ACS Medicinal Chemistry Letters

Letter

pubs.acs.org/acsmedchemlett

C-Glucopyranosyl-1,2,4-triazoles As New Potent Inhibitors of Glycogen Phosphorylase

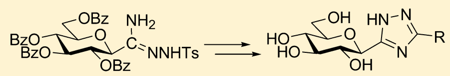
Éva Bokor,[†] Tibor Docsa,[‡] Pál Gergely,[‡] and László Somsák*[,†]

[†]Department of Organic Chemistry, University of Debrecen, POB 20, H-4010 Debrecen, Hungary

[‡]Department of Medical Chemistry, Medical and Health Science Centre, University of Debrecen, Egyetem tér 1, H-4032 Debrecen, Hungary

Supporting Information

ABSTRACT: Glycogen phosphorylase inhibitors are considered as potential antidiabetic agents. 3-(β-D-Glucopyranosyl)-5-substituted-1,2,4-triazoles were prepared by acylation of *O*-perbenzoylated *N*[1]-tosyl-*C*-β-D-glucopyranosyl formamidrazone and subsequent removal of the protecting groups. The best inhibitor was 3-(β-D-glucopyranosyl)-5-(2-naphthyl)-1,2,4-triazole (K_i = 0.41 μM against rabbit muscle glycogen phosphorylase b).



KEYWORDS: *1,2,4-Triazole, C-glucopyranosyl derivative, bioisoster, glycogen phosphorylase, inhibitor*

Inhibitors of enzymes are among classics of medicinal chemistry, and many drug molecules' activity is due to decreasing the efficiency of these catalytic proteins.[1] In a chemical biological approach, finding an enzyme inhibitor is the result of a good match of the biological and chemical spaces represented by a binding site of an enzyme and a small molecule, respectively, fitting to each other with considerable strength. Among several methods to design inhibitors, bioisosteric replacement of structural elements of existing molecules is widely applied and in many cases results in higher activity or other advantageous property of the new compound.[2]

Glycogen phosphorylase (GP) is the main regulatory enzyme of glycogen metabolism. GP, catalyzing the rate determining step of glycogen degradation in the liver by phosphorolysis, is directly responsible for the regulation of blood glucose levels. Therefore, GP has been a validated target in combating noninsulin-dependent or type 2 *diabetes mellitus* (T2DM), and its inhibitors are considered as potential antidiabetic agents. The biochemical and pharmacological background of this research has been thoroughly summarized in several reviews of the past decade; therefore, the reader is kindly referred to those papers.[3−5] Furthermore, possible application of GP inhibitors in intervention of other diseased states associated with GP activity (e.g., cardiovascular disorders,[6] ischemic lesions,[7,8] and tumorous growth[7]) has also been under investigations.

Several classes of compounds[9,10] were shown to be inhibitors of GP. The most widely studied group of molecules is that of glucose derivatives,[11,12] which bind primarily to the active site of GP.[13] The best glucose derivatives are submicromolar inhibitors of rabbit muscle GPb, the prototype of GPs.[14] Glucopyranosylidene-spiro-thiohydantoin (K_i = 29.8 μM against rat liver GP) was shown to exert considerable in vivo blood sugar diminishing activity.[15]

N-Acyl-β-D-glucopyranosylamines (compounds **1** in Chart 1) were among the first GP inhibitors,[16] and many analogous derivatives were investigated.[17−20] In this series, *N*-(2-naphthoyl)-β-D-glucopyranosylamine (**1** *R* = 2-naphthyl) was the best inhibitor,[18] which also served as a lead structure for bioisosteric replacements. As illustrated in Chart 1, enzymatic tests[21] as well as crystallographic studies[19] revealed high similarity of amide (**1**) and 1,2,3-triazole (**2**) type inhibitors both in binding strength and structural features of the enzyme−inhibitor complexes. Kinetic tests of bioisosteric oxadiazoles[22,23] **3**−**5** demonstrated that the constitution of the heterocycle had a strong bearing on the inhibition: the most efficient inhibitor in these series was 5-(β-D-glucopyranosyl)-3-(2-naphthyl)-1,2,4-oxadiazole (**5**), which had a similar efficiency to that of **1**.

Other investigations on *C*-glucopyranosyl heterocycles with condensed rings showed that benzothiazole **7** was much less efficient than benzimidazole **8**.[24] An X-ray crystallographic study of the RMGPb−**8** complex revealed a specific H-bond between NH of the heterocycle and the main chain C═O of His377,[25] and the stronger binding of **8** was attributed to this interaction, which cannot exist in the case of **7**.

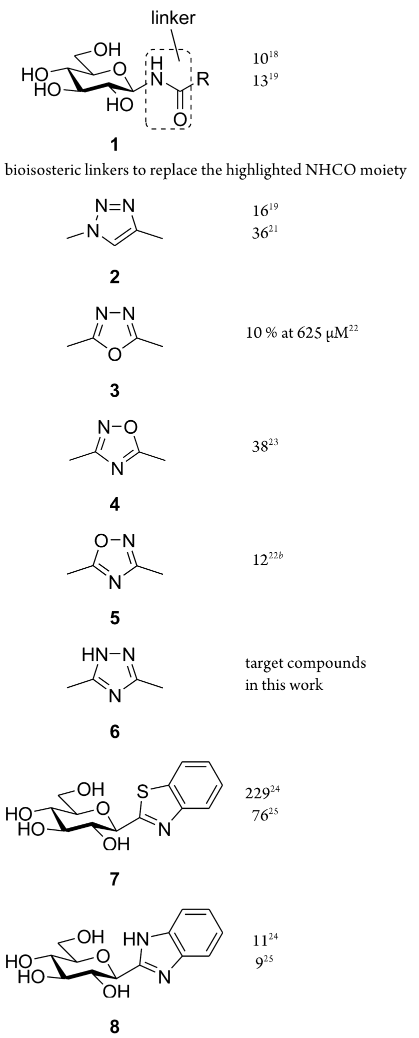
On the basis of these preliminaries, synthesis and study of 1,2,4-triazoles of type **6** were envisaged anticipating that the H-bond donor capacity of this heterocycle would result in stronger inhibitors of GP.

3-Glycosyl-5-substituted-1,2,4-triazoles were described in the literature mainly with furanoid rings in reactions of *C*-glycofuranosyl (thio)formimidates with hydrazide or amidrazone reagents[26−28] or transforming a 2,5-anhydro-D,L-allonolactone derivative with aminoguanidine.[29] 3-Glycopyranosyl-5-substituted-1,2,4-triazoles could not be located in the literature; the only *C*-glycopyranosyl-1,2,4-triazoles were 1,3,5-trisubstituted derivatives obtained from glycosyl cyanides with 1-aza-2-azoniaallene salts[30] or with hydrazonoyl chlorides in the presence of Yb(OTf)$_3$.[31]

Received: April 22, 2013
Accepted: May 17, 2013

dx.doi.org/10.1021/ml4001529 | ACS Med. Chem. Lett. XXXX, XXX, XXX−XXX

Chart 1. Selected Inhibitors of Glycogen Phosphorylase and Their Efficiency[a]

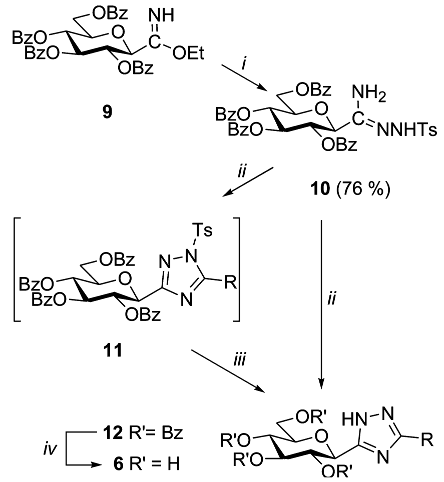


[a]K_i [μM] against RMGPb for R = 2-naphthyl. [b]A K_i value of 2.4 μM was measured independently by Oikonomakos and co-workers.[22]

Synthesis of the desired 3-glucopyranosyl-5-substituted-1,2,4-triazoles of type **6** was planned by adaptation of a literature protocol[32] in which acylation of N^1-tosylamidrazones gave 3,5-disubstituted-1-tosyl-1,2,4-triazoles. Removal of the *N*-tosyl group was foreseen under conditions usually applied for *N*-desulfonylation of nitrogen heterocycles.[33]

To start the syntheses, *O*-perbenzoylated *β*-D-glucopyranosyl formimidate[34] **9** was reacted with tosylhydrazide to give the necessary tosylamidrazone **10** in good yield (Scheme 1). Reaction of **10** with acetyl chloride furnished tosyl-triazole **11a**, which was *N*-detosylated by tetrabutylammonium fluoride (TBAF) to **12a**. With acetoxyacetyl chloride **10** gave a mixture of **11b** and **12b** indicating that the *N*-tosyl group is prone to splitting off under the acylation conditions. The crude mixture of **11b** and **12b** was treated with TBAF to produce **12b** in 61% yield for the two steps. Acylations of **10** with aromatic acid chlorides were accompanied by complete *N*-detosylation thereby simplifying the preparation of **12d–f**, which were obtained in good yields. Removal of the *O*-acyl protecting groups was effected under Zemplén conditions to give test compounds **6a** and **6c–f** in good to excellent yields.

Scheme 1. Synthesis of 3-(*β*-D-Glucopyranosyl)-5-substituted-1,2,4-triazoles (6)[a]



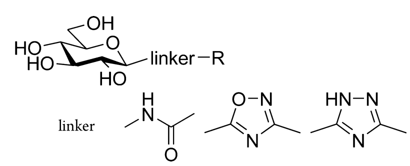
i) 1.5 equiv $TsNHNH_2$, dry CH_2Cl_2, rt; *ii*) 1.5 equiv RCOCl, 1.8 equiv pyridine, dry $CHCl_3$, 0 °C to rt; *iii*) TBAF, dry THF, reflux; *iv*) ~1M NaOMe in MeOH, rt.

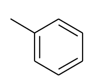
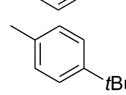
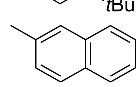
	R	Conditions and yields (%)					
			11		**12**[a]		**6**
a	-CH_3	*ii*	69	*iii*	88[b]	*iv*	73
b	-CH_2OCOCH_3	-	-	*ii, iii*	61[c]	-	-
c	-CH_2OH	-	-	-	-	*iv*	93[d]
d	-C_6H_5	-	-	*ii*	69	*iv*	62
e	-C_6H_4-4-*t*Bu	-	-	*ii*	58	*iv*	71
f	2-naphthyl	-	-	*ii*	56	*iv*	81

[a]From **10**. [b]From **11a**. [c]The crude mixture obtained from amidrazone **10** and acetoxyacetyl chloride was treated by TBAF. [d]From **12b**.

3-(*β*-D-Glucopyranosyl)-5-substituted-1,2,4-triazoles **6** were assayed against RMGPb as described earlier,[35] and the kinetic results, showing the compounds to be competitive inhibitors, are summarized in Table 1. Methyl (**6a**) and hydroxymethyl (**6c**) derivatives proved weak inhibitors in the micromolar

Table 1. Inhibition[a] of RMGPb by Compounds 6 and Comparison to Other Nonclassical Bioisosteres



R		1	5	6
-CH_3	a	32[16]	-	360[b]
-CH_2OH	c	18[19] 20[21]	-	105
	d	81[16] 144[17]	64[22]	7
 *t*Bu	e	-	-	207[b]
	f	10[18] 13[19]	12[22]	0.41

[a]K_i [μM] [b]Calculated from the IC_{50} value by using a web-based tool.[36]

range and were significantly less efficient than the parent amides **1a** and **1c**, respectively. Appending unsubstituted aromatic groups to the 1,2,4-triazole ring as in **6d** and **6f** led to a remarkable strengthening of the inhibition. While 1,2,4-oxadiazoles **5d** and **5f** were practically equipotent with the corresponding amides **1d** and **1f**, triazoles **6d** and **6f** inhibited the enzyme by ~1 order of magnitude stronger, respectively. This indicated that the possibility for the formation of a H-bond was advantageous for the binding, rendering compound **6f** to one of the most efficient glucose analogue inhibitors of GP known to date. Introduction of a *t*-butyl substituent in the 4-position of the phenyl group as in **6e** resulted in a much weaker inhibitor. This observation may reveal that the active site of GP, where these compounds may bind to, can not accommodate a bulky aliphatic moiety.

Further studies to establish the binding peculiarities of these inhibitors by X-ray crystallographic investigation of the enzyme−inhibitor complexes as well as molecular dockings to predict other efficient derivatives based on this skeleton are in progress.

In conclusion, a new method was elaborated for the synthesis of hitherto unknown 3-(β-D-glucopyranosyl)-5-substituted-1,2,4-triazoles. These compounds inhibited rabbit muscle GPb, and the 5-(2-naphthyl) derivative with its submicromolar inhibition proved one of the best inhibitors of the enzyme.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Representative synthetic procedures, enzyme kinetic measurements, and compound characterization. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*(L.S.) Tel: +3652512900, ext. 22348. Fax: +3652512744. E-mail: somsak@tigris.unideb.hu.

Funding

This work was supported by the Hungarian Scientific Research Fund (OTKA CK77712, CNK80709, and PD105808) as well as TÁMOP 4.2.1./B-09/1/KONV-2010-0007 and TÁMOP-4.2.2.A-11/1/KONV-2012-0025 projects implemented through the New Hungary Development Plan, cofinanced by the European Social Fund. T.D. thanks the Hungarian Academy of Sciences for a János Bolyai research fellowship.

Notes

The authors declare no competing financial interest.

■ REFERENCES

(1) Smith, H. J.; Simons, C. *Enzymes and Their Inhibition, Drug Development*; CRC Press: Boca Raton, FL, 2005.

(2) Lima, L. M. A.; Barreiro, E. J. Bioisosterism: a useful strategy for molecular modification and drug design. *Curr. Med. Chem.* **2005**, *12*, 23−49.

(3) Kurukulasuriya, R.; Link, J. T.; Madar, D. J.; Pei, Z.; Richards, S. J.; Rohde, J. J.; Souers, A. J.; Szczepankiewicz, B. G. Potential drug targets and progress towards pharmacologic inhibition of hepatic glucose production. *Curr. Med. Chem.* **2003**, *10*, 123−153.

(4) Ross, S. A.; Gulve, E. A.; Wang, M. H. Chemistry and biochemistry of type 2 diabetes. *Chem. Rev.* **2004**, *104*, 1255−1282.

(5) Agius, L. New hepatic targets for glycaemic control in diabetes. *Best Pract. Res., Clin. Endocrinol. Metab.* **2007**, *21*, 587−605.

(6) Baker, D. J.; Greenhaff, P. L.; Timmons, J. A. Glycogen phosphorylase inhibition as a therapeutic target: a review of the recent patent literature. *Expert Opin. Ther. Patents* **2006**, *16*, 459−466.

(7) Henke, B. R.; Sparks, S. M. Glycogen phosphorylase inhibitors. *Mini-Rev. Med. Chem.* **2006**, *6*, 845−857.

(8) Guan, T.; Qian, Y. S.; Tang, X. Z.; Huang, M. H.; Huang, L. F.; Li, Y. M.; Sun, H. B. Maslinic acid, a natural inhibitor of glycogen phosphorylase, reduces cerebral ischemic injury in hyperglycemic rats by GLT-1 up-regulation. *J. Neurosci. Res.* **2011**, *89*, 1829−1839.

(9) Somsák, L.; Czifrák, K.; Tóth, M.; Bokor, É.; Chrysina, E. D.; Alexacou, K. M.; Hayes, J. M.; Tiraidis, C.; Lazoura, E.; Leonidas, D. D.; Zographos, S. E.; Oikonomakos, N. G. New inhibitors of glycogen phosphorylase as potential antidiabetic agents. *Curr. Med. Chem.* **2008**, *15*, 2933−2983.

(10) Loughlin, W. A. Recent advances in the allosteric inhibition of glycogen phosphorylase. *Mini-Rev. Med. Chem.* **2010**, *10*, 1139−1155.

(11) Praly, J. P.; Vidal, S. Inhibition of glycogen phosphorylase in the context of type 2 diabetes, with focus on recent inhibitors bound at the active site. *Mini-Rev. Med. Chem.* **2010**, *10*, 1102−1126.

(12) Somsák, L. Glucose derived inhibitors of glycogen phosphorylase. *C. R. Chim.* **2011**, *14*, 211−223.

(13) Chrysina, E. D.; Chajistamatiou, A.; Chegkazi, M. From structure-based to knowledge-based drug design through X-ray protein crystallography: sketching glycogen phosphorylase binding sites. *Curr. Med. Chem.* **2011**, *18*, 2620−2629.

(14) Chrysina, E. D. The prototype of glycogen phosphorylase. *Mini-Rev. Med. Chem.* **2010**, *10*, 1093−1101.

(15) Docsa, T.; Czifrák, K.; Hüse, C.; Somsák, L.; Gergely, P. The effect of glucopyranosylidene-spiro-thiohydantoin on the glycogen metabolism in liver tissues of streptozotocin-induced and obese diabetic rats. *Mol. Med. Rep.* **2011**, *4*, 477−481.

(16) Watson, K. A.; Mitchell, E. P.; Johnson, L. N.; Cruciani, G.; Son, J. C.; Bichard, C. J. F.; Fleet, G. W. J.; Oikonomakos, N. G.; Kontou, M.; Zographos, S. E. Glucose analogue inhibitors of glycogen phosphorylase: from crystallographic analysis to drug prediction using GRID force-field and GOLPE variable selection. *Acta Crystallogr.* **1995**, *D51*, 458−472.

(17) Somsák, L.; Kovács, L.; Tóth, M.; Ősz, E.; Szilágyi, L.; Györgydeák, Z.; Dinya, Z.; Docsa, T.; Tóth, B.; Gergely, P. Synthesis of and a comparative study on the inhibition of muscle and liver glycogen phosphorylases by epimeric pairs of D-gluco- and D-xylopyranosylidene-spiro-(thio)hydantoins and *N*-(D-glucopyranosyl) amides. *J. Med. Chem.* **2001**, *44*, 2843−2848.

(18) Györgydeák, Z.; Hadady, Z.; Felföldi, N.; Krakomperger, A.; Nagy, V.; Tóth, M.; Brunyánszky, A.; Docsa, T.; Gergely, P.; Somsák, L. Synthesis of *N*-(β-D-glucopyranosyl)- and *N*-(2-acetamido-2-deoxy-β-D-glucopyranosyl) amides as inhibitors of glycogen phosphorylase. *Bioorg. Med. Chem.* **2004**, *12*, 4861−4870.

(19) Chrysina, E. D.; Bokor, É.; Alexacou, K.-M.; Charavgi, M.-D.; Oikonomakos, G. N.; Zographos, S. E.; Leonidas, D. D.; Oikonomakos, N. G.; Somsák, L. Amide-1,2,3-triazole bioisosterism: the glycogen phosphorylase case. *Tetrahedron: Asymm.* **2009**, *20*, 733−740.

(20) Kónya, B.; Docsa, T.; Gergely, P.; Somsák, L. Synthesis of heterocyclic *N*-(β-D-glucopyranosyl)carboxamides for inhibition of glycogen phosphorylase. *Carbohydr. Res.* **2012**, *351*, 56−63.

(21) Bokor, É.; Docsa, T.; Gergely, P.; Somsák, L. Synthesis of 1-(D-glucopyranosyl)-1,2,3-triazoles and their evaluation as glycogen phosphorylase inhibitors. *Bioorg. Med. Chem.* **2010**, *18*, 1171−1180.

(22) Tóth, M.; Kun, S.; Bokor, É.; Benltifa, M.; Tallec, G.; Vidal, S.; Docsa, T.; Gergely, P.; Somsák, L.; Praly, J.-P. Synthesis and structure−activity relationships of *C*-glycosylated oxadiazoles as inhibitors of glycogen phosphorylase. *Bioorg. Med. Chem.* **2009**, *17*, 4773−4785.

(23) Benltifa, M.; Vidal, S.; Fenet, B.; Msaddek, M.; Goekjian, P. G.; Praly, J.-P.; Brunyánszki, A.; Docsa, T.; Gergely, P. In the search of glycogen phosphorylase inhibitors: 5-substituted 3-*C*-glucopyranosyl-1,2,4-oxadiazoles from β-D-glucopyranosyl cyanides upon cyclization of *O*-acyl-amidoxime intermediates. *Eur. J. Org. Chem.* **2006**, 4242−4256.

(24) Hadady, Z.; Tóth, M.; Somsák, L. *C*-(β-D-glucopyranosyl) heterocycles as potential glycogen phosphorylase inhibitors. *Arkivoc* **2004**, *(vii)*, 140−149.

dx.doi.org/10.1021/ml4001529 | ACS Med. Chem. Lett. XXXX, XXX, XXX−XXX

(25) Chrysina, E. D.; Kosmopolou, M. N.; Tiraidis, C.; Kardarakis, R.; Bischler, N.; Leonidas, D. D.; Hadady, Z.; Somsák, L.; Docsa, T.; Gergely, P.; Oikonomakos, N. G. Kinetic and crystallographic studies on 2-(β-D-glucopyranosyl)-5-methyl-1,3,4-oxadiazole, -benzothiazole, and -benzimidazole, inhibitors of muscle glycogen phosphorylase *b*. Evidence for a new binding site. *Protein Sci.* **2005**, *14*, 873−888.

(26) Poonian, M. S.; Nowoswiat, E. F. Novel precursor for the synthesis of *C*-nucleoside analogues. Synthesis of the *C*-nucleoside analogues of ribavirin, bredinin, and related compounds. *J. Org. Chem.* **1980**, *45*, 203−208.

(27) Huynh-Dinh, T.; Igolen, J.; Bisagni, E.; Marquet, J. P.; Civier, A. Synthesis of *C*-nucleosides. Part 14. 5(3)-Glycosyl-1,2,4-triazole-3(5)-carboxamides as analogues of ribavirin. *J. Chem. Soc., Perkin Trans. 1* **1977**, 761−764.

(28) Vanek, T.; Farkas, J.; Gut, J. Synthesis of 3,5-disubstituted 1,2,4-triazole derivatives: an alternative preparation of the *C*-analogue of ribavirin. *Collect. Czech. Chem. Commun.* **1979**, *44*, 1334−1338.

(29) Just, G.; Ramjeesingh, M. C-Nucleosides and related compounds. 5. The synthesis of D,L-4(1β-ribofuranosyl)3-carboxamidopyrazole (V), D,L-5(1β-ribofuranosyl)2-amino-1,3,4-oxadiazole (VII) and D,L-5(1β-ribofuranosyl)2-amino-1,2,4-triazole (IX). *Tetrahedron Lett.* **1975**, 985−988.

(30) Al-Masoudi, N.; Hassan, N. A.; Al-Soud, Y. A.; Schmidt, P.; Gaafar, A.; Weng, M.; Marino, S.; Schoch, A.; Amer, A.; Jochims, J. C. Syntheses of *C*- and *N*-nucleosides from 1-aza-2-azoniaallene and 1,3-diaza-2-azoniaallene salts. *J. Chem. Soc., Perkin. Trans. 1* **1998**, 947−953.

(31) Al-Masoudi, N. A.; Al-Soud, Y. A.; Ali, I. A. I. Synthesis of 1,2,4-triazole *C*-nucleosides from hydrazonyl chlorides and nitriles. *Nucleosides, Nucleotides Nucleic Acids* **2007**, *26*, 37−43.

(32) Chouaieb, H.; Ben Mosbah, M.; Kossentini, M.; Salem, M. Novel method for the synthesis of 1,2,4-triazoles and 1,2,4-triazol-3-ones. *Synth. Commun.* **2003**, *33*, 3861−3868.

(33) Wuts, P. G. M.; Greene, T. W. *Greene's Protective Groups in Organic Synthesis*, 4th ed.; Wiley-Interscience: Hoboken, NJ, 2007.

(34) Bokor, É.; Szilágyi, E.; Docsa, T.; Gergely, P.; Somsák, L. Synthesis of substituted 2-(β-D-glucopyranosyl)-benzimidazoles and their evaluation as inhibitors of glycogen phosphorylase. *Carbohydr. Res.* **2013**, DOI: 10.1016/j.carres.2013.01.011.

(35) Ősz, E.; Somsák, L.; Szilágyi, L.; Kovács, L.; Docsa, T.; Tóth, B.; Gergely, P. Efficient inhibition of muscle and liver glycogen phosphorylases by a new glucopyranosylidene-spiro-thiohydantoin. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 1385−1390.

(36) Cer, R. Z.; Mudunuri, U.; Stephens, R.; Lebeda, F. J. IC_{50}-to-K_i: a web-based tool for converting IC_{50} to K_i values for inhibitors of enzyme activity and ligand binding. *Nucleic Acids Res.* **2009**, *37*, W441−W445.