STRUCTURAL MODIFICATION OF PIPERINE FROM PIPER NIGRUM L.

SUWICHADA JAIPEA

A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF
THE REQUIREMENTS FOR THE MASTER DEGREE OF SCIENCE
IN CHEMISTRY
FACULTY OF SCIENCE
BURAPHA UNIVERSITY
2022

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The Thesis of Suwichada Jaipea has been approved by the examining committee to be partial fulfillment of the requirements for the Master Degree of Science in Chemistry of Burapha University

Advisory Committee

Principal advisor

.....

(Associate Professor Rungnapha Saeeng)

Examining Committee

..... Principal examiner
(Associate Professor Dr. Puttinan Meepowpan)

..... Member
(Associate Professor Dr. Rungnapha Saeeng)

..... Member
(Assistant Professor Dr. Uthaiwan Sirion)

..... Member
(Assistant Professor Dr. Anan Athipornchai)

..... Dean of the Faculty of Science
(Associate Professor Dr. Usavadee Tuntiwaranuruk)
.....

This Thesis has been approved by Graduate School Burapha University to be partial fulfillment of the requirements for the Master Degree of Science in Chemistry of Burapha University

..... Dean of Graduate School
(Associate Professor Dr. Nujjaree Chaimongkol)
.....

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Piperine is a major alkaloid from seeds of black pepper (*Piper nigrum* L.), possess diverse biological properties such as anti-inflammatory,anti-oxidant, anti-microbial, and neuroprotective activities. This natural compound has gained the wide attention in the medical community. This work was aimed to modify the piperine structure to improve the efficacy for the treatment of Alzheimer's disease. The thesis was divided into two parts, the first part, a novel series of piperine amide analogues were designed and synthesized from piperine *via* aminolysis reaction to obtain the new 29 analogues in moderate to excellent yields. In the second part, novel 1,2,3-triazole piperine analogues were designed and synthesized by performing a key step click reaction through one-pot two-step to obtain new 25 analogues in low to excellent yields. All synthetic compounds were investigated for their antioxidant, acetylcholinesterase (AChE), and butyrylcholinesterase (BuChE) activities. Among the design compounds having a hydroxy group at the piperazine showed the most potent antioxidant activity (IC_{50} of 0.04 ± 0.00 μM). Its activity was also superior to that of standard ascorbic acid. Compound 7a had good anti-AChE activity with an IC_{50} of 37.37 ± 0.04 μM. Furthermore, compound 3z was found to be the most potent anti-BuChE derivative with an IC_{50} value of 4.60 ± 0.01μM and higher than galantamine as a standard drug up to 8-fold. Molecular modeling and kinetic studies showed that compounds 7a and 3z bound simultaneously to the peripheral anionic site (PAS) and catalytic sites (CAS) of the AChE and BChE. Thus, compounds 7a and 3z would be promising candidates as lead for further development as an anti-AChE and anti-BuChE agent for the treatment of Alzheimer's disease.

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Suwichada Jaipea

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CHAPTER 1

INTRODUCTION

Alzheimer’s disease (AD) is a chronic neurodegenerative disease that usually starts slowly and gradually worsens over time (Francis et al., 1999). As the disease progresses, a person with Alzheimer's disease will develop severe memory impairment and lose the ability to carry out everyday tasks. A central event in the pathogenesis of AD is the death of cholinergic neurons in the basal forebrain area which results in the deficit of acetylcholine (ACh) (Venkatasamy, Faas, Young, Raman, & Hider, 2004). Currently, cholinesterase inhibitors (ChEIs) can be increasing synaptic levels of ACh, thus used as drugs for the treatment of AD. Unfortunately, the success of drug treatment is very limited because the drug could only temporarily improve memory but cannot stop the process of neurodegeneration (Qizilbash et al., 2007). Thus, the development of novel multifunctional agents is necessary for future disease therapies.

Piper nigrum L., also commonly known as the black pepper, which a plant in the Piperaceae family. This traditional medicinal herb has been widely found in many Asian countries, especially in the Eastern part of Thailand. The black pepper has been generally used for the treatment and alleviation of pain, chills, rheumatism, influenza, muscular pains, chills, and fevers, including prevention of Alzheimer's disease in the elderly and also helps accelerate liver function to destroy toxins (Gupta et al., 2000). Piperine (**1**), as the most abundant alkaloid in black pepper, was the first isolated from the extract of pepper by Hans Christian Ørsted in 1819. Recently, it has been reported a broad spectrum of biological activities such as anti-Alzheimer, antioxidant, antimutagenic, anti-tumor (Anith, et al., 2018), antimicrobial antineoplastic agents (Venugopal D. et al., 2014), anti-inflammatory, anti-malarial, and cure for stomachache (Aziz D.M. et al., 2015). In addition, the structural modification of piperine for increasing the biological properties has been receiving attention from many researchers.

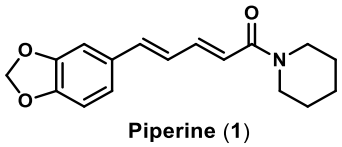


Figure 1 The chemical structure of piperine (**1**).

The piperazine as nitrogen-containing six-membered heterocyclic compounds have been found in the breakthroughs in Alzheimer's disease treatment, anticancer and antibacterial drugs (Meena et al., 2015).

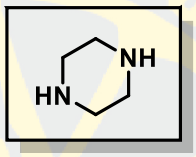


Figure 2 The chemical structure of piperazine.

Triazole is an important class of heterocyclic compounds that exhibited a wide range of pharmacological activities such as anti-HIV, anticancer, antitumor, anti-Alzheimer, antibacterial, antiviral, and antidiabetic, etc (Bozorov et al., 2019). In general, the formation of 1,2,3-triazole compounds *via* Cu(I)-catalyzed alkyne-azide 1,3-dipolar cycloaddition (CuAAC) reaction often referred to click chemistry, which has been widely used for the rapid assembly of heterocyclic molecules. (Wang et al., 2016). In recent years, a novel class of triazole-containing derivatives was synthesized and evaluated as lead compounds for diverse biological agents, including Alzheimer's disease activities (Xu et al., 2019).

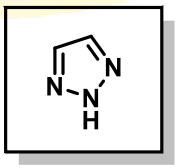
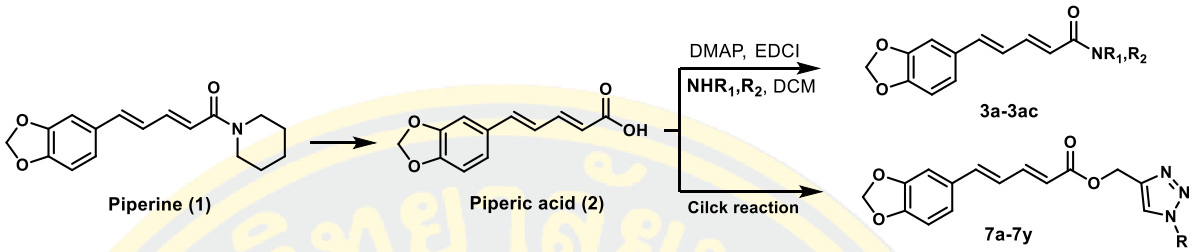


Figure 3 The chemical structure of triazole.

Base on the above-mention reports, we modified at amide position of piperine led to a new series of piperine analogues by substitution reaction with various types of piperazine and triazole through aminolysis to obtain piperine analogues **3** and *O*-

alkylation followed by CuAAC reaction to obtain piperine analogues **7**. All synthetic analogues were evaluated for Alzheimer's inhibitory activity.



Scheme 1 Strategies for structures modification of piperine.

1.1 Objectives

1. To modify the structure of piperine to new amide analogues *via* aminolysis.
2. To modify the structure of piperine to new 1,2,3-triazole-piperine analogues *via* click reaction in one-pot two-step process.
3. To study the antioxidant activity, acetylcholinesterase (AChE), and butyrylcholinesterase (BuChE) inhibitory activities, and kinetic studies of the synthetic compounds.

1.2 Contribution to knowledge

1. To establish convenient methods for modification of piperine.
2. To provide data of the bioactivities of synthetic piperine analogues for future drug development for anti-Alzheimer’s agents.

1.3 Scope of the study

1. Extraction and purification of piperine from *Piper nigrum* L.
2. Structural modification of piperine by chemical reaction.
3. Identification of the structure of all piperine analogues by spectroscopic methods.

4. Evaluation of the antioxidant activity, acetylcholinesterase (AChE), and butyrylcholinesterase (BuChE) inhibitory activities, kinetic studies of the resulting synthetic compounds.

CHAPTER 2

LITERATURE REVIEWS

2.1 Structure of alkaloid from *Piper nigrum* L.

Piperine (**1**)**,** extracted from *Piper nigrum* L. has been verified to display diverse biological activities such as anti-Alzheimer (Chonpathompikunlert, Wattanathorn, & Muchimapura, 2010), antidepressant, anticonvulsant, anti-ischemic (Wang et al., 2019), antioxidant, antimutagenic, anti-tumor (Anith et al., 2018), antimicrobial, antineoplastic (Venugopal et al., 2014), anti-inflammatory, anti-malarial and cure for stomachache (Aziz et al., 2015). The structure related to alkaloid such as piperyline (**3**), piperlonguminine (**4**) and piperettine (**5**) were isolated together with piperine (**1**) from this herb (Figure 4) (Dawid et al., 2013).

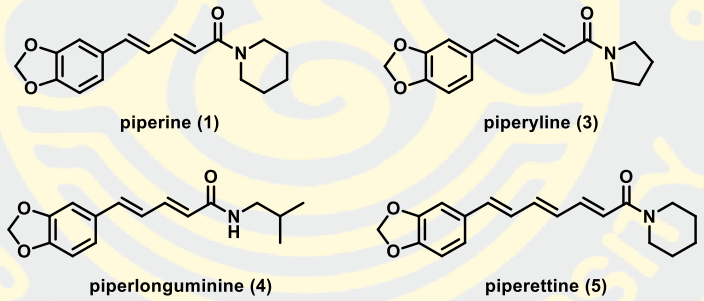


Figure 4 The structure of related alkaloid from *Piper nigrum* L.

2.2 Literature reviews of piperine in Alzheimer's disease.

Chonpathompikunlert et al., 2010 reported the importance of piperine in the prevention of neurodegeneration and cognitive impairment in animals with Alzheimer's disease. The results showed that piperine at dosages ranging from 5, 10, and 20 mg/kg BW, could significantly decrease the activity of acetylcholinesterase enzyme and improve neurodegeneration induced by direct orally intracerebroventricular administration of the ethylcholine aziridinium ion (AF64A). Moreover, piperine also demonstrated the increase of neuron density in the hippocampus, which played an important role in the treatment of Alzheimer's disease.

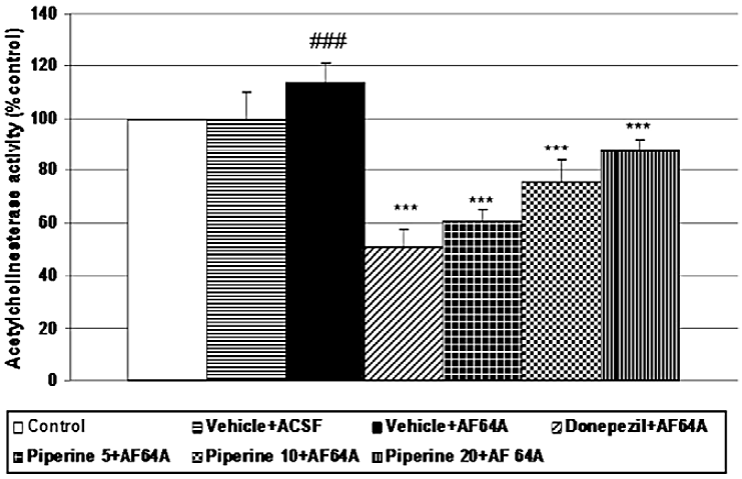


Figure 5 Effect of piperine on the activity of acetylcholinesterase enzyme in hippocampus.

Figueredo et al., 2015 showed the effect of piperine, acid **5**, and ester **6** on the inhibitory activity of acetylcholinesterase (AChE) at a concentration of 1 mM (Table 1) together with the molecular docking simulations of piperine and its derivatives within the binding pocket of AChE (Figure 6). The result docking simulation displays the possible electrostatic interactions of the acid **5** and ester **6** with amino acid Trp84 relate to the increased anticholinesterase activity. This amino acid is important in the interactions with AChE inhibitors currently approved for the treatment of Alzheimer's disease, as well as a key starting point for the study and develops novel AChE inhibitors.

Table 1 Percentage AChE inhibitory activity of piperine, piperic acid and piperic ester.

Compounds	**% Inhibition at 1 mM***
Piperine (**1**)	46.5
Piperic acid (**5**)	50.6
Piperic Ester (**6**)	63.6
Physostigmine	93.9

* Results are the mean (%) (n=3)

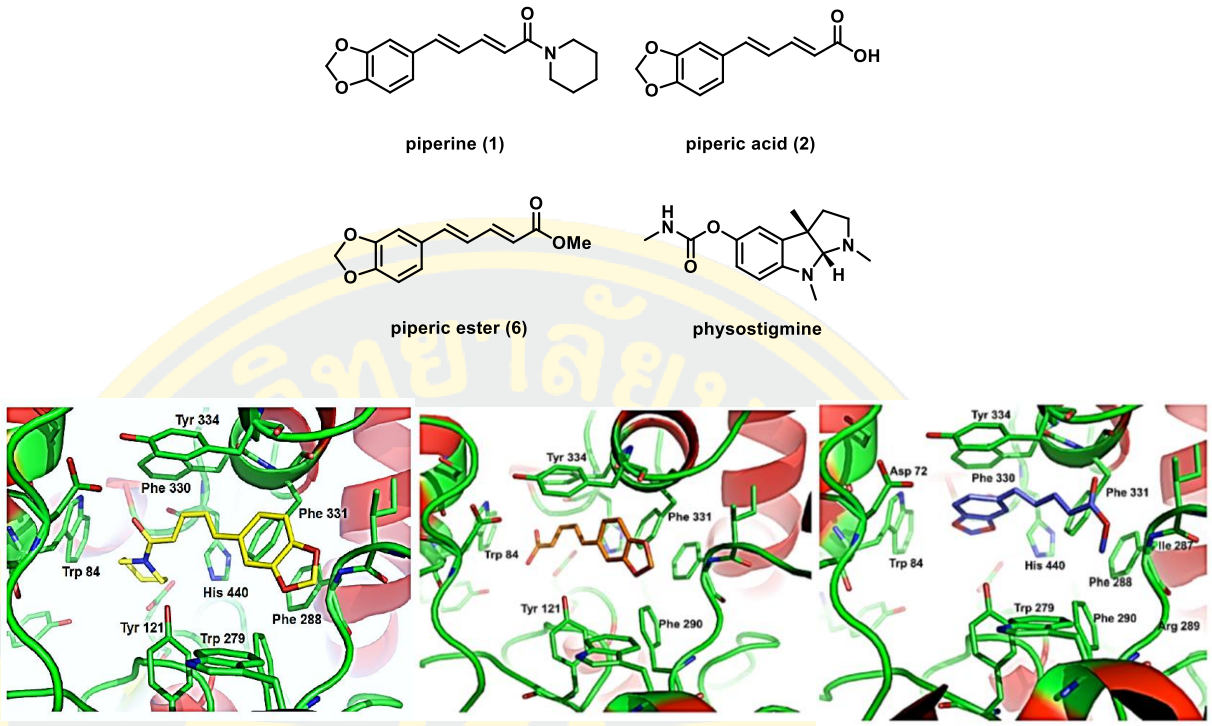


Figure 6 Binding mode suggested by docking for piperine **1**, piperic acid **5**, and piperic ester **6** (carbon atoms in yellow, orange and blue, respectively) with AChE, highlighting the main residues that contribute to the virtual interaction.

Table 2 Classification of the potential interaction types detected between each ligand and AChE residues in the best docking pose solution from the GOLD program.

Ligand	Electrostatic	π-π	Van der Waals
1	Phe288, Ser286, Arg289	-	Gly441, Glu199, Trp84 Ser200, His440, Gly118 Phe330, Tyr334, Tyr121 Phe331, Phe290, Trp279 Ile287, Gly335
5	Trp 84	Phe331	Phe330, Try334, Phe290 Tyr121
6	Trp84, His440 Phe331, Phe288 Gly335	Phe330	Tyr334, Asp72, Phe290 Ile287, Arg289, Trp279

Tu et al., 2015 isolated twenty-one known alkamides from the ethyl acetate extract of *P nigrum* fruit and investigated for their anticholinesterase and antioxidant activities (Figure 7). Piperine (**1**) was potent inhibitor with IC_{50} values of 63.16 μg/mL (AChE) and 25.11 μg/mL (BChE), respectively (Table 3). Whiles, compounds **11-14** featuring no methylenedioxyphenyl (MDP) ring presented no inhibitory activity against both enzymes which suggested that the MDP ring is an essential feature in ChE inhibitory activity. Moreover, compounds **18-20** with overlong carbon chain diminished the inhibition potency by reducing the enzyme affinity. Compound **21** showed more activity of BChE inhibition and higher selectivity (BChE IC_{50} = 12.88 μg/mL; AChE IC_{50} > 200 μg/mL) implied that the hydroxyl on phenyl ring was favorable for the BChE inhibition and selectivity. The anti-ChE activities of **5** were 6 times (AChE) and 1.5 times (BChE) higher than that of **1**, which indicated that three conjugated double bonds would benefit the inhibition. In addition, the best DPPH radical-scavenging potency (EC_{50} = 11.82 μg/mL) was provided by **7**, which is closer to that of ascorbic acid. Results indicated that some alkamides could be multifunctional lead candidates for Alzheimer's disease therapy.

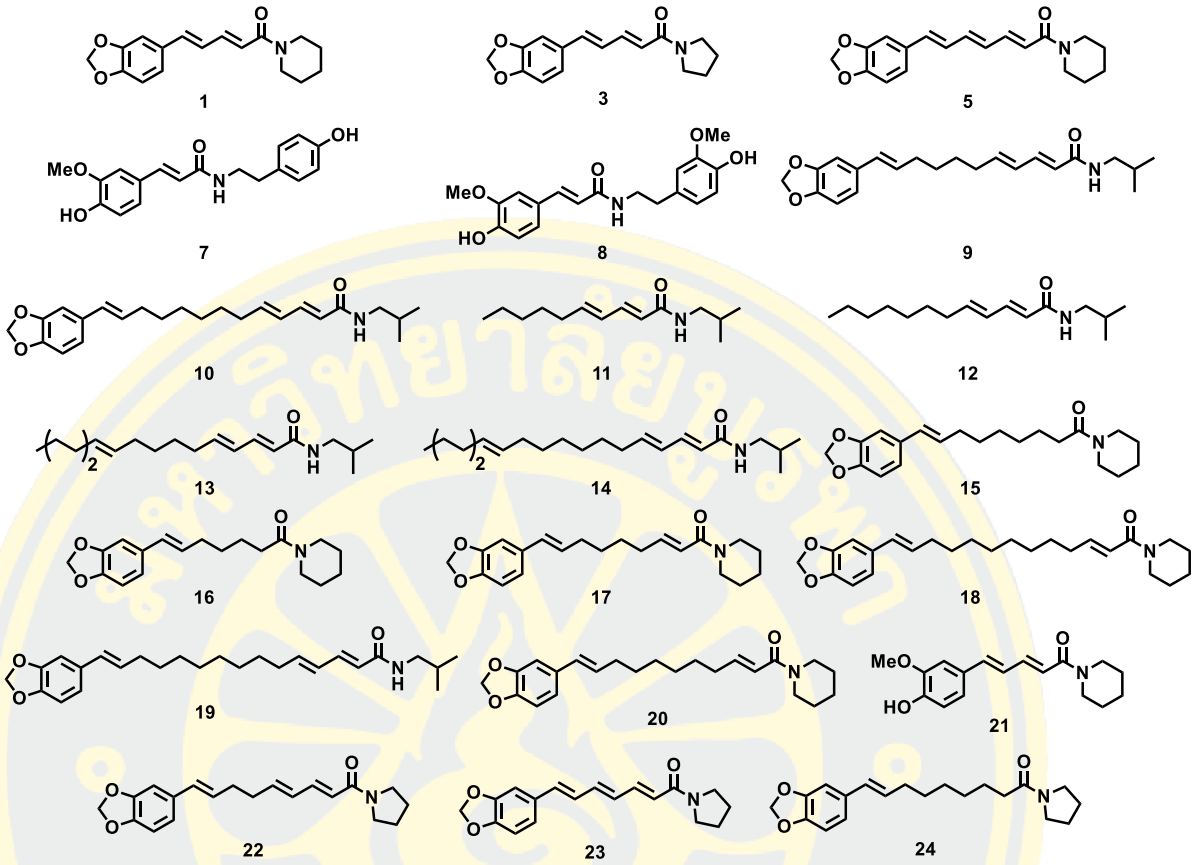


Figure 7 Compounds isolated from EtOAc extract of Piper nigrum L.

Table 3 Cholinesterase inhibitory and antioxidant activities of extracts and pure compounds of Piper nigrum L[a].

Samples	IC_{50} Values (µg/mL)		Selectivity for BChE[c]	DPPH radical scavenging activity		CLogP
	AChE	BuChE		200 µg/mL	EC_{50} (µg/mL)	
1	63.16 ± 1.09[b]	25.11 ± 0.21[b]	2.52	10.82 ± 1.60[b]	NT	3.31
2	69.88 ± 1.11[b]	36.19 ± 1.22[b]	1.93	12.92 ± 1.11[b]	NT	2.75
4	10.57 ± 1.23	14.89 ± 0.51	0.71	24.14 ± 3.49[b]	NT	3.92
7	NT	NT	/	86.92 ± 0.50	11.82 ± 1.58	2.42
8	NT	NT	/	66.06 ± 5.24[b]	108.62 ± 14.44[b]	2.27
9	119.23 ± 2.56[b]	107.66 ± 8.24[b]	1.11	12.53 ± 2.06[b]	NT	5.76
10	74.37 ± 3.08[b]	>200	<0.37	13.53 ± 2.81[b]	NT	6.82
11	NT	NT	/	13.61 ± 2.39[b]	NT	4.15
12	NT	NT	/	10.82 ± 1.46[b]	NT	5.21
13	NT	NT	/	NT[g]	NT	6.84
14	NT	NT	/	13.23 ± 2.54[b]	NT	7.90
15	112.40 ± 0.80[b]	74.90 ± 9.83[b]	1.50	15.48 ± 3.35[b]	NT	4.76
16	49.01 ± 2.96[b]	53.20 ± 5.10[b]	0.92	13.04 ± 2.54[b]	NT	3.71
17	84.35 ± 6.75[b]	21.43 ± 2.17	3.94	25.87 ± 6.25[b]	NT	5.28
18	NT	NT	/	22.28 ± 5.79[b]	NT	7.40
19	NT	NT	/	19.30 ± 1.58[b]	NT	7.88
20	NT	NT	/	13.22 ± 1.39[b]	NT	6.34
21	>200	12.88 ± 0.35	>15.53	42.84 ± 4.55[b]	NT	2.53
22	NT	NT	/	21.85 ± 2.62[b]	NT	4.30
23	11.80 ± 0.34	15.51 ± 0.29	0.76	29.68 ± 0.56[b]	NT	3.36
24	104.21 ± 3.43[b]	59.77 ± 1.37[b]	1.74	8.67 ± 2.89[b]	NT	4.20
Galantamine[e]	0.52 ± 0.01	4.27 ± 0.25	0.12	-	-	-
ascorbic acid [f]	-	-	-	96.61 ± 0.05	4.09 ± 0.10	-

[a]Data presented as Mean ± Standard error mean (SEM).
[b]p>0.05, being regarded as significant compared to control.
[c]Selectivity for BChE is defined as IC_{50}(AChE)/ IC_{50}(BChE).
[d]Sample was tested at 20 µg/mL due to turbidity at high concentration in assay.
[e]Standard drug for AD.
[f]Standard antioxidant.
[g]Amount of the compound 10 was not adequate to test.
NT, not tested.

Wang et al., 2019 investigated the therapeutic potential and neuroprotective mechanisms of piperine in an experimental mouse model by intracerebroventricular (ICV) infusion of streptozotocin (STZ), similar to neuropathological and biochemical alterations of Alzheimer's disease (Table 4). The piperine improved the elevated MDA level and significantly reduced SOD, CAT, and GSH levels compared to control, indicating a decreased oxidative stress in the hippocampus. Moreover, the elevation of hippocampal nitrite level, confirming reduced nitrative stress. The elevation of

hippocampal nitrite levels after treatment with **1**, confirming the reduced nitrative stress. This effect indicated that **1** can cause reverse neuroinflammation and restore abnormal neurotransmission induced by ICV-STZ, which also contributes to the treatment of Alzheimer's disease.

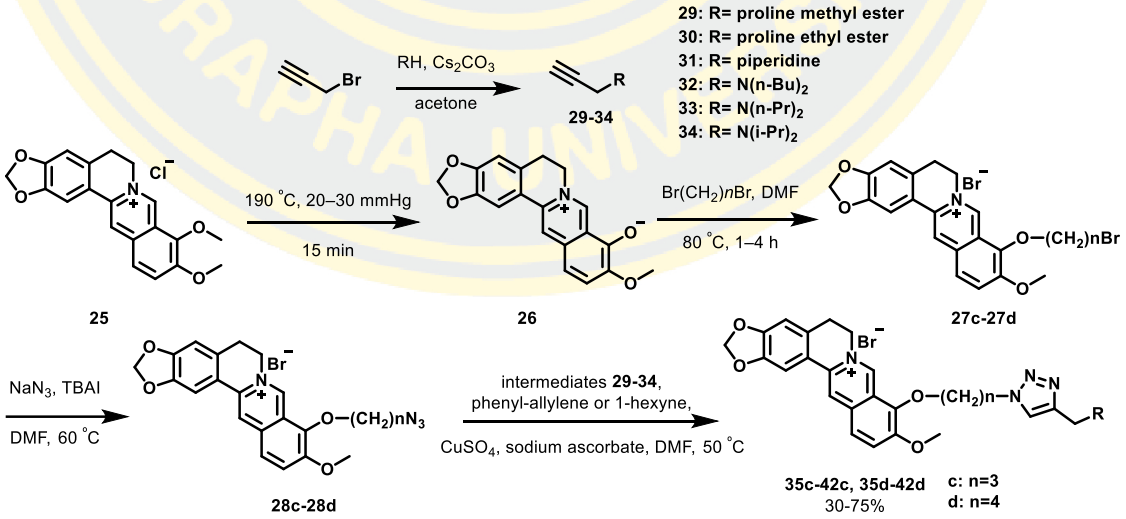
Table 4 Effect of piperine on MDA, SOD, CAT, GSH and nitrite levels in hippocampus of ICV-STZ-infused mice.

Groups	MDA (nmol/mg)	SOD (U/mg)	CAT (U/mg)	GSH (U/mg)	Nitrite (µmol/mg)
Control	2.5 ± 0.3	15.4 ± 0.74	4.3 ± 0.3	47.6 ± 2.2	2.3 ± 0.2
ICV-control	2.4 ± 0.2	14.9 ± 0.9	4.1 ± 0.4	45.7 ± 2.6	2.5 ± 0.4
ICV-STZ	8.8 ± 0.6**	4.7 ± 0.3**	1.9 ± 0.2**	14.9 ± 1.2**	6.8 ± 0.7**
ICV-STZ piperine2.5 (mg/kg)	6.2 ± 0.7**	8.4 ± 0.6**	2.5 ± 0.4*	30.1 ± 1.9*	5.2 ± 0.7**
ICV-STZ piperine5.0 (mg/kg)	3.9 ± 0.4##	11.9 ± 0.8##	3.6 ± 0.4#	38.6 ± 2.3##	3.9 ± 0.4##
ICV-STZ piperine10.0 (mg/kg)	2.8 ± 0.3##	14.4 ± 0.7##	4.1 ± 0.3##	44.2 ± 1.8##	2.8 ± 0.3##

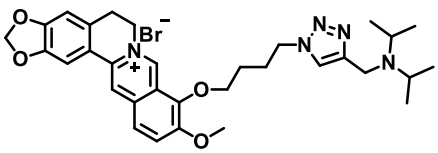
* Pb.05, ** Pb.01, compared with control; # Pb.05, ## Pb.01, compared with ICV-STZ- treated mice.

2.3 Literature reviews of triazole scaffold in the inhibitory activity of Alzheimer's disease.

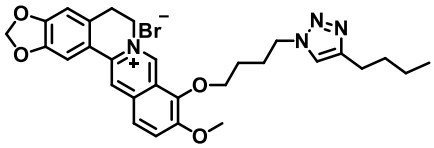
Shi et al., 2011 synthesized and evaluated acetylcholine (ACh) and β-amyloid inhibition of novel triazole containing berberine derivatives. The synthetic route started from demethylation of berberine **25** to obtain compound **26** in good yield. Alkylation of **26** with 1,3-dibromopropane or 1,4-dibromobutane in DMF gave compounds **27c-27d**, then azidation with sodium azide to provide compounds **28c-28d**. Finally, the design products **35c-42d** and **35c-42d** were obtained in 30-75% yields *via* click reaction (Scheme 2). All synthetic compounds were evaluated *in vitro* cholinesterase inhibitory activity. Among them, diisopropylamino substitution at the 4-position of the triazole ring **40d** showed the most potent activity against AChE with IC_{50} value of 0.044 ± 0.001 μM (Figure 8). Kinetic study of **40d** showed mixed-type inhibition with the binding at CAS and PAS regions of AChE. Molecular modeling studies showed π–π interactions between compound **40d** and amino acids Trp84 (4.09 Å) and Phe330 (4.33 Å) in catalytic sites (CAS) of AChE enzyme (Figure 9). Remarkable, compound **42d** possessing butyl at 4-position of triazole ring demonstrated the highest potency of β-amyloid aggregation (77.9% inhibition).



Scheme 2 Synthesis of triazole containing berberine derivatives **35c-42c** and **35d-42d.**



40d
IC_{50} AChE = 0.044 ± 0.001 μM



42d
β-amyloid aggregation inhibition = 77.9%

Figure 8 The most active compounds **40d** and **42d**

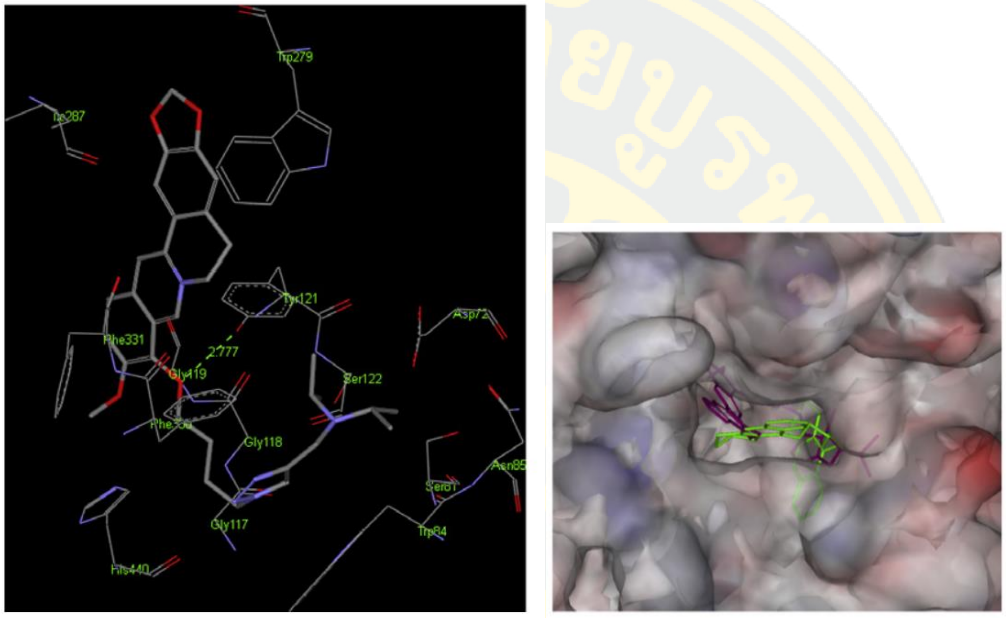
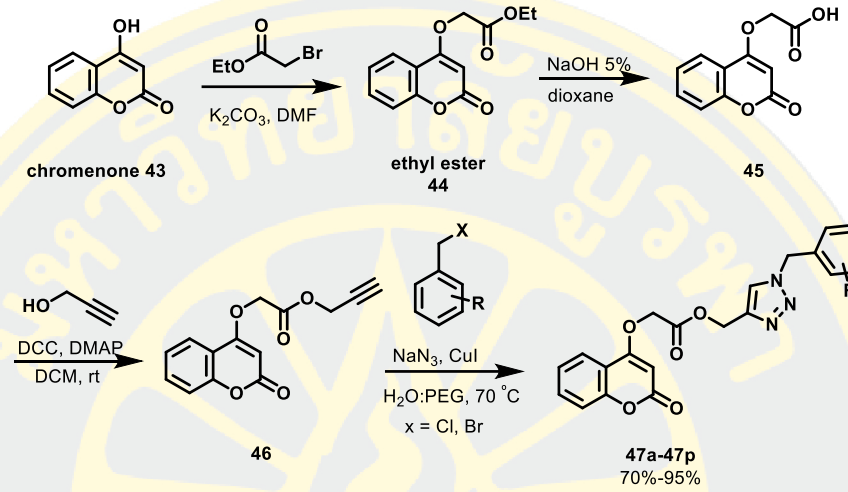


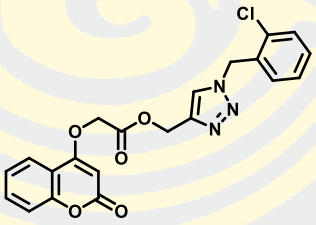
Figure 9 Docking models of the compound-enzyme complex. Representation of compound **40d** docked into the binding site of AChE. Hydrogen-bonding interaction between ligand and residues Tyr121 is shown with the green line.

Bagheri et al., 2015 synthesized 4-hydroxycoumarin containing benzyl-1,2,3-triazole scaffold for evaluated their anticholinergic activity (Scheme 3). This series were prepared from chromernone **43** reacted with ethyl 2-bromoacetate and K_2CO_3 in DMF to generate ethyl ester **44**. Next, hydrolysis of **44** with NaOH in dioxane provided carboxylic compound **45** followed by esterification with propargyl alcohol to generate compound **46**. Finally, the azidation and click reaction of compound **46** by using benzyl halide gave design products **47a-47p** in 70-95% yields. All synthetic compounds were investigated *in vitro* cholinesterase inhibitory activity. Among them, 2-chorobenzyl derivative **47k** showed the most potent activity against AChE with IC_{50} value of 0.18 μM higher than standard drug tacrine (IC_{50} = 0.35 μM) (Figure 10).

Kinetic study of **47k** showed non-competitive inhibition with K_i value of 1.27 μM. Molecular docking studied of the most potent inhibitor **47k** displayed π–π interaction between chlorophenyl moiety and amino acid Trp83 and the π–π stacking between coumarin phenyl ring and Tyr333 in active side of AChE (Figure 11).



Scheme 3 Synthesis of 4-hydroxycoumarin benzyl-1,2,3-triazole derivatives **47a-47p.**



47k, 80%
IC_{50} AChE = 0.18 μM

Figure 10 The most active compound **47k.**

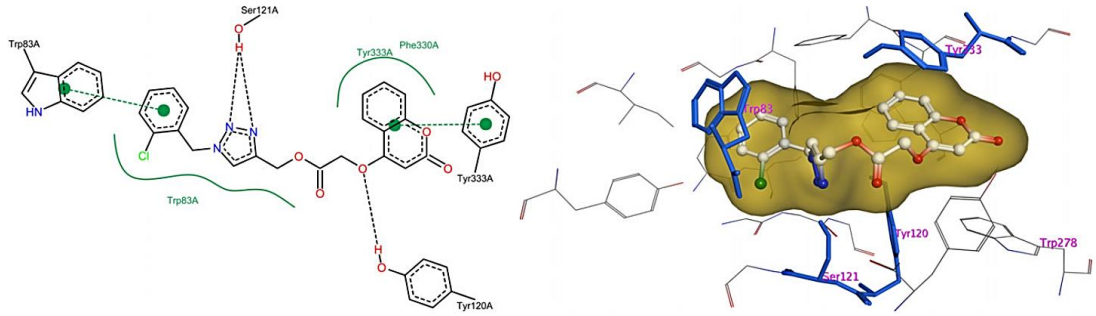
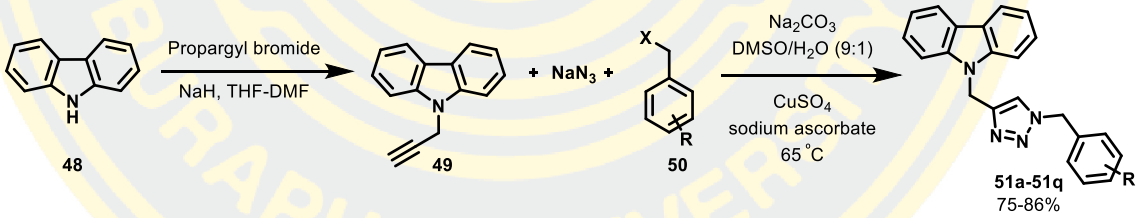
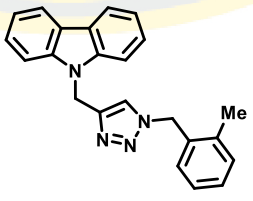


Figure 11 2D (left) and 3D (right) representation of proposed binding mode and notable interactions of compound **47k** in the active site of AChE.

Akrami et al., 2015 designed and synthesized triazole-containing carbazole derivatives as potential anti-Alzheimer's agents. The preparation of target compounds **51a-51q** were carried out between carbazole **48** reacted with propargyl bromide and sodium hydride (NaH) as a base to generate intermediate alkyne **49**. Next, copper-catalyzed azide-alkyne cycloaddition (CuAAC) of alkyne **49** with sodium azide and substituted benzyl halide **50** in presence of NaH in THF-DMF to obtain seventeen compounds **51a-51q** in 75-86% yields under one-pot synthesis concept (Scheme 4). Among all synthetic derivatives, compound **51c** bearing 2-methylbenzyl on the pendant 1,2,3-triazole group showed the most potent AChE inhibitory activity with IC_{50} value of 1.93 ± 0.11 µM (Figure 12). Compound **51c** could be served as a mixed type of inhibition AChE with a constant K_i value of 1.31 µM. Docking studies confirmed that carbazole moiety and triazole ring formed π–π interactions with amino acids Trp84 and Tyr334, respectively (Figure 13). The SAR studies revealed that the para position on the benzyl ring **51j-51n** reduced anti-AChE activity, whereas the ortho and meta positions on the benzyl ring **51a-51i** increased inhibitory activity and selectivity inhibition against AChE.



Scheme 4 Synthesis of triazole-containing carbazole derivatives **51a-51p.**



51c, 75%
IC_{50} AChE = 1.93±0.11 µM

Figure 12 The most active compound **51c.**

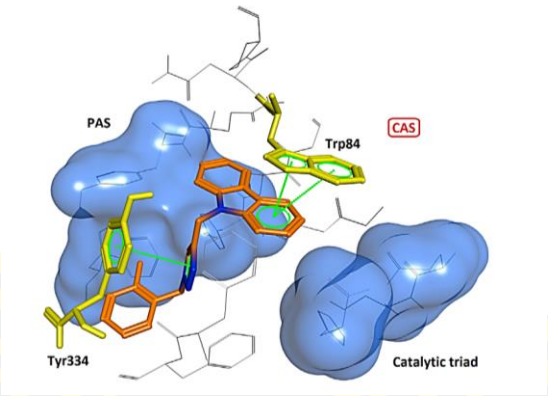
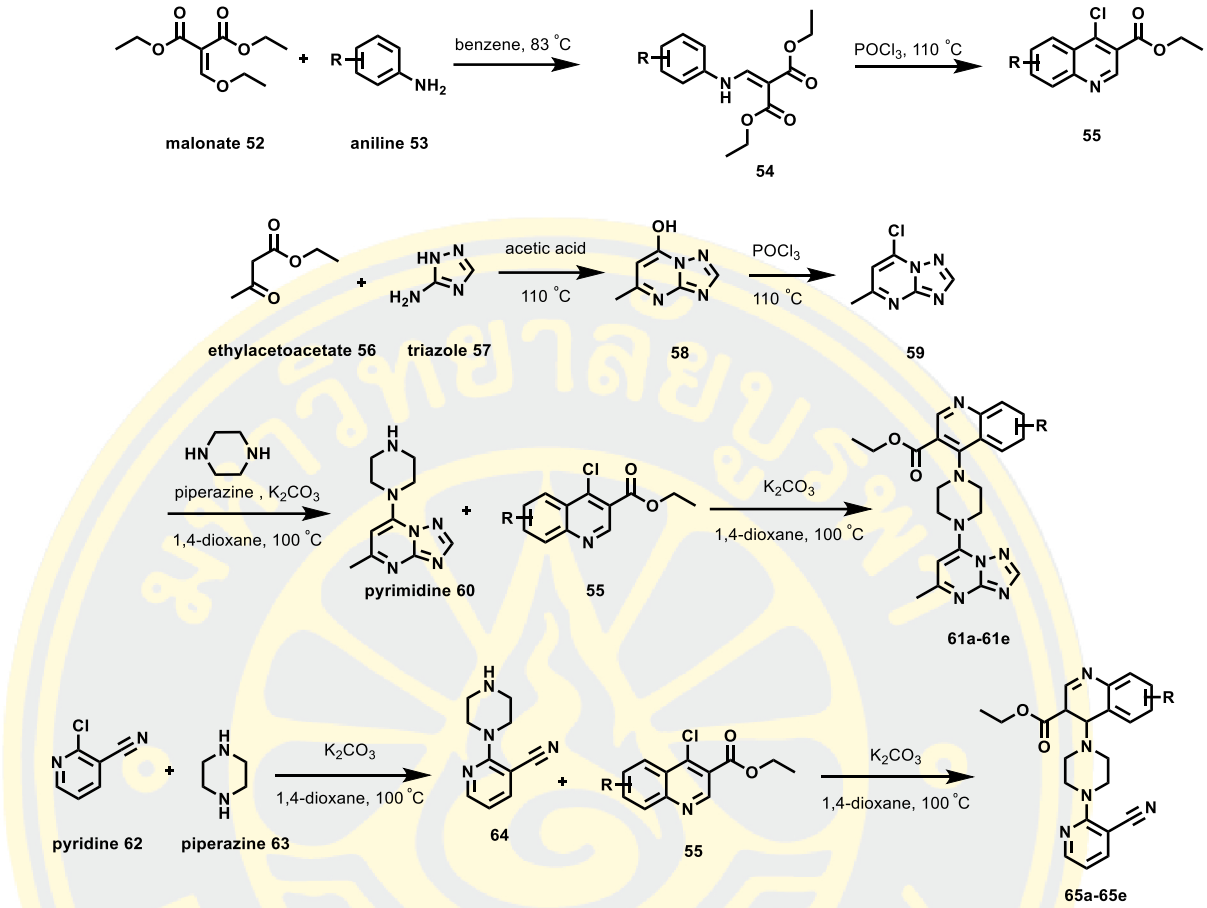


Figure 13 Schematic interaction of compound **51c** with the active site of AChE. π-π interactions are represented as green dashed lines.

Kumar et al., 2016 reported the synthesis and screening of triazolopyrimidine derivatives as multifunctional agents for Alzheimer' disease (Scheme 5). The substituted quinoline was prepared from malonate **52** reacted with substituted aniline, followed by intramolecular cyclization with phosphorous oxychloride to give substituted quinoline **55**. Next, ethyl acetoacetate **56** reacted with triazole **57** in acetic acid afforded compound **58**. Chlorination of **58** with phosphorous oxychloride rendered the corresponding chloride **59**, and then treated with piperazine to prepare pyrimidine **60**. The triazolopyrimidine derivatives **61a-61e** were produced by quinoline **55** reacted with pyrimidine **60** under basic conditions. Next series, starting by pyridine **62** reacted with piperazine **63** under basic condition to generate compound **64**. Finally, compound **64** reacts with substituted quinoline **55** in the presence of potassium carbonate to provide products **65a-65e**. All synthetic compounds were evaluated for cholinesterase inhibitory. Among all the synthetic analogues, compounds **61d** and **61e** have contained fluoride on quinoline ring exhibited good inhibitory activities against AChE and BuChE (Figure 14).



Scheme 5 synthesized of triazolopyrimidine derivatives **61a-61e**, **65a-65e**.

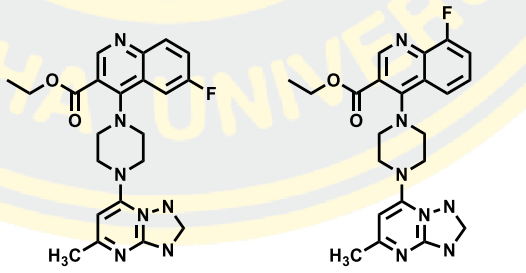
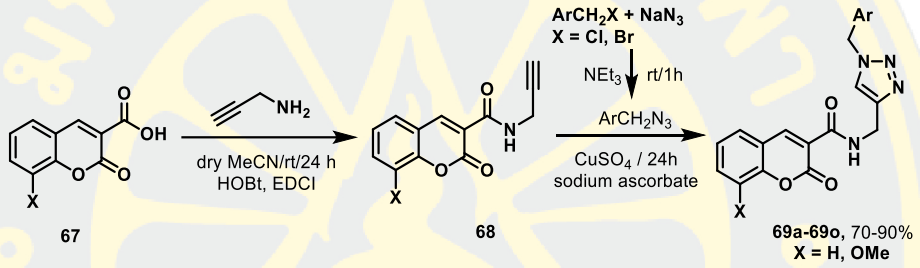


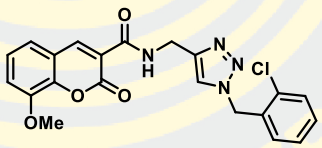
Figure 14 The most active compounds **61d** and **61e.**

Saeedi et al., 2016 synthesized novel chromenones linked to the 1,2,3-triazole ring system and evaluated for their biological activities against Alzheimer's disease. The target products **69a-69o** were prepared from coumarin-3-carboxylic acid **67** coupling with propargylamine follow by reacted with various organic azides,

generated *in situ* from the reaction between benzyl halides and sodium azide (Scheme 6). Investigation of anti-AChE and anti-BuChE activity of all synthesized compounds found that compound **69m** bearing methoxy group on the chromenone moiety and 2-chlorophenyl on the pendant 1,2,3-triazole group displayed good anti acetylcholinesterase activity with IC_{50} values of 15.42 ± 0.53 µM (Figure 15). Compound **69m** also showed a mixed type of inhibition, not showing activity against beta-secretase (BACE1) and neuroprotective effect against H_2O_2-induced cell death in PC12 neurons. Docking studies confirmed the binding activity of **69m** both the catalytic active site (CAS) and the peripheral anionic site (PAS) of AChE.



Scheme 6 Synthesis of novel chromenones linked to 1,2,3-triazole ring **69a-69o**.

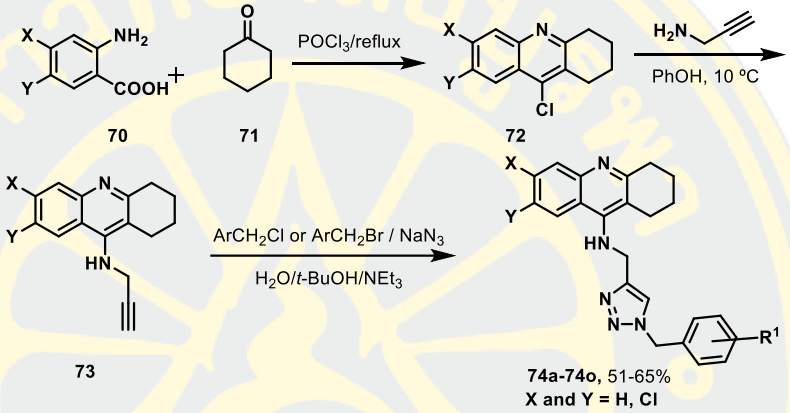


69m, 75%
IC_{50} AChE =15.42 ± 0.53 µM
IC_{50} BuChE = 96.13 ± 0.37 µM

Figure 15 The most active compound **69m.**

Najafi et al., 2017 reported a new series of tacrine-1,2,3-triazole hybrids derivatives **74a-74o** and evaluated for their both acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) inhibitory activity. The Friedel-Crafts cyclization reaction between anthranilic acid **70** and cyclohexanone **71** gave acridine **72**, then treated with propargylamine *via* propargylation follow by click reaction with *in situ* prepared azide derivatives gave the desired products **74a-74o** (Scheme 7). The *in vitro* AChE and BChE inhibitory activity of all compounds **74a-74o** were studied. The best anti-AChE was related to compound **74l** possessing chlorine at 7-position of acridine

moiety and 4-methoxyphenyl connected to 1,2,3-triazole ring (IC_{50} = 0.521 ± 0.025 µM). Compound **74j** without chlorine at 7-position of acridine moiety showed the most anti-BChE activity with IC_{50} = 0.055 ± 0.012 µM (Figure 16). *In vivo* evaluation of compound, **74l** confirmed the memory improvement in scopolamine-induced impairment. Molecular modeling and kinetics studies showed that compounds **74l** and **74j** bound to the catalytic site (CAS) and peripheral anionic site (PAS) of AChE and BChE, respectively.



Scheme 7 Synthesis of tacrine-1,2,3-triazole hybrids **74a-74o**.

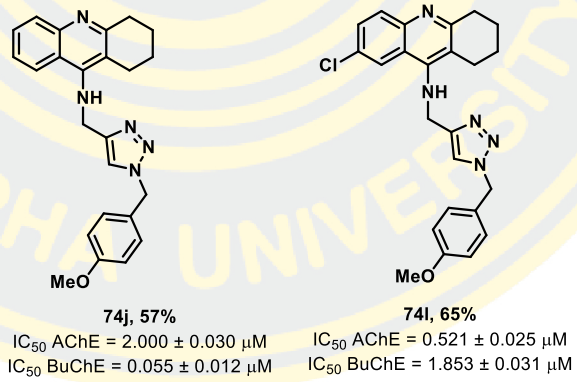
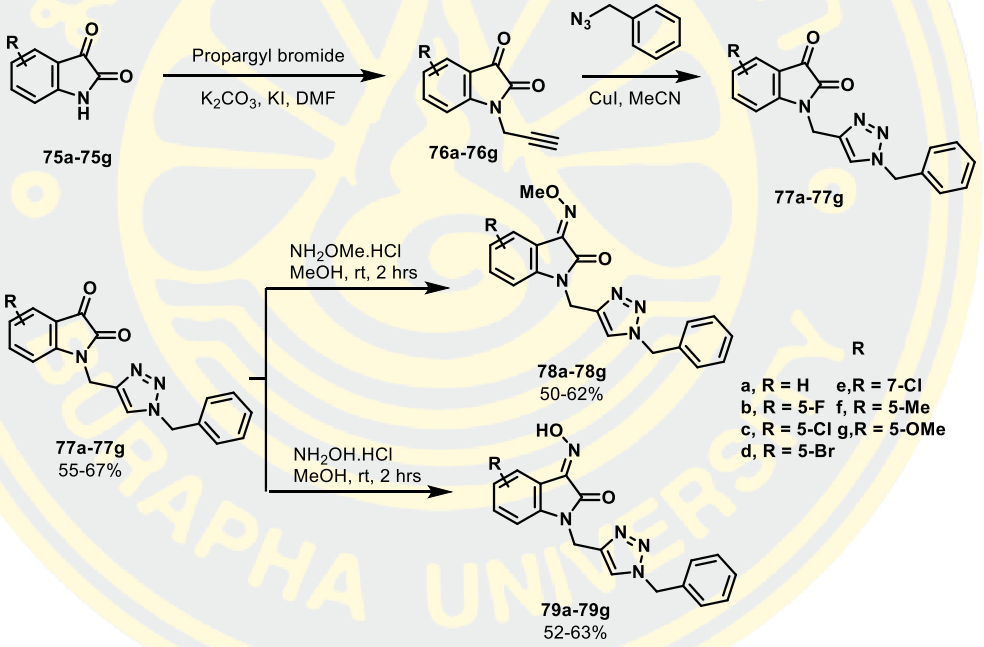


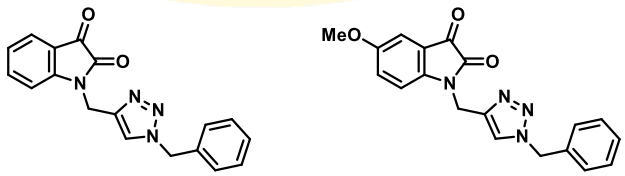
Figure 16 The most active compounds **74j** and **74l.**

Lan et al., 2019 reported the synthesis and evaluation of novel oxoindolin-2-one linked to 1,2,3-triazole ring derivatives. Initially isatins **75a-75g** reacted with propargyl bromide under basic condition via propargylation to produce intermediate **76a-76g**. Then, (azidomethyl)benzene was treated with CuI in acetonitrile to afford oxoindolin-2-one linked 1,2,3-triazole ring **77a-77g** in 55-67% yields. Compounds **77a-77g** were reacted with O-methylhydroxylamine hydrochloride in methanol to

give the target compounds **78a-78g** in 50-62% yields. Follow this condition, hydroxylamine hydrochloride was used instead of O-methylhydroxylamine hydrochloride to give the targeting compounds **79a-79g** in 52-63% yields (Scheme 8). The inhibitory activities of all synthetic compounds on AChE, DPPH scavenging, and cytotoxicity against three human cancer cell lines (SW620, human colon cancer; PC3, prostate cancer and NCI-H23, lung cancer) were evaluated. Among them, compounds **77a** and **77f** exhibited moderate inhibitory effect against AChE with %inhibition of 38.54 and 37.54%, respectively (Figure 17). Moreover, compounds **78c**, **78e**, **79c**, **79e**, and **79g** exhibited a strong cytotoxicity against human cancer cell lines with IC_{50} values in the range of 0.65-7.17 µM.



Scheme 8 Synthesis of oxoindolin-2-one linked to 1,2,3-triazole ring derivatives.



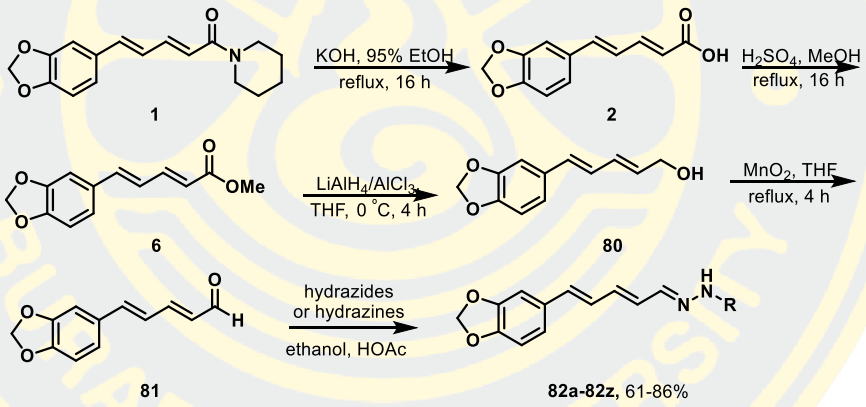
77a, 63%
%inhibition of AChE = 38.54

77f, 70%
%inhibition of AChE = 37.54

Figure 17 The most active compounds **77a** and **77f.**

2.4 Literature reviews of modification of piperine.

Qu et al., 2013 reported semi-synthesis and insecticidal activity of new piperine-based hydrazine derivatives **82a-82z**. Twenty-six new piperine-based hydrazone derivatives were generated from the basic hydrolysis reaction of piperine led to piperic acid **5**, then methyl piperate **6** was smoothly prepared by esterification of **5**. The reduction of ester **6** with $LiAlH_4$ and $AlCl_3$ gave alcohol **80** followed by oxidation with MnO_2 to produce aldehyde **81**. Finally, compound **81** was reacted with hydrazides or hydrazines to afford piperine-based hydrazone derivatives **82a-82z** in moderate to high yields (Scheme 9). Their insecticidal activity was evaluated against the pre-third-instar larvae of *M. separata in vivo*. Among them, **82b**, **82i**, and **82r** exhibited the pronounced insecticidal activity compared to toosendanin with a final mortality rate were 62.1%, 65.5%, and 65.5%, respectively (Figure 18).



Scheme 9 Synthetic route for the preparation of piperine-based hydrazone derivatives **82a-82z**.

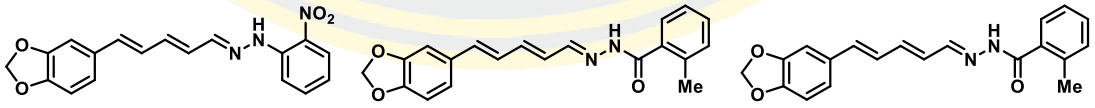
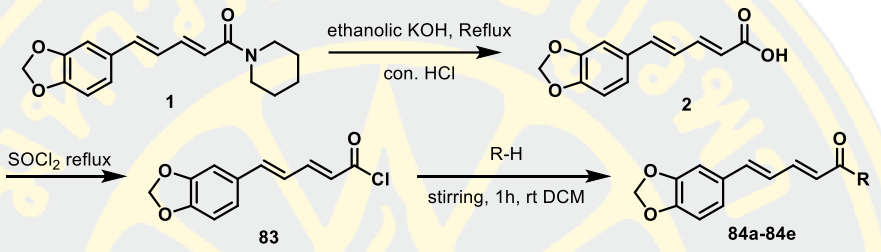


Figure 18 The most active compounds **82b, 82i**, and **82r** in the pronounced insecticidal activity.

Umadevi et al., 2013 synthesized piperine analogues and evaluated anticancer against human cancer cell lines (MCF-7, Breast Cancer cell line and Hela cervix cell line) and antibacterial activities. The preparation of target compounds **84a-84e** was

carried out *via* hydrolysis of **1** with KOH to afford piperic acid **5**, which then was converted into the acid chloride intermediate **83** by reaction with thionyl chloride. Finally, the nucleophilic substitution of **83** with substituted amines gave the piperine analogues **84a-84e** (Scheme 10). The bioactivities results demonstrated **84c** showed significant anticancer activity against Hela cervix cell line with IC_{50} value of 0.736 µM. (Figure 19). Piperine analogues demonstrated stronger antibacterial activity than piperine.



Scheme 10 Synthetic of piperine derivatives **84a-84e**.

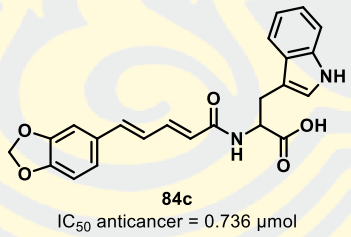
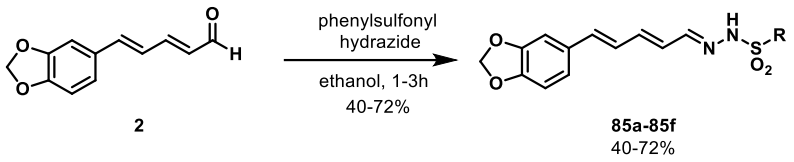


Figure 19 The most active compounds **84c** in anticancer activity.

Qu et al., 2015 prepared a series of piperine-based phenylsulfonylhydrazone derivatives 85a-85f for development to new insecticidal agents (Scheme 11). The aldehyde 5 reacted with different phenylsulfonyl hydrazides in ethanol to obtain piperine-based phenylsulfonylhydrazone derivatives 85a-85f in 40-72% yields. Among them, compound 85c (ND_{50} = 0.0074 µmol/larvae) and 85e (ND_{50} = 0.0075 µmol/larvae) were potent narcotic activity against the oriental armyworm, *M. separata*. Both compounds showed higher activity than wilfortrine (ND_{50} = 0.0207 µmol/larvae) and wilforgine (ND_{50} = 0.0086 µmol/larvae) (Figure 20).



Scheme 11 Synthetic route for preparation of piperine-based phenylsulfonyl-hydrazones **85a-85f**.

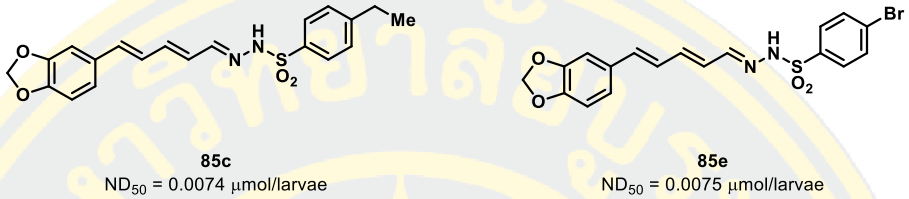
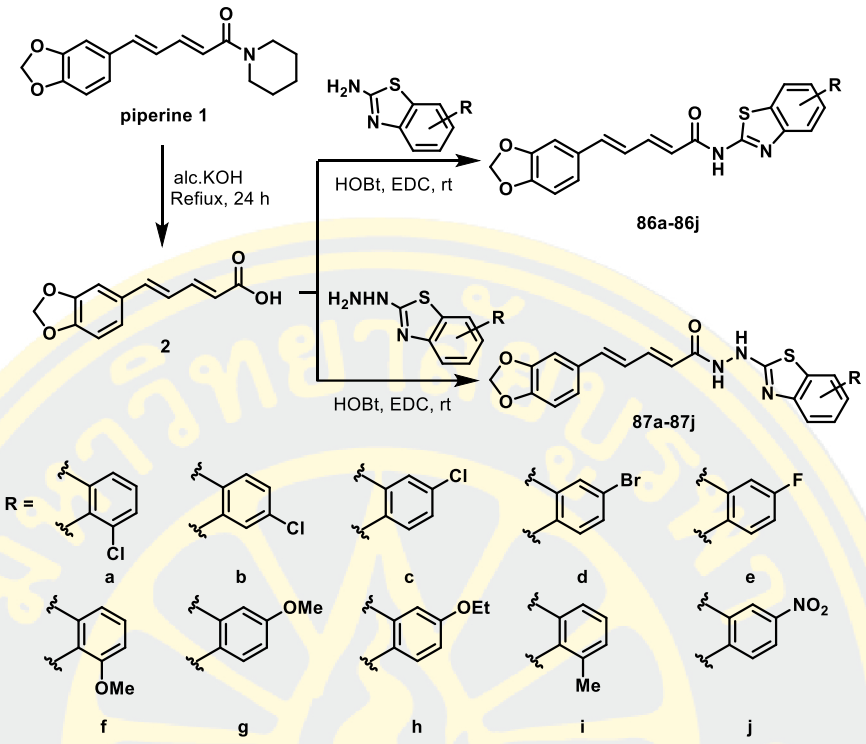


Figure 20 The most active compounds **85c** and **85e** in narcotic activity.

Kharbanda et al., 2016 synthesized twenty piperine derivatives containing benzothiazole moiety. The synthesis was carried out by hydrolysis of piperine under basic condition to obtain piperic acid and coupling with amine and hydrazine substituted benzothiazole to give piperine derivatives **86a-86j** and **87a-87j** (Scheme 11). The antidiabetic potential of twenty derivatives was studied and demonstrated that nine derivatives **86b,** and **87a-87h** exhibited better alleviation in plasma glucose levels than the reference rosiglitazone (Figure 21).



Scheme 12 Synthesis of novel piperine containing benzothiazole moiety.

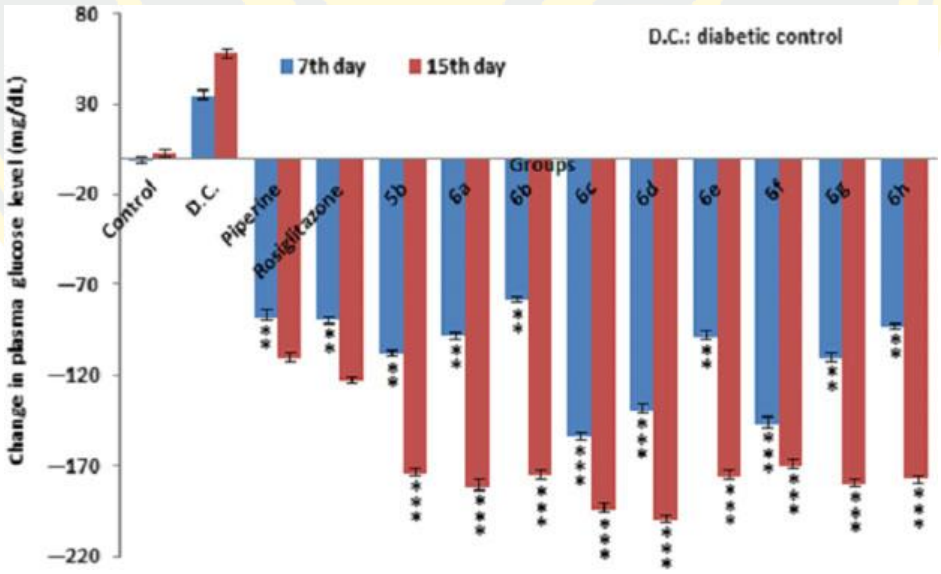
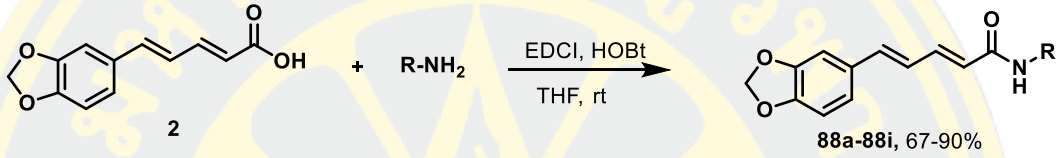


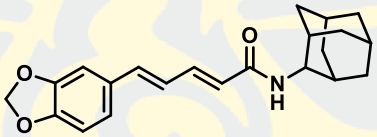
Figure 21 Antidiabetic effect of treating Wistar rats with synthesized compounds by oral glucose tolerance test.

Philipova et al., 2017 synthesized piperine amide analogs **88a-88i** by replacement of the piperidine moiety with different types of cyclic amines, including

adamantyl and monoterpene-derived fragments (Scheme 13). The *in vitro* antimycobacterial activity of all compounds was studied against reference strain *Mycobacterium tuberculosis* H37Rv. The results revealed that 3-exo-aminoisoborneol-derived amide **88g** showed the highest activity with MIC of 0.17 μM. The cytotoxicity against the human embryonal kidney cell line of **88g** was exerted moderate with IC_{50} value of 33.1 μM. Thus, this compound has low cytotoxicity with high antimycobacterial activities, represented by their selectivity index (SI > 194) (Figure 22).



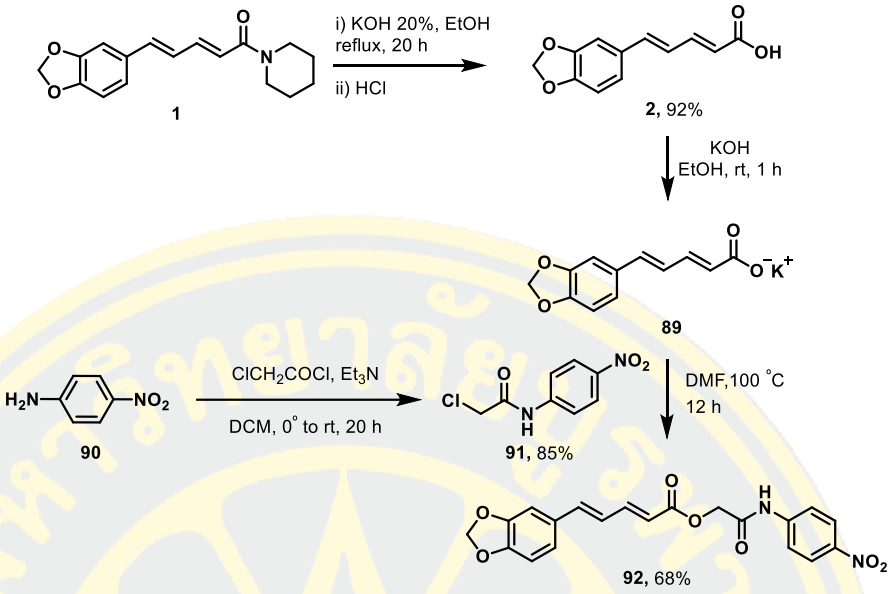
Scheme 13 Synthesis of piperine amide analogs.



88g, 67%
MTB MIC IC_{50} = 0.17 μM
IC_{50} = 33.1 ± 2.3 mM
SI = 194.7

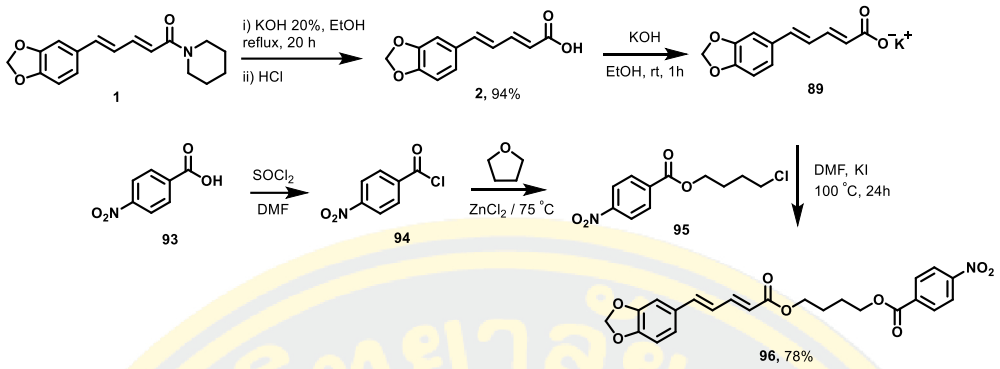
Figure 22 The most active compound **88g** of antimycobacterial activity.

Santos et al., 2018 designed and synthesized the target piperine analogues for evaluated their toxicological and antitumor activities. The reaction was started from basic hydrolysis of **1** to generate piperic acid **5** and transformed into design product *N*-(p-nitrophenyl)acetamide piperinoate (**92**) in good yield *via* nucleophilic substitution (Scheme 14). The acid chloride **91** using in the transformation was prepared by treatment of 4-nitroaniline **90** with chloroacetyl chloride in the presence of triethylamine. The toxicity was evaluated *in vitro* of *N*-(p-nitrophenyl)acetamide piperinoate (**92**) exhibited low toxicity and induced only 5.01% of hemolysis, which reduced the viability of RAW 264.7 cells by 49.75% at 1000 μg/m. The antitumor effect of *N*-(p-nitrophenyl)acetamide piperinoate (**92**) was involved modulation of the immune system to a cytotoxic Th1 profile.



Scheme 14 Synthesis of *N*-(p-nitrophenyl)acetamide piperinoate (HE-02).

Ferreira et al., 2020 prepared a series of piperine analogues for *in vivo* antitumor effect, antiangiogenic and immunomodulatory actions. Hydrolysis of piperine 1 under basic condition gave piperic acid 5 in excellent yield and converted to compound 89 by reaction with KOH. Next, 4-nitrobenzoic acid 93 reacted with thionyl chloride to give 4-nitrobenzoyl chloride 94, and then treated with furan in the presence of zinc chloride to afford substituted 4-chlorobutyl-4-nitrobenzoate 95. Finally, compound 89 reacted with 95 in the presence of KI in DMF to obtain product butyl 4-(4-nitrobenzoate)-piperinoate (**96**) in good yield (Scheme 15). Compound butyl 4-(4-nitrobenzoate)-piperinoate (**96**) demonstrated no genotoxicity in mice and the tumor model in 12.5, 25 or 50 mg/kg, which displayed a significant reduction in cell viability.



Scheme 15 Synthesis of butyl 4-(4-nitrobenzoate)-piperinoate (**DE-07**).

CHAPTER 3

RESEARCH METHODOLOGY

3.1 General Methods

All chemicals were purchased from commercial sources and used without further purification. ^{1}H and ^{13}C NMR spectra were recorded on a BRUKER AVANCE 400 MHz instrument. All spectra were measured in $CDCl_3$ solvent, and chemical shifts are reported as δ values in parts permillion (ppm) relative to tetramethylsilane (δ 0.00 ppm) or $CDCl_3$ δ_H 7.26 and δ_C 77.0 ppm as an internal standard. Data are reported as follows; chemical shift (multiplicity, integrated intensity or assignment, coupling constants in Hz, assignment). Hight-resolution mass spectra (HRMS) data were obtained with a Finnigan MAR95 at Mahidol University. Infrared spectra (IR) were determined on a PERKIN ELMER FT/IR-2000S spectrophotometer and are report in wave number (cm^{-1}). Analytical thin-layer chromatrography (TLC) was conducted on precoated TLC plates; silica gel 60F-254 (E. Merck, Darmstadt,Germany). Silica gel columns for open-column chromatrography utilized silica gel 60 (Grade 7729, < 230 mesh, 7734, 70-230 mash and 9385, 230-400 mesh, E. Merch, Darmstadt, Germany). Melting points were recorded using GALLENKAMP Melting point apparatus Griffin.

3.2 Isolation of piperine from black pepper.

The dried seeds of black pepper (*Piper nigrum* L.) used in the extraction were purchased from Tha Mai District, Chanthaburi province, Thailand. The seeds powder of *P. nigrum* (1,500 g) was marinated in 95% ethanol solutions 2.0 L for 3 days at room temperature. Then, the solution of 0.1% KOH in isopropanol 10 mL was added. The reaction mixture was stirred at room temperature for 1 h, then filtered using a Buchner funnel and water was added until a precipitate formed. The solution mixture was stored in a cool place overnight. The precipitate was filtered and purified by recrystallization with isopropanol to provide product as a yellow solid 25.4300g. The flowchart of piperine isolation was summarized as shown in Figure 23.

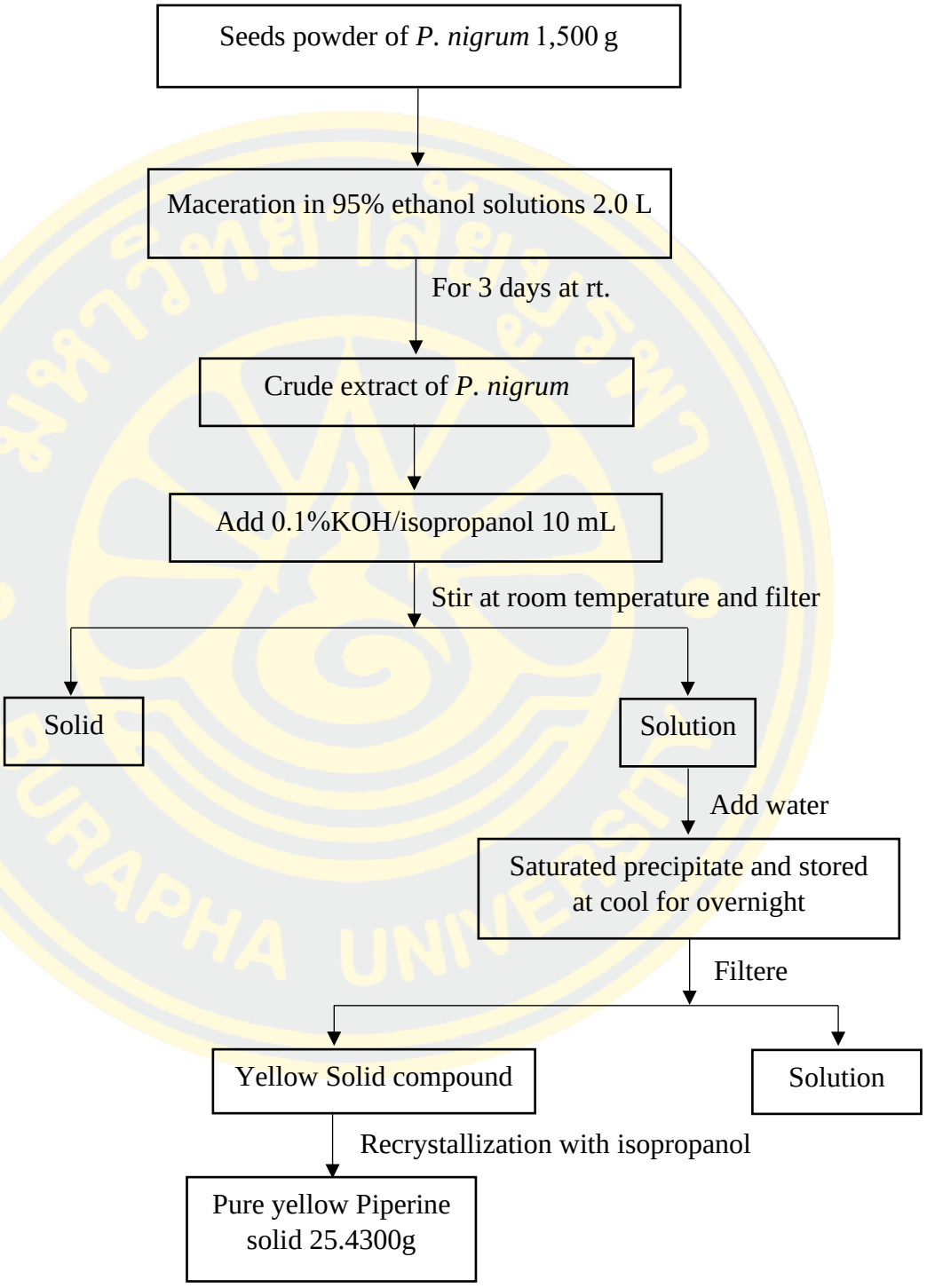
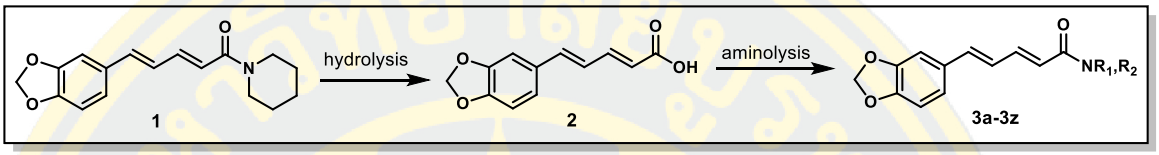


Figure 23 Flow chart isolation of piperine **1** from *Piper nigrum* L.

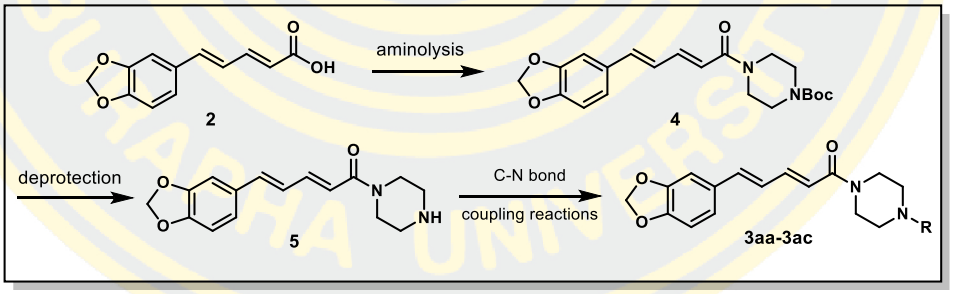
3.3 Methodology for the modification of piperine amide analogues

This research aims to design and synthesize the new analogs of piperine amide for study antioxidant activities, acetylcholinesterase (AChE), and butyrylcholinesterase (BuChE) inhibitory activity. The synthesis was carried out by hydrolysis of piperine **1** to generate piperic acid **2** then reacted with a various amine *via* aminolysis to afford piperine amide analogues **3a-3z** (Scheme 16).



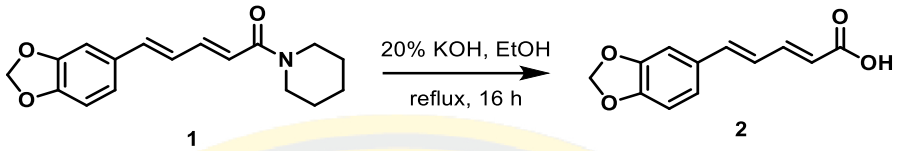
Scheme 16 The synthetic plan for preparation of piperine amide analogues **3a-3z**.

Moreover, the piperine amide analogues **3aa-3ac** were synthesized by aminolysis of **2** with 1-Boc-piperazine to provide compound **4**. Next, deprotection Boc of **4** under acid condition to give compound **5**. Finally, compound **5** reacted with various quinolines *via* C-N bond coupling reaction to afford target compounds **3aa-3ac** (Scheme 17).



Scheme 17 The synthetic plan for preparation of piperine amide analogues **3aa-3ac**.

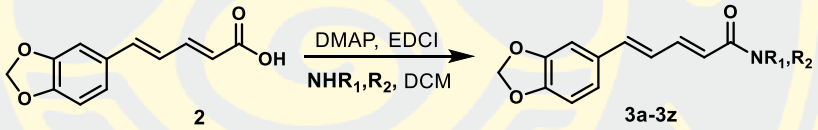
3.4 Preparation of piperic acid 2



The solution of piperine **1** (1.0 equiv.) and 20%KOH in ethanol was refluxed at 90 ^{0}C for 16 h then continuously stirring and cooled down to room temperature. The solution mixture was neutralized with 1 M HCl to pH 3.0 and transferred to a separatory funnel. The organic phase was collected and evaporated in *vacuo*. The crude product was washed with cool water (10 mL) and acetone (5 mL) to provide piperic acid **2** in a yield of 75% (592.4 mg) as a yellow solid.

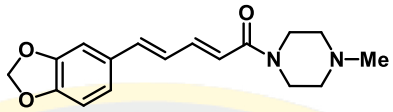
3.5 Synthesis of piperine amide analogues

General procedure A



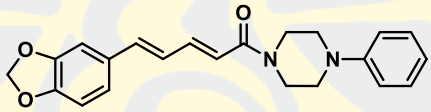
To a solution of piperic acid (100 mg, 0.4582 mmol) in dichloromethane (3 mL) and 4-dimethylaminopyridine (DMAP, 52.0 mg, 0.4582 mmol), 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDCI, 132.0 mg, 0.6873 mmol) was added at 0 °C. The reaction mixture was stirred for 30 min and warm up to room temperature. Next, substituted amine (0.6873 mmol) was added and continuous stirring for 30 min to 1 h. The reaction was monitored by TLC until the completion of the reaction. The reaction was quenched with saturated cool aqueous NH_4Cl and extracted three times with dichloromethane. The organic phase was collected and washed with brine, dried with anhydrous Na_2SO_4, filtered, and evaporated in *vacuo*. The crude product was purified by silica gel column chromatography to afford piperine amide analogues **3a-3z**.

3.5.1. Synthesis of 5-(benzo[1,3]dioxol-5-yl)-1-(4-methylpiperazin-1-yl)penta-2,4-dien-1-one (3a)



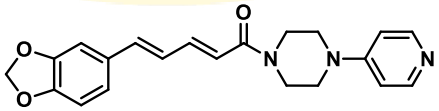
Following the general procedure A, 1-benzhydrylpiperazine (0.0762 mL, 0.6837 mmol) was employed in aminolysis reaction. The reaction was stirred at room temperature for 1 h. The crude product was purified by silica gel column chromatography (SiO_2, 10% MeOH/CH_2Cl_2) to provide piperine amide **3a** as a yellow oil (120.3 mg, 87%).

3.5.2. Synthesis of 5-(benzo[1,3]dioxol-5-yl)-1-(4-phenylpiperazin-1-yl)penta-2,4-dien-1-one (3b)



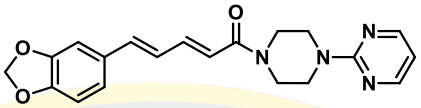
Following the general procedure A, 1-phenylpiperazine (0.104 mL, 0.6837 mmol) was employed in aminolysis reaction. The reaction was stirred at room temperature for 1 h. The crude product was purified by silica gel column chromatography (SiO_2, 40% EtOAc/*n*-Hexane) to provide piperine amide **3b** as a yellow oil (142.8 mg, 86%).

3.5.3. Synthesis of 5-(benzo[1,3]dioxol-5-yl)-1-(4-(pyridin-4-yl)piperazin-1-yl)penta-2,4-dien-1-one (3c)



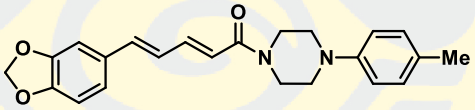
Following the general procedure A, 1-(4-pyridyl)piperazine (112.2 mg, 0.6837 mmol) was employed in aminolysis reaction. The reaction was stirred at room temperature for 45 min. The crude product was purified by silica gel column chromatography (SiO_2, 10% MeOH/CH_2Cl_2) to provide piperine amide **3c** as a orange solid (129.0 mg, 77%).

3.5.4. Synthesis of 5-(benzo[1,3]dioxol-5-yl)-1-(4-(pyrimidin-2-yl)piperazin-1-yl)penta-2,4-dien-1-one (3d)



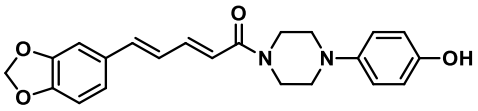
Following the general procedure A, 1-(2-pyrimidyl)piperazine (0.097 mL, 0.6837 mmol) was employed in aminolysis reaction. The reaction was stirred at room temperature for 45 min. The crude product was purified by silica gel column chromatography (SiO_2, 70% EtOAc/*n*-Hexane) to provide piperine amide **3d** as a yellow solid (124.4 mg, 74%).

3.5.5. Synthesis of 5-(benzo[1,3]dioxol-5-yl)-1-(4-(*p*-tolyl)piperazin-1-yl)penta-2,4-dien-1-one (3e)



Following the general procedure A, 1-(*p*-tolyl)piperazine (0.011 mL, 0.6837 mmol) was employed in aminolysis reaction. The reaction was stirred at room temperature for 45 min. The crude product was purified by silica gel column chromatography (SiO_2, 50% EtOAc/*n*-Hexane) to provide piperine amide **3e** as a yellow solid (139.2 mg, 80%).

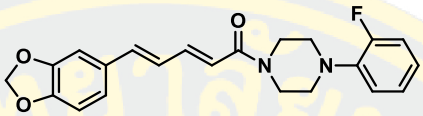
3.5.6. Synthesis of 5-(benzo[1,3]dioxol-5-yl)-1-(4-(4-hydroxyphenyl)piperazin-1-yl)penta-2,4-dien-1-one (3f)



Following the general procedure A, 1-(4-hydroxyphenyl)piperazine (122.5 mg, 0.6837 mmol) was employed in aminolysis reaction. The reaction was stirred at room temperature for 45 min. The crude product was purified by silica gel column

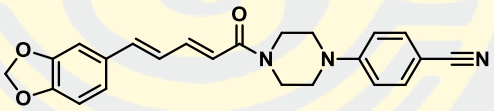
chromatography (SiO_2, 60% EtOAc/*n*-Hexane) to provide piperine amide **3f** as a yellow oil (94.9 mg, 55%).

3.5.7. Synthesis of 5-(benzo[1,3]dioxol-5-yl)-1-(4-(2-fluorophenyl)piperazin-1-yl) penta-2,4-dien-1-one (3g)



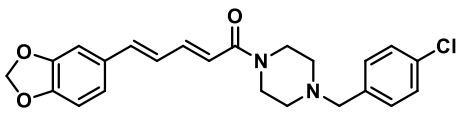
Following the general procedure A, 1-(2-fluorophenyl)-piperazine (151.4 mg, 0.6837 mmol) was employed in aminolysis reaction. The reaction was stirred at room temperature for 45 min. The crude product was purified by silica gel column chromatography (SiO_2, 50% EtOAc/*n*-Hexane) to provide piperine amide **3g** as a yellow solid (136.2 mg, 75%).

3.5.8. Synthesis of 4-(4-(-5-(benzo[1,3]dioxol-5-yl)penta-2,4-dienoyl)piperazin-1-yl)benzonitrile (3h)



Following the general procedure A, 4-(1-piperazinyl)benzonitrile (128.7 mg, 0.6837 mmol) was employed in aminolysis reaction. The reaction was stirred at room temperature for 45 min. The crude product was purified by silica gel column chromatography (SiO_2, 70% EtOAc/*n*-Hexane) to provide piperine amide **3h** as a yellow solid (106.0 mg, 60%).

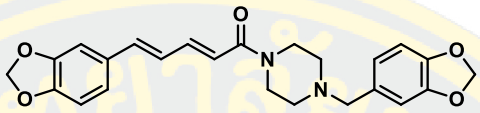
3.5.9. Synthesis of 5-(benzo[1,3]dioxol-5-yl)-1-(4-(4-chlorobenzyl)piperazin-1-yl)penta-2,4-dien-1-one (3i)



Following the general procedure A, 1-(4-chlorobenzyl)piperazine (0.11 mL, 0.6837 mmol) was employed in aminolysis reaction. The reaction was stirred at room temperature for 45 min. The crude product was purified by silica gel column

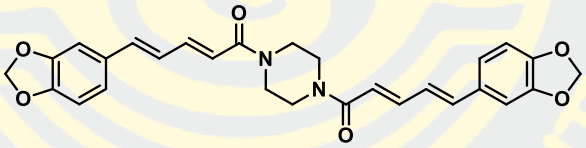
chromatography (SiO_2, 50% EtOAc/*n*-Hexane) to provide piperine amide **3i** as a brown solid (126.7 mg, 54%).

3.5.10. Synthesis of 5-(benzo[1,3]dioxol-5-yl)-1-(4-(benzo[1,3]dioxol-5-ylmethyl)piperazin-1-yl)penta-2,4-dien-1-one (3j)



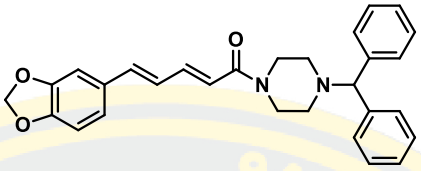
Following the general procedure A, 1-piperonylpiperazine (151.4 mg, 0.6837 mmol) was employed in aminolysis reaction. The reaction was stirred at room temperature for 1 h. The crude product was purified by silica gel column chromatography (SiO_2, 80% EtOAc/*n*-Hexane) to provide piperine amide **3j** as a yellow solid (149.7 mg, 78%).

3.5.11. Synthesis of 1,1'-(piperazine-1,4-diyl)bis(5-(benzo[1,3]dioxol-5-yl)penta-2,4-dien-1-one) (3k)



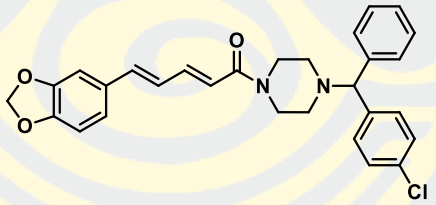
Following the general procedure A, piperazine anhydrous (52.9 mg, 0.6837 mmol) was employed in aminolysis reaction. The reaction was stirred at room temperature for 1 h. The crude product was purified by silica gel column chromatography (SiO_2, 70% EtOAc/*n*-Hexane) to provide piperine amide **3k** as a yellow solid (75.6 mg, 57%).

3.5.12. Synthesis of 1-(4-benzhydrylpiperazin-1-yl)-5-(benzo[1,3]dioxol-5-yl)penta-2,4-dien-1-one (3l)



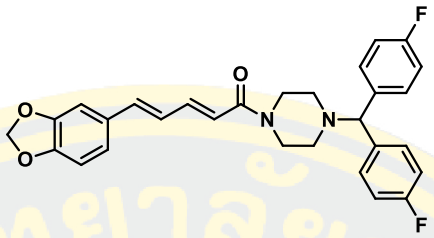
Following the general procedure A, 1-benzhydrylpiperazine (173.4 mg, 0.6837 mmol) was employed in aminolysis reaction. The reaction was stirred at room temperature for 1 h. The crude product was purified by silica gel column chromatography (SiO_2, 30% EtOAc/*n*-Hexane) to provide piperine amide **3l** as a yellow oil (181.7 mg, 88%).

3.5.13. Synthesis of 5-(benzo[1,3]dioxol-5-yl)-1-(4(4chlorophenyl)(phenyl)methyl)piperazin-1-yl)penta-2,4-dien-1-one (3m)



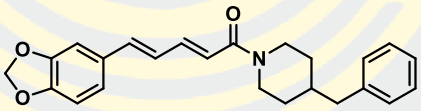
Following the general procedure A, 1-(4-chlorobenzhydry)piperazine (107.1 mg, 0.6837 mmol) was employed in aminolysis reaction. The reaction was stirred at room temperature for 2 h. The crude product was purified by silica gel column chromatography (SiO_2, 30% EtOAc/*n*-Hexane) to provide piperine amide **3m** as a yellow oil (191.8 mg, 71%).

3.5.14. Synthesis of 5-(benzo[1,3]dioxol-5-yl)-1-(4-(bis(4-fluorophenyl)methyl)piperazin-1-yl)penta-2,4-dien-1-one (5n)



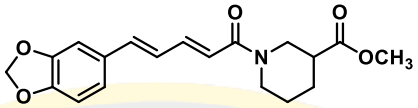
Following the general procedure A, 1-[bis(4-fluorophenyl)-methyl]piperazine (198.2 mg, 0.6837 mmol) was employed in aminolysis reaction. The reaction was stirred at room temperature for 45 min. The crude product was purified by silica gel column chromatography (SiO_2, 40% EtOAc/*n*-Hexane) to provide piperine amide **3n** as a yellow oil (194.7 mg, 87%).

3.5.15. Synthesis of 5-(benzo[1,3]dioxol-5-yl)-1-(4-benzylpiperidin-1-yl)penta-2,4-dien-1-one (3o)



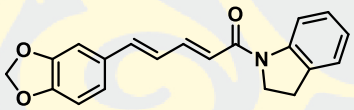
Following the general procedure A, 4-benzylpiperidine (0.12 mL, 0.6837 mmol) was employed in aminolysis reaction. The reaction was stirred at room temperature for 1 h. The crude product was purified by silica gel column chromatography (SiO_2, 30% EtOAc/*n*-Hexane) to provide piperine amide **3o** as a yellow oil (111.7 mg, 65%).

3.5.16. Synthesis of Methyl-1-(5-(benzo[1,3]dioxol-5-yl)penta-2,4-dienoyl)piperidine-3-carboxylate (3p)



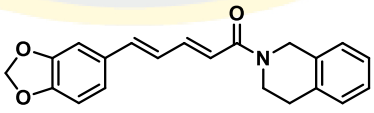
Following the general procedure A, methyl isonipecotate (0.092 mL, 0.6837 mmol) was employed in aminolysis reaction. The reaction was stirred at room temperature for 1 h. The crude product was purified by silica gel column chromatography (SiO_2, 80% EtOAc/*n*-Hexane) to provide piperine amide **3p** as a yellow oil (104.4 mg, 70%).

3.5.17. Synthesis of 5-(benzo[1,3]dioxol-5-yl)-1-(isoindolin-2-yl)penta-2,4-dien-1-one (3q)



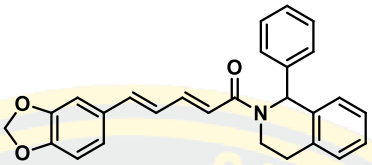
Following the general procedure A, indoline (0.076 mL, 0.6837 mmol) was employed in aminolysis reaction. The reaction was stirred at room temperature for 45 min. The crude product was purified by silica gel column chromatography (SiO_2, 30% EtOAc/*n*-Hexane) to provide piperine amide **3q** as a yellow solid (134.5 mg, 92%).

3.5.18. Synthesis of 5-(benzo[1,3]dioxol-5-yl)-1-(3,4-dihydroisoquinolin-2-yl)penta-2,4-dien-1-one (3r)



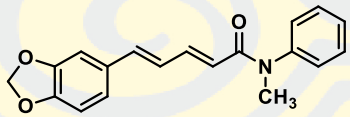
Following the general procedure A, 1,2,3,4-tetrahydroisoquinoline (0.011 mL, 0.6837 mmol) was employed in aminolysis reaction. The reaction was stirred at room temperature for 45 min. The crude product was purified by silica gel column chromatography (SiO_2, 40% EtOAc/*n*-Hexane) to provide piperine amide **3r** as a yellow oil (82.0 mg, 53%).

3.5.19 Synthesis of 5-(benzo[1,3]dioxol-5-yl)-1-(1-phenyl-3,4-dihydroisoquinolin-2-yl)penta-2,4-dien-1-one (3s)



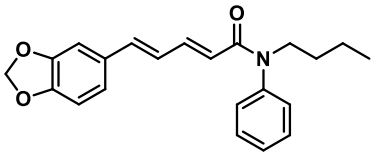
Following the general procedure A, 1-phenyl-1,2,3,4-tetrahydroisoquinoline (143.8 mg, 0.6837 mmol) was employed in aminolysis reaction. The reaction was stirred at room temperature for 1 h. The crude product was purified by silica gel column chromatography (SiO_2, 30% EtOAc/*n*-Hexane) to provide piperine amide **3s** as a orange oil (151.1 mg, 88%).

3.5.20. Synthesis of 5-(benzo[1,3]dioxol-5-yl)-*N*-methyl-*N*-phenylpenta-2,4-dienamide (3t)



Following the general procedure A, *N*-methylaniline (0.074 mL, 0.6837 mmol) was employed in aminolysis reaction. The reaction was stirred at room temperature for 1 h. The crude product was purified by silica gel column chromatography (SiO_2, 40% EtOAc/*n*-Hexane) to provide piperine amide **3t** as a yellow solid (111.5 mg, 79%).

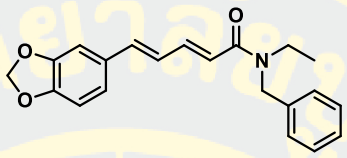
3.5.21. Synthesis of 5-(benzo[1,3]dioxol-5-yl)-*N*-butyl-*N*-phenylpenta-2,4-dienamide (3u)



Following the general procedure A, *N*-n-butylaniline (0.11 mL, 0.6837 mmol) was employed in aminolysis reaction. The reaction was stirred at room temperature

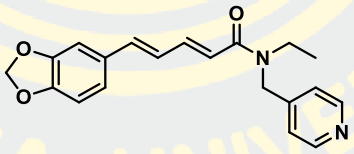
for 30 min. The crude product was purified by silica gel column chromatography (SiO_2, 40% EtOAc/*n*-Hexane) to provide piperine amide **3u** as a yellow oil (103.5 mg, 65%).

3.5.22 Synthesis of 5-(benzo[1,3]dioxol-5-yl)-*N*-benzyl-*N*-ethylpenta-2,4-dienamide (3v)



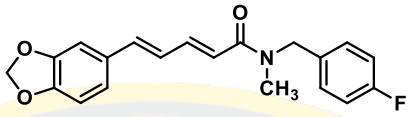
Following the general procedure A, *N*-ethylbenzylamine (0.10 mL, 0.6837 mmol) was employed in aminolysis reaction. The reaction was stirred at room temperature for 45 min. The crude product was purified by silica gel column chromatography (SiO_2, 40% EtOAc/*n*-Hexane) to provide piperine amide **3v** as a yellow oil (100.0 mg, 69%).

3.5.23. Synthesis of 5-(benzo[1,3]dioxol-5-yl)-*N*-ethyl-*N*-(pyridin-4-ylmethyl)penta-2,4-dienamide (3w)



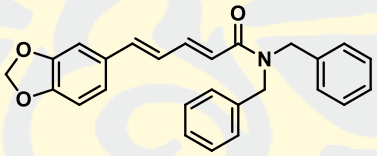
Following the general procedure A, 4-(ethylaminoethyl)pyridine (0.094 mL, 0.6837 mmol) was employed in aminolysis reaction. The reaction was stirred at room temperature for 45 min. The crude product was purified by silica gel column chromatography (SiO_2, 10% MeOH/CH_2Cl_2) to provide piperine amide **3w** as a yellow oil (78.9 mg, 75%).

3.5.24. Synthesis of 5-(benzo[1,3]dioxol-5-yl)-*N*-(4-fluorobenzyl)-*N*-methylpenta-2,4-dienamide (3x)



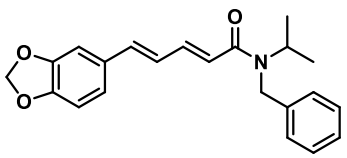
Following the general procedure A, 4-fluoro-*N*-methyl-benzylamine (0.091 mL, 0.6837 mmol) was employed in aminolysis reaction. The reaction was stirred at room temperature for 45 min. The crude product was purified by silica gel column chromatography (SiO_2, 40% EtOAc/*n*-Hexane) to provide piperine amide **3x** as a yellow oil (109.0 mg, 53%).

3.5.25. Synthesis of 5-(benzo[1,3]dioxol-5-yl)-*N*,*N*-dibenzylpenta-2,4-dienamide (3y)



Following the general procedure A, dibenzylamine (0.13 mL, 0.6837 mmol) was employed in aminolysis reaction. The reaction was stirred at room temperature for 45 min. The crude product was purified by silica gel column chromatography (SiO_2, 30% EtOAc/*n*-Hexane) to provide piperine amide **3y** as a yellow oil (162.6 mg, 98%).

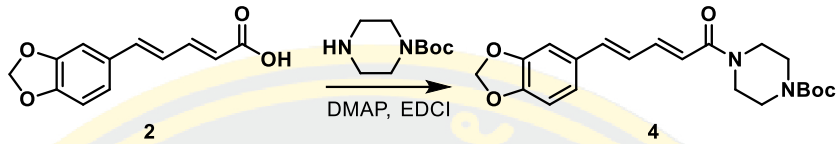
3.5.26. Synthesis of 5-(benzo[1,3]dioxol-5-yl)-*N*-benzyl-*N*-isopropylpenta-2,4-dienamide (3z)



Following the general procedure A, *N*-isopropylbenzylamine (0.11 mL, 0.6837 mmol) was employed in aminolysis reaction. The reaction was stirred at room temperature for 45 min. The crude product was purified by silica gel column

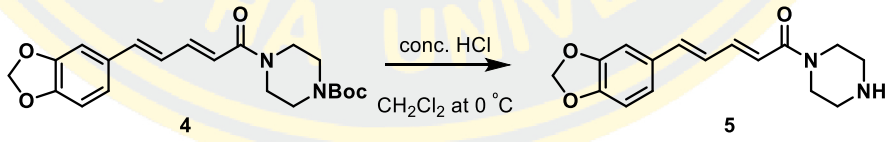
chromatography (SiO_2, 40% EtOAc/*n*-Hexane) to provide piperine amide **3z** as a yellow oil (110.1 mg, 69%).

3.6 Preparation of compound 4



To a solution of piperic acid (1 g, 4.5827 mmol) in dichloromethane (3 mL) at 0 °C, 4-dimethylaminopyridine (559.8 mg, 4.5827 mmol) and 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (1.32 g, 6.8741 mmol) were added. The reaction mixture was stirred for 30 min and warm up to room temperature. Next, *tert*-butyl piperazine-1-carboxylate (853.5 mg, 4.5827 mmol) was added and continuous stirred for 30 min. The reaction was monitored by TLC until the completion of the reaction. The reaction was quenched with saturated cool aqueous NH_4Cl and extracted three times with dichloromethane. The organic phase was collected and washed with brine, dried with anhydrous Na_2SO_4, filtered, and evaporated in *vacuo*. The crude product was purified by silica gel column chromatography (SiO_2, 60% EtOAc/*n*-Hexane) to afford compound **4** in a yield of 73% (1.3 g) as a yellow solid.

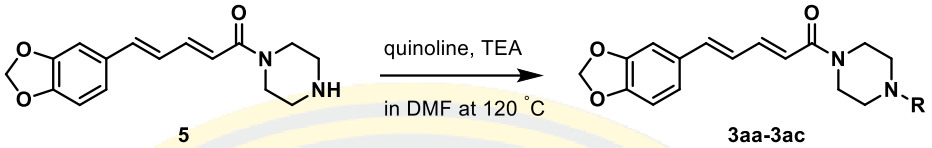
3.7 Preparation of compound 5



To a solution of compound **4** (1 g, 2.5894 mmol) in dichloromethane (5 mL) at 0 °C, conc. hydrochloric acid (5 mL) was added. The reaction was monitored by TLC until the completion of the reaction. The reaction was quenched with saturated cool aqueous $NaHCO_3$ and extracted three times with dichloromethane. The organic phase was collected and washed with brine, dried with anhydrous Na_2SO_4, filtered, and evaporated in *vacuo*. The crude product was purified by silica gel column chromatography (SiO_2, 10% MeOH/CH_2Cl_2) to afford compound **5** in a yield of 82% (1.1 g) as a yellow solid.

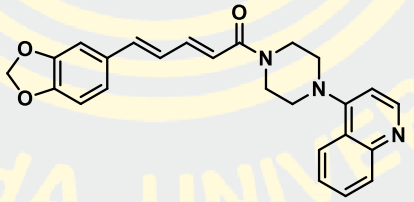
3.8 Synthesis of piperine amide analogues

General procedure B



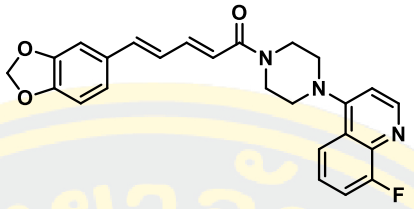
A mixture of **5** (100 mg, 0.3494) and triethylamine (TEA, 0.05 mL, 0.3494 mmol) in dimethylformamide (DMF, 1 mL) at 120 ºC, quinoline **aa-ac** (0.4192 mmol) was added. The reaction mixture was continuous stirred for 24 h and warm up to room temperature. The reaction was monitored by TLC until the completion of the reaction. The reaction was quenched with saturated cool aqueous NH_4Cl and extracted three times with dichloromethane. The organic phase was collected and washed with brine, dried with anhydrous Na_2SO_4, filtered, and evaporated in *vacuo*. The crude product was purified by silica gel column chromatography to afford piperine amide analogues **3aa-3ac**.

3.8.1. Synthesis of 5-(benzo[1,3]dioxol-5-yl)-1-(4-(quinolin-4-yl)piperazin-1-yl)penta-2,4-dien-1-one (3aa)



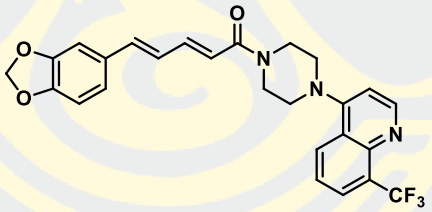
Following the general procedure B, 4-Chloroquinoline (82.0 mg, 0.4912 mmol) was used in reaction. The crude product was purified by silica gel column chromatography (SiO_2, 30% EtOAc/*n*-Hexane) to provide piperine-amide **3aa** as a yellow solid (75.4 mg, 52%).

3.8.4. Synthesis of 5-(benzo[1,3]dioxol-5-yl)-1-(4-(8-fluoroquinolin-4-yl)piperazin-1-yl)penta-2,4-dien-1-one (3ab)



Following the general procedure B, 4-Chloro-8-fluoroquinoline (76.0 mg, 0.4192 mmol) was used in reaction. The crude product was purified by silica gel column chromatography (SiO_2, 40% EtOAc/*n*-Hexane) to provide piperine-amide **3ab** as a yellow solid (55.0 mg, 37%).

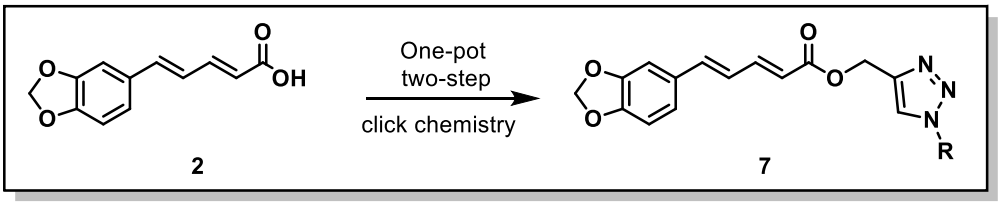
3.8.5. Synthesis of 5-(benzo[1,3]dioxol-5-yl)-1-(4-(8-(trifluoromethyl)quinolin-4-yl)piperazin-1-yl)penta-2,4-dien-1-one (3ac)



Following the general procedure B, 4-Chloro-8-(trifluoromethyl)quinoline (97 mg, 0.4192 mmol) was used in reaction. The crude product was purified by silica gel column chromatography (SiO_2, 40% EtOAc/*n*-Hexane) to provide piperine-amide **3ac** as a yellow solid (118.8 mg, 70%).

3.9 Methodology for the modification of 1,2,3-triazole-piperine analogues

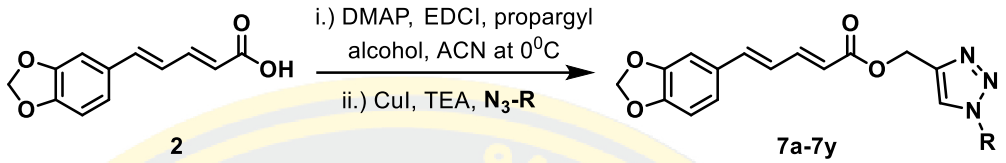
The synthesis was carried out by esterification of **2** to propargyl ester followed by click reaction with various substituted azides to afford 1,2,3-triazole-piperine analogues **7** in one-pot two-step reaction (Scheme 18).



Scheme 18 The synthetic plan for modification of 1,2,3-triazole-piperine analogues **7**.

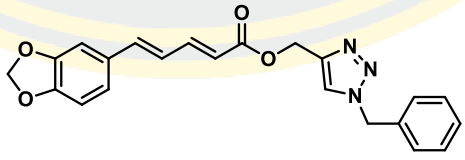
3.10 Synthesis of 1,2,3-triazole-piperine *via* one-pot two-step

General procedure C



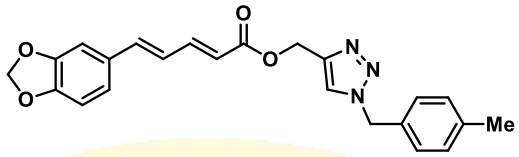
For the first step, a stirred solution of **2** (1 g, 4.5827 mmol) in acetonitrile (ACN, 2 mL) at 0 °C, 4-dimethylaminopyridine (52.0 mg, 0.4582 mmol) and 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (132.0 mg, 0.6874 mmol) were added. The reaction mixture was stirred for 30 min and warm up to room temperature. Next, propargyl alcohol (0.039 mL, 0.6874 mmol) was added and continuous stirred for 1 h. In the second step, the stirring was added triethylamine (TEA, 0.002 mL, 0.2291 mmol), CuI (65 mg, 0.3436 mmol), and substituted azide (1.3746 mmol) at room temperature. The reaction mixture was continuous stirred for 30 min to 1 h at room temperature. The reaction was monitored by TLC until the completion of the reaction. The reaction was quenched with saturated cool aqueous NH_4Cl and extracted three times with dichloromethane. The organic phase was collected and washed with brine, dried with anhydrous Na_2SO_4, filtered, and evaporated in *vacuo*. The crude product was purified by silica gel column chromatography to afford 1,2,3-triazole-piperine derivatives **7a-7y**.

3.10.1. Synthesis of *O*-((1-benzyl-1,2,3-triazol-4-yl)methyl)piperic ester (7a)



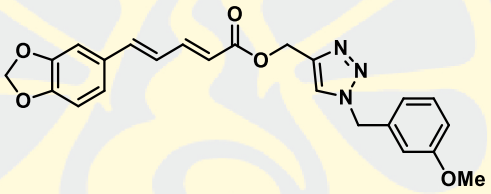
Following the general procedure C, 1-(azidomethyl)-benzene (262.0 mg, 1.3746 mmol) was used in click reaction. The reaction was stirred at room temperature for 30 min. The crude product was purified by silica gel column chromatography (SiO_2, 50% EtOAc/*n*-Hexane) to provide 1,2,3-triazole linked piperine **7a** as a yellow solid (70.7 mg, 79%).

3.10.2. *O*-((4-methylbenzyl-1,2,3-triazol-4-yl)methyl)piperic ester (7b)



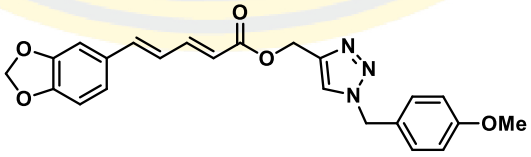
Following the general procedure C, 1-(azidomethyl)-4-methylbenzene (202.0 mg, 1.3746 mmol) was used in click reaction. The reaction was stirred at room temperature for 30 min. The crude product was purified by silica gel column chromatography (SiO_2, 40% EtOAc/*n*-Hexane) to provide 1,2,3-triazole linked piperine **7b** as a yellow solid (108.9 mg, 59%).

3.10.3. *O*-((3-methoxybenzyl-1,2,3-triazol-4-yl)methyl)piperic ester (7c)



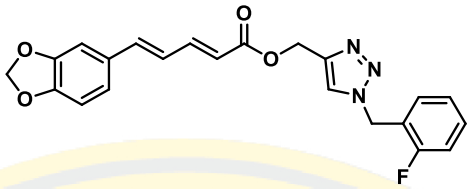
Following the general procedure C, 1-(azidomethyl)-3-methoxybenzene (224.1 mg, 1.3746 mmol) was used in click reaction. The reaction was stirred at room temperature for 1 h. The crude product was purified by silica gel column chromatography (SiO_2, 40% EtOAc/*n*-Hexane) to provide 1,2,3-triazole linked piperine **7c** as a yellow solid (79.9 mg, 42%).

3.10.4. *O*-((4-methoxybenzyl-1,2,3-triazol-4-yl)methyl)piperic ester (7d)



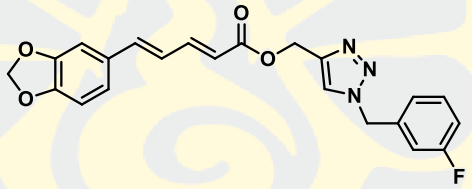
Following the general procedure C, 1-(azidomethyl)-4-methoxybenzene (224.1 mg, 1.3746 mmol) was used in click reaction. The reaction was stirred at room temperature for 30 min. The crude product was purified by silica gel column chromatography (SiO_2, 40% EtOAc/*n*-Hexane) to provide 1,2,3-triazole linked piperine **7d** as a yellow solid (148.8 mg, 77%).

3.10.5. *O*-((2-fluorobenzyl-1,2,3-triazol-4-yl)methyl)piperic ester (7e)



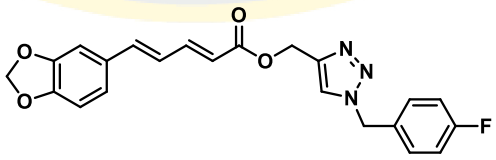
Following the general procedure C, 1-(azidomethyl)-2-fluorobenzene (207 mg, 1.3746 mmol) was used in click reaction. The reaction was stirred at room temperature for 1 h. The crude product was purified by silica gel column chromatography (SiO_2, 40% EtOAc/*n*-Hexane) to provide 1,2,3-triazole linked piperine **7e** as a yellow solid (142.1 mg, 76%).

3.10.6. *O*-((3-fluorobenzyl-1,2,3-triazol-4-yl)methyl)piperic ester (7f)



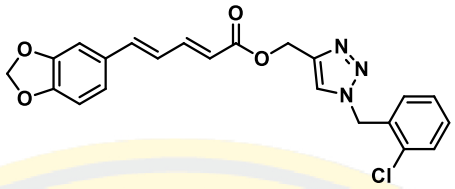
Following the general procedure C, 1-(azidomethyl)-3-fluorobenzene (207.6 mg, 1.3746 mmol) was used in click reaction step 2. The reaction was stirred at room temperature for 30 min. The crude product was purified by silica gel column chromatography (SiO_2, 40% EtOAc/*n*-Hexane) to provide 1,2,3-triazole linked piperine **7f** as a yellow solid (142.4 mg, 76%).

3.10.7. *O*-((4-fluorobenzyl-1,2,3-triazol-4-yl)methyl)piperic ester (7g)



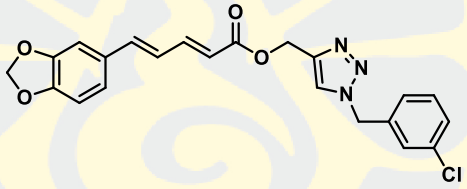
Following the general procedure C, 1-(azidomethyl)-4-fluorobenzene (207.6 mg, 1.3746 mmol) was used in click reaction. The reaction was stirred at room temperature for 1 h. The crude product was purified by silica gel column chromatography (SiO_2, 40% EtOAc/*n*-Hexane) to provide 1,2,3-triazole linked piperine **7g** as a yellow solid (60.9 mg, 64%).

3.10.8. *O*-((2-chlorobenzyl-1,2,3-triazol-4-yl)methyl)piperic ester (7h)



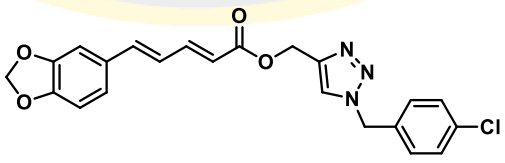
Following the general procedure C, 1-(azidomethyl)-2-chlorobenzene (211.1 mg, 1.3746 mmol) was used in click reaction. The reaction was stirred at room temperature for 1 h. The crude product was purified by silica gel column chromatography (SiO_2, 40% EtOAc/*n*-Hexane) to provide 1,2,3-triazole linked piperine **7h** as a yellow solid (141.7 mg, 72%).

3.10.9. *O*-((3-chlorobenzyl-1,2,3-triazol-4-yl)methyl)piperic ester (7i)



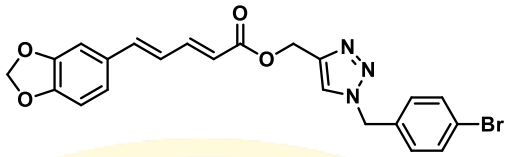
Following the general procedure C, 1-(azidomethyl)-3-chlorobenzene (211.1 mg, 1.3746 mmol) was used in click reaction. The reaction was stirred at room temperature for 1 h. The crude product was purified by silica gel column chromatography (SiO_2, 40% EtOAc/*n*-Hexane) to provide 1,2,3-triazole linked piperine **7i** as a yellow solid (145.6 mg, 75%).

3.10.10. *O*-((4-chlorobenzyl-1,2,3-triazol-4-yl)methyl)piperic ester (7j)



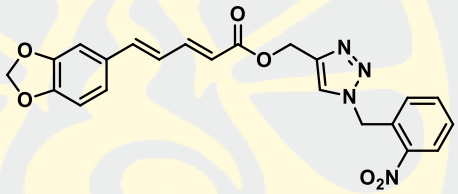
Following the general procedure C, 1-(azidomethyl)-4-chlorobenzene (211.1 mg, 1.3746 mmol) was used in click reaction. The reaction was stirred at room temperature for 1 h. The crude product was purified by silica gel column chromatography (SiO_2, 40% EtOAc/*n*-Hexane) to provide 1,2,3-triazole linked piperine **7j** as a yellow solid (97.2 mg, 50%).

3.10.11. *O*-((4-bromobenzyl-1,2,3-triazol-4-yl)methyl)piperic ester (7k)



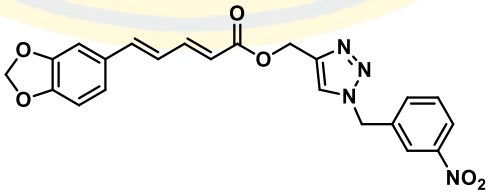
Following the general procedure C, 1-(azidomethyl)-4-bromobenzene (291 mg, 1.3746 mmol) was used in click reaction. The reaction was stirred at room temperature for 30 min. The crude product was purified by silica gel column chromatography (SiO_2, 40% EtOAc/*n*-Hexane) to provide 1,2,3-triazole linked piperine **7k** as a yellow solid (110.3 mg, 52%).

3.10.12. *O*-((2-nitrobenzyl-1,2,3-triazol-4-yl)methyl)piperic ester (7l)



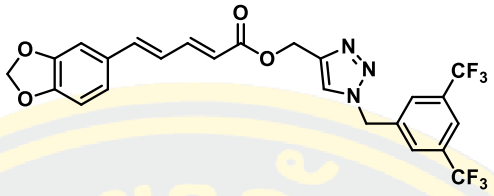
Following the general procedure C, 1-(azidomethyl)-2-nitrobenzene (244.7 mg, 1.3746 mmol) was used in click reaction. The reaction was stirred at room temperature for 1 h. The crude product was purified by silica gel column chromatography (SiO_2, 40% EtOAc/*n*-Hexane) to provide 1,2,3-triazole linked piperine **7l** as a yellow solid (133.1 mg, 67%).

3.10.13. *O*-((3-nitrobenzyl-1,2,3-triazol-4-yl)methyl)piperic ester (7m)



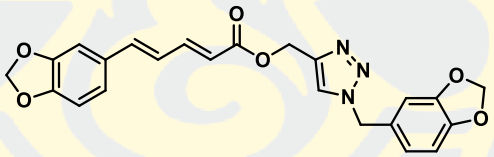
Following the general procedure C, 1-(azidomethyl)-3-nitrobenzene (244.7 mg, 1.3746 mmol) was used in click reaction. The reaction was stirred at room temperature for 30 min. The crude product was purified by silica gel column chromatography (SiO_2, 40% EtOAc/*n*-Hexane) to provide 1,2,3-triazole linked piperine **7m** as a yellow solid (69.5 mg, 35%).

3.10.14. *O*-((3,5-bis(trifluoromethyl)benzyl-1,2,3-triazol-4-yl)methyl)piperic ester (7n)



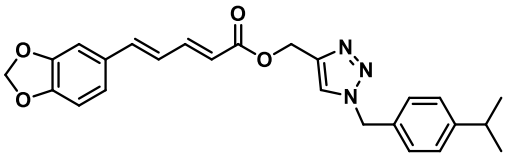
Following the general procedure C, 1-(azidomethyl)-3,5-bis(trifluoromethyl)benzene (369.8 mg, 1.3746 mmol) was used in click reaction. The reaction was stirred at room temperature for 30 min. The crude product was purified by silica gel column chromatography (SiO_2, 40% EtOAc/*n*-Hexane) to provide 1,2,3-triazole linked piperine **7n** as a yellow solid (134.1 mg, 62%).

3.10.15. *O*-((piperonylmethyl-1,2,3-triazol-4-yl)methyl)piperic ester (7o)



Following the general procedure C, 1-(azido)-piperonylmethane (243.3 mg, 1.3746 mmol) was used in click reaction. The reaction was stirred at room temperature for 30 min. The crude product was purified by silica gel column chromatography (SiO_2, 40% EtOAc/*n*-Hexane) to provide 1,2,3-triazole linked piperine **7o** as a yellow solid (105.7 mg, 57%).

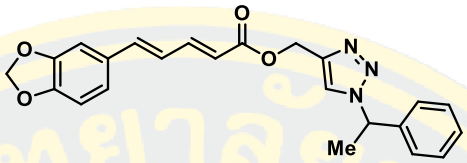
3.10.16. *O*-((4-isopropylbenzyl-1,2,3-triazol-4-yl)methyl)piperic ester (7p)



Following the general procedure C, 1-(azidomethyl)-4-isopropylbenzene (221.1 mg, 1.3746 mmol) was used in click reaction. The reaction was stirred at room temperature for 1 h. The crude product was purified by silica gel column

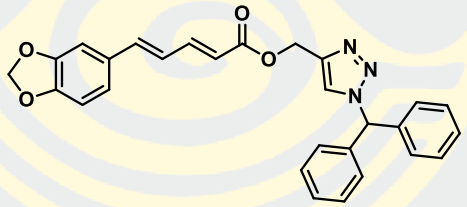
chromatography (SiO_2, 40% EtOAc/*n*-Hexane) to provide 1,2,3-triazole linked piperine **7p** as a yellow solid (124.4 mg, 66%).

3.10.17. *O*-((1-phenylethyl-1,2,3-triazol-4-yl)methyl)piperic ester (7q)



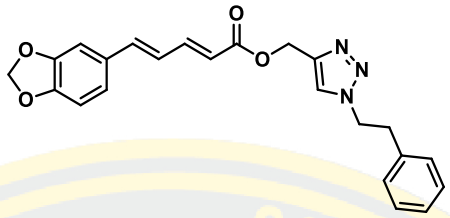
Following the general procedure C, 1-(azido)-1-phenylethane (202.2 mg, 1.3746 mmol) was used in click reaction. The reaction was stirred at room temperature for 1 h. The crude product was purified by silica gel column chromatography (SiO_2, 40% EtOAc/*n*-Hexane) to provide 1,2,3-triazole linked piperine **7q** as a yellow solid (106.2 mg, 53%).

3.10.18. *O*-((1-diphenylmethyl-1,2,3-triazol-4-yl)methyl)piperic ester (7r)



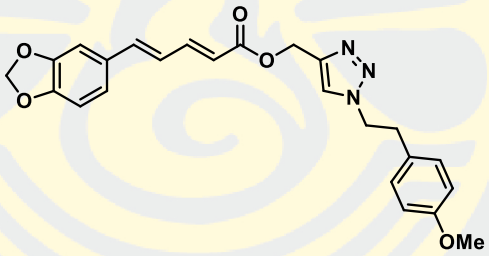
Following the general procedure C, 1-(azidomethyl)-diphenylmethane (346.8 mg, 1.3746 mmol) was used in click reaction. The reaction was stirred at room temperature for 1 h. The crude product was purified by silica gel column chromatography (SiO_2, 40% EtOAc/*n*-Hexane) to provide 1,2,3-triazole linked piperine **7r** as a yellow solid (62.0 mg, 30%).

3.10.19. *O*-((1-phenethyl-1,2,3-triazol-4-yl)methyl)piperic ester (7s)



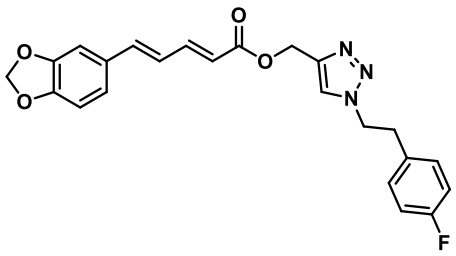
Following the general procedure C, 1-(azidoethyl)-benzene (202.2 mg, 1.3746 mmol) was used in click reaction. The reaction was stirred at room temperature for 30 min. The crude product was purified by silica gel column chromatography (SiO_2, 40% EtOAc/*n*-Hexane) to provide 1,2,3-triazole linked piperine **7s** as a yellow solid (86.3 mg, 69%).

3.10.20. *O*-((4-methoxyphenethyl-1,2,3-triazol-4-yl)methyl)piperic ester (7t)



Following the general procedure C, 1-(azidoethyl)-4-methoxybenzene (307.4 mg, 1.3746 mmol) was used in click reaction step 2. The reaction was stirred at room temperature for 1 h. The crude product was purified by silica gel column chromatography (SiO_2, 40% EtOAc/*n*-Hexane) to provide 1,2,3-triazole linked piperine **7t** as a yellow solid (162.0 mg, 76%).

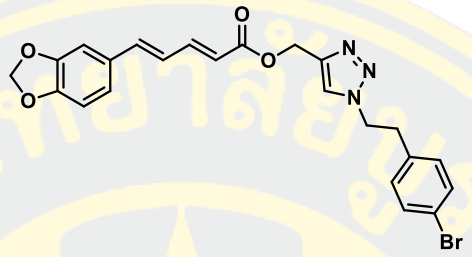
3.10.21. *O*-((4-fluorophenethyl-1,2,3-triazol-4-yl)methyl)piperic ester (7u)



Following the general procedure C, 1-(azidoethyl)-4-fluorobenzene (227.0 mg, 1.3746 mmol) was used in click reaction step 2. The reaction was stirred at room

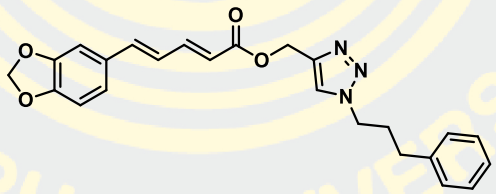
temperature for 1 h. The crude product was purified by silica gel column chromatography (SiO_2, 40% EtOAc/*n*-Hexane) to provide 1,2,3-triazole linked piperine **7u** as a yellow solid (117.1 mg, 61%).

3.10.22. *O*-((4-bromophenethyl-1,2,3-triazol-4-yl)methyl)piperic ester (7v)



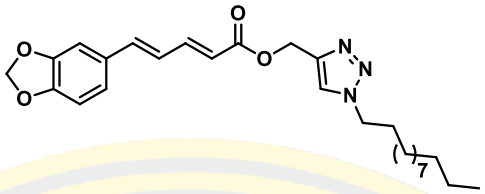
Following the general procedure C, 1-(azidoethyl)-4-bromobenzene (390.3 mg, 1.3746 mmol) was used in click reaction step 2. The reaction was stirred at room temperature for 30 min. The crude product was purified by silica gel column chromatography (SiO_2, 40% EtOAc/*n*-Hexane) to provide 1,2,3-triazole linked piperine **7v** as a yellow solid (125.8 mg, 57%).

3.10.23. *O*-((4-fluorophenethyl-1,2,3-triazol-4-yl)methyl)piperic ester (7w)



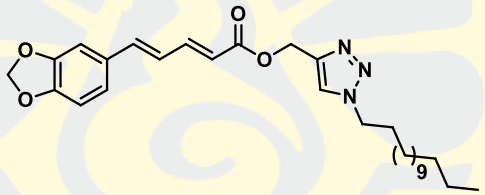
Following the general procedure C, 1-(azidoethyl)-4-fluorobenzene (221.4 mg, 1.3746 mmol) was used in click reaction step 2. The reaction was stirred at room temperature for 30 min. The crude product was purified by silica gel column chromatography (SiO_2, 40% EtOAc/*n*-Hexane) to provide 1,2,3-triazole linked piperine **7w** as a yellow solid (110.7 mg, 81%).

3.10.24. *O*-((1-tetradecyl-1,2,3-triazol-4-yl)methyl)piperic ester (7x)



Following the general procedure C, 1-azido-dodecane (290.3 mg, 1.3746 mmol) was used in click reaction. The reaction was stirred at room temperature for 1 h. The crude product was purified by silica gel column chromatography (SiO_2, 30% EtOAc/*n*-Hexane) to provide 1,2,3-triazole linked piperine **7x** as a yellow solid (95.3 mg, 79%).

3.10.25. *O*-((1-dodecyl-1,2,3-triazol-4-yl)methyl)piperic ester (7y)



Following the general procedure C, 1-azido-tetradecane (328.9 mg, 1.3746 mmol) was used in click reaction. The reaction was stirred at room temperature for 1 h. The crude product was purified by silica gel column chromatography (SiO_2, 30% EtOAc/*n*-Hexane) to provide 1,2,3-triazole linked piperine **7y** as a yellow solid (113.3 mg, 87%).

3.11 The biological activity studies

3.11.1 DPPH radical scavenging

2,2-diphenyl-1-picrylhydrazyl (DPPH) is an antioxidant assay based on electron transfer, that produces violet/purple color in methanol solution and fades to shades of yellow color in the presence of antioxidants. A solution of 0.15 mM DPPH in methanol was prepared, and 180 µL of this solution was mixed with 20 µL of the assayed sample dissolve in DMSO. The solution incubation for 30 min at room temperature in the dark, all experiments were carried out in triplicate**.** The absorbance of the mixture was measured spectrophotometrically at 517 nm. Vitamin C was used as a reference.

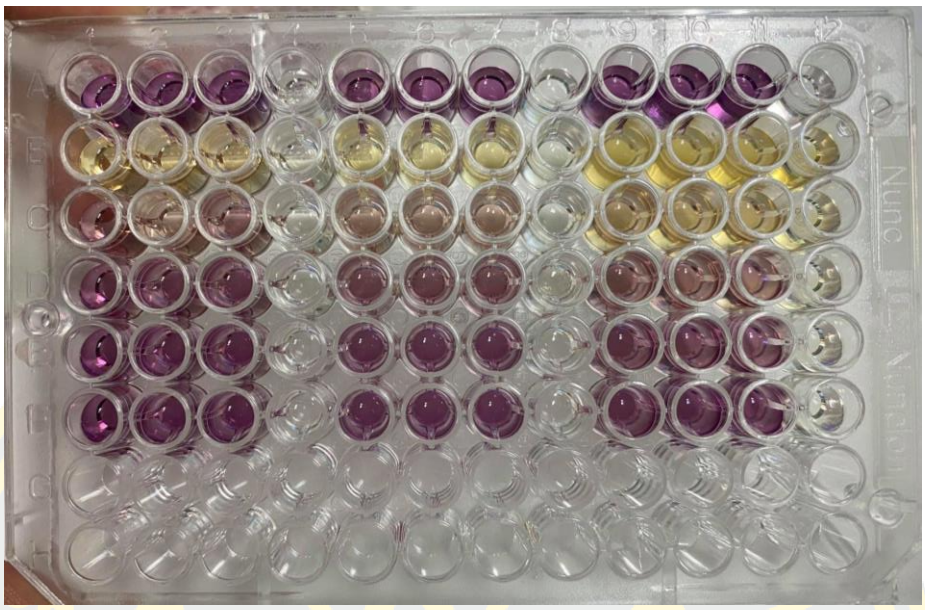


Figure 24 Decoloration of DPPH from purple to yellow on antioxidant activity evaluation.

3.11.2 Cholinesterase inhibition assay

The anti-ChE activity was measured by a microplate assay in Ellman's method. The inhibitory potency of target compounds against AChE (from electric eel) and BuChE (from equine serum) enzymes. A solution of 155 µL KH_2PO_4/K_2HPO_4 buffer (10 mM, pH 8.0) was added 5 µL of assayed sample dissolved in DMSO (from stock solution 200 mM). After that, were added 20 µL of enzyme solution (AChE or BuChE, 8 U/mL) to 96-well plates. The mixture solution was incubated for 15 min at room temperature. Finally, addition of 20 µL substrate (acetylthiocholine iodide (ATChI) or butyrylthiocholine iodide (BTChI) in 5.0 mM per well) and DTNB (5.0 mM per well) in the ratio of 3:1. The mixture solution was incubated for 5 min at room temperature, all experiments were carried out in triplicate. The absorbance of the mixture was measured spectrophotometrically at 405 nm. The standard galantamine and donepezil were used as a positive control.

3.11.3 Kinetic studies of AChE and BuChE inhibition

The kinetic study was carried out by using Ellman's method. The inhibition model and inhibition constant K_i plot between 1/velocity versus 1/substrate were produced with five different concentrations of the substrate acetylthiocholine iodide (0.3125, 0.625, 1.25, 2.5, and 5.0 mM). Following the experiment Cholinesterase

inhibition to afford curves were recorded at 405 nm. The type of inhibition was determined by Lineweaver-Burk. All rate measurements were performed in triplicate and data analysis was performed with Microsoft Excel 2010.

CHAPTER 4

RESULTS DISCUSSION & CONCLUSION

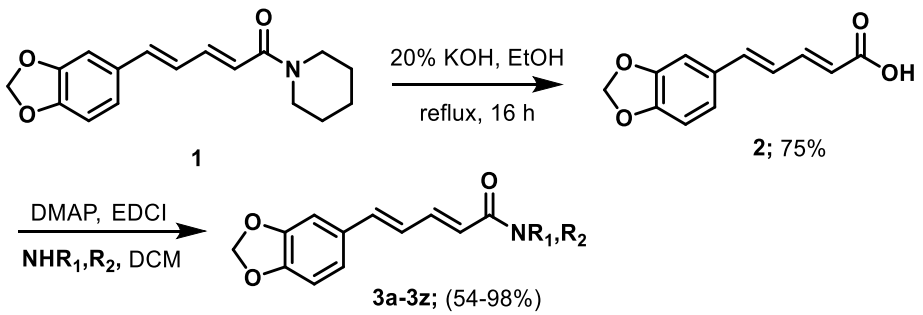
This research was aimed to modify and synthesize piperine analogues by various type of piperazine, amine and ester triazole at amide position. The fifty-four piperine analogues were investigated for inhibitory activity of antioxidant, acetylcholinesterase (AChE), and butyrylcholinesterase (BuChE) cause of Alzheimer's disease (AD).

4.1 Isolation of piperine from *Piper nigrum* L.

The dried seeds of black pepper (*Piper nigrum* L.) used in the extraction were purchased from Tha Mai District, Chanthaburi province, Thailand. The dried fruit powder of *P. nigrum* 1,500 g was macerated in 95% ethanol solutions 2.0 L at room temperature. The solution of 0.1% KOH in isopropanol 10 mL was added to the extract of *P. nigrum*. The reaction mixture was stirred at room temperature for 1 h, then filtered using a Buchner funnel and water was added until a precipitate formed. The solution mixture was stored in a cool place overnight. The precipitate was filtered and purified by recrystallization with isopropanol to piperine **1** in 4.0% (25.43g) as a yellow solid.

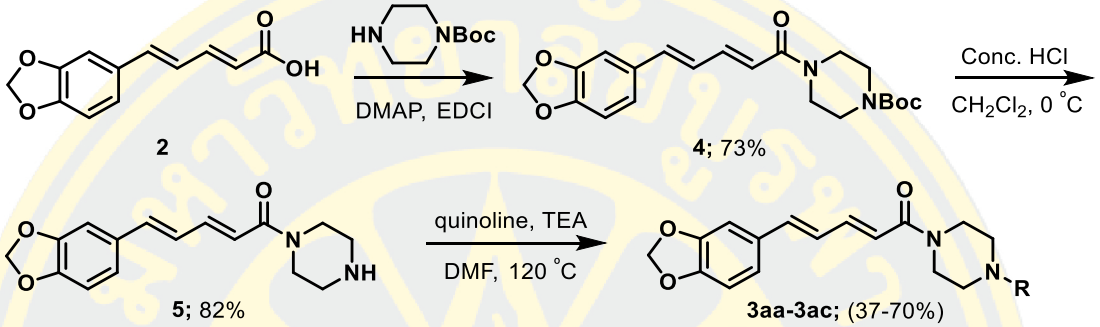
4.2 The synthesis of piperic acid and piperine amide analogues.

At first, piperine was converted to piperic acid **2** *via* hydrolysis with KOH in ethanol to obtain **2** in good yields (Scheme 19). Next step, aminolysis of **2** with various piperazine and amine derivatives using EDCI and DMAP in dichloromethane to afford twenty-six piperine amide analogues in moderate to excellent yields.



Scheme 19 The preparation of products **3a-3z**.

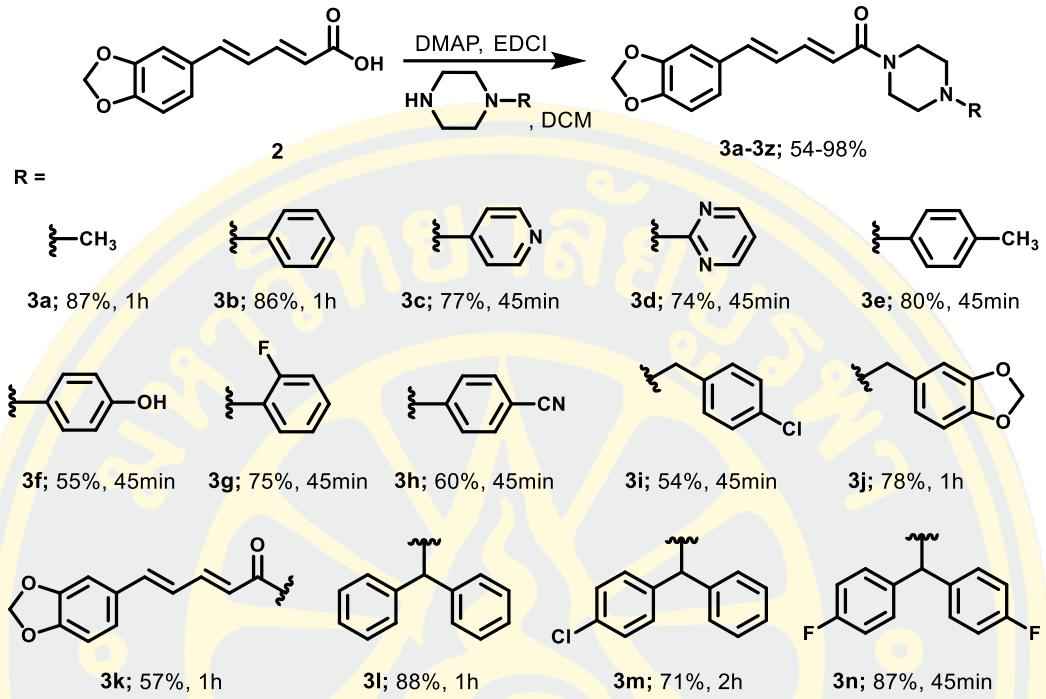
Furthermore, the piperine amide containing quinolones were synthesized by aminolysis of **2** with 1-Boc-piperazine to provide compound **4** in 73% yield. Removal of the Boc was accomplished with HCl to give compound **5** in excellent yield. Finally, compound **5** reacted with various quinolines *via* C-N bond coupling reaction to afford compounds **3aa-3ac** in low to good yields (37-70%) (Scheme 20).



Scheme 20 The preparation of products **3aa-3ac**.

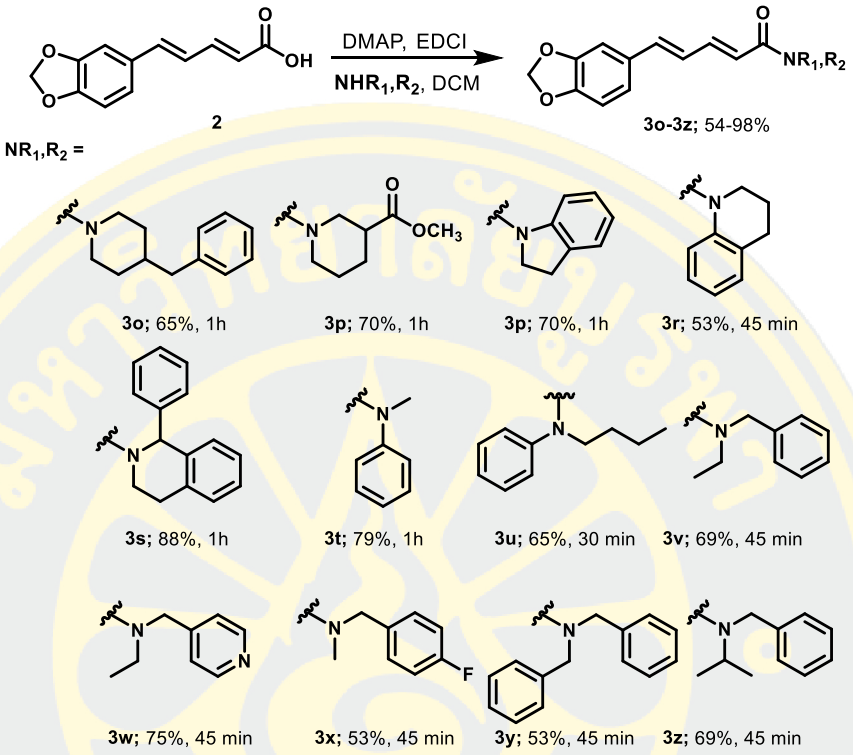
We successfully synthesized piperine amide analogues **3a-3ac** by a simple and rapid method as shown in Table 4.1 and 4.2. In a series of piperazine analogues **3a-3n**, the methyl-substituted piperazine **3a** could be converted to the desired product in a high yield (87%). For representing, aromatic group on piperazine **3b** showed the result in a satisfactory up to 86% yield. When piperazine bearing electron-donating or electron-withdrawing groups on aryl were employed, compunds **3c-3h** were obtained in moderate to high yields (55-80%). The reactions with benzyl substituted piperazine analogues afforded the products **3i** and **3j** in 54% and 78% yields, respectively. Surprisingly, piperazine anhydrous was substituted to generate **3k** in a moderate yield. Using Diarylmethyl piperazine, compounds **3l-3n** were produced in moderate to excellent yields (71-88%).

Table 5 The synthesis of piperine amide analogues **3a-3n**.



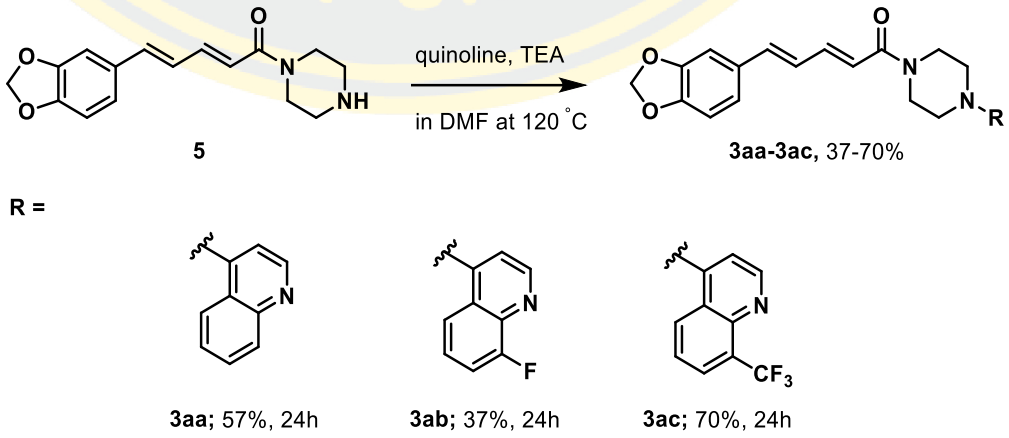
Gratifying, a series of piperine amide analogues **3o-3z** bearing various substituted secondary amine such as 4-benzylpiperidine, methyl piperidine, indoline, tetrahydroisoquinoline, and phenyltetrahydroisoquinoline were obtained in good to excellent yields (54-98%) from piperine using the same condition (Table 6).

Table 6 The synthesis of piperine amide analogues **3o-3z.**



A range of quinoline such as non-substituted quinoline 4-fluoroquinoline and 4-trifluoroquinoline was employed to react with piperine analog **5** under the basic conditions to afford the products **3aa-3ac** in low to good yields (37-70%) (Table 7).

Table 7 The synthesis of piperine amide analogues **3aa-3ac**.

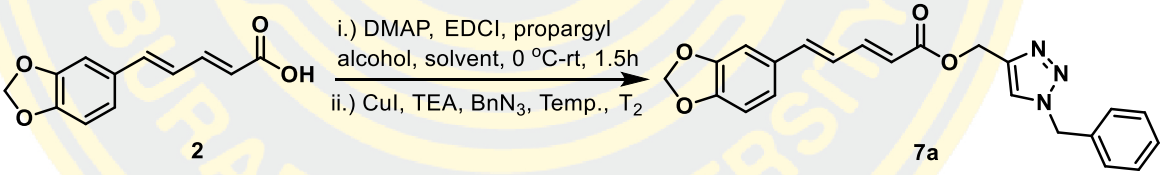


Moreover, a novel series of 1 ,2 ,3 -triazole piperine analogues was prepared using a sequential one-pot two-step procedure *via* esterification of **2** with propargyl alcohol followed by click reaction with various substituted benzyl azide to obtain products **7a-7y** in moderate to excellent yields.

4.3 Optimization of 1,2,3-triazole-piperine analogues.

To find an optimal reaction condition for the synthesis of 1,2,3-triazole-piperine analogues *via* one-pot two-step method, a model reaction was initially performed with benzyl azide as summarized in Table 4.4. The result showed that acetonitrile proved to be the best solvent over DMF, DMSO, DMA, DCM and THF, providing a high yield of product (67%) and shot reaction time (Table 8, entries 1-6). Moreover, the reaction at high temperatures (Table 8, entries 7-8) were found to give yields of **7a** lower than room temperature. Therefore, acetonitrile was chosen as an appropriate solvent and the reaction was carried out at room temperature for the optimized conditions.

Table 8 Optimization of the condition for synthesis 1,2,3-triazole-piperine analogues.

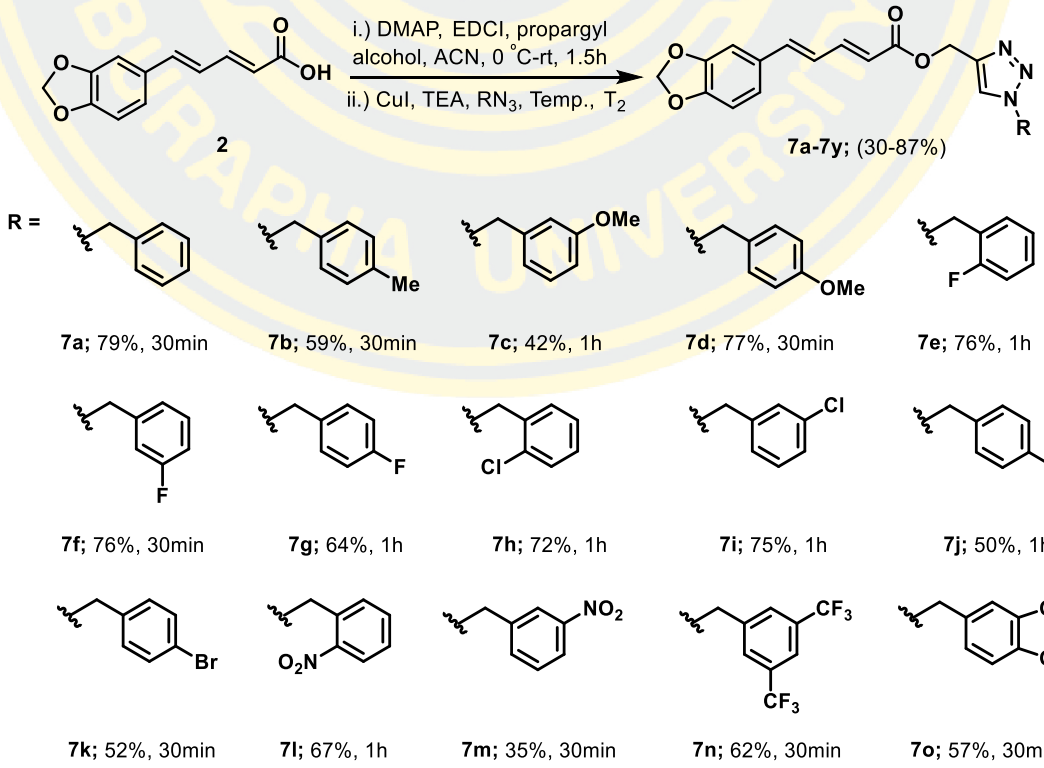


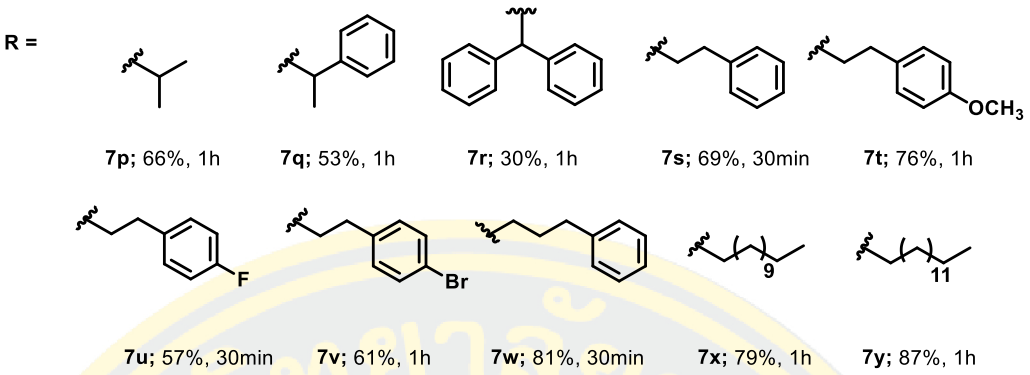
Entries	Solvent (1 mL)	Time$_2$ (min)	Temperature (°C)	Yields (%)
1	DMF	45	rt	67
2	DMSO	45	rt	56
3	DMA	45	rt	61
4	DCM	45	rt	56
5	THF	45	rt	72
6	ACN	15	rt	79
7	ACN	30	50	49
8	ACN	30	80	71

4.4 Synthesis of 1,2,3-triazole-piperine analogues 7a-7y.

With the optimized reaction conditions, a range of azide were examined to synthesize 1,2,3-triazole piperine analogues using the one-pot two-step reaction, and the results are summarized in Table 4.5. A variety of benzyl azide derivatives bearing electron-donating groups such as *p*-methyl, *m*-methoxy, *p*-methoxy and electron-withdrawing groups such as halides, nitro and trifluoromethyl gave the triazole piperine analogues **7a-7n** in 35-77% yields. The design product 5-(azidomethyl)benzodioxole **7o** was obtained in moderate yield. A series of **7p-7r** required longer reaction times and lower yield of products were observed due to the steric hindrance effects. When using Phenylethyl azides derivatives, **7s-7v** were obtained in higher yields than **7a** due to the elongation of carbon chain length which reducing the steric hindrance of phenyl group as well as phenylpropyl azide derivative to obtain **7w**. The reaction of **6** with substituted aliphatic azide gave compounds **7x** and **7y** in good yields under the established condition.

Table 9 Synthesis of 1,2,3-triazole-piperine analogues **7a-7y**.





7p; 66%, 1h **7q;** 53%, 1h **7r;** 30%, 1h **7s;** 69%, 30min **7t;** 76%, 1h

7u; 57%, 30min **7v;** 61%, 1h **7w;** 81%, 30min **7x;** 79%, 1h **7y;** 87%, 1h

4.5 Biological activity.

4.5.1 The inhibitory activities and IC_{50} values of piperine amide analogues against antioxidant, acetylcholinesterase (AChE), and butylcholinesterase (BuChE).

Twenty-nine piperine amide analogues were evaluated for antioxidant activity at the concentration 10 mM using ascorbic acid as a reference drug as shown in Table 10. Obviously, substituted 4-(piperidin-4-yl)phenol **3f** showed higher %inhibition than parent piperine, piperic acid, compound **4**, and compound **5** with an IC_{50} value of 0.04 mM. Moreover, compound **3f** showed more potent than standard drug ascorbic acid. While compounds **3a, 3b, 3c, 3j, 3l, 3o, 3q, 3s, 3v,** and **3x** demonstrate moderate antioxidant activity. Other piperine amide analogues in this series did not show antioxidant activities.

For evaluation of AChE and BuChE activities, the concentration of solution was increased to 1000 μM. Synthesized compounds **3a-3ac** were evaluated for inhibitory activity of AChE and galantamine and donepezil were used as positive controls.

Among synthesized compounds, **3c**, **3u** and **3y** showed better activity than the parent piperine and piperic acid with an IC_{50} value of 51.67, 54.95 and 71.12 μM, respectively. No inhibitory activity of AChE of piperazine containing electron-donating and electron-withdrawing aryl **3a-3h**, benzyl piperazine **3i** and **3j**, dimer piperine **3k**, diarylmethyl piperazine **3l-3n**, substituted amines **3o-3z** and quinolines **3aa-3ac** were observed.

Alkyl benzylamine compound **3z** exhibited high potent inhibitory activity of BuChE and better than galantamine up to 8-fold and close to donepezil with IC_{50} values of 4.60 µM. While compounds **3v** and **3x** bearing alkyl benzylamine as same as compound **3z** showed a significant increase in BuChE inhibitory activities (IC_{50} values of 12.66 and 15.43 µM, respectively). Substituted piperazine **3c** and substituted amine **3r** analogues showed moderate activity with IC_{50} values of 37.36 and 42.38 M, respectively. Other analogues did not show remarkable inhibitory activities. The designed compounds were selective inhibitors against BuChE.

Table 10 The inhibitory activities and IC_{50} values of piperine amide analogues against antioxidant, AChE, and BuChE.

compounds	**Antioxidant**		**Acetylcholinesterase (AChE)**		**Butyrylcholinesterase (BuChE)**	
	% Inhibition (10 mM)	**IC_{50} (mM ± SD)**	**% Inhibition (1000 µM)**	**IC_{50} (µM ± SD)**	**% Inhibition (1000 µM)**	**IC_{50} (µM ± SD)**
Piperine	12.92 ± 0.58	-	20.09 ± 0.70	-	80.92 ± 0.17	98.12 ± 1.29
Piperic acid	41.42 ± 0.31	13.64 ± 0.06	34.96 ± 0.36	-	91.60 ± 0.23	-
4	27.28 ± 0.06	-	29.81 ± 0.29	-	55.00 ± 0.50	853.07 ± 3.76
5	13.77 ± 0.28	-	19.87 ± 0.17	-	93.64 ± 0.35	207.92 ± 1.95
3a	70.94 ± 0.34	3.52 ± 0.03	6.02 ± 0.55	-	96.48 ± 0.19	135.66 ± 1.34
3b	57.97 ± 0.06	8.09 ± 0.02	8.28 ± 0.28	-	32.90 ± 0.64	-
3c	50.72 ± 0.40	10.04 ± 0.04	98.48 ± 0.27	51.67 ± 0.01	99.71 ± 0.35	37.36 ± 0.11
3d	44.02 ± 0.24	-	6.00 ± 0.22	-	44.78 ± 0.45	-
3e	30.00 ± 0.76	-	0.36 ± 0.08	-	31.64 ± 0.63	-
3f	95.13 ± 0.12	0.04 ± 0.00	82.14 ± 0.16	478.55 ± 1.20	88.68 ± 0.18	197.91 ± 1.66
3g	37.77 ± 0.27	-	78.09 ± 0.26	490.69 ± 0.64	59.71 ± 0.71	652.38 ± 3.82
3h	32.58 ± 0.86	-	6.69 ± 0.25	-	37.41 ± 0.29	-
3i	37.70 ± 0.88	-	80.86 ± 0.19	376.70 ± 0.55	25.16 ± 0.30	-

Table 10 (Continued).

compound	Antioxidant		Acetylcholinesterase (AChE)		Butyrylcholinesterase (BuChE)	
	% Inhibition (10 mM)	IC_{50} (mM ± SD)	% Inhibition (1000 μM)	IC_{50} (μM ± SD)	% Inhibition (1000 μM)	IC_{50} (μM ± SD)
3j	55.40 ± 0.11	8.66 ± 0.11	28.31 ± 0.68	-	25.13 ± 0.23	-
3k	8.21 ± 0.37	-	22.44 ± 0.07	-	63.69 ± 0.33	389.71 ± 2.73
3l	57.20 ± 0.28	8.78 ± 0.07	22.82 ± 0.48	-	3.06 ± 0.53	-
3m	39.92 ± 0.46	-	55.75 ± 0.41	899.25 ± 3.37	17.64 ± 0.24	-
3n	38.35 ± 0.10	-	21.22 ± 0.23	-	29.40 ± 0.22	-
3o	73.35 ± 0.32	4.62 ± 0.02	89.70 ± 0.21	182.50 ± 0.47	95.15 ± 0.44	46.83 ± 0.26
3p	43.33 ± 0.32	-	23.50 ± 0.22	-	100.19 ± 0.42	197.60 ± 1.44
3q	97.17 ± 0.31	1.64 ± 0.01	97.50 ± 0.33	185.47 ± 0.61	15.83 ± 0.09	-
3r	42.35 ± 0.16	-	35.70 ± 0.19	-	99.91 ± 0.09	42.38 ± 0.19
3s	53.30 ± 0.49	9.34 ± 0.06	25.68 ± 0.11	-	82.05 ± 0.25	346.08 ± 0.28
3t	23.24 ± 0.99	-	64.97 ± 0.23	833.98 ± 1.88	83.72 ± 0.20	158.13 ± 1.40
3u	7.06 ± 0.67	-	99.93 ± 0.07	54.95 ± 0.34	64.42 ± 0.05	624.39 ± 2.12
3v	55.06 ± 0.89	9.05 ± 0.04	56.35 ± 0.21	892.07 ± 1.56	83.39 ± 0.12	12.66 ± 0.08
3w	35.22 ± 0.42	-	4.53 ± 0.35	-	99.07 ± 0.25	126.86 ± 1.75
3x	73.94 ± 0.22	5.12 ± 0.02	41.39 ± 0.17	-	100.19 ± 0.34	15.43 ± 0.04
3y	2.06 ± 0.32	-	99.55 ± 0.22	71.12 ± 0.71	99.65 ± 0.24	88.64 ± 0.33
3z	30.70 ± 0.46	-	35.01 ± 0.23	-	95.79 ± 0.23	4.60 ± 0.01
3aa	10.38 ± 0.91	-	17.16 ± 0.27	-	74.47 ± 0.38	613.26 ± 2.36
3ab	28.71 ± 0.68	-	87.77 ± 0.10	216.23 ± 0.92	0.22 ± 0.28	-
3ac	6.68 ± 0.67	-	14.71 ± 0.23	-	74.68 ± 0.71	665.04 ± 1.82
Galantamine	-	-	98.52 ± 0.12	12.67 ± 0.07	96.21 ± 0.18	34.05 ± 0.32
Donepezil	-	-	100.15 ± 0.24	0.15 ± 0.001	100.04 ± 0.24	2.93 ± 0.02
Ascorbic acid	96.66 ± 0.42	0.054 ± 0.0002	-	-	-	-

4.5.2 The inhibitory activities and IC_{50} values of 1,2,3-triazole-piperine analogues against antioxidant, acetylcholinesterase (AChE), and butyrylcholinesterase (BuChE).

In vitro antioxidant activity of 1,2,3-triazole-piperine analogues is represented in Table 11 using ascorbic acid as a positive control. According to IC_{50} values, piperic ester compound **6** was the most active antioxidant activity with an IC_{50} value of 4.61 ± 0.03 mM. While compounds **7a, 7b, 7d, 7e, 7g, 7j, 7m, 7o, 7p, 7q** and **7s** showed good inhibitory activity with IC_{50} less than 20 mM.

The IC_{50} values of 1,2,3-triazole-piperine analogues against AChE and BChE were determined by Ellman's method. All results were compared with galantamine and donepezil as the reference drug. The corresponding data were summarized in Table 4.7. Compound **7a** possessing benzyl group connected to 1,2,3-triazole ring showed the best anti-AChE (IC_{50} = 37.37 □M). When comparing benzyl analog **7a** with substituted benzyl analogues **7b-7n** such as electron-donating groups (**7b-7d**), electron-withdrawing groups (**7e-7m**) and disubstituted **7n**, the inhibitory activity of AChE were diminished. Furthermore, disubstituted **7p-7r**, phenylethyl **7s-7v**, phenylethyl **7w** and aliphatic **7x** and **7y** showed low anti-AChE activity.

In part of BuChE activity, compound **7s** containing phenylethyl moiety displayed higher inhibitory activity than galantamine with IC_{50} values of 28.81 □M. While other compounds did not showed BuChE inhibitory activity. Based on the results, the steric effect of substituted benzyl triazole group on piperine plays an important role in these series and showed high selectivity against BChE activity.

Table 11 The inhibitory activities and IC_{50} values of 1,2,3-triazole-piperine analogues against antioxidant, AChE, and BuChE.

compound	Antioxidant		Acetylcholinesterase (AChE)		Butyrylcholinesterase (BuChE)	
	%Inhibition (10 mM)	IC_{50} (mM ± SD)	% Inhibition (1000 μM)	IC_{50} (μM ± SD)	% Inhibition (1000 μM)	IC_{50} (μM ± SD)
Piperine	12.92 ± 0.58	-	20.09 ± 0.70	-	80.92 ± 0.17	98.12 ± 1.29
Piperic acid	41.42 ± 0.31	13.64 ± 0.06	34.96 ± 0.36	-	91.60 ± 0.23	-
6	74.78 ± 0.14	4.61 ± 0.03	87.87 ± 0.15	71.07 ± 0.55	8.38 ± 0.75	-
7a	32.37 ± 0.12	17.49 ± 0.04	99.41 ± 0.30	37.37 ± 0.04	79.13 ± 0.31	238.63 ± 1.74
7b	62.16 ± 0.61	7.41 ± 0.02	90.78 ± 0.26	56.55 ± 0.32	99.86 ± 0.12	189.80 ± 7.80
7c	3.36 ± 0.26	-	51.35 ± 0.26	994.74 ± 3.96	19.51 ± 0.14	-
7d	72.87 ± 0.45	5.79 ± 0.03	48.34 ± 0.22	1056.33 ± 1.37	24.09 ± 0.09	-
7e	54.20 ± 0.25	9.07 ± 0.03	58.54 ± 0.66	672.06 ± 5.67	82.96 ± 0.25	197.53 ± 1.55
7f	9.37 ± 0.51	-	86.43 ± 0.16	228.02 ± 7.12	28.54 ± 0.28	-
7g	42.89 ± 1.02	14.05 ± 0.08	47.93 ± 0.26	1099.51 ± 0.95	61.67 ± 0.21	447.65 ± 1.45
7h	6.96 ± 0.18	-	24.75 ± 0.13	-	62.15 ± 0.16	423.00 ± 0.41
7i	4.71 ± 0.59	-	93.42 ± 0.47	336.50 ± 0.85	85.03 ± 0.12	261.93 ± 3.85
7j	36.37 ± 0.56	13.95 ± 0.11	46.06 ± 0.08	1225.89 ± 1.57	59.15 ± 0.18	711.50 ± 1.32
7k	19.20 ± 0.14	-	66.76 ± 0.07	672.23 ± 1.96	77.42 ± 0.26	340.81 ± 0.91
7l	1.93 ± 0.22	-	22.15 ± 0.13	-	29.87 ± 0.10	-
7m	53.09 ± 0.12	11.16 ± 0.03	18.08 ± 0.47	-	11.17 ± 0.46	-
7n	17.69 ± 0.55	-	6.37 ± 0.97	-	98.37 ± 0.27	230.19 ± 0.20
7o	40.68 ± 0.40	14.54 ± 0.01	35.16 ± 0.99	-	18.07 ± 0.18	-
7p	43.12 ± 0.55	13.94 ± 0.18	49.31 ± 0.25	1195.44 ± 7.68	32.61 ± 0.14	-
7q	48.84 ± 0.31	11.88 ± 0.09	95.64 ± 0.31	111.94 ± 0.33	96.83 ± 0.12	219.51 ± 1.41
7r	17.54 ± 1.07	-	0.02 ± 0.23	-	74.12 ± 0.06	204.56 ± 0.76

Table 11 (Continued).

compound	Antioxidant		Acetylcholinesterase (AChE)		Butyrylcholinesterase (BuChE)	
	%Inhibition (10 mM)	IC_{50} (mM ± SD)	% Inhibition (1000 μM)	IC_{50} (μM ± SD)	% Inhibition (1000 μM)	IC_{50} (μM ± SD)
7s	55.00 ± 0.38	8.82 ± 0.09	32.06 ± 0.75	-	94.94 ± 0.30	28.81 ± 0.34
7t	16.93 ± 0.70	-	36.46 ± 0.62	-	74.28 ± 1.35	370.03 ± 1.84
7u	0.82 ± 0.07	-	3.34 ± 0.08	-	23.09 ± 0.33	-
7v	18.73 ± 0.64	-	70.91 ± 0.20	627.44 ± 0.70	78.45 ± 0.17	320.72 ± 1.32
7w	19.55 ± 0.38	-	27.00 ± 1.95	-	17.28 ± 0.15	-
7x	9.86 ± 1.18	-	12.86 ± 0.57	-	34.63 ± 0.09	-
7y	0.77 ± 0.37	-	2.21 ± 0.69	-	35.65 ± 0.25	-
Galantamine	-	-	98.52 ± 0.12	12.67 ± 0.07	96.21 ± 0.18	34.05 ± 0.32
Donepezil	-	-	100.15 ± 0.24	0.15 ± 0.001	100.04 ± 0.24	2.93 ± 0.02
Ascorbic acid	96.66 ± 0.42	0.054 ± 0.0002	-	-	-	-

4.6 Kinetic study.

4.6.1 Kinetic study of piperine amide analogues against AChE inhibition.

The representative of piperine amide analogues was subjected to kinetic study to determine the mode of inhibition against AChE. The type of inhibition was determined by Lineweaver-Burk, while inhibition constant (K_i) plot between 1/velocity versus 1/substrate was produced with five different concentrations of the substrate acetylthiocholine iodide. The Lineweaver-Burk plot, demonstrated inhibition type and estimated inhibition constant (K_i) of compounds **3c**, **3u** and **3y** against AChE. The results are summarized in Table 12. The compounds **3c** and **3y** showed the interception of the lines at the y-axis to provide the competitive inhibitors and displayed inhibition constant (K_i) with 0.04829 and 0.03935 mM, respectively (Figures 25 and 27). In the other hand, compounds **3u** showed the interception of the lines at the x-axis to provide the non-competitive inhibitor with inhibition constant (K_i) of 0.01627 mM (Figure 26).

Table 12 Kinetic characterization of AChE inhibition.

compound	Type of inhibition	Ki (mM)	Kis (mM)
3c	competitive	0.04829	-
3u	non-competitive	0.01627	0.00720
3y	competitive	0.03935	-

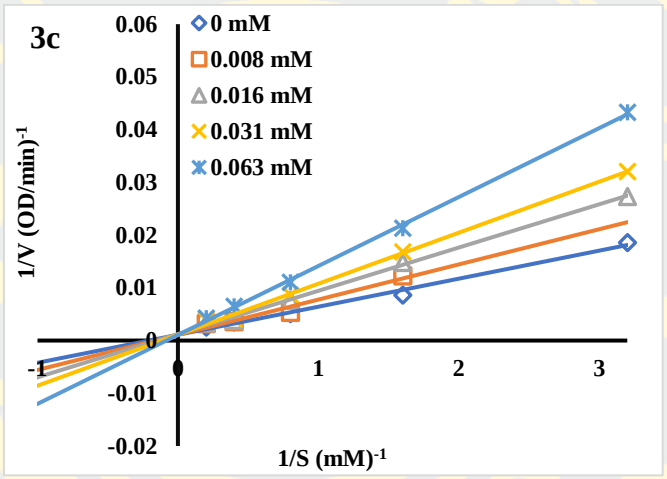


Figure 25 Lineweaver-Burk plots showing competitive inhibition of compound **3c** against AChE.

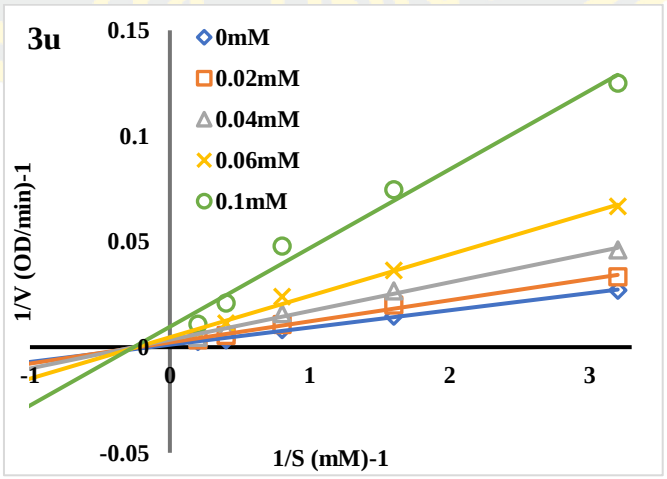


Figure 26 Lineweaver-Burk plots showing non-competitive inhibition of compound **3u** against AChE.

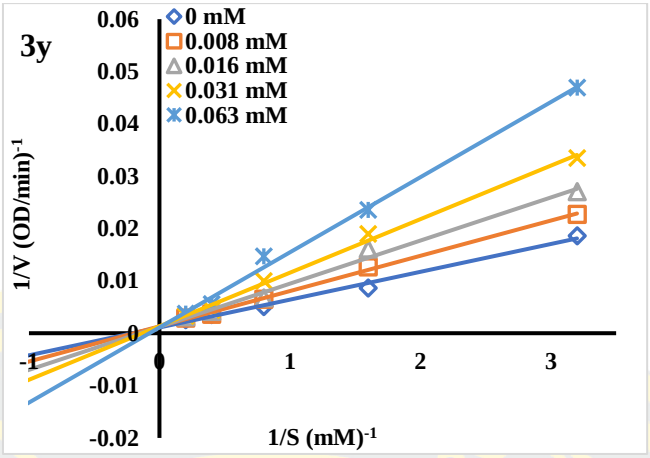


Figure 27 Lineweaver-Burk plots showing competitive inhibition of compound **3y** against AChE.

4.6.2 Kinetic study of piperine amide analogues against BuChE inhibition.

To determine the kinetic type of BChE inhibition, the most active compounds toward BChE were selected for kinetic studies of inhibition of enzymes. The graphical analysis of steady-state inhibition data for compounds **3c**, **3o**, **3r**, **3v**, **3x**, **3y** and **3z** are shown in Table 13 and Figures 28-34, respectively. The reciprocal plots confirmed that compounds **3o**, **3x**, and **3y** were the mixed types of inhibition for BChE and displayed the inhibition constant (K_i) of 0.15062, 0.03343 and 0.20608 mM, respectively (Figures 29, 32 and 33). The compounds **3v** and **3z** showed the non-competitive inhibitors with inhibition constant (K_i) of 0.01459 and 0.00391 mM, respectively (Figures 31 and 34). Compounds **3c** and **3r** demonstrated the competitive inhibitors and displayed an inhibition constant (K_i) with 0.01734 and 0.09897 mM (Figures 28 and 30).

Table 13 Kinetic characterization of BuChE inhibition.

compound	Type of inhibition	Ki (mM)	Kis (mM)
3c	competitive	0.01734	-
3o	mixed-type	0.15062	0.11017
3r	competitive	0.09897	-
3v	non-competitive	0.01459	0.01341
3x	mixed-type	0.03343	0.01308
3y	mixed-type	0.20608	0.12389
3z	non-competitive	0.00391	0.00343

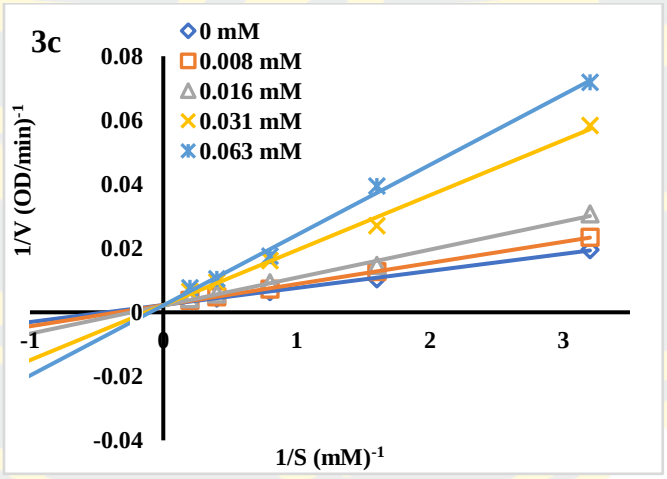


Figure 28 Lineweaver-Burk plots showing competitive inhibition of compound **3c** against BuChE.

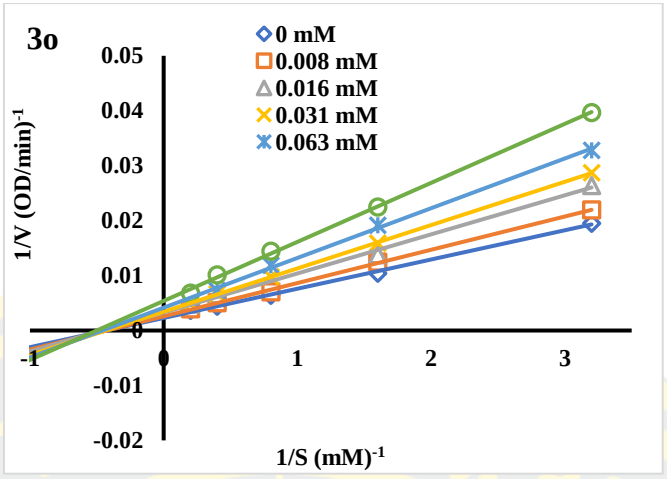


Figure 29 Lineweaver-Burk plots showing Mixed-type inhibition of compound **3o** against BuChE.

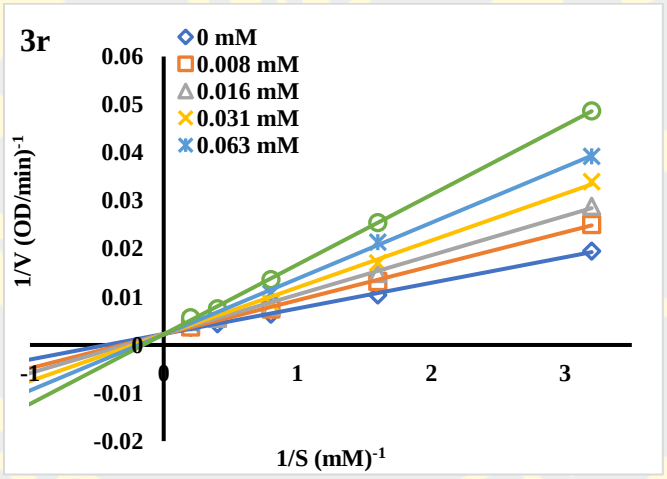


Figure 30 Lineweaver-Burk plots showing competitive inhibition of compound **3r** against BuChE.

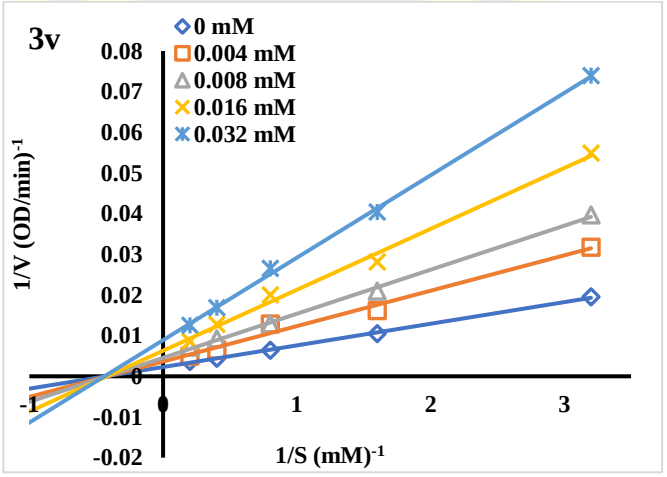


Figure 31 Lineweaver-Burk plots showing non-competitive inhibition of compound **3v** against BuChE.

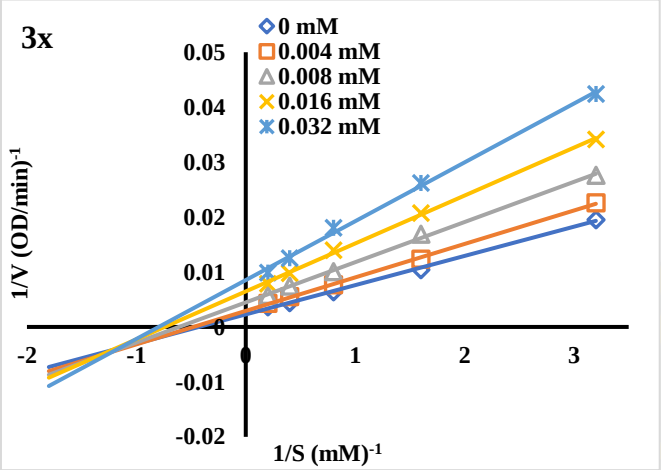


Figure 32 Lineweaver-Burk plots showing mixed-type inhibition of compound **3x** against BuChE.

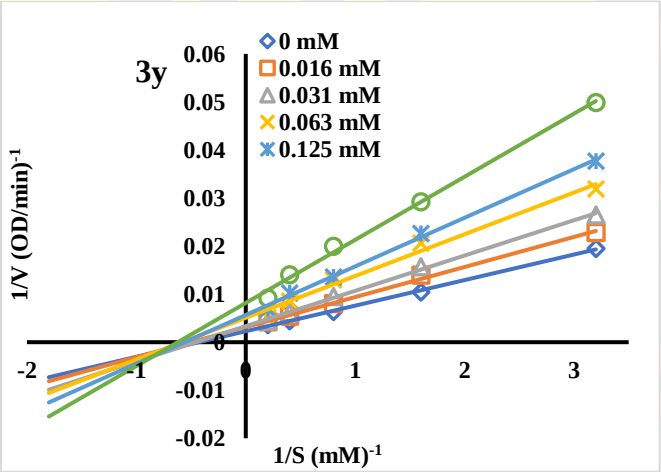


Figure 33 Lineweaver-Burk plots showing mixed-type inhibition of compound **3y** against BuChE.

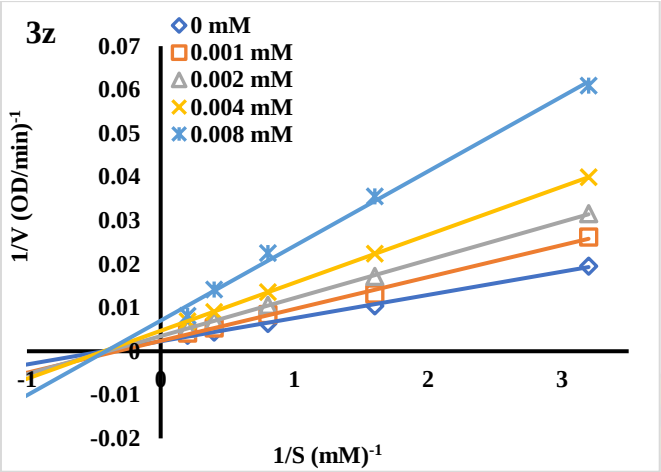


Figure 34 Lineweaver-Burk plots showing non-competitive inhibition of compound **3z** against BuChE.

4.6.3 Kinetic study of 1,2,3 triazole-piperine analogues against AChE inhibition.

To gain further insight into the mechanism of action, a kinetic study was carried out with the most potent AChE inhibitors (piperic ester **6**, compounds **7a** and **7b**) as shown in Table 14. All pattern indicates that piperic ester **6**, compounds **7a** and **7b** were the non-competitive inhibition. The calculated inhibitory constant (K_i) values based on the secondary plot of AChE inhibitors were 3.65217, 1.89130 and 4.45000 mM, respectively as shown in Figures 35-37.

Table 14 Kinetic characterization of AChE inhibition.

compounds	Type of inhibition	Ki (mM)	Kis (mM)
Piperic ester **6**	non-competitive	3.65217	3.20000
7a	partially non-competitive	1.89130	-
7b	non-competitive	4.45000	6.00000

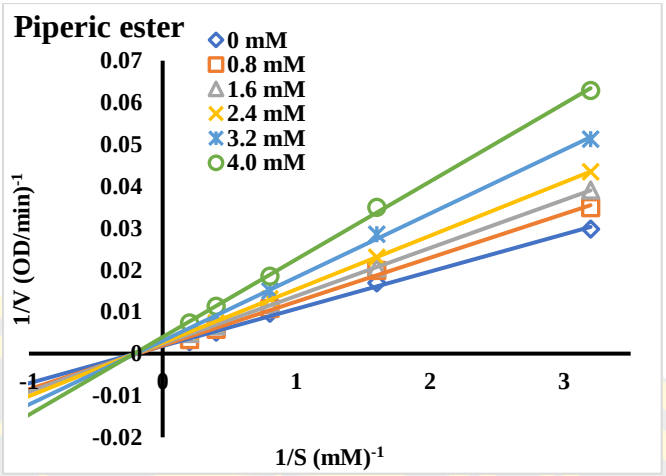


Figure 35 Lineweaver-Burk plots showing non-competitive inhibition of piperic ester against AChE.

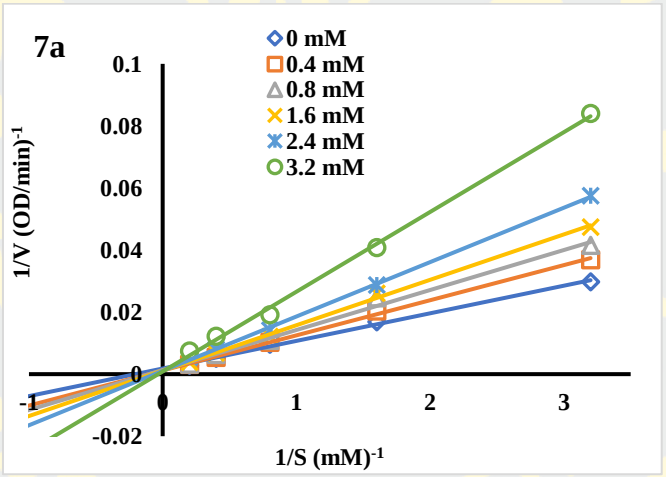


Figure 36 Lineweaver-Burk plots showing partially non-competitive of compound **7a** against AChE.

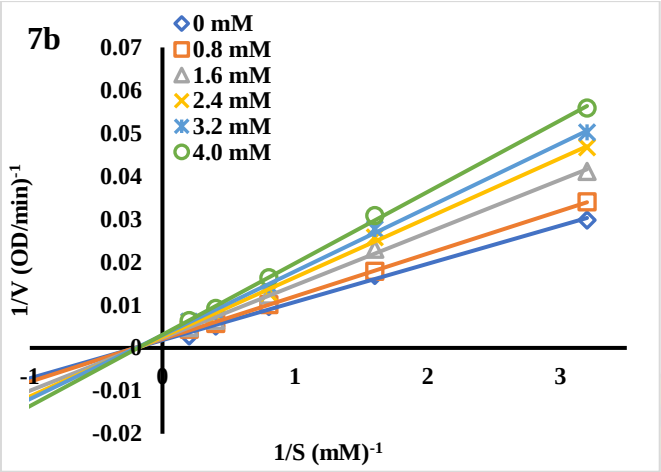


Figure 37 Lineweaver-Burk plots showing non-competitive inhibition of compound **7b** against AChE.

4.6.4 Kinetic study of 1,2,3 triazole-piperine analogues against BuChE inhibition.

In order to reveal the inhibition mode of BuChE induced by the most potent inhibitor **7s**, the graphical analysis of the Lineweaver-Burk reciprocal plots demonstrated a competitive inhibition (Figure 38), and the inhibition constant (K_i = 0.01235 mM) was shown in Table 15.

Table 15 Kinetic characterization of BuChE inhibition.

compound	Type of inhibition	Ki (mM)	Kis (mM)
7s	competitive	0.01235	-

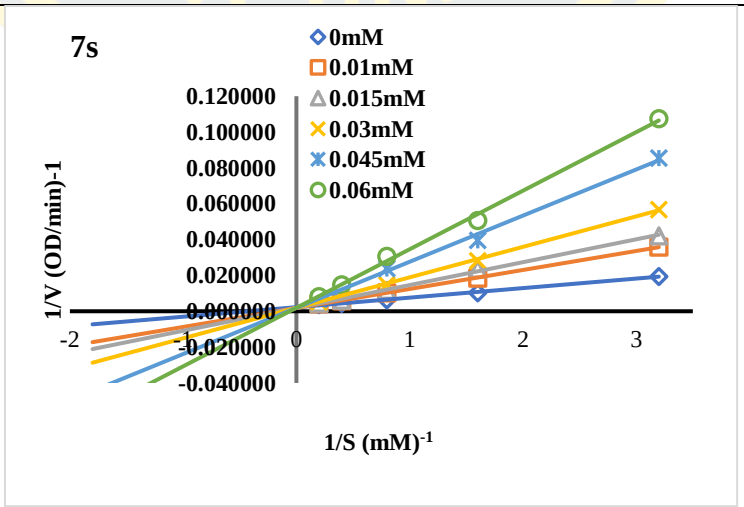
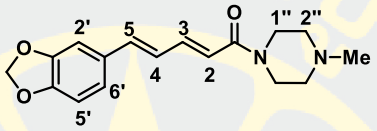


Figure 38 Lineweaver-Burk plots showing competitive inhibition of compound **7s** against BuChE.

4.7 Compounds characterization

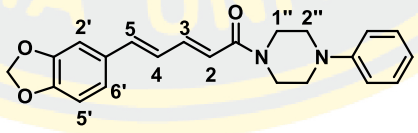
The products were characterized by spectroscopic methods (NMR and IR) and HRMS.

4.7.1 piperine amide analogues (3a-3ac, 4, and 5)



3a

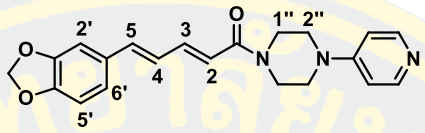
5-(benzo[1,3]dioxol-5-yl)-1-(4-methylpiperazin-1-yl)penta-2,4-dien-1-one; 87% yield (120.3 mg) as a yellow oil; R_f = 0.30 (10% MeOH/CH_2Cl_2); IR-ATR: ν_{max} 3196, 2896, 1610, 1631, 1585, 1488, 1440, 1249 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.40 (dd, J = 14.4, 9.6 Hz, 1H, H-3), 6.95 (s, 1H, H-2′), 6.87 (d, J = 8.0 Hz, 1H, H-Ar), 6.80-6.63 (m, 3H, H-5′, H-5, H-4), 6.38 (d, J = 14.4 Hz, 1H, H-2), 5.95 (s, 2H, O-CH_2-O), 3.80-3.50 (m, 4H, H-1″), 2.45-2.35 (m, 4H, H-2″), 2.29 (s, 3H, H-CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) 165.47, 148.15, 144.13, 143.01, 138.68, 130.80, 125.04, 122.53, 119.22, 108.41, 105.61, 101.22, 55.11, 54.59, 45.84, 45.47, 41.83; HRMS (ESI) m/z calcd for $C_{17}H_{20}N_2O_3$ $(M+H)^+$ 301.1552, found 301.1553.



3b

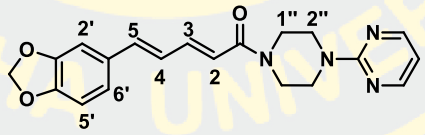
5-(benzo[1,3]dioxol-5-yl)-1-(4-phenylpiperazin-1-yl)penta-2,4-dien-1-one; 86% yield (142.8 mg) as a yellow oil; R_f = 0.25 (40% EtOAc/*n*-Hexane); IR-ATR: ν_{max} 2971, 2920, 1634, 1622, 1587, 1443, 1229 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.46 (ddd, J = 14.8, 9.2, 0.8 Hz, 1H, H-3), 7.55 (td, J = 7.2, 2.0 Hz, 2H, H-Ar), 6.98 (d, J = 2.8 Hz,1H, H-2′), 6.90 (dd, J = 14.4, 7.6 Hz, 4H, H-6′, H-Ar), 6.82-6.70 (m, 3H, H-5′, H-5, H-4), 6.44 (d, J = 14.8 Hz, 1H, H-2), 5.95 (s, 2H, O-CH_2-O), 3.9-3.60 (m, 4H, H-1″), 3.25-3.10 (m, 4H, H-2″); ^{13}C NMR (100 MHz, $CDCl_3$) 165.40,

150.76, 148.12, 148.07, 143.14, 138.80, 130.68, 129.07 (2C), 124.92, 122.53, 120.25, 118.98, 116.38 (2C), 108.34, 105.55, 101.16, 49.56, 49.19, 45.46, 41.78; HRMS (ESI) m/z calcd for $C_{22}H_{22}N_2O_3$ $(M+H)^+$ 363.1709, found 363.1708.



3c

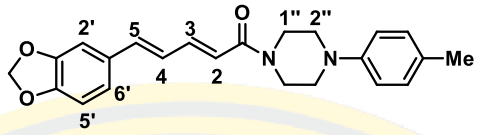
5-(benzo[1,3]dioxol-5-yl)-1-(4-(79uinolin-4-yl)piperazin-1-yl)penta-2,4-dien-1-one; 77% yield (129.0 mg) as a orange solid; R_f = 0.25 (10% MeOH/CH_2Cl_2); mp = 161-162 ^oC; IR-ATR: ν_{max} 3212, 2922, 1642, 1587, 1491, 1439, 1231 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 8.26 (s, 2H, H-Ar), 7.43 (dd, J = 14.4, 10.8 Hz, 1H, H-3), 6.94 (s, 1H, H-2′), 6.86 (d, J = 7.6 Hz, 1H, H-6′), 6.86-6.66 (m, 3H, H-5′, H-5, H-4), 6.62 (s, 2H, H-Ar), 6.38 (d, J = 14.4 Hz, 1H, H-2), 5.93 (s, 2H, O-CH_2-O), 4.00-3.60 (m, 4H, H-1″), 3.50-3.10 (m, 4H, H-2″); ^{13}C NMR (100 MHz, $CDCl_3$)165.51, 154.32, 149.99 (2C), 148.19, 148.05, 143.54, 139.19, 130.52, 124.70, 122.60, 118.49, 108.33 (2C), 108.20, 105.50, 101.16, 45.62 (2C), 44.65, 41.07; HRMS (ESI) m/z calcd for $C_{21}H_{21}N_3O_3$ $(M+H)^+$ 364.1661, found 364.1664.



3d

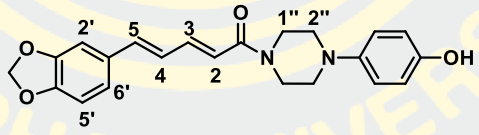
5-(benzo[1,3]dioxol-5-yl)-1-(4-(pyrimidin-2-yl)piperazin-1-yl)penta-2,4-dien-1-one; 74% yield (124.4 mg) as a yellow solid; R_f = 0.25 (70% EtOAc/*n*-Hexane); mp = 143-145 ^oC; IR-ATR: ν_{max} 3026, 2917, 1631, 1580, 1550, 1489, 1237 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 8.30 (d, J = 4.8 Hz, 2H, H-Ar), 7.46 (dd, J = 14.4, 9.2 Hz, 1H, H-3), 6.96 (s, 1H, H-2′), 6.87 (d, J = 8.0 Hz, 1H, H-6′), 6.80-6.67 (m, 3H, H-5′, H-5, H-4), 6.51 (s, 1H, H-Ar), 6.42 (d, J = 14.8 Hz, 1H, H-2), 5.94 (s, 2H, O-CH_2-O), 3.90-3.80 (m, 4H, H-1″), 3.80-3.50 (m, 4H, H-2″); ^{13}C NMR (100 MHz, $CDCl_3$) 165.63, 161.35, 157.63 (2C), 148.14, 148.09, 143.25, 138.87, 130.70,

124.94, 122.54, 119.02, 110.28, 108.36, 105.58, 101.18, 45.35, 43.59 (2C), 41.72; HRMS (ESI) m/z calcd for $C_{20}H_{20}N_4O_3$ $(M+H)^+$ 365.1614, found 365.1617.



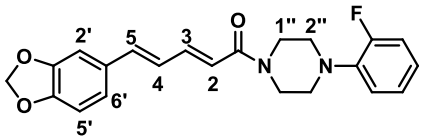
3e

5-(benzo[1,3]dioxol-5-yl)-1-(4-(*p*-tolyl)piperazin-1-yl)penta-2,4-dien-1-one; 80% yield (139.2 mg) as a yellow solid; R_f = 0.34 (50% EtOAc/*n*-Hexane); mp = 165-166 °C; IR-ATR: ν_{max} 2916, 2854, 1645 1622, 1591, 1502, 1455, 1442, 1232 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.46 (dd, J = 14.8, 9.6 Hz, 1H, H-3), 7.10 (d, J = 8.4 Hz, 2H, H-Ar), 6.99 (d, J = 0.8 Hz, 1H, H-2′), 6.90 (dd, J = 8.0, 1.2 Hz, 1H, H-6′), 6.85 (d, J = 8.4 Hz, 2H, H-Ar), 6.81-6.74 (m, 3H, H-5′, H-5, H-4), 6.45 (d, J = 14.4 Hz, 1H, H-2), 5.97 (s, 2H, O-CH_2-O), 4.00-3.64 (m, 4H, H-1″), 3.20-3.00 (m, 4H, H-2″) , 2.28 (s, 3H, H-CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) 165.48, 148.76, 148.19, 148.14, 143.19, 138.84, 130.78, 129.99, 129.67 (2C), 125.01, 122.59, 119.08, 116.88 (2C), 108.42, 105.62, 101.22, 50.33, 49.87, 45.64, 41.95, 20.37; HRMS (ESI) m/z calcd for $C_{23}H_{24}N_2O_3$ $(M+H)^+$ 377.1865, found 377.1866.



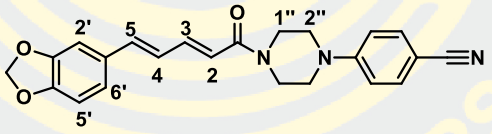
3f

5-(benzo[1,3]dioxol-5-yl)-1-(4-(4-hydroxyphenyl)piperazin-1-yl)penta-2,4-dien-1-one; 55% yield (94.9 mg) as a yellow oil; R_f = 0.19 (60% EtOAc/*n*-Hexane); IR-ATR: ν_{max} 2920, 1738, 1575, 1513, 1488, 1439, 1228, 1034 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.46 (dd, J = 14.8, 9.6 Hz, 1H, H-3), 6.86-6.37 (m, 9H, H-Ar, H-2′, H-6′, H-5′, H-5, H-4), 6.44 (d, J = 14.4 Hz, 1H, H-2), 5.98 (s, 2H, O-CH_2-O), 5.30 (s, 1H, OH), 3.89-3.70 (m, 4H, H-1″), 3.04 (s, 4H, H-2″); ^{13}C NMR (100 MHz, $CDCl_3$) 165.66, 150.40, 148.28 (2C), 148.22, 145.23, 143.42, 139.05, 130.84, 125.03, 122.69, 119.14, 119.03, 115.96, 108.52, 105.69, 101.31, 52.30, 43.04, 29.68; HRMS (ESI) m/z calcd for $C_{22}H_{22}N_2O_4$ $(M+Na)^+$ 401.1477, found 401.1479.



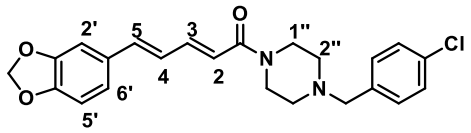
3g

5-(benzo[1,3]dioxol-5-yl)-1-(4-(2-fluorophenyl)piperazin-1-yl) penta-2,4-dien-1-one; 75% yield (136.2 mg) as a yellow solid; R_f = 0.32 (50% EtOAc/*n*-Hexane); mp = 125-126°C; IR-ATR: ν_{max} 2990, 2951, 1639, 1624, 1589, 1499, 1489, 1439, 1232 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.46 (dd, J = 14.8, 9.6 Hz, 1H, H-3), 7.10-7.00 (m, 2H, H-Ar, H-2′), 7.00-6.86 (m, 4H, H-Ar, H-6′), 6.82-6.69 (m, 3H, H-5′, H-5, H-4), 6.44 (d, J = 14.4 Hz, 1H, H-2), 5.96 (s, 2H, O-CH_2-O), 3.95-3.68 (m, 4H, H-1′), 3.16-3.00 (m, 4H, H-2′); ^{13}C NMR (100 MHz, $CDCl_3$) 165.55, 155.7 (d, J = 244 Hz, 1C, C-F), 148.21, 148.19, 148.17, 143.26, 139.53 (d, J = 8.0 Hz, 1C, C-F_o), 138.88, 130.80, 125.02, 124.50 (d, J = 3.0 Hz, 1C, C-F_p), 123.05 (d, J = 8.0 Hz, 1C, C-F_m), 122.60, 119.16 (d, J = 3.0 Hz, 1C, C-F_m), 119.07, 116.18 (d, J = 21.0 Hz, 1C, C-F_o), 108.45, 105.65, 101.25, 51.05, 50.32, 45.83, 42.10; HRMS (ESI) m/z calcd for $C_{22}H_{21}FN_2O_3$ $(M+H)^+$ 381.1614, found 381.1612.



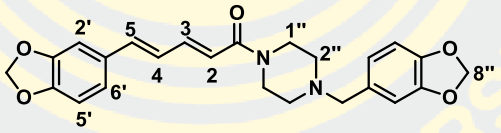
3h

4-(4-(5-(benzo[1,3]dioxol-5-yl)penta-2,4-dienoyl)piperazin-1-yl)benzonitrile; 60% yield (106.0 mg) as a yellow solid; R_f = 0.34 (70% EtOAc/*n*-Hexane); mp = 181-182 °C; IR-ATR: ν_{max} 2924, 2854, 1642, 1607, 1582, 1504, 1492, 1233 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.55-7.44 (m, 3H, H-Ar, H-3), 6.99 (d, J = 1.2 Hz, 1H, H-2′), 6.91 (dd, J = 8.4, 1.6 Hz, 1H, H-6′), 6.89-6.83 (d, J = 9.2, Hz, 2H, H-Ar), 6.82-6.74 (m, 3H, H-5′, H-5, H-4), 6.41 (d, J = 14.4 Hz, 1H, H-2), 5.99 (s, 2H, O-CH_2-O), 3.95-3.68 (m, 4H, H-1″), 3.45-3.31 (m, 4H, H-2″); ^{13}C NMR (100 MHz, $CDCl_3$) 165.73, 152.89, 148.39, 148.25, 143.83, 139.46, 133.58 (2C), 130.72, 124.83, 122.78, 119.74, 118.53, 114.38 (2C), 108.53, 105.69, 101.33, 101.07, 47.18 (2C), 46.55 (2C); HRMS (ESI) m/z calcd for $C_{23}H_{21}N_3O_3$ $(M+H)^+$ 388.1661, found 388.1662.



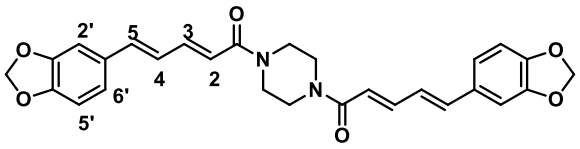
3i

5-(benzo[1,3]dioxol-5-yl)-1-(4-(4-chlorobenzyl)piperazin-1-yl)penta-2,4-dien-1-one; 54% yield (126.7 mg) as a brown oil; R_f = 0.20 (50% EtOAc/*n*-Hexane); IR-ATR: ν_{max} 3003, 2893, 1634, 1611, 1584, 1488, 1442, 1231, 1037, 851 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.42 (dd, *J* = 14.4, 9.6 Hz, 1H, H-3), 7.32-7.22 (m, 4H, H-Ar), 6.96 (d, *J* = 1.2 Hz, 1H, H-2′), 6.88 (dd, *J* = 8.0, 1.2 Hz, 1H, H-6′), 6.80-6.68 (m, 3H, H-5′, H-5, H-4), 6.39 (d, *J* = 14.8 Hz, 1H, H-2), 5.96 (s, 2H, O-CH_2-O), 3.80-3.50 (m, 4H, H-1″), 3.47 (s, 2H, H-CH_2), 2.50-2.30 (m, 4H, H-2″); ^{13}C NMR (100 MHz, $CDCl_3$) 165.50, 148.19 (2C), 143.08, 138.77, 136.14, 133.00, 130.61, 130.33 (2C), 128.48 (2C), 125.09, 122.60, 119.24, 108.48, 105.67, 101.27, 62.02, 53.15, 52.75, 45.64, 42.00; HRMS (ESI) m/z calcd for $C_{23}H_{23}ClN_2O_3$ (M+Na) + 433.1289; Found 433.1289.



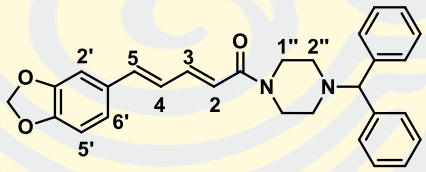
3j

5-(benzo[1,3]dioxol-5-yl)-1-(4-(benzo[1,3]dioxol-5-ylmethyl)piperazin-1-yl)penta-2,4-dien-1-one; 78% yield (149.7 mg) as a yellow solid; R_f = 0.27 (80% EtOAc/*n*-Hexane); mp = 137-138 ^oC; IR-ATR: ν_{max} 3063, 2895, 1634, 1610, 1590, 1487, 1435, 1247 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.40 (dd, *J* = 14.8, 9.6 Hz, 1H, H-3), 6.95 (s, 1H, H-2′), 6.88-6.81 (m, 2H, H-6′, H5″), 6.78-6.60 (m, 5H, H-10″, H-6″, H-5′, H-5, H-4), 6.37 (d, *J* = 14.8 Hz, 1H, H-2), 5.93 (d, *J* = 8.0 Hz, 4H, O-CH_2-O), 3.80-3.46 (m, 4H, H-1″), 3.40 (s, 2H, H-CH_2), 2.50-2.25 (m, 4H, H-2″); ^{13}C NMR (100 MHz, $CDCl_3$) 165.31, 148.06 (2C), 147.58, 146.62, 142.82, 138.52, 131.37, 130.76, 125.03, 122.45, 122.05, 119.27, 109.23, 108.34, 107.76, 105.55, 101.15, 100.79, 62.40, 52.98, 52.49, 45.56, 41.92; HRMS (ESI) m/z calcd for $C_{24}H_{24}N_2O_5$ $(M+H)^+$ 421.1763, found 421.1764.



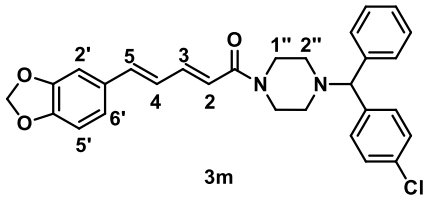
3k

1,1'-(piperazine-1,4-diyl)bis(5-(benzo[1,3]dioxol-5-yl)penta-2,4-dien-1-one); 57% yield (75.6 mg) as a yellow solid; R_f = 0.54 (70% EtOAc/*n*-Hexane); mp = 189-190 °C; IR-ATR: ν_{max} 3322, 2927, 2850, 1622, 1568, 1309, 1242 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.47 (dd, *J* = 10.4, 14.4 Hz, 2H, H-3), 6.99 (d, *J* = 1.2 Hz, 2H, H-2′), 6.91 (dd, *J* = 8.0, 1.6 Hz, 2H, H-6′), 6.84-6.70 (m, 6H, H-5′, H-5, H-4), 6.40 (d, *J* = 14.4 Hz, 2H, H-2), 5.99 (s, 4H, O-CH_2-O), 3.79-3.61 (m, 8H, H-1″, H-2″); ^{13}C NMR (100 MHz, $CDCl_3$) 165.61, 148.20 (4C), 143.02 (2C), 138.70 (2C), 130.89 (2C), 125.13 (2C), 122.59 (2C), 119.31 (2C), 108.49 (2C), 105.67 (2C), 101.28 (2C), 46.91, 46.45, 45.87, 43.21(2C) ; HRMS (ESI) m/z calcd for $C_{28}H_{26}N_2O_6$ $(M+Na)^+$ 509.1683, found 509.1684.



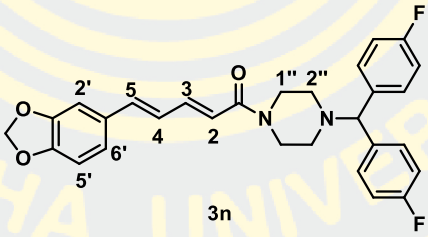
3l

1-(4-benzhydrylpiperazin-1-yl)-5-(benzo[1,3]dioxol-5-yl)penta-2,4-dien-1-one; 88% yield (181.7 mg) as a yellow oil; R_f = 0.31 (30% EtOAc/*n*-Hexane); IR-ATR: ν_{max} 3026, 2892, 1636, 1613, 1588, 1488, 1441, 1250, 1236 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.40 (dd, *J* = 15.6, 8.8 Hz, 5H, H-3, H-Ar), 7.27 (t, *J* = 7.2 Hz, 4H, H-Ar), 7.18 (t, *J* = 7.2 Hz, 2H, H-Ar), 6.95 (d, *J* = 1.6 Hz, 1H, H-2′), 6.85 (dd, *J* = 8.0, 1.6 Hz, 1H, H-6′), 6.78-6.63 (m, 3H, H-5′, H-5, H-4), 6.34 (d, *J* = 14.8 Hz, 1H, H-2), 5.92 (s, 2H, O-CH_2-O), 4.23 (s, 1H, H-CH), 3.80-3.40 (m, 4H, H- 1″), 2.50-2.20 (m, 4H, H-2″); ^{13}C NMR (100 MHz, $CDCl_3$) 165.20, 148.00 (2C), 142.70, 141.96 (2C), 138.44, 130.69, 128.41 (4C), 127.68 (4C), 126.96 (2C), 124.99, 122.40, 119.22, 108.27, 105.49, 101.07, 75.70, 51.96, 51.40, 42.62, 42.02; HRMS (ESI) m/z calcd for $C_{29}H_{28}N_2O_3$ $(M+H)^+$ 453.2178, found 453.2180.



3m

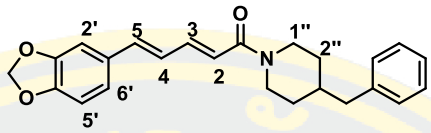
5-(benzo[1,3]dioxol-5-yl)-1-(4((4-chlorophenyl)(phenyl)methyl)piperazin-1-yl)penta-2,4-dien-1-one; 71% yield (191.8 mg) as a yellow oil; R_f = 0.31 (30% EtOAc/*n*-Hexane) ; IR-ATR: ν_{max} 2970, 1738, 1636, 1488, 1440, 1234 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.41 (dd, *J* = 14.4, 9.6 Hz, 1H, H-3), 7.39-7.32 (m, 4H, H-Ar), 7.27 (dd, *J* = 16.4, 7.2 Hz, 4H, H-Ar), 7.19 (t, *J* = 7.2 Hz, 1H, H-Ar), 6.95 (d, *J* = 1.6 Hz, 1H, H-2′), 6.86 (dd, *J* = 8.4, 1.6 Hz, 1H, H-6′), 6.78-6.60 (m, 3H, H-5′, H-5, H-4), 6.35 (d, *J* = 14.4 Hz, 1H, H-2), 5.93 (s, 2H, O-CH_2-O), 4.22 (s, 1H, H-CH), 3.80-3.50 (m, 4H, H-1′′), 2.50-2.25 (m, 4H, H-2′′); ^{13}C NMR (100 MHz, $CDCl_3$)165.33, 148.11, 148.09, 142.92, 141.44, 140.66, 138.64, 132.67, 130.75, 129.03 (2C), 128.68 (2C), 128.62 (2C), 127.68 (2C), 127.28, 125.01, 122.50, 119.14, 108.36, 105.58, 101.17, 75.05, 51.96, 51.42, 45.67, 42.06 ; HRMS (ESI) m/z calcd for $C_{29}H_{27}ClN_2O_3$ (M+H) + 487.1783; Found 487.1795.



3n

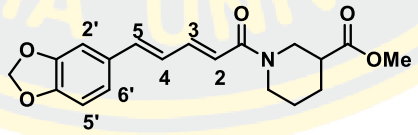
5-(benzo[1,3]dioxol-5-yl)-1-(4-(bis(4-fluorophenyl)methyl)piperazin-1-yl) penta-2,4-dien-1-one; 87% yield (194.7 mg) as a yellow oil; R_f = 0.30 (40% EtOAc/*n*-Hexane); IR-ATR: ν_{max} 2958, 2807, 1640, 1593, 1504, 1442, 1426, 1255 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.41 (dd, *J* = 14.8, 10.0 Hz, 1H, H-3), 7.34 (dd, *J* = 10.0, 5.6 Hz, 4H, H-Ar), 6.98 (t, *J* = 8.8 Hz, 5H, H-Ar, H-2′), 6.87 (dd, *J* = 8.0, 1.6 Hz, 1H, H-6′), 6.79-6.65 (m, 3H, H-5′, H-5, H-4), 6.36 (d, *J* = 14.8 Hz, 1H, H-2), 5.94 (s, 2H, O-CH_2-O), 4.23 (s, 1H, H-CH), 3.80-3.50 (m, 4H, H-1′′), 2.40-2.30 (m, 4H, H-2′′); ^{13}C NMR (100 MHz, $CDCl_3$) 165.35, 161.79 (d, *J* = 244 Hz, 2C, C-F), 148.13, 148.11, 142.97, 138.68, 137.55 (d, *J* = 12.0 Hz, 2C, C-F_m), 130.75, 129.14 (d,

J = 8.0 Hz, 4C, C-F_p), 125.00, 122.52, 119.12, 115.44 (d, *J* = 21.0 Hz, 4C, C-F_o), 108.38, 105.58, 101.18, 74.12, 51.92, 50.37, 45.67, 42.04; HRMS (ESI) m/z calcd for $C_{29}H_{26}F_2N_2O_3$ $(M+Na)^+$ 511.1809, found 511.1807.



3o

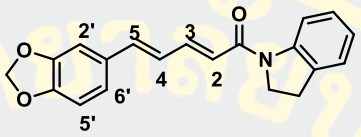
5-(benzo[1,3]dioxol-5-yl)-1-(4-benzylpiperidin-1-yl)penta-2,4-dien-1-one; 65% yield (111.7 mg) as a yellow oil; R_f = 0.30 (30% EtOAc/*n*-Hexane); IR-ATR: ν_{max} 3025, 2918, 1634, 1612, 1587, 1488, 1441, 1249 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.40 (ddd, *J* = 15.2, 8.8, 1.2 Hz, 1H, H-3), 7.32-7.24 (m, 2H, H-Ar), 7.20 (t, *J* = 14.8 Hz, 1H, H-Ar), 7.13 (d, *J* = 7.2 Hz, 2H, H-Ar), 6.97 (d, *J* = 1.6 Hz, 1H, H-2′), 6.87 (dd, *J* = 8.0, 1.6 Hz, 1H, H-6′), 6.80-6.68 (m, 3H, H-5′, H-5, H-4), 6.41 (d, *J* = 14.8 Hz, 1H, H-2), 5.95 (s, 2H, O-CH_2-O), 4.67 (d, *J* = 10.4 Hz, 1H, H-CH_2), 4.00 (d, *J* = 12.0 Hz, 1H, H-CH_2), 2.98 (t, *J* = 10.8 Hz, 1H, H-CH_2), 2.70-2.40 (m, 3H, H-CH_2, H-CH), 1.90-1.65 (m, 3H, H-CH_2, H-CH), 1.30-1.10 (m, 2H, H-CH_2); ^{13}C NMR (100 MHz, $CDCl_3$) 165.35, 148.12, 148.06, 142.53, 139.90, 138.25, 130.91, 129.00 (2C), 128.22 (2C), 125.96, 125.23, 122.44, 119.89, 108.41, 105.61, 101.20, 46.03, 42.88, 42.47, 38.26, 32.69, 31.76; HRMS (ESI) m/z calcd for $C_{24}H_{25}NO_3$ $(M+Na)^+$ 376.1913, found 376.1915.



3p

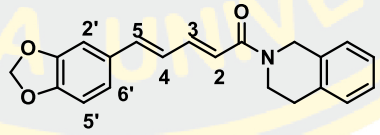
Methyl-1-(5-(benzo[1,3]dioxol-5-yl)penta-2,4-dienoyl)piperidine-3-carboxylate; 70% yield (104.4 mg) as a yellow oil; R_f = 0.34 (80% EtOAc/*n*-Hexane); IR-ATR: ν_{max} 2998, 2861, 1728, 1634, 1611, 1582, 1491, 1446, 1252 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.38 (ddd, *J* = 15.2, 8.8, 1.2 Hz, 1H, H-3), 6.95 (d, *J* = 1.6 Hz, 1H, H-2′), 6.86 (dd, *J* = 8.4, 1.6 Hz, 1H, H-6′), 6.78-6.68 (m, 3H, H-5′, H-5, H-4), 6.39 (d, *J* = 14.8 Hz, 1H, H-2), 5.94 (s, 2H, O-CH_2-O), 4.60-4.2 (m, 1H, H-CH_2), 4.20-4.10 (m, 1H, H-CH_2), 3.60 (s, 3H, H-CH_3), 3.30-3.00 (m, 1H, H-CH_2),

3.00-2.70 (m, 1H, H-CH_2), 2.62-2.40 (m, 1H, H-CH), 1.94 (dd, *J* = 13.2, 3.6 Hz, 2H, H-CH_2), 1.80-1.70 (m, 2H, H-CH_2); ^{13}C NMR (100 MHz, $CDCl_3$) 174.42, 165.37, 148.04 (2C), 142.82, 138.45, 130.73, 125.01, 122.41, 119.37, 108.31, 105.51, 101.14, 51.67, 44.88, 41.33, 40.76, 28.42, 27.72; HRMS (ESI) m/z calcd for $C_{19}H_{21}NO_5$ $(M+Na)^+$ 344.1498, found 344.1499.



3q

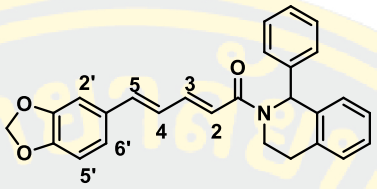
5-(benzo[1,3]dioxol-5-yl)-1-(isoindolin-2-yl)penta-2,4-dien-1-one; 92% yield (134.5 mg) as a yellow solid; R_f = 0.23 (30% EtOAc/*n*-Hexane); mp = 85-86 ^{o}C; IR-ATR: ν_{max} 3028, 2905, 1638, 1599, 1479, 1461, 1251 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.50 (dd, *J* = 14.8, 10.0 Hz, 1H, H-3), 7.25-7.10 (m, 2H, H-Ar), 7.05-6.95 (m, 3H, H-Ar, H-2′), 6.90 (dd, *J* = 8.0, 1.2 Hz, 1H, H-6′), 6.83-6.63 (m, 3H, H-5′, H-5, H-4), 6.34 (d, *J* = 14.0 Hz, 1H, H-2), 5.96 (s, 2H, O-CH_2-O), 4.16 (t, J = 8.0 Hz, 2H, H-CH_2) , 3.30-3.00 (m, 2H, H-CH_2); ^{13}C NMR (100 MHz, $CDCl_3$) 164.30, 148.22, 148.08, 143.21, 139.43, 130.63, 127.29, 124.87 (4C), 123.50, 122.69, 121.24, 117.21, 108.32, 105.61, 101.21, 47.85 (2C); HRMS (ESI) m/z calcd for $C_{20}H_{17}NO_3$ $(M+Na)^+$ 342.1106, found 342.1108.



3r

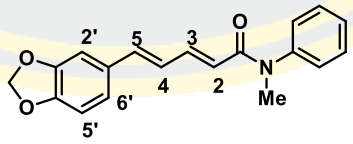
5-(benzo[1,3]dioxol-5-yl)-1-(3,4-dihydroisoquinolin-2-yl)penta-2,4-dien-1-one; 53% yield (82.0 mg) as a yellow oil; R_f = 0.40 (40% EtOAc/*n*-Hexane); IR-ATR: ν_{max} 3020, 2919, 1631, 1590, 1576, 1487, 1442, 1227 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.52-7.42 (m, 1H, H-3), 7.25-7.10 (m, 4H, H-Ar), 6.99 (d, *J* = 1.6 Hz, 1H, H-2′), 6.90 (dd, *J* = 8.0, 1.6 Hz, 1H, H-6′), 6.83-6.72 (m, 3H, H-5′, H-5, H-4), 6.49 (d, *J* = 14.4 Hz, 1H, H-2), 5.98 (s, 2H, O-CH_2-O), 4.88-4.73 (m, 2H, H-CH_2), 3.95-3.73

(m, 2H, H-CH_2), 2.92 (bs, 2H, H-CH_2); ^{13}C NMR (100 MHz, $CDCl_3$) 165.95, 148.15, 148.13, 142.96, 138.78, 130.81, 126.49, 125.08, 122.55 (4C), 119.66 (2C), 108.41, 105.62, 101.21, 45.98, 41.70, 29.58; HRMS (ESI) m/z calcd for $C_{20}H_{19}NO_3$ $(M+Na)^+$ 356.1263, found 356.1260.



3s

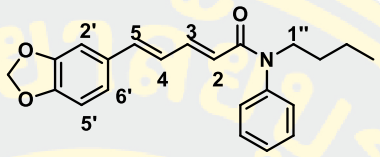
5-(benzo[1,3]dioxol-5-yl)-1-(1-phenyl-3,4-dihydroisoquinolin-2(1H)-yl)penta-2,4-dien-1-one; 88% yield (151.1 mg) as a orange oil; R_f = 0.32 (30% EtOAc/*n*-Hexane); IR-ATR: ν_{max} 3025, 2893, 1634, 1590, 1488, 1439, 1249 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.52 (ddd, J = 15.2, 8.4, 1.6 Hz, 1H, H-3), 7.40-7.15 (m, 9H, H-Ar), 7.12 (d, J = 6.4 Hz, 1H, H-CH), 6.97 (s, 1H, H-2′), 6.88 (dd, J = 8.0, 1.6 Hz, 1H, H-6′), 6.83-6.70 (m, 3H, H-5′, H-5, H-4), 6.47 (d, J = 14.8 Hz, 1H, H-2), 5.94 (s, 2H, O-CH_2-O), 3.91 (d, J = 12.8 Hz, 1H, H-CH_2), 3.60-3.30 (m, 1H, H-CH_2), 3.10-2.90 (m, 1H, H-CH_2), 2.90-2.60 (m, 1H, H-CH_2); ^{13}C NMR (100 MHz, $CDCl_3$) 165.44, 148.10, 148.06, 143.39, 142.28, 138.78, 135.35, 134.38, 130.72, 128.72, 128.51, 128.44 (2C), 128.08 (2C), 127.14, 126.90, 126.17, 125.02, 122.51, 119.41, 108.33, 105.57, 101.14, 55.44, 39.87, 29.53; HRMS (ESI) m/z calcd for $C_{27}H_{23}NO_3$ $(M+Na)^+$ 432.1576, found 432.1579.



3t

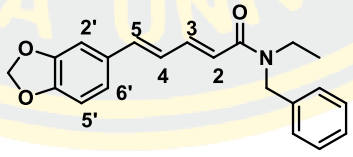
5-(benzo[1,3]dioxol-5-yl)-*N*-methyl-*N*-phenylpenta-2,4-dienamide; 79% yield (111.5 mg) as a yellow solid; R_f = 0.35 (40% EtOAc/*n*-Hexane); mp = 104-106 ^oC; IR-ATR: ν_{max} 2929, 1644, 1599, 1588, 1490, 1254 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.45-7.29 (m, 3H, H-Ar, H-3), 7.18 (d, J = 8.8 Hz, 2H, H-Ar), 6.86 (d, J = 1.6 Hz, 1H, H-2′), 6.80 (dd, J = 8.0, 1.6 Hz, 1H, H-6′), 6.73-6.60 (m, 3H, H-5′, H-5,

H-4), 6.48 (dd, *J* = 15.2, 11.2 Hz, 1H, H-2), 5.89 (d, *J* = 8.0 Hz, 2H, O-CH_2-O), 5.86 (d, *J* = 15.2 Hz, 1H, H-Ar), 3.35 (s, 3H, H-CH_3); ^{13}C NMR (100 MHz, $CDCl_3$)166.36, 148.14 (2C), 143.76, 141.95, 138.62, 130.91, 129.56 (2C), 127.45, 127.37 (2C), 125.21, 122.51, 121.19, 108.41, 105.66, 101.22, 37.43; HRMS (ESI) m/z calcd for $C_{19}H_{17}NO_3$ $(M+Na)^+$ 330.1106, found 330.1103.



3u

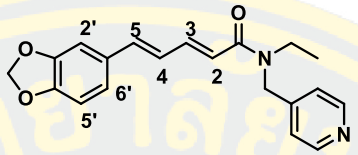
5-(benzo[1,3]dioxol-5-yl)-*N*-butyl-*N*-phenylpenta-2,4-dienamide; 65% yield (103.5 mg) as a yellow oil; R_f = 0.40 (40% EtOAc/*n*-Hexane); IR-ATR: ν_{max} 3063, 2927, 1641, 1614, 1586, 1253 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.45-7.37 (m, 3H, H-3, H-Ar), 7.37-7.32 (m, 1H, H-Ar), 7.21-7.13 (m, 2H, H-Ar), 6.87 (d, *J* = 1.6 Hz, 1H, H-2′), 6.90 (dd, *J* = 8.0, 1.2 Hz, 1H, H-6′), 6.75-6.65 (m, 2H, H-5′, H-5), 6.48 (dd, *J* = 15.6, 11.2 Hz, 1H, H-4), 5.91 (s, 2H, O-CH_2-O), 5.78 (d, *J* = 14.8 Hz, 1H, H-2), 3.78 (t, *J* = 7.6 Hz, 2H, H-1″), 1.57-1.46 (m, 2H, H-2″), 1.31 (dd, *J* = 15.2, 7.2 Hz, 2H, H-3″), 0.88 (t, *J* = 7.2 Hz, 3H, H-4″) ; ^{13}C NMR (100 MHz, $CDCl_3$) 165.77, 147.98, 142.26, 141.74, 138.31, 130.77, 129.36, 128.24, 127.44, 125.12, 122.35, 121.50, 108.24, 105.50, 101.08, 49.09, 29.84, 19.96, 13.69; HRMS (ESI) m/z calcd for $C_{22}H_{23}NO_3$ $(M+H)^+$ 350.1751, found 350.1751.



3v

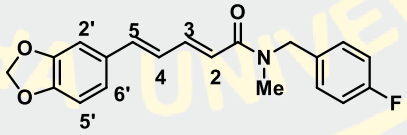
5-(benzo[1,3]dioxol-5-yl)-*N*-benzyl-*N*-ethylpenta-2,4-dienamide; 69% yield (100.0 mg) as a yellow oil; R_f = 0.28 (40% EtOAc/*n*-Hexane); IR-ATR: ν_{max} 2971, 2928, 1635, 1588, 1488, 1441, 1249 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.60-7.44 (m, 1H, H-3), 7.40-7.15 (m, 5H, H-Ar), 6.95 (d, *J* = 8.4 Hz, 1H, H-2′), 6.87 (dd, *J* = 18.4, 8.4 Hz, 1H, H-6′), 6.80-6.56 (m, 3H, H-5′, H-5, H-4), 6.34-6.19 (m, 1H, H-2), 5.93 (d, *J* = 8.4 Hz, 2H, O-CH_2-O), 4.64 (m, 2H, H-CH_2), 3.44 (dq, *J* = 52.0, 6.8 Hz,

2H, H-CH_2), 1.16 (t, *J* = 6.8 Hz, 3H, H-CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) 166.40, 148.07 (2C), 143.22, 138.73, 130.78, 128.70, 128.38, 127.92, 127.38, 127.09, 125.09 (2C), 122.48, 119.96, 108.33, 105.58, 101.15, 50.53, 41.70, 14.25; HRMS (ESI) m/z calcd for $C_{21}H_{21}NO_3$ $(M+Na)^+$ 358.1419, found 358.1420.



3w

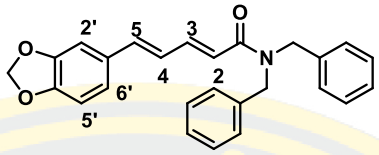
5-(benzo[1,3]dioxol-5-yl)-*N*-ethyl-*N*-(pyridin-4-ylmethyl)penta-2,4-dienamide; 75% yield (78.9 mg) as a yellow oil; R_f = 0.50 (10% MeOH/CH_2Cl_2); IR-ATR: ν_{max} 3026, 2969, 1635, 1610, 1587, 1442, 1250 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.60-7.43 (m, 1H, H-3), 7.40-7.15 (m, 4H, H-Ar), 6.96 (d, *J* = 8.0 Hz, 1H, H-2′), 6.88 (dd, *J* = 17.2, 8.0 Hz, 1H, H-6′), 6.81-6.58 (m, 3H, H-5′, H-5, H-4), 6.49-6.23 (m, 1H, H-2), 5.95 (d, *J* = 8.0 Hz, 2H, O-CH_2-O), 4.65 (m, 2H, H-CH_2), 3.44 (dq, *J* = 52.0, 6.8 Hz, 2H, H-CH_2), 1.17 (s, 3H, H-CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) 166.42, 148.11 (2C), 143.24, 138.75, 130.83, 128.74, 128.41, 127.96 (2C), 126.27, 125.14, 122.52, 120.01, 119.60, 108.38, 105.62, 101.18, 48.63, 41.74, 14.29; HRMS (ESI) m/z calcd for $C_{20}H_{20}N_2O_3$ $(M+H)^+$ 350.1751; Found 350.1751.



3x

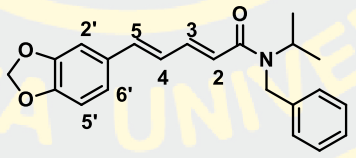
5-(benzo[1,3]dioxol-5-yl)-*N*-(4-fluorobenzyl)-*N*-methylpenta-2,4-dienamide; 53% yield (109.0 mg) as a yellow oil; R_f = 0.28 (40% EtOAc/*n*-Hexane); IR-ATR: ν_{max} 2928, 1673, 1592, 1488, 1504, 1444, 1216 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.50 (dd, *J* = 14.8, 10.0 Hz, 1H, H-3), 7.30-7.10 (m, 2H, H-Ar, H-2′), 7.10-6.92 (m, 3H, H-Ar, H-6′), 6.88 (t, *J* = 8.4 Hz, 1H, H-Ar), 6.82-6.50 (m, 3H, H-5′, H-5, H-4), 6.41 (dd, *J* = 14.8, 10.0 Hz, 1H, H-2), 5.96 (s, 2H, O-CH_2-O), 4.61 (d, *J* = 18.4 Hz, 2H, H-CH_2), 3.01 (s, 3H, H-CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) 171.36 (2C), 166.72, 148.18, 148.11, 143.39, 139.00, 132.14, 130.74, 129.63, 129.55, 126.00 (2C),

124.97, 115.30, 108.38, 105.61, 101.20, 50.42, 34.05; HRMS (ESI) m/z calcd for $C_{20}H_{18}FNO_3$ $(M+Na)^+$ 362.1168, found 362.1167.



3y

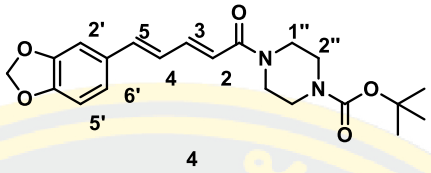
5-(benzo[1,3]dioxol-5-yl)-*N*,*N*-dibenzylpenta-2,4-dienamide; 98% yield (162.6 mg) as a yellow oil; R_f = 0.31 (30% EtOAc/*n*-Hexane); IR-ATR: ν_{max} 3026, 2920, 1633, 1584, 1462, 1438, 1251 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.50 (dd, *J* = 14.4, 10.8 Hz, 1H, H-3), 7.42-7.38 (m, 10H, H-Ar), 6.97 (d, *J* = 1.6 Hz, 1H, H-2′), 6.88 (dd, *J* = 8.0, 1.2 Hz, 1H, H-6′), 6.82-6.50 (m, 3H, H-5′, H-5, H-4), 6.49 (d, *J* = 14.4 Hz, 1H, H-2), 5.96 (s, 2H, O-CH_2-O), 4.61 (d, *J* = 18.8 Hz, 4H, H-CH_2); ^{13}C NMR (100 MHz, $CDCl_3$) 167.30, 148.19,148.11, 143.98, 139.08, 130.75, 128.84, 128.50, 128.30, 128.25 (4C), 128.06 (2C), 127.54, 126.85, 126.42, 125.05, 122.59, 119.44, 108.38, 105.66, 101.19, 53.04 (2C); HRMS (ESI) m/z calcd for $C_{26}H_{23}NO_3$ $(M+Na)^+$ 420.1576, found 420.1574.



3z

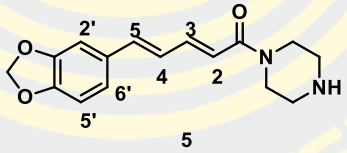
5-(benzo[1,3]dioxol-5-yl)-*N*-benzyl-*N*-isopropylpenta-2,4-dienamide; 69% yield (110.1 mg) as a yellow oil; R_f = 0.40 (40% EtOAc/*n*-Hexane); IR-ATR: ν_{max} 2970, 2929, 1636, 1590, 1488, 1441, 1416, 1249 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.49 (dd, *J* = 14.4, 12.0 Hz, 1H, H-3), 7.40-7.20 (m, 5H, H-Ar), 6.88 (s, 1H, H-2′), 6.82 (d, *J* = 8.0 Hz, 1H, H-6′), 6.80-6.65 (m, 3H, H-5′, H-5, H-4), 6.56 (dd, *J* = 14.8, 10.8 Hz, 1H, H-CH), 6.15 (d, *J* = 14.4 Hz, 1H, H-2), 5.90 (s, 2H, O-CH_2-O), 4.55 (s, 2H, H-CH_2), 1.62 (d, *J* = 6.8 Hz, 6H, H-CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) 167.25, 148.02, 143.06, 138.80, 138.53, 130.78, 128.58, 126.99, 126.00 (4C), 125.84, 125.17,

122.43, 121.06, 108.29, 105.56, 101.13, 45.79, 45.64, 20.17; HRMS (ESI) m/z calcd for $C_{22}H_{23}NO_3$ $(M+Na)^+$ 372.1576, found 372.1572.



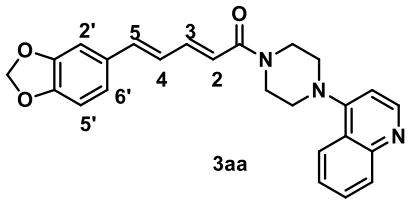
4

***tert*-butyl-4-(5-(benzo[1,3]dioxol-5-yl)penta-2,4-dienoyl)piperazine-1-carboxylate;** 73% yield (1.3 g) as a yellow solid; R_f = 0.73 (60% EtOAc/*n*-Hexane); mp = 85-87 °C; IR-ATR: ν_{max} 2979, 2906, 1622, 1685, 1583, 1420, 1244 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.44 (dd, J = 14.8, 10.4 Hz, 1H, H-3), 6.98 (s, 1H, H-2′), 6.89 (dd, J = 8.0 Hz, 1H, H-6′), 6.83-6.68 (m, 3H, H-5′, H-5, H-4), 6.38 (d, J = 14.4 Hz, 1H, H-2), 5.97 (s, 2H, O-CH_2-O), 3.72-3.51 (m, 4H, H-1′′), 3.45 (s, 4H, H-2′′), 1.47 (s, 9H, H-CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) 165.78, 154.57, 148.34, 148.25, 143.54, 139.15, 130.79, 124.95, 122.70, 118.88, 108.15, 105.69, 101.30, 80.33 (5C) 28.39 (3C); HRMS (ESI) m/z calcd for $C_{21}H_{26}N_2O_5$ $(M+H)^+$ 387.1914, found 387.1914.



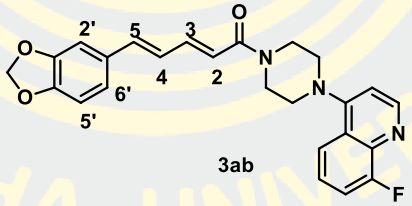
5

5-(benzo[1,3]dioxol-5-yl)-1-(piperazin-1-yl)penta-2,4-dien-1-one; 82% yield (1.1 g) as a yellow solid; R_f = 0.33 (10% MeOH/CH_2Cl_2); mp = 139-141 °C; IR-ATR: ν_{max} 2941, 2913, 1634, 1583, 1446, 1433, 1257 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.42 (dd, J = 14.8, 10.0 Hz, 1H, H-3), 6.98 (d, J = 1.6 Hz, 1H, H-2′), 6.89 (dd, J = 8.0, 1.6 Hz, 1H, H-6′), 6.81-6.68 (m, 3H, H-5′, H-5, H-4), 6.40 (d, J = 14.8 Hz, 1H, H-2), 5.98 (s, 2H, O-CH_2-O), 3.74-3.51 (m, 4H, H-1′′), 2.88 (t, J = 5.2 Hz, 4H, H-2′′); ^{13}C NMR (100 MHz, $CDCl_3$) 165.62, 148.21 (2C), 143.02, 138.71, 130.90, 125.14, 122.60, 119.33, 108.50, 105.68, 101.28, 46.94, 46.48, 45.88, 43.15; HRMS (ESI) m/z calcd for $C_{16}H_{18}N_2O_3$ $(M+Na)^+$ 309.1210, found 309.1206.



3aa

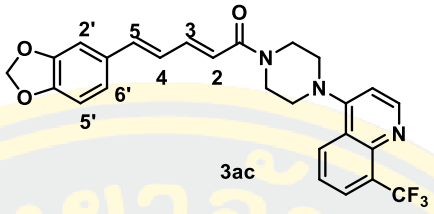
5-(benzo[1,3]dioxol-5-yl)-1-(4-(quinoline-4-yl)piperazin-1-yl)penta-2,4-dien-1-one; 52% yield (75.4 mg) as a yellow solid; R_f = 0.50 (30% EtOAc/*n*-Hexane); mp = 69-74 °C; IR-ATR: ν_{max} 2919, 1635, 1578, 1441, 1422, 1234 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 8.76 (d, J = 5.2 Hz, 1H, H-10″), 8.10 (d, J = 8.4 Hz, 1H, H-5″), 8.04 (d, J = 7.6 Hz, 1H, H-4″), 7.75-7.65 (m, 1H, H-7″), 7.57-7.44 (m, 2H, H-8″, H-9″), 6.99 (d, J = 1.2 Hz, 1H, H-2′), 6.91 (dd, J = 8.4, 1.6 Hz, 1H, H-6′), 6.88-6.84 (m, 1H, H-3), 6.81-6.74 (m, 3H, H-5′, H-5, H-4), 6.46 (d, J = 14.8 Hz, 1H, H-2), 5.98 (s, 2H, O-CH_2-O), 3.94 (bs, 4H, H-1″), 3.26 (t, J = 4.8 Hz, 4H, H-2″); ^{13}C NMR (100 MHz, $CDCl_3$) 165.85, 156.43, 150.61, 149.30, 148.33, 148.22, 143.74, 139.29, 130.75 (2C), 129.88, 129.36, 125.76, 124.91, 123.31, 123.25, 118.76, 109.02, 108.51, 105.68, 101.31, 52.26 (2C), 42.06 (2C); HRMS (ESI) m/z calcd for $C_{25}H_{23}N_3O_3$ $(M+H)^+$ 414.1812, found 414.1812.



3ab

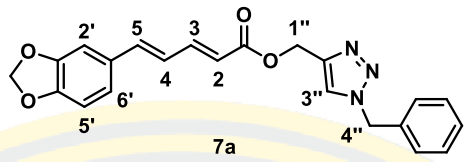
5-(benzo[1,3]dioxol-5-yl)-1-(4-(8-fluoroquinolin-4-yl)piperazin-1-yl)penta-2,4-dien-1-one; 37% yield (55.0 mg) as a yellow solid; R_f = 0.50 (40% EtOAc/*n*-Hexane); mp = 65-68 °C; IR-ATR: ν_{max} 2921, 1634, 1579, 1504, 1488, 1442, 1243 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 8.80 (d, J = 5.2 Hz, 1H, H-10″), 7.81 (d, J = 8.4 Hz, 1H, H-5″), 7.51 (d, J = 10.4 Hz, 1H, H-4″), 7.51-7.41 (m, 1H, H-8″), 7.41-7.33 (m, 1H, H-9″), 6.99 (d, J = 1.2 Hz, 1H, H-2′), 6.95-6.88 (m, 2H, H-6′, H-3), 6.79 (m, 3H, H-5′, H-5, H-4), 6.46 (d, J = 14.8 Hz, 1H, H-2), 5.98 (s, 2H, O-CH_2-O) 3.95 (bs, 4H, H-1″), 3.26 (t, J = 4.8 Hz, 4H, H-2″); ^{13}C NMR (100 MHz, $CDCl_3$) 165.87, 150.98, 148.38, 148.25 (2C), 143.84, 139.39, 130.75, 125.41, 125.32, 125.17, 124.89, 122.78, 119.00, 118.96, 118.68, 113.53, 113.34, 109.98, 108.54, 105.70, 101.34,

101.21, 52.16, 29.67; HRMS (ESI) m/z calcd for $C_{25}H_{22}FN_3O_3$ $(M+H)^+$ 432.1718, found 432.1718.



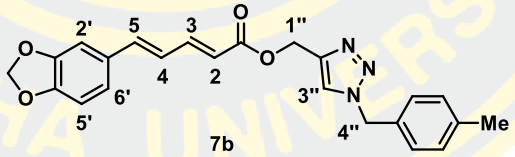
5-(benzo[1,3]dioxol-5-yl)-1-(4-(8-(trifluoromethyl)93uinoline-4-yl)piperazin-1-yl)penta-2,4-dien-1-one; 70% yield (118.8 mg) as a yellow solid; R_f = 0.67 (40% EtOAc/*n*-Hexane); mp = 158-161 °C; IR-ATR: ν_{max} 2923, 1637, 1584, 1419, 1317, 1238 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 8.90 (d, J = 4.8 Hz, 1H, H-10″), 8.26 (d, J = 8.4 Hz, 1H, H-5″), 8.05 (d, J = 7.2 Hz, 1H, H-4″), 7.57 (t, J = 8.0 Hz, 1H, H-8″), 7.94 (dd, J = 14.8, 10.4 Hz, 1H, H-9″), 6.99 (d, J = 1.2 Hz, 1H, H-2′), 6.95 (d, J = 5.2 Hz, 1H, H-6′), 6.93-6.87 (m, 1H, H-3), 6.86-6.70 (m, 3H, H-5′, H-5, H-4) 6.45 (d, J = 14.4 Hz, 1H, H-2), 5.98 (s, 2H, O-CH_2-O) 3.95 (bs, 4H, H-1″), 3.23 (t, J = 4.8 Hz, 4H, H-2″); ^{13}C NM(R (100 MHz, $CDCl_3$) 165.84, 156.50, 151.64, 148.37, 148.23, 146.20, 143.86, 139.39, 130.71, 127.90, 127.84, 124.86, 124.20, 124.07, 122.77 (2C), 118.62 (2C), 109.95, 108.52, 105.67, 101.32, 45.57 (2C), 42.01 (2C); HRMS (ESI) m/z calcd for $C_{26}H_{22}F_3N_3O_3$ $(M+H)^+$ 482.1686, found 482.1686.

4.7.2 1,2,3-triazole-piperine analogues (7a-7y)



7a

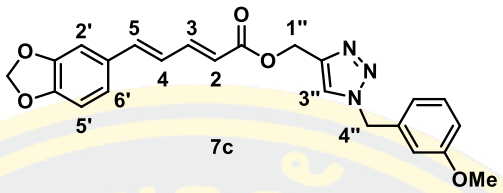
***O*-((1-benzyl-1,2,3-triazol-4-yl)methyl)piperic ester**; 79% yield (70.7 mg) as a yellow solid; R_f = 0.58 (50% EtOAc/*n*-Hexane), mp = 122-124 °C; IR-ATR: ν_{max} 3068, 2970, 1719, 1618, 1606, 1445, 1355, 1216 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.55 (s, 1H, H-3″), 7.41 (dd, *J* = 15.2, 10.8 Hz, 1H, H-3), 7.38-7.35 (m, 3H, H-Ar), 7.29-7.27 (m, 2H, H-Ar), 6.98 (d, *J* = 1.6 Hz, 1H, H-2′), 6.90 (dd, *J* = 8.0, 1.6 Hz, 1H, H-6′), 6.79 (d, *J* = 15.6 Hz, 1H, H-5), 6.78 (d, *J* = 8.0 Hz, 1H, H-5′), 6.66 (dd, *J* = 15.2, 10.8 Hz, 1H, H-4), 5.98 (s, 2H, OCH_2O), 5.92 (d, *J* = 15.6 Hz, 1H, H-2), 5.52 (s, 2H, H-4″), 5.28 (s, 2H, H-1″); ^{13}C NMR (100 MHz, $CDCl_3$) 166.68, 148.53, 148.15, 145.51, 143.34, 140.57, 134.33, 130.27, 128.99 (2C), 128.66, 128.00(2C), 124.17, 123.56, 122.94, 119.36, 108.38, 105.74, 101.28, 57.34, 54.04; HRMS (ESI) m/z calcd for $C_{22}H_{19}N_3O_4$ $(M+H)^+$ 390.1448, found 390.1449.



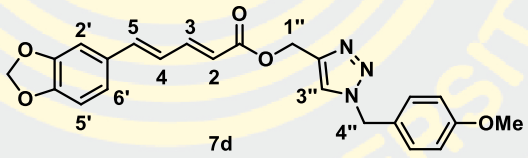
7b

***O*-((4-methylbenzyl-1,2,3-triazol-4-yl) methyl) piperic ester**; 59% yield (108.9 mg) as a yellow solid; R_f = 0.60 (40% EtOAc/*n*-Hexane), mp = 121-123°C; IR-ATR: ν_{max} 2970, 2920, 2852, 1700, 1619, 1606, 1441, 1372, 1255 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.53 (s, 1H, H-3″), 7.39 (dd, *J* = 15.2, 10.8 Hz, 1H, H-3), 7.17 (s, 4H, H-Ar), 6.96 (s, 1H, H-2′), 6.88 (d, *J* = 8.0 Hz, 1H, H-6′), 6.80-6.76 (m, 2H, H-5, H-5′), 6.65 (dd, *J* = 15.2, 10.8 Hz, 1H, H-4), 5.96 (s, 2H, OCH_2O), 5.90 (d, *J* = 15.2 Hz, 1H, H-2), 5.46 (s, 2H, H-4″), 5.26 (s, 2H, H-1″) 2.33 (s, 3H, H-CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) 166.78, 148.59, 148.22, 145.57, 143.32, 140.63, 138.69, 131.29, 130.35, 129.73 (2C), 128.14 (2C), 124.25, 123.47, 123.00, 119.44, 108.46, 105.81,

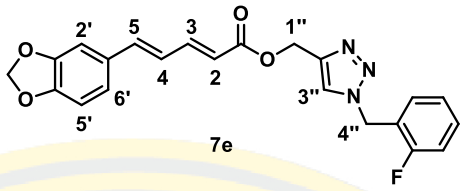
101.33, 57.40, 53.95, 21.08; HRMS (ESI) m/z calcd for $C_{23}H_{21}N_3O_4$ $(M+H)^+$ 404.1605, found 404.1605.



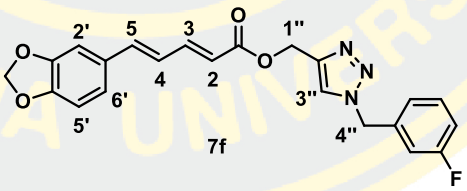
***O*-((3-methoxybenzyl-1,2,3-triazol-4-yl) methyl) piperic ester**; 42% yield (79.9 mg) as a yellow oil; R_f = 0.24 (40% EtOAc/*n*-Hexane); IR-ATR: ν_{max} 2924, 1701, 1618, 1605, 1447, 1369, 1132 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.55 (s, 1H, H-3″), 7.41 (dd, *J* = 15.2, 10.8 Hz, 1H, H-3), 7.32-7.24 (m, 1H, H-Ar), 6.98 (d, *J* = 1.6 Hz, 1H, H-2′), 6.92-6.76 (m, 6H, H-6′, H-5′, H-5, H-Ar), 6.67 (dd, *J* = 15.2, 10.8 Hz, 1H, H-4), 5.98 (s, 2H, OCH_2O), 5.92 (d, *J* = 15.2 Hz, 1H, H-2), 5.48 (s, 2H, H-1″), 5.28 (s, 2H, H-4″), 3.78 (s, 3H, OCH_3); ^{13}C NMR (100 MHz, $CDCl_3$) 166.78, 160.00, 148.57, 148.19, 145.61, 143.37, 140.66, 135.74, 130.32, 130.12, 124.21, 123.64, 122.99, 120.23, 119.36, 114.18, 113.65, 108.43, 105.79, 101.31, 57.36, 55.20, 54.04; HRMS (ESI) m/z calcd for $C_{23}H_{21}N_3O_5$ $(M+H)^+$ 420.1554, found 420.1554.



***O*-((4-methoxybenzyl-1,2,3-triazol-4-yl) methyl) piperic ester**; 77% yield (148.8 mg) as a yellow solid; R_f = 0.27 (40% EtOAc/*n*-Hexane), mp = 111-114°C; IR-ATR: ν_{max} 2924, 1736, 1710, 1609, 1442, 1366, 1126 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.53 (s, 1H, H-3″), 7.40 (dd, *J* = 15.2, 10.8 Hz, 1H, H-3), 7.27-7.23 (m, 2H, H-Ar), 6.97 (s, 1H, H-2′), 6.90-6.88 (m, 3H, H-6′, H-Ar), 6.79 (d, *J* = 15.6 Hz, 1H, H-5), 6.77 (d, *J* = 8.0 Hz, 1H, H-5′), 6.66 (dd, *J* = 15.2, 10.8 Hz, 1H, H-4), 5.97 (s, 2H, OCH_2O), 5.91 (d, *J* = 15.2 Hz, 1H, H-2), 5.44 (s, 2H, H-4″), 5.27 (s, 2H, H-1″), 3.80 (s, 3H, OCH_3); ^{13}C NMR (100 MHz, $CDCl_3$) 166.53, 159.62, 148.38, 147.99, 145.38, 143.01, 140.46, 130.10, 129.46 (2C), 126.20, 123.99, 123.29, 122.83, 119.16, 114.19 (2C), 108.22, 105.58, 101.16, 57.17, 55.01, 53.40; HRMS (ESI) m/z calcd for $C_{23}H_{21}N_3O_5$ $(M+Na)^+$ 420.1554, found 420.1554.

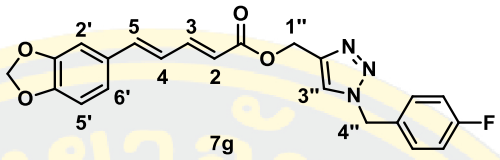


***O*-((2-fluorobenzyl-1,2,3-triazol-4-yl) methyl) piperic ester**; 76% yield (142.1 mg) as a yellow solid; R_f = 0.37 (40% EtOAc/*n*-Hexane), mp = 104-106°C; IR-ATR: ν_{max} 3027, 2970, 2925, 1705, 1620, 1609, 1448, 1369, 1231 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.64 (s, 1H, H-3″), 7.40 (dd, *J* = 15.2, 10.8 Hz, 1H, H-3), 7.36-7.25 (m, 2H, H-Ar), 7.16-7.11 (m, 2H, H-Ar), 6.97 (d, *J* = 1.6 Hz, 1H, H-2′), 6.87 (dd, *J* = 8.0, 1.6 Hz, 1H, H-6′), 6.78 (d, *J* = 15.6 Hz, 1H, H-5), 6.76 (d, *J* = 8.0 Hz, 1H, H-5′), 6.65 (dd, *J* = 15.2, 10.8 Hz, 1H, H-4), 5.96 (s, 2H, OCH_2O), 5.91 (d, *J* = 15.2 Hz, 1H, H-2), 5.57 (s, 2H, H-4″), 5.29 (s, 2H, H-1″); ^{13}C NMR (100 MHz, $CDCl_3$) 166.69, 160.38 (d, *J* = 246.0 Hz, C-F), 159.15, 148.51, 148.12, 145.55, 143.29, 140.59, 130.82 (d, *J* = 8.0 Hz, C_m-F), 130.49 (d, *J* = 3.0 Hz, C_m-F), 130.25, 124.70 (d, *J* = 3.0 Hz, C_o-F), 124.14, 123.77, 122.94, 121.60 (d, *J* = 15.0 Hz, C_p-F), 119.28, 115.70 (d, *J* = 21.0 Hz, C_o-F) , 108.36, 105.72, 101.26, 57.26, 47.56; HRMS (ESI) m/z calcd for $C_{22}H_{18}FN_3O_4$ $(M+H)^+$ 408.1354, found 408.1354.



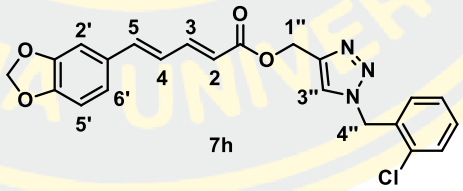
***O*-((3-fluorobenzyl-1,2,3-triazol-4-yl) methyl) piperic ester**; 76% yield (142.4 mg) as a yellow solid; R_f = 0.32 (40% EtOAc/*n*-Hexane), mp = 121-122°C; IR-ATR: ν_{max} 3064, 3030, 2930, 1705, 1618, 1606, 1489, 1449, 1370, 1121 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.58 (s, 1H, H-3″), 7.42 (dd, *J* = 15.2, 10.8 Hz, 1H, H-3), 7.39-7.30 (m, 1H, H-2′), 7.11-6.94 (m, 4H, H-Ar), 6.91 (dd, *J* = 8.4, 1.6 Hz, 1H, H-6′), 6.84-6.76 (m, 2H, H-5′, H-5), 6.68 (dd, *J* = 15.2, 10.8 Hz, 1H, H-4), 5.98 (s, 2H, OCH_2O), 5.93 (d, *J* = 15.2 Hz, 1H, H-2), 5.51 (s, 2H, H-4″), 5.30 (s, 2H, H-1″); ^{13}C NMR (100 MHz, $CDCl_3$) 166.75, 162.83 (d, *J* = 246.0 Hz, C-F), 148.55, 148.15, 145.64, 143.53, 140.69, 136.71 (d, *J* = 7.0 Hz, C_m-F), 130.67 (d, *J* = 9.0 Hz, C_m-F),

130.26, 124.14, 123.74, 123.52 (d, J = 3.0 Hz, C_p-F), 122.98, 119.25, 115.69 (d, J = 21.0 Hz, C_o-F), 1154.94 (d, J = 22.0 Hz, C_o-F), 108.39, 105.75, 101.29, 57.28, 53.37; HRMS (ESI) m/z calcd for $C_{22}H_{18}FN_3O_4$ $(M+H)^+$ 408.1354, found 408.1354.



7g

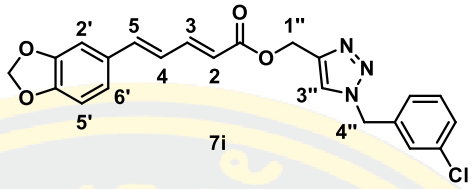
***O*-((4-fluorobenzyl-1,2,3-triazol-4-yl)methyl)piperic ester**; 64% yield (60.9 mg) as a yellow solid; R_f = 0.30 (40% EtOAc/*n*-Hexane), mp = 141-143°C; IR-ATR: ν_{max} 3140, 2970, 1502, 1702, 1603, 1434, 1368, 1217 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.56 (s, 1H, H-3″), 7.39 (dd, J = 15.2, 10.8 Hz, 1H, H-3), 7.28-7.24 (m, 4H, H-Ar),6.95 (bs, 1H, H-2′) 6.88 (d, J = 8.0 Hz, 1H, H-6′) 6.79-6.73 (m, 2H, H-5′, H-5) 6.64 (dd, J = 15.2, 10.8 Hz, 1H, H-4), 5.95 (d, J = 1.6 Hz, 2H, OCH_2O), 5.89 (d, J = 15.2 Hz, 1H, H-2), 5.47 (s, 2H, H-4″), 5.27 (s, 2H, H-1″); ^{13}C NMR (100 MHz, $CDCl_3$) 166.74, 162.76 (d, J = 247.0 Hz, C-F), 148.58, 148.20, 145.60, 143.52, 140.68, 130.30, 130.25 (d, J = 3.0 Hz, C_p-F), 129.94 (d, J = 8.0 Hz, 2×C_m-F), 124.18, 123.52, 123.00, 119.33, 116.04 (d, J = 21.0 Hz, 2×C_o-F), 108.43, 105.78, 101.32, 57.33, 53.32; HRMS (ESI) m/z calcd for $C_{22}H_{18}FN_3O_4$ $(M+H)^+$ 408.1354, found 408.1354.



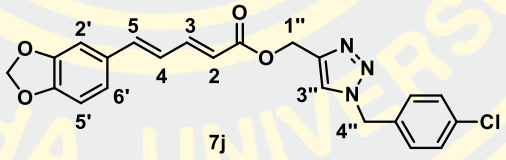
7h

***O*-((2-chlorobenzyl-1,2,3-triazol-4-yl) methyl) piperic ester**; 72% yield (141.7 mg) as a yellow solid; R_f = 0.55 (40% EtOAc/*n*-Hexane), mp = 120-122°C; IR-ATR: ν_{max} 3022, 2923, 1702, 1619, 1499, 1256 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.66 (s, 1H, H-3″), 7.47-7.24 (m, 4H, H-Ar), 7.21 (dd, J = 15.2, 10.8 Hz, 1H, H-3), 6.97 (s, 1H, H-2′), 6.90 (d, J = 8.0 Hz, 1H, H-6′), 6.85-6.73 (m, 2H, H-5, H-5′), 6.67 (dd, J = 15.6, 10.8 Hz, 1H, H-4) 5.98 (s, 2H, OCH_2O), 5.92 (d, J = 15.2 Hz, 1H, H-2), 5.66 (s, 2H, H-4″), 5.30 (s, 2H, H-1″); ^{13}C NMR (100 MHz, $CDCl_3$) 166.74, 148.59, 148.22, 145.58, 143.32, 140.64, 133.44, 132.20, 130.34, 130.32, 130.22, 129.87,

127.54, 124.25, 123.93, 122.99, 119.43, 108.45, 105.81, 101.33, 57.37, 51.31; HRMS (ESI) m/z calcd for $C_{22}H_{18}ClN_3O_4$ $(M+H)^+$ 424.1059, found 424.1059.

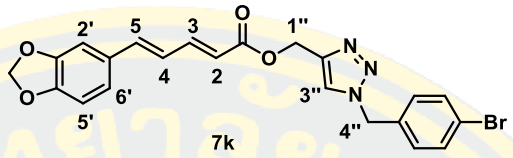


***O*-((3-chlorobenzyl-1,2,3-triazol-4-yl) methyl) piperic ester**; 75% yield (145.6 mg) as a yellow solid; R_f = 0.68 (40% EtOAc/*n*-Hexane), mp = 111-112°C; IR-ATR: ν_{max} 3069, 2891, 1718, 1488, 1445, 1249, 1036 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.59 (s, 1H, H-3″), 7.41 (dd, *J* = 15.2, 10.8 Hz, 1H, H-3), 7.36-7.23 (m, 3H, H-Ar), 7.19-7.12 (m, 1H, H-Ar), 6.98 (d, *J* = 1.6 Hz, 1H, H-2′), 6.90 (d, *J* = 1.6 Hz, 1H, H-6′), 6.83-6.74 (m, 2H, H-5, H-5′), 6.67 (dd, *J* = 15.6, 10.8 Hz, 1H, H-4) 5.98 (s, 2H, OCH_2O), 5.92 (d, *J* = 15.2 Hz, 1H, H-2), 5.49 (s, 2H, H-4″), 5.30 (s, 2H, H-1″); ^{13}C NMR (100 MHz, $CDCl_3$) 166.80, 148.63, 148.24, 145.67, 143.70 (2C), 140.72, 136.31, 134.97 (2C), 130.39, 130.36, 128.99, 128.12, 126.11, 124.25, 123.71, 123.03, 119.39, 108.48, 105.84, 101.36; HRMS (ESI) m/z calcd for $C_{22}H_{18}ClN_3O_4$ $(M+H)^+$ 424.1059, found 424.1059.



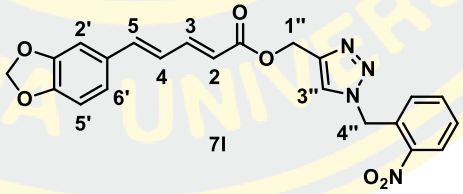
***O*-((4-chlorobenzyl-1,2,3-triazol-4-yl)methyl)piperic ester**; 50% yield (97.2 mg) as a yellow solid; R_f = 0.31 (40% EtOAc/*n*-Hexane), mp = 131-132°C; IR-ATR: ν_{max} 3144, 2917, 2850, 1702, 1602, 1447, 1368, 1226, 838 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.58 (s, 1H, H-3″), 7.41 (dd, *J* = 15.2, 10.8 Hz, 1H, H-3), 7.35-7.33 (m, 2H, H-Ar), 7.23-7.21 (m, 2H, H-Ar), 6.97 (s, 1H, H-2′), 6.90 (d, *J* = 8.0 Hz, 1H, H-6′), 6.79 (d, *J* = 15.6 Hz, 1H, H-5), 6.77 (d, *J* = 8.0 Hz, 1H, H-5′), 6.66 (dd, *J* = 15.2, 10.8 Hz, 1H, H-4), 5.97 (s, 2H, OCH_2O), 5.91 (d, *J* = 15.2 Hz, 1H, H-2), 5.49 (s, 2H, H-4″), 5.28 (s, 2H, H-1″); ^{13}C NMR (100 MHz, $CDCl_3$) 166.76, 148.57, 148.17, 145.65, 143.54, 140.72, 134.72, 132.84, 130.27, 129.37 (3C), 129.22, 124.15, 123.62, 123.01,

119.27, 108.42, 105.77, 101.31, 5.29, 53.31; HRMS (ESI) m/z calcd for $C_{22}H_{18}ClN_3O_4$ $(M+H)^+$ 424.1059, found 424.1059.



7k

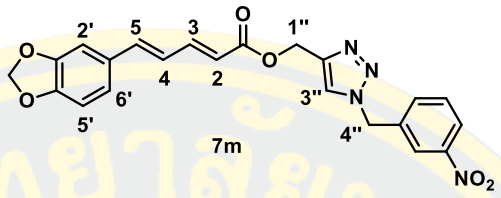
***O*-((4-bromobenzyl-1,2,3-triazol-4-yl) methyl) piperic ester**; 52% yield (110.3 mg) as a yellow solid; R_f = 0.31 (40% EtOAc/*n*-Hexane), mp = 126-127°C; IR-ATR: ν_{max} 3144, 2920, 2851, 1704, 1601, 1442, 1367, 1252, 528 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.56 (s, 1H, H-3″), 7.48 (d, J = 8.4 Hz, 2H, H-Ar), 7.39 (dd, J = 15.2, 10.8 Hz, 1H, H-3), 7.14 (d, J = 8.0 Hz, 2H, H-Ar), 6.96 (s, 1H, H-2′), 6.89 (d, J = 8.0 Hz, 1H, H-6′), 6.78 (d, J = 15.2 Hz, 1H, H-5), 6.76 (d, J = 8.0 Hz, 1H, H-5′), 6.65 (dd, J = 15.2, 10.8 Hz, 1H, H-4), 5.96 (s, 2H, OCH_2O), 5.90 (d, J = 15.2 Hz, 1H, H-2), 5.46 (s, 2H, H-4″), 5.27 (s, 2H, H-1″); ^{13}C NMR (100 MHz, $CDCl_3$) 166.78, 148.59, 148.20, 145.67, 143.59, 140.73, 133.36, 132.22 (2C), 130.29, 129.66 (2C), 124.18, 123.63, 123.03, 122.90, 119.30, 108.45, 105.80, 101.33, 57.32, 53.20; HRMS (ESI) m/z calcd for $C_{22}H_{18}N_4O_6$ $(M+H)^+$ 468.0553, found 468.0553.



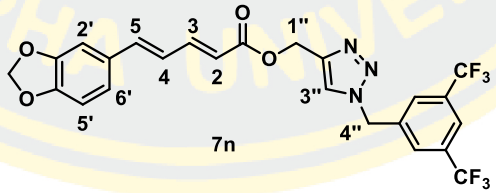
7l

***O*-((2-nitrobenzyl-1,2,3-triazol-4-yl)methyl)piperic ester**; 67% yield (133.1 mg) as a yellow solid; R_f = 0.25 (40% EtOAc/*n*-Hexane), mp = 143-145°C; IR-ATR: ν_{max} 3084, 2904, 1698, 1620, 1606, 1521, 1443, 1245 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 8.15 (dd, J = 1.2, 8.0 Hz, 1H, H-Ar), 7.79 (s, 1H, H-3″), 7.66-7.50 (m, 2H, H-Ar), 7.43 (dd, J = 15.2, 11.2 Hz, 1H, H-3), 7.12 (d, J = 7.6 Hz, 1H, H-Ar) 6.98 (d, J = 1.6 Hz, 1H, H-2′), 6.91 (dd, J = 1.6, 8.0 Hz, 1H, H-6′), 6.85-6.75 (m, 2H, H-5, H-5′), 6.68 (dd, J = 15.2, 10.8 Hz, 1H, H-4) 5.98 (s, 2H, OCH_2O), 5.95 (m, 3H, H-4″, H-2), 5.97-5.92 (m, 2H, H-1″); ^{13}C NMR (100 MHz, $CDCl_3$) 166.81, 148.68, 148.30,

145.76, 143.72, 140.80, 138.04, 134.37, 130.48, 130.42, 130.40, 129.73, 125.40, 124.73, 124.30, 123.08, 119.41, 108.53, 105.90, 101.41, 57.42, 50.81; HRMS (ESI) m/z calcd for $C_{22}H_{18}ClN_3O_4$ $(M+H)^+$ 435.1226, found 435.1226.

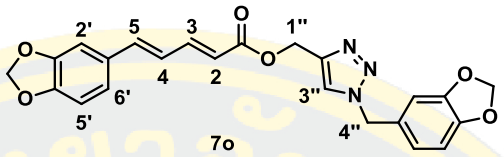


***O*-((3-nitrobenzyl-1,2,3-triazol-4-yl)methyl)piperic ester**; 35% yield (69.5 mg) as a yellow solid; R_f = 0.31 (40% EtOAc/*n*-Hexane), mp = 154-156°C; IR-ATR: ν_{max} 3035, 2969, 1698, 1617, 1524, 1453, 1379, 1223 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 8.23 (dt, J = 7.2, 2.0 Hz, 1H, H-Ar), 8.18 (bs, 1H, H-Ar), 7.66 (s, 1H, H-3′′), 7.62-7.56 (m, 2H, H-Ar), 7.42 (dd, J = 15.2, 10.8 Hz, 1H, H-3), 6.90 (dd, J = 8.4, 1.2 Hz, 1H, H-6′), 6.98 (d, J = 1.2 Hz, 1H, H-2′) 6.81 (d, J = 15.2 Hz, 1H, H-5), 6.78 (d, J = 8.0 Hz, 1H, H-5′), 6.67 (dd, J = 15.2, 10.8 Hz, 1H, H-4), 5.98 (s, 2H, OCH_2O), 5.92 (d, J = 15.2 Hz, 1H, H-2), 5.63 (s, 2H, H-4′′), 5.31 (s, 2H, H-1′′); ^{13}C NMR (100 MHz, $CDCl_3$) 166.87, 148.71, 148.57, 148.30, 145.83, 144.07, 140.89, 136.48, 133.89, 130.38, 130.33, 124.24, 123.90, 123.83, 123.11, 122.91, 119.32, 108.54, 105.89, 101.40, 57.36, 53.15; HRMS (ESI) m/z calcd for $C_{22}H_{18}N_4O_6$ $(M+H)^+$ 435.1299, found 435.1305.



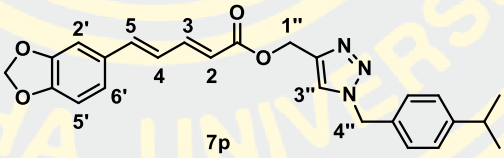
***O*-((3,5-bis(trifluoromethyl)benzyl-1,2,3-triazol-4-yl)methyl)piperic ester**; 62% yield (134.1 mg) as a yellow solid; R_f = 0.34 (40% EtOAc/*n*-Hexane), mp = 116-119°C; IR-ATR: ν_{max} 2970, 1737, 1621, 1608, 1445, 1353, 1276, 1124 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.85 (s, 1H, H-Ar), 7.74 (s, 3H, H-Ar, H-3′′), 7.37 (dd, J = 15.2, 10.8 Hz, 1H, H-3), 6.93 (s, 1H, H-2′), 6.86 (d, J = 7.6 Hz, 1H, H-6′), 6.75 (d, J = 15.2 Hz, 1H, H-5), 6.74 (d, J = 7.2 Hz, 1H, H-5′), 6.63 (dd, J = 15.2, 11.2 Hz, 1H, H-4), 5.94 (s, 2H, OCH_2O), 5.88 (d, J = 15.6 Hz, 1H, H-2), 5.66 (s, 2H, H-4′′), 5.29 (s, 2H, H-1′′); ^{13}C NMR (100 MHz, $CDCl_3$) 166.62, 148.53, 148.11, 145.67, 143.84, 140.72

(2C), 137.14, 132.21 (q, *J* = 66.0, 34.0 Hz, 2C-F) , 130.13, 128.05 (2C), 124.01 (2C), 122.76 (d, *J* = 38.0 Hz, 2C-F), 121.36, 119.01, 108.27, 105.63, 101.24, 57.11, 52.62; HRMS (ESI) m/z calcd for $C_{24}H_{17}N_3O_4$ $(M+H)^+$ 526.1196, found 526.1196.



7o

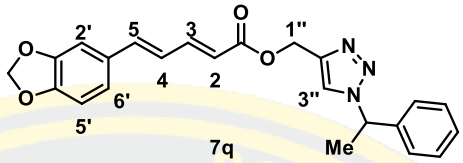
***O*-((piperonylmethyl-1,2,3-triazol-4-yl) methyl) piperic ester**; 57% yield (105.7 mg) as a yellow solid; R_f = 0.35 (40% EtOAc/*n*-Hexane), mp = 94-96°C; IR-ATR: ν_{max} 2923, 2845, 1713, 1621, 1607, 1443, 1369, 1246 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.53 (s, 1H, H-3″), 7.41 (dd, *J* = 15.2, 10.8 Hz, 1H, H-3), 6.98 (d, *J* = 1.6 Hz, 1H, H-2′), 6.90 (dd, *J* = 8.0, 1.6 Hz, 1H, H-6′), 6.80 (d, J = 15.6 Hz, 1H, H-5), 6.80-6.75 (m, 4H, H-5′, H-Ar), 6.67 (dd, *J* = 15.2, 10.8 Hz, 1H, H-4), 5.98 (s, 2H, OCH_2O), 5.97 (s, 2H, OCH_2O), 5.92 (d, *J* = 15.2 Hz, 1H, H-2), 5.41 (s, 2H, H-4″), 5.28 (s, 2H, H-1″); ^{13}C NMR (100 MHz, $CDCl_3$) 171.57, 148.71, 148.30, 147.22, 146.57, 145.86, 144.19, 143.39, 140.90, 137.01, 136.92, 134.89, 133.40, 132.82, 132.48, 128.10, 124.23, 123.10, 109.36, 108.53, 105.88, 101.40, 100.92; HRMS (ESI) m/z calcd for $C_{23}H_{19}N_3O_6$ $(M+H)^+$ 434.1347, found 434.1348.



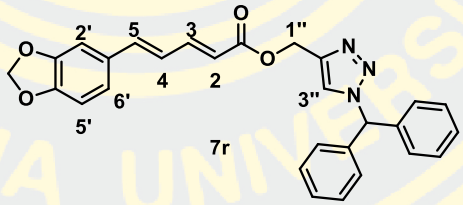
7p

***O*-((4-isopropylbenzyl-1,2,3-triazol-4-yl) methyl) piperic ester**; 66% yield (124.4 mg) as a yellow solid; R_f = 0.33 (40% EtOAc/*n*-Hexane), mp = 131-134°C; IR-ATR: ν_{max} 2957, 2925, 1737, 1695, 1607, 1493, 1449, 1134 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.56 (s, 1H, H-3″), 7.38 (dd, *J* = 15.2, 10.8 Hz, 1H, H-3), 7.20 (bs, 4H, H-Ar), 6.94 (s, 1H, H-2′), 6.87 (d, *J* = 7.6 Hz, 1H, H-6′), 6.75 (d, *J* = 15.6 Hz, 1H, H-5), 6.74 (d, *J* = 7.2 Hz, 1H, H-5′), 6.63 (dd, *J* = 15.2, 10.8 Hz, 1H, H-4), 5.93 (s, 2H, OCH_2O), 5.89 (d, *J* = 15.6 Hz, 1H, H-2), 5.46 (s, 2H, H-4″), 5.26 (s, 2H, H-1″), 2.88 (q, *J* = 6.8 Hz, 1H, CH), 1.21 (d, *J* = 6.8 Hz, 6H, $2CH_3$); ^{13}C NMR (100 MHz, $CDCl_3$) 166.67, 149.48, 148.51, 148.14, 145.47, 143.21, 140.54, 131.63, 130.26, 128.11 (2C), 127.03 (2C), 124.16, 123.49, 122.92, 119.37, 108.37, 105.73, 101.26, 57.34, 53.82,

33.68, 23.72 (2C); HRMS (ESI) m/z calcd for $C_{25}H_{25}N_3O_4$ $(M+H)^+$ 432.1918, found 432.1918.

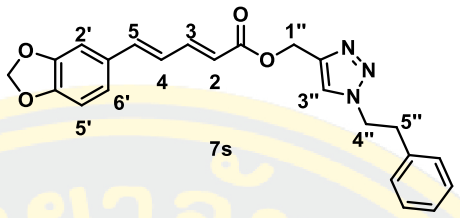


***O*-((1-phenylethyl-1,2,3-triazol-4-yl)methyl)piperic ester**; 53% yield (106.2 mg) as a yellow solid; R_f = 0.29 (40% EtOAc/*n*-Hexane), mp = 122-123°C; IR-ATR: ν_{max} 2892, 1711, 1618, 1607, 1444, 1378, 1234 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.56 (s, 1H, H-3″), 7.40 (dd, J = 15.2, 10.8 Hz, 1H, H-3), 7.37-7.26 (m, 5H, H-Ar), 6.96 (s, 1H, H-2′), 6.88 (d, *J* = 8.0 Hz, 1H, H-6′), 6.77 (d, *J* = 15.6 Hz, 1H, H-5), 6.76 (d, *J* = 7.2 Hz, 1H, H-5′), 6.65 (dd, *J* = 15.2, 10.8 Hz, 1H, H-4), 5.95 (s, 2H, OCH_2O), 5.91 (d, *J* = 15.2 Hz, 1H, H-2), 5.80 (q, *J* = 6.8 Hz, 1H, CH) 5.28 (s, 2H, H-1″), 1.97 (d, *J* = 15.2 Hz, 3H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) 166.54, 148.40, 148.02, 145.35, 142.71, 140.43, 139.53, 130.13, 128.76 (2C), 128.30, 126.27 (2C), 124.04, 122.83, 122.35, 119.26, 108.25, 105.61, 101.17, 60.09, 57.26, 21.04; HRMS (ESI) m/z calcd for $C_{23}H_{21}N_3O_4$ $(M+H)^+$ 404.1605, found 404.1605.

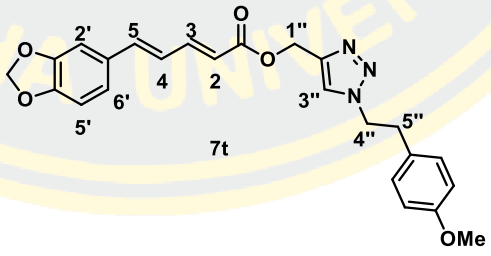


***O*-((1-diphenylmethyl-1,2,3-triazol-4-yl) methyl) piperic ester**; 30% yield (62.0 mg) as a yellow solid; R_f = 0.38 (40% EtOAc/*n*-Hexane), mp = 101-102°C; IR-ATR: ν_{max} 3070, 2898, 1708, 1618, 1606, 1448, 1373, 1260, 1131 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.50 (s, 1H, H-3″), 7.42 (dd, *J* = 15.2, 10.8 Hz, 1H, H-3), 7.37-7.34 (m, 6H, H-Ar, H-4″), 7.13-7.09 (m, 5H, H-Ar), 6.98 (d, *J* = 1.6 Hz, 1H, H-2′), 6.90 (dd, *J* = 8.4, 1.6 Hz, 1H, H-6′), 6.80 (d, *J* = 15.6 Hz, 1H, H-5), 6.78 (d, *J* = 8.0 Hz, 1H, H-5′), 6.67 (dd, *J* = 15.2, 10.8 Hz, 1H, H-4), 5.97 (s, 2H, OCH_2O), 5.93 (d, *J* = 15.2 Hz, 1H, H-2), 5.30 (s, 2H, H-1″); ^{13}C NMR (100 MHz, $CDCl_3$) 166.84, 148.68, 148.32, 145.65, 142.86, 140.71, 137.98 (3C), 130.47, 128.98 (4C), 128.65 (4C),

128.11 (2C), 124.37, 123.79, 123.08, 119.57, 108.56, 105.92, 101.43, 57.51; HRMS (ESI) m/z calcd for $C_{28}H_{23}N_3O_4$ $(M+H)^+$ 466.1761, found 466.1761.

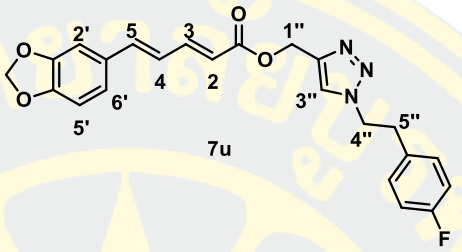


***O*-((1-phenethyl-1,2,3-triazol-4-yl) methyl) piperic ester**; 69% yield (86.3 mg) as a yellow solid; R_f = 0.35 (40% EtOAc/*n*-Hexane), mp = 122-123°C; IR-ATR: ν_{max} 3133, 3030, 2970, 1700, 1622, 1608, 1449, 1371, 1233 cm^{-1}; 69% yield; ^1H NMR (400 MHz, $CDCl_3$) δ 7.41 (dd, J = 15.2, 10.8 Hz, 1H, H-3), 7.38 (s, 1H, H-3″), 7.31-7.24 (m, 3H, H-Ar), 7.10-7.08 (m, 2H, H-Ar), 6.98 (d, J = 1.6 Hz, 1H, H-2′), 6.91 (dd, J = 8.4, 1.6 Hz, 1H, H-6′), 6.80 (d, J = 15.2 Hz, 1H, H-5), 6.78 (d, J = 8.0 Hz, 1H, H-5′), 6.68 (dd, J = 15.2, 10.8 Hz, 1H, H-4), 5.98 (s, 2H, OCH_2O), 5.92 (d, J = 15.2 Hz, 1H, H-2), 5.26 (s, 2H, H-1″), 4.58 (t, J = 7.6 Hz, 2H, H-4″), 3.20 (t, J = 7.6 Hz, 2H, H-5″); ^{13}C NMR (100 MHz, $CDCl_3$) 166.68, 148.55, 148.18, 145.48, 142.68, 140.57, 136.76, 130.29 (3C), 128.70, 128.53, 126.98, 124.20, 124.03, 122.96, 119.44, 108.40, 105.76, 101.29, 57.31, 51.52, 36.57; HRMS (ESI) m/z calcd for $C_{23}H_{21}N_3O_4$ $(M+H)^+$ 404.1605, found 404.1605.

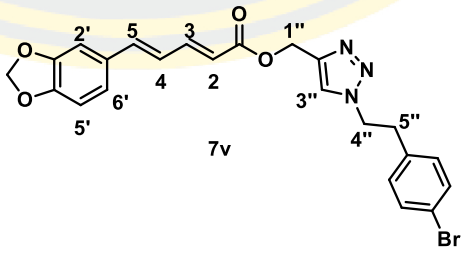


***O*-((4-methoxyphenethyl-1,2,3-triazol-4-yl)methyl)piperic ester**; 76% yield (162.0 mg) as a yellow solid; R_f = 0.40 (40% EtOAc/*n*-Hexane), mp = 111-112°C; IR-ATR: ν_{max} 3116, 3070, 2928, 1724, 1609, 1444, 1387, 1240 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.39 (s, 1H, H-3″), 7.38 (dd, J = 15.2, 10.8 Hz, 1H, H-3), 6.98 (s, 1H, H-2′), 6.96-6.95 (m, 2H, H-Ar), 6.88 (dd, J = 8.4, 1.6 Hz, 1H, H-6′), 6.80-6.74 (m, 4H, H-5′, H-5, H-Ar), 6.71 (dd, J = 15.4, 10.8 Hz, 1H, H-4), 5.94 (s, 2H, OCH_2O), 5.89 (d, J =

15.2 Hz, 1H, H-2), 5.24 (s, 2H, H-1″), 4.51 (t, J = 7.2 Hz, 2H, H-4″), 3.74 (s, 3H, OCH_3), 3.11 (t, J = 7.2 Hz, 2H, H-5″); ^{13}C NMR (100 MHz, $CDCl_3$) 166.56, 158.37, 148.42, 148.04, 145.38, 142.48, 140.48, 130.15, 129.43 (2C), 128.62, 124.05, 123.97, 122.86, 119.27 (2C), 113.95, 108.27, 105.62, 101.19, 57.20, 54.20, 51.60, 35.55; HRMS (ESI) m/z calcd for $C_{24}H_{23}N_3O_5$ $(M+H)^+$ 434.1710, found 434.1721.

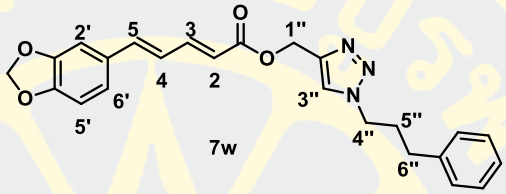


***O*-((4-fluorophenethyl-1,2,3-triazol-4-yl)methyl)piperic ester**; 61% yield (117.1 mg) as a yellow solid; R_f = 0.25 (40% EtOAc/*n*-Hexane), mp = 121-125°C; IR-ATR: ν_{max} 3137, 2925, 1738, 1698, 1622, 1609, 1500, 1450, 1388, 1218 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 77.41-7.34 (m, 1H, H-3), 7.38 (s, 1H, H-3″), 7.02-6.86 (m, 6H, H-2′, H-6′, H-Ar), 6.79-6.74 (m, 2H, H-5′, H-5), 6.65 (dd, J = 15.2, 10.8 Hz, 1H, H-4), 5.94 (s, 2H, OCH_2O), 5.88 (d, J = 15.2 Hz, 1H, H-2), 5.23 (s, 2H, H-1″), 4.52 (t, J = 7.2 Hz, 2H, H-4″), 3.15 (t, J = 7.2 Hz, 2H, H-5″); ^{13}C NMR (100 MHz, $CDCl_3$) 166.65, 161.75 (d, J = 244.0 Hz, C-F), 148.53, 148.15, 145.53, 142.73, 132.46 (d, J = 3.0 Hz, 2×C_m-F), 130.27, 130.04 (d, J = 8.0 Hz, 2×C_o-F), 124.16, 124.12, 122.96, 119.33, 115.63, 115.42, 108.38, 105.75, 101.28, 57.26, 51.48, 35.71; HRMS (ESI) m/z calcd for $C_{23}H_{20}FN_3O_4$ $(M+H)^+$ 422.1511, found 422.1512.

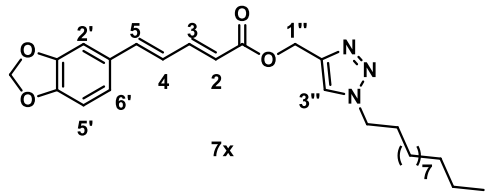


***O*-((4-bromophenethyl-1,2,3-triazol-4-yl)methyl)piperic ester**; 57% yield (125.8 mg) as a yellow solid; R_f = 0.30 (40% EtOAc/*n*-Hexane), mp = 143-151°C; IR-ATR: ν_{max} 2926, 1722, 1606, 1488, 1355, 1217, 506 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.43-7.36 (m, 3H, H-3, H-Ar), 7.39 (s, 1H, H-3″), 6.96 (s, 1H, H-2′), 6.93-

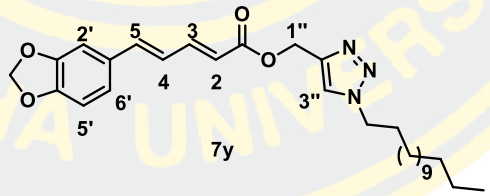
6.88 (m, 3H, H-6′, H-Ar), 6.81-6.75 (m, 2H, H-5′, H-5), 6.66 (dd, J = 15.2, 10.8 Hz, 1H, H-4), 5.95 (s, 2H, OCH_2O), 5.90 (d, J = 15.2 Hz, 1H, H-2), 5.25 (s, 2H, H-1″), 4.53 (t, J = 6.8 Hz, 2H, H-4″), 3.15 (t, J = 6.8 Hz, 2H, H-5″); ^{13}C NMR (100 MHz, $CDCl_3$) 166.79, 148.61, 148.23, 145.65, 142.87, 140.73, 135.75, 131.85 (2C), 130.30 (2C), 124.26, 124.20, 123.03, 121.02 (2C), 119.40, 108.47, 105.84, 101.34, 57.32, 51.27, 36.00; HRMS (ESI) m/z calcd for $C_{23}H_{20}BrN_3O_4$ $(M+H)^+$ 482.0710, found 482.0716.



***O*-((1-(3-phenylpropyl)-1,2,3-triazol-4-yl)methyl)piperic ester**; 81% yield (110.7 mg) as a yellow solid; R_f = 0.32 (40% EtOAc/*n*-Hexane), mp = 111-113°C; IR-ATR: ν_{max} 3062, 3028, 2969, 1738, 1682, 1601, 1443, 1365, 1227 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.61 (s, 1H, H-3″), 7.43 (dd, J = 15.2, 10.8 Hz, 1H, H-3), 7.34-7.27 (m, 2H, H-Ar), 7.22-7.16 (m, 3H, H-Ar), 6.97 (d, J = 1.2 Hz, 1H, H-2′), 6.89 (dd, J = 8.0, 1.2 Hz, 1H, H-6′), 6.79 (d, J = 15.2 Hz, 1H, H-5), 6.77 (d, J = 8.0 Hz, 1H, H-5′), 6.67 (dd, J = 15.2, 10.8 Hz, 1H, H-4), 5.96 (s, 2H, OCH_2O), 5.93 (d, J = 15.6 Hz, 1H, H-2), 5.31 (s, 2H, H-1″), 4.34 (t, J = 7.2 Hz, 2H, H-4″), 2.65 (t, J = 7.2 Hz, 2H, H-6″), 2.25 (q, J = 7.2 Hz, 2H, H-5″); ^{13}C NMR (100 MHz, $CDCl_3$) 166.82, 148.57, 148.19, 145.58, 142.97, 140.65, 139.96, 130.32, 128.52 (2C), 128.31 (2C), 126.27, 124.22, 123.71, 122.98, 119.43, 108.43, 105.79, 101.31, 57.41, 49.46, 32.37, 31.45; HRMS (ESI) m/z calcd for $C_{24}H_{23}N_3O_4$ $(M+H)^+$ 418.1761, found 418.1761.



***O*-((1-dodecyl-1,2,3-triazol-4-yl)methyl)piperic ester**; 79% yield (95.3 mg) as a yellow solid; R_f = 0.29 (30% EtOAc/*n*-Hexane), mp = 112-114°C; IR-ATR: ν_{max} 2955, 2917, 2848, 1708, 1621, 1609, 1446, 1368, 1233 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.60 (s, 1H, H-3″), 7.39 (dd, J = 15.2, 10.8 Hz, 1H, H-3), 6.94 (s, 1H, H-2′), 6.86 (d, J = 8.0 Hz, 1H, H-6′), 6.76 (d, J = 15.2 Hz, 1H, H-5), 6.74 (d, J = 7.6 Hz, 1H, H-5′), 6.64 (dd, J = 15.6, 10.8 Hz, 1H, H-4), 5.93 (s, 2H, OCH_2O), 5.90 (d, J = 15.2 Hz, 1H, H-2), 5.28 (s, 2H, H-1″), 4.30 (t, J = 7.6 Hz, 2H, N-CH_2), 1.86 (t, J = 6.4 Hz, 2H, CH_2), 1.28-1.21 (m, 18H, CH_2), 0.84 (t, J = 6.4 Hz, 3H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) 166.63, 148.45, 148.08, 145.37, 142.76, 140.47, 130.19, 124.08, 123.44, 122.86, 119.35, 108.27, 105.64, 101.21, 57.33, 50.16, 31.66, 30.02, 29.37, 29.28, 29.15 (2C), 29.10, 28.76, 26.26, 22.45, 13.89; HRMS (ESI) m/z calcd for $C_{27}H_{37}N_3O_4$ $(M+Na)^+$ 468.2857, found 468.2857.



***O*-((1-tetradecyl-1,2,3-triazol-4-yl)methyl)piperic ester**; 87% yield (113.3 mg) as a yellow solid; R_f = 0.20 (30% EtOAc/*n*-Hexane), mp = 111-115°C; IR-ATR: ν_{max} 2916, 2848, 1721, 1621, 1608, 1446, 1370, 1254 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.60 (s, 1H, H-3″), 7.39 (dd, J = 15.6, 10.8 Hz, 1H, H-3), 6.94 (s, 1H, H-2′), 6.86 (d, J = 8.0 Hz, 1H, H-6′), 6.76 (d, J = 15.6 Hz, 1H, H-5), 6.73 (d, J = 7.6 Hz, 1H, H-5′), 6.64 (dd, J = 15.6, 10.8 Hz, 1H, H-4), 5.93 (s, 2H, OCH_2O), 5.90 (d, J = 15.2 Hz, 1H, H-2), 5.28 (s, 2H, H-1″), 4.30 (t, J = 7.2 Hz, 2H, N-CH_2), 1.86 (t, J = 6.4 Hz, 2H, CH_2), 1.28-1.21 (m, 22H, CH_2), 0.84 (t, J = 6.4 Hz, 3H, CH_3); ^{13}C NMR (100

MHz, $CDCl_3$) 166.80, 148.64, 148.27, 145.56, 142.96, 140.66, 130.38, 124.27, 123.65, 122.83, 123.05, 119.54, 108.46, 105.83, 101.40, 57.52, 50.53, 31.88, 30.22, 29.64, 29.61, 29.58, 29.49, 29.35, 29.32, 28.96, 26.45, 22.65, 14.10; HRMS (ESI) m/z calcd for $C_{29}H_{41}N_3O_4$ $(M+H)^+$ 496.3170, found 496.3170.

4.8 Conclusion

We have successfully modified the structure of piperine isolated from *P nigrum*. A series of piperine amide analouges were performed by various amine *via* aminolysis to afford twenty-nine piperine amide analouges **3a**-**3ac** in low to excellent yields (37%-98%). Meanwhile, a series of 1,2,3-triazole-piperine analogues were performed by introducing various substituted azide groups through one-pot two-step to obtain twenty-five 1,2,3-triazole-piperine analouges **7a-7y** in low to excellent yields (30%-87%). All synthesized analouges were screened for *in vitro* antioxidant and acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE) activities. Among them, compound **3f** showed the highest antioxidant activity (IC_{50} value of 0.04 ± 0.00 μM) and better than ascorbic acid as a positive control. Compound **7a** had moderate anti-AChE activity, with an IC_{50} of 37.37 ± 0.04 μM. Furthermore, compound **3z** showed the most promising against BuChE with IC_{50} value of 4.60 ± 0.01μM and higher than galantamine up to 8-fold. Kinetic analysis indicates that these inhibitors exhibited non-competitive inhibition with target enzymes. Molecular docking studies showed that compounds **3z** and **7a** had a high binding energies against AChE and BuChE enzymes, respectively due to the presence of hydrogen bonding, hydrophobic and electrostatic interactions, compound **3z** has the potential as a lead compound to develop novel anti-BuChE agents and will be further developed for the treatment of Alzheimer's disease.

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มหาวิทยาลัยบูรพา
BURAPHA UNIVERSITY

BIOGRAPHY

NAME นางสาวสุวิชาดา ใจเปี้ย

DATE OF BIRTH ุ6 มกราคม พ. ศ. 2540

PLACE OF BIRTH จังหวัดกรุงเทพมหานคร

PRESENT ADDRESS บ้านเลขที่ 52/1 หมู่ 4 ตำบลในคลองบางปลากด อำเภอพระสมุทรเจดีย์ จังหวัดสมุทรปราการ