Synthesis, Molecular Docking And Anti-Diabetic Effect Of Oxazepine Clubbed Indole Derivatives On Alpha Glucosidase Enzyme

Section A-Research Paper

SYNTHESIS, MOLECULAR DOCKING AND ANTI-DIABETIC EFFECT OF OXAZEPINE CLUBBED INDOLE DERIVATIVES ON ALPHA GLUCOSIDASE ENZYME

Vijayshri Rokde[1*], Mohini Sihare[1]

Abstract:

In this investigation, we investigated the inhibitory effects of several indole-based compounds on the activity of intestinal and pancreatic alpha-glucosidase. It appears that antidiabetic medications must contain enzyme inhibitors that block the breakdown of carbohydrates. All analogues had good to moderate inhibitory interactions with alpha-glucosidase (IC50 = 3.08 to 7.37) compared to conventional acarbose (IC50 = 12.18). The activity potential for both enzyme inhibitory interactions and the analogues **3, 4, 6, 10, 12 and 16** were good. In order to suggest the impact of substituents on the inhibitory potential of analogues, structure activity relationships were carefully considered. Additional possible analogues and the enzyme active site interacted, according to docking studies.

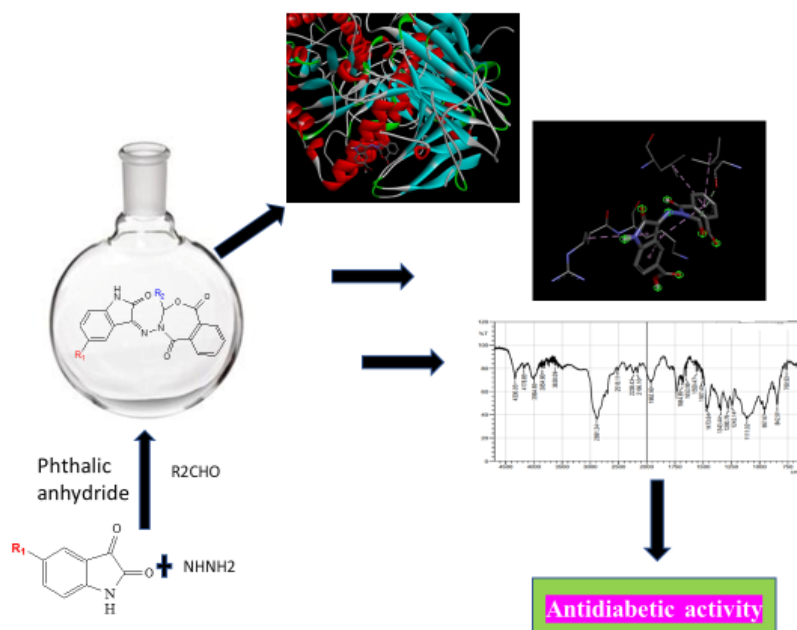**Keywords:** Pancreatic α-amylase, α-glucosidase, anti-diabetic, Indole, Benzopyrrole

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Graphical abstract

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1. INTRODUCTION

Hyperglycemia, particularly type 2 diabetes mellitus (T2DM), is a long-term metabolic disorder characterized by high blood glucose levels. This may cause due to insufficient insulin production or insulin resistance. Globally, it was found that in 2017, 425 million persons suffer from it and by 2040 it will be expected to increase up to 642 million. It is characterized by vascular disorders, diabetic eye disease, diabetic nephropathy, and nervous system disorders, and it is a significant risk factor for the onset of diabetes. These problems are indications of heart failure, seizure, and other chronic problems [1, 2].

Indole, also known as 2, 3 benzo-pyrrole, is a bicyclic heterocyclic moiety with the formula C_8H_7N, it is pervasive and features a six-membered aromatic ring tethered to a five-membered N having pyrrole ring and is used as a crucial building block system in medicinal chemistry. It contains 10 π electrons and hence follows the aromaticity rule that Huckels rules of aromaticity [3]. The enzymes alpha-amylase and alpha-glucosidase are responsible for hydrolyzing the carbohydrate into blood glucose. The pancreas and salivary glands release enzymes such alpha-glucosidase, which decrease the absorption of glucose from the small intestine by hydrolyzing the oligosaccharides into simple sugar. The inhibitors of alpha-amylase and alpha-glucosidase impede the process of absorbing glucose, leading to a reduction in postprandial blood glucose levels. This therapy strategy is believed to be effective in managing diabetes. Limiting glucose absorption by blocking this enzyme are the most widely used method of treating diabetes [4,5]. Starch, malt oligosaccharide, and maltodextrins are all hydrolyzed by the alpha-amylase enzyme, which releases glucose molecules in the process [6, 7]. Alpha-glucosidase catalyzes the hydrolytic process in this way to release glucose molecules from the carbohydrates [8, 9]. Examples of alpha-glucosidase and alpha-amylase inhibitors that reduce the incidence of type 2 diabetes include acarbose, miglitol and voglibose. Along with their potent therapeutic effects and rapid onset of action, oral diabetes medicines also have unfavourable side effects. Their main flaw is that their mode of action often alleviates the symptoms of diabetes rather than treating its underlying pathogenesis. In order to manage type 2 diabetes with minimal side effects, novel active ingredients need to be found [10, 11]. Indoles play a significant role in medicinal chemistry and are now recognized as a valuable source of synthons for the production of pharmaceuticals. Many biological characteristics, such as anti-cancer [12-15], antibacterial [16-20], antidiabetic [21, 22], antihistaminic [23], anti-cancer [24, 25], and anti-HIV effects [26, 27], have been seen in indole derivatives. Moreover, it is observed that the substitution of the indole ring at position 3 leads to the synthesis of more potent medicinal molecules, especially those possessing antimalarial, hypoglycemic, anti-inflammatory, and anticancer properties. Our team's continuous search for lead compounds has yielded a multitude of heterocyclic scaffolds with a range of biological potentials. We continue our work on diabetics by developing derivatives of oxazepine based on our previously reported indole thiourea.

2. MATERIAL AND METHOD
2.1 CHEMISTRY

All solvent-containing and chemicals were bought from E. Merck and Sigma-Aldrich. Using a Thermonik Precision Melting Point-Boiling Point Apparatus (C-PMB-2, Mumbai, India), melting points were identified in open capillaries. Utilizing the FT-IR-8400s spectrophotometer (Shimadzu, Japan), infrared spectra (KBr) were recorded. A Bruker Advance-II 400 spectrometer operating at 400 MHz was used to perform 1H-NMR, and tetramethylsilane (TMS) was used as an internal standard. Each and every chemical shift (ppm) value was recorded using it. Thin-layer chromatography methods (Merck) silica gel, HF254e361, Type 60, 0.25 mm, Darmstadt, Germany) were employed to achieve the material's purity. At his SAIF, University of Punjab, Chandigarh, mass spectra (ESI-MS) have been collected using a Waters Q-TOF-MS spectrometer (Waters, Micromass MS, USA). Thermo Fisher Scientific FLASH EA 112 CHN analyzer was used for elemental examinations.

Scheme 1 displays the synthesis pathway of the title compounds (1–17). From the starting material isatin, compound 2 was synthesized in high yield using the approach reported by Hassan M. *et al* (Indole-2,3-dione). Compound 2 was condensed with different substituted aromatic aldehydes to get a Schiff base in the solvent 100% ethanol. The preparation of pharmacophore was assisted by action of compound 3 with phthalic anhydride. All of the reaction products were achieved with a high rate of success. Elemental analysis, FT-IR (KBr, cm⁻¹), 1HNMR ((D6) DMSO, δ in ppm), and EI-MS (*m/z* [M+H]⁺) studies were carried out to assure structures of the newly synthesized compounds (1-17).

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2.2 SYNTHESIS OF COMPOUND 5-SUBSTITUTED -3-HYDRAZINYL IDENE INDOLE -2-ONE (2)

The reaction mixture of isatin (1 mmol) with hydrazine hydrate (99%, 0.055 g, 1.1 mmol), and absolute methanol (25 mL) was refluxed for 1 h followed by cooling to achieve room temperature. Finally, the hydrazones precipitate was filtered out and dried. Hydrazones were produced by recrystallizing the crude product from ethanol (2). Yield: 72%; m.p. 248–250°C; Rf 0.41 (Methanol: Toluene (1: 4)); IR (KBr, cm^{-1}): 3421–3320, (NH, NH2), 1685 (C=O), 1681 (C=N); 1H-NMR ((D6) DMSO, δ in ppm): δ 6.72–7.32 (m, 4H, Ar-H), 10.51 (s, 2H, NH2), 9.36 (s, 1H, NH); EI-MS: *m/z* [M+H]$^+$ 162.23.

Synthesis of compound 5-substituted-3-(substituted ethylidene hydrazinylidene indole 2-one (3)

Synthesis of compound 4-(5-substituted-2-oxoindolin-3-ylideneamino)-3,4-substituted benzo[e][1.3]oxazepane-1,5-dione (3)

2.3 MOLECULAR DOCKING STUDIES

Using the Autodock software [28], the active sites of alpha-amylase have been molecularly docked with the synthesized compounds. As well as the originally docked acarbose, X-ray coordinates for alpha-glucosidase was retrieved from the RCSB data bank website (5NN5 pdb for alpha-glucosidase). With a chain A sequence length of 913 amino acids for glucosidase. The original ligand, the medication acarbose, was re-docked into the active sites of alpha-glucosidase to demonstrate the validity of molecular docking. RMSD readings for this enzyme, show that the docked acarbose is accurately recreated with values of 0.99 and 1.06 Å. The HIS A584, ARG A608, LEU A868, VAL A867, LYS A760, VAL A763, ASP A 741, TRP A804, HIS A717, ARG A594, VAL A740, GLU A762, GLN A757, TRP A804, CYS A127, TRP A126, ASP A91, GLY A123 residues are the building blocks for the active site of alpha-glucosidase.

2.4 ANTIDIABETIC STUDIES
2.4.1 ALPHA-GLUCOSIDASE ASSAY [29]

The modified published approach was used to determine the level of -glucosidase activity inhibition. In 100 mL of phosphate buffer (pH 6.8) containing 200 mg of bovine serum albumin (Merck, German), one milligram of -glucosidase (S. cerevisiae, Sigma-Aldrich, USA) was dissolved. The reaction mixture, which contained 10 L of sample at concentrations ranging from 1 to 100 M, was pre-mixed with 490 L of phosphate buffer (pH 6.8) and 250 L of 5 mM p-nitrophenyl -D-glucopyranoside (Sigma-Aldrich, Switzerland) before being added to the reaction mixture. The addition of 250 L of alpha-glucosidase (0.15 unit/mL) was followed by a 5-minute preincubation at 37 °C and a 15-minute incubation period. The addition of 2000 L of 200 mM Na_2CO_3 stopped the process. By counting the amount of p-nitrophenol emitted from p-NPG, the amount of alpha-glucosidase activity was measured spectrophotometrically at 400 nm on a spectrophotometer UV-Vis. Acarbose served as the -glucosidase inhibitor's positive control. The IC50 value, or extract concentration needed to inhibit 50% of -glucosidase activity under test conditions, was determined. As shown, the percentage of inhibition was computed.

% Inhibition = (Absorbance of control − Absorbance of compound) × 100/Absorbance of control

2.4.2 STATISTICAL ANALYSIS

A non-linear regression graph was created using GraphPad Prism Software (Version 5) and plotted between percentage inhibition (x-axis) and concentrations (y-axis) to get the IC50 values, or concentrations needed to inhibit the alpha-glucosidase activities by 50%.

2.5 IN-VIVO ANTIDIABETIC ACTIVITY [30]

STZ-induced diabetic mice were weighed and randomly divided into seven groups of six mice per group per cage. Group 1 received vehicle (5% DMSO), serving as a diabetic control group. Group 2 received the standard drug, Glibenclamide, serving as a positive control group. Groups 3–7 were treated with synthesized compounds dissolved in DMSO, respectively. To calculate the fasting serum glucose levels on day 0, the mice were forced to fast for the entire night. One hundred milligram per kilogramme per day, for 21 days at a set time, was the dose that each test chemical received. A dose of 10 mg/kg body weight of Glibenclamide was given to the positive control group, while the same amount of the vehicle was given to the negative control group.

During the study, blood samples were taken from all mice on days 0, 7, 14 and 21, and percentage reduction in FBG was calculated. Blood samples were taken three to four times from the tail vein of each mouse on each day and the average value was determined. Glibenclamide was used as a reference drug in the determination of the in vivo antidiabetic activity of the synthesized compounds.
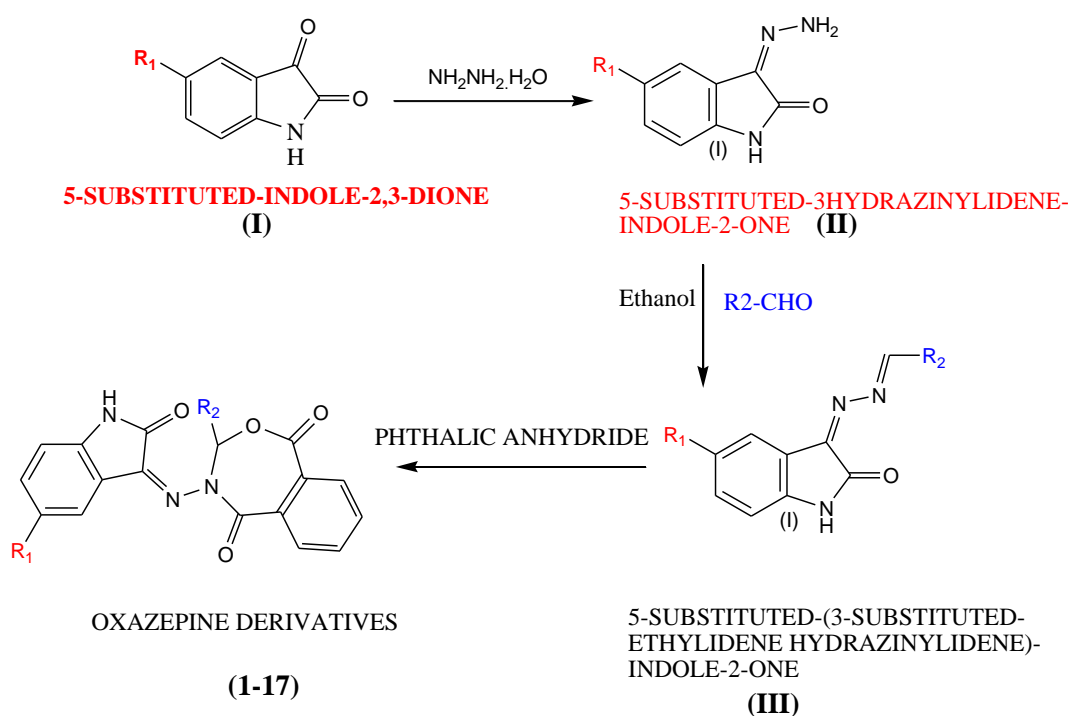
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3. RESULT AND DISCUSSION
3.1 SYNTHETIC CHEMISTRY

Compounds **(1-17)** will be prepared by reaction of compounds **(I)** with hydrazine hydrate and undergo nucleophilic substitution reaction. In the presence of ethanol as a solvent, the compounds **(II)** will react with different aromatic aldehydes to generate Schiff's bases **(III).** Further, Schiff's bases **(III)** undergo cyclization in the presence of phthalic anhydride will form the final proposed structure or compounds **(1-17)** given in scheme 1. All the structures of derivatives given in table 1.

The compounds will be purified by appropriate techniques such as recrystallization using appropriate solvents. The open tube capillary method will be used to determine the melting point of the compounds. The purity and homogeneity of the compounds will be determined by thin-layer chromatography (TLC) using precoated silica gel plates. The functional group's detection and structure prediction will be done based on IR and NMR spectra. Using mass spectroscopy (MS), the exact mass of the produced molecules will be determined and proportion of carbon, hydrogen, and nitrogen will be determined by elemental (C, H, N) analysis.
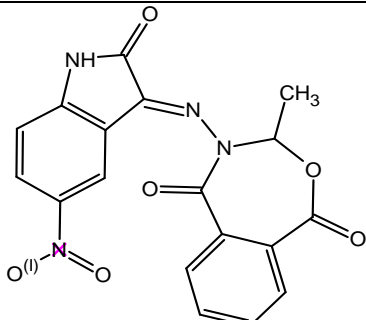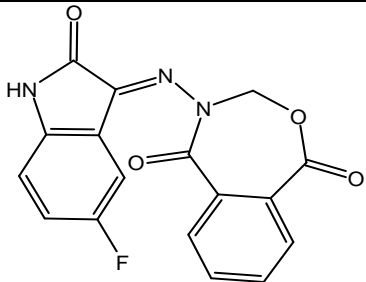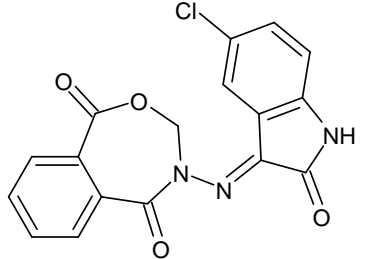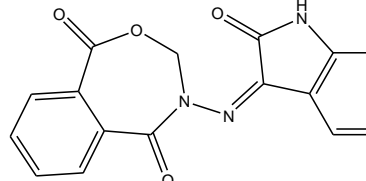

5-SUBSTITUTED-INDOLE-2,3-DIONE (I)

5-SUBSTITUTED-3HYDRAZINYLIDENE-INDOLE-2-ONE (II)

OXAZEPINE DERIVATIVES (1-17)

5-SUBSTITUTED-(3-SUBSTITUTED-ETHYLIDENE HYDRAZINYLIDENE)-INDOLE-2-ONE (III)

Scheme1: Strategy for Synthesis of indole fused oxazepine derivatives
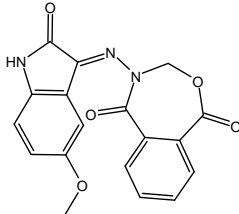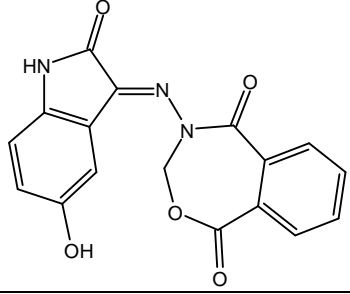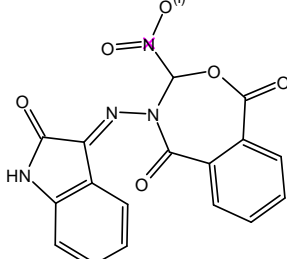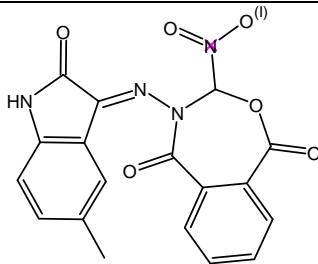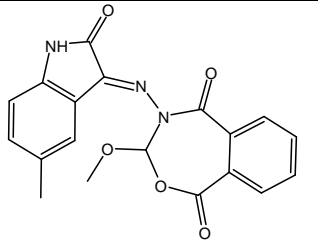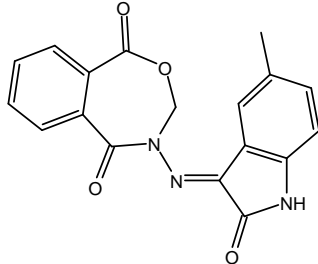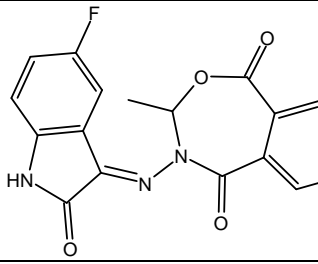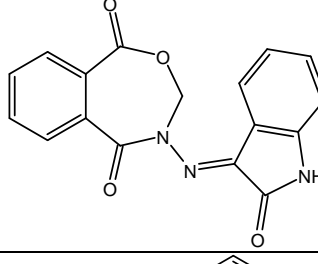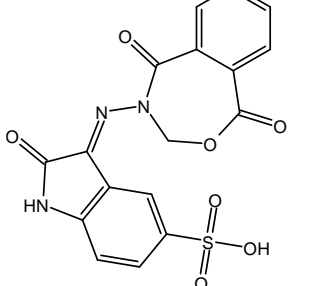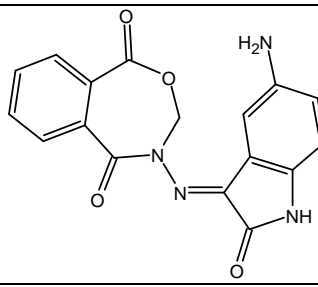
(By reacting substituted isatin **(I)** with hydrazine hydrate to form hydrazinylidene indole -2-one **(II)** then by reacting with Substituted aldehydes in ethanol, derivative **(III)** form and after cyclization with phthalic anhydride to form indole oxazepine **(1-17)** compounds)

Table 1: List of substitution of derivatives in scheme-I

S.NO	R1	R2	OXAZEPINE DERIVATIVES
1	NO$_2$	H	

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2	NO$_2$	CH$_3$	
3	F	H	
4	Cl	H	
5	Br	H	
6	OCH$_3$	H	
7	OH	H	
8	H	NO$_2$	

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9	CH₃	NO₂	
10	CH₃	OCH₃	
11	CH₃	H	
12	F	CH₃	
13	H	H	
14	SO₃H	H	
15	NH₂	H	

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16	CF₃	H	
17	COOH	H	

3.1.1 CHARACTERISATION STUDY OF SCHEME-1

3.1.1.1 4-(5-nitro-2-oxoindolin-3-ylideneamino)-3,4-dihydrobenzo[e][1.3]oxazepine-1,5-dione (1)

SMILES- [O-][N+](=O)C1=CC2=C(NC(=O)\C2=N\N2COC(=O)C3=CC=CC=C3C2=O)C=C1

Molecular Formula: $C_{17}H_{10}N_4O_6$, Mol wt.: 366.89, Yield 98%, mp: 241-244⁰C, Rf: 0.38 (Methanol: Toluene 1:4) IR (KBr, cm-1): 3402.05(N-H), 1738.22 (C=O), 1612.42(C=N), 2879.38(C-H), 1498.41(N=O), 1 H NMR (DMSO, ppm): σ 7.48-8.80 (m, 9H, Ar-H, 10.87 (s, 2H, N-H), 6.90 (d, J= 7.6Hz, 1H, CO-CH), 7.07 (d, J= 6.8 Hz, 1H, CH-Ar), EI-MS: m/z [M+H]⁺367.06. Ana. Calcd for: C, 55.74; H,2.75; N, 15.30 O, 26.21. Found: C, 58.81; H, 3.45; N, 16.25; O, 27.21

3.1.1.2 4-(5-nitro-2-oxoindolin-3-ylideneamino)-3,4-dihydro3methylbenzo[e][1.3]oxazepine-1,5-dione (2)

SMILES- CC1OC(=O)C2=CC=CC=C2C(=O)N1\N=C1/C(=O)NC2=C1C=C(C=C2)[N+]([O-])=O

Molecular Formula: $C_{18}H_{12}N_4O_6$, Mol wt.: 380.16, Yield 98%, mp: 241-244 ⁰C, Rf: 0.38 (Methanol: Toluene 1:4) IR (KBr, cm-1): 3206.18(N-H), 1738.22 (C=O), 1712.29(C=N), 2789.39(C-H), 1510.22 (N=O), 1 H NMR (DMSO, ppm): σ 6.90-8.73 (m, 8H, Ar-H, 10.77 (s, 2H, N-H), 6.92 (d, J= 7.4Hz, 1H, CO-CH), 7.27 (d, J= 6.7 Hz, 1H, CH-Ar), EI-MS: m/z [M+H]⁺ 381.08 Ana. Calcd for: C, 56.85; H, 3.18; N, 14.73, O, 25.24; Found: C, 58.90; H,4.18; N, 14.75; O, 25.24.

3.1.1.3 4-(5-fluoro-2-oxoindolin-3-ylideneamino)-3,4-dihydrobenzo[e][1.3]oxazepine-1,5-dione (3)

SMILES- FC1=CC2=C(NC(=O)\C2=N\N2COC(=O)C3=CC=CC=C3C2=O)C=C1

Molecular Formula: $C_{17}H_{10}N_3O_4$, Mol wt.: 339.28, Yield 98%, mp: 241-244 ⁰C, Rf: 0.38 (Methanol: Toluene 1:4) IR (KBr, cm-1): 3368.08(N-H), 1895.97 (C=O), 1653.00(C=N), 2804.50(C-H),1247.94(C-F), 1 H NMR (DMSO, ppm): σ 7.08-8.81 (m, 8H, Ar-H, 9.87 (s, 1H, N-H), 6.60 (d, J= 8.6Hz, 1H, CO-CH), 7.07 (d, J= 6.8 Hz, 1H, CH-Ar), EI-MS: m/z [M+H]⁺ 340.07 Ana. Calcd for: C, 60.18 ; H,2.97 ; F, 5.60; N, 12.39; O, 18.86;.Found: C, 61.18 ; H,2.97 ; F, 5.59; N, 13.29; O, 19.86.
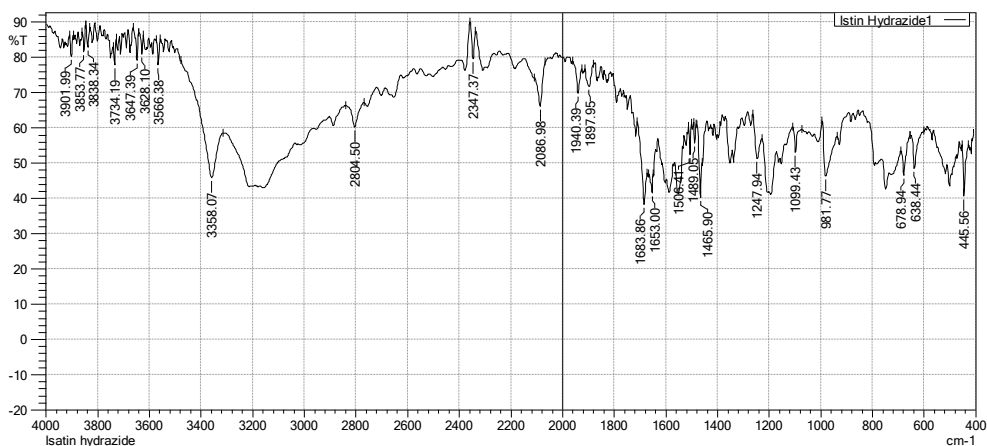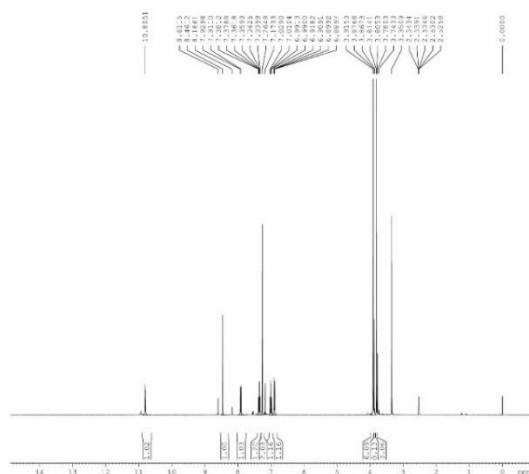
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Figure 1: IR spectra of derivative 3

Figure 2: NMR Spectra of derivative 3

Table 2: ADMET Study of derivative (3)

Physicochemical properties	
Formula	$C_{17}H_{10}FN_3O_4$
Molecular weight	339.28 g/mol
Num. heavy atoms	25
Num. arom. heavy atoms	12
Fraction Csp3	0.06
Num. rotatable bonds	1
Num. H-bond acceptors	6
Num. H-bond donors	1
Molar Refractivity	91.07
TPSA	88.07 Å2
Lipophilicity	
Log Po/w (iLOGP)	1.19
Log Po/w (XLOGP3)	2.17
Log Po/w (WLOGP)	1.22
Log Po/w (MLOGP)	1.92
Log Po/w (SILICOS-IT)	2.46

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Consensus Log *P*o/w	1.79
Water solubility	
Log *S* (ESOL)	− 3.00
Solubility	8.53 e -02 mg/ml; 2.51e-04 mol/l
Class	Soluble
Log *S* (Ali)	− 3.65
Solubility	7.55-02 mg/mL; 2.23e-04 mol/L
Class	Soluble
Log *S* (SILICOS-IT)	-5.37
Solubility	1.44e-03 mg/mL; 4.26e-06 mol/L
Class	Moderately soluble
Druglikeness	
Lipinski	Yes; 0 violation
Ghose	Yes
Veber	Yes
Egan	Yes
Muegge	Yes
Bioavailability score	0.55
Medicinal chemistry	
PAINS	1 alert, imine, 1-isatin
Brenk	1 alert imine, 1
Leadlikeness	Yes
Synthetic accessibility	3.24
Pharmacokinetics	
GI absorption	High
BBB permeant	No

3.1.1.4 4-(5-chloro-2-oxoindolin-3-ylideneamino)-3,4-dihydrobenzo[e][1.3]oxazepine-1,5-dione (4)

SMILES- ClC1=CC2=C(NC(=O)\C2=N\N2COC(=O)C3=CC=CC=C3C2=O)C=C1

Molecular Formula: $C_{17}H_{10}ClN_3O_4$, Mol wt.: 355.73, Yield 88%, mp: 241-244 ^{0}C, Rf: 0.38 (Methanol: Toluene 1:4) IR (KBr, cm-1): 3196.05(N-H), 1828.52 (C=O), 1616.36 (C=N), 2887.44(C-H),771.58(C-Cl), 1 H NMR (DMSO, ppm): ϭ 6.48-9.02 (m, 7H, Ar-H, 10.47 (s, 2H, N-H), 6.49 (d, J=8.4Hz, 1H, CO-CH), 6.47 (d, J= 8.2 Hz, 1H, CH-Ar), EI-MS: m/z [M+H]$^+$ 356.04. Ana. Calcd for: C, 57.40; H,2.83; Cl, 9.97; N, 11.81; O, 17.99. Found, C, 58.30; H,2.81; Cl, 10.97; N, 12.80; O, 18.89
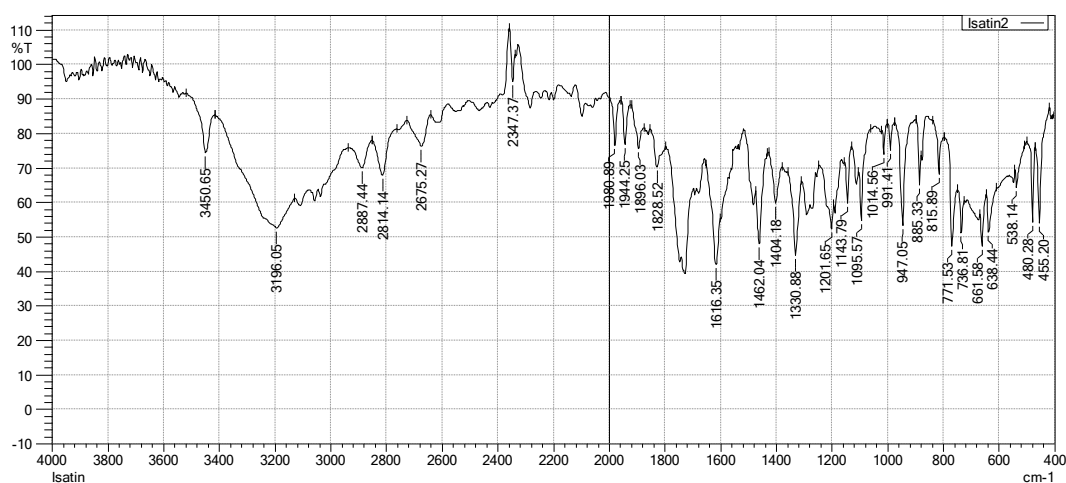

Figure 3: IR spectra of derivative 4

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Table 3: ADMET Study of Derivative (4)

Physicochemical properties	
Formula	$C_{17}H_{10}ClN_3O_4$
Molecular weight	355.73 g/mol
Num. heavy atoms	25
Num. arom. heavy atoms	12
Fraction Csp3	0.06
Num. rotatable bonds	1
Num. H-bond acceptors	6
Num. H-bond donors	1
Molar Refractivity	96.12
TPSA	88.07 Å2
Lipophilicity	
Log Po/w (iLOGP)	1.35
Log Po/w (XLOGP3)	2.70
Log Po/w (WLOGP)	1.31
Log Po/w (MLOGP)	2.04
Log Po/w (SILICOS-IT)	2.63
Consensus Log Po/w	2.03
Water solubility	
Log S (ESOL)	− 4.04
Solubility	3.28 e -02 mg/ml; 9.21e-05 mol/l
Class	Moderately Soluble
Log S (Ali)	− 4.20
Solubility	7.15-04 mg/mL; 2.01e-06 mol/L
Class	Moderately Soluble
Log S (SILICOS-IT)	-5.70
Solubility	7.15e-04 mg/mL; 2.01e-06 mol/L
Class	Moderately soluble
Druglikeness	
Lipinski	Yes; 0 violation
Ghose	Yes
Veber	Yes
Egan	Yes
Muegge	Yes
Bioavailability score	0.55
Medicinal chemistry	
PAINS	1 alert, imine, 1-isatin
Brenk	1 alert imine, 1
Leadlikeness	Yes
Synthetic accessibility	3.24
Pharmacokinetics	
GI absorption	High
BBB permeant	No

3.1.1.5 4-(5-bromo-2-oxoindolin-3-ylideneamino)-3,4-dihydrobenzo[e][1.3]oxazepine-1,5-dione (5)
SMILES-
BrC1=CC2=C(NC(=O)\C2=N\N2COC(=O)C3=CC=CC=C3C2=O)C=C1
Molecular Formula: $C_{17}H_{10}BrN_3O_4$, Mol wt.: 400.18, Yield 98%, mp: 251-254 ^{0}C, Rf: 0.37 (Methanol: Toluene 1:4) IR (KBr, cm-1): 3401.04(N-H), 1716.65 (C=O), 1595.13(C=N), 2889.37(C-H), 659.11(C-Br), 1 H NMR (DMSO,

ppm): ơ 6.88-8.20 (m, 8H, Ar-H, 10.77 (s, 2H, N-H), 6.47 (d, J= 7.7Hz, 1H, CO-CH), 7.67 (d, J= 6.8 Hz, 1H, CH-Ar), EI-MS: m/z [M+H]$^+$ 399.99. Ana. Calcd for: C, 51.02; H,2.52; Br, 19.97; N, 10.51; O, 15.99. Found, C, 52.12; H, 3.52; Br, 20.87; N, 11.50; O, 16.80.

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3.1.1.6 4-(5-methoxy-2-oxoindolin-3-ylideneamino)-3,4-dihydrobenzo[e][1.3]oxazepine-1,5-dione (6)
SMILES-
COC1=CC2=C(NC(=O)\C2=N\N2COC(=O)C3=CC=CC=C3C2=O)C=C1
Molecular Formula: $C_{18}H_{13}N_3O_5$, Mol wt.: 351.31, Yield 92%, mp: 249-252 ^{0}C, Rf: 0.42

(Methanol: Toluene 1:4) IR (KBr, cm-1): 3400.50(N-H), 1728.22 (C=O), 1612.49(C=N), 2833.25(C-H), 1 H NMR (DMSO, ppm): ℗ 6.48-8.210 (m, 9H, Ar-H, 10.67 (s, 2H, N-H), 6.49 (d, J= 7.8Hz, 1H, CO-CH), 7.65 (d, J= 6.9 Hz, 1H, CH-Ar), EI-MS: m/z [M+H]$^+$ 352.09. Ana. Calcd for: C, 61.54; H,3.73; N, 11.96; O, 22.77. Found, C, 63.64; H, 3.9; N, 12.16; O, 23.72.
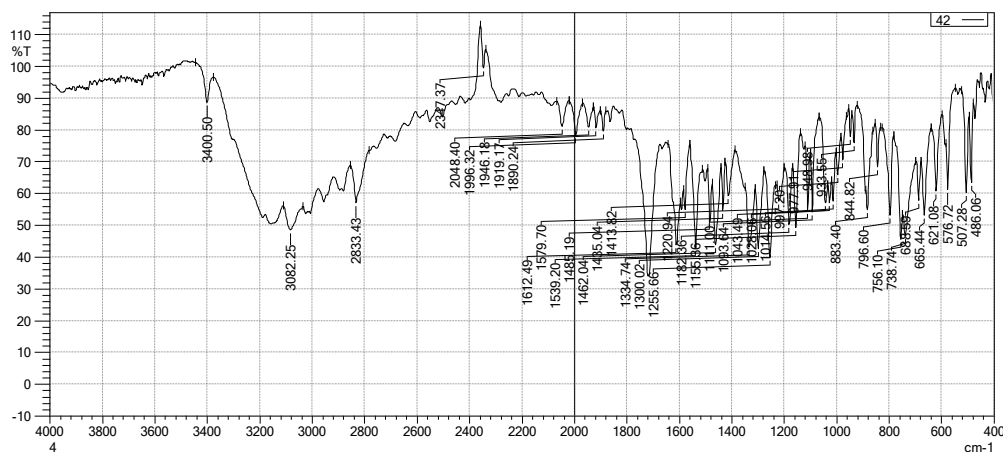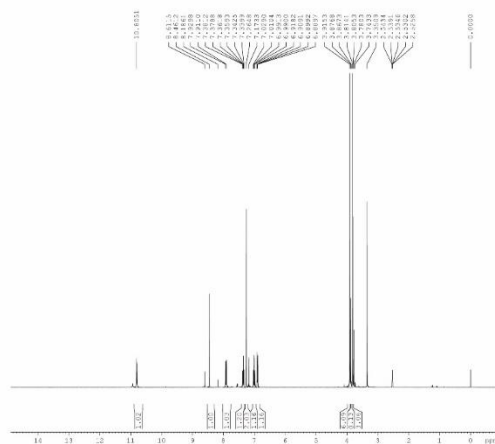

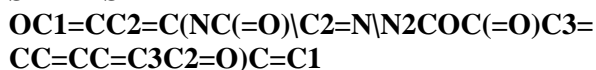**Figure 4:** IR spectra of derivative 6

Figure 5: NMR spectra of derivative 6

Table 4: ADMET Study of Derivative (6)

Physicochemical properties	
Formula	$C_{18}H_{13}N_3O_6$
Molecular weight	350.31 g/mol
Num. heavy atoms	26
Num. arom. heavy atoms	12
Fraction Csp3	0.11
Num. rotatable bonds	2
Num. H-bond acceptors	6
Num. H-bond donors	1

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Molar Refractivity	97.60
TPSA	97.30 Å2
Lipophilicity	
Log Po/w (iLOGP)	1.54
Log Po/w (XLOGP3)	2.04
Log Po/w (WLOGP)	0.67
Log Po/w (MLOGP)	1.25
Log Po/w (SILICOS-IT)	2.08
Consensus Log Po/w	1.52
Water solubility	
Log S (ESOL)	− 3.51
Solubility	1.08 e -01 mg/ml; 3.07e-04 mol/l
Class	Soluble
Log S (Ali)	−3.71
Solubility	6.83-02 mg/mL; 1.9e-04 mol/L
Class	Soluble
Log S (SILICOS-IT)	-5.21
Solubility	2.16e-03 mg/mL; 6.15e-06 mol/L
Class	Moderately soluble
Druglikeness	
Lipinski	Yes; 0 violation
Ghose	Yes
Veber	Yes
Egan	Yes
Muegge	Yes
Bioavailability score	0.55
Medicinal chemistry	
PAINS	1 alert, imine, 1-isatin
Brenk	1 alert imine, 1
Leadlikeness	No, 1violation MW>350
Synthetic accessibility	3.31
Pharmacokinetics	
GI absorption	High
BBB permeant	No

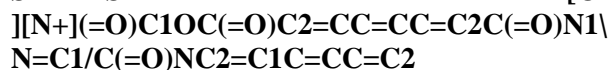**3.1.1.7.   4-(5-hydroxy-2-oxoindolin-3-ylideneamino)-3,4-dihydrobenzo[e][1.3]oxazepine-1,5-dione (7)**
SMILES-
OC1=CC2=C(NC(=O)\C2=N\N2COC(=O)C3=CC=CC=C3C2=O)C=C1
Molecular Formula: $C_{17}H_{11}N_3O_5$, Mol wt.: 337.29, Yield 82%, mp: 261-264 ^{0}C, Rf: 0.42 (Methanol: Toluene 1:4) IR (KBr, cm-1): 3292.78(N-H), 1722.43 (C=O), 1612.49(C=N), 2889.39(C-H), 3622.18(O-H), 1 H NMR (DMSO, ppm): ϭ 7.48.60 (m, 9H, Ar-H, 10.87 (s, 2H, N-H), 6.90 (d, J= 7.6Hz, 1H, CO-CH), 7.07 (d, J= 6.8 Hz, 1H, CH-Ar), EI-MS: m/z [M+H]$^+$ 338.07. Ana. Calcd for: C, 60.54; H, 3.29; N, 12.46; O, 23.72. Found, C, 61.44; H, 3.83; N, 12.96; O, 22.92.

3.1.1.8.   4-(2-oxoindolin-3-ylideneamino)-3,4-dihydro3-nitrobenzo[e][1.3]oxazepine-1,5-dione (8)
SMILES-   [O-][N+](=O)C1OC(=O)C2=CC=CC=C2C(=O)N1\N=C1/C(=O)NC2=C1C=CC=C2
Molecular Formula: $C_{17}H_{10}N_4O_6$, Mol wt.: 366.28, Yield 93%, mp: 231-234 ^{0}C, Rf: 0.39 (Methanol: Toluene 1:4) IR (KBr, cm-1): 3412.08(N-H), 1728.22 (C=O), 1612.49(C=N), 2809.22(C-H), 1577.27(N=O), 1 H NMR (DMSO, ppm): ϭ 7.18-8.23 (m, 9H, Ar-H, 10.77 (s, 2H, N-H), 6.80 (d, J= 6.6Hz, 1H, CO-CH), 9.07 (d, J= 6.2 Hz, 1H, CH-Ar), EI-MS: m/z [M+H]$^+$ 367.06, Ana. Calcd for: C, 55.74; H,2.75 ; N,15.30; O, 26.21; .Found: C, 55.74 ; H,2.75 ; N,15.30; O, 26.21;

3.1.1.9. 4-(5-methyl-2-oxoindolin-3-ylideneamino)-3,4-dihydro3-nitrobenzo[e][1.3]oxazepine-1,5-dione (9)

SMILES-
CC1=CC2=C(NC(=O)\C2=N/N2C(OC(=O)C3=CC=CC=C3C2=O)[N+]([O-])=O)C=C1

Molecular Formula: $C_{18}H_{12}N_4O_6$, Mol wt.: 380.31, Yield 92%, mp: 242-245 ^{0}C, Rf: 0.31 (Methanol: Toluene 1:4) IR (KBr, cm-1): 3356.14(N-H), 1735.93 (C=O), 1685.79(C=N), 2804.50(C-H), 1519.91(N=O), 1 H NMR (DMSO, ppm): ϭ 7.12-8.23 (m, 9H, Ar-H, 10.82 (s, 2H, N-H), 6.90 (d, J= 7.8Hz, 1H, CO-CH), 7.13 (d, J= 6.8 Hz, 1H, CH-Ar), EI-MS: m/z [M+H]$^+$ 381.08. Ana. Calcd for: C, 56.85; H, 3.18; N,14.73; O, 25.24; Found: C, 57.85; H, 3.20; N,14.83; O, 28.24.

3.1.1.10.4-(5-methyl-2-oxoindolin-3-ylideneamino)-3,4dihydro3methoxybenzo[e][1.3]oxazepine-1,5-dione (10)

SMILES-
COC1OC(=O)C2=CC=CC=C2C(=O)N1\N=C1/C(=O)NC2=C1C=C(C)C=C2

Molecular Formula: $C_{19}H_{15}N_3O_5$, Mol wt.: 365.34, Yield 98%, mp: 241-244 ^{0}C, Rf: 0.38 (Methanol: Toluene 1:4) IR (KBr, cm-1): 3412.08(N-H), 1728.22 (C=O), 1612.49(C=N), 2889.39(C-H), 1 H NMR (DMSO, ppm): ϭ 7.48.60 (m, 9H, Ar-H, 10.87 (s, 2H, N-H), 6.90 (d, J= 7.6Hz, 1H, CO-CH), 7.07 (d, J= 6.8 Hz, 1H, CH-Ar), EI-MS: m/z [M+H]$^+$ 366.10. Ana. Calcd for: C, 62.46; H, 4.14; N, 11.50; O, 21.90. Found: C, 67.80; H, 4.88; N, 15.23; O, 25.34.
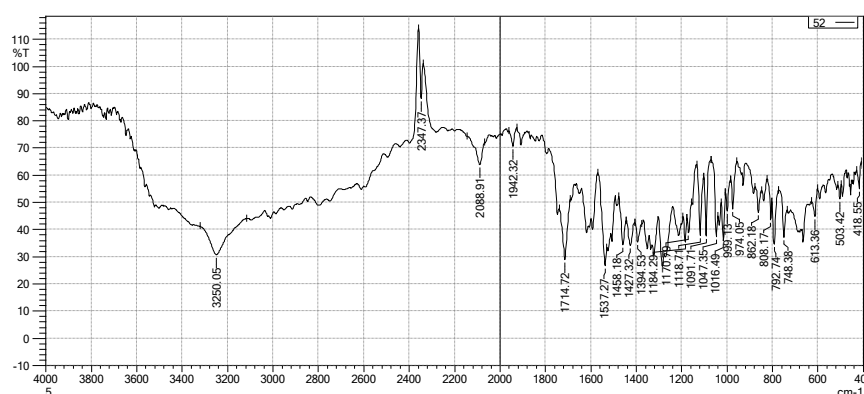

Figure 6: IR spectra of derivative 10

Table 5: ADMET Study of Derivative (10)

Physicochemical properties	
Formula	$C_{19}H_{15}N_3O_6$
Molecular weight	385.34 g/mol
Num. heavy atoms	27
Num. arom. heavy atoms	12
Fraction Csp3	0.16
Num. rotatable bonds	2
Num. H-bond acceptors	6
Num. H-bond donors	1
Molar Refractivity	101.97
TPSA	97.30 Å2
Lipophilicity	
Log Po/w (iLOGP)	2.23
Log Po/w (XLOGP3)	2.45
Log Po/w (WLOGP)	0.94
Log Po/w (MLOGP)	1.62
Log Po/w (SILICOS-IT)	2.18
Consensus Log Po/w	1.88
Water solubility	
Log S (ESOL)	− 3.85
Solubility	5.21 e -02 mg/ml; 1.43e-04 mol/l
Class	Soluble
Log S (Ali)	−4.14

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Solubility	5.67-02 mg/mL; 7.30e-05 mol/L
Class	Moderately Soluble
Log *S* (SILICOS-IT)	-5.36
Solubility	1.60e-03 mg/mL; 4.39e-06 mol/L
Class	Moderately soluble
Druglikeness	
Lipinski	Yes; 0 violation
Ghose	Yes
Veber	Yes
Egan	Yes
Muegge	Yes
Bioavailability score	0.55
Medicinal chemistry	
PAINS	1 alert, imine, 1-isatin
Brenk	1 alert imine, _1,
Leadlikeness	No, 1violation MW>350
Synthetic accessibility	3.95
Pharmacokinetics	
GI absorption	High
BBB permeant	No

3.1.1.11. 4-(5-methyl-2-oxoindolin-3-ylideneamino)-3,4-dihydrobenzo[e][1.3]oxazepine-1,5-dione (11) SMILES-CC1=CC2=C(NC(=O)\C2=N\N2COC(=O)C3=CC=CC=C3C2=O)C=C1

Molecular Formula: $C_{18}H_{13}N_3O_4$, Mol wt.: 335.31, Yield 89%, mp: 241-244 ^{0}C, Rf: 0.38 (Methanol: Toluene 1:4) IR (KBr, cm-1): 3412.08(N-H), 1728.22 (C=O), 1612.49(C=N), 2889.39(C-H), 1 H NMR (DMSO, ppm): ɕ 7.48.60 (m, 9H, Ar-H, 10.87 (s, 2H, N-H), 6.90 (d, J= 7.6Hz, 1H, CO-CH), 7.07 (d, J= 6.8 Hz, 1H, CH-Ar), EI-MS: m/z [M+H]$^+$ 336.09 Ana. Calcd for: C, 64.48; H, 3.91; N, 12.53; O, 19.09. Found: C, 68.80; H, 4.28; N, 15.23; O, 21.34.

3.1.1.12. 4-(5-fluoro-2-oxoindolin-3-ylideneamino)-3,4-dihydro3-methylbenzo[e][1.3]oxazepine-1,5-dione (12) SMILES-CC1OC(=O)C2=CC=CC=C2C(=O)N1\N=C1/C(=O)NC2=C1C=C(F)C=C2

Molecular Formula: $C_{18}H_{12}FN_3O_4$, Mol wt.: 353.30,Yield 98%, mp: 241-244 ^{0}C, Rf: 0.38 (Methanol: Toluene 1:4) IR (KBr, cm-1): 3412.08(N-H), 1728.22 (C=O), 1612.49(C=N), 2889.39(C-H), 1 H NMR (DMSO, ppm): ɕ 7.48.60 (m, 9H, Ar-H, 10.87 (s, 2H, N-H), 6.90 (d, J= 7.6Hz, 1H, CO-CH), 7.07 (d, J= 6.8 Hz, 1H, CH-Ar), EI-MS: m/z [M+H]$^+$ 354.08. Ana. Calcd for: C, 61.19; H, 3.42; F, 5.38; N, 11.89; O, 18.11. Found: C, 62.72; H, 3.47; F, 5.58; N, 12.79; O, 19.21.
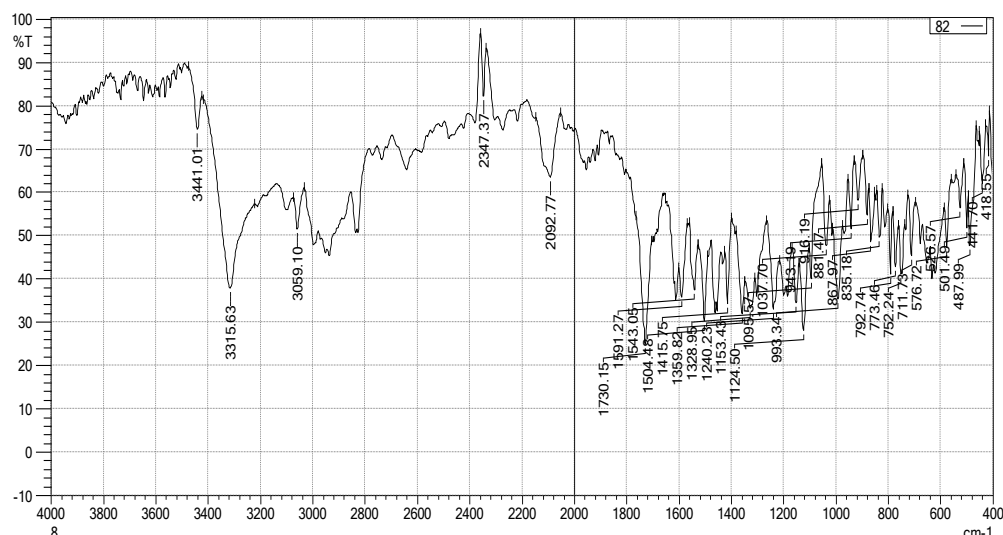

Figure 7: IR spectra of derivative 12

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Table 6: ADMET Study of Derivative (12)

Physicochemical properties	
Formula	$C_{18}H_{12}FN_3O_4$
Molecular weight	353.30 g/mol
Num. heavy atoms	26
Num. arom. heavy atoms	12
Fraction Csp3	0.11
Num. rotatable bonds	1
Num. H-bond acceptors	6
Num. H-bond donors	1
Molar Refractivity	95.88
TPSA	88.07 Å2
Lipophilicity	
Log Po/w (iLOGP)	1.96
Log Po/w (XLOGP3)	2.60
Log Po/w (WLOGP)	1.61
Log Po/w (MLOGP)	2.15
Log Po/w (SILICOS-IT)	2.55
Consensus Log Po/w	2.18
Water solubility	
Log S (ESOL)	− 3.94
Solubility	4.02e -02 mg/ml; 1.14e-04 mol/l
Class	Soluble
Log S (Ali)	−4.10
Solubility	2.81-02 mg/mL; 7.97e-06 mol/L
Class	Moderately Soluble
Log S (SILICOS-IT)	-5.52
Solubility	1.07e-03 mg/mL; 3.03e-06 mol/L
Class	Moderately soluble
Druglikeness	
Lipinski	Yes; 0 violation
Ghose	Yes
Veber	Yes
Egan	Yes
Muegge	Yes
Bioavailability score	0.55
Medicinal chemistry	
PAINS	1 alert, imine, 1-isatin
Brenk	1 alert imine, _1,
Leadlikeness	No, 1violation MW>350
Synthetic accessibility	3.79
Pharmacokinetics	
GI absorption	High
BBB permeant	No

3.1.1.13. 4-(2-oxoindolin-3-ylideneamino)-3,4-dihydrobenzo[e][1.3]oxazepine-1,5-dione (13)

SMILES-O=C1NC2=C(C=CC=C2)\C1=N/N1COC(=O)C2=CC=CC=C2C1=O
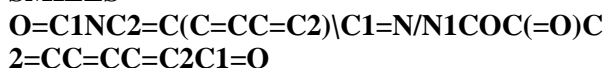
Molecular Formula: $C_{17}H_{11}N_3O_4$, Mol wt.: 321.29,Yield 89%, mp: 246-249 ^{0}C, Rf: 0.41 (Methanol: Toluene 1:4) IR (KBr, cm-1): 3412.08(N-H), 1728.22 (C=O), 1612.49(C=N), 2889.39(C-H), 1 H NMR (DMSO, ppm): ɓ 7.48.60 (m, 9H, Ar-H, 10.87 (s, 2H, N-H), 6.90 (d, J= 7.6Hz, 1H, CO-CH), 7.07 (d, J= 6.8 Hz, 1H, CH-Ar), EI-MS: m/z [M+H]⁺ 322.08. Ana. Calcd for: C, 63.55; H, 3.45; N, 13.08; O, 19.92. Found: : C, 63.55; H, 4.55; N, 13.12; O, 20.12.

3.1.1.14.(E)-3-(1,5-dioxobenzo[e][1,3]oxazepine-4(1H,3H,5H ylimino)-2-oxoindolin-sulphonic acid (14)

SMILES-OS(=O)(=O)C1=CC2=C(NC(=O)\C2=N\N2COC(=O)C3=CC=CC=C3C2=O)C=C1
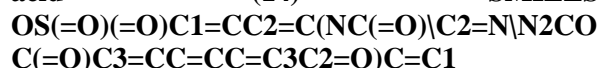
Molecular Formula: $C_{17}H_{11}N_3O_7S$, Mol wt.: 401.35, Yield 91%, mp: 241-244 ^{0}C, Rf: 0.39 (Methanol: Toluene 1:4) IR (KBr, cm-1): 3412.08(N-H), 1728.22 (C=O), 1622.13(C=N), 2839.22(C-H), 1 H NMR (DMSO, ppm): ɓ 7.48.60 (m, 9H, Ar-H, 10.87 (s, 2H, N-H), 6.90 (d, J= 7.6Hz, 1H, CO-CH), 7.07 (d, J= 6.8 Hz, 1H, CH-Ar), EI-MS: m/z [M+H]⁺ 402.03. Ana. Calcd for: C, 50.88; H, 2.76; N, 10.47; O, 27.90; S, 7.99. Found: , 51.75; H, 2.91 N, 11.27; O, 27.93; S, 8.12.

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3.1.1.15. 4-(5-amino-2-oxoindolin-3-ylideneamino)-3,4-dihydrobenzo[e][1.3]oxazepine-1,5-dione (15)

SMILES-
NC1=CC2=C(NC(=O)\C2=N\N2COC(=O)C3=CC=CC=C3C2=O)C=C1

Molecular Formula: $C_{17}H_{12}N_4O_4$, Mol wt.: 336.30, Yield 91%, mp: 251-254 ^{0}C, Rf: 0.42 (Methanol: Toluene 1:4) IR (KBr, cm-1): 3412.08(N-H), 1728.22 (C=O), 1612.49(C=N), 2889.39(C-H), 1 H NMR (DMSO, ppm): ℺ 7.48.60 (m, 9H, Ar-H, 10.87 (s, 2H, N-H), 6.90 (d, J= 7.6Hz, 1H, CO-CH), 7.07 (d, J= 6.8 Hz, 1H, CH-Ar), EI-MS: m/z [M+H]$^+$ 337.09. Ana. Calcd for: C, 60.71; H, 3.60; N, 16.66; O, 19.03. Found: C, 61.61; H, 4.20; N, 17.56; O, 19.82.

3.1.1.16.4-(5-(trifluoromethyl-2-oxoindolin-3-ylideneamino)-3,4-dihydrobenzo[e][1.3]oxazepine-1,5-dione (16)

SMILES-
FC(F)(F)C1=CC2=C(NC(=O)\C2=N/N2COC(=O)C3=CC=CC=C3C2=O)C=C1

Molecular Formula: $C_{18}H_{10}F_3N_3O_4$, Mol wt.: 389.88, Yield 94%, mp: 257-260 ^{0}C, Rf: 0.41 (Methanol: Toluene 1:4) IR (KBr, cm-1): 3408.22(N-H), 1728.22 (C=O), 1612.49(C=N), 2889.39(C-H), 1 H NMR (DMSO, ppm): ℺ 7.48.60 (m, 9H, Ar-H, 10.87 (s, 2H, N-H), 6.90 (d, J= 7.6Hz, 1H, CO-CH), 7.07 (d, J= 6.8 Hz, 1H, CH-Ar), EI-MS: m/z [M+H]$^+$ 390.06. Ana. Calcd for : C, 55.54 ; H, 2.59 ; F, 14.64; N, 10.79; O, 16.44;.Found: C, ; H, ;N, ; C, 55.54 ; H, 2.89 ; F, 15.60; N, 10.19; O, 17.44;.
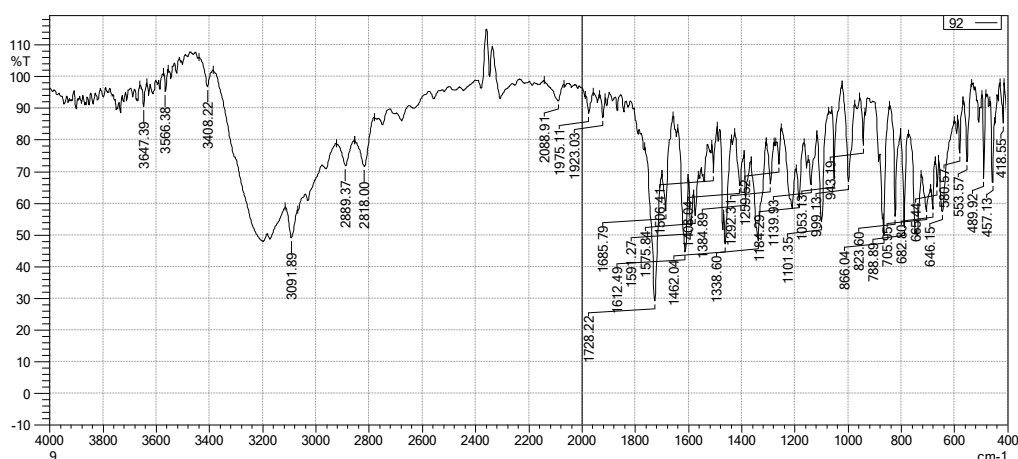

Figure 8: IR spectra of derivative 16

Table 7: ADMET Study of Derivative (16)

Physicochemical properties	
Formula	$C_{18}H_{10}F_3N_3O_4$
Molecular weight	389.88 g/mol
Num. heavy atoms	28
Num. arom. heavy atoms	12
Fraction Csp3	0.11
Num. rotatable bonds	2
Num. H-bond acceptors	8
Num. H-bond donors	1
Molar Refractivity	96.11
TPSA	88.07Å2
Lipophilicity	
Log Po/w (iLOGP)	1.44
Log Po/w (XLOGP3)	2.95
Log Po/w (WLOGP)	2.83
Log Po/w (MLOGP)	2.38
Log Po/w (SILICOS-IT)	3.09
Consensus Log Po/w	2.54
Water solubility	
Log S (ESOL)	− 4.30
Solubility	1.96e -02 mg/ml; 5.04e-05 mol/l
Class	Moderately Soluble
Log S (Ali)	−4.46

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Solubility	1.34-02 mg/mL; 3.45e-05 mol/L
Class	Moderately Soluble
Log *S* (SILICOS-IT)	-5.94
Solubility	4.46e-04 mg/mL; 1.15e-06 mol/L
Class	Moderately soluble
Druglikeness	
Lipinski	Yes; 0 violation
Ghose	Yes
Veber	Yes
Egan	Yes
Muegge	Yes
Bioavailability score	**0.55**
Medicinal chemistry	
PAINS	1 alert, imine, 1-isatin
Brenk	2 alert imine, _1
Leadlikeness	No, 1violation, MW>350
Synthetic accessibility	3.36
Pharmacokinetics	
GI absorption	High
BBB permeant	No

3.1.1.17.(E)-3-(1,5-dioxobenzo[e][1,3]oxazepine-4(1H,3H,5H-ylimino)-2-oxoindolin-5-carboxylic acid (17) SMILES-OC(=O)C1=CC2=C(NC(=O)\C2=N\N2COC(=O)C3=CC=CC=C3C2=O)C=C1

Molecular Formula: $C_{18}H_{11}N_3O_6$, Mol wt.: 365.30, Yield 97%, mp: 231-234 ^{0}C, Rf: 0.48 (Methanol: Toluene 1:4) IR (KBr, cm-1): 3412.08(N-H), 1728.22 (C=O), 1612.49(C=N), 2889.39(C-H), 1 H NMR (DMSO, ppm): σ 7.48.60 (m, 9H, Ar-H, 10.87 (s, 2H, N-H), 6.90 (d, J= 7.6Hz, 1H, CO-CH), 7.07 (d, J= 6.8 Hz, 1H, CH-Ar), EI-MS: m/z [M+H]$^+$ 366.06. Ana. Calcd for: C, 59.18; H, 3.04; N, 11.50; O, 26.28. Found: C, 61.10; H, 3.24; N, 11.50; O, 26.48.

3.2 *IN VITRO* BIOLOGICAL SCREENING

The absorption of carbohydrates that cause PPHG (postprandial hyperglycemia) is known to be significantly influenced by the enzyme alpha-glucosidase. Postprandial hyperglycemia (PPHG) is caused by the fast degradation of dietary carbohydrate. Since it has been established that the activity of HPA (human pancreatic alpha-amylase) in the small intestine is correlated with a rise in postprandial glucose

levels, its management is crucial in the management of type-2 diabetes. Pancreatic - amylase inhibitors slow down the breakdown of carbohydrates, which lowers the postprandial serum glucose levels and slows the rate of glucose absorption. Numerous sources, including plants and microorganisms, have been used to isolate and study glucosidase inhibitors. Inhibitors of intestinal disaccharidases and pancreatic -amylase were discovered in the 1970s, and it was discovered that these inhibitors could be used therapeutically

in the oral treatment of noninsulin-dependent diabetes mellitus (type-2 diabetes). As a result, the development of novel inhibitors is seen to be a crucial component of medical treatments that aim to control type II DM. Table 1 displays the inhibitory potentials of each of the synthesized Indole-based Analogues, numbered from 1 to 17.

When compared to the standard inhibitor acarbose (IC50 = 12.18 M), it was revealed that analogues 3, 4,6,10, 12 and 16 were effective at inhibiting alpha-amylase, with IC50 values of 7.30, 6.41, 6.89, 7.18, 6.79 and 7.37 M, respectively.

SAR study

The structure-activity relationship (SAR) investigation shown that the differences in the inhibitory activity of indole-based analogues 1–17 against alpha-glucosidase are due to different patterns of substitution on the phenyl ring of the molecule. Below is a discussion of a valid defence. The various functional groups on the phenyl ring are responsible for the variation in the alpha-glucosidase inhibitory capacity of indole analogues 1–17.

The activity of analogue 3 is improved, (IC50 = 7.30M), by substituting fluoro group on phenyl ring. Like this, analogue 4, alpha-glucosidase inhibitory activity was enhanced by the addition of fluoro (IC50 = 6.41M). On the other hand, the activity of analogue 6 with IC50 = 6.89 M was increased by the substitution of the methoxy group. However, methyl analogue 9 (IC50 = 3.70 M) showed the least amount of activity. The presence of methoxy group in analogue 10 shows moderate activity (IC50= 7.18) than others. Fluoro group on analogue 12 shows IC50= 6.37, three fluorine atoms on the phenyl ring may be the reason why the

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substitution of a trifluoromethyl group on the phenyl ring boosted the activity of analogue 16 (IC50 = 7.3 M).

The high electronegativity of the fluorine atom and the good interactions with the enzyme at the ortho, para, and meta substituents of the phenyl ring may explain the high inhibitory potential of fluorine analogues.

4. MOLECULAR DOCKING STUDY

Table 9 displays the IC50 values of the chosen analogues for their inhibition of alpha-glucosidase (3, 4, 6, 10 and 16). It is clear from Table 18 that

the functions in the substitution group R of the basic skeleton of analogues may vary in kind, number, and locations, which may affect the inhibitory potential of the chosen analogue. Molecular docking studies on all 17 analogues were carried out to define and justify the binding modes between the synthesized compounds by using ligand alpha -glucosidase

In Table18, the calculated intermolecular hydrogen bonds between the docked synthesized analogues and the active site residues of alpha-glucosidase are compiled along with the calculated binding energies of the stable complex's ligand, alpha-glucosidase.

Table 8: Inhibition concentrations, docking binding energies, hydrogen bonds and the number of closest residues to the docked selected analogues (1-17 and the acarbose drug)
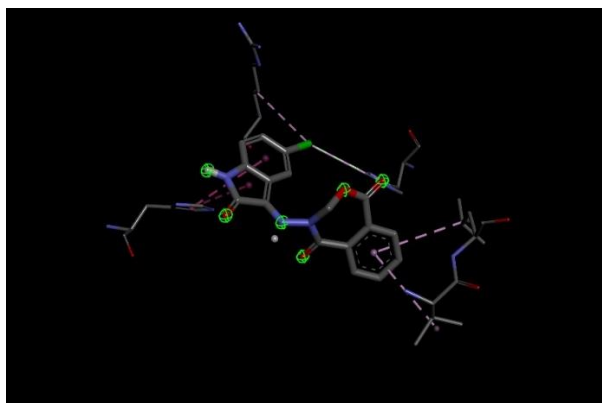
S. No.	Ligands	Docking scores (kcal mol^{-1})	Hydrogen bond interaction	IC50 ± SEM
1.	Derivative 1	-7.1	5	4.08± 0.10
2.	Derivative 2	-7.5	5	3.11± 0.10
3.	Derivative 3	**-8.0**	5	7.30± 0.10
4.	Derivative 4	**-8.1**	5	6.41± 0.10
5.	Derivative 5	-7.6	5	3.25± 0.10
6.	Derivative 6	**-8.8**	5	6.89± 0.10
7.	Derivative 7	-6.8	5	3.24± 0.10
8.	Derivative 8	-7.3	4	4.02± 0.10
9.	Derivative 9	-7.8	5	3.70± 0.10
10.	Derivative 10	**-9.1**	5	7.18± 0.10
11.	Derivative 11	-7.1	4	4.31± 0.10
12.	Derivative 12	**-8.2**	4	6.79± 0.10
13.	Derivative 13	-6.9	4	4.35± 0.10
14.	Derivative 14	-7.2	5	5.18± 0.10
15.	Derivative 15	-7.3	5	4.23± 0.10
16.	Derivative 16	**-9.2**	5	7.37± 0.10
17.	Derivative 17	-7.1	4	4.18± 0.10
18.	Acarbose	**-9.4**	10	12.18± 0.10

In this study, we evaluated the influence of fluorine location (ortho, meta, and para) and the effect of increasing halogen size (F, Cl, and Br) on the inhibition of alpha-glucosidase. In contrast to the chlorine, the fluorine at ortho and meta positions
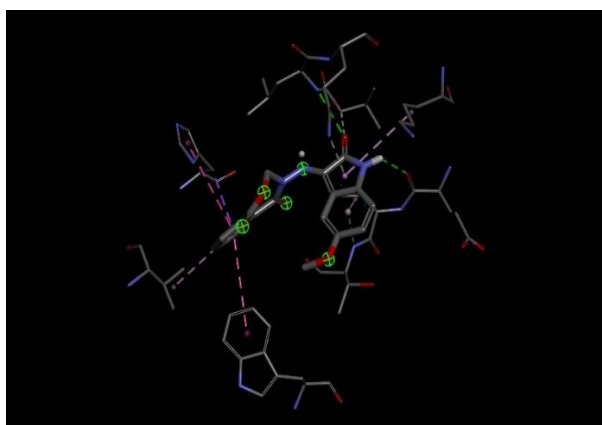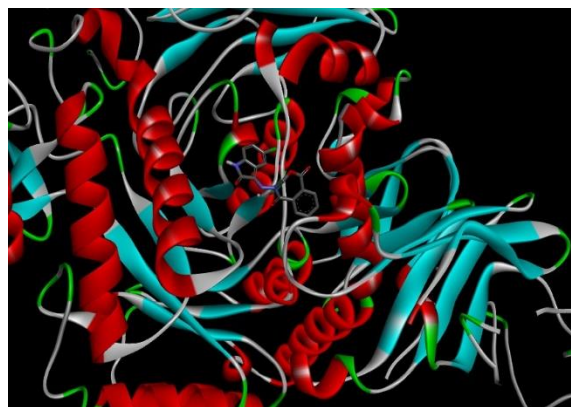
has enhanced inhibitory potential, as shown in Table 8. Figures 9 and 10 depict how the docked analogues interact with the active sites of alpha-glucosidase.
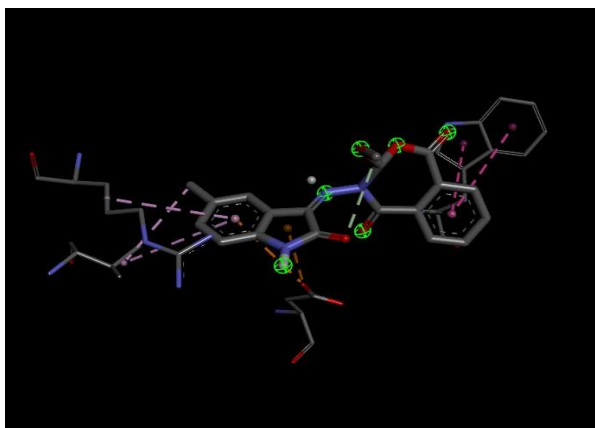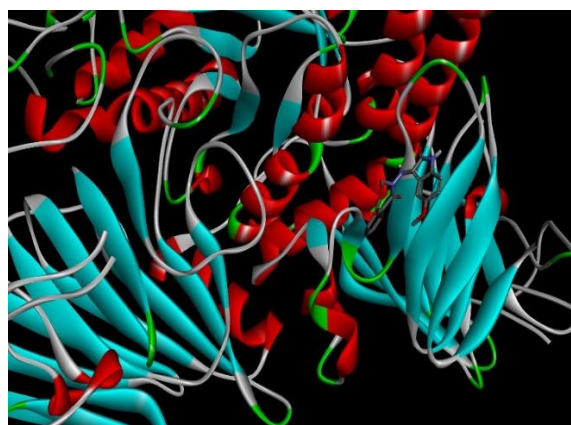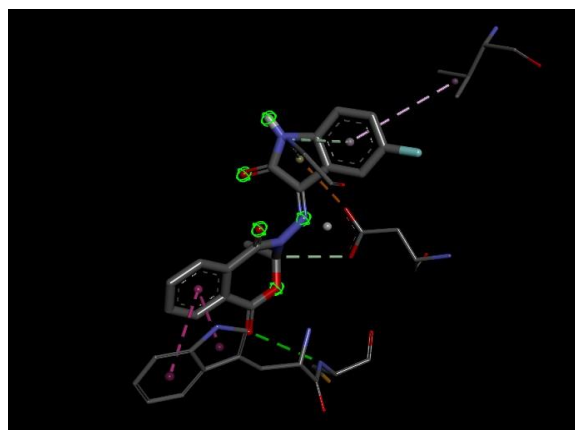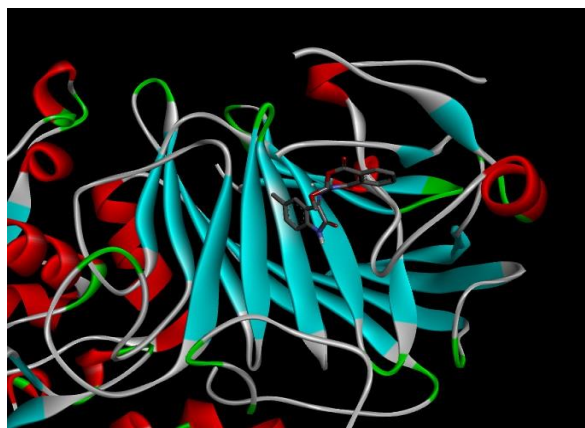

(Derivative 3)

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(Derivative 4)

(Derivative 6)

(Derivative 10)

(Derivative 12)

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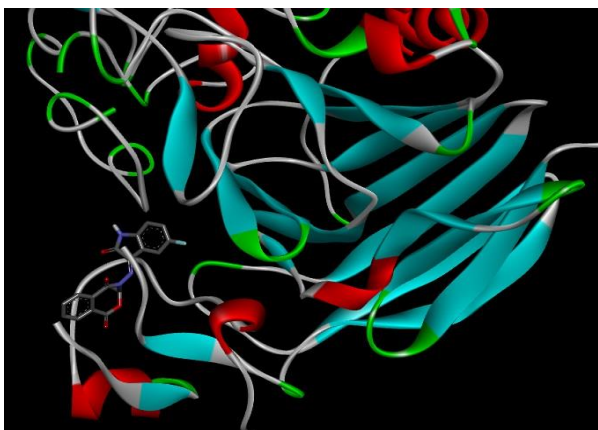
(Derivative 14)

(Derivative 16)

Figure 9: 3D Docking study of ligand-based images of derivative (3, 4, 6, 10, 12 and 16)

(Derivative 3)

(Derivative 40

(Derivative 6)
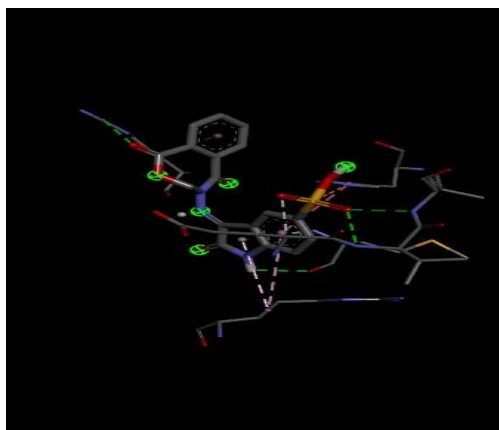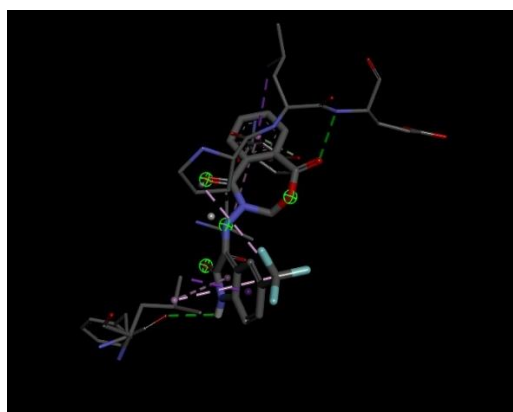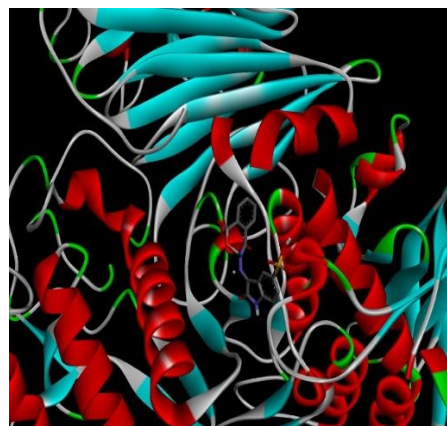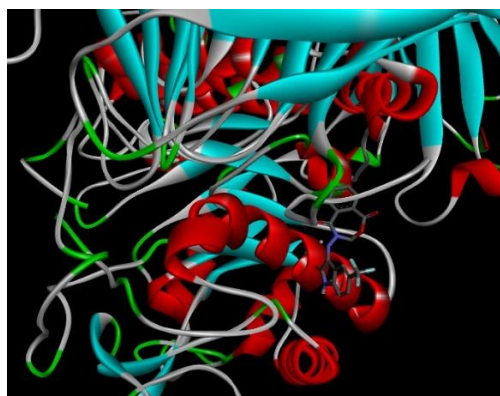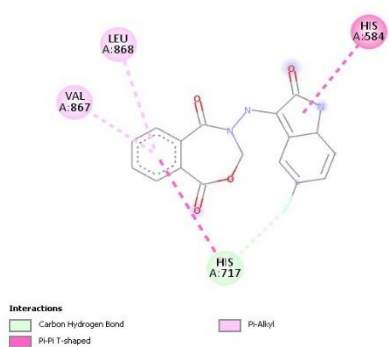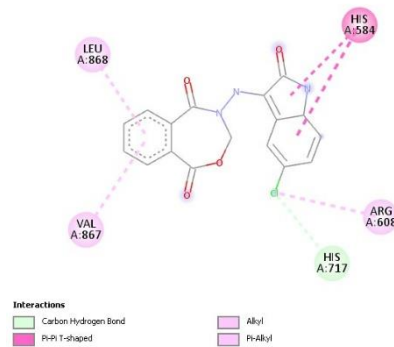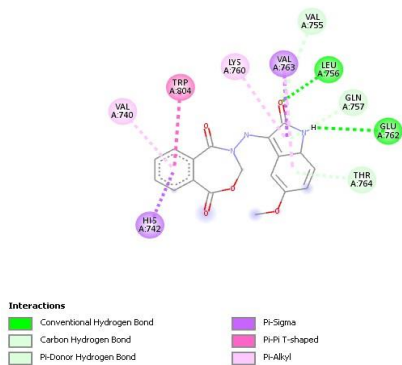

(Derivative 10)

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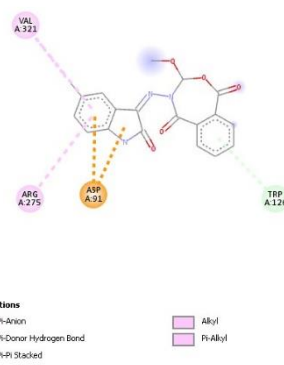
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(Derivative 12)

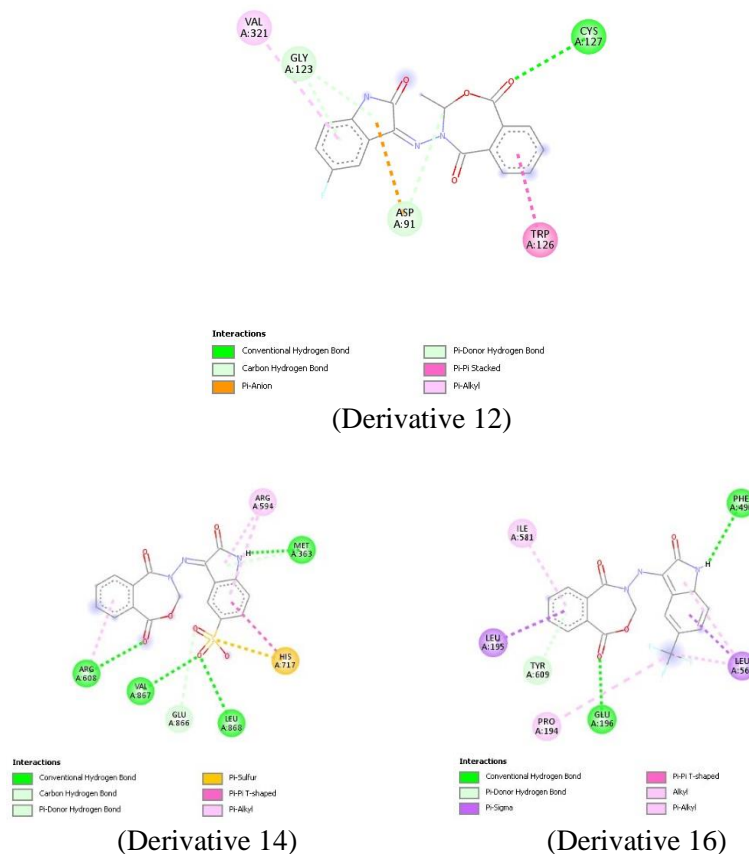
(Derivative 14)

(Derivative 16)

Figure 10: 2D closest interactions between active site residues of α-glucosidase and synthesized analogues 3, 4, 6, 10, 12, 14 and 16

In Figure 9, it is depicted how the substances 3, 4, 6, 10, 12 and 16 binds to the active site of alpha-glucosidase. While docking into the active site of enzyme, the lone pair of the Florine group of analogue 3 forms a weak hydrogen bond with amide group of HIS A 717 amino acid of 3.69. The alpha-glucosidase inhibitions are observed to increase experimentally as the electronegativity and size of the substituted halogens decrease. For example, the enzymatic inhibition of 3 with the substituted fluorine atom is higher than that of 4 with the substituted chlorine. Free binding energies of the stable complexes generated between positions 6, 10, 14 and 16 from one side and the residues of the active sites of -glucosidase vary little with variance smaller than 1.2 kcal mol-1. They also nearly shared interactions with the residues of the active site. For instance, five hydrogen bonds between VAL A755, LEU A756, GLN A757, GLU A762, and THR A764 in analogue 6 was found. One hydrogen bond in analogue 10 was TRP A 126. Four hydrogen bonds in analogue 14 were found to be MET A363, LEU A868, VAL A867 and ARG A608. Two strong hydrogen bonds are created between the active amino acids PHE A490 and GLU A196 in analogue 16.

In comparison to the reference drug acarbose, the alpha-glucosidase inhibition of the chosen is substantially less pronounced (Table 8). The outcome is consistent with the findings of molecular docking. With a binding energy of -9.4 kcal mol-1, acarbose alpha glucosidase complex was discovered to be more stable than the complexes produced between the docking chosen chemicals. The strength of the hydrogen bonds (2.5 d 3.1) formed between the functional groups of acarbose and the active amino acids of alpha glucosidase is largely what determines how stable docked acarbose is. In fact, the acarbose-alpha glucosidase combination forms twice as many hydrogen bonds as other complexes created with chemicals (Table 8 and Fig.9). Comparatively to other docked chosen compounds, the complex produced by the acarbose derivative and alpha-amylase demonstrated the maximum stability, which is mostly due to the number of hydrogens.

5. IN-VIVO STUDY

The relative long-term effect of the Benzopyrrole derivatives on (blood glucose) BGL was tested in vivo using STZ-induced diabetic mice. The test compounds and the standard drug were administered daily for 21 days at a fixed time after

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induction of diabetes. BGL was measured on days 0, 7, 14, and 21 at the same time and the results are shown in following table. Both Glibenclamide and the test compounds showed significant reductions in BGL on days 7, 14 and 21 compared to the diabetic control group. On each day, all test compounds showed a comparable reduction in fasting BGL compared to the standard drug (Glibenclamide), and the highest effect was seen on day 21. By comparison, compound 16 was found to show the highest antidiabetic activity (57.7 % reduction) followed by **14** (52.4 % reduction). Furthermore, compounds **3, 4**, **6** and **10** showed significant antidiabetic activities after 21 days of treatment and showed fasting BGL reductions of 47.7, 52.2, and 51.9 %, respectively. Glibenclamide (10 mg/kg) showed a 58.3 % reduction, and this value was not significantly different from those of the test compound (100 mg/kg) given in table 9 and 10.

Table 9: Effect of Synthesized Compounds (Each 100 Mg/Kg) on Fasting Blood Glucose Level of STZ-Induced Diabetic Mice

Treatment Groups	Fasting Blood Glucose Level (mg/dL) (Mean ± SEM)				Percent Change in BGL Between Day 0 and 21
	Day 0	Day 7	Day 14	Day 21	
Diabetic control	262.68 ± 11.14	288.34 ± 11.67	307.6 ± 10.75	324.84 ± 7.32	+24.9%
Glibenclamide (10 mg/kg)	268.68± 9.39	204.84 ± 6.57(−30.2%)*	152.1 ± 4.11 (−51.8%)*	115.68 ± 2.87(−65.7%)*	−58.3%
Compound 3	270.84 ± 12.18	211.68 ± 9.46 (−27.8%)*	166.6 ± 5.69 (−47.1%)*	132.34 ± 3.59 (−60.5%)*	−52.4%
Compound 6	254.34 ± 16.26	203.18 ± 7.13(−30.7%)*	164.34 ± 5.32 (−47.8%)*	136.34 ± 3.51 (−59.3%)*	−47.7%
Compound 10	281.6 ± 14.55	208.18 ± 6.13 (−28.8%)*	157.6 ± 6.12 (−50.1%)*	122.68 ± 2.72 (−63.5 %)*	−53.7%
Compound 12	266.18 ± 10.49	200.62 ± 6.68 (−31.6 %)*	163.18 ± 4.51 (−48.2%)*	130.68 ± 3.21 (−61.1%)*	−52.2%
Compound 16	267.37 ± 5.12	207.15 ± 6.19 (−29.4 %)*	165.18 ± 5.04 (−47.5 %)*	132.18 ± 4.21 (−60.6 %)*	−57.9%

The test compounds also did not show significant variation with each other in reducing fasting BGL in STZ-induced diabetic mice. The effects of the synthesized Benzopyrrole derivatives on the bodyweight of STZ-induced diabetic mice were also evaluated and the results are given in following table. Daily oral administration of test compounds for 21 days at 100 mg/kg dose showed improvements in body weight. Compared to the diabetic control group that showed significant weight reduction (11.3 %), Glibenclamide increased the bodyweight of the mice by about 12 %, whereas compounds **3, 6, 10, 12** and **16** increased body weight by 7.2, 4, 11.4, 7 and 9.2 %, respectively. The change in body weight caused by each of the rest test compounds was not significantly different from that of Glibenclamide.

Table 10: Effect of Synthesized Compounds (Each 100 Mg/Kg) on Body Weight of STZ-Induced Diabetic Mice

Treatment Groups	Bodyweight (g)				Change in Body Weight	
	Day 0	Day 7	Day 14	Day 21	Compared to DC	From day 0 to 21
Diabetic control	26.26 ± 0.84	25.23 ± 0.81	24.43 ± 0.97	23.68 ± 0.86	−	−11.3%***

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Glibenclamide (10 mg/kg)	26.39 ± 0.68	26.06 ± 0.57	26.26 ± 0.52	26.14 ± 0.48	+11.8%	−2.1%
Compound 3	25.79 ± 1.0	25.61 ± 1.01	25.18 ± 0.99	25.06 ± 0.97	+7.2%	−3.8%
Compound 6	23.6 ± 0.78	25.01 ± 0.78	24.79 ± 0.87	24.34 ± 0.85	+4.1%	−5.9%**
Compound 10	25.93 ± 0.57	25.84 ± 0.55	25.68 ± 0.72	26.00 ± 0.64	+11.4%	+1.4%
Compound 12	25.26 ± 0.94	24.88 ± 1.02	24.81 ± 0.98	25.03 ± 1.04	+7.1%	−1.8%
Compound 16	25.78 ± 0.85	25.39 ± 0.83	25.26 ± 0.99	25.51 ± 0.73	+9.2%	−2.2%

6. CONCLUSION

Novel indole analogues **1** through **17** were successfully synthesized to test their ability to inhibit glucosidase and amylase. Results in general showed that the type and position of substitutes play a significant influence in enzyme inhibition. The analogues **3, 4, 6, 10, 12, and 16** showed effective inhibitory actions against the alpha-glucosidase enzyme. Docking studies further reinforce the findings. The lead compounds identified in this study will be helpful in the future for developing medications to treat diabetes. To confirm the interactions of active substances with enzymes, we also performed a molecular docking study. The stability and ADMET property of the most active chemical, **16** was also calculated. With the help of this investigation, we have the chance to further assess compound **16**, which is the most active, in an animal model to see how it affects diabetes II inhibition.

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