Bioorganic & Medicinal Chemistry 22 (2014) 4028–4041

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry

journal homepage: www.elsevier.com/locate/bmc

Glucopyranosylidene-spiro-iminothiazolidinone, a new bicyclic ring system: Synthesis, derivatization, and evaluation for inhibition of glycogen phosphorylase by enzyme kinetic and crystallographic methods

Katalin Czifrák [a], András Páhi [a], Szabina Deák [a], Attila Kiss-Szikszai [a], Katalin E. Kövér [b], Tibor Docsa [c], Pál Gergely [c], Kyra-Melinda Alexacou [d], Maria Papakonstantinou [d], Demetres D. Leonidas [d,†], Spyros E. Zographos [d], Evangelia D. Chrysina [d,*], László Somsák [a,*]

[a] Department of Organic Chemistry, University of Debrecen, POB 20, H-4010 Debrecen, Hungary
[b] Department of Inorganic and Analytical Chemistry, University of Debrecen, POB 21, H-4010 Debrecen, Hungary
[c] Department of Medical Chemistry, Medical and Health Science Centre, University of Debrecen, Egyetem tér 1, H-4032 Debrecen, Hungary
[d] Institute of Biology, Medicinal Chemistry and Biotechnology, The National Hellenic Research Foundation, 48 Vassileos Constantinou Avenue, GR-116 35 Athens, Greece

ARTICLE INFO

Article history:
Received 17 March 2014
Revised 25 May 2014
Accepted 29 May 2014
Available online 16 June 2014

Keywords:
Anomeric spirocycle
Thiazolidinone
Type 2 diabetes
Glycogen phosphorylase
Inhibitor
X-ray protein crystallography
Structure-based drug design

ABSTRACT

The reaction of thiourea with *O*-perbenzoylated *C*-(1-bromo-1-deoxy-β-D-glucopyranosyl)formamide gave the new anomeric spirocycle 1*R*-1,5-anhydro-D-glucitol-spiro-[1,5]-2-imino-1,3-thiazolidin-4-one. Acylation and sulfonylation with the corresponding acyl chlorides (RCOCl or RSO_2Cl where R = *t*Bu, Ph, 4-Me-C_6H_4, 1- and 2-naphthyl) produced the corresponding 2-acylimino- and 2-sulfonylimino-thiazolidinones, respectively. Alkylation by MeI, allyl-bromide and BnBr produced mixtures of the respective *N*-alkylimino- and *N*,*N*′-dialkyl-imino-thiazolidinones, while reactions with 1,2-dibromoethane and 1,3-dibromopropane furnished spirocyclic 5,6-dihydro-imidazo[2,1-*b*]thiazolidin-3-one and 6,7-dihydro-5*H*-thiazolidino[3,2-*a*]pyrimidin-3-one, respectively. Removal of the *O*-benzoyl protecting groups by the Zemplén protocol led to test compounds most of which proved micromolar inhibitors of rabbit muscle glycogen phosphorylase *b* (RMGP*b*). Best inhibitors were the 2-benzoylimino- (K_i = 9 μM) and the 2-naphthoylimino-thiazolidinones (K_i = 10 μM). Crystallographic studies of the unsubstituted spiro-thiazolidinone and the above most efficient inhibitors in complex with RMGP*b* confirmed the preference and inhibitory effect that aromatic (and especially 2-naphthyl) derivatives show for the catalytic site promoting the inactive conformation of the enzyme.

1. Introduction

A large proportion of clinically used drugs are inhibitors of enzymes and many current drug discovery and development efforts aim at the quest of such compounds.[1] Finding efficient inhibitors for a particular enzyme, and especially increasing the efficacy of such compounds, usually makes use of the three dimensional structure of the enzyme in complex with substrate analogues, inhibitors, as well as the kinetic and mechanistic behaviour of the enzyme in their presence, an approach called rational inhibitor (or drug) design.

Inhibition of glycogen phosphorylase (GP), the main regulatory enzyme of glycogen metabolism, has been connected to type 2 diabetes mellitus,[2–5] and during the last decade also to other diseased states such as myocardial[6,7] and cerebral[8,9] ischemia as well as tumors[10–13] as amply discussed in recent reviews and primary research articles. Liver and muscle isoforms of GP have been thoroughly characterized, the structural features of the proteins and the binding sites are well known and have been surveyed,[14,15] therefore these enzymes are ideal targets for rational and structure-based inhibitor design.

Several structural classes of compounds for inhibition of GP have been designed and evaluated[2,5,16] (mostly with the prototype of GP[14] isolated from rabbit muscle (RMGP*b*)) among which the most populated group is that of the glucose analogues which bind

* Corresponding authors. Tel.: +30 2107273851; fax: +30 2107273831 (E.D.C.); tel.: +36 52512900x22348; fax: +36 52512744 (L.S.).
E-mail addresses: echrysina@eie.gr (E.D. Chrysina), somsak@tigris.unideb.hu (L. Somsák).
† Present address: Department of Biochemistry and Biotechnology, University of Thessaly, 26 Ploutonos Str., 41221 Larissa, Greece.

http://dx.doi.org/10.1016/j.bmc.2014.05.076
0968-0896/

mostly at the catalytic site.[14,15,17] The evolution of some glucose based compounds leading to the first low micromolar inhibitor of GP is exemplified in Chart 1 by compounds **A**–**C**. Thus, introduction of an acylamino type substituent in the β-anomeric position of **A** to give **B** resulted in enhanced binding by more than one order of magnitude. Formal ring closure of the substituents to yield spiro-hydantoin **C** gave an inhibitor even more efficient than **B**. Synthesis of the essentially equipotent spiro-thiohydantoin **D** proved much simpler[18] than that of **C**, and **D** was shown to have considerable *in vivo* effects in lowering blood sugar levels.[19]

The structural features responsible for the strong binding of the spiro-hydantoins **C** and **D** to RMGP*b* were established by X-ray crystallography, and summarized as follows:[20,21] (a) a hydrogen bond exists between the β-NH group of the hydantoin and the main chain oxygen of His377; (b) the rigid planar spiro-hydantoin moiety undergoes little loss of conformational entropy upon binding; (c) the hydrogen bonding capability of the hydantoin polar groups exploit existing water structure and recruit new waters to complete networks to protein atoms, thereby providing additional enthalpic interactions.

The first submicromolar glucose derived inhibitor of GP (**E** in Chart 1) was found among *N*-acyl-*N*′-β-D-glucopyranosyl ureas.[17,22] In this series X-ray crystallography of the enzyme-inhibitor complexes[2,23] indicated the absence of the above mentioned hydrogen bond between the β-NH group and His377 O; therefore, the stronger binding was attributed to extensive interactions with the large aromatic appendage of the inhibitor in the so-called β-channel of the protein. A variant of **E** with a 3,5-dimethyl-phenyl group in place of the 2-naphthyl moiety was shown to improve glucose tolerance and to rearrange hepatic metabolism in diabetic mice.[24]

These observations shifted the focus of inhibitor design towards interactions in the β-channel and led to new principles[25] stating that efficient inhibitors advantageously have a rigid spirobicyclic scaffold which should not necessarily have an H-bond donor towards His377 (although this can be beneficial if available), but a suitably oriented, large aromatic substituent must be present to fit into the β-channel. These principles were first validated by the synthesis and enzymatic evaluation of glucopyranosylidene-spiro-oxathiazolines[25,26] (e.g., **F** in Chart 1) and further corroborated by spiro-isoxazolines[27] **G** among which the 2-naphthyl derivatives proved to be nanomolar inhibitors.

Taking into account the above design principles and the importance of the hydrogen bond forming capacity of the polar groups in the inhibitor molecules as proven for the spiro-hydantoins, here we report on the synthesis of glucopyranosylidene-spiro-iminothiazolidinones (target compounds in Chart 1) which have, in comparison to **F** and **G**, additional polar moieties in the α-position of the sugar ring. These compounds have been tested for their inhibitory effects towards RMGP*b* by enzyme kinetic assays and, for selected inhibitors, also by crystallographic methods.

2. Results and discussion

2.1. Synthesis

Reactions of α-halogen carbonyl compounds with thioamides are generally used for the construction of the thiazole ring. As a variant of the method, α-halogen carboxylic acid derivatives (most often esters) and thiourea (or its substituted derivatives) can be reacted to give 2-imino-thiazolidin-4-ones or the tautomeric 2-amino-Δ^2-thiazolin-4-ones.[34] The latter reaction type is exemplified by some syntheses of spiro-thiazolidinones.[35–38] Related spirobicyclic systems containing a sugar ring are very scarce: to the best of our knowledge a ribofuranosylidene-spiro-thiazolidine-2,4-dione[39] and epimers of galactopyranosylidene-spiro-thiazolidine-2,4-dithione as well as galactopyranosylidene-spiro-2-amino-Δ^2-thiazolin-4-thione[40] were reported.

Since the above ribofuranosylidene-spiro-thiazolidine-2,4-dione[39] was prepared by reacting *O*-perbenzoylated 1-bromo-D-ribofuranosyl cyanide with thiourea, in our first attempts similar reactions of the analogous glucopyranosyl compound **1**[18] (Table 1) were tried. Unfortunately, no reaction took place under the reported conditions[39] (1.3–2 equiv of thiourea, EtOH–sulfolane solvent mixture, 100 °C) either with conventional or microwave heating. Changing the solvent to nitrobenzene or DMF and raising the reaction temperature to 150 °C brought about no transformation while in *N*-methylpyrrolidone or ethylene glycol only decomposition could be observed.

The next trials were performed with the conventionally used α-halogen ester type compounds **2** and **3**[41] (Table 1, entries 1–4). Under microwave heating conditions the desired spiro-thiazolidinone **5** was obtained albeit the conversion of the starting compounds was incomplete. The active ester **3** showed somewhat higher reactivity (entry 3). In a parallel experiment (entry 5) the α-bromo carboxamide type **4**[18] was also shown to be converted to **5**. Since the preparation of **4** is shorter than those of **2** and **3** further optimizations (entries 5–10) were carried out with this compound (as far as we know this type of cyclization with α-halogen

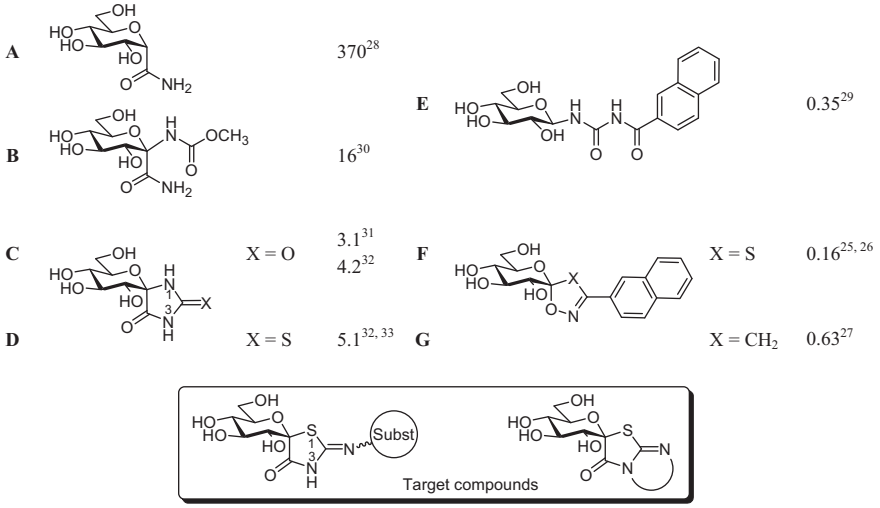

Chart 1. Inhibition of GP by selected derivatives of D-glucose (K_i [μM] against RMGP*b*) (see Refs. 25–33).