Article title: Molecular Docking and Admet Analyses of Photochemicals from *Nigella sativa* (blackseed), *Trigonella foenum-graecum* (Fenugreek) and *Anona muricata* (Soursop) on SARS-CoV-2 Target

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Preprint statement: This article is a preprint and has not been peer-reviewed, under consideration and submitted to ScienceOpen Preprints for open peer review.

Funder: NONE

DOI: 10.14293/S2199-1006.1.SOR-.PPKNVFY.v1

Preprint first posted online: 07 June 2020

Keywords: SARS-COV-2, 3CLpro, *Nigella sativa*, *Trigonella foenum-graecum*, *Anona muricata*, Molecular Docking, ADMET, COVID-19, coronavirus

MOLECULAR DOCKING AND ADMET ANALYSES OF PHYTOCHEMICALS FROM *Nigella sativa* (blackseed), *Trigonella foenum-graecum* (Fenugreek) and *Anona muricata* (Soursop) ON SARS-COV-2 TARGET

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ABSTRACT

The novel severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) responsible for the 2019 coronavirus disease (COVID-19) has caused a global health challenge. The SARS-COV-2 main protease, 3CLpro/Mpro plays a critical role in the viral gene expression and replication and has been a major target for inhibiting viral maturation and enhancing host innate immune responses against COVID-19. In this study, we screened a library of 38 phytochemicals from *Nigella sativa* (blackseed), *Trigonella foenum-graecum* (Fenugreek) and *Anona muricata* (Soursop) – potent medicinal plants with reported antiviral properties - in a molecular docking protocol on 3CLpro using Autodock4.0 tool implanted in PyRx followed by docking validation and insilico absorption, distribution, metabolism, excretion, and toxicology (ADMET) evaluations. The docking results were visualized using Accelrys Discovery Studio and Pymol software. Among the 38 ligands screened, 19 showed significant interaction through non-covalent hydrogen bonding, hydrophobic, and electrostatic interactions with binding affinities from -5.3kcal/mol to -8.1kcal/mol indicating significant binding interactions at the active site binding pocket. Another important interaction observed in the study which mostly involve the transfer of charges was pi-interactions such as Pi-Pi interaction, Pi-Alkyl interaction, Pi-Sulfur interaction, Pi- Sigma, and Pi-Pi stacking. The docking results revealed that phytochemicals from *T. foenum-graecum* showed more 3CLpro inhibitory potential compared to those from *N. sativa* and *A. muricata*. Insilico ADMET evaluations for drug-like and lead-like characteristics however demonstrated that only 8 ligands - apigenin, kaempferol, luteolin, dithymoquinone, naringenine, nornuciferine, quercetin and nigellidine were actually drug-like; showed best activities against 3CLpro, and lack hepatotoxicity effects while none was lead-like. Insilico results of this study further suggested that drug

repurposing candidates, remdesivir, indinavir, hydroxychloroquine, chloroquine and ritonavir, exhibited various interactions with 3CLpro. Hence, further *in vitro* and *in vivo* studies are proposed.

Keywords: SARS-COV-2, 3CLpro, *Nigella sativa*, *Trigonella foenum-graecum*, *Anona muricata*, Molecular Docking, ADMET

1. Introduction

The severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) responsible for the December 2019 coronavirus disease (COVID-19) first reported in Wuhan city of Hubei province in China was declared a pandemic by the world health organization on March 11th, 2020. As at 26th May, 2020, there were 5,370,375 cases of COVID-19 in 216 countries and 344,454 confirmed deaths [1].

The SARS-COV-2 follows earlier emergence of the severe acute respiratory syndrome coronavirus (SARS-CoV) and the Middle East respiratory syndrome coronavirus (MERS-CoV) in 2003 and 2012 respectively. These enveloped viruses come under the family known as Coronaviridae, Sub-order Coronidovirineae & Order Nidovirales [2] and have four genera: alpha, beta, gamma & delta genera and are known to often cause cold & respiratory diseases commonly in humans [3]. SARS-COV-2 like other coronaviruses, belongs to the beta genus genome and comprises a positive-sense single-stranded RNA having about thirty-thousand nucleotide in length and infects both animals and humans [3]. The genome of SARS-CoV-2 has fourteen open reading frames (ORFs) which have been elucidated to encode 27 proteins such that at the 5'-terminus, the orf1ab and orf1a code for two large polyproteins: pp1ab and pp1a respectively [4]. These large polyproteins altogether comprise about two-third of the genome having 16 non-structural proteins (nsps). The coronavirus main protease, chymotrypsin-like protease, 3CLpro also referred to as Mpro is found to be integral to the expression of viral gene and replication and is being encoded by the nsp5 in the viral genome [5]. Functional proteins including RNA-dependent RNA polymerase (RdRp) and Helicase (Hel) are generated when 3CLpro cleaves the two large polyproteins. Furthermore, the papain-like protease (PLpro) cleaves the two polyproteins generating proteins required for virus replication. It also helps to strip ubiquitin thereby making coronaviruses evade the innate immune responses of the host [6]. The other ORFs are important for encoding structural and accessory proteins such as membrane protein (M), spike surface glycoprotein (S), nucleocapsid protein (N), and small envelope protein (E) [5].

Currently, there is no approved drug for COVID-19 as at May 2020. However, drug repurposing and supportive strategies have been utilized in the treatment of COVID-19 patients. Such drugs include remdesivir, chloroquine, hydroxychloroquine, ritonavir, indinavir, ribavirin, & several others which are under clinical trials to ascertain their efficacies for use in treatments. The essential roles played by 3CLpro and PLpro make them good targets in inhibiting viral maturation and enhancing host innate immune responses against COVID-19 [7]. Computational methods are essential approaches often found useful in

economizing and accelerating drug discovery and development compared to the more time-consuming and costly process of experimental evaluations.. These methods have been utilized to screen several potential drug candidates and have played a role in predicting their pharmacological/pharmacokinetics profile which defines their absorption, distribution, metabolism and excretion, and toxicities (ADMET). In silico ADMET filters are fully considered now to be fully integrated right from the early stages of drug discovery and development [8].

The increasing advocacy for the use of herbal products in various diseased conditions has gained popular opinion in recent times. Several herbs and herbal products are also progressively being screened for their efficacies in the face of the current pandemic as the world thirst towards a cure for the disease. Medicinal plants or herbs have been used for thousands of years dating back to the times of the Bible and Quran. According to Ahmad *et al.* [9], *Nigella sativa* also known as blackseed or black cumin is an important medicinal plant that contains thymoquinone as the main active component. The other important constituents include dithymoquinone, p-cymene, thymohydroquinone, carvacrol, isoquinoline, 4-terpineol, sesquiterpene, longifolene, α -pinene, α -hederin, t-anethole, pyrazol alkaloids, and thymol [9]. *N. sativa* has been utilized as antiallergic [10-112], anticarcinogenic [9, 13-14], antidiabetic [15-19], antihypertensive [20], antimicrobial [9], antiviral [21] and antioxidative [9]. Another important medicinal plant commonly used in traditional medicine is *Trigonella foenum-graecum* (Fenugreek). Its seed and leaves are used for food and traditional medicine all over the world. Its biological actions include antiviral [21], hypoglycaemic and antidiabetic [22-23], antiulcerogenic [24] , hypocholesterolaemic [25-26], antinociceptive [27-28] and also possesses immunostimulatory effects [29]. It has also proven to be antioxidative [21, 30], antihypertensive [31], hepatoprotective [32], and chemoprotective [31, 33]. Soursop (*Annona muricata*) also known as graviola, gyanabama and sirsak is widely distributed in the tropical and subtropical parts of the world [34]. The plant have been used in traditional medicines against diseases such as arthritis [34-35], cancer [36-38], parasitic infections [39-41], antiviral [21, 43] neuralgia, diarrhea, fever, dysentery, malaria and to elevate the milk level of a mother after childbirth [34].

With the aforementioned biological activities of *Nigella sativa* (blackseed), *Trigonella foenum-graecum* (Fenugreek) and *Annona muricata* (Soursop), we aimed to perform a set of computational virtual screening techniques with molecular docking in addition to ADMET evaluations in this study to assess the inhibition of the SARS-COV-2 main protease, 3CLpro with small molecules from the medicinal plants. Compound interaction modes, binding energy at the active site, and ADMET evaluations were analyzed and ranked with the most promising compounds proposed as inhibitors of SARS-CoV-2 main protease.

2. Materials and Methods

2.1. Literature search and compound selection

Compounds from *Nigella sativa* (blackseed), *Trigonella foenum-graecum* (Fenugreek) and *Anona muricata* (Soursop) with biological activities including antiviral properties were searched for and selected both from the phytochemical and ethnobotanical databases (<https://phytochem.nal.usda.gov/phytochem>) [21], and Literatures [34, 42] and were then examined in the next stage of our study.

2.2. Preparation of protein/receptor and ligands.

Crystal structure of SARS-COV-2 main protease, 3CLpro (PDB ID: 6LU7) was retrieved from the Protein Data Bank (<http://www.rcsb.org>) [44]. Using the online tool, protein-ligand identifier profiler (PLIP) [45], the active site of the SARS-CoV-2 main protease was identified. The protein preparation step included the removal of water molecules using Accelrys Discovery Studio software [46]. This was followed by the making of the macromolecule using the PyRx software [47] which included addition of hydrogen atoms, updating and filling missing side chains residues, optimizing the hydrogen bonds, adding formal charges and then optimizing at neutral pH. The 3D SDF ligands (Figures 1-3) were downloaded from Pubchem database [48] while those of experimental and investigational drugs: hydroxychloroquine (DB01611), remdesivir (DB14761), ritonavir (DB00503), Indinavir (DB00224), and chloroquine (DB00608) were downloaded from the DrugBank [49]. They were minimized and converted into PDBQT in the PyRx virtual screening tool.

2.3 Molecular Docking and Validation of Docking Protocol

Using the AutoDock Vina wizard [50] implanted in PyRx for the docking procedure, the ligands and repurposed experimental drugs were screened for their interactions with the active site of the protease. A total of 9 ligand poses were assessed at the active site. The best pose was taken to be the pose with the lowest binding energy in kcal/mol. Docking results were exported away from PyRx, and receptor-ligand interactions analyzed in Discovery Studio and Pymol software [51]. Validation of docking protocol was done by re-docking of the co-crystallized ligand, N3 (N-[(5-METHYLISOXAZOL-3-YL)CARBONYL]ALANYL-L-VALYL-N-1-((1R,2Z)-4-(BENZYL OXY)-4-OXO-1-[(3R)-2-OXOPYRROLIDIN-3-YL]METHYL)BUT-2-ENYL)-L-LEUCINAMIDE) over 3CLpro.

2.4. Pharmacokinetics, Drug-like, Lead-like and ADMET Evaluations

Pubchem database [51] was utilized to obtain the canonical smiles of the various selected ligands. The pharmacokinetics of the ligands having the best poses were predicted using the online pkCSM software [52]. The major parameters associated with ADME properties such as the Lipinski's rule of five [Molecular weight, (MW) <500Da; octanol-water partition coefficient (log P) <5, HBond donor (HBD) ≤5, HBond

acceptor (HBA) ≤ 10]; Caco-2 permeability, topological surface area (TPSA) and water solubility (LogS) of the compounds were considered. Two sets of criteria were employed for assessing the suitability of ligands for lead discovery: “drug-like” and “lead-like”. The drug-like hits were based on the Muegge (Bayer) criteria [53] for which $200 \leq MW \leq 600$, $-2 \leq \text{LogP} \leq 5$, $TPSA \leq 150$, $HBD \leq 5$, $HBA \leq 10$, and $Rotatablebond, RotB \leq 15$. Whereas the lead-like criteria were $\text{LogP} < 3$, $MW < 300$, $HBD \leq 3$, $HBA \leq 3$, and $RotB \leq 3$. Using the pkCSM software, the hepatotoxicity, Minnow toxicity (Mtox), and *T. Pyriformis* toxicity (TPtox) were also considered.

3. Results and Discussion

A total of 38 compounds (Figure 1-3) from *T. foenum-graecum*, *N. sativa* and *A. muricata* were screened in this study for their SARS-COV-2 main protease, 3CLpro inhibitory potentials. 3CLpro inhibition was also studied for experimental and investigational drugs being considered for the treatment of COVID-19 - chloroquine, hydroxychloroquine, indinavir, remdesivir and ritonavir (Figure 4). The amino acids: THR25, THR26, HIS41, MET49, PHE140, LEU141, AASN142, GLY143, SER144, CYS 145, HIS163, HIS164, MET145, GLU166 and PRO168 were revealed to be significant binding site residues which could come into Hydrogen bond, electrostatic, and Hydrophobic interactions by the active site identification protocol for 3CL/Mpro using the online PLIP server [45]. Among the 38 ligands screened, compounds from *T. foenum-graecum* showed stronger inhibitions of 3CLpro/Mpro compared to those *N. sativa* and *A. muricata*. The docking result obtained in this study as presented in Table 1 showed that SARS-COV-2 main protease, 3CL/Mpro inhibition occurred with binding affinities ranging between -5.3kcal/mol to -8.1kcal/mol indicating significant binding interactions at the active site binding pocket. These interactions were stabilized through the formation of hydrogen bonds, electrostatic and hydrophobic interactions (Tables 1 and 3). Another important interaction observed which mostly involve the transfer of charges was pi-interactions such as Pi-Pi interaction, Pi-Alkyl interaction, Pi-Sulfur interaction, Pi- Sigma, and Pi-Pi stacking. These Pi interactions helped imbed the ligands in the active/binding site of the 3CLpro. Drug-like characteristics prediction using the pKCSM server [52] however demonstrated that only 8 of the ligands screened were actually drug-like while none was lead-like (Table 2). The drug-like ligands were apigenin, kaempferol, luteolin, dithymoquinone, naringenin, quercetin, nornuciferine, and nigellidine. Apigenin, a common potent dietary flavonoid with antiviral and antitumor activity showed a negative binding affinity of -6.8kcal/mol and four conventional hydrogen bonds with LEU141, SER144, HIS163, and GLU166 according to the docking result obtained (Tables 1 and 3). The stable binding interaction could further be linked to the Pi-sulfur interaction of apigenin ring with CYS145 in conjunction with the Pi-Alkyl interaction established with MET49 of the protease binding site. This is in contrast to chloroquine which hydrogen-bonded with LEU141 and HIS164 and in electrostatic interaction with GLU166 (Table 1). Kaempferol, a biologically active flavonoid with antiviral, anti-inflammatory, antioxidant, antimicrobial and anticancer activities exhibited

stable binding with a binding affinity of -7.1kcal/mol. This is attributed to its four conventional hydrogen bonds with GLU166, ASP187, GLU189, and SER144 together with electrostatic interaction with HIS 41. The stability of binding is further sustained by Pi-Stacked and Pi-alkyl hydrophobic interactions with HIS41 and MET49 respectively. The presence of Pi-Sulfur interactions with CYS145 and MET165 further enhanced binding at the enzyme's binding site. Luteolin, used traditionally in herbal medicines for treating inflammatory disorders, hypertension, and cancer demonstrated five conventional hydrogen bonding interactions with LEU141, GLY143, SER144, GLU166 and ARG188. Two different Pi-Alkyl hydrophobic interactions were additionally noted with MET165 and CYS145 which aided conformational stability of kaempferol at the 3CLpro receptor binding site. As noted for Apigenin and Kaempferol, these were noteworthy interactions over 3CLpro. The Pi-Alkyl typed hydrophobic interactions of dithymoquinone with HIS41, CYS145, MET165 and LEU167 (Tables 1 and 3) from the docking results were responsible for its inhibitory potential against the SARS-COV-2 main protease insilico. These interactions, majorly involving charge transfer helped intercalate the ligand at the protease binding site. Its binding affinity of -6.2kcal/mol is greater compared to those of ritonavir, indinavir and remdesivir: -7.7kcal/mol, -7.6kcal/mol and -6.8kcal/mol- respectively obtained in this study (Table 1). The Pi-alkyl hydrophobic interactions of naringenin, an anti-obesity, anti-diabetic, anti-dyslipidemic, and antifibrotic flavonoid from *T. foenum-graecum* with MET49 and CYS145 in addition to Hbond interactions with LEU141, CYS145, Ser144 and HIS163 contributed to the its stable conformational binding energy of -6.9kcal/mol. This is similar to the result obtained for quercetin but a Pi-sulfur hydrophobic interaction with CYS145 along with an additional Hbond with GLY143 were attributed to the favourable binding of quercetin at the receptor's binding site. Table 3 further revealed that the Pi-Cation interaction of nornuciferine from *A. muricata* with HIS41 was balanced by a Pi-donor Hydrogen bonding of the nornuciferine ring with CYS145. This was in addition to two conventional hydrogen bonding it formed with LEU141 and HIS164 of the receptor active site. Pi-Sigma and Pi-Sulfur interactions with HIS41 and MET49 respectively also contributed to the stability of binding at the binding site. Another compound from *N. sativa*, besides dithymoquinone, Nigellidine, exhibited significant binding interactions through its four Hbonds with THR26, GLY143, SER144 and CYS145. Further hydrophobic interactions with HIS41, MET49, HIS163 and HIS172 along with electrostatic interaction with HIS41 stabilized the binding of nigellidine at the 3CLpro. None of the drug-like compounds was lead-like having not satisfied the limits for lead-like characteristics: $\text{LogP} < 3$, $\text{MW} < 300$, $\text{HBD} \leq 3$, $\text{HBA} \leq 3$, and $\text{RotB} \leq 3$ (Table 2). However, they showed comparatively favourable predicted insilico absorption characteristics as indicated by the LogS (water solubility) and Caco-2 permeability values to the five experimental and investigational drugs utilized in this study (Table 2). Caco-2 permeability (Caco-2 cell line composed of human epithelial colorectal adenocarcinoma cells is an *in vitro* model of the human mucosa) is widely used to predict the absorption of orally administered drugs with high Caco-2 permeability being > 0.9 [ ]. The predictive results obtained in this study showed Caco-2 permeability of quercetin to be

very poor (-0.229) as also was the case with kaempferol (0.032) and luteolin (0.0916). The other ligands in contrast showed high Caco-2 permeability varying from 1.007 for apigenin to 1.47 for nornuciferine, a result lower than those of hydroxychloroquine (1.543) and chloroquine (1.624) but greater than those of indinavir (0.823), ritonavir (0.377) and remdesivir (0.635). These suggest good human mucosa absorption of these drug-like compounds. *T. pyriformis* is a protozoa bacteria whose toxicity is oftentimes used as a toxic endpoint [52]. The predicted results for *T. pyriformis* toxicity of the drug-like ligands varied from 0.288 for quercetin to 1.437 for nigelidine (Table 2). Also considered in this study are the predicted hepatotoxicity and Minnow toxicity (LC50) - representing the concentration of molecules to cause 50% death of the Flathead Minnows [52]. The drug-like phytochemicals lacked hepatotoxicities while naringenin had the lowest Minnows toxicity (0.039).

Docking of the N3 co-crystallized ligand showed interactions of the terminal ends of the N3 co-crystallized ligand through Hbonds with PHE140, GLU166, GLY143, CYS145, and LEU167 (Figure 5). Hydrophobic Alkyl and Pi-Alkyl interactions were also observed with MET165, MET49, and PRO168. This correlated favorably the interactions of the 8 drug-like ligands proposed as inhibitors of SARS-COV-2 main protease, 3CLpro in this study.

Results obtained in this study were different in several ways to other docking reports recently published. Zhang *et al.* [54] used a library consisting of 13 antiviral compounds which included quercetin and kaempferol from 26 Chinese traditional herbs in a docking study with 3CLpro, PLpro, and Spike proteins. Kaempferol and quercetin were reported to inhibit 3CLpro with binding affinities of -6.01kcal/mol and -6.25kcal/mol respectively. Bouchentouf and Missoum [55] reported that nigelidine from *N. sativa* also inhibited SARS-COV-2 but reported only one Hbond interaction with HIS163. The binding energy of the best pose in the docking protocol of N3 in this study (-6.5kcal/mol) was also different from that obtained (-8.1kcal/mol) in another study [56]. Differences in conformational search algorithms coupled with intrinsic randomness of conformational search could be responsible for such small differences which were not unexpected though. The predictive results of this study and other recently reported docking studies could be supported by further experimental *in vivo* studies using SARS-COV-2 animal models for use against SARS-COV-2.

Conclusion

The medicinal properties of traditional plants would be important considerations in the search for therapeutic solutions against lots of emerging diseases. Several approaches are involved in this among which bioinformatics offer a viable tool for screening many potential options. The molecular docking and ADMET evaluations of ligands from *N. sativa*, *T. foenum-graecum* and *A. muricata* considered in this study showed that 8 drug-like molecules from these medicinal plants were potent inhibitors of 3CLpro and lacked

hepatotoxicity effects while none was lead-like. Insilico results of this study further suggested that drug repurposing candidates, remdesivir, indinavir, hydroxychloroquine, chloroquine and ritonavir, exhibited various interactions with 3CLpro. Hence, further *in vitro* and *in vivo* studies are proposed.

Conflicts of Interest

None

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Figure 1: Compounds from *Nigella sativa*

Figure 2: Compounds from *A. muricata*

Naringenin

Quercetin

Tricin

Vitexin

Apigenin

beta-sitosterol

Carpaine

Diosgenin

Fenugreekine

Genistein

Gentianine

Gigogenin

Luteolin

Kaempferol

Neotogogenin

Quercitrin

Rutin

Stigmasterol

Yamogenin

Yuccagenin

Figure 3: Compounds from *T. foenum-graecum*

Chloroquine

Hydroxychloroquine

Remdesivir

Indinavir

Ritonavir

Figure 4: Experimental and Investigational Drugs

Table 1: Ligands Interactions Over SARS-COV-2 Main protease, 3CLpro/Mpro

Compound	Binding			
	Affinity (Kcal/mol)	HBond	Hydrophobic	Electrostatic
Apigenin	-6.8	LEU141-SER144-HIS163-GLU166	MET49	-
Gentianine	-5.3	GLY143-SER144-CYS145	HIS163-MET165	-
Gitogenin	-8.1	-	HIS41-MET49-CYS145	-
Kaempferol	-7.1	SER144-GLU166-GLN189-ASP187	HIS41-MET49	-
Luteolin	-7.1	LEU141-SER144-GLY143-GLU166-ARG188	CYS145-MET165	-
Neotigogenin	-7.3	HIS163	HIS41-MET49-HIS163	-
Procyanidin	-7.7	PHE140-HIS163	HIS41-MET49-CYS145	GLU166
Quarcitrin	-7.2	THR26-PHE140-GLU166	LEU141-CYS145	-
Rutin	-7.7	HIS41-PHE140-GLY143-GLU166-ASN142	CYS145	-
Yamogenin	-7.5	THR26	HIS41-CYS145-MET165-PRO168	-
Yuccagenin	-7.8	-	HIS41-CYS145	-
Dimothymoquinone	-6.2	-	HIS41-CYS145-MET165- LEU167	-
Stigmasterol	-7.0	THR26	HIS41-CYS145-MET165- PRO168	-
Naringenin	-6.9	LEU141-SER144-CYS145-HIS163	MET49-CYS145	-
Quarcetin	-7.2	LEU141-SER144-CYS145-HIS163	-	-
Nigellidine	-7.7	THR26-GLY143-SER144-CYS145	HIS41	-
Vitexin	-7.5	LEU141-SER144-HIS164	-	-
Fenugreekine	-7.9	THR26-LEU141-GLY143-CYS145-HIS163- GLU166-GLN189-HIS172	-	-
Nornuciferine	-6.6	LEU141-CYS145-HIS164	HIS41	HIS41
Indinavir	-7.6	THR26-ASN142-HIS164-GLN189	HIS41-CYS145	-
Ritonavir	-7.7	THR26-HIS41-GLU166-GLN189	HIS41-MET165-PRO168	HIS41-GLU166
Remdesivir	-6.8	ASN142-GLY143-HIS164	CYS145	HIS41
Hydroxychl-oroquine	-5.9	THR26-ASN142-SER144	HIS41	-
Chloroquine	-5.6	LEU141-HIS164	-	GLU166

Table 2: Predicted Pharmacokinetics of ligands

Compound	MW	LogP	RotB	HBA	HBD	TPSA	LogS	Caco2	Hepatotoxicity	T.Ptox	M.Ttox	Drug-like	Lead-like
Apigenin	270.24	2.577	1	5	3	112.519	-3.329	1.007	No	0.38	2.432	Yes	No
Gentianine	175.187	1.438	1	3	0	76.055	-1.27	-1739	Yes	0.249	1.468	No	No
Gitogenin	432.645	4.765	0	4	2	76.055	-5.01	1.272	No	0.339	0.821	No	No
Kaempferol	286.239	2.282	1	6	4	117.313	-3.04	0.032	No	0.312	2.885	Yes	No
Luteolin	286.239	2.282	1	6	4	117.313	-3.094	0.096	No	0.326	3.169	Yes	No
Neotigogenin	416.646	5.794	0	3	1	183.214	-5.485	1.301	No	0.375	0.281	No	No
Procyanidin	594.525	2.7327	4	13	10	242.064	-2.892	0.131	No	0.285	10.607	No	No
Quercitrin	448.38	0.4887	3	11	7	179.107	-2.903	0.048	No	0.285	4.954	No	No
Rutin	610.521	-1.6871	6	16	10	240.901	-2.892	-0.949	No	0.285	7.677	No	No
Yamogenin	414.63	5.7139	0	3	1	182.524	-5.539	1.293	No	0.399	0.247	No	No
Yuccagenin	430.629	4.6847	0	4	2	187.319	-5.096	1.264	No	0.351	0.787	No	No
Dithymoquinone	328.408	2.7134	2	4	0	141.923	-3.654	1.367	No	0.445	1.323	Yes	No
Stigmasterol	412.702	7.8008	5	1	1	186.349	-6.682	1.213	No	0.433	-1.675	No	No
Naringenin	272.256	2.510	1	5	3	114.235	-3.224	1.029	No	0.369	2.136	Yes	No
Quercetin	302.238	1.988	1	7	5	122.108	-2.925	-0.229	No	0.288	3.721	Yes	No
Vitexin	432.381	0.0917	3	10	7	173.994	-2.845	-0.956	No	0.285	4.897	No	No
Nornuciferine	281.355	3.1137	2	3	1	124.443	-3.597	1.47	Yes	0.282	0.039	Yes	No
Fenugreekine	663.43	-2.3659	11	18	8	245.744	-2.891	-0.711	No	0.285	10.164	No	No
Nigellidine	294.354	3.22942	1	4	1	128.116	-3.651	1.304	Yes	1.437	1.17	Yes	No
Indinavir	613.803	2.8669	11	7	4	266.218	-3.611	0.823	Yes	0.285	5.061		
Ritonavir	720.962	5.9052	17	9	4	302.058	-3.358	0.377	Yes	0.285	1.787		
Remdesivir	602.585	2.31218	13	13	4	242.488	-3.07	0.635	Yes	0.285	0.291		
Hydroxychloroquine	335.879	3.783	9	4	2	143.024	-3.627	1.543	Yes	1.061	1.325		
Chloroquine	319.88	4.8106	8	3	1	138.230	-4.249	1.624	Yes	1.558	0.747		

Table 3: Interactions of Drug-like Compounds

Drug-like Ligands	Hbond Interaction	Hydrophobic Interaction	2D Interaction	Distances		
Apigenin				Name	Distance (A ⁰)	Category
				LEU141	2.04466	Hydrogen Bond
				SER144	2.52292	Hydrogen Bond
				HIS163	2.35886	Hydrogen Bond
				GLU166	3.18882	Hydrogen Bond
				CYS145	5.57499	Pi-Sulfur
				CYS145	5.13709	Pi-Sulfur
				MET49	4.67203	Hydrophobic (Pi-Alkyl)
Kaempferol				Name	Distance (A ⁰)	Category
				ASP187	2.11646	Hydrogen Bond
				GLN189	2.08399	Hydrogen Bond
				SER144	2.34778	Hydrogen Bond
				HIS41:	4.98776	Electrostatic
				GLU166	3.1208	Hydrogen Bond
				CYS145	5.44914	Pi-sulphur
				CYS145	4.91477	Pi-sulphur
				MET165	5.4429	Pi-sulphur
				HIS41	4.68608	Hydrophobic (Pi-Pi Stacked)
				A:MET49	4.75162	Hydrophobic (Pi-Alkyl)

Luteolin

H-Bonds
Donor
Acceptor

Name	Distance (Å ⁰)	Category
LEU141	2.00409	Hydrogen Bond
SER144	2.41367	Hydrogen Bond
ARG188	2.44048	Hydrogen Bond
GLY143	2.67982	Hydrogen Bond
GLU166	2.97479	Hydrogen Bond
MET165	5.30064	Hydrophobic (Pi-Alkyl)
CYS145	4.92076	Hydrophobic (Pi-Alkyl)

Dithymoquinone

H-Bonds
Donor
Acceptor

Hydrophobicity
3.00
2.00
1.00
0.00
-1.00
-2.00
-3.00

Name	Distance (Å ⁰)	Category
HIS41	4.27848	Hydrophobic (Pi-Alkyl)
HIS41	5.04403	Hydrophobic (Pi-Alkyl)
CYS145	3.57497	Hydrophobic (Pi-Alkyl)
MET165	4.23175	Hydrophobic (Pi-Alkyl)
MET165	5.37228	Hydrophobic (Pi-Alkyl)
LEU167	5.12458	Hydrophobic (Pi-Alkyl)

Naringenin

H-Bonds
Donor
Acceptor

Hydrophobicity
3.00
2.00
1.00
0.00
-1.00
-2.00
-3.00

Name	Distance (Å ⁰)	Category
LEU141	2.59877	Hydrogen Bond
SER144	2.39831	Hydrogen Bond
SER144	2.23752	Hydrogen Bond
CYS145	2.33363	Hydrogen Bond
HIS163	2.32937	Hydrogen Bond
MET49	3.86659	Hydrophobic (Pi-Alkyl)
CYS145	5.28862	Hydrophobic

Querce- tin

Name	Distance (Å ⁰)	Category
LEU141	2.85742	Hydrogen Bond
SER144	2.79473	Hydrogen Bond
GLY143	2.17743	Hydrogen Bond
HIS163	2.65207	Hydrogen Bond
CYS145	4.07365	Hydrogen Bond
CYS145	5.30814	Pi-Sulfur

Nornu- ciferine

Name	Distance (Å ⁰)	Category
LEU141	2.16092	Hydrogen Bond
HIS164	3.47996	Hydrogen Bond
HIS41	4.11948	Electrostatic (Pi-Cation)
CYS145	4.17594	Hydrogen Bond
HIS41	3.64291	Hydrophobic (Pi-Sigma)
MET49	5.81965	Pi-sulfur

Nigelli- dine

Name	Distance (Å ⁰)	Category
THR26	1.94642	Hydrogen Bond
GLY143	1.92798	Hydrogen Bond
SER144	2.97969	Hydrogen Bond
CYS145	3.85792	Hydrogen Bond, other
HIS41	4.52642	Electrostatic
HIS41	4.55698	Hydrophobic
MET49	4.70489	Hydrophobic
HIS163	4.66967	Hydrophobic
HIS172	5.43984	Hydrophobic

Figure 5: Interactions of co-crystallized ligand, N3 over 3CLpro