In silico screening of Daphne tangutica Maxim plant for their bio-active phytochemicals as type 2 diabetes potential inhibitors

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Abstract

Ethnopharmacological relevance: Previously before the invention of virtual screening methods or techniques almost 80% of drugs were obtained from natural resources or compounds obtained from these sources. Plant species of the genus *Daphne* have historical background as a source of bioactive phytochemicals such as flavonoids including daphnodorins which have been found to possess significant chemotaxonomic value in drug discovery.

Aim of the study: Their comprehensive pharmacological, phytochemical, biological, various catalytic activities and clinical uses have prompted us to conduct the optimization and structure based virtual screening of its potent analogues. The aim of this work is to investigate the use of daphnodorins analogues for the first time as anti-diabetic inhibitors on the basis of significant features by computational analysis.

Materials and methods: A dataset of 38 compounds was selected from authentic database i.e., PubChem and ZINC database for computational analysis and quantitative structure activity relationship (QSAR). Optimized compounds were docked against various co-crystallized structures of inhibitors, antagonists and receptors were downloaded from PDB by implicating AutoDock Vina, Discovery studio 2020, PYMOL (Schrodinger). After performing molecular docking compounds of desired threshold were analyzed by ADMET studies through Swiss ADMET, ADMET SAR, ADMET lab and ProTox-II for investigating their drug-like nature. Elaboration of electronic structures of hit compounds was carried out using Gaussian 09W by intimating Density Functional Theory (DFT).

Results: At significant phases of drug design approaches regular use of molecular docking has helped to promote the separation of important representatives from 38 pharmaceutically active compounds and 14 compounds were selected as lead compounds which were analyzed through ADMET studies for identifying their drug like properties and toxicity. Dataset of these 14 hit compounds was subjected for measuring significant parameters such as dipole moment, electronic energy, energy of Frontier Molecular Orbitals (FMO) and hardness of molecules by performing DFT as a result, one compound was selected as lead compound for anti-diabetic agent.

1. Introduction

Medical engineering and scientific outcomes about endocrine metabolic disorders and their mechanisms of proliferation have been enhanced but contagious afflictions are still the foremost reason of morbidity and mortality in the entire world. Several diseases have been controlled through the regulation of digestive enzymes [1]. Any disturbance in their regulation process can cause serious infections. One of the most conventional, complex and progressive endocrine metabolic disorder is diabetes mellitus which is substantial health problem and widely distributed among all age groups of entire world [2]. According to World Health Organization increase in diabetic population would be 300 million or more by the year 2025 [3].

1.1. Therapeutic Molecular anti-diabetic targets

In our study we have selected five different remedial molecular targets which were reported for diabetes and virtually screened analogues of daphnodorins against these targets. Antidiabetic agents are stated as all the diverse forms of medicine with the omission of insulin that have been permitted to cure type 2 diabetes mellitus.

1.2. Alpha amylase and alpha-glucosidase inhibitors

Alpha amylase is the crucial enzyme among all digestive enzymes that participates in the breakdown of starch to maltose and conclusively into glucose [4]. It has been suggested that suppression of α-amylase by inhibitors can competently inhibit the arising level of blood glucose [5]. Acarbose, voglibose and miglitol are the FDA approved drugs which are used as α-amylase and α-glucosidase inhibitors with distasteful side effects like diarrhea and flatulence [4].

1.3. Dipeptidyl Peptidase-4 inhibitors

Glucagon-like peptide-1 (GLP-1) is a gastrointestinal hormone whose excretion is responsible for enrichment of pancreatic β-cell mass, lowering of glucagon secretion and elevation of muscle glucose uptake [6] Owing to degradation by serine protease dipeptidyl peptidase (DPP)-4 only 25% GLP-1 is available for entrance in circulation in its unbroken form [6] Suppression of DPP-4 by specific inhibitors can defend the deterioration of GLP-1 which sequentially enhance secretion of beta-cell insulin [7]. FDA approved DPP-4 inhibitors are sitagliptin, saxagliptin, linagliptin and alogliptin [8]. All these gliptins are proclaimed with serious infections like nasopharyngitis, upper respiratory tract infections and hypersensitive reactions [9].

1.4. Aldose Reductase

Aldose reductase is an enzyme which is present in most of the parts of the body which is convoluted in the catalyzation step of polyol pathway which is reliable for fructose formation from glucose. Action of this enzyme rises with the arising level of glucose in tissues of not insulin sensitive diabetic patients [10]. Aldose reductase inhibitor used in the diabetic therapy is fidarestat [11].

1.5. Emerging Role of Daphnodorins as Natural Products in Drug Discovery

The traditional drugs which are accessible in the market to cure diabetes generate distinct side effects. By considering these issues, demand of discovery of new drugs with reduced side effects has been increased. Natural products being chemically diverse compounds act as affluent pathway for the invention of new and versatile drugs under contemplation [12]. They have verified affluent modulators of antidiabetic targets. Plants have been used from ancient times due to their beneficial and curative properties [13]. Plant species of the genus *Daphne* which belongs to the Thymeleaceae family have excellent biological potential and remedial worth for pharmacologically effective compounds [14]. *Daphne tangutica Maxim* (Thymelaeaceae) is a shrub which is famous for its evergreen characteristics and turns out to be in Semitropical areas and Northern China. Roots of this shrub had been used to cure wounds and bruises as a folk medicine in China [15].

Daphnodorins and their analogues are contemplated as a pharmacologically significant scaffold which have demonstrated numerous biological activities such as antifungal and insecticidal activity [16] antibacterial, cytotoxic, antiviral, anti-HIV activity, antioxidant activity [17], inhibitory activity against ∝-glucosidase and angiotensin II formation [18]. Recent pharmacological studies have manifested their efficient and versatile anti-inflammatory, anti-oxidant and protein glycation inhibition activities [19]. Potential use of daphnodorins in food and pharmaceutical industry and their unique structural features have stimulated many chemists to carry out further research in depth [20].

Continuous discovery of drugs from nature creates a motivational force for researchers to create techniques for screening these metabolites and drugs obtained from natural products. So, new methodologies like combinatorial chemistry, virtual screening, computer aide drug designing and information technology have been exposed in drug discovery. These techniques have therapeutically potential to make use of structural complexity and unique binding modes. Identification and development of new drugs through their optimization has been increased due to reducing time for researchers rather than their synthesis [21].

1.6. Lead Compound Development

Virtual Screening is classified as rational drug discovery parameter, more direct, less time consuming, and economical from research point of view [22] [23]. Virtual screening tools help aide in evaluating the pharmacologically active compounds of daphnodorins [24].

1.6.1. Structure Based Designing

The aim of this paper is to investigate the use of daphnodorins analogues for the first time as anti-diabetic inhibitors on the basis of significant features by computational analysis [25]. The analyses accomplished included ADMET analysis of daphnodorins analogues, Virtual screening (VS) by way of molecular docking [26, 27] of daphnodorin derivative with targeted proteins of antidiabetic inhibitors and study of optimized molecular geometry for future derivatization through density functional theory (DFT) calculations [23]. ADMET are distinctive pharmacokinetic features of a ligand that contract mainly with its Absorption, Distribution, Metabolism, Excretion and toxicity.

VS is classified as rational drug discovery parameter, more direct, less time consuming, and economical from research point of view [22], [23], [24]. To elaborate the electronic features optimization of energy and molecular features of potential ligands density functional theory (DFT) was implemented [28],[29].

2. Result And Discussion

2.1. Evaluation Metrics for Molecular Docking

About 38 compounds library of daphodorin derivatives was subjected to virtual screening by using Autodock tools and interacted or analogized their binding energies. All compounds (Table 1) were originally docked through molecular docking for five different targets and the threshold which was used for their exposition was − 9.0 kcal/mol for anti-diabetic activity. This virtual screening categorized the compounds into active and inactive compounds. Subsequently by employing threshold it was recognized that 14 compounds proclaimed this threshold for antidiabetic activity [23]. Due to vast number of compounds and comprehensive docking studies we have selected compounds with desired binding energy. To evaluate our work, we preferred lowest energy and high binding affinity [30].

2.2. Molecular Docking for Antidiabetic Activity

2.2.1. Docking Study for Alpha amylase (PDB ID: 3BAJ)

Docking counterfeits of applicant ligands with alpha amylase co-crystal structure (PDB ID: 3BAJ) using Auto dock tools predicted that acarbose has value of binding affinity − 6.9 kcal/mol whereas daphnodorin E (**4**) and Daphnegiralin C2 (**38**) showed docking score of -10.0 kcal/mol and − 9.8 kcal/mol which is the best binding score with alpha amylase and these are selected as lead compounds [23]. Daphnodorin E (**4**) showed hydrogen bonding with HIS 299, HIS 305, ASP 300, ASP 197 and TRP 59 and hydrophobic interactions with TYR 62, HIS 305, LEU 162 with protease of alpha amylase (Fig. 1A). The detailed analysis along with types of bonds with their distances formed between the amino acids were provided in Table 2 (*supplementary material*: Fig. 1B & C) [31]. Drug-target interactions were assumed in provision of interfacing amino acids fragments. Hydrogen bonding, docking energy investigation, presumed binding sites and distinctions of active site amino acid residues. The docking evaluation of all ligands within active binding energies were depicted in Table 2. Total binding strength is a consequence of different kinds of bonds inclusive of ionic, hydrophobic interactions, Vander Waals forces and hydrogen bonds which are the promoter. Docking investigation explored that all lead compounds were capable of forming compatible hydrogen bond interactions and hydrophobic interactions [32].

Table 2
Molecular Docking data of studied molecules for alpha amylase (PDB ID: 3BAJ)

Ligand	Binding affinity, ΔG (Kcal/mol)	Hydrogen Bonding			Types of Interactions			Electrostatic	
					Hydrophobic				
		Amino Acids	Type	Distance	Amino acids	Distance	Type	Amino acids	Distance
Daphnodorin A (**1**)	-9.3	HIS 305 THR 163		2.664 2.458	TRP 59 TRP 59 LEU 165 TRP 59 LEU 162 LEU 165	3.716 3.782 5.122 5.366 5.253 4.982			
Daphnodorin B (**2**)	-9.1	HIS 305 ASP 197		3.001 2.433	TRP 59 TRP 59 HIS 305	3.786 4.315 5.564		ASP 300	4.320
Daphnodorin C (**3**)	-9.1	HIS 305 ASP 197		2.237 2.476	TRP 59 TRP 59 HIS 305	3.779 4.311 5.555		ASP 300	4.320
Daphnodorin E (**4**)	-10.0	HIS 299 HIS 305 ASP 300 ASP 197 TRP 59	Conventional Conventional Conventional Conventional Conventional	2.062 2.409 2.011 2.390 2.327	TYR 62 HIS 305 LEU 162	4.273 4.792 5.101			
Daphnogirin A (**7**)	-9.0	GLN 63 THR 163 ASP 197 ASP 300 HIS 201	H-Bonding H-Bonding H-Bonding H-Bonding Pi-cation	2.425 2.573 3.051 2.792	LEU 165 TYR 62 HIS 201 ALA 198 LYS 200 ILE 235	3.775 4.678 4.430 5.003 5.210 4.840	Pi-Sigma Pi Stacked Pi T-shaped Pi-Alkyl Pi-Alkyl Pi-Alkyl	GLU 233 ASP 300	4.338 3.707
Stelleranol (**9**)	-9.0				LEU 165 LEU 162	5.047 5.012	Pi-Alkyl Pi-Alkyl		
Daphanodorin H (**12**)	-9.5	ASP 300 HIS 305 HIS 305	Conventional C-H Bond Pi-Donor	2.338 3.789 2.973	LEU 162 LEU 165	4.986 4.917	Pi-Alkyl Pi-Alkyl		
Daphnegiralin A4 (**32**)	-9.0	GLN 63	Pi-Donor Hydrogen Bond	3.1983	TYR 62 TRP 59 TRP 59 TRP 59 TRP 59	3.625 5.817 4.863 4.434 4.465	Pi-Sigma Pi-Pi Stacked Pi-Pi Stacked Pi-Alkyl Pi-Alkyl		

Ligand	Binding affinity, ΔG (Kcal/mol)	Hydrogen Bonding			Types of Interactions			Electrostatic	
					Hydrophobic				
Daphegiralin B1 (**33**)	-9.0				TRP 59	3.636	Pi-Sigma		
					TRP 62	3.864	Pi-Sigma		
					TYR 62	4.214	Pi-Alkyl		
					LEU 162	5.485	Pi-Alkyl		
					LEU 165	5.457	Pi-Alkyl		
					LEU 162	5.354	Pi-Alkyl		
Daphnegiralin B4 (**36**)	-9.1	GLN 63	Conventional	2.544	TRP 59	3.839	Pi-Sigma		
					TYR 62	3.613	Pi-Sigma		
					TRP 59	5.022	Pi-Alkyl		
					TYR 62	4.457	Pi-Alkyl		
					HIS 299	4.304	Pi-Alkyl		
					LEU 162	4.862	Pi-Alkyl		
					LEU 165	5.370	Pi-Alkyl		
Daphnegiralin C1 (**37**)	-9.2	TYR 62	Pi-Donor	2.595	TRP 59	3.778	Pi-Pi Stacked		
			H-Bond		TRP 59	4.140	Pi-Pi Stacked		
					TRP 62	4.429	Pi-Pi Stacked		
Daphnegiralin C2 (**38**)	-9.8	GLU 233	Conventional	2.376	TRP 59	3.885	Pi-Pi Stacked	ASP 300	3.930
					TRP 59	4.327	Pi-Pi Stacked		
					TYR 62	4.302	Pi-Pi Stacked		
					TRP 59	5.372	Pi-Alkyl		
Acarbose (standard)	-6.9	HIS 305	Conventional	2.525					
		HIS 305	Conventional	2.389					
		HIS 305	Conventional	2.524					
		HIS 305	Conventional	2.253					
		HIS 305	Conventional	1.747					
		GLU 233	Conventional	2.861					
		ASP 300	C-H bond	2.973					
		HIS 305	Pi-Donor	2.415					

2.2.2. Docking Study for dipeptidyl peptidase-IV (PDB ID: 2P8S)

This study was basically performed to perceive the binding approaches of compounds into co-crystal structure DPP-IV (PDB ID: 2P8S) and to analyze active inhibitors from the analogues of daphnodorins as compared to sitagliptin which is the standard drug [33]. Results obtained after interpretation of all the desired compounds for their retardation activity against DPP-IV were articulated in Table 3 (Fig. 2A, *Supplementary material*: Fig. 2B & C). Daphnegiralin B1 **(33)** and B4 **(35)** have best binding affinity as compared to all hit compounds which have binding affinity score more than − 9.0 kcal/mol. Compound **35** showed conventional hydrogen bonding interactions with amino acid ARG 125 and electrostatic interaction with HIS 740 while Compound **33** did not show any type of such interactions [34] (Fig. 3). Compound **33** has promising van der Waals interactions with TYR 547, PHE 357, TYR 547, TYR 666 and **35** has convenient interactions with TYR 547 and TYR 585 [35] (Fig. 3).

Table 3
Molecular Docking data of studied molecules for dipeptidyl peptidase-IV (PDB ID: 2P8S)

Ligand	Binding affinity, ΔG (Kcal/mol)	Hydrogen Bonding			Types of Interactions			Electrostatic	
					Hydrophobic				
		Amino acids	Type	Distance	Amino acids	Distance	Type	Amino acids	Distance
Daphnodorin A **(1)**	-9.5	TYR 547	Conventional	2.903	TYR 547	5.402	Pi-Alkyl	ARG 125	3.929
		SER 630	Conventional	1.892					
		HIS 740	Conventional	2.984					
Daphnegiravone A **(21)**	-9.4				TYR 547	3.577	Pi-Sigma	ARG 125	3.952
						5.490			
					PHE 357	4.137	Pi-Pi T-shaped		
					TYR 547		Pi-Alkyl		
Daphnegiravone B **(22)**	-9.1	ARG 125	Pi-Cation; Pi-Donor Hydrogen Bond	3.419					
Daphnegiravone C **(23)**	-9.3	TYR 547	Conventional	2.342	ARG 125	4.975	Alkyl		
		SER 830	Conventional	2.193		5.201	Pi-Alkyl		
		ARG 125	Pi-Donor	3.653	HIS 126				
Daphnegiralin A1 **(29)**	-9.0	ARG 125	Pi-Cation;	4.172	TYR 547	4.167	Pi-Alkyl	ARG 125	4.715
			Pi-Donor			4.612	Pi-Alkyl		
					TYR 547				
Daphnegiralin B1 **(33)**	-9.8				TYR 547	5.77	Pi-Pi T-shaped		
						4.739	Pi-Alkyl		
					PHE 357	5.095	Pi-Alkyl		
					TYR 547	5.024	Pi-Alkyl		
					TYR 666				
Daphnegiralin B2 **(34)**	-9.5	ARG 125	Conventional	2.871	SER 552	3.950	Pi-Sigma		
		ARG 125	Conventional	1.995		4.809	Pi-Alkyl		
		ARG 125	Conventional	2.349	TYR 547				
Daphnegiralin B3 **(35)**	-9.6	ARG 125	Conventional	2.516	TYR 547	4.575	Pi-Alkyl		
		ARG 125	Conventional	1.828					
		CYS 551	Conventional	2.139					
Daphnegiralin B4 **(36)**	-9.9	ARG 125	Conventional	2.862	TYR 547	4.848	Pi-Alkyl	HIS 740	4.628
						5.219	Pi-Alkyl		
					TYR 585				
Daphnegiralin C1 **(37)**	-9.0	HIS 740	Conventional	2.958	TYR 547	3.497	Pi-Sigma		
		HIS 740	C-H bond	3.025		5.055			
					TYR 547		Pi-Pi Stacked		
Sitagliptin (standard)	-8.8	ARG 125	Conventional	1.920	PHE 357	4.738	Pi-Pi T-shaped		
		ARG 125	Conventional	2.508		5.106			
		ARG 125	Conventional	2.432	HIS 740		Pi-Alkyl		
		TYR 547	Conventional	1.930					
		TYR 585	Conventional	2.443					
		SER 630	Conventional	1.981					
		HIS 740	Conventional	2.768					

2.2.3. Docking Studies for Glycogen Synthase Kinase-3 (GSK-3) (PDB ID: 3SAY)

Numerous ligands were docked against the target protein GSK-3 (PDB ID: 3SAY) to perform docking inquisition. From this investigation binding affinity of various ligands was derivatized and we have selected compounds with desired binding energy of -9.0 kcal/mol. To evaluate our work, we preferred lowest energy and high binding affinity and it was recognized that 14 compounds proclaimed this threshold. Daphnodorin A **(1)**, Daphnegiralin A4 **(32)**, B1 **(33)**, B4 **(36)** and C1 **(37)** showed best docking score above − 10.0 kcal/mol among all these compounds. These ligands can be arranged in decreasing order of binding affinity in such a way:

(1) = (32) = (33) > (36) = (37)

Interactions among all these ligands and binding sites of receptor protein are superintended by hydrogen bonds and hydrophobic interactions (Fig. 3A, *Supplementary material*: Fig. 3B & C). Compound **32** showed four conventional types of hydrogen bonds as compared to **1** and **32** while numerous types of hydrophobic interactions such as pi-alkyl, alkyl and pi-sigma are present in both **32 and 33** compounds as compared to compound **1** which have pi-alkyl and alkyl type of hydrophobic interactions (Fig. 3A) [36]. All these interactions with certain amino acids occurred at different distances shown in Table 4.

Table 4
Molecular Docking data of studied molecules for Glycogen Synthase Kinase-3 (PDB ID: 3SAY)

Ligand	Binding affinity, ΔG (Kcal/mol)	Hydrogen Bonding			Types of Interactions			Electrostatic	
					Hydrophobic				
		Amino acids	Type	Distance	Amino acids	Distance	Type	Amino acids	Distance
Daphnodorin A (**1**)	-10.2	ARG 141	Conventional	1.999	ALA 83	4.228	Alkyl		
		ASN 186	Conventional	2.528	LEU 188	4.668	Alkyl		
		ASP 200	Conventional	2.684	ILE 62	5.051	Pi-Alkyl		
					VAL 110	5.118	Pi-Alkyl		
					LEU 132	4.225	Pi-Alkyl		
					LEU 188	5.315	Pi-Alkyl		
Daphnodorin B (**2**)	-9.0	ASN 64	Conventional	2.488	VAL 70	4.999	Pi-Alkyl	ASP 200	3.821
		LYS 183	Conventional	2.220	LEU 188	5.462	Pi-Alkyl		
		ASN 64	Conventional	2.615	CYS 199	4.752	Pi-Alkyl		
		GLN 185	Conventional	2.507					
		GLY 63	C-H bond	3.257					
		GLY 63	C-H bond	3.793					
		CYS 199	Pi-Donor	3.920					
Daphnegiravone A (**21**)	-9.2	ASN 64	Conventional	1.989	LEU 188	3.413	Pi-Sigma		
		VAL 135	Conventional	2.373	VAL 70	4.433	Alkyl		
					LYS 85	4.485	Alkyl		
					CYS 199	4.845	Alkyl		
					ALA 83	4.063	Pi-Alkyl		
					CYS 199	5.254	Pi-Alkyl		
					LEU 188	4.914	Pi-Alkyl		
Daphnegiravone B (**22**)	-9.9	VAL 135			VAL 70	3.887	Pi-Sigma	ARG 141	4.860
					VAL 70	4.613	Pi-Alkyl		
					ILE 62	4.966	Pi-Alkyl		
Daphnegiravone C (**23**)	-9.2	ARG 141	Conventional	2.466	ILE 62	4.832	Alkyl		
		ARG 141	Conventional	2.024	ALA 83	5.050	Alkyl		
		CYS 199	Conventional	2.923	LEU 188	5.245	Alkyl		
		ILE 62	Conventional	1.953	TYR 134	5.119	Pi-Alkyl		
					VAL 70	4.654	Pi-Alkyl		
					ALA 83	5.292	Pi-Alkyl		
					LYS 85	5.390	Pi-Alkyl		
					CYS 199	4.517	Pi-Alkyl		

Ligand	Binding affinity, ΔG (Kcal/mol)	Hydrogen Bonding			Types of Interactions			Electrostatic
					Hydrophobic			
Daphnegiralin A1 (**29**)	-9.0	LYS 85	C-H bond		TYR 134	5.973	Pi-Pi T-shaped	
					VAL 70	5.326	Alkyl	
					LYS 85	4.881	Alkyl	
					LEU 132	4.763	Alkyl	
					CYS 199	4.318	Alkyl	
					VAL 110	4.472	Alkyl	
					LEU 132	4.398	Alkyl	
					CYS 199	3.781	Alkyl	
					VAL 70	4.731	Pi-Alkyl	
					ILE 62	5.084		
							Pi-Alkyl	
Daphnegiralin A3 (**31**)	-9.2				TYR 134	5.819	Pi-Pi T-shaped	
					VAL 70	5.360	Alkyl	
					LYS 85	4.968	Alkyl	
					LEU 132	4.741	Alkyl	
					CYS 199	4.286	Alkyl	
					VAL 110	4.468	Alkyl	
					LEU 132	4.242	Alkyl	
					CYS 199	3.915	Alkyl	
					VAL 70	4.863	Pi-Alkyl	
					CYS 199	5.276	Pi-Alkyl	
					ILE 62	5.131	Pi-Alkyl	
Daphnegiralin A4 (**32**)	-10.2	ARG 141	Conventional	2.570	LEU 188	3.728	Pi-Sigma	
		ARG 141	Conventional	2.293	ALA 83	4.164	Alkyl	
		ARG 141	Conventional	2.400	LEU 188	4.415	Alkyl	
		ARG 141	Conventional	1.899	ILE 62	5.037	Pi-Alkyl	
					ALA 83	4.556	Pi-Alkyl	
					VAL 11	5.327	Pi-Alkyl	
					LEU 132	5.329	Pi-Alkyl	
					CYS 199	4.393	Pi-Alkyl	
Daphnegiralin B1 (**33**)	-10.2	ARG 141	Conventional	2.576	LEU 188	3.690	Pi-Sigma	
		ARG 141	Conventional	2.398	ALA 83	4.167	Alkyl	
		ASP 200	Conventional	2.869	LEU 188	4.371	Alkyl	
					ILE 62	5.048	Pi-Alkyl	
					ALA 83	4.561	Pi-Alkyl	
					VAL 110	5.251	Pi-Alkyl	
					LEU 132	5.282	Pi-Alkyl	
					CYS 199	4.368	Pi-Alkyl	

Ligand	Binding affinity, ΔG (Kcal/mol)	Hydrogen Bonding			Types of Interactions			Electrostatic
					Hydrophobic			
Daphnegiralin B2 (**34**)	-9.6				LEU 188	3.995	Pi-Sigma	
					LEU 188	3.787	Pi-Sigma	
					Val 70	4.759	Alkyl	
						4.865		
						4.975		
					ALA 83	4.932	Alkyl	
					LYS 85	5.186	Alkyl	
					LEU 132	4.788	Alkyl	
					CYS 199	5.496	Alkyl	
					CYS 199		Alkyl	
					ALA 83		Pi-Alkyl	
Daphnegiralin B3 (**35**)	-9.6	VAL 135	Conventional	2.091	VAL 70	5.224	Alkyl	
					ALA 83	4.896	Alkyl	
					LEU 132	4.904	Alkyl	
					CYS 199	4.407	Alkyl	
					LEU 188	4.734	Pi-Alkyl	
Daphnegiralin B4 (**36**)	-10.1	ARG 141	Conventional	2.0991	LEU 188	3.694	Pi-Sigma	
		ASP 200	Conventional	2.4391	ILE 62	4.965	Alkyl	
		GLN 185	Conventional	2.2609	ALA 83	4.112	Alkyl	
					LEU 188	4.357	Alkyl	
					ILE 62	4.826	Pi-Alkyl	
					ALA 83	4.707	Pi-Alkyl	
					VAL 110	4.978	Pi-Alkyl	
					LEU 132	5.051	Pi-Alkyl	
					CYS 199	4.198	Pi-Alkyl	
Daphnegiralin C1 (**37**)	-10.1	ARG 141	Conventional	3.053	LEU 188	3.704	Pi-Sigma	
		ARG 141	Conventional	2.098	ILE 62	4.936	Alkyl	
		ILE 62	Conventional	2.951	ALA 83	4.111	Alkyl	
		GLN 185	Conventional	2.100	LEU 188	4.357	Alkyl	
					ILE 62	4.823	Pi-Alkyl	
					ALA 83	4.715	Pi-Alkyl	
					VAL 110	4.978	Pi-Alkyl	
					LEU 132	5.046	Pi-Alkyl	
					CYS 199	4.192	Pi-Alkyl	
Daphnegiralin C2 (**38**)	-9.4				LEU 188	3.773	Pi-Sigma	
					VAL 70	4.190	Alkyl	
					LYS 85	5.086	Alkyl	
					CYS 199	5.174	Alkyl	
					ALA 83	3.755	Pi-Alkyl	
					CYS 199	5.471	Pi-Alkyl	

Ligand	Binding affinity, ΔG (Kcal/mol)	Hydrogen Bonding			Types of Interactions			Electrostatic
					Hydrophobic			
Indirubin (Standard)	-8.3	ASP 133	Conventional	2.432	LEU 188	3.367	Pi-Sigma	
		ASP 133	Conventional	2.242	ALA 83	4.008	Pi-Alkyl	
		CYS 199	Pi-donor	4.099	CYS 199	5.026	Pi-Alkyl	
						5.302		
						5.427		
					ALA 83	5.290	Pi-Alkyl	
					VAL 110	5.248	Pi-Alkyl	
					LEU 132	4.931	Pi-Alkyl	
					LEU 188	5.324	Pi-Alkyl	
					ILE 62	4.51	Pi-Alkyl	
					LEU 188		Pi-Alkyl	
					ILE 62		Pi-Alkyl	

2.2.4. Docking Results for Aldose Reductase (PDB ID: 1EKO)

Target receptor aldose reductase (PDB ID: 1EKO) binds siderostat on its active sites through hydrogen bonds through amino acid residues and hydrophobic interactions with amino acid residues (Fig. 4A, *Supplementary material*: Fig. 4B & C). From the docking analysis in Table 5, it is inspected that daphnegiralin B1 **(33)** and B3 **(35)** has comparable and better binging affinity as compared to standard molecule which specifies stabilized interaction between these compounds and target receptor (Fig. 4A). It can be perceived that two types of interactions with hydrogen and hydrophobic interactions are present between target protein and compounds **33** and **35**. Compound **33** interacts via three hydrogen bonds with amino acids TRP 20, TRP 111 and ALA 299 and make hydrophobic interactions with TRP 20, TRP 219, PRO 218 and LEU 300. While **35** interacts with amino acid residues TRP 20, CYS 298, ALA 299 and TRP 20 through four hydrogen bonds and various types of hydrophobic interactions can also be observed from Table 5.

Table 5
Molecular Docking data of studied molecules for Aldose Reductase (*PDB ID: 1EKO)*

Ligand	Binding affinity, ΔG (Kcal/mol)	Hydrogen Bonding			Types of Interactions			Electrostatic	
					Hydrophobic				
		Amino Acids	Type	Distance	Amino acids	Distance	Type	Amino acids	Distance
Daphnodorin A (**1**)	-9.2				TRP 20	5.305	Pi-Pi Stacked		
					PHE 122	3.885	Pi-Pi Stacked		
					TRP 219	3.914	Pi-Pi Stacked		
					TRP 219	5.615	Pi-Pi Stacked		
					TRP 219	3.798	Pi-Pi Stacked		
					TRP 219	5.975	Pi-Pi		
					PHE 122	4.601	Stacked		
					CYS 298	4.364	Pi-Pi Stacked		
					LEU 300	4.261	Pi-Pi T-shaped		
					TRP 219	4.496	Alkyl		
							Alkyl		
							Pi-Alkyl		
Daphnodorin B (**2**)	-9.2				TRP 20	5.317	Pi-Pi Stacked		
					PHE 122	3.856	Pi-Pi Stacked		
					TRP 219	3.911	Pi-Pi Stacked		
					TRP 219	5.624	Pi-Pi Stacked		
					TRP 219	3.793	Pi-Pi Stacked		
					TRP 219	5.991	Pi-Pi Stacked		
					PHE 122	4.584	Pi-Pi Stacked		
							Pi-Pi T-shaped		
Daphnegiravone B (**22**)	-9.7	TRP 20		2.57904	TRP 129	3.740	Pi-Sigma		
		HIS 110		1.93975	TRP 20	4.901	Pi-Pi T-shaped		
					TRP 20	4.607	Pi-Pi T-shaped		
Daphnegiravone C (**23**)	-9.4	TRP 20	Conventional	2.98608					
		VAL 297	Conventional	2.42064					

Ligand	Binding affinity, ΔG (Kcal/mol)	Hydrogen Bonding			Types of Interactions			Electrostatic
					Hydrophobic			
Daphnegiralin A4 (**32**)	-9.5				LEU 300	3.811	Pi-Sigma	
					TRP 20	3.962	Pi-Sigma	
					TRP 219	4.296	Pi-Pi Stacked	
					VAL 47	5.208	Alkyl	
					LEU 300	5.481	Alkyl	
					TRP 20	4.454	Pi-Alkyl	
					TRP 20	4.032	Pi-Alkyl	
					TRP 20	4.663	Pi-Alkyl	
					TYR 48	4.914	Pi-Alkyl	
					HIS 110	4.732	Pi-Alkyl	
						5.227	Pi-Alkyl	
						5.428	Pi-Alkyl	
					HIS 110	4.576	Pi-Alkyl	
					PHE 122	5.185	Pi-Alkyl	
					TRP 219			
					VAL 47			
Daphnegiralin B1 (**33**)	-9.9	TRP 20	Conventional	2.998	TRP 20	3.553		
		TRP 111	Conventional	2.192	TRP 219	3.961		
		ALA 299	Conventional	2.084	TRP 219	3.628		
					TRP 219	4.993		
					PRO 218	4.195		
					LEU 300	5.157		
					TRP 219	4.571		
					TRP 219	5.122		
Daphnegiralin B2 (**34**)	-9.0	TRP 20	Conventional	1.739	TRP 20	3.314	Pi-Sigma	
		HIS 110	Pi-Donor H-Bond	2.620	PHE 122	3.864	Pi-Sigma	
					PHE 122	3.849	Pi-Sigma	
					TRP 20	5.294	Pi-Pi Stacked	
					TRP 20	5.027	Pi-Pi Stacked	
					TRP 219	5.582		
					TRP 20	5.479	Pi-Pi Stacked	
					PRO 218	5.445	Pi-Alkyl	
							Pi-Alkyl	
Daphnegiralin B3 (**35**)	-9.8	TRP 20	Conventional	2.933	TRP 20	3.579	3.579	
		CYS 298	Conventional	3.676	TRP 219	3.945	3.945	
		ALA 299	Conventional	2.105	TRP 219	3.634	3.634	
		TRP 20	Pi-Donor	2.878	TRP 219	4.958	4.958	
					PRO 218	4.236	4.236	
					LEU 300	5.158	5.158	
					TRP 219	4.553	4.553	
					TRP 219	5.130	5.130	

Ligand	Binding affinity, ΔG (Kcal/mol)	Hydrogen Bonding			Types of Interactions			Electrostatic
					Hydrophobic			
Daphnegiralin B4 (**36**)	-9.3	HIS 110	Conventional	1.981	TRP 20	3.770	Pi-Sigma	
		TRP 111	Conventional	2.867	PHE 122	3.632	Pi-Sigma	
		CYS 298	Conventional	3.335	TRP 20	5.473	Pi-Pi Stacked	
		CYS 298	Conventional	2.360	TRP 20	4.889	Pi-Pi Stacked	
					VAL 47	4.980		
					TRP 20	4.713	Alkyl	
					PHE 122	4.691	Pi-Alkyl	
					TRP 219	4.933	Pi-Alkyl	
							Pi-Alkyl	
Daphnegiralin C1 (**37**)	-9.0	TYR 48		2.751	TYR 209	3.698	Pi-Sigma	
		HIS 110		2.454	TRP 20	4.813	Pi-Pi Stacked	
					TRP 20	5.002	Pi-Pi Stacked	
					TRP 219	4.105		
					CYS 298	4.887	Pi-Pi Stacked	
					LEU 300	4.721	Alkyl	
					TRP 219	4.348	Alkyl	
					VAL 47	4.985	Pi-Alkyl	
					LEU 300	5.275	Pi-Alkyl	
							Pi-Alkyl	
Fidarestat (standard)	-8.0	TYR 48	Conventional	2.878	TRP 20	4.444	Pi-Pi Stacked	
		HIS 110	Conventional	2.775	TRP 20	5.007	Pi-Pi Stacked	
		TRP 111	Conventional	2.230	VAL 47	5.011		
		TRP 20	Pi-Donor	3.095			Pi-Alkyl	
		TRP 20	Pi-Donor	2.454				

2.2.5. Molecular Docking analysis for alpha Glucosidase (PDB ID: 1VJT)

Alpha glucosidase inhibitory potential of selected compounds was assessed by docking daphnodorin derivatives into allosteric sites of crystal structure of alpha glucosidase (PDB ID: 1VJT). Daphnegiravone A (**21**) and Daphnegiralin B4 **(36)** exhibited various interrelations inclusive of hydrogen-bonding and hydrophobic interactions (Fig. 5A). Compound **(21)** interact with amino acids ARG 43, ASN 160, SER 94 and THR 158 and **36** with amino acids SER 10, ALA 159, TYR 85 through strong hydrogen bonding while **21** exhibited two hydrophobic interactions with amino acids ALA 159 and TYR 85 and **36** LEU 306, ARG 310, ARG 12, TYR 85. Daphnegiravone D **(24)** did not show any type of hydrogen bonding but displayed numerous hydrophobic interactions such as pi-sigma, alkyl and pi-alkyl with residual amino acids mentioned in Table 6 (Fig. 5A: *Supplementary material*) [37].

Table 6
Molecular Docking data of studied molecules for alpha glucosidase (PDB ID:1VJT)

Ligand	Binding affinity, ΔG (Kcal/mol)	Hydrogen Bonding			Types of Interactions			Electrostatic	
					Hydrophobic				
		Amino Acids	Type	Distance	Amino acids	Distance	Type	Amino acids	Distance
Daphnegiravone A (**21**)	-10.2	ARG 43	Conventional	2.705	ALA 159	3.903	Alkyl		
		ASN 160	Conventional	2.889	TYR 85	4.053	Pi-Alkyl		
		SER 94	Conventional	2.135					
		THR 158	Conventional	2.047					
Daphnegiravone D (**24**)	-9.2				TYR 85	3.696	Pi-Sigma		
					ALA 84	4.851	Alkyl		
					ALA 84	3.768	Alkyl		
					ALA 159	3.225	Alkyl		
					VAL 39	5.205	Alkyl		
					PRO 86	5.462	Alkyl		
					TYR 85	4.334	Pi-Alkyl		
Daphnegiralin B4 (**36**)	-9.1	SER 10	C-H bond	3.776	LEU 306	3.920	Alkyl		
		ALA 159	C-H bond	3.336	ARG 310	4.271	Alkyl		
		TYR 85	Pi-donor	2.999	ARG 12	4.353	Alkyl		
					TYR 85	5.079	Pi-Alkyl		
Acarbose (standard)	-6.6	THR 83	Conventional	1.92961					
		GLY 95	Conventional	1.63064					
		THR 125	Conventional	2.36349					
		THR 125	Conventional	2.43829					
		TYR 85	Conventional	2.02607					
		GLY 7	Conventional	2.32329					
		SER 10	Conventional	2.74075					
		TYR 85	Conventional	3.33697					

2.4. Docking Validation

Docking process is validated by using commonly employed method used for validation of docking i.e., *pose selection*. Best docked pose was selected and re-docked into co-crystal structure of active site. Keeping in view of preselected Root Mean Square Deviation RMSD value (usually 2 Å), if re-docked pose have RMSD value less than 2 Å, its mean that docking program is able to perform docking for given set of protein co-crystal structure [38, 39]. The RMSD values bar graph (Fig. 6) and overlaid structures (Fig. 7) is an indication of validation of docking of tested compounds.

2.5. Target Prediction

Docking analysis was performed for 38 compounds against five different anti-diabetic targets and it was observed that different compounds showed different docking scores. Compounds of desired threshold **(-9.0 kcal/mol)** were separated as diabetic inhibitors (hit compounds) after all docking results of all anti-diabetic targets and top results of **16 hit** compounds against multi-targets were sketched in the form of pie chart [40]. Pie chart was the graphical display which was the most favored presentation to summarize the docking results of these 16 hit compounds. Here is a pie chart (Fig. 8) with eight slices (A, B, C, D, E, F, G and H) which were identified via different colors [41] and each color was specified for specific anti-diabetic target or series of targets.

Each slice was further labelled by those hit compounds which give best threshold of binding affinity (-9.0 kcal/mol) against these single anti-diabetic targets or a series of anti-diabetic targets. For example, Daphnegiralin B2 **(34)**, B3 **(35)**, Daphnegirvone B **(22)** and C **(23)** were such compounds which have binding affinity against three anti-diabetic targets. Daphegiralin B4 **(36)** was the distinct compound which showed best binding affinity against all anti-diabetic targets Similarly, daphnodorin A (**1**), Daphnegiralin B1 **(33)** and C1 **(37)** were such compounds which exhibited required binding affinity score against four diabetic targets.

2.6. Scope of DFT

DFT is a computer-based approach used for elucidation and interpretation of reactivity and molecular structure to explore quantitative structure activity relationship [42]. Analysis of electronic characteristics of compounds through DFT plays essential role in determining their pharmacological properties [32]. For analyzing structure activity relationship, a variety of analogues have been inspected through implementation of DFT.

With the aim of study the effect of molecular structure on the inhibition proficiency and to investigate the reactivity properties considerable conformational analysis has been performed for hit compounds by implicating DFT [43]. The Density Functional Method (DFT) method has been used to carried out quantum chemical calculations and most stable conformation is obtained [44].

2.6.1. DFT Studies of hit Compounds

Daphnodorins which were isolated from stems, roots, barks and different parts of plants have structural diversity which enables their use for computational purposes. We had selected all hit compounds after docking studies for their further screening through DFT. Their electronic structures were categorically elaborated, and their results were revealed in the form of Table 7.

Table 7
Quantum chemical parameters based upon DFT computations for the purpose of SAR studies at DFT/B3LYP/3-21G

Sr#	Compounds	Parameters												
		E_{LUMO} (eV)	E_{HOMO} (eV)	ΔE (eV)	A (eV)	I (eV)	□ (eV)	μ (eV)	□ (eV)	S	□ (eV)	N (eV)	ΔN_{max} (eV)	d.p D
1	Daphnodorin A	-0.0775	-0.1795	0.10199	0.0775	0.1795	0.12854	−0.1285	0.050	10	0.1652	6.052	2.570	5.14
2	Daphnodorin B	-0.0709	-0.1652	0.09434	0.0709	0.1652	0.11805	-0.1180	0.0471	10.6	0.1478	6.765	2.682	8.23
7	Daphnogirin A	-0.0742	-0.2269	0.1527	0.0742	0.2269	0.15062	-0.1506	0.0763	6.54	0.0226	6.731	1.972	4.84
12	Daphnodorin H	-0.0744	-0.2063	0.1319	0.0744	0.2063	0.1403	-0.1403	0.0659	7.57	0.1493	6.697	2.127	4.12
21	Daphnegiravone A	-0.0795	-0.2112	0.1317	0.0795	0.2112	0.1096	-0.1096	0.0658	7.59	0.0911	10.96	1.664	2.45
22	Daphnegiravone B	-0.0831	-0.2147	0.1315	0.0831	0.1315	0.14891	-0.1489	0.0679	7.59	0.1685	5.934	1.668	4.75
23	Daphnegiravone C	-0.0632	-0.2060	0.1428	0.0632	0.2060	0.13466	-0.1346	0.0714	7.00	0.1269	7.880	1.885	1.84
29	Daphnegiralin A1	-0.0009	-0.2040	0.20497	0.0009	0.2040	0.10248	-0.1024	0.1015	4.92	0.0517	19.33	1.009	1.68
31	Daphnegiralin A3	-0.0107	-0.2127	0.20195	0.0107	0.2127	0.11725	-0.1172	0.1009	4.80	0.0618	16.16	1.106	4.27
32	Daphnegiralin A4	-0.0093	-0.2146	0.00935	0.2146	0.2053	0.11102	-0.1110	0.1016	4.91	0.0606	16.49	1.091	3.51
33	Daphnegiralin B1	-0.0105	-0.2082	0.19776	0.0105	0.2082	0.10939	-0.1093	0.1977	2.52	0.0302	33.11	0.553	5.23
34	Daphnegiralin B2	-0.0112	-0.2139	0.2027	0.0112	0.2139	0.1126	-0.1126	0.1013	4.93	0.0625	16	1.111	5.97
35	Daphnegiralin B3	-0.0117	-0.2126	0.20098	0.0117	0.2126	0.11119	-0.1111	0.1004	4.97	0.0596	16.75	0.553	2.86
36	Daphnegiralin B4	-0.0075	-0.2070	0.09974	0.0075	0.2070	0.1073	-0.1073	0.0997	5.01	0.0577	17.32	1.076	1.30
37	Daphnegiralin C1	-0.0191	-0.2234	0.20434	0.0191	0.2234	0.1213	-0.1213	0.1021	4.89	0.0720	13.8	1.187	4.40
38	Daphnegiralin C2	-0.0073	-0.2082	0.20092	0.0073	0.2082	0.10782	-0.1078	0.1377	3.63	0.0422	23.6	0.782	0.90

2.6.1.1. Frontier Molecular Orbitals (FMO)

Energies of the FMOs are desired quantum chemical descriptors which explain the reactivity, shape and binding behavior of a complete molecule as well as molecular substituents and fragments [45]. Molecules having small energy gap are more polarizable and generally affiliated with high chemical reactivity and designated as soft molecules. So, compounds having small energy gap are less stable, more reactive and most softer [46]. Hard molecules are those which have large energy gap and possessed high stability [47].

By summarizing the results of frontier molecular orbitals (FMOs) of compounds **(1), (2), (7), (12), (21), (22), (23), (29), (31), (32), (33), (34), (35), (36), (37)** and **(38)** which are highest occupied molecular orbitals (HOMO) and lowest unoccupied molecular orbitals (LUMO) we can arrange them according to their

stability order. From values of energy gap for all compounds which are mentioned in the Table 7 we can conclude that compounds **(32) and (36)** with smaller energy gap are considered as soft, most reactive and have smaller stability. While compound **(29)** with higher value of energy gap is considered as mostly hard, less reactive and more stable as compared to other compounds. So, stability order is as:

29 > 37 > 34 > 31 > 35 > 38 > 33 > 7 > 23 > 12 > 21 > 22 > 1 > 2 > 36 > 32

The HOMO orbitals of these compounds are mainly localized on oxygen atoms of carbonyl groups, hydroxyl groups and of 5-membered and 6-membered heterocyclic rings. While LUMO orbitals are centralized on double bonds (-C = C-) of phenyl rings. Hence, charge transfer in these compounds take place between pi(π) bonds and lone pairs [44].

2.6.1.2. Global Chemical Reactivity Descriptors

$E_{LUMO,}$ E_{HOMO} and their energy gap are used for prediction of favorable global reactivity parameters or descriptors which are useful for describing charge transfer, stability and chemical reactivity. Ionization potential of Daphnodorins is as a consequence of HOMO energies and electron affinities of the compounds are as a result of LUMO energies [48]. Electronegativity of a compound is essential descriptor because it controls the ability of a molecule to receive electrons. Molecules having lower electronegativity values have higher productivity of inhibition. From the values of the table, we can conclude that electronegativity values decrease in the following order:

7 > 22 > 12 > 23 > 1 > 37 > 2 > 31 > 34 > 35 > 32 > 21 > 33 > 38 > 36 > 29

Chemical hardness values are greater in **(33)** and **(37)** as compared to all other hit compounds and considered as least reactive. Value of chemical potential is inversely related to the reactivity and directly related to the stability [49].This parameter points out that **7** with highest value of chemical potential is less reactive and more stable. While compounds **(29)** and **(36)** with lowest value of chemical potential are considered as less stable and more reactive (Fig. 9A-C).

2.6.1.3. Molecular Electrostatic Potentials (MEP)

Molecular electrostatic potential surfaces which are also known as electrostatic potential maps are important to justify the reactivity of drugs as inhibitors [50]. These are three dimensional surfaces which are useful to analyze the charge distributions of different species [51]. In daphnodorins and their derivatives electronegative regions are detected in such parts where carbonyl and hydroxyl groups are present and most electropositive sections can be observed in conspicuously protonated regions [48]. The dark blue color displays most electropositive region while most electronegative region is represented by the dark red color. While green color illustrates neutral electrostatic potential [46]. Molecular electrostatic potential for all hit compounds have been mapped as shown in Fig. 10. MEP map in case of these compounds propose that red color signifies regions of negative potential around oxygen atoms of carbonyl groups and hydroxyl groups and are accountable for electrophilic interaction. Dark blue color indicates that hydrogen atoms possess maximum burden of positive potential and these regions are available for nucleophilic interaction. Most of the parts of these compounds bears green color which is responsible for neutral potential.

Electronegative atoms such as oxygen and nitrogen are the regions from which electrostatic forces of attraction started. Consequently, study of electrostatic potential on a continual electron density surface supported us to determine the reactivity of molecular species by indicating their electrophilic and nucleophilic components [47].

2.7. ADMET Prediction of Hit Compounds

ADMET (Absorption, Distribution, Metabolism, Excretion and toxicity) scanning aggregates pharmacokinetics and appreciable parameters of drug likeness such as mutagenicity and toxic masculinity dosage level of desired compounds for different tissues using free online servers which are helpful for exploring and evaluating ADME profiles of hit compounds obtained after molecular docking [36].

2.7.1 Pharmacokinetics Evaluation: While considering the instructiveness of compounds with enzymes it is inspected that these compounds must be use as a drug in the future. The appropriate use of compounds as a drug ADMET scrutiny shown in Table 8 of these molecules was done. The statistical values of the parameters achieved by this investigation must be within a certain criterion. If the numerical values of parameters do not follow the provided conditions it is assumed that the compound cannot be used as a drug [52]. Between the parameters studied after ADMET inspection most significant are the Lipinski Rule of 5 and the Jorgensen Rule of 3. Other constraints are solute as estimated Donor-Hydrogen bonds which is the predicted hydrogen bond sequence to be given to aqueous solution and solute as Donor-Hydrogen bonds which is the estimated number of hydrogen bonds to be approved by the component dissolved from water molecules in aqueous solution [53]. The solubility of a drug is characterized by log S which is a significant element for explaining the absorption process. Poor solubility consequences low absorption and bioavailability of drugs. The log S value of ordinary marketable drugs is bigger than − 4 and optimizing compounds for high activity on a biological objective with increasing molecular weights are inconceivable to absorbed and enter the site of action [34]

Table 8
ADMET analysis parameters to determine the drug likeness of compounds 1–38

Properties	Compounds						
		Daphnodorin A	**Daphnodorin B**	**Daphnogirin A**	**Daphnodorin H**	**Daphnegiravone B**	**Daphnegiravone C**
	Human intestinal Absorption	0.9860	0.9811		0.9727	0.9619	0.9954
Absorption	Caco-2	0.8723	-0.8913	-0.8832	-0.8208	-0.6855	0.6498
	P-Glycoprotein Substrate	-No	No	No	Yes	No	Yes
	P-glycoprotein inhibitor	Yes	Yes	Yes	Yes	Yes	Yes
	Skin permeability Log (Kp)	-5.51 cm/s	-6.29 cm/s	-6.65 cm/s	-6.18 cm/s	-6.21 cm/s	-5.17 cm/s
	Log S	-8.78	-8.20	-7.73	-8.73	-6.33	-6.44
Distribution	BBB permeability (Log BB)	+No	No	No	No	No	No
Metabolism	CYP2D6 substrate	No	No	No	No	No	No
	CYP3A4 substrate	No	Yes		Yes	Yes	Yes
	CYP1A2 inhibitor	No	No	No	No	No	No
	CYP2D6 inhibitor	No	No	No	No	No	No
	CYP3A4 inhibitor	No	N0	Yes	No	No	Yes
Excretion	Renal OCT2 inhibitor	No	No	No	No	Yes	No
Toxicity	Hepatoxicity	Yes	Yes	Yes	Yes	Yes	Yes
	Acute oral toxicity	2.523 kg/mol	2.38 kg/mol	2.933 kg/mol	2.83 kg/mol	2.516 g/mol	2.497 g/mol
Drug likeness (no. of violations)	Lipinski	2	2	2	3	0	0
	Ghose	2	2	2	2	Yes	Yes
	Veber	1	1	1	1	Yes	Yes
	Egan	1	1	1	1	Yes	Yes
Physiochemical Properties	Molecular weight	526.49 g/mol	542.49 g/mol	542.49 g/mol	568.48 g/mol	466.48 g/mol	408.49 g/mol
	No. of rotatable bonds	4	4	2	2	2	3
	No. of H-bond donors	6	7	6	6	3	2
	No. pf H-bond acceptors	9	10	10	11	8	5

2.7.2. Integrated Databases for Drug-likeness analysis

The drug-likeness of all hit compounds which were selected after molecular docking analysis was computed conferring to the Lipinski's Rule of five which is also considered as rule of good medicine and Jorgensen's rule of 3 [54]. According to the rule of good medicine molecular weight should be less than 500 g/mol, lipophilicity should be less than 5, competitor molecule must have 5 donor hydrogen bonds and 10 hydrogen bond receptors required [39]. The results of *in silico* interactions of compounds shown in Tables 2–6 to insight the prospective of compounds as drug contestants to overcome and treat diabetes and prescribe that out of 16 hit compounds **21, 22, 23, 29, 31, 32, 33**, **34, 35, 36** and **38** do not show considerable violations against significant physiochemical parameters and pharmacokinetic properties as indicated by Table 8. Because their values are within the required thresholds so, these drugs displayed acceptable oral bioavailability [32]. Daphnegiralin B4 **(36)** has values of Log S and BBB value between optimal range − 6.5 to 0.5. Distinctive dispensation parameters embrace renal organic cation transporter and P-glycoprotein non-impediment [55]. This compound did not offend Lipinski's rule of five and other rules and it has binding affinity against all diabetic targets within the required threshold which was 9.0 kcal/mol and was selected as lead compound [56].

3. Materials And Methods

3.1. Erecting of Phytochemical Library

For the purpose of making a library *(*Table 1) of compounds targeted molecules were selected from PubChem database (which comprise of 246 billion substances supported by U.S. National Library of Medicine), DNP, Coconut, Supera Natural II [57]. Similarity based search tool was applied to select daphnodorin derivatives. This led to the generation of library of compounds with 38b derivatives. They were downloaded in 3D conformer in structure-Data File (SDF) format and then loaded for optimization. These optimized compounds were retrieved in open babbled for making their pdb files which were converted into pdbqt format for molecular docking in Autodock Vina [58, 59] [60].

3.2. Protein preparation

Three-dimensional crystal structure of human pancreatic alpha-amylase in complex with acarbose (PDB ID: 3BAJ), human glycogen synthase kinase-3-beta in complex with inhibitor (PDB ID: 3SAY), human dipeptidyl peptidase-IV in complex with cyclohexalamine inhibitor (PDB ID: 2P8S), aldose reductase (PDB ID: 1EKO), alpha glucosidase (PDB ID: 1VJT) were inputted from protein data bank (PDB) (http://www.pdb.org/pdb/home.do,). Before initializing docking paralleling all water molecules and heteroatoms were removed [61]. Native ligands were removed with the aim of all compounds of our requirement would be docked in the functional site of the protein under consideration. The provision of the PDB was achieved by using Discovery Studio Visualizer v20. 1. 0. 19295 (Accelrys) [62]. The active sites of the targeted protein were anticipated and binding site sphere was made into these active sites and the resolution of targeted proteins is from 1.5–2.10 Å. Innovation of PDB file into PDBQT was achieved by Auto dock Vina by adding polar hydrogens and Kollman charges and Gasteiger charges [63].

3.3. Preparation of Ligand

For the purpose of making a library of compounds targeted molecules were selected from PubChem database [57], Coconut, DNP, Supera Natural II and ZINC database. They were downloaded in 3D conformer in SDF format and then loaded for optimization. These optimized compounds were retrieved in open babbled for making their pdb files which were converted into pdbqt format for molecular docking in Autodock Vina[55].

3.4. Studies and Grid preparation

All methodologies of completion regarding ligands and proteins for docking protocol were performed through MGL tools of Autodock vina by applying required steps. For Autodock vina study PDBQT files which are the elongated form of PDB were used which embraces atomic partial charges and atom types. Autogrid generated the rigid grid box, followed by AutoDock with Lamarckian genetic algorithm, responsible for generating best conformation in docking [64]. Output file and log file depict various poses and processes proposed during docking process [65].

3.5. Protein-Ligand Interaction

Protein ligand interactions are significant for inspecting the interaction styles between molecules. After completion of pdbqt files of proteins and ligands their results were speculated in the format of different poses of out files. These results in the form of pdbqt files were introduced to Discovery Studio Visualizer v20. 1. 0. 19295 (Accelrys) from where their 2D and 3D protein-ligand interactions were anticipated [66]. The interactions were categorized by the series of amino acids convoluted in the binding and variety of interactions between the ligand and the protein [67].

3.6. DFT (Density Functional Theory) Studies

Thus, purpose of this approach is to provide appreciative geometries for extensive range of systems. The speculative calculations have been carried out by DFT method using B3LYP 6-311G basis set [68]. For running significant chemical calculations such as molecular electrostatic potential surfaces, HOMO and LUMO surfaces and global reactivity parameters all input files of premeditated molecules were organized through Gauss View 06 program [69].

3.7. ADMET Prediction

ADMET are distinctive pharmacokinetic features of a ligand that contract mainly with its Absorption, Distribution, Metabolism, Excretion and toxicity. For evaluating these pharmacokinetic and mutagenic properties of sequestered compounds the ADMET SAR [55] and Swiss ADME servers was used. The structures of ligands and standard drugs were asserted in SMLIES format on these servers which speculates their pharmacokinetics, physiochemical characteristics along with whether a ligand has lead-likeness and drug-likeness characteristics by persecution the ligands to numerous constraints [65].

Conclusion

The traditional drugs which are accessible in the market to treat diabetes and oxidative effects caused by different enzymes generate distinct side effects. By considering these issues demand of discovery of new drugs with reduced side effects has been increased. Natural products being chemically diverse compounds act as affluent pathway for the invention of new and versatile drugs under contemplation. Daphnodorins and their analogues are contemplated as a pharmacologically significant scaffold which have verified affluent modulators for antidiabetic as well as anti-oxidant targets. These natural products embodied a prototypical pool of such incredible and chemically distinguishable molecules that have conclusively improved to collaborate with biological targets and provides us with incomparable lead compounds for drug design. This research work was purely computational approach using best utilities which were available.

Molecular docking approach was used to predict the binding energies and the interaction modes of 38 inhibitors derived from the Protein Data Bank. It was concluded that virtual screening of daphnodorins and their analogues through molecular docking represent a great set of 16 hit candidates. Quantum chemical calculations of hit compounds was performed at B3LYP/6311G basic set and their electronic structures were fully optimized to find their chemical reactivity through geometric parameters like MEP maps and contour diagrams of FMOs. After performing molecular docking and DFT studies compounds of

desired threshold (hit compounds) were analyzed by ADMET studies through Swiss ADMET, ADMET SAR, ADMET lab and ProTox-II for investigating their drug-like nature. By keeping these hit molecules as control, we have compared docking and ADMET properties against different targets. Our analysis took us to the conclusion that daphnegiralin B4 **(36)** among all ligands comes out to be a lead compound having drug like properties among 38 ligands being non-carcinogenic and non-cytotoxic for all anti-diabetic targets which would benefit medical community by providing significant weapons against.

Declarations

Conflict of Interest

There is no conflict of interest.

Supplementary Information: supplementary files available.

Data Availability Statement: All data generated or analysed during this study are included and available as its supplementary information files.

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Authors Contribution

Hafiza Ayesha Nazir[a]=Library preparation, Drafting, Molecular Docking; **Nusrat Shafiq**[a]*: conceptualization, supervision, Molecular docking study, Funding; **Uzma Arshad**[a:] Supervision, corrections, proof readings; **Rabia Zameer**[a]:Participatein paper writing;**Farah Yasmin**[a]: Drafting, library data analysis; **Fazeelat Imtiaz**[a]**:** proof reading, English check, Figure quality and resolution; **Maryam Rashid**[a]: Drafting, evaluation of results; **Shagufta Parveen**[a]: data analysis, Literature;**Bushra Nisar**[b]: verifying protocol and mechanisms;**Jallat Khan**[c]**:** ADME study evaluation, References check, formatting in Journal style

Supplementary Material: Available

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Tables

Table 1 is available in the Supplemental Files section.

Figures

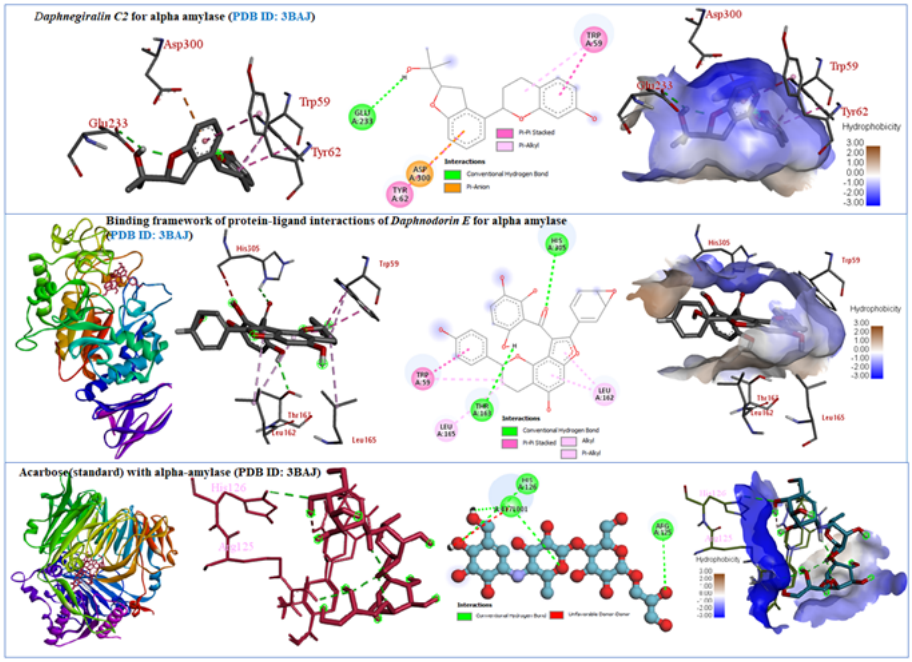

Figure 1

(A): 3D visualization of binding framework of protein-ligand interactions of *best compounds* for alpha amylase (PDB ID: 3BAJ)

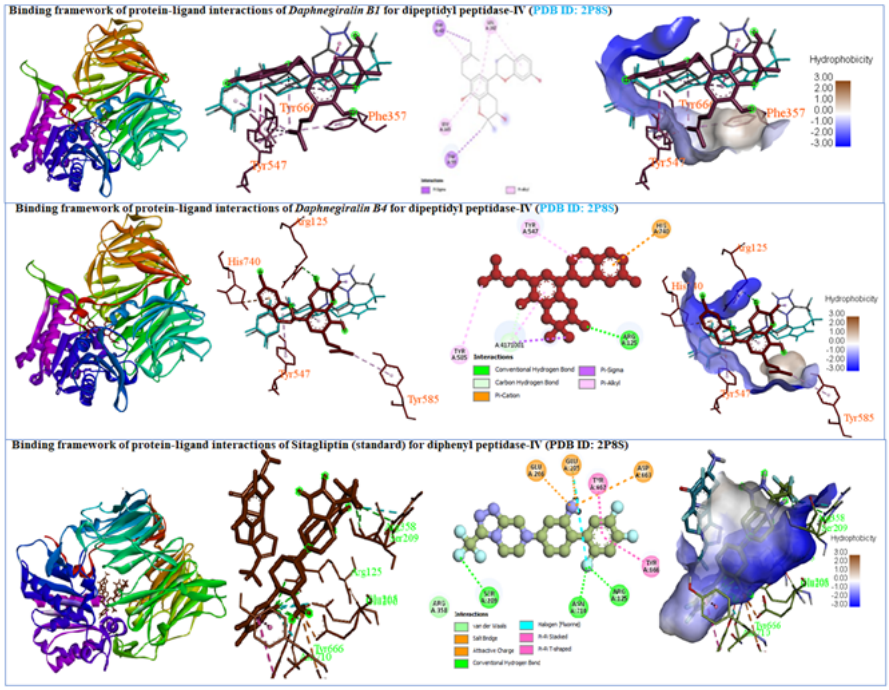

Figure 2

A:3D visualization, 2D visualization and hydrophobic interactions of binding framework of protein-ligand interactions of *best binding affinity molecules* for dipeptidyl peptidase-IV (PDB ID: 2P8S)

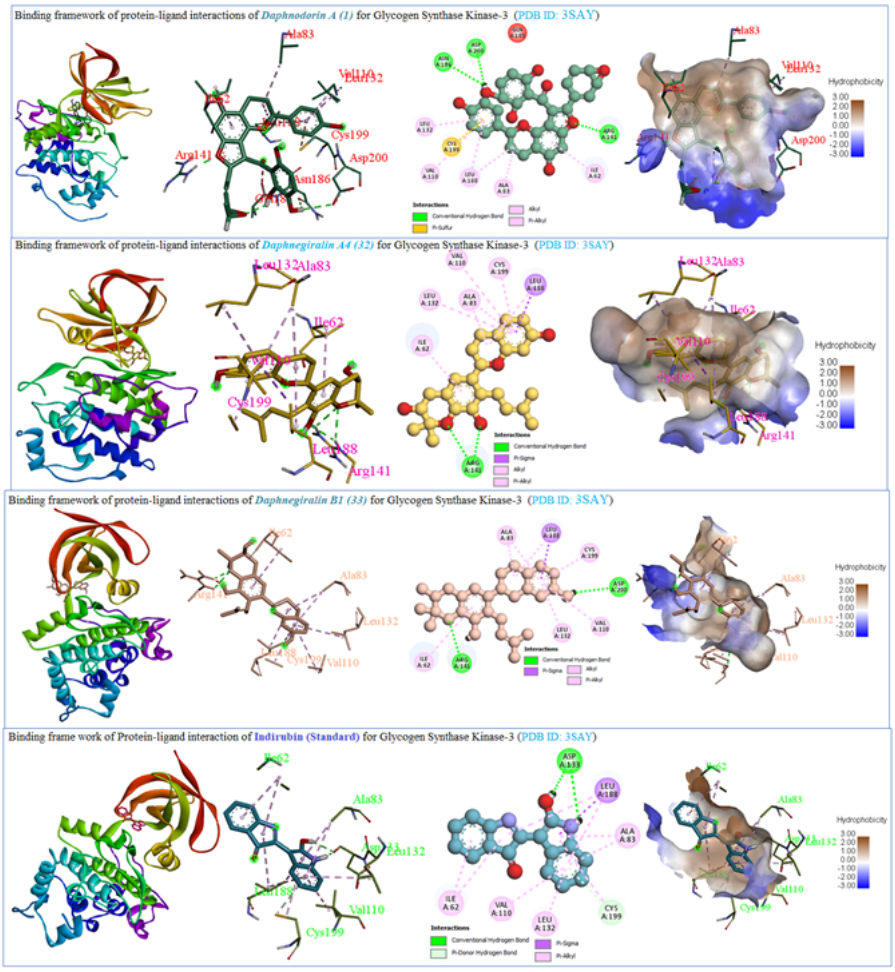

Figure 3

A:3D visualization, 2D visualization and hydrophobic interactions of binding framework of protein-ligand interactions of *best docked compounds* for Glycogen Synthase Kinase-3 (PDB ID: 3SAY)

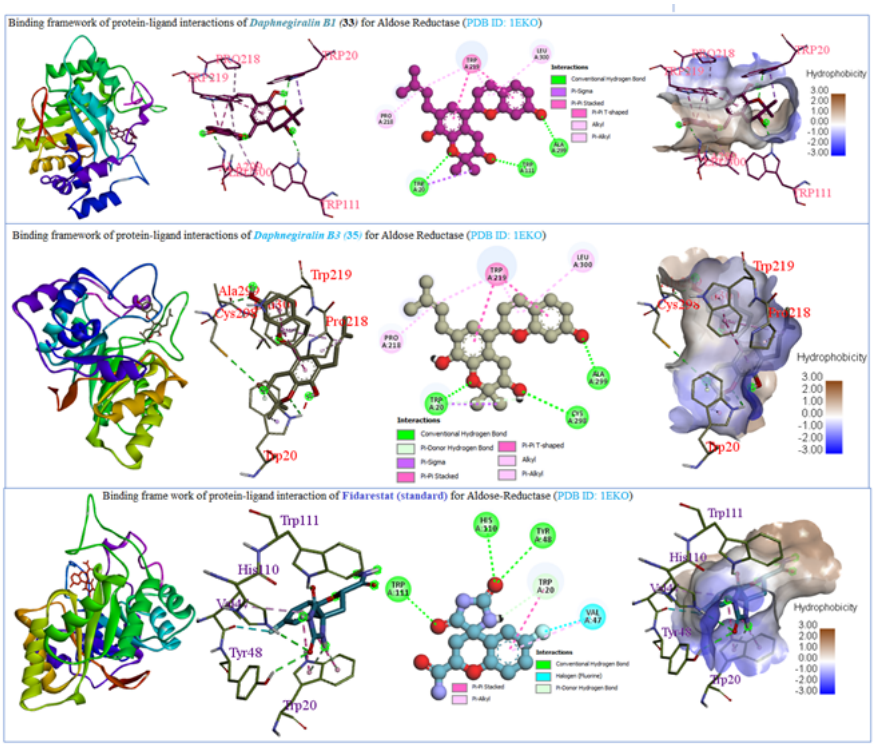

Figure 4

(A)*:* 3D visualization, 2D visualization and hydrophobic interactions of binding framework of protein-ligand interactions of ***best docked compound*** for Aldose Reductase (PDB ID: 1EKO)

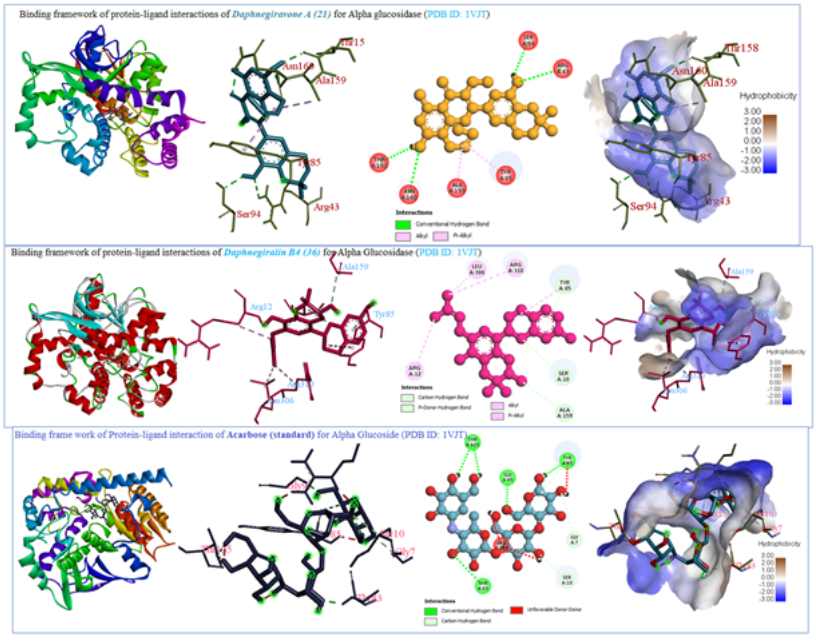

Figure 5

(A)*:* 3D visualization, 2D visualization and hydrophobic interactions of binding framework of protein-ligand interactions of *best docked compounds* for Aldose Reductase (PDB ID: 1VJT)

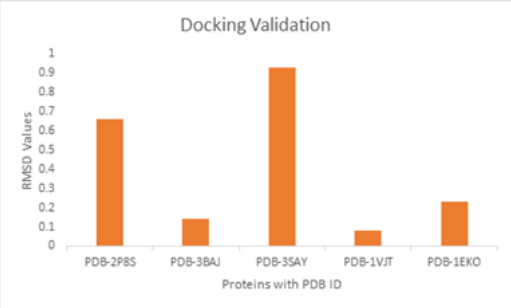

Figure 6

Representation of validation of proteins

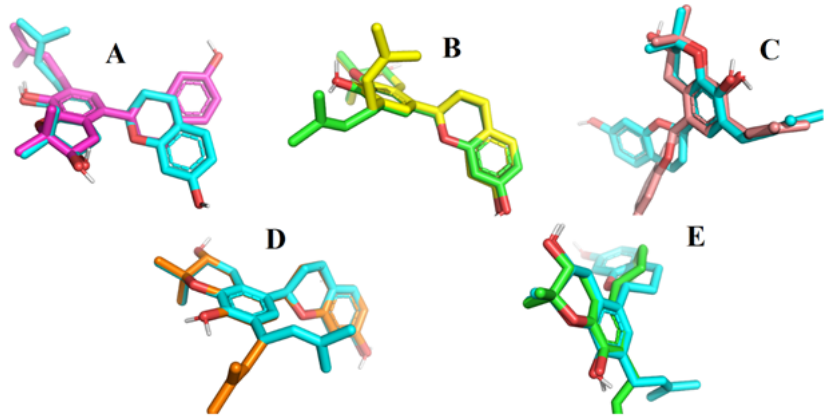

Figure 7

Redocked pose of ligand with its docked protein: (A) for protein PDB ID (2P8S); (B) for (3BAJ); (C) for (3SAY); (D) for (1EKO) and (E) for 1VJT

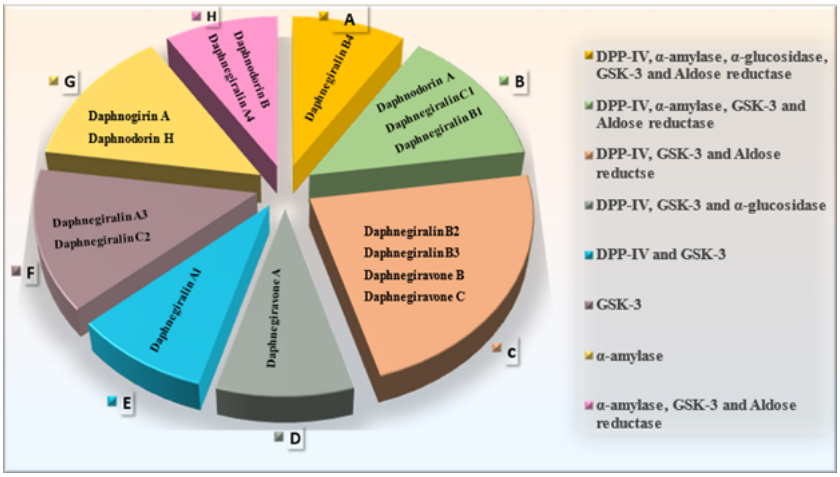

Figure 8

Pie Chart of top 16 hit compounds against predicted five anti-diabetic targets

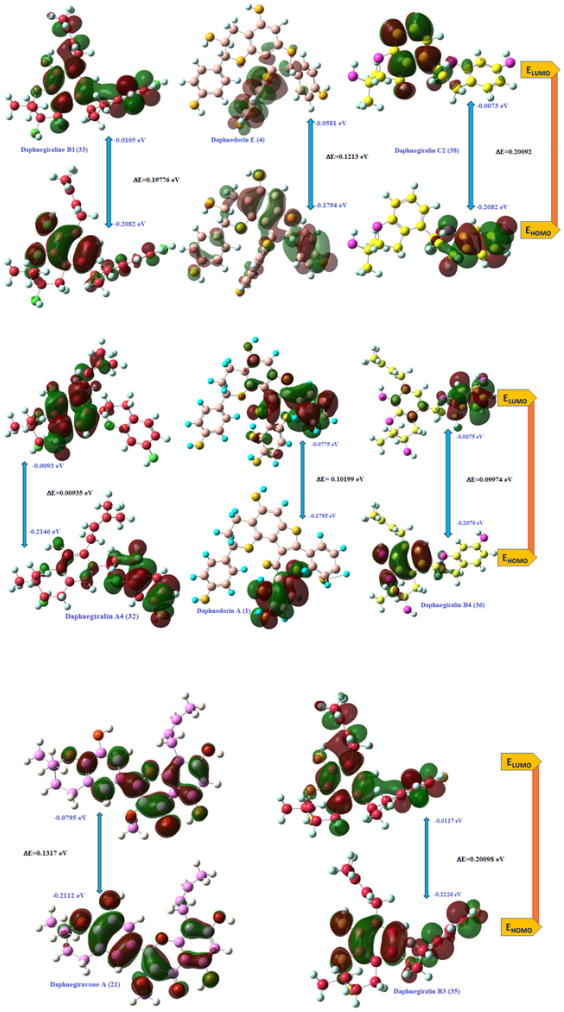

Figure 9

(A): Frontier Molecular Orbitals of Compounds (**38, 4, 33**)

(B): Frontier Molecular Orbitals of Compounds (**36, 1, 32**)

(C): Frontier Molecular Orbitals of Compounds (**35, 21**)

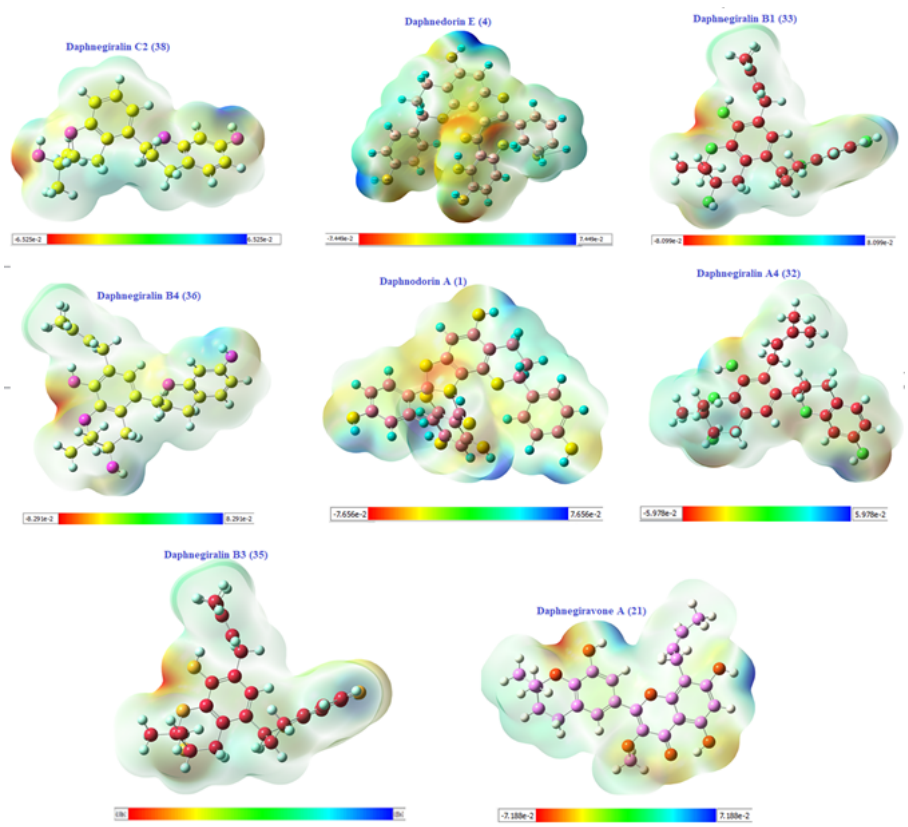

Figure 10

Molecular Electrostatic Potential (MEP) of compounds (G_1-G_4) based on DFT studies

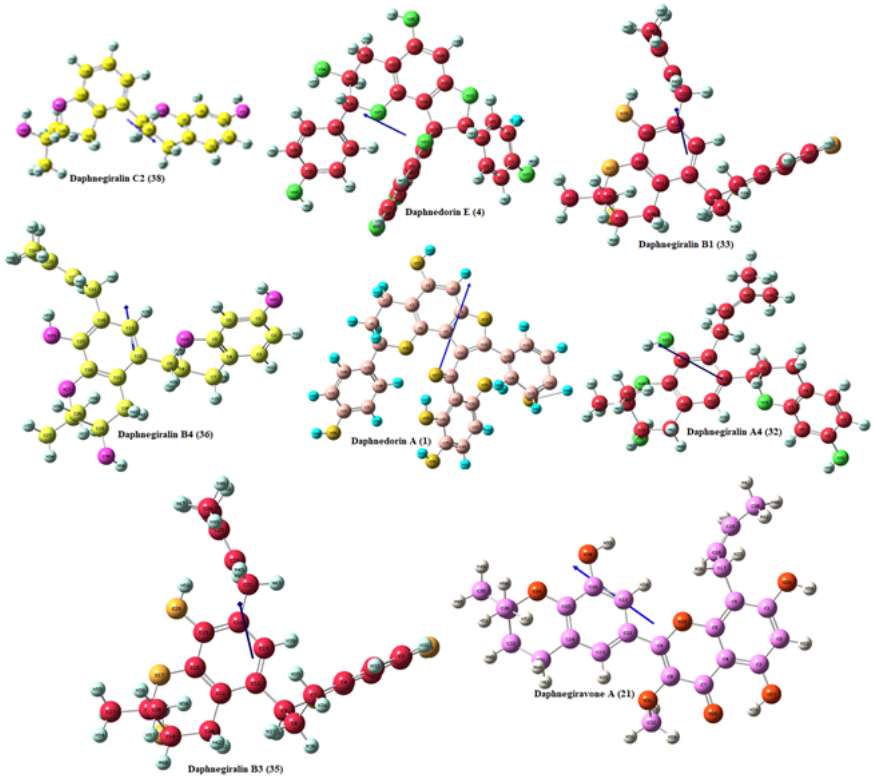

Figure 11

The optimized geometry, numbering system, and the vector of the dipole moment of compound (G_1-G_4) using B3LYP/3-21G(d)

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Table01.docx
- SupplementaryMaterial.docx