Available online at www.sciencedirect.com
ScienceDirect
Bioorganic & Medicinal Chemistry Letters 17 (2007) 3793–3797
Bioorganic & Medicinal Chemistry Letters

Design, synthesis, and binding studies of bidentate Zn-chelating peptidic inhibitors of glyoxalase-I

Swati S. More and Robert Vince*

Center for Drug Design, Academic Health Center, and Department of Medicinal Chemistry, College of Pharmacy, University of Minnesota, 8-123A WDH, 308 Harvard Street SE, Minneapolis, MN 55455, USA

Received 11 November 2006; revised 12 December 2006; accepted 13 December 2006
Available online 21 December 2006

Abstract—The known affinity of ethyl acetoacetate (ACC) toward divalent zinc prompted us to attempt its employment as a chelating moiety in the design of glyoxalase-I inhibitors. A practical synthetic route was developed to incorporate this pharmacophore into the side chain of glutamic acid, with flexibility to allow incorporation of additional functionality at the end-stage of the synthesis. Herein, the details of this synthetic approach as well as the evaluation of the resultant β-keto ester compounds are reported.

Substantial experimental evidence exists for the inhibitory effect of endogenous α-ketoaldehydes on cell growth.[1] Methylglyoxal, one of the simplest α-ketoaldehydes, is formed by the non-enzymatic and enzymatic fragmentation of dihydroxyacetone phosphate and glyceraldehyde 3-phosphate[2,3] and the catabolism of threonine.[4] But the therapeutic use of α-ketoaldehydes as potential anticancer agents is limited due to their detoxification to the corresponding aldonic acids by the glyoxalase system. This enzyme system requires reduced glutathione because the hemithioacetal formed non-enzymatically from reduced glutathione and α-ketoaldehyde is the substrate for glyoxalase-I and is further converted to D-lactic acid by glyoxalase-II (Fig. 1).[5]

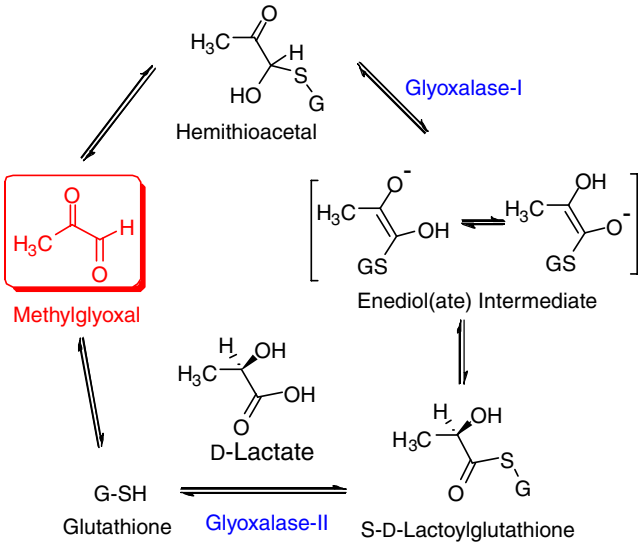

Figure 1. Glyoxalase pathway.

The major protective role played by the glyoxalase enzyme system prompted us to put forward a hypothesis that glyoxalase-I (Glx-I) inhibitors could be potential anticancer agents.[6] This was confirmed later through the development of numerous competitive and transition state analog inhibitors of Glx-I. The initial design of competitive inhibitors was based on taking advantage of the deep hydrophobic pocket in the active site of Glx-I. A large number of S-substituted alkylglutathiones[7] were synthesized; our S-*p*-bromobenzylglutathione (**1**)[8] being the most active amongst them. Further, the development of the enediol(ate) transition state mimics, S-(*N*-aryl-*N*-hydroxycarbamoyl)glutathiones (**3**), by Creighton and Murthy[9,10] started a new era of more potent tight-binding Glx-I inhibitors. Enhanced potency was due to possible chelation of the hydroxamate function with the divalent zinc in the active site. But the therapeutic use of these inhibitors as anticancer agents was precluded because of their charged nature, which limited their penetration inside cells and lability

Keywords: Methylglyoxal; Glyoxalase; Ethyl acetoacetate; Glutathione; β-Keto ester; Transition-state inhibitor.
* Corresponding author. Tel.: +1 612 6249911; fax: +1 612 6240139; e-mail: vince001@umn.edu

0960-894X/$ - see front matter
doi:10.1016/j.bmcl.2006.12.056

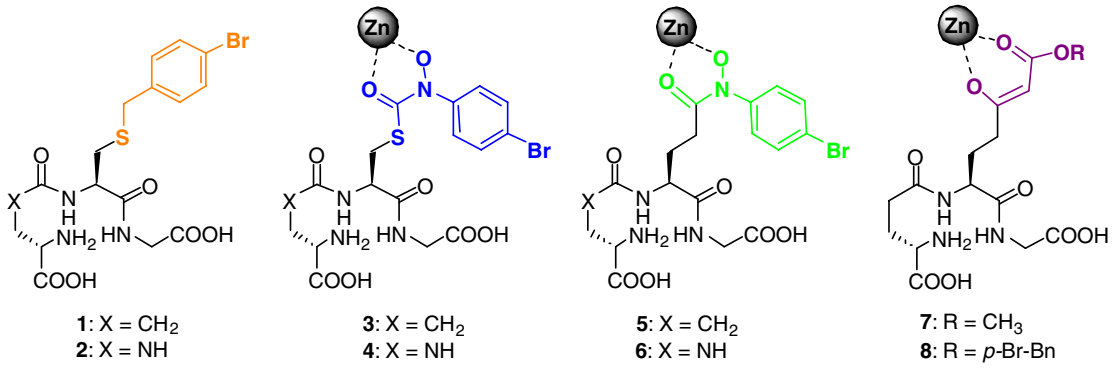

Figure 2. Development in the design of glyoxalase-I inhibitors.

to γ-glutamyltranspeptidase-mediated cleavage that led to their inactivation. The utilization of diester prodrugs of our S-*p*-bromobenzylglutathione inhibitor by Lo and Thornalley[11] overcame the cell-penetration problem. We recently reported upon the metabolic stability and inhibitory potency of a urea-isostere containing analog (**4**) (Fig. 2) of these tight-binding inhibitors.[12]

Recently, we developed a potent, tight-binding carbo-analog[13] of hydroxamate based transition state inhibitor **3**, compound **5** (K_i = 6.17 nM), and synthesized its metabolically stable analog **6** (K_i = 32.6 nM) (Fig. 2) which retained the inhibitory potency. The bioactivity of this series of compounds proved that the presence of sulfur is inconsequential to the ability of these glutathione analogs to inhibit Glx-I. Abolishing the Glx-II substrate similarity of these molecules would be expected to improve their in vivo stability against the hydrolysis of the thioester function, which is mediated by Glx-II. Removal of the sulfur atom also drastically reduced the synthetic complexity, thus facilitating further structural explorations.

The hydroxamate link, however, remained a foible that could render futile the aforementioned improvements. Although a strong ligand for zinc, this hydroxamide link is quite susceptible to hydrolytic breakdown. Indeed, the poor pharmacokinetics of hydroxamate-based enzyme inhibitors have plagued drug-development efforts in many an instance. We therefore deemed worthwhile the search for an alternative zinc-chelating group that would be more stable and also be amenable to substitutions for carrying out SAR at the hydrophobic substituent. The full 4s orbital and an empty 4p orbital of zinc allow for two covalent and two dative-coordinate bonds. As a consequence, Zn(II) salts (zinc chloride and zinc acetate) are known to form stoichiometric complexes with bidentate ligands.[14] In glyoxalase-I, Glu99 and Glu172 fulfill the covalent bonds, while two water molecules co-ordinate to this ion.[15] We sought to test the possibility of displacing the two water molecules by an ethyl acetoacetate (ACC)-like bidentate ligand (Fig. 3). ACC has been known to form stable complexes with divalent zinc, some of them being robust enough to be utilized as stabilizers in vinyl halide polymers.[14] Our choice of ACC was also based on the potential ease in variation of the terminal alkyl substituent. The initial

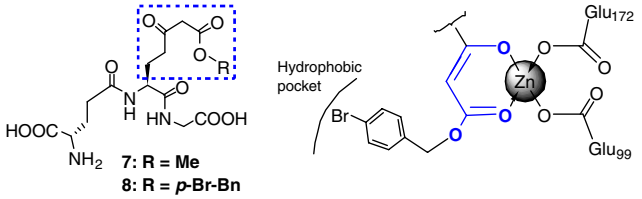

Figure 3. Rationale for the design of β-ketoester-based Glx-I inhibitors.

targets chosen were **7**, the methyl ester analog, and **8**, with the *p*-bromobenzyl substituent.

Synthesis of **7** is outlined in Scheme 1. EDC/HOBt-mediated coupling of the commercially available Boc-L-Glu(OBn)-OH with glycine *t*-butyl ester (as the HCl salt) afforded the dipeptide **10**. Selective removal of the Boc-function of dipeptide **10** in presence of the *t*-butyl ester was achieved by following the protocol of Hruby[16] (4 N HCl/dioxane at 0 °C). Amine **11** thus obtained was coupled to Boc-L-Glu(OH)-O*t*Bu (**19**) to obtain the tripeptide **12** in 84% yield. Compound **19** was prepared in four steps from L-glutamic acid. Regioselective side-chain methyl-esterification of glutamic acid **15** was achieved using chlorotrimethylsilane in methanol.[17] Amino acid **16** thus obtained was subjected to Boc protection using di-*tert*-butyl dicarbonate and *t*-butyl esterification of the carboxylic acid using *t*-BuOH/DCC-DMAP to obtain the orthogonally protected glutamic acid derivative **18**. Finally, hydrolysis of the side-chain methyl ester by lithium hydroxide gave the acid Boc-L-Glu(OH)-O*t*Bu (**19**).

Hydrogenolysis of the tripeptide **12** caused its quantitative conversion to the free acid **13**. Assembly of the β-keto ester[18] functionality was achieved by in situ conversion of acid **13** to its imidazolide with carbonyl diimidazole (CDI), and subsequent treatment with the Mg^{2+}-enolate of potassium monomethyl malonate to result in the formation of β-keto methyl ester precursor **14**. Global deprotection of protected β-keto ester **14** by treatment with trifluoroacetic acid lended us the title compound **7**.[19]

However, the same strategy was found to be inapplicable to the synthesis of β-ketobenzyl ester **8** (Scheme 2).