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Identification of Antidiabetic and Anti-inflammatory Potential Compounds of Ethylacetate Extract of *Tinospora cardifolia* (Wild) Identified by GC-MS and Spectral Analysis: A Computational Approach

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The present study was aimed to the phytochemical and GC-MS analysis for ethyl acetate extract of *Tinospora cardifolia*. The structure of the compounds was further confirmed by UV-spectroscopy and FTIR study. The *in silico* study like molecular, physico-chemical and drug likeliness property was carried out by computational approaches for the identified molecules. Further toxicity potential and pharmacokinetic profile were also determined. The study was carried out using OSIRIS data warrior and Swiss ADME tools. The docking analysis was carried out for the antidiabetic and anti-inflammatory profiles. The compounds were targeted for α-glucosidase, peroxisome proliferator-activated receptor, glucose transporter-1, cyclo-oxygenase-1 & 2 inhibitions. About 12 compounds were identified by GC-MS analysis. All the compounds exhibited moderate to good drug likeliness and pharmacokinetic potentials. The molecules showed a good bioactivity score against enzyme receptors. The ADMET prediction showed PGP and CYP-inhibitory effects with the least toxic profile. The docking analysis showed good binding affinity of that 1,4-*bis*(3,4,5-trimethoxy phenyl)hexahydrofuro[3,4-*c*]furan (Molecule-10) have strong binding affinity on targeted proteins under investigation.

Keywords: *Tinospora cardifolia*, Ethylacetate extract, Drug likeliness, Docking analysis, Anti-diabetic, Anti-inflammatory activity.

INTRODUCTION

Phytomedicines are herbal formulations made up of herbal extracts individually or in combinations used as medicines or cosmetics due to a wide range of therapeutic effects [1]. Herbal medicines are lost their popularity due to the increased use of allopathic medicines and their first effects [2]. But traditional medicines are using for more than 2000 years when the allopathic system was not started [3]. Attention is being focused on the investigation of the efficacy of plant-based drugs used in traditional medicine because they are economic, have few side effects and according to WHO, about 80% of the world population rely mainly on herbal remedies [4].

Guduchi or Amritha scientifically called *Tinospora cardifolia* (Willd.) Miers ex Hook. f. & Thoms and is widely distributed in different parts of India and China [5]. Around 1000 tonnes plants consume throughout the year in the form of medicines [6]. *T. cardifolia is* used in the form of tonics to treat different types of critical illnesses like jaundice, arthritis, diabetes and different types of skin diseases [7-9]. The major active constituent found in *T. cardifolia* are alkaloids, furano diterpenoids, clerodane norditerpenoids, sesquiterpenoids, phenolics, lignans, sterols, aliphatic compounds, polysaccharides, essential oil and fatty acids [10,11]. The whole plant possesses hepatoprotective, antiulcer and antioxidant properties, whereas the stems showed hepatoprotective, antipyretic, cytotoxic, immunomodulatory and antidiabetic activity [12-17]. Dried fruits are used for jaundice and rheumatism, whereas the leaves are used to treat diabetes [18] and the roots are employed for their powerful emetic, antistress, antioxidant, antiulcer and hypoglycemic properties as well as for the treatment of visceral obstructions [19-923]. Jain *et al*. [24] reported 40 compounds (ethyl acetate : 15, methanol: 14 and petroleum ether: 11) were detected using by GC-MS analysis in the different extracts of *T. cardifolia*. Thillaivanan & Samraj [25] identified 45 phytochemicals in the ethyl acetate extract.

In rational drug design major step is the identification and characterization of the bioactive molecules using advanced spectroscopic techniques like X-ray crystallography and nuclear magnetic resonance (NMR). The spectroscopy provides stereochemical information of molecules with the initiation of the structure-based drug design (SBDD) process. The application of *in silico* drug design is commonly based on background experimental information and computational methodologies [26]. Structure-based drug design describes the specificity and affinity of ligands with specifically targeted proteins [27]. The compound having high binding affinity and specificity is considered a biologically active molecule in respect to specific [28]. The foremost widely approaches used are molecular docking, molecular dynamics (MD), fragment-based drug design (FBDD) and pharmacophore modeling are referred to because of the commonest computational SBDD methods [27].

In silico approaches utilized in ligand-based drug design. It predicts molecular property, physico-chemical property, drug likeliness and ADMET prediction. The foremost widely used approach in ligand-based drug design (LBDD) is ligand chemical similarity, binding affinity and physico-chemical property with standard molecules. The other is pharmacophore mapping and quantitative structure-activity relationship (QSAR) [29]. The simulation of a biomolecular interaction is often achieved by molecular docking. It provides information regarding the affinity of every ligand [30]. The compounds with high relative molecular mass exhibit unsatisfied pharmacokinetic properties, because of poor solubility. A fragment-based drug design (FBDD) approach will be applied to overcome this problem. It is predicated on the identification of the molecules based on Lipinski rule five [31,32].

The present study was aimed to perform web and software-based SBDD, FBDD and LBDD design of the compounds present in ethyl acetate extract of *Tinospora cardifolia*. The presence of the compounds was determined by spectral and GC-MS analysis. Based on the literature present study, it was also aimed to target specific binding proteins and compounds of *T. cardifolia* responsible for antidiabetic and anti-inflammatory activity.

EXPERIMENTAL

Preparation of extracts of *Tinospora cardifolia*: The hydroalcoholic extract of *Tinospora cardifolia* was purchased from Herbal Creation, Nainital, India. Approximately 100 g of hydroalcoholic extract was suspended in 250 mL of petroleum ether to remove fatty components. Remove the petroleum ether and air-dried. To a dried extract added 250 mL of ethyl acetate and kept for 24 h. The resulting extracts were evaporated in the rotary flash evaporator to remove excess ethyl acetate. The extract was air dried in a desiccator and stored in an airtight glass container. The resulting extract was used for further analysis.

Phytochemical analysis: The alkaloids, glycosides, steroids, phenolics, aliphatic compounds, polysaccharides, furono diterpene glucoside, protein, calcium and phosphorus are major active chemical constituents of *T. cardifolia*. The hydroalcoholic and ethyl acetate extract was taken for different phytochemical analysis as reported procedure [33,34].

GC-MS full scan analysis: The ethyl acetate extract of *Tinospora cordifolia* was subjected to GC-MS full scan analysis. Accurately weighed 50 mg of ethyl acetate extract and dissolved in 100 mL of HPLC grade methanol. The resulting solution was further diluted to 10 mL to get the desired concentration of 30 µg/mL and used for analysis.

Determination of wavelength: Accurately weighed 100 mg of ethyl acetate extract and transferred into a 100 mL volumetric flask, dilute to 100 mL volume with phosphate buffer (pH 6.8). From the above solution taken 10 mL of solution into a100 mL volumetric flask diluted it to volume with diluents and mixed well (concentration: about 100 µg/mL). From the above stock solutions taken 3 mL of solution to 10 mL of volumetric flask and further diluted to 10 mL to get desired concentration of 30 µg/mL and used for analysis. The solution was scanned from 200-400 nm, the instrument was scanned in spectrum mode and determine the absorbance. The study was carried out in triplicate.

FTIR analysis: The FTIR analysis was carried out by Bruker (3000 Hyperion Microscope with Vertex 80 FTIR System), Germany associated with Micro ATR, Grazing angle. Approximately 1 mg of extract was placed on the sampling plate and was scanned at 4000-450 cm^{-1}.

Toxicity potential of compounds: The smile notation of compounds was entered in Orasis data warrior software and calculated molecular properties like shape index, molecular flexibility, molecular complexity of the scanned compounds found in GC-MS analysis. Similarly, physico-chemical properties such as molecular weight, partition coefficient (cLog P), Water solubility in moles/L (cLog S); hydrogen bond acceptors and donors, total surface area, relative polar surface area, topological polar surface area (TPSA) and violations of Lipinski's rule of five were calculated to evaluate the drug likeliness of the compounds and toxicity profile like mutagenic, tumorigenic, reproductive effective, irritant property was calculated. Fraction Csp3 and molar refractive index was calculated using the Swiss ADME online tool. The molecular, physico-chemical property and toxicity potential of the compounds were compared with the standard drugs. The absorption percentage (% Abs) was also determined by using the following formula:

$$\text{Absorbance (\%)} = 109 - (0.345 \times \text{TPSA})$$

Calculation of drug likeliness and bioactivity score: SMILES notations of the molecules were placed in the online tool Swiss ADME (http://www.swissadme.ch/index.php) to predict drug likeliness properties like Lipinski, Ghose, Veber, Egan, Muegge, bioavailability score and Molinspiration software version 2011.06 (www.molinspiration.com) to calculate the score for drug targets including enzymes and nuclear receptors, kinase inhibitors, GPCR ligands and ion channel modulators. Bioactivity rader of molecules and standards was prepared using the SWISS ADME tool.

Calculation of pharmacokinetic potential: The pharmacokinetic potential of the compounds was determined by the online tool to Swiss ADME (http://www.swissadme.ch/index.php). The pharmacokinetic properties like gastrointestinal (GI) absorption, BBB permeant, PGP substrate, CYP1A2 inhibitor,

CYP2C19 inhibitor, CYP2C9 inhibitor, CYP2D6 inhibitor, CYP3A4 inhibitor, Log Kp (skin permeation) was calculated. Based on the values determined boiled egg diagram was prepared using the SWISS ADME tool.

Docking analysis: Docking analysis of the molecules was carried out using Swiss dock (http://www.swissdock.ch/docking) and UCSF Chimera 1.5.3 was used for interactive visualization and analysis of molecular structures and related data, including density maps, trajectories and sequence alignments. The targeted proteins 5zcb (α-glucosidase), 5ycp (Human PPARγ ligand-binding domain complexed with Rosiglitazone), 4pyp (Crystal structure of the human glucose transporter GLUT1), 1eqg (The 2.6 Angstrom model of ovine Cox-1 complexed with ibuprofen), 3ln1 (structure of celecoxib bound at the COX-2 active site) was collected from RSCB protein data bank. The molecules were converted to Mole2 file format.

After preparation of proteins and molecules was submitted to the Swiss dock server for flexible docking. Docking analysis was carried out by UCSF Chimera 1.5.3 to analyze binding score, binging pose and binding residue. Out of 250 clusters least one is considered as best binding score.

RESULTS AND DISCUSSION

The present study was aimed to find out different types of biomolecules present in whole plant ethyl acetate extracts of *Tinospora cardifolia*. The different types of biomolecules were traced by GC-MS analysis. Further presence of structures was confirmed by UV-spectroscopic and FTIR studies. Further *in silico* analysis like molecular property, physico-chemical property, bioactivity score, toxicity potential, the pharmacokinetic property was carried out for the compounds identified in ethyl acetate extract.

Though different types of pharmacological activity were reported for the selected plant. The present study also focused on the antidiabetic and anti-inflammatory activity of the plant extract. The structure-based flexible docking analysis was carried out for compounds found in the extract. The main aim behind the study was to find out the molecules which one is selectively responsible for antidiabetic and anti-inflammatory activity.

Percentage yield and phytochemical analysis: The percentage yield was determined for ethyl acetate extract. It was found to be 12.8 %. The phytochemical analysis was carried out for the extract as per the literature procedure (Table-1). It shown presence of alkaloids, flavanoid, steroid, phenolic compound, lignin, terpenoids and aliphatic compounds.

TABLE-1
PHYTOCHEMICAL ANALYSIS

Phyto constituents	Hydroalcoholic extract	Ethylacetate extract
Alkaloid	Positive	Positive
Steroid	Positive	Positive
Flavanoid	Positive	Positive
Phenol	Positive	Positive
Lignan	Positive	Positive
Tannin	Positive	Negative
Saponin	Positive	Negative
Carbohydrates	Positive	Negative
Vitamins	Positive	Negative
Terpenoid	Positive	Positive
Aliphatic compounds	Positive	Positive

GC-MS analysis: The GC-MS chromatogram of ethyl acetate of *T. cardifolia* extract is shown in Fig. 1. The results showed a total of 12 compounds were identified in ethyl acetate extract (Table-1). The structure of compounds was confirmed by the NIST search library prepared during analysis. The name of compounds reported by the library is shown in Table-2. Out of all the compounds reported molecule 4 (15.90 %) and molecule 10 (13.23 %) were traced in higher concentrations at the retention time 21.45 and 32.37 min, respectively.

UV-spectroscopic and FTIR analysis: UV-spectroscopic study of ethyl acetate of *T. cardifolia* extract was carried out using phosphate buffer (pH 6.8) at 30 µg/mL (Fig. 2). The spectrum exhibited absorbance maxima at 222 nm. The UV-

TABLE-2
GC-MS ANALYSIS FOR ETHYL EXTRACT OF *Tinospora cardifolia*

Peak number	Time (min)	Peak area (%)	m.w.[a]	m.f.[b]	Name of compound	Probability
1	16.3975	5.148335324	168	$C_8H_8O_4$	4-Hydroxy-3-methoxy benzoic acid	43.5
2	18.5357	4.257378188	180	$C_{10}H_{12}O_3$	4-([1E]-3-Hydroxy-1-propenyl)-2-methoyphenol	17.2
3	20.1943	5.776540310	234	$C_{15}H_{22}O_2$	6-(3-Hydroxyprop-1-en-2-yl)-4,8a-dimethyl-1,5,6,7,8,8a-hexahydronaphthalen-2(3*H*)-one	25
4	21.4533	15.90186414	278	$C_{16}H_{22}O_4$	Dibutyl pthalate	15.1
5	26.2294	2.443181783	250	$C_{12}H_{14}N_2O_2S$	4-{3,4-Dimethoxy phenyl]-5-methyl-2-thiazoleamine	27.9
6	29.3003	3.143691736	358	$C_{20}H_{22}O_6$	Columbin	38
7	29.9997	3.345561769	250	$C_{16}H_{26}O_2$	3-Cyclopentyl-6-hydroxy-6-methyl-5-(1-methylethyl)-3,4,heptadiene-2-one	5.6
8	30.2395	2.159413103	428	$C_{27}H_{40}O_4$	4-Hydroxy-5',6a,8a,9-tetramethyl-1,2,2a,3,3',4,4',5,5',6,6a,6',8,8a,8b,9,12,12a-octadecahydrospiro[naphtho[2',1':4,5]indeno[2,1-b]furan-10,2'-pyran]-7(11aH)-one	27.3
9	30.8989	4.931841535	236	$C_{15}H_{24}O_2$	4,8a-Dimethyl-6-(prop-1-en-2-yl)-1,2,3,5,6,7,8,8a-octahydro naphthalene-2,3-diol	8.39
10	32.3777	13.23352017	446	$C_{24}H_{30}O_8$	1,4-*Bis*(3,4,5-trimethoxyphenyl)hexahydrofuro[3,4-c]furan	96.2
11	32.9706	4.114471969	344	$C_{21}H_{28}O_4$	5-Methoxy-13-methyl-2-oxo-5,6,7,8,9,11,12,13,14,15,16,17-dodecahydro-2H-cyclopenta[a]phenanthren-17-yl acetate	11.2
12	34.3894	4.090469369	392	$C_{19}H_{28}N_4O_5$	2-(5-Azido-3-nitropentyl)-1,3-di-tert-butyl-5-methoxybenzene	6.33

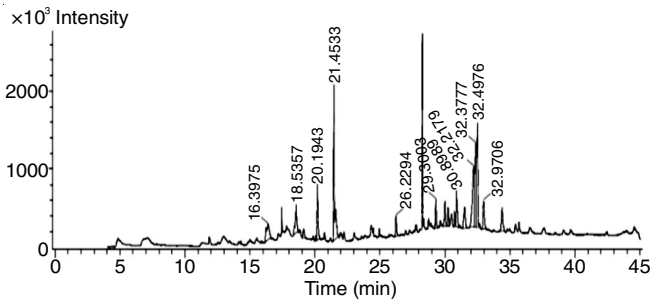


Fig. 1. GC-MS chromatogram for ethyl acetate extract of *Tinospora cardifolia*

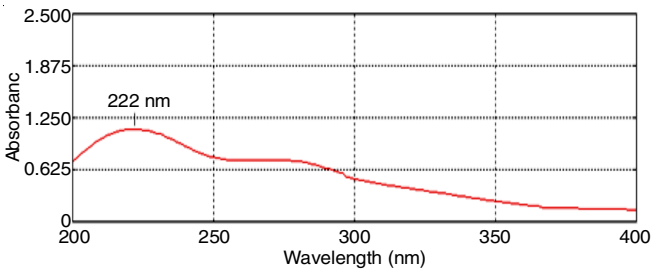


Fig. 2. UV-spectrum for ethyl extract of *Tinospora cardifolia*

spectrum of the extract showed allowed (π-π) transitions. The FTIR analysis was carried out on the extracts for the compounds found in GC-MS analysis and represented in Fig. 3. The FTIR peaks at 3422.60 cm^{-1} (-OH, *str.*); 2917.30 cm^{-1} (-OCH_3, *str.*); 2850.66 cm^{-1} (-CH_3, *str.*), 1736.40 cm^{-1} (C=O, *str.*); 1607.02

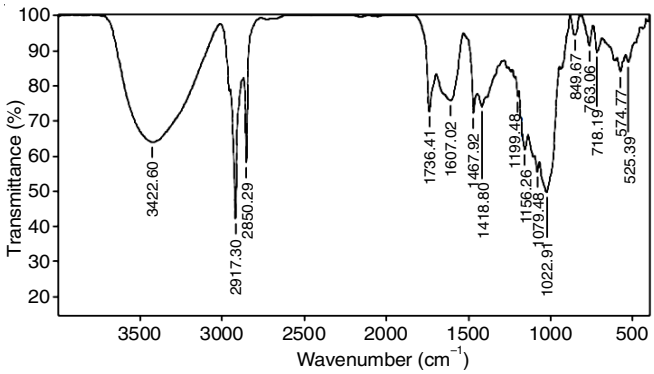
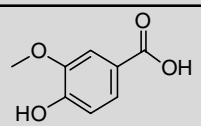
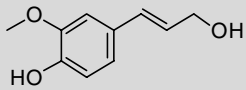
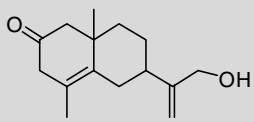
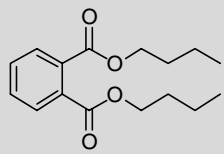
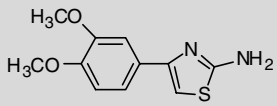
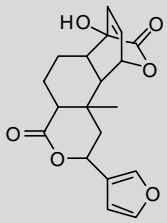


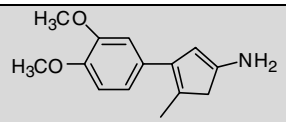
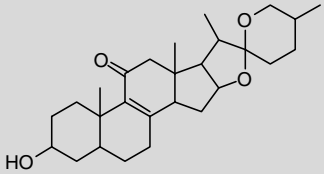
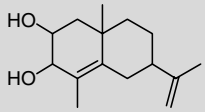
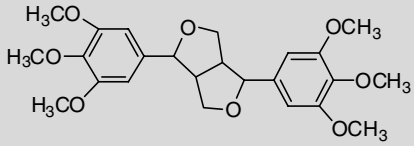
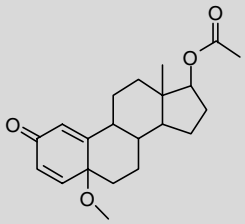
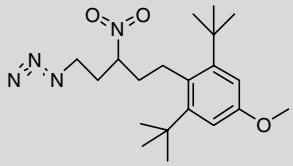
Fig. 3. IR spectra of ethyl acetate extract of *Tinospora cardifolia*

cm^{-1} (-C=C-, *str.*); 1418.80 cm^{-1} (-C-O-, *str.*) and 1199.48 cm^{-1} (-C-N, *str.*) exhibited the presence of reported compounds in GC-MS analysis. Based on the spectral analysis the structure of the compounds along with smile notation is represented in Table-3.

Molecular property: The shape index, molecular flexibility and molecular complexity play a vital role in drug action and binding with the receptor molecules. Generally, linear shape molecules are considered ideal drug molecules [35]. Whereas molecules with high flexibility and low molecular complexity are considered for proper binding affinity toward the receptors [36,37]. The molecular property of the molecules was determined by Orasis data warrior software and are given

TABLE-3
STRUCTURE OF MOLECULES ALONG WITH SMILE NOTATION FOR THE COMPOUNDS IDENTIFIED BY SPECTRAL ANALYSIS FOR ETHYL ACETATE EXTRACT OF *Tinospora cardifolia*

Compd. No.	Structure	Smiles
1		Oc1ccc(cc1OC)C(=O)O
2		Oc1ccc(cc1OC)\C=C\CO
3		C=C(CO)C1CC2=C(C)CC(=O)CC2(C)CC1
4		O=C(OCCCC)c1ccccc1C(=O)OCCCC
5		Nc1nc(c2cc(OC)c(OC)cc2)c(C)s1
6		O=C4OC(CC3(C)C4CCC1C3C2C=CC1(O)C(=O)O2)c5ccoc5

7		NC1=CC(c2cc(OC)c(OC)cc2)=C(C)C1
8		OC6CCC5(C)C(CCC1=C5C(=O)CC2(C)C4C(CC12)OC3(CCC(C)CO3)C4C)C6
9		C=C(C)C1CCC2(C)CC(O)C(O)C(C)=C2C1
10		COc1cc(cc(OC)c1OC)C4OCC3C4COC3c2cc(OC)c(OC)c(OC)c2
11		CC(=O)OC2CCC1C3CCC4(OC)C=CC(=O)C=C4C3CCC12C
12		COc1cc(c(CCC(CC\N=N#N)N(=O)=O)c(c1)C(C)(C)C)C(C)(C)C

in Table-4. The results shown molecules 6 and 11 are spherical in shape whereas molecules 1, 2, 3, 4, 5, 7, 8, 9, 10 and 12 are linear molecules. Except for molecule-12, all the molecules showed low molecular flexibility. Similarly, all the molecules showed higher molecular complexity compared to standards.

TABLE-4
MOLECULAR PROPERTY OF THE
MOLECULES IDENTIFIED IN GC-MS ANALYSIS

Molecules	Shape index[a]	Molecular flexibility[b]	Molecular complexity[c]
1	0.58333	0.28648	0.65854
2	0.69231	0.39309	0.66172
3	0.58824	0.49791	0.79217
4	0.70000	0.48056	0.70997
5	0.58824	0.35544	0.73199
6	0.44000	0.37897	0.96293
7	0.58824	0.36804	0.77373
8	0.51613	0.30951	1.00500
9	0.52941	0.44597	0.81145
10	0.50000	0.31125	0.88837
11	0.48000	0.36866	0.91361
12	0.51852	0.64369	0.73783
Ibuprofen	0.67000	0.62000	0.56000
Celicoxib	0.50000	0.47398	0.82757
Metformin	0.66667	0.79706	0.54931
Rosiglitazone	0.68000	0.53192	0.70181

[a]Molecular shape index (spherical ≤ 0.5 ≤ linear), [b]Molecular flexibility (low ≤ 0.5 ≤ high), [c]Molecular complexity (low ≤ 0.5 ≤ high)

Physico-chemical property: Physico-chemical properties like molecular weight, solubility, H-acceptors, H-donors, partition coefficient [38], total surface area, relative polar surface area, TPSA (Å^2) [39], percentage of absorption, fraction Csp3, molar refractive index [40] have great significance on biological activity and drug likeliness property of the molecules. The physico-chemical properties were calculated using Osiris data warrior and Csp3 and molar refractivity was calculate using the Swiss ADME tool (Table-5). All the molecules exhibited good drug likeliness characteristics with respect to the standard. Based on the molecular and the physico-chemical property bioactivity reader is drawn in Fig. 4.

Drug-likeness: The total drug likeliness characteristics like Drug likeness score, Lipinski, Ghose, Veber, Egan and Muegge rule. The bioavailability Score of all the molecules was also calculated by using the Swiss ADME tool (Table-6). All the molecules followed drug likeliness as per Lipinski, Ghose, Veber, Egan's rule. The molecule 8 does not follow drug likeliness as per Ghose rules. Similarly except for molecules 1, 2 and 12, all the molecules followed Muegge rules. The bioavailability score was found to be 0.55 in respect of all the compounds. Out of 12 molecules found in GC-MS analysis molecules 5, 6, 8 and 11 exhibited positive drug likeliness values 1.30, 0.73, 1.38 and 1.58, respectively. The results showed the good drug likeliness characteristics in comparison to standards.

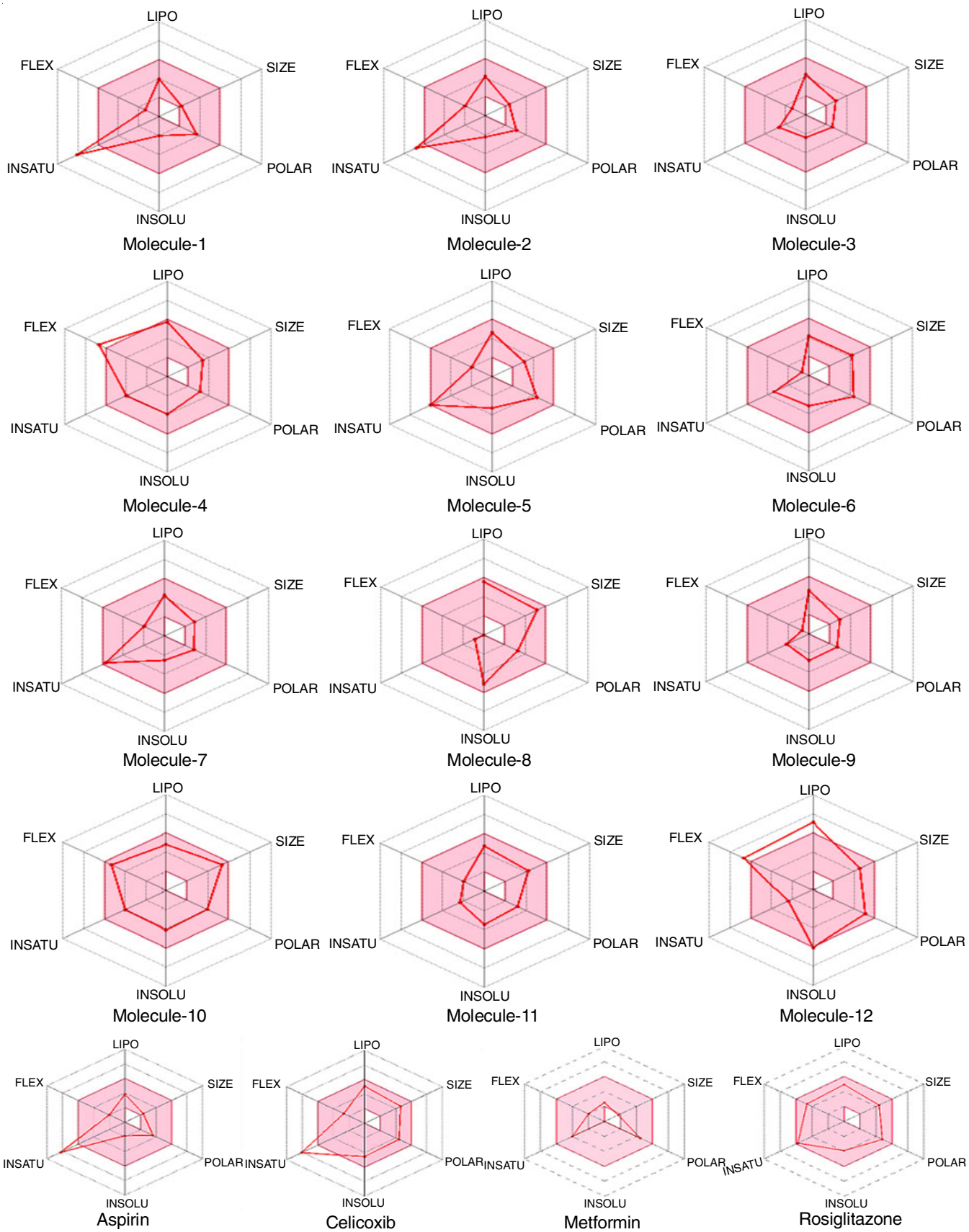


Fig. 4. Bioactivity radar of the molecules identified in GC-MS analysis in comparison to standards [The coloured zone is suitable physico-chemical space for oral bioavailability. LIPO (liphophilicity): $0.7 \leq \text{XLOGP3} \leq +5.0$; SIZE: $150 \text{ g/mol} \leq \text{MV}$; POLAR (polarity): $20 \text{ Å}^2 \leq \text{TPSA} \leq 130 \text{ Å}^2$; INSOLU (insolubility): $0 \leq \text{LOGS (ESOL)} \leq 5.0$; INSATU (insaturation): $0.25 \leq \text{fraction Csp}^3 \leq 1$; FLEX (flexibility): $0 \leq \text{number of rotatable bond} \leq 9$]

TABLE-5
PHYSICO-CHEMICAL PROPERTY OF THE MOLECULES IDENTIFIED IN GC-MS ANALYSIS

Molecules	m,.w.[a]	cLog P[b]	cLog S[c]	Solubility	H-acceptors	H-donors	Total surface area	Relative PSA[d]	TPSA[e] (Å²)	Abs (%)[f]	Fraction Csp3	MR[g]
1	168.15	0.7290	-1.351	Soluble	4	2	125.97	0.39089	66.76	85.96780	0.12	41.92
2	180.20	1.4078	-1.739	Soluble	3	2	148.48	0.24380	49.69	91.85695	0.20	51.02
3	234.34	3.1581	-2.771	Soluble	2	1	186.26	0.14034	37.30	96.13150	0.67	70.14
4	278.35	4.1158	-3.578	Moderately soluble	4	0	235.84	0.19539	52.60	90.85300	0.50	77.84
5	250.32	2.6439	-3.693	Moderately soluble	4	1	193.02	0.34504	85.61	79.46455	0.25	69.90
6	344.36	0.6348	-3.103	Soluble	6	1	234.16	0.31299	85.97	79.34035	0.58	85.55
7	231.29	1.8170	-2.548	Soluble	3	1	186.45	0.18917	44.48	93.65440	0.29	68.86
8	428.61	4.3439	-5.195	Moderately soluble	4	1	304.89	0.15133	55.76	89.76280	0.89	121.79
9	236.35	3.0890	-2.828	Soluble	2	2	184.60	0.14193	40.46	95.04130	0.73	71.11
10	446.49	2.5818	-3.074	Moderately soluble	8	0	337.10	0.23732	73.84	83.52520	0.50	116.82
11	344.45	2.9784	-3.744	Soluble	4	0	256.37	0.17974	52.60	90.85300	0.71	96.01
12	376.50	3.9866	-5.079	Poory soluble	7	0	305.68	0.23469	83.20	80.29600	0.70	108.59
Ibuprofen	206.30	3.0000	-2.890	Moderately soluble	2	1	172.90	0.15119	37.30	94.63000	0.12	89.96
Celicoxib	381.40	2.5888	-4.174	Moderately soluble	5	1	259.56	0.23767	86.36	79.20580	0.12	89.96
Metformin	129.20	-1.7137	0.827	Very soluble	5	4	108.92	0.56445	88.99	78.29850	0.50	36.93
Rosiglitazone	357.40	2.1619	-3.666	Moderately soluble	6	1	269.07	0.29565	96.83	75.59370	0.28	101.63

[a]Molecular weight; [b]P = [*n*-octanol]/[water]; [c]S = water solubility in mol/L at pH = 7.5 (25 °C); [d]Relative polar surface area; [e]Toplogical polar surface area; [f]Percentage of absorption; [g]Molar refractive index.

Bioactivity score: Based on the drug likeliness characters bioactivity score of the compounds was calculated by mole inspiration online tool (www.molinspiration.com). The bioactivity score was determined on GPCR ligand (G-Protein coupled receptor), ion channel modulator, a kinase inhibitor, nuclear receptor ligand, protease inhibitor and enzyme inhibitor. A bioactivity score of more than 0 is considered as a good, -0.50 to 0 consider as moderate and less than -0.5 is considered as inactive compounds [41]. Based on the result shown in Table-7 the bioactivity order for the molecules in respect to target receptors are enzyme inhibitor > nuclear receptor > ion channel modulator > GCPR ligand > protase inhibitor > kinase inhibitor. Out of 12 molecules isolated molecules 2, 3, 6, 8, 9, 10, 11 and 12 shown bioactivity score more than 0 in respect of different receptors.

Toxicity profiles: The toxicity potential of the molecules was determined for the mutagenic, tumorigenic, reproductive effects, irritant properties by Osiris data warrior. The result is shown in Table-8 shown molecules 1 and 4 shown high mutagenic effect, molecule 4 and 12 showed tumorigenic effect. Similarly 4 and 7 have a high reproductive effect, whereas molecules 2, 4 and 6 are irritant in nature.

Pharmacokinetics profiles: The pharmacokinetic profiles like GI absorption, BBB permeant, P-GP substrate, CYP inhibitory effect (1A2; 2C19; 2C9; 2D6, 3A4), Log Kp (skin permeation) was determined by the Swiss ADME tool. The results in Table-9

TABLE-6
DRUG LIKLINESS OF THE MOLECULES IDENTIFIED IN GC-MS ANALYSIS

Molecules	Druglikeness	Lipinski	Ghose	Veber	Egan	Muegge	Bioavailability score
1	-1.5442	Yes; 0 violation	Yes	Yes	Yes	No; 1 violation: m.w. < 200	0.55
2	0.5405	Yes; 0 violation	Yes	Yes	Yes	Yes	0.55
3	-1.5116	Yes; 0 violation	Yes	Yes	Yes	Yes	0.55
4	-4.7835	Yes; 0 violation	Yes	Yes	Yes	No; 2 violations: m.w. < 200, Heteroatoms < 2	0.55
5	0.7353	Yes; 0 violation	Yes	Yes	Yes	Yes	0.55
6	-3.4063	Yes; 0 violation	Yes	Yes	Yes	Yes	0.55
7	-0.886	Yes; 0 violation	Yes	Yes	Yes	Yes	0.55
8	-8.685	Yes; 0 violation	Yes	Yes	Yes	Yes	0.55
9	-7.9208	Yes; 0 violation	Yes	Yes	Yes	No; 1 violation: XLOGP3 > 5	0.55
10	-3.3599	Yes; 0 violation	Yes	Yes	Yes	Yes	0.55
11	-0.3236	Yes; 0 violation	Yes	Yes	Yes	Yes	0.55
12	2.7463	Yes; 0 violation	Yes	Yes	Yes	Yes	0.55
Ibuprofen	0.085	Yes; 0 violation	No; 1 violation: WLOGP > 5.6	Yes	Yes	Yes	0.55
Celicoxib	-8.1085	Yes; 0 violation	No; 1 violation: WLOGP > 5.6	Yes	Yes	Yes	0.55
Metformin	3.5915	Yes; 0 violation	No; 3 violations: m.w. < 160, WLOGP < -0.4, MR < 40	Yes	Yes	No; 2 violations: m.w. < 200, #C < 5	0.55
Rosiglitazone	7.5038	Yes; 0 violation	Yes	Yes	Yes	Yes	0.55

TABLE-7
BIOACTIVITY SCORE OF THE MOLECULES IDENTIFIED IN GC-MS ANALYSIS

Molecules	GPCR ligand	Ion channel modulator	Kinase inhibitor	Nuclear receptor ligand	Protease inhibitor	Enzyme inhibitor
1	-0.85	-0.42	-0.99	-0.61	-1.12	-0.35
2	-0.55	-0.05	-0.74	-0.3	-1	0
3	-0.38	-0.02	-0.94	0.4	-0.48	0.45
4	-0.16	-0.09	-0.27	-0.12	-0.25	-0.07
5	-0.43	-0.43	-0.38	-1.18	-0.89	-0.19
6	0.55	-0.17	-0.32	0.66	-0.13	0.47
7	-0.39	-0.23	-0.41	-0.24	-0.52	-0.07
8	-0.06	-0.19	-0.64	0.51	-0.06	0.53
9	-0.18	0.05	-0.83	0.85	-0.23	0.51
10	-0.03	-0.25	-0.19	-0.1	-0.16	0.01
11	0.2	0.07	-0.41	1.05	0.17	0.66
12	0.15	0.25	-0.04	0.15	0.1	0.17
Ibuprofen	-0.17	-0.01	-0.72	0.05	-0.21	0.12
Celicoxib	-0.06	-0.27	0.01	-0.28	-0.06	0.17
Metformin	-1.44	-0.82	-2.47	-3.48	-1.11	-1.59
Rosiglitazone	0.15	-0.65	-0.61	0.35	-0.21	-0.07

TABLE-8
TOXICITY PROFILES OF THE MOLECULES IDENTIFIED IN GC-MS ANALYSIS

Molecule	Mutagenic	Tumorigenic	Reproductive effective	Irritant
1	High	None	None	None
2	None	None	None	High
3	None	None	None	Low
4	High	High	High	High
5	None	None	None	None
6	None	None	None	High
7	None	None	High	None
8	None	None	None	None
9	None	None	None	None
10	None	None	None	None
11	None	None	None	None
12	None	High	None	None
Ibuprofen	None	None	None	None
Celicoxib	None	None	None	None
Metformin	High	None	High	None
Rosiglitazone	None	None	None	None

and Fig. 5 showed that all the molecules have GI-absorption capacity, Molecules 2, 3, 4, 7, 8, 9, 10, 11 & 12 have blood-brain barrier penetrability. Similarly for molecules 1, 5, 6, 10 and 12 Human intestinal absorptions (HIA) capacity is more. The molecules 6, 8 and 12 exhibited the PGP initiator effect whereas remaining molecules exhibited PGP inhibitory effect shown. The molecules 4, 5 6, 10 and 12 have a CYP-inhibitory effect against different CYP inhibitors. The results also reported skin permeability of the molecules in the acceptable range.

Docking analysis: From ligand-based approaches, it was found most of the molecules present in ethyl acetate extract are biologically active with good pharmacokinetics and therapeutic profile [42]. The literature reveals the antidiabetic and anti-inflammatory activity of *T. cardiflia*. Based on the fact preliminary docking analysis was carried out on different targeted proteins like 5zcb, 5ycp, 4pyp for α-glucosidase, PPARγ ligand binding and human glucose transporter GLUT1 inhibition and 1eqg, 3ln1 for COX-1 and COX-2 inhibitory

TABLE-9
PHARMACOKINETICS PROFILES OF THE MOLECULES IDENTIFIED IN GC-MS ANALYSIS

Molecules	GI absorption	BBB permeant	P-gp substrate	CYP1A2 inhibitor	CYP2C19 inhibitor	CYP2C9 inhibitor	CYP2D6 inhibitor	CYP3A4 inhibitor	Log Kp (skin permeation)
1	High	No	No	No	No	No	No	No	-6.31 cm/s
2	High	Yes	No	No	No	No	No	No	-6.13 cm/s
3	High	Yes	No	No	No	No	No	No	-6.34 cm/s
4	High	Yes	No	Yes	Yes	No	No	No	-4.80 cm/s
5	High	No	No	Yes	Yes	No	No	No	-5.97 cm/s
6	High	No	Yes	No	No	No	No	No	-7.15 cm/s
7	High	Yes	No	Yes	Yes	No	No	No	-6.33 cm/s
8	High	Yes	Yes	No	No	No	No	No	-5.94 cm/s
9	High	Yes	No	No	No	No	No	No	-6.00 cm/s
10	High	Yes	No	No	No	No	Yes	No	-6.98 cm/s
11	High	Yes	No	No	No	No	No	No	-6.47 cm/s
12	High	No	Yes	No	Yes	No	No	No	-3.66 cm/s
Ibuprofen	High	No	No	Yes	No	Yes	No	No	-6.21 cm/s
Celicoxib	High	No	No	Yes	No	Yes	No	No	-6.21 cm/s
Metformin	High	No	No	No	No	No	No	No	-7.84 cm/s
Rosiglitazone	High	No	No	No	Yes	Yes	Yes	Yes	-6.27 cm/s

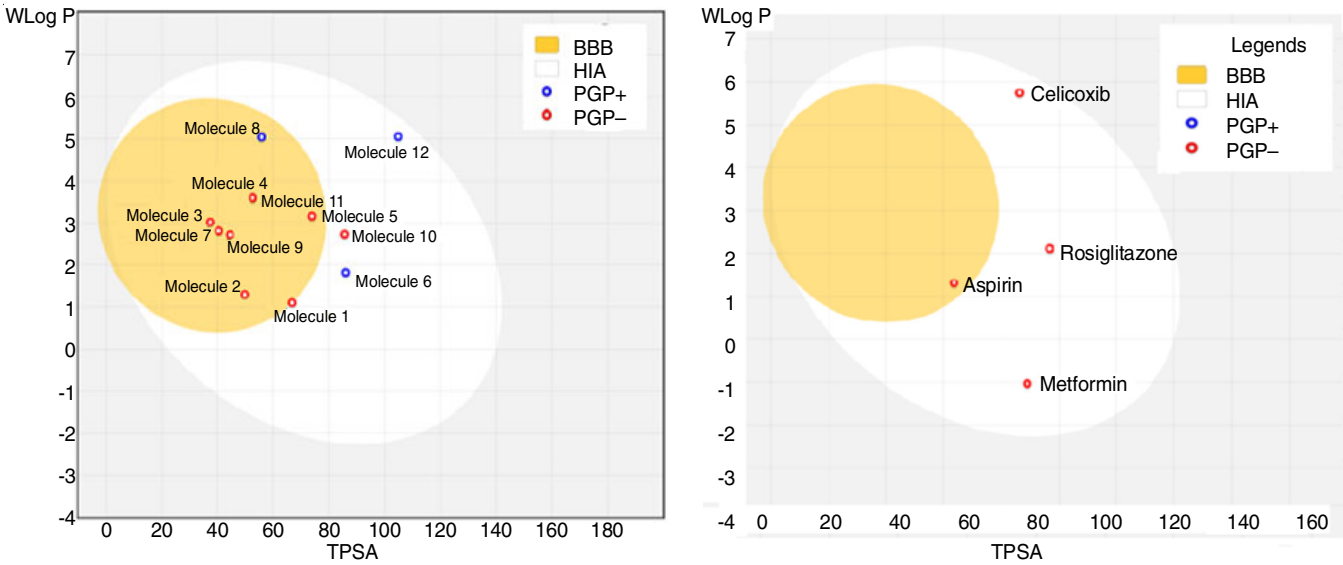


Fig. 5. Boiled egg diagram for the molecules identified in GC-MS analysis in comparison to standard

effects in respect of all the compounds and results were compared with standards metformin, rosiglitazone, ibuprofen and celecoxib.

The results in Table-10 represent the Full Fitness (kcal/mol) and binding energy ΔG (kcal/mol) of the molecules. The results show that the molecules 8 and 10 have good PPARγ ligand binding and GLUT-1 inhibition affinity. To anti-inflammatory activity molecules, 4 and 8 have COX1 and 4 and 10 have COX2 inhibition. The binding pose and binding residue of the molecules with good binding affinity represents in Figs. 6-9. From the analysis, it was found that out of all the compounds molecule-8 have potent COX-1 inhibitions where as molecule 10 has a good inhibitory action PPARγ, GLUT-1 and COX2 targeted proteins. The binding energies -6.0 to -7.0 consider as less binding affinity, -7.0 to -8.00 consider as moderate binding affinity and -8.00 to -10.00 consider as good binding affinity.

Conclusion

In conclusion, it was found that dibutyl phthalate (molecule 4) and 1,4-*bis*(3,4,5-trimethoxy phenyl)hexahydrofuro[3,4-*c*]-furan (molecule 10) is found in the highest concen-tration in ethyl acetate extract of *Tinospora cardifolia*. The compounds reported in GC-MS analysis shown good drug likeliness properties along with good toxicity potential and pharmacokinetic profiles except molecule-4. But due to the PGP and CYP-inhibitory effect, some development at the formulation level is required from a pharmaceutical point of view. In docking analysis, it was found that 4-hydroxy-5′,6a, 8a,9-tetramethyl-1,2,2a,3, 3′,4,4′,5,5′,6,6a,6′,8,8a,8b,9,12,12a-octadecahydro-spiro[naphtho[2′,1′:4,5]indeno[2,1-*b*] (molecule 8) have potent COX-1 inhibitions. Compound [1*S*-(1α, 3aα,4α,6aα)]-1*H*,3*H*-furo[3,4-*c*]furan tetrahydrophenyl (molecule 10) have strong binding affinity on PPARγ, GLUT-1 and COX2 targeted proteins.

TABLE-10
BINDING SCORES OF THE MOLECULES IDENTIFIED IN GC-MS ANALYSIS

Molecule	5ycp		4pyp		1eqg		3ln1	
	Full fitness (kcal/mol)	Binding energy, ΔG (kcal/mol)	Full fitness (kcal/mol)	Binding energy, ΔG (kcal/mol)	Full fitness (kcal/mol)	Binding energy, ΔG (kcal/mol)	Full fitness (kcal/mol)	Binding energy, ΔG (kcal/mol)
1	-1790.34	-6.54	-1197.17	-6.52	-2138.56	-6.75	-2271.01	-6.59
2	-1798.55	-6.96	-1199.25	-7.2	-2137.3	-7.16	-2282.12	-7.34
3	-1792.77	-7.43	-1199.43	-7.34	-2138.41	-7.17	-2284.21	-6.78
4	-1804.64	-7.47	-1208.59	-7.77	-2136.63	-8.04	-2284.21	-8.21
5	-1802.97	-7.58	-1208.02	-7.53	-2143.36	-7.83	-2282.55	-7.89
6	-1734.93	-7.57	-1138.89	-7.8	-2072.01	-7.01	-2209.37	-7.03
7	-1763.76	-7.28	-1170.98	-7.26	-2102.13	-7.22	-2245.47	-7.79
8	-1764.36	-8.15	-1176.79	-8.65	-2097.74	-8.02	-2239.42	-7.49
9	-1776.21	-7.14	-1179.52	-7.66	-2114.35	-7.04	-2251.4	-6.98
10	-1708.82	-8.42	-1127.52	-9.22	-2115.95	-7.04	-2189.36	-8.02
11	-1786.27	-7.8	-1189.89	-8.3	-2115.88	-7.6	-2254.05	-7.33
12	-1787.79	-7.97	-1184.42	-8.13	-2116.87	-7.3	-2262.15	-7.4
Metformin	–	–	-1389.93	-6.38	–	–	–	–
Rosiglitazone	-1849.54	-8.5	–	–	–	–	–	–
Ibuprofen	–	–	–	–	-2158.39	-7.74	–	–
Celicoxib	–	–	–	–	–	–	-2275.95	-10.3

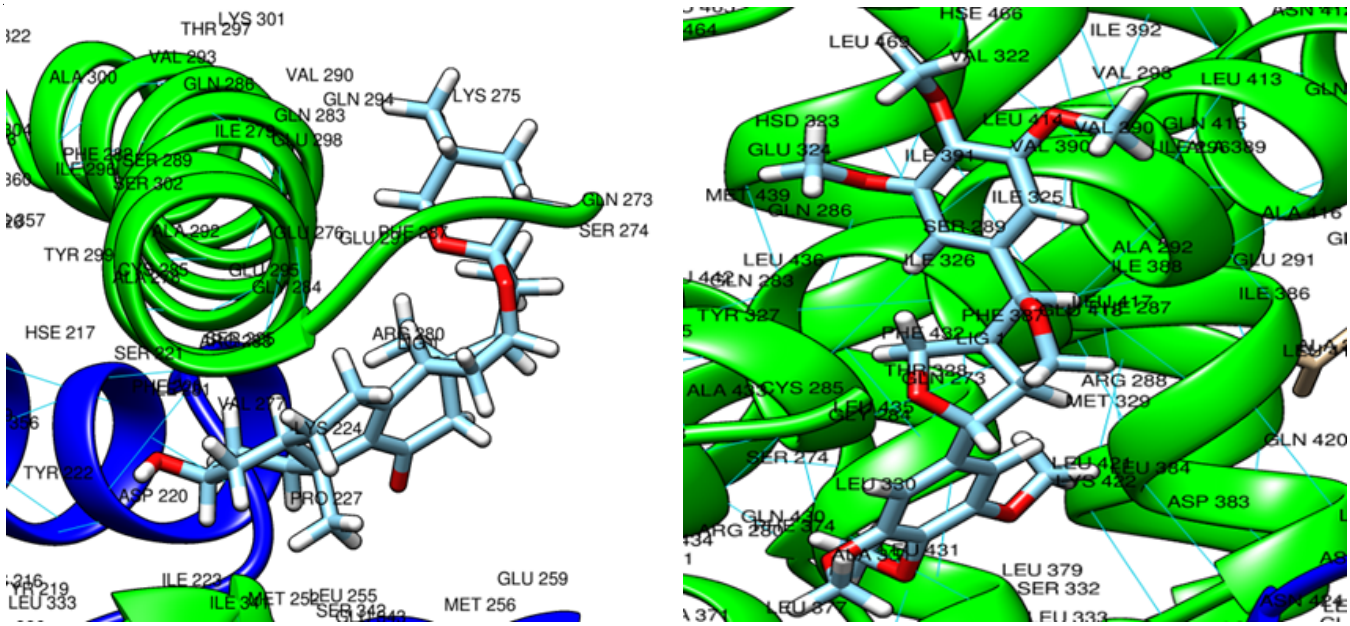


Fig. 6. Binding pose and amino acid residue for molecule 8 and 10 with 5ycp

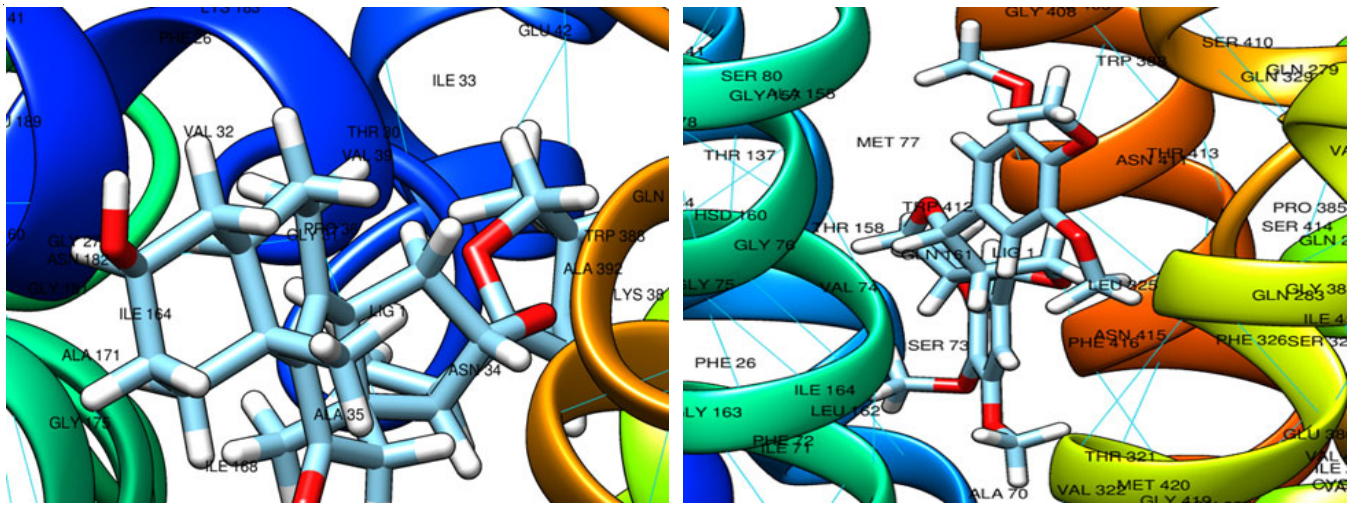


Fig. 7. Binding pose and amino acid residue for molecule 8 and 10 with 4pyp

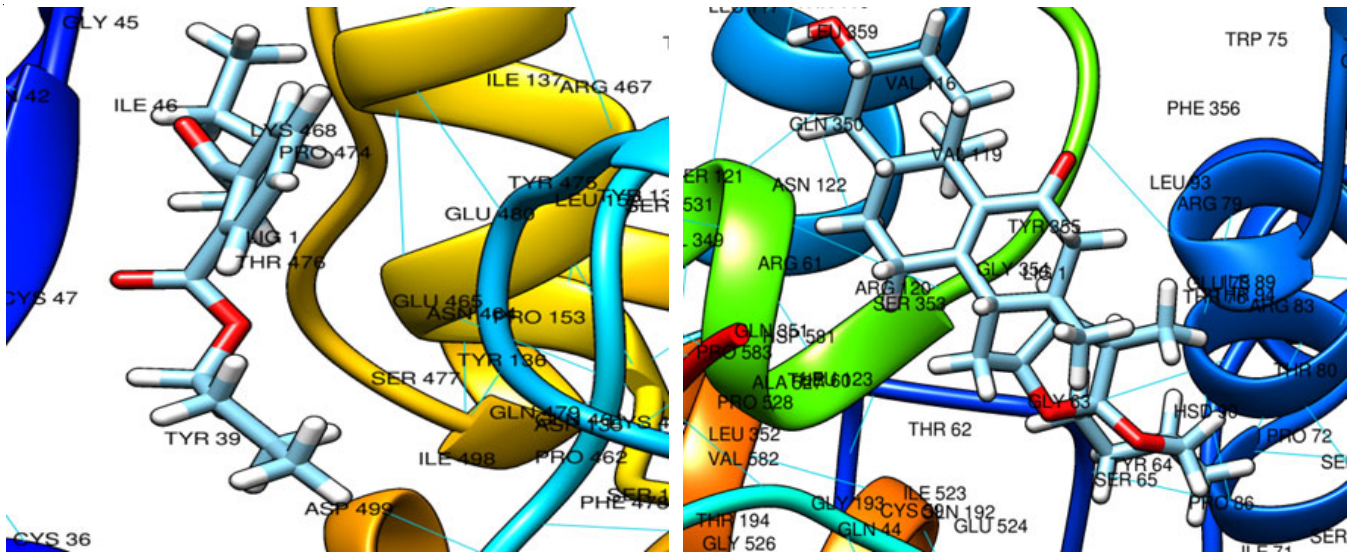


Fig. 8. Binding pose and aminoacid residue for molecule 4 and 8 with 1eqg

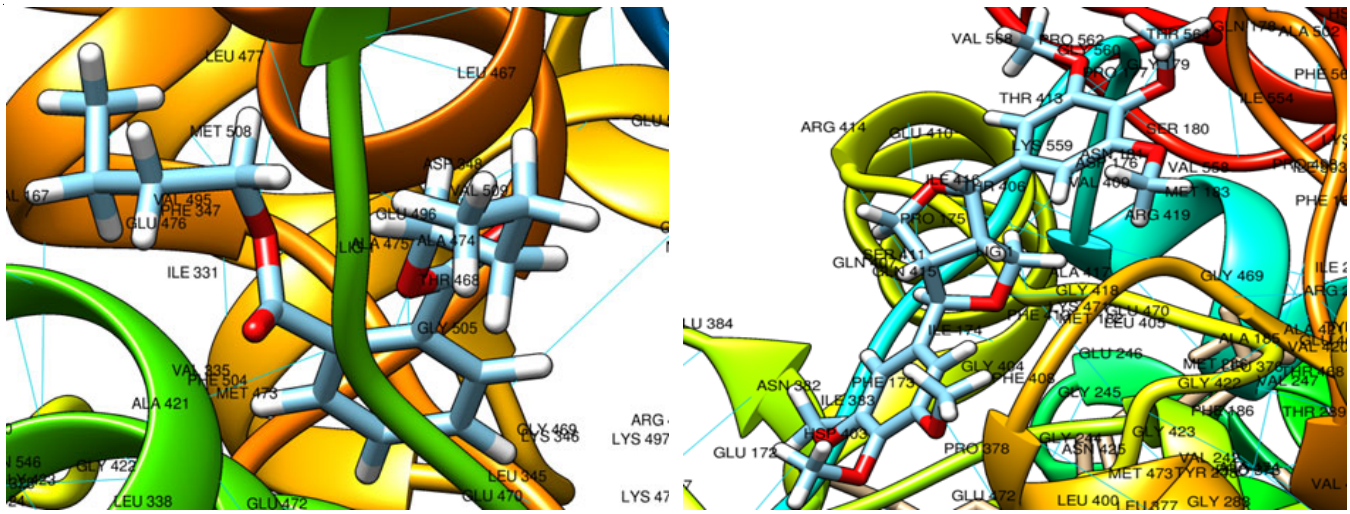


Fig. 9. Binding pose and amino acid residue for molecule 4 and 10 with 3ln1

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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