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Synthesis and Biological Activity of Pyridazinooxazines

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4-Chloro-5-(2-hydroxyethylamino)-3(2*H*)-pyridazinones were converted upon treatment with base to novel fused ring compounds, 3,4-dihydro-2*H*-pyridazino[4,5-*b*]-1,4-oxazin-8(7*H*)-ones. When a nitrogen atom in the hydroxyethylamino group or at the 2-position of the 3(2*H*)-pyridazinone ring had a remaining hydrogen, the ring closure reaction did not occur. 3,4-Dihydro-2*H*-pyridazino[4,5-*b*]-1,4-oxazine was similarly synthesized from corresponding precursor. The presence of a C-6 amino group in the precursor did not affect the ring formation mentioned above, but when the amino group was diazotized, ring formation took place in a different fashion, involving the C-6 diazonium moiety as a leaving group, to give another ring system, 6,7-dihydro-2*H*-pyridazino[3,4-*b*]-[1,4]oxazin-3(5*H*)-one. Similar phenomena were observed in the cases of precursors having a C-6 nitro group: cyclization occurred involving the elimination of the nitro group to give fused ring products. The method was applied to the formation of a tricyclic heterocycle, 2*H*-pyridazino[3,4-*b*][1,4]benzoxazin-3(5*H*)-one.

Some compounds thus obtained were found to have potent analgesic and significant anti-inflammatory activities in animal models.

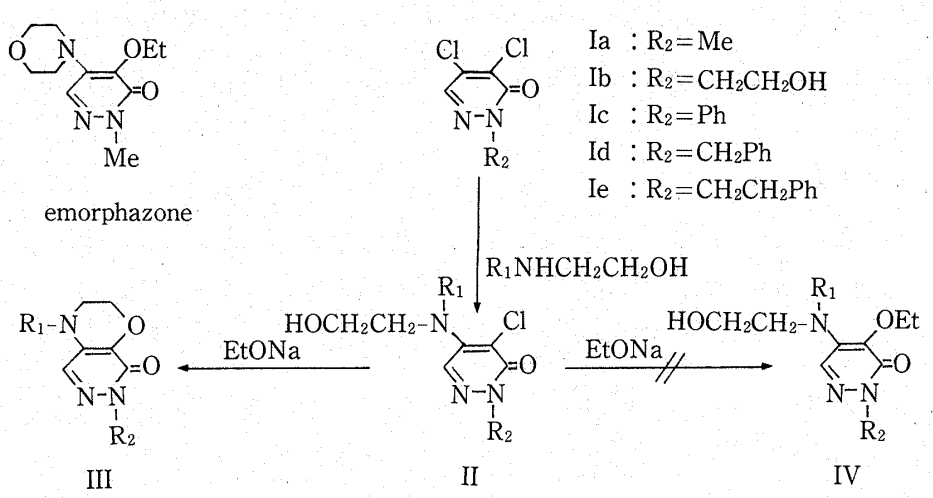
Keywords——3,4-dihydro-2*H*-pyridazino[4,5-*b*]-1,4-oxazin-8(7*H*)-one; 3,4-dihydro-2*H*-pyridazino[4,5-*b*]-1,4-oxazin-5(6*H*)-one; 3,4-dihydro-2*H*-pyridazino[4,5-*b*]-1,4-oxazine; 6,7-dihydro-2*H*-pyridazino[3,4-*b*][1,4]oxazin-3(5*H*)-one; 6,7-dihydro-2*H*-pyridazino[3,4-*b*][1,4]benzoxazin-3(5*H*)-one; intramolecular cyclization; nucleophilic substitution; analgesic activity; anti-inflammatory activity

During a synthetic study on urinary metabolites of emorphazone, a new non-steroidal analgesic anti-inflammatory drug,[1] Takaya *et al.*[2] found that the reaction of 4-chloro-5-(2-hydroxyethylamino)-2-methyl-3(2*H*)-pyridazinone (IIa or b) with sodium ethoxide did not give the desired product with a 4-ethoxy group (IV). Instead, a novel fused heterocycle, 3,4-dihydro-2*H*-pyridazino[4,5-*b*]-1,4-oxazin-8(7*H*)-one (IIIa or b), was obtained as a main product. This fused ring system was previously unknown, although one similar heterocycle, pyridazino[4,5-*d*][1,3]oxazin-4-one, has been reported.[3] In the present investigations, various pyridazino-1,4-oxazines were synthesized and their analgesic and anti-inflammatory activities were evaluated.

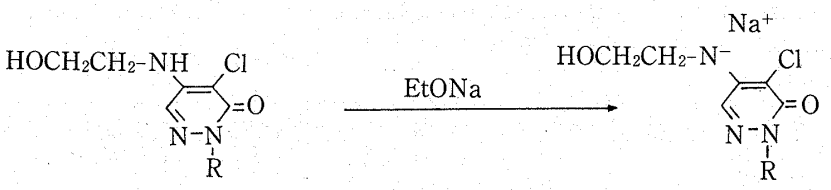
The synthetic route to III is shown in Chart 1. When two substituents, R_1 and R_2, in compounds II were variously changed, the corresponding fused products III were successfully obtained. The key intermediates II were prepared by the substitution reaction of 4,5-dichloro-3(2*H*)-pyridazinones (I) with *N*-substituted ethanolamines. The products thus obtained are summarized in Table I together with their yields and other data. These structures were elucidated on the basis of infrared (IR), nuclear magnetic resonance (NMR), and mass spectral data. In the NMR spectra, the chemical shifts of methylene protons on the 2- and 3-positions were δ 4.21—4.36 and 3.31—3.49, respectively, with coupling constants of 5—6 Hz. The IR spectra showed absorptions attributable to the carbonyl groups in the six-membered lactams at 1607—1640 cm^{-1}, and the mass spectra exhibited clear molecular ion peaks.

Compound V, with one proton on the nitrogen atom in the hydroxyethylamino group, did not give corresponding fused ring compounds. This result may be explained by the increased electron density at the 4-position caused by the stronger electron donating activity of the negatively charged nitrogen atom in the sodium salt of V (V′). Compound

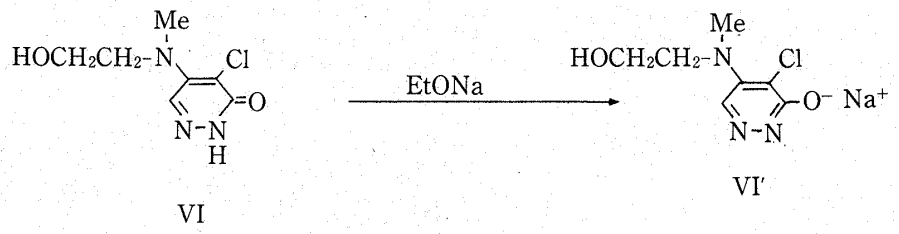
VI having no substituent at the 2-position of the 3 (2*H*)-pyridazinone ring also did not give the corresponding ring closure compound. Compound VI may be present as the enol salt (VI′) in the presence of sodium ethoxide, and the electron donating property of the anionic oxygen atom in VI′ might decrease the reactivity of the 4-position to nucleophiles. This may be the reason why VI did not cyclize.



	R_1	R_2		R_1	R_2
a	CH_2Ph	Me	f	Me	CH_2Ph
b	CH_2CH_2OH	Me	g	Me	CH_2CH_2Ph
c	Me	Me	h	Et	Me
d	Me	CH_2CH_2OH	i	CH_2CH_2OH	Ph
e	Me	Ph	j	CH_2Ph	Ph



Va : R=Me
Vb : R=Ph
Vc : R=CH_2Ph



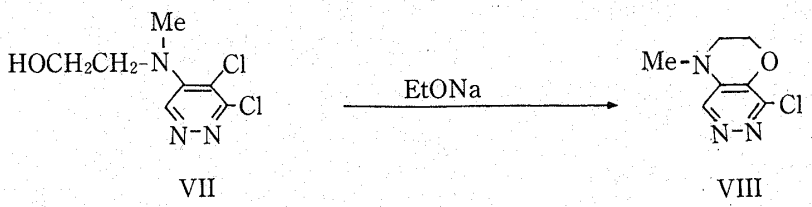


Chart 1

3,4-Dichloro-5-[(2-hydroxyethyl)methylamino]-pyridazine (VII), which did not have a lactam structure, gave colorless fine needles, mp 188.5—190°C, by the same procedure as in the case of the formation of III from II. The experimental formula, $C_7H_8ClN_3O$, of the product was obtained from the mass spectrum and elemental analysis, and the NMR and IR spectra suggested loss of the hydroxyl group. From these data, this product was identified as 8-chloro-3,4-dihydro-4-methyl-2*H*-pyridazino[4,5-*b*]-1,4-oxazine (VIII).

Table I. Yields and Physicochemical Data for 3,4-Dihydro-2*H*-pyridazino-[4,5-*b*]-1,4-oxazin-8(7*H*)-ones (III)

Compd. No.	Yields (%)	mp (°C)	Formula	Analysis (%) Calcd (Found) C	H	N
IIIc	81	108—109 (EtOH–IPE[a])	$C_8H_{11}N_3O_2$	53.03 (52.97	6.12 6.18	23.19 23.13)
IIId	75	144—146 (MeOH–AcOEt)	$C_9H_{13}N_3O_3$	51.17 (51.18	6.20 6.18	19.90 19.75)
IIIe	69	194—196 (EtOH)	$C_{13}H_{13}N_3O_2$	64.18 (63.89	5.39 5.47	17.28 17.06)
IIIf	88	132—133 (EtOH–IPE)	$C_{14}H_{15}N_3O_2$	65.35 (65.39	5.88 5.82	16.33 16.33)
IIIg	81	97—98 (CCl_4)	$C_{15}H_{17}N_3O_2$	66.40 (66.18	6.32 6.10	15.49 15.21)
IIIh	90	99—101 (EtOH–IPE)	$C_9H_{13}N_3O_2$	55.37 (55.32	6.71 6.71	21.53 21.38)
IIIi	67	179—180 (EtOH–IPE)	$C_{14}H_{15}N_3O_3$	61.53 (61.48	5.53 5.59	15.38 15.13)
IIIj	63	145—146 (EtOH–IPE)	$C_{19}H_{17}N_3O_2$	71.45 (71.51	5.37 5.22	13.16 13.11)

Compd. No.	MS *m/e* (M^+)	IR cm^{-1}	NMR (solvent) δ, ppm
IIIc	181	1615(CO)	($CDCl_3$); 3.02 (3H, s, 4-CH_3), 3.35 (2H, t, 3-CH_2), 3.76 (3H, s, 7-CH_3), 4.35 (2H, t, 2-CH_2), 7.54 (1H, s, CH)
IIId	211	3300(OH) 1615(CO)	($CDCl_3$); 3.03 (3H, s, CH_3), 3.36 (2H, t, 3-CH_2), 3.96 (3H, br, 7-$CH_2C\underline{H}_2O\underline{H}$), 4.35 (4H, t, 2-$CH_2$ and 7-$C\underline{H}_2CH_2O$), 7.61 (1H, s, CH)
IIIe	243	1615(CO)	($CDCl_3$); 3.02 (3H, s, CH_3), 3.35 (2H, t, 3-CH_2), 4.36 (2H, t, 2-CH_2), 7.48 (5H, m, Ph), 7.71 (1H,s, 5-CH)
IIIf	257	1607(CO)	($CDCl_3$); 2.97 (3H, s, CH_3), 3.31 (2H, t, 3-CH_2), 4.34 (2H, t, 2-CH_2), 5.34 (2H, s, $C\underline{H}_2Ph$), 7.32 (5H, m, Ph), 7.60 (1H, s, 5-CH)
IIIg	271	1610(CO)	($CDCl_3$); 2.98 (3H, s, CH), 3.09 (2H, t, $CH_2C\underline{H}_2$-Ph), 3.32 (2H, t, 3-CH_2), 4.34 (2H, t, 2-CH_2), 4.37 (2H, t, $C\underline{H}_2CH_2Ph$), 7.23 (5H, s, Ph), 7.52 (1H, s, 5-CH)
IIIh	195	1610(CO)	($CDCl_3$); 1.21 (3H, t, $CH_2C\underline{H}_3$), 3.35 (2H, t, 3-CH_2), 3.41 (2H, q, $C\underline{H}_2CH_3$), 3.73 (3H, s, 7-CH_3), 4.29 (2H, t, 2-CH_2), 7.53 (1H, s, CH)
IIIi	273	3370(OH) 1620(CO)	(DMSO-d_6); 3.46—3.64 (6H, m, 3-CH_2 and 4-$C\underline{H}_2C\underline{H}_2O$), 4.21 (2H, t, 2-$CH_2$), 4.81 (1H, t, OH), 7.40 (5H, m, Ph), 7.96 (1H, s, 5-CH)
IIIj	319	1640(CO)	($CDCl_3$); 3.41 (2H, t, 3-CH_2), 4.31 (2H, t, 2-CH_2), 4.51 (2H, s, $C\underline{H}_2Ph$), 7.35 (10H, m, Ph), 7.73 (1H, s, 5-CH)

a) IPE: isopropyl ether.

The reaction of 6-amino-4,5-dichloro-2-methyl-3(2*H*)-pyridazinone (IX) with *N*-methyl ethanolamine gave 6-amino-5-chloro-4-[(2-hydroxyethyl)methylamino]-2-methyl-3(2*H*)-pyridazinone (X) and its regioisomer (XI) in about equal amounts. The treatment of XI with sodium nitrite and hydrochloric acid gave colorless fine needles, which were identified from

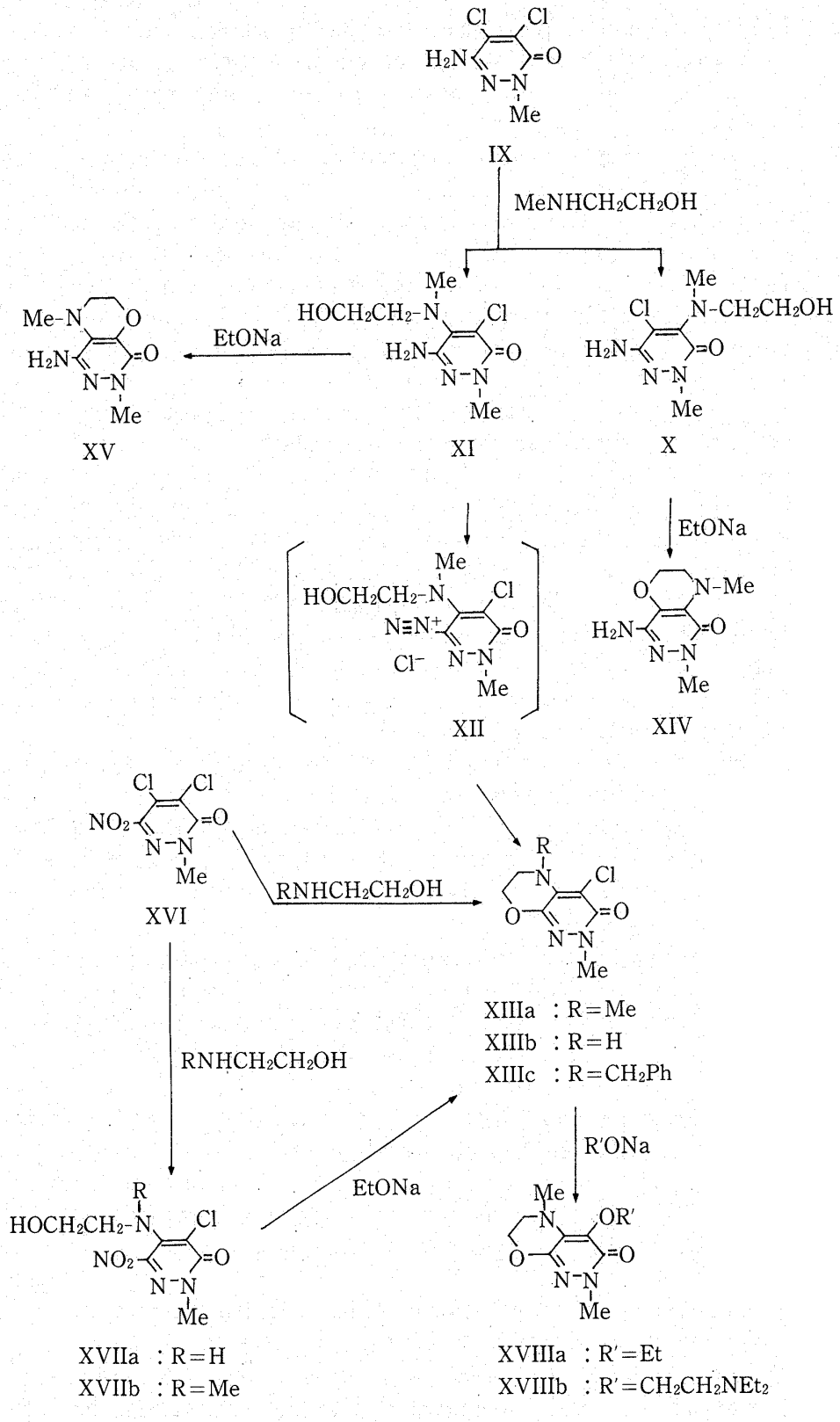


Chart 2

the NMR, IR, and mass spectral data as 4-chloro-6,7-dihydro-2,4-dimethyl-2*H*-pyridazino[3,4-*b*][1,4]oxazin-3(5*H*)-one (XIIIa), as shown in Chart 2. This product may reasonably be formed by nucleophilic ring closure of the diazotized intermediate (XII). These results lead to the structures X and XI shown in Chart 2.

It is known that the reaction of amines with 2-substituted 4,5-dihalo-3(2*H*)-pyridazinones generally results in the exclusive formation of the product monosubstituted in the 5-position.[4] In the case of IX, it is supposed that the electron-donating amino group at the 6-position increased the electron density of the 5-position, and consequently nucleophilic attack by the ethanolamine took place at both the 4- and 5-positions to give a mixture of 4-substituted and 5-substituted products.

Compound X was cyclized by heating with sodium ethoxide to the product corresponding to II, with the isomeric fused ring, 8-amino-3,4-dihydro-4,6-dimethyl-2*H*-pyridazino[4,5-*b*]-1,4-oxazin-5(6*H*)-one (XIV). The spectral data for this product indicated the absence of the hydroxyl group and the presence of a primary amino group and two methylenes in A_2B_2 system. The isomer of XIV (XV) was obtained quantitatively from XI by similar treatment.

The treatment of 4,5-dichloro-2-methyl-6-nitro-3(2*H*)-pyridazinone (XVI) with ethanolamine or *N*-methyl ethanolamine gave the corresponding hydroxyethylamino compound (XVIIa or XVIIb). Heating XVIIa with sodium ethoxide in ethanol did not yield the expected 6-nitro derivative of III, but another bicyclic fused ring product, 4-chloro-6,7-dihydro-2-methyl-2*H*-pyridazino[3,4-*b*][1,4]oxazin-3(5*H*)-one (XIIIb), was isolated instead in 57% yield as colorless needles with a melting point of 235—236°C. The structure of XIIIb was deduced on the basis of analytical and spectral data. Its IR and NMR spectra indicated the absence of the hydroxyl group and the presence of secondary amino, methyl, and two methylene groups. In a similar fashion, the 5-methyl analog of XIIIb (XIIIa), mp 122—123°C, was prepared from XVIIb in quantitative yield. On the other hand, when an aqueous mixture of XVI and *N*-methyl ethanolamine was heated for 5 h, the intermediate XVIIb could not be isolated, but XIIIa was directly obtained in 43% yield. The reaction of XVI with *N*-benzyl ethanolamine also directly afforded the cyclized product (XIIIc) as colorless needles, mp 122—123°C, in a yield of 52%.

The fused ring product thus obtained has a new heterocyclic skeleton, the reactivity of which, therefore, was briefly investigated. The chlorine atom at the 4-position could be replaced with an alkoxy group to give the ethoxy derivative (XVIIIa) or diethylaminoethoxy derivative (XVIIIb) by treatment with sodium alkoxide, but it could not be replaced with an amino group, such as diethylamino or morpholino group, under the conditions used (with sodium hydride at about 110°C).

In the example mentioned above we found that aliphatic ethanolamines readily achieved nucleophilic attack on compound XVI with elimination of the nitro group. It was expected that similar ring formation might take place between aromatic β-hydroxyamines and XVI to give tricyclic heterocycles. Indeed, we successfully constructed the 2*H*-pyridazino[3,4-*b*]-[1, 4]benzoxazin-3(5*H*)-one skeleton as shown in Chart 3. Thus, the reaction of XVI with 2-aminophenol provided 4-chloro-2-methyl-2*H*-pyridazino[3,4-*b*][1, 4]benzoxazin-3(5*H*)-one (XIX) in 67% yield as yellow needles which decomposed at 250°C with sublimation. The experimental formula, $C_{11}H_8ClN_3O_2$, was obtained from the mass spectrum and elemental analysis. Its IR and NMR spectra supported the assigned structure. It was previously reported that a tricyclic system was formed by the reaction of 3,4,6-trichloropyridazine with 2-aminophenols followed by treatment with acetic anhydride[5] or by the Vilsmeier reaction with phosphorus oxychloride in dimethylformamide.[6] Using the nitro compound XVI as a starting material, we could obtain the fused ring system in a single step.

The tricyclic compound XIX with two reactive sites, a secondary amine at the 5-position and a chlorine atom at the 4-position may be converted to various derivatives through alkylation of the sceondary amine or/and substitution of the chlorine atom by a wide variety

of reagents. Thus we examined the reactivity of these functional groups of XIX. On treatment with sodium hydride in xylene followed by boiling with aminoalkyl chlorides, XIX was converted to the corresponding 5-aminoalkyl derivative (XX). An ethanol solution of XXc was allowed to stand at room temperature in the presence of excess triethylamine, giving the 4-ethoxy product (XXI).

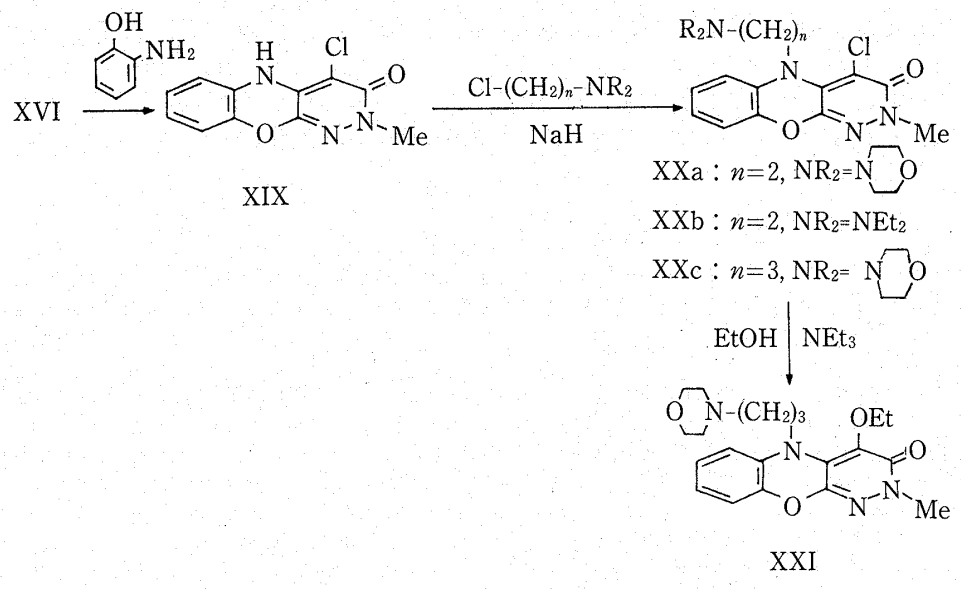


Chart 3

TABLE II. Analgesic and Anti-inflammatory Activities of the Products

Compd. No.	Analgesic activity: Haffner[a] Dose (mg/kg)	Haffner[a] Effective ratio	Stretching[b] Dose (mg/kg)	Stretching[b] Effective ratio	Anti-inflammatory activity[c] Dose (mg/kg)	Anti-inflammatory activity[c] Inhibition (%)
IIIa	100	8/8	100	7/7	100	14
IIIb	100	3/8	100	2/8	200	0
IIIc	100	1/8	100	2/8	200	9
IIId	100	2/8	100	2/8	200	5
IIIe	100	4/8	100	3/8	200	15
IIIf	100	6/8	100	7/8	200	22
IIIg	100	8/8	100	8/8	100	18
IIIh	100	7/8	100	7/8	200	−13[d]
IIIi	100	4/8	100	1/8	200	−9
IIIj	100	2/8	100	5/8	200	−22
VIII	100	2/8	100	1/8	200	2
XIIIa	100	5/8	100	1/8	200	16
XIV	100	5/8	100	6/8	200	18
XV	100	6/8	100	6/8	200	21
XVIIIa	100	4/8	100	4/8	200	10
XVIIIb	100	3/8	100	3/8	200	−8
XXI	100	7/8	100	8/8	200	14
Emorphazone	100	5/8	100	6/8	200	51
Aminopyrine	100	6/8	100	8/8	200	38

a) Modified Haffner's method (morphine 0.5 mg/kg, *s.c.*).[8]
b) Acetic acid-induced stretching method.[8]
c) Carrageenin-induced paw edema method.[9]
d) The minus sign indicates an increase in edema.

The synthetic experiments described above suggested that both the secondary amine in and the chlorine atom on the fused rings had normal reactivity and that these heterocycles were stable under usual synthetic conditions; therefore, they may be useful for the preparation of various modifications which might have potent biological activities.

The analgesic activities of the present compounds were evaluated by Haffner's method[7] with a slight modification[8] and by the acetic acid-induced stretching method.[8] Anti-inflammatory activity was determined by the method of Winter and his co-workers.[9] These results are summarized in Table II. Compounds IIIa, IIIf, IIIg, IIIh, XIV, XV, and XXI showed potent analgesic activity almost equal to those of the positive controls, emorphazone and aminopyrine, in both experimental models. Compounds IIIf and XV had significant anti-inflammatory activity, though they were less potent than the positive controls.

Experimental

All melting points were determined on a Yanagimoto micro-melting point apparatus and are uncorrected. NMR spectra were recorded on a JEOL JNM-PS-100 spectrometer using tetramethylsilane as an internal standard. IR spectra were taken with a JASCO DS-701G spectrometer and mass spectra were obtained on a JEOL JMS-01SG spectrometer.

General Procedure for the Preparation of 4-Chloro-5-(2-hydroxyethylamino)-3(2*H*)-pyridazinones (IIc—j)——A mixture of I (0.1 mol), *N*-substituted ethanolamine (0.3 mol), and MeOH or EtOH (about 200 ml) was heated under reflux for 3 h. The reaction mixture was concentrated to dryness under reduced pressure and the residue was extracted with $CHCl_3$ (about 100 ml). The extract was washed with water, dried over Na_2SO_4, and concentrated *in vacuo*. The residue was crystallized from a mixture of EtOH and isopropyl ether unless otherwise noted. Yields and the physicochemical data of the products are summarized in Table III.

General Procedure for the Preparation of 3,4-Dihydro-2*H*-pyridazino[4,5-*b*]-1,4-oxazin-8(7*H*)-ones (IIIc—j)——Compound II (0.2 mol) was added to an ethanolic solution of NaOEt prepared from 0.3 mol of metallic Na and 200 ml of EtOH. The mixture was refluxed for 5 h. The resulting precipitates were removed by filtration and the filtrate was concentrated to dryness under reduced pressure. $CHCl_3$ was added to the residue and the solution was washed with water, dried over Na_2SO_4, and concentrated *in vacuo*. Yields and other data are summarized in Table I.

4-Chloro-5-(2-hydroxyethylamino)-2-methyl-3(2*H*)-pyridazinone (Va)——A mixture of Ia (54 g, 0.3 mol), ethanolamine (56 g, 0.9 mol), and water (250 ml) was heated under reflux for 1 h. The reaction mixture was concentrated to about 150 ml and cooled in an ice bath. The resulting precipitates were collected, dried under a vacuum, and recrystallized from EtOH–isopropyl ether to give 40 g (66%) of Va, mp 133—134°C, as colorless plates. *Anal.* Calcd for $C_7H_{10}ClN_3O_2$: C, 41.29; H, 4.95; N, 20.64. Found: C, 41.47; H, 4.74; N, 20.61. NMR (CD_3OD) δ: 3.32 (2H, m, $NC\underline{H}_2CH_2O$), 3.52 (2H, t, $NCH_2C\underline{H}_2O$), 4.80 (3H, s, CH_3), 7.86 (1H, s, CH). IR cm^{-1}: 3210 (OH and NH), 1620 (CO). MS *m/e*: 203 (M^+).

4-Chloro-5-(2-hydroxyethylamino)-2-phenyl-3(2*H*)-pyridazinone (Vb)——A mixture of Ic (2.4 g, 10 mmol) ethanolamine (1.83 g, 30 mmol), and water (30 ml) was heated under reflux. After 3 h, the reaction mixture was allowed to stand at room temperature, giving 2.20 g (83%) of Vb as colorless precipitates, mp 169—170°C. *Anal.* Calcd for $C_{12}H_{12}ClN_3O_2$: C, 54.25; H, 4.55; N, 15.81. Found: C, 54.06; H, 4.49; N, 16.01. NMR (DMSO-d_6) δ: 3.47 (4H, m, $NC\underline{H}_2C\underline{H}_2O$), 4.87 (1H, br s, OH), 6.65 (1H, t, NH), 7.37 (5H, s, Ph), 7.99 (1H, s, 6-CH). IR cm^{-1}: 3375 (OH), 3260 (NH), 1630 (CO). MS *m/e*: 265 (M^+).

4-Chloro-5-[(2-hydroxyethyl)methylamino]-3(2*H*)-pyridazinone (VI)——A solution of 4,5-dichloro-3(2*H*)-pyridazinone (3.28 g, 20 mmol) and *N*-methyl ethanolamine (4.51 g, 60 mmol) in water (25 ml) was refluxed. After 4 h, the reaction mixture was concentrated under reduced pressure and the residue was recrystallized from MeOH–ethyl ether to give 2.57 g (63%) of VI, mp 142—143°C. *Anal.* Calcd for $C_7H_{10}ClN_3O_2$: C, 41.29; H, 4.95; N, 20.64. Found: C, 41.36; H, 4.91; N, 20.59. NMR (DMSO-d_6) δ: 3.15 (3H, s, CH_3), 3.60 (4H, m, $NC\underline{H}_2C\underline{H}_2O$), 3.84 (1H, br, OH), 7.90 (1H, s, CH), 12.74 (1H, br, NH). IR cm^{-1}: 3300 (OH and NH), 1592 (CO). MS *m/e*: 203 (M^+).

3,4-Dichloro-5-[(2-hydroxyethyl)methylamino]-pyridazine (VII)——A mixture of *N*-methyl ethanolamine (6.77 g, 90 mmol) and MeOH (25 ml) was added slowly to a solution of 3,4,5-trichloropyridazine (5.49 g, 30 mmol) in MeOH (25 ml). After the exothermic reaction had ceased, the solvent was distilled off under reduced pressure. The residue was extracted with $CHCl_3$. The extract was washed with water, dried over Na_2SO_4, and concentrated *in vacuo*. Recrystallization of the residual solid from AcOEt–isopropyl ether gave 3.44 g (52%) of VII as colorless needles, mp 72.0—72.5°C. *Anal.* Calcd for $C_7H_9Cl_2N_3O$: C, 37.86; H, 4.09; N, 18.92. Found: C, 37.98; H, 4.07; N, 18.68. NMR ($CDCl_3$) δ: 3.21 (3H, s, CH_3), 3.76 (2H, d, $NC\underline{H}_2CH_2O$), 3.86 (3H, m, $NCH_2C\underline{H}_2O$ and OH), 8.51 (1H, s, CH). IR cm^{-1}: 3290 (OH). MS *m/e*: 221 (M^+).

Table III. Yields and Physicochemical Data for 5-(2-Hydroxyethylamino)-4-chloro-3(2*H*)-pyridazinones (II)

Compd. No.	Yields (%)	mp (°C)	Formula	Analysis (%) Calcd. (Found) C	H	N
IIc	73	117—118 (EtOH)	$C_8H_{12}ClN_3O_2$	44.15 (44.14)	5.56 (5.62)	19.31 (19.24)
IId	51	102—103 (EtOH–IPE[a])	$C_9H_{14}ClN_3O_3$	43.64 (43.57)	5.70 (5.65)	16.97 (17.05)
IIe	76	107—108 (AcOEt)	$C_{13}H_{14}ClN_3O_2$	55.82 (55.88)	5.04 (4.96)	15.02 (14.80)
IIf	80	Oil: bp 210 (1 mmHg)	$C_{14}H_{16}ClN_3O_2$	57.24 (57.23)	5.49 (5.54)	14.30 (14.54)
IIg	76	90—91 (EtOH–IPE)	$C_{15}H_{18}ClN_3O_2$	58.54 (58.61)	5.89 (5.95)	13.65 (13.94)
IIh	68	70—71 (EtOH–IPE)	$C_9H_{14}ClN_3O_2$	46.66 (46.53)	6.09 (5.97)	18.14 (17.98)
IIi	67	96—97 (EtOH–IPE)	$C_{14}H_{16}ClN_3O_3$	54.29 (54.36)	5.21 (5.20)	13.57 (13.47)
IIj	81	135—138 (EtOH–IPE)	$C_{19}H_{18}ClN_3O_2$	64.14 (63.85)	5.10 (5.30)	11.81 (11.71)

Compd. No.	MS *m/e* (M^+)	IR cm^{-1}	NMR (solvent) δ, ppm
IIc	217	3350 (OH) 1597 (CO)	($CDCl_3$); 3.20 (4H, s, 5-NCH_3 and OH), 3.64 (2H, t, $NC\underline{H}_2CH_2O$), 3.71 (3H, s, 2-CH_3), 3.88 (2H, t, $NCH_2C\underline{H}_2O$), 7.72 (1H, s, CH)
IId	247	3300 (OH) 1600 (CO)	(DMSO-d_6); 3.08 (3H, s, CH_3), 3.53 (4H, m, 5-$NC\underline{H}_2C\underline{H}_2O$), 3.66 (2H, t, 2-$CH_2C\underline{H}_2O$), 4.03 (2-$C\underline{H}_2CH_2O$), 4.73 (2H, t, OH), 7.82 (1H, s, CH)
IIe	279	1615 (CO)	($CDCl_3$); 3.15 (3H, s, CH_3), 3.56 (3H, t, $NC\underline{H}_2CH_2O$ and OH), 3.72 (2H, t, $NCH_2C\underline{H}_2O$), 7.42 (5H, m, Ph), 7.81 (1H, s, 6-CH)
IIf	293	3370 (OH) 1600 (CO)	($CDCl_3$); 3.01 (3H, s, CH_3), 3.44 (2H, t, $NC\underline{H}_2CH_2O$), 3.70 ($NCH_2C\underline{H}_2O$), 4.26 (1H, br s, OH), 5.12 (2H, s, $C\underline{H}_2Ph$), 7.16 (5H, m, Ph), 7.69 (1H, s, 6-CH)
IIg	307	1605 (CO)	($CDCl_3$); 2.97 (2H, t, $CH_2C\underline{H}_2Ph$), 3.10 (3H, s, CH_3), 3.55 (2H, t, $NC\underline{H}_2CH_2O$), 3.80 (2H, t, $NCH_2C\underline{H}_2O$), 4.24 (2H, t, $C\underline{H}_2CH_2Ph$), 4.39 (1H, br, OH), 7.14 (5H, s, Ph), 7.68 (1H, s, 6-CH)
IIh	231	3400 (OH) 1600 (CO)	($CDCl_3$); 1.24 (3H, t, $CH_2C\underline{H}_3$), 3.19 (1H, br, OH), 3.45—3.80 (9H, m, $C\underline{H}_2CH_3$, $NC\underline{H}_2C\underline{H}_2O$, and 5-$NCH_3$), 7.68 (1H, s, CH)
IIj	309	3380 (OH) 1635 (CO)	($CDCl_3$); 3.69 (8H, s, $NC\underline{H}_2C\underline{H}_2O$), 4.29 (2H, br, OH), 7.44 (5H, m, Ph), 7.95 (1H, s, 6-CH)
IIi	355	3410 (OH) 1625 (CO)	($CDCl_3$); 2.98 (1H, s, OH), 3.58 (2H, d, $NC\underline{H}_2CH_2O$), 3.70 (2H, d, $NCH_2C\underline{H}_2O$), 4.68 (2H, s, $C\underline{H}_2Ph$), 7.32 (10H, m, Ph), 7.75 (1H, s, 6-CH)

a) IPE: isopropyl ether.

8-Chloro-3,4-dihydro-4-methyl-2*H*-pyridazino[4,5-*b*]-1,4-oxazine (VIII)——Compound VII (2.22 g, 10 mmol) was added to an ethanolic solution of EtONa prepared from 345 mg (15 mmol) of metallic Na and 10 ml of EtOH at room temperature. White precipitates were immediately formed. Water was added to the reaction mixture and the resulting insoluble colorless needles were collected on a filter and washed with water. The yield of VIII, mp 188.5—190.0°C, was 735 mg (40%). *Anal.* Calcd for $C_7H_8ClN_3O$: C, 45.30; H, 4.34; N, 22.64. Found: C, 45.32; H, 4.40; N, 22.53. NMR ($CDCl_3$) δ: 3.09 (3H, s, CH_3), 3.50 (2H, t, 3-CH_2), 4.45 (2H, t, 2-CH_2), 8.43 (1H, s, CH). IR cm^{-1}: 1578 (skeleton). MS *m/e*: 185 (M^+).

6-Amino-4,5-dichloro-2-methyl-3(2*H*)-pyridazinone (IX)——A mixture of 4,5-dichloro-2-methyl-6-nitro-3(2*H*)-pyridazinone (XVI) (1.54 g, 70 mmol), iron powder (2 g, excess), and acetic acid (12 ml) was

heated under reflux for 2 h. After the reaction mixture had been cooled to room temperature, the resulting precipitates were filtered off and the filtrate was concentrated *in vacuo*. The residue was dissolved in water and the solution was made basic with 30% NaOH, then extracted with $CHCl_3$. The extract was dried over Na_2SO_4 and the solvent was distilled off under reduced pressure. Recrystallization of the residue from $CHCl_3$ gave 750 mg (55%) of IX, mp 191.5—193.5°C (lit.[10] mp 191.5—193.0°C). *Anal.* Calcd for $C_5H_5ClN_3O$: C, 30.95; H, 2.60; N, 21.66. Found: C, 30.89; H, 2.63; N, 21.46.

The Preparation of 6-Amino-5-chloro-4-[(2-hydroxyethyl)methylamino]-2-methyl-3(2*H*)-pyridazinone (X) and 6-Amino-4-chloro-5-[(2-hydroxyethyl)methylamino]-2-methyl-3(2*H*)-pyridazinone (XI)——A mixture of IX (3.88 g, 20 mmol), *N*-methyl ethanolamine (4.50 g, 60 mmol), and water (70 ml) was heated under reflux for 3 h and the reaction mixture was extracted with $CHCl_3$. The extract was washed with water and dried over Na_2SO_4, then the solvent was removed by evaporation under reduced pressure. The residue was recrystallized from EtOH–isopropyl ether to give 1.49 g (31%) of XI, mp 187.5—188.5°C. *Anal.* Calcd for $C_8H_{13}ClN_4O_2$: C, 41.30; H, 5.63; N, 24.08. Found: C, 41.38; H, 5.48; N, 24.09. NMR (DMSO-d_6) δ: 2.79 (3H, s, 5-NCH_3), 3.08 (2H, t, $NC\underline{H}_2CH_2O$), 3.44 (3H, s, 2-CH_3), 3.54 (2H, t, $NCH_2C\underline{H}_2O$), 4.94 (1H, t, OH), 5.89 (2H, s, NH_2). IR cm^{-1}: 3410 (NH), 3310 (OH), 1595 (CO). MS *m/e*: 232 (M^+).

The mother liquor was concentrated *in vacuo* and the residue was recrystallized from isopropyl ether, giving 1.47 g (31%) of X, mp 110—112°C. *Anal.* Calcd for $C_8H_{13}ClN_4O_2$: C, 41.30; H, 5.63; N, 24.08. Found: C, 41.37; H, 5.66; N, 23.90. NMR (DMSO-d_6) δ: 2.95 (3H, s, 4-NCH_3), 3.42—3.50 (7H, m, 2-CH_3 and $NC\underline{H}_2$-$C\underline{H}_2O$), 4.61 (1H, br s, OH), 5.59 (2H, s, NH_2). IR cm^{-1}: 3440 (NH), 3350 (OH), 1620 (CO). MS *m/e*: 232 (M^+).

The Formation of 4-Chloro-6,7-dihydro-2,5-dimethyl-2*H*-pyridazino[3,4-*b*][1,4]oxazin-3(5*H*)-one (XIIIa) by the Diazotization of XI——Sodium nitrite (200 mg, 2.9 mmol) was slowly added to an ice-cooled solution of XI (606 mg, 2.6 mmol) in 50% HCl (2.4 ml). After 1 h, insoluble material was removed by filtration and the filtrate was extracted with $CHCl_3$. The extract was dried over Na_2SO_4 and the solvent was distilled off under reduced pressure. The residue was separated by thin-layer chromatography (Kieselgel GF_{254}, AcOEt, *Rf* 0.34), followed by extraction with MeOH, evaporation of the solvent, and recrystallization from EtOH–isopropyl ether, giving 80 mg (14%) of XIIIa, mp 122—123°C. *Anal.* Calcd for $C_8H_{10}ClN_3O_2$: C, 44.56; H, 4.67; N, 19.49. Found: C, 44.28; H, 4.63; N, 19.30. NMR (CCl_4) δ: 3.16 (3H, s, 5-CH_3), 3.60—3.68 (7H, m, $NC\underline{H}_2C\underline{H}_2O$ and 2-CH_3). IR cm^{-1}: 1640 (CO). MS *m/e*: 215 (M^+).

8-Amino-3,4-dihydro-4,6-dimethyl-2*H*-pyridazino[4,5-*b*]-1,4-oxazin-5(6*H*)-one (XIV)——Following the procedure described for the synthesis of III, 474 mg (2 mmol) of X was treated with 204 mg (3 mmol) of EtONa in 20 ml of EtOH to give 260 mg (66%) of XIV as colorless fine crystals, mp 128—129°C (EtOH–isopropyl ether). *Anal.* Calcd for $C_8H_{12}N_4O_2$: C, 48.97; H, 6.16; N, 28.55. Found: C, 49.07; H, 6.16; N, 28.28. NMR ($CDCl_3$) δ: 2.76 (3H, s, 4-NCH_3), 3.03 (2H, t, 3-CH_2), 3.60 (3H, s, 6-CH_3), 4.25 (4H, m, 2-CH_2 and NH_2). IR cm^{-1}: 3300 (NH), 3190 (NH), 1620 (CO). MS *m/e*: 196 (M^+).

5-Amino-3,4-dihydro-4,7-dimethyl-2*H*-pyridazino[4,5-*b*]-1,4-oxazin-8(7*H*)-one (XV)——Following the procedure described for the synthesis of III, XI (474 mg, 2 mmol) was treated with EtONa (204 mg, 3 mmol) in EtOH (20 ml) to give 200 mg (51%) of XV as colorless fine crystals, mp 168.5—169.5°C (EtOH–isopropyl ether). *Anal.* Calcd for $C_8H_{12}N_4O_2$: C, 48.97; H, 6.16; N, 28.55. Found: C, 48.82; H, 6.17; N, 28.31. NMR ($CDCl_3$) δ: 3.17 (3H, s, 4-NCH_3), 3.20 (2H, t, 3-CH_2), 3.56 (3H, s, 7-CH_3), 4.16 (4H, t, 2-CH_2 and NH_2). IR cm^{-1}: 3430 (NH), 3320 (NH), 1575 (CO). MS *m/e*: 196 (M^+).

4-Chloro-5-(2-hydroxyethylamino)-2-methyl-6-nitro-3(2*H*)-pyridazinone (XVIIa)——A mixture of XVI (8.8 g, 40 mmol), ethanolamine (7.3 g, 120 mmol), and EtOH (130 ml) was heated under reflux for 3 h. After the reaction mixture had been concentrated to about 50 ml, ethyl ether was added to the mixture to give 8.2 g (83%) of XVIIa as pale yellow crystals, mp 145—146°C. *Anal.* Calcd for $C_7H_9ClN_4O_4$: C, 33.82; H, 3.65; N, 22.54. Found: C, 33.88; H, 3.77; N, 22.53. NMR (DMSO-d_6) δ: 3.54 (3H, br s, $NC\underline{H}_2C\underline{H}_2O$), 3.70 (3H, s, CH_3), 4.97 (1H, br s, OH), 6.96 (1H, br s, NH). IR cm^{-1}: 3370 (OH and NH), 1640 (CO). MS *m/e*: 248 (M^+).

5-Chloro-4-[(2-hydroxyethyl)methylamino]-2-methyl-6-nitro-3(2*H*)-pyridazinone (XVIIb)——*N*-Methyl ethanolamine (4.5 g, 60 mmol) was added to an ice-cooled solution of XVI (4.4 g, 20 mmol) in EtOH (100 ml) and the mixture was gently warmed for a short time. After the reaction mixture had been concentrated, the residue was extracted with $CHCl_3$. The extract was washed with water, dried over Na_2SO_4, and concentrated *in vacuo*. Recrystallization of the residue from EtOH–isopropyl ether gave 1.76 g (34%) of XVIIb, mp 98—100°C. *Anal.* Calcd for $C_8H_{11}ClN_4O_4$: C, 36.58; H, 4.22; N, 21.33. Found: C, 36.71; H, 4.16; N, 21.19. NMR ($CDCl_3$) δ: 2.40 (1H, br s, OH), 2.93 (3H, s, 5-NCH_3), 3.35 (2H, t, $NC\underline{H}_2CH_2O$), 3.71 (2H, t, $NCH_2C\underline{H}_2O$), 3.79 (3H, s, 2-CH_3). IR cm^{-1}: 3450 (OH), 1650 (CO). MS *m/e*: 262 (M^+).

The synthesis of XIIIa by the Cyclization of XVIIb——XVIIb (526 mg, 2 mmol) was added to a solution of NaOEt (204 mg, 3 mmol) in EtOH (10 ml) at room temperature. Immediately, white precipitates were formed. The solvent was distilled off and the residue was extracted with $CHCl_3$. The extract was washed with water, dried over Na_2SO_4, and concentrated *in vacuo*. The residue was recrystallized from EtOH–isopropyl ether to give 420 mg (91%) of XIIIa as colorless fine needles. The physicochemical data of this product agreed completely with those of XIIIa described above.

4-Chloro-6,7-dihydro-2-methyl-2*H*-pyridazino[4,5-b][1,4]oxazin-3(5*H*)-one (XIIIb)——Compound XVIIa

(8.2 g, 33 mmol) was added to an ethanolic solution of NaOEt prepared from metallic Na (1.5 g, 66 mmol) and 100 ml of EtOH. After the mixture had been refluxed for 1 h, water was added to the reaction mixture to give crude XIIIb. Recrystallization of the crude product gave 4.3 g (65%) of colorless needles, mp 235—236°C. *Anal.* Calcd for $C_7H_8ClN_3O_2$: C, 41.70; H, 4.00; N, 20.84. Found: C, 41.93; H, 3.99; N, 20.68. NMR (DMSO-d_6) δ: 3.43 (2H, t, 6-CH_2), 3.48 (3H, s, CH_3), 4.30 (2H, t, 7-CH_2), 7.47 (1H, s, NH). IR cm^{-1}: 3320 (NH), 1620 (CO). MS *m/e*: 201 (M^+).

5-Benzyl-4-chloro-6,7-dihydro-2-methyl-2*H*-pyridazino[3,4-b][1,4]oxazin-3(5*H*)-one (XIIIc)——A mixture of XVI (1.12 g, 5 mmol), *N*-benzyl ethanolamine (2.27 g, 15 mmol), and EtOH (20 ml) was heated under reflux for 3 h. After the solvent had been distilled off, the residue was dissolved in $CHCl_3$. The solution was washed with water, dried over Na_2SO_4, and concentrated *in vacuo*. Recrystallization of the residual solid from CCl_4 gave 758 mg (52%) of XIIIc, mp 122—123°C. *Anal.* Calcd for $C_{14}H_{14}ClN_3O_2$: C, 57.64; H, 4.84; N, 14.40. Found: C, 57.55; H, 4.91; N, 14.38. NMR (CCl_4) δ: 3.25 (2H, t, 6-CH_2), 3.49 (3H, s, CH_3), 4.19 (2H, t, 7-CH_2), 4.84 (2H, s, CH_2Ph), 7.25 (5H, s, Ph). IR cm^{-1}: 1625 (CO). MS *m/e*: 291 (M^+).

6,7-Dihydro-2,5-dimethyl-4-ethoxy-2*H*-pyridazino[3,4-*b*][1,4]oxazin-3(5*H*)-one (XVIIIa)——Compound XIIIa (430 mg, 2 mmol) was added to a solution of NaOEt (408 mg, 6 mmol) in EtOH (15 ml). The mixture was heated in a sealed tube at 158°C for 18 h. The solvent was removed by evaporation, then water and $CHCl_3$ were added to the residue. The separated organic layer was washed with water and dried over Na_2SO_4. The solvent was evaporated off, and the residue was recrystallized from EtOH–isopropyl ether to give 180 mg (40%) of XVIIIa, mp 80—81°C. *Anal.* Calcd for $C_{10}H_{15}N_3O_3$: C, 53.32; H, 6.71; N, 18.65. Found: C, 53.19; H, 6.84; N, 18.75. NMR ($CDCl_3$) δ: 1.39 (3H, t, CH_2CH_3), 3.27 (3H, s, 5-CH_3), 3.31 (2H, t, 6-CH_2), 3.58 (3H, s, 2-CH_3), 4.25 (4H, m, CH_2CH_3 and 7-CH_2). IR cm^{-1}: 1635 (CO). MS *m/e*: 225 (M^+).

4-(2-Diethylaminoethyloxy)-6,7-dihydro-2,5-dimethyl-2*H*-pyridazino[3,4-b][1,4]oxazin-3(5*H*)-one (XVIIIb)——A 115 mg (5 mmol) portion of metallic Na was suspended in absolute dioxane (10 ml). 2-Diethylaminoethanol (1.76 g, 15 mmol) was added to the suspension with stirring. After the mixture had been stirred for 4 h at 50—60°C, XIIIa (1.08 g, 5 mmol) was added and the mixture was heated at 90—100°C for an additional 4 h. The resulting precipitates were filtered off and the filtrate was concentrated *in vacuo*. $CHCl_3$ was added to the residue, and the solution was washed with water, dried over Na_2SO_4, and concentrated under reduced pressure. The oily product (XVIIIb) was converted to the crystalline hydrochloride in the usual way. Yield 1.25 g (81%), mp 215—216°C. *Anal.* Calcd for $C_{14}H_{24}N_4O_2 \cdot HCl$: C, 50.52; H, 7.57; N, 16.83. NMR ($CDCl_3$) δ: 1.47 (6H, t, CH_2CH_3), 3.32 (3H, s, 5-CH_3), 3.40 (6H, m, CH_2CH_3 and 6-CH_2), 3.57 (3H, s, 2-CH_3), 3.64 (2H, br, $OCH_2CH_2N^+$), 4.32 (4H, m, $OCH_2CH_2N^+$ and 7-CH_2), 11.75 (1H, br s, N^+H). IR cm^{-1}: 2650 (N^+H), 2475 (N^+H), 1630 (CO). MS *m/e*: 332 (M^+).

4-Chloro-2-methyl-2*H*-pyridazino[3,4-*b*][1,4]benzoxazin-3(5*H*)-one (XIX)——A mixture of XVI (4.4 g, 20 mmol), 2-aminophenol (6.6 g, 60 mmol), and EtOH (100 ml) was heated under reflux for 4 h, then cooled. The resulting precipitates were collected and recrystallized from EtOH to give 3.35 g (67%) of XIX, which decomposed at 250°C with sublimation. *Anal.* Calcd for $C_{11}H_8ClN_3O_2$: C, 52.92; H, 3.23; N, 16.83. Found: C, 52.66; H, 3.35; N, 16.63. NMR (DMSO-d_6) δ: 3.02 (1H, br s, NH), 3.49 (3H, s, CH_3), 6.99 (3H, m, Ph), 7.24 (1H, m, Ph). IR cm^{-1}: 3150 (NH), 1655 (CO). MS *m/e*: 249 (M^+).

4-Chloro-2-methyl-5-(2-morpholinoethyl)-2*H*-pyridazino[3,4-*b*][1,4]benzoxazin-3(5*H*)-one (XXa)——NaH (500 mg, excess) was added to a solution of XIX (498 mg, 2 mmol) in xylene (20 ml), and the mixture was refluxed for 20 h. Then 2-morpholinoethyl chloride (330 mg, 2.2 mmol) was added and refluxing was continued for an additional 16 h. The solvent was distilled off and the residue was recrystallized from EtOH–isopropyl ether to give 400 mg (56%) of XXa as yellow needles, mp 134—135°C. *Anal.* Calcd for $C_{17}H_{19}ClN_4O_3$: C, 56.28; H, 5.28; N, 15.44. Found: C, 56.29; H, 5.10; N, 15.43. NMR ($CDCl_3$) δ: 2.26 (4H, t, morpholino), 2.48 (2H, t, 5-CH_2CH_2N), 3.42 (4H, t, morpholino), 3.65 (3H, s, CH_3), 4.15 (2H, t, 5-CH_2CH_2N), 7.00 (4H, s, Ph). IR cm^{-1}: 1660 (CO). MS *m/e*: 362 (M^+).

4-Chloro-5-(2-diethylaminoethyl)-2-methyl-2*H*-pyridazino[3,4-*b*][1,4]benzoxazin-3(5*H*)-one (XXb)——Following the procedure described for the synthesis of XXa, XXb was obtained from the reaction of XIX with 2-diethylaminoethyl chloride. Yield 47%, mp 102—104°C. *Anal.* Calcd for $C_{17}H_{21}ClN_4O_2$: C, 58.53; H, 6.07; N, 16.06. Found: C, 58.78; H, 6.08; N, 16.23. NMR ($CDCl_3$) δ: 0.76 (6H, t, CH_2CH_3), 2.31 (4H, q, CH_2CH_3), 2.52 (2H, t, 5-CH_2CH_2N), 3.62 (3H, s, 2-CH_3), 4.15 (2H, t, 5-CH_2CH_2N), 6.96 (4H, m, Ph). IR cm^{-1}: 1630 (CO). MS *m/e*: 348 (M^+).

4-Chloro-2-methyl-5-(3-morpholinopropyl)-2*H*-pyridazino[3,4-*b*][1,4]benzoxazin-3(5*H*)-one (XXc)——Following the procedure described for the synthesis of XXa, XXc was obtained from the reaction of XIX with 3-morpholinopropyl chloride. Yield 56%, mp 120—122°C. *Anal.* Calcd for $C_{18}H_{21}ClN_4O_3$: C, 57.37; H, 5.62; N, 14.87. Found: C, 57.17; H, 5.43; N, 14.75. NMR ($CDCl_3$) δ: 1.81 (2H, quintet, 5-$CH_2CH_2CH_2N$), 2.28 (6H, m, morpholino and 5-$CH_2CH_2CH_2N$), 3.60 (7H, m, morpholino and CH_3), 4.21 (2H, t, 5-$CH_2CH_2CH_2N$), 6.94 (4H, m, Ph). IR cm^{-1}: 1640 (CO). MS *m/e*: 376 (M^+).

4-Ethoxy-2-methyl-5-(3-morpholinopropyl)-2*H*-pyridazino[3,4-*b*][1,4]benzoxazin-3(5*H*)-one (XXI)——An ethanolic solution (25 ml) of XXc (1.51 g, 4 mmol) and triethylamine (1 ml, excess) was allowed to stand overnight at room temperature. The solvent was evaporated off and the residue was extracted with $CHCl_3$. The extract was washed with water, dried over Na_2SO_4, and concentrated *in vacuo*. The oily product (XXI) was converted to the crystalline hydrochloride in the usual way. Recrystallization from $CHCl_3$ gave the

hydrochloride of XXI in a yield of 940 mg (62%), mp 239—241°C. *Anal.* Calcd for $C_{18}H_{22}N_4O_2 \cdot HCl$: C, 56.80; H, 6.20; N, 13.25. Found: C, 56.61; H, 6.35; N, 13.18. NMR (DMSO-d_6) δ: 1.32 (3H, t, $CH_2C\underline{H}_3$), 2.11 (2H, br s, 5-$CH_2C\underline{H}_2CH_2N$), 3.10 (6H, br, morpholino and 5-$CH_2CH_2C\underline{H}_2N$), 3.40 (3H, s, 2-$CH_3$), 3.88 (4H, br, morpholino), 3.99 (2H, br, 5-$C\underline{H}_2CH_2CH_2N$), 4.25 (2H, q, $C\underline{H}_2CH_3$), 6.92—7.11 (4H, m, Ph), 11.32 (1H, br, N^+H). IR cm^{-1}: 2400—2600 (N^+H), 1610 (CO). MS *m/e*: 342 (M^+).

Analgesic Activity——The analgesic activities of the compounds synthesized in this study were evaluated by Haffner's method[7] with a slight modification.[8] Male ddY mice (Shizuoka Agricultural Cooperative for Laboratory Animals) weighing 18—22 g were given the test compounds (100 mg/kg) intraperitoneally 15 min after the subcutaneous injection of a threshold dose of morphine·HCl (0.5 ml/kg), and 30 min later the analgesic activity was tested. The activity was evaluated as positive when the animals failed to remove the clip applied to the base of the tail.

The acetic acid-induced stretching method[8] was also used for the evaluation. Male ddY mice were subcutaneously injected with the test compounds (100 mg/kg) and 30 min later 0.6% acetic acid solution (10 ml/kg) was given intraperitoneally. The activity was positive when the animals showed no stretching syndrome between 5 to 20 min after the injection of acetic acid.

The results are represented as the effective ratio, *i.e.* number of mice evaluated as positive/number of mice used.

Anti-inflammatory Activity——Anti-inflammatory activity was determined by the method reported previously.[9] Briefly, six male Wistar rats (Japan Clea Co. Ltd.) weighing 120—150 g were used for each group. Carrageenan (0.1 ml/animal, 1%) was subcutaneously injected into the plantar surface of the hind paw 30 min after the intraperitoneal injection of the test compound suspended in 1% gum arabic solution. Edema formation was measured 3 h after the injection and compared with that caused by carrageenan alone to evaluate the percent inhibition produced by the test compound.

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References

1) M. Sato, H. Tanizawa, T. Fukuda, and T. Yuizono, *Folia pharmacol. japon.*, **74**, 841 (1978); M. Sato, Y. Ishizuka, K. Seki, H. Tanizawa, T. Fukuda, and T. Yuizono, *ibid.*, **75**, 127 (1976).
2) M. Takaya, T. Yamada, and H. Shimamura, *Yakugaku Zasshi*, **98**, 1421 (1978).
3) S. Chimichi and R. Nesi, *J. Heterocyclic Chem.*, **14**, 1099 (1977).
4) D.L. Aldous and R.N. Castle, "The Chemistry of Heterocyclic Compounds," Vol. 28, ed. by R.N. Castle, John Wiley and Sons, Inc., New York, 1973, Chapter III.
5) T.V. Gortinskaya, V.G. Nyrkova, N.V. Savitskaya, M.L. Shchukina, A.I. Polezhaeva, and M.D. Mashkovskii, *Khim.-Farm. Zh.*, **6**, 8 (1972) [*Chem. Abs.*, **77**, 139924*n* 1972)].
6) N.V. Savitskaya, T.V. Gortinskaya, V.G. Nyrkova, and I.N. Fedorova, *Khim.-Farm. Zh.*, **11**, 30 (1977) [*Chem. Abs.*, **87**, 68269*w* (1977)].
7) F. Haffner, *Dtsch. Med. Wochenschr.*, **55**, 731 (1929).
8) M. Sato, H. Tanizawa, T. Fukuda, and T. Yuizono, *Japan. J. Pharmacol.*, **29**, 303 (1979).
9) C.A. Winter, E.A. Risley, and G.W. Nuss, *Proc. Soc. Exp. Biol. Med.*, **111**, 544 (1962).
10) T. Terai, M. Iizuka, N. Inokari, and S. Maeda, Japan. patent 5398 (1969).