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RESEARCH ARTICLE

# Design, synthesis and *in vivo/in vitro* screening of novel chlorokojic acid derivatives

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## Abstract

A series of novel Mannich bases of chlorokojic acid (2-chloromethyl-5-hydroxy-4*H*-pyran-4-one) were synthesized and their biological activities were investigated. Anticonvulsant activity results according to phase-I tests of Antiepileptic Drug Development (ADD) Program revealed that compound **13** was the most effective one at 4 h against subcutaneous pentylenetetrazole (scPTZ)-induced seizure test. Antimicrobial activities were evaluated *in vitro* against bacteria and fungi by using broth microdilution method. The antitubercular activities against *Mycobacterium tuberculosis* and *M. avium* were discussed with Resazurin microplate assay (REMA). The antimicrobial activity results indicated that compounds **1** and **12** (MIC: 8–16 µg/mL) showed higher activity against Gram negative bacteria while compound **12** had MIC: 4–16 µg/mL against Gram positive bacteria. Compound **1** was the most active one with MIC values of 8–32 µg/mL against fungi. Mannich bases also exhibit significant antitubercular activity in a MIC range of 4 to 32 µg/mL, especially compound **18** against *M. avium*.

**Keywords:** Chlorokojic acid, Mannich bases, anticonvulsant, antimicrobial, antimycobacterial

## Introduction

Kojic acid (5-hydroxy-2-hydroxymethyl-4*H*-pyran-4-one), is a biologically important fungal metabolite, produced by many species of *Aspergillus*, *Acetobacter* and *Penicillium*. Because of providing a promising skeleton for development of both new and more potent derivatives, it has been used by many researchers as a starting material in preparation of new compounds<sup>1–11</sup>. Chlorokojic acid (2-chloromethyl-5-hydroxy-4*H*-pyran-4-one), which is synthesized by chlorination of kojic acid exhibits distinct antibacterial and antifungal activities<sup>3–5</sup>. Moreover, these hydroxypyron derivatives are known as effective chelating agents forming complexes with metals<sup>5,12,13</sup> and this ability plays a significant role in antimicrobial activity<sup>14</sup>. Kojic acid and its derivatives also display a variety of biological activities, such as antiepileptic<sup>4,6–9</sup>, modest anti-inflammatory agent<sup>5</sup>, food additive<sup>15</sup>, antioxidant or antibrowning agent<sup>16</sup>, skin-lightening product in cosmetics as a result of inhibition of melanin production<sup>17</sup>, herbicidal<sup>18</sup>, anti-speck<sup>19</sup>, pesticide

and insecticide<sup>20</sup>, antitumor activity<sup>21</sup> and anti-diabetic agent<sup>22</sup>.

Epilepsy, one of the more common neurological disorders, affects a large section of people. Since available marketed antiepileptic drugs possess the risk of tolerance development, dose-related toxicity, and idiosyncratic side effects, the search for a new agent is still a popular investigation area of medicinal chemists worldwide<sup>23</sup>. In the literature many compounds bearing hydroxypyron ring such as kojic acid, kojic amine, maltol and etil maltol have been reported as anticonvulsant agents<sup>24–27</sup>. The lipid solubility of a drug is an important factor in connection with its transfer into the central nervous system. The increase of anticonvulsant effect in Mannich bases of kojic acid and allomaltol (5-hydroxy-2-methyl-4*H*-pyran-4-one) is attributed to increase of lipophilicity<sup>4,8,9</sup>.

Tuberculosis (TB), one of the earliest recorded human diseases and a chronic bacterial infection, continues to be an important global one with mortality worldwide killing more than 2 million people a year<sup>28,29</sup>. *Mycobacterium*

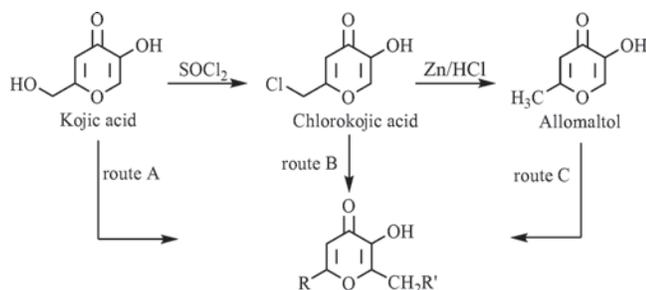
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*tuberculosis*, which causes TB, infects approximately one-third of the world's population and is comparable only to human immunodeficiency virus (HIV) as an infectious cause of death<sup>30</sup>. Also, *Mycobacterium avium* complex (MAC) is the most common cause of human infection due to nontuberculous mycobacteria. MAC is not only an opportunistic pathogen in acquired immunodeficiency syndrome (AIDS) patients but also cause of progressive pulmonary disease even in immunocompetent humans<sup>31</sup>. Clinical management of patients with *M. avium* infections is difficult, even with macrolides such as clarithromycin and azithromycin as first-line drugs in multidrug regimens<sup>32</sup>. The prevalence of multi-drug resistant and extensively drug resistant tuberculosis (MDR- and XDR-TB) strains is reported to be high in the countries where adequate supplies of the drugs are not available. The frightening TB is due to use and misuse of existing antibiotics and poor compliance with the long duration of current chemotherapy. The current trends suggest that TB will be among the 10 leading causes of global disease burden in the year 2020<sup>33</sup>. Thus, there is an urgent need for development of new antitubercular agents with improved properties such as enhanced activity against MDR and XDR strains, effectiveness against latent TB, shortened duration of therapy, reduced toxicity and rapid mycobactericidal mechanism of action<sup>34</sup>.

Although pharmaceutical industries have produced a number of new antibiotics in the last three decades, resistance by microorganisms has increased. Especially, widespread use of antibiotics has led many bacteria to evolve resistance to multiple versions of drugs such as methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant *Enterococcus* (VRE), and XDR-TB. Generally, bacteria have the genetic ability to transmit and acquire resistance to drugs, which are utilized as therapeutic agents and the concern is mainly on the number of patients in hospitals who have suppressed immunity, and due to new bacterial strains, which are multi-resistant<sup>35</sup>.

In our laboratory, a large number of Mannich bases of kojic acid (route A), chlorokojic acid (route B), and allomaltol (route C) were prepared (Scheme 1) for their various types of biological activities<sup>4,6-11</sup>. Most of these compounds seemed to be promising candidates for anticonvulsant, antimicrobial and antiviral agents. Mannich bases of kojic acid and allomaltol which



Scheme 1. Schematic representation of the synthetic route A, B and C.

contain lipophilic aryl portions were synthesized by our research group in order to increase the penetration to the blood-brain barrier<sup>4,6-9</sup>. These compounds demonstrated significant anticonvulsant activities *in vivo* against maximal electroshock (MES)- and subcutaneous pentylenetetrazole (scPTZ)-induced seizure tests. Also, in our recent studies Mannich bases of chlorokojic acid (route B) exhibited good antimicrobial and antiviral activities<sup>10-11</sup>.

Therefore in present work we planned to synthesize eighteen novel 6-chloromethyl-3-hydroxy-2-substituted 4*H*-pyran-4-one derivatives including piperazine ring and evaluate for their anticonvulsant and antimicrobial couplet with antitubercular activities (Scheme 2).

Adding a phenyl ring or equivalent hydrocarbon substitute, and a carbonyl or another electronegative group adjacent to the phenyl ring were expected to increase lipophilicity and biological effect as succeeded before.

## Materials-methods

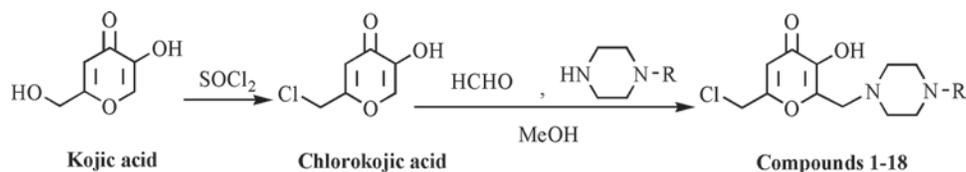
### Chemistry

All chemicals used for the synthesis of the compounds were supplied by Merck (Darmstadt, Germany) and Aldrich Chemical Co. (Steinheim, Germany). Melting points were determined by a Thomas Hoover Capillary Melting Point Apparatus (Philadelphia, PA, USA) and were uncorrected. IR spectra were recorded on a Perkin Elmer FT-IR-420 System, Spectrum BX spectrometer. <sup>1</sup>H-NMR and <sup>13</sup>C-NMR spectra were obtained with a Varian Mercury 400 MHz spectrophotometer in deuteriochloroform (CDCl<sub>3</sub>) and dimethylsulphoxide (DMSO-*d*<sub>6</sub>). Tetramethylsilane (TMS) was used as an internal standard (chemical shift in δ, ppm). Mass spectral analysis was carried out with a Micromass ZQ LC-MS with Masslynx Software Version 4.1 by using electrospray ionization (ESI+) method. The elemental analyses were performed with a Elementar Analysensysteme GmbH varioMICRO CHNS at The Scientific & Technological Research Council of Turkey-Ankara Testing and Analyses Laboratory (TUBİTAK-ATAL). For the compounds **9** and **17** elementary analysis were performed on Ankara University, Faculty of Pharmacy, Central Laboratory on CHNS-932 (LECO). The purity of the compounds was assessed by thin layer chromatography (TLC) on Kieselgel 60 F<sub>254</sub> (Merck, Darmstadt, Germany) chromatoplates.

Chlorokojic acid was synthesized as described in previous studies<sup>8-11</sup>. Yield 76%, m.p.: 166-167 °C.

### Preparation of Mannich bases of chlorokojic acid derivatives (1-18)

The secondary amine (substituted piperazine derivatives) and 37% formaline were dissolved in MeOH. Chlorokojic acid was added to the solution and the mixture was stirred vigorously for 15 to 25 min. The resulting precipitate was collected by filtration and washed with cold MeOH. All crude products recrystallized from appropriate solvent.

Scheme 2. General synthesis of compounds **1-18**.

6-(Chloromethyl)-3-hydroxy-2-[[4-phenylpiperazin-1-yl)methyl]-4H-pyran-4-one (**1**) IR  $\nu$  ( $\text{cm}^{-1}$ ): 1622 (C=O), 1455 (C=C), 1201 (C-O); 757 (C-Cl);  $^1\text{H-NMR}$  ( $\text{DMSO}-d_6$ , 400 MHz)  $\delta$  ppm: 2.62 (4H; t;  $J = 4.8$ ; piperazine- $H^2$ ,  $H^6$ ), 3.13 (4H; t;  $J = 4.8$ ; piperazine- $H^3$ ,  $H^5$ ), 3.63 (2H; s;  $-\text{CH}_2-$ ), 4.67 (2H; s;  $\text{ClCH}_2-$ ), 6.56 (1H; s;  $H^5$ ), 6.76 (1H; t;  $J = 7.2$ ; Ar- $H^a$ ), 6.91 (2H; d;  $J = 8.0$ ;  $H^{2'}$  and Ar- $H^{6'}$ ), 7.19 (2H; t;  $J = 7.8$ ;  $H^{3'}$  and Ar- $H^{5'}$ ), 9.24 (1H; brs; -OH);  $^{13}\text{C-NMR}$  (DEPT) ( $\text{CDCl}_3$ , 400 MHz)  $\delta$  ppm: 173.60, 112.08, 41.02, 49.13, 52.95, 55.26, 161.69, 150.99, 145.81, 129.17, 116.25, 120.09, 144.16;  $^{13}\text{C-NMR}$  (APT) ( $\text{CDCl}_3$ , 400 MHz)  $\delta$  ppm: -55.26, -52.95, -49.12, -41.22; ESI-MS ( $m/z$ ): 195 (100%), 335 ( $\text{M}^+\text{H}$ ), 337 ( $\text{M}^+\text{H}+2$ ), 357 ( $\text{M}^+\text{Na}$ ).

6-(Chloromethyl)-3-hydroxy-2-[[4-*o*-tolylpiperazin-1-yl)methyl]-4H-pyran-4-one (**2**) IR  $\nu$  ( $\text{cm}^{-1}$ ): 1622 (C=O), 1453 (C=C), 1196 (C-O); 761 (C-Cl);  $^1\text{H-NMR}$  ( $\text{CDCl}_3$ , 400 MHz)  $\delta$  ppm: 2.22 (3H; s;  $-\text{CH}_3$ ), 2.65 (4H; brs; piperazine- $H^2$ ,  $H^6$ ), 2.84 (4H; t;  $J = 4.4$ ; piperazine- $H^3$ ,  $H^5$ ), 3.65 (2H; s;  $-\text{CH}_2-$ ), 4.67 (2H; s;  $\text{ClCH}_2-$ ), 6.57 (1H; s;  $H^5$ ), 6.9-7.10 (4H; m; Ar- $H$ );  $^{13}\text{C-NMR}$  ( $\text{DMSO}-d_6$ , 400 MHz)  $\delta$  ppm: 17.43, 41.35, 51.19; 52.75; 53.39; 112.37; 118.64; 122.71; 126.40, 130.68, 131.62, 144.02, 147.59, 151.09, 161.13, 173.37; ESI-MS ( $m/z$ ): 349 ( $\text{M}^+\text{H}$ ), 351 ( $\text{M}^+\text{H}+2$ ), 371 (100%,  $\text{M}^+\text{Na}$ ).

6-(Chloromethyl)-3-hydroxy-2-[[4-*p*-tolylpiperazin-1-yl)methyl]-4H-pyran-4-one (**3**) IR  $\nu$  ( $\text{cm}^{-1}$ ): 1634 (C=O), 1456 (C=C), 1196 (C-O); 749 (C-Cl);  $^1\text{H-NMR}$  ( $\text{CDCl}_3$ , 400 MHz)  $\delta$  ppm: 2.19 (3H; s;  $-\text{CH}_3$ ), 2.61 (4H; t;  $J = 4.8$ ; piperazine- $H^2$ ,  $H^6$ ), 3.06 (4H; t;  $J = 5.0$ ; piperazine- $H^3$ ,  $H^5$ ), 3.62 (2H; s;  $-\text{CH}_2-$ ), 4.67 (2H; s;  $\text{ClCH}_2-$ ), 6.56 (1H; s;  $H^5$ ), 6.81 (2H; d;  $J = 8.4$ ; Ar- $H^{2'}$ , Ar- $H^{6'}$ ) 7.01 (2H; d;  $J = 8.8$ ; Ar- $H^{3'}$ , Ar- $H^{5'}$ ), 9.24 (1H; brs; -OH);  $^{13}\text{C-NMR}$  ( $\text{DMSO}-d_6$ , 400 MHz)  $\delta$  ppm: 19.93, 41.33, 48.57, 52.24, 53.32, 112.34, 115.58, 127.53, 129.22, 144.02, 147.46, 148.79, 161.14, 173.37; ESI-MS ( $m/z$ ): 349 ( $\text{M}^+\text{H}$ ), 351 ( $\text{M}^+\text{H}+2$ ), 371 ( $\text{M}^+\text{Na}$ , 100%).

6-(Chloromethyl)-2-[[4-(2,3-dimethylphenyl)piperazin-1-yl)methyl]-3-hydroxy-4H-pyran-4-one (**4**) IR  $\nu$  ( $\text{cm}^{-1}$ ): 1621 (C=O), 1454 (C=C), 1197 (C-O); 747 (C-Cl);  $^1\text{H-NMR}$  ( $\text{CDCl}_3$ , 400 MHz)  $\delta$  ppm: 2.13 (3H; s;  $-\text{CH}_3$ ), 2.19 (3H; s;  $-\text{CH}_3$ ), 2.64 (4H; brs; piperazine- $H^2$ ,  $H^6$ ), 2.79 (4H; t;  $J = 4.4$ ; piperazine- $H^3$ ,  $H^5$ ), 3.64 (2H; s;  $-\text{CH}_2-$ ), 4.68 (2H; s;  $\text{ClCH}_2-$ ), 6.56 (1H; s;  $H^5$ ), 6.8-7.0 (3H; m; Ar- $H$ ); ESI-MS ( $m/z$ ): 363 ( $\text{M}^+\text{H}$ ), 365 ( $\text{M}^+\text{H}+2$ ), 385 ( $\text{M}^+\text{Na}$ , %100).

2-[[4-(4-Acetylphenyl)piperazin-1-yl)methyl]-6-(chloromethyl)-3-hydroxy-4H-pyran-4-one (**5**) IR  $\nu$  ( $\text{cm}^{-1}$ ): 1622 (C=O), 1454 (C=C), 1199 (C-O); 747 (C-Cl);  $^1\text{H-NMR}$

( $\text{CDCl}_3$ , 400 MHz)  $\delta$  ppm: 2.44 (3H; s;  $\text{CH}_3\text{CO}-$ ), 2.61 (4H; t;  $J = 5.0$ ; piperazine- $H^2$ ,  $H^6$ ), 3.33 (4H; t;  $J = 4.8$ ; piperazine- $H^3$ ,  $H^5$ ), 3.63 (2H; s;  $-\text{CH}_2-$ ), 4.66 (2H; s;  $\text{ClCH}_2-$ ), 6.56 (1H; s;  $H^5$ ), 6.95 (2H; d;  $J = 9.6$ ; Ar- $H^{2'}$ ,  $H^{6'}$ ) 7.79 (2H; d;  $J = 9.2$ ; Ar- $H^{3'}$ ,  $H^{5'}$ ); ESI-MS ( $m/z$ ): 377 ( $\text{M}^+\text{H}$ ), 379 ( $\text{M}^+\text{H}+2$ ), 399 ( $\text{M}^+\text{Na}$ , 100%).

6-(Chloromethyl)-3-hydroxy-2-[[4-(4-nitrophenyl)piperazin-1-yl)methyl]-4H-pyran-4-one (**6**) IR  $\nu$  ( $\text{cm}^{-1}$ ): 1630 (C=O), 1458 (C=C), 1201 (C-O); 753 (C-Cl);  $^1\text{H-NMR}$  ( $\text{CDCl}_3$ , 400 MHz)  $\delta$  ppm: 2.48 (4H; t;  $J = 1.8$ ; piperazine- $H^2$ ,  $H^6$ ), 2.57 (4H; t;  $J = 4.8$ ; piperazine- $H^3$ ,  $H^5$ ), 3.61 (2H; s;  $-\text{CH}_2-$ ), 4.65 (2H; s;  $\text{ClCH}_2-$ ), 6.54 (1H; s;  $H^5$ ), 7.01 (2H; d;  $J = 9.6$ ; Ar- $H^{2'}$ ,  $H^{6'}$ ), 8.02 (2H; d;  $J = 9.2$ ; Ar- $H^{3'}$ ,  $H^{5'}$ ), 9.34 (1H; brs; -OH);  $^{13}\text{C-NMR}$  ( $\text{DMSO}-d_6$ , 400 MHz)  $\delta$  ppm: 42.09, 46.97, 52.51, 53.87, 113.12, 113.33, 126.35, 137.56, 144.83, 148.03, 155.35, 161.93, 174.15; ESI-MS ( $m/z$ ): 325 (100%), 380 ( $\text{M}^+\text{H}$ ), 382 ( $\text{M}^+\text{H}+2$ ), 402 ( $\text{M}^+\text{Na}$ ).

6-(Chloromethyl)-2-[[4-(4-cyclohexylpiperazin-1-yl)methyl]-3-hydroxy-4H-pyran-4-one (**7**) IR  $\nu$  ( $\text{cm}^{-1}$ ): 1630 (C=O), 1450 (C=C), 1197 (C-O); 740 (C-Cl);  $^1\text{H-NMR}$  ( $\text{CDCl}_3$ , 400 MHz)  $\delta$  ppm: 1.06-1.71 (5H; m; cyclohexane- $H$ ); 1.54-1.71 (5H; m; cyclohexane- $H$ ); 2.17 (1H; m; cyclohexane- $H$ ); 2.46 (8H; brs; piperazine- $H$ ), 3.53 (2H; s;  $-\text{CH}_2-$ ), 4.65 (2H; s;  $\text{ClCH}_2-$ ), 6.53 (1H; s;  $H^5$ ); ESI-MS ( $m/z$ ): 341 ( $\text{M}^+\text{H}$ , 100%), 343 ( $\text{M}^+\text{H}+2$ ), 363 ( $\text{M}^+\text{Na}$ ).

2-[[4-(4-Benzylpiperazin-1-yl)methyl]-6-(chloromethyl)-3-hydroxy-4H-pyran-4-one (**8**) IR ( $\text{cm}^{-1}$ ): 1627 (C=O), 1452 (C=C), 1200 (C-O); 748 (C-Cl);  $^1\text{H-NMR}$  ( $\text{CDCl}_3$ , 400 MHz)  $\delta$  ppm: 2.37 (4H; brs; piperazine- $H^2$ ,  $H^6$ ), 2.49 (4H; brs; piperazine- $H^3$ ,  $H^5$ ), 3.44 (2H; s;  $-\text{CH}_2-$ ), 3.55 (2H; s;  $-\text{CH}_2-\text{Ar}$ ), 4.65 (2H; s;  $\text{ClCH}_2-$ ), 6.54 (1H; s;  $H^5$ ), 7.2-7.3 (5H; m; Ar- $H$ ); ESI-MS ( $m/z$ ): 349 ( $\text{M}^+\text{H}$ , 100%), 351 ( $\text{M}^+\text{H}+2$ ), 371 ( $\text{M}^+\text{Na}$ ).

6-(Chloromethyl)-3-hydroxy-2-[[4-(2-methylbenzyl)piperazin-1-yl)methyl]-4H-pyran-4-one (**9**) IR  $\nu$  ( $\text{cm}^{-1}$ ): 1622 (C=O), 1455 (C=C), 1197 (C-O); 746 (C-Cl);  $^1\text{H-NMR}$  ( $\text{CDCl}_3$ , 400 MHz)  $\delta$  ppm: 2.29 (3H; s;  $-\text{CH}_3$ ); 2.38 (4H; brs; piperazine- $H^2$ ,  $H^6$ ), 2.47 (4H; t;  $J = 1.8$ ; piperazine- $H^3$ ,  $H^5$ ), 3.40 (2H; s;  $-\text{CH}_2-$ ), 3.55 (2H; s;  $-\text{CH}_2-\text{Ar}$ ), 4.65 (2H; s;  $\text{ClCH}_2-$ ), 6.54 (1H; s;  $H^5$ ), 7.10-7.20 (4H; m; Ar- $H$ );  $^{13}\text{C-NMR}$  (DEPT) ( $\text{CDCl}_3$ , 400 MHz)  $\delta$  ppm: 173.85, 112.74, 41.43, 53.05, 53.39, 56.12, 161.45, 146.01, 137.75, 130.51, 125.72, 136.32, 130.02, 127.35, 144.55, 60.80, 19.46;  $^{13}\text{C-NMR}$  (APT) ( $\text{CDCl}_3$ , 400 MHz)  $\delta$  ppm: -60.80, -56.12, -53.39, -53.05, -41.43; ESI-MS ( $m/z$ ): 363 ( $\text{M}^+\text{H}$ ), 365 ( $\text{M}^+\text{H}+2$ ), 385 ( $\text{M}^+\text{Na}$ , 100%).

6-(Chloromethyl)-3-hydroxy-2-[[4-(3-methylbenzyl)piperazin-1-yl]methyl]-4H-pyran-4-one (**10**) IR  $\nu$  ( $\text{cm}^{-1}$ ): 1624 (C=O), 1456 (C=C), 1197 (C-O); 747 (C-Cl);  $^1\text{H-NMR}$  ( $\text{CDCl}_3$ , 400 MHz)  $\delta$  ppm: 2.28 (3H; s;  $-\text{CH}_3$ ); 2.36-2.50 (8H; m; piperazine-H), 3.40 (2H; s;  $-\text{CH}_2-$ ), 3.56 (2H; s;  $-\text{CH}_2-\text{Ar}$ ), 4.63 (2H; s;  $\text{ClCH}_2-$ ), 6.52 (1H; s;  $H^F$ ), 7.03-7.19 (4H; m; Ar-H); ESI-MS (m/z): 363 ( $M^+ + \text{H}$ , 100%), 365 ( $M^+ + \text{H} + 2$ ), 385 ( $M^+ + \text{Na}$ ).

6-(Chloromethyl)-3-hydroxy-2-[[4-[3-(trifluoromethyl)benzyl]piperazin-1-yl]methyl]-4H-pyran-4-one (**11**) IR  $\nu$  ( $\text{cm}^{-1}$ ): 1623 (C=O), 1455 (C=C), 1197 (C-O); 749 (C-Cl);  $^1\text{H-NMR}$  ( $\text{CDCl}_3$ , 400 MHz)  $\delta$  ppm: 2.51 (8H; brs; piperazine-H), 3.56 (2H; s;  $-\text{CH}_2-$ ), 3.57 (2H; s;  $-\text{CH}_2-\text{Ar}$ ), 4.65 (2H; s;  $\text{ClCH}_2-$ ), 6.54 (1H; s;  $H^F$ ), 7.53-7.61 (4H; m; Ar-H); ESI-MS (m/z): 417 ( $M^+ + \text{H}$ , 100%), 419 ( $M^+ + \text{H} + 2$ ), 439 ( $M^+ + \text{Na}$ ).

6-(Chloromethyl)-3-hydroxy-2-[[4-[4-(trifluoromethyl)benzyl]piperazin-1-yl]methyl]-4H-pyran-4-one (**12**) IR  $\nu$  ( $\text{cm}^{-1}$ ): 1623 (C=O), 1457 (C=C), 1198 (C-O); 749 (C-Cl);  $^1\text{H-NMR}$  ( $\text{CDCl}_3$ , 400 MHz)  $\delta$  ppm: 2.40 (4H; brs; piperazine- $H^P$ ,  $H^F$ ), 2.50 (4H; t;  $J = 8.8$ ; piperazine- $H^S$ ,  $H^F$ ), 3.55 (2H; s;  $-\text{CH}_2-$ ), 3.57 (2H; s;  $-\text{CH}_2-\text{Ar}$ ), 4.66 (2H; s;  $\text{ClCH}_2-$ ), 6.55 (1H; s;  $H^F$ ), 7.51 (2H; d;  $J = 7.6$ ; Ar- $H^P$ ,  $H^F$ ), 7.66 (2H; d;  $J = 8.0$ ; Ar- $H^S$ ,  $H^F$ ); ESI-MS (m/z): 417 ( $M^+ + \text{H}$ , 100%), 419 ( $M^+ + \text{H} + 2$ ), 439 ( $M^+ + \text{Na}$ ).

6-(Chloromethyl)-2-[[4-(2,5-difluorobenzyl)piperazin-1-yl]methyl]-3-hydroxy-4H-pyran-4-one (**13**) IR  $\nu$  ( $\text{cm}^{-1}$ ): 1627 (C=O), 1460 (C=C), 1052 (C-O); 732 (C-Cl);  $^1\text{H-NMR}$  ( $\text{CDCl}_3$ , 400 MHz)  $\delta$  ppm: 2.38 (4H; brs; piperazine- $H^P$ ,  $H^F$ ), 2.47 (4H; t;  $J = 1.8$ ; piperazine- $H^S$ ,  $H^F$ ), 3.40 (2H; s;  $-\text{CH}_2-$ ), 3.55 (2H; s;  $-\text{CH}_2-\text{Ar}$ ), 4.65 (2H; s;  $\text{ClCH}_2-$ ), 6.54 (1H; s;  $H^F$ ), 7.10-7.20 (3H; m; Ar-H); ESI-MS (m/z): 363 ( $M^+ + \text{H}$ ), 365 ( $M^+ + \text{H} + 2$ ), 385 ( $M^+ + \text{Na}$ , 100%).

2-[[4-(4-Chlorobenzyl)piperazin-1-yl]methyl]-6-(chloromethyl)-3-hydroxy-4H-pyran-4-one (**14**) IR  $\nu$  ( $\text{cm}^{-1}$ ): 1621 (C=O), 1453 (C=C), 1196 (C-O); 746 (C-Cl);  $^1\text{H-NMR}$  ( $\text{CDCl}_3$ , 400 MHz)  $\delta$  ppm: 2.37 (4H; brs; piperazine- $H^P$ ,  $H^F$ ), 2.50 (4H; brs; piperazine- $H^S$ ,  $H^F$ ), 3.44 (2H; s;  $-\text{CH}_2-$ ), 3.56 (2H; s;  $-\text{CH}_2-\text{Ar}$ ), 4.65 (2H; s;  $\text{ClCH}_2-$ ), 6.54 (1H; s;  $H^F$ ), 7.29 (2H; d;  $J = 8.8$ ; Ar- $H^P$ ,  $H^F$ ); 7.35 (2H; d;  $J = 8.4$ ; Ar- $H^S$ ,  $H^F$ ), 9.18 (1H; brs; -OH);  $^{13}\text{C-NMR}$  ( $\text{DMSO}-d_6$ , 400 MHz)  $\delta$  ppm: 41.31, 52.26, 53.32, 60.87, 112.32, 128.00, 130.42, 131.27, 137.17, 143.93, 147.56, 161.06, 173.33; ESI-MS (m/z): 383 ( $M^+ + \text{H}$ , 100%), 385 ( $M^+ + \text{H} + 2$ , % 66.07), 405 ( $M^+ + \text{Na}$ ).

6-(Chloromethyl)-2-[[4-(2,6-dichlorobenzyl)piperazin-1-yl]methyl]-3-hydroxy-4H-pyran-4-one (**15**) IR  $\nu$  ( $\text{cm}^{-1}$ ): 1620 (C=O), 1454 (C=C), 1196 (C-O); 766 (C-Cl);  $^1\text{H-NMR}$  ( $\text{CDCl}_3$ , 400 MHz)  $\delta$  ppm: 2.49 (8H; brs; piperazine-H), 3.53 (2H; s;  $-\text{CH}_2-$ ), 3.54 (2H; s;  $-\text{CH}_2-\text{Ar}$ ), 4.64 (2H; s;  $\text{ClCH}_2-$ ), 6.53 (1H; s;  $H^F$ ), 7.32 (1H; t;  $J = 8.0$ ; Ar- $H^P$ ), 7.44 (2H; d;  $J = 7.6$ ; Ar- $H^S$ ,  $H^F$ ); ESI-MS (m/z): 325 (100%), 419 ( $M^+ + \text{H}$ ), 421 ( $M^+ + \text{H} + 2$ ), 441 ( $M^+ + \text{Na}$ ).

6-(Chloromethyl)-2-[[4-(2,4-dichlorobenzyl)piperazin-1-yl]methyl]-3-hydroxy-4H-pyran-4-one (**16**) IR  $\nu$  ( $\text{cm}^{-1}$ ): 1626 (C=O), 1454 (C=C), 1199 (C-O); 738 (C-Cl);  $^1\text{H-NMR}$  ( $\text{CDCl}_3$ , 400 MHz)  $\delta$  ppm: 2.50 (8H; brs; piperazine-H), 3.53 (2H; s;  $-\text{CH}_2-$ ), 3.57 (2H; s;  $-\text{CH}_2-\text{Ar}$ ), 4.65 (2H; s;  $\text{ClCH}_2-$ ), 6.54 (1H; s;  $H^F$ ), 7.38-7.55 (3H; m; Ar-H), 9.20-9.40 (1H; brs; -OH);  $^{13}\text{C-NMR}$  ( $\text{DMSO}-d_6$ , 400 MHz)  $\delta$  ppm: 42.09, 52.10, 54.08, 58.52, 113.11, 127.87, 129.31, 132.69, 134.74, 135.51, 144.74, 148.30, 161.85, 174.12; ESI-MS (m/z): 363 ( $M^+ + \text{H}$ ), 365 ( $M^+ + \text{H} + 2$ ), 385 ( $M^+ + \text{Na}$ , 100%).

6-(Chloromethyl)-2-[[4-(cyclohexylmethyl)piperazin-1-yl]methyl]-3-hydroxy-4H-pyran-4-one (**17**) IR  $\nu$  ( $\text{cm}^{-1}$ ): 1622 (C=O), 1456 (C=C), 1200 (C-O); 742 (C-Cl);  $^1\text{H-NMR}$  ( $\text{CDCl}_3$ , 400 MHz)  $\delta$  ppm: 0.78-1.70 (11H; m; cyclohexane-H), 2.04 (2H; d;  $J = 7.6$ ;  $-\text{CH}_2-\text{N}$ ), 2.31 (4H; brs; piperazine- $H^P$ ,  $H^F$ ), 2.46 (4H; brs; piperazine- $H^S$ ,  $H^F$ ), 3.54 (2H; s;  $-\text{CH}_2-$ ), 4.65 (2H; s;  $\text{ClCH}_2-$ ), 6.53 (1H; s;  $H^F$ ); ESI-MS (m/z): 355 ( $M^+ + \text{H}$ , 100%), 357 ( $M^+ + \text{H} + 2$ ), 377 ( $M^+ + \text{Na}$ ).

6-(Chloromethyl)-2-[[4-(cyclohexanecarbonyl)piperazin-1-yl]methyl]-3-hydroxy-4H-pyran-4-one (**18**) IR  $\nu$  ( $\text{cm}^{-1}$ ): 1638 (C=O), 1447 (C=C), 1216 (C-O); 738 (C-Cl);  $^1\text{H-NMR}$  ( $\text{CDCl}_3$ , 400 MHz)  $\delta$  ppm: 1.28-1.70 (8H; m; cyclohexane-H), 2.40-2.46 (3H; m; cyclohexane-H), 2.51 (4H; brs; piperazine- $H^P$ ,  $H^F$ ), 3.45 (4H; brs; piperazine- $H^S$ ,  $H^F$ ), 3.60 (2H; s;  $-\text{CH}_2-$ ), 4.65 (2H; s;  $\text{ClCH}_2-$ ), 6.55 (1H; s;  $H^F$ ), 9.21 (1H; brs; -OH);  $^{13}\text{C-NMR}$  ( $\text{DMSO}-d_6$ , 400 MHz)  $\delta$  ppm: 25.80, 26.25, 29.80, 41.59, 42.08, 45.42, 52.83, 53.50, 53.89, 113.11, 144.79, 148.08, 161.92, 174.03, 174.14; ESI-MS (m/z): 325 (100%), 326, 369 ( $M^+ + \text{H}$ ), 371 ( $M^+ + \text{H} + 2$ ), 391 ( $M^+ + \text{Na}$ ).

### Anticonvulsant activity

The compounds were tested for their anticonvulsant activity against maximal electroshock (MES)- and subcutaneous pentylenetetrazol (scPTZ)-induced seizure threshold tests. The acute neurological toxicity was determined in the rotorod test. All these tests were performed in male mice according to the phase-I tests of the Antiepileptic Drug Development (ADD) program which were developed by National Institutes of Health (NIH), National Institute of Neurological Disorders and Stroke (NINDS). Stimulator (Grass S88, Astro-Med. Inc. Grass Instrument Division, W. Warwick, RI, USA), constant current unit (Grass CCU1A, Grass Medical Instrument, Quincy, Mass., USA), and corneal electrodes were used for the evaluation of anticonvulsant activity against MES-induced seizure test. All synthesized compounds were suspended in 30% aqueous of PEG 400 and administered to the mice intraperitoneally in a volume of 0.01 mL/g at body weight. Twelve Swiss albino male mice ( $20 \pm 2$  g) were used for each compound. Mice were purchased from the Hacettepe University Animal Farm according to the ADD-NINDS program<sup>36</sup>. All the animals were acclimatized for a week before use. The animals were maintained in colony cages under a 12 h-light-and-12 h-dark cycle and kept under standard

(hygienic) conditions at an ambient temperature of  $22 \pm 3^\circ\text{C}$  and at a relative humidity between 50 to 60% and fed on standard laboratory diet and food and water was provided *ad libitum* except at the time they were brought out of the cage. All the experimental protocols were carried out with the permission from Hacettepe University, 'Laboratory Animals Ethic Committee' decision (02.01.2009 date 2008/80-4 number). Control animals received 30% aqueous PEG 400. Pentylenerazol was administered subcutaneously (s.c.) on the back of the neck. The rotorod toxicity test was performed on a 1 inch diameter knurled wooden rod, rotating at 6 rpm (the rotorod used in phase-I test was made by Hacettepe University Technical Department). MES tests were elicited with a 60-cycle alternating current of 50 mA intensity (5–7 times more than that required to elicit minimal seizures) delivered for 0.2 sec via corneal electrodes. A drop of 0.9% saline was instilled into the eye prior to application of the electrodes in order to prevent the death of the animal. Abolition of the hind limb tonic extension component of the seizure was defined as protection. 85 mg/kg of pentylenerazol (produces seizures in more than 95% of mice) was administered as a 0.5% solution s.c. into the posterior midline. The animal was observed for 30 min to decide whether the failure of the threshold seizure (a single episode of clonic spasms of at least 5 sec duration) could be defined as protection. The rotorod test was used to evaluate neurotoxicity. The animal was placed on a 1 inch diameter knurled wooden rod rotating at 6 rpm. Normal mice remain on a rod rotating at this speed indefinitely. Neurologic toxicity was defined as the failure of the animal to remain on the rod for 1 min.

### Antitubercular activity

The strains *Mycobacterium tuberculosis* H37Rv (ATCC 27294; American Type Culture Collection) reference strain and *M. avium* (ATCC 15769) were maintained on Lowenstein-Jensen medium and subcultured on Middlebrook 7H11 agar (Becton Dickinson) resuspended in 7H9-S broth medium supplemented with 10% [OADC; 0.1% casitone, 0.5% glycerol, supplemented oleic acid, albumin, dextrose, and catalase], 0.2% glycerol and 0.1% Bacto casitone (Difco). Suspensions were prepared in 0.04% (vol/vol) Tween 80–0.2%+bovine serum albumin so that adjusted to McFarland tube number 1. This was diluted to 1:20 and 100  $\mu\text{l}$  aliquot was used as inoculum. Reference antibacterial agents were purchased from Sigma Chemical Co. (St. Louis, MO, USA) and dissolved in dimethylsulphoxide (streptomycin), or in *d*-water (isoniazid, ethambutol). The stock solutions of the agents were prepared in medium according to the Clinical and Laboratory Standards Institute (CLSI M38-A2, formerly NCCLS)<sup>37,38</sup>. A stock solution of the resazurin sodium salt (Sigma) powder was prepared at 0.01% in sterile distilled water. It was filters-sterilized and kept at  $4^\circ\text{C}$ . One hundred microliters of Middlebrook 7H9 broth (0.1% casitone, 0.5% glycerol, and 10% OADC; Becton-Dickinson) was

dispensed in each well of a sterile flat-bottom 96-well plate, and serial twofold dilutions (256–0.06  $\mu\text{g}/\text{mL}$ ) of each compound were prepared directly in the plate. One hundred microliters of inoculum was added to each well. A growth control and a sterile control were also included for each stain. The plate was covered, and incubated at  $37^\circ\text{C}$  under a normal atmosphere. After 7 days of incubation, 10  $\mu\text{g}/\text{mL}$  of resazurin solution was added to each well, and the plate was reincubated overnight. A change in colour from blue (oxidized state) to pink (reduced) indicated the growth of bacteria, and the minimum inhibitory concentration (MIC) was defined as the lowest concentration of drug that prevented this change in color.

### Antibacterial and antifungal activities

The compounds of **1–18** were dissolved in dimethylsulphoxide:ethanol (80:20) and sterilized by filtration using 0.22  $\mu\text{m}$  Millipore (MA 01730, USA) and used as the stock solutions. Reference antibacterial agents were purchased from Sigma Chemical Co. (St. Louis, MO, USA) and dissolved in phosphate buffer solution (ampicillin, pH 8.0; 0.1 mol/mL), dimethylsulphoxide (ketoconazole), or in water (gentamicin, levofloxacin, fluconazole)<sup>10,11</sup>. Antibacterial activity test were carried out against standards; Gram negative standard strains of *Escherichia coli* ATCC 35218, *Pseudomonas aeruginosa* ATCC 10145, *Proteus mirabilis* ATCC 7002, *Klebsiella pneumoniae* RSKK 574, *Acinetobacter baumannii* RSKK 02026, and as Gram positive standard strains of *Staphylococcus aureus* ATCC 25923, *Enterococcus faecalis* ATCC 29212, *Bacillus subtilis* ATCC 6633 and their drug resistant isolates were used for the determination of antibacterial activity. *Candida albicans* ATCC 10231, *C. parapsilosis* ATCC 22019, *C. tropicalis* ATCC 13803 and *C. krusei* ATCC 6258 were used for the determination of antifungal activity. Mueller-Hinton Broth (MHB; Difco) and Mueller-Hinton Agar (MHA; Oxoid) were applied for growing and diluting of the bacteria suspensions<sup>39</sup>. The synthetic medium RPMI-1640 with L-glutamine was buffered to pH 7 with 3-[*N*-morpholino]-propansulfonic acid and culture suspensions were prepared as described previously<sup>40</sup>. The broth microdilution method was employed for antibacterial and antifungal activity tests. Media were placed into each 96 wells of the microplates. Extract solutions at 512  $\mu\text{g}/\text{mL}$  were added into first rows of microplates and twofold dilutions of the compounds (256–0.125  $\mu\text{g}/\text{mL}$ ) were made by dispensing the solutions to the remaining wells. The lowest concentration of the compounds that completely inhibit macroscopic growth was determined and MICs were reported as described previously study<sup>37,38,40</sup>.

## Results and discussion

### Chemistry

Treatment of kojic acid with thionyl chloride at room temperature yielded chlorokojic acid which after reduction with zinc and hydrochloric acid yielded allomaltol

(Scheme 1). These three compounds provided basis for our research area. Mannich bases of several hydroxypyranones including kojic acid and pyromeconic acid (3-hydroxy-4*H*-pyran-4-one) were synthesized before by different researchers<sup>41,42</sup>. They react with amines and formaline like phenols to produce the Mannich base as a result of aminoalkylation of the *ortho* position of the -OH group.

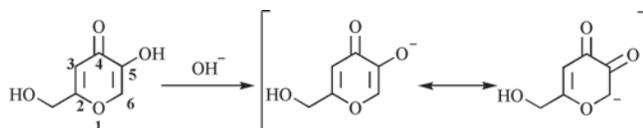
In an earlier study, it was reported that di-Mannich derivatives were obtained in an acidic medium from the reaction of kojic acid, formaline and aromatic amine<sup>43</sup>. On the other hand, although there were two open nuclear positions of kojic acid (3- and 6-), because of its phenol-like properties, the reaction occurred only at 6-position and mono-Mannich derivatives were obtained in basic medium (Scheme 3). When the enolic hydroxyl group converted to an ether group, 6-position was deactivated<sup>41</sup>. The mechanisms of this Mannich reaction both in an acidic and a basic medium were investigated and found that, enhanced reactivity in a basic medium is due to increase of the electronegativity at 6-position<sup>42</sup>.

It is well known that chlorokojic acid is an important compound from the chemical point of view and as the chlorine atom in the structure readily undergoes nucleophilic substitution<sup>44</sup>, it has been used as starting or intermediate material in many reactions<sup>2,4,6-11</sup>.

General synthesis of compounds is given in Scheme 2. Chlorokojic acid was gained by commercially available kojic acid as described methods from the published literature<sup>8-11</sup>. In order to obtain chlorokojic acid; 2-hydroxymethyl moiety of kojic acid was chlorinated by using thionyl chloride at room temperature.

Mannich type reactions are three component condensation reactions involving carbonyl compounds which exist as keto-enol tautomeric forms, formaline and a secondary amine. The amino alkylation of aromatic substrates by Mannich reaction is of considerable importance for the design, synthesis and modification of biologically active molecules. It has advantages ranging from lower reaction times, increased reaction rates to higher yields and reproducibility<sup>45</sup>. In order to investigate the influence of secondary amines moieties such as piperidine, morpholine and piperazine in the structure of Mannich bases, all of them are used in our previous studies (Scheme 2)<sup>4,6-11</sup>. Herein the basic substituent was introduced at the 6-position of chlorokojic acid via a Mannich type reaction using formaline and an appropriate substituted piperazine.

The physicochemical parameters of the synthesized compounds including melting point, yield, calculated



Scheme 3. Numeration and tautomers of kojic acid in basic medium.

logP (clogP) and elemental analysis data are presented in Table 1. clogP refers to calculated hydrophobicity of the compounds, respectively, clogP have been calculated from ACD/ChemSketch, Product version 12.01. The thumb rules for clogP values, to a drug like molecule must be lower than "5" to by-pass the cell barrier.

The structure of the compounds were clarified by IR, <sup>1</sup>H-NMR and mass spectroscopy in experimental part. Also, with a view to analyse the structures of compounds **1**, **6**, **9**, **14**, **16** and **18** <sup>13</sup>C-NMR spectroscopy was used. The selected diagnostic bands of IR spectra of chlorokojic acid derivatives provide useful information for determining structures. All the compounds exhibited absorption bands about 1620 cm<sup>-1</sup> due to ν (C=O) stretching of pyranone ring. Because of hydroxymethyl moiety showing two hydrogen bondings both intra- and intermolecular, (C=O) stretching gave signals at lower frequency. IR spectra of all compounds showed ν (C-Cl) at 732-766 cm<sup>-1</sup>, ν (C-O) at 1200-1195 cm<sup>-1</sup> and ν (C=C) at 1447-1460 cm<sup>-1</sup>. The formation of the Mannich bases of chlorokojic acid was further confirmed with the <sup>1</sup>H-NMR spectra. Assignments of the signals were based on the chemical shifts and intensity pattern. The <sup>1</sup>H-NMR spectra of the compounds exhibited triplet peaks for -CH<sub>2</sub>- at piperazine between 2.2 and 3.6 ppm. *J* values of these peaks were from 4.4 to 5.0 Hz. Phenyl ring's protons exhibit signals at 7.6 to 9.2 ppm. Compounds **7**, **17** and **18** which had cyclic protons in their structure instead of phenyl ring, showed peaks ranging 0.78 to 2.17 ppm as multiplets. Characteristic *H*<sup>5</sup> proton of the 4*H*-pyran-4-one ring were determined as singlet peaks between 6.52 and 6.57 ppm. Also, due to keto-enol tautomerisation of hydroxyl group on pyranone ring -OH peaks of compounds **2**, **4**, **5**, **7**, **9**, **10**, **11**, **12** and **13** were not observed. <sup>13</sup>C NMR spectra analysis were supported by DEPT and APT spectra which differentiate between -CH=, -CH<sub>2</sub>- and -CH<sub>3</sub> groups. The <sup>13</sup>C NMR signals of compounds **1**, **6**, **9**, **14**, **16** and **18** were in good agreement with proposed structures. Compounds displayed characteristic peaks of methylene group (ClCH<sub>2</sub>-) carbons at 41.02, 42.09, 41.43 41.31, 42.09 and 42.08 ppm, respectively. Carbonyl carbons of the 4*H*-pyran-4-one ring was found at a range of 161.06-161.93 ppm. Signals at 150.99-146.01 ppm were due to C<sub>3</sub> of the pyranone ring whereas 112.08-113.12 ppm belong to C<sub>5</sub>. The distinctive signals of all compounds were observed in the mass spectra which followed the similar fragmentation pattern. The entire spectrums showed molecular ion peaks, M<sup>+</sup>+23 (Na) peaks and isotope peaks owing to chlorine atom.

### Anticonvulsant activity

Seizures that are arising from discharging lesions of the cerebral cortex often occur as part of an epileptic syndrome. It is a group of signs and symptoms that customarily occur together. Identification of the syndrome helps to determine the appropriate therapy and the prognosis. The MES-induced seizure test is a predictor of compounds that are active against tonic-clonic (grand mal) seizures. The scPTZ-induced seizure test is used to detect

Table 1. Physicochemical parameters of the synthesized compounds.

Com no	R	Mol. Formula	M. p.	cLogP*	Log $\epsilon$ ( $\lambda$ max)nm	Yield (%)	Elemental analysis		
		(Mol. Wt.)	(°C)				Found % (calculated)		
							C	H	N
1	phenyl	C <sub>17</sub> H <sub>19</sub> ClN <sub>2</sub> O <sub>3</sub> (334.80)	155-6	2.67 ± 0.75	4.37 (205)	88	60.28 (60.99)	5.51 (5.72)	8.32 (8.37)
2	2-methylphenyl	C <sub>18</sub> H <sub>21</sub> ClN <sub>2</sub> O <sub>3</sub> (348.82)	151-2	3.14 ± 0.75	4.44 (207)	70	61.83 (61.98)	5.89 (6.07)	7.92 (8.03)
3	4-methylphenyl	C <sub>18</sub> H <sub>21</sub> ClN <sub>2</sub> O <sub>3</sub> (348.82)	168-9	3.14 ± 0.75	4.52 (203)	67	61.72 (61.98)	5.82 (6.07)	7.84 (8.03)
4	2,3-dimethylphenyl	C <sub>19</sub> H <sub>23</sub> ClN <sub>2</sub> O <sub>3</sub> (362.85)	164-5	3.60 ± 0.75	4.42 (211)	88	62.54 (62.89)	6.13 (6.39)	7.74 (7.72)
5	4-acetylphenyl	C <sub>19</sub> H <sub>21</sub> ClN <sub>2</sub> O <sub>4</sub> (376.83)	163-4	2.45 ± 0.75	4.34 (202)	85	59.46 (60.56)	5.40 (5.62)	7.43 (7.43)
6	4-nitrophenyl	C <sub>17</sub> H <sub>18</sub> ClN <sub>3</sub> O <sub>5</sub> (379.80)	178-9	3.12 ± 0.75	4.32 (202)	66	53.95 (53.76)	4.68 (4.78)	10.88 (11.06)
7	cyclohexyl	C <sub>17</sub> H <sub>25</sub> ClN <sub>2</sub> O <sub>3</sub> (340.85)	164-5	2.35 ± 0.75	4.17 (222)	75	59.71 (59.90)	7.19 (7.39)	8.08 (8.22)
8	benzyl	C <sub>18</sub> H <sub>21</sub> ClN <sub>2</sub> O <sub>3</sub> (348.82)	150-1	1.82 ± 0.75	4.33 (206)	63	61.44 (61.98)	6.19 (6.07)	7.46 (8.03)
9	2-methylbenzyl	C <sub>19</sub> H <sub>23</sub> ClN <sub>2</sub> O <sub>3</sub> (362.85)	152-3	2.28 ± 0.75	4.27 (202)	91	62.52 (62.89)	6.57 (6.39)	7.76 (7.72)
10	3-methylbenzyl	C <sub>19</sub> H <sub>23</sub> ClN <sub>2</sub> O <sub>3</sub> (362.85)	147-9	2.28 ± 0.75	4.27 (203)	89	63.16 (62.89)	6.08 (6.39)	7.74 (7.72)
11	3-trifluoromethylbenzyl	C <sub>19</sub> H <sub>20</sub> ClF <sub>3</sub> N <sub>2</sub> O <sub>3</sub> (416.82)	161-2	2.40 ± 0.75	4.24 (211)	78	54.69 (54.75)	4.45 (4.84)	6.73 (6.72)
12	4-trifluoromethylbenzyl	C <sub>19</sub> H <sub>20</sub> ClF <sub>3</sub> N <sub>2</sub> O <sub>3</sub> (416.82)	150-1	2.40 ± 0.75	3.97 (216)	83	54.59 (54.75)	4.75 (4.84)	6.71 (6.72)
13	2,5-difluorobenzyl	C <sub>18</sub> H <sub>19</sub> ClF <sub>2</sub> N <sub>2</sub> O <sub>3</sub> (384.81)	151-2	1.99 ± 0.75	3.99 (210)	75	55.88 (56.18)	4.91 (4.98)	7.28 (7.28)
14	4-chlorobenzyl	C <sub>18</sub> H <sub>20</sub> Cl <sub>2</sub> N <sub>2</sub> O <sub>3</sub> (382.90)	168-9	2.42 ± 0.75	4.35 (221)	92	56.08 (56.41)	5.10 (5.26)	7.31 (7.31)
15	2,6-dichlorobenzyl	C <sub>18</sub> H <sub>19</sub> Cl <sub>3</sub> N <sub>2</sub> O <sub>3</sub> (417.71)	167-9	3.03 ± 0.75	4.59 (203)	90	51.94 (51.76)	4.62 (4.58)	6.57 (6.71)
16	2,4-dichlorobenzyl	C <sub>18</sub> H <sub>19</sub> Cl <sub>3</sub> N <sub>2</sub> O <sub>3</sub> (417.71)	151-2	3.03 ± 0.75	4.42 (204)	85	51.57 (51.76)	4.58 (4.58)	6.66 (6.71)
17	cyclohexylmethyl	C <sub>18</sub> H <sub>27</sub> ClN <sub>2</sub> O <sub>3</sub> (354.87)	151-2	2.88 ± 0.75	4.17 (201)	76	60.89 (60.92)	7.41 (7.67)	7.98 (7.89)
18	cyclohexylcarbonyl	C <sub>18</sub> H <sub>25</sub> ClN <sub>2</sub> O <sub>4</sub> (368.85)	165-7	2.58 ± 0.75	4.32 (202)	75	57.87 (58.61)	6.65 (6.83)	7.33 (7.59)

\*cLogP: values are calculated theoretically by ACD/ChemSketch, Product version 12.01.

compounds useful in treating generalized absence (petit mal) seizures<sup>36</sup>.

According to our previous studies, when route A and C (Scheme 1) were examined, it was seen that substituted phenylpiperazine derivatives bearing 3-trifluoromethyl, 4-fluoro, 2-methoxy, 2-chloro and 4-chloro moieties were the most protective compounds in the Mannich series against convulsions at all doses. Also, kojic acid and allomaltol derivatives that carry hydroxypiperidine moiety were significantly more protective against scPTZ and MES tests at all doses<sup>9</sup> than other piperidine derivatives<sup>6,7</sup>.

Anticonvulsant activity tests were performed in male mice according to the phase-I tests of the ADD program which were developed by NIH and NINDS<sup>36</sup>. Herein, the anticonvulsant activities of the synthesized compounds were evaluated by MES and scPTZ-induced seizure tests performed at 0.5 and 4 h after administration with 30, 100 and 300 mg/kg doses using male Swiss albino mice (20 ± 2 g). The acute neurological toxicity was determined in the rotorod test. The results are presented in Table 2.

Chlorokojic acid was not found protective against scPTZ-induced seizure test; however, some series of

Table 2. Anticonvulsant and neurotoxicity screening data of the synthesized compounds.

Compounds	MES <sup>a</sup>						ScPTZ <sup>b</sup>						Toxicity <sup>c</sup>					
	0.5 h (mg/kg)			4 h (mg/kg)			0.5 h (mg/kg)			4 h (mg/kg)			0.5 h (mg/kg)			4 h (mg/kg)		
	30	100	300	30	100	300	30	100	300	30	100	300	30	100	300	30	100	300
<b>1</b>	0/1	0/1	0/1	0/1	0/1	<b>1/1</b>	0/1	0/1	0/1	0/1	0/1	0/1	0/4	0/4	0/4	0/2	0/2	0/2
<b>2</b>	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	<b>1/1</b>	0/4	0/4	0/4	0/2	0/2	0/2
<b>3</b>	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	<b>1/1</b>	0/1	0/1	0/1	0/4	0/4	0/4	0/2	0/2	0/2
<b>4</b>	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/4	0/4	0/4	0/2	0/2	0/2
<b>5</b>	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/4	0/4	0/4	0/2	0/2	0/2
<b>6</b>	0/1	0/1	0/1	0/1	0/1	<b>1/1</b>	0/1	0/1	0/1	0/1	0/1	<b>1/1</b>	0/4	0/4	0/4	0/2	0/2	0/2
<b>7</b>	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/4	0/4	0/4	0/2	0/2	0/2
<b>8</b>	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	<b>1/1</b>	0/1	0/1	0/1	0/4	0/4	0/4	0/2	0/2	0/2
<b>9</b>	0/1	0/1	0/1	0/1	<b>1/1</b>	<b>1/1</b>	0/1	0/1	0/1	0/1	0/1	0/1	0/4	0/4	0/4	0/2	0/2	0/2
<b>10</b>	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/4	0/4	0/4	0/2	0/2	0/2
<b>11</b>	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	<b>1/1</b>	0/4	0/4	0/4	0/2	0/2	0/2
<b>12</b>	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	<b>1/1</b>	<b>1/1</b>	0/4	0/4	0/4	0/2	0/2	0/2
<b>13</b>	0/1	0/1	0/1	0/1	0/1	<b>1/1</b>	0/1	0/1	<b>1/1</b>	<b>1/1</b>	<b>1/1</b>	<b>1/1</b>	0/4	0/4	0/4	0/2	0/2	0/2
<b>14</b>	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	<b>1/1</b>	0/1	<b>1/1</b>	<b>1/1</b>	0/4	0/4	0/4	0/2	0/2	0/2
<b>15</b>	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/4	0/4	0/4	0/2	0/2	0/2
<b>16</b>	0/1	0/1	0/1	0/1	<b>1/1</b>	<b>1/1</b>	0/1	0/1	0/1	0/1	0/1	0/1	0/4	0/4	0/4	0/2	0/2	0/2
<b>17</b>	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/4	0/4	0/4	0/2	0/2	0/2
<b>18</b>	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/4	0/4	0/4	0/2	0/2	0/2
<b>CKA</b>	0/1	0/1	<b>1/1</b>	0/1	0/1	<b>1/1</b>	0/1	0/1	0/1	0/1	0/1	0/1	0/4	0/4	0/4	0/2	0/2	0/2

CKA: Chlorokojic Acid, <sup>a</sup>MES: Maximal electroshock, <sup>b</sup>ScPTZ: Subcutaneous pentylenetetrazol, <sup>c</sup>Toxicity: Rotorod test, 0/1: no activity, 1/1: noticeable activity.

chlorokojic acid achieved notable activity when their Mannich bases were examined. Compounds **12** and **14** showed protection at both 300 mg/kg and 100 mg/kg doses at 4 h. It may be speculated that this is probably because of increment in lipophilicity. Also, when halogen substitution to benzyl ring is examined, at all doses, 2,5-difluorobenzyl (compound **13**) derivative was determined as the most active compound by scPTZ-induced seizure test at 4 h, but in contrast when 3,4-dichlorobenzyl derivative (compound **16**) was tested instead of fluoro atom, scPTZ protection was failed. However this time, selective MES protection was observed at 300 and 100 mg/kg. None of the compounds **4**, **5**, **7**, **10**, **15**, **17** and **18** showed anticonvulsant activity.

With respect to the results of the MES tests, chlorokojic acid showed protection at the 300 mg/kg dose at both at 0.5 and 4 h. The augmentation of this activity is aimed by preparing Mannich bases. Moreover, compounds **9** and **16** bearing 2-methylbenzyl and 2,4-dichlorobenzyl moiety, respectively, were found as the most effective molecules with selective protection at 100 and 300 mg/kg doses at 4 h in this series. Against the same test, compounds **1**, **6** and **13** exhibited activity at the 300 mg/kg. At the same dose, compounds **2**, **3**, **6**, **8** and **12** also showed protection against scPTZ-induced seizure test. None of the compounds showed neurotoxicity at any of the studied doses.

When the effect of aromaticity was examined, it was seen that, with the reduction of phenyl in compound **1** and benzyl group in compound **8** to change into cyclohexyl (compound **7**) and cyclohexylmethyl (compound **17**), respectively, their anticonvulsant activities disappeared. Also, compound **18** bearing cyclohexylcarbonyl was not

protective. Finally, anticonvulsant activities of Mannich bases were increased in some compounds while some were decreased when compared to chlorokojic acid. When the results of this study were compared to our previous studies<sup>4,8,9</sup> the expected increment in anticonvulsant activity of Mannich bases could not be observed. That shows us chlorokojic acid derivatives of Mannich bases have lower biological activities than kojic acid and allomaltol derivatives.

#### Antitubercular activity

The antitubercular activity of the compounds was performed as MICs against *M. tuberculosis* and *M. avium* by using Resazurin microplate assay procedure (REMA). Isoniazid, ethambutol and streptomycin were used as reference compounds (Table 3).

Mannich bases of allomaltol derivatives including 3-methyl, 4-methyl and 3,5-dimethyl piperidine and 2-methoxyphenyl piperazine containing piperazine and piperidine structure which were firstly synthesized by our research group were found to have antimycobacterial effect against *Mycobacterium smegmatis*<sup>46,47</sup>. However the main cause of TB is *M. tuberculosis*. It divides extremely slower than the other bacteria but can be cultured *in vitro* whereas the others can only grow within the cells of a host<sup>48</sup>. *M. avium* is a part of a nontuberculous mycobacteria group which causes pulmonary diseases resembling TB. Herein, the *in vitro* antitubercular activity of the compounds against *M. tuberculosis* and *M. avium* was evaluated and MIC values are demonstrated in Table 3. All of the Mannich bases have antitubercular activity and have shown promising *in vitro* antitubercular activity

Table 3. Screening for antimicrobial activity against Gram positive bacteria and *Mycobacterium* (MIC in µg/mL).

	Gram positive bacteria						Mycobacterium	
	<i>S. aureus</i>		<i>E. faecalis</i>		<i>B. subtilis</i>		<i>M. tuberculosis</i>	<i>M. avium</i>
	ATCC 25923	Isolated strain	ATCC 29212	Isolated strain	ATCC 6633	Isolated strain	ATCC 27294	ATCC 15769
<b>1</b>	16	128	32	128	16	32	32	16
<b>2</b>	16	128	32	128	16	32	32	16
<b>3</b>	16	128	32	128	8	32	8	8
<b>4</b>	16	128	32	128	8	32	16	16
<b>5</b>	16	128	32	128	8	32	16	16
<b>6</b>	16	128	32	128	8	32	32	16
<b>7</b>	16	128	32	128	8	32	16	8
<b>8</b>	16	128	32	128	8	32	32	32
<b>9</b>	16	128	32	128	8	32	32	32
<b>10</b>	16	128	32	128	8	32	32	32
<b>11</b>	16	128	32	128	8	32	16	8
<b>12</b>	8	128	16	128	4	16	16	8
<b>13</b>	16	128	32	128	8	32	32	8
<b>14</b>	32	128	64	128	16	64	16	8
<b>15</b>	32	128	64	128	16	64	16	8
<b>16</b>	32	128	64	128	16	64	16	8
<b>17</b>	64	128	64	128	32	64	32	8
<b>18</b>	32	128	64	128	16	64	16	4
<b>CKA</b>	32	128	16	128	8	64	16	8
<b>AMP</b>	<0.12	>128	0.5	>128	0.12	0.5		
<b>LVX</b>	0.25	128	0.5	32	-	-		
<b>INH</b>							0.125	0.125
<b>EMB</b>							2	2
<b>SM</b>							1	2

**CKA:** Chlorokojic acid, **AMP:** Ampicilline, **LVX:** Levofloxacin, **INH:** Isoniazid, **EMB:** Ethambutol, **SM:** Streptomycin  
Isolated strain of *S. aureus* (methicillin resist; MRSA), isolated strain of *E. faecalis* (cephalosporin resist), isolated strain of *B. subtilis* (ceftriaxon resist).

against *M. tuberculosis* in a MIC range of 8-32 µg/mL. Among the entire series the most effective one was compound **3** carrying 4-methylphenyl piperazine structure against *M. tuberculosis* (MIC: 8 µg/mL). The antitubercular activity against *M. avium* was generally stronger with MIC values of 4-32 µg/mL. Compound **18** bearing carboxyphenyl piperazine moiety showed the highest antitubercular activity (MIC: 4 µg/mL) against *M. avium* and comparable results to reference drug ethambutol and streptomycin (MIC: 2 µg/mL). Generally, compounds showed greater inhibition activity over *M. avium* growth than *M. tuberculosis* whereas compounds **3** and **8-10** had the same MIC values against both microorganisms. The existing antimycobacterial activity of chlorokojic acid was improved by synthesizing their Mannich bases at only compounds **3** and **18**. However when compared to reference drugs (isoniazid, ethambutol, streptomycin) antimycobacterial activity were not strong as expected and the scaffold should be developed in order to obtain more active compounds.

### Antibacterial and antifungal activity

The newly synthesized compounds (**1-18**) were tested for their *in vitro* antibacterial and antifungal activities according to the guidelines of the Clinical and

Laboratory Standards Institute (CLSI)<sup>10,11</sup>. For antibacterial activity assessment, standard strains (*Escherichia coli*, *Pseudomonas aeruginosa*, *Proteus mirabilis*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Staphylococcus aureus*, *Enterococcus faecalis* and *Bacillus subtilis*) and their drug-resistant isolates were tested; and for antifungal activity *Candida albicans* and *C. parapsilosis*, *C. tropicalis* and *C. krusei* were used.

Ampicillin, gentamicin, levofloxacin for antibacterial, ketoconazole and fluconazole for antifungal assays were also tested under identical conditions. The results are demonstrated in Tables 3-5 and expressed as MIC values in comparison to reference drugs and chlorokojic acid.

Some of the important antifungal agents (e.g. posaconazole, ketoconazole, itraconazole) that are being used for the treatment of fungal infections and antibiotics such as newly marketed linezolid, contain a piperazine and/or an azole ring in their structures<sup>11</sup>. Since the compounds we synthesized previously have remarkable antimicrobial activities, we aimed to assess the contribution of the substituents on the piperazine structure to the biological activity.

According to our previous studies<sup>10,11</sup>, 6-(chloromethyl)-3-hydroxy-2-[(3,4-dichlorobenzyl)piperazin-1-yl]methyl]-4H-pyran-4-one were significantly more active

than 3,4-dichlorophenyl derivative. However, in the compound containing fluorine atom, no meaningful changes were observed in activity (Figure 1). Our efforts for the synthesis of Mannich bases possessing antimicrobial activity led us to find out that compounds bearing chlorine atom were effective molecules. Also, to discover the effect of methylene linkage in compounds, we synthesized both the phenyl and the benzyl derivatives of the same substituents<sup>10,11</sup>. In the light of these facts, we examined the activities of designed Mannich bases bearing benzyl piperazine.

The synthesized compounds showed a broad spectrum of antibacterial activity against Gram negative and Gram positive standard strains with MIC values between 4 and 64 µg/mL (Table 3 and 4). In the meantime, the compounds showed activity against drug-resistant isolated both Gram positive and negative strains with MIC values of 16 to 128 µg/mL.

As observed for Gram negative bacteria, compounds **1** and **11**, bearing phenyl piperazine and 3-trifluoromethylbenzyl moieties respectively, were the most active compounds with the same MIC values (8–16 µg/mL). Both of them were four-folds more active than chlorokojic acid against *E. coli*. In this case, against the other Gram negative bacteria, activity increased two-folds. Structural modifications were not effective on activity, because the

other compounds (compounds **2–11**; **12–18**) had the same MIC values with chlorokojic acid (16–32 µg/mL).

As seen in Table 4, compound **12** having 4-trifluoromethylbenzyl in its structure was determined to have significantly high antibacterial potential against standard strains of *Staphylococcus aureus* and *Bacillus subtilis* with an inhibition between 4 and 8 µg/mL. In comparison of compound **12** with chlorokojic acid (MIC: 8–32 µg/mL) against both of mentioned bacteria, its activity increased four and two times, respectively. Moreover, compounds **1–12** and **13** also showed higher activity with MIC value 16 µg/mL than chlorokojic acid (MIC: 32 µg/mL) whereas compounds **14–16** and **18** had no difference in activity with chlorokojic acid. The least efficiency of the compounds among Gram positive bacteria was seen on *Enterococcus faecalis* with a concentration of 32–64 µg/mL except compound **13** (MIC: 16 µg/mL). Among the series of compounds (**14–16**) bearing chlorobenzylpiperazine the expected increment, like in 3,4-dichloro derivatives (Figure 1), was not observed in antimicrobial activity. Thus, it could be understood that within benzyl series the location of chlorine substituents affects the activity. As references ampicillin, gentamicin, and levofloxacin the tested compounds bearing slight activity against tested standard and their drug resistant isolates (*E. coli*, *P. aeruginosa*,

Table 4. Screening for antimicrobial activity against Gram negative bacteria (MIC in µg/mL).

	Gram negative bacteria									
	<i>E. coli</i>		<i>P. aeruginosa</i>		<i>P. mirabilis</i>		<i>K. pneumoniae</i>		<i>A. baumannii</i>	
	ATCC 35218	Isolated strain	ATCC 10145	Isolated strain	ATCC 7002	Isolated strain	RSKK 574	Isolated strain	RSKK 02026	Isolated strain
<b>1</b>	8	128	16	128	8	128	8	128	8	128
<b>2</b>	32	128	32	128	16	128	16	128	16	128
<b>3</b>	32	128	32	128	16	128	16	128	16	128
<b>4</b>	32	128	32	128	16	128	16	128	16	128
<b>5</b>	32	128	32	128	16	128	16	128	16	128
<b>6</b>	32	128	32	128	16	128	16	128	16	128
<b>7</b>	32	128	32	128	16	128	16	128	16	128
<b>8</b>	32	128	32	128	16	128	16	128	16	128
<b>9</b>	32	128	32	128	16	128	16	128	16	128
<b>10</b>	32	128	32	128	16	128	16	128	16	128
<b>11</b>	8	128	16	128	8	128	8	128	8	128
<b>12</b>	32	128	32	128	16	128	16	128	16	128
<b>13</b>	32	128	32	128	16	128	16	128	16	128
<b>14</b>	32	128	32	128	16	128	16	128	16	128
<b>15</b>	32	128	32	128	16	128	16	128	16	128
<b>16</b>	32	128	32	128	16	128	16	128	16	128
<b>17</b>	32	128	32	128	16	128	16	128	16	128
<b>18</b>	32	128	32	128	16	128	16	128	16	128
<b>CKA</b>	32	128	32	128	16	128	16	128	16	128
<b>AMP</b>	2	>128	-	-	2	>128	2	>128	2	>128
<b>LVX</b>	0.12	0.5	1	64	<0.12	1	0.12	1	0.12	64
<b>GM</b>	-	-	0.5	2	-	-	-	-	-	-

**CKA:** Chlorokojic acid, **AMP:** Ampicilline, **LVX:** Levofloxacin, **GM:** Gentamicine.

*E. coli* isolates; (+ESβLs enzyme), *P. aeruginosa* isolates (resist to Trimethoprim-sulfamethoxazole, tazobactam), *P. mirabilis* isolates (resist to Trimethoprim-sulfamethoxazole, amoxicillin clavulonate, ceftriaxone), *K. pneumoniae* isolates (resist to Trimethoprim-sulfamethoxazole, amoxicillin clavulonate, ceftriaxone), *A. baumannii* isolates (Trimethoprim-sulfamethoxazole resist).

*P. mirabilis*, *K. pneumoniae*, *A. baumannii*, *S. aureus*, *E. faecalis*, *B. subtilis*).

According to the obtained data (Table 5), compounds **1–3**, having phenyl, 2- and 4-methylphenyl in their structures respectively, possessed significant antifungal activity against *C. krusei* with MIC value of 32 µg/mL, even more active than the reference drug, fluconazole and chlorokojic acid, while compounds **4–18** had the same (MIC: 64 µg/mL). The MIC values of compounds **1** and **12** were 8 µg/mL with showing the highest activity against *C. albicans* in this series. Also, compounds **3–11**, **14** and **16** showed more remarkable antifungal activity with MIC value of 16 µg/mL than chlorokojic acid (MIC: 32 µg/mL). Compounds **1–3** inhibited the growth of *C. tropicalis* two-folds (MIC: 32 µg/mL) than chlorokojic acid and compounds **11**, **15** and **16** (MIC: 64 µg/mL). Compared with references all tested compounds shows weak antifungal activity against *Candida* species (*C. albicans*, *C. parapsilosis*, *C. tropicalis*) except from *C. krusei* with a MIC values of fluconazole 64 µg/mL.

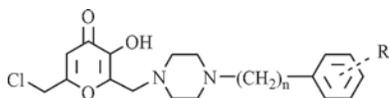


Figure 1. Phenyl and benzylpiperazine derivatives of Mannich bases of chlorokojic acid.

Table 5. Screening for antifungal activity of the compounds (MIC in µg/mL).

	Fungus			
	<i>C. albicans</i>	<i>C. parapsilosis</i>	<i>C. tropicalis</i>	<i>C. krusei</i>
	ATCC 10231	ATCC 90028	ATCC 13803	ATCC 6258
<b>1</b>	8	32	32	32
<b>2</b>	32	32	32	32
<b>3</b>	16	32	32	32
<b>4</b>	16	64	128	64
<b>5</b>	16	64	128	64
<b>6</b>	16	64	128	64
<b>7</b>	16	64	128	64
<b>8</b>	16	64	128	64
<b>9</b>	16	64	128	64
<b>10</b>	16	64	128	64
<b>11</b>	16	32	64	64
<b>12</b>	8	64	128	64
<b>13</b>	16	64	128	64
<b>14</b>	16	64	128	64
<b>15</b>	32	64	64	64
<b>16</b>	16	64	64	64
<b>17</b>	32	64	128	64
<b>18</b>	32	64	128	64
<b>CKA</b>	32	32	64	64
<b>KET</b>	0.5	1	2	4
<b>FLU</b>	2	4	4	64

**CKA:** Chlorokojic acid; **KET:** Ketaconazole; **FLU:** Fluconazole.

## Conclusion

In present study, Mannich bases of chlorokojic acid were synthesized and screened for their biological activities. Compounds were designed in such a way that the pyranone nucleus was substituted with different piperazine derivatives containing phenyl, benzyl or cyclohexyl groups. The existing anticonvulsant activity of chlorokojic acid, which was active against MES-induced seizure test, was aimed to increase by preparing more lipophilic agents as Mannich derivatives. The results of anticonvulsant evaluation revealed that compound **13**, bearing 2,4-difluorobenzyl moiety showed the highest protection against scPTZ-induced seizures. When antimicrobial activity was evaluated, compounds **1** and **11**, bearing phenyl piperazine and 3-trifluoromethylbenzyl moieties respectively, were found as the most active compounds with MIC values of 8–16 µg/mL against Gram negative bacteria. Compound **12**, carrying 4-trifluoromethylbenzyl group, was significantly effective among the synthesized compounds and chlorokojic acid against Gram positive bacteria. According to the antifungal activity results, the existing activity of chlorokojic acid increased, especially compound **1** was established as the most effective molecule against *Candida* spp. Beside this, compound **18** has been identified as a promising lead molecule for antimycobacterial activity with MIC value that is comparable to reference drugs. The endpoint in evaluation all of activity in piperazine containing Mannich bases of chlorokojic acid in this study is, these compounds have lower biological activities unexpectedly. However these compounds would represent a productive matrix for the development of new biologically important agents and deserve further investigation.

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## Declaration of interest

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