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Capsaicinoids from *Capsicum annuum* as an Alternative FabH Inhibitor of *Mycobacterium Tuberculosis*: *In Silico* Study

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Abstract

The number of tuberculosis (TB) cases worldwide reached 1.5 million in 2018; thus, TB is considered a deadly disease. TB is caused by *Mycobacterium tuberculosis* and involves lipid synthesis. Considering the importance of lipid metabolism in bacteria, FabH may be an essential protein target for repressing lipid synthesis. Capsaicinoids from *Capsicum annuum* demonstrate potent antibacterial activity. This study predicted the ability of capsaicinoid compounds to inhibit FabH. *In silico* analysis was performed by retrieving the structure of FabH from PDB and those of selected capsaicinoid derivatives from PubChem. The compounds were docked using AutoDock Vina in PyRx 0.8 software. The interactions of FabH and different capsaicinoid derivatives showed identical binding characteristics. The bonding type most frequently observed was hydrogen bonds. In conclusion, capsaicinoid derivatives could block lipid synthesis through FabH. The relevant mechanism and biological processes should be studied further.

Keywords: capsaicinoids, FabH, in silico, tuberculosis

Introduction

Tuberculosis (TB) is an infectious disease that had previously reached epidemic levels and then receded. While its behavior is similar to those of other infectious diseases, TB has killed more people than other diseases [1]. According to the World Health Organization, up to 1.5 million deaths due to TB were recorded in 2018. In the same year, the number of TB cases in Indonesia reached 1,020,000, which is the second of the world's highest cases [2]. TB is caused by the facultative pathogenic bacteria *Mycobacterium tuberculosis*. This bacteria has a four-layer structure, including an inner plasma membrane, a peptidoglycan–arabinogalactan layer, an outer membrane, and a capsule [3]. The inner and outer membranes of *M. tuberculosis* are constructed from a lipid bilayer and affected by the lipid synthesis of the bacterium [4]. The lipid synthesis of TB-causing bacteria plays a vital role in the bacterium's infection route, treatment, and therapy. Sulfolipids secreted by *M. tuberculosis* stimulate human neurons and cause cough [5].

The mechanism of lipid synthesis in microbes includes two processes, namely, initiation and elongation. During initiation, acetyl CoA, as a lipid precursor, is converted into malonyl-CoA under the catalysis of FabD to produce malonyl-ACP. Then, malonyl-CoA is condensed with 2-methylbutyryl CoA by FabH to produce β-ketoacyl ACP. During elongation, β-ketoacyl ACP is utilized to obtain Acyl ACP as a precursor for phosphatidic acid formation [6–8]. In general, the enzyme of the Fab family (β-ketoacyl-ACP synthase III) is the key to lipid synthesis in microbes [4, 9, 10]. Lipid synthesis in bacteria is considered the primary target in lipid degradation. Thus, some studies have tested the antibacterial activity of drugs and bioactive compounds via the lipid synthesis mechanism [8, 11, 12].

A previous study reported that some antibiotics, such as andrimid, moiramide B, platencin, platenmycin, thiolactomycin, triclosan, and AFN-1252, inhibit the lipid synthesis of microbes [11]. However, long-term consumption of antibiotics may lead to negative effects [13, 14]. Bioactive compounds from natural herbs have been proposed as possible treatment alternatives to minimize antibiotic consumption [6, 10, 15]. Chili (*Capsicum annuum*), which contains capsaicin as a main bioactive compound, has shown potential antibacterial [16, 17], antioxidant [18–20] anti-inflammatory [21],

and anti-virulence [17] activity. Different varieties of capsicum in Indonesia show different capsaicinoid contents [18, 19, 22, 23]. Capsaicinoids, including capsaicin, homocapsaicin, dihydrocapsaicin, norcapsaicin, and novamide, perform a number of biological functions [24, 25]. The antibacterial activity of capsaicin has been reported in *in vivo* and *in vitro* studies, but the mechanism of this compound in microbes at the cellular level remains unclear. Fatty acid synthesis has been reportedly as an alternative mechanism for antibacterial activity. One of the enzyme that catalyze in fatty acid synthesis for bacterial membrane formation is 3-ketoacyl-acyl carrier protein (ACP) synthase III (FabH) [10]. Capsaicinoids with the long carbon, also was generated from fatty acid group might inhibited FabH properly. Hence, this study aims to assess the antibacterial activity of capsaicin and its derivatives through an *in silico* study of the bioactive compounds with FabH of *M. tuberculosis*.

Methods

Protein structure retrieval and preparation. The 3D structures of the proteins and ligands were retrieved from RSCB PDB and PubChem, respectively. The protein structure of FabH of *M. tuberculosis* (2QO1) (http://www.rcsb.org/structure/2QO1) was prepared for docking using Discovery Studio Client 3.5. The structures of capsaicin (CID1548943), dihydrocapsaicin (CID107982), homocapsaicin (CID6442566), homodihydrocapsaicin (CID3084336), nonivamide (CID2998), nordihydrocapsaicin (CID168836), and nornordihydrocapsaicin (CID25200611) were downloaded from PubChem (https://pubchem.ncbi.nlm.nih.gov/) as SDF files. All compounds were energy-minimized and converted into PDB files by using PyRX 0.8 software [26].

Molecular docking. The FabH protein and capsaicinoids were docked using AutoDock Vina in PyRx 0.8 to determine their binding energy and interactions. The grid center of FabH protein was located at X = 21.25, Y = 35.42, Z = 29.27, and the dimensions of the grid were X = 71.01, Y = 66.86, and Z = 67.46.11-(Decyldithiocarbonyloxy)-undecanoic acid or 11-[(mercaptocarbonyl) oxy]undecanoic acid (CID 24778468), as a native ligand for FabH protein, were also docked with the protein to verify the capsaicinoid–FabH interactions. 3D and 2D visualization of the protein–ligand interactions was performed using Discovery Studio Client 3.5 [27, 28].

Bioactivity prediction. The SMILES of capsaicin, dihydrocapsaicin, homocapsaicin, homodihydrocapsaicin, nordihydrocapsaicin, nornordihydrocapsaicin, and nonivamide were retrieved from PubChem. The physicochemical properties and biological functions of the capsaicinoids were predicted using SuperPred and PASSOnline, respectively [29]. The toxicity of the capsaicinoids was also predicted by the ADMET online server at PKCSM (http://biosig.unimelb.edu.au/pkcsm/) [30]. The biological activities of the compounds, including their antibacterial and lipid synthesis inhibitory activities, were illustrated using a heatmap generated by the Heatmapper.ca webserver (http://heatmapper.ca/) with average linkage and Pearson similarity as parameters [31, 32].

Results and Discussion

Molecular interactions of compounds from capsicum with FabH of *mycobacterium tuberculosis*. A total of seven bioactive compounds from *Capsicum annuum* were docked to the FabH of *M. tuberculosis*, and the results are shown in Figure 1. Interestingly, all seven bioactive compounds of *C. annuum* targeted similar binding sites in the FabH protein. The capsaicinoids and FabH mainly interacted via the formation of hydrogen and electrostatic bonds with the same amino acid residue. Most of the *C. annuum* constituents bound to the active sites of FabH, such as SER190, THR80, GLN86, LEU85, and ASP107. Nornordihydrocapsaicin interacted with ASN81 of FabH. All of the capsaicinoid–FabH complexes revealed similar binding affinity. Thus, we assume that all *C. annuum* constituents selected in this study show similar binding strength. This finding contrasts the binding results of 11-[(mercaptocarbonyl) oxy] undecanoic acid and FabH protein. The binding affinity of 11-[(mercaptocarbonyl)oxy]undecanoic acid to FabH was greater than that observed in the capsaicinoid–FabH complexes (Figure 1; Table 1). The higher binding affinity of the capsaicinoids compared with that of the native ligand of FabH indicates that the former produce tighter interactions.

The results presented in Figure 1a–g show similar binding patterns. Van der Waals interactions could stabilize protein–ligand bonds. Among the interactions studied, the binding of capsaicin-FabH (Figure 1a, Table 1) and homocapsaicin–FabH (Figure 1c, Table 1) showed the lowest binding affinity (−6.7 kcal/mol) and mainly involved hydrogen bonds, which are fairly strong. The data suggest that capsaicin and its derivatives could potentially inhibit FabH. The similar active sites of capsaicinoids – FabH complexes indicated that capsaicinoid derivate compounds have similar inhibition activity of FabH.

Physicochemical, pharmacokinetic, and biological properties of capsaicinoids. The predicted physicochemical properties of capsaicinoids from *C. annuum* are shown in Figure 2a. Capsaicin and its derivatives do not satisfy Lipinski's rule, which performed molecular weight are more than 500, hydrogen acceptor more than 5, and hydrogen donor is less than 10. The data indicated that capsaicin and its derivatives may not be appropriate as main drugs but could be used as supplements.

Figure 2b presents the predicted biological functions of capsaicinoids as antibacterials and lipid synthesis inhibitors. The heatmap of the physiological functions of these compounds indicated that the latter could be classified into three groups. The first group includes capsaicin and homocapsaicin, both of which demonstrated excellent antibacterial and lipid peroxidase and CFAP synthase inhibitory activities. Nordihydrocapsaicin, dihydrocapsaicin, nornordihydrocapsaicin, and homodihydrocapsaicin form the second group. Nonivamide has a structure that differs from those of the other capsaicinoids and, thus, may be classified as an out-group. These results suggest that the

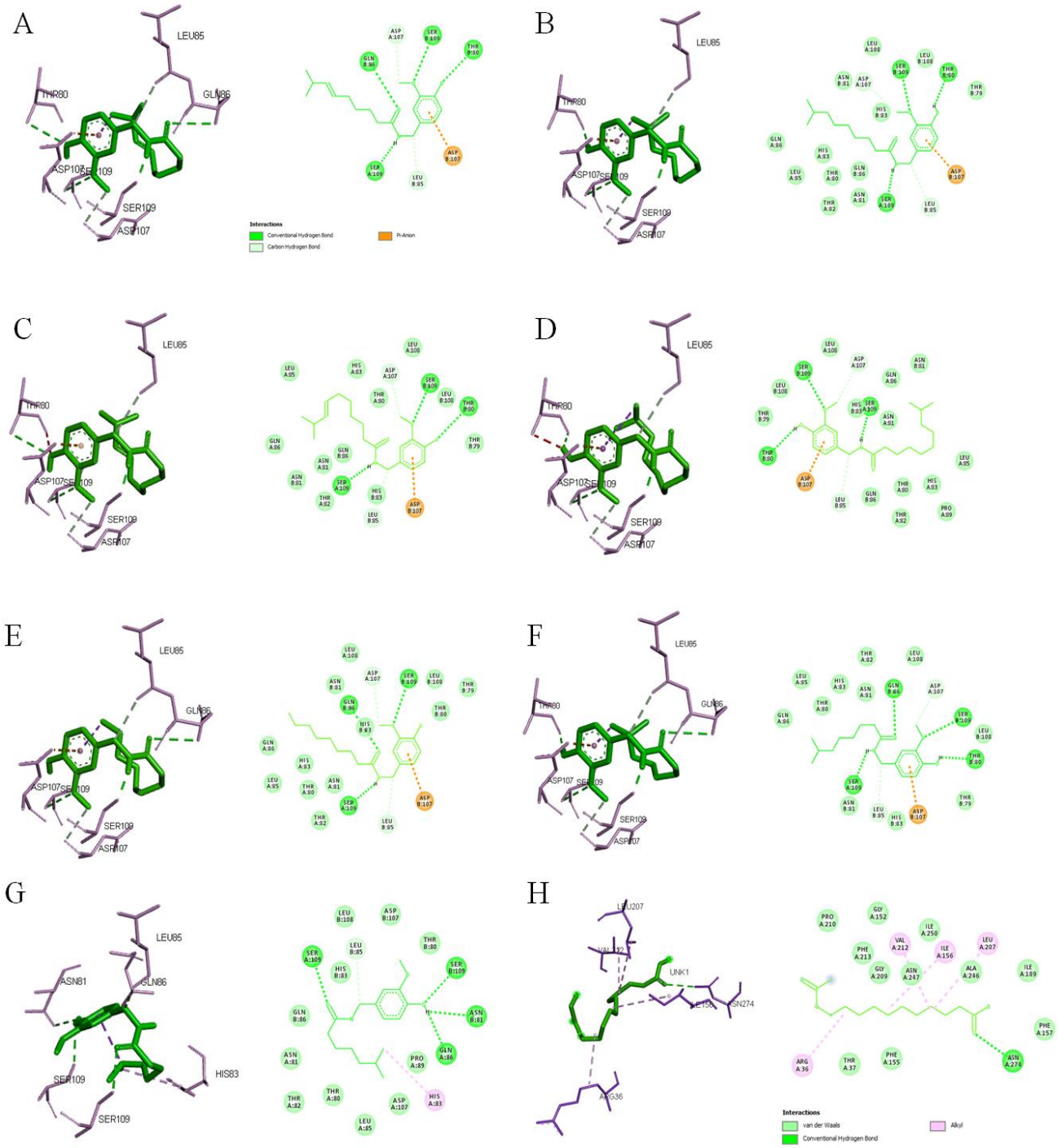

Figure 1. 3D and 2D Visualization of the Molecular Interactions of Capsaicinoids from Capsicum Annuum. (A) Capsaicin, (B) Dihydrocapsaicin, (C) Homocapsaicin, (D) Homodihydrocapsaicin, (E) Nonivamide, (F) Nordihydrocapsaicin, (G) Nornordihydrocapsaicin, and (H) 11-[(Mercaptocarbonyl)Oxy]Undecanoic Acid

Table 1. Interactions Between Capsaicinoid Compounds from Capsicum Annuum and 11-[(Mercaptocarbonyl)Oxy] Undecanoic Acid and Fabh Protein

Compounds	Amino acid	Category	Energy (kcal/mol)
11-(Decyldithiocarbonyloxy)-undecanoic acid	ASN274 ILE156, LEU207, VAL212, ARG35	Hydrogen Bond Hydrophobic	−4.7
Capsaicin	SER109, THR80, GLN86, LEU85, ASP107 ASP107	Hydrogen Bond Electrostatic	−6.7
Dihydrocapsaicin	SER109, THR80, LEU85, ASP107 ASP107	Hydrogen Bond Electrostatic	−6.5
Homocapsaicin	SER109, THR80, LEU85, ASP107 ASP107	Hydrogen Bond Electrostatic	−6.7
Homodihydrocapsaicin	SER109, THR80, LEU85, ASP107 ASP107	Hydrogen Bond Electrostatic	−6.6
Nonivamide	SER109, GLN86, LEU85, ASP107 ASP107	Hydrogen Bond Electrostatic	−6.3
Nordihydrocapsaicin	SER109, THR80, GLN86, LEU85, ASP107 ASP107	Hydrogen Bond Electrostatic	−6.3
Nornordihydrocapsaicin	GLN86, ASN81, SER109, LEU85 HIS83	Hydrogen Bond Hydrophobic	−6.1

classification of capsaicinoids is associated with their structure. Most of the capsaicinoids were hydrophobic compounds that could easily cross the cell membrane. The capsaicinoid structure consists of an aromatic ring, an amide bond, and hydrophobic side chains. The structure of capsaicinoid and its derivatives is differentiated based on the number of long carbon chains, 9 - 11 carbons with the number of double bonds located at different positions along the chain [24].

The pharmacokinetic properties of capsaicinoids contribute to these compounds' abilities for absorption, distribution, metabolism, excretion, and toxicity (ADMET). ADMET prediction is essential in drug design, especially that of drugs from natural bioactive compounds, to predict and screen the physiological effect of a compound [30]. The water solubility score of the capsaicinoids ranged from −3.52 log mol/L to −4.709 log mol/L. The Caco-2 score of the capsaicinoids was greater than 1. A Caco-2 permeability greater than 1.0 indicates high permeability in the Caco-2 cell line. The capsaicinoids also showed high (>80%) intestinal absorption. The test compounds showed moderate skin permeability with a standard logKp < −2.5. VDss values or the steady-state distribution volume of the capsaicinoids was fairly high (>2.8 L/kg). The capsaicinoids showed low unbound fraction values ranging from 0.125 to 0.233, while the blood brain barrier permeability of capsainoids compound demonstrated low value that were –0.31 until –0,168. The test compounds appeared to be unable to penetrate the central nervous system. Cytochrome p450 is an essential detoxification enzyme in the human body that could deactivate some drugs. CYP1A2, CYP2C19, CYP2C9, CYP2D6, and CYP3A4 are isoforms of cytochrome p450. Renal OCT2 substrate predicted the ability of compounds as substrate of renal organic cation transporter 2, identifying capsaicinoids were not a proper OCT2 substrate. The capsaicinoids could be excreted by the renal and hepatic routes. Toxicity tests indicated that the capsaicinoids are not mutagenic. The maximum allowable dose of capsaicinoids in humans is 0.46–0.552 log (mg/kg/day). Capsaicin, dihydro-capsaicin, homocapsaicin, and homodihydrocapsaicin are toxic to hepatocytes, but the other capsaicinoids do not show such toxicity. Homodihydrocapsaicin may lead to allergic contact dermatitis. All capsaicinoids showed toxicity to *T. pyriformis* and flathead minnows (Table 2).

Tuberculosis is a major health issue in some countries because the bacteria is resistant to antibiotics. Some studies have reported the development of resistance in TB bacteria to most of the frontline drugs used for TB treatment [10]. Lipid synthesis and elongation played an essential role in peptidoglycan membrane formation, which was dominated by 3-ketoacyl-acyl carrier protein (ACP) synthase (Fab) activity [7, 8, 11]. The enzymes of the Fab family, especially FabH, are critical in the lipid synthesis of *M. tuberculosis*. Therefore, FabH enzymes may be useful targets for TB treatment and therapy [9, 33]. Further research on lipid degradation mechanisms should be performed at the molecular level through *in silico* studies.

A previous *in silico* study reported that CYS112 is the essential reactive site for blocking FabH. The binding of plant/drug constituents to CYS112 amino acid residues influences lipid degradation [34, 35]. According to the

2D models of capsaicinoids–FabH complexes, *C. annuum* compounds bind to different amino acid residues of FabH. Capsaicinoids could interact with native ligands through different sites, which indicates that these compounds inhibit FabH at other active sites. The results of this study suggest that, despite their non-specific binding to reactive sites, capsaicin and its derivatives inhibit FabH allosterically and may have antibacterial activity.

a

Properties	Compounds						
	Capsaicin	Dihydrocapsaicin	Homocapsaicin	Nordihydrocapsaicin	Nornordihydrocapsaicin	Homodihydrocapsaicin	Nonivamide
PubChem ID	1548943	107982	6442566	168836	25200611	3084336	2998
Formula	$C_{18}H_{27}NO_3$	$C_{18}H_{29}NO_3$	$C_{19}H_{29}NO_3$	$C_{17}H_{27}NO_3$	$C_{16}H_{25}NO_3$	$C_{19}H_{31}NO_3$	$C_{17}H_{27}NO_3$
Molweight	305.412	307.428	319.438	293.401	279.375	321.454	293.401
xlogP	4.180	4.405	4.571	4.014	3.624	4.795	4.158
Heavy Atoms	22	22	23	21	20	23	21
Rotatable Bonds	10	11	11	10	9	12	11
H-bond Donors	2	2	2	2	2	2	2
H-bond Acceptors	3	3	3	3	3	3	3
Bonds	22	22	23	21	20	23	21
Rings	1	1	1	1	1	1	1
Polar Surface Area	58.560	58.560	58.560	58.560	58.560	58.560	58.560
Total Charge	0	0	0	0	0	0	0

b

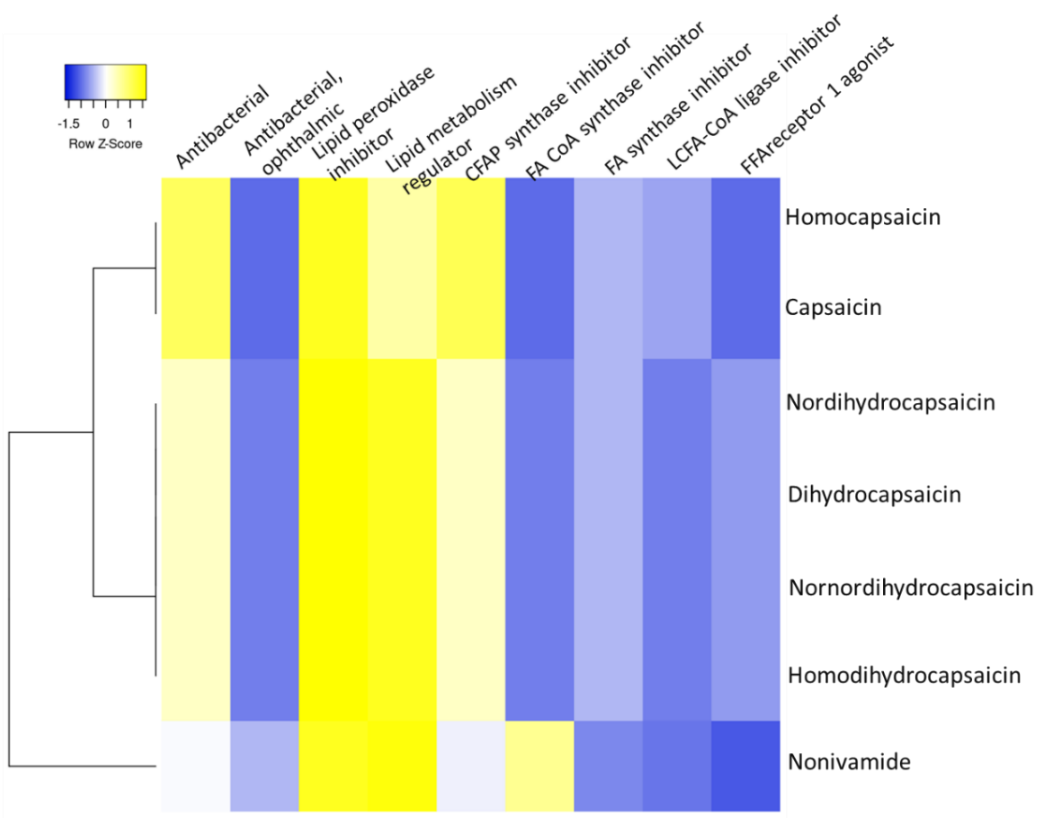

Figure 2. Physicochemical Properties and Predicted Biological Activities of Capsaicinoid and its Derivatives. (a) Physicochemical Characteristics of Capsaicinoid and its Derivatives. (b) Biological Activity and Phylogenetic Tree of Capsaicinoid and Its Derivatives. Compounds with Low Activity are Marked in Blue, While Those with High Activity are Marked in Yellow. The Color Intensity Indicates the Level of Biological Functions

Table 2. Pharmacokinetic Properties of the Capsaicinoids Selected in this Study

Parameter	Capsaicin	Dihydro capsaicin	Homo capsaicin	Homodihydro-capsaicin	Nonivamide	Nordihydro capsaicin	Nornordihydro-capsaicin
Water solubility (log mol/L)	−4.185	−4.321	−4.578	−4.709	−3.929	−3.919	−3.52
Caco2 permeability (log Papp in 10^{-6} cm/s)	1.364	1.375	1.382	1.392	1.369	1.357	1.338
Intestinal absorption (human) (% absorbed)	90.075	89.568	89.731	89.225	89.767	89.906	90.249
Skin permeability (lg Kp)	−2.825	−2.799	−2.795	−2.775	−2.818	−2.834	−2.883
P-glycoprotein substrate	Yes	Yes	Yes	Yes	Yes	Yes	Yes
P-glycoprotein I inhibitor	No	No	Yes	Yes	Yes	Yes	No
P-glycoprotein II inhibitor	No	No	No	No	No	No	No
VDss (human) (log L/kg)	0.391	0.435	0.425	0.468	0.453	0.393	0.341
Fraction unbound (human)	0.156	0.157	0.124	0.125	0.216	0.192	0.233
BBB permeability	−0.241	−0.268	−0.283	−0.31	−0.255	−0.225	−0.168
CNS permeability	−2.483	−2.535	−2.491	−2.544	−2.607	−2.525	−2.516
CYP2D6 substrate	No	No	No	No	No	No	No
CYP3A4 substrate	Yes	Yes	Yes	Yes	Yes	Yes	Yes
CYP1A2 inhibitor	Yes	Yes	Yes	Yes	Yes	Yes	Yes
CYP2C19 inhibitor	Yes	Yes	Yes	Yes	Yes	Yes	Yes
CYP2C9 inhibitor	Yes	Yes	Yes	Yes	Yes	Yes	No
CYP2D6 inhibitor	No	No	No	No	No	No	No
CYP3A4 inhibitor	No	No	No	No	No	No	No
Total clearance	1.298	1.245	1.327	1.275	1.37	1.215	1.185
Renal OCT2 substrate	No	No	No	No	No	No	No
AMES toxicity	No	No	No	No	No	No	No
Max. tolerated dose (human)	0.46	0.515	0.434	0.49	0.547	0.534	0.552
hERG I inhibitor	No	No	No	No	No	No	No
hERG II inhibitor	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Oral rat acute toxicity (LD50)	2.065	2.091	2.087	2.109	2.107	2.067	2.04
Oral rat chronic toxicity (LOAEL)	1.827	1.848	1.865	1.886	1.789	1.91	1.761
Hepatotoxicity	Yes	Yes	Yes	Yes	No	No	No
Skin sensitization	No	No	No	Yes	No	No	No
T. pyriformis toxicity	2.074	2.056	2.032	1.993	1.954	2.002	1.834
Minnow toxicity	0.44	0.312	0.164	0.036	0.596	0.568	0.781

Efficient ligand–protein binding is supported by various binding types and suitable binding energies [34, 35]. In this study, capsaicin and its derivatives mostly formed hydrogen and electrostatic bonds. Hydrogen bonds are important in protein–ligand binding because they improve binding stability. Electrostatic bonds could also help improve ligand binding efficiency [36]. Efficient ligand binding promotes the activity of *C. annuum* bioactive compounds. The antibacterial activity of capsaicin and its derivatives has been widely observed. Capsaicin performs its antibacterial action by damaging the cell membrane of bacteria. Capsaicin also shows anti-virulence activity, as evidenced by the high population of non-invasive bacterial strains of Group A streptococci after inoculation with capsaicin [17, 37]. The bioactive compounds of *C. annuum* could potentially inhibit bacterial activity [16, 17, 20]. This study demonstrates the promising application of capsaicinoids as a supplemental treatment for TB therapy. Administration of these substance may improve the immune system of TB patients and reduce the severity of the bacterial infection.

Conclusion

Capsaicinoids, including capsaicin, dihydrocapsaicin, homocapsaicin, homodihydrocapsaicin, nonivamide, nordihydrocapsaicin, and nornordihydrocapsaicin, demonstrated identical binding performance. The results of this study indicated that capsaicinoids from *C. annuum* could potentially inhibit FabH in *M. tuberculosis*. This study presents a new method to discover natural bioactive compounds with great application potential. Further study, such as molecular dynamic simulation, is required to assess the relevant mechanism and biological processes.

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