Available online at www.sciencedirect.com
SCIENCE DIRECT®
Bioorganic & Medicinal Chemistry Letters 15 (2005) 5375–5377
Bioorganic & Medicinal Chemistry Letters

N-Sulfonyl hydroxamate derivatives as inhibitors of class II fructose-1,6-diphosphate aldolase

Sabine Gavalda, Rémi Braga, Chantal Dax, Alain Vigroux and Casimir Blonski*

Laboratoire de Synthèse et de Physico-Chimie de Molécules d'intérêt Biologique (LSPCMIB), Groupe de Chimie Organique Biologique, UMR 5068, Université Paul Sabatier, Bât IIR1, 118 route de Narbonne, 31062 Toulouse Cedex 4, France

Received 30 June 2005; revised 1 September 2005; accepted 6 September 2005

Abstract—Dihydroxyacetone-phosphate and phosphonate derivatives were synthesized bearing a *N*-sulfonyl hydroxamate moiety. The phosphate derivatives represent competitive inhibitors for the class II-FBP aldolase catalyzed reaction, while the phosphonate isosteres are comparatively weaker inhibitors.

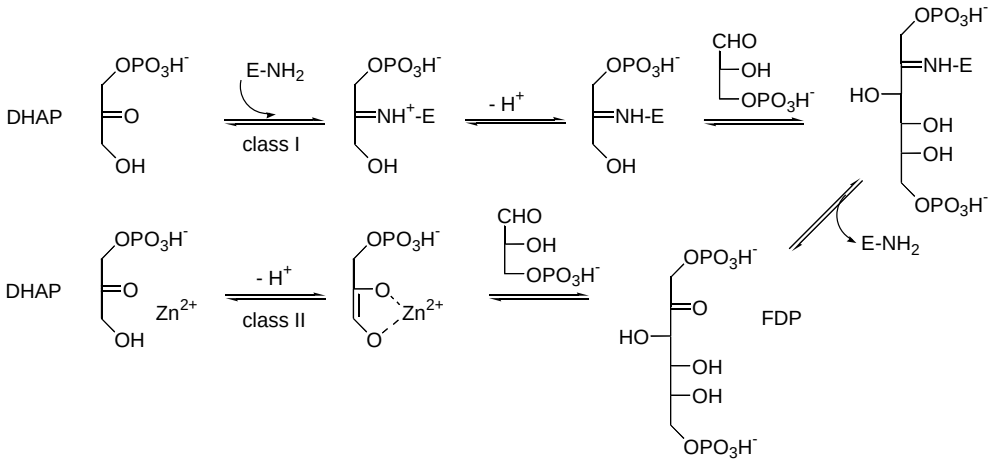
Aldolases are essential enzymes that catalyze carbon–carbon bond formation (or cleavage) in living organisms. Among the more extensively studied aldolases are fructose bisphosphate aldolases (EC 4.1.2.13) that participate in the metabolically important pathways of gluconeogenesis and glycolysis. In gluconeogenesis, these enzymes catalyze the aldol reaction of two triose-Ps, dihydroxyacetone-phosphate (DHAP) and D-glyceraldehyde 3-phosphate (G3P), to form fructose-1,6-bisphosphate (FBP). In glycolysis, FBP aldolases catalyze the reverse cleavage reaction.[1] Aldolases exist in two distinct classes;[2] class I aldolases are found in animals and higher plants, and catalyze the formation of a Schiff-base intermediate with substrate, whereas class II aldolases are found in algae, bacteria, and yeasts, and require a bivalent metal ion as cofactor. In class I aldolases, the reaction has the following features in the direction of FBP synthesis: (i) Schiff's base formation between DHAP and a lysyl residue at the active site; (ii) pro-*S* proton abstraction at C-3 in the immonium leading to an enamine (so-called carbanion) whose condensation with G3P yields FBP (new immonium ion intermediate) with an *S* configuration at C-3 (Scheme 1). In class II aldolase, a divalent cation (usually Zn^{2+}) functions as a Lewis acid to polarize the carbonyl bond of DHAP facilitating proton abstraction at C-3. The resulting endiolate, equivalent to the enamine intermediate, is stabilized by the zinc ion.

Class II aldolases are not present in mammals and as such they represent potential targets for the development of anti-bacterial and anti-fungal drugs.[3] Phosphoglycolohydroxamic acid (PGH) was, for more than 30 years, the only known potent inhibitor of class II aldolases.[4] Recently, two new derivatives of phosphoglycolic acid were synthesized and shown to be selective inhibitors of this family of enzymes.[3d] These studies substantiated the feasibility of developing new inhibitors of therapeutic interest based on the PGH motif while also affording insight into enzymology of glycolytic aldolases.

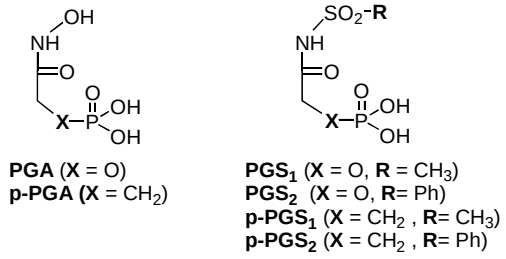
Based on these recent results, we prepared a new family of inhibitors possessing the phosphoglycolate skeleton bound to a sulfamate group (PGS, Scheme 2). Compounds with a NH moiety between carbonyl and sulfonyl groups are very acidic; their pK_a values are close to 2;[5] the presence of a negative charge at physiological pH at this position of PGS would be expected to improve the interaction with the active-site metal ion. Additionally, phosphonate isosteres of biologically important phosphates are of interest in biological chemistry, due to the exceptional stability of the phosphorus–carbon bond toward phosphatases as compared to a phosphorus–oxygen bond.[6] In order to gain understanding of the influence of such a structural modification on the binding of the *N*-sulfonyl hydroxamate inhibitors, we have also prepared their phosphonate isosteres (p-PGA and p-PGS, Scheme 2).

Keywords: Fructose bisphosphate aldolase; *N*-Sulfonyl hydroxamate derivatives; Inhibitors; Phosphonate.
* Corresponding author. Tel.: +33 5 61 55 6486; fax: +33 5 61 55 6080; e-mail: blonski@cict.fr

0960-894X/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2005.09.006



Scheme 1. Aldolase catalyzed reaction: class I and class II-FBP aldolases.

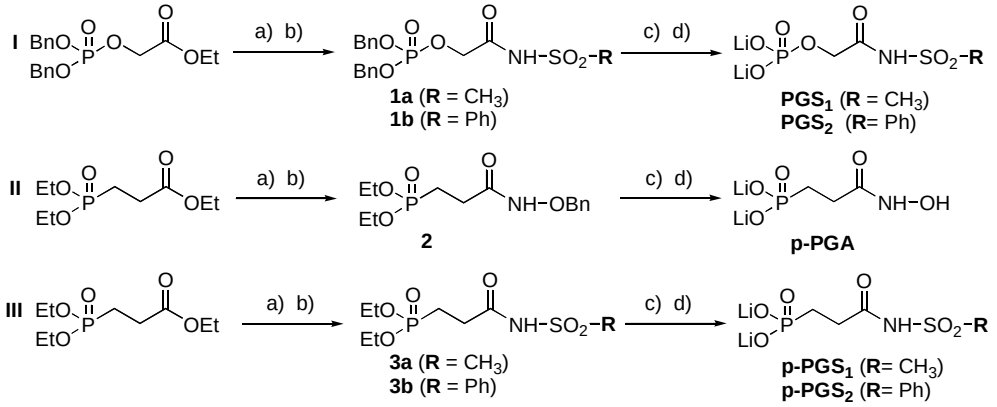


Scheme 2. Structures of inhibitors under study: phosphoglycolohydroxamic acid (PGH) and phosphoglycolosulfamate (PGS) and their phosphonate derivatives (p-PGH and p-PGS, respectively).

All compounds were synthesized in three steps starting from dibenzyl ethyl phosphoglycolate or triethyl 3-phosphonopropionate according to Scheme 3. In the first step, compounds are hydrolyzed under basic conditions to furnish the corresponding sodium salt derivatives. This is followed by reaction with the hydroxylamine or sulfonamide in the presence of carbodiimide to yield compounds **1a**,**b**, **2**, and **3a**,**b**. Deprotection of the phosphonate or phosphate group yields the expected compounds as the lithium salt.[7]

The interaction of these products with class II aldolase from *Escherichia coli* and class I aldolase from rabbit muscle was analyzed by enzyme kinetics.[8] Lineweaver–Burk plots of enzyme inhibition show clearly that PGS1 and PGS2 are fully competitive inhibitors for the reaction catalyzed by the class II aldolase (see Fig. 1 as an example). This result is an indication that the two inhibitors act at the active site and chelate the metal ion.[9] No inhibition effect is observed at a concentration up to 3 mM with aldolase from muscle or with other DHAP-dependent enzymes such as triose phosphate isomerase or glyceraldehyde 3-phosphate dehydrogenase used in the coupled enzymatic essays.

It is interesting to note that the PGS compounds are weaker inhibitors than those described previously. Their active-site affinity appears to be dependent on the chemical group used on the sulfonyl hydroxamate of the inhibitor, PGS2 being more efficient than PGS1 indicating a specific accommodation of the aromatic moiety by hydrophobic interaction of the enzyme toward the inhibitors (Table 1). This suggests chemical modification at the sulfamate level (e.g., aromatic ring bearing a reactive electrophile) may improve inhibitor efficiency or transform



Scheme 3. Synthesis of inhibitors under study. I: (a) NaOH, H_2O, (b) RSO_2NH_2, DCC, DMAP, CH_2Cl_2, (c) H_2, Pd/C, MeOH, (d) H_2O, LiOH; II: (a) NaOH, H_2O, (b) NH_2OBn, DCC, DMAP, CH_2Cl_2, (c) Me_3SiBr, (d) H_2O, LiOH; III: (a) NaOH, MeOH, (b) RSO_2NH_2, DMAP, DCC, CH_2Cl_2, (c) Me_3SiBr, (d) H_2O, LiOH.

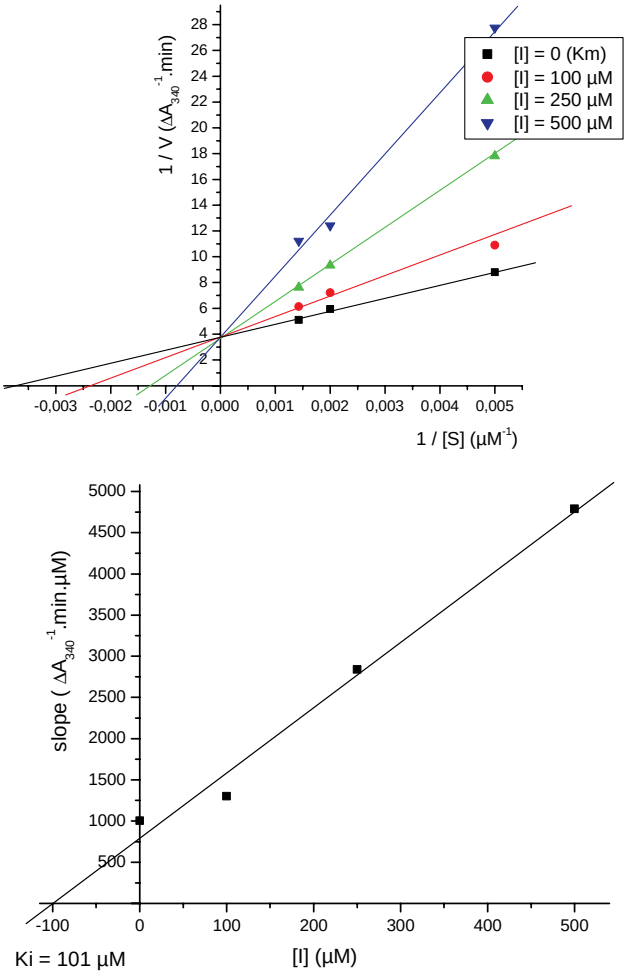


Figure 1. Lineweaver–Burk plots of class II-FBP aldolase inhibition by different concentrations of PGS_2.

them into specific irreversible inhibitors by taking advantage of the presence of nucleophilic residues (e.g., Asp-109 or Ser-61) in the active site or in its proximity.[10]

Surprisingly, the structural modification of converting a phosphate into phosphonate significantly weakened inhibitor binding (Table 1). These results strongly support the fact that stabilizing interactions made by active-site residues with the O_1 ester oxygen at the phosphate binding site are critical for inhibitor binding, which is not possible with a phosphonate moiety[11] and must be taken into account in the development of stable intracellular inhibitors against class II FBP aldolases.

Table 1. Kinetic parameter values (µM)

Aldolase	K_m	K_i					
	FBP	PGS1	PGS2	p-PGS1	p-PGS2	PGH	p-PGH
Rabbit	10	$>10^4$	$>10^4$	$>10^4$	$>10^4$	1	$>10^3$
E. coli	300	350	100	$>10^4$	$>10^4$	0.01	$>10^3$

Acknowledgment

We are thankful to Professor J. Sygusch for providing class I FBP aldolase from rabbit muscle and class II FBP aldolase from *E. coli*.

References and notes

1. (a) Horecker, B. L.; Tsolas, O.; Lai, C. Y. Aldolases. In *The Enzymes*, 3rd ed.; Boyer, P. D., Ed.; Academic Press: New York, 1972; Vol. 7, pp 213–258; (b) Gefflaut, T.; Blonski, C.; Perie, J.; Willson, M. *Prog. Biophys. Mol. Biol.* **1995**, *63*, 301.
2. Rutter, W. J. *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **1964**, *23*, 1248.
3. (a) Izard, T.; Sygusch, J. *J. Biol. Chem.* **2004**, *279*, 11825; (b) Blom, N.; Sygusch, J. *Nat. Struct. Biol.* **1996**, *3*, 856; (c) Fessner, W. D.; Schneider, A.; Held, H.; Sinerius, G.; Walter, C.; Hixon, M.; Schloss, J. V. *Angew. Chem., Int. Ed. Engl.* **1996**, *35*, 2219; (d) Fonvielle, M.; Weber, P.; Dabkowska, K.; Therisod, M. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 2923.
4. (a) Lewis, D. J.; Lowe, G. *J. Chem. Soc., Chem. Commun.* **1973**, 713; (b) Collins, K. D. *J. Biol. Chem.* **1974**, *249*, 136.
5. Blans, P.; Vigroux, A. *Chem. Eur. J.* **1999**, *5*, 1526.
6. (a) Engel, R. *Chem. Rev.* **1977**, *77*, 349; (b) Racha, S.; Li, Z.; El-Subbagh, H.; Abushanab, E. *Tetrahedron Lett.* **1992**, *33*, 5491; (c) Page, P.; Blonski, C.; Perie, J. *Tetrahedron Lett.* **1995**, *36*, 8027; (d) Page, P.; Blonski, C.; Perie, J. *Bioorg. Med. Chem.* **1999**, *7*, 1403.
7. All compounds gave satisfactory NMR (300 MHz), ESMS (negative mode), and CHN data.
8. Recombinant enzymes from rabbit muscle and from *E. coli* were furnished by Professor J. Sygusch, University of Montreal, Canada. Enzymatic assays were performed as described in Blonski, C.; De Moissac, D.; Perie, J.; Sygusch, J. *Biochem. J.* **1997**, *323*, 71.
9. Fortnagel, P.; Freese, E. *J. Biol. Chem.* **1968**, *243*, 5289.
10. Claustre, S.; Denier, C.; Lakhdar-Ghazal, F.; Lougare, A.; Lopez, C.; Chevalier, N.; Michels, P. A. M.; Perie, J.; Willson, M. *Biochemistry* **2002**, *41*, 10183.
11. Arth, H.-L.; Fessner, W.-D. *Carbohydrate Res.* **1998**, *305*, 313.