Novel, Highly Potent Aldose Reductase Inhibitors: (*R*)-(−)-2-(4-Bromo-2-fluorobenzyl)-1,2,3,4-tetrahydropyrrolo[1,2-*a*]pyrazine-4-spiro-3′-pyrrolidine-1,2′,3,5′-tetrone (AS-3201) and Its Congeners

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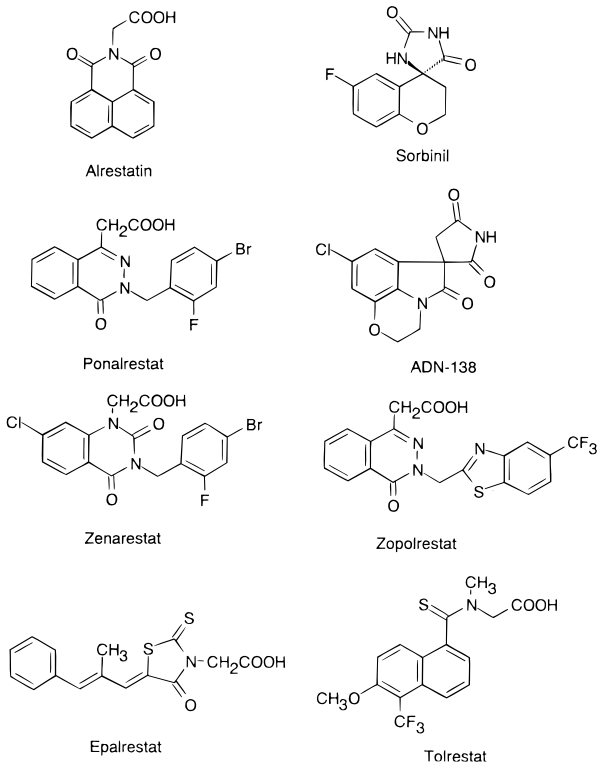
A series of novel tetrahydropyrrolo[1,2-*a*]pyrazine derivatives were synthesized and evaluated as aldose reductase inhibitors (ARIs) on the basis of their abilities to inhibit porcine lens aldose reductase (AR) in vitro and to inhibit sorbitol accumulation in the sciatic nerve of streptozotocin-induced diabetic rats in vivo. Of these compounds, spirosuccinimide-fused tetrahydropyrrolo-[1,2-*a*]pyrazine-1,3-dione derivatives showed significantly potent AR inhibitory activity. In the in vivo activity of these derivatives, 2-(4-bromo-2-fluorobenzyl)-1,2,3,4-tetrahydropyrrolo-[1,2-*a*]pyrazine-4-spiro-3′-pyrrolidine-1,2′,3,5′-tetrone (**23t**) (SX-3030) showed the best oral activity. The enantiomers of **23t** were synthesized, and the biological activities were evaluated. It was found that AR inhibitory activity resides in the (−)-enantiomer **43** (AS-3201), which was 10 times more potent in inhibition of the AR ($IC_{50} = 1.5 \times 10^{-8}$ M) and 500 times more potent in the in vivo activity ($ED_{50} = 0.18$ mg/kg/day for 5 days) than the corresponding (+)-enantiomer **44** (SX-3202). From these results, AS-3201 was selected as the candidate for clinical development. The absolute configuration of AS-3201 was also established to be (*R*)-form by single-crystal X-ray analysis. In this article we report the preparation and structure−activity relationship (SAR) of tetrahydropyrrolopyrazine derivatives including a novel ARI, AS-3201.

Introduction

Currently, a number of structurally diverse compounds possessing aldose reductase (AR) inhibitory activities have been synthesized as potential drugs for the treatment of diabetic complications such as cataract formation, retinopathy, neuropathy, and nephropathy. Most of these compounds contain acetic acid moieties or five-membered ring cyclic imides (hydantoin and succinimide), having an acidic proton[1] that appears to be necessary for the inhibition of AR (Chart 1). This is substantiated by the structures of tolrestat,[2] epalrestat,[3] and several inhibitors[4−8] which have been presently undergoing clinical trials. Thus, we focused our attention on molecules containing *N*-acylglycine moieties and planned to synthesize a series of heterocyclic compounds with those moieties.

In the course of synthesizing new compounds as aldose reductase inhibitors (ARIs), we unexpectedly found that 2-methyltetrahydropyrrolo[1,2-*a*]pyrazine-1,4-dione (**3**) had attractive biological activity. Although this compound possessed no acidic proton, in contrast to the above-mentioned ARIs, it showed not only AR inhibitory in vitro activity ($IC_{50} = 7.5 \times 10^{-6}$ M) but also in vivo activity ($ED_{50} = 68.4$ mg/kg). We also found that the pyrrolopyrazine **3** is more potent in vivo than the ring-opened compounds **2** and **4** (Table 1). These interesting observations suggested that the pyrrolopyrazine **3** possessed good oral absorption and efficient tissue penetration properties, and this furthered our interests to the unique structure of the tetrahydropyrrolo[1,2-*a*]pyrazine-1,4-dione ring system. However, compound **3** was unstable because of easy cleavage of the C−N bond between the 4- and 5-positions on the pyrazine ring under aqueous basic conditions. In an attempt to overcome this problem, we designed a novel and more stable tetrahydropyrrolo[1,2-*a*]pyrazine-1,3-

Chart 1



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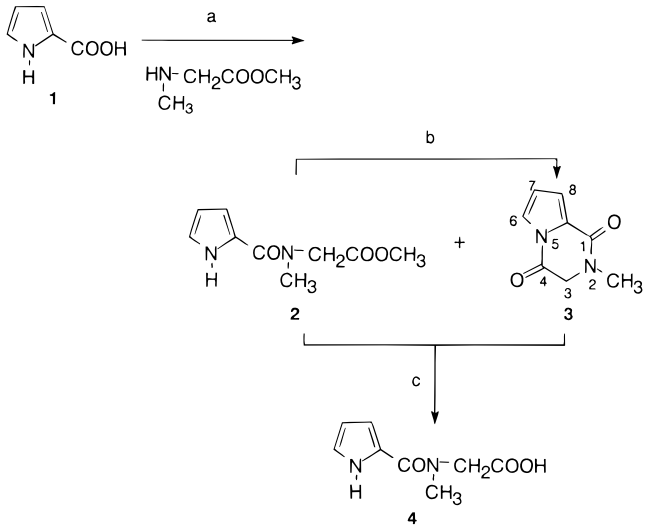
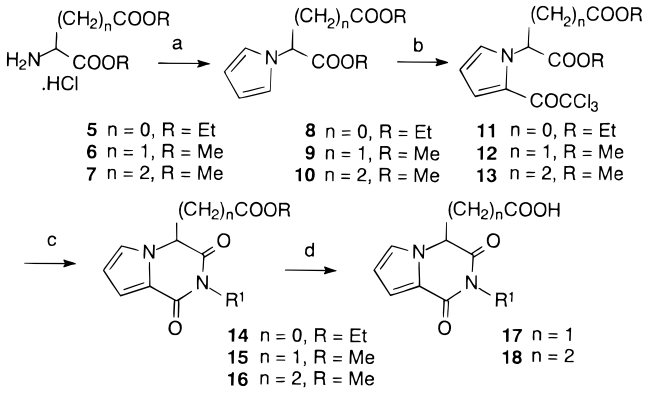
Scheme 1[a]



[a] Reagents: (a) WSC, Et_3N/CH_2Cl_2; (b) Et_3N/toluene; (c) 5% $NaHCO_3$−dioxane, WSC, 1-ethyl-3-(3-(dimethylamino)propyl)carbodiimide hydrochloride.

Scheme 2[a]



[a] Reagents: (a) 2,5-dimethoxy-THF/AcOH; (b) $CCl_3COCl/CHCl_3$; (c) R^1-NH_2, Et_3N/dry DMF; (d) 20% HCl aq/dioxane.

dione ring system and prepared many compounds having an acidic moiety to find more potent compounds than existing ARIs, such as ponalrestat[9] and tolrestat.[2]

Chemistry

The pyrrole derivatives were prepared according to the method shown in Scheme 1. Condensation of 2-pyrrolecarboxylic acid (**1**) with sarcosine methyl ester in the presence of triethylamine gave a mixture of *N*-methyl-*N*-(2-pyrrolylcarbonyl)glycine methyl ester (**2**) and 2-methyltetrahydropyrrolo[1,2-*a*]pyrazine-1,4-dione (**3**). Ester **2** was easily cyclized to pyrrolopyrazine **3** under basic conditions. Ester **2** and pyrrolopyrazine **3** were easily hydrolyzed in an aqueous sodium bicarbonate solution to give *N*-(2-pyrrolylcarbonyl)glycine (**4**).

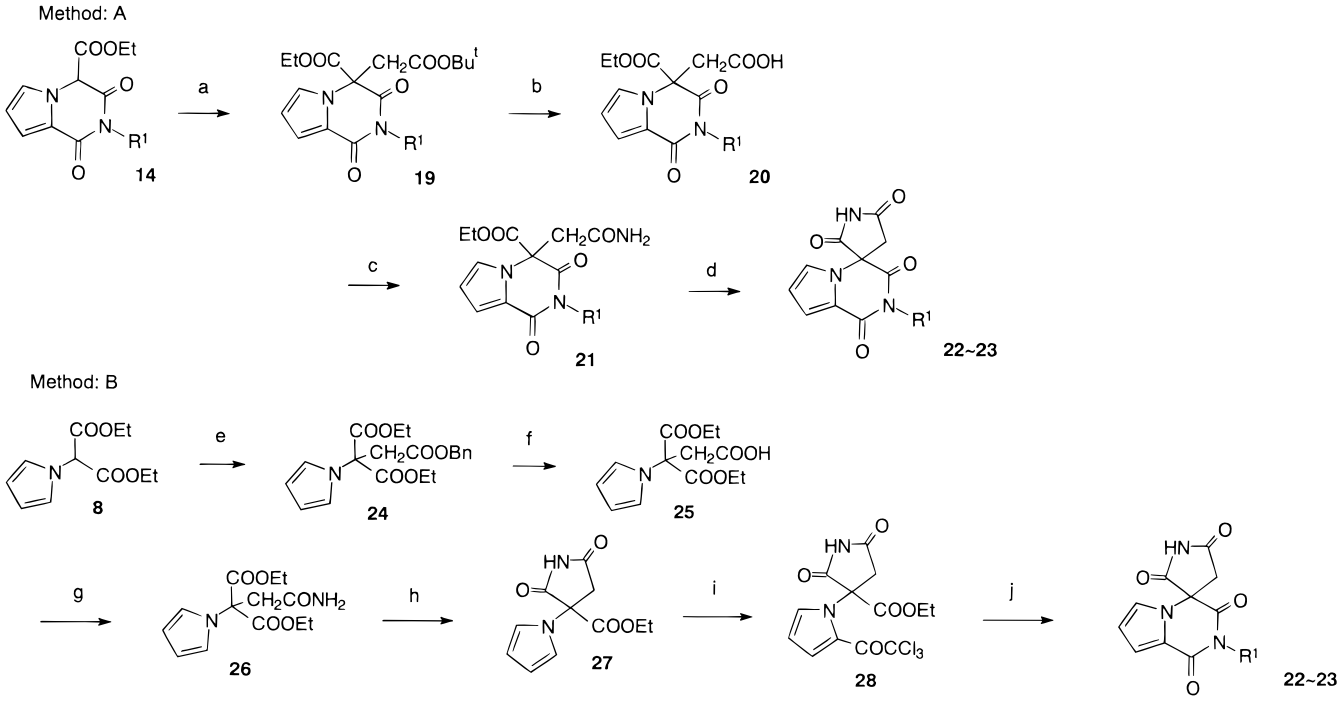
The 1,3-dioxotetrahydropyrrolo[1,2-*a*]pyrazine-4-carboxylic acid derivatives **17** and **18** were prepared according to the method shown in Scheme 2. Condensation of the appropriate amino acid diester **5**, **6**, or **7** with 2,5-dimethoxytetrahydrofuran,[10] followed by treatment with an excess of trichloroacetyl chloride, gave 2-trichloroacetylpyrrole[11] **11**, **12**, or **13**. These intermediates were subjected to ring closure by condensation with the required amine in the presence of triethylamine to give pyrrolopyrazine-4-carboxylate **14**, **15**, or **16**. Compound **15** or **16** was then hydrolyzed by 20% hydrochloric acid to give the desired tetrahydropyrrolo-[1,2-*a*]pyrazine-4-carboxylic acid **17** or **18**, respectively. Under the same conditions compound **14** underwent hydrolysis followed by decarboxylation.

The spirosuccinimide-fused tetrahydropyrrolo[1,2-*a*]-pyrazine-1,3-dione derivatives **22** and **23** were prepared according to two methods, A and B, shown in Scheme 3. In method A, pyrrolopyrazine-4-carboxylate (**14**) was alkylated with *tert*-butyl bromoacetate to give *tert*-butyl pyrrolopyrazineacetate **19**, which was deprotected with trifluoroacetic acid (TFA) to give acetic acid **20**. Treatment of **20** with thionyl chloride and subsequent reaction with a large excess of aqueous ammonium hydroxide gave acetamide **21**. Ring closure of **21** was accomplished by treatment with sodium hydride to give the final products **22** and **23**. In method B, the intermediate **27** was prepared from compound **8**. Alkylation of **8** with benzyl bromoacetate and subsequent deprotection by hydrogenolysis gave acetic acid **25**. Condensation of **25** with *N*-hydroxysuccinimide in the presence of 1-ethyl-3-(3-(dimethylamino)propyl)carbodiimide hydrochloride (WSC), followed by treatment of 7.8% ammonia acetonitrile solution, gave acetamide **26**. Cyclization of **26** with a catalytic amount of potassium carbonate smoothly proceeded to give succinimide **27** quantitatively. Conversion of **27** to the final products **22** and **23** was accomplished using the conditions described in the preparation of **14** from **8** shown in Scheme 2. Most of compounds **23** shown in Table 4 were prepared from the intermediate **28** using this method.

Compounds **31**, which have substituents on the pyrrole ring of **23t**, were prepared by two synthetic routes, A and B, shown in Scheme 4. In route A, halogenation[12] or acetylation[13] of **28** gave 4-halogenated or 4-acetylated trichloroacetylpyrroles **30a**−**c**, which were then condensed with 4-bromo-2-fluorobenzylamine in the presence of triethylamine to give the desired products **31a**−**c**. Direct bromination of **23t** in the presence of aluminum chloride shown in route B gave a mixture of 6-bromo (**31e**) and 6,7-dibromo (**31f**) derivatives. But chlorination of **23t** with sulfuryl chloride only gave 6-chloro derivative **31d**.

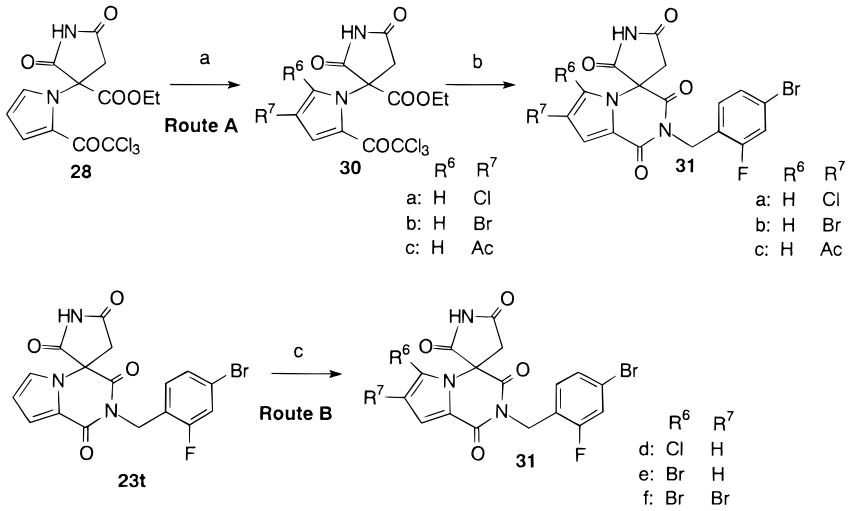
Preparation of Enantiomers of SX-3030 (AS-3201 and SX-3202). The enantiomers of **23t** (SX-3030), 2-(4-bromo-2-fluorobenzyl)-1,2,3,4-tetrahydropyrrolo[1,2-*a*]-pyrazine-4-spiro-3′-pyrrolidine-1,2′,3,5′-tetrone, were prepared according to the method shown in Scheme 5. Alkylation of ethyl [(benzyloxycarbonyl)amino]cyanoacetate[14] (**32**) with ethyl bromoacetate, followed by hydrolysis using hydrogen peroxide-sodium carbonate,[15] brought about ring closure to give succinimide **34.** Treatment of **34** with cinchonidine gave a cinchonidium salt, which was twice recrystallized from ethanol to give the diastereomerically enriched cinchonidium salt (>99.9% de). This salt was then treated with hydrochloric acid to liberate the (−)-enantiomer **35** (>99.5% ee). On the other hand, recrystallization of the residue which was obtained from the acidified mother liquors gave the pure (+)-enantiomer **36** (>99.4% ee). Each enantiomer (**35** or **36**) was then deprotected by hydrogenolysis with palladium carbon, followed by treatment with 2,5-dimethoxytetrahydrofuran, to give pyrrole

Scheme 3[a]



[a] Reagents: (a) (1) NaH, (2) $BrCH_2COO$-Bu^t/dry DMF; (b) trifluoroacetic acid/CH_2Cl_2; (c) (1) $SOCl_2/CH_2Cl_2$, (2) 25% aq NH_3/AcOEt; (d) NaH/dry DMF; (e) $BrCH_2COOBn$, K_2CO_3/Me_2CO or $BrCH_2COOBn$, NaH/dry DMF; (f) H_2, Pd−C/dioxane; (g) (1) *N*-hydroxysuccinimide, WSC/dry CH_2Cl_2, (2) 7% NH_3/CH_3CN; (h) K_2CO_3/Me_2CO; (i) $CCl_3COCl/CHCl_3$; (j) R^1-NH_2, Et_3N/DMF.

Scheme 4[a]



[a] Reagents: (a) Br_2 or SO_2Cl_2 or AcCl, $AlCl_3/CHCl_3$; (b) 4-Br-2F-Ph-CH_2NH_2, Et_3N/DMF; (c) Br_2, $AlCl_3$ or $SO_2Cl_2/CHCl_3$.

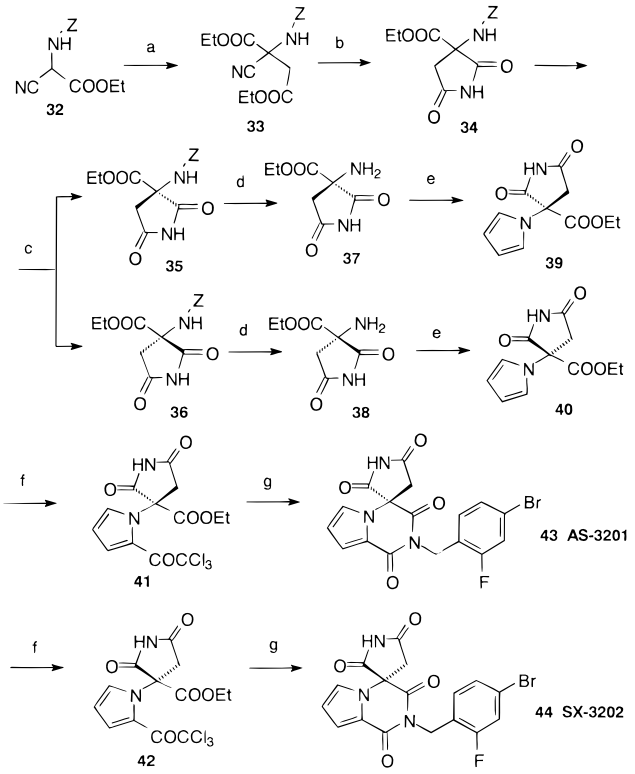
derivative (**39** or **40**), respectively. Conversion of **39** or **40** to the final product **43** (>99.4% ee) or **44** (>99.4% ee), respectively, was accomplished in the same manner as described in the preparation of **23** from **27** shown in Scheme 3 (method B). The optical purity of each enantiomer (**35**, **36**, **43**, or **44**) was determined by HPLC analysis on a chiral column (conditions described in Experimental Section). The absolute configuration of the (−)-enantiomer **43** (AS-3201) was established to be (*R*)-form by single-crystal X-ray analysis (Figure 1).

Pharmacological Results and Discussion

The AR inhibitory activity of the synthesized compounds was assessed by measuring the inhibition of enzymatic activity in a partially purified porcine lens preparation.[16] The inhibitory activity was expressed as the concentration (μM) of the test compound which inhibited the activity of AR by 50% (IC_{50}). The test compounds were also evaluated in vivo by measuring their ability to inhibit sorbitol accumulation in the sciatic nerve of streptozotocin-induced diabetic rats.[17] The ED_{50} value of test compounds represents the dose causing 50% decrease of the sorbitol accumulation.

Results for pyrrole derivatives are shown in Table 1. Pyrrolopyrazine **3**, which has no acidic moiety, showed almost the same potency as carboxylic acid **4** in the AR inhibitory activity ($IC_{50} = 7.5 \times 10^{-6}$ M). Furthermore, pyrrolopyrazine **3** potently inhibited the sorbitol accumulation in the sciatic nerve of the diabetic rats (56% inhibition at 100 mg/kg). Its in vivo activity was 2-fold

Scheme 5[a]



[a] Reagents: (a) $BrCH_2COOEt$, K_2CO_3/Me_2CO; (b) $H_2O_2-Na_2CO_3/H_2O-Me_2CO$; (c) (1) cinchonidine/EtOH, (2) HCl; (d) H_2, Pd−C/EtOH; (e) 2,5-dimethoxy-THF/AcOH; (f) CCl_3COCl/AcOEt; (g) 4-Br-2-F-Ph-CH_2NH_2, Et_3N/dry DMF.

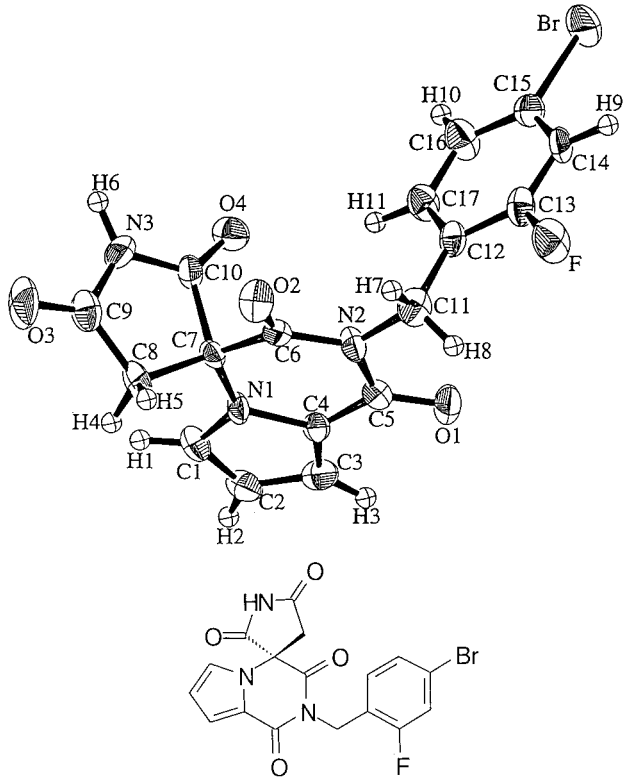


Figure 1. X-ray structure of AS-3201 (**43**).

better than that of carboxylic acid **4** (24% inhibition at 100 mg/kg). Ester **2** was inactive in the AR inhibition at 10^{-6} M, while its in vivo activity (38% inhibition at 100 mg/kg) was superior to that of carboxylic acid **4**. This may be attributed to formation of pyrrolopyrazine **3** in consequence of cyclization of the ester **2**. From these results, it appeared that pyrrolopyrazine **3** possessed good oral absorption and efficient tissue penetration.

Results for compounds with a carboxylic acid moiety at the C-4 position of the tetrahydropyrrolo[1,2-*a*]-pyrazine-1,3-dione ring are shown in Table 2. Acetic and propionic acid derivatives with either hydrogen or low alkyl substituents at the N-2 position of the pyrazine ring were inactive in the AR inhibition at 10^{-6} M, while these acetic acid derivatives produced weak inhibition of the sorbitol accumulation at 100 mg/kg in vivo (**17a**−**c**). Acetic and propionic acid derivatives with 3,4-dichloro or 4-bromo-2-fluorobenzyl groups at the N-2 position (**17d**,**e** and **18d**), with the exception of **18e**, gave potent AR inhibitory activity ($IC_{50} = 1.3-1.6 \times 10^{-7}$ M). Of these compounds, only **18d** produced weak inhibitory activity at a high dose of 100 mg/kg in vivo; however, these in vitro results were not satisfactorily reflected in the in vivo activities of the compounds. It may be attributed to poor oral absorption.

On the basis of this experience, we attempted to shift our attention to other acidic moieties. Replacement of the carboxylic acid moieties by spirosuccinimide at the C-4 position of the pyrrolopyrazine ring resulted in significant improvement of the in vivo activity. Therefore, we focused our efforts on the spirosuccinimide-fused tetrahydropyrrolopyrazine. To find beneficial substituents for the generation of optimal biological activity, compounds which have low alkyl, phenyl, benzyl, or phenethyl substituents at the N-2 position of the spirosuccinimide-fused pyrrolopyrazine ring were synthesized. As seen from Table 3, four of five compounds (**22b**−**d** and **23a**) showed good AR inhibitory activity. The best in the in vitro activity was observed for **23a** with a benzyl group ($IC_{50} = 9.9 \times 10^{-8}$ M). Compounds with methyl and benzyl groups (**22b** and **23a**) showed good in vivo activities (51% and 69% inhibition, respectively, at 30 mg/kg/day for 5 days). These results indicate that substituents at this position are important to produce inhibitors having good biological potency, particularly in vivo activity.

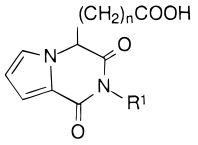
Thus, compounds with a variety of substituents on the benzyl group at the N-2 position of the pyrrolopyrazine were synthesized. These results are shown in Table 4. The AR inhibitory activities were significantly influenced by substituents on the benzyl group at the N-2 position of the pyrrolopyrazine. For instance, substitution by electron-withdrawing groups such as halogens produced more potent compounds than the parent **23a**, except for **23c**, in both the AR inhibition and in vivo activities. In particular, the monosubstituted 4-bromobenzyl derivative **23i** and the disubstituted 3,4-dichloro, 4-chloro-2-fluoro- and 4-bromo-2-fluorobenzyl derivatives **23r**−**t** showed excellent in vivo potency (ED_{50} = 0.3−0.7 mg/kg/day). Of these compounds, the best oral activity was observed for compound **23t** (SX-3030) having 4-bromo-2-fluorobenzyl group (ED_{50} = 0.3 mg/kg/day). On the other hand, substitution by electron-donating groups such as methoxy, methyl or amino group led to significant decrease in the in vivo activity (**23j**,**k**,**n**−**p**).

Table 1. Chemical and Biological Data of Pyrrole Derivatives

				aldose reductase inhibition	
				in vitro	in vivo
compd	mp, °C	recryst solvent	formula	porcine lens AR IC_{50} (μM)[a]	% inhibition[b] 100 mg/kg
2	96−98	AcOEt−hexane	$C_9H_{12}N_2O_3$	>10	38.0 ± 5.8
3	153−154	AcOEt−hexane	$C_8H_{10}N_2O_2$	7.5	55.7 ± 3.0
4	191−193	CH_3CN	$C_8H_{10}N_2O_3$	5.0	23.7 ± 4.2

[a] The concentration of test compounds required for 50% inhibition of AR. [b] Percent inhibition of sorbitol accumulation in the sciatic nerve of streptozotocin-induced diabetic rat. Test compounds were orally given at the single dose indicated. Values are mean ± SEM; mean of 4-6 rats.

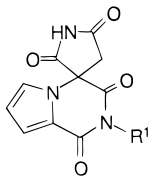
Table 2. Chemical and Biological Data of (2-Substituted-1,2,3,4-tetrahydro-1,3-dioxopyrrolo[1,2-*a*]pyrazin-4-yl)acetic Acids and -propionic Acids **17** and **18**



				aldose reductase inhibition	
				in vitro	in vivo
compd	*n*	R^1	mp, °C	porcine lens AR IC_{50} (μM)[a]	% inhibition[b] 100 mg/kg
17a	1	H	232−233	>10	12.5 ± 5.4
17b	1	CH_3	209−211	>10	12.8 ± 3.6
17c	1	$CH(CH_3)_2$	141−143	>10	12.9 ± 14.0
17d	1	3,4-Cl_2-C_6H_3-CH_2	213−215	0.131	NS
17e	1	2-F-4-Br-C_6H_3-CH_2	179−181	0.165	NS
18a	2	H	143−145	>10	NS
18b	2	CH_3	140−142	>10	NS
18c	2	$CH(CH_3)_2$	120−122	>10	NS
18d	2	3,4-Cl_2-C_6H_3-CH_2	146−148	0.132	17.2 ± 8.2
18e	2	2-F-4-Br-C_6H_3-CH_2	143−145	0.803	NS

[a] See footnote a in Table 1. [b] See footnote b in Table 1. NS, no significant inhibition.

Table 3. Chemical and Biological Data of 2-Substituted-1,2,3,4-tetrahydropyrrolo[1,2-*a*]pyrazine-4-spiro-3′-pyrrolidine-1,2′,3,5′-tetrones **22** and **23**



				aldose reductase inhibition	
				in vitro	in vivo
compd	R^1	method	mp, °C	porcine lens AR IC_{50} (μM)[a]	% inhibition[b] 30 mg/kg/day
22a	H	A	257−259	0.63	33.2 ± 9.3
22b	CH_3	A	285−287	0.28	51.2 ± 3.5
22c	C_6H_5	A	231−238	0.20	27.2 ± 10.6
23a	C_6H_5-CH_2-	A	157−159	0.099	69.1 ± 5.1
22d	C_6H_3-$(CH_2)_2$-	A	155−157	0.27	15.3 ± 10.3

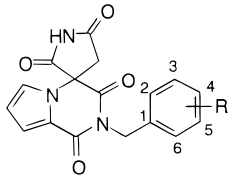
[a] See footnote a in Table 1. [b] Percent inhibition of sorbitol accumulation in the sciatic nerve of streptozotocin-induced diabetic rat. Test compounds were orally given once a day for 5 days at the indicated doses. Values are mean ± SEM; mean of 4-6 rats.

Compounds **31**, which have halogens or an acetyl group on the fused pyrrole ring of the pyrrolopyrazine, were synthesized. Results for these compounds are shown in Table 5. Compared to **23t**, halogens or acetyl substitution on the pyrrole ring retained the in vitro activity but yielded a loss of the in vivo activity at a dose of 1 mg/kg/day (**31a**−**f**).

AR had been reported to have a high enantioselectivity for spirohydantoins[18] and spirosuccinimides.[7] To examine enantiomeric preference for AR, the enantiomers of **23t** were prepared and evaluated for the AR inhibitory activity and in vivo potency. From the results in Table 6, the inhibitory activity was found to reside in essentially one isomer, the (−)-enantiomer **43** (AS-3201), which was 10 and 500 times more potent in vitro and in vivo, respectively, than the corresponding (+)-enantiomer **44** (SX-3202). Moreover, (−)-enantiomer **43** (AS-3201) was about 2 times more potent than the racemate **23t** (SX-3030) in both the AR inhibitory activity and in vivo activity.

AS-3201 was compared with tolrestat and ponalrestat to evaluate its pharmacokinetics in AR inhibitory activ-

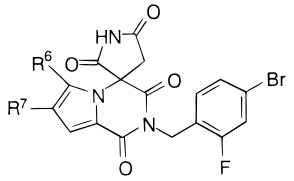
Table 4. Chemical and Biological Data of Compounds **23** with Substituents on the Benzyl Group



				aldose reductase inhibition		
				in vitro	in vivo	
compd	R	method	mp, °C	porcine lens AR IC_{50} (μM)[a]	% inhibition[b] 30 mg/kg/day	ED_{50}[c] mg/kg/day
23a	H	A	157−159	0.099	69.1 ± 5.1	
23b	2-F	B	172−175	0.061	98.0 ± 0.8	1.3
23c	4-F	B	189−190	0.120	58.8 ± 3.3	
23d	2-Cl	B	156−158	0.037	97.0 ± 2.1	5.8
23e	3-Cl	B	161−163	0.035	99.4 ± 1.3	3.8
23f	4-Cl	B	195−198	0.038	104.6 ± 1.2	1.5
23g	2-Br	B	226−229	0.052	82.6 ± 2.7	
23h	3-Br	B	152−153	0.037	102.1 ± 0.5	
23i	4-Br	B	165−167	0.047	108.1 ± 0.4	0.48
23j	4-CH_3	B	154−158	0.052	65.8 ± 4.4	
23k	4-OCH_3	B	184−185	0.046	46.7 ± 7.6	
23l	4-CF_3	B	182−183.5	0.080	88.4 ± 2.7	
23m	4-NO_2	B	213−215	0.110	102.2 ± 1.6	10.8
23n	4-NH_2	B	207−208	0.140	33.4 ± 3.3	
23o	2,4-$(OCH_3)_2$	B	172−174	0.350	NS	
23p	3,4-$(OCH_3)_2$	B	191−193	0.110	NS	
23q	2,4-F_2	B	221.5−223	0.072	98.0 ± 1.0	5.4
23r	3,4-Cl_2	A	221−223	0.023	111.8 ± 1.1	0.60
23s	2-F,4-Cl	B	196−199	0.041	109.5 ± 0.9	0.74
23t (SX-3030)	2-F,4-Br	A, B	192−193	0.045	114.6 ± 0.2	0.30

[a] See footnote a in Table 1. [b] See footnote b in Table 3. [c] The dose of test compounds required for 50% inhibition of sorbitol accumulation in the sciatic nerve of streptozotocin-induced diabetic rat. NS, no significant inhibition.

Table 5. Chemical and Biological Data of Compounds **31** with Substituents on the Pyrrole Ring



					aldose reductase inhibition	
					in vitro	in vivo
compd	R^6	R^7	route	mp, °C	porcine lens AR IC_{50} (μM)[a]	% inhibition[b] 1 mg/kg/day
31a	H	Cl	A	227−229	0.050	23.0 ± 7.3
31b	H	Br	A	112−114	0.10	28.8 ± 9.5
31c	H	CH_3CO	A	289−291	0.21	26.6 ± 7.6
31d	Cl	H	B	207−209	0.035	20.2 ± 6.3
31e	Br	H	B	212−214	0.040	NS
31f	Br	Br	B	253−254	0.052	NS
23t (SX-3030)	H	H		192−193	0.045	104.7 ± 2.2

[a] See footnote a in Table 1. [b] See footnote b in Table 3. NS, no significant inhibition.

ity and in vivo. AS-3201 inhibited porcine lens AR with an IC_{50} value of 15 nM. Its inhibitory potency was comparable to those of tolrestat (IC_{50} = 15 nM) and ponalrestat (IC_{50} = 21 nM). When AS-3201, ponalrestat, or tolrestat was given orally to the diabetic rats once a day for 5 days, AS-3201 inhibited sorbitol accumulation in the sciatic nerve of the diabetic rats with an ED_{50} value of 0.18 mg/kg/day. These results suggest that AS-3201 has higher membrane permeability to inhibit potently intracellular AR in target tissues in vivo in comparison with these AR inhibitors.

Table 6. Biological Data of Tolrestat, Ponalrestat, Racemate **23t**, and Its Enantiomers **43** and **44**

		aldose reductase inhibition	
		in vitro	in vivo
compd	enantiomer	porcine lens AR IC_{50} (μM)[a]	ED_{50}[b] mg/kg/day
23t (SX-3030)	racemate	0.029	0.30
44 (SX-3202)	(*S*)-(+)	0.19	>100
43 (AS-3201)	(*R*)-(−)	0.015	0.18
ponalrestat		0.021	11.0
tolrestat		0.015	26.7

[a] See footnote a in Table 1. [b] See footnote c in Table 4.

In conclusion, we have reported in this article a novel class of spirosuccinimide-fused tetrahydropyrrolo[1,2-*a*]pyrazine-1,3-dione derivatives having a high order of intrinsic biological activity to inhibit AR. Of those compounds, AS-3201 potently inhibited sorbitol accumulation in the sciatic nerve of the diabetic rats at low oral doses, and its activity was more potent than that of tolrestat or ponalrestat. AS-3201, which has highly potent in vivo activity, is being evaluated for its clinical efficacy in the prevention of diabetic complications.

Experimental Section

Melting points (mp) were determined on a Yanagimoto micromelting apparatus and are uncorrected. Proton nuclear magnetic resonance (^{1}H NMR) spectra were obtained on a Varian Gemini-200 spectrometer with tetramethylsilane as an internal standard. Chemical shifts are reported in δ value from internal tetramethylsilane. Splitting patterns are designated as follows: s, singlet; d, doublet; dd, double doublet; t, triplet; q, quartet; br s, broad singlet; m, multiplet. Coupling constants are reported in hertz (Hz). Infrared (IR) spectra were recorded on a Shimazu FTIR-8200PC spectrophotometer. Mass spectra (MS) were obtained with a Hitachi M-1000 LC API mass spectrometer. Elemental analyses were carried out on a Perkin-Elmer 2400 elemental analyzer, and results obtained for specified elements were within ±0.4% of the theoretical values. Optical rotations were determined on a Jasco DIP-370 model digital polarimeter (Japan Spectroscopic Co., Ltd.). Chromatographic separations were performed with silica gel 60 (Merck art. 7734; 70−230 mesh). Visualization was accomplished with UV light. Unless otherwise noted, all commercially available materials were used without further purification.

Reference Compound. Tolrestat was extracted from commercially available Alredase capsules (Wyeth Laboratories Co., Ltd.). Ponalrestat was prepared according to known procedures.[9]

***N*-Methyl-*N*-(2-pyrrolylcarbonyl)glycine Methyl Ester (2) and 1,2,3,4-Tetrahydro-2-methylpyrrolo[1,2-*a*]pyrazine-1,4-dione (3) (Scheme 1).** To a suspension of 2-pyrrolecarboxylic acid (**1**; 2.6 g, 23.4 mmol), sarcosine methyl ester hydrochloride (3.3 g, 23.6 mmol), and triethylamine (5.8 g, 57.3 mmol) in dry CH_2Cl_2 (50 mL) was added 1-ethyl-3-(3-(dimethylamino)propyl)carbodiimide hydrochloride (WSC; 5.4 g, 28.2 mmol), and the mixture was stirred at room temperature for 2 h. The reaction mixture was poured into water, acidified with HCl (10%), and then extracted with AcOEt. The extracts were washed with brine, dried over anhydrous $MgSO_4$, and evaporated in vacuo. The resulting crude product was purified by column chromatography on silica gel. Elution with AcOEt−hexane (1/2) gave a less polar compound **2** (2.1 g, 45.7%), and elution with AcOEt−hexane (1/1) gave a more polar compound **3** (1.0 g, 26.3%). Less polar compound **2**: mp 96−98 °C; ^{1}H NMR (DMSO-d_6) δ 3.25 (3H, br s), 3.67 (3H, s), 4.26 (2H, br s), 6.14 (1H, m), 6.60 (1H, br s), 6.91 (1H, m), 11.47 (1H, br s); IR (KBr, cm^{-1}) 3130 (NH), 1735 (CO), 1595 (CO); MS *m*/*z* 197 (MH$^+$). Anal. ($C_9H_{12}N_2O_3$) C, H, N. More polar compound **3**: mp 153−154 °C; ^{1}H NMR (DMSO-d_6) δ 2.95 (3H, s), 4.51 (2H, s), 6.57 (1H, t, J = 3.3 Hz), 6.95 (1H, dd, J = 3.4, 1.6 Hz), 7.56 (1H, dd, J = 3.2, 1.6 Hz); IR (KBr, cm^{-1}) 1720 (CO), 1630 (CO); MS *m*/*z* 165 (MH$^+$). Anal. ($C_8H_8N_2O_2$) C, H, N.

1,2,3,4-Tetrahydro-2-methylpyrrolo[1,2-*a*]pyrazine-1,4-dione (3, from 2). A mixture of *N*-methyl-*N*-(2-pyrrolylcarbonyl)glycine methyl ester (**2**; 0.5 g, 2.5 mmol), triethylamine (1.3 g, 12.8 mmol), and toluene (5 mL) was refluxed for 5 h. The reaction mixture was evaporated in vacuo. The crude product was purified by recrystallization from CH_3CN to give a white solid (0.34 g, 81.0%): mp 153−154 °C; ^{1}H NMR, IR, and MS were identical to the more polar compound **3** described above.

***N*-Methyl-*N*-(2-pyrrolylcarbonyl)glycine (4, from 2).** To a solution of *N*-methyl-*N*-(2-pyrrolylcarbonyl)glycine methyl ester (**2**; 1.2 g, 6.1 mmol) in dioxane (15 mL) was added 5% aqueous $NaHCO_3$ (15.4 mL), and the mixture was refluxed for 1 h. After cooling to room temperature, the reaction mixture was poured into ice−water containing concentrated HCl (1 mL) and extracted with AcOEt. The extracts were washed with brine and dried over anhydrous $MgSO_4$. Evaporation and recrystallization from CH_3CN gave a white solid (0.8 g, 72.7%): mp 191−3 °C; ^{1}H NMR (DMSO-d_6) δ 3.24 (3H, br s), 4.17 (2H, br s), 6.14 (1H, m), 6.60 (1H, br s), 6.90 (1H, m), 11.45 (1H, br s), 12.72 (1H, br s); IR (KBr, cm^{-1}) 3270 (NH), 1740 (CO), 1707 (CO); MS *m*/*z* 183 (MH$^+$). Anal. ($C_8H_{10}N_2O_3$) C, H, N.

***N*-Methyl-*N*-(2-pyrrolylcarbonyl)glycine (4, from 3).** To a stirred solution of 1,2,3,4-tetrahydro-2-methylpyrrolo[1,2-*a*]pyrazine-1,4-dione (**3**; 1.0 g, 6.1 mmol) in dioxane (20 mL) was added 5% aqueous $NaHCO_3$ (15.4 mL), and the mixture was stirred at room temperature for 6.5 h. The reaction mixture was poured into ice−water containing concentrated HCl (1 mL), and the precipitated white solid was collected by filtration. The solid was recrystallized from CH_3CN to give a white solid (0.8 g, 72.7%): mp 191−3 °C; ^{1}H NMR, IR, and MS were identical to the compound **4** described above.

2-(Pyrrol-1-yl)succinic Acid Dimethyl Ester (9, n = 1, R = Me) (Scheme 2). A mixture of dl-aspartic acid dimethyl ester hydrochloride (**6**; n = 1, R = Me; 19.2 g, 97.2 mmol), 2,5-dimethoxytetrahydrofuran (12.9 g, 97.6 mmol), and triethylamine (10.3 g, 101.8 mmol) in acetic acid (60 mL) was refluxed for 5 h. The reaction mixture was evaporated in vacuo. The residue was dissolved in AcOEt and washed with 5% aqueous $NaHCO_3$, water, and brine. The organic layer was dried over anhydrous $MgSO_4$ and evaporated in vacuo. The resulting crude product was purified by column chromatography on silica gel with AcOEt−hexane (1/4) to give a viscous oil (16.6 g, 81.0%): ^{1}H NMR (DMSO-d_6) δ 3.59 (3H, s), 3.64 (3H, s), 5.26 (1H, t, J = 7.3 Hz), 6.00 (2H, t, J = 2.1 Hz), 6.82 (2H, t, J = 2.1 Hz); IR (NaCl, cm^{-1}) 1735 (CO), 1725 (CO); MS *m*/*z* 212 (MH$^+$).

2-(2-(Trichloroacetyl)pyrrol-1-yl)succinic Acid Dimethyl Ester (12, n = 1, R = Me). A mixture of 2-(pyrrol-1-yl)succinic acid dimethyl ester (**9**, n = 1, R = Me; 14.6 g, 69.1 mmol), trichloroacetyl chloride (25.1 g, 138.2 mmol), and $CHCl_3$ (70 mL) was refluxed for 15 h. The reaction mixture was evaporated in vacuo. The residue was dissolved in AcOEt and washed with 10% aqueous $NaHCO_3$, water, and brine. The organic layer was dried over anhydrous $MgSO_4$ and evaporated in vacuo. The residue was recrystallized from AcOEt−hexane to give a white solid (21.6 g, 87.8%): mp 79−81 °C; ^{1}H NMR (DMSO-d_6) δ 3.57 (3H, s), 3.64 (3H, s), 6.01 (1H, br s), 6.39 (1H, dd, J = 4.4, 2.6 Hz), 7.56 (1H, dd, J = 4.4, 1.5 Hz), 7.61 (1H, t, J = 2.1 Hz); IR (KBr, cm^{-1}) 1745 (CO), 1730 (CO), 1650 (CO); MS *m*/*z* 356 (MH$^+$).

2-(1,2,3,4-Tetrahydro-2-methyl-1,3-dioxopyrrolo[1,2-*a*]-pyrazin-4-yl)acetic Acid Methyl Ester (15b, n = 1, R=R^1 = Me). To an ice-cooled solution of methylamine hydrochloride (0.38 g, 5.9 mmol) and triethylamine (0.57 g, 5.6 mmol) in dry DMF (8 mL) was added 2-(2-(trichloroacetyl)pyrrol-1-yl)succinic acid dimethyl ester (**12**, n = 1, R = Me; 1.0 g, 2.8 mmol), and the mixture was stirred at room temperature for 15 h. The reaction mixture was poured into ice−water containing concentrated HCl (1 mL) and extracted with AcOEt. The extracts were washed with brine and dried over anhydrous $MgSO_4$. Evaporation and purification by column chromatography on silica gel with hexane−AcOEt (5/1) gave a white solid (0.41 g, 62.1%): mp 61−62 °C; ^{1}H NMR (DMSO-d_6) δ 3.14 (3H, s), 3.42 (2H, m), 3.49 (3H, s), 5.36 (1H, s), 6.34 (1H, s), 6.90 (1H, t, J = 1.9), 7.36 (1H, s); IR (KBr, cm^{-1}) 1740 (CO), 1710 (CO), 1660 (CO); MS *m*/*z* 237 (MH$^+$).

2-(1,2,3,4-Tetrahydro-2-methyl-1,3-dioxopyrrolo[1,2-*a*]-pyrazin-4-yl)acetic Acid (17b, n = 1, R^1 = Me). To a stirred solution of 2-(1,2,3,4-tetrahydro-2-methyl-1,3-dioxopyrrolo[1,2-*a*]pyrazin-4-yl)acetic acid methyl ester (**15b**, n = 1, R=R^1 = Me; 0.7 g, 3.0 mmol) in dioxane (16 mL) was added 20% HCl (12.7 mL), and the mixture was refluxed for 4 h. The volatiles were removed in vacuo, water was added and the mixture was

extracted with AcOEt. The organic layer was washed with brine, dried over anhydrous $MgSO_4$, and evaporated in vacuo. The resulting crude product was purified by recrystallization from CH_3CN to give a white solid (0.46 g, 69.9%): mp 209−211 °C; ^{1}H NMR (DMSO-d_6) δ 3.16 (3H, s), 3.35 (2H, dd, J = 24, 18 Hz), 5.31 (1H, t, J = 4.0 Hz), 6.36 (1H, dd, J = 4.0, 2.6 Hz), 6.91 (1H, dd, J = 4.0, 2.0 Hz), 7.36 (1H, dd, J = 2.4, 2.0 Hz), 12.6 (1H, br s); IR (KBr, cm^{-1}) 3110 (OH), 1725 (CO), 1705 (CO), 1635 (CO); MS m/z 223 (MH^+). Anal. ($C_{10}H_{10}N_2O_4$) C, H, N.

3-(1,2,3,4-Tetrahydro-1,3-dioxo-2-isopropylpyrrolo[1,2-*a*]pyrazin-4-yl)propionic Acid (18c, *n* = 2, R^1 = iPr). The title compound was prepared from 3-(1,2,3,4-tetrahydro-1,3-dioxo-2-isopropylpyrrolo[1,2-*a*]pyrazin-4-yl)propionic acid methyl ester (**15c**, n = 2, R^1 = iPr; 1.5 g, 5.4 mmol) by the same manner as the compound **17b** (n = 1, R^1 = Me) described above. The resulting crude product was purified by recrystallization from AcOEt−hexane to give a white solid (0.8 g, 56.3%): mp 120−122 °C; ^{1}H NMR (DMSO-d_6) δ 1.38 (6H, dd, J = 6.8, 2.6 Hz), 1.96 (2H, m), 2.32 (2H, m), 4.99 (1H, dd, J = 14, 7.2 Hz), 5.22 (1H, t, J = 5.0 Hz), 6.40 (1H, dd, J = 8.0, 3.0 Hz), 6.93 (1H, dd, J = 4.0, 1.6 Hz), 7.3 (1H, dd, J = 2.6, 2.0 Hz), 12.2 (1H, br s); IR (KBr, cm^{-1}) 1735 (CO), 1695 (CO), 1625 (CO); MS m/z 265 (MH^+). Anal. ($C_{13}H_{16}N_2O_4$) C, H, N.

2-(2-(Trichloroacetyl)pyrrol-1-yl)malonic Acid Diethyl Ester (11, *n* = 0, R = Et) (Scheme 2). The title compound was prepared from 2-(pyrrol-1-yl)malonic acid diethyl ester[10] (**8**, n = 0, R = Et; 80 g, 0.36 mol) by the same manner as compound **12** (n = 1, R = Me) described in Scheme 2. The resulting crude product was purified by recrystallization from AcOEt−hexane to give a white solid (116 g, 88.1%): mp 67−69 °C; ^{1}H NMR ($CDCl_3$) δ 1.32 (6H, t, J = 7.2 Hz), 4.32 (4H, m), 6.38 (1H, dd, J = 4.4, 1.4 Hz), 6.77 (1H, s), 7.36 (1H, dd, J = 2.8, 1.6 Hz), 7.65 (1H, dd, J = 4.4, 1.6 Hz); IR (KBr, cm^{-1}) 1765 (CO), 1645 (CO); MS m/z 370 (MH^+).

2-[2-(4-Bromo-2-fluorobenzyl)-1,2,3,4-tetrahydro-1,3-dioxopyrrolo[1,2-*a*]pyrazin-4-yl]carboxylic Acid Ethyl Ester (14e, *n* = 0, R = Et, R^1 = 4-Br-2-F-C$_6$H$_3$-CH$_2$). The title compound was prepared from 2-(2-(trichloroacetyl)pyrrol-1-yl)malonic acid diethyl ester (**11**, n = 0, R = Et; 40.0 g, 0.11 mol) by the same manner as compound **15b** (n = 1, R=R^1 = Me) described above. Purification by column chromatography on silica gel with AcOEt−hexane (1/5) and recrystallization from AcOEt−hexane gave a white solid (38.0 g, 86.0%): mp 114−115 °C; ^{1}H NMR (DMSO-d_6) δ 1.13 (3H, t, J = 7.1 Hz), 4.19 (2H, q, J = 7.1 Hz), 5.00 (2H, s), 6.19 (1H, s), 6.46 (1H, dd, J = 4.0, 2.7 Hz), 7.07 (1H, dd, J = 3.9, 1.5 Hz), 7.15 (1H, t, J = 8.1 Hz), 7.30 (1H, dd, J = 2.6, 1.6 Hz), 7.37 (1H, dd, J = 8.2, 2.0 Hz), 7.55 (1H, dd, J = 9.9, 2.0 Hz); IR (KBr, cm^{-1}) 1730 (CO), 1710 (CO), 1670 (CO); MS m/z 409 (MH^+). Anal. ($C_{17}H_{14}BrFN_2O_4$) C, H, N.

2-[2-(4-Bromo-2-fluorobenzyl)-4-(ethoxycarbonyl)-1,2,3,4-tetrahydro-1,3-dioxopyrrolo[1,2-*a*]pyrazin-4-yl]-acetic Acid *tert*-Butyl Ester (19t, R^1 = 4-Br-2-F-C$_6$H$_3$-CH$_2$) (Scheme 3, Method A). To a cooled (0 °C) stirred solution of 2-[2-(4-bromo-2-fluorobenzyl)-1,2,3,4-tetrahydro-1,3-dioxopyrrolo[1,2-*a*]pyrazin-4-yl]carboxylic acid ethyl ester (**14e**, R^1 = 4-Br-2-F-C$_6$H$_3$-CH$_2$; 33.1 g, 80.9 mmol) in dry DMF (150 mL) was slowly added NaH (3.5 g, 87.5 mmol, 60% w/w dispersion in mineral oil), and the mixture was stirred at room temperature for 0.5 h. *tert*-Butyl bromoacetate (17.1 g, 87.7 mmol) was added dropwise, and the reaction mixture was stirred for a further 2 h. The reaction mixture was poured into ice−water and extracted with AcOEt. The extracts were washed with brine and dried over anhydrous $MgSO_4$. Evaporation and purification by column chromatography on silica gel with AcOEt−hexane (1/8) gave a semisolid (32.0 g, 75.7%): ^{1}H NMR (DMSO-d_6) δ 0.96 (3H, t, J = 7.0 Hz), 1.16 (9H, s), 3.70 (1H, d, J = 17.4 Hz), 3.82 (1H, d, J = 17.5 Hz), 4.08 (2H, m), 5.07 (2H, m), 6.48 (1H, dd, J = 4.0, 2.8 Hz), 7.12 (1H, dd, J = 3.9, 1.5 Hz), 7.19 (1H, t, J = 8.1 Hz), 7.37 (1H, dd, J = 8.2, 1.7 Hz), 7.56 (1H, dd, J = 7.7, 1.9 Hz), 7.58 (1H, d, J = 2.0 Hz); IR (NaCl, cm^{-1}) 1760 (CO), 1720 (CO), 1680 (CO).

2-[2-(4-Bromo-2-fluorobenzyl)-4-(ethoxycarbonyl)-1,2,3,4-tetrahydro-1,3-dioxopyrrolo[1,2-*a*]pyrazin-4-yl]-acetic Acid (20t, R^1 = 4-Br-2-F-C$_6$H$_3$-CH$_2$). To a stirred solution of 2-[2-(4-bromo-2-fluorobenzyl)-4-(ethoxycarbonyl)-1,2,3,4-tetrahydro-1,3-dioxopyrrolo[1,2-*a*]pyrazin-4-yl]acetic acid *tert*-butyl ester (**19t**, R^1 = 4-Br-2-F-C$_6$H$_3$-CH$_2$; 28.7 g, 54.8 mmol) in CH_2Cl_2 (100 mL) was added trifluoroacetic acid (TFA; 125 g, 1.1 mol), and the mixture was refluxed for 3 h. The reaction mixture was evaporated in vacuo. The resulting crude product was recrystallized from AcOEt−hexane to give a white solid (21.4 g, 83.6%): mp 142−144 °C; ^{1}H NMR (DMSO-d_6) δ 0.98 (3H, t, J = 7.1 Hz), 3.72 (1H, d, J = 18.0 Hz), 3.86 (1H, d, J = 18.0 Hz), 4.09 (2H, m), 5.05 (2H, m), 6.46 (1H, dd, J = 4.0, 2.8 Hz), 7.09 (1H, dd, J = 4.0, 1.5 Hz), 7.17 (1H, t, J = 8.1 Hz), 7.33 (1H, dd, J = 8.2, 2.2 Hz), 7.55 (1H, dd, J = 8.4, 1.9 Hz), 7.57 (1H, dd, J = 2.8, 1.5 Hz), 12.97 (1H, s); IR (KBr, cm^{-1}) 1745 (CO), 1700 (CO), 1665 (CO); MS m/z 469 (MH^+). Anal. ($C_{19}H_{16}BrFN_2O_6$) C, H, N.

2-[2-(4-Bromo-2-fluorobenzyl)-4-(ethoxycarbonyl)-1,2,3,4-tetrahydro-1,3-dioxopyrrolo[1,2-*a*]pyrazin-4-yl]-acetamide (21t, R^1 = 4-Br-2-F-C$_6$H$_3$-CH$_2$). To a stirred solution of 2-[2-(4-bromo-2-fluorobenzyl)-4-(ethoxycarbonyl)-1,2,3,4-tetrahydro-1,3-dioxopyrrolo[1,2-*a*]pyrazin-4-yl]acetic acid (**20t**, R^1 = 4-Br-2-F-C$_6$H$_3$-CH$_2$; 10.0 g, 21.4 mmol) was added dropwise $SOCl_2$ (6.2 g, 52.1 mmol), and the mixture was refluxed for 2 h. The volatiles were evaporated in vacuo, and the residue was dissolved in AcOEt (30 mL). This solution was added dropwise to a mixture of 25% aqueous NH_3 solution (29 mL) and AcOEt (30 mL) at 0 °C, and the mixture was stirred for 1 h at 0 °C. The organic layer was separated, washed with brine, dried over anhydrous $MgSO_4$, and evaporated in vacuo. The resulting crude product was recrystallized from CH_3CN to give a white solid (8.0 g, 80.0%): mp 190−192 °C; ^{1}H NMR (DMSO-d_6) δ 0.98 (3H, t, J = 7.2 Hz), 3.62 (2H, m), 4.09 (2H, m), 5.03 (2H, m), 6.44 (1H, dd, J = 4.4, 2.4 Hz), 7.04 (1H, dd, J = 4.0, 1.0 Hz), 7.08 (1H, br s), 7.24 (1H, t, J = 8.0 Hz), 7.32 (1H, dd, J = 10.4, 1.6 Hz), 7.36 (1H, dd, J = 3.0, 2.0 Hz), 7.54 (1H, dd, J = 10.0, 2.0 Hz), 7.58 (1H, d, J = 2.0 Hz); IR (KBr, cm^{-1}) 3410 (NH), 1745 (CO), 1705 (CO), 1680 (CO), 1650 (CO); MS m/z 466 (MH^+). Anal. ($C_{19}H_{17}BrFN_3O_5$) C, H, N.

2-(4-Bromo-2-fluorobenzyl)-1,2,3,4-tetrahydropyrrolo-[1,2-*a*]pyrazine-4-spiro-3′-pyrrolidine-1,2′,3,5′-tetrone (23t, R^1 = 4-Br-2-F-C$_6$H$_3$-CH$_2$, SX-3030). To a stirred solution of 2-[2-(4-bromo-2-fluorobenzyl)-4-(ethoxycarbonyl)-1,2,3,4-tetrahydro-1,3-dioxopyrrolo[1,2-*a*]pyrazin-4-yl]acetamide (**21t**, R^1 = 4-Br-2-F-C$_6$H$_3$-CH$_2$; 1.6 g, 3.6 mmol) in dry DMF (15 mL) was added NaH (0.3 g, 7.9 mmol, 63% w/w dispersion in mineral oil) portionwise at −20 °C (CH_3CN−dry ice). After stirring at −20 °C for 0.3 h, the reaction mixture was poured into ice−water containing concentrated HCl (1 mL) and extracted with AcOEt. The extracts were washed with brine, dried over anhydrous $MgSO_4$, and evaporated in vacuo. Purification by column chromatography on silica gel with AcOEt−hexane (1/3) and recrystallization from AcOEt−hexane gave a white solid (0.6 g, 40.0%): mp 192−193 °C; ^{1}H NMR (DMSO-d_6) δ 3.56 (2H, s), 5.00 (2H, m), 6.52 (1H, dd, J = 4.0, 2.8 Hz), 7.12 (1H, dd, J = 3.8, 1.4 Hz), 7.14 (1H, t, J = 8.2 Hz), 7.36 (1H, dd, J = 8.4, 2.0 Hz), 7.54 (1H, dd, J = 9.8, 2.0 Hz), 7.72 (1H, dd, J = 2.6, 1.6 Hz), 12.16 (1H, s); IR (KBr, cm^{-1}) 3230 (NH), 1790 (CO), 1730 (CO), 1705 (CO), 1660 (CO); MS m/z 422 (MH^+). Anal. ($C_{17}H_{11}BrFN_3O_4$) C, H, N.

2-[(Benzyloxycarbonyl)methyl]-2-(pyrrol-1-yl)malonic Acid Diethyl Ester (24) (Scheme 3, Method B). A stirred suspension of 2-(pyrrol-1-yl)malonic acid diethyl ester[10] (**8**, n = 0, R = Et; 36.4 g, 0.16 mol), anhydrous K_2CO_3 (22.1 g, 0.16 mol), and benzyl bromoacetate (40.3 g, 0.18 mol) in acetone (200 mL) was refluxed for 7 h. After cooling to room temperature, the reaction mixture was filtered off and the filtrate was evaporated in vacuo. The residue was dissolved in AcOEt, washed with brine, and dried over anhydrous $MgSO_4$. Evaporation of the solvent and purification by column chromatography on silica gel with AcOEt−hexane (1/10) gave a viscous oil (53.0 g, 87.9%): ^{1}H NMR ($CDCl_3$) δ 1.24 (6H, t, J

= 7.1 Hz), 3.52 (2H, s), 4.25 (4H, q, J = 6.4 Hz), 5.12 (2H, s), 6.18 (2H, t, J = 2.2 Hz), 6.82 (2H, t, J = 2.2 Hz), 7.34 (5H, m); IR (NaCl, cm^{-1}) 1740 (CO).

To a stirred solution of 2-(pyrrol-1-yl)malonic acid diethyl ester (**8**, n = 0, R = Et; 53.0 g, 0.24 mol) in dry DMF (300 mL) was added NaH (10.0 g, 0.26 mol, 63% w/w dispersion in mineral oil) portionwise at 0 °C. The mixture was stirred at 0 °C for 1 h. Benzyl bromoacetate (70.0 g, 0.31 mol) was added dropwise at 0 °C; the solution was warmed to room temperature and stirred overnight. The reaction mixture was poured into ice−water and extracted with AcOEt. The extracts were washed with brine and dried over anhydrous $MgSO_4$. Evaporation of the solvent gave a viscous brown oil (80.0 g, 91.0%): ^{1}H NMR, IR, and MS were identical to compound **24** described above.

2-(Carboxymethyl)-2-(pyrrol-1-yl)malonic Acid Diethyl Ester (25). To a solution of 2-[(benzyloxycarbonyl)methyl]-2-(pyrrol-1-yl)malonic acid diethyl ester (**24**; 53.0 g, 0.14 mol) in dioxane (300 mL) was added a catalytic amount of palladium carbon (5%, 2.5 g), and the mixture was hydrogenated at 40−50 °C under atmospheric pressure. After cooling to room temperature, the catalyst was filtered off and the filtrate was evaporated to give a white solid (36.0 g, 89.6%): mp 93−94 °C; ^{1}H NMR ($CDCl_3$) δ 1.28 (6H, t, J = 7.1 Hz), 3.52 (2H, s), 4.31 (4H, m), 6.20 (2H, t, J = 2.3 Hz), 6.84 (2H, t, J = 2.2 Hz); IR (KBr, cm^{-1}) 1740 (CO), 1720 (CO); MS m/z 284 (MH^+). Anal. ($C_{13}H_{17}NO_6$) C, H, N.

2-(Carbamoylmethyl)-2-(pyrrol-1-yl)malonic Acid Diethyl Ester (26). To a solution of 2-(carboxymethyl)-2-(pyrrol-1-yl)malonic acid diethyl ester (**25**; 48.7 g, 0.17 mol) and *N*-hydroxysuccinimide (21.8 g, 0.19 mol) in dry CH_2Cl_2 (200 mL) was added WSC (9.5 g, 0.21 mol), and the mixture was stirred at room temperature for 1 h. The mixture was added dropwise to a solution of 7.8% NH_3 in CH_3CN (130 mL) at 0 °C, and the solution was stirred for a further 1 h at 0 °C. The reaction mixture was poured into ice−water containing concentrated HCl (30 mL) and extracted with AcOEt. The organic extract was washed with brine and dried over anhydrous $MgSO_4$. Evaporation and purification by recrystallization from AcOEt−hexane gave a white solid (46.8 g, 96.5%): mp 94−95 °C; ^{1}H NMR ($CDCl_3$) δ 1.28 (6H, t, J = 7.0 Hz), 3.37 (2H, s), 4.31 (4H, q, J = 7.0 Hz), 5.45 (1H, br s), 5.58 (1H, br s), 6.19 (4H, t, J = 2.2 Hz), 6.86 (4H, t, J = 2.2 Hz); IR (KBr, cm^{-1}) 3400 (NH), 3210 (NH), 1730 (CO), 1720 (CO), 1680 (CO), 1665 (CO); MS m/z 283 (MH^+). Anal. ($C_{13}H_{18}N_2O_5$) C, H, N.

3-(Ethoxycarbonyl)-2,5-dioxo-3-(pyrrol-1-yl)pyrrolidine (27). A stirred suspension of 2-(carbamoylmethyl)-2-(pyrrol-1-yl)malonic acid diethyl ester (**26**; 1.0 g, 3.5 mmol) and a catalytic amount of anhydrous K_2CO_3 (49 mg, 0.35 mmol) in acetone (15 mL) was refluxed for 2 h. After cooling to room temperature, the reaction mixture was filtered off and the filtrate was evaporated in vacuo. The residue was dissolved in AcOEt and washed with cold 5% HCl, water, and brine. The organic layer was dried over anhydrous $MgSO_4$. Evaporation and purification by column chromatography on silica gel with AcOEt−hexane (1/2) gave a viscous oil (0.8 g, 95.2%): ^{1}H NMR ($CDCl_3$) δ 1.27 (3H, t, J = 3.5 Hz), 3.38 (1H, d, J = 17.9 Hz), 3.61 (1H, d, J = 13.0 Hz), 4.30 (4H, q, J = 7.0 Hz), 6.28 (2H, t, J = 2.2 Hz), 6.95 (2H, t, J = 2.2 Hz), 8.57 (1H, s); IR (NaCl, cm^{-1}) 3250 (NH), 1790 (CO), 1755 (CO), 1725 (CO). Anal. ($C_{11}H_{12}N_2O_4$) C, H, N.

3-(Ethoxycarbonyl)-2,5-dioxo-3-(2-(trichloroacetyl)-pyrrol-1-yl)pyrrolidine·$^1/_2$DMF (28). Trichloroacetyl chloride (42.0 g, 0.23 mol) was added to a solution of 3-(ethoxycarbonyl)-2,5-dioxo-3-(pyrrol-1-yl)pyrrolidine (**27**; 18.0 g, 76.2 mmol) in $CHCl_3$ (40 mL), and the mixture was refluxed for 16 h. The reaction mixture was evaporated in vacuo. The resulting brown gum was dissolved in AcOEt and washed with 10% aqueous $NaHCO_3$, water, and brine. The organic layer was dried over anhydrous $MgSO_4$ and evaporated in vacuo. The resulting crude product was recrystallized from AcOEt−hexane containing a theoretical amount of DMF to give a white solid (28.5 g, 89.3%): mp 94−97 °C; ^{1}H NMR (DMSO-d_6) δ 1.11 (3H, t, J = 7.1 Hz), 3.08 (1H, d, J = 18.2 Hz), 3.70 (1H, d, J = 18.2 Hz), 4.17 (2H, m), 6.44 (1H, dd, J = 4.5, 2.8 Hz), 7.55 (1H, dd, J = 2.8, 1.5 Hz), 7.68 (1H, dd, J = 4.4, 1.5 Hz), 12.42 (1H, s); IR (KBr, cm^{-1}) 3150 (NH), 1790 (CO), 1720 (CO), 1660 (CO); MS m/z 381 (MH^+). Anal. ($C_{13}H_{11}Cl_3N_2O_5$·$^1/_2$DMF) C, H, N.

2-(4-Bromo-2-fluorobenzyl)-1,2,3,4-tetrahydropyrrolo-[1,2-*a*]pyrazine-4-spiro-3′-pyrrolidine-1,2′,3,5′-tetrone (23t, R^1 = 4-Br-2-F-C_6H_3-CH_2, SX-3030) (Scheme 3, Method B). To a stirred solution of 4-bromo-2-fluorobenzylamine hydrochloride (10.2 g, 42.4 mmol) and triethylamine (9.0 g, 88.9 mmol) in dry DMF (70 mL) was added 3-(ethoxycarbonyl)-2,5-dioxo-3-(2-(trichloroacetyl)pyrrol-1-yl)pyrrolidine·$^1/_2$DMF (**28**; 14.8 g, 35.4 mmol), and the mixture was stirred at room temperature for 16 h. The reaction mixture was poured into ice−water containing concentrated HCl (10 mL) and extracted with AcOEt. The extracts were washed with brine and dried over anhydrous $MgSO_4$. Evaporation and purification by recrystallization from AcOEt−hexane gave a white solid (6.5 g, 43.6%): mp 192−193 °C; ^{1}H NMR, IR, and MS were identical to compound **23t** (R^1 = 4-Br-2-F-C_6H_3-CH_2), prepared according to method A shown in Scheme 3.

3-(4-Bromo-2-(trichloroacetyl)pyrrol-1-yl)-3-(ethoxycarbonyl)-2,5-dioxopyrrolidine (30b, R^6 = H, R^7 = Br) (Scheme 4, Route A). A solution of Br_2 (6.9 g, 43.2 mmol) in $CHCl_3$ (18 mL) was added dropwise to a stirred solution of 3-(ethoxycarbonyl)-2,5-dioxo-3-(2-(trichloroacetyl)pyrrol-1-yl)-pyrrolidine·$^1/_2$DMF (**28**; 6.0 g, 14.3 mmol) in $CHCl_3$ (30 mL) at 0 °C, and the mixture was stirred for a further 1 h at 0 °C. The reaction mixture was poured into ice−water (70 mL) and extracted with $CHCl_3$. The extracts were washed with brine, dried over anhydrous $MgSO_4$, and evaporated in vacuo. Purification of the resulting crude product by column chromatography on silica gel with $CHCl_3$−MeOH (2000/1) and recrystallization from AcOEt−hexane gave a white solid (2.4 g, 36.4%): mp 196−198 °C; ^{1}H NMR ($CDCl_3$) δ 1.25 (3H, J = 6.3 Hz), 1.62 (1H, s), 2.86 (1H, d, J = 19.2 Hz), 4.24 (1H, d, J = 19.2 Hz), 4.31 (2H, m), 7.39 (1H, d, J = 1.4 Hz), 7.70 (1H, d, J = 1.6 Hz), 8.48 (1H, s); IR (KBr, cm^{-1}) 3180 (NH), 1805 (CO), 1740 (CO), 1710 (CO), 1665 (CO); MS m/z 461 (MH^+). Anal. ($C_{13}H_{10}BrCl_3N_2O_5$) C, H, N.

3-(4-Chloro-2-(trichloroacetyl)pyrrol-1-yl)-3-(ethoxycarbonyl)-2,5-dioxopyrrolidine (30a, R^6 = H, R^7 = Cl). A solution of SO_2Cl_2 (4.8 g, 35.6 mmol) in $CHCl_3$ (5 mL) was added dropwise to a stirred solution of 3-(ethoxycarbonyl)-2,5-dioxo-3-(2-(trichloroacetyl)pyrrol-1-yl)pyrrolidine·$^1/_2$DMF (**28**; 6.0 g, 14.3 mmol) in $CHCl_3$ (50 mL) at 0 °C, and the mixture was stirred for a further 0.5 h at 0 °C. The reaction mixture was poured into ice−water (50 mL) and extracted with $CHCl_3$. The extracts were washed with brine, dried over anhydrous $MgSO_4$, and evaporated in vacuo. Purification of the crude oil by column chromatography on silica gel with AcOEt−hexane (1/2) gave a white solid (2.5 g, 41.7%): mp 182−185 °C; ^{1}H NMR ($CDCl_3$) δ 1.25 (3H, t, J = 5.5 Hz), 1.68 (1H, s), 2.86 (1H, d, J = 19.0 Hz), 4.22 (1H, d, J = 19.0 Hz), 4.31 (1H, m), 7.36 (1H, d, J = 1.6 Hz), 7.62 (1H, d, J = 2.0 Hz), 8.65 (1H, s); IR (KBr, cm^{-1}) 3180 (NH), 1800 (CO), 1750 (CO), 1710 (CO), 1665 (CO); MS m/z 417 (MH^+). Anal. ($C_{13}H_{10}Cl_4N_2O_5$) C, H, N.

3-(4-Acetyl-2-(trichloroacetyl)pyrrol-1-yl)-3-(ethoxycarbonyl)-2,5-dioxopyrrolidine (30c, R^6 = H, R^7 = Ac). To a stirred suspension of anhydrous aluminum chloride (4.8 g, 36.0 mmol) and 3-(ethoxycarbonyl)-2,5-dioxo-3-(2-(trichloroacetyl)pyrrol-1-yl)pyrrolidine·$^1/_2$DMF(**28**; 3.0 g, 7.2 mmol) in 1,2-dichloroethane (50 mL) was added acetyl chloride (3.0 g, 38.2 mmol) dropwise at room temperature. After stirring for 2 h, the reaction mixture was poured into ice−water and extracted with $CHCl_3$. The extracts were washed with brine and dried over anhydrous $MgSO_4$. Evaporation and purification by recrystallization from CH_3CN gave a white solid (2.5 g, 83.3%): mp 243−246 °C dec; ^{1}H NMR (DMSO-d_6) δ 1.12 (3H, t, J = 7.0 Hz), 2.43 (3H, s), 3.23 (1H, d, J = 18.2 Hz), 3.68 (1H, d, J = 18.2 Hz), 4.18 (2H, m), 7.91 (1H, d, J = 1.8 Hz), 8.13 (1H, d, J = 1.8 Hz), 12.48 (1H, s); IR (KBr, cm^{-1})

3140 (NH), 1790 (CO), 1730 (CO), 1680 (CO), 1640 (CO); MS *m*/*z* 423 (MH^+). Anal. ($C_{15}H_{13}Cl_3N_2O_6$) C, H, N.

7-Bromo-2-(4-bromo-2-fluorobenzyl)-1,2,3,4-tetrahydropyrrolo[1,2-*a*]pyrazine-4-spiro-3′-pyrrolidine-1,2′,3,5′-tetrone (31b, R^6 = H, R^7 = Br). The title compound was prepared from 3-(4-bromo-2-(trichloroacetyl)pyrrol-1-yl)-3-(ethoxycarbonyl)-2,5-dioxopyrrolidine (**30b**, R^6 = H, R^7 = Br; 2.1 g, 4.6 mmol) by the same manner as SX-3030 (**23t**, R^1 = 4-Br-2-F-C_6H_3-CH_2) described for method B in Scheme 3. Purification of resulting crude product by column chromatography on silica gel with $CHCl_3$ and recrystallization from CH_3CN gave a white solid (1.2 g, 52.2%): mp 112−114 °C; ^{1}H NMR (DMSO-d_6) δ 3.55 (2H, s), 4.99 (2H, m), 7.14 (1H, t, J = 8.2 Hz), 7.22 (1H, d, J = 1.8 Hz), 7.36 (1H, dd, J = 8.6, 2.2 Hz), 7.54 (1H, dd, J = 10.0, 2.2 Hz), 7.96 (1H, d, J = 1.8 Hz), 12.22 (1H, s); IR (KBr, cm^{-1}) 3220 (NH), 3120 (NH), 1790 (CO), 1720 (CO), 1670 (CO). Anal. ($C_{17}H_{10}Br_2FN_3O_4$) C, H, N.

2-(4-Bromo-2-fluorobenzyl)-6-chloro-1,2,3,4-tetrahydropyrrolo[1,2-*a*]pyrazine-4-spiro-3′-pyrrolidine-1,2′,3,5′-tetrone (31d, R^6 = Cl, R^7 = H) (Scheme 4, Route B). To a stirred solution of SX-3030 (**23t**; 0.9 g, 2.1 mmol) in $CHCl_3$ (350 mL) was added SO_2Cl_2 (0.68 g, 5.0 mmol), and the mixture was stirred at room temperature for 4 h. The reaction mixture was poured into water and extracted with AcOEt. The extracts were washed with brine and dried over anhydrous $MgSO_4$. Evaporation and purification by recrystallization from AcOEt−hexane gave a white solid (0.56 g, 57.7%): mp 212−215 °C; ^{1}H NMR (DMSO-d_6) δ 3.52 (2H, m), 5.01 (2H, m), 6.68 (1H, d, J = 4.2 Hz), 7.17 (1H, t, J = 8.2 Hz), 7.23 (1H, d, J = 4.4 Hz), 7.38 (1H, dd, J = 8.0, 1.8 Hz), 7.55 (1H, dd, J = 9.9, 1.8 Hz) 12.56 (1H, s); IR (KBr, cm^{-1}) 3440 (NH), 3200 (NH), 1805 (CO), 1720 (CO), 1675 (CO); MS *m*/*z* 456 (MH^+). Anal. ($C_{17}H_{10}BrClFN_3O_4$) C, H, N.

2-[(Benzyloxycarbonyl)amino]-2-cyanosuccinic Acid Diethyl Ester (33) (Scheme 5). A mixture of 2-[(benzyloxycarbonyl)amino]cyanoacetic acid ethyl ester[14] (**32**; 21.0 g, 80.1 mmol), anhydrous potassium carbonate (12.2 g, 88.3 mmol), ethyl bromoacetate (14.3 g, 85.6 mmol), and acetone (74 mL) was refluxed for 7 h. After cooling to room temperature, the reaction mixture was filtered off and the filtrate was evaporated in vacuo. The residue was dissolved in AcOEt and washed with 5% HCl, water, and brine. The organic layer was dried over anhydrous $MgSO_4$ and evaporated to give a viscous oil (27.5 g, 98.6%): ^{1}H NMR ($CDCl_3$) δ 1.25 (3H, t, J = 3.7 Hz), 1.34 (3H, t, J = 6.9 Hz), 3.18 (1H, d, J = 16.2 Hz), 3.56 (1H, br d, J = 16.1 Hz), 4.16 (2H, q, J = 7.1 Hz), 4.38 (2H, br q, J = 6.6 Hz), 6.31 (1H, s), 5.17 (2H, s), 7.37 (5H, s); IR (NaCl, cm^{-1}) 3340 (NH), 1740 (CO). The crude product was used without further purification in the next step.

3-[(Benzyloxycarbonyl)amino]-3-(ethoxycarbonyl)-2,5-dioxopyrrolidine (34). To a stirred solution of sodium carbonate (132.9 g, 1.25 mol) in water (1450 mL) was added 30% hydrogen peroxide (192 mL, 1.88 mol), and a solution of 2-[(benzyloxycarbonyl)amino]cyanosuccinic acid diethyl ester (**33**; 328 g, 0.94 mol) in acetone (1640 mL) was added dropwise at a temperature below 40 °C. The mixture was stirred for a further 2 h in the range of 25−40 °C. The reaction mixture was then cooled to below 10 °C in an ice bath, and a solution of 7% HCl (1220 mL) was added. The solution was stirred at room temperature until crystals precipitated. The crystals were collected by filtration and washed well with water. Recrystallization from AcOEt−hexane gave a white solid (181.3 g, 60.0%): mp 105−106 °C; ^{1}H NMR ($CDCl_3$) δ 1.29 (3H, t, J = 7.0 Hz), 3.18 (2H, m), 4.32 (2H, q, J = 6.9 Hz), 5.12 (2H, m), 6.26 (1H, s), 7.36 (5H, s), 8.19 (1H, s); IR (KBr, cm^{-1}) 1790 (CO), 1755 (CO), 1710 (CO); MS *m*/*z* 321 (MH^+). Anal. ($C_{15}H_{16}N_2O_6$) C, H, N.

Optical Resolution of 3-[(Benzyloxycarbonyl)amino]-3-(ethoxycarbonyl)-2,5-dioxopyrrolidine (34). (*R*)-(−)-3-[(Benzyloxycarbonyl)amino]-3-(ethoxycarbonyl)-2,5-dioxopyrrolidine (35). To a suspension of 3-[(benzyloxycarbonyl)amino]-3-(ethoxycarbonyl)-2,5-dioxopyrrolidine (**34**; 73.6 g, 0.23 mol) in EtOH (290 mL) was added cinchonidine (67.6 g, 0.23 mol), and the mixture was heated until dissolution occurred. The reaction mixture was left standing at room temperature until cinchonidium salt precipitated. The precipitate was collected by filtration and rinsed with EtOH. The crude cinchonidium salt was recrystallized twice from EtOH to give the pure cinchonidium salt (35.2 g, 24.9%, >99.9% de): mp 160−161 °C; $[\alpha]^{26}_D$ = −73.5° (*c* = 1.0, EtOH); IR (KBr, cm^{-1}) 1760 (CO), 1710 (CO), 1620 (CO).

The pure cinchonidium salt (4.3 g) was suspended in AcOEt (80 mL), and 5% aqueous HCl (40 mL) was added. The organic layer was separated, washed with brine, dried over anhydrous $MgSO_4$, and evaporated to give a white solid. Recrystallization from AcOEt−hexane gave an optically pure (−)-enantiomer (1.9 g, >99.5% ee): mp 117−118 °C; $[\alpha]^{27}_D$ = −31.8° (*c* = 1.0, MeOH); ^{1}H NMR, IR, and MS were identical to racemate **34**. Anal. ($C_{15}H_{16}N_2O_6$) C, H, N.

(*S*)-(+)-3-[(Benzyloxycarbonyl)amino]-3-(ethoxycarbonyl)-2,5-dioxopyrrolidine (36). The mother liquor of the cinchonidium salt as above-mentioned was concentrated in vacuo to give a dark gum (96 g), which was treated with 10% HCl and AcOEt. The organic layer was separated, washed with brine, dried over anhydrous $MgSO_4$, and evaporated in vacuo. The crude solid (43 g, 76.8% ee) was recrystallized three times from AcOEt−hexane to give the pure (+)-enantiomer (>99.4% ee, 21.7 g): mp 119−120 °C; $[\alpha]^{27}_D$ = +29.8° (*c* = 1.0, MeOH); ^{1}H NMR, IR, and MS were identical to racemate **34**.

The optical purity of the enantiomers **35** and **36** was established by HPLC analysis. HPLC conditions: instrument, Shimazu LC-6A system; column, CHIRAL PAK AS (250 × 4.6 mm i.d.; Daicel Chemical Industries, Ltd.); flow rate, 2 mL/min; mobile phase, EtOH−hexane (10/90); detector, UV 215, 254 nm; column temperature, 40 °C.

(*R*)-(−)-3-Amino-3-(ethoxycarbonyl)-2,5-dioxopyrrolidine (37). To a solution of (−)-3-[(benzyloxycarbonyl)amino]-3-(ethoxycarbonyl)-2,5-dioxopyrrolidine (**35**; 25.0 g, 78.1 mmol) in EtOH (140 mL) was added a catalytic amount of palladium carbon (5%, 1.0 g), and the mixture was hydrogenated at 40−50 °C under atmospheric pressure. After cooling to room temperature, the catalyst was filtered off and the filtrate was evaporated in vacuo. The crude product was recrystallized from iPrOH to give a white solid (13.9 g, 95.9%): mp 125−126 °C; $[\alpha]^{27}_D$ = −31.8° (*c* = 1.0, MeOH); ^{1}H NMR ($CDCl_3$) δ 1.30 (3H, t, J = 7.1 Hz), 1.64 (1H, br s), 2.20 (1H, br s), 2.76 (1H, d, J = 18.1 Hz), 3.18 (1H, d, J = 17.9 Hz), 4.29 (2H, q, J = 7.1 Hz), 8.31 (1H, br s); IR (KBr, cm^{-1}) 3360 (NH), 3300 (NH), 1755 (CO), 1705 (CO); MS *m*/*z* 187 (MH^+). Anal. ($C_7H_{10}N_2O_4$) C, H, N.

(*R*)-(−)-3-(Ethoxycarbonyl)-2,5-dioxo-3-(pyrrol-1-yl)-pyrrolidine (39). A mixture of (−)-3-amino-3-(ethoxycarbonyl)-2,5-dioxopyrrolidine (**37**; 12.0 g, 64.5 mmol), 2,5-dimethoxytetrahydrofuran (13.0 g, 98.4 mmol), and acetic acid (40 mL) was stirred at 70 °C for 1.5 h. The volatiles were removed in vacuo. The residue was dissolved in AcOEt and washed with 5% aqueous $NaHCO_3$, water, and brine. The organic layer was dried over anhydrous $MgSO_4$ and evaporated in vacuo. The crude product was purified by column chromatography on silica gel with AcOEt−hexane (1/3) to give a viscous oil (11.0 g, 72.4%): $[\alpha]^{26}_D$ = −59.5° (*c* = 1.0, MeOH); ^{1}H NMR, IR, and MS were identical to racemate **27**. Anal. ($C_{11}H_{12}N_2O_4$) C, H, N.

(*R*)-(−)-3-(Ethoxycarbonyl)-2,5-dioxo-3-(2-(trichloroacetyl)pyrrol-1-yl)pyrrolidine (41). The title compound was prepared from (−)-3-(ethoxycarbonyl)-2,5-dioxo-3-(pyrrol-1-yl)pyrrolidine (**39**; 7.0 g, 29.6 mmol) by the same manner as compound **28** described for method B in Scheme 3. Purification by column chromatography on silica gel with AcOEt−hexane (1/3) gave a semisolid (10.4 g, 91.9%): $[\alpha]^{27}_D$ = −426.4° (*c* = 1.0, MeOH); ^{1}H NMR, IR, and MS were identical to racemate **28**.

(*R*)-(−)-2-(4-Bromo-2-fluorobenzyl)-1,2,3,4-tetrahydropyrrolo[1,2-*a*]pyrazine-4-spiro-3′-pyrrolidine-1,2′,3,5′-tetrone (43, AS-3201). The title compound was prepared from (−)-3-(ethoxycarbonyl)-2,5-dioxo-3-(2-(trichloroacetyl)pyrrol-1-yl)pyrrolidine (**41**; 2.0 g, 5.2 mmol) by the same manner as

SX-3030 (**23t**, R^1 = 4-Br-2-F-C_6H_3-CH_2) described for method B in Scheme 3. Purification by column chromatography on silica gel with $CHCl_3$−MeOH (1000/1) and recrystallization from AcOEt−hexane gave a white solid (0.9 g, 45.0%, >99.4% ee): mp 192−193 °C; $[\alpha]^{28}_D = -5.4°$ (c = 1.0, MeOH); $[\alpha]^{28}_{405} = -33.0°$($c$ = 1.0, MeOH); ^{1}H NMR, IR, and MS were identical to racemate **23t**. Anal. ($C_{17}H_{11}BrFN_3O_4$) C, H, N.

(*S*)-(+)-2-(4-Bromo-2-fluorobenzyl)-1,2,3,4-tetrahydropyrrolo[1,2-*a*]pyrazine-4-spiro-3′-pyrrolidine-1,2′,3,5′-tetrone (44, SX-3202). Title compound was prepared from (*S*)-(+)-3-[(benzyloxycarbonyl)amino]-3-(ethoxycarbonyl)-2,5-dioxopyrrolidine (**36**) by the same manner as AS-3201 (**43**) described above. Purification by column chromatography on silica gel with $CHCl_3$−MeOH (1000/1) and recrystallization from AcOEt−hexane gave a white solid (>99.4% ee): mp 192−193 °C; $[\alpha]^{29}_D = +6.5°$ (c = 1.0, MeOH); $[\alpha]^{28}_{405} = +33.2°$ (c = 1.0, MeOH); ^{1}H NMR, IR, and MS were identical to racemate **23t.** Anal. ($C_{17}H_{11}BrFN_3O_4$) C, H, N.

The optical purity of enantiomers **43** and **44** was established by HPLC analysis. HPLC conditions: instrument, Shimazu LC-6A system; column, ULTRON ES-OVM (150 × 60 mm i.d.; Shinwa Chemical Industries, Ltd.); mobile phase, 20 mM KH_2PO_4/CH_3CN (80/20); flow rate, 1 mL/min; column temperature, 25 °C,; detection, UV 296, 261 nm.

Single-Crystal X-ray Analysis. X-ray Structure Determination of AS-3201, 43. Suitable crystals of AS-3201 were grown form ethanol solutions: $C_{17}H_{11}O_4N_3BrF$; M = 420.19; orthorhombic; $P2_12_12_1$; a = 13.207(3) Å; b = 19.253(3) Å; c = 6.280(2) Å; V = 1596.8(6) Å^3; Z = 4; ρ(calcd) = 1.748 g cm^{-1}; F(000) = 840.00; μ(Cu Kα) = 39.04 cm^{-1}; T = 293 K; crystal size, 0.5 × 0.3 × 0.8 mm.

All measurements were made on a Rigaku AFC5R diffractometer with graphite monochromated Cu Kα radiation (λ = 1.541 78 Å). The cell constants and an orientation matrix for data collection were determined from centered angles of 22 reflections in the range 47.83° ≤ 2θ ≤ 50.00°. The data were collected using the ω−2θ scan technique to a maximum 2θ value of 125.2°. Scans of (1.52 + 0.30 tan θ)° were made at a speed of 12.0°/min. Stationary background counts were recorded on each side of the reflections. A total of 1526 reflections was collected. The intensities of three representative reflections were measured after every 100 reflections. No decay correction was applied. The data were corrected for Lorentz and polarization effects. A correction for secondary extinction was applied. The structure was solved by direct methods (SIR92) and expanded using Fourier technique (DIRDIF 94). The non-hydrogen atoms were refined with anisotropic temperature factors. All hydrogen atoms were include at idealized positions but not refined. The final cycle of full-matrix least-squares refinement (SHELXL-93) was based on 1434 observed reflections and 236 variable parameters.

All the calculations were performed using the teXsan (Molecular Structure Co.). A refinement of the Flack's χ parameter was carried out to determine the absolute configuration. The value of refined χ [−0.06(7)] indicated that to be the correct absolute stereochemistry. The final R value was 0.057. The final difference Fourier was essentially featureless.

Biological Methods. (a) In Vitro Assay. Enzyme Preparation. Porcine lens AR was prepared according to the method of Hayman and Kinoshita[16] with slight modifications. The lenses were homogenized in 250 mM phosphate buffer (pH 7.4) containing 2 mM mercaptoethanol at 0−4 °C, and the homogenate was centrifuged at 20000g for 30 min. The supernatant was subjected to a 40−60% ammonium sulfate fractionation. The resultant precipitate was dissolved in 5 mM phosphate buffer (pH 7.4) containing 2 mM mercaptoethanol and used for enzyme assay. One unit (U) of AR was defined as the enzyme activity which oxidizes 1 μmol of NADPH/min under the assay conditions described below.

Enzyme Assay. The reaction mixture consisted of 100 mM phosphate buffer (pH 6.5), 0.2 mM NADPH, 1.5 mM D,L-glyceraldehyde, 0.4 M lithium sulfate, 7.0 mU/mL enzyme, and test compounds at various concentrations. The reaction mixture was incubated at 37 °C, and the absorbance at 340 nm was measured with a spectrophotometer (model 150-20; Hitachi Ltd., Japan). The enzyme activity was estimated on the basis of its ability to decrease in the absorbance over a period of 1 min. The concentration of compounds required for 50% inhibition of enzyme activity (IC_{50}) was estimated graphically from the log concentration−inhibition curve.

(b) In Vivo Assay. Male Wister rats (200−250 g) were rendered diabetic by an intravenous injection of streptozotocin (40 mg/kg), which had been freshly dissolved in physiological saline. After 7 days, the rats were divided into various groups, with 4−6 animals/group, and orally given test compounds, suspended in 0.5% tragacanth or an equivalent volume of 0.5% tragacanth, once a day for 5 days. The rats were sacrificed 4 h after the final administration of the test compounds. Tissue sorbitol level was determined according to the method of Clements et al.[17] with slight modifications.

The sciatic nerves sample (30−60 mg) were quickly dissected from the hind limb, placed into water (1.0 mL/40 mg of tissue), heated in boiling bath for 2 min, and then homogenized with a Polytron instrument in 6% perchloric acid (1 mL/10 mg of tissue). The homogenate was centrifuged at 1050g for 15 min at 4 °C. The supernatant was neutralized with 2 M K_2CO_3 and used as tissue extract for assay of sorbitol. Sorbitol was assayed by an enzymatic method, in which sorbitol dehydrogenase catalyzes the stoichiometric conversion of NAD by sorbitol to a fluorogenic product, NADH. The reaction mixture contained 30 mM glycine buffer (pH 9.4), 1.3 mM NAD, 1.3 U/mL sorbitol dehydrogenase, and 1.0 mL of the tissue extract in a total volume of 3 mL. After the mixture was allowed to stand for 60 min at room temperature, the fluorescence intensity was measured at 365-nm excitation wavelength and 430-nm emission wavelength using a fluorospectrophotometer (F3000, Hitachi Ltd., Japan). The sorbitol concentration was quantitated by comparison with standards of sorbitol. The sorbitol content in sciatic nerve of each animal was expressed as nmol/wet weight.

The activity of test compounds was expressed as the percent inhibition of sorbitol accumulation at a given dose, which was calculated according to the following equation:

$$\% \text{ inhibition} = (S - T)/(S - N) \times 100$$

where S is sorbitol content in sciatic nerve of untreated diabetic control rats, T is sorbitol content in sciatic nerve of diabetic rats given test compounds, and N is sorbitol content in sciatic nerve of age-matched nondiabetic control rats. The dose of compounds required for 50% inhibition of sorbitol accumulation (ED_{50}) was estimated from the log dose−inhibition curve.

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Supporting Information Available: X-ray crystallographic data (9 pages). Ordering information is given on any current masthead page.

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