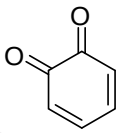
CHAPTER 1

INTRODUCTION

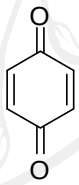
1.1 Quinone

A quinone is a class of organic compounds that are formally derived from aromatic compounds, such as benzene or naphthalene by conversion of an even number of –CH= groups into –C(=O)– groups with any necessary rearrangement of double bonds, resulting in a fully conjugated cyclic dione structure.[1] The class includes some heterocyclic compounds. The prototypical member of the class are 1,4-benzoquinone or cyclohexadienedione, often called simply quinine. Other important examples are 1,2-benzoquinone (*ortho*-quinone), 1,4-naphthoquinone and 9,10-anthraquinone (Figure 1.1). Quinones are oxidised derivatives of aromatic compounds and are often readily made from reactive aromatic compounds with electron-donating substituents such as phenols and catechol, also known as pyrocatechol or 1,2-dihydroxybenzene which increase the nucleophilicity of the ring and contributes to the large redox potential needed to break aromaticity (Quinones are conjugated but not aromatic). Quinones are electrophilic Michael acceptors stabilised by conjugation. Depending on the quinone and the site of reduction, reduction can either rearomatise the compound or break the conjugation. Conjugate addition nearly always breaks the conjugation. Derivatives of quinones are common constituents of biologically relevant molecules. Some serve as electron acceptors in electron transport chains such as those in photosynthesis

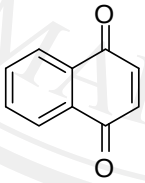
(plastoquinone, phylloquinone), and aerobic respiration (ubiquinone). Phylloquinone is also known as Vitamin K_1 as it is used by animals to help form certain proteins, which are involved in blood coagulation, bone formation and other processes. A natural example of the oxidation of hydroquinone to quinone is in the spray of bombardier beetles; hydroquinone is reacted with hydrogen peroxide to produce a fiery blast of steam, a strong deterrent in the animal world[2] and natural or synthetic quinones show a biological or pharmacological activity, and some of them show antitumoral activity possess a number of biological properties, including some claims in herbal medicine. These applications include purgative, antimicrobacterial, anti-tumor, inhibition of PGE2 (Prostaglandin E2) and anti-cardiovascular disease.[3]



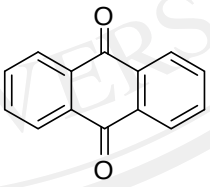
1,2-Benzoquinone (**1**)



1,4-Benzoquinone (**2**)



1,4-Naphthoquinone (**3**)



9,10-Anthraquinone (**4**)

Figure 1.1 Structure of quinones

1.1.1 Naphthoquinone

Napthoquinone is a class of natural phenols based on the C6-C4 skeleton. This double ketone (quinone) is a reactive metabolite of naphthalene and

given the derivatives of naphthalene are 1,2-napthoquinone, 1,4-naphthoquinone and 2,6-naphthoquinone.

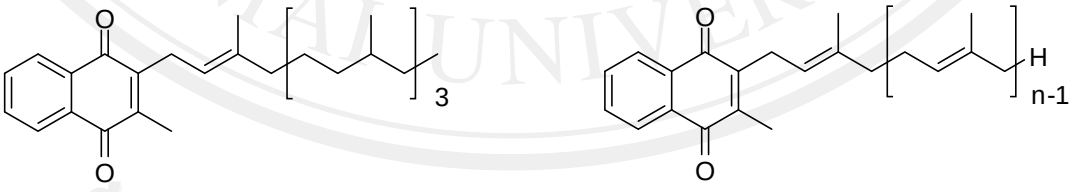
1. 1,2-Napthoquinone

1,2-Naphthoquinone or ortho-naphthoquinone is a polycyclic aromatic organic compound with formula $C_{10}H_6O_2$ and is found in diesel exhaust particles. The accumulation of this toxic metabolite in rats from doses of naphthalene has been shown to cause eye damage, including the formation of cataracts.[4]

2. 1,4-Naphthoquinone

1,4-Naphthoquinone forms yellow triclinic crystals and has an odor similar to benzoquinone. It is sparingly soluble in cold water, slightly soluble in petroleum ether, and freely soluble in most polar organic solvents. In alkaline solutions it produces a reddish-brown color. Because of their aromatic stability, 1,4-naphthoquinone derivatives are known to possess anti-bacterial and anti-tumor properties. Naphthoquinone forms the central chemical structure of many natural compounds, most notably the Vitamin K is a group of structurally similar, fat soluble vitamins that are needed for the posttranslational modification of certain proteins required for blood coagulation and in metabolic pathways in bone and other tissue. They are 2-methyl-1,4-naphthoquinone derivatives. This group of vitamins includes two natural vitamers: vitamin K_1 and vitamin K_2 (Figure 1.2). Vitamin K_1 is also known as vitamin K_j, phylloquinone or phytomenadione (also called phytonadione). Vitamin K_1 is required for blood coagulation and is synthesized by plants, is found in green leafy vegetables, and can be found in

soybean oil. Vitamin K_2 is involved in bone metabolism. Vitamin K_2 homologs (menaquinones) are characterized by the number of isoprenoid residues comprising the side chain. Menaquinones are abbreviated MK-n, where n represents the number of isoprenoid side chains. Thus, menaquinone-4 abbreviated MK-4, has 4 isoprene residues in the side chain. Bacteria can produce a range of vitamin K_2 forms, including the conversion of K_1 to K_2 (MK-7) by bacteria in the small intestines. No known toxicity exists for vitamins K_1 and K_2. Vitamin K_2 (menaquinone). In menaquinone the side chain is composed of a varying number of isoprenoid residues.Vitamin K_1 (phylloquinone). Both forms of the vitamin contain a functional naphthoquinone ring and an aliphatic side chain. Phylloquinone has a phytyl side chain. Naphthoquinone derivatives have significant pharmacological properties. They are cytotoxic, they have significant antibacterial, antifungal, antiviral, insecticidal, anti-inflammatory, and antipyretic properties. Plants with naphthoquinone content are widely used in China and the countries of South America, where they are used to treat malignant and parasitic diseases.[5,6]



Vitamin K1 (phylloquinone) (**5**) Vitamin K2 (menaquinone) (**6**)

Figure 1.2 Structure of Vitamin K_1 and Vitamin K_2

1.1.2 Hydroxy-1,4-naphthoquinone

A hydroxynaphthoquinone (formula: $C_{10}H_6O_3$) is any of several organic compounds that can be viewed as derivatives of a naphthoquinone through replacement of one hydrogen atom (H) by a hydroxyl group (-OH). In general, the term may mean any naphthoquinone derivative where any number *n* of hydrogens have been replaced by *n* hydroxyls, so that the formula is $C_{10}H_6O_{2+n}$. In this case the number *n* (which is between 1 and 6) is indicated by a multiplier prefix (mono-, di-, tri-, tetra-, penta-, or hexa-). The unqualified term "hydroxynaphthoquinone" usually means a derivative of 1,4-naphthoquinone. Other hydroxy- compounds can be derived from other isomers of the latter, such as 1,2-naphthoquinone and 2,6-naphthoquinone. The IUPAC nomenclature uses dihydronaphthalenedione instead of "naphthoquinone", with the necessary prefixes to indicate the positions of the carbonyl oxygens (=O) — as in 5,8-dihydroxy-1a,8a-dihydronaphthalene-1,4-dione (= 5,8-dihydroxy-1,4-naphthoquinone). The hydroxynaphtoquinones (in the particular or the general sense) include many biologically and industrially important compounds, and are a building-block of many medicinal drugs.[7-9]

1. 2-Hydroxy-1,4-naphthoquinone (Lawsone)

Lawsone is the phytochemical constituent of henna leaves, also known as hennotannic acid, is a red-orange dye present in the leaves of the henna plant (*Lawsonia inermis*) as well as jewelweed (*Impatiens balsamica*).[10] (and hence henna powder) that is responsible for creating the henna stain. The higher the lawsone content of the henna leaves, the deeper the resulting stain produced by the henna powder will be. On average the lawsone content of ‘good quality’ henna

powder is somewhere between 1% and 2%. The higher the temperatures where the henna is cultivated is directly proportionate to the lawsone content of the leaves. The higher the temperature, the higher the lawsone content percentage. This is why it is probably no surprise that the best quality henna powders come from some of the hottest regions of the world, such as tropical and subtropical regions of Africa, southern Asia, and northern Australia. Humans have used henna extracts containing lawsone as hair and skin pigments for more than 5000 years. Henna for body art has enjoyed a recent renaissance due to improvements in cultivation, processing, and the emigration of people from traditional henna-using regions.[11] Henna also acts as an anti-fungal[12] and a preservative for leather and cloth. The flowers of lawsone have been used to create perfume since ancient times. In an acidic solution, lawsone can react via Michael addition with the protein keratin in skin and hair, resulting in a strong permanent stain that lasts until the skin or hair is shed. Lawsone strongly absorbs UV light, and aqueous extracts can be effective, sunless tanning sunscreens. Chemically, lawsone is similar to juglone, which is found in walnuts.[13]

2. 5-Hydroxy-1,4-naphthoquinone (juglone)

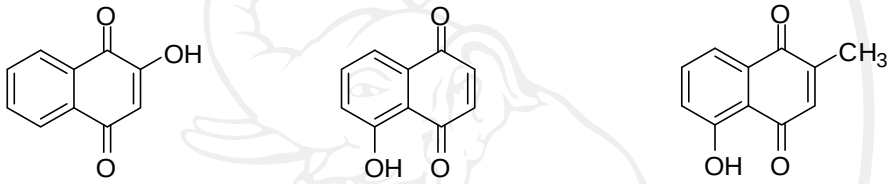
Juglone is an aromatic compound, formally derived from 1,4-naphthoquinone through the replacement of one hydrogen atom by a hydroxyl (OH) group. It is an isomer of lawsone with the molecular formula $C_{10}H_6O_3$. It is insoluble in benzene but soluble in dioxane, from which it crystallizes as yellow needles that melt at 162–163 °C.[14] Juglone is found naturally in the leaves, roots and bark of plants in the Juglandaceae family, particularly the black walnut

(*Juglans nigra*). Juglone is an allelopathic compound, meaning it is synthesized by one type of plant and affects the growth of another. In the case of juglone, it is toxic or growth-stunting to many types of plants. Landscapers have long known that gardening underneath or near black walnut trees can be difficult. Juglone exerts its affect by inhibiting certain enzymes needed for metabolic function. It is occasionally used as an herbicide. Juglone has also found use as a coloring agent for foods and cosmetics, such as hair dyes and has been used as a natural dye for clothing and fabrics, particularly wool, and as ink. Its other names are Iuglon, Juglane, Nucin, Regianin, Walnut extract, Yuglon, NCI 2323, Oil Red BS and 1,4-naphthoquinone. It is an isomer of Lawsone. It is highly toxic to many insect herbivores. Some of them, however, are capable of detoxification of juglone (and related naphthoquinones) to non-toxic 1,4,5-trihydroxynaphthalene.[15]

3. 5-Hydroxy-2-methyl-1,4-naphthoquinone (Plumbagin)

Plumbagin is an organic compound with the chemical formula $C_{11}H_8O_3$. Plumbagin is a yellow dye, formally derived from naphthoquinone. Plumbagin is found in the plants of Plumbaginaceae, Droseraceae, Ancestrocladaceae, and Dioncophyllaceae families. Plumbagin is also present along with a series of other structurally related naphthoquinones in the roots, leaves, bark, and wood of *Juglans regia* (English walnut, Persian walnut, and California walnut), *Juglans cinerea* (butternut and white walnut), and *Juglans nigra* (black walnut). Preparations derived from black walnut have been used as hair dyes and skin colorants in addition to being applied topically for the treatment of acne, inflammatory diseases, ring-worm, and fungal, bacterial, and viral infections. The root of *Plumbago*

zeylanica (also called Chitrak), a major source of plumbagin, has been used in traditional Indian medicine since 750 BC as an antiatherogenic, cardiotonic, hepatoprotective, and neuroprotective agent.[16,17] Plumbagin has been shown to exert several therapeutic biological effects including anticancer, antiproliferative, chemopreventive, chemotherapeutic, and radiosensitizing properties in experimental animals as well as in tumor cells in vitro.[18-20]



Lawsone (**7**) Juglone (**8**) Plumbagin (**9**)

Figure 1.3 Structure of hydroxy-1,4-naphthoquinone

Figure 1.4 Flowers and leaves of the henna plant (*Lawsonia inermis*)

1.2 Black pepper

Black pepper (*Piper nigrum*) is a flowering vine in the family *Piperaceae*, cultivated for its fruit, which is usually dried and used as a spice and seasoning. The fruit, known as a peppercorn when dried, is approximately 5 millimetres (0.20 in) in diameter, dark red when fully mature, and like all drupes, contains a single seed. Peppercorns, and the powdered pepper derived from grinding them, may be described simply as pepper, or more precisely as black pepper (cooked and dried unripe fruit), green pepper (dried unripe fruit) and white pepper (dried ripe seeds). Black pepper is native to South East Asia and China, and is extensively cultivated there and elsewhere in tropical regions. Currently Vietnam is the world's largest producer and exporter of pepper, producing 34% of the world's *Piper nigrum* crop as of 2008. Dried ground pepper has been used since antiquity for both its flavour and as a medicine. Black pepper is the world's most traded spice. It is one of the most common spices added to European cuisine and its descendants. The spiciness of black pepper is due to the chemical piperine. Black pepper is produced from the still-green unripe drupes of the pepper plant. The drupes are cooked briefly in hot water, both to clean them and to prepare them for drying. The heat ruptures cell walls in the pepper, speeding the work of browning enzymes during drying. The drupes are dried in the sun or by machine for several days, during which the pepper around the seed shrinks and darkens into a thin, wrinkled black layer. Once dried, the spice is called black peppercorn. On some estates, the berries are separated from the stem by hand and then sun dried without the boiling process. Once the peppercorns are dried, pepper spirit & oil can be extracted from the berries by crushing them. Pepper spirit is used in famous beverages like Coca-Cola

and many medicinal and beauty products. Pepper oil is also used as an ayurvedic massage oil and used in certain beauty and herbal treatments.

Black pepper was believed to cure illness such as constipation, diarrhea, earache, gangrene, heartdisease, hernia, hoarseness, indigestion, insect bites, insomnia, joint pain, liver problems, lung disease, oral abscesses, sunburn, tooth decay, and toothaches.[21] Various sources from the 5th century onward also recommend pepper to treat eye problems, often by applying salves or poultices made with pepper directly to the eye. Black pepper, either powdered or its decoction, is widely used in traditional Indian medicine and as a home remedy for relief from sore throat, throat congestion. Pepper is known to cause sneezing. Some sources say that piperine, a substance present in black pepper, irritates the nostrils, causing the sneezing.[22] It has been shown that piperine can dramatically increase absorption of selenium, vitamin B, beta-carotene and curcumin as well as other nutrients. Pepper contains small amounts of safrole, a mildly carcinogenic compound. Also, it is eliminated from the diet of patients having abdominal surgery and ulcers because of its irritating effect upon the intestines,[23] being replaced by what is referred to as a bland diet. However, extracts from black pepper have been found to have antioxidant properties[24] and anti-carcinogenic effects, especially when compared to chili.[25] Black pepper was shown in Figure 1.5

Figure 1.5 Black pepper

Black pepper contains about 3% essential oil, whose aroma is dominated (max. 80%) by monoterpene hydrocarbons: sabinene, β-pinene, limonene, furthermore terpinene, α-pinene, myrcene, Δ3-carene and monoterpene derivatives (borneol, carvone, carvacrol, 1,8-cineol, linalool). Sesquiterpenes make up about 20% of the essential oil: β-caryophyllene, humulene, β-bisabolone and caryophyllene oxide and ketone. Phenylether (eugenol, myristicin, safrole) are found in traces. Loss of monoterpenes due to bad storage conditions (especially for ground pepper) should be avoided. The most important odorants organoleptically in black pepper are linalool, α-phellandrene, limonene, myrcene and α-pinene; furthermore, branched-chain aldehydes were found (3-methylbutanal, methylpropanal). The musty flavour of old pepper is attributed to the formation of heterocyclic compounds (2-isopropyl-3-methoxypyrazine and 2,3-diethyl-5-methylpyrazine) in concentrations of about 1 ppb. The essential oil of white pepper has received less attention; the content of essential oil is lower (1%), and the most abundant compounds are monoterpene hydrocarbons: limonene, β-pinene, α-pinene and α-phellandrene. Organoleptically most important are linalool (although

occurring as a minor component), limonene, α-pinene and phenylpropanoids. The pungent principle in pepper is an alkaloid-analog compound, piperine; it is the amide of 5-(2,4-dioxymethylene-phenyl)-hexa-2,4-dienoic acid (piperinic acid) with azinane (piperidine); only the *trans,trans* conformer contributes to pepper's pungency. Several piperine-analogs have been isolated from black pepper where the acid carbon backbone is partially hydrogenated (piperanine) or two carbon atoms longer (piperettine); amides of piperinic acid with pyrrolidine (piperyline) or isobutylamine (piperlongumine) have also been isolated. Total content of piperine-analogs in black pepper is about 5%.[26]

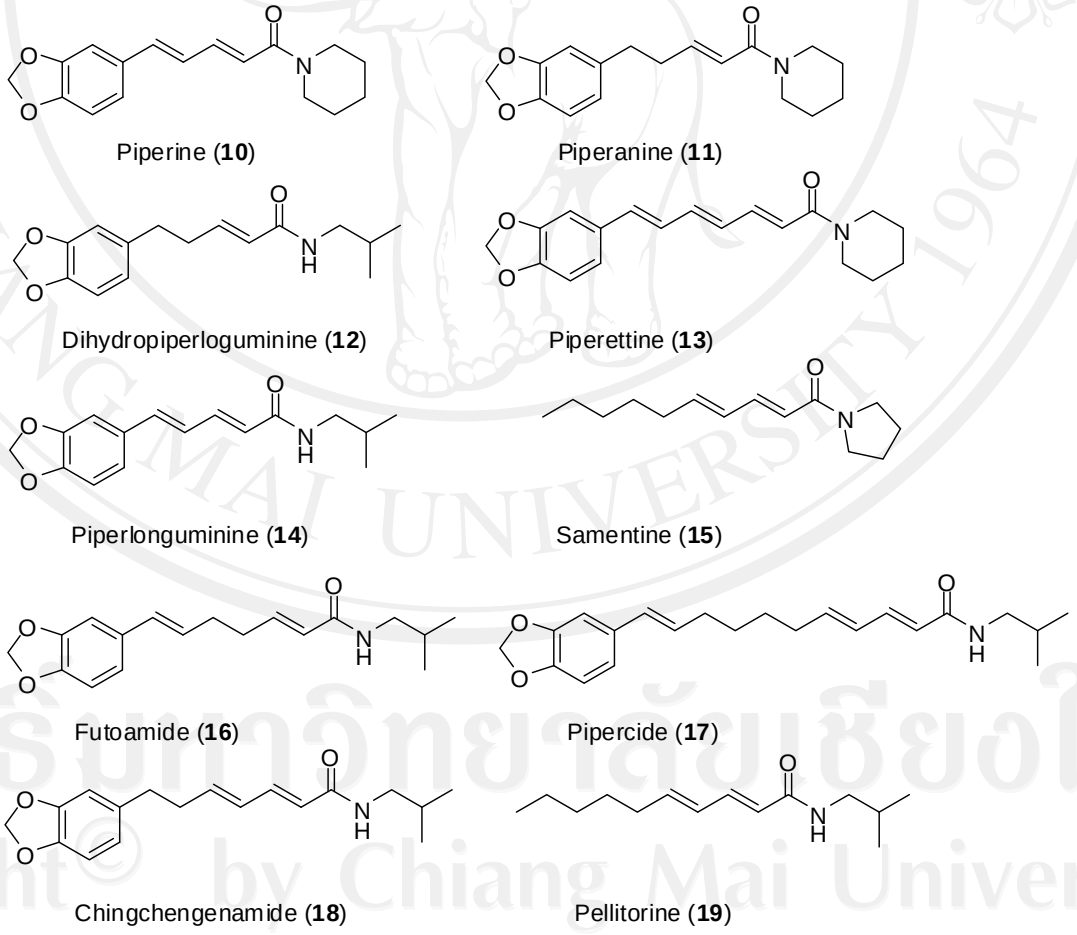


Figure 1.6 Chemical structures of some natural amides isolated from *Piper* sp.[27-28]

1.2.1 Piperine

Piperine is the alkaloid responsible for the pungency of black pepper and long pepper, along with chavicine (an isomer of piperine). It has also been used in some forms of traditional medicine and as an insecticide. Piperine forms monoclinic needles, is slightly soluble in water and more so in alcohol, ether or chloroform. The solution in alcohol has a pepper-like taste. Hydrolysed piperine by alkalis into a base and an acid, which were later named piperidine and piperic acid respectively. The alkaloid was synthesised by the action of piperoyl chloride on piperidine. Piperine is commercially available. If desired, it may be extracted from black pepper using dichloromethane.[28] Aqueous hydrotopes can also be used in the extraction to result in high yield and selectivity.[29] The amount of piperine varies from 1-2% in long pepper, to 5-9% in the white and the black peppers of commerce. The sharp flavour of freshly ground pepper is attributed to the compound chavicine, a geometric isomer (having the same molecular formula but differing in structure) of piperine. The loss of pungency of ground pepper on storage is associated with slow transformation of chavicine into piperine. The pungency of capsaicin and piperine is caused by activation of the heat and acidity sensing TRPV ion channel TRPV1 on nociceptors (pain sensing nerve cells).[30]

Piperine has also been found to inhibit human CYP3A4 and P-glycoprotein, enzymes important for the metabolism and transport of xenobiotics and metabolites.[31] In animal studies, piperine also inhibited other enzymes important in drug metabolism.[32] By inhibiting drug metabolism, piperine may increase the bioavailability of various compounds and alter the effectiveness of some medications. Researchers discovered that piperine can stimulate pigmentation in

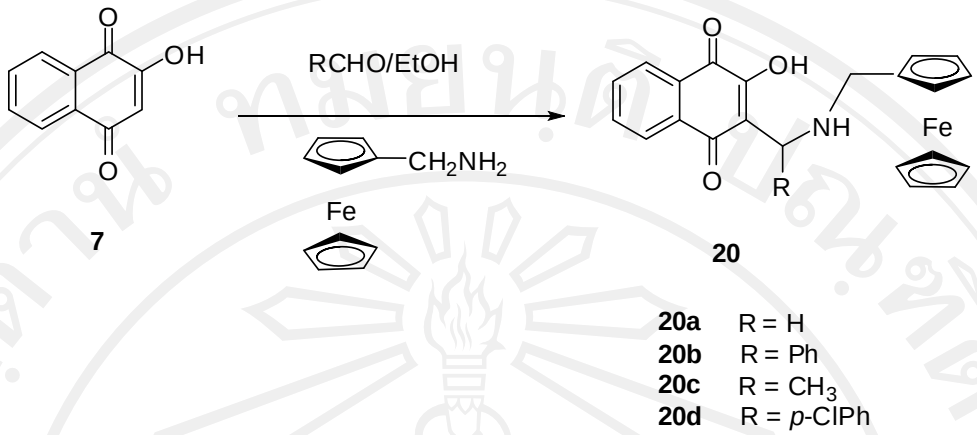
the skin, together with the exposure to UVB light.[33] Piperine has shown 'anti-depression like activity and cognitive enhancing effects in rats.[34]

More recently, piperine and its derivatives have been evaluated for inhibitory effects against epimastigote and amastigote forms of the protozoan parasite *Trypanosoma cruzi*.[27,35] In addition, piperine has been reported to show antioxidant action in experimental conditions both *in vivo* and *in vitro* through its radical quenching effect, insecticidal, and inhibition of liver metabolism.[36]

1.3 Literature Reviews

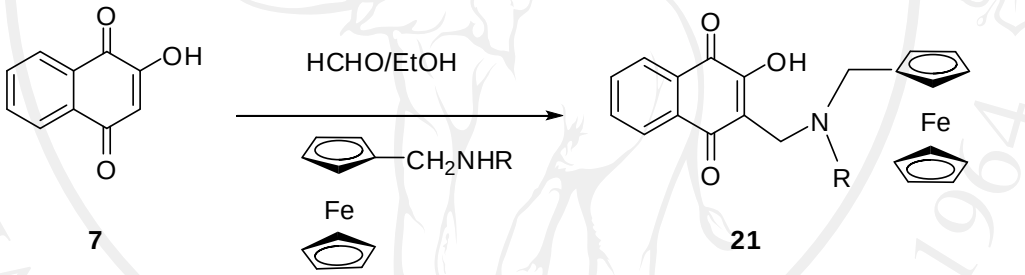
1.3.1 The application of hydroxynaphthoquinone derivatives have been reported especially as shown bellow.

In 2006, Baramee *et al*.[37] synthesized ferrocenic aminohydroxy-naphthoquinones for the use against *Toxoplasma gondii* and *Plasmodium falciparum*. Hydroxynaphthoquinone core with an amino-ferrocenic moiety, the high lipophilicity of ferrocene and its electrochemical behaviour render, were found to be significantly biological active against *T. gondii*. In this work, synthesis of thirteen novel ferrocenyl derivatives (**20a-d** and **21a-i**) which possess the hydroxynaphthoquinone backbone with different lateral chains, were shown in Scheme 1.1-1.3, three novel ferrocenyl derivatives **21d-21f** showing some significant activities against *T. gondii* with the IC_{50} values of 1.2±0.37, 2.1±0.5 and 3.0±0.4 for PLK strain and 1.4±0.27, 1.1±0.35 and 1.2±0.15 for ATO strain, respectively, and against *P. falciparum* with the IC_{50} values of 5±0.4, 2.5±0.3 and 6.25±1.5 for 3D7 strain and 2.5±0.3, 5.0±0.4 and 6.0±1.25 for Dd2 strain, respectively.



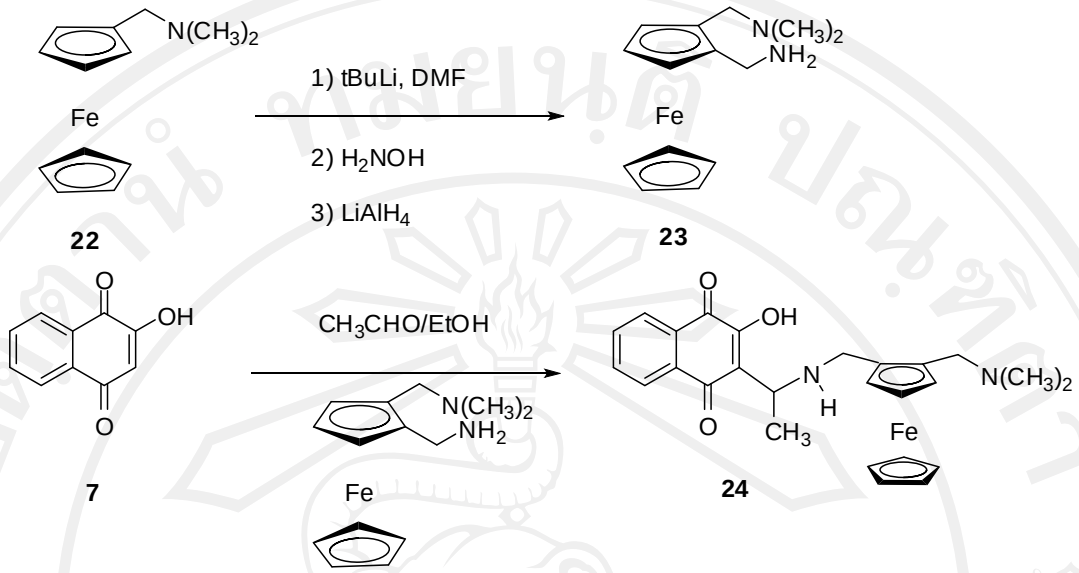
20a R = H
20b R = Ph
20c R = CH_3
20d R = *p*-ClPh

Scheme 1.1 Synthesis of ferrocenyl compounds **20a-20d**



21a R = CH_2CH_3
21b R = $(CH_2)_3CH_3$
21c R = $(CH_2)_3OH$
21d R = $(CH_2)_5CH_3$
21e R = $(CH_2)_6CH_3$
21f R = $(CH_2)_7CH_3$
21g R = $(CH_2)_8CH_3$
21h R = $(CH_2)_9CH_3$
21i R = $(CH_2)_{11}CH_3$

Scheme 1.2 Synthesis of ferrocenyl compounds **21a-21i**



Scheme 1.3 Synthesis of ferrocenyl compound **24**

Ngampong Kongkathip *et al.*[38] synthesized rhinacanthone (**25**), three 1,2-pyranonaphthoquinones (**26-28**), three 1,2-furanonaphthoquinones (**29-31**), three 1,4-pyranonaphthoquinones (**32-34**) and three 1,4-furanonaphthoquinones (**35-37**) for cytotoxicity against three cancer cell lines (KB, HeLa and $HepG_2$) it was found that rhinacanthone (**25**) and two 1,2-pyranonaphthoquinones (**26** and **27**) show very potent cytotoxicity with IC_{50} values of 0.92-9.63 µM, as shown in Figure 1.7 and Scheme 1.4-1.7.

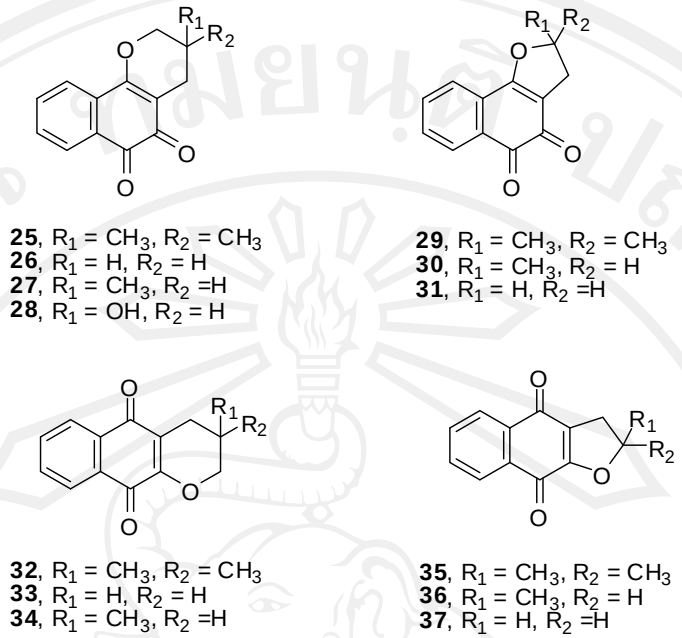
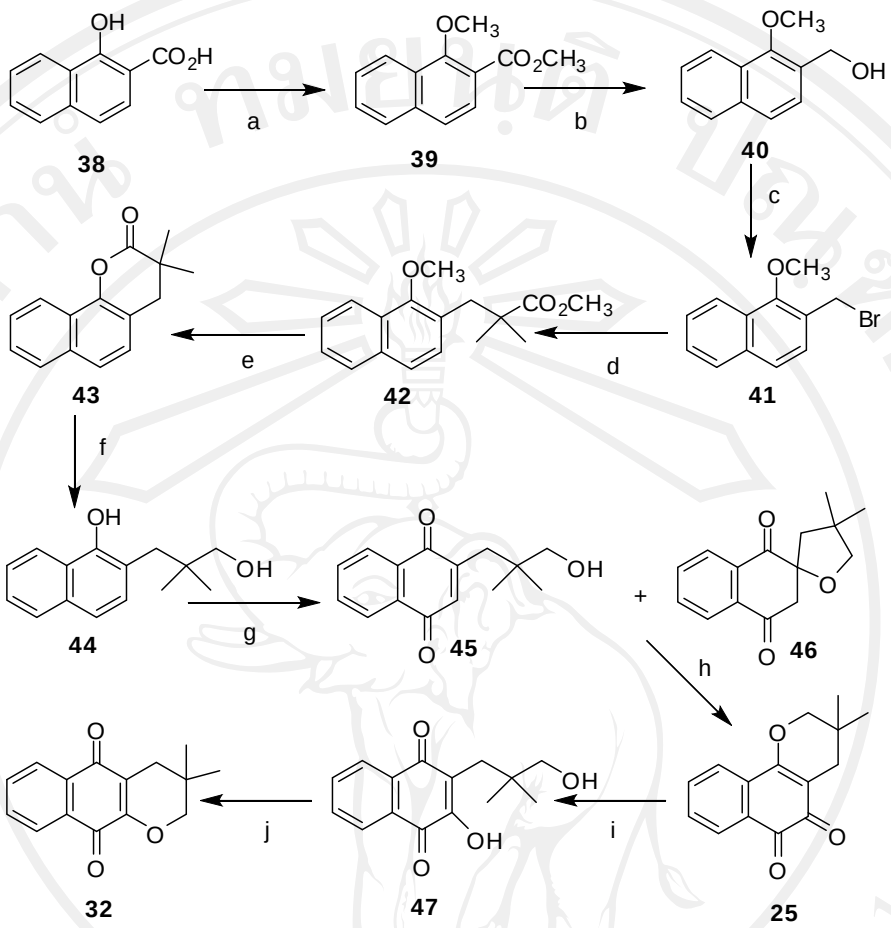
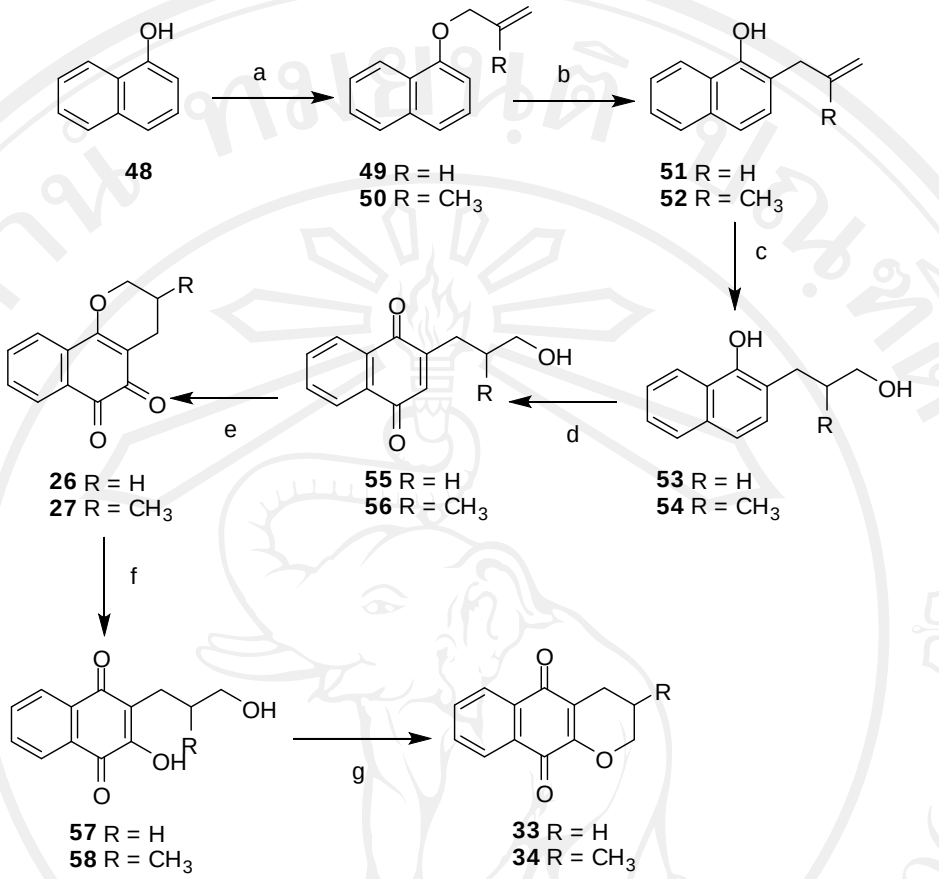
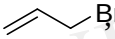
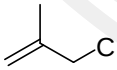


Figure 1.7 Rhinacanthone, 1,2-naphthoquinone and 1,4-naphthoquinone derivatives



Scheme 1.4 Synthesis of rhinacanthone (**25**) and 1,4-pyranonaphthoquinone (**32**) (a) MI, K_2CO_3, reflux, 12 h; (b) $LiAlH_4$, dry ether, rt, 1.5 h; (c) PBr_3, dry hexane-CH_2Cl_2 (1:1), rt 6 h; (d) LDA, methyl isobutylate, -78^{o}C, 2 h; (e) $AlCl_3$, chlorobenzene, reflux, 4 h; (f) $LiAlH_4$, dry ether, rt 2 h; (g) Fremy's salt, MeOH-DMF, 1 M NaOAc, rt 12 h; (h) DDQ, *p*-TsOH, benzene, reflux, 20 min; (i) 1% aq NaOH, reflux, 2 h; (j) 20% aq H_2SO_4, reflux, 5 h.



Scheme 1.5 Synthesis of 1,2-pyranonaphthoquinones (**26** and **27**) and 1,4-pyranonaphthoquinones (**33** and **34**) (a) (i)  , K_2CO_3, reflux, 3 h; (ii)  , K_2CO_3, reflux, 20 h; (b) 180°C, DMF, 6 h; (c) BH_3.THF and then H_2O_2/OH^-; (d) Fremy's salt, MeOH-DMF, 1 M NaOAc, rt, 12 h; (e) DDQ, *p*-TsOH, benzene, reflux, 20 min; (f) 1% aq NaOH, reflux, 2 h; (g) 20% aq H_2SO_4, reflux.

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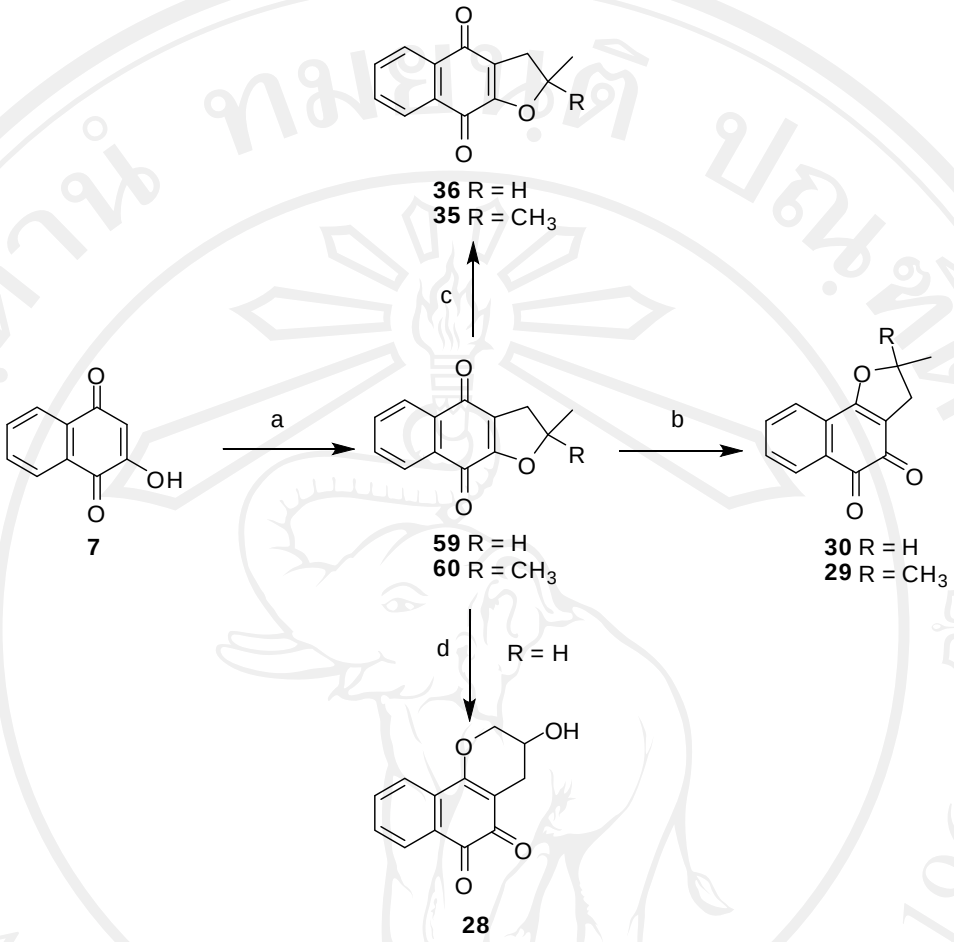
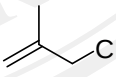
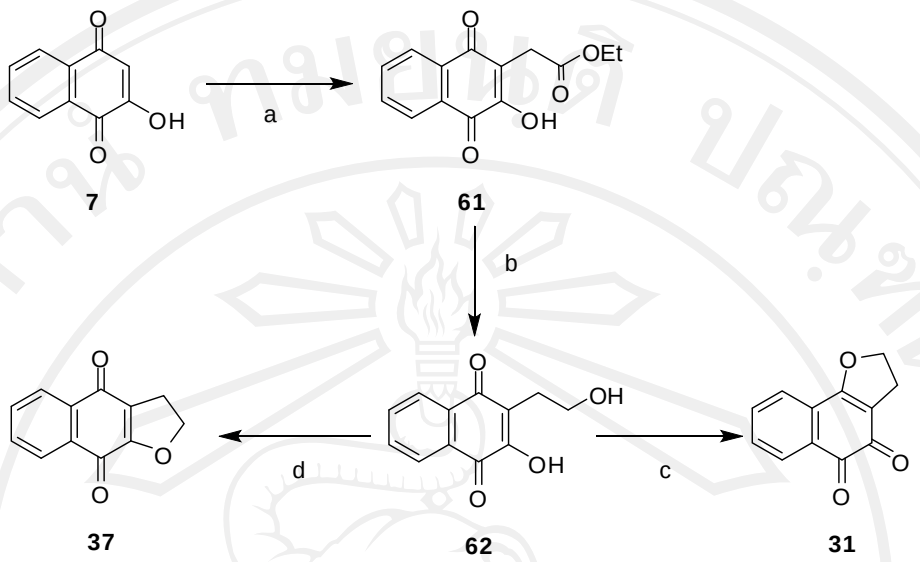
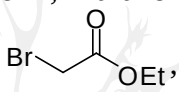


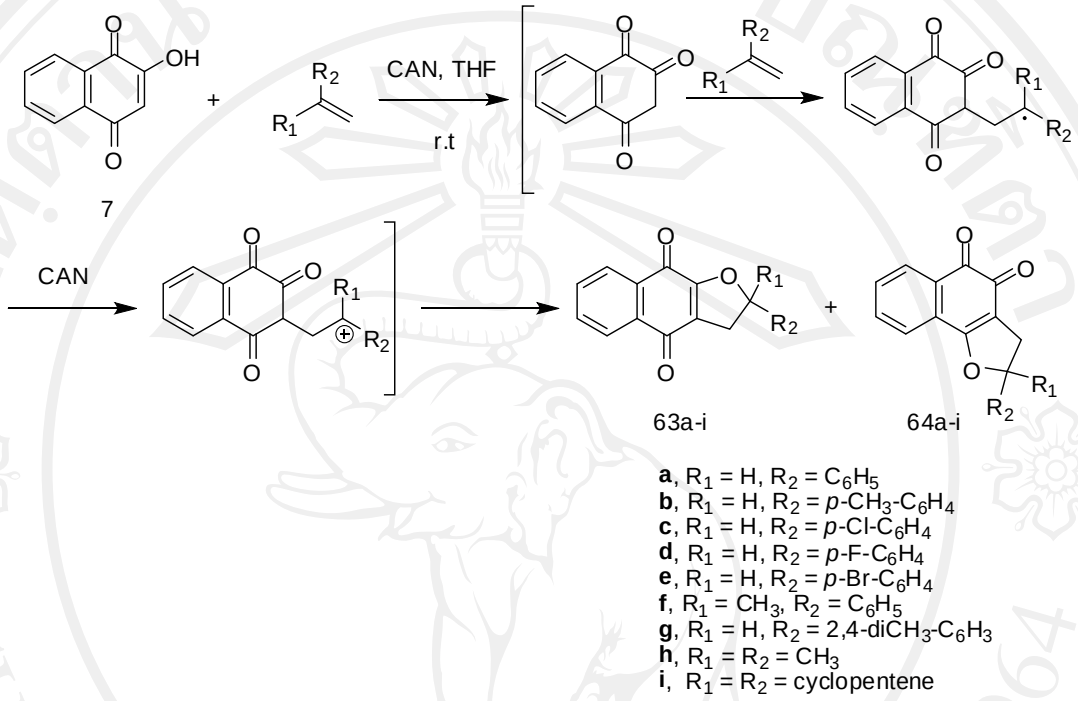
Scheme 1.6 Synthesis of 1,2-pyranonaphthoquinone (**28**), 1,2-furano naphthoquinones (**29** and **30**) and 1,4-furanonaphthoquinones (**35** and **36**) (a) (i) R = H; , K_2CO_3, DMF, reflux, 3 h; (ii) R = Me;  , K_2CO_3, KI, DMF, reflux, 3 h; (b) concd H_2SO_4, 0°C to rt, 30 min; (c) 20% aq H_2SO_4, reflux, 5 h; (d) *m*-CPBA, CH_2Cl_2, 0°C to rt, 24 h.



Scheme 1.7 Synthesis of 1,2-furanonaphthoquinone (**31**) and 1,4-furanonaphthoquinone (**37**) (a)  , Li2CO3, DMF, reflux, 3 h; (b) NaBH4, MeOH, reflux, 7 h; (c) concd H_2SO_4, 0^oC; (d) 20% aq H_2SO_4, reflux, 5 h.

Cristina *et al.*[39] synthesis a series of substituted α- and β-dihydrofuran naphthoquinones, **63a-i** and **64a-i**, and evaluated them as antifungal agents against six strains of *Candida* (*C.*): *C. albicans*, *C. krisei*, *C. parapsilosis*, *C. kefyr*, *C. tropicalis* and *C. dubliniensis*. The results indicated that **63h** was more active than commercially available drugs, itraconazole and fluconazole against *C. albicans*; **63i** also demonstrated good antifungal activity. Compounds **63h**, **63i** and **64i** exhibited a promising antifungal activity against strains *C. tropicalis*, *C. kefyr*, and *C. krisei* were sensitive to compounds **64a**, **64b**, **64c** and **64e**. Only compound **64e** demonstrated some antifungal activity against *C. parapsilosis*. Overall, some of the

α-furan naphthoquinones exhibited potent antifungal activity, with no hemolytic activity or cytotoxic effects.

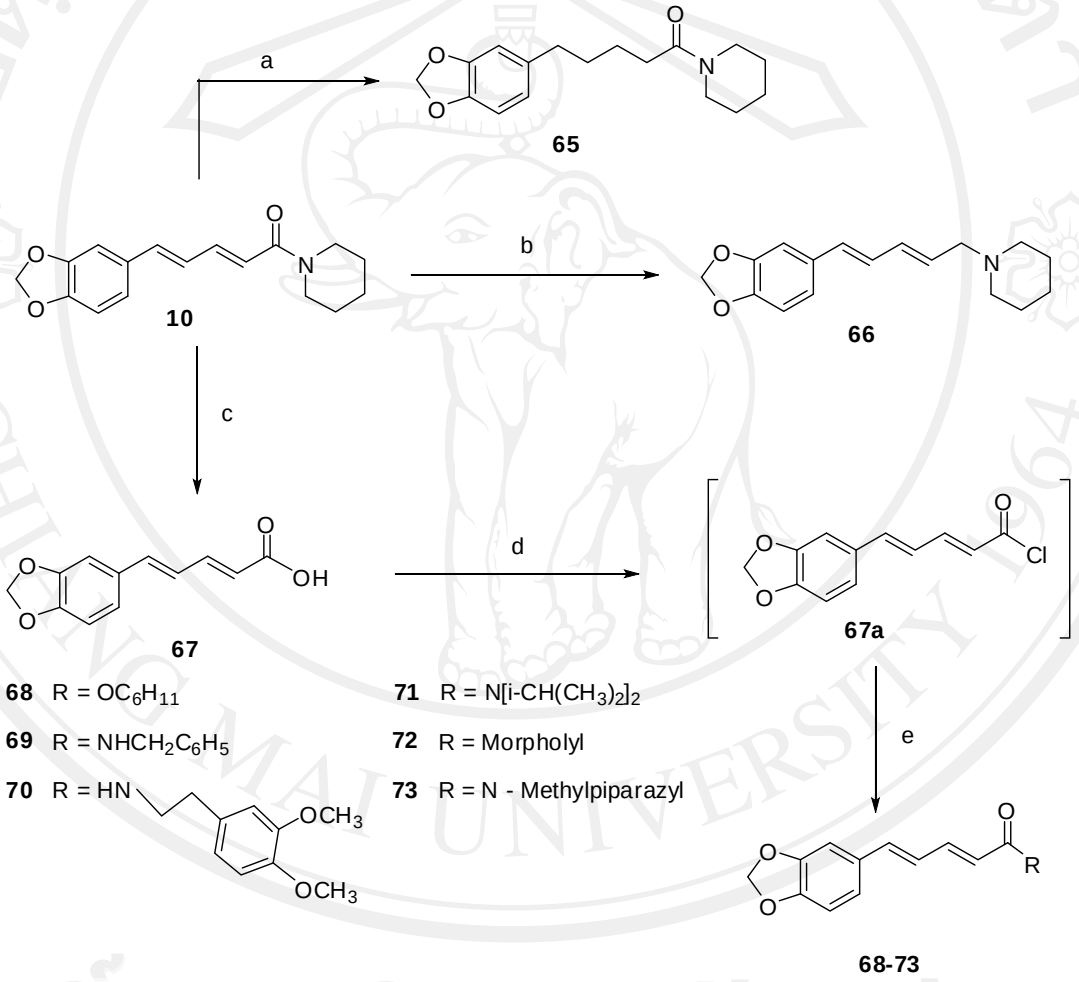


Scheme 1.8 Synthetic route used for the preparation of α- and β-furan naphthoquinones (**63a-i** and **64a-i**)

1.3.2 Piperine and their derivatives

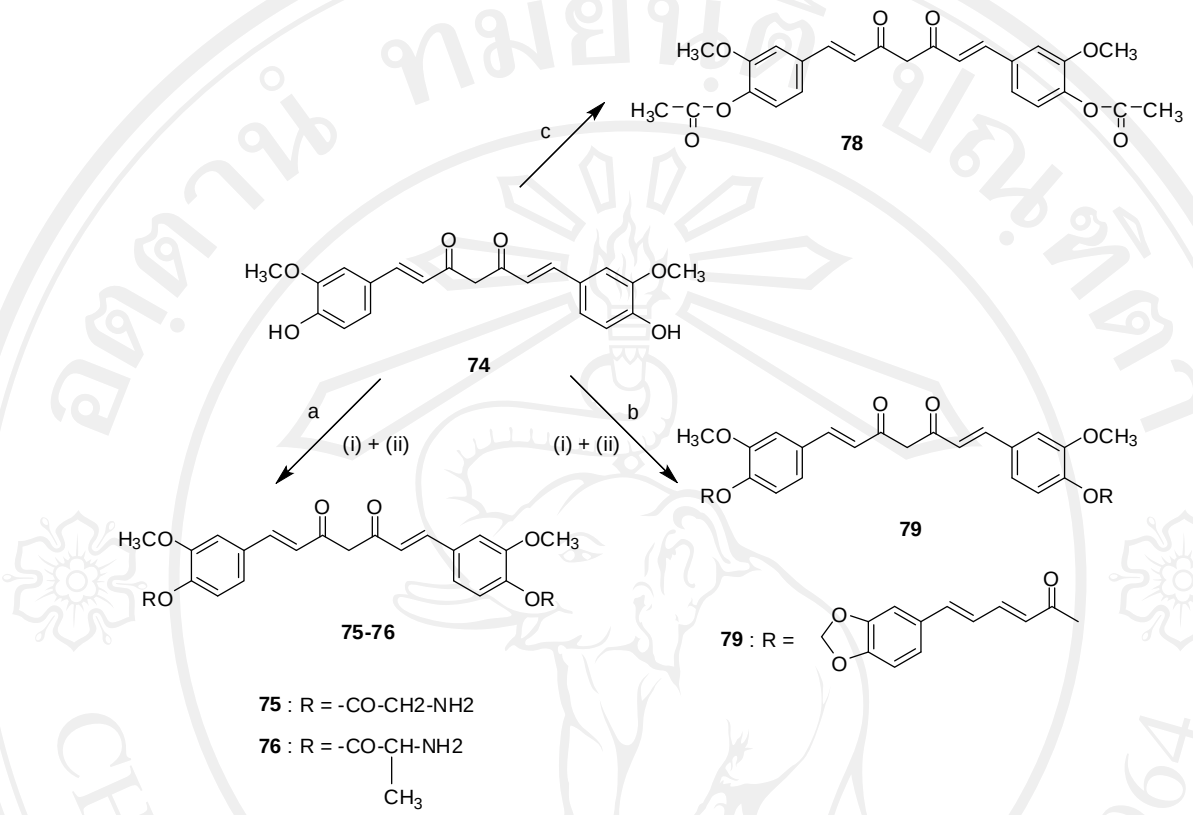
Ribeiro *et al*.[27] reported the toxicologies effects of piperine and its synthetic derivatives against the epimastigote and amastigote forms of the protozoan parasite *Trypanosoma cruzi*. Removal of the double bonds derivatives (**65**) did not interfere significantly with activity, suggesting that conjugation is not essential for trypanocidal activity and changing the piperidine moiety of the natural product for diisopropyl (**71**) or morpholyl groups (**72**) produces loss of activity on epimastigotes but does not interfere significantly with toxicity against intracellular

amastigotes. Finally, reduction of the carbonylamide group of piperine gave the allylic amine (**66**) which retained significant toxic effects against the parasites, showing that the carbonyl group is not important for the toxic effect. In conclusion, a series of piperine derivatives, which behave as potent inhibitors of the proliferation of *T. cruzi* parasites. Their synthetic pathway is shown in Scheme 1.9.

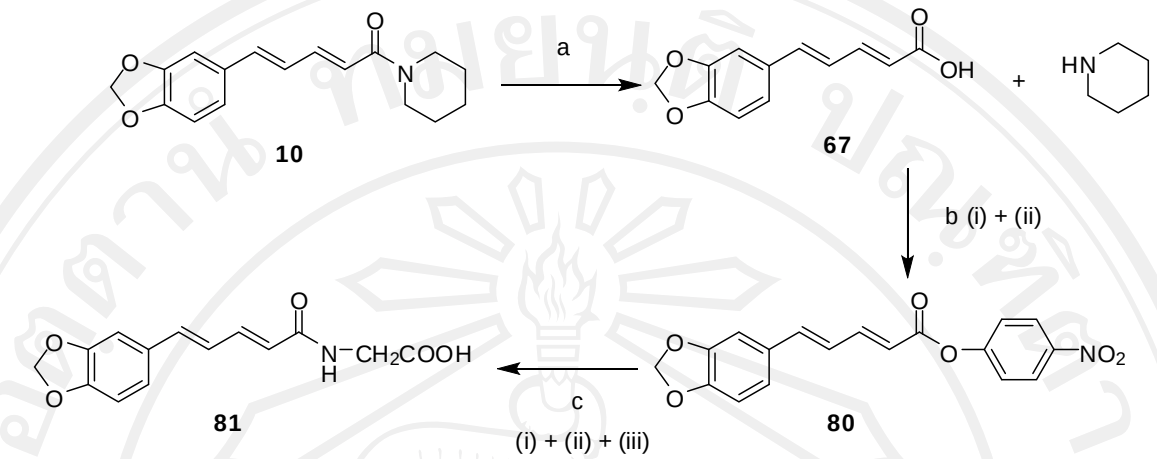


Scheme 1.9 Synthetic of piperine derivatives **65-73** (a) ethyl acetate, Pd/C, H_2, 2 h (90%); (b) DIBAL-H, toluene, -10^{o}C, 30 min (80%); (c) KOH, ethanol, reflux, 24 h; then HCl (aq), 0^{o}C (90%); (d) $(COCl)_2$, 25^{o}C, 30 min (100%); (e) CH_2Cl_2, alcohol or amine, 0^{o}C, 1 h (70-90%).

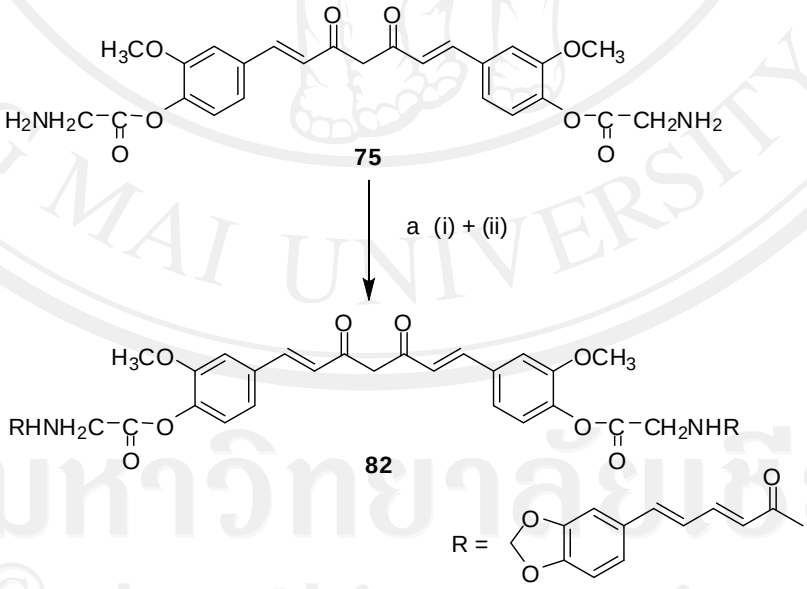
Mishra *et al.*[40] presented communication different curcumin bioconjugates viz. 4,4′-di-*O*-glycinoyl-curcumin (**75**), 4,4′-di-*O*-D-alaninoylcurcumin (**76**), 4,4′-di-*O*-(glycinoyl-di-N-piperoyl)-curcumin (**82**), 4,4′-di-*O*-piperoyl curcumin (**79**), curcumin-4,4′-di-*O*-β -D-glucopyranoside (**84**), 4,4′-di-*O*-acetyl-curcumin (**81**) along with piperoyl glycine. This makes these derivatives as potent prodrugs, which can get hydrolysed at the target sites. These bioconjugates were tested in vitro against different bacteria and fungi. The 4,4′-di-*O*-(glycinoyl-di-*N*-piperoyl)-curcumin (**82**) and 4,4′-di-*O*-acetyl-curcumin (**81**) are more effective than *Cefepime*, an antibacterial drug available in market, at the same concentration. The 4,4′-di-*O*-(glycinoyl-di-*N*-piperoyl)-curcumin (**82**) and 4,4′-di-*O*-piperoyl curcumin (**79**) had antifungal activity in vitro almost comparable with *fluconazole*, the most popular antifungal drug. The enhanced activity of these bioconjugates designed curcumin bioconjugates as potential antibacterial/antifungal drugs. The synthetic pathways are shown in scheme 1.10-1.13.



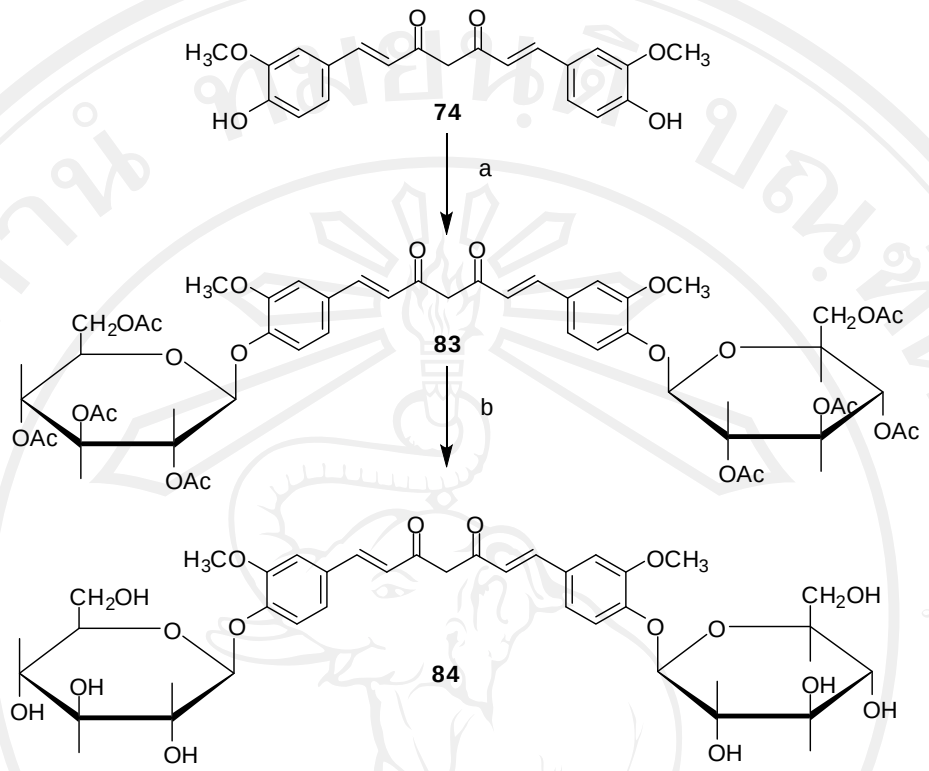
Scheme 1.10 Synthesis of 4,4′-di-*O*-glycinoyl-curcumin (**75**), 4,4′-di-*O*-D-alaninoylcurcumin (**76**), 4,4′-di-*O*-piperoyl curcumin (**79**) (a) (i) 10% NaOH, *N*-phthaloyl chloride, *N*-phthaloyl alaninoyl chloride, 6 h, 0^{o}C; (ii) NH_3-pyridine (9:1) v/v, 1 min. (b) (i) pyridine, piperoyl chloride (IX), rt, 6 h; (c) 10% NaOH, crushed ice, acetic anhydride.



Scheme 1.11 Synthesis of piperic acid (**67**), *p*-nitro phenyl ester of piperic acid (**80**) and piperoyl glycine (**81**) (a) Ethanolic KOH (2N), 2 h, reflux/HCl; (b) (i) *p*-nitrophenol, pyridine, TEA, (ii) DCC; (c) (i) Glycine, pyridine, (VI), (ii) DCC, DMAP.

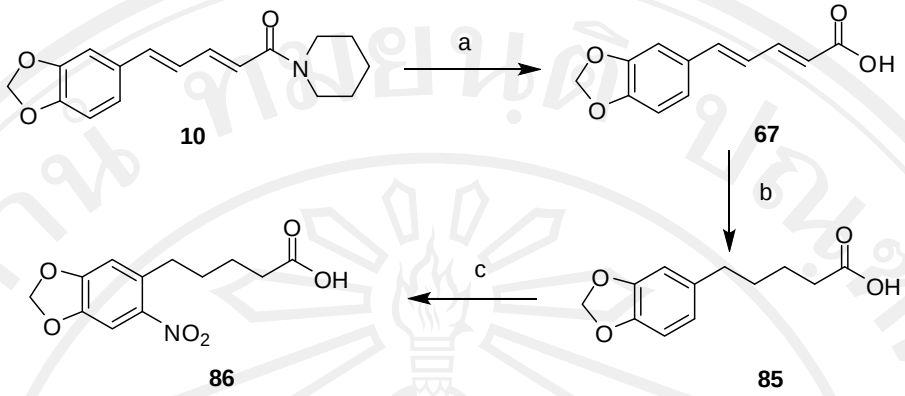


Scheme 1.12 Synthesis of 4,4′-di-*O*-(glycinoyl-di-*N*-piperoyl)-curcumin (**82**) (a) (i) *p*-nitro phenyl ester of piperic acid (VI); (ii) DCC, DMAP.

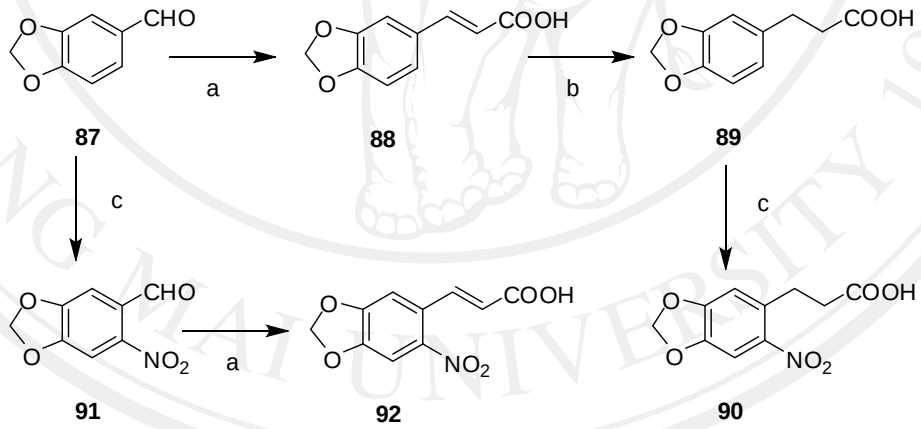


Scheme 1.13 Synthesis of curcumin-4,4′-di-*O*-β -D-glucopyranoside (**84**) (a) XII, pyridine; (b) NH_3-MeOH (1:1 v/v), 2 h at rt.

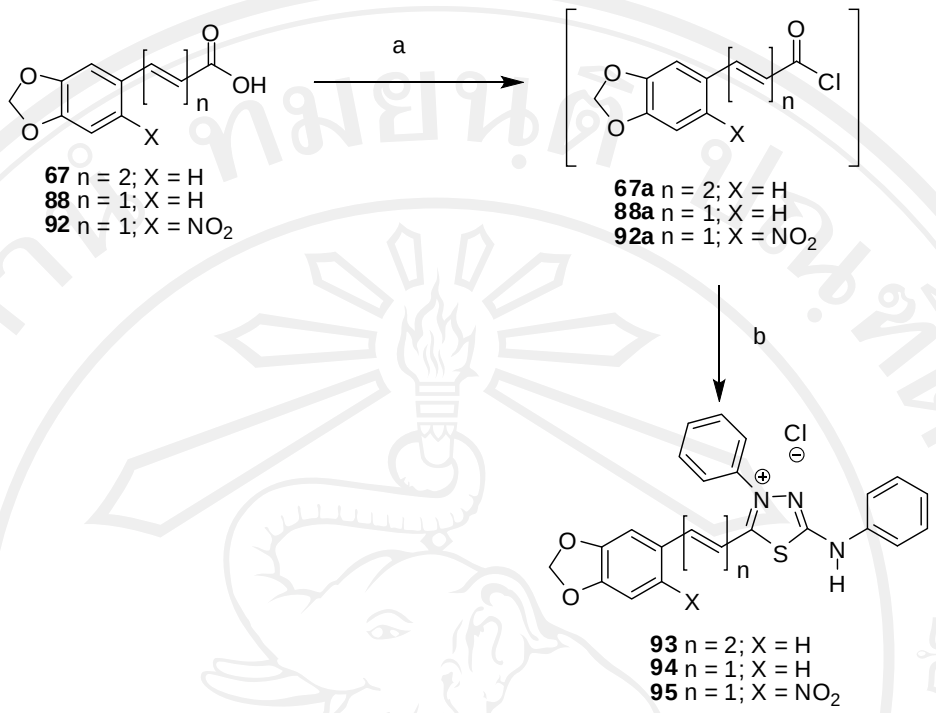
Ferreira *et al.*[41] reported that the synthesis and characterization of nine new 1,3,4-thiadiazolium-2-phenylamine chlorides derived from natural piperine and evaluate their toxic effects against the different form of *Trypanosoma cruzi*, and its cytotoxicity on murine macrophages. These analogues highlight the potential use of natural piperine as a precursor for new molecules which may be employed in the treatment of Chagas' disease. Compound **93** showed the best activity profile. Preparation of piperine analogues **85-86** and **90-101** were shown in Schemes 1.14, 1.15, 1.16, 1.17 and 1.18 respectively.



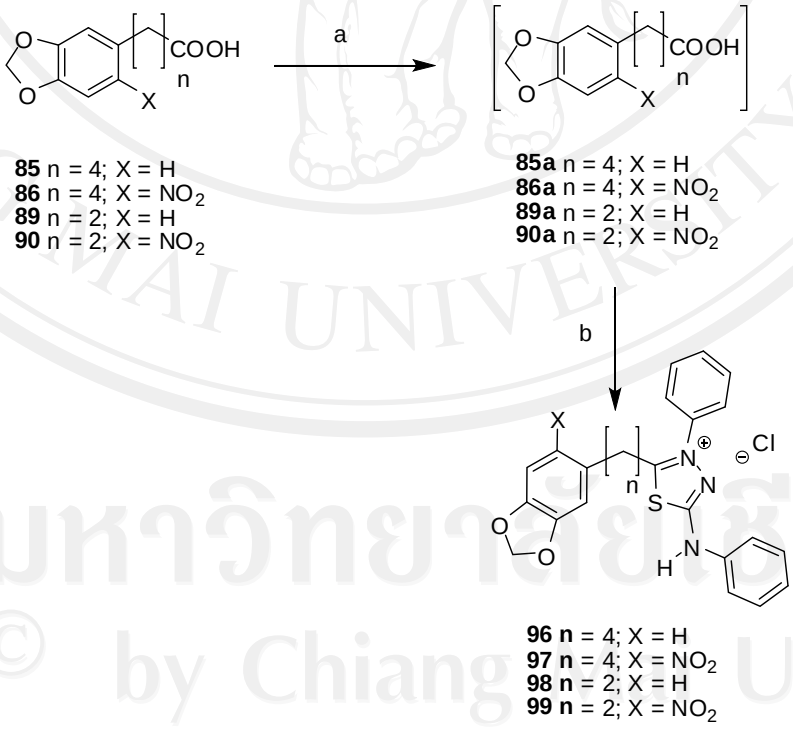
Scheme 1.14 Preparation of piperine analogues **85-86** (a) KOH, ethanol, reflux, 24 h, then HCl (aq), 0°C (93%); (b) ethyl acetate, Pd/C, H_2, 3 h (76%); (c) CH_3COOH (glacial), HNO_3 (concd) 3 h (77%).



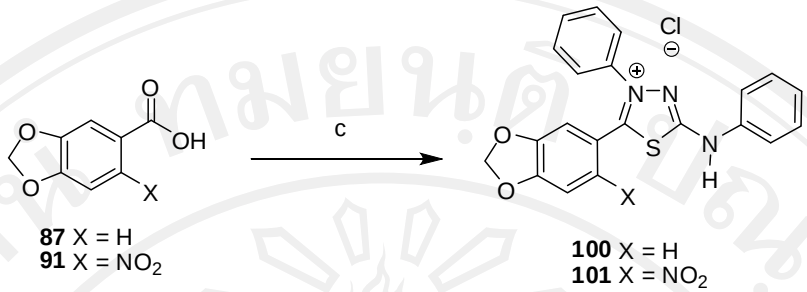
Scheme 1.15 Preparation of the precursor of the cinnamic series **90-92** (a) malonic acid, pyridine, piperidine, reflux, 8 h, then HCl (aq), 0°C (75-93%); (b) ethyl acetate, Pd/C, H_2, 3 h (80%); (c) CH_3COOH (glacial), HNO_3 (concd) 3 h (62-82%).



Scheme 1.16 Preparation of compounds **93-95** (a) $(COCl)_2$, 25°C, 2 h (100%); (b) 1,4-diphenylthiose-micarbazide, 1,4-dioxane, 25°C, 24-48 h (34-83%).



Scheme 1.17 Preparation of compounds **96-99** (a) $(COCl)_2$, 25°C, 2 h (100%); (b) 1,4-diphenylthiose-micarbazide, 1,4-dioxane, 25°C, 24-48 h (34-83%).

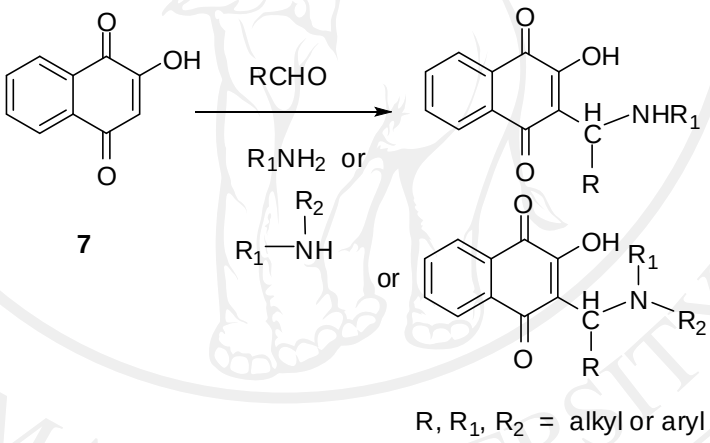


Scheme 1.18 Preparation of compounds **100-101** (c) 1,4-diphenylthio semicarbazide, TMSCl, DMF, 25^{o}C, 24 h (40-68%).

1.4 The research objectives

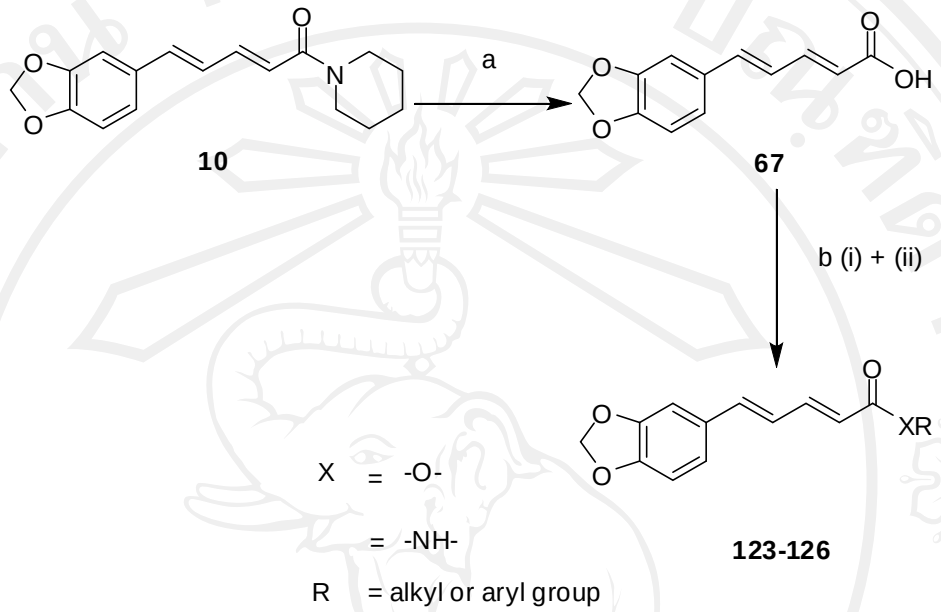
The aim of this research are to synthesize the naphthoquinone derivatives and piperine analogues for study their bioactivities against *Plasmodium falciparum*, K1 Strain (anti-malaria), *Mycobacterium tuberculosis* strain H37Ra (anti-TB) and anti-cancer cell lines (MCF7-breast cancer and NCI-H187-Small cell lung cancer). Moreover, the novel naphthoquinone derivatives and piperine analogues will be tested for their effective antioxidant property. The step of experiment including:

1. To synthesize naphthoquinone derivatives **102-122** as shown in Scheme 1.19



Scheme 1.19 The synthetic pathway of the naphthoquinone derivatives **102-122**

2. To synthesize the piperine analogues **123-126** as shown in Scheme 1.20



Scheme 1.20 The synthetic pathway of the novel piperine analogues **123-126** (a) KOH, ethanol, reflux, 24 h, then HCl (aq), 0^{o}C (b) (i) $(COCl)_2$, THF, 25^{o}C, 6 h; (ii) alcohol or amine, Et_3N, reflux, 5 h.

3. To examine the antimalarial activity against *Plasmodium falciparum* (K1 Strain) by Microculture Radioisotope Technique,[42,43] anti-Tuberculosis (*Mycobacterium tuberculosis* strain H37Ra) by Green fluorescent protein microplate assay (GFPMA)[44], anti-cancer cell line (MCF7-breast cancer and NCI-H187-Small cell lung cancer) by Resazurin Microplate assay (REMA)[45], cytotoxicity against Vero cells (African green monkey kidney) by Green Fluorescent Protein (GFP)-based assay[46] and their effective antioxidant property[47] of all derivatives.