Full Paper

Anticonvulsant and Neurotoxicity Evaluation of Some Novel Kojic Acids and Allomaltol Derivatives

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A series of new 3-hydroxy-6-hydroxymethyl/methyl-2-substituted 4*H*-pyran-4-ones were synthesized and prepared by the reaction of kojic acid or allomaltol with piperidine derivatives and formaline as potential anticonvulsant compounds. The structure of the synthesized compounds was confirmed using the elemental analysis results and the spectroscopic techniques such as IR, ^{1}H-NMR, and ESI-MS. Anticonvulsant activities were examined by maximal electroshock (MES) and subcutaneous Metrazol (scMet)-induced seizure tests. Neurotoxicity was determined by the rotorod toxicity test. All these tests were performed in accordance with the procedures of the Antiepileptic Drug Development (ADD) program. According to the activity studies and at all doses, 3-hydroxy-2-[(4-hydroxy-4-phenylpiperidin-1-yl)methyl]-6-methyl-4*H*-pyran-4-one (compound **1**), 2-{[4-(4-chlorophenyl)-3,6-dihydropyridin-1(2*H*)-yl]methyl}-3-hydroxy-6-methyl-4*H*-pyran-4-one (compound **6**), 2-[(4-acetyl-4-phenylpiperidin-1-yl)methyl]-3-hydroxy-6-(hydroxymethyl)-4*H*-pyran-4-one (compound **11**), and 2-{[4-(4-chlorophenyl)-3,6-dihydropyridin-1(2*H*)-yl]methyl}-3-hydroxy-6-hydroxymethyl-4*H*-pyran-4-one (compound **12**) were found to have anticonvulsant activity against MES-induced seizures at 4 h. Also, 2-{[4-(4-bromophenyl)-4-hydroxypiperidin-1-yl]methyl}-3-hydroxy-6-(hydroxymethyl)-4*H*-pyran-4-one (compound **8**) was determined to be the most active compound against scMet-induced seizures at all doses at 0.5 and 4 h. In the rotorod neurotoxicity screening, all compounds showed no toxicity at all doses.

Keywords: Allomaltol / Anticonvulsant activity / Kojic acid / Mannich reaction / Synthesis

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Introduction

Epilepsy, characterized by recurrent unprovoked seizures, is the most prevalent neurological disorder, affecting approximately 60 million people worldwide. More than 30% of patients with epilepsy have inadequate control of seizures with available medical therapies. The established conventional anti-epileptic drugs – though widely prescribed – exhibited undesired side-effect profiles ranging from drowsiness to megaloblastic anemia and failure to control seizures adequately [1 – 4].

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 maximal electroshock (MES)-induced seizure test is a predictor of compounds that are active against tonic-clonic (grand mal) seizures. The subcutaneous Metrazol (scMet)-induced seizure test is used to detect compounds useful in treating generalized absence (petit mal) seizures [5]. There is continuing demand for new anticonvulsant agents, as it has not been possible to control every kind of seizure with the currently available anti-epileptic drugs. To provide an improvement of the quality of life for people suffering from epilepsy, it is essential to search for new chemical entities with lower toxicity and fewer side effects for the treatment.

Previously, some Mannich base derivatives were prepared in our laboratory and were tested for their anticonvulsant and antimicrobial activities [6 – 9]. Among these

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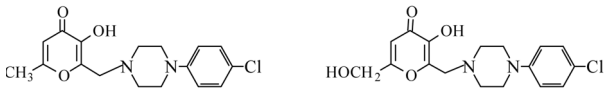


I **II**

Figure 1. Structure of 2-[4-(4-chlorophenyl)piperazin-1-ylmethyl]-3-hydroxy-6-methyl-4*H*-pyran-4-one **I** and 2-[4-(4-chlorophenyl)piperazin-1-ylmethyl]-3-hydroxy-6-hydroxymethyl-4*H*-pyran-4-one **II**.

compounds, 2-[4-(4-chlorophenyl)piperazin-1-ylmethyl]-3-hydroxy-6-methyl-4*H*-pyran-4-one (**I**; Fig 1) was found to be protective against MES-induced seizures at all doses at 0.5 h. By the way, 2-[4-(4-chlorophenyl)piperazin-1-ylmethyl]-3-hydroxy-6-hydroxymethyl-4*H*-pyran-4-one (**II**; Fig. 2) was determined to be the most active against MES-induced seizures at 0.5 and 4 h. In connection with these studies, we planned to modify the piperazine structure on the final Mannich base in order to develop new anticonvulsant agents. In this paper, synthesis and anticonvulsant activity of a series of 3-hydroxy-6-hydroxymethyl/methyl-2-substituted 4*H*-pyran-4-one derivatives (**1–12**) which are carrying various substituted piperidine derivatives at the 2-position are described.

Anticonvulsant activities of the synthesized compounds were examined by MES and scMet-induced seizure 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) and National Institute of Neurological Disorders and Stroke (NINDS), both USA [5]. This program was used for the screening of many compounds in various previous studies [6–10].

Results and discussion

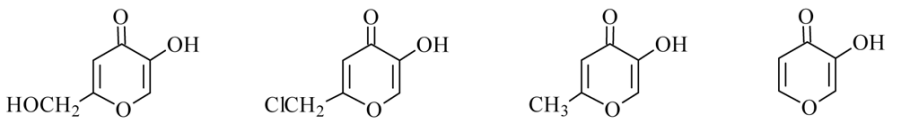
Chemistry

Kojic acid (5-hydroxy-2-hydroxymethyl-4*H*-pyran-4-one) provides a promising skeleton for the development of new and more potent derivatives such as chlorokojic acid (2-chloromethyl-5-hydroxy-4*H*-pyran-4-one), allomaltol (5-hydroxy-2-methyl-4*H*-pyran-4-one), and pyromeconic acid (3-hydroxy-4*H*-pyran-4-one) (Fig. 2). It contains a polyfunctional heterocyclic, an oxygen-containing ring with several important centers enabling additional reactions like oxidation and reduction, alkylation and acylation, nucleophilic and electrophilic substitution reactions, a ring opening of the molecule, and chelation. Therefore, many researchers used it as starting material for the preparation of new compounds [11–15].

Allomaltol was synthesized from commercially available kojic acid in a two-step reaction according to the literature [16–17]. Chlorination of the 2-hydroxymethyl moiety of kojic acid using thionyl chloride at room temperature afforded chlorokojic acid, with the ring hydroxyl being unaffected. Reduction of chlorokojic acid with zinc dust in concentrated hydrochloric acid resulted in the production of allomaltol [6–8, 18].

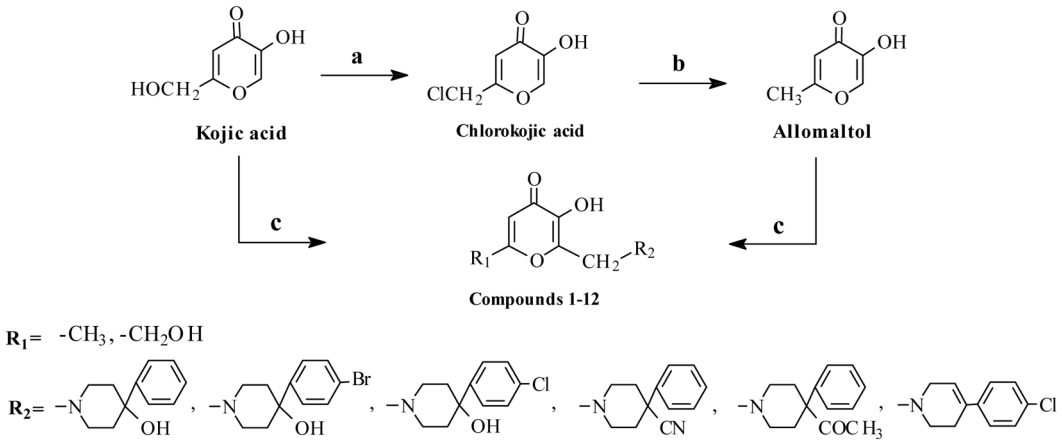
It is well known that hydroxypyranones can exist in cationic and anionic forms due to the protonation or deprotonation reactions, respectively. The hydroxyl group that is directly bound to the pyranone ring was probable more deprotonated than the hydroxymethyl group [19]. Quantum mechanical investigations on tautomeric equilibria of kojic acid were performed. Because of two intramolecular hydrogen bonds, the enolic structure of neutral kojic acid is expected to be the most stable one. One of these two bonds is located between the keto and hydroxyl group and the other hydrogen bond can be formed weakly between the hydroxymethyl moiety and the intra-ring oxygen [20]. The details of crystal structure analyses data of chlorokojic acid and allomaltol were investigated and their molecular geometry was compared to the geometry of their close analog kojic acid [21, 22]. In our previous studies, the structures of some Mannich bases of allomaltol derivatives were also determinated by X-ray analysis. The conformations of the molecules were determined by intra- and intermolecular hydrogen bonds. Some weak intramolecular interactions help to stabilize the structure [23, 24].

Multicomponent reactions are the major parts of the synthetic organic chemistry with advantages ranging from lower reaction times and temperatures to higher yields. Mannich-type reactions are three-component condensation reactions involving carbonyl compounds, existing as keto-enol tautomeric forms, formaline, and a primary or secondary amine. In 1912, Mannich and Krosche were the first to prepare some Mannich bases only



Kojic acid **Chlorokojic acid** **Allomaltol** **Pyromeconic acid**

Figure 2. Hydroxypyranone derivatives.



Reagents: a) $SOCl_2$; b) $Zn/HCl/H_2O$; c) related substituted piperidine/HCHO/MeOH.

Scheme 1. Synthesis of compounds **1–12**.

at the 6-position; this is the reactive position of kojic acid [25]. Due to its phenol-like properties, kojic acid readily undergoes aminomethylation in the Mannich reaction at room temperature, *ortho* to the enolic hydroxyl group. It is reported that di-Mannich derivatives can be obtained in an acidic medium from kojic acid, formaline, and an aromatic amine [25, 26]. Mannich bases of kojic acid derivatives, which show various biological activities, were prepared and evaluated for their activities by different researchers [6–9, 25, 27].

In this study, twelve new 3-hydroxy-6-hydroxymethyl/methyl-2-substituted 4*H*-pyran-4-one derivatives were synthesized as Mannich bases by the methodology shown in Scheme 1. The basic substituent can be introduced at the 6-position of allomaltol or kojic acid via a Mannich-type reaction, using formaline and an appropriate substituted piperidine in methanol at room temperature. The reaction proceeded very rapidly.

All the compounds were prepared as new products. Formation of the desired Mannich bases was confirmed on the basis of elemental analysis and the structures of the compounds were supported by spectral data. The IR, ^{1}H-NMR, ^{13}C-NMR, ^{13}C-DEPT, and ESI-MS are in agreement with the proposed structures. The results of elemental analysis for C, H, and N were within ± 0.4% of the theoretical values. Yields and melting points of the synthesized compounds are presented in Table 1.

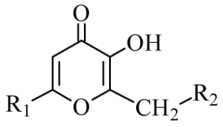
In the IR spectra, compounds **1–12** have O-H stretching (st) vibrations at 3400–3300 cm^{-1}. All compounds were associated with C=O (acetyl), C=O (pyranone), C=C, and C-O st at 1697, 1657–1618, 1594–1456, and 1232–1197 cm^{-1}, respectively. In the nuclear magnetic resonance spectra (^{1}H-NMR), the signals of the respective protons of the synthesized compounds were verified on the basis of their chemical shifts, multiplicities, and coupling constants. The ^{1}H-NMR spectra for all compounds demonstrated the presence of the characteristic singlet peaks of the 4*H*-pyran-4-one (H^5) ring proton in accordance with the literature [17]. Also, singlet peaks of the 6-methyl or 6-hydroxymethyl protons of the ring appeared in a region of 2.14 to 2.47 and 4.28 to 4.32 ppm, respectively. The methylene-group protons of compounds **1–12** were seen at 3.45 to 3.64 ppm as a singlet. ^{13}C-NMR spectra of compounds **2** and **4** have typical peaks for 6-methyl and carbonyl of the pyranone ring at 20.2 to 20.4 and 174.4 ppm, respectively. The characteristic peak for -CH_2- was observed at 54.3 to 56.1 ppm. The mass spectra of all compounds showed $[M^+ + 1]$ and $[M^+ + 23]$ peaks.

Anticonvulsant activity

In our former studies, evaluation of the anticonvulsant activity of the Mannich bases of allomaltol or kojic acid derivatives were studied. When the effects of different piperazine rings upon the activity were examined, kojic acid derivatives were found to be more active than allomaltol derivatives [6, 9]. Whenever mono-substituted piperidine derivatives or morpholine rings (They were also used instead of the piperazine ring) were used, the anticonvulsant activity of these Mannich bases of allomaltol derivatives decreased [7, 8].

In the view of previous study results, here, two Mannich base series were synthesized and compared to each other for anticonvulsant activity. These two series differ in the methyl (compounds **1–6**) or hydroxymethyl (compounds **7–12**) groups at the 6-positions of the substituted pyranone ring. Also, both Mannich bases, which have mono- or di-substituted piperidine rings, were designed (Scheme 1). It is generally accepted that the lipid solubil-

Table 1. Physicochemical data of the synthesized compounds.



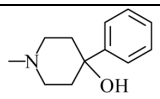
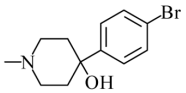
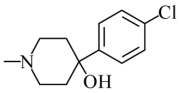
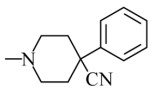
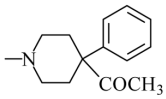
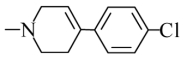
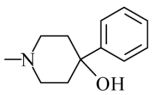
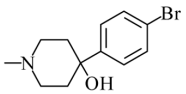
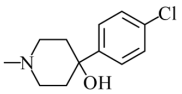
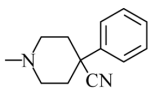
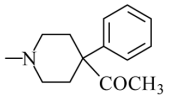
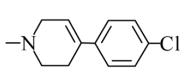
Compound	R_1	R_2	M.p. (°C)	Yield (%)	Emperical Formula	MW (g/mol)
1	-CH_3		192–193	52	$C_{18}H_{21}NO_4$	315.36
2	-CH_3		154–155	88	$C_{18}H_{20}BrNO_4$	394.26
3	-CH_3		121–122	81	$C_{18}H_{20}ClNO_4$	349.81
4	-CH_3		149–150	66	$C_{19}H_{20}N_2O_3$	324.37
5	-CH_3		191–192	67	$C_{20}H_{23}NO_4$	341.40
6	-CH_3		189–190	93	$C_{18}H_{18}ClNO_3$	331.79
7	-CH_2OH		126–127	62	$C_{18}H_{21}NO_5$	331.36
8	-CH_2OH		134–135	96	$C_{18}H_{20}BrNO_5$	410.26
9	-CH_2OH		141–142	94	$C_{18}H_{20}ClNO_5$	365.81
10	-CH_2OH		154–155	58	$C_{19}H_{20}N_2O_4$	340.37
11	-CH_2OH		166–167	85	$C_{20}H_{23}NO_5$	357.40
12	-CH_2OH		182–183	64	$C_{18}H_{18}ClNO_4$	347.80

Table 2. Phase-I anticonvulsant screening of the compounds.

Compound	MES[a]						scMet[b]						Toxicity[c]					
	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
1	0/1	0/1	**1/1**	**1/1**	**1/1**	**1/1**	**1/1**	**1/1**	**1/1**	0/1	0/1	0/1	0/4	0/4	0/4	0/2	0/2	0/2
2	0/1	0/1	**1/1**	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	**1/1**	0/4	0/4	0/4	0/2	0/2	0/2
3	0/1	0/1	0/1	0/1	0/1	**1/1**	0/1	0/1	0/1	0/1	0/1	**1/1**	0/4	0/4	0/4	0/2	0/2	0/2
4	0/1	**1/1**	**1/1**	0/1	0/1	**1/1**	**1/1**	**1/1**	**1/1**	0/1	0/1	0/1	0/4	0/4	0/4	0/2	0/2	0/2
5	0/1	0/1	0/1	0/1	**1/1**	**1/1**	0/1	0/1	0/1	0/1	0/1	0/1	0/4	0/4	4/4	0/2	0/2	0/2
6	0/1	**1/1**	**1/1**	**1/1**	**1/1**	**1/1**	0/1	0/1	0/1	**1/1**	**1/1**	**1/1**	0/4	0/4	0/4	0/2	0/2	0/2
7	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	**1/1**	**1/1**	**1/1**	**1/1**	0/4	0/4	0/4	0/2	0/2	0/2
8	0/1	0/1	**1/1**	0/1	0/1	0/1	**1/1**	**1/1**	**1/1**	**1/1**	**1/1**	**1/1**	0/4	0/4	0/4	0/2	0/2	0/2
9	0/1	0/1	**1/1**	0/1	0/1	0/1	0/1	0/1	0/1	**1/1**	**1/1**	**1/1**	0/4	0/4	0/4	0/2	0/2	0/2
10	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	**0/1**	**1/1**	**1/1**	**1/1**	0/4	0/4	0/4	0/2	0/2	0/2
11	0/1	0/1	0/1	**1/1**	**1/1**	**1/1**	**1/1**	**1/1**	**1/1**	0/1	0/1	**1/1**	0/4	0/4	0/4	0/2	0/2	0/2
12	0/1	0/1	0/1	**1/1**	**1/1**	**1/1**	0/1	**1/1**	**1/1**	0/1	0/1	**1/1**	0/4	0/4	0/4	0/2	0/2	0/2

[a] Maximal electroshock.
[b] Subcutaneous Metrazol.
[c] Rotorod test.0/1: No activity at dose level; **1/1**: noticeable activity at dose level; **0/4**: number of animals exhibited toxicity/number of animals tested for 0.5 h; **0/2**: number of animals exhibiting toxicity/number of animals tested for 4 h.

ity of a drug is an important factor in connection with its transfer into the central spinal fluid and the brain. Structure-activity relationship studies show that most of anticonvulsant drugs contain a phenyl ring and a carbonyl, or other electronegative groups adjacent to the phenyl ring [1]. Also, substitution of different lipophylic phenyl derivatives at the piperidine ring enables the penetration of the blood-brain barrier. Therefore, in this study, the effects of mono substitution of the phenyl group with electron-withdrawing groups at the *para*-position were examined. Moreover, the effect of six different piperidine rings on biological activity was investigated in both series. Different mono- or di-substitutions of the 4-position of the piperidine ring were also investigated for their influence on the activity.

The anticonvulsant activities of the compounds were initially evaluated against MES and scMet seizure tests induced at 0.5 and 4 h after administration with 30, 100, and 300 mg/kg doses using male Swiss albino mice (20 ± 2 g). Preliminary screening results are presented in Table 2.

With respect to the results of the anticonvulsant activity studies, 2-{[4-(4-bromophenyl)-4-hydroxypiperidin-1-yl]methyl}-3-hydroxy-6-(hydroxymethyl)-4*H*-pyran-4-one **8** was the most active compound against scMet-induced seizures at all doses at 0.5 and 4 h. In the same test, both of the non-substituted phenyl derivatives (compound **7** and **10**) were highly selective and were found to be active against -induced seizures at all doses after 4 h. Also, compound **9**, which is carrying a 4-chlorophenyl moiety, has same activity in the scMet test but is not selective. While compounds **2**, **3**, **11**, and **12** showed activity at 4 h, compound **7** was established to be protective at 0.5 h at the 300 mg/kg dose. Besides this, compound **12** showed activity at the 100 and 300 mg/kg doses after 0.5 h.

In the MES-induced seizure test in this series, compounds **1**, **6**, **11**, and **12** were determined to protect against seizures at all doses at 4 h. While compounds **6** and **12** are carrying the same moiety at the 2-position, they have differences at the 6-position on the pyran-4(1*H*)-one ring. Also, compounds **1** and **11** which are carrying a hydroxyl and acetyl moiety at the 4-position on the piperidine ring, respectively, were found to have anticonvulsant activity at the same doses. Some compounds like **3** and **4** exhibited activity at the 300 mg/kg dose and compound **5** was also active at 100 and 300 mg/kg doses at 4 h. Considering the results at 0.5 h, while compounds **1**, **2**, **8**, and **9** showed activity at the 300 mg/kg dose, compounds **4** and **6** were protective against seizures at 100 and 300 mg/kg doses.

In Mannich bases of allomaltol derivatives, 4-bromophenyl and 4-chlorophenyl derivatives (compounds **2** and **3**, respectively) were found to have lower activity than compound **1** which has a non-substituted phenyl ring. Conversely, in Mannich bases of kojic acid derivatives, compound **8** which is carrying a 4-bromophenyl moiety was the most active compound against scMet-induced seizure tests in this series. While the presence of an electron-rich group attached to the phenyl ring showed increased potency in scMet-induced seizure test in kojic acid derivatives (compounds **8** and **9**), this potency was decreased for allomaltol derivatives. On the other hand, when comparing the results of this study

between the two series, replacement of the hydroxymethyl with a methyl group at the 6-position of the pyranone ring increased the protective effect, because of the two hydrogen bonds of kojic acid, which are located between the keto and hydroxyl group and/or the hydroxymethyl moiety and the intra-ring oxygen. Besides this, compounds **6** and **12**, which are carrying the same moiety, have anticonvulsant activity.

In the series of kojic acid derivatives, the compounds of 4-hydroxypiperidine with substituted 4-bromophenyl and 4-chlorophenyl have higher activity against scMet-induced seizure tests than MES-induced seizure tests. Replacement of hydroxy with cyano or acetyl groups at the 4-position of the piperidine ring has a similar protective effect when compared with 4-hydroxy-4-phenypiperidine. None of the compounds showed neurotoxicity according to the rotorod test at any of the doses studied.

Conclusion

A series of novel Mannich bases of kojic acid or allomaltol, namely 3-hydroxy-6-hydroxymethyl/methyl-2-(substituted piperidin-1-yl)methyl-4*H*-pyran-4-ones were synthesized and studied for anticonvulsant activity in MES- and scMet-induced seizure tests. In this series, for all doses at 0.5 and 4 h, compound **8** was determined to be the most active against scMet-induced seizures. At all doses, compounds **1**, **4**, **6**, **7**, **9**, **10**, and **11** were also protective. Compound **1**, **4**, **11**, and **12** were found to have a significantly high anticonvulsant activity at 4 h against MES-induced seizures. In the rotorod neurotoxicity screening none of the compounds showed toxicity at any dose.

The results of this study revealed that the anticonvulsant activity of Mannich bases of allomaltol derivatives (compounds **1**–**6**) has effectively increased in comparison with our previous studies [6–8]. As for the present study, kojic acid derivatives were more active than allomaltol derivatives. It can be suggested that the increase in activity depends on the structure of kojic acid, because it includes two hydrogen bonds. Generally, most of the synthesized compounds (**1**, **4**, **6**, **7**, **8**, **9**, **10**, **11**, and **12**) seemed to be promising candidates for new anticonvulsant compounds.

Experimental

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 on a Thomas Hoover Capillary Melting Point Apparatus (Philadelphia, PA, USA) and were uncorrected. IR spectra were recorded on a Perkin Elmer FT-IR Spectrometer 1720 X (Perkin Elmer, Beaconsfield, UK) and Bruker Vector 22 IR as KBr disc (λ, in cm^{-1}; Opus Spectroscopic Software Version 2.0). ^{1}H-NMR spectra were obtained on a Bruker DPX 400 MHz High Performance Digital FT NMR (Bruker, Karlsruhe, Germany) and a Bruker Spectrospin Avance DPX-400 MHz Ultra Shield Superconducting NMR (Bruker, Rheinstetten, Germany) spectrophotometer using TMS as an internal standard (chemical shift in δ, ppm). ^{13}C-NMR and ^{13}C-DEPT spectra were recorded on a Bruker DPX 400 MHz High Performance Digital FT NMR spectrophotometer. Mass analysis was carried out with a Micromass ZQ LC-MS (Micromass, Manchester, UK) with Masslynx Software Version 4.1 by using ESI (+) method. The elemental analyses were performed with a Leco CHNS-932 (Leco, St. Joseph, MI, USA) at The Scientific & Technological Research Council of Turkey-Ankara Testing and Analyses Laboratory (TÜBITAK-ATAL). The purity of the compounds was assessed by thin layer chromatography (TLC) on Kieselgel 60 F_{254} chromatoplates (Merck, Darmstadt, Germany).

2-Chloromethyl-5-hydroxy-4H-pyran-4-one (Chlorokojic acid)

Chlorokojic acid was synthesized as described by Ellis *et al.* [17]. Yield: 76%; m.p.: 166–167°C.

2-Methyl-5-hydroxy-4H-pyran-4-one (Allomaltol)

Chlorokojic acid (30 g, 0.187 mol, 1 equiv.) was added to 100 mL of distilled water and heated to 50°C with stirring. Zinc dust (24.4 g, 0.375 mol, 2 equiv.) was added followed by the dropwise addition of concentrated hydrochloric acid (56.1 mL, 3 equiv.) over 1 h with vigorous stirring maintaining the temperature between 70–80°C. The reaction mixture was stirred for another 3 h at 70°C. The excess zinc was removed by hot filtration and the filtrate extracted with dichloromethane (3 × 200 mL). The combined organic extracts were dried over anhydrous sodium sulphate, filtered, and concentrated in vacuum to yield the crude product. Recrystallization from isopropanol afforded allomaltol as colourless plates (14.8 g, 63%). M.p.: 152–153°C (lit.: 153–155°C) [17]. IR (KBr disc) λ [cm^{-1}]: 1640 (C=O st), 1587 (C=C st), 1223, 1150 (C-O st); ^{1}H-NMR (DMSO-d_6) δ [ppm]: 2.25 (s, 3H, 2-CH_3), 6.10 (s, 1H, H^3), 6.30–7.15 (br, 1H, -O*H*), 7.80 (s, 1H, H^6).

*General preparation of Mannich bases of allomaltol derivatives **1–6***

Mannich bases were prepared by the reaction of substituted piperidine derivatives (0.01 mol) and allomaltol (0.01 mol) in methanol with 37% formaline. The mixture was stirred vigorously for 15 to 25 min. The resulting precipitate was collected by filtration and washed with cold methanol. The crude product was recrystallized from appropriate solvents.

*3-Hydroxy-2-[(4-hydroxy-4-phenylpiperidin-1-yl)methyl]-6-methyl-4H-pyran-4-one **1***

Recrystallization from methanol gave a white powder. IR (KBr disc) λ [cm^{-1}]: 1662 (C=O st), 1588, 1464 (C=C st), 1232, 1197 (C-O st); ^{1}H-NMR (DMSO-d_6) δ [ppm]: 1.78 (d, J = 12.34 Hz, 2H, piperidine), 2.21 (t, J = 12.60 Hz, 2H, piperidine), 2.47 (s, 3H, 6-CH_3), 2.71 (t, J = 10.75 Hz, 2H, piperidine), 2.77 (d, J = 10.40 Hz, 2H, piperidine), 3.53 (s, 1H, -O*H*), 3.73 (s, 2H, -CH_2-), 6.43 (s, 1H, H^5), 7.39 (t,

1H, phenyl $H^{4'}$), 7.50 (t, 2H, phenyl $H^{3'}$, $H^{5'}$), 7.67 (d, J = 7.49 Hz, 2H, phenyl $H^{2'}$, $H^{6'}$); MS (ESI) m/z: 316 [M^+ + 1] (100), 338 [M^+ + 23] (57).

2-{[4-(4-Bromophenyl)-4-hydroxypiperidin-1-yl]methyl}-3-hydroxy-6-methyl-4H-pyran-4-one ***2***

Recrystallization from chloroform/petroleum ether (40 – 60°C) gave a white powder. IR (KBr disc) λ [cm^{-1}]: 1619 (C=O st), 1464 (C=C st), 1225 (C-O st); ^{1}H-NMR ($CDCl_3$) δ [ppm]: 1.66 (d, J = 12.28 Hz, 2H, piperidine), 2.06 (t, J = 11.90 Hz, 2H, piperidine), 2.20 (s, 3H, 6-CH_3), 2.59 (t, J = 13.18 Hz, 2H, piperidine), 2.76 (d, J = 11.22 Hz, 2H, piperidine), 3.37 (s, 1H, -OH), 3.61 (s, 2H, -CH_2-), 6.09 (s, 1H, H^5), 7.26 (d, J = 8.60 Hz, 2H, phenyl), 7.36 (d, J = 8.58 Hz, 2H, phenyl); ^{13}C-NMR ($CDCl_3$ + DMSO-d_6) δ [ppm]: 20.44 (6-CH_3), 38.40 and 49.71 (piperidine -CH_2), 56.06 (-CH_2-), 69.98 (piperidine =C=), 77.90 and 104.91 (phenyl =C=), 112.10 (C_5), 120.64, 127.29 and 131.38 (phenyl -CH-), 144.26 (C_3), 148.96 (C_2), 165.13 (C_6), 174.47 (C_4); ^{13}C-DEPT ($CDCl_3$ + DMSO-d$_6$) δ: 20.44 (6-CH_3), 38.40 and 49.71 (piperidine -CH_2, −1.95, −1.98), 56.06 (-CH_2-, −0.50), 112.10, 127.29, 131.38 (-CH); MS (ESI) m/z: 394 [M^+] (98), 396 [M^+ + 2] (100), 417 [M^+ + 23] (25).

2-{[4-(4-Chlorophenyl)-4-hydroxypiperidin-1-yl]methyl}-3-hydroxy-6-methyl-4H-pyran-4-one ***3***

Recrystallization from chloroform/petroleum ether (40 – 60°C) gave a white powder. IR (KBr disc) λ [cm^{-1}]: 1618 (C=O st), 1464 (C=C st), 1221 (C-O st); ^{1}H-NMR ($CDCl_3$) δ [ppm]: 1.69 (d, J = 14.23 Hz, 2H, piperidine), 2.09 (t, J = 13.10 Hz, 2H, piperidine), 2.22 (s, 3H, 6-CH_3), 2.65 (t, J = 11.96 Hz, 2H, piperidine), 2.82 (d, J = 9.05 Hz, 2H, piperidine), 3.40 (s, 1H, -OH), 3.63 (s, 2H, -CH_2-), 6.12 (s, 1H, H^5), 7.22 – 7.36 (m, 4H, phenyl); MS (ESI) m/z: 350 [M^+ + 1] (100), 352 [M^+ + 3] (34), 372 [M^+ + 23] (13).

1-[(3-Hydroxy-6-methyl-4-oxo-4H-pyran-2-yl)methyl]-4-phenylpiperidine-4-carbonitrile ***4***

Recrystallization from chloroform/petroleum ether (40 – 60°C) gave a white powder. IR (KBr disc) λ [cm^{-1}]: 1621 (C=O st), 1497 (C=C st), 1217 (C-O st); ^{1}H-NMR (DMSO-d_6) δ [ppm]: 1.90 – 1.99 (m, 4H, piperidine), 2.14 (s, 3H, 6-CH_3), 2.36 (t, J = 11.37 Hz, 2H, piperidine), 2.88 (d, J = 12.03 Hz, 2H, piperidine), 3.48 (s, 2H, -CH_2-), 6.13 (s, 1H, H^5), 7.24 (t, 1H, phenyl ring $H^{4'}$), 7.31 (t, 2H, phenyl ring $H^{3'}$, $H^{5'}$), 7.41 (d, J = 7.47 Hz, 2H, phenyl $H^{2'}$, $H^{6'}$); ^{13}C-NMR (DMSO-d_6) δ [ppm]: 20.29 (6-CH_3), 36.29 and 50.95 (piperidine -CH_2), 42.58 (piperidine =C=), 54.31 (-CH_2-), 112.12 (C_5), 122.12 (phenyl =C=), 126.50, 128.91 and 129.87 (phenyl -CH-), 140.99 (-CN), 144.33 (C_3), 147.29 (C_2), 165.60 (C_6), 174.43 (C_4); ^{13}C-DEPT (DMSO-d_6) δ [ppm]: 20.29 (6-CH_3), 36.29 and 50.95 (piperidine -CH_2, −1.69, −1.60), 54.31 (-CH_2-, −0.90), 112.12, 126.50, 128.91, 129.87 (-CH); MS (ESI) m/z: 161 (100), 325 [M^+ + 1] (10), 347 [M^+ + 23] (38).

2-[(4-Acetyl-4-phenylpiperidin-1-yl)methyl]-3-hydroxy-6-methyl-4H-pyran-4-one ***5***

Recrystallization from chloroform/petroleum ether (40 – 60°C) gave a white powder. IR (KBr disc) λ [cm^{-1}]: 1697 (C=O st, carbonyl), 1657 (C=O st, pyranone), 1587, 1485 (C=C st), 1215 (C-O st); ^{1}H-NMR ($CDCl_3$) δ [ppm]: 1.83 (s, 3H, -COCH_3), 2.07 (t, J = 10.90 Hz, 2H, piperidine), 2.21 (s, 3H, 6-CH_3), 2.38 – 2.43 (m, 4H, piperidine), 2.78 (d, J = 11.46 Hz, 2H, piperidine), 3.53 (s, 2H, -CH_2-), 6.12 (s, 1H, H^5), 7.18 – 7.29 (m, 5H, phenyl); MS (ESI) m/z: 342 [M^+ + 1] (46), 347 [M^+ + 23] (100).

2-{[4-(4-Chlorophenyl)-3,6-dihydropyridin-1(2H)-yl]methyl}-3-hydroxy-6-methyl-4H-pyran-4-one ***6***

Recrystallization from chloroform/petroleum ether (40 – 60°C) gave a white powder. IR (KBr disc) λ [cm^{-1}]: 1655 (C=O st), 1585 (C=C st), 1217 (C-O st); ^{1}H-NMR (DMSO-d_6) δ [ppm]: 2.18 (s, 3H, -6-CH_3), 1.78 (t, J = 1.60 Hz, 2H, piperidine), 2.21 (t, J = 5.60 Hz, 2H, piperidine), 3.07 (d, J = 2.70 Hz, 2H, piperidine), 3.54 (s, 2H, -CH_2-), 6.10 (t, 1H, -CH=C=), 6.16 (s, 1H, H^5), 7.30 (d, J = 8.56 Hz, 2H, phenyl $H^{2'}$, $H^{6'}$), 7.36 (d, J = 8.62 Hz, 2H, phenyl $H^{3'}$, $H^{5'}$); MS (ESI) m/z: 161 (100), 332 [M^+ + 1] (36), 334 [M^+ + 3] (12), 354 [M^+ + 23] (44).

General preparation of Mannich bases of kojic acid derivatives ***7 – 12***

Mannich bases were prepared by the reaction of substituted piperidine derivatives (0.01 mol) and kojic acid (0.01 mol) in methanol with 37% formaline. The mixture was stirred vigorously for 15 to 25 min. The resulting precipitate was collected by filtration and washed with cold methanol. The crude product was recrystallized from appropriate solvents.

3-Hydroxy-6-(hydroxymethyl)-2-[(4-hydroxy-4-phenylpiperidin-1-yl)methyl]-4H-pyran-4-one ***7***

Recrystallization from methanol gave a white powder. IR (KBr disc) λ [cm^{-1}]: 1660 (C=O st), 1582, 1465 (C=C st), 1205 (C-O st); ^{1}H-NMR (DMSO-d_6) δ [ppm]: 1.58 (d, J = 12.60 Hz, 2H, piperidine), 1.95 (t, J = 12.56 Hz, 2H, piperidine), 2.59 (t, J = 10.85 Hz, 2H, piperidine), 2.66 (d, J = 10.35 Hz, 2H, piperidine), 3.17 (s, 1H, -OH), 3.56 (s, 2H, -CH_2-), 4.31 (s, 2H, HOCH_2-), 6.34 (s, 1H, H^5), 7.19 (t, J = 7.25 Hz, 1H, phenyl $H^{4'}$), 7.30 (t, J = 7.59 Hz, 2H, phenyl $H^{3'}$, $H^{5'}$), 7.46 (d, J = 7.54 Hz, 2H, phenyl $H^{2'}$, $H^{6'}$); MS (ESI) m/z: 332 [M^+ + 1] (100), 334 [M^+ + 3] (3), 354 [M^+ + 23] (28).

2-{[4-(4-Bromophenyl)-4-hydroxypiperidin-1-yl]methyl}-3-hydroxy-6-(hydroxymethyl)-4H-pyran-4-one ***8***

Recrystallization from chloroform gave a white powder. IR (KBr disc) λ [cm^{-1}]: 1659 (C=O st), 1464 (C=C st), 1203 (C-O st); ^{1}H-NMR (DMSO-d_6) δ [ppm]: 1.57 (d, J = 12.71 Hz, 2H, piperidine), 1.93 (t, J = 12.36 Hz, 2H, piperidine), 2.57 – 2.69 (m, 4H, piperidine $H^{2'}$, $H^{6'}$), 3.18 (s, 1H, -OH), 3.57 (s, 2H, -CH_2-), 4.32 (s, 2H, HOCH_2-), 6.34 (s, 1H, H^5), 7.42 (d, J = 8.48 Hz, 2H, phenyl $H^{3'}$, $H^{5'}$), 7.47 (d, J = 8.43 Hz, 2H, phenyl $H^{2'}$, $H^{6'}$); MS (ESI) m/z: 410 [M^+] (100), 412 [M^+ + 2] (95), 433 [M^+ + 23] (34).

2-{[4-(4-Chlorophenyl)-4-hydroxypiperidin-1-yl]methyl}-3-hydroxy-6-(hydroxymethyl)-4H-pyran-4-one ***9***

Recrystallization from chloroform gave a white powder. IR (KBr disc) λ [cm^{-1}]: 1660 (C=O st), 1581, 1486 (C=C st), 1203 cm^{-1} (C-O st); ^{1}H-NMR (DMSO-d_6) δ [ppm]: 1.56 (d, J = 12.46 Hz, 2H, piperidine), 1.90 (t, J = 12.65 Hz, 2H, piperidine), 2.50 – 2.67 (m, 4H, piperidine), 3.17 (s, 1H, -OH), 3.56 (s, 2H, -CH_2-), 4.31 (s, 2H, HOCH_2-), 4.93 (s, 1H, -OH), 6.33 (s, 1H, H^5), 7.35 (d, J = 8.59 Hz, 2H, phenyl $H^{3'}$, $H^{5'}$), 7.49 (d, J = 8.55 Hz, 2H, phenyl $H^{2'}$, $H^{6'}$); MS (ESI) m/z: 226 (100), 366 [M^+ + 1] (86), 368 [M^+ + 3] (28), 388 [M^+ + 23] (41).

1-{[3-Hydroxy-6-(hydroxymethyl)-4-oxo-4H-pyran-2-yl]methyl}-4-phenylpiperidine-4-carbonitrile ***10***

Recrystallization from chloroform/petroleum ether (40 – 60°C) gave a white powder. IR (KBr disc) λ [cm^{-1}]: 1654 (C=O st), 1594, 1468 (C=C st), 1209 (C-O st); ^{1}H-NMR (DMSO-d_6) δ [ppm]: 2.02 – 2.12

(m, 4H, piperidine), 2.52 (t, *J* = 11.94 Hz, 2H, piperidine), 2.99 (d, *J* = 12.14 Hz, 2H, piperidine), 3.63 (s, 2H, -CH_2-), 4.32 (s, 2H, HOCH_2-), 5.60–8.80 (br, 1H, -O*H*), 6.36 (s, 1H, H^5), 7.36 (t, *J* = 7.20 Hz, 1H, phenyl $H^{4'}$), 7.43 (t, *J* = 7.58 Hz, 2H, phenyl $H^{3'}$, $H^{5'}$), 7.53 (d, *J* = 7.59 Hz, 2H, phenyl $H^{2'}$, $H^{6'}$), 8.98 (s, 1H, -O*H*); MS (ESI) *m*/*z*: 177 (100), 341 [M^+ + 1] (36), 363 [M^+ + 23] (60).

2-[(4-Acetyl-4-phenylpiperidin-1-yl)methyl]-3-hydroxy-6-(hydroxymethyl)-4H-pyran-4-one ***11***

Recrystallization from chloroform/petroleum ether (40–60°C) gave a white powder. IR (KBr disc) λ [cm^{-1}]: 1697 (C=O st, carbonyl), 1652 (C=O st, pyranone), 1588, 1469 (C=C st), 1198 cm^{-1} (C-O st); ^{1}H-NMR (DMSO-d_6) δ [ppm]: 1.87 (s, 3H, -COCH_3), 1.96 (t, *J* = 10.09 Hz, 2H, piperidine), 2.30 (t, *J* = 10.71 Hz, 2H, piperidine), 2.37 (d, *J* = 13.22 Hz, 2H, piperidine), 2.62 (d, *J* = 11.09 Hz, 2H, piperidine), 3.45 (s, 2H, -CH_2-), 4.28 (s, 2H, HOCH_2-), 5.60–5.70 (br, 1H, -O*H*), 6.30 (s, 1H, H^5), 7.25–7.40 (m, 5H, phenyl); MS (ESI) *m*/*z*: 358 [M^+ + 1] (100), 380 [M^+ + 23] (80).

2-{[4-(4-Chlorophenyl)-3,6-dihydropyridin-1(2H)-yl]methyl}-3-hydroxy-6-hydroxymethyl-4H-pyran-4-one ***12***

Recrystallization from methanol gave a white powder. IR (KBr disc) λ [cm^{-1}]: 1652 (C=O st), 1558, 1456 (C=C st), 1199 (C-O st); ^{1}H-NMR (DMSO-d_6) δ [ppm]: 2.51 (br-s, 2H, piperidine), 2.73 (t, *J* = 5.48 Hz, 2H, piperidine), 3.16 (d, *J* = 1.95 Hz, 2H, piperidine), 3.64 (s, 2H, -CH_2-), 4.31 (s, 2H, HOCH_2-), 5.60–5.70 (br, 1H, -O*H*), 6.18 (t, 1H, -C*H*=C=, piperidine), 6.34 (s, 1H, H^5), 7.30 (d, *J* = 8.48 Hz, 2H, phenyl $H^{3'}$, $H^{5'}$), 7.36 (d, *J* = 8.51 Hz, 2H, phenyl $H^{2'}$, $H^{6'}$), 8.99 (s, 1H, O*H*); MS (ESI) *m*/*z*: 177 (100), 348 [M^+ + 1] (58), 350 [M^+ + 3] (18), 370 [M^+ + 23] (52).

Anticonvulsant assay

The compounds were tested for their anticonvulsant activity against maximal electroshock (MES)- and subcutaneous Metrazol (scMet)-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) [5]. This program was used for the screening of many compounds in various previous studies [6–10]. Stimulator (Grass S88, Astro-Med. Inc. Grass Instrument Division, W. Warwick, RI, USA), constant current unit (Grass CCU1A, Grass Medical Instrument, Quincy, MA, USA), and corneal electrodes were used for the evaluation of anticonvulsant activity against MES 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 ± 2 g) were used for each compound (mice were obtained from the Hacettepe University Animal Farm) according to the ADD-NINDS program [5]. The animals were kept under standard conditions at an ambient temperature of 25 ± 2°C and allowed free access to food and water 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 (17. 04. 2002 date 2002/ 24-3 number and 08. 04. 2003 date 2003/ 58-2 number). Control animals received 30% aqueous PEG 400. Metrazol 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).

Maximal electroshock (MES)-induced seizure test

MES seizures were elicited with a 60-cycle alternating current of 50 mA intensity (5 to 7 times more than that required to elicit minimal seizures) delivered for 0.2 s 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.

Subcutaneous Metrazol (scMet)-induced seizure test

85 mg/kg of Metrazol (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 s duration) could be defined as protection.

Neurotoxicity

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.

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References

[1] A. Korolkovas in *Essentials of Medicinal Chemistry*, John Wiley & Sons, New York **1988**, Second Edition.

[2] C. L. P. Deckers, P. Genton, G. J. Sills, D. Schmidt, *Epilepsy Res.* **2003**, *53*, 1–17.

[3] W. Löscher, *Trends Pharmacol Sci.* **2002**, *23*, 113–118.

[4] B. Malawska, *Curr. Top. Med. Chem.* **2005**, *5*, 69–85.

[5] R. L. Krall, J. K. Penry, B. G. White, J. H. Kupferberg, E. A. Swinyard, *Epilepsia* **1978**, *9*, 409–428.

[6] M. D. Aytemir, Ü. Çalış, M. Özalp, *Arch. Pharm.* **2004**, *337*, 281–288.

[7] M. D. Aytemir, Ü. Çalış, *FABAD J. Pharm. Sci.* **2006**, *31*, 23–29.

[8] M. D. Aytemir, Ü. Çalış, *HU J. Fac. Pharm.* **2007**, *27*, 1–10.

[9] M. D. Aytemir, E. Septioglu, Ü. Çalış, *Arzneim.-Forsch./Drug Res.* **2010**, *1*, 22–29.

[10] E. Septioglu, M. D. Aytemir, Ü. Çalış, *Arzneim.-Forsch./Drug Res.* **2005**, *55*, 259–264.

[11] J. Brtko, L. Rondahl, M. Fickova, D. Hudecova, *et al.*, *Cent. Eur. J. Public Health* **2004**, *12*, 16–18.

[12] M. D. Aytemir, D. D. Erol, R. C. Hider, M. Özalp, *Turk. J. Chem.* **2003**, *27*, 757 – 764.

[13] M. Uher, V. Konecny, O. Rajniakova, *Chem. Pap.–Chem. Zvesti.* **1994**, *48*, 282 – 284.

[14] W. Kandioller, C. G. Hartinger, A. A. Nazarov, J. Kasser, *et al.*, *J. Organomet. Chem.* **2009**, *694*, 922 – 929.

[15] G. Öztürk, D. D. Erol, M. D. Aytemir, T. Uzbay, *Eur. J. Med. Chem.* **2002**, *37*, 829 – 834.

[16] T. Yabuta, *J. Chem. Soc.* **1924**, *123*, 575 – 587.

[17] B. L. Ellis, A. K. Duhme, R. C. Hider, M. B. Hossain, *et al.*, *J. Med. Chem.* **1996**, *39*, 3659 – 3670.

[18] Z. D. Liu, S. Piyamongkol, D. Y. Liu, H. H. Khodr, *et al.*, *Bioorg. Med. Chem.* **2001**, *9*, 563 – 573.

[19] A. Beelik, C. B. Purves, *Can. J. Chem. Soc.* **1955**, *33*, 1361 – 1374.

[20] K. Zborowski, R. Grybos, L. M. Proniewicz, *J. Mol. Struct.* **2003**, *639*, 87 – 100.

[21] J. Lokaj, J. Kozisek, B. Koren, M. Uher, V. Vrabel, *Acta Cryst.* **1991**, *C47*, 193 – 194.

[22] K. Hryniewicz, K. Stadnicka, A. Pattek-Janczyk, *J. Mol. Struct.* **2009**, *919*, 255 – 270.

[23] N. O. Iskeleli, S. Isik, M. D. Aytemir, *Acta Cryst.* **2005**, *E61*, 1947 – 1949.

[24] Y. Köysal, S. Isik, M. D. Aytemir, *Acta Cryst.* **2004**, *E60*, 112 – 114.

[25] G. J. O'brien, M. Patterson, J. R. Meadow, *J. Org. Chem.* **1960**, *25*, 86 – 89.

[26] L. L. Woods, *J. Am. Chem. Soc.* **1946**, *68*, 2744 – 2745.

[27] L. S. Dehkordi, Z. D. Liu, R. C. Hider, *Eur. J. Med. Chem.* **2008**, *43*, 1035 – 1047.

