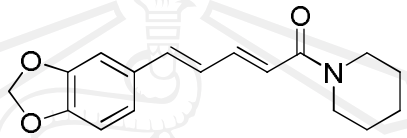
CHAPTER III

RESULTS AND DISCUSSION

3.1 Isolation of piperine (1) from black pepper



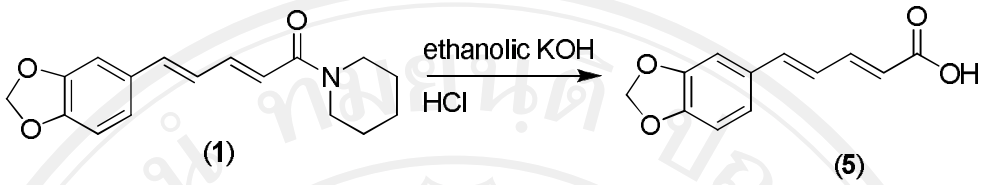
1

The black pepper powder was extracted with 95% ethanol and refluxed 24 h, followed by 10 % alcoholic potassium hydroxide, filtered and left it for one day to precipitate the crude. The crude product was purified by crystallization from dichloromethane: hexane (3:2) to obtain piperine in 1.13 % yield. The pure piperine as judged by ^{1}H-NMR technique as shown in Table 21. The ^{1}H NMR spectrum indicated a methylene proton at 6.00 ppm and the methylene protons of pyridine ring proton at 1.45-1.74 and 3.60 ppm.

Table 21 ^{1}H-NMR data of compound **1**

Compound	Physical property	m.p. (ºC)	Chemical shift (δ, ppm)
1	Yellow crystals	130.8-132.0	1.45-1.74 (m, 6H, H-3′,4′,5′), 3.60 (br, 4H, H-2′,6′), 6.00 (s, 2H, H-12), 6.51 (d, J = 14.6 Hz, 1H, H-2), 6.67-6.80 (m, 3H, H-5,10, 11), 6.90 (dd, J = 1.5, 8.0 Hz, 1H, H-4), 7.00 (s, 1H, H-7), 7.38-7.41 (m, 1H, H-3) ppm

3.2 Preparation of piperic acid (5) from piperine (1)



(1) (5)

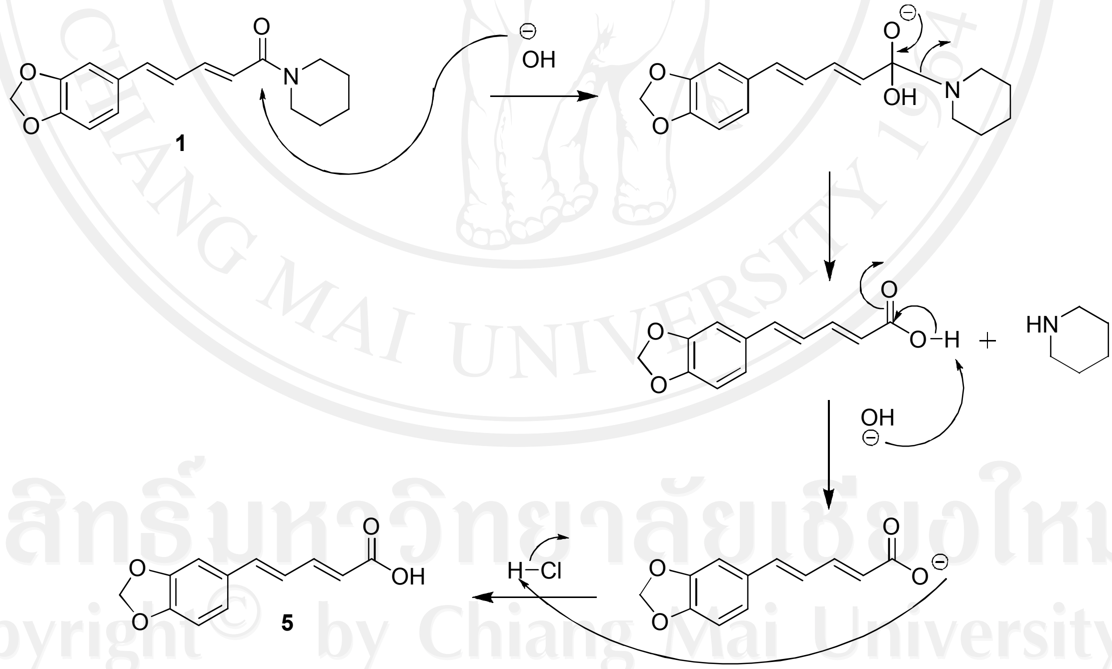
Treatment of piperine (**1**) with ethanolic KOH, refluxed for 25 h gave the corresponding solid potassium salt of piperic acid, then follow by dissolved in hot water 50 ml, acidified with 35% HCl to give the piperic acid (**5**). The crude product was purified by crystallization from ethanol to obtain piperic acid (**5**) in 98.98 % yield.

The structure of piperic acid (**5**) was characterized by ^{1}H NMR technique as shown in Table 22. The ^{1}H NMR spectrum indicated a methylene proton at 6.05 ppm and hydroxyl protons at 12.19 ppm.

The reaction mechanism for preparation of piperic acid (**5**) from piperine (**1**) by addition of hydroxide ion to the carbonyl group of piperine (**1**), followed by elimination of the piperidine, after that abstraction of a proton from hydrochloric acid to obtain piperic acid (**5**), as shown in Scheme 15.[33]

Table 22 [1]H-NMR data of compound **5**

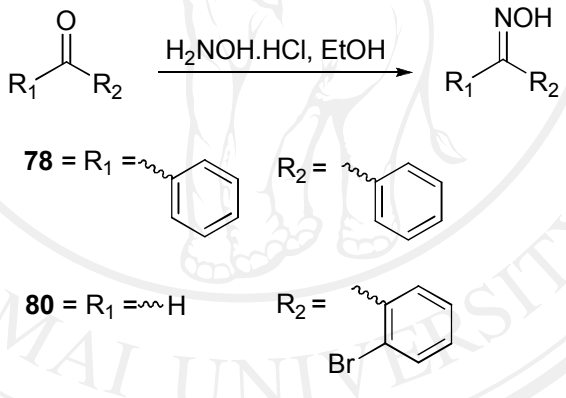
Compound	Physical property	m.p. (ºC)	Chemical shift (*δ,* ppm)
5	Yellow needles	217.0-217.8	5.92 (d, *J* = 15.1 Hz, 1H, H-2), 6.05 (s, 2H, H-12), 6.92 (d, *J* = 8.0 Hz, 1H, H-5), 6.96 (*s*, 1H, H-7), 6.97 (d, *J* = 3.5 Hz, 1H, H-5,10, 11), 7.00 (dd, *J* = 1.5, 8.0 Hz, 1H, H-4), 7.23 (d, *J* = 1.5 Hz, 1H, H-10), 7.25-7.32 (m, 1H, H-3), 12.19 (s, 1H, OH) ppm



Scheme 15 Mechanism of piperic acid (**5**) from piperine (**1**)[33]

3.3 Standard preparation of oxime

Ketone **77** and aldehyde **79** were reacted with hydroxylamine hydrochloride and sodium hydroxide in EtOH, was stirred at reflux for 3 h. After cooling, the mixture was neutralized and extracted by CH_2Cl_2 (3x100 mL). The organic layer was dried over Na_2SO_4. The reaction mixture was evaporated under vacuum pressure to give the crude product and then purified by column chromatography (silica gel; ethyl acetate in hexane, 1:9, as eluent) to obtain compound **78** and **80**, as shown in Scheme 16.

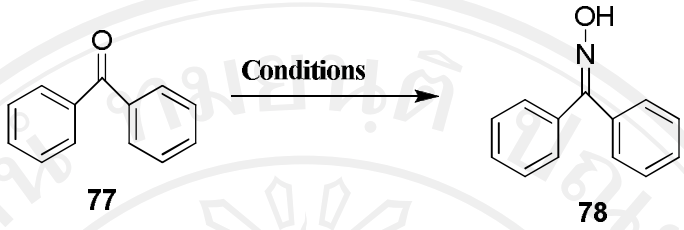


Scheme 16 Synthetic route for preparation of oximes of aldehyde or ketone

The results shown that obtained the oximes **78** and **80**, which correspond to the reaction of **77** and **79** with hydroxylamine hydrochloride, at high yield.

We were interested in preparation of oxime by various the amounts of hydroxylamine hydrochloride, sodium hydroxide and reaction time. The results are presented in Tables 6 and 23.

Table 23 The preparation of compound **78** by vary *n* equiv $NH_2OH.HCl$ and NaOH at reflux condition



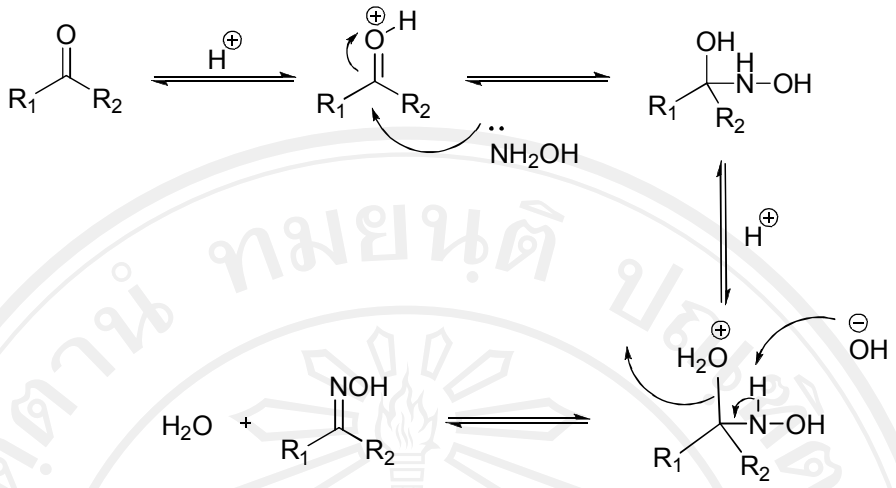
Entry	Conditions	Yield[a] (%)	Conversion (%)
1	1.5 equiv $NH_2OH.HCl$, 1.5 equiv NaOH, reflux 3 h	70	72
2	3.0 equiv $NH_2OH.HCl$, 6.0 equiv NaOH, reflux 3 h	98	99
3	3.0 equiv $NH_2OH.HCl$, 6.0 equiv NaOH, reflux 6 h	99	100

[a] Isolated yield

From the Tables 6 and 23, entry 2 and 3 gave high percent yield of compound **78**. However, when increase amount of $NH_2OH.HCl$ and NaOH and reaction time (entry 2 and 3), we found that the percent yield was nearly the same. In this research, was choosed the condition in entry 2 due to the results showed that the same as entry 3 and this condition utilized the lowest reaction time.

2-bromobenzaldehyde oxime **80** was prepared in a similar way, but the reaction of compound **80** gave moderate percent yield.

The purpose mechanism for preparation of oximes was shown in Scheme 17.



Scheme 17 Mechanism of oximes from aldehyde or ketone

The structure of these oximes was confirmed by the ^{1}H-NMR spectrum of oximes functional group at (δ 9.10-10.36 ppm).

The compound **78** was characterized by ^{1}H-NMR technique, as shown in Table 24, the oxime proton appeared as a singlet δ at 9.10 ppm.

Table 24 ^{1}H-NMR data of compound **78**

Compound	Physical property	m.p. (ºC)	Chemical shift (δ, ppm)
78	White crystals	217.0-217.8	7.31-7.41 (m, 3H, H-4,5,6), 7.41-7.43 (m, 2H, H-3,7), 7.45-7.50 (m, 5H, H-9,10,11,12,13), 9.10 (s, 1H, OH) ppm

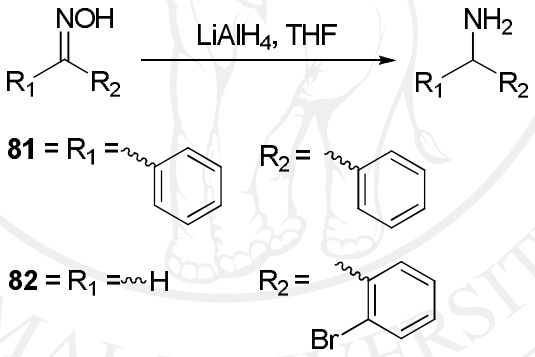
The compound **80** was characterized by ^{1}H-NMR technique, as shown in Table 25, the oxime proton appeared as a singlet δ at 10.36 ppm.

Table 25 ^{1}H-NMR data of compound **80**

Compound	Physical property	m.p. (ºC)	Chemical shift (δ, ppm)
80	White crystals	88.8-91.1	7.20-7.25 (m, 1H, H-5), 7.32 (t, J = 7.42 Hz, 1H, H-4), 7.57 (dd, J = 7.98, 1.19 Hz, 1H, H-6), 7.79 (dd, J = 7.79 Hz, 1H, H-3), 8.58(s, 1H, H-1), 10.36 (s, 1H, OH) ppm

3.4 Standard preparation of amine

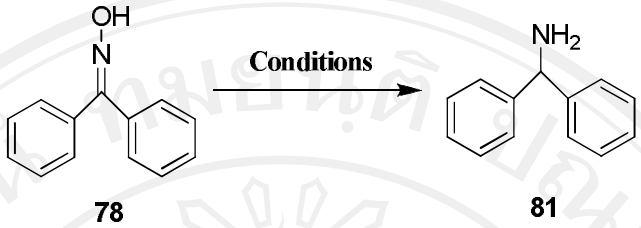
Oximes **78** and **80**, in anhydrous THF (10 mL), were reacted with $LiAlH_4$ in anhydrous THF (10 mL). The mixture was stirred under reflux for 3-6 h. After cooling, the mixture was hydrolyzed and extracted by CH_2Cl_2 (3x100 mL). The organic layer was dried over Na_2SO_4. The reaction mixture was evaporated under vacuum pressure to give the brown liquid product and then purified by preparative thin layer column chromatography (silica gel; ethyl acetate in hexane, 2:8, as eluent) to obtain compounds **81** and **82**, as show in Scheme 18.



Scheme 18 Synthetic route for preparation of amines from oximes

We were interested in preparation of amines by various reaction times. The results are presented in Tables 9 and 26.

Table 26 The preparation of compound **81** by vary reaction time at reflux condition



Entry	Conditions	Yield[a] (%)	Conversion (%)
1	1.0 equiv Benzophenone oxime, 4.0 equiv $LiAlH_4$, reflux 3 h	29	46
2	1.0 equiv Benzophenone oxime, 4.0 equiv $LiAlH_4$, reflux 6 h	34	52

[a] Isolated yield

From the Tables 9 and 26, entry 2 gave the highest percent yield of compound **81**. In this research, was choosed the condition in entry 2 due to the results showed that this condition gave the highest percent yield.

2-bromobenzylamine **82** was prepared in a similar way. In addition, the reaction gave compound **82** in 32 % yield and 48 % conversion from the starting material.

The structure of these amines was confirmed by the ^{1}H-NMR spectrum of amine (δ 1.86-2.03 ppm).

The compound **81** was characterized by ^{1}H-NMR technique, as shown in Table 27, the amine proton appeared as a singlet δ at 1.86 ppm.

Table 27 ^{1}H-NMR data of compound **81**

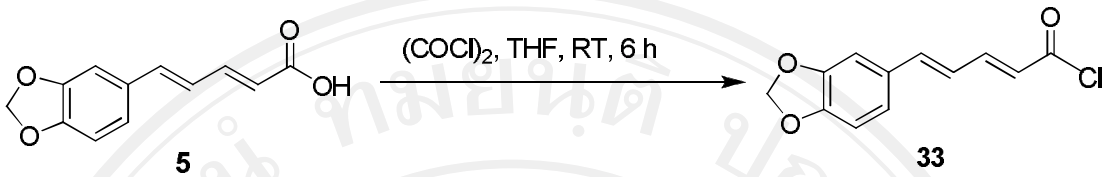
Compound	Physical property	m.p. (ºC)	Chemical shift (δ, ppm)
81	Colorless liquid	-	5.25 (s, 1H, H-1), 7.25-7.28 (m, 2H, H-5,11), 7.32-7.37 (m, 4H, H-4,6,10,12), 7.41-7.47 (m, 4H, H-3,7,9,13), 1.86 (s, 2H, NH) ppm

The compound **82** was characterized by ^{1}H-NMR technique, as shown in Table 28, the amine proton appeared as a singlet δ at 2.03 ppm.

Table 28 ^{1}H-NMR data of compound **82**

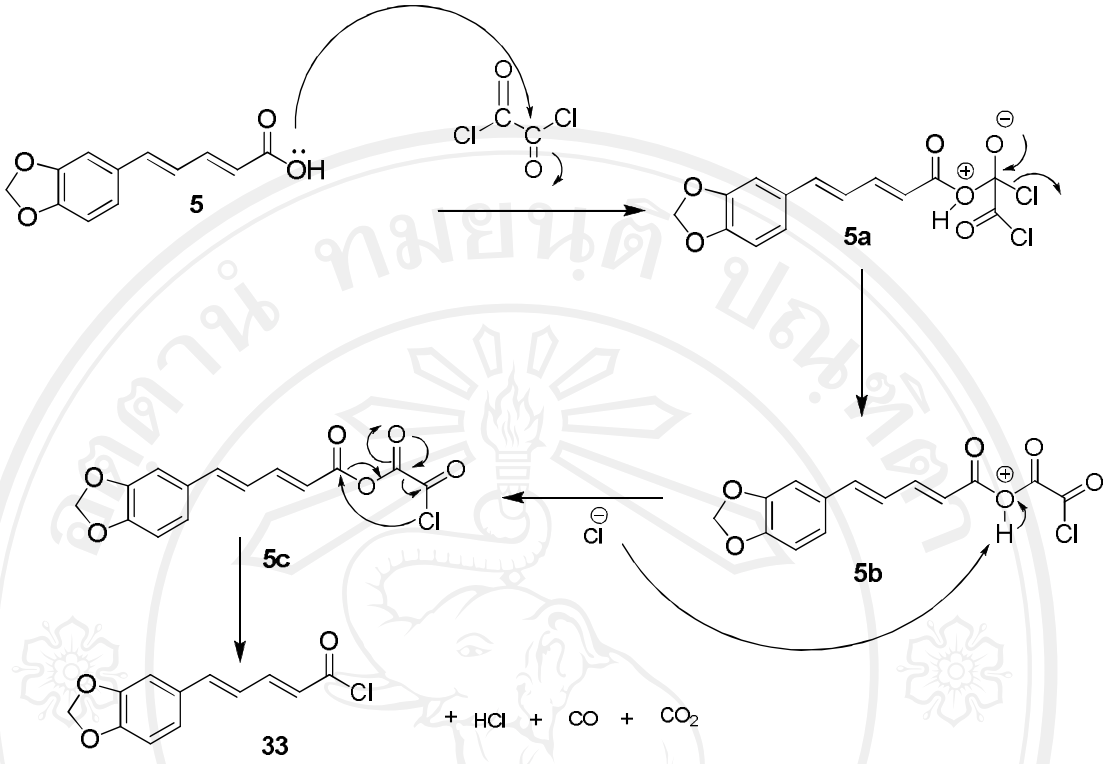
Compound	Physical property	m.p. (ºC)	Chemical shift (δ, ppm)
82	Slightly yellow liquid	-	5.34 (s, 2H, H-1) 7.25 (m, 2H, H-3,5), 7.37 (m, 1H, H-4), 7.78 (m, 1H, H-6), 2.03 (s, 2H, NH) ppm

3.5 Preparation of piperic acid chloride (33)



Piperic acid (**5**) was dissolved in dried THF and kept under nitrogen atmosphere. Oxalyl chloride was added dropwise in to the solution. The reaction mixture was stirred at room temperature for 6 h. Then, the excess oxalyl chloride was removed under reduced pressure to give acid chloride as an orange residue.

The purpose mechanism for preparation of piperic acid chloride (**33**) from piperic acid (**5**) by addition of hydroxide ion of piperic acid (**5**) to the carbonyl group of oxalyl chloride, followed by elimination of chloride ion to obtain the intermediate **5b**, then elimination of carbon monoxide and carbon dioxide to obtain piperic acid chloride (**33**). The reaction mechanism was shown in Scheme 19.[32,33,41]

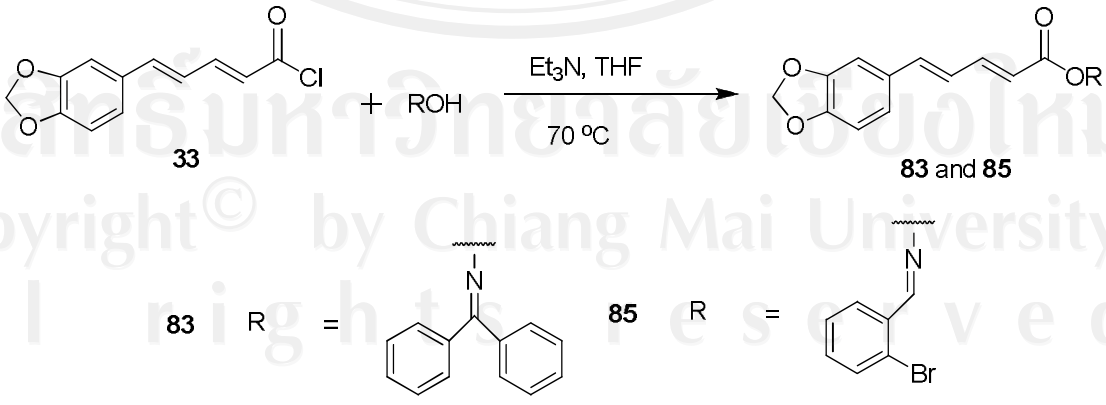


Scheme 19 Mechanism of piperic acid chloride (**33**)

3.6 Standard preparation of oxime-ester and amide derivatives of piperine (compounds 83, 84, 85, 86, 87 and 88)

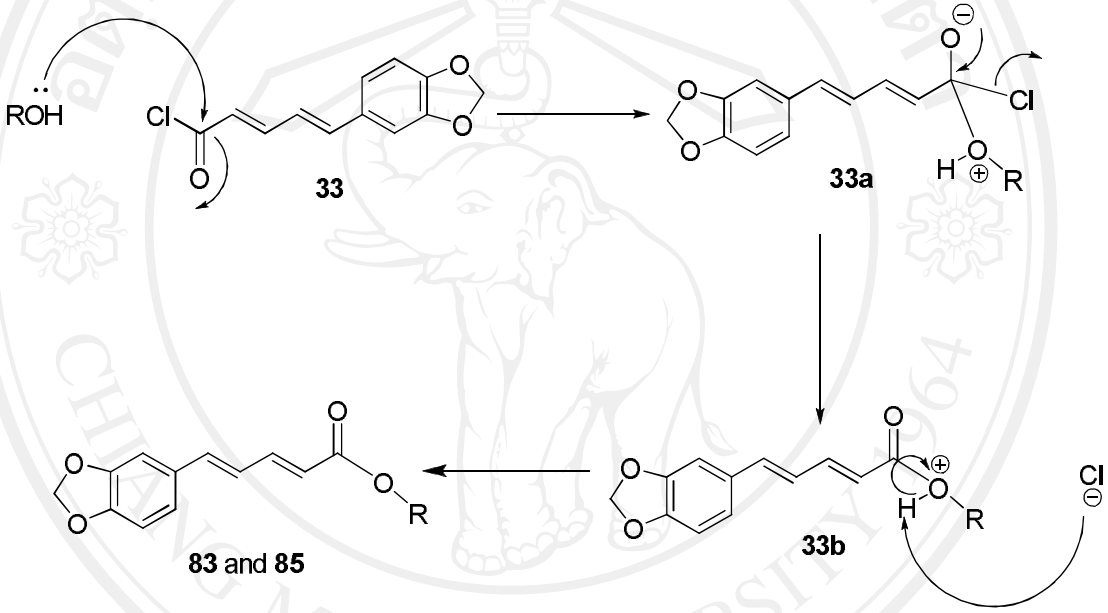
Piperic acid chloride (**33**) was dissolved in dried THF under nitrogen atmosphere and then added with benzophenone oxime (**78**), 2-bromobenzaldehyde oxime (**80**), diphenylmethanamine (**81**), 2-bromobenzylamine (**82**), allyl amine and 4-amino-pyridin solution, followed by triethylamine. The reaction mixture was stirred at 70 ºC for 5 h. The solvent in the reaction mixture was then removed under reduced pressure to give the yellow residue and was purified by column chromatography on silica gel using ethyl acetate and hexane, 1:9 or 2:1 as eluent, to obtain oxime-ester and amide derivatives of piperine (compounds **83, 84, 85, 86, 87** and **88**) in 28.13 – 58.91 % yield.

The synthetic route and the purpose mechanism for preparation of oxime-ester and amide derivatives of piperine were shown in Schemes 20, 21, 22 and 23, respectively.

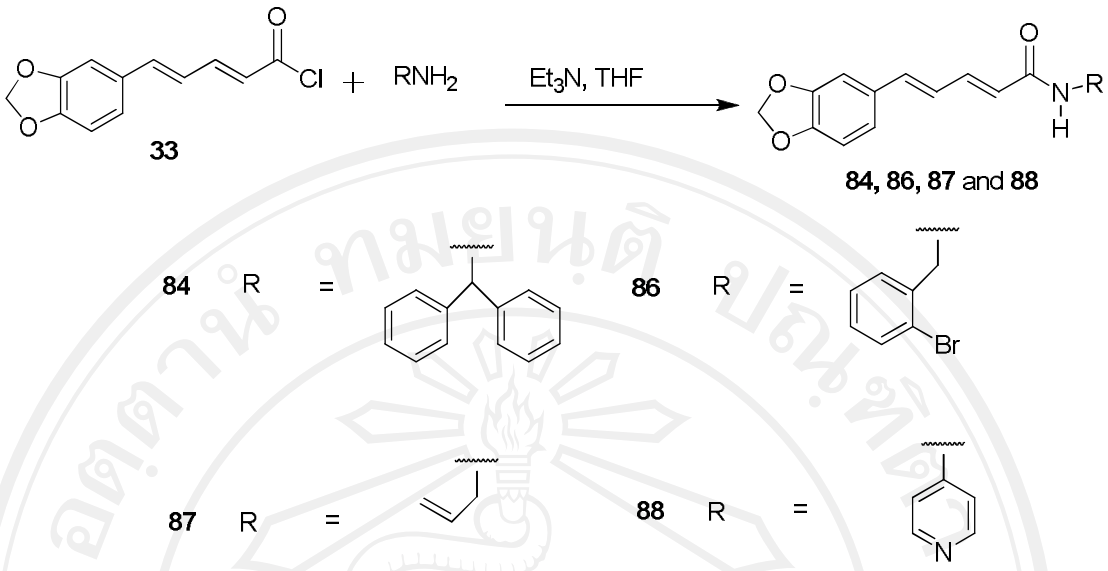


Scheme 20 Synthetic route for preparation of oxime-ester derivatives of piperine

The purpose mechanism for preparation of compounds **83** and **85** from piperic acid chloride (**33**) by addition of hydroxide ion of alcohol to the carbonyl group of piperic acid chloride (**33**), followed by elimination of chloride ion to obtain the intermediate 33b, then abstraction of proton by chloride ion to obtain oxime-esters **83** and **85**. The reaction mechanism was shown in Scheme 21.

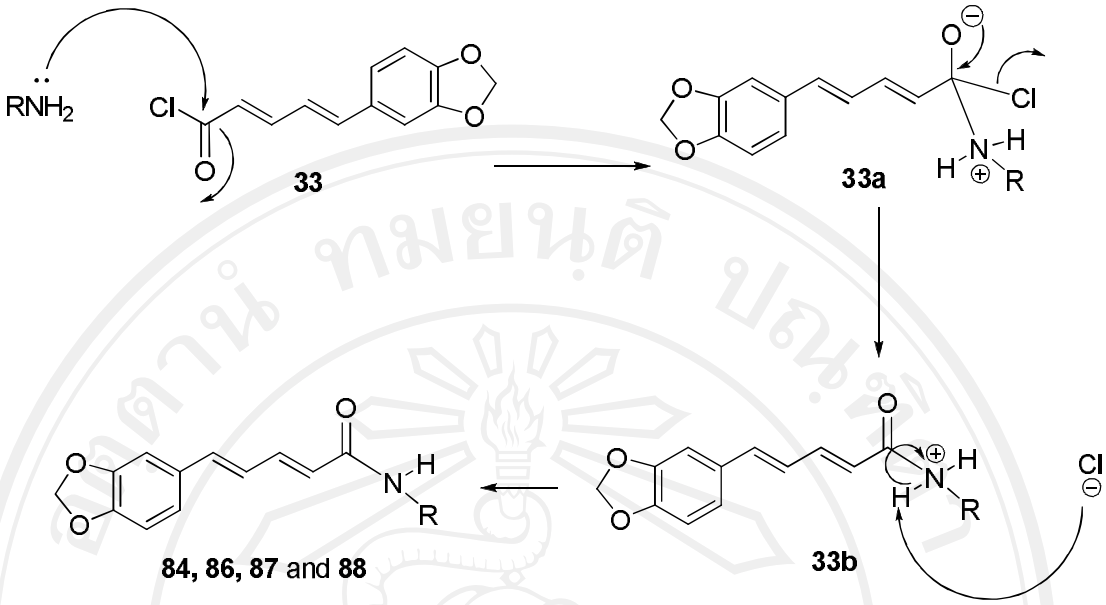


Scheme 21 Mechanism of oxime-ester derivatives of piperine



Scheme 22 Synthetic route for preparation of amide derivatives of piperine

The purpose mechanism for preparation of compounds **84**, **86**, **87** and **88** from piperic acid chloride (**33**) by addition of hydroxide ion of alcohol to the carbonyl group of piperic acid chloride (**33**), followed by elimination of chloride ion to obtain the intermediate 33b, then abstraction of proton by chloride ion to obtain amides **84**, **86**, **87** and **88**. The reaction mechanism was shown in Scheme 23.



Scheme 23 Mechanism of amide derivative of piperine

The structures of the ester and amide derivatives of piperine were elucidated by their spectroscopic data, ^{1}H-NMR, ^{13}C-NMR, 2D-NMR, IR and MS.

The compound **83** was characterized by ^{1}H-NMR technique, as shown in Table 29. The ^{1}H NMR spectrum indicated a methylene proton as a singlet δ at 5.97 ppm and benzene ring protons (H-4′, 5′, 6′, 7′, 8′, 10′, 11′, 12′, 13′, 14′) as a mutiplet δ at 7.32-7.38, 7.46-7.50 and 7.59-7.64 ppm corresponding with ^{13}C NMR spectral data revealed a methylene carbon at 101.35 ppm and a benzene ring carbon (C-4′, 5′, 6′, 7′, 8′, 10′, 11′, 12′, 13′, 14′) at 128.12, 128.29, 128.87, 129.02, 129.49 and 130.76 ppm.

Table 29 ^{1}H-NMR data of compound **83**

Compound	Physical property	m.p. (ºC)	Chemical shift (δ, ppm)
83	Yellow crystals	207.3-208.1	5.89 (d, J = 15.26 Hz, 1H, H-2), 5.97 (s, 2H, H-12), 6.62-6.70 (m, 1H, H-4), 6.73-6.78 (m, 2H, H-5,10), 6.89 (dd, J = 8.06, 1.58 Hz, 1H, H-11), 6.95 (d, J = 1.49 Hz, 1H, H-7), 7.32-7.38, 7.46-7.50, 7.59-7.64 (m, 10H, H-4′,5′,6′,7′,8′, 10′,11′,12′,13′,14′), 7.40-7.46 (m, 1H, H-3), ppm

The compound **84** was characterized by ^{1}H-NMR technique, as shown in Table 30. The ^{1}H NMR spectrum indicated a methylene proton as a singlet δ at 6.00 ppm, a amide proton as a singlet δ at 5.32 ppm and benzene ring protons (H-4′, 5′, 6′, 7′, 8′, 10′, 11′, 12′, 13′, 14′) as a singlet δ at 6.37-6.39, 7.26-7.31 and 7.33-7.37 ppm corresponding with ^{13}C NMR spectral data revealed a methylene carbon at 100.86 ppm, an amide carbon at 164.66 ppm and a benzene ring carbon (C-4′, 5′, 6′, 7′, 8′, 10′, 11′, 12′, 13′, 14′) at 126.98 and 128.18 ppm.

Table 30 ^{1}H-NMR data of compound **84**

Compound	Physical property	m.p. (ºC)	Chemical shift (δ, ppm)
84	Light yellow crystals	156.5-157.1	5.32 (s, 1H, NH), 5.99 (d, J = 2.00 Hz, 1H, H-2), 6.00 (s, 2H, H-12), 6.02 (s, 1H, H-2′), 6.65-6.73 (m, 1H, H-4), 6.79-6.86 (m, 2H, H-5,10), 7.00 (d, J = 1.20 Hz, 1H, H-7), 6.37-6.39, 7.26-7.31, 7.34-7.40 (m, 10H, H-4′,5′,6′,7′,8′,10′, 11′,12′,13′,14′), 7.42 (d, J = 14.81, 10.81 Hz, 1H, H-3) ppm

The compound **85** was characterized by ^{1}H-NMR technique, as shown in Table 31. The ^{1}H NMR spectrum indicated a methylene proton as a singlet δ at 6.00 ppm corresponding with ^{13}C NMR spectral data revealed a methylene carbon at 100.86 ppm.

Table 31 ^{1}H-NMR data of compound **85**

Compound	Physical property	m.p. (ºC)	Chemical shift (δ, ppm)
85	Yellow crystals	175.8-176.4	6.00 (s, 2H, H-12), 6.08 (d, J = 15.21 Hz, 1H, H-2), 6.76-6.88 (m, 3H, H-4,5,10), 6.90-6.98 (m, 1H, H-11), 7.03 (d, J = 1.60 Hz, 1H, H-7), 7.32-7.37 (m, 3H, H-5′,6′,7′), 7.61-7.63 (m, 1H, H-3), 8.09-8.15 (m, 1H, H-4′), 8.84 (s, 1H, H-2′) ppm

The compound **86** was characterized by ^{1}H-NMR technique, as shown in Table 32. The ^{1}H NMR spectrum indicated a methylene proton as a singlet δ at 5.97 ppm, an amide proton as a singlet δ at 3.74 ppm and the methylene protons of 2-bromobenzylamine , C-2′, as a doublet δ at 4.53 ppm corresponding with ^{13}C NMR spectral data revealed a methylene carbon at 101.32 ppm, an amide carbon at 166.06 ppm, and methylene carbon, C-2′, at 43.78 ppm.

Table 32 ^{1}H-NMR data of compound **86**

Compound	Physical property	m.p. (ºC)	Chemical shift (δ, ppm)
86	Yellow crystals	261.3-263.1	3.74 (br, 1H, NH), 4.53 (d, J = 5.60 Hz, 2H, H-2′), 5.93 (d, J = 14.81 Hz, 1H, H-2), 5.97 (s, 2H, H-12), 6.63-6.67 (m, 1H, H-4), 6.76-6.79 (m, 2H, H-5,10), 6.88 (d, J = 8.40 Hz, 1H, H-11), 6.97 (s, 1H, H-7), 7.27-7.34 (m, 3H, H-4′,5′,6′), 7.35-7.40 (m, 1H, H-3), 7.78 (d, J = 6.09 Hz, 1H, H-7′) ppm

The compound **87** was characterized by ^{1}H-NMR technique, as shown in Table 33. The ^{1}H NMR spectrum indicated a methylene proton as a singlet δ at 5.95 ppm, an amide proton as a doublet of doublet δ at 5.14 ppm and a methylene protons of allyl amine, C-2′, as a mutiplet δ at 5.82-5.92 ppm and C-4′ as a triplet δ at 3.98 ppm respectively, corresponding with ^{13}C NMR spectral data revealed a methylene carbon at 101.26 ppm, an amide carbon at 166.01 ppm, methylene carbons C-2′ and C-4′ at 42.03 and 116.41 ppm respectively.

Table 33 ^{1}H-NMR data of compound **87**

Compound	Physical property	m.p. (ºC)	Chemical shift (δ, ppm)
87	Slightly yellow crystals	181.8-182.5	3.98 (t, J = 5.76, 1.38 Hz, 2H, H-2′), 5.14 (dd, J = 8.87, 1.25 Hz, 1H, H-4′), 5.21 (dd, J = 17.15, 1.44 Hz, 1H, H-4′), 5.82-5.92 (m, 2H, NH, H-3′), 5.95 (s, d, J = 10 Hz, 3H, H-12,2), 6.63-6.71 (m, 1H, H-4), 6.74-6.77 (m, 2H, H-5,10), 6.86 (dd, J = 8.05, 1.49 Hz, 1H, H-11), 6.95 (d, J = 1.49 Hz, 1H, H-7), 7.36 (dd, J = 15, 10 Hz, 1H, H-3) ppm

The compound **88** was characterized by ^{1}H-NMR technique, as shown in Table 34. The ^{1}H NMR spectrum indicated a methylene proton as a singlet δ at 6.06 ppm and an amide proton as a singlet δ at 10.48 ppm corresponding with ^{13}C NMR spectral data revealed a methylene carbon at 101.38 ppm and an amide carbon at 164.88 ppm.

Table 34 ^{1}H-NMR data of compound **88**

Compound	Physical property	m.p. (ºC)	Chemical shift (δ, ppm)
88	Yellow crystals	171.5-172.4	6.06 (s, 1H, H-12), 6.28 (d, J = 14.92 Hz, 1H, H-2), 6.90-6.95 (m, 1H, H-4), 6.97-7.16 (m, 3H, H-5,10,11), 7.31 (d, J = 1.47 Hz, 1H, H-7), 7.35-7.41 (m, 1H, H-3), 7.61-7.69 (m, 2H, H-3′,6′), 8.42 (d, J = 5.27 Hz, 2H, H-4′,5′), 10.84 (s, 1H, NH) ppm

3.7 Biological activities

Antibacterial was determined by the paper disc method. The reference compound, gentamicin 0.75 mg/ml, exhibited clear zone values 2.3, 3.2, 1.8, and 2.4 cm (against *E. coli*, *S. aureus, P. aeruginosa* and *S. typhimurium*, respectively) and 100% DMSO. For the antibacterial assays, compounds **83, 84, 86, 87** and **88** against *E. coli* with clear zone values 0.8, 0.9, 1.0, 0.8 and 0.6 cm at 100, 100, 50, 10 and 10 ppm respectively, while all compounds were inactive to *S. aureus*. Compounds **84**, **86**, **87** and **88** showed against *P. aeroginosa*, values clear zone of 0.8, 0.7, 0.6 and 0.6 cm at 100, 10, 50, 50 and 10 ppm respectively. In addition, compounds **84, 86** and **88** were against *S. typhimurium* with clear zone values of 0.8, 0.9 and 0.8 cm at 10, 10, 100 ppm respectively.

Antifungal activity was assessed against *Candida albicans* and *Candida krusei* was determined by paper disc diffusion method. 100% DMSO and 0.75 mg/ml of gentamicin were used as a positive and a negative control respectively. For the antifungal assays, compounds **84, 86, 87** and **88** against *C. albicans* with clear zone values 1.0, 1.0, 0.7 and 1.2 cm at 50, 10, 50 and 50 ppm respectively, while these synthetic compounds were inactive to *C. krusei*. For the antioxidant assays, all compounds exhibited inactivity.

All these synthetic compounds were inactive antioxidant. Obviously, the compounds **84, 86, 87** and **88** are important for antibacterial and antifungal activities.

The biological activities of compounds **1, 5, 83, 84, 85, 86, 87** and **88** were shown in Table 35.

Table 35 The biological activities of compounds **1, 5, 83, 84, 85, 86, 87** and **88**

Compound	Conc. (ppm)	Size of zone inhibition (cm)					
		Antibacterial				Antifungal	
		E. coli	*S. aureus*	*P. aeroginosa*	*S. typhimurium*	*C. albicans*	*C. krusei*
1	10	-	-	-	-	-	-
	50	-	-	-	-	-	-
	100	-	-	0.7	-	-	-
5	10	-	-	-	-	-	-
	50	-	-	-	-	-	-
	100	-	-	-	-	-	-
83	10	-	-	-	-	-	-
	50	-	-	-	-	-	-
	100	0.8	-	-	-	-	-
84	10	-	-	0.8	0.8	-	-
	50	-	-	0.9	0.8	1	-
	100	0.9	-	1	0.8	1.1	-
85	10	-	-	-	-	-	-
	50	-	-	-	-	-	-
	100	-	-	-	-	-	-
86	10	-	-	-	0.9	1	-
	50	1	-	0.7	1	1	-
	100	1	-	0.8	1.1	1	-
87	10	0.8	-	-	-	-	-
	50	1	-	0.6	-	0.7	-
	100	1	-	0.7	-	0.8	-
88	10	0.6	-	0.6	-	-	-
	50	0.6	-	0.6	-	1.2	-
	100	1	-	0.6	0.8	1.3	-
DMSO	-	-	-	-	-	-	-
Gentamycin	-	2.3	3.2	1.8	2.4	-	-

Note

- Inactive