Eur J Med Chem (1992) 27, 391–394

Synthesis and *in vitro* antimalarial activity of some indolo[3,2-*c*]quinolines

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(Received 16 April 1991; accepted 9 January 1992)

Summary — A series of indolo[3,2-*c*]quinolines (**1b–e**) was synthesized by Fischer indolization of 7-chloro-1,2,3,4-tetrahydroquinolin-4-one with the appropriate hydrazines (**7b–e**). Evaluation of *in vitro* antimalarial activity was carried out against a chloroquine resistant strain of *Plasmodium falciparum*. Except for compound **1e** which lacked a basic side chain at position 9, the other indolo[3,2-*c*]quinolines **1b–d** were active. The most active compound was 3-chloro-8-methoxy-9-(4-methyl-1-piperazinylmethyl)-11H-indolo[3,2-*c*]quinoline (**1d**) trihydrochloride which was about 10^4 times more active than chloroquine *in vitro*. The effects of structural variation on antimalarial activity were discussed.

indolo[3,2-*c*]quinolines / *in vitro* antimalarial activity

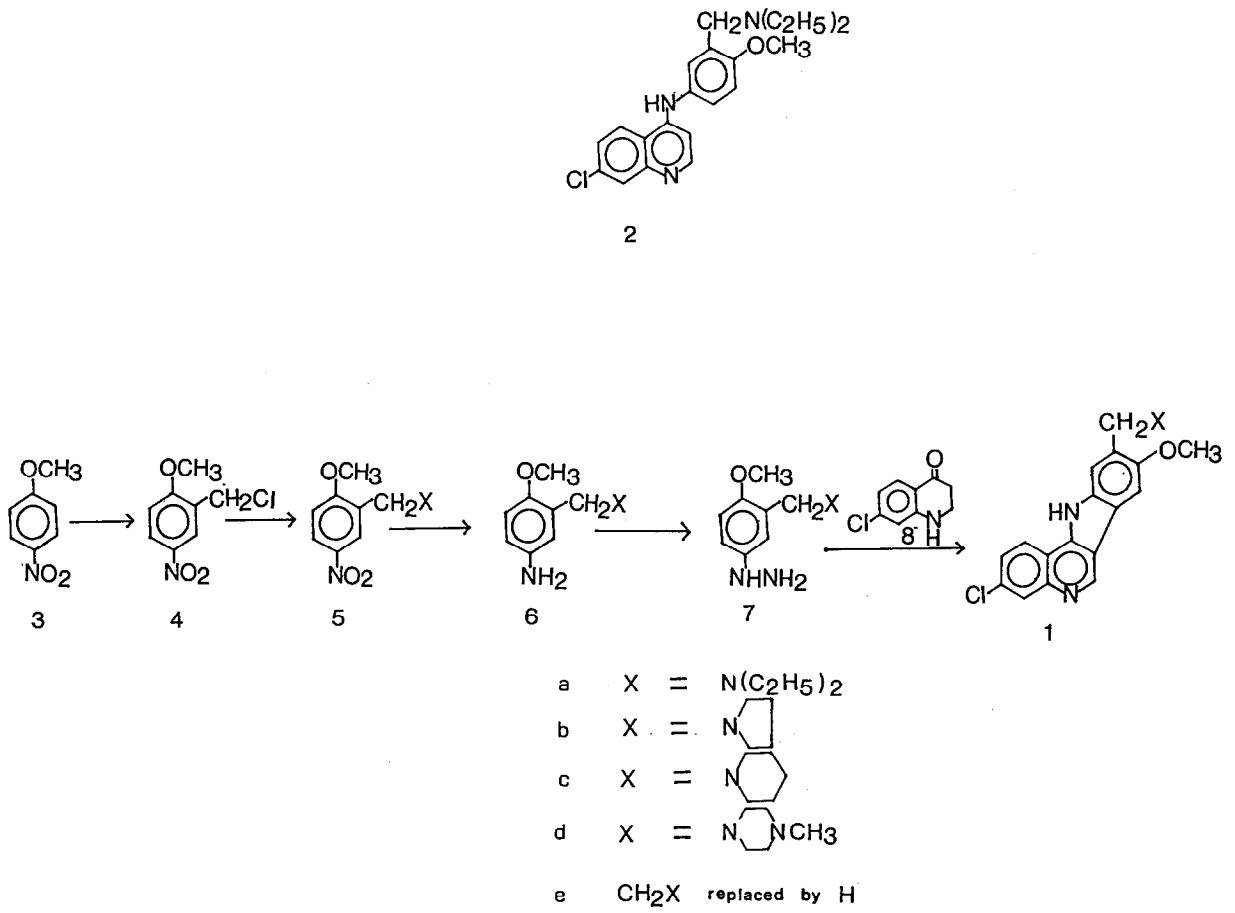
Introduction

A wide spectrum of biological activities has been observed with several derivatives of indolo[3,2-*c*]quinolines. These include antimalarial activity [1, 2], cytotoxic activity [3], DNA binding and RNA polymerase inhibitory activities [4, 5] and inhibition of zoxazolamine hydroxylase [6].

The synthesis of 3-chloro-9-*N*,*N*-diethylaminomethyl-8-methoxy-11H-indolo[3,2-*c*]quinoline (**1a**) was reported by Marquez *et al* [4], who showed that the planar structure of **1a** was responsible for its superior binding to DNA compared to its non-cyclized analogue, *O*-methylamodiaquine (**2**). *O*-Methylamodiaquine is an antimalarial agent comparable to amodiaquine in activity [7]. Compared to **2**, compound **1a** was reported to have diminished antimalarial activity in mice infected with *Plasmodium berghei* [5]. Since the amino side chain is known to be an essential moiety for antimalarial activity [7], it would be interesting to see if structural modification of the side chain of **1a** would result in enhanced antimalarial activity. This has led to the synthesis of the present series of compounds in which the conformationally flexible diethylamino function of **1a** has been replaced by the conformationally more restricted pyrrolidino, piperidino and piperazino rings, giving compounds **1b**, **1c** and **1d** respectively. For the purpose of comparison, compound **1e**, which has no side chain amino function at position 9, was also prepared.

Chemistry

The indolo[3,2-*c*]quinolines (**1b–e**) were synthesized by Fischer indolization of 7-chloro-1,2,3,4-tetrahydroquinolin-4-one (**8**) with the appropriate hydrazines (**7b–e**) (scheme 1). The hydrazines **7b–d** were prepared from *p*-nitroanisole (**3**) which on chloromethylation gave the chloromethyl derivative (**4**). Nucleophilic displacement of the chlorine atom of **4** by the heterocyclic bases pyrrolidine, piperidine and *N*-methylpiperazine in methanol furnished the corresponding amines (**5b–d**). Catalytic reduction of the aromatic nitro function of compounds **5b–d** over Raney nickel gave the corresponding aromatic amines (**6b–d**) which were used without purification in the subsequent step. The aromatic amines (**6b–d**) were converted to the diazonium salts with nitrous acid, generated *in situ* from sodium nitrite and acid, and the latter were immediately reduced with stannous chloride in acid to give the desired hydrazines (**7b–d**). The hydrazines, as bases, were reacted with 7-chloro-1,2,3,4-tetrahydroquinolin-4-one (**8**) in a Fischer indolization reaction to give the desired cyclized



Scheme 1.

compounds (**1b–d**) which were obtained as yellow solids from the reaction mixture. Compound **1e** was prepared in a similar manner from *p*-methoxyphenylhydrazine (**7e**), which was purchased commercially. The successful completion of the cyclization reaction was in all cases confirmed by NMR spectroscopy by the appearance of the aromatic C(6)-proton as a singlet at δ 8.5–8.8, when the adjacent ring nitrogen was protonated, and δ 9.2–9.6 when the latter was not. Furthermore, the C(7) and C(10) protons could be seen as singlets among other aromatic signals in the region of δ 7.0–7.5. Similar observations had previously been reported [4] for **1a**.

Results and discussion

The antimalarial activities of **1a–e** were evaluated by *in vitro* screening against a chloroquine resistant strain (K-1) of *P falciparum* according to the procedure of O'Neill *et al* [8]. The results of the biological evaluation are given in table I.

Table I. *In vitro* antimalarial activities of **1a–e** against *P falciparum* (K1, chloroquine resistant). Compounds **1a–e** were evaluated as the hydrochloride salts and chloroquine as the diphosphate salt. IC_{50} is the concentration of compound required to inhibit ^{3}H-hypoxanthine uptake by plasmodia by 50% compared to the control.

Compound	*IC_{50} (μM)*
1a	9.08×10^{-3}
1b	1.37×10^{-2}
1c	2.65×10^{-2}
1d	2.38×10^{-5}
1e	15.0
Chloroquine	0.27

With the exception of **1e**, the other indolo[3,2-*c*]quinolines (**1a–d**) exhibited good inhibitory activity against the chloroquine resistant strain of *P falciparum in vitro*. The poor activity of **1e**, which has no basic side chain at position 9, gives credence to the view that an amino group at the side chain is important for antimalarial activity.

Replacement of the diethylamino group of **1a** by the conformationally more rigid N-heterocycles had variable effects on activity. The antimalarial activity of the pyrrolidinyl derivative **1b** was comparable to that of **1a**, but the activity diminished in the case of the piperidinyl compound **1c**. Compound **1d** was the most active compound of this series. It was about 300 times more active than **1a** and approximately 10^4 times better than chloroquine to which the test organism was resistant. Unlike the other compounds of this series, **1d** has a polar dibasic piperazinyl moiety at the side chain. The greater activity of **1d** may be related to its lower hydrophobicity which may be optimal for activity. The improvement in antimalarial activity brought about by lowering the hydrophobicity of amodiaquine, chloroquine and quinacrine *via* N-oxidation of the ring nitrogen has been reported in the literature [9, 10]. The replacement of the diethylamino function of **1a** with a more polar piperazinyl moiety in **1d** may have achieved a similar result.

Despite the small compound base reported here, the evidence of potent *in vitro* inhibitory activity in the case of **1d** is interesting. The possibility of potent derivatives with dibasic side chains such as **1d** is a potential lead worthy of exploration. Further work will be directed towards the syntheses and evaluation of more indolo[3,2-*c*]quinolines with this structural feature.

Experimental protocols

Melting points were determined with a Gallenkamp melting point apparatus and were uncorrected. Infrared spectra were obtained in pressed KBr discs (unless otherwise stated) on a Jasco IR-810 spectrophotometer. Proton NMR spectra were recorded on a FT Jeol FX 90Q (90 MHz) instrument and chemical shifts were reported as δ ppm relative to tetramethylsilane (TMS) when taken in organic solvents, or sodium 3-(trimethylsilyl)propanesulphonate (DSS) in the case of D_2O solutions. Mass spectra were determined on a Micromass 7035 E double focussing mass spectrometer fitted with a mass spectrometry services solid state console and using Digital PDP 81a computer system for data capture and processing. Thin layer chromatography (TLC) was carried out on 5 x 20 cm Kieselgel G type 60 (Merck) plates. Elemental analyses (C, H, N) were performed on a Perkin-Elmer Auto-Analyser 240 and were found to be satisfactory.

The indoloquinoline 3-chloro-9-*N*,*N*-diethylamino-methyl-8-methoxy-11H-indolo[3,2-*c*]quinoline (**1a**) was prepared according to a reported procedure [4]. *p*-Methoxyphenylhydrazine hydrochloride and chloroquine diphosphate were purchased from Tokyo Kasei Chemical Company and Sigma Chemical Company respectively.

*General method for the preparation of amines **5b–d***

2-Chloromethyl-4-nitroanisole (**4**) [11] (0.026 mol) and an excess of the appropriate heterocyclic amine (0.135 mol) were refluxed for 18 h in methanol, after which the solvent was removed by evaporation under reduced pressure. The residue was dissolved in ether and the ethereal layer was washed with water until the aqueous phase was neutral. On drying over anhydrous Na_2SO_4, and removal of the solvent under reduced pressure, an oil was obtained. It was dissolved in dry ether and acidified by dropwise addition of freshly prepared ethanolic HCl which precipitated the product as the HCl salt.

*1-(2-Methoxy-5-nitrobenzyl)pyrrolidine (**5b**) hydrochloride*
The HCl salt was obtained in 80.2% yield, mp 195–196°C, after recrystallization from ethanol/dry ether. Found M^+ 236.1161 (61.52%). $C_{12}H_{16}N_2O_3$ requires 236.1161. IR, cm^{-1}: 2700–2450 (ν NH^+), 1620, 1595 (ν C = C). PMR: **5b** (HCl) (CD_3OD) δ ppm: 2.1 (4H, m, pyrrolidinyl CH_2), 3.3 (4H, m, pyrrolidinyl N-CH_2), 4.08 (3H, s, OCH_3), 4.50 (2H, s, Ar-CH_2), 7.2–8.5 (3H, aryl H).

*1-(2-Methoxy-5-nitrobenzyl)piperidine (**5c**) hydrochloride*
The HCl salt (84.5% yield) was recrystallized from ethanol and dry ether giving mp 212–213°C. Found M^+ 250.1300 (55.97%). $C_{13}H_{18}N_2O_3$ requires 250.1317. IR cm^{-1}: 2700–2500 (ν NH^+), 1620, 1600 (ν C = C). PMR: **5c** (HCl) (CD_3OD) δ ppm: 1.8 (6H, m, piperidinyl CH_2), 3.2 (4H, m, piperidinyl N-CH_2), 4.07 (3H, s, OCH_3), 4.41 (2H, s, Ar-CH_2), 7.2–8.5 (3H, aryl H).

*1-(2-Methoxy-5-nitrobenzyl)-4-methylpiperazine (**5d**) dihydrochloride*
The product **5d** (2HCl) was obtained in 65.5% yield and recrystallized from ethanol/dry ether, mp 211–212°C (lit [12] 81–83°C for base). Found M^+ 265.1422 (100%). $C_{13}H_{19}N_3O_3$ requires 265.1426. IR, cm^{-1}: 2700–2500 (ν NH^+), 1620, 1595 (ν C = C). PMR: **5d** (2HCl) (CD_3OD) ppm: 3.02 (3H, s, N-CH_3), 3.74 (8H, bs, piperazinyl CH_2), 4.08 (3H, s, OCH_3), 4.58 (2H, s, Ar-CH_2), 7.30–8.56 (3H, aryl H).

*General procedure for the preparation of arylamines **6b–d***

Each nitro compound (**5b–d**) (HCl) (0.007 mol) was dissolved in ethanol (50 ml) and hydrogenated at 60 psi, 28°C, in a Parr hydrogenator with 0.3 g Raney nickel (W2) as catalyst. When the uptake of hydrogen had ceased, the catalyst was removed by filtration through a sintered glass filter and the filtrate was acidified with ethanolic HCl. Removal of the solvent under reduced pressure gave arylamine as the HCl salt which was used without further purification for the next stage of reaction.

*General procedure for the preparation of hydrazines **7b–d***

Hydrazines **7b–d** were prepared according to a reported method [4]. Elemental analyses were unsatisfactory despite repeated attempts. This has also been noted in the literature [5].

*1-(5-Hydrazino-2-methoxybenzyl)pyrrolidine (**7b**)*
The hydrazine **7b** (2HCl) was obtained in 80% yield (1.69 g) and converted to the free base. IR, cm^{-1} (neat): 3500–3200 (ν $NHNH_2$). PMR: **7b** ($CDCl_3$) δ ppm: 1.73 (4H, m, pyrrolidinyl CH_2), 2.52 (4H, m, pyrrolidinyl N-CH_2), 3.56, 3.60 (2H, Ar-CH_2), 3.68, 3.71 (3H, OCH_3), 6.38–7.33 (3H, aryl H). Fine splittings of the Ar-CH_2 and O-CH_3 signals were observed. These were also noted in the spectrum of hydrazine **7c**. A previous report [4] on hydrazine **7a** had shown that these signals were singlets only after D_2O exchange. Other than a

possible solvent effect, the splitting may be due to conformational isomerism arising from the delocalization of the oxygen and nitrogen lone pairs of electrons to the aromatic ring.

*1-(5-Hydrazino-2-methoxybenzyl)piperidine (**7c**)*
The hydrazine **7c** (2HCl) was obtained in 85% yield, mp 200–203°C and was converted to the free base. IR, cm^{-1} (neat): 3500–3200 (ν $NHNH_2$). PMR: **7c** ($CDCl_3$) δ ppm: 1.48 (6H, m, piperidinyl CH_2), 2.3 (4H, m, piperidinyl CH_2), 3.18 (NH), 3.43, 3.46 (2H, Ar-CH_2), 3.66, 3.73 (3H, OCH_3), 6.43–7.26 (3H, aryl H).

*1-(5-Hydrazino-2-methoxybenzyl)-4-methylpiperazine (**7d**)*
The product (**7d**, 3HCl) was obtained in 75% yield, mp 215–216°C and was converted to the free base. IR, cm^{-1} (neat): 3500–3200 (ν $NHNH_2$). PMR: **7d** ($CDCl_3$) δ ppm: 2.21 (3H, s, NCH_3), 2.45 (8H, s, piperazinyl CH_2), 3.21 (NH), 3.45 (2H, s, Ar-CH_2), 3.66 (3H, s, OCH_3), 6.41–7.21 (3H, aryl H).

General procedure for the preparation of the indolo[3,2-c]quinolines 1b–e by Fischer indolization reaction

Indolization was carried out by reacting the hydrazine with 7-chloro-1,2,3,4-tetrahydroquinolin-4-one (**8**) [13] according to a reported method [4].

*3-Chloro-8-methoxy-9-(1-pyrrolidinylmethyl)-11H-indolo[3,2-c]quinoline (**1b**) dihydrochloride*
The product **1b** (2HCl) was obtained in 30% yield. Recrystallization from ethanol gave mp 330°C (with decomposition). Found M^+ 365.1295 (18.41%). $C_{21}H_{20}ClN_3O$ requires 365.1295. IR, cm^{-1}: 3000–2800 (ν NH^+), 1640, 1620 (ν C=C, δ NH), 1440 (δ CH). PMR: **1b** (2HCl) (D_2O) ppm: 2.20 (4H, m, pyrrolidinyl CH_2), 3.38, 3.55 (4H, m, pyrrolidinyl N-CH_2), 3.95 (3H, s, OCH_3), 4.40 (2H, s, Ar-CH_2), 6.96–7.4 (5H, m, aryl H), 8.78 (1H, s, aryl C(6)-H).

*3-Chloro-8-methoxy-9-(1-piperidinylmethyl)-11H-indolo[3,2-c]quinoline (**1c**) dihydrochloride*
The product **1c** (2HCl) was obtained in 30% yield and after recrystallization from ethanol gave mp 340°C (with decomposition). M^+ 379.1449 (21.98%). $C_{22}H_{22}ClN_3O$ requires 379.1451. IR, cm^{-1}: 3000–2700 (ν NH^+), 1620 (ν C=C, δ NH), 1440 (δ CH). PMR: **1c** (2HCl) (D_2O) δ ppm: 1.85 (6H, m, piperidinyl CH_2), 2.84–3.51 (4H, m, piperidinyl N-CH_2), 3.88 (3H, s, OCH_3), 4.26 (2H, bs, Ar-CH_2), 6.92–7.29 (5H, m, aryl H), 8.59 (1H, s, aryl C(6)-H).

*3-Chloro-8-methoxy-9-(4-methyl-1-piperazinylmethyl)-11H-indolo[3,2-c]quinoline (**1d**) trihydrochloride*
The product **1d** (3HCl) was obtained in 68% yield which on recrystallization from ethanol gave mp 350°C (with decomposition). Found M^+ 394.1560 (43.25%). $C_{22}H_{23}ClN_4O$ requires 394.1560. IR, cm^{-1}: 3000–2600 (ν NH^+), 1640, 1620 (ν C=C, δ NH), 1430 (δ CH). PMR: **1d** (2HCl) (D_2O) δ ppm: 3.11 (3H, s, N-CH_3), 3.75 (8H, bs, piperazinyl CH_2), 4.12 (3H, s, OCH_3), 4.66 (2H, s, Ar-CH_2), 7.69–8.19 (5H, m, aryl H), 9.35 (1H, s, aryl C(6)-H). The sample used had unprotonated ring nitrogen.

*3-Chloro-8-methoxy-11H-indolo[3,2-c]quinoline (**1e**) hydrochloride*
The product **1e** (HCl) was obtained in 68% yield. It was recrystallized from ethanol, giving mp 303–305°C. Found M^+ 282.0563 (100%). $C_{16}H_{11}ClN_2O$ requires 282.0560. IR, cm^{-1}: 2700–2600 (ν NH^+), 1640, 1610 (ν C=C, δ NH), 1430 (δ CH). PMR: **1e** (DMSO–d) δ ppm: 3.90 (3H, s, OCH_3), 6.90– 8.60 (6H, aryl H), 9.43 (1H, aryl C(6)-H).

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