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A Diverse Series of Substituted Benzenesulfonamides as Aldose Reductase Inhibitors with Antioxidant Activity: Design, Synthesis, and in Vitro Activity

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We have previously reported the successful replacement of a carboxylic acid functionality with that of a difluorophenolic group on the known aldose reductase inhibitors (ARIs) of 2-(phenylsulfonamido)-acetic acid chemotype. In the present work, based on bioisosteric principles, additional 2,6-difluorophenol and tetrazole, methylsulfonylamide, and isoxazolidin-3-one phenylsulfonamide derivatives were synthesized and tested in vitro in protocols primarily related to the long-term diabetic complications. Most of the compounds were found as ARIs at $IC_{50} < 100$ μM, while the introduction of the 4-bromo-2-fluorobenzyl group in a phenylsulfonamidodifluorophenol structure resulted in a compound (**4c**) presenting a submicromolar inhibitory profile. However, the derivatives of tetrazole, methylsulfonylamine, and the (*R*)-enantiomer of isoxazolidin-3-one did not exhibit appreciable ARI activity. The selectivity of the active ARIs is also discussed. Furthermore, the synthesized compounds exhibited potent antioxidant potential (homogeneous and heterogeneous systems).

Introduction

Aldose reductase enzyme (ALR2,[a] EC 1.1.1.21, AKR1B1) of the polyol metabolic pathway, and especially its inhibition by aldose reductase inhibitors (ARIs), has been gaining attention over the past years from the pharmaceutical community, as it appears to be a promising pharmacotherapeutic target. It was first found to be implicated in the etiology of the long-term diabetic complications, such as retinopathy, nephropathy, and neuropathy.[1] However, to date, emerging reports have suggested that under normal glucose concentration, ALR2 may be up-regulated by factors other than hyperglycemia and therefore be involved also in other pathological processes that have become major threats to human health in the 21st century such as cardiac disorders (including myocardial ischemia and ischemia−reperfusion injury, congestive heart failure, cardiac hypertrophy, and cardiomyopathy), inflammation, mood disorders, renal insufficiency, ovarian abnormalities, and human cancers such as liver, breast, ovarian, cervical, and rectal cancers.[2]

Intense efforts have been directed toward the development of effective ARIs, but still only one (epalrestat) is commercially available and only in Japan.[3] Thus, it is becoming important that novel chemotypes to be developed lacking the poor pharmacokinetic profile or the side effects of the various ARIs that have failed in the clinical trials.[4] Toward this direction, many studies have been reported to date, providing evidence that novel chemotypes, being neither carboxylic acid nor hydantoin derivatives, could be considered as promising hits or lead compounds for further development of potent ARIs with improved pharmacodynamic and pharmacokinetic properties.[5,6]

The present work involves the pharmacochemical study of novel ARIs. The design of the novel compounds was based on the concept of bioisosterism,[7,8] a strategy used for molecular modifications on the lead compound. The sulfonamide ($R_2SO_2NR_2$) functionality is an acceptable functional group in medicinal chemistry when incorporated into putative small-molecule therapeutics, as it has the potential to form several electrostatic interactions with proteins and other targets (during the period of 2006−2008, there were nine published patents in which all or most reported compounds, with a variety of biological responses, contained the sulfonamide functional group).[9] Furthermore, it has been reported that a series of compounds, derivatives of 2-(phenylsulfonamido)acetic acid, show important in vitro ALR2 inhibitory activity.[10−12] 2-(Phenylsulfonamido)acetic acid (compound **1**) was chosen as the lead compound and a series of nonclassical isosters to the acetic acid moiety were used for the latter's replacement in order for potent (with improved pharmacokinetic and pharmacodynamic characteristics) ARIs to be developed.

Diverse functional groups are known for their isosteric relationships with the carboxylate group. The methylsulfonylamine and the tetrazole group mimic the carboxylate group principally in terms of its physicochemical properties related to acidity, although tetrazole is more stable and lipophilic.[13] In order to further balance acidity with lipophilicity, the acetic acid moiety in the lead compound was replaced with that of difluorophenol, a reported bioisoster for carboxylate,[14,15] and a limited number of such compounds have already been published by our research group.[5] Furthermore, isoxazolidin-3-one with a calculated pK_a of 8.26[16] was studied as a putative isostere for the carboxylate group, as it has a structure relevant to that of hydantoin or succinimide derivatives (known classes of ARIs).

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[a] Abbreviations: AKR, aldo−keto reductase superfamily; ALR2, aldose reductase; ALR1, aldehyde reductase; ARIs, aldose reductase inhibitors; SAR, structure−activity relationship.
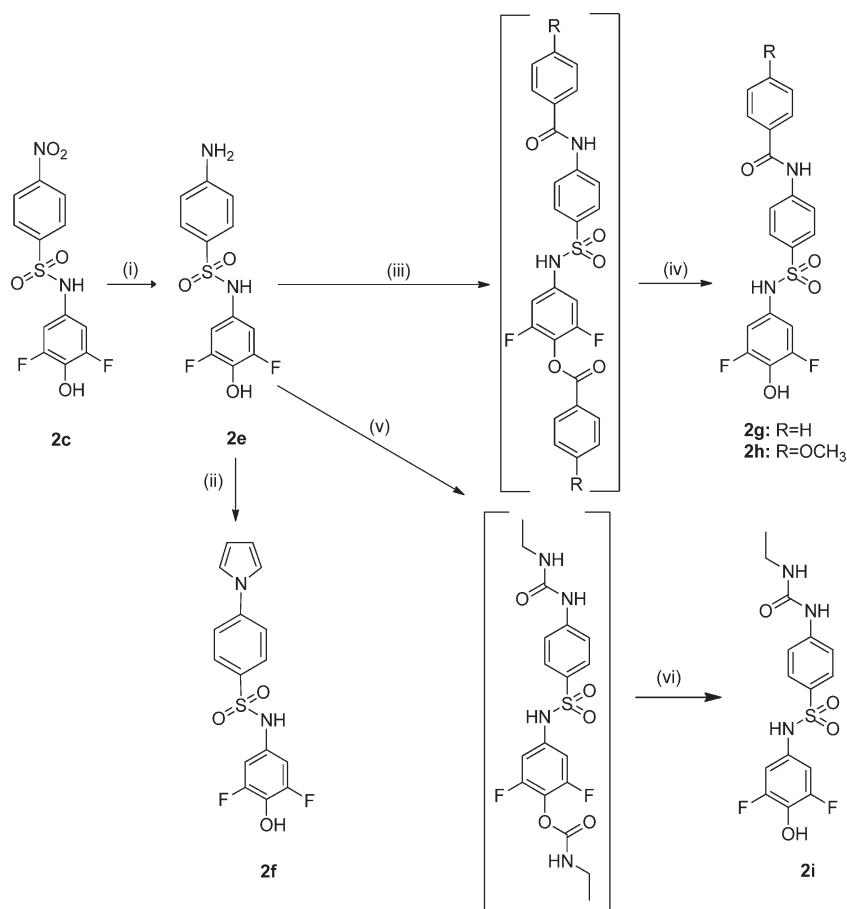
The data derived from the in vitro experiments revealed important bioisosterism in the case of the difluorophenol derivatives (compounds **2a–i**), as well as in the case of (*S*)-isoxazolidin-3-one derivative **9b** but not the (*R*)-isoxazolidin-3-one derivative **9a**. There is ample evidence that ALR2 may stereoselectively recognize the inhibitor. Some examples of the active isomers of known ARIs are sorbinil (S),[17] fidarestat (2S4S),[18,19] and ranirestat (R).[20,21] The phenylsulfonamides, derivatives of the tetrazole moiety (compounds **5** and **7**), and the methylsulfonylamine derivative (compound **8**) did not prove to be strong ARIs.

Scheme 1. Synthesis of *N*-(3,5-Difluoro-4-hydroxyphenyl)benzenesulfonamides **2a–d**[a]

2a: R=H
2b: R=OCH$_3$
2c: R=NO$_2$
2d: R=CF$_3$
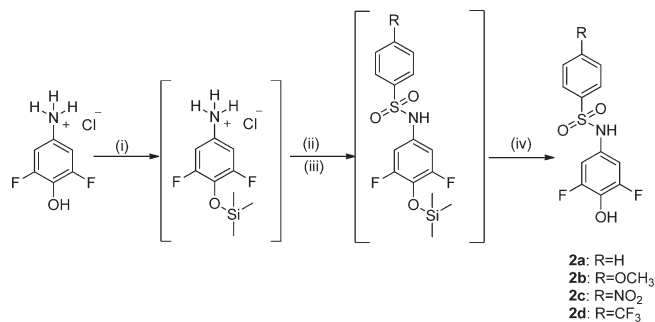
[a] Reagents and conditions: (i) (CH$_3$)$_3$SiCl/THF; (ii) ArSO$_2$Cl, DMAP/THF; (iii) Et$_3$N/THF; (iv) H$_2$O.

Reviews of the literature about ARIs under development and those already in clinical trials (for example, ranirestat, multicenter, phase II–III study, http://clinicaltrials.gov/ct2/show/NCT00927914) highlight the presence of the halogenated benzene ring as a substituent that importantly contributes to the improvement of the ALR2 inhibitory activity.[4,22] This observation, together with the increasing interest in the employment of fluorine at medical and pharmaceutical agents,[23] led to the choice of 4-bromo-2-fluoromethylbenzyl moiety as a potent "privileged scaffold"[24] that could be introduced to the phenylsulfonamidedifluorophenol derivatives (compounds **4a–c**). Furthermore, N-sulfonylation was also investigated (compound **4d**).

Regarding the desirable pharmacodynamic profile of a novel ARI, the following points are highlighted:

(a) ARIs that appear to be promising during in vitro studies often fail to proceed any further into clinical use because of side effects, which in many cases have been attributed to the lack of selectivity for other enzymes. ALR2 is a member of the aldo–keto reductase (AKR) superfamily,[25] and another member of the AKR superfamily is aldehyde reductase (ALR1, EC 1.1.1.2, AKR1A1). It is believed that the unfavorable profile of many ARIs in clinical trials is due to their concurrent inhibition of these closely related AKRs.[26]

(b) The limited efficacy of currently known ARIs may also be related to the post-translational modification of ALR2 activity, which is caused by the hyperglycemia-induced oxidative stress. This modification involves oxidation of a critical

Scheme 2. Synthesis of *N*-(3,5-Difluoro-4-hydroxyphenyl)benzenesulfonamides **2e–i**[a]

2g: R=H
2h: R=OCH$_3$

**2c**　　**2e**　　**2f**　　**2i**

[a] Reagents and conditions: (i) cyclohexene, Pd/C /(CH$_3$CH(OH)CH$_3$; (ii) 2,5-dimethoxytetrahydrofuran, 4-chloropyridine hydrochloride/1,4-dioxane; (iii) RC$_6$H$_4$COCl, Et$_3$N/1,4-dioxane; (iv) 5% aq NaOH; (v) ethyl isocyanate/1,4-dioxane; (vi) 5% aq NaOH.

Scheme 3. Synthesis of *N*-(4-Bromo-2-fluorobenzyl)-*N*-(3,5-difluoro-4-hydroxyphenyl)arylsulfonamides (**4a−c**) and *N*-(3,5-Difluoro-4-hydroxyphenyl)-*N*-(phenylsulfonyl)benzenesulfonamide (**4d**)[a]
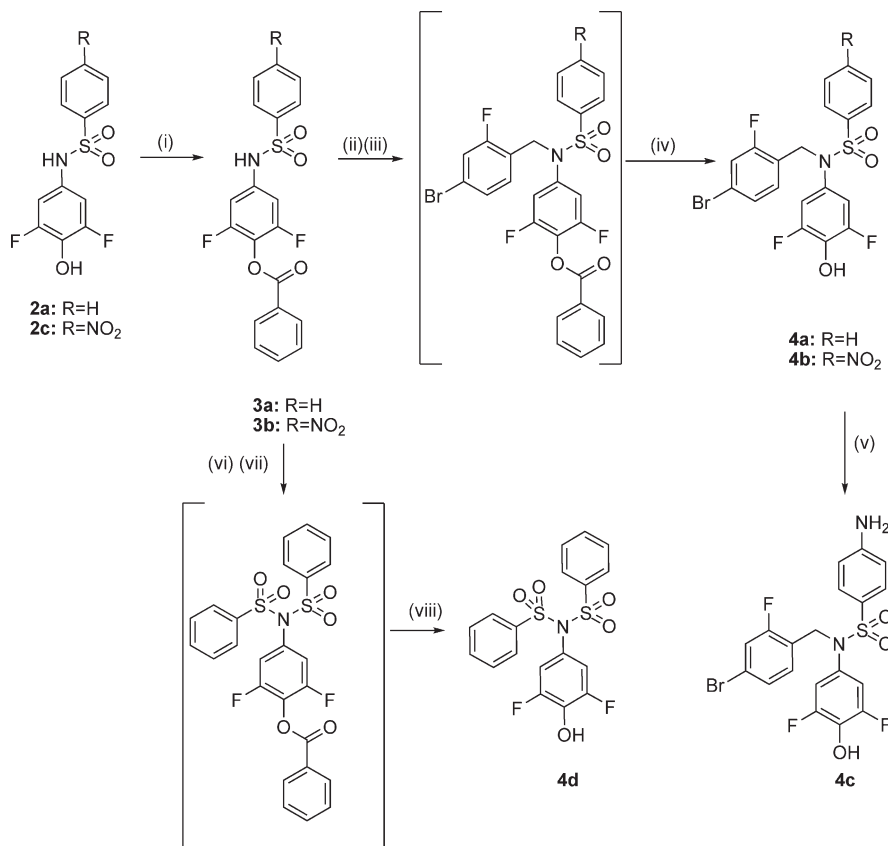
a Reagents and conditions: (i) PhCOCl, Et$_3$N/1,4-dioxane; (ii) NaH/DMF; (iii) 4-bromo-1-(bromomethyl)-2-fluorobenzene/DMF; (iv) 5% aq NaOH; (v) cyclohexene, Pd/C/(CH$_3$CH(OH)CH$_3$); (vi) NaH/THF; (vii) PhSO$_2$Cl/THF; (viii) 2.5% NaOH.

active cysteine thiol (Cys298), which regulates both substrate and inhibitor binding.[27,28] The resulting oxidized form of ALR2 shows an increase in K_m for aldehyde substrates and a marked reduction in sensitivity to ARIs. Additionally and taking into consideration the detoxification role of ALR2 against oxidative stress and its potential role as an antioxidant enzyme (catalysis of the reduction of a wide range of aldehydes generated from lipid peroxidation),[29] during treatment with ARIs the concurrent administration of antioxidants could contribute on the one hand in keeping the enzyme in its reduced form and on the other hand in counterbalancing its inhibition. Thus, a bifunctional compound that would combine ALR2 inhibitory activity and antioxidant potential could be of interest as a pharmacotherapeutic agent.

On the basis of the above presented data, the novel compounds developed were tested in vitro for their ALR2 and ALR1 inhibitory activity and their antioxidant potential in homogeneous and heterogeneous systems.

Chemistry

Compound **1** was prepared according to the standard method involving reaction of commercially available benzenesulfonyl choride with the appropriate amino acid (glycine) in aqueous NaOH, which has been described by De Ruiter et al.[10] In a similar manner compound **5** was also prepared using 1*H*-tetrazol-5-amine as the nucleophilic reactant but with a lower yield due to possible formation of sulfonylcarbamimidic azide as reported by Peet et al.[30]

The general synthetic procedure of the *N*-(3,5-difluoro-4-hydroxyphenyl)benzenesulfonamides **2a−d** is illustrated in Scheme 1. The presented compounds **2a−c** were prepared in a one-pot reaction as described previously,[5] and **2d** was prepared using the same procedure.

The synthesis of compounds **2e−i** are presented in Scheme 2. Compound **2e**, also reported previously,[5] was prepared by a heterogeneous catalytic hydrogen transfer reaction, and the formation of the pyrrol ring of compound **2f** was attained by a modified Clauson−Kaas type reaction with the use of 2,5-dimethoxytetrahydrofuran and 4-chloropyridine hydrochloride as a catalyst.[15] The synthetic route for the preparation of the sulfamoylphenylbenzamide derivatives **2g,h** involved a reaction between **2e** and commercially available benzenesulfonyl chlorides, followed by a selective hydrolysis in basic conditions of the intermediate esters. In a similar manner, the ureido derivative **2i** was prepared by using an excess of ethyl isocyanate for the urea formation,[31] followed by selective hydrolysis in basic conditions. Ultrasound irradiation in the urea formation step was found to increase the overall yield.

Phenyl benzoates (**3a**, **3b**) were prepared by phenol benzoylation catalyzed with triethylamine in dioxane[32] and were further treated with sodium hydride, followed by alkylation[33] with 4-bromo-1-(bromomethyl)-2-fluorobenzene and hydrolysis in basic conditions to give the *N*-(4-bromo-2-fluorobenzyl)-*N*-(3,5-difluoro-4-hydroxyphenyl)arylsulfonamides (**4a**, **4b**) as shown in Scheme 3. Compound **4c** was obtained from **4b** with a heterogeneous catalytic hydrogen transfer reaction. Furthermore, from **3a** and with treatment with

Scheme 4. Synthesis of Compounds **6a**, **6b**, and **7**[a]
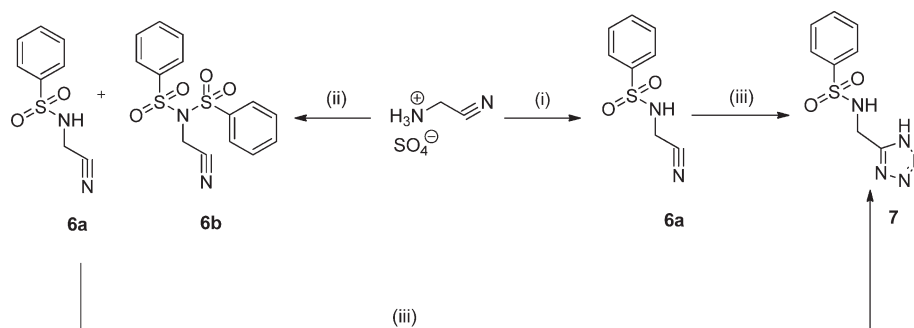
[a] Reagents and conditions: (i) PhSO$_2$Cl, Et$_3$N, DMAP/THF; (ii) PhSO$_2$Cl, DMAP/THF; (iii) (CH$_3$)$_3$SiN$_3$, (CH$_3$CH$_2$CH$_2$CH$_2$)$_2$Sn=O/toluene.

Scheme 5. Synthesis of *N*-(Methylsulfonyl)-2-(phenylsulfonamido)acetamide **8**[a]
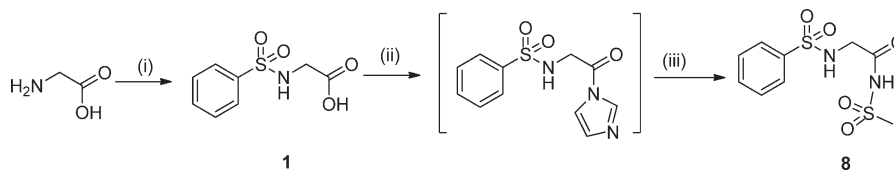
[a] Reagents and conditions: (i) PhSO$_2$Cl, aq NaOH; (ii) CDI/THF; (iii) CH$_3$SO$_2$NH$_2$, DBU/THF.

sodium hydride and N-sulfonylation with benzenesulfonyl chloride followed by hydrolysis in mild basic conditions in order to avoid the hydrolysis of the *N,N*-disulfonylated product,[34] **4d** was obtained.

For the synthesis of tetrazole **7** a reported one-step synthetic method for the transformation of nitriles into 5-substituted tetrazoles[35] was adopted. The method involves the use of trimethylsilyl azide as the azide source and the use of catalytic dialkyltin oxide. For the preparation of the appropriate nitrile **6a** commercial aminoacetonitrile sulfate, benzenesulfonyl chloride and a catalytic amount of triethylamine and *N,N*-dimethylpyridin-4-amine (DMAP) in tetrahydrofurane were used (method A). By modification of the reaction conditions (method B), improvement of the overall yield was achieved but *N*-(cyanomethyl)-*N*-(phenylsulfonyl)benzenesulfonamide (**6b**) was obtained as a side product. The previous synthetic routes mentioned are presented in Scheme 4.

The *N*-(methylsulfonyl)-2-(phenylsulfonamido)acetamide (**8**) was prepared according to a reported procedure by Yuan and Silverman[13] that involves treatment of **1** with carbonyldiimidazole followed by treatment with methanesulfonamide in the presence of 1,8-diazabicyclo[5.4.0]undec-7-ene as presented in Scheme 5.

For the synthesis of compounds **9a,b** commercial D- or L-cycloserine was allowed to react with benzenesulfonyl chloride in an aqueous buffer solution with pK_a = 10.[36]

Results and Discussion

Aldose Reductase Inhibitory Activity. The target compounds were tested for their ability to inhibit rat lenses ALR2. It has been shown that the human and rat sequences of this enzyme are characterized by 81% identity and 89% homology, while the proposed active sites of both enzymes are identical.[37] The performed assay was based on the spectrophotometric monitoring of NADPH oxidation, which is proven to be quite a reliable method.[38] Results are shown in Table 1.

The inhibitory activity of compounds **2a**, **5**, **7**, **8**, **9a**, and **9b**, which differ in the R$_3$ substitution and are all isosteres to

the acetic acid functionality of the lead compound **1**, indicates effective bioisosteric replacement in the case of the difluorophenol derivative **2a** (IC$_{50}$ = 32.9 μM). Compounds **5**, **7**, **8**, and **9a** are weak ARIs, while **9b** exhibits notable activity (IC$_{50}$ = 71.2 μM). It is of interest that there is significant difference in the inhibitory activity of the chiral compounds **9a** and **9b**. As the (*S*)-enantiomer **9b** was found to be 2 times more potent than the (*R*)-enantiomer **9a**, the stereocenter in the cycloserine moiety seems to have an impact on the ALR2 inhibitory activity and therefore chilarity could be considered as a useful tool in the lead optimization process, at least in the case of this class of ARIs.

Regarding the difluorophenol derivatives **2a−i** the R$_1$ substitution seems to play a significant role in the biological response. Methoxy (compound **2b**) and amino (compound **2e**) substituents increased ALR2 inhibitory activity with amino (IC$_{50}$ = 14.1 μM) being slightly superior to methoxy (IC$_{50}$ = 15.5 μM). In the case of substituents that are electron acceptors, as in the case of nitro (**2c**) and trifluoro (**2d**) substituent, no improvement in the inhibitory activity was noticed. A capability for structural modifications is provided by the amino substituent of the potent ARI **2e**. Initially, we introduced a pyrrole ring (compound **2f**) as an attempt to increase the total aromatic area of the inhibitor, but there was no improvement in the biological response. On the contrary, an improvement of efficacy was noticed in the case of introducing an additional benzene ring in compounds **2g** (IC$_{50}$ = 7.9 μM) and **2h** (IC$_{50}$ = 12.0 μM). In the case of **2g** and **2h** the presence of a carbonyl group between the two aromatic regions seems to be of significance because it may be involved in "charge-transfer interactions" in the active site of ALR2, as this has been previously postulated.[40,41]

The N-alcylation of the main sulfonamide scaffold with the "privileged" structure of 4-bromo-2-fluoromethylbenzene (R$_2$ substitution) resulted in the development of strong ARIs: **4a** (IC$_{50}$ = 5.7 μM), **4b** (IC$_{50}$ = 9.0 μM), and **4c** (IC$_{50}$ = 0.397 μM), with **4c** to be the most potent one of the whole series of the synthesized compounds in the present work. On the other hand, a farther N-sulfonylation (compound **4d**) did not significantly improve the inhibitory activity.
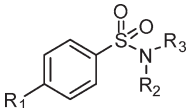
Table 1. ALR2 Inhibitory Activity Data

comp.	R_1	R_2	R_3	% inhibition $(\pm SD)^a$ (C=100μM)	$IC_{50} (\pm SD)^a$ μM
1	H	H	CH$_2$COOH	51.7(±1.8)	86.7(±4.12)[b]
2a	H	H	difluorohydroxyphenyl	71.3(±1.2)	32.9 (±0.04)[c]
2b	OCH$_3$	H	difluorohydroxyphenyl	79.5(±2.1)	15.5 (±0.12)[c]
2c	NO$_2$	H	difluorohydroxyphenyl	63.9(±0.8)	44.4 (±1.91)[c]
2d	CF$_3$	H	difluorohydroxyphenyl	55.8(±1.3)	91.5 (±3.24)
2e	NH$_2$	H	difluorohydroxyphenyl	84.1(±1.6)	14.1 (±0.46)[c]
2f	pyrrolyl	H	difluorohydroxyphenyl	61.1(±3.1)	57.1 (±2.90)
2g	NHCOPh	H	difluorohydroxyphenyl	87.7(±1.3)	7.9 (±0.72)
2h	NHCO-C$_6$H$_4$-pOCH$_3$	H	difluorohydroxyphenyl	77.9 (±0.8)	12.0 (±0.32)
2i	NHCONHCH$_2$CH$_3$	H	difluorohydroxyphenyl	63.6 (±1.8)	37.8 (±1.10)
4a	H	ethyl-fluoro-bromophenyl	difluorohydroxyphenyl	74.6(±3.6)	5.7(±0.05)
4b	NO$_2$	ethyl-fluoro-bromophenyl	difluorohydroxyphenyl	75.1(±2.4)	9.0 (±0.07)
4c	NH$_2$	ethyl-fluoro-bromophenyl	difluorohydroxyphenyl	87.4(±4.7)	0.397(±0.015)
4d	H	SO$_2$Ph	difluorohydroxyphenyl	65.1(±1.1)	28.1(±1.94)
5	H	H	tetrazolyl	44.8(±3.2)	
7	H	H	ethyl-tetrazolyl	23.8(±2.7)	
8	H	H	CH$_2$CONHSO$_2$CH$_3$	32.9(±2.1)	
9a	H	H	isoxazolidinone	25.7(±1.1)	
9b	H	H	isoxazolidinone	48.9(±1.1)	71.2(±1.3)
sorbinil				44.7(±4.37)% inhibition at C=0.25μM[d]	

$^a n = 3.$ ^{b} Reported IC_{50}: 134 μM.[10] ^{c} Reported by Alexiou et al.[5] ^{d} Reported IC_{50}: 0.25 μM.[39]

Aldehyde Reductase Inhibitory Activity. Another member of the AKR superfamily is aldehyde reductase (ALR1), and it is believed that the unfavorable profile of many ARIs in clinical trials is due to their concurrent inhibition of the closely related ALR1.[19] ALR1 and ALR2 share a high degree of amino acid sequence (∼65%) and structural homology.[42,43] The target compounds were tested for their ability to inhibit rat kidney ALR1, and the obtained data are presented in Table 2. Compounds **1**, **5**, **7**, **8**, **9a**, and **9b**, which were found to be weak ARIs, are also weak inhibitors of ALR1, with the notable example being **9b**, which possesses the highest selectivity ratio expressed as (% inhibition (*C* = 100 μM) of ALR2)/(% inhibition (*C* = 100 μM) of ALR1). As for the difluorophenol derivatives **2a–i** and **4a–d** strong ALR1 inhibitory activity was found in many cases, indicating lack of selectivity for these compounds.

Table 2. ALR1 Inhibitory Data and Selectivity Ratios

compd	% inhibition $\pm$ SD^a (*C* = 100 μM)	IC$_{50}$ $\pm$ SD,^{a} μM	(IC$_{50}$)^{ALR1}/ (IC$_{50}$)^{ALR2}	(% inh^{ALR2})/ (% inh^{ALR1})
1	30.52 $\pm$ 2.75			1.69
2a	79.23 $\pm$ 1.35	19.6 $\pm$ 1.4	0.60	0.89
2b	83.02 $\pm$ 0.88	23.8 $\pm$ 1.0	1.54	
2c	55.71 $\pm$ 1.21	132.9 $\pm$ 12.9	2.99	
2d	50.65 $\pm$ 1.22	105.2 $\pm$ 2.61	1.15	
2e	85.40 $\pm$ 1.31	10.3 $\pm$ 0.23	0.73	
2f	41.67 $\pm$ 2.35	280.7 $\pm$ 2.12	4.92	
2g	81.13 $\pm$ 2.05	6.9 $\pm$ 0.9	0.87	
2h	71.68 $\pm$ 0.76	12.4 $\pm$ 0.23	1.03	
2i	68.48 $\pm$ 3.07	25.5 $\pm$ 3.02	0.67	
4a	69.16 $\pm$ 0.44	11.9 $\pm$ 0.58	2.09	
4b	69.78 $\pm$ 0.44	15.42 $\pm$ 0.06	1.71	
4c	95.26 $\pm$ 0.26	0.323 $\pm$ 0.013	0.81	
4d	93.79 $\pm$ 0.69	9.7 $\pm$ 0.68	0.35	
5	21.71 $\pm$ 1.73			2.06
7	19.9 $\pm$ 0.72			1.20
8	12.24 $\pm$ 0.69			2.69
9a	22.43 $\pm$ 2.15			1.15
9b	2.67 $\pm$ 0.57			18.31
valproic acid		56.1 $\pm$ 2.7^b		

^{a} *n* = 3. ^{b} Reported IC$_{50}$: 50.1 μM.[44]

One of the primary roles of ALR1 in diabetes (as well as of ALR2) is the detoxification of tissues from the reactive α-oxoaldehyde glycating agents, like methylglyoxal and 3-deoxyglucosone.[45−47] However, other enzymes, such as glyoxalase-I, betaine aldehyde dehydrogenase, 2-oxoaldehyde dehydrogenase, and the dimeric dihydrodiol dehydrogenase could also act as the detotoxification enzymes for these glycating agents.[48,49] It is noted that the dimeric dihydrodiol dehydrogenase is not inhibited by the established ALR1 inhibitors (valproic acid and barbiturates) or the classical ARIs (sorbinil and tolrestat).[50] Furthermore, kidney polyols may be generated by both aldose and aldehyde reductase, with ALR1 significantly contributing to the polyol production in the kidney cortex, the predominant site of diabetes-linked kidney lesions.[51] It is noted that ALR1, but not ALR2, is localized in the kidney cortex.[52] Although a degree of selectivity toward ALR2 maybe important for a successful ARI, on the basis of the above points, this selectivity feature should not be considered as a negating factor for the identification of an initial lead ARI.

DPPH Scavenging Activity. The radical scavenging potential of the active ARIs **2a−i** and **4a−d** was assessed in vitro by using the model reaction with the stable free radical of 2,2-diphenyl-1-picrylhydrazyl (DPPH). The scavenging activity data are expressed as IC$_{50}$ values, EC$_{50}$ ratios, and an initial rate of interaction at equimolar concentration of DPPH and antioxidant (*C* = 0.2 mM). Results are summarized in Table 3. In this homogeneous system of the ethanol solution of DPPH (*C* = 0.2 mM), antioxidant activity stems from an intrinsic chemical reactivity toward radicals.[53] According to the derived data, the tested compounds possess lower antioxidant activity, but still significant, compared to the standard antioxidant Trolox. There is a considerable difference in the kinetics of the reaction with the DPPH between the tested molecules and Trolox. The reaction rate is significantly slower than that with Trolox. The steady state is achieved after 270 min, while for Trolox it is achieved after 30 min (reported time of 20 min by Silva et al.[54]) and remains constant for 270 min. Furthermore, the approximate stoichiometry of the reactions (σ, number of reduced DPPH molecules per one molecule of antioxidant), defined as 1/[(2)(EC$_{50}$)],[55] was found to be greater than 1 and apparently not equal to their available hydroxyl groups in all cases. A putative mechanism of a more complex reaction, justifying

Table 3. Antioxidant Activity Data

compd	homogeneous system of DPPH			heterogeneous system of DOPC liposomes
	IC$_{50}$ $\pm$ SD,^{a} μM	EC$_{50}$^{b}	initial rate^c $\pm$ SD^a	IC$_{50}$ $\pm$ SD,^{a} μM
2a	49 $\pm$ 0.01^d	0.25^d	0.498 $\pm$ 0.005^d	95.9 $\pm$ 8.5^d
2b	45.2 $\pm$ 0.01	0.23	0.661 $\pm$ 0.058	55.1 $\pm$ 2.6
2c	54.8 $\pm$ 0.08	0.27	0.465 $\pm$ 0.024	45.7 $\pm$ 0.7
2d	45.1 $\pm$ 0.2	0.23	0.490 $\pm$ 0.037	66.0 $\pm$ 1.3
2e	44.7 $\pm$ 0.13	0.22	0.777 $\pm$ 0.037	48.1 $\pm$ 4.3
2f	48.6 $\pm$ 0.23	0.24	0.489 $\pm$ 0.036	97.1 $\pm$ 3.4
2g	49.7 $\pm$ 0.1	0.25	0.817 $\pm$ 0.032	68.6 $\pm$ 1.6
2h	56.9 $\pm$ 0.2	0.26	0.995 $\pm$ 0.039	70.7 $\pm$ 3.9
2i	51.6 $\pm$ 0.1	0.28	0.859 $\pm$ 0.06	81.7 $\pm$ 4.3
4a	60.9 $\pm$ 0.1	0.30	1.000 $\pm$ 0.033	66.0 $\pm$ 6.1
4b	61.5 $\pm$ 0.1	0.31	0.974 $\pm$ 0.055	61.82 $\pm$ 5.42
4c	61.9 $\pm$ 0.06	0.31	0.977 $\pm$ 0.043	73.1 $\pm$ 6.7
4d	63.2 $\pm$ 0.3	0.32	0.967 $\pm$ 0.031	138.9 $\pm$ 13.1
Trolox	37 $\pm$ 0.09	0.19^e	1.809 $\pm$ 0.004	93.5^f

^{a} *n* = 3. ^{b} IC$_{50}$/C_{DPPH}. ^{c} Absorbance decrease at 517 nm after 30 s. ^{d} Reported data by Alexiou et al.[5] ^{e} Reported by Ancerewicz et al.[57] ^{f} Reported by Stefek et al.[58]

such a stoichiometry, could involve stabilization of the generated phenoxyl radicals of the tested compounds to the more stable sulfonamide/nitrogen centered radical due to the "capto-dative effect" present[56] and further reactions such as coupling, fragmentation, and intermolecular addition. In the case of compounds **4a−d** there is no possibility of further stabilization of the generated hydroxyl radical, and this is depicted on their higher IC_{50} values and on the higher initial velocity compared to compounds **2a−i** .

Inhibition of Lipid Peroxidation. In membranes, the relative antioxidant reactivity is probably different from a homogeneous system, since it is determined by additional factors, such as location of the antioxidant and radicals, and ruled to an extent by the partition ratios between water and lipophilic compartments. As an indicative heterogeneous assay, the antioxidant inhibitory efficiency of compounds **2a−i** and **4a−d** was evaluated in the system of unilamellar DOPC liposomes (C = 0.8 mM) oxidatively stressed by peroxyl radical generated in the aqueous phase by thermal decomposition of the hydrophilic azo initiator 2,2′-azobis-(2-amidinopropane) hydrochloride. Results are shown in Table 3. It is apparent from the inhibition data, which are expressed as IC_{50} values, that the tested compounds are strong antioxidants, comparable to that of the known antioxidant Trolox or even better in most cases.

Conclusion

The presented pharmacochemical study of a series of compounds, sulfonamides with diverse functional groups, shed light into the concept of the bioisterism and its application in drug design and indicated a series of ARIs with an IC_{50} of < 100 μM. N-Alcylation of the main difluorophenolic sulfonamide scaffold with the "privileged" structure of 4-bromo-2-fluoromethylbenzene resulted in a compound (**4c**) presenting submicromolar inhibitory profile (IC_{50} = 0.397 μM), antioxidant potential, and improved physicochemical profile. The latter bifunctional compound, although not selective, could be considered as a lead for further optimization, aimed for the design and development of potent ARIs with improved pharmacodynamic and pharmacokinetic profile. At this point, although a degree of selectivity toward ALR2 maybe important for a successful ARI, on the basis of the above-mentioned points, this selectivity feature should not be considered as a negating factor for the identification of an initial lead ARI.

Moreover, the data derived from the in vitro experiments revealed notable bioisosterism in the case of (*S*)-isoxazolidin-3-one derivative **9b** but not the (*R*)-isoxazolidin-3-one derivative **9a**. As the (*S*)-enantiomer **9b** was found to be 2 times more potent than the (*R*)-enantiomer **9a**, the stereocenter in the cycloserine moiety seems to have an impact on the ALR2 inhibitory activity, and therefore, chilarity could be considered as a useful tool in the lead optimization process, at least in the case of this class of ARIs that should be further explored.

Experimental Section

Chemistry. Unless otherwise stated, all commercial reagents were from Aldrich or Fluka. Melting points were determined in open glass capillaries using a Mel-Temp II apparatus. UV spectra were recorded either on a Perkin-Elmer 554 or on a Hitachi U-2001 spectrophotometer. Characterization of compounds was established by a combination of IR and NMR spectrometry techniques. IR spectra were obtained on a Shimadzu FTIR-8101M

spectrophotometer. ^{1}H NMR spectra were obtained on a Bruker AM-300 spectrometer with internal TMS standard. The purity of the compounds was determined by combustion analysis (elemental analysis) and was confirmed to be ≥95% for all compounds. Elemental analyses (C, H, N) were performed at Galbraith Laboratories, Inc., Knoxville, TN, or with a Perkin-Elmer 2400 CHN analyzer in the Department of Organic Chemistry, School of Chemistry, Aristotle University of Thessaloniki, Greece. All reactions were routinely checked by TLC on silica gel Merck 60 F$_{254}$, and flash chromatography was performed on silica gel Merck 9385. Compounds **2a−c** and **2e** have been previously synthesized by us.[5]

2-(Phenylsulfonamido)acetic Acid (1). Compound **1** was prepared by the standard method already reported.[10] Yield: 2.563 g (59%). Mp 169−170 °C (lit. 170−171 °C[59]). IR (Nujol): 3324 (COOH), 1735 (C=O) cm^{-1}. ^{1}H NMR (CDCl$_3$/DMSO-d_6): 3.7 (s, 2H, -CH_2COOH), 6.8−8 (m, 7H, -COOH, Ar-H, -SO$_2$NHCH_2-).

N-(3,5-Difluoro-4-hydroxyphenyl)-4-(trifluoromethyl)benzenesulfonamide (2d). To a suspension of 4-amino-2,6-difluorophenol hydrochloride (275 mg, 1.5 mmol) in THF (78 mL), chlorotrimethylsilane (0.25 mL, 1.9 mmol) was added. The mixture was vigorously stirred under a nitrogen atmosphere for 48 h. Then 4-trifluoromethylbenzenesulfonyl chloride (464.5 mg, 1.9 mmol) and N,N-dimethylpyridin-4-amine (DMAP, 48 mg, 0.4 mmol) were added and the mixture was vigorously stirred under a nitrogen atmosphere for 24 h. Triethylamine (0.21 mL, 1.5 mmol) in THF (11.5 mL) was then added dropwise, and the stirring was continued under a nitrogen atmosphere for 24 h. Finally, the reaction mixture was poured into a stirred water/ice mixture (~270 mL), and after 2 h of stirring it was extracted with CH$_2$Cl$_2$ (3 × 50 mL). The organic layer was collected, and the aqueous layer, after saturation with NaCl, was farther extracted with CH$_2$Cl$_2$ (2 × 20 mL). The combined organic phases were washed with saturated aqueous NaCl solution, dried over anhydrous Na$_2$SO$_4$, and evaporated under reduced pressure. The residue was flash-chromatographed with petroleum ether/EtOAc (2.5:1), and the title compound was obtained as a beige solid. An analytical sample was prepared by recrystallization from CH$_2$Cl$_2$/CH$_3$CN/petroleum ether. Yield: 320 mg (60%). Mp 187−188 °C. IR (Nujol): 3474-3175 (-NH- and -OH), 1374 (S=O), 1157 (S=O) cm^{-1}. ^{1}H NMR (CDCl$_3$/DMSO-d_6): δ 6.7 (d, 2H, difluorophenyl-2,6H, J = 8.2 Hz), 7.6−8.0 (m, 4H, trifluoromethylphenyl-H), 9.2−9.3 (br s, 1H, phenyl-OH), 9.8−10.0 (br s, 1H, -SO$_2$NH-). Anal. (C$_{13}$H$_8$F$_5$NO$_3$S) C, H, N.

N-(3,5-Difluoro-4-hydroxyphenyl)-4-(1H-pyrrol-1-yl)benzenesulfonamide (2f). To a solution of **2e** (202 mg, 0.67 mmol) in 1, 4-dioxane (10 mL) were added 2,5-dimethoxytetrahydrofuran (0.15 mL, 1.1 mmol) and 4-chloropyridine hydrochloride (154 mg, 1.03 mmol). The mixture was refluxed under a nitrogen atmosphere for 4 h with vigorous stirring, cooled to room temperature, and evaporated under reduced pressure. Most of the residue was dissolved in CH$_2$Cl$_2$ by the gradual addition of several portions of this solvent, dried over anhydrous Na$_2$SO$_4$, and concentrated under reduced pressure. The residue was flash-chromatographed with petroleum ether/EtOAc (3:1) to provide the title compound as a white solid. An analytical sample was prepared by recrystallization from CH$_2$Cl$_2$/CH$_3$CN/petroleum ether. Yield: 165 mg (70%). Mp 202−203 °C. IR (Nujol): 3340-3221 (-NH- and -OH), 1371 (S=O), 1153 (S=O) cm^{-1}. ^{1}H NMR (CDCl$_3$/DMSO-d_6): δ 6.2−6.5 (m, 4H, pyrrol-H), 6.7 (d, 2H, difluorophenol-2,6H, J = 8.7 Hz), 7.2−7.8 (m, 4H, 4-pyrrolylbenzenesulfonamidophenyl-H), 9.5−9.6 (br s, 1H, phenyl-OH), 9.8−10 (br s, 1H, -SO$_2$NH-). Anal. (C$_{16}$H$_{12}$F$_2$N$_2$O$_3$S·0.1CH$_2$Cl$_2$) C, H, N.

General Procedure for the Preparation of Sulfamoylphenylbenzamides (2g,h). To a solution of **2e** (295 mg, 0.97 mmol) in 1,4-dioxane (50 mL) were added triethylamine (0.27 mL, 1.94 mmol) and the appropriate aryl chloride (1.94 mmol) (benzoyl chloride, 0.23 mL; 4-methoxybenzoyl chloride, 0.27 mL). The mixture was refluxed under a nitrogen atmosphere for 4 h. After

removal of the solvent under reduced pressure, the residue was diluted in CH_2Cl_2 (50 mL), washed with H_2O (3 × 30 mL), brine (2 × 20 mL), and concentrated under reduced pressure. The crude residue was diluted in 1,4-dioxane (15 mL), and 5% cold aqueous solution of NaOH (15 mL) was added dropwise. The mixture was stirred vigorously at 0 °C (ice bath) under nitrogen atmosphere for 2 h. Without removing the ice bath, the reaction mixture was then acidified with an aqueous 10% HCl solution. The mixture was stirred with CH_2Cl_2 (100 mL) for 15 min, and after the two phases were separated, the aqueous phase was further extracted with CH_2Cl_2 (2 × 20 mL). The combined organic extracts were washed with H_2O (3 × 20 mL), saturated aqueous $NaHCO_3$ solution (2 × 20 mL), brine (2 × 20 mL), dried over anhydrous Na_2SO_4, and concentrated under reduced pressure. The crude residue was flash-chromatographed with a mixture of petroleum ether/EtOAc (3:1 and 2.5:1, respectively). Analytical samples were obtained by recrystallization from CH_2Cl_2/CH_3CN/petroleum ether as white solids.

N-(4-(N-(3,5-Difluoro-4-hydroxyphenyl)sulfamoyl)phenyl)-benzamide (2g). Yield: 133 mg (34%). Mp 244−245.5 °C. IR (Nujol): 3520−3345 (-NH-), 3160-3070 (-OH), 1670 (C=O), 1370 (S=O), 1160 (S=O) cm^{-1}. ^{1}H NMR (CDCl$_3$/DMSO-d_6): δ 6.75 (d, 2H, difluorophenol-2,6H, J = 8.7 Hz), 7.4−8.2 (m, 9H, aromatic-H), 9.4 (br s, 1H, phenyl-OH), 9.8 (s, 1H, -SO$_2$NH-), 10.4 (br s, 1H, -CONH-). Anal. (C$_{19}$H$_{14}$F$_2$N$_2$O$_4$S·0.11CH$_2$Cl$_2$) C, H, N.

N-(4-(N-(3,5-Difluoro-4-hydroxyphenyl)sulfamoyl)phenyl)-4-methoxybenzamide (2h). Yield: 108 mg (25%). Mp 229−231 °C. IR (Nujol): 3400−3196 (-NH- and -OH), 1670 (C=O), 1372 (S=O), 1162 (S=O), 1098 (Ar-O-CH$_3$) cm^{-1}. ^{1}H NMR (CDCl$_3$/DMSO-d_6): δ 3.8 (s, 3H, -OCH_3), 6.58−7.14 (m, 4H, difluorophenyl-2,6H and methoxyphenyl-3,5H), 7.54−8.2 (m, 6H, N-sulfamoylophenyl-H and methoxyphenyl-2,6H), 9.48 (br s, 1H, phenyl-OH), 9.87 (br s, 1H, -SO$_2$NH-), 10.24 (br s, 1H, -CONH-). Anal. (C$_{20}$H$_{16}$F$_2$N$_2$O$_5$S·0.2CH$_2$Cl$_2$) C, H, N.

N-(3,5-Difluoro-4-hydroxyphenyl)-4-(3-ethylureido)benzenesulfonamide (2i). Ethyl isocyanate (0.185 mL, 2.33 mmol) was added in a solution of **2e** (116 mg, 0.38 mmol) in 1,4-dioxane (30 mL), and the mixture was stirred under a nitrogen atmosphere and ultrasound irradiation for 8.5 h. More ethyl isocyanate (0.1 mL, 1.26 mmol) was added, and the reaction mixture was heated in 80 °C under nitrogen atmosphere and without ultrasound irradiation for 7 h. After removal of the solvent under reduced pressure the crude residue was diluted in 1,4-dioxane (10 mL) and a 5% aqueous solution of NaOH (10 mL) was added dropwise. The mixture was stirred under nitrogen atmosphere for 15 min in room temperature and then was cooled (ice bath) and acidified with an aqueous 10% HCl solution. The mixture was treated with CH_2Cl_2 (75 mL) for 15 min, and after that the two phases were separated. The aqueous phase was further extracted with CH_2Cl_2 (2 × 20 mL). The combined organic extracts were washed with H_2O, brine, dried over Na_2SO_4, and concentrated under reduced pressure. The crude residue was flash-chromatographed with a mixture of EtOAc/petroleum ether (1:1) to obtain a white solid. An analytical sample was obtained by recrystallization from CH_2Cl_2/CH_3CN/petroleum ether. Yield: 107 mg (74%). Mp 213−214.5 °C. IR (Nujol): 3581−3346 (-NH-), 3100−3050 (-OH), 1687 (C=O), 1370 (S=O), 1175 (S=O). ^{1}H NMR (CDCl$_3$/DMSO-d_6): δ 1.11−1.16 (t, 3H, -CH$_2$CH_3, J = 7.2 Hz), 3.18−3.27 (m, 2H, -NH-CH_2-CH$_3$), 5.94 (br s, 1H, -CO-NH-CH$_2$CH$_3$), 6.70 (d, 2H, difluorophenyl-2,6H, J = 8.5 Hz), 7.46−7.60 (m, 4H, phenyl-H), 8.54 (br s, 1H, phenyl-OH), 9.32 (br s, 1H, -SO$_2$NH-), 9.58 (s, 1H, -NH-CON-HCH$_2$CH$_3$). Anal. (C$_{15}$H$_{15}$F$_2$N$_3$O$_4$S·0.4CH$_2$Cl$_2$) C, H, N.

General Procedure for the Preparation of N-(4-Bromo-2-fluorobenzyl)-N-(3,5-difluoro-4-hydroxyphenyl)arylsulfonamides (4a,b). Sodium hydride (10.05 mg, 0.418 mmol) as a 60% suspension to mineral oil (16.75 mg) was added portionwise to a stirred solution of the intermediate benzoate (0.334 mmol) (**3a**, 130 mg; **3b**, 145.8 mg) in DMF (3 mL), and the mixture was stirred at room temperature for 30 min under nitrogen atmosphere. 4-Bromo-1-(bromomethyl)-2-fluorobenzene (125 mg,

0.468 mmol) was then added, and the reaction mixture was allowed to stir at room temperature and under nitrogen for 72 h. Then a 5% aqueous NaOH solution (3 mL) was added and the mixture was stirred under nitrogen overnight and then was cooled (ice bath) and acidified with concentrated HCl. The crude product was obtained as a thick precipitate and was isolated by filtration. Furthermore, the filtrate was treated with toluene (4 × 20 mL), washed with H_2O (3 × 30 mL), brine (2 × 20 mL), dried over Na_2SO_4, and concentrated under reduced pressure. The combined crude product was purified by flash chromatography and with a mixture of petroleum ether/EtOAc (10:1). Analytical samples were obtained by recrystallization from CH_2Cl_2/petroleum ether.

N-(4-Bromo-2-fluorobenzyl)-N-(3,5-difluoro-4-hydroxyphenyl)-benzenesulfonamide (4a). Yield: 93 mg (60%). Mp 195−196 °C. IR (Nujol): 3450−3229 (-OH), 1377 (S=O), 1153 (S=O) cm^{-1}. ^{1}H NMR (CDCl$_3$/DMSO-d_6): δ 4.64 (s, 2H, -CH_2-), 6.5 (d, 2H, difluorophenol-2,6H, J = 11.3 Hz), 6.98−7.90 (m, 9H, phenyl-H and phenyl-OH). Anal. (C$_{19}$H$_{13}$BrF$_3$NO$_3$S·0.4CH$_2$Cl$_2$) C, H, N.

N-(4-Bromo-2-fluorobenzyl)-N-(3,5-difluoro-4-hydroxyphenyl)-4-nitrobenzenesulfonamide (4b). Yield: 135 mg (78.3%). Mp 228−229 °C. IR (Nujol): 3563-3261 (-OH), 1377 (S=O), 1160 (S=O) cm^{-1}. ^{1}H NMR (CDCl$_3$−DMSO-d_6): δ 4.72 (s, 2H, -CH$_2$-), 6.44-6.66 (d, 2H, difluorophenol-2,6H, J = 17.9 Hz), 7.19−7.51 (m, 4H, Br,F-phenyl-H and phenyl-OH), 7.8 (d, 2H, 4-nitrophenyl-2,6H, J = 17.4 Hz), 8.28−8.51 (d, 2H, 4-nitrophenyl-3,5H, J = 17.4 Hz). Anal. (C$_{19}$H$_{12}$BrF$_3$N$_2$O$_5$S) C, H, N.

4-Amino-N-(4-bromo-2-fluorobenzyl)-N-(3,5-difluoro-4-hydroxy-phenyl)benzenesulfonamide (4c). To a solution of **4b** (42 mg, 0.08 mmol) in isopropanol (5 mL) were added cyclohexene (0.06 mL, 0.6 mmol) and Pd/C (21 mg). The mixture was refluxed under a nitrogen atmosphere for 7.5 h, cooled to room temperature, filtered through Celite, and evaporated under reduced pressure. The residue was recrystallized from CH_2Cl_2/petroleum ether to provide the **4c** as a white solid. Yield: 348 mg (77%). Mp 210−211 °C. IR (Nujol): 3400−3198 (-NH$_2$ and -OH), 1376 (S=O), 1146 (S=O) cm^{-1}. ^{1}H NMR (CDCl$_3$/DMSO-d_6): δ 4.34 (s, 2H, -CH_2-), 4.87 (s, 1H, -OH), 5.07 (br s, 2H, -NH$_2$-), 6.24 (d, 2H, difluorophenol-2,6H, J = 8.9 Hz), 6.37−7.17 (m, 7H, phenyl-H). Anal. (C$_{19}$H$_{14}$BrF$_3$N$_2$O$_3$S) C, H, N.

N-(3,5-Difluoro-4-hydroxyphenyl)-N-(phenylsulfonyl)benzenesulfonamide (4d). Under nitrogen atmosphere, a THF solution (4 mL) of **2g** (232 mg, 0.59 mmol) was added to a suspension of sodium hydride (135 mg, 2.25 mmol) as a 60% suspension in mineral oil (225 mg) in dry THF (25 mL), and the mixture was stirred for 1 h at room temperature. The mixture was cooled to 0 °C (ice bath), and benzenesulfonyl chloride (0.32 mL, 2.28 mmol) was added. After being stirred for 72 h at room temperature, the mixture was diluted with H_2O (50 mL) and extracted with AcOEt (3 × 60 mL). The AcOEt layer was dried over anhydrous NaSO$_4$, and the AcOEt was concentrated under reduced pressure. Then the residue was diluted in 1,4-dioxane (15 mL), a 2.5% aqueous NaOH solution (15 mL) was added, and the mixture was stirred for 5 h and then was cooled (ice bath) and acidified with concentrated HCl. The mixture was stirred with CH_2Cl_2 (100 mL) for 15 min, the two phases were separated, and the aqueous phase was further extracted with CH_2Cl_2 (2 × 20 mL). The combined organic phases were washed with H_2O (3 × 20 mL), brine (2 × 20 mL), dried over anhydrous Na_2SO_4, and concentrated under reduced pressure. The crude residue was flash-chromatographed with a mixture of petroleum ether/EtoAc (5:1), and the title compound was obtained as a white solid. An analytical sample was obtained by recrystallization from CH_2Cl_2/petroleum ether. Yield: 67 mg (26%). Mp 163−164 °C. IR (Nujol): 3226−3103 (-OH), 1373 (S=O), 1166 (S=O). ^{1}H NMR (CDCl$_3$/DMSO-d_6): δ 6.53 (d, 2H, difluorophenol-2,6H, J = 8.8 Hz), 7.28 (br s, 1H, phenyl-OH), 7.33−7.71 (m, 10H, phenyl-H). Anal. (C$_{18}$H$_{13}$F$_2$NO$_5$S) C, H, N.

N-(1H-Tetrazol-5-yl)benzenesulfonamide (5). Benzenesulfonyl chloride (2.55 mL, 20 mmol) was added dropwise in a warm (60−70 °C) solution of 1H-tetrazol-5-amine (1.7 g, 20 mmol)

and NaOH (1.6 g, 40 mmol) in water (20 mL). After 3 h of stirring and heating in the above-mentioned temperature, the mixture was left to cool down at room temperature. Then it was acidified with concentrated HCl (ice bath). The crude product was obtained as a thick precipitate, isolated by filtration, and recrystallized from EtOAc/petroleum ether to give the title compound. Yield: 1.8 g (40%). Mp 134−136 °C (lit. 132−134 °C[60]). IR (Nujol): 3324 (tetrazole N-H), 1384 (S=O), 1264 (tetrazole C-N), 1170 (S=O) cm^{-1}. ^{1}H NMR (CDCl$_3$/DMSO-d_6): δ 7.34−7.67 (m, 3H, phenyl-3,4,5-H), 7.85−8.12 (m, 2H, phenyl-2,6-H), 10.7 (br s, 2H, −SO$_2$NH- and tetrazole-H). Anal. (C$_7$H$_7$N$_5$O$_2$S) C, H, N.

N-((1H-Tetrazol-5-yl)methyl)benzenesulfonamide (7). To a solution of N-(cyanomethyl)benzenesulfonamide (70 mg, 0.356 mmol) and trimethylsilyl azide (0.093 mL, 0.713 mmol) in toluene (5 mL) was added dibutyltin oxide (8.88 mg, 0.0357 mmol), and the mixture was refluxed for 47 h under a nitrogen atmosphere. The reaction mixture was concentrated under reduced pressure. The residue was dissolved in MeOH and reconcentrated. The residue was partitioned between EtOAc (25 mL) and aqueous 10% NaHCO$_3$ solution (26 mL). The organic phase was extracted with an additional portion of aqueous 10% NaHCO$_3$ solution (25 mL). The combined aqueous extracts were acidified to pH 2 with 10% HCl and then extracted with EtOAc (2 × 25 mL). The combined organic extracts were dried over anhydrous Na$_2$SO$_4$, filtered, and concentrated to give the title tetrazole as a white solid. An analytical sample was obtained by recrystallization from CH$_2$Cl$_2$/petroleum ether. Yield: 45 mg (53%). Mp 181−182 °C. IR (Nujol): 3324 (tetrazole N-H), 1384 (-NH-), 1350-1324 (tetrazole C-N), 1170 (S=O) cm^{-1}. ^{1}H NMR (CDCl$_3$/DMSO-d_6): δ 4.2−4.4 (m, 2H, -NHCH_2-), 7.4−8.2 (m, 7H, phenyl-H, -SO$_2$NH- and tetrazole-H). Anal. (C$_8$H$_9$N$_5$O$_2$S·0.1CH$_2$Cl$_2$) C, H, N.

N-(Methylsulfonyl)-2-(phenylsulfonamido)acetamide (8). A solution of **1** (640 mg, 2.98 mmol) in THF (6 mL) was added dropwise to a stirred solution of carbonyldiimidazole (CDI) (726 mg, 4.46 mmol) in THF (7.5 mL) under a nitrogen atmosphere. The mixture was stirred for 30 min, refluxed for 1 h, and allowed to cool to room temperature. Methylsulfonamide (425 mg, 4.45 mmol) was added in one portion, and the mixture was stirred for 10 min before a solution of 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) (0.69 mL, 4.45 mmol) in THF (4.5 mL) was added dropwise. The resulting mixture was stirred for 72 h under a nitrogen atmosphere, poured into an ice-cold aqueous 1 N HCl solution (40 mL), and then was extracted with EtOAc (3 × 50 mL). After the two phases were separated, the aqueous phase was further washed with Et$_2$O (30 mL), acidified to pK_a = 2, and extracted with EtOAc (2 × 20 mL). The combined EtOAc extracts were dried over anhydrous Na$_2$SO$_4$ and concentrated under reduced pressure. An analytical sample was obtained by recrystallization from CH$_2$Cl$_2$/petroleum ether as a light yellow solid. Yield: 252 mg (29%). Mp 118−119 °C. IR (Nujol): 3282 (-NH-), 1340 (S=O), 1324 (S=O), 1166−1149 (S=O) cm^{-1}. ^{1}H NMR (CDCl$_3$/DMSO-d_6): δ 3.08 (s, 3H, -SO$_2$CH_3), 3.57−4.06 (br s, 3H, -SO$_2$NHCH_2-), 7.29−7.62 (m, 3H, phenyl 3,4,5-H), 7.65−8.08 (m, 2H, phenyl 2,6-H), 11,7 (br s, 1H, -CONHSO$_2$-). Anal. (C$_9$H$_{12}$N$_2$O$_5$S$_2$) C, H, N.

(R)-N-(3-Oxoisoxazolidin-4-yl)benzenesulfonamide (9a). To an ice-cold solution of D-cycloserine (204 mg, 2 mmol) in buffer NaHCO$_3$/Na$_2$CO$_3$, pH 10 (10 mL), benzenesulfonyl chloride (0.28 mL, 2.2 mmol) was added portionwise. The reaction mixture was stirred under nitrogen atmosphere and at 0−4 °C overnight. The crude product was obtained as a thick precipitate and purified as a white solid by flash chromatography and with a mixture of petroleum ether/EtOAc (2:1). Yield: 151 mg (39%). Mp 155−156 °C. IR (Nujol): 3324 (NH), 1380 (S=O), 1354−1328 (CN), 1162 (S=O) cm^{-1}. ^{1}H NMR (CDCl$_3$/DMSO-d_6): δ 3.961−4.52 (m, 3H, oxoisoxazolidinyl-H), 7.22−7.67 (m, 6H, phenyl-H and -SO$_2$NH-), 8.46 (br s, 1H, oxoisoxazolidinyl-NH). Anal. (C$_9$H$_{10}$N$_2$O$_4$S·1H$_2$O) C, H, N.

(S)-N-(3-Oxoisoxazolidin-4-yl)benzenesulfonamide (9b). To an ice-cold solution of L-cycloserine (250 mg, 2.45 mmol) in buffer NaHCO$_3$/Na$_2$CO$_3$, pH 10 (12.5 mL), benzenesulfonyl chloride (0.34 mL, 2.69 mmol) was added portionwise. The reaction mixture was stirred under nitrogen atmosphere and at 0−4 °C overnight. The crude product was obtained as a thick precipitate and purified as a white solid by flash chromatography and with a mixture of petroleum ether/EtOAc (2:1). Yield: 90 mg (17%). Mp 155−156 °C. IR (Nujol): 3324 (NH), 1384 (S=O), 1354−1328 (CN), 1170 (S=O) cm^{-1}. ^{1}H NMR (CDCl$_3$/DMSO-d_6): δ 3.961−4.52 (m, 3H, oxoisoxazolidinyl-H), 7.22−7.67 (m, 6H, aromatic-H and -SO$_2$NH-), 8.46 (br s, 1H, oxoisoxazolidinyl-NH). Anal. (C$_9$H$_{10}$N$_2$O$_4$S·1H$_2$O) C, H, N.

Biological Assays. In Vitro Aldose Reductase Enzyme Assay. The tested compounds and the reference compound sorbinil (C$_{11}$H$_9$FN$_2$O$_3$, Pfizer, Inc., Central Research Division, Groton, CT) were dissolved in 10% aqueous solution of DMSO. Lenses were quickly removed from Fischer-344 rats of both sexes following euthanasia. The experiments conform to the law for the protection of experimental animals (Republic of Greece) and are registered at the Veterinary Administration of the Republic of Greece. The enzyme preparation and assay were performed as previously described.[58] Compounds were tested at five concentrations, the log(dose)−response curves were then constructed from the inhibitory data, and the IC$_{50}$ values were calculated by least-squares analysis of the linear portion of the log(dose) vs response curves ($0.889 < r^2 < 0.994$). The experiments were performed in triplicate.

In Vitro Aldehyde Reductase Enzyme Assay. The tested compounds and the reference compound valproic acid were dissolved in 10% aqueous solution of DMSO. Kidneys were quickly removed from Fischer-344 rats of both sexes following euthanasia. The experiments conform to the law for the protection of experimental animals (Republic of Greece) and are registered at the Veterinary Administration of the Republic of Greece. The enzyme preparation and assay were performed as previously described.[44] Compounds were tested at five concentrations, the log(dose)−response curves were then constructed from the inhibitory data, and the IC$_{50}$ values were calculated by least-squares analysis of the linear portion of the log(dose) vs response curves ($0.863 < r^2 < 0.987$). The experiments were performed in triplicate.

DPPH Assay. To investigate the antiradical activity of the tested compounds in a homogeneous system, a method based on the scavenging of the stable free radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) was used.[53] The experimental protocol was applied as described previously.[5] The tested compounds and the reference compound 6-hydroxy-2,5,7,8-chroman-2-carboxylic acid (Trolox) were dissolved in ethanol, and the IC$_{50}$ values were determined by least-squares analysis of the linear portion of the log(dose)−percentage of scavenging of DPPH curves ($0.895 < r^2 < 0.988$) of nine concentrations of the tested compounds after 270 min (steady state). The effective concentration value (EC$_{50}$, i.e., the IC$_{50}$ value divided by the concentration of DPPH) was also calculated. Finally, the initial rate of the reaction was estimated from the approximately linear absorbance decrease during the initial 30 s, of equimolar concentrations (0.2 mM) of the tested compounds and DPPH. The experiments were performed in triplicate.

Liposome Preparation, Incubation, and LOOH Determination. Unilamellar L-α-phosphatidylcholine dioleoyl (C18:1, [cis]9, DOPC, 99% grade) liposomes were used as a heterogeneous system[61] for the evaluation of the antioxidant activity of the tested compounds in comparison with that of the standard Trolox. Peroxidation of the liposomal membrane was triggered by thermal decomposition of the hydrophilic azo compound 2,2-azobis(2-amidinopropane)hydrochloride (AAPH), and the procedure for the liposome preparation, incubation, and LOOH determination was applied as previously described.[5] The lipid peroxide value was determined using a calibration curve

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