.45**	**52.47±0.46**	**13.95±1.25**	**60.38±1.68**	**12.25±0.45**
E5	**64.52±1.15**	**19.67±0.60**	**56.34±0.59**	**17.46±1.40**	**58.96±1.34**	**15.60±0.60**
E6	**68.75±0.48**	**18.74±0.69**	**60.85±0.46**	**16.72±1.22**	**64.23±0.98**	**14.28±1.22**
E14	**72.36±1.34**	**20.41±0.52**	**66.74±1.04**	**16.23±0.20**	**56.64±0.78**	**11.08±1.74**
E15	**47.46±0.46**	**15.80±1.15**	**42.25±1.13**	**14.76±0.41**	**41.63±0.40**	**12.55±1.795**
E17	**71.46±0.28**	**17.20±1.41**	**61.28±1.20**	**15.90±0.35**	**54.85±0.25**	**13.25±1.31**
E19	**64.76±1.08**	**14.13±1.37**	**55.76±0.49**	**13.28±0.65**	**45.72±0.25**	**12.68±0.55**
Simvastatin	**40.75±0.10**	**12.10±0.24**	**35.46±1.10**	**14.46±0.05**	**24.45±2.50**	**12.55±0.45**
Fenofibrate	**37.85±0.05**	**11.68±1.25**	**32.91±1.24**	**10.64±0.16**	**20.55±3.80**	**10.02±0.74**

Values are expressed as mean ± S.D. (n = 6). Triglyceride and VLDL concentrations are estimated by the standard method and the values are expressed as mg/dl serum; $P < 0.05$, $P < 0.01$, $P < 0.001$, when compared with the control group; Simvastatin; Fenofibrate;

Table No 10.3Effects of benzopyran-3-carbonyl derivatives on high-density lipid levels in triton-induced hyperlipidemic rats

Compounds	*Serum HDL (mg/dl)*		
	6hrs	***24hrs***	***48hrs***
Control	***42.58±0.25***	***37.62±0.40***	***31.74±0.60***
E2	**43.64±0.30**	**38.69±0.20**	**29.41±0.65**
E3	**44.10±0.55**	**37.56±0.35**	**28.52±0.40**
E5	**43.67±0.40**	**34.55±0.60**	**25.74±0.50**
E6	**45.71±0.56**	**35.95±0.45**	**26.82±0.45**
E14	**34.20±0.64**	**30.43±0.50**	**22.46±0.75**
E15	**44.37±0.57**	**34.59±0.60**	**26.60±0.46**
E17	**42.78±0.68**	**35.86±0.40**	**27.85±0.45**
E19	**38.56±0.08**	**37.62±0.64**	**27.60±0.40**
Simvastatin	**39.46±0.20**	**30.05±0.55**	**20.35±0.34**
Fenofibrate	**38.46±0.15**	**29.46±0.71**	**19.55±0.70**

Values are expressed as mean ± S.D. (n = 6). HDL concentrations are estimated by the standard method and the values are expressed as mg/dl serum; *P* < 0.05; *P* < 0.01; *P* < 0.001 when compared with the control group; Simvastatin; Fenofibrate;

Hyperlipidemia is one of the important risk factors involved in the development of cardiovascular diseases. Atherosclerosis and congestive heart diseases are strongly associated with disorders of lipid metabolism and plasma lipoproteins. Triton WR-1339-treated rats are considered to be a useful acute hyperlipidemic model associated with inactive lipoprotein lipase. Triton WR-1339 acts as a surfactant to block the uptake of lipoprotein from the circulation by extra hepatic tissues resulting in an increase in the level of circulatory lipoproteins. Triton WR-1339-induced hyperlipidemic rats treated with benzopyran-3-carbonyl derivatives produced reversal of increase in serum cholesterol and triglycerides and LDL from the 6 h up to 48 h and VLDL from 24 h.

Increase in triglyceride level was evident in control animals due to inhibition of lipoprotein lipase (LPL) by Triton. Treatment with benzopyran-3-carbonyl derivatives resulted in reduction of triglyceride levels and lowered the serum triglyceride level by activating LPL. LPL is a prime enzyme related to triglyceride metabolism. Further VLDL levels were reduced significantly at 24 and 48 h.

CONCLUSION

The docking study, ADME report, and in-vivo activity results strongly suggest that most of molecules synthesized in this study may indeed be promising drug candidates with interesting pharmacological profile and most of these derivatives could be a fruitful for further development of better anti-hyperlipidimic activity.

Acknowledgment: We are thankful to the principal, management and colleagues for their support and guidance.

Conflict of Interest: The authors states that no conflict of interests

Financial Support: Self support

REFERENCE

1) *Natarajan kiruthiga, thangaveluprabha, chellappaselvinthanuja, kulandaivelsrinivasan, thangavelsivakumar , synthesis, biological evaluation, and docking study of novel 2-phenyl-1benzopyran-4-one derivatives - as a potent cyclooxygenase-2 inhibitor Asian Journal of Pharmaceutical and Clinical Research, 2019; 12 (3):304-10,*
2) *Koppula PK, Purohit N. Bis-(1 H-2-benzopyran-1-one) derivatives: Synthesis and antimicrobial evaluation. Journal of Chemical Sciences. 2013 Nov; 125:1535-42.*
3) *Miky JA, Sharaf HH. Synthesis and biological activities of 5H-furo [3, 2-g] [1] benzopyran-5-one derivatives. Indian Journal of Chemistry 1998, Vol. 37B, January pp. 68 – 72*
4) *Kaur A, Sharma V, Budhiraja A, Kaur H, Gupta V, Kant R, Ishar MP. Synthesis and evaluation of substituted 4, 4a-Dihydro-3H, 10H-pyrano [4, 3-b][1] benzopyran-10-one as antimicrobial agent. International Scholarly Research Notices. 2013; 2013.*
5) *Zhang Q, Wei H, Li J, Zhao X, Luo J. One-pot synthesis of benzopyrans catalyzed by silica supported dual acidic ionic liquid under solvent-free conditions. Heterocyclic Communications. 2017 Dec 1;23(6):411-4.*
6) *Wang S, Zhu S, Tanzeng Y, Zhang Y, Li C, Ma M, Lu W. Design, Synthesis, and Evaluation of Near-Infrared Fluorescent Molecules Based on 4H-1-Benzopyran Core. Molecules. 2021 Jan;26(22):6986.*
7) *Du F, Zhou Q, Fu X, Shi Y, Chen Y, Fang W, Yang J, Chen G. Synthesis and biological evaluation of 2, 2-dimethylbenzopyran derivatives as potent neuroprotection agents. RSC advances. 2019;9(5):2498-508.*
8) *Som S, Jayaveera KN. Synthesis and evaluation of antibacterial activity of some Benzopyran-4-one derivatives. Asian Journal of Research in Chemistry. 2012;5(3):414-7.*
9) *Ahmed HA. Synthesis of some novel benzopyranes derivatives and evaluation their biological activity. SYNTHESIS. 2015;8(4).*

10) *Kumar P, Rahman MA, Wal P, Rawat P, Singh K. Design, Synthesis, and Anticancer Evaluation of Novel Benzopyran 1, 3, 4-oxadiazole Derivatives. Indian J. Heterocyclic Chemistry. 2020 Jul 1; 30:395-402.*

11) *Vawhal PK, Jadhav SB. Design, Synthesis, and Biological Evaluation of 3-Chloro-2-Oxo-N-(Arylcarbamoyl)-2H-1-Benzopyran-6-Sulfonamide Derivatives as Potential DPP-IV Inhibitors. Int. J. Health Sci. 2022; 6:373-92.*

12) *Kaushik R, Chand M, Rashid M, Jain SC. Synthesis of novel 2-acetamidothiazoles tethered with 1, 2, 3triazole and pyridine pharmacophores. Heteroatom Chemistry. 2018 Jul; 29(4):e21447.*

13) *Thumar NJ, Patel MP. Synthesis and in vitro antimicrobial evaluation of 4H-pyrazolopyran,-benzopyran and naphthopyran derivatives of 1H-pyrazole. Arkivoc. 2009 Jan 1; 13:363-80.*

14) *Shailesh K, Devdutt C, Devi Prasad S. Synthesis and Antimicrobial Activity of Novel 3,7-Disubstituted 2H-1- Benzopyran-2-Ones. , Med Chem (Los Angeles) 2017, 7:4; 857-864*

15) *Naik MD, Bodke YD, Kumar AA, Naik JK, Shastri SL. Synthesis, characterization and biological evaluation of novel series of 2-(benzylamino)-2-oxoethyl]-2-oxo-2H-1-benzopyran-3-carboxamide derivatives. Indian Journal of Chemistry. 2020, 59:B; 406-419*

16) *Hoti R, Vehapi I, Mulliqi Osmani G, Ismaili H, VEPRIM T. Synthesis of some New Heteroarylamino-3-nitro-2H-[1]-Benzopyran-2-ones and their antibacterial activity. American Scientific Research Journal for Engineering, Technology, and Sciences. 2016;22(1):1-9.*

17) *Hoti R, Troni N, Vehapi I, Mulliqi-Osmani G, Ismaili H, Thaçi V. Synthesis of some novel derivatives of substituted 2H-[1]-benzopyran-2-ones and their antimicrobial activity. Journal of Chemistry. 2016 Dec;4(2):45-59.*

18) *Joseph M. 4 methyl benzyl amine substituted benzopyran, antimicrobial activity. The Pharma Innovation Journal 2018; 7(12): 01-04*