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Original article

Hexadentate 3-hydroxypyridin-4-ones with high iron(III) affinity: Design, synthesis and inhibition on methicillin resistant *Staphylococcus aureus* and *Pseudomonas strains*

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ABSTRACT

A range of hexadentate 3-hydroxypyridin-4-ones have been synthesized. These compounds were found to possess a high affinity for iron(III), with $\log K_1$ values of about 34 and pFe values over 30. Antimicrobial assays indicated that they can inhibit the growth of three clinical isolates of methicillin resistant *Staphylococcus aureus* (MRSA) and three clinical isolates of *Pseudomonas*, suggesting that hexadentate 3-hydroxypyridin-4-ones have potential application in the treatment of wound infections.

1. Introduction

Antibiotic-resistant bacterial infections are becoming more and more common in the clinical setting [1–3]. After urinary tract infections, wound infections are probably the next most commonly treated condition, accounting for 10–16 % of all nosocomial infections [4,5]. The condition can range from merely superficial, as seen in cases of cellulitis, to severe infections such as staphylococcal scalded skin syndrome. In the United Kingdom, up to 10% of all hospital admissions are related to wound infections, prolonging hospital stay by as much as 6.5 days per patient [6]. It is estimated that this costs the National Health Service (NHS) approximately one billion pounds per year [7]. Therefore, the economic and social burden resulting from the mismanagement of chronic wound conditions is huge. Consequently, there is an urgent need for the development of novel types of antimicrobial agents targeting unique mechanisms and pathways.

Wound infections involve a complex interaction between host, pathogen and the environment [8]. Infected wounds are considered to be those with ~10^5 colony-forming units (CFUs) per gram of viable tissue [9]. Therefore, understanding the molecular mechanisms involved in these human–microorganism interactions is crucial. To date, the two main bacteria identified in these infections are *Staphylococcus aureus* and *Pseudomonas aeruginosa*. The overuse and misuse of antibiotics has led to the emergence of the multidrug-resistant bacteria, methicillin resistant *S. aureus* (MRSA), which has been directly associated with severe wound infections and has resulted in both an increase in morbidity and mortality rate. Treatment is problematic, principally because of the elevated levels of bacterial virulence factors and the urgent requirement for novel and alternative treatment protocols.

Abbreviations: hydroxypyridinone, HPO; methicillin resistant *Staphylococcus aureus*, MRSA; 1-hydroxybenzotriazole, HOBt; 1,3-dicyclohexylcarbodiimide, DCC; *N*,*N*-dimethylformamide, DMF; high-resolution mass spectra, HRMS; tetramethylsilane, TMS; electrospray ionization mass spectra, ESI-MS; thin-layer chromatography, TLC; Cystine Lactose Electrolyte Deficient, CLED; colony-forming unit, CFU.

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Iron is an essential cofactor of many biochemical pathways in both prokaryotic and eukaryotic species [10]. Hence, decreasing the availability of iron should, in principle, inhibit microbial growth [11]. Many microorganisms have evolved strategies to scavenge and absorb iron from the environment by the production and secretion of siderophores, which possess a high affinity and selectivity for iron(III) [12]. Thus, *S. aureus* has been demonstrated to use two hydroxycarboxylate-type siderophores, staphyloferrin A and staphyloferrin B, via the transporters Hts and Sir, respectively, to access the transferrin iron pool [13,14]. Such uptake can be interrupted by the introduction of high-affinity iron-selective chelating agents [15]. To be effective the iron affinity of these agents must be extraordinarily high, so that they can efficiently out-compete the native siderophores for iron. Once an iron complex is formed with high affinity hexadentate hydroxypyridinone, siderophores will be unable to scavenge this iron at measurable rates [12]. Most siderophores are structured around one of three types of chelator, namely hydroxamate, catecholate and α-hydroxycarboxylate. Thus, chelators designed for an antimicrobial purpose should ideally not contain these structures, otherwise the iron–chelator complex may well be able to supply iron to the microorganism via iron-siderophore transporters [16]. Recently, we have demonstrated that hexadentate 3-hydroxypyridin-4-ones (HPO) can inhibit the growth of both Gram-positive and Gram-negative bacteria [17–19]. The hexadentate hydroxypyridinones developed in these studies have a different structure from all known siderophores, as the chelating moiety is a hydroxypyridinone, so, unlike ferroxamine, the iron complex is predicted not to gain access to the bacteria by receptor-mediated transport.

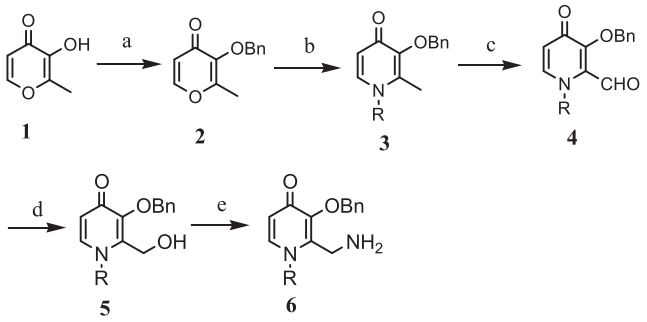
A number of 'Trojan Horse' antibiotics have incorporated 3-hydroxypyridin-4-one moieties [20–22], with the goal of such conjugates utilising the monocatecholato outer membrane carriers, Cir and Fiu [22,23]. These carriers probably utilise the 3:1 (ligand:iron) complex [24], which has a net negative charge in contrast to the iron(III)-hexadentat hydroxypyridinones described in this work. The bidentate ligand-containing conjugates form a range of complexes, including the 1:1, 2:1 and 3:1 ligand:iron species; however at the low concentrations utilised, the 1:1 complex is likely to be the dominant species [24] and this is the species transported by the monocatecholate carriers, Cir and Fiu. These bidentate complexes will bear a net positive charge, in contrast to the iron(III)-hexadentate hydroxypyridinones described in this work, where the iron(III) is completely complexed by the hydroxypyridinone rings and the resulting complex has a net charge of zero [17,25].

Herein we describe the synthesis of a range of hexadentate 3-hydroxypyridin-4-ones with high affinity for iron(III) and report their antimicrobial activity against MRSA and *P. aeruginosa* isolates.

2. Chemistry

2.1. Synthesis of benzyl protected bidentate ligands (*6*)

Hexadentate ligands can be formed by conjugating three bidentate ligands onto a suitable tripodal backbone. However, in order for the ligand to adopt the correct geometry for iron(III) binding, it is essential that the backbone be connected to the ring at the ortho position relative to one of the chelating oxygen anions [26], namely position-2 or -5 on the pyridinone ring. In this work, we firstly synthesized a range of benzyl protected bidentate HPOs containing a free amino group at position-2 (Scheme 1). The benzylation of the starting material, 3-hydroxy-2-methyl-4*H*-pyran-4-one (**1**) was achieved by the treatment with benzyl chloride to obtain **2** in 86% yield. Treatment of **2** with primary amines under basic conditions provided the HPO derivatives **3** in good yield



Scheme 1. Reagents and conditions: (a) BnCl, NaOH, MeOH, reflux, 8 h; (b) RNH_2, NaOH, CH_3OH/H_2O, reflux, 2 h; (c) SeO_2, $CH_3COOH/(CH_3CO)_2O$, 90–100 °C; (d) $NaBH_4$, ethanol, rt,2 h; (e) i) $SOCl_2$, rt, overnight; ii) $NH_3 \cdot H_2O$, CH_3OH, 3–4 h.

(85–90%), which underwent selective oxidation of the methyl group at position-2 with selenium dioxide in acetic anhydride to generate the aldehyde **4** in moderate yield. Reduction of **4** was carried out using sodium borohydride as a reducing agent, producing alcohol **5** in good yield. Compound **5** was treated with thionyl chloride to obtain the corresponding chlorinated product, which was then treated with ammonia in methanol without purification, generating benzyl protected bidentate ligands with a free amino group at position-2 (**6**) in 58–62 % yield.

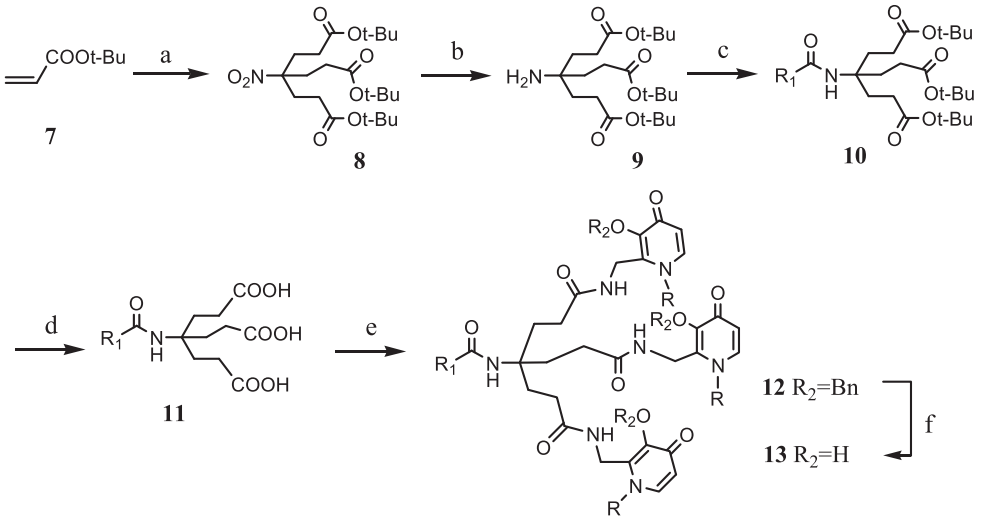
2.2. Synthesis of hexadentate hydroxypyridinones (*13*)

The synthetic route for the synthesis of hexadentate hydroxypyridinones (**13**) is outlined in Scheme 2. Firstly we synthesized tripodal compounds (triacids **11**) starting from tert-butyl acrylate (**7**). The reaction of **7** and nitromethane in the presence of tetrabutylammonium bromide provided Michael addition product **8**, which was reduced by hydrogenation in the presence of Raney nickel to produce amine **9** [27]. Compounds **10a** and **10b** were prepared by treating **9** with acetyl chloride and butyryl chloride, respectively, in over 80% yields. Compound **10c** was prepared by coupling 2-(2-(2-methoxyethoxy)ethoxy)acetic acid to amine **9** in the presence of 1-hydroxybenzotriazole (HOBt) and 1,3-dicyclohexylcarbodiimide (DCC) in 82% yield. Treatment of **10** with formic acid provided the triacids **11** in quantitative yield. Amines **6** were then conjugated to the triacids **11** via amide bonds in the presence of HOBt and DCC in *N*,*N*-dimethylformamide (DMF) at room temperature, providing the protected hexadentate ligand **12** in moderate to good yields. Deprotection of benzyl groups on **12** was achieved by hydrogenation in the presence of palladium/charcoal, generating the hexadentate chelators **13** as hydrochloride salts in excellent yield. All the hexadentate chelators were fully characterized by ^{1}H NMR, ^{13}C NMR, mass spectrometry and high-resolution mass spectrometry. In comparison with our previously reported hexadentate HPOs [17,18,25], structures of the hexadentate HPOs synthesized in this article could be changed by introducing different substituents on the side chain and position-1 on pyridinone ring. Thus, a large number of hexadentate HPO analogues possessing different partition coefficients can be prepared, which is useful in the establishment of a structure–activity relationship. Furthermore, the present synthetic method has the advantages of a shorter synthetic procedure.

3. Results and discussion

3.1. Physico-chemical properties of hexadentate chelators *13*

In order to demonstrate the iron(III) affinity of hexadentate



Scheme 2. Reagents and conditions: (a) CH_3NO_2, $N^+(n\text{-}Bu)_4Br^-$; (b) H_2, Raney Ni; (c) R_1COCl, Et_3N, DCM; or R_1COOH, DCC, HOBt, DMF; (d) HCOOH; (e) **6**, HOBt, DCC, DMF, rt, 2days; (f) H_2, 5%Pd–C, methanol, rt, 8–10 h。.

chelator **13**, we selected a representative group of the hexadentate chelators, and evaluated their pKa values and stability constants for the corresponding iron(III) complexes using an automated titration system [28,29]. The titration data were analysed with pHab [30].

3.1.1. pKa values

The pH dependence of the UV spectra of **13a** (Fig. 1) was recorded between 250 and 390 nm over the pH range 4.0–11.0 for the free ligand. The speciation spectra demonstrate a clear shift in λ_{max} from 280 to 310 nm, which reflects the pH dependence of the ligand ionization equilibrium. The hexadentate ligand **13a** can be considered as a trimer of the corresponding bidentate ligands and therefore it possesses two sets of intrinsic pKa values. Using the spectrophotometric titration method, the pKa values of **13a** obtained from nonlinear least-squares regression analysis were found to be 2.75, 3.26, 3.69, 8.46, 8.88 and 9.32. Of the six pKa values, the three lower values correspond to the 4-oxo function and the higher three correspond to the 3-hydroxyl function. The pKa values of chelators **13c**, **13h** and **13n** were determined using the same method as that for **13a** and in similar fashion, all were found to possess six pKa values (Table 1).

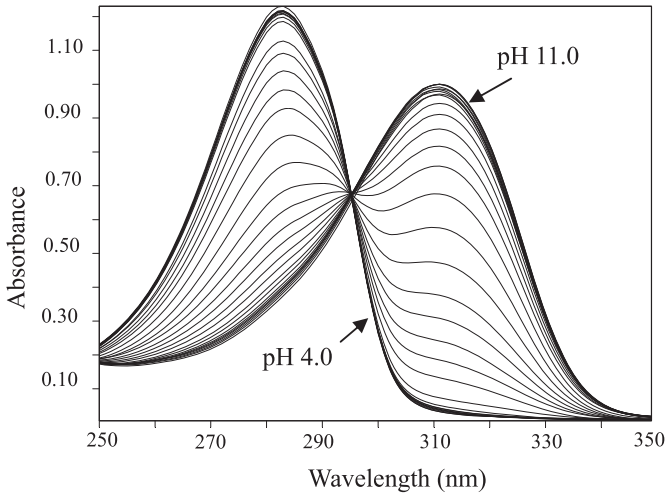


Fig. 1. UV spectra of **13a**. [**13a**] = 38.0 μM, pH was changed from 4.0 to 11.0 by the addition of KOH in 20.0 mL of 0.1 M KCl at 25 °C.

3.1.2. Iron(III) affinity

The stability constant of an iron–ligand complex is one of the key parameters related to the chelation efficacy of a ligand. The log stability constants of the series of chelator-iron(III) complexes were determined by spectrophotometric titration against the hydroxyl anion. The $\log K_1$ values were found to be similar and close to 34 (Table 1). As an example, a series of UV spectra of **13a** in the presence of iron at different pH values is shown in Fig. 2. The pFe^{3+} value, defined as the negative logarithm of concentration of the free iron(III) in solution (when $[Fe^{3+}]_{total} = 10^{-6}$ M; $[Ligand]_{total} = 10^{-5}$ M; pH = 7.4) is a more suitable factor for comparison under physiological conditions than the stability constant, since this parameter takes into account the effect of ligand basicity, denticity, and the degree of protonation. The pFe^{3+} values of **13** (**13a**, **13c**, **13h** and **13n**), calculated using the measured stability constants and pKa values, are all extremely high. These values are close to 30, suggesting that substituent in the 1-position of the pyridinone ring does not appreciably influence the iron(III) affinity. This is because the group at position-1 has less effect on the pKa value of 3-hydroxyl than the group at position-2, and the electron-withdrawing ability does not vary much amongst the introduced substituents.

3.2. Antimicrobial assay

3.2.1. Inhibition on MRSA and Pseudomonas

Three hydroxypyridinones (**13a**, **13l** and **13i**) were selected to investigate the inhibitory effect on three strains of MRSA and three strains of *Pseudomonas* in comparison with deferiprone, a clinically used bidentate iron chelator [31]. The viable cell numbers were determined after incubation of bacteria for 6 and 24 h in the

Table 1
pKa values and iron affinities of hexadentate ligands.

Ligands	pKa	Log K	pFe^{3+}
13a	9.32, 8.88, 8.46, 3.69, 3.26, 2.75	33.95 ± 0.033	30.4
13c	9.4, 8.95, 8.25, 3.71, 3.27, 2.81	34.16 ± 0.04	30.7
13h	9.11, 8.49, 7.83, 3.65, 3.11, 2.55	33.86 ± 0.054	31.4
13n	9.25, 8.76, 8.11, 3.72, 3.20, 2.71	33.99 ± 0.014	30.9.

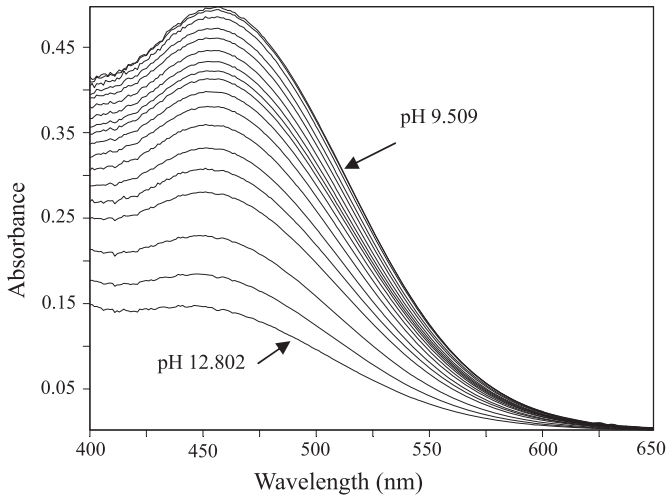


Fig. 2. UV spectra of **13a** with iron(III). [**13a**] = 29.5 μM, [Fe^{3+}] = 26.9 μM with addition of KOH, start in 20.130 mL of 0.1 M KCl at 25 °C, pH from 9.509 to pH 12.802.

presence or absence of iron chelators (**13a**, **13i** and **13l**). **13l** was found to exhibit the strongest inhibitory activity against MRSA1 amongst the tested chelators after both 6 and 24 h incubation (Figs. 3 and 4). At 6 h incubation, hexadentate chelators **13a** and **13i** showed stronger inhibition on MRSA-1 than deferiprone (Fig. 3). At 24 h incubation, **13a** and deferiprone exhibited similar inhibitory activity, while **13i** was much weaker. In the cases of MRSA-2 and MRSA-3, similar results were found, chelator **13l** exhibiting the strongest inhibitory activity throughout. After 24 h incubation, the inhibition rates of **13 l** at a concentration of 400 μg/mL against MRSA-1, MRSA-2 and MRSA-3 were determined to be 96.8%, 95.6% and 97.2%, respectively, while the inhibition rates of deferiprone at the same concentration against MRSA-1, MRSA-2 and MRSA-3 were determined as 80.1%, 83.1% and 87.6%, respectively (Table 2). Overall, the *Pseudomonas* strains were found to be more sensitive to the iron chelators than the MRSA bacterial strains. Surprisingly, at concentrations less than 200 μg/mL, all three compounds (**13a**, **13i** and **13l**) failed to exhibit superior inhibitory activity against the three strains of *Pseudomonas* when compared with deferiprone (Figs. 5 and 6). However, at higher concentrations, these three compounds were found to exhibit a stronger inhibitory effect. After incubation for 24 h, all the three strains of *Pseudomonas* were killed when the concentration of hexadentate chelator (**13a**, **13i** and **13l**) was over 400 μg/mL, while the bacterial inhibition rates of deferiprone at a concentration of 400 μg/mL against *P. aeruginosa*-1, *P. aeruginosa*-2 and *Pseudomonas* sp.-1 were determined to be 93.1%, 92.3% and 92.1%, respectively (Table 3).

Recently, Thompson et al. reported antibacterial activities of iron chelators against common nosocomial pathogens including two *S. aureus* strains and two *P. aeruginosa* strains [10]. Deferiprone was reported to have a MIC value of 256–512 μg/mL against *S. aureus* and *P. aeruginosa* strains in both CAMHB and RPMI 1640 medium, whereas the iron chelator VK28 was reported to have a MIC value of 256 μg/mL against two *S. aureus* strains and >512 μg/mL against two *P. aeruginosa* strains in CAMHB medium. Furthermore VK28 exhibited a stronger antibacterial activity in RPMI 1640 medium with a MIC value of 16–32 μg/mL against *S. aureus* strains and a MIC value of 16 μg/mL against *P. aeruginosa* strains. The different behaviour of deferiprone and VK28 can be attributed to their ready ability to penetrate membranes by simple different [32].

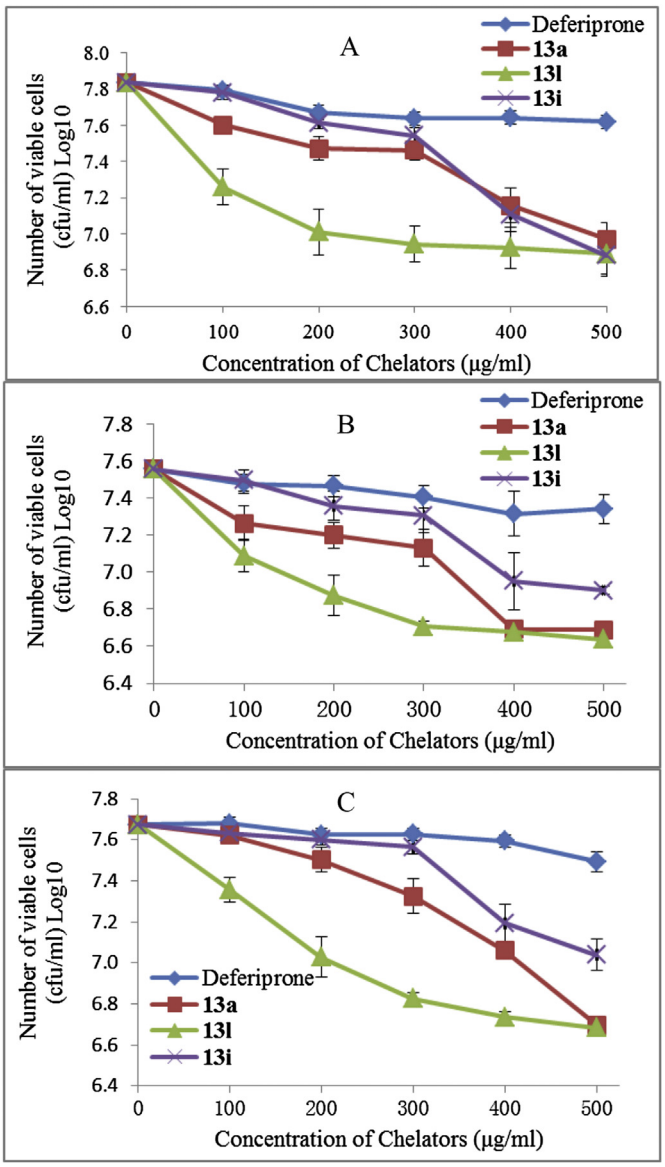


Fig. 3. Antimicrobial effect of chelators (◊, Deferiprone; □, **13a**; Δ, **13l**; ×,**13i**) on three strains of MRSA (Gram-positive microorganism) after 6 h, (A) MRSA-1, (B) MRSA-2, and C) MRSA-3. Results are the mean of three independent experiments performed in duplicate. Error bars represent standard error.

3.2.2. Structure–activity relationship investigation

In order to further explore the structure-antimicrobial activity relationship, a range of hexadentate chelators were evaluated for their inhibitory activity against one strain of MRSA and one strain of *P. aeruginosa* (Fig. 7). Three compounds (**13j**, **13k** and **13l**) which contain hexyl groups at position-1 on the pyridinone rings were found to possess stronger inhibitory activity against MRSA than the other tested compounds, as assessed by the bacterial inhibition rates. In contrast, compounds **13d** and **13e**, which contain ethyl at position-1 on the pyridinone rings, were found to be relatively weak inhibitors of MRSA growth. Significantly, **13j**, **13k** and **13l** are the most hydrophobic amongst the tested compounds (the calculated partition coefficients (ClogP) values [33] are 2.44, 3.83 and 1.97, respectively) (Table 4). Some of the more hydrophilic molecules, for instance **13n** and **13r** also possess appreciable antibacterial activity. Presumably, all these chelators inhibit bacterial growth by scavenging iron in the immediate environment around

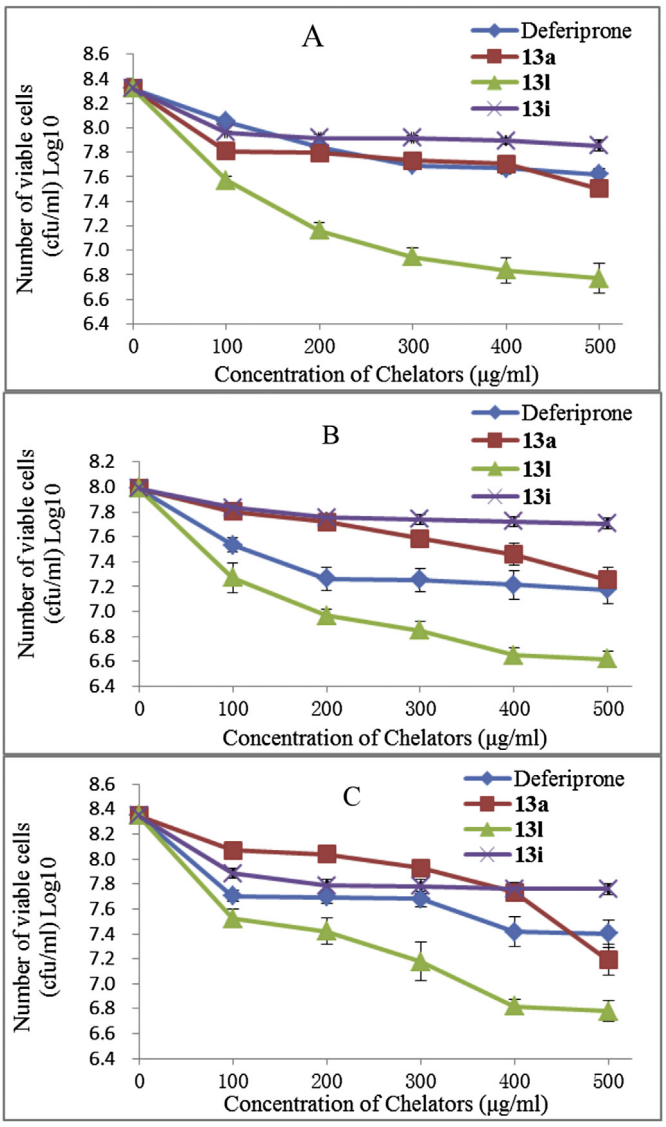


Fig. 4. Antimicrobial effect chelators (◊, Deferiprone; □, **13a**; Δ, **13l**; ×, **13i**) on three strains of MRSA (Gram-positive microorganism) after 24 h, (A) MRSA-1, (B) MRSA-2, and C) MRSA-3. Results are the mean of three independent experiments performed in duplicate. Error bars represent standard error.

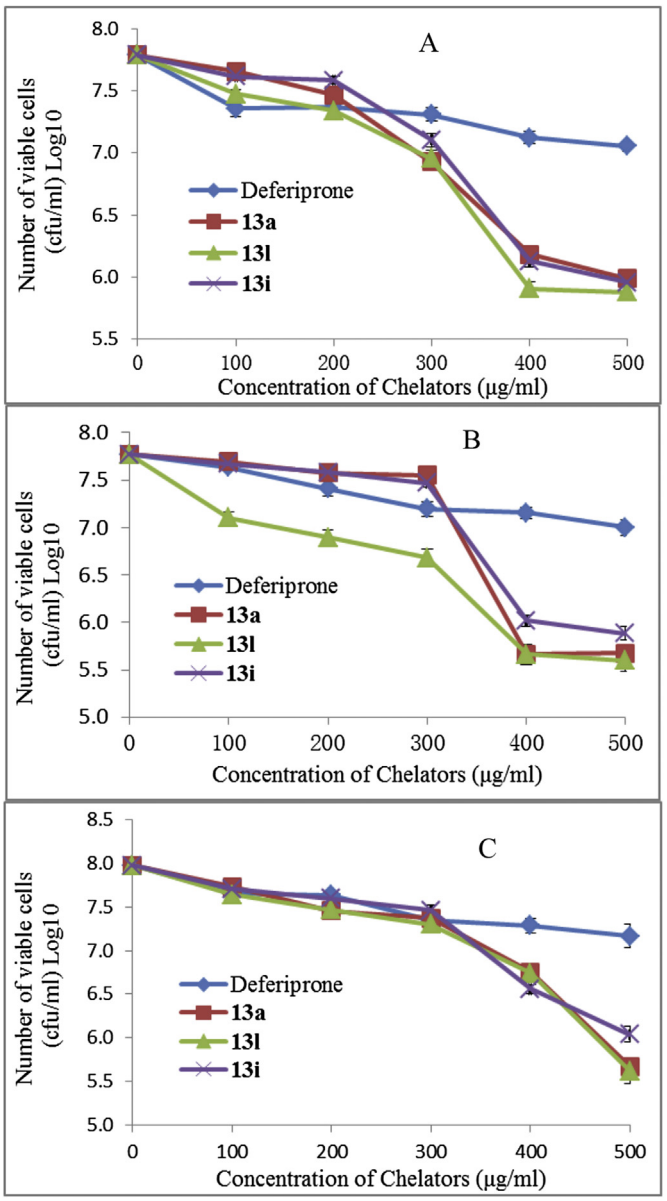


Fig. 5. Antimicrobial effect chelators (◊, Deferiprone; □, **13a**; Δ, **13l**; ×, **13i**) on three strains of *Pseudomonas* (Gram-negative microorganism) after 6 h, (A) P. aeruginosa-1, (B) P. aeruginosa-2, and C) *Pseudomonas* sp.-1. Results are the mean of three independent experiments performed in duplicate. Error bars represent standard error.

Table 2
Bacterial inhibition rate (%) of iron chelators on MRSA strains after 6 h and 24 h incubation at concentration of 500 μg/ml.

	MRSA-1		MRSA-2		MRSA-3	
	6 h	24 h	6 h	24 h	6 h	24 h
Deferiprone (3.60 mM)	38.8	80.1	37.4	83.1	33.2	87.6
13a (0.62 mM)	85.5	84.4	86.6	80.0	89.4	92.1
13l (0.44 mM)	87.3	96.8	88.1	95.6	89.7	97.2
13i (0.48 mM)	88.0	65.6	78.2	46.9	75.8	74.0

the bacteria. Surprisingly, in the case of *P. aeruginosa*, the two most hydrophobic compounds (**13j**, **13k**) and highly hydrophilic compound (**13f**) were found to be equally effective at inhibiting bacterial growth. After incubation with these chelators at a concentration of 300 μg/mL for 24 h, *P. aeruginosa* was completely killed (Fig. 7B). Compound **13n** with a high hydrophilicity (ClogP value −4.88) was also found to exhibit a relatively strong inhibitory effect on *P. aeruginosa*. The minimum bactericidal concentrations (MBC) and minimum inhibitory concentrations (MIC) of the hexadentate chelators on MRSA and *P. aeruginosa* were determined (Table 5). Both the MBC and MIC data are in agreement with those for structure–activity relationship investigation. **13k** was found to have a lowest MIC value of 384 μg/mL against MRSA, while **13k**, **13j** and **13f** possess the lowest MIC value of 256 μg/mL against *P. aeruginosa*.

4. Conclusion

Iron chelators have been used in treatment of iron overload disorders associated thalassaemia and haemochromatosis, in cosmetic therapy and in the treatment of nail infections. The iron chelators, deferiprone and deferasirox, which were primarily designed to treat systemic iron overload, also exert a deleterious

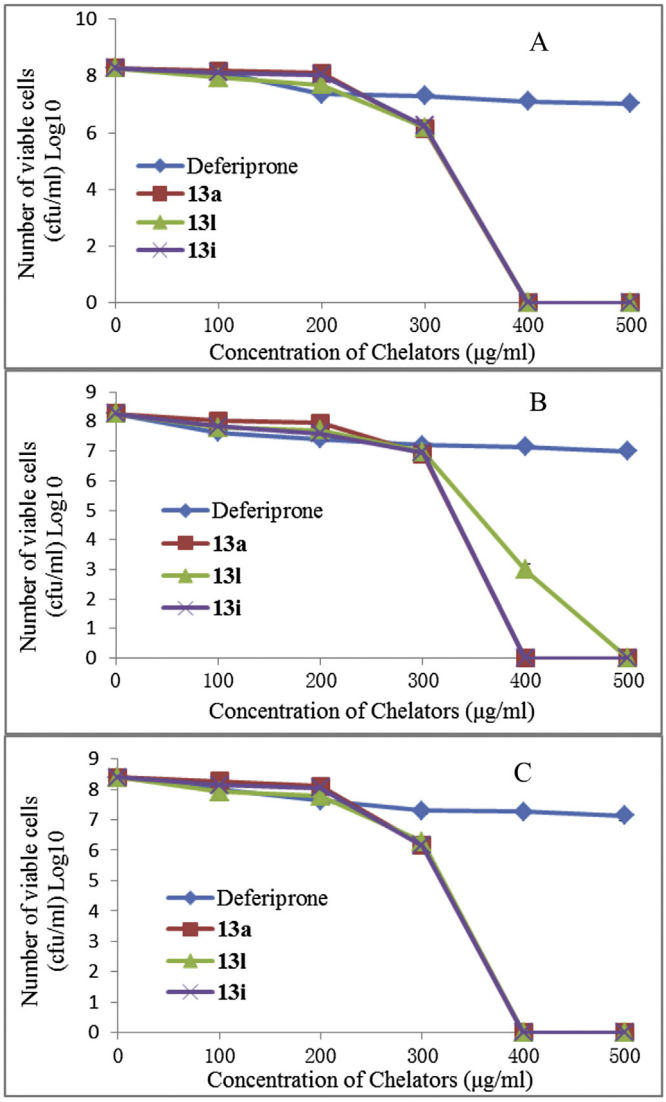


Fig. 6. Antimicrobial effect chelators (◊, Deferiprone; □, **13a**; Δ, **13l**; ×, **13i**) on three strains of *Pseudomonas* (Gram-negative microorganism) after 24 h, (A) *P. aeruginosa*-1, (B) P.aeruginosa-2, and C) *Pseudomonas* sp.-1. Results are the mean of three independent experiments performed in duplicate. Error bars represent standard error.

Table 3
Bacterial inhibition rate (%) of iron chelators on *Pseudomonas* strains after 6 h and 24 h incubation at concentration of 400 μg/ml.

	P. aeruginosa-1		P. aeruginosa-2		*Pseudomonas* sp.-1	
	6 h	24 h	6 h	24 h	6 h	24 h
Deferiprone (2.88 mM)	78.1	93.1	75.4	92.3	78.7	92.1
13a (0.50 mM)	97.5	100	99.2	100	94.1	100
13l (0.35 mM)	98.7	100	99.2	100	94.2	100
13i (0.38 mM)	97.8	100	98.2	100	96.1	100

effect on fungal growth both in vitro and in animal models [34]. Iron chelators were also found to exhibit potential in the treatment of mucormycosis and invasive aspergillosis [34]. Thus, the inhibition of microorganism iron uptake represents a promising alternative area of research for the design of new antibacterial and antifungal agents. The hexadentate 3-hydroxypyridin-4-ones described in this study were found to possess moderate

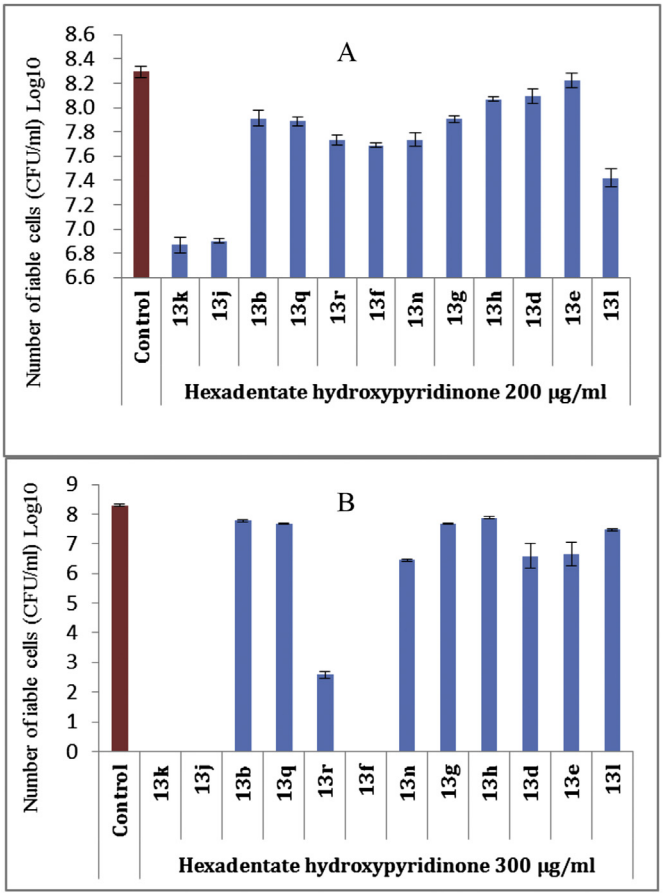


Fig. 7. Antimicrobial effect of chelators on (A) MRSA at 200 μg/mL after 24 h, and (B) *P. aeruginosa* at 300 μg/mL after 24 h. Results are the mean of three independent experiments performed in duplicate. Error bars represent standard error.

inhibitory activity against the growth of both Gram-positive bacteria MRSA and Gram-negative bacteria *P. aeruginosa*. Lipophilicity of iron chelator favours an increase of the inhibitory effect against MRSA. Compounds **13j** and **13k** possess strongest inhibitory activity against MRSA. Compounds **13f**, **13j** and **13k** possess strongest inhibitory activity against *P. aeruginosa*. These hexadentate chelators have potential as antimicrobial agents, particularly in the treatment of external infections, such as those located in wounds.

5. Experimental section

5.1. General

All chemicals were of AR grade and used without any further purification. Melting points were determined using an SGW X-4A Digital Melting Point Apparatus and were uncorrected. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker Avance 500 spectrometer with TMS as an internal standard. Electrospray ionization (ESI) mass spectra were obtained by infusing samples into an LCQ Deca XP ion trap instrument. High resolution mass spectra (HRMS) were determined on Waters QTOF micro.

5.2. Chemistry

5.2.1. General procedure for preparation of *3*

A mixture of **2** (5 g, 23.15 mmol), amine RNH_2 (25.5 mmol), sodium hydroxide (2 g, 50 mmol) in methanol/water (20 mL/

Table 4
Investigation of antimicrobial activity-structure of hexadentate chelators.[a]

Compounds	R_1 (substituents at side chain)	R (substituents at position-1 in pyridinone ring)	Inhibition rate for MRSA (%)	Inhibition rate for *P. aeruginosa* (%)	ClogP
13b	n-C_3H_7	CH_3	56.9	70.5	−3.51
13d	CH_3	C_2H_5	35.3	87.9	−3.78
13e	n-C_3H_7	C_2H_5	12.7	88.4	−2.38
13f	$CH_3O(CH_2CH_2O)_2CH_2$	C_2H_5	76.0	100	−4.22
13g	CH_3	n-C_4H_9	60.3	76.8	−0.59
13h	n-C_3H_7	n-C_4H_9	42.2	63.3	0.80
13j	CH_3	n-C_6H_{13}	96.1	100	2.44
13k	n-C_3H_7	n-C_6H_{13}	96.1	100	3.83
13l	$CH_3O(CH_2CH_2O)_2CH_2$	n-C_6H_{13}	85.8	85.5	1.97
13n	n-C_3H_7	CH_2CH_2OH	71.6	98.6	−4.88
13q	n-C_3H_7	$CH_2CH_2OCH_3$	61.3	77.3	−3.56
13r	$CH_3O(CH_2CH_2O)_2CH_2$	$CH_2CH_2OCH_3$	72.5	100	−4.88

[a] Bacterial inhibition rates were determined after incubation for 24 h. In cases of MRSA, the concentration of iron chelator was 200 μg/mL. In cases of *P. aeruginosa*, the concentration of iron chelator was 300 μg/mL.

Table 5
MBC and MIC values of hexadentate chelators (μg/mL, mM in parentheses).[a]

Compounds	MRSA		*P. aeruginosa*	
	MBC	MIC	MBC	MIC
13b	>500 (0.60)	768 (0.92)	500 (0.60)	512 (0.61)
13d	>500 (0.59)	>768 (0.90)	>500 (0.59)	768 (0.90)
13e	>500 (0.57)	>768 (0.88)	500 (0.57)	512 (0.58)
13f	>500 (0.52)	512 (0.53)	300 (0.31)	256 (0.26)
13g	>500 (0.54)	>768 (0.82)	>500 (0.54)	512 (0.55)
13h	>500 (0.52)	>768 (0.80)	>500 (0.52)	768 (0.80)
13j	500 (0.49)	512 (0.50)	300 (0.29)	256 (0.25)
13k	400 (0.38)	384 (0.37)	300 (0.29)	256 (0.24)
13n	>500 (0.54)	768 (0.83)	400 (0.43)	384 (0.41)
13q	>500 (0.52)	>768 (0.79)	500 (0.52)	512 (0.53)
13r	>500 (0.47)	768 (0.73)	400 (0.38)	384 (0.36)
13a	>500 (0.62)	>768 (0.95)	400 (0.50)	512 (0.63)
13l	>500 (0.44)	512 (0.45)	400 (0.35)	512 (0.45)
13i	>500 (0.48)	>768 (0.73)	400 (0.38)	512 (0.49)
Deferiprone	>500 (3.60)	>768 (5.53)	>500 (3.60)	>768 (5.53)

[a] Concentrations used for MBC are: 100, 200, 300, 400 and 500 (μg/mL); concentrations used for MIC are: 192, 256, 348, 512 and 768 (μg/mL).

20 mL) was refluxed. The reaction was monitored by TLC. After completion of the reaction (about 2 h), the reactant was concentrated under reduced pressure to about half volume. Extracted with dichloromethane (3 × 40 mL), the combined organic layers were washed with brine twice and dried over anhydrous sodium sulphate. After removal of the solvent, the crude product **3** was obtained as a brown oil.

*5.2.1.1. 3-(Benzyloxy)-1,2-dimethylpyridin-4(1H)-one (**3a**).* Yield: 85% (4.50 g, 19.67 mmol). ^{1}H NMR ($CDCl_3$) δ 2.08 (s, 3H, CH_3), 3.48 (s, 3H, NCH_3), 5.17 (s, 2H, $PhCH_2$), 6.32 (d, J = 7.5 Hz, 1H, Pyridinone C5-H), 7.17 (d, J = 7.5 Hz, 1H, Pyridinone C6-H), 7.28–7.39 (m, 5H, Ar). ESI-MS: m/z 230 ($[M+H]^+$).

*5.2.1.2. 3-(Benzyloxy)-1-ethyl-2-methylpyridin-4(1H)-one (**3b**).* Yield: 87% (4.89 g, 20.14 mmol). ^{1}H NMR ($CDCl_3$) δ 1.10 (t, J = 7.5 Hz, 3H, CH_3), 1.95 (s, 3H, CH_3), 3.65 (q, J = 7.5 Hz, CH_2), 5.03 (s, 2H, $PhCH_2$), 6.25 (d, J = 7.5 Hz, Pyridinone C5-H), 7.10 (d, J = 7.5 Hz, Pyridinone C6-H), 7.17-7.27 (m, 5H, Ar). ESI-MS: m/z 244 ($[M+H]^+$).

*5.2.1.3. 3-(Benzyloxy)-1-butyl-2-methylpyridin-4(1H)-one (**3c**).* Yield: 85% (5.34 g, 19.68 mmol). ^{1}H NMR ($CDCl_3$) δ 0.92 (t, J = 7.0 Hz, 3H, CH_3), 1.27 (m, 2H, CH_2), 1.57 (m, 2H, CH_2), 2.06 (s, 3H, CH_3), 3.74 (t, J = 7.5 Hz, 2H, NCH_2), 5.16 (s, 2H, $PhCH_2$), 6.42 (d, J = 7.5 Hz, 1H, Pyridinone C5-H), 7.25 (d, J = 7.5 Hz, 1H, Pyridinone C6-H), 7.29–7.38 (m, 5H, Ar). ESI-MS: m/z 272 ($[M+H]^+$).

*5.2.1.4. 3-(Benzyloxy)-1-hexyl-2-methylpyridin-4(1H)-one (**3d**).* Yield: 88% (6.09 g, 20.37 mmol). ^{1}H NMR ($CDCl_3$) δ 0.89 (t, J = 7.0 Hz, 3H, CH_3), 1.28 (m, 6H, CH_2), 1.60 (m, 2H, CH_2), 2.07 (s, 3H, CH_3), 3.71 (t, J = 7.5 Hz, 2H, NCH_2), 5.22 (s, 2H, $PhCH_2$), 6.41 (d, J = 7.5 Hz, 1H, Pyridinone C5-H), 7.17 (d, J = 7.5 Hz, 1H, Pyridinone C6-H), 7.29–7.41 (m, 5H, Ar). ESI-MS: m/z 300 ($[M+H]^+$).

*5.2.1.5. 3-(Benzyloxy)-1-(2-methoxyethyl)-2-methylpyridin-4(1H)-one (**3e**).* Yield: 85% (5.37 g, 19.67 mmol). ^{1}H NMR ($CDCl_3$) 1.99 (s, 3H, CH_3), 3.14 (s, 3H, OCH_3), 3.39 (t, J = 5.0 Hz, 2H, CH_2), 3.80 (t, J = 5.0 Hz, 2H, CH_2), 5.06 (s, 2H, $PhCH_2$), 6.25 (d, J = 7.5 Hz, 1H, Pyridinone C5-H), 7.26 (d, J = 7.5 Hz, 1H, Pyridinone C6-H), 7.15–7.29 (m, 5H, Ar.) ESI-MS: m/z 274 ($[M+H]^+$).

*5.2.1.6. 3-(Benzyloxy)-1-(2-hydroxyethyl)-2-methylpyridin-4(1H)-one (**3f'**).* Yield: 85% (5.10 g, 19.68 mmol). ^{1}H NMR ($CDCl_3$) δ 2.11 (s, 3H, CH_3), 3.84 (m, 4H, CH_2), 4.95 (s, 2H, $PhCH_2$), 6.15 (d, J = 7.5 Hz, 1H, Pyridinone C5-H), 7.29–7.35 (m, 5H, Ar), 7.38 (d, J = 7.5 Hz, 1H, Pyridinone C6-H). ESI-MS: m/z 260 ($[M+H]^+$).

*5.2.1.7. 3-(Benzyloxy)-1-(2-(benzyloxy)ethyl)-2-methylpyridin-4(1H)-one(**3f**).* To a solution of **3f'** (10 g, 38.6 mmol) in dry THF (80 mL) was added sodium hydride (1.39 g, 57.9 mmol) and benzyl chloride (5.0 g, 40 mmol). The mixture was refluxed for 3 h. Water was added dropwise cautiously to quench the reaction. The reactant was concentrated and then was dissolved in dichloromethane, washed with brine twice and dried over anhydrous sodium sulphate. After removal of the solvent, 12.1 g (34.75 mmol, 90% yield) of crude product **3f** was obtained as a brown solid. ^{1}H NMR ($CDCl_3$) δ 2.07 (s, 3H, CH_3), 3.58 (t, J = 5.0 Hz, 2H, CH_2), 3.92 (t, J = 5.0 Hz, 2H, CH_2), 4.42 (s, 2H, CH_2), 5.19 (s, 2H, $PhCH_2$), 6.38 (d, J = 7.5 Hz, 1H, Pyridinone C5-H), 7.22 (d, J = 7.5 Hz, 1H, Pyridinone C6-H), 7.15–7.40 (m, 10H, Ph). ESI-MS: m/z 350 ($[M+H]^+$).

*5.2.2. General procedure for preparation of **5***

A mixture of **3** (10 mmol), SeO_2 (30 mmol) in acetic acid/acetic anhydride (20 mL/20 mL) was heated at 90–100 °C for 3–4 h. After removal of the solvent, the residue was purified by silica gel column chromatography using ethyl acetate/methanol (50:1 ~ 20:1) as an eluent to provide aldehyde **4** as a brown oil. To a solution of **4** (5 mmol) in ethanol (5 mL) was added $NaBH_4$ (0.138 g, 3.75 mmol). The mixture was stirred at room temperature for 2 h. The solution was concentrated and purified by silica gel column chromatography (CH_2Cl_2/MeOH, 50:1 ~ 10:1) to give product **5** as a white powder.

*5.2.2.1. 3-(Benzyloxy)-2-(hydroxymethyl)-1-methylpyridin-4(1H)-one (**5a**).* 1.50 g (6.1 mmol), yield: 61% (2 steps). ^{1}H NMR ($CDCl_3$) δ 3.73 (s, 3H, NCH_3), 3.85 (s, 2H, OCH_2), 5.16 (s, 2H, $PhCH_2$), 6.19 (d, J = 9.5 Hz, 1H, Pyridinone C5-H), 7.29–7.45 (m, 5H, Ar), 7.63 (d, J = 9.5 Hz, 1H, Pyridinone C6-H). ESI-MS: m/z 246 ($[M+H]^+$).

*5.2.2.2. 3-(Benzyloxy)-1-ethyl-2-(hydroxymethyl)pyridin-4(1H)-one (**5b**).* 1.51 g (5.8 mmol), yield: 58% (2 steps). ^{1}H NMR ($CDCl_3$) δ 1.32 (CH_3), 3.99 (CH_3CH_2), 4.43 (CH_2NH_2), 5.11 (s, 2H, $PhCH_2$), 6.53 (d, J = 8.0 Hz, 1H, Pyridinone C5-H), 7.20 (d, J = 8.0 Hz, 1H, Pyridinone C6-H), 7.27–7.40 (m, 5H, Ar). ESI-MS: m/z 260 ($[M+H]^+$).

*5.2.2.3. 3-(Benzyloxy)-1-butyl-2-(hydroxymethyl)pyridin-4(1H)-one (**5c**).* 1.75 g (6.1 mmol), yield: 61% (2 steps). ^{1}H NMR ($CDCl_3$) δ 0.92 (t, J = 7.5 Hz, 3H, CH_3), 1.30 (m, 2H, CH_2), 1.63 (m, 2H, CH_2), 3.87 (t, J = 7.5 Hz, 2H, NCH_2), 4.37 (s, 2H, CH_2), 5.20 (s, 2H, $PhCH_2$), 6.42 (d, J = 7.5 Hz, 1H, Pyridinone C5-H), 7.16 (d, J = 7.5 Hz, 1H, Pyridinone C6-H), 7.34 (m, 5H, Ar). ESI-MS: m/z 288 ($[M+H]^+$).

*5.2.2.4. 3-(Benzyloxy)-1-hexyl-2-(hydroxymethyl)pyridin-4(1H)-one (**5d**).* 1.96 g (6.2 mmol), yield: 62% (2 steps). ^{1}H NMR ($CDCl_3$) δ 0.88 (t, J = 6.0 Hz, 3H, CH_3), 1.26 (m, 6H, CH_2), 1.63 (m, 2H, CH_2), 3.91 (t, J = 7.5 Hz, 2H, NCH_2), 4.43 (s, 2H, CH_2), 5.07 (s, 2H, $PhCH_2$), 6.44 (d, J = 7.5 Hz, 1H, Pyridinone C5-H), 7.13 (d, J = 7.5 Hz, 1H, Pyridinone C6-H), 7.30 (m, 5H, Ar). ESI-MS: m/z 316 ($[M+H]^+$).

*5.2.2.5. 3-(Benzyloxy)-2-(hydroxymethyl)-1-(2-methoxyethyl)pyridin-4(1H)-one (**5e**).* 1.88 g (6.5 mmol), yield: 65% (2 steps). ^{1}H NMR ($CDCl_3$) δ 3.22 (s, 3H, OCH_3), 3.52 (t, J = 7.0 Hz, 2H, CH_2), 4.13 (t, J = 7.0 Hz, 2H, CH_2), 4.53 (s, 2H, CH_2), 5.00 (s, 2H, $PhCH_2$), 6.31 (d, J = 7.5 Hz, 1H, Pyridinone C5-H), 7.20 (d, J = 7.5 Hz, 1H, Pyridinone C6-H), 7.27–7.32 (m, 5H, Ar). ESI-MS: m/z 290 ($[M+H]^+$).

*5.2.2.6. 3-(Benzyloxy)-1-(2-(benzyloxy)ethyl)-2-(hydroxymethyl) pyridin-4(1H)-one (**5f**).* 2.16 g (5.9 mmol), yield: 59% (2 steps). ^{1}H NMR ($CDCl_3$) δ 3.62 (t, J = 5.0 Hz, 2H, CH_2), 4.14 (t, J = 5.0 Hz, 2H, CH_2), 4.39 (s, 2H, CH_2), 4.49 (s, 2H, CH_2), 5.01 (s, 2H, $PhCH_2$), 6.34 (d, J = 7.5 Hz, 1H, Pyridinone C5-H), 7.20 (d, J = 7.5 Hz, 1H, Pyridinone C6-H), 7.14–7.30 (m, 10H, Ar). ESI-MS: m/z 366 ($[M+H]^+$).

*5.2.3. General procedure for preparation of **6***

Thionyl chloride (30 mL) was added slowly to a flask containing **5** (20 mmol). The resulting solution was stirred at room temperature overnight. The reaction mixture was concentrated to dryness under reduced pressure. The residue was dissolved in methanol (50 mL), followed by the addition of ammonia solution (37%, 10 mL). The resulting solution was stirred for 4 h. After removal of the solvent, the residue was purified by column chromatography (CH_2Cl_2/MeOH, 20:1 to 5:1) to provide amine **6** as a brown oil.

*5.2.3.1. 2-(Aminomethyl)-3-(benzyloxy)-1-methylpyridin-4(1H)-one (**6a**).* 2.98 g (12.2 mmol), **y**ield: 61%. ^{1}H NMR ($CDCl_3$) δ 3.68 (s, 2H, CH_2), 3.70 (s, 3H, CH_3), 5.30 (s, 2H, $PhCH_2$), 6.41 (d, J = 6.0 Hz, 1H, Pyridinone C5-H), 7.18 (d, J = 6.0 Hz, 1H, Pyridinone C6-H), 7.32–7.38 (m, 5H, Ph). ESI-MS: m/z 245 ($[M+H]^+$).

*5.2.3.2. 2-(Aminomethyl)-3-(benzyloxy)-1-ethylpyridin-4(1H)-one (**6b**).* 2.99 g (11.6 mmol), yield: 58%. ^{1}H NMR ($CDCl_3$) δ 1.33 (t, J = 7.5 Hz, 3H, CH_3), 3.65 (s, 2H, CH_2), 4.00 (q, J = 7.5 Hz, 2H, CH_2), 5.29 (s, 2H, $PhCH_2$), 6.43 (d, J = 7.5 Hz, 1H, Pyridinone C5-H), 7.23 (d, J = 7.5 Hz, 1H, Pyridinone C5-H), 7.29–7.39 (m, 5H, Ph). ESI-MS: m/z 259 ($[M+H]^+$).

*5.2.3.3. 2-(Aminomethyl)-3-(benzyloxy)-1-butylpyridin-4(1H)-one (**6c**).* 3.50 g (12.2 mmol), yield: 61%. ^{1}H NMR ($CDCl_3$) δ 0.90 (t, J = 7.5 Hz, 3H, CH_3), 1.26 (m, 2H, CH_2), 1.56 (m, 2H, CH_2), 3.82 (t, J = 7.5 Hz, 2H, CH_2), 4.19 (d, J = 5.5 Hz, 2H, CH_2), 5.18 (s, 2H, $PhCH_2$), 6.36 (d, J = 7.5 Hz, 1H, Pyridinone C5-H), 7.14 (d, J = 7.5 Hz, 1H, Pyridinone C6-H), 7.29–7.31 (m, 5H, Ph). ESI-MS: m/z 287 ($[M+H]^+$).

*5.2.3.4. 2-(Aminomethyl)-3-(benzyloxy)-1-hexylpyridin-4(1H)-one (**6d**).* 3.90 g (12.4 mmol), yield: 62%. ^{1}H NMR ($CDCl_3$) δ 0.87 (t, J = 7.5 Hz, 3H, CH_3), 1.27 (m, 6H, CH_2), 1.65 (m, 2H, CH_2), 3.65 (s, 2H, CH_2), 3.91 (t, J = 7.5 Hz, 2H, NCH_2), 5.31 (s, 2H, $PhCH_2$), 6.42 (d, J = 7.5 Hz, 1H, Pyridinone C5-H), 7.20 (d, J = 7.5 Hz, 1H, Pyridinone C6-H), 7.28–7.38 (m, 5H, Ph). ESI-MS: m/z 315 ($[M+H]^+$).

*5.2.3.5. 2-(Aminomethyl)-3-(benzyloxy)-1-(2-methoxyethyl)pyridin-4(1H)-one (**6e**).* 3.40 g (11.8 mmol), yield: 59%. ^{1}H NMR ($CDCl_3$) δ 3.19 (s, 3H, OCH_3), 3.35 (t, J = 5.0 Hz, 2H, CH_2), 3.46 (t, J = 5.0 Hz, 2H, CH_2), 3.88 (s, 2H, CH_2), 5.23 (s, 2H, $PhCH_2$), 6.39 (d, J = 7.5 Hz, 1H, Pyridinone C5-H), 7.23 (d, J = 7.5 Hz, 1H, Pyridinone C6-H), 7.27–7.29 (m, 5H, Ph). ESI-MS: m/z 289 ($[M+H]^+$).

*5.2.3.6. 2-(Aminomethyl)-3-(benzyloxy)-1-(2-(benzyloxy)ethyl)pyridin-4(1H)-one (**6f**).* 4.30 g (11.8 mmol), yield: 59%. ^{1}H NMR ($CDCl_3$) δ 3.64 (t, J = 5.0 Hz, 2H, CH_2), 3.67 (s, 2H, CH_2), 4.15 (t, J = 5.0 Hz, 2H, NCH_2), 4.42 (s, 2H, $PhCH_2$), 5.27 (s, 2H, $PhCH_2$), 6.40 (d, J = 6.5 Hz, 1H, Pyridinone C5-H), 7.32 (d, J = 6.5 Hz, 1H, Pyridinone C6-H), 7.15–7.37 (m, 10H, Ph). ESI-MS: m/z 365 ($[M+H]^+$).

*5.2.4. Procedure for the preparation of **10a** and **10b***

To a solution of amine **7** (2.07 g, 5 mmol) in anhydrous dichloromethane (30 mL) cooled on an ice-bath was added triethylamine (0.8 mL), followed by the addition of acyl chloride (5 mmol). The solution was stirred at 0 °C for 3 h and then at room temperature for an additional 3 h. The reaction mixture was washed with dilute hydrochloric acid and brine and dried over anhydrous sodium sulphate. After removal of the solvent, the residue was purified by column chromatography (cyclo-hexane/ethyl acetate, 2:1 ~ 1:1) to provide product as a white solid.

*5.2.4.1. (**10a**).* 1.88 g (4.1 mmol), yield: 82%. ^{1}H NMR ($CDCl_3$) δ 1.35 (s, 27H , CH_3), 1.72 (m, 6H, CH_2), 1.85 (s, 3H, CH_3), 2.03 (m, 6H, CH_2), 7.25 (s, 1H, NH). ESI-MS: m/z 458 ($[M+H]^+$).

*5.2.4.2. (**10b**).* 1.94 g (4.0 mmol), yield: 80%. ^{1}H NMR ($CDCl_3$) δ 0.93 (t, J = 7.5 Hz, 3H, CH_3), 1.43 (s, 27H, CH_3), 1.63 (m, 2H, CH_2), 1.97 (t, J = 7.5 Hz, 2H, CH_2), 2.08 (t, J = 7.5 Hz, 6H, CH_2), 2.22 (t, J = 7.5 Hz, 6H, CH_2), 5.80 (s, 1H, NH). ESI-MS: m/z 486 ($[M+H]^+$).

*5.2.5. Procedure for the preparation of **10c***

A mixture of 2-(2-(2-methoxyethoxy)ethoxy)acetic acid (2.136 g, 12 mmol), amine **2** (4.15 g, 10 mmol), HOBt (12 mmol) and DCC (12 mmol) in DMF (40 mL) was stirred at room temperature overnight. The reaction mixture was filtered to remove the resulting white precipitate and the filtrate was concentrated under high vacuum. The residue was dissolved in dichloromethane (150 mL), washed with 5% $NaHCO_3$, brine, 5% cold HCl and brine successively and dried over anhydrous Na_2SO_4. After removal of the solvent, the residue was purified by chromatography using ethyl acetate/hexane (1:1 to 2:1) as an eluent to provide pure product **10c** (4.72 g, 8.2 mmol, 82%) as a colourless oil. ^{1}H NMR ($CDCl_3$) δ 1.43 (s, CH_3, 27H), 1.98 (m, CH_2, 6H), 2.19 (m, CH_2, 6H), 3.38 (s, CH_3, 3H), 3.57 (m, CH_2, 2H), 3.68 (m, CH_2, 6H), 3.90 (s, CH_2, 2H), 6.46 (s, NH, 1H). ESI-MS: m/z 576 ($[M+H]^+$).

*5.2.6. General procedure for preparation of **11***

A solution of **10** in formic acid was stirred at room temperature

for 24 h. After removal of formic acid, toluene was added and then rotary evaporated to remove residual formic acid. The crude product **11** was obtained as a white solid in quantitative yield.

*5.2.6.1. (**11a**).* ^{1}H NMR (DMSO-d_6) 1.78 (s, 3H, CH_3), 1.81 (t, J = 7.5 Hz, 6H, CH_2), 2.11 (t, J = 7.5 Hz, 6H, CH_2), 7.18 (s, 1H, NH), 12.22 (br, 3H, COOH).

*5.2.6.2. (**11b**).* ^{1}H NMR (DMSO-d_6) 0.84 (t, J = 7.5 Hz, 3H, CH_2), 1.48 (m, 2H, CH_2), 1.82 (t, J = 7.5 Hz, 6H, CH_2), 2.03 (t, J = 7.5 Hz, 2H, CH_2), 2.10 (t, J = 7.5 Hz, 6H, CH_2), 7.12 (s, 1H, NH), 12.08 (br, 3H, COOH).

*5.2.6.3. (**11c**).* ^{1}H NMR (DMSO-d_6) δ 1.87 (m, CH_2, 6H), 2.13 (m, CH_2, 6H), 3.24 (s, CH_3, 3H), 3.45 (m, CH_2, 2H), 3.55 (m, CH_2, 6H), 3.81 (s, CH_2, 2H), 6.84 (s, NH, 1H), 12.11 (br, 3H, COOH).

*5.2.7. General procedure for preparation of **12***

A mixture of triacid **11** (1 mmol), amine **6** (3.3 mmol), HOBt (0.51 g, 3.3 mmol) and DCC (0.68 g, 3.3 mmol) in dry DMF (15 mL) was stirred at room temperature for 2 days. After filtration, the filtrate was concentrated, the residue was purified by column chromatography using CH_2Cl_2/MeOH (20:1 to 5:1) as an eluent to give the desired product **12** as a pale yellow powder.

*5.2.7.1. **12a** (R_1=CH_3, R=CH_3).* 0.71 g (0.73 mmol), yield: 73%. ^{1}H NMR ($CDCl_3$) δ 1.82 (s, 3H, CH_3), 1.99 (m, 6H, CH_2), 2.22 (m, 6H, CH_2), 3.48 (s, 9H, CH_3), 4.28 (s, 6H, NCH_2), 4.98 (s, 6H, $PhCH_2$), 6.21 (d, J = 7.5 Hz, 3H, Pyridinone C5-H), 7.05 (d, J = 7.5 Hz, 3H, Pyridinone C6-H), 7.12 (s, 1H, NH), 7.27–7.29 (m, 15H, Ph), 7.46 (s, 3H, NH); ^{13}C NMR δ 24.04 (*C*H_3CO), 30.67 (*C*H_2), 31.74 (*C*H_2), 34.40 (CONH*C*), 41.57 (N*C*H_3), 57.68 (NH*C*H_2-pyridinone), 73.15 (Ph*C*H_2), 117.03 (*C*-5H in pyridinone), 128.35 (Ph), 128.52 (Ph), 128.73 (Ph), 136.97 (Ph), 140.26 (*C*-2 in pyridinone), 140.61 (*C*-3 in pyridinone), 147.36 (*C*-6H in pyridinone), 170.33 (*C*-4 in pyridinone), 173.30 (*C*O), 173.49 (*C*O). ESI-MS: m/z 968 ([M+H]$^+$).

*5.2.7.2. **12b** (R_1=n-C_3H_7, R=CH_3).* 0.82 g (0.82 mmol), yield: 82%. ^{1}H NMR (DMSO-d_6) δ 0.79 (t, J = 7.5 Hz, 3H, CH_3), 1.42 (m, 2H, CH_2), 1.79 (m, 6H, CH_2), 2.00 (t, J = 7.5 Hz, 2H, CH_2), 2.07 (m, 6H, CH_2), 3.90 (s, 9H, CH_3), 4.43 (d, J = 6.5 Hz, 6H, CH_2), 5.15 (s, 6H, $PhCH_2$), 6.23 (d, J = 6.5 Hz, 3H, Pyridinone C5-H), 7.18 (s, 1H, NH), 7.33–7.38 (m, 15H, Ph), 7.59 (d, J = 6.5 Hz, 3H, Pyridinone C6-H), 8.57 (s, 3H, NH); ^{13}C NMR δ 13.51 (*C*H_3CH$_2$), 18.81 (CH$_3$*C*H_2), 29.33 (*C*H_2CO), 30.05 (CO*C*H_2*C*H_2), 34.37 (CO*C*H_2), 37.79 (NH*C*), 42.73 (N*C*H_3), 56.59 (NH*C*H_2-pyridinone), 73.62 (Ph*C*H_2O), 114.14 (*C*-5H in pyridinone), 128.17 (Ph), 128.32 (Ph), 128.48 (Ph), 136.73 (Ph), 142.46 (*C*-2 in pyridinone), 144.95 (*C*-3 in pyridinone), 145.10 (*C*-6H in pyridinone), 167.10 (*C*-4 in pyridinone), 171.76 (*C*O), 172.48 (*C*O). ESI-MS: m/z 996 ([M+H]$^+$), 1018 ([M+Na]$^+$).

*5.2.7.3. **12c** (R_1=$CH_3O(CH_2CH_2O)_2CH_2$, R=CH_3).* 0.80 g (0.74 mmol), yield: 74%. ^{1}H NMR ($CDCl_3$) δ 1.99 (m, 6H, CH_2), 2.17 (m, 6H, CH_2), 3.29 (s, 3H, CH_3), 3.48 (s, 9H, CH_3), 3.58 (m, 8H, CH_2), 3.85 (s, 2H, CH_2), 4.27 (d, J = 7.0 Hz, 6H, CH_2), 4.98 (s, 6H, $PhCH_2$), 6.19 (d, J = 6.5 Hz, 3H, Pyridinone C5-H), 6.35 (s, 1H, NH), 7.04 (d, J = 6.5 Hz, 3H, Pyridinone C6-H), 7.26 (s, 3H, NH), 7.28–7.31 (m, 15H, Ph); ^{13}C NMR δ 30.30 (CO*C*H_2*C*H_2), 31.22 (CO*C*H_2), 34.36 (NH*C*), 41.55 (N*C*H_3), 57.56 (NH*C*H_2-pyridinone), 58.82 (*C*H_3O), 70.06 (*C*H_2O), 70.30 (*C*H_2O), 70.60 (*C*H_2O), 70.95 (*C*H_2O), 71.68 (*C*H_2OCO), 73.10 (Ph*C*H_2O), 117.05 (*C*-5H in pyridinone), 128.37 (Ph), 128.52 (Ph), 128.50 (Ph), 136.99 (Ph), 140.13 (*C*-2 in pyridinone), 140.42 (*C*-3 in pyridinone), 147.23 (*C*-6H in pyridinone), 169.13 (*C*-4 in pyridinone), 172.38 (*C*O), 173.38 (*C*O). ESI-MS: m/z 1086 ([M+H]$^+$).

*5.2.7.4. **12d** (R_1=CH_3, R=C_2H_5).* 0.53 g (0.71 mmol), yield: 71%. ^{1}H NMR (DMSO-d_6) δ 1.19 (t, J = 7.0 Hz, 9H, CH_3), 1.74 (s, 3H, CH_3), 1.76 (m, 6H, CH_2), 2.00 (m, 6H, CH_2), 3.85 (q, J = 7.0 Hz, 6H, CH_2), 4.26 (d, J = 5.0 Hz, 6H, CH_2), 5.10 (s, 6H, $PhCH_2$), 6.23 (d, J = 7.5 Hz, 3H, Pyridinone C5-H), 7.13 (s, 1H, NH), 7.31–7.43 (m, 15H, Ph), 7.65 (d, J = 7.5 Hz, 3H, Pyridinone C5-H), 8.09 (t, J = 5.0 Hz, 3H, NH); ^{13}C NMR δ 16.36 (*C*H_3CH$_2$), 23.53 (*C*H_3CO), 29.19 (*C*H_2), 29.83 (*C*H_2), 33.51 (CONH*C*), 47.40 (N*C*H_2CH$_3$), 56.58 (NH*C*H_2-pyridinone), 72.05 (Ph*C*H_2), 116.88 (*C*-5H in pyridinone), 127.81 (Ph), 128.16 (Ph), 128.39 (Ph), 137.59 (Ph), 139.36 (*C*-2 in pyridinone), 139.47 (*C*-3 in pyridinone), 146.67 (*C*-6H in pyridinone), 168.72 (*C*-4 in pyridinone), 172.07 (*C*O), 172.33 (*C*O). ESI-MS: m/z 740 ([M+H]$^+$), 762 ([M+Na]$^+$).

*5.2.7.5. **12e** (R_1=n-C_3H_7, R=C_2H_5).* 0.77 g (0.74 mmol), yield: 74%. ^{1}H NMR (DMSO-d_6) δ 0.79 (t, J = 7.5 Hz, 3H, CH_3), 1.18 (t, J = 7.5 Hz, 9H, CH_3), 1.44 (m, 4H, CH_2), 1.76 (m, 6H, CH_2), 1.97 (t, J = 7.5 Hz, 2H, CH_2), 2.01 (m, 6H, CH_2), 3.83 (q, J = 7.5 Hz, 6H, CH_2), 4.25 (d, J = 5.0 Hz, 6H, CH_2), 5.09 (s, 6H, $PhCH_2$), 6.22 (d, J = 7.5 Hz, 3H, Pyridinone C5-H), 7.29–7.42 (m, 15H, Ph), 7.63 (d, J = 7.5 Hz, 3H, Pyridinone C6-H), 8.05 (t, J = 5.0 Hz, 3H, NH); ^{13}C NMR δ 15.64 (*C*H_3CH$_2$), 16.33 (*C*H_3CH$_2$N), 18.77 (CH$_3$*C*H_2CH$_2$), 29.16 (CH$_3$CH$_2$*C*H$_2$), 29.84 (*C*H_2), 33.50 (*C*H_2), 37.92 ((NH*C*), 47.36 (N*C*H_2CH$_2$), 56.48 (NH*C*H_2-pyridinone), 72.03 (Ph*C*H_2), 116.88 (*C*-5H in pyridinone), 127.80 (Ph), 128.15 (Ph), 128.38 (Ph), 137.61 (Ph), 139.34 (*C*-2 in pyridinone), 139.42 (*C*-3 in pyridinone), 146.67 (*C*-6H in pyridinone), 171.59 (*C*-4 in pyridinone), 172.07 (*C*O), 172.16 (*C*O). ESI-MS: m/z 1038 ([M+H]$^+$), 1060 ([M+Na]$^+$).

*5.2.7.6. **12f** (R_1=$CH_3O(CH_2CH_2O)_2CH_2$, R=C_2H_5).* 0.83 g (0.74 mmol), yield: 74%. ^{1}H NMR (DMSO-d_6) δ 1.18 (t, J = 6.0 Hz, 9H, CH_3), 1.80 (m, 6H, CH_2), 2.02 (m, 6H, CH_2), 3.18 (s, 3H, OCH_3), 3.38–3.49 (m, 8H, CH_2), 3.74 (s, 2H, CH_2), 3.84 (q, J = 6.0 Hz, 6H, CH_2), 4.25 (d, J = 6.0 Hz, 6H, CH_2), 5.09 (s, 6H, $PhCH_2$), 6.22 (d, J = 7.5 Hz, 3H, Pyridinone C5-H), 6.78 (s, 1H, NH), 7.64 (d, J = 7.5 Hz, 3H, Pyridinone C6-H), 7.30–7.42 (m, 15H, Ph), 8.09 (t, J = 5.0 Hz, 3H, NH); ^{13}C NMR δ 16.29 (*C*H_3CH$_2$), 29.09 (CO*C*H_2*C*H_2), 29.93 (CO*C*H_2), 33.52 (NH*C*), 47.37 (N*C*H_2CH$_3$), 56.71 (NH*C*H_2-pyridinone), 57.93 (*C*H_3O), 69.43 (*C*H_2O), 69.47 (*C*H_2O), 69.76 (*C*H_2O), 70.32 (*C*H_2O), 71.13 (*C*H_2OCO), 72.03 (Ph*C*H_2O), 116.87 (*C*-5H in pyridinone), 127.81 (Ph), 128.11 (Ph), 128.35 (Ph), 137.59 (Ph), 139.35 (*C*-2 in pyridinone), 139.38 (*C*-3 in pyridinone), 146.67 (*C*-6H in pyridinone), 168.39 (*C*-4 in pyridinone), 171.86 (*C*O), 172.17 (*C*O). ESI-MS: m/z 1128 ([M+H]$^+$), 1150 ([M+Na]$^+$).

*5.2.7.7. **12g** (R_1=CH_3, R=n-C_4H_9).* 0.80 g (0.73 mmol), yield: 73%. ^{1}H NMR ($CDCl_3$) δ 0.90 (t, J = 7.5 Hz, 9H, CH_3), 1.25 (m, 6H, CH_2), 1.55 (m, 6H, CH_2), 1.84 (s, 3H, CH_3), 1.92 (m, 6H, CH_2), 2.08 (m, 6H, CH_2), 3.82 (t, J = 7.5 Hz, 9H, CH_2), 4.19 (d, J = 5.5 Hz, 6H, CH_2), 5.18 (s, 6H, $PhCH_2$), 6.36 (d, J = 7.5 Hz, 3H, Pyridinone C5-H), 6.51 (s, 3H, NH), 7.11 (s, 1H, NH), 7.14 (d, J = 7.5 Hz, 3H, Pyridinone C6-H), 7.29–7.32 (m, 15H, Ph); ^{13}C NMR δ 13.69 (*C*H_3CH$_2$), 19.60 (CH$_3$*C*H_2), 24.02 (*C*H_3CO), 30.47 (*C*H_2), 31.39 (*C*H_2), 33.66 (CONH*C*), 34.12 (*C*H_2), 53.83 (N*C*H_2CH$_2$), 57.38 (NH*C*H_2-pyridinone), 72.86 (Ph*C*H_2), 117.77 (*C*-5H in pyridinone), 128.49 (Ph), 128.58 (Ph), 129.22 (Ph), 137.05 (Ph), 138.99 (*C*-2 in pyridinone), 140.31 (*C*-3 in pyridinone), 147.04 (*C*-6H in pyridinone), 170.05 (*C*-4 in pyridinone), 172.87 (*C*O), 173.47 (*C*O). ESI-MS: m/z 1094 ([M+H]$^+$).

*5.2.7.8. **12h** (R_1=n-C_3H_7, R=n-C_4H_9).* 0.87 g (0.77 mmol), yield: 77%. ^{1}H NMR (500 MHz, $CDCl_3$) ^{1}H NMR ($CDCl_3$) δ 0.81 (t, J = 7.5 Hz, 3H, CH_3), 0.90 (t, J = 7.5 Hz, 9H, CH_3), 1.25 (m, 6H, CH_2), 1.54 (m, 8H, CH_2), 1.99 (m, 6H, CH_2), 2.04 (t, J = 7.5 Hz, 2H, CH_2), 2.17 (m, 6H, CH_2), 3.78 (t, J = 7.5 Hz, 6H, CH_2), 4.22 (s, 6H, CH_2), 5.10 (s, 6H, $PhCH_2$), 6.31 (t, J = 7.5 Hz, 3H, Pyridinone C5-H), 7.01 (t, J = 7.5 Hz, 3H, Pyridinone C6-H), 7.13–7.30 (m, 15H, Ph); ^{13}C NMR δ 13.46

(CH_3), 13.61 (CH_3), 18.78 (CH_3CH_2), 19.03 (CH_3CH_2), 29.21 (CH_2), 29.92 (CH_2), 33.03 (CH_2), 33.48 (CONHC), 37.97 ($COCH_2$), 51.99 (NCH_2), 56.38 ($NHCH_2$-pyridinone), 71.90 ($PhCH_2$), 116.54 (C-5H in pyridinone), 127.81 (Ph), 128.13 (Ph), 128.48 (Ph), 137.52 (Ph), 139.61 (C-2 in pyridinone), 139.81 (C-3 in pyridinone), 146.60 (C-6H in pyridinone), 171.59 (C-4 in pyridinone), 172.03 (CO), 172.17 (CO). ESI-MS: *m/z* 1122 ($[M+H]^+$), 1144 ($[M+Na]^+$).

5.2.7.9. ***12i*** *(R_1=$CH_3O(CH_2CH_2O)_2CH_2$, R=n-C_4H_9).* 0.83 g (0.68 mmol), yield: 68%. ^{1}H NMR (DMSO-d_6) δ 0.84 (t, *J* = 7.0 Hz, 9H, CH_3), 1.19 (m, 6H, CH_2), 1.52 (m, 6H, CH_2), 1.80 (m, 6H, CH_2), 2.01 (m, 6H, CH_2), 3.18 (s, 3H, CH_3), 3.38 (t, *J* = 5.0 Hz, 2H, CH_2), 3.50 (m, 6H, CH_2), 3.75 (s, 2H, CH_2), 3.79 (t, *J* = 7.0 Hz, 6H, CH_2), 4.22 (d, *J* = 5.0 Hz, 6H, CH_2), 5.10 (s, 6H, $PhCH_2$), 6.20 (d, *J* = 7.5 Hz, 3H, Pyridinone C5-H), 6.78 (s, 1H, NH), 7.63 (d, *J* = 7.5 Hz, 3H, Pyridinone C6-H), 7.30–7.41 (m, 15H, Ph), 8.09 (t, *J* = 5.0 Hz, 3H, NH); ^{13}C NMR δ 13.42 (CH_3CH_2), 19.02 (CH_3CH_2), 24.47 ($CH_3CH_2CH_2$), 29.14 (CH_2), 30.00 (CH_2), 33.45 (CONHC), 52.08 (OCH_3), 56.67 ($NHCH_2$-pyridinone), 57.96 ($COCH_2O$), 69.44 (NCH_2), 69.48 (OCH_2), 69.76 (OCH_2), 70.32 (OCH_2), 71.15 (OCH_2), 71.97 ($PhCH_2$), 116.54 (C-5H in pyridinone), 127.81 (Ph), 128.12 (Ph), 128.48 (Ph), 137.51 (Ph), 139.59 (C-2 in pyridinone), 139.82 (C-3 in pyridinone), 146.61 (C-6H in pyridinone), 168.38 (C-4 in pyridinone), 171.83 (CO), 172.18 (CO). ESI-MS: *m/z* 1212 ($[M+H]^+$), 1234 ($[M+Na]^+$).

5.2.7.10. ***12j*** *(R_1=CH_3, R=n-C_6H_{13}).* 0.85 g (0.72 mmol), yield: 72%. ^{1}H NMR ($CDCl_3$) δ 0.86 (t, *J* = 7.0 Hz, 9H, CH_3), 1.24 (m, 18H, CH_2), 1.56 (m, 6H, CH_2), 1.84 (s, 3H, CH_3), 1.99 (m, 6H, CH_2), 2.07 (m, 6H, CH_2), 3.82 (t, *J* = 7.0 Hz, 6H, CH_2), 4.19 (d, *J* = 6.0 Hz, 6H, CH_2), 5.18 (s, 6H, $PhCH_2$), 6.36 (d, *J* = 7.5 Hz, 3H, Pyridinone C5-H), 6.50 (s, 3H, NH), 7.14 (d, *J* = 7.5 Hz, 3H, Pyridinone C6-H), 7.29–7.31 (m, 15H, Ph); ^{13}C NMR δ 13.98 (CH_3CH_2), 20.40 ($CH_3CH_2CH_2$), 24.01 (CH_3CO), 25.95 (CH_2), 30.50 (CH_2), 31.29 (CH_2), 31.47 (CH_2), 31.62 (CH_2), 34.09 (CONHC), 57.27 (NCH_2CH_2), 57.32 ($NHCH_2$-pyridinone), 72.83 ($PhCH_2$), 117.73 (C-5H in pyridinone), 128.47 (Ph), 128.56 (Ph), 129.17 (Ph), 137.05 (Ph), 138.95 (C-2 in pyridinone), 140.27 (C-3 in pyridinone), 147.04 (C-6H in pyridinone), 170.03 (C-4 in pyridinone), 172.85 (CO), 173.48 (CO). ESI-MS: *m/z* 1178 ($[M+H]^+$), 1200 ($[M+Na]^+$).

5.2.7.11. ***12k*** *(R_1=n-C_3H_7, R=n-C_6H_{13}).* 0.92 g (0.77 mmol), yield: 77%. ^{1}H NMR ($CDCl_3$) δ 0.86 (m, 12H, CH_3), 1.24 (m, 18H, CH_2), 1.56 (m, 8H, CH_2), 1.93 (m, 6H, CH_2), 2.10 (m, 6H, CH_2), 2.01 (t, *J* = 7.5 Hz, 2H, CH_2), 3.81 (t, *J* = 7.5 Hz, 6H, CH_2), 4.19 (d, *J* = 5.5 Hz, 6H, CH_2), 5.16 (s, 6H, $PhCH_2$), 7.35 (d, *J* = 7.5 Hz, 3H, Pyridinone C5-H), 6.70 (s, 3H, NH), 7.14 (d, *J* = 7.5 Hz, 3H, Pyridinone C6-H), 7.04 (s, 1H, NH), 7.27–7.31 (m, 15H, Ph); ^{13}C NMR δ 13.87 (CH_3), 13.92 (CH_3), 19.21 (CH_2), 22.39 (CH_2), 25.94 (CH_2), 30.59 (CH_2), 31.29 (CH_2), 31.60 (CH_2), 34.05 (CH_2), 39.21 ($COCH_2$), 50.61 (CONHC), 53.86 (NCH_2), 57.22 ($NHCH_2$-pyridinone), 72.87 ($PhCH_2$), 117.63 (C-5H in pyridinone), 128.42 (Ph), 128.52 (Ph), 129.06 (Ph), 136.99 (Ph), 138.98 (C-2 in pyridinone), 140.35 (C-3 in pyridinone), 147.08 (C-6H in pyridinone), 172.95 (C-4 in pyridinone), 173.08 (CO), 173.47 (CO).ESI-MS: *m/z* 1206 ($[M+H]^+$), 1228 ($[M+Na]^+$).

5.2.7.12. ***12l*** *(R_1=$CH_3O(CH_2CH_2O)_2CH_2$, R=n-C_6H_{13}).* 0.77 g (0.60 mmol), yield: 60%. ^{1}H NMR ($CDCl_3$) δ 0.87 (t, *J* = 6.5 Hz, 9H, CH_3), 1.24 (m, 18H, CH_2), 1.56 (m, 6H, CH_2), 1.92 (m, 6H, CH_2), 2.03 (m, 6H, CH_2), 3.27 (s, 3H, CH_3), 3.47 (t, *J* = 4.5 Hz, 2H, CH_2), 3.58 (m, 6H, CH_2), 3.81 (t, *J* = 7.0 Hz, 6H, CH_2), 3.84 (s, 2H, CH_2), 4.18 (d, *J* = 6.0 Hz, 6H, CH_2), 5.18 (s, 6H, $PhCH_2$), 6.33 (d, *J* = 7.5 Hz, 3H, Pyridinone C5-H), 6.41 (s, 3H, NH), 6.49 (s, 1H, NH), 7.13 (d, *J* = 7.5 Hz, 3H, Pyridinone C6-H), 7.30-7.33 (m, 15H, Ph); ^{13}C NMR δ 13.95 (CH_3), 22.43 (CH_2), 25.98 (CH_2), 30.14 (CH_2), 30.85 (CH_2), 31.27 (CH_2), 31.60 (CH_2), 34.06 (CONHC), 53.96 (OCH_3), 57.42 ($NHCH_2$-pyridinone), 58.78 ($COCH_2O$), 70.05 (NCH_2), 70.30 (OCH_2), 70.59 (OCH_2), 70.99 (OCH_2), 71.66 (OCH_2), 72.84 ($PhCH_2$), 117.72 (C-5H in pyridinone), 128.49 (Ph), 128.57 (Ph), 129.24 (Ph), 137.12 (Ph), 138.92 (C-2 in pyridinone), 140.14 (C-3 in pyridinone), 146.97 (C-6H in pyridinone), 169.06 (C-4 in pyridinone), 172.31 (CO), 173.31 (CO).ESI-MS: *m/z* 1296 ($[M+H]^+$).

5.2.7.13. ***12m*** *(R_1=CH_3, R=CH_2CH_2OBn).* 0.91 g (0.68 mmol), yield: 68%. ^{1}H NMR (500 MHz, DMSO-d6) δ 1.74 (s, 3H, CH_3), 1.77 (m, 6H, CH_2), 2.00 (m, 6H, CH_2), 3.60 (t, *J* = 5.0 Hz, 6H, CH_2), 4.09 (t, *J* = 5.0 Hz, 6H, CH_2), 4.30 (d, *J* = 5.0 Hz, 6H, CH_2), 4.43 (s, 6H, $PhCH_2$), 5.10 (s, 6H, $PhCH_2$), 6.21 (d, *J* = 7.5 Hz, 3H, Pyridinone C5-H), 7.12 (s, 1H, NH), 7.19–7.46 (br, 30H, Ar), 7.60 (d, *J* = 7.5 Hz, 3H, Pyridinone C5-H), 8.03 (t, *J* = 5.0 Hz, 3H, NH). ^{13}C NMR δ 23.48 (CH_3CO), 29.24 (CH_2), 29.91 (CH_2), 33.72 (CONHC), 52.01 (OCH_2CH_2), 56.58 ($NHCH_2$-pyridinone), 69.05 (NCH_2), 72.04 ($PhCH_2$), 72.09 ($PhCH_2$), 116.33 (C-5H in pyridinone), 127.10 (Ph), 127.43 (Ph), 127.80 (Ph), 128.14 (Ph), 128.22 (Ph), 128.39 (Ph), 137.58 (Ph), 137.92 (Ph), 140.02 (C-2H in pyridinone), 140.36 (C-3H in pyridinone), 146.49 (C-6H in pyridinone), 168.74 (C-4H in pyridinone), 172.15 (CO), 172.38 (CO). ESI-MS: *m/z* 1328 ($[M+H]^+$), 1350 ($[M+Na]^+$).

5.2.7.14. ***12n*** *(R_1=n-C_3H_7, R=CH_2CH_2OBn).* 0.99 g (0.73 mmol), yield: 73%. ^{1}H NMR (500 MHz, DMSO-d_6) δ 0.80 (t, *J* = 7.5 Hz, 3H, CH_3), 1.45 (br, 2H, CH_2), 1.80 (m, 6H, CH_2), 2.01 (m, 8H, CH_2), 3.61 (t, *J* = 7.0 Hz, 6H, CH_2), 4.10 (t, *J* = 7.0 Hz, 6H, CH_2), 4.32 (s, 6H, CH_2), 4.43 (s, 6H, $PhCH_2$), 5.11 (s, 6H, $PhCH_2$), 6.22 (d, *J* = 7.5 Hz, 3H, Pyridinone C5-H), 7.09 (s, 1H, NH), 7.21–7.43 (br, 30H, Ar), 7.61 (d, *J* = 7.5 Hz, 3H, Pyridinone C6-H), 8.05 (s, 3H, NH). ^{13}C NMR δ 13.62 (CH_3CH_2), 18.73 ($CH_3CH_2CH_2$), 24.42 ($CH_3CH_2CH_2$), 29.25 (CH_2), 29.97 (CH_2), 33.74 (CONHC), 52.01 (OCH_2CH_2), 56.45 ($NHCH_2$-pyridinone), 69.06 (NCH_2), 72.05 ($PhCH_2$), 72.10 ($PhCH_2$), 116.34 (C-5H in pyridinone), 127.10 (Ph), 127.43 (Ph), 127.80 (Ph), 128.14 (Ph), 128.22 (Ph), 128.39 (Ph), 137.58 (Ph), 139.92 (Ph), 140.05 (C-2H in pyridinone), 140.36 (C-3H in pyridinone), 146.50 (C-6H in pyridinone), 171.66 (C-4H in pyridinone), 172.19 (CO), 172.40 (CO). ESI-MS: *m/z* 1356 ($[M+H]^+$), 1378 ($M+Na]^+$).

5.2.7.15. ***12o*** *(R_1=$CH_3O(CH_2H_2O)_2CH_2$, R=CH_2CH_2OBn).* 1.03 g (0.71 mmol), yield: 71%. ^{1}H NMR (500 MHz, DMSO-d_6) δ 1.81 (m, 6H, CH_2), 2.00 (m, 6H, CH_2), 3.17 (s, 3H, OCH_3), 3.37 (t, *J* = 5.0 Hz, 2H, CH_2), 3.48 (br, 4H, CH_2), 3.60 (t, *J* = 5.0 Hz, 6H, CH_2), 3.75 (s, 2H, CH_2), 4.08 (t, J = 5.0 Hz, 6H, CH_2), 4.30 (d, *J* = 5.0 Hz, 6H, CH_2), 4.43 (s, 6H, $BnOCH_2$), 5.09 (s, 6H, $PhCH_2$), 6.21 (d, *J* = 7.5 Hz, 3H, Pyridinone C5-H), 6.77 (s, 1H, NH), 7.19–7.42 (br, 30H, Ar), 7.60 (d, *J* = 7.5 Hz, 3H, Pyridinone C5-H), 8.04 (t, *J* = 5.0 Hz, 3H, NH). ^{13}C NMR δ 29.13 (CCH_2), 29.99 ($COCH_2CH_2$), 30.75 ($COCH_2$), 33.73 (NHC), 51.99 (OCH_2CH_2), 56.75 ($NHCH_2$-pyridinone), 57.94 (CH_3O), 69.05 (NCH_2), 69.43 (CH_2O), 69.47 (CH_2O), 70.33 (CH_2O), 71.12 (CH_2O), 72.03 ($PhCH_2$), 72.08 ($PhCH_2$), 116.33 (C-5H in pyridinone), 127.09 (Ph), 127.42 (Ph), 127.80 (Ph), 128.13 (Ph), 128.22 (Ph), 128.38 (Ph), 137.56 (Ph), 137.92 (Ph), 139.98 (C-2H in pyridinone), 140.35 (C-3H in pyridinone), 146.48 (C-6H in pyridinone), 168.40 (C-4H in pyridinone), 171.94 (CO), 172.36 (CO). ESI-MS: *m/z* 1446 ($[M+H]^+$), 1468 ($[M+Na]^+$).

5.2.7.16. ***12p*** *(R_1=CH_3, R=$CH_2CH_2OCH_3$).* 0.86 g (0.77 mmol), yield: 77%. ^{1}H NMR (500 MHz, DMSO-d6) δ 1.74 (s, 3H, CH_3CO), 1.76 (m, 6H, CH_2), 1.92 (m, 6H, CH_2), 3.19 (s, 9H, CH_3), 3.49 (t, *J* = 5.0 Hz, 6H, CH_2), 4.02 (t, *J* = 5.0 Hz, 6H, CH_2), 4.26 (d, *J* = 5.0 Hz, 6H, CH_2), 5.10 (s, 6H, $PhCH_2$), 6.20 (d, *J* = 7.5 Hz, 3H, Pyridinone C5-H), 7.12 (s, 1H, NH), 7.30–7.42 (br, 15H, Ar), 7.58 (d, *J* = 7.5 Hz, 3H, Pyridinone C6-H), 8.03 (t, *J* = 5.0 Hz, 3H, NH). ^{13}C NMR δ 23.46 (CH_3CO), 29.19 (CH_2), 29.82 (CH_2), 33.67 (CONHC), 48.56 (OCH_3), 51.77 (OCH_2CH_2), 56.54 ($NHCH_2$-pyridinone), 71.29 (NCH_2CH_2), 71.98 ($PhCH_2$), 116.32

(*C*-5H in pyridinone), 127.81 (Ph), 128.15 (Ph), 128.42 (Ph), 137.57 (Ph), 139.94 (*C*-2H in pyridinone), 140.32 (*C*-3H in pyridinone), 146.40 (*C*-6H in pyridinone), 168.70 (*C*-4H in pyridinone), 172.07 (*C*O), 172.33 (*C*O). ESI-MS: *m*/*z* 1110 ([M+H]$^+$).

5.2.7.17. ***12q*** *(R_1=n-C_3H_7, R=$CH_2CH_2OCH_3$).* 0.83 g (0.74 mmol), yield: 74%. ^{1}H NMR (500 MHz, DMSO-d_6) δ 0.79 (t, *J* = 7.5 Hz, 3H, CH_3), 1.44 (m, 2H, CH_2), 1.77 (m, 6H, CH_2), 1.98 (t, *J* = 7.5 Hz, 2H, CH_2), 2.04 (m, 6H, CH_2), 3.22 (s, 9H, CH_3), 3.66 (t, *J* = 4.5 Hz, 6H, CH_2), 4.50 (d, *J* = 4.5 Hz, 6H, CH_2), 4.68 (s, 6H, CH_2), 5.12 (s, 6H, PhCH_2), 7.17 (s, 1H, NH), 7.27 (d, *J* = 7.0 Hz, 3H, Pyridinone C5-H), 8.15 (d, *J* = 7.0 Hz, 3H, Pyridinone C6-H), 8.67 (t, *J* = 7.5 Hz, 3H, NH). ^{13}C NMR δ 13.64 (CH_3), 19.68 ($CH_3CH_2CH_2$), 24.34 (CH_2CO), 29.05 (CH_2), 30.55 (CH_2), 33.48 (CONH*C*), 48.83 (OCH_3), 51.84 (OCH_2), 56.68 (NHCH_2-pyridinone), 72.44 (NCH_2CH_2), 73.17 (PhCH_2), 117.18 (*C*-5H in pyridinone), 127.99 (Ph), 128.22 (Ph), 129.18 (Ph), 137.22 (Ph), 138.98 (*C*-2H in pyridinone), 142.97 (*C*-3H in pyridinone), 146.04 (*C*-6H in pyridinone), 171.16 (*C*-4H in pyridinone), 173.01 (*C*O), 173.92 (*C*O).ESI-MS: *m*/*z* 1128 ([M+H]$^+$).

5.2.7.18. ***12r*** *(R_1=$CH_3O(CH_2CH_2O)_2CH_2$, R=$CH_2CH_2OCH_3$).* 0.85 g (0.70 mmol), yield: 70%. ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.80 (m, 6H, CH_2), 2.01 (m, 6H, CH_2), 3.21 (s, 3H, OCH_3), 3.38 (t, *J* = 6.0 Hz, 2H, CH_2), 3.48 (t, *J* = 7.0 Hz, 6H, CH_2), 3.51 (m, 6H, CH_2), 3.75 (s, 2H, CH_2), 4.02 (t, *J* = 5.0 Hz, 6H, CH_2), 4.27 (6H, *J* = 5.0 Hz, NHCH_2), 5.10 (s, 6H, PhCH_2), 6.21 (d, *J* = 7.5 Hz, 3H, Pyridinone C5-H), 6.78 (s, 1H, NH), 7.58 (d, *J* = 7.5 Hz, 3H, Pyridinone C6-H), 8.04 (t, *J* = 5.0 Hz, 3H, NH). ^{13}C NMR δ 28.99 (CCH_2), 30.76 ($COCH_2CH_2$), 33.35 (NH*C*), 50.02 (OCH_3), 53.00 (OCH_2), 56.71 (NHCH_2-pyridinone), 59.05 (CH_3O), 68.14 (CH_3OCH_2), 68.56 (NCH_2), 68.99 (OCH_2), 69.39 (OCH_2), 69.85 (OCH_2), 71.11 (OCH_2),72.84 (PhCH_2), 116.71 (*C*-5H in pyridinone), 127.86 (Ph), 128.15 (Ph), 128.92 (Ph), 137.50 (Ph), 139.13 (*C*-2H in pyridinone), 141.29 (*C*-3H in pyridinone), 145.68 (*C*-6H in pyridinone), 171.91 (*C*-4H in pyridinone), 172.93 (*C*O), 173.35 (*C*O). ESI-MS: *m*/*z* 1218 ([M+H]$^+$).

5.2.8. General procedure for preparation of ***13***

To a suspension of **12** (**10a**–**r**) (1 mmol) and concentrated hydrochloric acid (1 mL) in MeOH (30 mL) was added 5% Pd/C (0.15 g). Hydrogenation was carried out at 30 psi H_2 for 5–6 h. After filtration to remove the catalyst, the filtrate was concentrated to dryness. The residue was purified by crystallization from methanol/acetone. Hydrochlorides of the hexadentate ligand class **13** were obtained as white solids.

5.2.8.1. ***13a*** *(R_1=CH_3, R=CH_3).* 0.70 g (0.87 mmol), yield: 87%. M.p. 112–114 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ 1.75 (s, 3H, CH_3), 1.77 (m, 6H, CH_2), 2.06 (m, 6H, CH_2), 4.11 (s, 9H, CH_3), 4.50 (d, *J* = 5.0 Hz, 6H, CH_2), 7.19 (s, 1H, NH), 7.33 (d, *J* = 7.0 Hz, 3H, Pyridinone C5-H), 8.25 (d, *J* = 7.0 Hz, 3H, Pyridinone C6-H), 8.81 (s, 3H, NH). ^{13}C NMR (DMSO-d_6) δ 23.44 (CH_3CO), 29.20 (CH_2), 29.95 (CH_2), 34.43 (CONH*C*), 43.84 (NCH_3), 56.62 (NHCH_2-pyridinone), 111.43 (*C*-5H in pyridinone), 139.47 (*C*-2 in pyridinone), 139.68 (*C*-3 in pyridinone), 144.33 (*C*-6H in pyridinone), 160.06 (*C*-4 in pyridinone), 168.62 (*C*O), 173.28 (*C*O). ESI-MS: *m*/*z* 698 ([M+H]$^+$), 720 ([M+Na]$^+$). ESI-HRMS: calcd. for $C_{33}H_{44}O_{10}N_7$ 698.3150, found 698.3135 ([M+H]$^+$); calcd. for $C_{33}H_{43}O_{10}N_7Na$ 720.2969, found 720.2947 ([M+Na]$^+$).

5.2.8.2. ***13b*** *(R_1=n-C_3H_7, R=CH_3).* 0.74 g (0.89 mmol), yield: 89%. ^{1}H NMR (500 MHz, DMSO-d_6) δ 0.81 (t, *J* = 7.0 Hz, 3H, CH_3), 1.44 (m, 2H, CH_2), 1.78 (m, 6H, CH_2), 1.99 (t, *J* = 7.0 Hz, 2H, CH_2), 2.06 (m, 6H, CH_2), 4.06 (s, 9H, CH_3), 4.48 (d, *J* = 5.0 Hz, 6H, CH_2), 7.11 (s, 1H, NH), 7.18 (d, *J* = 7.0 Hz, 3H, Pyridinone C5-H), 8.12 (d, *J* = 7.0 Hz, 3H, Pyridinone C6-H), 8.71 (s, 3H, NH). ESI-MS: *m*/*z* 726 ([M+H]$^+$). ESI-HRMS: calcd. for $C_{35}H_{47}O_{10}N_7Na$ 748.3282, found 748.3263 ([M+Na]$^+$).

5.2.8.3. ***13c*** *(R_1=$CH_3O(CH_2CH_2O)_2CH_2$, R_1=CH_3).* 0.87 g (0.94 mmol), yield: 94%. ^{1}H NMR (500 MHz, DMSO-d_6) δ 1.83 (m, 6H, CH_2), 2.09 (m, 6H, CH_2), 3.20 (s, 3H, CH_3), 3.40 (m, 2H, CH_2), 3.54 (m, 6H, CH_2), 3.77 (s, 2H, CH_2), 4.08 (s, 9H, CH_3), 4.49 (d, *J* = 5.0 Hz, 6H, CH_2), 6.81 (s, 1H, NH), 7.27 (d, *J* = 6.5 Hz, 3H, Pyridinone C5-H), 8.21 (d, *J* = 6.5 Hz, 3H, Pyridinone C6-H), 8.75 (s, 3H, NH). ^{13}C NMR δ 29.19 (CH_2), 30.10 (CH_2), 34.46 (CONH*C*), 43.98 (NCH_3), 56.88 (NHCH_2-pyridinone), 57.99 (CH_3O), 69.40 (CH_2CO), 69.45 (CH_2O), 69.69 (CH_2O), 70.27 (CH_2O), 71.10 (CH_2O), 111.32 (*C*-5H in pyridinone), 139.48 (*C*-2 in pyridinone), 139.86 (*C*-3 in pyridinone), 144.14 (*C*-6H in pyridinone), 159.88 (*C*-4 in pyridinone), 168.61 (*C*O), 173.29 (*C*O). ESI-MS: *m*/*z* 816 ([M+H]$^+$). ESI-HRMS: calcd. for $C_{38}H_{54}O_{13}N_7$ 816.3780, found 816.3751 ([M+H]$^+$); calcd. for $C_{38}H_{53}O_{13}N_7Na$ 838.3599, found 838.3568 ([M+Na]$^+$).

5.2.8.4. ***13d*** *(R_1=CH_3, R=C_2H_5).* 0.74 g (0.87 mmol), yield: 87%. ^{1}H NMR (500 MHz, DMSO-d_6) δ 1.38 (t, *J* = 7.0 Hz, 9H, CH_3), 1.77 (m, 9H, $3CH_2$ and buried CH_3), 2.05 (m, 6H, CH_2), 4.40 (m, 6H, CH_2), 4.51 (br, 6H, CH_2), 7.04 (s, 1H, NH), 7.28 (d, *J* = 7.0 Hz, 3H, Pyridinone C5-H), 8.26 (d, *J* = 6.5 Hz, 3H, Pyridinone C6-H), 8.57 (s, 3H, NH). ^{13}C NMR δ 16.51 (CH_3CH_2), 23.46 ($COCH_3$), 29.30 (CH_2), 32.83 (CH_2), 33.67 (CONH*C*), 49.41 (NCH_2CH_3), 56.60 (NHCH_2-pyridinone), 111.67 (*C*-5H in pyridinone), 138.07 (*C*-2 in pyridinone), 138.28 (*C*-3 in pyridinone), 145.56 (*C*-6H in pyridinone), 164.49 (*C*-4 in pyridinone), 169.08 (*C*O), 172.92 (*C*O). ESI-MS: *m*/*z* 740 ([M+H]$^+$), 762 ([M+Na]$^+$). ESI-HRMS: calcd. for $C_{36}H_{50}O_{10}N_7$ 740.3619, found 740.3597 ([M+H]$^+$).

5.2.8.5. ***13e*** *(R_1=n-C_3H_7, R=C_2H_5).* 0.76 g (0.87 mmol), yield: 87%. M.p. 149–151 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ 0.81 (t, *J* = 7.5 Hz, 3H, CH_3), 1.38 (t, *J* = 7.5 Hz, 9H, CH_3), 1.44 (m, 2H, CH_2), 1.80 (m, 6H, CH_2), 1.99 (t, *J* = 7.5 Hz, 2H, CH_2), 2.06 (m, 6H, CH_2), 4.43 (t, *J* = 7.5 Hz, 6H, CH_2), 4.52 (d, *J* = 5.0 Hz, 6H, CH_2), 7.13 (s, 1H, NH), 7.38 (br, 3H, Pyridinone C5-H), 8.31 (d, *J* = 7.5 Hz, 3H, Pyridinone C6-H), 8.78 (s, 3H, NH). ESI-MS: *m*/*z* 768 ([M+H]$^+$). ESI-HRMS: calcd. for $C_{38}H_{54}O_{10}N_7$ 768.3932, found 768.3900 ([M+H]$^+$); calcd. for $C_{38}H_{53}O_{10}N_7Na$ 790.3752, found 790.3723 ([M+Na]$^+$).

5.2.8.6. ***13f*** *(R_1=$CH_3O(CH_2CH_2O)_2CH_2$, R=C_2H_5).* 0.88 g (0.91 mmol), yield: 91%. ^{1}H NMR (500 MHz, DMSO-d_6) δ 1.37 (t, *J* = 7.0 Hz, 9H, CH_3), 1.83 (m, 6H, CH_2), 2.07 (m, 6H, CH_2), 3.21 (s, 3H, OCH_3), 3.41 (t, *J* = 5.0 Hz, 2H, CH_2), 3.52 (m, 6H, CH_2), 3.77 (s, 2H, CH_2), 4.41 (m, 6H, CH_2), 4.51 (d, *J* = 5.0 Hz, 6H, CH_2), 6.81 (s, 1H, NH), 7.31 (br, 3H, Pyridinone C5-H), 8.26 (br, 3H, Pyridinone C6-H), 8.73 (s, 3H, NH). ^{13}C NMR δ 16.40 (CH_3), 29.09 (CH_2), 29.92 (CH_2), 33.90 (CONH*C*), 50.88 (CH_2CO), 56.81 (NHCH_2-pyridinone), 58.00 (OCH_3), 69.45 (NCH_2), 69.49 (OCH_2), 69.75 (OCH_2), 70.34 (OCH_2), 71.14 (OCH_2), 111.87 (*C*-5H in pyridinone), 138.24 (*C*-2 in pyridinone), 138.48 (*C*-3 in pyridinone), 144.65 (*C*-6H in pyridinone), 160.65 (*C*-4 in pyridinone), 168.50 (*C*O), 172.87 (*C*O). ESI-MS: *m*/*z* 858 ([M+H]$^+$), 880 ([M+Na]$^+$). ESI-HRMS: calcd. for $C_{41}H_{59}O_{13}N_7Na$ 880.4069, found 880.4039 ([M+Na]$^+$).

5.2.8.7. ***13g*** *(R_1=CH_3, R=n-C_4H_9).* 0.83 g (0.89 mmol), yield: 89%. M.p. 147–150 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ 0.89 (t, *J* = 7.5 Hz, 9H, CH_3), 1.31 (br, 6H, CH_2), 1.69–1.79 (m, 15H, $6CH_2$ and buried CH_3), 2.07 (m, 6H, CH_2), 4.40 (t, *J* = 7.5 Hz, 6H, CH_2), 4.51 (d, *J* = 5.0 Hz, 6H, CH_2), 7.24 (s, 1H, NH), 7.46 (m, 3H, Pyridinone C5-H), 8.32 (m, 3H, Pyridinone C6-H), 8.87 (s, 3H, NH). ^{13}C NMR δ 13.40 (CH_3CH_2), 19.01 ($CH_3CH_2CH_2$), 23.44 ($COCH_3$), 29.26 (CH_2), 30.01 (CH_2), 32.86 (CH_2), 33.91 (CONH*C*), 55.54 (NCH_2), 56.58 (NHCH_2-pyridinone), 111.67 (*C*-5H in pyridinone), 138.70 (*C*-2 in pyridinone), 139.40 (*C*-3 in pyridinone), 144.50 (*C*-6H in pyridinone),

159.92 (*C*-4 in pyridinone), 168.88 (*C*O), 173.15 (*C*O). ESI-MS: *m/z* 824 ($[M+H]^+$). ESI-HRMS: calcd. for $C_{42}H_{61}O_{10}N_7Na$ 846.4378, found 846.4362 ($[M+Na]^+$).

5.2.8.8. ***13h*** *(R_1=n-C_3H_7, R=n-C_4H_9).* 0.87 g (0.91 mmol), yield: 91%. ^{1}H NMR (500 MHz, DMSO-d_6) δ 0.81 (t, *J* = 7.5 Hz, 3H, CH_3), 0.89 (t, *J* = 7.5 Hz, 9H, CH_3), 1.31 (m, 6H, CH_2), 1.45 (m, 2H, CH_2), 1.71 (m, 6H, CH_2), 1.78 (m, 6H, CH_2), 1.99 (t, *J* = 7.5 Hz, 2H, CH_2), 2.06 (m, 6H, CH_2), 4.36 (t, *J* = 7.5 Hz, 6H, CH_2), 4.50 (d, *J* = 5.0 Hz, 6H, CH_2), 7.14 (s, 1H, NH), 7.31 (m, 6H, Pyridinone C5-H and 3OH), 8.25 (d, *J* = 7.0 Hz, 3H, Pyridinone C5-H), 8.75 (br, 3H, NH). ^{13}C NMR δ 13.39 (*C*H_3CH$_2$), 13.59 (*C*H_3CH$_2$), 18.81 (*C*H_2), 18.98 (*C*H_2), 29.31 (*C*H_2CO), 30.09 (*C*H_2), 32.85 (*C*H_2), 33.93 (CONH*C*), 37.88 (*C*H_2), 55.52 (N*C*H_2), 56.55 (NH*C*H_2-pyridinone), 111.61 (*C*-5H in pyridinone), 138.76 (*C*-2 in pyridinone), 139.38 (*C*-3 in pyridinone), 144.43 (*C*-6H in pyridinone), 159.90 (*C*-4 in pyridinone), 171.91 (*C*O), 173.27 (*C*O). ESI-MS: *m/z* 852 ($[M+H]^+$). ESI-HRMS: calcd. for $C_{44}H_{66}O_{10}N_7$ 852.4871, found 852.4837 ($[M+H]^+$).

5.2.8.9. ***13i*** *(R_1=$CH_3O(CH_2CH_2O)_2CH_2$, R=n-C_4H_9).* 0.99 g (0.94 mmol), yield: 94%. M.p. 127–128 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ 0.89 (t, *J* = 7.5 Hz, 9H, CH_3), 1.31 (m, 6H, CH_2), 1.72 (m, 6H, CH_2), 1.82 (m, 6H, CH_2), 2.08 (m, 6H, CH_2), 3.20 (s, 3H, CH_3), 3.40 (t, *J* = 5.0 Hz, 2H, CH_2), 3.53 (m, 6H, CH_2), 3.77 (s, 2H, CH_2), 4.39 (t, *J* = 7.5 Hz, 6H, CH_2), 4.51 (d, *J* = 5.0 Hz, 6H, CH_2), 6.81 (s, 1H, NH), 7.38 (d, *J* = 7.0 Hz, 3H, Pyridinone C5-H), 8.29 (d, *J* = 7.0 Hz, 3H, Pyridinone C6-H), 8.79 (t, *J* = 5.0 Hz, 3H, NH). ^{13}C NMR δ 13.40 (*C*H_3CH$_2$), 19.01 (CH$_3$*C*H$_2$), 29.13 (*C*H_2), 30.01 (*C*H_2), 32.87 (*C*H_2), 33.93 (CONH*C*), 55.48 (N*C*H_2CH$_2$), 56.74 (NH*C*H_2-pyridinone), 58.00 (O*C*H_3), 69.45 (O*C*H_2CO), 69.49 (*C*H_2), 69.74 (*C*H_2), 70.33 (*C*H_2), 71.15 (*C*H_2), 111.66 (*C*-5H in pyridinone), 138.67 (*C*-2 in pyridinone), 139.09 (*C*-3 in pyridinone), 144.58 (*C*-6H in pyridinone), 160.11 (*C*-4 in pyridinone), 168.49 (*C*O), 172.91 (*C*O). ESI-MS: *m/z* 942 ($[M+H]^+$). ESI-HRMS: calcd. for $C_{47}H_{72}O_{13}N_7$ 942.5188, found 942.5154 ($[M+H]^+$).

5.2.8.10. ***13j*** *(R_1=CH_3, R=n-C_6H_{13}).* 0.92 g (0.90 mmol), yield: 90%. M.p. 132–135 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ 0.84 (t, *J* = 7.0 Hz, 9H, CH_3), 1.26 (m, 18H, CH_2), 1.71–1.79 (m, 15H, 6CH_2 and CH_3 at 1.74), 2.05 (t, *J* = 7.0 Hz, 6H, CH_2), 4.35 (t, *J* = 7.5 Hz, 6H, CH_2), 4.50 (d, *J* = 5.5 Hz, 6H, CH_2), 7.20 (s, 1H, NH), 7.28 (d, *J* = 7.0 Hz, 3H, Pyridinone C5-H), 8.25 (d, *J* = 7.0 Hz, 3H, Pyridinone C6-H), 8.73 (t, *J* = 5.0 Hz, 3H, NH). ^{13}C NMR δ 13.78 (*C*H_3CH$_2$), 21.90 (*C*H_2), 23.43 (*C*H_3CO). 25.33 (*C*H_2), 29.24 (*C*H_2), 29.98 (*C*H_2), 30.67 (*C*H_2), 30.90 (*C*H_2), 33.90 (CONH*C*), 55.49 (N*C*H_2), 56.55 (NH*C*H_2-pyridinone), 111.62 (*C*-5H in pyridinone), 138.48 (*C*-2 in pyridinone), 138.65 (*C*-3 in pyridinone), 144.71 (*C*-6H in pyridinone), 160.61 (*C*-4 in pyridinone), 168.81 (*C*O), 173.05 (*C*O). ESI-MS: *m/z* 908 ($[M+H]^+$). ESI-HRMS: calcd. for $C_{48}H_{74}O_{10}N_7$ 908.5497, found 908.5469 ($[M+H]^+$); calcd. for $C_{48}H_{73}O_{10}N_7Na$ 930.5317, found 930.5286 ($[M+Na]^+$).

5.2.8.11. ***13k*** *(R_1=n-C_3H_7, R=n-C_6H_{13}).* 0.99 g (0.95 mmol), yield: 95%. M.p. 134–136 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ 0.81 (t, *J* = 7.5 Hz, 3H, CH_3), 0.84 (t, *J* = 7.0 Hz, 9H, CH_3), 1.29 (m, 18H, CH_2), 1.45 (m, 2H, CH_2), 1.72 (m, 6H, CH_2), 1.78 (m, 6H, CH_2), 1.99 (t, *J* = 7.5 Hz, 6H, CH_2), 2.06 (m, 6H, CH_2), 4.36 (t, *J* = 7.5 Hz, 6H, CH_2), 4.50 (d, *J* = 5.0 Hz, 6H, CH_2), 7.15 (s, 1H, NH), 7.31 (d, *J* = 7.0 Hz, 3H, Pyridinone C5-H), 8.26 (d, *J* = 7.0 Hz, 3H, Pyridinone C6-H), 8.76 (t, *J* = 5.0 Hz, 3H, NH). ^{13}C NMR δ 13.64 (*C*H_3), 13.78 (*C*H_3), 18.81 (*C*H_2), 21.90 (*C*H_2), 25.33 (*C*H_2), 29.26 (*C*H_2), 30.05 (*C*H_2CO), 30.67 (*C*H_2), 30.89 (*C*H_2), 33.90 (CONH*C*), 37.94 (*C*H_2), 55.49 (N*C*H_2), 56.47 (NH*C*H_2-pyridinone), 111.62 (*C*-5H in pyridinone), 138.51 (*C*-2 in pyridinone), 138.64 (*C*-3 in pyridinone), 144.70 (*C*-6H in pyridinone), 160.56 (*C*-4 in pyridinone), 171.71 (*C*O), 173.09 (*C*O). ESI-MS: *m/z* 936 ($[M+H]^+$). ESI-HRMS: calcd. for $C_{50}H_{78}O_{10}N_7$ 936.5810, found 936.5791 ($[M+H]^+$); calcd. for $C_{50}H_{77}O_{10}N_7Na$ 958.5630, found 958.5609 ($[M+Na]^+$).

5.2.8.12. ***13l*** *(R_1=$CH_3O(CH_2CH_2O)_2CH_2$, R=n-C_6H_{13}).* 1.04 g (0.92 mmol), yield: 92%. M.p. 106–108 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ 0.84 (t, *J* = 7.0 Hz, 9H, CH_3), 1.27 (m, 18H, CH_2), 1.72 (br, 6H, CH_2), 1.82 (m, 6H, CH_2), 2.07 (m, 6H, CH_2), 3.21 (s, 3H, CH_3), 3.40 (t, *J* = 5.0 Hz, 2H, CH_2), 3.52 (m, 6H, CH_2), 3.77 (s, 2H, CH_2), 4.35 (t, *J* = 7.5 Hz, 6H, CH_2), 4.49 (d, *J* = 5.0 Hz, 6H, CH_2), 6.80 (s, 1H, NH), 7.24 (d, *J* = 6.5 Hz, 3H, Pyridinone C5-H), 8.22 (d, *J* = 6.5 Hz, 3H, Pyridinone C6-H), 8.71 (t, *J* = 5.5 Hz, 3H, NH). ^{13}C NMR δ 13.77 (*C*H_3CH$_2$), 21.90 (*C*H_2), 25.33 (*C*H_2), 29.13 (*C*H_2), 30.00 (*C*H_2), 30.67 (*C*H_2), 30.90 (*C*H_2), 33.90 (CONH*C*), 55.41 (N*C*H_2CH$_2$), 56.73 (NH*C*H_2-pyridinone), 58.00 (O*C*H_3), 69.47 (O*C*H_2CO), 69.50 (O*C*H_2), 69.74 (O*C*H_2), 70.35 (O*C*H_2), 71.16 (O*C*H_2), 111.60 (*C*-5H in pyridinone), 138.17 (*C*-2 in pyridinone), 138.62 (*C*-3 in pyridinone), 144.79 (*C*-6H in pyridinone), 160.83 (*C*-4 in pyridinone), 168.46 (*C*O), 172.81 (*C*O). ESI-MS: *m/z* 1026 ($[M+H]^+$). ESI-HRMS: calcd. for $C_{53}H_{83}O_{13}N_7Na$ 1048.5947, found 1048.5929 ($[M+Na]^+$).

5.2.8.13. ***13m*** *(R_1=CH_3, R=CH_2CH_2OH).* 0.78 g (0.87 mmol), yield: 87%. ^{1}H NMR (500 MHz, DMSO-d_6) δ 1.73 (s, 3H, CH_3), 1.75 (m, 6H, CH_2), 2.02 (m, 6H, CH_2), 3.78 (t, *J* = 7.5 Hz, 6H, CH_2O), 4.51 (t, *J* = 7.5 Hz, 6H, CH_2N), 4.53 (d, *J* = 6.5 Hz, 6H, CH_2NH), 7.21 (s, 1H, NH), 7.34 (d, *J* = 7.5 Hz, 3H, Pyridinone C5-H), 8.20 (d, *J* = 7.5 Hz, 3H, Pyridinone C6-H), 8.77 (t, *J* = 7.5 Hz, 3H, NH). ^{13}C NMR δ 23.89 (CH_3CO), 30.77 (NH*C*), 31.26 (CH_2), 33.35 (CH_2), 52.83 (OCH_2), 55.15 (NCH_2), 56.37 (NH*C*H_2-pyridinone), 111.74 (*C*-5H in pyridinone), 138.56 (*C*-2 in pyridinone), 139.71 (*C*-3 in pyridinone), 145.48 (*C*-6H in pyridinone), 159.98 (*C*-4 in pyridinone), 168.40 (CO), 171.21 (CO). ESI-MS: *m/z* 788 ($[M+H]^+$). ESI-HRMS: calcd. for $C_{36}H_{50}O_{13}N_7$ 788.3467, found 788.3442 ($[M+H]^+$).

5.2.8.14. ***13n*** *(R_1=n-C_3H_7, R=CH_2CH_2OH).* 0.81 g (0.88 mmol), yield: 88%. ^{1}H NMR (500 MHz, DMSO-d_6) δ 0.81 (t, *J* = 7.5 Hz, 3H, CH_3), 1.44 (m, 2H, CH_2), 1.77 (m, 6H, CH_2), 1.99 (t, *J* = 7.5 Hz, 7.5 Hz, 2H, CH_2), 2.05 (m, 6H, CH_2), 3.73 (t, *J* = 5.0 Hz, 6H, CH_2), 4.51 (br, 6H, CH_2), 4.54 (d, *J* = 5.0 Hz, 6H, CH_2), 7.11 (s, 1H, NH), 7.27 (d, *J* = 6.0 Hz, 3H, Pyridinone C5-H), 8.18 (d, *J* = 6.0 Hz, 3H, Pyridinone C6-H), 8.71 (s, 3H, NH). ESI-MS: *m/z* 816 ($[M+H]^+$). ESI-HRMS: calcd. for $C_{38}H_{54}O_{13}N_7$ 816.3780, found 816.3751 ($[M+H]^+$).

5.2.8.15. ***13o*** *(R_1=$CH_3O(CH_2CH_2O)_2CH_2$, R=CH_2CH_2OH).* 0.93 g (0.92 mmol), yield: 92%. ^{1}H NMR (500 MHz, DMSO-d_6) δ 1.81 (m, 6H, CH_2), 2.06 (m, 6H, CH_2), 3.20 (s, 3H, CH_3), 3.52 (m, 8H, CH_2), 3.73 (t, *J* = 4.5 Hz, 6H, CH_2), 3.77 (s, 2H, CH_2), 4.54 (m, 12H, CH_2), 6.80 (s, 1H, NH), 7.31 (d, *J* = 7.0 Hz, 3H, Pyridinone C5-H), 8.18 (d, *J* = 7.0 Hz, 3H, Pyridinone C6-H), 8.74 (br, 3H, NH). ^{13}C NMR δ 29.06 (CH_2), 29.92 (CH_2), 34.19 (CONH*C*), 56.77 (N*C*H_2CH$_2$), 57.66 (NH*C*H_2-pyridinone), 57.98 (*C*H_3O), 60.31 (CO*C*H_2O), 69.44 (*C*H_2OH), 69.48 (*C*H_2O), 69.72 (*C*H_2O), 70.32 (*C*H_2O), 71.13 (*C*H_2O), 111.38 (*C*-5H in pyridinone), 137.16 (*C*-2 in pyridinone), 139.49 (*C*-3 in pyridinone), 144.36 (*C*-6H in pyridinone), 168.41 (*C*-4 in pyridinone), 171.98 (*C*O), 173.04 (*C*O). ESI-MS: *m/z* 906 ($[M+H]^+$). ESI-HRMS: calcd. for $C_{41}H_{59}O_{16}N_7Na$ 928.3916, found 928.3884 ($[M+Na]^+$).

5.2.8.16. ***13p*** *(R_1=CH_3, R=$CH_2CH_2OCH_3$).* 0.87 g (0.93 mmol), yield: 93%. ^{1}H NMR (500 MHz, DMSO-d_6). ^{1}H NMR (500 MHz, DMSO-d_6) δ 1.75 (s, 3H, CH_3), 1.77 (m, 6H, CH_2), 2.05 (m, 6H, CH), 3.23 (s, 9H, CH_3), 3.67 (t, *J* = 5.0 Hz, 6H, CH_2),4.51 (d, *J* = 5.0 Hz, 6H, CH_2), 4.62 (t, *J* = 5.0 Hz, 6H, CH_2), 7.18 (s, 1H, NH), 7.29 (d, *J* = 7.0 Hz, 3H, Pyridinone C5-H), 8.16 (d, *J* = 7.0 Hz, 3H, Pyridinone C6-H), 8.71 (s, 3H, NH). ^{13}C NMR δ 23.97 (CH_3CO), 33.35 (CNH), 35.14 (CH_2), 36.62 (CH_2), 54.33 (OCH_3), 55.59 (OCH_2), 56.54 (NCH_2), 58.69 (NH*C*H_2-pyridinone), 112.41 (*C*-5H in pyridinone), 138.32 (*C*-2 in

pyridinone), 138.80 (*C*-3 in pyridinone), 144.31 (*C*-6H in pyridinone), 159.12 (*C*-4 in pyridinone), 170.12 (CO), 170.53 (CO). ESI-MS: *m/z* 830 ($[M+H]^+$). ESI-HRMS: calcd. for $C_{39}H_{55}O_{13}N_7Na$ 852.3756, found 852.3721 ($[M+Na]^+$).

5.2.8.17. ***13q*** *(R_1=n-C_3H_7, R=$CH_2CH_2OCH_3$).* 0.88 g (0.91 mmol), yield: 91%. ^{1}H NMR (500 MHz, DMSO-d_6) δ 0.81 (t, *J* = 7.5 Hz, 3H, CH_3), 1.44 (m, 2H, CH_2), 1.77 (m, 6H, CH_2), 1.98 (t, *J* = 7.5 Hz, 2H, CH_2), 2.04 (m, 6H, CH_2), 3.23 (s, 9H, OCH_3), 3.66 (t, *J* = 5.0 Hz, 6H, CH_2), 4.50 (d, *J* = 5.0 Hz, 6H, CH_2), 4.58 (br, 6H, CH_2), 7.10 (s, 1H, NH), 7.18 (br, 3H, Pyridinone C5-H), 8.11 (br, 3H, Pyridinone C6-H), 8.64 (br, 3H, NH). ^{13}C NMR δ 13.63 (CH_3CH_2), 18.82 ($CH_3CH_2CH_2$), 29.25 (CH_2CO), 30.02 (CH_2), 34.03 (CH_2), 37.91 (CONHC), 54.80 (OCH_3), 56.57 ($NHCH_2$-pyridinone), 58.38 (CH_3OCH_2), 70.73 (NCH_2CH_2), 111.30 (*C*-5H in pyridinone), 138.15 (*C*-2 in pyridinone), 139.38 (*C*-3 in pyridinone), 144.62 (*C*-6H in pyridinone), 161.72 (*C*-4 in pyridinone), 171.82 (*C*O), 173.18 (*C*O). ESI-MS: *m/z* 858 ($[M+H]^+$). ESI-HRMS: calcd. for $C_{41}H_{59}O_{13}N_7Na$ 880.4069, found 880.4039 ($[M+Na]^+$).

5.2.8.18. ***13r*** *(R_1=$CH_3O(CH_2CH_2O)_2CH_2$, R=$CH_2CH_2OCH_3$).* 0.95 g (0.90 mmol), yield: 90%. ^{1}H NMR (500 MHz, DMSO-d_6) δ 1.82 (m, 6H, CH_2), 2.07 (m, 6H, CH_2), 3.21 (s, 3H, CH_3), 3.23 (s, 9H, CH_3), 3.40 (t, *J* = 5.0 Hz, 2H, CH_2), 3.52 (m, 6H, CH_2), 3.66 (t, *J* = 5.0 Hz, 6H, CH_2), 3.77 (s, 2H, CH_2), 4.51 (d, *J* = 5.0 Hz, 6H, CH_2), 4.60 (br, 6H, CH_2), 6.79 (s, 1H, NH), 7.23 (d, *J* = 7.5 Hz, 3H, Pyridinone C5-H), 8.13 (s, 3H, NH), 8.69 (d, *J* = 7.5 Hz, 3H, Pyridinone C6-H). ^{13}C NMR δ 29.26 (CH_2CO), 30.09 (CH_2), 34.22 (CH_2), 54.46 (OCH_2), 55.15 (NCH_2CH_2), 56.90 ($NHCH_2$-pyridinone), 58.07 (CH_3O), 58.48 (CH_3OCH_2), 69.51 (OCH_2), 69.72 (CH_2O), 70.36 (CH_2O), 70.73 (CH_2O), 71.15 (CH_2O), 111.26 (*C*-5H in pyridinone), 138.73 (*C*-2 in pyridinone), 139.32 (*C*-3 in pyridinone), 144.33 (*C*-6H in pyridinone), 160.88 (*C*-4 in pyridinone), 168.46 (*C*O), 172.89 (*C*O). ESI-MS: *m/z* 948 ($[M+H]^+$). ESI-HRMS: calcd. for $C_{44}H_{65}O_{16}N_7Na$ 970.4385, found 970.4343.

5.3. Physico-chemical properties of hexadentate HPOs

5.3.1. pKa determination

The titration system used in this determination comprised an autoburette (Metrohm Dosimat 765 l mL syringe) and a HP 8453 UV–visible spectrophotometer. 0.1 M KCl electrolyte solution was used to maintain the ionic strength. The temperature of the test solutions was maintained in a thermostatic cuvette holder at 25 ± 0.1 °C using a Cary 1 controller, An argon atmosphere was applied to the entire titration equipment. The initial sample concentration was approximately 7×10^{-5} M. pKa values were analysed from these data by pHab [30].

5.3.2. Determination of iron(III) affinity

The automatic titration system used in this study comprised of an autoburette (Metrohm Dosimat 765 l ml syringe) and Mettler Toledo MP230 pH meter with Metrohm pH electrode (6.0133.100) and a reference electrode (6.0733.100). 0.1 M KCl electrolyte solution was used to maintain the ionic strength. The temperature of the test solutions was maintained in a thermostatic jacketed titration vessel at 25 °C ± 0.1 °C by using a Techne TE-8J temperature controller. The solution under investigation was stirred vigorously during the experiment. A Gilson Mini-plus#3 pump with speed capability (20 mL/min) was used to circulate the test solution through a Hellem quartz flow cuvette. For stability constant determinations, a 50 mm path length cuvette was used and for pKa determinations, a cuvette path length of 10 mm was used. The flow cuvette was mounted on an HP 8453 UV–visible spectrophotometer. All instruments were interfaced to a computer and controlled by a Visual Basic program. Automatic titration and spectral scans adopted the following strategy: the pH of a solution was increased by 0.1 pH unit by the addition of KOH from the autoburette; when pH readings varied by <0.001 pH unit over a 3 s period, an incubation period was activated. For pKa determinations, a period of 1 min was adopted; for stability constant determinations, a period of 5 min was adopted. At the end of the equilibrium period, the spectrum of the solution was then recorded. The cycle was repeated automatically until the defined end point pH value was achieved. All the titration data were analysed with the pHab program [34]. The species plot was calculated with the HYSS program [35]. Analytical grade reagent materials were used in the preparation of all solutions.

5.4. Determination of antimicrobial activity

5.4.1. Bacterial culture

All chemicals were purchased from Sigma laboratories (Poole, Dorset, England), unless otherwise stated. Three clinical isolates of MRSA and three clinical isolates of *P. aeruginosa* (non-mucoid), isolated from patients with wound infections were used in the present study. These bacteria have been identified by using biochemical tests as the mentioned Standard Operating Procedures. They were kindly donated by Dr Tony Elston (Medical microbiology Department in Colchester Hospital University Foundation NHS Trust). Bacteria were grown without shaking in 10 mL Luria–Bertani (LB) broth medium overnight in a O_2 incubator at 37 °C. The Optical Density (OD) was measured at 595 nm to give a colony-forming unit (CFU)/ml of 10^8 [36].

5.4.2. Antimicrobial assays

To determine the antimicrobial activity of the iron chelators on the inhibition of bacterial growth, antimicrobial MBCs [17], and MICs assays were performed. In brief, bacterial isolates were grown in LB broth overnight at 37 °C and the OD was measured as described above. Experiments were performed in 96-well plates containing ~1×10^7 CFU/mL, either in the absence, or presence, of various concentrations of the iron chelators (for MBCs: 100, 200, 300, 400 and 500 μg/mL; and for MICs: 192, 256, 348, 512 and 768 μg/mL). Plates were incubated in the 37 °C O_2 incubator for 6 and 24 h and the number of viable bacteria was determined by their growth on a Cystine Lactose Electrolyte Deficient (CLED) agar. The bacterial inhibition rate was calculated as follows:

$$R = \frac{X_0 - X_t}{X_0} \times 100\%$$

where R is the bacterial inhibition rate, X_0 the number of bacteria without an iron chelator (control), and X_t the number of bacteria following treatment with an iron chelator.

The MBCs defined as the lowest concentration of antimicrobial that will prevent the growth of an organism after sub-culture on to antibiotic-free media, were determined by looking for the growth of bacterial colonies (normally around 100 bacteria). The MICs endpoint was determined as the lowest concentration of antimicrobial that will inhibit the visible growth of a micro-organism after overnight incubation using microdilution methodology recommended by the BSAC method [37,38]. Both MBC and MIC measurement were carried out in quadruplicate.

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