GLYOXYL ANALOGS OF INDOLE PHYTOALEXINS: SYNTHESIS AND ANTICANCER ACTIVITY

Peter KUTSCHY[a1,*], Andrej SÝKORA[b], Zuzana ČURILLOVÁ[a2], Mária REPOVSKÁ[a3], Martina PILÁTOVÁ[c1], Ján MOJŽIŠ[c2], Roman MEZENCEV[d], Pavel PAZDERA[e] and Tatiana HROMJÁKOVÁ[a4]

[a] *P. J. Šafárik University, Faculty of Science, Institute of Chemical Sciences, Department of Organic Chemistry, Moyzesova 11, 040 01 Košice, Slovak Republic; e-mail: [1] peter.kutschy@upjs.sk, [2] zuzana.curillova@upjs.sk, [3] repovska@gmail.com, [4] tatiana.hromjakova@gmail.com*

[b] *University of Prešov, Faculty of Health Care, Partizánska 1, 080 01 Prešov, Slovak Republic; e-mail: sykora@unipo.sk*

[c] *P. J. Šafárik University, Faculty of Medicine, Department of Pharmacology, SNP 1, 040 66 Košice, Slovak Republic; e-mail: [1] martinapilatova@yahoo.co.uk, [2] jan.mojzis@upjs.sk*

[d] *Georgia Institute of Technology, College of Science, School of Biology, 315 Ferst Drive, Atlanta, GA 30332, USA; e-mail: roman.mezencev@biology.gatech.edu*

[e] *Centre for Syntheses at Sustainable Conditions and Their Management, Department of Chemistry, Masaryk University, Kotlářská 2, 611 37 Brno, Czech Republic; e-mail: pazdera@chemi.muni.cz*

Received May 13, 2010
Accepted June 28, 2010
Published online August 31, 2010

Glyoxyl analogs of indole phytoalexins brassinin, 1-methoxybrassinin, brassitin, 1-methoxybrassitin and 1-methoxybrassenin B were prepared, using (1*H*-indol-3-yl)-, (1-methoxyindol-3-yl)- and [1-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)indol-3-yl]glyoxyl chlorides as starting compounds. Synthesized products were examined for their antiproliferative activity against human cancer cell lines Jurkat (T-cell acute lymphoblastic leukemia), MCF-7 (breast adenocarcinoma, estrogen receptor-positive), MDA-MB-231 (breast adenocarcinoma, estrogen receptor-negative), HeLa (cervical adenocarcinoma), CCRF-CEM cell line (T-cell acute lymphoblastic leukemia) and A-549 cell line (lung adenocarcinoma), and their activity compared with natural phytoalexins and corresponding (1*H*-indol-3-yl)acetic acid derivatives. The highest potency with IC_{50} 3.3–66.1 μmol l^{-1} was found for glyoxyl analogs of 1-methoxybrassenin B.
Keywords: Heterocycles; Indoles; Natural products; Phytoalexins; Glyoxylindoles; Antiproliferative activity.

(1*H*-Indol-3-yl)glyoxyl and (1*H*-indol-3-yl)oxoacetyl derivatives are valuable intermediates in the synthesis of anticancer indolo[2,3-*a*]carbazole alka-

© 2010 Institute of Organic Chemistry and Biochemistry
doi:10.1135/cccc2010048

loids[1a], natural bis(indole) alkaloids harmacanthins[1b] and spongotine A [1c], or other compounds. Indolylglyoxyl moiety itself appears in several marine natural products. Thus hyrtiosin B (**1**) possessing cytotoxic activity against epidermoid carcinoma human KB cells was isolated from Okinawan marine sponge *Hyrtios erecta*[2a] or homofascaplysin B (**2**) with HIV reverse transcriptase inhibitory activity, from the sponge *Fascaplysinopsis reticulata* collected in the Benga lagoon, Fiji Islands[2b]. L-Arginine derived leptoclinidamines A (**3a**) and B (**3b**) were isolated from Australian ascidian *Leptoclinides durus*. Leptoclinidamine A (**3a**) was examined for antimalarial, antitrypanosomal and cytotoxic activity but was not bioactive[2c]. Interestingly, (1*H*-indol-3-yl)glyoxylic acid was suggested as an intermediate in biotransformation of indole-3-acetic acid to indole-3-carboxaldehyde in *Brassica oleracea*[2d]. Synthetic indolylglyoxyl amides were identified as a new group of microtubule destabilizing anticancer agents, with the most active

1

2

3a, R = H
3b, R = OH

4

5a, R = H, X = CH_2, Y = S
5b, R = OCH_3, X = CH_2, Y = S
5c, R = H, X = CO, Y = S
6a, R = H, X = CH_2, Y = O
6b, R = OCH_3, X = CH_2, Y = O

7a, R = OCH_3
7b, R = H

derivative *N*-(pyridine-4-yl)-[1-(4-chlorobenzyl)indol-3-yl]glyoxylamide (Indibulin, D-24851, **4**) possessing the promising in vitro activity against SKOV3 ovarian cancer, U87 glioblastoma and ASPC-1 pancreatic cancer cells[3a]. Recently, substituted indolylglyoxylamides were found to exhibit anxiolytic[3b,3c], antiprion[3d] and anti HIV [3e] activity.

Within our continuing research in the synthesis and anticancer activity of indole phytoalexins and their analogs[4] it was also decided to study the synthesis and antiproliferative activity of glyoxyl analogs of natural phytoalexins brassinin (**5a**), 1-methoxybrassinin (**5b**), brassitin (**6a**), 1-methoxybrassitin (**6b**) and 1-methoxybrassenin B (**7a**), having the CH_2 or CO group replaced by CO–CO grouping. The aim of this study was also to compare their antiproliferative activities with corresponding natural phytoalexins. Among them, 1-methoxybrassenin B (**7a**) is the only indole phytoalexin with a side chain attached to indole position 3 via C=O group[4g]. We have also studied glyoxyl analogs of 1-(β-D-glucopyranosyl)-brassinin and 1-(β-D-glucopyranosyl)brassenin B, since 1-(β-D-glucopyranosyl)-brassinin was isolated as detoxification product of brassinin, produced by fungus *Sclerotinia sclerotiorum*[5] although after its synthesis it was found not to exhibit antitumor activity[4h].

RESULTS AND DISCUSSION

Chemistry

To synthesize glyoxyl analogs **10**, **12** and **13**, indolylglyoxyl chlorides **9** were selected as starting compounds, and their use as acylating reagents in the reactions with methyl carbamodithioate (**A**)[6a] and dimethyl carbon-imidodithioate hydroiodide (**B**)[6b] were studied (Scheme 1). Indole (**8a**) reacts smoothly with oxalyl chloride in diethyl ether without catalyst with the formation of moderately stable (1*H*-indol-3-yl)glyoxyl chloride (**9a**) in 92% yield[7a]. Analogous acylation of 1-methoxyindole (**8b**)[7b] and 1-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)indole (**8c**)[7c] afforded highly unstable chlorides **9b** [7d] and **9c** that were used in the next reaction as crude products immediately after their preparation.

Acylation of dithiocarbamate **A** with acid chlorides **9a** and **9b** resulted in the low yields of **10a** (15%) and **10b** (6%). It is supposed that in the first stage, the *S*-acylation takes place and the rearrangement with the migration of acyl group to nitrogen atom occurs in the second stage, resulting in final *N*-acylation. To facilitate the rearrangement, prolongation of reaction time and increased temperature (up to 60 °C) were examined, however the yield

did not improved. Therefore another approach, consisting in coupling of (1*H*-indol-3-yl)glyoxylic acid (**11a**)[7a] and (1-methoxyindol-3-yl)glyoxylic acid (**11b**)[7d] with dithiocarbamate **A** in the presence of dicyclohexylcarbodiimide (DCC) was examined. The reaction of acid **11a** in chloroform or dichloromethane was complicated by its very low solubility. This problem was solved, using the *N*,*N*-dimethylformamide as a co-solvent. Activation of

$(COCl)_2$
Et_2O
9a: 92%
9b, **9c** used as crude products
8a-8c
9a-9c
A
H_2N SCH_3
MeCN/pyridine
6-26% (from **8a-8c**)
For **11a**, **11b**
NaOH
Et_2O/H_2O
64-78% (from **8a**, **8b**)
10a-10c
A
DCC
For **11a**: CH_2Cl_2/DMF
For **11b**: CH_2Cl_2
40-41%
11a, **11b**
For **10a**, **10b**
MNO
MeCN
62-99%
12a, **12b**
$HN{=}C(SCH_3)_2.HI$
B
9a-9c
MeCN/pyridine
34-75%
13a-13c

8a, **9a**, **10a**, **11a**, **12a**, **13a**: R = H
8b, **9b**, **10b**, **11b**, **12b**, **13b**: R = OCH_3
8c, **9c**, **10c**, **13c**: R = 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl

SCHEME 1

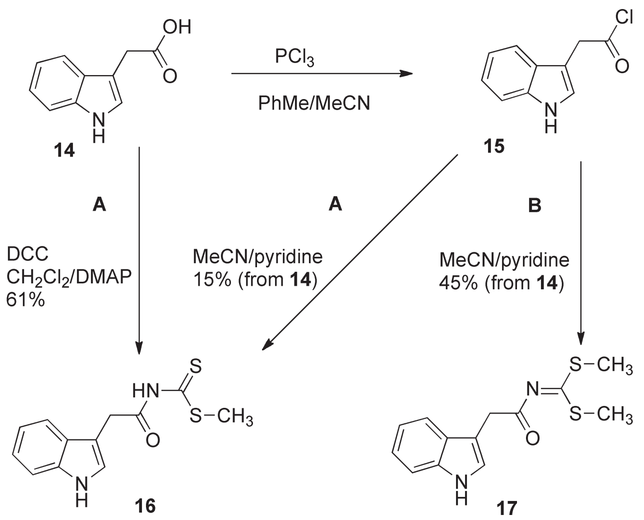
carboxylic group with DCC resulted in improved yields of target brassinin analogs **10a** and **10b** (Scheme 1). Although brassitin and its derivatives, examined to date, appeared to be devoid of antitumor activity[4i], we decided to verify the activity of corresponding glyoxyl analogs. Preparation of glyoxylbrassitins **12a** and **12b**, isosteric with brassinin analogs **10a** and **10b**, was achieved by treatment of **10a** and **10b** with mesityl nitrile oxide (MNO)[8] in 99% (**12a**) and 62% (**12b**) yields. Finally, the analogs of 1-methoxybrassenin B **13a–13c** were successfully prepared by treatment of glyoxylchlorides **9a–9c** with imide **B**, using the previously desribed procedure[4h].

The attempts for removal of acetyl groups from tetraacetyl-β-D-glucopyranosyl derivatives **10c** and **13c** have failed, and only intractable mixture of decomposition products was formed, using sodium methoxide[9a] or potassium carbonate[9b] in methanol, triethyl amine in methanol–water mixture[9c] and ammonia in methanol or dichlormethane[9d]. Although partial deprotection, hydrolysis, aminolysis or methanolysis of glyoxyl group may be expected, we did not succeed in isolation of such products.

Structure of the synthesized compounds was confirmed by spectral methods. In the IR spectra, two absorption bands of glyoxylic carbonyl groups were present at 1589–1642 and 1678–1754 cm^{-1}, that were in the case of brassitin and 1-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl) derivatives overlapped by absorption bands of thiocarbamate and acetyl carbonyls. Corresponding signals of C=O carbons in ^{13}C NMR spectra appeared at 157–161 and 176–179 ppm for brassinin (**10a–10c**) and brassitin analogs (**12a**, **12b**), whereas at 171–173 and 180–182 ppm for brassenin B analogs **13a–13c**. In the spectra of brassinin analogs **10a–10c**, the signals of thiocarbonyl group at 203–204 ppm were present, while for brassitin (**12a**, **12b**) or brassenin B analogs (**13a–13c**), the signals of corresponding carbonyl carbons of $COSCH_3$ grouping and C=N bond were observed at 169 and 178–179 ppm, respectively. In the case of 1-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl) derivatives **10c** and **13c**, their β-anomeric structure was determined by vicinal *trans*-diaxial coupling constant $J(1',2')$ = 8.7 Hz.

In order to appreciate the influence of glyoxyl group on biological activity, also the methyl 2-(1*H*-indol-3-yl)acetylcarbamodithioate (**16**) and homobrassenin B (**17**) were synthesized from (1*H*-indol-3-yl)acetic acid (**14**; Scheme 2). Acid **14** can be transformed to corresponding acid chloride **15** by treatment with oxalyl chloride[10a], thionyl chloride[10b] or phosphorous pentachloride[10c]. We have found that the use of phosphorous trichloride[11] in toluene is more convenient. The chloride **15** prepared in this way was used as a crude product in the acylation of dithiocarbamate **A**. Because of the low 15% yield, product **16** was prepared by DCC activation of carboxyl-

ic acid **14**. Reaction in refluxing chloroform afforded 32% of dithiocarbamate **16**, however acylation in dichloromethane in the presence of catalytic amount of 4-dimethylaminopyridine[10d] proceeded more effectively with the formation 2-(1*H*-indol-3-yl)acetylcarbamodithioate (**16**) in 61% yield. Homobrassenin B (**17**) was obtained in 45% yield by acylation of imine **B** with acid choride **15** (Scheme 2).



SCHEME 2

Biological Results

Cytotoxicity data demonstrate that all glyoxyldithiocarbamates **10a**, **10b** and **10c**, and glyoxylthiocarbamates **12a** and **12b** are inactive (IC_{50} > 100 μmol l^{-1}) in all used cell lines, irrespective of substitution on indole nitrogen (Table I).

On the other hand, compounds **13a**, **13b** and **13c** that are *S*-methyl derivatives of **10a**, **10b**, and **10c** demonstrate cytotoxicity with IC_{50} values in the micromolar range (Fig. 1). While average IC_{50} values across all tested cell lines are very similar for these three compounds (27.2, 35.1 and 32.0 μmol l^{-1}, respectively), there are considerable differences in their average IC_{50} values among different cell lines with CCRF-CEM being most sensitive and A-549 being most resistant cell lines. Interestingly, Jurkat cells demonstrated remarkably lower sensitivity to all 3 agents than another T-cell lymphoblastic leukemia cell line CCRF-CEM and this finding differs from previously reported very similar sensitivities of these two cell lines to

methotrexate, doxorubicin and daunorubicin and much higher sensitivity of Jurkat cells to the cytotoxic effect of vincristine[12a]. As a result, compounds **13a**, **13b** and **13c** are unlikely to share their mode of action with that of methotrexate, antracyclines or vincristine. Pearson correlation coefficients (PCC) between IC_{50} values of evaluated compounds and doxo-

TABLE I
Antiproliferative activities of indole phytoalexins, their glyoxyl analogs and related compounds

5a-5c,10a-10c,12a, 12b, 16 **7a, 7b, 13a-13c, 17**

Compd.	R	X	Y	IC_{50}, μmol l^{-1}					
				Jurkat	MCF7	MDA-MB23	HeLa	CCRF-CEM	A-549
5a[a]	H	CH_2	S	>100	>100	>100	>100	90.2	>100
5b[a]	OCH_3	CH_2	S	37.5	>100	>100	>100	63.5	>100
5c[a]	H	CO	S	>100	>100	>100	>100	>100	>100
7a[a]	OCH_3	CO	–	26.6	63.6	82.8	54.6	39.0	67.5
7b[a]	H	CO	–	70.4	35.9	100	31.4	36.0	100
10a	H	CO-CO	S	>100	>100	>100	>100	>100	>100
10b	OCH_3	CO-CO	S	>100	>100	>100	>100	>100	>100
10c	G[b]	CO-CO	S	>100	>100	>100	>100	>100	>100
12a	H	CO-CO	O	>100	>100	>100	>100	>100	>100
12b	OCH_3	CO-CO	O	>100	>100	>100	>100	>100	>100
13a	H	CO-CO	–	39.1	22.9	36.9	20.1	12.9	31.5
13b	OCH_3	CO-CO	–	63.4	24.3	30.3	23.2	3.3	66.1
13c	G[b]	CO-CO	–	35.8	25.5	36.7	35.8	15.8	42.3
16	H	CH_2-CO	S	>100	>100	>100	>100	>100	>100
17	H	CH_2-CO	–	>100	>100	>100	>100	>100	>100
Doxorubicin				0.078	0.5	0.2	0.2	0.09	1.9
Cisplatin				12.0	11.4	14.7	7.7	4.4	12.2
Etoposide				1.2	10.9	21.2	3.9	1.1	14.3

[a] Prepared according to literature procedures: **5a** [11a], **5b** [4e], **5c** [11b], **7a** [4d], **7b** [11b]; [b] 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl.

rubicin, cisplatin and etoposide (Table II) also suggest that compounds **13a**, **13b** and **13c** do not share the same mode of action with doxorubicin and etopiside; however, high value of PCC for compound **13a** and cisplatin indicates that DNA-damaging effect is probably involved in the cytotoxicity of this compound. On the other hand, compound **7a** likely exerts the same mode of action than etoposide, since activity profiles of these two agents have high PCC value (0.937) that would be obtained by chance with very low probability (in our set of 12,000 random 6-dimensional vectors, PCC ≥ 0.937 was found only for 71 random vectors, which corresponds to probability of 0.592%).

TABLE II
Pearson correlation coefficients (PCC) calculated for IC_{50} values of pairs of compounds across 6 cell lines (only compounds with at least one IC_{50} < 100 μmol l^{-1} are included). *p*-Values (two-tailed *t*-test) < 0.05 for PCC ≥ 0.812

Compound	Doxorubicin	Cisplatin	Etoposide
5b	0.431	0.239	0.691
7a	0.389	0.538	0.937
7b	0.509	0.538	0.693
13a	0.168	0.889	0.454
13b	0.568	0.639	0.176
13c	0.499	0.704	0.458

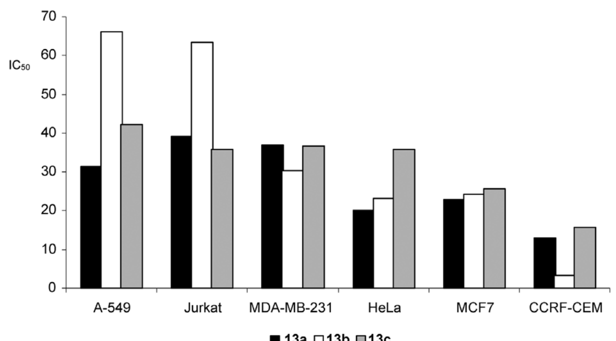


FIG. 1
Cell lines are organized from highest to lowest average IC_{50} value for *S*-methyl dithiocarbamates **13a–13c**

Considering similarity of its profile with etoposide[12b], compound **7a** is likely an inhibitor of topoisomerase II. This analysis is further supported by the scatterplot for IC_{50} values between **7a** and etoposide (ETO; Fig. 2), which shows strong positive correlation between these two variables without false positivity introduced by outliers. Similarly, the scatterplot for IC_{50} values of **13a** and cisplatin (CPT; Fig. 2) does not indicate bias introduced by outliers and PCC determined for these two compounds (0.889) would be observed by chance only in about 1.3% cases.

Effect of introduction of CH_3O group to the indole nitrogen in compound **13a** is not consistent across different cell lines. While it dramatically decreases cytotoxic potency for A-549 and Jurkat cell lines, it results in almost 4-fold increase in potency for CCRF-CEM cells. Furthermore, the effect of introduction of CH_3O group is inconsistent for different structural types even for the same cell lines. For example, in Jurkat cells, introduction of this group results in increased potency for compounds **5b** and **7a**, compared to their respective parent compounds **5a** and **7b**, but it decreases potency of **13b** in comparison with its parent compound **13a**. These inconsistencies suggest that (i) some tested compounds induce cytotoxocity via multiple drug targets with different expressions across different cell types, and (ii) different structural types of tested compounds differ in their mode of action against the same cell lines.

The lack of activity of compound **17** may indicate that cytotoxicity of studied imines in this panel of cell lines requires single carbonyl or glyoxyl group linking indole and dimethyl carbonimidodithioate moieties.

In summary, glyoxyl analogs of brassinin and brassitin, representing a special kind of glyoxylamides, and glyoxyl analogs of 1-methoxybrassenin B, possessing glyoxylimine moiety were prepared. Examination of antiproli-

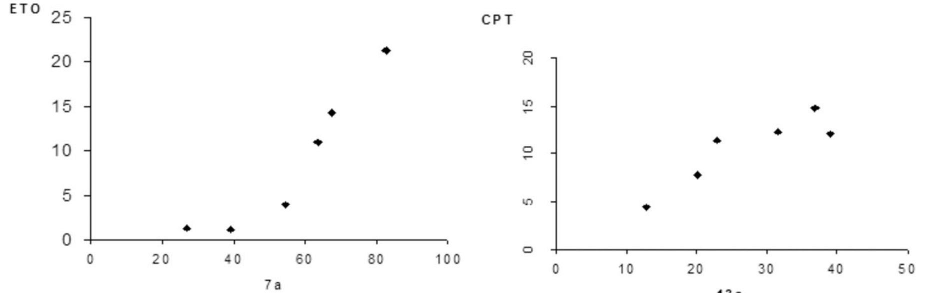


FIG. 2
Scatterplots of IC_{50} values for etoposide (ETO) vs **7a** (left) and cisplatin (CPT) vs **13a** (right) against panel

ferative activity of synthesized substances against human cancer cell lines revealed higher anticancer potency of glyoxylimines compared to glyoxylamides under study, with the highest potency in individual cell line displayed by glyoxyl analogue of 1-methoxybrassenin B (**13b**) against CCRF-CEM cell line. Glyoxylimine **13a** is likely a DNA-damaging agent while natural compound **7a** is predicted to be a topoisomerase II inhibitor.

EXPERIMENTAL

Biological Materials and Methods

Jurkat (human T-cell acute lymphoblastic leukemia), HeLa (human cervical adenocarcinoma), MCF-7 (human breast adenocarcinoma), MDA-MB-231 (human breast adenocarcinoma) and A-549 cell lines (human lung adenocarcinoma) were kindly provided by Dr. Hajdúch (Olomouc, Czech Republic). CCRF-CEM cell line (human T-cell acute lymphoblastic leukemia) was obtained from the German Collection of Microorganisms and Cell Cultures (Braunschweig, Germany). The cells were routinely maintained in RPMI 1640 medium with L-Glutamine and HEPES (Jurkat, HeLa and CCR-CEM) or Dulbecco's modified Eagle's medium with Glutamax-I (MCF-7, MDA-MB-231 and A-549) supplemented with 10% fetal calf serum, penicillin (100 IU ml^{-1}) and streptomycin (100 μg ml^{-1}) (all from Invitrogen, USA), in humidified air with 5% CO_2 at 37 °C. Before each cytotoxicity assay, the cell viability was determined by the trypan blue exclusion method and found to be greater than 95%. The cytostatic/cytotoxic effects of compounds were studied using the colorimetric microculture assay with the MTT end-point[12]. Briefly, 3×10^3 (A-549, MCF-7, MDA-MB-231), 5×10^3 (HeLa) or 1×10^4 (Jurkat and CEM) cells were plated per well in 96-well polystyrene microplates (Sarstedt, Germany) in the culture medium containing tested chemicals at final concentrations of 1×10^{-4}, 5×10^{-5}, 1×10^{-5}, 5×10^{-6} and 1×10^{-6} mol l^{-1}. After 72 hours of incubation, 10 μl of MTT (5 mg ml^{-1}) (Sigma, Germany) were added in each well. After additional 4 h, during which insoluble formazan was produced, 100 μl of 10% sodium dodecylsulfate were added in each well and another 12 h were allowed for the dissolution of formazan. The absorbance was measured at 540 nm using the automated MRX microplate reader (Dynatech Laboratories, UK). The blank-corrected absorbance of the control wells was taken as 100% and the results were expressed as a percentage of the control. IC_{50} values (concentrations of tested agents that inhibited growth of cell cultures to 50% of the untreated control) were determined by GraphPad Prism for Windows version 5.01 (GraphPad Software, Inc.).

Chemical Synthesis

Melting points were determined on a Koffler micro melting point apparatus and are uncorrected. IR spectra were recorded on an IR-75 spectrometer (Zeiss Jena); wave numbers (ν) are given in cm^{-1}. ^1H (400 MHz) and ^{13}C (100 MHz) NMR spectra were measured on a Varian Mercury Plus spectrometer. Chemical shifts (δ) are reported in ppm downfield from TMS as internal standard and coupling constants (*J*) are given in Hz. The signals of individual protons and carbons were assigned on the basis of 2D NMR experiments (H,H COSY, HSQC and HMBC). Microanalyses were performed with a Perkin–Elmer, Model 2400 analyzer. EI mass

spectra were recorded on a GC-MS Trio 1000 (Fison Instruments) spectrometer at ionization energy 70 eV, whereas MALDI-TOF spectrum of compound **13b** was measured on a MALDI IV (Shimadzu, Kratos Analytical). The progress of chemical reactions was monitored by a thin layer chromatography, using Macherey–Nagel plates Alugram®Sil G/UV254. Preparative column chromatography was performed on Kieselgel 60 Merck Type 9385 (0.040–0.063 mm). Indole, oxalyl chloride and 2-(1*H*-indol-3-yl)acetic acid (Merck), and indoline and D-glucose (Avocado) were used as obtained.

2-[1-(2′,3′,4′,6′-Tetra-*O*-acetyl-β-D-glucopyranosyl)-1*H*-indol-3-yl]oxoacetylchloride (**9c**)

To a solution of 1-(tetra-*O*-acetyl-β-D-glucopyranosyl)-1*H* indole[7c] (**8c**; 224 mg, 0.5 mmol) in a mixture of dry diethyl ether (8 ml) and dry acetonitrile (2 ml) cooled to 0–5 °C, a solution of oxalyl chloride (190 mg, 0.129 ml, 1.5 mmol) in dry diethyl ether (4 ml) was added dropwise under nitrogen atmosphere. The reaction mixture was stirred overnight, the solvent evaporated and the obtained yellow oil of unstable crude product immediately used in the next reaction.

Methyl 2-(1*H*-Indol-3-yl)-2-oxoacetylcarbamodithioate (Glyoxylbrassinin; **10a**)

Method A. To a solution of freshly prepared (1*H*-indol-3-yl)glyoxyl chloride[7a] (**9a**; 190 mg, 0.92 mmol) in dry acetonitrile (17 ml), a solution of methyl carbamodithioate[6a] (**A**; 98 mg, 0.92 mmol) in dry pyridine (2.5 ml) was added and the reaction mixture was stirred at room temperature for 2 h. The resulted mixture of intensive red color was poured into ice-cool water (50 ml) and the product extracted with diethyl ether (30 ml). The extract was washed with saturated solution of sodium hydrogen carbonate (2 × 20 ml), dried over sodium sulfate, the solvent evaporated and the obtained residue re-evaporated with toluene to remove residual pyridine. Chromatography of the obtained residue on silica gel (40 g, dichloromethane as an eluent) afforded product **10a**. Yield 42 mg (15%).

Method B. To a solution of (1*H*-indol-3-yl)glyoxylic acid[7a] (**11a**; 95 mg, 0.5 mmol) in a mixture of dry dichloromethane (4 ml) and DMF (0.2 ml), methyl carbamodithioate (**A**; 54 mg, 0.5 mmol) and DCC (103 mg, 0.5 mmol) were added and the reaction mixture was stirred at room temperature for 24 h. After evaporation of dichloromethane, the obtained residue was diluted with DMF (1 ml) and poured into cold water (50 ml). The separated precipitate was filtered with suction, dried and submitted to column chromatography on silica gel (10 g, dichloromethane as an eluent; for a good separation a column diameter should be 6 cm). Yield 56 mg (40%) of yellow crystals, m.p. 210–212 °C (ethanol), R_F 0.5 (dichloromethane). IR (KBr): 3378 and 3310 (N–H), 1689 and 1598 (C=O). ^{1}H NMR (400 MHz, $CDCl_3$): 12.46 s, 1 H (N-H); 12.32 s, 1 H (N-H); 8.58 d, 1 H, *J* = 3.2 (H-2); 8.19–8.15 m, 1 H (H-4); 7.57–7.55 m, 1 H (H-7); 7.32–7.29 m, 2 H (H-6, H-5); 2.63 s, 3 H (SCH_3). ^{13}C NMR (100 MHz, $CDCl_3$): 204.2 (C=S), 179.5 (C=O), 161.3 (C=O), 138.7 (C-2), 136.6 (C-7a), 125.7 (C-3a), 124.0 (C-6), 123.0 (C-5), 121.2 (C-4), 112.9 (C-7), 111.7 (C-3), 20.0 (SCH_3). EIMS, *m/z* (%): 278 (3) [$M^{\bullet+}$], 231 (11), 159 (7), 144 (100), 116 (22), 89 (19), 63 (7). For $C_{12}H_{10}N_2O_2S_2$ (278.4) calculated: 51.52% C, 3.62% H, 10.06% N; found: 51.52% C, 3.85% H, 10.31% N.

Methyl 2-(1-Methoxy-1*H*-indol-3-yl)-2-oxoacetylcarbamodithioate
(1-Methoxyglyoxylbrassinin; **10b**)

Method A. According to the previous procedure, using acid chloride **9b** [7d] freshly prepared from 1-methoxyindole **8b** [7b], product **10b** was obtained after chromatography on silica gel (25 g, cyclohexane–acetone as an eluent, 3:1). Yield 18 mg (6%), R_F 0.42 (cyclohexane–acetone, 3:1).

Method B. To a solution of (1-methoxy-1*H*-indol-3-yl)glyoxylic acid (**11b**; 88 mg, 0.4 mmol) in dry dichloromethane (4 ml), methyl carbamodithioate (**A**; 44 mg, 0.4 mmol) and DCC (84 mg, 0.4 mmol) were added and the reaction mixture was stirred at room temperature for 24 h. After evaporation of dichloromethane, the obtained residue was chromatographed on silica gel (10 g, dichloromethane as an eluent). Yield 50 mg (41%) of yellow amorphous solid, m.p. 161–165 °C, R_F 0.94 (dichloromethane). IR (KBr): 3314 (N–H), 1715 and 1633 (C=O). ^{1}H NMR (400 MHz, $CDCl_3$): 11.14 s, 1 H (N-H); 9.07 s, 1 H (H-2); 8.44–8.41 m, 1 H (H-4); 7.53–7.51 m, 1 H (H-7); 7.42–7.40 m, 2 H (H-6, H-5); 4.23 s, 3 H (OCH_3); 2.71 s, 3 H (SCH_3). ^{13}C NMR (100 MHz, $CDCl_3$): 202.9 (C=S), 176.7 (CONH), 157.7 (C=O), 134.7 (C-2), 131.9 (C-7a), 125.0 (C-6), 124.6 (C-5), 123.91 (C-3a), 122.8 (C-4), 109.0 (C-7), 107.3 (C-3), 67.1 (OCH_3), 20.4 (SCH_3). EIMS, *m/z* (%): 308 (15) [$M^{\bullet+}$], 261 (78), 174 (100), 159 (54), 143 (24), 47 (36), 45 (39). For $C_{13}H_{12}N_2O_3S_2$ (308.4) calculated: 50.63% C, 3.92% H, 9.08% N; found: 50.91% C, 3.62% H, 8.83% N.

Methyl 2-[1-(2′,3′,4′,6′-Tetra-*O*-acetyl-β-D-glucopyranosyl)-1*H*-indol-3-yl]-2-oxoacetylcarbamodithioate (1-(2′,3′,4′,6′-Tetra-*O*-acetyl-β-D-glucopyranosyl)-methoxyglyoxylbrassinin; **10c**)

To a solution of glyoxyl chloride **9c** freshly prepared from 1-(2′,3′,4′,6′-tetra-*O*-acetyl-β-D-glucopyranosyl)-1*H*-indole[7c] (**8c**; 224 mg, 0.5 mmol) in dry acetonitrile (11 ml), a solution of methyl carbamodithioate[6a] (**A**; 54 mg, 0.5 mmol) in dry pyridine (2 ml) was added and the reaction mixture was stirred at room temperature for 5 h. The resulted mixture was poured into a mixture of ice and water (150 ml) and the product extracted with diethyl ether (3 × 40 ml). The collected extract was washed with a saturated solution of sodium chloride (40 ml), dried over sodium sulfate, the solvent was evaporated and the obtained residue re-evaporated with toluene to remove the residual pyridine. Chromatography of the obtained residue on silica gel (25 g, cyclohexane–acetone as an eluent, 2:1) afforded product **10c**. Yield 80 mg (26%) of light yellow crystals, m.p. 186–188 °C (acetone–cyclohexane), R_F 0.19 (cyclohexane–acetone, 2:1), $[\alpha]_D^{20}$ –43.2 (*c* 0.24, chloroform). IR ($CHCl_3$): 3327 (N–H), 1754 and 1642 (C=O). ^{1}H NMR (400 MHz, $CDCl_3$): 11.09 s, 1 H (NH); 9.09 s, 1 H (H-2); 8,41–8.39 m, 1 (H-4); 7.55 –7.52 m, 1 H (H-7); 7.42–7.37 m, 2 H (H-5, H-6); 5.67 d, 1 H *J*(1′,2′) = 8.7 (H-1′); 5.54 dd, 1 H, *J*(2′,1′) = 8.7, *J*(2′,3′) = 9.4 (H-2′); 5.48 dd, 1 H, *J*(3′,4′) = 8.9, *J*(3′,2′) = 9.4 (H-3′); 5.33 dd, 1 H, *J*(4′,3′) = 9.0, 10.1 (H-4′); 4.33 dd, 1 H, *J*(6′b,5′) = 5.0, *J*(6′b,6′a) = 12.6 (H-6′b); 4.24 dd, 1 H, *J*(6′a,5′) = 2.2, *J*(6′a,6′b) = 12.6 (H-6′a); 4.05 ddd, 1 H, *J*(5′,6′a) = 2.3, *J*(5′,6′b) = 4.9, *J*(5′,4′) = 10.1 (H-5′); 2.72 s, 3 H (SCH_3); 2.12 s, 3 H, 2.10 s, 3 H, 2.03 s, 3 H and 1.68 s, 3 H (4 × CH_3CO). ^{13}C NMR (100 MHz, $CDCl_3$): 203.0 (C=S), 177.7 (CONH), 157.3 (C=O), 170.5, 170.0, 169.2 and 168.4 (4 × CH_3**C**O), 138.3 (C-2), 135.6 (C-7a), 127.3 (C-3a), 124.9 (C-5), 124.5 (C-6), 123.0 (C-4), 113.2 (C-3), 110.8 (C-7), 84.2 (C-1′), 75.3 (C-5′), 72.7 (C-3′), 70.8 (C-2′), 67.8 (C-4′), 61.7 (C-6′), 20.5 (SCH_3), 20.7, 20.5, 20.4 and 20.0 (4 × **C**H_3CO). For $C_{26}H_{28}N_2S_2O_{11}$ (608.7) calculated: 50.31% C, 4.64% H, 4.60% N; found: 50.58% C, 4.87% H, 4.25% N.

S-Methyl 2-(1*H*-Indol-3-yl)-2-oxoacetylcarbamothioate
(Glyoxylbrassitin; **12a**)

To a solution of glyoxylbrassinin (**10a**; 18 mg, 0.065 mmol) in dry acetonitrile (1.5 ml), solution of mesitylnitrile oxide (MNO; 21 mg, 0.13 mmol) in dry acetonitrile (1.5 ml) was added and the mixture was stirred at room temperature for 12 h. After evaporation of solvent, the obtained residue was submitted to column chromatography on silica gel (3 g, hexane–ethyl acetate as an eluent, 5:1) to afford product **12a**. Yield 17 mg (99%) of yellow amorphous solid, m.p. 181–184 °C, R_F 0.43 (cyclohexane–acetone, 2:1). IR (KBr): 3304 (N–H), 1745 and 1626 (C=O). ^{1}H NMR (400 MHz, $CDCl_3$): 11.54 s, 1 H (N-H); 10.15 s, 1 H (N-H); 8.95 d, 1 H, J = 3.4 (H-2); 8.38–8.36 m, 1 H (H-4); 7.50–7.48 m, 1 H (H-7); 7.35–7.29 m, 2 H (H-5, H-6); 2.42 s, 3 H (SCH_3). ^{13}C NMR (100 MHz, $CDCl_3$): 176.9 (C=O), 169.3 (**C**$OSCH_3$), 160.4 (C=O), 139.1 (C-2), 136.3 (C-3a), 126.5 (C-7a), 124.0 (C-6), 123.3 (C-5), 122.1 (C-4), 112.2 (C-7), 112.0 (C-3), 12.5 (SCH_3). For $C_{12}H_{10}N_2O_3S$ (262.3) calculated: 54.95% C, 3.48% H, 10.68% N; found: 54.57% C, 4.13% H, 10.31% N.

S-Methyl 2-(1-Methoxy-1*H*-indol-3-yl)-2-oxoacetylcarbamothioate
(1-Methoxyglyoxylbrassitin; **12b**)

Product **12b** was obtained according to previous procedure, using hexane–ethyl acetate, 4:1 as an eluent for column chromatography. Yield 12 mg (62%) of light yellow crystals, m.p. 123–125 °C, R_F 0.41 (cyclohexane–acetone, 2:1). IR ($CHCl_3$): 3107 (N–H), 1713 and 1627 (C=O). ^{1}H NMR (400 MHz, $CDCl_3$): 9.94 s, 1 H (N-H); 9.05 s, 1 H (H-2); 8.42–8.40 m, 1 H (H-4); 7.53–7.51 m, 1 H (H-7); 7.41–7.38 m, 2 H (H-5, H-6); 4.22 s, 3 H (OCH_3); 2.43 s, 3 H (SCH_3). ^{13}C NMR (100 MHz, $CDCl_3$): 176.4 (C=O), 169.4 (**C**$OSCH_3$), 160.1 (C=O), 134.8 (C-2), 131.9 (C-7a), 125.0 (C-6), 124.6 (C-5), 123.9 (C-3a), 122.8 (C-4), 109.0 (C-7), 107.0 (C-3), 67.1 (OCH_3), 12.6 (SCH_3). EIMS, *m/z* (%): 292 (44) [$M^{\bullet+}$], 174 (100), 159 (83), 143 (69), 115 (52), 75 (53), 47 (28). For $C_{13}H_{12}N_2O_4S$ (392.3) calculated: 53.42% C, 4.14% H, 9.58% N; found: 53.28% C, 4.30% H, 9.75% N.

Dimethyl 2-(1*H*-Indol-3-yl)-2-oxoacetylcarbonimidodithioate
(Glyoxylbrassenin B; **13a**)

To a solution of freshly prepared (1*H*-indol-3-yl)glyoxyl chloride[7a] (**9a**; 190 mg, 0.92 mmol) in dry acetonitrile (17 ml), a solution of dimethyl carbonimidodithioate hydroiodide[6b] (**B**; 228 mg, 0.92 mmol) in dry pyridine (2.5 ml) was added and the reaction mixture was stirred at room temperature for 1 h. The reaction mixture was slowly poured into ice-cold water (50 ml) and set aside at 5 °C overnight. The separated precipitate was filtered with suction, washed with cold water, dried and crystallized from methanol. Yield 200 mg (75%) of yellow crystals, m.p. 162–164 °C, R_F 0.31 (cyclohexane–acetone, 2:1). IR (KBr): 3248 (N–H), 1678 and 1600 (C=O). ^{1}H NMR (400 MHz, $CDCl_3$): 12.36 s, 1 H (NH); 8.39 d, 1 H, $J(1,2)$ = 3.1 (H-2); 8.17 dd, 1 H, $J(4,6)$ = 1.7, $J(4.5)$ = 7.1 (H-4); 7.55 dd, 1 H, $J(7,5)$ = 1.6, $J(7,6)$ = 7.7 (H-7); 7.29 ddd, 1 H, $J(6,4)$ = 1.7, $J(6,5)$ = 6.7, $J(6,7)$ = 7.7 (H-6); 7.26 ddd, 1 H, $J(5,7)$ = 1.5, $J(5,6)$ = 6.7, $J(5,4)$ = 7.2 (H-5); 2.57 s, 6 H (2 × SCH_3). ^{13}C NMR (100 MHz, $CDCl_3$): 182.2 (C=O), 178.2 (C=N), 172.9 (C=O), 137.7 (C-2), 136.6 (C-7a), 125.6 (C-3a), 123.7 (C-6), 122.7 (C-5), 121.2 (C-4), 112.7 (C-7), 112.3 (C-3), 15.7 (2 × SCH_3). EIMS, *m/z* (%): 293 (2) [$M^{\bullet+}$], 144 (100) [M^+ – $CONC(SCH_3)_2$], 116 (50) [M^+ – $COCONC(SCH_3)_2$], 89 (48), 75 (14), 74 (15),

47 (13), 45 (15), 47 (15). For $C_{13}H_{12}N_2O_2S_2$ (292.4) calculated: 53.40% C, 4.14% H, 9.58% N; found: 53.68% C, 4.27% H, 9.31% N.

Dimethyl 2-(1-Methoxy-1*H*-indol-3-yl)-2-oxoacetylcarbonimidodithioate (1-Methoxyglyoxylbrassenin B; **13b**)

To a solution of (1-methoxy-1*H*-indol-3-yl)glyoxyl chloride (**9b**)[7d], freshly prepared from 1-methoxyinole[7b] (**8b**; 147 mg, 1 mmol), in dry acetonitrile (10 ml), dimethyl carbonimidodithioate hydroiodide[6b] (**B**; 249 mg, 1 mmol) in dry pyridine (2.5 ml) was added and the reaction mixture was stirred at room temperature overnight. Then the reaction mixture was slowly poured into ice-cold water (50 ml) and the product extracted with diethyl ether (1 × 30 ml). The collected extract was washed with saturated sodium hydrogen carbonate solution (2 × 20 ml), dried over sodium sulfate, the solvent was evaporated and the obtained residue re-evaporated with toluene to remove residual pyridine. The residue was dissolved in ethyl acetate, a small amount of silica gel was added, the solvent was evaporated and the crude product preabsorbed on silica gel submitted to column chromatography on silica gel (15 g, hexane–ethyl acetate as an eluent, 3:1). Yield 110 mg (34%) of yellow crystals, m.p. 82–84 °C (ethyl acetate–hexane), R_F 0.38 (hexane–ethyl acetate, 3:1). IR ($CHCl_3$): 1689 and 1598 (C=O). ^{1}H NMR (400 MHz, $CDCl_3$): 8.60 s, 1 H (H-2); 8.48–8.45 m (H-4); 7.51–7.48 m, 1 H (H-7); 7.38–7.33 m, 2 H (H-5, H-6); 4.21 s, 3 H (OCH_3); 2.59 s, 6 H (SCH_3). ^{13}C NMR (100 MHz, $CDCl_3$): 180.6 (C=O), 178.6 (C=N), 171.8 (C=O), 133.2 (C-2), 132.0 (C-3a), 124.5 (C-5), 123.8 (C-6), 123.5 (C-7a), 122.8 (C-7), 108.7 (C-4), 108.6 (C-3), 66.9 (OCH_3), 16.2 (SCH_3). EIMS, *m/z* (%): 174 (100) [M^+ – $CONC(SCH_3)_2$], 159 (53), 148 (26), 143 (50), 115 (44), 75 (34). For $C_{14}H_{14}N_2O_3S_2$ (322.4) calculated: 52.16% C, 4.38% H, 8.69% N; found: 52.40% C, 4.18% H, 8.55% N.

Dimethyl 2-[1-(2′,3′,4′,6′-Tetra-*O*-acetyl-β-D-glucopyranosyl)-1*H*-indol-3-yl]-2-oxoacetylcarbonimidodithioate (1-(2′,3′,4′,6′-Tetra-*O*-acetyl-β-D-glucopyranosyl)-glyoxylbrassenin B; **13c**)

To a solution of glyoxyl chloride **9c**, freshly prepared from 1-(2′,3′,4′,6′-*O*-acetyl-β-D-glucopyranosyl)-1*H*-indole (**8c**; 224 mg, 0.5 mmol), in dry acetonitrile (5 ml), dimethyl carbonimidodithioate hydroiodide[6b] (**B**; 125 mg, 0.5 mmol) in dry pyridine (2 ml) was added and the reaction mixture was stirred at room temperature for 3.5 h. Then the reaction mixture was slowly poured into a mixture of ice and water (75 ml) and the product extracted with diethyl ether (4 × 25 ml). The collected extract was washed with saturated sodium chloride solution (25 ml), dried over sodium sulfate, the solvent was evaporated and the obtained residue re-evaporated with toluene to remove residual pyridine. The residue was submitted to column chromatography on silica gel (15 g, cyclohexane–acetoneas an eluent, 2:1). Yield 203 mg (65%) of light yellow amorphous solid, m.p. 73–75 °C, R_F 0.24 (cyclohexane–acetone, 2:1), $[\alpha]_D^{20}$ –57.9 (*c* 0.86, chloroform). IR: 1744 (C=O), 1620 (O=C–N=C). ^{1}H NMR (400 MHz, $CDCl_3$): 8.54 s, 1 H (H-2); 8.45–8.43 m, 1-H (H-4); 7.53–7.50 m, 1 H (H-7); 7.38–7.34 m, 2 H (H-6, H-5); 5.65 d, 1 H, *J*(1′,2′) = 8.7 (H-1′); 5.52 dd, 1 H, *J*(2′,3′) = 9.2, *J*(2′,1′) = 8.7 (H-2′); 5.46 dd, 1 H, *J*(3′,2′) = 9.2, *J*(3′,4′) = 9.4 (H-3′); 5.33 dd, 1 H, *J*(4′,3′) = 9.5, *J*(4′,5′) = 10.0 (H-4′); 4.32 dd, 1 H, *J*(6′b,5′) = 4.9, *J*(6′b,6′a) = 12.5 (H-6′b); 4.22 dd, 1 H, *J*(6′a,5′) = 2.1, *J*(6′a,6′b) = 12.6 (H-6′a); 4.03 ddd, 1 H, *J*(5′,6′a) = 2.2, *J*(5′,6′b) = 4.9, *J*(5′,4′) = 10.0 (H-5′); 2.59 s, 6 H (2 × SCH_3); 2.11 s, 3 H, 2.09 s, 3 H, 2.03 s and 3 H, 1.70 s, 3 H (4 ×

CH_3CO). ^{13}C NMR (100 MHz, $CDCl_3$): 181.7 (CON), 179.3 (C=N), 171.5 (C=O), 170.5, 170.1, 169.3 and 168.4 (4 × $CH_3\mathbf{C}O$), 136.6 (C-2), 135.9 (C-7a), 127.3 (C-3a), 124.4 (C-5), 123.9 (C-6), 123.1 (C-4), 114.5 (C-3), 110.6 (C-7), 84.3 (C-1′), 75.2 (C-5′), 72.9 (C-3′), 70.7 (C-2′), 67.9 (C-4′), 61.7 (C-6′), 20.7, 20.6, 20.5 and 20.0 (4 × $\mathbf{C}H_3CO$), 16.2 (2 × SCH_3). EIMS, *m/z* (%): 474 (19) $[M - CONC(SCH_3)_2]^{•+}$, 169 (53), 206 (100), 148 (16), 144 (22), 109 (24), 75 (13). For $C_{27}H_{30}N_2O_{11}S_2$ (622.7) calculated: 52.08% C, 4.86% H, 4.50% N; found: 52.47% C, 4.51% H, 4.92% N.

2-(1*H*-indol-3-yl)acetyl Chloride (**15**)

To a suspension of acid **14** (0.175 g, 1 mmol) in dry toluene (4 ml) and dry acetonitrile (0.6 ml), phosphorus trichloride (0.174 ml, 0.275 g, 2 mmol) was added and the mixture was stirred at 40 °C for 30 min. The resulting solution was decanted from phosphorus acid deposited on the flask walls, the flask washed with dry toluene (5 ml) and the obtained solution concentrated to approximately 1/4 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.

Methyl 2-(1*H*-Indol-3-yl)acetylcarbamodithioate (**16**)

Method A. To a stirred solution of crude acid chloride **15**, freshly prepared from 1 mmol of acid **14** in dry toluene (1 ml), a solution of methyl carbamodithioate (**A**; 0.107 g, 1 mmol) in pyridine (4 ml) was added and the mixture was stirred at room temperature overnight. After pouring into water (60 ml), the product was extracted with diethyl ether (3 × 30 ml), the extract washed with saturated hydrogen carbonate solution (2 × 60 ml), dried over sodium sulfate, the solvent was evaporated and the residue chromatographed on silica gel (20 g, hexane–ethyl acetate as an eluent, 3:1). Yield 0.040 g (15%, from acid **14**).

Method B. To a stirred solution of acid **14** (0.88 g, 0.5 mmol) in chloroform (3 ml), a solution of DCC (0.113 g, 0.55 mmol) and methyl carbamodithioate (**A**; 0.059 g, 0.55 mmol) in chloroform (3 ml) was added and the reaction mixture was stirred at room temperature for 1 h, and then refluxed for 20 h. The solvent was evaporated and the obtained residue chromatographed on silica gel (10 g, hexane–ethyl acetate as an eluent, 3:1). Yield 0.044 g (32%).

Method C. To a stirred suspension of acid **14** (0.175 g, 1 mmol) in dry dichloromethane (9 ml), a solution of DCC (0.206 g, 1 mmol), methyl carbamodithioate[6b] (**A**; 0.107 g, 1 mmol) and a catalytic amount of DMAP was added and the reaction mixture was stirred at room temperature for 2 h. After adding a small amount of silica gel, the solvent was evaporated and the residue preabsorbed on silica gel was purified by chromatography on silica gel (20 g, hexane–ethyl acetate as an eluent, 3:1). Yield 0.160 g (61%) of light yellow crystals (ethyl acetate–hexane), m.p. 130–132 °C. IR ($CHCl_3$): 3020 (N–H), 1700 (C=O). ^1H NMR (400 MHz, $CDCl_3$): 9.59, 1 H (N-H); 8.28, 1 H (N-H); 7.57 dd, 1 H, *J*(4,5) = 7.9, *J*(4,6) = 1.0 (H-4); 7.42 dd, 1 H, *J*(7,6) = 8.1, *J*(7,5) = 0.9 (H-7); 7.27 ddd, 1 H, *J*(6,7) = 8.1, *J*(6,5) = 7.1, *J*(6,4) = 1.1 (H-6); 7.22 d, 1 H, *J* = 2.5 (H-2); 7.19 ddd, 1 H, *J*(5,4) = 8.0, *J*(5,6) = 7.1, *J*(5,7) = 1.0 (H-5); 3.89 s, 2 H (CH_2); 2.59 s, 3 H (SCH_3). ^{13}C NMR (400 MHz, $CDCl_3$): 204.5 (C=O), 168.4 (C=N), 136.4 (C-7a), 126.6 (C-3a), 123.9 (C-2), 123.0 (C-6), 120.5 (C-5), 118.3 (C-4), 111.6 (C-7), 106.7 (C-3), 34.0 (CH_2), 20.5 (SCH_3). EIMS, *m/z* (%): 264 (7) $[M^{•+}]$, 216 (35), 157 (61), 131 (15), 129 (100), 103 (19), 102 (24), 77 (29). For $C_{12}H_{12}N_2OS_2$ (264.4) calculated: 54.52% C, 4.58% H, 10.60% N; found: 54.31% C, 4.73% H, 10.43% N.

Dimethyl 2-(1*H*-Indol-3-yl)acetylcarbonimidodithioate (**17**)

To a stirred solution of crude acid chloride **15**, freshly prepared from 1 mmol of acid **14**, a solution of dimethyl carbonimidodithioate hydroiodide[6b] (**B**; 0.249 g, 1 mmol) in pyridine (4 ml) was added and the reaction mixture was stirred at room temperature for 30 min. After pouring into water (80 ml), the product was extracted with diethyl ether (4 × 30 ml), the extract washed with saturated solution of sodium hydrogen carbonate (3 × 100 ml), dried over sodium sulfate, the solvent was evaporated and the residue chromatographed on silica gel (30 g, hexane–ethyl acetate as an eluent, 3:1). Yield 0.124 g (45%, from acid **14**) of colorless crystals (acetone–hexane), m.p. 138–140 °C. IR ($CHCl_3$): 3000 (N–H), 1653 (C=O), 1566 (C=N). ^{1}H NMR (400 MHz, $CDCl_3$): 8.16 s, 1 H (N-H); 7.62 dd, 1 H, *J*(4,5) = 7.8, *J*(4,6) = 1.1 (H-4); 7.33 dd, 1 H, *J*(7,6) = 8.1, *J*(5,7) = 0.9 (H-7); 7.19 ddd, 1 H, *J*(6,7) = 8.0, *J*(6,5) = 7.1, *J*(6,4) = 1.1 (H-6); 7.15 s, 1 H (H-2); 7.11 ddd, 1 H, *J*(5,4) = 8.0, *J*(5,6) = 7.1, *J*(5,7) = 1.0 (H-5); 3.98 s, 2 H (CH_2); 2.40 s, 6 H (SCH_3). ^{13}C NMR (400 MHz, $CDCl_3$): 182.7 (C=O), 172.4 (C=N), 136.1 (C-7a), 127.6 (C-3a), 123.3 (C-2), 122.0 (C-6), 119.4 (C-5),119.0 (C-4), 111.1 (C-7), 108.8 (C-3), 35.6 (CH_2), 15.6 (SCH_3). EIMS, *m/z* (%): 278 (8) [$M^{\bullet+}$], 230 (18), 157 (15), 148 (18), 130 (45), 129 (100), 102 (22), 77 (22), 75 (21), 47 (18). For $C_{13}H_{14}N_2OS_2$ (278.4) calculated: 56.09% C, 5.07% H, 10.06% N; found: 56.37% C, 5.18% H, 10.25% N.

This work was supported by the Slovak Research and Development Agency under the contract No. APVV-0514-06. This work was also supported by the State NMR Programme No. 2003SP200280203.

REFERENCES

1. a) Roy S., Eastman A., Gribble G. W.: *Synth. Commun.* **2005**, *35*, 595; b) Guinchard X., Vallée Y., Denis J.-N.: *Org. Lett.* **2007**, *9*, 3761; c) Murai K., Morishita M., Nakatani R., Kubo O., Fujioka H., Kita Y.: *J. Org. Chem.* **2007**, *72*, 8947.
2. a) Kobayashi J., Murayama T., Ishibashi M., Kosuge S., Takamatsu M., Ohizumi Y., Kobayashi H., Ohta T., Nozoe S., Sasaki T.: *Tetrahedron* **1990**, *46*, 7699; b) Jimenez C., Qoinoa E., Adamczeski M., Hunter L. M., Crews P.: *J. Org. Chem.* **1991**, *56*, 3403; c) Carroll A. R., Avery V. M.: *J. Nat. Prod.* **2009**, *72*, 696; d) Devys M., Barbier M.: *Phytochemistry* **1991**, *30*, 389.
3. a) Bacher G., Nickel B., Emig P., Vanhoefer U., Seeber S., Shandra A., Klenner T., Beckers T.: *Cancer Res.* **2001**, *61*, 392; b) Da Settimo F., Simorini F., Taliani S., La Motta C., Marini A. M., Salerno S., Bellandi M., Novellino E., Greco G., Cosimelli B., Da Pozzo E., Costa B., Simola N., Morelli M., Martini C.: *J. Med. Chem.* **2008**, *51*, 5798; c) Taliani S., Cosimelli B., Da Settimo F., Marini A. M., La Motta C., Simorini F., Salerno S., Novellino E., Greco G., Cosconati S., Marinelli L., Salvetti F., Lábbate G., Trasciatti S., Montali M., Costa B., Martini C.: *J. Med. Chem.* **2009**, *52*, 3723; d) Thompson M. J., Borsenberger V., Louth J. C., Judd K. E., Chen B.: *J. Med. Chem.* **2009**, *52*, 7503; e) Wang T., Yin Z., Zhang Z., Bender J. A., Yang Z., Johnson G., Yang Z., Zadjura L. M., D'Arienzo C. J., DiGiugno Parker D., Gesenberg C., Yamanaka G. A., Gong Y-F., Ho H-T., Fang H., Zhou N., McAuliffe B. V., Eggers B. J., Fan L., Nowicka-Sans B., Dicker I. B., Gao Q., Colonno R. J., Lin P-F., Meanwell N. A., Kadow J. F.: *J. Med. Chem.* **2009**, *52*, 7778.
4. a) Mezencev R., Mojžiš J., Pilátová M., Kutschy P.: *Neoplasma* **2003**, *50*, 239; b) Pilátová M., Šarišský M., Kutschy P., Miroššay A., Mezencev R., Čurillová Z., Suchý M., Monde K.,

Miroššay L., Mojžiš J.: *Leukemia Res.* **2005**, *29*, 415; c) Mezencev R., Kutschy P., Salayová A., Čurillová Z., Mojžiš J., Pilátová M., McDonald J.: *Chemotherapy* **2008**, *54*, 372; d) Čurillová Z., Kutschy P., Solčániová E., Pilátová M., Mojžiš J., Kováčik V.: *Arkivoc* **2008**, *viii*, 85; e) Kutschy P., Salayová A., Čurillová Z., Kožár T., Mezencev R., Mojžiš J., Pilátová M., Balentová E., Pazdera P., Sabol M., Zburová M.: *Bioorg. Med. Chem.* **2009**, *17*, 3698; f) Mezencev R., Kutschy P., Salayová A., Updegrove T., McDonald J. F.: *Neoplasma* **2009**, *56*, 321; g) Kutschy P., Mezencev R. in: *Targets in Heterocyclic Systems – Chemistry and Properties* (O. A. Attanasi and D. Spinelli, Eds), Vol. 12, p. 120 (2008). Italian Society of Chemistry, Urbino 2009; h) Kutschy P., Sabol M., Marušková R., Čurillová Z., Dzurilla M., Géci I., Alföldi J., Kováčik V.: *Collect. Czech. Chem. Commun.* **2004**, *69*, 850; i) Kutschy P., Čurillová Z., Mojžiš J., Pilátová M.: Unpublished results.

5. Pedras M. S. C., Ahiahonu P. W. K., Hossain M.: *Phytochemistry* **2004**, *65*, 2685.
6. a) Braun V.: *Ber. Dtsch. Chem. Ges.* **1902**, *35*, 3378; b) Masataka Y., Hidekatsu H.: *Synthesis* **1985**, 891.
7. a) Shaw K. N. F., McMillan A., Gudmundson A. G., Armstrong M. D.: *J. Org. Chem.* **1958**, *23*, 1171; b) Kawasaki T., Kodama A., Nishida T., Shimizu K., Somei M.: *Heterocycles* **1991**, *32*, 221; c) Walton E., Holly F. M., Jenkins S. R.: *J. Org. Chem.* **1968**, *33*, 192; d) Acheson R. M., Hunt P. G., Littlewood D. M., Murrer B. A., Rosenberg H. E.: *J. Chem. Soc., Perkin Trans. 1* **1978**, 1117.
8. Corsaro A., Pistará V.: *Tetrahedron* **1998**, *54*, 15027.
9. a) Zemplén G., Gerecs A., Hadacsy J.: *Ber. Dtsch. Chem. Ges.* **1936**, *69*, 1927; b) Perrin C. L., Fabian A., Brunckova J., Brian K. O.: *J. Am. Chem. Soc.* **1999**, *121*, 6911; c) Sawaki M., Takeda T., Ogihara Y., Shibata S.: *Chem. Pharm. Bull.* **1984**, *32*, 3698; d) Kaneko T., Clardy J., Wong H., Okamoto T. K.: *Tetrahedron Lett.* **1985**, *26*, 4015.
10. a) Cuevas-Yañez E., Muchowski M., Cruz-Almanza R.: *Tetrahedron* **2004**, *60*, 1505; b) Ando R., Kawamuro M., Chiba N.: *J. Med. Chem.* **2001**, *44*, 4468; c) Raucher S., Bray B., Lawrence R.: *J. Am. Chem. Soc.* **1987**, *109*, 442; d) Pedemonte N., Sonawane N. D., Taddei A., Hu J., Zegerra-Moran O., Suen Y. F., Robins L. I., Dicus C. W., Willenbring D., Nantz M. H., Kurth M. J., Galietta L. J. V., Verkman A. S.: *Mol. Pharmacol.* **2005**, *67*, 1797.
11. a) Kutschy P., Dzurilla M., Takasugi M., Török M., Achbergerová I., Homzová R., Rácová M.: *Tetrahedron* **1998**, *54*, 3549; b) Kutschy P., Dzurilla M., Takasugi M., Sabová A.: *Collect. Czech. Chem. Commun.* **1999**, *64*, 348.
12. a) da Silva C. P., de Oliveira C. R., da Conceição M., de Lima P.: *Biochem. Pharmacol.* **1996**, *51*, 1331; b) Hande K. R.: *Eur. J. Cancer* **1998**, *41*, 1514.
13. Mosmann T.: *J. Immunol. Methods* **1983**, *65*, 55.