9,10-(3',4'-PYRROLIDINO) -9,10-DIHYDROANTHRACENE AND STRUCTURALLY RELATED COMPOUNDS AS SYNERGISTIC ANTIMALARIAL DRUGS

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Abstract : 9,10-(3',4'-pyrrolidino)-9,10-dihydroanthracene and amino derivatives of 9,10-dihydro-9,10-ethano and 9,10-ethenoanthracenes have been synthesized and evaluated for their intrinsic antimalarial activity and their capability to induce antimalarial synergy with chloroquine as well.

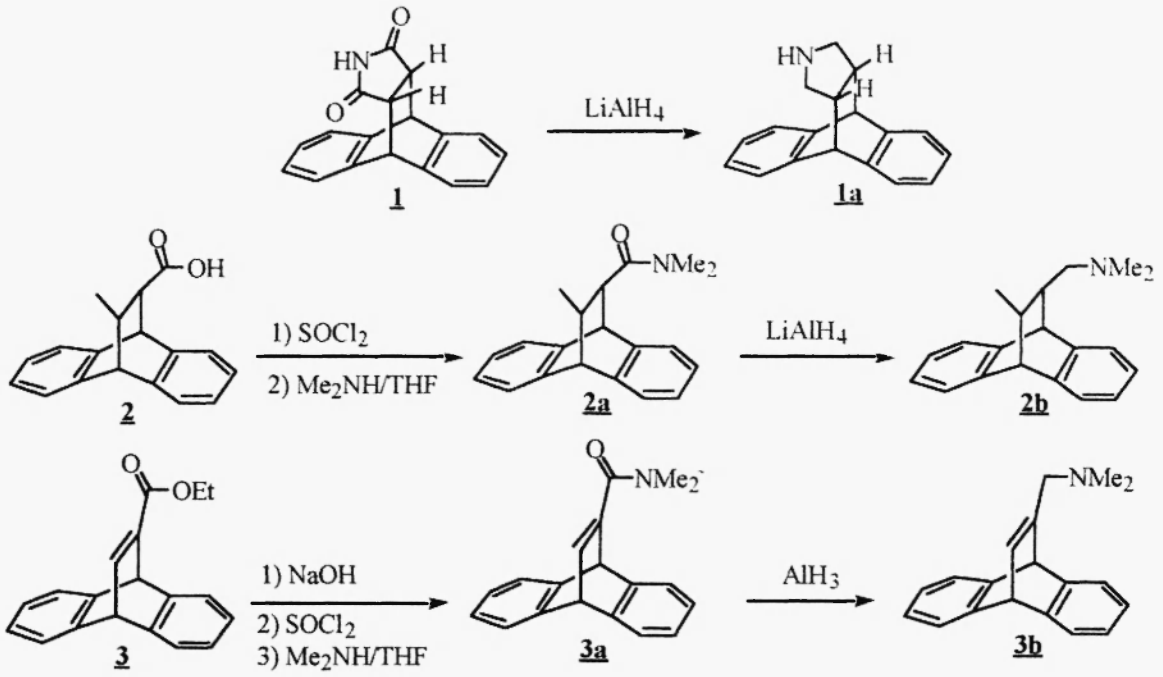
INTRODUCTION

The dramatic expansion of *Plasmodium falciparum* strains resistant to chloroquine greatly reduces clinical efficacy of this compound (1). Thus, the search and the development of alternative drugs against chloroquine resistant parasite strains is urgently needed. With respect to this, reversal of resistance by compounds with poor antimalarial activity is a possible chemotherapeutic approach. Referring to this, several compounds like verapamil (2-5), desipramine (6, 7) and antihistaminic drugs (8, 9) demonstrated in the past decade promising capability to reverse the chloroquine resistance in parasite isolates in vitro, in animal models (10- 12) and human infections (13) as well.

Owing to the antihistaminic (antiH$_1$) activity (14) of 9,10-dihydro-9,10-ethano or 9,10-ethenoanthracenes (DEEA), a new pyrrolidino derivative and related compounds belonging to this series were prepared and tested as antiH$_1$ (15), antimalarial drugs and chemosensitizers : 9,10-(3',4'-pyrrolidino)-9,10-dihydroanthracene **1a**, 11-methyl-12-(N,N-dimethylaminomethyl)-9,10-dihydro-9,10-ethanoanthracenes **2b** and 11-(N,N-dimethylaminomethyl)-9,10-dihydro-9,10-ethenoanthracene **3b**.

RESULTS AND DISCUSSION

Compounds **1**, **2** and **3** were obtained by a Diels Alder reaction (16-18) between anthracene and olefines or alkynes substituted in various ways. Intermediate compounds **1**, **2** and **3** finally give **1a**, **2b** and **3b** respectively as shown in scheme 1.



SCHEME 1

Increase in antimalarial activity of chloroquine combined with verapamil or compounds **1a**, **2b** and **3b** was quantitatively analysed by comparing percentage of growth inhibition for several fixed concentrations of chloroquine alone and in the presence of several fixed, subinhibitory concentrations of sensitizers. Effects of each fixed concentration of these sensitizers on the response of the parasites to chloroquine is expressed as the fractional inhibitory concentration (FIC). The FIC is calculated by the following formula : FIC = % inhibition of the A + B combination / (% inhibition of A + % inhibition of B). A FIC of 1.0 represents no change in the response of chloroquine combined with sensitizers while FIC values > 1.0 represent the degree of potentiation and mean synergism. In contrast FIC values less than 1.0 mean antagonism.

The FIC values are in the range from 1.0 to 73.0 for **1a**, from 1.0 to 4.2 for **2b** and from 1.0 to 2.5 for **3b** . Added to this, **1a** and **2b** show synergy with chloroquine at lower concentration than **3b** does.

Results were plotted by using isobologram analysis based on the IC_{50} values of each compound which shows synergism with chloroquine : (IC_{50} of compounds in combination with chloroquine/ IC_{50} of compounds alone) on the x-axis and (IC_{50} of chloroquine in combination with compounds/ IC_{50} of chloroquine alone) on the y-axis. The 1 value on axes refers to chloroquine alone or to one of the compounds alone and the line represents no change in the activity of chloroquine. Concave curve of the isobologram indicates synergism ; in contrast a convexe curve indicates antagonism. Verapamil, taken as reference, reverses the chloroquine resistance.

Compounds	D6 strain IC_{50} (μM)	W2 strain IC_{50} (μM)
chloroquine	0.012	0.4
1a	22	17
2b	11	4
3b	15	12

Table 1 : Antimalarial activity of the compounds studied.

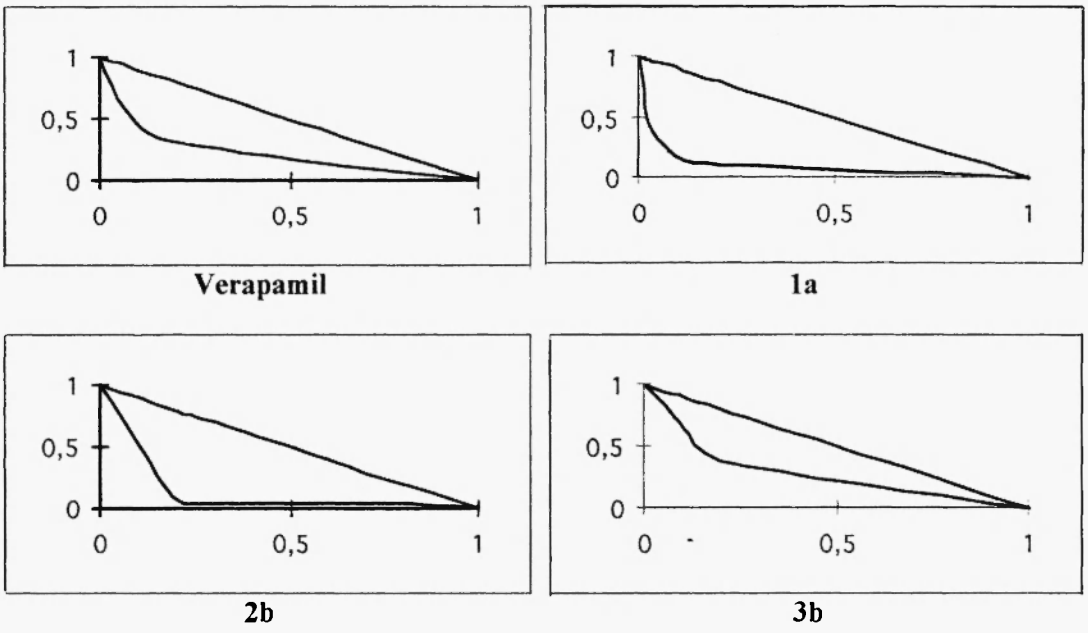


Figure 1 : Isobologram analysis

CONCLUSION

The results demonstrate that compounds under evaluation and chiefly **1a** are potent modulators of the chloroquine resistance in *Plasmodium falciparum* clones. Synthesis of symmetric or unsymmetric amido and amino derivatives of DEEA and their evaluation on other chloroquine resistant clones would enable to report structure activity relationships with a view to optimize compounds investigated.

EXPERIMENTAL

Biology :

The chloroquine susceptible West African clone D6 (Sierra Leone) and the multidrug-resistant Indochina clone W2 were used as reference parasite. The activities of chloroquine, verapamil,

compounds **1a**, **2b** and **3b** were evaluated in vitro against clones of *Plasmodium falciparum*, using an isotopic, micro, drug susceptibility test, in which the hematocrit was 1.5% and the initial parasitemia was 0.5% to 0.8%. This was described previously (19).

Chemistry :

Melting points were determined on a Büchi apparatus and are given uncorrected. ^{1}H and ^{13}C NMR spectra were performed on a Brüker ARX200 spectrometer with TMS as internal reference. Liquid chromatography was performed on silica gel 60 (70-230 Mesh) and TLC on silica gel 60 F_{254}.

i) The starting imide **1** (16) (22mmol) was added to a slurry of $LiAlH_4$ (110mmol) in anhydrous THF (100ml). After one day at room temperature, water (4.2ml), NaOH 1.25N (4.2ml) and water (8.4ml) were successively added. Suspension was filtered over celite. Filtrates were concentrated. The residue was extracted with diethylether and water (pH=1), the aqueous phase was basified (pH=10) and extracted with $CHCl_3$. The organic phase was dried over drierite.The solution was filtered and filtrates were concentrated under vacuum to obtain **1a**

ii) Acid **2** (17) (10mmol) and $SOCl_2$ (7ml) in methylene chloride (40ml) were heated under reflux during 3 h. After elimination under vacuum of $SOCl_2$ in excess, the acid chloride was dissolved in THF and added to a 2N solution of dimethylamine in THF (25ml).The mixture was stirred at room temperature. The solvent was eliminated under vacuum. The residue was dissolved in methylene chloride, washed with water (pH=10) before the organic phase was dried over drierite. After removal of the solvent, amide **2b** is used in the next step without other treatment.

iii) Amino derivative **2b** was obtained in the same way as **1a**.

iv) Ester **3** (18) (43mmol), NaOH (4.3g), H_2O (66ml), and MeOH (44ml) were heated under reflux during 3 days. After elimination of MeOH, the residue was dissolved in basic water (pH=10). After filtration over celite, the acid derivative was precipitated with HCl (10N). Then the acid obtained was treated in the same way as **2** leading to **3a**.

v) H_2SO_4 96% was cautiously added to a slurry of $LiAlH_4$ (23mmol) in THF (80ml). After stirring 1h at room temperature, the amido derivative **3a** (12mmol) in THF (40ml) was added dropwise at 0°C and after one hour more, water (0.9ml), NaOH 1.25N (0.9ml) and water (1.8ml) were successively added. The suspension was filtered over celite. Filtrates were concentrated. **3b** was purified chromatographically (ether/ethylacetate, v/v 7 : 3). The reduction of **3a** was performed with AlH_3 leading to the pure expected compound **3b** (a tentative using $LiAlH_4$ (20) gave a mixture of ethano and etheno derivatives).

	Yield %	m.p.°C	(solvent) ^{1}H NMR δppm (multiplicity, J[Hz])	(solvent) ^{13}CNMR δppm (multiplicity)
1	97	>300 lit (16) : 303	(DMSO-d_6) : 10.79 (s, 1H) ; 7.33 (m, 2H) ; 7.27 (m, 2H) ; 7.14 (m, 4H) ; 4.73 (s, 2H) ; 3.20 (s, 2H).	(DMSO-d_6) : 178.1 (s) ; 142.1 (s) ; 139.5 (s) ; 126.5 (d) ; 124.7 (d) ; 124.2 (d) ; 47.6 (d) ; 44.4 (d).

	Yield %	m.p.°C	(solvent) ^{1}H NMR δppm (multiplicity, J[Hz])	(solvent) ^{13}CNMR δppm (multiplicity)
1a	80	>300	(DMSO-d6) : 7.34 (m, 4H) ; 7.18 (m, 2H) ; 7,00 (m, 2H) ; 4.34 (s, 2H) ; 3.35 (m, 2H) ; 2.71 (br.s, 2H) ; 1.92 (m, 2H).	(DMSO-d6) : 143.3 (s) ; 140.6 (s) ; 126.4 (d) ; 126.3 (d) ; 126.1 (d) ; 124.1 (d) ; 47.2 (t) ; 44.8 (d) ; 43.2 (d).
2a	55	145-7	($CDCl_3$) : 7.58 (m, 4H) ; 7.13 (m, 4H) ; 4.14 (d, 1H, J=1.8) ; 3.95 (d, 1H, J=2.1) ; 3.09 (br. s, 3H) ; 2.87 (br. s, 3H) ; 2.59 (m, 1H) ; 2.35 (dd, 1H, J=2.0, 5.4) ; 0.89 (d, 3H, J=6.9).	($CDCl_3$) : 172.3 (s) ; 144.1 (s) ; 142.8 (s) ; 141.3 (s) ; 138.6 (s) ; 126.1 (d) ; 125.8 (d) ; 125.7 (d) ; 125.6 (d) ; 124.6 (d) ; 122.8 (d) ; 122.4 (d) ; 50.8 (d) ; 49.9 (d) ; 47.8 (d) ; 36.8 (d) ; 37.3 (q) ; 36.0 (q) ; 20.7 (q).
2b	88	235-7	($CDCl_3$) : 12.3 (br. s, 1H) ; 7.64 (m, 1H) ; 7.31 (m, 1H) ; 7.16 (m, 2H) ; 7.03 (m, 4H) ; 4.85 (d, 1H, J=1.7) ; 3.87 (d, 1H, J=2.1) ; 2.68 (s, 6H) ; 2.50 (d, 2H, J=8.1) ; 1.59 (m, 1H) ; 1.38 (m, 1H) ; 0.83 (d, 3H, J=6.8).	($CDCl_3$) : 144.3 (s) ; 142.2 (s) ; 140.2 (s) ; 138.4 (s) ; 126.7 (d) ; 126.6 (d) ; 126.4 (d) ; 126.2 (d) ; 126.1 (d) ; 125.8 (d) ; 124.0 (d) ; 123.3 (d) ; 61.9 (t) ; 50.5 (d) ; 45.4 (d) ; 43.6 (q) ; 39.8 () ; 20.3 (q)
3a	81	115	($CDCl_3$) : 7.32 (m, 4H) ; 7.12 (dd, 1H, J=1.9, 6.1) ; 6.97 (m, 4H) ; 5.37 (d, 1H, J=1.7) ; 5.21 (d, 1H, J=6.0) ; 2.91 (s, 6H).	($CDCl_3$) : 146.1 (s) ; 145.9 (s) ; 145.7 (s) ; 142.8 (s) ; 126.2 (d) ; 126.0 (d) ; 124.4 (d) ; 124.2 (d) ; 59.4 (t) ; 53.6 (d) ; 51.4 (d) ; 43.1 (q).
3b (HCl)	70	280	(D_2O) : 7.32 (m, 4H) ; 7.13 (d, 1H, J=6) ; 6.95 (m, 4H) ; 5.28 (d, 1H, J=6.1) ; 5.18(d, 1H, J=1.9) ; 3.84 (s, 2H) ; 2.53 (s, 6H).	(D_2O) : 146.1 (s) ; 145.9 (s) ; 145.7 (s) ; 142.8 (s) ; 126.2 (d) ; 124.3 (d) ; 124.1 (d) ; 59.3 (t) ; 53.6 (d) ; 51.4 (d) ; 43.1 (q).

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