Synthesis and antiproliferative activity of 1-methoxy-, 1-(α-D-ribofuranosyl)- and 1-(β-D-ribofuranosyl)brassenin B

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Dedicated to Professor Arlette Solladié-Cavallo on her 70th birthday

Abstract
Syntheses of indole phytoalexin 1-methoxybrassenin B and a linear synthesis of its nucleoside analogs 1-(α-D-ribofuranosyl)brassenin B and 1-(β-D-ribofuranosyl)brassenin B are reported from the corresponding 1-substituted indole-3-carboxaldehydes and carboxylic acids as key intermediates. Examination of the antiproliferative activity of synthesized compounds on human tumor cell lines Jurkat, CEM, CEM-VCR, MCF-7 and HeLa revealed the highest activity for 1-methoxybrassenin B, whereas activity of the nucleoside analogs decreased and appeared to be dependent on lipophilicity.

Keywords: Indole, phytoalexins, nucleoside analogs, 1-methoxybrassenin B, antiproliferative activity

Introduction

Indole phytoalexins are inducible phytochemicals produced by Brassica plants in response to several forms of stress, including microbial infection.[1] However, their isolation from plants requires complicated procedures and does not afford sufficient quantities for biological studies. With respect to the well known anticarcinogenicity of Brassica vegetables,[1c,2] there is a strong need to investigate the synthetic approaches to 1-methoxyindole phytoalexins and their analogs,

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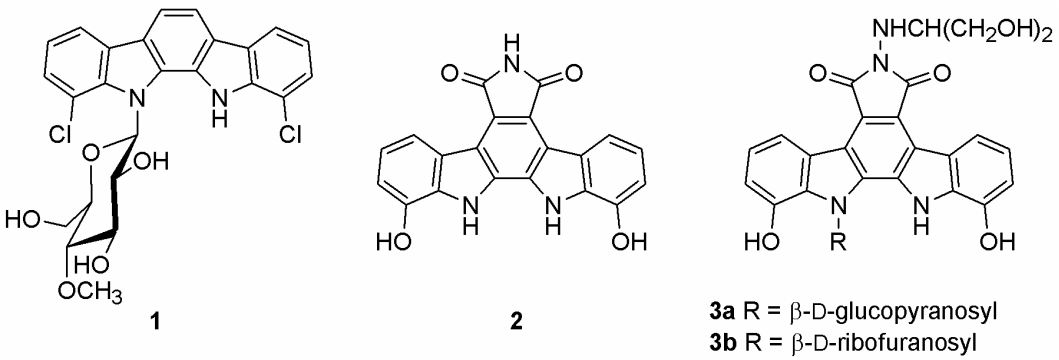
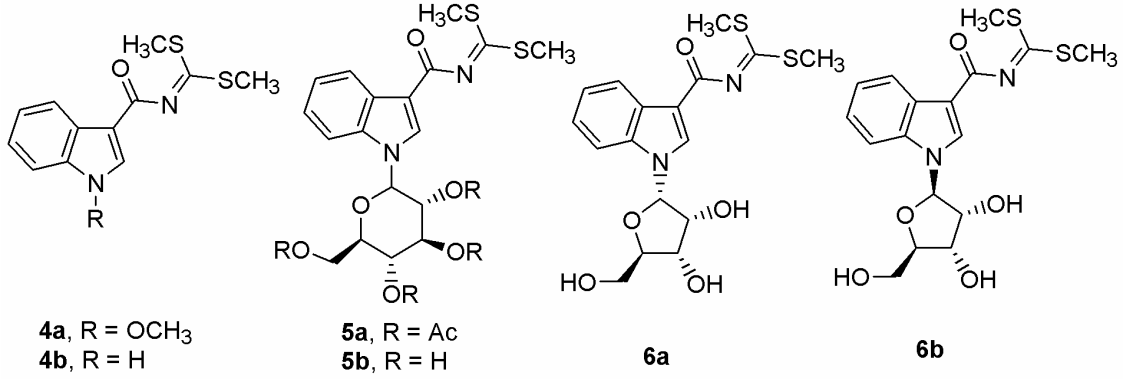
including *N*-glycosylated ones. Indole nucleosides represent a rare type of natural products with interesting biological properties. Among them, nucleoside antibiotic rebeccamycin (**1**)[3a] and its analogs have been identified as attractive cancer chemotherapy agents.[3b] Glycosylation of the natural indolocarbazole BE 13793C (**2**)[4a] and its further modification lead to the improvement of its biological properties and yielded a potent anticancer drug J-107,088 (**3a**), active against human stomach cancer cells MKN-45 implanted to mice.[4b,c] From the series of sugar analogues of J-107,088 (**3a**), the β-*D*-ribofuranoside J-107,534 (**3b**) was found to be 6 times more potent than **3a** at inhibiting topoisomerase I, an attractive target for cancer chemotherapy.[4d] A series of recently described 2,3-substituted-5,6-dichloro-1-(β-D-ribofuranosyl)indole compounds exhibit activity against human cytomegalovirus and herpes simplex virus type I, but this activity was not well separated from cytotoxicity.[5]



Within our continuing investigation of the synthesis of cruciferous phytoalexins and their nucleoside analogs,[6] we have focused our effort also towards the synthesis and biological activity of 1-methoxybrassenin B (**4a**), isolated in 1991 from cabbage (*Brassica oleracea* var. *capitata*) inoculated with *Pseudomonas cichorii*,[7] and its ribofuranosyl analogs **6a** and **6b**.

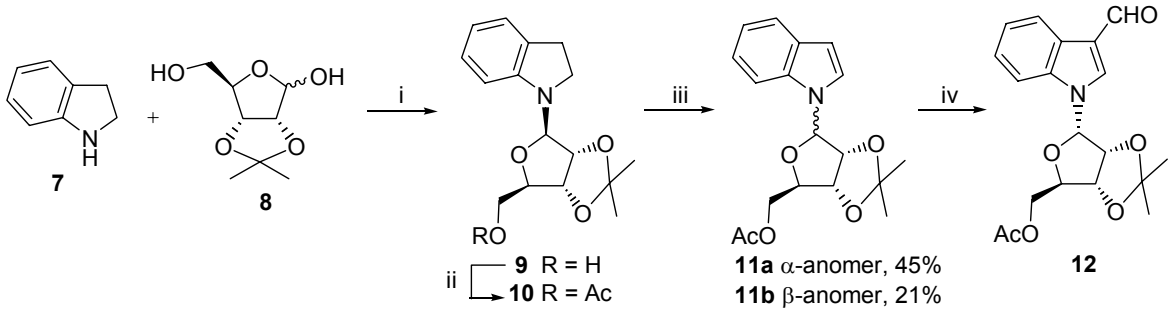


Results and Discussion

Synthesis of brassenin B (**4b**)[8] (the demethoxy analog of **4a**) and its 1-(β-D-glucopyranosyl) analog (**5b**)[6a] have created the starting position for the present investigation of the preparation of 1-methoxybrassenin B and convergent or linear synthesis of its *N*-ribofuranosides respectively. We started our study by the preparation of 1-(β-D-ribofurosyl)brassenin B (**6b**) from brassenin B (**4b**), using a convergent approach. Although, the nucleophilicity of indole nitrogen in general is low, and standard methods used in nucleoside chemistry fail to afford indole nucleosides, several successful ribosylations of indoles are described. 1-(β-D-Ribofuranosyl)indoles were obtained by glycosylation of sodium salts of 2,5,6-trichloroindole and 2-bromo-5,6-dichloroindole[5] or 5-nitroindole[9] with 5-*O*-*tert*-butyldimetylsilyl-2,3-*O*-isopropylidene-α-D-ribofuranosyl chloride. In our case, however, this method failed to afford the desired product, probably because of the low reactivity of starting brassenin B, and only decomposition products were observed. Also unsuccessful was the reaction of brassenin B with 5-*O*-*tert*-butyldimethylsilyl-2,3-*O*-isopropylidene-α-D-ribofuranose *via* Mitsunobu conditions[10] (PPh_3, diethyl azodicarboxylate) resulting in acylation of brassenin B with the formation of 1-ethoxycarbonylbrassenin B. Therefore, in the subsequent approaches to the synthesis of the target compounds we focused our attention on linear synthesis, using the indoline-indole method, which offers a possibility to take advantage of the enhanced nucleophilicity of indoline compared to the less nucleophilic indole. In this method a 2,3-dihydroindole (indoline) derivative is reacted in ethanol at room temperature or under reflux in the presence of catalytic amount of acetic acid with a suitable glycosyl donor (frequently a per-*O*-acetylated saccharide) to form an intermediate glycosyl indoline, which is then oxidized to the glycosylindole derivative.[11] Using this synthetic strategy, the corresponding glycosylated indole-3-carboxaldehydes **12** and **16** were selected as the key intermediates for the linear synthesis of **6a** and **6b**.

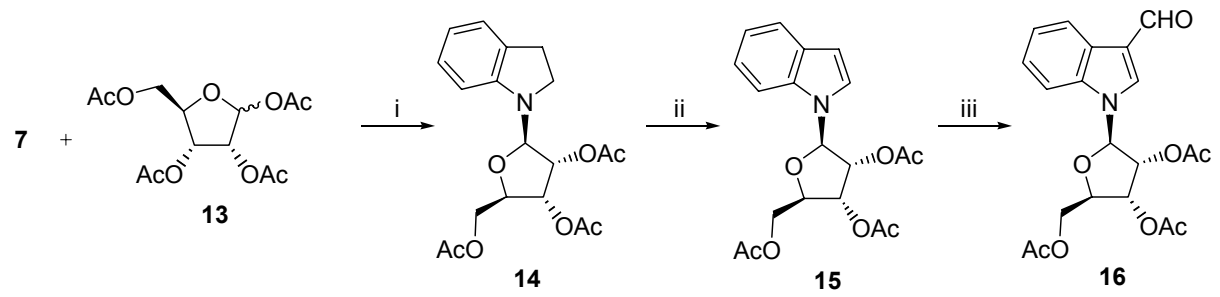
At the time we started this study, literature procedures reported 1-acetylindoline as the sole reaction product between per-*O*-acetylated ribofuranose with indoline.[11] On the other hand, formation of an *N*-glycosidic bond can be achieved through the reaction of 2,3-*O*-isopropylidene-D-ribofuranose (**8**) with indoline (**7**) as reported.[12] In agreement with literature where it was described that reaction of 2,3-isopropylidene-D-ribofuranose (**8**) with indoline (**7**) in anhydrous methanol leads to β-D-ribofuranosylamine **9** as a major product as well as α-D-ribofuranosylamine and ribopyranosyl derivative in the ratio (78 : 8 : 14),[12] we isolated 1-(2,3-*O*-isopropylidene-β-D-ribofuranosyl)indoline (**9**, Scheme 1) in 64% yield. After the protection of the 5′ hydroxyl with the acetyl group, the obtained indoline **10** was oxidized with DDQ to corresponding indole **11**. Oxidation produced a mixture of α- and β-ribofuranosylindoles **11a** and **11b** in a ratio 68 : 32 (determined by ^{1}H NMR spectroscopy) obtained in 45 % and 21% yield after chromatography. The anomeric configurations of **11a** and **11b** were determined by NOE-difference experiments (Figure 1 and 2). A similar anomerization had been observed at oxidation of 5-fluoro-(5-*O*-trityl-2,3-di-*O*-acetyl-β-D-ribofuranosyl)indoline, which yielded the corresponding indole β- and α-ribosides (35% and 8%).[11] Although the mechanism of formation

of the α-anomer is not known, it was suggested that DDQ eliminated a hydride ion from the anomeric center to form a carbocation, which can be stabilized by conjugation with the unshared electron pair of the neighboring nitrogen and oxygen atoms. A proton is then eliminated and a hydrogen transferred from the pyrrole ring to the anomeric carbon of the planar carbonium ion from either face to produce a mixture of β- and α-anomers.[11] The unexpected formation of α-ribofuranosylindole **11a** opened the way to synthesis of **6a**. Indole **11a** was subjected to a Vilsmeier reaction and aldehyde **12** was obtained in 86% yield.



Scheme 1. (i) MeOH, reflux, 5 h, 64%. (ii) Ac_2O, py, 20 h, 73%. (iii) DDQ, toluene, 10 min. (iv) from **11a** $POCl_3$, DMF, 20 min, 86%.

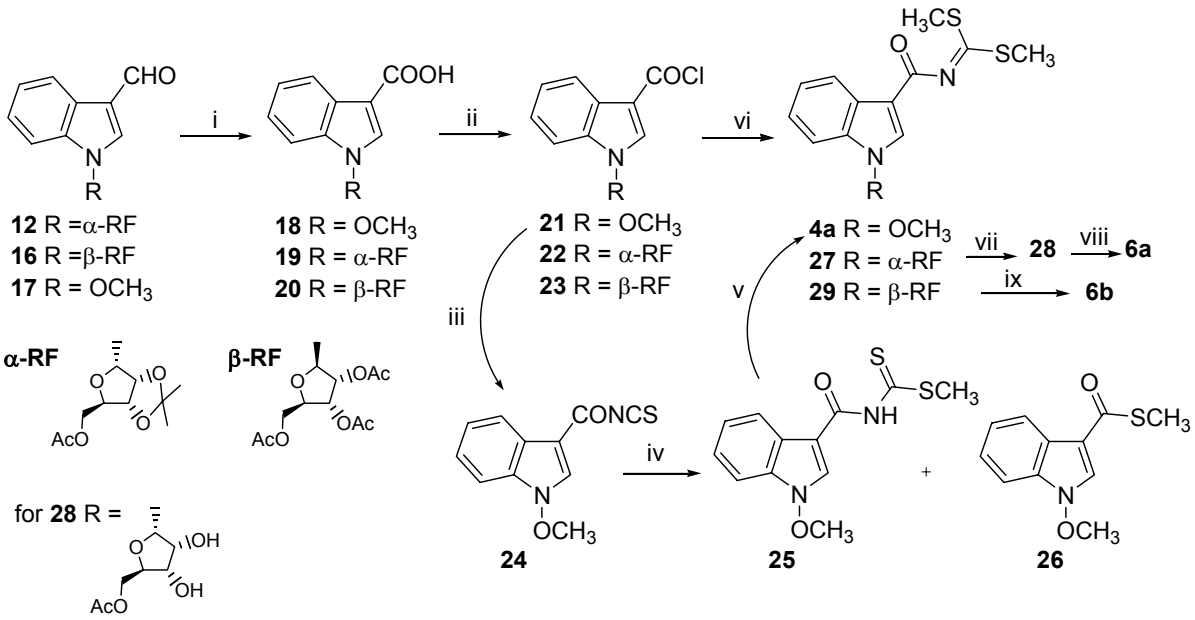
Because of the low yield of β-anomeric indole **11b**, we have been searching for another way to obtain suitably protected 1-(β-D-ribofuranosyl)indole-3-carboxaldehyde. Consequently, we decided to verify the information that the reaction of peracetylated ribofuranose **13** with indoline (**7**) produces only 1-acetylindoline.[11] It was found that condensation of protected ribose **13** with 3 equivalents of indoline under reflux during 6 hours afforded the desired 1-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)indoline (**14**) in 63% yield after column chromatography in addition to the 1-acetylindoline (Scheme 2). We presented this result in 2003 on the 10th Blue Danube Symposium on Heterocyclic Chemistry in Vienna.[6e] In the same year, an analogous preparation of **14** in 55% yield after preparative TLC was published using 2 equivalents of indoline under reflux for 27 hours.[13] Oxidation of **14** with DDQ afforded 1-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)indole (**15**) of very high purity in 98% yield without chromatography. Vilsmeier formylation of **15** gave the required intermediate aldehyde **16** in 61% yield.



Scheme 2. (i) AcOH, EtOH, reflux, 6 h, 63%. (ii) DDQ, toluene, 1 h, 98%. (iii) $POCl_3$, DMF, 75 min, 61%.

In analogy to the linear synthesis of target nucleoside analogs **6a** and **6b**, we decided to use 1-methoxyindole-3-carboxaldehyde (**17**)[14] as the key synthetic intermediate in the synthesis of 1-methoxybrassenin B (**4a**). Aldehyde **17** was prepared by Vilsmeier-Haack formylation of 1-methoxyindole that was obtained by Somei's method, based on Na_2WO_4 catalyzed oxidation of indoline to 1-hydroxyindole with H_2O_2 and subsequent *O*-methylation with dimethyl sulphate.[15] With the main intermediates in hand, we continued in the synthesis of target compounds by oxidation of aldehydes **17**, **12** and **16** to carboxylic acids **18**-**20** (Scheme 3). First we tried $KMnO_4$ as in the synthesis of glucopyranosylindole-3-carboxylic acid.[6a] In this way, 1-methoxyindole-3-carboxylic acid **18** was prepared in 50% yield. However, oxidation with $NaClO_2$ in a mixture of *t*-BuOH and 2-methylbut-2-ene over 39 hours returned an excellent 95% yield in accordance with the literature.[16] Application of a $NaClO_2$ oxidation to α- and β-ribofuranosylindol-3-carbaldehydes **12** and **16** afforded acids **19** and **20** in 91% and 81% yield with shorter reaction times of 8.5 and 5.5 hours respectively. Owing to the low solubility of aldehydes **12** and **16** in *t*-BuOH and 2-methylbut-2-ene, dioxane had to be added to achieve complete dissolution of starting compounds. In the next step, treatment of acids **18**-**20** with PCl_3 afforded the unstable acid chlorides **21**-**23,** which were used in subsequent reactions as crude products. In the synthesis of 1-methoxybrassenin B (**4a**), we examined an approach *via* the acyl isothiocyanate used in the preparation of its demethoxyanalog **4b**.[8] Transformation of acid chloride **21** to acyl isothiocyanate **24** (Scheme 3) proceeded smoothly and the product could be isolated by column chromatography and crystallization or preferably used in the next step as a crude product. Reaction of isothiocyanate **24** with NaSH and methyl iodide afforded dithiocarbamate **25** (23% yield from acid **18**) and thioester **26** (25% yield from acid **18**) as a side product. Methylation of **25** afforded 1-methoxybrassenin B (**4a**, 81%). The efficiency of this method was low, because the important intermediate **25** was formed only in 23% yield. Finally, the target phytoalexin **4a** was advantageously prepared in 47% yield by the acylation of dimethyl carbonimidodithioate hydroiodide[17] with acid chloride **21** in pyridine. This reaction was previously used in the case of pyridine-4-carbonyl chloride[18] and a protected derivative of glucopyranosylindol-3-carbonyl chloride[6a]. Application of this method to ribofuranosyl acid chlorides **22** and **23** afforded the α- and β-anomers of protected ribofuranosyl derivatives of **27**

and **29** in 46 and 39% yield from the acids **19** and **20**, respectively. The target nucleosides derived from 1-methoxybrassenin B were obtained by final deprotection. The removal of the isopropylidene group of **27** with trifluoroacetic acid and subsequent treatment of 5'-*O*-acetyl derivative **28** with sodium methoxide afforded 1-(α-D-ribofuranosyl)brassenin B (**6a**). The deprotection of peracetylated compound **29** with catalytic amount of sodium methoxide in methanol smoothly afforded target compound **6b**.



Scheme 3. (i) $NaClO_2$, $NaH_2PO_4 \cdot 2H_2O$, t-BuOH, 2-methylbut-2-ene, for **17** 39h, 95%[16], for **12** dioxane, 8.5 h, 91%, for **16** dioxane, 5.5 h, 81%. (ii) PCl_3, dry toluene, dry CH_3CN, for **18** and **19** 1 h, for **20** 2.5 h. (iii) KSCN, acetone, 1.5 h. (iv) NaSH, CH_3I, dry THF, DMF, 10 min, 23% of **25** and 25% of **26**. (v) CH_3I, K_2CO_3, dry acetone, 1 h, 81%. (vi) $(SCH_3)_2C{=}NH \cdot HI$, py, for **21** 20 min, 47%, for **22** 1 h, 46%, for **23** 1.5 h, 39%. (vii) TFA, CH_2Cl_2, 20 h, 84%. (viii) MeONa, MeOH, 20 min, 89%. (ix) MeONa, MeOH, 20 min, 83%.

The structures of all nucleoside analogs were confirmed by spectral methods. In their ^1H and ^{13}C NMR spectra, the signals were assigned on the basis of 2D COSY and hetero-correlated HSQC spectra. The structures of α- and β-ribofuranosylindole **11a** and **11b** were determined by NOE experiments depicted on Figure 1 and 2. Irradiation of H-1′ signal enhanced the signal of H-2′ (8.7%) for **11a** and signal H-4′for **11b** (2.0%), in agreement with their respective α- and β-configurations. The observed NOE between H-1′and H-2′, H-3′ (Figure 3) in the 1-(5-*O*-acetyl-α-D-ribofuranosylbrassenin B (**28**) confirmed its α-anomeric structure. The β-anomeric configuration of 1-(β-D-ribofuranosyl)brassenin B (**6b**) was confirmed by interaction between H-1′ and H-4′ (Figure 4). The conformation of the aglycone relative to the sugar moiety was suggested on the basis of strong interaction between H-1′ and H-7 as well as H-1′ and H-2 signals (Figure 1-4).

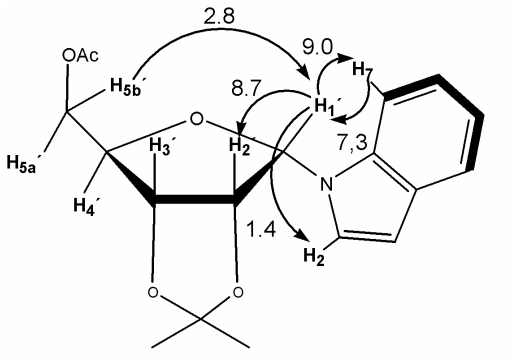


Figure 1. NOE enhancements (%) for **11a.**

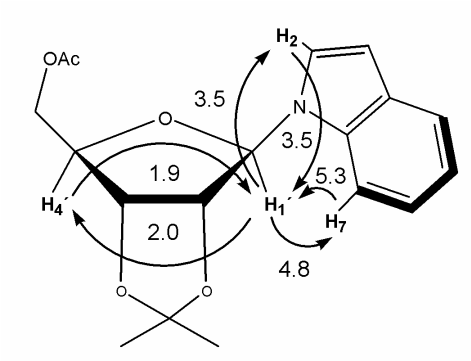


Figure 2. NOE enhancements (%) for **11b.**

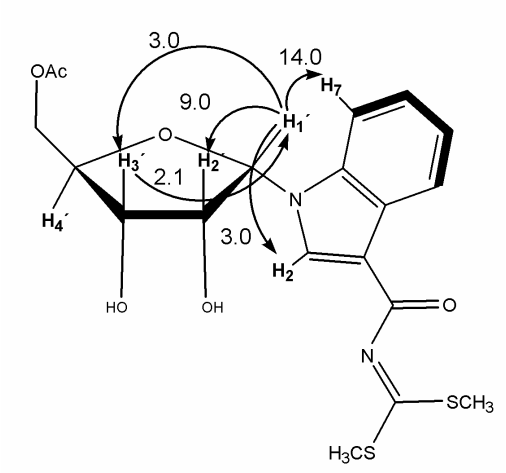


Figure 3. NOE enhancements (%) for **28.**

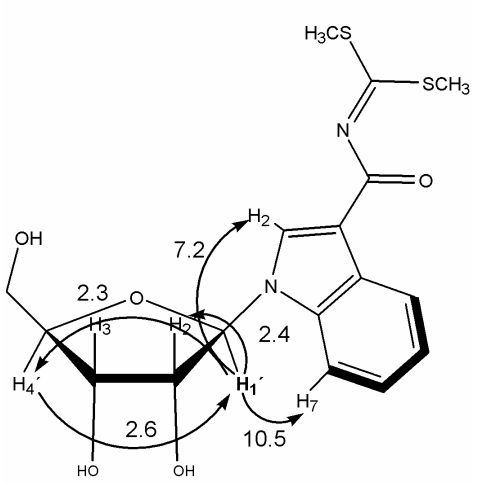
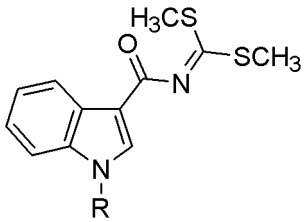


Figure 4. NOE enhancements (%) for **6b.**

It was our final goal to obtain information about antiproliferative activities of the synthesized compounds and compare them with phytoalexin aglycons as well as previously prepared 1-(β-D-glucopyranosyl) derivatives **5a** and **5b**. The antiproliferative activities were examined towards Jurkat cells (acute T-lymphoblastic leukemia), CEM (acute T-lymphoblastic leukemia), CEM-VCR (acute T-lymphoblastic leukemia, VCR-resistant), MCF-7 (mammary gland adenocarcinoma, estrogen receptor expressed), HeLa (cervix carcinoma) by an MTT (thiazolyl blue) test[19] in a culture medium containing the tested chemicals at a concentration of 10^{-4} mol×L^{-1} after 72 h incubation. The determined activity of compounds is given in Table 1 as the percent of living cells compared to solvent control (100%). The highest activity was found with indole phytoalexin 1-methoxybrassenin B (**4a**), whereas its demethoxy analog **4b** was less active in all tested cell lines. Replacement of the methoxy group by a protected glycosyl moiety resulted in a small decrease of activity, with ribofuranosyl derivatives **27** and **29** being generally more active compared to glucopyranosyl derivative **5a**. Nucleoside analogs **5b**, **6a** and **6b** obtained after removal of the protecting groups almost completely lost antiproliferative activity, probably because of decreased lipophilicity. Compounds with a marked activity at 10^{-4} mol×L^{-1} were examined at the concentrations 10^{-5} - 10^{-9} mol×L^{-1}. A weak activity was found only in Jurkat cells, where compounds **4a**, **4b**, **5a** and **29** at a concentration of 10^{-6} mol×L^{-1} inhibited proliferation to 80-90%.

Table 1. Antiproliferative activity[a] of 1-methoxybrassenin B and its analogs



Comp.	R	Jurkat	CEM	CEM-VCR	MCF-7	HeLa
4a	OCH_3	7	5	18	18	17
4b	H	22	26	40	55	41
5a	tetraacetyl-β-D-glucopyranosyl	9	100	62	87	86
5b	β-D-glucopyranosyl	100	100	100	100	100
27	5-*O*-acetyl-2,3-*O*-isopropylidene-α-D-ribofuranosyl	11	4	22	20	43
6a	α-D-ribofuranosyl	90	100	100	90	58
29	triacetyl-β-D-ribofuranosyl	14	32	27	22	38
6b	β-D-ribofuranosyl	70	100	90	90	90

[a]Antiproliferative activity is given as the percent of living cells compared to solvent control (100%) at a concentration 10^{-4} mol×L^{-1} after 72 h incubation.

Experimental Section

General Procedures. Melting points were determined on a Kofler micro melting point apparatus and are uncorrected. IR spectra were recorded on an IR-75 spectrometer (Zeiss Jena). ^{1}H and ^{13}C NMR spectra were measured on a Varian Gemini 2000 spectrometer using TMS as an internal standard. All new compounds were characterized by ^{1}H, ^{13}C NMR as well as ^{1}H-^{1}H and ^{1}H-^{13}C correlation experiments. Specific optical rotations were measured on a digital polarimeter P3002 (Kruess) in a 1 dm cell and are given in 10^{-1}deg×cm^2×g^{-1}; concentration is given in g/100 ml. Microanalyses were performed with a Perkin-Elmer, Model 2400 analyzer. The EI mass spectra were recorded on a Finigan SSQ 700 spectrometer at ionization energy of 70 eV, whereas MALDI-TOF mass spectra were measured on a MALDI IV (Shimadzu, Kratos Analytical). The samples were ionized with a N_2-laser (λ = 337 nm). The progress of chemical reactions was monitored by thin layer chromatography, using Macherey-Nagel plates Alugram®Sil G/UV254. Preparative column chromatography was performed on Kieselgel Merck Typ 9385 (40-63 μm).

1-(2,3-*O*-Isopropylidene-β-D-ribofuranosyl)indoline (9)

To a solution of 2,3-*O*-isopropylidene-D-ribofuranose[20] (**8**, 6.243 g, 32.82 mmol) in anhydrous methanol (10 mL) was added indoline (**7**, 3.68 mL, 3.911g, 32.82 mmol) and reaction mixture was stirred under reflux for 5 h, then concentrated in vacuum and the obtained residue chromatographed on silica gel (150 g, hexane/acetone 3/1) to give **9** (6.1 g, 64%), as light yellow oil. Spectral data were identical with the described compound.[12]

1-(2,3-*O*-Isopropylidene-5-*O*-acetyl-β-D-ribofuranosyl)indoline (10)

To a cooled (0 °C) and stirred solution of 1-(2,3-*O*-isopropylidene-β-D-ribofuranosyl)indoline **9** (6.1 g, 20.9 mmol) in pyridine (10 mL) was added dropwise acetic anhydride (5.2 mL) within 10 min, with cooling to 0 °C. The reaction mixture was stirred for 2 h at room temperature and set aside overnight. Then the obtained solution was poured into water (150 mL), extracted with diethyl ether (2×60 mL), collected organic layers washed with 1M HCl (70 mL), dried over Na_2SO_4, concentrated in vacuum and the obtained residue was subjected to column chromatography on silica gel (80 g, hexane/ethyl acetate 5/1) to give **10** (5.1 g, 73%) as light yellow oil. $[\alpha]_D^{20}$ –51.9 (*c* 0.37, $CHCl_3$); IR ($CHCl_3$) λ (cm^{-1}) 1733 (C=O); ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm) 7.09-7.06 (m, 2H, $H_{arom.}$), 6.76- 6.72 (m, 2H, $H_{arom.}$), 5.41 (d, $J_{1',2'}$ = 3.7 Hz, 1H, H-1′), 4.83 (dd, $J_{2',1'}$ = 3.7 Hz, $J_{2',3'}$ = 6.6 Hz, 1H, H-2′), 4.59 (dd, $J_{3',2'}$ = 6.6 Hz, $J_{3',4'}$ = 3.9 Hz, 1H, H-3′), 4.32-4.17 (m, 3H, H-4′, H-5′$_a$, H-5′$_b$), 3.53 (t, *J* = 8.4 Hz, 2H, $CH_{2\ indoline}$), 3.01 (t, *J* = 8.4Hz, 2H, $CH_{2\ indoline}$), 2.08 (s, 3H, CH_3CO), 1.59 (s, 3H, $C(CH_3)_2$), 1.37 (s, 3H, $C(CH_3)_2$); ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm) 170.87 ($CH_3\underline{C}O$), 149.75 (q), 130.60 (q), 127.47, 124.97, 119.64 and 108.90 ($C_{arom.}$), 114.76 ($\underline{C}(CH_3)_2$), 93.29 (C-1′), 81.63 (C-2′), 80.84 (C-3′), 80.46 (C-4′), 64.32 (C-5′), 47.51 and 28.52 (C-2, C-3), 27.59 and 25.69 ($C(\underline{C}H_3)_2$), 21.02 ($\underline{C}H_3CO$); EI MS, *m/z* (%): 333 [M^+] (100), 173 (16), 147 (90), 119 (21), 118 (44), 68 (16), 43 [Ac^+] (62); Anal. Calcd for $C_{18}H_{23}NO_7$ (333.4): C 64.85 H 6.95 N 4.20. Found: C 65.02 H 6.71 N 4.38.

Oxidation of 10 with DDQ

To a solution of **10** (3.622 g, 10.86 mmol) in anhydrous toluene (57 ml) was added dropwise within 15 min a solution of DDQ (2.7 g, 11.95 mmol) in anhydrous toluene (57 ml). Reaction mixture was stirred for 10 min at room temperature and then poured into 4% aqueous solution of K_2CO_3 (100 mL). The product was extracted with $CHCl_3$ (100 and 50 mL). The combined extracts were washed with water (100 and 50 mL), dried over Na_2SO_4 and concentrated under reduced pressure. Chromatography on silica gel (75 g, cyclohexane/ethyl acetate 20/1) of the obtained residue afforded compounds **11a** (1.627 g, 45%) and **11b** (0.75 g, 21%) as colorless oils**.**

1-(2,3-*O*-Isopropylidene-5-*O*-acetyl-α-D-ribofuranosyl)indole (11a)

R_f = 0.34 (cyclohexane/acetone 4/1); $[\alpha]_D^{20}$ –70.5 (*c* 0.28, $CHCl_3$); IR ($CHCl_3$) λ (cm^{-1}) 1740 (C=O); ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm) 7.61 (d, $J_{4,5}$ = 7.5 Hz, 1H, H-4), 7.52 (d, $J_{2,3}$ = 3.3 Hz, 1H, H-2), 7.33 (d, $J_{7,6}$ = 8.2 Hz, 1H, H-7), 7.20 (ddd, $J_{6,7}$ = 8.0 Hz, $J_{6,5}$ = 7.1, *J* = 1.1 Hz, 1H, H-6), 7.12 (ddd, $J_{5,4}$ = 7.9 Hz, $J_{5,6}$ = 6.9 Hz, *J* = 1.1 Hz, 1H, H-5), 6.56 (d, $J_{3,2}$ = 3.3 Hz, 1H, H-

3), 6.28 (d, $J_{1',2'}$ = 3.9 Hz, 1H, H-1′), 4.87 (dd, $J_{2',1'}$ = 3.8 Hz, $J_{2',3'}$ = 6.0 Hz, 1H, H-2′), 4.81 (dd, $J_{3',2'}$ = 6.0 Hz, $J_{3',4'}$ = 1.1 Hz, 1H, H-3′), 4.53 (t, J = 4.4 Hz, 1H, H-4′), 4.34 (dd, $J_{5'b,\ 5'a}$ = 11.9 Hz, $J_{5'b,\ 4'}$ = 5.3 Hz, 1H, H-5′$_b$), 4.19 (dd, $J_{5'a,\ 5'b}$ = 11.9 Hz, $J_{5'a,\ 4'}$ = 4.2 Hz, 1H, H-5′$_a$), 2.16 (s, 3H, CH_3CO), 1.53 (s, 3H, $C(CH_3)_2$), 1.31 (s, 3H, $C(CH_3)_2$); ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm) 170.61 ($CH_3\underline{C}O$), 136.11 (q), 128.98 (q), 127.07, 121.94, 121.27, 120.33, 109.23 and 102.87 ($C_{arom.}$), 114.10 ($\underline{C}(CH_3)_2$), 86.59 (C-1′), 82.08 (C-3′), 80.30 (C-4′), 80.04 (C-2′), 64.58 (C-5′), 26.21 and 24.67 ($C(\underline{C}H_3)_2$), 21.17 ($\underline{C}H_3CO$); Difference NOE spectra ($CDCl_3$): irr. at δ 6.28 (H-1′) enhanced signals (H-2′, 8.7 %) and (H-7, 9.0 %) and (H-2, 1.4 %), irr. at δ 7.33 (H-7) enhanced signals (H-1′, 7.3 %); EI MS, *m/z* (%): 331 [M^+] (100), 145 (26), 117 (32), 43 [Ac^+] (58); Anal. Calcd for $C_{18}H_{21}NO_7$ (331.4): C 65.24 H 6.39 N 4.23. Found: C 65.51 H 6.02 N 4.49.

1-(2,3-*O*-Isopropylidene-5-*O*-acetyl-β-D-ribofuranosyl)indole (11b)

R_f = 0.41 (cyclohexane/acetone 4/1); $[\alpha]_D^{20}$ –49.4 (*c* 0.71, $CHCl_3$); IR ($CHCl_3$) λ (cm^{-1}) 1740 (C=O); ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm) 7.62 (d, $J_{4,5}$ = 7.7 Hz, 1H, H-4), 7.48 (d, $J_{7,6}$ = 8.1 Hz, 1H, H-7), 7.24 (ddd, $J_{6,7}$ = 8.1 Hz, $J_{6,5}$ = 7.3 Hz, J = 1.1 Hz, 1H, H-6), 7.20 (d, $J_{2,3}$ = 3.1 Hz, 1H, H-2), 7.17-7.12 ($J_{5,4}$ = 7.9 Hz, $J_{5,6}$ = 7.0 Hz, J = 1.0 Hz, 1H, H-5), 6.55 (d, $J_{3,2}$ = 3.3 Hz, 1H, H-3), 6.08 (d, $J_{1',2'}$ = 3.1 Hz, 1H, H-1′), 4.97 (dd, $J_{2',1'}$ = 2.9 Hz, $J_{2',3'}$ = 6.6 Hz, 1H, H-2′), 5.84 (dd, $J_{3',2'}$ = 6.6 Hz, $J_{3',4'}$ = 3.8 Hz, 1H, H-3′), 4.39-4.34 (m, 2H, H-4′, H-5′$_b$), 4.22 (dd, $J_{5'a,\ 5'b}$ = 13.0 Hz, $J_{5'a,4'}$ = 5.7 Hz, 1H, H-5′$_a$), 2.08 (s, 3H CH_3CO), 1.64 (s, 3H, $C(CH_3)_2$), 1.36 (s, 3H, $C(CH_3)_2$); ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm) 170.76 ($CH_3\underline{C}O$), 135.48 (q), 129.68 (q), 124.84, 122.37, 121.42, 120.65, 110.48 and 103.53 ($C_{arom.}$), 115.23 ($\underline{C}(CH_3)_2$), 92.08 (C-1′), 84.60 (C-3′), 82.45 (C-4′), 80.90 (C-2′), 63.98 (C-5′), 27.46 and 25.58 ($C(\underline{C}H_3)_2$), 20.98 ($\underline{C}H_3CO$); Difference NOE spectra ($CDCl_3$): irr. at δ 6.08 (H-1′) enhanced signals (H-7, 4.8 %) and (H-2, 3.5 %) and (H-4′, 2 %), irr. at δ 7.20 (H-2) enhanced signals (H-3, 3.9 %) and (H-1′, 3.5 %) and (H-2′, 2.2 %), irr. at δ 7.48 (H-7) enhanced signals (H-1′, 5.3 %) and (H-2′, 2.0 %) and (H-3′, 1.3 %); EI MS, *m/z* (%): 331 [M^+] (100), 145 (22), 117 (26), 69 (13), 43 [Ac^+] (50); Anal. Calcd for $C_{18}H_{21}NO_7$ (331.4): C 65.24 H 6.39 N 4.23. Found: C 65.53 H 6.18 N 3.96.

1-(2,3-*O*-Isopropylidene-5-*O*-acetyl-α-D-ribofuranosyl)indole-3-carboxldehyde (12)

To a solution of indole **11a** (2.57 g, 7.76 mmol) in dry DMF (8.2 mL) cooled to 0 °C was added dropwise a mixture of $POCl_3$ (2.06 mL, 3.45 g, 22.50 mmol) in dry DMF (6.5 mL) prepared at 0 °C. Reaction mixture was stirred for 20 min at room temperature, until the starting material was consumed (TLC monitoring, eluent cyclohexane/ethyl acetate 3/1). Reaction mixture was poured into crushed ice (60 mL), neutralized with 4% aqueous solution of K_2CO_3 (330 ml), extracted with diethyl ether (3×60 mL) and dried over Na_2SO_4. After removal of the solvent, the residue was crystallized from propan-2-ol to give **12** (2.40 g, 86%) as colorless crystals. $[\alpha]_D^{20}$ –87.9 (*c* 1.14, $CHCl_3$); mp 101-103 °C; IR ($CHCl_3$) λ (cm^{-1}) 1743 a 1657 (C=O); ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm) 10.04 (s, 1H, CHO), 8.33 –8.31 (m, 1H, H-4), 8.09 (s, 1H, H-2), 7.35-7.30 (m, 3H, H-5, H-6, H-7), 6.30 (d, $J_{1',2'}$ = 4.1 Hz, 1H, H-1′), 4.96 (dd, $J_{2',1'}$ = 4.1 Hz, $J_{2',3'}$ = 5.9 Hz, 1H, H-2′), 4.86 (d, $J_{3',2'}$ = 6.0 Hz, 1H, H-3′), 4.65 (t, J = 4.4 Hz, 1H, H-4′), 4.37 (dd, $J_{5'b,\ 5'a}$ = 12.0 Hz, $J_{5'b,\ 4'}$ = 5.0 Hz, 1H, H-5′$_b$), 4.23 (dd, $J_{5'a,\ 5'b}$ = 12.0 Hz, $J_{5'a,\ 4'}$ = 3.9 Hz, 1H, H-5′$_a$), 2.17 (s, 3H, CH_3CO), 1.40 (s, 3H, $C(CH_3)_2$), 1.29 (s, 3H, $C(CH_3)_2$); ^{13}C NMR (75 MHz, $CDCl_3$) δ

(ppm) 185.12 (CHO), 170.29 ($CH_3\underline{C}O$), 137.80 (C-2), 136.54 (q, $C_{arom.}$), 125.26 (q, $C_{arom.}$), 124.17, 123.28 and 109.57 (C-5, C-6, C-7), 122.43 (C-4), 118.92 (q, $C_{arom.}$), 114.20 ($\underline{C}(CH_3)_2$), 87.10 (C-1′), 81.97 (C-3′), 80.57 (C-4′), 79.90 (C-2′), 64.42 (C-5′), 25.86 and 24.42 ($C(\underline{C}H_3)_2$), 21.00 ($\underline{C}H_3CO$); EI MS, *m/z* (%): 359 [M^+] (100), 215 (22), 144 (15), 69 (16), 43 [Ac^+], (62); Anal. Calcd for $C_{19}H_{21}NO_6$ (359.4): C 63.50 H 5.89 N 3.90. Found: C 63.21 H 6.17, N 3.75.

1-(2,3,5-Tri-*O*-acetyl-β-D-ribofuranosyl)indoline (14)

To a suspension of peracetylated β-D-ribofuranose **13** (2 g, 6.28 mmol) in dry ethanol (16 ml) were added indoline **7** (2.25 g, 2.11 mL, 18.84 mmol) and 97% acetic acid (0.25 mL). Reaction mixture was refluxed for 6 h, solvent evaporated and the residue chromatographed on silica gel (200 g, hexane/ethyl acetate 3/1; after removal of unreacted indoline; eluent was changed to hexane/ethyl acetate 1/1) to give **14** (1.48 g, 63%) as light yellow oil. $[\alpha]_D^{20}$ –35.4 (*c* 0.37, $CHCl_3$); IR ($CHCl_3$) λ (cm^{-1}) 1753 (C=O); ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm) 7.10 – 7.05 (m, 2H, $H_{arom.}$), 6.76 – 6.70 (m, 2H, $H_{arom.}$), 5.64 (d, $J_{1',2'}$ = 7.3 Hz, 1H, H-1′), 5.45 (dd, $J_{2',1'}$ = 6.9 Hz, $J_{2',3'}$ = 5.8 Hz, 1H, H=2′), 5.28 (dd, $J_{3',2'}$ = 5.2 Hz, $J_{3',4'}$ = 2.3 Hz, 1H, H-3′), 4.26 – 4.21 (m, 3H, H-4′, H-5′$_a$, H-5′$_b$), 3.59 (t, *J* = 9.6 Hz, 2H, $CH_{2\ indoline}$); 3.02 (t, *J* = 9.5 Hz, 2H, $CH_{2\ indoline}$), 2.14 (s, 3H, CH_3CO), 2.10 (s, 3H, CH_3CO), 2.09 (s, 3H, CH_3CO); ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm) 170.48 ($CH_3\underline{C}O$), 169.89 ($CH_3\underline{C}O$), 169.75 ($CH_3\underline{C}O$), 149.55 (q), 130.24 (q), 127.35, 125.04, 119.65 and 107.94 ($C_{arom.}$), 87.66 (C-1′), 77.93 and 64.01 (C-4′, C-5′), 71.29 (C-3′), 69.77 (C-2′), 45.15 and 28.14 (C-2, C-3), 20.82 ($\underline{C}H_3CO$), 20.74 ($\underline{C}H_3CO$), 20.63 ($\underline{C}H_3CO$); EI MS, *m/z* (%): 377 [M^+] (82), 304 (28), 259 (22), 174 (27), 148 (97), 139 (46), 118 (32), 43 [Ac^+] (100); Anal. Calcd for $C_{19}H_{23}NO_7$ (377.4): C 60.47 H 6.14 N 3.71. Found: C 60.12 H 6.03 N 3.95.

1-(2,3,5-Tri-*O*-acetyl-β-D-ribofuranosyl)indole (15)

To a solution of indoline **14** (1.48 g, 3.93 mmol) in anhydrous toluene (20 mL) was added dropwise a solution of DDQ (0.98 g, 4.32 mmol) in dry toluene (20 mL) within 10 min. Reaction mixture was stirred at room temperature for 1 h and then poured into 4% aqueous solution of K_2CO_3 (40 mL). The product was extracted with $CHCl_3$ (2×30 mL), combined extracts washed with water (2×75 mL), dried over Na_2SO_4 and concentrated in vacuum. The crude product **15** (1.45 g, 98%) was used in the next reaction as colorless oil. $[\alpha]_D^{20}$ –9.5 (*c* 0.28, $CHCl_3$); IR ($CHCl_3$) λ (cm^{-1}) 1753 (C=O); ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm) 7.62 (d, *J* = 7.9 Hz, 1H) and 7.50 (d, *J* = 8.1 Hz, 1H, H-4, H-7), 7.28 (d, $J_{2,3}$ = 3.3 Hz, 1H, H-2); 7.25-7.13 (m, 2H, H-5, H-6), 6.60 (d, $J_{3,2}$ = 3.2 Hz, 1H, H-3), 6.18 (d, $J_{1',2'}$ = 6.3 Hz, 1H, H-1′), 5.59 (dd, $J_{2',1'}$ = 6.1 Hz, $J_{2',3'}$ = 5.8 Hz, 1H, H-2′), 5.47 (dd, $J_{3',2'}$ = 5.4 Hz, $J_{3',4'}$ = 3.3 Hz, 1H, H-3′), 4.37 (m, 3H, H-4′, H-5′$_a$, H-5′$_b$), 2.16 (s, 6H, 2 x CH_3CO), 2.03 (s, 3H, CH_3CO); ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm) 170.43 ($CH_3\underline{C}O$), 169.73 ($CH_3\underline{C}O$), 169.43 ($CH_3\underline{C}O$), 136.18 (q), 129.33 (q), 123.90, 122.42, 121.30, 120.69, 109.81 a 104.32 ($C_{arom.}$), 86.81 (C-1′), 79.49 and 63.46 (C-4′, C-5′), 73.05 (C-3′), 70.79 (C-2′), 20.85 ($\underline{C}H_3CO$), 20.68 ($\underline{C}H_3CO$), 20.44 ($\underline{C}H_3CO$); EI MS, *m/z* (%): 375 [M^+] (52), 259 (24), 139 (29), 117 (18), 97 (15), 43 [Ac^+] (100); Anal. Calcd for $C_{19}H_{21}NO_7$ (375.4): C 60.79 H 5.64 N 3.73. Found: C 60.98 H 5.40 N 3.51.

1-(2,3,5-Tri-*O*-acetyl-β-D-ribofuranosyl)indole-3-carboxaldehyde (16)

To a solution of indole **15** (1.45 g, 3.86 mmol) in dry DMF (4 mL) cooled to 0 °C was added dropwise a mixture of $POCl_3$ (1.7 g, 1.02 mL, 11.1 mmol) in dry DMF (3.4 mL) prepared at 0 °C. Reaction mixture was stirred for 15 min at 0 °C and 1 h at room temperature, then poured into crushed ice (100 ml), neutralized with 4% aqueous solution of K_2CO_3 (160 mL), extracted with ethyl acetate (2×50 mL) and dried over Na_2SO_4. After removal of the solvent, the residue was crystallized from diethyl ether to give **16** (0.96 g, 61%) as colorless crystals. $[\alpha]_D^{20}$ 53.1 (*c* 0.89, $CHCl_3$); mp 107-108 °C; IR ($CHCl_3$) λ (cm^{-1}) 1740 and 1653 (C=O); ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm) 10.06 (s, 1H, CHO), 8.33 –8.30 (m, 1H, H-4), 8.03 (s, 1H, H-2), 7.55 (dd, $J_{7,6}$= 7.2 Hz, J =1.1 Hz, 1H, H-7), 7.37-7.34 (m, 2H, H-5,H-6), 6.17 (d, $J_{1',2'}$ = 5.3 Hz, 1H, H-1′), 5.56 (t, J = 5.4 Hz, 1H, H-2′), 5.44 (dd, $J_{3',2'}$ = 5.2 Hz, $J_{3',4'}$ = 5.0 Hz, 1H, H-3′), 4.50-4.43 (m, 3H, H-4′, H-5′$_a$, H-5′$_b$), 2.18 (s, 3H, CH_3CO), 2.16 (s, 3H, CH_3CO), 2.10 (s, 3H, CH_3CO); ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm) 184.81 (CHO), 170.25 ($CH_3\underline{C}O$), 169.60 ($CH_3\underline{C}O$), 169.31 ($CH_3\underline{C}O$), 136.59 (q, $C_{arom.}$), 134.49 (C-2); 125.79 (q, $C_{arom.}$), 124.63 and 123.68 (C-5, C-6), 122.38 (C-4), 119.81 (q, $C_{arom.}$), 110.44 (C-7), 87.67 (C-1′), 80.09 (C-4′), 73.74 (C-2′), 70.18 (C-3′), 62.85 (C-5′), 20.85 ($\underline{C}H_3CO$), 20.59 ($\underline{C}H_3CO$), 20.45 ($\underline{C}H_3CO$); EI MS, *m/z* (%): 403 [M^+] (37), 259 (44), 144 (11),139 (36), 97 (16), 43 [Ac^+] (100); Anal. Calcd for $C_{20}H_{21}NO_8$ (403.4): C 59.55 H 5.25 N 3.47. Found: C 59.37 H 5.48 N 3.27.

1-(2,3-*O*-Isopropylidene-5-*O*-acetyl-α-D-ribofuranosyl)indole-3-carboxylic acid (19)

A solution of 80% $NaClO_2$ (3.46 g, 30.61 mmol) and $NaH_2PO_4.2H_2O$ (3.58 g, 22.95 mmol) in water (15 mL) was added at 0 °C to a suspension of aldehyde **12** (0.55 g, 1.53 mmol) in *tert*-butyl alcohol (15 mL) and 2-methylbut-2-ene (15 mL). Dioxane (5 mL) was added to dissolve starting aldehyde **12** and the mixture was stirred at room temperature for 8.5 h (TLC, monitoring, eluent cyclohexane/acetone 2/1). After dilution with water (45 mL), the product was extracted with chloroform (2×30 ml), extract washed with brine (60 mL) and water (40 mL), dried over Na_2SO_4, solvent evaporated under reduced pressure and the residue submitted to chromatography on silica gel (15 g, cyclohexane/acetone 2/1) to give **19** (0.52 g, 91%) as colorless amorphous compound. $[\alpha]_D^{20}$ –55.2 (*c* 0.96, $CHCl_3$); mp 146-149°C; IR ($CHCl_3$) λ (cm^{-1}) 3153 (O-H), 1740 and 1663 (C=O); ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm) 8.28 (s, 1H, H-2), 8.26 –8.23 (m, 1H, H-4), 7.34-7.28 (m, 3H, H-5, H-6, H-7), 6.30 (d, $J_{1',2'}$ = 4.2 Hz, 1H, H-1′), 4.94 (dd, $J_{2',1'}$ = 4.2 Hz, $J_{2',3'}$ = 6.0 Hz, 1H, H-2′), 4.85 (dd, $J_{3',2'}$ = 6.0 Hz, $J_{3',4'}$ = 0.8 Hz, 1H, H-3′), 4.65 (t, 1H, J = 4.2 Hz, H-4′), 4.37 (dd, $J_{5'b,\ 5'a}$ = 12.0 Hz, $J_{5'b,\ 4'}$ = 4.9 Hz, 1H, H-5′$_b$), 4.23 (dd, $J_{5'a,\ 5'b}$ = 12.0 Hz, $J_{5'a,\ 4'}$ = 3.9 Hz, 1H, H-5′$_a$), 2.18 (s, 3H, CH_3CO), 1.44 (s, 3H, $C(CH_3)_2$), 1.29 (s, 3H, $C(CH_3)_2$); ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm) 170.51 and 170.43 (COOH and $CH_3\underline{C}O$), 136.15 (q, $C_{arom.}$), 134.89 (C-2), 127.03 (q, $C_{arom.}$), 123.20, 122.69, 122.21 (C-6, C-5, C-4), 114.44 ($\underline{C}(CH_3)_2$), 109.73 (C-7), 107.63 (q, $C_{arom.}$), 87.30 (C-1′), 82.16 (C-3′), 80.55 (C-4′), 80.10 (C-2′), 64.77 (C-5′), 25.99 and 24.68 ($C(\underline{C}H_3)_2$), 21.24 ($\underline{C}H_3CO$); MALDI-TOF MS, *m/z* (%): 414 [M+K]$^+$ (24), 398.3 [M+Na]$^+$ (58), 375.8 [M+H]$^+$ (88), 358 [M-H_2O]$^+$ (100); Anal. Calcd for $C_{19}H_{21}NO_7$ (375.4): C 60.79 H 5.64 N 3.73. Found: C 60.52 H 5.79 N 3.59.

1-(2,3,5-Tri-*O*-acetyl-β-D-ribofuranosyl)indole-3-carboxylic Acid (20)

A solution of 80% $NaClO_2$ (4.52 g, 40 mmol) and $NaH_2PO_4.2H_2O$ (4.68 g, 30 mmol) in water (20 mL) was added at 0 °C to a suspension of aldehyde **16** (0.81 g, 2 mmol) in *tert*-butyl alcohol (20 mL) and 2-methylbut-2-ene (20 mL). Dioxane (10 mL) was added to dissolve starting aldehyde **16** and the mixture was stirred at room temperature for 5.5 h. After dilution with water (60 mL), the product was extracted with chloroform (2 x 40 mL), extract washed with brine (60 mL) and water (60 mL), dried over Na_2SO_4, and solvent was evaporated under reduced pressure. Residue was crystallized from ethyl acetate/hexane to give **20** (0.68 g, 81%) as colorless crystals. $[\alpha]_D^{20}$ –44.41 (*c* 0.97, $CHCl_3$); mp 174-177 °C; IR ($CHCl_3$) λ (cm^{-1}) 3126 (O-H), 1740 and 1663 (C=O); ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm) 8.26 (s, 1H, H-2), 8.25-8.24 (m, 1H, H-4.), 7.55 (dd, 1H, J = 6.7 Hz, J = 1.8 Hz, 1H, H-7), 7.35-7.33 (m, 2H, H-5, H-6), 6.19 (d, $J_{1',2'}$ = 5.0 Hz, 1H, H-1′), 5.57 (t, J = 5.2 Hz, 1H, H-2′); 5.47 (dd, $J_{3',2'}$ = 5.2 Hz, $J_{3',4'}$ = 4.9 Hz, 1H, H-3′), 4.47-4.43 (m, 3H H-4′, H-5′$_a$, H-5′$_b$), 2.27 (s, 3H, CH_3CO), 2.15 (s, 3H, CH_3CO), 2.11 (s, 3H CH_3CO); ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm) 170.56, 169.92, 169.60 and 169.34 (COOH, 3 x $CH_3\underline{C}O$); 136.15 (q, $C_{arom.}$), 131.91, 127.24 (q, $C_{arom.}$), 123.68, 123.04, 122.20 and 110.35 ($C_{arom.}$), 108.77 (q, $C_{arom.}$), 87.65 (C-1′), 80.02 (C-4′), 74.00 (C-3′), 70.18 (C-2′), 62.71 (C-5′), 20.83 ($\underline{C}H_3CO$), 20.60 ($\underline{C}H_3CO$), 20.47 ($\underline{C}H_3CO$); EI MS, *m/z* (%): 419 [M^+] (23), 259 (48), 144 (12), 139 (45), 97 (20), 43 [Ac^+] (100); Anal. Calcd for $C_{20}H_{21}NO_9$ (419.4): C 57.28 H 5.05 N 3.34. Found: C 57.49 H 4.82 N 3.46.

General procedure for the preparation of acid chlorides 21-23

To a suspension of acid (0.5 mmol of **18,** or 1 mmol of **19** or **20**) in dry toluene (2 mL, or 8 mL) and dry acetonitrile (0.3 mL, or 1.2 mL) was added phosphorus trichloride (0.5 mmol for **18**, 1 mmol for **19** and 2 mmol for **20**) and the mixture was stirred at room temperature for 1 h (**18** and **19**) or 2.5 h (**20**). The resulting solution was decanted from phosphorus acid deposited on the flask walls, the flask washed with dry toluene (3 ml) and obtained solution concentrated to approximately ¼ of its original volume to remove the excess of phosphorus trichloride. The obtained solution of unstable crude product was immediately used in the next reaction.

1-Methoxyindole-3-ylcarbonyl Isothiocyanate (24)

To a solution of crude chloride **21** freshly prepared from 0.5 mmol of acid **18** was added a solution of KSCN (0.048 g, 0.5 mmol) in dry acetone (1 mL). The reaction mixture was stirred at room temperature for 1 h, solvent evaporated and the obtained crude isothiocyanate was immediately used in next reaction or isolated by flash chromatography on silica gel (5g, cyclohexane/acetone 3/1) to give **24** (0.042 g, 36%) as unstable colorless crystals, mp 53-60 °C (acetone/hexane). IR ($CHCl_3$) λ (cm^{-1}) 1966 (NCS), 1680 (C=O).

Reaction of 24 with NaSH and CH_3I

To a solution of crude isothiocyanate **24,** obtained from 0.5 mmol of acid **18,** in dry THF (2 mL) and dry DMF (0.5 mL) was added methyl iodide (0.16 g, 0.07 mL, 1.1 mmol) and then $NaSH\times H_2O$ (0,148 g; cca 2 mmol) under cooling with cold water. Reaction mixture was stirred

at room temperature for 10 min, then poured into water (10 mL), extracted with ethyl acetate (3×15 mL), collected extracts washed with brine (2×15 mL), dried over Na_2SO_4 and evaporated. Chromatography on silica gel (30 g, hexane/ethyl acetate 3/1) of the obtained residue afforded compounds **25** (0.033 g, 23% from acid **18**) as light yellow crystals and **26** (0,028 g, 25% from acid **18**) as colorless crystals.

1-Methoxyoxobrassinin (25). R_f = 0,46 (hexane/ethyl acetate 3/1); mp = 145-455 °C (acetone/hexane); ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm) 10.02 (s, 1H, NH), 8.19 (d, *J* = 7.3 Hz, 1H, $H_{arom.}$), 7.99 (s, 1H, H-2), 7.53 (d, *J* = 7.3 Hz, 1H, $H_{arom.}$), 7.36-7.40 (m, 2H, $H_{arom.}$), 4.20 (s, 3H, OCH_3), 2.72 (s, 3H SCH_3); ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm) 205.2 (C=S), 160.1 (C=O), 132.6 (q), 126.9, 124.6, 123.6, 122.4 (q), 121.2, 109.4 and 104.8 (q) ($C_{arom.}$), 67.2 (OCH_3), 20.8 (SCH_3); EI MS, *m/z* (%): 280 $[M+H]^+$ (4), 249 (12), 232 (34), 174 (100), 143 (38), 115 (43), 159 (43), 47 (42); Anal. Calcd for $C_{12}H_{12}N_2O_2S_2$ (280,37): C 51.41 H 4.31 N 9.99. Fond: C 51.16 H 4.58 N 10.23.

Methyl 1-methoxyindole-3-carbothioate (26). R_f = 0,61 (hexane/ethyl acetate 3/1); mp 65-67 °C (diethyl ether/petroleum ether); IR ($CHCl_3$) λ (cm^{-1}) 1633 (C=O); ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm) 8.29 (dd, *J* = 6.9 Hz, *J* = 1.9 Hz, 1H, $H_{arom.}$), 8.04 (s, 1H H-2), 7.48 (dd, *J* = 6.6 Hz, *J* = 1.9 Hz, 1H, $H_{arom.}$), 7.31-7.35 (m, 2H, $H_{arom.}$), 4.17 (s, 3H, OCH_3), 2.51 (s, 3H, SCH_3); ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm) 185.2 (C=O), 132.3 (q), 127.4, 124.1, 123.2, 122.3, 121.9 (q), 112.3 (q) and 108.8 ($C_{arom.}$), 67.0 (OCH_3), 11.2 (SCH_3); EI MS, *m/z* (%): 221 [M+H] (19), 174 (100), 159 (54), 143 (47), 115 (39), 114,88 (16), 63 (11), 62 (15), 47 (15), 45 (18), 43 (12), 32 (49); Anal. Calcd for $C_{11}H_{11}N_2O_2S$ (221.28): C 59.71 H 5.01 N 6.33. Found: C 59.40 H 5.27 N 6.58.

Synthesis of 1-methoxybrassenin B (4a) by methylation of 25

To the solution of **25** (0.033 g, 0.12 mmol) in dry acetone (1 mL) was added methyl iodide (0.022 mL, 0.5 g, 0.36 mmol) and powdered K_2CO_3 (0.017 g, 0.12 mmol) and mixture was stirred at room temperature for 1 h. Reaction mixture was then poured into cold water (20 mL), extracted with ethyl acetate (10 and 2×5 mL) and dried over Na_2SO_4. After evaporation of solvent the residue was crystallized from ethyl acetate/hexane to give **4a** (0.028 g, 81%) as colorless crystals, mp 71-73 °C; lit.[7] 73-74 °C, spectral data are identical with natural 1-methoxybrassenin B.[7]

General procedure for preparation of 1-methoxybrassenin B (4a) and its analogs 27, 29

To a stirred solution of crude acid chloride **21**, **22** or **23** freshly prepared from 0.5 mmol of acid **18**, or 1 mmol of **19** or **20**, was added a solution of dimethyl carbonimidothioate hydroiodide (0.5 mmol for **4a** and 1 mmol for **27** or **29**) in pyridine (2 mL or 8 mL) and reaction mixture was stirred at room temperature for 30 min (**4a**), 1 h (**27**) and 1.5 h (**29**). After pouring into water (50 mL or 150 mL), the product was extracted with diethyl ether (3×20 mL or 3×75 mL), the extract washed with saturated solution $NaHCO_3$ (3×60 mL or 3×70 mL), dried over Na_2SO_4, solvent evaporated and the residue chromatographed on silica gel.

1-Methoxybrassenin B (4a). After chromatography on silica gel (12 g, hexane/ethyl acetate 3/1) and crystallization from ethyl acetate/hexane; compound **4a** (0,069 g, 47% from acid **18**) was obtained as colorless crystals, mp 71-73 °C; lit.[7] 73-74 °C; spectral data are identical with the natural 1-methoxybrassenin B.[7]

1-(2,3-*O*-Isopropylidene-5-*O*-acetyl-α-D-ribofuranosyl)brassenin B (27). After chromatography on silica gel (20 g, hexane/ethyl acetate 2/1) compound **27** (0,22 g, 46% from acid **19**) was isolated as colorless amorphous substance. $[\alpha]_D^{20}$ –13.1 (*c* 0.81, $CHCl_3$); mp 45-50 °C; IR ($CHCl_3$) λ (cm^{-1}) 1737 a 1620 (C=O); ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm) 8.36 (dd, *J* = 6.6 Hz, *J* = 1.1 Hz, 1H, H-4), 8.21 (s, 1H, H-2), 7.36-7.27 (m, 3H, H-5, H-6, H-7), 6.30 (d, $J_{1',2'}$ = 3.8 Hz, 1H, H-1′), 4.92 (dd, $J_{2',1'}$ = 4.1 Hz, $J_{2',3'}$ = 6.0 Hz, 1H, H-2′), 4.85 (dd, $J_{3',2'}$ = 6.0 Hz, $J_{3',4'}$ = 0.8 Hz, 1H, H-3′), 4.63 (t, *J* = 4.4 Hz, 1H, H-4′), 4.37 (dd, $J_{5'b,\,5'a}$ = 12.1 Hz, $J_{5'b,\,4'}$ = 5.2 Hz, 1H, H-5′$_b$), 4.21 (dd, $J_{5'a,\,5'b}$ = 12.1 Hz, $J_{5'a,\,4'}$ = 4.0 Hz, 1H, H-5′$_a$), 2.58 (s, 6H, 2 x SCH_3), 2.17 (s, 3H, CH_3CO), 1.46 (s, 3H, $C(CH_3)_2$) and 1.29 (s, 3H, $C(CH_3)_2$); ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm) 174.36 and 172.94 (O=C-N=C), 170.39 ($CH_3\underline{C}O$), 136.52 (q, $C_{arom.}$) 135.02 (C-2), 127.10 (q, $C_{arom.}$), 123.29, 122.68, 122.61 (C-6, C-5, C-4), 114.22 ($\underline{C}(CH_3)_2$), 113.81 (q, $C_{arom.}$), 109.45 (C-7), 86.95 (C-1′), 82.13 (C-3′), 80.42 (C-4′), 80.01 (C-2′), 64.59 (C-5′), 26.04 and 24.66 ($C(\underline{C}H_3)_2$), 21.22 ($\underline{C}H_3CO$), 16.14 (double intensity, SCH_3); Difference NOE spectra ($CDCl_3$): irr. at δ 6.30 (H-1′) enhanced signals (H-2′, 11%) and (H-7, 14%) and (H-2, 2%), irr. at δ 4.92 (H-2′) enhanced signals (H-1′, 10%); MALDI-TOF MS, *m/z* (%): 517.5 $[M+K]^+$ (28), 501.4 $[M+Na]^+$ (14), 479 $[M+H]^+$ (9), 357.7 $[M-NC(SCH_3)_2]^+$ (100); Anal. Calcd for $C_{22}H_{26}N_2O_7S_2$ (478.6): C 55.21 H 5.48 N 5.85. Found: C 55.48 H 5.37 N 5.63.

1-(2,3,5-Tri-*O*-acetyl-β-D-ribofuranosyl)brassenin B (29). After chromatography on silica gel (40 g, hexane/ethyl acetate 1/1) and crystallization from diethyl ether/hexane; compound **29** (0.12 g, 39% from acid **20**) was obtained as colorless crystals. $[\alpha]_D^{20}$ –88.3 (*c* 1.08, $CHCl_3$), mp 127-128 °C; IR ($CHCl_3$) λ (cm^{-1}) 1753 (C=O), 1630 (O=C-N=C); ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm) 8.30 (dd, *J* = 7.1 Hz, *J* = 1.8 Hz, 1H, H-4), 8,12 (s, 1H, H-2), 7.54-7.51 (m, 1H, H-7), 7.33-7.30 (m, 2H, H-5, H-6), 6.17 (d, $J_{1',2'}$ = 5.7 Hz, 1H, H-1′), 5.67 (dd, $J_{2',1'}$ = 5.7 Hz, $J_{2',3'}$ = 5.5 Hz, 1H, H-2′), 5.45 (dd, $J_{3',2'}$ = 5.5 Hz, $J_{3',4'}$ = 4.2 Hz, 1H, H-3′), 4.47 (ddd, $J_{4',3'}$ = 4.2 Hz, $J_{4',5'b}$ = 2.8 Hz, $J_{4',5'a}$ = 3.3 Hz, 1H, H-4′), 4.43 (dd, $J_{5'b,5'a}$ = 12.1 Hz, $J_{5'b,4'}$ = 2.8, 1H, H-5′$_b$), 4.38 (dd, $J_{5'a,5'b}$ = 12.1 Hz, $J_{5'a,4'}$ = 3.3 Hz, 1H, H-5′$_a$), 2.60 (s, 6H, 2 x SCH_3), 2.17 (s, 3H, CH_3CO), 2.16 (s, 3H, CH_3CO), 2.08 (s, 3H, CH_3CO); ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm) 175.92 and 172.58 (O=C-N=C), 170.48, 169.71 and 169.39, (3 x $CH_3\underline{C}O$), 136.65 (q, $C_{arom.}$), 131.81 (C-2), 127.48 (q, $C_{arom.}$), 123.75, 123.04 (C-5, C-6), 122.62 (C-4), 115.20 (q, $C_{arom.}$), 110.49 (C-7), 87.58 (C-1′), 80.30 (C-4′), 73.70 (C-2′), 70.72 (C-3′), 63.19 (C-5′), 20.96 ($\underline{C}H_3CO$), 20.79 ($\underline{C}H_3CO$), 20.62 ($\underline{C}H_3CO$), 16.20 (double intensity, SCH_3); MALDI-TOF MS, *m/z* (%): 562.4 $[M+K]^+$ (48), 549.3 $[M+Na]^+$ (100), 523.3 $[M+H]^+$ (9), 402.7 (20); Anal. Calcd $C_{23}H_{26}N_2O_8S_2$ (522.6): C 52.86 H 5.01 N 5.36. Found: C 53.05 H 4.82 N 5.15.

1-(5-*O*-Acetyl-α-D-ribofuranosyl)brassenin B (28). To a cooled (0 °C) and stirred solution of **27** (0.07 g, 0.15 mmol) in CH_2Cl_2 (1 mL) was added TFA (1.13 mL, 1.67 g, 14.63 mmol) and reaction mixture was stirred at room temperature for 4 h and then set aside overnight. Then 10%

solution of $NaHCO_3$ (12.3 mL, 14.63 mmol) was added and product was extracted with CH_2Cl_2 (2×20 mL). Collected organic layers were dried over Na_2CO_3, solvent evaporated and the residue chromatographed on silica gel (4 g, cyclohexane/acetone 1/1) to give **28** (0.055 g, 84%) as amorphous colorless solid. $[\alpha]_D^{20}$ +13.0 (*c* 0.91, $CHCl_3$); mp 50-59 °C; IR ($CHCl_3$) λ (cm^{-1}) 3280, 3140 (O-H),1720 (C=O), 1620 (O=C-N=C);^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm) 8.24 (s, 1H, H-2), 8.12 (dd, *J* = 6.3 Hz, *J* = 1.3 Hz, 1H, H-4), 7.33 (dd, *J* = 6.9 Hz, *J* = 1.9 Hz, 1H, H-7), 7.24-7.22 (m, 2H, H-5, H-6), 6.23 (d, $J_{1',2'}$ = 4.1 Hz, 1H, H-1′), 4.46 (d, $J_{2',1'}$ = 4.1 Hz, 1H, H-2′), 4.43 (dd, $J_{4',3'}$ = 5.7 Hz, $J_{4',5'b}$ = 2.8 Hz, $J_{4',5'a}$ = 5.2 Hz, H-4′), 4.33 (dd, $J_{5'b,\ 5'a}$ = 12.1 Hz, $J_{5'b,\ 4'}$ = 2.8 Hz, 1H, H-5′$_b$), 4.28-4.25 (m, 1H, H-3′), 4.17 (dd, $J_{5'a,\ 5'b}$ = 12.1 Hz, $J_{5'a,\ 4'}$ = 5.2 Hz, 1H, H-5′$_a$), 3.81 (s, 1H, CD_3COOD exchangeable, OH), 3.57 (s, 1H, CD_3COOD exchangeable, OH), 2.53 (s, 6H, 2 x SCH_3), 2.09 (s, 3H, CH_3CO); ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm) 175.70 and 173.58 (O=C-N=C), 171.03 ($CH_3\underline{C}O$), 136.77 (q, $C_{arom.}$), 135.50 (C-2), 126.79 (q, $C_{arom.}$), 123.30, 122.67, 122.05 (C-6, C-5, C-4), 113.48 (q, $C_{arom.}$), 110.20 (C-7), 86.73 (C-1′), 81.75 (C-4′), 71.96 (C-3′), 71.88 (C-2′), 64.25 (C-5′), 21.11 ($\underline{C}H_3CO$), 16.29 (double intensity, SCH_3); Difference NOE spectra ($CDCl_3$): irr. at δ 6.23 (H-1′) enhanced signals (H-7, 14%) and (H-2′, 9%) and (H-2, 3%) and (H-3′, 3%), irr. at δ 4.26 (H-3′) enhanced signals (H-1′, 2%) and (H-2′, 5%), irr. at δ 8.24 (H-2) enhanced signals (H-1′, 2%) and (H-2′, 2%);. MALDI-TOF MS, *m/z* (%): 477.7 $[M+K]^+$ (22), 461.5 $[M+Na]^+$ (90), 439.2 $[M+H]^+$ (84), 318.1 $[M\text{-}HN{=}C(SCH_3{+}H)]^+$ (100). Anal. Cald for $C_{19}H_{22}N_2O_6S_2$ (438.5): C 52.04 H 5.06 N 6.39. Found: C 51.82 H 5.29 N 6.03.

1-(α-D-Ribofuranosyl)brassenin B (6a)

To a stirred suspension of **28** (0.06 g, 0.137 mmol) in dry methanol (2 mL) was added 0.1 M methanolic solution of MeONa (0.14 mL, 0.014 mmol) and stirring was continued at room temperature for 20 min. After neutralization with Amberlite IR-75 H^+ (0.07 g) and stirring for 5 min, the resin was filtered off, washed with methanol, the filtrate evaporated and obtained residue chromatographed on silica gel (3 g, CH_2Cl_2/MeOH 19/1) to give **6a** (0.048 g, 89%) as colorless amorphous solid. $[\alpha]_D^{20}$ +38,2 (c =0,463, $CHCl_3$); mp 53-75 °C; IR (KBr) λ (cm^{-1}) 3380, 3280 (O-H), 1627 (O=C); ^{1}H NMR (300 MHz, $CDCl_3$, 50°C) δ (ppm) 8.24 (s, 1H, H-2), 8.20 (dd, *J* = 7.4 Hz, *J* = 1.4 Hz, 1H, H-4), 7.34-7.22 (m, 3H, H-7, H-6, H-5), 6.22 (d, $J_{1',2'}$ = 4.4 Hz, 1H, H-1′), 4.45 –4.42 (m, 1H) and 4.36-4.28 (m 2H, H-2′, H-3′, H-4′), 3.85 (d, $J_{5'b,\ 5'a}$ = 12.4 Hz, 1H, H-5′$_b$), 3.74 (d, $J_{5'a,\ 5'b}$ = 11.8 Hz, 1H, H-5′$_a$), 3.11 (s, 1H, CD_3COOD exchangeable, OH), 3.01 (s, 1H, CD_3COOD exchangeable, OH), 2.54 (s, 6H, 2 x SCH_3), 2.14 (s, 1H, CD_3COOD exchangeable, OH); ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm) 175.71 and 173.68 (O=C-N=C), 136.77 (C-2), 135.45 (q, $C_{arom.}$), 126.77 (q, $C_{arom.}$), 123.33, 122.68, 122.06 (C-6, C-5, C-4), 113.39 (q, $C_{arom.}$), 110.31 (C-7), 86.80 (C-1′), 84.34 (C-4′), 72.13 (C-3′), 71.44 (C-2′), 62.46 (C-5′), 16.27 (double intensity, 2 x SCH_3); MALDI-TOF HS, *m/z* (%): 435.8 $[M+K]^+$ (18), 420.1 $[M+Na]^+$ (62), 397.5 $[M+H]^+$ (42), 276.3 $[M\text{-}HN{=}C(SCH_3{+}H)]^+$ (48); Anal. Calcd for $C_{17}H_{20}N_2O_5S_2$ (396.5): C 51.50 H 5.08 N 7.07. Found: C 51.74 H 4.82 N 7.39.

1-(β-D-Ribofuranosyl)brassenin B (6b)

To a stirred solution of triacetyl derivative **29** (0.1 g, 0.20 mmol) in dry methanol (2 mL) was added 0.1 M methanolic solution of MeONa (0.20 mL, 0.020mmol) and stirring was continued at room temperature for 20 min. After neutralization with Amberlite IR-75 H^+ (0.1 g) and stirring for another 5 min, the resin was filtered off, washed with methanol, filtrate evaporated and obtained residue chromatographed on silica gel (3g, CH_2Cl_2/methanol 19/1) to give **6b** (0.063g, 83%) as colorless amorphous compound. $[\alpha]_D^{20}$ –27.0 (*c* 0.22, $CHCl_3$); mp 57-67 °C; IR ($CHCl_3$) λ (cm^{-1}) 3427 (O-H), 1623 (C=O); ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm) 8.25 (s, 1H, H-2), 8.00 (d, 1H, *J* = 7.2 Hz, H-4), 7.42 (d, *J* = 7.2 Hz, 1H, H-7), 7.21-7.14 (m, 2H, H-5, H-6), 5.95 (d, $J_{1',2'}$ = 3.1 Hz, 1H, H-1′), 4.32-4.29 (m, 3H, H-2′, H-3′, CD_3COOD exchangeable OH), 4.08-4.02 (m, 2H, H-4′, CD_3COOD exchangeable OH), 3.87-3.71 (m, 2H, H-5′$_a$, H-5′$_b$), 3.38 (s, 1H, CD_3COOD exchangeable OH), 2.45 (s 6H, 2 x SCH_3); ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm) 175.98 and 173.89 (O=C-N=C), 136.54 (q, $C_{arom.}$), 133.18 (C-2), 126.69 (q, $C_{arom.}$), 123.39 and 122.71 (C-5, C-6), 121.69 (C-4), 113.48 (q, $C_{arom.}$), 110.70 (C-7), 90.00 (C-1′), 83.92 (C-4′), 75.06 (C-2′), 70.13 (C-3′), 61.63 (C-5′), 15.95 (double intensity, 2 x SCH_3); Difference NOE spectra ($CDCl_3$): irr. at δ 5.95 (H-1′) enhanced signals (H-7, 11%) and (H-2, 7%) and (H-2′, 2.4%) and (H-4′, 2.3%), irr. at δ 4.26 (H-4′) enhanced signals (H-1′, 2.6%); MALDI-TOF MS, *m/z* (%): 435.7 $[M+K]^+$ (30), 419.6 $[M+Na]^+$ (69), 397.0 $[M+H]^+$ (82), 276.0 (100); Anal. Calcd for $C_{17}H_{20}N_2O_5S_2$ (396.5): C 51.50 H 5.08 N 7.07. Found: C 51.74 H 4.82 N 7.39.

Cell culture

CEM (human T-cell acute lymphoblastic leukemia cells) and drug-resistant sublines CEM/VCR (vincristine-selected) as well as Jurkat cells (human acute T-lymphoblastic leukemia cells), MCF-7 cells (breast cancer) and HeLa cells (cervix cancer) were kindly provided by Dr. M. Hajdúch (Olomouc, Czech Republic). Cells were maintained in RPMI 1640 medium (CEM, CEM-VCR, Jurkat and HeLa cells) or Dulbeco´s medium (MCF-7 cells) with Glutamax-I supplemented with 10% fetal calf serum, penicillin (100 IU×mL^{-1}) and streptomycin (100 μg×mL^{-1}) (all from Invitrogen, UK), in the atmosphere 5% CO_2 in humidified air at 37 °C. Cell viability, estimated by trypan blue exclusion, was greater than 95% before each experiment.

Assessment of antiproliferative activity by MTT assay

The antiproliferative activity of the synthesized compounds was examined by MTT (thiazolyl blue) test[19] using the selected human cancer cell lines. Briefly, 1×10^4 cells were plated per well in 96-well polystyrene microplates (Sarstedt, Germany) in the culture medium containing the tested chemicals at final concentrations 10^{-4}-10^{-9} mol×L^{-1}. After 72 h incubation, 10 μL of MTT (5 mg×mL^{-1}) were added in each well. After additional 4 h, during which insoluble formazan was produced, 100 μl of 10% sodium dodecylsulphate were added in each well and another 12 h were allowed the formazan to be dissolved. The absorbance was measured at 540 nm using the automated MRX microplate reader (Dynatech laboratories UK). Absorbance of control wells was taken as 100%, and the results were expressed as a percent of control.

Statistical Analysis

For all experiments, mean values and standard deviations (from 3 experiments) were calculated using the ArcusQuickstat software package. To evaluate the statistical significance observed between groups, Student's *t*-test was employed. The statistical significance was considered to be present if $P<0.05$.

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