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Synthesis of Some Novel Mannich Bases Derived From Allomaltol and Evaluation of Their Anticonvulsant Activities

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Introduction

In recent years, antiepileptic drug development has been one of the most prominent research areas. Although several new anticonvulsants are already in clinical use, some types of seizures are still not adequately treated with current therapy and have limitations intolerable side effects [1-3]. In response to these limitation, the development of new drugs to optimally manage seizures has been strongly advocated. Thus the search for new anticonvulsant drugs continues to be an active area of investigation in medicinal chemistry[4-6].

Recent studies revealed that Mannich bases having piperazine groups on the allomaltol (5-hydroxy-2-methyl-4H-pyran-4-one) structure showed anticonvulsant activity in the maximal electroshock (MES) and subcutaneous Pentylenetetrazole (scMet) screens[7]. Moreover, structure of some compounds were determined by X-Ray analysis [8-10].

As a continuation of our studies concerning different piperidine derivatives, we aimed to prepare Mannich bases of allomaltol derivatives in order to investigate the influence of replacing the piperidine moiety by phenylpiperazine derivatives on anticonvulsant activity. The structures of the synthesized compounds were confirmed by IR, ^{1}H-NMR, mass spectra and elemental analysis. Anticonvulsant activities of the compounds 1-7 were examined by MES and scMet tests. The compounds were suspended in 30 % aqueous of PEG 400 and administered intraperitoneally in a volume of 0.01 ml/g at body weight to the mice. Seizure assays and

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neurotoxicity were determined according to the phase I tests of the Antiepileptic Drug Development (ADD) program protocol which were developed by the National Institute of Neurological Disorders and Stroke (NINDS), National Institutes of Health (NIH)[7, 11-15].

Materials and Methods

Chemistry

All chemicals used in this study were supplied by Merck (Darmstadt, Germany) and Aldrich Chemical Co. (Steinheim, Germany). Melting points were determined with 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) as KBr disc (γ, cm-1). 1H-NMR spectra were obtained on a Bruker AC 80 MHz spectrophotometer and Bruker GMBH DPX-400 MHz High Performance Digital FT NMR spectrophotometer (Karlsruhe, Germany) using TMS as an internal standard (chemical shift in δ, ppm). Mass spectra were recorded on Agilent 6890 Network GC System and Agilent 5973 Network Mass Selective Detector. 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 Analyses Laboratory (TÜBİTAK-ATAL). The purity of the compounds was assessed by TLC on Kieselgel 60 F254 (Merck, Darmstadt, Germany).

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

It was prepared by using the procedure already described by Yabuta[16].

5-Hydroxy-2-methyl-4H-pyran-4-one (Allomaltol)

Recrystallisation from isopropanol afforded allomaltol as colourless plates (14.8 g, 63 %): mp 152-153 °C (lit. value17 153-155 °C). IR (KBr disc) 3300-3100 (O-H st), 1640 (C=O st), 1587, 1384 (C=C st), 1223, 1150, 1050 cm^{-1} (C-O st). ^{1}H-NMR (DMSO-d_6, 60 MHz) δ 2.25 (3H; s; 2-CH_3), 6.10 (1H; s; H3), 6.30-7.15 (broad, 1H, 5-OH), 7.80 (1H; s; H6).

General synthesis of Mannich bases (Compounds 1-7).

Mannich bases were prepared by the reaction of substituted piperidine derivatives (0.01 mol) and allomaltol (0.01 mol) in methanol (15 mL) with 37% formaline (1 mL). The reaction mixture was stirred vigorously for 15

to 25 min at room temperature. The resulting precipitate was collected by filtration and washed cold methanol. The crude product recrystallized from appropriate solvents.

3-Hydroxy-6-methyl-2-(piperidin-1-ylmethyl)-4H-pyran-4-one (1).

Recrystallization from methanol gave a white powder. IR (KBr disc) 3300-3100 (O-H st), 1626 (C=O st) and 1460 cm^{-1} (C=C st). ^{1}H-NMR δ ($CDCl_3$, 80 MHz) 1.40-2.00 (6H; m; piperidine), 2.20 (3H; s; 6-CH_3), 2.40 (4H; d; piperidine), 3.50 (2H; s; -CH_2-), 6.20 ppm (1H; s; H_5). Anal. Cal. for $C_{12}H_{17}NO_3$ M.W.: 223 Cal. C: 64.55H: 7.67 N: 6.27 Found C: 63.98 H: 7.17 N: 6.02

3-Hydroxy-6-methyl-2-((3-methylpiperidin-1-yl)methyl)-4H-pyran-4-one (2).

Recrystallization from ethyl acetate gave a white crystalline solid. IR (KBr disc) 3295-3100 (O-H st), 1673 (C=O st) and 1460 cm^{-1} (C=C st). ^{1}H-NMR δ ($CDCl_3$, 80 MHz) 0.80 (3H; d; -CH_3), 1.40-2.00 (7H; m; piperidine), 2.20 (3H; s; 6-CH_3), 2.40 (2H; d; piperidine), 3.50 (2H; s; -CH_2-), 6.20 ppm (1H; s; H_5). GC(MS) m/e 98, 84 (base peak), 70, 56. Anal. Cal. for $C_{13}H_{19}NO_3$ M.W.: 237 Cal. C: 65.79 H: 8.07 N: 5.90 Found C: 65.48 H: 8.39 N: 5.92

3-Hydroxy-6-methyl-2-((4-methylpiperidin-1-yl)methyl)-4H-pyran-4-one (3).

Recrystallization from chloroform/petroleum ether (40-60 °C) gave a white crystalline solid. IR (KBr disc) 3200-2927 (O-H st), 1640 (C=O st) and 1459 cm^{-1} (C=C st). ^{1}H-NMR δ ($CDCl_3$, 80 MHz) 1.00 (3H; d; -CH_3), 1.20-2.00 (5H; m; piperidine), 2.30 (3H; s; 6-CH_3), 3.00 (4H; d; piperidine), 3.70 (2H; s; -CH_2-), 6.20 ppm (1H; s; H_5). GC(MS) m/e 98 (base peak), 84, 70, 56. Anal. Cal. for $C_{13}H_{19}NO_3$ M.W.: 237 Cal. C: 65.79 H: 8.07 N: 5.90 Found C: 65.60 H: 8.45 N: 5.83

2-((3,5-Dimethylpiperidin-1-yl)methyl)-3-hydroxy-6-methyl-4H-pyran-4-one (4).

Recrystallization from ethyl acetate gave a white crystalline solid. IR (KBr disc) 3298-2940 (O-H st), 1622 (C=O st) and 1458 cm^{-1} (C=C st). 1H-NMR δ ($CDCl_3$, 400 MHz) 0.88 (6H; d; J= 5.71; -CH_3), 1.60-1.80 (4H; m; piperidine H_3', H_4', H_5'), 2.30 (5H; s; 6-CH_3 and piperidine H_6'), 2.95 (2H; d;

J= 8.69, piperidine H_2), 3.65 (2H; s; -CH_2-), 6.20 ppm (1H; s; H_5). GC(MS) m/e 113, 98 (base peak), 84, 70, 56. Anal. Cal. for $C_{14}H_{21}NO_3$ M.W.: 251 Cal. C: 66.90 H: 8.42 N: 5.57 Found C: 67.01 H: 8.25 N: 5.60

3-Hydroxy-2-{(4-(4-hydroxyethyl)piperidin-1-yl)methyl}-6-methyl-4H-pyran-4-one (5).

Recrystallization from chloroform/petroleum ether (40-60 °C) gave a white crystalline solid. IR (KBr disc) 3408-3000 (O-H st), 1626 (C=O st) and 1456 cm^{-1} (C=C st). ^{1}H-NMR δ ($CDCl_3$, 80 MHz) 1.10-1.90 (5H; m; piperidine), 2.00-2.20 (2H; m; -CH_2CH_2OH), 2.30 (3H; s; 6-CH_3), 3.00 (4H; d; piperidine), 3.50-3.90 (4H; m; -CH_2- and -CH_2CH_2OH), 6.10 ppm (1H; s; H5). GC(MS) m/e 128, 112, 98, 84, 70, 56 (base peak). Anal. Cal. for $C_{14}H_{21}NO_4$.H2O M.W.: 385 Cal. C: 58.93 H: 8.12 N: 4.90 Found C: 58.83 H: 8.51 N: 4.81

3-Hydroxy-2-{(2-(2-hydroxyethyl)piperidin-1-yl)methyl}-6-methyl-4H-pyran-4-one (6).

Recrystallization from chloroform/petroleum ether (40-60 °C) gave a orange crystalline solid. IR (KBr disc) 3242-2937 (O-H st), 1622 (C=O st) and 1461 cm^{-1} (C=C st). ^{1}H-NMR δ ($CDCl_3$, 80 MHz) 2.00-2.25 (7H; m; piperidine and 6-CH_3), 2.30-2.60 (2H; m; -CH_2CH_2OH), 3.00-3.60 (7H; m; piperidine and -CH_2-), 4.00 (2H; t; -CH_2CH_2OH), 6.20 ppm (1H; s; H_5). GC(MS) m/e 128, 112, 98, 84, 70, 56 (base peak). Anal. Cal. for $C_{14}H_{21}NO_4$.H_2O M.W.: 385 Cal. C: 58.93 H: 8.12 N: 4.90 Found C: 59.16 H: 7.19 N: 4.93

2-((4-Benzylpiperidin-1-yl)methyl)-3-hydroxy-6-methyl-4H-pyran-4-one (7).

Recrystallization from chloroform/petroleum ether (40-60 °C) gave a white crystalline solid. IR (KBr disc) 3100-2879 (O-H st), 1633 (C=O st) and 1460 cm^{-1} (C=C st). ^{1}H-NMR δ ($CDCl_3$, 80 MHz) 1.10-2.00 (5H; m; piperidine), 2.20 (3H; s; 6-CH_3), 2.40 (2H; d; -CH_2-), 2.85 (4H; d; piperidine), 3.50 (2H; s; -CH_2-), 6.10 (1H; s; H_5), 6.90-7.30 ppm (5H; m; phenyl). GC(MS) m/e 171, 148, 127 (base peak), 112, 98, 85, 56. Anal. Cal. for $C_{19}H_{22}NO_3$ M.W.: 312 Cal. C: 73.05 H: 7.09 N: 4.48 Found C: 72.76 H: 7.40 N: 4.47

Anticonvulsant Activity

The compounds were tested for their anticonvulsant activity against

MES and scMet induced seizures. The rotarod toxicity test was performed for neurological toxicity according to the phase I tests of ADD (Antiepileptic Drug Development) program protocol[11] which has also been used for evaluation in various previous studies[7, 14, 15, 17, 18]. 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 test. All synthesized compounds were suspended in 30% aqueous of PEG 400 and administered intraperitoneally in a volume of 0.01 ml/g at body weight to the mice. 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 NINDS-ADD program protocol[11] and used according to the Hacettepe University, 'Laboratory Animals Ethic Committee' 17. 04. 2002 date 2002/ 24-3 number decision). Control animals received 30% aqueous PEG 400. Pentylenetetrazole (metrazol) was administered s.c. on the back of the neck. The rotarod toxicity test was performed on a 1 inch diameter knurled wooden rod; rotating at 6 rpm (the rotarod used in Phase I test was made by Hacettepe University Technical Department).

Maximal Electroshock Seizure (MES) test

Maximal electroshock seizures were elicited with a 60-cycle alternating current of 50 mA intensity (five to seven times that necessary to elicit minimal seizures) delivered for 0.2 s via corneal electrodes. A drop of 0.9 % saline was instilled in each 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 Pentylenetetrazole (Metrazol) (ScMet) test

85 mg/kg of Pentylenetetrazole (produces seizures in more than 95 % of mice) was administered as a 0.5 % solution subcutaneously into the posterior midline. The animal was observed for 30 min. The absence of even a threshold seizure (a single episode of clonic spasms of at least 5 s duration) was defined as protection.

Neurotoxicity

The rotarod test was used to evaluate the neurotoxicity. The animal was placed on a 1 inch diameter knurled wooden rod rotating at 6 rpm.

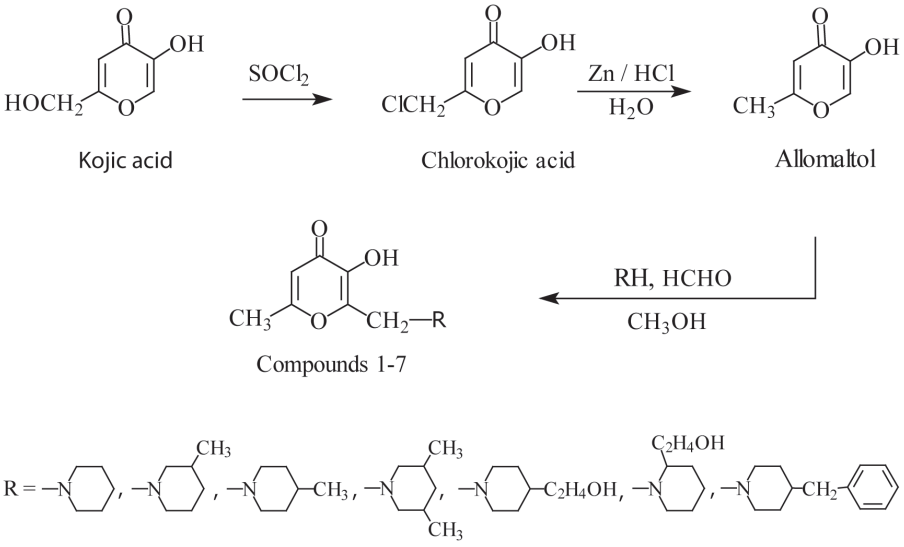
Normal mice remain on a rod rotating at this speed indefinitely. Neurologic toxicity was defined as the failure of the animal to remain on the rod for 1 min.

Results and Discussion

Chemistry

Allomaltol was synthesized from commercially available 5-hydroxy-2-hydroxymethyl-4H-pyran-4-one (kojic acid) in a two-step reaction according literature[7, 16, 19]. Chlorination of the 2-hydroxymethyl moiety of kojic acid using neat thionyl chloride afforded 2-chloromethyl-5-hydroxy-4H-pyran-4-one (chlorokojic acid), with quite significant yield (76%), with the ring hydroxyl being unaffected. Allomaltol was produced by reduction of chlorokojic acid with zinc dust in concentrated hydrochloric acid[7]. In the hydroxypyran form, only one enolic hydroxyl group is present and the position ortho to this hydroxyl group is readily subject to aminomethylation in the Mannich reaction at room temperature[20, 21].

In this study, seven new 3-hydroxy-6-methyl-2-substituted 4H-pyran-4-one derivatives were synthesized as a Mannich bases. Compounds 1-7 were prepared by the reaction of appropriate substituted piperidine derivatives with allomaltol and formaldehyde at room temperature in methanol (Scheme 1).

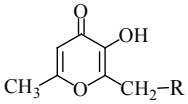


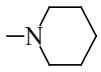
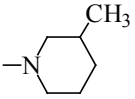
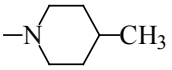
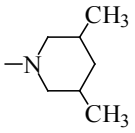
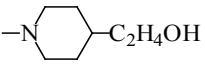
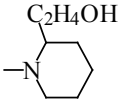
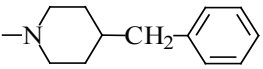
Scheme 1
Synthesis and structure of the compounds 1-7.

The structures of the synthesized compounds 1-7 were confirmed by IR, ^{1}H-NMR, mass spectra and elementary analysis. Yields and the melting point properties of the synthesized compounds were presented in Table I. In the IR spectra, compounds 1-7 have O-H stretching bands at 3400-2879 cm^{-1}. All compounds were associated with C=O and C=C stretching bands at 1673-1622 and 1461-1456 cm^{-1} respectively. With ^{1}H-NMR spectra, H_5 protons of the 4H-pyran-4-one ring were found as a singlet peak in the region 6.13-7.20 ppm in accordance with literature[7]. 6-Methyl-4H-pyran-4-one derivatives showed methyl group protons as a singlet at 2.20-2.30 ppm. The methylene group protons of compounds 1-7 appeared as a singlet at 3.50-3.70 ppm.

TABLE I

The Structures, Melting Points and Yields of the Synthesized Compounds



Compound Number	R	M. p. (°C)	Yield (%)
1		150-1	30
2		140-1	56
3		146-7	39
4		154-5	41
5		108-9	59
6		107-8	36
7		130-1	35

Anticonvulsant Activity

The anticonvulsant activities of the compounds were initially evaluated against MES and scMet induced seizures using Swiss albino male mice (20 ± 2 g). The results are shown in Table II. According to the activity studies, 2-((3,5-dimethylpiperidin-1-yl)methyl)-3-hydroxy-6-methyl-4H-pyran-4-one (compound 4) was determined to be most active against scMet at 100 and 300 mg/kg dose at half an hour. 3-Hydroxy-2-{(4-(4-hydroxy ethyl)piperidin-1-yl)methyl}-6-methyl-4H-pyran-4-one (compound 5) was found to have anticonvulsant activity against scMet seizures at 300 mg/kg dose at the fourth hour and MES seizures at 300 mg/kg dose at half an hour. Compound 7 was protective against scMet at 300 mg/kg at the fourth hour. None of the compounds 1, 2, 3 and 6 showed anticonvulsant activity. Neurotoxicity was observed in compound 3 which was administered to mice at 300 mg/kg dose level.

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Summary

Synthesis of Some Novel Mannich Bases Derived From Allomaltol and Evaluation of Their Anticonvulsant Activities

In this study, new 3-hydroxy-6-methyl-2-substituted 4H-pyran-4-one derivatives were synthesized and their anticonvulsant activities were evaluated. Mannich bases were prepared by the reaction of suitable piperidine derivatives with allomaltol and formaline. The structure of the synthesized compounds was confirmed by IR, ^{1}H-NMR, Mass and elementary analysis. Anticonvulsant activities of the compounds were examined by maximal electroshock (MES) and subcutaneous Pentylenetetrazole (scMet). Neurotoxicity was determined by rotarod toxicity test. All these tests were performed in mice according to procedures of the Antiepileptic Drug Development (ADD) program protocol of the National Institutes of Health (NIH). According to the activity studies, 2-((3,5-dimethylpiperidin-1-yl)methyl)-3-hydroxy-6-methyl-4H-pyran-4-one (compound 4) was determined to be most active against scMet at 100 and 300 mg/kg dose at half an hour. 3-Hydroxy-2-{(4-(4-hydroxyethyl)piperidin-1-yl)methyl}-6-methyl-4H-pyran-4-one (compound 5) and 2-((4-benzylpiperidin-1-yl)methyl)-3-hydroxy-6-methyl-4H-pyran-4-one (compound 7) were found to have anticonvulsant activity against scMet seizures at 300 mg/kg dose. Only compound 5 was shown to be protective against MES at 300 mg/kg dose in this series.

Key words: Allomaltol, 3-hydroxy-6-methyl-2-substituted 4H-pyran-4-one derivatives, Mannich bases, anticonvulsant activity

Özet

Allomaltolden Türetilen Bazı Yeni Mannich Bazlarının Sentezi ve Antikonvülsan Aktivitelerinin Değerlendirilmesi

Bu çalışmada, yeni 3-hidroksi-6-metil-2-sübstitüe 4H-piran-4-on türevleri sentezlenmiş ve antikonvülsan aktivitelerini değerlendirilmiştir. Mannich bazları, uygun piperidin türevleri ile allomaltol ve formaldehitin reaksiyona sokulması sonucu hazırlanmıştır. Sentezlenen bileşiklerin yapıları IR, ^{1}H-NMR, Mass ve elementel analiz ile aydınlatılmıştır. Maksimal elektroşok (MES) ve subkutan pentilentetrazol (scMet) testleri ile bileşiklerin antikonvülsan aktiviteleri araştırılmıştır. Rotarod toksisite testi ile nörotoksisiteleri saptanmıştır. Bütün bu testler, farelerde Amerika Ulusal Sağlık Enstitüsü'nün (NIH) Antiepileptik İlaç Geliştirme (ADD) program protokolüne göre yapılmıştır. Aktivite çalışmasına göre, 2-((3,5-dimetilpiperidin-1-ilmetil)-3-hidroksi-6-metil-4H-piran-4-on (4) bileşiği scMet nöbetlerine karşı yarım saatde 100 ve 300 mg/kg dozlarda en aktif bileşik olduğu saptanmıştır. 3-Hidroksi-2-{(4-(4-hidroksietil)piperidin-1-il)metil}-6-metil-4H-piran-4-on (5) ve 2-((4-benzilpiperidin-1-il)metil)-3-hidroksi-6-metil-4H-piran-4-on (7) bileşikleri scMet nöbetlerine karşı 300 mg/kg dozda antikonvülsan aktiviteye sahip olduğu bulunmuştur. Bu seride sadece bileşik 5'in, 300 mg/kg dozda MES'e karşı koruyucu özellik göstermiştir.

Anahtar Kelimeler: Allomaltol, 3-hidroksi-6-metil-2-sübstitüe-4H-piran-4-on türevleri, Mannich bazları, antikonvülsan aktivite

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