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Synthesis of 4(1*H*)-pyridinone derivatives and investigation of analgesic and antiinflammatory activities

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Abstract

This paper describes recent results of a research program aimed at the synthesis and pharmacological evaluation of new 4(1*H*)-pyridinone derivatives belonging to the 1,3-disubstituted series (**4**–**11**). These compounds were structurally planned by applying the molecular hybridization strategy on previously described 1,2-disubstituted-4(1*H*)-pyridinone derivatives, considered as lead compounds, which present potent analgesic properties (M.D. Aytemir, T. Uzbay, D.D. Erol, Arzneim. Forsch. (Drug Res.) 49 (1999) 250). Their chemical structures have been proved by means of their IR and ^{1}H NMR data and by elemental analysis. The analgesic profile of the title compounds (**4**–**11**), evaluated by the model of abdominal constrictions induced by acetic acid, showed that all the 4(1*H*)-pyridinone derivatives were active, exhibiting an analgesic activity comparable with that of aspirin (acetyl salicylic acid) used as a standard. The antiinflammatory profile of the synthesized compounds, evaluated by the model of carrageenan rat paw edema, showed that all compounds were active and were comparable with indomethacin used as a standard.

Keywords: 4(1*H*)-Pyridinones; Analgesic properties; Antiinflammatory agents; Kojic acid derivatives

1. Introduction

The class of pyridinones includes compounds endowed with pharmacological activities in different animal tests. Molecules containing the 4(1*H*)-pyridinone nucleus possess antibacterial [2,3], antifungal [4], antimalarial [5,6], cardiotonic [7], antineoplastic [8–11], antiinflammatory [1], analgesic [1,12–15] activity and they are used for the treatment of Parkinson's disease [16,17].

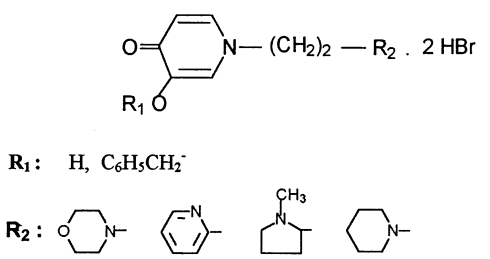

Fig. 1. 1,3-Disubstituted 4(1*H*)-pyridinone·2HBr derivatives.

Among the pharmacological properties of 4(1*H*)-pyridinone derivatives, the analgesic effects [1,18] and the antiinflammatory activity in the carrageenan-induced rat paw edema could suggest inhibition of prostaglandin E and arachidonic acid synthesis. On the other hand, the 4(1*H*)-pyridinone based structures do not feature molecular characteristics of known analgesics and antiinflammatory agents.

These results led us to design new structurally related derivatives, keeping the 4(1*H*)-pyridinone framework and modifying the nature of the substituent on the basic moiety. In this paper we describe the synthesis, structural properties and pharmacological evaluation of two new series of 1,3-disubtituted-4(1*H*)-pyridinone derivatives (Fig. 1). The analgesic or antiinflammatory activities were investigated for the 4(1*H*)-pyridinone derivatives keeping acetyl salicylic acid and indomethacin as comparison compounds.

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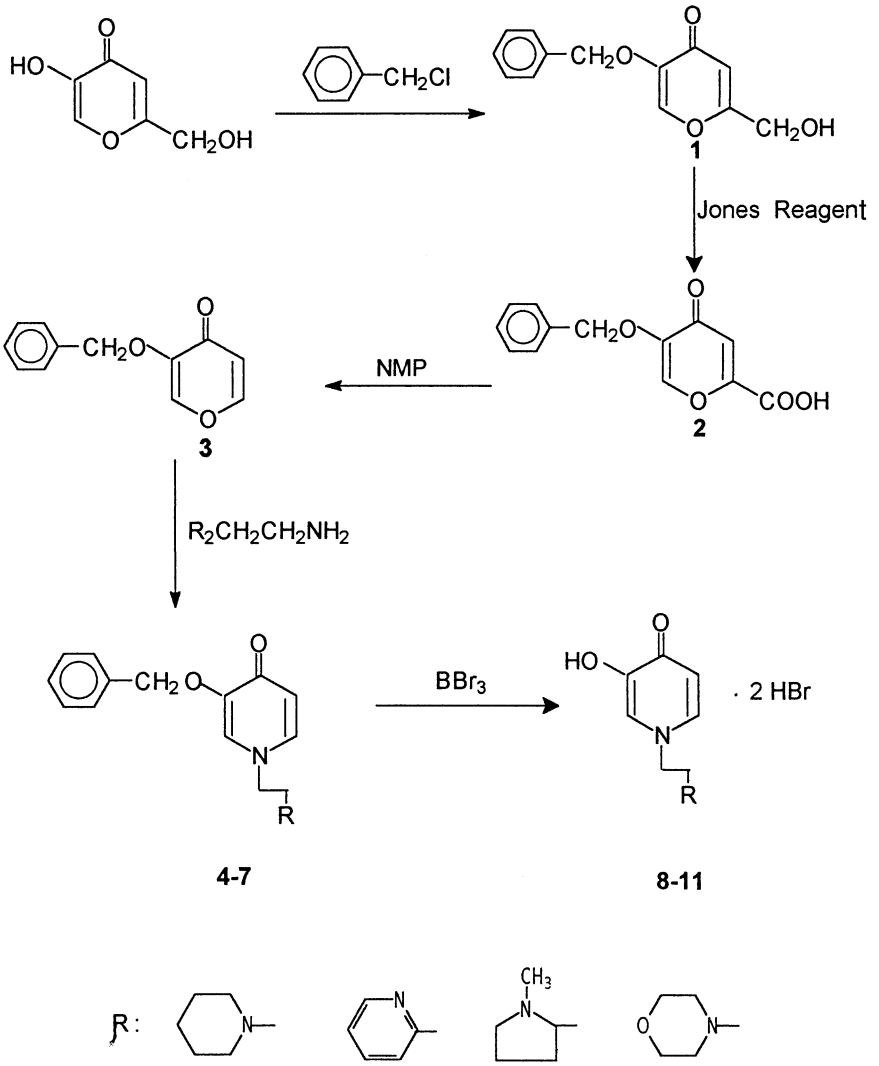


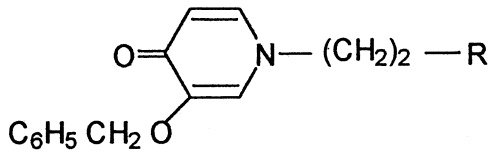
Scheme 1. Synthesis of 1,3-disubstituted-4(1*H*)-pyridinone·2HBr derivatives.

2. Chemistry

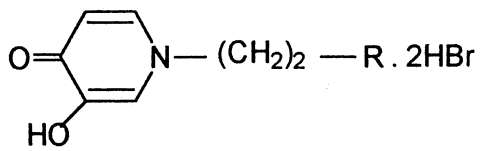
4(1*H*)-pyridinone derivatives (**4**–**11**) were prepared as illustrated in Scheme 1 by means of nucleophilic substitution on 4-pyrone derivatives by suitable cyclic amines such as piperidine, pyridine, pyrrolidine and morpholine.

The synthesized compounds (**4**–**7**) were obtained by reacting primary amines with 4-pyrone derivatives in absolute ethanol. The desired substituted-4(1*H*)-pyridinone derivatives (**8**–**11**) were obtained by cleavage with BBr_3 in dichloromethane. Since a convenient route to 4-pyridones is the condensation of 4-pyrones and primary amines, commercially available kojic acid was selected as the starting material for the synthesis of the 4-pyridone analogs (Scheme 1). Condensation of the benzyl ether of the starting material with primary amines afforded the desired pyridinones. The Jones' oxidation of the hydroxymethyl group required conversion to benzyl kojic acid, as a protection of the phenolic function. Pyronecarboxylic acid **2** could be converted to the benzyloxy-4-pyrone **3** by heating in *N*-methylpyrrolidone that caused thermal decarboxylation. Formula, melting point and yield (%) of the compounds are shown in Table 1. Susceptibility of the free bases to air oxidation necessitated preparation of the corresponding hydrobromide salts for storage and biological studies. The structures of the newly prepared compounds were assigned according to the reaction mechanisms and analytical data and were confirmed by IR and ^{1}H-NMR spectral data (Table 2). Thus all these compounds showed in their IR spectra a strong band at 1632 cm^{-1} assignable to a C=O group, and a band at 1234 cm^{-1} that is characteristic of a C–O–C group. The ethylene group protons in the ^{1}H-NMR spectra of all compounds appeared as a triplet at 4.70–3.50 ppm for –N–CH_2– and a triplet signal at 3.90–2.90 ppm for –N–CH_2–CH_2–. The characteristic doublets of pyridones were observed at aromatic fields.

Table 1
Structures and chemical data of N-substituted-3-benzyloxy-4(1*H*)-pyridinones (**4**–**7**) and N-substituted-3-hydroxy- 4(1*H*)-pyridinones (**8**–**11**)



Compound No.	R	Yield (%)[a]	Melting Point (°C)	Molecular Formula	Analysis
4		75	Liquid	$C_{19}H_{24}N_2O_2$	C,H,N
5		59	Liquid	$C_{19}H_{18}N_2O_2$	C,H,N
6		63	Liquid	$C_{19}H_{24}N_2O_2$	C,H,N
7		78	Liquid	$C_{18}H_{22}N_2O_3$	C,H,N



8		51	220 dec.	$C_{12}H_{18}N_2O_2.2HBr$	C,H,N
9		45	204 dec.	$C_{12}H_{18}N_2O_2.2HBr$	C,H,N
10		64	270 dec.	$C_{12}H_{12}N_2O_2.2HBr$	C,H,N
11		48	213 dec.	$C_{11}H_{16}N_2O_3.2HBr$	C,H,N

Yields are of the products obtained with a first crystallization from ethanol-ether

3. Chemical experimental section

Melting points were determined in open glass capillaries on a Thomas-Hoover (Philadelphia, USA) apparatus and are uncorrected. The infrared spectra were recorded on a Perkin–Elmer FT IR 1720 X IR (Beaconsfield, UK) spectrophotometer using samples in potassium bromide disks. ^{1}H-NMR spectra were measured on a Perkin–Elmer R 32 90 MHz and Bruker AC 200 MHz FT NMR (Karlsruhe, Germany) tetramethylsilane using as an internal standard. Chemical shift values were reported as δ (ppm) values. Analyses indi-

Table 2
Spectral data of N-substituted-3-benzyloxy-4(1*H*)-pyridinones (**4**–**7**) and N-substituted-3-hydroxy-4(1*H*)-pyridinones·2HBr (**8**–**11**) [a]

Comp. no.	IR (cm^{-1})		^1H NMR (ppm)				
	C=O	C–O–C/OH	N–CH_2 (t)	CH_2 (t)	O–CH_2 (s)	$H^{3,2}$ (s,d)	H^6 (s)
4	1634	1121	3.50	3.05	5.00	6.10–5.30	7.70
5	1635	1227	3.40	2.85	5.20	7.05–6.40	7.20
6	1632	1224	3.85	3.00	5.00	5.95–6.50	7.90
7	1635	1234	4.10	3.70	5.20	5.95–6.30	7.70
8	1615	3400–3300	4.65	3.20		7.00	8.15
9	1614	3400–3300	4.75	3.05		7.00–7.85	8.15
10	1634	3100–3300	4.70	3.60		6.90–7.90	8.40
11	1617	3500–3200	4.70	3.65		7.10–7.90	8.20

[a] s: singlet; d: doublet; t: triplet. Compounds **4**–**7** were dissolved in $CDCl_3$ and compounds **8**–**11** in DMSO-d_6.

cated by elemental symbols were within $\pm 0.4\%$ of the theoretical values and were performed by Butterworth UK. The purity of the compounds was determined by TLC on silica gel HF 254 (Merck) (chloroform–methanol 95:5). All chemicals were obtained from Aldrich Chemical Co. (Steinheim, Germany).

*3.1. 5-Benzyloxy-2-hydroxymethyl-4-pyrone (**1**)*

Anhydrous K_2CO_3 (276.2 g, 2 mol) was suspended in a solution of kojic acid (142 g, 1 mol) and benzyl chloride (253.2 g, 2 mol) in dimethylformamide (700 ml). The temperature of the reaction mixture was raised to 110°C and maintained for 3 h. The dark reaction mixture was allowed to cool, poured into ice water (100 ml) and extracted with dichloromethane. The organic phase was washed with water, dried with Na_2SO_4 and evaporated. Recrystallization of the resulting solid from chloroform provided compound **1** as white needles (125 g, 54%), m.p. 131–133°C [3].

*3.2. 5-Benzyloxy-4-pyrone-2-carboxylic acid (**2**)*

Compound **1** (10 g, 43.2 mmol) was dissolved in acetone (500 ml), cooled in an icebath and Jones reagent was added (25 ml). The inorganic material was removed by filtration and the filtrate evaporated to dryness. Pure compound **2** was obtained by recrystallization from methanol: (8.89 g, 89%) m.p. 194–196°C [3].

*3.3. 3-Benzyloxy-4-pyrone (**3**)*

Benzylcomenic acid (**2**) (10 g, 40.6 mmol) was dissolved in *N*-methyl pyrrolidone and refluxed overnight. After cooling 25 ml of dimethylformamide was added to the reaction mixture and the solvent was evaporated to give a residue, which was extracted with dichloromethane. The organic layer was washed with 5% sodium hydroxide and water, dried and evaporated. The residue was treated with charcoal in ethanol and the resulting yellow solid was recrystallized from toluene: (4.68 g, 56%), m.p. 85–86°C [3].

*3.4. General procedure for N-substituted-4(1H)-pyridinones (**4**–**7**)*

Compound **3** (1 mol) was dissolved in ethanol and the appropriate primary amine was added. The mixture was heated under reflux for 40 h, the solvent evaporated and the residue poured into the ice water and extracted with chloroform. Organic phase was dried with Na_2SO_4, filtered and evaporated in vacuo to yield the title compounds as orange oil (Table 1).

*3.5. General procedure for N-substituted hydroxy-4(1H)-pyridinones (**8**–**11**)*

Compounds (**4**–**7**) were dissolved in dry dichloromethane. BBr_3 in dichloromethane was added dropwise under nitrogen and the reaction was stirred for 3 h. The excess of BBr_3 was destroyed at 15°C by the addition of cold methanol and stirring was continued in an ice bath for 30 min. The mixture was concentrated to dryness in vacuum and the residue was dissolved several times in methanol and evaporated. Recrystallization from ethanol–ether gave the pure 4-pyridinone derivatives as yellow–white powder (Table 1).

4. Pharmacological experimental section

Male Swiss Webster mice (20 ± 2 g) were purchased (local breed) from the University of Hacettepe Animal House and maintained at a temperature between 20 and 23°C. The animals were housed in groups of eight, with food and water ad libitum and allowed to get accustomed to their environment for at least 2 days before the test. Motor coordination was measured according to the method of Gross et al. [19]. The effects were evaluated 15, 30, 45, 60 and 75 min after the adminis-

tration of the investigated compounds. Animal experiments have been practiced under the supervision of the Veterinary Faculty of the Ankara University, which is responsible for the observation of the rules of guidelines of animal experiments.

4.1. Analgesic activity

Modified acetic acid writhing test for screening analgesic activity was employed [18,20]. Each compound (**4**–**11**) was i.p. administered to mice in groups of eight at a dose level of 100 mg/kg. One hour later, 3% (w/w) solution of acetic acid (300 mg/kg) was administered i.p. Control groups ($n = 8$) received an equal volume of 0.9% NaCl (10 ml/kg). Animals were placed in glass cages 5 min after the acetic acid injection and the number of 'writhes' induced in each mouse was observed for a 10-min period. Acetylsalicylic acid was used as a reference analgesic drug and administered according to the test protocol (100 mg/kg).

The analgesic activity was expressed in terms of % inhibition;

$$\%\ \text{Analgesic activity} = n - n'/n\,100$$

where n = mean number of writhes of control group, n' = mean number of writhes of the test group.

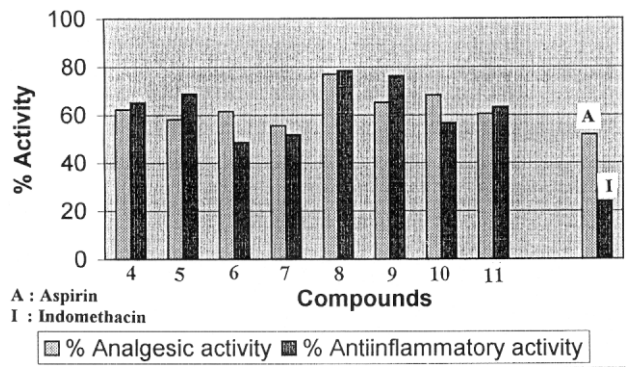


Fig. 2. Analgesic and antiinflammatory activities of 4-pyridinone derivatives.

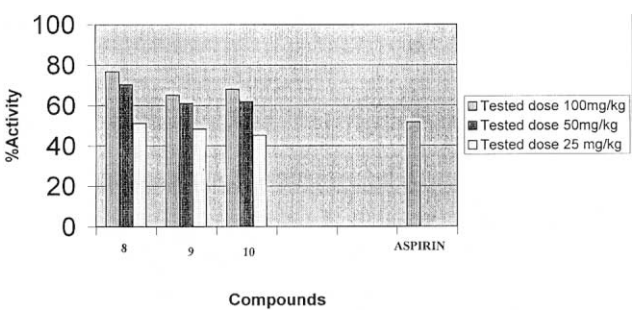


Fig. 3. Action profile of 4-pyridinones.

4.2. Antiinflammatory activity

Carrageenan-induced mouse paw edema was measured using Peacock Dial Thickness gauge (0.01–10 mm). Eight mice per group were used. Sixty minutes after i.p. administration of the compound (100 mg/kg) 2% carrageenan (0.01 ml) was injected s.c. into the plantar surface of the right hind paw. Two hours later the volume of the edema was measured. Indomethacin (100 mg/kg i.p. injection) was used as a positive control [20].

4.3. Statistical analysis

Student's t-test and two-factor analysis of variance, (Pharmacologic calculation system version 4.1) was employed.

5. Results and discussion

The evaluation of the analgesic profile of all 1,3-disubstituted-4(1*H*)-pyridinone derivatives (**4**–**11**) were performed using the classical acetic acid-induced mice abdominal contractions test with aspirin as the standard. In the present study, acetic acid 3% (300 mg/kg) was used to induce abdominal contractions (writhing) in the mice. Koster et al. [21] used very low dose of acetic acid (60 mg/kg) for evaluating writhing response in mice. In our preliminary studies, we did not observe marked and acceptable writhing responses using this low dose of acetic acid. In some previous studies, acetic acid 3% (300 mg/kg) has also been used for evaluating writhing responses in mice [19,20,22]. Using different mice species may be responsible for the discrepancy. With regard to the analgesic activity, all the compounds were shown to be equally potent in comparison with aspirin. The degree of protection ranged from 65 to 79% at a dose of 100 mg/kg. Compounds **8**–**10** were most active and displayed a significant analgesic effect at dose of 50 mg/kg (70, 61 and 61%, respectively) and 25 mg/kg (51, 48 and 45%, respectively). Compound **8** is the more promising one and slightly more potent than the others.

Antiinflammatory activities of all the synthesized compounds were screened using the carrageenan hindpaw edema test with indomethacin as the standard. These results are shown in Fig. 2. The edema inhibition of all compounds was significant when compared to the inhibition obtained by indomethacin. In Fig. 2 the activity of compound **8** is shown to be significantly higher and more potent in comparison with indomethacin. The results obtained indicate that the investigated 4(1*H*)-pyridinone derivatives have a profile of action corresponding to new analgesic and antiinflammatory agents (Fig. 3).

Acknowledgements

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