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4*H*-Pyran-4-one derivatives:; leading molecule for preparation of compounds with antimycobacterial potential

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A series of 3-hydroxy-6-methyl-2-((4-substitutedpiperazin-1-yl)methyl)-4H-pyran-4-one structured compounds were synthesized by reacting 5-hydroxy-2-methyl-4H-pyran-4-one with suitable piperazine derivatives using Mannich reaction conditions. Antibacterial activities of the compounds for *E. coli, S. paratyphi, S. flexneri, E. gergoviae*, and *M. smegmatis* were assessed in vitro by using broth dilution for determination of the minimum inhibitory concentration (MIC). In addition, their inhibitory effects over DNA gyrase enzyme were evaluated using a DNA gyrase supercoiling assay. Among the synthesized compounds; compound **7** showed a 4 μg/mL MIC value for *M. smegmatis*, whereas the other compounds demonstrated moderate to high activity. 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.

Key Words: Antimycobacterial activity; DNA gyrase activity; hydroxy-4*H*-pyran-4-one.

Introduction

Hydroxypyranone derivative compounds display antimicrobial,[1–5] antitumor,[6,7] anticonvulsant,[8–10] and tyrosinase inhibitory effects.[11,12] These effects are generally based on their high iron chelating capacities and

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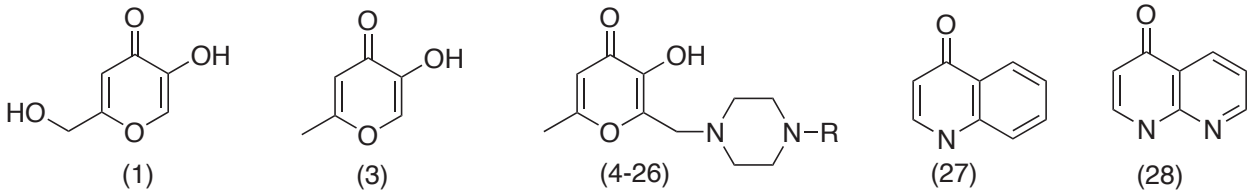
highly lipophilic characters.[13,14] Since Yabuta first synthesized allomaltol from kojic acid, which is a metabolic product of several species of the genus *Aspergillus*,[15] many researchers studied the Mannich bases of kojic acid (1), maltol, and allomaltol (3) derivatives to improve these properties with less toxic side effects[3,4,8−10,15−20] (Scheme 1).



Scheme 1. Structures of the compounds.

Mycobacterium is a genus of Actinobacteria, given its own family, the *Mycobacteriaceae*. This genus includes saprophytic species that are widespread in nature as well as the causative pathogens of the major human disease complexes such as *M. tuberculosis, M. bovis, M. africanum*, and *M. microti* for tuberculosis and *M. leprae* for leprosy.[21]

Mycobacteria that are neither tuberculosis nor leprosy bacteria are categorized as typical mycobacteria (old designation), nontuberculous mycobacteria (NTM), or mycobacteria other than tubercle bacilli (MOTT). These subtypes, such as *M. smegmatis, M. abscessus, M. fortuitum*, and *M. kansasii*, can cause pulmonary disease resembling tuberculosis, lymphadenitis, skin and soft tissue infections, bone, joint, and tendon infections, and disseminated diseases in immunocompromised patients.[21]

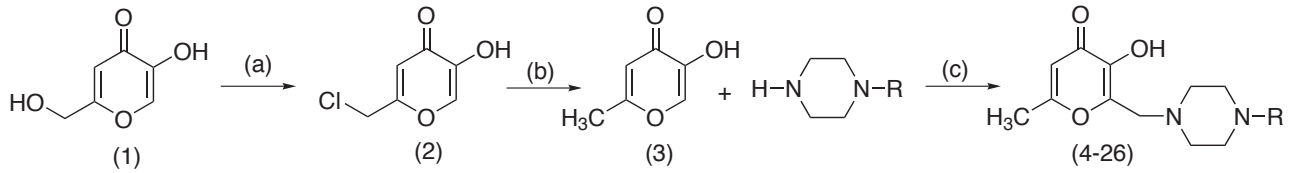
Generally it is assumed that for many gram-negative bacteria DNA gyrase is the target, whereas topoisomerase IV is the target for many gram-positive bacteria. Mycobacteria do not contain endospores or capsules. They are neither gram-positive nor gram-negative in the traditional sense; they are classified 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.[21]

For the treatment of Mycobacterium infections with the inconsistency of Gram determination, chemical structures which inhibit the bacterial DNA gyrase or the topoisomerase subtype enzymes are used, thereby inhibiting DNA replication and transcription.

Different Mannich bases of hydroxypyranones with piperidine and piperazine side chains have structural resemblances with quinolone (27) and naphthyridone (28) derivatives (Scheme 1), major treatments of Mycobacteria infections.[22] However, rapidly increasing multi-drug resistance is the limitation of usage in these agents.

Taken as a whole, hydroxypyranone derivatives can be suitable leads for mycobacterial infections by means of their high lipophilic characters and structural similarities with the therapeutics in use if they accomplish either DNA gyrase or the topoisomerase enzyme inhibition.

With this perspective, we propose to combine and produce a series of twenty-three 3-hydroxy-6-methyl-2-((4-substitutedpiperazin-1-yl)methyl)-4H-pyran-4-one (**4-26**) structured compounds: 12 of them (compounds **4-9, 11, 14, 16, 23-25**) have been previously described,[8,10,17,18] while 11 of them have been newly synthesized by our group (Schemes 1 and 2). All these compounds have been characterized in our laboratory using physicochemical, spectral (IR, ^{1}H-NMR), and elemental analysis data.



Reagents and conditions; (a) $SOCl_2$, 2h, rt ; (b) Zn/HCl,H_2O, 3h, 70° C; (c) HCHO, MeOH

Scheme 2. General synthesis of the compounds.

Further, to establish their antimycobacterial and antimicrobial activities over *M. smegmatis* ATCC 14468, *E. coli* ATCC 25922, *S. paratyphi* ATCC BAA-1250, *S. flexneri* ATCC 12022, and *E. gergoviae* ATCC 33426, broth dilution was performed to determine the minimum inhibitory concentration (MIC) (Table). This series of tested compounds was preferred to constitute a homolog series as they were also screened for their DNA gyrase enzyme inhibition for determining their type of activity by using a DNA gyrase supercoiling assay.

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 uncorrected. IR spectra (KBr) were recorded on a Perkin Elmer 1720X FT-IR spectrometer (Beaconsfield, UK). ^{1}H-NMR spectra were obtained by a 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 controlled 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.

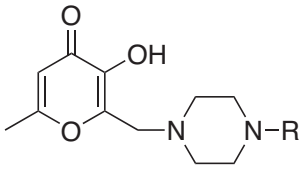
General method for synthesis of 2-(chloromethyl)-5-hydroxy-4H-pyran-4-one (Compound 2)

Kojic acid (142 g, 2 mol) **(1)** was dissolved in thionyl chloride (237 g, 2 mol), followed by stirring for 2 h at room temperature. The yellow solid was filtered and washed with cold petroleum ether. Recrystallization from water gave light-yellow crystals (115 g, 72%), melting point (mp) 146-147 °C.

General method for synthesis of 5-hydroxy-2-methyl-4H-pyran-4-one (Compound 3)

Compound **2** (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. Conc. HCl (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, extracted with dichloromethane, dried with Na_2SO_4, and evaporated to dryness. Recrystallization

Table. MIC (μg/mL) of the compounds over *E. coli* ATCC 25922, *S. paratyphi* ATCC BAA-1250, *S. flexneri* ATCC 12022, *E. gergoviae* ATCC 33426, and *M. smegmatis* ATCC 14468.



				MIC (μg/ml)		
Compound	R	*E. coli*	*S. paratyphi*	*S. flexneri*	*E. gergoviae*	*M. smegmatis*
4	phenyl	64	32	64	64	8
5	2-chlorophenyl	32	128	128	128	16
6	2-fluorophenyl	128	128	64	128	8
7	2-methoxy	32	32	64	64	4
8	3-chlorophenyl	128	128	64	128	8
9	3-methoxy	32	32	64	64	16
10	4-methylphenyl	64	128	64	64	8
11	4-chlorophenyl	>128	>128	>128	>128	8
12	4-(4-trifluoromethyl)phenyl	64	32	64	64	16
13	4-cyanophenyl	>128	>128	>128	>128	16
14	4-nitrophenyl	128	128	64	128	8
15	cyclohexyl	>128	>128	>128	>128	16
16	pyridine-2-yl	64	32	64	64	8
17	pyridine-4-yl	64	32	64	64	16
18	phenylcarbonyl	64	64	64	64	8
19	furan-2-ylcarbonyl	64	64	64	64	8
20	tetrahydrofuran-2-ylmethyl	64	64	64	64	8
21	cyclohexyl methyl	>128	>128	>128	>128	8
22	N-methylpiperidin-4-yl	>128	>128	>128	>128	16
23	tert-butoxycarbonyl	>128	>128	>128	>128	16
24	ethoxycarbonyl	64	64	64	64	8
25	2-hydroxyethyl	64	32	64	64	8
26	2-(dimethylamino)ethyl	64	32	64	64	8

of the resulting yellow solid from isopropanol provided compound **3** as light-yellow needles (10.2 g, 64%), mp 125-127 °C.

General method for synthesis of 3-hydroxy-6-methyl-2-((4-substitutedpiperazin-1-yl)methyl)-4H-pyran-4-one derivatives (Compounds 4-26)

A mixture of 0.01 mol of substituted piperazine and 0.01 mol of compound **3** 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 crystallized from suitable solvents.

3-Hydroxy-6-methyl-2-{[4-(4-methylphenyl)piperazin-1-yl]methyl}-4H-pyran-4-one (Compound 10)

Recrystallized from methanol. IR (KBr) (υ, cm^{-1}), 3722 (O-H stretching), 3024 (aromatic C-H stretching), 3001 (alkene C-H stretching), 2923-2821 (alkane C-H stretching), 1623 (C=O stretching), 1339 (C-N stretching), 1201 (C-O stretching); ^{1}H-NMR, δ (ppm), 6.8-7 (m, 4H, aromatic CH), 6.2 (s, 1H, C=CH), 3.5 (s, 2H, methylene), 2.6-3 (m, 8H, piperazine CH_2), 2.3 (s, 3H, Ar-CH_3), 2.2 (s, 3H, C=C-CH_3). Anal. Calcd. For $C_{18}H_{22}N_2O_3$: C 68.77, H 7.05, N 8.91. Found: C 68.67, H 6.621, N 8.933. mp 176.7 °C.

3-Hydroxy-6-methyl-2-{[4-(4-trifluoromethyl)phenyl]piperazin-1-yl]methyl}-4H-pyran-4-one (Compound 12)

Recrystallized from methanol. IR (KBr) (υ, cm^{-1}), 3698 (O-H stretching), 2955-2838 (alkane C-H stretching), 1636 (C=O stretching), 1333 (C-N stretching), 1104 (C-O stretching); ^{1}H-NMR, δ (ppm), 7.5-7.0 (m, 4H, aromatic CH), 6.2 (s, 1H, C=CH), 3.6 (s, 2H, methylene), 3.3-2.6 (m, 8H, piperazine CH_2), 2.3 (s, 3H, C=C-CH_3). Anal. Calcd. For $C_{18}H_{19}F_3N_2O_3$-0.25HOH: C 57.98, H 5.27, N 7.52. Found: C 57.96, H 5.519, N 7.587. mp 177.6 °C.

4-{4-[(3-Hydroxy-6-methyl-4-oxo-4H-pyran-2-yl)methyl]piperazin-1-yl} benzonitrile (Compound 13)

Recrystallized from methanol/diethyl ether. IR (KBr) (υ, cm^{-1}), 3739 (O-H stretching), 3025 (aromatic C-H stretching), 2956-2850 (alkane C-H stretching), 2208 (C≡N stretching), 1632 (C=O stretching), 1357 (C-N stretching), 1205 (C-O stretching); ^{1}H-NMR, δ (ppm), 7.6-7.0 (m, 4H, aromatic CH), 6.2 (s, 1H, C=CH), 3.6 (s, 2H, methylene), 3.4-2.6 (m, 8H, piperazine CH_2), 2.2 (s, 3H, C=C-CH_3). Anal. Calcd. For $C_{18}H_{19}N_3O_3$-0.35HOH: C 65.18, H 5.97, N 12.67. Found: C 65.18, H 6.122, N 12.62. mp 166.4 °C.

2-[(4-Cyclohexylpiperazin-1-yl)methyl]-3-hydroxy-6-methyl-4H-pyran-4-one (Compound 15)

Recrystallized from methanol/diethyl ether. IR (KBr) (υ, cm^{-1}), 3734 (O-H stretching), 2922-2812 (alkane C-H stretching), 1619 (C=O stretching), 1341 (C-N stretching), 1198 (C-O stretching); ^{1}H-NMR, δ (ppm), 6.2 (s, 1H, C=CH), 3.5 (s, 2H, methylene), 2.2-2.4 (m, 8H, piperazine CH_2), 1.7-1.5-1.1 (m, 11H, cyclohexyl). Anal. Calcd. For $C_{17}H_{26}N_2O_3$: C 66.64, H 8.55, N 9.14. Found: C 66.45, H 7.913, N 9.138. mp 180.6 °C.

3-Hydroxy-6-methyl-2-{[4-(pyridin-4-yl)piperazin-1-yl]methyl}-4H-pyran-4-one (Compound 17)

Recrystallized from methanol/diethyl ether. IR (KBr) (υ, cm^{-1}), 3708 (O-H stretching), 3135 (aromatic C-H stretching), 3023 (alkene C-H stretching), 2815 (alkane C-H stretching), 1621 (C=N stretching), 1602 (C=O stretching), 1348 (C-N stretching), 1214 (C-O stretching); ^{1}H-NMR, δ (ppm), 8.3-6.6 (m, 4H, pyridinyl C-H), 6.2 (s, 1H, C=CH), 3.7 (s, 2H, methylene), 3.4-2.7 (m, 8H, piperazine CH_2), 2.3 (s, 3H, C=C-CH3). Anal. Calcd. $C_{16}H_{19}N_3O_3$-0.1HOH: C 63.39, H 6.38, N 13.86. Found: C 63.28, H 6.349, N 13.76. mp 180.4 °C.

3-Hydroxy-6-methyl-2-{[4-(phenylcarbonyl)piperazine-1-yl]methyl}-4H-pyran-4-one (Compound 18)

Recrystallized from methanol. IR (KBr) (υ, cm^{-1}), 3708 (O-H stretching), 3055 (aromatic C-H stretching), 3018 (alkene C-H stretching), 2946-2839 (alkane C-H stretching), 1616 (C=O stretching), 1340 (C-N stretching), 1218 (C-O stretching); ^{1}H-NMR, δ (ppm), 7.4 (m, 5H, aromatic CH), 6.1 (s, 1H, C=CH), 3.5 (s, 2H, methylene), 3.3-2.5 (m, 8H, piperazine CH_2), 2.2 (s, 3H, C=C-CH3). Anal. Calcd. For $C_{18}H_{20}N_2O_4$-1.25HOH: C 61.61, H 6.46, N 7.98. Found: C 61.68, H 6.401, N 7.815. mp 115.3 °C.

2-{[4-(Furan-2-ylcarbonyl)piperazin-1yl]methyl}-3-hydroxy-6-methyl-4H-pyran-4-one (Compound 19)

Recrystallized from methanol. IR (KBr) (υ, cm^{-1}), 3689 (O-H stretching), 3135 (aromatic C-H stretching), 2923-2811 (alkane C-H stretching), 1607 (C=O stretching), 1222 (C-O stretching); ^{1}H-NMR, δ (ppm), 7.8-7.0-6.6 (m, 3H, furyl CH), 6.2 (s, 1H, C=CH), 3.5 (s, 2H, methylene), 3.6-2.5 (m, 8H, piperazine CH_2), 2.2 (s, 3H, C=C-CH_3). Anal. Calcd. $C_{16}H_{18}N_2O_5$-0.25HOH: C 59.52, H 5.77, N 8.68. Found: C 59.24, H 5.211, N 8.616. mp 145.9 °C.

3-Hydroxy-6-methyl-2-{[4-(tetrahydrofuran-2-ylmethyl)piperazin-1-yl]methyl}-4H-pyran-4-one (Compound 20)

Recrystallized from methanol. IR (KBr) (υ, cm^{-1}), 3697 (O-H stretching), 3055 (alkene C-H stretching), 2924-2837 (alkane C-H stretching), 1655 (C=O stretching), 1457 (alkane C-H bending), 1341 (C-N stretching), 1077 (C-O stretching); ^{1}H-NMR, δ (ppm), 6.4 (s, 1H, C=CH), 3.45 (s, 2H, methylene), 3.35-2.4 (m, 8H, piperazine CH_2), 2.4 (s, 3H, C=C-CH_3). Anal. Calcd. For $C_{16}H_{24}N_2O_4$: C 62.32, H 7.84, N 9.08. Found: C 62.04, H 8.068, N 8.998. mp 150.1 °C.

3-Hydroxy-6-methyl-2-{[4-(cyclohexylmethyl)piperazine]methyl}-4H-pyran-4-one (Compound 21)

Recrystallized from methanol. IR (KBr) (υ, cm^{-1}), 3349 (O-H stretching), 2918-2825 (alkane C-H stretching), 1636 (C=O stretching), 1453 (alkane C-H bending), 1228 (C-N stretching), 1197 (C-O stretching); ^{1}H-NMR, δ (ppm), 6.2 (s, 1H, C=CH), 3.5 (s, 2H, methylene), 3.3-2.5 (m, 8H, piperazine CH_2), 2.2 (s, 3H, C=C-CH_3). Anal. Calcd. For $C_{18}H_{28}N_2O_3$: C 67.47, H 8.81, N 8.74. Found: C 67.43, H 8.319, N 8.778. mp 181.4 °C.

3-Hydroxy-6-methyl-2-{[4-(1-methylpiperidin-4-yl)piperazin-1-yl]methy}-4H-pyran-4-one (Compound 22)

Recrystallized from methanol. IR (KBr) (υ, cm^{-1}), 3671 (O-H stretching), 3053 (alkene C-H stretching), 2970-2801 (alkane C-H stretching), 1629 (C=O stretching), 1451 (alkane C-H bending), 1226 (C-N stretching), 1132 (C-O stretching); ^{1}H-NMR, δ (ppm), 6.4 (s, 1H, C=CH), 3.5 (s, 2H, methylene), 3.3-2.5 (m, 8H, piperazine CH_2), 2.2 (s, 3H, C=C-CH_3). Anal. Calcd. For $C_{17}H_{27}N_3O_3$-0.25HOH: C 62.65, H 8.50, N 12.89. Found: C 62.70, H 8.795, N 12.72. mp 178.8 °C.

2-({4-[2-(Dimethylamino)ethyl]piperazin-1-yl}methyl)-3-hydroxy-6-methyl-4H-pyran-4-one (Compound 26)

Recrystallized from methanol/diethyl ether. IR (KBr) (υ, cm^{-1}), 3350 (O-H stretching), 2958-2828 (alkane C-H stretching), 1621 (C=O stretching), 1458 (alkane C-H bending), 1251 (C-N stretching), 1204 (C-O stretching);^{1}H-NMR, δ (ppm), 6.4 (s, 1H, C=CH), 3.45 (s, 2H, methylene), 3.3-2.5 (m, 8H, piperazine CH_2), 2.2 (s, 3H, C=C-CH_3). Anal. Calcd. For $C_{15}H_{25}N_3O_3$-1.25HOH: C 56.67, H 8.72, N 13.22. Found: C 56.96, H 8.698, N 12.98. mp 78.4 °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 X 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. Reactions were stopped with 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 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).

Results and Discussion

The desired compounds were synthesized as follows. Initially, commercial kojic acid (compound **1**) was reacted with thionyl chloride at room temperature to obtain 2-(chloromethyl)-5-hydroxy-4H-pyran-4-one (compound

2).[15] Then compound **2** was reduced to allomaltol (compound **3**) using zinc dust suspension in water and concentrated hydrochloric acid at 70-80 °C in 4.5 h without affecting the hydroxyl group at the fifth position of compound **2**.[8,9,10,15] After purification, finally, allomaltol was reacted with suitable piperazine derivatives in methanol/formalin media until the formation of the desired 3-hydroxy-6-methyl-2-((4-substitutedpiperazin-1-yl)methyl)-4H-pyran-4-one derivatives (compounds **4-26**) as precipitates. In the Mannich reaction; substitution for the second position of the ring system is preferred due to the presence of a free enolic hydroxyl group in the third position.[20] Compounds were crystallized from appropriate solvents in moderate yields (Scheme 2).

IR, ^{1}H-NMR, mass, and elementary analysis characterized the proposed structure. In the IR spectra; all compounds displayed strong, round O-H stretching bands between 3350 and 3700 cm^{-1}. The C=O stretch band belongs to the fourth position of the pyran ring generally seen between 1600 and 1655 cm^{-1}. C-H stretching belongs to the methyl group at the sixth position of the pyran and piperazine ring system observed between 2800 and 2900 cm^{-1}, and C-O-C stretching of the pyran system was noticeable between 1100 and 1200 cm^{-1}.

In the ^{1}H-NMR spectra of compounds, piperazine ring protons are obvious at δ 2.2-3.6 ppm as triplets. Vinylic protons of the pyran ring system are observed as singlets at δ 6.1-6.4 ppm. The methylene linkage protons between pyran and piperazine rings were between δ 3.45 and 3.75 ppm as singlets. The removal of the 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-((4-substituted piperazin-1-yl)methyl)-4H-pyran-4-one structures. All the other protons were according to the expected chemical shift and integral values.[8,9,23]

Biological evaluation

All the bacteria that were tested for MIC values were gram negative except for *Mycobacterium smegmatis*. The results show that in general 3-hydroxy-6-methyl-2-((4-substituted piperazin-1-yl)methyl)-4H-pyran-4-one derivatives are selectively effective over gram positive bacteria either by their metal chelating properties or by their selective enzyme inhibiting activities such as DNA gyrase and topoisomerases with their high lipophilic character. Compound **7** has the greatest inhibition activity, whereas the rest of the compounds have slightly lower but nevertheless still significant inhibition over *M. smegmatis* growth (Table). However, these compounds, not even compound **7**, did not show serious growth inhibitory activity over the other gram negative organisms tested (Table).

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[21] and thus the growth inhibitory effect could be due to DNA-gyrase activity and/or topoisomerase activity inhibition by these compounds.

In this work, we have chosen to concentrate on the inhibitory effect of these compounds on DNA gyrase activity in order to explain the significance of the MIC results. DNA gyrase is a topoisomerase that 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-((4-substitutedpiperazin-1-yl)methyl)-4H-pyran-4-one series of compounds

were analyzed for any effect on the supercoiling activity of DNA gyrase, very little, if any, effect was observed. The DNA gyrase was actively supercoiling the relaxed, open circle plasmid and increasing amounts of most of the compounds did not significantly inhibit this activity. Only a few of the compounds, namely compounds **13** and **14** and to a certain extent **24**, show activity over enzyme (Figure).

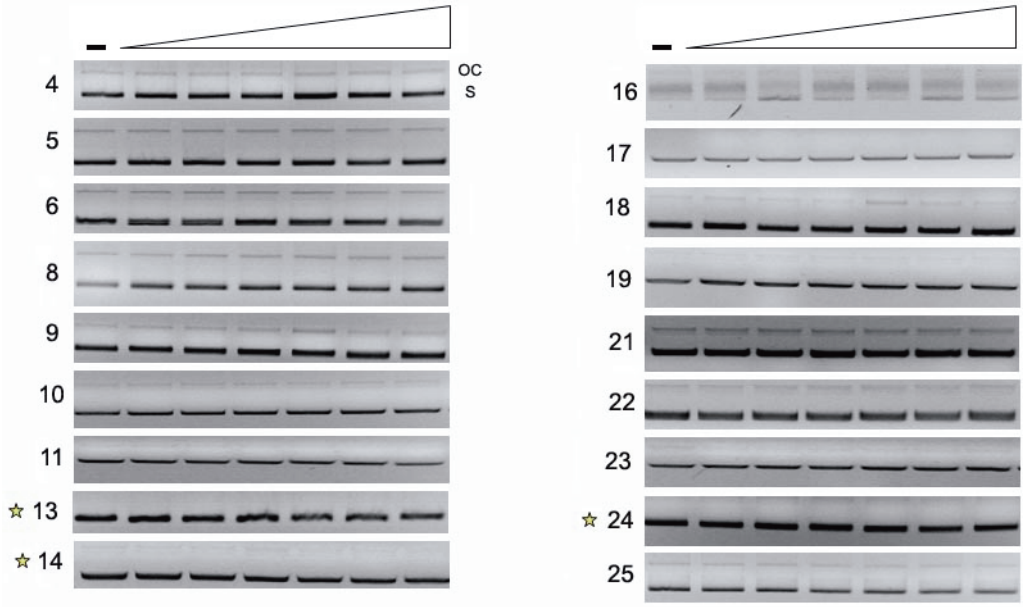


Figure. Effect of synthetic compounds of 3-hydroxy-6-methyl-2-((4-substituted piperazin-1-yl)methyl)-4H-pyran-4-one series on DNA gyrase supercoiling activity. The bands in the agarose gel pictures indicate the topoisomers of pBR322 plasmid. OC and S indicate open circular and supercoiled pBR322 plasmid DNA, respectively; -, empty vehicle control; compounds are titrated at 2, 4, 8, 16, 32, 64 μg/mL concentration). Most compounds show either no effect on DNA gyrase activity, or a slight increase. Only the compounds indicated by a star show minor inhibition of DNA gyrase activity.

Compounds of different chemical family had previously been shown to inhibit DNA gyrase activity;[24] however, those tested in this assay had at best a very mild inhibition of the enzyme, although they had very high growth inhibition in the MIC assay. The fact that they did not have more than a mild inhibitory effect on DNA gyrase enzyme does not rule out the possibility that they may still inhibit bacterial growth through interfering with the function of other topoisomerases, which will be investigated in future studies.

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 chromosomes during replication. However, it should be noted that for *Mycobacterium* in particular there are not even any topoisomerase IV genes, but instead topoisomerase II,[25] and for those species only we are concentrating our current efforts on studying the inhibition of these compounds on topoisomerase II in particular with their effects over multi-drug resistant and non resistant Mycobacterium species, mainly in *Mycobacterium tuberculosis*.

References

1. Kayahara, H.; Shibata, N.; Tadasa, K.; Maeda, H.; Kotani, T.; Ichimoto, I. *Agric. Biol. Chem.* **1990,** *54,* 2441-2442.
2. Kotani, T.; Ichimoto, I.; Tatsumi, C.; Fujita, T. *Agric. Biol. Chem.* **1975,** *39,* 1311-1317.
3. Aytemir, M. D.; Hider, R. C.; Erol, D. D.; Özalp, M. and Ekizoğlu, M. *Turk. J. Chem.* **2003,** *27,* 445-452.
4. Aytemir, M. D.; Erol, D. D.; Hider, R. C.; Özalp, M. *Turk. J. Chem.* **2003,** *27,* 757-764.
5. Wan, H. M.; Chen, C. C.; Giridhar, R.; Chang, T. S.; Wu, W. T. *J. Ind. Microbiol. Biotechnol.* **2005,** *32,* 227-233.
6. Yamato, M.; Yasumoto, Y.; Sakai, J.; Luduena, R. F.; Baneerjee, A.; Tashiro, T. *J. Med. Chem.* **1987,** *30,* 1897-1900.
7. Tamura, T.; Mitsumori, K.; Totsuka, Y.; Wakabayashi, K.; Kido, R.; Kasai, H.; Nasu, M.; Hirose, M. *Toxicology* **2006,** *222,* 213-224.
8. Aytemir, M. D., Çalış, Ü.; Özalp, M. *Arch. Pharm. Pharm. Med. Chem.* **2004,** *337,* 281-288.
9. Aytemir, M. D.; Çalış, Ü. *Hacettepe University Journal of the Faculty of Pharmacy* **2007,** *27,* 1-10.
10. Aytemir, M. D.; Çalış, Ü.; Fabad J. *Pharm. Sci.* **2006,** *31,* 23-29.
11. Cabanes, J.; Chazarra, S.; Garcia-Carmona, F. *J. Pharm. Pharmacol.* **1994,** *46,* 982-985.
12. Kim, H.; Choi, J.; Cho, J. K.; Kim, S. Y.; Lee, Y. S. *Bioorg. Med. Chem. Lett.* **2004,** *14,* 2843-2846.
13. Moggia, F.; Brisset, H.; Fages, F.; Chaix, C.; Mandrand, B.; Dias, M.; Levillain, E. *Tetrahedron Lett.* **2006,** *47,* 3371-3374.
14. Zborowski, K.; Grybos, R.; Proniewicz, L. M. *Inorg. Chem. Commun.* **2005,** *8,* 76-78.
15. Yabuta, T. *J. Chem. Soc.* **1924,** *125,* 575-587.
16. Köysal, Y; Işık Ş; Aytemir, M. D. *Acta Cryst.* **2004**, *E60*, o112-o114.
17. Ocak, N; Işık, Ş., Aytemir, M. D. *Acta Cryst.* **2004***, E60*, o561-o563.
18. İskeleli, N. O.; Işık, Ş., Aytemir, M. D. *Acta Cryst.* **2005**, *E61*, o1947-o1949.
19. 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.
20. O'Brien, G.; Patterson, J. M.; Meadow, J. R. *J. Org. Chem.* **1960,** *25,* 86-89.
21. Kayser, F. H. Medical microbiology, Georg Thieme Verlag, Stuttgart, 2005.
22. Schaumann, R.; Rodloff, A. C. *Anti-Infective Agents in Medicinal Chemistry* **2007,** *6,* 49-56.
23. Liu, Z. D.; Piyamongkol, S.; Liu, D. Y.; Khodr, H. H.; Lu, S. H.; Hider, R. C. *Bioorg. Med. Chem.* **2001,** *9,* 563-573.
24. Mdluli, K.; Ma, Z. *Infectious Disorders - Drug Targets* **2007,** *7,* 159-168.
25. Aubry, A.; Fisher, L. M.; Jarlier, V.; Cambau, E. *Biochem. Biophys. Res. Commun.* **2006,** *348,* 158-165.