CHAPTER 2

LITERATURE REVIEW

Acetylcholine (ACh) is a neurotransmitter of many neuronal synapses. It is synthesized in nerve terminals from acetyl CoA and choline in a reaction catalysed by choline acetyltransferase (CAT) and stored in vesicle in the presynaptic neuron. When the nerve impulse arrives at the nerve ending, ACh, stored there in vesicles, is released and combines with a receptor molecule (muscarinic and nicotinic cholinergic receptor) in the postsynaptic membrane or the end-plate membrane of a muscle fibre [1]. A summary of ACh synthesis and degradation is shown in Figure 2.1

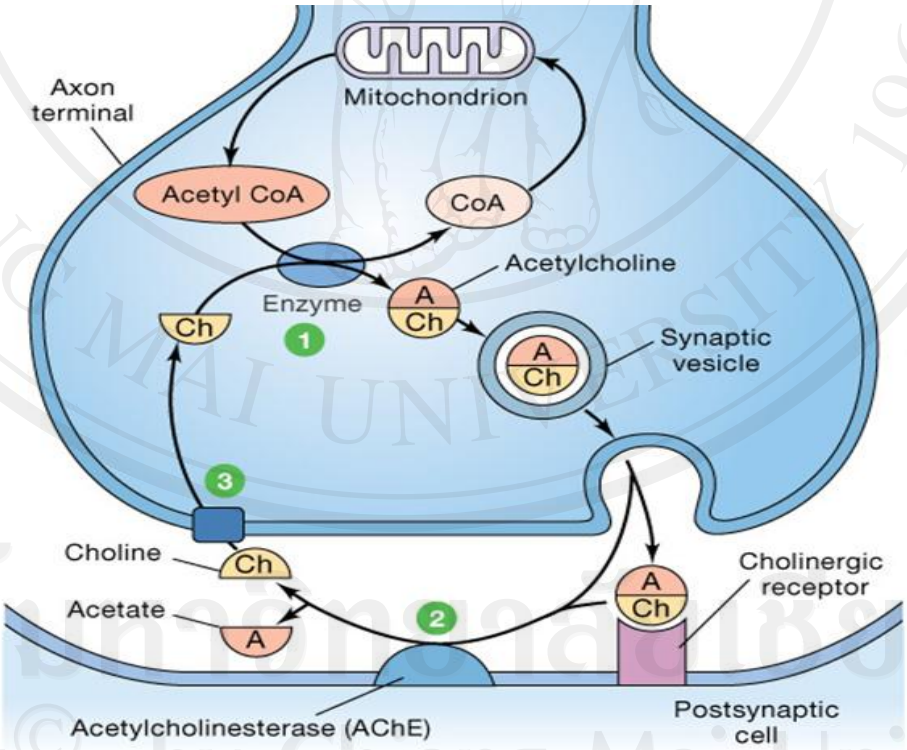


Figure 2.1 A summary of acetylcholine synthesis and degradation (http://faculty.pasadena.edu/dkwon/chap%208_files/textmostly/slide58.html)

Consequently, inhibition of acetylcholinesterase (AChE), the key enzyme in the breakdown of acetylcholine in the nervous system, enhances the accumulation of ACh in insects and causes hyper-stimulation of neuron, resulting in death. This property led to the development of inhibitors of this enzyme for the purposes for insecticides [2].

Acetylcholinesterase inhibitors (AChEI) are used as pharmaceuticals and as pesticides, especially against insects and other arthropod vertebrates. In medicine, AChEI are employed mostly for correcting the effects of insufficient levels of ACh. Inhibition of AChE serves as a strategy for the treatment of Alzheimer's disease (AD), senile dementia, ataxia, myasthenia gravis and Parkinson's disease [3]. In agriculture, AChEIs are used for the control of insects and some other arthropod pests. Insecticidal activity is based on the overstimulation of the cholinergic system in the insect and including muscular weakness and twitching, discontinued breathing, paralysis and death of insect.

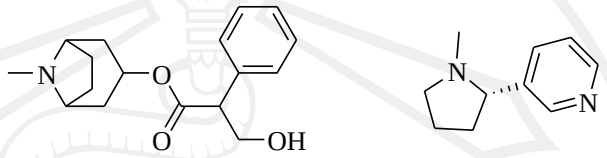
Several compounds have been reported AChE inhibitory activity such as alkaloids, flavonoids and xanthones. However, alkaloids are the most effective compounds.

2.1 Alkaloids

Alkaloids are a group of naturally occurring chemical compounds that contain mostly basic nitrogen atoms. Most alkaloids contain oxygen; those compounds are usually colorless crystals at ambient conditions and some alkaloids are colored such as berberine (yellow) and sanguinarine (orange). Alkaloids are weak bases and poorly soluble in water but readily dissolve in organic solvents. These compounds are produced by a large variety of organisms, including bacteria, fungi, plants, and animals, and are part of the group of natural products. Many alkaloids are toxic to other organisms. They often

have pharmacological effects and are used as medications and recreational drugs. The alkaloids are classified into the following major groups.

- **True (typical) alkaloids** that are derived from amino acids and have nitrogen in a heterocyclic ring such as atropine nicotine, and morphine as shown in Figure 2.2.



Atropine Nicotine

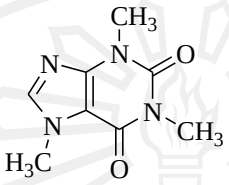
Figure 2.2 Structures of true alkaloids

- **Protoalkaloids** that are derived from amino acids and do not have nitrogen in a heterocyclic ring such as ephedrine and adrenaline as shown in Figure 2.3.

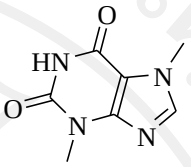
Ephedrine Adrenaline

Figure 2.3 Structures of protoalkaloids

- **Pseudo alkaloids** that are not derived from amino acids but have nitrogen in a heterocyclic ring such as caffeine and theobromine as shown in Figure 2.4.



Caffeine



Theobromine

Figure 2.4 Structures of pseudo alkaloids

Additionally, alkaloids are divided base on the chemical structure into 2 groups [4], as shown in Figure 2.5:

I. Non-heterocyclic alkaloids,

II. Heterocyclic alkaloids, divided into 14 groups according to their ring structure.

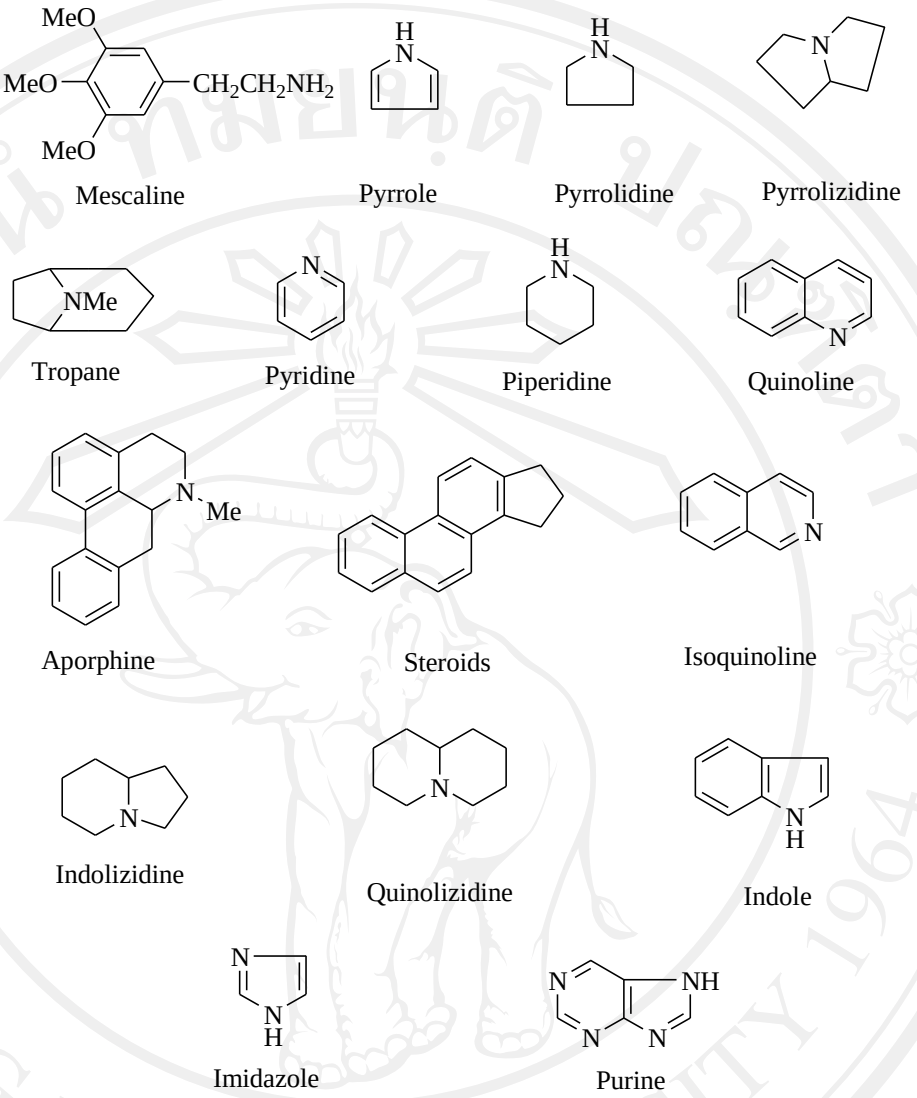
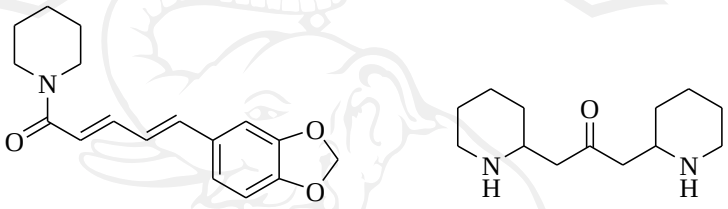


Figure 2.5 Some core structures of alkaloids.

Some alkaloids have apparently pharmaceuticals properties such as piperidine, pyrrolidine, pyrrolizidine, pyridine, quinoline and indole.

Piperidine is true alkaloids. The structure of this compound consists of a six-membered ring containing five methylene units and one nitrogen atom. Piperidine has been obtained from black pepper. Piperidine is also commonly used in chemical degradation reactions, such as the sequencing of DNA in the cleavage of particular modified nucleotides. Several alkaloids are in piperidine alkaloids such as lobeline, anaferine and piperine as shown in Figure 2.6.



Piperine Anaferine

Figure 2.6 Structures of some piperidine alkaloids

Pyrrolidine is a cyclic secondary amine with a five-membered ring containing four carbon atoms and one nitrogen atom. Pyrrolidine found naturally in the leaves of tobacco and carrot. The pyrrolidine ring structure is present in numerous natural alkaloids such as nicotine and hygrine. Nicotine used in medicine and as an insecticide as shown in Figure 2.7.

Nicotine Hygrine

Figure 2.7 Structures of some pyrrolidine alkaloids

Pyrrolizidine is known to be toxic for humans and animals. The most important toxicological feature in vertebrates is their hepatotoxicity and pneumotoxicity). Furthermore, pyrrolizidine alkaloids exhibit a substantial mutagenicity, carcinogenicity and embryotoxicity and a weak virustatic and antileucemic activity. Many alkaloids have pyrrolizidine ring in the structure such as laburnine and heliotridine as shown in Figure 2.8.

Laburnine Heliotridine

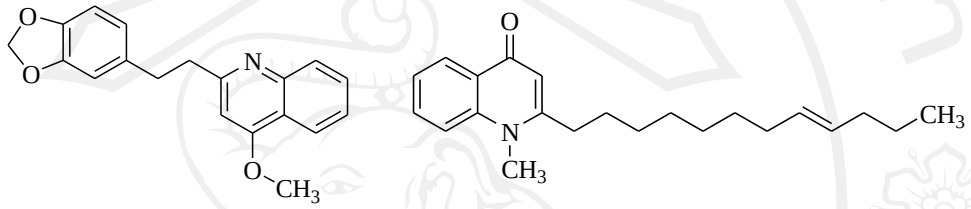
Figure 2.8 Structures of some pyrrolizidine alkaloids

Pyridine is a basic heterocyclic organic compound. The structure of pyridine is related to benzene, with one C-H group replaced by a nitrogen atom. Pyridine is found among the volatile components of black tea and in the leaves and roots of *Atropa belladonna* [5]. Pyridine is widely used as a solvent in organic chemistry and in industrial practice. Pyridine is an effective, basic solvent that is relatively unreactive, which makes it a good acid scavenger. Several alkaloids are in pyridine alkaloids such as nicotine, anabasine and actindine as shown in Figure 2.9.

Anabasine Actinidine

Figure 2.9 Structures of some pyridine alkaloids

Quinoline is a colourless hygroscopic liquid with a strong odour. This compound is mainly used as a building block to other specialty chemicals and used in manufacturing of dyes, preparation of hydroxyquinoline sulfate and niacin. In 1975, Vitzthum *et.al* found that quinoline in the volatile components of black tea [5]. The quinoline ring structure is present in numerous natural alkaloids such as cusparine and evocarpine as shown in Figure 2.10.



Cusparine evocarpine

Figure 2.10 Structures of some quinoline alkaloids

Indole is an aromatic heterocyclic organic compound the structure of indole alkaloids consisting of a six-membered benzene ring fused to a five-membered nitrogen-containing pyrrole ring. Indole is a popular component of essential oils and the substrate to many pharmaceuticals. In 1997, Porter *et.al* reported that tree indole alkaloids were reported from cultures of *Balansia epichloe* (Weese) [6]. Several alkaloids are in indole alkaloids such as physostigmine and bufotenin. Physostigmine were used in treatment of Alzheimer's disease and glaucoma as shown in Figure 2.11.

Physostigmine Bufotenin

Figure 2.11 Structures of some indole alkaloids

2.2 AChE inhibitory activity of alkaloids

Many investigations have described the AChE inhibition of alkaloids. Roddick (1989) have reported that steroidal glycoalkaloids α-solanine and α-chaconine from *Solanum* sp. significantly inhibited bovine and human acetylcholinesterase at a concentration of 100 μM [7].

In addition, Lopez *et al*., (2002) studies AChE inhibitory activity of alkaloids from *Amaryllidaceae* family found that seven alkaloids, sanguinine, galanthamine, 11-hydroxygalanthamine, epinorgalanthamine, oxoassoanine, assoanine and pseudolycorine, exhibited AChE inhibitory activity with IC_{50} values of 0.10 ± 0.01, 1.07± 0.18, 1.61 ± 0.21, 9.06 ± 0.65, 47.21± 1.13, 3.87 ± 0.24, 152 ± 32.06 [μM], respectively [8].

Kim *et al.* (2002) indicated corynoline, an alkaloids from extract of methanolic extract of the aerial parts of *Corydalis incisa* (Papaveraceae), had significant inhibitory effects on AChE with the IC_{50} value 30.6 μM [9]. In 2004, four isoquinoline alkaloids, corynoxidine, protopine, palmatine, and berberine have been isolated from the methanolic extract of the aerial parts of *Corydalis speciosa*. The IC_{50} values of corynoxidine, protopine, palmatine, and berberine were 89.0, 16.1, 5.8, and 3.3 μM, respectively [10].

On the other hand Andrade *et al.* (2005) isolated ten alkaloids, coronaridine, voacangine, voacangine hydroxyindolenine, rupicoline, ibogamine, ibogaine, ibogaline, desethyl-voacangine, voachalotine, and affinisine, from the chloroform extract of stalk of *Tabernaemontana australis* and these compounds showed AChEI activity at the same concentration with physostigmine and galanthamine (detection limit of 0.01 mM) by TLC assay using the modified Ellman's method [11].

Moreover, Langjae *et al.* (2007) reported that new stigmastane-type steroidal alkaloid, 4-acetoxy-plakinamine B, was isolated from the Thai sponge *Corticium* sp. and showed AChE inhibitory with IC_{50} values of 3.75±1.69 µM [12].

Additionally, many investigations have described some *Stemona* alkaloids and synthetic compounds exhibited AChE inhibitory activity. Wang *et al.* (2007) isolated alkaloids from the roots of *Stemona sessilifolia* (*S. sessilifolia*) and found that two new *Stemona* alkaloids, sessilistemonamines A and sessilistemonamines B which were moderately active on AChE with IC_{50} values of 68.8±9.5 and 17.1 ± 2.5 µM, respectively [13].

Baird *et al.* (2009) indicated that (11Z)-1′,2′-didehydrostemofoline from an unidentified *Stemona* sp. showed the highest inhibitory activity of AChE with a minimum inhibitory requirement (MIR) of 5 ng, followed by, (3′*S*)-hydroxystemofoline with a MIR of 10 ng. Whereas, methylstemofoline had a significantly higher activity than its corresponding *E*-isomer, (11*E*)-methylstemofoline with MIR values of 100 and 500 ng, respectively [14].

Mungkornasawakul *et al.* (2009) studied the AChE inhibitory activity of the root extract of *S. aphylla* and found that the crude extract had higher activity than stemaphylline and stemaphylline-*N*-oxide [15].

Sastraruji *et al.* (2010) reported the semisynthesis of the four known *Stemona* alkaloids i.e. oxystemofoline, methoxystemofline, (1´*R*)-hydroxystemofoline and (1´*S*)-hydroxystemofoline. In a TLC bioautographic assay, (1´*R*)-hydroxystemofoline was found to be the most active AChE inhibitor with a MIR of 5 ng, while (1´*S*)-hydroxystemofoline exhibited slightly weaker inhibitory activity with a MIR 10 ng [16].

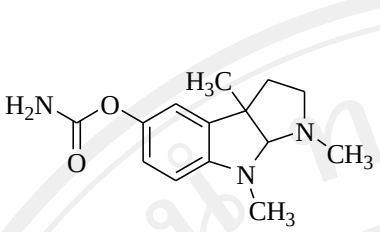
Chaiyong *et al* (2010) found that the alkaloids from *S.curtisii*, stemocurtisine, stemocurtisinol, oxyprotostemonine and stemocurtisine *N*-oxide were relatively inactive as AChE inhibitors, with MIR of 500-1000 ng [17].

Sastraruji *et al* (2011) isolated a new stemofoline alkaloid, (2´*S*)-hydroxy-(11*S*,12*R*)-dihydrostemofoline and known compounds stemofoline and (2´*S*)-hydroxystemofoline from the root extracts of *S. aphylla*. Stemofoline and (2´*S*)-hydroxystemofoline were the most active with MIR of 10 ng [18].

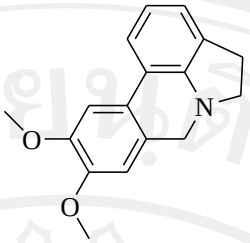
Summary of alkaloid compounds having significant AChE inhibitory activity is provided in the Table 2.1 and the structures of these compounds are shown in Figure 2.12 [19].

Table 2.1 Alkaloids having acetylcholinesterase inhibitory activity

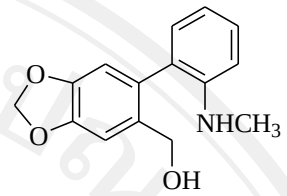
Name of alkaloids	Class	Sources	Activity	Reference
Assoanine	Steroidal alkaloid	*Narcissus assoanus*	50% inhibition at 3.87±0.24 μM	[8]
Buxamine B	Steroidal alkaloid	*Bucus hyrcana Bucus papillosa*	50% inhibition at 7.56±0.008 μM	[19]
Coronaridine	Indole alkaloid	*Tabernaemontana australis*	Minimum concentration of 25 μM to produce detectable spot in TLC	[19]
N, *N*-dimethyl buxapapine	Steroidal alkaloid	*Bucus papillosa*	50% inhibition at 7.28±0.06 μM	[10]
Epinorgalantamine	Steroidal alkaloid	Narcissus confuses N. perezchiscanoi *Narcissus leonensis N. legionensis Narcissus poeticus*	50% inhibition at 9.60±0.65 μM	[9]
Galantamine	Steroidal alkaloid	Galanthus nivalis *Narcissus confuses Lycorus radiate*	50% inhibition at 1.07±0.18 μM	[19]
(-)-Huperzine A	Quinolizidine alkaloid	*Huperzia serrata Huperzia dalhousieana*	50% inhibition at 10^{-4} μM	[19]
11-Hydroxygalantamine	Steroidal alkaloid	*Narcissus poeticus*	50% inhibition at 1.61±0.21 μM	[8]
Oxoassoanine	Steroidal alkaloid	*Narcissus assoanus*	50% inhibition at 47.21±1.13 μM	[8]
Physostigmine	Indole alkaloid	*Physostigma venenosum*	50% inhibition at $6x10^{-4}$ μM	[19]
Sanguinine	Steroidal alkaloid	*Eucharis grandiflora*	50% inhibition at 0.10±0.01 μM	[8]
Sarsalignone	Steroidal alkaloid	*Sarcococca saligna*	50% inhibition at 7.023±0.007 μM	[19]
Vaganine	Steroidal alkaloid	*Sarcococca saligna*	50% inhibition at 8.59±0.155 μM	[19]
Voacangine	Indole alkaloid	*Tabernaemontana australis*	Minimum concentration of 25 μM to produce detectable spot in TLC	[10]



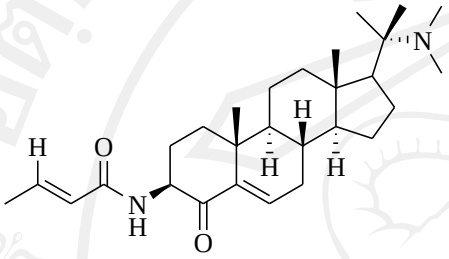
Physostigmine



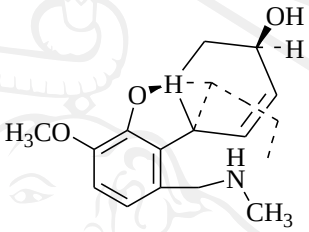
Assoanine



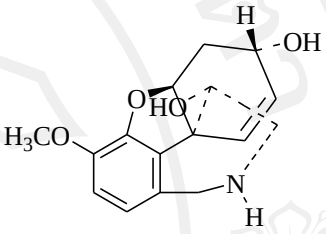
Oxoassoanine



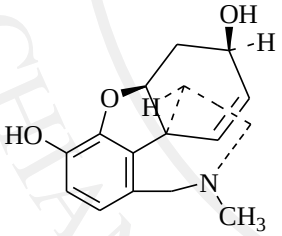
Sarsalignone



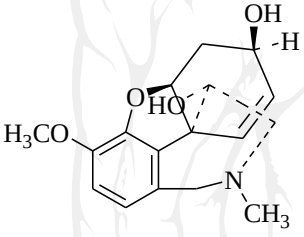
Galantamine



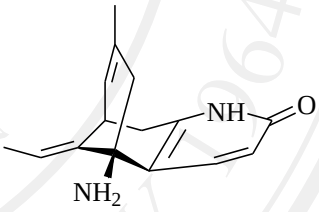
Epinorgalantamine



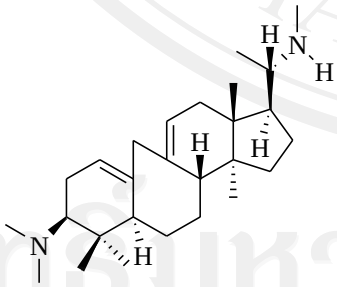
Sanguinine



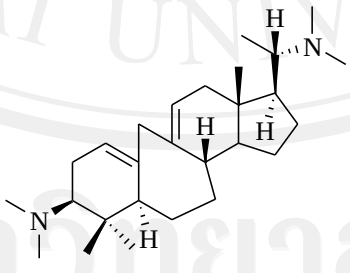
11-Hydroxygalantamine



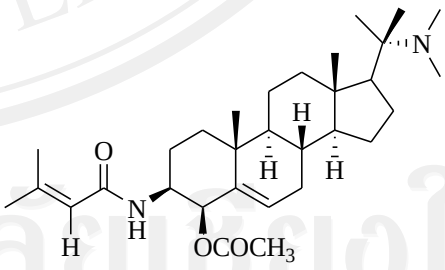
(-)-Huperzine A



Buxamine B



N,*N*-Dimethyl buxapapine



Vaganine

Figure 2.12 Structures of some acetylcholinesterase inhibitors obtained from plants.

2.3 Other AChEIs

Several investigations have reported non-alkaloid compounds can inhibit AChE. Brühlmann *et al* (2004) screened of non-alkaloidal natural compounds as AChEI found that six of seven compounds active for AChEI xanthones. This report is the first that a promising potential for AChE inhibition exists in such non-nitrogenous natural compounds [20].

In the same time, Urbain *et al* isolated four xanthones, bellidin, bellidifolin, bellidin 8-*O*-β-glucopyranoside (norswertianolin), and bellidifolin 8-*O*-β-glucopyranoside (swertianolin) from methanol extract of the leaves of *Gentiana campestris* and studies AChEI activity by TLC bioautographic method. The result showed that, bellidifolin exhibited similar activity to galanthamine in this method [21].

Moreover, In 2007 Jung *et al* isolated four flavonoid, tiliroside, 3-methoxy quercetin, quercitrin and quercetin, from ethyl acetate extract of whole plants of *Agrimonia pilosa ledeb* and studied AChEI activity. This report is the first time that all four flavonoids showed significant inhibitory effects on AChE. Tiliroside, 3-methoxy quercetin, quercitrin and quercetin exhibited AChE inhibitory activity with IC_{50} values of 23.5, 37.9, 66.9 and 19.8 μM, respectively [22].

In addition, In 2009 Khan *et al* examined *in vitro* AChE and butyrylcholinesterase (BChE) inhibitory activities of four flavonoid derivatives,quercetin, rutin, kaempferol 3-*O*-β-d-galactoside and macluraxanthone. Macluraxanthone showed to be the most potent and specific inhibitor of both the enzymes having the IC_{50} values of 8.47 and 29.8 μM, respectively [23].

2.4 AChEIs assay

Several investigation of AChEIs assay to find a new inhibitor is important such as assay base on colorimetric methods, assay base on fluorimetric methods, assay base on a radiometric method and assay base on mass spectrometric detection. Assay base on colorimetric methods is suitable for screening natural product compounds can inhibit acetylcholinesteras. These assays consist of TLC assay and HPLC assay. TLC assay divided into 2 methods; Ellman's method and TLC bioautography [24].

Ellman's method

The samples are spotted on the TLC plate before standard development. A solution of 5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB) and Acetylthiocholine (ATCI) is first sprayed until the silica is saturated with the solvent and then an enzyme solution is applied. A yellow coloration should appear after about 5 min with white spots for inhibitory compounds. The reaction of this method as shown in Figure 2.13. This provides an extremely rapid method to screen large numbers of samples to discover new inhibitors of AChE. However, this method is known to give a number of false-positive effects. In order to prove that the spots are effectively due to an inhibition of the enzyme and not to a chemical reaction between DTNB and thiocholine, AChE was premixed with ATCI to form thiocholine. The TLC layer was first sprayed with a solution of DTNB and then with thiocholine. The white spots observed were compared to those recorded for the enzyme inhibition.

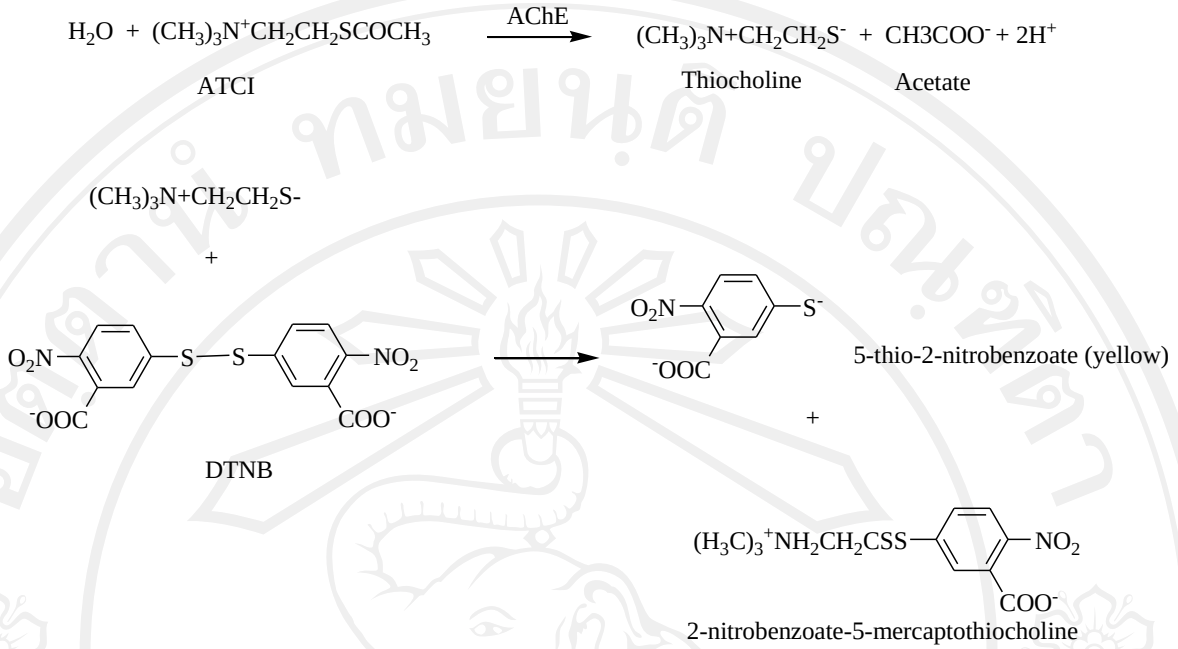


Figure 2.13 The reaction of Ellman's method

TLC bioautography

TLC bioautography is suitable for small amount of sample. This method is based on the reaction of AChE with a-naphthyl acetate and the subsequent formation of a purple dye with a-naphthol and Fast Blue Salt B as shown in Figure 2.14. The samples are spotted on the plate before standard development. An enzyme solution is sprayed first on the plate. It is then incubated at 37°C for 20 min before spraying a mixture of a-naphthyl acetate and Fast Blue Salt B. A purple coloration appears after 1–2 min and the inhibitory compounds are shown by white spots on the plate. The inhibition is easier to see with this method given that the contrast with the background is stronger than when using Ellman's method.

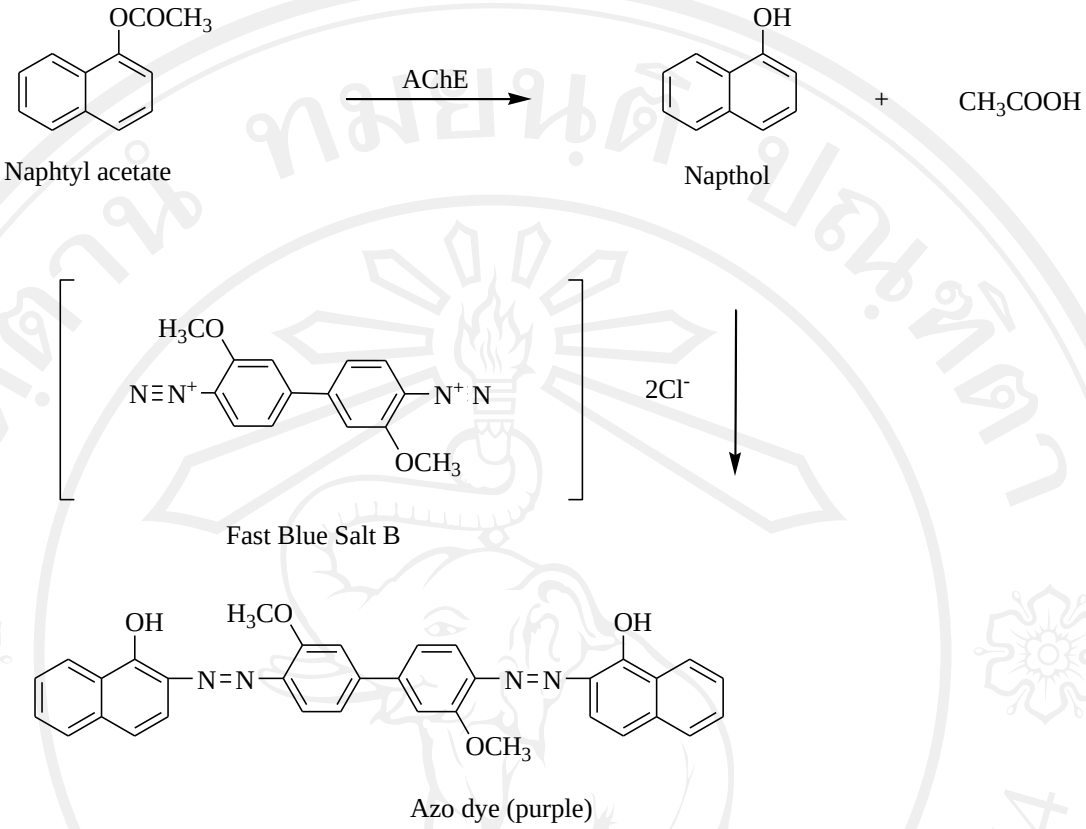


Figure 2.14 Reaction of AChE with naphthyl acetate and subsequent formation of a purple dye.

2.5 *Dasymaschalon*

Annonaceae is a large family (about 120 genera and more than 2000 species) found in tropical regions, and about 50 species of Annonaceae were used in folk medicine. A number of isolated natural compounds from Annonaceae exhibit potent biological activities such as cytotoxic, antitumor, antiparasitic, antimicrobial, antiplatelet aggregation and immunosuppressive activities [25].

Dasymaschalon is a small genus in the family of Annonaceae. About 40 species in the genus *Dasymaschalon* are widely distributed throughout Southeast Asia, particularly in

Thailand and Malaysia Peninsular. Twelve species, *D. acuminatum, D. angustifolium, D. dasymaschalum, D. echinatum, D. filipes, D. glaucum, D. grandiflorum, D. lomentaceum, D. macrocalyx, D. obtusipetalum, D. sootepense,* and *D. wallichii*, were reported in Thailand. *Dasymaschalon* is known in Thailand as "bu rong." The chemical constituents of the plants in this genus have been reported to contain various types of secondary metabolites which are alkaloids, acetogenins, xanthones, flavonoids and flavonol glycosides. Some of the compounds are promising anti-cancer and cancer chemopreventive agents [25].

There are many reports described the phytochemicals and their biological activity were found in this genus.

2.5.1 *D. sootepense*

Morphology: Small trees, to 10 m tall, distribute in Northern Thailand (Chiang Mai and Uttaradit Provinces) and China (Yunnan)

Phenology: Available data are sparse. Flowering specimens collected in April and June; fruiting specimens collected in February, June, September and October.

Thai Vernacular Name: Bu-rong Suthep [26]

In 1998, Sinz *et al* isolated acetogenins , sootepensin A , sootepensin B, tonkinin C and tonkinesin C from the leaves of *D. sootepense*. The result showed that four acetogenins were found to be highly cytotoxic against the L1210 tumor cell line [27]. Then, two flavonol glycosides have been isolated from a methanolic extract of the leaves of *D. sootepense* [28].

In 2008, Hongtong *et al* have reported that a new phenanthrene alkaloid namely, 7-hydroxydehydrodicentrine, along with five known alkaloids, nordicentrine, dicentrinone,

sinactine, epiberberine and aristolactam AII were isolated from the ethyl acetate fraction partitioned from the methanol extract of the leaves and twigs of *D. sootepense*, the structure of these compounds are shown in Figure 2.15 [29].

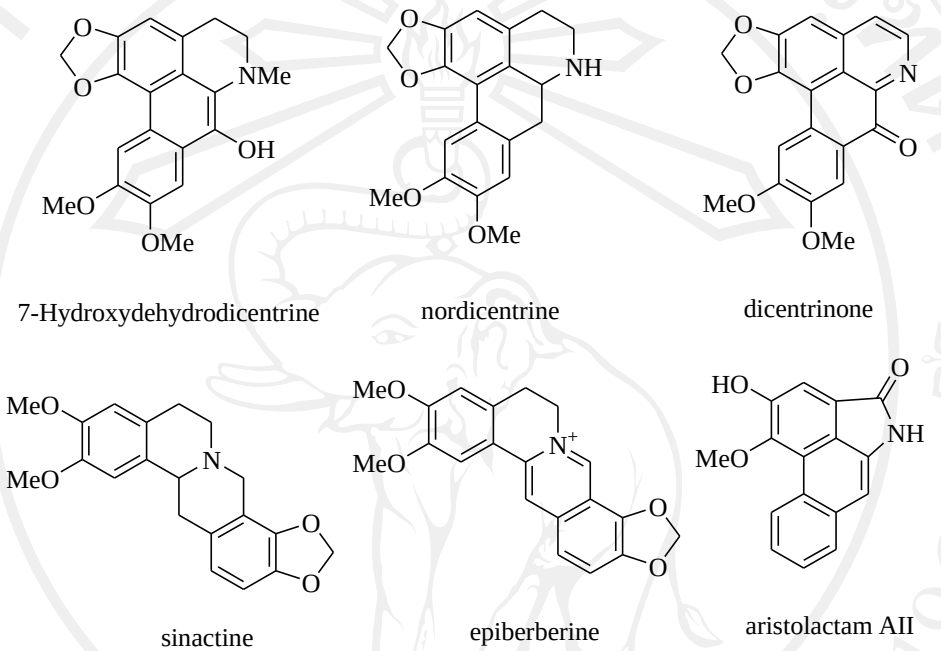


Figure 2.15 Some alkaloids structure from the leaves and twigs of *D. sootepense*

2.5.2 *D. blumei*

Chanakul *et al* (2011) isolated four aristololactam alkaloids, including the hitherto unknown 3,5-dihydroxy-2,4-dimethoxyaristolactam, as well as the three known compounds, aristolactam BI, goniopedaline, and griffithinam from the stems of *D. blumei* and oxoaporphine alkaloids, oxodiscoguattine, dicentrinone, and duguevalline from the combined leaves and twigs of the same plant. All isolates were evaluated for cytotoxicity against a panel of mammalian cancer cell lines and a noncancerous human embryonic

kidney cell Hek 293. The structures of alkaloids from *D. blumei* are shown in Figure 2.16 [30].

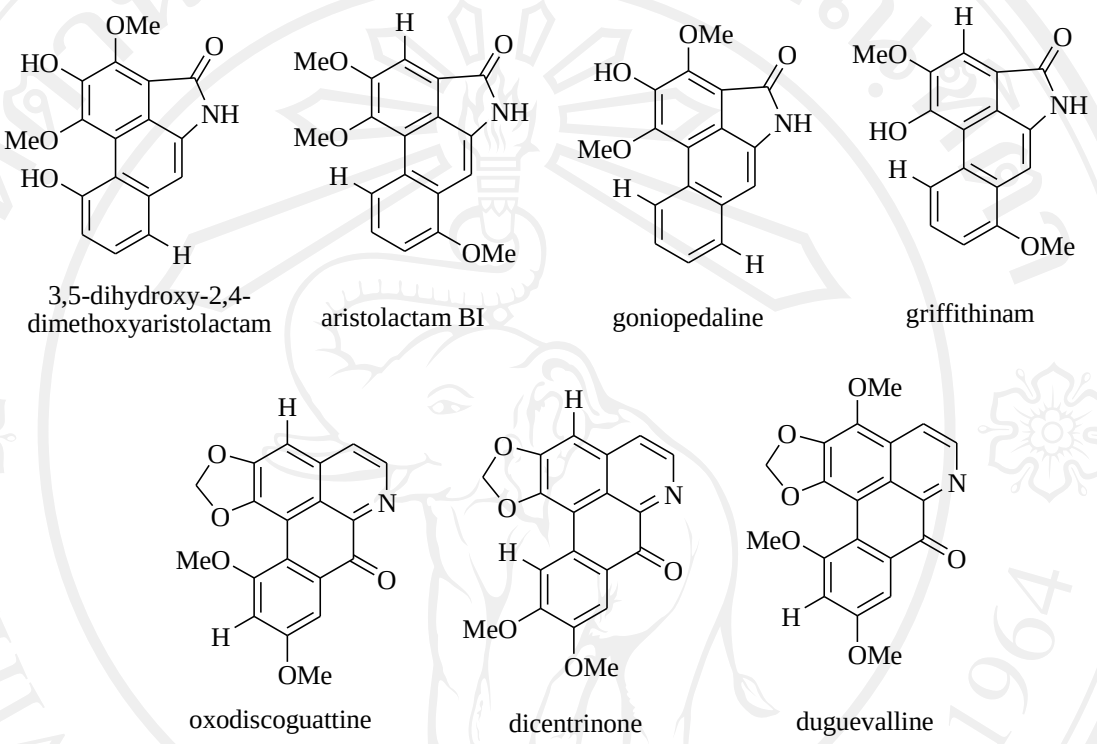


Figure 2.16 Alkaloids structures from the stems of *D. blumei*

2.5.3 *D. glaucum* [26]

Morphology: Small trees, to 8 m tall, 13 cm d.b.h. Young branches glabrous to hairy. Leaf laminas 10.5–21 cm long, 4–8 cm wide. Flowers on young growth; flowering pedicels 17–19 mm long, 1–1.6 mm in diameter, sparsely hairy. Sepals 2.5–3 mm long, 2.5–3 mm wide, triangular, 0.2–0.5 mm thick, glabrous adaxially, hairy abaxially. Petals connivent at maturity, 26–42 mm long, 8–10 mm wide, densely hairy abaxially, purplish-red; floral chamber ca. 24 mm long. Stamen number unknown, 1.6–2 mm long;

connectives truncate to rounded, 0.3–0.6 mm long, 0.6– 1.1 mm wide, glabrous; pollen exine echinate. Carpel number unknown; ovaries 1.6–2 mm long, 0.5–0.6 mm wide, Fruiting pedicels 7–55 mm long, 1–3.1 mm wide, glabrous to sparsely hairy. Monocarps 6–28 per fruit, 16–55 mm long, 5–7.5 mm wide, ellipsoid (1-seeded) or moniliform (> 2-seeded), glabrous to sparsely hairy, red or brown; constrictions 1.2–3.5 mm wide. Seeds 1–6 per monocarp, 6.5–13 mm long, 4.8–7 mm wide, length/width ratio 1.6–2.3, ellipsoid, yellow. Morphology of flower and fruits of *D. glaucum* as shown in Figure 2.15.

Distribution and Habitat: Northern, North-Eastern and South-Western Thailand (Chiang Mai, Nong Khai, Prachuap Khiri Khan and Ratchaburi Provinces), China (Hainan, Guangxi) Laos, and Vietnam. Montane forests over limestone and sandstone; 200–2400 m. The geographical distributions of *D. glaucum* in Thailand and neighboring regions as shown in Figure 2.17.

Phonology: Flowering in May and June; fruiting between May and December.

Thai Vernacular Name: Bu-rong Bai Nuan.

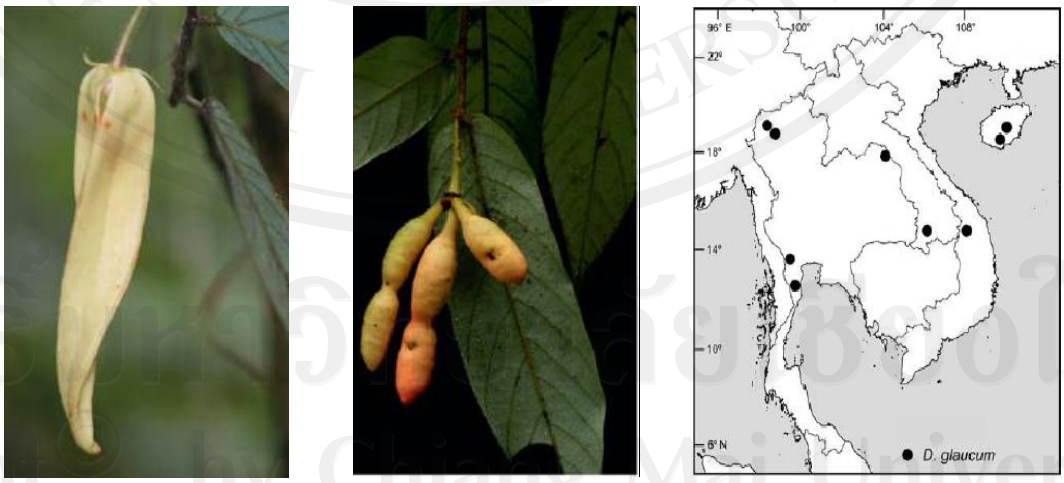
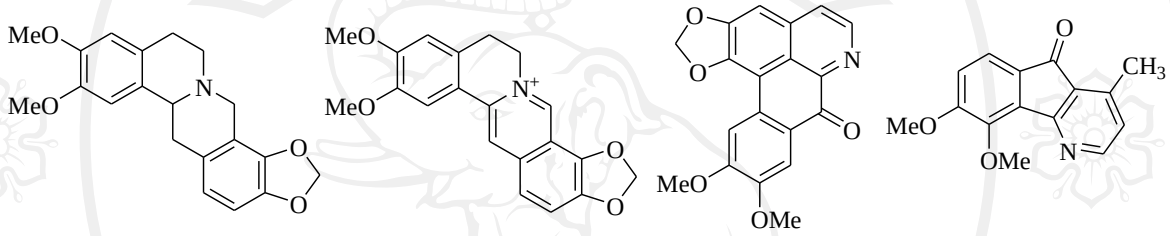


Figure 2.17 Flower and fruits of *D. glaucum* and geographical distributions of *D. glaucum* in Thailand and neighboring regions [26].

In previous studies, Karntanakrit *et al* (2011) were found four known alkaloids in the methanol extract from leaves and twigs of *D.glaucum*, tetrahydroepiberberine, epiberberine , dicentrinone and isoursuline, the structure of these compounds are shown in Figure 2.18 [25].



tetrahydroepiberberine epiberberine dicentrinone isoursuline

Figure 2.18 Structures of alkaloids from the leaves and twigs of *D.glaucum*