Potent, Orally Active Aldose Reductase Inhibitors Related to Zopolrestat: Surrogates for Benzothiazole Side Chain

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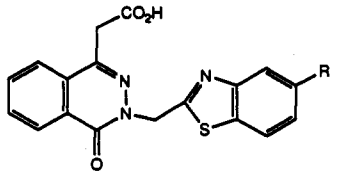
A broad structure–activity program was undertaken in search of effective surrogates for the key benzothiazole side chain of the potent aldose reductase inhibitor, zopolrestat (1). A structure-driven approach was pursued, which spanned exploration of three areas: (1) 5/6 fused heterocycles such as benzoxazole, benzothiophene, benzofuran, and imidazopyridine; (2) 5-membered heterocycles, including oxadiazole, oxazole, thiazole, and thiadiazole, with pendant aryl groups, and (3) thioanilide as a formal equivalent of benzothiazole. Several benzoxazole- and 1,2,4-oxadiazole-derived analogues were found to be potent inhibitors of aldose reductase from human placenta and were orally active in preventing sorbitol accumulation in rat sciatic nerve, in an acute test of diabetic complications. 3,4-Dihydro-4-oxo-3-[(5,7-difluoro-2-benzoxazolyl)methyl]-1-phthalazineacetic acid (**124**) was the best of the benzoxazole series ($IC_{50} = 3.2 \times 10^{-9}$ M); it suppressed accumulation of sorbitol in rat sciatic nerve by 78% at an oral dose of 10 mg/kg. Compound **139**, 3,4-dihydro-4-oxo-3-[[(2-fluorophenyl)-1,2,4-oxadiazol-5-yl]methyl]-1-phthalazineacetic acid, with $IC_{50} < 1.0 \times 10^{-8}$ M, caused a 69% reduction in sorbitol accumulation in rat sciatic nerve at an oral dose of 25 mg/kg. The thioanilide side chain featured in 3-[2-[[3-(trifluoromethyl)phenyl]amino]-2-thioxoethyl]-3,4-dihydro-4-oxo-1-phthalazineacetic acid (**195**) proved to be an effective surrogate for benzothiazole. Compound **195** was highly potent in vitro ($IC_{50} = 5.2 \times 10^{-8}$ M) but did not show oral activity when tested at 100 mg/kg. Additional structure–activity relationships encompassing a variety of heterocyclic side chains are discussed.

The role of aldose reductase (AR) mediated glucose metabolism in the etiology of diabetic complications and the therapeutic potential of aldose reductase inhibitors (ARIs) have been extensively reviewed.[1] Among the clinically important ARIs, sorbinil was the first one to enter broad scale clinical testing and it has been shown to demonstrate efficacy in diabetic painful neuropathy.[2] In a previous publication,[3] we have described the design, synthesis and pharmacological evaluation of the potent, orally active ARI, 1 (zopolrestat), which is currently being tested



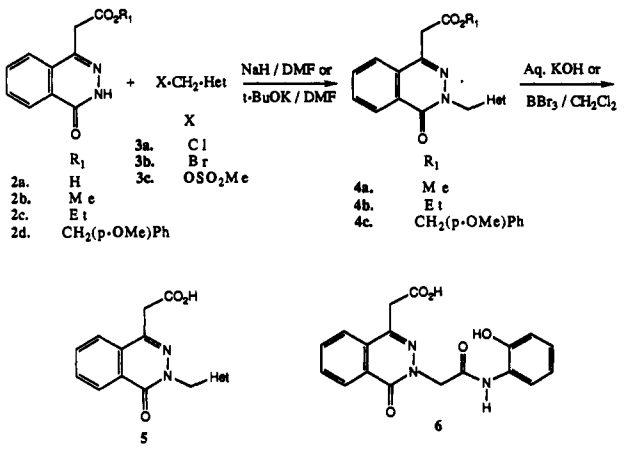
1 R = CF_3 (Zopolrestat)
1a R = H

in the clinic for treatment of diabetic complications. We have also given an extensive account of the structure–activity relationships (SAR) pertaining to 1 with a special focus on the benzothiazole side chain. In the zopolrestat series, the best combination of in vitro and in vivo potency was found in members featuring an acetic acid side chain at the 1-position of the phthalazinone ring, a methylene spacer between the phthalazinone ring and the benzothiazole side chain and 5- or 7-fluoro, -chloro, -bromo, and -trifluoromethyl or 5,7-difluoro or -dichloro substituents on the benzothiazole ring. From a medicinal chemistry perspective, we were interested in two additional important SAR issues: (1) what other heterocycles can serve as surrogates for the benzothiazole side chain and (2) can benzothiazole broadly function as an ARI potentiating group when appended to other backbones? We will address the first issue in this paper and the second in a subsequent paper (manuscript in preparation).

Chemistry

Scheme I illustrates the general method employed for the preparation of target phthalazinone acetic acids **5**.

Scheme I



Exposure of methyl or ethyl 3,4-dihydro-4-oxo-1-phthalazineacetate **2b** or **2c**[4] dissolved in dimethylform-

† Department of Medicinal Chemistry.
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(1) For recent reviews, see (a) Kador, P. F.; Robinson, W. G.; Kinoshita, J. H. The Pharmacology of Aldose Reductase Inhibitors. *Ann. Rev. Pharmacol. Toxicol.* **1985**, *25*, 691–714. (b) Kador, P. F.; Kinoshita, J. H.; Sharpless, N. E. Aldose Reductase Inhibitors: A Potential New Class of Agents for the Pharmacological Control of Certain Diabetic Complications. *J. Med. Chem.* **1985**, *28*, 841–849. (c) Harrison, H. E.; Stribling, D.; Armstrong, F. M. Aldose Reductase in the Etiology of Diabetic Complications: 1. Introduction. *J. Diabetic Complications* **1989**, *3*, 6–11. (d) Stribling, D.; Armstrong, F. M.; Harrison, H. E. Aldose Reductase Reductase in the Etiology of Diabetic Complications: 2. Nephropathy. *J. Diabetic Complications* **1989**, *3*, 70–76. (e) Stribling, D.; Perkins, C. M.; Armstrong, F. M.; Smith, J. C. Aldose Reductase in the Etiology of Diabetic Complications: 3. Neuropathy. *J. Diabetic Complications* **1989**, *3*, 139–148. (f) Stribling, D.; Armstrong, F. M.; Hardman, M.; Perkins, C. M. Aldose Reductase in the Etiology of Diabetic Complications: 4. Retinopathy. *J. Diabetic Complications* **1990**, *4*, 102–107.

(2) (a) Greene, D. A.; Porte, D.; Brie, V.; Clements, R. S.; Shamoon, H.; Ziedler, A.; Peterson, M. J.; Munster, E.; Pfeifer, M. A. Clinical Response to Sorbinil in Diabetic Neuropathy. *Diabetalogia* **1989**, 32–493A. (b) Jaspan, J.; Malone, J.; Nikolai, R.; Bergman, M. Clinical Response to Sorbinil (S) in Painful Diabetic Neuropathy. *Diabetes* **1989**, *38* (suppl. 2), 14A.

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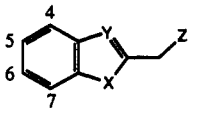
Table I. Physical Constants of 2-Halomethyl Derivatives of Benzoxazoles, Benzothiophenes, and Other 5/6-Fused Heterocycles

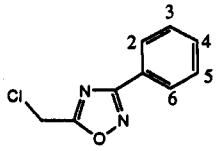


compd	X	Y	Z	subst	mp, °C; and/or ^{1}H NMR[a]
30	O	N	Br		*b*
31	O	N	Cl	5-Cl	53–56; δ 4.8 (s, 2 H), 7.3 (d, 8, 1 H), 7.5 (dd, 2 and 8, 1 H), 7.7 (d, 2, 1 H)
32	O	N	Br	5-Br	63–65; δ 4.8 (s, 2 H), 7.3–7.5 (m, 2 H), 7.9 (d, 2, 1 H)
33	O	N	Cl	5-CF_3	51–53; δ 4.78 (s, 2 H), 7.68 (s, 2 H), 8.02 (s, 1 H)
34	O	N	Br	6-Br	83; δ 4.6 (s, 2 H), 7.6 (m, 2 H), 7.8 (d, 2, 1 H)
35	O	N	Cl	5,7-F_2	33–36;[c] δ 4.7 (s, 2 H), 6.8 (m, 1 H), 7.4 (dd, 2 and 4, 1 H)
36	O	N	Cl	5,7-Cl_2	52–53; δ 4.72 (s, 2 H), 7.31 (d, 4, 1 H), 7.57 (d, 4, 1 H)
37	O	N	Br	5-phenyl	*b*; δ 4.5 (s, 2 H), 7.2–7.5 (m, 7 H), 7.8 (d, 2, 1 H)
38	O	N	Cl	5,6-benzo	*d*
39	S	CH	Cl		*b,e*; δ 4.6 (s, 2 H), 7.1–7.4 (m, 3 H), 7.5–7.8 (m, 2 H)
40	S	CH	Cl	4-Cl	63–65; 4.7 (s, 2 H), 7.1 (d, 8, 1 H), 7.2 (s, 2 H), 7.4 (d, 8, 1 H), 7.6 (dd, 6 and 8, 1 H)
41	S	CH	Cl	5-F	37–40; δ 4.85 (s, 2 H), 7.0 (m, 1 H), 7.2 (s, 1 H), 7.6 (m, 1 H), 7.8 (m, 1 H)
42	S	CH	Cl	5-Cl	*b*; δ 4.8 (s, 2 H), 7.2 (m, 2 H), 7.7 (m, 2 H)
43	S	CH	Cl	5-Br	65–67; δ 4.8 (s, 2 H), 7.1 (s, 1 H), 7.4 (d, 9, 1 H), 7.6 (dd, 4 and 9, 1 H), 7.8 (d, 4, 1 H)
44	S	CH	Cl	5-NO_2	96–99; δ 4.85 (s, 2 H), 7.4 (s, 1 H), 7.9 (d, 9, 1 H), 8.2 (dd, 4 and 9, 1 H), 8.6 (d, 4, 1 H)
45	S	CH	Cl	7-aza	47–49; δ 4.8 (s, 2 H), 7.2 (d, 2, 1 H), 7.3 (m, 1 H), 7.9 (dd, 6 and 8, 1 H), 8.5 (dd, 2 and 6, 1 H)
46	O	CH	Cl	5-Cl	4.6 (s, 2 H), 6.5 (s, 1 H), 6.8–7.5 (m, 3 H)
47	NMe	N	Cl		94–95 (lit. 95)[f]

[a] Spectra were taken in $CDCl_3$. [b] Melting point not taken. [c] Melting point of this compound in ref 7 is in error. [d] Reference 7. [e] Cf. *J. Chem. Soc. (C)* **1967**, 731. [f] *J. Am Chem. Soc.* **1943**, *65*, 1854.

amide to sodium hydride or potassium *tert*-butoxide followed by the desired heterocyclic alkylating agents **3** gave N-alkylated esters **4a** or **4b**. The specific alkylating agents used in this program and the N-alkylated esters derived from them are listed in Tables I–III and Tables IV–VI, respectively. Base-promoted hydrolysis of esters **4a** and **4b** yielded target acids **5** (Tables VII–IX). However, esters (**4a** or **4b**) with pendant Het = 2-benzoxazolyl moieties,

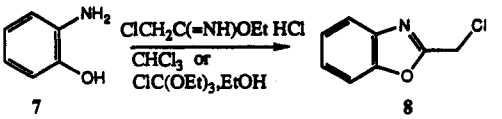
Table II. Physical Constants of (Chloromethyl)-1,2,4-oxadiazoles



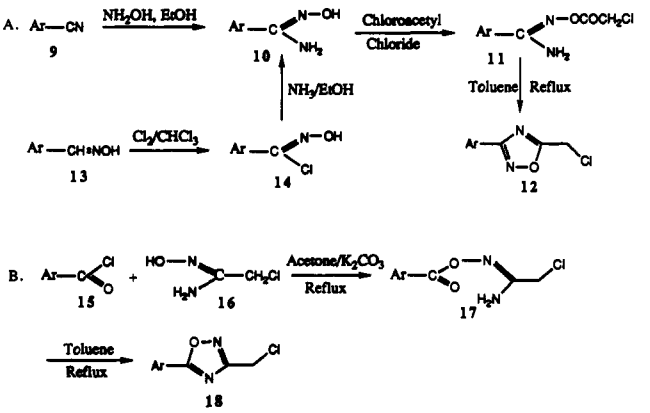
compd	subst	mp, °C; and/or ^{1}H NMR[a]
48		*b*
49	2-F	32–34; δ 4.6 (s, 2 H), 7.0–7.4 (m, 3 H), 7.9 (m, 1 H)
50	2-Cl	*b*; δ 4.8 (s, 2 H), 7.4 (m, 3 H), 7.9 (m, 1 H)
51	2-Br	*b*; δ 4.7 (s, 2 H), 7.2–7.4 (m, 2 H), 7.6–7.9 (m, 2 H)
52	2-Me	liquid; δ 2.6 (s, 3 H), 4.7 (s, 2 H), 7.3 (m, 3 H), 8.0 (m, 1 H)
53	2-CF_3	liquid; δ 4.8 (s, 2 H), 7.8 (m, 4 H)
54	2-OMe	59–62; δ 4.0 (s, 3 H), 4.7 (s, 2 H), 6.9–7.1 (m, 2 H), 7.2–7.4 (m, 1 H), 7.9 (dd, 2 and 8, 1 H)
55	4-Br	55–60; δ 4.7 (s, 2 H), 7.6 (d, 9, 2 H), 7.9 (d, 9, 2 H)
56	2,3-F_2	40–42
57	2,4-F_2	34–35; δ 4.7 (s, 2 H), 6.8–7.3 (m, 2 H), 8.1 (m, 1 H)
58	2-Cl, 6-F	*c*; δ 4.8 (s, 2 H), 6.9–7.6 (m, 3 H)
59	2-aza	*b*
60	2-Cl	liquid; δ 4.7 (s, 2 H), 7.45 (m, 3 H), 8.1 (m, 1 H)
61	2-CF_3	*c*; δ 4.6 (s, 2 H), 7.5–8.0 (m, 4 H)

[a] Spectra were taken in $CDCl_3$. [b] Reference 8. [c] Melting point not taken.

Scheme II



Scheme III



upon hydrolysis by either acid or base gave not only the expected acids **5** (Het = 2-benzoxazolyl), but also varying proportions of acids with hydroxy anilide side chains **6**, resulting from hydrolytic scission of the benzoxazole ring. It is known that, in contrast to benzothiazoles, benzoxazoles are more sensitive to ring cleavage under hydrolytic conditions.[5] Consequently, purification of the desired acids with benzoxazolyl side chains was quite difficult and cumbersome. A solution to this problem was found by

(3) Mylari, B. L.; Larson, E. R.; Beyer, T. A.; Zembrowski, W. J.; Aldinger, C. E.; Dee, M. F.; Siegel, T. W.; Singleton, D. H. Novel, Potent Aldose Reductase Inhibitors: 3,4-Dihydro-4-oxo-3-[[5-(trifluoromethyl)-2-benzothiazolyl]-methyl]-1-phthalazineacetic acid (Zopolrestat) and Congeners. *J. Med. Chem.* **1991**, *34*, 108–122.

(4) Foldeak, K. Phthalazines and Related Heterocycles. X. Derivatives of 3-Substituted 4-Phthalazon-1-ylacetic Acids. *Chem. Abstr.* **1970**, *73*, 77173y.

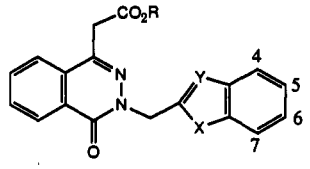
(5) Thiazoles and Their Benzo Derivatives. *Comprehensive Heterocyclic Chemistry*; Katritsky, A. R., Rees, C. W., Eds.; Pergamon Press Ltd.: Elmsford, NY, 1984; Vol. 6, pp 192, 258.

Table III. Physical Constants of Alkylating Agents Derived from Oxazoles, Thiazoles, Isothiazoles, Etc.

compd	chemical name	mp, °C; and/or ^{1}H NMR[a]
62	2-(bromomethyl)-3-phenyl-1,2,4-thiadiazole	88–91
63	2-(chloromethyl)-3-(2-chlorophenyl)-1,2,4-thiadiazole	59–62
64	2-(chloromethyl)-4-phenyloxazole	50–52[b]
65	2-(chloromethyl)-5-phenyloxazole	64–66 (lit. 64–65)[c]
66	2-(bromomethyl)-4,5-diphenyloxazole	108 (lit. 104–106)[d]
67	4-(chloromethyl)-2-phenylthiazole	53 (lit. 55.5–56)[e]
68	4-(chloromethyl)-2-(2-fluorophenyl)thiazole	140–143[f]
69	2-(hydroxymethyl)-4-phenylthiazole	80[g]
70	4-(chloromethyl)-3-phenylisothiazole mesylate	liquid; 4.5 (s, 2 H), 7.4 (m, 3 H), 7.7 (m, 2 H)
71	5-(chloromethyl)-3-phenylisothiazole	liquid; δ 4.8 (s, 2 H), 7.4 (m, 3 H), 7.7 (m, 2 H)
72	5-(chloromethyl)-2-phenyl-1,3,4-oxadiazole	116–118 (lit. 120)[h]
73	2-(chloromethyl)-7-chloroimidazo[1,2-*a*]pyridine	122–124[i]
74	3-(bromomethyl)benzisothiazole	δ 4.8 (s, 2 H), 8.0 (m, 2 H)[j]

[a] Spectra were taken in $CDCl_3$. [b] Cf. *Chem. Ber.* **1953**, *86*, 96. [c] *Il Farmaco Ed. Sci.* **1958**, *13*, 177. [d] *J. Org. Chem.* **1960**, *25*, 1151. [e] *J. Chem. Soc.* **1961**, 405. [f] Cf. U.S. Patent 4,307,105, 1981. [g] Cf. *J. Am. Chem. Soc.* **1931**, *53*, 1470 for the preparation of precursor 2-(hydroxymethyl)-4-phenylthiazole. [h] *Chem. Ber.* **1969**, *96*, 1049. [i] Cf. *Il Farmaco Ed. Sci.* **1975**, *30*, 815. [j] Cf. Gillham, Jr., R. A. Ph.D. Thesis, California Institute of Technology, 1969.

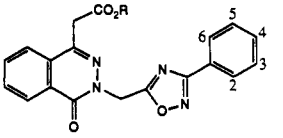
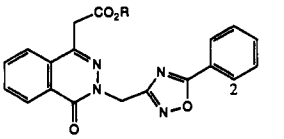
Table IV. Physical Data for Phthalazinones Esters with Benzoxazole, Benzothiophene, and Other 5/6 Fused Heterocyclic Side Chains



compd	X	Y	subst	R	mp, °C; or NMR
75	O	N		CH_2(*p*-OMe)Ph	(DMSO, 300 MHz) δ 3.8 (s, 3 H), 4.0 (s, 2 H), 5.1 (s, 2 H), 5.68 (s, 2 H), 6.8 (d, 9, 2 H), 7.0 (d, 9, 2 H), 7.3 (d, 8, 1 H), 7.25–7.82 (m, 8 H), 8.5 (dd, 2 and 6, 1 H)
76	O	N	5-Cl	Et	*a*
77	O	N	5-Br	Et	126–130
78	O	N	5-CF_3	Et	110–112
79	O	N	5-CF_3	CH_2(*p*-OMe)Ph	125–127
80	O	N	6-Br	Et	*a*
81	O	N	5,7-F_2	Et	68–69
82	O	N	5,7-F_2	CH_2(*p*-OMe)Ph	133–134
83	O	N	5,7-Cl_2	CH_2(*p*-OMe)Ph	146–147
84	O	N	5-phenyl	Et	167–170
85	O	N	5,6-benzo	Et	182–184
86	S	CH		Me	118–120
87	S	CH	4-Cl	Me	139–142
88	S	CH	5-F	Et	135–136
89	S	CH	5-Cl	Me	168–170
90	S	CH	7-aza	Me	145–146
91	O	CH	5-Cl	Me	129–131
92	NMe	N		Me	111–118

[a] Not isolated.

Table V. Physical Data for Phthalazinone Esters with 1,2,4-Oxadiazole Side Chains

compd	subst	R	mp, °C; or ^{1}H NMR
			
93		Et	*a*
94	2-F	Et	105
95	2-Cl	Et	*a*
96	2-Br	Et	($CDCl_3$, 60 MHz) δ 1.3 (t, 8, 3 H), 4.0 (s, 2 H), 4.2 (v, 8, 2 H), 7.2 (m, 2 H), 7.8 (m, 4 H), 8.4 (m, 1 H)
97	2-Me	Et	*a*
98	2-CF_3	Et	115
99	2-OMe	Et	*a*
100	4-Br	Me	160–162
101	2,3-F_2	Me	156–157
102	2,4-F_2	Et	($CDCl_3$, 60 MH) δ 1.3 (t, 8, 3 H), 4.0 (s, 2 H), 4.2 (v, 8, 2 H), 5.8 (s, 2 H), 7.3 (m, 3 H), 7.8 (m, 3 H), 8.4 (m, 1 H)
103	2-Cl, 6-F	Et	138–140
104	2-aza	Et	123
			
105	2-Cl	Et	($CDCl_3$, 60 MHz) δ 1.2 (t, 8, 3 H), 4.0 (s, 3 H), 4.2 (v, 8, 2 H), 5.6 (s, 2 H), 7.4 (m, 3 H), 7.8 (m, 4 H), 8.4 (dd, 2 and 8, 1 H)
106	2-CF_3	Et	90–93

[a] Not isolated.

starting from the *p*-methoxybenzyl ester **2d**, which was prepared by reacting a dimethylformamide solution of **2a** with triethylamine, followed by *p*-methoxybenzyl chloride. Alkylation of **2d** with 2-(halomethyl)benzoxazoles (e.g. **8**) according to Scheme I gave **4c** (Het = 2-benzoxazolyl), which upon exposure to boron tribromide in methylene chloride resulted in a smooth and exclusive scission of the *p*-methoxybenzyl ester group and gave the desired acids **5** (Het = 2-benzoxazolyl) in high yield.

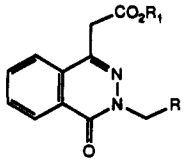
The requisite array of heterocyclic alkylating agents **3** were prepared by adapting or modifying extant literature procedures. 2-(Chloromethyl)benzoxazoles **8** were prepared by condensing 2-aminophenols **7** with 2-chloro-1,1,1-triethoxyethane[6] (Scheme II).

3-Substituted (5-(chloromethyl)-1,2,4-oxadiazoles[7] were prepared according to Scheme IIIA. Amidoximes **10**,[8]

(6) Mylari, B. L.; Scott, P. J.; Zembrowski, W. J. 2-Chloro-1,1,1-Triethoxyethane and its Use in a Versatile Synthesis of Substituted 2-Chloromethyl Heterocycles Including Benzothiazole and Benzoxazole. *Synth. Commun.* **1989**, *19*, 2921–2924.

(7) Palazzo, G.; Tavella, M.; Strani, G.; Silvestrini, B. 1,2,4-Oxadiazoles-IV. Synthesis and Pharmacological Properties of a Series of Substituted Aminoalkyl-1,2,4-Oxadiazoles. *J. Med. Pharm. Chem.* **1961**, *4*, 351–367.

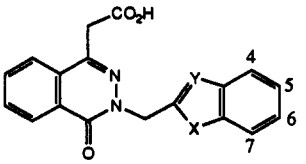
Table VI. Physical Data for Phthalazinone Esters with Oxazole, Thiazole, Isothiazole, Etc. Side Chains



compd	R	R_1	mp, °C; or ^{1}H NMR
107	3-phenyl-1,2,4-thiadiazol-5-yl	Et	($CDCl_3$, 60 MHz) δ 1.2 (t, 8, 3 H), 4.0 (s, 2 H), 4.2 (v, 8, 2 H), 5.9 (s, 2 H), 7.3 (m, 3 H), 7.8 (m, 3 H), 8.4 (m, 3 H)
108	3-(2-chlorophenyl)-1,2,4-thiadiazol-5-yl	CH_2(p-OMe)Ph	133–135
109	4-phenyl-2-oxazolyl	Et	130–131
110	5-phenyl-2-oxazolyl	Et	115–117
111	4,5-diphenyl-2-oxazolyl	Et	75
112	2-phenyl-4-thiazolyl	Et	104–106
113	2-(2-fluorophenyl)-4-thiazolyl	Et	104–107
114	4-phenyl-2-thiazolyl	Et	120–124
115	3-phenyl-4-isothiazolyl	Et	($CDCl_3$, 60 MHz), 1.2 (t, 8, 3 H), 3.9 (s, 2 H), 4.2 (v, 8, 2 H), 5.4 (s, 2 H), 7.6 (m, 7 H), 8.4 (m, 1 H), 8.8 (s, 1 H)
116	3-phenyl-5-isothiazolyl	Et	98–104
117	7-chloroimidazo[1,2,4]pyridine-2-yl	Me	188–189
118	3-benzisothiazolyl	Me	*a*

[a] Not isolated.

Table VII. Physical Constants and Aldose Reductase Inhibition Data for 3,4-Dihydro-4-oxo-phthalazine-1-acetic Acids with Benzoxazole, Benzothiophene and Other 5/6-Fused Heterocyclic Side Chains

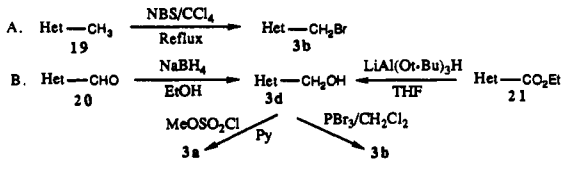


compd	X	Y	subst	formula	mp, °C	IC_{50},[a] M	inhibition of sorbitol accumulation in vivo[b] dose, mg/kg	% inhibition
				(zopolrestat)				
1						3.1×10^{-9}	10	80[b]
119	O	N		$C_{18}H_{13}N_3O_4$	156–157	8.9×10^{-8}		NT[c]
120	O	N	5-Cl	$C_{18}H_{12}ClN_3O_4$	198–200	7.8×10^{-8}	10	72
121	O	N	5-Br	$C_{18}H_{12}BrN_3O_4$	190–192	2.7×10^{-8}	10	67
122	O	N	5-CF_3	$C_{19}H_{12}F_3N_3O_4$	173–174	3.1×10^{-8}	10	32
123	O	N	6-Br	$C_{18}H_{12}BrN_3O_4$	173	5.4×10^{-7}		NT
124	O	N	5,7-F_2	$C_{18}H_{11}F_2N_3O_4$	175–176	3.2×10^{-9}	10	78
125	O	N	5,7-Cl_2	$C_{18}H_{11}Cl_2N_3O_4$	202–203	3.2×10^{-8}	10	63
126	O	N	5-phenyl	$C_{24}H_{17}N_3O_4$	116–120	6.1×10^{-6}		NT
127	O	N	5,6-benzo	$C_{22}H_{15}N_3O_4$	215–216	3.0×10^{-6}		NT
128	S	CH		$C_{19}H_{14}N_2O_3S$	189–190	1.2×10^{-7}	100	77
129	S	CH	4-Cl	$C_{19}H_{13}ClN_2N_2O_3S$	204–207	1.1×10^{-7}		NT
130	S	CH	5-F	$C_{19}H_{13}FN_2O_3S$	200–201	1.5×10^{-17}	25	49
131	S	CH	5-Cl	$C_{19}H_{13}ClN_2O_3S$	205–206	2.5×10^{-7}	10	NS
132	S	CH	5-Br	$C_{19}H_{13}BrN_2O_3S$	211–212	1.0×10^{-6}		NT
133	S	CH	5-NO_2	$C_{19}H_{13}N_3O_3S$	204	2.2×10^{-6}	10	NS[d]
134	S	CH	7-aza	$C_{18}H_{13}N_3O_3S$	201–202	4.2×10^{-7}	25	NS
135	O	CH	5-Cl	$C_{19}H_{13}ClN_2O_4$	184–185	1.5×10^{-7}		NT
136[e]	NH	CH		$C_{19}H_{14}H_3O_3$	178–180	8.7×10^{-7}		NT
137	NMe	N		$C_{19}H_{16}N_4O_3$	230 (d)	4.2×10^{-6}		NT

[a] IC_{50}s were calculated with a log linear regression analysis. Sorbinil was used as a positive control and its inhibition values, including range, are as follows: 10^{-5}, 87 ± 9%; 10^{-6} M, 70 ± 10%; 10^{-7} M, 36 ± 12%; n = 120. Its average IC_{50}, based on 120 determinations, is 3.47×10^{-7} M with SEM = 0.25×10^{-7} M. [b] Reference 3. [c] NT = not tested. [d] NS = not significant at $p < 0.05$ (Student's t test). [e] We thank our colleague Mr. H. R. Howard for preparation of this compound.

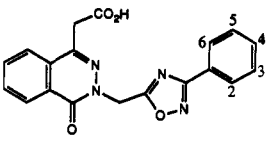
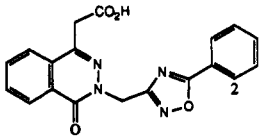
prepared from either nitriles **9** or aldoximes **13**, were condensed with chloroacetyl chloride to directly obtain the desired chloromethyl oxadiazoles **12** or to obtain intermediate O-acylated amidoximes **11**, which were subsequently cyclized by refluxing in toluene to obtain **12**. Scheme IIIB illustrates the synthetic route for the preparation of 5-substituted 3-(chloromethyl)-1,2,4-oxadiazoles **18**. This time chloromethyl amidoxime **16** was condensed with benzoyl chlorides **15** and the resulting O-acylated amidoximes **17** were cyclized in refluxing toluene to obtain the desired alkylating agents **18**.[9]

Scheme IV



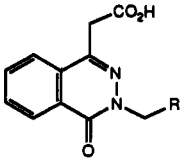
(8) Eloy, F.; Lenaers, R. The Chemistry of Amidoximes and Related Compounds. *Chem. Rev.* 1962, *62*, 155–183.

Table VIII. Physical Constants and Aldose Reductase Inhibition Data for 3,4-Dihydro-4-oxophthalazine-1-acetic Acids with 1,2,4-Oxadiazole Side Chains

compd	subst	formula	mp, °C	IC_{50},[a] M	inhibition of sorbitol accumulation in vivo[b]	
					dose, mg/kg	% inhibition
						
138		$C_{19}H_{14}N_4O_4$	202–205	1.6×10^{-7}		NT[c]
139	2-F	$C_{19}H_{13}FN_4O_4$	210–211	$<1.0 \times 10^{-8}$ [d]	25	69
140	2-Cl	$C_{19}H_{13}ClN_4O_4$	164–167	2.3×10^{-8}	10	NS[e]
					25	75
141	2-Br	$C_{19}H_{13}BrN_4O_4$	171–173	6.5×10^{-9}	10	27
142	2-Me	$C_{20}H_{16}N_4O_4$	182–184	6.4×10^{-8}	10	NS
143	2-CF_3	$C_{20}H_{13}F_3N_4O_4$	132–134	2.2×10^{-7}	25	60
144	2-OMe	$C_{20}H_{16}N_4O_5$	174–175	4.7×10^{-7}	25	NS
145	4-Br	$C_{19}H_{13}BrN_4O_4$	193–195	4.6×10^{-6}		NT
146	2,3-F_2	$C_{19}H_{12}F_2N_4O_4$	199–200	1.9×10^{-8}	25	89
147	2,4-F_2	$C_{19}H_{12}F_2N_4O_4$	219–221	6.3×10^{-7}	25	46
148	2-Cl, 6-F	$C_{19}H_{12}ClFN_4O_4$	179–182	2.4×10^{-7}		NT
149	2-aza	$C_{18}H_{13}N_5O_4$	196–200	1.56×10^{-7}	10	NS
						
150	2-Cl	$C_{19}H_{13}ClN_4O_4$	163–165	2.8×10^{-8}	25	74
151	2-CF_3	$C_{20}H_{13}F_3N_4O_4$	*f*	4.7×10^{-7}		NT

[a–c] See Table VII. [d] Compound not titrated below 10^{-8} M. [e] Not statistically significant. [f] Melting point not taken.

Table IX. Physical and Biological Data for 3,4-Dihydro-4-oxophthalazine-1-acetic Acids with Oxazole, Thiazole, Isothiazole, Etc. Side Chains



compd	R	formula	mp, °C	IC_{50},[a] M
152[b]	3-phenyl-1,2,4-thiadiazol-5-yl	$C_{19}H_{14}N_4O_3S$		2.2×10^{-6}
153	3-(2-chlorophenyl)-1,2,4-thiadiazol-5-yl	$C_{19}H_{13}ClN_4O_3S$	163–164	1.1×10^{-6}
154	2-phenyl-1,3,4-oxadiazol-5-yl	$C_{18}H_{14}N_4O_4$	260	3.1×10^{-6}
155	4-phenyl-2-oxazolyl	$C_{20}H_{15}N_3O_4$	181–184	3.2×10^{-6}
156	5-phenyl-2-oxazolyl	$C_{20}H_{15}N_3O_4$	159–162	6.6×10^{-7}
157	4,5-diphenyl-2-oxazolyl	$C_{26}H_{19}N_3O_4$	197–200	4.5×10^{-6}
158	2-phenyl-4-thiazolyl	$C_{20}H_{15}N_3O_3S$	164–165	8.2×10^{-6}
159	2-(2-fluorophenyl)-4-thiazolyl	$C_{20}H_{14}FN_3O_3S$	184–185	4.6×10^{-7}
160	4-phenyl-2-thiazolyl	$C_{20}H_{15}FN_3O_3S$	183–185	3.4×10^{-6}
161	3-phenyl-4-isothiazolyl	$C_{20}H_{15}N_3O_3S$	218	1.0×10^{-5}
162[c]	3-phenyl-5-isothiazolyl	$C_{20}H_{15}N_3O_3S$	169–170	6.7×10^{-7}
163[d]	7-chloroimidazo[1,2-*a*]pyridine-2-yl	$C_{18}H_{13}ClN_4O_3$	219–220	3.7×10^{-8}
164[e]	3-benzisothiazolyl	$C_{18}H_{13}N_2O_3S$	168	7.2×10^{-7}
165[f]	2-quinolyl	$C_{20}H_{15}N_3O_3$	193–194	5.9×10^{-8}

[a] See Table VII. [b] Compounds 150–158 and 162 were not tested in vivo. [c] Compound tested in vivo at 25 mg/kg, but was not active. [d] Compound tested in vivo at 10 mg/kg, but was not active. [e] Compound was not active in vivo at 100 mg/kg (41% inhibition of sorbitol accumulation in rat sciatic nerve). [f] We thank Dr. E. R. Larson and Mr. M. G. Evans for preparation of this compound.

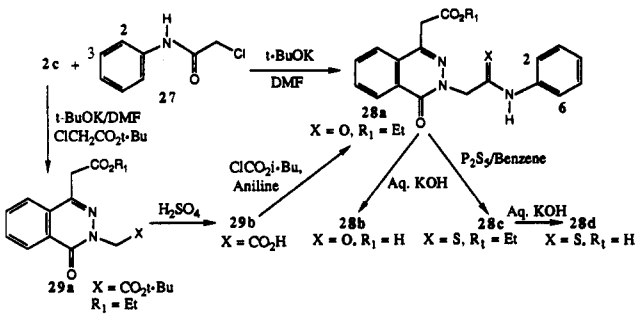
A number of other alkylating agents were prepared according to Scheme IV. Bromomethyl compounds **3b** were prepared from **19** via a standard NBS reaction (Scheme IVA). Aldehyde (**20**) or ester (**21**) derivatives of heterocycles were reduced with sodium borohydride, lithium aluminum hydride, or lithium tri-*tert*-butoxy aluminohydride to alcohols **3d**. Exposure of alcohols **3d** to 2 equiv of methanesulfonyl chloride in pyridine gave chloromethyl heterocycles **3a**. Specific starting materials ethyl benzo[*b*]thiophenecarboxylates (**22b**, X = S),[10] 2-formylthieno[2,3-*b*]pyridine (**23a**),[11] ethyl 5-chlorobenzo[*b*]furan-

(9) (a) Weidinger, H.; Kranz, J. Synthese von 1,3,4-Thiadiazolen. *Chem. Ber.* **1963**, *96*, 1059–1063. (b) Palazzo, G.; Baiocchi, L.; Picconi, G. 1,2,4-Oxadiazoles. X (1). Aryl-1,2,4-Oxadiazolecarbaldehydes. *J. Heterocycl. Chem.* **1979**, *16*, 1469–1475.

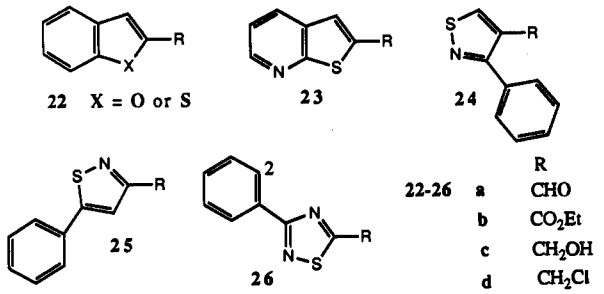
(10) (a) Iddon, B.; Scrowston, R. M. Recent Advances in the Chemistry of Benzo[*b*]thiophenes. *Adv. Heterocycl. Chem.* **1970**, *11*, 177. (b) U.S. Patent 3,910,955, Oct 7, 1975.

(11) Reichstein, T.; Oppenauer, R.; Grussner, A.; Hirt, R.; Rhyner, L.; Glatthaar, C. Synthesen von Cumaron-2-carbonsauren und von Oxy-coumaronen. *Helv. Chim. Acta* **1935**, *18*, 816–830.

Scheme V



carboxylate (**22b**, X = O, 5-chloro),[12] ethyl 3-phenyl-1,2-thiazole-4-carboxylate and ethyl 5-phenyl-1,2-thiazole-3-carboxylate (**24b** and **25b**, respectively),[13] and ethyl 3-(2-chlorophenyl)-1,2,4-thiadiazole-5-carboxylate (**26b**)[14] were prepared according to literature methods.



Phthalazinone esters with anilide and thioanilide side chains **28a** and **28c** (Scheme V) were prepared according to the method described earlier[3] (Table X). Alternatively, a more efficient method consisted of alkylating **2c** with *tert*-butyl chloroacetate to obtain the diester **29a**, which could be selectively hydrolyzed to the monoacid **29b**. Exposure of **29b** to isobutyl chloroformate followed by desired anilines gave the anilides **28a**.

Results and Discussion

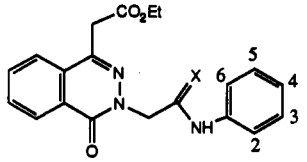
AR inhibition was measured both in vitro and in vivo. The enzyme isolated from human placenta was used for in vitro evaluation with DL-glyceraldehyde as the substrate and NADPH as the cofactor. A streptozotocin-induced diabetic rat model was used to assess the ability of orally administered compounds to prevent the high glucose induced rise in accumulation of sorbitol in the sciatic nerve. Testing protocols have been fully described elsewhere.[3] Compounds with IC_{50} at least equal to 10^{-6} M against the placental enzyme were selected for in vivo testing. Unless explicitly stated, discussion of activity or potency refers to in vitro results.

Following the discovery of zopolrestat (**1**), we undertook an expanded SAR program in order to examine the potential of other heterocycles to serve as surrogates for the benzothiazole moiety. Except for the classical replacement of -S- by vinyl group[16] there is a paucity of knowledge in the area of benzothiazole bioisosteres. Therefore, we embarked on a structure-driven approach which encompassed exploration of (1) a variety of 5/6-fused heterocycles including benzoxazole, benzothiophene, benzofuran, indole, and imidazopyridine as S and/or N replacements, (2) substituted 5-membered heterocycles, including oxadiazole, oxazole, thiazole, and thiadiazole, which may be visualized as derivatives arising from scission of one of the ring junction bonds of benzothiazole, and (3) thioanilides as ring-opened equivalents of benzothiazole.

S and/or N Replacements. Benzoxazole, benzothiophene, and benzofuran were pursued as replacements for benzothiazole to cover a wide range of lipophilicity. Their respective calculated log *P* (*C* log *P*) values are 1.43, 3.17, and 2.70.[16] Our objective was to assess how well these heterocycles could serve the role of benzothiazole in **1** in conferring AR inhibition activity and, if encouraging, to exploit SAR developed in the zopolrestat series to obtain ARIs more potent than zopolrestat.

The parent benzoxazole **119** was sufficiently potent in vitro to pursue SAR within the series which paralleled those in the benzothiazole class. As expected from our previous experience,[3] compounds with 5-Cl (**120**), -Br (**121**), and -CF_3 (**122**) and 5,7-F_2 (**124**) and 5,7-Cl_2 (**125**) substituents were as potent or more potent than **119**. Steric bulk at the 5 (cf. **126**) and 5,6 (cf. **127**) positions had an unfavorable effect on in vitro activity. In general, the benzoxazole analogues were about 5–10× less potent than the corresponding benzothiazole analogs, in vitro (**1** vs **122**). The above benzoxazoles (**120–122**, **124** and **125**) were also active in vivo by the oral route. The 5,7-F_2 analogue (**124**) showed the best activity, both in vitro and in vivo. While its efficacy in inhibiting sciatic nerve sorbitol accumulation in our diabetic rat model at 10 mg/kg was indistinguishable from that of zopolrestat at the same dose, a rigorous comparison with zopolrestat is not possible at this time because we have not done appropriate dose–response studies in both acute and chronic models[3] of diabetic complications. However, the 5-CF_3 compound (**122**) was significantly less potent than either **121** or zopolrestat. While benzoxazoles are labile to both acid and base (cf. Chemistry section), qualitatively **122** appeared to undergo faster hydrolytic cleavage to the hydroxy anilide **190**, which is practically devoid of AR inhibition activity. Therefore, we surmise that the lower than expected in vivo potency

Table X. Physical Data for Ethyl 3*H*-dihydro-4-oxo-1-phthalazine Acetates with Anilide and Thioanilide Side Chain



compd	X	subst	mp, °C
166	O		187
167	O	2-Cl	200–201
168	O	2-CF_3	208–209
169	O	3-F	180–181
170	O	3-Cl	175–176
171	O	3-CF_3	143
172	O	4-Cl	232–233
173	O	4-CF_3	196–197
174	O	2-Br, 5-CF_3	160
175	O	3,5-$(CF_3)_2$	206–207
176	O	3,4,5-$(OMe)_3$	175–176
177	S	3-Cl	109–111
178	S	3-CF_3	109–110
179	S	2-Br, 5-CF_3	140

(12) Klemm, L. H.; Merrill, R. E. Chemistry of Thienopyridines. XVIII. Lithiation as a Route to 2- and 3-Substituted Thieno[2,3-*b*]pyridines (1). *J. Heterocyclic Chem.* **1974**, *11*, 355–361.
(13) Franz, J. E.; Black, L. L. Thermolysis and Photolysis of 1,3,4-Oxathiazole-2-ones: I. *Tetrahedron Lett.* **1970**, *16*, 1381–1384.
(14) Howe, R. K.; Franz, J. E. Nitrile Sulfides. Synthesis of 1,2,4-Thiadiazoles. *J. Org. Chem.* **1974**, *39*, 962–968.
(15) Relation of Chemical Structure and Biological Activity. *Medicinal Chemistry, Part I*: Burger, A., Ed.; Wiley-Interscience: New York, 1970; p 77.
(16) MedChem Software, Release 3.54; Chemical Information Systems, Inc.: Caremont, CA, Jan 1989, p 14.1.

of 122 may be a reflection of its diminished oral bioavailability, because a portion of the administered compound could be deactivated during transit through the acidic rat stomach.

Wherever direct comparisons could be made, members of the benzothiophene series were less potent than those of the benzoxazole series. For example, 128, 131, and 132 were less potent than 119, 120, and 121. As expected, 120 was more potent than 131 in vivo. Incorporation of a N atom at the 7-position of benzothiophene led to 134, which was about as potent as the parent, 128.

The high in vitro potency of 165 is in agreement with the anticipated bioisosteric relationship between benzothiazole and quinoline.[15] The indole (136) and benzimidazole (137) analogues were less potent than either benzoxazole (119) or benzothiophene (128) analogues. Since we did not prepare the target acid derived from parent imidazo[1,2-a]pyridine, we do not have a rigorous comparison of the corresponding target with targets from other parent heterocycles, e.g., 119. However, the 7-chloroimidazo[1,2-a]pyridine congener 163 was quite potent. The only benzofuran investigated (135) was no more potent than the corresponding benzothiophene (131).

In vitro potency differences among compounds with various 5/6 heterocyclic side chains could not be solely attributed to lipophilicity differences. Compounds 123, 131, 134, and 135 with *C* log *P*s 1.63, 3.21, 1.06, and 2.72,[16] respectively, were very similar in potency. However, it is noteworthy that the two best side chains, benzothiazole and benzoxazole, are more susceptible than other side chains to any potential nucleophilic interaction at C-2,[5] by amino acid residues at the inhibitor site. Susceptibility of benzothiazole to nucleophiles is also indicated by quantum mechanical calculations.[17]

5-Membered Heterocycles. Fortuitously, the first target that we chose to pursue was oxadiazole, specifically 3-phenyl-1,2,4-oxadiazole. Other than benzoxazole, this ring system yielded the most number of highly potent compounds (Table VIII). Analogues with 2-F (139), -Cl (140), and -Br (141) and 2,3-F_2 (146) substituents on the phenyl ring were as potent as the best compounds in the benzoxazole series (cf. 139 vs 124). Substitution at the 4-position of the phenyl ring had a deleterious effect on potency (cf. 141 vs 145). This was further confirmed in 147 where addition of even a 4-F substituent to a highly potent 2-F congener 139, resulted in significant loss of potency. While all the potent oxadiazole analogues (139–141 and 146) were active in vivo, they were less potent than expected based on their in vitro potency. For example, 141 gave a low response at 10 mg/kg and while 140 was active in vivo at 25 mg/kg, it showed no significant activity at 10 mg/kg. This phenomenon may be attributed to (1) lower lipophilicity of 3-phenyl-1,2,4-oxadiazole (*C* log *P*, 0.83) relative to that of either benzoxazole or benzothiazole (*C* log *P*, 1.43 and 2.03, respectively) and/or (2) bioavailability problem. Because 1,2,4-oxadiazoles, like benzoxazoles, are susceptible to hydrolysis by acids, they may not be fully intact for absorption during passage through the gastrointestinal tract.

There was very little change in either in vitro or in vivo potency when the 1,2,4-oxadiazole ring was connected to the phthalazinone backbone through the alternate 5-position. For example, the 5-linked oxadiazoles 150 and 151 were indistinguishable from their 3-linked counterparts 140 and 143. Replacement of 1,2,4-oxadiazole by a 1,3,4-oxadiazole side chain led to a less potent compound (154 vs 138).

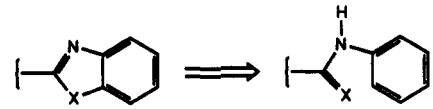


Figure 1.

Analogues with oxazole (155–157), thiazole (158–160), and isothiazole (161 and 162) side chains were moderately active when compared to benzoxazole (119) and benzothiazole (1a[3]) counterparts. The obvious difference in molecular geometry between the 5/6-fused systems and 5-membered rings with flexible side chains, particularly the extent of deviation from coplanarity between the 5-membered heterocycles and the pendant phenyl ring, could account for the gradation in potency. The potency of 1,2,4-thiadiazole analogue 152 was more akin to that of either thiazole 160 or oxazole 155, rather than to that of 1,2,4-oxadiazole 138. While this result was unexpected, further confirmation of the difference between 1,2,4-oxadiazole and 1,2,4-thiadiazole systems was obtained when 153 was found to be significantly less potent than 140. In retrospect and interestingly, 1,2,4-oxadizol-3-yl and -5-yl systems proved to be the best surrogates for the benzothiazole side chain of zopolrestat among the 5-membered heterocycles that we prepared.

Anilides and Thioanilides. A formal relationship between anilides/thioanilides with benzoxazoles/benzothiazoles can be visualized through a retrosynthetic scheme (see Figure 1). We were gratified that activity was widespread among the anilides. Several members had IC_{50}s around 10^{-8} M, especially those that were visualized as formal equivalents of 5-substituted benzoxazoles. This was also true of thioanilides (cf. 195 and 1). Two representative compounds from both the anilide (184 and 185) and thioanilide (195 and 196) series were tested by the oral route at 100 mg/kg, but were found not to be active. Poor oral absorption, a short plasma half-life due to susceptibility to amidases and/or inefficient penetration into sciatic nerve may have contributed to the observed poor oral activity. Nevertheless, the anilide and thioanilide moieties functioned well as bioisosteres of benzoxazole and benzothiazole, respectively, in inhibiting aldose reductase in vitro.

Conclusion

We have discovered that benzoxazole and 3-aryl-1,2,4-oxadiazole side chains are effective surrogates for the benzothiazole side chain of zopolrestat. Several compounds with new side chains are potent, orally active ARIs. In particular, 3,4-dihydro-4-oxo-3-[(5,7-difluoro-2-benzoxazolyl)methyl]-1-phthalazineacetic acid (124) is a highly potent ARI both in vitro and in vivo. Thioanilide moiety visualized as a retrosynthetic equivalent of benzothiazole also proved to be an effective bioisostere of benzothiazole. 3-[2-[[3-(Trifluoromethyl)phenyl]amino]-2-thioxoethyl]-3,4-dihydro-4-oxophthalazineacetic acid (195) was highly potent in vitro (IC_{50} = 5.2 × 10^{-8} M).

Experimental Section

Melting points were taken on a Thomas-Hoover melting point apparatus and are uncorrected. Structure of all new compounds were confirmed by NMR and/or MS spectra. ^{1}H NMR spectra were obtained on Bruker (AM300) or Varian (XL250 or T60) instruments. Chemical shifts are expressed in ppm downfield from internal TMS. ^{1}H NMR spectra are tabulated in the following order: chemical shift, multiplicity, coupling constant(s)

(17) McCracken, R. O.; Lipkowitz, K. B. Structure–Activity Relationships of Benzothiazole and Benzimidazole Anthelmintics: A Molecular Modeling Approach to In Vivo Drug Efficacy. *J. Parasitol.* 1990, *76*, 863–864.

in Hertz, number of protons. High-resolution mass spectra were run on a Kratos (MS30) high-resolution mass spectrometer. Satisfactory elemental analysis was obtained on all target carboxylic acids except as noted.

2-(Hydroxymethyl)thieno[2,3-*b*]pyridine (23c). To a solution of 2-formylthieno[2,3-*b*]pyridine[13] (**23a**; 1.63 g, 10 mmol) in EtOH (20 mL) was added $NaBH_4$ (190 mg, 5 mmol). After 30 min, the solution was evaporated and then was extracted with CH_2Cl_2 (50 mL). The organic extract was washed with H_2O (50 mL), dried over anhydrous $MgSO_4$ and evaporated to obtain a light amber colored liquid (87%): ^1H NMR ($CDCl_3$, 60 MHz) δ 4.9 (s, 1 H), 6.3 (b, 1 H), 6.9 (s, 1 H), 7.1 (d, 2, 1 H), 7.3 (dd, 2 and 8, 1 H), 7.9 (dd, 8 and 8, 1 H), 8.4 (dd, 6 and 6, 1 H).

2-(Hydroxymethyl)-3-phenyl-1,2,4-thiadiazole (26c). A solution of ethyl 3-phenyl-1,2,4-thiadiazolecarboxylate[14] (**26b**; 3.0 g, 13 mmol) in THF (20 mL) was added to a mixture of lithium tri-*tert*-butoxyaluminohydride (6.5 g, 26 mmol) and THF (50 mL). The reaction mixture was stirred for 30 min and then cautiously quenched with H_2O (1 mL). Et_2O (100 mL) was added to the mixture and filtered, and the filtrate was washed with H_2O (2 × 20 mL). The organic portion was collected, dried, and evaporated to obtain a yellow-orange solid. This crude solid was crystallized from cyclohexane to obtain the title product as a white solid (98%): mp, 97–100 °C; ^1H NMR ($CDCl_3$, 60 MHz) δ 5.2 (s, 2 H), 7.5 (m, 3 H), 8.2 (dd, 4 and 10, 2 H).

2-(Hydroxymethyl)-3-(2-chlorophenyl)-1,2,4-thiadiazole (cf. 26c). A solution of ethyl 3-(2-chlorophenyl)-1,2,4-thiadiazolecarboxylate (**26b**; 17 g, 63 mmol) in THF (100 mL) was added to a mixture of lithium tri-*tert*-butoxyaluminohydride (32 g, 126 mmol) and THF (225 mL). The reaction mixture was stirred for 30 min and poured cautiously over ice–water (200 mL), and a sufficient quantity of dilute HCl was added to adjust the pH to around 2.0. It was then filtered, and the filtrate was extracted with Et_2O (500 mL). The organic layer was collected, dried, and evaporated. The residue was chromatographed over silica gel and eluted with a 9:1 mixture of CH_2Cl_2 and EtOAc to obtain a tan solid (91%): mp, 87–89 °C; ^1H NMR ($CDCl_3$, 250 MHz) δ 5.2 (s, 2 H), 7.4 (m, 2 H), 7.5 (dd, 4 and 10, 1 H), 7.8 (dd, 4 and 10, 1 H).

2-(Bromomethyl)-3-(2-chlorophenyl)-1,2,4-thiadiazole (cf. 26, R = CH_2Br). Phosphorous tribromide (2 mL, 22 mmol) was added dropwise to a solution of 2-(hydroxymethyl)-3-(2-chlorophenyl)-1,2,4-thiadiazole (vide supra) (5.0 g, 22 mmol) in CH_2Cl_2 (75 mL). After 20 min the reaction mixture was cautiously added to ice–water (20 mL). The CH_2Cl_2 layer was collected and was washed successively with Na_2CO_3 solution (5%) and H_2O. The CH_2Cl_2 extract was dried and evaporated to obtain a dark red solid. It was chromatographed over silica gel. Elution with CH_2Cl_2 and evaporation of the fractions gave a white solid (40%): mp, 112 °C; ^1H NMR ($CDCl_3$, 250 MHz), δ 4.7 (s, 2 H), 7.2 (m, 3 H), 8.2 (dd, 4 and 10, 2 H).

2-(Chloromethyl)-3-(2-chlorophenyl)-1,2,4-thiadiazole (cf. 26d, R = CH_2Cl). To a solution of 2-(hydroxymethyl)-3-(2-chlorophenyl)-1,2,4-thiadiazole (vide supra) (8.08 g, 35.6 mmol) in CH_2Cl_2 (50 mL) was added pyridine (7.05 g, 89 mmol) followed by methanesulfonyl chloride (10.21 g, 89 mmol) and stirred at room temperature overnight. It was poured into H_2O (200 mL) containing concentrated HCl (5 mL), and the CH_2Cl_2 layer was collected. The CH_2Cl_2 extract was washed with H_2O (2 × 20 mL), collected, and evaporated. The resulting crude solid was purified by flash chromatography over silica gel using CH_2Cl_2 as the eluent (58%): mp, 59–62 °C; ^1H NMR ($CDCl_3$, 250 MHz) δ 5.2 (s, 2 H), 7.4 (m, 2 H), 7.5 (dd, 4 and 10, 1 H), 7.9 (dd, 4 and 10, 1 H).

Ethyl 3,4-Dihydro-4-oxo-3-[(5-bromo-2-benzoxazolyl)methyl]-1-phthalazineacetate (77). A mixture of **2c** (2.3 g, 10 mmol) and NaH (720 mg, 15 mmol) in DMF (30 mL) was stirred at room temperature for 30 min. To this was added 5-bromo-2-(bromomethyl)benzoxazole (**32**, 3.2 g, 11 mmol), and the resulting mixture was stirred for 30 min. It was then poured over ice–water (100 mL); sufficient 10% HCl was added to adjust the pH to about 4.0. The precipitated solid was extracted with EtOAc (100 mL) and the extract was dried and evaporated to obtain a gummy solid. This was chromatographed over silica gel. Elution with a 1:1 mixture of CH_2Cl_2 and EtOAc gave the product as a white solid (40%): mp, 126–130 °C; ^1H NMR ($CDCl_3$, 250 MHz) δ 1.3 (t, 8, 3 H), 4.0 (s, 2 H), 4.2 (q, 8, 2 H), 5.7 (s, 2 H), 7.6 (dd, 2 and 9, 1 H), 7.7 (d, 9, 1 H), 8.0 (m, 4 H), 8.3 (d, 8, 1 H).

3,4-Dihydro-4-oxo-3-[(5-bromo-2-benzoxazolyl)methyl]-1-phthalazineacetic Acid (121). Compound **78** (1.3 g, 2.9 mmol) was dissolved in THF (10 mL) and to it was added aqueous KOH (20%, 1 mL). The reaction mixture was stirred for 30 min at room temperature and then evaporated to dryness under vacuum. The residue was dissolved in H_2O (5 mL) and acidified with 10% HCl (1 mL) to pH of about 2.0. The precipitated solid was crystallized from benzene (77%): mp, 190–192 °C; ^1H NMR (DMSO, 300 MHz) δ 4.0 (s, 2 H), 5.68 (s, 2 H), 7.58 (dd, 9 and 2, 1 H), 7.72 (d, 9, 1 H), 8.0 (m, 4 H), 8.3 (d, 8, 1 H).

3,4-Dihydro-4-oxo-[[5-(trifluoromethyl)-2-benzoxazolyl]methyl]-1-phthalazineacetic Acid (122). The ethyl ester **79a** (0.88 g, 2.0 mmol) was hydrolyzed according to the above procedure. Analysis of the crude product by reverse-phase HPLC (Waters 990 instrument with CH_3CN, pH 7.4 buffer at a flow rate of 1 mL/min) indicated it to be a mixture of two compounds in the ratio 5.5:4.5. Separation of this mixture by thick-layer chromatography with a 9:1 mixture of EtOAc and MeOH gave two products. The less polar compound (**122**) was obtained as a white solid (32%): mp, 173–174 °C; ^1H NMR (DMSO, 300 MHz) δ 4.04 (s, 2 H), 5.73 (s, 2 H), 7.73 (dd, 1.3 and 8.5, 1 H), 7.96 (d, 9, 1 H), 7.85–8.05 (m, 3 H), 8.28 (d, 1, 1 H), 8.3 (dd, 1 and 8); ^{13}C NMR 38.248, 48.618, 112.098, 117.372, 122.596, 126.112, 126.311, 126.894, 129.403, 132.288, 133.963, 140.788, 142.501, 152.326, 158.453, 164.189, 171.133. The more polar compound (**190**) was also obtained as a white solid (40%): mp, 219–220 °C; ^1H NMR (DMSO, 300 MHz), 4.2 (s, 2 H), 5.2 (s, 2 H), 7.05 (d, 9, 1 H), 7.3 (dd, 1 and 9, 1 H), 7.85–8.05 (m, 3 H), 8.3 (dd, 1 and 6, 1 H), 8.4 (s, 1 H), 9.8 (s, 1 H).

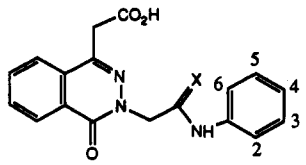
(*p*-Methoxyphenyl)methyl 3,4-Dihydro-4-oxo-1-phthalazineacetate (2d). To a solution 3,4-dihydro-4-oxo-1-phthalazineacetic acid (**2a**; 20.4 g, 0.1 mol) in DMF (100 mL) was added NaI (1.5 g, 10 mmol) followed by triethylamine (14 mL, 0.1 mol). *p*-Methoxybenzyl chloride (15.6 g, 0.1 mol) was then added, and the reaction was stirred for 3 h. The reaction mixture was poured into H_2O (50 mL) and extracted with EtOAc (2 × 50 mL). The extract was washed with aqueous Na_2CO_3 (10%) and water and then dried. Evaporation of the solvent gave a solid, which was crystallized from benzene (42%): mp, 141 °C; ^1H NMR ($CDCl_3$, 250 MHz), δ 3.8 (s, 3 H), 4.0 (s, 2 H), 5.1 (s, 2 H), 6.8 (d, 10, 2 H), 7.2 (d, 10, 2 H), 7.6 (m, 1 H), 7.8 (m, 2 H), 8.5 (dd, 2 and 8, 1 H).

(*p*-Methoxyphenyl)methyl 3,4-Dihydro-4-oxo-3-[[5-(trifluoromethyl)-2-benzoxazolyl]methyl]-1-phthalazineacetate (79). To a solution of **2d** (1.57 g, 4.8 mmol) in DMF (20 mL) was added potassium *tert*-butoxide (0.7 g, 6.3 mmol). After stirring the reaction for 30 min at room temperature, 2-(chloromethyl)-5-(trifluoromethyl)benzoxazole (**33**, 1.14 g, 4.8 mmol) dissolved in DMF (5 mL) was added to it. The reaction was stirred for another hour and was then poured into ice–water (50 mL); sufficient 10% HCl was added to adjust the pH to about 2.0. The precipitated solid was collected and purified by chromatography over silica gel. Elution with a 1:1 mixture of EtOAc and CH_2Cl_2 gave the product as a white solid (66%): mp 125–127 °C; ^1H NMR ($CDCl_3$, 300 MHz) δ 3.75 (s, 3 H), 3.98 (s, 2 H), 5.65 (s, 2 H), 6.77 (d, 6, 2 H), 7.17 (d, 6, 2 H), 7.55 (d, 2, 1 H), 7.65 (m, 1 H), 7.75 (m, 3 H), 7.94 (s, 1 H), 8.45 (dd, 6 and 2, 1 H); ^1H NMR ($CDCl_3$, 250 MHz) δ 3.75 (s, 3 H), 5.1 (s, 2 H), 5.68 (s, 2 H), 5.8 (d, 10, 2 H), 7.2 (d, 10, 2 H), 7.6 (m, 2 H), 7.7 (m, 1 H), 7.8 (m, 2 H), 7.92 (s, 1 H), 8.4 (dd, 2 and 8).

3,4-Dihydro-4-oxo-3-[[5-(trifluoromethyl)-2-benzoxazolyl]methyl]-1-phthalazineacetic Acid (122). The ester **79** (127 mg, 0.24 mmol) was dissolved in CH_2Cl_2 (15 mL) and placed in a dry ice–acetone bath. To this solution was added a solution of boron tribromide (60 mg, 0.24 mmol) in CH_2Cl_2 (5 mL). After 30 min, the reaction was allowed to warm up to room temperature and then quenched with ice–water (10 mL). The CH_2Cl_2 layer was washed with aqueous $NaHCO_3$ (10%) and water (10 mL), and the organic extract was evaporated to dryness. The resulting solid was crystallized from benzene to obtain the title product (75%): mp, 173–174 °C; ^1H NMR (DMSO, 250 MHz) δ 4.02 (s, 2 H), 5.75 (s, 2 H), 7.78 (dd, 2 and 8, 1 H), 8.0 (m, 4 H), 8.15 (s, 1 H), 8.3 (dd, 2 and 8, 1 H).

3-[2-[[2-Hydroxy-5-(trifluoromethyl)phenyl]amino]-2-oxoethyl]-3,4-dihydro-4-oxo-1-phthalazineacetic Acid (190).

Table XI. Physical and in Vitro Aldose Reductase Inhibition Data for 3,4-Dihydro-4-oxophthalazine-1-acetic Acids with Anilide and Thioanilide Side Chains



compd	X	subst	formula	mp, °C	IC_{50},[a] M
180	O		$C_{18}H_{15}N_3O_4$	203	4.9×10^{-6}
181	O	2-Cl	$C_{19}H_{14}ClN_3O_4$	192–193	4.3×10^{-7}
182	O	2-CF_3	$C_{19}H_{14}F_3N_3O_4$	208–209	5.9×10^{-6}
183	O	3-F	$C_{18}H_{14}FN_3O_4$	202–203	2.2×10^{-8}
184	O	3-Cl	$C_{18}H_{14}ClN_3O_4$	204	1.3×10^{-8}
185	O	3-CF_3	$C_{19}H_{14}F_3N_3O_4$	182–183	1.9×10^{-8}
186	O	4-Cl	$C_{18}H_{14}ClN_3O_4$	209–210	4.4×10^{-7}
187	O	4-CF_3	$C_{19}H_{14}F_3N_3O_4$	201–202	2.1×10^{-6}
188	O	2-Br, 5-CF_3	$C_{19}H_{13}BrF_3N_3O_4$	200–202	2.8×10^{-8}
189	O	3,5-$(CF_3)_2$	$C_{20}H_{16}F_6N_3O_4$	275	1.1×10^{-6}
190	O	2-OH, 5-CF_3	$C_{19}H_{14}F_3N_3O_5$	219–220	2.0×10^{-7}
191	O	2-OH, 3,5-F_2	$C_{18}H_{13}F_2N_3O_5$	202	1.7×10^{-7}
192	O	2-Br, 3,5-$(CF_3)_2$	$C_{20}H_{12}BrF_6N_3O_4$	216–217	2.2×10^{-6}
193	O	3,4,5-$(OMe)_3$	$C_{21}H_{21}N_3O_7$	163–164	*b*
194	S	3-Cl	$C_{18}H_{14}ClN_3O_3S$	179–180	5.0×10^{-8}
195	S	3-CF_3	$C_{19}H_{14}F_3O_3S$	168–169	5.2×10^{-8}
196	S	2-Br, 5-CF_3	$C_{19}H_{13}BrF_3N_3O_3S$	176–177	2.0×10^{-7}

[a] See Table VII. [b] 20% inhibition at 10^{-5} M.

Ester **78** (1.5 g, 3.76 mmol) was dissolved in a 2:1 mixture of EtOH–THF (20 mL), and aqueous KOH (10%, 5 mL) was added. After the reaction was stirred for 2 h at room temperature, excess solvents were removed and the residue was dissolved in H_2O (10 mL). To the resulting solution was added sufficient dilute HCl (10%) to adjust the pH to about 2.0. The precipitated solid was collected and crystallized from MeOH (40%): mp, 202 °C; ^1H NMR (DMSO, 250 MHz) δ 4.0 (s, 2 H), 5.1 (s, 2 H), 6.95 (m, 1 H), 7.75 (m, 1 H), 7.9 (m, 3 H), 8.45 (d, 8, 1 H), 9.95 (s, 1 H).

Preparation of 29b. To a solution of ester **2c** (46.9 g, 0.2 mol) in DMF (200 mL) was added potassium *tert*-butoxide (24.7 g, 0.22 mol) and stirred for 30 min at room temperature. To this was added *tert*-butyl bromoacetate (42.9 g, 0.22 mol), and the reaction was stirred for another hour. The mixture was poured into ice–water (500 mL), sufficient dilute HCl (10%) was added to adjust the pH to about 2.0, and then the mixture was extracted with EtOAc (2 × 200 mL). The organic extract was dried and evaporated to obtain the diester **29a** as an oil (95%). This was taken directly into the next step by dissolving it in concentrated H_2SO_4 (60 mL). After stirring the reaction for 1 h, it was cautiously poured into ice (500 g). The precipitated solid was collected and crystallized from EtOAc (68%): mp, 171 °C; ^1H NMR ($CDCl_3$, 300 MHz) δ 1.14 (t, 8, 3 H), 4.08 (s, 2 H), 4.12 (q, 8, 2 H), 4.79 (s, 2 H), 7.9 (m, 3 H), 8.32 (dd, 2 and 8, 1 H).

Ethyl 3-[2-(Phenylamino)-2-oxoethyl]-3,4-dihydro-4-oxo-1-phthalazineacetate (166). To a solution of isobutyl chloroformate (1.37 g, 10 mmol) in $CHCl_3$ (25 mL) was added triethylamine (1.01 g, 10 mmol) followed by **29b** (2.9 g, 10 mmol), and the reaction was stirred for 30 min at 0 °C. A solution of aniline (0.93 g, 10 mmol) in $CHCl_3$ (5 mL) was added to the reaction, and the reaction was slowly warmed to room temperature. Excess $CHCl_3$ was removed, and the resulting solid was crystallized from EtOH (78%): mp, 87 °C; ^1H NMR ($CDCl_3$, 300 MHz) δ 1.14 (t, 8, 3 H), 4.12 (s, 2 H), 4.2 (q, 8, 2 H), 5.0 (s, 2 H), 7.02 (m, 1 H), 7.25 (m, 2 H), 7.9 (m, 3 H), 8.39 (dd, 2 and 8, 1 H).

Ethyl 3-[2-[[3-(Trifluoromethyl)phenyl]amino]-2-oxoethyl]-3,4-dihydro-4-oxo-1-phthalazineacetate (171). Ester **2c** (4.03 g, 17.3 mmol) was alkylated according to procedure for **79**, using 3-(trifluoromethyl)-2-chloroacetanilide (cf. **27**, 4.1 g, 17.3 mmol). The desired product was crystallized from EtOH (60%): mp, 143 °C; ^1H NMR ($CDCl_3$, 250 MHz) δ 1.2 (t, 8, 3 H), 4.1 (s, 2 H), 4.25 (q, 8, 2 H), 5.45 (s, 2 H), 7.4 (m, 2 H), 7.9 (m, 4 H), 8.1 (s, 1 H), 8.2 (dd, 2 and 8, 1 H), 8.4 (dd, 2 and 8, 1 H).

Ethyl 3-[2-[[3-(Trifluoromethyl)phenyl]amino]-2-thioxoethyl]-3,4-dihydro-4-oxo-1-phthalazineacetate (178). A mixture of **171** (3.5 g, 8.1 mmol), benzene (100 mL), and phosphorus pentasulfide (7.0 g, 16.2 mmol) was heated at 70 °C for 3 h. The mixture was cooled and filtered, and the filtrate was evaporated to a crude solid. This solid was chromatographed over silica gel (eluent, 9:1 $CHCl_3$–EtOAc) to obtain the title product as a light yellow solid (45%): mp, 109–110 °C; ^1H NMR ($CDCl_3$, 250 MHz) δ 1.2 (t, 8, 3 H), 4.0 (s, 2 H), 4.2 (q, 8, 2 H), 5.4 (s, 2 H), 7.4 (m, 2 H), 7.8 (m, 3 H), 8.0 (m, 1 H), 8.1 (s, 1 H), 8.2 (dd, 2 and 8, 1 H).

Biological Methods. The procedures employed for isolation of human placental AR and in vitro AR inhibition assays have been described in our earlier publication.[3]

In vivo evaluation was conducted as follows. Rats (n = 4) were made diabetic by a single iv injection of streptozotocin (86 mg/kg). The inhibitor was then administered by oral gavage at the indicated doses at 4, 7, and 24 h. At 27 h the animals were sacrificed and the sciatic nerve and lens were removed for sorbitol determination. Inhibition is calculated on the basis of comparison to untreated diabetic animals (n = 4) and significance was calculated by using Student's t test ($p < 0.05$).

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Registry No. 2a, 25947-11-9; **2c**, 25947-13-1; **2d**, 138129-45-0; **23a**, 53174-98-4; **26b**, 50483-79-9; **26b** chloro deriv., 138128-75-3; **26c**, 138128-76-4; **26c** chloro deriv., 138128-77-5; **26d** (R = CH_2Br), 138128-78-6; **26d** (R = CH_2Cl), 138128-79-7; **29b**, 138129-46-1; **30**, 73101-74-3; **31**, 63842-22-8; **32**, 133122-57-3; **33**, 131337-75-2; **34**, 138128-80-0; **35**, 131337-74-1; **36**, 50710-33-3; **37**, 138128-81-1; **38**, 41014-41-9; **39**, 2076-88-2; **40**, 131337-71-8; **41**, 131337-70-7; **42**, 55810-81-6; **43**, 50638-17-0; **44**, 119198-20-8; **45**, 124168-58-7; **46**, 74136-78-0; **47**, 4760-35-4; **48**, 1822-94-2; **49**, 110704-45-5; **50**, 50737-32-1; **51**, 90224-62-7; **52**, 60580-24-7; **53**, 110704-47-7; **54**, 110704-43-3; **55**, 110704-42-2; **56**, 131337-73-0; **57**, 131337-72-9; **58**, 110704-44-4; **59**, 90002-06-5; **60**, 110704-33-1; **61**, 133144-89-5; **62**, 138128-82-2; **63**, 138128-83-3; **64**, 110704-37-5; **65**, 64640-13-7; **66**, 81819-14-9; **67**, 4771-31-7; **68**, 138128-84-4; **69**, 65384-99-8; **70**, 138128-85-5; **71**, 138128-86-6; **72**, 33575-83-6; **73**, 124168-59-8; **74**, 59057-83-9; **75**, 138128-87-7; **76**, 138128-88-8; **77**, 138128-89-9; **78**, 131337-21-8; **79**, 138128-90-2; **80**, 138128-91-3; **81**, 131337-22-9; **82**, 138128-92-4; **83**, 138128-93-5; **84**, 138128-94-6; **85**, 110722-33-3; **86**, 131337-48-9; **87**, 138128-95-7; **88**, 138128-96-8; **89**, 110703-92-9; **90**, 124168-24-7; **91**, 131337-54-7; **92**, 138128-97-9; **93**, 138128-98-0; **94**, 110722-41-3; **95**, 138128-99-1; **96**, 110722-39-9; **97**, 138129-00-7; **98**, 110704-54-6; **99**, 138129-01-8; **100**, 138129-02-9; **101**, 131337-26-3; **102**, 138129-03-0; **103**, 110722-38-8; **104**, 110704-53-5; **105**, 138128-99-1; **106**, 138129-04-1; **107**, 138129-05-2; **108**, 138129-06-3; **109**, 110703-88-3; **110**, 110703-87-2; **111**, 138129-07-4; **112**, 131337-45-6; **113**, 131337-46-7; **114**, 110703-91-8; **115**, 110703-81-6; **116**, 110703-80-5; **117**, 138129-08-5; **118**, 110703-79-2; **119**, 110722-34-4; **120**, 110722-35-5; **121**, 110749-07-0; **122**, 131337-23-0; **123**, 138129-09-6; **124**, 131337-24-1; **125**, 110722-36-6; **126**, 138129-10-9; **127**, 110722-37-7; **128**, 131337-35-4; **129**, 131337-40-1; **130**, 131337-37-6; **131**, 110703-78-1; **132**, 131337-39-8; **133**, 131337-38-7; **134**, 124168-21-4; **135**, 131337-42-3; **136**, 138151-13-0; **137**, 110703-66-7; **138**, 110722-43-5; **139**, 110703-57-6; **140**, 110749-08-1; **141**, 110703-55-4; **142**, 110722-45-7; **143**, 110721-48-7; **144**, 110722-46-8; **145**, 110722-44-6; **146**, 131337-28-5; **147**, 131337-27-4; **148**, 110749-09-2; **149**, 110703-54-3; **150**, 131337-29-6; **151**, 138129-11-0; **152**, 138129-12-1; **153**, 138129-13-2; **154**, 110703-63-4; **155**, 110703-74-7; **156**, 110703-73-6; **157**, 110703-64-5; **158**, 131337-32-1; **159**, 131337-33-2; **160**, 110703-77-0; **161**, 112065-65-3; **162**, 110703-62-3; **163**, 138129-14-3; **164**, 110703-60-1; **165**, 110703-59-8; **166**, 138129-15-4; **167**, 138129-16-5; **168**, 138129-17-6; **169**, 138129-18-7; **170**, 138129-19-8; **171**, 138129-20-1; **172**, 138129-21-2; **173**, 138129-22-3; **174**, 138129-23-4; **175**, 138129-24-5; **176**, 138129-25-6; **177**, 138129-26-7; **178**, 138129-27-8; **179**, 138129-28-9; **180**, 138129-29-0; **181**, 138129-30-3; **182**, 138129-31-4; **183**, 138129-32-5; **184**, 138129-33-6; **185**, 138129-34-7; **186**, 138129-35-8; **187**, 138129-36-9; **188**, 138129-37-0; **189**, 138151-14-1; **190**, 138129-38-1; **191**, 138129-39-2; **192**, 138129-40-5; **193**, 138129-41-6; **194**, 138129-42-7; **195**, 138129-43-8; **196**, 138129-44-9; aldose reductase, 9028-31-3; *p*-methoxybenzyl chloride, 824-94-2; 3-(trifluoromethyl)-2-fluoroacetanilide, 2339-83-5.