Synthesis of New 4(1H)-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(1H)-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(1H)-pyridinone have potential pharmacological usefulness, one of the most important research topic is the chelating of this structures. 3-hydroxy-4-pyriones are bidentate chelating compounds used for removal therapies of this structures. 3-hydroxy-4-pyriones are the most effective in the neutral phase and N-substituted cyclic amino propylene 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 (Philadelphia, PA, USA) and are uncorrected. Infrared spectra were recorded on a Bruker Vector 22 (Opus Spectroscopic Software Version 2.0) (Bruker Analytische Messtechnik, Karlsruhe, Germany), using potassium bromide pellets, the frequencies are expressed in cm⁻¹. The 1H-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, or dimethyl sulphoxide-d6 (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 CNS 932 analyzer (Philadelphia, 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 and refluxed for 24 h. When the reaction completed, pH of the reaction mixture was arranged to pH=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 Na₂SO₄, 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(1H)-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 μm millipore filters. Antimicrobial tests were then carried out by disc diffusion method using 100 μL of suspension containing 10^6 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. Microbial 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 3.97 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(1H)-pyridinone and 2-ethyl-3-benzyloxy-N-substituted-4(1H)-pyridinone, 3-hydroxy-2-methyl-N-substituted-4(1H)-pyridinone and 2-ethyl-3-hydroxy-N-substituted-4(1H)-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 1H-NMR spectral data. The IR spectrum of compounds displayed a strong band in range of 1630-1657 cm⁻¹ assignable to C=C 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. 1H-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-CH₂-, a multiplet signal at 3.36-1.18 ppm for N-CH₂C₂H₅ and a triplet signal at 4.26-2.18 ppm for N-CH₂CH₂C₂H₅. The results of elemental analysis were within ± 0.4% of the theoretical values.

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\text{R}_1 = \text{CH}_3, \text{C}_2\text{H}_5
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Scheme 1. The general synthesis of the compounds
3-Benzoyloxy-2-methyl-4-pyrole (1)
Yield: 80 %. M.p. 166-167 °C. H-NMR (80 MHz; CDCl3): δ = 2.00-2.10 (s; 3H, -CH3), 1.50-1.70 (s; 2H, -OCH2), 6.31-6.50 (m; 2H, pyrone H3), 7.30-7.40 (m; 5H, -CH2), 7.50-7.70 (m; 5H, -CH2).

3-Benzoyloxy-2-ethyl-4-pyrole (2)
Yield: 75%. M.p. 23-24 °C. H-NMR (80 MHz; CDCl3): δ = 2.00-2.10 (s; 3H, -CH3), 1.50-1.70 (s; 2H, -OCH2), 5.10-5.20 (s; 2H; pyrone H3), 6.01-6.40 (m; 2H; -OCH2), 7.50-7.70 (m; 5H, -CH2).

2-Methyl-1,1-[3-(2-oxopyridinol-1-yl)-propyl]-3-benzoyloxy-4(1H)-pyridinone (3)
Yield: 84 %. Liq. 1H-NMR (400 MHz; CDCl3): δ = 1.74-1.81 (m; 2H, -CH2), 1.87-1.94 (m; 2H; pyrrolidine H2), 2.16 (s; 3H, -CH3), 2.19-2.23 (t; 2H; pyrrolidine H2), 2.5 (s; 3H, pyrrolidine H2), 3.18-3.21 (t; 2H; N-CH(2), 3.81-3.85 (t; 2H; N-CH(2), 5.02 (s; 2H, -OCH2), 6.13-6.15 (m; 1H; pyridinone H6), 7.31-7.41 (m; 5H; C(6)), 7.62-7.64 (m; 1H; pyridinone H5).

3-Benzoyloxy-2-methyl-1,1-[3-(2-piperidinol-1-yl)-propyl]-4(1H)-pyridinone (4)
Yield: 48 %. Liq. 1H-NMR (400 MHz; CDCl3): δ = 2.04 (s; 3H, -CH3), 2.32-2.37 (m; 2H; pyrrolidine H2), 2.42-2.55 (m; 2H; pyrrole H2), 2.79-2.87 (m; 4H; pyrrole H2 and H4), 3.22-3.27 (m; 2H, N-CH(2),CH(2)), 3.44-3.47 (t; 2H; N-CH(2),CH(2)), 4.0-4.45 (t; 2H; N-CH(2),CH(2)), 5.06 (s; 2H, -OCH2), 6.07-6.09 (m; 1H; pyridinone H6), 7.26-7.37 (m; 5H; C(6)), 7.60-7.62 (m; 1H; pyridinone H5).

3-Benzoyloxy-2-methyl-1,1-[3-(2-methylpiperidine-1-yl)-propyl]-4(1H)-pyridinone (5)
Yield: 39 %. Liq. 1H-NMR (400 MHz; CDCl3): δ = 0.90 (s; 3H, piperidine 2-CH), 1.16-1.22 (m; 5H; N-CH(2),CH(2), and 2-CH2), 1.45-1.70 (m; 8H; piperidine H2, H3, H4, H5, H6, H7, H8), 2.47-2.53 (m; 2H, N-CH(2),CH(2)), 2.61-2.65 (q; 1H; piperidine 2-CH2), 3.54-3.65 (m; 2H; N-CH(2),CH(2)), 5.17 (s; 2H, -OCH2), 6.30-6.22 (m; 1H; pyridinone H6), 7.18-7.23 (m; 5H; C(6)), 7.32-7.34 (d; 1H; pyridinone H5).

3-Benzoyloxy-1,1-[3-(imidazole-1-yl)-propyl]-2-methyl-4(1H)-pyridinone (6)
Yield: 67 %. Liq. 1H-NMR (400 MHz; CDCl3): δ = 1.87-1.91 (s; 3H, -CH3), 2.14-2.21 (m; 2H; N-CH(2),CH(2) and CH2), 3.62-3.66 (t; 2H; N-CH(2),CH(2) and CH2), 3.84-3.87 (t; 2H; N-CH(2),CH(2) and CH2), 5.18 (s; 2H, -OCH2), 6.29-6.31 (d; 1H; pyridinone H6), 6.75 (s; 1H; imidazolyl H4), 6.88-6.90 (d; 1H; pyridinone H5), 7.09 (s; 1H; imidazolyl H1), 7.19-7.26 (m; 5H; C(6)), 7.37 (s; 1H; imidazolyl H2).

3-Benzoyloxy-2-methyl-1,1-[3-(morpholine-4-yl)-propyl]-4(1H)-pyridinone (7)
Yield: 21 %. Liq. 1H-NMR (80 MHz; CDCl3): δ = 1.60-1.80 (m; 2H; N-CH(2),CH(2) and morfillinyl H2), 2.10 (s; 3H, -CH3), 3.50-3.70 (m; 6H, N-CH(2),CH(2) and morfillinyl H2, H3), 3.80-4.00 (m; 2H; N-CH(2),CH(2)), 5.10 (s; 2H, -OCH2), 6.20 (d; 1H; pyridinone H6), 7.07-7.30 (m; 5H; C(6)), 7.40 (d; 1H; pyridinone H5).

3-Benzoyloxy-2-ethyl-1,1-[3-(2-oxopyridinol-1-yl)-propyl]-4(1H)-pyridinone (8)
Yield: 86 %. Liq. 1H-NMR (400 MHz; CDCl3): δ = 0.98-1.02 (t; 3H, -CH3), 1.61-1.84 (m; 2H; N-CH(2),CH(2), 1.87-1.95 (m; 2H; pyridinone H2), 2.19-2.23 (t; 2H; pyridinone H2), 2.50-2.51 (t; 2H; pyridinone H2), 2.54-2.60 (q; 2H, -CH2,CH2), 3.19-3.23 (t; 2H; N-CH(2),CH(2)), 3.81-3.85 (t; 2H; N-CH(2),CH(2)), 5.09 (s; 2H, -OCH2), 6.15-6.17 (d; 1H; pyridinone H7), 7.29-7.42 (m; 5H; C(6)), 7.62-7.64 (d; 1H; pyridinone H5).

3-Benzoyloxy-2-ethyl-1,1-[3-(pyridinol-1-yl)-propyl]-4(1H)-pyridinone (9)
Yield: 32 %. Liq. 1H-NMR (400 MHz; CDCl3): δ = 1.98 (s; 3H, -CH3), 2.22-2.26 (m; 2H; pyridinone H2), 2.42-2.47 (m; 2H; pyridinone H3), 2.71-2.80 (m; 4H; pyridinone H2, H3).
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. 1H-NMR (400 MHz; DMSO-d₆) δ = 2.24-2.31 (m; 2H; N-CH₂-CH₂), 2.41 (s; 3H; -CH₃), 4.42-4.26 (t; 2H; N-CH₂(CH₂)₂), 4.32-4.36 (t; 2H; N-CH₂(CH₂)₂), 7.05-7.07 (d; 1H; pyridinone H), 7.64 (s; 1H; imidazolyl H), 7.77 (s; 1H; imidazolyl H), 8.17-8.19 (d; 1H; pyridinone H), 9.14 (s; 1H; imidazolyl H). %C₁₂H₁₇Br₂N₃O₂ (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. 1H-NMR (400 MHz; DMSO-d₆) δ = 1.99-2.07 (m; 2H; N-CH₂-CH₂), 2.30 (s; 3H; -CH₃), 3.00-3.04 (t; 2H; N-CH₂(CH₂)₂), 3.04-3.24 (m; 4H; morpholinyl H₃, H₅), 3.56-3.76 (m; 4H; morpholinyl H₂, H₆) 4.22-4.26 (t, 2H, N-CH₂(CH₂)₂), 6.92-6.94 (d; 1H; pyridinone H₅), 8.06-8.08 (d; 1H; pyridinone H₆), 9.14 (s; 1H; imidazolyl H). %C₁₃H₂₂Br₂N₂O₃ (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. 1H-NMR (400 MHz; DMSO-d₆) δ = 1.84-1.88 (m; 2H; N-CH₂-CH₂), 1.90-1.94 (m; 2H; pyrolidinone H₄), 2.20-2.24 (t; 2H; pyrolidinone H₃), 2.68-2.74 (q; 2H; -CH₂CH₃), 3.22-3.25 (t; 2H; N-CH₂(CH₂)₂), 3.32-3.35 (t; 2H; pyrolidinone H₅), 3.94-3.98 (t; 2H; N-CH₂(CH₂)₂), 6.28-6.30 (d; 1H; pyridinone H), 7.71-7.73 (d; 1H; pyridinone H). %C₁₄H₂₂Br₂N₂O₃ (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, 1643, 1285 O-H / N-= / C=O /C-O cm⁻¹. 1H-NMR (80 MHz; DMSO-d₆) δ = 1.23-1.27 (t; 3H; -CH₂CH₃), 1.77-1.98 (m; 2H; pyridinone H), 2.03-2.11 (m; 2H; pyridinone H), 2.21-2.29 (m; 2H; pyridinone H), 2.99-3.04 (q; 2H; -CH₂CH₃), 3.06-3.11 (q; 2H; pyridinone H), 3.31-3.36 (m; 2H; N-CH₂-CH₂-), 3.51-3.61 (q; 2H; N-CH₂CH₂), 4.48-4.51 (t; 2H; N-CH₂CH₂), 7.72-7.23 (d; 1H; pyridinone H), 8.35-8.40 (d; 1H; pyridinone H), 9.99 (O-H). %C₁₄H₂₄Br₂N₂O₂ (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. 1H-NMR (400 MHz; DMSO-d₆) δ = 0.92-0.95 (t; 3H; -CH₂CH₃), 1.08-1.10 (d; 3H; piperidine 2-CH₂), 1.53-1.62 (m; 6H; piperidine H, H†), 1.91-1.95 (t; 2H; piperidine H), 2.67-2.73 (q; 2H; -CH₂CH₃), 2.90-2.99 (m; 4H; N-CH₂CH₂), 3.94-3.98 (q; 1H; piperidine 2-CH₂), 4.15-4.18 (t; 2H; N-CH₂CH₂), 6.86-6.87 (d; 1H; pyridinone H), 8.08-8.10 (d; 1H; pyridinone H), 9.34 (O-H). %C₁₆H₂₈Br₂N₂O₂ (440.22): calcd. C 39.65, H 6.41, N 6.36; found C 39.14, H 7.01, N 5.93.

2-Ethyl-3-hydroxy-1-[3-(2-oxopyridin-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. 1H-NMR (80 MHz; DMSO-d₆) δ = 2.40-2.60 (s; 3H; -CH₃), 3.30-3.70 (m; 2H; -CH₂CH₂), 4.20-4.60 (m; 6H; -CH₂CH₂), 7.00-7.20 (d; 1H; pyridinone H), 7.60-7.90 (d; 2H; imidazolyl H, H†), 8.20-
The antimicrobial activity of all the synthesized compounds was 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(1H)-pyridinone 3-12 or 3-hydroxy-2-methyl/ethyl-N-substituted-4(1H)-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(1H)-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 Fe3+ ions. For the series of 3-benzyloxy-4(1H)-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.

References


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