Eur J Med Chem (1992) 27, 779–789

Synthesis and aldose reductase inhibitory activity of substituted 2(1*H*)-benzimidazolone- and oxindole-1-acetic acids

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(Received 10 December 1991; accepted 17 March 1992)

Summary — Potent *in vitro* inhibition of the enzyme aldose reductase (AR) was observed with several members of a series of 3-alkylated 2(1*H*)-benzimidazolone-1-acetic acids, as well as with analogs from a structurally-related series of oxindole-1-acetic acids with 3-alkyl or 3-alkylidene substituents. Intrinsic activity against AR was, in general, greatest in compounds from the second series, especially with analogs which contain alkylidene side chains, with typical IC_{50} values of ≤ 1 μM. However, in a streptozotocin-diabetic rat model, the best compounds from either series failed to prevent sorbitol accumulation in lens or sciatic nerve to the degree observed with AR inhibitors such as ponalrestat or zopolrestat.

aldose reductase / diabetes / 2(1*H*)-benzimidazolone-1-acetic acid / oxindole-1-acetic acid

Introduction

An increase in the flux of glucose through the polyol pathway, associated with diabetic hyperglycemia, is implicated in the development of chronic complications in diabetes, especially neuropathy, retinopathy, nephropathy and cataract formation [1–3]. A key component of this process is aldose reductase (AR, EC 1 1 1 21), an NADPH-dependent enzyme which converts glucose to sorbitol and consequently leads to the cellular damage associated with the above conditions [4, 5]. Efficient inhibition of AR should minimize such cell damage, resulting in an improvement in the duration and quality of life for many diabetic patients [6–12].

The structural classes which display the most potent AR inhibitory activity are predominantly those in which the compounds contain an acidic functional group, although non-acidic compounds have been reported [13, 14]. In addition to hydantoins (imidazolidin-2,4-diones) such as sorbinil and imirestat [15–19], oxazolidin-2,4-diones [20], thiazolidin-2,4-diones [21] and some phenols [22, 23], the most extensively studied class of compounds which possesses intrinsic activity contains a carboxylic acid moiety [24–29]. Agents such as ponalrestat, tolrestat and more recently zopolrestat (fig 1) have received considerable attention due to their ability to suppress the activity of AR both *in vitro* and *in vivo* and they have undergone extensive study to determine their clinical efficacy [30–37]. Indeed, tolrestat is currently marketed and/or approved in several countries outside the US for the treatment of diabetic complications.

We prepared the two series described in this paper to explore the effect of ring modification on activity in our previously discovered group of quinoxalinone-1-acetic acid AR inhibitors [38]. Interestingly, all three

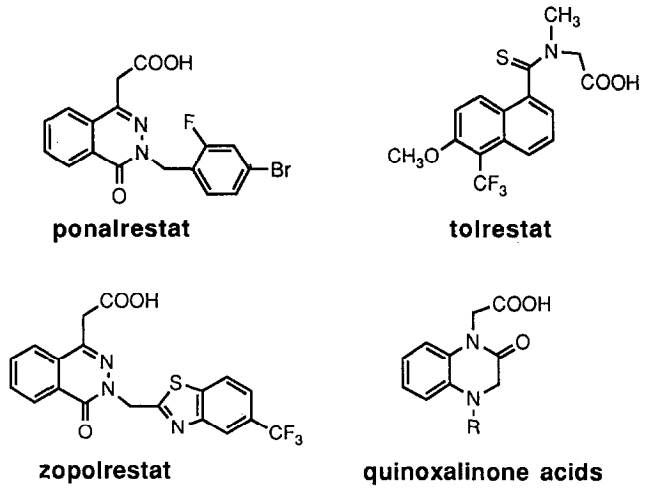


Fig 1. Formulae for ponalrestat, tolrestat, zopolrestat and quinoxalinone acids.

series contain an *N*-aryl glycine moiety and an adjacent carbonyl group, two components proposed by Kador *et al* as essential for interaction with the enzyme [39].

Chemistry

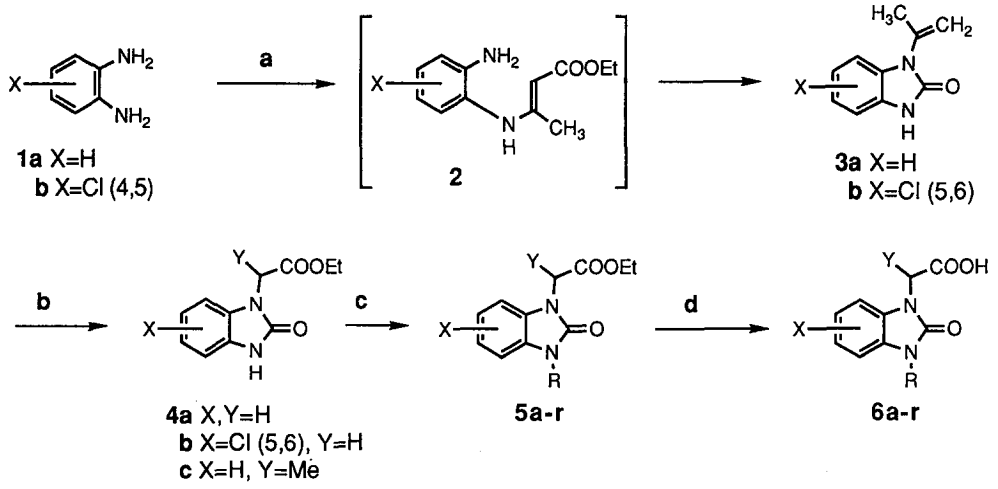
The synthesis of the benzimidazolone series was performed as depicted in scheme 1. Beginning with a suitably substituted *o*-phenylenediamine **1**, the conversion to the isopropenyl intermediate **3** could be achieved in excellent yield using freshly distilled ethyl acetoacetate according to the procedure of Davoll *et al* [40]. Alkylation of **3** with ethyl bromoacetate (or ethyl 2-bromopropionate) in ethanolic sodium ethoxide, followed by cleavage of the labile isopropenyl group, provided sufficient **4** for subsequent conversion to the desired esters **5** and the corresponding acids **6**.

The preparation of the oxindole acetic acids was accomplished *via* two general processes. As shown in scheme 2, the 3,3-bis-substituted oxindoles and related spiro analogs were prepared by alkylation of an *N*-acetyl oxindole **8** [41–44], deacetylation of **9** to give **10**, N-alkylation with benzyl bromoacetate and hydrogenation over palladium on carbon. The acids **12** were purified by conversion to their dicyclohexylammonium salts. The 3-monoalkylated oxindole acids **15** were prepared, as shown in scheme 3, by condensation of oxindoles **7** with the requisite aldehydes, alkylation of the intermediate alkylidenes **13** with benzyl bromoacetate (or benzyl 2-bromopropionate) and hydrogenation of the esters **14** to generate the desired acids. Alternatively, the sodium salt of **13** was treated with *tert*-butyl bromoacetate and the resulting ester **14** was hydrolyzed with trifluoroacetic acid to give acid **16**, retaining the double bond. Compound **12d** was prepared in a more lengthy manner (scheme 4) which, nevertheless, employed the same processes to incorporate the desired substituents.

All of the chiral compounds (**6k**, **12c–d**, **15h**, **17g**) thus prepared were tested as their racemic mixtures. The configuration of the individual alkylidene acids **16a–f** (or their corresponding esters) was assigned based upon their chromatographic behavior, the *cis*-(*Z*) isomer being less polar than their corresponding *trans*-(*E*) isomer [45]. These assignments were confirmed using ^{1}H-NMR spectral data; *eg*, esters **14a** (fig 2) display a slight down-field shift of the vinyl proton singlet in the *trans* isomer relative to that of the *cis* isomer (δ 7.53 *vs* 7.42, respectively).

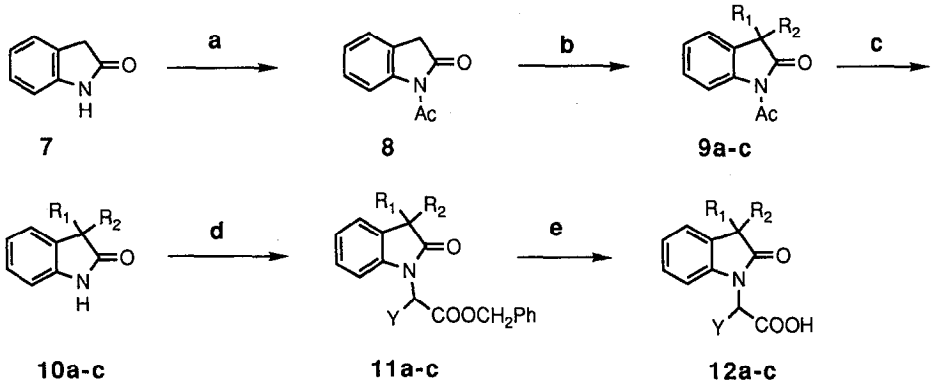
Pharmacology

These compounds were evaluated for their *in vitro* inhibitory activity against aldose reductase isolated from human placenta (or, in the case of compounds **6i**, **6p** and **6q**, from bovine lens) as well as for their ability to inhibit sorbitol accumulation in lens and sciatic nerve of a streptozotocin-diabetic rat model after oral administration [46].



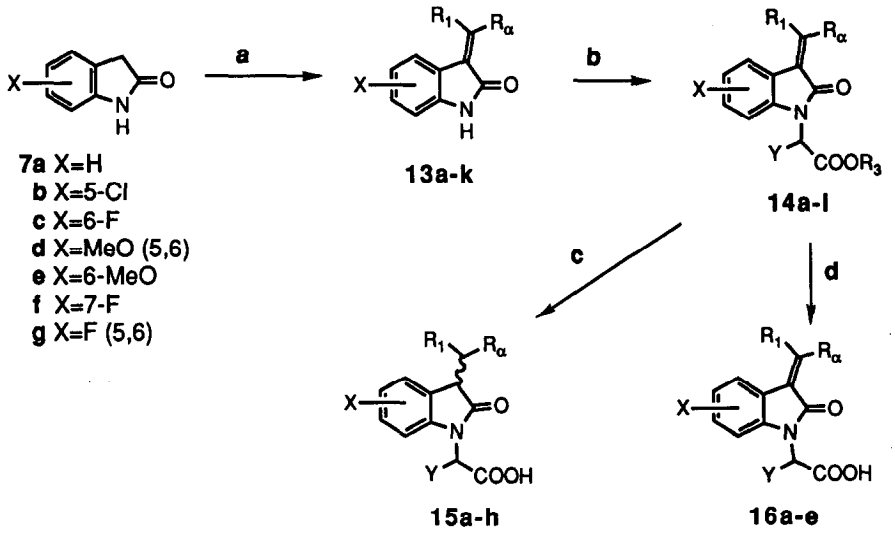
a) ethyl acetoacetate, xylenes, reflux b) BrCH(Y)COOEt (Y=H, Me), NaOEt
c) RZ (R=aralkyl, benzothiazolylmethyl; Z=Cl, Br), NaH, DMF d) NaOH, aq EtOH.

Scheme 1.



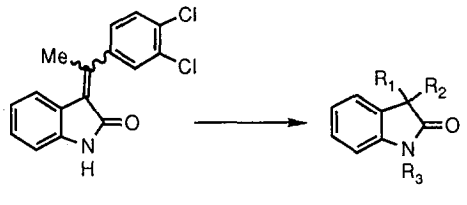
a) Ac_2O, reflux **b**) aralkyl bromide, K_2CO_3, DMF **c**) NaOH, aq. MeOH, reflux
d) $BrCH_2COOCH_2Ph$, NaH, DMF **e**) H_2, Pd/C, EtOAc, HOAc

Scheme 2.



a) $R_1C(O)R_\alpha$ (R_α=H,Me), MeOH, pyrrolidine, reflux **b**) $BrCH(Y)COOR_3$, NaH, DMF
c) H_2, Pd/C, EtOAc **d**) CF_3COOH, 25°C

Scheme 3.



	R_1	R_2	R_3
18	CH(Me)Ph(3,4-Cl_2)	H	H
19	"	H	Ac
20	"	Me	Ac
10d	"	Me	H
11d	"	Me	$CH_2CO_2CH_2\phi$
12d	"	Me	CH_2CO_2H

Scheme 4.

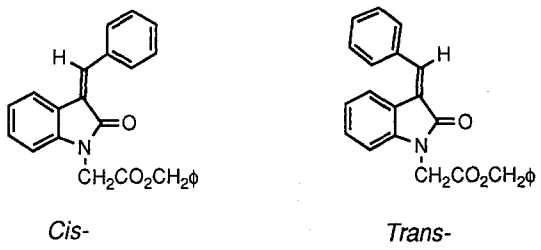


Fig 2. Esters **14a**.

Biological results and discussion

The biological data are summarized in tables II, IV and V. There are many potent *in vitro* inhibitors of aldose reductase among the benzimidazolone derivatives (table II), the most potent ones being **6f**, **6i**, **6q** and **6r**, with IC_{50}'s well below 1 μM. It is apparent that lipophilic groups such as substituted benzyl and naphthylmethyl enhance activity, while halogen substitution in the benzimidazole ring enhances potency in the non-halogenated derivatives **6a** and **6l**. Testing of representative compounds in the streptozotocin-diabetic rat model proved disappointing: only compounds **6f** and **6r** showed weak, but statistically significant effects on sorbitol accumulation in the sciatic nerve, none significantly influenced lens sorbitol levels. Clearly, none of these compounds approach the *in vivo* activity of ponalrestat or zopolrestat.

In the oxindole series (table IV) there is an even greater number of potent *in vitro* inhibitors of aldose reductase. While dibenzyl substitution (*eg*, **12a**) is detrimental to activity, the 3-mono substituted compounds or the spiro-compound **12b** generally show good inhibition of aldose reductase. Alkyl substitution α to the carboxylate (*ie*, **15h**) reduces binding affinity approximately 30-fold, but the same modification to the benzyl moiety produces no change in activity. The arylidene derivatives **16a–f** are particularly potent and approach or surpass the potency of ponalrestat [47] and zopolrestat [37]; no difference was detected between *cis* and *trans* isomers (*ie*, **16b** and **16c**) when tested separately. *In vivo*, acids **12b** and **15c** (table IV), show modest *in vivo* effects on sciatic nerve sorbitol, whereas **15a** reduces lens sorbitol accumulation without apparent effect on sciatic nerve sorbitol levels. Clearly, at the 25 mg/kg oral dose level employed, these compounds are less efficacious AR inhibitors than either ponalrestat or zopolrestat, or such non-carboxylic acid AR inhibitors as sorbinil [37].

We were disappointed to observe that the compounds prepared did not express the level of *in vivo* activity we had expected based on their observed *in vitro* potency. This poor efficacy may be a consequence of poor pharmacokinetics (poor absorption, poor tissue penetration or a short half-life) [48]. Since poor pharmacokinetics of aldose reductase inhibitors have been correlated with low pK_a [48], we prepared compounds **17a–g** listed in table V as potentially less acidic bioisosteres of compound **6f**. However, none of these agents is as potent as **6f** *in vitro*, and the one compound tested *in vivo* (**17b**) shows only modest activity at 100 mg/kg *po*.

Conclusion

We have discovered potent *in vitro* inhibitors of aldose reductase in a series of substituted 2(1*H*)-benzimidazolone- and oxindole-1-acetic acids. Potency in this series is greatly enhanced by substituents such as halogenated benzyl [48] and benzothiazolylmethyl [37] which are known to lead to good activity in the phthalazinone series. The SAR in the current series suggests that these compounds may interact at a binding site similar to that of the phthalazinone series, but that additional factors (*eg*, poor pharmacokinetics) prevent them from being superior as orally active AR inhibitors.

Experimental protocols

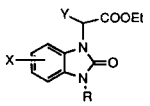
Chemistry

Melting points were determined on a Thomas–Hoover melting point apparatus using open capillary tubes and are uncorrected. Chromatography was performed using Merck Kieselgel 60 (F254, 0.25 mm) plates for TLC or Woelm silica gel (32–63 μm) for flash chromatography. ^{1}H-NMR spectra were recorded on Varian T-60, Bruker WM-250 or Bruker AM-300 spectrometers using tetramethylsilane as an internal standard when required. Chemical shifts are expressed in units (ppm) and the splitting patterns are abbreviated as follows: s: singlet; d: doublet; t: triplet; q: quartet; m: multiplet. Mass spectra were obtained using a Finnigan 4510 mass spectrometer for low resolution and an AEI MS-30 instrument for high resolution determinations. Microanalyses were performed by the Pfizer analytical department and agree within ± 0.4% of the calculated values unless otherwise noted. Reagent grade materials were purchased from commercial sources and were used without further purification (except as noted).

Method A. Preparation of N-isopropenyl benzimidazolones. 1-Isopropenyl-2(1H)-benzimidazolone ***3a***

In a 1-l round-bottomed flask fitted with a Dean-Stark trap, a mixture of *o*-phenylenediamine (65 g, 0.60 mol, Aldrich Chem Co) in 300 ml xylenes under N_2 was refluxed while freshly distilled ethyl acetoacetate (100 g, 0.77 ml) was added dropwise over a 30-min period. After 3 h, at which point *ca* 12 ml H_2O had collected in the trap, the clear solution was allowed to cool to an almost solid mass. After filtering and washing with xylenes (100 ml) and hexanes (500 ml) the resulting white

Table I. Physical constants of benzimidazoline esters.



No	*X*	*Y*	*R*	*Yield (%)*	*mp (°C)*	*Solv*[a]	*Formula*
5a	H	H	$C_6H_5CH_2$	43	122–124	b	$C_{18}H_{18}N_2O_3$
b	H	H	4-$MeC_6H_4CH_2$	79	103–105	1	$C_{19}H_{20}N_2O_3$
c	H	H	4-$ClC_6H_4CH_2$	75	134–137	b	$C_{18}H_{17}ClN_2O_3$
d	H	H	4-$MeOC_6H_4CH_2$	75	123–125	2	$C_{19}H_{20}N_2O_4$
e	H	H	2,4-$Cl_2C_6H_3CH_2$	50	124–126	3	$C_{18}H_{16}Cl_2N_2O_3$
f	H	H	3,4-$Cl_2C_6H_3CH_2$	45	124–126	4	$C_{18}H_{16}Cl_2N_2O_3$
g	H	H	4-Br, 2-$FC_6H_3CH_2$	94	113–115	b	$C_{18}H_{16}BrFN_2O_3$
h	H	H	(1-Naphthyl)CH_2	47	116–118	5	$C_{22}H_{20}N_2O_3$
i	H	H	(2-Naphthyl)CH_2	81	Oil	b	$C_{22}H_{20}N_2O_3$
j	H	H	2,4-$Cl_2C_6H_3COCH_2$	18	Oil	b	$C_{19}H_{16}N_2O_4$
k	H	Me	3,4-$Cl_2C_6H_3CH_2$	84	Oil	b	$C_{19}H_{18}Cl_2N_2O_3$
l	H	H	(2-BTZ)[c]CH_2	98	116–119	b	$C_{19}H_{17}N_3O_3S$
m	H	H	(6-Cl-2-BTZ)CH_2	90	133–135	b	$C_{19}H_{16}ClN_3O_3S$
n	H	H	(6-MeO-2-BTZ)CH_2	85	128–130	b	$C_{20}H_{19}N_3O_4S$
o	5,6-Cl_2	H	$C_6H_5CH_2$	32	Oil	b	$C_{18}H_{16}Cl_2N_2O_3$
p	5,6-Cl_2	H	4-$ClC_6H_4CH_2$	21	Oil	b	$C_{18}H_{15}Cl_3N_2O_3$
q	5,6-Cl_2	H	3,4-$Cl_2C_6H_3CH_2$	69	152–154	3	$C_{18}H_{14}Cl_4NO_3$
r	5,6-Cl_2	H	(2-BTZ)CH_2	48	Oil	b	$C_{19}H_{15}Cl_2N_3O_3S$

[a]Recrystallization solvents: 1. hexane; 2. aqueous EtOH; 3. EtOH; 4. EtOAC: hexane; 5. Et_2O: hexane; [b]Chromatographed on silica gel (230–400 mesh); [c]2-BTZ = 2-benzothiazolyl.

needles were dried *in vacuo* at 25°C to give **3a** (56.4 g, 54%), mp 110–112°C (lit mp 121°C [40]*); TLC (R_f 0.65, $CHCl_3$: CH_3OH, 9:1).

Similarly, 4,5-dichloro-*o*-phenylenediamine was converted to 4,5-dichloro-1-isopropenyl-2(1*H*)-benzimidazolone **3b**, mp 232–235°C dec.

Method B. Preparation of N-alkanoic acid esters

Ethyl 2-oxo-11-benzimidazolineacetate ***4a***. Under N_2 in a flame-dried flask, 200 ml EtOH was treated with Na metal (3.96 g, 0.172 mol) followed by **3a** (30 g, 0.172 mol). After 30 min at 25°C, the solution was treated with ethyl bromoacetate (26.7 ml, 0.24 mol), refluxed for 3 h and allowed to cool to room temperature overnight. Sulfuric acid (18 N, 17.5 ml) was added to produce a thick precipitate which was filtered, washed with cold water (200 ml) and air-dried to a white solid, **4a** (16.3 g, 43%), mp 173–175°C (mp 175–176°C [49]**).

In the same manner, **3b** was converted to ethyl 5,6-dichloro-2-oxo-1-benzimidazolineacetate, **4b** (48%), mp 277–278°C dec.

Ethyl 2-(2-oxo-1-benzimidazoline)propionate ***4c***. Under N_2, **3a** (0.348 g, 0.002 mol) in 10 ml of dry DMF was treated with NaH (0.096 g, 0.002 mol, 50% oil dispersion) at 25°C. After 15 min, ethyl 2-bromopropionate (0.362 g, 0.002 mol) was added and stirring was continued for 30 min. The resulting solution was poured into 100 ml H_2O and extracted with EtOAc. The organic extract was dried ($MgSO_4$), concentrated *in vacuo* to an oil and refluxed with 1.0 ml concentrated H_2SO_4 for 3 h. This was then cooled and evaporated to dryness, and the residue was redissolved in EtOAc, washed with 1 N HCl, 1 N NaOH and finally H_2O and then dried with $MgSO_4$. The EtOAc was removed *in vacuo* to give crude **4c** as an oil (0.308 g, 66%).

Method C. Alkylation of benzimidazoline esters. Ethyl 3-benzyl-2-oxo-1-benzimidazolineacetate ***5a***

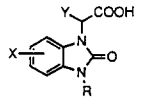
Under N_2, a solution of **4a** (0.880 g, 0.004 mol) in 20 ml dry DMF at 25°C was treated with NaH (0.184 g, 0.004 mol, 50% oil dispersion). After 15 min, benzyl bromide (0.684 g, 0.004 mol) was added, the reactants were stirred for a further 30 min and finally poured over 300 ml H_2O. The resulting solids were filtered, washed well with H_2O and air-dried. Recrystallization from EtOH gave pure **5a** (0.535 g, 43%), mp 122–124°C. Anal $C_{18}H_{18}N_2O_3$ (C, H, N).

In the same manner, the benzimidazolinone esters of table I (**5b–r**) were prepared from **4a–c**. The oxindole esters of table III (**11a–d**, **14a-l**) were similarly prepared from intermediates **10a–d** *(Method F)* and **13a–k** *(Method G)*, respectively, using benzyl or *t*-butyl bromoacetate in lieu of benzyl bromide.

*Similar results were obtained using gold label (99+%, Aldrich Chem Co) ethyl acetoacetate without further purification.

**Obtained as an oil (bp 128–137°C (0.6 mm Hg)).

Table II. Physical and biological data for 3-substituted benzimidazoline-1-acetic acids.



No	*X*	*Y*	*R*	*Yield (%)*	*mp (°C)*	*Solv*[a]	*Formula*[b]	*IC_{50}*[c] *(μM)*	*In vivo (% inhibition)*[d] *Dose (mg/kg)*	*Nerve*	*Lens*
6a	H	H	$C_6H_5CH_2$	60	200–202	2	$C_{16}H_{14}N_2O_3$	2.7 (0.6–12)			
b	H	H	4-$MeC_6H_4CH_2$	92	196–198	4	$C_{17}H_{16}N_2O_3$	3.9 (1.2–13)			
c	H	H	4-$ClC_6H_4CH_2$	40	201–203	2	$C_{16}H_{13}ClN_2O_3$	0.9 (0.3–3)			
d	H	H	4-$MeOC_6H_4CH_2$	36	200–202	2	$C_{17}H_{16}N_2O_4 \cdot 0.25H_2O$	1.9 (0.6–6)			
e	H	H	2,4-$Cl_2C_6H_3CH_2$	63	210–212	2	$C_{16}H_{12}Cl_2N_2O_3$	1.2 (0.4–3)			
f	H	H	3,4-$Cl_2C_6H_3CH_2$	73	183–185	2	$C_{16}H_{12}Cl_2N_2O_3$	0.15 (0.07–0.3)	25	29*	
g	H	H	4Br-2F-$C_6H_3CH_2$	42	194–196 dec	2	$C_{16}H_{12}BrFN_2O_3 \cdot 0.5H_2O$	1.0 (0.5–2)	100	16	26
h	H	H	(1-Naphthyl)CH_2	53	208–210	4	$C_{20}H_{16}N_2O_3$	1.0 (0.4–3)			
i	H	H	(2-Naphthyl)CH_2	23	209–211	3	$C_{20}H_{16}N_2O_3$	0.12 (0.03–0.4)[e]	25	8	
j	H	H	2,4-$Cl_2C_6H_3COCH_2$	21	230–232 dec	2	$C_{17}H_{12}Cl_2N_2O_4 \cdot 0.25H_2O$	3.4 (1–11)	25	14	
k	H	Me	3,4-$Cl_2C_6H_3CH_2$	87	176–178	4	$C_{17}H_{14}Cl_2N_2O_3$	34 (7–154)	25	9	
l	H	H	(2-BTZ)[f]CH_2	7	205–206 dec	3	$C_{17}H_{13}N_3O_3S \cdot H_2O$	0.8 (0.3–2)	100	0	12
m	H	H	(6-Cl-2-BTZ)CH_2	84	258–260 dec	6[g]	$C_{17}H_{12}ClN_3O_3S \cdot 0.25H_2O$	0.8 (0.3–2.4)			
n	H	H	(6-MeO-2-BTZ)CH_2	70	217–218 dec	3	$C_{18}H_{15}N_3O_4S \cdot 0.25H_2O$	3.5 (1.1–12)			
o	5,6-Cl_2	H	$C_6H_5CH_2$	55	211–213	4	$C_{16}H_{12}Cl_2N_2O_3 \cdot 0.5C_2H_5OH$	1.0 (0.3–3)			
p	5,6-Cl_2	H	4-$ClC_6H_4CH_2$	20	213–216	4	$C_{16}H_{11}Cl_3N_2O_3$	1.9 (0.4–9)[e]	14	0	
q	5,6-Cl_2	H	3,4-$Cl_2C_6H_3CH_2$	72	212–214	4	$C_{16}H_{10}Cl_4N_2O_3$	0.18 (0.05–0.6)[e]	25	25	
r	5,6-Cl_2	H	(2-BTZ)CH_2	15	261–264	7[g]	$C_{17}H_{11}Cl_2N_3O_3S \cdot 0.25H_2O$	0.25 (0.09–0.7)	50	21*	0

[a]See footnote [a] in table I; [b]Analyses for C, H, N were within ± 0.4% of theoretical values except where noted; [c]IC_{50} with 95% confidence limits for the inhibition of human placental aldose reductase (HPAR). The standard agents sorbinil (s), ponalrestat (p) and zopolrestat (z) showed the following IC_{50} (M) values: (s) $3.47 \times 10^{-7} \pm 0.02 \times 10^{-7}$ ($n = 120$), (p) $1.10 \times 10^{-7} \pm 0.01 \times 10^{-7}$ ($n = 6$) and (z) $4.90 \times 10^{-8} \pm 0.08 \times 10^{-8}$ ($n = 46$); [d]The percentage inhibition of sorbitol accumulation *versus* untreated diabetic controls in nerve and lens of streptozotocinized rat. Streptozotocin (85 mg/kg, iv) was administered at 0 h, the test compounds were given orally at 4, 7 and 24 h at the doses indicated and sorbitol was assayed at 27 h. Inhibition values for ponalrestat in three separate tests were 44, 47 and 57% in the sciatic nerve at 5 mg/kg and ED_{50} values for sorbinil and zopolrestat were 4.4 mg/kg and 3.6 mg/kg, respectively. In instances where the value was < 0, the value is presented as zero; [e]These values are for compounds tested *vs* bovine lens aldose reductase (BLAR). IC_{50} (M) values for sorbinil using preparations of HPAR and BLAR were $3.47 \times 10^{-7} \pm 0.02 \times 10^{-7}$ ($n = 120$) and $3.10 \times 10^{-7} \pm 1.00 \times 10^{-7}$ ($n = 3$), respectively; [f]See footnote [c] in table I; [g]6. $CHCl_3$: EtOH; 7. $CHCl_3$; *Significantly different from untreated diabetic controls at $P < 0.05$.

Method D. Preparation of benzimidazolone alkanoic acids. 3-Benzyl-2-oxo-1-benzimidazolineacetic acid ***6a***

A solution of **5a** (0.425 g, 0.0014 mol) in 10 ml EtOH was treated with 1.4 ml 1 N NaOH and 2.0 ml H_2O at room temperature. After stirring for another 30 min the solution was diluted with 5 ml H_2O and acidified with 3 N HCl to precipitate the crude acid (0.345 g, 88%), mp 200–203°C. Recrystallization from EtOH produced pure **6a**, mp 200–202°C. Anal $C_{16}H_{14}N_2O_3$ (C, H, N).

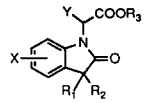
The same procedure was employed to produce the remaining acids **6b–r** (table II).

Method E. Alkylation of N-acetyl oxindoles. 1-Acetyl-3,3-bis-(3,4-dichlorobenzyl)oxindole monohydrate ***9a***

A mixture of *N*-acetyloxindole [41] (**8**, 5.0 g, 0.0306 mol), K_2CO_3 (9.8 g, 0.071 mol) and KI (11.6 g, 0.07 mol) in 80 ml dry DMF was stirred under N_2 at 25°C while a solution of 3,4-dichlorobenzyl chloride (8.9 ml, 0.0643 mol) in 20 ml dry DMF was slowly added. After stirring for 72 h at room temperature, the mixture was diluted with Et_2O, washed with H_2O and saturated aqueous NaCl and dried over $MgSO_4$. Concentration *in vacuo* provided **9a** as the monohydrate (5.75 g, 38%), mp 177–179°C; MS, *m/e* 497(M^{+4}), 495(M^{+2}), 493(M^+). Anal $C_{24}H_{17}Cl_4NO_2 \cdot H_2O$ (C, H, N).

By a similar procedure, the following compounds were also made (yield, mp, recrystallization solvent): 1'-acetyl-1,2-dihydro-spiro[inden-2,3'-indolin]-2'-one hemihydrate (**9b**, 87%, 159°C dec, DMF-H_2O); 1'-acetyl-5(6)-chloro-1,2-dihydro-spiro[inden-2,3''-indolin]-2'-one (**9c**, 57%, 108–110°C, DMF-H_2O; by replacing 3,4-dichlorobenzyl chloride with 1 equiv of 1,2-bis(bromomethyl) benzene and with 1 equiv of 1,2-bis(bromomethyl)-4-chlorobenzene [50]) respectively.

Table III. Physical constants for oxindole-1-acetic acid esters.



No	*X*	*R_1*	*R_2*	*R_3*	*Y*	*Yield (%)*	*Solv*[a]	*mp (°C)*	*Formula*
11a	H	3,4-$Cl_2C_6H_3CH_2$	3,4-$Cl_2C_6H_3CH_2$	CH_2Ph	H	48	[b]	Oil[c]	$C_{31}H_{23}Cl_4NO_3$
b	H	1,2-($CH_2C_6H_4CH_2$)		CH_2Ph	H	93	3	141–143	$C_{25}H_{21}NO_3 \cdot 0.5H_2O$
c	H	4-Cl-1,2-($CH_2C_6H_3CH_2$)		CH2Ph	H	48	7	136–140	$C_{25}H_{20}ClNO_3$
d	H	3,4-$Cl_2C_6H_3CHMe$	Me	CH_2Ph	H	79	[b]	Oil[d]	$C_{26}H_{23}Cl_2NO_3$
14a	H	*cis*-3,4-$Cl_2C_6H_3CH$ =		CH_2Ph	H	52	7	190–192	$C_{24}H_{17}Cl_2NO_2 \cdot H_2O$
	H	*trans*-3,4-$Cl_2C_6H_3CH$ =		CH_2Ph	H	34	7	160–162	$C_{24}H_{17}Cl_2NO_2 \cdot 0.5H_2O$
b	5-Cl	3,4-$Cl_2C_6H_3CH$ =		CH_2Ph	H	82	8	196–198	$C_{24}H_{16}Cl_3NO_3 \cdot 0.25H_2O$
c	6-F	3,4-$Cl_2C_6H_3CH$ =		CH_2Ph	H	78	9	198–200	$C_{24}H_{16}Cl_2FNO_3 \cdot 0.5H_2O$
d	5,6-$(OMe)_2$	3,4-$Cl_2C_6H_3CH$ =		CH_2Ph	H	93	9	213–215	$C_{26}H_{21}Cl_2NO_5 \cdot 0.5H_2O$
e	6-OMe	3,4-$Cl_2C_6H_3CH$ =		CH_2Ph	H	83	9	146–148 dec	$C_{25}H_{19}Cl_2NO_4$
f	H	3,4-$Cl_2C_6H_3CMe$ =		CH_2Ph	H	18	[b]	120–122	$C_{25}H_{19}Cl_2NO_3$
g	H	(2-BTZ)CH =		*t*-Bu	H	97	[b]	144–146 dec	$C_{22}H_{20}N_2O_3S$
h	5-Cl	(2-BTZ)CH =		*t*-Bu	H	99	[b]	200–202 dec	$C_{22}H_{19}ClN_2O_3S$
i	6-F	(2-BTZ)CH =		*t*-Bu	H	99	[b]	169–171 dec	$C_{22}H_{19}FN_2O_3S$
j	7-F	(2-BTZ)CH =		*t*-Bu	H	58	[b]	Gum[e]	$C_{22}H_{19}FN_2O_3S$
k	5,6-F_2	(2-BTZ)CH =		*t*-Bu	H	90	[b]	Gum[f]	$C_{22}H_{18}F_2N_2O_3S$
l	H	3,4-$Cl_2C_6H_3CH$ =		CH_2Ph	Me	13	[b]	Gum[g]	$C_{25}H_{19}Cl_2NO_3$

[a]Recrystallization solvents, see footnote [a] in table I. Also: 7. $CHCl_3$, 8. EtOAc, 9. DMF: H_2O; [b]see footnote [b] in table I; [c]high resolution mass spectrum: calcd 597.0730; found 597.0556; [d]MS (%), *m/e* 467 (5, M^+), 295 (100), 204(73); [e]MS (%), *m/e* 410 (17, M^+), 310 (27), 309 (100), 57 (92); [f]MS (%), *m/e* 428 (1, M^+), 297 (5), 168 (29), 57 (100); [g]high resolution mass spectrum: calcd 451.0742; found 451.0727.

Method F. Deacetylation of N-acetyl oxindoles. 3,3-bis-(3,4-Dichlorobenzyl)oxindole ***10a***

A mixture of **9a** (7.8 g, 0.0158 mol) in 100 ml CH_3OH was treated with 100 ml 4% NaOH and heated for 15 min in a steam bath. The resulting solution was cooled to 25°C, acidified to pH 2 with concentrated HCl and the solids were filtered. The crude yellow solid product was washed well with H_2O and dried *in vacuo* for 18 h to give **10a** (6.2 g, 87%), mp 175–178°C; MS, *m/e* 451(M^{+2}), 449(M^+). Anal $C_{22}H_{15}Cl_4NO$.

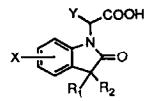
By a similar method, the following compounds were prepared (yield, mp, recrystallization solvent): 1,2-dihydro-spiro[inden-2,3'-indolin]-2'-one hemihydrate (**10b**, 90%, 186–189°C, EtOH-H_2O); 5(6)-chloro-1,2-dihydrospiro[inden-2,3'-indoline]-2'-one (**10c**, 61%, 160–163°C dec, high resolution mass spectrum: calcd. 271.0577; found 271.0546 (^{37}Cl); and 3-(3,4-dichloro-α-methylbenzyl)-3-methyloxindole (**10d**, 97%, gum, ^1H-NMR ($CDCl_3$, δ) 1.17–1.47 (m, 6H), 3.23 (q, 1H), 6.70–7.42 (m, 7H), 8.72 (s, 1H)).

Method G. Alkylidene formation. 3-(3,4-Dichlorobenzylidene)-oxindole ***13a***

To a well stirred suspension of oxindole (12.5 g, 0.091 mol) and 3,4-dichlorobenzaldehyde (16.0 g, 0.091 mol) in 30 ml CH_3OH was added 7.6 ml pyrrolidine in a dropwise manner (caution–exothermic!). The mixture was heated on a steam bath for 15 min, cooled to room temperature and the orange solid product filtered. After washing with 30 ml cold CH_3OH the solids were dried *in vacuo* to give pure **13a** (21.4 g, 81%), mp 183–186°C. Anal $C_{15}H_{19}Cl_2NO$ (C, H, N).

Using the same method, the following 3-substituted oxindoles were prepared (yield, mp, recrystallization solvent): 5-chloro-3-(3,4-dichlorobenzylidene) (**13b**, 82%, 272–275°C, CH_3OH); 3-(3,4-dichlorobenzylidene)-6-fluoro hemihydrate, (**13c**, 84%, 217–220°C, CH_3OH); 3-(3,4-dichlorobenzylidene)-5,6-dimethoxy (**13d**, 88%, 248–250°C dec, CH_3OH); 3-(3,4-dichlorobenzylidene)-6-methoxy (**13e**, 85%, 189–191°C, CH_3OH); 3-(3,4-dichloro-α-methylbenzylidene) (**13f**, 82%, 233–235°C, CH_3OH); 3-(2'-benzothiazolylmethylidene) (**13g**,

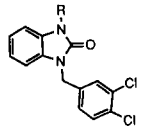
Table IV. Physical and biological data for oxindole-1-acetic acids.



No	*X*	R_1	R_2	*Y*	*Yield*[a]	*mp (°C)*	*Solv*[b]	*Formula*[c]	IC_{50} [d] (μM)	*In vivo (% inhibition)*[e] *dose (mg/kg)*	*Nerve*	*Lens*
12a	H	3,4-$Cl_2C_6H_3CH_2$	3,4-$Cl_2C_6H_3CH_2$	H	65	185–186	5	$C_{24}H_{17}Cl_4NO_3$•$C_{12}H_{23}N$	149 (18–1240)			
b	H	1,2-($CH_2C_6H_4CH_2$)		H	13	185–187	3	$C_{18}H_{15}NO_3$•$C_{12}H_{23}N$	0.9 (0.3–3)	100	21*	0
c	H	4-Cl-1,2-($CH_2C_6H_3CH_2$)		H	89	135–136 dec	11	$C_{18}H_{14}ClNO_3$•$C_{12}H_{23}N$ •0.5H_2O	0.5 (0.2–1)			
d	H	3,4-$Cl_2C_6H_3CH_2$	Me	H	72	151–153 dec	10	$C_{19}H_{17}Cl_2NO_3$•$C_{12}H_{23}N$	4.1 (1.4–11)			
15a	H	3,4-$Cl_2C_6H_3CH_2$	H	H	73	168–171	2	$C_{17}H_{13}Cl_2NO_3$	0.3 (0.1–1)	100	2	38*
b	5-Cl	3,4-$Cl_2C_6H_3CH_2$	H	H	18	148–150	3	$C_{17}H_{12}Cl_3NO_3$•$C_{12}H_{23}N$	0.9 (0.3–3)			
c	6-F	3,4-$Cl_2C_6H_3CH_2$	H	H	28	157–160	5	$C_{17}H_{12}Cl_2FNO_3$•$C_{12}H_{23}N$	0.5 (0.2–2)	100	29*	0
d	5,6-$(MeO)_2$	3,4-$Cl_2C_6H_3CH$	H	H	17	222–223 dec	10	$C_{19}H_{17}Cl_2NO_5$•$C_{12}H_{23}N$	6.4 (0.8–51)			
e	6-MeO	3,4-$Cl_2C_6H_3CH_2$	H	H	81	182–183	10	$C_{18}H_{15}Cl_2NO_4$•$C_{12}H_{23}N$	1.1 (0.4–3)	100	20	3
f	H	3,4-$Cl_2C_6H_3CMeCH_2$	H	H	50	175–177 dec	2	$C_{18}H_{15}Cl_2NO_3$	0.3 (0.15–0.6)	100	0	0
g	6-OH	3,4-$Cl_2C_6H_3CH_2$	H	H	65	141–144 dec	10	$C_{17}H_{13}Cl_2NO_4$•$C_{12}H_{23}N$	1.4 (0.5–4)	100	29	7
h	H	3,4-$Cl_2C_6H_3CH_2$	H	Me	57	94–96 dec	10	$C_{18}H_{15}Cl_2NO_3$•$C_{12}H_{23}N$ •0.5H_2O	95 (12–784)			
16a	H	(2-BTZ) CH = [f]		H	31	257–259 dec	12	$C_{18}H_{12}N_2O_3S$•0.25H_2O	0.14 (0.05–0.4)	25	0	0
b	5-Cl	(2-BTZ) CH = [f]		H	47	276–278 dec	2	$C_{18}H_{11}ClN_2O_3S$•0.5H_2O	0.10 (0.04–0.3)	25	26	17
c	5-Cl	(2-BTZ) CH = [g]		H	26	192–195 dec	2	$C_{18}H_{11}ClN_2O_3S$•0.75H_2O	0.12 (0.05–0.3)	25	23	0
d	6-F	(2-BTZ) CH = [f]		H	73	285–287 dec	2	$C_{18}H_{11}FN_2O_3S$	0.05 (0.02–0.1)	25	13	10
e	7-F	(2-BTZ) CH = [f]		H	65	249–251 dec	2	$C_{18}H_{11}FN_2O_3S$•0.75H_2O	0.13 (0.05–0.3)	25	0	12
f	5,6-F_2	(2-BTZ) CH = [f]		H	52	198–202 dec	2	$C_{18}H_{10}F_2N_2O_3S$•0.5H_2O	0.10 (0.03–0.3)	25	19	10
Ponalrestat[e]									[d]	[e]		
Zopolrestat[e]									[d]	[e]		

[a]Compounds **12a–d** and **15a–h** were prepared *via Method H*, **16a–f** *via Method I*; [b]for recrystallization solvents 1–9 see footnote [a] in tables I and III and footnote [e] in table II. Additional solvents: 10. Et_2O; 11. EtOH: pentane; 12. toluene:pentane; [c]see footnote [b] in table II; [d]see footnote [c] in table II; [e]see footnote [d] in table II; [f]*cis* isomer; [g]*trans* isomer; *significantly different from untreated diabetic controls at $P < 0.05$.

Table V. Physical and biological data for replacement of the carboxylate group in compound **6f**.



No	*R*	*Yield (%)*	*mp (°C)*	*Formula*[a]	IC_{50}[b] (μM)
17a	$CH_2CONHOCH_3$	28	213–215	$C_{17}H_{15}Cl_2N_3O_3$	163 (19–1360)
b	$CH_2CONHOH$	29	187–189 dec	$C_{16}H_{13}Cl_2N_3O_3$	3 (1–9)[c]
c	$CH_2CON(OH)CH_3$	38	208–211 dec	$C_{17}H_{15}Cl_2N_3O_3$	79 (10–653)
d	CH_2CONH-G_1§	26	172–174	$C_{21}H_{16}Cl_2N_4O_2$	29 (4–240)
e	CH_2CONH-G_2§	11	291–293	$C_{20}H_{16}C1_2N_4O_2S$	57 (16–200)
f	CH_2CONH-G_3§	8	270–272 dec	$C_{17}H_{13}Cl_2N_7O_2$•0.5H_2O	32 (4–263)
g	$CH_2CH(OH)CONH_2$	43	158–160	$C_{17}H_{15}Cl_2N_3O_3$	[d]
6f	CH_2COOH				0.15 (0.07–0.3)

[a]See footnote [b] in table II; [b]see footnote [c] in table II; [c]at 100 mg/kg *po*, this compound produced an 18% (NS) inhibition of nerve sorbitol and 23% (NS) inhibition of lens sorbitol accumulation in the rat. [d]not calculated; at 10^{-5} M the inhibition of enzyme was 3%; NS = not statistically significant; § G_1 = 2-pyridyl; G_2 = 3-methyl-5-isothiazolyl; G_3 = 5-tetrazolyl.

58%, 231–234°C, CH_3OH); 3-(2'-benzothiazolylmethylidene)-5-chloro (**13h**, 63%, >285°C); 3-(2'-benzothiazolylmethylidene)-6-fluoro (**13i**, 50%, 273–276°C dec); 3-(2'-benzothiazolylmethylidene)-7-fluoro (**13j**, 46%, 293–295°C dec); 3-(2'- benzothiazolylmethylidene)-5,6-difluoro (**13k**, 29%, 265–267°C).

Method H. Hydrogenation of benzyl esters. 3-(3,4-Dichlorobenzyl)oxindole-1-acetic acid ***15a***

A suspension of *cis*-3-(3,4-dichlorobenzylidene)oxindole-1-acetic acid benzyl ester (**14a**, 2.28 g, 0.0052 mol), prepared by *Method C*, and 1 g of 5% Pd on carbon in 50 ml EtOAc was treated with 2 ml AcOH and hydrogenated at atmospheric pressure until H_2 uptake ceased (*ca* 3 h). A TLC ($CHCl_3$:CH_3OH, 9:1) showed only the desired product at R_f 0.05. After filtering through diatomaceous earth the solvent was evaporated *in vacuo* to a yellow foam which crystallized from EtOH:H_2O, giving **15a** as a white solid (1.325 g, 73%), mp 168–171°C; MS, *m/e* 351(M^{+2}), 349(M^+). Anal $C_{17}H_{13}Cl_2NO_3$ (C, H, N).

Compounds **12a–d**, **15b–f** and **15h** (from **14l**) were prepared in the same manner (table IV). Their dicyclohexylammonium salts were prepared by treatment of an Et_2O solution of the acid with dicyclohexylamine in Et_2O; the salts thus formed would often precipitate as an analytically pure substance or would require a single recrystallization to meet purity requirements.

3-(3,4-Dichlorobenzyl)-6-hydroxyoxindole-1-acetic acid dicyclohexylamine salt ***15g***

A solution of 3-(3,4-dichlorobenzyl)-6-methoxyoxindole-acetic acid (**15e**, 1.8 g, 0.00473 mol) in 60 ml CH_2Cl_2 was cooled to 0°C and treated with 9.5 ml (0.0095 mol) of 1 M boron tribromide in CH_2Cl_2. After 15 min at 0°C the reaction was allowed to stir for 1 h at 25°C to give a red solution. At this point an additional 10 ml of the BBr_3 solution was added, the reactants were stirred overnight at 25°C and then poured over 100 ml ice water. The aqueous layer was extracted with EtOAc and the combined organic layers were washed with H_2O and saturated NaCl. After drying ($MgSO_4$) the solvent was removed *in vacuo* to an orange oil (1.7 g). The oil was redissolved in 150 ml Et_2O and treated with dicyclohexylamine (2.4 ml, 0.0118 mol) to produce the title compound as a crystalline white salt (1.7 g, 65%), mp 141–144°C dec. Anal $C_{17}H_{13}Cl_2NO_4$•$C_{12}H_{13}N$•0.5H_2O (C, H, N).

Method I. Hydrolysis of t-butyl esters. 3-(2-Benzothiazolylmethylidene)oxindole-1-acetic acid ***16a***

Under N_2 a solution of 3-(2-benzothiazolylmethylidene)oxindole-1-acetic acid *tert*-butyl ester (**14g**, 1.00 g, 0.0025 mol) in 20 ml CF_3COOH was stirred at 25°C for 48 h, after which a TLC ($CHCl_3$:CH_3OH, 9:1) showed the product at R_f 0.1. After dilution with $CHCl_3$ the organics were washed (H_2O, saturated aqueous NaCl), dried ($MgSO_4$) and concentrated *in vacuo* to an orange solid. Recrystallization from toluene:pentane gave **16a** as the quarterhydrate (0.26 g, 31%), mp 257–259°C dec; MS, *m/e* 337(M^{+1}), 336(M^+). Anal $C_{18}H_{12}N_2O_3S$•0.25H_2O (C, H, N).

Compounds **16b–f** (table IV) were similarly prepared. The *trans* isomer **16c** was isolated by concentration of the mother liquor from the recrystallization of the *cis* isomer **16b**.

Method J. Carboxylate replacement. 3-(3,4-Dichlorobenzyl)-N-methoxy-2-oxo-1-benzimidazolineacetamide ***17a***

Under N_2 in a flame dried flask a solution of **6f** (1.0 g, 0.00285 mol) and triethylamine (0.40 ml, 0.00285 mol) in 5 ml dry THF was cooled to –18°C. With good stirring, ethyl chloroformate (0.27 ml, 0.00285 mol) was added, followed after 20 min by methoxylamine hydrochloride (0.486 g, 0 0057 mol) in 6 ml pyridine. The reactants were allowed to warm to 25°C over 24 h and the resulting precipitate was then filtered, washed with H_2O and air-dried to give **17a** (0.299 g, 28%), mp 213–215°C. Anal $C_{17}H_{15}Cl_2N_3O_3$ (C, H, N).

Compounds **17b–f** were prepared in a similar manner, substituting the appropriate amine for methoxylamine HCl in this step.

3-(3-(3,4-Dichlorbenzyl)-2-oxo-1-benzimidazolinyl)-2-hydroxypropionamide ***17g***

To a suspension of oil free NaH (2.4 g of 50% NaH in oil dispersion, 50 mmol) was added 100 ml of anhydrous DMF under N_2. To this was added **3a** (8.71 g, 50 mmol) portionwise over a 2-min period (caution: vigorous gas evolution; the use of an efficient mechanical stirrer is recommended). After 30 min, potassium iodide (8.3 g, 50 mmol) and 3,4-dichlorobenzyl chloride (9.8 g, 50 mmol) were added and stirring was continued at 25°C overnight. The reaction mixture was diluted with H_2O (100 ml) and EtOAc (100 ml), the aqueous layer extracted twice with 50-ml portions of EtOAc and the organics were combined, washed (H_2O, satd NaCl) and dried over $MgSO_4$. Concentration *in vacuo* produced a yellow liquid (20.3 g) which was dissolved in 200 ml of EtOH, treated with 10 ml of H_2O and 10 ml of concentrated H_2SO_4 and stirred at room temperature for 18 h. The resulting white solids were filtered, washed well with H_2O and Et_2O and dried to give 1-(3,4-dichlorobenzyl)-2-benzimidazolinone, 8.35 g (57%), mp 170–172°C; MS, *m/e* 292 (M^+).

Three grams (10.2 mmol) of the above material was added to a stirred suspension of oil-free NaH (0.49 g of 50% dispersion, 10.2 mmol) and 30 ml of anhydrous DMF under N_2. After 25 min, bromoacetaldehyde diethyl acetal (1.59 ml, 10.2 mmol) was added and the mixture was stirred at 25°C for 24 h, then at 110–115°C for another 18 h. The reaction was cooled to 25°C, diluted with Et_2O and washed with H_2O and satd NaCl, then dried ($MgSO_4$). The solvent was removed *in vacuo* to give 3-(3,4-dichlorobenzyl)-2-oxo-1-benzimidazolineacetaldehyde diethyl acetal as a pale yellow oil (4.2 g, 100%); MS, *m/e* 412, 410, 408(M^+). The acetal (4.1 g, 10 mmol) in 100 ml of *p*-dioxane was treated with 100 ml of 3 N HCl in a steam bath for 40 min. The cooled mixture was poured over 300 g crushed ice and extracted with EtOAc (3 x 100 ml). The organics were washed (satd $NaHCO_3$, satd NaCl), dried over $MgSO_4$ and concentrated to give 3-(3,4-dichlorobenzyl)-2-oxo-1-benzimidazolineacetaldehyde (3.1 g, 93%).

Under N_2, a mixture of this crude aldehyde (3.1 g, 9.25 mmol) and trimethylsilyl cyanide (6.0 ml, 45 mmol) was cooled in an ice bath and treated with anhydrous zinc iodide (0.07 g, 0.22 mmol). After 30 min, the bath was removed and stirring was continued at 25°C for another 18 h. The mixture was diluted with 100 ml of $CHCl_3$, washed with satd $NaHCO_3$ and satd NaCl, dried over $MgSO_4$ and concentrated to give 3-(3-(3,4-dichlorobenzyl)-2-oxo-1-benzimidazolinyl)-2-(trimethylsilyloxy)propionitrile as a pale yellow viscous oil (3.65 g, 91%); MS, *m/e* 437, 435, 433(M^+).

The preceding nitrile (1.85 g, 4.26 mmol) in 3 ml of Et_2O was added to 3 ml of concentrated HCl which was cooled to 0°C. The mixture was saturated with HCl gas for 10 min and stirred at 25°C for 18 h to give a suspension which was poured over 200 ml of ice/H_2O. The resulting gum was dissolved in 100 ml of $CHCl_3$, washed with satd $NaHCO_3$ and satd NaCl, dried over $MgSO_4$ and concentrated to a white foam. Recrystallization from MeOH:Et_2O:hexanes provided pure title compound **17g** as white crystals (0.34 g, 21%), mp 158–159.5°C; MS, *m/e* 379(M^+), 361, 335, 305, 159. Anal $C_{17}H_{15}Cl_2N_3O_3$ (C, H, N).

1-Acetyl-3-(3,4-dichloro-α-methylbenzyl)-3-methyloxindole ***20***

A suspension of 3-(3,4-dichloro-α-methylbenzylidene)oxindole (**13f**, 5.0 g, 0.0164 mol), prepared according to *Method G*, and 1.0 g of 5% Pd on C in 100 ml EtOH and 100 ml EtOAc was hydrogenated in a Parr apparatus at 50 psi for 4 h, after which H_2 uptake had ceased. After filtration through diatomaceous earth the solvent was removed *in vacuo* to give 3-(3,4-dichloro-α-methylbenzyl)oxindole **18** as a red-orange gum which solidified on standing (5.02 g, 100%, mp 127–129°C; ^{1}H-NMR (60 MHz, $CDCl_3$, δ), 1.3 (t, 3H), 3.7 (m, sH), 6.5–7.4 (m, 7H), 9.3 (bs, 1H).

The above compound (5.0 g, 0.0164 mol) in 100 ml acetic anhydride was refluxed for 5 h, cooled to room temperature, concentrated *in vacuo* to an oil which was redissolved in 150 ml EtOAc and washed with H_2O and saturated aqueous NaCl. The solvent was dried over $MgSO_4$ and removed *in vacuo* to provide 1-acetyl-3-(3,4-dichloro-α-methylbenzyl)-oxindole **19** as an oil (5.5 g, 97%); ^{1}H-NMR (60 MHz, $CDCl_3$, δ) 1.2–1.6 (m, 3H), 2.6 (d, 3H), 3.6–3.8 (m, 2H), 6.6–7.4 (m, 6H), 8.1 (dd, 1H).

Under N_2, NaH (0.76 g, 0.0158 mol, 50% oil dispersion) was washed with pentane and covered with 100 ml dry DMF. A solution of 5.5 g (0.158 mol) of **19** in 50 ml DMF was added dropwise over 2 min with vigorous evolution of H_2. After 30 min at room temperature, the dark brown solution was treated with CH_3I (1.0 ml, 0.016 mol) and stirred another 18 h, after which the reaction was quenched by pouring slowly over 200 ml ice water. The oily residue was extracted into Et_2O and washed (H_2O, saturated NaCl), dried ($MgSO_4$) and concentrated to give **20** as a yellow oil (4.05 g, 71%); MS, *m/e* 364(M^{+2}), 362(M^+). This product was used without purification in the preparation of compound **10d** *(Method F)*, which was then converted via ester **11d** *(Method C)* to acid **12d**.

Biological methods

Enzyme preparation

Aldose reductase was partially purified from human placentae by a modification of Hayman and Kinoshita's [51] purification of rat lens aldose reductase. Freshly obtained human placentae were homogenized in 3 volumes of 0.1 M potassium phosphate buffer, pH 7.0, containing 5 mM 2-mercaptoethanol and centrifuged for 20 min at 33 000 *g* at 4°C. The supernatant was subjected to a 50–75% ammonium sulfate fractionation and the resulting pellets were pooled, resuspended in a minimum volume of buffer and dialyzed overnight. The dialysate was chromatographed on a DEAE-cellulose column (2 cm x 25 cm) and aldose reductase was eluted with a linear salt gradient (0–1 M NaCl). Peak fractions containing aldose reductase activity were pooled and aliquots stored frozen.

Aldose reductase was also partially purified (50%–75% ammonium sulfate fraction) from bovine lenses and its enzymatic activity was determined by the method of Hayman and Kinoshita [51] .

Assay procedure

Enzyme activity was assayed using an Abbott VP bichromatic clinical analyzer which measured the rate of NADPH oxidation at 340 nm at 25°C over 10 min in a reaction mixture of 0.25 ml of 50 mM potassium phosphate buffer (pH 7.1) containing 0.4 M ammonium sulfate, 0.067 mM NADPH, and 1.0 mM DL-glyceraldehyde. Sufficient enzyme was added to produce a rate of NADPH oxidation equal to 4 milliunits (unit equal to one μmol of NADPH oxidized at 25°C per min) . The coefficient of variation for this assay over a four-year period was approximately 12%.

In vivo analysis

According to the method of Peterson *et al* [46], rats were made diabetic by the injection of 85 mg/kg of streptozotocin at 0 h, test compounds were administered by oral gavage at 4, 7 and 24 h. The sorbitol content of sciatic nerves and lenses were determined at 27 h. Results were expressed as the mean

(± SEM) percent inhibition of sorbitol accumulation *versus* untreated diabetic controls. Statistical significance was calculated on the basis of the absolute levels of sorbitol in the treated and untreated diabetic groups using Student's *t*-test.

Statistical analysis

The IC_{50} value for each compound was calculated according to the method of Sarges *et al* [51].

Acknowledgements

The authors wish to acknowledge the expert technical assistance of D Beebe in performing the *in vitro* analyses of AR inhibitory activity and C Aldinger for the *in vivo* analyses. In addition, the assistance of E Whipple and R Ware with structural determinations is greatly appreciated.

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