Synthesis and anticonvulsant activity of new kojic acid derivatives

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Abstract

A series of new 3-hydroxy-6-hydroxymethyl-2-substituted 4H-pyran-4-one derivatives were synthesized as potential anticonvulsant compounds. Mannich compounds were prepared by the reaction of appropriate substituted piperazine derivatives with kojic acid and formaldehyde. The structure of the synthesized compounds was confirmed using the elementary analysis results and spectroscopic techniques such as IR, 1H-NMR and ESI-MS. Anticonvulsant activities of the synthesized compounds were examined by maximal electroshock (MES) and subcutaneous Metrazol (scMet) induced seizure tests. Neurotoxicity was determined by the rotord toxicity test. All these tests were performed according to procedures of the Antiepileptic Drug Development (ADD) program. According to the activity studies, 2-(4-(chlorophenyl)piperazin-1-l)methyl-3-hydroxy-6-hydroxymethyl-4H-pyran-4-one (compound 11) against MES seizures and 3-hydroxy-6-hydroxymethyl-2-(4-(2-methoxyphenyl)piperazin-1-l)methyl-4H-pyran-4-one (compound 7) against scMet seizures were determined to be the most active compounds at all doses without neurotoxicity.

1. Introduction

Since the early twentieth century, kojic acid (5-hydroxy-2-hydroxymethyl-4H-pyran-4-one) has been known as an additive to prevent browning of food materials in the food industry, as an antioxidant in order to preserve their freshness and to inhibit discoloration. A biologically important natural substance, it is an antibiotic produced by many species of fungi or bacteria, such as Aspergillus oryzae, Penicillium or Acetobacter spp. in an aerobic process from a wide range of carbon sources [1–3]. It has played an important role in iron-overload diseases such as β-thalassemia or anemia, since it possesses iron chelating activity [2, 4–6]. It also formed stable complexes that the metal kofates were prepared by reaction of kojic acid with metal acetate salts [3, 7, 8]. Additionally, kojic acid and its derivatives have shown to possess various bioactivities such as antimicrobial [2, 3, 9–13], cosmetic skin-whitening [1, 3, 14], herbicidal [9, 15], anti-speck [16], pesticide and insecticide [17, 18], antitumor [19, 20], anti-diabetic [21] and antiproliferative activities [22].

Epilepsy, the most common serious neurological disorder characterized by recurrent unprovoked seizures, is estimated to affect approximately 60 million people worldwide. Especially, the majority of cases is in developing countries. More than 30% of patients with epilepsy have inadequate control of seizures with available medical therapies. The established conventional antiepileptic drugs like phenytoin, carbamazepine, ethosuximide, valproic acid and barbiturates, though widely prescribed, exhibited unfavorable side effect profiles such as drowsiness, ataxia, hepatotoxicity, gingival hyperplasia, and megaloblastic anemia and failed to control seizures adequately. In recent years, several new antiepileptic drugs such as lamotrigine, oxcarbazepine, felbamate, gabapentin, topiramate, fosphenytoin sodium, tiragabine, zonisamide and levetiracetam have been approved. Some of the mechanisms of new antiepileptic drugs include potentiation of γ-aminobutyric acid (GABA)-ergic transmission, blockage of voltage-dependent sodium channels, attenuation of excitatory neurotransmission, and modulation of voltage-sensitive calcium channels [23–26]. There is continuing demand...
for new anticonvulsant agents, as it has not been possible to control every kind of seizure with the currently available antiepileptic drugs. To provide improvement of the quality of life of people suffering from epilepsy, it is essential to search for newer chemical entities with lower toxicity and fewer side effects for the treatment of epilepsy.

Kojic amine (2-aminomethyl-5-hydroxy-4H-pyran-4-one) has been found to possess GABA agonist activity (Fig. 1) [27, 28]. The early neuropharmacological profile of kojic amine resulted in its classification as a GABA_A receptor agonist [29, 30]. When compared with the structure of GABA, the similarity of muscimol, a GABA-like agent, and kojic amine is apparent.

Kojic acid has weaker activity than ethylmalto (2-ethyl-3-hydroxy-4H-pyran-4-one) (Fig. 2) against the convulsions induced by metrazole and strychnine. The increase of the inhibitory effect of 2-alkyl-3-hydroxy-4H-pyran-4-ones on metrazol-induced convulsion with increasing carbon number of the alkyl group might be due to enhancement of lipid solubility [31, 32]. In our previous studies, we reported that Mannich bases of alломalto derivatives showed anticonvulsant and antifungal activities [13, 31, 32].

In continuation of our earlier work, the present paper reports on the synthesis, anticonvulsant and neurotoxicity evaluation of some new kojic acid derivatives. 3-Hydroxy-6-hydroxymethyl-2-substituted 4H-pyran-4-one derivatives (1–15) which are carrying various substituted piperazine derivatives at the 2nd position (Fig. 2) are designed as lipophilic agents. Therefore these compounds might be transferred into the central spinal fluid and brain easily.

2. Material and methods

2.1 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 Spectrometer 1720 X (Beaconsfield, UK) and Bruker Vector 22 IR (Opus Spectroscopic Software Version 2.0) as KBr disc (γ, cm⁻¹). ¹H-NMR spectra were obtained on a Bruker DPX-400 MHz High Performance Digital FT NMR (Karlsruhe, Germany) and a Bruker Spectrospin Avance DPX-400 MHz Ultra Shield Superconducting NMR (Germany-Switzerland) spectrophotometer using TMS as an internal standard (chemical shift in δ, ppm). Mass analysis was carried out with a Micromass ZQ LC-MS with Masslynx Software Version 4.1 by using ESI (+) method. The elemental analyses were performed with a Leco CHNS-932 (St. Joseph, MI, USA) at The Scientific & Technological Research Council of Turkey- Ankara Testing and Analyzes Laboratory (TÜBİTAK-ATAL). The purity of the compounds was assessed by thin layer chromatography (TLC) on Kieselgel 60 F₂₅₄ (Merck) chromatoplastes.

Fifteen compounds were synthesized according to the procedures shown below.

2.1.1 General synthesis of Mannich bases (Scheme 1)

Mannich bases were prepared by the reaction of substituted piperazine 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 methanol or chloroform.

2.1.2 Analytical data for some compounds

3-Hydroxy-6-hydroxymethyl-2-[4-(3-fluoromethylphenyl)piperazin-1-ylmethyl]-4H-pyran-4-one (4)

IR (KBr disc) 1648 (C=O st), 1503 (C=C st), 1113 cm⁻¹ (C=O st). ¹H-NMR δ (DMSO-d₆, 400 MHz) 2.61 (4H; t; piperezine), 3.20 (4H; t; piperezine), 3.58 (2H; s; −CH₂−), 4.31 (2H; d; J = 1.91; −HOCH₂−), 5.68 (1H; t; −OH), 6.34 (1H; s; H₂), 7.05 (1H; d; J = 7.58; phenyl ring H²), 7.14 (1H, s; phenyl ring H²), 7.20 (1H; d; J = 8.42; phenyl ring H²), 7.40 (1H; t; J = 7.98; phenyl ring H³), 9.05 ppm (1H; s; −OH). ESI m/e 177 (100 %), 385 (M+1, 50.59 %), 407 (M+23).

2-[4-(4-Fluorophenyl)piperazin-1-ylmethyl]-3-hydroxy-6-hydroxymethyl-4H-pyran-4-one (6)

IR (KBr disc) 1613 (C=O st), 1514, 1461 (C=C st), 1205 cm⁻¹ (C=O st). ¹H-NMR δ (DMSO-d₆, 400 MHz) 2.59 (4H; t; piperezine), 3.05 (4H; t; piperezine), 3.57 (2H; s; −CH₂−), 4.31 (2H; s; HOCH₂−), 5.67 (1H; t; −OH), 6.33 (1H; s; H²), 6.92 (2H; dd; J = 4.71; phenyl ring H², H³), 7.03 (2H; d; J = 8.72; phenyl ring H², H³), 9.03 ppm (1H; s; −OH). ESI m/e 177 (100 %), 335 (M+1, 43.45 %), 357 (M+23).
2.2 Pharmacology

The compounds were tested for their anticonvulsant activity against maximal electroshock (MES) and subcutaneous Metrazol (sC Met) 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 the National Institutes of Health (NIH) and National Institute of Neurological Disorders and Stroke (NINDS) [35]. This program was used for the screening of many compounds in various previous studies [13, 33, 34]. Stimulator (Grass S88, Astro-Med. Inc. Grass Instrument Division, W. Warwick, RI, USA), constant current unit (Grass CCG1A, Grass Medical Instrument, Quincy, MA, USA), and corneal electrodes were used for the evaluation of anticonvulsant activity in the 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 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 [35] and used according to the Hacettepe University, "Laboratory Animals Ethic Committee" decision (8 April 2003/no. 58-2). 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 the Phase I test was made by Hacettepe University Technical Department).

2.2.1 Maximal electroshock (MES) induced seizure test

MES seizures 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 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.

2.2.2 Subcutaneous Metrazol (sC Met) 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.

2.2.3 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.

3. Results

3.1 Chemistry

Kojic acid contains a polynuclear heterocyclic, oxygen containing ring with several important centers enabling additional reactions like oxidation and reduction, alkylation and acylation, substitution nucleophilic reac-
tions, substitution electrophilic reactions, a ring opening of the molecule, and chelation [2, 36–39]. Since kojic acid is freely soluble in water, ethanol, acetone, and sparingly soluble in ether, ethylacetate, and chloroform, its various derivatives were prepared quite easily [1, 2, 40].

It is well known that hydroxypryanones 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 probably more deprotonated than the hydroxymethyl group. 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 groups and the other hydrogen bond can be formed weakly between the hydroxymethyl moiety and intra-ring oxygen [5].

In our previous studies, the structures of some Mannich bases were determined by x-ray analysis. The conformation of the molecule is determined by intra- and intermolecular hydrogen bonds. Some weak intramolecular interactions helped to stabilize the structure. The piperazine ring displayed an almost perfect chair conformation [41, 42].

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 a three-component condensation reaction involving carbonyl compounds, which exist 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 in the 6th position, which is the reactive position of kojic acid [37]. Due to phenol-like properties kojic acid readily undergoes aminomethylation in the Mannich reaction ortho to the enolic hydroxyl group at room temperature. Later, di-Mannich derivatives of kojic acid were synthesized in an acid medium by Woods [43]. Mannich bases of kojic acid derivatives which show various biological activities were prepared and evaluated for their activities by different researchers [13, 33, 34, 37, 39].

In this study, the synthesis of 15 3-hydroxy-6-hydroxymethyl-2-substituted 4H-pyrane-4-ones derivatives were prepared as Mannich bases by the method shown in Scheme 1. The commercially available kojic acid was used as starting material. The Mannich bases of kojic acid were formed by the reaction of appropriately substituted piperazine derivatives with kojic acid and 37% formaline at room temperature in methanol. Mannich bases obtained at the 2nd position because of the hydroxyl group at the 3rd position on the 4H-pyrane-4-one ring. The reaction proceeded very rapidly.

All the compounds were prepared as new products except compound 1 (CAS 303033-62-7) is a commercial product in “Science Finder 2007”. Since there is no information in the literature for the preparation and structural characteristics, this compound has been included in our research programme and characterized by spectral data).

Formation of the desired Mannich bases was confirmed on the basis of elementary analysis and structures of compounds were supported by spectral data. The IR, 1H-NMR and ESI-MS are in agreement with the proposed structures. Yields and the melting points of the synthesized compounds are presented in Table 1.

In the IR spectra, compounds 1–15 have O–H stretching bands at 3 400–3 300 cm⁻¹. All compounds were associated with C=O, C=C and C–O stretching bands at 1 658–1 603, 1 588–1 460 and 1 251–1 113 cm⁻¹, respectively. With 1H-NMR spectra, characteristic singlet peaks of the methylene group and H₂ protons of the 6-hydroxymethyl-4H-pyrane-4-one ring were found in the region 4.30–4.32 and 6.32–6.35 ppm, respectively, in accordance with the literature [13]. Also, peaks for -CH₂– protons of compounds 1–15 appeared as singlets at 3.54–3.60 ppm. Substituted piperazone ring protons were found at 2.39–2.63 and 2.96–3.72 ppm as two triplet peaks. The mass spectra of all compounds showed M+1 and M+23 peaks.

3.2 Anticonvulsant activity

In the present study, some new derivatives of kojic acid have been synthesized as potential anticonvulsant compounds according to previous studies [13, 33, 34]. The anticonvulsant activities of the compounds were initially evaluated in MES and scMet seizure tests induced 0.5 and 4 h after administration at 30, 100 and 300 mg/kg doses using Swiss albino male mice (20 ± 2 g). Preliminary screening results are presented in Table 2.

According to the results of the anticonvulsant activity studies, 3-hydroxy-6-hydroxymethyl-2-[4-(2-methoxyphenyl)piperazin-1-ylmethyl]-4H-pyrane-4-one (compound 7) was highly selective and found to be the most active compound against scMet seizures at all doses at 0.5 and 4 h. In the same test, while compound 6, which is carrying a 4-fluorophenyl moiety, was protective at all doses at 0.5 h and at the 100 and 300 mg/kg doses at 4 h, compound 10, which is carrying a 3-chlorophenyl moiety, was found to have anticonvulsant activity at all doses at 4 h and at the 300 mg/kg dose at 0.5 h. Compound 2 showed activity against scMet seizures at 100 and 300 mg/kg at 4 h and 300 mg/kg at 0.5 h. Compounds 3, 4, 5, 11 and 13 were protective against scMet seizures 300 mg/kg at 4 h. Some compounds like 3, 8 and 11 were also active at 100 and 300 mg/kg doses at 0.5 h. Compounds 4 and 9 exhibited activity at the 100 mg/kg dose at 0.5 h.

In the MES test the most active compound of the group of tested compounds was 2-[4-(4-chlorophenyl)-piperazin-1-ylmethyl]-3-hydroxy-6-hydroxymethyl-4H-pyrane-4-one (compound 11) at all doses at 0.5 h and 4 h. Considering the results at 4 h, compounds 4, 9 and
Table 1: Physicochemical data of the synthesized compounds.

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15, which are carrying a trifluoromethyl, 2-chlorophenyl and tert-butyl carboxylate moiety at the 2nd position on the pyran-4(1H)-one ring, respectively, were determined to protect against seizures at all doses in this series. Also, compound 14 which is carrying a 4-acetyl moiety showed selective activity at all doses at 0.5 h and at the 300 mg/kg dose at 4 h. At the 300 mg/kg dose, while compounds 1, 4, 10 and 13 showed activity at 0.5 h, compounds 2, 5, 6 and 14 were established to be protective at 4 h. Besides this, compound 8 was having activity at 100 and 300 mg/kg doses at 0.5 h. While 2-fluorophenyl (5) and 4-fluorophenyl (6) derivatives were protective against MES seizures at 4 h to a similar degree, compound 6 showed greater activity than compound 5 against scMet seizures. Only compound 12 did not show anticonvulsant activity in any test.
Compounds with a substituted piperazine ring such as phenyl (1), 2-pyridine (12) and pyrimidine (13) rings were found to have lower or no activity than the others which have a piperazine ring containing substituted phenyl (2–11), acetyl (14) and tert-butyl carboxylate (15). Presence of an electron rich group attached to the phenyl ring showed increased potency in both seizure tests. In addition, while the 2-fluorophenyl derivative (5) was not protective, the 4-fluorophenyl derivative (6) showed activity against scMet seizures at all dose at 0.5 h. Also, only 2- (9) and 4-chlorophenyl (11) derivatives were effective in the MES test at 4 h; the 3-chlorophenyl (10) derivative was not active beside the chloro series. Conversely, in the scMet test at 4 h, compound 10 was the most active compound in this series. 2-Methoxy (7) derivative showed selective activity against scMet seizures, but the 3-methoxy (8) derivative was not selective and was less active than compound 7. Also, compounds 14 and 15 were selective protective compounds in the MES tests. It seems that the compounds in the series of substituted phenylpiperazine with 3-trifluromethyl, 2- and 4-chloro are more suitable for the MES tests, while derivatives with 4-fluoro, 2-methoxy and 3-chloro yielded the most active compounds in the scMet tests. None of the compounds showed neurotoxicity according to the rotord test at any of the doses studied.

### 4. Discussion

In our previous studies, 3-hydroxy-6-methyl-2-substituted 4H-pyran-4-one derivatives were evaluated for their anticonvulsant activity. It is generally accepted that the lipid solubility of a drug is an important factor in connection with its transfer into the central spinal fluid and brain. Also, substitution of different lipophilic phenyl derivatives at the 4<sup>th</sup> position of the piperazine ring enables penetration of the blood-brain barrier. The effects of mono substitution of the phenyl group with an electron donating electron withdrawing group at the ortho, meta and para position were examined. According to the results, these compounds, especially 4-chlorophenyl and 3-trifluoromethylphenyl derivatives, had marked anticonvulsant activity in scMet and MES tests [13]. When substituted piperidine derivatives and morpholine ring at the 2<sup>nd</sup> position of allomaltol (Fig. 2) were used instead of the piperazine ring, the anticonvulsant activity of these Mannich bases was decreased [33, 34].

Instead of allomaltol, used as starting material in former studies, in this study kojic acid was used. These two starting materials differ only in the methyl or hydroxymethyl groups at the 6<sup>th</sup> position of the pyranone ring. Both kojic acid and allomaltol derivatives, including...
4-chloro and 3-trifluoromethylphenylpiperazine, were protective against all seizures. When the effects of different piperazine rings upon activity were examined, kojic acid derivatives were found to be more active than alloanortol derivatives. On the other hand, when the results of this study were compared with those of our previous studies, replacement of hydroxymethyl with a methyl group at the 6th position of the pyranone ring increased the protective effect in both tests, because of two hydrogen bonds of kojic acid, which are located between the keto and hydroxyl group and/or hydroxymethyl moiety and intra-ring oxygen.

Our structure-activity relationship (SAR) studies showed that the promising anticonvulsant drugs contain electronegative groups adjacent to the phenyl ring, including nitro, acetyl, trifluoromethyl, fluoro, chloro and methoxy. The other active derivatives include a carbonyl (acetyl) or ester (tert-butylcarboxylate) instead of the phenyl ring.

5. Conclusion

A series of novel Mannich bases of kojic acid, namely 3-hydroxy-6-hydroxymethyl-2-(substituted piperazin-1-yl)methyl-4H-pyran-4-ones were synthesized and studied for anticonvulsant activity in MES and scMet tests. In the Mannich bases series, for all doses, at 0.5 and 4 h, while compound 7 was found to have significantly high selective anticonvulsant activity against scMet seizures, compound 11 was determined to be the most active against MES seizures. At all doses, compounds 4, 9, 11, 14 and 15 were found to have anticonvulsant activity against MES seizures and compounds 6, 7 and 10 were protective against scMet seizures. In the rotorod neurotoxicity screening none of the compounds showed toxicity at any dose.

The results of this study revealed that anticonvulsant activity of Mannich bases has effectively increased in comparison with our previous studies. It can be suggested that the increase in activity depends on the structure of kojic acid, used as starting material, which includes two hydrogen bonds. Generally, most of the synthesized compounds (4, 6, 7, 9, 10, 11, 14 and 15) seemed to be promising candidates as new anticonvulsant compounds. In brief, especially compounds 7 and 11 are the most active compounds in these series; they might be potentially useful in the treatment of grand mal and Petit mal epilepsies.

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References


