CHAPTER II

LITERATURE REVIEWS

Orthosiphon grandiflorus

O. grandiflorus Bold, known locally as Yaa nuat maeo, Kidney tea plant or Java tea, is a medicinal plant in family Labiatae. *O. grandiflorus* is used traditionally for treatment of kidney diseases, dysuria with urinary stones, back and joint pain, diuretic, antihypertensive and antidiabetic (นันทวัน บุณยะประภัศร, 2529). It has been recommended that about 4 g of aerial parts of *O. grandiflorus* are ground, macerating with 750 ml of boiling water and the water is administered for the periods of 1-6 months for treatment of renal stones (สุนทรี สิงหบุตรา, 2542).

Morphological characteristic of *O. grandiflorus*

O. grandiflorus is square-stalked plant grow to height of around one meter. *O. grandiflorus* leaves are lanceolate and alternate cross-wise. The pale violet flowers are arranged in whorl and long filaments that project outside the flowers. Fruits contain 4 dry 1 seeded nutlets. Nutlet is obovate, rather flattened and rugose (นันทวัน บุณยะประภัศร, 2529) (Figure 1).

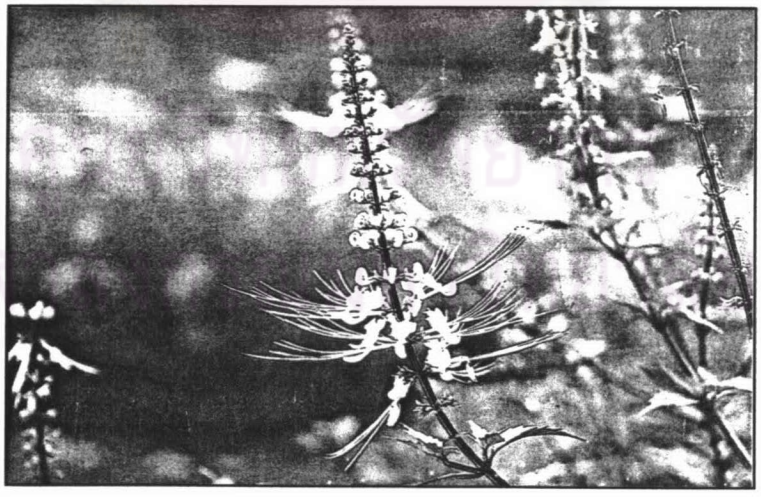


Figure 1 Morphology of *O. grandiflorus*.

Chemical constituents found in *O. grandiflorus* include (Banskota, A.H., *et al.*, 2003; Awale, S., *et al.*, 2002).

1. Diterpenes:
    1.1. Neoorthosiphols A and B
    1.2. Nororthosiphonolide A
    1.3. Norstaminols A-C
    1.4. Norstaminolactone A
    1.5. Orthosiphols A-J
    1.6. Orthosiphonones A and B
    1.7. Secoorthosiphols A-C
    1.8. Siphonols A-E
    1.9. Staminols A and B
    1.10. Staminolactones A and B
2. Flavonoids:
    2.1. Cirsimaritin
    2.2. Eupatorin
    2.3. 5-Hydroxy-6,7,3',4'-tetramethoxyflavone
    2.4. 6-Hydroxy-5,7,4'-trimethoxyflavone
    2.5. Ladanein
    2.6. Pillion
    2.7. Rhamnazin
    2.8. Salvigenin
    2.9. Sinensetin
    2.10. Tetramethylscutellarein
    2.11. 5,7,3',4'-Tetramethoxyflavone
    2.12. 5,7,4'-Trimethoxyflavone
    2.13. 7,3',4'-Tri-*O*-methylluteolin

3. Others:

    3.1 Acetovanillochromene

    3.2 Aurantiamide acetate

    3.3 Benzochromene

    3.4 Betulinic acid

    3.5 Caffeic acid

    3.6 2-Caffeoyltartaric acid

    3.7 Cichoric acid

    3.8 Coumarin

    3.9 2,3-Decaffeoyltartaric acid

    3.10 Methylripariochromene A

    3.11 Oleanolic acid

    3.12 Orthochromene A

    3.13 Rosmarinic acid

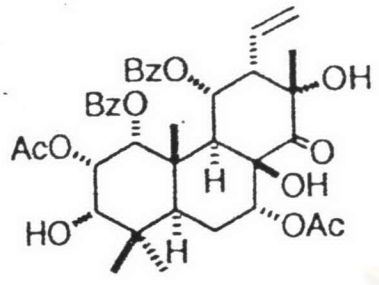
    3.14 β-Sitosterol

    3.15 Ursolic acid

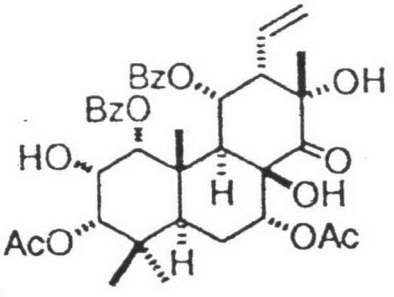
    3.16 Vomifoliol

The chemical structures of some chemical constituents found in *O. grandiflorus* were shown in figure 2.

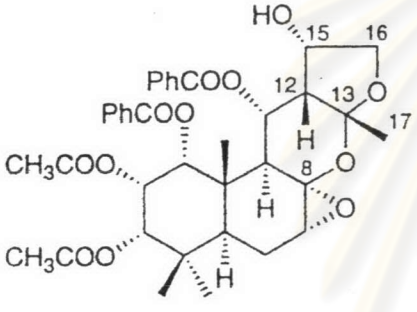
Diterpenes



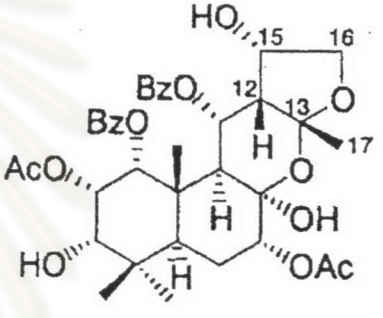
Neoorthosiphol A



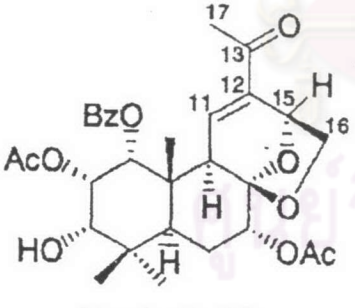
Neoorthosiphol B



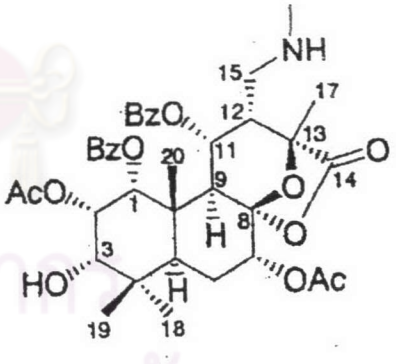
Norstaminol A



Norstaminol B



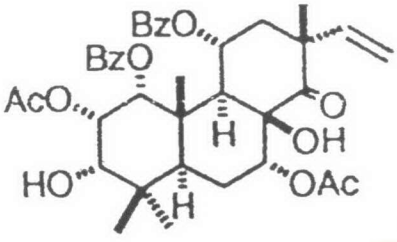
Norstaminol C



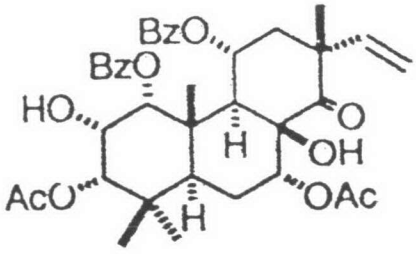
Norstaminolactone A

Figure 2 Chemical constituents found in *O. grandiflorus* (Shibuya, H. *et al.*, 1999; Ohashi, K., *et al.* 2000; Tezuka, Y. *et al.*, 2000; Awale, S. *et al.*, 2002, 2003; Olah, N. *et al.*, 2003; Akowuah, G.A. *et al.*, 2004; Nguyen, M.T.T. *et al.*, 2004).

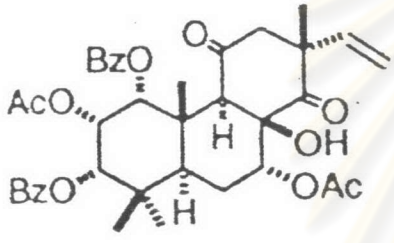
Diterpenes



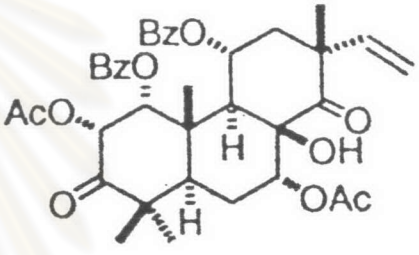
Orthosiphol A



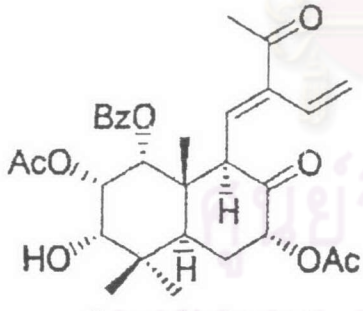
Orthosiphol B



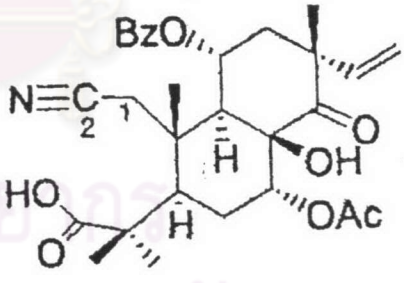
Orthosiphonone A



Orthosiphonone B



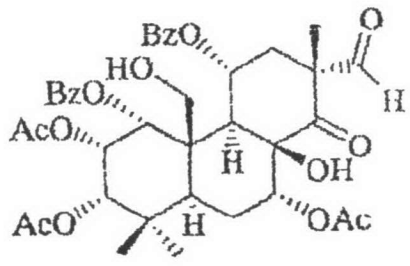
Secoorthosiphol B



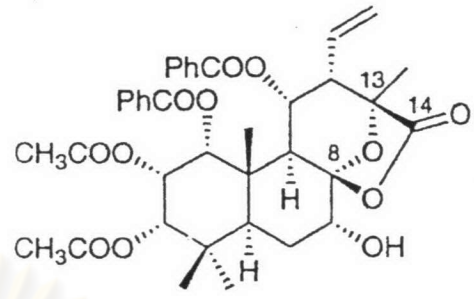
Secoorthosiphol C

Figure 2 *(Continued)* Chemical constituents found in *O. grandiflorus* (Shibuya, H. *et al.*, 1999; Ohashi, K., *et al.* 2000; Tezuka, Y. *et al.*, 2000; Awale, S. *et al.*, 2002, 2003; Olah, N. *et al.*, 2003; Akowuah, G.A. *et al.*, 2004; Nguyen, M.T.T. *et al.*, 2004).

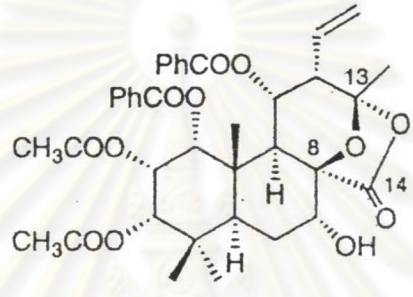
Diterpenes



Siphonol E

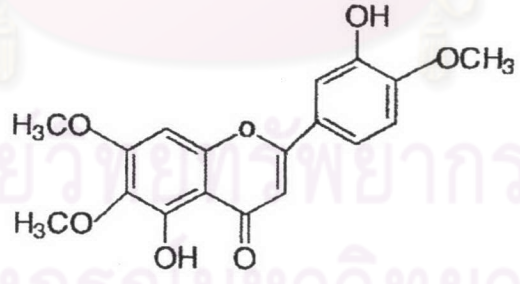


Staminolactone A



Staminolactone B

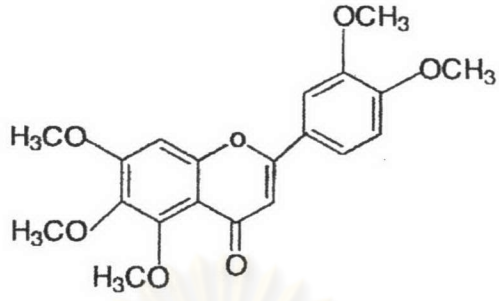
Flavonoids



Eupatorin

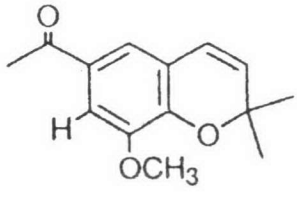
Figure 2 *(Continued)* Chemical constituents found in *O. grandiflorus* (Shibuya, H. *et al.*, 1999; Ohashi, K., *et al.* 2000; Tezuka, Y. *et al.*, 2000; Awale, S. *et al.*, 2002, 2003; Olah, N. *et al.*, 2003; Akowuah, G.A. *et al.*, 2004; Nguyen, M.T.T. *et al.*, 2004).

Flavonoids

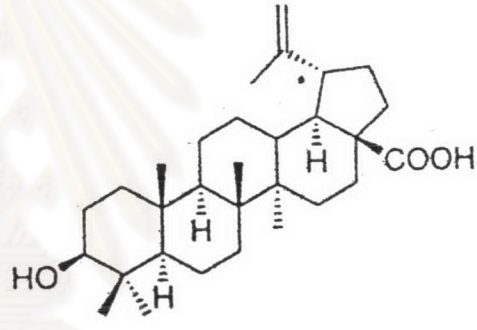


Sinensetin

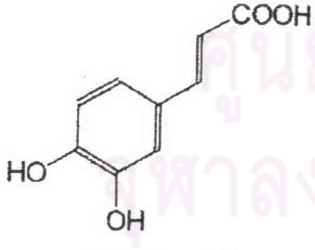
Others



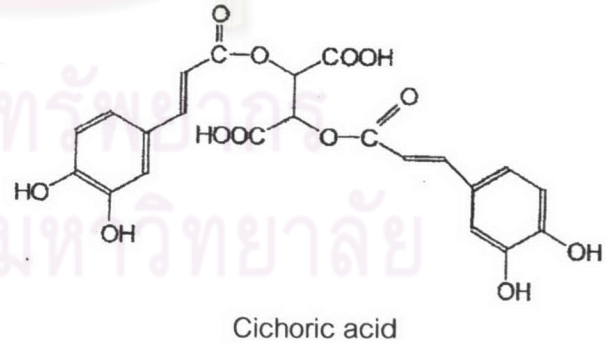
Acetovanillochromene



Betulinic acid



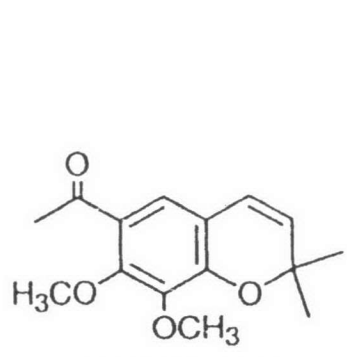
Caffeic acid



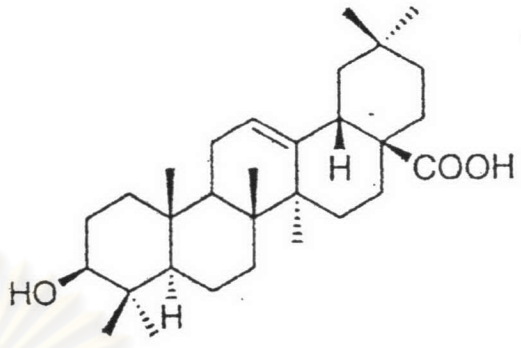
Cichoric acid

Figure 2 *(Continued)* Chemical constituents found in *O. grandiflorus* (Shibuya, H. *et al.*, 1999; Ohashi, K., *et al.* 2000; Tezuka, Y. *et al.*, 2000; Awale, S. *et al.*, 2002, 2003; Olah, N. *et al.*, 2003; Akowuah, G.A. *et al.*, 2004; Nguyen, M.T.T. *et al.*, 2004).

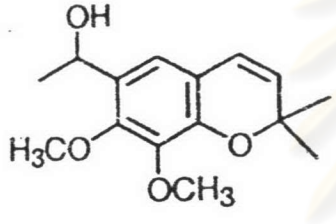
Others



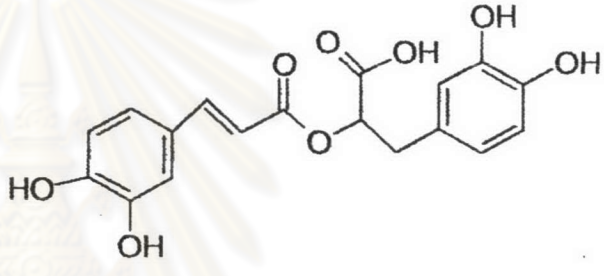
Methylripariochromene A



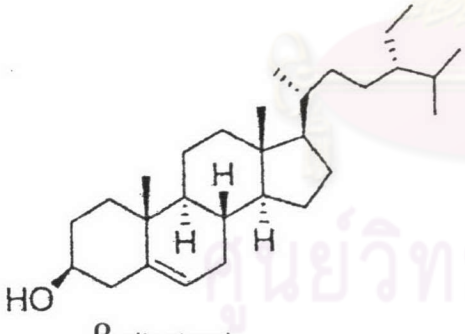
Oleanolic acid



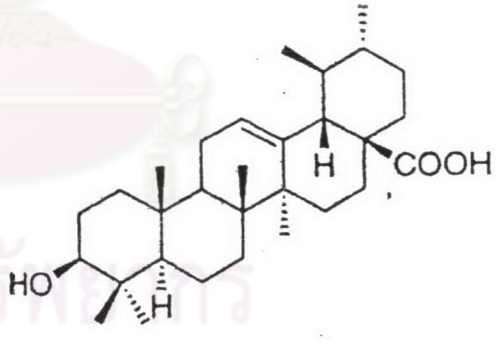
Orthochromene A



Rosmarinic acid



β-sitosterol



Ursolic acid

Figure 2 *(Continued)* Chemical constituents found in *O. grandiflorus* (Shibuya, H. *et al.*, 1999; Ohashi, K., *et al.* 2000; Tezuka, Y. *et al.*, 2000; Awale, S. *et al.*, 2002, 2003; Olah, N. *et al.*, 2003; Akowuah, G.A. *et al.*, 2004; Nguyen, M.T.T. *et al.*, 2004).

Pharmacological effects

1. Diuretic effect

Rats were given orally with *O. grandiflorus* aqueous extract at the doses of 125 mg/kg, 750 mg/kg and 1 g/kg whereas rats in the other two control groups receiving water and furosemide, respectively. The results showed that *O. grandiflorus* aqueous extract was active on the diuretic system in rodents and the optimum activity which was comparable to that of the furosemide control group, was found in rat receiving the extract at the dosage of 750 mg/kg (Englert, J. and Harnischfeger, G., 1991).

Rats were given orally with 1 ml water/rat, 30 mg/kg of furosemide, 700 mg/kg of tincture A and 700 mg/kg of tincture B (Tincture A & B were prepared from 100 g of dry leaves of *O. grandiflorus* extracted with 500 g of 50% & 70% ethanol, respectively). The results showed that the tincture A possessed a better diuretical action than the tincture B. The tincture A eliminated sodium better than the tincture B or furosemide, usefully diuretic, but it had preserved potassium for body better than furosemide or the tincture B. The experiments on same tincture A indicated a very good elimination of uric acid (Olah, N., *et al.*, 2003).

2. Renal stone effect

Effect of *O. grandiflorus* on the prevention of urolithiasis was studied in 16 patients who had renal stone with a diameter of 0.3-2 cm. Each patient received 300 ml of tea (prepared by 4 g of dry leaves of *O. grandiflorus* in 750 ml of water) for 1-10 months. The results showed that 4 patients had the stone removed into the urine and pH of the urine was trended to be increased. (**วีระสิงห์ เมืองมั่น และ มยุรี เนิดน้อย**, 2535).

Effect of *O. grandiflorus* on various parameters which were the promoters and inhibitors of renal stone precipitating was studied in 6 healthy male volunteers aged between 27-45 years old. They received 1 liter/day in 4 divided dose of 0.53%w/v of *O. grandiflorus* macerating solution. Their urine samples were then collected at 3 interval times during 24 hours for analysis of various chemistry values. The results showed that their urine pH was increased and the titratable acidity was lower as compared to those of the

water control group. There were no changes of urine volume, osmolarity, creatinine, sodium, potassium and chloride. However, an increase of the promoters of renal stone such as oxalate, calcium was observed. It was recommended that *O. grandiflorus* may be beneficial for increasing the alkalinity of the urine. Thus, it could be used to lower risk factors of uric acid renal stone. In contrast, it showed a tendency to increase risk of calcium oxalate urolithiasis (**มยุรี เนิดน้อย และวีระสิงห์ เมืองมั่น**, 2535).

A long-term study on the efficacy of *O. grandiflorus* in renal calculi treatment was performed comparing to sodium potassium citrate, a day widely used for the management of uric acid nephrolithiasis. Forty-eight patients with nephrolithiasis were randomly divided into 2 groups. Patients in group I was received 2 cups of *O. grandiflorus* tea containing 5 g of *O. grandiflorus* and patients in group II received 5-10 g of granular sodium potassium citrate for 18 months. The results showed that rates of stone size reduction per year was not statistically different between both groups which were 28.6 ± 16.0% and 33.8 ± 23.6% for patient group I and II, respectively. After the treatments, more than 90% of the subjects in both groups reported an easing of the clinical symptoms of the disease. None of the subjects in group I had any adverse effects from *O. grandiflorus* whereas 26.3% of the subjects in group II reported feeling of fatigue and loss of appetite (Premgamone, A., *et al.*, 2001).

Effect of *O. grandiflorus* treatment on 45 patients with renal stones was performed. The patients were divided into 3 groups. They were received tea made of 2, 3 and 4 tea bags of *O. grandiflorus* (2.5 g/bag) per day for group 1, 2 and 3, respectively for 15 days. The results showed that *O. grandiflorus* at various dosages caused little effects on serum and urine parameters related to stone risk factors as well as urinary pH. However, higher doses and larger duration of *O. grandiflorus* administration clearly caused an increase of urinary volume (**อมร เปรมกมล และคณะ**, 2545).

3. Antihypertensive effect

Methylripariochromene A (MRC) was isolated from leaves of *O. grandiflorus* and subjected to the examination of antihypertensive activity using conscious male stroke-prone

spontaneously hypertensive rats. The results indicated that MRC caused a continuous decrease in systolic blood pressure and decrease in heart rate after subcutaneous administration MRC at doses of 50 mg/kg and 100 mg/kg as compared to the control receiving 0.5% Tween 80 solution which was used as a vehicle of MRC. MRC exhibited the concentration-dependent suppression of contractions induced by high K^+, *l*-phenyleprine or prostaglandin $F_{2\alpha}$ in endothelium-denuded rat thoracic aorta. In addition, MRC increased urinary volume and the excretion of Na^+, K^+ and Cl^- at 3 hours after oral administration at the dose of 100 mg/kg of MRC (Matsubara, T., *et al.*, 1999).

Neoorthosiphols A and B, orthosiphol A and B, orthosiphonone A and B, methylripariochromene A, acetovanillochromene, orthochromene A, tetramethylscutellarein and sinensetin were isolated from leaves of *O. grandiflorus*. They were found to exhibit concentration-dependent suppression of contractions induced by K^+ in endothelium-denuded rat thoracic aorta (Ohashi, K., *et al.*, 2000).

4. Nitric oxide (NO) inhibitory effect

Several diterpene derivatives obtained from methanolic extract of *O. grandiflorus* such as staminane-type diterpines, isopimarane-type diterpenes etc. were tested for their inhibitory activities against NO production by lipopolysaccharide (LPS)-activated macrophage-like J774.1 cells. The results showed that the isolated diterpenes significantly inhibited nitric oxide production in LPS-activated macrophage-like J774.1 cells in a dose-dependent manner (Awale, S. *et al.*, 2003; Nguyen, M.T.T. *et al.*, 2004).

5. Anti-herpes simplex virus type 2 effect

Effect of *O. grandiflorus* extract was evaluated against herpes simplex virus type 2 strain Baylor 186 comparing to the standard antiviral drug, acyclovir. In that study, *O. grandiflorus* was extracted into five fractions including ethanol fraction, hexane fraction, aqueous-ethanol fraction, ethyl acetate fraction and aqueous-ethyl acetate fraction. The results showed that *O. grandiflorus* hexane, aqueous-ethanol and aqueous-ethyl acetate

extracts were shown to possess anti-herpes simplex virus activity (สกุลรัตน์ รัตนาเกียรติ์, 2545).

6. Antiproliferative effect

Orthosiphols D, E, O, P and Q, nororthosiphonolide A and orthosiphonone A were isolated from the aerial parts of *O. grandiflorus*. They displayed mild antiproliferative activities against highly liver metastatic colon 26-L5 carcinoma and human HT-1080 fibrosarcoma cell lines (Awale, S., *et al.*, 2002).

7. Antihistamine effect

An aqueous extract of *O. grandiflorus* was studied *in vitro* in isolated tracheal chain of guinea pig preparation. The test solutions (aqueous extract of *O. grandiflorus* 0, 2, 6 and 8 mg/ml, histamine 2×10^{-3} M and theophylline 4×10^{-3} M) were added one by one into the chamber and the changing of tracheal chain tension was recorded. The results showed that aqueous extract at the concentration of 8 mg/ml caused a relaxation of trachea smooth muscle and also could completely block the histamine-induced contraction of trachea smooth muscle (Chanarat, N., *et al.*, 1997).

8. Hypoglycemic effect

The hypoglycemic effect of *O. grandiflorus* aqueous extract was studied in normal, alloxan-induced diabetics (50 mg/kg, 3 days) and glucose orally-loaded (1 g/kg) rats. Each group of rats were treated with normal saline solution, tolbutamide at doses of 1 g/kg and *O. grandiflorus* at doses of 400 mg/kg. Blood glucose levels at various times were compared with base-line control. The result showed that *O. grandiflorus* aqueous extract caused a reduction of blood glucose in all groups of rats but its effect was lower than tolbutamide (Chanarat, N., *et al*, 1997).

Hypoglycemic effect of *O. grandiflorus* extract on oral glucose tolerance was studied in normal rats. Rats were given orally with *O. grandiflorus* ethanol extract at doses of 125, 250, 500 mg/kg, or chlorpropamide at the dosage of 40 mg/kg. The result showed

that *O. grandiflorus* ethanol extract at the dose of 125 mg/kg reduced blood glucose more than the control rats (กัลยา อนุลักขณาปกรณ์, อรษา ปานเจริญ และ จารีย์ บันสิทธิ์, 2543).

9. Antiinflammatory effect

Anti-inflammatory activity of orthosiphol A and B was measured on mouse ears using a tumor promoter, TPA (12-*O*-tetradecanolphorbol-13-acetate, 2 μg). Orthosiphol A and B showed potent inhibitory activity against the inflammation induced by TPA, on mouse ears (Masuda, T., *et al*., 1992).

10. Transforming growth factor-beta 1 (TGF-β1) antagonistic activity

Aqueous and methanolic extract of *O. grandiflorus* as well as triterpenoidal constituents, ursolic and oleanolic acids, were tested for the TGF-β1 antagonistic activity. The results showed that both aqueous and methanol extracts exhibited a dose-dependent inhibitory activity towards the binding of on TGF-β1 to its receptor in Balb/c 3T3 cells. Triterpenoidal constituents were also the potent *in vitro* TGF-β1 antagonists (Yoshimura, H., *et al*, 2003).

Toxicological effects

Acute toxicity

Acute toxicity study was performed in mice and rats using an aqueous extract of dried leaves of *O. grandiflorus* at doses of 0.5, 1 and 2 g/kg body weight. The median lethal doses (LD_{50}) in g/kg of the extract in animals after single intraperitoneal injection were 0.93 (male rats), 0.81 (female rats), 0.70 (male mice) and 0.84 (female mice); but no lethal effect was found after single feeding of up to 5 g/kg body weight in both rats and mice (ยุวดี วงษ์กระจ่าง และคณะ, 2533).

Subacute toxicity

A subacute toxicity study was performed in mice and rats using an aqueous extract of dried leaves of *O. grandiflorus* at doses of 0.5, 1 and 2 g/kg body weight given orally for

30 days. The results showed that no changes of body weight, clinical blood chemistry and histopathology of major visceral organs, i.e., kidney, liver, heart, lung and spleen as compared to the control group (ยุวดี วงษ์กระจ่าง และคณะ, 2533).

Chronic toxicity

A chronic toxicity study was performed in rats using an aqueous extract of dried aerial parts of *O. grandiflorus* at doses of 0.9, 9.0 and 18.0 g/kg/day given orally for 6 months. The results showed that there were no differences in growth and food consumption between groups throughout the study. Generally, clinical signs revealed no abnormalities. Only at the dose of 18.0 g/kg/day that platelet number was increased significantly ($p<0.05$). Serum sodium levels decreased ($p<0.05$) in all treatment groups. Histopathological findings showed no evidence of lesions related to the drug toxicity (ทรงพล ชีวะพัฒน์ และคณะ, 2536).

A chronic toxicity study was performed in rats using an aqueous extract of dried aerial parts of *O. grandiflorus* at doses of 0.96, 2.40 and 4.80 g/kg/day given orally for 6 months. There were no differences in body weight or food consumption between groups throughout the study. Hematological examination, clinical blood chemistry, gross and histopathological examinations of the visceral organs were determined after the completion of 6 month study. Platelet counts of female rats receiving the highest dose (4.80 g/kg/day) of the extract was significantly higher than that of the control, but was still in the normal range. Serum sodium levels decreased ($p<0.05$) in all treatment groups. Alkaline phosphatase level of the male rats treated with the extract were higher than that of the control. Incidence of glomerular hydrocalyx in male rats receiving highest dose (4.80 g/kg/day) of the extract was significantly higher than that of the control (นาถฤดี สิทธิสมวงศ์ และคณะ, 2542).

Xenobiotics metabolism

Xenobiotic are chemical compounds that do not belong to the normal composition of the human body. These compounds enter the body via diet, air and medication. The principal route of elimination of xenobiotics from the body is biotransformation (Taavitsainen, P., 2001). Biotransformation is the conversion of lipophilic xenobiotics into more hydrophilic, water-soluble metabolites, thereby serving to reduce the biological half-life of the xenobiotics, reduce the duration of the exposure to xenobiotics, avoid accumulation of the parent xenobiotics in the organism, change the biological activity of the xenobiotics and change the duration of the biological activity of the xenobiotics (Ioannides, C., 1996). Drug metabolizing enzymes are a diverse group of proteins that are responsible for metabolizing a vast array of xenobiotic compounds including drugs, environmental pollutants, and endogenous compounds such as steroids and prostaglandins. Drug metabolism is normally divided into two phases, phase I (or functionalisation reactions) and phase II (or conjugative reactions) (Table1).

Table 1 Reactions classed as phase I or phase II metabolism (Gibson, G. G. and Skett, P., 1994)

Phase I	Phase II
Oxidation	Glucuronidation/glucosidation
Reduction	Sulfation
Hydrolysis	Methylation
Hydration	Acetylation
Dethioacetylation	Amino acid conjugation
Isomerisation	Glutathione conjugation
	Fatty acid conjugation
	Condensation

The reactions of phase I are thought to act as a preparation of the phase II reactions, resulting in conjugation with endogenous substrates, increased water solubility and polarity, and drug elimination or excretion from the body. Liver is the main organ responsible for phase I and phase II drug metabolism reactions.

Phase I metabolism includes oxidation, reduction, hydrolysis and hydration reactions. Oxidation performed by the microsomal mixed-function oxidase system (cytochrome P450 dependent). The mixed-function oxidase system is found in microsomes of many cells such as liver, kidney and intestine.

Cytochrome P450 (CYP)

The CYP superfamily is divided into families and subfamilies on the basis of their nucleotide sequence homology. The CYP superfamily of microsomal hemoproteins catalyses the monooxygenation of a large number of endogenous and exogenous compounds. They play a key role in the metabolism of a wide variety of xenobiotics, drugs, alcohols, aromatic organic compounds, including many environmental pollutants and natural plant products. Some of these are chemical carcinogens or mutagens. Most biotransformation of xenobiotics is done by enzymes from the families CYP1, 2 and 3. Other families are mainly involved in the metabolism of endogenous compounds, such as fatty acids, bile acids and hormones. About 70% of the CYP enzymes in the human liver belong to the families which participate in drug metabolism. Of these, CYP3A4 represents about 30% and CYP2C about 20% of the total CYP enzymes. The regulation of CYP enzymes has been most extensively studied using rat, mouse and rabbit as model systems. Species differences have been noted in the expression of CYP gene. Table 2 presents sequential homology between rat and human CYP isoforms. Sequential homology of cDNA and amino acid sequence between rat and human CYP isoforms is high in similarily, approximately 70% (Soucek, P. and Gut, I., 1992).

CYP1A subfamily

The human CYP1A subfamily consists of two members, CYP1A1 and CYP1A2. CYP1A2 is mainly expressed in the liver, while CYP1A1 is primarily expressed in extrahepatic tissues. Evolutionary conservation the CYP1A subfamily throughout mammals suggests that this subfamily of P450 may play an important role in the metabolism of critical environmental chemicals (Table 3) (Ioannides, C., 1996).

CYP1A1 is induced by polycyclic aromatic hydrocarbons (PAHs), such as 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), benzo[*a*]pyrene, methylcholanthrene (MC) and β-naphthoflavone (BNF) (Table 4) (Oinonen, T. and Lindros, K.O., 1998).

The constitutively expressed CYP1A2 in human liver is inducible by, for example, environmental compounds, such as constituents of cooked meat, cruciferous vegetables, cigarette smoke, PAHs and polychlorinated biphenyls. CYP1A2 represents about 15% of total CYP enzymes in the human liver.

CYP2B subfamily

The expression of the highly similar CYP2B1 and 2B2 forms is low in rat liver, but both are up to 50-fold induced by phenobarbital. CYP2B1 metabolizes various lipophilic drugs and steroids more efficiently than CYP2B2 (Oinonen, T. and Lindros, K., 1998). Rat CYP2B1 is analogous to human CYP2B6 that is found in liver and extrahepatic tissues but are generally expressed at low levels in the absence of inducers such as phenobarbital (Gonzalez, F. and Gelboin, H., 1994).

CYP2E subfamily

CYP2E1 is constitutively expressed in human liver and in rodents. It has been shown to be inducible by ethanol and a number of other small compounds that are substrates for the enzyme. CYP2E1 is capable of metabolizing numerous low molecular weight chemicals, including benzene, carbon tetrachloride, acrylonitrile, *N*-nitrosodimethylamine, and others. The ubiquitous nature of CYP2E1 substrates in the industrial workplace, environment, tobacco smoke, and diet has made human health implications (Gonzalez, F. and Gelboin,

H., 1994). CYP2E1 expression is regulated by many factors. For example, fasting elevates level of the protein by increasing the transcription of the gene and diabetes by stabilizing the mRNA, while isoniazid increases the translation efficiency and affects the enzyme stabilization similarly to ethanol and imidazole (Taavitsainen, P., 2001).

CYP3A subfamily

CYP3A subfamily represents about 30% of the total P450 content in the human liver, although levels of the protein may vary 40-fold among individuals. In human, CYP3A subfamily consists of three members. CYP3A4 is the most abundant CYP enzyme in human liver and expressed in several tissues, but the expression in the liver and in the small intestine is of major interest in view of the metabolism of drugs and other xenobiotic chemicals. CYP3A5 is a minor, polymorphic CYP3A enzyme, which is expressed in lungs and colon. About 20% of individual adults express CYP3A5 at a high level in the liver. CYP3A7 is expressed in fetal liver and in the adult endometrium and placenta (Taavitsainen, P., 2001). Members of this family metabolize steroids and selected drugs. The expression of *CYP3A1* is extremely low in normal rat liver and *CYP3A2* is only expressed in male rats (Oinonen, T. and Lindros, K.O., 1998).

CYP3A is inducible by many drugs, for example, rifampicin, dexamethasone, carbamazepine and phenobarbital type inducers. The inhibitors of CYP3A have a wide variety of chemical structures. For example, azole-type fungicides, ketoconazole and itraconazole are the potent inhibitor (Taavitsainen, P., 2001).

Table 2 Sequential homology between rat and human P450 forms (Soucek, P. and Gut, I., 1992)

P450 form		Sequential homology[a] (%)
Rat	Human orthologue	
CYP1A1	CYP1A1	80 (78)
CYP1A2	CYP1A2	75 (70)
CYP2A1	----------	----[b]
CYP2A2	----------	----[b]
CYP2A3	CYP2A6	(85)
---------	CYP2A7	----[b]
CYP2B1	CYP2B6	78 (74)
	CYP2B7	(76)
CYP2B2	----------	----[b]
CYP2B3	----------	----[b]
CYP2B18	----------	----[b]
CYP2C6	CYP2C10	(75)
CYP2C7	----------	----[b]
CYP2C11	CYP2C9	80 (77)
CYP2C12	----------	----[b]
CYP2C13	CYP2C8	74 (68)
CYP2D1	CYP2D6	(71)
CYP2D2	----------	----[b]
CYP2D3	----------	----[b]
CYP2D4	----------	----[b]
CYP2D5	----------	----[b]
----------	CYP2D7	----[b]
----------	CYP2D8	----[b]
CYP2E1	CYP2E1	75 (78)

Table 2 (*continuted*) Sequential homology between rat and human P450 forms (Soucek, P. and Gut, I., 1992)

P450 form		Sequential homology[a] (%)
Rat	Human orthologue	
----------	CYP2F1	----[b]
CYP3A1	CYP3A3	(78)
----------	CYP3A4	(73)
CYP3A2	CYP3A5	(71)
	CYP3A7	(65)
CYP3A9	----------	----[b]
CYP4A1	CYP4A9	----[b]
CYP4A2	----------	----[b]
CYP4A3	----------	----[b]
CYP4A8	----------	----[b]
CYP4B1	CYP4B1	(>80)
CYP7	CYP7	(82)
CYP11A1	CYP11A1	79 (76)
CYP11B1	CYP11B1	(67)
CYP17	CYP17	74
CYP19	CYP19	80 (78)
---------	CYP21A2	----[b]
CYP27	CYP27	----[b]

[a] Similarity of cDNA and amino acid (in parentheses) sequence stated.

[b] No data available regarding existence of orthogous form.

Table 3 Carcinogens and toxins metabolized by human P450s (Gonzalez, F. and Gelboin, H., 1994)

P450	Carcinogen/toxin
CYP1A1	Benzo(a)pyrene, dimethylbenz(a) anthracene, 6-nitrochrysene
CYP1A2	1-Aminofluorene, 2- acetylaminoflurene, aflatoxin B_1, 4-aminobiphenyl, 2-aminoanthracene, 2-amino-3-methylimidazo[4,5*f*]quinoline (IQ), 2-amino-3,8-dimethylimidazo[4,5*f*]quinoline (MeIQ), 2-amino-3,8-dimethylimidazo[4,5*f*]quinoxaline ($MeIQ_x$) 2-amino-3,4,8-trimethylimidazo[4,5*f*]quinoxaline ($DiMeIQ_x$), 2-amino-6-methyldipyrido[1,2-*a*:3',2'-*d*]imidazole (Glu P-1), 2-aminodipyrido[1,2-*a*:3',2'-*d*]imidazole (Glu P-2), 3-amino-1,4-dimethyl-5*H*-pyridol[4,3-*b*] indole (Trp P-1), 3-amino-1-methyl-5*H*-pyridol[4,3-*b*]indole (Trp P-2), 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP), 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NKK), 6-nitrochrysene
CYP2A6	Aflatoxin B_1, *N*-nitrosodiethylamine, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK)

Table 3 (*continuted*) Carcinogens and toxins metabolized by human P450s (Gonzalez, F. and Gelboin, H., 1994)

P450	Carcinogen/toxin
CYP2B6	6-Aminochrysene
CYP2C8, 9, 18	None known
CYP2D6	4-(Methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK)
CYP2E1	Acetaminophen, acrylonitrile, benzene, carbon tetrachloride, chloroform, 1,2-dichloropropane, ethyl carbonate, ethylene dibromide, ethylene dichloride, methyl chloride, methylene chloride, N-nitrosodiethylamine, styrene, vinyl carbamate, vinyl bromide, vinyl chloride, 1,11-trichloroethane, trichloroethylene
CYP2F1	None known
CYP3A4	Aflatoxin B_1, aflatoxin G_1, 6-aminochrysene, benzo(a)pyrene-7,8 diol, 6-nitrochrysene, 1-nitropyrene, *tris*-(2,3-dibromopropyl) phosphate, sterigmatocystin, serecionine
CYP4A11	None known
CYP4B1	None known

Table 4 Regulation of P450 gene expression (Soucek, P. and Gut, I., 1992)

CYP	Mechanism and regulation of gene expression[a]
1A1	Inducible (all tissues) – MC, TCDD, ACLR, ISF, BNF: transcriptional activation + mRNA stabilization
1A2	Constitutive (liver): transcriptional activation + MRNA stabilization
	Inducible (all tissues) - MC, TCDD, ACLR, ISF, BNF: transcriptional activation + mRNA stabilization
2A1	Constitutive (liver) – age, sex, growth hormone, diabetes, hypertension: activation
	Inducible – MC, PB, ACLR, BNF, PCN, ISF: transcription activation
2A2	Constitutive (liver) – age, sex (♂), growth hormone: transcriptional activation
2A3	Inducible (lung) – MC
2B1	Constitutive (lung, testis) – age, strain, colony, growth hormone: transcriptional activation
	Inducible (all tissues) – PB, AE, AC, ISF, PCN, starvation: transcriptional activation + mRNA stabilization

Table 4 (*Continued*) Regulation of P450 gene expression (Soucek, P. and Gut, I., 1992)

CYP	Mechanism and regulation of gene expression[a]
2B2	Constitutive (liver) – age, growth hormone
	Inducible – PB, ACLR, ISF, PCN, DEX, starvation: transcriptional activation + mRNA stabilization
2B3	Constitutive (liver)
2B12	Constitutive (liver, lung, kidney, testis)
	Inducible – PB: transcriptional activation
2C6	Constitutive (liver, brain) – age: transcriptional activation
	Inducible – PB
2C7	Constitutive – age, sex (♂/♀ = 1/2), diabetes: transcriptional activation
	Inducible – PB, AE
2C11	Constitutive (liver) – age, sex (♂), growth hormone, diabetes, hypertension: transcriptional activation
2C12	Constitutive (liver) – age, sex (♀), growth hormone, diabetes: transcriptional activation
2C13	Constitutive – age, sex (♂), strain, diabetes: transcriptional activation
	Inducible – AE

Table 4 (*Continued*) Regulation of P450 gene expression (Soucek, P. and Gut, I., 1992)

CYP	Mechanism and regulation of gene expression[a]
2D1	Constitutive (liver, kidney) – age, sex, strain: transcriptional activation Inducible – MC, PB: transcriptional activation
2D2	Constitutive (liver, kidney) – age: transcriptional activation
2D3-5	Constitutive – age
2E1	Constitutive (liver, kidney) – age, growth hormone: transcriptional activation; diabetes, starvation; transcriptional activation + mRNA stabilization Inducible (liver, lung) – ISN, AE, AC, B: protein stabilization
2G1	Constitutive (olfactory neuroepithelium)
3A1	Inducible – PCN, DEX, PB, hypertension: transcriptional activation + mRNA stabilization; TAO : protein stabilization
3A2	Constitutive (liver, intestine) – age, sex (♂), growth hormone, diabetes, starvation: transcriptional activation Inducible – PB: activation; TAO: protein stabilization
4A1-3	Constitutive (liver, kidney): transcriptional activation

Table 4 (*Continued*) Regulation of P450 gene expression (Soucek, P. and Gut, I., 1992).

CYP	Mechanism and regulation of gene expression[a]
4A1-3	Inducible – clofibrate, starvation: transcriptional activation
4B1	Constitutive (lung): transcriptional activation

[a] Abbreviation used : AC – acetone, ACLR – Aroclor 1254, AE – ethanol, B – benzene, BNF - β-naphthoflavone, DEX – dexamethasone, ISF – isosafrole, ISN – isoniazid, MC – 3-methylchlanthrene, PB – Phenobarbital, PCN – pregnenolone-16α-carbonitrile, TAO – triacetyloleandomycin, TCDD – tetrachlorodibenzo-*p*-dioxin.

Physiological factors: age, sex (♂, male; ♀, female specificity), diabetes, growth hormone, hypertension, starvation; suggested mechanism of gene expression: protein/mRNA stabilization, transcriptional activation.

Mechanism of induction of CYPs

Induction is defined as an increase in enzyme activity associated with an increase in intracellular enzyme concentration. Induction of drug metabolism contributes to interindividual and intraindividual variation in drug efficacy and potential toxicity associated with drug-drug interactions (Woolf, T.F., 1999).

A considerable diversity has been depicted in the mechanisms of regulation of P450 (Figure 3). Mechanisms of induction include gene transcription through receptors, mRNA processing, mRNA stabilization and enzyme stabilisation which are shown in Table 5.

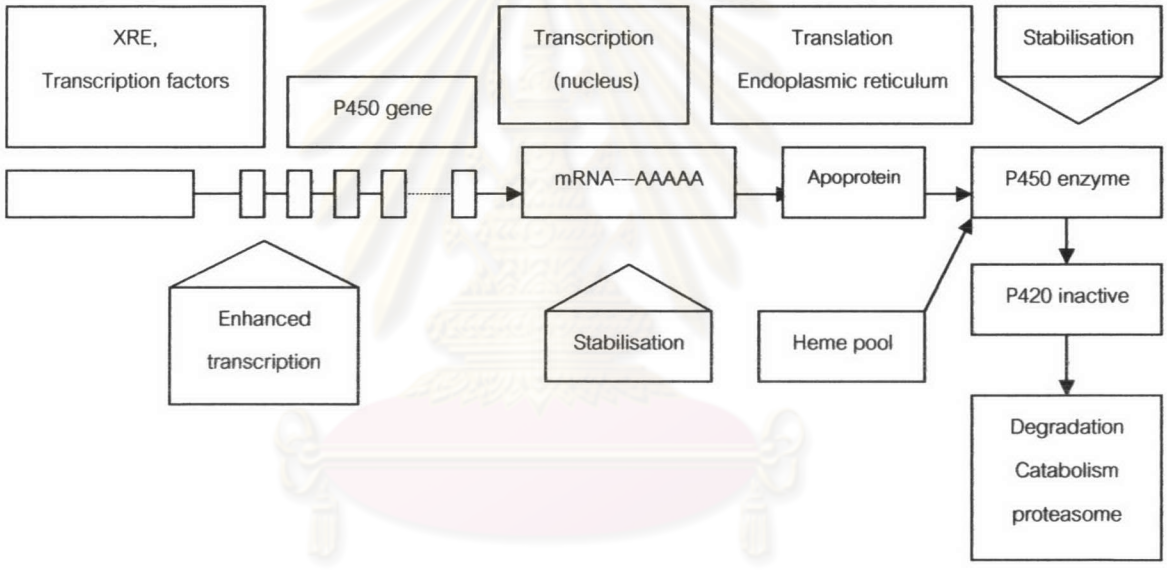


Figure 3 Steps in P450 expression (Berthou, F., 2001).

Table 5 Mechanisms of induction known to date for difference P450s (Berthou, F., 2001)

Induction mechanism	P450s known to be induced or regulated
Gene transcription through receptors	1A1 (cytosolic AhR), 1A2, 1B1 2A6, 2B6 (CAR), 2C8, 2C9, 2C18, 2C19 3A4, 3A5 (nuclear receptors PXR and SXR) 4A11 (PPARα)
mRNA processing	1A2
mRNA stabilisation	1A1, 2E1, 3A4
Enzyme stabilisation	2E1

Abbreviation used: AhR-aryl hydrocarbon receptor, CAR-Constitutive androstane receptor, PPAR-peroxisome proliferators activated receptor, PXR-pregnane (or pregnenolone)-receptor and SXR-Steroid-xenobiotic-receptor.

Mechanism of inhibition of CYPs

(Benedetti, M., 1998; Berthou, F., 2001; Gibson, G. and Skett, P., 1994; Lin, J.H. and LU, A.Y.H., 1998; Woolf, T., 1999)

Inhibition of P450 means either a decrease of metabolism of a particular xenobiotic by another xenobiotic simultaneously present in the active site of the enzyme. There are many known selective inhibitors of various P450s isoforms, and there are several different mechanisms by which a P450 isoform may be inhibited. This inhibition of drug metabolism by drugs or xenobiotics can take place in several ways including the destruction of pre-existing enzymes, inhibition of enzyme synthesis or by complexing and thus inactivating the drug-metabolising enzyme. Inhibition of drug metabolism may result in drug toxicity or induce carcinogens. Mechanism of CYPs inhibition can be classified into three categories.

1. Reversible inhibition

Reversible inhibition is probably the most common mechanism responsible for the documented drug interactions. Reversible interactions arise as a result of competition at the CYP active site and probably involve only binding of the substrate to the ferric form

of the enzyme. Many of the potent reversible CYP inhibitors are nitrogen-containing drugs, including imidazoles, pyridines and quinolines. These compounds can not only bind to the prosthetic haem iron, but also to the lipophilic region of the protein. The potency of an inhibitor is determined both by its lipophilicity and by the strength of the bond between its nitrogen lone electron pair and the prosthetic haem iron.

2. Quasi-irreversible inhibition via metabolic intermediate complexation

A large number of drugs, including methylenedioxybenzenes, alkylamines, macrolide antibiotics and hydrazines, undergo metabolic activation by CYP enzymes to form inhibitory metabolites. These metabolites can form stable complexes with the prostatic haem of CYP, called metabolic intermediate (MI) complex, so that the CYP is sequestered in a functionally inactive state. MI complexation can be reversed, and the catalytic function of ferric CYP can be restored by *in vitro* incubation with highly lipophilic compounds that displace the metabolic intermediate from the active site. The *in vivo* situations, the MI complex is so stable that the CYP involved in the complex is unavailable for drug metabolism, and synthesis of new enzymes is only means by which activity can be restored.

3. Mechanism-based inhibition ("suicide")

Mechanism-based inhibitors are compounds which are converted into an active form by P450 and are then able to be covalently bound or complexed, usually irreversibly, with either the heme moiety or to the protein within the active site of the enzyme.