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Original Article

Anticancer potential of secondary metabolites of *Piper nigrum* against MOA B, AIF, CYP and EGFR proteins: *In silico* study

[Potencial anticancerígeno de los metabolitos secundarios de *Piper nigrum* frente a las proteínas MOA B, AIF, CYP y EGFR: Estudio *in silico*]

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Abstract

Context: Searching for novel anticancer-leading drugs is a current major concern for providing better treatment outcomes. *Piper nigrum* is one of the spice plants used as an anticancer in traditional medicine. Many studies reported the secondary metabolite content of *P. nigrum*. Some have been evaluated as anticancer agents, but many still need to be studied.

Aims: To evaluate the *in silico* anticancer activity of *P. nigrum* secondary metabolites.

Methods: Cheminformatics and bioinformatics analysis were used to investigate drug-likeness, toxicity, pharmacokinetics, and drug interaction against enzymes involved in reactive oxygen species production and cell growth.

Results: Ten out of twenty-five compounds qualify as drugs according to the rules of Lipinski, Veber, Egan, Ghose, and Muegge, and they met the pharmacokinetic parameters. A molecular docking study showed that seven compounds showed a good affinity for enzymes involved in triggering cellular oxidative stress and cell death. Furthermore, piperine, piperidine derivatives, and piperettin may inhibit the cancer cell's growth due to their affinity with epidermal growth factor receptor (EGFR) compared to erlotinib. Of all these compounds, (2E,4E,8E)-9-(benzo[d][1,3]dioxol-5-yl)-1-(piperidine-1-yl)nona-2,4,8-trien- 1-one showed the best affinity with EGFR, monoamine oxidase B, apoptosis-inducing factor, and cytochrome P450.

Conclusions: Besides piperine and piperidine derivatives, other compounds in *P. nigrum,* such as piperamide; piperamine; sarmentine; trichosta; piperettine; and pyrrolidine, 1-[5-(1,3-benzodioxol-5-yl)- 1 - oxo -2,4-pentadienyl]-,(E,E)-, also have promising potential as an anticancer.

Keywords: anticancer; *in silico*; molecular docking; oxidative stress; *Piper nigrum*; spices.

Resumen

Contexto: La búsqueda de nuevos fármacos anticancerígenos líderes es una de las principales preocupaciones actuales para proporcionar mejores resultados de tratamiento. *Piper nigrum* es una de las plantas especiadas utilizadas como anticancerígeno en la medicina tradicional. Muchos estudios informaron sobre el contenido de metabolitos secundarios de *P. nigrum*. Algunos han sido evaluados como agentes anticancerígenos, pero muchos aún necesitan ser estudiados.

Objetivos: Evaluar la actividad anticancerígena *in silico* de los metabolitos secundarios de *P. nigrum*.

Métodos: Se utilizaron análisis quimioinformáticos y bioinformáticos para investigar la semejanza de los fármacos, la toxicidad, la farmacocinética y la interacción farmacológica contra las enzimas implicadas en la producción de especies reactivas de oxígeno y el crecimiento celular.

Resultados: Diez de los veinticinco compuestos califican como fármacos según las reglas de Lipinski, Veber, Egan, Ghose y Muegge, y cumplieron con los parámetros farmacocinéticos. Un estudio de acoplamiento molecular mostró que siete compuestos mostraron una buena afinidad por las enzimas involucradas en el desencadenamiento del estrés oxidativo celular y la muerte celular. Además, la piperina, los derivados de la piperidina y la piperetina pueden inhibir el crecimiento de la célula cancerosa debido a su afinidad con el receptor del factor de crecimiento epidérmico (EGFR) en comparación con el erlotinib. De todos estos compuestos, (2E,4E,8E)-9-(benzo[d][1,3]dioxol-5-il)-1-(piperidina-1-il)nona-2,4,8-trien-1-ona mostró la mejor afinidad con EGFR, monoaminooxidasa B, factor inductor de apoptosis y citocromo P450.

Conclusiones: Además de la piperina y los derivados de piperidina, otros compuestos en *P. nigrum*, como la piperamida; piperamina; sarmentina; trichosta; piperetina; y la pirrolidina, 1-[5-(1,3-benzodioxol-5-il)-1-oxo-2,4-pentadienil]-,(E,E)-, también tienen un potencial prometedor como anticancerígeno.

Palabras Clave: acoplamiento molecular; anticancerígeno; especias; estrés oxidativo; *in silico*; *Piper nigrum*.

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Abbreviations: ACAT: acyl-coenzyme A cholesterol acyltransferase; AChE: acetylcholinesterase enzyme; AIF: apoptosis-inducing factor; BBB: blood-brain barrier; CYP: cytochrome P450; EGFR: epidermal growth factor receptor; FDA: Food and Drug Administration; GI: gastrointestinal; MOA B: monoamine oxidase B; P-gp: P-glycoprotein; ROS: reactive oxygen species; SH-SY5Y: human neuroblastoma cells; VEGFR-1 and VEGFR-2: vascular endothelial growth factors 1 and 2.

INTRODUCTION

Cancer is one of the most devastating diseases in the World since it is the second leading cause of mortality in humans. World Health Organization (WHO) stated that in 2020, there were 10 million deaths cases caused by various cancers, including breast (2.26 million cases), lung (2.21 million cases), colon and rectum (1.93 million cases), prostate (1.41 million cases), skin (non-melanoma) (1.20 million cases), and stomach (1.09 million cases) (Shaik et al., 2022). Therefore, searching for novel cancer prevention and treatment drugs is a major concern in current research. Natural products have remarkable chemical diversity with chemopreventive or therapeutic action. More than 50% of Food and Drug Administration (FDA) approved drugs are based on natural sources (Gu et al., 2013), even though some synthetic chemical drugs commonly use natural compounds as backbones (Dehelean et al., 2021). For example, camptothecin is a compound isolated from the wood and bark of *Camptotheca acuminata; it* has been known as one of the most common drugs used in cancer clinical treatment. Further, camptothecin-based semisynthetic derivatives called topotecan and irinotecan were also FDA-approved drugs for the treatment of various cancers (Huang et al., 2021).

Inducing oxidative stress by using prooxidative drugs could be used to induce apoptosis in cancer cells. Oxidative stress will cause the accumulation of reactive oxygen species (ROS), leading to the damage of various cellular components such as lipids, nucleic acids, and proteins, resulting in cell death (Van Loenhout et al., 2020). There are several prooxidative drugs used in preclinical studies, such as piperlongumine, resveratrol, oleanolic acid, plumbagin, and celastrol. In clinical trials, such as choline tetrathiomolybdate (ATN-224), 2-methoxyoestradiol, curcumin and its derivatives, and clinical use such as cisplatin and doxorubicin (Nizami et al., 2023). Metastatic events resulting in low survival rates of cancer patients also challenge cancer treatment (Tometich et al., 2020). Therefore, finding novel compounds that target proteins in the cell proliferation pathway is important. One of them is the epidermal growth factor receptor (EGFR). This protein is widely reported to be upregulated in solid tumors and is involved in the growth of cancer cells and metastasis (Mustofa et al., 2022; Sasaki et al., 2013; Tobita et al., 2003; Yan and Efferth, 2019). Targeting EGFR with molecule inhibitors such as erlotinib, pyrotinib, olmutinib, and lapatinib has been approved in cancer therapy (Abourehab et al., 2021). Other targeted proteins are vascular endothelial growth factors 1 and 2 (VEGFR-1 and VEGFR-2), important enzymes in the process of angiogenesis and initiation of cancer cell metastasis (Behelgardi et al., 2020; Guo et al., 2021; Saharinen et al., 2011). Currently, FDA has approved nine VEGFR-2 targeted inhibitors for anticancer drugs, such as sorafenib, sunitinib, pazopanib, and vandetanib (Xiu-Juan et al., 2023).

Many studies reported the isolated compounds of *Piper nigrum* L. (family *Piperaceae*) because this plant has advantages as an anticancer in traditional use (Kammath et al., 2021; Wang et al., 2014). The *P. nigrum* root is applied against abdominal tumors in Thailand (Chaveerach et al., 2008). In China, the fruit of this plant is also used to treat respiratory or gastric cancers (Wang et al., 2014). Many *in vitro* and *in vivo* studies have also been conducted to evaluate the potential of crude extracts and fractions of *P. nigrum* as anticancer (Wang et al., 2014; Wu et al., 2023). Some isolated compounds have been evaluated as anticancer agents, but many still need to be studied. (Zheng et al., 2016) For example, piperine and piperidine were reported to inhibit cell proliferation/migration or induce cell death in cancer cell lines and in cancer animal models (de Almeida et al., 2020; Turrini et al., 2020). Piperine and piperidine are the most widely evaluated compounds as anticancers from *P. nigrum* (Wiraswati et al., 2024). However, other important compounds such as pipene, piperamine, and piperamide are reported to have pharmacological activity, but their anticancer studies are still limited (Ahmad et al., 2012; Barata et al., 2021; Febriyanti et al., 2018; Zied et al., 2013). *P. nigrum,* with its secondary metabolites as one of the most commonly used spices, has been reported to have antibacterial, antimalaria, antiapoptotic, antidepressant, anti-colon toxin, antidiarrhoeal, antifungal, antimutagenic, anti-inflammatory, antioxidative, anti-metastatic activity, antispasmodic, antipyretic, antispermatogenic, anti-tumor, antithyroid, ciprofloxacin potentiator, cold extremities, hepatoprotective, gastric ailments, intermittent fever, contraceptive agent, insecticidal activity and larvicidal activity (de Almeida et al., 2020; Ekaputri et al., 2018; Khairani et al., 2022; Nisar et al., 2012).

Therefore, this study aims to investigate the anticancer potential of isolated compounds of *P. nigrum* through an *in silico* approach. We employed web-based tools to evaluate drug-likeness, pharmacokinetics, and toxicity. Then, we conducted a molecular

docking analysis to predict the interaction between *P. nigrum* compounds and targeted receptors. We use an *in silico* approach to simplify the drug screening process before running *in vitro/in vivo* evaluations that are often restricted by time, resources, and budget.

MATERIAL AND METHODS

Compound sources

The studied compounds were collected from metabolite profiling of previous findings. Methanol extract of *P. nigrum* containing piperine, copaene, adamantane, 3-heptadecen-5-yne, (Z)-, and benzene,1,3-diisocyanatomethyl- (Zahin et al., 2021). Acetone extract of *P. nigrum* containing piperine, piperolein B, piperamide, guineensine, and piperettine (Singh et al., 2004). Ethyl acetate extract of *P. nigrum* containing piperidine, 1-[5-(1,3-benzodioxol-5-yl)-1-oxo-2,4-pentadienyl]-,(Z,Z)-, piperine, (E)-9- (benzo[d][1,3] dioxol-5-yl)-1-(piperidine), (E)-5-(benzo[d][1,3]dioxol-5-yl)-1-(piperidine), and caryophyllene (Sharma et al., 2023). Ethanol extract of *P. nigrum* containing piperidine, 1-[5-(1,3-benzodioxol-5-yl)-1-oxo-2,4-pentadienyl]-,(Z,Z)-, caryophyllene, (E)-5-(benzo[d][1,3] dioxol-5-yl)-1-(piperidine), (2E,6E)-7-(benzo [d][1,3] dioxol-5-yl)-1-(piperidine-1-yl) hepta-2,6-dien-1-one and pyrrolidine, 1-[5-(1,3-benzodioxol-5-yl)-1-oxo-2,4-pentadienyl]-, (E,E)- (Arsana et al., 2022). n-Hexane extract of *P. nigrum* containing (E)-9- (benzo[d][1,3] dioxol-5-yl)-1-(piperidine), (2E,4E,8E)-9-(benzo[d][1,3] dioxol-5-yl)-1-(piperidine-1-yl)nona-2,4,8-trien-1-one, piperidine, 1-[5-(1,3-benzodioxol-5-yl)-1-oxo-2,4-penta-dienyl]-,(Z,Z)-, (2E,4E,10E)-N-isobutyl-hexadeca-2,4,10-trienamide and (E)-5-(benzo[d][1,3] dioxol-5-yl)-1-(piperidine-1-yl)pent-2-en-1-one (Sharma et al., 2023). Petroleum ether extract of *P. nigrum* containing methyl benzene, caryophyllene, β–bisabolene, α–copane, and δ–cadinene (Gupta et al., 2013). After excluding similar compounds found in each solvent, 26 compounds were collected for further *in silico* study.

Drug-likeness and pharmacokinetic analysis

Drug likeness and pharmacokinetic analyses of each compound were analyzed using the SwissADME server (http://www.swissadme.ch/) (accessed on 8 May 2022). Criteria of Lipinski, Ghose, Veber, Egan, and Muegge were applied for drug-likeness analysis. Several pharmacokinetic parameters such as bioavailability score, gastrointestinal (GI) absorption, blood-brain barrier (BBB) permeant, P-glycoprotein (P-gp) substrate, cytochrome P-450 (CYP) inhibitor, Log -Kp, and synthetic accessibility were also estimated by using the SwissADME server.

Ligand's structure construction and Swisstarget prediction

The structures of ligands were built and energy-optimized using MarvinSkecth software (https://chemaxon.com/marvin). Protein targets of each ligand were predicted using the Swisstarget web interface (http://www.swisstargetprediction.ch/) (Daina et al., 2019).

Docking protocol validation

Using PyRx 0.8 (Dallakyan and Olson, 2015) and the Vina wizard, the native ligand was docked to the protein's apo structure in order to validate the docking approach (Eberhardt et al., 2021). The native ligand's structure and the docked native ligand's structure were then compared.

Molecular docking study

Protein structures of apoptosis-inducing factor (AIF) (PDBid: 3GD4), cytochrome P450 (PDBid: 3UA1), epidermal growth factor receptor (EGFR) (PDBid: 7LGS), vesicular endothelial growth factor receptor 2 (VEGFR2) (PDBid: 4AG8) and monoamine oxidase B (MAO B) (PDBid: 3PO7) were downloaded from protein database https://www.rcsb.org/, ligands were built by using Marvinskecth software. Protein and native ligand structures were separated by using UCSF Chimera (Pettersen et al., 2004). Virtual screening was conducted using PyrX software (Dallakyan, 2015) and Vina software version 1.2.5 (Eberhardt et al., 2021; Trott and Olson, 2010) using a specific docking approach. The grid box of each protein was set as follows. FAD binding site of AIF: center_x = -38.2452366018, center_y = 32.0100280352, center_z = -66.5351499558, size_x = 20.920884835, size_y = 22.3906239317, and size_z = 20.0342296282. NAD binding site of AIF: center_x = -53.111355318, center_y = 35.3289032063, center_z = -62.8228636709, size_x = 16.6638416826, size_y = 16.5291746822, and size_z = 16.9067035181. P450: center_x = 22.4352469091, center_y = 15.7724166091, center_z = -11.7903410354, size_x = 18.0732745812, size_y = 18.3984997202, and size_z = 16.9671603273. EGFR: center_x = 35.908672876, center_y = -16.7581889732, center_z = 5.54734097564, size_x = 15.497640582, size_y = 16.3811798781, and size_z = 15.502662544. VEGFR2: center_x = 19.7198546085, center_y = 25.3581113307, center_z = 39.2354490812, size_x = 20.704665871, size_y = 13.2245054733, and size_z = 14.8866176788. MAO B: center_x = 52.9745, center_y = 147.1606, center_z = 24.3551, size_x = 25, size_y = 25, and size_z = 25. Ligand interaction with protein was visualized by using Ligplot plus software (Laskowski and Swindells, 2011).

In silico data analysis

Analysis of *in silico* data for drug discovery required a number of applications and webservers. The SwissADME server (http://www.swissadme.ch/) was used to examine each compound's pharmacokinetic and drug-likeness evaluations, and then the data was displayed in the form of tables. The Swisstarget web interface (http://www.swisstargetprediction.ch/) was used to predict the protein targets of each ligand (Daina et al., 2019), and then the data was displayed in the form of tables. The MarvinSkecth software (https://chemaxon.com/marvin) was used to construct and optimize the energy of the ligand structures; then the ligand files were saved in .pdb format. Protein databases (https://www.rcsb.org/) were used to download protein structures. UCSF Chimera was used to separate the native ligand structure from the protein (Pettersen et al., 2004). Vina software version 1.2.5 (Eberhardt et al., 2021; Trott and Olson, 2010) and PyrX software (Dallakyan and Olson, 2015) were used for virtual screening utilizing a specific docking technique. After that, the selected ligand model was saved in .pdb format by using UCSF Chimera (Pettersen et al., 2004). Ligplot Plus software was used to visualize the interaction between the ligand and the protein (Laskowski and Swindells, 2011), and the interaction was displayed in the form of figures.

RESULTS AND DISCUSSION

Literature study of compounds isolated from *Piper nigrum* by using different organic solvents

Various compounds have been isolated from *P. nigrum* using different organic solvents from polar solvent such as methanol to nonpolar solvent such as n-hexane (Arsana et al., 2022; Gupta et al., 2013; Sharma et al., 2023; Singh et al., 2004; Zahin et al., 2021). Using methanol, some compounds have been successfully isolated and identified with the most abundant as follows: Piperine (46.1%), copaene (12.25%), adamantane (8.07%), 3-heptadecen-5-yne, (Z)- (5.72%) and benzene,1,3-diisocyanatomethyl- (5.20%). The methanol extract exerted inhibitory activity against methicillin-resistant *S. aureus* (MRSA), and showed weak antioxidant activity and antimutagenic activity (Zahin et al., 2021). Similar to methanol extract, acetone extract also exhibited piperine as the most abundant compound (33.53%) might be caused by similar solvent polarity, followed by piperolein B (13.73%), piperamide (3.43%), guineensine (3.23%) and piperettine (2.76%). The acetone extract inhibited 100% mycelial growth of *Penicillium viridcatum* and *Aspergillus ochraceus* in the food-poisoning technique (Singh et al., 2004). Using the less polar solvent such as ethyl acetate and ethanol piperidine, 1-[5-(1,3-benzodioxol-5-yl)-1-oxo-2,4-pentadienyl]-,(Z,Z)- was identified as the most abundant compound (69.4 and 36,8% respectively) (Arsana et al., 2022; Sharma et al., 2023). However, piperine was detected in low abundance in the ethyl acetate extract (3.5%) (Sharma et al., 2023) and ethanol extract (3.45%) (Arsana et al., 2022). The ethyl acetate extract exerted potent anti-glycation, showed significant anti-Aβ fibrilization activities, exhibited a competitive inhibition of acetylcholinesterase enzyme (AChE), and displayed neuroprotective potential in hydrogen peroxide-induced oxidative stress in human neuroblastoma cells (SH-SY5Y) (Sharma et al., 2023). Using n-hexane as a nonpolar solvent (E)-9- (benzo[d][1,3] dioxol-5-yl)-1-(piperidine) was identified as the most abundant compound (16.67%). The n-hexane extract displayed potent anti-glycation, presented significant anti-Aβ fibrilization activities, was a competitive inhibitor of AChE, and had neuroprotective potential in hydrogen peroxide-induced oxidative stress in SH-SY5Y (Sharma et al., 2023). Using another nonpolar solvent (petroleum ether), other compounds were identified, and methyl benzene comprised the most abundant compound (84.67%) (Gupta et al., 2013). Other compounds such as piperamine, pipericide, sarmentosine, sarmentine, and trichosta also have been known as important derivatives of *P. nigrum* (Ahmad et al., 2012). These compounds are displayed in Fig. 1 and used in further *in silico* studies.

Drug-likeness and pharmacokinetic analysis

Lipinski determines a compound as a drug with the following criteria: molecular weight <500 Dalton, hydrogen bond donor <5, hydrogen bond acceptor <10, log-P <5. Muegge-based drug-likeness criteria on several parameters: 200≤ molecular weight ≤400, -2≤ log-P ≤5, TPSA ≤150 A2, hydrogen bond donor <5, hydrogen bond acceptor <10, rotatable bond <15, rings <7 and at least four carbon atom and one heteroatom. Veber set drug criteria as follows: topological polar surface area (TPSA) <140 A2 and rotatable bond <10. Ghose utilizes four parameters to qualify a molecule as a drug: 160g ≤ molecular weight ≤ 480, -0.4≤ log-P ≤5.6, 40≤molar refractivity ≤130, and 20≤ total atom number ≤70. Egan considered three criteria: TPSA <132 A2 and -1≤ log-P ≤6. Solubility character was measured using the ESOL model, log-S value ≤-6 is considered a poorly soluble compound, and log-S value ≤-10 is considered an insoluble compound. Additionally, a compound with 0.55≤ bioavailability score ≤0.85, and 0.25≤ Csp3 (degree of flexibility) score ≤1 is qualified as orally bioavailable (Bogari et al., 2022; Wiraswati et al., 2023a). Based on results in Table 1 several compounds such as piper-

ine, piperamide, piperamine, sarmentine, trichosta, piperettine, (E)-5-(benzo[d][1,3] dioxol-5-yl)-1-(piperidine), pyrrolidine, 1-[5-(1,3-benzodioxol-5-yl)-1-oxo-2,4-pentadienyl]-, (E,E)-, (2E,4E,8E)-9-(benzo[d][1,3]dioxol-5-yl)-1-(piperidine-1-yl)nona-2,4,8-trien-1-one, piperidine, 1-[5-(1,3-benzodioxol-5-yl)-1-oxo-2,4-pentadienyl]-,(Z,Z)- were qualified as drug based on Lipinski, Ghose, Veber, Egan and Muegge criteria. All the compounds also have appropriate solubility, bioavailability, and flexibility degree.

Each compound's pharmacokinetic parameters (Table 2) were anticipated based on GI absorption, permeability to the BBB, and skin permeability (log Kp). The lower the negative log Kp, the less permeable the molecule is to the skin. Another aspect that defines pharmacokinetics is a molecule's interaction with certain proteins, such as the permeability of P-gp and CYP. P-gp is a critical factor in active efflux toward biological membranes, whereas CYP is a protein involved in drug biotransformation. The synthetic accessibility score has ranged from 1 (very simple) to 10 (extremely difficult) (Bogari et al., 2022; Wiraswati et al., 2023a). According to Table 2, the previously mentioned compounds have high GI absorption, are not considered substrates of P-gp protein, and have easy synthetic accessibility.

Swisstarget prediction

Swisstarget prediction web interface (www.swisstargetprediction.ch) was used to predict the probable protein target of compounds using the structural similarity of query compounds with active protein ligands present in the database (Daina et al., 2019). The results are displayed in Table 3. Among the compounds, six compounds (piperine, piperettine, piperidine, 1-[5-(1,3-benzodioxol-5-yl)-1-oxo-2,4-pentadienyl]-,(Z,Z)-, pyrrolidine, 1-[5-(1,3-benzodioxol-5-yl)-1-oxo-2,4-pentadienyl]-, (E,E)-, sarmentine and trichosta) have probability in targeting MAO B. Other compounds such as piperamide may interact with serotonin transporter; piperamine and (E)-5-(benzo[d][1,3] dioxol-5-yl)-1-(piperidine) may interact with serotonin 1α receptor; (2E,4E,8E)-9-(benzo[d][1,3]dioxol-5-yl)-1-(piperidine-1-yl)nona-2,4,8-trien-1-one may interact with acyl-coenzyme A cholesterol acyltransferase (ACAT).

MAO B has catalytic activity in oxidative deamination of bioamines such as 2-phenylethylamine and benzylamine, dopamine, and tyramine. During the oxidation of monoamine substrate, ROS will be produced (Aljanabi et al., 2021). It has been reported that in Parkinson's disease there is irregular expression of the enzyme, leading to unnecessary metabolism of monoamine neurotransmitter. One of current treatment of this disease is by inhibiting the activity of MAO B by using particular drugs such as rasagiline, selegiline and safinamide (Baweja et al., 2023). Multiple cancer cells also have been reported to overexpress the enzyme (Aljanabi et al., 2021; Baweja et al., 2023). Exploration of MAO B inhibitors or activated prodrugs is also a promising cancer treatment (Shih, 2018). It has been reported that in glioblastoma, N,N-bis(2-chloroethyl)-2-(1-methyl-1,2,3,6-tetrahydropyridin-4-yl) propenamide (MP-MUS) is oxidized by MAO B leading to mitochondrial damage and apoptosis induction (Raghavan et al., 2020). Meanwhile, ACAT is an enzyme that plays a role in cholesterol homeostasis by supporting cell membrane stability. The overexpression of ACAT has been reported to present in various types of cancer cells (Ayyagari et al., 2020). Inhibition of this enzyme might be a promising way for cancer treatment (Zabielska et al., 2019). It has been reported that avasimibe, a potent inhibitor of acyl-CoA cholesterol acyltransferase-1 (ACAT-1), effectively suppressed the proliferation of pancreatic ductal adenocarcinoma (Li et al., 2018).

***In silico* anticancer assay**

An *in silico* study was attempted in this report to reveal the molecular action of dominant compounds contained in *P. nigrum*. MAO B has been reported to be highly expressed in several types of cancer, such as colorectal, breast, lung (Aljanabi et al., 2021), and brain cancer (Levin et al., 2017; Sharpe and Baskin, 2016). Previous *in silico* study has been conducted by using piperlonguminine, a compound isolated from *P. nigrum* as a potential anticancer drug, and shows that this compound has a high affinity against MAOB, with an affinity energy of -8.6 kcal/mol (Suwardi et al., 2023). In previous studies, we have also performed anticancer *in vitro* or *in silico* studies by using AIF (Wiraswati et al., 2017; Wiraswati et al., 2023a) and CYP, EGFR, and VEGFR2 (Wiraswati et al., 2023b) as protein targets. Hence, in this study, we are performing molecular docking of compounds contained in *P. nigrum* on MAO B, AIF, P450, EGFR, and VEGFR2.

Molecular docking validation was performed by comparing the orientation of the native ligand and the docked native ligand. Fig. 2 shows that the orientation of the native ligand and docked native ligand were almost similar. Therefore, we can conclude that the molecular docking method was valid.

Molecular docking with the native ligand of each protein was attempted to validate the grid box. The study was also performed on established anticancer drugs such as thiodione (Wiraswati et al., 2016), tamoxifen (Singh et al., 2011), erlotinib (Sever et al., 2020), sorafenib, (Taghour et al., 2022) and L-deprenyl (Szende, 2001) on AIF, CYP, EGFR, VEGFR2, and

MAO B, respectively. Table 4 shows the affinity of ten compounds for enzymes involved in ROS production (such as AIF, CYP, MAO B) and cell growth (such as EGFR and VEGFR2). The results showed that all compounds have a higher affinity for MAO B than the positive control. Except for piperamide, piperamine, and sarmentine, other compounds have higher binding to AIF on the NAD binding domain. However, only three compounds (piperine, piperettine, and (2E,4E,8E)-9-(benzo[d][1,3]dioxol-5-yl)-1-(piperidine-1-yl)nona-2 ,4,8-trien-1-one), which have a higher affinity towards all enzymes involved in ROS formation. (2E,4E,8E)-9-(benzo[d][1,3]dioxol-5-yl)-1-(piperidine-1-yl)nona-2,4,8-trien-1-one shows the best affinity for MAO B compared to other compounds, positive controls, or original ligands.

Apart from that, *in silico* research revealed that piperine, piperidine derivatives, and piperettin may play a role in inhibiting the growth of cells, which showed a higher affinity with EGFR than the positive control. Of all these compounds (2E,4E,8E)-9-(benzo[d][1,3]dioxol-5-yl)-1-(piperidine-1-yl)nona-2,4,8-trien-1-one seems to have the most potential as an EGFR inhibitor because its affinity is better than the positive control and the native ligand. Other compounds must overcome the affinity threshold (e.g., increasing dosage) to compete with the native ligand of each enzyme, as does the positive control.

Fig. 3A-E visualizes the molecular interaction of CYP with native ligand, tamoxifen, (2E,4E,8E)-9-(benzo[d][1,3]dioxol-5-yl)-1-(piperidine-1-yl)nona-2,4,8-trien-1-one, and piperettine. The best binding score on CYP was performed by (2E,4E,8E)-9-(benzo[d][1,3] dioxol-5-yl)-1-(piperidine-1-yl)nona-2,4,8-trien-1-one with affinity energy -10.399 kcal/mol. This compound forms two hydrogen bonds with CYP involving Ser119 and Thr224 (Fig. 3D). Thr224 is one of the important residues in CYP (Sevrioukova and Poulos, 2012). Fig. 4A-E visualizes the molecular interaction of MOA B with native ligand, L-deprenyl, (2E,4E,8E)-9-(benzo[d][1,3] dioxol-5-yl)-1-(piperidine-1-yl)nona-2,4,8-trien-1-one, and piperine. (2E,4E,8E)-9-(benzo[d][1,3] dioxol-5-yl)-1-(piperidine-1-yl)nona-2,4,8-trien-1-one poses a very high binding score with MAO B with affinity energy -11.537 kcal/mol. This compound forms five hydrogen bonds with MAO B involving Ile14, Ser15, Arg42, Leu171, and Thr426 (Fig. 4D). Piperine forms three hydrogen bonds involving Ser15, Ser59, and Tyr60 (Fig. 4E). Leu171 was reported as one of the active residues in MAO B (Binda et al., 2011). Fig. 5A-E visualize molecular interaction of NAD binding domain of AIF with native ligand, thiodione, (2E,4E,8E)-9-(benzo[d][1,3] dioxol-5-yl)-1-(piperidine-1-yl)nona-2,4,8-trien-1-one, and E-5-(benzo[d][1,3] dioxol-5-yl)-1-(piperidine). (2E,4E,8E)-9-(benzo[d][1,3] dioxol-5-yl)-1-(piperidine-1-yl)nona-2,4,8-trien-1-one also poses very high binding score with AIF. This compound forms two hydrogen bonds involving Phe309 and Val368 (Fig. 5D). Phe349 is one of the essential residues on AIF (Sevrioukova, 2009). Another piperidine derivative, ((E)-5-(benzo[d][1,3] dioxol-5-yl)-1-(piperidine)) also has a high binding score by forming two hydrogen bonds involving Arg284 and Lys341 (Fig. 5E). Fig. 6A-E visualize molecular interaction of EGFR with native ligand, erlotinib, (2E,4E,8E)-9-(benzo[d][1,3] dioxol-5-yl)-1-(piperidine-1-yl)nona-2,4,8-trien-1-one, and piperettine. However, (2E,4E,8E)-9-(benzo[d][1,3] dioxol-5-yl)-1-(piperidine-1-yl)nona-2,4,8-trien-1-one and piperettine show high binding score on EGFR by forming one hydrogen bond involving Met796 (Fig. 5D). Met796 is one of the essential binding sites of erlotinib (Fig. 5C). Furthermore, the residue also is an essential binding site of another EGFR inhibitors such as sunvozertinib (Wang et al., 2022) and 4-anilinoquinazoline derivatives (Moghadam et al., 2020).

Piperine and piperidine are the most widely evaluated compounds as anticancers from *P. nigrum* (Wiraswati et al., 2024). However, other important compounds such as piperamine, and piperamide are reported to have pharmacological activity, but their anticancer studies are still limited (Ahmad et al., 2012; Barata et al., 2021; Febriyanti et al., 2018; Zied et al., 2013). Here we also report that other than piperine and piperidine derivatives, another compound such as piperamide, piperamine, sarmentine, trichosta, piperettine, and pyrrolidine, 1-[5-(1,3-benzodioxol-5-yl)-1-oxo-2,4-pentadienyl]-, (E,E)- also has potential as anticancer drug. The compounds also qualified as drugs based on Lipinski, Veber, Egan, Ghose, Muegge and have good pharmacokinetics (Tables 1 and 2).

The above *in silico* study suggested that most compounds have good affinity with enzymes involved in oxidative stress (AIF, CYP, and MAO B) and cell growth (EGFR). This finding supported previous analysis stating that one of the main molecular actions of *P. nigrum* extract is inducing oxidative stress (Valdelúcia Maria et al., 2016). The main compound of *P. nigrum*, piperine, exerts antioxidant activity at low doses. Meanwhile, it induces apoptosis by stimulating the production of ROS in many cancers at higher doses (Acharya et al., 2010; Rather and Bhagat, 2018; Turrini et al., 2020). AIF is a bifunctional flavoprotein exhibiting different actions by maintaining mitochondrial morphology and energy metabolism or activating the apoptotic process in a caspase-independent manner (Letkovska et al., 2021).

Table 1. Drug-likeness results of the identified compounds from *Piper nigrum*.

Molecule	MW	H-Bond		Fraction Csp3	Rotatable bonds	MR	TPSA	Consensus Log-P	ESOL Log-S	ESOL Class	Drug-likeness				
		Acceptors	Donors								Lipinski	Ghose	Veber	Egan	Muegge
Adamantane	136.23	0	0	1.00	0	43.84	0	3.34	-3.36	Soluble	Yes	No	Yes	Yes	No
Benzene,1,3-diisocyanatomethyl-	188.18	4	0	0.20	4	50.50	58.86	2.14	-3.20	Soluble	Yes	Yes	Yes	Yes	No
β–Bisabolene	204.35	0	0	0.60	4	70.68	0	4.83	-4.89	Moderately soluble	Yes	Yes	Yes	Yes	No
Copaene/ α-copaene	204.35	0	0	0.87	1	67.14	0	4.30	-3.86	Soluble	Yes	Yes	Yes	Yes	No
Caryophyllene	232.40	0	0	0.76	0	77.88	0	4.90	-4.63	Moderately soluble	Yes	Yes	Yes	Yes	No
δ-Cadinene	204.35	0	0	0.73	1	69.04	0	4.08	-3.61	Soluble	Yes	Yes	Yes	Yes	No
Guineensine	383.52	3	1	0.46	13	116.75	47.56	5.58	-5.81	Moderately soluble	Yes	Yes	No	Yes	No
3-Heptadecen-5-yne, (Z)-	234.42	0	0	0.76	10	81.52	0	6.21	-5.55	Moderately soluble	Yes	No	Yes	No	No
Methyl benzene	92.14	0	0	0.14	0	31.41	0	2.51	-2.77	Soluble	Yes	No	Yes	Yes	No
Piperine	285.34	3	0	0.35	4	85.48	38.77	3.03	-3.74	Soluble	Yes	Yes	Yes	Yes	Yes
Piperolein B	343.46	3	0	0.57	9	105.18	38.77	4.45	-4.86	Moderately soluble	Yes	Yes	Yes	Yes	No
Piperamide	304.43	3	1	0.59	7	99.14	38.82	1.58	-2.37	Soluble	Yes	Yes	Yes	Yes	Yes
Piperamine	301.38	4	0	0.50	7	89.96	38.77	3.18	-3.67	Soluble	Yes	Yes	Yes	Yes	Yes
Pipericide	397.55	3	1	0.48	14	121.56	47.56	5.93	-6.16	Poorly soluble	Yes	No	No	No	No
Sarmentosine	263.42	1	0	0.71	10	87.78	20.31	4.33	-4.19	Moderately soluble	Yes	Yes	Yes	Yes	No
Sarmentine	313.39	3	0	0.42	7	95.09	38.77	3.66	-4.19	Moderately soluble	Yes	Yes	Yes	Yes	Yes
Trichosta	271.31	3	0	0.31	6	80.67	38.77	2.75	-3.44	Soluble	Yes	Yes	Yes	Yes	Yes

Table 1. Drug-likeness results of the identified compounds from *Piper nigrum* (continued...)

Molecule	MW	H-Bond		Fraction Csp3	Rotatable bonds	MR	TPSA	Consensus Log-P	ESOL Log-S	ESOL Class	Drug-likeness				
		Acceptors	Donors								Lipinski	Ghose	Veber	Egan	Muegge
Piperettine	315.41	3	0	0.53	7	95.56	38.77	3.79	-4.15	Moderately soluble	Yes	Yes	Yes	Yes	Yes
(E)-9- (Benzo[d][1,3] dioxol-5-yl)-1-(piperidine)	343.46	3	0	0.57	6	105.18	38.77	4.45	-4.86	Moderately soluble	Yes	Yes	Yes	Yes	No
(E)-5-(Benzo[d][1,3] dioxol-5-yl)-1-(piperidine)	286.37	3	0	0.50	6	83.15	35.53	4.02	-4.44	Moderately soluble	Yes	Yes	Yes	Yes	Yes
Pyrrolidine, 1-[5-(1,3-benzodioxol-5-yl)-1-oxo-2,4-pentadienyl]-, (E,E)-	271.31	3	0	0.31	6	80.67	38.77	2.75	-3.44	Soluble	Yes	Yes	Yes	Yes	Yes
(2E,6E)-7-(Benzo [d][1,3] dioxol-5-yl)-1-(piperidine-1-yl) hepta-2,6-dien-1-one	311.42	2	0	0.40	6	99.22	21.70	4.40	-4.81	Moderately soluble	Yes	Yes	Yes	Yes	No
(2E,4E,8E)-9-(Benzo[d][1,3]dioxol-5-yl)-1-(piperidine-1-yl)nona-2,4,8-trien-1-one	339.43	3	0	0.38	7	104.23	38.77	4.17	-4.62	Moderately soluble	Yes	Yes	Yes	Yes	Yes
Piperidine, 1-[5-(1,3-benzodioxol-5-yl)-1-oxo-2,4-pentadienyl]-,(Z,Z)-	285.34	3	0	0.35	4	85.48	38.77	3.00	-3.74	Soluble	Yes	Yes	Yes	Yes	Yes
(2E,4E,10E)-N-Isobutylhexadeca-2,4,10-trienamide	305.50	1	1	0.65	14	99.83	29.10	5.56	-5.04	Moderately soluble	Yes	Yes	No	Yes	No

Table 2. Pharmacokinetic results of the identified compounds from *Piper nigrum*.

Molecule	Bioavailability score	GI Absorption	BBB permeant	P-gp substrate	CYP inhibitor					Log -Kp (cm/s)	Alerts		Leadikeness violation	Synthetic accessibility
					1A2	2C19	2C9	2D6	3A4		PAINS	BRENK		
Adamantane	0.55	Low	Yes	No	No	No	Yes	No	No	-4.12	0	0	2	3.80
Benzene,1,3-diisocyanatomethyl-	0.55	High	Yes	No	Yes	Yes	No	No	No	-5.03	0	2	1	1.62
β–Bisabolene	0.55	Low	No	No	No	No	Yes	No	No	-2.98	0	1	2	3.90
Copaene/ α-copaene	0.55	Low	Yes	No	Yes	Yes	Yes	No	No	-4.37	0	1	2	4.62
Caryophyllene	0.55	Low	No	No	No	Yes	Yes	No	No	-3.94	0	1	2	4.73
δ-Cadinene	0.55	Low	No	No	No	Yes	Yes	No	No	-4.65	0	1	2	4.35
Guineensine	0.55	High	Yes	No	No	No	Yes	Yes	Yes	-3.80	0	2	3	3.80
3-Heptadecen-5-yne, (Z)-	0.55	Low	No	No	Yes	No	No	No	No	-2.19	0	1	3	4.63
Methyl benzene	0.55	Low	No	No	Yes	No	No	No	No	-4.92	0	0	1	1
Piperine	0.55	High	Yes	No	Yes	Yes	Yes	No	No	-5.58	0	2	0	2.92
Piperolein B	0.55	High	Yes	No	No	No	Yes	Yes	Yes	-4.67	0	0	2	3.18
Piperamide	0.55	High	Yes	Yes	No	No	No	Yes	Yes	-7.14	1	0	0	2.20
Piperamine	0.55	High	Yes	No	Yes	Yes	No	Yes	No	-5.63	0	1	1	2.94
Pipericide	0.55	High	No	Yes	No	No	No	Yes	Yes	-3.51	0	2	3	3.91
Sarmentosine	0.55	High	Yes	No	Yes	No	No	Yes	No	-4.10	0	2	2	3.16
Sarmentine	0.55	High	Yes	No	Yes	Yes	Yes	No	Yes	-5.20	0	1	0	3.06
Trichosta	0.55	High	Yes	No	Yes	Yes	No	No	No	-5.75	0	2	0	2.84
Piperettine	0.55	High	Yes	No	No	Yes	Yes	Yes	No	-5.26	0	0	1	2.94
(E)-9- (Benzo[d][1,3] dioxol-5-yl)-1-(piperidine)	0.55	High	Yes	No	No	No	Yes	Yes	Yes	-4.67	0	0	2	3.18
(E)-5-(Benzo[d][1,3] dioxol-5-yl)-1-(piperidine)	0.55	High	Yes	No	Yes	Yes	Yes	Yes	No	-4.73	0	1	1	2.86
Pyrrolidine, 1-[5-(1,3-benzodioxol-5-yl)-1-oxo-2,4-pentadienyl]-, (E,E)-	0.55	High	Yes	No	Yes	Yes	No	No	No	-5.75	0	2	0	2.84
(2E,6E)-7-(Benzo [d][1,3] dioxol-5-yl)-1-(piperidine-1-yl) hepta-2,6-dien-1-one	0.55	High	Yes	No	No	Yes	Yes	Yes	Yes	-4.54	0	1	1	3.44

Table 2. Pharmacokinetic results of the identified compounds from *Piper nigrum* (continued...)

Molecule	Bioavailability score	GI Absorption	BBB permeant	P-gp substrate	CYP inhibitor					Log -Kp (cm/s)	Alerts		Leadikeness violation	Synthetic accessibility
					1A2	2C19	2C9	2D6	3A4		PAINS	BRENK		
(2E,4E,8E)-9-(Benzo[d][1,3]dioxol-5-yl)-1-(piperidine-1-yl)nona-2,4,8-trien-1-one	0.55	High	Yes	No	No	Yes	Yes	No	Yes	-5.03	0	2	1	3.35
Piperidine, 1-[5-(1,3-benzodioxol-5-yl)-1-oxo-2,4-pentadienyl]-,(Z,Z)-	0.55	High	Yes	No	Yes	Yes	Yes	No	No	-5.58	0	2	0	2.92
(2E,4E,10E)-N-Isobutylhexadeca-2,4,10-trienamide	0.55	High	Yes	No	Yes	No	Yes	No	Yes	-3.40	0	3	2	3.63

Table 3. Swisstarget prediction of compounds contained in ethanol extract of *Piper nigrum*.

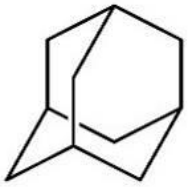
No	Compound's name	Swisstarget	Probability (%)
1	Piperine	Monoamine oxidase B	100
2	Piperamide	Serotonin transporter	11.15
3	Piperamine	Serotonin 3a (5-HT3a) receptor	11.15
4	Sarmentine	Monoamine oxidase B	10.99
5	Trichosta	Monoamine oxidase B	55.90
6	Piperettine	Monoamine oxidase B	10.16
7	(E)-5-(Benzo[d][1,3] dioxol-5-yl)-1-(piperidine)	Serotonin 1a (5-HT1a) receptor	11.43
8	Pyrrolidine, 1-[5-(1,3-benzodioxol-5-yl)-1-oxo-2,4-pentadienyl]-, (E,E)-	Monoamine oxidase B	55.90
9	(2E,4E,8E)-9-(Benzo[d][1,3]dioxol-5-yl)-1-(piperidine-1-yl)nona-2,4,8-trien-1-one	Acyl coenzyme A: cholesterol acyltransferase 1	13.07
10	Piperidine, 1-[5-(1,3-benzodioxol-5-yl)-1-oxo-2,4-pentadienyl]-,(Z,Z)-	Monoamine oxidase B	73.22

Table 4. Molecular docking result of compounds against MAO B, AIF, CYP, EGFR and VEGFR2. Compound with high affinity higher than positive control are marked in bold.

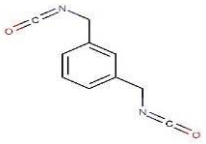
No.	Compound	AIF (3GD4)		CYP (3UA1)	EGFR (7LGS)	VEGFR2 (4AG8)	MAO B (3PO7)
		FAD binding site	NAD binding site				
1	Native ligand	-14.264	-8.561	-14.391	-8.262	-11.568	-11.025
2	Positive control (thiodione)	-10.349	-7.668	n.d	n.d	n.d	n.d
3	Positive control (tamoxifen)	n.d	n.d	-9.199	n.d	n.d	n.d
4	Positive control (erlotinib)	n.d	n.d	n.d	-7.416	n.d	n.d
5	Positive control (sorafenib)	n.d	n.d	n.d	n.d	-10.223	n.d
6	Positive control (L-deprenyl or selegiline)	n.d	n.d	n.d	n.d	n.d	-7.234
7	Piperine	-9.619	**-7.796**	**-9.339**	**-7.785**	-9.968	**-10.288**
8	Piperamide	-8.04	-6.049	-7.677	-6.17	-7.845	**-8.616**
9	Piperamine	-8.271	-7.572	-7.969	-7.184	-8.327	**-9.248**
10	Sarmentine	-7.331	-6.303	-6.836	-6.119	-7.49	**-7.65**
11	Trichosta	-9.427	**-7.714**	-9.128	-7.246	-9.683	**-9.902**
12	Piperettine	-9.863	**-7.907**	**-9.519**	**-7.981**	-9.075	**-10.2**
13	(E)-5-(Benzo[d][1,3] dioxol-5-yl)-1-(piperidine)	-9.072	**-7.94**	-8.766	**-7.482**	-9.265	**-9.982**
14	Pyrrolidine, 1-[5-(1,3-benzodioxol-5-yl)-1-oxo-2,4-pentadienyl]-, (E,E)-	-9.434	**-7.825**	-9.126	**-7.450**	-9.775	**-9.947**
15	(2E,4E,8E)-9-(Benzo[d][1,3]dioxol-5-yl)-1-(piperidine-1-yl)nona-2,4,8-trien-1-one	-9.781	**-8.688**	**10.399**	**-8.549**	-9.37	**-11.537**
16	Piperidine, 1-[5-(1,3-benzodioxol-5-yl)-1-oxo-2,4-pentadienyl]-,(Z,Z)-	-9.669	**-8.187**	-8.762	**-7.814**	-9.394	**-9.355**

n.d: not determined

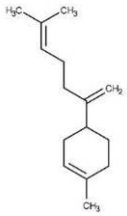
Adamantane



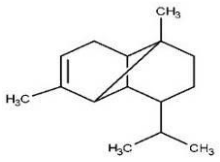
Benzene,1,3-diisocyanatomethyl-



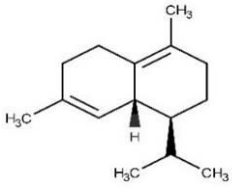
β–bisabolene



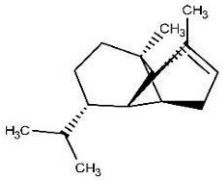
α-copane



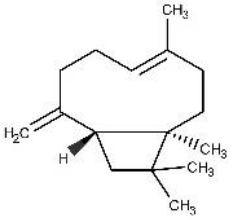
δ-cadinene



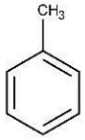
Copaene



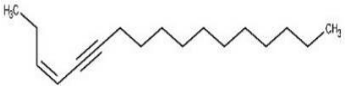
Caryophyllene



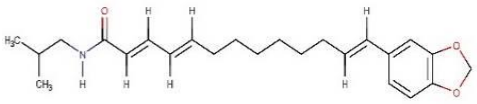
Methyl benzene



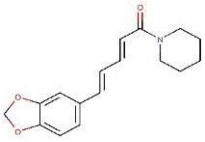
3-Heptadecen-5-yne, (Z)-

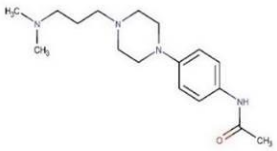


Guineensine



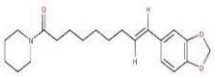
Piperine



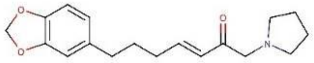


Piperamide

Piperolein B



Piperamine



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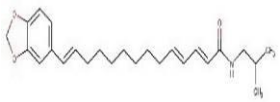
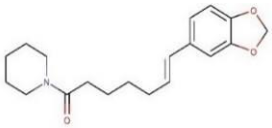
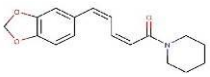


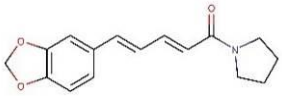
Piperettine



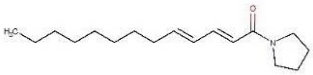
Piperidine, 1-[5-(1,3-benzodioxol-5-yl)-1-oxo-2,4-pentadienyl]-,(Z,Z)-



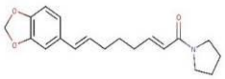
Pyrrolidine, 1-[5-(1,3-benzodioxol-5-yl)-1-oxo-2,4-pentadienyl]-, (E,E)-



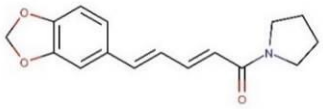
Sarmentine



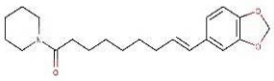
Sarmentosine



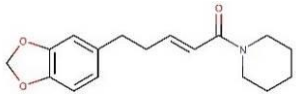
Trichosta



(E)-9- (Benzo[d][1,3] dioxol-5-yl)-1-(piperidine)

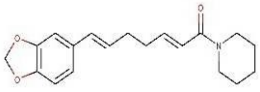


(E)-5-(Benzo[d][1,3] dioxol-5-yl)-1-(piperidine)



(2E,4E,10E)-N-Isobutylhexadeca-2,4,10-trienamide

(2E,6E)-7-(Benzo [d][1,3] dioxol-5-yl)-1-(piperidine-1-yl) hepta-2,6-dien-1-one



(2E,4E,8E)-9-(Benzo[d] [1,3] dioxol-5-yl)-1-(piperidine-1-yl) nona-2,4,8-trien-1-one

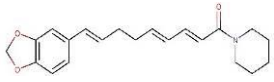


Figure 1. Structure of most abundant or important compounds identified in *Piper nigrum*.

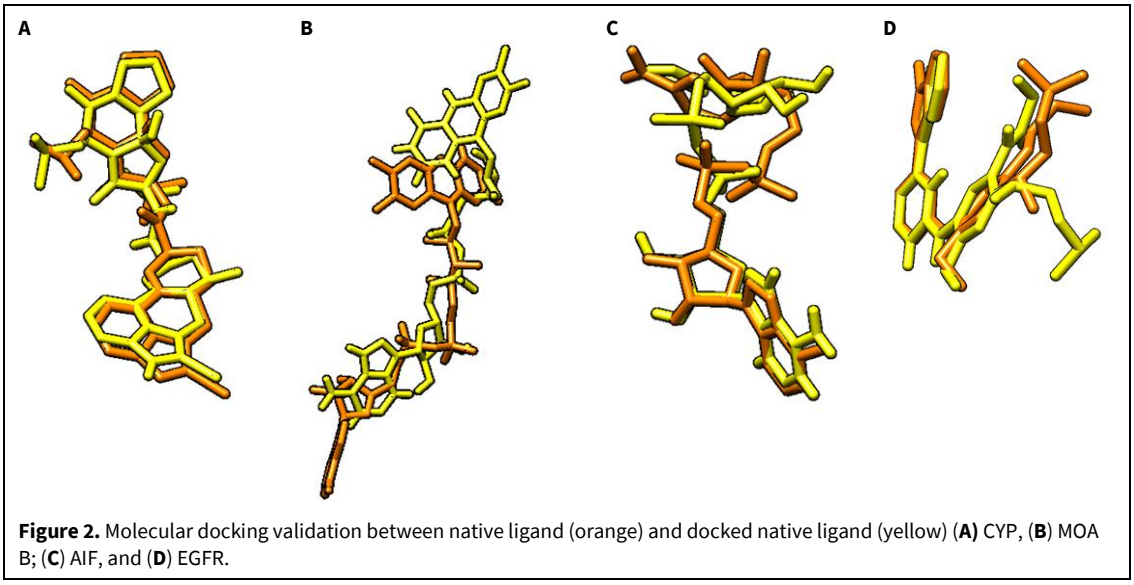


Figure 2. Molecular docking validation between native ligand (orange) and docked native ligand (yellow) (**A)** CYP, (**B**) MOA B; (**C**) AIF, and (**D**) EGFR.

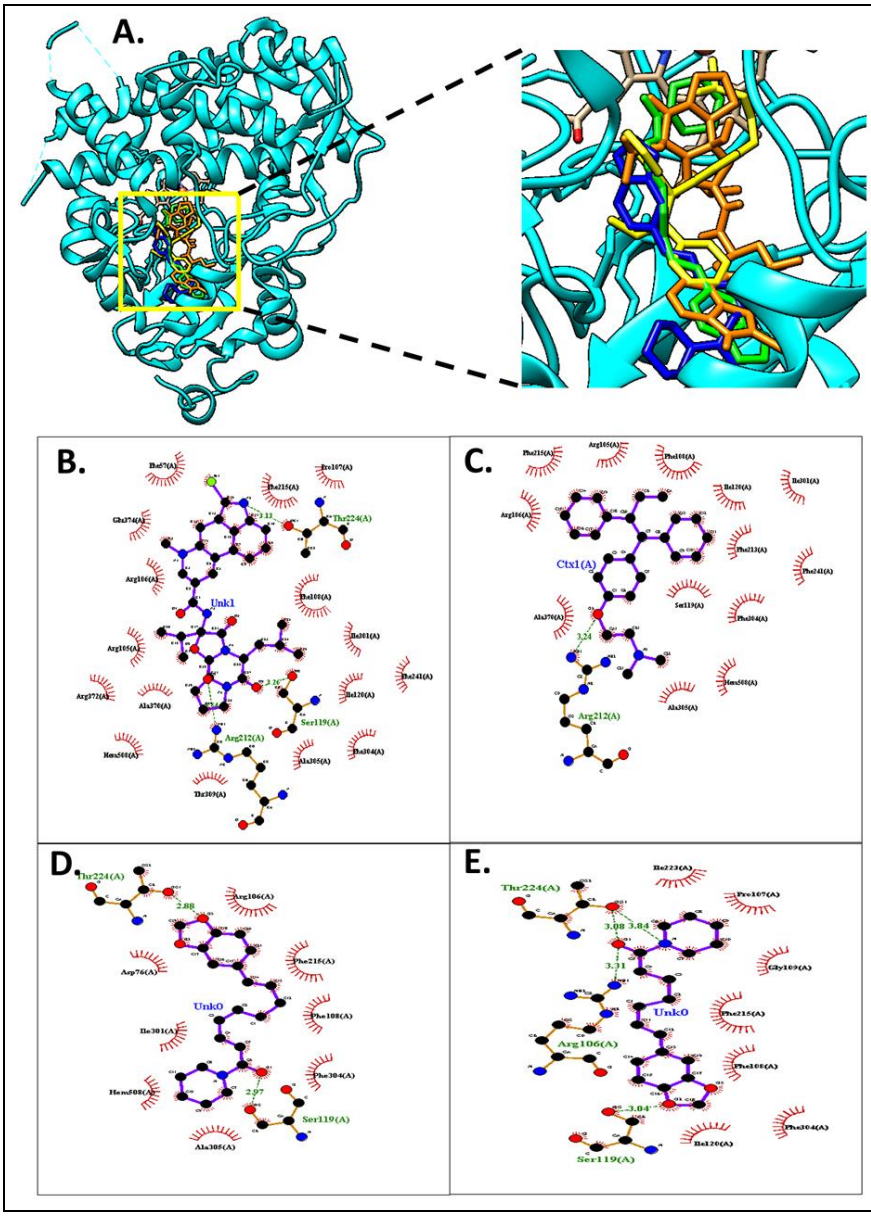


Figure 3. Ligand molecular interaction with CYP.

(A) native ligand, **(B)** tamoxifen, **(C)** (2E,4E,8E)-9-(benzo[d][1,3]dioxol-5-yl)-1-(piperidine-1-yl)nona-2,4,8-trien-1-one, and **(D)** piperettine.

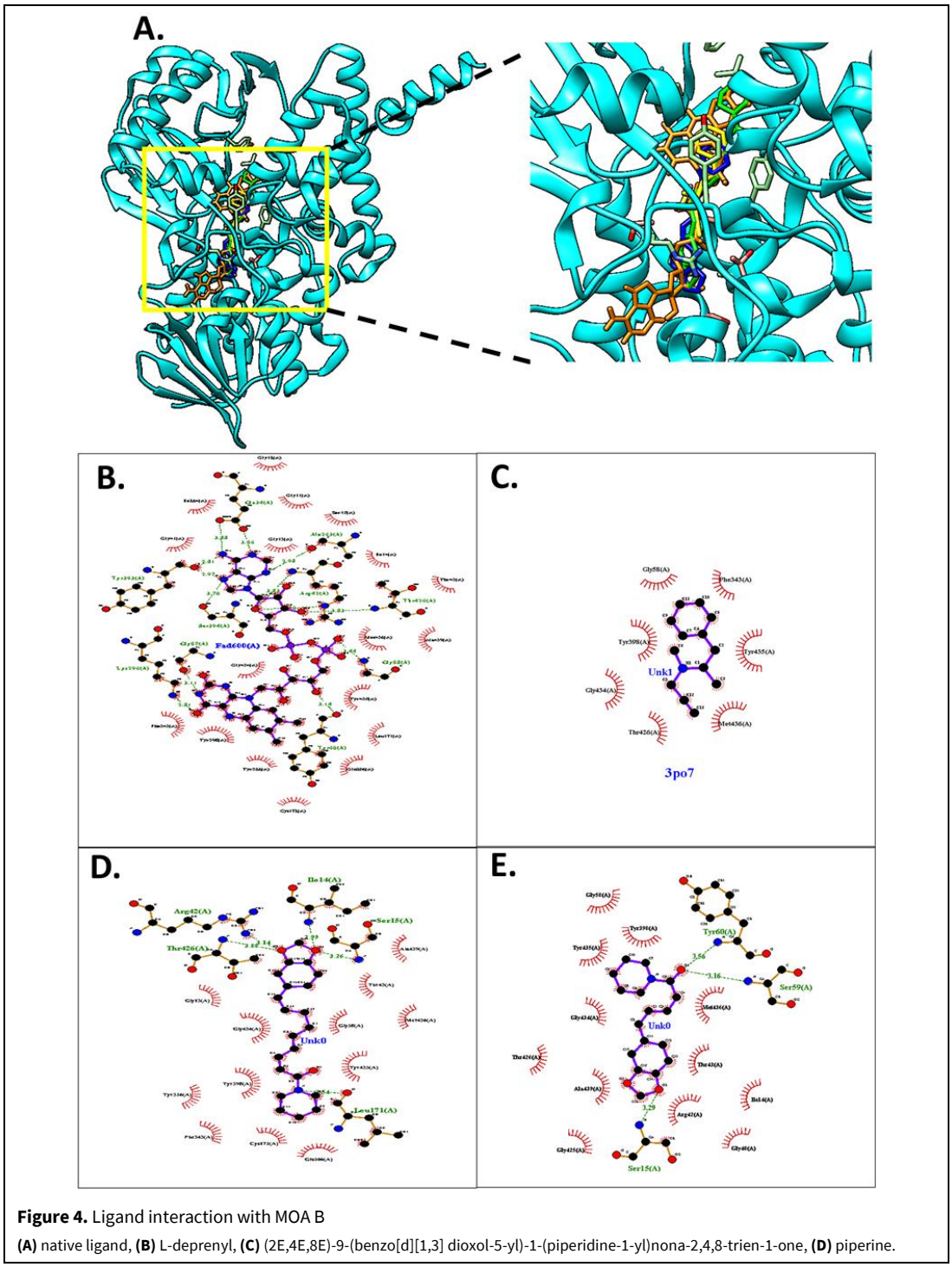


Figure 4. Ligand interaction with MOA B

(A) native ligand, **(B)** L-deprenyl, **(C)** (2E,4E,8E)-9-(benzo[d][1,3] dioxol-5-yl)-1-(piperidine-1-yl)nona-2,4,8-trien-1-one, **(D)** piperine.

Previous *in silico* and *in vitro* studies show that AIF mediates lethal redox stress induced by menadione (Wiraswati et al., 2017; Wiraswati et al., 2016). The affinity of *P. nigrum* compounds with AIF indicated its potential to kill cancer cells. However, they must first overcome the binding constraints, given that the affinity is lower than the native ligand. CYP is an enzyme involved in the metabolism of various anticancer drugs (Fujita, 2006). The drugs will undergo oxidation by the enzyme, leading to the production of ROS that will cause oxidative damage to cancer cells (Sharmistha et al., 2016). Monoamine oxidase B is a FAD-dependent mitochondrial enzyme catalyzing oxidative deamination of several amines. This enzyme catalyzes monoamine substrate oxidation to produce ROS (Aljanabi et al., 2021). All the above findings suggested that the main anticancer action of *P. nigrum* compounds is by triggering oxidative stress that may be associated with cell growth inhibition or cell death induction.

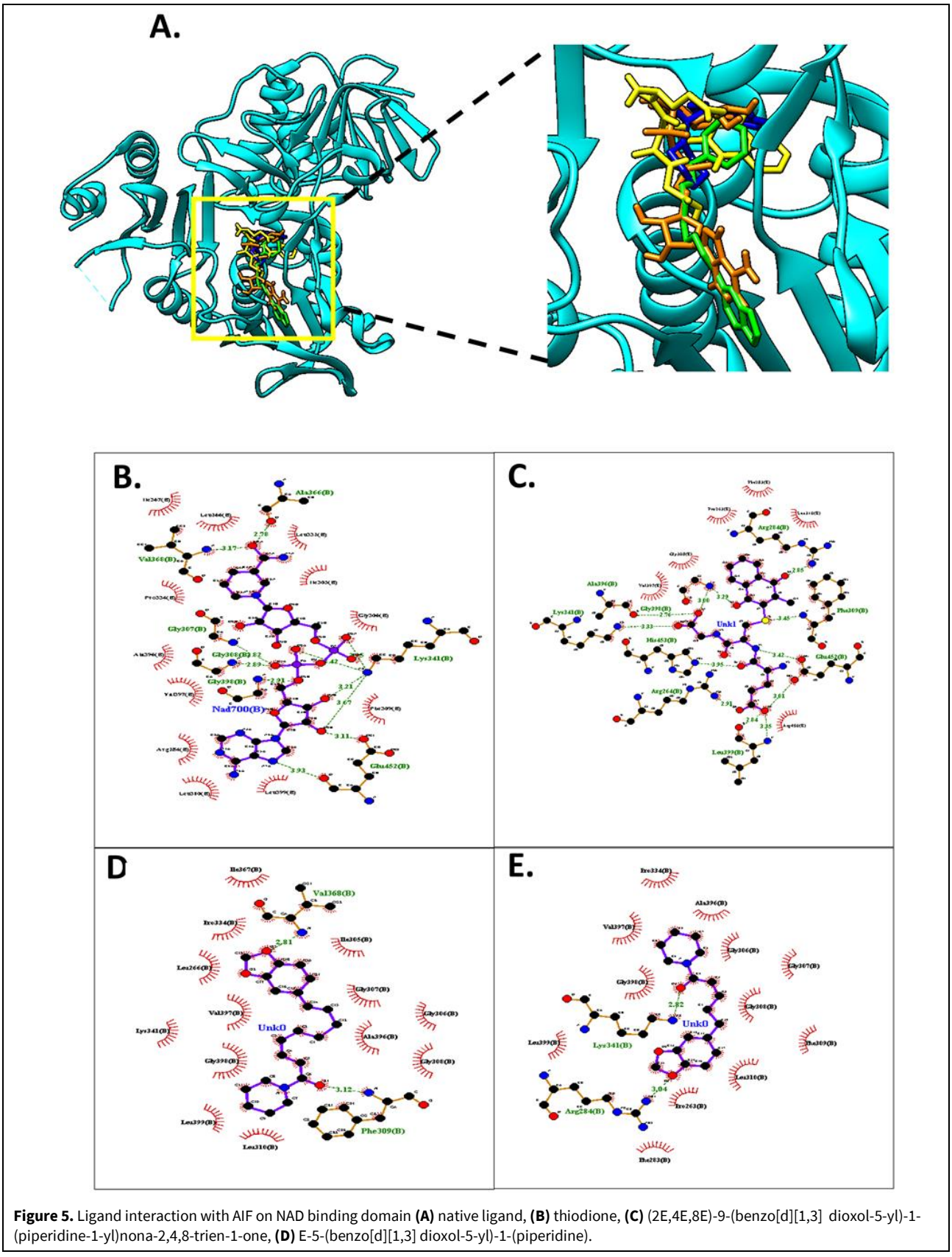


Figure 5. Ligand interaction with AIF on NAD binding domain **(A)** native ligand, **(B)** thiodione, **(C)** (2E,4E,8E)-9-(benzo[d][1,3] dioxol-5-yl)-1-(piperidine-1-yl)nona-2,4,8-trien-1-one, **(D)** E-5-(benzo[d][1,3] dioxol-5-yl)-1-(piperidine).

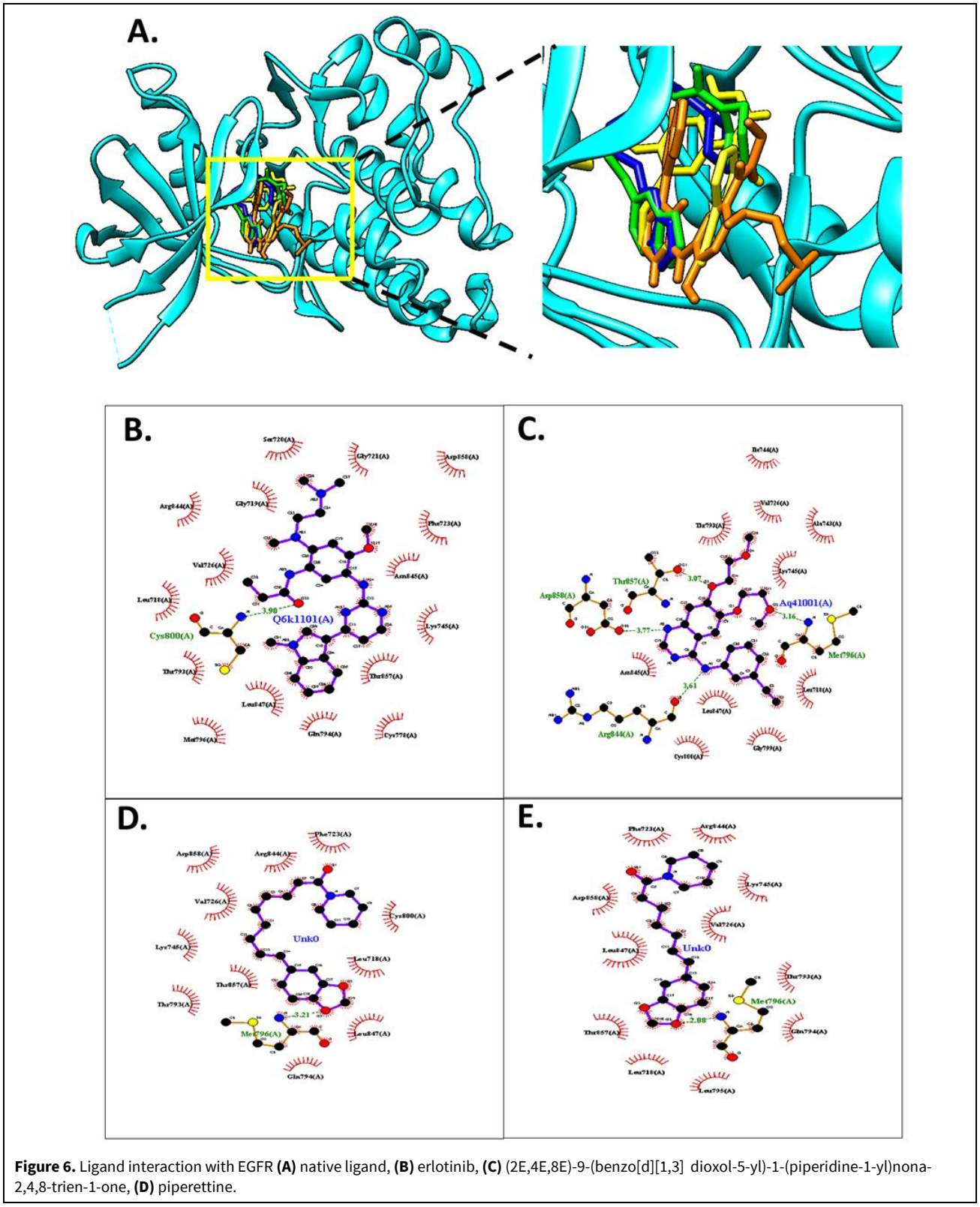


Figure 6. Ligand interaction with EGFR **(A)** native ligand, **(B)** erlotinib, **(C)** (2E,4E,8E)-9-(benzo[d][1,3] dioxol-5-yl)-1-(piperidine-1-yl)nona-2,4,8-trien-1-one, **(D)** piperettine.

CONCLUSION

In silico studies show that ten of the 25 compounds isolated from *Piper nigrum* qualify as drugs according to Lipinski, Veber, Egan, Ghose, Muegge and have good pharmacokinetics. The ten compounds are piperine; piperamide; piperamine; sarmentine; trichosta; piperettine; (E)-5-(benzo[d][1,3] dioxol-5-yl)-1-(piperidine); pyrrolidine, 1-[5-(1,3-benzodioxol-5-yl)-1-oxo-2,4-pentadienyl]-, (E,E)-; (2E,4E,8E)-9-(benzo[d][1,3]dioxol-5-yl)-1-(piperidine-1-yl)nona-2,4,8-trien-1-one, and piperidine, 1-[5-(1,3-benzodioxol-5-yl)-1-oxo-2,4-pentadienyl]-,(Z,Z)-. All ten compounds showed a higher affinity with MOA B

compared to an anticancer drug L-deprenyl. Some of them also showed good affinity with other stress oxidative enzymes (CYP or AIF), namely piperine; trichosta; piperettine; pyrrolidine, 1-[5-(1,3-benzodioxol-5-yl)-1-oxo-2,4-pentadienyl]-, and piperidine derivates. Furthermore, we showed that piperine, piperidine derivatives, and piperettin may contribute to cancer cell growth inhibition, which was shown by their binding score with EGFR. Of all these compounds (2E,4E,8E)-9-(benzo[d][1,3]dioxol-5-yl)-1-(piperidine-1-yl)nona-2,4,8-trien-1-one seems to have the most potential to bind with EGFR, MOA B, AIF, and CYP. All the *in silico* studies above show that besides piperine and piperidine derivatives, other compounds in *P. nigrum* such as piperamide, piperamine, sarmentine, trichosta, piperettine, and pyrrolidine, 1-[5-(1,3-benzodioxol-5-yl)- 1-oxo-2,4-pentadienyl]-, (E,E)-, also has potential as an anticancer drug.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

ACKNOWLEDGMENTS

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AUTHOR CONTRIBUTION:

Contribution	Wiraswati HL	Rohmawaty E	Ramadhanti J	Achadiyani	Khairani S	Berbudi A	Ma'ruf IF
Concepts or ideas	x						x
Design	x						x
Definition of intellectual content	x						x
Literature search		x		x			
Experimental studies	x		x	x			x
Data acquisition					x	x	
Data analysis			x		x	x	
Statistical analysis							
Manuscript preparation	x						x
Manuscript editing	x			x			x
Manuscript review	x	x	x	x	x	x	x

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