SYNTHESIS OF SOME ANALOGS OF INDOLE PHYTOALEXINS BRASSININ AND METHOXYBRASSENIN B AND THEIR POSITIONAL ISOMERS

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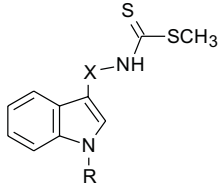
Dedicated to the memory of Dr Miroslav Protiva.

Treatment of indole-3-carboxylic acid with phosphorus trichloride and subsequent reaction of the obtained acid chloride with potassium thiocyanate afforded indol-3-ylcarbonyl isothiocyanate (**13**). Its treatment with sodium hydrogensulfide in the presence of methyl iodide lead to the corresponding methyl dithiocarbamate, an oxo derivative of brassinin (oxobrassinin, **14**), which by methylation with methyl iodide afforded brassenin B (**8**). Corresponding 2-isomers, **21** and **23**, were obtained by an analogous sequence, starting from indole-2-carboxylic acid. During the preparation of isooxobrassinin (**21**), which appeared to be unstable in the basic reaction medium, also an imidazo[3,4-*a*]indole derivative **22** has been isolated as an unexpected side product. Related oxobrassinin analogs and their 2-isomers were prepared by treatment of indol-3- and indol-2-ylcarbonyl isothiocyanate with methanol and amines. In the case of isothiocyanate **13**, besides the expected products of nucleophilic addition to NCS group (monothiocarbamate **25a** and thiourea derivatives **25b**–**25g**), also the substitution products were obtained. Their formation could be explained by partial decomposition of the starting isothiocyanate to an unstable ketene, which reacts with methanol and amines to afford the corresponding methyl carboxylate **26a** and carboxamides **26b**–**26g**. Antifungal activity of the prepared compounds has been examined, using the fungus *Bipolaris leersiae*. All of the compounds exhibited lower activity than phytoalexin brassinin.

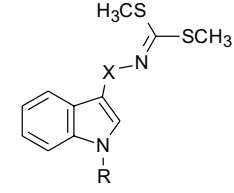
Key words: Indoles; Phytoalexins; Brassinin; Methoxybrassenin B; Isothiocyanates.

Indole phytoalexins are an interesting group of natural products, exhibiting antimicrobial[1] and cancer chemopreventive[2,3] activity. Since 1986, when the first three indole phytoalexins, named brassinin (**1**), methoxybrassinin (**2**) and cyclobrassinin (**9**) were isolated from Chinese cabbage[1,4], more than

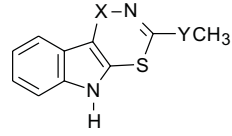
twenty related compounds have been isolated from the plant family *Cruciferae*, including many economically important vegetables. Their isolation, structure and biological properties have been summarized in two review articles[5,6]. The unique structural feature of these compounds is the presence of indole ring and a side chain or another heterocycle, containing a nitrogen atom and one or two sulfur atoms. Usually, these moieties are linked to the 3-position of indole nucleus *via* CH_2 group. In the case of methoxybrassenin A (**5**, ref.[7]) and cyclobrassinin (**9**, ref.[1]), also the corresponding oxo derivatives, namely methoxybrassenin B (**6**, ref.[7]) and cyclobrassinon (**10**, ref.[8]) were isolated from cabbage and kohlrabi, respectively. The compound **3** (oxobrassinin) has not been found yet in cruciferous plants. Isolation from plants does not afford sufficient quanti-



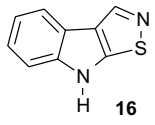
	X	R
1	CH_2	H
2	CH_2	OCH_3
3	CO	H
4	$(CH_2)_2$	H



	X	R
5	CH_2	OCH_3
6	CO	OCH_3
7	CH_2	H
8	CO	H



	X	Y
9	CH_2	S
10	CO	O



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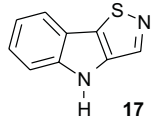

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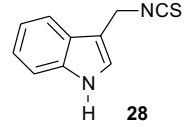

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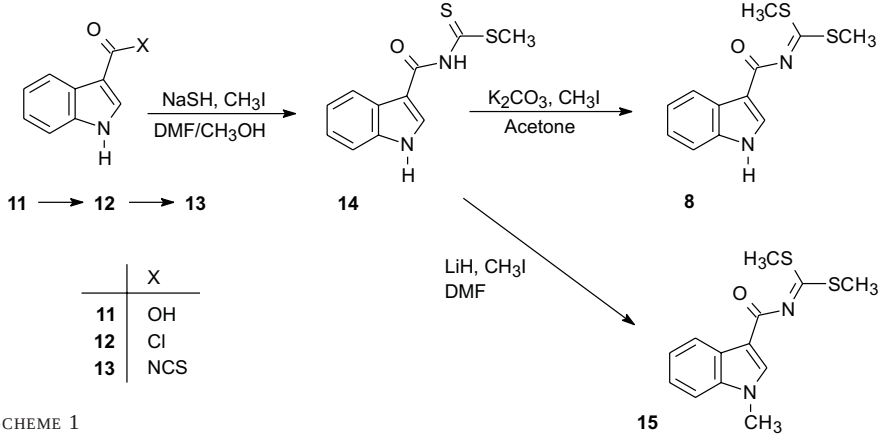
ties of indole phytoalexins for biological screening and therefore it is quite important to investigate the synthesis of these compounds and their analogs. In some cases, an analog can be more active than natural phytoalexin. It was recently found, that homobrassinin (**4**) exhibits a higher antifungal activity than brassinin (**1**, ref.[9]). Preparation of indole phytoalexins and their analogs is often complicated by instability of intermediates and low yields of final products, particularly, when 1-unsubstituted indoles are used. Brassinin and its derivatives were synthesized in 12–72% overall yields from substituted indoles and indole-3-carboxaldehydes *via* (indol-3-ylmethyl)amine derivatives, which

were treated with carbon disulfide and methyl iodide[1,3,4,10–12]. Brassinin and its derivatives can be cyclized with pyridinium bromide perbromide[1,4] or *N*-bromosuccinimide[3,11–14] to cyclobrassinin and its derivatives in 34–61% yields. Methoxybrassenin A (**5**, ref.[7]) and corresponding demethoxy analog **7** (brassenin A, ref.[12]) were obtained by methylation of methoxybrassinin (**2**) and brassinin (**1**) with methyl iodide in methanol or acetone in the presence of potassium carbonate in 87 and 40% yield, respectively. On the other hand, no attention has been devoted yet to the synthesis of the indole phytoalexins or their analogs having the side chain or another heterocycle linked to the 3-position of indole ring *via* C=O group. The aim of the present study was to synthesize the oxobrassinin (**3**), brassenin B (**8**), their isomers with the side chain in 2-position, as well as related monothiocarbamates and thioureas, and to compare the antifungal activity of synthesized compounds with phytoalexin brassinin. To achieve this goal, we decided to build up the dithiocarbamate and dithiocarbonate side chain, present in target compounds, *via* indol-3- and indol-2-ylcarbonyl isothiocyanates (**13** and **20**) as the key intermediates and by using the carboxylic acids **11** and **18** as starting compounds. In the first step, transformation of acids **11** and **18** to acid chlorides **12** and **19** was required. Previously described procedures, using thionyl chloride[15–17] are suitable for the preparation of dilute solutions of crude chlorides, used in the reaction with amines, where the acidic residues are neutralized by an excess of reagent. Because of their high instability, acid chlorides **12** and **19** cannot be purified by distillation or crystallization to remove acidic residues, which are undesired in the next step. We have found, that the difficulties, which are frequently associated with the synthesis of heteroarenecarboxylic acid chlorides[18] can be solved by using phosphorus trichloride. Indole-3-carbonyl chloride (**12**) can be advantageously prepared by treatment of acid **11** with an excess of phosphorus trichloride in dry benzene in the presence of acetonitrile at room temperature for 20 min, according to the slightly modified method, recently described for the preparation of 3-(indol-3-yl)propenoyl chloride[19]. The excess of phosphorus trichloride was removed by concentration of the obtained solution, and the reaction of crude chloride **12** with potassium thiocyanate in dry acetone afforded unstable indol-3-ylcarbonyl isothiocyanate (**13**). Infrared spectrum of crude isothiocyanate **13**, taken immediately after evaporation of acetone exhibited characteristic absorption bands ν(N=C=S) at 1 970 cm^{-1} and ν(N–H) at 3 467 cm^{-1}. Treatment of freshly prepared isothiocyanate **13** with sodium hydrogensulfide in the presence of methyl iodide resulted in the formation of oxobrassinin (**14**) in 31% yield (Scheme 1). Methylation of **14** with

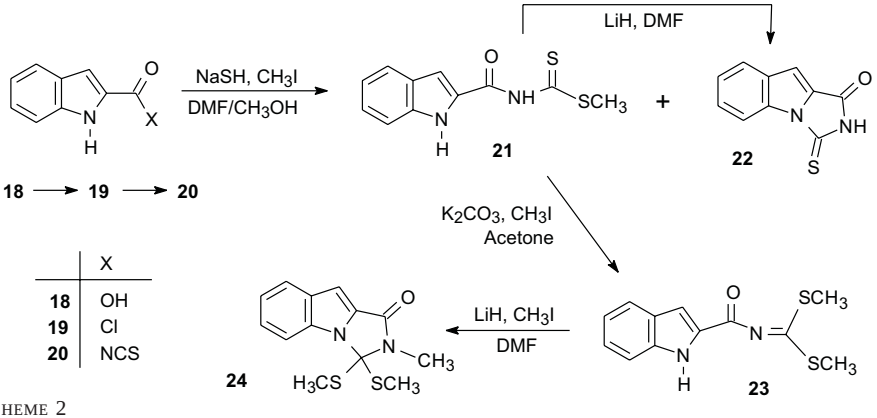
methyl iodide in dry acetone in the presence of potassium carbonate afforded brassenin B (**8**), a demethoxy analog of methoxybrassenin B (**6**) in 87% yield. Another analog, interesting from the point of view of its possible biological activity is methylbrassenin B (**15**). It could be prepared by methylation of methyloxobrassinin, using 1-methylindole-3-carboxylic acid as starting compound. However, it was attractive to examine, if **15** could be obtained by a twofold methylation of **14**. It was found, that by treatment of **14** with an excess of methyl iodide in dimethylformamide in the presence of two equivalents of lithium hydride, methylbrassenin B (**15**) is formed in 91% yield.



SCHEME 1

Positional isomers in general differ in their biological activities. This was also observed in the case of indole phytoalexin brassilexin (**16**), and its analog isobrassilexin (**17**), where the latter exhibits a higher cytotoxic activity[20]. Therefore, we have decided to study the preparation of isooxobrassinin (**21**) and isobrassenin B (**23**, Scheme 2). The synthetic procedures, leading to **21** and **23**, were expected to be identical with those, used for the preparation of **8** and **14**. However, the acid **18** appeared to be significantly less reactive toward phosphorus trichloride in the presence of acetonitrile in benzene at room temperature than indole-3-carboxylic acid (**11**) and, under these conditions, no chloride **19** was formed. The desired transformation was successfully brought about by heating for one hour in benzene at 85–90 °C without addition of acetonitrile. Reaction of an unstable chloride **19** with potassium thiocyanate in dry acetone afforded isothiocyanate **20**, which was used in the next reaction as an unstable crude intermediate. Sur-

prisingly, treatment of **20** with sodium hydrogensulfide in the presence of methyl iodide resulted in the formation of a mixture of expected dithiocarbamate **21** (isooxobrassinin) in 19% yield, together with a 9% yield of unexpected imidazo[3,4-*a*]indole derivative **22**. It was assumed, that dithiocarbamate **21** cyclized to **22** in the slightly basic reaction me-

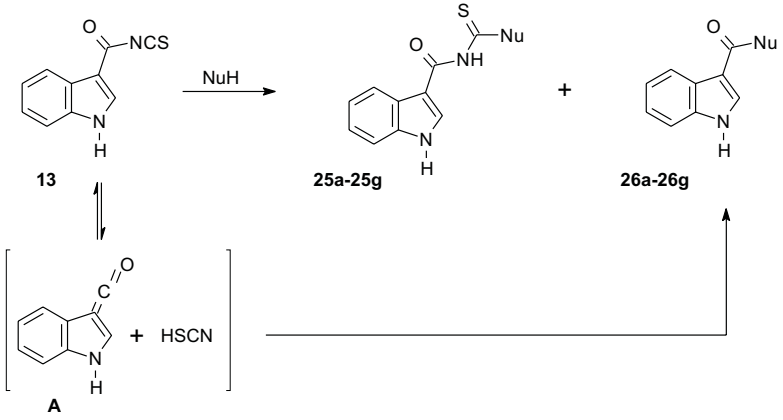


SCHEME 2

dium. This assumption was confirmed by independent cyclization of **21** to **22** with lithium hydride in dimethylformamide in 99% yield. Methylation of dithiocarbamate **21** with methyl iodide in dry acetone in the presence of potassium carbonate afforded isobrassenin B (**23**), a positional isomer of brassenin B (**8**). The attempts to prepare methylisobrassenin B (positional isomer of **15**) by methylation of **23** have failed. Using methyl iodide in acetone in the presence of potassium carbonate, no reaction occurred, even after prolonged heating, whereas in dimethylformamide with lithium hydride and methyl iodide, a competitive cyclization took place, followed by methylation with the formation of derivative **24**. Structure of the compounds **22** and **24** was confirmed by spectral methods. In ^{1}H NMR spectrum of the derivative **22**, only the signals of aromatic protons at 7.38–8.28 ppm are present, whereas the spectrum of **24** exhibits also the signals of two SCH_3 groups at 1.50 ppm and a signal of NCH_3 group at 3.15 ppm. In the mass spectra, the *m/z* values of molecular ions at 202 (100%, **21**) and 278 (4%, **24**) are in accord with the expected molecular weights.

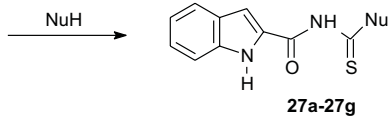
To compare the antifungal activity of previously described brassinin analogs, having the methylsulfanyl group replaced by an alkoxy or amino group[12], with corresponding oxo derivatives, we have also studied the reactions of isothiocyanate **13** with methanol and piperidine. Surprisingly, in

both cases we have obtained a mixture of addition and substitution products, *i.e.* monothiocarbamate **25a** (8%) or thiourea **25f** (17%), and ester **26a** (29%) or amide **26f** (20%, Scheme 3). To study the generality of this unexpected behaviour of isothiocyanate **13** in a reaction with nucleophilic reagents, a series of amines was used, affording in all cases a mixture of thiourea derivatives (**25b**–**25g**) and amides (**26b**–**26g**, Scheme 3, Table I).



In the formulae **25-27**:

	Nu
a	CH_3O
b	CH_3NH
c	$PhCH_2NH$
d	$(CH_2)_5CHNH$
e	$4\text{-}CH_3\text{-}C_6H_4\text{-}NH$
f	$(CH_2)_5N$
g	$O(CH_2)_4N$



SCHEME 3

Preparation of compounds **25b**–**25g** required the use of an excess of amine to bring the reaction to completition. Most probably, the thiocyanic acid, liberated during the formation of amides, is bonded to hitherto unreacted amine, to produce corresponding ammonium thiocyanates. Previously, in the reaction of pent-4-ynoyl isothiocyanate with amines, we were able to isolate these salts because the reactions were performed in benzene, in which the insoluble salts precipitated, whereas the other products (thioureas and amides) remained in solution[21]. Indol-3-ylcarbonyl isothiocyanate (**13**) is insoluble in benzene and its reactions with nucleophiles were performed in acetone. Isolation of products required the

use of column chromatography after evaporation of acetone; hence, the highly polar salts could not be isolated. To obtain more information about nucleophilic additions to hitherto unknown indolylcarbonyl isothiocyanates, also the reactions of indol-2-ylcarbonyl isothiocyanate (**20**) with the same nucleophiles has been carried out. In this case, however, the only products obtained in 22–45% yield were the corresponding monothiocarbamate **27a** and thiourea derivatives **27b**–**27g** (Scheme 3, Table I). These observations indicate a specific reactivity of indol-3-ylcarbonyl isothiocyanate (**13**). A dramatic difference between isothiocyanates **13** and **20** cannot be explained on the basis of the present experience about the selectivity of nucleophilic addition to and substitution at isothiocyanatocarbonyl group[21]. Substitution of NCS group was observed to proceed simultaneously with nucleophilic addition only in the reactions of some aliphatic, but not aromatic acyl isothiocyanates with amines. Even in the case of pent-4-ynoyl isothiocyanate, with the prevailing tendency to the formation of substitution products with amines, treatment with methanol afforded corresponding monothiocarbamate as the sole reaction product[21]. On the contrary, the formation of the substitution product in the case of isothiocyanate **13** is most pronounced in the reaction with methanol. This reaction is preferably performed at 70–75 °C, however the same ratio of products **25a** and **26a** was obtained at room temperature, whereas the reaction time was prolonged from 10 min to 24 h. Based on the above mentioned findings, the formation of ester **26a** and amides **26b**–**26g** is not likely to proceed by direct substitution of NCS group. It can be assumed, that the reason consists in the activation effect of indole nitrogen lone pair into 3-position, resulting in partial decomposition of isothiocyanate **13** to thiocyanic acid and unstable ketene intermediate (**A**, Scheme 3). The reverse reaction is also possible and thus both isothiocyanate **13** and ketene **A** can be simultaneously present in the reaction mixture. Consequently, the ester **26a** and amides **26b**–**26g** are formed by the addition of a nucleophile to ketene **A**, whereas monothiocarbamate **25a** and thiourea derivatives **25b**–**25g** are produced by nucleophilic addition to isothiocyanate **13**. Electron-withdrawing character of carbonyl group in the 3-position enhances the stability of indol-3-ylcarbonyl isothiocyanate (**13**), when compared with indol-3-ylmethyl isothiocyanate (**28**), a postulated intermediate in the biosynthesis of indole phytoalexins[28,29], which cannot be isolated or synthesized due to its intrinsic instability[12]. An interaction between the indole nitrogen lone pair and carbonyl group in the 3-position was suggested for the explanation of infrared spectrum of 2,3-dihydrocarbazol-4(1*H*)-one, showing no absorption in the 1 600–1 700 cm^{-1} region[30]. Ketene **A** was pro-

TABLE I
Physicochemical properties of products **25a–27g**

Compound	Formula M.w.	M.p., °C Solvent[b]	Yield[a] %	Calculated/Found		
				% C	% H	% N
25a	$C_{11}H_{10}N_2O_2S$ 234.3	174–176 A–C	8	56.40 56.12	4.30 4.15	11.96 11.68
25b	$C_{11}H_{11}N_3OS$ 233.3	205–207 A–W	24	56.63 56.41	4.75 4.56	18.01 17.83
25c	$C_{17}H_{15}N_3OS$ 309.4	185–187 A–W	18	66.00 66.13	4.89 4.97	13.58 13.52
25d	$C_{16}H_{19}N_3OS$ 301.4	199–201 D–H	38	63.76 63.89	6.35 6.09	13.94 14.12
25e	$C_{11}H_{11}N_3OS$ 309.4	183–185 E–W	12	66.00 66.28	4.89 5.02	13.58 13.32
25f	$C_{15}H_{17}N_3OS$ 287.4	178–180 A–H	17	62.69 62.76	5.99 6.11	14.62 14.37
25g	$C_{14}H_{15}N_3O_2S$ 289.4	196–198 A–C	4	58.11 58.40	5.23 5.48	14.52 14.68
26a	$C_{10}H_9NO_2$ 175.2	148–150[c] M–W	29	68.56 68.32	5.18 5.21	7.99 7.75
26b	$C_{10}H_{10}N_2O$ 174.2	166–168 A–W	9	68.95 68.76	5.79 5.90	16.08 15.81
26c	$C_{16}H_{14}N_2O$ 250.3	175–177[d] A–W	22	76.78 76.52	5.64 5.89	11.19 11.02
26d	$C_{15}H_{18}N_2O_2$ 242.3	201–202[e] A–C	35	74.35 74.55	7.49 7.29	11.56 11.63
26e	$C_{16}H_{18}N_2O$ 250.3	199–200[f] E–W	24	76.78 76.61	5.64 5.78	11.19 10.95
26f	$C_{14}H_{16}N_2O$ 228.3	159–161[g] A–C	18	73.66 73.48	7.06 6.89	12.27 12.31
26g	$C_{13}H_{14}N_2O$ 230.3	220–222 A–C	35	67.81 67.69	6.13 6.04	12.17 12.31
27a	$C_{11}H_{10}N_2O_2S$ 233.3	175–177 M–W	45	56.40 56.25	4.30 4.49	11.96 11.80

TABLE I
(*Continued*)

Compound	Formula M.w.	M.p., °C Solvent[b]	Yield[a] %	Calculated/Found		
				% C	% H	% N
27b	$C_{11}H_{11}N_3OS$	228–230	22	56.63	4.75	18.01
	233.3	E–W		56.39	4.92	17.90
27c	$C_{17}H_{15}N_3OS$	199–201	39	66.00	4.89	13.58
	309.4	CH–P		66.19	5.05	13.48
27d	$C_{19}H_{19}N_3OS$	182–183	25	63.76	6.35	13.94
	301.4	M–W		63.51	6.44	14.17
27e	$C_{17}H_{15}N_3OS$	195–197	23	66.00	4.89	13.58
	309.4	E		65.83	5.11	13.39
27f	$C_{15}H_{17}N_3OS$	159–161	45	62.69	5.96	14.62
	287.4	E–W		62.77	6.08	14.83
27g	$C_{14}H_{15}N_3O_2S$	175–177	25	58.11	5.23	14.52
	289.4	E–W		58.35	5.13	14.41

[a] Calculated from corresponding carboxylic acid **11** or **18**. [b] A Acetone, C cyclohexane, CH chloroform, D dichloromethane, E ethanol, H hexane, P light petroleum, W water. [c] Ref.[22] 145 °C. [d] Ref.[23] 178 °C. [e] Ref.[24] 205–207 °C. [f] Ref.[25] 200.9–201.1 °C. [g] Ref.[26] 164 °C, Ref.[27] 161–163 °C.

posed as an intermediate in the synthesis of indole-3-carboxylates[22] and indole-3-carboxamides[23] by photolysis of 3-diazoquinolin-4(3*H*)-one in the presence of alcohols and amines. It may be also involved in the chemistry of 3-trifluoroacetylindole, which with lithium dialkyl amides affords corresponding indole-3-carboxamides[27]. Since isothiocyanates are less reactive toward alcohols than toward amines, prevailing formation of ester **26a** in the reaction of isothiocyanate **13** with methanol can be explained by effective trapping of ketene **A** with methanol.

Antifungal activity of oxobrassinin (**14**), brassenin B (**8**) and their analogs (**15**, **25a**–**25g**) as well as their positional isomers (**21**, **23**, **27a**–**27g**) was examined by TLC bioassay, using the fungus *Bipolaris leersiae*. The com-

pounds, showing a distinct antifungal spots were tested quantitatively and compared with brassinin (**1**), which completely inhibited the conidial germination of the fungus at a concentration of 0.1 mmol l^{-1}. All of the substances exhibited lower activity than brassinin. Interestingly, the highest activity was showen by the monothiocarbamate **27a**, a positional isomer having the SCH_3 group replaced by OCH_3 group, which completely inhibited the germination at 0.25 mmol l^{-1}, and by thiourea derivative **27f** (1 mmol l^{-1}). The other compounds exhibited very low or no activity. Therefore, the preparation of further analogs of methoxybrassenin B by methylation of **25a**–**25g** or **27a**–**27g** has not been studied. Similarly, low activities have been recently found by investigation of the related analogs of brassinin and methoxybrassenin A (ref.[12]). It can be expected that analogs of this type will probably not lead to compounds with significant antifungal activities.

EXPERIMENTAL

The infrared absorption spectra were recorded on an IR-75 spectrometer (Zeiss, Jena) in chloroform except compounds **25c**, **25e**–**25g**, **26c** and **26e** which were measured in KBr discs; the wavenumbers are given in cm^{-1}. ^1H NMR spectra were measured on a TESLA BS 487A (80 MHz) spectrometer in hexadeuterioacetone (compounds **14**, **25b**–**25e**, **26g**, **27a** and **27b**), deuteriochloroform (compounds **8**, **15**, **23**, **24**, **25a**, **26a**, **26f** and **27d**), hexadeuteriodimethyl sulfoxide (compound **22**), in a mixture of hexadeuteriodimethyl sulfoxide and hexadeuterioacetone (compounds **25f**, **25g**, **26d** and **27f**) and in a mixture of hexadeuteriodimethyl sulfoxide and deuteriochloroform (compounds **21**, **27c**, **27e** and **27g**). Chemical shifts (δ) are reported in ppm downfield from tetramethylsilane, coupling constants (*J*) in Hz. The mass spectra were recorded on a JMS-100D spectrometer (Jeol) at ionization energy 70 eV. The reaction course was monitored by thin layer chromatography, using the Silufol plates (Kavalier). The preparative column chromatography was performed on Kieselgel Merck, Typ 9385, 230–400 mesh. Antifungal activity was examined according to previously described procedure[12]. Indole-3-carboxylic acid (**11**) was prepared by oxidation of indole-3-carbaldehyde with potassium permanganate[31].

Indol-3-ylcarbonyl Isothiocyanate (**13**)

To a stirred suspension of indole-3-carboxylic acid (**11**; 806 mg, 5 mmol) in a mixture of dry benzene (25 ml) and dry acetonitrile (3 ml), phosphorus trichloride (610 mg, 440 µl, 5 mmol) was added and the mixture was stirred for 25 min at room temperature. The resulting solution was decanted from the phosphorous acid, deposited on the flask walls, the flask washed with dry benzene (5 ml) and obtained solution concentrated to approximately 1/5 of its original volume (bath temperature up to 30 °C), to remove the excess of phosphorus trichloride. The obtained solution of acid chloride **12** was diluted with dry acetone (10 ml) and added in one portion to a solution of potassium thiocyanate (0.48 g, 5 mmol) in dry acetone (30 ml). The mixture was stirred for 30 min at room temperature, filtered with the aid of charcoal and the flask washed with dry acetone (10 ml). The obtained acetone solution of

crude isothiocyanate **13** can be used for the next reaction. Evaporation of the solvent afforded unstable crude product. IR: 3 467 (NH), 1 970 (NCS), 1 686 (C=O).

Methyl *N*-(Indol-3-ylcarbonyl)dithiocarbamate (Oxobrassinin, **14**)

An acetone solution of crude isothiocyanate **13** (from 5 mmol of acid **11**) was evaporated to dryness (bath temperature up to 30 °C) and the obtained semi-solid residue was immediately dissolved in dimethylformamide (12 ml), containing methyl iodide (1.06 g, 470 μl, 7.5 mmol). A freshly prepared solution of sodium hydrogensulfide (430 mg, 5 mmol) in methanol (10 ml) was then added dropwise into this solution during 2–3 min with stirring and water cooling. The reaction mixture was then diluted with cold water (100 ml) and after standing at 5 °C for 30 min the separated precipitate was filtered off with suction, washed with water and dried. Yield 390 mg (31%), m.p. 190–193 °C (decomp., acetone–cyclohexane). For $C_{11}H_{10}N_2OS_2$ (250.3) calculated: 52.78% C, 4.03% H, 11.19% N; found: 52.60% C, 4.18% H, 11.37% N. IR: 3 467 and 3 400 (NH), 1 680 (C=O), 1 453 (NHCS). ^{1}H NMR: 2.67 s, 3 H (SCH_3); 7.29 m, 2 H, 7.59 m, 1 H, 8.29 m, 1 H and 8.58 m, 1 H (H-arom.); 11.17 s, 1 H (NH).

Dimethyl *N*-(Indol-3-ylcarbonyl)dithiocarbonimidate (Brassenin B, **8**)

To a solution of dithiocarbamate **14** (250 mg, 1 mmol) in dry acetone (10 ml) was added methyl iodide (426 mg, 187 μl, 3 mmol) and anhydrous potassium carbonate (138 mg, 1 mmol) and the mixture was stirred for 165 min at room temperature. After pouring into 50 ml of cold water and standing at 5 °C for 30 min, the separated precipitate was filtered off with suction, washed with water and dried. Yield 230 mg (87%), m.p. 143–145 °C (acetone–water). For $C_{12}H_{12}N_2OS_2$ (264.4) calculated: 54.52% C, 4.58% H, 10.60% N; found: 54.73% C, 4.32% H, 10.43% N. IR: 3 474 (NH), 1 620 (C=N–C=O). ^{1}H NMR: 2.58 s, 6 H (2 × SCH_3); 7.33 m, 3 H, 7.98 d, 1 H, J = 3 and 8.25 m, 1 H (H-arom.); 11.30 s, 1 H (NH).

Dimethyl *N*-[(1-Methylindol-3-yl)carbonyl]dithiocarbonimidate (Methylbrassenin B, **15**)

To a suspension of lithium hydride (16 mg, 2 mmol) in dimethylformamide (10 ml) was added oxobrassinin (**14**, 250 mg, 1 mmol) and the mixture was stirred at room temperature for 20 min. Methyl iodide (568 mg, 249 μl, 4 mmol) was then added and stirring was continued for 1 h. Reaction mixture was slowly poured into 100 ml of cold water with intensive stirring. After standing overnight at 5 °C, the separated precipitate was filtered off with suction, washed with water and dried. Yield 240 mg (91%), m.p. 82–84 °C (dichloromethane–hexane). For $C_{13}H_{14}N_2OS_2$ (278.4) calculated: 56.09% C, 5.07% H, 10.06% N; found: 55.95% C, 4.93% H, 9.89% N. IR: 1 620 (C=N–C=O). ^{1}H NMR: 2.60 s, 6 H (2 × SCH_3); 3.85 s, 3 H (NCH_3); 7.30 m, 3 H, 7.82 s, 1 H and 8.28 m, 1 H (H-arom.).

Indol-2-ylcarbonyl Isothiocyanate (**20**)

To a suspension of indole-2-carboxylic acid (**18**; 806 mg, 5 mmol) in dry benzene (25 ml) was added phosphorus trichloride (610 mg, 440 μl, 5 mmol) and the mixture was stirred for 1 h at 85–90 °C (bath temperature). The subsequent procedure, identical with the procedure for the preparation of **13**, afforded unstable crude isothiocyanate **20**. IR: 3 471 (NH), 1 960 (NCS), 1 676 (C=O).

Methyl *N*-(Indol-2-ylcarbonyl)dithiocarbamate (Isooxobrassinin, **21**) and 1-Thioxo-1*H*-imidazo[3,4-*a*]indol-3(2*H*)-one (**22**)

The crude indol-2-ylcarbonyl isothiocyanate (**20**; from 5 mmol of acid **18**) was treated with sodium hydrogensulfide in the presence of methyl iodide, according to the procedure for **14**. The crude product was chromatographed on 60 g of silica gel, eluent benzene, affording 235 mg (19%) of dithiocarbamate **21** and 92 mg (9%) of compound **22**.

Methyl N-(indol-2-ylcarbonyl)dithiocarbamate (**21**). M.p. 171–173 °C (chloroform–light petroleum). For $C_{11}H_{10}N_2OS_2$ (250.3) calculated: 52.78% C, 4.03 %H, 11.19% N; found: 52.94% C, 3.82% H, 10.96% N. IR: 3 455 and 3 393 (NH), 1 685 (C=O), 1 470 (NHCS). ^{1}H NMR: 2.68 s, 3 H (SCH_3); 7.02–7.75 m, 5 H (H-arom.); 9.21 s, 1 H (NH).

1-Thioxo-1H-imidato[3,4-a]indol-3(2H)-one (**22**). M.p. 256–259 °C (acetone–hexane). For $C_{10}H_6N_2OS$ (202.2) calculated: 59.39% C, 2.99% H, 13.85% N; found: 59.53% C, 2.78% H, 14.06% N. IR: 3 434 (NH), 1 763 (C=O). ^{1}H NMR: 7.38 s, 1 H, 7.70 m, 3 H and 8.28 m, 1 H (H-arom.). MS, *m/z* (%): 202 (M^+, 100), 143 (95), 115 (52).

Cyclization of Dithiocarbamate **21** to Imidazo[3,4-*a*]indole (**22**)

To a stirred suspension of lithium hydride (8 mg, 1 mmol) in dimethylformamide (2 ml) was added dithiocarbamate **21** (50 mg, 0.2 mmol). The mixture was stirred at room temperature for 10 min, poured into cold water (12 ml) and neutralized with diluted hydrochloric acid (1 : 1). Separated precipitate of **22** was filtered off with suction, washed with water and dried. Yield 40 mg (99%).

Dimethyl *N*-(Indol-2-ylcarbonyl)dithiocarbonimidate (Isobrassenin B, **23**)

To a solution of dithiocarbamate **21** (125 mg, 0.5 mmol) in dry acetone (5 ml) was added methyl iodide (213 mg, 94 µl, 1.5 mmol) and anhydrous potassium carbonate (69 mg, 0.5 mmol) and the mixture was stirred for 100 min at room temperature. After pouring into 50 ml of cold water, the separated precipitate was filtered off with suction, washed with water and dried. Yield 110 mg (83%), m.p. 160–162 °C (acetone–water). For $C_{12}H_{12}N_2OS_2$ (264.4) calculated: 54.52% C, 4.58% H, 10.60% N; found: 54.46% C, 4.41% H, 10.71% N. IR: 4 370 (NH), 1 620 (C=N–C=O). ^{1}H NMR: 2.58 s, 6 H (2 × SCH_3); 7.05–7.75 m, 5 H (H-arom.); 9.40 s, 1 H (NH).

2-Methyl-1,1-bis(methylsulfanyl)-1*H*-imidazo[3,4-*a*]indol-3(2*H*)-one (**24**)

To a stirred suspension of lithium hydride (16 mg, 2 mmol) in dimethylformamide (10 ml) at room temperature was added isobrassenin B (**23**, 254 mg, 0.96 mmol). After 20 min, methyl iodide (567 mg, 294 µl, 4 mmol) was added and stirring was continued for 50 min. The mixture was then poured into cold water (100 ml) with intensive stirring and set aside for 30 min at 5 °C. The separated precipitate was filtered off with suction, washed with water and dried. Yield 240 mg (90%), m.p. 138–139 °C (acetone–water). For $C_{13}H_{14}N_2OS_2$ (278.4) calculated: 56.09% C, 5.07% H, 10.06% N; found: 55.94% C, 5.24% H, 9.90% N. IR: 1 693 (C=O). ^{1}H NMR: 1.50 s, 6 H (2 × SCH_3); 3.15 s, 3 H (NCH_3); 6.95 s, 1 H, 7.32 m, 2 H and 7.81 m, 2 H (H-arom.). MS, *m/z* (%): 278 (M^+, 4), 231 (100), 216 (7), 143 (14), 115 (10), 88 (49).

Methyl *N*-(Indol-3-ylcarbonyl)thiocarbamate (**25a**) and Methyl Indole-3-carboxylate (**26a**)

An acetone solution of crude isothiocyanate **13** (from 2 mmol of acid **11**) was evaporated to dryness, the residue dissolved in 10 ml of dry methanol and the obtained solution was heated with stirring at 70–75 °C (bath temperature) for 10 min. After pouring into 100 ml of cold water and standing for 2 days at 5 °C, the separated precipitate was filtered off with suction, washed with water, dried and chromatographed on 25 g of silica gel, eluent cyclohexane–acetone (2 : 1), affording compounds **25a** and **26a** (Table I).

Methyl N-(indol-3-ylcarbonyl)thiocarbamate (**25a**). IR: 3 466 and 3 433 (NH), 1 687 (C=O), 1 487 (NHCS). ^{1}H NMR: 4.09 s, 3 H (OCH_3); 7.35 m, 2 H, 7.62 m, 1 H, 8.36 m, 1 H and 8.50 m, 1 H (H-arom.).

3-Substituted 1-(Indol-3-ylcarbonyl)thioureas (**25b**–**25g**) and *N*-Substituted Indol-3-carboxamides (**26b**–**26g**)

To an acetone solution of crude isothiocyanate **13** (from 2 mmol of acid **11**) was added methylamine (992 mg of 25% aqueous solution, 8 mmol), benzylamine (429 mg, 437 µl, 4 mmol), cyclohexylamine (298 mg, 343 µl, 3 mmol), *p*-toluidine (429 mg, 4 mmol), piperidine (340 mg, 396 µl, 4 mmol) or morpholine (348 mg, 348 µl, 4 mmol) and the mixture was stirred for 20 min at room temperature. After evaporation of the solvent, the residue was chromatographed on 40 g of silica gel, eluent cyclohexane–acetone (2 : 1) (**25b**, **26b**; **25c**, **26c**; **25d**, **26d**; **25g**, **26g**), or 80 g (**25e**, **26e**) and 50 g (**25f**, **26f**) of silica gel, using a mixture benzene–acetone (7 : 1) as eluent. The obtained products were crystallized from a suitable solvent (Table I).

1-(Indol-3-ylcarbonyl)-3-methylthiourea (**25b**). IR: 3 466 and 3 260 (NH), 1 656 (C=O), 1 497 (NHCS). ^{1}H NMR: 3.80 d, 3 H, J = 5 (NCH_3); 7.25 m, 2 H, 7.55 m, 1 H, 8.22 m, 1 H and 8.55 m, 1 H (H-arom.); 9.70 s, 1 H and 10.90 s, 2 H (NH).

1-Benzyl-3-(indol-3-ylcarbonyl)thiourea (**25c**). IR: 3 497 and 3 342 (NH), 1 623 (C=O), 1 543 (NHCS). ^{1}H NMR: 5.00 d, 2 H, J = 5 (CH_2); 7.20–7.65 m, 8 H, 8.22 m, 1 H and 8.60 s, 1 H (H-arom.); 9.75 s, 1 H (NH).

1-Cyclohexyl-3-(indol-3-ylcarbonyl)thiourea (**25d**). IR: 3 466 and 3 250 (NH), 1 656 (C=O), 1 500 (NHCS). ^{1}H NMR: 1.20–1.87 m, 10 H ((CH_2)$_5$); 4.30 m, 1 H (CH); 7.25 m, 2 H, 7.55 m, 1 H, 8.25 m, 1 H and 8.55 d, 1 H, J = 3 (H-arom.); 9.50 s, 1 H and 11.14 s, 2 H (NH).

1-(Indol-3-ylcarbonyl)-3-(4-methylphenyl)thiourea (**25e**). IR: 3 444 and 3 357 (NH), 1 633 (C=O), 1 507 (NHCS). ^{1}H NMR: 2.37 s, 3 H (CH_3); 7.55 m, 4 H, 7.67 m, 3 H, 8.32 m, 1 H and 8.65 s, 1 H (H-arom.); 9.81 s, 1 H (NH).

1-[N-(Indol-3-ylcarbonyl)thiocarbamoyl]piperidine (**25f**). IR: 3 390 and 2 314 (NH), 1 653 (C=O), 1 514 (NHCS). ^{1}H NMR: 1.71 m, 6 H ((CH_2)$_3$); 3.92 m, 4 H (CH_2NCH_2); 7.20 m, 2 H, 7.48 m, 1 H, 8.25 m, 1 H and 8.43 m, 1 H (H-arom.); 9.38 s, 1 H (NH).

4-[N-(Indol-3-ylcarbonyl)thiocarbamoyl]morpholine (**25g**). IR: 3 453 and 3 394 (NH), 1 610 (C=O), 1 515 (NHCS). ^{1}H NMR: 3.78 m, 8 H ((CH_2)$_4$); 7.20 m, 2 H, 7.48 m, 1 H, 8.20 m, 1 H and 8.42 s, 1 H (H-arom.); 9.78 s, 1 H (NH).

3-(Methylaminocarbonyl)indole (**26b**). IR: 3 474 and 3 283 (NH), 1 633 (C=O). ^{1}H NMR: 2.90 d, 3 H, J = 4 (CH_3); 7.15 m, 2 H, 7.49 m, 1 H, 7.79 m, 1 H and 8.25 m, 1 H (H-arom.).

4-(Indol-3-ylcarbonyl)morpholine (**26g**). IR: 3 480 (NH), 1 607 (C=O). ^{1}H NMR: 3.70 m, 8 H ((CH_2)$_4$); 7.15 m, 2 H, 7.50 m, 1 H and 7.76 m, 2 H (H-arom.).

Methyl *N*-(Indol-2-ylcarbonyl)thiocarbamate (**27a**)

An acetone solution of crude isothiocyanate **20** (from 5 mmol of acid **18**) was evaporated to dryness, the residue dissolved in 25 ml of dry methanol and the obtained solution was stirred at 70–75 °C (bath temperature) for 10 min. The mixture was poured into 150 ml of cold water and after standing for 20 min, the separated precipitate was filtered off with suction, dried and crystallized (Table I). IR: 3 453 and 3 407 (NH), 1 700 (C=O), 1 486 (NHCS). ^{1}H NMR: 4.12 s, 3 H (OCH_3); 7.05–7.75 m, 5 H (H-arom.); 10.75 s, 1 H and 11.02 s, 1 H (NH).

3-Substituted 1-(Indol-2-ylcarbonyl)thioureas (**27b**–**27g**)

To an acetone solution of isothiocyanate **20** (from 5 mmol of acid **18**) was added methylamine (992 mg of 25% aqueous solution, 8 mmol), benzylamine (563 mg, 573 µl, 5 mmol), cyclohexylamine (496 mg, 572 µl, 5 mmol), *p*-toluidine (536 mg, 5 mmol), piperidine (511 mg, 594 µl, 6 mmol) or morpholine (523 mg, 524 µl, 6 mmol) in benzene (5 ml) and the mixture was stirred for 10 min or 40 min (**27e**). In the case of thiourea **27b**, the resulted solution was concentrated to approximately one half of its original volume and water was added until permanent turbidity. After standing at 5 °C for 30 min, the separated precipitate was filtered off with suction, washed with water and dried. In the case of thioureas **27c**–**27g**, the reaction mixture was evaporated to dryness and the oily residue triturated with hexane (20 ml). The formed solid was filtered off, washed with hexane, dried and crystallized from a suitable solvent (Table I).

1-(Indol-2-ylcarbonyl)-3-methylthiourea (**27b**). IR: 3 456 and 3 266 (NH), 1 650 (C=O), 1 494 (NHCS). ^{1}H NMR: 3.22 d, 3 H, J = 5 (NCH_3); 7.00–7.75 m, 5 H (H-arom.); 10.80 s, 2 H and 11.62 s, 1 H (NH).

1-Benzyl-3-(indol-2-ylcarbonyl)thiourea (**27c**). IR: 3 460 and 3 266 (NH), 1 660 (C=O), 1 493 (NHCS). ^{1}H NMR: 4.93 d, 2 H, J = 6 (CH_2); 7.10–7.73 m, 10 H (H-arom.); 10.30 s, 1 H and 11.30 s, 2 H (NH).

1-Cyclohexyl-3-(indol-2-ylcarbonyl)thiourea (**27d**). IR: 3 460 and 3 260 (NH), 1 660 (C=O), 1 507 (NHCS). ^{1}H NMR: 1.15–2.30 m, 10 H ($(CH_2)_5$); 4.30 m, 1 H (CH); 7.10–7.78 m, 5 H (H-arom.); 8.98 s, 1 H, 9.40 s, 1 H and 10.55 s, 1 H (NH).

1-(Indol-2-ylcarbonyl)-3-methylphenylthiourea (**27e**). IR: 3 453 and 3 230 (NH), 1 666 (C=O), 1 513 (NHCS). ^{1}H NMR: 2.37 s, 3 H (CH_3); 7.00–7.75 m, 9 H (H-arom.); 10.54 s, 1 H, 11.42 s, 1 H and 12.71 s, 1 H (NH).

1-[N-(Indol-2-ylcarbonyl)thiocarbamoyl]piperidine (**27f**). IR: 3 460 and 3 400 (NH), 1 674 (C=O), 1 517 (NHCS). ^{1}H NMR: 1.69 m, 6 H ($(CH_2)_3$); 3.95 m, 4 H (CH_2NCH_2); 7.00–7.75 m, 5 H (H-arom.); 9.97 s, 1 H and 11.25 s, 1 H (NH).

4-[N-(Indol-2-ylcarbonyl)thiocarbamoyl]morpholine (**27g**). IR: 3 456 and 3 400 (NH), 1 684 (C=O), 1 510 (NHCS). ^{1}H NMR: 3.78 m, 8 H ($(CH_2)_4$); 7.00–7.72 m, 5 H (H-arom.); 10.20 s, 1 H and 11.10 s, 1 H (NH).

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