Novel Spirosuccinimides with Incorporated Isoindolone and Benzisothiazole 1,1-Dioxide Moieties as Aldose Reductase Inhibitors and Antihyperglycemic Agents

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Compounds from two novel series of spirosuccinimides were prepared. Analogs of series **2** possessed a spiro-fused isoindolone moiety while those of series **3** contained a spiro-fused benzisothiazole S,S-dioxide group. These compounds were evaluated as aldose reductase inhibitors (ARI) in vitro by their ability to inhibit glyceraldehyde reduction using a partially purified bovine lens aldose reductase preparation and in vivo as inhibitors of galactitol accumulation in the lens, sciatic nerve, and diaphragm of galactose-fed rats. Many members from the isoindolone series **2**, particularly those containing an isoindolone *N*-methyl moiety, showed good in vitro and in vivo potency. The most potent member, the 6-chloro analog **32**, was resolved, and aldose reductase activity was found to reside almost exclusively in the (+)-enantiomer. Compound **32** was approximately equipotent in the sciatic nerve of the galactose-fed rat to other cyclic imide ARI's of similar in vitro activity, namely sorbinil and ADN-138 and also to tolrestat, an acetic acid-based ARI (ED_{50}'s 4–8 mg/kg). Compounds from both series, **2** and **3**, were also found to lower plasma glucose levels of genetically obese db/db and ob/ob mice with potency similar to that of ciglitazone. However, members from these series failed to lower insulin levels of the ob/ob mouse at the doses tested.

Aldose reductase (EC 1.1.1.21) is an NADPH-dependent, intracellular enzyme which catalyzes the conversion of glucose to sorbitol in the first step of the polyol pathway. In diabetes mellitus, plasma glucose levels are elevated and this excess glucose is metabolized within many tissues by the polyol pathway. The increased flux of glucose through this pathway has been linked to the progression of a number of diabetic complications, including neuropathy, nephropathy, retinopathy, and cataract formation. In animal experiments[1-4] and in recent clinical studies[5-13] compounds which inhibit aldose reductase and block the entry of glucose into the polyol pathway have reduced neural sorbitol levels and have halted the accelerated loss of neural function and axonal degeneration.

Structurally diverse classes of compounds have been found to inhibit aldose reductase.[14] However, potent, orally active compounds have been limited to members containing acetic acid moieties or five-membered cyclic imides,[15] although recently, (arylsulfonyl)nitromethanes have emerged as a new class of orally potent aldose reductase inhibitors.[16] The acetic acid class of inhibitors, which includes tolrestat,[17] contains many members that

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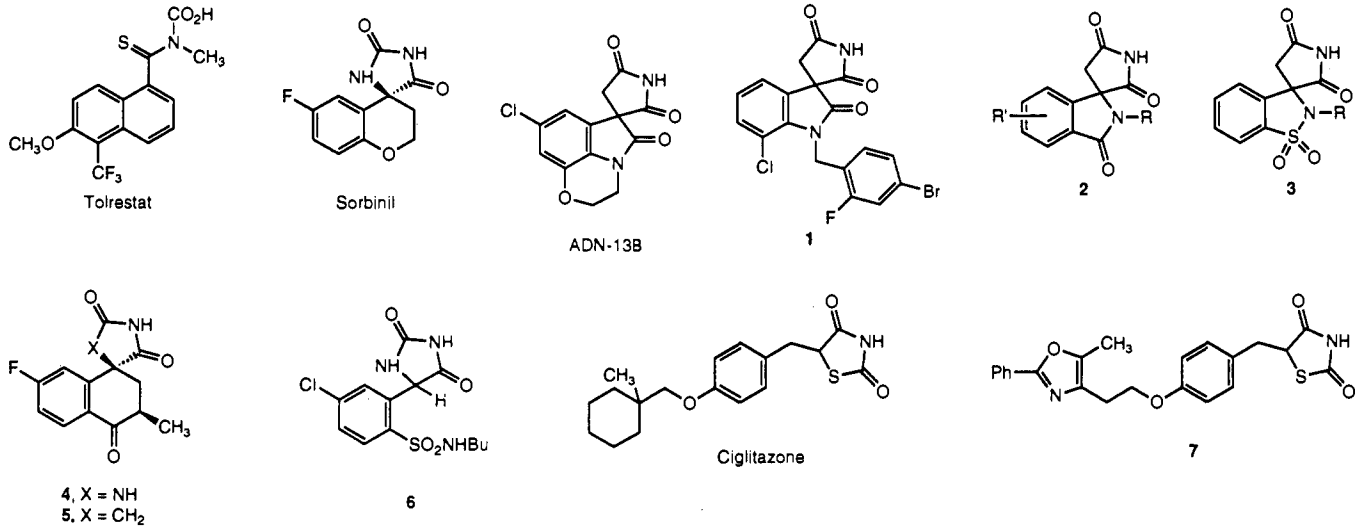
Chart I



show high intrinsic activity, but relatively few of these analogs have shown appreciable in vivo potency.[15,18-21] This has been attributed to their low pK_a values which causes the carboxylate moiety of these compounds to exist in the ionized form at physiological pH, in turn leading to their poor ability to passively diffuse through biological membranes.[18] On the contrary, the five-membered ring cyclic imides, primarily spirohydantoins, of which sorbinil[22] is the prototypical example, are largely un-ionized at physiological pH, readily penetrate nerve cell membranes and have a high correlation of in vivo potency with in vitro activity.[18,23] Spirocyclic oxazolidinediones,[24] thiazolidinediones,[25] and succinimides[26] are also members of the latter category.

Several series of succinimides that are spiro-fused to an indolone framework have been reported to show strong oral activity, including ADN-138[26] and 1.[27] Furthermore ADN-138 was devoid of the anticonvulsant effects that often plague the hydantoin class.[26,28] We recently identified two novel spirosuccinimide series 2 and 3, which possess spiro-fused isoindolone and benzisothiazole *S,S*-dioxide moieties, respectively. The ring system of 2 differs from the ring system of 1 in an amide transposition (CON to NCO). The isoindolone carbonyl of 2 is also spatially oriented relative to its acidic heterocyclic ring in a manner similar to the ketone carbonyl relationship found in spirohydantoin 4[29] (a compound reported to be more potent than sorbinil[2]) and spirosuccinimide 5.[30] On the other hand, benzisothiazole 3 is related to 2 by a replacement of the isoindolone carbonyl (CO) with a sulfonyl moiety (SO_2). Other aldose reductase inhibitors containing sulfonamide groups include 2-[(aminosulfonyl)phenyl]-hydantoins (e.g. 6).[31] Here we report the in vitro and in vivo aldose reductase inhibition results for analogs of 2 and 3.

In the course of investigating new compounds as antihyperglycemic agents, we discovered that members from both 2 and 3 appreciably lowered plasma glucose levels in the db/db mouse,[32] a model of type 2 diabetes. These mice are obese, glucose intolerant, and have fasting hyperglycemia sometimes accompanied by hyperinsulinemia.[32] Traditional hypoglycemic agents, the sulfonylureas, which exert their effect primarily through stimulation of insulin release, are not effective in this model, even at high doses.[33]

Ciglitazone, a thiazolidine-2,4-dione, is the prototypical agent active in this model and other animal models of insulin resistance.[34,35] Furthermore, ciglitazone was not

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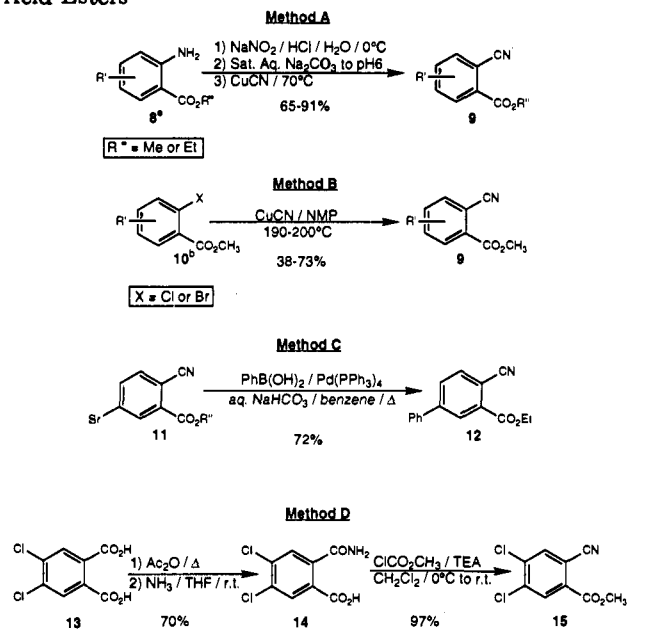
active in non-diabetic animals and, therefore, it did not possess the liability to induce hypoglycemia, which is a problem encountered using the sulfonylureas. Other antihyperglycemic agents possessing the 2,4-thiazolidinedione moiety have appeared,[36-40] including the highly potent Takeda compound 7, which also possesses a 4-linked oxazole moiety.[41] Closely related 2,4-oxazolidinediones have also been described.[42] However, except for a few other series, including tetrazoles[43] and 1,2,3,5-oxathiadiazole 2-oxides,[44] there was not a wealth of new structural types active in insulin-resistant animal models. Therefore we decided to further investigate analogs of 2 and 3 as antihyperglycemic agents.

Chemistry

The isoindolone-based spirosuccinimides 2 were prepared according to method E in Scheme II, and their requisite starting materials, substituted cyanobenzoic acid esters, were generated by methods A–D shown in Scheme I. The benzisothiazole-based analogs 3 were prepared from N-substituted saccharins using method H in Scheme IV, and the saccharins, in turn, were prepared using either method F or G in Scheme III. The substituted 2-cyanobenzoic acid esters 9 were generally generated by two methods. One method (A) involved diazotization of substituted anthranilic acid esters 8 followed by reaction with cuprous cyanide. Alternatively, 2-chloro- or 2-bromobenzoic acid esters 10 were reacted with cuprous cyanide in *N*-methylpyrrolidinone at elevated temperatures (method B). 5-Phenyl-2-cyanobenzoic acid ethyl ester (12) was prepared from the 5-bromo analog 11 by palladium-catalyzed cross-coupling with phenylboronic acid[45] (method C), and 5,6-dichloro-2-cyanobenzoic acid methyl ester (15) was prepared from phthalic acid derivative 13, by standard conversion to the monoamide 14, followed by dehydration–esterification using methyl chloroformate/triethylamine[46] (method D).

Scheme I. Synthesis of Substituted 1,2-Cyanobenzoic Acid Esters



[a] Esters were commercially available or prepared from commercially available carboxylic acids via Fischer esterification (HCl/R"OH). [b] Esters were commercially available or prepared from commercially available carboxylic acids by treatment with CH_3I/K_2CO_3/DMF/at room temperature.

The cyanoesters 9, 12, or 15 were cyclized using ammonia in methanol[47] to the appropriate 3-iminoisoindolin-1-one 16 (Scheme II). Compounds 16 were further reacted with excess ethyl cyanoacetate at 180 °C[48] to afford the cyano esters 17. N-Alkylation of 17 employing an alkyl or aralkyl halide or tosylate and potassium carbonate in DMF at 100 °C proceeded smoothly. The product 18 was generally a mixture of geometrical isomers. Conversion to the spirosuccinimide derivative 2 was accomplished using standard conditions: conjugate addition of cyanide to form the dinitrile 19; formation of the diimino ether with HCl in methanol followed by cyclization to 20; and hydrolysis/decarboxylation of the 4'-carbomethoxy moiety. Chemical data for analogs of 2 are shown in Table I.

An analog of 2, compound 32 was resolved using a procedure similar to one reported for the resolution of compound 1.[27] The methylquinidinium salt was prepared by reacting 32 with 1 equiv of methylquinidinium hydroxide. The diastereomers were separated via recrystallization from ether–acetonitrile. Each diastereomer was then treated with aqueous acid to liberate the enantiomerically enriched form of 32. The (+)-enantiomer had an ee = 98%, and the enantiomeric excess of the (−)-enantiomer was determined to be 78%.

The N-substituted saccharin analogs 22, which were starting materials for benzisothiazole-based spirosuccinimides 3, were prepared using one of two methods shown in Scheme III. Method F involved N-alkylation of sodium saccharin in DMF at 100 °C using the appropriate alkyl

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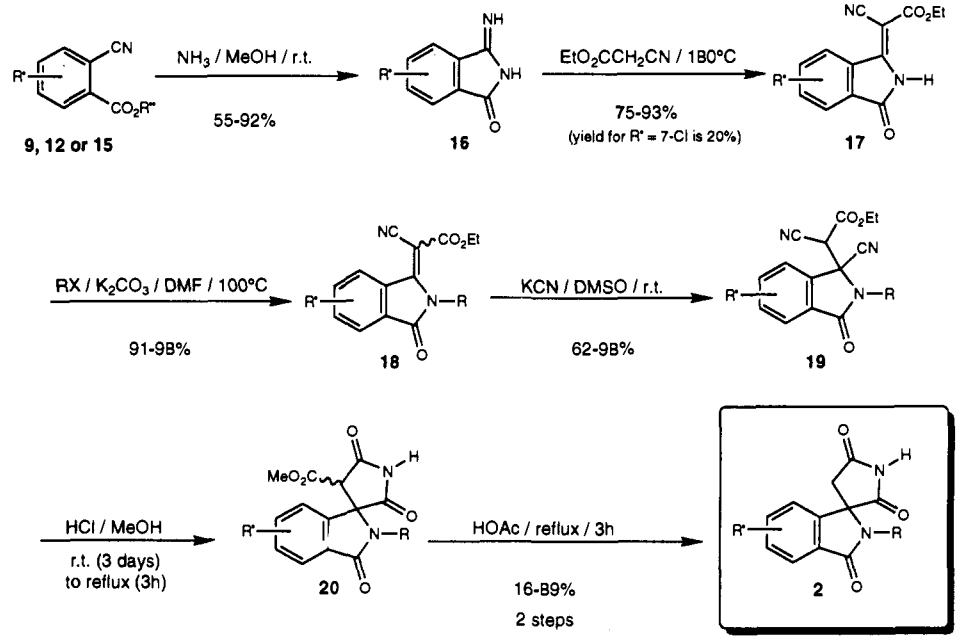
Scheme II. Method E. Preparation of Isoindolone-Based Spirosuccinimides



or aralkyl halide or tosylate. Alternatively, **22** could be synthesized by reacting methyl 2-(chlorosulfonyl)benzoate **23**, with the requisite alkyl, aralkyl, or aryl amine, followed by base-promoted, thermal cyclization. Then, according to method H in Scheme IV, the N-substituted saccharin **22** was converted to acylcyanoacetate **24** by condensation with ethyl cyanoacetate and concomitant ring opening. Cyclization/dehydration employing acetic anhydride or acetic anhydride/pyridine afforded the cyanoester **25**, which was generally a mixture of geometrical isomers. Compounds **25** were then converted to spirosuccinimides **3** using the conditions described in the preparation of **2** from **18**. Two analogs of **2**, the *N*-(*p*-aminobenzyl) **83** and *N*-(*p*-acetylaminobenzyl) derivative **84** were prepared from the nitro compound **78** via tin(II) chloride reduction[49] to **83** followed by acetylation to **84** (Scheme V). Chemical data for analogs of **3** are shown in Table II.

Biological Results and Discussion

The aldose reductase inhibitor activity of these compounds was assessed in vitro by measuring the NADPH-dependent inhibition of glyceraldehyde reduction in a partially purified bovine lens preparation. Many compounds were further evaluated in vivo by measuring their ability to inhibit galactitol accumulation in the lens, sciatic nerve, and diaphragm of galactose-fed rats. These assays were previously described in detail.[18] The biological results for **2** and **3** are reported in Table III and IV, respectively.

Although analogs of the isoindolone series **2** showed promising results in our assays, the members of the benzisothiazole series **3** had poor activity in vitro and were at least 1 order of magnitude less active than the corresponding members of series **2**. For example, compare **28** with **59** and **31** with **66**. Two analogs of the benzisothiazole series, namely **66** and **71**, were further tested in vivo and showed reduced potency relative to members of series **3**; thus we focused our efforts on the isoindolone series **2**.

Within this series, it quickly became apparent that increasing the size of the R-group on nitrogen resulted in decreasing intrinsic and oral activity (compare **28–30** in ring unsubstituted sequence where R′ = H; **32** with **33** in R′ = 6-Cl sequence; and **38** with **39** in R′ = 5,6-Cl_2 sequence). Surprisingly, the 4-bromo-2-fluorobenzyl moiety, which is a group largely responsible for the high potency of statil and related compounds,[2,50] did very little for our analogs. For instance, while 4-bromo-2-fluorobenzyl derivative **31** was more potent in vitro than the benzyl analog **50**, its intrinsic activity did not improve over methyl analog **28**. Furthermore, these 4-bromo-2-fluorobenzyl-containing analogs, **31** or **34**, showed substantially reduced activity in vivo at the high dose of 100 mg/kg. By contrast, the carbonyl transposition congener **1**[27] was highly active in vitro (83% at 10^{-7} M) and showed significant activity in the sciatic nerve of the galactose-fed rat at 1 mg/kg.

Isoindole ring substitution had a modest effect on in vitro potency. In general, substitution in positions 4 through 7 of **2** tended to increase activity slightly. All four chlorine-containing positional isomers (**32**, **35–37**) were prepared and position 6 (compounds **32** and **38**) appeared optimal for this substituent. Halogen substitution at this position was also shown to be preferable for sorbinil and its analogs.[2,23] The naphthyl (**45**) and 5-phenyl (**46**) analogs were also among the more potent compounds in vitro.

The most potent compounds in vitro were also among the more potent compounds in the galactose-fed rat model. Compounds active in the sciatic nerve, the therapeutically relevant target tissue, at or below 10 mg/kg were **32** (6-Cl), **36** (7-Cl), **38** (5,6-Cl_2), **42** (4-CH_3), **45** (5,6-(–CH=CH–CH=CH–)), and **46** (5-Ph). Compound **32** was deemed to have the best oral activity and was therefore resolved in order to demonstrate possible enantiomeric preferences. The data, shown in Table V, indicated that activity resided in essentially one isomer, the (+)-enantiomer. Although the absolute configuration of this enantiomer was not determined, on the basis of the precedent with sorbinil

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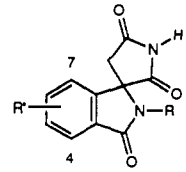
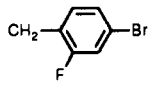
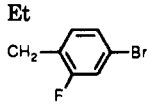
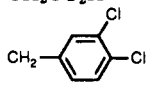
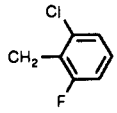
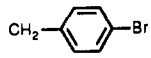
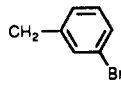
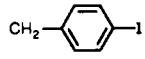
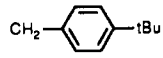
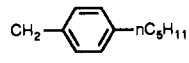
Table I. Spiro[1*H*-isoindole-1,3′-pyrrolidine]-2′,3′,5′(2*H*)-triones **2**. Chemical Data



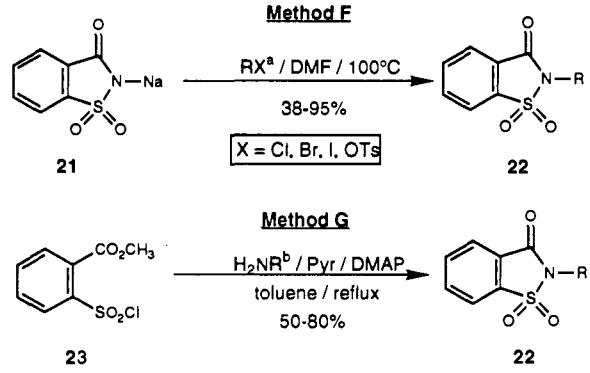
compd	R	R′	synthesis method	mp, °C (recryst solvent)	formula[a]
28	CH_3	H	E	289–302 (EtOH)	$C_{12}H_{10}N_2O_3$
29	Et	H	E	231–239 dec	$C_{13}H_{12}N_2O_3$
30	nBu	H	E	182–186	$C_{15}H_{14}N_2O_3$
31		H	E	230–237 (EtOH)	$C_{18}H_{12}BrFN_2O_3$
32	CH_3	6-Cl	A,E	339–343 dec	$C_{12}H_9ClN_2O_3$
33	Et	6-Cl	A,E	297–298 dec	$C_{13}H_{11}ClN_2O_3$
34		6-Cl	A,E	218–222	$C_{18}H_{11}BrClFN_2O_3$
35	CH_3	5-Cl	A,E	318–329 dec	$C_{12}H_9ClN_2O_3$
36	CH_3	7-Cl	A,E	310–311	$C_{12}H_9ClN_2O_3$
37	CH_3	4-Cl	A,E	352–356	$C_{12}H_9ClN_2O_3$
38	CH_3	5,6-di-Cl	D,E	289–293	$C_{12}H_8Cl_2N_2O_4$
39	nPr	5,6-di-Cl	D,E	248–250	$C_{14}H_{12}Cl_2N_2O_4$
40	CH_3	6-F	B,E	322–326	$C_{12}H_9FN_2O_3$
41	CH_3	5-Br	A,E	316–322	$C_{12}H_9BrN_2O_3$
42	CH_3	4-CH_3	B,E	327–329	$C_{13}H_{12}N_2O_3$
43	CH_3	5-CH_3	A,E	276–283	$C_{13}H_{12}N_2O_3$
44	CH_3	6-CO_2CH_3	A,E	273–278	$C_{14}H_{12}N_2O_5$
45	CH_3	5,6- 	A,E	295–297	$C_{16}H_{12}N_2O_3$
46	CH_3	5-Ph	A,C,E	273–275	$C_{18}H_{14}N_2O_3$
47	CH_3	5-OCH_3	B,E	226–232 (EtOH)	$C_{13}H_{12}N_2O_4$
48	CH_3	5-SCH_3	B,E	243–253 (EtOH)	$C_{13}H_{12}N_2O_3S$
49	CH_3	6-NO_2	B,E	357 dec	$C_{12}H_9N_3O_5$
50	CH_2Ph	H	E	222–225	$C_{18}H_{14}N_2O_3$
51[b]	CH_2CO_2H	H	E	285–293	$C_{13}H_{10}N_2O_3$
52		H	E	207–209	$C_{18}H_{12}Cl_2N_2O_3$
53		H	E	207–209 (EtOH)	$C_{18}H_{12}ClFN_2O_3$
54		H	E	231–232 (EtOH)	$C_{18}H_{13}BrN_2O_3$
55		H	E	239–240 (EtOH)	$C_{18}H_{13}BrN_2O_3$
56[c]		H	E	243–245	$C_{18}H_{13}IN_2O_3$
57		H	E	224–225 (EtOH)	$C_{22}H_{22}N_2O_3$
58		H	E	182–183 (EtOH)	$C_{23}H_{24}N_2O_3$

[a] Analyses (C, H, N) were within ±0.4% unless otherwise indicated. [b] Anal. calcd C, 56.94; found C, 56.43. [c] The starting material, RBr, was prepared from commercially available carboxylic acid.

and its analogs[23] including **4**[2] and **5**,[30] the most likely candidate would be the *R*-enantiomer.

Compound **32** had in vivo activity (ED_{50} ~ 4–10 mg/kg per nerve) comparable to the other cyclic imides of similar in vitro potency, sorbinil and ADN-138 (45–80% at 10^{-6} M). The carboxylic acid, tolrestat, although having far better intrinsic activity (65% at 10^{-7} M), had approximately the same oral potency as these spirocyclic imides. The 4-bromo-2-fluorobenzyl indolone-based spirosuccinimide **1** was similar to tolrestat as an inhibitor of aldose reductase in vitro; however, it was more potent in vivo with an ED_{50} approaching 1 mg/kg as opposed to 6 mg/kg in the sciatic nerve of the galactose-fed rat for tolrestat. All these results are consistent with the observations that five-membered ring cyclic imide inhibitors are more bioavailable than carboxylic acid-based inhibitors and that in vivo activity correlates better with in vitro activity in the imide series than in the carboxylic acid series.

Scheme III. Preparation of N-Substituted Saccharins



[a] Alkyl and aralkyl halides and tosylates were commercially available or prepared from commercially available or known alcohols (PBr_3/Et_2O/0 °C or TsCl/pyr/DMAP), commercially available or known aldehydes ($NaBH_4$/MeOH/0 °C then PBr_3/Et_2O/0 °C), or commercially available carboxylic acids (BH_3·THF then PBr_3/Et_2O/0 °C). [b] Aryl, alkyl, or aralkylamines were commercially available or known.

With regard to the antihyperglycemic activity, analogs from series **2** and **3** were evaluated in the db/db mouse at 100 mg/kg (see Tables III and IV for results). In this assay, plasma glucose levels of the drug-treated group were measured relative to a vehicle-treated control group. A 50–60% decrease in plasma glucose levels is equivalent to the levels of non-diabetic animals. While ciglitazone caused a 30% decrease in plasma glucose at 100 mg/kg, the Takeda compound **7** normalized (52% decrease) glucose levels at 5 mg/kg. Several of our analogs had potency approximately equal to ciglitazone; however, beyond this point there were few structure–activity relationship (SAR) factors evident. There was a clear separation of SAR for series **2** and **3** between their ability to inhibit aldose reductase and lower plasma glucose in the db/db mouse. Only one compound, the *N*-methyl-5,6-dichloroisoindolone **38**, showed good oral activity in both areas (94% inhibition of galactitol accumulation in the nerve of the galactose-fed rat at 50 mg/kg and 31% decrease in plasma glucose in the db/db mouse at 100 mg/kg).

The limited data suggested that the isoindolone and benzisothiazole 1,1-dioxide frameworks of **2** and **3**, respectively, were bioisosteric since many analogs of **2** and **3** with the same R group had approximately the same activity (compare **31** with **66** and **52** with **67**). Other features worth noting include the finding that compounds containing branched chain R groups, **62** and **63**, showed activity, while those containing linear chained alkyl groups showed substantially reduced potency (see **28**, **30**, **59–61**). Also several *N*-aralkyl compounds were active, especially analogs containing *p*-chloro or *p*-bromobenzyl moieties (compounds **31**, **50**, **52**, **66–68**, **70**, **82**, **85**, **90**). However, even minor changes to these active compounds generally led to a large drop in activity (compare **31** with **34**, **54**, and **55**; **68** with **54**; **66** with **91–93**). With the thought that the benzisothiazole *S*,*S*-dioxide spirosuccinimide moiety of **3** might be functioning as a 2,4-thiazolidinedione surrogate, we appended several oxazole fragments of the Takeda compound **7** onto benzisothiazole nitrogen of series **3**. However this change was not successful at generating analogs (**75–77**) with superior potency.

Two of the more active compounds, both 4-bromo-2-fluorobenzyl analogs (**31** and **66**), were further evaluated in another model of insulin-resistance, the ob/ob mouse[32,51] (Table VI). Although both of these compounds caused a significant decrease in plasma glucose at 100 mg/kg, neither compound lowered insulin levels at this dose, as opposed to the control, ciglitazone. Our drug design efforts with regard to these analogs are hampered by a lack of knowledge concerning the antihyperglycemic (and molecular) mechanism of action for these, as well as other series. This factor, a lack of potency beyond that of ciglitazone, and an inability to lower insulin levels led us to discontinue our search for antihyperglycemic agents based on the structures of **2** and **3**.

In summary, isoindolone and benzisothiazole 1,1-dioxide-based spirosuccinimides were evaluated as aldose reductase inhibitors and antihyperglycemic agents. Many members of the isoindolone series **2**, particularly those with isoindole ring *N*-methyl groups, showed good in vitro and in vivo potency. The most potent, the 6-chloro analog **32**, was resolved, and the (+)-enantiomer was found to be the active one. Compounds from both series **2** and **3** were also found to lower plasma glucose levels of db/db and ob/ob mice, with potency similar to that of ciglitazone; however, these analogs do not appear to lower insulin levels in the ob/ob mouse model at the doses tested.

Experimental Section

Partially Purified Enzyme Preparation and Galactose-Fed Rat Model. Previously described procedures were used.[18] For the galactose-fed rat model, all data are reported as mean values for three to eight drug-treated tissue samples. The Dunnett's multiple comparison test was used to assess the statistical significance between means of compound-treated tissues compared to the nontreated group ($p < 0.05$ was statistically significant). Thus all percentage inhibition values given in the tables for each compound are statistically significant unless otherwise indicated.

Antihyperglycemic Assays. Effect of drugs on blood glucose levels of diabetic mice [male, db/db (C57BL/KsJ), Jackson Laboratories] was determined as previously described.[44]

The procedure using ob/ob mice was as follows: In each study, mice [male (C57 BI/6J), Jackson Laboratories, ages 2–5 months (10–65 g)] are randomized according to body weight into four groups of 10 mice. The mice are housed five per cage and are maintained on normal rodent chow with water ad libitum. Mice received compound daily by gavage (suspended in 0.5 mL of 0.5% methyl cellulose). The dose of compounds given was 100 mg/kg of body weight per day. The dose was calculated based on the fed weekly body weight and is expressed as active moiety. Control mice received vehicle only.

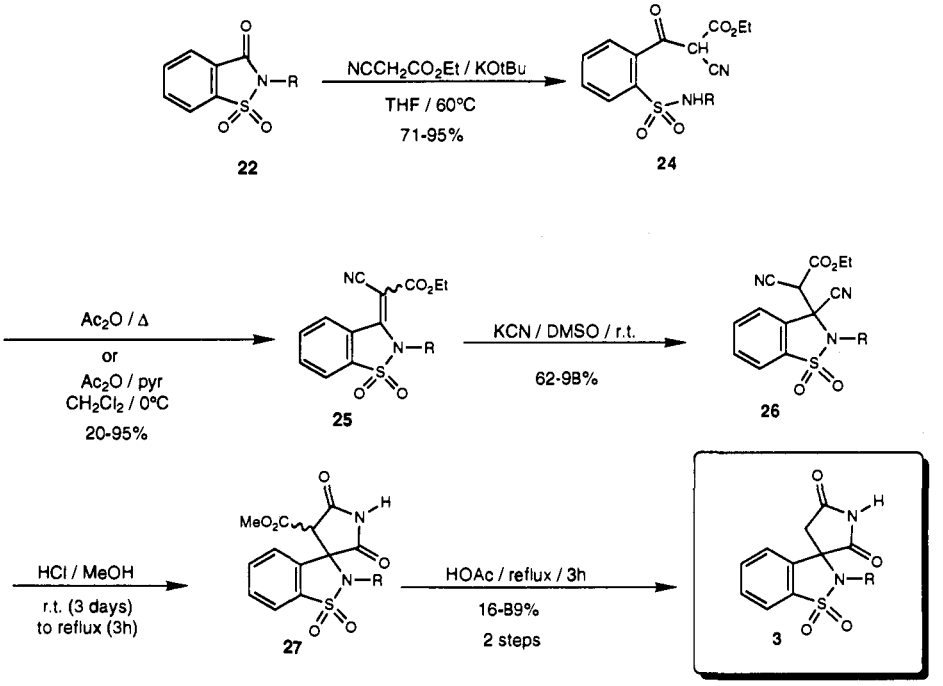
On the morning of day 4 two drops of blood (approximately 50 μL) were collected into sodium fluoride containing tubes after decapitation 4 hours after compound administration. The plasma was isolated by centrifugation, the concentration of glucose was measured enzymatically on an Abbott V.P. Analyzer, and plasma insulin was quantitated by radioimmunoassay.[52]

For each mouse, the percentage change in plasma glucose on day 4 was calculated relative to the mean plasma glucose of the vehicle treated mice. Analysis of variance followed by Dunett's Comparison Test (one-tailed) were used to estimate the significant difference between the plasma glucose values from the control group and the individual compound-treated groups. A compound was considered active if the difference has a $p < 0.05$.

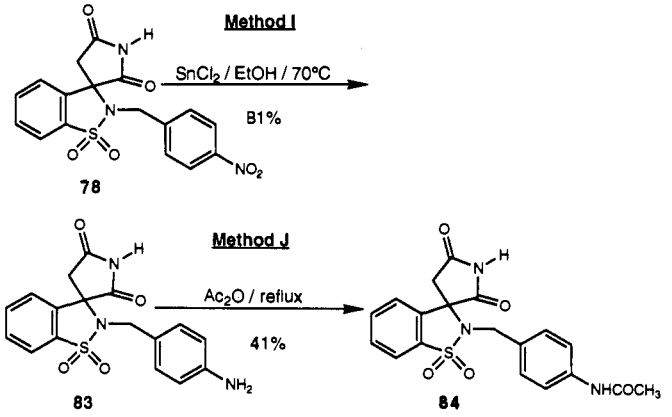
Chemistry. Melting points were determined on an Electrothermal capillary melting point apparatus and are not corrected. Proton magnetic resonance (^1H NMR) spectra were recorded at 200 MHz (Varian XL-200), 400 MHz (Bruker AM-400), or at 80 MHz (Varian CFT-20). Infrared spectra were obtained on either

(51) Coleman, D. Obese and Diabetes: Two Mutant Genes Causing Diabetes-Obesity Syndromes in Mice. *Diabetologia* 1978, *14*, 141–148.
(52) Heding, L. G. Determination of Total Serum Insulin (IRI) in Insulin-treated Diabetic Patients. *Diabetalogia* 1972, *8*, 260–266.

Scheme IV. Method H. Preparation of Benzoisothiazole *S,S*-Dioxide-Based Spirosuccinimides



Scheme V



a Beckman Accu Lab 2 or a Perkin-Elmer Model 781 spectrophotometer as KBr pellets, thin films on sodium chloride plates, or as solutions in chloroform and are reported as reciprocal centimeters (cm^{-1}). Mass spectra were recorded on either a Finnigan model 8230 or a Hewlett-Packard Model 5995A spectrometer. Analyses (C, H, N) were carried out on a modified Perkin-Elmer Model 240 CHN analyzer. Analytical results for elements were within ±0.4% of the theoretical values. Flash chromatography was carried out according to the procedure of Still.[53] Thin-layer analyses were done on E. Merck Silica Gel 60 F-254 plates of 0.25-mm thickness. Enantiomeric purities of (+)- and (−)-**32** were determined on a Waters Model 6000 HPLC using a Chirosphere 25 cm × 4.1 mm reverse-phase chiral column with 100% isopropyl alcohol as mobile phase at 0.2 mL/min flow rate.

Method A. 4-Chloro-2-cyanobenzoic Acid Methyl Ester (9, R′ = 4-Cl, R″ = Me). Water (540 mL) was added to a stirred suspension of 2-amino-4-chlorobenzoic acid methyl ester (**8**, R′ = 4-Cl, R″ = Me, 20.0 g, 0.108 mol) in concentrated aqueous HCl (65 mL), and this solution was cooled, with stirring, below 5 °C. A solution of sodium nitrite (7.4 g, 0.108 mol) in water (20 mL) was added dropwise over a 10-min period at 0–5 °C until complete dissolution occurred. This diazonium solution was then brought to pH 6 with saturated aqueous sodium bicarbonate.

In a separate reaction vessel, a solution of copper sulfate pentahydrate (32.3 g, 0.129 mol) in water (130 mL) was added dropwise to a stirred 0–5 °C solution of potassium cyanide (32.3 g, 0.496 mol) in 65 mL of water. Benzene (100 mL) was added to the resulting brown suspension and this biphasic mixture was heated in a 60 °C oil bath.

The previously prepared diazonium solution was added dropwise to the brown copper(I) cyanide solution at 60 °C over a 40-min period. The reaction mixture was heated at 70 °C for 1 additional hour and cooled to room temperature, and ethyl acetate (1 L) was added. The biphasic mixture was filtered through Celite. The layers were separated, and the ethyl acetate phase was washed with brine and dried ($MgSO_4$). The solvent was removed, and the solid was triturated with petroleum ether to provide the product (15.8 g, 75%) as a tan solid. A small portion was recrystallized from petroleum ether–chloroform to afford colorless plates: mp 115–117 °C; NMR ($CDCl_3$ 200 MHz) δ 3.99 (s, 3 H, CH_3), 7.63 (dd, 1 H, J = 2, 9 Hz, Ar*H*-3), 7.77 (d, 1 H, J = 2 Hz, Ar*H*-5), 8.08 (d, 1 H, J = 9 Hz, Ar*H*-2); IR ($CHCl_3$) 2220, 1730 cm^{-1}. Anal. ($C_9H_6ClNO_2$) C, H, N.

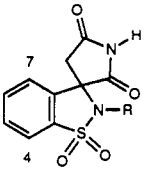
Method B. 2-Cyano-4-fluorobenzoic Acid Methyl Ester (9, R′ = 4-F). Copper(I) cyanide (5.22 g, 58.3 mmol) was added to a stirred solution of 2-chloro-4-fluorobenzoic acid methyl ester (**10**, R′ = 4-F, X = Cl, 10.0 g, 53.0 mmol) and 1-methyl-2-pyrrolidinone (30 mL) under a dry N_2 atmosphere. The suspension was heated to 195 °C for 1.5 h and then cooled to room temperature. The reaction mixture was diluted with water (800 mL) and filtered. The solid cake was added to a stirred solution of sodium cyanide (3 g) in water (110 mL), and this suspension was stirred rapidly at room temperature for 50 min. Ethyl acetate (300 mL) was added, and the biphasic mixture was filtered through Celite. The layers were separated, and the aqueous layer was extracted with ethyl acetate (300 mL). The combined ethyl acetate phase was washed with brine and dried ($MgSO_4$). The solvent was removed to provide the product (6.9 g, 73%) as an off-white solid. A 100-mg sample was purified by flash chromatography (9:1 CH_2Cl_2–petroleum ether) to provide a white solid (88 mg): mp 104–106 °C; NMR ($CDCl_3$ 200 MHz) δ 3.98 (s, 3 H, CH_3), 7.37 (ddd, 1 H, J = 3, 8, 9 Hz, Ar*H*-3), 7.49 (dd, 1 H, J = 3, 8 Hz, Ar*H*-5), 8.17 (dd, 1 H, J = 5,9 Hz, Ar*H*-2); IR ($CHCl_3$): 2220, 1735 cm^{-1}. Anal. ($C_9H_6FNO_2$) C, H, N.

Method C. 2-Cyano-5-phenylbenzoic Acid Ethyl Ester (12). A solution of phenylboronic acid (4.69 g, 38.5 mmol) in ethanol (20 mL) was added rapidly to a stirred suspension of

(53) Still, W. C.; Kahn, M.; Mitra, A. Rapid Chromatographic Technique for Preparative Separations with Moderate Resolution. *J. Org. Chem.* **1978**, *43*, 2923–2825.

(54) Lau, C. K.; Tardif, S.; Dufreare, C.; Scheigetz, J. Reductive Deoxygenation of Aryl Aldehydes and Ketones by *tert*-Butylamine-Borane and Aluminum Chloride *J. Org. Chem.* **1989**, *54*, 491–494.

Table II. Spiro[1,2-benzisothiazole-3(2*H*),3′-pyrrolidine]-2′,5′-dione 1,1-Dioxides **3**. Chemical Data



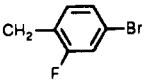
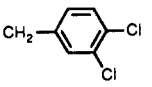
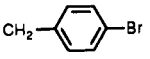
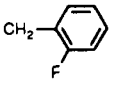
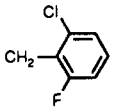
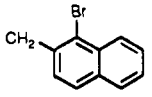
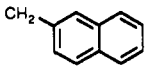
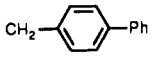
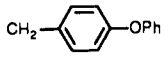
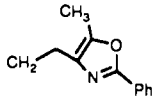
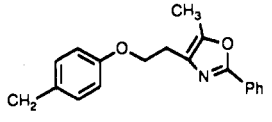
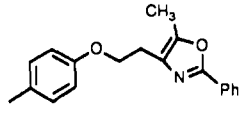
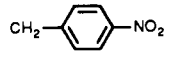
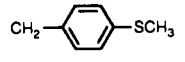
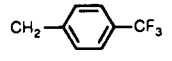
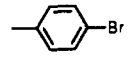
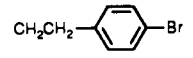
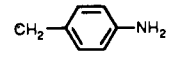
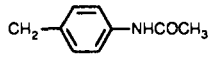
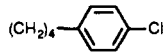
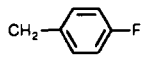
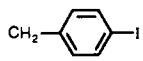
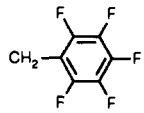
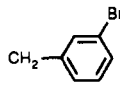
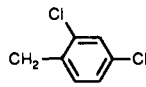
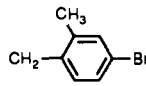
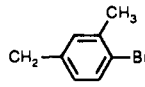
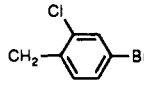
compd	R	synthesis method	mp, °C (recryst solvent)	formula[a]
59	CH_3	F,H	288–293 (EtOH)	$C_{11}H_{10}N_2O_4S \cdot {}^1/_4H_2O$
60	Et	F,H	216–220 (EtOH)	$C_{12}H_{12}N_2O_4S$
61	nBu	F,H	215–218 (EtOH)	$C_{14}H_{16}N_2O_4S$
62	$CH(CH_3)_2$	F,H	217–220	$C_{13}H_{14}N_2O_4S$
63	$CH(CH_2CH_3)_2$	G,H	93–97	$C_{16}H_{20}N_2O_4S$
64	CH_2CO_2H	F,H	254–258	$C_{12}H_{10}N_2O_6S$
65	CH_2Ph	F,H	205–208	$C_{17}H_{14}N_2O_4S$
66		F,H	120–123	$C_{17}H_{12}BrFN_2O_4S$
67		F,H	197–198	$C_{17}H_{12}Cl_2N_2O_4S$
68		F,H	210–214	$C_{17}H_{13}BrN_2O_4S$
69		F,H	203–205	$C_{17}H_{13}FN_2O_4S$
70		F,H	267–270	$C_{17}H_{12}BrFN_2O_4S$
71		F,H	250–252	$C_{21}H_{15}BrN_2O_4S$
72		F,H	135–155	$C_{21}H_{16}N_2O_4S$
73		F,H	220–240	$C_{23}H_{18}N_2O_4S$
74[b]		F,H	175–180	$C_{23}H_{18}N_2O_5S$
75[c]		F,H	235–237	$C_{22}H_{19}N_3O_5S$
76[d]		F,H	118–120	$C_{29}H_{25}N_3O_6S$
77[e]		G,H	126–132	$C_{28}H_{23}N_3O_6S$
78		F,H	244–245	$C_{17}H_{13}N_3O_6S$
79[f]		F,H	166–170	$C_{18}H_{16}N_2O_4S_2$
80		F,H	118–119	$C_{18}H_{13}F_3N_2O_4S$
81		G,H	278–293 dec	$C_{16}H_{11}BrN_2O_4S$
82		G,H	100–110	$C_{18}H_{15}BrN_2O_4S$
83		F,H,I	137–151 dec	$C_{17}H_{15}N_3O_4S$

Table II. (Continued)

compd	R	synthesis method	mp, °C (recryst solvent)	formula[a]
84		F,H,I,J	235–240	$C_{19}H_{17}N_3O_5S$
85[g]		F,H	160–161 (toluene:petroleum ether)	$C_{20}H_{19}ClN_2O_4S$
86		F,H	190–192 (CH_2Cl_2)	$C_{17}H_{13}FN_2O_4S$
87[h]		F,H	133–135 dec (CH_2Cl_2)	$C_{17}H_{13}IN_2O_4S$
88		F,H	111–120	$C_{17}H_9F_5N_2O_4S$
89		F,H	191	$C_{17}H_{13}BrN_2O_4S$
90		F,H	211–216	$C_{17}H_{12}Cl_2N_2O_4S$
91[h]		F,H	237–241	$C_{18}H_{15}BrN_2O_4S$
92[h]		F,H	212–215	$C_{18}H_{15}BrN_2O_4S$
93[h]		F,H	203–205	$C_{17}H_{12}BrClN_2O_4$

[a] Analyses (C, H, N) were within ±0.4%. [b] Starting material, RBr, prepared from commercially available alcohol. [c] Starting material, RBr, prepared from known alcohol.[41] [d] Starting material, RBr, prepared from known aldehyde.[41] [e] Starting material, RNH_2, is known.[41] [f] Starting material, RBr, prepared from commercially available alcohol. [g] Starting material, ROTs, prepared from known alcohol.[54] [h] Starting material, RBr, prepared from commercially available acid.

5-bromo-2-cyanobenzoic acid ethyl ester (**11**, prepared according to method A, 8.9 g, 35 mmol), tetrakis(triphenylphosphine)-palladium (1.21 g, 1.05 mmol), benzene (70 mL), and 2.0 M aqueous Na_2CO_3 (35 mL). This biphasic mixture was heated to reflux and stirred for 4 h under a dry N_2 atmosphere. The reaction mixture was cooled to room temperature, 30% hydrogen peroxide (16 mL) was added, and the suspension was stirred at room temperature for 1 h. The product was extracted with ether (3 × 100 mL) and the combined ether phase was washed with brine (200 mL) and dried ($MgSO_4$). Silica gel (150 mL) was added, and the solvent was removed. The silica adsorbate was flash chromatographed (9:1 petroleum ether–ethyl acetate) to provide the product as a white solid (6.37 g, 72%): mp 69–74 °C; NMR (DMSO-d_6, 200 MHz) δ 1.37 (t, 3 H, J = 7 Hz, CH_3), 4.41 (q, 2 H, J = 7 Hz, CH_2), 7.51 (m, 3 H, Ar*H*), 7.76 (dd, 2 H, J = 2, 8 Hz, Ar*H*), 8.08 (m, 2 H, Ar*H*), 8.30 (d, 1 H, J = 2 Hz, Ar*H*); IR (KBr) 2210, 1720 cm^{-1}. Anal. ($C_{16}H_{13}NO_2$) C, H, N.

Method D. 2-Cyano-4,5-dichlorobenzoic Acid Methyl Ester (15). A suspension of 4,5-dichlorophthalic acid (**13**) and acetic anhydride (70 mL) was heated to reflux until dissolution occurred. A distilling head was then attached, and 30 mL of distillate was removed. The solution was cooled to room temperature whereupon a precipitate appeared. This solid was filtered, washed with anhydrous ether, and dried in vacuo to provide 4,5-dichlorophthalic anhydride (40.4 g, 87%) as a tan solid, mp 189–192 °C.

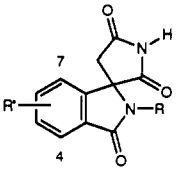
This anhydride (20.0 g, 92.2 mmol) was dissolved in dry THF (400 mL) and cooled in an ice bath. Ammonia gas was then passed through this solution for 10 min to afford a precipitate. The THF was removed and water (400 mL) was added. This aqueous solution was brought to acidic pH with 10% aqueous HCl whereupon a precipitate appeared. The solid was collected, washed with water, and dried in vacuo to provide 2-carbamoyl-4,5-dichlorobenzoic acid (**14**, 17.3 g, 80%) as a tan solid, mp 197–201 °C.

Triethylamine (25.4 mL, 0.182 mol) was added dropwise over a 20-min period to a cold (0–5 °C), stirred suspension of **14** (21.3 g, 91 mmol) in CH_2Cl_2 (110 mL). Methyl chloroformate (15.5. mL 0.2 mol) was then added dropwise over a 30-min period. The reaction mixture was warmed to room temperature and stirred overnight. The solvents were removed and water (200 mL) was added. The water phase was filtered, and the precipitate was washed well with water and dried in vacuo to provide the title compound (20.5 g, 97%) as a tan solid: mp 120–123 °C; NMR (DMSO-d_6, 200 MHz) δ 3.91 (s 2 H, CH_3), 8.26 (s, 1 H, Ar*H*), 8.46 (s, 1 H, Ar*H*); IR (KBr) 2225, 1735 cm^{-1}. Anal. ($C_9H_5NO_2$) C, H, N.

Method E. General Procedure for the Preparation of 2,3-Dihydro-3-imino-1*H*-isoindol-1-ones 16. Preparation of 5-Chloro-2,3-dihydro-3-imino-1*H*-isoindol-1-one (16, R′ = 5-Cl). Ammonia gas was passed through a solution of 4-chloro-2-cyanobenzoic acid methyl ester (**9**, R′ = 4-Cl, R″ = Me, 16.9 g, 86.5 mmol) in methanol (800 mL) over a 30-min period. The solution was then stored at room temperature for 3 days. The resulting precipitate was collected, washed with ether, and dried in vacuo to provide the title compound (12.5 g, 80% as a tan solid: mp 295–296 °C dec; NMR (DMSO-d_6, 200 MHz) δ 7.71 (s, 2 H, Ar*H*), 8.14 (s, 1 H, Ar*H*); IR (KBr) 3260, 1720, 1670, 1605 cm^{-1}; M_r 180.01630 (calcd for $C_8H_5ClN_2O$ 180.0090).

General Procedure for the Preparation of Cyano-(2,3-dihydro-3-oxoisoindol-1-ylidene)acetic Acid Ethyl Esters 17. Preparation of Cyano-(6-chloro-2,3-dihydro-3-oxoisoindol-1-ylidene)acetic Acid Ethyl Ester (17, R′ = 6-Cl). A suspension of 5-chloro-3-imino-1-oxoisoindoline (**16**, R′ = 5-Cl, 5.0 g, 27.6 mmol) in ethyl cyanoacetate (11.7 mL, 0.110 mol) was placed in a 180 °C oil bath and heated for 10 min, whereupon the internal temperature reached 160 °C. The reaction mixture was cooled to room temperature, and the solid was collected, broken up, and triturated with ether to provide the title compound (7.1 g, 93%) as an off-white solid: mp 179–181 °C; NMR (DMSO-

Table III. Spiro[1*H*-isoindole-1,3′-pyrrolidine]-2′,3′,5′-(2*H*)-triones **2**. Biological Data



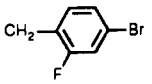
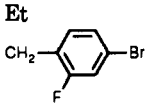
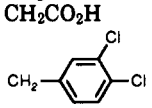
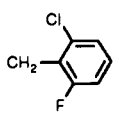
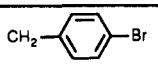
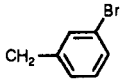
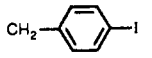
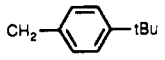
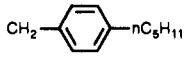
			aldose reductase inhibition						antihyperglycemic activity:[c] % decrease in plasma glucose at 100 mg/kg per day
					in vivo[b]				
			in vitro[a] % inhibition at		dose, mg/kg per day	% inhibition			
compd	R	R′	10^{-6} M	10^{-7} M		lens	nerve	diaphragm	
28	CH_3	H	45	11	66	32	63	58	*e*
29	Et	H	21	*g*	73	25	47	62	NT
30	nBu	H	*g*		NT				*e*
31		H	54	7	101	NS	NS	30	36 ± 8[d]
32	CH_3	6-Cl	80	37	79	81	80	92	*e*
					53	69	92	90	
					25	51	84	81	
					6	NS	28	53	
					3	9	38	43	
33	Et	6-Cl	37	14	3	NS	NS	NS	*e*
34		6-Cl	70	13	97	NS	24	30	*e*
35	CH_3	5-Cl	72	21	81	54	77	81	*e*
					10	NS	NS	34	
					6	NS	NS	31	
					3	NS	NS	NS	
36	CH_3	7-Cl	57	19	10	13	32	47	NT
					6	7	21	37	
					3	NS	NS	23	
37	CH_3	4-Cl	65	29	9	NS	NS	NS	*e*
					6	NS	NS	NS	
					3	NS	21	NS	
38	CH_3	5,6-Cl_2	83	38	50	71	94	90	31 ± 5[d]
					9	10	25	57	
					6	NS	NS	44	
					3	NS	NS	25	
39	nPr	5,6-Cl_2	*g*		4	NS	NS	NS	*e*
40	CH_3	6-F	74	29	52	26	71	68	*e*
					3	NS	NS	15	
41	CH_3	5-Br	71	27	10	NS	NS	39	*e*
					6	NS	NS	27	
					3	NS	NS	NS	
42	CH_3	4-CH_3	59	11	10	29	63	69	NT
					6	28	50	57	
					3	NS	NS	37	
43	CH_3	5-CH_3	61	26	10	12	NS	47	*e*
					6	13	NS	39	
					3	NS	20	27	
44	CH_3	6-CO_2CH_3	49	14	10	NS	NS	16	*e*
					6	NS	NS	NS	
					3	NS	NS	NS	
45	CH_3	5,6- 	82	37	10	14	47	59	*e*
46	CH_3	5-Ph	81	37	9	NS	34	55	*e*
					6	NS	27	43	
					3	NS	NS	29	
47	CH_3	5-OCH_3	56	*g*	3	NS	NS	NS	*e*
48	CH_3	5-SCH_3	60	*g*	3	NS	NS	NS	*e*
49	CH_3	6-NO_2	77	32	3	NS	NS	NS	*e*
50	CH_2Ph	H	*g*		NT				22 ± 15
51	CH_2CO_2H	H	*g*		46	17	NS	NS	NT
52		H	45	18	109	NS	NS	33	21 ± 2[d]
53		H	*g*		NT				*e*

Table III. (Continued)

compd	R	R′	aldose reductase inhibition: in vitro[a] % inhibition at 10^{-6} M	10^{-7} M	in vivo[b]: dose, mg/kg per day	% inhibition: lens	nerve	diaphragm	antihyperglycemic activity:[c] % decrease in plasma glucose at 100 mg/kg per day
54		H	NT		NT				*e*
55		H	NT		NT				*e*
56		H	NT		NT				*e*
57		H	NT		NT				*e*
58		H	NT		NT				*e*
tolrestat			94	65	6	NS	53	80	NT
sorbinil			45	10	4	NS	55	69	NT
ADN-138			66	32	10	20	57	88	NT
					1	NS	NS	64	
1			92	83	10	36	86	84	*e*
					3	23	67	77	
					1	NS	46	49	
ciglitazone			9	*g*	94	NS	NS	NS	32 ± 9[f]
7			NT		NT				52 ± 4 (at 5 mg/kg)[e]
									40 ± 8 (at 1 mg/kg)[e]

[a] Inhibition of enzymatic activity in a partially purified bovine lens preparation (mean of two determinations). [b] Inhibition of galactitol accumulation in the lens, sciatic nerve, or diaphragm of rats ($n = 6$) fed 20% galactose for 4 days; compounds were administered in the diet. NS = not significant inhibition of polyol accumulation ($p > 0.05$) at the given dose. NT = not tested. [c] Values (mean ± SE) are percent decrease relative to vehicle-treated group with use of 4–6 db/db mice per group. [d] $p < 0.05$. [e] Less than 15% decrease at the 100 mg/kg dose. [f] Mean ± SD of 38 experiments. [g] No inhibitory activity at the given concentration.

d_6, 200 MHz) δ 1.30 (t, 3 H, $J = 7.0$, CH_3), 4.33 (q, 2 H, $J = 7.0$, CH_2) 7.93 (s, 2 H, Ar*H*) 8.36 (s, 1 H, Ar*H*) 11.24 (s, 1 H, N*H*); IR (KBr) 3300, 2220, 1750, 1710, 1695, 1610 cm^{-1}. Anal. ($C_{13}H_9ClO_3N_2$) C, H, N.

General Procedure for the Preparation of 2-Substituted-cyano-(2,3-dihydro-3-oxoisoindol-1-ylidene)acetic Acid Ethyl Esters 18. Preparation of Cyano-(6-chloro-2,3-dihydro-2-methyl-3-oxoisoindol-1-ylidene)acetic Acid Ethyl Ester (18, R = Me, R′ = 6-Cl). A suspension of **17** (R′ = 6-Cl, 7.8 g, 28 mmol), iodomethane (2.3 mL, 36.4 mmol), and potassium carbonate (3.87 g, 28.0 mmol) in dry DMF (39 mL) was heated, with stirring, under a dry N_2 atmosphere in a 100 °C oil bath for 1 h. The reaction mixture was cooled to room temperature and added to water (500 mL). The water phase was extracted with ether (3 × 200 mL). The combined ether phase was washed with brine, dried ($MgSO_4$), and concentrated to provide the title compound (7.4 g, 92%) as a light yellow solid and as a mixture of double bond regioisomers: NMR (DMSO-d_6, 200 MHz) δ 1.31, 1.32 (2 t, 3 H, CH_2CH_3), 3.17, 3.52, (2 s, 3 H, NCH_3), 4.33, 4.37 (2 q, 2 H, CH_2CH_3), 7.18–8.0 (m, 2 H, Ar*H*), 8.33, 8.40 (2 s, 1 H, ArH).

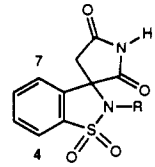
General Procedure for the Preparation of Spiro[1*H*-isoindole-1,3′-pyrrolidine]-2′,3,5′(2*H*)-triones 2. Preparation of 6-Chloro-2-methylspiro[1*H*-isoindole-1,3′-pyrrolidine]-2′,3,5′(2*H*)-trione (32). Potassium cyanide (1.75 g, 26.7 mmol) was added to a stirred, room temperature suspension of **18** (R = Me, R′ = 6-Cl, 7.40 g, 25.5 mmol) in dry DMSO (180 mL). After 2 h, the reaction mixture was added to water (1 L) and extracted with ether (3 × 25 mL). The aqueous phase was cautiously acidified (Caution!, HCN) with 10% aqueous HCl to pH 1. The resulting precipitate was collected, washed with water, and dried in vacuo to give (6-chloro-1-cyano-2-methyl-3-oxo-1-isoindolinyl)-cyanoacetic acid ethyl ester (**19**, R = Me, R′ = 6-Cl, 7.1 g, 88%) which was used immediately without purification.

A suspension of this compound (7.1 g, 22.5 mmol) in dry methanol (225 mL) was cooled in an ice bath. Hydrogen chloride gas was passed through this suspension and within 5 min dissolution occurred. After an additional 15 min, the solution was warmed to room temperature and stored for 3 days. The solution was then heated to reflux for 4 h and cooled to room temperature. The methanol was removed, water (200 mL) was added, and the organics were extracted with ethyl acetate (3 × 100 mL). The combined extracts were dried ($MgSO_4$) and concentrated to provide a yellow solid (7.7 g) containing 6-chloro-4′-(methoxycarbonyl)-2-methylspiro[1*H*-isoindole-1,3′-pyrrolidine]-2′,3,5′(2*H*)-trione (**20**, R = Me, R′ = 6-Cl).

The above solid was dissolved in glacial acetic acid and heated to reflux for 4.5 h. After cooling to room temperature overnight, the reaction mixture was filtered and the solid product was washed well with ether to provide the title compound (4.45 g, 60% from **18**) as a white solid: mp 339–343 °C dec; NMR (DMSO-d_6, 400 MHz) δ 2.89 (s, 3 H, NCH_3) 3.24 (d, 1 H, $J = 19$ Hz, CH_2-proton closer to aromatic ring, as determined by NOE experiment), 3.40 (d, 1 H, $J = 19$ Hz, CH_2-proton closer to NCH_3, as determined by NOE experiment), 7.63 (dd, 1 H, $J = 2, 7$ Hz, Ar5*H*), 7.73 (d, 1 H, $J = 7$ Hz, Ar4*H*), 7.95 (d, 1 H, $J = 2$ Hz, Ar7*H*), 12.11 (s, 1 H, N*H*); IR (KBr) 3195, 1800, 1730, 1700, 1615 cm^{-1}; MS (EI, *m/e*) 266 (3%, MI), 264 (10%, MI), 235 (6%), 195 (31%), 194 (100%), 166 (11%), 164 (33%). Anal. ($C_{12}H_9ClN_2O_3$) C, H, N.

Resolution of 6-Chloro-2-methylspiro[1*H*-isoindole-1,3′-pyrrolidine]-2′,3,5′(2*H*)-trione (32). Preparation of Methylquinidinium Hydroxide in Methanol. Iodomethane (1.1 mL, 17.22 mmol) was added to a stirred, room temperature suspension of quinidine (5.0 g, 14.96 mmol), anhydrous potassium carbonate (12.5 g, 91.3 mmol), and acetone (185 mL). The suspension was stirred for 3 h and added to water (500 mL) and petroleum ether (200 mL), and the biphasic mixture was rapidly stirred for 15 min. The solid was filtered and recrystallized from ethanol (60 mL) to provide methylquinidinium iodide as a white crystalline solid (2.2 g, 31%). A solution of this solid (2.18 g) in methanol (60 mL) was passed through a column of Amberlite IRA-400 (OH−) anion exchange resin (30 mL) and eluted with methanol. The UV-active fractions were collected. Three 5.00-mL samples were each diluted with water (35 mL) and titrated against 0.100 N aqueous HCl using phenolphthalein as indicator. The normality of the methylquinidinium hydroxide solution was 0.0406 N in methanol.

Table IV. Spiro[1,2-benzisothiazole-3(2*H*),3′-pyrrolidine]-2′,5′-dione 1,1-Dioxides **3**. Biological Data



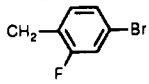
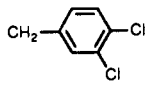
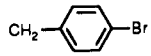
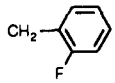
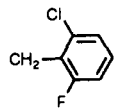
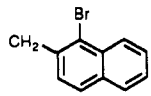
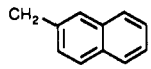
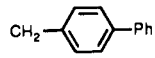
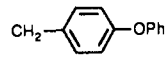
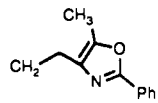
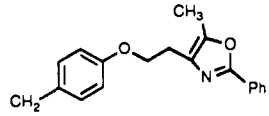
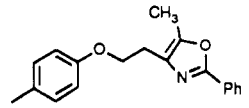
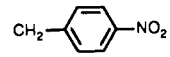
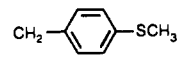
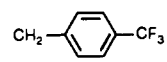
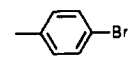
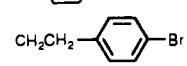
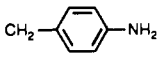
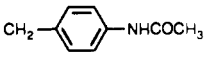
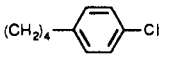
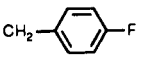
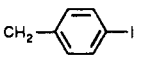
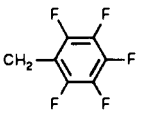
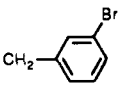
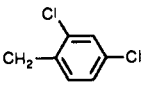
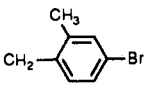
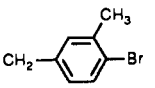
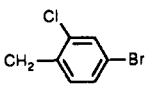
compd	R	aldose reductase inhibition:[a] % inhibition in vitro[b] at 10^{-5} M	aldose reductase inhibition:[a] % inhibition in vitro[b] at 10^{-6} M	antihyperglycemic activity:[c] % decrease in plasma glucose at 100 mg/kg per day
59	CH_3	40	*f*	*e*
60	Et	NT		*e*
61	nBu	NT		*e*
62	$CH(CH_3)_2$	NT		27 ± 3[d] at 87 mg/kg
63	$CH(CH_2CH_3)_2$	NT		31 ± 6[d]
64	CH_2CO_2H	*f*		NT
65	CH_2Ph	45	*f*	*e*
66		52	*f*	48 ± 4[d] (<15 at 20 mg/kg)
67		63	*f*	27 ± 4[d]
68		NT		37 ± 5[d]
69		NT		*e*
70		NT		17 ± 7[d]
71		59	25	*e*
72		57	20	*e*
73		54	15	*e*
74		67	24	*e*
75		NT		*e*
76		NT		*e*
77		NT		*e*
78		37	*f*	*e*
79		NT		*e*
80		34	*f*	*e*
81		*f*		*e*
82		*f*		25 ± 10

Table IV. (Continued)

compd	R	aldose reductase inhibition:[a] % inhibition in vitro[b] at 10^{-5} M	aldose reductase inhibition:[a] % inhibition in vitro[b] at 10^{-6} M	antihyperglycemic activity:[c] % decrease in plasma glucose at 100 mg/kg per day
83		NT		e
84		NT		e
85		NT		20 ± 8[d]
86		NT		e
87		NT		e
88		NT		e
89		NT		e
90		NT		19 ± 6
91		NT		e
92		NT		e
93		NT		e

[a] Compounds 66 and 71 were tested in the 4-day galactose-fed rat model (in vivo aldose reductase screen) and did not show significant reduction of galactitol levels in the three tissues (lens, nerve, and diaphragm) at 100 mg/kg per day. No other compounds were tested in vivo in this series. [b] Inhibition of enzymatic activity in a partially purified bovine lens preparation (mean of two determinations). [c] Values (mean ± SE) are percent decrease relative to vehicle-treated group with use of 4–6 db/db mice per group. [d] $p < 0.05$. [e] Less than 15% decrease at the 100 mg/kg dose. [f] No inhibitory activity at given dose; NT = not tested.

Table V. Aldose Reductase Activity of Enantiomers of 32

	aldose reductase inhibition					
				in vivo[b]		
	in vitro[a] % inhibition at		dose, mg/kg	% inhibition		
compd	10^{-6} M	10^{-7} M	per day	lens	nerve	diaphragm
(±)-32	80	37	6	NS	28	54
(+)-32	82	39	6	21	58	67
(−)-32	33	c	6	NS	NS	24

[a] Inhibition of enzymatic activity in a partially purified bovine lens preparation (mean of two determinations). [b] Inhibition of galactitol accumulation in the lens, sciatic nerve, or diaphragm of rats (n = 6) fed 20% galactose for 4 days; compounds were administered in the diet. NS = no significant inhibition of polyol accumulation ($p > 0.05$) at the given dose. [c] No inhibitory activity at given concentration.

(+)- and (−)-6-Chloro-2-methylspiro[1*H*-isoindole-1,3′-pyrrolidine]-2′,3,5′(2*H*)-trione (32). Racemic **32** (1.02 g, 3.86 mmol) was added to a solution of 0.0406 N methylquinidinium hydroxide in methanol (95.00 mL, 3.86 mmol), and the resulting solution was stirred for 10 min and then concentrated. The solid was triturated with ether, dried in vacuo, and recrystallized from 1:1 ether–acetonitrile (300 mL) to provide white needles (0.75 g). These needles were dissolved in methanol (10 mL) and treated with 10% aqueous HCl (3 mL). The resulting precipitate was filtered and washed well with water. The methanol–aqueous HCl phase was concentrated and diluted with water (20 mL) to afford a second precipitate which was filtered and washed well with water. These combined precipitates were dried in vacuo at 100 °C to afford 200 mg (20%) of the (+)-enantiomer as a white solid in 98% ee as determined by reverse-phase HPLC on a chiral column (conditions described earlier in Experimental Section). This enantiomer was the slower of the two eluting enantiomers: mp 273–275 °C; NMR, IR, and MS were identical to racemic material; $[\alpha]^{25}_D = +71.7°$ (c = 1, DMF). Anal. ($C_{12}H_9ClN_2O_3$) C, H, N.

The mother liquor from the ether:acetonitrile recrystallization contained the remaining methylquinidinium salt of **32** (approximately 1.6 g). This was concentrated and recrystallized from 55:45 ether–acetonitrile (100 mL). The mother liquor from this recrystallization was again concentrated (approximately 1.3 g) and again recrystallized from 2:1 ether–acetonitrile (30 mL). The mother liquor was concentrated and the resulting off-white solid

Table VI. ob/ob Mouse Data for 31 and 66[a]

compound	% decrease in plasma glucose[b]	% decrease in insulin[b]
31	27 ± 5	c
66	27 ± 7	c
ciglitazone	43 ± 5	39 ± 5

[a] Compounds were tested at 100 mg/kg per day × 4. [b] Values (mean ± SE) are percent decrease relative to vehicle-treated group with use of 4–6 ob/ob mice per group ($p < 0.05$). [c] Less than 15% decrease at the 100 mg/kg dose.

(1.2 g) was suspended in 10% aqueous HCl (20 mL) and stirred at room temperature for 24 h. The solid was filtered, triturated with ether, and dried in vacuo at 100 °C to afford 297 mg (29%) of the (−)-enantiomer as a white solid in 78% ee as determined by reverse-phase HPLC on a chiral column: mp 270–273 °C; NMR, IR, and MS were identical to racemic material; $[\alpha]^{25}_D$ = −55.3° (c = 1, DMF). Anal. ($C_{12}H_9ClN_2O_3$) C, H, N.

Method F. 2-[(4-Bromo-2-fluorophenyl)methyl]benzoisothiazolin-3-one 1,1-Dioxide [22, R = (4-Bromo-2-fluorophenyl)methyl]. A stirred suspension of sodium saccharin (21, 10.0 g, 48.7 mmol) and 4-bromo-2-fluorobenzyl bromide (12.9 g, 48.7 mmol) in anhydrous DMF (50 mL) was heated in a 100 °C oil bath under a dry nitrogen atmosphere for 1 hour. Dissolution occurred within 10 min, and a solid eventually reprecipitated. The reaction mixture was cooled to room temperature and was added to water (600 mL). The solid was filtered, washed well with water, and dried in vacuo to give the title compound as a white solid (17.1 g, 95%): mp 177–182 °C; NMR ($CDCl_3$, 200 MHz) δ 4.93 (s, 2 H, NCH_2), 7.35 (m, 3 H, ArH), 7.90 (m, 3 H, ArH), 8.11 (m, 1 H, ArH); IR ($CHCl_3$) 1730, 1600. Anal. ($C_{14}H_9BrFNO_3S$) C, H, N.

Method G. 2-[2-(4-Bromophenyl)ethyl]benzisothiazolin-3-one 1,1-Dioxide (22, R = (2-(4-Bromophenyl)ethyl). 4-Bromophenethylamine (10.4 g, 51.8 mmol), pyridine (5.0 mL, 62.2 mmol), and chloroform (35 mL) were cooled in an ice bath under a dry nitrogen atmosphere. Methyl 2-(chlorosulfonyl)benzoate (23, 12.2 g, 51.8 mmol) was added in six equal portions over a period of ca. 45 min. The ice bath was removed, and the thick reaction mixture was allowed to stir overnight at room temperature. The chloroform was removed, and the residue was treated twice with 10% HCl (60 mL) and once with water (100 mL). The aqueous phase was removed by decantation and extracted with ethyl acetate. The residue was also extracted with ethyl acetate. All extracts were combined, dried ($MgSO_4$), and concentrated to give the sulfonamide as a yellow solid (14.5 g, 70%) which was used immediately without further purification.

A suspension of this sulfonamide (14.3 g, 35.9 mmol), pyridine (0.58 mL, 7.17 mmol), and 4-(dimethylamino)pyridine (0.88 g, 7.17 mmol) in xylenes (121 mL) was heated to reflux under a dry nitrogen atmosphere. After 19 h additional 4-(dimethylamino)-pyridine (0.87 g, 7.17 mmol) was added and heating was continued for another 6 h. The xylenes were then removed, and the yellow solid was washed with 10% aqueous HCl and water and then dried in vacuo to provide the title compound as a yellow solid (10.3 g, 78%): NMR (DMSO-d_6, 200 MHz) δ 3.0 (t, 2 H, J = 7 Hz, NCH_2CH_2Ar), 3.95 (t, 2 H, J = 7 Hz, NCH_2CH_2), 7.24 (d, 2 H, J = 9 Hz, ArH), 7.46 (d, 2 H, J = 9 Hz, ArH), 8.05 (m, 3 H, ArH), 8.30 (m, 1 H, ArH).

Method H. General Procedure for the Preparation of 2-(Aminosulfonyl)-α-cyano-β-oxobenzenepropanoic Acid Ethyl Esters 24. Preparation of 2-[[[(4-Bromo-2-fluorophenyl)methyl]amino]sulfonyl]-α-cyano-β-oxobenzenepropanoic Acid Ethyl Ester (24, R = (4-Bromo-2-fluorophenyl)-methyl). Ethyl cyanoacetate (14.9 mL, 140 mmol) was added dropwise, over a 20-min period, to a stirred, room temperature suspension of potassium *tert*-butoxide (15.1 g, 134 mmol) in dry THF (85 mL) under a dry N_2 atmosphere. This suspension was then heated in a 60 °C oil bath for 45 min and then cooled to room temperature. To this suspension was added a solution of 22 [R = (4-Bromo-2-fluorophenyl)methyl, 16.5 g, 44.7 mmol] in dry THF (190 mL), dropwise over a 10-min period. The resulting suspension was heated to reflux temperature for 3 h and then cooled to room temperature. The reaction mixture was diluted with water (2 L), acidified to pH 1 with concentrated aqueous HCl, and then heated on a hot plate with rapid stirring for 10 min. The cooled suspension was filtered, and the white solid was washed with water and dried in vacuo to provide the title compound (19.8 g, 91%): mp 123–124 °C; NMR (DMSO-d_6, 300 MHz. Compound is in the enolic form and is a mixture of geometrical isomers. Larger peak is listed first.) δ 0.92 and 1.25 (t, 3 H, J = 7 Hz, CH_2CH_3), 3.80 and 4.21 (q, 2 H, J = 7 Hz, CH_2CH_3), 3.91 and 4.04 (s, 2 H, $NHCH_2$), 7.34 and 7.48 (m, 4 H, ArH), 7.67 (m, 2 H, ArH), 7.86 (m, 1 H, ArH), 8.05 (broad peak, 1 H, OH); IR (KBr) 3300, 2210, 1680, 1602, 1577, 1560 cm^{-1}. Anal. ($C_{19}H_{16}BrFN_2SO_5$) C, H, N.

General Procedure for the Preparation of [2,3-Dihydrobenz[*d*]isothiazol-3-ylidene]-α-cyanoacetic Acid Ethyl Ester 1,1-Dioxides 25. Preparation of [2,3-Dihydro-2-[(4-bromo-2-fluorophenyl)methyl]benz[*d*]isothiazol-3-ylidene]-α-cyanoacetic Acid Ethyl Ester 1,1-Dioxide (25, R = (4-Bromo-2-fluorophenyl)methyl). A solution of 24 ((4-bromo-2-fluorophenyl)methyl, 17.6 g, 36.4 mmol) in acetic anhydride was heated to reflux for 50 min and then cooled to room temperature. The reaction mixture was added to water and stirred for 15 min. The suspension was filtered, and the solid was washed with water and dried in vacuo. The solid product was flash chromatographed (4:1 petroleum ether:ethyl acetate on silica gel) to provide the product (12.4 g, 73%) as a white solid. A small portion was again flash chromatographed (1:1 petroleum ether–dichloromethane) to provide an analytical sample of the title compound as a white solid: mp 161–164 °C; NMR (DMSO-d_6, 300 MHz. Mixture of geometrical isomers. Larger peak listed first.) δ 1.2 and 1.3 (t, 3 H, CH_2CH_3) 4.11 and 4.35 (q, 2 H, CH_2CH_3), 5.21 and 5.42 (s, 2 H, NCH_2), 7.13 and 7.24 (t, 1 H, J = 8 Hz, ArH), 7.44 (dd, 1 H, J = 2, 6.5 Hz, ArH), 7.63 (dd, 1 H, J = 2, 8 Hz, ArH), 8.12 (m, 2 H, ArH), 8.50 (m, 1 H, ArH), 8.85 (dd, 1 H, J = 2, 5 Hz, ArH); IR (KBr) 3440 broad peak, 2200, 1710, 1590, 1565 cm^{-1}; MS (CI, *m/e*) 467 (78%), 465 (72%), 402 (12%), 400 (12%), 189 (100%), 187 (100%). Anal. ($C_{19}H_{14}BrFN_2O_4S$) C, H, N.

General Procedure for the Preparation of (3-Cyano-1,1-dioxo-2,3-dihydrobenz[*d*]isothiazol-3-yl)-α-cyanoacetic Acid Ethyl Esters 26. Preparation of [2-[(4-Bromo-2-fluorophenyl)methyl]-3-cyano-1,1-dioxo-2,3-dihydrobenz[*d*]isothiazol-3-yl]-α-cyanoacetic Acid Ethyl Ester (26, R = (4-Bromo-2-fluorophenyl)methyl). Potassium cyanide (1.40 g, 21.4 mmol) was added to a suspension of 25 (R = (4-bromo-2-fluorophenyl)-methyl, 9.5 g, 20.4 mmol) in dry DMSO (44 mL) under a dry nitrogen atmosphere. After stirring at room temperature for 1 h 25 min, the reaction mixture was diluted with water (500 mL) and extracted with ether (3 × 200 mL). The extracts were discarded. After filtering a small amount of white solid, the aqueous phase was acidified with concentrated HCl to pH 1. The resulting oil was extracted with ether (3 × 200 mL). The extracts were combined, dried over $MgSO_4$, and concentrated to give the title compound as a solid (6.83 g, 68%): NMR ($CDCl_3$, 200 MHz) δ 1.42 (t, 3 H, J = 7 Hz, CH_2CH_3), 4.26 (d, 2 H, J = 6 Hz, NCH_2), 4.46 (q, 2 H, J = 7 Hz, CH_2CH_3), 5.36 (t, 1 H, 7 Hz, $CNCHCH_2CH_3$), 7.18 (m, 3 H, ArH), 7.44 (m, 1 H, ArH), 7.70 (m, 2 H, ArH), 7.97 (m, 1 H, ArH).

General Procedure for the Preparation of Spiro[benzisothiazole-3,3′-pyrrolidine]-2′5′-dione 1,1-Dioxides 3. Preparation of 2-[(4-Bromo-2-fluorophenyl)methyl]spiro[benzisothiazole-3,3′-pyrrolidine]-2′5′-dione 1,1-Dioxide (66). A solution of 26 [R = (4-bromo-2-fluorophenyl)methyl, 6.62 g, 13.4 mmol] in dry methanol (125 mL) was cooled in an ice bath, and hydrogen chloride was passed through to saturate the solution. After 50 min the solution was allowed to warm to room temperture and left standing for 5 days. The solution was then heated to reflux for 4 h under a dry nitrogen atmosphere and cooled to room temperature. The methanol was removed, water (100 mL) was added to the solid residue, and the organics were extracted with ethyl acetate (1 × 200 mL, 2 × 100 mL). The combined extracts were dried ($MgSO_4$) and concentrated to provide a white foamy solid (6.54 g) containing 2-[(4-bromo-2-fluorophenyl)-methyl]-4′-(methoxycarbonyl)-spiro[benzisothiazole-3,3′-pyrrolidine]-2′5′-dione 1,1-dioxide [27, R = (4-bromo-2-fluorophenyl)-methyl], which was used immediately without purification.

This solid was dissolved in glacial acetic acid (114 mL) and heated to reflux for 4 h. After cooling to room temperature overnight, the reaction mixture was concentrated. The residue was partitioned between 0.5 N NaOH (100 mL) and ether (100 mL). The aqueous layer was separated and extracted again with ether (2 × 50 mL.) The aqueous layer was acidified to pH 1 with concentrated HCl, and the resulting dark oil was extracted with ethyl acetate (3 × 100 mL). The extracts were dried ($MgSO_4$), filtered, and combined with silica gel (60 mL). The solvent was removed and the adsorbate was flash chromatographed (eluant 3:2 petroleum ether–ethyl acetate to give 3.18 g of product which was triturated with ether to provide the title compound as a white solid (2.52 g, 43%): mp 120–123 °C; NMR (DMSO-d_6, 300

MHz) δ 3.3 (q, 2 H, J = 9 Hz, NCH_2), 4.54 (dd, 2 H, J = 12, 16 Hz, CH_2), 7.44 (m, 2 H, ArH), 7.55 (dd, 1 H, J = 1, 8 Hz, ArH), 7.66 (d, 1 H, J = 8 Hz, ArH), 7.73 (t, 1 H, J = 7 Hz, ArH), 7.82 (t, 1 H, J = 7 H, ArH), 7.99 (d, 1 H, J = 8 Hz, ArH), 12.05 (s, 1 H, NH); IR (KBr) 3240 broad band, 1795, 1730, 1610, 1580 cm^{-1}; MS (EI, m/e) 441 (5%), 439 (6%), 376 (7%), 374 (7%), 265 (7%), 187 (100%), 189 (96%). Anal. ($C_{17}H_{12}BrFN_2O_4S$) C, H, N.

Method I. Preparation of 2-[(2-Aminophenyl)methyl]spiro[benzisothiazole-3(2*H*),3′-pyrrolidine]-2′5′-dione 1,1-Dioxide (83). A suspension of 78 (1.60 g, 4.13 mmol) and finely ground stannous chloride dihydrate (4.70 g, 20.9 mmol) in absolute ethanol (85 mL) was heated at 72 °C for ca. 19 h. Dissolution was almost complete. The reaction mixture was allowed to cool and was filtered. The filtrate was concentrated, and water (400 mL) was added to the residual oil. A solid formed immediately, and the mixture was carefully basified with saturated aqueous sodium bicarbonate to pH 7. The organics were extracted with ethyl acetate (1 × 300 mL, 1 × 200 mL). The extracts were combined and dried (brine, Na_2SO_4) to give a yellow solid (1.42 g). The solid was adsorbed onto silica gel and flash chromatographed (9:1 methylene chloride:methanol) to give the title compound as a white solid (1.20 g, 81%): mp 137–151 °C dec: NMR (DMSO-d_6, 400 MHz) δ 3.00 (d, 1 H, J = 9 Hz, CH_2), 3.11 (d, 1 H, J = 9 Hz, CH_2), 4.11 (d, 1 H, J = 15 Hz, NCH_2Ar), 4.40 (d, 1 H, J = 15 Hz, NCH_2Ar), 5.32 (broad, 2 H, NH_2), 6.50 (d, 2 H, J = 8 Hz, ArH), 7.01 (d, 2 H, J = 8 Hz, ArH), 7.62 (d, 1 H, J = 8 Hz, ArH), 7.74 (dt, 2 H, J = 8, 10 Hz, ArH), 7.98 (d, 1 H, J = 7 Hz, ArH), 11.9 (s, 1 H, NH); IR (KBr) 3400 (broad), 1805, 1740, 1635, 1530; MS (FAB$^+$, m/e) 357 (15%), 237 (15%), 131 (35%), 91 (100%). Anal. ($C_{17}H_{15}N_3O_4S$) C, H, N.

Method J. Preparation of *N*-[4-[[2′,5′-Dioxospiro[1,2-benzisothiazole-3(2*H*),3′-pyrrolidin]-2-yl]methyl]phenyl]-acetamide *S*,*S*-Dioxide (84). Compound 83 (0.400 g, 1.12 mmol) and acetic anhydride (2 mL) were stirred together for ca. 19 h with cooling in an ice bath under a dry nitrogen atmosphere. The acetic anhydride was removed. The solid residue was treated with saturated aqueous sodium bicarbonate (5 mL) and then was filtered and air-dried. The solid was dissolved in THF, and silica gel (5 mL) was added. The solvent was removed, and the adsorbate was flash chromatographed (95:5 methylene chloride–methanol) and dried at 106 °C for 48 h to give the product as a white solid (0.18 g, 41%): mp 235–240 °C; NMR (DMSO-d_6, 400 MHz) δ 2.03 (s, 3 H, OCH_3), 3.16 (q, 2 H, J = 10 Hz, CH_2), 4.32 (d, 1 H, J = 6 Hz, NCH), 4.50 (d, 1 H, J = 6 Hz, NCH), 7.30 (d, 2 H, J = 8 Hz, ArH), 7.72 (t, 1 H, ArH), 7.79 (t, 1 H, ArH), 7.65 (d, 1 H, J = 8 Hz, ArH), 7.72 (t, 1 H, J = 8 Hz, ArH), 7.79 (t, 1 H, J = 8 Hz, ArH), 8.00 (d, 1 H, J = 8 Hz, ArH), 9.97 (s, 1 H, NH), 11.9 (s, 1 H, NH); IR (KBr) 3385, 3200, 3100, 1800, 1725, 1675, 1610, 1540 cm^{-1}; MS (Cl$^+$, m/e) 400 (21%), 336 (20%), 253 (25%), 148 (100%). Anal. ($C_{19}H_{17}N_3O_5S$) C, H, N.

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