Synthesis and Exploitation of the Biological Profile of Novel Guanidino Xylofuranose Derivatives

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Abstract

The synthesis of novel guanidino sugars as potential mimetics of nucleosides and their biological evaluation is described. 5-Guanidino xylofuranoses containing different *O*-substituents at C-3, including saturated/unsaturated hydrocarbon chains and aromatic-containing moieties, were accessed from 5-azido xylofuranose precursors through reduction followed by guanidinylation of the obtained amines with *N*,*N*′-bis(*tert*-butoxycarbonyl)-*N*″-triflylguanidine. A 5-azido 3-*O*-methyl-branched *N*-benzyltriazole isonucleoside was converted into the corresponding 5-guanidino-containing isonucleoside, whose structure includes both the guanidine and triazole moieties as nucleobase-like motifs connected to the xylofuranose template. In alternative, this structurally new type of compound was synthesized *via* cycloaddition between a 5-guanidino-3-*O*-propargyl xylofuranose derivative and benzyl azide in the presence of a CuI/Amberlyst A-21 catalytic system, along with the 5-iodotriazole derivative as a secondary product, which, in turn was the sole product when using equimolar CuI and a catalytic amount of 4-dimethylaminopyridine. A guanidinomethyltriazole 3′-*O*-dodecyl xylofuranos-5′-yl isonucleoside, which comprise a novel isonucleosidic framework having a guanidine system appended on the sugar-linked triazole motif, was obtained from the related aminomethyltriazole 5'-isonucleoside via guanidinylation. Bioactivity screening revealed 2 compounds as selective inhibitors of acetylcholinesterase (AChE), namely the guanidinomethyltriazole derivative, with moderate inhibition ($K_i = 22.87\ \mu M$)

and the 3-O-dodecyl (N-Boc)guanidino xylofuranose, which was the most active compound ($K_i = 7.49\,\mu M$) acting as a non-competitive inhibitor. The latter also displayed moderate antiproliferative effects in chronic myeloid leukemia (K562, $GI_{50} = 31.02\,\mu M$) and in breast cancer (MCF-7, $GI_{50} = 26.89\,\mu M$) cells. The aminomethyltriazole 5'-isonucleoside was the most potent molecule with single-digit micromolar GI_{50} values against both cells ($GI_{50} = 6.33\,\mu M$ and $8.45\,\mu M$), similar to that of the standard drug 5-fluorouracil against MCF-7 cells.

Keywords: guanidino sugars; isonucleosides; guanidinylation: antiproliferative activity; cholinesterase inhibitors

1. Introduction

The guanidine moiety has attracted significant interest in medicinal chemistry, due to its presence in several bioactive natural and synthetic compounds of pharmaceutical potential [1–3]. The stability of this functionality, its basic nature, the ability for cation-π [4] and hydrogen bond interactions [1], being a pre-requisite for its for recognition by enzymes or receptors, are among the aspects that motivate its inclusion into new potential bioactive structures. Guanidine-containing molecules have been reported to possess anticancer [5,6], antimicrobial [7–10], antiviral [11,12], anti-inflammatory [13,14], or antiparasitic [8,15] properties and to display inhibitory abilities towards therapeutically-relevant enzymes [16–19]. Various guanidine derivatives reached clinical application, from which the antidiabetic metformin [20] or the antimalarial chloroguanide [21] can be highlighted. The guanidine system is present in structural components of biomolecules, namely in the amino acid arginine or in the nucleobase guanine. It is also embodied in the nitrogenous aromatic moiety of various bioactive compounds, such as in the 2-aminopurine motif, which is contained in the antibiotic natural nucleosides amipurimycin and miharamycins [22,23], and in various synthetic nucleosides exhibiting anticancer [24] and antiviral properties [25], or in the 2-aminopyrimidine moiety, which is present in the anticancer drug imatinib [26]. On the other hand, the amidine (or imidamide) system (NH_2-C=NR) contained in the guanidine group is present in the cytosine and adenine-based motifs, which are found in various nucleoside analogues used as chemotherapeutic agents [27,28]. Carbohydrate-based molecules possessing guanidine groups have also shown their importance as therapeutic compounds, with important clinically-used molecules such as the natural pseudotrisaccharide antibiotic streptomycin [29] and the antiviral sialic acid mimetic zanamivir [30]. Research reports on guanidino sugars also revealed the ability of iminosugar guanidines to act as glycosidase inhibitors [31–35], while N-glycosyl guanidines containing N′-4-arylthiazol-2-yl groups were described to possess inhibitory activity towards HIV-1 protease and nitric oxide synthase [36]. Other reported guanidino-containing sugar derivatives of biological interest included analogues of N-glucosyl arginine [37] and β-(1→6)-guanidine-linked glucooligomers, which were
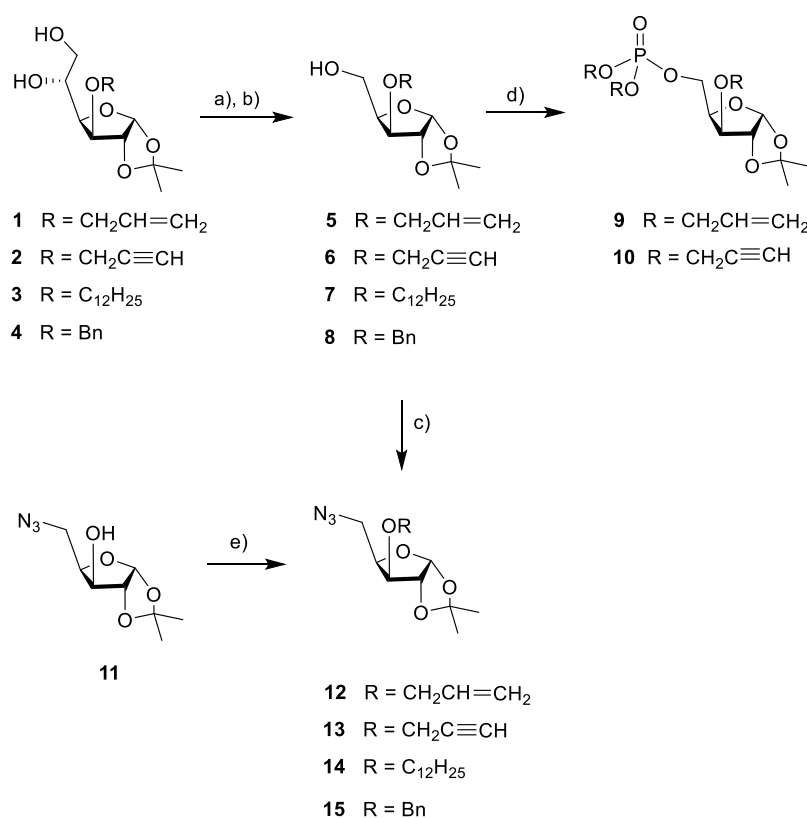
shown to be able to interact with phosphate anions in water through the pseudo interglycosidic guanidine linkage [38]. Oligonucleotide analogues comprising bridging guanidine groups replacing the phosphodiester bond have been synthesized, showing the ability to bind complementary DNA or RNA sequences and resistance to nucleases [39-42]. Motivated by the biological profile of guanidine-containing molecules, namely those based on carbohydrate templates, and within our interest in the access to novel bioactive nucleoside analogues and simpler carbohydrates derivatives comprising functional groups contained in nucleoside partial structures, we report herein on the synthesis and biological evaluation of novel 5-guanidino xylofuranose derivatives. These molecules can be foreseen as potential isonucleoside mimetics, in which a system contained in aminopurine/cytosine nucleobases, *i.e.* the guanidine/amidine group, is linked to a furanose unit at a non-anomeric position. Moreover, a related compound comprising a 1,2,3-triazole unit between C-5 of the sugar moiety and the guanidine group, *i.e.* guanidinomethyltriazole 5′-isonucleoside, a previously unreported type of nucleoside analogue, was accessed. Different *O*-substituents were installed at C-3 in the furanose moiety, *i.e.* from small saturated/unsaturated to long hydrocarbon chains and aromatic-containing systems, including 1,2,3-triazole units, enabling varying the types of interactions that the molecules may establish with a biological target. The molecules were then evaluated for their ability to inhibit cholinesterases (ChEs) and for their anticancer potential. ChEs are therapeutic targets for the symptomatic treatment of Alzheimer's disease (AD) since these enzymes hydrolyze the neurotransmitter acetylcholine, whose level is low in AD patients [43]. Few examples of guanidine-containing molecules exhibiting cholinesterase inhibitory ability were reported [16,17,44]. Moreover, we have previously found the inhibitory effects of aminopurine and guanine isonucleosides on the activity of acetylcholinesterase [45,46], which further motivated us to evaluate the anticholinesterasic activities of simpler guanidine-containing sugar derivatives such as the synthesized 5-guanidino furanoses. All compounds were assessed for their antiproliferative effects on cancer cells.

2. Results and Discussion

2.1. Chemistry

The synthesis of 3-*O*-substituted 5-guanidino xylofuranose derivatives involved the access to 5-azido precursors, which could be achieved by different pathways depending on the 3-*O*-substituent (Scheme 1). One pathway involved the oxidative cleavage of 3-*O*-allyl, -propargyl [47], -dodecyl [48] and -benzyl [49] 1,2-*O*-isopropylidene glucofuranose derivatives (**1-4**) with sodium metaperiodate, followed by reduction with sodium borohydride to afford the corresponding xylofuranose derivatives **5-8** for further installation of the azide functionality. This was firstly attempted by a sequence of tosylation (tosyl chloride/pyridine) and further nucleophilic replacement with sodium

azide, by which the 3-*O*-dodecyl and 3-*O*-benzyl 1,2-*O*-isopropylidene xylofuranoses **7**-**8** were converted into **14-15** as previously reported [47,48]. In the case of the allyl and propargyl derivatives **5-6**, the corresponding intermediate 5-tosylates were reverted to the 5-hydroxyl derivatives upon treatment with the azide anion. The treatment of **5-6** with diphenylphosphoryl azide in the presence of 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) proved also not to be feasible for the access to the corresponding 5-azido derivatives, leading solely to the xylofuranosyl phosphates **9** and **10** [47] that did not evolve towards the desired azides even after long reaction times (16-24 h), heating (40 ºC) and addition of sodium azide. Hence, the 3-*O*-allyl-5-azido furanose derivative **12** was synthesized by 3-*O*-allylation of known 5-azido-1,2-*O*-isopropylidene xylofuranose (**11**) [50] with allyl bromide in the presence of sodium hydride in 87% yield, similarly as for the 3-*O*-propargylated counterpart **13** which was obtained via *O*-propargylation of **11** as previously reported [47].

1 R = CH$_2$CH═CH$_2$
2 R = CH$_2$C≡CH
3 R = C$_{12}$H$_{25}$
4 R = Bn

5 R = CH$_2$CH═CH$_2$
6 R = CH$_2$C≡CH
7 R = C$_{12}$H$_{25}$
8 R = Bn

9 R = CH$_2$CH═CH$_2$
10 R = CH$_2$C≡CH

11

12 R = CH$_2$CH═CH$_2$
13 R = CH$_2$C≡CH
14 R = C$_{12}$H$_{25}$
15 R = Bn

Scheme 1. Reagents and conditions: (a) NaIO$_4$, THF (60-75% aq. soln.), r. t., 2.5-4 h; (b) NaBH$_4$, EtOH/H$_2$O (2/1), r. t., 1 h, 58 (**5**), 57% (**6**) [47], 64% (**7**) [48], 82% (**8**) [49], 2 steps; (c) TsCl, CH$_2$Cl$_2$/pyridine (1:1, from **7**), pyridine (from **8**), r. t., 16 h; (d) NaN$_3$, DMF, 80 °C, 16 h, 77 % (**14**) [48], 80% (**15**) [47], 2 steps; (d) DPPA, DBU, toluene, r. t., 16 h (from **5**), 24 h, then 40°C, 16 h (from **6**), 98% (**9**), 68 % (**10**) [47]; (e) RBr, NaH, DMF, r. t., 16 h (for R = C$_3$H$_5$), 5 min. (for R = C$_3$H$_3$), 87% (**12**), 95 % (**13**) [47].

The 5-azido xylofuranose derivatives **12**-**15** were then engaged in reduction/guanidinylation (Scheme 2). A reported one pot-two step procedure, in which the azido sugar is subjected to hydrogenation in the presence of the guanidinylating reagent *N,N'*-bis(*tert*-butoxycarbonyl)-*N''*-triflylguanidine and diisopropylethylamine (DIPEA) [51,52] was firstly used. While this protocol allowed obtaining the 5-guanidino xylofuranose derivatives containing 3-*O*-dodecyl and 3-*O*-benzyl groups (**19** and **20**) in high yields (86-89%), the latter not being removed under the conditions used, when applied to the 3-*O*-allyl-5-azido sugar **12** it led to the 5-guanidino 3-*O*-propyl xylofuranose **16** in 60% yield. Hence, to avoid the reduction of the allyl and eventually of the propargyl moiety, an alternative method consisting of the selective reduction of the azide group by the Staudinger reaction with triphenylphosphane in tetrahydrofuran (THF)/water, followed by guanidinylation, was employed. The 3-*O*-allyl and 3-*O*-propyl 5-guanidino sugars **17**-**18** were thus obtained from the azido derivatives **12**-**13**, respectively, by this methodology in very good yields (88-95%). The 3-*O*-dodecyl and 3-*O*-benzyl 5-azido xylofuranoses **14**-**15** were also subjected to the Staudinger/guanidinylation protocol leading to the corresponding guanidine sugars **19**-**20** in comparable yields as those obtained by the one-pot hydrogenation/guanydinilation method.

12 R = CH₂CH=CH₂	**16** R = CH₂CH₂CH₃
13 R = CH₂C≡CH	**17** R = CH₂CH=CH₂
14 R = C₁₂H₂₅	**18** R = CH₂C≡CH
15 R = Bn	**19** R = C₁₂H₂₅
	20 R = Bn

Scheme 2. Reagents and conditions: (a) *N,N'*-diBoc-*N''*-triflylguanidine, H₂, 10% Pd/C, DIPEA, AcOEt, 16 h, **16** (60%), **19** (86%), **20** (89%); (b) PPh₃, THF/H₂O (13/1), r. t., 4 h (for 12-15), 24 h (for **21**); (c) *N,N'*-diBoc-*N''*-triflylguanidine, DIPEA, AcOEt, r. t., 45 min (for 12-15), 10 h (for **21**), **17** (88%), **18** (95%), **19** (81%), **20** (83%), 2 steps, **22 22** (54%); (d) propargylamine, toluene, 110°C, 24 h, 20% [47].

The access to a 3-*O*-dodecyl xylofuranose derivative including a 1,2,3-triazole moiety, which is a stable unit prompted to establish hydrogen-bond and π-stacking interactions with amino acid-residues, between the sugar and the guanidino moieties was then accomplished. Nucleoside analogues containing a 3-*O*-dodecyl xylofuranose system were previously described as possessing antiproliferative activities in cancer cells [48]. Hence the aminomethyltriazole **21**, whose synthesis was previously described *via* thermal cycloaddition between the 5-azido 3-*O*-dodecyl xylofuranose **14** and propargylamine [47] was subjected to guanidinylation leading to the guanidinomethyltriazole 5′-isonucleoside **22** in 54% yield.

A 5-guanidino sugar containing a 3-*O*-substituent combining a benzyl moiety with a triazole motif was also synthesized (Scheme 3). Hence, the previously reported 5-azido-3-*O*-(benzyltriazolyl)methyl xylofuranose derivative **23**, accessed from **13** *via* 1,3-dipolar cycloaddition with benzyl azide and further azidation [47], was subjected to Staudinger reduction followed by guanidinylation leading to the 5-guanidino 3′-*O*-methyl-branched *N*-benzyltriazole isonucleoside **24** in 84% yield. Alternatively, **24** could be obtained through "click" cycloaddition between **18** with benzyl azide in the presence of copper iodide/Amberlyste A-21 [53] in 38% yield with the 5-iodotriazole **25** being also formed as secondary product in 10% yield. Identification of **25** was based on its NMR data, with the absence of the signal corresponding to H-3′ on the ^{1}H NMR spectrum and the chemical shift difference between the triazole quaternary carbon signals in the ^{13}C NMR spectrum resonating at $\delta = 148.3$ (C-2′) and $\delta = 80.8$ (C-3′) ppm. The formation of iodotriazoles has been reported in CuI-catalyzed azide-alkyne cycloadditions in the presence of organic bases [54-56]. The Amberlyst A-21 resin, onto which CuI is supported, appears to be able to elicit triazole iodination due to its dimethylamino groups. The use of copper iodide as a promoter in an equimolar amount relatively to **18** and a catalytic amount of 4-dimethylaminopyridine (DMAP) as base, conditions previously reported to increase triazole iodination [56], in a dilute dichloromethane solution at 30 ºC, led solely to the iodotriazole **25** in 66% yield.
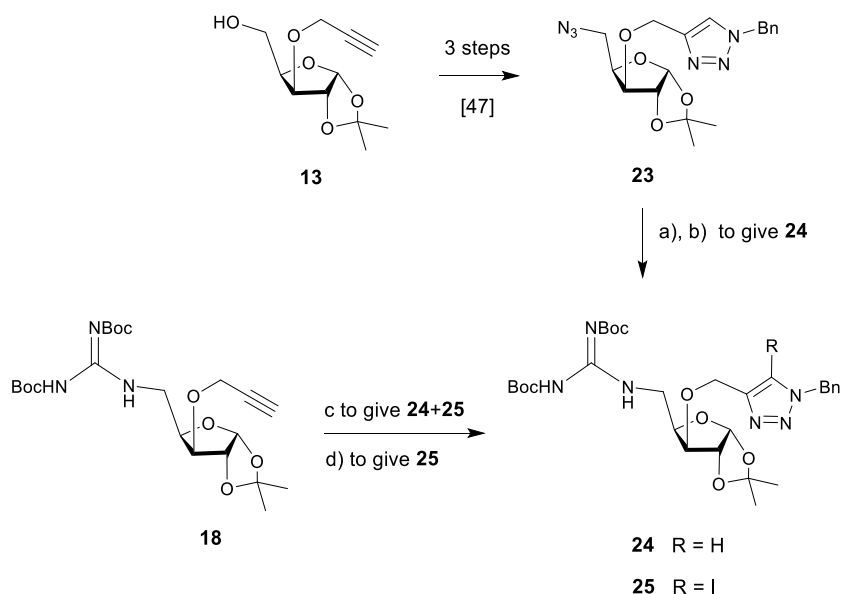
Scheme 3. (a) PPh$_3$, THF/H$_2$O (10/1), r. t., 4 h; (b) *N,N'*-diBoc-*N''*-triflylguanidine, DIPEA, AcOEt, r. t., 2 h, 84%, 2 steps. (c) BnN$_3$, cat. CuI/A-21, CH$_2$Cl$_2$, 4 d, **22** (38%), **23** (10%). (d) BnN$_3$, CuI, cat. DMAP, CH$_2$Cl$_2$, 30 ºC, 72 h, 66%.

The 3-*O*-benzyl and 3-*O*-dodecyl 5-guanidino xylofuranoses **19** and **20** were subjected to *t*-butoxycarbonyl group (Boc) and 1,2-*O*-acetonide removal through treatment with trifluoroacetic acid (TFA) in the presence of triethylsilane acting as *tert*-butyl cation scavenger [57-58]. While both carbamate functions of the 3-*O*-benzyl derivative **20** were cleaved, leading to the deprotected 5-guanidino xylofuranose **27** in 36% yield, the 3-*O*-dodecyl counterpart underwent selective removal of imino N-Boc group under similar conditions to afford the N-Boc-monoprotected guanidine **26** in 26% yield. Both compounds were obtained as anomeric mixtures with α/β ratios close to 1:1. No products resulting from cyclization involving the guanidine amino groups and the anomeric center, *i.e.* guanidine-type iminosugar derivatives [33, 52, 59], were detected.
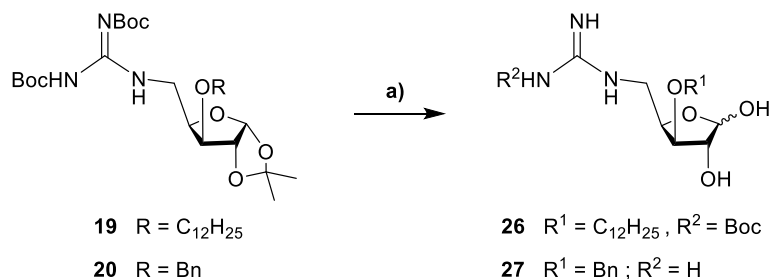
Scheme 4. (a) TFA/H$_2$O/triethylsilane 48:1:1, r.t., 2 h, **26** (33%), **27** (36%).

2.2. Biological Evaluation

All newly synthesized guanidine derivatives (**16**-**20**, **22**, **24**-**27**) as well as the aminomethyltriazole isonucleoside **21**, precursor of guanidine **22**, were subjected to biological evaluation, focusing on the study of their ability to inhibit cholinesterases and on their antiproliferative effects in cancer cells.

2.2.1. Cholinesterase Inhibition

The inhibitory effects of the compounds on the activities of acetylcholinesterase (AChE, from *Electrophorus electricus*) and butyrylcholinesterase (BChE, from equine serum) were evaluated by the Ellman's assay. The cholinesterase inhibitor galantamine hydrobromide (GH), which is commonly used in AD symptomatic therapy, was included as a reference. The inhibition constants, which were determined for the significantly active molecules, *i.e.,* those that showed more than 50% inhibition of the enzymatic activity at 10 µM concentration, and their types of inhibition are presented in Table 1.

Table 1. Significant results on the evaluation of the inhibitory effects of the compounds on the activity of cholinesterases.; inhibition constants K_i (for competitive inhibition) and $K_{i'}$ (for uncompetitive inhibition) are reported in µM; the results are mean values resulting from triplicate experiments.

	AChE		BChE	
Compound	% Inhibition	K_i [K_i'] (type of inhibition)	% Inhibition	K_i [K_i'] (type of inhibition)
GH	96.7	0.54 ± 0.01 (competitive)	86.3	9.37 ± 0.67 (competitive)
22	79.53	22.87 ± 3.83 [89.18 ± 1.8] (mixed-type)	21.69	n.d.
26	72.16	7.49 ± 0.92 [7.69 ± 1.62] (non-competitive)	37.76	n.d.

The guanidinomethyltriazole 5′-isonucleoside **22** and the 3-*O*-dodecyl (*N*-Boc)guanidino xylofuranose derivative **26** were the active compounds of the series, exhibiting selectivity towards AChE. The isonucleoside **22** showed moderate mixed-type AChE inhibition, the competitive component of which is more pronounced than the uncompetitive one ($K_i =$ 22.87 µM, $K_i' =$ 89.18 µM, Figure 1). Since the aminomethyltriazole isonucleoside **21** did

not show significant AChE inhibition, it appears that the presence of the guanidine moiety in **22** is important for its inhibitory effect.
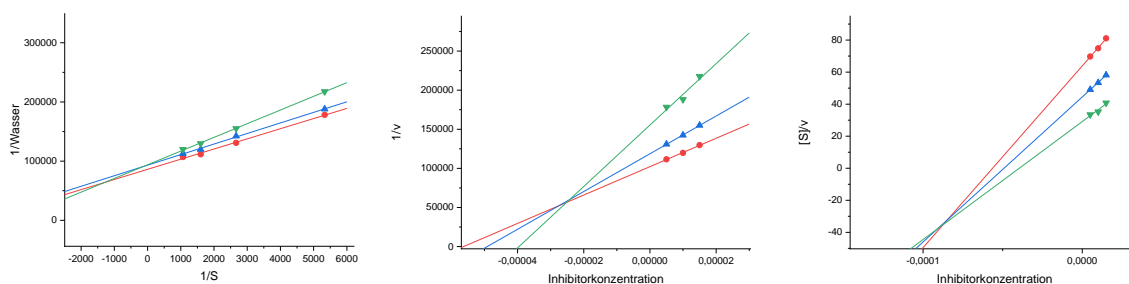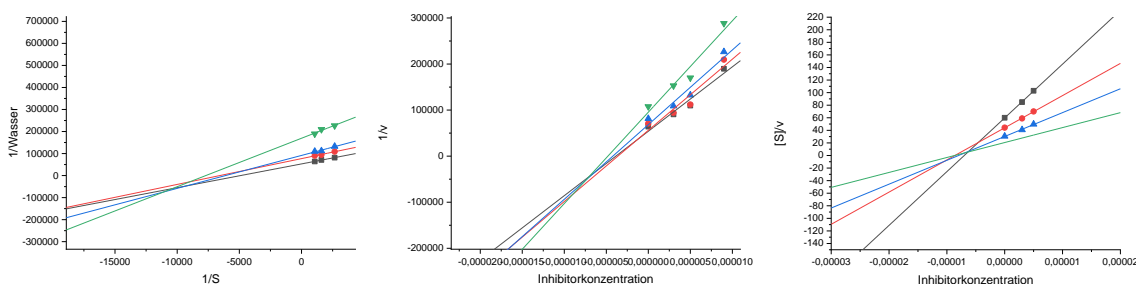

Figure 1. Lineweaver–Burk (left), Dixon (center) and Cornish-Bowden (right) plots for the inhibition of AChE by compound **22**.

The isonucleoside **26** was the best AChE inhibitor with single-digit micromolar values of inhibition constants and exhibiting a non-competitive effect (K_i = 7.49 µM, K_i' = 7.69 µM, Figure 2).

Figure 2. Lineweaver–Burk (left), Dixon (center) and Cornish-Bowden (right) plots for the inhibition of AChE by compound **26**.

2.2.2. Antiproliferative Activity

The antiproliferative activities of the compounds were evaluated in K562 (chronic myeloid leukemia) and in MCF-7 (breast adenocarcinoma) cell lines by the rezasurin (Alamar Blue) assay. The 3-*O*-dodecyl-5-(*N'*,*N'*-di-Boc)guanidino-1,2-*O*-isopropylidene xylofuranose derivative **19**, the aminomethyltriazole isonucleoside **21** and the 3-*O*-dodecyl (*N*-Boc)guanidino xylofuranose **26** were the compounds that showed significant effects. The 3-*O*-dodecyl guanidine **19** exhibited modest and similar activities towards K562 (GI$_{50}$ = 47.5 µM) and MCF-7 (GI$_{50}$ = 41.68 µM) cells. Removal of the imine Boc group and of the 1,2-acetonide function of **19** leading to **26** enabled a 1,5-fold increase in the antiproliferative activities in both K562 and MCF-7 cell lines, with GI$_{50}$ values for **26** of 31.02 µM and 26.89 µM, respectively. The most effective compound in the panel was

the aminomethyltriazole derivative **21** displaying low micromolar antiproliferative activities with GI$_{50}$ values of 6.33 μM and 8.45 μM in K562 and in MCF-7 cells, respectively. Noteworthy to mention is that the GI$_{50}$ value of **21** in MCF-7 cells is similar to that of the reference anticancer drug 5-fluorouracil.

Table 1. Compounds' antiproliferative activities in K562 and MCF-7 cell lines.

Compound	GI$_{50}$ (μM)*	
	K562	**MCF-7**
16	>50	>50
17	>100	>100
18	>6.25	>6.25
19	47.53	41.68
20	>25	>25
21	6.33	8.45
22	>25	>25
24	>6.25	>6.25
25	>6.25	>6.25
26	31.02	26.89
27	>100	>100
imatinib	0.47 ± 0.02	26.8 ± 2.0
5-fluorouracil	>100	9.7 ± 0.0

tested at least in duplicate.

3. Conclusions

A diversity of guanidine-containing xylofuranose derivatives were synthesized from azido or amino precursors using the guanidinylation with *N*,*N′*-bis(*tert*-butoxycarbonyl)-*N″*-triflylguanidine as key step. The panel of synthesized molecules included 5-guanidino 3-*O*-substituted xylofuranoses (**16**-**20**, **24**-**25**), including 5-guanidino 3-*O*-methyl-branched *N*-benzyltriazole isonucleosides (**24**-**25**), a novel type of structural framework, and the guanidinomethyltriazole xylofuranos-5′-yl isonucleoside **22**, which also comprises a previously unreported type of structure. These molecules may be foreseen as potential nucleoside mimetics since the guanidine function is present in nucleobase derivatives and due to the presence of the triazole system as well in some of them. Conditions for click chemistry cycloaddition from the 5-guanidino-3-*O*-propargyl xylofuranose precursor **18** were established for obtaining predominantly the 5-guanidino *N*-benzyltriazole isonucleoside **24**, by using a CuI/Amberlyst A-21 catalytic system, or for accessing solely its related 5-iodotriazole derivative **25**, by using an equimolar amount of CuI and catalytic DMAP.

From the molecules tested, two demonstrated significant AChE inhibitory abilities and three showed antiproliferative effects in K526 and MCF-7 cells. The most active compounds exhibited K_i and GI_{50} values in the low micromolar order of concentration, namely the (*N*-Boc)guanidino xylofuranose **26**, the best AChE inhibitor and the aminomethyltriazole 5'-isonucleoside **21**, precursor of the guanidinomethyltriazole isonucleoside **22**, as the most potent antiproliferative compound. Its activity against the MCF-7 cell line is similar to that of the reference drug 5-fluorouracil, which turns it a promising anticancer lead molecule. Although the antiproliferative activity of the guanidinomethyltriazole 5'-isonucleoside **22** could not be determined ($GI_{50} > 25$ µM), the lack of activity of this compound at concentrations below 25 µM clearly indicates that the inclusion of a guanidine moiety in **21** leads to a decrease in the antiproliferative effect of the structure. On the other hand, the isonucleoside **22**, unlike its precursor aminomethyltriazole **21**, displayed moderate selective inhibition of AChE, which indicates the importance of the guanidine moiety for recognition by the enzyme and shows the interest of this structural framework in the search for new AChE inhibitors.

Given that all significant active compounds in the biological screening comprise a 3-*O*-dodecyl substituent, the presence of this hydrophobic chain appears to be crucial for the detected effects, reinforcing the therapeutic interest of 3-*O*-dodecyl xylofuranosyl nucleoside analogues/mimetics.

To the best of our knowledge, this contribution presents the first reported examples of guanidine-containing sugar derivatives displaying AChE inhibition and antiproliferative effects in cancer cells, demonstrating, therefore, the potential of such structures for the discovery of lead molecules for AD and cancer.

4. Materials and Methods

4.1. Chemistry

4.1.1. General Methods

Chemical reactants were purchased from *Sigma-Aldrich* and *Alfa Aesar*. The reactions were followed by TLC using Merck 60 F_{254} silica gel aluminium plates. Spots were visualized under UV light (254 nm) and/or after immersion in a 10% (v/v) ethanolic H_2SO_4 solution or in the Hanessian's stain (solution of cerium (IV) sulfate (0.2% w/v) and ammonium molybdate (5% w/v) in H_2SO_4 (6% aq.)) followed by charring with a heat gun (200 ºC). Compounds were purified by flash column chromatography on silica gel 60 G (0.040–0.063 mm, E. Merck). NMR experiments were carried out using a BRUKER Avance 400 spectrometer operating at 400.13 MHz (for ^{1}H NMR), 100.62 MHz (for ^{13}C NMR) or at 161.91 MHz (for ^{31}P NMR). Spectra were referenced to internal TMS, in the case of ^{1}H NMR spectra in CDCl₃, or to the respective solvent peak. ^{31}P NMR Spectra

were referenced following the IUPAC recommendations for chemical shift referencing [60]. 2D NMR experiments (COSY, HSQC, HMBC) were performed for supporting signal assignment. Chemical shifts are given in parts per million and coupling constants (J) are reported in Hertz (Hz). High-resolution mass spectrometry (HRMS) data were obtained on a High Resolution QqTOF Impact II mass spectrometer from Bruker Daltonics equipped with an electrospray ion source (ESI). Spectra were recorded in positive mode with external calibration. Melting points were determined with a Stuart SMP30 instrument. Optical rotations (589 nm, sodium D line, 20 ºC) were measured using a Perkin–Elmer 343 polarimeter.

Synthesis of compounds **6-8** [47-49], **10** [47], and **13**-**15** [47,48] was previously described.

4.1.2. 3-*O*-Allyl-1,2-*O*-isopropylidene-α-D-xylofuranose (**5**)

To a solution of 3-*O*-allyl-1,2-*O*-isopropylidene-α-D-glucofuranose (**1**, 0.5 g, 1.92 mmol) in THF/H_2O (8 mL, 3:1), at 0 °C, sodium metaperiodate (0.95 g, 4.44 mmol) was added. The mixture was stirred for 4 h at room temperature. Then, it was diluted with EtOAc and filtered over a pad of Celite. The phases were separated, the aqueous phase was extracted with AcOEt (2×) and the combined organic layers were dried with anhydrous $MgSO_4$. After filtration and concentration under vacuum, the crude residue was dissolved in EtOH/H_2O (15 mL, 3:1) and at. 0 °C, $NaBH_4$ (61m g, 1.58 mmol) was added. The mixture was stirred at room temperature for 1 h. Then, EtOAc was added. The mixture was washed with brine soln. and the aqueous phase was extracted with EtOAc (2×). The combined organic layers were dried with anhydrous $MgSO_4$, filtered and concentrated. The residue was subjected to column chromatography (AcOEt/hexane, 1:4) to afford **5** (0.256 g, 58%, 2 steps) as a yellow oil. $[\alpha]_D^{20} = -36$ (c = 1, in CH_2Cl_2). ^1H NMR (CDCl$_3$, 400 MHz): δ = 5.95 (d, 1 H, H-1, $^3J_{1,2}$ = 3.8), 5.84 (m, 1 H, H-2′), 5.27 (dq, 1 H, H-3′a, $J_{2',3'a}$ = 17.2, $J_{1'a,3'a} = J_{1'b,3'a} = J_{3'a,3'a}$ = 1.4), 5.21 (dq, 1 H, H-3′b, $J_{2',3'b}$ = 10.5, $J_{1'a,3'b} = J_{1'b,3'b} = J_{3'a,3'a}$ = 1.4), 4.56 (d, 1 H, H-2), 4.27 (ddd, 1 H, H-4), 4.14 (ddt, 1 H, H-1′a, $J_{1'a,1'b}$ = 13.0, $J_{1'a,2'}$ = 5.2, $J_{1'a,3'a} = J_{1'a,3'b}$ = 1.4), 4.01-3.89 (m, 3 H, H-1′b, H-3, H-5a, $J_{3,4}$ = 3.4, $J_{4,5a}$ = 5.2, $J_{5a,5b}$ = 12.1), 3.85 (dd, part B of ABX system, H-5b, $J_{4,5b}$ = 4.7), 2.30 (br.s, 1 H. OH-5), 1.47 (s, 3 H, CH_3, *i*-Pr), 1.30 (s, 3 H, CH_3, *i*-Pr) ppm. ^{13}C NMR (CDCl$_3$, 400 MHz): δ = 133.7 (C-2′), 118.2 (C-3′), 111.8 (Cq, *i*-Pr), 105.1 (C-1), 83.0 (C-3), 82.6 (C-2), 80.1 (C-4), 71.0 (C-1′), 61.1 (C-5), 26.9 (CH$_3$, *i*-Pr), 26.4 (CH$_3$, *i*-Pr) ppm. HRMS: calcd for $C_{11}H_{18}O_5$ [M + H]$^+$ 231.1227, found 231.1235; calcd for $C_{11}H_{18}O_5$ [M + Na]$^+$ 253.1046, found 253.1058.

4.1.3. Diphenyl (3-*O*-allyl-5-deoxy-1,2-*O*-isopropylidene-α-D-xylofuranos-5-yl)phosphate (**9**)

To a solution of 3-*O*-allyl-1,2-*O*-isopropylidene-α-D-xylofuranose (**5**, 0.71 g, 3.08 mmol) in toluene (16 mL) at 0 °C and under nitrogen atnosphere, diphenylphosphoryl azide (DPPA, 0.9 mL, 4.18 mmol) and 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU (0.65 mL, 4.35 mmol) were added and the mixture was stirred at room temperature for 16 h. The mixture was then washed twice with 2 M HCl soln. and water. The organic phase was dried with anhydrous magnesium sulfate, filtered and the solvent was evaporated under vacuum. The crude residue was subjected to column chromatography (EtOAc/hexane, 1:4) to afford **9** (1.40 g, 98%) as a colourless oil. $[\alpha]_D^{20} = -43$ (c = 1, in CH_2Cl_2). ^{1}H NMR (CDCl$_3$, 400 MHz,): δ = 7.42-7.08 (m, 10 H, C*H*, 2 × Ph), 5.92 (d, 1 H, H-1, $J_{1,2}$ = 3.7), 5.79 (m, 1 H, H-2′), 5.23 (dq, 1 H, H-3′a, $J_{2',3'a}$ = 17.2, $J_{1'a,3'a} = J_{1'b,3'a} = J_{3'a,3'a}$ = 1.4), 5.16 (dq, 1 H, H-3′b, $J_{2',3'b}$ = 10.4, $J_{1'a,3'b} = J_{1'b,3'b} = J_{3'a,3'a}$ = 1.4), 4.58-4.39 (m, 4 H, H-2, H-4, H-5a, H-5b), 4.06 (ddt, 1 H, H-1′a, $J_{1'a,1'b}$ = 12.8, $J_{1'a,2'}$ = 5.2, $J_{1'a,3'a} = J_{1'a,3'b}$ = 1.4), 3.94-3.84 (m, 2 H, H-1′b, H-3, $J_{1'b,2'}$ = 5.7, $J_{3,4}$ = 2.3), 1.46 (s, 3 H, C*H*$_3$, *i*-Pr), 1.30 (s, 3 H, C*H*$_3$, *i*-Pr) ppm. ^{13}C NMR (CDCl$_3$, 100 MHz,): δ = 150.40 (d, Cq, Ph, $^2J_{C,P}$ = 7.2), 150.38 (d, Cq, Ph, $^2J_{C,P}$ = 7.2), 133.6 (C-2′), 130.0, 129.8, 126.1, 125.4, 120.2, 120.0 (C*H*, 2 × Ph, $^3J_{C,P}$ = 4.8, $^3J_{C,P}$ = 4.9), 117.9 (C-3′), 111.9 (Cq, *i*-Pr), 105.2 (C-1), 82.1 (C-2), 81.1 (C-3), 78.5 (d, C-4, $^3J_{C-4,P}$ = 8.6), 71.0 (C-1′), 66.1 (d, C-5, $^2J_{C-5,P}$ = 5.8), 26.8 (C*H*$_3$, *i*-Pr), 26.2 (C*H*$_3$, *i*-Pr) ppm. ^{31}P NMR (162 MHz, CDCl$_3$): δ = − 12.10 ppm. HRMS: calcd for $C_{23}H_{27}O_8P$ [M + H]$^+$ 463.1516, found 463.1527; calcd for $C_{23}H_{27}O_8P$ [M + Na]$^+$ 485.1336, found 485.1348.

4.1.4. 3-O-Allyl-5-azido-5-deoxy-1,2-O-isopropylidene-α-D-xylofuranose (**12**)

To a solution of 5-azido-5-deoxy-1,2-*O*-isopropylidene-α-D-xylofuranose (**11**, 380 mg, 1.76 mmol) in DMF (10 mL), at 0° C and under nitrogen atmosphere, sodium hydride (60% in mineral oil, 84 mg, 2.1 mmol) was added. After stirring during 10 min, allyl bromide (0.18 mL, 2.1 mmol) was added. The reaction was stirred at room temperature overnight. Then, the mixture was diluted with diethyl ether and water was added. The phases were separated, and the aqueous phase was extracted with diethyl ether (2 ×). The combined organic layers were dried with anhydrous MgSO$_4$. After filtration and evaporation of the solvent in vacuum, the crude residue was subjected to column chromatography (AcOEt/hexane, 1:4) to afford **12** (391 mg, 87%) as a yellow oil. $[\alpha]_D^{20} = -28$ (c = 1, in CH_2Cl_2). ^{1}H NMR (CDCl$_3$, 400 MHz): δ = 5.92-5.80 (m, 2 H, H-1, H-2′, $^3J_{1,2}$ = 3.6), 5.28 (dq, 1 H, H-3′a, $J_{2',3'a}$ = 17.2, $J_{1'a,3'a} = J_{1'b,3'a} = J_{3'a,3'a}$ = 1.4), 5.21 (dq, 1 H, H-3′b, $J_{2',3'b}$ = 10.3, $J_{1'a,3'b} = J_{1'b,3'b} = J_{3'a,3'a}$ = 1.4), 4.57 (d, 1 H, H-2), 4.28 (td, 1 H, H-4, $J_{3,4}$ = 3.4, $J_{4,5a} = J_{4,5b}$ = 6.7), 4.14 (ddt, 1 H, H-1′a, $J_{1'a,1'b}$ = 12.8, $J_{1'a,2'}$ = 5.3, $J_{1'a,3'a} = J_{1'a,3'b}$ = 1.4), 3.98 (ddt, 1 H, H-1′b, $J_{1'b,2'}$ = 5.9, $J_{1'b,3'a} = J_{1'b,3'b}$ = 1.4), 3.88 (d, 1 H, H-3), 3.58-3.45 (m, 2 H, H-5a, H-5b, $J_{5a,5b}$ = 12.4), 1.48 (s, 3 H, C*H*$_3$, *i*-Pr), 1.30 (s, 3 H, C*H*$_3$, *i*-Pr) ppm. ^{13}C NMR (CDCl$_3$, 400 MHz): δ = 133.7 (C-2′), 118.0 (C-3′), 111.9 (Cq, *i*-Pr), 105.1

(C-1), 82.2 (C-2), 81.4 (C-3), 78.8 (C-4), 71.0 (C-1′), 49.1 (C-5), 26.9 (CH_3, *i*-Pr), 26.3 (CH_3, *i*-Pr) ppm. HRMS: calcd for $C_{11}H_{17}N_3O_4$ [M + H]$^+$ 256.1292, found 256.1299.

4.1.5. General procedures for the reduction-guanidinylation of azido sugars.

Method A: To a solution of azido sugar (0.13 mmol) in AcOEt (3 mL) under nitrogen atmosphere, *N*,*N*′-bis(*tert*-butoxycarbonyl)-*N*″-triflylguanidine (51 mg, 0.13 mmol), 10% Pd/C (spatula tip, approx. 20% w/w) and *N*,*N*-diisopropylethylamine (DIPEA, 1.5 equiv., 0.03 mL, 0.2 mmol) were added. Three vacuum/nitrogen purge cycles were applied, and the mixture was then left stirring under hydrogen atmosphere overnight. The mixture was filtered over a short plug of Celite and the Celite pad was washed twice with AcOEt. The filtrate was concentrated under reduce pressure and the crude residue was subjected to column chromatography.

Method B: To a solution of azido sugar (0.26 mmol) in THF/H_2O (2.8 mL, 13:1), triphenylphosphane (2 equiv., 140 mg, 0.52 mmol) was added and the mixture was stirred at room temperature for 4 h. The solvents were evaporated, and the crude residue was dried under vacuum. The residue was then dissolved in AcOEt (3.5 mL) and to the resulting solution, under nitrogen atmosphere, *N*,*N*′-bis(*tert*-butoxycarboyl)-*N*″-triflylguanidine (102 mg, 0.26 mmol) and DIPEA (2 equiv., 0.09 mL, 0.52 mmol) were added. The solution was stirred at room temp. for 45 min and it was then concentrated under reduced pressure (unless otherwise stated). The crude residue was subjected to column chromatography.

4.1.5.1. 5-[*N*′,*N*″-bis(*tert*-butoxycarbonyl)]guanidino-5-deoxy-1,2-*O*-isopropylidene-3-*O*-propyl-α-D-xylofuranose (**16**)

Obtained according to the general procedure for reduction-guanidinylation (method A), starting from starting from 5-azido-1,2-*O*-isopropylidene-3-*O*-propargyl-α-D-xylofuranose (**13**, 150 mg, 0.59 mmol) and using *N*,*N*′-bis(*tert*-butoxycarboyl)-*N*″-triflylguanidine (232 mg, 0.59 mmol) and DIPEA (0.15 mL, 0.89 mmol). Purification by column chromatography (AcOEt/hexane 1:10) afforded **16** (168 mg, 60%) as a yellow oil. $[\alpha]_D^{20} = -16$ (c = 1, in CH_2Cl_2). ^{1}H NMR (CDCl$_3$, 400 MHz): δ = 11.45 (s, 1 H, N*H*), 8.60 (br.s, 1 H, N*H*), 5.91 (d, 1 H, H-1, $J_{1,2}$ = 3.8), 4.54 (d, 1 H, H-2), 4.34 (ddd, 1 H, H-4), 3.88 (dt, 1 H, H-5a, $J_{5a,5b}$ = 13.8, $J_{4,5a}$ = $J_{5a,NH}$ = 6.0), 3.79 (d, 1 H, H-3, $J_{3,4}$ = 3.6), 3.65 (ddd, 1 H, H-5b, $J_{4,5b}$ = 6.8, $J_{5b,NH}$ = 4.9), 3.55 (dt, 1 H, H-1′a, $J_{1'a,1'b}$ = 8.8, $J_{1'a,CH2-2'}$ = 6.7), 3.38 (dt, 1 H, H-1′b, $J_{1'b,CH2-2'}$ = 6.7), 1.66-1.53 (m, 2 H, C*H*2-2′), 1.52 (s, 3 H, C*H*3, *i*-Pr), 1.48, 1.47 (2 s, 18 H, 6 × C*H*3, 2 × Boc), 1.30 (s, 3 H, C*H*3, *i*-Pr), 0.9 (t, 3 H, C*H*3-3′, J = 7.4) ppm. ^{13}C NMR (CDCl$_3$, 400 MHz): δ = 163.6 (*C*O, =NBoc), 156.4 (Cq, GN), 152.9 (*C*O, NHBoc), 111.5 (Cq, *i*-Pr), 105.2 (C-1), 83.0, 82.9 (C-3, Cq, Boc), 82.2 (C-2), 79.2 (Cq, Boc), 78.0 (C-4), 72.0 (C-1′), 39.3 (C-5), 28.3, 28.1 (6 × C*H*3, Boc), 26.9

(CH_3, i-Pr), 26.3 (CH_3, i-Pr), 22.9 (C-2′), 10.6 (C-3′) ppm. HRMS: calcd for $C_{22}H_{39}N_3O_8$ [M + H]$^+$ 474.2810, found 474.2812.

4.1.5.2.   3-O-Allyl-5-[N',N''-bis($tert$-butoxycarbonyl)]guanidino-5-deoxy-1,2-O-isopropylidene-α-D-xylofuranose (**17**)

Obtained according to the general procedure for reduction-guanidinylation (method B), starting from 3-O-allyl-5-azido-5-deoxy-1,2-O-isopropylidene-α-D-xylofuranose (**12**, 60 mg, 0.235 mmol) and using triphenylphosphane (123 mg, 0.47 mmol), N,N'-bis($tert$-butoxycarboyl)-N''-triflylguanidine (92 mg, 0.235 mmol) and DIPEA (0.47 mmol, 0.08 mL). Purification by column chromatography (AcOEt/hexane 1:8) afforded **17** (98 mg, 88%) as a colorless oil. $[\alpha]_D^{20}$ = −12 (c = 1, in CH_2Cl_2). ^{1}H NMR (CDCl$_3$, 400 MHz): δ = 8.60 (br.s, 1 H, NH), δ = 5.96-5.82 (m, 2 H, H-1, H-2′, $^3J_{1,2}$ = 3.8), 5.27 (dq, 1 H, H-3′a, $J_{2',3'a}$ = 17.3, $J_{1'a,3'a}$ = $J_{1'b,3'a}$ = $J_{3'a,3'a}$ = 1.4), 5.19 (dq, 1 H, H-3′b, $J_{2',3'b}$ = 10.3, $J_{1'a,3'b}$ = $J_{1'b,3'b}$ = $J_{3'a,3'a}$ = 1.4), 4.56 (d, 1 H, H-2), 4.34 (ddd, 1 H, H-4), 4.13 (ddt, 1 H, H-1′a, $J_{1'a,1'b}$ = 12.6, $J_{1'a,2'}$ = 5.4, $J_{1'a,3'a}$ = $J_{1'a,3'b}$ = 1.4), 3.98 (ddt, 1 H, H-1′b, $J_{1'b,2'}$ = 6.0, $J_{1'b,3'a}$ = $J_{1'b,3'b}$ = 1.4), 3.92-3.82 (m, 2 H, H-3, H-5a, $J_{3,4}$ = 3.1), 3.64 (ddd, 1 H, H-5b, $J_{5a,5b}$ = 13.4, $J_{4,5b}$ = 7.2, $J_{5b,NH}$ = 4.5), 1.52 (s, 3 H, CH_3, i-Pr), 1.48, 1.47 (2 s, 18 H, 6 × CH_3, 2 × Boc), 1.30 (s, 3 H, CH_3, i-Pr) ppm. ^{13}C NMR (CDCl$_3$, 400 MHz): δ = 163.6 (CO, =NBoc), 156.5 (Cq, GN), 153.0 (CO, NHBoc), 133.9 (C-2′), 118.1 (C-3′), 111.7 (Cq, i-Pr), 105.2 (C-1), 83.1 (Cq, Boc), 82.4, 82.1 (C-2, C-3,), 79.4 (Cq, Boc), 78.1 (C-4), 71.1 (C-1′), 39.5 (C-5), 28.4, 28.2 (6 × CH_3, Boc), 26.8 (CH_3, i-Pr), 26.3 (CH_3, i-Pr) ppm. HRMS: calcd for $C_{22}H_{37}N_3O_8$ [M + H]$^+$ 472.2653, found 472.2655.

4.1.5.3. 5-[N',N''-bis($tert$-butoxycarbonyl)]guanidino-5-deoxy-1,2-O-isopropylidene-3-O-propargyl-α-D-xylofuranose (**18**)

Obtained according to the general procedure for reduction-guanidinylation (method B), starting from 5-azido-5-deoxy-1,2-O-isopropylidene-3-O-propargyl-α-D-xylofuranose (**13**, 65 mg, 0.26 mmol) and using triphenylphosphane (140 mg, 0.52 mmol), N,N'-bis($tert$-butoxycarboyl)-N''-triflylguanidine (102 mg, 0.26 mmol) and DIPEA (0.09 mL, 0.52 mmol). Purification by column chromatography (AcOEt/hexane 1:8) afforded **18** (115 mg, 95%) as a colorless oil. $[\alpha]_D^{20}$ = −13 (c = 1, in CH_2Cl_2). ^{1}H NMR (CDCl$_3$, 400 MHz): δ = 8.59 (br.s, 1 H, NH), 5.92 (d, H-1, $^3J_{1,2}$ = 3.7), 4.65 (d, 1 H, H-2), 4.37 (ddd, 1 H, H-4), 4.29 (dd, part A of ABX system, H-1′a, $J_{1'a,1'b}$ = 16.0, $J_{1'a,3'}$ = 2.3), 4.23 (dd, part B of ABX system, H-1′b, $J_{1'b,3'}$ = 2.3), 4.10 (d, 1 H, H-3, $J_{3,4}$ = 3.1), 3.93 (ddd, 1 H, H-5a, $J_{5a,5b}$ = 13.8, $J_{4,5a}$ = 6.4, $J_{5a,NH}$ = 5.2), 3.59 (ddd, 1 H, H-5b, $J_{4,5b}$ = 7.2, $J_{5b,NH}$ = 4.6), 2.47 (t, 1 H, H-3′, $J_{1'a,3'}$ = $J_{1'b,3'}$ = 2.3), 1.49, 1.48 (2 s, 21 H, 6 × CH_3, 2 × Boc, CH_3, i-Pr), 1.31 (s, 3 H, CH_3, i-Pr) ppm. ^{13}C NMR (CDCl$_3$, 400 MHz): δ = 163.6 (CO, =NBoc), 156.5 (Cq, GN), 153.0 (CO, NHBoc), 111.9 (Cq, i-Pr), 105.1 (C-1), 83.2 (Cq, Boc), 82.3, 81.9 (C-2, C-3), 79.4 (Cq, Boc), 79.0 (C-2′), 78.2 (C-4), 75.6 (C-3′), 57.6 (C-1′), 39.4 (C-5), 28.4, 28.2 (6 × CH_3, Boc), 26.8 (CH_3, i-Pr), 26.4 (CH_3, i-Pr) ppm.

HRMS: calcd for $C_{22}H_{35}N_3O_8$ $[M + H]^+$ 470.2497, found 470.2494. calcd for $C_{22}H_{35}N_3O_8$ $[M + Na]^+$ 492.2316, found 492.2307.

4.1.5.4. 5-[N',N''-bis(*tert*-butoxycarbonyl)]guanidino-5-deoxy-3-O-dodecyl-1,2-O-isopropylidene-α-D-xylofuranose (**19**)

Obtained according to the general procedure for reduction-guanidinylation (method A), starting from starting from 5-azido-5-deoxy-3-O-dodecyl-1,2-O-isopropylidene-α-D-xylofuranose (**14**, 50 mg, 0.13 mmol) and using N,N'-bis(*tert*-butoxycarboyl)-N''-triflylguanidine (51 mg, 0.13 mmol) and DIPEA (0.03 mL, 0.2 mmol). Purification by column chromatography (AcOEt/petroleum ether 1:20) afforded **19** (67 mg, 86%) as a colorless oil.

Alternatively, the title compound could be obtained through method B, starting from **14** (70 mg, 0.18 mmol), and using triphenylphosphane (96 mg, 0.37 mmol), N,N'-bis(*tert*-butoxycarboyl)-N''-triflylguanidine (71 mg, 0.18 mmol) and DIPEA (0.06 mL, 0.37 mmol), in 81% yield (88 mg). $[\alpha]_D^{20} = -15$ (c = 1, in CH_2Cl_2). ^{1}H NMR (CDCl$_3$, 400 MHz): δ = 11.45 (s, 1 H, NH), 8.57 (br.s, 1 H, NH), 5.89 (d, H-1, $^3J_{1,2} = 3.6$), 4.52 (d, 1 H, H-2), 4.32 (br. ddd, 1 H, H-4), 3.84 (dt, 1 H, H-5a, $J_{5a,5b} = 13.5$, $J_{4,5a} = J_{5a,NH} = 5.9$), 3.76 (d, 1 H, H-3, $J_{3,4} = 2.9$), 3.69-3.49 (m, 2 H, H-5b, H-1′a), 3.43-3.33 (m, 1 H, H-1′b), 1.58-1.39 (m, 23 H, CH_2-2′, 6 × CH_3, 2 × Boc, CH_3, *i*-Pr), 1.34-1.15 (m, 21 H, CH_3, *i*-Pr, CH_2-3′ to CH_2-11′), 0.84 (t, 3 H, CH_3-12′, $^3J = 6.5$) ppm. ^{13}C NMR (CDCl$_3$, 400 MHz): δ = 163.6 (CO, =NBoc), 156.5 (Cq, GN), 153.0 (CO, NHBoc), 111.6 (Cq, *i*-Pr), 105.2 (C-1), 83.0, 83.0 (C-3, Cq, Boc), 82.3 (C-2), 79.2 (Cq, Boc), 78.1 (C-4), 70.6 (C-1′), 39.4 (C-5), 32.0, 29.7, 29.7, 29.7, 29.6, 29.4 (C-2′ to C-9′), 28.4, 28.1 (6 × CH$_3$, Boc), 26.8 (CH$_3$, *i*-Pr), 26.3 (CH$_3$, *i*-Pr), 26.2 (C-10′), 22.8 (C-11′), 14.2 (C-12′) ppm. HRMS: calcd for $C_{31}H_{57}N_3O_8$ [M + H]+ 600.4218, found 600.4216.

4.1.5.5. 3-O-Benzyl-5-[N',N''-bis(*tert*-butoxycarbonyl)]guanidino-5-deoxy-1,2-O-isopropylidene-α-D-xylofuranose (**20**)

Obtained according to the general procedure for reduction-guanidinylation (method A), starting from starting from 5-azido-3-O-benzyl-5-deoxy-1,2-O-isopropylidene-α-D-xylofuranose (**15**, 60 mg, 0.2 mmol) and using N,N'-bis(*tert*-butoxycarboyl)-N''-triflylguanidine (77 mg, 0.2 mmol) and DIPEA (0.05 mL, 0.3 mmol). Purification by column chromatography (AcOEt/petroleum ether 1:20) afforded **20** (91 mg, 89%) as a colorless oil.

Alternatively, the title compound could be obtained through method B, starting from **15** (60 mg, 0.2 mmol), and using triphenylphosphane (103 mg, 0.39 mmol), N,N'-bis(*tert*-butoxycarboyl)-N''-triflylguanidine (77 mg, 0.2 mmol) and DIPEA (0.07 mL, 0.39 mmol), in 83% yield (85 mg). $[\alpha]_D^{20} = -23$ (c = 1, in CH_2Cl_2). ^{1}H NMR (CDCl$_3$, 400 MHz): δ = 8.63 (br.s, 1 H, NH), 7.39-7.26 (m, 5 H, CH, Ph), 5.95 (d, H-1, $^3J_{1,2} = 3.8$), 4.67 (d, part A of AB system, 1 H, H-a from CH_2Ph, $^2J_{a,b} = 11.9$), 4.62 (d, 1 H, H-2), 4.52 (d, part

B of AB system, 1 H, H-b from CH_2Ph), 4.34 (ddd, 1 H, H-4), 3.95 (d, 1 H, H-3, $J_{3,4}$ = 3.5), 3.88 (dd, part A of ABX system, 1 H, H-5a, $J_{5a,5b}$ = 14.1, $J_{4,5a}$ = 4.8), 3.64 (dd, part B of ABS system, 1 H, H-5b, $J_{4,5b}$ = 7.2), 1.51-1.42 (m, 21 H, 6 × CH_3, 2 × Boc, CH_3, *i*-Pr), 1.30 (s, 3 H, CH_3, *i*-Pr) ppm. ^{13}C NMR (CDCl$_3$, 400 MHz): δ = 163.3 (CO, =NBoc), 156.5 (Cq, GN), 153.1 (CO, NHBoc), 137.2 (Cq, Ph), 128.6, 128.1, 128.0 (CH, Ph), 112.0 (Cq, *i*-Pr), 105.1 (C-1), 83.4 (Cq, Boc), 82.2, 82.2 (C-2, C-3), 79.7 (Cq, Boc), 78.2 (C-4), 72.0 (CH$_2$Ph), 39.4 (C-5), 28.3, 28.1 (6 × CH$_3$, Boc), 26.8 (CH$_3$, *i*-Pr), 26.2 (CH$_3$, *i*-Pr) ppm. HRMS: calcd for C$_{26}$H$_{39}$N$_3$O$_8$ [M + H]$^+$ 522.2810, found 522.2810.

4.1.5.6. 3-*O*-(1-Benzyl-1*H*-1,2,3-triazol-4-yl)methyl-5-[*N′*,*N″*-bis(*tert*-butoxycarbonyl)]guanidino-5-deoxy-1,2-*O*-isopropylidene-α-D-xylofuranose (**24**) and 3-*O*-(1-Benzyl-5-iodo-1*H*-1,2,3-triazol-4-yl)methyl-5-[*N′*,*N″*-bis(*tert*-butoxycarbonyl)]guanidino-5-deoxy-1,2-*O*-isopropylidene-α-D-xylofuranose (**25**)

Compound **24** was obtained according to the procedure for reduction-guanidinylation (method B), starting from 5-[*N′*,*N″*-bis(*tert*-butoxycarbonyl)]guanidino-5-deoxy-1,2-*O*-isopropylidene-3-*O*-propargyl-α-D-xylofuranose (**18**, 251 mg, 0.65 mmol) and using triphenylphosphane (341 mg, 1.3 mmol), *N*,*N′*-bis(*tert*-butoxycarboyl)-*N″*-triflylguanidine (127 mg, 0.65 mmol) and DIPEA (0.08 mL, 0.46 mmol). The guanidinylation step was completed within 2 h; then the mixture was diluted with AcOEt and washed with water (2×). The organic phase was dried with anhydrous MgSO$_4$, filtered, the solvent was evaporated under vacuum and the residue was purified by column chromatography (AcOEt/hexane, 1:1) afforded **24** (328 mg, 84%) as a colorless oil.

In addition, **24** could be accessed, along with **25**, starting from **18** and using the following procedure:

To a solution of 5-[*N′*,*N″*-bis(*tert*-butoxycarbonyl)]guanidino-5-deoxy-1,2-*O*-isopropylidene-3-*O*-propargyl-α-D-xylofuranose (**18**, 250 mg, 0.53 mmol) in AcOEt (10 mL) under nitrogen atmosphere, benzyl azide (1.7 mL, 12.8 mmol) and CuI/Amberlyst A-21 (0.5 mmol.g-1, 0.1 mmol CuI, 200 mg) were added. The mixture was stirred at room temperature for 4 d. The catalyst was filtered off, the solvent was evaporated, and the crude residue was subjected to column chromatography (AcOEt/hexane, 1:3) to afford **24** (121 mg, 38%) and **25** (40 mg, 10%) as yellow oil.

Compound **25** could be solely obtained by reacting **18** (34 mg, 0.072 mmol) in CH$_2$Cl$_2$ (10 mL) with benzyl azide (0.05 mL, 0.4 mmol) and CuI (14 mg, 0.072 mmol) in the presence of DMAP (2.7 mg, 0.022 mmol). The mixture was stirred at 30 ºC for 72 h. It was then diluted with CH$_2$Cl$_2$ (10 ml) and washed with 1 M HCl (2 × 2 ml) and water (2 ml). The organic phase was dried with anhydrous MgSO$_4$, filtered, and the solvent was evaporated under vacuum. The residue was purified by column chromatography (AcOEt/hexane 1:4) to afford **25** (35 mg, 66%).

Data for 24: $[\alpha]_D^{20} = -65$ (c = 1, in CH$_2$Cl$_2$). ^{1}H NMR (CDCl$_3$, 400 MHz): δ = 11.46 (s, 1 H, N*H*), 8.57 (br. t, 1 H, N*H*), 7.58 (s, 1 H, H-3′), 7.42-7.24 (m, 5 H, C*H*, Ph), 5.89 (d, H-1, $^3J_{1,2}$ = 3.7), 5.55, 5.50 (2 d, AB system, 2 H, C*H*$_2$Ph, $^2J_{a,b}$ = 15.2), 4.77 (d, 1 H, part A of AB system, H-1′a, $^2J_{1'a,1'b}$ = 11.9), 4.67-4.60 (m, 2 H, H-1′b, H-2), 4.34 (ddd, 1 H, H-4), 4.00 (d, 1 H, H-3, $J_{3,4}$ = 3.1), 3.83 (dt, 1 H, H-5a, $J_{5a,5b}$ = 13.7, $J_{4,5a} = J_{5a,NH}$ = 5.8), 3.59 (ddd, 1 H, H-5b, $J_{4,5b}$ = 7.2, $J_{5b,NH}$ = 5.0), 1.48, 1.45 (2 s, 21 H, 6 × C*H*$_3$, 2 × Boc, C*H*$_3$, *i*-Pr), 1.30 (s, 3 H, C*H*$_3$, *i*-Pr) ppm. ^{13}C NMR (CDCl$_3$, 400 MHz): δ = 163.6 (*C*O, =NBoc), 156.4 (Cq, GN), 153.0 (*C*O, NHBoc), 144.7 (C-2′), 134.6 (Cq, Ph), 129.3, 128.9, 128.2 (*C*H, Ph), 122.9 (C-3′), 111.8 (Cq, *i*-Pr), 105.2 (C-1), 83.2 (Cq, Boc), 82.8 (C-3), 82.1 (C-2), 79.4 (Cq, Boc), 78.0 (C-4), 63.8 (C-1′), 54.3 (*C*H$_2$Ph), 39.4 (C-5), 28.4, 28.2 (6 × C*H*$_3$, Boc), 26.8 (*C*H$_3$, *i*-Pr), 26.3 (*C*H$_3$, *i*-Pr) ppm. HRMS: calcd for C$_{29}$H$_{42}$N$_6$O$_8$ [*M* + H]$^+$ 603.3137, found 603.3146.

Data for 25: $[\alpha]_D^{20} = -27$ (c = 1, in CH$_2$Cl$_2$). ^{1}H NMR (CDCl$_3$, 400 MHz): δ = 11.42 (s, 1 H, N*H*), 8.55 (br. t, 1 H, N*H*), 7.40-7.23 (m, 5 H, C*H*, Ph), 5.91 (br. d, H-1), 5.58 (br. s, 2 H, C*H*$_2$Ph), 4.73-4.65 (m, 2 H, H-1′a, H-2), 4.61 (d, 1 H, part B of AB system, H-1′b, $^2J_{1'a,1'b}$ = 11.9), 4.38 (br.ddd, 1 H, H-4), 4.03 (br.d, 1 H, H-3), 3.93 (br.ddd, 1 H, H-5a, $J_{5a,5b}$ = 13.8), 3.57 (ddd, 1 H, H-5b, $J_{4,5b}$ = 7.2, $J_{5b,NH}$ = 5.0), 1.48, 1.47 (2 s, 21 H, 6 × C*H*$_3$, 2 × Boc, C*H*$_3$, *i*-Pr), 1.30 (s, 3 H, C*H*$_3$, *i*-Pr) ppm. ^{13}C NMR (CDCl$_3$, 400 MHz): δ = 163.6 (*C*O, =NBoc), 156.4 (Cq, GN), 153.0 (*C*O, NHBoc), 148.3 (C-2′), 134.2 (Cq, Ph), 129.1, 128.7, 128.0 (*C*H, Ph), 111.8 (Cq, *i*-Pr), 105.2 (C-1), 83.1 (Cq, Boc), 82.6 (C-3), 82.3 (C-2), 80.8 (C-3′), 79.3 (Cq, Boc), 78.2 (C-4), 63.5 (C-1′), 54.4 (*C*H$_2$Ph), 39.8 (C-5), 28.4, 28.2 (6 × C*H*$_3$, Boc), 26.8 (*C*H$_3$, *i*-Pr), 26.4 (*C*H$_3$, *i*-Pr) ppm. HRMS: calcd for C$_{29}$H$_{41}$IN$_6$O$_8$ [*M* + H]$^+$ 729.2103, found 729.2117.

4.1.6. 5-Deoxy-5-(4-{[*N′,N″*-bis(*tert*-butoxycarbonyl)]guanidino}methyl-1*H*-1,2,3-triazol-1-yl)-3-*O*-dodecyl-1,2-*O*-isopropylidene-α-D-xylofuranose (**22**)

To a solution of 5-(4-aminomethyl-1H-1,2,3-triazol-1-yl)-5-deoxy-3-*O*-dodecyl-1,2-*O*-isopropylidene-α-d-xylofuranose (**21**, 11 mg, 0.025 mmol) in AcOEt (1 mL), *N,N′*-bis(tert-butoxycarbonyl)-*N″*-triflylguanidine (9.8 mg, 0.026 mmol mmol), and DIPEA (0.01 mL, 0.057 mmol) were added and the solution was stirred at at room temperature under nitrogen atmosphere for 18 h. The solvent was removed under reduced pressure. The residue was purified by column chromatography (AcOEt/hexane 1:6) to afford **22** (9.2 mg, 54%) as a colorless oil. ^{1}H NMR (CDCl$_3$, 400 MHz): δ = 11.46 (s, 1 H, N*H*), 8.79 (br. t, 1 H, N*H*-5), 7.64 (s, 1 H, H-5), 5.94 (d, H-1′, $^3J_{1',2'}$ = 3.7), 4.78-4.63 (m, 3 H, C*H*$_2$N, H-5′a, $J_{a,b}$ = 11.5, $J_{a,NH}$ = 5.0, $J_{a,NH}$ = 5.1), 4.59 (d, 1 H, H-2), 3.84 (d, 1 H, H-3′, $J_{3',4'}$ = 3.1), 4.56-4.43 (m, 2 H, H-4′, H-5′b), 3.68-3.58 (m, 1 H, H-1″a), 3.47-3.37 (m, 1 H, H-1″b), 1.63-1.53 (m, 23 H, C*H*$_2$-2″, 6 × C*H*$_3$, 2 × Boc, C*H*$_3$, *i*-Pr), 1.34-1.15 (m, 21 H, C*H*$_3$, *i*-Pr, C*H*$_2$-3″ to C*H*$_2$-11″), 0.87 (t, 3 H, C*H*$_3$-12″, ^3J = 6.5) ppm. ^{13}C NMR (CDCl$_3$, 400 MHz): δ = 163.5 (*C*O, =NBoc), 156.1 (Cq, GN), 153.1 (*C*O, NHBoc), 144.0 (C-4), 123.4 (C-5), 112.2 (Cq, *i*-Pr), 105.4 (C-1), 83.4 (Cq, Boc), 82.5, 82.1 (C-2, C-3), 79.6 (Cq, Boc), 79.0 (C-4), 70.7 (C-1″), 49.4 (C-5′), 36.6 (*C*H$_2$N), 32.1, 29.8, 29.8, 29.7, 29.6,

29.5 (C-2″ to C-9″), 28.4, 28.2 (6 × CH_3, Boc), 26.9 (CH_3, i-Pr), 26.4 (CH_3, i-Pr), 26.3 (C-10″), 22.8 (C-11″), 14.3 (C-12″) ppm. HRMS: calcd for $C_{34}H_{60}N_6O_8$ [M + H]+ 681.4551, found 681.4557.

4.1.7. General procedures for t-butoxycarbonyl group (Boc) and 1,2-O-acetonide removal

A solution of 5-[N',N''-bis(Boc)]guanidino-3-O-benzyl/dodecyl-1,2-O-isopropylidene-α-D-xylofuranose (0.15 mmol) in TFA/H_2O/triethylsilane 48:1:1 (2 mL) was stirred at room temperature under nitrogen atmosphere for 2 h. The solvent was removed under reduced pressure. The residue was purified by column chromatography.

4.1.7.1. 5-[N'-($tert$-butoxycarbonyl)]guanidino-5-deoxy-3-O-dodecyl-1,2-O-isopropylidene-α-D-xylofuranose (26)

Obtained according to the general procedure for t-butoxycarbonyl group (Boc) and 1,2-O-acetonide removal, starting from 5-[N',N''-bis($tert$-butoxycarbonyl)]guanidino-5-deoxy-3-O-dodecyl-1,2-O-isopropylidene-α-D-xylofuranose (19, 91 mg, 0.15 mmol). Purification by column chromatography (dichloromethane/methanol 10:1) afforded 26 (23 mg, 33%) as a colorless oil. ^{1}H NMR (MeOD, 400 MHz): 5.29 (d, 1 H, H-1 α, $^3J_{1,2}$ = 3.8), 5.15 (br. s, 0.9 H, H-1 β), 4.50-4.40 (m, H-4 β, H-4 α), 4.09-3.98 (m, 2.9 H, H-2 α, H-2 β*, H-3 α), 3.89 (dd, 0.9 H, H-3 β), 3.79-3.41 (m, 7.6 H, H-5a α, H-5 b α, H-5a β, H-5 b β, H-1′a α, H-1′b α, H-1′a β, H-1′b β), 1.68-1.57 (m, 3.8 H, CH_2-2′ α, CH_2-2′ β), 1.57-1.49 (br.s, 17.1 H, 3 × CH_3, Boc α, 3 × CH_3, Boc β), 1.42-1.21 (br.s, 34.2 H, CH_3, CH_2-3′ to CH_2-11′, α, β), 0.90 (t, 5.7 H, CH_3-12′, α, β, ^3J = 6.8). ^{13}C NMR (MeOD, 400 MHz): δ = 156.5 (Cq, GN), 153.6, 153.5 (CO, NHBoc)*, 104.4 (C-1 β), 97.6 (C-1 α), 86.2 (C-3 β), 85.8, 85.7 (Cq, Boc), 85.2 (C-3 α), 80.1 (C-2 β), 79.7 (C-4 β), 77.3 (C-2 α), 76.4 (C-4 α), 71.8, 71.7 (C-1′, α, β), 43.4, 42.8 (C-5 α, β), 33.1, 30.9, 30.8, 30.8, 30.6, 30.5 (C-2′ to C-9′, α, β), 28.1 (6 × CH_3, Boc, α, β), 27.3, 27.3 (C-10′, α, β), 23.8 (C-11′, α, β), 14.5 (C-12′, α, β) ppm. HRMS: calcd for $C_{23}H_{46}N_3O_6$ [M + H]+ 460.3381, found 460.3385.

4.1.7.2. 3-O-Benzyl-5-guanidino-5-deoxy-1,2-O-isopropylidene-α-D-xylofuranose (27)

Obtained according to the general procedure for t-butoxycarbonyl group (Boc) and 1,2-O-acetonide removal, starting from 3-O-benzyl-5-[N',N''-bis($tert$-butoxycarbonyl)]guanidino-5-deoxy-1,2-O-isopropylidene-α-D-xylofuranose (20, 82 mg, 0.157 mmol). Purification by column chromatography (AcOEt/methanol 10:1) afforded 27 (16 mg, 36%) as a colorless oil. ^{1}H NMR (MeOD, 300 MHz): δ = 7.46-7.25 (m, 10 H, CH, Ph, α, β), 5.34 (d, 1 H, H-1 α, $^3J_{1,2\ (α)}$ = 4.2), 5.16 (d, 1 H, H-1 β, $^3J_{1,2\ (β)}$ = 1.5), 4.78, 4.74 (2 d, 2 × part A of AB system, 2 H, H-a from CH_2Ph, $^2J_{a,b}$ = 11.9, α, β), 4.59, 4.55 (2 d, part B of AB system, 1 H, H-b from CH_2Ph, α, β), 4.41-4.27 (m, 2 H, H-4, α, β), 4.19-4.14 (m, 2 H, H-2 α, H-2 β) 4.11 (dd, 1 H, H-3 α, $J_{2,3\ (α)}$ = 4.1, $J_{3,4\ (α)}$ = 5.7) 3.98 (dd, 1 H, H-3 β, $J_{2,3\ (β)}$ = 2.4, $J_{3,4\ (β)}$ = 5.6), 3.51-3.41 (m, 3 H, CH$_2$-5 β, H-5 a α, $J_{5a,5b}$

$_{(\alpha)}$ = 14.3, $J_{4,5a\,(\alpha)}$ = 4.1), 3.36 (dd, part B of ABX system, 1 H, H-5b α, $J_{4,5b\,(\alpha)}$ = 6.4) ppm. ^{13}C NMR (MeOD, 400 MHz): δ = 163.1 (Cq, GN), 139.3, 139.1 (Cq, Ph, α, β), 129.5 129.1, 129.0, 128.9, 128.9 (CH, Ph, α, β), 104.4 (C-1 β), 97.5 (C-1 α), 84.8, 84.5 (C-3 α, β) 80.5, 80.3 (C-2 β, C-4 β), 77.3, 77.0 (C-2 α, C-4 α), 73.2, 73.1 (CH$_2$Ph α, β), 43.8, 43.0 (C-5 α, β) ppm . HRMS: calcd for C$_{13}$H$_{19}$N$_3$O$_4$ [M + H]+ 282.1448, found 282.1445.

4.2. Biological Assays

4.2.1. Cholinesterase Inhibition Assays

A BMG Labtech Spectrostar Omega working in the slow kinetics mode and measuring the absorbance at a distinct wavelength of λ = 412 nm with center scanning was used for the enzymatic studies. In short: A mixture of a DTNB solution (125 µL, 3 mM in 50 mM Tris-HCl buffer, pH 8), enzyme solution (25 µL, 2 U/mL) and compounds solutions (25 µL, 3 different concentrations and water as a blank) was incubated at 30 °C for 20 min. The substrate (25 µL, [ATChI] = 0.9375 mM, 0.625 mM, 0.325 mM, 0.1875 mM) was added to start the enzymatic reaction. The absorbance data was recorded under a controlled temperature of 30 °C for 30 min at 1 min intervals at λ = 412 nm. The relative inhibition was determined as the quotient of the slopes (compound divided by blank) of the linear ranges. The used substrate concentration was 0.625 mM.
The mode of inhibition as well as K_i and K_i' values were determined using Lineweaver–Burk, Dixon and Cornish-Bowden plots.

4.2.2. Antiproliferative Assays

Human cancer cell lines were obtained from the European Collection of Authenticated Cell Cultures and were cultivated according to the provider's instructions. Briefly, MCF-7 and K562 cell linea were cultivated in DMEM medium supplemented with 10% fetal bovine serum. All media were supplemented with penicillin (100 U/mL) and streptomycin (100 mg/mL) and cell lines were cultivated at 37 °C in 5% CO$_2$. For the viability assays, cells were seeded into 96-well plates in the appropriate densities and after 24 h preincubation period, were treated in triplicates with six different doses of each compound for 72 hours. After treatments, resazurin (Merck) solution was added for 4 h, and fluorescence of resorufin corresponding to live cells was measured at 544 nm/590 nm (excitation/emission) using a Fluoroskan Ascent microplate reader (Labsystems). The GI$_{50}$ value, the drug concentration lethal to 50% of the cells, was calculated from the dose response curves that resulted from the assays. Imatinib and 5-fluorouracil were purchased from Merck.

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