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In-Silico Study, Synthesis and Pharmacological Screening of Novel Benzopyran-3-Carbonyl Derivatives as Antidiabetic Agents

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KEYWORDS

Anti-diabetic activity, Molecular docking studies, Benzopyran

ABSTRACT:

Introduction: In the current work, in-silico docking investigation for 60 benzopyran-3-carbonyl derivatives was conducted since benzopyran derivatives play a vital role in many different research disciplines. Since benzopyran derivatives are important in many different study fields, an in-silico docking analysis for 60 benzopyrancarbonyl derivatives was carried out in the current work. A docking study was carried out to investigate the antidiabetic efficacy of the protein α-amylase (PDB ID: 1OSE) crystal structure. Significant docking scores exhibits anti-diabetic action from D1, D4, D10, D12, D13, D16 and D19. The starting material for the synthesis of novel benzopyran-3-carbonyl derivatives was 3,4-diaminobenzoic acid resulting in a final product like 1-(6-chloro-2-oxo-2H-1-benzopyran-3-carbonyl)-1H-benzotriazole-5-carbohydrazide (D1–D20). At 100 g/ml, the benzopyran-3-carbonyl derivatives exhibited 24.67% (D10) α-amylase inhibitory activity, with an IC50 value of 72.55 g/ml. Benzopyran-3-carbonyl derivatives significantly inhibited α-amylase activity when compared to acarbose. The results show moderate to poor antihyperglycemic action in STZ-induced diabetic rats, with benzopyran-3-carbonyl derivatives showing notably lower blood glucose AUC. The most significant change between the standard and benzopyran-3-carbonyl derivatives was a 24-hour drop in blood glucose of 155 mg/dl.

1. Introduction

Because of its lipophilicity, the benzopyran derivative exhibits assisting penetration character. Research indicates that one important chemical synthon that functioned as a bioactive agent was the variety of substituted benzopyrans. Bioactive benzopyran derivatives are necessary to stimulate the immunity of the living system and are used as a treatment agent for a variety of disorders an oxygen atom with carbons in a heterocyclic ring is referred to as a "pyran." "Benzopyran" is a hetero-ring fused with a benzene ring. The majority of synthetic and natural pharmacological building blocks are pyran heterocyclic molecules[1].

2. Material and Methods

PyRX, PyMOL, Biovia Discovery Studio 2020, and Autodock vina were used for the docking research.

A protein docking study was conducted for antidiabetic efficacy, specifically focusing on the crystal structures of α-amylase (PDB ID: 1OSE). Using an AMD Ryzen 7 3700U CPU, an HP 15s-eq0132au laptop was used to execute the computational job.

Protein preparation

The RCSB Protein Data Bank provided the crystal structures of α-amylase (PDB ID: 1OSE), which has antidiabetic effect. The proteins were processed by eliminating any additional ligands using the Swiss PDB viewer, and then stored in PDB format[2-3.]

Ligand preparation

Chemsketch was used to create the ligands' three-dimensional structures, which were then uploaded into BIOVIA Discovery Studio Visualizer-2020. Ligand minimization was completed and saved as a cluster sdf file using the BIOVIA Discovery Studio Visualizer-2020's "SMALL MOLECULE" procedure.

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Docking studies

The significance of docking studies increases in order to minimise false positives and determine the ideal ligand orientation within the protein's active site. PyRx-Virtual Screening Tool was used for the docking process. Using PyRx-Virtual Screening Tool, convert all ligands to pdbqt, then pick them as the ligands in the Vina wizard. the synthesised proteins were loaded and chosen as a macro molecule in the PyRx-Virtual Screening Tool. The calculation of the binding amino acid involved in the interaction energy (interaction between the ligand and receptor) was done.

Drug Likeliness Studies

DruLiTO was used to load the suggested phytochemicals in sdf file and perform the drug likeliness test.

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 in Table. 1.

Table.1: List of compounds and their binding affinity

Compound	Binding Affinity
D1	-10.5
D2	-9.2
D3	-9.4
D4	-10.7
D5	-10.4
D6	-10.2
D7	-10.3
D8	-10.4
D9	-10.4
D10	-10.7
D11	-10.7
D12	-11.1
D13	-9.4
D14	-10.8
D15	-10.6
D16	-11
D17	-8.1
D18	-10.2
D19	-9.9
D20	-10.4
Glimepiride	-9.6

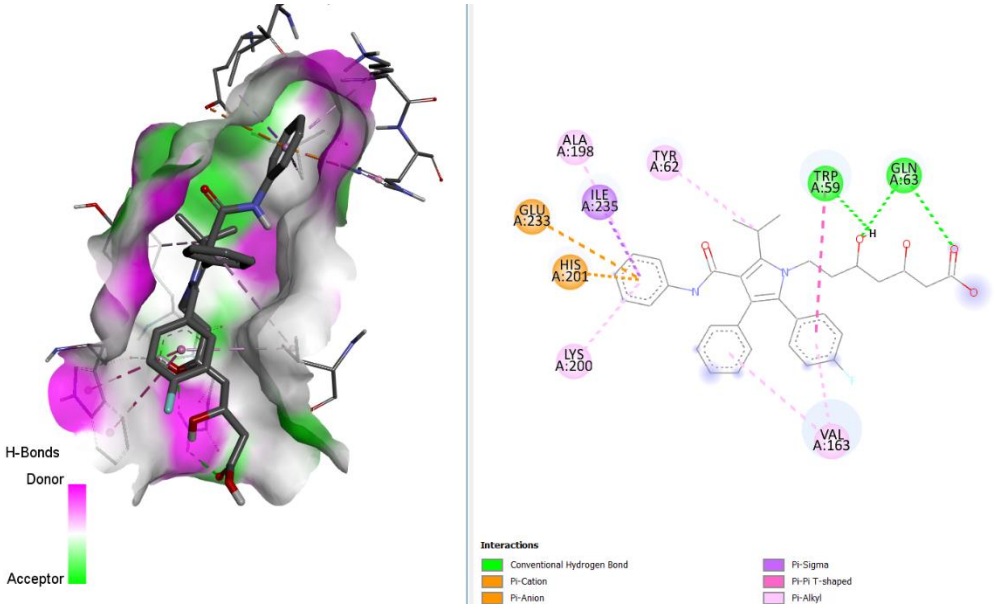

Fig. 1: 3D & 2D interactions of Compounds with Protein α-amylase (PDB ID: 1OSE)

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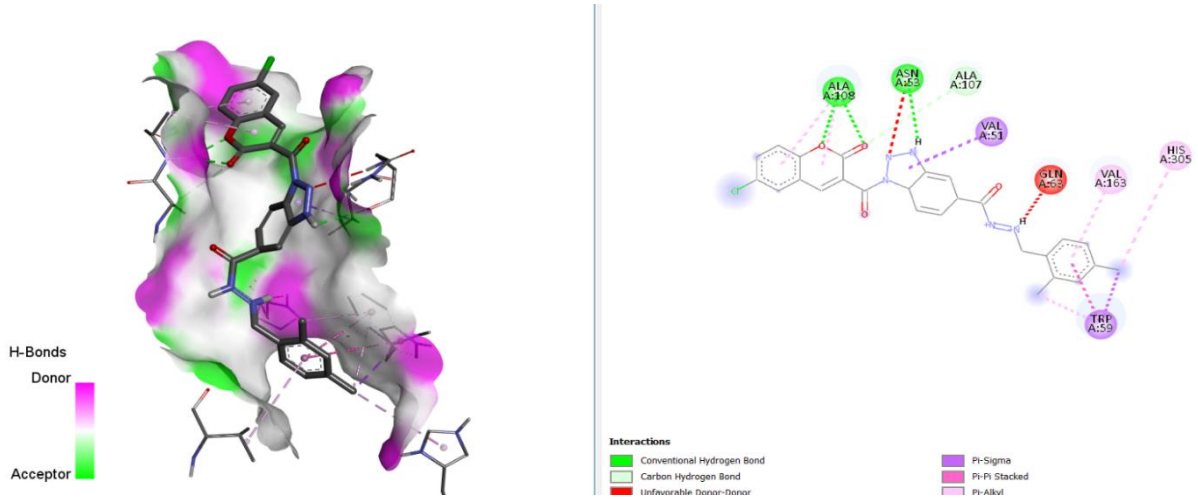

Fig. 2: 3D & 2D interactions of D10 with 1OSE

Table.2: Drug Likeliness Studies

Compound Code	Mol.Wt	LogP	Rotatable Bonds	Acceptors	Donors	Lipinki's Violations
D1	471.86	3.6435	4	8	1	0
D2	487.859	3.3491	4	9	2	0
D3	501.886	3.65752	4	9	2	1
D4	522.304	4.0025	4	9	2	1
D5	536.331	4.3055	5	9	1	1
D6	501.886	3.6521	5	9	1	1
D7	486.875	3.2257	4	9	2	1
D8	502.874	2.9313	4	10	3	1
D9	500.902	3.53412	4	9	2	1
D10	499.914	4.26034	4	8	1	0
D11	503.877	4.09102	4	8	1	1
D12	489.85	3.7826	4	8	1	0
D13	504.865	3.3648	4	9	2	1
D14	534.847	3.6908	5	10	1	1
D15	551.302	4.2051	5	10	1	1
D16	595.753	4.3142	5	10	1	1
D17	516.857	3.5517	5	10	1	1
D18	532.856	3.2573	5	11	2	1
D19	530.884	3.86012	5	10	1	1
D20	531.872	3.1339	5	11	2	1
Glimepride	490.626	3.074	7	5	3	0

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Table.3: In-Silico Absorption Studies

Compound code	Water solubility	Caco2 permeability	Intestinal absorption (human)	Skin Permeability	P-glycoprotein substrate	P-glycoprotein I inhibitor	P-glycoprotein II inhibitor	VDss (human)	Fraction unbound (human)	BBB permeability	CNS permeability
D1	-4.94	0.368	100	-2.734	Yes	Yes	Yes	-0.255	0.173	-1.012	-2.106
D2	-4.09	-0.077	94.511	-2.738	Yes	Yes	Yes	-0.417	0.177	-1.175	-2.952
D3	-4.108	-0.089	94.979	-2.738	Yes	Yes	Yes	-0.314	0.149	-1.143	-2.913
D4	-4.112	-0.109	95.687	-2.737	Yes	Yes	Yes	-0.314	0.143	-1.318	-2.901
D5	-4.843	0.27	100	-2.735	Yes	Yes	Yes	-0.146	0.179	-1.388	-2.867
D6	-4.797	0.292	99.102	-2.735	Yes	Yes	Yes	-0.232	0.199	-1.25	-2.92
D7	-4.071	-0.054	95.681	-2.74	Yes	Yes	Yes	-0.402	0.169	-1.127	-2.271
D8	-3.619	-0.204	87.24	-2.736	Yes	Yes	Yes	-0.702	0.085	-1.709	-3.144
D9	-4.11	-0.054	96.083	-2.74	Yes	Yes	Yes	-0.35	0.166	-1.104	-2.185
D10	-5.033	0.28	100	-2.734	Yes	Yes	Yes	-0.154	0.192	-1.014	-1.949
D11	-4.904	0.322	100	-2.734	Yes	Yes	Yes	-0.224	0.216	-1.222	-2.778
D12	-4.836	0.319	100	-2.734	Yes	Yes	Yes	-0.297	0.218	-1.246	-2.85
D13	-4.085	-0.034	93.9	-2.738	Yes	Yes	Yes	-0.424	0.159	-1.201	-2.987
D14	-4.815	-0.192	97.975	-2.735	Yes	Yes	Yes	-0.635	0.231	-1.659	-2.978
D15	-4.767	-0.216	100	-2.735	Yes	Yes	Yes	-0.405	0.193	-1.626	-2.227
D16	-4.782	-0.221	100	-2.735	Yes	Yes	Yes	-0.39	0.192	-1.634	-2.204
D17	-4.801	-0.263	99.521	-2.735	Yes	Yes	Yes	-0.461	0.179	-1.438	-2.336
D18	-3.877	-0.293	91.477	-2.735	Yes	Yes	Yes	-0.692	0.18	-1.518	-3.068
D19	-4.803	-0.196	99.996	-2.735	Yes	Yes	Yes	-0.409	0.197	-1.451	-2.267
D20	-3.712	-0.291	92.447	-2.735	Yes	Yes	Yes	-0.568	0.162	-1.47	-2.491

Table.4: In-Silico Distribution Studies

Compound code	VDss (human)	Fraction unbound (human)	BBB permeability	CNS permeability
D1	-0.255	0.173	-1.012	-2.106
D2	-0.417	0.177	-1.175	-2.952
D3	-0.314	0.149	-1.143	-2.913
D4	-0.314	0.143	-1.318	-2.901
D5	-0.146	0.179	-1.388	-2.867
D6	-0.232	0.199	-1.25	-2.92
D7	-0.402	0.169	-1.127	-2.271
D8	-0.702	0.085	-1.709	-3.144
D9	-0.35	0.166	-1.104	-2.185
D10	-0.154	0.192	-1.014	-1.949
D11	-0.224	0.216	-1.222	-2.778

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D12	-0.297	0.218	-1.246	-2.85
D13	-0.424	0.159	-1.201	-2.987
D14	-0.635	0.231	-1.659	-2.978
D15	-0.405	0.193	-1.626	-2.227
D16	-0.39	0.192	-1.634	-2.204
D17	-0.461	0.179	-1.438	-2.336
D18	-0.692	0.18	-1.518	-3.068
D19	-0.409	0.197	-1.451	-2.267
D20	-0.568	0.162	-1.47	-2.491

Table. 5: In-Silico Metabolism Studies

Compound code	CYP2D6 substrate	CYP3A4 substrate	CYP1A2 inhibitor	CYP2C19 inhibitor	CYP2C9 inhibitor	CYP2D6 inhibitor	CYP3A4 inhibitor
D1	No	Yes	No	Yes	Yes	No	Yes
D2	No	Yes	Yes	Yes	Yes	No	Yes
D3	No	Yes	Yes	Yes	Yes	No	Yes
D4	No	Yes	Yes	Yes	Yes	No	Yes
D5	No	Yes	No	Yes	Yes	No	Yes
D6	No	Yes	No	Yes	Yes	No	Yes
D7	No	Yes	Yes	Yes	Yes	No	Yes
D8	No	Yes	Yes	Yes	Yes	No	Yes
D9	No	Yes	Yes	Yes	Yes	No	Yes
D10	No	Yes	No	Yes	Yes	No	Yes
D11	No	Yes	No	Yes	Yes	No	Yes
D12	No	Yes	No	Yes	Yes	No	Yes
D13	No	Yes	Yes	Yes	Yes	No	Yes
D14	No	Yes	No	Yes	Yes	No	Yes
D15	No	Yes	No	Yes	Yes	No	Yes
D16	No	Yes	No	Yes	Yes	No	Yes
D17	No	Yes	No	Yes	Yes	No	Yes
D18	No	Yes	Yes	Yes	Yes	No	Yes
D19	No	Yes	No	Yes	Yes	No	Yes
D20	No	Yes	Yes	Yes	Yes	No	Yes

Table. 6: In-Silico Excretion Studies

Compound code	Total Clearance	Renal OCT2 substrate
D1	-0.274	No
D2	-0.501	No
D3	-0.544	No
D4	-0.385	No
D5	-0.237	No
D6	-0.352	No

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D7	-0.506	No
D8	-0.439	No
D9	-0.604	No
D10	-0.43	No
D11	-0.516	No
D12	-0.417	No
D13	-0.448	No
D14	-0.359	No
D15	-0.261	No
D16	-0.381	No
D17	-0.195	No
D18	-0.328	No
D19	-0.247	No
D20	-0.358	No

Table. 7: In-Silico Toxicity Studies

Compound code	AMES toxicity	Max. tolerated dose	hERG I inhibitor	hERG II inhibitor	Oral Rat Acute Toxicity	Oral Rat Chronic Toxicity (LOAEL)	Hepatotoxicity	Skin Sensitisatio	T.Pyriformis toxicity	Minnow toxicity
D1	No	0.402	No	Yes	2.817	1.244	Yes	No	0.288	-1.174
D2	No	0.442	No	Yes	2.867	2.466	Yes	No	0.286	-0.698
D3	No	0.331	No	Yes	2.951	2.366	Yes	No	0.286	-0.825
D4	No	0.341	No	Yes	2.917	2.413	Yes	No	0.286	-1.043
D5	No	0.305	No	Yes	2.947	-0.008	Yes	No	0.286	-1.721
D6	No	0.331	No	Yes	2.937	0.206	Yes	No	0.286	-1.485
D7	No	0.411	No	Yes	2.766	2.686	Yes	No	0.286	-0.395
D8	Yes	0.379	No	Yes	2.643	3.818	Yes	No	0.285	-0.186
D9	Yes	0.415	No	Yes	2.761	2.571	Yes	No	0.286	-0.628
D10	No	0.305	No	Yes	2.845	1.191	Yes	No	0.288	-1.427
D11	No	0.359	No	Yes	2.876	0.203	Yes	No	0.287	-1.317
D12	No	0.353	No	Yes	2.894	0.17	Yes	No	0.286	-1.368
D13	No	0.347	No	Yes	2.829	2.77	Yes	No	0.285	-0.655
D14	Yes	0.2	No	Yes	2.749	0.019	Yes	No	0.285	-1.208
D15	Yes	0.153	No	Yes	2.88	1.516	Yes	No	0.285	-1.377
D16	Yes	0.155	No	Yes	2.886	1.488	Yes	No	0.285	-1.523
D17	Yes	0.227	No	Yes	2.802	1.626	Yes	No	0.286	-0.994
D18	Yes	0.322	No	Yes	2.496	3.104	Yes	No	0.285	-0.661
D19	Yes	0.146	No	Yes	2.866	1.642	Yes	No	0.286	-1.159
D20	Yes	0.31	No	Yes	2.535	3.322	Yes	No	0.285	-0.484

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3. Synthesis

Thin layer chromatography and melting/boiling point measurements were performed to verify the purity of the starting materials employed in the reactions. All the chemicals were produced by Sigma Aldrich, Merck, and CDH laboratory chemical suppliers[4-7].

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

Melt 1.5 ml of hydrochloric acid and 5 ml of water in a beaker and dissolved in a beaker containing 1.3 g of 3,4-diaminobenzoic acid. Stir until the solid dissipates and if needed, reheat slightly. Thaw the mixture to 15˚C. Add a solution of 2g sodium nitrite in 2ml water and stir thoroughly. In two to three minutes, the reaction mixture warms up to a temperature of about 85˚C before starting to cool. Pale brown replaces the strong red colour. Continue to stir for 15 minutes till the temperature drops about 35-40˚C. 1H-benzotriazole-5-carboxylic acid (a) is obtained by thoroughly chilling the product in an ice bath for 30 minutes, filtering it, and then washing it with cold water. From aqueous ethanol, the crude product was recrystallized. A single spot-on TLC revealed the product's purity.

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

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 with water, and finally purified by recrystallization in ethanol. The purity of the product was confirmed by a single spot-on TLC.

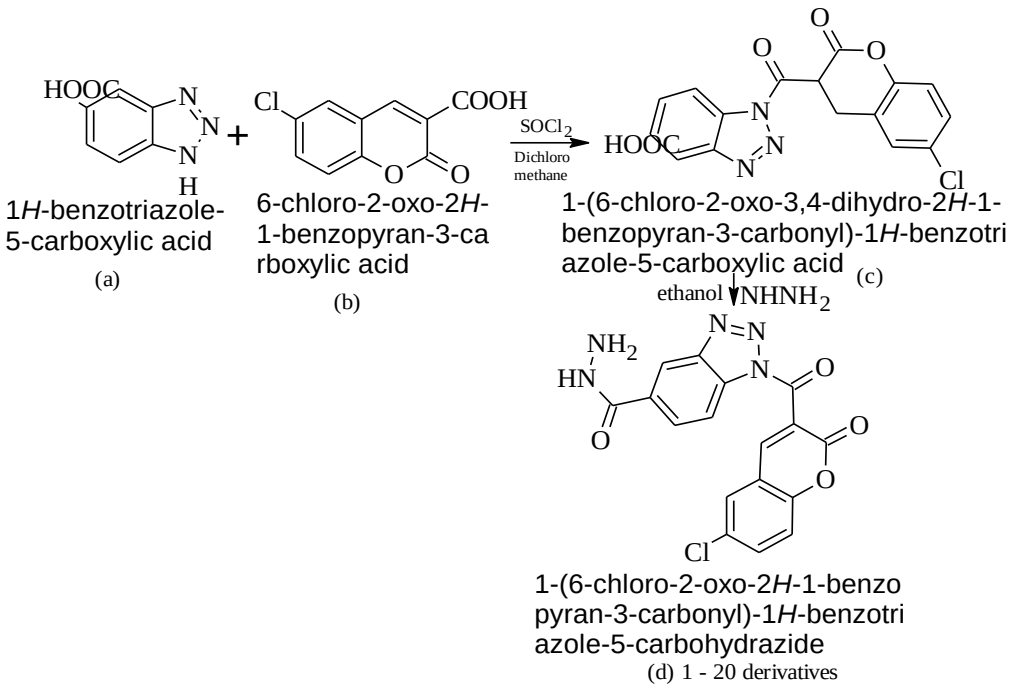

Fig. 3: Synthetic scheme of 1-(6-Chloro-2-Oxo-2H-1-benzopyran-3-carbonyl)-1H-benzotriazole-5-carbohydrazide

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

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 from

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aqueous ethanol. The purity of the product was confirmed by a single spot-on TLC.

(d) Step-IV Preparation of Derivatives of compound 1-(6-chloro-2-oxo-2*H*-1-benzopyran-3-carbonyl)-1*H*-benzotriazole-5-carbohydrazide (D1-D20)

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 C °) to this a mixture of 0.01mole aromatic aldehyde and few drops of glacial acetic acid in ethanol 30 ml refluxed for 5 hours, the residue was stirred with ice cold water 50 ml 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 (D1-D20). The purity of the product was confirmed by a single spot-on TLC. IR Spectra of compound D10 is 2950-3050 N-H cm^{-1}Stretch Of 1^0 amine, 2900-2950cm^{-1} N-H stretch 2° or 3° amine, 2900-2850cm^{-1} Aromatic C-H Stretch, 2700 cm^{-1}Aliphatic -C-H Stretch, 1450, 1500, 1595 cm^{-1}C = O Stretch, 830cm^{-1} -CH, CH_3, CH_2 Stretch 955cm^{-1}. ^{1}HNMR Spectra in δ is 3.783 -NH (1H), 0.932, 1.170, 1.511, 1.529, 2.745, 2.780, 3.27-$(CH3)_2$, CH(7H), 6.5-8.5 Ar-H (multiplate). ^{13}CNMR 2C –CH_3 22.51 37.30, 1C CH 13.47 20C from Aromatic ring 90-130 3C from C=O 148.26 151.10 155.17 and M^+ Peaks (Mass Peak) at m/z 467.3 and Base Peak is 499.90.

In vitro α-Amylase Inhibitory Assay

The assay was carried out following the standard protocol with slight modifications. Starch azure (2 mg) was suspended in 0.2 ml of 0.5M Tris–HCl buffer (pH 6.9) containing 0.01 M $CaCl_2$ (substrate solution). The tubes containing substrate solution were boiled for 5 min and then pre-incubated at 37°C for 5 min. benzopyran-3-carbonyl derivatives were dissolved in DMSO in order to obtain concentrations of 10, 20, 40, 60, 80, and 100µg/ml. Then, 0.2 ml of benzopyran-3-carbonyl derivatives of particular concentration was added to the tube containing the substrate solution. In addition, 0.1 ml of porcine pancreatic amylase in Tris–HCl buffer (2units/ml) was added to the tube containing the benzopyran-3-carbonyl derivatives and substrate solution. The reaction was carried out at 37°C for 10 min. The reaction was stopped by adding 0.5 ml of 50% acetic acid in each tube. The reaction mixture was centrifuged at 3000 rpm for 5 min at 4°C. The absorbance of resulting supernatant was measured at 590 nm using spectrophotometer (Perkin Elmer Lambda 25 UV–VIS spectrophotometer). Same procedure was followed for all the fifteen derivatives to test their α-amylase inhibitory effects. Acarbose, a known α-amylase inhibitor was used as a standard drug. The experiments were repeated thrice. The α-amylase inhibitory activity was calculated by using following formula: [8-11]

The α-amylase inhibitory activity = (Ac +) − (Ac −) − (As − Ab)/ (Ac +) − (Ac −) × 100

***In-Vivo* Anti-diabetic activity Streptozotocin (STZ)-induced diabetes Animals**

About 3–4-week-old male albino wistar rats with body weight 160 ± 20 g were procured. All the rats were kept in animal housing facility by maintaining the standard conditions of optimum temperature (22 °C), relative humidity and a 12 h light/dark cycle with free access to diet and water unless stated otherwise. All rats were acclimatized for 4 days in laboratory conditions before start of experiment.

STZ was dissolved in Citrate buffer (0.1 M) and injected intraperitoneally in overnight fasted 80 Albino Wistar rats of body weight 160 ± 20 g at the dose of 60 mg/kg body weight. After 48 h of injection, fasting blood glucose level of animals was measured from cut tail vain using glucometer (Accu-Chek). Animals exhibiting fasting blood glucose level between 300-450 mg/dl were considered diabetic and further divided into desired experimental groups containing 8 animals per group. Each group received oral doses of fine suspension of test samples prepared in 1% gum acacia at a dose of 250 mg/kg of benzopyran-3-carbonyl derivatives. Metformin was used as standard antidiabetic drugs at a dose of 250 mg/kg body weight. During experiments blood of animals were measured at time interval of 30, 60, 90, 120, 180, 240, 300 min and at 1440 min posttest sample/standard drug or vehicle treatment. To determine the percentage blood glucose lowering by test substance or standard drug Blood glucose level (mg/dl) vs time (min) were plotted on Graph Pad Prism. Area under the curve (AUC) between 0-300 min and 0-1440 min was calculated from the graph and by comparing the AUC of test substance

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treated/standard drug treated groups to show the improvement in hyperglycemia.[12-14]

4. Results

In vitro α-Amylase Inhibitory Assay

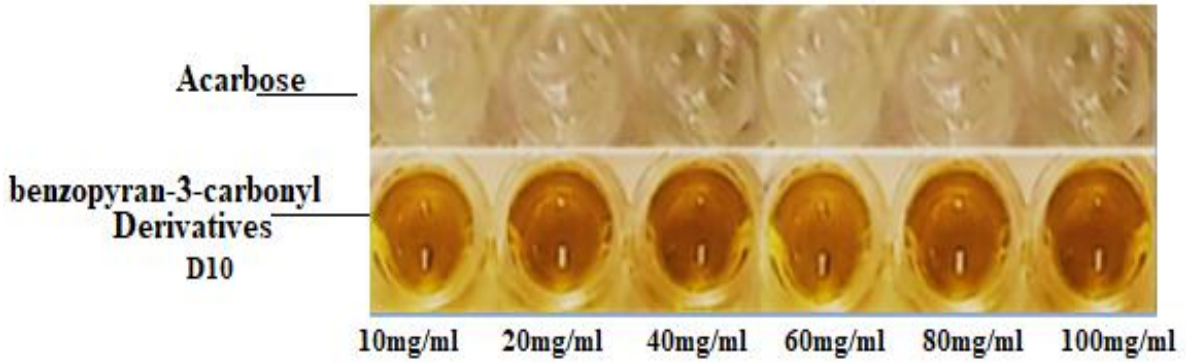

Fig. 4: α-amylase inhibitory activity

Where Ac+, Ac−, As, and Ab are defined as the absorbance of 100% enzyme activity (only solvent with enzyme), 0% enzyme activity (only solvent without enzyme), a test sample (with enzyme), and a blank (a test sample without enzyme), respectively. The concentration of acarbose and benzopyran-3-carbonyl derivatives required to inhibit 50% of α-amylase activity under the conditions was defined as the IC_{50} value. The α-amylase inhibitory activities of benzopyran-3-carbonyl derivatives and acarbose were calculated, and its IC_{50} values were determined. All values were expressed mean ± SD. Statistical difference and linear regression analysis were performed using Graphpad prism 5 statistical software.

Acarbose (at a concentrations 100µg/ml) showed 42.58% inhibitory effects on the α-amylase activity with an IC_{50} value 84.36±1.52µg/ml. The benzopyran-3-carbonyl derivatives(at a concentration 100µg/ml) exhibited 24.67% (D10) of α-amylase inhibitory activity with an IC_{50} values 72.55±1.50µg/ml. the benzopyran-3-carbonyl derivatives showed appreciable α-amylase inhibitory effects when compared with acarbose as shown in table no 8 and figure no 4.

Table.8: Alpha-amylase inhibitory effects of benzopyran-3-carbonyl derivatives and standard acarbose (standard α-amylase inhibitor)

Compounds	*% of Inhibition at different Concentration (µg/mL) of various*benzopyran-3-carbonyl derivatives						*IC_{50} value (µg/mL)*
	10	20	40	60	80	100	
D1	2.54	5.48	12.45	20.42	26.74	33.45	45.21±2.41
D4	2.41	5.10	11.85	19.46	25.34	32.46	39.56±1.85
D10	1.90	3.58	10.46	16.74	21.89	24.67	72.55±1.50
D12	2.16	5.10	11.35	18.37	23.62	29.87	44.28±2.10
D13	2.98	6.18	14.10	20.97	24.75	29.34	55.40±1.65

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D16	2.75	5.94	12.75	18.64	23.80	28.75	48.85±2.75
D19	3.15	6.52	14.09	20.43	24.10	30.46	59.64±2.50
Standard Acarbose	4.58	9.12	20.34	29.74	36.74	42.58	84.36±1.52

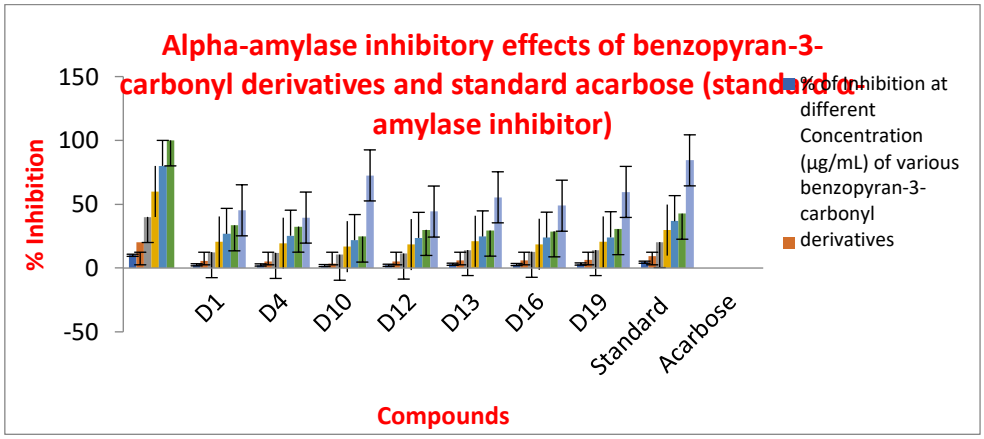

Fig.5: Alpha-amylase inhibitory effects of benzopyran-3-carbonyl derivatives and standard acarbose (standard α-amylase inhibitor).

Fig. 6: Measurement of blood glucose level through tail vein

A pool of β-cell destruction in STZ-induced diabetic rats results into severe insulin deficiency followed by elevation in fasting blood glucose level beyond the normal value. All the animals were treated with benzopyran-3-carbonyl derivatives. Results show moderate to low antihyperglycemic activity in blood glucose AUC in single dose animal experiments except significant lowering with benzopyran-3-carbonyl derivatives. The most significant effect shown by benzopyran-3-carbonyl derivatives reduced blood glucose level at 24hrs, 155 mg/dl as compared with standard.

Table No 9: Anti hyperglycemic activity of benzopyran-3-carbonyl derivatives on STZ-induced diabetic rats at the dose of 250 mg/kg).

Compounds	Glucose Value in mg/dl 24hrs					
	0 min	*5hrs*	*10hrs*	*15hrs*	*20hrs*	*24hrs*
Control	**435±20**	**422±15**	**415±10**	**410±06**	**410±05**	**408±05**
D1	**436±19**	**410±05**	**365±06**	**325±10**	**240±15**	**180±18**
D4	**433±12**	**390±10**	**345±12**	**290±08**	**212±10**	**166±05**
D10	**434±10**	**400±10**	**362±08**	**280±22**	**214±10**	**155±12**
D12	**435±14**	**370±15**	**335±10**	**295±12**	**255±15**	**184±10**

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D13	**435±15**	**385±13**	**320±12**	**274±10**	**234±10**	**170±10**
D16	**432±12**	**405±12**	**370±13**	**295±15**	**240±10**	**210±12**
D19	**434±15**	**400±13**	**384±15**	**310±12**	**264±12**	**225±12**
Metformin	**433±12**	**210±10**	**160±10**	**146±10**	**140±10**	**115±10**

Table.10: AUC (Area under curve) values showing the effect of benzopyran-3-carbonyl derivativeson 18 h fasted STZ-induced diabetic rats over a period of 5 h comparing with the standard anti-diabetic drugs (metformin). AUC values are presented as mean ± SD and significance was calculated as comparison to control group.

Compounds	**Blood Glucose AUC**	
	0 min	***5hrs***
Control	1.5462	1.5123
D1	0.9856	0.8542
D4	0.7854	0.6524
D10	0.7958	0.6246
D12	0.8742	0.8123
D13	0.8861	0.8261
D16	0.9541	0.8795
D19	0.8956	0.8245
Metformin	0.7828	0.4562

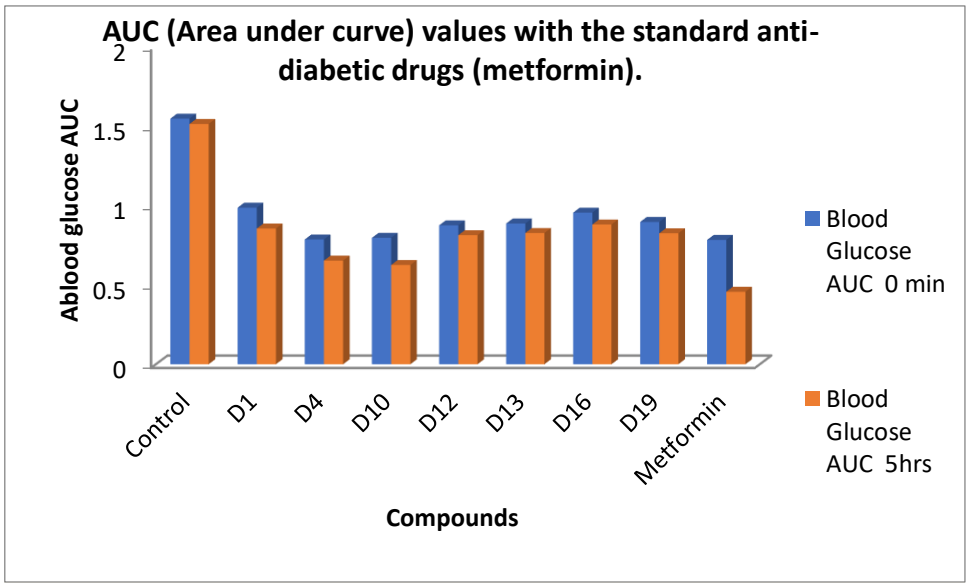

Fig.7: AUC (Area under curve) values showing the effect of benzopyran-3-carbonyl derivatives on 18 h fasted STZ-induced diabetic rats over a period of 5 h comparing with the standard anti-diabetic drugs (metformin). AUC values are presented as mean ± SD and significance was calculated as comparison to control group.

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In conclusion, our study revealed that the synthesized novel derivatives of benzopyran-3-carbonyl derivatives exhibited significant lowering of blood sugar in STZ induced albino wistar rats. Among them, *D10*and F6 possesses strong *in vitro* as well as *in vivo* antidiabetic effects which may be responsible for their hypoglycemic property.

5. Discussion

The docking study, *in vitro* 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-diabetic activity.

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