Synthesis, Antitumor Activity and Structure-activity Studies of Novel Pyridoxine-based Structural Analogs of Saccharumoside-B

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Abstract

In this work, a series of novel pyridine- and pyridoxine-based saccharumoside-B derivatives were synthesized, and their antitumor activity on nine human tumor cell lines (MCF-7, MDA-MB-231, A-498, SNB-19, M-14, NCI-H322M, HCT-115, HCT-116, PC-3) and cytotoxicity on three conditionally normal cell lines (HEK-293, Chang Liver, MSC) were studied in comparison with camptothecin, doxorubicin and saccharumoside-B. Structure-activity patterns, and in particular the contribution to antitumor activity of the peripheral fragments of the studied pharmacophore were analyzed. Our synthetic explorations also allowed us to propose a new efficient approach to the synthesis of saccharumoside B, which was used as a reference drug.

Introduction

The chemically multifunctional pyridoxine scaffold (vitamin B6) provides a useful structural platform for the design of modified analogs of physiologically active compounds [1]. A good illustration is a series of pyridoxine-based bioisosteric analogs of estradiol described in our papers [2–4]. The leading compounds from this series demonstrated expressed activity against the estrogen-dependent MCF-7 breast cancer cell line (IC_{50} 2–8 μ M) and good selectivity for a panel of normal and tumor cells, including the estrogen receptor negative MDA-MB-231 breast cancer cells. Another example is a library of bioisosteric analogs of a natural biologically active compound, dehydrosingerone (DZG), which were synthesized by replacing the aromatic fragment of DZG with the pyridoxine ring [5]. The most active compounds had pronounced cytotoxicity comparable to doxorubicin with improved selectivity towards tumor cells. We also used pyridoxine scaffold as a structural basis for the synthesis of novel potent inhibitors of acetylcholinesterase, mimetics of pyridostigmine, with improved toxicity profile [6].

The same approach can be used to develop structurally modified analogs of saccharumoside B (Fig. 1A). This phenolic glycoside from sugar maple (*Acer saccharum*) bark is of great interest to medicinal chemists because of its unique biological properties [7], including promising anti-tumor activity [7–8]. For example, saccharumoside-B and its derivatives (Fig. 1B) showed high antiproliferative activity against various tumor cell lines (MCF-7, HL-60, MIA PaCa-2, DU145, HeLa, CaCo-2, MCF10A) with IC_{50} values ranged from 4.4 ± 0.4 to 49.6 ± 3.6 μ M, and low cytotoxicity to normal cells ($IC_{50} > 100$ μ M). A leading compound containing a *p*-fluorobenzoyl fragment exhibited the highest antiproliferative activity against MCF-7 breast cancer and HL-60 leukemia cells with IC_{50} 6.1 ± 0.6 and 4.4 ± 0.4 μ M, respectively, and was equipotent to camptothecin.

In our recent work, we obtained 11 new pyridoxine-based analogs of saccharumoside B (Fig. 1C) containing different substituents R1 and R2 in the benzoyl fragment and R3 in the pyridine ring [9]. The synthesized compounds were studied for cytotoxicity on nine human tumor cell lines (MCF-7, MDA-MB-231, A-498, SNB-19, M-14, NCI-H322M, HCT-115, HCT-116, PC-3) and on three conditionally normal cell lines (HEK-293, Chang Liver, MSC). Several compounds (for example, **13d**, Fig. 1D) showed high antitumor activity (IC_{50} 1.5–52 μ M), comparable to camptothecin (IC_{50} 0.7–96) and doxorubicin (IC_{50}

1.5–76), but with significantly higher selectivity toward tumor cells. The selectivity index for pyridoxine-based saccharumoside B analogs ranged from 1 to 31, whereas it ranged within 0.3–4.2 and 0.4–1.4 for camptothecin and doxorubicin, respectively. Analysis of the structure-activity patterns revealed that R1 and R2 substituents in the benzoyl fragment significantly influenced the antitumor activity, and that the most active compounds had H-bond accepting groups, such as methoxy, hydroxy or benzyloxy, in these positions [9].

As a continuation of these studies, in this work we have synthesized a new series of pyridoxine-based saccharumoside B analogs (Fig. 1E) and studied their *in vitro* antitumor activity and cytotoxicity. In particular, we investigated the question of whether the peripheral fragments of the studied pyridoxine-based pharmacophore, namely the hydroxymethyl substituent in the sixth position of pyridine ring, the benzoyl fragment, the ketal group, and the dioxepin cycle (all these fragments are highlighted in bold in the structure of **13d**, Fig. 1D), contribute to target activity.

Our synthetic explorations also allowed us to develop a new efficient approach to the synthesis of saccharumoside B, which was used as a reference drug.

Results And Discussion

Synthesis

The synthetic routes to the title compounds are depicted in Schemes 1–3.

The synthesis of the debenzoylated analogs of saccharumoside-B based on 6-substituted pyridoxine derivatives is shown in Scheme 1. The key glycoside intermediate **2** was obtained in five steps according to our recently described method [9]. The aldehyde group of **2** was reduced by the treatment with NaBH₄ in MeOH to give the corresponding alcohol **3** in a yield of 89%. Acid hydrolysis of **3** led to compound **4** in a yield of 95%. Removal of the acetonide protecting group of compound **2** under similar conditions, bypassing the stage of reduction of the aldehyde group, led to the formation of hemiacetal **5** in 97% yield.

The synthesis of another pyridoxine-based structural analog of saccharumoside-B without a substituent in the sixth position of the pyridine ring is shown in Scheme 2. The intermediate seven-membered cyclic ketal of pyridoxine **6** was obtained by the treatment of pyridoxine hydrochloride **1** with acetone in the presence of hydrochloric acid using our reported method [10]. Pyridoxine-containing O-glycoside **7** was prepared in 56% yield by a phase-transfer reaction of α -D-acetobromoglucose with compound **6** in the presence of K₂CO₃ and TBAB in CHCl₃:H₂O (1:1, v/v). Removal of the acetyl groups with a methanol-water solution (10:1, v/v) of potassium carbonate led to tetraol **8** in 92% yield. Regioselective O-6 benzoylation of pyridoxine β -D-glucopyranoside **8** using 4-methylbenzoyl chloride and Me₂SnCl₂ as a catalyst according to literature methods with minor modifications [11–13] gave compound **9** in 53% yield.

The synthesis of another structural analog of saccharumoside-B based on a 3-hydroxy-2-methylpyridine scaffold is shown in Scheme 3. Compounds **11** and **12** were obtained by the same methods as described

above for compounds **7** and **8** in Scheme 2. However, all our attempts to obtain O-6 monobenzoylelated product **13** were unsuccessful. The reason was very low solubility of compound **12** in all organic solvents except DMSO, and therefore the benzylation reaction had to be carried out under heterophase conditions. However, heterophase conversion of compound **12** into O-6 monobenzoylelated product **13** was much slower (complete reaction occurred within 1 month) than homophase conversion of **13** into dibenzyolated product **14**. As a result, only the dibenzyolated compound **14** could be obtained using the described method. Our attempts to perform a regioselective cleavage of the ester bonds of **14** were also unsuccessful.

In this work, we have also developed a novel synthetic approach to saccharumoside-B which was used as a reference drug. The first method of synthesis of saccharumoside-B was reported by Rayavarapu et al [8], but it proved difficult to reproduce. Our novel method shown in Scheme 4 was based on a previously described approach to the synthesis of phenolic glycosides vitexnegheteroin A and ovatoside D [13]. Compounds **17** and **18** were obtained from vanillin **15** according to previously described literature methods. Thus, the vanillin-containing O-glycoside **17** was obtained by reaction of vanillin **15** with D-acetobromoglucose under basic conditions, followed by mild deprotection of hydroxyl groups of the resulting tetraester **16** by the treatment with K_2CO_3 in methanol-water solution (10:1, v/v) [13]. Vanillic acid **18** was obtained by oxidation of vanillin **15** with silver oxide under alkaline conditions [14]. Vanillic acid **18** was then reacted with allyl bromide under basic conditions to give 4-allyloxyvanillic acid **19**, and the latter was quantitatively converted to the corresponding acyl chloride **20** by refluxing with $SOCl_2$. The regioselective O-6 benzylation of glucopyranoside **17** with chloride **20** in the presence of $MeSnCl_2$ as a catalyst led to the corresponding monobenzyolated adduct **21**. At the last stage, one-pot treatment of **21** with $Pd(PPh)_3$ and $NaBH_4$ in methanol resulted in removal of allyl group and reduction of aldehyde group leading to saccharumoside-B in 91% yield. The described synthesis represents a novel approach to the target compound which has such advantages as simple synthetic and purification procedures, inexpensive reagents, good reproducibility, and high total yield (46% starting from **15**).

The structures of the synthesized compounds were confirmed by HSQC, COSY, 1H and ^{13}C NMR spectroscopy, and mass spectrometry.

Cytotoxicity evaluation

The synthesized pyridoxine-based structural analogs of saccharumoside-B were evaluated for *in vitro* cytotoxicity against a panel of human tumor and normal cell lines using MTT assay. Doxorubicin, camptothecin, saccharumoside-B, and one of the most active pyridoxine-containing derivatives of saccharumoside-B **13d** (Fig. 1D) obtained and described in our previous work [9] were used as reference drugs. The screening results are shown in Table 1.

According to Table 1, all new compounds exhibited moderate *in vitro* cytotoxic activity against the studied tumor cell lines. Based on direct comparison with reference drugs, all these compounds were comparable to saccharumoside-B, but at the same time they were quite inferior to doxorubicin, camptothecin and **13d**. At the same time, all our compounds showed toxicity comparable to

saccharumoside-B and **13d** on conventionally normal cells and were much less toxic to normal cells than camptothecin and doxorubicin.

Table 1

In vitro antitumor activity and cytotoxicity (IC₅₀ and CC₅₀, μ M, mean $\pm$ SD) of the synthesized compounds and reference drugs^a

Antitumor activity, IC ₅₀ , μ M						
Compound	PC-3	MDA-MB-231	MCF-7	M-14	SNB-19	HCT-116
3	>200	59.81 $\pm$ 3.73	13.14 $\pm$ 1.54	35.74 $\pm$ 1.62	117.69 $\pm$ 6.31	84.29 $\pm$ 4.81
4	94.78 $\pm$ 3.13	62.00 $\pm$ 3.08	16.99 $\pm$ 3.16	51.05 $\pm$ 2.07	122.86 $\pm$ 6.67	81.82 $\pm$ 4.56
5	67.65 $\pm$ 3.96	49.08 $\pm$ 0.84	41.37 $\pm$ 2.69	55.43 $\pm$ 2.16	74.18 $\pm$ 2.48	65.32 $\pm$ 4.75
9	61.38 $\pm$ 3.44	55.96 $\pm$ 3.73	22.61 $\pm$ 1.78	39.61 $\pm$ 1.03	112.27 $\pm$ 7.08	77.26 $\pm$ 3.23
14	67.39 $\pm$ 3.82	51.71 $\pm$ 2.94	26.12 $\pm$ 1.75	36.13 $\pm$ 1.58	76.82 $\pm$ 4.35	72.94 $\pm$ 4.89
13d ^b	6.11 $\pm$ 0.31	2.14 $\pm$ 0.42	3.09 $\pm$ 0.18	5.46 $\pm$ 0.28	14.36 $\pm$ 2.52	4.09 $\pm$ 0.49
Saccharumside-B	51.35 $\pm$ 3.00	48.94 $\pm$ 2.77	34.19 $\pm$ 2.89	50.01 $\pm$ 0.76	82.71 $\pm$ 4.95	67.10 $\pm$ 3.71
Camptothecin	1.94 $\pm$ 0.22	1.98 $\pm$ 0.26	1.54 $\pm$ 0.04	0.74 $\pm$ 0.02	96.39 $\pm$ 4.97	1.41 $\pm$ 0.17
Doxorubicin	1.66 $\pm$ 0.25	4.29 $\pm$ 0.44	2.04 $\pm$ 0.40	1.62 $\pm$ 0.28	75.67 $\pm$ 6.57	1.49 $\pm$ 0.29
Antitumor activity, IC ₅₀ , μ M				Cytotoxicity, CC ₅₀ , μ M		
Compound	HCT-115	A498	NCI-H322M	HEK-293	MSC	Chang Liver
3	74.26 $\pm$ 3.91	43.47 $\pm$ 2.94	28.72 $\pm$ 1.27	65.76 $\pm$ 3.11	51.19 $\pm$ 2.12	56.74 $\pm$ 2.19
4	66.76 $\pm$ 3.54	39.86 $\pm$ 2.56	29.32 $\pm$ 1.13	64.95 $\pm$ 3.61	68.14 $\pm$ 2.32	77.72 $\pm$ 2.37
5	59.20 $\pm$ 1.01	54.25 $\pm$ 2.11	61.61 $\pm$ 2.39	45.48 $\pm$ 3.04	48.02 $\pm$ 3.67	66.92 $\pm$ 4.85
9	60.26 $\pm$ 2.71	32.49 $\pm$ 1.44	29.09 $\pm$ 1.06	64.18 $\pm$ 2.58	68.78 $\pm$ 3.67	86.82 $\pm$ 2.09

^a The average value of the three independent experiments

^b Reported in ref. [9]

Antitumor activity, IC ₅₀ , μ M						
14	45.34 $\pm$ 3.07	20.62 $\pm$ 1.94	24.45 $\pm$ 1.77	56.53 $\pm$ 3.03	42.89 $\pm$ 2.52	58.02 $\pm$ 2.12
13d^b	3.55 $\pm$ 0.11	8.14 $\pm$ 1.28	8.02 $\pm$ 1.60	48.02 $\pm$ 6.37	67.37 $\pm$ 5.29	~200
Saccharumoside-B	64.31 $\pm$ 3.95	40.45 $\pm$ 2.01	43.86 $\pm$ 2.62	61.86 $\pm$ 3.34	88.89 $\pm$ 4.61	106.36 $\pm$ 6.75
Camptothecin	4.25 $\pm$ 0.44	0.72 $\pm$ 0.07	1.65 $\pm$ 0.22	1.28 $\pm$ 0.14	3.11 $\pm$ 0.36	1.94 $\pm$ 0.07
Doxorubicin	2.91 $\pm$ 0.52	4.60 $\pm$ 0.79	2.71 $\pm$ 0.34	1.74 $\pm$ 0.03	2.02 $\pm$ 0.07	1.69 $\pm$ 0.14
^a The average value of the three independent experiments						
^b Reported in ref. [9]						

The results of biological studies elucidated the importance of the peripheral fragments of the studied pyridine- and pyridoxine-based bioisosteric analogs of saccharumoside-B for antitumor activity. In our previous work we found that the replacement of the vanillin moiety (aglycon) with 6-hydroxymethylpyridoxine or 6-formylpyridoxine led to increased cytotoxicity against a number of tumor cell lines compared to unmodified saccharumoside-B, while varying the substituents in the benzoyl moiety of the studied pyridoxine-based saccharumoside-B derivatives had only a minor effect on the target activity [9]. Compounds containing toluoyl or 4-hydroxy-3-methoxy-benzoyl fragments showed the highest activity and selectivity towards tumor cell lines [9]. The results of the present work have demonstrated that all peripheral fragments of the studied pharmacophore make a significant contribution to antitumor activity. The removal of any one or more fragments of this pharmacophore, namely, the hydroxymethyl group in the sixth position of pyridoxine (compounds **9** and **14**), the ketal and benzoyl fragments (compounds **3**, **4**, **5**), and the dioxepin cycle (compound **14**), resulted in a significant, up to 10-30-fold decrease in the target activity as compared to that of the leading compound **13d**. In general, the antitumor activity of the synthesized compounds against the studied tumor cell lines was comparable to that of saccharumoside-B.

Conclusions

In conclusion, in this paper we report an efficient method of synthesis of five novel pyridine and pyridoxine-based structural analogs of saccharumoside-B. The obtained compounds were evaluated *in vitro* for antitumor activity against a panel of nine human cancer cell lines. Cytotoxicity of all compounds against three normal human cell lines was also evaluated. In our previous work we have found that the replacement of the vanillin moiety with 6-hydroxymethylpyridoxine leads to a significant increase in

cytotoxicity against the studied tumor cell lines compared to saccharumoside-B, and that varying the nature of substituents in the benzoyl fragment and/or replacing the hydroxymethyl group in the sixth position of pyridoxine by the formyl group has little effect on the target activity [9]. As a continuation of this study, in the present work we have observed that the removal of any one or more fragments from the structure of the lead compound **13d**, namely, the hydroxymethyl group in the sixth position of pyridoxine, the ketal and benzoyl fragments, and the dioxepin cycle, leads to a significant decrease in the target activity to values comparable with those of saccharumoside-B. Therefore, it can be concluded that these peripheral fragments of the studied pharmacophore are important for antitumor activity. It was also shown that the synthesized compounds exhibit cytotoxicity comparable to saccharumoside-B on conditionally normal cell lines. We have also developed a new approach to the synthesis of saccharumoside-B itself, which has such advantages as simple synthetic and purification procedures, inexpensive reagents, good reproducibility, and high total yield.

Experimental/ Material And Methods

Chromatographic purification of compounds was carried out using column chromatography on Acros silica gel (60-200 mesh). Reaction progress and purity of compounds were monitored by TLC on Sorbfil PTLC-AF-A-UF plates. Melting points of the products were determined using a Stanford Research Systems MPA-100 OptiMelt appliance. ^1H , ^{13}C , HSQC, COSY NMR spectra were recorded on a Bruker Avance 400 spectrometer (400.17 and 100.62 MHz) of Spectral-Analytical Center of FRC Kazan Scientific Center of the RAS. Signals of chloroform-*d* (δ_{H} 7.24, δ_{C} 77.23), dimethyl sulfoxide-*d*₆ (δ_{H} 2.50, δ_{C} 39.51) were used as references in the ^1H and ^{13}C NMR spectra. Coupling constants (*J*) are reported in Hz (splitting abbreviations: s, singlet; d, doublet; t, triplet; m, multiplet; br, broad; and combinations thereof). HPLC/MS-experiment was carried out using a TripleTOF 5600, AB Sciex superhigh resolution mass spectrometer (Germany) from the solution in methanol by turboionic spray (TIS) ionization method with the collision energy with nitrogen molecules of 10 eV. For analyzing optically active substances, "ADP 440" (B + S) polarimeter was used.

Synthesis

*(6-(Hydroxymethyl)-3,3,8-trimethyl-1,5-dihydro-[1, 3]dioxepino[5,6-*c*]pyridin-9-yl)-β-D-glucopyranoside (3)*: NaBH₄ (0.05 g, 1.25 mmol) was added portion wise to a solution of compound **2** (0.50 g, 1.25 mmol) in 30 mL of methanol at 0°C. After stirring at room temperature for 20 min, the solution was neutralized with an aqueous solution of hydrochloric acid to pH = 7. Then the solvent was removed in vacuo, and the residue was purified by column chromatography (eluent ethanol – methylene chloride 1:3, v/v). It was obtained as a white amorphous solid, yield 89% (0.45 g); $[\alpha]^{25}_{\text{D}} + 10.5$ (*c* 1.0, methanol); ^1H NMR (DMSO-*d*₆, 400 MHz) δ 1.41 (6H, s, C(CH₃)₂), 2.45 (3H, s, CH₃), 3.05-3.16 (2H, m, 2CHO), 3.20-3.30 (2H, m, 2CHO), 3.42 (1H, dt, *J* = 11.5, 5.8 Hz, CH₂OH), 3.64 (1H, ddd, *J* = 11.5, 4.6, 1.8 Hz, CH₂OH), 4.39 (1H, dd, *J* = 5.8, 4.6 Hz, CH₂OH), 4.41 (1H, d, *J* = 7.2 Hz, OCHO), 4.46 (2H, d, *J* = 5.5 Hz, CH₂OH), 4.84 (1H, d, *J* = 16.0 Hz, CH₂O), 4.86 (1H, d, *J* = 16.8 Hz, CH₂O), 4.89 (1H, d, *J* = 16.0 Hz, CH₂O), 4.99 (1H, d, *J* = 4.9 Hz, CHOH),

5.09 (1H, d, J = 4.6 Hz, CHOH), 5.10 (1H, t, J = 5.5 Hz, CH₂OH), 5.11 (1H, d, J = 16.8 Hz, CH₂O), 5.56 (1H, d, J = 4.8 Hz, CHOH); ¹³C NMR (DMSO-*d*₆, 101 MHz) δ 19.3 (CH₃), 23.6 (C(CH₃)₂), 23.7 (C(CH₃)₂), 59.2 (CH₂O), 59.8 (CH₂O), 61.2 (CH₂O), 63.5 (CH₂O), 70.0 (CHO), 73.8 (CHO), 76.2 (CHO), 76.8 (CHO), 101.9 (C(CH₃)₂), 105.0 (OCHO), 131.4 (C_{Pyr}), 142.1 (C_{Pyr}), 147.4 (C_{Pyr}), 148.6 (C_{Pyr}), 151.3 (C_{Pyr}); HRMS (ESI⁺) m/z calcd for C₁₈H₂₈NO₉ 402.1759, found 402.1764 (M+H⁺).

(4,5,6-Tris(hydroxymethyl)-2-methylpyridin-3-yl)- β -D-glucopyranoside (4): Concentrated HCl (0.80 mL) was added to a solution of compound **3** (0.40 g; 1.00 mmol) in water (30 mL). After stirring at room temperature for 3 hours, the solution was neutralized with NaHCO₃. Then the solvents were evaporated under reduced pressure at 45 °C. The product was recrystallized from water. It was obtained as a white solid, yield 95% (0.34 g); mp 204°C; [α]_D²⁵ - 8.0 (*c* 0.5, H₂O); ¹H NMR (DMSO-*d*₆, 400 MHz) δ 2.52 (3H, s, CH₃), 3.05-3.12 (2H, m, 2CHO), 3.21-3.27 (1H, m, CHO), 3.29-3.35 (1H, m, CHO), 3.39 (1H, m, CH₂OH), 3.66 (1H, m, CH₂OH), 4.51 (1H, d, J = 7.7 Hz, OCHO), 4.56-4.80 (7H, m, 2CH₂OH+CH₂OH), 4.93 (1H, dd, J = 7.8, 4.5 Hz, CH₂OH), 4.98 (1H, t, J = 5.6 Hz, CH₂OH), 5.03 (1H, d, J = 5.0 Hz, CHOH), 5.07 (1H, t, J = 5.4 Hz, CH₂OH), 5.13 (1H, d, J = 4.9 Hz, CHOH), 5.64 (1H, d, J = 4.9 Hz, CHOH); ¹³C NMR (DMSO-*d*₆, 101 MHz) δ 19.7 (CH₃), 54.4 (CH₂O), 56.0 (CH₂O), 61.4 (CH₂O), 62.8 (CH₂O), 70.4 (CHO), 73.9 (CHO), 76.3 (CHO), 76.6 (CHO), 104.5 (OCHO), 132.7 (C_{Pyr}), 142.4 (C_{Pyr}), 148.2 (C_{Pyr}), 150.5 (C_{Pyr}), 154.3 (C_{Pyr}); HRMS (ESI⁺) m/z calcd for C₁₅H₂₄NO₉ 362.1446, found 362.1451 (M+H⁺).

*(7-Hydroxy-4-(hydroxymethyl)-2-methyl-5,7-dihydrofuro[3,4-*b*]pyridin-3-yl)- β -D-glucopyranoside (a mixture of two diastereomers) (5)*: concentrated HCl (0.5 mL) was added to a solution of compound **2** (0.40 g; 1.00 mmol) in water (20 mL). After stirring at room temperature for 3 hours, the solution was neutralized with NaHCO₃. Then the solvents were evaporated under reduced pressure at 45 °C. The dry residue was dissolved in ethanol and the precipitate was filtered off. The filtrate was evaporated under reduced pressure. The dry residue was dissolved in water and purified using preparative reverse phase liquid chromatography (gradient elution with water–methanol). It was obtained as a white amorphous solid; yield 97% (0.35 g); ¹H NMR (DMSO-*d*₆, 400 MHz) δ 2.51 (3H, s, CH₃), 2.52 (3H, s, CH₃), 3.03-3.12 (4H, m, 4CHO), 3.19-3.30 (4H, m, 4CHO), 3.36-3.42 (2H, m, 2CH₂OH), 3.62-3.66 (2H, m, 2CH₂OH), 4.45 (1H, d, J = 7.4 Hz, OCHO), 4.47 (1H, d, J = 7.4 Hz, OCHO), 4.53-4.83 (6H, m, 2CH₂OH + 2CH₂OH), 4.96-5.15 (10H, m, 2CH₂O + 4CHOH + 2CH₂OH), 5.61 (2H, d, J = 4.5 Hz, 2CHOH), 6.02 (1H, s, OCHOH), 6.03 (1H, s, OCHOH), 6.76 (2H, br s, 2OCHOH); ¹³C NMR (DMSO-*d*₆, 101 MHz) δ 19.79 (CH₃), 19.86 (CH₃), 56.92 (CH₂OH), 61.21 (CH₂OH), 61.26 (CH₂OH), 68.55 (CH₂O), 68.62 (CH₂O), 70.10 (CHO), 70.15 (CHO), 73.83 (CHO), 76.29 (CHO), 76.74 (CHO), 76.79 (CHO), 97.92 (OCHOH), 97.96 (OCHOH), 104.57 (OCHO), 104.69 (OCHO), 130.33 (C_{Pyr}), 139.12 (C_{Pyr}), 147.79 (C_{Pyr}), 152.52 (C_{Pyr}), 152.59 (C_{Pyr}), 154.43 (C_{Pyr}); HRMS (ESI⁺) m/z calcd for C₁₅H₂₂NO₉ 360.1289, found 360.1295 (M+H⁺).

*(3,3,8-Trimethyl-1,5-dihydro-[1,3]dioxepino[5,6-*c*]pyridin-9-yl)-2,3,4,6-tetra-O-acetyl- β -D-glucopyranoside (7)*: phase transfer catalyst tetrabutylammonium bromide (TBAB) (0.92 g, 2.87 mmol), α -D-

acetobromoglucose (2.90 g, 7.06 mmol) and a solution of K_2CO_3 (0.97 g, 7.06 mmol) in 15 mL of water were added sequentially to a solution of compound **6** (1.20 g, 5.74 mmol) in 15 mL of $CHCl_3$. The reaction mixture was stirred for 3 hours at 45 °C. Then the organic part was separated and dried in vacuo. The dry residue was dissolved in methylene chloride and purified using column chromatography (eluent ethyl acetate). It was obtained as a white solid; yield 56% (1.74 g); mp 88-89 °C; $[\alpha]^{25}_D + 16.0$ (c 2.0, methanol); 1H NMR ($CDCl_3$, 400 MHz) δ 1.45 (3H, s, $C(CH_3)_2$), 1.48 (3H, s, $C(CH_3)_2$), 2.03 (3H, s, $CH_3C(O)O$), 2.04 (6H, s, $2CH_3C(O)O$), 2.14 (3H, s, $CH_3C(O)O$), 2.47 (3H, s, CH_3), 3.58 (1H, ddd, $J = 12.3, 5.5, 2.3$ Hz, $CHCH_2O$), 4.08 (1H, dd, $J = 12.3, 2.3$ Hz, $CHCH_2O$), 4.19 (1H, dd, $J = 12.3, 5.5$ Hz, $CHCH_2O$), 4.68 (1H, d, $J = 15.1$ Hz, CH_2O), 4.72 (1H, d, $J = 7.8$ Hz, $OCHO$), 4.89 (1H, d, $J = 16.8$ Hz, CH_2O), 4.92 (1H, d, $J = 15.1$ Hz, CH_2O), 4.98 (1H, d, $J = 16.8$ Hz, CH_2O), 5.13 (1H, t, $J = 9.5$ Hz, CHO), 5.25 (1H, t, $J = 9.5$ Hz, CHO), 5.31 (1H, dd, $J = 9.5, 7.8$ Hz, CHO), 8.05 (1H, s, CH_{Pyr}); ^{13}C NMR ($CDCl_3$, 101 MHz) δ 19.3 (CH_3), 20.7 ($CH_3C(O)O$), 20.9 ($CH_3C(O)O$), 23.5 ($C(CH_3)_2$), 23.9 ($C(CH_3)_2$), 59.8 (CH_2O), 61.8 (CH_2O), 61.9 (CH_2O), 68.3 (CHO), 71.6 (CHO), 72.0 (CHO), 72.8 (CHO), 101.72 ($C(CH_3)_2$), 102.75 ($OCHO$), 133.7 (C_{Pyr}), 142.6 (C_{Pyr}), 143.3 (C_{Pyr}), 147.3 (C_{Pyr}), 150.6 (C_{Pyr}), 169.3 ($CH_3C(O)O$), 169.6 ($CH_3C(O)O$), 170.4 ($CH_3C(O)O$), 170.7 ($CH_3C(O)O$); HRMS (ESI⁺) m/z calcd for $C_{25}H_{34}NO_{12}$ 540.2076, found 540.2081 ($M+H^+$).

(3,3,8-Trimethyl-1,5-dihydro-[1, 3]dioxepino[5,6-*c*]pyridin-9-yl)- β -D-glucopyranoside (**8**): a solution of K_2CO_3 (0.70 g, 5.04 mmol) in 1.5 mL of water was added to a solution of compound **7** (1.36 g, 2.52 mmol) in 15 mL of methanol. The reaction mixture was stirred at room temperature for 1.5 h. Then the solvents were evaporated under reduced pressure at 45 °C, and the product was purified by column chromatography (eluent ethanol – methylene chloride 1:4, v/v). It was obtained as a white amorphous solid; yield 92% (0.86 g); $[\alpha]^{25}_D + 13.9$ (c 3.9, methanol); 1H NMR ($DMSO-d_6$, 400 MHz) δ 1.41 (6H, s, $C(CH_3)_2$), 2.46 (3H, s, CH_3), 3.06-3.15 (2H, m, 2CHO), 3.20-3.29 (2H, m, 2CHO), 3.41 (1H, br m, CH_2OH), 3.65 (1H, ddd, $J = 11.9, 4.7, 1.6$ Hz, CH_2OH), 4.40 (1H, dd, $J = 5.8, 4.7$ Hz, CH_2OH), 4.44 (1H, d, $J = 7.3$ Hz, $OCHO$), 4.70 (1H, d, $J = 15.2$ Hz, CH_2O), 4.81 (1H, d, $J = 15.2$ Hz, CH_2O), 4.88 (1H, d, $J = 17.0$ Hz, CH_2O), 4.98 (1H, d, $J = 4.8$ Hz, $CHOH$), 5.07 (1H, d, $J = 4.7$ Hz, $CHOH$), 5.12 (1H, d, $J = 17.0$ Hz, CH_2O), 5.54 (1H, d, $J = 4.7$ Hz, $CHOH$), 8.00 (1H, s, CH_{Pyr}); ^{13}C NMR ($DMSO-d_6$, 101 MHz) δ 19.4 (CH_3), 23.4 ($C(CH_3)_2$), 23.6 ($C(CH_3)_2$), 59.3 (CH_2O), 60.8 (CH_2O), 61.2 (CH_2O), 69.9 (CHO), 73.8 (CHO), 76.2 (CHO), 76.7 (CHO), 102.0 ($C(CH_3)_2$), 104.7 ($OCHO$), 133.0 (C_{Pyr}), 141.0 (C_{Pyr}), 142.1 (C_{Pyr}), 147.7 (C_{Pyr}), 150.9 (C_{Pyr}); HRMS (ESI⁺) m/z calcd for $C_{17}H_{26}NO_8$ 372.1653, found 372.1658 ($M+H^+$).

(3,3,8-Trimethyl-1,5-dihydro-[1, 3]dioxepino[5,6-*c*]pyridin-9-yl)-6-*O*-(4-methylbenzoyl)- β -D-glucopyranoside (**9**): Me_2SnCl_2 (0.029 g, 0.13 mmol) was added to a solution of compound **8** (0.50 g, 1.35 mmol) in 33 mL of THF and 3 mL of water. The reaction mixture was stirred for 10 min at room temperature, then DIPEA (0.47 mL, 2.70 mmol) and *p*-toluoyl chloride (0.18 mL, 1.35 mmol) were sequentially added at 0 °C. After stirring at 0 °C for 1 h and then at room temperature for 1 h, the reaction was quenched with methanol, and then the resulting mixture was evaporated under reduced pressure. The product was purified by column chromatography (eluent methanol – methylene chloride 1:12, v/v). It was obtained as a white

amorphous solid; yield 53% (0.35 g); $[\alpha]^{25}_D$ - 23.9 (*c* 1.0, methanol); ^1H NMR (DMSO- d_6 , 400 MHz) δ 1.23 (3H, s, C(CH₃)₂), 1.35 (3H, s, C(CH₃)₂), 2.38 (3H, s, CH₃), 2.42 (3H, s, CH₃), 3.28-3.32 (3H, m, 3CHO), 3.45 (1H, m, CHO), 4.17 (1H, dd, *J* = 11.8, 6.3 Hz, CHCH₂O), 4.57 (1H, dd, *J* = 11.8, 1.5 Hz, CHCH₂O), 4.59 (1H, d, *J* = 7.0 Hz, OCHO), 4.70 (1H, d, *J* = 15.3 Hz, CH₂O), 4.79 (1H, d, *J* = 15.3 Hz, CH₂O), 4.88 (1H, d, *J* = 16.8 Hz, CH₂O), 5.01 (1H, d, *J* = 16.8 Hz, CH₂O), 5.24 (1H, d, *J* = 3.3 Hz, CHOH), 5.37 (1H, d, *J* = 4.6 Hz, CHOH), 5.66 (1H, d, *J* = 4.4 Hz, CHOH), 7.29 (2H, d, *J* = 8.1 Hz, 2CH_{Ar}), 7.75 (2H, d, *J* = 8.1 Hz, 2CH_{Ar}), 8.02 (1H, s, CH_{Pyr}); ^{13}C NMR (DMSO- d_6 , 101 MHz) δ 19.4 (CH₃), 21.2 (CH₃), 23.3 (C(CH₃)₂), 23.5 (C(CH₃)₂), 59.0 (CH₂O), 60.9 (CH₂O), 63.8 (CH₂O), 69.9 (CHO), 73.6 (CHO), 73.9 (CHO), 75.9 (CHO), 102.0 (C(CH₃)₂), 104.4 (OCHO), 126.9, 129.2, 133.1, 141.0, 142.3, 143.6, 147.4, 150.9 (5C_{Pyr} + 6C_{Ar}), 165.6 (C(O)O); HRMS (ESI⁺) *m/z* calcd for C₂₅H₃₂NO₉ 490.2072, found 490.2077 (M+H⁺).

(2-Methylpyridin-3-yl)-2,3,4,6-tetra-O-acetyl- β -D-glucopyranoside (11): phase transfer catalyst tetrabutylammonium bromide (TBAB) (1.57 g, 4.86 mmol), α -D-acetobromoglucose (6.00 g, 14.6 mmol) and a solution of K₂CO₃ (2.01 g, 14.6 mmol) in 30 mL of water were added sequentially to a solution of compound **10** (1.06 g, 9.72 mmol) in 30 mL of CHCl₃. The reaction mixture was stirred for 3 hours at 45 °C. Then the organic part was separated and dried in vacuo. The dry residue was dissolved in methylene chloride and purified using column chromatography (eluent ethyl acetate). It was obtained as a white solid; yield 74% (3.16 g); mp 129-130°C; $[\alpha]^{25}_D$ - 35.3 (*c* 1.0, methanol); ^1H NMR (CDCl₃, 400 MHz) δ 2.03 (3H, s, CH₃C(O)O), 2.04 (3H, s, CH₃C(O)O), 2.06 (3H, s, CH₃C(O)O), 2.07 (3H, s, CH₃C(O)O), 2.41 (3H, s, CH₃), 3.85 (1H, ddd, *J* = 12.3, 5.3, 2.5 Hz, CHCH₂O), 4.17 (1H, dd, *J* = 12.3, 2.5 Hz, CHCH₂O), 4.27 (1H, dd, *J* = 12.3, 5.3 Hz, CHCH₂O), 4.99 (1H, d, *J* = 7.6 Hz, OCHO), 5.17 (1H, m, CHO), 5.27-5.36 (2H, m, 2CHO), 7.08 (1H, dd, *J* = 8.2, 4.8 Hz, CH_{Pyr}), 7.27 (1H, dd, *J* = 8.2, 1.5 Hz, CH_{Pyr}), 8.21 (1H, dd, *J* = 4.8, 1.5 Hz, CH_{Pyr}); ^{13}C NMR (CDCl₃, 101 MHz) δ 19.3 (CH₃), 20.7 (3CH₃C(O)O), 20.8 (CH₃C(O)O), 62.0 (CH₂O), 68.3 (CHO), 71.1 (CHO), 72.3 (CHO), 72.6 (CHO), 99.5 (OCHO), 121.9 (C_{Pyr}), 122.5 (C_{Pyr}), 143.8 (C_{Pyr}), 150.1 (C_{Pyr}), 151.3 (C_{Pyr}), 169.2 (CH₃C(O)O), 169.5 (CH₃C(O)O), 170.3 (CH₃C(O)O), 170.6 (CH₃C(O)O); HRMS (ESI⁺) *m/z* calcd for C₂₀H₂₆NO₁₀ 440.1551, found 440.1559 (M+H⁺).

(2-Methylpyridin-3-yl)- β -D-glucopyranoside (12): a solution of K₂CO₃ (0.48 g, 3.46 mmol) in 2.0 mL of water was added to a solution of compound **11** (0.76 g, 1.73 mmol) in 20 mL of methanol. The reaction mixture was stirred at room temperature for 1.5 h. The formed precipitate was filtered off, washed twice with water and dried in vacuo. It was obtained as a white solid; yield 91% (0.43 g); mp 248°C (decomp.); $[\alpha]^{25}_D$ - 53.0 (*c* 0.5, DMSO); ^1H NMR (DMSO- d_6 , 400 MHz) δ 2.40 (3H, s, CH₃), 3.14-3.20 (1H, m, CHO), 3.24-3.30 (2H, m, 2CHO), 3.31-3.36 (1H, m, CHO), 3.41 (1H, dt, *J* = 11.6, 5.7 Hz, CH₂OH), 3.69 (1H, ddd, *J* = 11.6, 5.7, 1.8 Hz, CH₂OH), 4.56 (1H, t, *J* = 5.7 Hz, CH₂OH), 4.85 (1H, d, *J* = 7.5 Hz, OCHO), 5.04 (1H, d, *J* = 5.2 Hz, CHOH), 5.10 (1H, d, *J* = 4.3 Hz, CHOH), 5.36 (1H, d, *J* = 3.5 Hz, CHOH), 7.17 (1H, dd, *J* = 8.2, 4.9 Hz, CH_{Pyr}), 7.42 (1H, dd, *J* = 8.2, 1.2 Hz, CH_{Pyr}), 8.21 (1H, dd, *J* = 4.9, 1.2 Hz, CH_{Pyr}); ^{13}C NMR (DMSO- d_6 , 101 MHz) δ 19.2 (CH₃), 60.7 (CH₂O), 69.7 (CHO), 73.2 (CHO), 76.5 (CHO), 77.1 (CHO), 100.7 (OCHO), 121.3

(C_{Pyr}), 122.0 (C_{Pyr}), 141.6 (C_{Pyr}), 148.3 (C_{Pyr}), 151.6 (C_{Pyr}); HRMS (ESI⁺) *m/z* calcd for C₁₂H₁₈NO₆ 272.1129, found 272.1134 (M+H⁺).

(2-Methylpyridin-3-yl)-2,6-di-O-(4-methylbenzoyl)-β-D-glucopyranoside (14): Me₂SnCl₂ (0.024 g, 0.11 mmol) was added to a suspension of compound **12** (0.30 g, 1.11 mmol) in 20 mL of THF. The reaction was stirred for 10 min at room temperature, then DIPEA (0.38 mL, 2.21 mmol) and *p*-toluoyl chloride (0.15 mL, 1.11 mmol) were sequentially added at 0 °C. After stirring at 0 °C for 1 h and then at room temperature for 1 month, the reaction was quenched with methanol, and then the resulting mixture was evaporated under reduced pressure. The product was purified by column chromatography (eluent methanol – methylene chloride 1:12, v/v). It was obtained as a white amorphous solid; yield 82% (0.23 g); [α]²⁵_D - 8.3 (c 0.3, methanol); ¹H NMR (DMSO-*d*₆, 400 MHz) δ 2.06 (3H, s, CH₃), 2.37 (3H, s, CH₃), 2.41 (3H, s, CH₃), 3.49 (1H, td, *J* = 9.1, 5.7 Hz, CHO), 3.74 (1H, td, *J* = 9.1, 5.9 Hz, CHO), 3.97 (1H, ddd, *J* = 9.1, 7.2, 1.4 Hz, CHO), 4.35 (1H, dd, *J* = 11.9, 7.2 Hz, CHCH₂O), 4.67 (1H, dd, *J* = 11.9, 1.4 Hz, CHCH₂O), 5.14 (1H, dd, *J* = 9.1, 8.1 Hz, CHO), 5.36 (1H, d, *J* = 8.1 Hz, OCHO), 5.66 (1H, d, *J* = 5.9 Hz, CHOH), 5.73 (1H, d, *J* = 5.7 Hz, CHOH), 7.34 (2H, d, *J* = 8.1 Hz, 2CH_{Ar}), 7.39 (2H, d, *J* = 8.1 Hz, 2CH_{Ar}), 7.89 (4H, d, *J* = 8.1 Hz, 4CH_{Ar}), 6.99 (1H, dd, *J* = 8.3, 4.8 Hz, CH_{Pyr}), 7.43 (1H, d, *J* = 8.3 Hz, CH_{Pyr}), 8.04 (1H, d, *J* = 4.8 Hz, CH_{Pyr}); ¹³C NMR (DMSO-*d*₆, 101 MHz) δ 18.6 (CH₃), 21.16 (CH₃), 21.19 (CH₃), 63.8 (CH₂O), 70.2 (CHO), 73.5 (CHO), 73.8 (CHO), 74.0 (CHO), 98.3 (OCHO), 121.5, 121.8, 126.9, 127.0, 129.2, 129.3, 129.4, 142.3, 143.7, 143.8, 147.9, 150.9 (5C_{Pyr} + 12C_{Ar}), 165.0 (C(O)O), 165.5 (C(O)O); HRMS (ESI⁺) *m/z* calcd for C₂₈H₃₀NO₈ 508.1966, found 508.1971 (M+H⁺).

4-(Allyloxy)-3-methoxybenzoic acid (19): K₂CO₃ (11.04 g, 80.0 mmol) and allyl bromide (5.3 mL, 60.0 mmol) were sequentially added to a solution of 4-hydroxy-3-methoxybenzoic acid (3.36 g, 20.0 mmol) in 100 mL DMF. The reaction mixture was stirred for 3 hours at 40 °C. Then the precipitate was filtered off, and the filtrate was evaporated under reduced pressure. The dry residue was dissolved in 100 mL of EtOH/H₂O (3:1, w/w), and NaOH (1.60 g, 40.0 mmol) was added. The resulting reaction mixture was refluxed for 1 h. The solution was then evaporated to dryness, then the solid residue was dissolved in water (100 mL), and the resulting solution was acidified slowly with 50% H₂SO₄ (6 mL) under stirring. Then the crystalline precipitate was filtered off, washed with water and dried in vacuo. It was obtained as a white solid; yield 92% (3.82 g); mp 174 °C; ¹H NMR (DMSO-*d*₆, 400 MHz) δ 3.81 (3H, s, CH₃O), 4.62 (2H, d, *J* = 5.3 Hz, CH₂=CH-CH₂O), 5.27 (1H, d, *J* = 10.5 Hz, CH₂=CH-CH₂O), 5.40 (1H, dd, *J* = 17.2, 1.1 Hz, CH₂=CH-CH₂O), 6.04 (1H, ddd, *J* = 22.4, 10.5, 5.3 Hz, CH₂=CH-CH₂O), 7.04 (1H, d, *J* = 8.5 Hz, CH_{Ar}), 7.45 (1H, d, *J* = 1.6 Hz, CH_{Ar}), 7.54 (1H, dd, *J* = 8.5, 1.6 Hz, CH_{Ar}), 12.69 (1H, s, C(O)OH); ¹³C NMR (DMSO-*d*₆, 101 MHz) δ 55.5 (CH₃O), 68.9 (CH₂=CH-CH₂O), 112.1, 112.3, 118.0, 123.0, 123.1, 133.3, 148.5, 151.5, (CH₂=CH-CH₂O + 6C_{Ar}), 167.1 (C(O)OH).

4-(Allyloxy)-3-methoxybenzoyl chloride (20): 4-(Allyloxy)-3-methoxybenzoic acid (3.00 g) was dissolved in 12 mL of SOCl₂, and the resulting mixture was refluxed for 2 h. The excess of SOCl₂ was removed under

vacuum, and the obtained residue was used at the next step without further purification.

(4-Formyl-2-methoxyphenyl)-6-O-(4-(allyloxy)-3-methoxybenzoyl)-β-D-glucopyranoside (21): Me_2SnCl_2 (0.05 g, 0.22 mmol) was added to a solution of compound **17** (0.68 g, 2.17 mmol) in 30 mL of THF and 3 mL of water. The reaction was stirred for 10 min at room temperature, then DIPEA (0.75 mL, 4.33 mmol) and 4-(allyloxy)-3-methoxybenzoyl chloride **20** (0.49 g, 2.17 mmol) were sequentially added at 0 °C. After stirring at 0 °C for 1 h and then at room temperature for 4 h, the reaction was quenched with methanol, and then the resulting mixture was evaporated under reduced pressure. The product was purified by column chromatography (eluent methanol – methylene chloride 1:15, v/v). It was obtained as a white amorphous solid; yield 73% (0.80 g); $[\alpha]^{25}_{\text{D}} - 40.4$ (*c* 0.5, methanol); ^1H NMR ($\text{DMSO}-d_6$, 400 MHz) δ 3.22 - 3.28 (1H, m, CHO), 3.30 - 3.38 (2H, m, 2CHO), 3.77 (3H, s, CH_3O), 3.83 (3H, s, CH_3O), 3.81-3.87 (1H, m, CHO), 4.20 (1H, dd, $J = 11.8, 7.7$ Hz, CHCH_2O), 4.58 (1H, dd, $J = 11.8, 1.3$ Hz, CHCH_2O), 4.67 (2H, d, $J = 5.3$ Hz, $\text{CH}_2=\text{CH}-\text{CH}_2\text{O}$), 5.20 (1H, d, $J = 7.2$ Hz, OCHO), 5.28-5.31 (2H, m, $\text{CH}_2=\text{CH}-\text{CH}_2\text{O}+\text{CHOH}$), 5.41-5.45 (2H, m, $\text{CH}_2=\text{CH}-\text{CH}_2\text{O}+\text{CHOH}$), 5.49 (1H, d, $J = 4.2$ Hz, CHOH), 6.07 (1H, ddd, $J = 22.5, 10.6, 5.3$ Hz, $\text{CH}_2=\text{CH}-\text{CH}_2\text{O}$), 7.08 (1H, d, $J = 8.5$ Hz, CH_{Ar}), 7.26 (2H, br s, 2CH_{Ar}), 7.41 (2H, m, 2CH_{Ar}), 7.54 (1H, dd, $J = 8.4, 1.8$ Hz, CH_{Ar}), 9.81 (1H, s, C(O)H); ^{13}C NMR ($\text{DMSO}-d_6$, 101 MHz) δ 55.57 (CH_3O), 55.60 (CH_3O), 64.0 (CH_2O), 68.9 ($\text{CH}_2=\text{CH}-\text{CH}_2\text{O}$), 70.1 (CHO), 72.9 (CHO), 73.9 (CHO), 76.6 (CHO), 98.9 (OCHO), 110.8, 112.2, 112.4, 114.5, 118.1, 122.0, 123.1, 124.6, 130.5, 133.2, 148.6, 149.2, 151.3, 151.9 ($\text{CH}_2=\text{CH}-\text{CH}_2\text{O} + 12\text{C}_{\text{Ar}}$), 165.2 (C(O)O), 191.4 (C(O)H); HRMS (ESI^+) m/z calcd for $\text{C}_{25}\text{H}_{28}\text{NaO}_{11}$ 527.1524, found 527.1529 ($\text{M}+\text{Na}^+$).

(4-(Hydroxymethyl)-2-methoxyphenyl)-6-O-(4-hydroxy-3-methoxybenzoyl)-β-D-glucopyranoside (SACCHARUMOSIDE-B): $\text{Pd}(\text{PPh}_3)_4$ (0.03 g, 0.025 mmol) was added to a solution of compound **21** (0.25 g, 0.50 mmol) in 12 mL of CH_3OH . The reaction was stirred for 5 min at room temperature, then NaBH_4 (0.06 g, 1.49 mmol) was added. After stirring at room temperature for 1 h, the solution was neutralized with concentrated hydrochloric acid, and the resulting mixture was evaporated under reduced pressure. The product was purified by column chromatography (eluent methanol – methylene chloride 1:9, v/v). It was obtained as a white amorphous solid; yield 91% (0.21 g); $[\alpha]^{25}_{\text{D}} - 38.0$ (*c* 0.5, methanol); ^1H NMR ($\text{DMSO}-d_6$, 400 MHz) δ 3.20 - 3.35 (3H, m, 3CHO), 3.69-3.76 (1H, m, CHO), 3.73 (3H, s, CH_3O), 3.78 (3H, s, CH_3O), 4.14 (1H, dd, $J = 11.8, 7.4$ Hz, CHCH_2O), 4.38 (2H, d, $J = 5.7$ Hz, CH_2OH), 4.56 (1H, dd, $J = 11.8, 1.2$ Hz, CHCH_2O), 4.94 (1H, d, $J = 7.1$ Hz, OCHO), 5.09 (1H, t, $J = 5.7$ Hz, CH_2OH), 5.22 (1H, d, $J = 4.1$ Hz, CHOH), 5.32 (1H, d, $J = 4.7$ Hz, CHOH), 5.36 (1H, d, $J = 5.3$ Hz, CHOH), 6.62 (1H, dd, $J = 8.3, 1.3$ Hz, CH_{Ar}), 6.88 (1H, d, $J = 8.3$ Hz, CH_{Ar}), 6.92 (1H, d, $J = 1.3$ Hz, CH_{Ar}), 7.01 (1H, d, $J = 8.3$ Hz, CH_{Ar}), 7.41 (1H, d, $J = 1.8$ Hz, CH_{Ar}), 7.45 (1H, dd, $J = 8.3, 1.8$ Hz, CH_{Ar}), 10.00 (1H, s, OH); ^{13}C NMR ($\text{DMSO}-d_6$, 101 MHz) δ 55.5 (CH_3O), 55.7 (CH_3O), 62.7 (CH_2O), 63.9 (CH_2O), 70.2 (CHO), 73.2 (CHO), 73.8 (CHO), 76.7 (CHO), 99.8 (OCHO), 111.0, 112.7, 114.9, 115.2, 118.3, 120.4, 123.5, 136.4, 145.0, 147.4, 148.7, 151.7 (12C_{Ar}), 165.4 (C(O)O); HRMS (ESI^-) m/z calcd for $\text{C}_{22}\text{H}_{25}\text{NO}_{11}$ 465.1402, found 465.1397 ($\text{M}-\text{H}^+$).

Cytotoxicity evaluation

The cytotoxicity was evaluated on MCF-7 (human breast cancer cells), MDA-MB-231 (human breast cancer cells), A-498 (human kidney cancer cell), SNB-19 (human glioblastoma cell), M-14 (melanoma cell), NCI-H322M (lung cancer cell), HCT-15 (colon cancer cell), HCT-116 (colon cancer cell), PC-3 (prostate cancer), HEK 293 (human embryonic kidney), Chang Liver (human liver), MSC (human mesenchymal stem cells) by using MTT assay. Cells were cultured in a-MEM (minimum essential medium Eagle) / DMEM (Dulbecco's modified Eagle's medium) supplemented with 10% FBS, 2 mM L-glutamine, 100 µg/mL penicillin and 100 µg/mL streptomycin. Cells were seeded in 96-well plates at the density of 10,000 cells per well and grown overnight at 37°C and 5% CO₂ in humidified atmosphere. Then the medium was changed to the fresh one containing compounds to be tested in concentration of 0.1-100 µg/mL. After 72 h of cultivation, the cultural fluid was discarded, and MTT solution (in DPBS) was added to the fresh media until final concentration of 0.5 mg/mL. After 2 h the liquid was replaced with dimethyl sulfoxide (Sigma-Aldrich, St. Louis, MO) to dissolve formazan crystals, and absorption was measured on Tecan Infinite 200Pro at 557 nm with reference 700 nm. Based on data obtained, the CC₅₀ values (concentrations decreasing the metabolic activity by 2-fold) were calculated. Experiments were carried out in biological triplicates (i.e. newly prepared cultures and medium) with independent repeats in each one. The IC₅₀ and CC₅₀ were calculated by plotting the log doses vs inhibition (expressed as a percentage relative to the control) – variable slope in Prism 6 (GraphPad Software Inc.).

Declarations

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Compliance with ethical standards

Conflict of Interest

The authors declare that they have no conflict of interest.

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Schemes

Schemes 1 to 4 are available in the Supplemental Files section.

Figures

Figure 1

Saccharumoside-B (**A**); structural analogs of saccharumoside-B [8] (**B**); pyridoxine-based analogs of saccharumoside-B described in our previous work (**C**), and the leading compound **13d** from this series [9] (**D**); compounds synthesized in this work (**E**)

Supplementary Files

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