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Novel and Potent Aldose Reductase Inhibitors: 4-Benzyl- and 4-(Benzothiazol-2-ylmethyl)-3,4-dihydro-3-oxo-2*H*-1,4-benzothiazine-2-acetic Acid Derivatives[1)]

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A number of 1,4-benzothiazine-2-acetic acid derivatives (1, 2 and 3) and their bioisosteres (15b, 16, 18 and 20b) were synthesized and evaluated *in vitro* for the ability to inhibit aldose reductase (AR) in porcine lens. The compounds which exhibited potent activity *in vitro* were also assayed *in vivo* for inhibitory activity against sorbitol accumulation in the erythrocytes, sciatic nerve and lens of streptozotocin-diabetic rats. The 4-(substituted benzothiazol-2-ylmethyl)-1,4-benzothiazine-2-acetic acid derivatives (2 and 3) showed more potent AR inhibitory activity than did the 4-(4-bromo-2-fluorobenzyl)-1,4-benzothiazine-2-acetic acid derivatives (1). 4-(4,5,7-Trifluorobenzothiazol-2-ylmethyl)-3,4-dihydro-3-oxo-2*H*-1,4-benzothiazine-2-acetic acid (2q, SPR-210) showed not only a potent AR-inhibitory activity *in vitro* (IC_{50} 9.5×10^{-9} M) but also a significant reduction in sorbitol accumulation in rat sciatic nerve (ID_{50} 0.1 mg/kg) and lens (ID_{50} 9.8 mg/kg). Optical resolution of the racemic SPR-210 was achieved by means of a diastereomer salt method using (−)-brucine. The biological activities of both enantiomers, (+)- and (−)-SPR-210, were comparable to that of the racemate.

Keywords aldose reductase inhibitor; 1,4-benzothiazine-2-acetic acid; sorbitol accumulation; benzothiazole; bioisostere; optical resolution

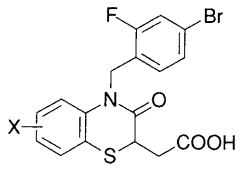
In hyperglycemia, aldose reductase (AR) catalyzes the conversion of an excess of glucose into sorbitol, and the accumulation of intracellular sorbitol is considered to cause the development of diabetic complications such as neuropathy, retinopathy, nephropathy, and cataracts.[3)] Aiming at treatment of these diseases, several acetic acid derivatives, for example, tolrestat,[4)] epalrestat,[5)] zenarestat,[6)] AD-5467,[7)] and zopolrestat[8)] have been developed as AR inhibitors.

In the course of our studies in search of new AR inhibitors, we synthesized 4-(4-bromo-2-fluorobenzyl)-1,4-benzothiazine-2-acetic acid derivatives whose structures are similar to that of ponalrestat[9)] and found that some of these compounds exhibited potent AR-inhibitory activity. Some AR inhibitors possessing 1,4-benzothiazine-4-acetic acid structure have been reported,[7,10)] but their activity is weak. It has also been reported that the structure of zopolrestat[8)] is characterized by a substituted benzothiazol-2-ylmethyl moiety. These results prompted us to synthesize a series of 4-(substituted benzothiazol-2-yl)methyl-1,4-benzothiazine-2-acetic acid derivatives.

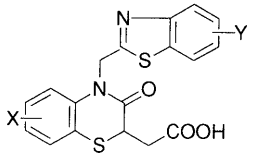
This paper describes the synthesis and biological activity of 4-(4-bromo-2-fluorobenzyl)- and 4-(substituted benzothiazol-2-yl)methyl-3,4-dihydro-3-oxo-2*H*-1,4-benzothiazine-2-acetic acids (**1**, **2** and **3**) including a novel orally active AR inhibitor, SPR-210 (Chart 1), and their bioisosteres (**15b**, **16**, **18** and **20b**) (Chart 4). We also report optical resolution of racemic SPR-210 and the biological activity of both enantiomers.

Chemistry

The starting 2-aminothiophenols (**8**) and their hydrochlorides (**13**) were prepared according to the literature.[8,11)] Exceptionally, 2-amino-3,4,6-trifluorothiophenol (**7**) was newly prepared as shown in Chart 2. Acetylation of 2,3,5,6-tetrafluoroaniline **4** with acetic anhydride followed by thionization with phosphorus pentasulfide afforded the thioacetanilide (**5b**). The presence of two rotamers was observed in the proton NMR spectrum of **5b**, which exhibited two signals of methyl protons at δ 2.48 and 2.79. This phenomenon might be due to an intramolecular hydrogen bond between the thioamide

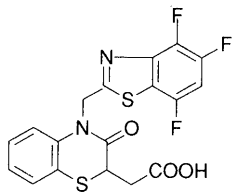


1: X = F, Cl, Me, OMe



2: X = H
Y = F, Cl, Br, OMe, di-F, di-Cl, tri-F *etc.*

3: X = F, Cl, Me, OMe, CF_3
Y = di-F, di-Cl, tri-F



SPR-210 (**2q**)

Chart 1

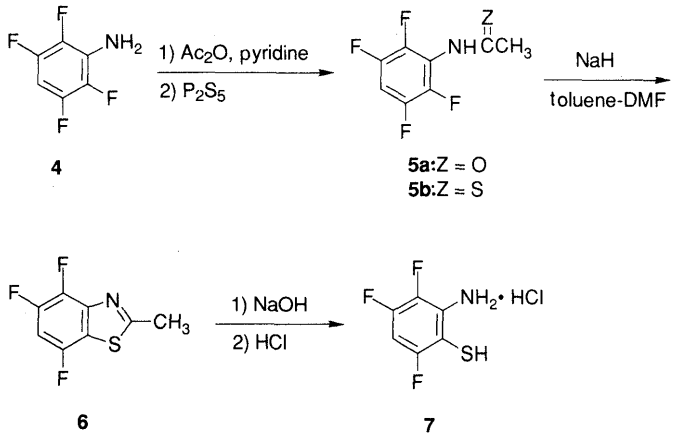


Chart 2

proton and the fluorine atom.[12] Cyclization of **5b** by treatment with sodium hydride in a mixture of dimethylformamide (DMF) and toluene gave the benzothiazole **6** which was finally hydrolyzed to the desired 2-amino-3,4,6-trifluorothiophenol **7**. The 4-benzyl derivatives **1** and the 4-(benzothiazol-2-yl)methyl derivatives **2** and **3** of 1,4-benzothiazine-2-acetic acid were prepared by the synthetic route outlined in Chart 3. The required intermediates, ethyl 3,4-dihydro-3-oxo-2*H*-1,4-benzothiazine-2-acetates (**9**) (Table I), were prepared by reacting 2-aminothiophenols **8** with diethyl fumarate according to Bourdais.[13] Treatment of **9** with 4-bromo-2-fluorobenzyl bromide **10** at room temperature in the presence of sodium hydride in DMF provided the 4-benzylbenzothiazine derivatives **11** which were hydrolyzed to the corresponding acids **1**. On the other hand, the intermediates **9** were treated with bromoacetonitrile in the presence of potassium

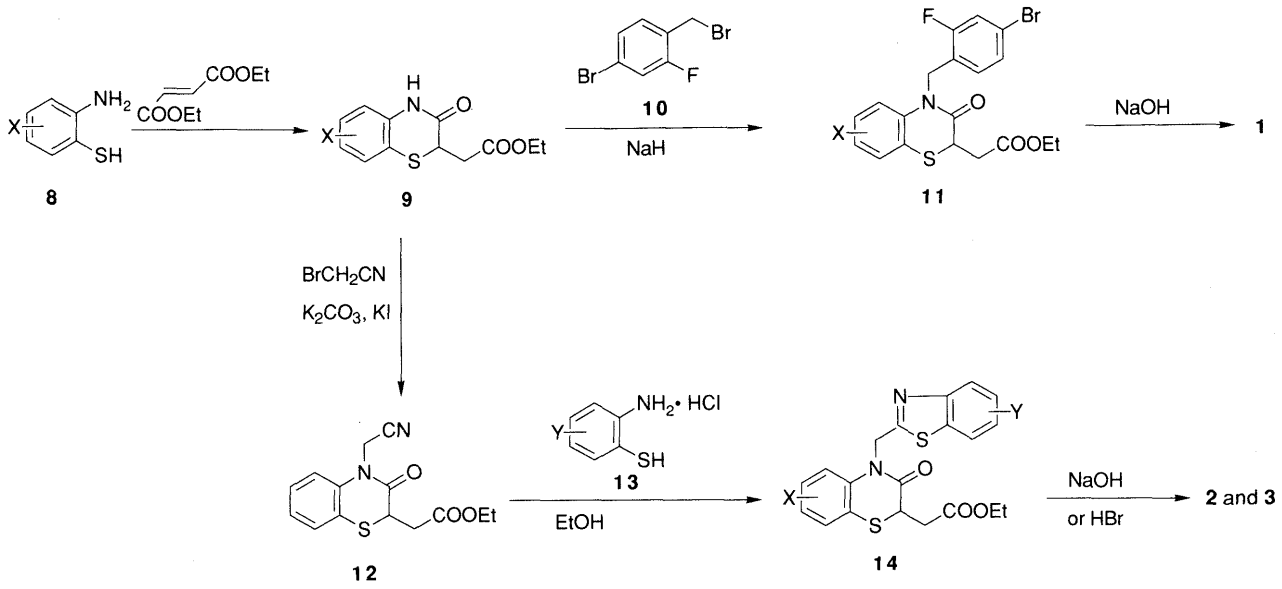
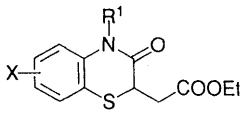


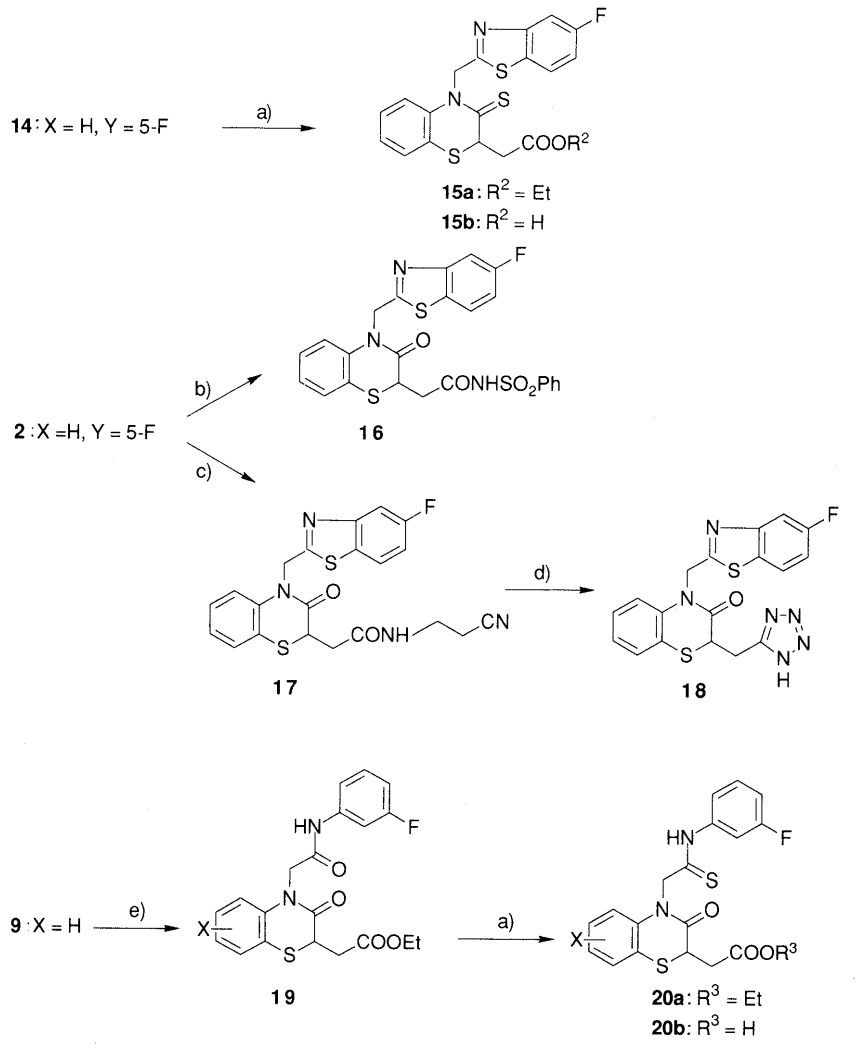
Chart 3

TABLE I. 1,4-Benzothiazine-2-acetates **9** and Their 4-Cyanomethyl Derivatives **12**



X[a]	R^1	Recrystn. solvent[b]	mp (°C)	Yield (%)	X[a]	R^1	Recrystn. solvent[b]	mp (°C)	Yield (%)
H	H	E	128	92	6-OMe	H	E	119—120	50
5-F	H	E-I	111—112	67	7-OMe	H	E	123—124	40
6-F	H	E-I	123—124	48	7-CF_3	H	E-I	188—190	86
7-F	H	E-I	147—148	75	H	CH_2CN	E-I	86—88	72
8-F	H	E	180—182	70	6-F	CH_2CN	E-I	95—96	74
5-Cl	H	E	108—109	47	7-F	CH_2CN	E-I	209—210	65
7-Cl	H	E	166—167	24	8-F	CH_2CN	E-I	72—73	64
8-Cl	H	E	118	50	7-Cl	CH_2CN	E-I	116—117	78
5-Me	H	E	139—140	82	6-Me	CH_2CN	E-I	103—104	70
6-Me	H	E	126—127	52	7-Me	CH_2CN	E	97—99	65
7-Me	H	E	154	89	6-OMe	CH_2CN	E	82—83	72
8-Me	H	E	95—97	46	7-OMe	CH_2CN	E	85	49
5-OMe	H	E	103—106	45	7-CF_3	CH_2CN	E-I	80—82	73

a) Structures of all new compounds were confirmed by NMR and MS. *b*) E, EtOH; I, isopropyl ether.



a) i) P_2S_5 ; ii) NaOH. b) $PhSO_2NH_2$, WSC•HCl, HBT. c) 3-aminopropionitrile, WSC•HCl, HBT. d) i) $TMSN_3$, DEAD, PPh_3 ; ii) NaOH. e) 3-F-$PhNHCOCH_2Cl$, NaH

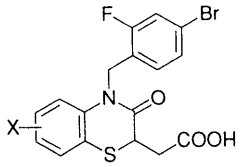
Chart 4

carbonate and a catalytic amount of potassium iodide in dimethyl sulfoxide to give the 4-cyanomethyl derivatives **12** (Table I), which were converted to 4-(substituted benzothiazol-2-yl)methyl-1,4-benzothiazine-2-acetates (**14**) through acid-catalyzed cyclization with 2-aminothiophenol hydrochlorides **13** in boiling anhydrous ethanol. The compounds **14** were further hydrolyzed to the corresponding acids **2** and **3**. Physical data for **1**, **2** and **3** are listed in Tables II and III, respectively.

We also synthesized **15b**, **16**, **18** and **20b** as shown in Chart 4. The thiolactam **15b** was obtained by treatment of **14** (X=H, Y=5-F) with phosphorus pentasulfide, followed by alkaline hydrolysis. Condensation of **2c** (X=H, Y=5-F) with benzenesulfonamide in the presence of 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (WSC·HCl) and 1-hydroxybenzotriazole provided the sulfonamide **16**. The tetrazole **18** was synthesized according to Duncia, *et al.*,[14] through condensation of **2c** with 3-aminopropionitrile using WSC to afford the *N*-(2-cyanoethyl)amide **17** and subsequent transformation of **17** to the tetrazole **18** employing triphenylphosphine, diethyl azodicarboxylate (DEAD) and trimethylsilylazide ($TMSN_3$). Alkylation of **9** (X=H) with *N*-(3-fluorophenyl)chloroacetamide afforded the acetamide **19** which was converted to the corresponding thioamide **20b** by treatment with phosphorus pentasulfide, followed by hydrolysis. Compound **20b** has the ring-opened structure of the benzothiazole moiety of **2c**.

The optical resolution of the racemic SPR-210 was effected by means of a diastereomer salt method using a chiral amine. Thus, a solution of racemic SPR-210 and (−)-brucine in methanol was kept at room temperature to deposit (−)-SPR-210·(−)-brucine salt as a crystalline solid, which was purified by recrystallization from methanol. The free acid (−)-SPR-210 was obtained by treatment of the salt with 2 N hydrochloric acid. Similarly, the free acid (+)-SPR-210 was isolated from the (+)-SPR-210·(−)-brucine salt which was contained in the methanolic solution. Optical purities (>98%) of the enantiomers were determined by means of HPLC using a

TABLE II. Physical Data and AR Inhibitory Activity *in Vitro* of 4-(4-Bromo-2-fluorobenzyl)-1,4-benzothiazine-2-acetic Acids **1**



Compound	X	Formula[a]	Recrystn. solvent[b]	mp (°C)	Yield (%)	IC_{50} (M)[c]
1a	H	$C_{17}H_{13}BrFNO_3S$	Ac-H	138—139	75	6.1×10^{-8}
1b	5-F	$C_{17}H_{12}BrF_2NO_3S\cdot1/2H_2O$	Ac-H	146—149	87	6.3×10^{-8}
1c	6-F	$C_{17}H_{12}BrF_2NO_3S$	I-H	147—148	70	6.8×10^{-8}
1d	7-F	$C_{17}H_{12}BrF_2NO_3S$[d]	Ac-I	136—138	53	6.1×10^{-8}
1e	5-Cl	$C_{17}H_{12}BrClFNO_3S$	Ac-H	147—149	72	2.0×10^{-7}
1f	7-Cl	$C_{17}H_{12}BrClFNO_3S$	Ac-I	164—166	58	5.7×10^{-8}
1g	8-Cl	$C_{17}H_{12}BrClFNO_3S$	I-H	158—159	60	4.1×10^{-8}
1h	5-Me	$C_{18}H_{15}BrFNO_3S$	Ac	192—194	76	4.5×10^{-7}
1i	6-Me	$C_{18}H_{15}BrFNO_3S$	I-H	134—135	66	3.5×10^{-8}
1j	7-Me	$C_{18}H_{15}BrFNO_3S$	Ac-I	152—154	89	4.0×10^{-8}
1k	8-Me	$C_{18}H_{15}BrFNO_3S$	I	72—73	90	4.6×10^{-8}
1l	5-OMe	$C_{18}H_{15}BrFNO_4S$	E-I	96—98	36	1.8×10^{-7}
1m	7-OMe	$C_{18}H_{15}BrFNO_4S$	E	168—170	43	4.1×10^{-8}

a) Elemental analysis data (C, H, N) were within ±0.4% of the theoretical values unless otherwise indicated. *b*) Ac, AcOEt; E, EtOH; I, isopropyl ether; H, hexane. *c*) IC_{50} value for porcine lens AR. *d*) *Anal.* Calcd: C, 47.57, Found: C, 46.86. HRMS Calcd: 426.9689, Found: 426.9681.

chiral column.

Biological Results and Discussion

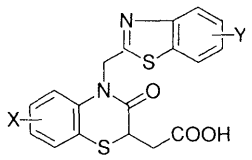
Compounds **1**, **2** and **3** were evaluated *in vitro* for their ability to inhibit porcine lens AR with DL-glyceraldehyde as the substrate.[15] IC_{50} values of **1** are shown in Table II. The compounds **1** having a substituent such as F, Cl, Me and OMe at the 6, 7 and 8 positions of the benzothiazine nucleus (**1c**, **d**, **f**, **g**, **i**, **j**, **k** and **m**) as well as the non-substituted derivative (**1a**) exhibited potent AR-inhibitory activity (IC_{50} 3.5—6.8×10^{-8} M). However the compounds **1** with a substituent such as Cl, Me and OMe at the 5 position (**1e**, **h** and **l**) showed somewhat lower activity. It is interesting that **1b** possessing a less bulky F at the 5-position retained inhibitory activity (IC_{50} 6.3×10^{-8} M) comparable to that of **1a**. These results are probably due to the peri-interaction between the bulky substituents and the 4-benzyl group that may bind effectively with the enzyme in a suitable orientation.

The compounds **2** with the halogen(s)-substituted benzothiazole ring exhibited potent AR-inhibitory activity *in vitro*, as listed in Table III. The IC_{50} values of **2j** (X=H, Y=4,5-di-F), **2q** (X=H, Y=4,5,7-tri-F) and **2r** (X=H, Y=4,5,7-tri-Cl) were of the order of 10^{-9} M, being superior to those of the positive controls, tolrestat, zenarestat and zopolrestat, in our assay system. However, **2k** (X=H, Y=5,6-di-F) was less potent. The 6-methoxy- and 5,7-dimethylbenzothiazole derivatives (**2i** and **2p**, respectively) also showed decreased potency. The introduction of a substituent such as F, Cl, Me and OMe onto the 1,4-benzothiazine nucleus of the highly potent trifluoro derivative **2q** did not significantly influence the inhibitory activity, as seen in **3d**—**i**. It seems, therefore, that the substituent effect on the benzothiazole ring is more important than that on the 1,4-benzothiazine nucleus as a factor governing the inhibitory activity.

We also examined the AR-inhibitory activities of **15b**, **16**, **18** and **20b** obtained as bioisosteres of **2c**. The IC_{50} values are listed in Table IV. The inhibitory activities of these compounds were exceedingly reduced, except for **15b**. These results suggest that the carboxylic acid moiety and the benzothiazole ring were crucial for potent inhibitory activity.

The compounds **2** and **3** with sufficient potency ($IC_{50}<2\times10^{-8}$ M) *in vitro* were also assessed *in vivo* for inhibitory activity against sorbitol accumulation in the erythrocytes, sciatic nerve and lens of streptozotocin-diabetic rats.[16] Many compounds exhibited inhibitory effects superior to that of the positive controls at an oral dose of 30 mg/kg, as shown in Table III. The 4,5-difluoro- and 4,5,7-trifluorobenzothiazolyl derivatives (*e.g.*, **2j**, **2q**, **3a**, **3e**, **3g**, and **3i**) gave particularly promising results. Compound **2q** (X=H, Y=4, 5, 7-tri-F; SPR-210) was significantly active in the erythrocytes and sciatic nerve at an oral dose of 3 mg/kg and in the lens at 10 mg/kg. Acetic acid derivatives generally have insufficient AR-inhibitory activity in the lens.[8] It is worth noting that the present series of compounds having a dihalo-, trihalo-, or tetrafluorobenzothiazole nucleus exhibited good inhibitory activity in the lens. Compounds **3b** and **3c** having a 7-CF_3 and a 6-OMe on the benzothiazine nucleus, respectively, were considerably less active than the corresponding non-substituted derivatives (**2j**, **n**). The derivatives substituted by a halogen atom on the benzothiazine nucleus (**3d**—**g**) showed excellent activities, comparable to that of the non-substituted **2q**. Compounds **3i** possessing a 6-Me as a substituent also exhibited good inhibitory activities. It is characteristic that the introduction of CF_3 and OMe onto the benzothiazine nucleus considerably decreased the activity *in vivo*, but did not change the activity *in vitro*. Based on the data listed in Table III, it is difficult to estimate clearly the contributions

TABLE III. Physical and Biological Data for 4-(Benzothiazol-2-yl)methyl-1,4-benzothiazin-2-ylacetic Acids **2** and **3**



Compd.	X	Y	Formula[a]	Method[b]	Crystn. solvent[c]	mp (°C)	Yield (%)	IC_{50} (M)[d]	% inhibn. of sorbitol accum. *in vivo*, 30 mg/kg, *p.o.*[e]		
									Erythrocyte	Nerve	Lens
2a	H	H	$C_{18}H_{14}N_2O_3S_2$	A	E-I	182—184	69	1.8×10^{-7}		NT	
2b	H	4-F	$C_{18}H_{13}FN_2O_3S_2$	A	Ac-E	204—207	87	1.8×10^{-8}	95.5[g]	95.5[g]	9.0
2c	H	5-F	$C_{18}H_{13}FN_2O_3S_2$	A	Ac-E	181—183	77	1.5×10^{-8}	77.6[g]	51.3[g]	−4.1
2d	H	6-F	$C_{18}H_{13}FN_2O_3S_2$	A	Ac-I	209—210	95	5.8×10^{-8}		NT	
2e	H	7-F	$C_{18}H_{13}FN_2O_3S_2$	A	C-H	195—197	88	3.6×10^{-8}		NT	
2f	H	4-Cl	$C_{18}H_{13}ClN_2O_3s_2$	A	I-N	121—125	72	2.1×10^{-8}		NT	
2g	H	5-Cl	$C_{18}H_{13}ClN_2O_3S_2$	A	C-H	176—178	66	2.5×10^{-8}	38.2	30.8[g]	−22.9
2h	H	5-Br	$C_{18}H_{13}BrN_2O_3S_2$	A	E-I	96—99	87	4.9×10^{-8}		NT	
2i	H	6-OMe	$C_{19}H_{16}N_2O_4S_2$	A	I	202—205	85	1.8×10^{-6}		NT	
2j	H	4,5-F_2	$C_{18}H_{12}F_2N_2O_3S_2$	B	E	173—175	63	9.9×10^{-9}	83.4[g]	98.9[g]	69.3[g]
2k	H	5,6-F_2	$C_{18}H_{12}F_2N_2O_3S_2$	A	E-H	196—197	64	3.2×10^{-7}		NT	
2l	H	5,7-F_2	$C_{18}H_{12}F_2N_2O_3S_2$	A	E	155—157	78	1.4×10^{-8}	98.7[g]	91.3[g]	59.4[g]
2m	H	6,7-F_2	$C_{18}H_{12}F_2N_2O_3S_2$	A	E	208—209	78	1.4×10^{-8}	100.0[g]	93.0[g]	63.2[g]
2n	H	4,5-Cl_2	$C_{18}H_{12}Cl_2N_2O_3S_2\cdot3/2H_2O$	A	C-H	184—186	77	1.5×10^{-8}	97.7[g]	81.4[g]	41.0[f]
2o	H	6,7-Cl_2	$C_{18}H_{12}Cl_2N_2O_3S_2$	A	C-I	194—199	64	1.4×10^{-8}	68.1[g]	53.7[g]	44.9[g]
2p	H	5,7-Me_2	$C_{20}H_{18}N_2O_3S_2$	A	E-O	226—227	66	8.5×10^{-7}		NT	
2q	H	4,5,7-F_3	$C_{18}H_{11}F_3N_2O_3S_2$	A	E-W	141—142	88	9.5×10^{-9}	74.2[g]	96.9[g]	60.9[g]
								[ID_{50} (mg/kg)[h]	1.0	0.1	9.8]
2r	H	4,5,7-Cl_3	$C_{18}H_{11}Cl_3N_2O_3S_2$	A	D-H	190—192	85	9.1×10^{-9}	74.5[g]	44.1[g]	41.2[g]
2s	H	4,5,6,7-F_4	$C_{18}H_{10}F_4N_2O_3S_2$	A	Ac-H	107—111	85	1.2×10^{-8}	82.2[g]	73.2[g]	45.1[g]
3a	6-Me	4,5-F_2	$C_{19}H_{14}F_2N_2O_3S_2$	A	D-H	177—178	81	9.8×10^{-9}	87.3[g]	97.1[g]	57.1[g]
3b	7-CF_3	4,5-F_2	$C_{19}H_{11}F_5N_2O_3S_2$	A	Ac-I	154—156	38	8.2×10^{-9}	87.7[g]	55.5[g]	25.0
3c	6-OMe	4,5-Cl_2	$C_{19}H_{14}Cl_2N_2O_4S_2$	A	D-H	183—185	81	6.8×10^{-9}	74.9[g]	8.7	5.2
3d	6-F	4,5,7-F_3	$C_{18}H_{10}F_4N_2O_3S_2\cdot1/2H_2O$	B	D-H	155—156	79	1.2×10^{-8}	73.8[g]	97.2[g]	74.4[g]
3e	7-F	4,5,7-F_3	$C_{18}H_{10}F_4N_2O_3S_2$[i]	A	Ac-H	94—96	91	9.7×10^{-9}	84.3[g]	100.0[g]	67.5[g]
3f	8-F	4,5,7-F_3	$C_{18}H_{10}F_4N_2O_3S_2\cdot1/2H_2O$	B	D-H	164—165	75	8.7×10^{-9}	77.1[g]	100.0[g]	68.5[g]
3g	7-Cl	4,5,7-F_3	$C_{18}H_{10}ClF_3N_2O_3S_2$	A	Ac-I	167—170	57	8.7×10^{-9}	85.9[g]	96.4[g]	43.6[f]
3h	7-OMe	4,5,7-F_3	$C_{19}H_{13}F_3N_2O_4S_2$	A	E-I	82	67	1.2×10^{-8}	83.9[g]	73.0[g]	41.0[g]
3i	6-Me	4,5,7-F_3	$C_{19}H_{13}F_3N_2O_3S_2\cdot1/2H_2O$	A	E-W	173—176	92	8.4×10^{-9}	74.6[g]	98.5[g]	65.4[g]
Tolrestat								1.7×10^{-8}	100.0[g]	79.9[g]	30.4
Zenarestat								1.2×10^{-8}	100.0[g]	89.3[g]	29.9
Zopolrestat								1.3×10^{-8}	99.5[g]	97.3[g]	28.3

a) See footnote a, Table II. *b*) A, NaOH; B, 47% HBr. *c*) Ac, AcOEt; C, $CHCl_3$; D, CH_2Cl_2; E, EtOH; H, hexane; I, isopropyl ether; N, acetonitrile; O, dioxane; W, H_2O. *d*) IC_{50} value for porcine lens AR. *e*) NT: not tested, *f*) $p<0.05$, *g*) $p<0.01$. *h*) ID_{50} value was determined from the dose response data (3, 10 and 30 mg/kg). i) *Anal.* Calcd: C, 49.69, Found: C, 48.87. HRMS Calcd: 442.0069. Found, 442.0078.

TABLE IV. *In Vitro* AR-Inhibitory Activity of the Bioisosteres

Compound	IC_{50} (M)[a]
15b	4.2×10^{-8}
16	6.7×10^{-6}
18	3.8×10^{-6}
20b	4.2×10^{-6}

a) IC_{50} value for porcine lens AR.

TABLE V. Biological Data for Racemate and Enantiomers of SPR-210 (**2q**)

Compound	IC_{50} (nM)[a]	% inhibition of sorbitol accum., 3 mg/kg, *p.o.*[b]		
		Erythrocyte	Nerve	Lens
±	9.7	99.4[b]	89.4[b]	31.1
+	10.1	94.0[b]	93.4[b]	46.7[b]
−	10.6	97.7[b]	90.1[b]	32.1

a) IC_{50} value for porcine lens AR. *b*) $p<0.01$.

of the substituents (X and Y) in compounds **2** and **3** to the activity. However, the benzothiazole moiety substituted with halogens, particularly with fluorine, seems to be an effective pharmacophore.

The biological activities of (+)-SPR-210 and (−)-SPR-210 were examined . The IC_{50} values and the inhibitory effect against sorbitol accumulation *in vivo* of both enantiomers were comparable to those of racemic SPR-210 (Table V). Similar results were reported with chiral rhodaninacetic acid derivatives, potent AR inhibitors, whose enantiomers exhibited activity equipotent to that of the racemate *in vitro*.[17]

In conclusion, we have found a novel class of AR inhibitors, the 4-(substituted benzothiazol-2-ylmethyl)-1,4-benzothiazine-2-acetic acid derivatives. Among them, compound **2q**, SPR-210, was considered to be of potential

therapeutic value as a potent, orally active AR inhibitor. Further pharmacological evaluations of the compounds exhibiting high potency *in vivo*, including SPR-210, are in progress.

Experimental

Melting points were determined in open capillaries with a Mettler FP62 melting point apparatus and are uncorrected. ^{1}H-NMR spectra were measured on a JEOL JNM-GSX270 spectrometer with chemical shifts given in δ values with tetramethylsilane (TMS) as an internal standard. MS were obtained with a JEOL JMS-SX102 instrument, and optical rotation was measured with a Horiba SEPA-200 polarimeter. HPLC analysis for determination of optical purity was performed using a system consisting of a JASCO 880PU pump, 875UV UV detector (254 nm) and a Chiralcel OD column, 4.6 × 250 mm (Dicel Chemical Industries, Ltd.). The mobile phase was hexane–isopropanol–trifluoroacetic acid (95 : 5 : 0.3), and the flow rate was 1.0 ml/min.

2′,3′,5′,6′-Tetrafluoroacetanilide (5a) A mixture of 2,3,5,6-tetrafluoroaniline (**4**) (20.77g, 126 mmol), pyridine (9.97 g, 126 mmol) and acetic anhydride (12.87 g, 126 mmol) was heated at 120 °C for 2 h. The mixture was diluted with water to deposit **5a** (20.20 g, 78%) as a colorless crystalline powder, mp 138—140°C (lit.[18] 138.5—139 °C). MS *m/z*: 207 (M^+), 165.

2′,3′,5′,6′-Tetrafluorothioacetanilide (5b) A mixture of the acetanilide **5a** (23.75 g, 114.7 mmol) and phosphorus pentasulfide (25.49 g, 114.7 mmol) in benzene (350 ml) was refluxed for 15 h. The mixture was cooled and filtered. The insoluble material was washed with a small volume of ether, and the combined filtrate was extracted with 10% NaOH. The aqueous layer was acidified (to pH 4) with hydrochloric acid and the precipitated product was collected to give **5b** (16.70 g, 65%), mp 86—88 °C. ^{1}H-NMR ($CDCl_3$) δ: 2.79 (2.48) (3H, s), 7.05—7.26 (1H, m), 8.72 (8.10) (1H, s). MS *m/z*: 223 (M^+), 204.

4,5,7-Trifluoro-2-methylbenzothiazole (6) Compound **5b** (1.78 g, 8.0 mmol) in toluene (43 ml) was treated with sodium hydride (384 mg, 60% in oil, 9.6 mmol) at 0 °C. Stirring was continued at room temperature for 30 min, then the mixture was refluxed for 30 min. To the hot suspension was added DMF (8 ml), then the mixture was refluxed for a further 2 h, cooled to room temperature and poured into ice-water. The mixture was extracted with AcOEt and washed with brine. The organic layer was dried and evaporated to dryness. The residue was extracted with hexane, and the hexane-soluble fraction was evaporated to give **6** (1.25 g, 77%), mp 89—90 °C. ^{1}H-NMR ($CDCl_3$) δ: 2.88 (3H, s), 6.94—7.06 (1H, m). MS *m/z*: 203 (M^+), 186, 162.

2-Amino-3,4,6-trifluorothiophenol Hydrochloride (7) A mixture of **6** (1.25 g, 6.2 mmol) and aqueous 30% NaOH (15 ml) in ethylene glycol (15 ml) was refluxed under Ar for 3 h. The reaction mixture was cooled in an ice bath and acidified (to pH 3) with hydrochloric acid. The mixture was extracted with ether, and the organic layer was washed with water, dried, acidified with a 10% methanolic solution of hydrogen chloride (5 ml) and evaporated to dryness. The residue was triturated with cold diisopropyl ether (IPE) to give **7** (1.03 g, 77%), mp 122—124 °C. ^{1}H-NMR ($CDCl_3$–D_2O) δ: 6.32—6.43 (1H, m). MS *m/z*: 356 (disulfide), 179 (M^+), 178.

Typical Procedure for the Synthesis of Ethyl 3,4-Dihydro-3-oxo-2*H*-1,4-benzothiazine-2-acetates (9) A mixture of 2-amino-5-fluorothiophenol (**8**, X = 5-F) (12.4 g, 87 mmol) and diethyl fumarate (15.0 g, 87 mmol) was heated at 170 °C for 2 h under Ar. The mixture was diluted with IPE and the resulting precipitate was collected. Recrystallization from EtOH gave **9** (X = 7-F) (18.3 g, 75%) as colorless needles, mp 147—148 °C. ^{1}H-NMR ($CDCl_3$) δ: 1.28 (3H, t, J = 7.1 Hz), 2.59 (1H, dd, J = 8.0, 16.3 Hz), 3.05 (1H, dd, J = 6.6, 16.3 Hz), 4.01 (1H, dd, J = 6.6, 8.0 Hz), 4.21 (2H, q, J = 7.1 Hz), 6.86—7.07 (3H, m), 9.09 (1H, s). MS *m/z*: 269 (M^+), 195.

Typical Procedure for the Synthesis of Ethyl 4-(4-Bromo-2-fluorobenzyl)-3,4-dihydro-3-oxo-2*H*-1,4-benzothiazine-2-acetates (11) Sodium hydride (88 mg, 60% in oil, 2.2 mmol) was suspended in DMF (1.5 ml). A solution of **9** (X = 5-OMe) (562 mg, 2.0 mmol) in DMF (4 ml) was added dropwise to the sodium hydride suspension with stirring, and then a solution of 4-bromo-2-fluorobenzyl bromide **10** (536 mg, 2.0 mmol) in DMF (1 ml) was added. The mixture was stirred for 2 h at room temperature and diluted with a saturated aqueous solution of NH_4Cl. The resulting mixture was extracted with AcOEt and the organic layer was washed with brine, dried and evaporated to give an oily residue, which was purified by chromatography on a silica gel column (hexane–AcOEt, 4 : 1). The product was crystallized from IPE to give **11** (X = 5-OMe) (598 mg, 64%), mp 116—118 °C. ^{1}H-NMR ($CDCl_3$) δ: 1.27 (3H, t, J = 7.3 Hz), 2.59 (1H, dd, J = 6.6, 16.6 Hz), 3.10 (1H, dd, J = 7.8, 16.6 Hz), 3.77 (3H, s), 3.84 (1H, dd, J = 6.6, 7.8 Hz), 4.18 (2H, q, J = 7.3 Hz), 5.20 (1H, d, J = 16.2 Hz), 5.30 (1H, d, J = 16.2 Hz), 6.73—7.12 (6H, m). MS *m/z*: 469 (M^+), 467, 280.

Typical Procedure for the Hydrolysis of the Ester (7) to 4-(4-Bromo-2-fluorobenzyl)-3,4-dihydro-3-oxo-2*H*-1,4-benzothiazine-2-acetic Acids (1) The ester **11** (X = 5-OMe) (468 mg, 1.0 mmol) was dissolved in dioxane–MeOH (2 : 1, 9 ml), and then 1.6% NaOH (3 ml) was added to the solution. The mixture was stirred for 2 h at room temperature, then diluted with water. The aqueous solution was acidified with 7% HCl and extracted with AcOEt. The organic layer was dried and evaporated to dryness. The residue was recrystallized from EtOH–IPE to give **1** (X = 5-OMe) (195 mg, 44%) as a colorless powder, mp 96—98 °C. ^{1}H-NMR ($CDCl_3$) δ: 2.66 (1H, dd, J = 5.9, 17.0 Hz), 3.14 (1H, dd, J = 8.8, 17.0 Hz), 3.78 (3H, s), 3.83 (1H, dd, J = 5.9, 8.8 Hz), 5.21 (1H, d, J = 15.9 Hz), 5.31 (1H, d, J = 15.9 Hz), 6.74—7.12 (6H, m). MS *m/z*: 441 (M^+), 439, 252. *Anal.* Calcd for $C_{18}H_{15}BrFNO_4S$: C, 49.10; H, 3.43; N, 3.18. Found: C, 49.15; H, 3.52; N, 3.12.

Typical Procedure for the Synthesis of Ethyl 4-Cyanomethyl-3,4-dihydro-3-oxo-2*H*-1,4-benzothiazine-2-acetates (12) A mixture of **9** (X = H) (5.0 g, 20 mmol), bromoacetonitrile (3.57 g, 30 mmol), K_2CO_3 (4.14 g, 30 mmol), KI (370 mg), and dimethyl sulfoxide (DMSO) was stirred at room temperature for 24 h under Ar. The mixture was diluted with water and extracted with AcOEt. The organic layer was washed with brine, dried, and evaporated *in vacuo*. The oily residue was extracted with benzene (50 ml × 2), and the total benzene fractions were concentrated. The residue was recrystallized from EtOH–IPE to give **12** (X = H) (3.54 g, 61%), mp 86—88 °C. ^{1}H-NMR ($CDCl_3$) δ: 1.27 (3H, t, J = 7.3 Hz), 2.58 (1H, dd, J = 7.3, 16.6 Hz), 3.03 (1H, dd, J = 6.8, 16.6 Hz), 3.94 (1H, dd, J = 6.8, 7.3 Hz), 4.18 (2H, q, J = 7.3 Hz), 4.76 (1H, d, J = 17.6 Hz), 4.94 (1H, d, J = 17.6 Hz), 7.10—7.41 (4H, m). MS *m/z*: 290 (M^+), 216.

Typical Procedure for the Synthesis of Ethyl 4-(Substituted benzothiazol-2-ylmethyl)-3,4-dihydro-3-oxo-2*H*-1,4-benzothiazine-2-acetates (14) A mixture of 2-amino-3,4,6-trifluoroaminothiophenol hydrochloride (**7**) (4.31 g, 20 mmol) and **12** (X = H) (5.8 g, 20 mmol) in anhydrous EtOH (40 ml) was refluxed for 17 h under Ar. The mixture was concentrated *in vacuo*, and the residue was diluted with water. The resulting mixture was extracted with AcOEt. The organic layer was dried and evaporated to dryness and the residue was triturated with IPE to obtain **14** (X = H, Y = 4,5,7-tri-F) (5.85 g, 65%) as a colorless powder, mp 109—110 °C. ^{1}H-NMR ($CDCl_3$) δ: 1.29 (3H, t, J = 7.3 Hz), 2.65 (1H, dd, J = 16.4, 7.1 Hz), 3.12 (1H, dd, J = 16.4, 6.9 Hz), 4.06 (1H, dd, J = 6.9, 7.1 Hz), 4.22 (2H, q, J = 7.3 Hz), 5.55 (1H, d, J = 16.6 Hz), 5.62 (1H, d, J = 16.6 Hz), 6.99—7.41 (5H, m). MS *m/z*: 452 (M^+), 378, 250.

Typical Procedure for the Hydrolysis of the Ester (14) to 4-(Substituted benzothiazol-2-ylmethyl)-3,4-dihydro-3-oxo-2*H*-1,4-benzothiazine-2-acetic Acids (2 and 3) a) A 2% NaOH solution (22 ml) was added dropwise to a solution of the ester **14** (X = H, Y = 4,5,7-tri-F) (4.2 g, 9.3 mmol) in MeOH–dioxane (1 : 2, 69 ml). The mixture was stirred for 2 h at room temperature, then diluted with water. The aqueous solution was extracted with AcOEt. The organic layer was dried and evaporated to dryness, and the residue was recrystallized from EtOH–H_2O (2 : 1) to give **2q** (X = H, Y = 4,5,7-tri-F; SPR-210) (3.95 g, 88%) as a colorless crystalline powder, mp 140—142 °C. ^{1}H-NMR ($CDCl_3$) δ: 2.71 (1H, dd, J = 7.1, 16.6 Hz), 3.17 (1H, dd, J = 7.3, 16.6 Hz), 4.02 (1H, dd, J = 7.1, 7.3 Hz), 5.54 (1H, d, J = 16.4 Hz), 5.63 (1H, d, J = 116.4 Hz), 6.98—7.41 (5H, m). MS *m/z*: 424 (M^+), 222, 178. *Anal.* Calcd for $C_{18}H_{11}F_3N_2O_3S_2$: C, 50.94; H, 2.61; N, 6.60. Found; C, 51.17; H, 2.61; N, 6.90.

b) A mixture of **14** (X = H, Y = 4,5-di-F) (1.9 g, 4.4 mmol) and 47% HBr (30 ml) was refluxed for 1 h and then poured into ice-water. The precipitated solid was collected and recrystallized from EtOH to give **2j** (X = H, Y = 4,5-di-F) (1.12 g, 63%) as colorless crystalline powder, mp 173—175 °C. ^{1}H-NMR ($CDCl_3$) δ: 2.72 (1H, dd, J = 6.9, 17.0 Hz), 3.18 (1H, dd, J = 7.3, 17.0 Hz), 4.02 (1H, dd, J = 6.9, 7.3 Hz), 5.55 (1H, d, J = 16.3 Hz), 5.65 (1H, d, J = 16.3 Hz), 7.04—7.51 (6H, m). MS *m/z*: 406 (M^+), 222. *Anal.* Calcd for $C_{18}H_{12}F_2N_2O_3S_2$: C, 53.20; H, 2.98; N, 6.89. Found: C, 53.11; H, 3.02; N, 6.82.

Ethyl 4-(5-Fluorobenzothiazol-2-ylmethyl)-3,4-dihydro-3-thioxo-2*H*-1,4-benzothiazine-2-acetate (15a) A mixture of **2c** (X = H, Y = 5-F) (722 mg, 1.73 mmol) and phosphorus pentasulfide (770 mg, 3.46 mmol)

in toluene (10 ml) was refluxed for 4 h. The insoluble matter was removed by filtration, and the filtrate was evaporated to dryness. The residue was purified by chromatography on a silica gel column (hexane–AcOEt, 10 : 1) to give **15a** (535 mg, 72%). ^{1}H-NMR ($CDCl_3$) δ: 1.26 (3H, t, J=7 Hz), 2.61 (1H, dd, J=9, 16 Hz), 3.06 (1H, dd, J=5, 16 Hz), 4.18 (2H, q, J=7 Hz), 4.61 (1H, dd, J=5, 9 Hz), 5.92 (1H, d, J=16 Hz), 6.46 (1H, d, J=16 Hz), 7.09—7.79 (7H, m). MS m/z: 432 (M^+).

4-(5-Fluorobenzothiazol-2-ylmethyl)-3,4-dihydro-3-thioxo-2*H*-1,4-benzothiazine-2-acetic Acid (15b) The ester **15a** (433 mg, 1 mmol) was dissolved in dioxane–MeOH (2 : 1, 6 ml), and then 8% NaOH (0.6 ml, 1.2 mmol) was added to the solution dropwise at 0 °C. The mixture was stirred for 2 h at room temperature, then diluted with water. The aqueous solution was acidified with 10% HCl and extracted with AcOEt. The organic layer was washed with brine, dried and evaporated to dryness. The residue was recrystallized from $CHCl_3$–hexane to give **15b** (205 mg, 51%) as a colorless powder, mp 240—241 °C. ^{1}H-NMR ($CDCl_3$–DMSO-d_6) δ: 2.57 (1H, dd, J=10, 17 Hz), 3.01 (1H, dd, J=5, 17 Hz), 4.61 (1H, dd, J=5, 10 Hz), 5.90 (1H, d, J=16 Hz), 6.49 (1H, d, J=16 Hz), 7.10—7.82 (7H, m). MS m/z: 404 (M^+).

***N*-Phenylsulfonyl-[4-(5-fluorobenzothiazol-2-ylmethyl)-3,4-dihydro-3-oxo-2*H*-1,4-benzothiazin-2-yl]acetamide (16)** A solution of 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (WSC·HCl) (192 mg, 1.0 mmol), **2c** (X=H, Y=5-F) (388 mg, 1.0 mmol) and 1-hydroxybenzotriazole (HOBt) (126 mg, 1.0 mmol) in CH_2Cl_2 (20 ml) was stirred at room temperature under Ar for 1 h, and then benzenesulfonamide (158 mg, 1.0 mmol) and triethylamine (101 mg, 1.0 mmol) were added. The mixture was stirred further for 15 h, then poured into water. The resulting mixture was extracted with CH_2Cl_2 and the organic layer was washed with brine, dried and evaporated to dryness. The residue was purified by chromatography on a silica gel column (CH_2Cl_2–MeOH, 97 : 3) to give **16** (194 mg, 37%) as colorless powder, mp 145—147 °C. ^{1}H-NMR ($CDCl_3$) δ: 2.69 (1H, dd, J=4.9, 15.1 Hz), 3.02 (1H, dd, J=8.3, 15.1 Hz), 3.87 (1H, dd, J=4.9, 8.3 Hz), 5.41 (1H, d, J=16.6 Hz), 5.59 (1H, d, J=16.6 Hz), 7.03—8.06 (12H, m), 9.48 (1H, br). MS m/z: 527 (M^+), 342, 204. *Anal.* Calcd for $C_{24}H_{18}FN_3O_4S_3$: C, 54.64; H, 3.44; N, 7.96. Found: C, 54.54; H, 3.41; N, 7.94.

***N*-2-Cyanoethyl-[4-(5-fluorobenzothiazol-2-ylmethyl)-3,4-dihydro-3-oxo-2*H*-1,4-benzothiazin-2-yl]acetamide (17)** A solution of WSC·HCl (192 mg, 1.0 mmol), **2c** (X=H, Y=5-F) (388 mg, 1.0 mg), and HOBt (135 mg, 1.0 mol) in CH_2Cl_2 (10 ml) was stirred at room temperature for 1 h under Ar, then a solution of 3-aminopropionitrile (70 mg, 1.0 mmol) in CH_2Cl_2 (3 ml) and triethylamine (101 mg, 1.0 mmol) was added. The mixture was stirred for 24 h, then poured into water. The resulting mixture was extracted with CH_2Cl_2 and the organic layer was washed with water, dried and evaporated to dryness. The residue was recrystallized from AcOEt–EtOH to give **17** (370 mg, 84%) as a colorless powder, mp 192—194 °C. ^{1}H-NMR ($CDCl_3$) δ: 2.53—2.70 (3H, m), 2.95 (1H, dd, J=7.8, 14.4 Hz), 3.47—3.55 (2H, m), 4.02 (1H, dd, J=5.9, 7.8 Hz), 5.55 (2H, s), 6.66 (1H, br), 7.04—7.42 (5H, m), 7.66—7.88 (2H, m). MS m/z: 440 (M^+), 290.

5-[4-(5-Fluorobenzothiazol-2-ylmethyl)-3,4-dihydro-3-oxo-2*H*-1,4-benzothiazin-2-ylmethyl]-1*H*-tetrazole (18) A mixture of **17** (350 mg, 0.8 mmol), triphenylphosphine (419 mg, 1.6 mmol), diethylazodicarboxylate (278 mg, 1.6 mmol) and trimethylsilyl azide (184 mg, 1.6 mmol) in THF (8 ml) was stirred at room temperature for 24 h under Ar. The reaction mixture was diluted with water and extracted with AcOEt. The organic layer was dried and evaporated to give an oily residue which was purified by chromatography on a silica gel column (hexane–AcOEt, 3 : 1). The oily product (150 mg) was treated in a mixture of 1.6% NaOH (1.5 ml), dioxane (3 ml) and MeOH (1.5 ml) at room temperature for 3 h. The reaction mixture was diluted with water and extracted with AcOEt. The organic layer was dried and evaporated to dryness. The residue was triturated with IPE to give **18** (90 mg, 34%) as a colorless powder, mp 220—222 °C. ^{1}H-NMR ($CDCl_3$–CD_3OD) δ: 3.39 (1H, dd, J=7.3, 15.6 Hz), 3.66 (1H, dd, J=7.3, 15.6 Hz), 4.10 (1H, t, J=7.3 Hz), 5.55 (1H, d, J=16.6 Hz), 5.60 (1H, d, J=16.6 Hz), 7.07—7.41 (5H, m), 7.69—7.84 (2H, m). MS m/z: 412 (M^+), 186. *Anal.* Calcd for $C_{18}H_{13}FN_6OS_2$: C, 52.42; H, 3.18; N, 20.38. Found: C, 52.19; H, 3.20; N, 20.15.

Ethyl 4-[2-(3-Fluorophenylamino)-2-oxoethyl]-3,4-dihydro-3-oxo-2*H*-1,4-benzothiazine-2-acetate (19) Sodium hydride (124 mg, 60% in oil, 3.1 mmol) was added to a solution of **9** (X=H) (754 mg, 3.0 mmol) in DMF (7 ml). The mixture was stirred at room temperature for 10 min, then a solution of 3′-fluoro-2-chloroacetanilide (578 mg, 3.1 mmol) in DMF (3 ml) was added dropwise at 0 °C. The reaction mixture was stirred at room temperature for 2 h, then diluted with water and extracted with AcOEt. The organic layer was washed with brine, dried and evaporated to dryness. The residue was purified by chromatography on a silica gel column (toluene–AcOEt, 9 : 1) to give **19** (405 mg, 34%). ^{1}H-NMR ($CDCl_3$) δ: 1.25 (3H, t, J=7 Hz), 2.71 (1H, dd, J=6, 16 Hz), 2.94 (1H, dd, J=8, 16 Hz), 4.01 (1H, dd, J=6, 8 Hz), 4.15 (2H, dq, J=2, 7 Hz), 4.60 (1H, d, J=16 Hz), 4.81 (1H, d, J=16 Hz), 6.74—6.82 (1H, m), 7.01—7.51 (7H, m), 8.67 (1H, br s). MS m/z: 402 (M^+).

Ethyl 4-[2-(3-Fluorophenyl)amino-2-thioxoethyl]-3,4-dihydro-3-oxo-2*H*-1,4-benzothiazine-2-acetate (20a) A mixture of **19** (402 mg, 1.0 mmol) and phosphorus pentasulfide (222 mg, 1.0 mmol) in benzene (7 ml) was heated at 70 °C for 4 h. The insoluble matter was removed by filtration, and the filtrate was evaporated to dryness. The residue was purified by chromatography on a silica gel column (hexane–AcOEt, 9 : 1) to give **20a** (370 mg, 88%). ^{1}H-NMR ($CDCl_3$) δ: 1.24 (3H, t, J=7 Hz), 2.72 (1H, dd, J=6, 16 Hz), 2.93 (1H, dd, J=9, 16 Hz), 4.02 (1H, dd, J=6, 9 Hz), 4.14 (2H, dq, J=2, 7 Hz), 5.05 (1H, br d, J=16 Hz), 5.22 (1H, br d, J=16 Hz), 6.91—7.81 (8H, m), 10.33 (1H, br s). MS m/z: 418 (M^+).

4-[2-(3-Fluorophenyl)amino-2-thioxoethyl]-3,4-dihydro-3-oxo-2*H*-1,4-benzothiazine-2-acetic Acid (20b) The ester **20a** (34 mg, 0.08 mmol) was dissolved in dioxane–MeOH (2 : 1, 1.5 ml). A 2% NaOH solution (0.2 ml, 0.10 mmol) was added dropwise at 0 °C, and the mixture was stirred at room temperature for 1.5 h, then diluted with water. The mixture was acidified with 10% HCl and extracted with AcOEt. The organic layer was washed with brine, dried and evaporated to dryness. The residue was recrystallized from hexane–EtOH to give **20b** as a colorless powder (20 mg, 64%), mp 174—175 °C. ^{1}H-NMR ($CDCl_3$–DMSO-d_6) δ: 2.69 (1H, dd, J =7, 16 Hz), 2.93 (1H, dd, J=8, 16 Hz), 4.03 (1H, dd, J=7, 8 Hz), 4.99 (1H, d, J=17 Hz), 5.23 (1H, d, J=17 Hz), 6.88—7.76 (8H, m). HRMS Calcd for $C_{18}H_{15}FN_2O_3S_2$ 390.0508, Found 390.0520.

Optical Resolution of Racemic SPR-210 Racemic SPR-210 (**2q**, 30.0 g) and (−)-brucine (27.9 g) were dissolved in hot MeOH (1500 ml) and the solution was allowed to stand at room temperature for 24 h. The resulting colorless crystals were collected and recrystallized three times from MeOH to yield the (−)-SPR-210·(−)-brucine salt (14.5 g), mp 193—194 °C, $[\alpha]_D$ −25° (c =0.5, MeOH).

The methanol solution after separation of the (−)-SPR-210·(−)-brucine salt was concentrated to one-third of its original volume and allowed to stand at room temperature for 24 h to give colorless crystals. Recrystallization from MeOH was repeated three times to give the (+)-SPR-210·(−)-brucine salt (11.0 g), mp 163—165 °C, $[\alpha]_D$ −2° (c=0.5, MeOH).

A suspension of the (−)-SPR-210·(−)-brucine (10.0 g) salt in water (50 ml) was acidified with 2 N HCl at 0 °C and extracted with AcOEt. The organic layer was dried and evaporated to give a viscous oil which was triturated with hexane–AcOEt to give (−)-SPR-210 (5.1 g, 98%) as colorless crystals, mp 99—100 °C, $[\alpha]_D$ −38° (c=1.0, MeOH), optical purity 98.5% ee (HPLC). *Anal.* Calcd for $C_{18}H_{11}F_3N_2O_3S_2$: C, 50.94; H, 2.61; N, 6.60. Found: C, 50.66; H, 2.64; N, 6.77.

(+)-SPR-210 was also obtained using the same procedure as described for the (−)-enantiomer, mp 98—100 °C, $[\alpha]_D$ +38° (c=1.0, MeOH), optical purity 99.4% ee (HPLC). *Anal.* Calcd for $C_{18}H_{11}F_3N_2O_3S_2$: C, 50.94; H, 2.61; N, 6.60. Found: C, 50.74; H, 2.58; N, 6.38.

Biological Methods **A) *In Vitro* Assay** Porcine lens AR was homogenized in a Teflon homogenizer with 3 volumes of cold distilled water and then centrifuged at 10000 × ***g*** for 15 min to remove insoluble materials. The supernatant was fractionated with 0—40% ammonium sulfate and centrifuged at 10000 × ***g*** for 10 min, and the precipitate was discarded. The supernatant was dialyzed overnight against 0.05 M sodium chloride. The dialyzed solution was stored at −80 °C prior to use.

AR activity was assayed spectrophotometrically with an auto-analyzer, COBAS FARA II (F. Hoffman-La Roche Ltd.) using a minor modification of the method of Hayman and Kinoshita.[15] For this assay, we measured the absorbance of NADPH at 340 nm at 25 °C for 2 min after starting the reaction by addition of substrate. All test compounds were dissolved in DMSO to make 10 mM solutions and diluted with distilled water. The assay was carried out in 0.25 ml volumes of 40 mM sodium phosphate buffer (pH 6.2) containing 0.4 M lithium sulfate, 0.1 mM NADPH, the enzyme, various concentrations of a test compound and 3 mM DL-glyceraldehyde as a substrate. IC_{50} values were calculated from

the least-squares regression line of the log concentration–response curve.

B) *In Vivo* Assay Male Sprague-Dawley rats (5 weeks of age, $n=4$—5) were made diabetic by a single i.v. injection of streptozotocin (STZ, 60 mg/kg). The inhibitors were then orally administered 4, 8 and 24 h after STZ injection. Rats were anesthetized with ether 3 h after final dosing of the inhibitor. The erythrocytes, the sciatic nerves and the lenses were collected to measure the sorbitol contents.[16] Inhibition was calculated on the basis of comparison with untreated diabetic controls and the significance of differences was calculated by using Student's t test. The ED_{50} values were calculated by least-squares linear regression analysis.

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