Appendices

APPENDIX A

Cytotoxicity and *in vitro* antimalarial activity tests of Prof. Yusuke Wataya' Labolatory

(Kim *et al.*, 1999)

Toxicity against mammalian cell line: FM3A cells grew with a doubling time of about 12 h. Prior to exposure to drugs, cell density was adjusted to 5 x 10^4 cell/mL. A cell suspension of 995 μL was dispensed to the test plate, and compound at various concentrations suspended in dimethyl sulfoxide (5 μL) was added to individual wells of a multidish, 24 wells. The plates were incubated at 37 °C in a 5 % CO_2 atmosphere for 48 h. All of the test compounds were assayed in duplicate at each concentration. Cell numbers were measured using a microcell counter CC-130 (Toa Medical Electric Co., Japan). All data points represent the mean of three experiments. The EC_{50} value refers to the concentration of the compound necessary to inhibit the increase in cell density at 48 h by 50 % of control. Selectivity refers to the mean of EC_{50} value for FM3A cells per the mean of EC_{50} value for *P. falciparum*.

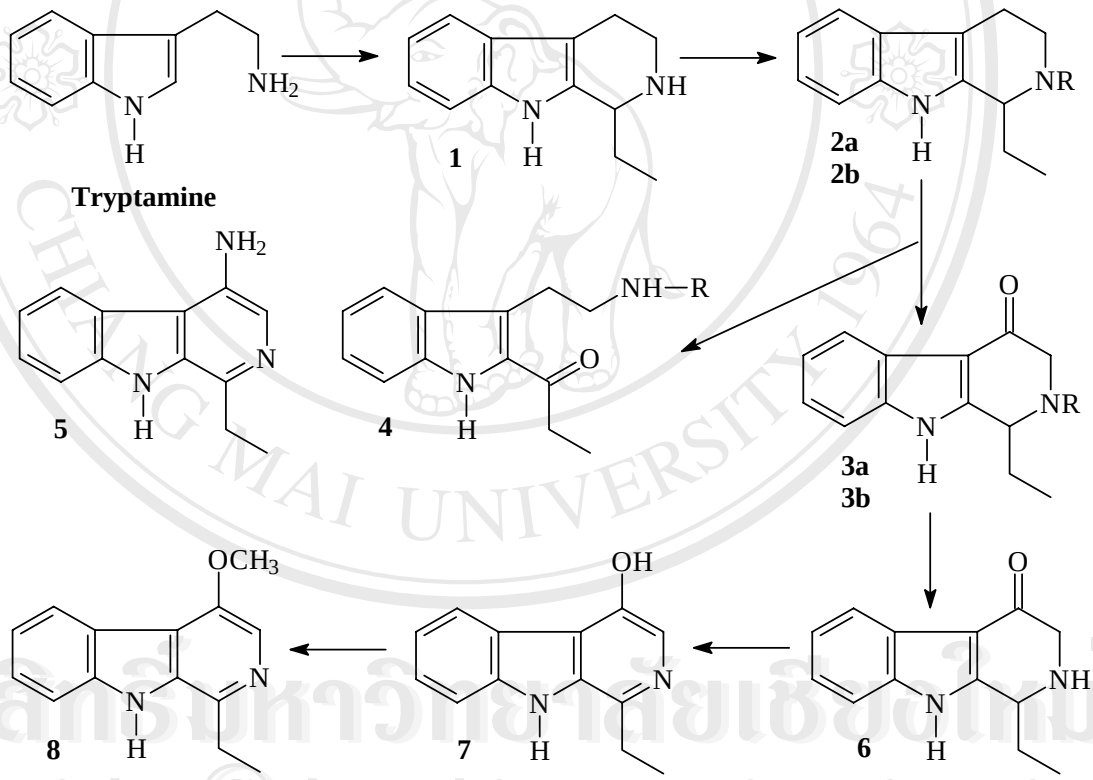
***In vitro* antimalarial activity:** Asynchronously cultivated *P. falciparum* were used. Various concentrations of compounds in dimethyl sulfoxide were prepared. Five microliters of each solution was added to individual wells of a multidish, 24 wells. Erythrocytes with 0.3 % parasitemia were added to each well containing 995 μL of culture medium to give a final hematocrit level of 3 %. The plates were incubated at 37 °C for 72 h in a CO_2-O_2-N_2 incubator (5 % CO_2, 5 % O_2, and 90 % N_2 atmosphere). To evaluate the antimalarial activity of test compound, we prepare thin blood films from each culture and stained them with Giemsa (E. Merck, Germany). Total 1 x 10^4 erythrocytes/1 thin blood film were examined under microscopy. All of the test compounds were assayed in duplicate at each concentration. Drug-free control cultures were run simultaneously. All data points represent the mean of three experiments. Parasitemia in control reached between 4 % and 5 % at 72 h. The EC_{50} value refers to the concentration of the compound necessary to inhibit the increase in parasite density at 72 h by 50 % of control.

APPENDIX B

Synthesis routes for 1-ethyl-4-methoxy-β-carboline (Crenatine) reported

1. Prof. Cook' method

Method 1 (Cain *et al.*, 1982)



a; R = C(O)Ph, b; R = C(O)CCl$_3$

1-Ethyl-1,2,3,4-tetrahydro-*9H*-pyrido[3,4]indole (**1**) was prepared in 96 % yield by a Pictet-Spengler reaction of tryptamine with propionaldehyde and subsequently converted into the corresponding amides **2a** and **2b** on treatment with benzoyl chloride or trichloroacetyl chloride, respectively. It was necessary to protect the amine **1** as an amide since charge-transfer complexes are known to from between DDQ and amines, thus rendering the reagent less effective. Treatment of benzamide **2a** with DDQ in an aqueous medium under a variety of conditions always furnished a mixture of the desired 3-acyl-indole 3a and keto amide **4**, the product of attack at C-1 of the tetrahydro-β-carboline. Generally the ratio of **3a** to **4** increased with decreasing temperature (ca. 1:1 at room temperature, 2:1 at 0 °C, and 5:1 at –78 °C), and it is clear that lowering the temperature at which the DDQ oxidation is carried out favors oxidation of the tetrahydro-β-carboline at C-4 in preference to reaction at C-1.

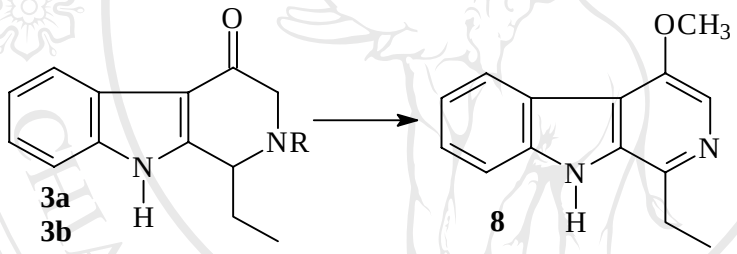
The related trichloroamide **2b** was oxidized with DDQ in methanol at –78 °C to provide the desired ketone **3b** in only 29 % yield; however, when a solution of acetone/water (9:1) cooled to –78 °C was dropped into a mixture of solid DDQ and **2b**, a 77 % yield of the 3-acylindole **3b** was obtained. Ethyl -β-carboline **5** was also isolated from this process in 6 % yield; the lability of the trichloramide toward hydrolysis, as compared to the benzamide, may account for the formation of **5**. In spite of the propensity for 2a to provide significant amounts of the amide **4**, the 3-acylindoles **3a** and **3b** returned only starting material when subjected to a similar oxidation. The usual blue coloration observed when DDQ is dissolved with indoles in solution was not observed with **3a** or **3b**. Since electron-withdrawing groups inhibit the formation of charge-transfer complexes with DDQ, it is not surprising that the ketones **3a** and **3b** (vinylogous amides) are inert to DDQ oxidation. Similarly, the 2-acylindole **4** could not be oxidized with DDQ, and this reaction mixture did not take on a blue color at anytime. Furthermore, a carbonyl substituent located on the indole nitrogen (amide), such as that contained in hexahydrocanthin-6-one, likewise prevents the formation of the necessary charge-transfer complex and results in the recovery of the starting hexahydrocanthin-6-one derivative.

Removal of the protecting groups (amides) from **3a** or **3b** was best carried out in base with the high dilution as a result of the limited solubility of these two compounds; furthermore, attempts to hydrolyze **3a** or **3b** under acidic conditions afforded an inseparable mixture of compounds. Although the trichloroamide function of **2b** can be easily cleaved in refluxing

hydrazine to give **1**, use of these conditions with keto amide **3b** gave instead 1-ethyl-4-amino-β-carboline (**5**) in 68 % yield.

The 3-acylindole **6** was converted into the desired 1-ethyl-4-hydroxy-β-carboline (**7**) on heating with sulfur in xylene. This method proved superior to the use of either palladium on carbon or chloranil for oxidation and has been used previously in their laboratory to prepare benzodiazepine receptor antagonists. Finally, the phenol **7** was then methylated with diazomethane to provide the natural product crenatine (**8**) in 63 % yield.

Method 2 (Hagen *et al.*, 1989)



3a
3b

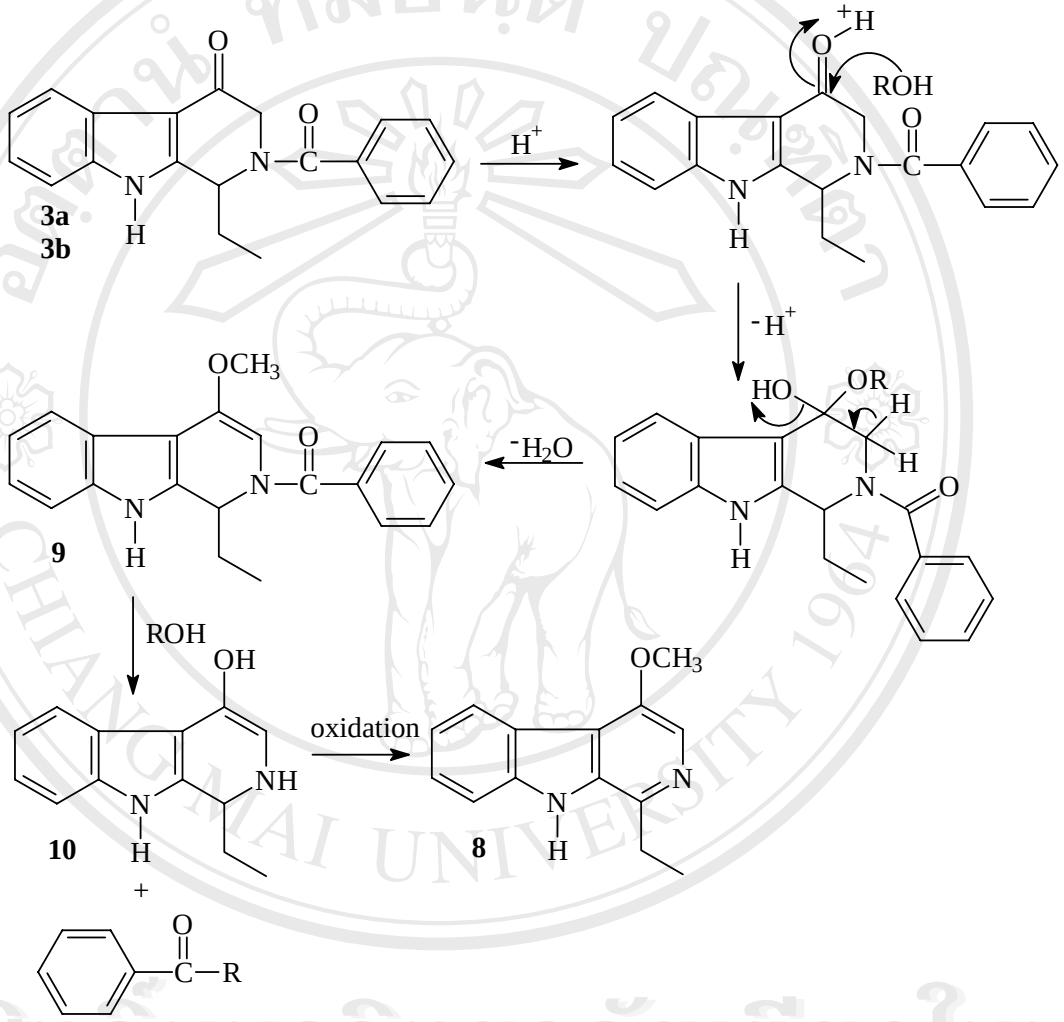
8

a; R = C(O)Ph, b; R = C(O)CCl$_3$

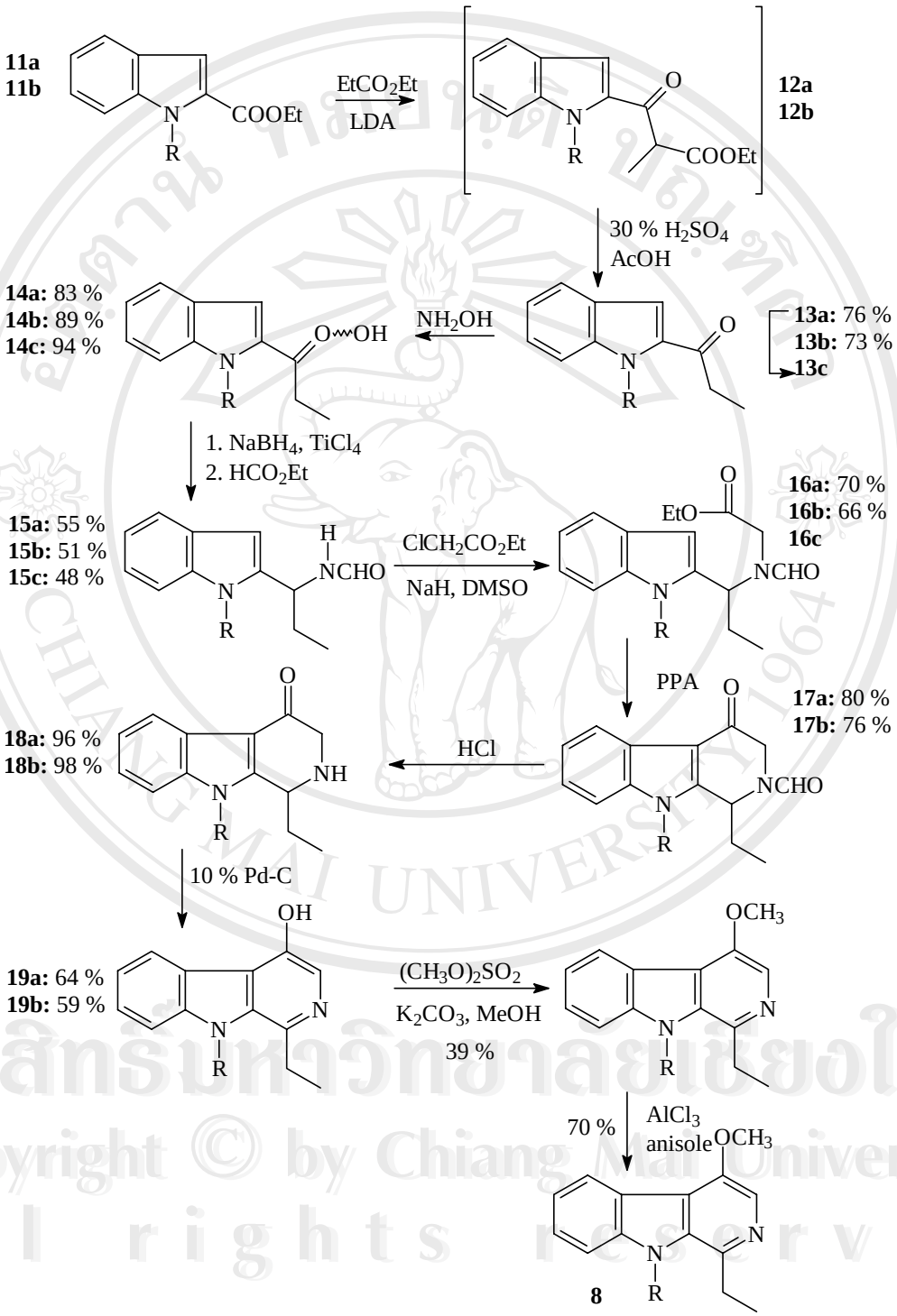
When 1-ethyl-4-oxo-1,2,3,4-tetrahydro-β-carboline (**3a**) was heated in methanol and trimethyl orthoformate in the presence of *p*TSA, **8** was obtained in 42 % yield; moreover, reaction of the 4-oxo-2-trichloroacetamide derivative **3b** under similar condition [CH_3OH, $(CH_3O)_3CH$, H_2SO_4) gave **8** in 48 % yield.

A proposed mechanism for this transformation was as follow described. Formation of the hemiketal of 3a, followed by loss of water, would generate the desired enol ether **9**. The amide, which is now activated to hydrolysis, could undergo reaction with methanol to provide 1,2-dihydro-β-carboline **10**. Intermediates of this type are known to undergo oxidation-disproportionation to provide β-carbolines in related systems. It is also conceivable that 1,2-

dihydro-β-carboline **10** may initially undergo oxidation-disproportionation to generate an acylpyridinium intermediate, followed by hydrolysis to give **8**.



2. Synthesis of crenatine from ethyl 1-benzylindole-2-carboxylate (Murakami *et al.*, 1991)



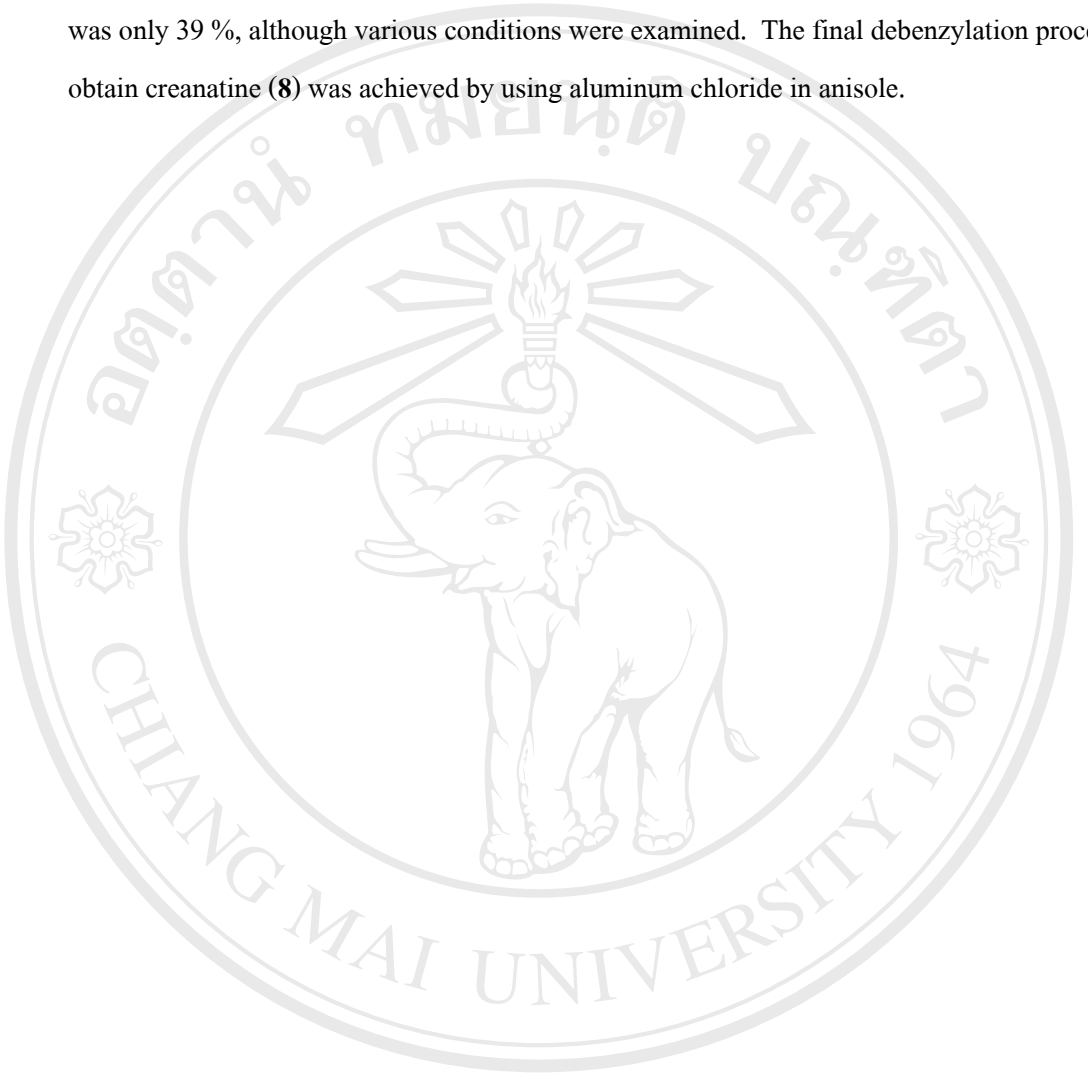
a: R = PhCH$_2$, b: R = Me, c: R = H

The strategy involves the use of the 2-carboethoxy group of ethyl indole-2-carboxylates (**11**) as a one-carbon unit, and cyclization of the elongated C_2-substituent to the nucleophilic 3-position. It is an advantage that the cyclization also results in the introduction of an oxygen functionality at the 4-position of the β-carboline skeleton. However, this cyclization reaction has a problem as to the direction of cyclization. Thus, ethyl 1-benzyl-1*H*-indole-2-carboxylate was used as a substrate in order to protect the 1-position. The reason for use of the benzyl group is that the electron-donating 1-benzyl group should make the C_3-position more nucleophilic and that the benzyl group can be removed at any time after cyclization by means of a new and mild method which them had developed for debenzylation of 2-acylindoles.

The 1-benzylindole (**11a**) was allowed to react with ethyl propionate under Claisen condensation conditions to give the keto-ester (**12a**). The keto-ester (**12a**) was, without purification, treated with sulfuric acid to give 1-benzyl propionyl-1H-indole (**13a**) (ketone degradation). Conversion of **13a** into the glycinate (16a) by treatment with ethyl glycinate *via* formation of the Schiff's base or reductive amination was unsuccessful. The introduction of a nitrogen functionality into the 2-propionylindole (**13a**) to obtain the formamide (15a) was achieved by employing the Leuckart reaction under high temperature and pressure, but the yield was variable (8-54 %), and the autoclaving procedure was inconvenient. For improvement of this step, the propionyl indole (**13a**) was converted to the oxime (**14a**). The oxime (**14a**) was separable into two geometric isomers [(E)- and (Z)-**14a**] but their configuration was not determined. The oxime [**14a**, a mixture of (E)- and (Z)-] was reduced with sodium borohydride-titanium tetrachloride and the resulting amine was formylated with ethyl formate to give the same formamide (**15a**). This reaction was better than the above-mentioned Leuckart reaction in the viewpoints of easy handling and average yield. The *N*-alkylation of the formamide (**15a**) with ethylchloroacetate under basic conditions smoothly gave the glycinate (**16a**). Although the glycinate (**16a**) showed a clear single spot on TLC and sharp melting point, ^{1}H-NMR of **16a** showed apparently an equimolar mixture of two isomers. This can be explained in terms of rotational isomerism due to the formamide moiety. The glycinate (**16a**) was then cyclized to polyphosphoric acid (PPA) to give the cyclic ketone (**17a**).

The hydrolysis of the cyclic ketone (**17a**) with hydrochloric acid gave the NH-compound (**18a**), which was in turn aromatized with 10 % palladium on carbon to give the 4-hydroxy-β-

carboline (**19a**) in a reasonable yield. The methylation of compound **19a** was carried out with diazomethane or dimethyl sulfate-base to give the desired methyl ether (**20**). But the best yield was only 39 %, although various conditions were examined. The final debenzylation process to obtain creanatine (**8**) was achieved by using aluminum chloride in anisole.



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Drug Synthesis and Development Using Thai Medicinal Plants as an Origin

Master degree thesis title: Synthesis of Morin Esters