Review Article

Aldose Reductase Inhibitors

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Aldose reductase ([EC1.1.1.21]: AR) acts on the first step of the polyol metabolic pathway to catalyze the reduction of glucose to sorbitol with NADPH as a coenzyme. Hyperactivity of the pathway in individuals with high blood glucose level is closely related to the onset or progression of diabetic complications. AR inhibitors have therefore been noted as possible pharmacotherapeutic agents for the treatment of diabetic complications. One AR inhibitor has been on the market in Japan, while some potent inhibitors are in clinical trials. Reviewed are the physiological roles of AR, the chemical structures of AR inhibitors, interactions of AR inhibitors with AR using X-ray studies, and the following potencies of AR inhibitors: *in vitro* activities for AR, *in vitro* selectivities between AR and aldehyde reductase, their pharmacological effects *in vivo*, and their effectiveness in clinical trials. Also discussed are directions for the design of future AR inhibitors.

Keywords: Aldose reductase inhibitor; Diabetic complication; X-ray crystallography; α/β TIM barrel; Polyol pathway; Neuropathy

INTRODUCTION

Glucose is metabolized mostly *in vivo* by the glycolytic system, but an extremely small part is metabolized through the polyol pathway.[1] The polyol pathway consists of two steps, i.e. one step converting glucose into sorbitol and the other step converting sorbitol into fructose.[2] The rate-limiting enzyme of this pathway is aldose reductase ([EC1.1.1.21]: AR), which belongs to the aldo-keto reductase family and requires NADPH as a coenzyme.[1,2] AR is distributed widely throughout the body, including areas such as the testis,[3] adrenal gland,[4] arterial smooth muscle,[5] ovary[6] and target organs of diabetic complications such as the lens,[5,7] retina,[7] kidney[5,7] and peripheral nerves.[3,5] As physiological roles, AR regulates osmotic pressure in the kidney medulla.[8] Further, steroid metabolism in the adrenal gland and reproductive organs,[4,9] detoxication of aldehyde compounds such as 4-hydroxynonenal (HNE) in the vascular wall in case of giant cell arteritis,[10] and hormone regulation in the ovary[6] have been reported as roles.

In a hyperglycemic state, the glucose metabolism through the polyol pathway is accelerated.[2] In the peripheral nerves, the decrease of *myo*-inositol content implicated in the excessive

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accumulation of sorbitol has been observed,[11] and the decrease of *myo*-inositol content results in a decrease in Na^+, K^+-ATPase activity, which is essential for nerve conduction.[12,13] In addition, the excessive consumption of NADPH associated with the enhanced enzymatic reaction of AR results in suppression of NO synthesis[14] and reduced glutathione (GSH) production,[15] both of which require NADPH as a coenzyme. The decrease in the amount of NO acting as a vasodilating mediator causes circulatory disorders. The decrease in the amount of GSH leads to an increase in reactive oxygen species, resulting in impairment of endothelial function. Hence, acceleration of the polyol pathway

metabolism of glucose is an important factor in the onset and progression of diabetic complications such as neuropathy and vascular disorders. Thus, various AR inhibitors have been developed to correct this metabolic abnormality.

STRUCTURES AND *IN VITRO* ACTIVITIES OF POTENT INHIBITORS

The developed potent AR inhibitors can be structurally classified into two main groups; acetic acid compounds and spirohydantoins (Fig. 1). Alrestatin, which is an acetic acid compound, is the first orally effective AR
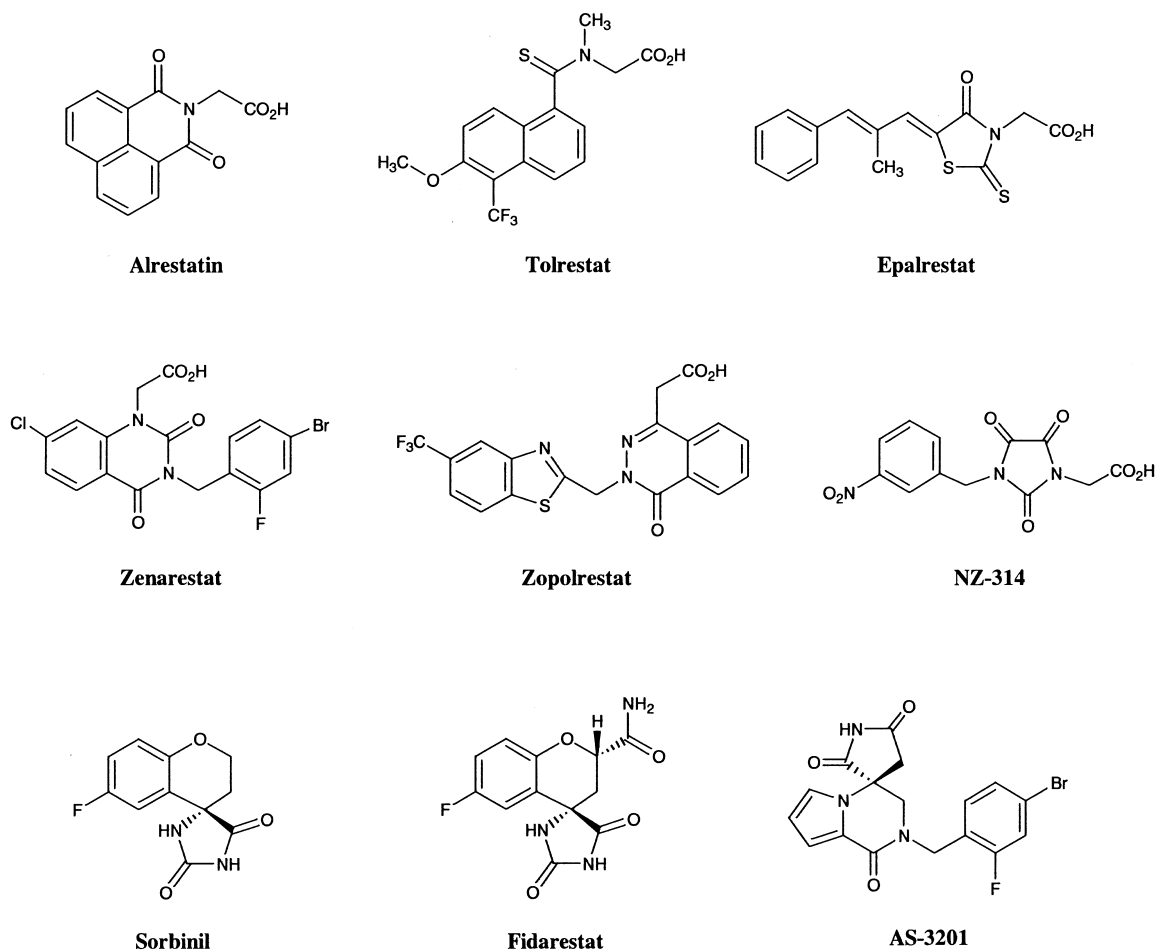

FIGURE 1   Chemical structures of aldose reductase (AR) inhibitors.

inhibitor.[16] This compound was synthesized in consideration of the hydrolysis-resistance of an active compound found by screening focused on carboxylic acids.[17] Tolrestat[18] was developed from alrestatin by means of molecular modification, where the imido ring was cleaved to afford naphthoylglycine derivatives.[17,18] Epalrestat was developed by introducing of the substituent onto the 5-position in rhodanine-N-acetic acid.[19] The isomeric form of epalrestat has been revealed by X-ray crystal analysis; however, epalrestat easily isomerizes in solution even under natural light.[20] Zenarestat was chosen among approximately 150 synthetic quinazoline derivatives from a barbital compound selected as a AR inhibitor prototype.[21] Zopolrestat was discovered in a series of phthalazinoneacetic acid compounds having a benzothiazole group, a group which was determined by another program to be an effective pharmacophore.[22] NZ-314 was obtained as a result of the introduction of various substituted phenyl groups into parabanic acid having a carboxyl group.[23]

Regarding the other main class, hydantoins, sorbinil was developed by a systematic exploration of a spirohydantoin compound which was a relative of a lead compound discovered in a high-throughput screen.[24] The absolute configuration of sorbinil was determined to be 4S by single-crystal X-ray analysis; the activity of the R isomer is much lower than sorbinil.[25] Fidarestat, which has a carbamoyl group on the 2-position of sorbinil, was selected among various 2-substituted derivatives.[26] The absolute configuration of fidarestat was determined by single-crystal X-ray analysis;[27] fidarestat, 2S,4S isomer, is the most potent among the four isomers.[26] AS-3201, a spirosucinimide compound, is also much more active than its enantiomer.[28]

These compounds have a hydrophilic moiety and a hydrophobic moiety, and structurally they are not highly flexible. The hydrophilic moieties, i.e. the carboxyl group or the hydantoin ring, are essential for AR inhibitory activity. In vitro activities of the inhibitors shown in Fig. 1 are listed in Table I. These compounds, except for alrestatin and sorbinil which are the earliest developed compounds in each class, have similarly high inhibitory activities against AR.

Interactions of Inhibitors With AR by X-ray Studies

The X-ray crystal analyses of human AR[30,31] and pig AR[32] were reported in 1992. These analyses showed that the structure is a single domain

TABLE I   *In vitro* IC$_{50}$ values of potent inhibitors against aldose reductase (AR)

Classes	Inhibitors	IC$_{50}$ (μM)[a]				
Acetic acid compounds	Alrestatin	1[b]				
	Tolrestat		0.015[c]			
	Epalrestat			0.012[d]	0.021[e]	
	Zenarestat			0.011[d]		
	Zopolrestat					0.041[f]
	NZ-314				0.062[e]	
Spirohydantoins	Sorbinil	0.26[b]		0.28[d]		
	Fidarestat			0.018[d]		
Succinimide	AS-3201		0.015[c]			

[a] DL-Glyceraldehyde used as substrate.
[b] Against calf lens AR: data from Sarges *et al.*[24]
[c] Against porcine lens AR: data from Negoro *et al.*[28]
[d] Against rat lens AR: data from Mizuno *et al.*[29]
[e] Against rat lens AR: data from Ishii *et al.*[23]
[f] Against rat lens AR: data from Mylari *et al.*[22]

composed of a α/β TIM barrel; a core of the eight parallel β strands is surrounded by the eight helices with the two extra helices. A coenzyme lies across the barrel with the nicotinamide ring of the coenzyme being centered on the barrel.[30−32]

The reduction mechanism of the substrate by the enzyme containing NADPH was proposed based on the complexed structure with citrate.[33] The reduction proceeds in either a stepwise or concerted fashion by means of a nucleophilic attack of a hydride produced at the 4-position of the nicotinamide ring of the coenzyme followed by a protonation of the hydrogen atom on the Oη of Tyr48.[33]

Regarding complexes with potent inhibitors, each structure complexed with zopolrestat (PDB code: 1MAR), tolrestat (1AH3), alrestatin (1AZ1), sorbinil (1AH0), and fidarestat (1EF3) has been elucidated in regard to its interactions with AR.[27,34−36] These inhibitors are located in the active site, though these are not competitive inhibitors. Figure 2 shows the superimposition of the structures with zopolrestat, tolrestat, and fidarestat with Cα atoms. These inhibitors bind to the active site by hydrophobic interactions; the site is surrounded by the hydrophobic side chains such as Trp20, Trp111, Phe122, Trp219, and Leu300, and by hydrophilic interactions

illustrated in Fig. 2, where dotted lines indicate hydrogen bonds. The carboxyl groups and hydantoin rings are located in the common space with the hydride source (C4 of the nicotinamide ring of the coenzyme) and proton source (Oη of Tyr48).

The interactions of zopolrestat with human AR were first revealed in the potent inhibitors; the carboxylic acid group forms hydrogen bonds with Oη of Tyr48, Nε2 of His110, and Nε1 of Trp111.[34] This analysis also clarified other hydrogen bonds between the nitrogen atom of the phthalazinone ring and Sγ of Cys298, between the nitrogen atom of the benzothiazole ring and the nitrogen atom of Leu300, and between fluorine atoms of the trifluoromethyl group and Oγ1 of Thr113.[34] The structure revealed the movement of the loop (residues 121–135) and the segment (298–303),[34] which are close to the inhibitor binding site, to be induced by zopolrestat. Next, each complex structure of pig AR containing either tolrestat or sorbinil was reported.[35] The carboxylic acid group of tolrestat forms the same hydrogen bonds as that of zopolrestat, and tolrestat has further hydrogen bonds between the fluorine atoms of the trifluoromethyl group and Oγ of Ser302.[35] The direction of the naphthalene ring of tolrestat is almost the same as that of the benzothiazole ring
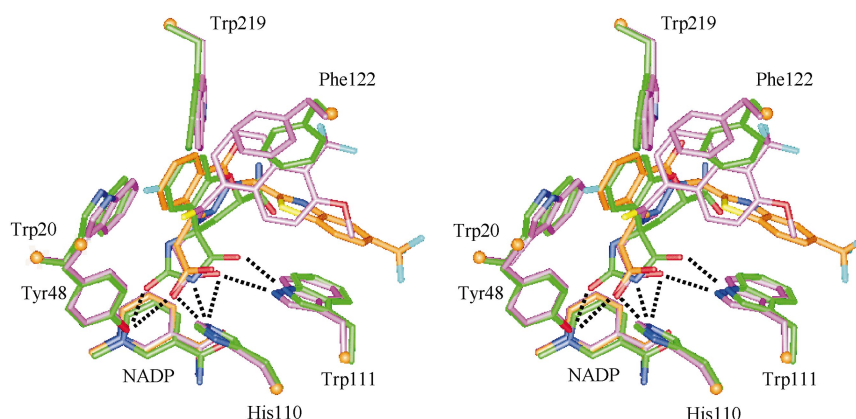

FIGURE 2 The superimposition of the AR complexes with zopolrestat (orange), tolrestat (pink), and fidarestat (green) with coordinates of Cα atoms from Protein Data Bank (codes are 1MAR, 1AH3 and 1EF3). These inhibitors bind to the active site by hydrophobic and hydrophilic interactions. In this figure, hydrogen bonds of carboxyl groups or hydantoin ring with aldose reductase (AR) are represented by dotted lines.

of zopolrestat; however, the faces of these aromatic groups are nearly vertical to each other (Fig. 2). Accordingly, the naphthalene ring of tolrestat permits the CH/π interactions[37] with the side chains of Trp111, while the benzothiazole ring of zopolrestat induces stacking (π/π) interactions. The spirohydantoin ring of sorbinil forms hydrogen bonds with AR in a manner similar to that of the carboxyl groups of zopolrestat and tolrestat; the carbonyl group on the 2'-position forms a hydrogen bond with the Oη of Tyr48, and the other carbonyl group on the 5'-postition forms two hydrogen bonds with the Nϵ1 of Trp111 and the Nϵ2 of His110.[35] This observation clarified that the hydantoin ring is bioisosteric with the carboxyl groups of the AR inhibitors.

The authors have represented the interactions of fidarestat with AR. The spirohydantoin ring binds to AR through hydrogen bonds considered to be similar to those of sorbinil.[27] The dissimilarity is that the 1'-position nitrogen atom in the spirohydantoin ring of fidarestat forms a hydrogen bond with the Nϵ2 of His110. Furthermore, the hydrogen bond between the oxygen atom of the carbamoyl group in fidarestat and the main chain nitrogen atom of Leu300 is clarified. The authors proposed that the presence of the hydrogen bond is one of the factors causing fidarestat's higher affinity than sorbinil.[27] Contributions of hydrogen bonds to binding energies have been investigated by experiments with protein engineering[38] and by data analyses of 200 drugs and enzyme inhibitors.[39]

The high resolution structure of a mutant of human AR containing alrestatin showed that two molecules of alrestatin bind to the active site.[36] The acetic acid group of one molecule of the alrestatin double-decker bind in a manner similar to the binding of the acetic acid groups of zopolrestat and tolrestat.[36] The acetic acid group of the other molecule interacts with the main chain nitrogen atoms of Ala299 and Leu301. The two molecules are stacked through the interaction of the aromatic rings.[36]

Quite recently, the complex structure of AR with zenarestat was clarified and registered in Protein Data Bank (PDB code: 1IEI).

ENZYME SELECTIVITIES OF INHIBITORS

AR belongs to the aldo-keto reductase superfamily. Human AR has a 65% identity to human aldehyde reductase (ALR), and has significant identities to 2,5-diketogluconic acid reductase, frog p-crystallin, and prostaglandin F synthase.[40] The phylogenetic relationships among 45 members of this family were determined using the neighbor-joining method.[41]

ALR, having the closest homology to AR, is a major enzyme which detoxifies 3-deoxyglucosone (3-DG)[42] which is one of the intermediates in the Maillard reaction (a non-enzymatic glycation). Since the reaction forms advanced glycation end products (AGEs), which have been proposed as one of the causes or promoters of diabetic complications, the selectivity between AR and ALR is indispensable to AR inhibitors. As shown in Table II, the selectivities of almost all the AR inhibitors described in Fig. 1 are high except for that of sorbinil. The three-dimensional structures of human and porcine ALR are composed of α/β TIM-barrels, and are similar

TABLE II Selectivities of inhibitors towards aldose reductase (AR) and aldehyde reductase (ALR)

Inhibitors	IC$_{50}$ (μM)[a]		Ratio of IC$_{50}$ (ALR/AR)
	AR	ALR	
Alrestatin[b]	1	148	148
Tolrestat[b]	0.01	0.72	72
Zenarestat[b]	0.4	14.5	36
Zopolrestat[b]	0.06	27.0	450
Sorbinil[b]	2	5.4	2.7
Epalrestat[c]	0.021	1.5	71
NZ-314[c]	0.062	>100	>1613
Fidarestat[d]	0.009	1.3	144

[a] DL-Glyceraldehyde used as substrate.
[b] Against human AR and human ALR: data from Ehrig et al.[47] and Barski et al.[48]
[c] Against rat lens AR and rat kidney ALR: data from Ishii et al.[23]
[d] Against human AR and human ALR: data from Mizuno et al.[29]

to that of AR.[43,44] The structure of porcine ALR with tolrestat showed that the residues forming hydrogen bonds with the carboxyl group of tolrestat and the hydrophobic residues surrounding the inhibitor are highly conserved between AR and ALR. A significantly different interaction is expressed in the trifluoromethyl group of tolrestat. This group contacts the side chains of Arg312 and Asp313 in ALR;[45] however, it forms hydrogen bonds in AR. Interestingly, the mutation Arg312Ala in ALR induces 130-fold tighter bindings of tolrestat.[45]

The superimposition of AR with zopolrestat and ALR with tolrestat illustrates zopolrestat's differing interactions between these two enzymes.[46] Regarding the hydrophilic interactions of zopolrestat, three residues (Cys298, Leu300, and Thr113) participate in hydrogen bondings with the atoms on the phthalazinone ring and benzothiazole ring in AR, as described in the previous section; however, the corresponding residues (isoleucine, proline, and tyrosine, respectively) in ALR cannot form hydrogen bonds.[46] The authors also proposed that the correspondence of Leu300 in AR to proline in ALR is one of the major causes of the high selectivity of fidarestat, because the oxygen atom of the carbamoyl group in fidarestat is able to form a hydrogen bond with the main chain nitrogen atom of Leu300 in AR, but not with proline in ALR.[27]

Further X-ray studies could clarify the differences of these interactions in order to design more potent and selective AR inhibitors. With elucidation of the physiological significance of other enzymes belonging to the aldo-keto reductase superfamily, further studies will also be needed regarding the selectivities not only with ALR but with other enzymes as well.

PHARMACOLOGICAL EFFECTS *IN VIVO*

AR inhibitors inhibit the decrease of the *myo*-inositol content in the nerves of diabetic animals and the decrease in activity of Na^+,K^+-ATPase, and improve the slowing of nerve conduction velocity.[13,49,50] In a long-term diabetic state, histological changes are observed in addition to functional disturbances of the nervous system. Fidarestat is effective against demyelination and axonal degeneration seen in the peripheral nerve,[51] and sorbinil exhibits an effect against progression of axonal dystrophy seen in the autonomic nerve.[52]

Diabetic animals show not only a slowing of nerve conduction velocity but also a decrease in nerve blood flow.[14] WAY121509, 2-[(4-bromo-2-fluorophenyl)methyl]-6-fluorospiro[isoquino-line-4(1*H*), 3′-pyrrolidine]-1,2′,3,5′(2*H*)-tetrone, exhibits the effects both on nerve conduction velocity and decrease in nerve blood flow.[14] Since this effect is diminished on coadministration of N^G-nitro-L-arginine, an NO synthase inhibitor, it has been shown that the polyol metabolism and the NO metabolism are linked.[14]

Recently, non-enzymatic glycation and oxidative stress have received attention as other factors considered to be linked with the polyol metabolism.[53,54] Fructose which increases with the enhanced polyol metabolism is, in itself, a potent glycating substance,[55,56] and its metabolites such as fructose-3-phosphate, methyl-glyoxal (MG), and 3-DG are also potent glycating substances.[57] An increase in 3-DG is observed in the erythrocytes of diabetes patients, and this increase is corrected by epalrestat.[58] On the other hand, the amount of GSH[15,59] and the activity of antioxidant enzymes[60] are changed when the polyol pathway is in a hyperactive state. MG, which is a metabolite of fructose as mentioned above, has a high capacity to produce reactive oxygen species. When the polyol pathway metabolism is increased, oxidative stress is caused by a decrease in the active oxygen-scavenging effect and an increase in the production of reactive oxygen species, and the cells are considered to be continually exposed to a cytotoxic environment. Sorbinil has been proven to suppress the decrease in GSH[59] and

suppress formation of malonaldehyde,[61] a per-oxidation product of lipid, and it has been confirmed that AR inhibitors have an antioxidant effect. On the other hand, it has also been reported that AR is expressed in the vascular wall in the case of giant cell arteritis for detoxifying HNE, a peroxidation product of lipid, and that suppression of AR by sorbinil increases cell apoptosis.[10] There may be a complicated relation between oxidative stress and the polyol metabolism. Non-enzymatic glycation and oxidative stress have been reported to be the important factors in the onset and progression of retinopathy and nephropathy. The fact that AR inhibitors exhibit an effect on these phenomena strongly suggests the usefulness of AR inhibitors for treating microangiopathic complications.

On removal of the endothelial cells from the aorta, the smooth muscle starts growing, and at this time, AR expression is enhanced.[62] An *in vitro* experiment showed that epalrestat exhibits a suppressive effect on the smooth muscle growth caused by such factors as PDGF under a high glucose concentration.[63] Thus, AR inhibitors are possibly effective also against macroangiopathy. In addition, some AR inhibitors show a favorable effect on abnormal bone metabolism,[64] cancer cachexia,[65] and resistance to anticancer drugs,[66] suggesting that AR inhibitors are applicable to various diseases.

CLINICAL TRIALS OF AR INHIBITORS

The clinical efficacy of AR inhibitors have been demonstrated in clinical trials involving patients with diabetic neuropathy.

One-year administration of sorbinil alleviated subjective symptoms and functional disturbances in motor nerve conduction, and increased the number of regenerated nerve fibers.[67] Tolrestat shows a similar effect in both patients with Type-I diabetes (IDDM) and Type-II diabetes (NIDDM).[68] Recent studies have demonstrated that both of zenarestat[69] and fidarestat[70] are effective against neuropathy when administered for 1 year. In particular, since fidarestat exhibits an effect on hypoesthesia, which is a risk factor leading to diabetic foot, fidarestat is considered to be effective also regarding the patient's quality of life. In Japan, epalrestat having an effect on subjective symptoms is on the market. AR inhibitors have shown various effects, while many adverse reactions have also been reported. Marketing or development was withdrawn due to skin disorders frequently seen in the case of sorbinil[71] and due to serious hepatotoxicity in the case of tolrestat.[72] The development of zenarestat in the US was abandoned due to its irreversible nephrotoxicity.

Among clinical trials other than those regarding diabetic neuropathy, the Sorbinil Retinopathy Trial (follow-up ranged from 12 to 56 months) has been reported.[73] In 21 months of follow-up, sorbinil decreased the number of microaneurysms in patients, but at maximal follow-up, it showed no significant effect on the number of microaneurysms. However, the Diabetes Control and Complications Trial (DCCT) began to show a benefit only at 3 years, and the most significant changes were not seen until after 5–8 years of follow-up.[74] The sorbinil study could easily have been stopped before the possible benefit became apparent. It is uncertain whether sorbinil is effective against diabetic retinopathy. On the other hand, ponalrestat, 3-(4-bromo-2-fluorobenzyl)-4-oxo-3*H*-phthalazin-1-ylacetic acid, had no effect on the course of nonproliferative diabetic retinopathy compared to a placebo.[75]

FUTURE DESIGN OF AR INHIBITORS

The three-dimensional structures of complexes of AR with various inhibitors are available for structure-based design. Computer modeling[76–78] and three-dimensional database searches[79] have been performed based on X-ray structures. Thus

novel inhibitors being structurally quite different from already-known inhibitors can be discovered by this means; furthermore, a drastic modification of a known inhibitor may also lead to more effective inhibitors.

The multi-addition of tolrestat to AR holo-enzyme has been revealed by electrospray mass spectrometry.[80] New inhibitors can be found that act against sites other than that elucidated by X-ray studies.

Though the present clinical trials of AR inhibitors have been focused on diabetic neuropathy, AR inhibitors can be expected to be efficacious against diabetic nephropathy and retinopathy. For that, taking into the consideration pharmacokinetics determined by ADME (absorption, distribution, metabolism and excretion) profiles, it is hoped that AR inhibitors which are highly organ-specific will be designed.

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