VIETNAM ACADEMY OF SCIENCE AND TECHNOLOGY
UNIVERSITY OF SCIENCE AND TECHNOLOGY OF HANOI

FINAL REPORT
GROUP PROJECT

TITLE:

In English: ***Docking studies of chemical constituents from Cannabis Sativa for potential antimalarial activities***

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I. INTRODUCTION

Malaria is a serious disease in the world through parasite that outbroke in the 20th century and has spread in more than 90 countries worldwide at the same time [(1)]. According to the World Health Organization, 228 million malaria cases and 405,000 deaths worldwide has been recorded in 2019, with the South East Asia region taking up roughly 3.4% [(2)]. The prevalence of Malaria has been a major cause of mortality in infants and adults globally. In reality, the parasite is transmitted from the bites of infection of mosquitoes to humans. Protozoan of the genus plasmodium is the major antecedent of the infection and humans can be siphoned through the saliva of Anopheles mosquito [(3)]. In reality, 4 types of Plasmodium Malaria spread to humans, such as *Plasmodium vivax, Plasmodium ovale, Plasmodium malariae, Plasmodium falciparum*. However, *Plasmodium falciparum* is the major cause of mortality and the most prevalent malaria parasite in 2018 [(2)]. Therefore, it is the force of scientist's motivation to research or discover new drugs with high efficacy against this disease.

In the periods of treatment methods, disruption folate metabolism was believed to be the best solution, including 2 different important enzymes *dihydrofolate reductase-thymidinesynthase* (DHFR-TS) and *dihydropteroate synthase* (DHPS). The inhibition of these two enzymes might abrogate essential folate cofactors for DNA synthesis and metabolism of several amino acids [(4)(5)]. At this time, there were many antifolate drugs, which were mass-produced for the treatment. However, these drugs proved futile due to the increased resistance in the malarial virus. Mutations happen in the active site of two key enzymes DHFR-TS and DHPS directly affect the binding ability of drugs, resulting in the diminishing of long-term efficiency [(6)]. Furthermore, the current state of development of the pharmaceutics industry has yet to give an answer to the side effects this virus can do to other parts of our body, such as bone marrow, skin, and hair [(7)]. As such, it is crucial to discover novel drugs, which limit the side effects, play a role in the pharmaceutical industry with high efficacy and longer useful therapeutic life.

To this day, more than 500 compounds have identified in *Cannabis sativa L.*, including cannabinoids and flavonoids which are known for potential antimalarial properties [(8)] [(9)]. The present work conducts molecular docking studies of these compounds on enzyme pfDHFR-TS to investigate in combination with analyzing drug-like and pharmacokinetic properties to find potential inhibition candidates.

Objectives

The ultimate purpose of this research is to find a new drug against the malarial parasite known as *Plasmodium falciparum* that has proven immune to many of the common drugs. Particularly, the study has the following sub-objectives:

- To find the protein with the lowest binding energy to dock with ligands found in *Cannabis Sativa* L.
- To find possible docking ligands for the protein we found before.
- To compare results from various in silico docking tools.

Main questions:

During our research, a few notable questions were addressed:

- How accurate would the experiments be compared to lab works?
- How do we simulate our work progress on a computer platform?
- What programs and web servers are needed to conduct the experiments?
- How do we confirm the drug's effectiveness without actual lab tools and test subjects?

II. Theoretical background and state of current research

Hemp (or *Cannabis Sativa* L.) belongs to the Cannabaceae family and Cannabis genus. It has square body, with sharp but staggered leaves. Native to Central Asia, the plant has long been cultivated in Asia, Europe, and China. Now a widespread tropical, temperate and subarctic cultivar and waif.

Image: *Cannabis Sativa* L.

Table 1. Table representing the constituents of *Cannabis Sativa* L. in 2015

No.	Group	Number of Known Compounds
1	CBG Type	17
2	CBC Type	8
3	CBD Type	8
4	Δ^9 – THC type	18
5	Δ^8 – THC type	2
6	CBL type	3
7	CBE type	5
8	CBN type	10
9	CBND type	2
10	CBT type	9
11	Miscellaneous	22
12	Total Canabinoids	104
13	Total Non-Canabinoids	441
	Total	**545**

Compounds isolated from *C. Sativa* have a typical terpenophenolic C_{21} frame called "cannabinoids". This compound class has derivatives and modified structures which are also considered to be cannabinoids. Since the beginning of the investigation on *C. sativa*, 120 types of cannabinoids have been isolated so far, which can be classified into 11 categories: (-)-9-trans-tetrahydrocannabinol (Δ^9- THC), (-)-Δ^8-trans-tetrahydrocannabinol (Δ^8-THC), cannabigerol (CBG), cannabichromene (CBC), cannabidiol (CBD), cannabinodiol (CBND), cannabielsoin (CBE), cannabicyclol (CBL), cannabinol (CBL) CBN), cannabitriol (CBT) and others.

Cannabis sativa seeds are chiefly used to make hemp seed oil which can be used for cooking, lamps, lacquers, or paints. They can also be used as caged-bird feed, as they provide a source of nutrients for most animals. Medically, cannabis is somewhat effective in chemotherapy-induced nausea and vomiting (CINV) and may be a reasonable option in those who do not improve following preferential treatment. Comparative studies have found cannabinoids to be more effective than some conventional antiemetics such as prochlorperazine, promethazine, and metoclopramide in controlling CINV, but these are used less frequently because of side effects including dizziness, dysphoria, and hallucinations. They can also be used for curing long-term pain. A review in 2014 found limited and weak evidence that smoked cannabis was effective for chronic non-cancer pain [(22)]. A meta-analysis found that inhaled medical cannabis was effective in reducing neuropathic pain in the short term for one in five to six patients [(23)]. Another review found limited evidence that medical cannabis was effective for neuropathic pain when combined with traditional analgesics [(24)].

In recent times, there have been a number of studies in the world recording the effects of *Cannabis sativa L*. in the treatment of malaria, which is attracting increasing attention from scientists around the world.

III. Scientific methods and materials

1. Ligand preparation

From *Cannabis Sativa L*., 125 compounds (**Figure A1**) were collected and constructed by ChemDraw, Chem3D and Marvin to be used for drawing, displaying and characterizing chemical structures, substructures and reactions(https://chemaxon.com/). Moreover, Pymol 2.2.2 [(11)] was used for building up the 3D structure of compounds and Gabedit 2.5.0 played an important role in energy minimization [(12)]. For evaluating drug-like properties and the acute toxicity for all compounds, Lipinski's rule of five(http://www.scfbio-iitd.res.in/) was used for basic evaluation of the chemical compounds which can be suitable to being drug, Molinspiration is considered for evaluating miLog P (Octanol-water partition coefficient logP), TPSA (Total polar surface area) and enzyme inhibitor. On the other hand, ProTox-II evaluated toxicity prediction.

2. Protein Modeling

In searching the information of enzyme pfDHFR-TS via Protein Data Bank (RCSB PDB), there are 14 structures in total. However, this is due to the fact that the best structure with PDB ID: 1J3I as evaluation is good resolution 2.33 Å [(13)]**.** The protein structure was prepared using the Graphical User Interface program named AutoDock tools and Chimera 1.13.1 in order to obtain the correct ionization and tautomeric states of amino acid residues [(14)]. Two different soft wares can remove the water molecule surround and add polar hydrogen atoms. The Kollman united atom partial charges and salvation parameters were assigned.

Moreover, it needs to be changed with being a PDBQT file which contains the atomic coordinates by Grix Box construction as an important process in detecting the effect location surrounding protein via AutoGrid and AutoDock.

3. Validation docking

AutoDock4.2.6 was utilized to perform validation using PDB protein 1J3I. For the examination, the two existing inhibitors including Cycloguanil and WR99210 were chosen from various literature studies [13] [15]. The two inhibitors were docked within the active site of enzyme pfDHFR-TS formed by Ile14, Asp54, Ser108, Ser111, Ile164 and Tyr170 residues, respectively.

4. Molecular docking

All the docking runs were performed in Intel ® Core TM i7-9700K CPU @ 3.60 GHz, with 32 GB DDR4 RAM. The outputs from docking studies were analyzed using PyMOL[11], Discovery Studio Visualizer[16], LigPlus[17] and Maestro[18] to calculate the distances of hydrogen bonds as measured between the hydrogen and its assumed binding partner.

5. Docking using AutoDock 4.2.6

AutoDock 4.2.6 was compiled and run under Ubuntu-Linux 14.04.6 LTS operating system [14]. The location and dimensions of the grid box for enzyme was chosen such that it incorporates the amino acids domain involved in binding with standard inhibitor which was enclosed in a box with the number of grid points in x × y × z size directions (62 × 68 × 72) and a grid spacing of 0.375 Å. The pre-calculated binding affinity of each ligand's atom type was prepared using AutoGrid. AutoDock 4.2.6 was utilized for the molecular docking simulation. The parameters of the Lamarckian Genetic Algorithm (LGA) were: 50 runs; elitism of 1; the mutation rate of 0.02; the population size of 300; a crossover rate of 0.80; number of generations of 27,000; the energy evaluations of 50,000,000 and the root-mean-square (RMS) cluster tolerance was set to 2.0 Å in each run. The ligand conformation with the lowest free energy of binding, chosen from the most favored cluster, was selected for further analysis.

6. Docking using AutoDock vina

AutoDock Vina in molecular docking was planned with the exhaustiveness of 400. The maximum energy difference between the worst and best docking modes was set to 7 kcal/mol. The grid center was selected chosen such that it incorporates the amino acids domain involved in binding with standard. The grid size was set to 15×19×14 $Å^3$, which is enough to cover the entire target active site [19].

IV. Result and discussion

1. Docking of standard solutions

Cycloguanil and WR99210 were chosen as positive controls due to their ability to inhibit the protein, according to the online Protein Data Bank (PDB). They both showed to form major hydrogen bond interactions with Ile14, Asp54 and Ile164, indicating that it is necessary for a compound to have interaction with at least one of these 3 in order for inhibition to occur.

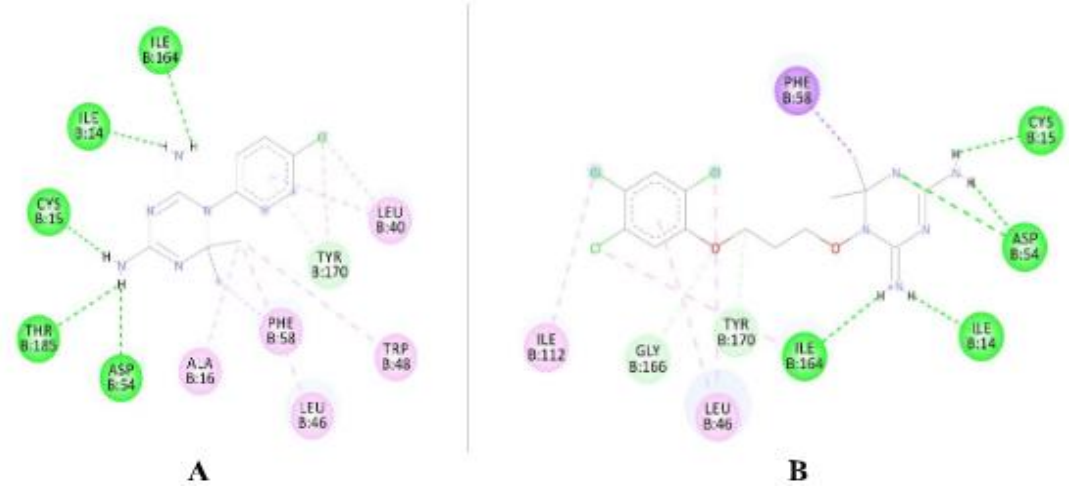


Figure 1. Hydrogen bonding patterns of Cycloguanil and WR99210 inhibitors with enzyme pfDHFR-TS (PDB ID: 1J3I). (A) Cycloguanil; (B) WR99210.

2. Drug-likeness evaluation

Assessment of 125 compounds were performed via the Lipinski's Rule of Five, which states that a compound in nature is a potential drug if its molecular weight is less than 500 Da, has no more than 5 hydrogen bond donors or 10 hydrogen bond acceptors, and octanol-water partition coefficient (LogP) is less than 5.

Table 2. List of compounds with drug-like properties suitable with Lipinski's rule of five. (*Red letters: unsatisfactory characteristics)

ID	Compound	MW	HBD	HBA	LogP
1	(-)-(7R)-Cannabicoumarononic acid	356	0	4	5.601800
2	(-)-7-hydroxycannabichromane	330	2	3	5.921300
3	(+-)-4-acetoxycannabichromene	372	1	4	5.960900
4	(+-)-3''-hydroxy-delta4''-cannabichromene	328	1	2	6.524121
5	(1'S)-hydroxycannabinol	342	3	4	4.691619
6	3,3'-demethyl-heliotropamide	580	6	9	4.399299
7	4,5-dihydroxy-2,3,6-trimethoxy-9,10-dihydrophenanthrene	302	2	5	2.656499
8	4,7-dimethoxy-1,2,5-trihydroxyphenanthrene	286	3	5	2.738699
9	4-acetoxy-2-geranyl-5-hydroxy-3-n-pentylphenol	374	2	4	5.991001
10	4-hydroxy-2,3,6,7-tetramethoxy-9,10-dihydrophenanthrene	374	2	4	5.991001
11	5-acetoxy-6-geranyl-3-n-pentyl-1,4-benzoquinone	372	0	4	5.544899
12	5'-Methyl-4-pentylbiphenyl-2,6,2'-triol	286	3	3	4.511519
13	6-prenylapigenin	338	3	5	3.928298
14	7-methoxycannabispirone	260	0	3	3.030899
15	7-oxo-9a-hydroxyhexahydrocannabinol	346	2	4	4.109598
16	8,9-Dihydroxy-delta-6a-tetrahydrocannabinol	346	3	4	3.955198
17	8-hydroxycannabinol	298	2	3	4.420519
18	8-hydroxycannabinolic acid A	326	2	4	4.233019
19	8-oxo-delta9-THC	328	1	3	4.914799
20	9,10-dihydro-2,3,5,6-tetramethoxyphenanthrene-1,4-dione	330	0	6	1.827100
21	9a-hydroxy-10-oxo-delta6a,10a-tettetrahydrocannabinol	344	2	4	4.163399
22	9a-hydroxyhexahydrocannabinol	332	2	3	4.930599
23	9ß,10ß-epoxyhexahydrocannabinol	330	1	3	4.947000
24	10aa-hydroxy-10-oxo-delta8-tetrahydrocannabinol	344	2	4	4.018798
25	10a-hydroxy-delta9,11-hexahydrocannabinol	330	2	3	4.706599

26	10a-hydroxyhexahydrocannabinol	332	2	3	4.786500
27	10aR-hydroxyhexahydrocannabinol	332	2	3	4.786500
28	10-Ethoxy-9-hydroxy-delta-6a-tetrahydrocannabinol	374	2	4	4.999400
29	11-acetoxy-delta 9-tetrahydrocannabinolic acid A	388	1	5	5.498900
30	Apigenin-6,8-di-C-ß-D-glucopyranoside	594	11	15	-3.090901
31	Cannabichromanones B	362	2	5	3.824898
32	Cannabichromanones C	344	0	4	4.327498
33	Cannabichromanones D	314	0	3	5.075499
34	Cannabicitran	314	0	2	5.658700
35	Cannabicoumaronone	328	0	3	5.309000
36	Cannabielsoin (CBE)	330	2	3	4.706599
37	Cannabielsoin acid A (CBEA-A)	330	3	4	4.764500
38	Cannabigerol (CBG)	316	2	2	6.065701
39	Cannabigerol monomethylether (CBGM)	330	1	2	6.368701
40	Cannabigerolic acid (CBGA)	360	3	4	5.763900
41	Cannabigerolic acid monomethylether (CBGAM)	374	2	4	6.066900
42	Cannabigerovarin (CBGV)	288	2	2	5.285501
43	Cannabigerovarinic acid (CBGVA)	332	3	4	4.983699
44	Cannabinodiol (CBND)	310	2	2	5.839022
45	Cannabidivarin (CBVD)	286	2	2	5.066300
46	Cannabinol (CBN)	310	1	2	5.727821
47	Cannabinol methylether (CBNM)	324	0	2	6.030820
48	Cannabinol-C2	272	1	2	4.565499
49	Cannabinol-C4	300	1	2	5.345700
50	Cannabinolic acid (CBNA)	354	2	4	5.426020
51	Cannabiorcool (CBN-C1)	258	1	2	4.445219
52	Cannabisin A	594	8	10	4.685401
53	Cannabisin M	624	6	10	4.389099
54	Cannabisin N	509	5	9	2.960398
55	Cannabisin O	624	6	10	4.711001
56	Cannabisol	312	5	6	-0.053101
57	Cannabitriol (CBT)	346	3	4	3.955198
58	Cannabivarin (CBV)	282	1	2	4.947619
59	Cannflavin A	436	3	6	5.663403
60	Cannflavin C	436	3	6	5.663402
61	Carmagerol	350	4	4	4.231299

62	Catechin	290	5	6	1.546100
63	Cannabichromene (CBC-C5)	314	1	2	6.035602
64	Cannabichromanone-C3 (CBCN-C3)	304	1	4	3.683798
65	CBCN-C5	332	1	4	4.463999
66	CBDA-C5	328	2	3	5.268900
67	CBD-C1	258	2	2	4.422219
68	CBD-C4	300	2	2	5.456401
69	CBD-C5	314	2	2	5.846501
70	CBDM-C5	328	1	2	6.149501
71	CBDVA-C3	314	2	3	4.878800
72	CBDV-C3	286	2	2	5.066300
73	CBEA-C3 B	330	2	4	3.738898
74	CBEA-C5 A	358	2	4	4.519099
75	CBEA-C5 B	358	2	4	4.519099
76	CBE-C3	302	2	3	3.926398
77	CBE-C5	330	2	3	4.706599
78	CBF-C5	310	1	2	6.456122
79	CBGA	360	3	4	5.763900
80	CBG-C5	316	2	2	6.065701
81	CBL	314	1	2	5.666720
82	CBLA	342	1	3	5.479220
83	CBLV	286	1	2	4.886519
84	CBM	346	3	4	4.076199
85	CBN	310	1	2	5.727821
86	CBNA	354	2	4	5.426020
87	CBND-C3	282	1	2	4.759510
88	CBND-C5	310	1	2	5.539711
89	CBR	348	3	4	3.901399
90	CBTT	362	4	5	2.925499
91	CBX	306	0	2	6.032722
92	Chrysin	254	2	4	2.713999
93	Chrysoeriol	300	3	6	2.428199
94	Cis-delta9-THC	314	1	2	5.735801
95	**Cycloguanil**	**251**	**4**	**5**	**1.525500**
96	DCBF-C5	308	1	2	6.365822
97	Delta 9 -THC	314	1	2	5.735801
98	Delta 8-THC	314	1	2	5.735800
99	Delta 8-THC A	342	1	3	5.548300

100	Delta-9-tetrahydrocannabinol-C4(THC-C4)	300	1	2	5.345700
101	Delta-9-tetrahydrocannabinolic acid A (THCA-A)	358	2	4	5.433999
102	Delta-9-tetrahydrocannabinolic acid-C4 (THCA-C4)	358	2	4	5.712933
103	Delta9-THC aldehyde	341	2	5	6.329080
104	Docosanoic acid methyl ester	430	1	3	7.186965
105	Isocannabispiradienone	242	1	3	2.279900
106	Isolated 4-terpenyl cannabinolate	462	1	4	7.396723
107	Isoselachoceric acid	340	1	2	7.892902
108	Isovitexin	432	7	10	-0.065500
109	Kaempferol	286	4	6	2.305299
110	Lariciresinol	360	3	6	2.653699
111	Luteolin	286	4	6	2.125199
112	Mannitol	174	2	2	1.515400
113	OH-iso-HHCV-C3	298	2	3	4.779920
114	10-oxo-Δ6a(10a)-tetrahydrocannabinol (OTHC)	328	1	3	5.048499
115	Pyrimethamine	248	4	4	2.523800
116	Quebrachitol	194	5	6	-3.180501
117	Quercetin	304	5	7	1.186300
118	Secoisolariciresinol	362	4	6	2.117200
119	Sesquicannabigerol	384	2	2	7.792204
120	Sophoroside	342	8	11	-5.397200
121	ß-sitosterol	414	1	1	8.024803
122	Tetrahydrocannabinolic acid (THCA)	358	2	4	5.434000
123	Tetrahydrocannabivarin (THCV)	286	1	2	4.955600
124	Uracil	112	2	4	0.660500
125	Vitexin	432	7	10	0.065500
126	8,9-Di-OH-CBT-C5	362	4	5	2.926000
	WR99210	**393**	**4**	**4**	**2.834000**

The outcomes indicate that amongst studied molecules, 62 candidates were sorted out as favorable for drug development with at fewer than two neglectable violations of the five rules.

3. Pharmacokinetics assessment and toxicity prediction

The compounds above are then put on Molinspiration and Protox-II for evaluation of pharmacokinetic attributes and toxicity prediction. Molinspiration is an online web server for the prediction of drug-likeness score including G protein-coupled receptors (GPCR) ligands, ion channel modulators, kinase inhibitors, nuclear receptor ligands, protease inhibitors and other enzyme targets, while Protox-II is a popular and freely available server for in silico toxicity predictions.

Table 3. Pharmacokinetic parameters and toxicity prediction.

ID	Name	Molinspiration			Protox-II	
		miLogP	TPSA	Enzyme inhibitory level	LD_{50} (mg/kg)	Toxicity level
5	(1'S)-hydroxycannabinol	5.49	69.92	0.29	13500	6
7	4,5-dihydroxy-2,3,6-trimethoxy-9,10-dihydrophenanthrene	2.93	68.16	0.15	1000	4
8	4,7-dimethoxy-1,2,5-trihydroxyphenanthrene	3.27	79.15	0.23	550	4
12	5'-Methyl-4-pentylbiphenyl-2,6,2'-triol	5.68	60.68	0.05	2000	4
13	6-prenylapigenin	4.71	90.89	0.46	3919	5
14	7-methoxycannabispirone	2.37	35.54	0.20	500	4
15	7-oxo-9a-hydroxyhexahydrocannabinol	4.59	66.76	0.3	1000	4
16	8,9-Dihydroxy-delta-6a-tetrahydrocannabinol	4.61	69.92	0.32	750	4
17	8-hydroxycannabinol	5.67	49.69	0.20	400	4
18	8-hydroxycannabinolic acid A	5.38	66.76	0.24	400	4
19	8-oxo-delta9-THC	5.59	46.53	0.28	500	4
20	9,10-dihydro-2,3,5,6-tetramethoxyphenanthrene-1,4-dione	2.17	71.08	0.34	150	3
21	9a-hydroxy-10-oxo-delta6a,10a-tettetrahydrocannabinol	4.42	66.76	0.12	750	4
22	9a-hydroxyhexahydrocannabinol	5.69	49.69	0.41	1000	4
23	9ß,10ß-epoxyhexahydrocannabinol	5.89	41.99	0.49	1000	4
24	10aa-hydroxy-10-oxo-delta8-tetrahydrocannabinol	5.01	66.76	0.27	500	4

25	10a-hydroxy-delta9,11-hexahydrocannabinol	5.56	49.69	0.41	860	4
26	10a-hydroxyhexahydrocannabinol	5.72	49.69	0.35	1000	4
27	10aR-hydroxyhexahydrocannabinol	6.06	49.69	0.23	1000	4
28	10-Ethoxy-9-hydroxy-delta-6a-tetrahydrocannabinol	5.60	58.92	0.19	750	4
31	Cannabichromanones B	4.21	83.83	0.39	2647	5
32	Cannabichromanones C	4.11	60.45	0.17	2647	5
33	Cannabichromanones D	5.66	35.54	0.17	860	4
36	Cannabielsoin (CBE)	5.79	49.69	0.31	500	4
42	Cannabigerovarin	6.78	40.46	0.54	730	4
43	Cannabigerovarinic acid (CBGVA)	6.07	77.75	0.60	1000	4
44	Cannabinodiol (CBND)	6.63	40.46	0.08	1500	44
57	Cannabitriol (CBT)	4.61	69.92	0.28	750	4
60	Cannflavin C	6.38	100.13	0.38	3919	5
61	Carmagerol	5.46	80.91	0.69	500	4
62	Catechin	1.37	110.37	0.47	10000	6
64	Cannabichromanone-C3 (CBCN-C3)	3.79	63.60	0.27	2647	5
65	CBCN-C5	4.86	63.60	0.29	2647	5
67	CBD-C1	5.22	40.46	0.21	2000	4
71	CBDVA-C3	5.59	57.53	0.34	1000	4
73	CBEA-C3 B	4.24	66.76	0.32	3	1
74	CBEA-C5 A	5.51	66.76	0.34	3	1
75	CBEA-C5 B	5.30	66.76	0.33	3	1
76	CBE-C3	4.73	49.69	0.29	500	4
77	CBE-C5	5.79	49.69	0.31	500	4
83	CBLV	5.57	29.46	0.31	1000	4
84	CBM	5.07	77.75	0.25	1000	4
87	CBND-C3	5.56	40.46	0.04	2200	5
89	CBR	4.78	69.92	0.37	1000	4
90	CBTT	4.25	90.15	0.45	500	4
92	Chrysin	2.94	70.67	0.26	2500	5
93	Chrysoeriol	2.28	100.13	0.21	4000	5
105	Isocannabispiradienone	1.79	46.53	0.35	530	4
109	Kaempferol	2.17	111.12	0.26	3919	5

110	Lariciresinol	2.33	88.39	0.23	1500	4
111	Luteolin	1.97	111.12	0.28	3919	5
112	Mannitol	2.03	40.46	-0.14	710	4
113	8-hydroxy-isohexahydrocannabivirin	5.06	53.6	0.25	1000	4
114	10-oxo-Δ6a(10a)-tetrahydrocannabinol (OHTC)	5.37	46.53	0.21	750	4
116	Quebrachitol	-1.99	110.37	-0.05	804	4
117	Quercetin	0.71	127.44	0.29	2000	4
118	Secoisolariciresinol	2.08	99.38	0.08	2000	4
122	Tetrahydrocannabinolic acid (THCA)	5.98	66.76	0.51	500	4
123	Tetrahydrocannabivarin (THCV)	5.62	29.46	0.43	482	4
124	Uracil	-0.90	65.72	-2.68	6000	5
126	8,9-Di-OH-CBT-C5	4.61	69.92	0.32	750	4
	Cycloguanil	**0.9**	**80.01**	**0.31**	**310**	**4**
	WR99210	**2.33**	**98.48**	**0.33**	**310**	**4**

In general, the calculated properties of studied compounds are quite interesting in consideration of toxicity scale. Most candidates are positioned from rank 4 to 6 (Low toxicity to non-toxic), with the exception of compounds 73, 74, 75 (rank 1) and 20 (rank 3). On the other hand, it is observed that three compounds 116 and 124 had their miLogP value fall below zero (-1.99 and -0.9 respectively) which suggest they are unable to bind with pfDHFR-TS enzyme. In addition, the enzyme inhibitory potential value of compound 112 was -0.14, meaning these compounds are not likely to exhibit inhibition activity to the target enzyme. Thus, these 3 ligands were excluded from docking studies.

4. Docking studies

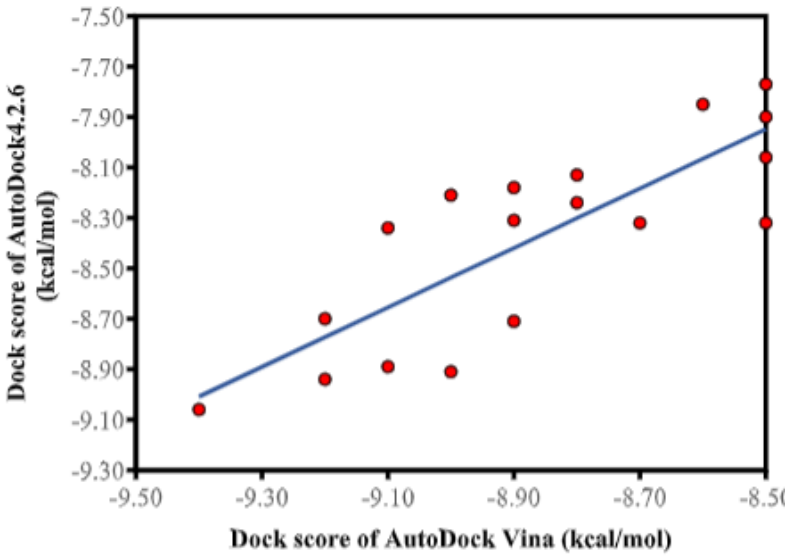
To determine the inhibition potential of the selected candidates with our protein model, AutoDock 4.2.6 and AutoDock Vina were chosen to conduct the simulations. Cycloguanil and WR99210 were used as standard inhibitors with dock score of AutoDock4.2.6 were -7.77 kcal/mole and -8.91 kcal/mole and dock score of AutoDock Vina were -8.50 kcal/mole and -9.10 kcal/mole, respectively. Thus, any ligand has docking energy fall within these range or more negative would be consider as potential inhibitor of pfDHFR-TS.

Table 4. The docking score results of studied compounds on enzyme pfDHFR-TS.

ID	Name	AutoDock4	AutoDock Vina
15	7-oxo-9a-hydroxyhexahydrocannabinol	-9.06	-9.40
16	8,9-Dihydroxy-delta-6a-tetrahydrocannabinol	-8.32	-8.50
19	8-oxo-delta9-THC	-8.89	-9.10
21	9a-hydroxy-10-oxo-delta6a,10a-tettetrahydrocannabinol	-8.18	-8.90
24	10aa-hydroxy-10-oxo-delta8-tetrahydrocannabinol	-8.13	-8.80
25	10a-hydroxy-delta9,11-hexahydrocannabinol	-8.21	-9.00
26	10a-hydroxyhexahydrocannabinol	-8.34	-9.10
27	10aR-hydroxyhexahydrocannabinol	-8.10	-9.20
31	Cannabichromanones B	-7.85	-8.60
33	Cannabichromanones D	-8.71	-8.90
57	Cannabitriol (CBT)	-8.32	-8.70
74	CBEA-C5 A	-8.06	-8.50
90	CBTT	-7.90	-8.50
111	Luteolin	-8.31	-8.90
113	8-hydroxy-isohexahydrocannabivirin	-8.24	-8.80
114	10-oxo-Δ6a(10a)-tetrahydrocannabinol (OTHC)	-8.94	-9.20
	Cycloguanil	**-7.77**	**-8.50**
	WR99210	**-8.91**	**-9.10**

According to obtained results, 11 out of 62 screened compounds were assumed as potential inhibitors (Table 4). Compound 7 and 126 are the top two ligands with their docking energy far more exceeding than standard compounds. The rest 14 hits presented dock scores matching with the selection criteria with dock score ranges from -7.98 kcal/mol to –8.81 kcal/mol for AutoDock4.2.6 and from -8.29 kcal/mol to -9.53 kcal/mol for AutoDock Vina, respectively. On the other hand, a high correlation coefficient between the docking energy of AutoDock4.2.6 and AutoDock Vina was recorded with the value of R = 0.82 (Figure 2) that support for the accuracy of the docking study.

Figure 2. Results from AutoDock4 and AutoDock Vina in comparison to each other (R = 0.82).



For inhibition to occur, interaction is needed on key amino acid residues Ile14, Asp54 and Ile164 at the active site of the enzyme. The hydrogen bonding patterns and stereo view of binding mode of 10 potential pfDHFR-TS inhibition compounds are shown in Figure 3.

The potential hits were further analyzed for ligand efficiency and binding poses (Table 5). Ligand efficiency (LE) is a useful metric for the selection of lead compounds in drug discovery, it is a measurement of the binding energy of the ligand per atom, which is calculated according to the equation 1.

$$\Delta g = \frac{\Delta G}{N_{non-hydrogen\ atoms}} \qquad \text{(Equation 1*)}$$

*Δg: Ligand efficiency; ΔG: docking energy

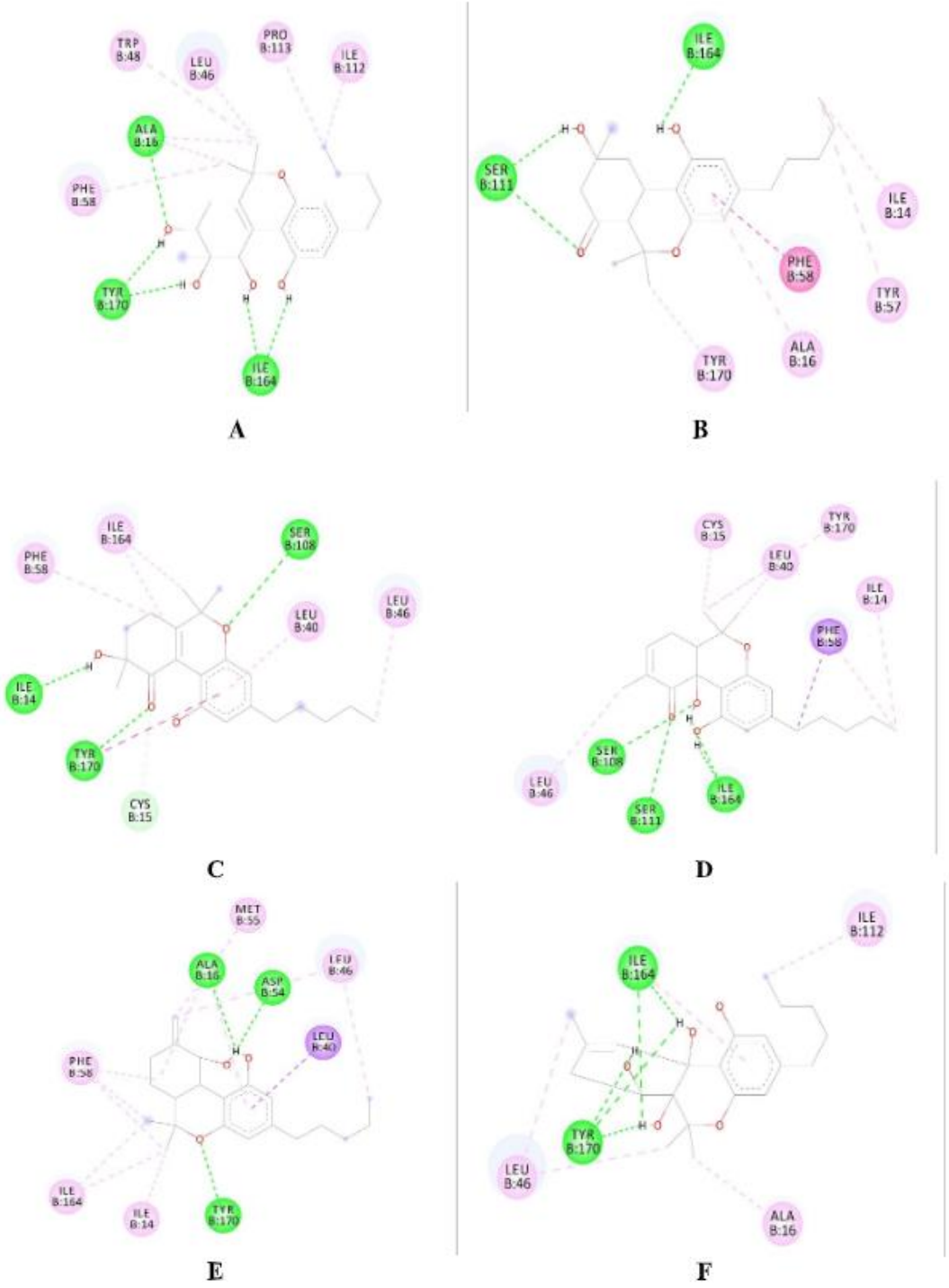
Statistically, compounds with LE varies within 0.3 < LE < 0.5 is more potential for further optimization (Chen et al., 2015). The LE in this study was calculated using AutoDock4.2.6, as a result, all 16 hits compounds showed promising drug ability with LE score varies from -0.39 to -0.47.

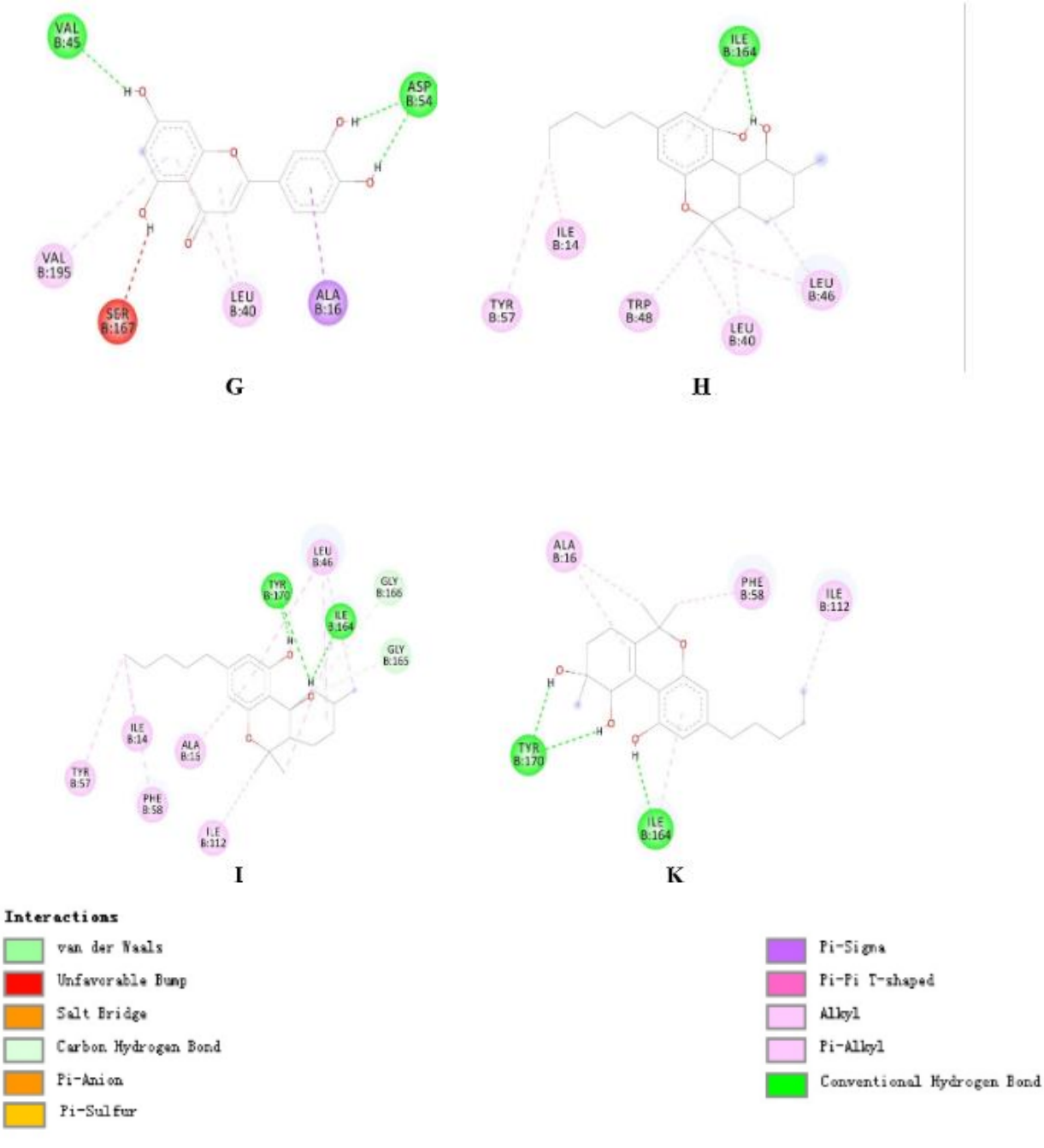
Table 5. The ligand efficacy and hydrogen bonds interactions between potential compounds and enzyme pfDHFR-TS

ID	Compound name	Ligand efficiency	No. of H-bonds	Interacting residues
15	7-oxo-9a-hydroxyhexahydrocannabinol	-0.39	3	Ser111; Ile164
16	8,9-dihydroxy-delta6a,10a-tetrahydrocannabinol	-0.41	5	Ala16; Tyr170; Ile164
19	8-oxo-delta9-tetrahydrocannabinol	-0.39	1	Ala16
21	9a-hydroxy-10-oxo-delta6a,10a-tettetrahydrocannabinol	-0.42	3	Ser108; Ile14; Tyr170
24	10aa-hydroxy-10-oxo-delta8-tetrahydrocannabinol	-0.42	3	Ser108; Ser111; Ile164
25	10a-hydroxy-delta9,11-hexahydrocannabinol	-0.42	3	Ala16; Asp54; Tyr170
26	10a-hydroxyhexahydrocannabinol	-0.45	1	Ile164
27	10aR-hydroxyhexahydrocannabinol	-0.43	3	Tyr170; Ile164
31	Cannabichromanones B	-0.40	4	Thr107; Ser108; Ser167
33	Cannabichromanones D	-0.41	1	Ser167
57	Cannabitriol	-0.45	3	Tyr170; Ile164
74	Cannabielsoic acid A	-0.41	3	Ala16; Leu40; Ile164
90	Cannabitetrol	-0.43	5	Ile164; Tyr170
111	Luteolin	-0.46	3	Val45; Asp54
113	8-hydroxy-isohexahydrocannabivirin	-0.47	3	Tyr170; Ala16
114	10-oxo-delta6a,10a-tetrahydrocannabinol	-0.43	2	Tyr170
	Cycloguanil	**-0.45**	**5**	**Ile164; Ile14; Cys15; Thr185; Asp54**
	WR99210	**-0.38**	**5**	**Cys15; Asp54; Ile14; Ile164**

For inhibition to occur, interaction is needed on key amino acid residues Ile14, Asp54 and Ileu164 at the active site of the enzyme [20]. As indicated from Table 4, although better docking energy than standard Cycloguanil and WR99210, compounds **19**, **27**, **90**, **113** and **114** did not formed hydrogen bonds with any of three key residues, therefore, these ligands were assumpted as non-potential for pfDHFR-TS inhibition. Regarding to toxicity prediction (Table 3), compound **33** was excluded due to high toxicity (LD_{50} value 3 mg/kg). Compound **31** and **113** were highlighted with their safety class of toxic (LD_{50} value 3919 mg/kg and 2647 mg/kg, respectively). The hydrogen bonding patterns and stereo view of binding mode of 10 potetial pfDHFR-TS inhibition compounds are shown in Figure 3 and Figure A2.

Figure 3. Hydrogen bonding patterns of 10 potential pfDHFR-TS inhibition compounds. (A): Compound 16; (B) Compound 15; (C): Compound 21; (D): Compound 24; (E): Compound 25; (F): Compound 90; (G): Compound 111; (H): Compound 26; (I): Compound 27; (K): Compound 57





V. Scientific conclusions

In our research, computational molecular simulation and drug-like properties assessment were used to gain insight into the binding ability of phytoconstituents of *Cannabis Sativa L.* on enzyme pfDHFR-TS. Finally, 10 out of 125 studied compounds including compound 15, 16, 21, 24, 25, 26, 27, 57, 90 and 111 were identified as potential candidates for inhibiting function of pfDHFR-TS at the active site through hydrogen bonds with Ile14, Asp54 and Ile 164 residues. These finding shed light on the potential anti-malaria activity of compounds isolated from *Cannabis sativa L.*

IV. Management of the project at the end

No.	Plan	Done	Details	Person in charge
1	Structure (2D, 3D)	Successfully compiled 125 chemical compounds from Cannabis Sativa in reliable sources. The structures were built in ChemDraw, Chem3D and displayed and characterized in Marvin.	In total, there are about 400-500 chemical compounds inside *Cannabis Sativa*. Although we found only 125, all of these are confirmed in reliable sources (textbook, articles)	Nam + Kiệt
2	Ligand preparation (Drug potential assessment)	Using Lipinski's rule of five, found the online software to detect and evaluate drug-like properties and acute toxicity of all research compounds.	Finding successfully the software of evaluation Lipinski's rule of five, Molinspiration and ProTox II.	Nam
3	Protein modeling	Filtration of protein 1i3j (remove unacceptable chain and the water molecule surround; add polar hydrogen atoms)	AutoDock tools and Chimera 1.13.1	Nam
4	Docking studies - AutoDock 4.2.6	Docking studies via Ubuntu-Linux 14.04.6 LTS operating system after having done ligand preparation and protein modeling	AutoDock 4.2.6, Ubuntu-Linux 14.04.6 LTS, Cygwin 64 terminal	Nam
5	Docking studies - AutoDock vina	Docking via PyRx after having done ligand preparation and protein modeling	PyRx	Nam
6	Image Collection	After docking studies, tasks of image collection include showing the results of the abilities of Hydrogen binding from ligands to protein.	Maestro 11.9, LigPlus, AutoDock 4.2.6, Discovery Studio 2017 R2 Client.	Nam + Kiệt

IV. Key references:

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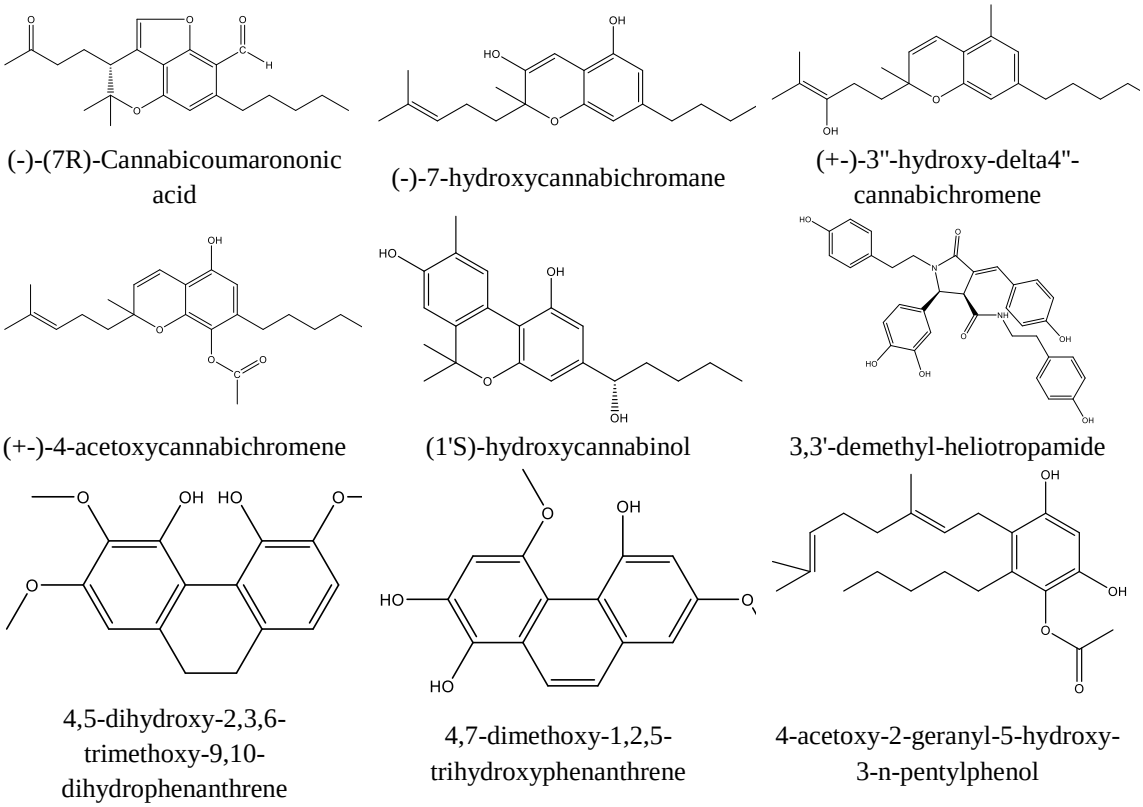
V. Appendix

Figure A1. Structure of 125 compounds isolated from *Cannabis Sativa*



(-)-(7R)-Cannabicoumarononic acid

(-)-7-hydroxycannabichromane

(+-)-3''-hydroxy-delta4''-cannabichromene

(+-)-4-acetoxycannabichromene

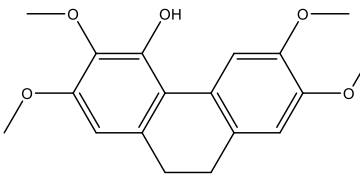
(1'S)-hydroxycannabinol

3,3'-demethyl-heliotropamide

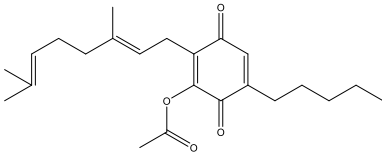
4,5-dihydroxy-2,3,6-trimethoxy-9,10-dihydrophenanthrene

4,7-dimethoxy-1,2,5-trihydroxyphenanthrene

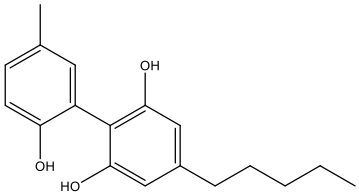
4-acetoxy-2-geranyl-5-hydroxy-3-n-pentylphenol



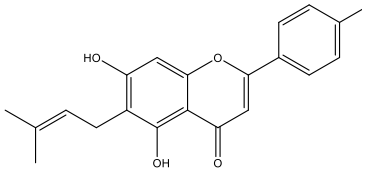
4-hydroxy-2,3,6,7-tetramethoxy-9,10-dihydrophenanthrene



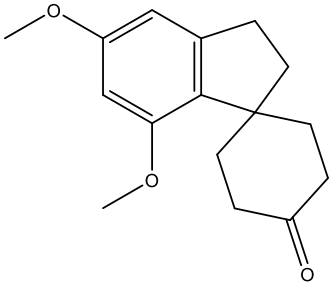
5-acetoxy-6-geranyl-3-n-pentyl-1,4-benzoquinone



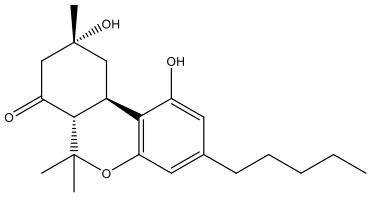
5'-Methyl-4-pentylbiphenyl-2,6,2'-triol



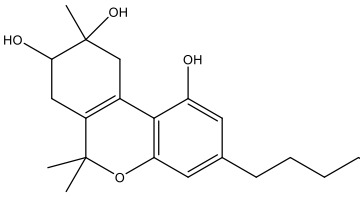
6-prenylapigenin



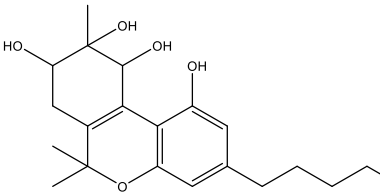
7-methoxycannabispirone



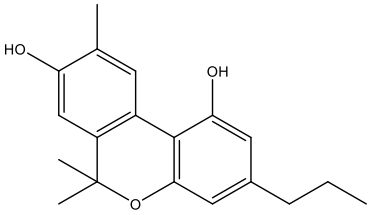
7-oxo-9a-hydroxyhexahydrocannabinol



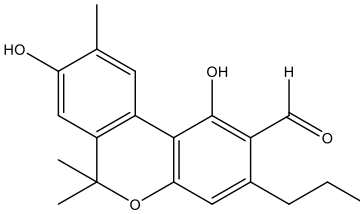
8,9-Dihydroxy-delta-6a-tetrahydrocannabinol



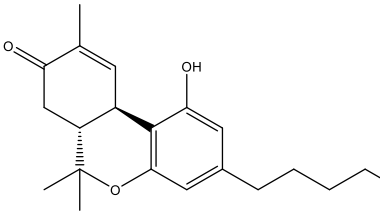
8,9-dihydroxy-delta6a,10a-tetrahydrocannabinol (8,9-Di-OH-CBT-C5)



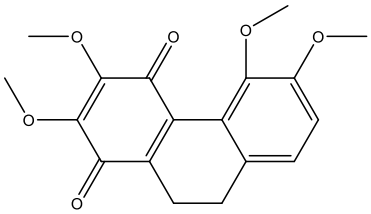
8-hydroxycannabinol



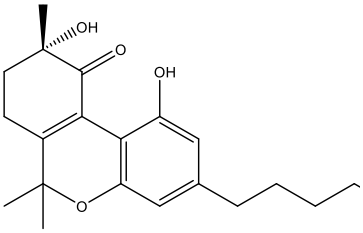
8-hydroxycannabinolic acid A



8-oxo-delta9-tetrahydrocannabinol (8-oxo-delta9-THC)



9,10-dihydro-2,3,5,6-tetramethoxyphenanthrene-1,4-dione



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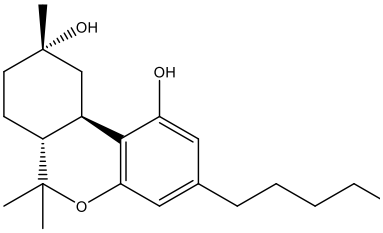
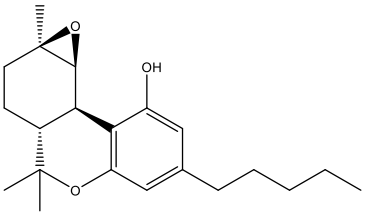
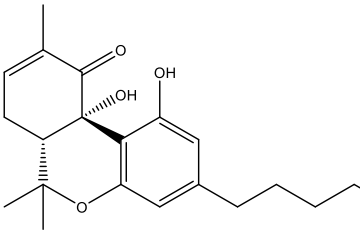


9a-hydroxyhexahydrocannabinol



9β,10β-epoxyhexahydrocannabinol



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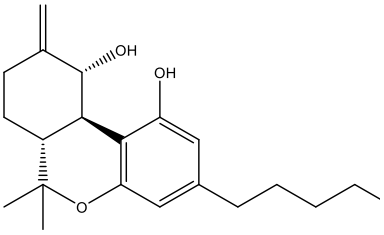
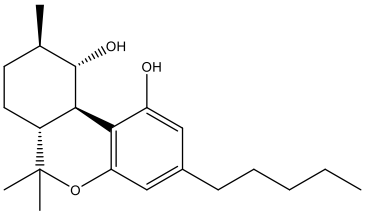
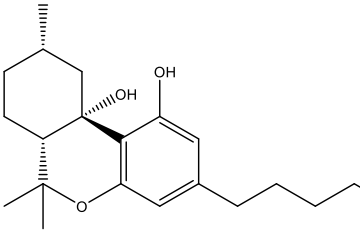


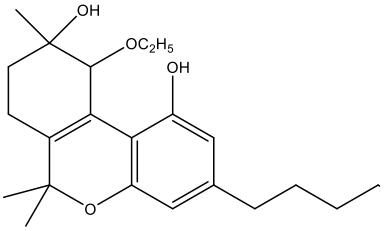
10a-hydroxy-delta9,11-hexahydrocannabinol



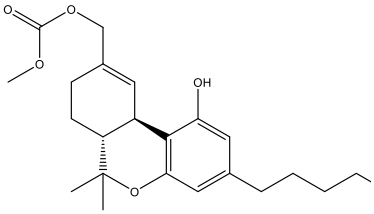
10a-hydroxyhexahydrocannabinol



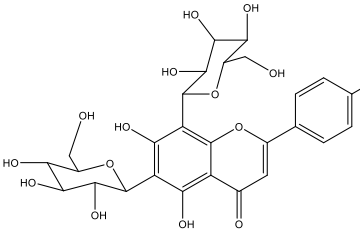
10aR-hydroxyhexahydrocannabinol



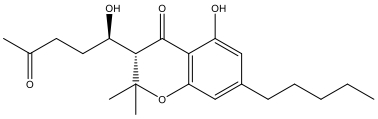
10-Ethoxy-9-hydroxy-delta-6a-tetrahydrocannabinol



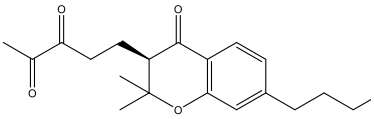
11-acetoxy-delta 9-tetrahydrocannabinolic acid A



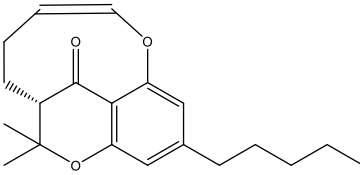
Apigenin-6,8-di-C-β-D-glucopyranoside



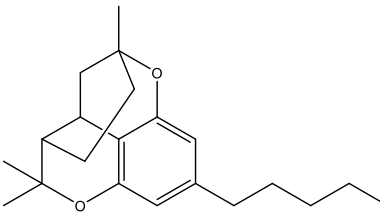
Cannabichromanones B



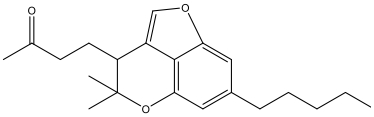
Cannabichromanones C



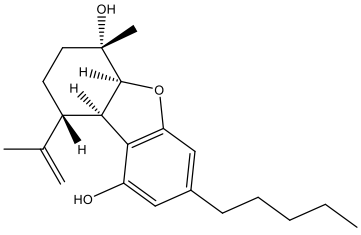
Cannabichromanones D



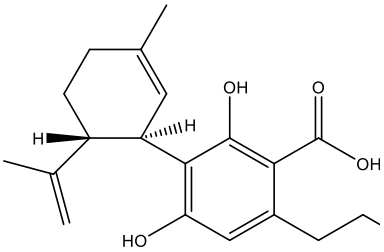
Cannabicitran



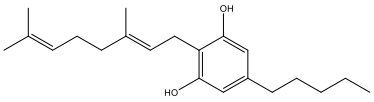
Cannabicoumaronone



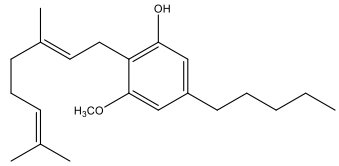
Cannabielsoin (CBE)



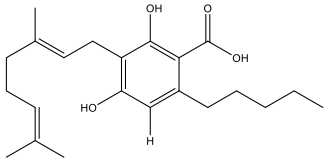
Cannabielsoin acid A (CBEA-A)



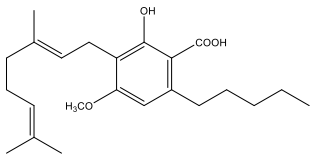
Cannabigerol (CBG)



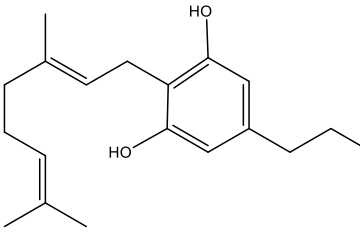
Cannabigerol monomethylether (CBGM)



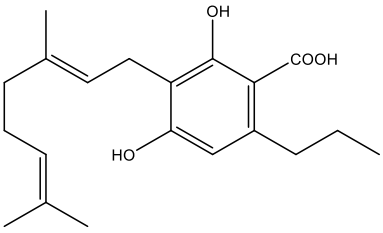
Cannabigerolic acid (CBGA)



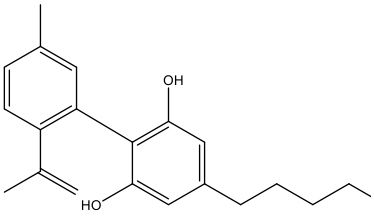
Cannabigerolic acid monomethylether (CBGAM)



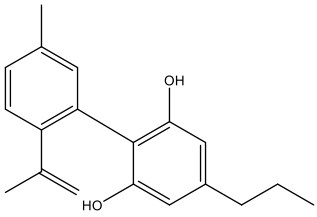
Cannabigerovarin (CBGV)



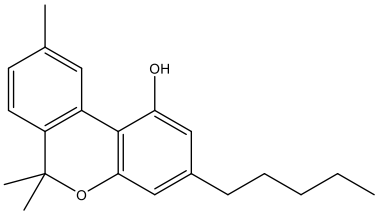
Cannabigerovarinic acid (CBGVA)



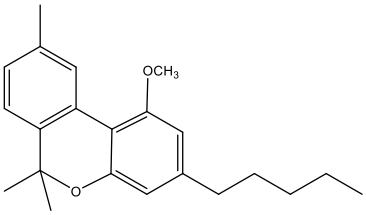
Cannabinodiol (CBND)



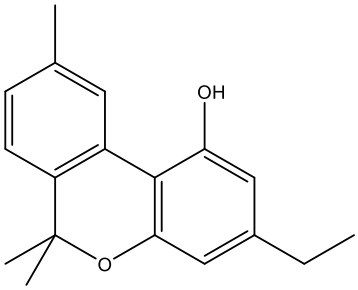
Cannabinodivarin (CBVD)



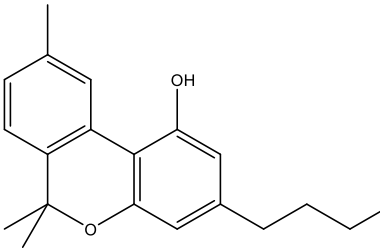
Cannabinol (CBN)



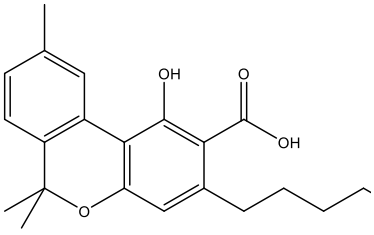
Cannabinol methylether (CBNM)



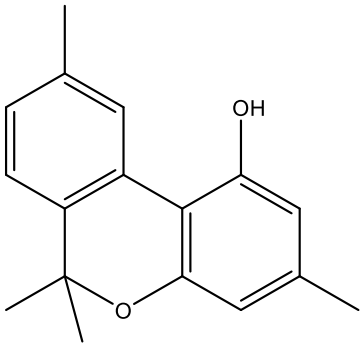
Cannabinol-C2 (CBN-C2)



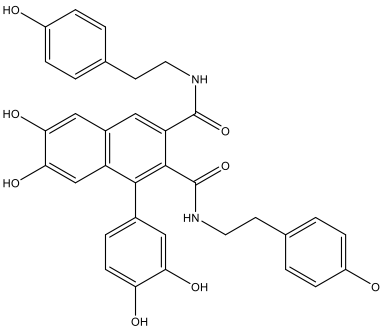
Cannabinol-C4 (CBN-C4)



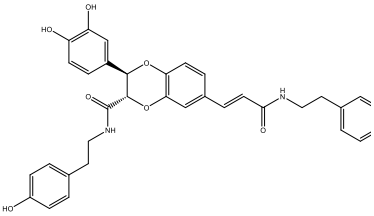
Cannabinolic acid (CBNA)



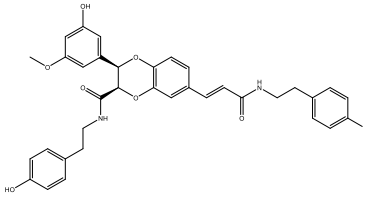
Cannabiorcool (CBN-C1)



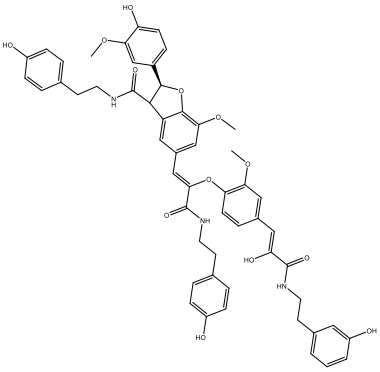
Cannabisin A



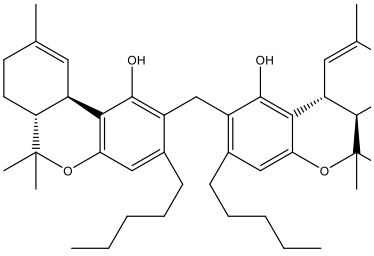
Cannabisin M



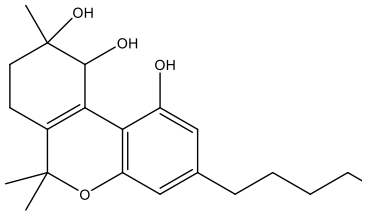
Cannabisin N



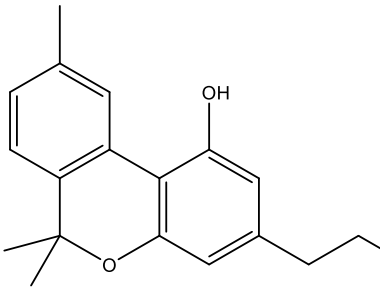
Cannabisin O



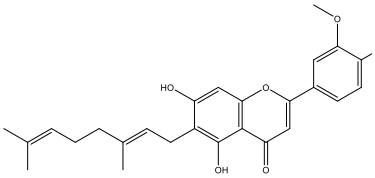
Cannabisol



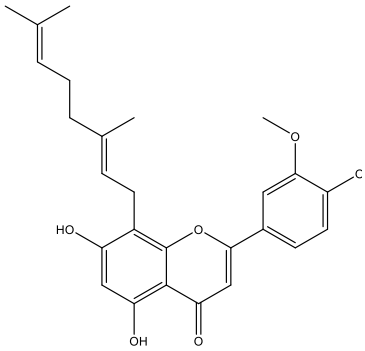
Cannabitriol (CBT)



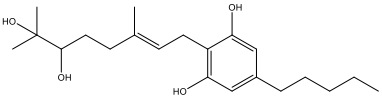
Cannabivarin (CBV)



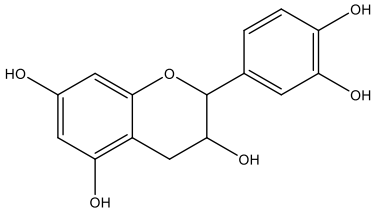
Cannflavin A



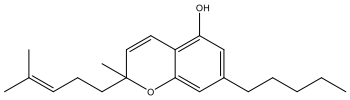
Cannflavin C



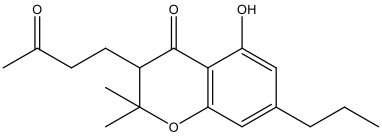
Carmagerol



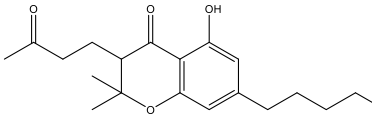
Catechin



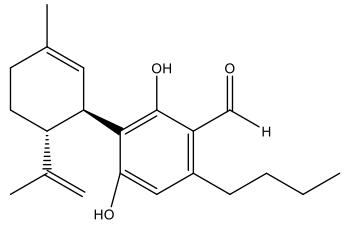
Cannabidiol-C5 (CBC-C5)



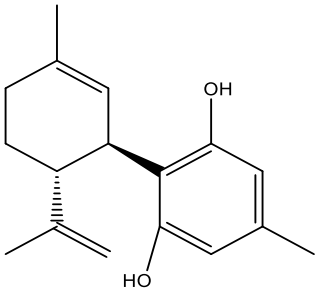
Cannabichromanone-C_3 (CBCN-C3)



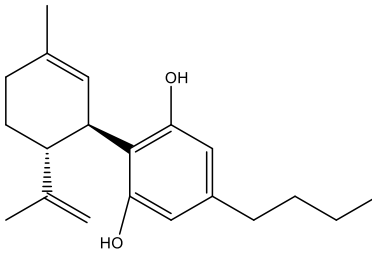
Cannabichromanone-C_5 (CBCN-C5)



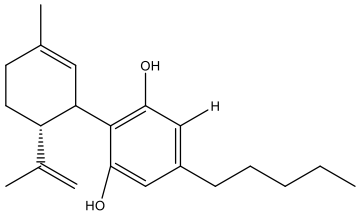
Cannabidiolic acid-C5 (CBDA-C5)



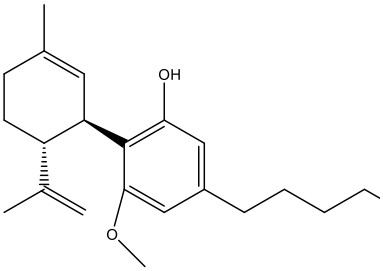
Cannabidiocol (CBD-C1)



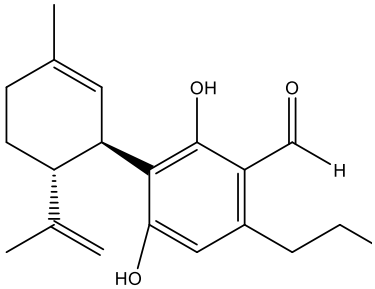
Cannabidiol-C_4 (CBD-C4)



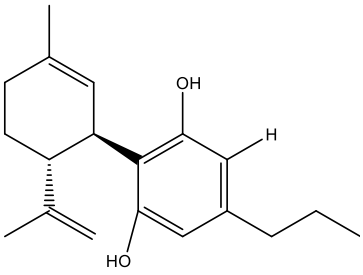
(-)-cannabidiol (CBD-C5)



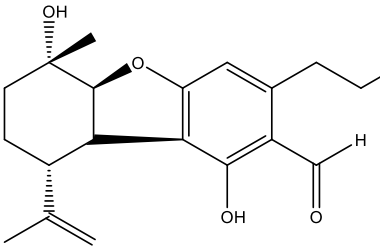
Cannabidiol monomethyl ether (CBDM-C5)



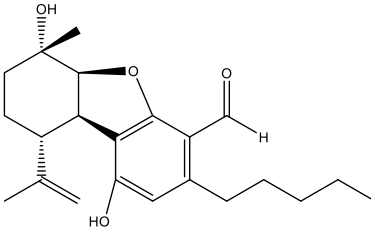
Cannabidivarinic acid (CBDVA-C3)



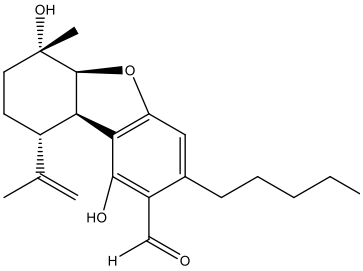
(-)-cannabidivarin (CBDV-C3)



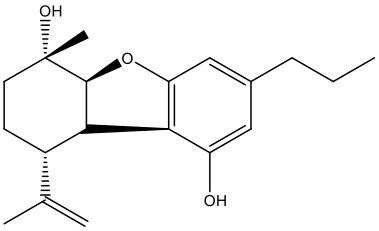
Cannabielsoic acid B-C3 (CBEA-C3 B)



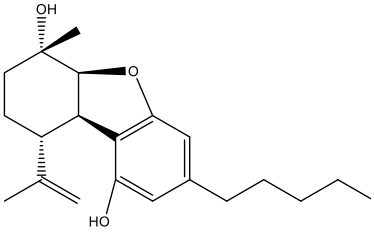
Cannabielsoic acid A (CBEA-C5 A)



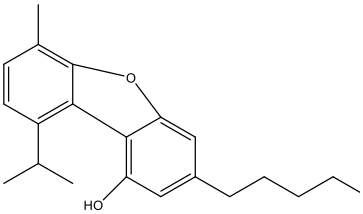
Cannabielsoic acid B (CBEA-C5 B)



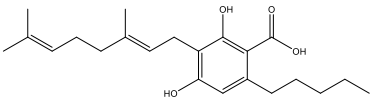
C3-cannabeilsoin (CBE-C3)



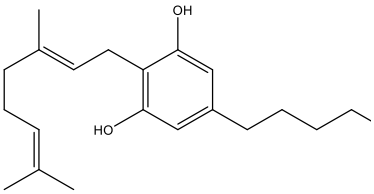
Cannabielsoin (CBE-C5)



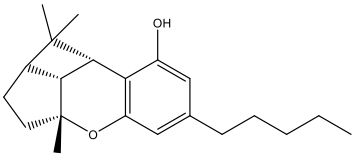
Cannabifuran (CBF-C5)



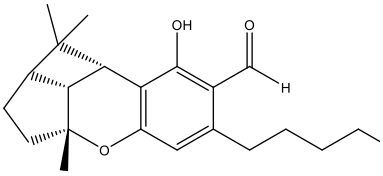
Cannabigerolic acid (CBGA)



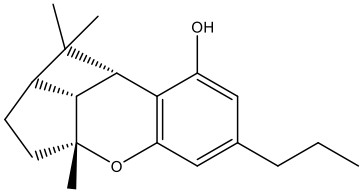
Cannabigerol (CBG-C5)



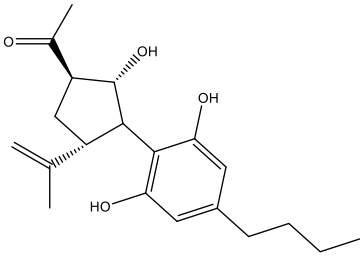
Cannabicyclol (CBL)



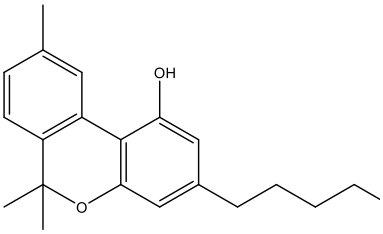
Cannabicyclolic acid (CBLA)



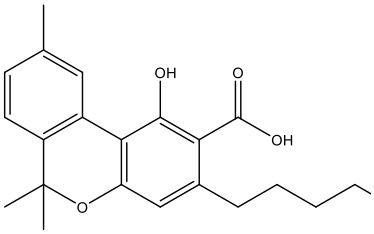
Cannabicyclovarin (CBLV)



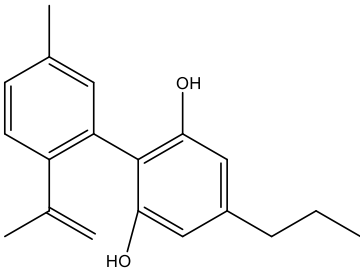
Cannabimovone (CBM)



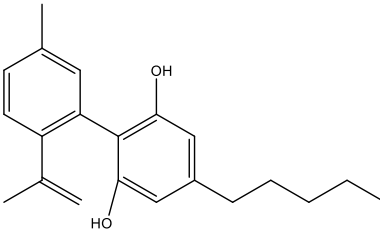
Cannabinol (CBN)



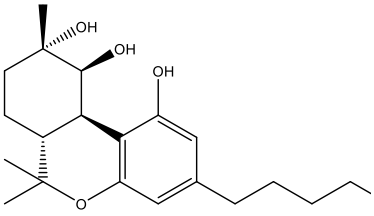
Cannabinolic acid (CBNA)



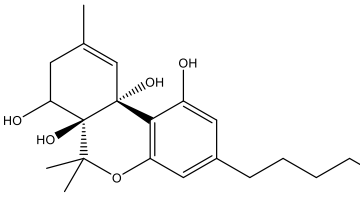
Cannabinodivarin (CBND-C3)



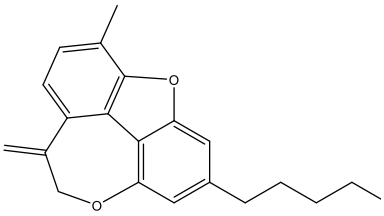
Cannabinodiol (CBND-C5)



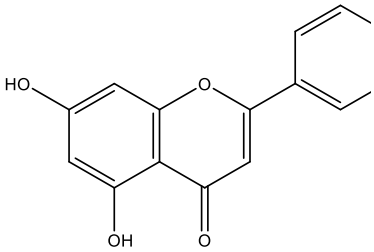
Cannabiripsol (CBR)



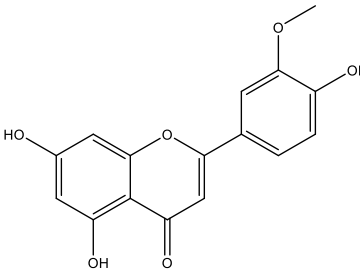
Cannabitetrol (CBTT)



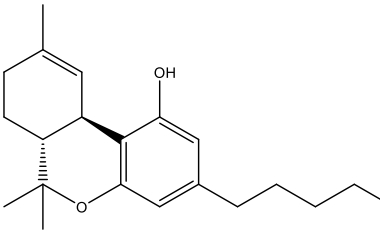
Tetracyclic cannabinoid cannabioxepane (CBX)



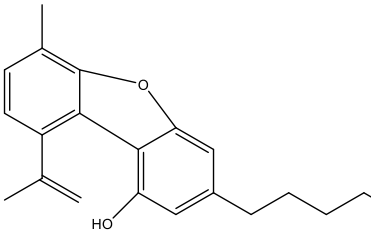
Chrysin



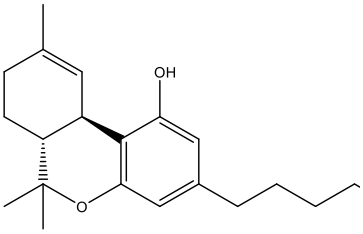
Chrysoeriol



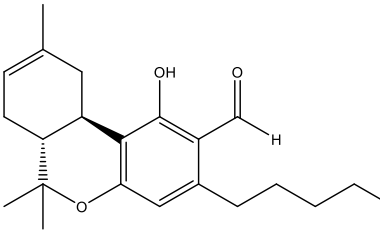
(-)-Δ^9-cis-(6aS,10aR)-tetrahydrocannabinol (cis-delta9-THC)



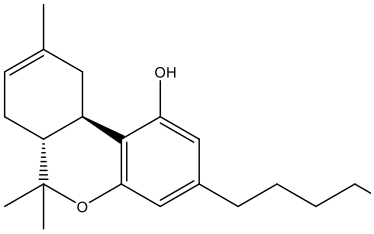
Dehydrocannabifuran (DCBF-C5)



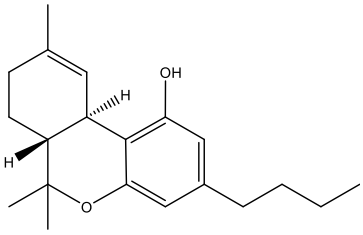
(-)-Δ^9-cis-(6aS,10aR)-tetrahydrocannabinol (delta 9 – THC)



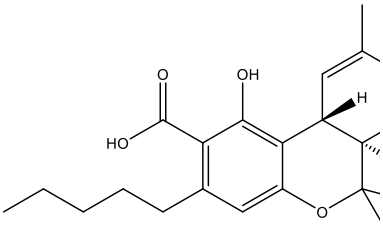
Δ^8-trans-tetrahydrocannabinolic acid A (delta8-THC A)



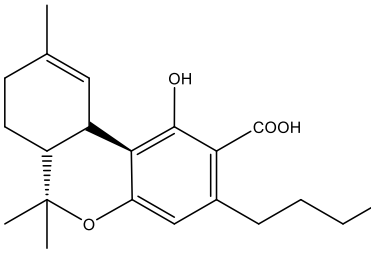
(-)-Δ^8-trans-Tetrahydrocannabinol (delta8-THC)



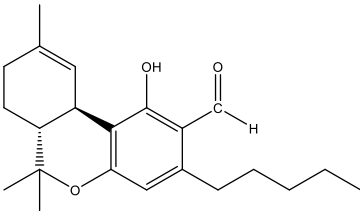
Delta-9-tetrahydrocannabinol-C4 (THC-C4)



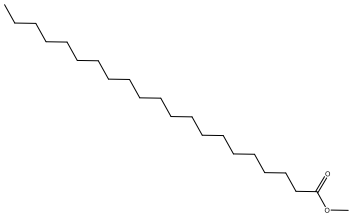
Delta-9-tetrahydrocannabinolic acid A (THCA-A)



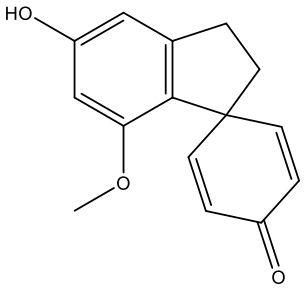
Delta-9-tetrahydrocannabinolic acid-C4 (THCA-C4)



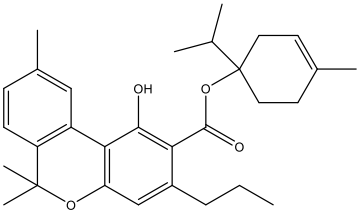
(-)-Δ^9-trans-tetrahydrocannabinol aldehyde (delta9-THC aldehyde)



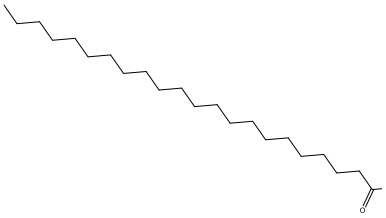
Docosanoic acid methyl ester



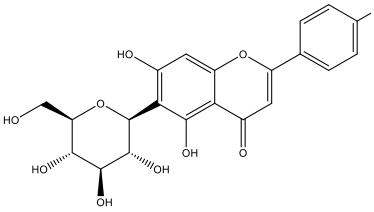
Isocannabispiradienone



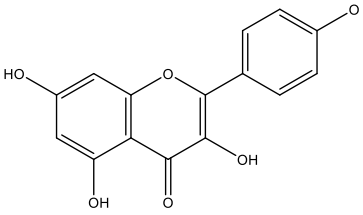
4-terpenyl cannabinolate



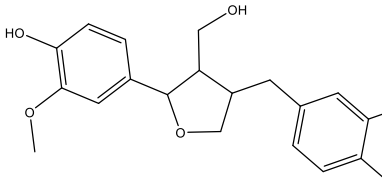
Isoselachoceric acid



Isovitexin



Kaempferol



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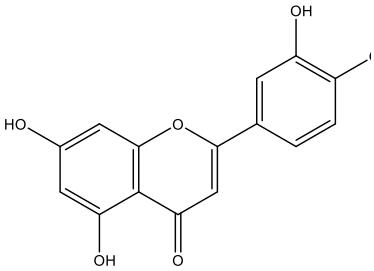
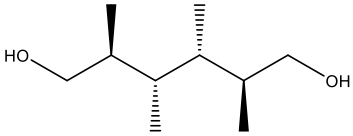
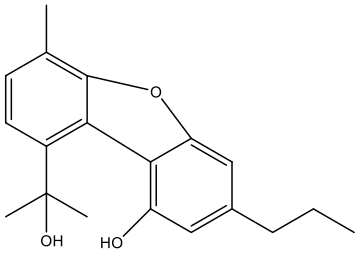


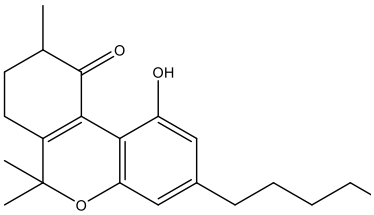
Luteolin



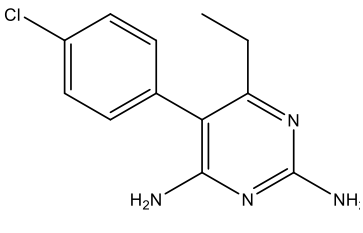
Mannitol



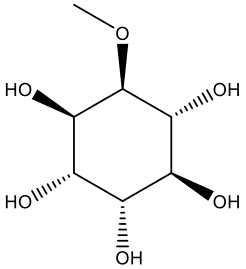
8-hydroxy-isohexahydrocannabivirin (OH-iso-HHCV-C3)



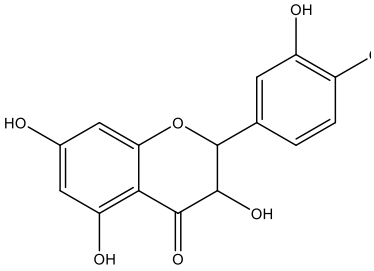
10-oxo-delta6a,10a-tetrahydrocannabinol (OTHC)



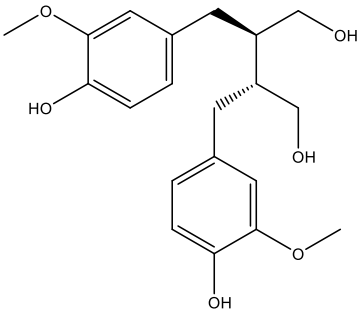
Pyrimethamine



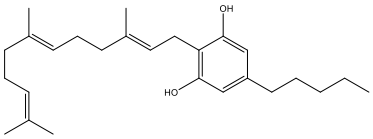
Quebrachitol



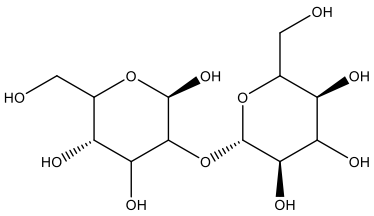
Quercetin



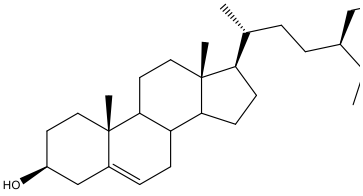
Secoisolariciresinol



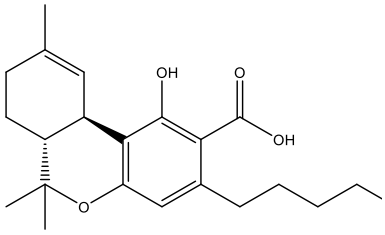
Sesquicannabigerol



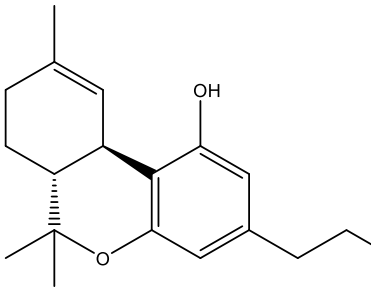
Sophoroside



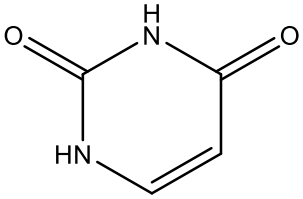
β-sitosterol



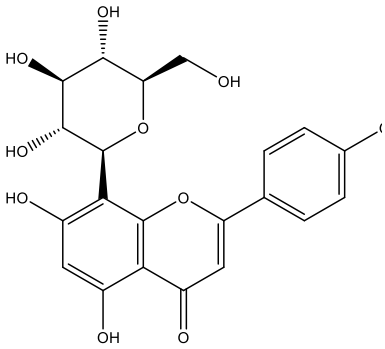
Tetrahydrocannabinolic acid (THCA)



Tetrahydrocannabivarin (THCV)

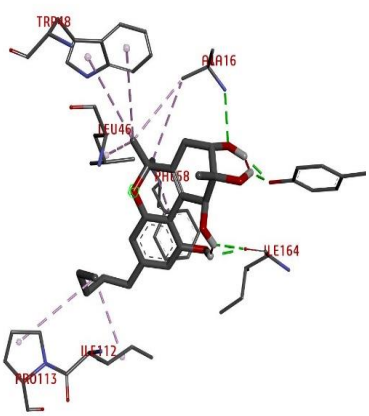


Uracil

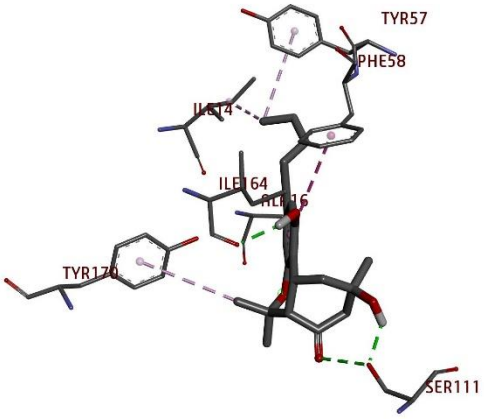


Vitexin

Figure A2. Stereoview of the binding mode of ten potential inhibitors of pfDHFR-TS enzyme (A): Compound 16; (B) Compound 15; (C): Compound 21; (D): Compound 24; (E): Compound 25; (F): Compound 90; (G): Compound 111; (H): Compound 26; (I): Compound 27; (K): Compound 57



A



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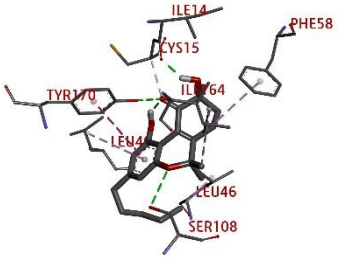

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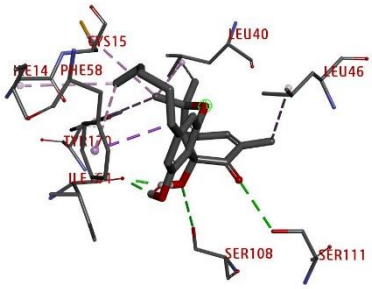

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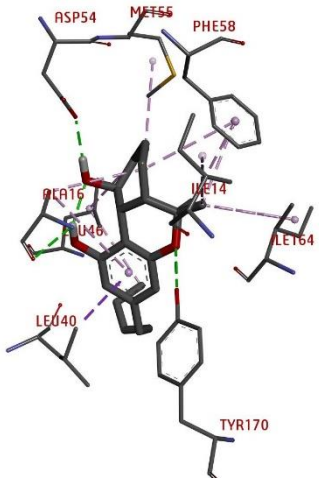
ASP54
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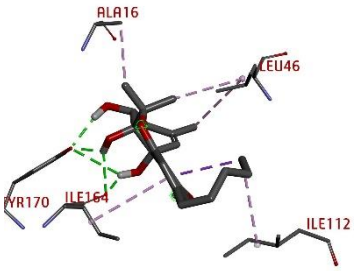
ALA16
ILE14
LEU46
ILE164
LEU40
TYR170

E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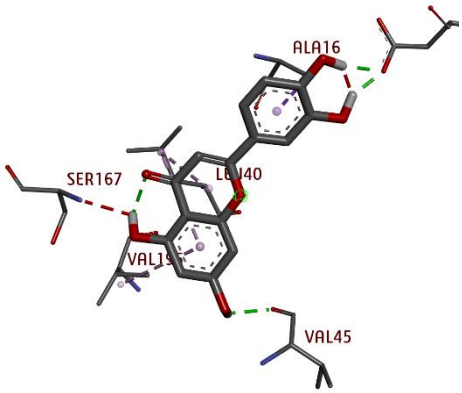
LEU46
TYR170
ILE164
ILE112

F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LEU40
VAL19
VAL45

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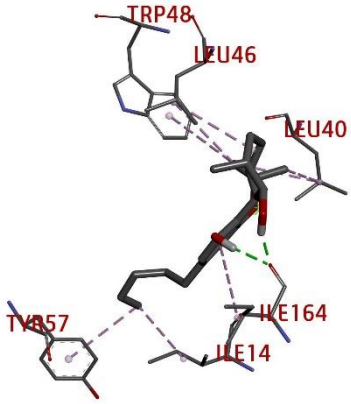
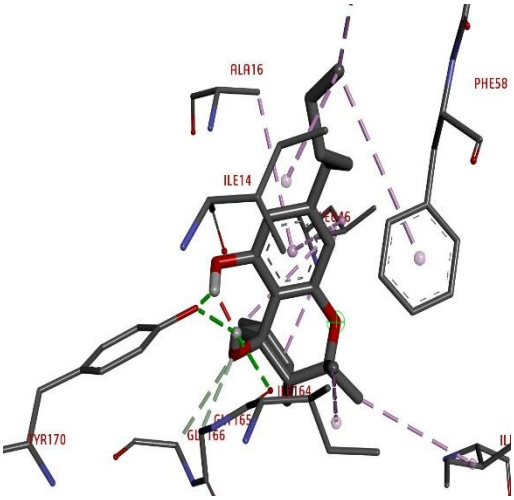

TRP48
LEU46
LEU40
ILE164
ILE14

H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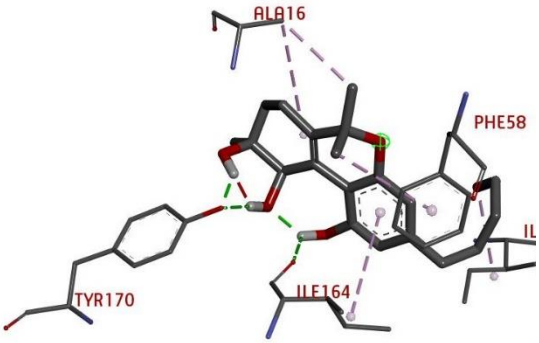
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