Synthesis and Evaluation of Anti-Inflammatory and Antitussive Activity of Hydantion Derivatives

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Abstract: 1-Methylhydantion is a compound, which was isolated from Oviductus Ranae for the first time. In our study, we found that it showed good antitussive and anti-inflammatory activity. It is also the first report which illustrates the antitussive activity of hydantion derivative. A series of hydantion derivatives were synthesized and evaluated for their anti-inflammatory and antitussive activity *in vivo*. The pharmacological tests showed that compounds **7a, 7c** and **7d** have good anti-inflammatory and antitussive activity compared to Ibuprofen and codeine. Compound **7a** in particular showed two-fold stronger anti-inflammatory activity than Ibuprofen.

Keywords: Anti-inflammatory activity, Antitussive activity, Biological activity, Hydantoin, Ibuprofen, Synthesis.

INTRODUCTION

Hydantoin (1, 3-imidazolidinedione) was discovered in 1861 by Baeyer, who isolated it as one of the reduction products of allantoin during the study of uric acid. Hydantoin derivatives display diverse and interesting pharmacological properties. Such as phenytoin, mephenytoin, norantoin, and some 5-substituted hydantoins are well-known anticonvulsive drugs [1, 2, 3]. Hydantoins also exhibit antidepressant, antiviral, antitumoral and antithrombotic activities, as well as inhibitory activity against some enzymes such as human aldose reductase and human leucocyte elastase [4].

Oviductus Ranae is the dry oviductus of the tely-Rana temporaria chensinensis David, which has many pharmacological actions, such as enhancement of immune and stress performance, antioxidant and anti-aging, blood fat regulation, the effect of growth and sexual function, anti-hypoxia and anti-anxiety, anti-fatigue, antitussive expectoration and ataxia regulation [5]. 1-Methylhydantion, a hydantion derivative, for the first time has been isolated from Oviductus Ranae [6]. In our study, it shows good antitussive activity and anti-inflammatory activity. It is the first report which shows that hydantion derivative has antitussive activity.

In this paper, we report our efforts towards the synthesis and investigation of the antitussive activity of a series of novel hydantoin derivatives. We have also optimized the compound to enhance the anti-inflammatory activity of the hydantion derivatives. This allows the compound to possess both good antitussive activity and good anti-inflammatory activity. Ibuprofen, a common non-steroidal anti-inflammatory drug (NSAIDs) is used primarily for fever, pain, dysmenorrhea and inflammatory diseases [7]. Here we combined the structure of Ibuprofen and hydantoin into one molecule. A series of hydantion derivatives were synthesized and evaluated for their anti-inflammatory and antitussive activity *in vivo*. The pharmacological tests showed that compounds **7a, 7c** and **7d** have good anti-inflammatory and antitussive activity compared to Ibuprofen and codeine. Compound **7a** even showed two-fold stronger anti-inflammatory activity than Ibuprofen.

METHODS

Evaluation of Anti-Inflammatory Activity [8]

Anti-inflammatory activity of these hydantion derivatives was evaluated using xylene-induced mouse ear edema model. Kunming male mice (20-22g) were divided into ten groups randomly (n=10 for each group). One group was kept as control and the others were given test compounds including one group given Ibuprofen as standard. 1 hour after the last oral treatment, 0.02mL xylene was applied to the right ear of each mouse. Mice were killed by cervical dislocation 1 hour after xylene application. Ear disk of 8.0 mm in diameter was punched out and weighed. The difference in weight of the ears from each group was used to analyze the anti-inflammatory function.

Evaluation of Antitussive Activity [9]

The antitussive activity was evaluated in mouse after cough induction by ammonia aerosol stimulation and the number of cough was detected in 2 min. Kunming male mice (20-22g) were divided into ten groups randomly (n=10 for each group). One group was kept as control and the others were given test compounds including one group given codeine as standard. 1 hour after the last oral treatment, mice cough was induced by ammonia aerosol stimulation. The antitussive effects of the target compounds on mice were evaluated by the frequency of cough caused by ammonia and the latent period of cough after administration.

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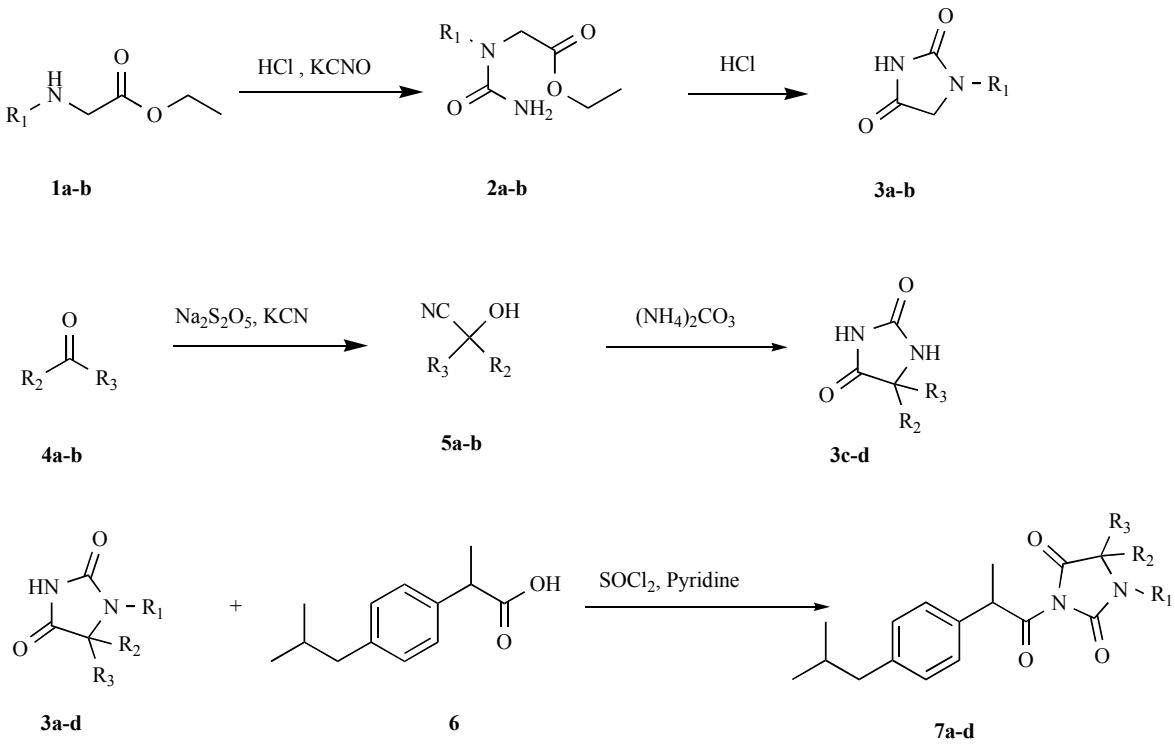

3a: R_1= -CH_3

3b: R_1= -CH_2Ph

3c: R_2 = H, R_3 = H

3d: R_2= -CH_3, R_3 = -CH_3

7a: R_1= -CH_3 ,R_2=H , R_3=H

7b: R_1=-CH_2Ph ,R_2=H , R_3=H

7c: R_1=H ,R_2=H , R_3=H

7d: R_1= -CH_3 ,R_2=-CH_3 , R_3=-CH_3

Scheme 1. Synthesis of hydantion derivatives.

RESULTS AND DISCUSSION

Synthesis

A general synthetic route for the hydantion derivatives used in this study is shown in Scheme **1**. In order to reduce the toxicity dangerous, compounds **3a-d** were synthesized by one pot method. Hydantoins **3a-b** were prepared by acid-catalyzed cyclization of N-substituted glycine ethylesters **1a-b** [10]. N-substituted glycine ethylesters **1a-b** were first reacted with potassium cyanate to obtain **2a-b,** Compounds **2a-b** were then cyclized under acidic condition to get **3a-b.** 5-Substituted hydantoins **3c-d** were synthesized through Bucherer-Berg method [11, 12]. Acetone or formaldehyde were first reacted with potassium cyanide to get cyanohydrin derivatives **5a-b**, Compounds **5a-b** were then treated with ammonium carbonate to obtain **3c-d**. Ibuprofen was first treated with $SOCl_2$ to obtain the acyl chloride, and then reacted with hydantoins **3a-d** in pyridine to give the target compounds **7a-d**. The structures of compounds were confirmed by MS, ^{1}H NMR, ^{13}C NMR and Elemental analysis.

Biological Activity

The anti-inflammatory activity and antitussive activity of hydantion derivatives were summarized in Tables **1** and **2**. The results showed that compounds **7a**, **7c** and **7d** have both good anti-inflammatory and antitussive activity. It should be noted that compound **7a** has two-fold stronger anti-inflammatory activity than Ibuprofen. Compounds **3a**, **3c** and **3d** showed good antitussive activity**,** but anti-inflammatory activity was weak. Compounds **3b** and **7b** that substituted benzyl at N_1 position of hydantoin have nearly no activity.

These SAR studies revealed that the substitution of methyl or hydrogen at the N_1 position of hydantoin had effective influence on antitussive activity. Especially, N_1 substituted 1-methyl hydantoin **7a** had better anti-inflammatory activity than Ibuprofen. However, with benzyl group at N1 position only showed weak anti-inflammatory and antitussive activity. Because compound **7b** didn't show any anti-inflammatory activity, so the mechanism of anti-inflammatory action may not be as that the hydantion derivatives first hydrolyze to Ibuprofen, then Ibuprofen plays

Table 1. Anti-Inflammatory Activity of the Target Compounds

Compound	Dose(mg/kg)	Ear swelling degree [a] (mg)	Inhibition rate [b] (%)
control	5%CMC	12.36±0.52*	
Ibuprofen	100	8.89±0.33**	28.07
3a	100	10.58±0.32*	14.35
3b	100	12.15±0.42*	1.70
3c	100	10.60±0.44*	14.19
3d	100	10.50±0.58*	15.08
7a	100	5.61±0.42**	54.61
7b	100	11.38±0.46**	7.93
7c	100	9.33±0.51*	24.51
7d	100	9.27±0.40**	25.00

[a] Ear swelling degree was calculated as (the weight of right ear)-(the weight of left ear)

[b] Inhibition rate was calculated as (1-the ear swelling degree of the compound/the ear swelling degree of the control) ×100%

Standard: Ibuprofen, each value is the mean ± SEM for ten mice,* $P < 0.05$;** $P < 0.01$ compared with control.
Data analyzed by one-way ANOVA followed by Student's t-test

Table 2. Antitussive Effect of the Target Compounds

Compound	Dose(mg/kg)	Latency (s)	Frequency of cough
control	5%CMC	27.3±5.8*	42±3.1*
codeine	30	53±6.2*	17±2.7**
3a	100	39.5±9.2*	16.5±2.5*
3b	100	29.5±6.29*	45.5±3.5*
3c	100	36.3±5.3*	17.5±4.5*
3d	100	33.5±4.6*	17.9±3.8*
7a	100	40±4.8*	15.5±3.5**
7b	100	33.3±7.1**	29.2±3.3*
7c	100	77±6.7*	18.3±3.6**
7d	100	38.3±5.2*	18.6±2.9**

Standard: codeine, each value is the mean ± SEM for ten mice,* $P < 0.05$;** $P < 0.01$ compared with control.
Data analyzed by one-way ANOVA followed by Student's t-test.

the anti-inflammatory action. Further extensive studies need to be carried out to determine their mechanism of anti-inflammatory action.

SYNTHESIS EXPERIMENTAL SECTION

All reagents were of commercial quality and were used as received. Solvents were dried and purified by using standard techniques. Reactions were monitored by TLC. The elemental analysis was performed on a Carlo Erba 1106 analyzer. The ^{1}H NMR and ^{13}C NMR spectra were recorded on a UNITY-400 NMR spectrometer with TMS as the internal standard. The MS spectra were taken on an LCQ electrospray mass spectrometer.

General Procedure for the Compounds 3a-b

N-substituted glycine ethylester **1** (0.019 mol) was added into conc. HCl (2.4 mL, 0.028 mol). With continued stirring at room temperature, a solution of potassium cyanate (2.3 g, 0.028 mol) in 3.24 mL of water was added dropwise. The mixture was stirred at room temperature for 20 h and then extracted with CH_2Cl_2. The organic layer was dried under reduced pressure to get the crude N-substituted N-carbethoxymethylureas **2**. 12.50 mL 25% HCl was then added into the mixture and heated under reflux for 4 h and then cooled in ice. The precipitate was collected and recrystallized with hot water to get pure product **3a-b** as white solid.

1-Methylimidazolidine-2, 4-Dione (3a)

Yield 62%; ^{1}H-NMR ($CDCl_3$, 400 MHz) δ: 2.98 (s, 3H, CH_3), 3.93 (s, 2H, CH_2), 8.74 (s, 1H, NH); ^{13}C-NMR ($CDCl_3$, 100 MHz) δ: 170.22, 156.66, 52.93, 29.37.

1-Benzylimidazolidine-2, 4-Dione (3b)

Yield 68%; ^{1}H-NMR ($CDCl_3$, 400 MHz) δ:3.77 (s, 2H, CH_2), 4.52 (s, 2H, CH_2), 7.24-7.29 (m, 2H, 2×ArH), 7.31-7.37 (m, 3H, 3×ArH), 9.27 (s, 1H, NH); ^{13}C-NMR ($CDCl_3$,

100 MHz) δ: 171.44, 156.96, 136.52, 128.53, 127.42, 127.42, 127.33, 127.33, 50.38, 45.26.

General Procedure for the Compounds 3c-d

Sodium metabisulphite (14.6 g, 0.077 mol), compound **4** (0.077 mol) and potassium cyanide (5.0 g, 0.077 mol) was dissolved in 20 mL water, stirred at room temperature for 0.5 h to get cyanohydrin derivatives **5**. Ammonium carbonate (14.8 g, 0.154 mol) was then added. The mixture was heated to 50℃ for half an hour, and the temperature was raised to 90℃ and heated for another 3 h. The solvent was evaporated under reduced pressure. The crude product was recrystallized with hot water to get pure product **3c-d** as white solid.

Imidazolidine-2, 4-Dione (3c)

Yield 42%; ^{1}H-NMR (DMSO-d_6, 400 MHz) δ: 3.84 (s, 2H, CH_2), 7.67 (s, 1H, NH), 10.59 (s, 1H, NH); ^{13}C-NMR (DMSO-d_6, 100 MHz) δ: 174.26, 158.74, 47.56.

5, 5-Dimethylimidazolidine-2, 4-Dione (3d)

Yield 48%; ^{1}H-NMR (DMSO-d_6, 400 MHz) δ: 1.44 (s, 6H, 2×CH_3), 7.82 (s, 1H, NH), 10.68 (s, 1H, NH); ^{13}C-NMR (DMSO-d_6, 100 MHz) δ: 181.78, 158.49, 61.35, 24.26.

General Procedure for the Compounds 7a-d

Ibuprofen (0.5 g, 0.002 mol), $SOCl_2$(0.48 g, 0.004 mol) added into CH_2Cl_2 10 ml, stirred at reflux for 2 h, then added into compound 3 (0.002 mol) were dissolved in pyridine 10 mL, reacted at room temperature overnight. By evaporating the solvent, compound **7** was obtained through flash chromatography (methanol / chloroform 1/20).

3-(2-(4-Isobutylphenyl)Propanoyl)-1-Methylimidazolidine-2,4-Dione (7a)

White solid, yield 82%; ^{1}H-NMR ($CDCl_3$, 400 MHz) δ: 0.89 (d, J = 6.8 Hz, 6H, 2×CH_3), 1.49 (d, J = 6.8 Hz, 3H, CH_3), 1.83-1.85 (m, 1H, CH), 2.42 (d, J = 7.2 Hz, 2H, CH_2), 2.93 (s, 3H, CH_3), 3.77 (s, 2H, CH_2), 4.85 (q, J = 6.8 Hz, 1H, CH), 7.07 (d, J = 8.0 Hz, 2H, 2×ArH), 7.16 (d, J = 8.0 Hz, 2H, 2×ArH); ^{13}C-NMR ($CDCl_3$, 100 MHz) δ: 172.58, 166.93, 152.86, 140.88, 136.68, 129.57, 129.57, 127.64, 127.64, 50.85, 46.62, 44.97, 30.06, 29.52, 22.32, 22.32, 18.57; ESI-MS (M^++H), *m/z*: 303.3; Anal. Calcd. for $C_{17}H_{22}N_2O_3$: C 67.53, H 7.33, N 9.26; Found C 67.55, H 7.32, N 9.25.

1-Benzyl-3-(2-(4-isobutylphenyl)Propanoyl)Imidazolidine-2,4-Dione (7b)

Oil, yield 90%; ^{1}H-NMR ($CDCl_3$, 400 MHz) δ: 0.89 (d, J = 6.56 Hz, 6H, 2×CH_3), 1.50 (d, J = 6.92 Hz, 3H, CH_3), 1.84-1.86 (m, 1H, CH), 2.44 (d, J = 7.16 Hz, 2H, CH_2), 3.60 (dd, J_1 = 17.72 Hz, J_2 = 25.0 Hz, 2H, CH_2), 4.46 (dd, J_1 = 14.96 Hz, J_2 = 6.4 Hz, 2H, CH_2), 4.87 (q, J = 6.96 Hz, 1H, CH), 7.11-7.18 (m, 6H, 6×ArH), 7.32 (m, 3H, 3×ArH); ^{13}C-NMR ($CDCl_3$, 100 MHz) δ: 172.60, 166.88, 152.84, 140.93, 136.69, 134.42, 129.60, 129.60, 129.02, 129.02, 128.33, 128.00, 128.00, 127.68, 127.68, 48.24, 46.83, 46.44, 45.00, 30.10, 22.34, 22.34, 18.39; ESI-MS (M^++H), *m/z*: 379.4; Anal. Calcd. for $C_{23}H_{26}N_2O_3$: C 72.99, H 6.92, N 7.40; Found C 72.96, H 6.91, N 7.41.

3-(2-(4-Isobutylphenyl)propanoyl)Imidazolidine-2,4-Dione (7c)

White solid, yield 75%; ^{1}H-NMR ($CDCl_3$, 400 MHz) δ: 0.87 (d, J = 6.64 Hz, 6H, 2×CH_3), 1.49 (d, J = 6.96 Hz, 3H, CH_3), 1.83-1.85 (m, 1H, CH), 2.42 (d, J = 7.16 Hz, 2H, CH_2), 3.86 (d, J = 4.76 Hz, 2H, CH_2), 4.81 (q, J = 6.96 Hz, 1H, CH), 5.94 (s, 1H, NH), 7.07 (d, J = 8.12 Hz, 2H, 2×ArH), 7.15 (d, J = 8.12 Hz, 2H, 2×ArH); ^{13}C-NMR ($CDCl_3$, 100 MHz) δ: 172.72, 168.52, 154.86, 141.07, 136.43, 129.66, 129.66, 127.61, 127.61, 46.96, 45.71, 44.99, 30.08, 22.35, 22.35, 18.44; ESI-MS (M^++H), *m/z*: 289.3; Anal. Calcd. for $C_{16}H_{20}N_2O_3$: C 66.65, H 6.99, N 9.72; Found C 66.66, H 6.99, N 9.74.

3-(2-(4-Isobutylphenyl)propanoyl)-5,5-Dimethylimidazolidine-2,4-Dione (7d)

White solid, yield 85%; ^{1}H-NMR ($CDCl_3$, 400 MHz) δ: 0.85 (d, J = 6.6 Hz, 6H, 2×CH_3), 1.23 (d, J = 11.2 Hz, 6H, 2×CH_3), 1.48 (d, J = 6.96 Hz, 3H, CH_3), 1.80-1.82 (m, 1H, CH), 2.42 (d, J = 7.16 Hz, 2H, CH_2), 4.75 (q, J = 6.96 Hz, 1H, CH), 6.08 (s, 1H, NH), 7.06 (d, J = 8.0 Hz, 2H, 2×ArH), 7.11 (d, J = 8.0 Hz, 2H, 2×ArH); ^{13}C-NMR ($CDCl_3$, 100 MHz) δ: 174.39, 172.89, 152.60, 141.01, 136.75, 129.58, 129.58, 127.62, 127.62, 58.02, 47.17, 44.93, 30.1, 24.92, 24.92, 22.24, 22.24, 17.93; ESI-MS (M^++H), *m/z*: 317.4, Anal. Calcd. for $C_{18}H_{24}N_2O_3$: C 68.33, H 7.65, N 8.85; Found C 68.34, H 7.64, N 8.86.

CONCLUSION

In summary, a series of novel hydantion derivatives were synthesized and evaluated for their anti-inflammatory and antitussive activity. These SAR studies revealed that the substitution of methyl and hydrogen at the N_1 position of hydantoin had pronounced influence on antitussive activity. It's noteworthy to mention that compound **7a** showed two-fold stronger anti-inflammatory activity than Ibuprofen. Further extensive studies are required to be carried out to determine their mechanism of action.

CONFLICT OF INTEREST

Declared none.

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