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Orienting the heterocyclic periphery: a structural model for chloroquine's antimalarial activity†

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The antimalarial drug chloroquine binds to gallium proto-porphyrin-IX in methanol and in the solid state and represents a unique drug–heme model.

Chloroquine, the potent antimalarial drug, has found new life as a chemotherapy agent.[1] The origin of chloroquine's remarkable antimalarial activity inspired an intense sustained medicinal chemistry effort which has led to thousands of quinoline derivatives having been prepared and assayed for their antimalarial activity.[2] Although chloroquine resistance is now widespread, and this limits its use for single agent antimalarial therapy, its use in cancer therapy highlights the continued uncertainty of its targets and of its drug action mechanisms. Unfortunately the effort to understand its anti-malarial pharmacology is haunted by continued absence of any precise structural data for its biochemical interactions.[3] This is a poor position to begin efforts to develop chloroquine and the related quinoline family of antimalarials as antineoplastic agents.

Chloroquine's antimalarial activity is widely attributed to its disruption of heme processing in the digestive vacuole of *Plasmodia*. Warhurst's original hypothesis[4] of chloroquine activity has evolved to a model of drug inhibition of heme crystallization into the ultimate product of heme processing, hemozoin or malaria pigment.[5] The insolubility, paramagnetism, and nanocrystalline character of hemozoin has caused considerable difficulties in working with this unusual heme product[6] and its adducts with chloroquine. Direct observation of drug–hemozoin(or heme) binding has not been possible and some of the best evidence for its operation *in vivo* is the co-localization of radio-labeled drug with the heme crystals.[7] A heme–chloroquine complex has been observed by UV spectroscopy,[8] and binding mechanisms based upon π–π complexation have been proposed based on nuclear magnetic resonance (NMR)[9] and Raman spectroscopy studies.[10] A number of Q.M. studies have attempted to shed further light on these interactions,[11] but, in the absence of a well defined structural basis to begin this modeling, the results of these efforts have been ambiguous.

To solve these problems, we have developed two new soluble models for hemozoin: the first being based on ferric *meso*- and deutero-protoporphyrin-IX,[12] and the second being a gallium protoporphyrin-IX model.[13] Both form soluble and crystallographically characterized hemozoin-like propionate bridged dimers. Gallium(III) has a similar ionic radius as that of ferric iron, 0.62 Å *vs.* 0.65 Å, respectively,[14] and both share similar coordination chemistries of the trivalent oxidation state.[15] Ga(III)/Fe(III) mimicry has been used extensively[16] to understand difficult heme and non-heme biochemistry such as that in ^{1}H NMR determination of the diastereomerically controlled axial ligation of the pyropheophorbide A unit of chlorophyll.[17] Herein we demonstrate that, in solution and in the solid state, chloroquine forms a distinct complex with [Ga(III)(PPIX)]$_2$ and that these provide the first experimental structural model for this critical heme–drug interaction.

Chloroquine and Ga(PPIX) form a well-defined complex in solution as monitored by ^{1}H NMR, Fig. 1 and 2, and Fig. S3–S5 (ESI†). In solution, this interaction is in dynamic equilibrium

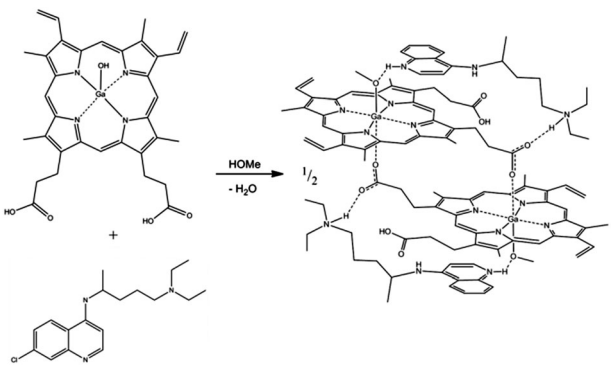

Fig. 1 Reaction of Ga(PPIX)(OH) with chloroquine free base to give [Ga(PPIX)(OMe)(CQ)]$_2$ (**1**). For numbering scheme see Fig. S1 (ESI†).

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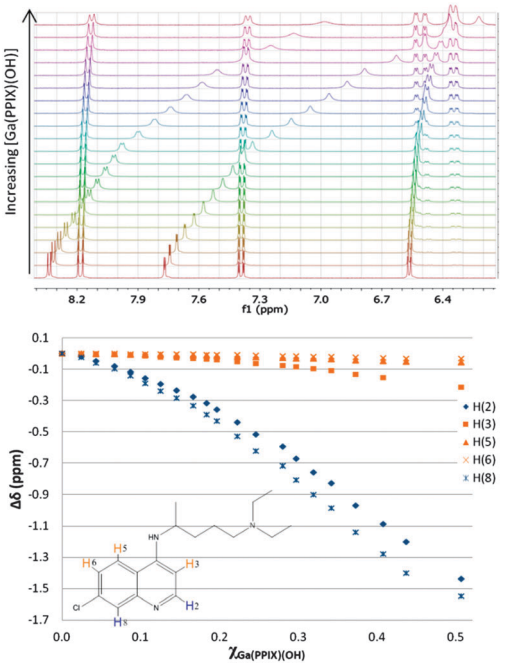

Fig. 2 Above, ^{1}H NMR titration for CQ added to Ga(PPIX)(OH); below, $\Delta\delta$ of CQ quinoline ring peaks with increasing Ga(PPIX) mole fraction corresponding to the stacked spectra shown.

that is fast on the NMR timescale, and the peaks observed are the average of those of all species. Large upfield peak shifts occur for the protons on the N-edge of the quinoline ring of the chloroquine and the protons near the terminus of the side chain show dramatic shifts as well. There is also a very large upfield shift and broadening of the signal of the methine proton H(20) of the porphyrin ring, which rests between the propionate groups, and a lesser shift and further broadening of the signals of the methylene protons of the propionic acid groups themselves. In the dimerized form observed crystallographically (see below), one of these propionic acid groups becomes a bridging propionate and also interacts with the terminal N of the bound chloroquine. A Job plot analysis, Fig. S5 (ESI†), fits well to either a 2 : 2 or 1 : 1 stoichiometry with an apparent binding constant of chloroquine to Ga(PPIX) of $K_{eq} = 1.48(5) \times 10^4\ M^{-1}$, assuming the 1 : 1 stoichiometry and ignoring dimerization and axial ligand exchange. However, this is at best an estimate of what is a multi-step and possibly cooperative series of equilibria.

Upon standing or concentration, solutions of gallium(III) protoporphyrin IX dimer and chloroquine crystallize as a 2 : 2 metalloporphyrin/chloroquine ensemble which preserves the solution interactions. Needle-shaped crystals of the drug–dimer complex suitable for X-ray diffraction grow well in methanol solutions containing ratios of two or more molecules of racemic free base chloroquine per molecule of Ga(PPIX)(OH). X-ray diffraction results in the model shown in Fig. 3 where views of the asymmetric unit and the key drug–porphyrin interactions are shown. As in malaria pigment, there is an inversion center of symmetry relating the two metalloporphyrin units, with the two enantiomers of chloroquine selectively bound to either one of the two chiral faces of dimer. The planes of the quinoline and porphyrin rings are oblique by 14.17°, and there is little overlap when viewed orthogonally (Fig. 3B). Chloroquine binds to gallium protoporphyrin IX with three E–H drug bonds oriented to the macrocycle's π-bonds over the N–C bonds of

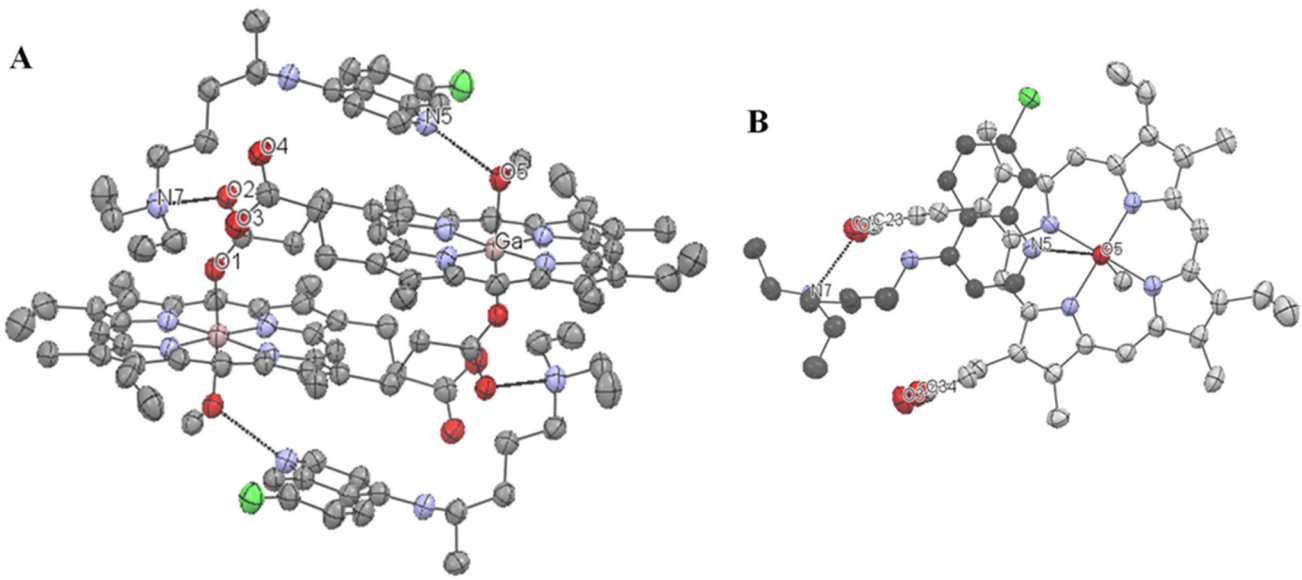

Fig. 3 Crystal structure of $[Ga(PPIX)(OMe)(CQ)]_2$. **A** Propionate bridged dimer generated by inversion symmetry with the two enantiomers of chloroquine hydrogen bound to the propionate carboxylate, N(7)–O(2) and methanol solvate, N(5)–O(5). **B** View down the Ga–O bond perpendicular to the porphyrin plane. Key metric parameters (Å) include: Ga–O(1) 2.010(6), Ga–O(5) 2.079(6), Ga–N(1) 2.002(6), Ga–N(2) 2.018(7), Ga–N(3) 2.011(7), Ga–N(4) 2.027(7), O(1)–C(23) 1.259(9), O(2)–C(23) 1.254(9), O(3)–C(34) 1.244(10), O(4)–C(34) 1.240(10). R_1 = 0.0642. Data measured at 112 K, thermal ellipsoids correspond to 30% probability, hydrogens omitted for clarity.

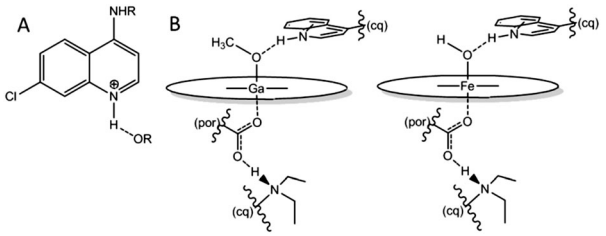

Fig. 4 (A) Substrate as chloroquine–alkoxide complex. (B) Configuration at the key binding site in the gallium complex, and proposed configuration of the analogous iron(III) protoporphyrin IX hydroxide complex.

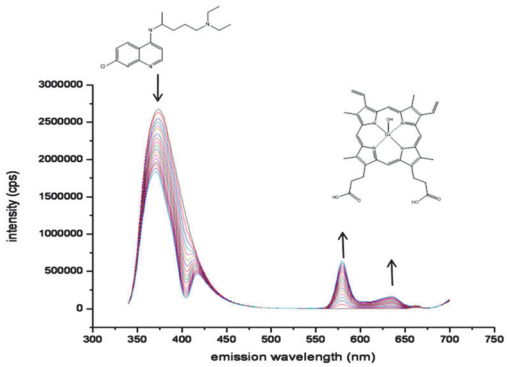

Fig. 5 Major fluorescence emission peak of chloroquine (373 nm) decreases in intensity upon addition of Ga(PPIX)(OH). Minor peak (417 nm) does not change. Ga(PPIX)(OH) peaks are observed due to direct excitation of the porphyrin at the excitation wavelength.

the porphyrin's pyrrole rings. This combination of C–H, and N–H aromatic interactions, with long ring–ring separations, (3.40–3.63 Å) results in a unique tilted but oriented edge interaction with relatively minor and weak electron donor/acceptor arene π-stacking. The quinoline ring nitrogen of the chloroquine hydrogen bonds to a coordinated methanol on a six-coordinate gallium, and there is an extensive hydrogen bonding and solvation network, Fig. S6 and S7 (ESI†).

The inclusion of a hydrogen bonded methanol or water molecule in the coordination sphere of the gallium, giving it an in-plane six coordinate geometry, is distinct from the solid state structures of hematin anhydride (β-hematin)[18] and malaria pigment[19] which are out of plane and five coordinate. Although Ga(PPIX) forms a condensed phase that is analogous to malaria pigment,[16f] in the presence of CQ it does not form. In general, the monomer and 6-coordinate species of Ga(PPIX) are considerably more soluble. In the case of **1** chloroquine hydrogen bonding to the methanol will generate a stronger Ga–OMe linkage which in turn stabilizes a planar six coordinate gallium. We propose that by analogy with hematin in water, the six coordinate hydroxide-like complex in Fig. 4B would be stabilized by the a high field ligand driving the metal to a lower spin state. Ferric heme proteins with coordinated hydroxides are often $S = 1/2$ and six coordinate.[20] Alkoxide and phenoxide antimalarials also have a high affinity for iron(III), as seen in a halofantrine-heme structure reported recently which have Fe–O bonds between the heme and the drug.[21] The geometry for $[Ga(PPIX)(OMe)(CQ)]_2$ in Fig. 3 could represent a drug–substrate interaction for heme in solution, possibly as $[Fe(PPIX)(H_2O{:}CQ)]_2$ (Fig. 4), prior to crystallization. Such an interaction would inhibit the growth of hemozoin, and thus account for the drug action of chloroquine.[22] This structure poises both the diethylamine and quinoline ring nitrogens in positions with hydrogen bonds to suitable acceptors. This corresponds to the expected protonation state of these antimalarials in the digestive vacuole and nicely solves the conundrum poised by the theoretical prediction of drug binding to the surface of the (001) growing face of malarial pigment without a second proton acceptor for the quinoline ring nitrogen hydrogen bond.[22a,23] In solution, and with a solvent bound or associated with the iron, this is no longer a problem.

In an effort to expand upon our solution observations to include biomimetic concentrations, we explored the electronic interactions of the species in solution, using the nascent fluorescent properties of both chloroquine and the gallium porphyrin. Titration of Ga(PPIX)(OH) or Ga(OEP)(OMe) against chloroquine in methanol gives a dramatic reduction in intensity of the 365 nm emission of chloroquine, Fig. 5, that is not evident in titration against acetic acid alone. A weaker peak at 417 nm, previously obscured, remains at constant intensity throughout. The absence of any change in quantum yield on addition of acid discounts simple pH effects on the quantum yield of the chloroquine in the ranges observed.

High-spin iron(III) porphyrins are, in general, fluorescence quenchers, while gallium porphyrins are highly fluorescent molecules themselves, and are currently being developed for use as photosensitizers in photodynamic therapy.[24] Any quenching of quinoline fluorescence emission that takes place must be due to close-range interactions between the drug and porphyrin molecules. The reaction between the drug and the porphyrin is slower than the excitation/emission pathway, and the quenching observed is therefore directly related to the amount of drug which is complexed to metalloporphyrin in the solution. This effect is readily quantifiable by fitting the data to a simple linear Stern–Volmer plot, after adjusting for concentration of the drug and the small absorption by the Ga(PPIX)(OH) (Fig. S9, ESI†).

From these data we can determine an approximation of the drug binding constant using the fluorescence intensities to be $K_{association} = 6.67 \times 10^4$ based on an assumption of 1:1 interaction. Photoexcitation is known to increase the basicity of the quinoline ring N *via* promotion of the stability of the amidine tautomer, whose pK_a is significantly higher,[25] which could account for the discrepancy between the NMR and fluorescence results. Regardless of the origin of these excited state dynamics, the practical implications for utilizing these models in high throughput antimalarial drug discovery screens is compelling.

To conclude, we have determined the unambiguous structure of the bound chloroquine–gallium(III) protoporphyrin IX reciprocal dimer complex by crystallography and established that key aspects of this structure are maintained in solution. The structure includes multiple sites of binding interactions of the drug to the metallo-protoporphyrin IX species, with quinoline–porphyrin stacking

interactions and two sites of hydrogen bonding interactions between each drug–porphyrin subunit leading to a very stable structure in which Van der Waals interactions with the porphyrin itself, rather than the central metal, dominate the interactions between the heme model and the drug. The structure is consistent with many known structure activity relationships for chloroquine: either enantiomer alone is active,[26] while changing the length or bulk of the side chain reduces activity.[27]

Recent years have seen leaps and bounds in the improvement of our understanding of the quinoline family of anti-malarial agents and their interactions with free heme.[2a,3,21] With these results it is clear that chloroquine may bind to heme in a manner distinct from that of the quinoline alkoxides such as quinine or quinidine which directly bind heme through the drug oxygen.[21] Thus the different quinoline sub-classes may target heme detoxification in different ways.[2a] This is excellent news, as it exemplifies the fragility of the hemozoin formation pathway in the parasite and its susceptibility to many kinds of interruption and opens us to the possibilities of exploring the diverse mechanisms of activities of each of these mini-classes of drugs to branch out in the development of new antimalarials into a much more diverse pool of compounds, taking advantage of these different pathways.

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Notes and references

1 V. R. Solomon and H. Lee, *Eur. J. Pharmacol.*, 2009, **625**, 220–233.
2 (*a*) A. P. Gorka, A. de Dios and P. D. Roepe, *J. Med. Chem.*, 2013, **56**, 5231–5246; (*b*) P. E. Thompson and L. M. Werbel, *Antimalarial Agents*, Academic Press, New York, 1972, p. 395; (*c*) G. R. Coatney, W. C. Cooper, N. B. Eddy and J. Greenberg, *Survey of Antimalarial Agents*, US Government Printing Office, Washington, 1952, p. 323.
3 T. J. Egan, *Targets*, 2003, **2**, 115–124.
4 D. C. Warhurst, C. A. Homewood, W. Peters and V. C. Baggaley, *Proc. Helminthol. Soc. Wash.*, 1972, **39**, 271–278.
5 A. C. Chou, R. Chevli and C. D. Fitch, *Biochemistry*, 1980, **400**, 1543.
6 (*a*) D. S. Bohle, B. J. Conklin, D. Cox, S. K. Madsen, S. Paulson, P. W. Stephens and G. T. Yee, *ACS Symp. Ser.*, 1994, **572**, 497–515; (*b*) A. F. G. Slater, W. J. Swiggard, B. R. Orton, W. D. Flitter, D. E. Goldberg, A. Cerami and G. B. Henderson, *Proc. Natl. Acad. Sci. U. S. A.*, 1991, **88**, 325–329.
7 D. J. Sullivan, I. Y. Gluzman, D. G. Russell and D. E. Goldberg, *Proc. Natl. Acad. Sci. U. S. A.*, 1996, **93**, 11865–11870.
8 (*a*) T. J. Egan, W. W. Mavuso, D. C. Ross and H. M. Marques, *J. Inorg. Biochem.*, 1997, **68**, 137–145; (*b*) C. H. Kaschula, T. J. Egan, R. Hunter, N. Basilico, S. Parapini, D. Taramelli, E. Pasini and D. Monti, *J. Med. Chem.*, 2002, **45**, 3531–3539.
9 (*a*) S. Moreau, B. Perly, C. Chachaty and C. Deleuze, *Biochim. Biophys. Acta*, 1985, **840**, 107–116; (*b*) I. Constantinidis and J. D. Satterlee, *J. Am. Chem. Soc.*, 1988, **110**, 4391–4395; (*c*) A. Leed, K. DuBay, L. M. B. Ursos, D. Sears, A. C. de Dios and P. D. Roepe, *Biochemistry*, 2002, **41**, 10245–10255.
10 G. T. Webster, D. McNaughton and B. R. Wood, *J. Phys. Chem. B*, 2009, **113**, 6910–6916.
11 C. Portela, C. M. M. Afonso, M. M. M. Pinto and M. Joao Ramos, *Bioorg. Med. Chem. Lett.*, 2004, **12**, 3313–3321.
12 D. S. Bohle, L. Dodd Erin, J. Kosar Aaron, L. Sharma, W. Stephens Peter, L. Suarez and D. Tazoo, *Angew. Chem., Int. Ed.*, 2011, **50**, 6151–6154.
13 D. S. Bohle and E. L. Dodd, *Inorg. Chem.*, 2012, **51**, 4411–4414.
14 R. D. Shannon, *Acta Crystallogr., Sect. A: Cryst. Phys., Diffr., Theor. Gen. Crystallogr.*, 1976, **32**, 751–767.
15 R. B. Martin, *Met. Ions Biol. Syst.*, 1988, **24**, 1–57.
16 (*a*) E. Vo, H. C. Wang and J. P. Germanas, *J. Am. Chem. Soc.*, 1997, **119**, 1934–1940; (*b*) G. Kubal, A. B. Mason, S. U. Patel, P. J. Sadler and R. C. Woodworth, *Biochemistry*, 1993, **32**, 3387–3395; (*c*) W. R. Harris and V. L. Pecoraro, *Biochemistry*, 1983, **22**, 292–299; (*d*) M. Merkx and B. A. Averill, *Biochemistry*, 1998, **37**, 8490–8497; (*e*) E. A. Fadeev, M. Luo and J. T. Groves, *J. Am. Chem. Soc.*, 2004, **126**, 12065–12075; (*f*) D. S. Bohle, E. L. Dodd, T. B. J. Pinter and M. J. Stillman, *Inorg. Chem.*, 2012, **51**, 10747–10761.
17 S. Sasaki, T. Mizoguchi and H. Tamiaki, *Bioorg. Med. Chem. Lett.*, 2006, **16**, 1168–1171.
18 D. S. Bohle, E. L. Dodd and P. W. Stephens, *Chem. Biodiversity*, 2012, **9**, 1891–1902.
19 N. Klonis, R. Dilanian, E. Hanssen, C. Darmanin, V. Streltsov, S. Deed, H. Quiney and L. Tilley, *Biochemistry*, 2010, **49**, 6804–6811.
20 A. Feis, M. P. Marzocchi, M. Paoli and G. Smulevich, *Biochemistry*, 1994, **33**, 4577–4583.
21 K. A. de Villiers, H. M. Marques and T. J. Egan, *J. Inorg. Biochem.*, 2008, **102**, 1660–1667.
22 (*a*) R. Buller, M. L. Peterson, O. Almarsson and L. Leiserowitz, *Cryst. Growth Des.*, 2002, **2**, 553–562; (*b*) I. Solomonov, M. Osipova, Y. Feldman, C. Baehtz, K. Kjaer, I. K. Robinson, G. T. Webster, D. McNaughton, B. R. Wood, I. Weissbuch and L. Leiserowitz, *J. Am. Chem. Soc.*, 2007, **129**, 2615–2627.
23 K. Y. Fong and D. W. Wright, *Future Med. Chem.*, 2013, **5**, 1437–1450.
24 (*a*) C. Litwinski, S. Tannert, A. Jesorka, M. Katterle and B. Röder, *Chem. Phys. Lett.*, 2006, **418**, 355–358; (*b*) Y. Nakae, E.-i. Fukusaki, S.-i. Kajiyama, A. Kobayashi, S. Nakajima and I. Sakata, *J. Photochem. Photobiol., A*, 2005, **172**, 55–61; (*c*) B. C. Robinson, I. M. Leitch, S. Greene and S. Rychnovsky, *WO Pat.*, 20022096366A2, To Miravant Pharmaceuticals, Inc, USA, 2002.
25 (*a*) N. Mataga, Y. Kaifu and M. Koizumi, *Bull. Chem. Soc. Jpn.*, 1956, **29**, 373–379; (*b*) P. J. Kovi, A. C. Capomacchia and S. G. Schulman, *Anal. Chem.*, 1972, **44**, 1611–1615; (*c*) S. G. Schulman, R. M. Threatte, A. C. Capomacchia and W. L. Paul, *J. Pharm. Sci.*, 1974, **63**, 876–880.
26 P. Augustijns and N. Verbeke, *Clin. Pharmacokinet.*, 1993, **24**, 259.
27 R. T. Berliner and T. Butler, in *Summary of data on the durgs tested in man*, ed. F. Y. Wiselogle and J. V. Edwards, U. Michigan, Ann Arbor, 1946, pp. 221–390.

