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Malaria continues to be a major endemic infectious disease in many developing countries, especially in Asia, Africa and South America. It is estimated that nearly half of the world population are at risk, with fatal rates being extremely high among young children under 5 years of age. The disease is caused by *Plasmodium sp.*, especially *Plasmodium falciparum*, the pathogen responsible for the life threatening form of the disease. Lately the problems have been further complicated by many factors such as:

a)     The rapid emergence of drug resistant parasites, resulting in the drug's obsolescence at an alarming rate.

b)     Spreading of the disease to new grounds where they were hitherto unknown. This is due to the ease of communication and mobility of the population to and from the infected areas.

c)     Lack of global research funds.

d)     Being a "poor man's disease" malaria is categorized by pharmaceutical companies as low priority and therefore not much attention has been paid to the development of vaccines and new drugs against the parasites.

Concerted efforts from scientists, especially those in the endemic areas, in research towards finding new drugs or vaccines to fight malaria are required. Hence, novel antimalarials from synthesis, or from bioresources are urgently needed [1].

As part of an ongoing multidisciplinary research program on antimalarials conducted at Mahidol University and the National Center for Genetic Engineering and Biotechnology (BIOTEC) we have been searching for new antimalarials from plants and microorganisms by employing the activity guided separation and purification techniques. Also, in parallel, work has been carried out on the organic synthesis of new chemical entities guided by the understanding of the mechanism-of-action. Several compounds possessing impressive antimalarial activities, with some bearing novel structural architectures, have been obtained for further development, and examples of these are outlined here.

Antimalarials from Bioresources.

Earlier work on the leaves of *Azadirachta indica* var siamensis, commonly consumed by Thais as a vegetable, led to the isolation of the bitter principle which was identified as the triterpene **1**. The structural integrity of nimbolide (**1**) was proved by chemical correlations with the known limonoids, *i.e.* nimbin and nimbic acid. Nimbolide exhibits moderate antimalarial activity against *P. falciparum* in culture but is inactive *in vivo* [2]. Since **1** is available in large quantity from the leaves of the plant, we are now employing this compound as the starting material in our semisynthetic chemical modification program.
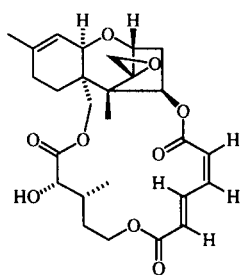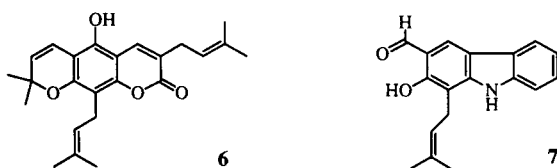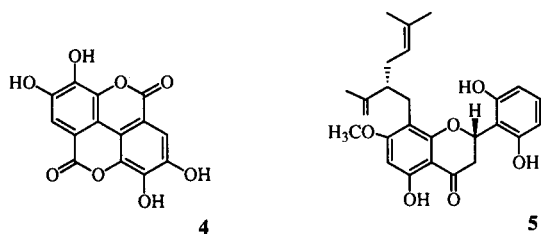


**1**
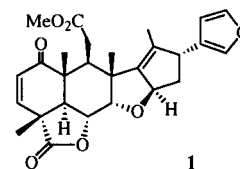
Activity guided isolation of antimalarial principles from other higher plants have yielded a wide range of chemical structures. Novel endoperoxide **2**, ($EC_{50}$ = 8 x $10^{-7}$ g/ml) was isolated from *Ammomum krervanh* Pierre [3], a widely used spice commonly known as *Round Siam Cardamom* in the spice market. Another sesquiterpene endoperoxide **3** was from *Cyperus rotundus* [4]. Interestingly, the endoperoxide functionality has recently been recognized as responsible for the activity against malarial parasites [5] and it should be noted that both *Ammomum krervanh* and *Cyperus rotundus*, the sources of compounds **2** and **3**, have long been documented and prescribed in Thai folklore medicines as a cure for "high fever".
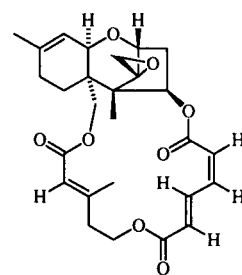


**2**                    **3**
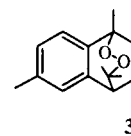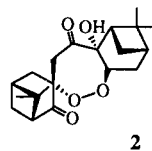
The screening of Thai medicinal plants used in primary health care [6] with alleged antimalarial activities has been carried out employing activity guided isolation and purification techniques and has also yielded active anti-
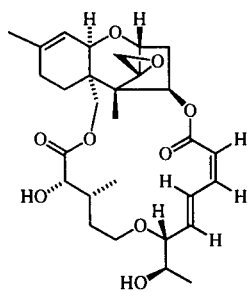
malarial principles. A few examples are shown below, ranging from the well known ellagic acid **4** ($EC_{50}$ = 1 x $10^{-8}$ g/ml), from *Harrisonia perforata* and *Irvingia malayanna*, to the flavanone, **5** ($EC_{50}$ = 4.6 x $10^{-6}$ g/ml), from *Artemisia indica* [7], to the coumarin **6** ($EC_{50}$ = 4 x $10^{-7}$ g/ml) and alkaloid **7** ($EC_{50}$ = 4.5 x $10^{-6}$ g/ml), from *Clausena harmandiana* [8].
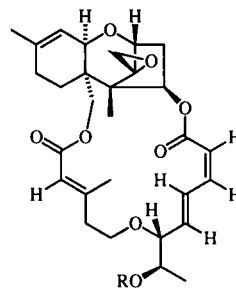
In our ongoing effort to identify novel naturally occurring antimalarial agents, fermentation extracts of microorganisms collected from various parts of Thailand, and deposited at the BIOTEC culture collection, have been subjected to activity screening, purification and structural elucidation.
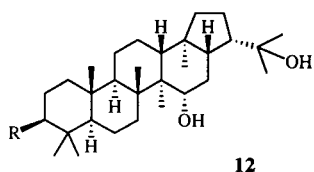
The strain *Myrothecium verrucaria* BCC 112, identified from the soil sample of Kanchanaburi province, western Thailand, provided a very reactive crude extract against *P. falciparum* in our *in vitro* screening assay. Activity guided isolation and purification resulted in the identification of several trichothecene derivatives **8-11**. Macrocyclic trichothecenes are known as mycotoxins, out of which, roridin E acetate (**11b**) deserves special mention. This compound was formerly synthesized from roridin E (**11a**), but has since been conclusively proved to be a natural product produced by *M. verrucaria* BCC 112. It's antimalarial and cytotoxic activities are shown in Table 1. It can be seen that the acetate **11b** is much more reactive against *P. falciparum* in culture when compared with the parent compound **11a**, or artemisinin, the now in use antimalarial drug. It is also far less toxic in various cell lines [9].



**4**

**5**

**6**

**7**

**8** Verrucarin A

($EC_{50}$ = 9 x $10^{-10}$ g/ml)

**9** Verrucarin J

($EC_{50}$ = 2 x $10^{-10}$ g/ml)

**10** Roridin A

($EC_{50}$ = 3.1 x $10^{-10}$ g/ml)

**11a** Roridin E ( R = H )

($EC_{50}$ = 1.5 x $10^{-10}$ g/ml)

**11b** Roridin E acetate ( R = Ac )

($EC_{50}$ = 0.6 x $10^{-10}$ g/ml)

Table 1

The Antimalarial and Cytotoxic Activities of Roridin E (**11a**) and Roridin E Acetate (**11b**)

| Compound | Antimalarial Activity (EC$_{50}$, ng/ml) P. falciparum | Cytotoxicity (EC$_{50}$, ng/ml) | | |
|---|---|---|---|---|
| | | KB Cells [a] | BCI Cells [b] | Vero Cells [c] |
| Roridin E (**11a**) | 0.15 | 0.5 | 0.7 | 0.4 |
| Roridin E Acetate (**11b**) | 0.06 | 14 | 15 | 4.1 |
| Artemisinin (**24**) | 1.1 | | | |

[a] Human epidermoid carcinoma in the mouth (oral cavity); [b] Human breast cancer cells; [c] African green monkey kidney fibroblast.

Triterpenes **12**, bioxanthracenes **13** and quinones **14** were obtained from insect pathogenic fungi *Aschersonia tubulata* I6850, *Cordyceps pseudomilitaris* BCC 1620 and *Cordyceps unilateralis* I3711, respectively [8].
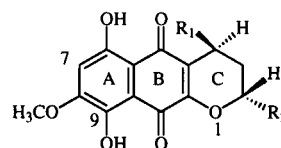


**14**

| | R$_1$ | R$_2$ |
|---|---|---|
| a | OH | (acetyl, C(=O)CH$_3$) |
| b | H | (acetyl, C(=O)CH$_3$) |
| c | OCH$_3$ | (acetyl, C(=O)CH$_3$) |
| d | OH | (CH(OH)CH$_3$) |
| e | H | (CH(OH)CH$_3$) |

EC$_{50}$ ~ 10$^{-6}$ g/ml



**12**

a: Dustanin, R = H
b: 3-Hydroxydustanin, R = OH
c: Dustanin-3-acetate, R = OAc (novel)

EC$_{50}$ ~ 10$^{-6}$ g/ml
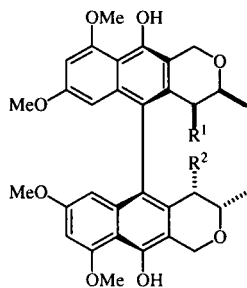


**13**

| R$^1$ | R$^2$ | Name | Antimalarial Activity EC$_{50}$, 10$^{-6}$ g/ml |
|---|---|---|---|
| OH | OH | ES-242-*4* | 4.7 |
| OH | OAc | ES-242-*3* | 8.1 |
| OAc | OAc | ES-242-*2* | 2.2 |
| OH | H | ES-242-*5* | 3.7 |
| OAc | H | ES-242-*1* | 5.2 |
| H | H | ES-242-*6* | 8.4 |

Wood-decayed fungus, *Xylaria sp.* BCC 1067, provided compounds **15-18**, while **19-23** were isolated from the marine mangrove fungus, *Aigialus parvus* [8].

Endoperoxide Derivatives.

Following the discovery of the antimalarial agent artemisinin (**24**) from the Chinese herb *Artemisia annua* Linn. [5], efforts have been directed toward the synthesis of its derivatives and other endoperoxides [10] for a better understanding of its mechanism-of-action [5,11-13]. Most of the artemisinin derivatives, including dihydroartemisinin (**25**), artemether (**26**), arteether (**27**) and sodium artesunate (**28**), all of which are now in clinical trials (clinical use !), are obtained by chemical manipulations at C-10 on the lactone carbonyl moiety of artemisinin, to increase solubility and bioavailability of the drug. A report that caught our attention was when artemisitene (**29**), an oxidized form of artemisinin, was found as a minor constituent in
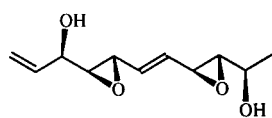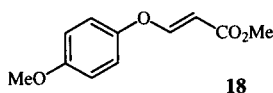
**15** (-)-Depudecin
EC$_{50}$ 1.0 x 10$^{-6}$ g/ml
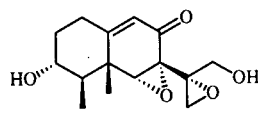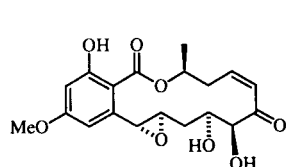
**16** (+)-Phaseolinone
EC$_{50}$ 0.50 x 10$^{-6}$ g/ml
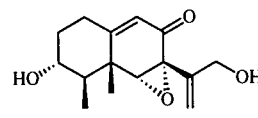
**17** (+)-Phomenone
EC$_{50}$ 0.32 x 10$^{-6}$ g/ml

**18**
EC$_{50}$ 19 x 10$^{-6}$ g/ml

**19** Hypothemycin
EC$_{50}$ 2.2 x 10$^{-6}$ g/ml

**20**
EC$_{50}$ >20 x 10$^{-6}$ g/ml

**21**
EC$_{50}$ >20 x 10$^{-6}$ g/ml

**22**
EC$_{50}$ 6.6 x 10$^{-6}$ g/ml
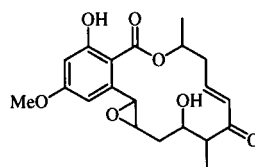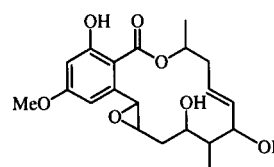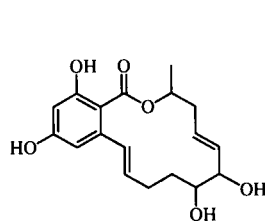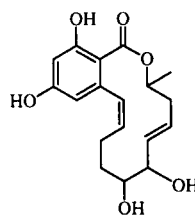
**23**
EC$_{50}$ >20 x 10$^{-6}$ g/ml

an American variant of *Artemisia annua* [14]. The unique structural feature of **29**, bearing an exocyclic methylene lactone moiety, presented an opportunity for nucleophilic attack *via* a Michael addition reaction. The overall process, which would finally provide the Michael adduct, *e.g.*, **30**, would be equivalent to a straightforward derivatization of the artemisinin molecule at the C-16 methyl carbon, and with further manipulation at C-10 would finally furnish **31** (Scheme 1).

Scheme 1



**25**

**26**, R = Me
**27**, R = Et
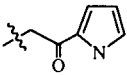**28**, R = COCH$_2$CH$_2$COO$^-$Na$^+$

**24**
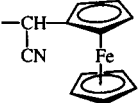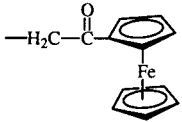
C-10
C-16

**31**

**29**

**30**
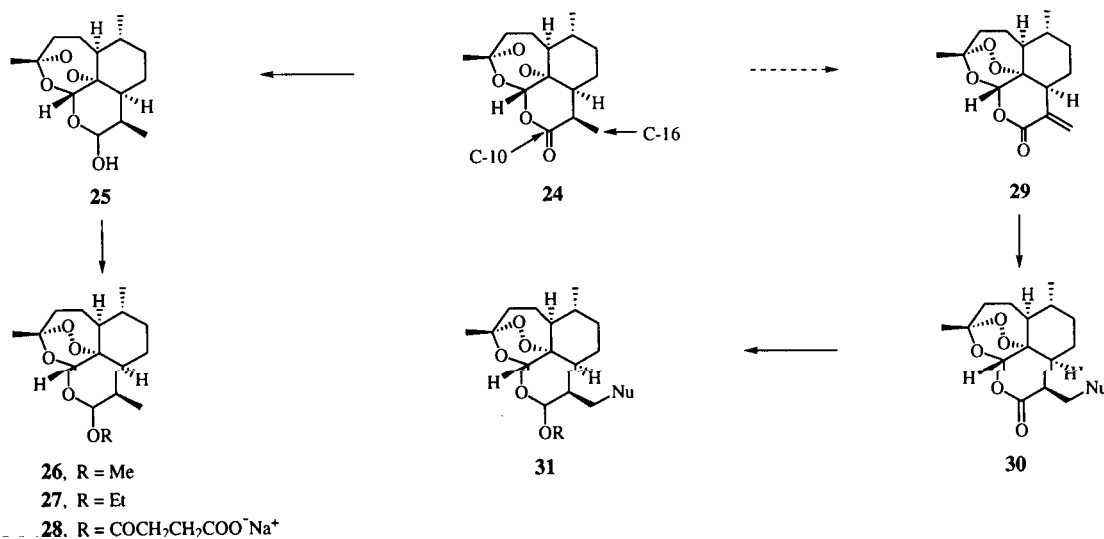
However, due to the fact that artemisitene (**29**) is found to co-occur in very small quantities with its parent compound **24** and that its extraction and purification are not straightforward, we have opted to develop a short one-pot synthesis of **29** from **24** [15]. We have employed the former as a Michael acceptor in nucleophilic addition reactions.

Numerous derivatives of the general structure **30** have been synthesized and, in certain cases, reduced to the lactol **31**. All of the compounds described (Table 2) display *in vitro* antimalarial activity against *P. falciparum* comparable to artemether (**26**) which was used as the standard in the screening.

Table 2



**30**
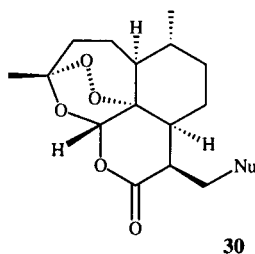
| Nu | EC$_{50}$ 10$^{-9}$ g/ml |
|---|---|
| *n*-Bu | 1.9 |
| —CMe$_2$CO$_2$Me | 0.1 |
| —CH(S$_2$CNEt$_2$)$_2$ | 1.12 |
|  | 9.65 |
| SPh | 0.2 |
|  | 1 |
|  | 2.1 |
|  | 0.32 |

Of particular interest is the dimer **33** obtained from the reaction of artemisitene (Michael acceptor) with the anion **32** (Michael donor), derived from artemisinin **24**. The stereochemical structure of the dimer was confirmed by X-ray crystalography which also indicated that the oxygen atoms in **33** are engulfed by the hydrophobic part of the molecule (Figure 1) [8]. The $EC_{50}$ value of **33** against *P. falciparum* in culture was found to be $5.7 \times 10^{-10}$ *M* as compared to $3.9 \times 10^{-9}$ *M* and $1.2 \times 10^{-8}$ *M* of artemisinin (**24**) and artemisitene (**29**), respectively. *In vivo* testings have also shown that compound **33** is active against both *P. yoelii* and *P. berghei*.
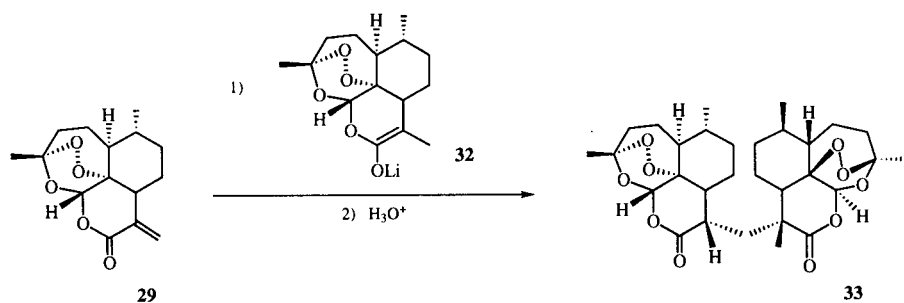


A computer generated space-filling diagram of **33** from X-ray crystallographic data.

● Oxygen    ◎ Carbon    ○ Hydrogen

Figure 1.

Conclusion.

Research on natural products provides bioactive chemical entities which can furnish leads and structural models for further development in the various fields, notably in the agricultural and pharmaceutical sciences. The selected research work on bioactive natural products described above serve as examples.

Acknowledgement.

REFERENCES AND NOTES

[1]    J. D. Phillipson and C. W. Wright, *Planta Med. Suppl. 1*, **57**, S53 (1991).

[2a]    S. Rochanakij, Y. Thebtaranonth, C. Yenjai and Y. Yuthavong, *Southeast Asian J. Trop. Med. Pub. Hlth.*, **16**, 66 (1985); [b] O. H. Bray, J. D. Connolly, W. Peters, J. D. Phillipson, B. L. Robinson, A. Tella, Y. Thebtaranonth, D. C. Warhurst and Y. Yuthavong, *Trans. Roy. Soc. Trop. Med. Hyg.*, **79**, 426 (1985).

[3]    S. Kamchonwongpaisan, C. Nilanonta, B. Tarnchompoo, C. Thebtaranonth, Y. Thebtaranonth, Y. Yuthavong, P. Kongsaeree and J. Clardy, *Tetrahedron Letters*, **36**, 1821 (1995).

[4]    C. Thebtaranonth, Y. Thebtaranonth, S. Wanauppathamkul and Y. Yuthavong, *Phytochemistry*, **40**, 125 (1995).

[5]    D. L. Klayman, *Science*, **228**, 1049 (1985).

[6]   N. R. Farnsworth and N. Bunyapraphatsara, eds., Thai Medicinal Plants Recommended for Primary Health Care System, Medicinal Plants Information Center, Mahidol University, 1992, p 48.

[7]   R. Chanphen, Y. Thebtaranonth, S. Wanauppathamkul and Y. Yuthavong, *J. Nat. Prod.*, **61**,1146 (1998).

[8]   Unpublished results.

[9]   M. Isaka, J. Punya, Y. Lertwerawat, M. Tanticharoen and Y. Thebtaranonth, *J. Nat. Prod.*, **62**, 329 (1999).

[10]   See for examples: [a] R. K. Haynes and S. C. Vonwiller, *J. Chem. Soc., Chem. Commun.*, 451 (1990); [b] G. H. Posner, C. H. Oh, L. Gerena and W. K. Milhous, *J. Med. Chem.*, **35**, 2459 (1992).

[11]   R. K. Haynes, H. Pai and A. Voerste, *Tetrahedron Letters*, **40**, 4715 (1999).

[12]   S. R. Meshnick, T. E. Taylor and S. Kamchonwongpaisan, *Microbiol. Rev.*, **60**, 301 (1996).

[13]   S. Kamchonwongpaisan, S. Paitayatat, Y. Thebtaranonth, P. Wilairat and Y. Yuthavong, *J. Med. Chem.*, **38**, 2311 (1995).

[14]   N. Acton and D. L. Klayman, *Planta Med.*, 441 (1985).

[15]   S. Paitayatat, B. Tarnchompoo, Y. Thebtaranonth and Y. Thebtaranonth, *J. Med. Chem.*, **40**, 633 (1997); see also F. S. El-Feraly, A. Ayalp, M. A. Al-Yahya, D. R. McPhail and A. T. McPhail, *J. Nat. Prod.*, **53**, 66 (1990).