Available online at www.sciencedirect.com
SCIENCE DIRECT®
Tetrahedron Letters 45 (2004) 7663–7666
Tetrahedron Letters

Amidomethylation of amodiaquine: antimalarial *N*-Mannich base derivatives

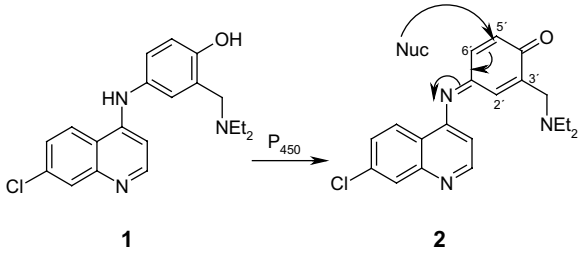
Francisca Lopes,[a] Rita Capela,[a] José O. Gonçaves,[a] Peter N. Horton,[b] Michael B. Hursthouse,[b] Jim Iley,[c,*] Catarina M. Casimiro,[d] Joana Bom[d] and Rui Moreira[a]

[a]*CECF, Faculdade de Farmácia, Universidade de Lisboa, Av. Forças Armadas, 1600-083 Lisboa, Portugal*
[b]*EPSRC National Crystallography Service, School of Chemistry, University of Southampton, Highfield, Southampton SO17 1BJ, UK*
[c]*Chemistry Department, The Open University, Milton Keynes MK7 6AA, UK*
[d]*Centro de Malária e Outras Doenças Tropicais, IHMT, Rua da Junqueira, 67, 1000 Lisboa, Portugal*

Received 25 June 2004; revised 12 August 2004; accepted 17 August 2004

Abstract—Novel *N*-Mannich base-type derivatives of the antimalarial drug amodiaquine were synthesised by reaction with tertiary *N*-chloromethylamides. With the exception of the derivative of ethyl hippurate, all the so-formed (1-amidomethyl-1*H*-quinolin-4-ylidene)arylamines displayed high chemical and enzymatic stability. These compounds displayed antimalarial activity against the multi-drug resistant *Plasmodium falciparum* strain Dd2 (IC_{50} values 15–31 nM) and demonstrated no significant loss in activity compared to amodiaquine (IC_{50} 30 nM).

Amodiaquine, **1**, is an effective antimalarial against both chloroquine-sensitive and chloroquine-resistant strains of *Plasmodium falciparum*.[1–3] The prophylactic clinical use of amodiaquine has been discontinued due to reports of hepatotoxicity and agranulocytosis.[4,5] These toxic side-effects have been ascribed to a cytochrome P_{450}-catalysed oxidation of the 4-hydroxyanilino moiety to a quinone imine, **2** (Scheme 1).[6–8] This metabolite reacts with proteins and peptides such as glutathione via nucleophilic addition to C-5′; its generation and binding to cellular macromolecules could affect cellular function directly or by immunological mechanisms that trigger hypersensitivity reactions.[8] The amidomethyl group has been suggested as a pro-drug protecting group for phenolic drugs.[9] We therefore decided to explore the reaction of amodiaquine with tertiary *N*-chloromethylamides and herein report that this leads to the *N*-amidomethylation of the quinoline nitrogen atom, rather than phenolic *O*- or amino *N*-alkylation. The resulting (1-amidomethyl-1*H*-quinolin-4-ylidene)arylamines, **3** are



1 **2**

Scheme 1.

kinetically rather stable ($t_{1/2}$ 3 to 11 d at pH 0.3 and 5 to >30 d at pH 7.4) (Table 1) and have useful antimalarial activity.

Compounds **3** were synthesised, albeit in moderate yields, by reacting the appropriate tertiary *N*-chloromethylamide with the sodium salt of amodiaquine, itself prepared from amodiaquine and sodium hydroxide or, alternatively, generated in situ with sodium hydride (1 mol equiv) (Scheme 2).[10] *N*-Chloromethylamides were prepared as previously reported,[11] by reacting the appropriate secondary amide with paraformaldehyde and chlorotrimethylsilane.

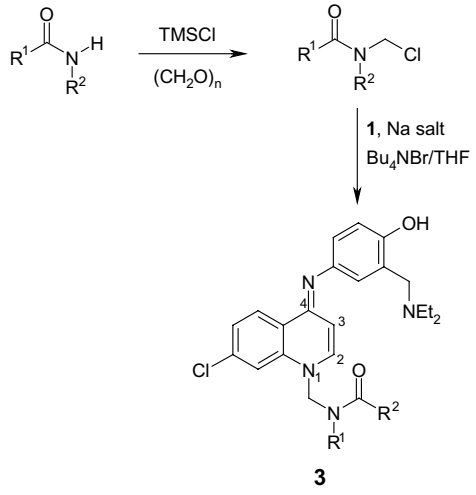
Keywords: Amodiaquine; Amidomethylation; Antimalarial.
* Corresponding author. Tel.: +44 01908 652513; fax: +44 01908 858327; e-mail: j.n.iley@open.ac.uk

0040-4039/$ - see front matter © 2004 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tetlet.2004.08.093

Table 1. Reactivity at 37 °C and antimalarial activity against the Dd2 *P. falciparum* strain of *N*-Mannich base derivatives of amodiaquine

Compound	R^1	R^2	Yield (%)	$t_{1/2}$ (h)			$IC_{50} \pm SD$[a] (nM)
				pH 0.3	pH 7.4	80% Human plasma	
3a	Me	Me	18	ND[b]	ND	ND	ND
3b	Me	4-$MeOC_6H_4$	47	102	469	Stable	19 ± 2.7
3c	Me	C_6H_5	31	207	559	Stable	(75)[c]
3d	Me	4-ClC_6H_4	55	256	>700	Stable	31 ± 1.6
3e	Me	4-$NO_2C_6H_4$	19	266	>700	Stable	15 ± 2.4
3f	CH_2CO_2Et	C_6H_5	53	81	129	19	25 ± 2.6
Chloroquine	—	—	—	—	—	—	270 ± 0.6
Amodiaquine	—	—	—	—	—	—	30 ± 2.4

[a] SD: standard deviation.
[b] ND: not determined.
[c] Percentage of inhibition of parasite growth at 1 nM.



Scheme 2.

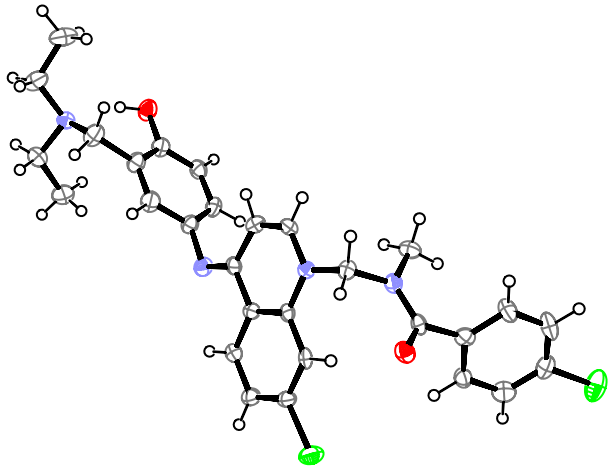
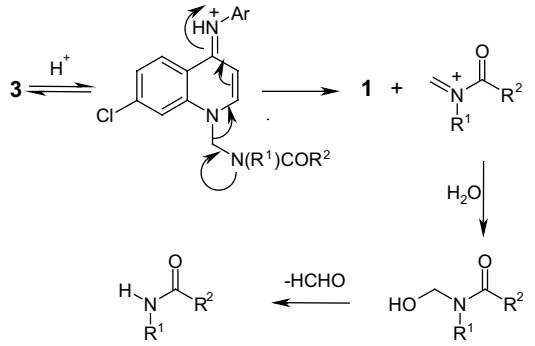


Figure 1. The Ortep plot of the X-ray crystal structure of **3d**.

The structural assignment of the *N*-amidomethylation products, **3**, is based on spectroscopic data, including DEPT, COSY and HMQC techniques. A characteristic ^1H NMR feature of compounds **3** is the resonance of the NCH_2N group, which appears as a singlet at 5.4–6.0 ppm; these are within the range reported for *N*-Mannich bases derived from amides and sulfonamides.[12] Further confirmation of the structure of **3** comes from the C2–H and C3–H resonances and corresponding vicinal coupling constant.[13] The C2–H resonance appears as a doublet at ca. 7.0 ppm (^3J = 8.1 Hz), which represents a dramatic upfield shift compared to the value of 8.5 ppm for the C2–H in **1** (^3J = 5.3 Hz).[14] The C3–H resonance in **3** appears at ca. 6.1 ppm, also upfield from the value of 6.6 ppm in **1**. Confirmation of the structure of the aminomethylated products as **3** came from the X-ray structure of **3d** (Fig. 1),[15] which shows inter alia an intramolecular hydrogen bond between the phenolic OH proton and the adjacent tertiary amino nitrogen atom.

Alkylation of aminoquinolines on the ring nitrogen is known.[16] However, we were surprised by such reaction for the anion of amodiaquine. The estimated pK_a values in the nonaqueous solvent DMSO for the relevant functional groups are: pyridyl *N*- ca. 5.1;[17] anilinium *N*- ca. −0.2,[18] aniline *N*- ca. 20–21[19] and phenolic *O*- ca. 20–21.[20] Thus, the pyridyl group is predicted to be more nucleophilic than the aniline, but less reactive than either the phenoxide or anilide anions. The pK_a values of the latter two groups make it is very difficult to predict, which anion is formed, *O*- or *N*-. The chemoselectivity of amodiaquine alkylation appears not to be thermodynamically driven since the heats of formation for the phenol *O*-, aniline *N*- and quinoline *N*-acetamidomethylated derivatives were calculated by the PM3 method within MOPAC to be −23.6, −31.9 and −24.3 kcal mol^{-1}, respectively (i.e., alkylation of the aniline functionality produces the most stable product). We suspect the lack of reaction at the phenol reflects either the steric hindrance exerted by the neighbouring diethylaminomethyl group or the preservation of the intramolecular hydrogen bond involving the phenol in the products **3**.

Using an HPLC method,[21] we found that compounds **3b–e** are very slowly converted to amodiaquine in pH 7.4 buffer (Table 1). However, the reactivity of compounds **3b–e** increases significantly at pH 0.3. Electron-donating substituents in the benzamide moiety enhance the rate of the acid-catalysed pathway (e.g., **3b** > **3c** > **3d** ≈ **3e**). These results are consistent with protonation of the 4-imino nitrogen atom followed by alkyl C–N bond scission via an S_N1 mechanism, leading to the formation of amodiaquine and the corresponding



Scheme 3.

secondary amide (Scheme 3), which was also detected by HPLC. Compounds **3b–e** are very stable in human plasma, with no significant degradation occurring during 48 h of incubation. In contrast, the ethyl hippurate derivative **3f** is rapidly hydrolysed in human plasma to yield amodiaquine. Possibly, the ethyl ester functionality is rapidly hydrolysed and the corresponding hippurate intermediate cyclises to expel amodiaquine.

Compounds **3** were assayed, using a microdilution assay,[22] for their inhibitory activity against intraerythrocytic forms of the Dd2 *P. falciparum* strain, a strain resistant to several common antimalarial drugs such as chloroquine but not to amodiaquine. All compounds tested displayed useful activity against this multi-drug resistant strain, with compounds **3b** and **3e** being significantly more active than amodiaquine. The results suggest that the physicochemical properties of the amide moiety do not contribute significantly to the antimalarial activity. For example, the 4-MeO and the 4-NO_2 benzamide derivatives (**3b** and **3e**, respectively) are equipotent despite significant differences in electronic and lipophilic properties between the two substituents ($\sigma_{p\mathrm{MeO}} = -0.27$, $\pi_{\mathrm{MeO}} = -0.02$, $\sigma_{p\mathrm{NO_2}} = 0.78$, $\pi_{\mathrm{NO_2}} = -0.28$). Moreover, a larger amino acid-based amide moiety, that is **3f**, also does not significantly affect the antimalarial activity.

In summary, amodiaquine reacts with tertiary *N*-chloromethylamides to form a novel antimalarial type of compound, **3**, that contains the 1*H*-quinolin-4-ylideneamine core structure. Compounds **3** are stable *N*-Mannich base-type derivatives and display excellent activity against a multi-drug resistant *P. falciparum* strain.

Acknowledgements

This project was supported by the contract POCTI/FCB/39218/2001. The authors are greatly indebted to Prof. Virgílio do Rosário (CMDT) for helpful discussions on the antimalarial assay results.

References and notes

1. Watkins, W. M.; Spencer, H. C.; Kariuki, D. M.; Sixsmith, D. G.; Boriga, D. A.; Kipingor, T.; Koech, D. K. *Lancet* **1984**, *22*, 357–359.
2. Muller, O.; Boele van Hensbroek, M.; Jaffar, S.; Drakeley, C.; Okorie, C.; Joof, D.; Pinder, M.; Greenwood, B. *Trop. Med. Int. Health* **1996**, *1*, 124–132.
3. Brasseur, P.; Guiguemde, R.; Diallo, S.; Guiyedi, V.; Kombila, M.; Ringwald, P. *Trans. R. Soc. Trop. Med. Hyg.* **1999**, *93*, 645–650.
4. Hatton, C. S. R.; Bunch, C.; Peto, T. E. A.; Pasvol, G.; Russell, S. G.; Singer, C. R. J.; Edwards, G.; Winstanley, P. *Lancet* **1986**, 411–414.
5. Neftel, K. A.; Woodtly, W.; Schmid, M.; Frick, P. G.; Fehr, J. *Br. Med. J.* **1986**, *292*, 721–723.
6. Maggs, J. L.; Tingle, M. D.; Kitteringham, N. R.; Park, B. K. *Biochem. Pharmacol.* **1988**, *37*, 303–311.
7. Harrison, A. C.; Kitteringham, N. R.; Clarke, J. B.; Park, B. K. *Biochem. Pharmacol.* **1992**, *43*, 1421–1430.
8. Naisbitt, D. J.; Williams, D. P.; O'Neill, P. M.; Maggs, J. L.; Willock, D. J. *Chem. Res. Toxicol.* **1998**, *11*, 1586–1595.
9. Getz, J. J.; Prankerd, R. J.; Sloan, K. B. *J. Org. Chem.* **1992**, *57*, 1702–1706.
10. General procedure for the preparation of **3**: a CH_2Cl_2 solution of the *N*-chloromethylamide (1 mmol in 2 mL) was added to a suspension of tetrabutylammonium bromide (1 mmol) and the sodium salt of **1** (1 mmol) in tetrahydrofuran (10 mL). Upon completion of the reaction (TLC), the solution was evaporated to dryness and the residue submitted to column chromatography on silicagel, using CH_2Cl_2/methanol (varying proportions) containing 1% of triethylamine as the eluent.
11. Moreira, R.; Mendes, E.; Calheiros, T.; Bacelo, M. J.; Iley, J. *Tetrahedron Lett.* **1994**, *35*, 7107–7110.
12. Iley, J.; Lopes, F.; Moreira, R. *J. Chem. Soc., Perkin Trans. 2* **2001**, 749–753.
13. Compounds **3** were fully characterised by ^{1}H and ^{13}C NMR methods and elemental analysis. For example: **3d**: δ_H (300 MHz, $CDCl_3$) 1.08 (6H, t, $J = 7.1$ Hz, CH_3CH_2), 2.60 (4H, q, $J = 7.1$ Hz, CH_3CH_2), 2.86 (3H, s, N–Me), 3.71 (2H, s, CH_2Ar), 5.52 (2H, s, NCH_2N), 6.04 (1H, d, $J = 8.1$ Hz, C3–H), 6.56 (1H, d, $J = 2.4$ Hz, C2′–H), 6.69 (1H, dd, $J = 2.2$, 8.4 Hz, C6′–H), 6.76 (1H, d, $J = 8.4$ Hz, C5′–H), 6.94 (1H, d, $J = 8.1$ Hz, C2–H), 7.22 (1H, dd, $J = 1.8$, 8.6 Hz, C6–H), 7.33–7.43 (5H, m, C8–H and C_6H_4Cl), 8.46 (1H, d, $J = 8.7$ Hz, C5–H); δ_C (75 MHz, $CDCl_3$) 11.32 (CH_3), 35.00 (NCH_3), 46.38 (CH_2), 57.01 (ArCH_2N), 63.05 (NCH_2N), 102.11 (C3), 114.64 (C8), 116.51 (C5′), 120.64 (C6′), 121.33 (C2′), 122.96 (*C*Ar), 124.10 (*C*Ar), 124.54 (C6), 127.85 (C5), 128.64 (*C*HAr), 129.07 (*C*HAr), 133.07 (*C*Ar), 136.83 (*C*Ar), 137.44 (*C*Ar), 138.98 (C2), 143.48 (*C*Ar), 153,61 (*C*Ar), 153.87 (*C*Ar), 170.95 (CO). Anal. ($C_{29}H_{30}Cl_2N_4O_2$) Calcd C, 64.81; H, 5.63; N, 10.42. Found C, 64.68; H, 5.66; N, 10.38.
14. Based on COSY ^{1}H NMR, the following assignment was made for amodiaquine: δ_H (300 MHz, $CDCl_3$) 1.14 (6H, t, $J = 7.0$ Hz, CH_3CH_2), 2.65 (4H, q, $J = 7.0$ Hz, CH_3CH_2), 3.78 (2H, s, CH_2Ar), 6.63 (1H, d, $J = 5.3$ Hz, C3–H), 6.67 (1H, br s, NH), 6.86 (1H, d, $J = 8.5$ Hz, C5′–H), 6.93 (1H, d, $J = 2.6$ Hz, C2′–H), 7.09 (1H, dd, $J = 2.6$, 8.5 Hz, C6′–H), 7.41 (1H, dd, $J = 2.2$, 9.0 Hz, C6–H), 7.84 (1H, d, $J = 9.0$ Hz, C5–H), 7.99 (1H, d, $J = 2.2$ Hz, C8–H), 8.46 (1H, d, $J = 5.3$ Hz, C2–H).
15. Crystallographic data (excluding structure factors) for the structure in this paper, has been deposited with the Cambridge Crystallographic Data Centre as supplementary publication numbers 242788. Copies of the data can be obtained, free of charge, on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (Fax: +44(0)-1223-336033 or e-mail: deposit@ccdc.cam.ac.uk). Crystal data: Yellow plates, $C_{29}H_{30}Cl_2N_4O_2$, Mr = 537.47,

$T = 120(2)$ K, monoclinic, space group P_1/n, $a = 8.8163(3)$ Å, $b = 30.2471(17)$ Å, $c = 10.2526(5)$ Å, $\beta = 108.359(2)°$, $V = 2594.9(2)$ Å^3, $\rho_{(calcd)} = 1.376$ Mg/m^3, $\mu = 0.285$ mm^{-1}, $Z = 4$, reflections collected: 14,764, independent reflections: 4926 ($R_{int} = 0.1147$), final R indices [$I > 2\sigma(I)$]: $R_1 = 0.0740$, $wR_2 = 0.1234$, R indices (all data): $R_1 = 0.2208$, $wR_2 = 0.1667$.

16. (a) Glen, W. L.; Sutherland, M. M. J.; Wilson, F. J.; Browning, C. H.; Browning, P.; Robb, J. V. M. *J. Chem. Soc.* **1939**, 489–492; (b) Simpson, J. C. E.; Wright, P. H. *J. Chem. Soc.* **1948**, 1707–1709.
17. The aqueous pK_a for the pyridyl *N*- in amodiaquine is 7.08 (Hawley, S. R.; Bray, P. G.; O'Neill, P. M.; Park, B. P.; Ward, S. A. *Biochem. Pharmacol.* **1996**, *52*, 723–733); however, the pK_a of 4-methylpyridine in DMSO (4.1, http://www.chem.wisc.edu/areas/reich/pkatable/index.htm) is ca. 2 pK_a units lower than in water (6.14, Chrystiuk, E.; Williams, A. *J. Am. Chem. Soc.* **1987**, *109*, 3040–3046).
18. The aqueous pK_a for diphenylamine is 0.77 (Pytela, O.; Vetesnik, V. *Coll. Czech. Chem. Commun.* **1983**, *48*, 2368–2375); however, the pK_a of aniline in DMSO (3.6, http://www.chem.wisc.edu/areas/reich/pkatable/index.htm) is ca. 1 pK_a unit lower than in water (4.62, Gervasini, A.; Aurou, A. *J. Phys. Chem.* **1993**, *97*, 2628–2639).
19. The pK_a in DMSO for diphenylamine acting as an acid is 24.9, while the presence of the pyridyl *N*- in the 4-position has the effect of lowering the pK_a by ca. 4 units (cf. aniline, pK_a 30.6, and 4-aminopyridine, pK_a 26.5) (Bordwell, F. G.; Algrim, D. J. *J. Am. Chem. Soc.* **1988**, *110*, 2964–2968); Alternatively, perfluoro 4-phenylaminopyridine has a pK_a in DMSO of 9.4, while 4-phenylamino-2,3,5,6-tetrafluoropyridine has a pK_a in DMSO of 15.1 (Vlasov, V. M.; Os'kina, I. A.; Starichenko, V. F. *Russ. J. Org. Chem.* **1997**, *33*, 660–664,). If we use these values to assume the effect of an F atom is to lower the pK_a by 1.2 units, then a value for the pK_a of 4-phenylaminopyridine of 19.9 can be estimated.
20. Data for phenol pK_as in DMSO (Bordwell, F. G.; McCallum, R. J.; Olmstead, W. N. *J. Org. Chem.* **1984**, *49*, 1424–1427, http://www.chem.wisc.edu/areas/reich/pkatable/index.htm) afford a Hammett equation, p$K_a = -5.96\rho + 18.2$, that allows calculation of a pK_a of 19.9 for 4-aminophenol and 20.86 for 4-phenylaminophenol.
21. Furtado, T.; Iley, J.; Jarvinen, T.; Mendes, E.; Moreira, R.; Neres, J.; Rautio, J. *Bioorg. Med. Chem.* **2002**, *10*, 809–816.
22. (a) Jensen, J. B.; Trager, W. *J. Parasitol.* **1977**, *63*, 883–886; (b) Fairlamb, A. H.; Warhurst, D. C.; Peters, W. *Ann. Trop. Med. Parasitol.* **1985**, *79*, 379–384.