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Short communication

Synthesis of novel functionalized 5-nitroisoquinolines and evaluation of *in vitro* antimalarial activity

P Rathelot[1], P Vanelle[1], M Gasquet[2], F Delmas[2], MP Crozet[3], P Timon-David[2], J Maldonado[1]*

[1]*Laboratoire de chimie organique, Faculté de pharmacie;*
[2]*Laboratoire de parasitologie, Faculté de pharmacie, 27, boulevard J-Moulin, 13385 Marseille Cedex 05;*
[3]*Radicaux libres et synthèse, CNRS URA 1412, Faculté des sciences et techniques de Saint-Jérôme, Université d'Aix-Marseille III, BP 562, 13397 Marseille Cedex 20, France*

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Summary — Novel aldimine and hydrazone isoquinoline derivatives were obtained after subjecting 1-formyl-5-nitroisoquinoline to classical reactions. Some of these compounds were found to have activity against a chloroquine-resistant *Plasmodium falciparum* strain (ACC Niger).

isoquinoline / antimalarial drug / hydrazone / aldimine

Introduction

Malaria is endemic throughout much of the tropics and sub-tropics placing at risk some 40% of the world's population. More than 100 million clinical cases of the disease are thought to occur annually resulting in 1–2 million deaths [1]. This situation is currently worsening due to the emergence and extension of drug-resistant parasites. The quinoline nucleus is a common component found in many available antimalarial drugs, quinine, chloroquine, amodiaquine and mefloquine, and various heterocyclic aldehyde derivatives have antimalarial properties [2, 3]. Our interest in the isoquinoline series led us to prepare new potentially antiinfective agents that undergo electron transfer in their mode of action [4, 5], by involving the bioisosterism and functionalization of 1-formyl-5-nitroisoquinoline [6] by classical organic aldimine and hydrazone formation.

Chemistry and pharmacology

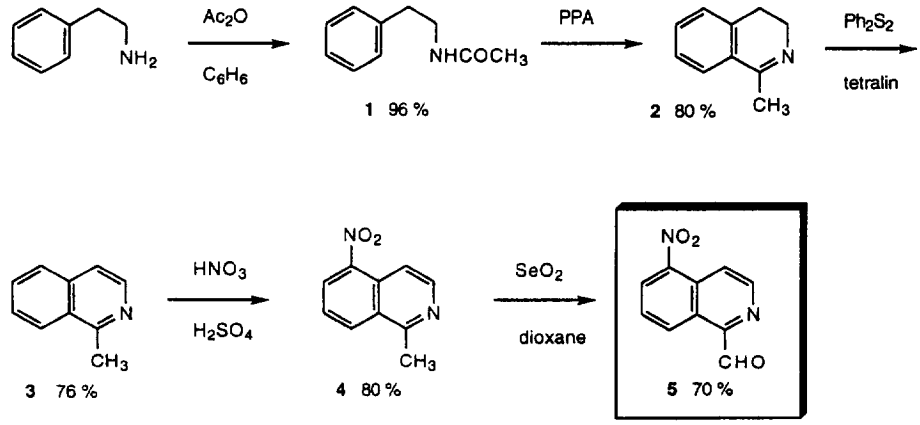
1-Formyl-5-nitroisoquinoline **5** has been prepared in five steps from the inexpensive and commercially available 2-phenylethylamine (scheme 1). The required starting material produced 2-phenylethylacetamide **1** by acetylation. The synthesis of 1-methyl-3,4-dihydroisoquinoline was carried out utilizing the Bischler–Napieralski reaction [7], which was modified by use of polyphosphoric acid (PPA) as the dehydrating reagent instead of $POCl_3$. This modification resulted in an increase in the yield of **2** from 30 to 80%. Derivative **2** was then dehydrogenated with diphenyl disulfide as reported previously [8]. Nitration of 1-methylisoquinoline with HNO_3/H_2SO_4 [6] resulted in just one compound, 1-methyl-5-nitroisoquinoline **4**. Oxidation of **4** with selenium dioxide [6] gave 1-formyl-5-nitroisoquinoline **5** in 70% yield.

The hydrazones and aldimines were prepared using classical methods (scheme 2, tables I and II). The new derivatives were identified using NMR (tables III and IV) and their purity checked by thin-layer chromatography and elemental analysis C, H, N. These compounds were tested *in vitro* against a chloroquine-resistant *Plasmodium falciparum* strain (ACC Niger).

Results and discussion

The *in vitro* test results (table V) indicate that the aldimines **7** are more effective than the hydrazones **6**. The functionalization of 1-formyl-5-nitroisoquinoline is thus favorable for the activity of isoquinoline derivatives. For the hydrazones, the antimalarial activity is greater for six- or seven-membered rings than for a

*Correspondence and reprints

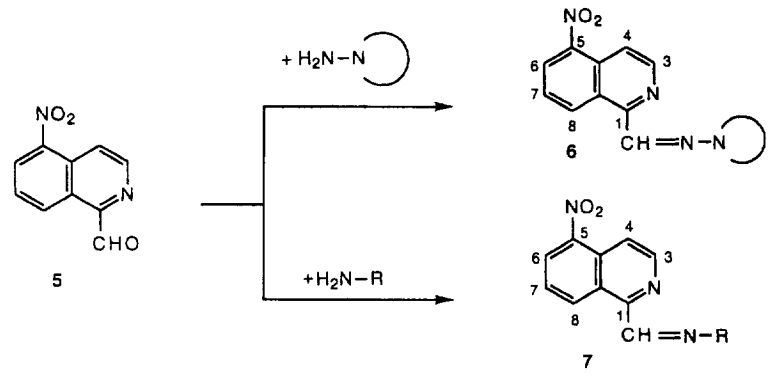


Scheme 1.

five-membered ring. The presence of an oxygen atom in the six-membered ring of the morpholine nucleus has a negative effect on the *in vitro* activity. When a nitrogen atom is present in the six-membered ring of morpholine nucleus, substitution by a hydroxyethyl group rather than a methyl group leads to a significant loss of activity.

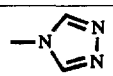
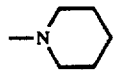
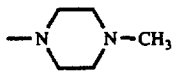
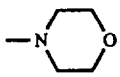
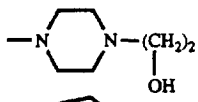
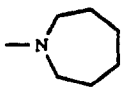
In the aldimine series, aromatic ring substitution by halogen groups (Cl or Br) increases the antimalarial activity. The presence of a methyl or trifluoromethyl group in place of halogen group is defavorable, except for the *ortho* substitution of the methyl group. The *o*-hydroxybenzylamine skeleton is an essential part of active compounds, such as bialamicol or amodiaquine, and so we have incorporated one methylene group between the aromatic ring and the imine function. This incorporation favors antimalarial activity. For example, compound **7k** was twice as active as compound **7f**. The position of the methyl group on the aromatic ring does not modify the antimalarial activity. Substitution by an electron-withdrawing group such as CF_3 in compound **7h** increases the antimalarial activity. Derivative **7l** was the most active with an ED_{50} of 0.7 μg/ml and was 10 times more active than derivative **7h**. This confirms the importance of CF_3 aromatic substitution. This has been demonstrated for a series of 5-(aryloxy)-4-methylprimaquine analogues [9] and for the structures of mefloquine and halofantrine, which are the most promising of new antimalarial drugs in regions of chloroquine resistance and have a CF_3 group in their structure. Replacing the methylene group by an ethylene group or the introduction of a methyl group on this methylene group was detrimental for activity.

In conclusion, we have described the preparation of new isoquinoline derivatives which exhibit promising *in vitro* antimalarial activity. Further studies of these agents, particularly the influence of electron-with-



Scheme 2.

Table I. Physical properties of hydrazones.

Compound	—N◯	Yield (%)	MW	Mp (°C) (ethanol)
6a		80	267.20	244
6b		96	284.32	109
6c		89	299.34	137
6d		91	286.29	168
6e		62	329.36	243
6f		58	298.35	87

drawing groups, hydrophobicity and ionization in the side chain on the antimalarial activity are in progress.

Experimental protocols

Chemistry

Melting points were recorded on a Büchi apparatus using a glass capillary and are uncorrected. ^1H NMR spectra were recorded on a Bruker 200 MHz instrument and chemical shifts are reported in δ units (ppm) relative to internal TMS. Microanalyses for C, H, N were performed by the Microanalytical Section of Saint-Jérôme Faculty and results were within ±0.4% of theoretical values.

Preparation of 1-formyl 5-nitroisoquinoline

2-Phenylethylacetamide 1. To a solution of 2-phenylethylamine (36.4 g, 0.3 mol) in 150 ml benzene was added dropwise acetic anhydride (31.5 ml, 0.3 mol). The reaction mixture was refluxed for 2 h. The benzene was removed under vacuum, and the remaining oil was distilled, bp 119–120°C (0.1 mbar), to yield 47.2 g (96%) of 2-phenylethylacetamide **1**. ^1H NMR ($CDCl_3$) δ 1.92 (s, 3H); 2.81 (t, J = 7.1 Hz, 2H); 3.48 (q, J = 6.3 Hz, 2H); 6.06 (broad s, 1H); 7.16–7.35 (m, 5H).

1-Methyl-3,4-dihydroisoquinoline 2. The acetamide derivative **1** (32.7 g, 0.2 mol) was heated with 275 g polyphosphoric acid (PPA) at 180°C for 3 h. The reaction mixture was carefully added to ice water and the solution was neutralized with NaOH solution (20%). After cooling to room temperature, the resulting oil was extracted with ethyl ether. The organic extracts were dried over magnesium sulfate and the solvent evaporated under reduced pressure. The residual oil was distilled, bp 67–69°C (0.3 mbar), to yield 23.3 g (80%) of **2** (lit [10]). ^1H NMR ($CDCl_3$) δ 2.36 (t, J = 1.5 Hz, 3H); 2.67 (t, J = 7.3 Hz, 2H); 3.63 (td, J = 7.3 and 1.5 Hz, 2H); 7.13 (dd, J = 7.3 and 1.7 Hz, 1H); 7.24 (ddd, J = 7.3, 6.6 and 1.7 Hz, 1H); 7.31 (ddd, J = 7.3, 6.6 and 1.7 Hz, 1H); 7.43 (dd, J = 7.3 and 1.7 Hz, 1H).

Table II. Physical properties of aldimines.

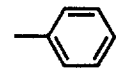
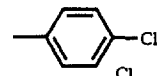
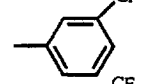
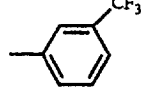
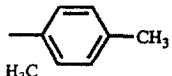
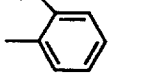
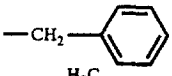
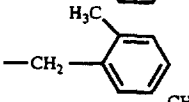
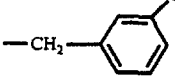
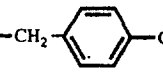
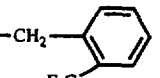
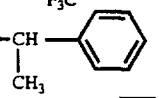
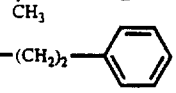
Compound	R	Yield (%)	MW	Mp (°C) (ethanol)
7a		89	277.29	102
7b		95	356.19	161
7c		75	311.74	165
7d		79	311.74	132
7e		71	345.29	109
7f		80	291.31	129
7g		76	291.31	131
7h		80	291.31	111
7i		80	306.35	126
7j		80	306.35	105
7k		80	306.35	107
7l		83	360.32	122
7m		70	306.35	103
7n		98	306.35	81

Table III. ^{1}H NMR data for hydrazones.

Compound	H_3[a]	H_4[b]	H_6[c]	H_7[d]	H_8[e]	$CH{=}N$[f]	—N⟩
6a	8.96	8.46	8.71	8.02	9.54	9.61	9.43 (s, 2H)
6b	8.70	8.26	8.46	7.70	9.80	8.02	1.62–1.72 (m, 2H); 1.80–1.91 (m, 4H); 3.42–3.47 (m, 4H)
6c	8.71	8.28	8.47	7.70	9.76	8.02	2.43 (s, 3H); 2.70 (t, J = 5.1 Hz, 4H); 3.48 (t, J = 5.1 Hz, 4H)
6d	8.73	8.31	8.48	7.71	9.75	8.09	3.44 (t, J = 5.0 Hz, 4H); 3.99 (t, J = 5.0 Hz, 4H)
6e	8.72	8.30	8.48	7.71	9.77	8.04	1.74 (broad s, 1H); 2.70 (t, J = 5.2 Hz, 2H); 2.82 (t, J = 5.1 Hz, 4H); 3.49 (t, J = 5.1 Hz, 4H); 3.73 (t, J = 5.2 Hz, 2H)
6f	8.65	8.16	8.44	7.67	9.68	7.69	1.65–1.69 (m, 4H); 1.90 (broad s, 4H); 3.72–3.78 (m, 4H)

Chemical shifts ($CDCl_3$/TMS) δ in ppm. [a](d, J = 6.1 Hz); [b](d, J = 6.1 Hz); [c](d, J = 7.7 Hz); [d](dd, J = 8.7 and 7.7 Hz); [e](d, J = 8.7 Hz); [f](s).

1-Methylisoquinoline 3. To a solution of **2** (14.5 g, 0.1 mol) in tetralin (80 ml) was added 25 g diphenyldisulfide. The reaction mixture was heated at 200°C with stirring for 20 h. After cooling, the solution was brought to pH 1 with 1 N HCl (150 ml). The aqueous layer was washed with ethyl ether and made alkaline with 1 N NaOH. The brown oil obtained was extracted with ethyl ether. After drying over magnesium sulfate, the solvent was removed under vacuum and the remaining oil was distilled, bp 71–73°C (0.1 mbar) to yield 11.6 g (81%) of **3** (lit [11] bp = 81°C/1 mmHg). ^{1}H NMR ($CDCl_3$) δ 2.93 (s, 3H); 7.53 (d, J = 5.9 Hz, 1H); 7.61 (td, J = 6.8 and 1.2 Hz, 1H); 7.70 (td, J = 6.8 and 1.2 Hz, 1H); 7.82 (dd, J = 6.8 and 1.2 Hz, 1H); 8.13 (dd, J = 6.8 and 1.2 Hz, 1H); 8.38 (d, J = 5.9 Hz, 1H).

1-Methyl-5-nitroisoquinoline 4. Compound **3** (16.4 g, 0.115 mol) was dissolved in 40 ml concentrated H_2SO_4 at −10°C and then 16 ml concentrated HNO_3 (d = 1.38) cooled to −10°C was added dropwise. After the addition, the solution was heated at 60°C for 2 h and then poured slowly over crushed ice. The solution was made alkaline with an NH_4OH solution (10%). The obtained precipitate was filtered, washed with water, dried and recrystallized from ethanol to yield 17 g (79%) of **4**, mp 150°C (lit [4] mp = 150–151°C). ^{1}H NMR ($CDCl_3$) δ 3.06 (s, 3H); 7.72 (dd, J = 8.0 and 8.1 Hz, 1H); 8.28 (d, J = 6.2 Hz, 1H); 8.49 (d, J = 8.0 Hz, 1H); 8.49 (d, J = 8.1 Hz, 1H); 8.61 (d, J = 6.2 Hz, 1H).

1-Formyl-5-nitroisoquinoline 5. A solution of **4** (3 g, 15.9 mmol) in 100 ml 1,4-dioxane was treated with 1.8 g (15.9 mmol) of selenium dioxide (freshly resublimed) and the mixture was refluxed for 3 h. The precipitated selenium was removed by filtration, and the filtrate was flash evaporated. The residue was dissolved in chloroform. The chloroformic layer was extracted with HCl (6 x 100 ml of 1 N HCl and 100 ml of 5 N HCl). The aqueous solution was alkalinized with 2.5 N NaOH. The precipitate was purified by chromatography on a silica-gel column eluting with chloroform and recrystallized from ethanol to yield 2.25 g (70%) of **5**, mp 175–176°C [4]. ^{1}H NMR ($CDCl_3$) δ 7.89 (dd, J = 7.6 and 8.6 Hz, 1H); 8.59 (d, J = 7.6 Hz, 1H); 8.74 (d, J = 6.1 Hz, 1H); 9.00 (d, J = 6.1 Hz, 1H); 9.77 (d, J = 8.6 Hz, 1H); 10.41 (s, 1H).

General procedure for preparation of hydrazones 6 and aldimines 7

To a solution of 1-formyl-5-nitroisoquinoline **5** (0.4 g, 2 mmol) in boiling ethanol (30 ml), was added 3 mmol of hydrazine or amine derivative and the reaction mixture was refluxed for 1 h (for the aldimines) or 3 h (for the hydrazones). After cooling to room temperature, the precipitate was isolated by filtration, washed with water and recrystallized from ethanol.

Biology

In vitro antimalarial test

An ACC Niger chloroquine resistant *P falciparum* strain was used. The parasite was cultured according to Trager and Jensen [12], on glucose-enriched RPMI 1640 medium supplemented with HEPES and 10% human serum. The test procedure followed the method of Trager and Polonsky [13]. Each concentration was tested in triplicate. Giemsa-stained thin blood smears were examined under 1000 x magnification, and the percentage of parasited red blood cells was counted on at least 9000 red blood cells observed for each concentration. Percentage growth inhibition of the parasite was calculated by the following formula:

$$\frac{\text{Parasitemia in solvent} - \text{parasitemia with drugs}}{\text{parasitemia in solvent}} \times 100$$

Table IV. ^{1}H NMR data of aldimines.

Compound	H_3[a]	H_4[b]	H_6[c]	H_7[d]	H_8[e]	*CH=N*	*R*
7a	8.93	8.57	8.59	7.87	10.27	9.05[f]	7.35–7.57 (m, 5H)
7b	8.95	8.60	8.60	7.89	10.24	9.04[f]	7.34 (d, J = 8.7 Hz, 2H); 7.66 (d, J = 8.7 Hz, 2H)
7c	8.93	8.58	8.58	7.87	10.22	9.03[f]	7.35 (d, J = 8.6 Hz, 2H); 7.49 (d, J = 8.6 Hz, 2H)
7d	8.94	8.59	8.59	7.88	10.21	9.02[f]	7.28–7.50 (m, 4H)
7e	8.95	8.59	8.61	7.89	10.24	9.06[f]	7.55–7.68 (m, 4H)
7f	8.87	8.53	8.51	7.82	10.24	9.03[f]	2.42 (s, 3H); 7.26–7.36 (m, 4H)
7g	8.93	8.57	8.59	7.87	10.36	8.97[f]	2.53 (s, 3H); 7.15–7.38 (m, 4H)
7h	8.87	8.52	8.54	7.77	10.10	8.93[g]	5.06 (d, J = 1.3 Hz, 2H); 7.33–7.50 (m, 5H)
7i	8.86	8.52	8.54	7.76	10.11	8.94[g]	2.52 (s, 3H); 5.15 (d, J = 1.3 Hz, 2H); 7.26–7.40 (m, 4H)
7j	8.86	8.52	8.53	7.77	10.09	8.93[g]	2.42 (s, 3H); 5.02 (d, J = 1.3 Hz, 2H); 7.15–7.37 (m, 4H)
7k	8.86	8.52	8.53	7.76	10.09	8.91[g]	2.41 (s, 3H); 5.01 (d, J = 1.3 Hz, 2H); 7.24 (d, J = 8.0 Hz, 2H); 7.35 (d, J = 8.0 Hz, 2H)
7l	8.88	8.53	8.54	7.79	10.07	8.93[g]	5.23 (d, J = 1.3 Hz, 2H); 7.43 (dd, J = 7.5 and 6.8 Hz, 1H); 7.57 (dd, J = 7.8 and 6.8 Hz, 1H); 7.62 (d, J = 7.5 Hz, 1H); 7.74 (d, J = 7.8 Hz, 1H)
7m	8.84	8.52	8.54	7.80	10.14	8.91[f]	1.76 (d, J = 6.6 Hz, 3H); 4.75 (q, J = 6.6 Hz, 1H); 7.32–7.58 (m, 5H)
7n	8.83	8.51	8.54	7.76	9.88	8.74[g]	3.19 (t, J = 7.3 Hz, 2H); 4.12 (td, J = 7.3 and 1.3 Hz, 2H); 7.23–7.41 (m, 5H)

Chemical shifts ($CDCl_3$/TMS) δ in ppm. [a](d, J = 6.1 Hz); [b](d, J = 6.1 Hz); [c](d, J = 7.7 Hz); [d](dd, J = 8.7 and 7.7 Hz); [e](d, J = 8.7 Hz); [f](s); [g](t, J = 1.3 Hz).

Table V. *In vitro* antimalarial activity against ACC Niger *P falciparum* strain.

Compound	ED_{50} (μg/ml)
5	5.0
6a	21.9
6b	4.3
6c	4.5
6d	40.8
6e	22.5
6f	4.4
7a	7.8
7b	4.2
7c	5.3
7d	3.9
7e	16.2
7f	15.2
7g	3.5
7h	6.8
7i	6.5
7j	4.5
7k	7.5
7l	0.7
7m	10.5
7n	11.3
Chloroquine	0.1

ED_{50} = effective dose 50 (concentration of a compound which inhibits 50% of *P falciparum*, compared with the controls).

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