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Synthesis, Physicochemical Properties and Biological Evaluation of Aromatic Ester Prodrugs of 1-(2′-Hydroxyethyl)-2-ethyl-3-hydroxypyridin-4-one (CP102): Orally Active Iron Chelators with Clinical Potential

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Abstract

The synthesis of seven aromatic ester derivatives of 1-(2′-hydroxyethyl)-2-ethyl-3-hydroxypyridin-4-one is described. These ester prodrugs have been designed to target iron chelators to the liver, the major iron storage organ. In principle this should improve chelation efficacy and minimize toxicity.

The distribution coefficients of these ester prodrugs between 1-octanol and MOPS buffer pH 7·4 were measured together with their rates of hydrolysis at pH 2 and pH 7·4, in rat blood and liver homogenate. Esters with heteroaromatic acid moieties were found to be less stable than benzoyl analogues. The in-vivo iron mobilisation efficacy of these ester prodrugs has been compared with that of the parent drug using a ^{59}Fe-ferritin loaded rat model. Many prodrugs were found to enhance the ability of the parent hydroxypyridinone to facilitate ^{59}Fe excretion. However, not all prodrugs provided increased efficacy, demonstrating that lipophilicity is not the only factor which influences drug efficacy. Furthermore, no clear correlation between efficacy and susceptibility to hydrolysis was detected.

The picolinic and nicotinic ester derivatives appear to offer the best potential as prodrugs as they have a relatively low LogP value and yet lead to enhanced efficacy over the parent hydroxypyridinone.

Transfusion-dependent patients such as those suffering from β-thalassaemia develop a fatal secondary haemosiderosis and consequently a selective iron chelator must be used to relieve such iron overload (Pippard et al 1978a). Currently, desferrioxamine is the only clinically useful drug which is widely available for this purpose. Unfortunately patient compliance is poor, because of the lack of oral activity and rapid plasma clearance (Pippard et al 1978a, b). Consequently there is an urgent need for an orally active iron chelating agent.

3-Hydroxypyridin-4-ones are currently one of the main candidates for the development of orally active iron chelators (Tilbrook & Hider 1998). To date, several 3-hydroxypyridin-4-one ligands have been widely investigated for iron chelation, both in iron-overloaded animal models and in thalassaemic patients. Most results have shown that excretion of iron can be enhanced via both the urinary and biliary routes, and some compounds have potential as clinically useful chelators (Porter et al 1989). Unfortunately, the majority of effort with human studies has centred on the 1,2-dimethyl analogue **1** (L1, Deferiprone; Table 1), which is a relatively inefficient chelator under in-vivo conditions and consequently high doses must be utilised in order to achieve clinically useful levels of iron excretion. One of the major reasons for the limited efficacy of **1** in clinical use is that it undergoes extensive phase II metabolism in the liver (Singh et al 1992a). The 3-hydroxyl functionality, which is crucial for scavenging iron, is also a prime target for phase II conjugation. Urinary recovery studies conducted on **1** in both rats and man have shown that, respectively, >44% and >85% of the administered dose is recovered in the urine as the non-chelating 3-*O*-glucuronide conjugate (Figure 1) (Singh et al

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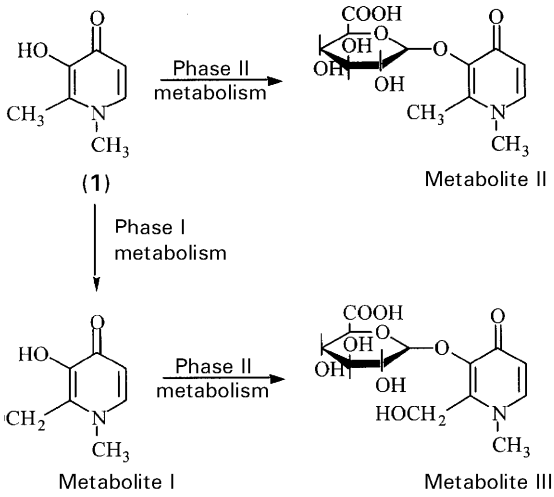


Figure 1. Metabolism of **1** in rat and man to form the corresponding non-chelating glucuronide conjugates.

1992a). This extensive biotransformation of **1** limits its ability to mobilise excess body iron in thalassaemic patients (Oliveri et al 1992).

The 1,2-diethyl analogue **2** (Table 1) has also been introduced into man. This chelator has been found to be more efficient at iron removal than **1** in several mammalian species, for example the rat (Porter et al 1990a, b). A likely explanation for this greater efficacy in the rat model is the difference in metabolism of **2** and **1** (Figure 2) (Singh et al 1992a). The 3-*O*-glucuronide of **2**, unlike that of **1**, is only a minor metabolite in the rat. The major metabolic pathway for **2** in the rat is phase I metabolism which leads to the formation of the 2-(1′-hydroxyethyl) metabolite (metabolite I; **3**). This metabolite does not undergo further phase II metabolism to form a glucuronide conjugate and hence retains the ability to chelate iron (Figure 2). Promising results obtained in rat models led to the limited clinical evaluation of **2** in thalassaemic patients (Porter et al 1994). Unfortunately, the metabolism of **2** in man did not parallel that in the rat. The main urinary metabolite of **2** in man is the 3-*O*-glucuronide conjugate (>85%; Porter et al 1993). Extensive conversion to this metabolite was found to severely limit the clinical efficacy of **2**.

This critical dependence of chelator efficacy on metabolic behaviour has led to a concept of ligand design which minimises conjugation reactions with glucuronic acid. Despite the limited efficacy of **2** in man, the superior extracellular and intracellular

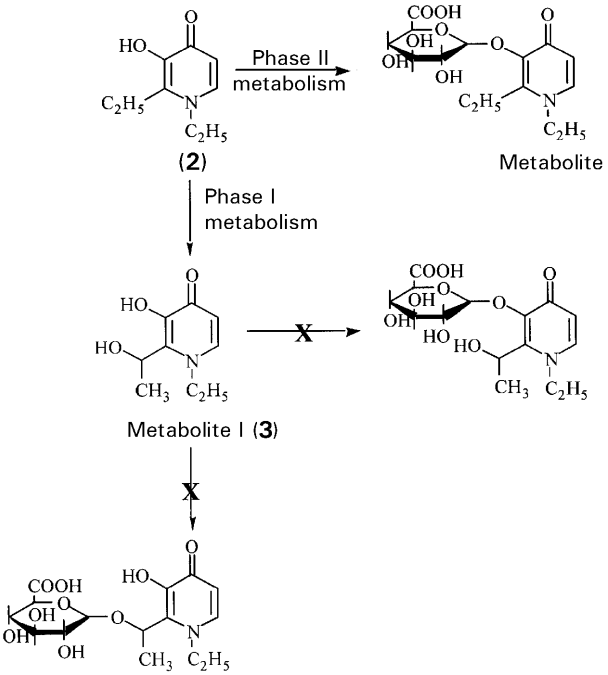
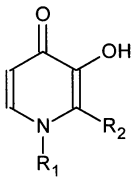


Figure 2. Metabolism of **2** via phase I and phase II metabolic pathways. Metabolite I (**3**) is the major metabolite in rat; metabolite II is the major metabolite in man.

Table 1. Chemical structure of selected 3-hydroxypyridin-4-ones and aliphatic ester derivatives of CP102.



Compound	R1	R2	MW	$D_{7.4}$
1	CH_3	CH_3	139	0·17
2	CH_2CH_3	CH_2CH_3	167	1·78
3	CH_2CH_3	$CH(OH)CH_3$	183	0·25
CP102 (**4**)	CH_2CH_2OH	CH_2CH_3	183	0·22
5	$CH_2CH_2OCOCH_3$	CH_2CH_3	225	0·58
6	$CH_2CH_2OCOCH(CH_3)_2$	CH_2CH_3	253	5·06
7	$CH_2CH_2OCOC(CH_3)_3$	CH_2CH_3	267	14·5

iron mobilisation ability in the rat provided important information for chelator design. The lack of glucuronidation of the 2-(1′-hydroxyethyl) metabolite of **2** led to the investigation of the possibility of developing structurally related compounds. Indeed, 1-hydroxyalkyl derivatives of 3-hydroxypyridin-4-ones such as 1-(2′-hydroxyethyl)-2-ethyl analogue CP102 (**4**; Table 1), which are not extensively metabolised via phase II reactions, have been identified (Singh et al 1992b, 1996). Efficacy studies of CP102 in rat indicated that the iron excretion was at least twice that seen with **1** (Jin et al 1993). Further investigation in primates confirmed that CP102 is even more effective; urinary and biliary iron excretion being almost three times greater than that of **1** (Jin et al 1993).

Although the use of CP102 may offer a significant improvement over previously evaluated 3-hydroxypyridin-4-ones, the disadvantage of this hydrophilic compound ($D_{7.4} = 0.22$) is poor oral absorption and insufficient extraction by the liver, which is the major iron storage organ. The development of hydrophobic ester prodrugs of CP102 is one route which has been considered for improving both drug absorption and hepatic extraction. A range of 1-positioned aliphatic ester derivatives of CP102 has been prepared by Rai et al (1999) (Table 1). In-vitro esterase studies indicate that **7**, which is the pivaloyl ester of CP102, may partially fulfil the requirements for relatively efficient liver extraction. This is due to its resistance towards hydrolysis in pH 2 and 7·4 buffer and a much lower hydrolysis rate in plasma than the liver, whereas **5** and **6** show much faster hydrolysis rates in plasma (Choudhury 1995). Preliminary pharmacokinetic and absorption studies of **7** in the rat have demonstrated that selective targeting of the liver is possible (Choudhury et al 1997). Results obtained indicate that **7** is rapidly absorbed from the gastrointestinal tract in the intact form and subsequently undergoes extensive first pass metabolism. Unfortunately, pivalic acid possesses undesirable side effects in man when used in high doses. For instance, the carnitine cycle is inhibited by branched acids which can lead to serious muscle side effects (Melegh et al 1987; Holme et al 1989, 1992). In order to overcome this problem, alternate ester prodrug derivatives of CP102 need to be identified.

In this study we report the synthesis and properties of aromatic esters of CP102. The advantage of using aromatic moieties is that they do not interfere with the carnitine transport system. Indeed, benzoic acid is used clinically to treat hyperammonaemia caused by a genetic defect in urea synthesis (Brusilow et al 1979; Batshaw et al 1982; Poon & Pang 1995). It is well established that benzoic acid is conjugated with glycine to form hippuric acid in humans and that this conjugate is not toxic (Ansel & Levy 1969; Borshop et al 1989). By selecting different aromatic acid moieties, it is possible to synthesise a range of ester derivatives with differing lipophilicity and possibly differing biological activity.

Materials and Methods

Chemistry

Melting points were determined using an Electrothermal IA 9100 Digital Melting Point Apparatus and are uncorrected. ^{1}H-NMR spectra were recorded using a Perkin-Elmer (60 MHz) NMR spectrometer. Chemical shifts (δ) are reported in ppm downfield from the internal standard tetramethylsilane (TMS). Elemental analyses were performed by Micro Analytical Laboratories, Department of Chemistry, The University of Manchester, Manchester, M13 9PL, UK. The common synthetic pathway of the ester prodrugs of CP102 is summarised in Figure 3.

*2-Ethyl-3-benzyloxypyran-4(1*H*)-one* (**8**). To a solution of ethyl maltol (56 g, 0·4 mol, 1 equivalent) in methanol (500 mL) was added sodium hydroxide (17·6 g, 0·44 mol, 1·1 equivalent) dissolved in water (40 mL) followed by benzyl chloride (55·7 g, 0·44 mol, 1·1 equivalents) and the mixture was refluxed for 6 h. After removal of solvent by rotary evaporation, the residue was mixed with water (200 mL) and extracted with dichloromethane (3 × 100 mL). The combined extracts were washed with 5% aqueous sodium hydroxide (2 × 200 mL), followed by water (2 × 200 mL). The organic fraction was then dried over anhydrous sodium sulphate, filtered and rotary evaporated to yield an orange oil which solidified on cooling. Recrystallization from diethyl ether gave the pure product (**8**) as colourless needles (78 g, 84·8%), mp 33–34°C. ^{1}H-NMR ($CDCl_3$) δ: 1·0 (t, 3H, 2-CH_2 CH_3), 2·55 (q, 2H, 2-CH_2CH_3), 5·13 (s, 2H, CH_2Ph), 6·3 (d, 1H, 5- *H*(pyranone)), 7·35 (s, 5H, *Ar*), 7·6 (d, 1H, *6-H* (pyranone)).

*2-[2-Ethyl-3-benzyloxypyridin-4(1*H*)-one-1-yl]-ethanol* (**9**). To a solution of (**8**) (25 g, 0·11 mol, 1 equivalent) in ethanol (200 mL)/water (200 mL) was added ethanolamine (10 g, 0·165 mol, 1·5 equivalent) followed by 2 N sodium hydroxide solution until pH 13·5 was obtained. The mixture was refluxed overnight. After adjustment to pH 1 with concentrated hydrochloric acid, the solvent

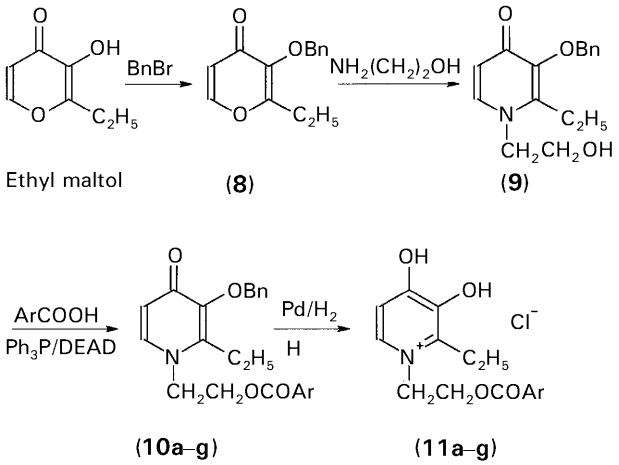


Figure 3. The synthesis of aromatic ester derivatives of CP102.

was removed by rotary evaporation before addition of water (200 mL) and washing with diethyl ether (2 × 200 mL). Subsequent adjustment of the aqueous fraction to pH 7 with 10 N sodium hydroxide solution was followed by extraction into dichloromethane (4 × 200 mL). The combined organic layers were dried over anhydrous sodium sulphate, filtered and rotary evaporated to give a yellow solid. Recrystallization from ethyl acetate gave the pure product (**9**) as yellow needles (17·85 g, 60%), mp 120–122°C. ^{1}H-NMR ($CDCl_3$) δ: 1·0 (t, 3H, 2-CH_2CH_3), 2·65 (q, 2H, 2-CH_2CH_3), 3·8 (br., s, 4H, NCH_2CH_2O), 5·0 (s, 2H, CH_2Ph), 5·3–6·2 (br., 1H, *OH*), 6·1 (d, 1H, *5-H* (pyridinone)), 7·0–7·5 (m, 6H, *Ar* & *6-H* (pyridinone)). Anal. calcd for $C_{16}H_{19}NO_3$: C 70·31, H 7·01, N 5·12%. Found: C 70·58, H 6·89, N 4·96%

*General method for the preparation of benzylated ester derivatives of CP102 (**10a–g**).* A solution of triphenyl phosphine (2·88 g, 11 mmol, 1·1 equivalents) and benzylated CP102 (**9**) (2·73 g, 10 mmol, 1 equivalents) in dry tetrahydrofuran (75 mL) was added dropwise to a solution of diethyl azodicarboxylate (1·914 g, 11 mmol, 1·1 equivalents) and the corresponding aromatic acid (10 mmol, 1 equivalent) in dry tetrahydrofuran (15 mL) at room temperature. After stirring the mixture overnight at room temperature, the solvent was removed under reduced pressure. The residue thus obtained was purified by column chromatography on silica gel (eluant, methanol–chloroform, 8 : 92) to afford the product (**10a–g**) as a yellow oil.

*General method for the preparation of ester derivatives of CP102 (**11a–g**).* Solutions of the benzylated ester derivatives of CP102 (**10a–g**) (2–3 g) in *N*,*N*-dimethylformamide (40–50 mL) were subjected to hydrogenolysis in presence of 5% Pd/C (5–10% w/w of the compound) catalyst for 12 h. The mixtures were warmed and filtered. The filtrates were acidified using hydrogen chloride gas followed by rotary evaporation to give white solids. Recrystallization from methanol/diethyl ether gave the pure products (**11a–g**) as white powders.

Distribution coefficient determination

Distribution coefficients were determined using an automated continuous flow technique as described by Rai et al (1998). The system comprised an IBM compatible PC running the Omniferous Personal Computer Auto-Titrator (TOPCAT) program, which controlled both a Metrohm 665 Dosimat autoburette and a Pye-Unicam Lambda 5 UV/vis spectrophotometer, as well as performing all calculations of distribution coefficients. All distribution coefficient determinations were performed using analytical-grade reagents under a nitrogen atmosphere using a flat-based glass vessel equipped with a sealable lid at 25°C. The aqueous and octanol phases were presaturated with respect to each other before use. The filter probe consisted of a polytetrafluoroethylene plunger associated with a gel-filtration column. The aqueous phase (50 mM MOPS buffer, pH 7·4, prepared using Milli-Q water) was separated from the two-phase system (1-octanol–MOPS buffer, pH 7·4) by means of a hydrophilic cellulose filter (5 μm diameter), 589/3 Blauband filter paper, Schleicher and Schuell) mounted in the gel-filtration column adjuster (SR 25/50, Pharmacia). A known volume (normally 25–50 mL) of MOPS buffer (saturated with octanol) was taken in the flat base mixing chamber. After a base line was obtained, the solution was used for reference absorbance. The compound to be examined was dissolved in buffer (saturated with octanol) so as to give an absorbance of between

0·5–1·5 absorbance units at the preselected wavelength (~280 nm). The flow rate of the aqueous circuit was limited to 1 mL min^{-1}, as greater flow rates would result in the overloading of the hydrophilic filter and subsequent octanol contamination of the aqueous phase. The on-line spectrophotometer allowed continuous assessment of the equilibrium of the aqueous phase. Once a stable UV absorbance was obtained, an aliquot of octanol was added and re-equilibration assessed. This cycle was repeated until a pre-defined total volume of added octanol was reached. The distribution coefficient was calculated for each octanol addition.

Ester hydrolysis

In order to identify a lead prodrug by which specific drug delivery could be achieved, it was essential to determine the stability of the ester under different conditions. The hydrolysis rates of all aromatic ester derivatives were compared with the pivaloyl ester analogue **7** at different pH values (pH 2·0 and pH 7·4), in rat blood and in the presence of liver homogenate.

Each ester prodrug, 10 μmol in 0·1 mL of acetonitrile solution (50% v/v in water), was added to 0·9 mL of phosphate buffer (100 mM, pH 7·4 or pH 2·0). The samples were incubated at 37°C for various time-intervals up to 24 h. At the end of the incubation, the samples were removed from the incubator and immediately subjected to extraction as described below. Fresh heparinized rat whole blood (0·5 mL) was added to the phosphate buffer (0·5 mL, 0·139 M, pH 7·4) containing the ester substrate (2·0 μmol). Subsequently the samples were incubated at 37°C for 0, 10, 20, 40 or 60 min. The hydrolytic reaction was terminated by removing the samples onto an ice bath and extracting the hydrolytic product immediately. For studies utilising liver homogenate, rats were killed by cervical dislocation and the livers removed and immersed in ice-cold phosphate buffer (100 mM, pH 7·4) in order to remove excess blood. The livers were blotted dry, weighed and cut into small pieces. Thereafter the tissue was homogenised in phosphate buffer (100 mM, pH 7·4) in the ratio of 1 g liver tissue to 4 mL buffer. Each ester (2 μmol) was added to 1·0 mL of liver homogenate, which was diluted before use with phosphate buffer (100 mM, pH 7·4) to a concentration of 25 mg liver tissue in 10 mL of incubation mixture. The incubation was carried out at 37°C for 0, 10, 20, 40 or 60 min and termination of the hydrolysis was achieved by immediate extraction.

Extraction and HPLC procedures for analysis of hydrolytic product. All of the incubated samples, in phosphate buffer, whole blood and liver homogenate, were added to 5 mL organic solvent (acetonitrile–isopropanol, 80 : 20 v/v). Solid NaCl (0·5 g) was added and the mixture was acidified using 2 N HCl (100 μL). The samples were then mixed and centrifuged. The entire upper organic layer (4–5 mL) was separated, evaporated and analysed by an established HPLC method. A Hewlett Packard Model 1090M Series-II HPLC system, complete with auto injector, auto sampler and diode array detector, linked to a HP 900-300 data station was used for analysis. A polymer PLRP-S column (15 cm × 0·46 cm) and a gradient mobile phase system, utilising PBS (10 mM, pH 2·90, containing 2 mM EDTA) and acetonitrile, were used for the separation of the analytes. Eluents were monitored at 285 nm.

Efficacy study

In-vivo iron mobilisation efficacy of ester prodrugs has been compared with **1** and the parent compound CP102 in a 59Fe-ferritin loaded rat model (Liu et al 1999). Hepatocytes of normal fasted rats (190–230 g) were labelled with ^{59}Fe by administration of ^{59}Fe-ferritin by the tail vein. One hour later, each rat was orally administered with chelator (450 μmol kg^{-1}). Due to the poor water solubility associated with several of the hydrophobic prodrug molecules, 70% 1,2-propanediol in water (v/v) was adopted as the solvent. This compound did not influence the efficacy of **1** (data not shown). Control rats were administered with an equivalent volume of 70% 1,2-propanediol. Rats were placed in individual metabolic cages and urine and faeces collected. Rats were allowed access to food 1 h after oral administration of chelator. There was no restriction of water throughout the study period. The investigation was terminated 24 h after ^{59}Fe-ferritin administration. Rats were killed and the liver, gastrointestinal tract (including contents) and faeces were removed for gamma counting.

Results

Chemistry

The properties of seven aromatic ester derivatives of CP102 (**11a–g**) and the ^{1}H-NMR spectra of the corresponding benzyl ethers (**10a–g**) are presented in Tables 2, 3 and 4. The diethyl azodicarboxylate/triphenylphosphine system (Mitsunobu 1981)

Table 2. Synthesis of benzylated ester derivatives of CP102 (**10a–g**).

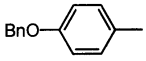
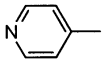
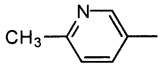
Compound	Ar	Yield (%)	^{1}H-NMR ($CDCl_3$) δ ppm
10a		68	1·0 (t, 3H,2-CH_2CH_3), 2·55 (q, 2H, 2-CH_2CH_3), 4·0 (t, 2H, NCH_2CH_2O), 4·4 (t, 2H, NCH_2CH_2O), 5·1 (s, 2H,CH_2Ph), 5·2 (s, 2H, CH_2Ph), 6·25 (d, 1H, 5-*H* (pyridinone)), 6·8–7·8 (m, 15H, *Ar* & 6-*H*(pyridinone))
10b		73	1·2 (t, 3H,2-CH_2CH_3), 2·65 (q, 2H, 2-CH_2CH_3), 4·1 (t, 2H, NCH_2CH_2O),4·4 (t, 2H, NCH_2CH_2O), 5·1 (s, 2H,CH_2Ph), 5·23 (s, 2H,CH_2Ph), 6·4 (d, 1H, *5-H*(pyridinone)), 6·95 (d, 1H, *6-H*(pyridinone)), 7·1 (d, 2H, *Ar*, H-*meta* to the carboxyl group), 7·15–7·65 (m, 10H, *Ar*),7·9 (d, 2H, *Ar*, H-*ortho* to the carboxyl group)
10c		89	1·0 (t, 3H, 2-CH_2CH_3), 2·6 (q, 2H, 2-CH_2CH_3), 4·1 (t, 2H, NCH_2CH_2O), 4·4 (t, 2H, NCH_2CH_2O), 5·18 (s, 2H, CH_2Ph), 6·45 (d, 1H, *5-H*(pyridinone)), 7·0–8·1 (m, 11H,*Ar* & *6-H*(pyridinone))
10d		82	1·0 (t, 3H,2-CH_2CH_3), 2·65 (q, 2H, 2-CH_2CH_3), 4·15 (t, 2H, NCH_2CH_2O), 4·55 (t, 2H, NCH_2CH_2O), 5·21 (s, 2H, CH_2Ph), 6·45 (d, 1H, *5-H*(pyridinone)), 7·1–8·2 (m, 9H, *Ar* & 6-*H*(pyridinone)), 8·75 (d, 1H, *6-H*(pyridine))
10e		74	1·0 (t, 3H,2-CH_2CH_3), 2·5 (q, 2H, 2-CH_2CH_3), 4·0 (t, 2H, NCH_2CH_2O), 4·35 (t, 2H, NCH_2CH_2O), 5·0 (s, 2H, CH_2Ph), 6·12 (d, 1H, *5-H* (pyridinone)), 6·8–7·4 (m, 7H, *Ar*;*5-H*(pyridine) & *6-H*(pyridinone)), 7·9 (m, 1H, *4-H*(pyridine)), 8·5 (d, 1H, *6-H*(pyridine)), 8·8 (s, 1H, *2-H*(pyridine))
10f		72	1·0 (t, 3H,2-CH_2CH_3), 2·55 (q, 2H, 2-CH_2CH_3), 4·0 (t, 2H, NCH_2CH_2O), 4·35 (t, 2H, NCH_2CH_2O), 5·05 (s, 2H, CH_2Ph), 6·1 (d, 1H, *5-H*(pyridinone)), 6·8–7·3 (m, 6H, *Ar* &*6-H*(pyridinone)), 7·5 (d, 2H, *3,5-H*(pyridine)), 8·5 (d, 2H, *2,6-H*(pyridine))
10g		81	1·0 (t, 3H, 2-CH_2CH_3), 2·6 (s, 3H, 6-CH_3), 2·65 (q, 2H, 2-CH_2CH_3), 4·1 (t, 2H, NCH_2CH_2O), 4·45 (t, 2H, NCH_2CH_2O), 5·2 (s, 2H,CH_2Ph), 6·35 (d, 1H, *5-H*(pyridinone)), 7·1–7·6 (m, 7H, *Ar*; *5-H*(pyridine) & *6-H*(pyridinone)), 8·05 (m, 1H, *4-H*(pyridine)), 9·05 (s, 1H, *2-H*(pyridine))

Table 3. Aromatic ester derivatives CP102 (**11a–g** hydrochloride) and their distribution coefficients between an aqueous phase buffered at pH 7·4 and octanol (n = 5).

Compound Ar	mp (°C)	Yield (%)	Formula	$D_{7\cdot4}$	Log P
11a	166–168	92	$C_{16}H_{17}NO_5$.HCl	50·3 ± 0·8	1·70
11b	159–161	88	$C_{16}H_{17}NO_5$.HCl	17·7 ± 0·7	1·25
11c	155–158	93	$C_{16}H_{17}NO_4$.HCl	32·8 ± 1·7	1·52
11d	160–163	86	$C_{15}H_{16}N_2O_4$.2HCl	1·3 ± 0·1	0·10
11e	188–190	90	$C_{15}H_{16}N_2O_4$.2HCl	1·8 ± 0·1	0·25
11f	196–197	92	$C_{15}H_{16}N_2O_4$.2HCl	1·8 ± 0·2	0·25
11g	198–201	85	$C_{16}H_{18}N_2O_4$.2HCl	4·9 ± 0·1	0·69

was selected due to the mild and neutral reaction conditions required and the excellent yields.

Distribution coefficients

The UV spectra associated with compound **11f** (Figure 4) are presented as an example. The distribution coefficient values of the ester prodrugs covered the range 1·3–50·3 (Table 3), which suggested that all the compounds had the potential to be orally active. The para-hydroxybenzoyl ester derivative of CP102 (**11b**), had a lower partition coefficient value than the unsubstituted analogue, **11c**. In contrast, **11a**, the ortho-hydroxybenzoyl ester derivative of CP102, was more lipophilic than **11c**, probably due to intramolecular hydrogen bonding between the phenolate group and the carbonyl function (Figure 5), resulting in both the hydroxyl and carbonyl groups being less exposed to the aqueous solvent. The picolinic, nicotinic and

Table 4. ^{1}H-NMR spectra and elemental analysis of **11a–g** hydrochloride.

Compound	^{1}H-NMR (DMSO-d_6) δ ppm		Elemental analysis (%)		
			C	H	N
11a	1·2 (t, 3H, 2-CH_2CH_3), 2·9 (q, 2H, 2-CH_2CH_3), 4·6 (br., s, 4H, NCH_2CH_2O), 6·5–7·6 (m, 5H, *Ar* & *5-H*(pyridinone)), 8·1 (d, 1H, *6-H*(pyridinone)), 7·5–10·0 (br., 3H, *OH*)	Calcd (%) Found (%)	56·56 56·39	5·34 5·10	4·12 4·02
11b	1·12 (t, 3H, 2-CH_2CH_3), 2·96 (q, 2H, 2-CH_2CH_3), 4·6 (br., s, 4H, NCH_2CH_2O), 6·85 (d, 2H, *Ar* 1H, H-*meta* to the carboxyl group), 7·35 (d, 1H, *5-H*(pyridinone)), 7·7 (d, 2H, *Ar*, H-*ortho* to the carboxyl group), 8·3 (d, 1H, *6-H*(pyridinone)), 8·5–10·2 (br., 3H, *OH*)	Calcd (%) Found (%)	56·56 56·43	5·34 5·21	4·12 3·96
11c	0·95 (t, 3H, 2-CH_2CH_3), 2·8 (q, 2H, 2-CH_2CH_3), 4·5 (br., s, 4H, NCH_2CH_2O), 7·10–7·95 (m, 6H, *Ar* & *5-H*(pyridinone)), 8·2 (d, 1H, *6-H*(pyridinone)), 7·9–9·6 (br., 2H,*OH*)	Calcd (%) Found (%)	59·35 59·58	5·60 5·47	4·33 4·25
11d	1·15 (t, 3H, 2-CH_2CH_3), 3·03 (q, 2H, 2-CH_2CH_3), 4·77 (br., s, NCH_2CH_2O), 7·5 (d, 1H, *5-H*(pyridinone)), 7·6–8·3 (m, 3H, *3,4,5-H*(pyridine)), 8·45 (d, 1H, *6-H*(pyridinone)), 8·75 (d, 1H, 6-*H*(pyridine))	Calcd (%) Found (%)	49·88 49·63	5·02 4·95	7·76 7·85
11e	1·2 (t, 3H, 2-CH_2CH_3), 3·0 (q, 2H, 2-CH_2CH_3), 4·7 (br., s, 4H, NCH_2CH_2O), 7·3 (d, 1H, *5-H*(pyridinone)), 7·6–8·0 (m, 1H, *6-H*(pyridinone)), *5-H*(pyridine)), 8·1–8·65 (m, 2H,*4-H*(pyridine) 8·8(d, 1H, *6-H*(pyridine)), 9·0 (s, 1H, *5-H*,*2-H*(pyridine))	Calcd (%) Found (%)	49·88 50·15	5·02 5·09	7·76 7·69
11f	1·15 (t, 3H, 2-CH_2CH_3), 2·95 (q, 2H, 2-CH_2CH_3), 4·7 (br., s, 4H, NCH_2CH_2O), 7·3 (d, 1H, *5-H*(pyridinone)), 7·95 (d, 2H, *3,5-H*(pyridine)), 8·25 (d, 1H, *6-H*(pyridinone)), 8·8 (d, 2H, *2,6-H*(pyridine)), 9·9 (s, br., 3H, *OH* & *NH*)	Calcd (%) Found (%)	49·88 49·79	5·02 5·24	7·76 7·57
11g	1·2 (t, 3H, 2-CH_2CH_3), 2·7 (s, 3H, *6-*CH_3), 3·05 (q, 2H, 2-none)), CH_2CH_3), 4·8 (br., s, 4H, NCH_2CH_2O), 7·5 (d, 1H, *5-H*(pyridinone-7·8 (d, 1H, *5-H*(pyridine)), 8·3–8·85 (m, 2H, *4-H*(pyridine) & *6-H* (pyridinone)), 9·05 (s, 1H, *2-H*(pyridine))	Calcd (%) Found (%)	51·21 51·21	5·37 5·24	7·47 7·35

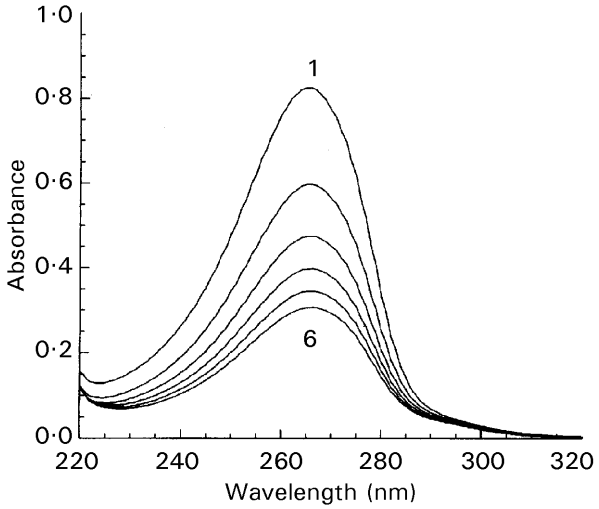


Figure 4. UV spectra for the determination of the distribution coefficient (pH 7·4) of **11f**. Spectra 1–6 are of the aqueous phase and result from the sequential addition of 5-mL aliquots of 1-octanol.

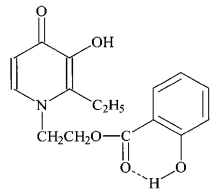


Figure 5. Proposed intramolecular hydrogen bonding of **11a**.

isonicotinic ester derivatives, **11d**, **11e** and **11f**, had much lower partition coefficients than the benzoyl ester derivatives.

Ester hydrolysis studies

The stability of the pivaloyl ester **7** was compared with the aromatic esters (**11a–11g**) (Table 5). The stability of these esters at both pH 7·4 and pH 2·0 varied quite appreciably, the range of values being 0·2–105·2 nmol mL^{-1} h^{-1} (pH 2·0) and 0·1–

Table 5. Hydrolysis of CP102 ester prodrugs at different pH values, in rat blood and in rat liver homogenate.

Compound	Rate of hydrolysis			
	pH 2·0 (nmol $mL^{-1} h^{-1}$)	pH 7·4 (nmol $mL^{-1} h^{-1}$)	Rat blood (nmol $mL^{-1} h^{-1}$)	Rat liver (nmol $g^{-1} h^{-1}$)
7	5·0 ± 1·1	<0·1	185·6 ± 27·2	27 600 ± 700
11a	0·2 ± 0·1	17·3 ± 1·4	16·8 ± 0·8	7400 ± 3600
11b	0·5 ± 0·1	<0·1	38·4 ± 0·8	15 900 ± 2100
11c	1·9 ± 0·5	0·8 ± 0·3	591·2 ± 16·0	180 400 ± 5700
11d	97·7 ± 5·6	57·5 ± 0·5	3541·6 ± 93·6	432 800 ± 2900
11e	30·4 ± 4·6	28·5 ± 1·0	794·4 ± 12·0	43 400 ± 900
11f	105·2 ± 15·0	97·9 ± 1·2	1313·6 ± 182·4	95 200 ± 8200
11g	17·1 ± 1·0	16·7 ± 0·2	563·2 ± 62·4	153 500 ± 2200

Values are expressed as means ± s.d. (n = 3).

97·9 nmol $mL^{-1} h^{-1}$ (pH7·4). In particular, esters containing a nitrogen atom in the aromatic moiety (i.e. **11d–f**) were found to be less stable than the corresponding benzoyl analogue (**11c**). All esters were hydrolysed more rapidly in rat blood, activities falling in the range 16·8–3541·6 nmol $mL^{-1} h^{-1}$. However, some esters, such as **11a** and **11b**, possessed appreciable stability in rat whole blood, with plasma esterase activities of 16·8 and 38·4 nmol $mL^{-1} h^{-1}$, respectively. The nitrogen-containing esters (e.g. **11d–f**) demonstrated higher hydrolysis rates when compared with the other esters. Without exception, the rate of hydrolysis was found to be much greater in the presence of liver homogenate, activities ranging from 7400 nmol (g tissue)$^{-1} h^{-1}$ (**11a**) to 432 800 nmol (g tissue)$^{-1} h^{-1}$ (**11d**). Generally the hydrolytic rates in the presence of liver homogenate were several-hundred-fold faster than those observed in the rat blood.

Efficacy study

The iron mobilisation efficacy of ester prodrugs was compared with the parent compound (CP102) and the 1,2-dimethyl derivative **1** (Table 6). All the ester prodrugs were found to be superior to **1**. Several, such as **11a**, **11d** and **11e**, with $D_{7.4}$ values of 50·3, 1·3 and 1·8, respectively, provided a significant improvement over CP102 ($D_{7.4} = 0·22$) in the efficacy model. The optimal effect was observed with the picolinic ester derivative **11d**, which provided 70% improvement in the ability of facilitating iron excretion over that of the parent drug (CP102). However, not all the prodrugs were associated with an increased efficacy; **11b**, **11c** and **11f** were less effective than the parent compound (CP102), suggesting that lipophilicity was not the only factor which influenced drug efficacy. There was no correlation between efficacy and ester hydrolysis in the liver. Thus, the four most effective compounds at removing iron from the liver were **7**, **11a**, **11d** and **11e** despite the hydrolysis rate being spread over the range 7400–432 800 nmol (g tissue)$^{-1} h^{-1}$ (Table 5). Clearly as long as hydrolysis occurred at an appreciable rate, then iron chelation occurred with acceptable efficacy. In a similar fashion $D_{7.4}$ values were not critical, **11a** being hydrophobic ($D_{7.4} = 50·3$) and **11c** being relatively hydrophilic ($D_{7.4} = 1·8$).

Table 6. Iron mobilization studies of CP102 ester prodrugs and the parent compound in the ^{59}Fe-ferritin loaded rat model.

Chelator	% Faecal iron excretion
Control	3·87 ± 1·0
1	13·2 ± 5·2
CP102	16·8 ± 2·7
7	23·0 ± 7·3
11a	26·3 ± 6·2
11b	15·1 ± 4·9
11c	13·5 ± 6·8
11d	28·4 ± 3·5
11e	27·6 ± 8·8
11f	13·4 ± 3·7
11g	22·2 ± 2·6

All chelators were given orally (450 mmol kg^{-1}). Values are expressed as means ± s.d. (n = 5).

Discussion

In order to obtain selective delivery of the chelator CP102 to the major chelatable iron pool in the body – the liver, it is essential that prodrug esters are absorbed intact from the gastrointestinal tract, and are reasonably stable in the plasma, thereby providing sufficient time for the prodrug to perfuse the liver. Subsequently, rapid hydrolysis by hepatic carboxyesterases will generate hydrophilic metabolites within hepatocytes. Hydrolysis studies conducted at selected pH values provide useful information relating to the likely in-vivo stability of the corresponding compounds at different sites in the gastrointestinal

tract. Although the stability of these aromatic esters was found to vary quite appreciably, they were found to be relatively stable in aqueous solution at the pH values of 2·0 and 7·4 (Table 5). That the esters with heteroaromatic acid moieties (e.g. **11d–f**) are less stable than the benzoyl analogue is probably due to the aromatic nitrogen exerting a negative inductive effect and thereby rendering the ester bond more susceptible to hydrolysis.

Ideally, ester prodrugs which are designed to selectively deliver CP102 to the liver, should undergo minimal pre-systemic hydrolysis in the plasma relative to the rate of hydrolysis in the liver. This would ensure that the ester reaches the liver intact where extensive intracellular hydrolysis will occur to produce the more hydrophilic CP102. Extensive extracellular hydrolysis will reduce the effectiveness of the hydrophobic ester by virtue of the poor extraction of the hydrophilic compound by the liver. However, it is also essential that there is some esterase activity within the systemic circulation in order to ensure that the small quantities of ester which are not extracted by the liver during the first pass are hydrolysed to the hydrophilic metabolite. The ratio between the hydrolytic rates of the esters in the presence of liver homogenate and that in the rat blood varies from 50 to 450.

In-vivo iron mobilisation efficacies of the ester prodrugs were investigated with ^{59}Fe-ferritin loaded rats. In this model the chelator was administered at a time when ^{59}Fe released by lysosomal degradation of ferritin was maximally available (Pippard et al 1981; Liu et al 1999). Since a major reason for developing ester prodrugs of hydroxypyridinones is to target the liver iron pool, the ^{59}Fe-ferritin/rat model was considered to be an ideal in-vivo test system. Preliminary analysis of the results shows that in many cases the ester prodrug leads to superior iron excretion via the bile than does the corresponding alcohol (CP102) (Table 6). Several aromatic esters, for instance **11a**, **11d** and **11e** were found to be even more effective than the pivaloyl ester analogue (**7**). Such enhancement is almost certainly due to the increased lipophilicity of these ester prodrugs as compared with the parent compound, which
indicates that by analogy with **7**, selective delivery of the drug to the liver has been achieved. Pharmacokinetic studies have demonstrated that **7** is rapidly cleared by the liver and undergoes extensive hydrolysis to generate the hydrophilic metabolite CP102 (Choudhury et al 1997).

Some interesting features emerge from the structure–activity studies, for instance the efficacy of the para-hydroxybenzoyl ester derivative of CP102 (**11a**), is twice that of the ortho-hydroxybenzoyl analogue (**11b**), which suggests that the position of the substitution on the aromatic ring has a dominating influence on efficacy. The comparison between **11e** and **11f** is particularly significant as, although their log P values are identical (Table 3), their efficacies are markedly different (Table 6). This confirms that lipophilicity is not the only factor which influences the drug efficacy. Furthermore, no clear correlation between the hydrolytic rates and efficacy of iron removal could be identified for this series of compounds, indicating that other factors are important for controlling their in-vivo disposition. A likely additional factor is prodrug metabolism. Although CP102 does not undergo extensive glucuronic conjugation (Singh et al 1992b, 1996), the more lipophilic ester derivatives may do so. Although the esters are predicted to be rapidly hydrolysed in the liver, a competition may exist between ester hydrolysis and phase II metabolism. Detailed metabolism studies need to be undertaken in order to verify this hypothesis.

In summary, the selective delivery of CP102 to target organs such as the liver can be achieved using aromatic ester prodrugs. Although several aromatic ester prodrugs provide a significant improvement over the parent hydroxyalkyl compound, the selection of ester moieties is critical. The nature and position of the substituent on the aromatic ring has an appreciable influence on the efficacy of the compound.

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