POLYCYCLIC N-HETERO COMPOUNDS. XXXI. SYNTHESIS AND ANTI-PLATELET AGGREGATION ACTIVITY OF 4-SUBSTITUTED 5,6-DIHYDROBENZO[h]QUINAZOLINES

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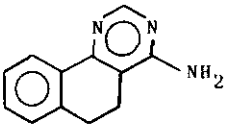
Abstract - 4-Substituted 5,6-dihydrobenzo[h]quinazolines (IV) were synthesized by the reaction of 4-chloro-5,6-dihydrobenzo[h]-quinazoline (II) with amines (III) and their inhibitory activity against platelet aggregation was investigated.

4-Amino-5,6-dihydrobenzo[h]quinazoline (I) was originally reported by us as a precursor of 11,13,15-triazasteroid.[1] Its pharmacological activity was not investigated except for an antireserpine activity in mice.[2] In the course of the random screenig of I for a research on other biological activities, it was found that this compound had an inhibitory activity against collagen-induced platelet aggregation and the potency of its activity was stronger than that of aspirin which was familiar as an anti-platelet agent. Platelet is one of the constituent of blood and a protective cell for bleeding of the organism, and it also acts an important role in the formation of hemostasis and thrombosis, so that, the anti-platelet therapy recently became main one of the some antithrombotic therapies.

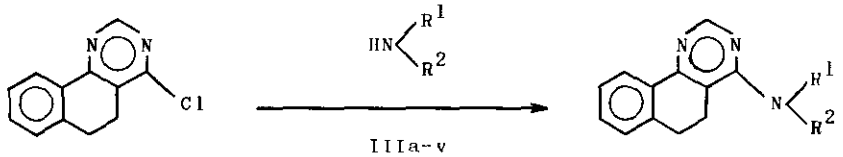


I

Furthermore, concerning to the inhibitory activity against platelet aggregation, there are no reports of compounds having a benzo[h]quinazoline skeleton. These facts suggested us that the investigation on I and its analogue linked to a development of new inhibitory agent against platelet aggregation. Therefore,

we were interested in carring out the chemical modification of I in the hope that a more effective inhibitory agent could be found. This paper deals with the synthesis of some 4-substituted 5,6-dihydrobenzo[h]quinazolines and evaluation of them on inhibitory activity against platelet aggregation.

As shown in Scheme 1, alkylamines (IIIb-q) were allowed to react with 4-chloro-5,6-dihydrobenzo[h]quinazoline[3] (II) to obtain 4-alkylamino-5,6-dihydrobenzo-[h]quinazolines (IVb-q). Syntheses of several compounds of 4-alkylamino derivatives were already reported (IVb,e-n,r,s).[2-5] 4-Hydrazino derivative (IVa) was prepared by the reaction of II with hydrazine hydrate (IIIa) in methyl cellosolve at room temperature. N-(5,6-Dihydro-4-benzo[h]quinazolinyl)-2-methyl-



II IV

IVa: R^1 = H, R^2 = NH_2
b: R^1 = H, R^2 = Me
c: R^1 = R^2 = Me
d: R^1 = H, R^2 = Et
e: R^1 = H, R^2 = $(CH_2)_2OMe$
f: R^1 = H, R^2 = $(CH_2)_2NEt_2$
g: R^1 = H, R^2 = $(CH_2)_2$N O
h: R^1 = H, R^2 = $CH_2CH(OH)Me$
i: R^1 = H, R^2 = $(CH_2)_3OH$
j: R^1 = H, R^2 = $(CH_2)_3OEt$
k: R^1 = H, R^2 = $CH_2\overset{\overset{Me}{|}}{C}CH_2OH$ ($\overset{|}{Me}$)
l: R^1 = H, R^2 = $CH_2CH(OH)CH_2OH$
m: R^1 = H, R^2 = $(CH_2)_3NMe_2$
n: R^1 = H, R^2 = $(CH_2)_3$N O
o: $R^1 \sim R^2$ = 
p: $R^1 \sim R^2$ = 
q: $R^1 \sim R^2$ = 
r: R^1 = H, R^2 = $(CH_2)_2COOH$
s: R^1 = H, R^2 = $CH{=}C(CO_2Et)_2$
t: R^1 = H, R^2 = $CH_2CH(Me)COOH$
u: R^1 = H, R^2 = $(CH_2)_3COOH$
v: $R^1 \sim R^2$ = 

Scheme 1

β-alanine (IVt) was prepared by the reaction of II with 2-methyl-β-alanine (IIIt) in the presence of potassium carbonate. N-(5,6-Dihydro-4-benzo[h]-quinazolinyl)-γ-aminobutyric acid (IVu) was also prepared by the similar reaction of II with γ-aminobutyric acid (IIIu). 4-(2-Oxo-1-pyrrolidinyl)-5,6-dihydrobenzo[h]quinazoline (IVv) was obtained by the cyclization of IVu with acetic anhydride and pyridine. *Reaction conditions, appearances, melting points,* and yields of alkylamino derivatives (IVc,d,o,p,q) are listed in Table I and instrumental analyses of the products which were newly synthesized in this paper are listed in Tables II-III.

The inhibitory activity against rabbit platelet aggregation of the above compounds (I and IV) was screened by a turbidimetric method developed by Born *et al.*[6] using an aggregometer. The maximum aggregation rate (MAR) was calculated from an aggregation response curve obtained by equation 1, and then inhibition rate of the test compound at each concentration was calculated by equation 2.

Equation 1:

$$\text{MAR} = \frac{\text{X - optical transmission of PRP}}{\text{optical transmission of PPP - optical transmission of PRP}} \times 100$$

where X was maximum optical transmission on the aggregation response curve.

Equation 2:

$$\text{Inhibition rate} = \left(1 - \frac{\text{MAR of test compound-treated PRP}}{\text{MAR of vehicle-treated PRP}}\right) \times 100$$

The inhibitory activity against rabbit platelet aggregation of aspirin was also screened as a positive control. Only when the inhibition rate of a test compound (final concentration was 50 μmol/l and this is as same as that of aspirin) was significantly different from that of aspirin at $p<0.05$ on statistical analysis using Student's *t*-test, the amounts of the test compound which was required to produce a 50% inhibition (IC_{50}) of rabbit platelet aggregation induced by collagen was calculated by a probit method. Inhibition rates and IC_{50} values of the test compounds and aspirin are shown in Table IV.

Many compounds produced a potent and dose-dependent inhibition against rabbit platelet aggregation induced by collagen. Their structure-activity relationships may be summerized as follows. First, comparison of their potency showed IVd > IVc, IVm, IVb > I, IVn, IVa, IVe, IVh > IVu, IVj, IVq > IVp > IVk, IVi > IVl> IVs, IVv, IVr, IVg, IVt, IVf, IVo. There is no different between 4-amino derivative (I) and 4-hydrazino derivative (IVa) on an inhibitiory activity and

Table I Reaction Conditions, Appearances, Melting Points, and Yields of IVc,d,o,p,q

Compd	React. condition		Appearance	Mp (°C)	Yield (%)
	Temp. (°C)	Time (h)	(Recryst. solv.)		
IVc	20	2.5	colorless needles (benzene-n-hexane)	70 - 71	78
IVd	20	10	colorless needles (ethanol)	184 - 186	94
IVo	50	0.5	colorless plates (ethanol-water)	104.5 - 105	68
IVp	70	0.5	colorless prisms (n-hexane)	127 - 129	79
IVq	70	1	colorless plates (methanol-water)	117 - 118	86

Table II Elemental Analyses and Ms and Ir Spectral Data of Products

Compd	Formula	Analysis (%); Calcd (Found)			Ms (m/z)	Ir[a] (cm^{-1})
		C	H	N		
IVa	$C_{12}H_{12}N_4$	67.90	5.70	26.40	212 (M^+)[b]	3330, 3290,
		(67.92	5.67	26.28)		3200
IVc	$C_{14}H_{16}ClN_3$[c]	64.25	6.12	16.06	225 (M^+)[b]	——
		(64.01	6.16	16.05)		
IVd	$C_{14}H_{15}N_3$	74.63	6.71	18.65	225 (M^+)[b]	3250
		(74.48	6.77	18.57)		
IVo	$C_{16}H_{17}N_3$	76.46	6.81	16.72	252 (M + H)[d]	——
		(76.20	6.83	16.51)		
IVp	$C_{17}H_{20}N_4$	72.82	7.19	19.99	281 (M + H)[d]	——
		(73.05	7.30	19.84)		
IVq	$C_{16}H_{17}N_3O$	71.88	6.41	15.72	268 (M + H)[d]	——
		(71.61	6.44	15.48)		
IVt	$C_{16}H_{17}N_3O_2$	67.82	6.05	14.83	265	3350, 1710
		(67.70	6.12	14.71)	($M^+ - H_2O$)[b]	
IVu	$C_{16}H_{17}N_3O_2$	67.82	6.05	14.83	283 (M^+)[b]	3350, 2950
		(67.91	6.07	14.79)		1610
IVv	$C_{16}H_{15}N_3O$	72.43	5.70	15.84	265 (M^+)[b]	1700
		(72.40	5.68	15.75)		

a) Absorption bands due to N-H, O-H, and/or C=O; measured in KBr disks.
b) EI ms. c) As monohydrochloride. d) FAB ms.

this result suggested that hydrazino group plays a similar role to amino group in an inhibitory action against aggregation of platelet. The compounds bearing dimethylamino group (IVc,m) showed higher potency, whether that group directly attached to the 4-position of the nucleus or not. Introduction of methyl group

Table III Nmr Spectral Data of Products

Compd[a]	Chemical Shifts, δ (J in Hz)				
	Side Chain Protons	Ring Protons			
		2-H[b]	5,6-H	7,8,9-H[c]	10-H[c]
IVa	3.60 and 6.12(2H and 1H, each br, D_2O exchangeable, NH_2 and NH),	8.70	2.60[d] 2.99[d]	7.33	8.28
IVc[e]	3.02(6H, s, 2 x NCH_3),	8.68	2.86(br s)	7.30	8.20
IVd	1.30(3H, t, J = 7, CH_3), 3.58 (2H, m, NCH_2), 6.80(1H, br, exchangeable with D_2O, NH)	8.63	2.60[f] 2.93[f]	7.28	8.27
IVo	1.94(4H, m, $NCH_2C\underline{H}_2C\underline{H}_2$), 3.68 (4H, t, J = 6.5, 2 x NCH_2)	8.65	2.93(m)	7.36	8.26
IVp	2.36(3H, s, CH_3), 2.55(4H, t, J = 5, 2 x $C\underline{H}_2NCH_3$), 3.43(4H, t, J = 5, 2 x $NC\underline{H}_2CH_2NCH_3$)	8.78	2.83(br s)	7.35	8.24
IVq	3.40(4H, t, J = 5, 2 x NCH_2), 3.87(4H, t, J = 5, 2 x OCH_2)	8.82	2.85(br s)	7.37	8.26
IVt	1.10(3H, d, J = 7, CH_3), 2.78 (2H, m, overlapped on 5,6-H, CH_2), 3.60(1H, m, $C\underline{H}CH_3$), 7.00 (1H, br, exchangeable with D_2O, NH)	8.45	2.78(m)	7.30	8.13
IVu	1.85(2H, m, $CH_2C\underline{H}_2CH_2$), 2.31 (2H, t, J = 7, CH_2CO), 3.45(2H, m, changed to triplet after addition of D_2O, J = 7, NCH_2), 6.96(1H, br, exchangeable with D_2O, NH), 12.00(1H, br, exchangeable with D_2O, OH)	8.43	2.80(m)	7.31	8.14
IVv	2.30(2H, m, $CH_2C\underline{H}_2CH_2$), 2.60 (2H, t, J = 7, CH_2CO), 4.10(2H, t, J = 7, NCH_2)	8.93	3.86(br s)	7.33	8.30

a) Measured in $CDCl_3$ except for IVt and IVu (DMSO-d_6). b) All signals were singlet. c) All signals were multiplet. d) Each 2H, each t, J = 8 Hz. e) Measured as free base. f) Each 2H, m.

Table IV Maximum Inhibition Rate and IC_{50} on Platelet Aggregation Induced by Collagen

Compd	Max. inhibit. rate[a]	IC_{50}[b]	Compd	Max. inhibit. rate[a]	IC_{50}[b]
I	81.5 ± 3.6**	24.2 (17.3 - 39.4)	IVl	36.0 ± 1.8*	114.0 (83.9 - 170.6)
IVa	80.0 ± 3.3**	25.0 (18.4 - 38.6)	IVm	80.6 ± 4.6**	19.6 (15.4 - 26.1)
IVb	81.4 ± 5.7**	21.2 (14.6 - 36.6)	IVn	70.8 ± 1.3**	24.3 (17.1 - 40.8)
IVc	90.8 ± 1.5**	19.3 (14.6 - 25.8)	IVo	16.1 ± 1.1	——
IVd	94.1 ± 1.4**	15.8 (11.0 - 22.6)	IVp	39.8 ± 3.0**	83.5 (62.7 - 122.1)
IVe	77.7 ± 2.5**	25.2 (19.9 - 33.9)	IVq	58.1 ± 4.1**	49.7 (27.6 - 206.5)
IVf	19.0 ± 7.3	——	IVr	28.9 ± 0.2	——
IVg	28.7 ± 1.6	——	IVs	33.1 ± 7.1	——
IVh	77.5 ± 6.9**	26.9 (21.1 - 37.0)	IVt	25.3 ± 0.3	——
IVi	38.6 ± 1.8**	95.5 (61.0 - 194.3)	IVu	57.2 ± 7.8**	41.9 (31.7 - 61.9)
IVj	54.5 ± 3.6**	45.5 (37.6 - 57.6)	IVv	30.9 ± 2.8	——
IVk	36.5 ± 4.3*	93.6 (67.9 - 145.0)	aspirin	23.9 ± 1.7	142.7 (115.5 - 199.5)

a) Value is expressed as % and the mean ± S.E. of at least 3 experiments at final concentration of 50 μmol/l. Significantly different from aspirin at $p<0.05$ (*) and $p<0.01$ (**). b) Figures in upper lines and lower lines for each compound represent the IC_{50} values (μmol/l) and 95% confidence limits (μmol/l - μmol/l), respectively. Experiments were repeated at least each 3 times at final concentrations of 5, 10, and 50 μmol/l (in the case of aspirin, final concentrations were 50, 250, and 500 μmol/l).

(IVb) and ethyl group (IVd) to the amino group on the 4-position of the nucleus resulted in increasing the activity. Especially, compound IVd had the highest potency among all derivatives listed in Table IV. A comparison of the inhibitory activity of compound IVd with that of aspirin in terms of IC_{50} indicates that compound IVd was about 10 times more potent than aspirin. On the other hand, further introduction of methoxy group (IVe), diethylamino group (IVf), or

morpholino group (IVg) to the terminal carbon in the side chain of compound IVd resulted in reducing the activity. In particular, the introduction of dialkylamino group such as compounds IVf,g greatly reduced the potency, but, that of dialkylaminomethyl group to the same position such as compounds IVm,n did a little. The compounds bearing N-cyclic group directly at the 4-position of the nucleus were less active, especially, compounds IVo,v which contained pyrrolidine ring were much less active. The compounds bearing carboxyl group or ester group at the terminal position in the side chain were less active, except for compound IVu. This compound, IVu, showed higher potency than aspirin and contained three carbon atoms between nitrogen atom and carboxyl group in the side chain, while other compounds (IVr,s,t) contained two carbon atoms between them.

EXPERIMENTAL

Mps were determined on a Yanagimoto micro melting point apparatus and are uncorrected. Elemental analyses were performed on a Yanagimoto MT-2 CHN Corder elemental analyzer. The ir spectra were obtained with a Japan Spectroscopic A-102 diffraction grating infrared spectrophotometer. The nmr spectra were measured on a Hitachi R-22FTS FT-NMR spectrometer (90 MHz). The chemical shifts (δ) in ppm are measured relative to tetramethylsilane as an internal standard, and the signals are designated as follows: s, singlet; d, doublet; t, triplet; m, multiplet; br, broad. The EI ms spectra were taken with a Shimadzu LKB-9000 instrument at 70 eV, and FAB ms spectra were done with a VG 11-250 instrument at 70 eV. Amines used as reagents are commercially available, and DL-amino acids were used in these experiments.

General Procedure for the Preparation of 4-Alkylamino-5,6-dihydrobenzo[h]-quinazolines (IVb-q)

A mixture of 1 mmol of 4-chloro-5,6-dihydrobenzo[h]quinazoline (II) and 5 - 8 mmol of a suitable alkylamine (III) was stirred at 20 - 100 °C until the starting material disappeared (checked by a thin layer chromatography). After evaporation of excess amine, ca. 50 ml of water was added and precipitated crystalline solid was collected by suction and recrystallized from an appropriate solvent. When the residue did not solidify on addition of water, it was extracted with chloroform. The organic layer was washed with water, dried over anhydrous sodium sulfate, and evaporated to dryness. The resulting residue was

recrystallized from an appropriate solvent. When the residue thus obtained could not be recrystallized, the oily residue was crystallized as hydrochloride.

Preparation of 4-Hydrazino-5,6-dihydrobenzo[h]quinazoline (IVa)

To a solution of 7.55 g (34.9 mmol) of II in 50 ml of methyl cellosolve was added 17 ml (350 mmol) of hydrazine hydrate and the resulting mixture was stirred under N_2 gas stream for 1 h at room temperature. After concentration of the reaction mixture, the precipitated crystalline solid was collected by suction, washed with water, and recrystallized from benzene to give 7.04 g (96%) of the titled product as colorless needles, mp 173 - 175 °C.

Preparation of N-(5,6-Dihydro-4-benzo[h]quinazolinyl)-2-methyl-β-alanine (IVt)

A mixture of 216 mg (1.0 mmol) of II, 242 mg (2.3 mmol) of 2-methyl-β-alanine, 221 mg of potassium carbonate, 15 ml of dioxane, and 15 ml of water was refluxed for 2 days. After evaporation of the solvent, a small amount of water was added to dissolve the residue and the resulting solution was acidified with acetic acid. The precipitated crystalline solid was collected by suction and was recrystallized from diluted ethanol to give 118 mg (42%) of the titled product as colorless needles, mp 213 - 215 °C.

Preparation of N-(5,6-Dihydro-4-benzo[h]quinazolinyl)-γ-aminobutyric Acid (IVu)

To a solution of 648 mg (3 mmol) of II in 10 ml of dioxane was added a solution of 618 mg (6 mmol) of γ-aminobutyric acid and 663 mg (4.8 mmol) of potassium carbonate in 10 ml of water and the resulting solution was refluxed for 24 h. After cooling of the reaction mixture, the solvent was evaporated and the residue was dissolved in a small amount of water. The solution was acidified with acetic acid. Precipitated crystalline solid was collected by suction and recrystallized from ethanol to give 493 mg (58%) of the titled product as colorless needles, mp 216 - 218 °C.

Preparation of 4-(2-Oxo-1-pyrrolidinyl)-5,6-dihydrobenzo[h]quinazoline (IVv)

To a solution of 400 mg (1.4 mmol) of IVu in 0.4 ml of dry pyridine was added 0.42 ml (4.4 mmol) of acetic anhydride, and the resulting solution was allowed to stand for 5 days at room temperature. After evaporation of the solvent, a small amount of xylene was added and the mixture was furthermore evaporated to dryness. Resulting crystalline residue was recrystallized from ethanol-cyclohexane to give 268 mg (72%) of the titled product as colorless fine needles, mp 129 - 131 °C.

Preparation of Platelet

Blood was collected from male albino rabbit with 0.1 volume of 3.8% sodium citrate as the anticoagulant. After mixing, platelet rich plasma was obtained by removing erythrocytes and leukocytes by slow centrifugation (160 x g, 10 min). Platelet poor plasma was prepared by further centrifugation at 2000 x g for 10 min. The platelet rich plasma and platelet poor plasma thus obtained were used for the aggregation.

Measurement of Platelet Aggregation

Platelet aggregation was measured by continuous recording of light transmission through platelet rich plasma using a aggregometer (Aggrecoder II PA-3220, Kyoto Daiichi Kagaku Co. Ltd., Kyoto) at 37 °C. Twenty five microliter of 10% DMSO-1 M tris-HCl buffer (pH 7.4) solution containing inhibitory agents (aspirin) or test compound (final concentration, 50 μmol/l) were added to 325 μl aliquots of platelet rich plasma in siliconized glass cuvettes. After incubation for 2 min, collagen was added as a aggregating agent (final concentration, 20 μg/ml). Continuous magnetic stirring was used to ensure adequate mixing and to prevent platelet sedimentation.

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