This is a repository copy of *Gluco-1 H-imidazole : A New Class of Azole-Type β-Glucosidase Inhibitor*.

White Rose Research Online URL for this paper:
http://eprints.whiterose.ac.uk/129628/

Version: Accepted Version

**Article:**
Schröder, Sybrin P, Wu, Liang orcid.org/0000-0003-0294-7065, Artola, Marta et al. (9 more authors) (2018) *Gluco-1 H-imidazole : A New Class of Azole-Type β-Glucosidase Inhibitor*. Journal of the American Chemical Society. pp. 1-4. ISSN 0002-7863

https://doi.org/10.1021/jacs.8b02399

---

**Reuse**
Items deposited in White Rose Research Online are protected by copyright, with all rights reserved unless indicated otherwise. They may be downloaded and/or printed for private study, or other acts as permitted by national copyright laws. The publisher or other rights holders may allow further reproduction and re-use of the full text version. This is indicated by the licence information on the White Rose Research Online record for the item.

**Takedown**
If you consider content in White Rose Research Online to be in breach of UK law, please notify us by emailing eprints@whiterose.ac.uk including the URL of the record and the reason for the withdrawal request.
Gluco-1H-imidazole: a new class of azole-type β-glucosidase inhibitor

Sybrin P. Schröder,‡ Liang Wu,§ Marta Artola,† Thomas Hansen,† Wendy A. Offen,§ Maria J. Ferraz,‡ Kah-Yee Li,‡ Johannes M. F. G. Aerts,† Gijsbert A. van der Marel,† Jeroen D. C. Codée,† Gideon J. Davies§ and Herman S. Overkleeft§

†Department of Bioorganic Synthesis and ‡Department of Medical Biochemistry, Leiden Institute of Chemistry, Leiden University, Einsteinweg 55, 2333 CC Leiden, The Netherlands.
§Department of Chemistry, York Structural Biology Laboratory, University of York, Heslington, York, YO10 5DD, U.K.

Supporting Information Placeholder

ABSTRACT: Gluco-azoles competitively inhibit glucosidases by transition-state mimicry and their ability to interact with catalytic acid residues in glucosidase active sites. We noted that no azole-type inhibitors described, to date, possess a protic nitrogen characteristic for 1H-imidazoles. Here, we present gluco-1H-imidazole, a gluco-azole bearing a 1H-imidazole fused to a glucopyranose-configured cyclitol core, and three close analogues as new glucosidase inhibitors. All compounds inhibit human retaining β-glucosidase, GBA1, with the most potent ones inhibiting this enzyme (deficient in Gaucher disease) on a par with glucosidase. None inhibit glucosylceramide synthase, cytosolic β-glucosidase GBA2 or α-glucosidase GAA. Structural, physical and computational studies provide first insights into the binding mode of this conceptually new class of retaining β-glucosidase inhibitors.

Glycosidases catalyze the hydrolysis of interglycosidic linkages, and glucosidase inhibitors are widely regarded as promising therapeutic entities. Azole-containing glycopyranoside mimics are a major class of competitive glucosidase inhibitors. The natural product, nagstatin (1), is a potent N-acetyl-β-D-glucosaminidase (NAG) inhibitor. Glucopyranose mimics bearing tetrazole (2), triazole (3, 4) and imidazole (5) moieties have been conceived as β-glucosidase inhibitors by Tatsuta and Vasella, with inhibitory potencies against sweet almond β-glucosidase ranging from low micromolar (5) to high micromolar (4, Figure 1a). The reaction itinerary followed by many β-glucosidases proceeds via a H transition state conformation, matching one of the thermodynamically most favored conformations of gluco-azoles (local minima calculated for 5 are H1 and H2). This conformational transition state mimicry contributes considerably to the inhibitory potency of gluco-azoles. Vasella concluded, from extensive studies on gluco-azoles (including compounds 2-5), that an azole nitrogen at the position corresponding to the exocyclic oxygen in a β-glucoside substrate is essential for inhibitory potency, as this ‘exocyclic’ nitrogen participates in lateral coordination with the catalytic acid/base of anti-protonating glucosidases. Remarkably, no azole-type inhibitors synthesized to date are protic with respect to the azole ring (such as 1H-imidazoles), and we reasoned that such position-al isomers would be attractive alternatives to the reported gluco-azoles. Herein, we report on the synthesis and biochemical evaluation of gluco-1H-imidazole 6 and its analogues 7-9 as conceptually new retaining β-glucosidase inhibitors (Figure 1b).

Figure 1. (a) Known cyclitol-azole type glycosidase inhibitors and their inhibitory potencies against porcine NAG (1) and sweet almond β-glucosidase (2-5). (b) 1H-Imidazoles subject of this study.

The synthesis of gluco-1H-imidazole 6 commenced with cyclohexene 10, which was transformed into the vicinal diimine following a modified procedure from Llebaria. Rapid conversion of 10 was accomplished using RuCl3/NaIO4, affording a separable, near equimolar mixture of 1,2-cis-dihydroxy isomers 11a (SS) and 11b (RR). Bismesylation and subsequent azide substitution afforded diazido cyclitols 12a,b. Platinum oxide catalyzed hydrogenolysis afforded diazino cyclitols 13a,b, which were condensed with trimethyl orthoformate in hexafluoroisopropanol (HFIP) to afford imidazolines 14a.b. Protected 1H-imidazole 15 was obtained by oxidation of either isomer of 14 with IBX/DMSO, and final hydrogenolysis afforded gluco-1H-imidazole 6. Derivatization of the imidazole ring of gluco-1H-imidazoles was achieved by variation of the trimethyl orthoester employed. Thus, reaction of 13a,b with trimethyl orthoformate followed by oxidation and deprotection furnished 2-buty1-1H-imidazole 7 via 16a,b and 17. Utilizing tetrabenzyl mycosaminocyclitol as starting material yielded conduritol B-1H-imidazoles 8 and 9 (see Supporting Information SI).
We determined competitive kinetic constants and IC\textsubscript{50} values for 1H-imidazoles 6–9 as compared with glucimidazole 5 for inhibition of the retaining glucosidases \textit{Tm}GH1 from \textit{Thermotoga maritima},\textsuperscript{26} \textit{Tx}GH116 from \textit{Thermoanaerobacterium xylanolyticum},\textsuperscript{21} sweet almond \(\beta\)-glucosidase (GHA1), human lysosomal acid \(\beta\)-glucosylceramidase (GBA1, GH30), human cytosolic \(\beta\)-glucosidase (GAA, GH31), murine glucosylceramide synthase (GCS) and human acid \(\alpha\)-glucosidase (GAA, GH31). Gluco-1H-imidazole 6 displayed micromolar inhibitory activity against most \(\beta\)-glucosidases tested, with \(K_i\) values ranging from \(\sim 3.9 \mu M\) against GBA1 to \(\sim 69 \mu M\) against \(Tx\)GH116 (Table 1). Remarkably, given their apparent structural similarity, 1H-imidazole 6 proved to be a weaker \(\beta\)-glucosidase inhibitor than glucimidazole 5, which inhibited \(\beta\)-glucosidases at nanomolar concentrations. Attachment of a butyl moiety, as in 7, increased the inhibitory potency of the gluco-1H-imidazole scaffold. Whilst this increase in potency was modest for \textit{Tx}GH116 and \textit{Tm}GH116, 7 inhibited sweet almond \(\beta\)-glucosidase and GBA1 with nanomolar potency. Notably, the biological role of GBA1 is breakdown of amphiphilic glucosylceramide.\textsuperscript{22} Connduritol-1H-imidazole 8 did not effectively inhibit any glucosidases tested except for GBA1, consistent with a loss of interactions at the O6 position, but also with the fact that GBA1 is effectively inhibited by conduritol B epoxide (CBE).\textsuperscript{23} As with the gluco-1H-imidazoles, addition of a butyl moiety to the conduritol-1H-imidazole scaffold increased activity against sweet almond \(\beta\)-glucosidase and GBA1, rendering 9 a nanomolar inhibitor of these enzymes. No compounds displayed any inhibitory activity against GBA2, GCS or \(\alpha\)-glucosidase GAA under our assay conditions. Competitive, selective GBA1 inhibitors are considered attractive starting points for the development of pharmacological chaperones: compounds that stabilize the fold of mutant, partially malfunctioning GBA1 in Gaucher patients.\textsuperscript{24,25} Compounds 7 and 9 (as well as known glucimidazole 5) are in our opinion attractive starting points for this purpose.

One striking result displayed in Table 1 is that 1H-imidazole 6 is a comparatively much weaker retaining \(\beta\)-glucosidase inhibitor than glucimidazole 5. Since the structures are closely related, we elected to investigate this result in more depth. Literature studies have shown that the inherent basicity of the azole ring in compounds 5 and 6 are almost identical (5: \(\text{pK}_a\) 6.2, lit. 6.1\textsuperscript{26}; 6: \(\text{pK}_a\) 6.0; Figure S1, S2). We also explored the interaction of gluco-1H-imidazoles with \textit{Tm}GH1 and \textit{Tx}GH116 by isothermal titration calorimetry (ITC) at pH 5.8 (activity optimum\textsuperscript{11–13}) and pH 6.8 (where 5 shows strongest inhibition)\textsuperscript{11}. In line with the \(K_i\) data (Table 1), ITC titrations at both pH values showed nanomolar binding affinities for 5, and micromolar affinities for 6 and 7 (Table S3). The enthalpic contributions in active site binding for 1H-azoles are smaller than those observed for 5, and only partially compensated for by increased entropic contributions to the binding energy. In line with what has been reported for 5,\textsuperscript{11} the presence of a hydrophobic moiety in 7 reduced its enthalpy of binding compared to 6, which was counterbalanced by an increase in favorable entropic contributions to binding.

Table 1. Inhibition constants (\(K_i\)) and IC\textsubscript{50} values in \(\mu M\) for compounds 5–9.

| \(Tm\)GH1\textsuperscript{a} | \(Tx\)GH116\textsuperscript{c} | sweet almond \(\text{GHA1}\)\textsuperscript{d} | GBA1\textsuperscript{e} | GBA2\textsuperscript{e} | GCS\textsuperscript{c} | GAA\textsuperscript{c} |
|-----------------|-----------------|----------------|-----------|-----------|--------|--------|
| 6               | 58 ± 1          | 69 ± 12        | 23 ± 1    | 3.9 ± 2   | >100   | >50    | >100    |
| 7               | 39 ± 14         | 46 ± 5         | 0.039 ± 0.006 | 0.133 ± 0.040 | >100   | >50    | >100    |
| 8               | >100\textsuperscript{f} | >100\textsuperscript{f} | >100\textsuperscript{f} | 13.7 ± 6 | >100   | >50    | >100    |
| 9               | >100\textsuperscript{f} | >100\textsuperscript{f} | 0.221 ± 0.004 | 0.079 ± 0.001 | >100   | >50    | >100    |
| 5               | 0.026 ± 0.001   | 0.165 ± 0.006  | 0.067 ± 0.004\textsuperscript{f} | 0.070 ± 0.005 | >100   | >50    | >100    |

Values are mean ± SD from triplicate experiments. Assays measured at pH 6.8 using \(\beta\)-p-NPG substrate; \(^*\)pH 5.2 using \(\beta\)-2,4-DNPG substrate; \(^f\) Apparent IC\textsubscript{50}. \(^6\text{Lit.}\) \(K_i\) = 0.100 \(\mu M\).\n
![Scheme 1. Synthesis of 1H-imidazoles 6 and 7.]*

\*Reagents and conditions: a) RuCl\textsubscript{3}-H\textsubscript{2}O (7 mol%), NaIO\textsubscript{4}, EtOAc, MeCN, H\textsubscript{2}O, 0 °C, 90 min, 11a 40%, 11b 32%; b) MsCl, \(N\)-methyl-imidazole, Et\textsubscript{3}N, CHCl\textsubscript{3}, rt, 16 h, then Na\textsubscript{2}SO\textsubscript{4}, DMF, 100 °C, 16h, 12a 74%, 12b 58%; c) PO\textsubscript{3}, H\textsubscript{2}O, THF, rt, 16h, 13a 80%, 13b 96%; d) trimethyl orthoformate, HFIP, rt, 16h, 14a 76%, 14b 87%; or trimethyl orthovalerate, HFIP, rt, 16h, 16a 74%, 16b 78%; e) For 15: IBX, DMSO, 45 °C, 16h, 75% from 14a, 71% from 14b; for 17: (COCl)\textsubscript{2}, DMSO, DCM, -60 °C, 1h, 76% from 16a, 70% from 16b; f) Pd(OH)\textsubscript{2}/C, H\textsubscript{2}, HCl, MeOH, quant. 6, quant. 7.
azole is in a neutral state served for complexes with may be bound less strongly. No strong B-factor trend was ob-

Next, the X-ray structure of in complex with 5,1H-imidazole 6 was determined to 1.7 Å (PDB: 5OSS), and compared to the structure of the same enzyme complexed with 5 (PDB: 2CES).

We observed a single molecule of 6 in the active site after ligand soaking, adopting a 'E' conformation, similar to the complex of 5 and TmGH1 (Figure 2a). H-bonding interactions made by 6 to active site residues were identical to those observed with 5, albeit with a slight 'upwards' tilt for 6 compared to 5 (~0.4 or 0.5 Å 'upward' shifts at the apical imidazole carbon, compared with ligands in chains A or B of 2CES respectively; Figure 2b, Figure S3). Crystal structures in TgxGH16 at 2.1 Å resolution (Figure 2c,d) also revealed similar binding modes and a ~0.5 Å 'upwards' tilt for 6 compared to 5 (PDB: SOST and 5BX4, respectively). For both TmGH1 and TgxGH16 complexes, B-factors in the 'imidazole' portion of 6 were markedly higher when compared to the 'glucose' portion of the molecule, indicating the imidazole of 6 was more disordered in the crystal structure and may be bound less strongly. No strong B-factor trend was observed for complexes with 5 (Figure S3).

The underlying cause for the reduced potency of gluco-1H-imidazole 6 compared to 5 is most likely the combination of a number of factors. We propose that repositioning of the N1 atom (from the bridgehead position in 5 to the position in 6) brings two major consequences that together reduce the binding affinity of 6 compared to 5. Firstly, considering the situation where the imidazole is in a neutral state, the free lone pair of the N2 atom in 5 can laterally coordinate to the acid/base residue of the bound glucosidase in typical anti-protonation glucosidases (although TgxGH16 lacks this interaction due to the unusual placement of its acid/base residue).

Figure 2. (a) Gluco-1H-imidazole 6 in complex with TmGH1, with direct H-bonding interactions shown. (b) Overlay of 5 (pink) and 6 (cyan) within the TmGH1 active site (chain B from each structure). (c) Gluco-1H-imidazole 6 in complex with TgxGH16. (d) Overlay of 5 (salmon) and 6 (blue) within the TgxGH16 active site. Electron densities are REFMAC maximum-likelihood/structure). (a) Prototropic tautomerism of 6. (b) Positive charge is delocalized onto the 'apical' carbon in protonated 6. (c) In 5, positive charge is delocalized onto the anomic equivalent carbon, ideally located for charge-charge interaction with the nucleophile residue. Mulliken charges are annotated in red.

Figure 3. Interactions of gluco-1H-imidazole 6 and classical glucoimidazole 5 with the catalytic residues. (a) Prototropic tautomerism of 6. (b) Positive charge is delocalized onto the 'apical' carbon in protonated 6. (c) In 5, positive charge is delocalized onto the anomic equivalent carbon, ideally located for charge-charge interaction with the nucleophile residue. Mulliken charges are annotated in red.

In conclusion, we have described a new class of competitive β-glucosidase inhibitors: the 1H-gluco-azoles. The synthetic route is flexible regarding substituents on the imidazole ring, and can likely be transferred to configurational isomers by applying this route to configurational isomers of cyclohexene. Our compounds resemble to some extent the 1H-imidazoles reported by Li and Byers, and Field et al., whose simple, achiral molecules inhibit several glucosidases as well, though likely by a different mode of action. 1H-Imidazole 6 appeared a poorer inhibitor than compound 5, and we hypothesize this is caused by delocalization...
of the lone pair on the nitrogen atom due to tautomerism and/or impaired δ+ charge development at the anomeric center. Introduction of an alkyl substituent on the 1H-imidazole ring (as in 7) as well as ‘deletion’ of the methylene carbon (as in 9) yielded potent competitive inhibitors of the human lysosomal glucosylceramide GBA1, selective over human cytosolic GBA2 and GCS. These and related compounds we believe have potential to be developed into pharmacological chaperone candidates for Gaucher disease and perhaps also neuropathological disorders (in particular, Parkinsonism) that are characterized by partial deficiency in GBA.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website.

Experimental data and procedures, enzyme kinetics, DFT calculations, crystallographic data (PDF).

AUTHOR INFORMATION

Corresponding Author

* h.s.overkleeft@lic.leidenuniv.nl

Notes

The authors declare no competing financial interests.

ACKNOWLEDGMENT

We thank the Netherlands Organization for Scientific Research (NWO-CW ChemiThem grant to JMFGA and HSO), the European Research Council (ERC-2011-AdG-290836 ‘Chembiosphing’ to HSO, and ERC-2012-AdG-322942 ‘Glycopoise’ to GJD), Sanofi Genzyme (research grant to JM FGA and HSO and postdoctoral contract to MA) and Diamond Light Source (beamline I03, proposal number mx-13587) for provision of data collection facilities. GJD thanks the Royal Society for the Ken Murray Research Professorship.

REFERENCES

(1) N. Asano, Glycobiology 2003, 13, 93.
(2) V. H. Lilleyhund, H. H. Jensen, X. Liang, M. Bols, Chem. Rev. 2002, 102, 515.
(3) T. Aoyagi, H. Suda, K. Uotani, F. Kojima, T. Aoyama, K. Horiguchi, M. Hamada, T. Takeuchi, J. Antibiolt. 1992, 45, 1404.
(4) K. Tatsuta, S. Miura, S. Ohita, H. Gunji, Tetrahedron Lett. 1995, 36, 1085.
(5) P. Ermert, A. Vasella, Helv. Chim. Acta 1991, 74, 2043.
(6) T. Granier, N. Panday, A. Vasella, Helv. Chim. Acta 1997, 80, 979.
(7) T. D. Heightman, M. Locatelli, A. Vasella, Helv. Chim. Acta 1996, 79, 2190.
(8) G. Speciale, A. J. Thompson, G. J. Davies, S. J. Williams, Chem. Soc. Rev. 2014, 23, 1.
(9) A. Tankrathok, J. Iglesias-Fernández, R. J. Williams, S. Penghaisong, S. Baiya, Z. Hakki, R. C. Robinson, M. Hrnova, C. Rovira, S. J. Williams, J. R. Ketudat Cairns, ACS Catal. 2015, 5, 6041.
(10) A. Varrot, M. Schülein, M. Pippeier, A. Vasella, G. J. Davies, J. Am. Chem. Soc. 1999, 121, 2621.
(11) T. M. Gloster, S. Roberts, G. Perugini, M. Rossi, M. Moracci, N. Panday, M. Terinek, A. Vasella, G. J. Davies, Biochemistry 2006, 45, 11879.
(12) V. Notenboom, S. J. Williams, R. Hoos, S. G. Withers, D. R. Rose, Biochemistry 2000, 39, 11553.
(13) M. Hrnova, V. A. Streltsov, B. J. Smith, A. Vasella, N. J. Varghese, G. B. Fincher, Biochemistry 2005, 44, 16529.
(14) T. D. Heightman, A. T. Vasella, Angew. Chem. Int. Ed. 1999, 38, 2060.
(15) F. G. Hansen, E. Bundgaard, R. Madsen, J. Org. Chem. 2005, 70, 10139.
(16) A. Tranero, A. Llebaria, ACS Med. Chem. Lett. 2011, 2, 614.
(17) M. Artola, L. Wu, M. J. Ferraz, C. L. Kuo, L. Raich, I. Z. Breen, W. A. Offen, J. D. C. Codée, G. A. van der Marel, C. Rovira, J. M. F. G. Aerts, G. J. Davies, H. S. Overkleeft, ACS Cent. Sci. 2017, 3, 784.
(18) S. Khaksar, A. Heydari, M. Takaibekah, S. M. Vahdat, J. Fluor. Chem. 2019, 11, 1377.
(19) K. C. Nicolosiu, C. J. N. Mathison, T. Montagnon, J. Am. Chem. Soc. 2004, 126, 5192.
(20) L. Liu, Z. Zeng, G. Zeng, M. Chen, Y. Zhang, J. Zhang, X. Fang, M. Jiang, L. Lu, Bioorg. Med. Chem. Lett. 2012, 22, 837.
(21) R. Charoenwattanasatien, S. Pengthaisong, I. Breen, R. Matoth, S. Sansenya, Y. Hua, A. Tankrathok, L. Wu, C. Songziriththugil, H. Tanaka, S. J. Williams, G. J. Davies, G. Kurisu, J. R. K. Cairns, ACS Chem. Biol. 2016, 11, 1891.
(22) M. Abdul-Hammed, B. Breiden, G. Schwarzmann, K. Sandhoff, J. Lipid Res. 2017, 58, 563.
(23) G. A. Grabowski, K. Osięcki-Newman, T. Dinur, D. Fabbro, G. Legler, S. G., R. J. Desnica, J. Biol. Chem. 1996, 261, 8263.
(24) A. R. Sawkar, W.-C. Cheng, E. Beutler, C.-H. Wong, W. E. Balch, J. W. Kelly, Proc. Natl. Acad. Sci. U. S. A. 2002, 99, 15428.
(25) R. A. Steet, S. Chung, B. Wustman, A. Powe, H. Do, S. A. Komfield, Proc. Natl. Acad. Sci. U. S. A. 2006, 103, 13813.
(26) N. Panday, A. Vasella, Synthesis 1999, 1459.
(27) N. Panday, Y. Canac, A. Vasella, Helv. Chim. Acta 2000, 83, 58.
(28) B. Wang, J. I. Olsen, B. W. Laursen, J. C. Navarro Poulsen, M. Bols, Chem. Sci. 2017, 8, 7383.
(29) T. D. Heightman, A. Vasella, K. E. Tsitsanou, S. E. Zographos, V. T. Skamnaki, N. G. Oikonomakos, Helv. Chim. Acta 1998, 81, 853.
(30) Y. Harrak, C. M. Bara, A. Delgado, A. R. Castaño, A. Llebaria, J. Am. Chem. Soc. 2011, 133, 12079.
(31) Y.-K. Li, L. D. Byers, J. Med. Chem. 1998, 41, 2279.
(32) A. Trapero, A. Llebaria, J. Lipid Res. 2014, 1. 1.
(33) S. J. Williams, J. R. Ketudat Cairns, ACS Med. Chem. Lett. 2011, 2, 12079.
(34) M. E. Gegg, D. Burke, S. J. R. Heales, J. M. Cooper, J. Hardy, N. W. Wood, A. H. Schapira, Ann. Neurol. 2012, 72, 455.
SYNOPSIS TOC (Word Style “SN_Synopsis_TOC”). If you are submitting your paper to a journal that requires a synopsis graphic and/or synopsis paragraph, see the Instructions for Authors on the journal’s homepage for a description of what needs to be provided and for the size requirements of the artwork.

Insert Table of Contents artwork here