Search for therapeutics against COVID 19 targeting SARS-CoV-2 papain-like protease: an *in silico* study

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Research Article

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Abstract

Background: The global pandemic of novel coronavirus disease 2019 (COVID-19) caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is an enveloped, positive-sense, single-stranded RNA betacoronavirus of the family Coronaviridae. Papain-like protease (PLpro) of SARS CoV-2 is an important target of COVID-19 because it is a multifunctional cysteine protease essential for coronaviral replication.

Large numbers of phytochemicals with varied chemical structures isolated from medicinal plants have been shown to possess antiviral activity. Some of these phytochemicals have been chosen on the basis of literature survey for this study. Reported inhibitors of the papain-like protease are taken as control and for QSAR study.

Methods: Three dimensional structure of target was downloaded from Protein Data Bank and docked with phytochemicals & inhibitors by using software FlexX. Inhibitors of the papain-like protease were taken from binding database and QSAR analysis was performed by using EasyQSAR software.

Results: Six phytochemicals: Baicalin, Rutin, Biopterin, Licoleafol, Luteolin and Quercetin shows stable bonding pattern with the target in compare to known inhibitors as it shows least score in docking, forms maximum number of hydrogen bonds with the active residues of the receptor. The predicted IC50 values of the phytochemicals are also better than the known inhibitors.

Conclusion: Based on present observation of docking score of both phytochemicals and known inhibitors, IC50 value of known inhibitors and predicted IC50 of phytochemicals, we suggests above mentioned six phytochemicals may be the Papain-like protease (PLpro) targeted potent drug leads against Covid-19.

Introduction

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) causes the global pandemic of novel coronavirus disease 2019 (COVID-19) began in Wuhan, China, in December 2019 and has since spread worldwide (1).

In human being COVID 19 experience as a mild to moderate respiratory problems and can be improved without any particular cure but senior peoples with diabetes, chronic respiratory diseases, cancer and cardiovascular disease are prone to high risk in this infection. The clinical practitioners report says patients with COVID-19 showed sign of sore throat, cough, fever, muscle pain, tiredness and viral pneumonia. This virus spread from diseased person to other through coughing and sneezing and can be avoided by keeping a proper distance with others and sanitizing hands with alcohol regularly. So practicing personal sanitation and public distancing is the only means to prevent from this deadly pandemic (2, 3). Several countries have enforced lockdown which is helping in confining the spread of the disease, however it has not been totally successful. In addition to loss of human lives, COVID-19 is causing rigorous economic losses to both developed and developing nations. According to WHO report as

of May 31, 2020, the virus has infected 5934936 people in more than 215 countries including a shocking 367166 deaths (2).

SARS-CoV-2 is a new member of betacorona virus in the Coronaviridae family (4). The virion of SARS-CoV-2 is consists of crown-shaped peplomers, 80-160 nm in diameter (5).

HCoVs generally are positive-sense single-stranded RNA (30kb) viruses. HCoVs are characterize by two groups of protein; structural such as Spike (S), Nucleocapsid (N) Matrix (M) and Envelope (E), and non-structural proteins such as RNA dependent RNA polymerase (RdRp) (nsp12) the Papain-like protease (PLpro) and 3C-like protease (3CLpro). PLpro is a crucial enzyme in the life cycle of RNA viruses, comprising coronaviruses. PLpro is a multifunctional cysteine protease that processes the viral polyprotein and host cell proteins by hydrolysing the peptide and isopeptide bonds in viral and cellular substrates leading to the virus replication. It is responsible for the cleavages of N-terminus of the replicase poly-protein to release Nsp1, Nsp2 and Nsp3, which is essential for correcting virus replication. PLpro also antagonize the host's innate immunity. As a vital enzyme in the process of coronavirus replication and infection of the host, PLpro is an accepted target for coronavirus inhibitors. It is very important for targeting PLpro to treat coronavirus infections (6, 7). Stripping ubiquitin and ISG15 from host-cell proteins to assist coronaviruses in their evasion of the host innate immune responses is an added function of PLpro. Inhibiting viral replication and inhibiting the dysregulation of signaling cascades in infected cells leading to cell death in surrounding and uninfected cells may be achieved by targeting PLpro (8). Therefore, the papain-like protease (PLpro) is an important target for antiviral drug design (9).

At present, there is no evidence from randomized clinical trials (RCTs) that any possible therapy improves outcomes in patients with either suspected or confirmed COVID-19, therefore, there is an urgent need for effective drugs (10).

Plants have naturally developed over the years in diverse weather conditions on earth and have been bestowed with rich composite of secondary metabolites/phytochemicals with wide pharmacokinetic spectrum. Around 2500 medicinal plant species have been recognized worldwide to treat a myriad of inflictions and ailments (11, 12). A large number of compounds of varied chemical structures isolated from medicinal plants possess antiviral activity (13-19) (Table 1).

Experimental approaches for the study of interactions between drug compounds and target proteins are expensive and time consuming. *In silico* approaches propose techniques to examine hypotheses of new putative drugs by reducing the cost and shortening the time.

Therefore, the present study was conducted to identify potential inhibitors of SARS-CoV-2 papain-like protease from natural compounds using *in silico* approaches. Reported inhibitors of the Papain-like protease are taken as control and for QSAR study.

Table 1: Some antiviral phytochemicals

Sl. No.	Phytochemicals	Plant (part)
1	Calanolide A	*Calophyllum lanigerum*
2	Curcumin	Turmeric etc.
3	Eugenol	*Syzygium aromaticum*
4	Collinin	*Zanthoxylum schinifolium*
5	Ellagic acid	*Phyllanthus urinaria*
6	Resveratrol	grapes, blueberries, raspberries, mulberries and peanuts
7	Galangin	*Helichrysum aureonitens* (shoots)
8	Leachianone G	*Morus alba* L.
9	Kaempferol	apples, grapes, tomatoes, green tea, potatoes, onions, broccoli, squash, cucumbers, lettuce, green beans, peaches, blackberries, raspberries, and spinach etc.
10	epigallocatechin gallate	*Camellia sinensis*
11	epigallocatechin	*Camellia sinensis*
12	epicatechin gallate	*Camellia sinensis*
13	epicatechin	*Camellia sinensis*
14	catechin	*Camellia sinensis*
15	Camptothecin	*Ophiorrhiza mungos* (leaves)
16	Caffeine	*Theobroma cacao* L. and *Coffea* sp.
17	Emetine	*Cephaelis ipecacuanha*
18	Oliverine	*Polyathia oliveri*
19	Schumannificine	*Schumanniophyton magnificum* (bark)
20	Afromosin	*Wisteria brachybotrys*
21	Formononetin	*Wisteria brachybotrys*
22	Ternatin	*Evodia madagascariensis*
23	Wogonin	*Scutellaria baicalensis*
24	Podophyllotoxin	*Podophyllum peltatum*
25	Cochinolide	*Homalium cochinchinesis* (root bark)
26	Dolabellane	*Dolabella californica*
27	Sageone	*Salvia officinalis*
28	Silymarin	*Silybum marianum*
29	Cyanidol	*Silybum marianum*
30	Salaspermic acid	*Triterygium wilfordii*
31	Platanic acid	*Syzigium claviflorum* (leaves)
32	Baicalin	*Scutellaria baicalensis* (roots)
33	Chalcones	*Glycyrrhiza inflate* (roots)

34	Dammarenolic acid	*Aglaia* sp. (bark)
35	Decanoylphorbol-13 acetate	*Croton mauritianus* (leaves)
36	Excoecarianin	*Phyllanthus urinaria* (whole plant)
37	Loliolide	*Phyllanthus urinaria* (whole plant)
38	Honokiol	Magnolia tree (roots, bark)
39	Jubanines	*Ziziphus jujuba* (roots)
40	Limonoids	*Swietenia macrophylla* (stem)
41	Oleanane	*Camellia japonica* (flowers)
42	Quercetin	*Embelia ribes* (seeds)
43	Saikosaponins	*Bupleurum kaoi* (roots)
44	Sennoside A	*Rheum palmatum* (roots)
45	Silvestrol	*Aglaia foveolata* (leaves, bark)
46	SJP-L-5	*Schisandra micrantha* (roots)
47	Spiroketalenol	*Tanacetum vulgare* (rhizome)
48	Swerilactones	*Swertia mileensis* (whole plant)
49	Xanthohumol	*Humulus lupulus* (whole plant)
50	Oxyresveratrol	*Artocarpus lakoocha* (Heartwood)
51	Saikosaponin B2	*Bupleurum kaoi* (Root)
52	Tangeretin	*Citrus reticulate* (Pericarps)
53	Nobiletin	*Citrus reticulate* (Pericarps)
54	Jatrophane ester	*Euphorbia amygdaloides* spp. (Whole plant)
55	Glycyrrhizic acid	*Glycyrrhiza radix* (Roots)
56	Quercetin 3-rhamnoside	*Houttuynia cordata* (Aerial parts)
57	Samarangenin B	*Limonium sinense* (Root)
58	LPRP-Et-97543	*Liriope platyphylla* (Root)
59	Pterocarnin A	*Pterocarya stenoptera* (Bark)
60	Chalepin	*Ruta angustifolia* (Leaves)
61	Pseudane IX	*Ruta angustifolia* (Leaves)
62	Manassantin B	*Saururus chinensis* (Root)
63	Dicaffeoylquinic acids	*Schefflera heptaphylla* (Leaf stalks)
64	Scopadulcic acid B	*Scoparia dulcis* L. (Whole plant)
65	5,7,4' trihydroxy-8-methoxyflavone (F36)	*Scutellaria baicalensis* (Root)
66	Naringin	grape and orange (skin)

67	Myricetin	*Myrica cerifera*
68	Inophyllum_B	*Calophyllum inophyllum*
69	Inophyllum_P	*Calophyllum inophyllum*
70	Pericalline	*Catharanthus roseus* / *C. lanceus*
71	Chrysophanic acid	*Dianella longifolia*
72	Nordihydroguaiaretic acid	*Larrea divaricata*
73	Retrojusticidin B	*Phyllanthus myrtifolius*
74	Emodin	*Rheum sp.* and *Polygonum sp.*
75	Gingerol	Zingiberis rhizome
76	Anthraquinone	*Dianella longifolia*
77	Methyl rosmarinate	*Hyptis atrorubens Poit*
78	Licoleafol	*Glycyrrhiza uralensis*
79	Amaranthin	*Amaranthus tricolor*
80	Calceolarioside B	*Fraxinus sieboldiana*
81	Actinophnine	*Actinodaphne hookeri*
82	Biopterin	*Crithidia fasciculata*
83	Buchapine	*Euodia roxburghiana*
84	Caribine	*Hymenocallis arencola*
85	Lycorine	*Clivia miniata*
86	Fisetin	*Rhus spp.*
87	Morin	*Chlorophora tinctoria* L. Gaud
88	Luteolin	*Matricaria inodora* L.
89	Rutin	*Fagopyrum esculentum*
90	Taxifolin	*Acacia catechu*
91	Oleanolic acid	*Prosopis glandulosa*
92	Betulinic acid	*Syzigium claviflorum*

Materials And Methods

The Ligands

Antiviral phytochemicals were taken based on literature survey and known inhibitors of the papain-like protease of SARS CoV-2 were taken from the Binding Database (20). The structure of these phytochemicals and known inhibitors of the Papain-like protease were retrieved from PubChem Compound and by drawing using ChemOffice tools. The three dimensional structure of these compounds in sdf format were generated using OpenBabel software (21).

The receptor

The crystal structure of the papain-like protease of SARS CoV-2 was downloaded from RCSB Protein Data Bank (http://www.rcsb.org). It was deposited by Osipiuk, J et al. on 22nd March 2020 and released on 1st April 2020. The protein has three chains (Chain A, B and C) of 317 residues determined by X-ray diffraction method at a resolution of 2.70 Å. The PDB id of the protein is 6W9C.

Active site identification

The active sites of the receptor were identified by the FlexX software during receptor preparation process.

Protein – Ligand interaction using FlexX

Docking is a term used for computational schemes that attempt to find the best matching between two molecules: a receptor and ligand (22). The receptor was docked with known inhibitors of the Papain-like protease and phytochemicals using software FlexX (23). The active site amino acids were defined in the target molecule during the target preparation. The SDF file of all the compounds was loaded in FlexX as docking library. The output file gave the energy values in Kcal/mol. For each docked molecule, this value was noted down.

Quantitative Structure Activity Relationship (QSAR) studies

The QSAR analysis (24) was performed by taking the known inhibitors of the papain-like protease. The QSAR descriptors *viz.* Molar Refractivity, Molar volume, Parachor, Polarizability and Monoisotopic mass were generated for each of the molecule using ACD ChemSketch softwares. The activities have been calculated by taking the inverse logarithm of IC50 values. The descriptors were tabulated in a MS Excel Sheet against their bioactivities (log IC_{50}^{-1}). The descriptors and activities were loaded in Easy QSAR software for multiple linear regression analysis. From the regression, the QSAR equation was generated and the IC50 values of best docked phytochemicals were was predicted.

Results

Interaction energies between ligand and receptor play the most crucial role in drug designing. In this work, the papain-like protease of SARS CoV-2 (PDB ID: 6W9C) was selected as drug target and the interactions of the compounds were studied using FlexX software. The docking results of phytochemicals with target are described in table 2 and the docking results of papain-like protease inhibitors with target are described in table 3. The docking poses of best docked phytochemicals and inhibitors are shown in Figures (Figure 1 – Figure 9). Phytochemicals: Baicalin, Quercetin, Licoleafol, Biopterin, Luteolin and Rutin show much more binding affinity with the target in comparison to the reported inhibitors of the papain-like protease.

Table 2: Docking results of Phytochemicals with Papain-like protease of SARS CoV-2

Phytochemicals	Docking Score (Kcal/mol)	Residues involved in the hydrogen bonding
Calanolide A	-5.9827	ARG138, ASN146
Curcumin	-11.9951	ASP12, ASN13
Eugenol	-10.6228	ARG138, ASN146, TYR83
Collinin	-3.9488	ARG138, ASN146, ASN13
Ellagic acid	-14.0599	LYS105, TRP106, ASP286, ALA288
Resveratrol	-11.7275	TYR56, TYR72, ALA131
Galangin	-16.4712	ASN109, CYS111, TYR112, GLY163, GLN269, GLY271
Leachianone G	-11.2836	THR74, THR75, AN156
Kaempferol	-15.1889	ASN109, CYS111, TYR112, GLY163, GLN269, GLY271
Epigallocatechin gallate	-13.9114	HIS89, LYS92, TRP93, TRP106, ASP108, CYS155, ASN156, LYS157
Eepigallocatechin	-18.7043	TYR72, TYR83, ALA131, ARG138, ASN146
Epicatechin gallate	-13.6344	ASP12, ASN13, TYR83, ARG138, ASN146
Epicatechin	-16.2121	TYR83, ALA131, ARG138, ASN146
Catechin	-16.2121	TYR83, ALA131, ARG138, ASN146
Camptothecin	-10.1170	TRP106, ASP286, ALA288
Caffeine	-7.9904	THR74, THR75
Emetine	-12.2723	THR74, ASP76, LYS92, ASN156
Oliverine	-14.6060	LYS105, ASP108, GLU167
Schumannificine	-17.5028	LYS92, ASP108, LYS157, GLU161
Afromosin	-10.6251	TYR83, ARG138, ASN146
Formononetin	-11.5464	TYR72, ARG138
Ternatin	-8.2402	LYS105, ASP108, TYR264
Wogonin	-17.5758	ASP12, TYR83, ARG138, ASN146
Podophyllotoxin	-8.0189	THR74, THR75, ASN156
Cochinolide	-14.2751	ASN13, TYR83, ARG138, ASN146
Dolabellane	-7.4013	THR74, THR75, ASP76
Sageone	-11.6264	ASN13, TYR56, ARG138, ASN146
Silymarin	-13.3862	ASN13, TYR72, ALA131, ALA135, ARG138
Cyanidol	-12.8967	HIS89, TRP106, LYS157, LEU162
Salaspermic acid	NOT DOCKED	
Platanic acid	-7.3902	THR74, THR75, ASP76
Baicalin	**-34.3309**	ASN13, TYR71, TYR83, ASP134, ARG138
Dammarenolic acid	-0.1305	ASN13, ARG138
Excoecarianin	NOT DOCKED	
Loliolide	-10.4500	ASN13, ARG138, ASN146

Honokiol	-7.9547	TYR56, ALA131, ASN146
Oleanane	NOT DOCKED	
Quercetin	**-24.9869**	ASP108, ASN109, CYS111, TYR112, GLY163, GLN269, GLY271
Sennoside A	-8.1751	ASN109, VAL159, LEU162, GLN269
Silvestrol	-1.8664	ASN13, TYR71, TYR72, ARG138
SJP-L-5	-13.2672	ASN13, ARG138, ASN146
Xanthohumol	-9.1494	LYS92, LYS105, TRP106, LYS157, GLU161
Spiroketalenol	-12.9512	THR74, THR75, ASP76
Licochalcone	-10.8747	ALA131, ASN146
Chalcone	-14.1573	ARG138
Decanoylphorbol-13 acetate	6.3441	LYS92, LYS105, TRP106, LYS157, GLU161
Jubanine A	-1.8017	LYS157, GLU167
Jubanine B	NOT DOCKED	
3-Hydroxy Caruilignan C	-8.3390	ASN13, TYR56, ARG138
Limonin	NOT DOCKED	
Oxyresveratrol	-14.8726	ASN13, TYR56, TYR72, ALA131
Saikosaponin B2	NOT DOCKED	
Tangeretin	-6.8313	ASN13, ARG138, ASN146
Nobiletin	-6.0527	ASN13, ARG138, ASN146
Jatrophane ester	NOT DOCKED	
Glycyrrhizic acid	NOT DOCKED	
Quercetin 3-rhamnoside	-20.3139	ASN13, TYR56, TYR72,TYR83, ARG138, ASN146
LPRP-Et-97543	-13.2511	ASP108, GLU167, GLY271
Chalepin	-7.9836	ASN13, TYR56
Manassantin B	-8.8858	THR74, THR75, TRP93, ASN156, LYS157, GLU161
Dicaffeoylquinic acid	-12.6497	ASN13, TYR56, TYR72, ASP134, ARG138, ASN146
Scopadulcic acid B	NOT DOCKED	
Naringin	-9.1107	ASP12, ASN13, TYR71, ASP134
Myricetin	-14.3847	ASP108, ASN109, CYS111, TYR112, GLY163, GLN269, GLY271
Inophyllum_B	-11.4238	ARG138, ASN146
Inophyllum_P	-11.4238	ARG138, ASN146
Pericalline	-12.1713	TRP106, GLU167
Chrysophanic acid	-22.4865	ASP12, ASN13, TYR56, ARG138
Nordihydroguaiaretic acid	-9.8957	TRP106, GLY163, TYR268, ASP286, ALA288
Retrojusticidin B	-12.5426	LYS105, TRP106, ALA288
Emodin	-23.1325	ASN13, TYR56, ALA131

Gingerol	-5.3377	TYR71, TYR83, ASP134, ARG138, ASN146
Anthraquinone	-11.6131	ASN13, ARG138
Methyl rosmarinate	-13.9423	LYS105, ASP108, ASP286, ALA288
Licoleafol	**-26.5293**	LYS92, LYS105, TRP106, LYS157, LEU162, GLU167
Amaranthin	-20.2696	ASP12, ASN13, TYR56, ASP134, ARG138, GLU143, ASN146
Calceolarioside B	-15.0832	ASN13, TYR56, TYR72, ARG138
Papaverine	-8.1881	LYS45, THR74, THR75, ASP76
Biopterin	**-26.9995**	ASN109, GLY160, GLN269
Buchapine	-7.3976	THR74, THR75
Caribine	-14.7955	LYS105, LYS157, GLU167
Lycorine	-15.1457	LYS105, ASP108, GLU161, GLU167
Fisetin	-16.3059	TYR83, ALA131, ARG138
Morin	-17.0390	ASN109, GLY160, LEU162, GLN269
Luteolin	**-25.9438**	ASP108, ASN109, CYS111, TYR112, LY163, GLN269, GLY271
Rutin	**-27.0507**	ASP12, ASN13, TYR83, ASP134, ARG138, ASN146
Taxifolin	-15.0153	ASN13, TYR72, ASP134, ARG138
Oleanolic acid	NOT DOCKED	
Betulinic acid	NOT DOCKED	
5,7,4' trihydroxy-8-methoxyflavone	-15.7340	TYR71, TYR83, ARG138, ASN146
Swerilactone A	NOT DOCKED	
Swerilactone B	NOT DOCKED	

Table 3: Docking results of Known inhibitors with Papain-like protease of SARS CoV-2

Inhibitors (ID)	Docking Score (Kcal/mol)	Residues involved in the hydrogen bonding
BDBM31524	-16.0618	TYR56, ARG138
BDBM31531	-17.4198	TYR56, TYR71, ALA131, ARG138
BDBM31523	**-20.2673**	ASN13, TYR56, ARG138
BDBM50007789	-12.5947	ASP108, LYS157, LEU162, GLU167
BDBM31530	-19.0533	TYR56, ASP134, ARG138
BDBM50007789	-12.5947	ASP108, LYS157, LEU162, GLU167
BDBM31530	-19.0533	TYR56, ASP134, ARG138
BDBM31528	-16.2484	TYR56, ARG138
BDBM154574	-11.6219	ASP108, LYS157, LEU162, GLU167
BDBM31520	-16.3075	TYR56, ARG138
BDBM31524	-16.0614	TYR56, ARG138
BDBM31527	-16.5289	TYR56, ARG138
BDBM31529	-16.4990	ASN13, TYR56, ARG138
BDBM31526	-16.6231	TYR56, ARG138
BDBM31508	-15.3900	TYR56, ARG138
BDBM154573	-12.0729	ASN13, TYR83, ASN146
BDBM31525	-12.0982	TYR56, ARG138
BDBM31514	-12.1381	TYR56, ARG138
BDBM31512	-14.7425	TYR56, ARG138
BDBM31509	-15.6676	TYR56, ARG138
BDBM31521	-10.6302	THR74, THR75
BDBM31522	**-24.0149**	TYR56, TYR71, ALA131, ARG138
BDBM31510	-11.8777	TYR56, ARG138
BDBM31516	**-21.3324**	TYR56, TYR71, ALA131, ARG138
BDBM31511	-18.2742	ASN13, TYR56, ARG138
BDBM31513	-9.5794	TYR56, ARG138

There was significant correlation with R square value of 82% (The Rsq value should be definitely high for a good QSAR equation, Higher Rsq means higher fitting of the equation to the given data, hence better predictions it will provide for new test data). The Adjusted Rsq is 73 % therefore the difference between Rsq and adjusted Rsq is less (High difference in Rsq and Adjusted Rsq indicates weaker overall prediction). The F statistics value of the test is 5.01 and the critical F value is 2.20 (The F statistics of the test should be greater than Critical F otherwise the generated equation is inefficient).

The equation generated out of QSAR analysis is as follows:

Activity = -6.36683 + 40.54242 (Molar refractivity) + -0.02928 (Molar volume) + 0.012697 (Parachor) + -1.02268E+26 (Polarizability) + 0.003573 (Monoisotopic mass)

From the above QSAR equation the IC 50 value of Baicalin, Quercetin, Licoleafol, Biopterin, Luteolin and Rutin were predicted and shown in table 4. The multiple regression plot (linear) of QSAR analysis is shown in figure 10.

Table 4: Comparison of IC 50 values of Papain-like protease inhibitors and best docked phytochemicals

Inhibitors (ID)	IC $_{50}$ (nM)	Phytochemicals	Predicted IC $_{50}$ (nM)
BDBM31524	230	Baicalin	69.1831
BDBM31531	460	Quercetin	933.2543
BDBM31523	560	Licoleafol	1548.817
BDBM50007789	980	Biopterin	19498.45
BDBM31530	1180	Luteolin	977.237
BDBM50007789	1200	Rutin	0.18197
BDBM31530	1300		
BDBM31528	1400		
BDBM154574	2260		
BDBM31520	2300		
BDBM31524	2640		
BDBM31527	2700		
BDBM31529	4800		
BDBM31526	5200		
BDBM31508	8700		
BDBM154573	10900		
BDBM31525	11100		
BDBM31514	12100		
BDBM31512	13500		
BDBM31509	14800		
BDBM31521	22600		
BDBM31522	24800		
BDBM31510	29100		
BDBM31516	46100		
BDBM31511	90000		
BDBM31513	149000		

Discussion

The least score in docking was preferred for considering better ligand as it indicates more stability in binding (22). The interactions of phytochemicals and the Papain-like protease inhibitors with target were screened based on hydrogen bonding based prediction (25). Among the inhibitors, three inhibitors: BDBM31523, BDBM31522 and BDBM31516 show more binding affinity with target. The docking score of BDBM31523 is -20.2673 Kcal/mol and forms four hydrogen bonds with active site residues. BDBM31522

forms five hydrogen bonds with the residues of binding pocket with a docking score of -24.0149 Kcal/mol. BDBM31516 binds with the target with a docking score of -21.3324 Kcal/mol and forms five hydrogen bonds.

Some phytochemicals exhibited better binding efficacy with the target. Among them Baicalin, Rutin, Biopterin, Licoleafol, Luteolin and Quercetin binds more strongly with the target in comparison to the inhibitors and other phytochemicals.

Baicalin a flavonoid obtained from roots of the plant *Scutellaria baicalensis* interferes and inhibits dengue virus (DENV-2) at various stages of the virus replication cycle (26) has the highest docking score (-34.3309 Kcal/mol) with the receptor among all the phytochemicals and inhibitors also forms seven hydrogen bonds with the receptor.

Rutin have antiviral effect against avian influenza strain H5N1 (27), a naturally occurring flavonoid found in many foods, especially buckwheat (*Fagopyrum esculentum*) strongly docked with the target forming ten hydrogen bonds with a docking score of -27.0507 Kcal/mol.

Biopterin isolated from *Crithidia fasciculata* possessing antiviral activity (14) shows strong binding affinity with the receptor, forms eight hydrogen bonds with a docking score of -26.9995 Kcal/mol.

Licoleafol a prenylated antiviral flavanone isolated from *Glycyrrhiza uralensis* (28) which forms eight hydrogen bonds with target and binds with a docking score of -26.5293 Kcal/mol.

Luteolin an antiviral flavone against herpes and poliomelytis viruses isolated from *Matricaria inodora* L. plant (14) has binding efficacy with the target with a docking score of -25.9438 Kcal/mol and forms eight hydrogen bonds with the receptor.

Quercetin exhibit remarkable activities against picornaviruses and vesicular stomatitis virus (14) a potent antioxidant flavonoid found mostly in onions, grapes, berries, cherries, broccoli, and citrus fruits shows good binding affinity with the target, forms eight hydrogen bonds with a docking score of -24.9869 Kcal/mol.

The predicted IC50 values of above mentioned phytochemicals were much less than the most of the inhibitors (Table 4).

The Papain-like protease (PLpro) is a multifunctional cysteine protease that processes the viral polyprotein and host cell proteins by hydrolysing the peptide and isopeptide bonds in viral and cellular substrates leading to the virus replication. Targeting PLpro with antiviral drugs may have an advantage in not only inhibiting viral replication but also inhibiting the dysregulation of signaling cascades in infected cells that may lead to cell death in surrounding, uninfected cells (6, 7 and 8).

Six phytochemicals: Baicalin, Rutin, Biopterin, Licoleafol, Luteolin and Quercetin shows stable bonding pattern with the target in compare to known inhibitors as it shows least score in docking, forms

maximum number of hydrogen bonds with the active residues of the receptor. The predicted IC50 values of the phytochemicals are also better than the known inhibitors. Therefore, these six phytochemicals have more potentiality to inhibit the Papain-like protease.

Conclusion

Based on present observation of docking score of both phytochemicals and known inhibitors, IC50 value of known inhibitors and predicted IC50 of phytochemicals, we suggests six phytochemicals: Baicalin, Rutin, Biopterin, Licoleafol, Luteolin and Quercetin may be the Papain-like protease (PLpro) targeted potent drug leads against Covid-19. However, further studies are required to validate the same in vivo or in vitro.

Declarations

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Figures

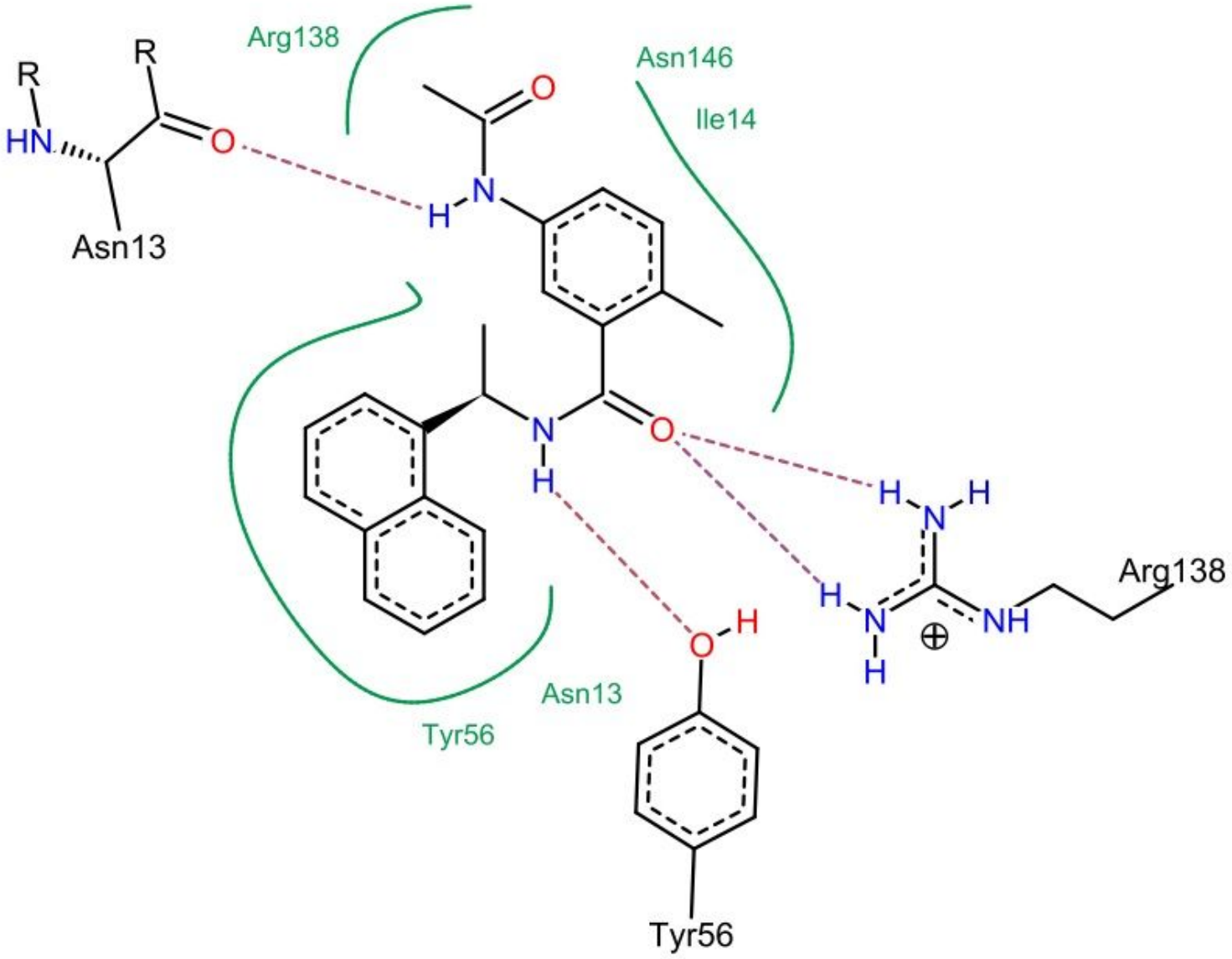

Figure 1

Docking pose of inhibitor BDBM31523 with target

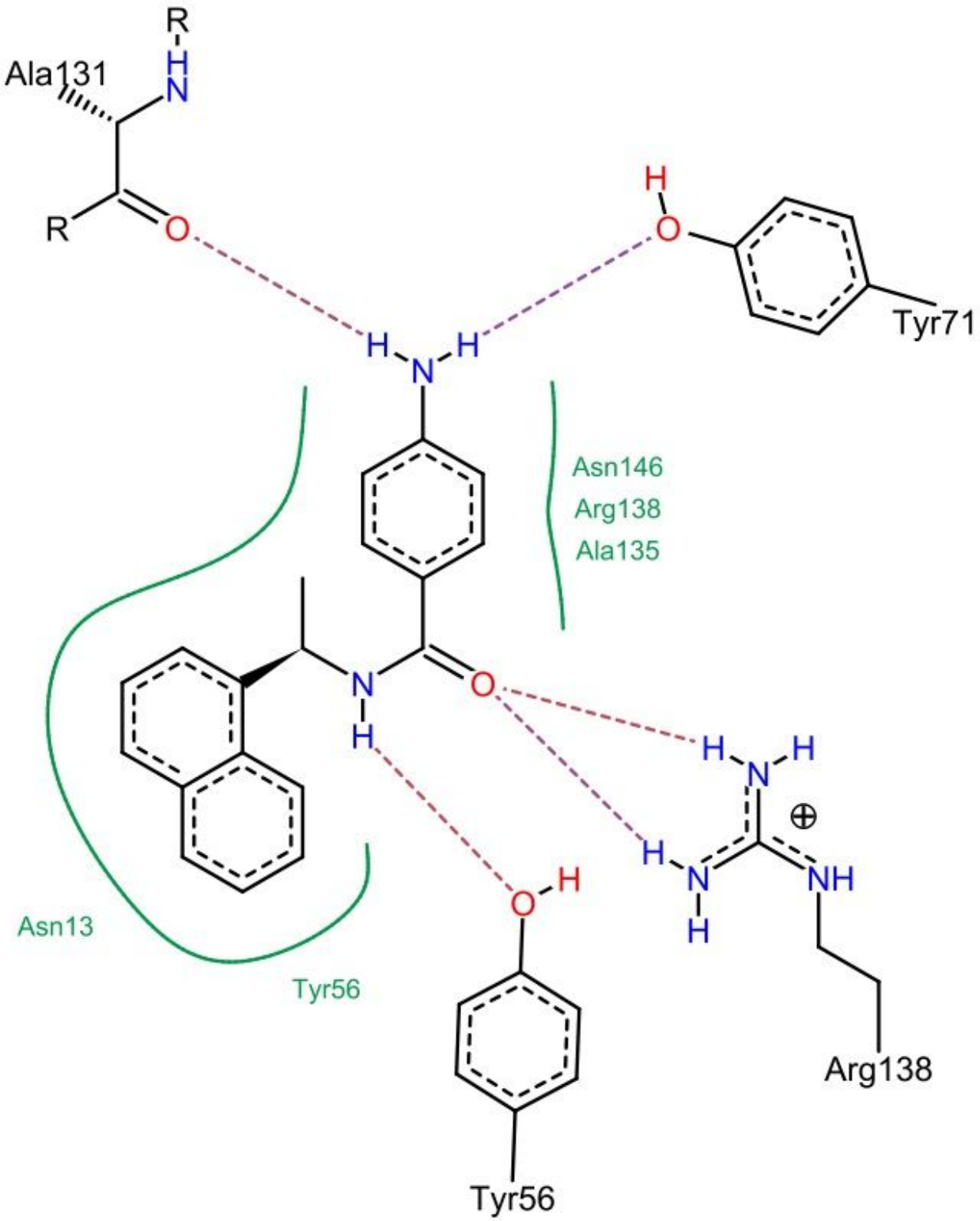

Figure 2

Docking pose of inhibitor BDBM31522 with target

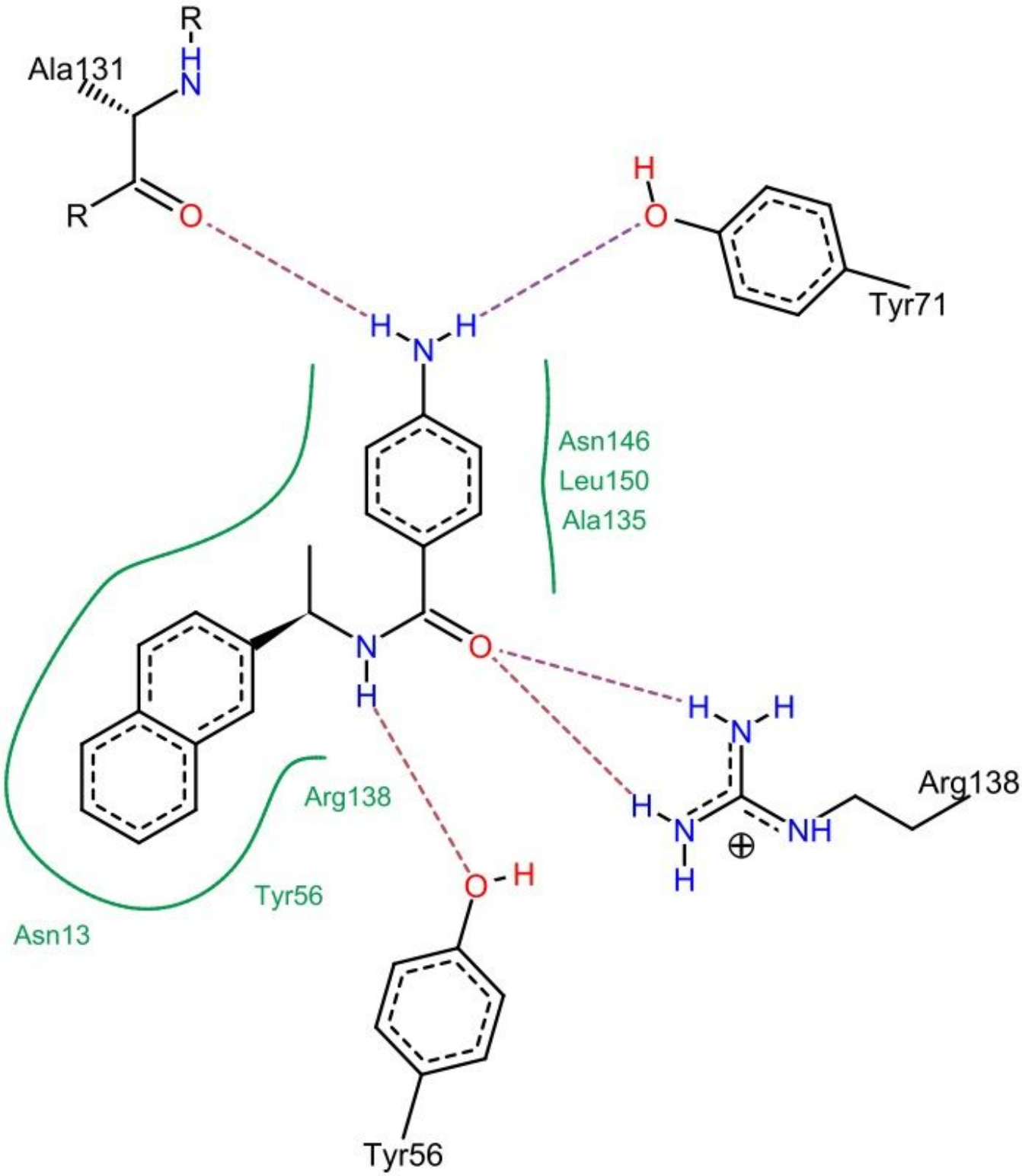

Figure 3

Docking pose of inhibitor BDBM31516 with target

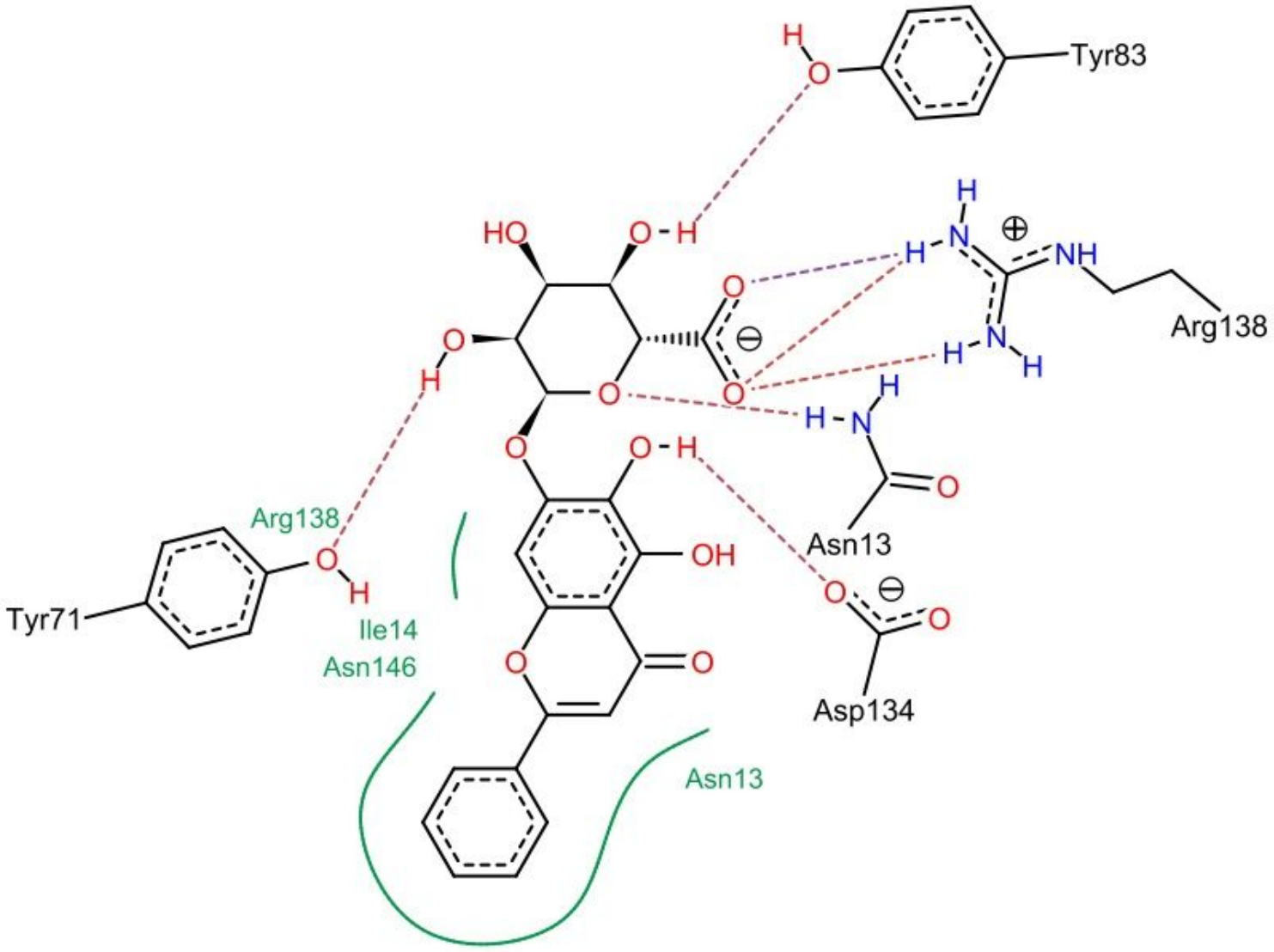

Figure 4

Docking pose of phytochemical Baicalin with target

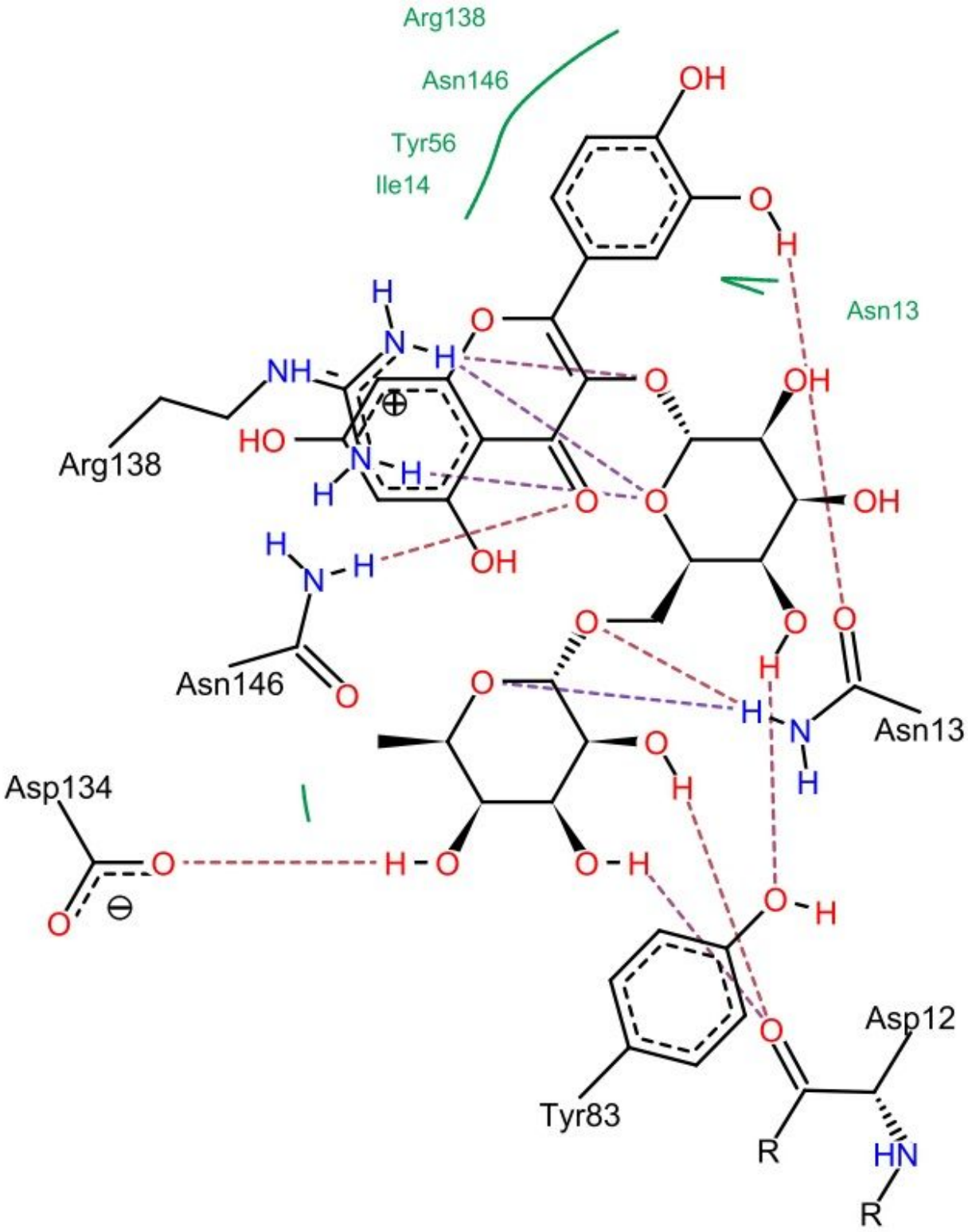

Figure 5

Docking pose of phytochemical Rutin with target

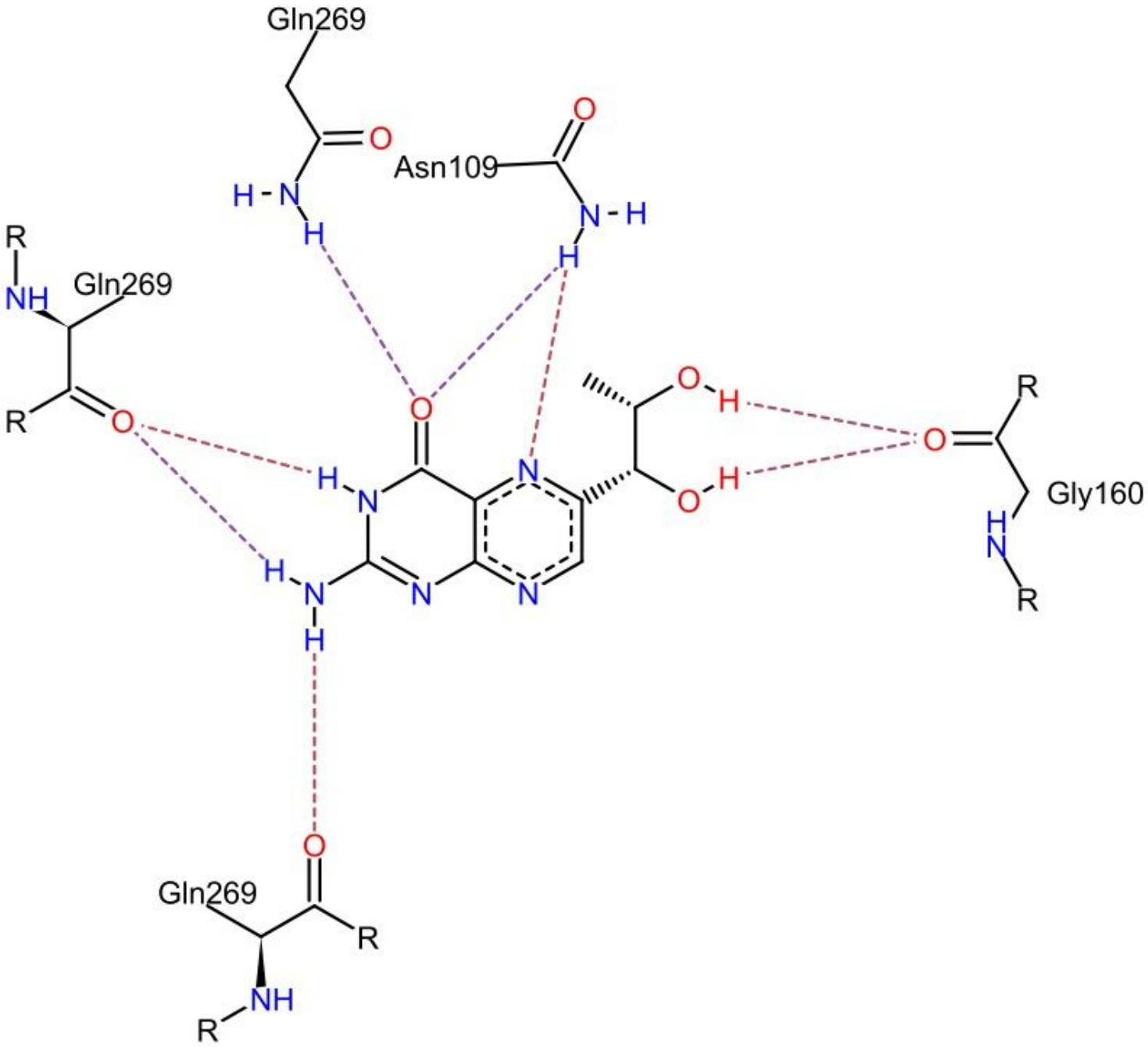

Figure 6

Docking pose of phytochemical Biopterin with target

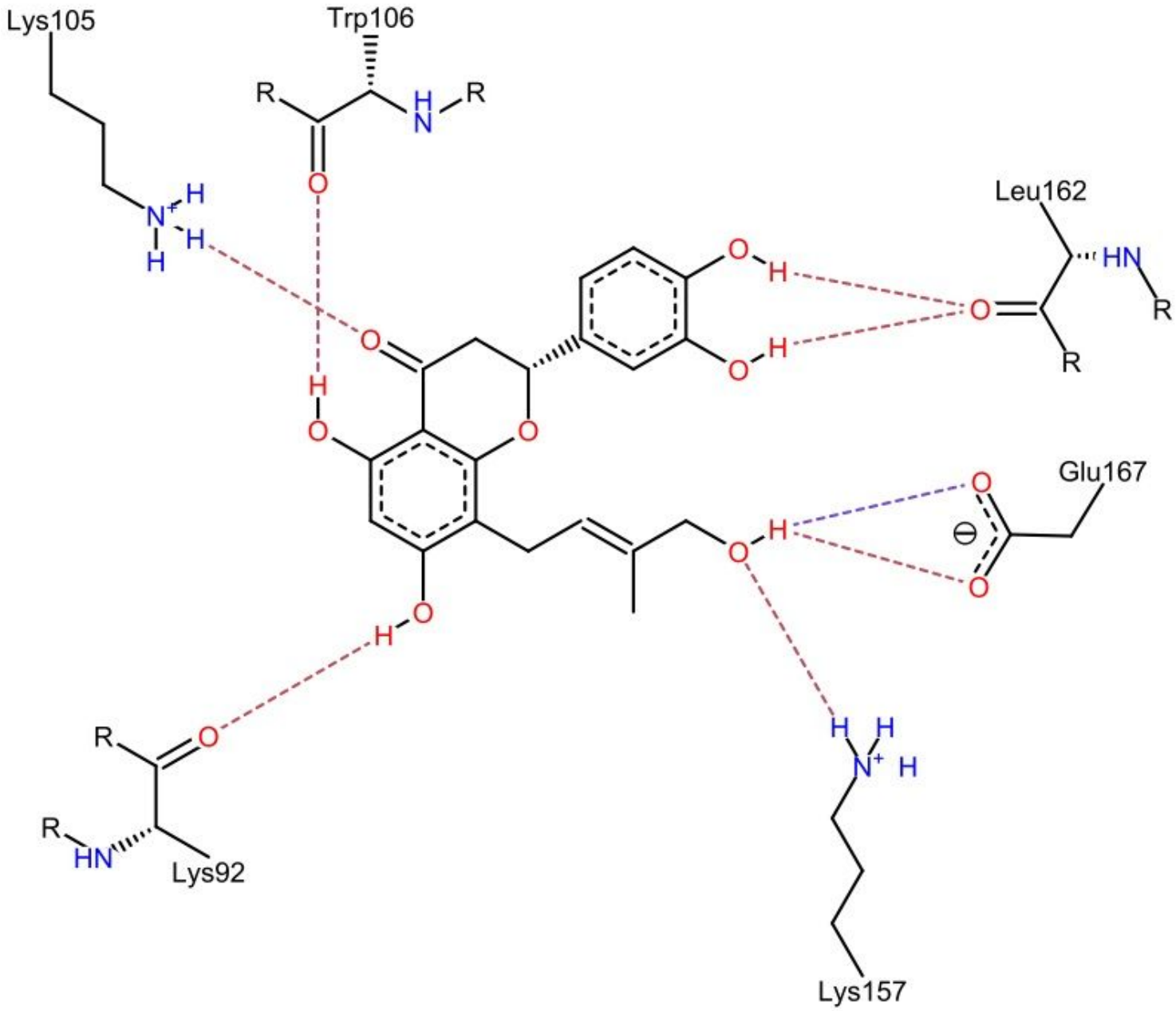

Figure 7

Docking pose of phytochemical Licoleafol with target

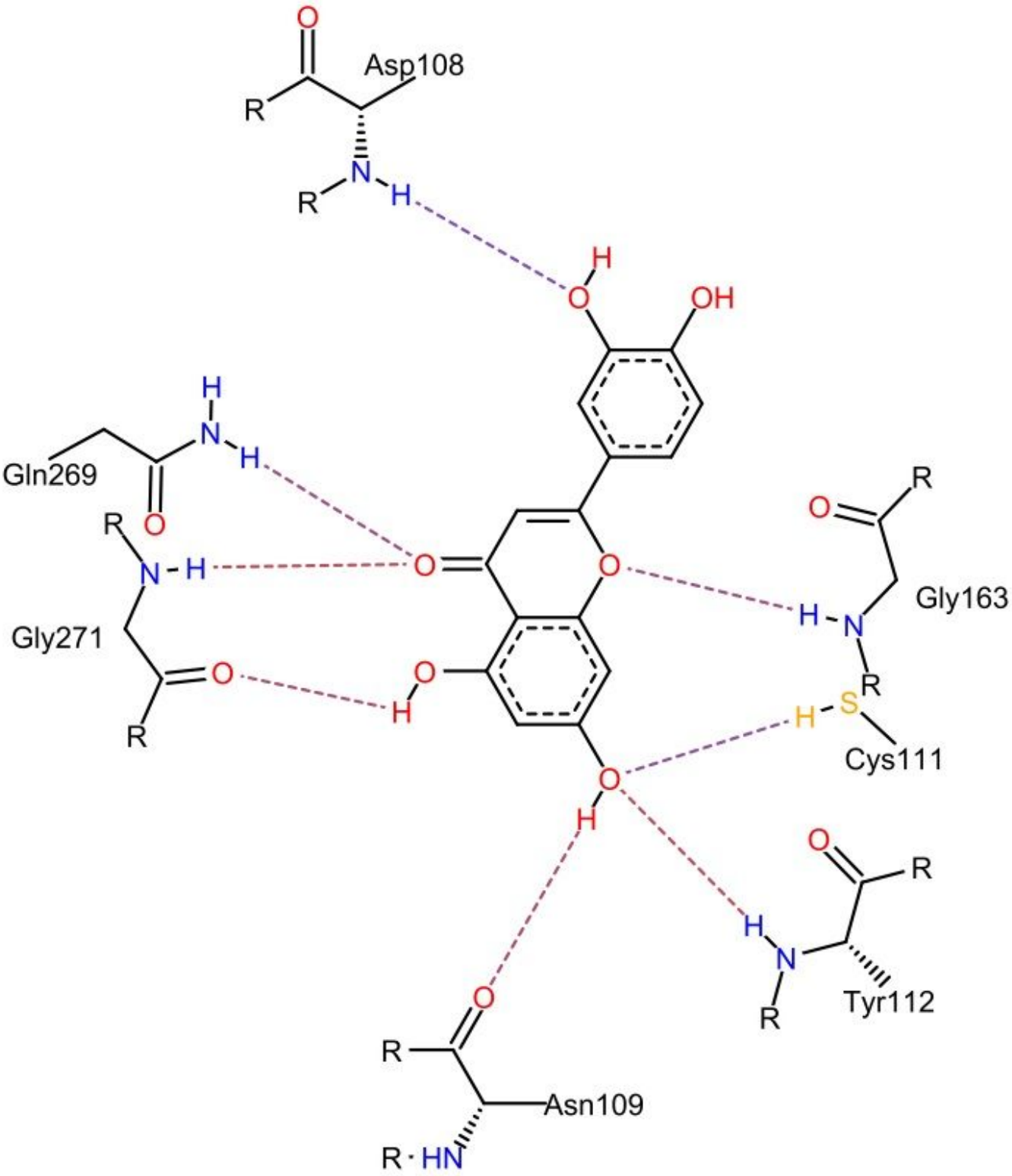

Figure 8

Docking pose of phytochemical Luteolin with target

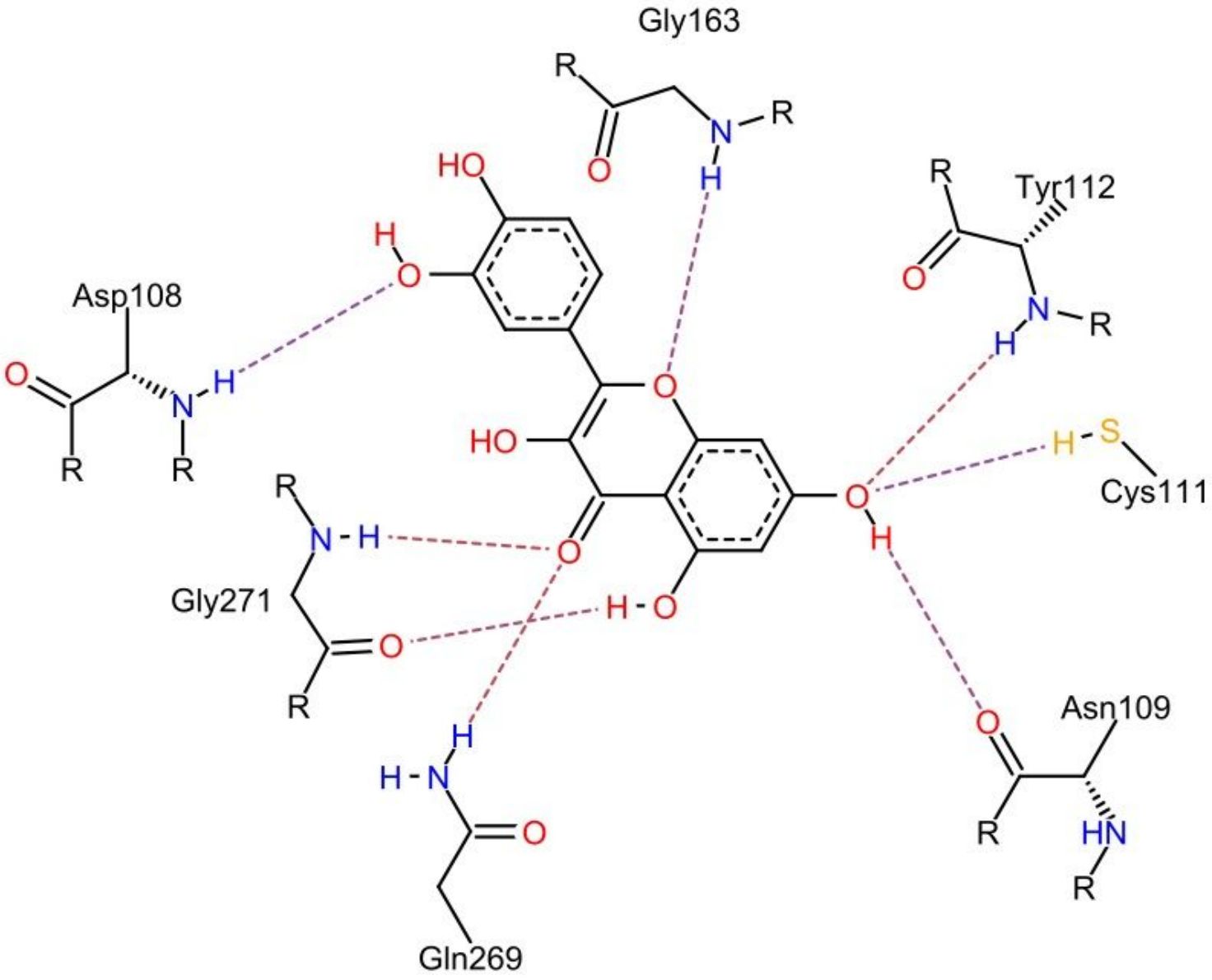

Figure 9

Docking pose of phytochemical Quercetin with target

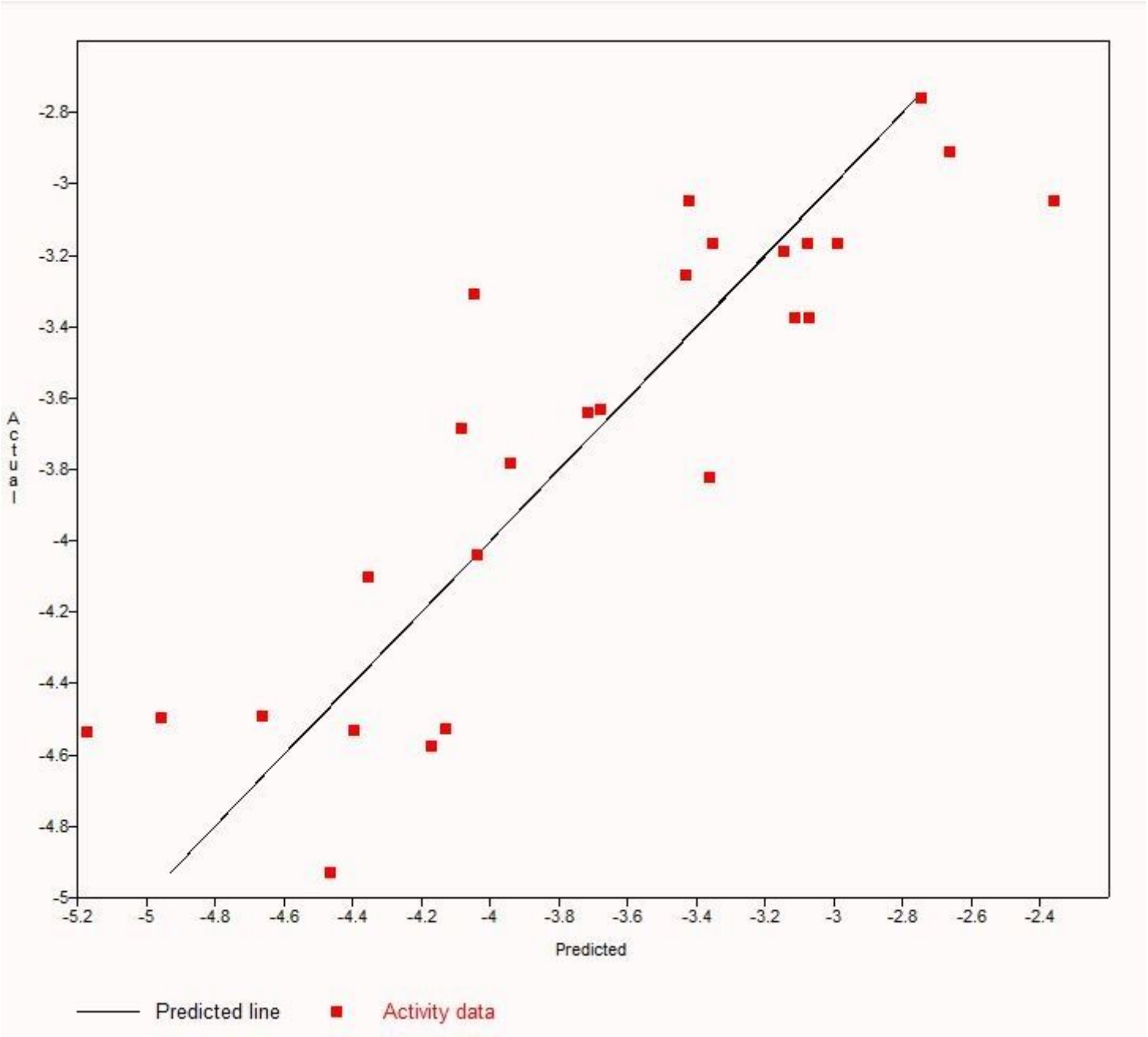

Figure 10

The multiple regression plot (linear) for inhibitors