Pergamon

Bioorganic & Medicinal Chemistry Letters 9 (1999) 277–278

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

Specific, Uncompetitive Inhibition of β-Galactosidases by a 5,6-Isopropylidenedioxyfuro[2,3-d]isoxazole-3-methanol Derivative.

Christophe Schaller, Raynald Demange, Sylviane Picasso and **Pierre Vogel***

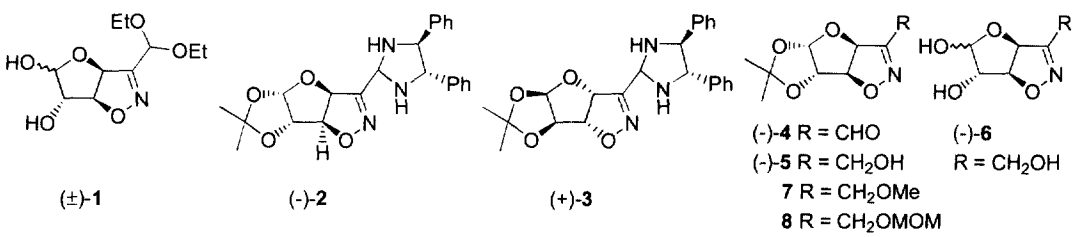
Institut de chimie organique de l'Université de Lausanne, BCH, CH-1015 Lausanne-Dorigny, Switzerland

Received 6 November 1998; accepted 7 December 1998

Abstract: (-)-(3a*S*,5*S*,6*S*,6a*R*)-3a,5,6,6a-Tetrahydro-5,6-isopropylidenedioxyfuro[2,3-d]isoxazole-3-methanol ((-)-**5**) has been tested toward 25 glycohydrolases and found to inhibit β-galactosidase from *Aspergillus niger* (K_i = 18 μM) and that from *Aspergillus orizae* (K_i = 72 μM). Hydrolysis of the acetonide or exchange of CH_2OH group for a CHO, CH_2OMe or a CH_2OMOM group suppresses the inhibitory activity.

Keywords: acetonide, β-galactosidases, inhibitors, isoxazoles

Glycosidases are involved in several biological processes.[1] Compounds that imitate the charge and steric information of transition or intermediate structures of the hydrolytical process have shown promising inhibitory activities.[2] We show that other types of compounds (not rationally designed) can be potent and selective glycosidase inhibitors. The intermediates (±)-**1**, (-)-**2**, (+)-**3**, (-)-**4**, (+)-**4**, (-)-**5**, (+)-**5**, (-)-**6** and (+)-**6** in our synthesis of 1,5-dideoxy-1,5-iminoalditols[3] have been tested for their inhibitory activity toward two α-L-fucosidases (from bovine epididymis, human placenta), three α-galactosidases (*Aspergillus niger*, *E. coli*, coffee beans), five β-galactosidases (*Aspergillus niger*, *Aspergillus orizae*, *E. coli*, bovine liver, jack beans), two maltases (yeasts, rice), one isomaltase (baker yeasts), two amyloglucosidases (*Asp. niger*, *Rhizopus* mold), two β-glucosidases (almonds, *Caldocellum saccharolyticum*), two α-mannosidases (jack beans, almonds), one β-mannosidase (*Helix pomatia*), one β-xylosidase (*Aspergillus niger*), one α-N-acetylgalactosaminidase (chicken liver) and three β-N-acetylglucosaminidases (jack beans, bovine epididymis A and B).



None of these compounds had inhibitory activity at 1 mM concentration, except for (-)-**5** which showed 90% (IC_{50}: 43 μM, K_i: 18 μM), 80% (IC_{50}: 150 μM, K_i: 72 μM), 50% (IC_{50}: 1 mM) and 67% (IC_{50}: 550 μM, pH 5) inhibition of β-galactosidase form *Aspergillus niger*, from *Asp. orizae*, from jack beans and of β-glucosidase from *Caldocellum saccharolyticum*, respectively. Furanose (-)-**6** showed a weak inhibitory activity toward rice α-glucosidase at pH 4 (59% at 1 mM, IC_{50}: 805 μM).[4] The β-galactosidase inhibition of (-)-**5** requires both the isopropylidenedioxy and the alcohol moieties as we find that (±)-**7** and (±)-**8** derived

* Fax: 0041 21 692 39 75; E-mail: pierre.vogel@ico.unil.ch

0960-894X/99/$ - see front matter
PII: S0960-894X(98)00722-7

from (±)-**5**[3] had no activity. As shown by the Lineweaver and Burk plots (Fig. 1A, B) (-)-**5** behaves as an uncompetitive inhibitor because both V_{max} and K_M values were affected by increasing concentrations of (-)-**5**.

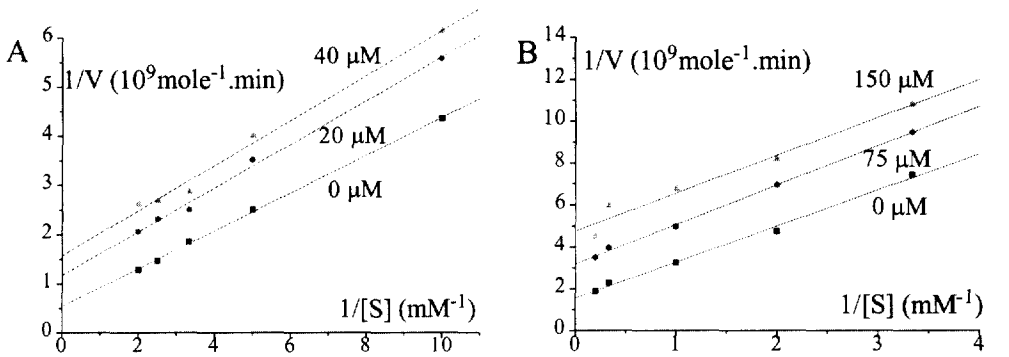
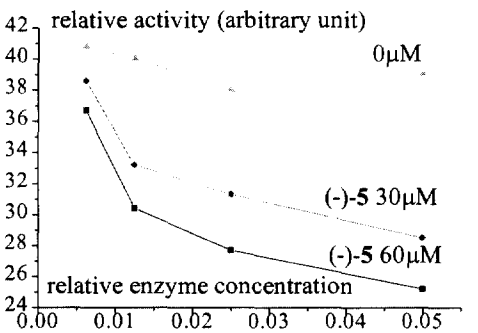


Fig.1: Effect of *p*-nitrophenyl β-galactoside (*S*) concentration on the inhibition by (-)-**5** of β-galactosidase from (A) *Aspergillus niger*, (B) from *Aspergillus orizae*.



The activity of β-galactosidase form *Aspergillus niger* was moderately increased with dilution indicating (-)-**5** to be a reversible, tightly bound inhibitor (Fig. 2).[5] Inhibition studies as a function of the pH showed that inactivation was maximum at pH 4.8 which is also the optimum pH of the enzyme. The 1H-NMR spectrum of (-)-**5** did not vary between pH 3.3 to pH 12.

Fig.2: Reversibility of *Asp. niger* β-galactosidase inhibition by (-)-**5**

Our results show that simple compounds[6] that are not sugar analogues can be selective glycosidase inhibitors. Acetonide (-)-**5** is a potent, specific uncompetitive inhibitor of fungal β-galactosidases.

Acknowledgements: We are grateful to the *Swiss National Science Foundation* (Bern) for financial support.

References

1. See e.g.: Hendrix, M.; Wong, C.-H. *Pure & Appl. Chem.* **1996**, *68*, 2081-2087; Ganem, B. *Acc. Chem. Res.* **1996**, *29*, 340-347; Platt, F. M.; Reinkensmeier, G.; Dwek, R. A.; Butters, T. D. *J. Biol. Chem.* **1997**, *272*, 19365-19372; Kolter, T. *Angew. Chem., Int. Ed. Engl.* **1997**, *36*, 1955-1959.
2. See e.g.: Bols, M. *Acc. Chem. Res.* **1998**, *31*, 1-8; Kraehenbuehl, K.; Picasso, S.; Vogel, P. *Helv. Chim. Acta* **1998**, *81*, 1439-1479 and ref. cited therein.
3. Schaller, C; Vogel, P.; Jäger, V. *Carbohydr. Res.* in print.
4. Appropriate *p*-nitrophenyl glycoside substrates buffered to the optimum pH of the enzymes were used, for details see: Brandi, A.; Cicchi, S.; Cordero, F.M.; Frignoli, B.; Goti, A.; Picasso, S.; Vogel, P. *J. Org. Chem.* **195**, *60*, 6806-6812.
5. For other inhibitors of this β-galactosidase, see: Lee, Y. C. *Biochem. Biophys. Res. Commun.* **1981**, *35*, 161-167; Miyake, Y.; Ebata, M. *J. Antibiot.* **1987**, *40*, 122-123; Miyake, Y.; Ebata, M. *Agric. Biol. Chem.* **1988**, *52*, 153-158; Kameda, Y.; Kawashima, K.; Takeuchi, M.; Ikeda, K.; Asano, N.; Matsui, K. *Carbohydr. Res.* **1997**, *300*, 259-264; Qian, X.; Moris, V. F.; Wong, C.-H. *Bioorg. Med. Chem. Lett.* **1996**, *6*, 1117-1122; Picasso, S.; Chen, Y.; Vogel, P. *Carbohydr. Lett.* **1994**, *1*, 1-8.
6. Glucosidases are inhibited by imidazoles, see: Li, Y. K.; Byers, L. D. *Biochim. Biophys. Acta.* **1989**, *999*, 227-232; Field, R. A.; Haines, A. H.; Chrystal, E. J.; Luszniak, M. C. *Biochem. J.* **1991**, *274*, 885-889.