Research Article

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*Cannabis sativa L. chemical compositions as potential plasmodium falciparum dihydrofolate reductase-thymidinesynthase enzyme inhibitors: An in silico study for drug development

https://doi.org/10.1515/chem-2021-0102
received December 11, 2020; accepted November 2, 2021

Abstract: This study contributes to anti-malarial research effort by conducting in silico assessment of 125 compounds originated from Cannabis sativa L. against plasmodium falciparum dihydrofolate reductase-thymidinesynthase (pfDHFR-TS) enzyme for potential inhibition activity. Drug-like and pharmacokinetic criteria were used to assess the drug-like properties of the studied compounds. AutoDock4.2.6 and AutoDock Vina software were used to calculate the possible binding pose of the studied compounds to pfDHFR-TS enzyme. The docking procedure was validated using two known inhibitors cycloguanil and WR99210. 65 out of 125 compounds violated no more than 2 of Lipinski’s rule of five and were sorted out as favorable for drug development. Amongst these 65 compounds, pharmacokinetic properties and toxicity evaluation identified 60 compounds that meet the criteria of drug-like properties and were subjected to further docking studies. Docking outcomes identified 10 compounds including compounds 4, 9, 19, 22, 23, 25, 30, 42, 43, and 59 as potential candidates for inhibiting the function of pfDHFR-TS at the active site through hydrogen bonds with Ile14, Asp54, and Ile 164 residues. Compound 9 is considered as the top “hit” with docking energy far more exceeding those of the standard compounds. High correlation coefficient between the docking energy of AutoDock4.2.6 and AutoDock Vina was recorded with the value of $R^2 = 0.74$.

Keywords: virtual screening, molecular docking, pfDHFR-TS, anti-malarial compounds, Cannabis sativa L., Plasmodium falciparum

1 Introduction

Malaria represents a considerable threat to public health, not only in the past, but also in recent period where the disease was reported to be responsible for approximately 405,000 death worldwide [1,2]. The etiologies of malaria is explored due to the development of protozoan parasite of the genus plasmodium in erythrocytes through a bite of female anopheles mosquito [3]. According to literature studies, it is well recognized that Plasmodium falciparum and P. vivax (accounts for 99.7% of all cases in 2018) have developed resistance to nearly all the currently available antimalarial drugs, such as sulfadoxine/pyrimethamine,
mefloquine, halofantrine, and quinine, thus posing significant threat to malarial control and results in increased malarial morbidity and mortality.

Amongst current treatment methods, folate metabolism is known as one of the best targets for drug development [4]. It includes two important enzymes, dihydrofolate reductase-thymidinesynthase (DHFR-TS) and dihydropterotate synthase. The inhibition of these two enzymes might abrogate essential folate cofactors for DNA synthesis and metabolism of several amino acids [5]. Up to date, many antifolate drugs have been developed for the treatment; however, it is increasingly susceptible to the resistance in malaria parasite. Mutations occur in the active site of the two enzymes, directly affecting the binding ability of drugs and resulting in diminished long-term efficiency [6]. In addition, these types of drugs were reported to have several side effects on bone marrow, skin, and hair [7]. Due to the burden this disease causes to the health and social systems, more novel agents are still in urgent need, especially the one originated from natural sources with less side effects during treatment. Cannabis sativa L. has been well studied in terms of chemical composition with hundreds of compounds being identified so far and they are rich in cannabinoids, flavonoids, sophoroside, etc. Amongst them, several flavonoids were reported in previous studies for potential antimalarial activity [8,9], thus, suggesting this species is an interesting natural source for further investigation. In this study, a set of 125 compounds from Cannabis sativa L. were assessed for drug-like and pharmacokinetic properties and then, molecular docking study was conducted against enzyme pfDHFR-TS to find potential inhibitors for further drug development.

2 Experimental methods

2.1 Protein and ligand preparation

Among 14 structures of enzyme pfDHFR-TS available from Protein Data Bank (RCSB PDB), the best structure with PDB ID: 1J3I was selected for research with a resolution of 2.33 Å [10,11]. The protein structure was prepared using the Graphical User Interface program named AutoDock Tools to produce accurate representation of amino acid residues in terms of ionization and tautomeric states [12]. Procedures of the protein preparation process included removal of water molecules, addition of polar hydrogen atoms, and assignment of Kollman united atom partial charges and salvation parameters. Obtained atomic coordinates of the protein were then exported into a PDBQT file which will be used for execution of AutoGrid and AutoDock.

The chemical structure of 125 compounds isolated from Cannabis Sativa L. were collected from published literature [8,9,13] (Figure S1). Chemical structures of these compounds were visualized using Marvin software. The 3D structure of the compounds were built using Pymol 2.2.2 [14]. The energy minimization was carried out using Gabedit 2.5.0 [15]. Open bioactivity prediction online server Molinspiration and ProTox-II were utilized to evaluate the drug-like properties and the acute toxicity of all the research compounds.

2.1.1 Docking using AutoDock4.2.6

A computer equipped with Intel® Core™ i7-9700K CPU @ 3.60 GHz, with 32 GB DDR4 RAM, was utilized to perform docking runs. Docking results were analyzed by different packages including PyMOL [14], Discovery Studio Visualizer [16], LigPlus [17], and Maestro [18]. Distances of hydrogen bonds between the hydrogen and its assumed binding partner were calculated based on the analyzed result.

Compilation of AutoDock4.2.6 and docking was performed under Ubuntu-Linux 14.04.6 LTS operating system [11]. The grid box that encloses amino acid domains had dimensions of $62 \times 68 \times 72$ Å ($x \times y \times z$) with grid spacing of 0.375 Å. AutoGrid and AutoDock was used to calculate the pre-calculated binding affinity of each ligand’s atom type and to perform molecular docking simulation, respectively. The parameters of the Lamarckian genetic algorithm 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 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.

2.1.2 Docking using AutoDock Vina

AutoDock Vina was employed to perform molecular docking with global searching exhaustiveness of 400. Difference in energy between the worst and best docking modes was allowed to vary up to 7 kcal/mol. The grid center was selected such that it incorporates the amino acids domain involved in binding with standard ligand. The grid size was set to $15 \times 19 \times 14$ Å$^3$, which is large enough to cover the entire target active site [19].
3 Results and discussion

3.1 Validation docking

Two co-crystallized ligand cycloguanil and WR99210 redocked as references with pfDHFR-TS resulted in dock score of $-9.32$ and $-9.92$ kcal/mol, respectively. These two inhibitors were both shown to form hydrogen bonds with Ile14, Asp54, and Ile164, as indicated previously in the literature [20] (Figure 1).

Obtained results show that these inhibitors share the common residues interaction similar to previously published studies, suggesting that the procedure and the set parameters were suitable for docking simulation and are reproducible.

3.2 Drug-like and pharmacokinetic properties assessment

As part of the in silico screening, the drug-like properties of 125 compounds were assessed by subjecting them to Lipinski’s rule of five (Table S1). This rule includes criteria that determine which compound is considered to be drug-like in nature, such as molecular weight (MW) < 500 Da, number of hydrogen bond donors (HBD) ≤5, number of hydrogen bond acceptors (HBA) ≤10, octanol-water partition coefficient (log $P$) < 5, and molar refractivity (MR) value between 40–130. Compounds that satisfy these rules would be considered to be drug-like in nature. The obtained results help in providing essential information regarding the development and discovery of new drugs.

The outcomes indicate that amongst the studied molecules, 65 candidates were sorted out as favorable for drug development (Table 1). These compounds were then further evaluated for pharmacokinetic properties and toxicity prediction using Molinspiration and ProTox-II cheminformatic server (Table S2) [21]. In general, the studied compounds showed interesting results regarding calculated toxicity. From Table S2, compounds 5, 54, and 60 were classified as non-toxic with very high LD$_{50}$ (6,000, 10,000 and 13,500 mg/kg, respectively). Ten compounds were positioned at rank 5 and could be considered as safe. Forty-five compounds were classified as compounds with low toxicity (rank 4) which is equivalent to the toxic prediction of cycloguanil and WR99210. On the other hand, it is observed that three compounds 5, 17, and 63 had their milog $P$ value lain in minus values ($-0.9$, $-1.99$, and $-4.3$, respectively) which suggest their inability to bind with pfDHFR-TS enzyme. In addition, the enzyme inhibitory potential value of compounds 29 and 62 were −0.14 and −0.32, respectively, indicating that these compounds are not likely to exhibit inhibition activity toward target enzyme. Thus, these five ligands were excluded and

![Figure 1: Hydrogen bonding patterns of cycloguanil and WR99210 inhibitors with enzyme pfDHFR-TS (PDB ID: 1J3J). (a) Cycloguanil and (b) WR99210.](image-url)
Table 1: List of compounds with drug-like properties satisfying with Lipinski's rule

| ID  | Compound name                                      | MW  | HBD | HBA | log P  | MR    |
|-----|---------------------------------------------------|-----|-----|-----|--------|-------|
| 1   | (1’S)-Hydroxycannabinol                           | 342 | 3   | 4   | 4.69   | 97.15 |
| 2   | 4,5-Dihydroxy-2,3,6-trimethoxy-9,10-dihydrophenanthrene | 302 | 2   | 5   | 2.66   | 81.34 |
| 3   | 4,7-Dimethoxy-1,2,5-trihydroxyphenanthrene        | 286 | 3   | 5   | 2.74   | 77.97 |
| 4   | 8,9-Dihydroxy-delta6α,10α-Tetrahydrocannabinol    | 362 | 4   | 5   | 2.93   | 100.22|
| 5   | Uracil                                            | 112 | 2   | 4   | -0.66  | 25.81 |
| 6   | 5’-Methyl-4-pentylphenyl-2,6,2’-triol             | 286 | 3   | 3   | 4.51   | 84.84 |
| 7   | 6-Prenylapigenin                                  | 338 | 3   | 5   | 3.93   | 93.95 |
| 8   | 7-Methoxycannabiniprone                           | 260 | 0   | 3   | 3.03   | 73.02 |
| 9   | 7-oxo-9α-Hydroxyhexahydrocannabinol               | 346 | 2   | 4   | 4.11   | 97.14 |
| 10  | 8,9-Dihydroxy-delta-6α-tetrahydrocannabinol      | 346 | 3   | 4   | 3.96   | 98.83 |
| 11  | 8-Hydroxycannabinol                               | 298 | 2   | 3   | 4.42   | 86.66 |
| 12  | 8-Hydroxycannabinolic acid A                     | 326 | 2   | 4   | 4.23   | 92.05 |
| 13  | 8-oxo-Delta9-tetrahydrocannabinol                 | 328 | 1   | 3   | 4.91   | 95.65 |
| 14  | Tetrahydrocannabinabirin                          | 286 | 1   | 2   | 4.96   | 86.03 |
| 15  | Secoisolariciresin                                | 362 | 4   | 6   | 2.12   | 97.88 |
| 16  | Quercetin                                         | 304 | 5   | 7   | 1.19   | 73.25 |
| 17  | Quebrachitol                                      | 194 | 5   | 6   | -3.18  | 40.83 |
| 18  | 9,10-Dihydro-2,3,5,6-tetramethoxyphenanthrene-1,4-dione | 330 | 0   | 6   | 1.83   | 84.91 |
| 19  | 9α-Hydroxy-10-oxo-deltaα,10α-tetrahydrocannabinol | 344 | 2   | 4   | 4.16   | 97.83 |
| 20  | 9β,10β-Epoxyhexahydrocannabinol                   | 330 | 1   | 3   | 4.95   | 94.79 |
| 21  | 9a-Hydroxyhexahydrocannabinol                     | 332 | 2   | 3   | 4.93   | 96.75 |
| 22  | 10α-Hydroxy-10-oxo-delta8-tetrahydrocannabinol   | 344 | 2   | 4   | 4.02   | 96.81 |
| 23  | 10α-Hydroxy-delta9,11-hexahydrocannabinol        | 330 | 2   | 3   | 4.71   | 96.65 |
| 24  | Chrysin                                           | 254 | 2   | 4   | 2.71   | 69.15 |
| 25  | Cannabitetrol                                     | 362 | 4   | 5   | 2.93   | 99.27 |
| 26  | Cannabiripsol                                     | 348 | 3   | 4   | 3.90   | 98.14 |
| 27  | Pyrimethamine                                    | 248 | 4   | 4   | 2.52   | 70.68 |
| 28  | 8-Hydroxy-isohexahydrocannabivirin                | 298 | 2   | 3   | 4.78   | 89.36 |
| 29  | Mannitol                                          | 174 | 2   | 2   | 1.52   | 50.83 |
| 30  | Luteolin                                          | 286 | 4   | 6   | 2.13   | 72.48 |
| 31  | Chrysoeriol                                       | 300 | 3   | 6   | 2.43   | 77.37 |
| 32  | Lariciresin                                       | 360 | 3   | 6   | 2.65   | 95.78 |
| 33  | Isocannabspiradienone                             | 242 | 1   | 3   | 2.28   | 67.94 |
| 34  | Cannabinodivarin                                  | 282 | 1   | 2   | 4.76   | 86.65 |
| 35  | Cannabimovone                                     | 346 | 3   | 4   | 4.08   | 98.84 |
| 36  | Cannabicyclovarian                                | 286 | 1   | 2   | 4.89   | 83.34 |
| 37  | Kaempferol                                        | 286 | 4   | 6   | 2.31   | 72.39 |
| 38  | Cannabielsoin                                     | 330 | 2   | 3   | 4.71   | 96.65 |
| 39  | C3-Cannabielsoin                                  | 302 | 2   | 3   | 3.93   | 87.42 |
| 40  | Cannabielsoic acid B                              | 358 | 2   | 4   | 4.52   | 102.04|
| 41  | Cannabielsoic acid A                              | 358 | 2   | 4   | 4.52   | 102.04|
| 42  | 10α-Hydroxyhexahydrocannabinol                    | 332 | 2   | 3   | 4.79   | 96.68 |
| 43  | 10αR-Hydroxyhexahydrocannabinol                   | 332 | 2   | 3   | 4.79   | 96.68 |
| 44  | 10-Ethoxy-9-hydroxy-delta-6α-tetrahydrocannabinol| 374 | 2   | 4   | 5.00   | 108.23|
| 45  | Cannabichromanones B                              | 362 | 2   | 5   | 3.82   | 99.79 |
| 46  | Cannabichromanones C                              | 344 | 0   | 4   | 4.33   | 97.12 |
| 47  | Cannabielsoic acid B-C3                           | 330 | 2   | 4   | 3.74   | 92.81 |
| 48  | Cannabidivarinic acid                            | 314 | 2   | 3   | 4.88   | 93.19 |
| 49  | Cannabielsoin                                     | 330 | 2   | 3   | 4.71   | 96.65 |
| 50  | Cannabielsoin acid A                              | 330 | 3   | 4   | 4.76   | 94.76 |
| 51  | Cannabidiocol                                     | 258 | 2   | 2   | 4.42   | 78.54 |
| 52  | Cannabichromanone-C5                             | 332 | 1   | 4   | 4.46   | 93.78 |
| 53  | Cannabichromanone-C7                             | 304 | 1   | 4   | 3.68   | 84.54 |
| 54  | Catechin                                         | 290 | 5   | 6   | 1.55   | 72.62 |
| 55  | Cannabigerovarinic acid                          | 332 | 3   | 4   | 4.98   | 96.85 |
| 56  | Carmagerol                                        | 350 | 4   | 4   | 4.23   | 102.00|
only 60 amongst 65 compounds possess pharmacokinetic properties and toxic ranking suitable for further docking studies.

3.3 Docking studies

To explore the inhibition potential of 60 selected ligands with enzyme pfDHFR-TS model, AutoDock4.2.6 and AutoDock Vina were utilized for docking studies. Table 2 exhibits the dock score of the studied compounds.

| ID | Compound name                                                                 | MW  | HBD | HBA | log P | MR     | AutoDock4.2.6 | AutoDock Vina |
|----|-------------------------------------------------------------------------------|-----|-----|-----|-------|--------|---------------|---------------|
| 4  | 8,9-Dihydroxy-delta6a,10a-tetrahydrocannabinol                                | 268 | 1   | 2   | 4.56  | 81.31  | −8.32         | −8.50         |
| 9  | 7-oxo-9a-Hydroxyhexahydrocannabinol                                          | 254 | 1   | 2   | 4.30  | 76.67  | −8.91         | −9.10         |
| 13 | 8-oxo-Delta9-tetrahydrocannabinol                                            | 346 | 3   | 4   | 3.96  | 98.83  | −8.77         | −9.00         |
| 19 | 9a-Hydroxy-10-oxo-delta6a,10a-tetrahydrocannabinol                           | 346 | 3   | 4   | 3.96  | 98.83  | −9.18         | −9.40         |
| 21 | 10a-Hydroxy-10-oxo-delta8-tetrahydrocannabinol                               | 346 | 3   | 4   | 3.96  | 98.83  | −8.77         | −9.00         |
| 22 | 10a-Hydroxy-delta9,11-hexahydrocannabinol                                    | 346 | 3   | 4   | 3.96  | 98.83  | −9.18         | −9.50         |
| 25 | Cannabitetrol                                                                | 282 | 1   | 2   | 4.95  | 85.93  | −7.77         | −8.91         |
| 28 | 8-Hydroxy-isohexahydrocannabinivirin                                         | 282 | 1   | 2   | 4.95  | 85.93  | −7.77         | −8.91         |
| 30 | Luteolin                                                                     | 30  | 0   | 3   | 5.08  | 91.20  | −7.77         | −8.91         |
| 41 | Cannabielsoic acid A                                                         | 30  | 0   | 3   | 5.08  | 91.20  | −7.77         | −8.91         |
| 42 | 10a-Hydroxyhexahydrocannabinol                                               | 30  | 0   | 3   | 5.08  | 91.20  | −7.77         | −8.91         |
| 43 | 10aR-Hydroxyhexahydrocannabinol                                              | 30  | 0   | 3   | 5.08  | 91.20  | −7.77         | −8.91         |
| 45 | Cannabichromanones B                                                         | 30  | 0   | 3   | 5.08  | 91.20  | −7.77         | −8.91         |
| 59 | Cannabidriol                                                                 | 30  | 0   | 3   | 5.08  | 91.20  | −7.77         | −8.91         |
| 61 | 10-oxo-Delta6a,10a-tetrahydrocannabinol                                      | 30  | 0   | 3   | 5.08  | 91.20  | −7.77         | −8.91         |
| 65 | Cannabichromanones D                                                         | 30  | 0   | 3   | 5.08  | 91.20  | −7.77         | −8.91         |

The bold rows indicate data for standard ligands.

Cycloguanil and WR99210 were used as reference inhibitors with dock score obtained from AutoDock4.2.6 being −7.77 and −8.91 kcal/mol and dock score of AutoDock Vina being −8.50 and −9.10 kcal/mol, respectively. Thus, ligand has docking energy fall within these range or more negative would be considered as potential inhibitor of pfDHFR-TS. In general, according to the obtained results, 16 out of 60 screened compounds were identified as potential inhibitors (Table 2). Compounds 9 and 61 are the top two ligands with docking energy far more exceeding those of standard compounds. The rest 14 hits presented dock
scores that matched the selection criteria and had dock score ranging from $-7.85$ to $-9.06$ kcal/mol, and from $-8.5$ to $-9.4$ kcal/mol, obtained from AutoDock and 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 $R^2 = 0.74$ (Figure 2) that supports for the accuracy of the docking study.

The potential hits were further analyzed for ligand efficiency (LE) and binding poses (Table 3). LE is a useful metric for the selection of lead compounds in drug discovery and has been widely used as a measurement for the binding energy of the ligand per atom, which is calculated according to the equation (1):

$$\Delta g = \frac{\Delta G}{N_{\text{non-hydrogen atoms}}}.$$

where $\Delta g$ is the ligand efficiency and $\Delta G$ is the docking energy.

Statistically, compounds with LE varying within $0.3 < \text{LE} < 0.5$ are more potential for further optimization. Calculated LE of 16 hit compounds in this study ranged from $-0.39$ to $-0.47$, suggesting their considerable potential for drug development.

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 in Table 3, although having better docking energy than standard cycloguanil and WR99210, compounds 13, 28, 45, 61, and 65 did not form hydrogen bonds with any of three key residues, therefore, these ligands were assumed as non-specific for pDHFR-TS inhibition potential. Regarding the toxicity prediction (Table 2), compound 41 was excluded due to high toxicity (LD$_{50}$ value of 3 mg/kg). Compounds 30 and 61 were highlighted for their safe class of toxicity (LD$_{50}$ value of 3,919 and 2,647 mg/kg, respectively) (Table S1). The hydrogen bonding patterns and stereo view of binding mode of ten potential pDHFR-TS inhibition compounds are shown in Figures S2 and S3.

### Table 3: The LE and hydrogen bond interactions between 16 potential compounds and pDHFR-TS

| ID | Compound name                                      | LE    | No. of H-bonds | Interacting residues         |
|----|---------------------------------------------------|-------|----------------|------------------------------|
| 4  | 8,9-Dihydroxy-delta6a,10a-tetrahydrocannabinol    | $-0.41$ | 5              | Ala16; Tyr170; Ile164        |
| 9  | 7-oxo-9a-Hydroxyhexahydrocannabinol               | $-0.39$ | 3              | Ser111; Ile164               |
| 13 | 8-oxo-Delta9-tetrahydrocannabinol                 | $-0.39$ | 1              | Ala16                        |
| 19 | 9a-Hydroxy-10-oxo-delta6a,10e-tetrahydrocannabinol| $-0.42$ | 3              | Ser108; Ile14; Tyr170        |
| 22 | 10aa-Hydroxy-10-oxo-delta8-tetrahydrocannabinol   | $-0.42$ | 3              | Ser108; Ser111; Ile164       |
| 23 | 10o-Hydroxy-delta9,11-hexahydrocannabinol         | $-0.42$ | 3              | Ala16; Asp54; Tyr170         |
| 25 | Cannabitetrol                                     | $-0.43$ | 5              | Ile164; Tyr170               |
| 28 | 8-Hydroxy-isohexahydrocannabinivirin              | $-0.47$ | 3              | Tyr170; Ala16                |
| 30 | Luteolin                                          | $-0.46$ | 3              | Val145; Asp54                |
| 41 | Cannabielsoic acid A                              | $-0.41$ | 3              | Ala16; Leu40; Ile164         |
| 42 | 10o-Hydroxyhexahydrocannabinol                    | $-0.45$ | 1              | Ile164                       |
| 43 | 10oR-Hydroxyhexahydrocannabinol                   | $-0.43$ | 3              | Tyr170; Ile164               |
| 45 | Cannabichromanones B                              | $-0.40$ | 4              | Thr107; Ser108; Ser167       |
| 59 | Cannabinol                                       | $-0.45$ | 3              | Tyr170; Ile164               |
| 61 | 10-oxo-Delta6a,10e-tetrahydrocannabinol           | $-0.43$ | 2              | Tyr170                       |
| 65 | Cannabichromanones D                              | $-0.41$ | 1              | Ser167                       |
| 66 | Cycloguanil                                       | $-0.45$ | 5              | Ile164; Ile14; Cys15; Thr185; Asp54 |
|    | WR99210                                           | $-0.38$ | 5              | Cys15; Asp54; Ile14; Ile164   |

The bold rows indicate data for standard ligands.
4 Conclusion

In this study, 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. Among 125 studied compounds, 10 compounds including compounds 4, 9, 19, 22, 23, 25, 30, 42, 43, and 59 were identified as potential candidates for inhibiting the function of pfDHFR-TS at the active site through hydrogen bonds with Ile14, Asp54, and Ile 164 residues. Compound 9 is considered as the top “hit” regarding binding affinity to target enzyme and drug-like properties. The LE value of these compounds ranged from −0.39 to −0.47, suggesting their promising opportunities for further optimization in drug development. These findings shed light on the potential anti-malarial activity of compounds isolated from Cannabis sativa L.

Acknowledgements: The authors are thankful to Prof. Pham Quoc Long and Prof. Pham Thi Hong Minh for further optimization in drug development. These compounds isolated from Cannabis sativa L. on enzyme pfDHFR-TS enzyme inhibitors from Cannabis sativa L.

Author contributions: P.M.Q. and H.H.P.T. conceived and designed the study. P.H.N., N.T.K., N.X.H., and D.T.T.L. performed data collection and data analysis. L.T.T.H., T.Q.T., and N.P.H. performed drug-like and pharmacokinetic properties assessment. P.M.Q., T.N.T.A., P.L.S., and H.H.P.T. performed docking studies and wrote the manuscript. All authors have read and approved the final version of the manuscript.

Conflict of interest: The authors declare no conflict of interest.

Ethical approval: The conducted research is not related to either human or animal use.

Data availability statement: All data generated or analyzed during this study are included in this published article.

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