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Original article

Basic 3-hydroxypyridin-4-ones: Potential antimalarial agents

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Abstract

3-Hydroxypyridin-4-ones selectively bind iron under biological conditions and one such compound has found application in the treatment of thalassaemia-linked iron overload. Related molecules have also been demonstrated to possess an antimalarial effect at levels which are non-toxic to mammalian cells. In an attempt to improve the efficiency of such molecules we have investigated the effect of introducing basic nitrogen centres into 3-hydroxypyridin-4-ones in an attempt to achieve targeting to lysosomes and other intracellular acidic vacuoles. Several of the compounds reported in this communication possess enhanced antimalarial activity over that of the simple hydroxypyridinone class.

Keywords: Hydroxypyridinones; Iron chelators; Malaria; Lysosomotrophic

1. Introduction

Malaria is one of the most prevalent and devastating human parasitic diseases. It is estimated that over 2000 million people, living in more than 100 countries, are exposed to the risk of malaria and that some 270 million of these are infected with malarial parasites [1,2]. Annual deaths are estimated to approximately 2 million, the majority being children under the age of five. Due to the increasing resistance of *Plasmodium falciparum* to current antimalarial drugs such as the antifolate pyrimethamine and the quinine family [3,4], the search for new chemotherapeutic agents has become highly demanding. Iron chelation offers one possible approach to control malaria infections [5].

Iron is an essential micronutrient required by the malaria parasite for many biochemical reactions involved in growth and multiplication. The iron source for *P. falciparum* is not of extracellular origin [6]. In fact the growth rate of the parasite is independent of host iron status. When parasites invade erythrocytes they generate a low molecular weight iron pool which originates from haemoglobin digestion. The use of powerful iron chelators could, in principle, limit the size of this pool thereby reducing the rate of incorporation of iron into critical metalloenzymes which can lead to parasite death [7]. It is clear from previous studies that iron chelators have the capacity to clear parasites [8,9].

3-Hydroxypyridin-4-ones (HPOs) have been extensively studied as orally active iron(III) chelating agents [10] and have been investigated for antimalarial activity [11,12]. Unfortunately, many bidentate 3-hydroxypyridin-4-ones possessing good antimalarial activity are also toxic to mammalian cells due to their relatively high lipophilicity [13]. In order to improve drug efficacy and/or decrease drug toxicity, it will be necessary to design chelators which can be efficiently and selectively delivered to the malaria-infected erythrocyte.

Studies by several investigators have demonstrated that intracellular vesicles of the malaria parasite reach pH values in the vicinity of 5.0 [14]. Estimates of red cell cytoplasmic and parasite cytoplasmic pH are 7.0–7.2 and 6.8–7.0, respectively [15]. 3-Hydroxypyridin-4-ones containing basic side chains are

Abbreviations: HPO, 3-hydroxypyridin-4-one; DR, distribution ratio; MOPS, morpholinopropane sulphonic acid.

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predicted to accumulate in such acidic organelles. It is assumed that only the neutral form of weak base (L) can readily diffuse across both plasma and vesicle membranes and that their concentrations are equal in all three compartments. In contrast the protonated forms (LH^+) will not readily cross membranes and so basic hydroxypyridinones will be accumulated in acidic vesicles. Such an elevated concentration of chelator would be predicted to increase the efficiency of iron scavenging, particularly with bidentate ligands. Furthermore, the marked pH dependence of haemoglobin digestion may provide an additive inhibitory effect for basic iron chelators, as an accumulation of such molecules will influence the vesicle pH value.

This concept led to the design and synthesis of a series of basic hydroxypyridinones with pK_a values covering the range 4.9–7.5 (**4a**–**18**) (Table 1). It was anticipated that an ideal chelator for this purpose should have a pK_a value in the range of 6.0–8.0. Such molecules are almost fully protonated in acid intracellular compartment (pH 5.5), whereas at the cytoplasmic pH value of 7 the neutral species make an appreciable contribution. Thus, it would be expected that these ligands accumulate within acid intracellular compartments.

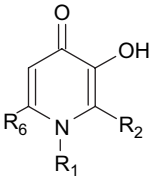
In this work we describe the synthesis, physico-chemical properties and iron mobilization efficacy of a wide range of novel bidentate 3-hydroxypyridin-4-ones containing different basic substituents at pyridinone ring positions 1, 2 and 6. Preliminarily *in vitro* antimalarial activities are also reported.

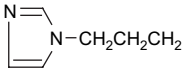
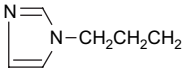
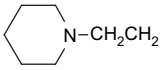
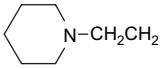
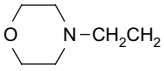
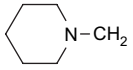
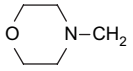
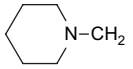
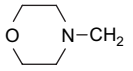
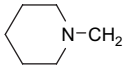
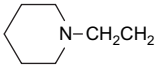
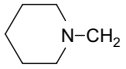
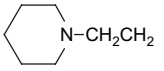
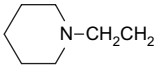
2. Synthesis of basic hydroxypyridin-4-ones

The 3-hydroxypyridin-4-one derivatives are conveniently prepared from the corresponding 3-hydroxypyran-4-ones in three steps through protection of hydroxyl group. The protected compound is then reacted with a primary amine R_1NH_2, in which R_1 represents the group on the nitrogen atom of the desired 3-hydroxypyridin-4-ones, in the presence of base (Scheme 1) [16,17]. Although the 3-hydroxy substituents of 3-hydroxypyran-4-ones can also be protected by methyl ether formation [18,19,20,21], the corresponding 2-alkyl-3-methoxy-4-pyrones are oils which are less convenient to work with than the crystalline 2-alkyl-3-benzyloxy-4-pyrones [10,22,23]. Furthermore, the benzyl protecting group can be removed by hydrogenation under acidic, neutral or basic conditions. For these reasons the benzyl group was selected for the work described in this paper.

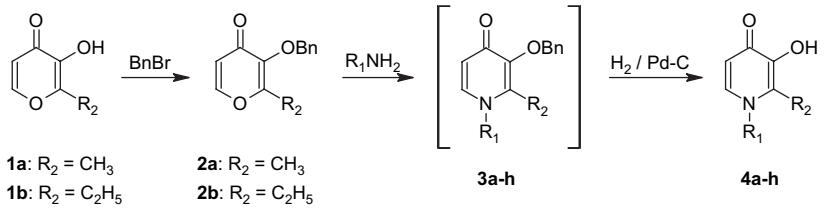
The conversion of 4-pyrone to 4-pyridinone involves an initial Michael reaction followed by ring opening and ring closure. Mesomerisation of α,β-unsaturated carbonyl compound causes the β-carbon to be electron deficient and therefore susceptible to nucleophilic attack. When the nucleophile is a primary amine, double attack at both α,β-unsaturated functions of the 4(1*H*)-pyranone leads to the formation of 4*H*-pyridin-4-one with the loss of a water molecule [24,25]. Conversion of 3-hydroxypyran-4-one derivatives to the corresponding pyridine-4-one analogues can be achieved without protection of the 3-hydroxyl group [26]. However, the synthetic utility of this reaction is limited to small primary amines since larger amines result in yields less than 10% [27].

Table 1
The structure of 3-hydroxypyridin-4-ones



Compd.	R_1	R_2	R_6
4a		CH_3	H
4b		C_2H_5	H
4c	$CH_2CH_2N(CH_3)_2$	CH_3	H
4d	$CH_2CH_2N(CH_3)_2$	C_2H_5	H
4e	$CH_2CH_2N(C_2H_5)_2$	CH_3	H
4f		CH_3	H
4g		C_2H_5	H
4h		CH_3	H
9a	CH_3		CH_3
9b	CH_3		CH_3
9c	CH_3		CH_2OH
9d	CH_3		CH_2OH
13a	CH_3	H	
13b		H	
18a		CH_2OH	H
18b		$CH(OH)CH_3$	H
19a	CH_3	CH_3	H
19b	C_2H_5	C_2H_5	H
20	C_2H_5	$CH(OH)CH_3$	H

The general methodology adopted for the synthesis of *N*-basic substituted 3-hydroxypyridin-4-ones **4a**–**h** is summarised in Scheme 1 [10,28]. The commercially available maltol **1a** or ethyl maltol **1b** was benzylated to give **2a** and **2b**. Reaction of **2a** or **2b** with primary amines gave the benzylated pyridinones **3a**–**h**, which were subsequently subjected to catalytic hydrogenation to remove the protecting group, yielding the corresponding bidentate chelators **4a**–**h** as hydrochloride salts.



Scheme 1. Synthesis of *N*-substituted 3-hydroxypyridin-4-ones.

The basic substituent can also be introduced at the 2-position of allomaltol **5a** or kojic acid **5b** *via* a Mannich-type reaction, using formaldehyde and a secondary amine in 95% aqueous ethanol at room temperature (Scheme 2). The desired 2-basic substituted 3-hydroxypyridin-4-ones **9a–d** were then prepared by the reaction of the corresponding benzylated pyranone **7a–d** with methylamine, followed by acidic hydrolysis to remove the protecting group (Scheme 2). The methodology adopted for introduction of basic substituents at the 6-position (2-position of the 5-hydroxypyridin-4-ones) is presented in Scheme 3. Chlorination of protected kojic acid **10** in neat thionyl chloride below 0 °C afforded benzylated chlorokojic acid **11**. The chlorine was then substituted by reaction with a secondary amine (piperidine) in the presence of tributylamine in DMF. The reactions of the protected pyranone **12** with an excess of primary amines were performed by refluxing in 50% aqueous ethanol in the presence of a catalytic amount of sodium hydroxide. After acidic reflux the bidentate chelators **13a** and **13b** were isolated as hydrochloride salts.

Two 2-(1′-hydroxyalkyl)-*N*-basic substituted 3-hydroxypyridin-4-ones **18a** and **18b** were also synthesised (Scheme 4). The 2-position of pyromeconic acid **14** can be functionalised in an analogous fashion to the aldol condensation whereby the pyrone anion aldehyde, under alkaline aqueous conditions, furnishes, on acidic work up, the corresponding 2-(1′-hydroxyalkyl)-3-hydroxypyranone **15** in high yields (80–90%). The pH of the reaction solution was found to be critical, since highly alkaline conditions resulted in an extensive aldehyde polymerisation. The optimal pH for this reaction, as adopted in this study, was found to be 10.5. The protection of the 2-(1′-hydroxyl) function proved to be essential since without protection marked decomposition was observed, which consequently resulted in low yield (<10%). In this work, both the 3-hydroxyl and the 2-(1′-hydroxyl) groups were protected in one step by reacting the corresponding pyran-4-ones **15** with benzaldehyde dimethyl acetal in *N*,*N*-dimethylformamide in the presence of a catalytic amount of toluene-*p*-sulphonic acid. The desired pyridinone products **18** were then prepared by the reaction of the protected pyranone **16** with the 2-piperidinoethylamine, followed by hydrogenation to remove the protecting group. The introduction of a 1′-hydroxyalkyl group at the 2-position has been previously found to produce appreciable increases in the pFe^{3+} values [29].

3. Results

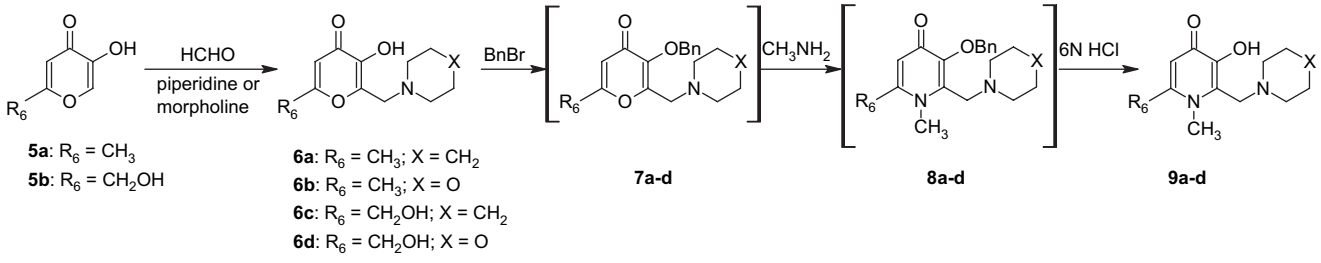
3.1. Physico-chemical characterisation

3.1.1. pK_a values of basic hydroxypyridinones and their corresponding iron(III) complexes

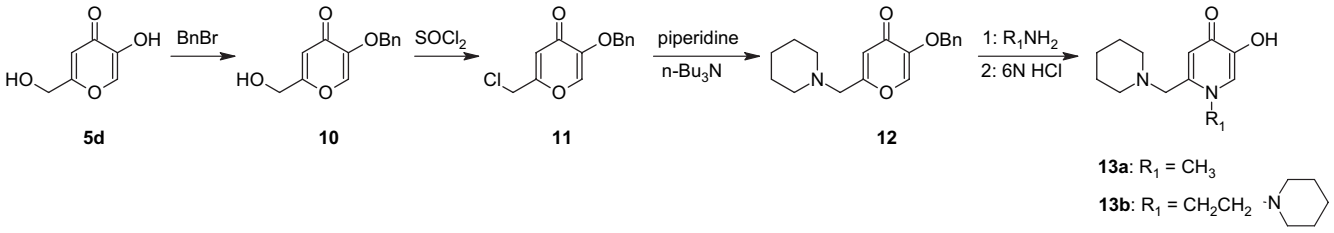
All ligands were investigated by simultaneous spectrophotometric and potentiometric titrations. Most of the basic 3-hydroxypyridin-4-ones in this current work possess three pK_a values. The pK_{a1} values correspond to the protonation of the 4-hydroxy group, the pK_{a2} values to the deprotonation of the basic substituent and pK_{a3} to the dissociation of the 3-hydroxyl group (Scheme 5). The only exception is **13b** which has an additional $pK_{a2'}$ associated with the second basic substituent (Scheme 5). The optimized pK_a values obtained from non-linear least-square regression analysis [30] are shown in Table 2. The pK_a values obtained from spectrophotometric titration were in good agreement with those calculated from the potentiometric titration. The observed pK_a values of the basic side chain cover the range of 4.8–7.6.

3.1.2. Distribution and partition coefficients of basic hydroxypyridinones

The distribution coefficients ($D_{7.4}$) between 1-octanol and MOPS (3-(*N*-morpholino)propanesulphonic acid) buffer (pH 7.4) were determined *via* the automated filter-probe system [28]. As a result of ionization at pH 7.4, the fraction of the



Scheme 2. Synthesis of 2-basic substituted 3-hydroxypyridin-4-ones (**9a–d**).



Scheme 3. Synthesis of mono- and di-basic substituted 3-hydroxypyridin-4-ones (**13a** and **13b**).

neutral species (F_n) can be calculated by employing the pK_a values determined above (Eq. (1)). The partition coefficient (P) of each compound can then be calculated from Eq. (2) and the resulting values are given in Table 3. The distribution coefficients of the Fe-complexes ($D_{7.4}$ Fe-complex) can be calculated from Eq. (3) [28]. This equation holds for ligand $\log D_{7.4}$ values > 0.1. For those ligands which possess $\log D_{7.4}$ values < 0.1 (the more hydrophilic ligands), Eq. (4) provides a superior estimate [28].

$$\text{Neutral fraction}(F_n) = \frac{1}{1 + 10^{pK_{a1}+pK_{a2}-2pH} + 10^{pK_{a2}-pH} + 10^{pH-pK_{a3}}} \quad (1)$$

$$P = \frac{D_{7.4}}{F_n} = D_{7.4} \times (1 + 10^{pK_{a1}+pK_{a2}-2pH} + 10^{pK_{a2}-pH} + 10^{pH-pK_{a3}}) \quad (2)$$

$$\log D_{7.4}\,\text{Fe-complex} = 2.53 \log D_{7.4}\,\text{ligand} - 0.80 \quad (3)$$

$$\log D_{7.4}\,\text{Fe-complex} = 0.49 \log D_{7.4}\,\text{ligand} - 2.45 \quad (4)$$

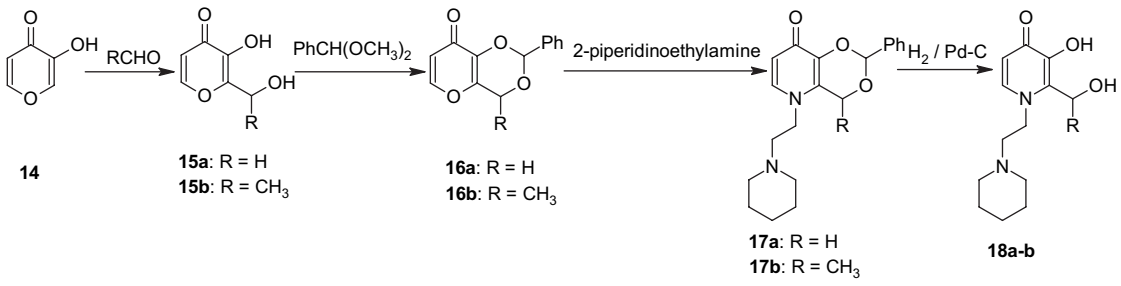
3.1.2.1. Stability constants of ligand–iron(III) complexes. Bidentate 3-hydroxypyridin-ones are capable of forming a number of complexes with iron(III) so that aqueous solutions equilibrate to give a mixture of species, the composition of which depends on the metal ion, ligand and hydrogen ion concentrations. In this current work, the stepwise equilibrium constants (K_1, K_2 and K_3) of selected basic 3-hydroxypyridinones were evaluated using an automated spectrophotometric titration system [30]. The cumulative constant β_3 is the multiple of these three constants. The optimized β_3 values are presented in Table 4.

The pK_a values of two selected Fe-complexes (**9a**–iron(III) and **9b**–iron(III)) were also determined. Bidentate 3-hydroxypyridin-4-ones form 3:1 iron(III) complexes at neutral and alkaline pH values. Thus these 3:1 complexes possess three pK_a values, corresponding to the protonation of the basic side chain at 2-position of each of the ligands bound to iron(III). The determined pK_a values of **9a**–iron(III) complex are 8.49, 7.14 and 5.21 whereas for the morpholine-containing **9b**–iron(III) complex the corresponding values are 5.02, 4.47 and 4.01.

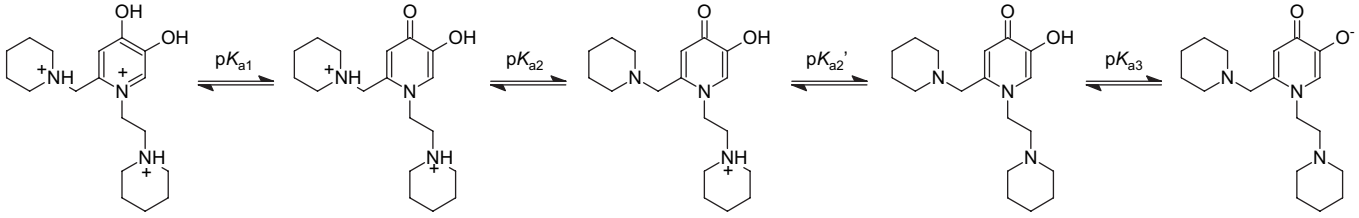
3.1.3. Biological evaluation

3.1.3.1. Iron mobilization efficacy of basic hydroxypyridinones. The *in vivo* iron scavenging ability of the basic hydroxypyridinones is compared with those of the dialkylpyridinones (Table 5). Several hydroxypyridinone derivatives such as **4a** and **4b** were found to be relatively poor scavengers of iron under *in vivo* conditions. However, the substituted 2-(1′-hydroxyalkyl) derivatives, **18a** and **18b** were found to be superior to the dialkylpyridinones **19a** and **19b**, with efficacies close to 20%. The ligands **4c** and **4e** were found to be as equally effective as **19a**. The most effective basic hydroxypyridinone was **4g** with an associated efficacy of 26.5%. All the basic compounds were found to be less effective than the non-basic ligands **19b** and **20**.

3.1.3.2. In vitro antimalarial activity. The *in vitro* antimalarial activity of the basic HPOs was compared with that associated with pyridinones **19a**, **19b** and **20** (Table 5). All iron chelators inhibited the growth of *P. falciparum*. The basic hydroxypyridinones, **4a**–**d** with a mean IC_{50} around 80, were found to have the



Scheme 4. Synthesis of 1-(2′-piperidinoethyl)-2-(1′-hydroxyalkyl)-3-hydroxypyridin-4(1*H*)-ones (**18a** and **18b**).



Scheme 5. Protonation equilibria of 1-(2′-piperidinoethyl)-2-piperidinomethyl-5-hydroxypyridin-4(1*H*)-one (**13b**).

highest IC_{50} values in comparison with **19b** and **20**. Significantly, several hydroxypyridinones such as **4e** and **4h** exhibited similar activity to that of **19b** and **20**. The IC_{50} values of **18a** and **18b** were close to 10 μM, the most potent of the group and significantly lower than the value associated with the analogous non-basic "high pFe^{3+}"compound **20**.

4. Discussion

4.1. Physico-chemical properties of hydroxypyridinones

In order to design a basic compound with a useful acid compartment accumulation effect, it is necessary to select optimal substituents. The distribution ratio (DR) (Table 3) of basic hydroxypyridinones between cytoplasm (pH_A) and parasite lysosome (pH_B) can be calculated from Eq. (5). Although compounds with pK_a values above 8.0 have a greater chance of accumulating efficiently within the organelle, the penetration rate of such compounds will be slower due to the high proportion of ionized species present at pH 7.0. Ideally, drugs designed for accumulating in acidic intracellular vesicles related to the parasite should not only possess an appreciable distribution ratio but must also be able to penetrate the cell at a sufficiently high rate. It is predicted that an ideal hydroxypyridin-4-one chelator for this purpose should have a pK_{a2} value in the range of 6.0–8.0 (Fig. 1).

Distribution Ratio(DR)

$$= \frac{\left(1 + 10^{pK_{a1}+pK_{a2}-2pH_B} + 10^{pK_{a2}-pH_B} + 10^{pH_B-pK_{a3}}\right)}{\left(1 + 10^{pK_{a1}+pK_{a2}-2pH_A} + 10^{pK_{a2}-pH_A} + 10^{pH_A-pK_{a3}}\right)} \quad (5)$$

In the current study, a wide range of bidentate hydroxypyridinone iron chelators with different basic substituents have been synthesised. The pK_a value of the basic function in the molecule (pK_{a2}) varies not only with the nature of the substituent but also with the position of the substitution (Table 2). A particularly marked influence was noted between the pairs **9a** and **13a**, with pK_{a1} values of 2.73 and 2.16 and the pK_{a3} values of 10.28 and 8.86, respectively. Interestingly, the pK_{a3} values fall either side of the typical values obtained with the dialkylpyridinones **19a** and **19b**. The reason for these differences is the existence of the electron withdrawal influence by the positive moiety at the 6-position in **13a** which assists in the stabilisation of the phenoxide anion, whereas with **9a** there is

Table 2
Spectrophotometric and potentiometric determined pK_a values for ligands

Compd.	pK_as (potentiometric)			pK_as (spectrophotometric)		
	pK_{a1}	pK_{a2}	pK_{a3}	pK_{a1}	pK_{a2}	pK_{a3}
4a	3.08	6.71	9.82	3.13	6.60	9.92
4b	3.15	6.47	9.66	3.09	6.55	9.71
4c	2.86	7.02	9.58	2.74	7.05	9.75
4d	2.68	7.16	9.58	2.62	6.88	9.66
4e	2.66	7.50	9.60	2.63	7.53	9.65
4f	2.88	7.47	9.71	2.77	7.42	9.63
4g	2.74	7.38	9.70	2.73	7.34	9.67
4h	2.64	4.91	9.68	2.67	4.89	9.71
9a	2.73	6.98	10.28	2.50	7.07	10.81
9b	2.43	4.82	9.72	2.38	4.89	10.07
9c	2.41	6.67	10.38	2.14	6.74	10.51
9d	2.14	4.99	9.75	1.98	5.10	9.77
13a	2.16	6.72	8.86	2.15	6.74	8.85
13b	2.04	5.57; 7.60	8.95	1.16	5.66; 7.58	8.83
18a	2.20	7.43	9.24	2.25	7.35	9.28
18b	2.14	7.35	8.73	2.17	7.37	8.70
19a	3.68	–	9.77	3.56	–	9.64
19b	3.81	–	9.93			
20	3.03	–	8.77	3.11	–	8.74

Table 3
Distribution ratio, distribution coefficient values of ligands and their corresponding iron(III) complexes between an aqueous phase buffered at pH 7.4 and 1-octanol

Compd.	$D_{7.4}$ ligand ($n = 5$)	F_n (pH 7.4)	log *P* ligand	$D_{7.4}$ Fe-complex	DR
4a	0.344 ± 0.005	0.85	−0.392	0.012	31.8
4b	0.420 ± 0.019	0.89	−0.326	0.018	25.2
4c	0.154 ± 0.005	0.70	−0.657	0.001	52.5
4d	0.538 ± 0.047	0.70	−0.114	0.033	51.6
4e	0.493 ± 0.005	0.43	0.059	0.027	76.8
4f	0.960 ± 0.029	0.47	0.310	0.143	73.9
4g	4.591 ± 0.031	0.52	0.946	7.493	69.9
4h	0.122 ± 0.001	0.99	−0.909	0.001	1.92
9a	2.536 ± 0.012	0.70	0.559	1.670	51.8
9b	0.364 ± 0.003	0.99	−0.435	0.012	1.76
9c	1.636 ± 0.059	0.83	0.295	0.551	36.2
9d	0.170 ± 0.001	0.99	−0.765	0.002	1.95
13a	3.493 ± 0.056	0.80	0.640	3.752	34.2
13b	26.11 ± 0.450	0.38	1.837	608.8	424.7
18a	0.501 ± 0.018	0.48	0.019	0.028	71.3
18b	0.908 ± 0.023	0.52	0.242	0.124	69.9
19a	0.170 ± 0.010	0.99	−0.770	0.003	1.0
19b	1.790 ± 0.010	0.99	0.257	0.240	1.0
20	0.266 ± 0.002	0.96	−0.557	0.006	1.0

Table 4
Hydroxypyridinone affinity constants for Fe(III)

Compd.	log β_3	pFe^{3+} (at pH 7.45)
9a	38.0	19.0
9b	38.0	19.0
9c	38.6	20.0
9d	38.6	19.9
13a	35.7	21.9
13b	35.7	22.5
18a	35.3	20.4
19a	36.5	19.4
19b	36.7	19.7
20	35.4	21.3

strong hydrogen bonding possible with the 2-substituent which has the opposite influence of stabilising the protonated phenolic form **21**. In supporting this hypothesis, **9c** has a similar pK_{a3} value to that of **9a** and **13b** has a similar pK_{a3} value to that of **13a**. The pK_{a2} values for the aliphatic amine side chains at positions 1, 2 and 6 are all markedly lower than that of the corresponding free amines. This results from the electron deficient pyridinone ring which exerts an electron withdrawing effect on the side chains, irrespective of the position. As a result of electron withdrawal associated with the morpholine ring oxygen, the pK_{a2} values of the compounds **4h**, **9b** and **9d** are lower than that of the corresponding piperidine-containing compounds, **4g**, **9a** and **9c**.

The introduction of a hydroxyl function on the 2-substituent (**18b**) is associated with a decrease in the pK_{a3} value; 8.73 as compared with 9.70 of **4g**. This is due to the favourable hydrogen bonding with the phenoxide anion **22** [29]. Not surprisingly, the pK_{a2} value is largely unaffected by this substitution, the pK_{a2} values for **18a** and **4g** being 7.35 and 7.38, respectively.

Table 5
In vitro antimalarial activities and iron mobilization efficacy of basic hydroxypyridinones

Compd.	IC_{50} (μM)	Iron mobilization (%)	Efficacy of iron removal (%)
Control	–	3.9 ± 1.0	0.0
4a	85	5.3 ± 1.9	1.4
4b	65	6.8 ± 1.5	2.9
4c	80	13.0 ± 2.5	9.1
4d	85	20.3 ± 6.8	16.4
4e	50	12.4 ± 1.9	8.5
4f	35	22.7 ± 3.3	18.8
4g	25	30.4 ± 5.3	26.5
4h	50	–	–
9a	23	–	–
9b	50	–	–
9c	29	–	–
9d	35	–	–
13a	31	–	–
13b	21.5	–	–
18a	10	23.3 ± 3.4	19.4
18b	8	23.1 ± 3.9	19.2
19a	67.5	13.4 ± 5.2	9.5
19b	55	58.3 ± 9.4	54.4
20	45	48.4 ± 7.2	44.5

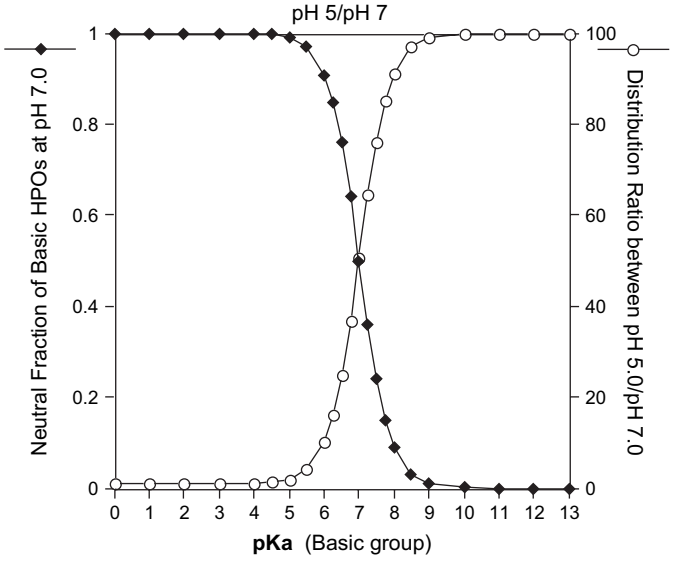
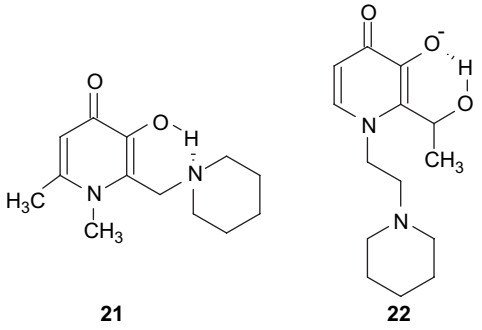


Fig. 1. The relationship between the pK_a value of hydroxypyridinones and (○) distribution ration (DR) between two phases at pH values 5 and 7 and (●) the fraction of non-ionized species present at pH 7.0.



21 **22**

The ligands reported in this study cover a wide range of distribution coefficients (0.1–26.0, Table 3). As all the $D_{7.4}$ values exceeded 0.1, it was anticipated that the compounds would be orally active and some ($D_{7.4} > 1.0$) may well be susceptible to efficient first-pass extraction by the liver. The calculated $D_{7.4}$ values of the (3:1) iron(III) complexes covered the range 0.001–26 (Table 3). In most cases the iron(III) complexes are predicted to be more hydrophilic than their corresponding free ligands. However, this trend does not hold for compounds which have $D_{7.4}$ values greater than 3 (**4g**, **13a** and **13b**). In these cases the iron(III) complexes were predicted to be more hydrophobic than their corresponding free ligands [28].

With regards to chelation of iron(III), a suitable comparator is the pFe^{3+} value, defined as the negative logarithm of the concentration of the free iron(III) in solution under defined conditions. Typically pFe^{3+} values are calculated for $[ligand]_{total} = 10^{-5}$ M and $[iron]_{total} = 10^{-6}$ M at pH 7.4. Comparison of ligands using this parameter is useful, since the pFe^{3+} value, unlike the corresponding stability constant, takes into account the effects of ligand basicity, denticity and degree of protonation, as well as differences in metal–ligand stoichiometries [29]. Chelators with high pFe^{3+} values are predicted not only to scavenge iron

more effectively at low ligand concentrations, but also to dissociate less readily and therefore form lower concentrations of the partially coordinated complexes. The compounds of this study (Table 4) fall into two broad classes: one with pFe^{3+} values < 20 (which are similar to the parent **19a**) and the other group, with pFe^{3+} values > 20. It has been previously established that the introduction of an 1-hydroxyalkyl group at the 2-position of 3-hydroxypyridinones reduces the pK_a values of the ligand and hence increases the pFe^{3+} values, due to the formation of a stable intramolecular hydrogen bond between the 2-(1′-hydroxyl) group and the adjacent 3-hydroxyl function, as indicated in **22** [29].

The introduction of an aliphatic amine group at the 6-position also leads to a marked reduction of pK_{a1} and pK_{a3} values and therefore to a significant improvement in the pFe^{3+} values, for example **13a** and **13b** (Table 4). This effect was found to be marginally greater with the di-substituted compound (**13b**, $pFe^{3+} = 22.5$) than with the mono-substituted compound (**13a**, $pFe^{3+} = 21.9$). The compound **13a** has a pFe^{3+} value which is in the region of the three orders magnitude larger than that of **19a** (deferiprone). For the strongly basic chelators such as **9a** the 3:1 neutral complex was found to be the dominant species over the pH range 6.0–10.0. Thus at the cytoplasmic pH value of 7.0, the 3:1 iron(III) complex of **9a** forms a mixture of the monopositive and dipositive charged species. In contrast at pH 5.5 the dominant species is the dipositive cation. Thus although the free ligand is predicted to be accumulated in acidic intracellular compartments (Fig. 1), if it chelates iron(III) within such a compartment it will form a highly charged iron complex which is unlikely to partition through membranes. In contrast, the pK_a values of the morpholine-containing iron complex $(\mathbf{9b})_3 \cdot$Fe(III) remain largely uncharged at both pH 5.5 and 7.0.

4.2. Biological properties of hydroxypyridinones

The *in vivo* iron mobilization efficacy of selected ligands was evaluated in a non-iron overloaded rat model [31]. [^{59}Fe]ferritin was used to label the liver iron pool, and this was followed by a challenge with a test chelator at a time when the iron released by lysosomal degradation of ferritin was maximally available [31,32]. This [^{59}Fe]ferritin-loaded rat model can be used to assess oral bioavailability and to compare the ability of chelators to remove iron from liver. Many of the basic hydroxypyridinones led to superior iron excretion *via* the bile as compared to deferiprone (**19a**) (Table 5). There was no obvious correlation between efficacy and the pK_{a2} value of the side chain, although those with pK_{a2} values > 7.0 tended to be more efficient than those with pK_{a2} values < 7.0. The imidazole-containing molecules were much less effective than the tertiary amine derivatives. Comparison of the tertiary amino-containing pyridinones indicates that **4g** is worthy of further investigation. It is likely that this high relative efficacy is associated with the high log *P* value (0.943), which would be predicted to favour efficient extraction by the liver.

Surprisingly the basic pyridinones which also possessed a hydroxylated side chain on the 2-substituent (**18a** and **18b**) were found to be less effective than the non-basic analogue **20**. Clearly the introduction of a basic function does not necessarily enhance the ability of a pyridinone to scavenge iron under *in vivo* conditions. This finding is likely to be associated with the formation of the highly charged iron complexes in acidic intracellular compartments, such as the lysosome.

The data obtained, from the *in vitro* antimalarial study, indicate that all of the tested iron chelators inhibit the growth of the parasites (Table 5). The hydrophobic basic chelators, such as **4g**, **9a**, **13a** and **13b**, were more active against *P. falciparum* than the dialkyl hydroxypyridinones (**19a** and **19b**). In contrast, the hydrophilic and imidazole-containing molecules (**4a**, **4b**, **4c** and **4d**) demonstrated a weak inhibitory action.

In addition to lipophilicity, it is clear that the basic strength of 3-hydroxypyridinones is an important factor for antimalarial activity. For instance, the IC_{50} values of the morpholine-containing ring compounds, **4h** and **9b**, are close to those corresponding to the neutral 3-hydroxypyridinones when compared with the corresponding piperidine-containing ring ligands **4g** and **9a** (Table 5). Interestingly, the basic 2-(1′-hydroxyethyl)-3-hydroxypyridinone **18b** is more active against malarial parasites than the neutral analogues such as **20**, although it is not as effective in iron removal. Compound **18a** is predicted to be strongly accumulated in acidic organelles, but will form highly charged iron complexes which will not readily escape from the organelle. It appears that this accumulation effect has a dominant influence on antimalarial properties. The iron chelator **13b** which possesses two basic side chains has the highest pFe^{3+} value and log *P* value of the entire group, and yet is not as effective as an antimalarial when compared with the monobasic hydrophilic chelators, **18a** and **18b**. This is probably due to its high net charge which renders it less able to penetrate membranes by non-facilitated diffusion.

The comparison of the basic pyridinones highlights **18a** and **18b** as the most promising candidates for further investigation. These two compounds apparently possess suitable partition coefficients to facilitate their penetration of intracellular organelles and yet are sufficiently hydrophilic to lack the general toxicity associated with more lipophilic hydroxypyridinones [13].

5. Experimental section

5.1. Materials and general procedures

Maltol and ethyl maltol were purchased from Pfizer (Widnes, UK). All other chemicals were obtained from Aldrich Chemical Co. (Gillingham, UK). 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 parts per million downfield from the internal standard tetramethylsilane (TMS). Mass spectra (EI) were recorded on a Joel AX505W. Elemental analyses were performed by Micro Analytical Laboratories, Department of Chemistry, The University of Manchester, Manchester M13 9PL.

Compounds **19a** (1,2-dimethyl-3-hydroxypyridin-4-one), **19b** (1,2-diethyl-3-hydroxypyridin-4-one) and **20** (1-ethyl-2-(1′-hydroxyethyl)-3-hydroxypyridin-4-one) were synthesised from maltol **1a**, ethyl maltol **1a** and pyromeconic acid **14**,

respectively, as previously described [10,29]. 3-Benzyloxy-2-methylpyran-4(1*H*)-one (**2a**) and 3-benzyloxy-2-ethylpyran-4(1*H*)-one (**2b**) were synthesised from maltol **1a** and ethyl maltol **1b**, respectively, following the methodology as described by Dobbin et al. [10].

5.2. General procedure for the preparation of N-basic substituted 3-hydroxypyridin-4-ones *4a–h*

To a solution of (**2a**) or (**2b**) (20 mmol, 1 eq.) in ethanol (50 mL)/water (50 mL) was added the selected amine (30 mmol, 1.5 eq.) followed by 2 N sodium hydroxide solution until pH 13.5 was obtained. The mixture was then refluxed for 12 h. After adjustment to pH 1 with concentrated hydrochloric acid, the solvent was removed by rotary evaporation prior to addition of water (50 mL) and washing with diethyl ether (2 × 50 mL). Subsequent adjustment of the aqueous fraction to pH 9 with 10 N sodium hydroxide solution was followed by extraction into dichloromethane (4 × 50 mL). The combined organic layers were dried over anhydrous sodium sulphate, filtered, and rotary evaporated to give the benzylated hydroxypyridinones **3a–h** (Scheme 1) as a yellow oil, which were subsequently dissolved in ethanol (90 mL)/water (10 mL) and subjected to hydrogenolysis in the presence of 5% Pd/C (5–10% w/w of the compound) catalyst for 2 h. Following the filtration, the pH of the solution was adjusted to 1 using concentrated hydrochloric acid and the solvent was removed *in vacuo* to yield the crude product. Recrystallization from methanol/diethyl ether gave **4a–h** as a white or yellow solid.

5.2.1. 3-Hydroxy-1-[3′-(imidazol-1-yl)propyl]-2-methylpyridin-4(1H)-one dihydrochloride (*4a*)

Yield 69%; mp 160–161 °C; δ_H (360 MHz; DMSO-d_6; Me_4Si) 2.2–2.75 (2H, m, $CH_2CH_2CH_2$), 2.6 (3H, s, 2-CH_3), 4.1–4.8 (4H, m, $CH_2CH_2CH_2$), 7.4 (1H, dd, $J = 2.6$, 6.7 Hz, 5-*H*(pyridinone)), 7.7 (1H, t, $J = 1.6$ Hz, 5-*H*(imidazole)), 7.92 (1H, d, $J = 1.5$ Hz, 6-*H*(imidazole)), 8.4 (1H, dd, $J = 2.4$, 6.8 Hz, 6-*H*(pyridinone)), 9.38 (1H, s, 2-*H*(imidazole)), 6.1–8.8 (3H, br, *OH* and *NH*). Analysis $C_{12}H_{17}N_3O_2Cl_2 \cdot H_2O$ (C, H, N were within 0.4% of the theoretical values).

5.2.2. 2-Ethyl-3-hydroxy-1-[3′-(imidazol-1-yl)propyl]-pyridin-4(1H)-one dihydrochloride (*4b*)

Yield 59%; mp 189–191 °C; δ_H (60 MHz; DMSO-d_6; Me_4Si) 1.03 (3H, t, 2-CH_2CH_3), 1.85–2.55 (2H, m, $CH_2CH_2CH_2$), 2.8 (2H, q, 2-CH_2CH_3), 3.9–4.8 (4H, m, $CH_2CH_2CH_2$), 7.4 (1H, d, 5-*H*(pyridinone)), 7.65 (1H, d, 5-*H*(imidazole)), 7.9 (1H, d, 6-*H*(imidazole)), 8.35 (1H, d, 6-*H*(pyridinone)), 9.35 (1H, s, 2-*H*(imidazole)). Analysis $C_{13}H_{19}N_3O_2Cl_2$ (C, H, N were within 0.4% of the theoretical values).

5.2.3. 1-[2′-(Dimethylamino)ethyl]-3-hydroxy-2-methylpyridin-4(1H)-one dihydrochloride (*4c*)

Yield 65.7%; mp 251–252 °C; δ_H (60 MHz; D_2O; Me_4Si) 2.58(3H, s, 2-CH_3), 3.0 (6H, s, $N(CH_3)_2$), 3.4–3.9 (2H, m, $N-CH_2CH_2N(CH_3)_2$), 4.75 (2H, t, $N-CH_2CH_2N(CH_3)_2$), 7.1 (1H, d, 5-*H*(pyridinone)), 8.1 (1H, d, 6-*H*(pyridinone)). Analysis $C_{10}H_{18}N_2O_2Cl_2$ (C, H, N were within 0.4% of the theoretical values).

5.2.4. 1-[2′-(Dimethylamino)ethyl]-2-ethyl-3-hydroxypyridin-4(1H)-one dihydrochloride (*4d*)

Yield 52%; mp 234–236 °C; δ_H (60 MHz; D_2O; Me_4Si) 1.28 (3H, t, 2-CH_2CH_3), 2.75–3.3 (2H, q, 2-CH_2CH_3), 3.05 (6H, s, $N(CH_3)_2$), 3.5–3.9 (2H, m, $N-CH_2CH_2N(CH_3)_2$), 4.74 (t, $N-CH_2CH_2N(CH_3)_2$), 7.15 (2H, d, 1H, 5-*H*(pyridinone)), 8.1 (d, 6-*H*(pyridinone)). Analysis $C_{11}H_{20}N_2O_2Cl_2 \cdot H_2O$ (C, H, N were within 0.4% of the theoretical values).

5.2.5. 1-[2′-(Diethylamino)ethyl]-3-hydroxy-2-methylpyridin-4(1H)-one dihydrochloride (*4e*)

Yield 85.5%; mp 244–247 °C (dec.); δ_H (360 MHz; DMSO-d_6; Me_4Si) 1.26 (6H, t, $J = 7.2$ Hz, $N(CH_2CH_3)_2$), 2.6 (3H, s, 2-CH_3), 2.9–3.8 (6H, m, $N(CH_2CH_3)_2$ and $N-CH_2CH_2N(C_2H_5)_2$), 4.9 (2H, t, $J = 7.9$ Hz, $N-CH_2CH_2N(C_2H_5)_2$), 7.4 (1H, d, $J = 7.0$ Hz, 5-*H*(pyridinone)), 8.45 (1H, d, $J = 7.0$ Hz, 6-*H*(pyridinone)), 9.1–10.4 (3H, br, *OH* and *NH*). Analysis $C_{12}H_{22}N_2O_2Cl_2$ (C, H, N were within 0.4% of the theoretical values).

5.2.6. 3-Hydroxy-2-methyl-1-(2′-piperidinoethyl)-pyridin-4(1H)-one dihydrochloride (*4f*)

Yield 75.6%; mp 213–215 °C; δ_H (60 MHz; D_2O; Me_4Si) 1.3–2.4 (6H, m, br, $-CH_2CH_2CH_2-$(piperidine ring)), 2.64 (3H, s, 2-CH_3), 2.7–4.2 (6H, m, $-CH_2-N-CH_2-$(piperidine ring) and (pyridinone)$N-CH_2CH_2N$(piperidine)), 4.8 (2H, t, (pyridinone)$N-CH_2CH_2N-$(piperidine)), 7.18 (1H, d, 5-*H*(pyridinone)), 8.18 (1H, d, 6-*H*(pyridinone)). Analysis $C_{13}H_{22}N_2O_2Cl_2 \cdot H_2O$ (C, H, N were within 0.4% of the theoretical values).

5.2.7. 2-Ethyl-3-hydroxy-1-(2′-piperidinoethyl)-pyridin-4(1H)-one dihydrochloride (*4g*)

Yield 65%; mp 207–209 °C; δ_H (60 MHz; D_2O; Me_4Si) 1.22 (3H, t, 2-CH_2CH_3), 1.4–2.5 (6H, m, br, $-CH_2CH_2CH_2-$(piperidine ring)), 3.0 (2H, q, 2-CH_2CH_3), 3.1–3.9 (6H, m, $-CH_2-N-CH_2-$(piperidine ring) and (pyridinone)$N-CH_2CH_2N$(piperidine)), 4.8 (2H, t, (pyridinone)$N-CH_2CH_2N$(piperidine)), 7.18 (1H, d, 5-*H*(pyridinone)), 8.18 (1H, d, 6-*H*(pyridinone)). Analysis $C_{14}H_{24}N_2O_2Cl_2 \cdot H_2O$ (C, H, N were within 0.4% of the theoretical values).

5.2.8. 3-Hydroxy-2-methyl-1-(2′-morpholinoethyl)-pyridin-4(1H)-one dihydrochloride (*4h*)

Yield 69.7%; mp 214–216 °C; δ_H (360 MHz; D_2O; Me_4Si) 2.65 (3H, s, 2-CH_3), 3.4–4.2 (12H, m, $-CH_2OCH_2-$ and $-CH_2-N-CH_2-$(morpholine ring) and CH_2CH_2), 7.1 (1H, d, $J = 7.1$ Hz, 5-*H*(pyridinone)), 8.1 (1H, d, $J = 7.1$ Hz, 6-*H*(pyridinone)). Analysis $C_{12}H_{20}N_2O_3Cl_2$ (C, H, N were within 0.4% of the theoretical values).

5-Hydroxy-2-methylpyran-4(1*H*)-one (allomaltol) (**5a**) was synthesised from kojic acid (**5b**) in two steps using the established procedure [28].

5.3. General procedure for the preparation of 2-basic substituted 3-hydroxypyran-4(1H)-one **6a–d**

To a solution of piperidine or morpholine (40 mmol) and 40% aqueous formaldehyde (3 mL) in 95% ethanol (40 mL) was slowly added **5a** or **5b** (30 mmol). The mixture was stirred for 1 h and then cooled and refrigerated. The resulting crystals were collected by suction filtration and washed with ethanol and diethyl ether to give **6a–d** as a white or yellow crystalline solid.

5.3.1. 3-Hydroxy-6-methyl-2-piperidinomethylpyran-4(1H)-one (**6a**)

Yield 74%; mp 146–148 °C; δ_H (60 MHz; DMSO-d_6; Me_4Si) 1.1–1.6 (6H, s, br, $-CH_2CH_2CH_2-$(piperidine ring)), 2.1–2.4 (7H, m, 6-CH_3 and $-CH_2-N-CH_2-$(piperidine ring)), 3.3 (2H, s, 2-CH_2), 6.1 (1H, s, 5-*H*(pyranone)).

5.3.2. 3-Hydroxy-6-methyl-2-morpholinomethylpyran-4(1H)-one (**6b**)

Yield 79%; mp 132–136 °C; δ_H (60 MHz; DMSO-d_6; Me_4Si) 2.2–3.0 (7H, m, $-CH_2-N-CH_2-$(morpholine ring) and 6-CH_3), 3.5–4.0 (6H, m, $-CH_2OCH_2-$(morpholine ring) and 2-CH_2), 6.4 (1H, s, 5-*H*(pyranone)).

5.3.3. 3-Hydroxy-6-hydroxymethyl-2-piperidinomethylpyran-4(1H)-one (**6c**)

Yield 82%; mp 163–165 °C; δ_H (60 MHz; DMSO-d_6; Me_4Si) 1.3–1.7 (6H, m, br, $-CH_2CH_2CH_2-$(piperidine ring)), 2.2–2.6 (4H, m, br, $-CH_2-N-CH_2-$(piperidine ring)), 3.4 (2H, s, 2-CH_2), 4.2 (2H, s, 6-CH_2OH), 6.2 (1H, s, 5-*H*(pyranone)).

5.3.4. 3-Hydroxy-6-hydroxymethyl-2-morpholinomethylpyran-4(1H)-one (**6d**)

Yield 88%; mp 156–158 °C; δ_H (60 MHz; DMSO-d_6; Me_4Si) 2.6–32.8 (4H, m, $-CH_2-N-CH_2-$(morpholine ring)), 3.6–3.9 (6H, m, $-CH_2OCH_2-$(morpholine ring) and 2-CH_2), 4.5 (2H, s, 6-CH_2OH), 6.5 (1H, s, 5-*H*(pyranone)).

5.4. General procedure for the preparation of 2-basic substituted 3-hydroxypyridin-4-ones **9a–d**

To a solution of **6a–d** (20 mmol, 1 eq.) in methanol (50 mL) was added sodium hydroxide (25 mol, 1.25 eq.) dissolved in water (5 mL) and the mixture was heated to reflux. Benzyl bromide (25 mol, 1.25 eq.) was added dropwise over 30 min and then refluxed for 6 h. After removal of solvent by rotary evaporation, the residue was mixed with water (50 mL) and extracted with dichloromethane (3 × 50 mL). The combined organic fraction was washed with 5% aqueous sodium hydroxide (2 × 50 mL) and water (2 × 50 mL), dried over anhydrous sodium sulphate, filtered and rotary evaporated to yield the benzylated pyranones **7a–d** (Scheme 2), which were subsequently dissolved in ethanol (10 mL) followed by addition of 40% aqueous methylamine (10 mL). The reaction mixture was sealed in a thick-walled glass tube and stirred at 70 °C for 12 h. After adjustment to pH 1 with concentrated hydrochloric acid, the solvent was removed by rotary evaporation prior to addition of water (50 mL) and washed with diethyl ether (2 × 50 mL). Subsequent adjustment of the aqueous fraction to pH 9 with 10 N sodium hydroxide solution was followed by extraction into dichloromethane (4 × 50 mL), the combined organic layers then being dried over anhydrous sodium sulphate, filtered, and rotary evaporated to give the benzylated pyridinones **8a–d** (Scheme 2), which were then dissolved in 6 N hydrochloric acid (40 mL) and refluxed for 4 h. After removal of the solvent by rotary evaporation, the residue was purified by recrystallization from methanol/diethyl ether to afford **9a–d** as a white solid.

5.4.1. 1,6-Dimethyl-3-hydroxy-2-piperidinomethylpyridin-4(1H)-one dihydrochloride (**9a**)

Yield 54%; mp 186–188 °C; δ_H (60 MHz; D_2O; Me_4Si) 1.2–2.2 (6H, m, br, $-CH_2CH_2CH_2-$(piperidine ring)), 2.5 (3H, s, 6-CH_3), 3.0–3.5 (6H, m, $-CH_2-N-CH_2-$(piperidine ring) and 2-CH_2), 3.95 (3H, s, $N-CH_3$), 7.2 (1H, s, 5-*H*(pyridinone)). Analysis $C_{13}H_{22}N_2O_2Cl_2\cdot H_2O$ (C, H, N were within 0.4% of the theoretical values).

5.4.2. 1,6-Dimethyl-3-hydroxy-2-morpholinomethylpyridin-4(1H)-one dihydrochloride (**9b**)

Yield 47%; mp 196–200 °C; δ_H (60 MHz; D_2O; Me_4Si) 2.5 (3H, s, 6-CH_3), 3.1–3.6 (4H, m, $-CH_2-N-CH_2-$(morpholine ring)), 3.7–4.0 (7H, m, $-CH_2OCH_2-$(morpholine ring) and $N-CH_3$), 4.7 (2H, s, 2-CH_2), 6.95 (1H, s, 5-*H*(pyridinone)). Analysis $C_{12}H_{20}N_2O_3Cl_2$ (C, H, N were within 0.4% of the theoretical values).

5.4.3. 3-Hydroxy-6-hydroxymethyl-1-methyl-2-piperidinomethylpyridin-4(1H)-one dihydrochloride (**9c**)

Yield 34%; mp 194–197 °C; δ_H (60 MHz; D_2O; Me_4Si) 1.5–2.1 (6H, m, br, $-CH_2CH_2CH_2-$(piperidine ring)), 3.1–3.75 (4H, m, $-CH_2-N-CH_2-$(piperidine ring)), 3.9 (3H, s, $N-CH_3$), 4.65 (2H, s, 2-CH_2), 4.75 (2H, s, 6-CH_2OH), 7.2 (1H, s, 5-*H*(pyridinone)). Analysis $C_{13}H_{22}N_2O_3Cl_2\cdot 1/2H_2O$ (C, H, N were within 0.4% of the theoretical values).

5.4.4. 3-Hydroxy-6-hydroxymethyl-1-methyl-2-morpholinomethylpyridin-4(1H)-one dihydrochloride (**9d**)

Yield 37%; mp 190–1940 °C; δ_H (60 MHz; D_2O; Me_4Si) 3.2–3.7 (4H, m, $-CH_2-N-CH_2-$(morpholine ring)), 3.75–4.1 (7H, m, $-CH_2OCH_2-$(morpholine ring) and $N-CH_3$), 4.7 (4H, s, 2-CH_2 and 6-CH_2OH), 7.15 (1H, s, 5-*H*(pyridinone)). Analysis $C_{12}H_{20}N_2O_4Cl_2$ (C, H, N were within 0.4% of the theoretical values).

5.4.5. 2-Chloromethyl-5-benzyloxypyran-4(1H)-one (**11**)

To a well stirred thionyl chloride (50 mL) at 0 °C was added 4.92 g (20 mmol) of benzylated kojic acid **10** and the reaction was stirred at 0 °C for 2 h. The mixture was then poured into ice and extracted with chloroform (3 × 100 mL). The extracts were combined and washed successively with saturated sodium hydrogen carbonate solution and brine. After drying over anhydrous sodium sulphate, the solvent was removed to give the crude product. Recrystallization from dichloromethane/petroleum ether

(40–60 °C) afforded a white solid (4.13 g, 77%); mp 97–99 °C; δ_H (60 MHz; DMSO-d_6; Me_4Si) 4.3 (2H, d, 2-CH_2Cl), 5.1 (2H, s, CH_2Ph), 6.4 (1H, s, 3-*H*(pyranone)), 7.3 (5H, s, *Ph*) 7.5 (1H, s, 6-*H*(pyranone)). Analysis $C_{13}H_9O_4Cl$ (C, H, Cl were within 0.4% of the theoretical values).

*5.4.6. 5-Benzyloxy-2-piperidinomethylpyran-4(1H)-one (**12**)*

To a solution of piperidine and tributylamine in *N*,*N*-dimethylformamide (50 mL) was added 4.4 g (17.7 mmol) of **11** and the reaction mixture was allowed to stir at room temperature overnight. The mixture was then diluted with water (250 mL) and extracted with dichloromethane (3 × 150 mL). The extracts were combined, washed with water, dried over anhydrous sodium sulphate, filtered and the solvent removed *in vacuo*. The residue was purified by column chromatography on silica gel (eluant: methanol/chloroform; 10:90 v/v) to afford a light yellow oil (4.9 g, 93%); δ_H (60 MHz; $CDCl_3$; Me_4Si) 1.4–1.9 (6H, m, br, $-CH_2CH_2CH_2-$(piperidine ring)), 2.3–2.6 (4H, m, br, $-CH_2-N-CH_2-$(piperidine ring)), 3.25 (2H, s, 2-CH_2N), 5.0 (2H, s, CH_2Ph), 6.35 (1H, s, 3-*H*(pyranone)), 7.3 (5H, s, *Ph*) 7.45 (1H, s, 6-*H*(pyranone)). Analysis $C_{18}H_{19}NO_4$ (C, H, N were within 0.4% of the theoretical values).

*5.4.7. 5-Hydroxy-1-methyl-2-piperidinomethylpyridin-4(1H)-one dihydrochloride (**13a**)*

To a solution of **12** (1.6 g, 5 mmol, 1 eq.) in ethanol (10 mL) was added 10 mL of 40% aqueous methylamine and the mixture was sealed in a thick-walled glass tube and stirred at 70 °C for 6 h. After removal of the solvent, the residue was purified by column chromatography on silica gel (eluant: methanol/chloroform; 15:85 v/v) to afford a yellow oil, which was then dissolved in 6 N hydrochloric acid (40 mL) and refluxed for 4 h. After removal of the solvent by rotary evaporation, the residue was purified by recrystallization from methanol/diethyl ether to afford **13a** as a white solid (0.92 g, 62%); mp 225–227 °C; δ_H (60 MHz; D_2O; Me_4Si) 1.4–2.0 (6H, s, br, $-CH_2CH_2CH_2-$(piperidine ring)), 3.0–3.5 (4H, br, $-CH_2-N-CH_2-$(piperidine ring)), 3.95 (3H, s, $N-CH_3$), 4.4 (2H, s, 2-CH_2N), 7.2 (1H, s, 3-*H*(pyridinone)), 8.0 (1H, s, 6-*H*(pyridinone)). Analysis $C_{12}H_{20}N_2O_2Cl_2$ (C, H, N were within 0.4% of the theoretical values).

*5.4.8. 5-Hydroxy-1-(2′-piperidinoethyl)-2-piperidinomethylpyridin-4(1H)-one trihydrochloride (**13b**)*

To a solution of **12** (3.2 g, 10 mmol, 1 eq.) in ethanol (50 mL)/water (50 mL) was added 2-piperidinoethylamine (2.56 g, 20 mmol, 2 eq.) followed by 2 N sodium hydroxide solution until pH 12.5 was obtained. The mixture was then refluxed for 12 h. After adjustment to pH 1 with concentrated hydrochloric acid, the solvent was removed by rotary evaporation prior to addition of water (50 mL) and washed with diethyl ether (2 × 50 mL). Subsequent adjustment of the aqueous fraction to pH 9 with 10 N sodium hydroxide solution was followed by extraction into dichloromethane (4 × 50 mL). The combined organic layers were then dried over anhydrous sodium sulphate, filtered, and concentrated to yield a light brown oil which was purified by column chromatography on silica gel (eluant: methanol/chloroform; 20:80 v/v). The resulting light yellow oil was then dissolved in 6 N hydrochloric acid (40 mL) and refluxed for 4 h. After removal of the solvent by rotary evaporation, the residue was purified by recrystallization from methanol/diethyl ether to afford **13b** as a yellow solid (1.2 g, 28%); mp 190–194 °C; δ_H (60 MHz; D_2O; Me_4Si) 1.4–2.0 (14H, m, br, 2 × $-CH_2CH_2CH_2-$(piperidine ring) and (pyridinone)N$-CH_2CH_2$N(piperidine)), 3.0–3.5 (10H, br, 2 × $-CH_2-N-CH_2-$(piperidine ring) and (pyridinone)N$-CH_2CH_2$N(piperidine)), 4.34 (2H, s, 2-CH_2N), 6.9 (s, 1H, 3-*H*(pyridinone)),7.8 (1H, s, 6-*H*(pyridinone)). Analysis $C_{18}H_{32}N_3O_2Cl_3 \cdot 3H_2O$ (C, H, N were within 0.4% of the theoretical values).

*5.4.9. 2-Hydroxymethyl-3-hydroxypyran-4(1H)-one (**15a**)*

Sodium hydroxide (4 g, 100 mmol) dissolved in distilled water (10 mL) was added to a solution of **14** (8.96 g, 80 mL, 1 eq.) in methanol (50 mL) and allowed to stir at room temperature for 5 min; 35% formaldehyde solution (16 mL) was added dropwise over 15 min and the solution was stirred overnight. After adjustment to pH 1 with 37% hydrochloric acid, the reaction mixture was concentrated *in vacuo* to dryness and the resulting solid was extracted with isopropanol (2 × 100 mL) at 90 °C. The isopropanol extracts were concentrated to yield the crude products. Recrystallization from isopropanol afforded a white crystalline solid (9.7 g, 85.4%); mp 154–156 °C (lit. value [33]: 154–155 °C); δ_H (60 MHz; DMSO-d_6; Me_4Si) 4.4 (2H, s, 2-CH_2OH), 4.6–5.7 (1H, br, 2-CH_2OH), 6.34 (1H, d, 5-*H*), 8.1 (1H, d, 6-*H*), 9.0 (1H, br, s, 3-*OH*).

*5.4.10. 2-(1′-Hydroxyethyl)-3-hydroxypyran-4(1H)-one (**15b**)*

Pyromeconic acid (**12**) (5.6 g, 50 mmol) was added to water (50 mL) and the pH of the solution was adjusted to 10.5 using 50% aqueous sodium hydroxide. Acetaldehyde (2.64 g, 60 mmol) dissolved in water (20 mL) was slowly added dropwise over 1 h and the solution allowed to stir overnight. The reaction mixture was acidified to pH 1 with 37% hydrochloric acid and concentrated *in vacuo* to dryness. The residue was extracted with isopropanol (2 × 70 mL) at 90 °C. The isopropanol extracts were combined and concentrated to yield, after recrystallization from toluene, a pale yellow crystalline solid (3.7 g, 47.4%); mp 131–132 °C (lit. value [33]: 130–131 °C); δ_H (60 MHz; DMSO-d_6; Me_4Si) 1.3 (3H, d, 2-CH_2CH_3), 5.03 (1H, q, 2-$CHCH_3$), 6.38 (1H, d, 5-*H*), 8.2 (1H, d, 6-*H*).

*5.4.11. 8-Oxo-4,8-dihydro-2-phenyl-4H-pyrano[3,2-d]-m-dioxin (**16a**)*

A solution of **15a** (2.84 g, 20 mmol), benzaldehyde dimethyl acetal (6.08 g, 40 mmol), and toluene-*p*-sulphonic acid monohydrate (0.04 g, cat) in *N*,*N*-dimethylformamide (50 mL) was rotated under aspirator pressure at 80 °C for 3 h. The solvent was removed under high vacuum and the residue taken into dichloromethane (100 mL). The organic solution was washed successively with 5% sodium hydrogen carbonate solution and brine. After drying over magnesium sulphate, the solvent was removed to give the crude product. Recrystallization from dichloromethane/petroleum ether (40–60 °C) afforded a white crystalline solid (3.77 g, 82%); mp 141–143 °C; δ_H (60 MHz; $CDCl_3$; Me_4Si) 4.7 (2H, d, CH_2O), 5.88 (1H, s, 2-*CH*Ph), 6.35

(1H, d, 7-*H*(pyranone)), 7.2–7.9 (6H, m, Ar and 6-*H*(pyranone)); *m/z*, 230 (M^+).

Analogous synthesis starting with **15b** gave compound **16b** as shown in Scheme 4.

5.4.12. 8-Oxo-4,8-dihydro-4-methyl-2-phenyl-4H-pyrano-[3,2-d]-m-dioxin (*16b*)

Mp 112–113 °C; δ_H (60 MHz; $CDCl_3$; Me_4Si) 1.55 (3H, d, $CHCH_3$), 5.0 (1H, q, $CHCH_3$), 5.8 (1H, s, *CH*Ph), 6.25 (1H, d, 7-*H*(pyranone)), 7.10–7.75 (6H, m, *Ph* and 6-*H*(pyranone)); *m/z*, 244 (M^+).

5.4.13. 8-Oxo-4,8-dihydro-2-phenyl-5-(2′-piperidinoethyl)-4H-pyridino[3,2-d]-m-dioxin (*17a*)

To a solution of **16a** (3.45 g, 15 mmol, 1 eq.) in ethanol (50 mL)/water (50 mL) was added 2-piperidinoethylamine (3.84 g, 30 mmol, 2 eq.) followed by 2 N sodium hydroxide solution until pH 12.5 was obtained. The mixture was then refluxed for 3 h. TLC analysis (eluant: methanol/chloroform; 10:90 v/v) showed that no starting material was present. After removal of solvent by rotary evaporation, the residue was purified by column chromatography on silica gel (eluant: methanol/chloroform; 20:80 v/v) to afford a yellow crystalline solid (2.6 g, 51%); mp 137–140 °C; δ_H (60 MHz; $CDCl_3$; Me_4Si) 1.2–1.9 (6H, br, m, $-CH_2CH_2CH_2-$(piperidine ring)), 2.0–2.9 (6H, m, $-CH_2-N-CH_2-$(piperidine ring) and (pyridinone)N$-CH_2CH_2$N(piperidine)), 3.65 (2H, t, (pyridinone)N$-CH_2CH_2$N(piperidine)), 4.9 (2H, s, CH_2O), 5.85 (1H, s, *CH*Ph), 6.3 (1H, d, 7-*H*(pyridinone)), 7.0–7.8 (6H, m, *Ph* and 6-*H*(pyridinone)).

5.4.14. 8-Oxo-4,8-dihydro-2-phenyl-4-methyl-5-(2′-piperidinoethyl)-4H-pyridino[3,2-d]-m-dioxin (*17b*)

An analogous procedure to the preparation of **17a** using **7b** (1.83 g, 7.5 mmol, 1 eq.) afforded **17b** (1.24 g, 46.7%) as a light brown oil; δ_H (60 MHz; $CDCl_3$; Me_4Si) 1.0–1.8 (9H, m, $CHCH_3$ and $-CH_2CH_2CH_2-$(piperidine ring)), 1.9–2.8 (6H, m, $-CH_2NCH_2-$(piperidine ring) and (pyridinone)N$-CH_2CH_2$N(piperidine)), 3.4–4.0 (2H, m, (pyridinone)N$-CH_2CH_2$N(piperidine)), 5.1 (1H, q, $CHCH_3$), 5.5 (1H, s, *CH*Ph), 6.2 (1H, d, 7-*H*(pyridinone)), 7.0–7.8 (6H, m, *Ph* and 6-*H*(pyridinone)).

5.4.15. 2-(1′-Hydroxymethyl)-3-hydroxy-1-(2′-piperidinoethyl)-pyridin-4(1H)-one dihydrochloride (*18a*)

A solution of **17a** (2.55 g, 7.5 mmol) in ethanol (30 mL) was adjusted to pH 1 with concentrated hydrochloric acid prior to hydrogenolysis for 3 h in the presence of 5% Pd/C catalyst (0.5 g). Filtration followed by rotary evaporation gave the crude product as a white solid. Recrystallization from methanol/diethyl ether gave a white crystalline solid (1.98 g, 81.2%); mp 278–280 °C (dec.); δ_H (360 MHz; D_2O; Me_4Si) 1.0–2.4 (6H, m, br, $-CH_2CH_2CH_2-$(piperidine ring)), 2.7–3.9 (6H, m, $-CH_2NCH_2-$(piperidine ring) and (pyridinone)N$-CH_2CH_2$N(piperidine)), 4.8 (2H, t, $J = 7.7$ Hz, (pyridinone)N$-CH_2CH_2$N(piperidine)), 4.95 (2H, s, CH_2O), 7.18 (1H, d, $J = 6.9$ Hz, 5-*H*(pyridinone)), 8.2 (1H, d, $J = 7.0$ Hz, 6-*H*(pyridinone)). Analysis $C_{13}H_{22}N_2O_3Cl_2$ (C, H, N were within 0.4% of the theoretical values).

5.4.16. 2-(1′-Hydroxyethyl)-3-hydroxy-1-(2′-piperidinoethyl)-pyridin-4(1H)-one dihydrochloride (*18b*)

An analogous hydrogenation procedure to the preparation of **18a** using **17b** (1.24 g, 3.5 mmol) and 5% Pd/C catalyst (0.3 g) yielded 0.92 g of the title compound (77.5%) after recrystallization from methanol/diethyl ether, as a white solid; mp 191–193 °C; δ_H (360 MHz; DMSO-d_6; Me_4Si) 1.55 (3H, d, $J = 6.9$ Hz, $CHCH_3$), 1.3–2.4 (6H, m, br, $-CH_2CH_2CH_2-$(piperidine ring)), 2.7–4.1 (6H, m, $-CH_2-N-CH_2-$(piperidine ring) and (pyridinone)N$-CH_2CH_2$N(piperidine)), 4.5–5.4 (2H, m, (pyridinone)N$-CH_2CH_2$N(piperidine)), 5.6 (1H, q, $J = 6.9$ Hz, $CHCH_3$), 7.4 (1H, d, $J = 7.0$ Hz, 5-*H*(pyridinone)), 8.5 (1H, d, $J = 7.0$ Hz, 6-*H*(pyridinone)), 7.1–9.0 (2H, br, *OH* and *NH*). Analysis $C_{14}H_{24}N_2O_3Cl_2 \cdot 1/2H_2O$ (C, H, N were within 0.4% of the theoretical values).

5.5. Determination of physico-chemical properties

5.5.1. pK_a determination of ligands

Equilibrium constants of protonated ligands were determined using an established automated titration system [30]. The pK_a values were obtained to an accuracy of ±0.02 pH unit.

5.5.2. Iron(III) affinity constant determination

The cumulative iron(III) stability constants (β_3) for the ligands were determined by spectrophotometric titration of iron(III)–ligand complexes using an automated system [30]. The iron(III) complexes of the ligand were prepared in 5:1 molar ratio in 0.1 M KCl. This solution was acidified to pH 1.5 using 0.2 M HCl and then titrated against 0.2 M KOH. The resulting spectrophotometric data were analysed using COMPT1 program and the cumulative stability constant was obtained to an accuracy of ±0.2 log unit.

The stepwise stability constants (K_1, K_2 and K_3) of the ligands were optimized from the spectrophotometric titration of the iron(III)–ligand [30].

5.5.3. Distribution coefficient determination

Distribution coefficients were determined using an automated continuous flow technique [28]. The aqueous and octanol phases were presaturated with respect to each other before use. The aqueous phase (50 mM MOPS buffer, pH 7.4) was separated from the two phase system (1-octanol/MOPS buffer, pH 7.4) by means of a hydrophilic cellulose mounted in the gel-filtration column adjuster. A known volume of MOPS buffer is 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 so as to give an absorbance between 0.5–1.5 absorbance units at the preselected wavelength.

5.5.4. pK_a determination of iron(III) complexes

The pK_a values of the iron(III) complexes were determined by spectrophotometric titration using an automated system

[30]. The iron(III) complexes of the ligand (10^{-5} M) were prepared in a 5-fold molar excess of ligand in 0.1 M KCl. This solution was acidified to pH 1.5 using 0.2 M HCl and then titrated against 0.2 M KOH. The resulting spectrophotometric data were analysed using the STABOPT program [34]. The pK_a values were obtained to an accuracy of ±0.2 log unit.

5.6. Biological experiments

5.6.1. Animals

Male Wistar rats were purchased from Tuck & Son (Battlesbridge, Essex SSI, UK) and housed in the Biological Service Unit, King's College London. The animals were maintained at 20–23 °C with food and water freely available. All animal experiments performed were specified in project licence PPL 70/4561, authorized by the Secretary of State (England) under Animals Act 1986.

5.6.2. Iron mobilization efficacy study in [^{59}Fe]ferritin loaded rat

Hepatocytes of normal fasted rats (190–230 g) were labelled with ^{59}Fe by administration of [^{59}Fe]ferritin to the tail vein [31]. One hour later, each rat was administered orally with chelator. Control rats were administered with an equivalent volume of water. The 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 the [^{59}Fe]ferritin administration, rats were killed, and the liver and gastrointestinal tract (including its content and faeces) were removed for gamma counting. The ''iron mobilization'' and ''efficacy'' were calculated according to Eqs. (6) and (7).

$$\text{Iron mobilization}(\%) = \frac{^{59}\text{Fe-activity}_{(\text{gut and faeces})}}{^{59}\text{Fe-activity}_{(\text{gut and faeces})} + ^{59}\text{Fe-activity}_{(\text{liver})}} \times 100 \quad (6)$$

$$\text{Efficacy}(\%) = \text{iron mobilization}(\%) - \text{control}(\%) \quad (7)$$

5.6.3. In vitro antimalarial activity

A chloroquine-resistant clone of *P. falciparum* (TM267TR) was obtained from a stock of a continuous line maintained in human red blood cells in RPMI 1640 medium (Gibco) containing 10% human serum, 25 mM HEPES (Sigma), and 25 mM $NaHCO_3$. Parasite growth was synchronized by a sorbitol lysis method [35]. Before use, the parasites were washed twice with warm RPMI 1640 medium, and diluted with normal red blood cells to final hematocrit and parasitemia of 1% and 0.5%, respectively. Tests for drug susceptibility were performed by an established method [36], whereby 200 μl of a parasitized red blood cell suspension was incubated with 50 μl of the iron chelator alone or in combination with pyridines at various concentrations in 96-well microtiter plates. The plates were incubated at 37 °C in 5% O_2, 5% CO_2 and 90% N_2. After 24 h, the cultures were pulsed with ^{3}H-hypoxanthine (specific activity 1 Ci/mL by adding 0.6 μCi of isotope to each well). Microtiter plates were kept in the incubation chamber for an additional 18 h. Then, each plate was harvested onto glass fiber discs using a TOMTEC MASH II cell harvester. Scintillation cocktail (Omnifluor, New England Nuclear Research Products, Boston) was added and radioactivity was determined by a Betaplate liquid scintillation counter (Wallac, Finland). The IC_{50} values of individually tested agents were obtained from dose–response curves generated from serial dilutions conducted in triplicate by a computerized, non-linear regression analysis.

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Supplementary data

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