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Studies on Dihydropyridines. III.[1] Synthesis of 4,7-Dihydrothieno[2,3-*b*]-pyridines with Vasodilator and Antihypertensive Activities

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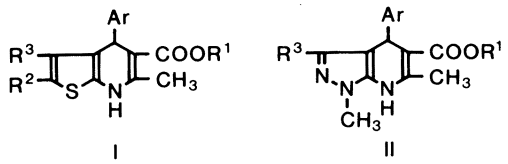
A series of 4-aryl-4,7-dihydrothieno[2,3-*b*]pyridine-5-carboxylate derivatives (I) was synthesized and tested for binding affinity to Ca^{2+} channels in rat cerebral cortex membranes, coronary vasodilator effect in isolated guinea pig hearts, and antihypertensive activity in spontaneously hypertensive rats. Several compounds had potent coronary vasodilator and antihypertensive activities. The structure–activity relationships of the series indicated that a lipophilic 3-alkyl substituent with moderate bulkiness was effective for enhancing the pharmacological potencies. Among them, methyl 4,7-dihydro-3-isobutyl-6-methyl-4-(3-nitrophenyl)thieno[2,3-*b*]-pyridine-5-carboxylate (S-312) was selected as a promising cardiovascular agent. The relationship between the absolute configuration of S-312 and its biological activities is also presented.

Keywords——thieno[2,3-*b*]pyridine; calcium antagonist; vasodilator activity; antihypertensive activity

In continuing our studies on the chemistry and utilization of 1,4-dihydropyridine derivatives,[2] we tried to synthesize a series of alkyl 4-aryl-4,7-dihydrothieno[2,3-*b*]pyridine-5-carboxylates (I). This system I consists of a new type of 1,4-dihydropyridines with a fused thiophene nucleus, structurally resembling 4-aryl-4,7-dihydropyrazolo[3,4-*b*]pyridine derivatives (II). Some of II have been reported in our preceding paper to possess potent antihypertensive and vasodilator activities due to a calcium (Ca) blocking effect.[2b]



I II

Chart 1

Chemistry

The synthesis of I was accomplished by the Michael addition reaction of 2-aminothiophenes (**1**—**11**) to α,β-unsaturated ketones (**12**—**18**), followed by cyclocondensation. The reaction of 2-aminothiophene (**1**) with methyl 3-nitrobenzylideneacetoacetate (**12**) in *tert*-butanol at 80 °C for 3 h provided a complex mixture, from which five products (**19** and **52a**—**55a**) were isolated in low yields by means of silica gel column chromatography (Chart 2 and Table I). Similar reaction of some 5-unsubstituted 2-aminothiophenes (**2**—**4**) with **12** provided low yields of the corresponding 4,7-dihydrothieno[2,3-*b*]pyridine derivatives (**20**—**22**) together with some side products (**53b**—**d**—**55b**—**d**), as shown in Chart 2 and Table I. On the other hand, when the 5-substituted 2-aminothiophenes (**5**—**7**) were treated with **12** in *tert*-

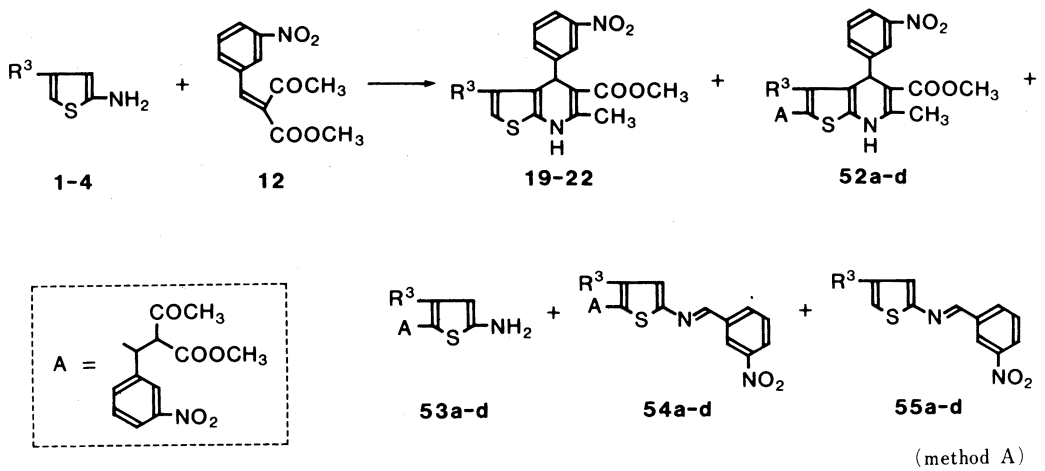


Chart 2

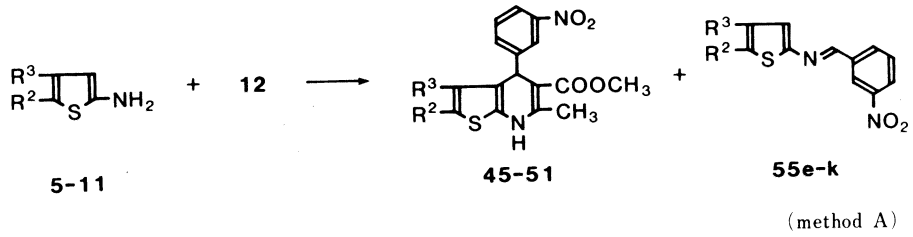


Chart 3

TABLE I. Yields of the 4,7-Dihydrothieno[2,3-*b*]pyridine Derivatives (**19**—**22** and **45**—**48**) and the Side Products (**52**—**55**)

Entry No.	R^2	R^3	No.[b]	Y (%)	No.[b]	Y (%)	No.[b]	Y (%)	No.[b]	Y (%)	No.[b]	Y (%)
1	H	H	**19**	29.4	**52a**	14.4	**53a**	8.4	**54a**	4.4	**55a**	10.9
2	H	CH_3	**20**	9.7	**52b**	—[a]	**53b**	24.1	**54b**	3.5	**55b**	—
3	H	C_6H_5	**21**	12.4	**52c**	—	**53c**	41.5	**54c**	5.2	**55c**	3.7
4	H	Cyclohexyl	**22**	13.3	**52d**	—	**53d**	10.9	**54d**	27.5	**55d**	—
5	CH_3	H	**45**	59.6	**52e**	—	**53e**	—	**54e**	—	**55e**	26.2
6	iso-C_3H_7	H	**46**	61.4	**52f**	—	**53f**	—	**54f**	—	**55f**	24.1
7	CH_3	CH_3	**47**	58.8	**52g**	—	**53g**	—	**54g**	—	**55g**	26.0
8	CH_3	Cyclohexyl	**48**	—	**52h**	—	**53h**	—	**54h**	—	**55h**	66.7

a) Not isolated. *b*) Compound No. Y = yield.

butanol, the desired 4,7-dihydrothieno[2,3-*b*]pyridine derivatives (**45**—**47**) were obtained in good yields, together with small amounts of Schiff bases (**55e**—**g**) as side products (Chart 3 and Table I). Similarly, reactions of some 4,5-disubstituted 2-aminothiophenes (**9**—**11**) with **12** provided **49**—**51** as shown in Table II (method A). In the reaction of **8** (R^3 = cyclohexyl; R^2 = methyl) with **12**, no thieno[2,3-*b*]pyridine derivative (**48**) was obtained probably because of the lowered reactivity at the C-3 position of **8** due to the steric effect of a bulkier C-4 substituent beside the 5-methyl group.

As shown in Table I, 2-aminothiophenes (**1**—**11**) act as both enamine and amine toward the electrophiles (**12**) and mainly provide the 4,7-dihydrothieno[2,3-*b*]pyridines (**19**—**22** and

45—**47**) and the Schiff bases (**55**), respectively.[3] In the case of 5-unsubstituted 2-aminothiophenes (**1**—**4**), another reaction involving electrophilic attack at the C-5 position occurred to provide the products **52**—**54**, the formation of which not only resulted in lower yield, but also complicated the isolation of the desired products (**19**—**22**). In order to prevent the formation of such side products (**52**—**54**), we tried to conduct the reaction of **12** with the 2-aminothiophene protected with a *tert*-butoxycarbonyl substituent at the C-5 position. Deprotection of the products, 2-*tert*-butoxycarbonyl-4,7-dihydrothieno[2,3-*b*]-pyridine derivatives, with acid may proceed readily to provide the desired compounds (method B in Chart 4). Eleven 2-amino-5-*tert*-butoxycarbonylthiophenes (**58**—**68**) were prepared by the thiation of *tert*-butyl 3-R^3-3-cyanomethylidenepropionates (**57a**—**k**),[4] which were obtained by the Knoevenagel reaction of *tert*-butyl acylacetates (**56a**—**k**) with cyanoacetic acid in the presence of piperidine and acetic acid.[5] Besides **12**, several kinds of alkyl arylmethylideneacetoacetates (**13**—**18**)[2b] were used to synthesize the desired compounds. Reactions of 2-amino-5-*tert*-butoxycarbonylthiophenes (**58**—**68**) with α, β-unsaturated ketones (**12**—**18**) in *tert*-butanol were carried out at 85 °C for 60 h and gave the 2-*tert*-butoxycarbonyl-4,7-dihydrothieno[2,3-*b*] pyridine derivatives (**69**—**93**) in reasonable yields, together with the Schiff bases (**94a**—**n**). Deprotection of the products (**69**—**93**) with trifluoroacetic acid smoothly proceeded to provide the desired 2-unsubstituted 4,7-dihydrothieno[2,3-*b*]pyridine derivatives (**20**—**44**) in excellent yields (Chart 4 and Table II). Among them, **20** and **21** (R^3 = methyl and phenyl) were identical with the compounds synthesized *via* method A in Chart 2. The reaction of **60** (R^3 = cyclohexyl) with **12** did not provide the 4,7-dihydrothieno[2,3-*b*]pyridine derivative (**71**), giving only the Schiff base (**94c**). The yields, melting points, and results of elementary analysis of the compounds (**19**—**51**) obtained are

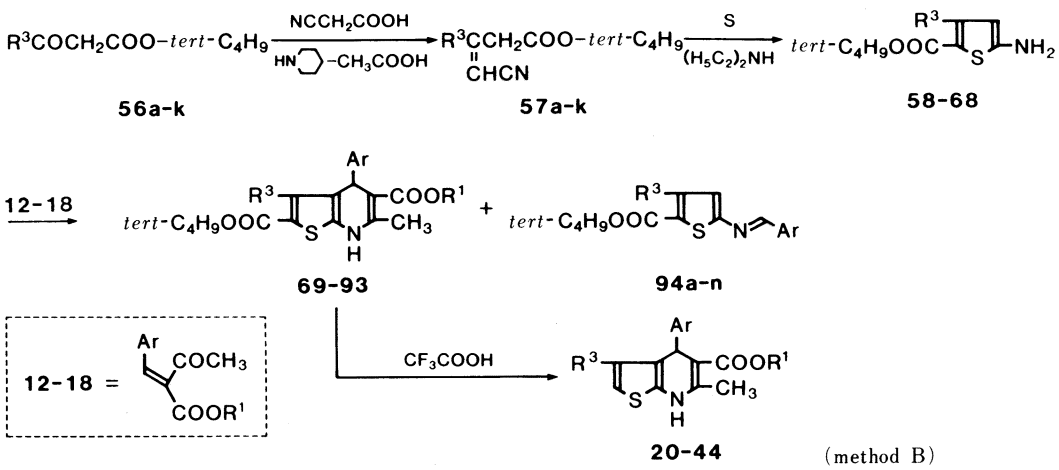


Chart 4

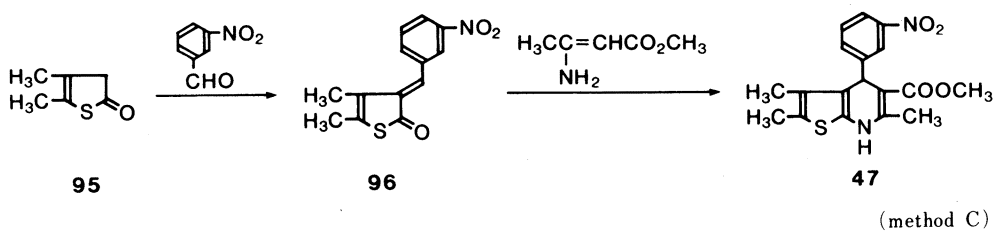


Chart 5

TABLE II. Alkyl (R^1) 4-Aryl-2-R^2-3-R^3-4,7-dihydro-6-methylthieno[2,3-*b*]pyridine-5-carboxylates (**19**—**51**)

Compd. No.	Ar	R^1	R^2	R^3	Method[a]	**69**—**93** Yield (%)	**69**—**93** (Compd. No.)	**19**—**51** Yield (%)	mp (°C)	Recrystn. solvent	Formula	Analysis (%) Calcd (Found) C	H	N
19	3-$NO_2C_6H_4$	CH_3	H	H	A			29.4	190—192	MeOH	$C_{16}H_{14}N_2O_4S$	58.17	4.27	8.46
												(57.96	4.32	8.38)
20	3-$NO_2C_6H_4$	CH_3	H	CH_3	A			9.7	205—208	MeOH	$C_{17}H_{16}N_2O_4S$	59.29	4.68	8.13
					B	65.0	(**69**)	84.8				(59.24	4.57	8.10)
21	3-$NO_2C_6H_4$	CH_3	H	C_6H_5	A			12.4	213—215	MeOH	$C_{22}H_{18}N_2O_4S$	65.01	4.46	6.89
					B	72.8	(**70**)	80.9				(64.83	4.34	6.93)
22	3-$NO_2C_6H_4$	CH_3	H	Cyclohexyl	A			13.3	180—182	iso-Pr_2O	$C_{22}H_{24}N_2O_4S$	64.06	5.86	6.79
					B	—	(**71**)					(64.07	5.80	6.73)
23	3-$NO_2C_6H_4$	CH_3	H	iso-C_3H_7	B	3.8	(**72**)	52.6	193—195	MeOH	$C_{19}H_{20}N_2O_4S$	61.28	5.41	7.52
												(61.25	5.44	7.48)
24	3-$NO_2C_6H_4$	CH_3	H	C_4H_9	B	60.4	(**73**)	92.5	155—156	MeOH	$C_{20}H_{22}N_2O_4S$	62.16	5.74	7.25
												(61.92	5.73	7.26)
25	3-$NO_2C_6H_4$	CH_3	H	iso-C_4H_9	B	68.9	(**74**)	94.3	189—190	MeOH	$C_{20}H_{22}N_2O_4S$	62.16	5.74	7.25
												(61.97	5.54	7.30)
(+)-**25**	3-$NO_2C_6H_4$	CH_3	H	iso-C_4H_9				19.5[b]	173—175	EtOH	$C_{20}H_{22}N_2O_4S$	62.16	5.74	7.25
												(62.08	5.78	7.16)
(−)-**25**	3-$NO_2C_6H_4$	CH_3	H	iso-C_4H_9				13.5[b]	173—175	EtOH	$C_{20}H_{22}N_2O_4S$	62.16	5.74	7.25
												(62.12	5.87	7.25)
26	3-$NO_2C_6H_4$	CH_3	H	Cyclopentyl-methyl	B	67.6	(**75**)	92.1	166—168	MeOH	$C_{22}H_{24}N_2O_4S$	64.06	5.86	6.79
												(63.87	5.94	6.71)
27	3-$NO_2C_6H_4$	CH_3	H	Cyclohexyl-methyl	B	62.2	(**76**)	78.6	164—166	MeOH	$C_{23}H_{26}N_2O_4S$	64.77	6.14	6.57
												(64.73	6.00	6.37)
28	3-$NO_2C_6H_4$	CH_3	H	4-Cl-C_6H_4	B	67.5	(**77**)	92.7	229—231	THF–EtOH	$C_{22}H_{17}ClN_2O_4S$	59.93	3.89	6.35
												(59.92	4.17	6.30)
29	3-$NO_2C_6H_4$	CH_3	H	3,4-$(CH_3O)_2$ $=C_6H_3$	B	68.6	(**78**)	92.2	220—222	THF–MeOH	$C_{24}H_{22}N_2O_6S$	61.79	4.75	6.00
												(61.56	4.67	5.85)
30	3-$NO_2C_6H_4$	CH_3	H	2-Thienyl	B	74.9	(**79**)	89.4	216—218	EtOH	$C_{20}H_{16}N_2O_4S_2$	58.23	3.90	6.79
												(58.17	3.93	6.65)
31	3-$NO_2C_6H_4$	iso-C_3H_7	H	C_4H_9	B	64.2	(**80**)	89.3	116—117	iso-Pr_2O	$C_{22}H_{26}N_2O_4S$	63.75	6.32	6.76
												(63.71	6.38	6.78)
32	3-$NO_2C_6H_4$	iso-C_3H_7	H	iso-C_4H_9	B	68.9	(**81**)	90.0	80—84	iso-Pr_2O	$C_{22}H_{26}N_2O_4S \cdot 1/2$ iso-Pr_2O	64.49	7.14	6.02
												(64.62	7.00	6.00)

33	3-$NO_2C_6H_4$	CH_3O–CH_2CH_2	H	C_4H_9	B	56.7	(**82**)	87.9	134—136	iso-Pr_2O	$C_{22}H_{26}N_2O_5S$	61.38 6.09 6.51 (61.41 6.18 6.54)
34	3-$NO_2C_6H_4$	CH_3O–CH_2CH_2	H	iso-C_4H_9	B	60.4	(**83**)	90.0	128—130	iso-Pr_2O	$C_{22}H_{26}N_2O_5S$	61.38 6.09 6.51 (61.43 6.10 6.50)
35	3-$NO_2C_6H_4$	CH_3O–CH_2CH_2	H	2-Thienyl	B	63.5	(**84**)	88.2	180—181	MeOH	$C_{22}H_{20}N_2O_5S_2$	57.87 4.41 6.13 (57.79 4.43 6.16)
36	3-$NO_2C_6H_4$	C_6H_5–CH_2CH_2	H	iso-C_4H_9	B	57.6	(**85**)	89.1	125—127	iso-Pr_2O	$C_{27}H_{28}N_2O_4S$	68.04 5.92 5.88 (68.15 5.81 5.95)
37	3-$NO_2C_6H_4$	C_6H_5–CH_2CH_2	H	C_6H_5	B	55.3	(**86**)	91.1	147—150	EtOH	$C_{29}H_{24}N_2O_4S$	70.14 4.87 5.64 (70.20 4.91 5.68)
38	3-$NO_2C_6H_4$	C_6H_5–CH_2CH_2	H	4-Cl–C_6H_4	B	53.5	(**87**)	82.4	169—170	EtOH	$C_{29}H_{23}ClN_2O_4S$	65.59 4.37 5.28 (65.44 4.53 5.19)
39	3-$NO_2C_6H_4$	C_6H_5–CH_2CH_2	H	2-Thienyl	B	47.1	(**88**)	90.3	192—194	EtOH	$C_{27}H_{22}N_2O_4S_2$	64.52 4.41 5.57 (64.32 4.42 5.64)
40	3-$NO_2C_6H_4$	4-C_6H_5-piperidinoethyl	H	C_4H_9	B	18.6	(**89**)	84.6	156—158	iso-Pr_2O	$C_{32}H_{37}N_3O_4S$	68.67 6.66 7.51 (68.58 6.51 7.42)
41	2-$NO_2C_6H_4$	CH_3	H	iso-C_4H_9	B	14.8	(**90**)	70.3	165—167	iso-Pr_2O	$C_{20}H_{22}N_2O_4S$	62.16 5.74 7.25 (62.24 5.59 7.28)
42	2-$NO_2C_6H_4$	CH_3	H	C_6H_5	B	9.6	(**91**)	70.1	213—215	EtOH	$C_{22}H_{18}N_2O_4S$	65.01 4.46 6.89 (64.97 4.58 6.83)
43	3-Pyridyl	CH_3	H	C_4H_9	B	43.8	(**92**)	94.9	231—232	MeOH	$C_{19}H_{22}N_2O_2S$	66.64 6.48 8.18 (66.35 6.47 8.08)
44	3-Pyridyl	CH_3	H	iso-C_4H_9	B	46.5	(**93**)	64.0	215—217	MeOH	$C_{19}H_{22}N_2O_2S$	66.64 6.48 8.18 (66.55 6.49 8.09)
45	3-$NO_2C_6H_4$	CH_3	CH_3	H	A			59.6	213—215	THF–MeOH	$C_{17}H_{16}N_2O_4S$	59.29 4.68 8.13 (59.18 4.57 8.15)
46	3-$NO_2C_6H_4$	CH_3	iso-C_3H_7	H	A			61.4	132—134	iso-Pr_2O	$C_{19}H_{20}N_2O_4S$	61.28 5.41 7.52 (61.29 5.46 7.49)
47	3-$NO_2C_6H_4$	CH_3	CH_3	CH_3	A			58.8	250—252	THF	$C_{18}H_{18}N_2O_4S$	60.32 5.06 7.82 (60.46 4.96 7.67)
49	3-$NO_2C_6H_4$	CH_3	CH_3	C_6H_5	A			49.7	196—197	THF–MeOH	$C_{23}H_{20}N_2O_4S$	65.70 4.79 6.66 (65.55 4.66 6.89)
50	3-$NO_2C_6H_4$	CH_3	C_3H_7	C_4H_9	A			39.6	150—152	EtOH	$C_{23}H_{28}N_2O_4S$	64.46 6.59 6.54 (64.21 6.29 6.48)
51	3-$NO_2C_6H_4$	CH_3	–$(CH_2)_4$–		A			69.5	231—233	THF–MeOH	$C_{20}H_{20}N_2O_4S$	62.48 5.24 7.29 (62.54 5.20 7.32)

a) A: the method shown in Charts 2 and 3. B: the method shown in Chart 4. *b*) Overall yield from **25**.

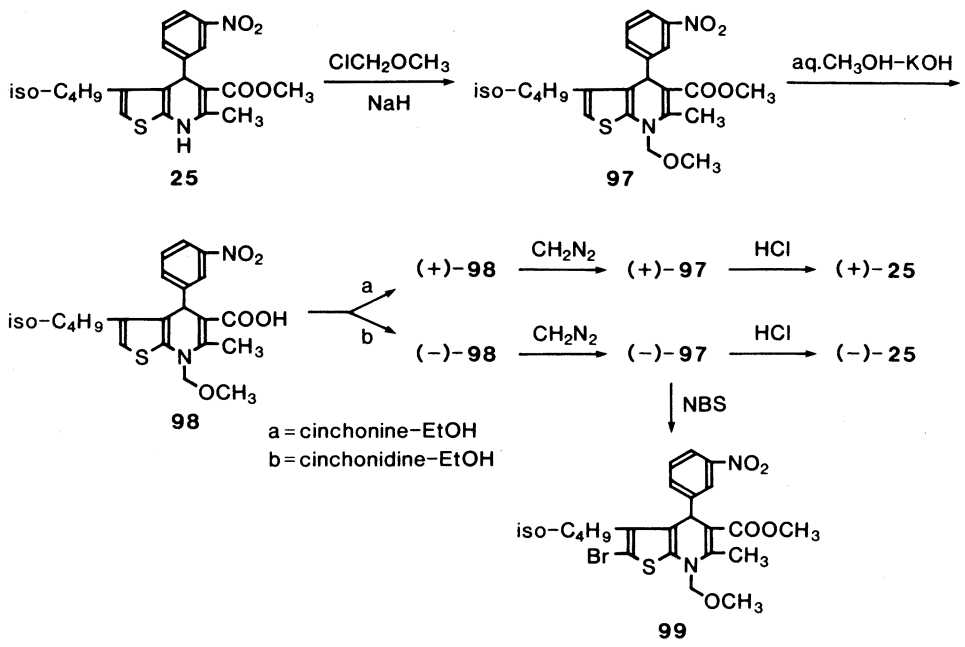


Chart 6

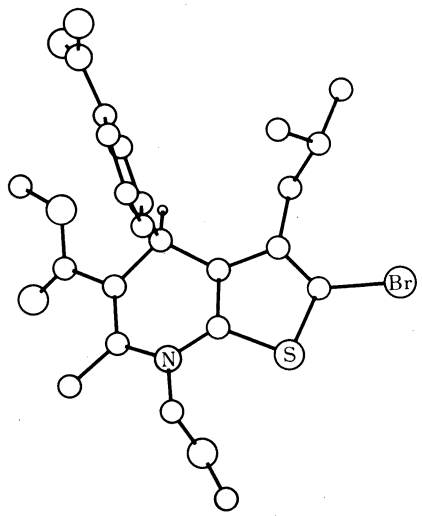


Fig. 1. X-Ray Crystallographic Structure of *R*-(−)-Methyl 2-Bromo-4,7-dihydro-3-isobutyl-7-methoxymethyl-6-methyl-4-(3-nitrophenyl)-thieno[2,3-*b*]pyridine-5-carboxylate (**99**)

listed in Table II, together with the yields of the intermediates (**69**—**93**). The infrared (IR) and proton nuclear magnetic resonance (^{1}H-NMR) data for the compounds (**19**—**51**) are summarized in Table V. Furthermore, the structures of the 4,7-dihydrothieno[2,3-*b*]-pyridines (**19**—**51**) obtained were confirmed by an unequivocal synthesis of **47** by means of the modified Hantzsch method[6] as shown in Chart 5 (method C).

Opticl isomers exist for the compounds (**19**—**51**). Among those having higher Ca-blocking and coronary vasodilator activities, compound **25** was resolved as shown in Chart 6. Compound **25**, unstable toward alkali, was converted to the 7-methoxymethyl derivative (**97**), which was hydrolyzed with methanolic potassium hydroxide solution to give the carboxylic acid **98**. This was successfully resolved by using cinchonine and cinchonidine to provide both

enantiomers (**98a** and **b**). Treatment with diazomethane gave the esters (**97a** and **b**), demethoxymethylation of which provided the desired products, *S*-(+)-**25** and *R*-(−)-**25**, respectively. Their absolute configurations were established by X-ray crystallographic analysis of the 2-bromo derivative (**99**) obtained from **97b**, which was assigned the *R* configuration (Fig. 1).

Pharmacological Results and Discussion

[^{3}H]Nitrendipine ([^{3}H]NTD) has been employed to directly identify the putative Ca^{2+} channels in various tissue membranes.[7] The Ca-blocking activity of each test compound was evaluated in terms of its inhibitory effect on specific [^{3}H]-NTD binding in rat cerebral cortex membranes.[8] Individual K_i values were calculated from the observed IC_{50} values and are summarized in Table III together with those of the three reference Ca-blockers, nifedipine, nicardipine hydrochloride and methyl 3-cyclopentyl-4,7-dihydro-1,6-dimethyl-4-(3-nitrophenyl)pyrazolo[3,4-*b*]pyridine-5-carboxylate (8363-S).[2b] Compound **19**, [methyl 4,7-dihydro-6-methyl-4-(3-nitrophenyl)-thieno[2,3-*b*]pyridine-5-carboxylate], without any substituent at the C-2 and C-3 positions, exhibited a weak binding affinity to specific [^{3}H]NTD binding sites. The introduction of an alkyl or aryl substituent at the C-3 position increased its potency. In particular, the 3-cyclohexyl (**22**), 3-butyl (**24**), 3-isobutyl (**25**) and 3-cyclopentylmethyl (**26**) analogues exhibited markedly strong binding affinity, comparable to those of the reference compounds. The 3-phenyl (**21**) and 3-(2-thienyl) (**30**) derivatives showed moderate potency. On the other hand, the introduction of a 2-alkyl substituent into **19** resulted in a decrease of the activity, as seen in the 2-methyl (**45**), 2-isopropyl (**46**) and 2-propyl-3-butyl (**50**) analogues. Replacement of the 5-methyl ester group in compounds **24** and **25** with other alkyl esters such as isopropyl, methoxymethyl, phenethyl and 4-phenylpiperidinoethyl esters did not enhance the activity. Coronary vasodilator activity was measured in isolated guinea pig heart by Langendorff's method.[9] The values shown in Table III were obtained by measuring the drops of coronary arterial perfusates in 1 min after intracoronary administration of the test compounds. At the same time, the effects of the test compounds on the cardiac contraction and heart rate were recorded. As shown in Table III, the coronary vasodilator activity of **19** was very weak, but increased coronary arterial flow was induced by the 3-substituted derivatives (**20**—**44**), in proportion to their binding affinity to the specific [^{3}H]NTD binding sites. The duration of action of almost all the compounds tested was more lasting than those of nifedipine and nicardipine hydrochloride. Compounds **22** and **24**—**26** exhibited remarkably strong potency with long duration of action. However, the cardiac contraction and heart rate were not influenced at the doses that increased coronary arterial flow. On the other hand, the compounds (**45**—**51**) which had a 2-alkyl substituent showed weak potency in coronary vasodilator activity.

Antihypertensive activity was evaluated in conscious spontaneously hypertensive rats (SHR). Systolic blood pressure (SBP), recorded indirectly from the tail, was determined before dosing and at various time intervals during the ensuing 24 h after intraperitoneal administration of each test compound.[10] A decrease in SBP was observed with most of the compounds which exhibited the coronary vasodilator and Ca-blocking activities. The order of antihypertensive potency was consistent with that of the binding affinity to specific [^{3}H]NTD binding sites. Maximal decreases in SBP for the compounds, **22**, **24**—**26**, **31**, **32** and **34**, observed at 1—2 h after administration were comparable to that caused by nifedipine (Table III).

Although a precise relationship can not yet be established between the chemical structure and biological activity, fusion of the thiophene nucleus to 1,4-dihydropyridine seems to satisfy the structural requirements for enhancing the Ca-blocking activity. The results also

TABLE III. Ca-Blocking, Coronary Vasodilator and Antihypertensive Activities of 4,7-Dihydrothieno[2,3-*b*]pyridine Derivatives (**19**—**51**)

Compd. No.	Affnity to Ca^{2+} channel[a] Inhibition of [^{3}H]NTD binding K_i ($\times 10^{-9}$ M)	CVD effect[b] max. increase of CPF (%) (0.1 μg, i.c.)	Anti-HT activity[c] max. decrease of SBP (mmHg) (3 mg/kg, i.p.)	Acute toxicity[d] LD_{50} (mg/kg, *p.o.*)
19	8.0	9.0	0	
20	2.3	30.1	18	>1000
21	0.86	91.3 (40)	14	>1000
22	0.54	105.8 (42)	57	>1000
24	0.30	87.5 (45)	54	>1000
25	0.19	103.3 (50)	34	>1000
26	0.34	95.3 (55)	47	>1000
27	0.54	46.7 (50)	23	
28	3.7	20.3 (15)	19	
29	1.3	36.9 (8)	18	
30	2.0	39.2 (15)	26	
31	0.85	57.2 (80)	49	
32	1.4	74.6 (34)	56	>500
33	0.56	84.8 (14)	33	>1000
34	0.50	96.8 (17)	39	>1000
35	3.0	66.7 (10)	0	
36	1.2	68.2 (100)	29	
37	1.5	24.0	26	
38	11.7	0	19	
39	10.5	30.1	25	
40		33.1	28	
43		28.4 (5)	26	
44		45.4 (6)	29	
45	10.6	28.0	13	
46	258	0	0	
47	6.4	30.4	0	
49	1.1	69.0	21	
50	11	44.8	0	
51	7.3	52.5	9	
Nifedipine	1.28	70.4 (7)	45	562
Nicardipine–HCl	0.20	91.0 (13)	53	
8363-S[e]	0.56	47.1 (20)	66	524

Abbreviations used are: [^{3}H]NTD, [^{3}H]nitrendipine; CVD effect, coronary vasodilator effect; CPF, coronary perfusion flow; anti-HT activity, antihypertensive activity; SBP, systolic blood pressure. *a*) The K_i values were calculated from the equation, $K_i = IC_{50}/(1 + c/K_d)$, where IC_{50} = the concentration causing 50% inhibition of specific [^{3}H]NTD binding, c = [^{3}H]NTD concentration (0.2 nM) and K_d = dissociation constant (0.2—0.23 nM). Usually, 2 preparations were used for the determination of the K_i values of test compounds. *b*) The test compounds were administered into coronary artery at a dose of 0.1 μg. Two to 4 preparations for each compound were used for the determination of CVD effect. The values in parentheses are the times (min) required for 50% recovery of the maximum change of CPF. *c*) For the determination of antihypertensive activity, test compounds were intraperitoneally administered at a dose of 3 mg/kg in 2 SHRs. Four SHRs were used for **24**, **25** and nifedipine. *d*) LD_{50} values were determined after the oral administration of test compounds to 5 to 6 male slc, ddY mice. *e*) Methyl 3-cyclopentyl-4,7-dihydro-1,6-dimethyl-4-(3-nitrophenyl)pyrazolo[3,4-*b*]pyridine-5-carboxylate.[2b]

indicate that the potency is enhanced in those compounds which have a lipophilic alkyl group with moderate bulkiness at the C-3 position, but lack a substituent at the C-2 position. Replacement of the 5-methoxycarbonyl group with a bulkier ester moiety, such as isopropyl, methoxyethyl and phenethyl esters, did not increase the potency.

The acute toxicity in mice was determined for several compounds which showed potent coronary vasodilator and antihypertensive activities. The LD_{50} values were calculated by the

TABLE IV. Effects of *S*-(+)- and *R*-(−)-S-312 on Ca-Blocking, Coronary Vasodilator and Antihypertensive Activities

Compd. No.	Inhibition of [^{3}H]NTD binding[a] K_i ($\times 10^{-9}$ M)	K^+-Contracture RC_{50}[d] ($\times 10^{-9}$ M)	CVD effect[b] max. increase of CPF (%) (0.1 μg, i.c.)	Anti-HT activity[c] max. decrease of SBP (mmHg) (3 mg/kg, *p.o.*)
S-(+)-S-312	0.14	0.52	137.1 (64)	51
R-(−)-S-312	7.7	11.0	43.9 (10)	0

Abbreviations used are: [^{3}H]NTD, [^{3}H]nitrendipine; CVD effect, coronary vasodilator effect; CPF, coronary perfusion flow; anti-HT activity, antihypertensive activity; SBP, systolic blood pressure *a*, *b*) See footnotes *a*) and *b*) in Table III. *c*) For the determination of antihypertensive activity, test compounds were orally administered at a dose of 3 mg/kg in 4 SHRs. *d*) RC_{50} values are the concentrations required for 50% relaxation of the contracture of isolated rabbit femoral arteries by KCl at 5×10^{-2} M. Six preparations were used for the determination of RC_{50} of test compounds.

Bliss method[11] for the 24 h after oral administration. As shown in Table III, all the compounds tested exhibited weak toxicity.

On the basis of these results, compound **25** (S-312), which exhibited potent coronary vasodilator action as well as strong binding affinity to the specific [^{3}H]NTD binding sites, and was less toxic in mice, was selected as a candidate for further development.[12] S-312 had qualitatively similar actions to those of a pyrazolo[3,4-*b*]pyridine derivative (8363-S) reported in our preceding paper.[2b] However, there were some differences in potency between the two compounds: S-312 showed much greater potency in coronary vasodilator effect and less toxicity than 8363-S.

Our interest was then focused on the relationship between the absolute configuration of S-312 and its biological activities. The affinity of (+)-S-312 with the *S* configuration to the specific [^{3}H]NTD binding sites was much greater than that of the *R*-(−)-enantiomer (Table IV). The potency of each enantiomer in terms of the maximum decrease of SBP in SHR and the increase of coronary perfusion flow in isolated guinea pig hearts after oral and intracoronary administration, respectively, were consistent with the binding affinities, as shown in Table IV. Moreover, the Ca-blocking activity of both enantiomers was evaluated in terms of the inhibitory effect on the K^+-contracture of isolated rabbit femoral arteries.[13] The RC_{50} values of each enantiomer are shown in Table IV. The potency of *S*-(+)-S-312 was stronger than that of the *R*-(−)-enantiomer. Thus, the potent coronary vasodilator and antihypertensive activities found in S-312 (**25**) reside in the (+)-enantiomer with the *S* configuration.

Experimental

All melting and boiling points are uncorrected. IR spectra were measured on a JASCO IRA-I spectrometer, and absorption data are given in cm^{-1}. ^{1}H-NMR spectra were recorded with a Varian EM390 spectrometer in deuteriochloroform ($CDCl_3$) with tetramethylsilane as an internal standard. The chemical shifts and coupling constants (*J*) are given in δ and Hz, respectively, and the abbreviations of signal patterns are as follows: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; and br, broad. Optical rotations were measured with a Perkin-Elmer 241 polarimeter. High performance liquid chromatography (HPLC) was performed on a Shimadzu LC-4A with a CHIRALPAK OT (+) (0.46 × 25 cm) column (MeOH, 1 ml/min, 26 atm pressure). Thin-layer chromatography (TLC) was conducted on Merck Silica gel F_{254} plates. Standard work-up procedures were as follows: the reaction mixture was partitioned between the indicated solvent and water, and the organic extract was washed successively with water, $NaHCO_3$ solution, NaOH solution and hydrochloric acid solution, and then dried over $MgSO_4$, filtered and evaporated *in vacuo*. Chromatographic separation was carried out on Merck Silica gel 60 using the indicated eluents.

2-Aminothiophene (**1**),[14] 4-methyl- and 4-phenyl-2-amino-thiophenes (**2** and **3**),[15] and 2-amino-4,5,6,7-tetrahydrobenzothiophene (**11**)[15b] were prepared by the literature methods. 2-Amino-3-cyclohexylthiophene (4) was prepared in a manner similar to that described for **2**. Liquid, IR ($CHCl_3$): 3190, 3310 cm^{-1}. ^{1}H-NMR δ: 1.80 (11H, m), 3.58 (2H, br s), 6.07 (2H, s). 2-Amino-5-R^2-4-R^3-thiophenes (**5**—**10**) were prepared in a similar manner to that described for 2-amino-5-methylthiophene (**5**) in the literature.[4,16] (R^2, R^3): **6** (Me, Me): bp 75 °C (0.6 mmHg). IR (neat): 3310, 3190 cm^{-1}. ^{1}H-NMR δ: 1.98 (3H, s), 3.50 (2H, br s), 5.90 (1H, s). **7** (Me, Ph): liquid. IR (neat): 3350, 3280 cm^{-1}. ^{1}H-NMR δ: 2.33 (3H, s), 3.60 (2H, br s), 6.17 (1H, s), 7.33 (5H, m). **8** (iso-Pr, H): bp 83 °C (0.9 mmHg). IR (neat): 3310, 3190 cm^{-1}. ^{1}H-NMR δ: 1.25 (6H, d, J=4 Hz), 6.34 (1H, m). **9** (Me, cyclohexyl): liquid, IR ($CHCl_3$): 3400, 3340 cm^{-1}. ^{1}H-NMR δ: 1.69 (11H, m), 2.18 (3H, s), 3.53 (2H, br s), 5.98 (1H, s), **10** (Pr, Bu): liquid, IR (neat): 3300, 3175 cm^{-1}. ^{1}H-NMR δ: 1.41 (16H, m), 3.50 (2H, br s), 5.90 (1H, s).

Alkyl (R^1) 3- or 2-nitrobenzylideneacetoacetates (**12**—**17**) and methyl 3-pyridylmethylideneacetoacetate (**18**) were prepared by the usual method.[2b]

tert-Butyl 4-R^3-2-aminothiophene-5-carboxylates (**58**—**68**) were prepared in a manner similar to that described for ethyl 2-amino-4-methylthiophene-5-carboxylate in the literature.[4] **58**—**68** (R^3): **58** (Me): 35.0% yield from *tert*-Bu acetoacetate (**56a**), liquid. ^{1}H-NMR δ: 1.53 (9H, s), 2.35 (3H, s), 4.32 (2H, br s), 5.85 (1H, s). **59** (Ph): 25.7% yield from *tert*-Bu benzoylacetate (**56b**), mp 110—116 °C. ^{1}H-NMR δ: 1.34 (9H, s), 4.22 (2H, br s), 6.03 (1H, s), 7.37 (5H, m). **60** (cyclohexyl): 7.5% yield from *tert*-Bu cyclohexylcarbonylacetate (**56c**), liquid. ^{1}H-NMR δ: 1.57 (10H, m), 1.54 (9H, s), 3.50 (1H, m), 4.38 (2H, br s), 6.05 (1H, s). **61** (iso-Pr): 30.7% yield from *tert*-Bu isobutanoylacetate (**56d**), liquid. ^{1}H-NMR δ: 1.16 (6H, d, J=7 Hz), 1.52 (9H, s), 3.80 (1H, m), 4.15 (2H, br s), 6.04 (1H, s). **62** (Bu): 42.1% yield from *tert*-Bu pentanoylacetate (**56e**), liquid. ^{1}H-NMR δ: 1.30 (16H, m), 2.81 (2H, t, J=7 Hz), 4.13 (2H, br s), 5.93 (1H, s). **63** (iso-Bu): 24.9% yield from *tert*-Bu 3-methylbutanoylacetate (**56f**), mp 84—87 °C. ^{1}H-NMR δ: 0.90 (6H, d, J=6 Hz), 1.52 (9H, s), 1.88 (1H, m), 2.70 (2H, d, J=7 Hz), 4.15 (2H, br s), 5.91 (1H, s). **64** (cyclopentylmethyl): 28.3% yield from *tert*-Bu cyclopentylacetoacetate (**56g**), mp 98—99 °C. ^{1}H-NMR δ: 1.65 (18H, m), 2.83 (2H, d, J=7 Hz), 4.17 (2H, br s), 5.94 (1H, s). **65** (cyclohexylmethyl): 10.2% yield from *tert*-Bu cyclohexylacetoacetate (**56h**), mp 100—103 °C. ^{1}H-NMR δ: 1.79 (20H, m), 2.73 (2H, d, J=7 Hz), 4.13 (2H, br s), 5.91 (1H, s). **66** (4-Cl-Ph): 53.4% yield from *tert*-Bu 4-chlorobenzoylacetate (**56i**), mp 152—154 °C. ^{1}H-NMR δ: 1.39 (9H, s), 4.23 (2H, br s), 6.00 (1H, s), 7.32 (4H, s). **67** (3,4-di-MeO-Ph): 52.8% yield from *tert*-Bu 3,4-dimethoxybenzoylacetate (**56j**), mp 157—159 °C. ^{1}H-NMR δ: 1.38 (9H, s), 3.87 (6H, s), 4.27 (2H, br s), 6.04 (1H, s), 6.93 (3H, m). **68** (2-thienyl): 37.6% yield from *tert*-Bu 2-thenoylacetate (**56k**), mp 96—98 °C. ^{1}H-NMR δ: 1.50 (9H, s), 4.17 (2H, br s), 6.19 (1H, s), 7.23 (3H, m).

Reaction of 1 with 12——Method A: A mixture of **1** (0.53 g, 5.35 mmol) and **12**[2b] (1.28 g, 5.35 mmol) in *tert*-BuOH (10 ml) was stirred at 80 °C for 3 h, and then evaporated. The residue was chromatographed on silica gel with the following eluants. The 1st fraction eluted with CH_2Cl_2 gave 2-(3-nitro-benzylidene)aminothiophene (**55a**, 0.136 g, 10.9%), yellow prisms, mp 94—95 °C (MeOH). IR (Nujol): 1348 cm^{-1}. ^{1}H-NMR δ: 7.08 (3H, s), 7.60 (1H, t, J=8 Hz), 8.20 (2H, m), 8.49 (1H, s), 8.67 (1H, t, J=2 Hz). *Anal.* Calcd for $C_{11}H_8N_2O_2S$: C, 56.89; H, 3.47; N, 12.06. Found: C, 56.86; H, 3.55; N, 11.98. The 2nd fraction eluted with CH_2Cl_2 gave **1** (48 mg, 9.0%). The 3rd fraction eluted with CH_2Cl_2 gave methyl 2-acetyl-3-[2-(3-nitrobenzylidene)amino-5-thienyl]-3-(3-nitrophenyl)propionate (**54a**, 0.113 g, 4.4%), which was a diastereoisomeric mixture according to its ^{1}H-NMR spectrum. IR ($CHCl_3$): 1348, 1715, 1742 cm^{-1}. ^{1}H-NMR δ: 2.16, 2.29 (3H, s), 3.54, 3.70 (3H, s), 4.53 (1H, d, J=12 Hz), 5.13 (1H, d, J=12 Hz), 8.07 (9H, m). The 4th fraction eluted with CH_2Cl_2 gave methyl 4,7-dihydro-6-methyl-4-(3-nitrophenyl)thieno[2,3-*b*]pyridine-5-carboxylate (**19**, 0.52 g, 29.4%) listed in Tables II and V. The 5th fraction eluted with CH_2Cl_2–CH_3CN (10:1) gave 5-[2-acetyl-2-methoxycarbonyl-1-(3-nitrophenyl)]ethyl-**1** (**53a**, 0.157 g, 8.4%) as a diastereoisomeric mixture. IR ($CHCl_3$): 1345, 1715, 1742, 3350, 3420 cm^{-1}. ^{1}H-NMR δ: 2.10, 2.23 (3H, s), 3.60 (2H, br s), 3.50, 3.68 (2H, s), 4.40, 4.43 (1H, d, J=12 Hz), 4.93 (1H, d, J=12 Hz), 5.93 (1H, d, J=4 Hz), 6.47, 6.49 (1H, d, J=4 Hz), 7.79 (4H, m). The 6th fraction eluted with CH_2Cl_2–CH_3CN (10:1) gave 2-[2-acetyl-2-methoxycarbonyl-1-(3-nitrophenyl)]ethyl-**19** (**52a**, 0.447 g, 14.1%) as a diastereoisomeric mixture. IR ($CHCl_3$): 1345, 1685, 1715, 1743, 3425 cm^{-1}. ^{1}H-NMR δ: 2.07, 2.09 (3H, s), 2.38 (3H, s), 3.41, 3.43 (3H, s), 3.57 (3H, s), 4.53 (1H, d, J=12 Hz), 4.90 (1H, d, J=12 Hz), 5.27 (1H, s), 6.33 (1H, d, J=4 Hz), 6.58 (1H, br s), 7.74 (8H, m).

Reaction of 2 with 12——Method A: A mixture of **2** (1.0 g, 8.85 mmol) and **12** (2.35 g, 8.85 mmol) in *tert*-BuOH (10 ml) was treated in a manner similar to that described above. Purification by chromatography on silica gel with benzene–ethyl acetate (AcOEt) (9:1) gave the following products. The 1st fraction, a diastereomeric mixture of 4-methyl-**53a** (**53b**, 0.772 g, 24.1%). ^{1}H-NMR δ: 2.12, 2.22 (3H, s), 2.05 (3H, s), 3.48, 3.62 (3H, s), 3.78 (2H, br s), 4.95 (1H, d, J=12 Hz), 5.08, 5.12 (1H, d, J=12 Hz), 5.73 (1H, s), 7.79 (4H, m). The 2 nd fraction, a diastereomeric mixture of 4-methyl-**54a** (**54b**, 0.153 g, 3.5%). ^{1}H-NMR δ: 2.18, 2.25 (3H, s), 2.30 (3H, s), 3.55, 3.67 (3H, s), 4.55 (1H, d, J=12 Hz), 5.23, 5.27 (1H, d, J=12 Hz), 6.85 (1H, s), 8.03 (8H, m). The 3rd fraction, 3-methyl-**19** (**20**, 0.285 g, 9.7%), listed in Tables II and V.

Reaction of 3 with 12——Method A: A mixture of **3** (1.0 g, 5.71 mmol) and **12** (1.42 g, 5.71 mmol) in *tert*-BuOH (10 ml) was treated in a manner similar to that described above. Purification by chromatography on silica gel with benzene–AcOEt (9:1) gave the following products. The 1st fraction, 4-phenyl-**55a** (**55c**, 0.066 g, 3.7%), yellow plates, mp 124—125 °C (ether). IR (Nujol): 1347 cm^{-1}. ^{1}H-NMR δ: 7.43 (8H, m), 8.18 (2H, m), 8.43 (1H, s), 8.63 (1H, t, J=2 Hz). *Anal.* Calcd for $C_{17}H_{12}N_2O_2S$: C, 66.22; H, 3.92; N, 9.08. Found: C, 66.16; H, 3.87; N, 9.20. The 2nd

TABLE V. IR and ^{1}H-NMR Data for 4,7-Dihydrothieno[2,3-*b*]pyridine Derivatives (**19**—**51**)

Compd. No.	IR (Nujol) (cm^{-1}) NH	CO	NO_2	^{1}H-NMR (in $CDCl_3$) δ
19	3345	1663	1337	2.45 (3H, s), 3.57 (3H, s), 5.35 (1H, s), 6.43 (1H, brs), 6.47 (1H, d, J=5 Hz), 6.58 (1H, d, J=5 Hz), 7.71 (4H, m)
20	3290	1640	1350	1.88 (3H, s), 2.40 (3H, s), 3.62 (3H, s), 5.22 (1H, s), 6.22 (1H, s), 6.45 (1H, brs), 7.65 (4H, m)
21	3300	1632	1350	2.45 (3H, s), 3.58 (3H, s), 5.35 (1H, s), 6.48 (1H, s), 6.48 (1H, brs), 7.43 (9H, m)
22	3310	1630	1340	1.65 (14H, m), 3.67 (3H, s), 5.31 (1H, s), 6.22 (1H, s), 6.70 (1H, brs), 7.65 (4H, m)
23	3285	1639	1338	0.96 (6H, d, J=7 Hz), 2.38 (3H, s), 2.61 (1H, m), 3.67 (3H, s), 5.33 (1H, s), 6.29 (1H, s), 6.63 (1H, brs), 7.70 (4H, m)
24	3305	1630	1345	1.50 (9H, m), 2.40 (3H, s), 3.65 (3H, s), 5.27 (1H, s), 6.23 (1H, s), 6.55 (1H, brs), 7.70 (4H, m)
25	3300	1636	1340	0.78 (6H, d, J=6 Hz), 1.60 (1H, m), 2.07 (2H, d, J=7 Hz), 2.37 (3H, s), 3.63 (3H, s), 5.25 (1H, s), 6.22 (1H, s), 7.67 (4H, m)
26	3315	1633	1348	1.53 (11H, m), 2.38 (3H, s), 3.66 (3H, s), 5.27 (1H, s), 6.24 (1H, s), 6.62 (1H, brs), 7.69 (4H, m)
27	3315	1630	1340	1.09 (11H, m), 2.09 (2H, d, J=7 Hz), 2.38 (3H, s), 3.67 (3H, s), 5.27 (1H, s), 6.20 (1H, s), 6.77 (1H, brs), 7.71 (4H, m)
28	3290	1627	1350	2.43 (3H, s), 3.60 (3H, s), 5.31 (1H, s), 6.50 (1H, s), 6.57 (1H, brs), 7.47 (8H, m)
29	3350	1630	1343	2.45 (3H, s), 3.62 (3H, s), 3.72 (3H, s), 3.91 (3H, s), 5.37 (1H, s), 6.50 (1H, s), 6.73 (1H, brs), 7.25 (7H, m)
30	3320	1630	1343	2.32 (3H, s), 3.53 (3H, s), 5.50 (1H, s), 7.45 (8H, m)
31	3300	1627	1341	1.48 (15H, m), 2.40 (3H, s), 5.00 (1H, m), 5.27 (1H, s), 6.23 (1H, s), 6.47 (1H, brs), 7.72 (4H, m)
32	3260	1620	1350	0.79 (6H, d, J=6 Hz), 1.60 (1H, m), 2.08 (2H, d, J=7 Hz), 2.38 (3H, s), 4.98 (1H, m), 5.26 (1H, s), 6.20 (1H, s), 6.86 (1H, brs), 7.71 (4H, m)
33	3280	1627	1339	1.20 (9H, m), 2.38 (3H, s), 3.35 (3H, s), 3.55 (2H, t, J=7 Hz), 4.18 (2H, t, J=7 Hz), 5.27 (1H, s), 6.22 (1H, s), 6.53 (1H, brs), 7.68 (4H, m)
34	3315	1635	1350	0.78 (6H, d, J=6 Hz), 1.58 (1H, m), 2.08 (2H, d, J=7 Hz), 2.39 (3H, s), 3.38 (3H, s), 3.60 (2H, m), 4.22 (2H, m), 5.30 (1H, s), 6.22 (1H, s), 7.73 (4H, m)
35	3275	1630	1347	2.32 (3H, s), 3.20 (3H, s), 3.47 (2H, t, J=7 Hz), 4.08 (2H, t, J=7 Hz), 5.52 (1H, s), 7.45 (8H, m)
36	3325	1640	1341	0.79 (6H, d, J=6 Hz), 1.57 (1H, m), 2.03 (2H, d, J=7 Hz), 2.32 (3H, s), 2.93 (2H, t, J=7 Hz), 4.30 (2H, m), 5.15 (1H, s), 6.20 (1H, s), 6.52 (1H, brs), 7.52 (9H, m)
37	3305	1628	1345	2.36 (3H, s), 2.82 (2H, t, J=7 Hz), 4.22 (2H, m), 5.29 (1H, s), 6.47 (1H, s), 6.63 (1H, brs), 7.40 (14H, m)
38	3285	1623	1340	2.37 (3H, s), 2.85 (2H, t, J=7 Hz), 4.27 (2H, m), 5.22 (1H, s), 6.48 (1H, s), 6.67 (1H, brs), 7.92 (13H, m)
39	3285	1630	1340	2.37 (3H, s), 2.92 (2H, t, J=7 Hz), 4.32 (2H, m), 5.48 (1H, s), 6.60 (1H, brs), 6.65 (1H, s), 7.36 (12H, m)
40	3300	1635	1345	1.05 (7H, m), 2.42 (16H, m), 2.39 (3H, s), 4.20 (2H, t, J=6 Hz), 5.27 (1H, s), 6.21 (1H, s), 6.98 (1H, brs), 7.65 (9H, m)
41	3270	1637	1338	0.78 (6H, d, J=6 Hz), 1.60 (1H, m), 2.17 (1H, m), 2.43 (1H, m), 2.32 (3H, s), 3.50 (3H, s), 5.98 (1H, s), 6.20 (1H, s), 6.73 (1H, brs), 7.42 (4H, m)
42	3275	1625	1340	2.35 (3H, s), 3.45 (3H, s), 6.00 (1H, s), 6.43 (1H, s), 6.63 (1H, brs), 7.23 (9H, m)
43		1677		1.55 (12H, m), 3.63 (3H, s), 5.13 (1H, s), 6.20 (1H, s), 7.79 (5H, m)
44		1680		0.78 (6H, d, J=6 Hz), 1.62 (1H, m), 2.08 (2H, m), 2.37 (3H, s), 3.65 (3H, s), 5.17 (1H, s), 6.19 (1H, s), 7.82 (5H, m)
45	3275	1632	1340	2.26 (3H, d, J=1 Hz), 2.42 (3H, s), 3.55 (3H, s), 5.25 (1H, s), 6.13 (1H, q, J=1 Hz), 6.48 (1H, brs), 7.70 (4H, m)
46	3280	1628	1338	1.72 (3H, s), 2.13 (3H, s), 2.28 (3H, s), 3.48 (3H, s), 5.09 (1H, s), 6.63 (1H, brs), 7.74 (4H, m)
47	3335	1662	1338	2.10 (3H, s), 2.41 (3H, s), 3.50 (3H, s), 5.07 (1H, s), 6.52 (1H, brs), 7.32 (9H, m)
49	3300	1630	1350	1.20 (6H, d, J=7 Hz), 2.43 (3H, s), 3.58 (3H, s), 2.95 (1H, m), 5.28 (1H, s), 6.18 (1H, s), 6.45 (1H, brs), 7.69 (4H, m)
50	3300	1632	1345	1.20 (12H, m), 2.35 (4H, m), 2.36 (3H, s), 3.63 (3H, s), 5.22 (1H, s), 6.55 (1H, brs), 7.65 (4H, m)
51	3310	1640	1350	2.08 (11H, m), 3.58 (3H, s), 5.13 (1H, s), 6.42 (1H, brs), 7.65 (4H, m)

fraction, a diastereomeric mixture of 4-phenyl-**53a** (**53c**, 1.01 g, 41.5%). ^{1}H-NMR δ: 2.23 (3H, s), 3.43, 3.65 (3H, s), 3.72 (2H, br s), 4.37, 4.42 (1H, d, J=12 Hz), 5.97 (1H, s), 7.51 (9H, m). **53c**-Benzamide, liquid. IR ($CHCl_3$): 1350, 1660, 1720, 1745 cm^{-1}. ^{1}H-NMR δ: 2.03, 2.23 (3H, s), 3.43, 3.60 (3H, s), 4.55 (1H, d, J=12 Hz), 5.27, 5.30 (1H, d, J=12 Hz), 6.21 (1H, s), 7.49 (14H, m). The 3rd fraction, a diastereomeric mixture of 4-phenyl-**54a** (**54c**, 0.165 g, 5.2%). ^{1}H-NMR δ: 2.08, 2.30 (3H, s), 3.45, 3.67 (3H, s), 4.48, 4.51 (1H, d, J=12 Hz), 5.26, 5.32 (1H, d, J=12 Hz), 6.92 (1H, s), 7.79 (14H, m). The 4th fraction, 3-phenyl-**19** (**21**, 0.287 g, 12.4%) listed in Tables II and V.

Reaction of 4 with 12——Method A: A mixture of **4** (1.0 g, 5.52 mmol) and **12** (1.46 g, 5.52 mmol) in *tert*-BuOH (10 ml) was treated in a manner similar to that described above. Purification by chromatography on silica gel with benzene–AcOEt (9 : 1) gave the following products. The 1st fraction, a diastereomeric mixture of 4-cyclohexyl-**53a** (**53d**, 0.259 g, 10.9%). ^{1}H-NMR δ: 2.13, 2.25 (3H, s), 2.77 (1H, m), 3.55, 3.68 (3H, s), 3.73 (2H, br s), 4.13 (1H, d, J=12 Hz), 5.20, 5.23 (1H, d, J=12 Hz), 5.95 (1H, s), 7.87 (4H, m). The 2nd fraction, a diastereomeric mixture of 4-cyclohexyl-**54a** (**54d**, 0.855 g, 27.5%). ^{1}H-NMR δ: 1.54 (10 H, m), 2.17, 2.30 (3H, s), 2.82 (1H, m), 3.58, 3.68 (3H, s), 4.60 (1H, d, J=12 Hz), 5.35 (1H, d, J=12 Hz), 7.00 (1H, s), 8.10 (3H, m). The 3rd fraction, 3-cyclohexyl-**19** (**22**, 0.302 g, 13.3%) listed in Tables II and V.

Reaction of 5 with 12——Method A: A mixture of **5** (1.0 g, 8.85 mmol) and **12** (2.35 g, 8.85 mmol) in *tert*-BuOH (10 ml) was treated in a manner similar to that described above. Purification by chromatography on silica gel with benzene–AcOEt (9 : 1) gave the following products. The 1st fraction, 5-methyl-**55a** (**55e**, 0.57 g, 26.2%), yellow needles, mp 113—114 °C (MeOH). IR ($CHCl_3$): 1350 cm^{-1}. ^{1}H-NMR δ: 2.45 (3H, d, J=1 Hz), 6.63 (1H, m), 6.98 (1H, d, J=3 Hz), 7.55 (1H, d, J=8 Hz), 8.17 (2H, m), 8.30 (1H, s), 8.60 (1H, t, J=1 Hz). *Anal.* Calcd for $C_{12}H_{10}N_2O_2S$: C, 58.52; H, 4.09; N, 11.37. Found: C, 58.54; H, 4.05; N, 11.34. The 2nd fraction, 2-methyl-**12** (**45**, 1.814 g, 59.6%), listed in Tables II and V.

Reaction of 7 with 12——Method A: A mixture of **7** (0.7 g, 5.50 mmol) and **12** (1.37 g 5.50 mmol) in *tert*-BuOH (10 ml) was treated in a manner similar to that described above. Purification by chromatography on silica gel with $CHCl_3$ gave the following products. The 1st fraction, 4,5-dimethyl-**55a** (**55g**, 0.372 g, 26.0%), yellow needles, mp 111—113 °C (MeOH). IR (Nujol): 1350 cm^{-1}: ^{1}H-NMR δ: 2.07 (3H, s), 2.29 (3H, s), 6.78 (1H, s), 7.99 (5H, m). *Anal.* Calcd for $C_{13}H_{12}N_2O_2S$: C, 59.98; H, 4.65; N, 10.76. Found: C, 60.07; H, 4.46; N, 10.82. The 2nd fraction, 2,3-dimethyl-**19** (**47**), 1.16 g, 58.8%) listed in Tables II and V.

Preparation of 47——Method C: A mixture of 2,3-dihydro-4,5-dimethyl-2-oxothiophene (**95**,[17] 4.61 g, 36 mmol) and 3-nitrobenzaldehyde (5.435 g, 36 mmol) in EtOH (20 ml) was saturated with HCl under ice cooling. After stirring for 5 h at 0 °C, the precipitated crystalline product was collected by filtration, washed with 50% aqueous EtOH, and recrystallized from EtOH to give the benzylidene derivative **96** (6.19 g, 65.8%) as yellow needles, mp 155—156 °C. IR (Nujol): 1350, 1672 cm^{-1}. ^{1}H-NMR δ: 1.98 (3H, s), 2.21 (3H, s), 6.78 (1H, s), 8.04 (4H, m). *Anal.* Calcd for $C_{13}H_{11}NO_3S$: C, 59.76; H, 4.24; N, 5.36. Found: C, 60.04; H, 4.11; N, 5.26. A mixture of **96** (0.2 g, 0.77 mmol) and methyl 3-aminocrotonate (0.18 g, 1.56 mmol) in propionic acid (3 ml) was refluxed for 5 h, and then evaporated. The residue was chromatographed on silica gel with benzene–AcOEt (9 : 1) to give yellow crystals (18 mg, 6.5%). This product was identical with **47**, prepared by method A described above, based on a comparison of their IR spectra.

Methyl 2-R^2-3-R^3-4,7-dihydro-6-methyl-4-(3-nitrophenyl)-thieno[2,3-*b*] pyridine-5-carboxylates (**46** and **49**—**51**) were prepared in a manner similar to that described for **47**, and are listed in Tables II and V.

Preparation of 20 by the Reaction of 12 with 58——Method B: A mixture of **12** (3.86 g, 15 mmol) and **58** (3.3 g, 15 mmol) in *tert*-BuOH (30 ml) was refluxed for 90 h, and then evaporated. The residue was chromatographed on silica gel with CH_2Cl_2 to give the following products. The 1st fraction, *tert*-butyl 4-methyl-2-(3-nitrobenzylidene)aminothiophene-5-carboxylate (**94a**, 0.638 g, 12.3%), a viscous liquid. IR ($CHCl_3$): 1350, 1683 cm^{-1}. ^{1}H-NMR δ: 1.58 (9H, s), 2.35 (3H, s), 6.98 (1H, s), 8.20 (5H, m). The 2nd fraction, 2-*tert*-butoxycarbonyl-**20** (**69**, 4.33 g, 65.0%), yellow prisms, mp 131—134 °C (ether). IR (Nujol): 1348, 1635, 1697, 3320 cm^{-1}. ^{1}H-NMR δ: 1.51 (9H, s, *tert*-Bu), 5.22 (1H, s, C-4 H). *Anal.* Calcd for $C_{23}H_{24}N_2O_6S$: C, 59.44; H, 5.44; N, 6.30. Found: C, 59.25; H, 5.42; N, 6.11. A mixture of **69** (1.0 g, 2.25 mmol) and trifluoroacetic acid (4 ml) was stirred at 20 °C for 2 h, and then evaporated. The residue was chromatographed on silica gel with $CHCl_3$ to give **20**, methyl 4,7-dihydro-3,6-dimethyl-4-(3-nitrophenyl)thieno[2,3-*b*]pyridine-5-carboxylate (0.656 g, 84.8%), which was shown to be identical with the sample prepared by method A, by comparison of their IR spectra.

Alkyl (R^1) 4-aryl-3-R^3-4,7-dihydro-6-methylthieno[2,3-*b*]pyridine-5-carboxylates (**21**—**44**) were prepared in a manner similar to that described for **20** (method B). Their yields, melting points, elementary analyses and spectral data are listed in Tables II and V.

Methyl 4,7-Dihydro-3-isobutyl-7-methoxymethyl-6-methyl-4-(3-nitrophenyl)thieno[2,3-*b*]pyridine-5-carboxylate (97)——A solution of **25** (41.9 g, 0.108 mol) in tetrahydrofuran (THF) (210 ml) was added dropwise to a mixture of 60% NaH–oil dispersion (6.5 g, 0.163 mol) in THF (200 ml) under ice cooling. The mixture was stirred for 20 min, then methoxymethyl chloride (12.4 ml, 0.163 mol) was added dropwise at 3—8 °C. The mixture was stirred for a further 1 h, quenched with aqueous NH_4Cl, and evaporated. The residue was extracted with ether, and the extract was washed with water, dried, and evaporated. The residue was chromatographed on silca gel with CH_2Cl_2 to give **97** (37.5 g, 80.5%) as a viscous liquid. IR ($CHCl_3$): 1347, 1690 cm^{-1}. ^{1}H-NMR δ: 0.80 (6H, d, J=7 Hz), 1.62 (1H, m), 2.11 (2H, m), 2.55 (3H, s), 3.50 (3H, s), 3.70 (3H, s), 5.02 (1H, d, J=11 Hz), 5.27 (1H, s), 6.36 (1H, s), 7.71 (4H, m).

Hydrolysis of 97——A solution of **97** (35.72 g, 0.083 mol) with KOH (16.8 g, 0.258 mol) in 80% aqueous MeOH (670 ml) was refluxed for 25 h. The aqueous solution obtained after removal of MeOH and addition of water (100 ml) was washed with ether, and acidified with HCl. The precipitated crystalline solid was collected by filtration, washed with water, and recrystallized from MeOH to give 4,7-dihydro-3-isobutyl-7-methoxymethyl-6-methyl-4-(3-nitrophenyl)thieno[2,3-*b*]pyridine-5-carboxylic acid (**98**, 20.7 g, 60.2%) as yellow plates, mp 149 °C (dec). IR (Nujol): 1345, 1660, 2650 cm^{-1}. ^{1}H-NMR δ: 0.80 (6H, d, J=7 Hz), 1.63 (1H, m), 2.13 (2H, m), 2.60 (3H, s), 3.50 (3H, s), 5.05 (1H, d, J=11 Hz), 5.20 (1H, d, J=11 Hz), 5.30 (1H, s), 6.40 (1H, s), 7.72 (4H, m). *Anal.* Calcd for $C_{21}H_{22}N_2O_5S$: C, 60.86; H, 5.35; N, 6.76. Found: C, 60.47; H, 5.75; N, 6.79.

Resolution of 98——A solution of **98** (16.65 g, 40.2 mmol) and cinchonine (11.83 g, 40.2 mmol) in CH_2Cl_2 (100 ml), after dilution with EtOH (150 ml), was concentrated *in vacuo* to 137 g in total. The precipitated crystals (12.81 g), after standing overnight, were collected by filtration, and dissolved in CH_2Cl_2–EtOH (1 : 2) (300 ml). The solution, after concentration *in vacuo* to 150 g in total, was allowed to stand for 3 d. The precipitated yellow needles [(+)-**98**-cinchonine salt] (10.55 g, 37.0%) were collected by filtration. mp 202—203 °C, $[\alpha]_D^{23}$: +124.2±1.7° (c=0.977, $CHCl_3$). The salt (4.0 g, 5.64 mmol) was treated with 0.1 N HCl (72 ml) in ether (60 ml). The organic layer was separated, washed with water, dried, and evaporated to give (+)-**98** (2.36 g) as a yellow amorphous solid, $[\alpha]_D^{24}$: +166.6±2.0° (c=1.017, EtOH). The filtrates were collected and evaporated. The residue was treated with 0.1 N HCl in ether, and the organic layer was separated, washed with water, dried, and evaporated to give a yellow viscous material (8.1 g). This was dissolved in a solution of cinchonidine (5.61 g, 19.1 mmol) in EtOH (40 ml). The resulting solution was left to stand for 3 d, and the precipitated yellow needles, (−)-**98**-cinchonidine salt (8.32 g, 29.2%), were collected by filtration. mp 171—172 °C, $[\alpha]_D^{23}$: −81.2 ±1.3° (c=0.962, $CHCl_3$). The salt (4.0 g, 5.64 mmol) was treated with 0.1 N HCl (72 ml) in ether (60 ml). The organic layer was separated, washed with water, dried, and evaporated to give (−)-**98** (2.33 g) as a yellow amorphous solid, $[\alpha]_D^{24}$: −168.5±2.2° (c=0.922, EtOH).

(+)-Methyl 4,7-Dihydro-3-isobutyl-6-methyl-4-(3-nitrophenyl)thieno[2,3-*b*]pyridine-5-carboxylate [(+)-25]——A solution of (+)-**98** (2.324 g, 5.64 mmol) in ether (30 ml) was added dropwise to a solution of diazomethane (14 mmol) in ether (15 ml) at room temperature. The mixture, after being stirred for 0.5 h, was quenched with acetic acid, washed with water, dried, and evaporated to give (+)-7-methoxymethyl-**25** [(+)-**97**, 2.41 g, 99.7%] as a viscous liquid, $[\alpha]_D^{25}$: +153.4±1.6° (c=1.023, EtOH); HPLC t_R: 9.09 min. A solution of (+)-**97** (2.39 g, 5.55 mmol) with 1 N HCl (13 ml) in acetone (65 ml) was stirred at 25 °C for 1 h. After removal of the solvent, the residue was chromatographed on silica gel with CH_2Cl_2–CH_3CN (50 : 1) to give (+)-**25** (1.738 g, 81.0%) as a yellow crystalline solid. Recrystallization from EtOH gave yellow plates, mp 173—175 °C, $[\alpha]_D^{24}$: +276.4±3.1° (c=1.013, EtOH); HPLC t_R: 5.40 min. IR (Nujol): 1342, 1630, 3305 cm^{-1}. *Anal.* Calcd for $C_{20}H_{22}N_2O_4S$: C, 62.16; H, 5.74; N, 7.25. Found: C, 62.08; H, 5.78; N, 7.16.

(−)-Methyl 4,7-Dihydro-3-isobutyl-6-methyl-4-(3-nitrophenyl)thieno[2,3-*b*]pyridine-5-carboxylate [(−)-25]——A solution of (−)-**98** (2.326 g, 5.61 mmol) in ether (30 ml) was treated with diazomethane in a manner similar to that described above to give (−)-7-methoxymethyl-**25** [(−)-**97**] (2.356 g, 97.6%) as a viscous liquid, $[\alpha]_D^{23}$: −153.4±1.4° (c=1.071, EtOH). HPLC t_R: 7.03 min. A solution of (−)-**97** (2.345 g, 5.45 mmol) and 1 N HCl (13 ml) in acetone (65 ml) was treated in a manner similar to that described above to give (−)-**25** (1.525 g, 72.4%) as yellow plates, mp 173—175 °C, $[\alpha]_D^{24}$: −275.9±3.2° (c=0.976, EtOH): HPLC t_R: 5.03 min; IR (Nujol): 1342, 1630, 3305 cm^{-1}. *Anal.* Calcd for $C_{20}H_{22}N_2O_4S$: C, 62.16; H, 5.74; N, 7.05. Found: C, 62.12; H, 5.87; N, 7.25.

***R*-(−)-Methyl 2-Bromo-4,7-dihydro-3-isobutyl-7-methoxymethyl-6-methyl-4-(3-nitrophenyl)thieno[2,3-*b*]pyridine-5-carboxylate (99)**——A solution of (−)-**97** (0.65 g, 1.41 mmol) and *N*-bromosuccinic imide (0.3 g, 1.69 mmol) in CCl_4 (7 ml) was refluxed for 0.5 h. The mixture, after removal of the precipitates by filtration, was evaporated and the residue was chromatographed on silica gel with CH_2Cl_2 to give **99** (0.543 g, 73.7%) as a yellow crystalline solid. Recrystallization from MeOH gave pale yellow plates, mp 110—111 °C, $[\alpha]_D^{26}$: −232.4±2.5° (c=1.088, MeOH). IR (Nujol): 1352, 1695 cm^{-1}. ^{1}H-NMR δ: 0.86 (6H, d, J=6 Hz), 1.98 (3H, m), 2.52 (3H, s), 3.47 (3H, s), 3.69 (3H, s), 4.93 (1H, d, J=11 Hz), 5.07 (1H, d, J=11 Hz), 5.23 (1H, s), 7.69 (4H, m). *Anal.* Calcd for $C_{22}H_{25}BrN_2O_5S$: C, 51.87; H, 4.95; Br, 15.69; N, 5.50. Found: C, 51.76; H, 4.98; Br, 15.87; N, 5.51.

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References and Notes

1) A part of this work was presented at the Japanese-United States Congress of Pharmaceutical Sciences 1987, Honolulu, December 1987.
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group, followed by thermal cleavage of the amino-adducts, may provide the Schiff bases. The mechanism of the reactions has been discussed in our preceding paper.[2a)]

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