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ENZYME SELECTIVITY ANALYSES OF ARYLSULFONYLAMINO ACID ALDOSE REDUCTASE INHIBITORS

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Arylsulfonylamino acids, displaying a wide range of inhibitory activities versus rat lens aldose reductase (RLAR), were analyzed for enzyme selectivity in several test systems. These RLAR inhibitors were found not to produce significant inhibition of genetically-linked reductases (aldehyde reductase, ALR), catalytically similar reductases (*Pachysolen tannophilus* xylose reductase, PTXR), functionally distinct oxidoreductases (glutathione reductase, GR, lactate dehydrogenase, LDH, and γ-transaminase, GABA-T), and thymidylate synthase (TS). These data suggest that aldose reductase differs significantly from other oxidoreductases in its inhibitor binding domain(s). Furthermore, the aldose reductase selectivity demonstrated by the arylsulfonylamino acids suggests that these compounds may not inhibit other key metabolic transformations in various cell types and that they may function as selective probes for studies of the relationship between aldose reductase mediated biochemical changes and the pathologies of chronic diabetes.

KEY WORDS: Aldose reductase inhibitors, enzymes, IC_{50}, percent inhibition, selectivity.

INTRODUCTION

Aldose reductase (AR; alditol: $NADP^+$ oxidoreductase; EC 1.1.1.21), an enzyme of the polyol pathway, catalyzes the NADPH-dependent reduction of glucose to sorbitol in a variety of mammalian tissues. During hyperglycemia there is increased flux of glucose through the polyol pathway in tissues such as the lens, retina, nerve, and kidney and this is associated with several biochemical changes including intracellular sorbitol accumulation and myoinositol depletion. A significant body of evidence has emerged to suggest that these AR-mediated intracellular changes ultimately are expressed as chronic or late-onset diabetic pathologies such as cataract, retinopathy, neuropathy, and nephropathy.[1–4]

Over the past several decades, a number of compounds including sorbinil, tolrestat, ponalrestat, have been developed as inhibitors of AR (ARIs), and these compounds

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have demonstrated some efficacy in the prevention of certain diabetic pathologies in animal models and human studies.[1-4] In previous publications, we described the synthesis and rat lens aldose reductase (RLAR) inhibitory activity of a number of arylsulfonylamino acids.[5-7] Of the compounds studied to date, the highest degree of inhibitory activity is produced by members of the 4-[(benzoylamino)phenylsulfonyl]-glycine (4-BAPSG) and the 2-(naphthyl)sulfonylglycine (2-NAPSG) structural subclasses (Figure 1). Competitive binding and multiple inhibition studies indicate that the high AR affinity of these compounds, particularly the 4-BAPSG derivatives, results from multiple site interactions with the enzyme. Furthermore, this multiple site interaction appears to be highly structure-dependent since modest variations in the structures of BAPSG arylsulfonylamino acids results in significant decreases in inhibitory potential. For example, all of the following structural modifications result in significantly reduced RLAR affinity: (1) altering the position of the 4-benzoylamino moiety of 4-BAPSG, as in the 2- and 3-BAPSG regioisomers, (2) elimination of the 4-benzoylamino moiety or replacing it with simply alkyl amide functionalities such as acetylamino (4-AAPSG), (3) amino acid modifications such as aryl or alkyl substitution on nitrogen or the α-carbon. The one exception to this trend is α-phenyl substitution when the α-carbon has the *S*-configuration. For example, 4-S-BAPS-2-PG has inhibitory activity comparable to the parent 4-BAPSG (Figure 1). Although not studied in the same detail as the 4-BAPSG analogs, similar structural changes in the 2-NAPSG series result in comparable decreases in inhibitory activity.

While there are numerous reports regarding the AR inhibitory potential of a variety of compounds (sorbinil, tolrestat, and ponalrestat) and the mechanisms by which these compounds inhibit this enzyme, relatively few studies have been published concerning the enzyme selectivities of ARIs. The limited number of studies of this type published to date have focused primarily on the ability of ARIs to inhibit the related aldehyde reductase family of enzymes. Based on this observation, and the high level of RLAR inhibitory activity observed for a number of the arylsulfonylamino acids, as well as their multiple mode of interaction with the enzyme, the present studies were undertaken to measure selectivities in several enzyme systems. Enzymes representing key metabolic and biochemical pathways in various cell types were selected, including rat kidney aldehyde reductase (RKALR), *Pachysolen tannophilus* xylose reductase (PTXR), glutathione reductase (GR), γ-aminobutyric acid trans-aminase/succinic semialdehyde dehydrogenase (GABASE), lactate dehydrogenase (LDH), and thymidylate synthase (TS). The arylsulfonylamino acids selected for this study included compounds with a wide range of inhibitory activities (IC_{50} values of 0.36 to 230 μM) in the RLAR assay (Table 1).

MATERIALS AND METHODS

Synthesis of Inhibitors

The syntheses of the arylsulfonylamino acids utilized in this study were described earlier.[5-7] Representative members were chosen to include compounds with a wide range of partially purified RLAR inhibitory activities (0.41–230 μM) based upon the aromatic ring and amino acid substitution patterns (Figure 1).

Table 1 IC_{50} values and enzyme selectivities

Compound	IC_{50} μM		% Inhibition at 100 μm				
	RLAR	RK ALR	PTXR	GR	GABASE	TS	LDH
4-AAPSG	4.2	>100	13	17	0.0	6.9	8.0
4-AAAPSG	5.5	>100	1.0	12	0.0	0.0	4.4
4-BAPSG	0.41	>100	0.0	24	13	0.0	3.0
4-BAPSSAR	2.2	42	24	10	0.0	3.1	6.0
4-R-BAPS-2-PG	140	ND	5.5	14	0.0	0.0	4.9
4-S-BAPS-2-PG	0.60	>100	17	10	0.0	0.0	9.0
2-NAPSG	0.36	>100	1.0	5.8	15	0.0	3.0
2-NAPS-N-PG	4.5	81	20	14	0.0	0.0	6.0
S-2-NAPS-2-PG	2.5	>100	4.5	16	0.0	0.0	11
R-2-NAPS-2-PG	230	>100	0.0	3.0	0.0	2.8	10

[1]The different enzymes utilized in this study were: RLAR—Rat lens aldose reductase; RK ALR—Rat kidnet aldehyde reductase; PTXR—*Pachysolen tannophilus* NADPH-dependent xylose reductase; GR—Glutathione reductase isolated from Baker's yeast: GABASE—γ-Aminobutyric acid transaminase/succinic semialdehyde dehydrogenase; TS—Thymidylate synthase isolated from *Lactobacillus casei*; LDH—L-lactate dehydrogenase isolated from rabbit muscle.

INHIBITOR	R	R'	R''
AAPSG	CH_3CO-	H	H
AAAPSG	N_3CH_2CO-	H	H
4-BAPSG	PhCO-	H	H
4-BAPSSAR	PhCO-	CH_3	H
4-R-BAPS-2-PG	PhCO-	H	Ph (R)
4-S-BAPS-2-PG	PhCO-	H	Ph (S)
2-NAPSG		H	H
2-NAPS-N-PG		Ph	H
R-2-NAPS-2-PG		H	Ph (R)
S-2-NAPS-2-PG		H	Ph (S)

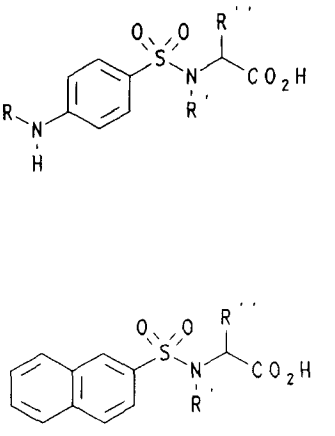

Figure 1 Structures of aldose reductase inhibitors.

Preparation of the Enzymes and Assays

Rat lens aldose reductase

Rat lens aldose reductase (RLAR) was isolated from the lens of adult (90 day old) Sprague-Dawley rats of mixed sex (Auburn University Animal Control Facility). Enzyme isolation began with the removal of the eyes from freshly sacrificed rats (via O_2 deprivation), followed by freezing (−80°C). Careful dissection of the lenses from partially thawed eyes was accomplished by the posterior approach. Preparation of the crude or partially purified enzyme began with the homogenization of 100 lenses in 10 ml of doubly-distilled water in a 20 ml Wheaton Dunce tissue grinder, followed

by centrifugation at 12,000 × g (15 min, 5°C) to sediment the particulate matter. The addition of an ammonium sulfate fractionation (40%) wth subsequent centrifugation at 12,000 × g (15 min, 5°C) allowed for the partial elimination of extraneous proteins.

Aldose reductase activities of the partially purified rat lens enzyme preparations were spectrophotometrically determined by ascertaining the decrease in absorbance at 340 nm, per 3 min, with a Shimadzu UV-160 spectrophotometer equipped with a thermally-controlled multi-cell positioner at 30°C. The control cuvette reaction contained the following components: 0.0104 mM NADPH (Type I, Sigma) in 0.1 M potassium phosphate buffer, pH 6.2; 10 mM D,L-glyceraldehyde (Sigma), 0.2 ml of the crude enzyme solution, and doubly-distilled water to give a total cuvette volume of 2 ml. All of the reagents, except for the substrate, were included as a blank to correct for any nonspecific oxidation of NADPH. For each RLAR assay, the enzyme reaction rate was adjusted with doubly-distilled water to give a change of -0.0200 ± -0.0030 absorbance units/min following a 45 s lag period.

Aldehyde reductase

Aldehyde reductase (ALR, RKALR, alcohol:$NADP^+$ oxidoreductase; EC 1.1.1.2) was purified from rat kidney and assayed as described by Sato *et al.*[8]

NADPH-Dependent Pachysolen tannophilus *xylose reductase*

Pachysolen tannophilus was obtained from the stock cultures available from the Auburn University Microbiology Department. The growth conditions utilized for isolating the yeast xylose reductase (PTXR) have been described in an earlier publication.[9] Aeration of the *P. tannophilus* culture induced an NADPH-dependent xylose reductase when grown in the presence of D-glucose.

NADPH-Dependent xylose reductase (PTXR-NADPH dependent, xylitol $NADP^+$ oxidoreductase; EC 1.1.1.139) was assayed with 100 mM D,L-glyceraldehyde, 0.2 ml crude enzyme supernatant, 0.15 mM NADPH (Type I, Sigma), and doubly-distilled water, to give a total volume of 2.0 ml. A reaction blank, which contained all of the reagents, except the substrate, was included to account for nonspecific cofactor oxidation. Overall activities were monitored for 3 min at 340 nm (30°C) by noting either a decrease (upon addition of the inhibitors) or an increase (control) in the activity.

Glutathione reductase

Glutathione reductase (GR, oxidized glutathione:NADPH oxidoreductase; EC 1.6.4.2) was obtained from the yeast *Saccharomyces cerevisae* (Type III, Baker's Yeast, Sigma) and assayed using the general procedure of Carlberg and Mannervik.[10] With this method, the oxidation of NADPH was followed spectrophotometrically for 3 min (340 nm) with a constant temperature regulation of 30°C. The reaction mixture contained 200 mM potassium phosphate, pH 7.0; 4 mM NADPH in 10 mM Tris-HCl, pH 7.0; 20 mM glutathione disulfide (GSSG), and GR in a total volume of 2.0 ml. These cuvette concentrations for NADPH and GSSG ensured that the reaction operated at saturating conditions. GR was added to the mixture to initiate the reaction, resulting in activities of -0.0120 ± -0.0020 absorbance units/min. A blank, containing all of reagents except GSSG, was included to account for NADPH-nonspecific oxidation.

GABA transaminase

γ-Aminobutyric acid transaminase (GABA-T or aminotransaminase; EC 4.1.1.15) was isolated from the microorganism *Pseudomonas fluorescens* (Sigma) and assayed by the procedure of Anlezark *et al.*[11] The control reaction included the incubation of 1.8 mM $NADP^+$ in 75 mM sodium phosphate buffer, pH 7.2; 50 mM 2-mercaptoethanol, 75 mM α-ketoglutarate in 100 mM sodium pyrophosphate buffer, pH 8.6; 90 mM GABA prepared in the same pyrophosphate buffer; and the reconstituted enzyme preparation. Since monitoring of the GABASE activity is a coupled reaction, the GABA-T and succinic semialdehyde dehydrogenase enzymes are reconstituted together as a 1:10 dilution in 10% glycerol and 75 mM sodium phosphate buffer (pH 7.2), giving a control activity of -0.0090 ± -0.0010 for 3 min. The addition of a blank, which contained all of the components above, except α-ketoglutarate, was included for any background oxidation not associated with the enzymatic reaction.

Thymidylate synthase

The purified thymidylate synthase enzyme (TS; 5,10-methylenetetrahydrofolate:dUMP C-methyltransferase; EC 2.1.1.45) was prepared according to the procedure by Lyon *et al.*[12] Approximately 200 g of cultured *Lactobacillus casei* cells were suspended in a buffer containing 100 mM potassium phosphate, pH 7.0; and 25 mM 2-mercaptoethanol. After adjustment of the pH to 7.0–7.4 with 6 M KOH, the resulting slurry was stirred during sonication in an ice bath (4 × 5 min bursts). This suspension was adjusted to a pH of 7.2–7.4 with 6 M KOH and centrifuged at 10,000 × g (40 min, 5°C). Application of two ammonium sulfate fractionations (35 and 65%) allowed for further purification of the crude enzyme preparation; for each of the fractionations, centrifugation at 10,000 × g for 60 min (35%) and 40 min (65%) provided partially purified TS as a small pellet.

The TS pellet was suspended in 100 mM potassium phosphate buffer, pH 6.8, and 25 mM 2-mercaptoethanol and dialyzed (twice) for 24 h against a buffer containing 50 mM Tris-HCl, pH 6.5; 10 mM KCl, 1 mM EDTA, and 10 mM 2-mercaptoethanol. This dialyzed preparation was then applied to a carboxymethyl Sephadex (CM-Seph) column (5 × 100 cm; Sigma) previously equilibrated with dialysis buffer. Washing the CM-Seph column with 2 L of dialysis buffer eluted extraneous enzymes without affecting the bound dihydrofolate reductase (DHFR) and TS. These bound enzymes were eluted upon application of a linear KCl gradient (700 mM) and monitored at 280 nm. The broad DHFR peak, followed by a sharp TS peak, are observed late in the elution profile. Those fractions that contained TS were pooled and dialyzed against 6 L of 5 mM potassium phosphate, pH 6.8; and 10 mM 2-mercaptoethanol for 12 h at 5°C. The dialyzed TS solution was then applied to a hydroxylapatite column (AHA; 2.5 × 50 cm; BioRad) previously equilibrated with a 5 mM potassium phosphate, pH 6.8; and 10 mM 2-mercaptoethanol buffer. Elution of the bound TS was achieved via application of a linear gradient containing 500 mM potassium phosphate, pH 6.8; and 10 mM 2-mercaptoethanol. Fractions containing appreciable TS activity were pooled, assayed for enzymatic activity, and checked for homogeneity by SDS-PAGE.

Thymidylate synthase activity was measured spectrophotometrically at 340 nm (3 min) by observing an increase in the absorbance as a result of dihydrofolate formation.[12] The reaction cuvette contained 1 mM deoxyridine-5′-monophosphate (dUMP), 1 mM 5,10-methylenetetrahydrofolate (5,10-CH_2-THF) prepared in the

cofactor buffer (below), 500 mM potassium phosphate, the TS enzyme concentration necessary for obtaining a change of -0.0200 ± -0.0020 absorbance units/min, and water to give a total cuvette volume of 2 ml. A blank, containing all of the reagents listed above, except for the cofactor, was included to account for any nonspecific oxidation.

The cofactor utilized in the TS assay, 5,10-CH_2-THF, was prepared as an epimeric ($\pm$) mixture by the catalytic hydrogenation of folic acid in acetic acid and stored as a lyophilized powder at $-80°C$ until needed.[13] Either 6 mg of ($\pm$)-tetrahydrofolate or 3 mg of ($-$)-tetrahydrofolate are dissolved in a cofactor buffer solution containing 0.5 ml of 0.5 M sodium bicarbonate, pH 8.1; 1.25 ml of 0.27 M formaldehyde, 1.25 ml of 1.0 M 2-mercaptoethanol, and 2.0 ml of doubly-distilled water.

Lactate dehydrogenase

Rabbit muscle lactate dehydrogenase (LDH, L-lactate:NAD^+ oxidoreductase; EC 1.1.1.27; Type I, Sigma) was assayed according to the procedure by Eichner.[14] The assay mixture contained 30.0 mM sodium pyruvate in 100 mM potassium phosphate buffer, pH 7.5; 8.1 mM NADH, and the LDH enzyme to give a total volume of 3 ml. Each reaction was monitored for 3 min at 340 nm (25°C) to give a change of -0.0260 ± -0.0020 absorbance units/min. A blank cuvette, containing all of the reagents except for pyruvate, was included for any extraneous activity associated with the reversible reduction step or any nonspecific oxidation.

Screening of ARIs

Each arylsulfonylamino acid was prepared as an aqueous solution with a small amount of $NaHCO_3$ and sonicated and warmed to facilitate dissolution. The percentage inhibition by these compounds was determined by an average of multiple determinations, with the activity expressed as compared to a control. The initial cuvette concentration of inhibitors utilized in the enzyme assays was 100 μM. For IC_{50} determinations, each inhibitor was tested at no less than four different concentrations with a minimum of two determinations at each concentration. Inhibition IC_{50} values were acquired by plotting percent inhibition versus log concentration and least squares analyses of the linear portion of a log-dose response curve.[15]

RESULTS AND DISCUSSION

The arylsulfonylamino acids chosen for enzyme selectivity studies represent those major structural subclasses identified by earlier structure-inhibition analyses. Thus, arylsulfonylamino acids ARIs of high inhibitory activity (4-BAPSG, 4-S-BAPS-2-PG and 2-NAPSG with IC_{50} values of 0.3 to 0.6 μM), intermediate inhibitory activity (4-AAPSG, 4-AAAPSG, 4-BAPSSAR, 2-NAPS-N-PG, S-2-NAPS-2-PG with IC_{50} values of 2 to 5 μM), and low inhibitory activity (4-R-BAPS-2-PG and R-2-NAPS-2-PG with $IC_{50} > 100$ μM) were included in the enzyme selectivity testing (Figure 1, Table 1). Compounds with a wide range of RLAR inhibitory activities were included in an attempt to identify divergent structure-inhibitory trends in the RLAR assay versus other enzymes.

Both aldose reductase and aldehyde reductase are members of a class of enzymes

referred to as the monomeric NADPH-dependent carbonyl reductases. These two enzymes originate from the same genetic locus and have overlapping substrate specificities, including the ability to convert glucose to sorbitol. Therefore, it has been proposed that both of these enzymes may play a role in the development of chronic diabetic pathologies.[16] Most ARIs developed date have been found to inhibit both aldose reductase and aldehyde reductase. For example, alconil is a more potent inhibitor of aldehyde reductase (IC_{50} of 0.027 μM) than aldose reductase (IC_{50} of 0.3 μM), with a selectivity index (IC_{50} ALR/IC_{50} AR) of 0.1.[16] Also, while tolrestat and sorbinil are more potent inhibitors of aldose reductase IC_{50} values of 0.029 and 0.28 μM, respectively) than aldehyde reductase (IC_{50} values of 0.54 μM and 1.9 μM, respectively), their selectivity indices are less than 20.[16] Only the ARIs epalrestat and EBPC (1-benzyl-3-hydroxy-2(5H)-oxopyrrole-4-carboxylate) are reported to have aldehyde reductase/aldose reductase selectivity indices greater than 100; EBPC appears to be the most selective inhibitor known with a selectivity index > 4000.[16] These observations prompted analysis of selected arylsulfonylamino acids in the rat kidney aldehyde reductase assay (RKALR). Of the compounds tested, none were found to inhibit RKALR at concentrations that produce significant inhibition of RLAR (Table 1). In this assay, 4-BAPSSAR (IC_{50} = 42 μM) and 2-NAPS-N-PG (IC_{50} = 81 μM), produced the highest level of RKALR inhibition, but only at concentrations approximately 20 times greater than those found to produce 50% inhibition of RLAR. The remaining arylsulfonylamino acids, including the more potent RLAR inhibitors 4-BAPSG, 4-S-BAPS-2-PG and 2-NAPSG, have IC_{50} values greater than 100 μM versus RKALR. The limited solubility of these compounds prevented reliable testing beyond concentrations of 100 μM, thus, IC_{50} values could not be determined for most compounds in this assay. Nonetheless, the low degree of inhibition produced by 4-BAPSG, 4-S-BAPS-2-PG and 2-NAPSG demonstrates that these compounds have enzyme selectivity indices (IC_{50} RKALR/IC_{50} RLAR) greater than 150, and these indices are comparable to those reported earlier for the more AR selective inhibitors epalrestat and EBPC.[16] These data provide additional support for the hypothesis that aldose reductase and aldehyde reductase, in spite of their catalytic similarities, differ significantly in their inhibitor binding domain(s). Furthermore, the separation in inhibitory activities observed for some members of the arylsulfonylamino acid series may prove useful for *in vivo* determinations of the relative contributions of both of these reductases to sorbitol production in diabetic tissues.

The yeast *P. tannophilus* is capable of converting xylose to ethanol by a series of fermentation reactions initiated by xylose reductases (PTXRs). To date two distinct xylose reductases have been identified in this cell line; a nonspecific NAD(P)H xylose reductase and a NADPH-specific xylose reductase.[17] Based on their abilities to catalyze the reduction of a variety of sugars using NADPH as a cofactor, these enzymes have been classified as aldose reductases.[9,18] This classification prompted an earlier study to determine the ability of a variety of structurally diverse RLAR inhibitors to inhibit the NADPH-specific PTXR.[9] The inhibitors studied included tolrestat, sorbinil, benzopyran-4-acetic acids, quinolone-1-acetic acids and N-benzoyl- and N-arylglycines with RLAR IC_{50} values in the 10 μM to 0.04 μM range. When tested against NADPH-specific PTXR, none of these compounds were found to produce greater than 30% inhibition of PTXR at inhibitor concentrations of 50 μM. As a continuation of this investigation, the present study evaluated the arylsulfonylamino acids as inhibitors of the NADPH-specific PTXR. Of these compounds, only one

(4-BAPSSAR) was found to produce more than 20% inhibition of PTXR at a concentration of 100 μM (Table 1). However, the two most potent RLAR inhibitors, 4-BAPSG and 2-NAPSG, did not produce any significant degree of PTXR inhibition. These data are consistent with the hypothesis that, in spite of their catalytic similarities, PTXR does not appear to possess the inhibitor binding site(s) present on RLAR. These data also suggest that PTXR would not serve as a reasonable enzyme source for the screening or study of ARIs developed to inhibit animal or human aldose reductases.

The oxidoreductase glutathione reductase (GR) indirectly promotes the metabolism of peroxides by catalyzing the NADPH-dependent reduction of glutathione disulfide (GSSG) to glutathione (GSH). Once the GSH pools are formed, certain tissues (erythrocytes), in conjunction with glutathione peroxidase, can metabolize hydrogen peroxides to water, thus preventing oxidative damage to the surrounding tissues. Assessment of the arylsulfonylamino acids revealed that this NADPH-requiring oxidoreductase also was not significantly inhibited by these ARIs (Table 1), suggesting that GR also lacks the inhibitor binding site(s) present on RLAR.[9] The lack of GR inhibition by ARIs, however, contrasts with the inhibition produced by sulfhydryl modifying reagents (*p*-mercuribenzoate, *N*-ethylmaleinide) and various nucleotides ($NADP^+$) in which the IC_{50} values typically ranged from 3.7–4.7 μM (modifying reagents) to 50 μM for the $NADP^+$ cofactor.[19] These observations, coupled with an absence of inhibition with PTXR and ALR suggests that the arylsulfonylamino amino ARIs would not interfere with the catalytic activities of these biodiverse oxidoreductase systems.

Lactate dehydrogenase (LDH), an enzyme normally found in skeletal and heart muscle, catalyzes the NADH-mediated reduction of pyruvate to L-lactate. Ultimately, the presence of LDH allows for the conversion of lactate, the end-product of anoxic glycosis, into the more biocompatible glucose. In the interim, NAD^+ is also generated for use in the glycolytic pathway, i.e. for the oxidation of glyceraldehyde-3-phosphate to 1,3-bisphosphoglycerate. High levels of LDH activity are associated with a number of neoplasms (Hodgkin's lymphoma), liver afflictions (viral hepatitis), and anemias. Several compounds, including carboxylic acids (oxalic acid), are known to compete with either lactate or pyruvate. Others have been developed in anticipation of inhibiting LDH for control of various neoplasms. For example, several investigators have reported the inhibition of LDH by benzopyran, quinoline, and quinazoline carboxylic acids with most IC_{50} values between 30–100 μM.[20–24] However, these compounds are not selective for LDH since enzymes including glutathione, glyceraldehyde phosphate, and malate dehydrogenases are equally affected. Based on the inhibitory activity of a number of carboxylic acids versus LDH, the arylsulfonylamino acids also were screened against this enzyme. None of these compounds, however, exhibited any significant degree of LDH inhibition at 100 μM (Table 1), supporting the conclusion that these compounds would not suppress endogenous LDH activity.

Inhibitors of GABA-aminotransferase are of clinical significance since inhibition of the GABA-T enzyme is proposed to increase brain GABA levels thus promoting sedation in certain epileptic seizures. However, most of these compounds, including valproic acid, ethanolamine-*O*-sulfate, and gabaculine, are of limited clinical applicability since they are nonspecific, do not cross the blood-brain barrier, or are relatively toxic. Because of the *in vitro* inhibitory observed with valproate (IC_{50} = 3.8 mM)[25] and other organic acids, the arylsulfonylamino acid ARIs were

examined to determine their ability to inhibit GABASE activity, a coupled enzyme system of GABA-T and SSD (succinic semialdehyde dehydrogenase). The only compounds producing measurable inhibition of this enzyme systems were 4-BAPSG and 2-NAPSG, two of the more potent RLAR inhibitors. These data demonstrate that the arylsulfonylamino acids would not inhibit the normal GABA-T catalyzed transamination conversion of GABA to succinic semialdehyde (SSA), or the oxidation of SSA.

During DNA biosynthesis, TS catalyzes the conversion of deoxyuridine monophosphate (dUMP) and 5,10-methylenetetrahydrofolate (5,10-CH_2-THF) to its deoxythymidine monophosphate (dTMP) and dihydrofolate (DHF) counterparts. Due to its role in the *de novo* production of dTMP for DNA biosynthesis, TS became an ideal target for drug intervention in cancer chemotherapy, leading to the design of effective antimetabolites such as 5-fluorodeoxyuridine monophosphate (5-FdUMP). From the data exhibited in Table 1, it is evident that the arylsulfonylamino acid ARIs do not significantly inhibit TS at concentrations of 100 μM in contrast to the intrinsic cofactor pyridoxal phosphate ($K_i = 0.8\ \mu M$) and 5-fluorouracil.[26] Thus, it is reasonable to assume that these ARIs would not reduce the levels of background DNA synthesis observed with normal cell growth.

In conclusion, arylsulfonylamino acid ARIs appear to be relatively selective in their enzyme inhibition profile. Arylsulfonylamino acids, displaying a wide range of inhibitory activites in the RLAR assay system, do not significantly inhibit genetically-linked reductases (ALR), catalytically similar reductases (PTXR), functionally distinct oxidoreductases (GR, LDH, GABA-T), or enzymes involved with DNA biosynthesis (TS). The AR selectivity demonstrated by these compounds suggests that they may not inhibit other key metabolic transformations in various cell types, and, therefore, function as selective probes for *in vivo* and *in vitro* studies of the relationship between AR-mediated biochemical changes and the late-onset pathologies of chronic diabetes.

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