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BENZOTHIAZOL-2-YLCARBOXYLIC ACIDS WITH DIVERSE SPACERS : A NOVEL CLASS OF POTENT, ORALLY ACTIVE ALDOSE REDUCTASE INHIBITORS

Tomoji Aotsuka,* Naoki Abe, Kanako Fukushima, Naoki Ashizawa, and Motoyuki Yoshida

Research and Development Division, GRELAN Pharmaceutical Co., Ltd.,3-4-3,
Sakae-cho, Hamura, Tokyo, Japan

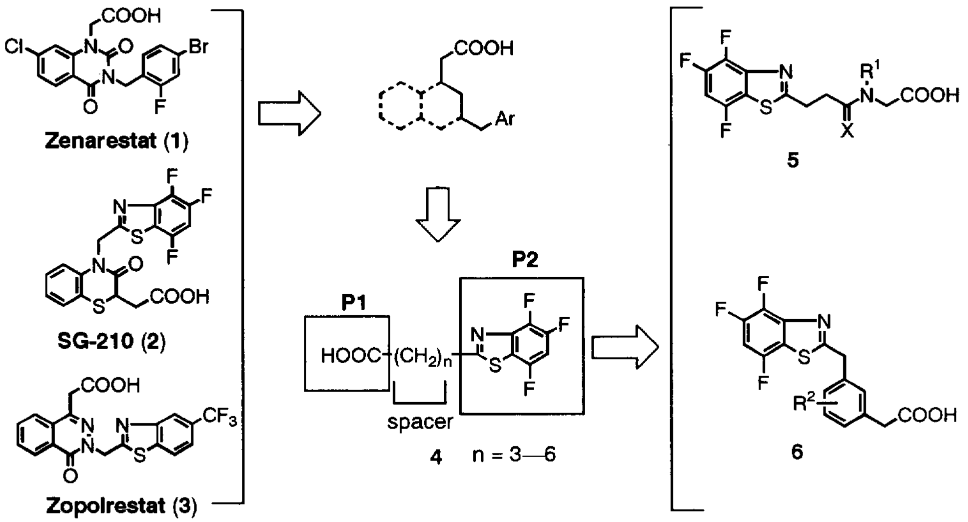
Abstract. The benzothiazol-2-ylcarboxylic acids, with diverse spacers between the carboxy group and the benzothiazole ring, were synthesized and evaluated for their ability to inhibit aldose reductase (AR). The phenylacetic acids **6** which have a less complex structure than that of existing AR inhibitors were found to be highly potent, orally active AR inhibitors. © 1997 Elsevier Science Ltd.

In hyperglycemia, aldose reductase (AR) catalyzes the conversion of excess glucose into sorbitol, and the accumulation of intracellular sorbitol is implicated in the development of diabetic complications such as neuropathy, retinopathy, nephropathy, and cataracts.[1] Inhibition of AR should therefore provide a pharmacological approach to the prevention and treatment of these complications. On the basis of this hypothesis, several AR inhibitors with diverse structures have been developed for clinical use.

Carboxylic acid derivatives such as zenarestat (**1**),[2] SG-210 (**2**)[3] and zopolrestat (**3**)[4] are among the most potent AR inhibitors. Studies of structure-activity relationships for these compounds indicated that both the carboxylic acid moiety (P1) and the lipophilic aromatic or benzothiazole ring (P2) were important for increasing the inhibitory activity. A hypothesis that the distance between the P1 and the P2 crucially influences AR inhibitory activity prompted us to synthesize some 4,5,7-trifluorobenzothiazol-2-yl carboxylic acids (**4**) in which the P1 was separated from the P2 by 3 to 6 methylene units. The number with the most potent activity was found to be five methylene units similar to compounds **1**, **2** and **3**. This observation encouraged us to modify the methylene spacer in **4c** in an attempt to further increase the inhibitory activity of the compounds (figure 1).

This paper describes the synthesis and biological activity of **4a-d**, (benzothiazol-2-yl)propionylaminoacetic acids (**5a-g**), (benzothiazol-2-yl)methylphenylacetic acids (**6a-i**), and their congeners **14**, **20**, **22a,b**, including a novel orally active AR inhibitor, **GP-1447**.

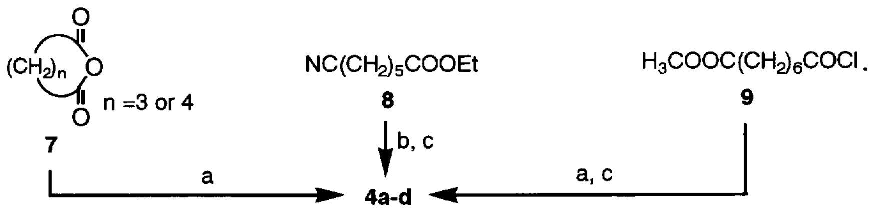
Figure 1.



Chemistry.

Compounds **4a-d** were prepared as described in Scheme 1. The cyclic acid anhydrides **7** were treated with 2-amino-3,4,6-trifluorobenzenethiol (**10**)[3] in *N*-methylpyrrolidinone (NMP) at 100°C to give **4a** and **4b**.[5] Treatment of the half acid chloride **9** in a similar manner for **7** followed by ester hydrolysis afforded **4d**.[6] The cyanohexanoate **8** was converted to **4c** through acid-catalyzed cyclization[4] with the hydrochloride of **10** in the presence of a small amount of ethanol in boiling xylene.

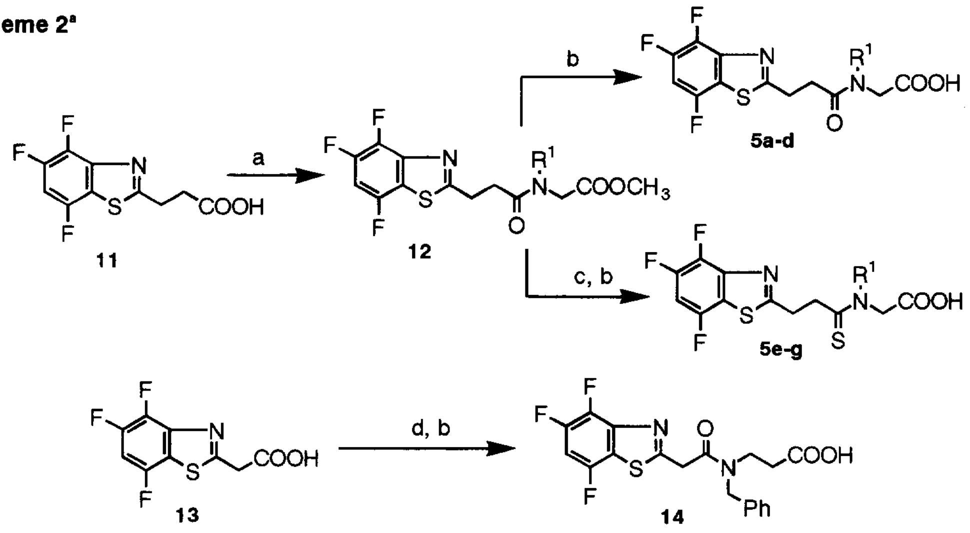
Scheme 1[a]



[a]Reaction conditions; (a) 2-amino-3,4,6-trifluorobenzenethiol (**10**), NMP, 100°C; (b) **10** · **HCl**, EtOH, Xylene; (c) NaOH.

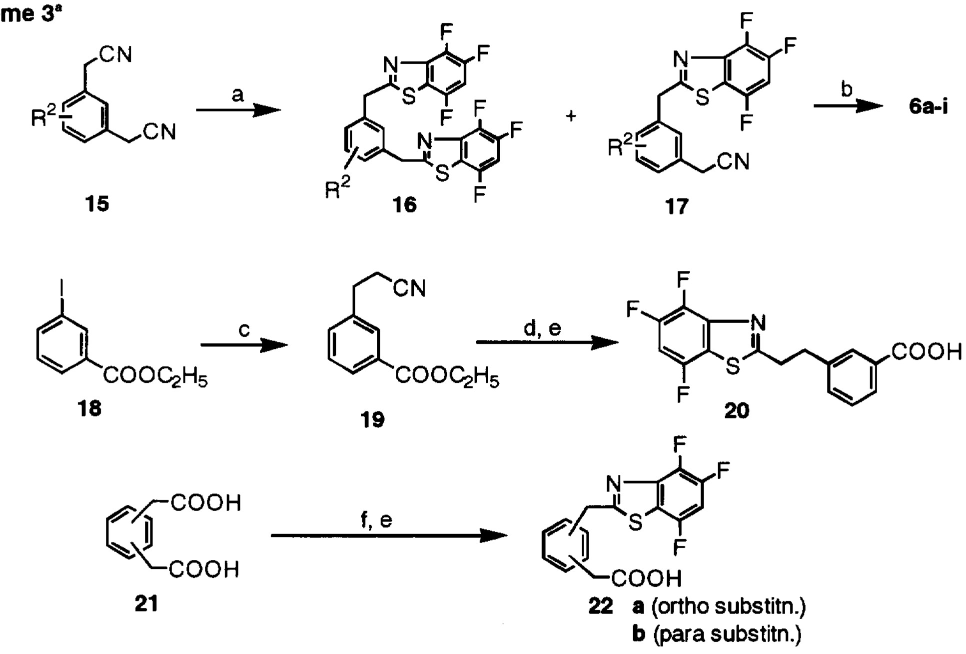
The amino acid derivatives **5a-g** and **14** were prepared by the synthetic route outlined in Scheme 2. Condensation of the benzothiazolylpropionic acid **11** with the *N*-substituted glycine esters using 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (WSC) provided the acylglycine esters **12** which were hydrolyzed to **5a-d.** Compounds **12** were converted into the corresponding thioamides **5e-g** by treatment with phosphorus pentasulfide, followed by hydrolysis. A similar sequence of reactions using the benzothiazoleacetic acid **13** in place of **11** afforded the *N*-acyl-β-alanine derivative **14**.

Scheme 2[a]



[a]Reaction conditions; (a) $R^1NHCH_2COOCH_3$, WSC; (b) NaOH; (c) P_4S_8; (d) $HN(CH_2Ph)CH_2CH_2COOEt$, WSC.

Scheme 3[a]



[a]Reaction conditions: (a) **10** • HCl, EtOH, 170°C, in sealed tube; (b) 50% H_2SO_4; (c) i) acrylonitrile, $Pd(OAc)_2$, NEt_3; ii) H_2/Pd-C; (d) **10**, $ZnCl_2$; (e) NaOH; (f) i) $SOCl_2$; ii) **10**, NMP, 100°C.

The phenylacetic acid derivatives **6a-i** and **22a,b**, and the benzoic acid derivative **20** were prepared as shown in Scheme 3. Acid-catalyzed cyclization of the biscyanomethylbenzenes **15** gave the benzothiazolylmethylphenylacetonitriles **17** as the major product and the bisbenzothiazolyl derivatives **16** as a by-product. Hydrolysis of **17** in acidic conditions provided the desired compound **6a-i**. Pd-catalyzed vinylation[7] of the iodobenzoate **18** with acrylonitrile followed by hydrogenolysis gave the intermediate **19**, which was converted into the benzothiazolylethylenebenzoic acid **20** through Lewis acid-catalyzed cyclization[8] and subsequent hydrolysis. The phenylenediacetic acids **21** were converted into the corresponding acid chlorides, which upon cyclization with **10** afforded **22a** (*ortho* isomer) and **22b** (*para* isomer), respectively, as positional isomers of **6a** (R^2=H).

Results and discussion.

Compounds **4**, **5**, **6** and their congeners **14**, **20**, **22** were evaluated *in vitro* for their ability to inhibit porcine lens AR with DL-glyceraldehyde as the substrate.[9] The AR inhibitory activity of **4** was dramatically affected by the number of methylene units as shown in Table 1. The distance between the P1 and the P2 appeared to be the crucial element, however, the most potent compound **4c** (IC_{50} 50nM) with five methylene units was less active than the existing AR inhibitors, **1**, **2**, **3** (IC_{50} 9—12nM). We believe that these results may be explained as follows. 1) The free rotation of the methylene units disturbs the favorable conformation for interaction with the enzyme. 2) The hydrophilic character of amides may confer enzyme affinity upon the AR inhibitors, whereas in the case of **4** this effect can not be expected.

Table 1.

Compd.	n	% inhibition at 10^{-7}M[a)]
4a	3	1
4b	4	35
4c	5	71 (IC_{50} 50nM)
4d	6	11

a : AR inhibitory activity in porcine lens.

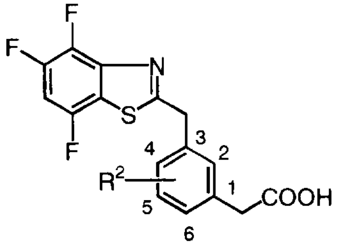
Table 2.

Compd.	R^1	X	IC_{50} (nM)[a)]	nerve[b)]	lens[b)]
5a	CH_3	O	13	61**	30*
5b	Ph	O	8.3	54**	80*
5c	CH_2Ph	O	9.7	75**	54**
5d	CH_2CH_2Ph	O	21	65**	-2
5e	CH_3	S	11	48**	58*
5f	Ph	S	8.4	64**	12
5g	CH_2Ph	S	23	65**	23
Tolrestat			16	84**	36
Zenarestat			12	89**	30

*$p<0.05$, **$p<0.01$ vs. diabetic control. a : AR inhibitory activity in porcine lens. b : % inhibition of sorbitol accumulation (preventive effect[10]), rat, 30mg/kg, p.o.

The spacers of **5a-g** and **14** were expected to have both conformational rigidity due to the planarity of the amides and the enzyme affinity due to the hydrophilicity of amides. The amide derivatives **5a-d** exhibited increased AR inhibitory activity (IC_{50} 8.3—21nM) compared with **4c** as shown in Table 2. Variation of the substituent, groups such as methyl, phenyl and benzyl on the amide nitrogen did not significantly influence the inhibitory activity. The thioamide derivatives **5e-g** also exhibited good activity (IC_{50} 8.4—23nM). In contrast, the β-alanine derivative **14** corresponding to the isomer of **5c** showed a significant reduction in activity (IC_{50} >100nM), therefore the position of the amide moiety in the spacer appears to be crucial to the inhibitory activity.

Table 3.



Compd.	R^2	IC_{50} (nM)[a)]	nerve[b)]	lens[b)]
6a	H	6.6	1.5	>10
6b	5-Me	9.6	0.25	1.6
6c	5-F	8.8	0.32	10
6d	5-Cl	7.0	0.46	7.8
6e	5-Br	9.4	0.68	9.8
6f	5-I	11	>10	>10
6g	5-NMe_2•HCl	12	0.53	>10
6h	5-COOH	8.7	>10	>10
6i	2-Cl	9.3	0.12	0.71
Zenarestat		12	76%[c)]	21%[c)]
SG-210		9.5	1.8	>10

a : AR inhibitory activity in porcine lens. b : ED_{50} for the inhibition of sorbitol accumulation in diabetic rats, p.o. (curative effect[12]). c : % inhibition at 30mg/kg

The introduction of a benzene ring into the spacer led to more potent compounds **6a-i** (IC_{50} 6.6—12nM) as shown in Table 3. It is noteworthy that the series of compounds having a simple hydrophobic spacer expressed highly potent activity. Various substituents were introduced on the benzene ring such as methyl, halogen, dimethylamino, and carboxy group, however substituent effects were not clearly observed. Whereas, the benzoic acid derivative **20** corresponding to the isomer of **6a** had decreased potency (IC_{50} >100nM). Interestingly compounds **22a** and **22b** (IC_{50} 19nM and 38nM) corresponding to the *ortho* or *para* isomer of **6a**, respectively, showed potent inhibitory activity which was somewhat weaker than **6a-i**. Compounds **6** and **22** which exhibited potent activity both have the benzothiazolylmethyl and acetic acid moieties as common structural features. On the basis of these results, in order to elicit potent inhibitory activity, the conformational regulation of the spacer is a more important factor rather than presence of a hydrophilic spacers such as amides.

Compounds **5a-g** were evaluated *in vivo* for their preventive effect[10] on sorbitol accumulation in the tissues of streptozotocin(STZ)-induced diabetic rats at the dose of 30mg/kg. All the compounds were significantly active in the sciatic nerve, but less active than the positive controls, tolrestat[11] and zenarestat. Compounds **5a**, **5b**, **5c**, and **5e** exhibited significant inhibition in the lens, while the positive controls had insufficient activity. Although **5** were further evaluated for their curative effect[12] on sorbitol accumulation at the dose of 10mg/kg, no significant reduction in sorbitol accumulation was observed.

The most promising result was obtained in the evaluation for the curative effect of **6a-i**. Many compounds exhibited inhibitory effects superior to that of SG-210 and zenarestat. Compound **6i** (R^2 = 2-Cl) was found to have the best activity in the sciatic nerve with ED_{50} values of 0.12mg/kg and in the lens with ED_{50} values of 0.71mg/kg. The introduction of a substituent such as Me, F, Cl, Br, and NMe_2 at the 5 position of the phenyl group increased the activity *in vivo* compared with that of non-substituted **6a**. The dicarboxylic acid **6h** was far less potent than non-substituted **6a**, in spite of its potent inhibitory activity *in vitro*.

Among these compounds, the 5-methylphenylacetic acid **6b** (**GP1447**) was selected as a candidate for further pharmacological evaluations. It shows not only potent AR inhibitory activity but also a favorable pharmacological profile such as an enzyme selectivity toward AR,[13, 14] sufficient oral absorption, and low toxicity.

In conclusion, We have found that simple methylene units play an important role as the spacer between P1 and P2. Compound **4c** with five methylene units showed optimum potency in the **4** series. Further modification of the spacing group revealed that **5** having the amide group and **6** having the benzene ring as a spacer exhibited potent AR inhibitory activity. The phenylacetic acid **GP1447** displayed favorable pharmacological properties as a highly potent AR inhibitor.

Acknowledgment

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