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Original Article

Synthesis and physico-chemical properties of a series of bidentate 3-hydroxypyridin-4-ones iron chelating agents

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Abstract

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. 3-Hydroxypyridin-4-ones are selective for iron(III) under most biological conditions, but unlike desferrioxamine, are efficiently absorbed when administered orally. In this study, the synthesis and determination of partition coefficients (K_{part}) of a range of 1-substituted-2-ethyl-3-hydroxypyridin-4-ones, as orally active iron chelators, are described. All of the 1-substituted-2-ethyl-3-hydroxypyridin-4-ones were synthesized *via* a three step synthetic pathway. The commercially available 2-ethyl-3-hydroxypyran-4-one (ethyl maltol) was benzylated in aqueous methanol. The reaction product of the benzylated ethyl maltol with an excess of the suitable primary aryl amines was heated in a thick-walled sealed glass tube at 150-160 ^{o}C to give 1-aryl-2-ethyl-3-benzyloxypyridin-4-one derivatives which were isolated as the free-bases. Removal of the benzyl group under acidic conditions was performed by catalytic hydrogenation to yield the bidentate chelators as HCl salt in good yield. In this work, final following compounds of 1-phenyl-2-ethyl-3-hydroxypyridin-4-one, 1-(4-methylphenyl)-2-ethyl-3-hydroxypyridin-4-one, 1-(4-methoxylphenyl)-2-ethyl-3-hydroxypyridin-4-one, and 1-(4-nitrophenyl)-2-ethyl-3-hydroxypyridin-4-one were synthesized. Identif-cation and structural elucidation of ligands were achieved by ^{1}HNMR, IR, elemental analysis, mass spectra and through physical experiments. The K_{part} values of the compounds were also determined in an aqueous/octanol system using an automated continuous flow method (a filter probe method).

Keywords: Hydroxypyridinones; Partition coefficient; Iron chelator; Iron overload

INTRODUCTION

The most frequent treatment of inherited hematological diseases such as β-thalassaemia is to maintain high levels of hemoglobin by regular blood transfusion (1). Repeated transfusion leads to elevated body iron levels due to the inability of humans to excrete iron *via* the kidney. Excess iron is mainly located within the liver and other highly perfused organs leading to tissue damage, organ failure and eventually death (2). Complications associated with elevated iron levels can be largely avoided by the use of iron-specific chelating agents and in particular desferrioxamine (DFO) (Fig. 1). Unfortunately, the major limiting factor of DFO is that it is not orally active and has to be administered parentally. This in turn leads to poor patient compliance (3,4).

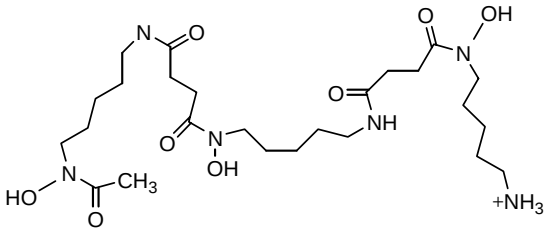

Fig. 1. Structure of desferrioxamine (DFO)

Hydroxypyridinones (HPOs) are currently one of the main candidates for the development

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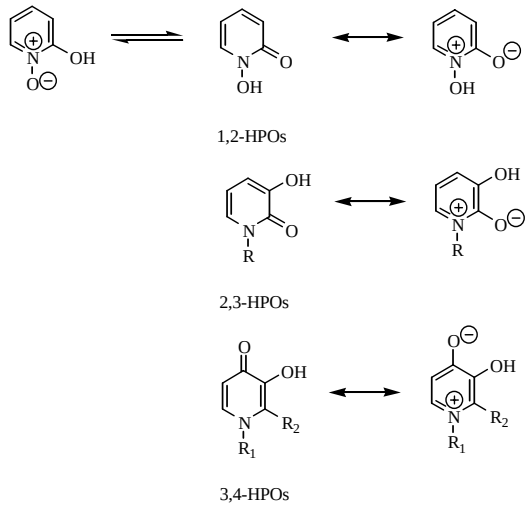

Fig. 2. Tautomerism and mesomerism of the three main classes of hydroxypyridinone ligands: 1-hydroxy-pyridin-2-ones (1,2-HPOs); 3-hydroxypyridin-2- ones (3,2-HPOs); 3-hydroxypyridin-4-ones (3,4-HPOs)

of orally active iron chelator alternatives to DFO. In fact, there are three classes of hydroxypyridinones: 1-hydroxypyridin-2-ones (1,2-HPOs) (5), 3-hydroxypyridin-2-ones (3,2-HPOs) (6) and 3-hydroxypyridin-4-ones (3,4-HPOs) (7) (Fig. 2). Of the three classes of HPO ligands, the 3-hydroxypyridin-4-one class possesses the highest affinity for Fe(III) that is 10^{37}. This is the direct consequence of the elevated pK_a value associated with the 4-oxo group compared with the 2-oxo congeners (Table 1) (8).

The HPOs form five-membered chelate rings in which the metal (iron) is coordinated by two vicinal oxygen atoms. The hydroxy-pyridinones are monoprotic acids and thus form neutral tris Fe(III) complexes (Fig. 3) (8). The deprotonated forms of the HPOs have a zwitterionic aromatic resonance form. This is due to the delocalization of the nitrogen electron lone pair with the crabonyl oxygen to afford a resonance structure whereby two negative charges are distributed between the two donor oxygens. The resulting charge associated with these oxygen atoms is therefore greater than one but less than two

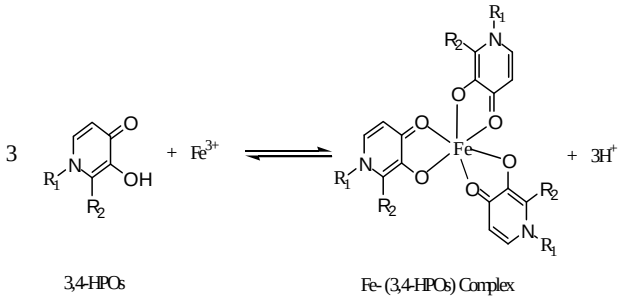

Fig. 3. Formation of Fe(III) complex of 3-hydroxypyridin-4-one ligands

the extent of electron-pair delocalization from the nitrogen onto the cabonyl oxygen will dictate the hardness of that donor atom and hence influence overall chelate stability (9).

3,4-HPOs are selective for iron(III) (Table 2) under most biological conditions, but unlike DFO, are efficiently absorbed when administered orally (10). So far, several 3-hydroxy pyridin-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 urinary and biliary routs, and some compounds have potential as clinically useful chelators (11). The majority of effort with human studies has centered on the simple 1,2-dialkyl derivatives, such as 1,2-dimethyl-3-hydroxy pyridin-4-one (Deferiprone, L1)- (marketed by Apotex Inc. Toronto, Canada, as Ferripox^{TM}). L1 is effective at removing iron from iron overloaded animals including man (12,13) but is associated with some disadvantages (14,15). One of the major reasons for the limited efficacy of L1 in clinical use is that it conjugates rapidly with glucuronic acid under *in vivo* conditions (Fig. 4) (16) and consequently high doses must be utilized in order to achieve clinically useful levels of iron excretion (17).

Despite these limitations, the ability of these compounds to relieve iron overload in man has made it clear that this class of the chela-

Table 1. pK_a Values and Fe(III) affinity constants for bidentate ligands

Ligand	pK_{a1}	pK_{a2}	Log β_3 (Fe^{3+})
1-Hydroxypyridin-2-ones (1,2-HPOs)	-	5.8	27
3-Hydroxypyridin-2-ones (3,2-HPOs)	0.2	8.6	32
3-Hydroxypyridin-4-ones (3,4-HPOs)	3.6	9.9	37

Table 2. Logarithms of overall stability constants for desferrioxamine (DFO) and 3-Hydroxypyridin-4-ones (3,4-HPOs) with selected metal ions.

Metal ion	DFO	3,4-HPOs
Fe (III)	31	37
Cu (II)	14	17
Zn (II)	11	12.5
Mg (II)	4	7
Ca (II)	2.3	4.5

tors has considerable potential as orally active iron chelators (18,19). In addition to the potential treatment of iron overload in thalassaemic patients, hydroxypyridin-4-ones may well find other clinical applications centered on iron removal. The hydroxypyridones are being investigated for the treatment of malaria (20), antimicrobial activity (21) and aluminum removal especially aluminum mobilization in renal dialysis patients (22,23).

In order to investigate further ligands which are able to scavenge iron effectively at low concentrations, it was decided to synthesize other derivatives of this type of compounds namely 1-aryl-3-hydroxypyridin-4-one derivatives (4a-d). In this study, synthesis of these compounds and their partition coefficients (K_{part}) were discussed.

The partition coefficients (K_{part}) of the compounds were determined in an aqueous/octanol system using an automated continuous flow method (a filter probe method) (24).

MATERIALS AND METHODS

Materials

All the chemicals used in this project were obtained from Aldrich (Gillingham, UK). Melting points were determined using an Electrothermal IA 9100 Digital melting point. IR spectra were recorded on a perkin-Elmer 1420. Proton NMR spectra were determined with EM-390 (80 MHz). Mass spectra were taken using a Vacuum Generaters 16F (35eV). Elemental analysis (Leco CHNCl-932) was performed by micro analytical laboratories, (University of Manchester, UK).

Chemistry

Synthesis of benzyl ethyl maltol (compound 2)

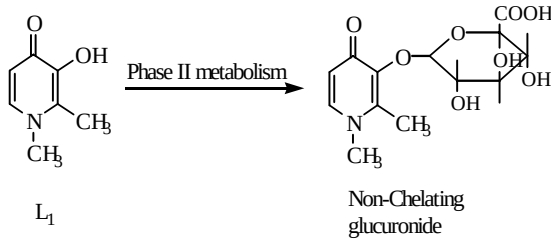

Fig. 4. Phase II metabolism of 1,2-dimethyl-3-hydroxypyridin-4-one (L_1) leading to the formation of non-chelating glucuronide conjugation

Sodium hydroxide (6.0 g, 0.15 mol) dissolved in water (20 ml) followed by benzyl chloride (19.0 g, 0.15 mol) were added to a solution of ethyl maltol (compound 1) (21.0 g, 0.15 mol) in methanol (180 ml) and the mixture was refluxed for 12 h. After removal of solvent by rotary evaporation, the residue was mixed with water (75 ml) and extracted into dichloromethane (2 × 75 ml). The combined extracts were washed with 5% sodium hydroxide (3 × 150 ml) and then with water (2 × 150 ml). The organic fraction was 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 as colourless needles, 27.30 g (79%); mp 32-33 ^{o}C.

Synthesis of 1-phenyl-2-ethyl-3-benzyloxy pyridin-4-one (compound 3a)

Aniline (2 ml, 0.02 mol) was added to a solution of benzyl ethyl maltol (compound 2) (2.30 g, 0.01 mol) in ethanol (20 ml)/water (20 ml). The mixture was heated in a thick-walled sealed glass tube at 150-160 ^{o}C for 24 h. After removal of solvent by rotary evaporation, the residue was mixed with water (40 ml) and extracted into dichloromethane (3 × 40 ml). The organic layers were then dried over anhydrous sodium sulphate, filtered and rotary evaporated to yield a brown oil. The resulting oil was purified by column chromatography on silica gel (eluent = 5% methanol/chloroform, Rf = o.45) to give a yellow oil (1.22 g, 40%).

Synthesis of 1-phenyl-2-ethyl-3-hydroxy pyridin-4-one hydrochloride (compound 4a)

The solution of 1-phenyl-2-ethyl-3-benzyloxypyridin-4-one (compound 3a) (1.53 g,

0.005 mol) in ethanol (27 ml)/water (3 ml) was adjusted to pH 1 with hydrochloric acid prior to hydrogenolysis for 4 h in the presence of 5% Pd/C catalyst (0.3 g). Filtration followed by rotary evaporation gave a white solid, recrystallization from ethanol/diethyl ether yielded a white powder (1.01 g, 80%); mp 211-212 ^{o}C.

Synthesis of 1-(4-methylphenyl)-2-ethyl-3-benzyloxypyridin-4-one (compound 3b)

Benzyl ethyl maltol (compound 2) (2.30 g, 0.01 mol) and p-toluidine (4-methylaniline) (2.14 g, 0.02 mol) were reacted as describe for compound 3a to afford a brown oil (compound 3b). The resulting oil was purified by column chromatography on silica gel (eluent = 10% methanol/chloroform, Rf = 0.50) to give a yellow oil (1.31 g, 41%).

Synthesis of 1-(4-methylphenyl)-2-ethyl-3-hydroxypyridin-4-one hydrochloride (compound 4b)

An analogous hydrogenation procedure for preparation of lignd compound 4a using compound 3b (1.60 g, 0.005 mol) and 5% Pd/C catalyst (0.3 g) yielded 1.02 g of the title compound (77%) after recrystallization from ethanol/diethyl ether, as a white powder; mp 245-246 ^{o}C.

Synthesis of 1-(4-methoxyphenyl)-2-ethyl-3-benzyloxypyridin-4-one (compound 3c)

Benzyl ethyl maltol (compound 2) (2.30 g, 0.01 mol) and p-anisidine (4-metoxylaniline) (2.46 g, 0.02 mol) were reacted as describe for compound 3a to obtain a brown oil (compound 3c). The resulting oil was purified by column chromatography on silica gel (eluent = 10% methanol/chloroform, Rf = 0.48) to give a yellow oil (1.31 g, 39%).

Synthesis of 1-(4-methoxylphenyl)-2-ethyl-3-hydroxypyridin-4-one hydrochloride (compound 4c)

An analogous hydrogenation procedure for preparation of lignd compound 4a using compound 3c (1.68 g, 0.005 mol) and 5% Pd/C catalyst (0.3 g) yielded 1.06 g of the title compound (75%) after recrystallization from ethanol/diethyl ether, as a white powder; mp 242-243 ^{o}C.

Synthesis of 1-(4-nitrophenyl)-2-ethyl-3-benzyloxypyridin-4-one (compound 3d)

Benzyl ethyl maltol (compound 2) (2.30 g, 0.01 mol) and 4-nitroaniline (2.76 g, 0.02 mol) were reacted as described for compound 3a to afford a brown oil (compound 3d). The resulting oil was purified by column chromatography on silica gel (eluent = 10% methanol/chloroform, Rf = 0.48) to give a yellow oil (0.88 g, 25%).

Synthesis of 1-(4-nitrophenyl)-2-ethyl-3-hydroxypyridin-4-one hydrochloride (compound 4d)

An analogous hydrogenation procedure for preparation of ligand compound 4a using compound 3d (1.75 g, 0.005 mol) and 5% Pd/C catalyst (0.3 g) gave 1.05 g of the title compound (71%) after recrystallization from ethanol/diethyl ether, as a white powder; mp 281-282 ^{o}C.

Determination of partition coefficients using the filter probe method.

Partition coefficients (K_{part}) of the ligands were determined using the automated continuous flow method technique as previously described (24). The system was comprised of an IBM compatible PC running the "TOPCAT" program which controlled both Metrohm 665 Dosimat autoburette and a Pye-Unicam Lambda 5 UV/Vis spectro-photometer, as well as performing all calculations of partition coefficients. All K_{part} values were achieved using analitical grade reagents under nitrogen atmosphere in a sealed titration vessel (250 ml) at a laboratory constant temperature (25 ± 0.5 °C). The two phases used, were MOPS [3-(N-morpholino)-propane sulphonic acid] buffer (50 mM, pH 7.4), prepared by the use of distilled water and n-octanol, each of which was pre-equilibrated with the other phase before use due to the limited solubility of water in n-octanol (2.3 M) (25). The buffer (100 µl) was circulated through a spectrophotometric flow-cell, which was returned to the mixing chamber with the aid of a peristaltic pump at a flow rate of 1 ml/min.

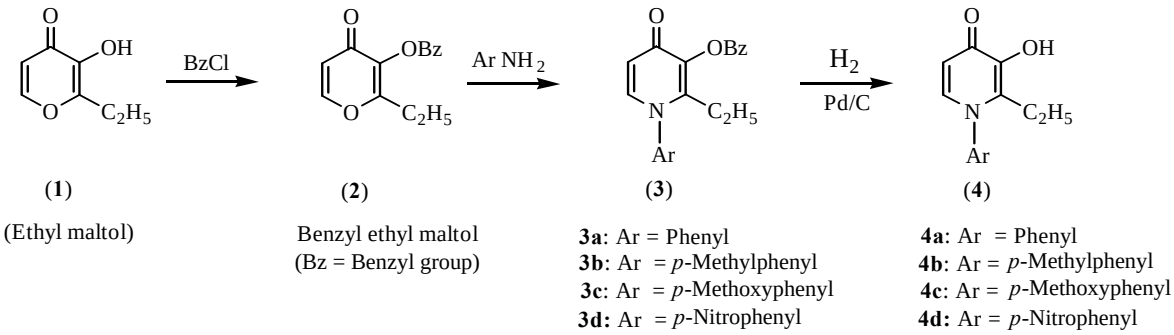

Fig. 5. Synthesis of 1-aryl-3-hydroxypyridin-4-ones *via* the three steps synthetic pathway.

The filter probe consisted of a polytetrafluoroethylene (PTFE) plunger associated with a gel-filtration column. The aqueous phase was separated from two-phase system (n-octanol/MOPS) by means of a hydrophilic cellulose filter (5- diameter, 589/3 blue band filter paper, Schleicher and Schuell) mounted in the gel-filtration column adjuster SR 25/50, (Pharmacia). A known volume (normally 20-100 ml) of MOPS buffer (saturated with n-octanol) was taken in the flat-based glass-mixing chamber. A base-line absorption value of the solution was used as a reference absorbance. A 0.1 mM solution of the ligand was prepared in the aqueous phase (typically 40 ml) to give an absorbance between 1.5-2.0 at the preselected wavelength (~ 280 nm). Upon commencement of the computer program, absorbance measurements were automatically recorded at preselected time intervals, usually 1 s When the absorbance readings were stabilized, as determined by the computer from equilibrium conditions selected by the operator (typically a constant absorbance is where the absorbance changes by less than 0.002 absorbance units over a minimum of 10 min), a suitable volume of n-octanol was added to the aqueous phase from the automatic dispenser. Absorbance readings were subsequently recorded until the system reached to the equilibrium again, at which point a further aliquot of n-octanol was added. This cycle was repeated for at least five additions of n-octanol. The computer program calculates the partition coefficient for each n-octano addition. Finally, a mean partition coefficient value and standard deviation were calculated.

RESULTS

In this work, compounds 1-phenyl-2-ethyl-3-hydroxypyridin-4-one, 1-(4-metylphenyl)-2-ethyl-3-hydroxypyridin-4-one, 1-(4-methoxyphenyl)-2-ethyl-3-hydroxypyridin-4-one and 1-(4-nitrophenyl)-2-ethyl-3-hydroxypyridin-4-one were synthesized by the following methodology as described by Rai and co-workers (25) (Fig. 5). The commercially available 2-ethyl-3-hydroxypyran-4-one (ethyl maltol), 1 was benzylated in 90% aqueous methanol to give compound 2 as a white crystalline solid. Heating compound 2 with aryl amines in aqueous ethanol, in a thick-walled sealed glass tube at 150-160 ^{o}C gave the benzylated aryls compounds 3a-d which were isolated as the free-bases. Finally, the benzyl protecting group was removed by hydroge-nation under acidic conditions to give the corresponding 3-hydroxypyridin-4-ones compounds 4a-d as HCl salt in good yield.

Identification and structural elucidation of ligands were achieved by ^{1}HNMR, IR, elemental analysis, mass spectra and through physical experiments:

Compound 2 (Fig. 5).

^{1}H NMR (DMSO-d_6): δ 1.10 (t, 3H, 2-CH_2-CH_3), 2.50 (q, 2H, 2-CH_2-CH_3), 5.10 (s, 2H, O-CH_2-Ph), 6.40 (d, 1H, 5-H (pyranone ring)), 7.23-7.48 (m, 5H, Ph), 8.05 (d, 1H, 6-H (pyranone ring)).

MS (EI): m/z = 230 (M^+), 201 (M-C_2H_5), 139 (M- CH_2Ph).

IR (KBr): 1640 (C=O), 1578 (C=C) cm^{-1}

Anal. Calcd for $C_{14}H_{14}O_3$: C, 73.06; H, 6.08%. Found: C, 72.91; H, 6.10%.

Compound 3a (Fig. 5).

^{1}H NMR ($CDCl_3$): δ1.20 (t, 3H, 2-CH_2-CH_3), 2.91 (q, 2H, 2-CH_2-CH_3), 5.18 (s, 2H, O-CH_2-Ph), 6.45 (d, 1H, 5-H (pyridinone ring)), 7.11-7.80 (m, 11H, O-CH_2-Ph, N-Ph & 6-H (pyridinone ring)).
MS (EI): m/z = 305 (M^+), 276 (M-C_2H_5), 228 (M- Ph), 214 (M- CH_2Ph)), 137 (M- Ph, -CH_2Ph).
IR (KBr): 1630 (C=O), 1580 (C=C), 1300 (C-N) cm^{-1}.
Anal. Calcd for $C_{20}H_{19}NO_2$: C, 78.70; H, 6.23; N, 4.59%. Found: C, 78.85; H, 6.25; N, 4.61%.

Compound 4a (Fig. 5).

^{1}H NMR (DMSO-d_6): δ 1.28 (t, 3H, 2-CH_2-CH_3), 3.0 (q, 2H, 2-CH_2-CH_3), 5.2-5-8 (br, 1H, 3-OH), 6.54 (d, 1H, 5-H), 7.25-7.75 (m, 6H, N-Ph & 6-H (pyridinone ring)).
MS (EI): m/z = 215 (M-HCl), 214 (M-H, HCl), 138 (M-HCl, Ph).
MS (EI)): m/z = 139 (M), IR (KBr): 3200 (OH), 1635 (C=O, for free base) cm^{-1}.
Anal. Calcd for $C_{13}H_{14}NO_2Cl$: C, 62.06; H, 5.57; N, 5.57; Cl, 14.09%. Found: C, 62.21; H, 5.59; N, 5.55; Cl, 14.04%.

Compound 3b (Fig. 5).

^{1}H NMR ($CDCl_3$): δ 1.15 (t, 3H, 2-CH_2-CH_3), 2.60 (s, 3H, N-C_6H_4-CH_3), 2.85 (q, 2H, 2-CH_2-CH_3), 5.10 (s, 2H, O-CH_2-Ph), 6.35 (d, 1H, 5-H ((pyridinone ring)), 7.25-7.8 (m, 10H, O-CH_2-Ph, N-C_6H_4-CH_3 & 6-H (pyridinone ring)).

MS (EI): m/z = 319 (M^+), 304 (M-CH_3), 290 (M-C_2H_5), 228 (M- CH_2Ph).
IR (KBr): 1635 (C=O), 1587 (C=C), 1300 (C-N) cm^{-1}.
Anal. Calcd for $C_{21}H_{21}NO_2$: C, 79.01; H,6.58; N,4.39%. Found: C, 79.30; H, 6.55; N, 4.42%.

Compound 4b (Fig. 5).

^{1}H NMR (DMSO-d_6): δ 1.20 (t, 3H, 2-CH_2-CH_3), 2.70 (s, 3H, N-C_6H_4-CH_3), 3.05 (q, 2H, 2-CH_2-CH_3), 4.2-5-1 (br, 1H, 3-OH), 6.60 (d, 1H, 5-H(pyridinone ring)), 7.2-7.6 (d, 3H, N-C_6H_4-CH_3 (H-*ortho* to the methyl group) & 6-H (pyridinone ring)). 7.8 (d, 2H, N-C_6H_4-CH_3 (H-*meta* to the methyl group)
MS (EI): m/z = 229 (M-HCl), 228 (M-H, HCl), 214 (M- HCl, -CH_3), 138 (M-HCl, CH_3-Ph).
IR (KBr): 3200 (OH), 1640 (C=O,for free base) cm^{-1}.
Anal. Calcd for $C_{14}H_{16}NO_2Cl$: C, 63.31; H, 6.02; N, 5.27; Cl, 13.35 %. Found: C, 63.11; H, 6.04; N, 5.25; Cl, 13.40%.

Compound 3c (Fig. 5).

^{1}H NMR ($CDCl_3$): δ 1.20 (t, 3H, 2-CH_2-CH_3), 2.90 (q, 2H, 2-CH_2-CH_3), 3.84 (s, 3H, -OCH_3), 5.15 (s, 2H, O-CH_2-Ph), 6.36 (d, 1H, 5-H ((pyridinone ring)), 7.25-7.9 (m, 10H, O-CH_2-Ph, N-C_6H_4-OCH_3 & 6-H (pyridinone ring)).
MS (EI): m/z = 335 (M^+), 306 (M-C_2H_5), 304 (M-CH_3), 228 (M- $C_6H_4OCH_3$).
IR (KBr): 1630 (C=O), 1580 (C=C), 1305 (C-N) cm^{-1}.
Anal. Calcd for $C_{21}H_{21}NO_3$: C, 75.24; H,6.26; N,4.18%. Found: C, 75.50; H, 6.29; N, 4.16%.

Compound 4c (Fig. 5).

^{1}H NMR (DMSO-d_6): δ 1.24 (t, 3H, 2-CH_2-CH_3), 2.95 (q, 2H, 2-CH_2-CH_3), 3.90 (s, 3H, O-CH_3), 5.1-5-9 (br, 1H, 3-OH), 6.54 (d, 1H, 5-H(pyridinone ring)), 7.3-8.0 (m, 5H, N-C_6H_4-OCH_3 & 6-H (pyridinone ring)).
MS (EI): m/z = 245 (M-HCl), 244 (M-H, HCl), 214 (M-HCl, -OCH_3), 138 (M-HCl, -$C_6H_4OCH_3$).
IR (KBr): 3200 (OH), 1635 (C=O, for free base), 1580 (C=C) cm^{-1}.
Anal. Calcd for $C_{14}H_{14}NO_3Cl$: C, 59.71; H, 5.68; N, 4.97; Cl, 12.59 %. Found : C, 59.53; H, 5.66; N, 4.99; Cl, 12.63%.

Compound 3d (Fig. 5).

^{1}H NMR ($CDCl_3$): δ 1.15 (t, 3H, 2-CH_2-CH_3), 2.90 (q, 2H, 2-CH_2-CH_3), 5.05 (s, 2H, O-CH_2-Ph), 6.30 (d, 1H, 5-H ((pyridinone ring)), 7.26-8.2 (m, 10H, O- CH_2-Ph, N-C_6H_4-NO_2 & 6-H (pyridinone ring)).
MS (EI): m/z = 350 (M^+), 321 (M-C_2H_5), 304 (M-NO_2), 259 (M- CH_2Ph).
CH_2-Ph, N-C_6H_4-NO_2 & 6-H (pyridinone ring)

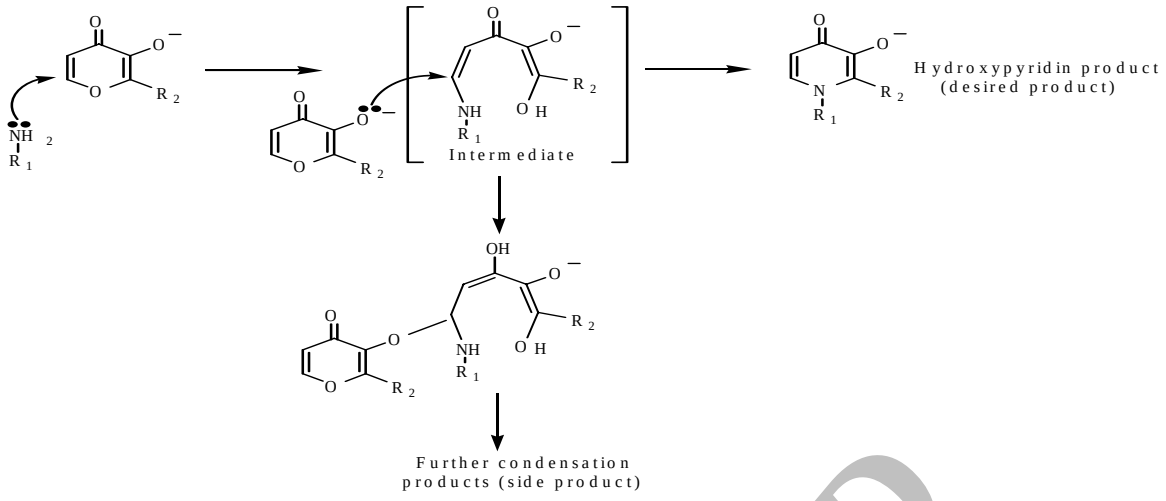

Fig. 6. A possible condensation product in the synthesis of bidentate pyridin-4-ones from reaction of unprotected maltol (ethyl maltol) with primary amines (under basic conditions)

MS (EI): m/z = 350 (M^+), 321 (M-C_2H_5), 304 (M-NO_2), 259 (M- CH_2Ph).
IR (KBr): 1633 (C=O), 1585 (C=C), 1300 (C-N) cm^{-1}.
Anal. Calcd for $C_{20}H_{18}N_2O_4$: C, 68.59; H, 5.14; N,8.00%. Found: C, 68.80; H, 5.16; N, 7.97 %.

Compound 4d (Fig. 5).

^{1}H NMR (DMSO-d_6): δ 1.25 (t, 3H, 2-CH_2-CH_3), 3.05 (q, 2H, 2-CH_2-CH_3), 5.2-6.0 (br, 1H, 3-OH), 6.55 (d, 1H, 5-H (pyridinone ring)), 7.60 (d, 1H, 6-H (pyridinone ring), 7.80 (d, 2H, H-*meta* to the nitro group), 8.36 (d, 2H, H-*ortho* to the nitro group).
MS (EI): m/z = 260 (M-HCl), 259 (M-H, HCl), 231 (M-HCl, -C_2H_5). 213 (M- HCl, -H, -OCH_3)
IR (KBr): 3200 (OH), 1632 (C=O, for free base), 1578 (C=C) cm^{-1}.
Anal. Calcd for $C_{13}H_{13}N_2O_4Cl$: C, 52.64; H, 4.38; N, 9.44; Cl, 11.95 %. Found: C, 52.75; H, 4.37; N, 4.41; Cl, 11.99%

DISCUSSION

The 3,4-HPO ligands are synthesized from the 2-ethyl-3-hydroxypyran-4-one (ethyl maltol, 1) in three steps through protection of hydroxyl group. The protected compound is then reacted with an aryl amine $ArNH_2$ to give desired N-aryl pyridin-4-ones (Fig. 5) (24). Although the 3-hydroxy substituents of 3-hydroxypyran-4-ones can also be protected by methyl ether formation, the corresponding 3-methoxy-2-ethyl-4-pyrone (methoxy ethyl maltol) is oil which is less convenient to work with than the crystalline 3-benzyloxy-2-ethyl-4-pyron (benzyl ethyl maltol). Furthermore, the benzyl protecting group can be removed by hydrogenation under acidic, neutral or basic conditions. For these reasons the benzyl group was selected herein.

The conversion of pyran-4-one to pyridin-4-one 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 pyran-4-one leads to formation of pyridin-4-one with the loss of a water molecule (9). The protection of the 3-hydroxyl function proved to be essential since under the basic conditions employed in the amination reaction it is likely that the unprotect-ed hydroxyl group undergoes a michael-type reaction with intermediates formed during the amination step (Fig. 6). Further condensation products lead to significant consumption of starting material and therefore influence the overall yield.

Conversion of maltol with aryl amines can be achieved without protection of the 3-hydroxyl group in acidic conditions (26) (Fig.7).

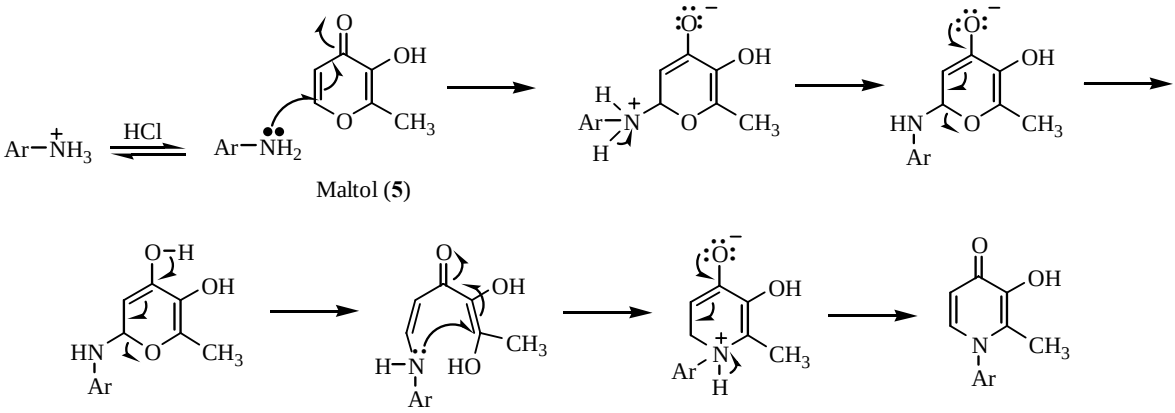

Fig. 7. Formation of 1-aryl-3- hydroxypyridin-4-ones from reaction of unprotected maltol with aryl amines under acidic conditions

Under the acidic conditions employed in the amination reaction, it is unlikely that the unprotected hydroxyl function could undergo a Michael-type reaction with intermediates formed during the amination step. It should be noted that, in acidic condition, aryl amines, unlike alkyl amines, are not completely protonated and a small fraction of amine is as an un-protonated specious. The nitrogen atom of this specious would be sufficiently nucleophilic to explain attack at $C_{(6)}$ [or $C_{(2)}$] of the maltol (Fig. 7).

This investigation, prompted us to attempt a direct one-step reaction of ethyl maltol (1) (instead of maltol) with aniline in dilute hydrochloric acid, which consequently resulted in yield of less than 10% (the yield for reaction of maltol with aniline was 22%) (Fig. 8). This may be attributed to the introduction of a bigger substituted group (namely ethyl group) at 2-position of pyranone ring providing a steric barrier to ring closure resulting in high yields of side products. In order to improve the yield, the three-step reaction was adopted (Fig. 5). The amination step with benzylated ethyl maltol was accomplished under two different conditions, either at normal reflux conditions or at elevated temperatures. Unfortunately, a reaction period of 72 h at normal reflux resulted in low yields again (<20%). In contrast, the reaction of benzyl protected ethyl maltol with related aryl amines in a thick-walled sealed glass tube at 150-160 ^{o}C for 24 h afford the desired benzylated 2-ethyl-3-benzyloxypyridin-4-ones compounds 3a-d in good yields (25-41%).

Table 3. The partition coefficient values (K_{part}) of ligands (4a-d) and their corresponding iron (III) complexes between n-octanol and MOPS buffer at pH 7.4. Number of determinations = 6

Ligand	K_{part} of Ligand	K_{part} of Fe-Complex
4a	18.2 ± 0.20	223.8
4b	30.0 ± 0.40	765.1
4c	25.2 ± 0.40	513.0
4d	7.1 ± 0.20	22.1

The K_{part} values of ligands: The partition coefficient of the free ligands and their iron(III) complexes between an aqueous phase buffered at pH 7.4 and 1-octanol are presented in Table 3. The K_{part} values of bidentate ligands were determined by using the filter probe method (24), and the theoritical partition coefficients of their corresponding Fe-complexes (K_{part}Fe-complex) were calculated from equation1 (9) (Table 3). log K_{part}Fe-complex = -2.46 log K_{part}Ligand- 0.75 (Eq. 1)

In general, as expected the introduction of a more hydrophobic substituted group on the heterocyclic nitrogen results in an increase in the K_{part} values of both the ligands and the iron complexes. In previous studies, we have shown that in most cases iron(III) complexes are more hydrophilic than their corresponding free ligands. However, this trend did not hold for those compounds which have K_{part} values greater than 3 (i.e., compounds 4a-d). Among the ligands, compound 4b and compound 4d possess the highest and the lowest K_{part} values respectively, and not surprisingly they form

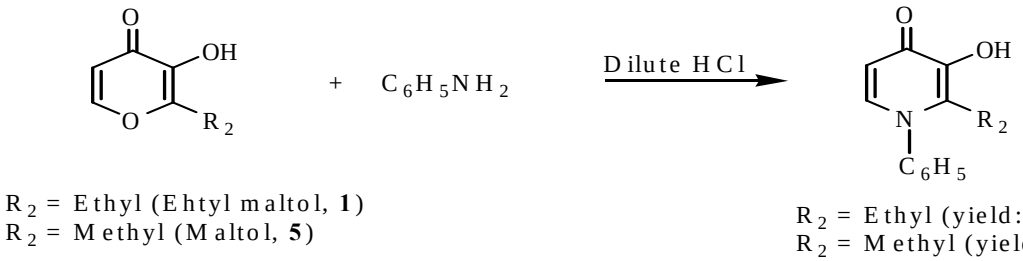

Fig. 8. Synthesis of 1-phenyl-3-hydroxypyridin-4-one using unprotected maltol and ethyl maltol *via* single step synthetic pathway**.**

the most hydrophobic and hydrophilic iron(III) complexes respectively.

CONCLUSIONS

In this study, four derivatives of 3-hydroxypyridin-4-ones (compounds 4a-d) were synthe-sized *via* a three-step synthetic pathway. Identification and structural elucidation of these ligands were achieved by ^{1}HNMR, IR, elemental analysis and mass spectra. The K_{part} values of the compounds were also determined. The results showed that the K_{part} values of iron(III) complexes are greater than the K_{part} values of their corresponding free ligands.

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