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Studies on Benzoheterocyclic Derivatives. XVII.[1] Analgesic Activity of Dihydrobenzofuran Derivatives

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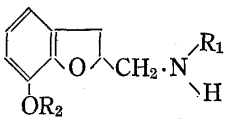
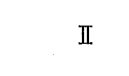
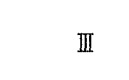
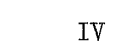
Analgesic activity of ten 2-(N-substituted amino)methyl-7-methoxy-2,3-dihydroben zofurans was examined by the acetic acid method and the Haffner tail pinching method. Some of them showed a potent analgesic activity. In order to investigate the true nature of their analgesic activity, the activity of morphine and of levallorphan combined with the test compounds were studied. None of the test compounds had any antagonistic activity against morphine and levallorphan. The analgesic activity of these compounds seems to be non-narcotic.

In our earlier articles[3,4] a series of 2-(N-substituted amino)methyl-2,3-dihydrobenzofuran derivatives were prepared and screened for their analgesic potency. It was found that 2-(alkylamino)methyl-7-methoxy analogs (VIII—X) were the most active of this series, but unfortunately they showed undesirable effects such as restlessness, tremor, vocalization, and reddening of eyelids, ears, tail, and limbs in mice.

In order to obtain compounds which possessed potent analgesic activity without undesirable activities, a series of 2-(N-substituted amino)methyl-7-methoxy analogs were synthesiz-

Table I. Chemical Structure of Test Compounds 2-(N-Substituted amino)methyl-7-methoxy-2,3-dihydrobenzofurans and related compounds



Compound No.	R_1	R_2	Salt	mol. wt.
I	$CH_2CH{=}CH_2$	CH_3	maleate	335.4
II		CH_3	maleate	349.4
III		CH_3	maleate	411.6
IV		CH_3	maleate	349.4
V	$CH_2CH_2C_6H_5$	CH_3	maleate	375.4
VI	$CH_2CH_2CH_2C_6H_5$	CH_3	hydrochloride	333.9
VII	$CH_2CH_2CH_2C_6H_5$	H	hydrobromide	364.4
VIII	H	CH_3	hydrochloride	215.7
IX	CH_3	CH_3	maleate	309.3
X	C_2H_5	CH_3	maleate	323.3
Pentazocine				285.4

1) Part XVI: N. Hirose, S. Kuriyama, and S. Toyoshima, *Chem. Pharm. Bull.* (Tokyo) **24**, 2661 (1976).
2) Location: *Koishikawa 4–6–10, Bunkyo-ku, Tokyo, 112 Japan.*
3) T. Ohgoh, N. Hirose, S. Sohda, and S. Toyoshima, *Yakugaku Zasshi*, **91**, 603 (1971).
4) T. Ohgoh, N. Hirose, N. Hashimoto, A. Kitahara, and K. Miyao, *Japan. J. Pharmacol.*, **21**, 119 (1971).

ed and screened for their analgesic activity.[1] From the result of the preliminary screening test, several potent compounds (I—VI) were picked out and screened intensively for their analgesic potency.

This paper describes further studies on their pharmacological activities, comparing their pharmacological profile with that of the parent compounds (VIII—X) or reference standard (Pentazocine).

Materials and Methods

Animals——Male mice (dd strain, weighing 17—23 g) and male rats (Wistar strain, weighing 150—300 g) were used.

Compounds——2-(N-Substituted amino)methyl-7-methoxy-2,3-dihydrobenzofurans (I—VI) were synthesized according to the methods reported previously,[1,3] and (VII), a demethylated form of VI, was newly prepared in this work. All the test compounds and the reference substance were dissolved in 0.9% NaCl solution for the test of analgesic activity or suspended in 5% acacia gum for the test of acute toxicity. In the experiments of hypothermic activity they were dissolved in pyrogen-free 0.9% NaCl solution.

Experimental

7-Hydroxy-2-(3-phenylpropylamino)methyl-2,3-dihydrobenzofuran (VII)——A mixture of 2.0 g of 7-methoxy-2-(3-phenylpropylamino)methyl-2,3-dihydrobenzofuran (VI) and 20 ml of 48% HBr was refluxed for 1 hr. The clear reaction mixture was concentrated to dryness under a reduced pressure, and the residual white solid was recrystallized from EtOH to give colorless needles (1.5 g, HBr salt of VII), mp 254° (decomp.). IR ν_{max}^{Nujol} 3290 cm^{-1} (OH). *Anal.* Calcd. for $C_{18}H_{21}O_2N \cdot HBr$: C, 59.32; H, 6.10. Found: C, 59.45; H, 6.16.

Analgesic Activity——a) Acetic Acid Method: A modification of the method of Costa[5] was employed. A group of 10 male mice was used for each dosage level. All the test compounds were administered orally to the group at a logarithmic series of doses, ranging from 25 to 100 mg/kg. Thirty minutes later, each mouse was given intraperitoneal injection of acetic acid (0.5%, 0.1 ml/10 g). The mice were placed in an observation cage and the number of stretching or writhing was countered for 10 min for each mouse. The compound was considered effective at a specific dose when stretching or writhing syndrome was not observed.

b) Haffner Tail-pinching Method: A modified method[6,7] of Haffner was employed. Mice were given the test compound orally or intraperitoneally at doses ranging from 200 to 12.5 mg/kg. Each dose group consisted of 10 mice. After 10, 20, 30, 45, and 60 min, the base of the tail of each mouse was pinched by an artery clip with 500 g pressure. Protection was defined as failure of a mouse to respond to the tail pinching.

Interaction with Other Analgesics——Combined experiments were made using 5 mice per group employing the acetic acid method. The analgesic activity of morphine (4 mg/kg) was compared with the effect produced by the simultaneous administration of morphine (4 mg/kg) and the test compound (I-VI and VIII) (5 mg/kg). For comparison the interaction of morphine (4 mg/kg) with levallorphan (5 mg/kg) or Pentazocine (5 mg/kg) was tested. Similar experiments were made to examine the effect of levallorphan (5 mg/kg) or pentazocine (5 mg/kg) on the analgesic activities of the test compounds (I–VI and VIII) (25 mg/kg).

Hypothermic Activity——A group of 3 male rats was used for the test. Before the experiment, normal rectal temperature of the rats was measured, and the rats showing the rectal temperature of 37.0—38.0° were selected and used in this experiment. The test compounds (100 mg/kg) were administered orally and the rectal temperature of the rats was measured by a thermister 40 mm inside the anus. The temperature was measured at 30-min intervals for 2 hr, then at 1-hr intervals thereafter.

Inhibition of Carrageenin-induced Edema——This experiment was carried out according to the method of Winter,[8] with a group of 3 male rats. The rats were given 0.1 ml of 0.1% solution of carrageenin(in 0.9% NaCl solution) subcutaneously at their hind paw. After 1 hr each rat was given the test compound (100 mg/kg) orally, and the volume of the hind paw was measured at 1-hr intervals for 5 hr.

Acute Toxicity and Behavioral Observation——A group of 10 male mice was used. The test compounds suspended in 5% acacia gum were injected intraperitoneally. The LD_{50} value was calculated from the lethal rate of the mice for 24 hr after the administration by the method of Litchfield and Wilcoxon.[9] Mice were observed for their behavioral changes.

5) P.J. Costa and D.D. Bonnycastle, *J. Pharmacol. Exptl. Therap.*, **113**, 310 (1955).
6) F. Haffner, *Deut. Med. Wochschr.*, **18**, 71 (1929).
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8) C.A. Winter, *Proc. Soc. Exptl. Biol. Med.* **111**, 544 (1962).
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Results

Analgesic Activity

a) Acetic Acid Method——As shown in Table II, seven 2-(N-substituted amino)methyl-7-methoxy-2,3-dihydrobenzofuran derivatives (I—VII) were tested for their analgesic potency by the oral administration, and their activities were compared with those of the parent compounds (VIII—X) and pentazocine. Compounds I and VI were the most potent of this series, though their activity was lower than that from the intraperitoneal administration described in the previous report.[1] The parent compounds (VIII—X) showed a marked potency, but analgesic activity of pentazocine was considerably weaker than that of VI. As VI showed the most potent analgesic activity, 7-hydroxy analog (VII), the demethylated form of VI, was prepared in expectation of a more potent activity but VII was considerably less potent than the parent compound (VI). The AD_{50} of I and VI was calculated[9] as shown in Table II.

Table II. Analgesic Activities of 2-(N-Substituted amino)-methyl-7-methoxy-2,3-dihydrobenzofurans and Related Compounds (Inhibition of acetic acid-induced writhing)

Compound No.	Inhibition of writhing (%) Dose (mg/kg, *p.o.*)			AD_{50}[a] (mg/kg, *p.o.*)	Confidence limits ($p=0.05$)
	100	50	25		
I	100	0	—	72	63— 82
II	60	20	—		
III	60	20	—		
IV	20	—	—		
V	0	—	—		
VI	100	60	20	70	63— 78
VII	0[b]	0	—		
VIII	100	20	—		
IX	100	80	70		
X	100	60	40		
Pentazocine	20	0	—	160	138—186

a) AD_{50} values were calculated by the Litchfield-Wilcoxon method.[8]
b) Inhibition of writhing by intraperitoneal administration showed 100% (50 mg/kg) and 60% (25 mg/kg).

Table III. Analgesic Activities of 2-(N-Substituted amino)-methyl-7-methoxy-2,3-dihydrobenzofurans (Haffner tail-pinching method)

Compound No.	Route of administration					
	p.o. (mg/kg)			*i.p.* (mg/kg)		
	100	100	50	50	25	12.5
I	—	100	0	—	80	40
II	60	20	—	20	0	—
III	20	0	—	0	0	—
IV	20	20	—	60	40	—
V	20	0	—	0	—	—
VI	40	40	—	80	20	—
VIII	—	100	80	—	60	60
IX	—	100	80	—	100	60
X	—	100	20	—	100	40
Pentazocine	0	0	—	20	—	—

b) **Haffner Tail-pinching Method**——In this method, the parent compounds (VIII—X) produced a significant activity. Most of the test compounds (II—VI) showed only a weak activity but I was approximately equipotent to the parent compounds (VIII—X). The results obtained are shown in Table III.

Interaction with Other Analgesics

Effect of the test compounds on analgesic potency of morphine was examined. As shown in Table IV, the potency of morphine was antagonized by the simultaneous administration of levallorphan but not by any of the test compounds. Therefore, they do not seem to have any antagonistic activity against morphine. Examination of the effect of levallorphan on the analgesic activity of the test compounds indicated, as shown in Table V, that levallorphan had no effect on analgesic potency of the test compounds. These results suggest that analgesic properties of these dihydrobenzofuran derivatives may differ from those of morphine-like analgesic drugs.

Table IV. Analgesic Activities of Morphine combined with I, II, VI, VIII, and Levallorphan (Inhibition of acetic acid-induced writhing)

	Compound (Simultaneous use)	Inhibition of acetic acidind-uced writhing (%)
Morphine (4 mg/kg, *i.p.*) plus	None	90
	I (5 mg/kg, *i.p.*)	100
	II (5 mg/kg, *i.p.*)	80
	VI (5 mg/kg, *i.p.*)	100
	VIII (5 mg/kg, *i.p.*)	100
	Levallorphan (5 mg/kg, *s.c*)	20

Table V. Analgesic Activities of Some Dihydrobenzofuran Derivatives combined with Levallorphan (Inhibition of acetic acid-induced writhing)

	Compound (Simultaneous use)	Inhibition of acetic acid-induced writhing (%)
Levallorphan (5 mg/kg, *s.c.*) plus	none	0
	I (25 mg/kg, *i.p.*)	100
	II (25 mg/kg, *i.p.*)	100
	IV (25 mg/kg, *i.p.*)	100
	VI (25 mg/kg, *i.p.*)	80
	VIII (25 mg/kg, *i.p.*)	100
	IX (25 mg/kg, *i.p.*)	100
	X (25 mg/kg, *i.p.*)	100

Hypothermic Activity

Compounds I and VI showed a significant hypothermic activity, but VII did not. The maximum effect of I appeared at 0.5—1.0 hr after its administration and the degree of the restoration of the rectal temperature was relatively fast. On the other hand, the maximum fall of rectal temperature was produced by VI 1 hr after the administration, and the temperature recovered very slowly. At an oral dose of 100 mg/kg, this compound kept hypothermic effect of 1° at 5 hr after dosing. Hypothermic activity of I, VI, and VII in rats is shown in Fig. 1.

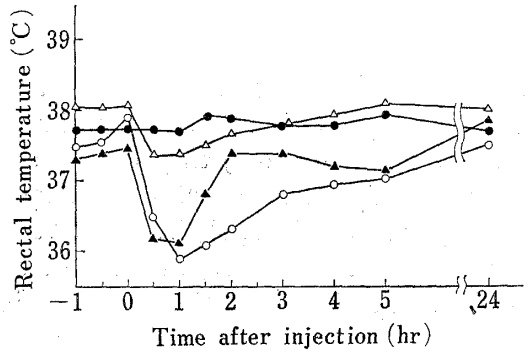

Fig. 1. Hypothermic Effects of I, VI, and VII in Rats

●—●: saline
▲—▲: I 100 mg/kg, *p.o.*
○—○: VI 100 mg/kg, *p.o.*
△—△: VII 100 mg/kg, *p.o.*

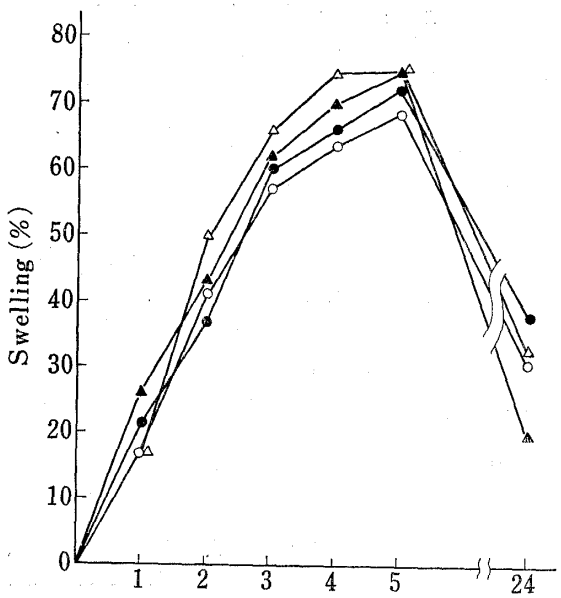

Fig. 2. Effect of I, VI, and VII on Edema of hind paw in rats induced by carrageenin

●—●: saline
▲—▲: I 100 mg/kg, *p.o.*
○—○: VI 100 mg/kg, *p.o.*
△—△: VII 100 mg/kg, *p.o.*

Inhibition of Carrageenin-induced Edema

Three compounds (I, VI, and VII) were screened for their inhibitory activity on the carrageenin-induced rat paw edema by an oral administration of 100 mg/kg but they showed no anti-inflammatory activity in this test. The results of this test are shown in Fig. 2.

Acute Toxicity and Behavioral Observation

Compound I and the parent compounds (VIII—X) produced the signs of violent restlessness, tremor, and reddening of eyelids, ears, tail and limbs in mice generally at a sublethal dose. On the other hand, most of the test compounds (II—VI) did not show any behavioral changes observed in I and in VIII to X, but produced mild sedation and ataxia in a sublethal

Table VI. Acute Toxicity in Mice

Compd. No.	Mortality[a] (%) (200 mg/kg, *i.p.*)	LD_{50} (mg/kg, *i.p.*)	Confidence limits ($p=0.05$)	Signs observed
I	60	130	118—143	restlessness, vocalization, tremor, and reddening of eyelids, ears, tail, and limbs
II	100			mild sedation and ataxia
III	100			
IV	0			
V	20			
VI	60	91	76—108	mild sedation, loss of righting reflex, ataxia, and Straub tail
VIII	20			restlessness, vocalization, tremor, and reddening of eyelids, ears, tail, and limbs
IX	0			
X	80			
Pentazocine	100	85	71—102	loss of righting reflex, ataxia, and Straub tail

a) Mortality at 24 hr after intraperitoneal administration.

dose. In particular, VI produced signs of loss of righting reflex, ataxia, and Straub tail in sublethal dose, and pentazocine produced similar behavioral changes as observed with VI. Mortality of mice from these compounds at 24 hr after their intraperitoneal administration (200 mg/kg) and LD_{50} values of I and VI are given in Table VI.

Discussion

As described in the previous report[1] I and VI showed 40% and 70% inhibition against acetic acid-induced writhing by an intraperitoneal administration of 12.5 mg/kg. In the present work, acetic acid method and Haffner method by an oral administration were carried out to investigate their analgesic activity in detail. Acetic acid test showed that their analgesic potency by an oral administration was lower than the intraperitoneal administration, but a significant potency was still observed in I and VI. As is well known for the relationship between morphine and codeine, replacement of a methoxyl group with hydroxyl group brings a marked increase in the analgesic activity. In order to investigate a similar relation in this series, the activity of VII, a demethylated analog of VI, was compared with that of VI. Contrary to our expectation, demethylation markedly decreased the potency, and only an extremely weak activity was observed in VII. Compared with the parent compounds (VIII—X), VI had a lower analgesic potency but had no untoward actions (restlessness, reddening, *etc.*) which were observed in the parent compounds. This fact seems to indicate that our objective of trying to find a compound possessing a potent analgesic activity without any untoward activities was achieved. On the other hand, it was observed that I produced some degree of restlessness and reddening of eyelids, ears, *etc.* in mice at a toxic dose, like the parent compounds. The AD_{50} value of I was 72 mg/kg, and that of VI was 70 mg/kg by an oral administration.

The analgesic effect of morphine was compared with the effect of the simultaneous administration of morphine and the test compounds or levallorphan, and it was found that the potency of morphine was antagonized by levallorphan, but not by the test compounds (I, II, VI, and VIII). Levallorphan had no effect on the analgesic activity of the test compounds, that is, their analgesic activity was not antagonized by levallorphan. These results indicate that all the test compounds have no morphine antagonistic activity and no morphine-like analgesic activity. Among the test compounds, I and VI showed a significant hypothermic action. There is an apparent difference between the time course of hypothermia of I and that of VI; the former is short acting and the latter is long acting, while VII showed no hypothermic activity. It appears that demethylation deprives VI of almost all its biological activities.

The LD_{50} value of I and VI were 130 and 91 mg/kg (*i.p.*) respectively in the mouse, and these values mean them equitoxic with or less than that of pentazocine (85 mg/kg). As regards the gross behavioral changes caused by intraperitoneal administration of 200 mg/kg of the test compounds in mice, I and the parent compounds (VIII—X) exhibited vocalization, restlessness (suggestive of CNS stimulation), and reddening of eyelids, ears, tail, and limbs (suggestive of peripheral vasodilation). However, II to VI did not show such behavioral changes but rather exhibited mild sedation and ataxia. The analgesic activity of VI will be discussed from different angles in detail in later papers.