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Synthesis and chloroquine-enhancing activity of N_a-deacetyl-ferrocenoyl-strychnobrasiline

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Abstract—Several strychnobrasiline derivatives have been synthesized to overcome the lack of in vivo reversal activity of the parent compound. In the present study, N_a-deacetyl-ferrocenoyl-strychnobrasiline was synthesized by condensing N_a-deacetyl-strychnobrasiline with ferrocenic acid previously treated with oxalyl chloride. While the in vitro antiplasmodial activity of the test compound (IC_{50} = 4.83 μg/mL) was increased 15-fold compared to that of strychnobrasiline, and the in vitro enhancing activity was found to be similar to that of the parent compound, the compound was devoid of any in vivo potentiating effect, and an antagonistic effect was even observed at higher doses. Based on the overall results on the hemisynthesis of strychnobrasiline derivatives for better reversal activity, this strategy has appeared to be of little value for useful drugs.

Drug resistance is an adverse factor for the efficacy of several chemotherapeutic treatments in infectious diseases. One useful strategy to overcome this phenomenon is the search of drugs called chemosensitizers that could reverse this resistance. Referring to malaria, several synthetic and naturally-occurring chemosensitizers have been found to restore chloroquine sensitivity to resistant strains of *Plasmodium* malaria.[1] Two of the most active, namely desipramine and cyproheptadine, reached the stage of the preliminary clinical trials, but the negative results obtained have somewhat decreased the therapeutic interest of this approach.[2,3] Consequently, their uses have been mainly restricted to biochemical tools that might help contribute to the understanding of chloroquine resistance and its reversal. However, the encouraging results recently obtained in the clinical trial of another chemosensitizer, chlorpheniramine, have given a renewed interest in this approach.[4]

Strychnobrasiline (**1**) is a naturally-occurring chemosensitizer previously isolated as the major constituent from *Strychnos myrtoides*[5] One striking point in the biological activities of this alkaloid is the lack of in vivo chloroquine-potentiating effect, probably because of its hydrophilic property.[6] Deacetylation of **1** led to a slight appearance of in vivo activity, and we, therefore, assumed that the introduction of adequate substitutions in the indolinic nitrogen might substantially increase the in vivo activity. Our previous results suggested that the Nb,C(21)-secocuran skeleton of strychnobrasiline derivatives is necessary for synergistic activity which is significantly influenced by the basicity of the indolinic atom.[7] Our choice was first directed to the ferrocenyl group because, in a previous study, it was found that the intercalation of this functional group in the side chain of chloroquine remarkably enhanced the antimalarial activity of the synthetic compound.[8] This paper reports on the synthesis as well as the in vitro and in vivo

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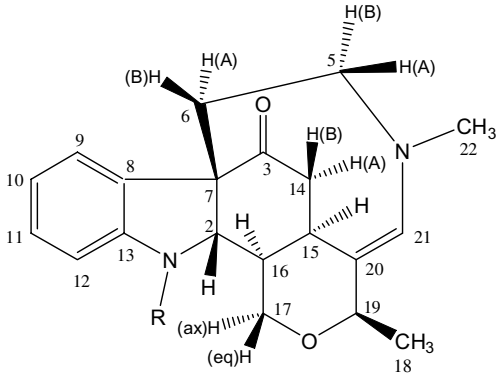
chloroquine-enhancing activity of N_a-deacetyl-ferrocenoyl-strychnobrasiline (**2**).

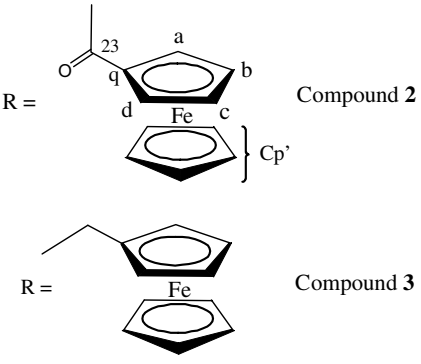
Strychnobrasiline (**1**) was extracted from stem barks of *S. myrtoides* by classical acid–alkaline methods and purified from the crude alkaloid extract by crystallization as hydrochloride in methanol/ether with 1.4% yield. Compound **1** was then deacetylated with 1 N aqueous hydrochloric acid under reflux for one hour to afford N_a-deacetyl-strychnobrasiline with 81% yield. Its physical and spectral data were in all points identical to a reference compound.[7]

Structures of compounds referred in this study are given in Figure 1. Attempts to synthesize first N_a-deacetyl-ferrocenylethyl-strychnobrasiline (**3**) by condensation of ferrocene carbaldehyde with N_a-deacetyl-strychnobrasiline followed by reduction with $NaBH_3CN$ failed because of the probablity of a steric hindrance. The synthesis of N_a-deacetyl-ferrocenoyl-strychnobrasiline (**2**) required a more powerful reagent. To this end, **2** was synthesized by condensation of N_a-deacetyl-strychnobrasiline with an acyl chloride involving ferrocenic acid and oxalyl chloride, with an overall yield of 22%.[9] Based on the knowledge of the ^1H and ^{13}C chemical shifts of strychnobrasiline,[10] the concerted interpretation of the 1D and 2D NMR spectra of compound **2** led to the full assignment of its ^1H and ^{13}C chemical shifts.[11] The HMBC spectrum was recorded at 333 K to refine the proton signals of the ferrocene group and thus observing unambiguous long distance correlations. Two NOESY spectra were recorded, one at 300 K with the aim of observing maximum correlations, and another one at 333 K to sharpen the proton signals of the cyclopentadienyl group. Because of the rotation barrier of the N_a-acyl group, strychnobrasiline appeared as a mixture of two isomers *E* and *Z* in practically equal proportions.[9] Regarding compound **2**, examination of the 1D proton signals indicated one predominant isomer, and the observation of correlation between H-2 and Ha suggested a *Z* isomer.



R = Acetyl : Strychnobrasiline **1**

R = H : N_a-deacetylstrychnobrasiline



Compound **2**

Compound **3**

Figure 1. Structures of the compounds referred to this study.

The in vitro and in vivo antiplasmodial tests, respectively, based on the inhibition of [^{3}H]-hypoxanthine uptake by *P. falciparum* cultured in human blood, and the 4 days suppressive test of Peters, were conducted as previously described.[6] In vitro, compound **2** displayed moderate antiplasmodial activity with 4.83 ± 0.17 μg/mL and 22.73 ± 4.12 μg/mL as IC_{50} and IC_{90}, respectively, while the parent compound **1** had a low antiplasmodial activity with IC_{50} and IC_{90} values of 73.08 ± 2.93 and 429.15 ± 11.41 μg/mL, respectively.[5] Compound **2** also showed significant in vitro synergistic effect against the chloroquine resistant strain FCM29 as evidenced by the isobologramme curve depicted in Figure 2. However, the in vivo results were disappointing because compound **2** was devoid of both intrinsic antimalarial and chloroquine-enhancing activities (Table 1), and an

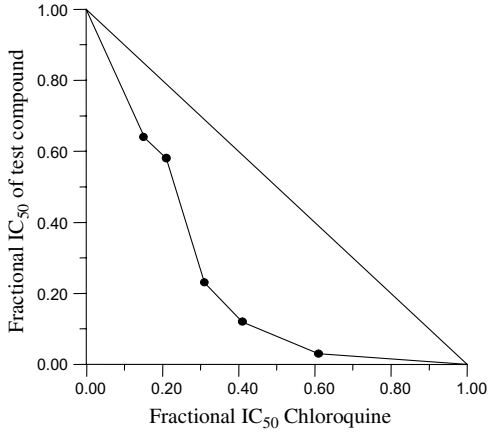


Figure 2. Isobologramme of in vitro interaction between chloroquine and N_a-deacetyl-ferrocenoyl-strychnobrasiline against the chloroquine resistant *P. falciparum* strains FCM29. The concave curve indicates synergism.

Table 1. In vivo antimalarial effect of chloroquine (**CQ**), compound **2** and their combination

Test compounds (doses), route of administration	Parasitemia, %	Inhibition, %
Controls, oral	38.9 (±12)	0
2 (5 mg/kg), oral	36.1 (±3)	7.2
2 (10 mg/kg), oral	38.4 (±10)	1.3
CQ (0.75 mg/kg), sub-cutaneous	32.2 (±6)	17.2
CQ (10 mg/kg), oral	0.1 (±0)	99.8
CQ (0.75 mg/kg), sub-cutaneous + **2** (10 mg/kg), oral	36.4 (±4)	6.4
CQ (0.75 mg/kg), sub-cutaneous + **2** (50 mg/kg), oral	38.1 (±2.1)	2.1

antagonistic effect was observed even at 50 mg/kg. It can be concluded from the overall work devoted to the hemisynthesis of strychnobrasiline derivatives that this way might not lead to any useful chemosensitizers, and it may be profitable to carry on further work on the transformation of strychnobrasiline into malagashanine derivatives which showed in vivo chemosensitizing activities.[12]

Acknowledgements

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9. Preparation of N_a-deacetyl-ferrocenoyl-strychnobrasiline: A solution of ferrocenic acid (189.6 mg, 0.8 mM) in anhydrous dichloromethane was added to 88.4 μL oxalyl chloride under nitrogen atmosphere. The mixture was stirred at room temperature for 30 min, then heated at 60 °C for 20 min. A dichloromethane solution of N_a-deacetyl-strychnobrasiline (200 mg, 0.616 mM) and 88.6 μL triethylamine were added dropwise. The mixture was stirred at room temperature for 1 h, and then heated under reflux for 5 h. The mixture was made alkaline with K_2CO_3 and extracted several times with dichloromethane. The organic phase was washed with water, then dried over Na_2SO_4 and evaporated to dryness under reduced pressure. The residue was purified by column chromatography on silica gel (dichloromethane/methanol 9:1) to yield yellow crystals of N_a-deacetyl-ferrocenoyl-strychnobrasiline.
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11. Data for compound **2**: Yield = 22%, mp = 80 °C; $[\alpha]_D^{22}$ +0.92 (c = 4.2, $CHCl_3$). MS (EI+) *m/z* (relative intensity) 536 (M^+, 55.6), 213 (100), 185 (24.9), 120 (22.9), 97 (8.9). δ_H, (400 MHz, $CDCl_3$) 5.07 (1H, d, *J* 10.2) H-2, 2.70 (1H, dd, *J* 8.0, 14.2) H-5A, 2.92 (1H, m) H-5B, 1.95 (1H, dd *J* 6.4, 12.7) H-6A, 3.05 (1H, m) H-6B, 7.56 (1H, br d, *J* 7.5) H-9, 7.01 (1H, dd, *J* 7.5, 7.4) H-10, 7.08 (1H, dd, *J* 7.4, 7.2) H-11, 7.33 (1H, br m) H-12, 2.19 (1H, dd *J* 5.4, 17.5) H-14A, 2.55 (1H, m) H-14B, 2.55 (1H, m) H-15, 1.80 (1H, br d, *J* 10.2) H-16, 3.56 (1H, br d, *J* 11.4) H-17_{ax}, 4.10 (1H, br m) H-17_{eq}, 1.27 (3H, d *J* 6.0) H-18, 3.88 (1H, q, *J* 6.0) H-19, 5.95 (1H, s) H-21, 2.24 (3H, s) H-22, 4.23 (1H, m) Cp-c, 4.30 (1H, m) Cp-b, 4.50 (1H, br m) Cp-d, 4.63 (1H, br m) Cp-a, 4.17 (5H, s) Cp′. δ_C 64.7 (C-2), 190.1 (C-3), 53.9 (C-5), 40.5 (C-6), 57.2 (C-7), 133.8 (C-8), 125.9 (C-9), 124.4 (C-10), 127.6 (C-11), 118.4 (C-12), 142.2 (C-13), 40.1 (C-14), 40.9 (C-15), 42.6 (C-16), 69.1 (C-17), 16.8 (C-18), 76.4 (C-19), 137.6 (C-20), 132.5 (C-21), 42.3 (C-22), 169.6 (C-23), 69.1 (Cp-c), 69.1 (Cp-b), 71.1 (Cp-d), 70.9 (Cp-a), 79.6 (Cp-q), 70.3 (Cp′).
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