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4-(Sulfonylamino)phenyl α-D-Glucopyranosides as Competitive Inhibitors of Yeast α-Glucosidase

Josie C. Briggs, Alan H. Haines* and Richard J. K. Taylor*†

School of Chemical Sciences, University of East Anglia, Norwich, UK NR4 7TJ

Certain members of a series of 4-(sulfonylamino)phenyl α-D-glucopyranosides are extremely good competitive inhibitors of yeast α-glucosidase and, remarkably, 4-(4-nitrophenylsulfonylamino)phenyl and 4-(2-naphthylsulfonylamino)phenyl α-D-glucopyranoside (K_i 4.8 and 3.1 μmol dm^{-3}, respectively) are superior to 1-deoxynojirimycin (K_i 14.6 μmol dm^{-3}) against this enzyme.

Recently, we reported[1] the preparation of 2-chloromethyl-4-nitrophenyl α-D-glucopyranoside and its ability to act as a highly effective enzyme-activated irreversible inhibitor of yeast α-glucosidase. Glycosidase inhibitors are of considerable current interest, particularly because of the anti-HIV activity shown by nojirimycin, castanospermine, and some of their derivatives,[2] which act competitively against such enzymes. In an attempt to extend the class of enzyme-activated irreversible inhibitors for glycosidases, we have now synthesised a series of 4-(sulfonylamino)phenyl α-D-glucopyranosides. We reasoned, on the basis of recent observations regarding the formation of 1,4-benzoquinones,[3] that enzymic liberation of the aglycone 4-$RSO_2NHC_6H_4O^-$ from these glycosides might be followed by ejection of a sulfinate anion RSO_2^- with concomitant formation of 4-iminoquinone **1** which would then undergo attack by a nucleophilic centre in the enzyme-active site, leading to enzyme deactivation. Although the glycosides appear not to act in the manner desired, we have observed that some of them are remarkably efficient *competitive* inhibitors of yeast α-glucosidase, and that this property is dependent on the structure of the sulfonyl-containing moiety in the aglycone.

4-Aminophenyl α-D-glucopyranoside tetraacetate **2**, prepared by reduction of the corresponding 4-nitrophenyl glycoside derivative **3** with hydrogen over palladium-on-charcoal, was treated in pyridine solution separately with methane-, 4-nitrobenzene-, 4-methylbenzene-, 2,4,6-triisopropylbenzene-, and 2-naphthalene-sulfonyl chloride and the glycoside tetraacetates so obtained were treated with sodium methoxide in methanol to afford compounds **4–8**, respectively.‡ Incubation experiments with glycosides **5** and **6** and yeast α-glucosidase (pH 6.8, 30 °C, 0.1–1.0 mmol dm^{-3} in glycoside) led to only slow loss of enzyme activity (*ca.* 1% min^{-1}), the rate of which did *not* depend on the inhibitor concentration. This result stands in contrast to our earlier experiments[1] under the same conditions with 2-chloromethyl-4-nitrophenyl α-D-glucopyranoside.

Competitive inhibition studies on **4–8** towards yeast α-glucosidase were conducted at pH 6.8 and 30 °C with 4-nitrophenyl α-D-glucopyranoside as substrate. In contrast to glycoside **4**, which showed no significant inhibitory properties under these conditions, glycoside **5** proved to be an extremely effective competitive inhibitor.§ A Lineweaver–Burk plot for this experiment [Fig. 1(*A*)], afforded K_i = 4.8 μmol dm^{-3}. This value should be compared with the reported values[4–6] for 1-deoxynojirimycin, a potent inhibitor of this enzyme, of 12.6, 8.7 and 14.6 μmol dm^{-3}. Similar plots from experiments involving glycosides **6–8** gave, respectively, values for K_i of 28, 18 and 3.1 μmol dm^{-3}. TLC and optical rotation measurements indicated that the glycosides were relatively inert to the enzyme over the time scale of the experiment and under

† *Present address*: Department of Chemistry, University of York, Heslington, York, UK Y01 5DD.

‡ Satisfactory analytical and spectral data were obtained for all new compounds.

§ Compound **5** did not inhibit almond β-glucosidase.

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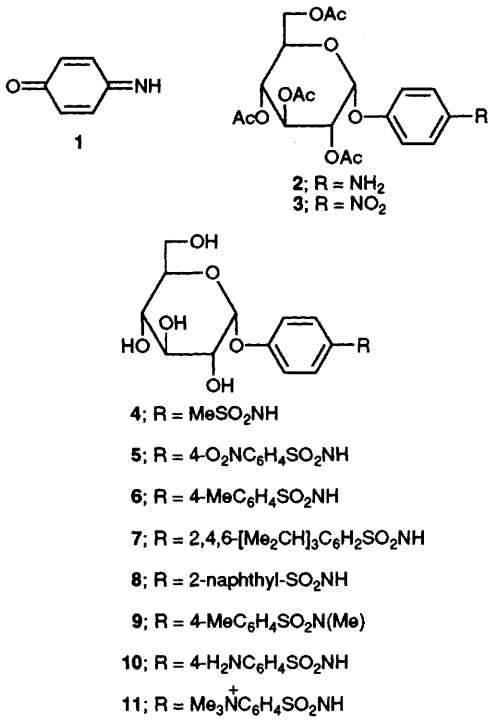

conditions which led to enzymic liberation of D-glucose from 4-nitrophenyl α-D-glucopyranoside.

Apart from the slow enzymic hydrolysis of the sulfonylamino glycosides, a possible rationale for their failure to act as irreversible inhibitors could lie in the relatively high acidity of the imino group in the aglycone. Thus, elimination of a sulfinate residue from a liberated aglycone which was ionised at this centre might reasonably be expected to be disfavoured. We were prompted to investigate, therefore, the *N*-methyl derivative of a member of this series. Treatment[7] of **6** with methanol, diethyl diazodicarboxylate and triphenylphosphine led to the required *N*-methyl compound **9**. Despite this structural change, incubation experiments with the α-glycosidase showed **9** not to be an irreversible inhibitor. However, the chemical transformation of **6** into **9** changed completely the nature of inhibition observed with the two compounds, which for **9** now appeared to be of an uncompetitive type [Fig. 1(*B*)]. In this case the compound binds reversibly to the enzyme–substrate complex affording an inactive enzyme–substrate–inhibitor complex but the inhibitor does not bind to the free enzyme.[8] This result suggests that the efficiency of the 4-(sulfonylamino)phenyl glycosides **5–8** as inhibitors might be due, at least in part, to the acidity of the imino function, an observation supported by the fact that the 4-nitrophenylsulfonylamino derivative **5** [Hammett substituent constant[9] $\sigma_p(NO_2) = 0.78$] and the 2-naphthylsulfonylamino compound **8** {σ_p (3,4-[CH]$_4$) = 0.17} are more effective inhibitors than the 4-methylphenylsulfonylamino compound **6** [σ_p (Me) = −0.17]. However, this argument is too simplistic since **8** has similar inhibitor properties to **5** and the 4-aminophenylsulfonylamino derivative **10** [σ_p (NH_2) = −0.66], made by reduction (Pd–C/H_2) of **5**, proved to be as effective an inhibitor as **5** and **8**, with a K_i value of 3.2 μmol dm^{-3}.

Attempts to prepare a more effective inhibitor than **5** by forming the 4-trimethylammonium compound **11** [σ_p (Me_3N^+) = 0.88] were thwarted by our inability to quaternise the *N*,*N*-dimethyl derivative of **10** on treatment with methyl iodide.

In an anti-HIV screen, compound **8** showed weak activity in reducing the virus (HIV-1 IIIB) progeny in infected cell (C8166) cultures by 50% at 40 μmol dm^{-3}, but the other compounds showed negligible activity or were toxic to the cells.

We thank Dr N. Mahmood of the MRC Collaborative Centre for conducting the anti-HIV experiments, and the MRC for financial support of the work through their AIDS Directed Programme.

Received, 2nd June 1993; Com. 3/03127F

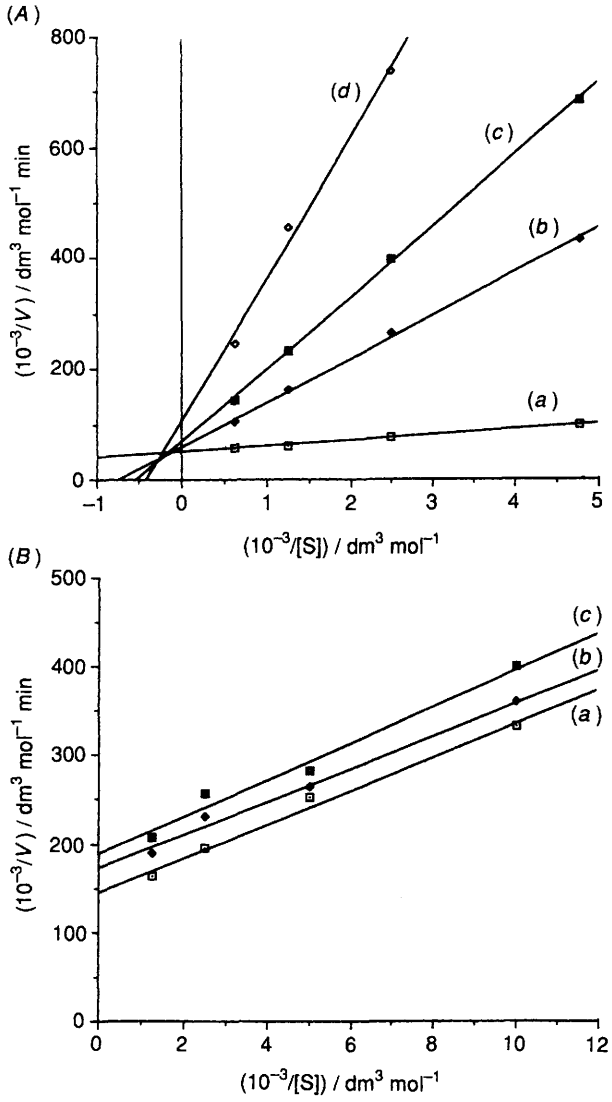

Fig. 1 (*A*) Lineweaver–Burk plot for the inhibition of yeast α-glucosidase with **5**. Assays were performed in PIPES [piperazine-*N*,*N*′-bis(ethanesulfonic acid)] buffer, pH 6.8, at 30 °C, with inhibitor concentrations of (*a*) 0, (*b*) 0.01, (*c*) 0.02 and (*d*) 0.05 mmol dm^{-3}. (*B*) Lineweaver–Burk plot for the inhibition of yeast α-glucosidase with **9**. Assays were performed as in (*A*) except that inhibitor concentrations were (*a*) 0, (*b*) 0.012 and (*c*) 0.036 mmol dm^{-3}.

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