Phenolic Marine Natural Products as Aldose Reductase Inhibitors[§]

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Four different types of marine natural compounds isolated from tunicates were found to inhibit human aldose reductase. They all are characterized by a heterocyclic system, and at least two phenolic groups are present in the structure. Two of the compounds tested showed an inhibitory potency 5/6-fold higher than that of the known AR inhibitor sorbinil. One notable structural feature of these active compounds is the lack of either the carboxylic acid or the spiro-hydantoin commonly present in the principal classes of currently used inhibitors.

Aldose reductase (alditol/$NADP^+$ oxidoreductase, E.C.1.1.1.21, ALR2), the first enzyme in the polyol pathway, catalyzes the reduction of the aldehyde form of D-glucose to D-sorbitol with concomitant conversion of NADPH to $NADP^+$.[1−5]

It is commonly accepted that this polyol pathway plays an important role in the development of some degenerative complications of diabetes,[6−10] such as neuropathy, nephropathy, retinopathy, cataract formation, and cardiovascular disease. The elevated blood glucose levels that are characteristic of diabetes mellitus cause a significant flux of glucose through the polyol pathway in those tissues where glucose uptake is independent of insulin such as nerves, retina, lens, and kidney. In the Western world, the incidence of diabetes mellitus is increasing at an almost epidemic rate, and because of its high incidence and the associated morbidity and mortality, this disease has become a major health hazard. The Diabetes Control and Complications Trial (DCCT)[11] undertaken in the United States in 1993, the United Kingdom Prospective Diabetes Study (UKPDS)[12] in 1998, and a Japanese 6-year trial[13] have all demonstrated that strict and sustained control of glucose excursions through interventions including intensive insulin therapy reduces the risk of developing these complications in diabetic patients, thereby showing the association between hyperglycemia and the development of long-term complications. However, because close control is difficult to maintain, considerable efforts have been made to find novel, effective antidiabetic agents that act by mechanisms independent of those that control blood glucose levels. Thus, ALR2 inhibitors (ARIs) seem to offer the possibility of preventing or arresting the progression of these long-term diabetic complications, despite high blood glucose levels, and with no risk of hypoglycemia, since they have no effect on plasma glucose.[14]

A large variety of structurally diverse compounds have been identified to date as potent in vitro ARIs.[3,15−17] The most potent and better-characterized orally active ARIs belong to two main chemical classes: (i) carboxylic acid derivatives such as tolrestat, epalrestat, and zenarestat, and (ii) spiro-hydantoins such as sorbinil (Figure 1).

Even though numerous clinical trials for the treatment of some of the diabetic complications have been conducted with synthetic ARIs, except for epalrestat, which is marketed only in Japan, there are no drugs to directly treat these diabetic complications. Thus, an urgent need for new ARIs remains an important goal in pharmacological research.

The number of ARIs isolated from marine natural sources reported to date is very limited.[17−22] In our continuing search for new such ARIs, about two thousand marine natural products from our compound library were systematically screened.[23] We have previously published that the marine natural polybrominated diphenyl ether **1** (Figure 2), isolated from the marine sponge *Dysidea herbacea*, is a potent AR inhibitor.[24] Here we report the human aldose reductase (h-ALR2) inhibitory activity of four different types of compounds (Figure 2), all isolated from marine tunicates and characterized by a heterocyclic system to which at least two phenolic groups are bound. Other natural phenolic compounds, mainly flavones, chalcones, and coumarins, have been previously studied as ARIs.[17,25−27]

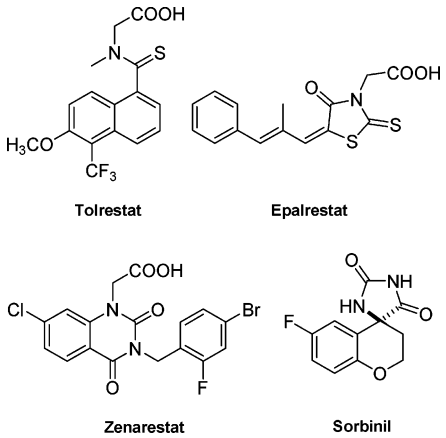

Figure 1. Some orally active ALR2 inhibitors.

The biological assay employed is based on the quantification of NADPH consumption that takes place when h-ALR2 catalyzes the conversion of glyceraldehyde into glycerol.[28] This assay was carried out in 96-well microtiter plates at 37 °C in 100 mM sodium phosphate buffer pH 6.2, 400 mM ammonium sulfate, 5 mU/mL of recombinant h-ALR2 (1 mU of activity was defined as a change in absorbance of 0.012 units per minute), and 0.1 mM NADPH. The final reaction volume was 200 μL per well. The compounds to be assayed were dissolved in dimethyl sulfoxide, and the corresponding solution was added to the well and preincubated for 5 min at 37 °C prior to addition of the substrate. The reaction was initiated by addition of 10 mM glyceraldehyde, and the decrease in optical density at 340 nm was monitored for 6 min at 37 °C in a microtiter plate reader (MRX-TCII, Dynex Technologies) in three intervals of 2 min each.

The known alkaloids **2** and **3**, isolated by us from the red ascidian *Botryllus leachi* as previously described,[29] were found to show modest inhibitory activity against h-ALR2 (Table 1). Both kinds of compounds, imidazole derivative **2** and pyrazine derivatives **3a** and **3b**, have in common a central nitrogenous heterocycle core

[§] This paper is dedicated to the memory of P. J. Scheuer (Heilbronn, Germany 1915−Hawaii 2003), a pioneer in marine drug research.

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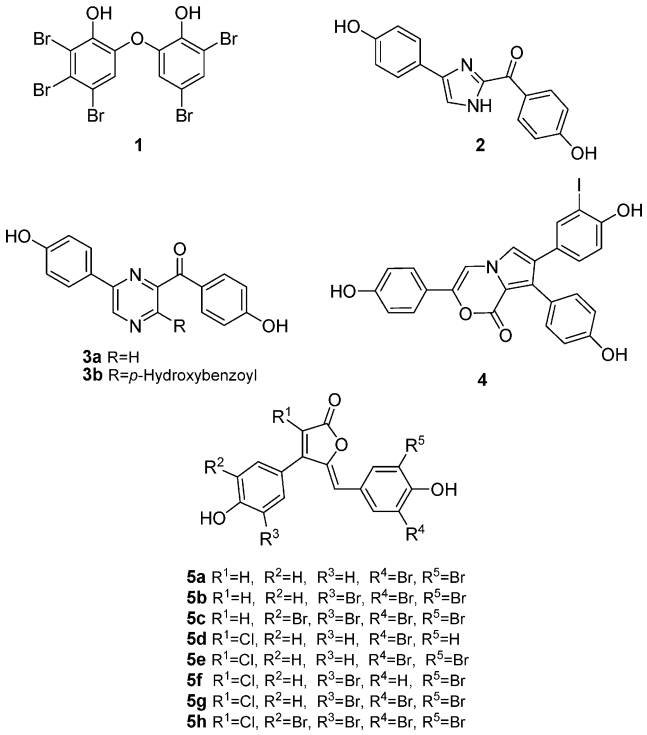

Figure 2. Marine natural products with in vitro AR inhibitory activity.

Table 1. Human ALR2 Inhibitory Activities of the Test Compounds and Sorbinil as Standard Inhibitor

compd	IC_{50} (μM)[a]	compd	IC_{50} (μM)[a]
2	21.4	**5d**	46.2
3a	41.4	**5e**	0.8
3b	19.4	**5f**	16.9
4	0.6	**5g**	12.7
5a	>57	**5h**	18.7
5b	48.1		
5c	19.8	sorbinil	3.6

[a] IC_{50} values represent the concentration (μM) required to produce 50% enzyme inhibition. Human recombinant ALR2 was used, and all values are the mean of at least three experiments.

with both *p*-hydroxybenzoyl and *p*-hydroxyphenyl rings present in their structure. From the activity data for these compounds, **2** versus **3a**, it can inferred that when only a *p*-hydroxybenzoyl and a *p*-hydroxyphenyl ring are present in the structure, the compound with imidazole as the heterocycle core showed better activity than the compound with pyrazine. When the pyrazine derivative has a second *p*-hydroxybenzoyl group, **3b** versus **3a**, the compound is more active than that with only one *p*-hydroxybenzoyl group.

Another known alkaloid, lukianol B (**4**), isolated by us as the minor constituent of a small sample of unidentified encrusting tunicate as previously described,[30] with two *p*-hydroxyphenyl and a 3-iodo-*p*-hydroxyphenyl group linked to an *N*-alkylpyrrole-2-carboxilic acid moiety, was found to be the most active ARI among all the phenolic derivatives assayed. To reach the IC_{50}, only one-sixth of the amount of this substance is needed compared to the quantity needed in the case of sorbinil.

Rubrolides **5a** through **5h** are a known non-nitrogenous family of compounds isolated from the ascidians *Ritterella rubra*[31] and *Synoicum blochmanni*.[32] These rubrolides have two *p*-hydroxyphenyl rings linked to a γ-lactone ring, and they all have halogen atoms. Among all the rubrolides assayed, it is worthy to stress the potent inhibitory activity of rubrolide **5e**. To reach the IC_{50}, only one-fifth of the amount of this substance is needed compared to the quantity needed in the case of sorbinil, and the IC_{50} is similar to that of lukianol B (**4**). To assess the importance of the chlorine group in the γ-lactone ring, we pairwise compared the activities of compounds **5a**/**5e**, **5b**/**5g**, and **5c**/**5h**. From these results (Table 1) it can be inferred that replacement of hydrogen at the α-carbon (R^1) with chlorine significantly improves potency. For the rubrolides **5a**−**c**, the inhibitory activity increases with the number of replacements of hydrogen with bromine. However, there is no apparent correlation between the degree of bromination and the inhibitory activity among the chlorinated rubrolides.

In conclusion, we report here the human aldose reductase (h-ALR2) inhibitory activity of four different types of compounds, all of them isolated from marine tunicates. They all are characterized by a heterocyclic system to which at least two phenolic groups are attached. Two of the 12 compounds assayed are very promising, as they showed inhibitory activities about 5-fold more potent than that of the known AR inhibitor sorbinil. One notable structural feature of these two very active compounds is the lack of either the carboxylic acid or the spiro-hydantoin group commonly present in the principal classes of currently used AR inhibitors.

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