CHAPTER III

EXPERIMENTATION

A. Isolation and Antimalarial activity of *P. javanica*

Instruments

Nuclear magnetic resonance: JMR-A500

GC-MS: Varian GC Star 3400CX coupled with Varian SATRUM 2000

Chemicals

Methanol: Lab-Scan, AR; 99.8 % (GC)

Chloroform: Merck, pro analysi; 99.0-99.4 % (GC)

n-Hexane: Merck, pro analysi; 99 % (GC)

Ethyl acetate: Merck, pro analysi; 99 % (GC)

Plant materials

Wood and stembark of *P. javanica* were collected from Queen Sirikit Botanical Garden, Chiang Mai, Thailand in July, 2000, and were identified by comparing with the references deposited there, and at Pharmacy Faculty, Chiang Mai University.

Preparation of crude extracts

About 100 g (Table 5) of dried ground of plant materials were separately macerated in 600 mL methanol, chloroform, hexane for three days or boiled with 1.5 L of water for 10 hours. Then, they were filtered and evaporated to dryness under reduced pressure. The residue plant materials were extracted again using the same process. The second extracts were pooled together with the first corresponding extracts.

Table 5: Preparation of crude extracts

Part used	**Amount of *P. javanica* used in each solvent (g)**			
	methanol	chloroform	*n*-hexane	water
wood	121.120	105.574	102.952	111.605
stembark	129.465	103.678	119.626	116.069

Isolation of hexane extract from stembark

Preparation of hexane crude extracts

2,206.36 g dried ground *P. javanica* stembark was macerated in 6 L of hexane for three days. Then, it was filtered and evaporated to dryness under reduced pressure. The residue plant material were extracted again using the same process. The second extract was pooled together with the first corresponding extract.

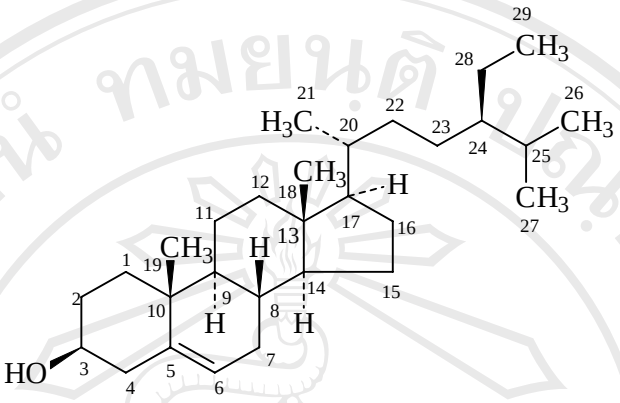
Isolation of hexane crude extract

12.152 g of hexane crude extract was mixed with kieseguhr (12 g) and chromatographed on a silica gel 60 (230-400 mesh, 300 g) column using hexane-ethyl acetate (9:1, 7:3, 5:5, 3:7, 1:9, 0:10) as eluent. Ten fractions were collected based on their chromatogram on silica gel 60 GF_{254} TLC plate using hexane-ethyl acetate (7:3) as the solvent system. These were designated as Fraction I, II, III, …, X.

Purification of fraction with antimalarial activity

Based on antimalarial activity of each fraction, Fraction V (0.7593 g) was further purified by column chromatography (SiO_2 60 g, eluents; hexane, hexane-ethyl acetate 9:1, hexane-ethyl acetate 8:2, chloroform-ethyl acetate 19:1, ethyl acetate, methanol) to give six isolated fractions, designated as Fraction V-1, V-2,…,V-6. Fraction V-3 was crystallized in methanol to provide white crystal of 102.1 mg.

Structure elucidation of Fraction V-3



The chemical structure of Fraction V-3 was elucidated from ^{1}H- and ^{13}C-NMR spectra. These spectra were performed with JEOL JMN-A 500 spectrometer. The solvent for these spectra was deuterated chloroform using tetramethylsilane as the internal reference standard. The chemical shift were reported in ppm scale: ^{1}H-NMR ($CDCl_3$, 500 MHz) δ 0.66-0.98, 1.00-2.29, 3.50 (m), 5.33 (m); ^{13}C-NMR ($CDCl_3$, 125 MHz) δ 11.83 (C-18), 11.96 (C-29), 18.76 (C-21), 19.01 (C-27), 19.37 (C-19), 19.79 (C-26), 21.06 (C-11), 23.04 (C-28), 24.29 (C-15), 26.04 (C-23), 28.22 (C-16), 29.11 (C-25), 31.61 (C-2), 31.87 (C-7 and C-8), 33.91 (C-22), 36.11 (C-20), 36.48 (C-10), 37.22 (C-1), 39.75 (C-12), 42.25 (C-4 and C-13), 45.80 (C-24), 50.10 (C-9), 56.02 (C-17), 56.73 (C-14), 71.78 (C-3), 121.69 (C-6), 140.71 (C-5).

Isolation of chloroform crude extract from stembark

Preparation of chloroform crude extracts

1,628.33 g dried ground *P. javanica* stembark was macerated in 4 L of chloroform for seven days. Then, it was filtered and evaporated to dryness under reduced pressure. The residue plant material were extracted again using the same process. The second extract was pooled together with the first corresponding extract.

Isolation of chloroform crude extract

21.901 g of the chloroform crude extract was dissolved in chloroform (100 ml) and extracted with 2% sulfuric acid (5 x 100 ml). The combined acid extract was made alkaline with 25% ammonia solution and repeatedly extracted with five portion of chloroform (400, 4 x 200 ml), dried with anhydrous sodium sulfate, filtered and dried under reduced pressure to provide an alkaloidal portion. Purification of alkaloidal portion using preparative TLC was performed on 5 mm coated of silica gel 60 254F, 20x20 glass plate using hexane-ethyl acetate (7-3) as mobile phase.

Structure elucidation of alkaloidal portion

The chemical structure of major compound in alkaloidal portion was elucidated from mass spectra and also compared with the data from literature. GC chromatogram and mass spectra of extracts were carried on Varian GC Star 3400CX coupled with Varian SATRUM 2000 GC-MS system using J&W Scientific 30 m x 0.251 mm DB-5MS column with 0.25 μm film thickness and helium as carrier gas. The temperatures of the GC-MS instrument were set as 280 °C at the injector, 260 °C at the transfer line and GC oven was programmed as followed: the temperature was initially held at 100 °C for 1 min, increased at 16.6 °C/min to 150 °C and held for 1 min, increased at 22 °C/min to 260 °C and held for 15 min. The first run, mass analysis parameters was set for electron impact (EI) mode. After 17 days, the second run was performed. The holding time at 260 °C was decreased to 10 min and mass analysis parameter was set for chemical ionization (CI) mode using methane as reagent gas.

***In vitro* anti-malarial activity test (by Kamchonwongpaisan's group)**

The antimalarial activity of extracts against *P. falciparum* K1 infected red cell was measured by using the [^{3}H]hypoxanthine incorporation method reported by Desjardins *et al*. (1979) and modified by Kamchonwongpaisan *et al*. (1995). Briefly, extract was dissolved in dimethyl sulfoxide (DMSO) and diluted with the culture medium to the required concentration. A mixture of 25 μL of the medium containing a sample and 200 μL of 1.5 % cell suspension with 1-2 % parasitemia at ring stage was cultured for 24 h, after which 25 μL of 0.25 μCi [^{3}H]hypoxanthine was added. After an addition 18 h in culture, the cells were harvested onto

glass-fiber filters (Unifilter®, Packard, USA). The filters were air-dried and 20 μL liquid scintillation fluid (Microscint, Packard) was added. The radioactivity on the filters was then measured using a microplate scintillation counter (Topcount, Packard, USA). The IC_{50}s, the concentrations required for 50 % reduction of the radioactivity as compared to control without the sample, of the sample against these infected cells were obtained from dose-response curves.

B. Synthesis, cytotoxicity and antimalarial activity of 1-substituted-4-oxygenated-β-carbolines

Instruments

Nuclear magnetic resonance spectrometer: Varian Gemini 2000 (300 MHz FT-NMR)

Mass spectrometer: JEOL-JMS-O1SG-2, JEOL-DX-300

Chemicals

Tryptamine: Aldrich; 98 %

Acetadehyde: Merch; 99 % (GC)

Propionadehyde: Wako; 95.0 % (GC)

H_2SO_4: Wako; 95+% (Ti)

Benzoyl chloride: TCI; >98.0 % (T)

DDQ: aldrich; 98 %, TCI; > 97 % (T)

$(CH_3O)CH$: Wako; 98 % (GC)

DMAP: TCI; 99.0+ %(T)

Triethylamine: Wako; 98 % (GC)

Methyl iodide: TCI; 99.5+ % (GC)

p-Chloranil: TCI; 95.0+ % (T)

Sulfolane: nacalai tesque; 99 %

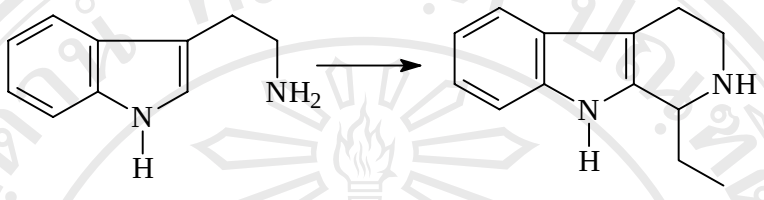
p-Toluenesulfonic acid monohydrate: TCI; 98.0+ % (T)

$(CH_3O)_2C(CH_3)_2$: TCI; 98+ % (GC)

Siliga gel: Chromatorex chromatography silica gel 100-200 mesh

Synthesis and structure elucidation of 1-substituted-4-oxygenated- β-carbolines

1-ethyl-1,2,3,4-tetrahydro-β-carboline (4)



Tryptamine **4**

RUN 1

To the mixture of tryptamine (2.0099 g, 12.54 mmol) and 10 % MeOH (12.50 mL) was added propionaldehyde (1.35 mL, 18.83 mmol), and then conc. H_2SO_4 (0.70 mL, 6.78 mmol). Refluxed for 6.5 h, the reaction mixture was evaporated to remove MeOH, basified with 28 % ammonia solution (2 mL) and extracted with EtOAC (4 x 10 mL). The extract was washed with 10 % NaCl solution (1 x 20 mL), dried (K_2CO_3) and evaporated to yield sticky brown product **4** (1.5708 g, 62.61 %).

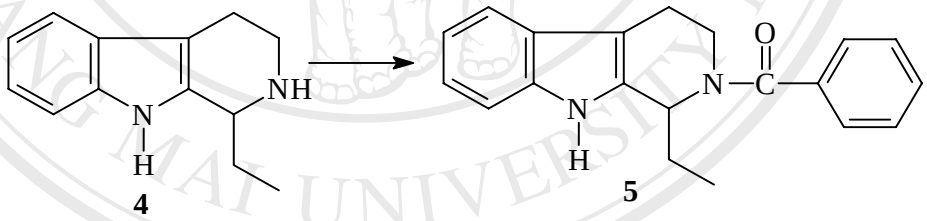
RUN 2

To the mixture of tryptamine (2.0144 g, 12.57 mmol) and 10 % MeOH (12.50 mL) was added conc. H_2SO_4 (0.70 mL, 6.78 mmol), and then propionaldehyde (1.35 mL, 18.83 mmol). Refluxed for 1.5 h, second portion of propionaldehyde (1.35 mL, 18.83 mmol) was added and continued to reflux up to 6.5 h. The reaction mixture was evaporated to remove MeOH, basified with 28% ammonia solution (2 mL) and extracted with EtOAc (4 x 10 mL). The EtOAc extract was washed with 10 % NaCl solution (1 x 20 mL), dried (K_2CO_3) and evaporated to yield sticky brown product **4** (2.5145 g, 125.64 %).

RUN 3

To the mixture of tryptamine (7.0083 g, 43.74 mmol) and 10 % MeOH (45.00 mL) was added conc. H_2SO_4 (2.40 mL, 23.25 mmol), and then propionaldehyde (9.50 mL, 132.51 mmol). The mixture was refluxed for 6.5 h, epavorated to remove MeOH, basified with 28 % ammonia solution (6.9 mL), and extracted with EtOAc (4 x 60 mL). The EtOAc extract was washed with 10 % NaCl solution (1 x 50 mL), dried (K_2CO_3), evaporated to yield sticky brown product (10.1251 g, 115.66 %) that was crystallized in Et_2O to provide white fine crystal (2.0526 g). The filtrate obtained from crystallization was concentrated and chromatographed through silica gel column (column dimeter 2.5 inch, silica gel 60 g) with suction using EtOAc (100 mL and $CHCl_3$-MeOH (1-1) as eluent, the result provided pale yellow product (5.5637 g). Total yield of **4** was 7.6152 g (86.99 %).

1-ethyl-2-benzoyl-1,2,3,4-tetrahydro-β-carboline (5)



4 **5**

RUN 4

To the solution of **4** (1.5708 g, 7.85 mmol) in pyridine-benzene (11.75-23.50 mL) was added benzoyl chloride (1.65 mL, 14.18 mmol) and then gradually from ambient temperature to 60 °C, and maintained heating at this temperature; total time was 30 min. After the reaction mixture was cooled, quenched with water (95 mL), two layers were separated. Aqueous layer was extracted with benzene (2 x 40). Combined organic layer was washed with water (2 x 50 mL), saturated Na_2CO_3 solution (3 x 50 mL), water (4 x 95 mL), and 10 % NaCl solution (2 x 50 mL). And then dried (Na_2SO_4) and evaporated to yield sticky oil brown product (2.7836 g,

116.39 %). This product was chromatographed on silica gel column using EtOAc as eluent, and finally crystallized in MeOH to yield white fine crystal **5** (1.1385 g, 47.69 %).

RUN 5

To the solution of **4** (1.8884, 9.44 mmol) in pyridine-benzene (14.2-28.27 mL) was added benzoyl chloride and then gradually heated to 60 °C, and maintained heating at this temperature; total time was 30 min. After the reaction mixture was cooled, quenched with water (100 mL), two layers were separated. Aqueous layer was extracted with EtOAc (2 x 50 mL). Combined organic layer was evaporated to provide sticky brown product that was chromatographed through silica gel column. Thus the result provided brown product that was chromatographed through silica gel column again to provide white fine crystal **5** (0.7119 g, 24.81 %).

RUN 6

Benzoyl chloride (0.24 mL, 2.0829 mmol) was added to the mixture of **4** (204.1 mg, 1.0198 mmol), dichloromethane (10.00 mL), DMAP (13.0 mg, 0.1064 mmol), and triethylamine (0.17 mL, 1.2096 mmol) and then gradually heated to 60 °C, and maintained heating at this temperature with total time of 30 min. After the reaction was cooled, 1 *N* NaOH solution (10 mL) was added and two layers were separated. Then aqueous layer was extracted with EtOAc (2 x 10 mL). Combined organic layer was dried (Na_2CO_3), and evaporated to yield white powder (419.3 mg) that was crystallized from MeOH resulted in white fine crystal **5** (0.1287 mg, 41.49 %).

RUN 7

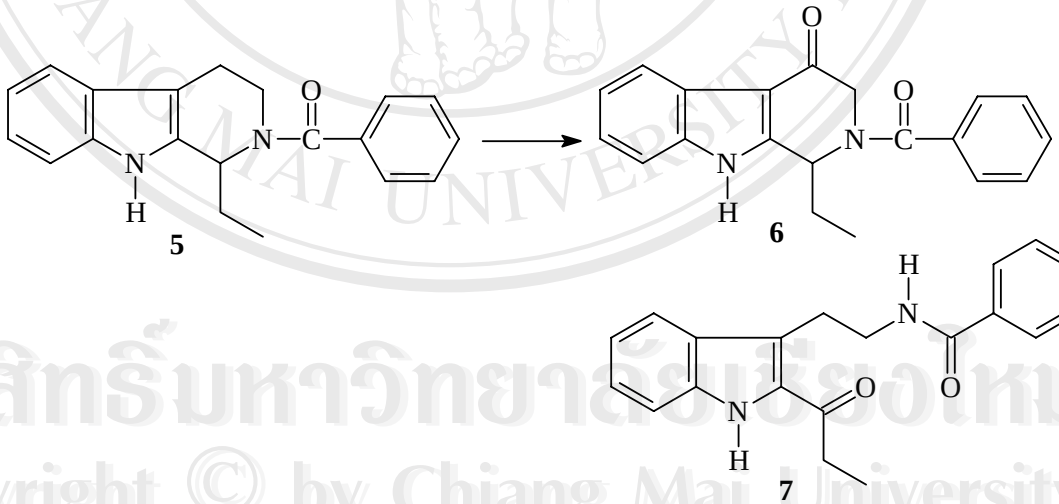
Benzoyl chloride was added to the mixture of **4** (3.6625 g, 18.30 mmol), dichloromethan (50.00 mL), DMAP (217.2 mg, 1.78 mmol), and triethylamine (3.00 mL, 21.35 mmol) and then gradually heated to 60 °C, and maintained heating at this temperature; total time was 30 min. After the reaction mixture was cooled, added 1 *N* NaOH solution (30 mL) and two layers were separated. Then aqueous layer was extracted with dichloromethan (2 x 30 mL). Combined organic layer was dried (Na_2CO_3), and evaporated resulted in sticky brown product (9.3911 g) that was crystallized in MeOH to provided pale yellow fine crystal (2.7383 g). The supernatant

was let to provide pale yellow fine crystal (1.9951 g). The total amount of **5** was 4.7334 g (85.04 %).

RUN 8

Benzoyl chlolride was added to the mixture of **4** (1.9032 g, 9.5098 mmol), dichloromethane (50.00 mL), DMAP (125.7 mg, 1.0289 mmol), and triethylamine (1.70 mL, 12.0961 mmol) and then stirred at ambient temperature for 1 h. The reaction mixture was added 1 *N* NaOH (30 mL) and two layer were separated. Aqueous layer was extracted with chloroform (2 x 50 mL). Combined organic layer was washed with water (2 x 100 mL), dried (Na_2SO_4) and evaporated to yield brown product that was crystallized in MeOH to provide white fine crystal **5** (2.0859 g, 72.12 %).

1-ethyl-2-benzoyl-4-oxo-1,2,3,4-tetrahydro-β-carboline (6)



RUN 9

To the solution of **5** (0.1066 g, 0.35 mmol) in THF-H_2O (9-1, 4 mL) sank in ice bath was added solution of DDQ (0.1601 g, 0.71 mmol) in THF (1.1 mL) and stirred for 1 h. Then the blue mixture was stirred at ambient temperature for 2.5 h. The red reacted mixture was added 1 *N*

NaOH (15 mL) and extracted with EtOAc (3 x 30 mL). The extract was washed with 10 % HCl solution (1 x 15 mL), dried (Na_2CO_3) and evaporated to yield sticky brown product (0.1362 g). This product was chromatographed on Al_2O_3 column using $CHCl_3$-EtOAc (19-1) as eluent, the result provided compound **6** (51.0 mg, 35.96 %) and side product **7** (40.1 mg).

RUN 10

To the mixture of **5** (0.1010 g, 0.33 mmol) and DDQ (0.1600 g, 0.70 mmol) sank in dry ice-acetone bath was added slurry of THF-H_2O (9-1, 5 mL). Then the blue mixture was stirred and allowed to warm to ambient temperature for 6 h. The red reacted mixture was added 1 *N* NaOH solution (15 mL) and extracted with EtOAc (3 x 30 mL). The extract was washed with 10 % HCl solution (1 x 15 mL), dried (Na_2CO_3) and evaporated to yield sticky brown product (0.1004 g). This product was chromatographed on silica gel column using $CHCl_3$-EtOAc (19-1) as eluent. The result provided **6** (18.0 mg, 17.0 %), side product **7** (11.7 mg) and the mixture of **6** and side product **7** (29.2 mg).

RUN 11

To the mixture of **5** (0.1024 g, 0.34 mmol) and DDQ (0.1640 g, 0.72 mmol) sank in dry ice-acetone bath was added slurry of THF-H_2O (9-1, 5 mL) and stirred for 3 h. Then the blue mixture was stirred and allowed to ambient temperature for 16 h. The red reacted mixture was added 1 *N* NaOH solution (15 mL) and extracted with EtOAc (3 x 30 mL). The extract was washed with 10 % HCl solution (1 x 15 mL), dried (Na_2CO_3) and evaporated to obtain brown product (0.1032g, 96.35 %). This product was chromatographed on Al_2O_3 column using $CHCl_3$-EtOAc (19-1) as eluent. The result provided **6** (38.1 mg, 35.57 %) and side product **7** (27.6 mg).

RUN 12

To the mixture of **5** (0.1024 g, 0.34 mmol) and DDQ (0.1950 g, 0.86 mmol) sank in dry ice-acetone bath was added slurry (-10 °C) of THF-H_2O (9-1, 5 mL) and stirred for 1.25 h. Then the blue mixture was stirred at ambient environment for 19 h. The red reacted mixture was added 1 *N* NaOH solution (15 mL) and extracted with EtOAc (3 x 30 mL). The extract was washed with 10 % HCl solution (1 x 15 mL), dried (Na_2CO_3) and evaporated to obtain brown product

(0.1158 g). This product was chromatographed on silica gel column using EtOAc-Hexane (2-1), EtOAc, and MeOH as eluent. The result provided **6** (56.6 mg, 52.84 %) and side product **7** (13.9 mg).

RUN 13

To the mixture of **5** (0.5438 g, 1.79 mmol) and DDQ (0.8818 g, 3.88 mmol) sank in dry ice-acetone bath was added slurry (-10 °C) of THF-H_2O (9-1, 50 mL) and stirred for 3 h. Then the blue mixture was stirred at ambient environment for 18 h. The red reacted mixture was poured in to 1 *N* NaOH solution (50 mL) and extracted with EtOAc (3 x 60 mL). The extract was washed with 10 % HCl solution (1 x 50 mL), dried (Na_2CO_3) and evaporated to obtain brown product (0.6583 g). This product was chromatographed on silica gel column using EtOAc-Hexane (2-1) as eluent. The result provided **6** (0.3175 g) that was crystallized in MeOH to yield white fine crystal (275.4 mg, 48.42 %) and side product **7** (93.4 mg).

RUN 14

To the mixture of **5** (0.5069 g, 1.67 mmol) and DDQ (0.8347 g, 3.68 mmol) sank in dry ice-acetone bath was added slurry (-10 °C) of THF-H_2O (9-1, 50 mL) and stirred for 2.5 h. Then the blue mixture was stirred at ambient environment for 6 h. The red reacted mixture was evaporated, added 1 *N* NaOH solution (30 mL) and extracted with EtOAc (4 x 30 mL). The extract was washed with 10 % HCl solution (1 x 30 mL), dried (Na_2CO_3) and evaporated to yield brown product (0.5246 g). This product was chromatographed on silica gel column using EtOAc-Hexane (2-1) as eluent. The result provided **6** (0.2619 g, 49.38 %) and side product **7** (0.1417 g).

RUN 15

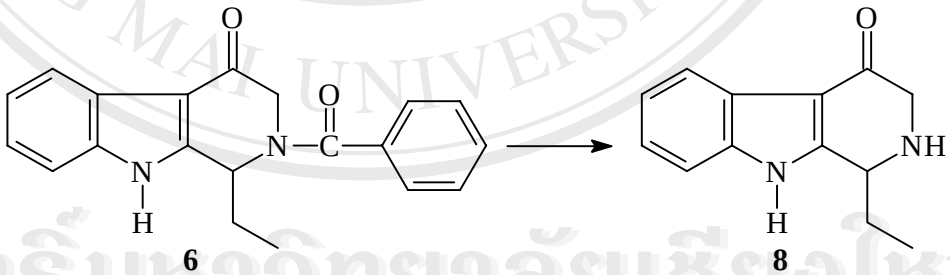
To the mixture of **5** (0.7249 g, 2.35 mmol) and DDQ (1.0555 g, 4.65 mmol) sank in dry ice-acetone bath was added slurry (-10 °C) of THF-H_2O (9-1, 50 mL) and stirred for 3 h. Then the blue mixture was stirred at ambient environment for 12 h. The red reacted mixture was evaporated to reduced solvent volumn to 5 mL and dissolved in EtOAc (150 mL). The EtOAc layer was washed with 1 *N* NaOH solution (2 x 50 mL), dried (Na_2CO_3) and evaporated to obtain yellow product (0.7957 g). This product was chromatographed on silica gel column using

EtOAc-Hexane (2-1) as eluent. The result provided **6** (0.3203 g, 42.80 %) and side product **7** (0.0967 g).

RUN 16

To the mixture of **5** (2.0195 g, 6.64 mmol) and DDQ (3.2673 g, 14.39 mmol) sank in dry ice-acetone bath was added slurry (-10 °C) of THF-H_2O (9-1, 50 mL) and stirred for 3 h. Then the blue mixture was stirred at ambient environment for 18 h. The red reacted mixture was poured in to 1 *N* NaOH solution (50 mL) and extracted with EtOAc (4 x 100 mL). The extract was washed with 10 % HCl solution (1 x 50 mL), dried (Na_2CO_3) and evaporated to obtain brown product (1.9307 g). This product was chromatographed on silica gel column using EtOAc-Hexane (2-1) as eluent. The result provided **3** (1.1358 g) crystallized in MeOH to yield white crystal (0.7986 g, 37.81 %) and side product **7** (0.2853 g) crystallized in MeOH to yield white crystal (0.1673 g, 7.8 %).

1-ethyl-4-oxo-1,2,3,4-tetrahydro-β-carboline (8)



6 **8**

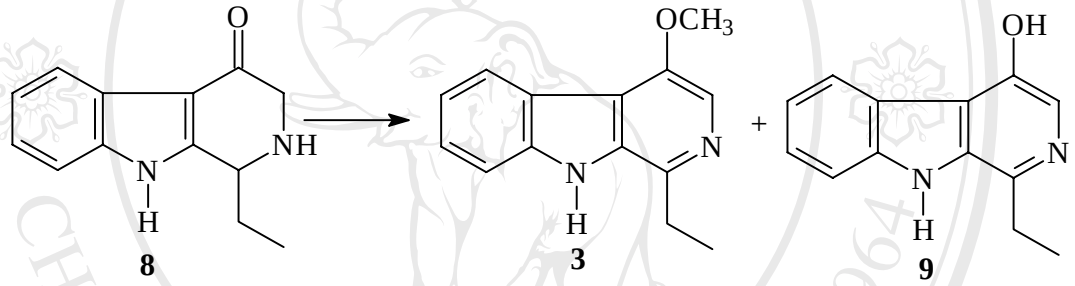
RUN 17

The mixture of **6** (0.0825 g, 0.26 mmol) and 6 *N* NaOH solution (30 mL) was refluxed for 3 h. After cooling, the reacted mixture was extracted with EtOAc (5 x 30 mL). The extract was dried (Na_2CO_3) and evaporate to yield white fine crystal **8** (0.0473 g, 85.19 %).

RUN 18

The mixture of **6** (0.2619 g, 0.82 mmol) and 6 *N* NaOH solution (30 mL) was refluxed for 3 h. After cooling, the reacted mixture was extracted with EtOAc (5 x 40 mL). The extract was evaporate to yield white solid (0.2537 mg) that was crystallized in MeOH to provide white fine crystal **8** (0.1600 mg, 90.78 %).

1-ethyl-4-methoxy-β-carboline (3) from (8)

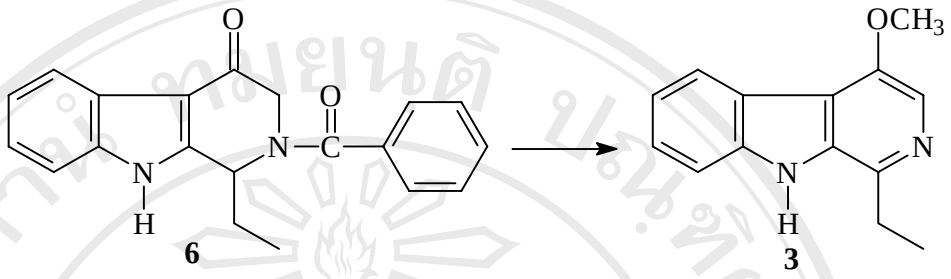


8 **3** **9**

RUN 19

A solution composed of (**8**) (0.1455 g, 0.68 mmol) in methanol (10.0 mL), trimethyl orthoformate (2.0 mL) and *p*-toluenesulfonic acid (0.250 g, 1.45 mmol) was refluxed for 24 h. Evaporation of the reaction solution gave a residue which was disolved in EtOAc (30 mL). The organic fraction was washed with saturated aqueous $NaHCO_3$ (20 mL) and aqueous 2 *N* HCl (4 x 20 mL). The acidic aqueous extracts were combined, and the pH of the solution was adjusted to 8 (solid Na_2CO_3), followed by extraction with EtOAc (3 x 40 mL). The EtOAc extracted were combined, dried (Na_2CO_3) and evaporated to provide crude product 0.0134 mg which was chromatographed through a silica gel column (EtOAc-MeOH) 9-1 as eluent, Al_2O_3 column (EtOAc-Hexane 9-1 as eluent) and then preparative TLC plate (EtOAc as eluent) to provide 0.0022 mg (1.53 %) of (**3**) and 0.0020 mg (1.30 %) of 1-ethyl-4-hydroxy-1,2,3,4-tetrahydro-β-carboline (**9**).

1-ethyl-4-methoxy-β-carboline (3) from 6



6 **3**

RUN 20

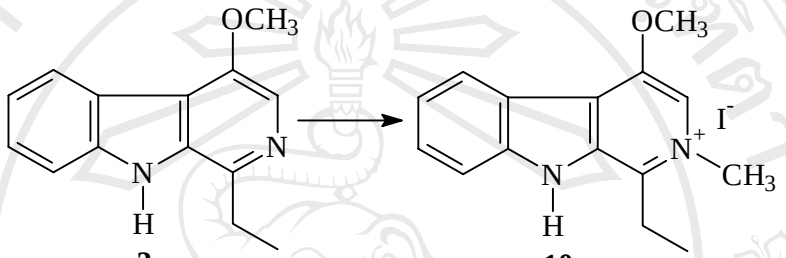
The mixture of benzene (7.0 mL) and *p*-toluenesulfonic acid monohydrate (0.0328 g, 0.17 mmol) was refluxed in Dean-Stark apparatus for 1 h and cooled to ambient temperature. The reaction mixture was added **6** (0.0502 g, 0.16 mmol). Then the green mixture was added dimethoxypropane (0.06 mL, 0.49 mmol) and stirred for 1 h. After that the red mixture was added *p*-chloranil (0.0788 g, 0.32 mmol) and stirred for 20 h. The reacted mixture was added 1 *N* NaOH solution (20 mL) and extract with chloroform (3 x 30 mL). The extract was dried (Na_2CO_3) and evaporated to yield brown product. This product was chromatographed [silica gel column, $CHCl_3$-MeOH (19-1)] to obtain brown product that was future chromatographed [silica gel column, EtOAc-Hexane (9-1)] to provide white fine crystal **3** (0.0187 g, 52.41 %).

RUN 21

The mixture of benzene (55.0 mL) and *p*-toluenesulfonic acid monohydrate (0.4606 g, 2.42 mmol) was refluxed in Dean-Stark apparatus for 1 h and cooled to ambient temperature. The reaction mixture was added **6** (0.7014 g, 2.20 mmol). Then the green mixture was added dimethoxypropane (0.81 mL, 6.61 mmol) and stirred for 1 h. After that the red mixture was added *p*-chloranil (1.0884 g, 4.43 mmol) and stirred for 20 h. The reacted mixture was poured into 5 % NaOH solution (60 mL) and extracted with $CHCl_3$ (3 x 100 mL). The extract was washed with 10 % NaCl (1 x 100 mL), dried (Na_2CO_3) and evaporated to yield brown product (0.8835 g). This

product was chromatographed [silica gel column, $CHCl_3$-MeOH (19-1)] to obtain brown product that was crystallize in MeOH to provide pale yellow fine crystal **3** (0.3909 g, 78.41 %).

1-ethyl-2-methyl-4-methoxy-β-carboline (10)

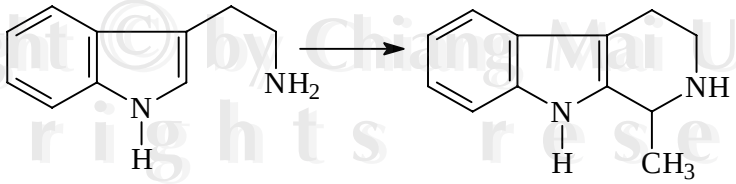


3 **10**

RUN 22

The mixture of **3** (0.0230 g, 0.10 mL), tetramethylene sulfone (0.9 mL) and methyl iodide (0.72 mL, 11.56 mmol) was heated at 50 °C for 17 h. The reacted mixture was cooled and added ether-MeOH (3 mL-2 drop) and $CHCl_3$ (3 x 1 mL). The result provided white fine crystal **10** (0.0286 g, 76.61 %)

1-methyl-1,2,3,4-tetrahydro-β-carboline (11)



Tryptamine **11**

RUN 23

To the mixture of tryptamine (1.0043 g, 6.27 mmol) and 10% MeOH (6.40 mL) was added conc. H_2SO_4 (0.35 mL, 3.39 mmol) and acetadehyde (0.70 mL, 12.48 mmol). Then refluxed (100 °C, 2 h), added the second portion acetadehyde (0.70 mL) and continued refluxing up to 6.5 h. The reaction mixture was evaporated, basified with 28% ammonia solution (1 mL) and decanted. The residue was dissolved in ether. The ether solution was dried and evaporated to provide yellow product that did not dissolve well in $CHCl_3$. This product and the residue that did not dissolve in ether was combined and extracted again with ether. The extract was dried (K_2CO_3) and evaporated to yield yellow product **11** (0.0588 g, 5.04%).

RUN 24

To the 10% acetadehyde (20.00 mL, 35.41 mmol) was added the mixture of tryptamine (1.0012 g, 6.25 mmol), water (20 mL), and 2 *N* H_2SO_4 (3.2 mL, 6.40 mEq). Refluxed (110 °C, 50 min) and cooled, the reaction mixture was added satd. Na_2CO_3. The precipitate was dissolved in 5% HCl and then filtered. The filtrate was treated with NaOH and the precipitate occurred was extracted with ether (3 x 60 mL). The extract was dried (Na_2SO_4) and evaporated to yield yellow product **11** (0.0605 g, 5.20%).

RUN 25

To the 20% acetadehyde (10.00 mL, 35.43 mmol) was added the mixture of tryptamine (0.5127 g, 3.20 mmol), water (15 mL), and 2 *N* H_2SO_4 (2.40 mL, 4.80 mEq). The reaction mixture was (i) stepwise heated to 100 °C, and maintained heating at this temperature; total heating time was 45 min., (ii) cooled and (iii) added satd. Na_2CO_3. The precipitate was dissolved in 5% HCl (30 mL) and then filtered. The filtrate was treated with NaOH until the precipitate was not occurred well. The precipitate was extracted with ether (4x30 mL). The extract was washed with water (40 mL), dried (K_2CO_3) and evaporated to yield yellow product **11** (0.3481 g, 58.56%).

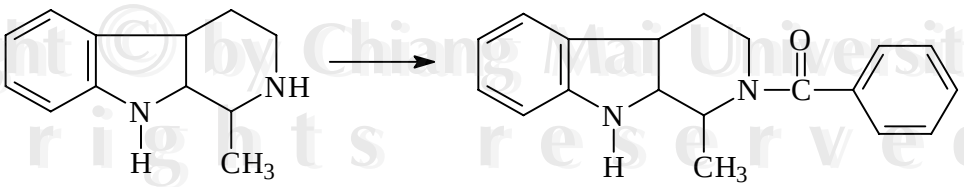
RUN 26

To the 20% acetadehyde (20.00 mL, 70.83 mmol) was added the mixture of tryptamine (1.0060 g, 6.28 mmol), water (20 mL), and 2 *N* H_2SO_4 (3.20 mL, 6.40 mEq). The reaction mixture was (i) stepwise heated to 100 °C, and maintained heating at this temperature; total heating time was 70 min., (ii) cooled and (iii) added satd. Na_2CO_3. The precipitate was dissolved in 5% HCl (30 mL) and then filtered. The filtrated was treated with NaOH until the precipitate was not occurred. The precipitate was extrated with ether (4 x 30 mL). The extract was washed with water (40 mL), dried (K_2CO_3) and evaporated to yield yellow product **11** (0.9029 g, 77.80%).

RUN 27

To the 20% acetadehyde (40.00 mL, 141.66 mmol) was added the mixture of tryptamine (2.0211 g, 12.61 mmol), water (40 mL), and 2 *N* H_2SO_4 (6.40 mL, 12.80 mEq). The reaction mixture was (i) stepwise heated to 100 °C, and maintained heating at this temperature; total heating time was 45 min., (ii) cooled and (iii) added satd. Na_2CO_3. The precipitate was dissolved in 5% HCl (50 mL) and then filtered. The filtrated was treated with NaOH until the precipitate did not occur. The precipitate was extracted with ether (4 x 40 mL). The extract was washed with water (40 mL), dried (K_2CO_3) and evaporated to yield yellow product **11** (2.0454 g, 87.12%).

1-methyl-2-benzoyl-1,2,3,4-tetrahydro-β-carboline (12)



11 **12**

RUN 28

Benzoyl chloride (0.64 mL, 5.55 mmol) was added to the solution of **11** (0.5169 g, 2.78 mmol) in pyridine/benzene (4.50 / 9.00 mL). Then the reaction mixture was heated to 60 °C, and maintained heating at this temperature; total heting time was 30 min., cooled and quenched with water (30 mL). The aqueous layer was extracted with EtOAc (3 x 30 mL). Combined organic layer was washed with water (2 x 15 mL); satd. Na_2CO_3 (3 x 15 mL); water (4 x 30 mL); and 10% NaCl (2 x 15 mL), dried (Na_2SO_4) and evaporated to yield sticky brown product. This product was chromatographed (column, silica gel, ethyl acetate) to provide brown product (0.7528 g) that was crystallized in MeOH to obtain white fine crystal **12** (0.2001 g, 24.83%).

RUN 29

Benzoyl chloride (0.72 mL, 6.25 mmol) was added to the mixture of **11** (0.5694 g, 3.06 mmol), dichloromethane (20.00 mL), triethylamine (0.52 mL, 3.70 mmol) and DMAP (0.0385 g, 0.31 mmol). Then the reaction mixture was heated to 60 °C, and maintained heating at this temperature; total heating time was 2 h, cooled and quenched with 1 *N* NaOH (30 mL). The aqueous layer was extracted with EtOAc (3 x 30 mL). Combined organic layer was dried (Na_2CO_3) and evaporated to yield sticky brown product (1.1254 g). This product was chromatographed (column, silica gel, EtOAc) to provide brown product that was crystallized in MeOH to obtain pale yellow fine crystal **12** (0.3095 g, 34.87%).

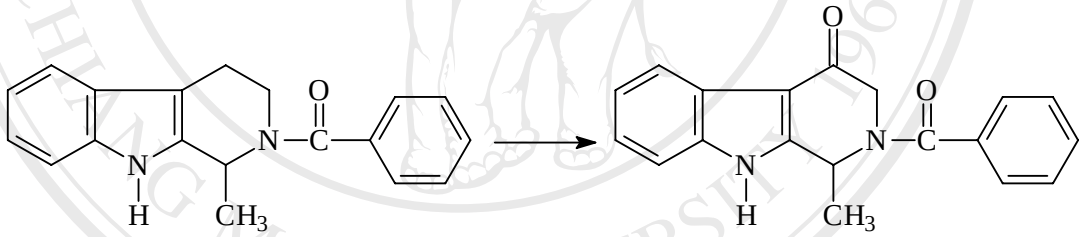
RUN 30

Benzoyl chloride (2.00 mL, 17.38 mmol) was added to the mixture of **11** (1.5159 g, 8.14 mmol), dichloromethane (40.00 mL), DMAP (0.1077 g, 0.88 mmol) and triethylamine (1.40 mL, 9.96 mmol). Then the reaction mixture was heated to 60 °C, and maintained heating at this temperature; total heating time was 2 h, cooled and quenched with 1 *N* NaOH (30 mL). The aqueous layer was extracted with dichloromethane (30 mL). Combined organic layer was dried (Na_2CO_3) and evaporated to yield sticky brown product (3.5141 g). This product was chromatographed (column, silica gel, EtOAc) to provide product that was crystallized in MeOH to obtain pale yellow fine crystal **12** (0.9445 g, 37.97%).

RUN 31

Benzoyl chloride (6.30 mL, 54.68 mmol) was added to the mixture of **11** (5.02 g, 26.97 mmol), dichloromethane (80.00 mL), DMAP (0.3303 g, 2.70 mmol) and triethylamine (4.50 mL, 32.02 mmol). The reaction mixture was stirred at ambient temperature for 1 h and then quenched with 1 *N* NaOH (80 mL). The aqueous layer was extracted with chloroform (2 x 10 mL). Combined organic layer was washed with water (2 x 150 mL), dried (Na_2SO_4) and evaporated to yield sticky brown product (9.6 g). This product was chromatographed [column, silica gel, EtOAc-hexane (9-1)] to provide product that was crystallized in acetone to obtain pale yellow fine crystal **12** (1.0205 g, 13.04%).

1-methyl-2-benzoyl-4-oxo-1,2,3,4-tetrahydro-β-carboline (13)



RUN 32

To the mixture of **12** (0.1030 g, 0.35 mL) and DDQ (0.1685 g, 0.74 mmol) sank in dry ice – acetone bath was added slurry (10 °C) of THF-H_2O (9-1, 5 mL) and stirred for 3 h. Then the blue mixture was stirred at environmental temperature for 11 h. The red reacted mixture was evaporated, added 1 *N* NaOH and extracted with ethyl acetate. The extract was washed with 10% HCl, dried (Na_2CO_3) and evaporated to obtained brown product (0.1224 g). This product was chromatographed [column, silica gel, EtOAc – hexane (2-1)] to provide pale yellow fine crystal **13** (0.0275 g, 25.47%).

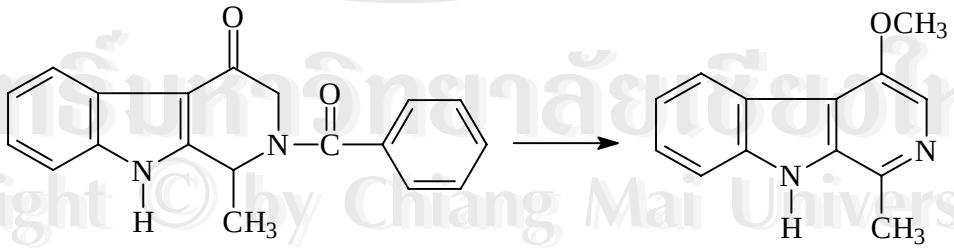
RUN 33

To the mixture of **12** (0.9795 g, 3.38 mL) and DDQ (1.5217 g, 6.70 mmol) sank in dry ice – acetone bath was added slurry (10 °C) of THF-H_2O (9-1, 50 mL) and stirred for 3 h. Then the blue mixture was stirred at environmental temperature for 12 h. The red reacted mixture was evaporated, added EtOAc (150 mL), washed with 1 *N* NaOH (2 x 50 mL), dried (Na_2CO_3) and evaporated to obtain brown product (1.1849 g). This product was chromatographed [column, silica gel, EtOAc – hexane (2-1)] to provide pale yellow fine crystal **13** (0.4200 g, 40.91%).

RUN 34

To the mixture of **12** (0.2200 g, 0.76 mL) and DDQ (0.3467 g, 1.53 mmol) sank in dry ice – acetone bath was added slurry (10 °C) of THF-H_2O (9-1, 10 mL) and stirred for 3 h. Then the blue mixture was stirred at environmental temperature for 12 h. The red reacted mixture was evaporated, added EtOAc (100 mL), washed with 1 *N* NaOH (2 x 50 mL), washed with water (50 mL), dried (Na_2SO_4) and evaporated to obtain brown product (0.2913 g). This product was chromatographed [column, silica gel, EtOAc – hexane (2-1)] to provide pale yellow fine crystal IV (0.1195 g) that was crystallized in EtOAc to yield white fine crystal **13** (0.0876 g, 40.00%).

Synthesis of 1-methyl-4-methoxy-β-carboline (14)



13 **14**

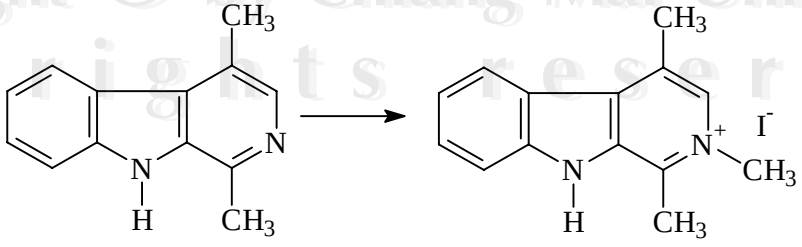
RUN 35

The mixture of benzene (12.00 mL) and *p*-toluenesulfonic acid monohydrate (0.0340 g, 0.18 mmol) was refluxed (120 °C) in Dean-Stark apparatus for 1 h and cooled to ambient temperature. The reaction mixture was added **13** (0.0484 g, 0.16 mmol). Then the green mixture was added dimethoxypropane (0.060 mL, 0.49 mmol) and stirred for 1 h. After that the red mixture was added *p*-chloranil (0.0785 g, 0.32 mmol) and stirred for 20 h. The reacted mixture was added 1 *N* NaOH (30 mL), and extracted with CHCl3 (2 x 50 mL). The extract was washed with water (50 mL), dried (Na_2SO_4) and evaporated to yield brown product (0.0690 g). This product was chromatographed [column, Al_2O_3, EtOAC – hexane (2-1)] to provide white fine crystal **14** (0.0300 g, 88.88%).

RUN 36

The mixture of benzene (20.00 mL) and *p*-toluenesulfonic acid monohydrate (0.0506 g, 0.27 mmol) was refluxed (120 °C) in Dean-Stark apparatus for 1 h and cooled to ambient temperature. The reaction mixture was added **13** (0.0506 g, 0.17 mmol). Then the green mixture was added dimethoxypropane (0.065 mL, 0.53 mmol) and stirred for 1 h. After that the red mixture was added *p*-chloranil (0.0818 g, 0.33 mmol) and stirred for 20 h. The reacted mixture was added 1 *N* NaOH (30 mL), and extracted with CHCl3 (2 x 50 mL). The extract was washed with water (50 mL), dried (Na_2SO_4) and evaporated to yield brown product (0.0676 g). This product was chromatographed [column, Al_2O_3, EtOAc – hexane (2-1)] to provide white fine crystal **14** (0.0286 g, 81.05%).

1, 2-dimethyl-4-methoxy-β-carboline (15)



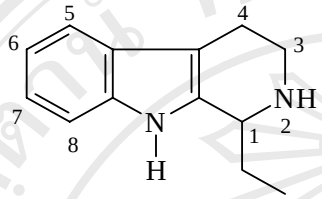
14 **15**

RUN 15

The mixture of **14** (0.0230 g, 0.11 mmol), tetramethylene sulfone (1 mL) and methyl iodide (0.74 mL, 11.89 mmol) was stirred at ambient temperature for 15 h. The reacted mixture was added ether-MeOH (4 mL - 3 drop). The precipitated occurred was washed with EtOAc (2 x 3 mL) and $CHCl_3$-MeOH (3 mL - 3 drop). The result provided white fine crystal **15** (0.0284 g, 73.97%).

Structure elucidation

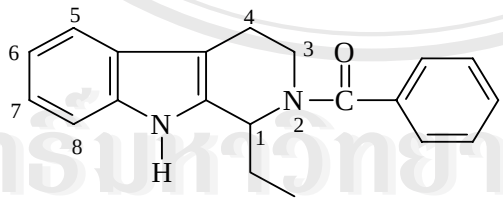
1-ethyl-1,2,3,4-tetrahydro-β-carboline (4)



EI-MS *m/z* 200 (M^+, 75.40%), 183 (3.93%), 171 (100%), 156 (29.35%), 144 (30.32%), 85 (38.75%)

^{1}H NMR ($CDCl_3$) δ 1.07 (t, *J* = 7.42 Hz, 3H, 1-$CH_2\underline{CH_3}$), 1.63-1.76 (m, 1H, 4-CH_2), 1.87-1.98 (m, 1H, 4-CH_2), 2.72-2.78 (m, 2H, 1-$\underline{CH_2}CH_3$), 2.99-3.08 (m, 1H, 3-CH_2), 3.34-3.41 (m, 1H, 3-CH_2), 4.00-4.04 (m, 1H, 1-H), 7.07-7.18 (m, 2H, 6-H/7-H), 7.30-7.33 (d, 1H, *J* = 7.97 Hz, 5-H or 8-H), 7.50 (d, *J* = 7.14 Hz, 1H, 5-H or 8-H), 7.77 (s br, 1H, NH)

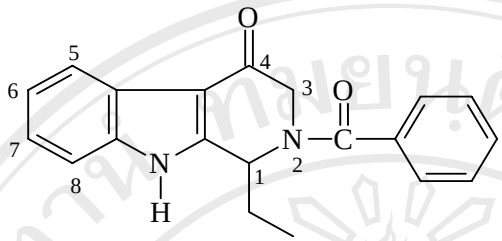
1-ethyl-2-benzoyl-1,2,3,4-tetrahydro-β-carboline (5)



EI-MS *m/z* 304 (M^+, 11.98%), 275 (38.05%), 105 (100%)

^{1}H NMR ($CDCl_3$) δ 1.17 (t, *J* =7.42 Hz, 3H, 1-$CH_2\underline{CH_3}$), 1.92-2.09 (m, 2H, 4-CH_2), 2.66-2.82 (m, 2H, 1-$\underline{CH_2}CH_3$), 3.49-3.58 (m, 1H, 3-CH_2), 3.90-3.97 (m, 1H, 3-CH_2), 5.86 (t, *J* = 6.87 Hz, 1H, 1-H), 7.08-7.20 (m, 2H, 6-H/7-H), 7.32-7.34 (d, 1H, *J* = 7.97, 8-H), 7.45 (s br, 6H, 5-H or 8-H/Ph-H), 8.13 (s br, 1H, NH)

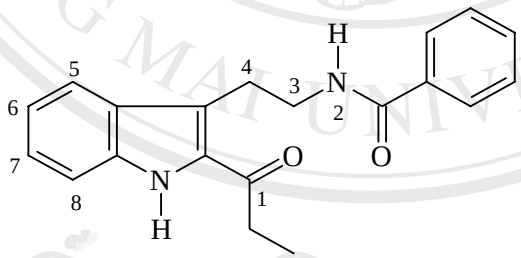
1-ethyl-2-benzoyl-4-oxo-1,2,3,4-tetrahydro-β-carboline (6)



EI-MS *m/z* 318 (M^+, 26.64%), 289 (10.95%), 213 (38.19%), 105 (100%)

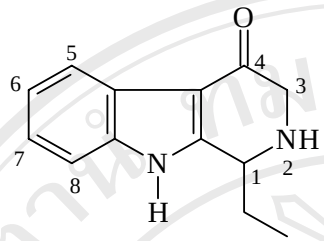
^{1}H NMR ($CDCl_3$) δ 1.18 (t, *J* =7.42 Hz, 3H, 1-$CH_2\underline{CH_3}$), 2.06-2.15 (m, 2H, 1-$\underline{CH_2}CH_3$), 4.19 (d, *J* = 17.58, 1H, 3-CH_2), 4.41 (d, *J* = 17.58, 1H, 3-CH_2), 6.40 (t, *J* = 6.87, 7.97 Hz, 1H, 1-H), 7.15-7.28 (m, 4H include $CHCl_3$) 7.41-7.56 (m, 6H), 8.11 (d, J = 8.24, 1H, 5-H or 8-H), 10.65 (s br, 1H, C=O)

Side product (7)



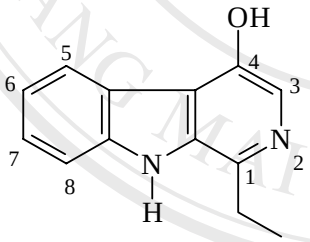
^{1}H NMR ($CDCl_3$) δ 1.25 (t, 3H, *J* =7.14, 7.14 Hz), 3.02 (q, 2H, *J* = 7.14, 7.14, 7.42 Hz), 3.47 (t, 2H, *J* = 6.59, 6.87 Hz), 3.81 (q, 2H, *J* = 6.59, 5.77, 6.59 Hz), 6.77 (s br, 1H), 7.15 (t, 1H, *J* = 7.97 Hz), 7.27-7.49 (m, 5H), 7.68 (d, 2H, *J* = 9.61 Hz), 7.76 (d, 1H, *J* = 8.24 Hz), 8.90 (s br, 1H)

1-ethyl-4-oxo-1,2,3,4-tetrahydro-β-carboline (8)



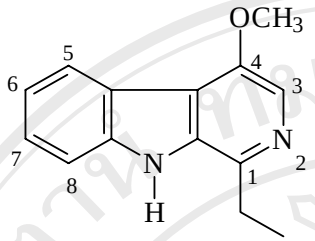
^{1}H NMR (DMSO) δ 1.01 (t, *J* = 7.42, 7.14 Hz, 3H, 1-$CH_2\underline{CH_3}$), 2.49-1.70 (m, 2H, 1-$\underline{CH_2}CH_3$), 3.26 (d, *J* = 16.21 Hz, 1H, 3-CH_2), 3.38 (d, *J* = 16.48 Hz, 1H, 3-CH_2), 4.02-3.99 (m, 1H, 1-H), 7.16-7.10 (m, 2H, 6-H/7-H), 7.42 (d, *J* = 8.24, 1H, 5-H or 8-H), 7.91 (d, *J* = 7.14 Hz, 5-H or 8-H).

1-ethyl-4-hydroxy-β-carboline (9)



^{1}H NMR ($CDCl_3$) δ 1.48 (t, *J* =7.42, 7.69 Hz, 3H, 1-$CH_2\underline{CH_3}$), 3.15 (q, *J* = 7.42, 7.69, 7.69 Hz, 2H, 1-$\underline{CH_2}CH_3$), 7.28-7.32 (m, 1H, 5-H or 8-H), 7.52-7.58 (m, 2H, 6-H/7-H), 7.83 (d, *J* = 5.49 Hz, 1H, 3-H), 8.12 (d, *J* = 7.97 Hz, 1H, 5-H or 8-H), 8.24 (s br, 1H, NH), 8.40 (d, *J* = 5.49 Hz, 1H, 4-OH)

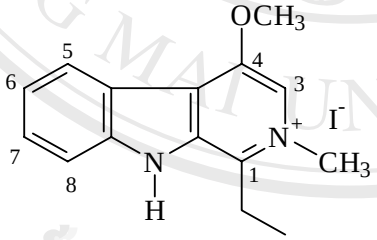
1-ethyl-4-methoxy-β-carboline (3)



EI-MS *m/z* 226 (M^+, 100%), 211 (70.68%), 198 (11.51%), 183 (47.04%)

^{1}H NMR ($CDCl_3$) δ 1.45 (t, *J* = 7.69 Hz, 3H, 1-$CH_2\underline{CH_3}$), 3.08 (q, *J* = 7.69 Hz, 2H, 1-$\underline{CH_2}CH_3$), 4.13 (s, 3H, -OCH_3, small spit on the top of peak; J = 0.82 Hz), 7.30-7.32 (m, 1H, 5-H or 8-H), 7.50-7.52 (m, 2H, 6-H/7-H), 8.01 (s, 1H, 3-H), 8.18 (s br, 1H, NH), 8.33 (d, J = 7.69, 1H, 5-H or 8-H)

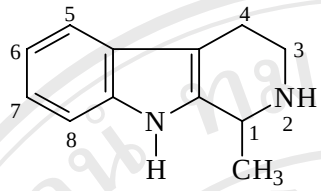
1-ethyl-2-methyl-4-methoxy-β-carboline (10)



EI-MS *m/z* 240 (M^+, 100%), 225 (65.59%), 211 (51.87%), 197 (34.69%), 142 (67.78%)

^{1}H NMR (DMSO) δ 1.34 (t, 3H, *J* = 7.42, 7.97 Hz, 1-$CH_2\underline{CH_3}$), 3.39-3.45 (m, 2H, 1-$\underline{CH_2}CH_3$), 4.16 (s, 3H, -OCH_3), 4.35 (s, 3H, N^+CH_3), 7.40-7.45 (m, 1H, 5-H or 8-H), 7.75-7.77 (m, 2H, 6-H/7-H), 8.31 (d, 1H, *J* = 8.24 Hz, 1H, 5-H or 8H), 8.43 (s, 1H, 3-H)

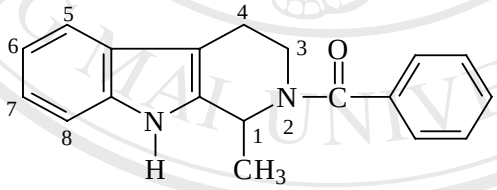
1-methyl-1,2,3,4-tetrahydro β–carboline (11)



EI-MS: *m/z* 186 (M^+, 59.80%), 171 (100%), 157 (46.46%)

^{1}H NMR ($CDCl_3$) δ 1.45 (d, *J* = 6.59 Hz, 3H, 1-CH_3), 2.69-2.84 (m, 2H, 4-CH_2), 3.01-3.10 (m, 1H, 3-CH_2), 3.34-3.41 (m, 1H, 3-CH_2), 4.16 (q, J = 6.59, 6.87, 6.59 Hz, 1-H), 7.07-7.19 (m, 2H, 6-H/7-H), 7.31 (d, 1H, *J* = 7.14 Hz, 5-H or 8-H), 7.49 (d, 1H, *J* = 7.15 Hz, 5-H or 8-H), 7.75 (s br, 1H, NH)

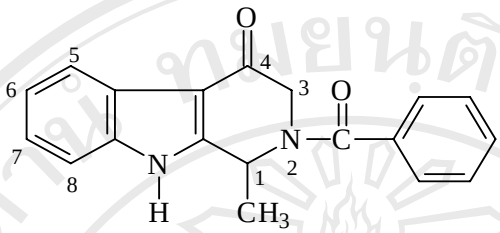
2-benzoyl-1-methyl-1,2,3,4-tetrahydro β–carboline (12)



EI-MS: *m/z* 290 (M^+, 67.21%), 275 (49.86%), 185 (22.07%)

^{1}H NMR ($CDCl_3$): δ 1.60 (d, *J* = 5.49 Hz, 3H), 2.68-2.96 (m, 2H, 4-CH_2), 3.46-3.48 (m, 1H, 3-CH_2), 3.92-4.95 (m, 1H, 3-CH_2), 5.88 (s br, 1H, 1-H), 7.08-7.19 (m, 2H, 6-H/7-H), 7.29 (d, 1H, *J* = 7.69 Hz, 5-H or 8-H), 7.46 (s br, 6H, 5-H or 8-H/Ph-H), 8.38 (s br, 1H, NH)

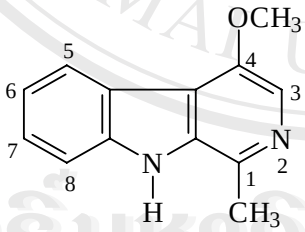
2-benzoyl-1-methyl-4-oxo-1,2,3,4-tetrahydro β–carboline (13)



EI-MS: *m/z* 304 (M^+, 38.11%), 199 (100%), 171 (31.05%), 143 (39.73%), 115 (26.82%), 105 (78.29%), 77 (56.89%)

^{1}H NMR ($CDCl_3$): δ 1.73 (d, *J* = 6.59 Hz, 3H, 1-CH_3), 4.19 (d, *J* = 18.13 Hz, 1H, 3-CH_2), 4.39 (d, *J* =16.76 Hz, 1H, 3-CH_2), 6.48 (s br, 1H, 1-H), 7.14-7.29 (m, 4H include $CHCl_3$), 8.12-8.14 (m, 6H), 10.76 (s br, 1H, C=O)

4-methoxy-1-methyl-β–carboline (14)

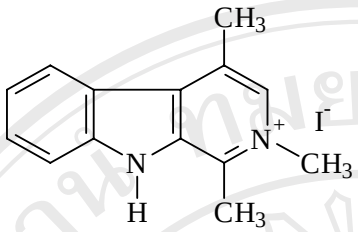


EI-MS: *m/z* 212 (M^+, 96.24%), 197 (6.05%), 182 (9.79%), 169 (100%)

CHN (%): calc. 73.56, 5.70, 13.20; found 72.31, 5.80, 11.29

^{1}H NMR ($CDCl_3$): δ 2.56 (s, 3H, 1-CH_3), 4.13 (s, 3H, OCH_3), 7.27-7.32 (m, 1H, 5-H or 8-H), 7.49-7.52 (m, 2H, 6-H/7-H), 7.97 (s, 1H, 3-H), 8.25 (s br, 1H, NH), 8.31 (d, *J* = 7.69 Hz, 1H, 5H or 8-H)

1, 2-dimethyl-4-methoxy-β–carboline (15)



EI-MS: *m/z* 226 (M^+, 100%), 211 (35%), 183 (86%)

CHN (%): calc. 47.47, 4.27, 7.91; found 47.36, 4.50, 5.56

^{1}H NMR (DMSO): δ 2.98 (s, 3H, 1-CH_3), 4.16 (s, 3H, OCH_3), 4.35 (s, 3H, N^+CH_3), 7.43-7.48 (m, 1H, 5-H or 8-H), 7.75-7.76 (m, 2H, 6-H/7-H), 8.30 (d, J = 7.97 Hz, 5-H or 8-H), 8.46 (s, 1H, 3-H)

Cytotoxicity and *In vitro* antimalarial activity of synthesized compounds (by Wataya' group.)

The *in vitro* antimalarial activities against *P. falciparum* (chloroquine sensitive FCR-3 strain) of the synthesized compounds and their cytotoxicities against mouse mammary tumor FM3A were evaluated. Selective toxicities, defined by the ration EC_{50}(FM3A)/EC_{50}(*P. falciparum*), were determined (Kim *et al*., 1999; see in appendix A).