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Mannich base derivatives of 3-hydroxy-6-methyl-4*H*-pyran-4-one with antimicrobial activity

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A series of 3-hydroxy-6-methyl-2-[(substitutedpiperidine-1-yl)methyl]-4*H*-pyran-4-one structured compounds were synthesized by reacting 5-hydroxy-2-methyl-4*H*-pyran-4-one with suitable piperidine derivatives using Mannich reaction conditions. Antibacterial activities of the compounds for *E. coli ATCC 25922*, *S. paratyphi ATCC BAA-1250*, *S. flexneri ATCC 12022*, *E. gergoviae ATCC 33426*, and *M. smegmatis ATCC 14468* were assessed in vitro by the broth dilution method for determination of minimum inhibitory concentration (MIC). In addition, their inhibitory effects over DNA gyrase enzyme were evaluated using a DNA gyrase supercoiling assay. All the synthesized compounds showed a MIC value of either 8 or 16 μg/mL for *M. smegmatis*, whereas minimum to moderate activity was achieved for the others. Those tested in the supercoiling assay had at best a very mild inhibition of the enzyme. This series deserves further attention for testing over *Mycobacterium* species and topoisomerase II inhibition to develop new lead drugs to treat non-tuberculous mycobacterial infections.

Key Words: Antimycobacterial and antibacterial activity; DNA gyrase activity; Mannich bases of 3-hydroxy-4*H*-pyran-4-one.

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Introduction

The genus *Mycobacterium* has 140 well characterized species with its own family *Mycobacteriaceae.*[1] This genus includes saprophytic species that are widespread in nature, as well as causative pathogens of major human diseases like *M. tuberculosis, M. bovis, M. africanum*, and *M. microti*, which cause tuberculosis, and *M. leprae*, which causes leprosy.[2] Mycobacteria referred to as atypical mycobacteria, non-tuberculous mycobacteria (NTM), or mycobacteria other than tuberculous bacillus (MOTT) can produce localized disease in the lungs, lymph glands, skin, wounds, or bone, especially in susceptible/immunocompromised individuals.[3,4] Infections and diseases due to non-tuberculous mycobacteria (NTM) are rising.[5] There are no genome sequence data and understanding of the biology of these 'atypical mycobacteria' is limited.[6]

Mycobacteria do not contain endospores or capsules. They are neither gram-positive nor gram-negative in the traditional sense; they are classified as acid-fast gram-positive bacteria due to a lack of outer cell membrane. All *Mycobacterium* species share a characteristic cell wall, thicker than in many other bacteria, which is hydrophobic, waxy, and rich in mycolic acids/mycolates.[7] Transportation of compounds through the cell wall of mycobacteria is one major factor relevant to the design of new antimycobacterial agents. This is difficult since mycolic acids and surface associated lipids of these organisms form a transport barrier compared to the cell wall of other eubacteria.[8]

Quinolones **(1)**, naphthyridones **(2)**, and coumarins **(3)** inhibit bacterial type II topoisomerases, deoxyribonucleic acid (DNA) gyrase, and topoisomerase IV, and are essential enzymes that maintain the supercoils in DNA both in gram-positive and gram-negative bacteria[9,10] (Figure 1).

Topoisomerase IV genes that have been reported in most bacterial species, are absent in a few bacteria such as *Treponema* spp., *Helicobacter* spp., and notably *Mycobacterium* spp.[11] and this is another factor that limits the antimycobacterial design. Consequently, these bacteria are unusual in producing a unique type II topoisomerase.[10]

Quinolones **(1)**, naphthyridones **(2)**, and coumarins **(3)** are relatively functional in *Mycobacterium* therapy with their low lipid solubility and rapidly increasing multi-drug resistance. Different Mannich bases of hydroxypyranones by means of piperidine and piperazine side chains have structural resemblance with these stated structures and might be an alternative for therapy.

Since Yabuta first synthesized allomaltol from kojic acid, which is a metabolic product of several species of the genus *Aspergillus*,[12] many researchers have studied Mannich bases of kojic acid **(4)**, maltol, and allomaltol **(6)** derivatives to improve their antimicrobial,[13–17] antitumor,[18,19] anticonvulsant,[20–22] tyrosinase inhibitory,[23,24] and toxic side effects.[12,15,16,20–22,25,26] These are generally based on their high iron chelating capacities and high lipophilic characters[27,28] (Figure 1).

Mannich bases of hydroxypyranones have high lipid solubility and structural likeness to the above-mentioned compounds that inhibit bacterial type II topoisomerases, DNA gyrase, and topoisomerase IV. We have focused our research to combine and produce a series of 3-hydroxy-6-methyl-2-[(substitutedpiperidine-1-yl)methyl]-4*H*-pyran-4-one (compounds **7a-t**) structured compounds. This series is composed of a total of 19 compounds; 6 of which have previously been described (compounds **7a, 7c, 7d, 7f, 7p, 7s**),[21,22] while our group has synthesized 13 of them for the first time (Figures 1 and 2). All these compounds have been characterized in our laboratory with physicochemical, spectral (IR, ^{1}H-NMR), and elemental analysis data.

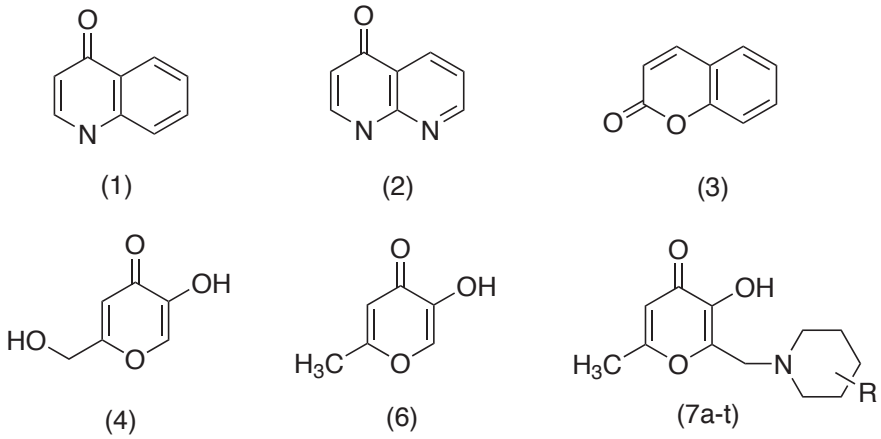


Figure 1. Structures of the compounds.

Further, the broth dilution method was used to determine minimum inhibitory concentration (MIC), and to establish their antituberculostatic and antimicrobial activities over *M. smegmatis*, *E. coli*, *S. paratyphi*, *S. flexneri*, and *E. gergoviae* (Table). This series of compounds was chosen to constitute a homolog series as they were also screened for their DNA gyrase enzyme inhibition to determine their activity using a DNA gyrase supercoiling assay.

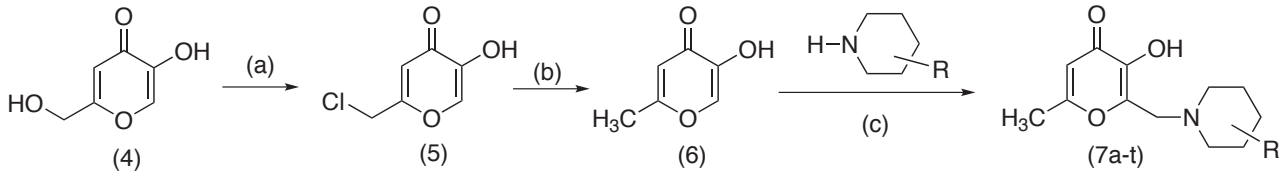


Figure 2. General synthesis of the compounds.

Experimental

All reagents were obtained from commercial sources. Solvents were dried and purified with known conventional methods. DNA gyrase assay kit 3 (1000U-K0003) and DNA gyrase enzyme (100U-G1001) were purchased from Inspiralis Co. (Norwich, UK). Melting points were detected with a Mettler-Toledo FP-62 melting point apparatus (Columbus, OH, USA) and are uncorrected. IR spectra (KBr) were recorded on a Perkin Elmer 1720X FT-IR spectrometer (Beaconsfield, UK). ^{1}H-NMR spectra were obtained by Varian Mercury 400, 400 MHz High Performance Digital FT-NMR using DMSO-$d6$ and tetramethylsilane as internal standard. All chemical shift values were recorded as δ (ppm). The purity of the compounds was measured by thin-layer chromatography on silica gel-coated aluminum sheets (Merck, 1.005554, silica gel HF254–361, Type 60, 0.25 mm; Darmstadt, Germany). The elemental analyses of the compounds were performed on a Leco CHNS 932 analyzer (Leco Corp., MI, USA). Elemental analysis for C, H, and N were within $\pm 0.4\%$ of theoretical

values. ^{1}H-NMR spectra and elemental analysis were performed at the Central Analysis Laboratory of Ankara University, Faculty of Pharmacy, in Ankara, Turkey.

General method for synthesis of 2-(chloromethyl)-5-hydroxy-4*H*-pyran-4-one (5)

Kojic acid (142 g, 2 mol) **(4)** was dissolved in thionyl chloride (237 g, 2 mol), followed by stirring for 2 h at room temperature. The yellow solid was filtered, and then washed with cold petroleum ether. Recrystallization from water gave light-yellow crystals (115 g, 72%), melting point (mp) 146-147 °C (lit. value[29] 146-147 °C).

General method for synthesis of 5-hydroxy-2-methyl-4*H*-pyran-4-one (6)

Compound **5** (20 g, 0.12 mol) was suspended in water (500 mL). The temperature of the reaction mixture was raised to 50 °C. Zinc dust (16 g, 0.24 mol) was added and stirred at 70 °C for 0.5 h. Concentrated hydrochloric acid (13.6 g, 3 mol) was added dropwise followed by stirring for 4 h at 70-80 °C. The solution was filtered, poured into ice-water and extracted with dichloromethane, dried with anhydrous sodium sulfate, and evaporated to dryness. Recrystallization of the resulting yellow solid from isopropanol provided compound **6** as light-yellow needles (10.2 g, 64%), mp 125-127 °C (lit. value[29] 125-127 °C).

General method for synthesis of 3-hydroxy-6-methyl-2-[(substitutedpiperidine-1-yl)methyl]-4*H*-pyran-4-one derivatives (7a-t)

A mixture of 0.01 mol of substituted piperidine and 0.01 mol of compound **6** in 20 mL of methanol with 0.012 mol of 37% formalin was stirred at room temperature until a solid mass precipitated. The compound was filtered and washed with cold water, dried under vacuum, and recrystallized from suitable solvents.

3-Hydroxy-6-methyl-2-[(2-methylpiperidine-1-yl)methyl]-4*H*-pyran-4-one (7b)

Recrystallized from methanol/diethyl ether. IR (KBr) (υ, cm^{-1}, stretching) 1618 (C=O); ^{1}H-NMR δ (ppm), 6.2 (s, 1H), 3.55 (s, 2H), 3.1 (d, 1H), 2.7 (m, 2H), 2.3 (s, 3H), 1.8, 1.7 and 1.5 (m, 6H), 1.05 (m, 3H, piperidine -CH_3). Anal. Calcd. For $C_{13}H_{19}NO_3$: C 65.8, H 8.07, N 5.90. Found: C 66.2, H 8.10, N 5.96. mp 148-149 °C.

2-[(2,6-Dimethylpiperidine-1-yl)methyl]-3-hydroxy-6-methyl-4*H*-pyran-4-one (7e)

Recrystallized from methanol/diethyl ether. IR (KBr) (υ, cm^{-1}, stretching), 1607 (C=O); ^{1}H-NMR δ (ppm), 6.2 (s, 1H), 3.50 (s, 2H), 2.6 (m, 2H), 2.3 (s, 3H), 1.75 (m, 1H), 1.6 (m, 2H), 1.35 (m, 1H), 1.1 (m, 6H, piperidine -CH_3), 1.0 (m, 2H). Anal. Calcd. For $C_{14}H_{21}NO_3$-0.1HOH: C 66.43, H 8.44, N 5.53. Found: C 66.54, H 8.44, N 5.54. mp 156-157 °C.

2-[(5-Ethyl-2-methylpiperidine-1-yl)methyl]-3-hydroxy-6-methyl-4*H*-pyran-4-one (7g)

Recrystallized from methanol/diethyl ether. IR (KBr) (υ, cm^{-1}, stretching), 1618 (C=O); ^{1}H-NMR δ (ppm), 6.2 (s, 1H), 3.5 (s, 2H), 3.0 (m, 1H), 2.4 (s, 3H), 1.9-1.2 (m, 10H), 0.9 (m, 5H, piperidine-C_2H_5 and -CH_3). Anal. Calcd. For $C_{15}H_{23}NO_3$-0.25HOH: C 66.76, H 8.78, N 5.19. Found: C 66.65, H 8.90, N 5.14. mp 144-145 °C.

3-Hydroxy-6-methyl-2-[(2-propylpiperidine-1-yl)methyl]-4*H*-pyran-4-one (7h)

Recrystallized from methanol/diethyl ether. IR (KBr) (υ, cm^{-1}, stretching), 1605 (C=O); ^{1}H-NMR δ (ppm), 6.2 (s, 1H), 3.50 (s, 2H), 2.95 (m, 1H), 2.45 (m, 2H), 2.25 (s, 3H), 1.8-1.2 (m, 10H, piperidine and

-C_3H_7), 0.9 (t, 3H, propyl). Anal. Calcd. For $C_{15}H_{23}NO_3$-0.4HOH: C 66.10, H 8.80, N 5.14. Found: C 66.01, H 9.20, N 5.09. mp 165-166 °C.

3-Hydroxy-6-methyl-2-[(4-propylpiperidine-1-yl)methyl]-4*H*-pyran-4-one (7i)

Recrystallized from methanol/diethyl ether. IR (KBr) (υ, cm^{-1}, stretching), 1612 (C=O); ^{1}H-NMR δ (ppm), 6.2 (s, 1H), 3.6 (s, 2H), 3.0 (d, 2H), 2.3 (s, 3H), 2.15 (t, 2H), 1.7 (d, 2H), 1.4-1.1 (m, 7H, piperidine and -C_3H_7), 0.9 (t, 3H, -C_3H_7). Anal. Calcd. For $C_{15}H_{23}NO_3$-0.1HOH: C 67.44, H 8.75, N 5.24. Found: C 67.42 H 8.76, N 5.16. mp 157-158 °C.

3-Hydroxy-2-[(3-hydroxypiperidine-1-yl)methyl]-6-methyl-4*H*-pyran-4-one (7j)

Recrystallized from methanol/diethyl ether. IR (KBr) (υ, cm^{-1}, stretching), 1608 (C=O); ^{1}H-NMR δ (ppm), 6.2 (s, 1H), 3.85 (m, 1H), 3.65 (s, 2H), 2.7-2.5 (m, 4H), 2.30 (s, 3H), 1.9-1.5 (m, 4H). Anal. Calcd. For $C_{12}H_{17}NO_4$: C 60.24, H 7.16, N 5.85. Found: C 59.91, H 7.32, N 5.80. mp 136-137 °C.

3-Hydroxy-2-[(4-hydroxypiperidine-1-yl)methyl]-6-methyl-4*H*-pyran-4-one (7k)

Recrystallized from methanol/diethyl ether. IR (KBr) (υ, cm^{-1}, stretching) 1612 (C=O); ^{1}H-NMR δ (ppm), 6.2 (s, 1H), 3.50 (s, 2H), 3.45 (m, 1H), 2.7 (m, 2H), 2.25 (s, 3H), 2.15 (m, 2H), 1.7 and 1.4 (m, 4H). Anal. Calcd. For $C_{12}H_{17}NO_4$-0.25HOH: C 59.12, H 7.24, N 5.75. Found: C 58.97, H 7.07, N 5.70. mp 155-156 °C.

3-Hydroxy-2-{[3-(hydroxymethyl)piperidine-1-yl]methyl}-6-methyl-4*H*-pyran-4-one (7l)

Recrystallized from methanol/diethyl ether. IR (KBr) (υ, cm^{-1}, stretching), 1618 (C=O); ^{1}H-NMR δ (ppm), 6.2 (s, 1H), 3.65 (s, 2H), 3.5 (m, 2H, methanol CH_2), 3.0-2.85 (m, 2H), 2.3 (s, 3H), 2.15-1.6 (m, 6H), 1.1 (m, 1H). Anal. Calcd. For $C_{13}H_{19}NO_4$: C 61.64, H 7.56, N 5.53. Found: C 61.71, H 7.87, N 5.52. mp 147-148 °C.

3-Hydroxy-2-{[4-(hydroxymethyl)piperidine-1-yl]methyl}-6-methyl-4*H*-pyran-4-one (7m)

Recrystallized from methanol/diethyl ether. IR (KBr) (υ, cm^{-1}, stretching), 1626 (C=O); ^{1}H-NMR δ (ppm), 6.2 (s, 1H), 3.60 (s, 2H), 3.50 (d, 2H, methanol CH_2), 3.0 (d, 2H), 2.3 (s, 3H), 2.2 (t, 2H), 1.73-1.63 (m, 4H). Anal. Calcd. For $C_{13}H_{19}NO_4$-0.5HOH: C 59.53, H 7.69, N 5.34. Found: C 59.34, H 7.98, N 5.30. mp 152-153 °C.

3-Hydroxy-6-methyl-2-[(4-phenylpiperidine-1-yl)methyl]-4*H*-pyran-4-one (7n)

Recrystallized from methanol. IR (KBr) (υ, cm^{-1}, stretching), 1614 (C=O); ^{1}H-NMR δ (ppm), 7.3-7.1 (m, 5H, aromatic CH), 6.1 (s, 1H), 3.55 (s, 2H), 2.95 (d, 3H), 2.3 (s, 3H), 1.6 and 1.8 (m, 4H). Anal. Calcd. For $C_{18}H_{21}NO_3$: C 72.22, H 7.07, N 4.68. Found: C 72.03, H 6.89, N 4.72. mp 167-168 °C.

3-Hydroxy-2-{[4-(4-hydroxyphenyl)piperidine-1-yl]methyl}-6-methyl-4*H*-pyran-4-one (7o)

Recrystallized from methanol. IR (KBr) (υ, cm^{-1}, stretching), 1615 (C=O); ^{1}H-NMR δ (ppm), 7.5-7.3 (m, 4H, aromatic CH), 6.1 (s, 1H), 4.88 (s, 1H, Ar-OH), 3.55 (s, 2H), 2.5-2.7 (m, 5H), 2.25 (s, 3H), 1.9 and 1.6 (m, 4H). Anal. Calcd. For $C_{18}H_{21}NO_4$: C 68.55, H 6.71, N 4.44. Found: C 68.35, H 6.54, N 4.49. mp 177-178 °C.

3-Hydroxy-6-methyl-2-{[4-(morpholin-4-yl)piperidine-1-yl]methyl}-4*H*-pyran-4-one (7r)

Recrystallized from chloroform/petroleum ether. IR (KBr) (υ, cm^{-1}, stretching), 1620 (C=O); ^{1}H-NMR δ (ppm), 6.2 (s, 1H), 3.55 (t, 4H, morpholine CH_2), 3.45 (s, 2H), 2.85 (m, 2H), 2.4 (t, 4H, morpholine CH_2), 2.25 (s, 3H), 2.05 (m, 3H), 1.7 and 1.35 (m, 4H). Anal. Calcd. For $C_{16}H_{24}N_2O_4$-0.2HOH: C 61.60, H 7.88, N 8.98. Found: C 61.54, H 7.49, N 8.82. mp 157-158 °C.

Ethyl 1-[(3-hydroxy-6-methyl-4-oxo-4*H*-pyran-2-yl)methyl]piperidine-4-carboxylate (7t)

Recrystallized from chloroform/petroleum ether. IR (KBr) (υ, cm^{-1}, stretching), 1726 (C=O), 1616 (C=O); ^{1}H-NMR δ (ppm), 6.1 (s, 1H), 4.05 (q, 2H), 3.5 (s, 2H), 2.8 (m, 2H), 2.3 (m, 1H), 2.25 (s, 3H), 2.15 (m, 2H), 1.8 and 1.55 (m, 4H), 1.2 (t, 3H). Anal. Calcd. For $C_{15}H_{21}NO_5$-0.1HOH: C 60.54, H 7.2, N 4.71. Found: C 60.56, H 6.87, N 4.73. mp 127-128 °C.

Microbiology

Broth Dilution Method for Minimum Inhibitory Concentration (MIC)

MICs of compounds prepared in tubes were determined by broth dilution using Middlebrook 7H10 agar supplemented with 10% oleic acid-albumin-dextrose-catalase for mycobacteria and Tryptic Soy Broth for other species. The tested dilutions ranged from 128 to 0.5 μg/mL using dimethyl sulfoxide (DMSO) as solvent for all compounds. Mycobacteria were suspended in Middlebrook 7H10 broth and the others in Tryptic Soy Broth to match the turbidity of 1 McFarland standard (2×10^8 cfu/mL). The tube slants were inoculated with undiluted or 1/100 diluted bacterial suspensions and incubated at 37 °C. The slants were examined until visible colonies were seen in the control tube. The controls prepared with the amounts of DMSO used in the dilutions did not show any inhibitory activity under these circumstances. The MIC value of each isolate was the lowest concentration of the compound that inhibited visible bacterial growth.

DNA gyrase supercoiling assay

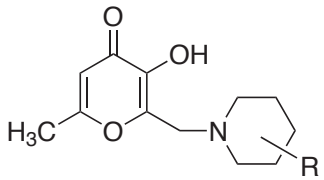
DNA gyrase supercoiling assays were performed with a gyrase supercoiling assay kit (Inspiralis) according to the manufacturer's instructions and analyzed by monitoring the conversion of relaxed pBR322 plasmid to its supercoiled form using DNA gel electrophoresis. Essentially, 1 U *E. coli* DNA gyrase was first diluted in 5× gyrase buffer and incubated in an assay buffer (35 mM Tris.HCl (pH 7.5), 24 mM KCl, 4 mM $MgCl_2$, 2 mM DTT, 1.8 mM Spermidine, 1 mM ATP, 6.5% (w/v) glycerol, and 0.1 mg/mL BSA), with 0.5 μg of pBR322 plasmid and a series of synthesized compound dilutions at 37 °C for 30 min. The reactions were stopped by the addition of Stop Dye (40% sucrose, 100 mM Tris.HCl (pH 7.5), 1 mM EDTA, 0.5 mg/mL bromophenol blue) and loaded on a TAE agarose gel (1%). Gels were visualized using a gel documentation system (BIORAD Chemi Doc).

Since high levels of DMSO are known to also affect DNA gyrase activity, titration was used to determine the minimum amount of DMSO to be used in the assays and 5% DMSO (with negligible or no effect on the gyrase) was chosen to dilute the compounds (data not shown).

Result and discussion

Final compounds that we synthesized were as follows: kojic acid (compound **4**) was reacted with thionyl chloride at room temperature to achieve compound **5**.[12] Subsequently, this intermediate (compound **5**) was reduced to allomaltol (compound **6**) using zinc dust suspension in water and concentrated hydrochloric acid at 70-80 °C over 4.5 h. This reaction exclusively affects methyl chlorine and leaves the carbonyl group likewise.[12,20–22,30] After purification, to conclude, allomaltol was reacted with suitable piperidine derivatives in methanol/formalin medium until the formation of desired 3-hydroxy-6-methyl-2-[(substitutedpiperidine-1-yl)methyl]-4*H*-pyran-4-one derivatives (compounds **7a-t**) as precipitates. In the Mannich reaction; substitution for the second position of the ring system is favored due to the existence of a free enolic hydroxyl group in the third position for this ring system.[26] Compounds were crystallized from appropriate solvents in moderate yields (Figure 2).

Table. MIC (μg/mL) of the compounds over *E. coli*, *S. paratyphi*, *S. flexneri*, *E. gergoviae*, and *M. smegmatis*.



	R	*E. coli*	*S. paratyphi*	*S. flexneri*	*E. gergoviae*	*M. smegmatis*
7a	hydrogen	32	128	128	128	16
7b	2-methyl	64	128	64	64	16
7c	3-methyl	128	128	128	128	8
7d	4-methyl	64	128	128	64	8
7e	2,6-dimethyl	32	64	64	64	8
7f	3,5-dimethyl	64	32	64	64	8
7g	5-ethyl-2-methyl	128	128	64	128	16
7h	2-propyl	32	64	128	64	16
7i	4-propyl	32	64	128	64	8
7j	3-hydroxy	64	32	64	128	8
7k	4-hydroxy	32	32	64	128	16
7l	3-(hydroxymethyl)	32	64	32	64	16
7m	4-(hydroxymethyl)	64	32	32	64	16
7n	4-phenyl	32	128	64	128	8
7o	4-(4-hydroxyphenyl)	64	64	64	64	8
7p	4-piperidin-1-yl	64	32	64	64	16
7r	4-(4-morpholino)	64	64	64	64	16
7s	4-benzyl	32	32	64	64	16
7t	4-ethoxycarbonyl	64	128	64	64	8

IR, ^{1}H-NMR, and elemental analysis characterized the proposed structure. In the ^{1}H-NMR spectra of compounds, piperidine ring protons are dispersed between δ 1.1 to 3.45 ppm in various integrals according

to their position toward ring nitrogen and their neighboring replacements of different electronic environments. Vinylic protons of the pyran ring system are observed as singlets at δ 6.1-6.2 ppm. The methylene linkage protons between pyran and piperidine rings occur between δ 3.45 and 3.55 ppm as singlets. The deletion of a singlet around 9-9.5 ppm belonging to the proton at the sixth position of the allomaltol structure and the formation of these new singlets confirmed the desired 3-hydroxy-6-methyl-2-[(substitutedpiperidine-1-yl)methyl]-4*H*-pyran-4-one structures. Spectral data of 6 compounds (compounds **7a, 7c, 7d, 7f, 7p, 7s**) are compatible with the data previously described for those.[21,22] All the other protons were seen according to the expected chemical shift and integral values.[20−22,29]

Biological evaluation

Every compound belonging to the series was tested over *E. coli, S. paratyphi, S. flexneri,* and *E. gergoviae* as gram-negative and *M. smegmatis* as gram-positive type bacteria for their MIC values. All 3-hydroxy-6-methyl-2-[(substitutedpiperidine-1-yl)methyl]-4*H*-pyran-4-one derivatives showed serious inhibitory activity over *M. smegmatis* growth in concentrations of 8-16 μg/mL, showing moderate to minimum activity over other bacteria types. What distinguishes Mycobacteria from the other species tested is that, although *Mycobacterium* species can still be considered gram-positive, they are not truly gram-positive in the traditional sense, and indeed some researchers classify them as acid-fast gram-positive bacteria[17] and thus the growth inhibitory effect could be due to DNA gyrase activity and/or topoisomerase activity inhibition by these or similar compounds. Moreover, *Mycobacterium* species share a characteristic cell wall, thicker than that in many other bacteria, with a hydrophobic character.[7]

These results indicated that these compounds were selectively effective over gram-positive bacteria either by their high lipophilic properties or by their selective DNA gyrase and topoisomerases enzyme inhibitor activities like quinolones (**1**), naphthyridones (**2**), and coumarins (**3**).

In this work, we focused on the DNA gyrase inhibitory effect of these compounds to explain the significance of the MIC. DNA gyrase is a topoisomerase, which can convert relaxed pBR322 plasmid to its supercoiled topoisomer; therefore DNA gyrase activity can be monitored as 2 bands on an agarose gel. The upper band is open-circular (nicked) plasmid, whereas the faster running band is negatively supercoiled (closed circular) plasmid. Using this assay, we can analyze the effect of various compounds on DNA gyrase activity.

When the 3-hydroxy-6-methyl-2-[(substitutedpiperidine-1-yl)methyl]-4*H*-pyran-4-one series of compounds were analyzed for any effect on the supercoiling activity of DNA gyrase, we observed a minimal effect. For most compounds, even in increasing amounts, did not inhibit the supercoiling activity of DNA gyrase. Only a few compounds (compounds **7a**, **c**, **f**, and **n**) showed activity over this enzyme to a certain extent (Figure 3).

The fact that they did not have more than a mild inhibitory effect on DNA gyrase enzyme, however, does not rule out the possibility that they may still inhibit bacterial growth through interfering with the function of other topoisomerases. Indeed, topoisomerase IV is the subtype commonly found in many gram-positive bacteria and has a specialized function in mediating the decatenation of interlocked daughter chromosome during replication. However, it should be noted that, for *Mycobacterium* in particular, there are not even any topoisomerase IV genes, but instead topoisomerase II. [10] Our current research effort is focused on studying the inhibitory role of these compounds on topoisomerase II, and in particular on their effects over multi-drug resistant and nonresistant *Mycobacterium* species, especially *Mycobacterium tuberculosis.*

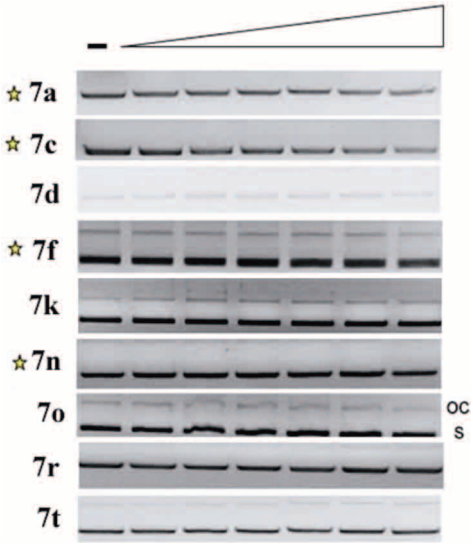


Figure 3. Effect of some 3-hydroxy-6-methyl-2-[(substitutedpiperidine-1-yl)methyl]-4*H*-pyran-4-one derivatives on DNA gyrase supercoiling activity. The bands in the agarose gel pictures indicate the topoisomers of pBR322 plasmid. OC and S indicate open circular and supercoiled pBR322 plasmid DNA, respectively. Empty vehicle control; compounds are titrated at 2, 4, 8, 16, 32, 64 μg/mL concentration). Most compounds showed either no effect on DNA gyrase activity, or a slight increase. Only these compounds indicated (*) show minor inhibition of DNA gyrase activity, where some non-effective ones were also given as examples.

References

1. Euzeby JP 2003. List of bacterial names withstanding in nomenclature - Genus *Mycobacterium*. http://www.bacterio.cict.fr/m/mycobacterium.html
2. Katoch, V. M. *Indian J. Med. Res.* **2004,** 120, 290-304.
3. Wallace, R. J. Jr.; O'Brein, R.; Glassroth, J.; Raleigh, J.; Dutta, A. *Am. Rev. Respir. Dis.* **1990,** 142, 940-953.
4. Katoch, V. M.; Mohan, K. T. A typical mycobacterial infections. Sharma, S. K., Eds.; *Tuberculosis,* Jaypee Brothers Medical Publishers (P) Ltd, New Delhi, 2001.
5. Khan, K.; Wang, J.; Marras, T. K. *Am. J. Respir. Crit. Care Med.* **2007,** 176, 306-313.
6. Behr, M. A. *Microbes Infect.* **2008,** 10, 968-972.
7. Kayser, F. H. *Medical Microbiology,* Georg Thieme Verlag, Stuttgart, 2005.
8. Besra, G. S.; Chatterjee, D. Lipids and carbohydrates of *Mycobacterium tuberculosis;* Bloom, B. R., Ed.; *Tuberculosis*, ASM Press, Washington, DC, 1994.
9. Maxwell, A. *Trends Microbiol.* **1997,**5, 102-109.
10. Aubry, A.; Fisher, L. M.; Jarlier, V.; Cambau, E. *Biochem. Biophys. Res. Commun.* **2006,** 348, 158-165.
11. Cole, S. T.; Brosch, R.; Parkhill, J.; Garnier, T.; Churcher, C.; Harris, D.; Gordon, S. V.; Eiglmeier, K.; Gas, S.; Barry, C. E.; Tekaia, F.; Badcock, K.; Basham, D.; Brown, D.; Chillingworth, T.; Connor, R.; Davies, R.; Devlin,

K.; Feltwell, T.; Gentles, S.; Hamlin, N.; Holroyd, S.; Hornsby, T.; Jagels, K.; Krogh, A.; McLean, J.; Moule, S.; Murphy, L.; Oliver, K.; Osborne, J.; Quail, M. A.; Rajandream, M. A.; Rogers, J.; Rutter, S.; Seeger; K.; Skelton, J.; Squares, R.; Squares, S.; Sulston, J. E.; Taylor, K.; Whitehead, S.; Barrell, B. G. et al. *Nature* **1998,** 393, 537-544.

12. Yabuta, T. *J. Chem. Soc.* **1924,** 125, 575-587.
13. Kayahara, H.; Shibata, N.; Tadasa, K.; Maeda, H.; Kotani, T.; Ichimoto, I. *Agric. Biol. Chem.* **1990,** 54, 2441-2442.
14. Kotani, T.; Ichimoto, I.; Tatsumi, C.; Fujita, T. *Agric. Biol. Chem.* **1975,** 39, 1311-1317.
15. Aytemir, M. D.; Hider, R. C.; Erol, D. D.; Özalp, M.; Ekizoğlu, M. *Turk J. Chem.* **2003,** 27, 445-452.
16. Aytemir, M. D.; Erol, D. D.; Hider, R. C.; Özalp, M. *Turk J. Chem.* **2003,** 27, 757-764.
17. Wan, H. M.; Chen, C. C.; Giridhar, R.; Chang, T. S.; Wu, W. T. *J. Ind. Microbiol. Biotechnol.* **2005,** 32, 227-233.
18. Yamato, M.; Yasumoto, Y.; Sakai, J.; Luduena, R. F.; Baneerjee, A.; Tashiro, T. *J. Med. Chem.* **1987,** 30, 1897-1900.
19. Tamura, T.; Mitsumori, K.; Totsuka, Y.; Wakabayashi, K.; Kido, R.; Kasai, H.; Nasu, M.; Hirose, M. *Toxicology* **2006,** 222, 213-224.
20. Aytemir, M. D.; Çalış, Ü.; Özalp, M. *Arch. Pharm. Pharm. Med. Chem.* **2004,** 337, 281-288.
21. Aytemir, M. D.; Çalış, Ü. *Hacettepe University Journal of the Faculty of Pharmacy* **2007,** 27, 1-10.
22. Aytemir, M. D.; Çalış, Ü. *FABAD J. Pharm. Sci.* **2006,** 31,23-29.
23. Kim, H.; Choi, J.; Cho, J. K.; Kim, S. Y.; Lee, Y. S. *Bioorg. Med. Chem. Lett.* **2004,** 14, 2843-2846.
24. Moggia, F.; Brisset, H.; Fages, F.; Chaix, C.; Mandrand, B.; Dias, M.; Levillain, E. *Tetrahedron Lett.* **2006,** 47, 3371-3374.
25. Ellis, B. L.; Duhme, A. K.; Hider, R. C.; Hossain, M. B.; Rizvi, S.; Van Der Helm, D. *J. Med. Chem.* **1996,** 39, 3659-3670.
26. O'Brien, G.; Patterson, J. M.; Meadow, J. R. *J. Org. Chem.* **1960,** 25, 86-89.
27. Moggia, F.; Brisset, H.; Fages, F.; Chaix, C.; Mandrand, B.; Dias, M.; Levillain, E. *Tetrahedron Lett.* **2006,** 47, 3371-3374.
28. Zborowski, K.; Grybos, R.; Proniewicz, L. M. *Inorg. Chem. Commun.* **2005,** 8, 76-78.
29. Erol, D. D.; Yuluğ, N. *Eur. J. Med. Chem.* **1994,** 29, 893-897.
30. Liu, Z. D.; Piyamongkol, S.; Liu, D. Y.; Khodr, H. H.; Lu, S. H.; Hider, R. C. *Bioorg. Med. Chem.* **2001,** 9, 563-573.