Synthesis of New 4(1*H*)-Pyridinone Derivatives and Their Antibacterial Activity

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*A series of 4(1H)-pyridinone derivatives were carried out by starting the reaction from 3-hydroxy-2-methyl/ethyl-4-pyrone. The structures of the synthesized compounds were confirmed by analytical and spectral data. All synthesized compounds were tested for their antibacterial activity against different microorganisms and compared with reference drugs. Compounds **13**, **14**, **16**, **17**, **19** and **22** were identified as effective against a variety of tested microorganisms.*

Keywords: Antimicrobial activity, ethyl maltol, maltol, 4(1H)-pyridinone, 4-pyrone

4(1*H*)-Pyridinone derivatives have attracted more attention due to their interesting pharmacological properties. Molecules containing this general structure possess antibacterial [1-3], antifungal [3, 4], antimalarial [5,6], cardiotonic [7], antineoplastic [8-10], analgesic-antiinflammatory [1, 11-14] activities, and they are used for the treatment of Parkinson's [15, 16] and Thalassemia's [17, 18] diseases. Although the compounds having 4(1*H*)-pyridinone have potential pharmacological usefulness, one of the most important research topic is the chelating of this structures. 3-hydroxy-4-pyridinones are bidentate chelating compounds used for removal therapies of unbalanced toxic hard metal ions in the body (e.g.: Fe^{3+}, Al^{3+})[19-21] and also for clinical diagnosis and chemotherapy through their complexes with radionuclides (e.g.: $^{111}In^{3+}$, $^{67}Ga^{3+}$) [22, 23].

It has been known that iron has a vital role in microorganism life cycle to survive the existence. Compounds that have capability of forming chelates have removed the iron ion in environment and caused the extermination. The hydroxypyridinones (N-heterocycles with a hydroxyl group *ortho* to the ketone group) are known to have a strong interaction with Fe^{3+}. Among them, the 3-hydroxy-4-pyridinones are the most effective in the neutral *p*H range [24-26]. One of those compounds (1,2-dimethyl-3-hydroxy-4-pyridinone (DMHP), Deferiprone®) has even been proposed as an orally active clinical chelating agent [27].

In view of these facts we designed a series of new antimicrobial compounds carrying 4(1*H*)-pyridinone framework and N-substituted cyclic amino propylene moiety. Three of the compounds **16, 17, 21** were synthesized before in literature [28, 29], but for the integrity of the antimicrobial effects of this structural serial we incorporated these compounds to our synthesis procedure.

Experimental part

Materials and methods

All chemicals were supplied from Aldrich and Merck Chemicals Co. Melting points (°C) were determined in a Thomas Hoover capillary melting point apparatus (Philadephia, PA, USA) and are uncorrected. Infrared spectra were recorded on a Bruker Vector 22 (Opus Spectroscopic Software Version 2.0) (Bruker Analytische Messtechnik, Karlruhe, Germany), using potassium bromide pellets, the frequencies are expressed in cm^{-1}. The ^{1}H-NMR spectra were obtained by Bruker AC 80 MHz (Karlsruhe, Germany) and Bruker Avance DPX-400 MHz FT NMR (Bruker, Rheinstetten, Germany) instruments using chloroform-d_1 or dimethylsulphoxide-d_6 (Merck) as solvent and tetramethylsilane as internal standard. Splitting patterns were designated as follows; s: singlet, d: doublet, t: triplet, q: quartet and m: multiplet. All chemical shift values were recorded as δ (ppm). The purity of the compounds was controlled by thin layer chromatography (Merck, silica gel, HF254 Typ 60). Elemental analyses were performed on a Leco CHNS 932 analyzer (Philadephia, PA, USA) at Scientific and Technical Research Council of Turkey, Instrumental Analysis Laboratory in Ankara.

Synthesis of 2-alkyl -3-benzyloxy-4-pyrone (1, 2)

2-Methyl/ethyl-3-hydroxy-4-pyrone (0.01 mol) and benzyl chloride (0.02 mol) were dissolved in methanol which become basic with 2 M sodium hydroxide, and refluxed for 5 h. At the end of this period, dark colored reaction mixture was allowed to cool, condensed in evaporator, poured into water, extracted with dichloromethane (3x30 mL). The organic phase was washed with water (3x10 mL) and sodium hydroxide solution (%5) (1x10 mL) respectively, dried with anhydrous Na_2SO_4 and evaporated. Recrystallization of the resulting solid from ethanol-ether mixture provided compound **1-2** [1, 30, 31].

Synthesis of 1,2-disubstituted-3-benzyloxy-4(1H)-pyridinones (3-12)

2-Methyl/ethyl-3-benzyloxy-4-pyrone **1, 2** (0.0043 mol) and appropriate primary amine (0.0065 mol) were dissolved in 50 mL of ethanol:water (1:1) mixture. The *p*H of the solution was adjust to *p*H= 13.5 with 2 M sodium hydroxide and refluxed for 24 h. When the reaction completed, *p*H of the reaction mixture was arranged to *p*H=1 by adding glacial hydrochloric acid, then evaporated to dryness. 50 mL of water was added to the residue and washed with ether (2x50 mL). The pH of the water phase was fixed to 9 with 10 M sodium hydroxide and then extracted with dichloromethane (4x50 mL). Organic phases were collected, dried with anhydrous Na_2SO_4, filtered and evaporated in vacuum. Yielded brown oily compound was purified by using column chromatography (mobile phase, chloroform: methanol, 8:2).

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Synthesis of N-substituted-2-methyl/ethyl-3-hydroxy-4(1H)-pyridinones (13-22)

10 mL of hydrobromic acid (% 48) solution was added to 1, 2-disubstituted-3-benzyloxy-4(1*H*)-pyridinone derivative (0.01 mol) and refluxed for 4 h. Then hydrogen bromide solution was removed under vacuum. The brown colour residue was treated with charcoal in ethanol, heated, filtered and evaporated to dryness. Recrystallization of the product from appropriate solvent gave pure N-substituted-2-methyl/ethyl-3-hydroxy-4(1H)-pyridinone derivative.

Microbiology

The antibacterial activities of the synthesized compounds against various bacteria were tested by using disc-diffusion [32] and microdilution assay [33].

Disc diffusion assay

The synthesized and lyophilized compounds were dissolved in dimethylsulfoxide (DMSO) to a final concentration of 20 mg/mL and sterilized by filtration by 0.45 m millipore filters. Antimicrobial tests were then carried out by disc diffusion method using 100 µL of suspension containing 10^8 CFU/mL of bacteria spread on nutrient agar (NA). The discs (6 mm in diameter) were impregnated with 15 mL of each compounds (300 mg/disc) at the concentration of 20 mg/ mL and placed on the inoculated agar. DMSO impregnated discs were used as negative controls. Ofloxacin (10 µg/disc), sulbactam (30 µg)+cefoperazone (75 µg) (105 µg/disc) and/or netilmicin (30 mg/disc) were used as positive reference standards to determine the sensitivity of one strain/isolate in each microbial species tested. The inoculated plates were incubated at 37°C for 24 h for bacterial strains. Plant associated microorganisms were incubated at 27°C. Antimicrobial activity in disc diffusion assay was evaluated by measuring the zone of inhibition against the test organisms. Each assay in this experiment was repeated twice.

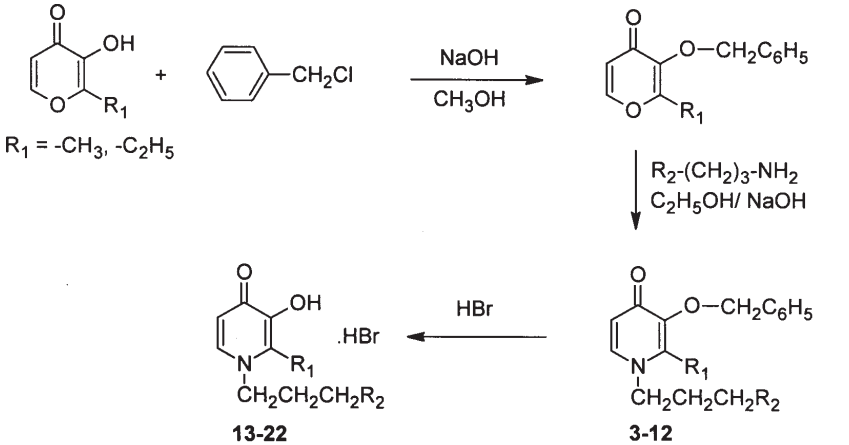
Microdilution assays

The minimal inhibition concentration (MIC) values were also studied for the microorganisms the compounds which show inhibiton in disc diffusion assay. The inocula of microorganisms were prepared from 12 h broth cultures and suspensions were adjusted to 0.5 McFarland standard turbidity. The test compounds dissolved in dimethylsulfoxide (DMSO) were first diluted to the highest concentration (600 µg/mL) to be tested, and then serial twofold dilutions were made in a concentration range from 9.37 to 600 µg/mL in 10 mL sterile test tubes containing nutrient broth. MIC values of each compound against bacterial strains were determined based on a micro-well dilution method with some modifications. The 96-well plates were prepared by dispensing into each well 95 µL of nutrient broth and 5 µL of the inoculum. A 100 µL from each of the test compounds initially prepared at the concentration of 600 µg/mL was added into the first wells. Then, 100 mL from their serial dilutions was transferred into six consecutive wells. The last well containing 195 µL of nutrient broth without compound and 5 µL of the inoculum on each strip was used as negative control. The final volume in each well was 200 µL. Maxipime (Bristol-Myers Squibb) at the concentration range of 500-7.8 µg/mL was prepared in nutrient broth and used as standard drug for positive control. The plate was covered with a sterile plate sealer. Contents of each well were mixed on plate shaker at 300 rpm for 20 s and then incubated at appropriate temperatures for 24 h. Microbial growth was determined by absorbance at 600 nm using the ELx 800 universal microplate reader (Biotek Instrument inc, Highland Park, Vermont, USA). All of the compounds tested in this study were screened two times against each microorganism. The MIC was defined as the lowest concentration of the compounds to inhibit the growth of microorganisms.

Results and discussion

A series of novel 3-benzyloxy-N-substituted-4(1*H*)-pyridinone and 2-ethyl-3-benzyloxy-N-substituted-4(1*H*)-pyridinone, 3-hydroxy-2-methyl-N-substituted-4(1*H*)-pyridinone and 2-ethyl-3-hydroxy-N-substituted-4(1*H*)-pyridinone were synthesized by using ANRORC (addition nucleophile ring opening ring closure) mechanism (scheme 1).

The basic structures of the isolated compounds two of which were starting and twenty of which were target compounds were characterized by IR and ^{1}H-NMR spectral data. The IR spectrum of compounds displayed a strong band in range of 1630-1657 cm^{-1} assignable to C=O group, a 1298-1252 cm^{-1} band, characteristic of C-O-C group and a broad 3462-3178 cm^{-1} band indicative C-OH functional group. ^{1}H-NMR spectra of all compounds showed the characteristic doublets of pyridone at aromatic fields. N-alkyl side chain placed as a triplet at 4.51-3.30 ppm for N-CH_2-, a multiplet signal at 3.36-1.18 ppm for N-CH_2CH_2-CH_2-, a triplet signal at 4.26-2.18 ppm for N-CH_2CH_2-CH_2. The results of elemental analysis were within ± 0,4% of the theoretical values.



R_2 = pyrolidine, 2-oxopyrolidine, 2-methylpiperidine, morpholine and imidazole

Scheme 1. The general synthesis of the compounds

***3-Benzyloxy-2-methyl-4-pyrone* (1)**

Yield: 80 %. M.p. 166-167 °C. ^{1}H-NMR (80 MHz; $CDCl_3$) δ= 2.00-2.10 (s; 3H; -CH_3), 5.10-5.20 (s; 2H; -OCH_2-), 6.30-6.50 (d; 2H; pyrone H^5, H^6), 7.30-7.80 (m; 5H; -C_6H_5).

***3-Benzyloxy-2-ethyl-4-pyrone* (2)**

Yield: 75%. M.p. 23-24 °C. ^{1}H-NMR (80 MHz; $CDCl_3$) δ= 2.00-2.10 (s; 3H; -CH_2CH_3), 3.10-3.20 (q; 2H, -CH_2CH_3), 5.10-5.20 (s; 2H; -OCH_2-), 6.10-6.40 (d; 2H; pyrone H^5, H^6), 7.50-7.90 (m; 5H; -C_6H_5).

***2-Methyl-1-[3-(2-oxopyrolidine-1-yl)propyl]-3-benzyloxy-4(1H)-pyridinone* (3)**

Yield: 84 %. Liq. ^{1}H-NMR (400 MHz; $CDCl_3$) δ= 1.74-1.81 (m; 2H, N-$CH_2CH_2CH_2$), 1.87-1.94 (m; 2H; pyrrolidone H^4), 2.16 (s; 3H; -CH_3), 2.19-2.23 (t; 2H; pyrrolidone H^5), 2.5 (s; 3H; pyrrolidone H^3), 3.18-3.21 (t; 2H; N-$CH_2CH_2CH_2$), 3.81-3.85 (t; 2H; N-$CH_2CH_2CH_2$), 5.02 (s; 2H; -OCH_2-), 6.13-6.15 (d; 1H; pyridinone H^5), 7.31-7.41 (m; 5H; C_6H_5), 7.62-7.64 (d; 1H; pyridinone H^6).

***3-Benzyloxy-2-methyl-1-[3-(pyrolidine-1-yl)propyl]-4(1H)-pyridinone* (4)**

Yield: 48 %. Liq. ^{1}H-NMR (400 MHz; $CDCl_3$) δ= 2.04 (s; 3H; -CH_3), 2.32-2.37 (m; 2H; pyrolidine H^4), 2.42-2.5 (m; 2H; pyrolidine H^3), 2.79-2.87 (m; 4H; pyrolidine H^2 and H^5), 3.22-3.27 (m; 2H, N-$CH_2CH_2CH_2$-), 3.44-3.47 (t; 2H; N-$CH_2CH_2CH_2$), 4.0-4.5 (t; 2H; N-$CH_2CH_2CH_2$),5.06 (s; 2H; -OCH_2-) 6.07-6.09 (d; 1H; pyridinone H^5), 7.26-7.37 (m; 5H; C_6H_5), 7.60-7.62 (d; 1H; pyridinone H^6).

***3-Benzyloxy-2-methyl-1-[3-(2-methylpiperidine-1-yl)propyl]-4(1H)-pyridinone* (5)**

Yield: 39 %. Liq. ^{1}H-NMR (400 MHz; $CDCl_3$) δ= 0.90 (s; 3H; piperidine 2-CH_3), 1.16-1.22 (m; 5H; N-CH_2-CH_2-CH_2 and 2-CH_3), 1.45-1.70 (m; 8H; piperidine H^3, H^4, H^5, H^6), 2.47-2.53 (m; 2H; N$CH_2CH_2CH_2$-), 2.61-2.65 (q; 1H; piperidine 2-C*H*), 3.54-3.65 (m; 2H; N-$CH_2CH_2CH_2$), 5.17 (s; 2H; OCH_2), 6.20-6.22 (d; 1H; pyridinone H^5), 7.18-7.23 (m; 5H; C_6H_5), 7.32-7.34 (d; 1H; pyridinone H^6)

***3-Benzyloxy-1-[3-(imidazole-1-yl)propyl]-2-methyl-4(1H)-pyridinone* (6)**

Yield: 67 %. Liq. ^{1}H-NMR (400 MHz; $CDCl_3$) δ= 1.87-1.91 (s; 3H, -CH_3), 2.14-2.21 (m; 2H; N-CH_2-CH_2-CH_2), 3.62-3.66 (t; 2H; N $CH_2CH_2CH_2$-), 3.84-3.87 (t; 2H; N-$CH_2CH_2CH_2$), 5.18 (s; 2H; OCH_2), 6.29-6.31 (d; 1H; pyridinone H^5), 6.75 (s; 1H; imidazolyl H^4), 6.88-6.90 (d; 1H; pyridinone H^6), 7.09 (s; 1H; imidazolyl H^5), 7.19-7.26 (m; 5H; C_6H_5), 7.37 (s; 1H; imidazolyl H^2)

***3-Benzyloxy-2-methyl-1-[3-(morpholine-4-yl)propyl]-4(1H)-pyridinone* (7)**

Yield: 21 %. Liq. ^{1}H-NMR (80 MHz; $CDCl_3$) δ= 1.60-1.80 (m; 2H; N-CH_2-CH_2-CH_2- and morpholinyl H^3, H^5), 2.10 (s; 3H; -CH_3), 3.50-3.70 (m, 6H, N $CH_2CH_2CH_2$-and morpholinyl H^2, H^6), 3.80-4.00 (t; 2H; N-$CH_2CH_2CH_2$) 5.10 (s; 2H; OCH_2), 6.20 (d; 1H; pyridinone H^5), 7.10-7.30 (m; 5H; C_6H_5), 7.40 (d; 1H; pyridinone H^6)

***3-Benzyloxy-2-ethyl-1-[3-(2-oxopyrolidine-1-yl)propyl]-4(1H)-pyridinone* (8)**

Yield: 86 %. Liq. ^{1}H-NMR (400 MHz; $CDCl_3$) δ= 0.98-1.02 (t; 3H; -CH_2-CH_3), 1.76-1.84 (m; 2H; N-CH_2-CH_2-CH_2-), 1.87-1.95 (m; 2H; pyrolidinone H^4), 2.19-2.23 (t; 2H; pyrolidinone H^5), 2.50-2.51 (t; 2H; pyrolidinone H^3), 2.54-2.60 (q; 2H; -CH_2-CH_3), 3.19-3.23 (t; 2H; N-$CH_2CH_2CH_2$), 3.81-3.85 (t; 2H; N-$CH_2CH_2CH_2$), 5.09 (s; 2H; OCH_2), 6.15-6.17 (d; 1H; pyridinone H^5), 7.29-7.42 (m; 5H; C_6H_5), 7.62-7.64 (d; 1H; pyridinone H^6)

***3-Benzyloxy-2-ethyl-1-[3-(pyrolidine-1-yl)propyl]-4(1H)-pyridinone* (9)**

Yield: 32 %. Liq. ^{1}H-NMR (400 MHz; $CDCl_3$) δ= 1.98 (s; 3H; -CH_3), 2.22-2.26 (m; 2H; pyrolidine H^4), 2.42-2.47 (m; 2H; pyrolidine H^3), 2.71-2.80 (m; 4H; pyrolidine H^2, H^5), 3.18-3.23 (m; 2H; N-CH_2-CH_2-CH_2-), 3.46-3.50 (t, 2H, N $CH_2CH_2CH_2$-), 3.94-3.99 (t; 2H; N-$CH_2CH_2CH_2$), 5.03 (s; 2H; OCH_2), 6.08-6.10 (d; 1H; pyridinone H^5), 7.23-7.35 (m; 5H; C_6H_5), 7.58-7.56 (d; 1H; pyridinone H^6)

***3-Benzyloxy-2-ethyl-1-[3-(2-methylpiperidine-1-yl)propyl]-4(1H)-pyridinone* (10)**

Yield: 35 %. Liq. ^{1}H-NMR (400 MHz; $CDCl_3$) δ= 0.93 (s; 3H; piperidine 2-CH_3), 0.95-0.99 (t; 3H; -CH_2-CH_3), 1.18-1.23 (m; 2H; N-CH_2-CH_2-CH_2-), 1.49-1.75 (m; 8H; piperidine H^3, H^4, H^5, H^6), 2.08-2.13 (q; 2H; -CH_2-CH_3), 2.52-2.58 (m; 2H; N-$CH_2CH_2CH_2$), 2.65-2.69 (q; 1H; piperidine 2-CH), 3.66-3.79 (m; 2H; N-$CH_2CH_2CH_2$), 5.21 (s; 2H; OCH_2), 6.33-6.35 (d; 1H; pyridinone H^5), 7.17-7.27 (m; 5H; C_6H_5), 7.36-7.38 (d; 1H; pyridinone H^6)

***3-Benzyloxy-2-ethyl-1-[3-(imidazole-1-yl)propyl]-4(1H)-pyridinone* (11)**

Yield: 79 %. Liq. ^{1}H-NMR (400 MHz; $CDCl_3$) δ= 0.86-0.90 (t; 3H; -CH_2CH_3), 2.06-2.13 (m; 2H; N-CH_2-CH_2-CH_2-), 2.29-2.34 (q; 2H; -CH_2-CH_3), 3.62-3.66 (t; 2H; N-$CH_2CH_2CH_2$), 3.89-3.92 (t; 2H; N-$CH_2CH_2CH_2$), 5.21 (s; 2H; OCH_2), 6.33-6.35 (d; 1H; pyridinone H^5), 6.82 (s; 1H; imidazole H^4), 6.97-6.99 (d; 1H; pyridinone H^6), 7.06 (s; 1H; imidazole H^5), 7.21-7.27 (m; 5H; C_6H_5), 7.39 (s; 1H; imidazole H^2)

***3-Benzyloxy-2-ethyl-1-[3-(morpholine-4-yl)propyl]-4(1H)-pyridinone* (12)**

Yield: 26 %. Liq. ^{1}H-NMR (400 MHz; DMSO-d_6) δ= 0.98-1.01 (t; 3H; -CH_2CH_3), 1.73-1.79 (m; 2H; N-CH_2-CH_2-CH_2-), 2.18-2.21 (t; 2H; N-$CH_2CH_2CH_2$), 2.28-2.43 (m; 4H, morpholine H^3, H^5), 2.58-2.63 (q; 2H; -CH_2-CH_3), 3.46-3.50 (m; 4H, morpholine H^2, H^6), 3.88-3.91 (t; 2H; N-$CH_2CH_2CH_2$), 5.08 (s; 2H; OCH_2), 6.18-6.20 (d; 1H; pyridinone H^5), 7.32-7.40 (m; 5H; C_6H_5), 7.60-7.62 (d; 1H; pyridinone H^6)

***3-Hydroxy-2-methyl-1-[3-(2-oxopyrolidine-1-yl)propyl]-4(1H)-pyridinone hydrobromide* (13)**

Yield: 73 %. M.p. 190-191°C. IR (KBr): ν= 3410, 2553 1657, 1261 O-H/ -N^+= / C=O /C-O cm^{-1}. ^{1}H-NMR (400 MHz; DMSO-d_6) δ= 1.89-1.99 (m; 4H; N-CH_2-CH_2-CH_2- and pyrolidine H^4), 2.21-2.25 (t; 2H; pyrolidine H^3), 2.53 (s; 3H; -CH_3), 3.24-3.27 (t; 2H; N-$CH_2CH_2CH_2$), 3.33-3.37 (t; 2H; pyrolidine H^5), 4.27-4.31 (t; 2H; N-$CH_2CH_2CH_2$), 7.10-7.11 (d; 1H; pyridinone H^5), 8.25-8.27 (d; 1H; pyridinone H^6). %$C_{10}H_{20}Br_2N_2O_3$ (412.12): calcd. C 37.89, H 4.89, N 6.80; found C 37.52, H 4.71, N 7.24.

***3-Hydroxy-2-methyl-1-[3-(pyrolidine-1-yl)propyl]-4(1H)-pyridinone hydrobromide* (14)**

Yield: 44 %. M.p. 239-240 °C. IR (KBr): ν = 3395, 2702, 1637, 1283 O-H/ -N^+= / C=O /C-O cm^{-1}. ^{1}H-NMR (400 MHz; DMSO-d_6) δ = 1.89-1.92 (m; 2H; pyrolidine H^5), 1.96-2.04 (m; 2H; pyrolidine H^4), 2.12-2.20 (m; 2H; pyrolidine H^3), 2.51 (s; 3H; -CH_3), 2.99-3.03 (m; 2H; pyrolidine H^2), 3.19-3.21 (m; 2H; N-CH_2-CH_2-CH_2-), 3.43-3.53 (q; 2H; N-$CH_2CH_2CH_2$), 4.39-4.43 (t; 2H; N-$CH_2CH_2CH_2$), 7.07-7.09 (d; 1H; pyridinone H^5), 8.21-8.23 (d; 1H; pyridinone H^6), 9.81 (O-H). %$C_{13}H_{22}Br_2N_2O_2$ (398.14): calcd. C 39.22, H 5.57, N 7.04; found C 39.59, H 5.75, N 7.10.

***3-Hydroxy-2-methyl-1-[3-(2-methylpiperidine-1-yl)propyl]-4(1H)-pyridinone hydrobromide* (15)**

Yield: 42 %. M.p. 186-187 (dec.) °C. IR (KBr): ν = 3384, 2673, 1639, 1285 O-H/ -N^+= / C=O /C-O cm^{-1}. ^{1}H-NMR (400 MHz; DMSO-d_6) δ = 1.12 -1.19 (d; 3H; piperidine 2-CH_3), 1.31-1.78 (m; 6H; piperidine H^3, H^4, H^5), 1.93-2.04 (m; 2H; piperidine H^6), 2.26 (s; 3H; -CH_3), 2.82-2.90 (q; 1H; piperidine 2-CH), 2.94-3.02 (m; 2H; N-CH_2-CH_2-CH_2-), 3.07-3.20 (m; 2H; N-$CH_2CH_2CH_2$), 3.30-3.41 (m; 2H; N-$CH_2CH_2CH_2$), 6.54-6.56 (d; 1H; pyridinone H^5), 7.84-7.86 (d; 1H; pyridinone H^6), 9.07 (O-H). %$C_{15}H_{26}Br_2N_2O_2$ (426.19):

calcd. C 42.27, H 6.15, N 6.57; found C 41.80, H 6.63, N 6.35.

***3-Hydroxy-1-[3-(imidazole-1-yl)propyl]-2-methyl-4(1H)-pyridinone hydrobromide* (16)**

Yield: 71 %. M.p. 179-180 °C. IR (KBr): ν = 3462, 2547, 1637, 1290 O-H/ -N$^+$= / C=O /C-O cm^{-1}. ^{1}H-NMR (400 MHz; DMSO-d$_6$) δ= 2.24-2.31 (m; 2H; N-CH$_2$-C*H$_2$*-CH$_2$), 2.41 (s; 3H, -CH$_3$), 4.22-4.26 (t; 2H; N CH$_2$CH$_2$C*H$_2$*-), 4.32-4.36 (t; 2H; N-C*H$_2$*CH$_2$CH$_2$), 7.05-7.07 (d; 1H; pyridinone H^5), 7.64 (s; 1H; imidazolyl H^4), 7.77 (s; 1H; imidazolyl H^5),8.17-8.19 (d; 1H; pyridinone H^6), 9.14 (s; 1H; imidazolyl H^2). %$C_{12}H_{17}Br_2N_3O_2$ (395.09): calcd. C 36.48, H 4.34, N 10.64; found C 36.92, H 4.73, N 10.97.

***2-Ethyl-3-hydroxy-1-[3-(morpholine-4-yl)propyl]-4(1H)-pyridinone hydrobromide* (17)**

Yield: 65 %. M.p. 248-249(dec.) °C. IR (KBr): ν = 2724, 1637, 1263 O-H/ -N$^+$= / C=O /C-O cm^{-1}. ^{1}H-NMR (400 MHz; DMSO-d$_6$) δ = 1.99-2.07 (m; 2H; N-CH$_2$-C*H$_2$*-CH$_2$-), 2.30 (s; 3H; -CH$_3$), 3.00-3.04 (t; 2H; N-CH$_2$CH$_2$C*H$_2$*), 3.04-3.24 (m; 4H; morpholinyl H^3, H^5), 3.56-3.76 (m; 4H; morpholinyl H^2, H^6) 4.22-4.26 (t, 2H, N-C*H$_2$*CH$_2$CH$_2$ -), 6.92-6.94 (d; 1H; pyridinone H^5), 8.06-8.08 (d; 1H; pyridinone H^6), 9.89 (O-H). %$C_{13}H_{22}Br_2N_2O_3$ (414.13): calcd. C 37.70, H 5.35, N 6.76; found C 37.52, H 5.14, N 6.35.

***2-Ethyl-3-hydroxy-1-[3-(2-oxopyrolidine-1-yl)propyl]-4(1H)-pyridinone hydrobromide* (18)**

Yield: 69 %. M.p. 260-262 °C. IR (KBr): ν = 3387, 1657, 1630, 1252 O-H/ -N$^+$= / C=O /C-O cm^{-1}. ^{1}H-NMR (400 MHz; DMSO-d$_6$) δ = 1.84-1.88 (m; 2H; N-CH$_2$-C*H$_2$*-CH$_2$-), 1.90-1.94 (m; 2H; pyrolidinone H^4), 2.20-2.24 (t; 2H; pyrolidinone H^3), 2.68-2.74 (q; 2H; -C*H$_2$*-CH$_3$), 3.22-3.25 (t; 2H; N-CH$_2$CH$_2$C*H$_2$*), 3.32-3.35 (t; 2H; pyrolidinone H^5), 3.94-3.98 (t; 2H; N-C*H$_2$*CH$_2$CH$_2$), 6.28-6.30 (d; 1H; pyridinone H^5), 7.71-7.73 (d; 1H; pyridinone H^6). %$C_{14}H_{22}Br_2N_2O_3$ (426.15): calcd. C 39.46, H 5.20, N 6.57; found C 40.01, H 5.66, N 6.75.

2-Ethyl-3-hydroxy-1-[3-(pyrolidine-1-yl)propyl]-4(1H)-pyridinone hydrobromide (19)

Yield: 55 %. M.p. 208-209 °C. IR (KBr): ν = 3178, 2684, 1636, 1298 O-H/ -N$^+$= / C=O /C-O cm^{-1}. ^{1}H-NMR (400 MHz; DMSO-d$_6$) δ = 1.23-1.27 (t; 3H; -CH$_2$C*H$_3$*), 1.77-1.98 (m; 2H; pyrolidine H^5), 2.03-2.11 (m; 2H; pyrolidine H^4), 2.21-2.29 (m; 2H; pyrolidine H^3), 2.99-3.04 (q; 2H; -C*H$_2$*CH$_3$), 3.06-3.11 (q; 2H; pyrolidine H^2), 3.31-3.36 (m; 2H; N-CH$_2$-C*H$_2$*-CH$_2$-), 3.51-3.61(q; 2H; N-CH$_2$CH$_2$C*H$_2$*), 4.48-4.51 (t; 2H; N-C*H$_2$*CH$_2$CH$_2$), 7.22-7.23 (d; 1H; pyridinone H^5), 8.35-8.40 (d; 1H; pyridinone H^6), 9.99 (O-H). %$C_{14}H_{24}Br_2N_2O_2$ (412.16): calcd. C 39.09, H 6.09, N 6.51; found C 39.18, H 5.77, N 6.59.

2-Ethyl-3-hydroxy-1-[3-(2-methylpiperidine-1-yl)propyl]-4(1H)-pyridinone hydrobromide (20)

Yield: 40 %. M.p. 197-198 °C. IR (KBr): ν = 3278, 2675, 1643, 1292 O-H/ -N$^+$= / C=O /C-O cm^{-1}. ^{1}H-NMR (400 MHz; DMSO-d$_6$) δ = 0.92-0.95 (t; 3H; 2-CH$_2$C*H$_3$*), 1.08-1.10 (d; 3H; piperidine 2-CH$_3$), 1.53-1.62 (m; 6H; piperidine H^3, H^4, H^5), 1.91-1.95 (t, 2H, piperidine H^6), 2.67-2.73 (q; 2H; -C*H$_2$*-CH$_3$), 2.90-2.99 (m; 4H; N-CH$_2$C*H$_2$*C*H$_2$*), 3.94-3.98 (q; 1H; piperidine 2-CH), 4.15-4.18 (t; 2H; N-C*H$_2$*CH$_2$CH$_2$), 6.86-6.87 (d; 1H; pyridinone H^5), 8.08-8.10 (d; 1H; pyridinone H^6), 9.34 (O-H). %$C_{16}H_{28}Br_2N_2O_2$ (440.22): calcd. C 43.65, H 6.41, N 6.36; found C 43.14, H 7.01, N 5.93.

2-Ethyl-3-hydroxy-1-[3-(imidazole-1-yl)propyl]-4(1H)-pyridinone hydrobromide (21)

Yield: 53 %. M.p. >260 °C. IR (KBr): ν = 3457, 2544, 1635, 1285 O-H/ -N$^+$= / C=O /C-O cm^{-1}. ^{1}H-NMR (80 MHz; DMSO-d$_6$) δ = 2.40-2.60 (s; 3H, -CH$_3$), 3.30-3.70 (m; 2H, -C*H$_2$*CH$_3$), 4.20-4.60 (m; 6H; -C*H$_2$*C*H$_2$*C*H$_2$*), 7.00-7.20 (d; 1H; pyridinone H^5), 7.60-7.90 (d; 2H; imidazolyl H^4, H^5), 8.20-

Table 1

ANTIBACTERIAL ACTIVITY RESULTS OF THE ACTIVE COMPOUNDS AGAINST THE BACTERIAL STRAINS TESTED

Test microorganisms	Compounds												Antibiotics[a]	
	13		14		16		17		19		22		DD[b]	MIC[c, d]
Acinetobacter baumanii-A8													18 (OFX)	31.25
Acinetobacter lwoffi F1													24 (OFX)	62.50
Bacillus macerans A199			10	250									19 (OFX)	15.62
Bacillus megaterium A59													9 (SCF)	15.62
*Bacillus subtilis*ATCC6633	8	250	12	250					10	250	8	250	28 (OFX)	62.50
Bacillus subtilis A57													28 (OFX)	125
Brucella abortus A77													12 (SCF)	62.50
Burkholdria cepacia A225													22 (SCF)	125
Clavibacter michiganense A227			10	125			8	500					25 (SCF)	16.62
Cedecea davisae F2													14 (OFX)	62.50
Enterobacter cloacae A135													20 (NET)	31.25
Enterococcus faecalis ATCC29122													18 (SCF)	31.25
Ecsherichia coli A1			10	250					8	500			- (OFX)	62.50
Klebsiella pneumoniae F3			8	250			8	250	8	250			12 (OFX)	125
Klebsiella pneumoniae A137							10	250					12 (OFX)	125
Morgonella morganii F4			12	125									14 (OFX)	125
Proteus vulgaris A161			10	250	10	250			8	500			12 (OFX)	125
Proteus vulgaris KUKEM1329													13 (OFX)	125
Pseudomonas aeruginosa ATCC9027			12	125					10	250			22 (NET)	31.25
Pseudomonas aeruginosa ATCC27859													22 (NET)	15.62
Pseudomonas aeruginosa F5	10	250	12	62.5					10	250	10	250	18 (NET)	125
Pseudomonas pseudoalkaligenes F6													18 (NET)	125
Pseudomonas syringae pv. tomato A35													24 (OFX)	125
Salmonella cholerasuis arizonae F7			10	125									14 (NET)	250
Salmonella enteritidis ATCC13076													27 (SCF)	62.50
Serratia plymuthica F8													16 (NET)	125
Shigella sonnei F9			10	250									24 (NET)	31.25
Staphylococcus aureus A215			12	125					10	250			22 (SCF)	31.25
Staphylococcus aureus ATCC29213													22 (SCF)	62.50
Staphylococcus epidermis A233			10	125									- (SCF)	15.62
Staphylococcus hominis F10			12	62.5									- (SCF)	15.62
Streptococcus pyogenes ATCC176													10 (OFX)	62.50
Streptococcus pyogenes KUKEM 676			10	250									13 (OFX)	31.25
Xanthamonas campestris A235													20 (SCF)	31.25
Yersinia enterocolitica F11													16 (OFX)	62.50

[a]OFX = Ofloxacin (10 μg/disc); SCF = sulbactam (30 μ)+cefoperazone (75 μg) (105 μg/disc) and NET = netilmicin (30 μg/disc) were used as positive reference standards

[b]Inhibition zone in diameter (mm) around the disc

[c]Minimal inhibitory concentrations as (μg/mL)

[d]Maxipine (μg/mL) was used as reference antibiotic in micro well dilution assay.

8.40 (d; 1H; pyridinone H^6), 9.10-9.30 (s; 1H; imidazolyl H^2). %$C_{13}H_{19}Br_2N_3O_2$ (409.12): calcd. C 38.16, H 4.68, N 10.27; found C 38.46, H 4.93, N 10.54.

2-Ethyl-3-hydroxy-1-[3-(morpholine-4-yl)propyl]-4(1H)-pyridinone hydrobromide (22)

Yield: 65 %. M.p. 224-225 (dec.)°C. IR (KBr): ν = 2722, 1637, 1265 O-H/ $-N^+=$ / C=O /C-O cm^{-1}. ^{1}H-NMR (400 MHz; DMSO-d_6) δ = 0.98-1.02 (s; 3H, $-CH_3$), 1.96-2.03 (m; 2H; N-CH_2-CH_2-CH_2-), 2.67-2.72 (q; 2H; -CH_2-CH_3), 2.92-3.04 (t; 2H; N-$CH_2CH_2CH_2$), 3.13-3.26 (m; 4H, morpholine H^3, H^5), 3.59-3.78 (m; 4H, morpholine H^2, H^6), 4.08-4.12 (m; 2H; N-$CH_2CH_2CH_2$), 6.62-6.63 (d; 1H; pyridinone H^5), 7.85-7.87 (d; 1H; pyridinone H^6), 9.83 (O-H). %$C_{14}H_{24}Br_2N_2O_3$ (428.16): calcd. C 39.27, H 5.65, N 6.54; found C 39.75, H 5.41, N 6.61.

The antimicrobial activity of all the synthesized compounds were evaluated against standards. Inhibition zone and minimal inhibitory concentrations (MIC) of the active compounds are given in the table 1.

Conclusion

In this study compounds having 3-benzyloxy-2-methyl/ethyl-N-substituted-4(1*H*)-pyridinone **3-12** or 3-hydroxy-2-methyl/ethyl-N-substituted-4(1*H*)-pyridinone hydrobromide **13-22** structures were synthesized and examined according to their antibacterial activities. For the antibacterial activity screening, all the synthesized compounds were evaluated by disc diffusion and microdilution methods against the various strains. Among the synthesized 4(1*H*)-pyridinones, compounds **13**, **14**, **16**, **17**, **19** and **22** showed significant inhibition than standards in disc diffusion assay. However, MIC values of the compounds were not less than reference maxipine except compound **14**. Due to these results and taking the chemical structures of the active compounds into consideration, it is clear that compounds having 3-OH and 4-carbonyl groups can form selective chelates between the oxygen atoms of groups and Fe^{3+} ions. For the series of 3-benzyloxy-4(1*H*)-pyridinones **3-12**, it is clear that none of the compounds showed significant antibacterial activity that can explain because of losing chelate formation property with the effect of disappearance of free OH.

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