Pergamon

SCIENCE DIRECT®

Bioorganic & Medicinal Chemistry Letters 13 (2003) 1059–1062

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

Alkylation of Manganese(II) Tetraphenylporphyrin by Antimalarial Fluorinated Artemisinin Derivatives

Montserrat Rodriguez,[b] Danièle Bonnet-Delpon,[a] Jean-Pierre Bégué,[a,*] Anne Robert[b] and Bernard Meunier[b,*]

[a]*Molécules Fluorées, BIOCIS-CNRS, Faculté de Pharmacie, rue J.-B. Clément, 92296 Châtenay-Malabry, France*
[b]*Laboratoire de Chimie de Coordination du CNRS, 205, route de Narbonne, 31077 Toulouse Cedex 4, France*

Received 22 July 2002; revised 1 October 2002; accepted 15 January 2003

Abstract—The alkylating properties of two artemisinin derivatives bearing a trifluoromethyl substituent at C10 were evaluated toward manganese(II) tetraphenylporphyrin, considered as a heme model. Chlorin-type covalent adducts were obtained by alkylation of the porphyrin ring by C-centered radicals derived from reductive activation of the peroxide function of the drugs.

From the discovering of artemisinin[1] **1a** (Fig. 1), the use of peroxide-based drugs for the treatment of multi-drug resistant malaria has been a promising approach. Artemisinin and its currently used hemisynthetic derivatives artemether **1b** and artesunate **1c** are rapidly absorbed, resulting in the more rapid parasite clearance among the available antimalarial drugs.[2] However, they are also rapidly eliminated[3] (half-life in plasma <2 h) resulting in an incomplete elimination of parasites leading to recrudescences when these drugs are used alone. Another drawback of artemisinin itself is its poor solubility in both water and oil that hampers its bioavailability.

To improve the pharmaceutical profile of artemisinin, a lot of derivatives have been synthesized, mainly by reduction of the lactone at C10 followed by functionalization of the obtained lactol.[4] Compounds bearing a fluoroalkyl substituent at C10 are of special interest owing to the hemiketal function stabilization by an electron withdrawing group, preventing a rapid metabolism. In addition, fluoroalkyl groups increase the lipophilicity of the compound (log P = 4.36, vs 3.36 for artemether). Another motive for preparing fluorinated artemisinin derivatives is that they can be detected and localized in vivo by several imaging techniques.[5]

The synthesis of 10α-(trifluoromethyl)hydroartemisinin **2** and 10β-(trifluoromethyl)trimethylsilyloxyartemisinin **3** was recently reported.[6–8] Compound **2** is active against the chloroquine resistant W-2 Indochina *Plasmodium falciparum* strain (IC_{50} value is 2.6, compared to 5.4 nM for β-artemether) and, in vivo, protects well mice from infection.[8b] Conversely, compound 3 has a poor activity in vitro ($IC_{50} = 72$ nM) as well as in vivo.[9]

The heme promoted reductive homolysis of the peroxide bond of artemisinin and related trioxanes leading to C4-centered alkylating radicals has been documented[10–12] and considered as pharmacologically relevant.[10,13] Similar C4-centered radicals were probable intermediates of the thermal decomposition of artemisinin previously reported.[14] Alkylation of heme,[12] or of a synthetic metalloporphyrin, manganese(II) tetraphenylporphyrin ($Mn^{II}TPP$) used as a heme model, by artemisinin,[15] artemether,[16] or synthetic antimalarial trioxanes[17] has been reported, and the resulting porphyrin–drug covalent adducts have been characterized.

Alkylation of $Mn^{II}TPP$ by Trioxane 2

To check if the alkylating ability was maintained with pharmacologically active trifluoromethyl derivatives of artemisinin **2** and **3**, we investigated their reactivity toward manganese(II) tetraphenylporphyrin in conditions that has been previously used for artemisinin and artemether.[15] For this purpose, trioxane **2** was incubated

*Corresponding author. Fax: +33-5-6155-3003; e-mail: bmeunier@lcc-toulouse.fr

0960-894X/03/$ - see front matter © 2003 Elsevier Science Ltd. All rights reserved.
doi:10.1016/S0960-894X(03)00076-3

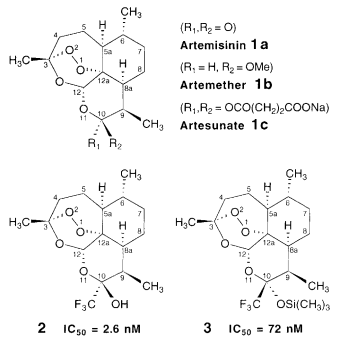

Figure 1. Structures of artemisinin **1a**, artemether **1b**, and artesunate **1c**. Structures of the 10-trifluoromethyl derivatives of artemisinin **2** and **3**. IC_{50} values were measured on W-2 Indochina *P. falciparum* strain (ref 7 for **2**, previously unpublished for **3**).

with $Mn^{II}TPP$ generated in situ from $Mn^{III}(TPP)Cl$ and borohydride in degassed dichloromethane at room temperature for 2 hrs ($[Mn^{III}(TPP)Cl] = 13.9$ mM, $[\mathbf{2}] = 19.3$ mM, $[n Bu_4N^+ BH_4^-] = 113$ mM, molar ratio $Mn^{III}(TPP)Cl/[\mathbf{2}]/BH_4^- = 1/1.4/8$). This reaction provided, after demetallation of the macrocycle (with cadmium nitrate followed by aqueous acetic acid 10 vol%)[15] and purification by chromatography, the chlorin-type adduct **H_2TPC–2** (Fig. 2, yield = 20%), as expected from the reaction of $Mn^{II}TPP$ with the parent drug artemisinin.

The UV–visible spectrum of the demetallated adduct **H_2TPC–2** was characteristic of a chlorin-type macrocycle [λ_{max} (nm) (rel. intens.) 372 (19), 406_{sh} (70), 420 (Soret, 100), 518 (8), 546 (6), 598, (3), 650 (13), with a ratio $\varepsilon_{650}/\varepsilon_{420} = 0.13$]. In the ^1H NMR spectrum, the multiplet signals corresponding to the aromatic protons (8.59–7.72 ppm) indicated a loss of the C_4 symmetry. The two intracyclic NH protons were detected at −1.51 ppm, deshielded by 1.3 ppm with respect to tetraphenylporphyrin, indicating a lower ring current effect. Three dihydropyrrole protons were detected at 4.69 ppm (dd, 1H, H2′α), 4.38 ppm (dd, 1H, $^2J = 17$ Hz, $^3J = 9$ Hz, H3′α), and 4.03 ppm (d, 1H, $^2J = 17$ Hz, H3′β). The proton H2′α (4.69 ppm) appeared as a doublet of doublets, corresponding to the coupling with H3′α($^3J = 9$ Hz) on one hand, and with one of the diastereotopic protons of the methylene C4 on the other hand ($^3J = 8$ Hz). This latter coupling provided confirmation of the C-alkylation at position C2′. Among the protons of the drug-derived moiety, the resonances of the methyl substituents at C6 and C9 were detected at 0.68 and 0.92 ppm, respectively, instead of 0.97 and 1.07 in the starting compound **2**.

The mass spectrum (ES^+) of **H_2TPC–2** exhibited a parent peak at $m/z = 911.6$ (MH^+), corresponding to the molecular mass. It should be noted that the reduction of C12 by BH_4^- was a general feature, as in the case of artemisinin. Even when the mass analysis was performed before purification by chromatography, only a trace amount of the 'complete' adduct **H_2TPC–2-OAc**, bearing the acetate at C12, was detected ($m/z = 969.4$, MH^+, Fig. 2).

In addition, MS analysis of the crude demetallated product (before chromatography) exhibited a peak at $m/z = 1207.6$ (20% with respect to the parent peak at $m/z = 911.6$), corresponding to a a- or b-tetrahydroporphyrin dialkylated by **2** (a- and b-tetrahydroporphyrins are reduced porphyrin derivatives with two dihydropyrrole rings, either adjacent or opposite,

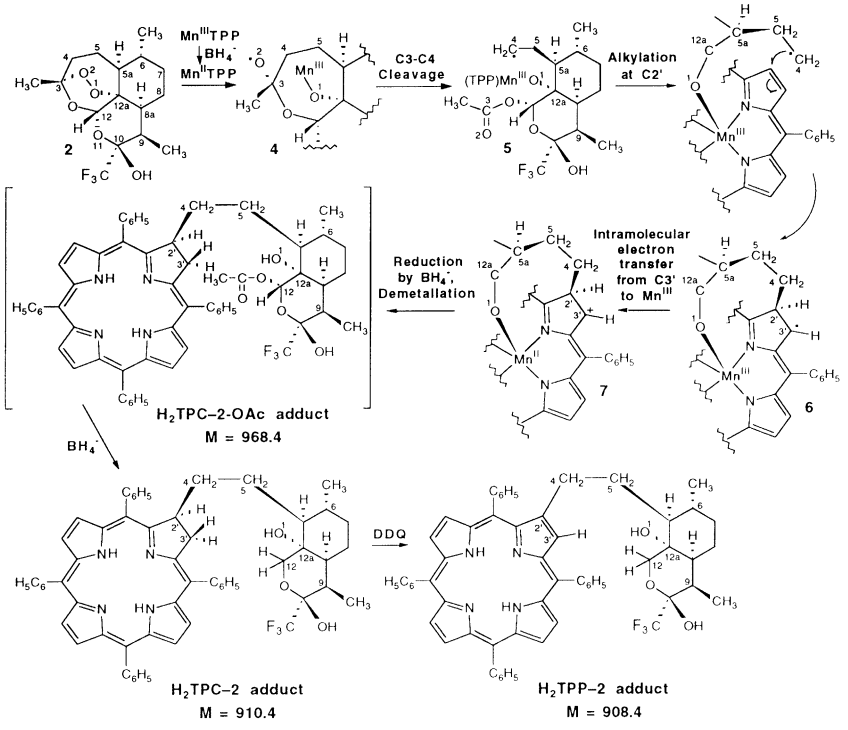

Figure 2. Mechanism of alkylation of $Mn^{II}TPP$ by 10α-trifluoromethylhydroartemisinin **2**.

respectively. These structures still retain conjugation and aromaticity).[18] This (or these) dialkylated tetrahydroporphyrin(s) were not isolated and fully characterized. However, this result, obtained with only 1.4 mol equiv of drug with respect to Mn^{II}TPP, indicates the possibility of efficient polyalkylation of the porphyrin ring in the used conditions.

Mechanism of the Alkylation Reaction

The formation of the adduct **H_2TPC–2** can be explained by the mechanism proposed in Figure 2. The reductive activation of the peroxide bond of **2** by Mn^{II}TPP produced the alkoxy radical **4** which quickly rearranged by homolytic cleavage of the C3–C4 bond to the non-sterically hindered C-centered radical **5**. The intramolecular addition of **5** on a β-pyrrolic carbon of the porphyrin ring allowed, after an intramolecular electron transfer from C3′ to the manganese(III), the generation of a carbocation at C3′. The attack of borohydride at this position led to the dihydropyrrole ring. Borohydride also mediated the reduction occurring at C12 of the drug fragment and the loss of the acetate function. The in situ demetallation under mild acidic conditions (transmetallation from Mn^{II} complex to its Cd^{II} analogue, followed by in situ demetallation of the cadmium complex) provided the covalent adduct **H_2TPC–2** as main product.

The purified demetallated chlorin-type adduct **H_2TPC–2** can be oxidized to the corresponding porphyrin adduct **H_2TPP–2** in the presence of a quinone derivative (2,3-dichloro-5,6-dicyanobenzoquinone, DDQ, 10 mol equiv). The product, recovered after chromatography (neutral alumina, dichloromethane/methanol 99.5/0.5 v/v), presented the expected UV–visible spectrum for a porphyrin ligand [λ_{max} (nm) (rel. intens.) 418 (Soret, 100), 514 (4), 586 (2), no absorption at 650]. The mass spectrum (ES^+) of **H_2TPP–2** was m/z (rel. intens.) 909.4 (MH^+), 910.5 (67), 911.5 (26). The intensity of the peak at $m/z = 911.5$ indicated that no chlorin-type adduct remained. The ^{1}H NMR spectrum confirmed the structure of a porphyrin-type covalent adduct: the NH resonances were detected at −2.86 ppm, and seven β-pyrrolic protons were identified at 8.90 (AB, 2H), 8.83 (d, 1H), 8.77 (2×d, 2H), 8.72 (br s, 1H) and 8.64 ppm (d, 1H)], the broad singlet at 8.72 being assigned to the C3′ proton. It should be noted that the NMR spectra of both chlorin and porphyrin adducts **H_2TPC–2** and **H_2TPP–2** are very similar to those previously reported for the analogue adducts obtained from Mn^{II}TPP and artemisinin itself.

Alkylation of Mn^{II}TPP by Trioxane 3

The reaction of 10β-(trifluoromethyl)trimethylsilyloxyartemisinin **3** with Mn^{II}TPP under the same conditions as reported above, yielded after demetallation to the same adduct **H_2TPC–2**, isolated in the reaction with trioxane **2**: all the analyses of the covalent adduct obtained from Mn^{II}TPP and **3** were consistent with those obtained for **H_2TPC–2** and **H_2TPP–2**. Then, we performed the DDQ-mediated oxidation without purification of the intermediate chlorin adduct. After chromatographic purification, **H_2TPP–2** ($m/z = 909.4$, MH^+) was characterized as the major adduct resulting from the alkylation of Mn^{II}TPP by **3**. Traces of an adduct bearing an acetate at C12 and a trimethylsilyl at C10 were detected by ES-MS ($m/z = 1039.4$, MH^+, 3% with respect to the parent peak; another adduct bearing the trimethylsilyl, but a reduced C12 position was also present at $m/z = 981.4$). It should be mentioned that, when **3** was treated with borohydride in the absence of Mn(TPP)Cl for 2 h, the yield of desilylation was below 10–20%. In these conditions, the alkylation reaction of the porphyrin cycle by a preliminary desilylated derivative of **3** is not probable.

However, the main result is that the trifluoromethyl substituent at position C10 did not disturb the alkylating ability expected for artemisinin derivatives. In addition, the presence of the bulky-$Si(CH_3)_3$ fragment, directed in α with respect to the mean drug plane (i.e., on the same side than the peroxide function) did not preclude the interaction between the metal complex and the peroxide function of the drug which is necessary for the reductive activation step (on this point of view, one can notice that α-arteether and β-arteether exhibit the same antimalarial activity against the D-6 clone of *P. falciparum*[19]). A similar effect has been reported with synthetic analogues of artemisinin lacking the lactone cycle: compounds bearing an α- or β-methoxy substituent at C12 were both biologically active and able to alkylate Mn^{II}TPP.[17]

In conclusion, both antimalarial 10-trifluoromethyl artemisinin derivatives are able to behave as alkylating agents after reductive activation of the peroxide in vitro by a synthetic heme model. Such reaction occurring in vivo with heme itself produced by digestion of hemoglobin within *P. falciparum* may lead to heme–drug adducts toxic for the parasite.

References and Notes

1. Klayman, D. L. *Science* **1985**, *228*, 1049.
2. White, N. J.; Nosten, F.; Looareesuwan, S.; Watkins, W. M.; Marsh, K.; Snow, R. W.; Kokwaro, G.; Ouma, J.; Hien, T. T.; Molyneux, M. E.; Taylor, T. E.; Newbold, C. I.; Ruebush, T. K.; Danis, M.; Greenwood, B. M.; Anderson, R. M.; Olliaro, P. *Lancet* **1999**, *353*, 1965.
3. Duc, D. D.; de Vries, P. J.; Nguyen, X. K.; Le Nguyen, B.; Kager, P. A.; van Boxtel, C. J. *Am. J. Trop. Med. Hyg.* **1994**, *51*, 785.
4. Vroman, J. A.; Alvim-Gaston, M.; Avery, M. A. *Curr. Pharm. Des.* **1999**, *5*, 101.
5. Pu, Y. M.; Torok, D. S.; Ziffer, H.; Pan, X.-Q.; Meshnick, S. R. *J. Med. Chem.* **1995**, *38*, 4120.
6. Abouabdellah, A.; Bégué, J.-P.; Bonnet-Delpon, D.; Gantier, J.-C.; Thanh Nga, T. T.; Thac, T. D. *Bioorg. Med. Chem. Lett.* **1996**, *6*, 2717.
7. Thanh Nga, T. T.; Ménage, C.; Bégué, J.-P.; Bonnet-Delpon, D.; Gantier, J.-C. *J. Med. Chem.* **1998**, *41*, 4101.
8. (a) Grellepois, F.; Chorki, F.; Crousse, B.; Ourévitch, M.; Bonnet-Delpon, D.; Bégué, J.-P. *J. Org. Chem.* **2002**, *67*, 1253.

(a) Chorki, F.; Grellepois, F.; Crousse, B.; Hoang, V. D.; Van Hung, N.; Bonnet-Delpon, D.; Begue, J. P. *Org Lett*. **2002**, *4*, 757.
9. Pradines, B.; Parzy, D.; Grellier, P.; Bonnet-Delpon, D.; Bégué, J.-P. Unpublished results.
10. Posner, G. H.; Oh, C. H.; Wang, D.; Gerena, L.; Milhous, W. K.; Meshnick, S. R.; Asawamahasakda, W. *J. Med. Chem*. **1994**, *37*, 1256.
11. Robert, A.; Dechy-Cabaret, O.; Benoit-Vical, F.; Meunier, B. *Acc. Chem. Res*. **2002**, *35*, 167.
12. Robert, A.; Coppel, Y.; Meunier, B. *Chem. Commun*. **2002**, 414.
13. Meshnick, S. R.; Taylor, T. E.; Kamchonwongpaisan, S. *Microbiol. Rev*. **1996**, *60*, 301.
14. Lin, A. J.; Klayman, D. L.; Hoch, J. M. *J. Org. Chem*. **1985**, *50*, 4504.
15. Robert, A.; Meunier, B. *J. Am. Chem. Soc*. **1997**, *119*, 5968.
16. Robert, A.; Meunier, B. *Chem. Eur. J*. **1998**, *4*, 1287.
17. Cazelles, J.; Robert, A.; Meunier, B. *J. Org. Chem*. **2002**, *67*, 909.
18. Smith, K. M., In *Porphyrins and Metalloporphyrins*; Smith, K. M., Ed.; Amsterdam, 1975; p 3.
19. Bhattacharjee, A. K.; Karla, J. M. *Chem. Res. Toxicol*. **1999**, *12*, 422.