Studies on Cardiotonic Agents. VII.[1] Potent Cardiotonic Agent KF15232 with Myofibrillar Ca^{2+} Sensitizing Effect

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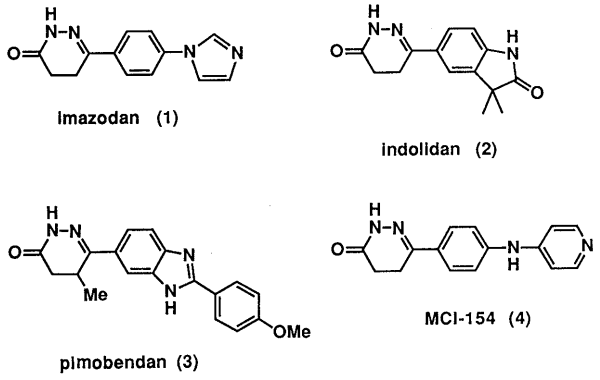
Received August 3, 1990

A series of novel 4,5-dihydro-5-methyl-6-(2 or 4-substituted 7-quinazolinyl)-3(2*H*)-pyridazinones was synthesized and examined for cardiotonic activity in anesthetized dogs. The 4-substituted aminoquinazolines generally showed potent and long-lasting inotropic activity. Fall in the activity was observed on the introduction of substituent at the 2-position of the quinazoline ring. The 3-substituted 4 (3*H*)-quinazolinimines generally exhibited weak activity. Ca^{2+} sensitizing effect of the 4-substituted amino derivatives was also examined in chemically skinned fiber from papillary muscle of guinea pig. The alkylamino derivatives exhibited small sensitizing effect, while the benzylamino derivatives exhibited large effect. Among them, KF15232 (Ix) was found to have the most potent cardiotonic and Ca^{2+} sensitizing activities.

Keywords cardiotonic agent; calcium ion^{2+} sensitizing effect; structure–activity relationship; pyridazinone; quinazoline

The extensive research effort to find a non-glycoside, non-catecholamine digitalis substitute resulted in the discovery of several new cardiotonic agents.[2] Some of these new drugs, including 4,5-dihydro-3(2*H*)-pyridazinone derivatives imazodan (**1**)[3] and indolidan (**2**)[4] showed combined inotropic–vasodilatory properties. These agents demonstrated an inhibitory activity of the cardiac low-Km, cyclic adenosine 3′,5′-monophosphate (c-AMP)-specific phosphodiesterase (PDE III) which was believed to be the principal component of the intropic–vasodilatory action.

Recently, a new mechanism of positive inotropic action was proposed for some 4,5-dihydro-3(2*H*)-pyridazinones, such as pimobendan (**3**) and MCI-154 (**4**).[5,6] These compounds showed a Ca^{2+} sensitizing effect in the contractile protein system, and the effect was responsible, at least in part, for the mechanism of the inotropic action.[7,8] It can be speculated that the risk of arrhythmogenic action of compounds which showed a Ca^{2+} sensitizing effect in addition to a cardiac PDE inhibitory effect might be lower than that of pure PDE inhibitors.[9] Little work has been reported on the structure–activity relationships for Ca^{2+} sensitizing effect.

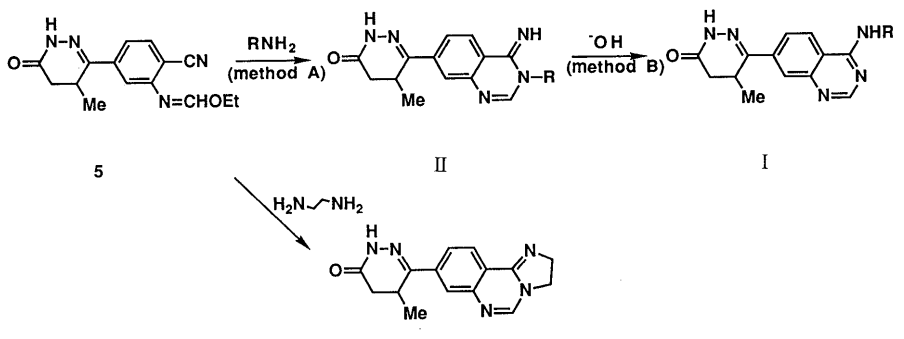


We have recently reported that 4,5-dihydro-5-methyl-6-(4-methylamino-7-quinazolinyl)-3(2*H*)-pyridazinone (Ib)[1] showed potent inotropic activity in anesthetized dogs. As an extension of our studies, we have attempted to modify Ib by replacement of the 4-methylaminoquinazolinyl group with other quinazolinyl groups.

The present paper describes the synthesis and the structure–activity relationships for inotropic and Ca^{2+} sensitizing effect of a series of novel 4,5-dihydro-5-methyl-6-(2 or 4-substituted 7-quinazolinyl)-3-(2*H*)-pyridazinones.

Chemistry Previously,[1] Ib was synthesized from the ethoxymethylenimine (**5**) *via* IIb by the route shown in Chart 1. In order to prepare the compounds which have another amino group at the 4-position of the quinazoline ring, we first attempted the reaction of **5** with various primary amines.

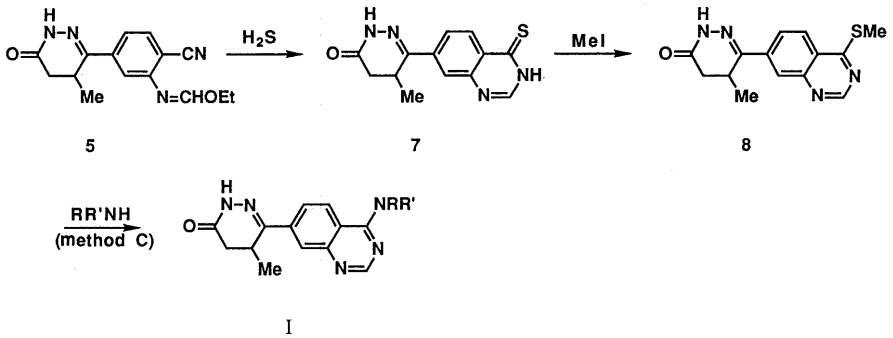
Reaction of **5** with various amines in methanol at 40—60 °C for 0.5—5 h afforded II (method A) and the rearrangement of II[1] in 2 N NaOH and dimethylformamide (DMF) at 60—100 °C for 0.5—5 h afforded I (method B). The results from the experiments are summarized in



5 II I

6

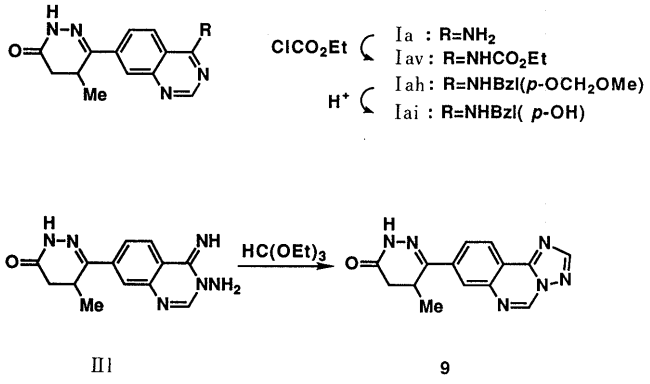
Chart 1



5 7 8

I

Chart 2



III 9

Chart 3

Tables I—II.

Reaction of **5** with *tert*-butylamine was very slow, and the longer reaction time resulted in degradation of **5**. Reaction of **5** with ethylenediamine did not afford IIv but the imidazoquinazoline (**6**).

Rearrangement of II to I proceeded without any unusual occurrence. Consequently, the method shown in Chart 1 is generally applicable for preparation of the compounds which have an amino group at the 4-position of the quinazoline ring.

We also investigated the more generally applicable method for preparation of I. An alternative synthetic route to I is shown in Chart 2. The thione (**7**), which was synthesized from **5** by treatment with H_2S in pyridine in the presence of Et_3N,[10] was converted to the thioether (**8**) by alkylation with MeI. Reaction of **8** with various amines in dimethylsulfoxide (DMSO) or in the absence of solvent at 50—170 °C afforded I (method C). These results are also summarized in Table I.

In the reaction of **8** with cyclopropylamine, the starting **8** was recovered probably due to high volatility of the amine.

Compound Ia reacted with ethyl chloroformate to give the carbamate (Iav). Demethoxymethylation of Iah with 3 N HCl afforded the hydroxybenzyl derivative (Iai). The 3-amino-4(3*H*)-quinazolinimine (III) reacted with triethyl orthoformate to give the triazoloquinazoline (**9**) (Chart 3).

Next, we investigated introduction of the carbon functional group into the 4-position of the quinazoline ring. Treatment of **8** with NaCN in DMF at 100 °C did not afford the desired cyanide (**10**). Sulfur extrusion reaction[11] of **11** prepared from **7** by alkylation with diethyl bromomalonate afforded **12** (Chart 4). The proton nuclear magnetic resonance (^{1}H-NMR) spectrum of **12** at pD 5.6 suggested that **12** was a mixture of tautomeric isomers (**12a** and **12b**) in a ratio of 1 : 1. Compound **12a** showed the proton signals of the 3-position of the quinazoline ring at δ 12.91 ppm and the 2-position of the quinazoline ring at δ 8.22 ppm, while **12b** showed the methine proton of malonic acid group at δ 6.20 ppm and the 2-position of the quinazoline ring at δ 9.30 ppm, respectively. At pD 14.9, the signals due to **12a** were almost non-existent. Therefore, the **12b** form was exclusively dominant at pD 14.9 (see Experimental section).

Hydrolysis under alkaline condition and subsequent acidification gave the 4-methyl derivative (**13**). Reaction of **12** with MeI in the presence of Et_3N followed by hydrolysis did not afford the desired 4-ethyl derivative (**14**) but a complexed mixture (Chart 4).

Desulfurization of **8** with Raney-Ni in DMF afforded the dihydroquinazoline (**15**) which was oxidized with MnO_2 to give the quinazoline (**16**) (Chart 5).

Finally, we investigated the synthesis of 2,4-disubstituted quinazoline derivatives.

The 2-oxo derivatives were synthesized by the method shown in Chart 6. Key intermediate **18** was prepared by reaction of the anthranilonitrile (**17**) with ethyl chloroformate. Reaction of **18** with methylamine in DMSO at 150°C gave the quinazolinimine (**19**), with NH_3 gave the quinazolinone, (**20**) and with ethylenediamine gave no quinazolinimine (**21**) but did give the imidazoquinazoline (**22**).

The synthetic sequences leading to the compounds which have an alkylthio and an alkylamino group at the 2- or 4-position of quinazoline ring are shown in Chart 7. Reaction of **17** with CS_2[12] in pyridine afforded the 2,4-dimercaptoquinazoline (**23**), and followed by alkylation with MeI in basic medium gave the 2,4-bismethylthioquinazoline (**24**). Reaction of **24** with methylamine and propylamine afforded selectively 4-methylamino-2-methylthio and 4-propylamino-2-methylthio derivatives (**25**, **26**), respectively. The structure of **25** was confirmed by the nuclear Overhauser effect (NOE) which was observed between the methyl proton at the 4-position (δ 3.01 ppm as doublet) and the ring proton at the 5-position of quinazoline (δ 8.12 ppm as doublet). In oxidation reaction of **26** with potassium permanganate, a new spot probably due to the formation of the 2-methylsulfonyl-4-propylamino derivative (**27**), was detected on thin layer chromatography analysis. Compound **27** could not be isolated because of its instability. Treatment of the $CHCl_3$ extract of the reaction mixture with methylamine gave the 2-methylamino-4-

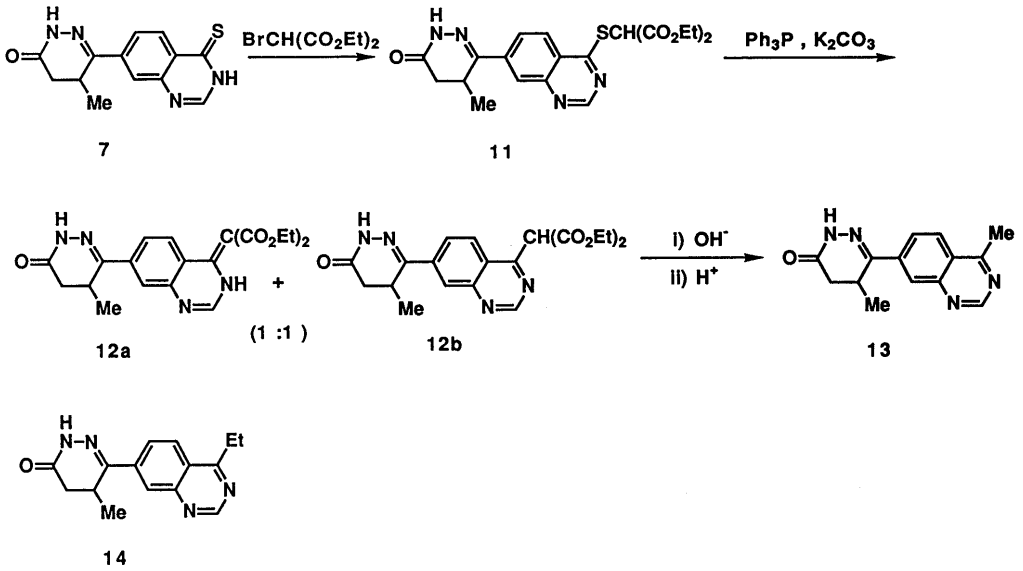


Chart 4

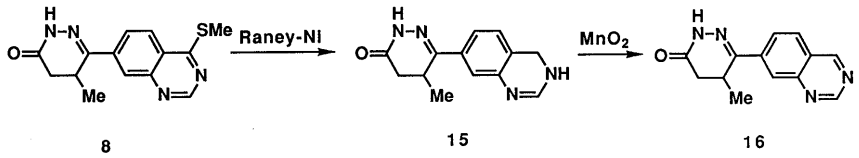


Chart 5

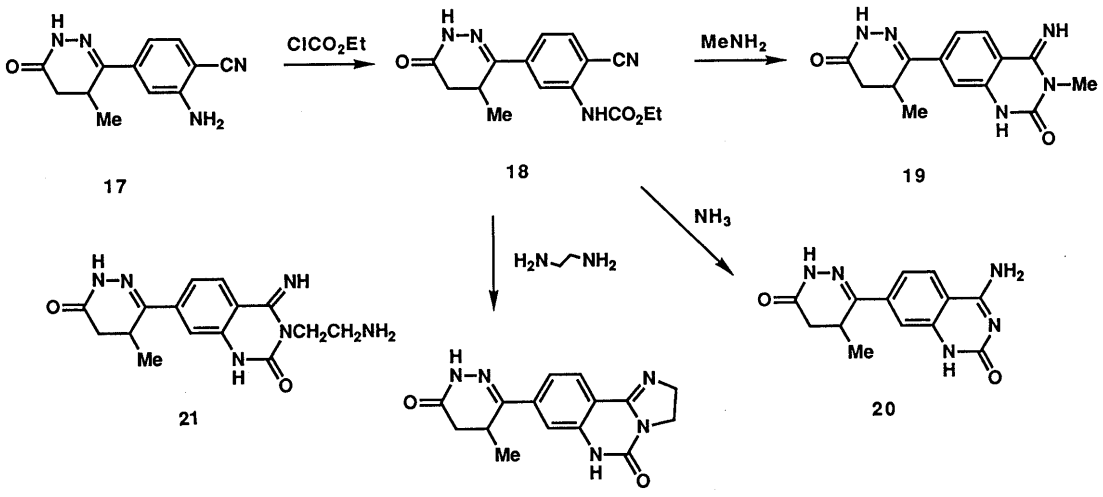


Chart 6

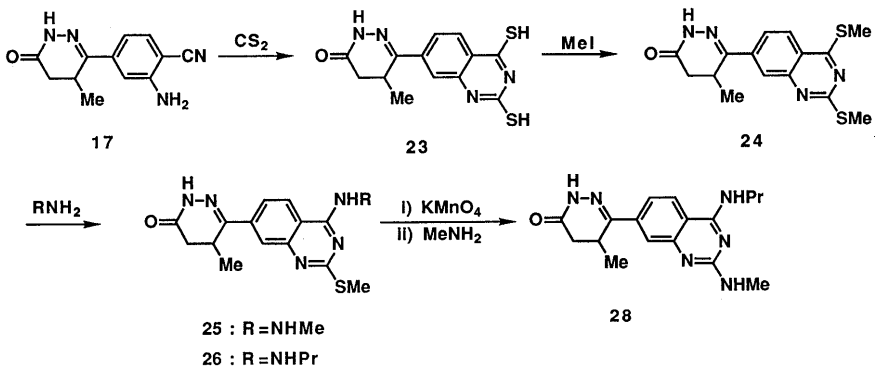
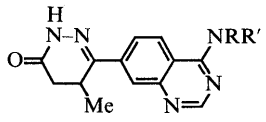


Chart 7

TABLE I.



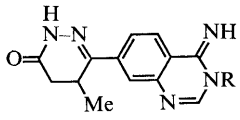
Compd. No.	R, R′	Method Yield (%)	mp (°C) (Crystn.[a] solv.)	Formula	Analysis (%) Calcd (Found)		
					C	H	N
Ia[b]	H, H	B 59	>300	$C_{13}H_{13}N_5O$	61.16 (60.92)	5.13 5.00	27.43 27.15)
Ib[b]	Me, H	B 76	296—302 (DMSO–H_2O)	$C_{14}H_{15}N_5O$	62.44 (62.37	5.61 5.62	26.01 25.62)
Ic	Et, H	B 60	261—266	$C_{15}H_{17}N_5O$	63.59 (63.38	6.05 5.91	24.72 24.69)
Id	Pr, H	B 64	263—269	$C_{16}H_{19}N_5O$	64.63 (64.60	6.44 6.50	23.55 23.55)
Ie	iso-Pr, H	B 71	259—261	$C_{16}H_{19}N_5O$	64.63 (64.73	6.44 6.22	23.55 23.20)
If	cyclo-Pr, H	B 21 C 0	286—287 (dec.)	$C_{16}H_{17}N_5O \cdot 1/2H_2O$	63.14 (63.52	5.96 5.76	23.01 23.26)
Ig	Bu, H	B 21 C 21	225—231	$C_{17}H_{21}N_5O$	65.57 (65.20	6.80 7.12	22.49 22.61)
Ih	iso-Bu, H	C 53	285—286	$C_{17}H_{21}N_5O$	65.57 (65.55	6.80 6.73	22.49 22.32)
Ii	*sec*-Bu, H	C 28	>300	$C_{17}H_{21}N_5O \cdot 1/2H_2O$	63.72 (64.10	6.93 6.90	21.85 21.98)
Ij	*tert*-Bu, H	C 0					
Ik	Pentyl, H	C 57	254—255	$C_{18}H_{23}N_5O$	66.44 (66.53	7.12 7.35	21.52 21.89)
Il	Isopentyl, H	C 46	292—293	$C_{18}H_{23}N_5O$	66.44 (66.59	7.12 7.20	21.52 21.26)
Im	Cyclopentyl, H	C 82	262 (dec.)	$C_{18}H_{21}N_5O$	66.85 (66.80	6.56 6.43	21.65 21.38)
In	Hexyl, H	C 46	254—255	$C_{19}H_{25}N_5O$	67.23 (67.29	7.42 7.53	20.63 20.41)
Io	Cyclohexyl, H	C 65	288—291 ($CHCl_3$–MeOH–Et_2O)	$C_{19}H_{23}N_5O$	67.63 (67.23	6.88 6.92	20.75 20.35)
Ip	Heptyl, H	C 67	234—238	$C_{20}H_{27}N_5O$	67.95 (68.29	7.71 7.75	19.80 19.52)
Iq	Octyl, H	C 71	224—225	$C_{21}H_{29}N_5O$	68.63 (68.80	7.95 8.02	19.06 18.96)
Ir	Cyclooctyl, H	C 31	289—290 (MeOH–H_2O)	$C_{21}H_{27}N_5O \cdot 1/2H_2O$	67.34 (67.53	7.55 7.28	18.69 19.01)
Is	$HOCH_2CH_2$, H	C 43	234—236 (MeOH–H_2O)	$C_{15}H_{17}N_5O_2 \cdot H_2O$	56.77 (56.99	6.03 5.92	22.06 21.83)
It	$Me_2NCH_2CH_2$, H	C 74	243—245	$C_{17}H_{22}N_6O \cdot 1/2H_2O$	60.88 (60.94	6.91 6.75	25.06 24.78)
Iu	3-Morpholinopropyl, H	C 69	234—236	$C_{20}H_{26}N_6O_2 \cdot H_2O$	59.97 (60.30	7.06 6.85	20.97 21.29)
Iw	Allyl, H	C 27	252—255	$C_{16}H_{17}N_5O \cdot 1/2H_2O$	63.14 (63.26	5.97 6.03	23.00 22.95)
Ix	Bzl, H	B 81 C 46	248	$C_{20}H_{19}N_5O \cdot H_2O$	66.10 (66.08	5.82 5.93	19.27 19.44)
Iy	(*o*-Me)Bzl, H	C 12	287 (dec.)	$C_{21}H_{21}N_5O \cdot H_2O$	66.82 (66.95	6.15 6.29	18.55 18.35)
Iz	(*p*-Me)Bzl, H	C 40	268—271	$C_{21}H_{21}N_5O$	70.17 (69.84	5.89 6.00	19.48 19.53)
Iaa	(*p*-F)Bzl, H	C 29	264	$C_{20}H_{18}FN_5O \cdot H_2O$	62.97 (63.31	5.30 5.24	18.35 18.45)
Iab	(*o*-Cl)Bzl, H	C 36	219—224	$C_{20}H_{18}ClN_5O \cdot 1/2H_2O$	61.77 (61.43	4.93 4.89	18.00 17.66)
Iac	(*m*-Cl)Bzl, H	C 68	237—242	$C_{20}H_{18}ClN_5O \cdot H_2O$	60.37 (60.75	5.08 4.95	17.59 17.48)
Iad	(*p*-Cl)Bzl, H	C 34	237—242	$C_{20}H_{18}ClN_5O$	63.24 (63.01	4.78 4.77	18.44 18.40)
Iae	(*o*-MeO)Bzl, H	C 27	>300	$C_{21}H_{21}N_5O_2$	67.18 (67.00	5.64 5.89	18.65 18.48)
Iaf	(*m*-MeO)Bzl, H	C 30	296 (dec.)	$C_{21}H_{21}N_5O_2$	67.18 (66.89	5.64 5.53	18.65 18.57)

TABLE I. (continued)

Compd. No.	R, R′	Method Yield (%)	mp (°C) (Crystn.[a] solv.)	Formula	Analysis (%) Calcd (Found)		
					C	H	N
Iag	(*p*-MeO)Bzl, H	C 46	256—257	$C_{21}H_{21}N_5O_2$	67.18 (67.20)	5.64 5.93	18.65 18.83)
Iah	(*p*-$MeOCH_2O$)Bzl, H	C	192—195	$C_{22}H_{23}N_5O_3$	65.17 (65.25)	5.72 5.43	17.27 17.51)
Iai[c]	(*p*-HO)Bzl, H	[d] 77	187—190	$C_{20}H_{19}N_5O_2 \cdot HCl \cdot 3/2H_2O$	56.54 (56.59)	5.46 5.15	16.48 16.13)
Iaj	(4-Pyridyl)CH_2, H	C 16	284—285	$C_{19}H_{18}N_6O$	65.88 (65.90)	5.24 5.32	24.26 24.37)
Iak	(3-Pyridyl)CH_2, H	C 23	284—285	$C_{19}H_{18}N_6O \cdot 1/2H_2O$	64.21 (64.53)	5.40 5.35	23.64 23.62)
Ial	Ph, H	B 75 C 69	286—289	$C_{19}H_{17}N_5O$	68.87 (68.63)	5.17 5.17	21.13 21.01)
Iam	(*m*-Cl)Ph, H	C 37	293—294	$C_{19}H_{16}ClN_5O$	62.38 (62.26)	4.41 4.33	19.14 18.93)
Ian	(*p*-Cl)Ph, H	C 18	>300	$C_{19}H_{16}ClN_5O$	62.38 (62.40)	4.41 4.37	19.14 18.87)
Iap	$PhCH_2CH_2$, H	C 48	264—265	$C_{21}H_{21}N_5O$	70.17 (70.33)	5.89 5.90	19.48 19.57)
Iaq	(3,4-Di-MeO)$PhCH_2CH_2$, H	C 46	249—250	$C_{23}H_{25}N_5O_3$	65.85 (65.72)	6.02 6.13	16.69 16.90)
Iar	(2-Pyridyl)CH_2CH_2, H	C 20	245—247	$C_{20}H_{20}N_6O$	66.65 (66.64)	5.59 5.63	23.32 22.95)
Ias	Me, Me	C 51	235—237	$C_{15}H_{17}N_5O$	63.59 (63.33)	6.05 6.02	24.72 24.53)
Iat[c]	$-(CH_2)_2-NH-(CH_2)_2-$	C 35	209—211 (MeOH–Et_2O)	$C_{17}H_{20}N_6O \cdot 2HCl \cdot H_2O$	49.16 (48.83)	5.82 6.10	20.23 20.01)
Iau	$-(CH_2)_5-$	C 80	268—270	$C_{18}H_{21}N_5O$	66.85 (66.56)	6.55 6.71	21.66 21.60)
Iav	$EtCO_2$, H	[d] 82	261—265	$C_{16}H_{17}N_5O_3$	58.71 (58.37)	5.23 5.39	21.39 21.18)

a) Recrystallized from DMF–H_2O except where otherwise noted in parentheses. *b*) Ref. 1. *c*) As HCl salt. *d*) See Experimental section.

TABLE II.



Compd. No.	R	Yield[a] (%)	mp (°C)	Formula	Analysis (%) Calcd			Found		
					C	H	N	C	H	N
IIb[b]	Me	63	253—255	$C_{14}H_{15}N_5O$	62.44	5.61	26.01	62.39	5.55	25.83
IIc	Et	53	238—244	$C_{15}H_{17}N_5O$	63.59	6.05	24.72	63.93	6.18	24.94
IId	Pr	57	239—240	$C_{16}H_{19}N_5O$	64.63	6.44	23.55	64.82	6.44	23.76
IIe	iso-Pr	70	205	$C_{16}H_{19}N_5O$	64.63	6.44	23.55	64.85	6.50	23.54
IIf	cyclo-Pr	65	232 (dec.)	$C_{16}H_{17}N_5O \cdot 1/2H_2O$	63.14	5.96	23.01	63.00	5.82	22.78
IIg	Bu	35	198—200	$C_{17}H_{21}N_5O$	65.57	6.80	22.49	65.52	6.59	22.20
IIj	*tert*-Bu	0								
IIx	$PhCH_2$	82	216	$C_{20}H_{19}N_5O \cdot H_2O$	66.10	5.82	19.27	66.40	5.86	19.34
IIv	$H_2NCH_2CH_2$	0[c]								
IIal	Ph	77	274—275 (dec.)	$C_{19}H_{17}N_5O$	68.87	5.17	21.13	68.78	5.16	20.92
IIao	(4-MeO)Ph	40	268	$C_{20}H_{19}N_5O_2$	66.47	5.30	19.38	66.20	5.32	19.06
IIax	NH_2	70	193	$C_{13}H_{14}N_6O$	57.77	5.22	31.09	57.42	5.01	30.89

a) All compounds were prepared by Method A, and recrystallized from DMF–H_2O. *b*) Ref. 1. *c*) Compound **6** was obtained (see Experimental section).

propylamino derivarive (**28**).

We also investigated the synthesis of the 2-methyl-4-alkylamino derivatives (**30**, **31**). Breukink[13] reported the synthesis of 4-methoxy-2-methylquinazoline by reaction of *o*-acetamidobenzonitrile with sodium methoxide. Acetylation of **17** with acetyl chloride in pyridine, followed by treatment with potassium carbonate in methanol afforded the 4-methoxy-2-methylquinazoline (**29**). Reaction of **29**

TABLE III. Biological Activities of Some Quinazolinylpyridazinone Derivatives

Compd. No.	Cardiotonic activity[a]				Ca^{2+} sensitizing effect[e]	
	Dose (mg/kg i.v.)	LVdP/dt max[b] (Δ%)	Relative[c] potency	Duration[d] (min)	Tension in skinned fiber (Δ%)[f] Drug concn. 10^{-4} M	10^{-5} M
Ia[g]	0.01	56.6±19.8	0.99	>60	7.5±2.3	
Ib[g]	0.01	51.3±9.6	1.31	>60	9.8±1.8	
Ic[g]	0.01	49.5±1.7	1.18	>60	NT[h]	
Id[g]	0.01	55.7±3.9	1.40	>60	NT	
Ie[d]	0.01	40.6±4.3	0.93	>60	20.4±3.3	
If	0.01	41.0±6.3	0.80	>60	35.3±4.8	
Ig	0.01	67.7±10.4	1.56	>60	52.2±8.6 (4)	
Ih	0.01	49.8±10.3	1.03	>60	NT	
Ii	0.01	47.6±6.4	1.41	>60	37.3±2.9	
Ik	0.01	46.9±2.3	0.95	>60	33.6±3.1	
Il	0.01	37.7±12.4	0.93	60	22.8±3.7	
Im	0.01	35.8±11.2	0.95	50	30.5±4.5	
In	0.01	20.2 (2)	0.50	20	16.8±1.0	
Io	0.01	35.5±7.3	1.01	>60	NT	
Ip	0.01	22.2±8.2	0.48	30	NT	
Iq	0.01	8.1 (2)	—	—	NT	
Ir	0.01	21.2 (2)	0.47	>60	NT	
Is	0.01	22.9±5.3	0.68	>60	NT	
It	0.01	4.6 (2)	—	—	NT	
Iu	0.01	28.6 (2)	0.63	>60	16.9±2.0	
Iw	0.01	45.9±8.7	1.05	>60	33.8±2.7	
Ix	0.01	54.5±3.0	1.61	>60	176.4±53.9 (5)	36.7±2.5 (4)
Iy	0.01	59.5±11.0	1.32	>60		16.0±4.2
Iz	0.01	43.0±7.4	1.27	>60		16.8±0.3
Iaa	0.01	42.5±6.8	1.09	>60		27.9±5.5 (4)
Iab	0.01	31.5±3.4	0.87	>50		15.4±2.6 (4)
Iac	0.01	43.5±5.4	1.20	>60		9.4±0.5 (4)
Iad	0.01	33.2±5.0	0.82	>60		10.7±2.6 (4)
Iae	0.01	61.5±10.2	1.45	>60		14.5±2.7
Iag	0.01	44.1±11.0	0.96	>60		10.0±3.1 (4)
Iaj	0.01	21.1±7.4	0.56	>60		14.4±0.8 (4)
Iak	0.01	30.4±3.2	0.69	>60	NT	
Ial	0.01	26.7±8.7	0.63	>60	36.5±5.3	
Iam	0.01	17.2 (2)	0.42	10	NT	
Ian	0.01	10.4 (2)	0.25	10	28.6±1.7	
Iap	0.01	54.1±9.5	1.12	>60		15.0±3.5
Iaq	0.01	2.9 (2)	—	—	NT	
Iar	0.01	39.1±7.9	0.96	>60	NT	
Ias	0.01	38.8±1.4	0.90	>60	19.0±3.2	
Iat[g]	0.01	16.2 (2)	0.35	40	NT	
Iau	0.01	40.3±9.6	0.89	>60	NT	
Iav	0.01	12.9 (2)	0.29	30	NT	
IIb	0.03	12.7 (2)	0.29	15	NT	
IIc	0.01	10.6 (2)	0.22	5	NT	
IId	0.01	3.3 (2)	—	—	NT	
IIe	0.01	4.6 (2)	—	—	NT	
IIg	0.03	42.3±5.1	1.09	>60	NT	
IIx	0.01	9.2±1.9	0.22	5	NT	
IIal	0.01	9.0 (2)	—	—	NT	
IIao	0.01	5.5 (2)	—	—	NT	
6	0.01	11.8 (2)	0.30	30	NT	
7	0.01	9.1 (2)	—	—	NT	
8	0.01	52.0±3.8	1.20	30	NT	
9	0.03	18.8 (2)	0.43	30	NT	
11	0.01	11.0±2.9	0.26	20	NT	
12	0.01	28.7±11.5	0.67	40	NT	
13	0.01	29.0±3.1	0.68	>60	NT	
15	0.01	5.2 (2)	—	—	NT	
16	0.01	64.7±3.1	1.60	30	NT	
19	0.01	4.8 (2)	—	—	NT	
20	0.01	−3.8 (2)	—	—	NT	
22	0.01	3.3 (2)	—	—	NT	
23	0.01	2.4 (2)	—	—	NT	
24	0.01	4.3 (2)	—	—	NT	
25	0.01	9.0 (2)	0.19	10	NT	

TABLE III. (continued)

Compd. No.	Cardiotonic activity[a]				Ca^{2+} sensitizing effect[e]	
	Dose (mg/kg i.v.)	LV$dP/dt$ max[b] (Δ%)	Relative[c] potency	Duration[d] (min)	Tension in skinned fiber (Δ%)[f] Drug concn. 10^{-4} M	10^{-5} M
26	0.01	14.3 (2)	0.30	20	NT	
28	0.01	11.4 (2)	0.25	10	NT	
29	0.01	17.2 (2)	0.50	10	NT	
30	0.01	22.8±5.1	0.56	60	NT	
31	0.01	13.7 (2)	0.40	30	NT	
PM[i]	0.03	8.2±1.6	—	—	68.2±6.8	16.1±1.5
Vehicle					10.1±2.7[j]	6.4±1.8[k]

a) In anesthetized dogs. *b*) Each value represents the mean±standard error of triplicate experiments except where otherwise noted in parentheses. *c*) Compared to the percent increase in LV$dP/dt$ max observed with Milrinone (0.03 mg/kg i.v.) in the same dog. *d*) Time (min) that an agent increases in LV$dP/dt$ more than 10% from the pretreatment control value. *e*) In skinned muscle fibers (see Experimental section). *f*) Each value represents the mean±standard error of triplicate experiments except where otherwise noted in parentheses. *g*) As HCl salt. *h*) NT: not tested. *i*) PM: pimobendan (**3**). *j*) 10 μg/ml of PEG 400. *k*) 5 μg/ml of PEG 400.

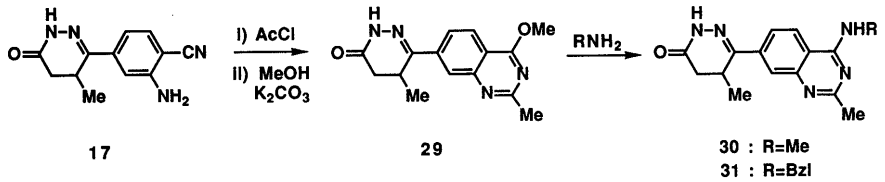


Chart 8

with methylamine and benzylamine gave the 2-methylamino (**30**) and the 2-benzylamino (**31**) derivatives, respectively (Chart 8).

Biological Results We initially examined the cardiotonic activities of these compounds in anesthetized open chest dogs using the procedures previously described.[14] The results of the test are shown in Table III. The cardiotonic activity of the compounds was determined by measuring percent increase in maximum dP/dt left ventricular pressure (LV$dP/dt$ max, Δ%) after i.v. administration (0.01 or 0.03 mg/kg) in anesthetized mongrel dogs of either sex (8—15 kg). The potency of cardiotonic activity of the test compounds was compared with milrinone (0.03 mg/kg).[15] Relative potency was calculated as the LV$dP/dt$ max of each compound to that of milrinone (milrinone = 1) in the same dogs.

The 3-substituted 4(3*H*)-quinazolinimines (II) generally exhibited weak activity except the *n*-butyl derivative (IIg).

We then examined the effects of the substituents at the 4-position of quinazolines on the cardiotonic activity. The methylthio (**8**) and the H (**16**) derivatives showed potent activity with short duration, and the thione (**7**) showed weak activity. Potent and long-lasting activity was observed on introduction of substituted amino groups (I). In a series of alkylamino quinazolines, introduction of an alkyl group having 1—5 carbon atoms (Ib, Ic, *etc.*) into the amino group led to potent and long-lasting activity. The activity of compounds having 6—8 carbon atoms (In, Ip, Iq, Ir) decreased with increasing length of the alkyl chain, except that of the cyclohexyl derivative (Io). The dimethyl derivative (Ias) retained the activity. Marked fall in the activity was observed on introduction of hydrophilic substituents such as hydroxy (Is), dimethylamino (It) and morpholino group (Iu) into the alkyl group. The benzyl derivatives (Ix—Iag) generally showed potent and long-lasting activity. Of these, Ix was one of the most potent (relative potency = 1.61) compounds in the series. The phenethyl (Iap) and pyridylethyl (Iar) derivatives retained the activity. Phenyl (Ial-Ian) and ethoxycarbonyl (Iav) groups led to relatively weak compounds.

Compounds **30** and **31**, which are the 2-methyl analogue of Ib and Ix, respectively, showed weak activity. Generally, introduction of substituents into the 2-position of the quinazoline ring led to diminished activity (**19**, **20**, **22**—**26**, **30**, **31**).

Consequently, the quinazolines having alkylamino and benzylamino groups at the 4-position generally showed potent and long-lasting cardiotonic activity.

Next, we examined the Ca^{2+} sensitizing effect of the quinazolinamines (I) in skinned muscle fibers[16] from guinea pig papillary muscles. The effects of the test (10^{-4} and 10^{-5} M) on the tension development (increased tension, Δ%) induced by pCa ($-\log[Ca^{2+}]$M) 5.8 in chemically skinned fibers are shown in Table III (method D). In this experiment, we used skinned fibers which had been pretreated with 250 μg/ml of saponin. It has been reported[17] that higher concentrations of saponin (> 150 μg/ml) destroy not only the surface membrane but also the sarcoplasmic reticulum (SR) membrane. Therefore, the influence by the Ca^{2+} uptake and release activities of the SR should have been minimal.

Effects of substituents at the 4-position of the quinazoline ring on Ca^{2+} sensitizing effect were also examined. In a series of 4-alkylamino derivatives, introduction of amino (Ia), methylamino (Ib), dimethylamino (Ias) and hexylamino (In) groups led to weak activity. An alkylamino group with 3—5 carbon atoms provided moderately potent compounds (Ie—Ig and Ii—Im). In these compounds, Ig showed the most potent activity, but the activity was weaker than pimobendan. Anilino (Ial, Ian) substituents

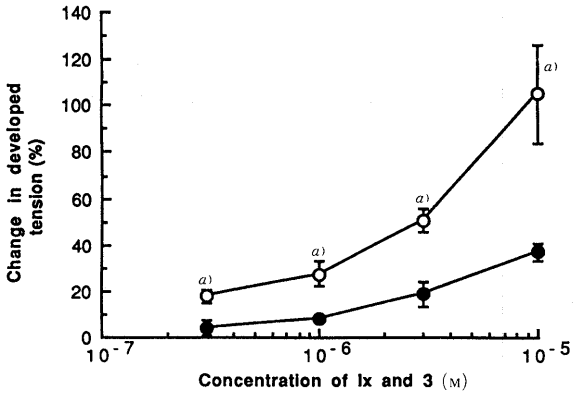


Fig. 1. Concentration–Response Curve for the Effect of Ix and **3** on Developed Tension

pCa = 6.0 (see Experimental section). Results are presented as mean ± SEM. —○—, Ix (KF15232); —●—, **3** (pimobendan). *a*) $p < 0.05$, when compared to **3** (Mann–Whitney U-test, $n = 5$).

also led to moderately active compounds.

The benzylamino derivative (Ix) increased the tension development by about 180% and 40% at the concentration of 10^{-4} and 10^{-5} M, respectively. Decrease of the activity was observed on introduction of substituents on benzyl moiety. Picolyl (Iaj) and phenethyl (Iap) derivatives also showed reduced activity.

The concentration-response curve for the effects of Ix and **3**[5] on the tension development induced by pCa 6.0 is shown in Fig. 1 (method E). The tension development was significantly increased by Ix (3×10^{-7} to 10^{-5} M) in a concentration-dependent manner. The activity of Ix was about 10 times more potent than 3. It seems likely that the concentrations of Ix required for this effect were similar to that for positive inotropic effect.

These results indicated that Ix exhibited the most potent inotropic and Ca^{2+} sensitizing effect. But it must be noted that the compounds which showed potent inotropic activity did not always exhibit potent Ca^{2+} sensitizing effect: there are differences of structural requirement between the two activities. Further studies are necessary to elucidate the definite mechanisms of the positive inotropic action of the compounds.

In summary, this paper has described structure–activity relationships of a novel series of 4,5-dihydro-5-methyl-6-(2- or 4-substituted 7-quinazolinyl)-3(2*H*)-pyridazinones for cardiotonic activity and Ca^{2+} sensitizing effect. Among these compounds, KF-15232 (Ix) was found to have the most potent inotropic and Ca^{2+} sensitizing activities. Further pharmacological and biochemical studies on this compound are in progress and will be reported elsewhere.

Experimental

All melting points were determined on a Büchi 510 micro melting point apparatus and are uncorrected. Infrared (IR) spectra were measured on a JASCO IR810 spectrophotometer. ^{1}H-NMR spectra were measured on a Hitachi R-90H and a JEOL JNM-GX-270 spectrometer using tetramethylsilane (TMS) as an internal standard. Mass spectra (MS) were run on a JEOL-JMS-01SG-2 and a JMS-SX102 spectrometer.

4,5-Dihydro-6-(3,4-dihydro-4-thioxo-7-quinazolinyl)-5-methyl-3(2H)-pyridazinone (7) H_2S gas was passed into a solution of **5**[1] (19.5 g, 68.6 mmol) and Et_3N (25 ml, 179 mmol) in pyridine (250 ml) for 30 min at room temperature with stirring. The precipitated crystals were collected by filtration, washed with water and dried to afford crude **7** (13.0 g, 70%). The crystals were used in the next reaction without further purification. An analytical sample was prepared by recrystallization from DMF–water, mp > 300 °C. *Anal.* Calcd for $C_{13}H_{12}N_4OS$: C, 57.34; H, 4.44; N, 20.57. Found: C, 57.60; H, 4.45; N, 20.60. IR (KBr): 1670, 1590, 1560 cm^{-1}. NMR (DMSO-d_6) δ: 13.89 (1H, br, SH), 11.25 (1H, s, NH), 8.56 (1H, d, J = 9 Hz, Ar-H), 8.20 (1H, s, Ar-H), 8.05 (2H, m, Ar-H), 3.53 (1H, m, CH), 2.80 (1H, dd, J = 7, 17 Hz, CH′H), 2.31 (1H, d, J = 17 Hz, CH′H), 1.11 (3H, d, J = 7 Hz, CH_3). MS *m/z*: 272 (M^+).

4,5-Dihydro-5-methyl-6-(4-methylthio-7-quinazolinyl)-3(2*H*)-pyridazinone (8) A mixture of **7** (13.0 g, 47.7 mmol), MeI (4.0 ml, 64.2 mmol) and 2N NaOH (30.0 ml) in DMF (100 ml) was stirred for 30 min at room temperature. The reaction mixture was concentrated under reduced pressure and mixed with water. The precipitated crystals were collected by filtration, washed with water and dried to afford crude **8** (10.3 g, 75%). The crystals were used in the next reaction without further purification. An analytical sample was prepared by recrystallization from DMF–water, mp 235 °C. *Anal.* Calcd for $C_{14}H_{14}N_4OS$: C, 58.72; H, 4.93; N, 19.57. Found: C, 58.85; H, 5.06; N, 19.80. IR (KBr): 1680, 1610, 1550 cm^{-1}. NMR (DMSO-d_6) δ: 11.12 (1H, s, NH), 8.96 (1H, s, Ar-H), 8.12 (3H, m, Ar-H), 3.55 (1H, m, CH), 2.85 (1H, dd, J = 7, 17 Hz, CH′H), 2.68 (3H, s, SCH_3), 2.28 (1H, d, J = 17 Hz, CH′H), 1.11 (3H, d, J = 7 Hz, $CHCH_3$). MS *m/z*: 286 (M^+).

General Procedure for the Synthesis of 4,5-Dihydro-5-methyl-6-(3-substituted 3,4-Dihydro-4-imino-7-quinazolinyl)-3(2*H*)-pyridazinones. Method A. 4,5-Dihydro-6-(3,4-dihydro-4-imino-3-methyl-7-quinazolinyl)-5-methyl-3(2*H*)-pyridazinone (11b)[1] A mixture of **5** (1.5 g, 5.3 mmol) and 40% $MeNH_2$/MeOH (40 ml) was stirred for 30 min at 50 °C. The precipitated crystals were collected by filtration, washed with MeOH and dried to give crude IIb (0.9 g, 63%) which was recrystallized from DMF–water for analysis.

4,5-Dihydro-6-(2,3-dihydroimidazo[1,2-*c*]quinazolin-8-yl)-5-methyl-3(2*H*)-pyridazinone (6) Compound **6** (0.60 g, 61%) was prepared from **5** (1.0 g, 3.6 mmol) with ethylenediamine (3.0 ml) as described for IIb, mp 280—282 °C. *Anal.* Calcd for $C_{15}H_{15}N_5O \cdot 1/2H_2O$: C, 62.06; H, 5.55; N, 24.12. Found: C, 61.84; H, 5.93; N, 23.97. IR (KBr): 1680, 1620, 1600 cm^{-1}. NMR (DMSO-d_6) δ: 11.20 (1H, s, NH), 8.01 (1H, s, Ar-H), 7.90 (1H, d, J = 8 Hz, Ar-H), 7.78 (1H, d, J = 8 Hz, Ar-H) 7.73 (1H, s, Ar-H), 4.13 (2H, t, J = 8 Hz, CH_2), 3.95 (2H, t, J = 8 Hz, CH_2), 3.47 (1H, m, CH), 2.78 (1H, dd, J = 7, 17 Hz, CHCH′H), 2.30 (1H, d, J = 17 Hz, CHCH′H), 1.12 (3H, d, J = 8 Hz, CH_3). MS *m/z*: 281 (M^+).

General Procedure for the Synthesis of 4,5-Dihydro-5-methyl-6-(4-substituted Amino-7-quinazolinyl)-3(2*H*)-pyridazinones. Method B. 4,5-Dihydro-5-methyl-6-(4-methylamino-7-quinazolinyl)-3(2*H*)-pyridazinone (Ib)[1] A mixture of IIb (0.29 g, 1.1 mmol) and 2N-NaOH (10 ml) was stirred for 5 h at 100 °C. The reaction mixture was neutralized with 1N HCl and the precipitated crystals were collected by filtration, washed successively with water and MeOH and dried to give crude Ib (0.22 g, 76%) which was recrystallized from DMSO–water for analysis.

Method C. 4,5-Dihydro-6-(4-benzylamino-7-quinazolinyl)-3(2*H*)-pyridazinone (Ix) A solution of **8** (0.29 g, 1.0 mmol) and benzylamine (0.33 ml, 3.0 mmol) in DMSO (10 ml) was stirred for 14 h at 170 °C under N_2 atmosphere. Water (20 ml) was added to the mixture and the precipitated crystals were collected by filtration, washed with water and dried to afford crude Ix (0.16 g, 46%) which was recrystallized from DMF–water for analysis. IR (KBr): 1680, 1620, 1580 cm^{-1}. NMR (DMSO-d_6) δ: 11.18 (1H, s, CONH), 8.85 (1H, br, NH), 8.44 (1H, s, Ar-H), 8.29 (1H, d, J = 8 Hz, Ar-H), 7.96 (2H, m, Ar-H), 7.30 (5H, br, benzyl), 4.78 (2H, d, J = 7 Hz, CH_2), 3.55 (1H, m, CH), 2.78 (1H, dd, J = 7, 17 Hz, CHCH′H), 2.25 (1H, d, J = 17 Hz, CHCH′H), 1.12 (3H, d, J = 8 Hz, CH_3). MS *m/z*: 345 (M^+).

4,5-Dihydro-5-methyl-6-(4-ethoxycarbonylamino-7-quinazolinyl)-3(2*H*)-pyridazinone (Iav) Ethyl chloroformate (0.10 ml, 1.6 mmol) was added to a solution of Ia (0.20 g, 0.78 mmol) in pyridine (5 ml) and the resultant solution was stirred for 3 h at room temperature, then concentrated. The residue was mixed with water and the precipitated crystals were collected by filtration, washed with water and dried to afford crude Iav (0.21 g, 82%) which was recrystallized from DMF–water for analysis. IR (KBr): 1690, 1620, 1600 cm^{-1}. NMR (DMSO-d_6) δ: 11.22 (1H, s, NH), 10.73 (1H, s, OCONH), 8.98 (1H, s, Ar-H), 8.36 (1H, d, J = 8 Hz, Ar-H), 8.18 (1H, s, Ar-H), 8.13 (1H, d, J = 8 Hz, Ar-H), 4.27 (2H, q, J = 7 Hz, CH_2CH_3), 3.62 (1H, m, CH), 2.78 (1H, dd, J = 7, 17 Hz, CHCH′H), 2.32 (1H, d, J = 17 Hz, CHCH′H), 1.31 (3H, t, J = 7 Hz, CH_2CH_3), 1.15 (3H, d, J = 8 Hz, CH_3). MS *m/z*: 328 ($M^+ + 1$) (Fast atom bombardment method).

4,5-Dihydro-5-methyl-6-[4-(*p*-hydroxybenzylamino)quinazolin-7-yl]-3-(2*H*)-pyridazinone (Iai) A suspension of Iah (0.40 g, 0.99 mmol) in 3N-HCl (3 ml) was stirred for 8 h at 60 °C. The precipitated crystals were

collected by filtration, washed with water and dried to give crude Iai (0.36 g, 93%) as HCl salt. An analytical sample was prepared by recrystallization from DMF–water. IR (KBr): 1690, 1620, 1600 cm^{-1}. NMR (DMSO-d_6) δ: 11.15 (1H, s, NH), 10.50 (1H, br, OH), 8.88 (1H, s, Ar-H), 8.75 (1H, d, J=8 Hz, Ar-H), 8.08 (1H, d, J=8 Hz. Ar-H), 8.25 (1H, s, Ar-H), 7.22 (2H, d, J=8 Hz, benzyl), 6.73 (2H, d, J=8 Hz, benzyl), 4.80 (2H, br, NHC$\underline{H}_2$), 3.47 (1H, m, CH), 2.78 (1H, dd, J=7, 17 Hz, CHC$\underline{H}$'H), 2.32 (1H, d, J=17 Hz, CHCH'$\underline{H}$), 1.15 (3H, d, J=8 Hz, CH_3). MS m/z: 361 (M^+).

4,5-Dihydro-5-methyl-6-(9-*s*-triazolo[1,5-*c*]quinazolinyl)-3(2*H*)-pyridazinone (9) A suspension of IIax (0.35 g, 1.3 mmol) in triethyl orthoformate (10 ml) was stirred for 3 h at 150 °C, then concentrated. Water was added to the residue and the precipitated crystals were collected by filtration, washed with water and dried to afford crude **9** (0.28 g, 77%), which was recrystallized from DMF–water for analysis, mp 271—273 °C. *Anal.* Calcd for $C_{14}H_{12}N_6O$: C, 59.99; H, 4.32; N, 29.98. Found: C, 60.05; H, 4.40; N, 29.89. IR (KBr): 1700, 1640, 1620 cm^{-1}. NMR (DMSO-d_6) δ: 11.24 (1H, s, NH), 9.68 (1H, s, Ar-H), 8.72 (1H, s, Ar-H), 8.48 (1H, d, J=8 Hz, Ar-H), 8.23 (1H, d, J=8 Hz. Ar-H), 8.32 (1H, s, Ar-H), 3.67 (1H, m, CH), 2.83 (1H, dd, J=7, 17 Hz, C$\underline{H}$'H), 2.38 (1H, d, J=17 Hz, CH'$\underline{H}$), 1.18 (3H, d, J=8 Hz, CH_3). MS m/z: 280 (M^+).

Diethyl [7-(2,3,4,5-Tetrahydro-5-methyl-3-oxo-6-pyridazinyl)-4-quinazolinyl]thiomalonate (11) Diethyl bromomalonate (2.4 ml, 14 mmol) was added to a suspension of **7** (3.7 g, 14 mmol) and Et_3N (13.8 ml, 100 mmol) in DMF (100 ml) and the reaction mixture was stirred for 2 h at room temperature, then concentrated under reduced pressure. The resultant residue was partitioned between water and $CHCl_3$, and the organic layer was washed with water, dried over $MgSO_4$ and evaporated to dryness. The residue was purified by column chromatography (SiO_2, 150 g, 10% MeOH–$CHCl_3$) to afford **11** (3.4 g, 59%). An analytical sample was prepared by recrystallization from DMF–water, mp 164 °C. *Anal.* Calcd for $C_{20}H_{22}N_4O_5S$: C, 55.79; H, 5.16; N, 13.01. Found: C, 56.01; H, 5.35; N, 12.65. IR (KBr): 1740, 1690 cm^{-1}. NMR (DMSO-d_6) δ: 11.30 (1H, s, NH), 9.00 (1H, s, Ar-H), 8.25 (2H, m, Ar-H), 8.18 (1H, d, J=8 Hz, Ar-H), 5.82 (1H, s, COCHCO), 4.26 (4H, q, J=7 Hz, C$\underline{H}_2$CH$_3$), 3.61 (1H, m, CH), 2.80 (1H, dd, J=7, 17 Hz, CHC$\underline{H}$'H), 2.32 (1H, d, J=17 Hz, CHCH'$\underline{H}$), 1.24 (6H, t, J=7 Hz, CH$_2$C$\underline{H}_3$), 1.18 (3H, d, J=8 Hz, CH_3). MS m/z: 430 (M^+).

Diethyl [7-(2,3,4,5-Tetrahydro-5-methyl-3-oxo-6-pyridazinyl)-4-quinazolinyl]malonate (12) A mixture of **11** (2.7 g, 6.3 mmol), Ph_3P (1.9 g, 7.2 mmol) and K_2CO_3 (5.7 g, 41 mmol) in DMF (20 ml) was stirred for 3 h at 120 °C. After cooling, the reaction mixture was partitioned between AcOEt and water, then the water layer was acidified to pH 4.0 with 1 N-HCl. The precipitated crystals were collected by filtration, washed with water and dried to afford crude **12** (1.4 g, 56%) which was recrystallized from EtOH–Et_2O for analysis, mp 173—175 °C. *Anal.* Calcd for $C_{20}H_{22}N_4O_5$: C, 60.29; H, 5.57; N, 14.06. Found: C, 60.66; H, 5.68; N, 14.26. IR (KBr): 1720, 1680 cm^{-1}. NMR (DMSO-d_6) (pD=5.6) δ: 12.91 (0.5H, br s, quinazoline NH of **12a**), 11.30, 11.20 (0.5H, each s, pyridazinone CONH of **12a**, **12b**), 9.30 (0.5H, s, quinazoline 2-H of **12b**), 8.32 (0.5H, s, quinazoline 8-H of **12b**), 8.24, 8.16 (0.5H, each d, J=9 Hz, quinazoline 5, 6-H of **12b**), 8.22 (0.5H, s, quinazoline 2-H of **12a**), 7.89, 7.62 (0.5H, each d, J=9 Hz, quinazoline 5, 6-H of **12a**), 6.20 (0.5H, s, COCHCO of **12b**), 4.24, 4.22 (2H, each q, J=7 Hz, C$\underline{H}_2$CH$_3$), 3.66, 3.49 (0.5H, each m, C$\underline{H}$CH$_3$), 2.82, 2.76 (0.5H, each dd, J=7, 17 Hz, CHC$\underline{H}$'H), 2.33, 2.28 (0.5H each, d, J=17 Hz, CHCH'$\underline{H}$), 1.24, 1.19 (3H each, t, J=7 Hz, CH$_2$C$\underline{H}_3$), 1.15, 1.10 (1.5H, each d, J=7 Hz, CHC$\underline{H}_3$). NMR (DMSO-d_6–D_2O–NaOD) (pD=14.9) δ: 8.88 (1H, s, quinazoline 2-H of **12b**), 8.12 (1H, s, quinazoline 8-H of **12b**), 8.00, 7.98 (1H, d, J=9 Hz, quinazoline 5,6-H of **12b**), 3.94 (4H, q, J=7 Hz, C$\underline{H}_2$CH$_3$ of **12b**), 3.54 (1H, m, CH of **12b**), 2.83 (1H, dd, J=7, 17 Hz, CHC$\underline{H}$'H of **12b**), 2.43 (1H, d, J=17 Hz, CHCH'$\underline{H}$ of **12b**), 1.22 (3H, d, J=7 Hz, CH_3 of 12b), 1.00 (6H, t, J=7 Hz, CH$_2$C$\underline{H}_3$ of **12b**). MS m/z: 398 (M^+).

4,5-Dihydro-5-methyl-6-(4-methyl-7-quinazolinyl)-3(2*H*)-pyridazinone (13) A mixture of **12** (0.77 g, 1.9 mmol) and 2 N NaOH (7.0 ml) in EtOH (20 ml) was stirred for 2 h at 90 °C. The reaction mixture was neutralized with 1 N HCl, then concentrated. The resultant residue was partitioned between $CHCl_3$ and water, then the organic layer was dried over $MgSO_4$ and concentrated to give residual crystals of **13** which were recrystallized from DMF–water to afford pure **13** (0.34 g, 70%), mp 218—221 °C. *Anal.* Calcd for $C_{14}H_{14}N_4O$: C, 66.12; H, 5.55; N, 22.03. Found: C, 66.31; H, 5.71; N, 21.98. IR (KBr): 1690, 1620 cm^{-1}. NMR (DMSO-d_6) δ: 11.14 (1H, s, NH), 9.08 (1H, s, Ar-H), 8.18 (3H, s, Ar-H), 3.60 (1H, m, CH), 2.90 (3H, s, CH_3), 2.78 (1H, dd, J=7, 17 Hz, C$\underline{H}$'H), 2.32 (1H, d, J=17 Hz, CH'$\underline{H}$), 1.15 (3H, d, J=8 Hz, CHC$\underline{H}_3$). MS m/z: 254 (M^+).

4,5-Dihydro-6-(3,4-dihydro-7-quinazolinyl)-5-methyl-3(2*H*)-pyridazinone (15) A mixture of **8** (1.0 g, 3.5 mmol) and Raney-Ni (W-2) (5.0 g) in DMF (10 ml) was stirred for 1 h at 60 °C. After removal of the catalyst by filtration, the mixture was concentrated under reduced pressure. The precipitated crystals were collected by filtration, washed with water and dried to afford crude **15** (0.22 g, 26%) which was recrystallized from DMF–water for analysis, mp 263 °C (dec.). *Anal.* Calcd for $C_{13}H_{14}N_4O$: C, 64.45; H, 5.82; N, 23.12. Found: C, 64.63; H, 5.96; N, 22.98. IR (KBr): 1680, 1600 cm^{-1}. NMR (DMSO-d_6) δ: 10.88 (1H, s, CONH), 8.20 (1H, br s, NH), 7.27 (1H, d, J=8 Hz, Ar-H), 7.12 (2H, s, Ar-H), 6.89 (1H, d, J=8 Hz, Ar-H), 4.48 (2H, s, NHC$\underline{H}_2$), 3.28 (1H, m, CH), 2.68 (1H, dd, J=7, 17 Hz, CHC$\underline{H}$'H), 2.20 (1H, d, J=17 Hz, CHCH'$\underline{H}$), 1.06 (3H, d, J=8 Hz, CH_3). MS m/z: 242 (M^+).

4,5-Dihydro-5-methyl-6-(7-quinazolinyl)-3(2*H*)-pyridazinone (16) A mixture of **15** (0.25 g, 1.0 mmol) and MnO_2 (2.0 g) in $CHCl_3$ (20 ml) was stirred for 1 h at room temperature. The oxidant was filtered off, then the resultant solution was concentrated. The residue was crystallized from Et_2O to afford crude **16** which was recrystallized from DMF–water to give pure **16** (0.20 g, 83%), mp 224—225 °C. *Anal.* Calcd for $C_{13}H_{12}N_4O$: C, 64.98; H, 5.04; N, 23.31. Found: C, 65.23; H, 4.97; N, 23.02. IR (KBr): 1680, 1620 cm^{-1}. NMR (DMSO-d_6) δ: 11.22 (1H, s, NH), 9.60 (1H, s, Ar-H), 9.29 (1H, s, Ar-H), 8.20 (3H, m, Ar-H), 3.62 (1H, m, CH), 2.78 (1H, dd, J=7, 17 Hz, C$\underline{H}$'H), 2.37 (1H, d, J=17 Hz, CH'$\underline{H}$), 1.16 (3H, d, J=8 Hz, CH_3). MS m/z: 240 (M^+).

4-(2,3,4,5-Tetrahydro-5-methyl-3-oxo-6-pyridazinyl)-*N*-(ethoxycarbonyl)anthranilonitrile (18) Ethyl chloroformate (2.3 ml, 24 mmol) was added to a solution of **17** (4.5 g, 20 mmol) in pyridine (40 ml), then the solution was stirred for 30 h at room temperature. The reaction mixture was concentrated and mixed with water. The precipitated crystals were collected by filtrateion to afford crude **18** (3.0 g, 50%). An analytical sample was prepared by recrystallization from DMF–water, mp 178–181 °C. *Anal.* Calcd for $C_{15}H_{16}N_4O_3$: C, 59.98; H, 5.38; N, 18.65. Found: C, 59.72; H, 5.42; N, 18.23. IR (KBr): 2205, 1740, 1680 cm^{-1}. NMR (DMSO-d_6) δ: 11.21 (1H, s, NH), 9.67 (1H, s, NH), 7.90 (1H, s, Ar-H), 7.79 (1H, d, J=7 Hz, Ar-H), 7.68 (1H, d, J=7 Hz, Ar-H), 4.17 (2H, q, J=7 Hz, C$\underline{H}_2$CH$_3$), 3.50 (1H, m, CH), 2.72 (1H, dd, J=7, 17 Hz, C$\underline{H}$'H), 2.30 (1H, d, J=17 Hz, CH'$\underline{H}$), 1.28 (3H, t, J=7 Hz, CH$_2$C$\underline{H}_3$), 1.12 (3H, d, J=8 Hz, CHC$\underline{H}_3$). MS m/z: 300 (M^+).

4,5-Dihydro-5-methyl-6-(1,2,3,4-tetrahydro-4-imino-3-methyl-2-oxo-7-quinazolinyl)-3(2*H*)-pyridazinone (19) To a solution **18** (0.30 g, 1.0 mmol) in DMSO (5 ml) was added dropwise 40% $MeNH_2$–MeOH solution (1.0 ml) within a 2 h period at 150 °C with stirring. After cooling, water (20 ml) was added to the solution and the precipitated crystals were collected by filtration, washed with water and dried to afford crude **19** which was recrystallized from DMF–water to give pure **19** (0.20 g, 74%), mp 271—273 °C. *Anal.* Calcd for $C_{14}H_{15}N_5O_2 \cdot H_2O$: C, 55.43; H, 5.66; N, 23.08. Found: C, 55.68; H, 5.45; N, 23.06. IR (KBr); 1680, 1620 cm^{-1}. NMR (DMSO-d_6) δ: 11.12 (1H, s, NH), 10.81 (1H, br, NH), 8.88 (1H, br, NH), 8.12 (1H, d, J=9 Hz, Ar-H), 7.51 (2H, m, Ar-H), 3.40 (1H, m, CH), 3.34 (3H, s, NCH_3), 2.75 (1H, dd, J=7, 17 Hz, C$\underline{H}$'H), 2.30 (1H, d, J=17 Hz, CH'$\underline{H}$), 1.09 (3H, d, J=7 Hz, CH_3). MS m/z: 285 (M^+).

4,5-Dihydro-6-(1,2-dihydro-4-amino-2-oxo-7-quinazolinyl)-5-methyl-3(2*H*)-pyridazinone (20) Compound **20** (0.20 g, 74%) was prepared from **18** (0.30 g, 1.0 mmol) with ammonia gas (passes into the reaction mixture) as described for **19**, mp 230—236 °C. *Anal.* Calcd for $C_{13}H_{13}N_5O_2 \cdot H_2O$: C, 53.97; H, 5.23; N, 24.21. Found: C, 53.85; H, 5.63; N, 24.01. IR (KBr): 1680, 1620 cm^{-1}. NMR (DMSO-d_6) δ: 11.10 (1H, s, NH), 7.98 (1H, d, J=8 Hz, Ar-H), 7.80 (3H, br, NH_2, NH), 7.50 (1H, s, Ar-H), 7.48 (1H, d, J=8 Hz, Ar-H), 3.40 (1H, m, CH), 2.70 (1H, dd, J=7, 17 Hz, C$\underline{H}$'H), 2.31 (1H, d, J=17 Hz, CH'$\underline{H}$), 1.08 (3H, d, J=8 Hz, CH_3). MS m/z: 271 (M^+).

4,5-Dihydro-5-methyl-6-(2,3,5,6-tetrahydro-5-oxo-8-imidazo[1,2-c]quinazolinyl)-3(2*H*)-pyridazinone (22) Compound **22** (0.20 g, 67%) was prepared from **18** (0.30 g, 1.0 mmol) with ethylenediamine (1.0 ml) at described for **19**, mp >300 °C. *Anal.* Calcd for $C_{15}H_{15}N_5O_2$: C, 60.60; H, 5.09; N, 23.56. Found: C, 60.83; H, 5.25; N, 23.34. IR (KBr): 1700, 1620 cm^{-1}. NMR (DMSO-d_6) δ: 10.88 (1H, s, NH), 7.90 (1H, br s, NH), 7.85 (1H, d, J=8 Hz, Ar-H), 7.50 (2H, m, Ar-H), 3.92 (4H, m, CH_2CH_2), 3.45 (1H, m, CH), 2.68 (1H, dd, J=7, 17 Hz, CHC$\underline{H}$'H), 2.30 (1H, d, J=17 Hz, CHCH'$\underline{H}$), 1.12 (3H, d, J=8 Hz, CH_3). MS m/z: 297 (M^+).

4,5-Dihydro-6-(2,4-dimercapto-7-quinazolinyl)-5-methyl-3(2*H*)-pyridazinone (23) A mixture of **17** (4.5 g, 20 mmol) and CS_2 (5.0 ml) in pyridine (40 ml) was stirred for 4 h at 100 °C. Water (150 ml) was added to the reaction mixture, then precipitated crystals were collected by filtration, washed with water and dried to give crude **23** (5.5 g, 89%). An

analytical sample was prepared by recrystallization from DMF–water, mp>300°C. *Anal.* Calcd for $C_{13}H_{12}N_4OS_2 \cdot 1/2H_2O$: C, 49.82; H, 4.19; N, 17.87. Found: C, 50.13; H, 4.03; N, 17.85. IR (KBr); 1670, 1620 cm^{-1}. NMR (DMSO-d_6) δ: 13.58 (1H, s, SH), 13.01 (1H, s, SH), 11.22 (1H, s, NH), 8.26 (1H, d, J=8 HZ, Ar-H), 7.73 (1H, s, Ar-H), 7.68 (1H, d, J=8 Hz, Ar-H), 3.40 (1H, m, CH), 2.78 (1H, dd, J=7, 17 Hz, CH'H), 2.34 (1H, d, J=17 Hz, CH'H), 1.14 (3H, d, J=7 Hz, CH_3). MS m/z: 304 (M^+).

4,5-Dihydro-6-(2,4-bismethylthio-7-quinazolinyl)-5-methyl-3(2*H*)-pyridazinone (24) MeI (0.40 ml, 6.4 mmol) was added to a mixture of **23** (0.9 g, 2.9 mmol) and 2N NaOH (2.0 ml) in MeOH (30 ml), then the mixture was stirred for 1 h at room temperature. Water (60 ml) was added to the mixture and the precipitated crystals were collected by filtration, washed with water and dried to give crude **24** (0.87 g, 89%). An analytical sample was preapared by recrystallization from DMF–water, mp 245—246°C. *Anal.* Calcd for $C_{15}H_{16}N_4OS_2$: C, 54.19; H, 4.85; N, 16.85. Found: C, 53.92; H, 4.87; N, 16.68. IR (KBr): 1700, 1610 cm^{-1}. NMR (DMSO-d_6) δ: 11.18 (1H, s, NH), 7.92 (3H, s, Ar-H), 3.50 (1H, m, CH), 2.70 (1H, dd, J=7, 17 Hz, CH'H), 2.62, 2.59 (3H, each s, SCH_3), 2.32 (1H, d, J=17 Hz, CH'H), 1.09 (3H, d, J=8 Hz, $CHCH_3$). MS m/z: 332 (M^+).

4,5-Dihydro-5-methyl-6-(4-methylamino-2-methylthio-7-quinazolinyl)-3(2*H*)-pyridazinone (25) A mixture of **24** (4.9 g, 16 mmol) in 40% $MeNH_2$–MeOH solution (40 ml) was stirred for 30 min at 60°C. The reaction mixture was concentrated under reduced pressure, then the residue was mixed with weter. The precipitated crystals were collected by filtration, washed with water and dried to give crude **25** which was recrystallized from DMF–water to give pure **25** (1.4 g, 29%), mp 137—138°C. *Anal.* Calcd for $C_{15}H_{17}N_5OS \cdot H_2O$: C, 54.04; H, 5.74; N, 21.00. Found: C, 54.35; H, 5.51; N, 21.13. IR (KBr): 1680, 1620 cm^{-1}. NMR (DMSO-d_6) δ: 11.10 (1H, s, NH), 8.35 (1H, br, NH), 8.12 (1H, d, J=8 Hz, 5-H of quinazoline), 7.78 (1H, d, J=8 Hz, 6-H of quinazoline), 7.76 (1H, s, 8-H of quinazoline), 3.51 (1H, m, CH), 3.01 (3H, d, J=6 Hz, $NHCH_3$), 2.74 (1H, dd, J=7, 17 Hz, CH'H), 2.53 (3H, s, SCH_3), 2.30 (1H, d, J=17 Hz, CH'H), 1.12 (3H, d, J=8 Hz, $CHCH_3$), NOE 13% NCH_3 (δ 3.01)—5-H of quinazoline (δ 8.12). MS m/z: 315 (M^+).

4,5-Dihydro-5-methyl-6-(2-methylthio-4-propylamino-7-quinazolinyl)-3(2*H*)-pyridazinone (26) Compound **26** (0.12 g, 39%) was prepared from **24** (0.40 g, 1.2 mmol) with 40% propylamine–MeOH (50 ml) as described for **25**, mp 199°C. *Anal.* Calcd for $C_{17}H_{24}N_5OS$: C, 58.93; H, 6.98; N, 20.21. Found: C, 59.28; H, 6.78; N, 20.19. IR (KBr): 1670, 1620 cm^{-1}. NMR (DMSO-d_6) δ: 11.14 (1H, s, NH), 8.37 (1H, t, J=6 Hz, NH), 8.18 (1H, d, J=9 Hz, Ar-H), 7.83 (1H, d, J=9 Hz, Ar-H), 7.81 (1H, s, Ar-H), 3.50 (3H, m, CH, NCH_2), 2.68 (1H, dd, J=7, 17 Hz, CHCH'H), 2.52 (3H, s, SCH_3), 2.28 (1H, d, J=17 Hz, CHCH'H), 1.68 (2H, m, CH_2CH_3), 1.12 (3H, d, J=8 Hz, $CHCH_3$), 0.95 (3H, t, J=7 Hz, CH_2CH_3). MS m/z: 343 (M^+).

4,5-Dihydro-5-methyl-6-(2-methylamino-4-propylamino-7-quinazolinyl)-3(2*H*)-pyridazinone (27) A mixture of **26** (0.40 g, 1.2 mmol) and $KMnO_4$ (0.50 g, 3.2 mmol) in 50% AcOH (20 ml) was stirred for 10 min at 5—10°C. Water was added to the mixture, then the mixture was extracted with $CHCl_3$ (20 ml × 5). The extract was added to 40% $MeNH_2$–MeOH solution (50 ml) and the whole was stirred for 10 h at 60°C. The mixture was concentrated under reduced pressure, then the resultant residue was partitioned between $CHCl_3$ and water. The organic layer was washed with water, dried over $MgSO_4$ then evaporated. The residual crystals were recrystallized from DMF–water to give pure **27** (0.27 g, 69%), mp 225—231°C. *Anal.* Calcd for $C_{17}H_{22}N_6O \cdot 1/2H_2O$: C, 60.88; H, 6.91; N, 25.06. Found: C, 61.14; H, 6.98; N, 24.70. IR (KBr): 1680, 1600 cm^{-1}. NMR (DMSO-d_6) δ: 11.13 (1H, s, NH), 7.90 (1H, d, J=8 Hz, Ar-H), 7.75 (1H, s, NH), 7.45 (1H, s, Ar-H), 7.40 (1H, d, J=8 Hz, Ar-H), 6.41 (1H, br, NH), 3.40 (6H, m, CH, $NHCH_3$, $NHCH_2$), 2.67 (1H, dd, J=7, 17 Hz, CHCH'H), 2.28 (1H, d, J=17 Hz, CHCH'H), 1.63 (2H, m, CH_2CH_3), 1.09 (3H, d, J=8 Hz, $CHCH_3$), 0.90 (3H, t, J=8 Hz, CH_2CH_3). MS m/z: 326 (M^+).

4,5-Dihydro-6-(4-methoxy-2-methyl-7-quinazolinyl)-5-methyl-3(2*H*)-pyridazinone (29) Acetyl chloride (3.0 ml, 42 mmol) was added to a solution of **17** (3.0 g, 14 mmol) in pyridine (30 ml), then the mixture was stirred for 3 h at 80°C. The reaction mixture was concentrated and the residue was partitioned between $CHCl_3$ and water. The organic layer was washed with water, dried over $MgSO_4$ and evporated. A mixture of K_2CO_3 (2.0 g, 14 mmol) in 50% MeOH (100 ml) was added to the residue, then the whole was stirred for 3 h at 80°C. The reaction mixture was concentrated under reduced pressure and the precipitated crystals were collected by filtration, washed with water and dried to give crude **29** (1.1 g, 28%). An analytical sample was prepared by recrystallization from DMF–water, mp 232°C (dec.). *Anal.* Calcd for $C_{15}H_{16}N_4O_2$: C, 63.36; H, 5.68; N, 19.70. Found: C, 63.02; H, 5.73; N, 19.82. IR (KBr): 1690, 1620 cm^{-1}. NMR (DMSO-d_6) δ: 11.22 (1H, s, NH), 8.09 (3H, s, Ar-H), 4.12 (3H, s, OCH_3), 3.58 (1H, m, CH), 2.76 (1H, dd, J=7, 17 Hz, CH'H), 2.64 (3H, s, CH_3), 2.30 (1H, d, J=17 Hz, CH'H), 1.09 (3H, d, J=8 Hz, $CHCH_3$). MS m/z: 284 (m^+).

4,5-Dihydro-6-(4-benzylamino-2-methyl-7-quinazolinyl)-5-methyl-3-(2*H*)-pyridazinone (31) A mixture of **29** (0.76 g, 2.7 mmol) and benzylamine (1.5 ml) in DMSO (15 ml) was stirred for 3 h at 150°C. After cooling, the mixture was concentrated and the residue was partitioned between $CHCl_3$ and water. The organic layer was washed with water, dried over $MgSO_4$ and evaporated. The resultant residue was purified by column chromatography (SiO_2, 100 g, 8% MeOH–$CHCl_3$) to afford **31** (0.31 g, 32%) which was recrystallized from DMF–water for analysis, mp 115°C (dec.). *Anal.* Calcd for $C_{21}H_{21}N_5O \cdot H_2O$: C, 66.82; H, 6.15; N, 18.55. Found: C, 66.53; H, 6.18; N, 18.39. IR (KBr): 1680, 1570 cm^{-1}. NMR (DMSO-d_6) δ: 10.88 (1H, s, NH), 8.67 (1H, br s, NH), 8.22 (1H, d, J=8 Hz, Ar-H), 7.86 (2H, m, Ar-H), 7.31 (5H, br, benzyl), 4.86 (2H, d, J=8 Hz, $NHCH_2$), 3.52 (1H, m, CH), 2.68 (1H, dd, J=7, 17 Hz, CHCH'H), 2.43 (3H, s, CH_3), 2.27 (1H, d, J=17 Hz, CHCH'H), 1.12 (3H, d, J=8 Hz, $CHCH_3$). MS m/z: 359 (M^+).

4,5-Dihydro-5-methyl-6-(2-methyl-4-methylamino-7-quinazolinyl)-3(2*H*)-pyridazinone (30) Compound **30** (0.38 g, 76%) was prepared from **29** (0.50 g, 1.8 mmol) with 40% methylamine–MeOH (20 ml) as described for **31**, mp 151—153°C (dec.). *Anal.* Calcd for $C_{15}H_{17}N_5O \cdot H_2O$: C, 59.79; H, 6.36; N, 23.24. Found: C, 59.75; H, 6.48; N, 22.94. IR (KBr): 1720, 1590 cm^{-1}. NMR (DMSO-d_6) δ: 11.13 (1H, s, NH), 8.23 (1H, br, NH), 8.14 (1H, d, J=8 Hz, Ar-H), 7.88 (1H, s, Ar-H), 7.85 (1H, d, J=8 Hz, Ar-H), 3.52 (1H, m, CH), 3.00 (3H, d, J=6 Hz, $NHCH_3$), 2.74 (1H, dd, J=7, 17 Hz, CH'H), 2.48 (3H, s, CH_3), 2.28 (1H, d, J=17 Hz, CH'H), 1.09 (3H, d, J=8 Hz, $CHCH_3$). MS m/z: 283 (M^+).

Ca^{2+} Sensitizing Effect 1) Method D[16]: Male guinea pigs (Hartley) weighing 300—400 g were killed by a blow on the head, and the hearts were removed. The papillary muscle was isolated from the right ventricle in a bath containing "relaxing solution (Ca^{2+} free)" of the following composition (mM): KSO_3CH_3, 118; Mg $(SO_3CH_3)_2$, 5; ATP-Na_2, 5.3; ethyleneglycolbis (β-aminoethylether)-*N*,*N*'-tetraacetic acid (EGTA), 2; piperazine-*N*,*N*'-bis (2-ethanesulfonic acid) (PIPES), 20; adjusted to pH 7.0 with KOH. The small bundle (about 0.1—0.2 mm in diameter and 2—3 mm in length) was prepared from the papillary muscle, then one end of the bundle was connected to a strain gauge transducer (Nihon Koden TB-611T) for measurement of isometric tension, and the other end was immersed in a bath containing relaxing solution. To obtain skinned fiber, the small bundle was treated with the relaxing solution containing 250 μg/ml of saponin for 30 min. At the beginning of the experiment, the fiber was stretched in relaxing solution until resting tension was 1—2 mg, then the external solution was replaced by "activating solution" (pCa (-log $[Ca^{2+}]_M$) = 5.8, KSO_3CH_3 77, Mg $(SO_3CH_3)_2$ 5, Ca $(SO_3CH_3)_2$, 8.3; ATP-Na_2, 5.4; EGTA, 10; PIPES, 20; (mM) adjusted to pH 7.0 with KOH). The test compounds were added to this solution at a concentration of 10^{-4} or 10^{-5} M, using polyethyleneglycol (PEG) 400 as vehicle. The final concentration of PEG 400 was 10 μl/ml (drug concentration of 10^{-4} M) or 5 μl/ml (drug concentration of 10^{-5} M). The Ca^{2+} sensitizing activity of the compounds was determined by measuring percent increase in the tension development after addition of the test compounds. Experiments were performed at 24—25°C.

2) Method E: The fiber described above was stretched in relaxing solution (KSO_3CH_3, 129; Mg $(CO_3CH_3)_2$, 5.1; ATP-Na_2, 4.2; EGTA, 2; PIPES, 20; (mM), adjusted to pH 7.0 with KOH) until resting tension was 1—2 mg, then the relaxing solution was replaced by activating solution (pCa = 6.0, KSO_3CH_3, 90.2; Mg $(SO_3CH_3)_2$, 5; Ca $(SO_3CH_3)_2$, 7.18; ATP-Na_2, 4.14; EGTA, 10; PIPES, 20; (mM), adjusted to pH 7.0 with KOH) containing PEG 400 (2.5 μl/ml). When the contraction reached a plateau (pre-drug value), the solution was replaced by PEG 400 solution (5 μl) of Ix or **3** in activating solution (2 ml) (drug concentration of 3×10^{-7}, 10^{-6}, 3×10^{-6} and 10^{-5} M). The tension development induced by the compounds was expressed as percent increase from the pre-drug value. Each concentration of one compound was examined in the same skinned fiber.

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