SYNTHESIS OF 4,6-DISUBSTITUTED-2-(1*H*-INDOL-3-YL)-BENZOTHIAZOLES

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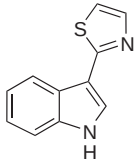
A new four-step synthesis of substituted 2-(1*H*-indol-3-yl)benzothiazoles is described, using N^3-phenyl-1*H*-indole-3-carbothioamides as key intermediates. The structure of the obtained products was determined by IR, ^{1}H, ^{13}C NMR and MS spectral methods.

Keywords: Indoles; Indolylbenzothiazoles; Phytoalexins; Heterocycles; Plant hormones; Camalexin; Natural products.

Pronounced cytotoxic activity of numerous benzothiazole derivatives suggests their potential use as antitumor agents[1–4]. Interesting is also their *in vitro* and *in vivo* antimicrobial activity, particularly against *Mycobacterium tuberculosis*[5].

In the present work we focused our attention on the synthesis of analogs of indole phytoalexin camalexin (**1**), namely benzocamalexin (**2**) and its derivatives.



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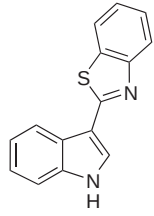

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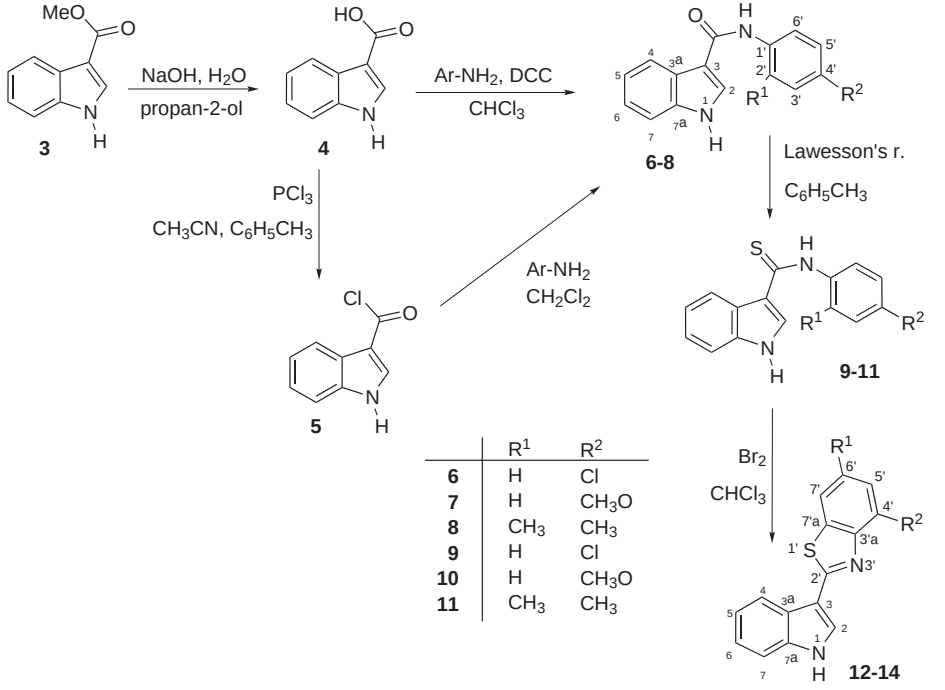
Camalexin, a cruciferous phytoalexin produced by *Arabidopsis Thaliana*[6] and *Camelina sativa*[7] has antifungal activities against *Alternaria brassicae Cladosporium sp.* and *Alternaria brassicicola*[6,7]. Camalexin and other indolyl thiazoles exhibit significant cytotoxic activity against human brest cancer cell line SKBr3 (ref.[8]). Benzothiazolyl derivatives in position 1 of the indole ring have been prepared by irradiation of indole-1-carbothioanilides in methanol at ambient temperature[9]. Reaction of methyl 1-methylindole-2-carboxylate with 2-aminobenzene-1-thiol in the presence of $Al(Me)_3$ catalyst afforded 2-(benzothiazol-2-yl)-1-methylindole[10]. Benzocamalexin (**2**) was previously prepared by cyclocondensation of indole-3-carbaldehyde with 2-aminobenzene-1-thiol in a solution of aqueous HCl and ethanol under reflux[11]. It was also obtained by cyclocondensation of 1-(*tert*-butoxycarbonyl)-1*H*-indole-3-carbaldehyde with 2-aminobenzene-1-thiol in a mixture of methanol and benzene (1:2) at ambient temperature and subsequent removal of the *tert*-butoxycarbonyl protecting group with sodium methoxide in methanol[12].

For the synthesis of 4,6-disubstituted-2-(1*H*-indol-3-yl)benzothiazoles, the cyclocondensation of indole-3-carbaldehyde is not convenient, because corresponding derivatives of 2-aminobenzene-1-thiol are not easily available. Therefore we designed a new synthetic methodology, using commercially available methyl indole-3-carboxylate (**3**) and substituted anilines as suitable starting compounds. The synthetic strategy included preparation of indole-3-carbanilides, their transformation to the corresponding thioanilides and final cyclization of *N*-arylthioamide moiety to benzothiazole derivatives (Scheme 1).

Attempts to achieve direct transformation of methyl indole-3-carboxylate (**3**) to the corresponding amides by its reaction with anilines at higher temperature with Lewis acids or microwave irradiation failed. Therefore, ester **3** was hydrolyzed to indole-3-carboxylic acid[13] (**4**) with a solution of NaOH in aqueous propan-2-ol at 85–90 °C. Amides **6**–**8** were obtained by reaction of acid **4** with anilines in the presence of dicyclohexylcarbodiimide (DCC) in chloroform, or by reaction of anilines with indole-3-carbonyl chloride (**5**). Chloride **5** prepared from acid **4** by treatment with phosphorus trichloride in a mixture of toluene and acetonitrile (2:1) is an unstable compound[14]. Therefore after its preparation the reaction mixture was decanted from phosphorous acid separated on the flask walls, concentrated to a quarter of its original volume and the obtained solution was treated with anilines for 3 h at room temperature. This approach afforded N^3-(4-chlorophenyl)-1*H*-indole-3-carboxamide (**6**) in 80% yield, whereas amides **7** and **8** were obtained in yields lower then 50%. Amides **7** and **8** can be more advanta-

geously prepared by direct coupling of acid **4** with 4-methoxyaniline and 2,4-dimethylaniline, respectively, in the presence of DCC in boiling chloroform[15]. Although products **7** and **8** were formed quantitatively, their separation from dicyclohexylurea was rather difficult. It was found that amides are well soluble in dioxane, whereas dicyclohexylurea is insoluble. Therefore a mixture of an amide and the urea was stirred in dioxane for 15 min at room temperature, the insoluble urea filtered off, and amide was obtained in 80–88% yield after evaporation of dioxane and crystallization.



	R^1	R^2
6	H	Cl
7	H	CH_3O
8	CH_3	CH_3
9	H	Cl
10	H	CH_3O
11	CH_3	CH_3

SCHEME 1

In the next step, synthetic transformation of amides **6**–**8** to thioamides **9**–**11** using P_4S_{10} or Lawesson's reagent as sulfuration reagents was studied. Lawesson's reagent appeared to be more effective and by reflux in toluene, thioamides **9**–**11** were obtained in 78–90% yieds. In ^{13}C NMR spectra of the thioamides, the signals of C=S carbon atoms appeared at 190–192 ppm, instead of C=O signals present in the spectra of amides at 163 ppm. In the mass spectra of thioamides, their molecular peaks were present at higher *m/z* values (by 16), compared with molecular ions of the corresponding amides.

The key step in the synthesis of camalexin analogs **12**–**14** is the cyclization of thioamides **9**–**11** to the benzothiazole ring. For this purpose, we examined two reagents. By treatment of a dioxane solution of thioamides with freshly prepared dioxane solution of dioxane dibromide, no reaction took place (monitored by TLC). Therefore we performed the cyclization under the conditions of the Huggershoff reaction (reaction of thioamides with bromine in chloroform)[16,17]. It is assumed that this reaction proceeds *via* oxidative formation of disulfide giving corresponding sulfenyl bromide, in which the sulfur atom has electrophilic properties. The electrophilic attack of sulfenyl bromide on *ortho*-position of the phenyl ring then results in the formation of the benzothiazole ring. Using commercial chloroform, we obtained from thioamides only the corresponding amides, probably because of the hydrolysis of sulfenyl bromides with a small amount of water present in the solvent. Using dry chloroform, the cyclization of thioamides proceeded smoothly at room temperature and desired benzocamalexin derivatives **12**–**14** were obtained, after chromatographic isolation and crystallization, in 32–64% yields.

EXPERIMENTAL

Infrared absorption spectra of compounds (**4**, **6**–**14**) were recorded on an IR 75 spectrometer (Zeiss, Jena) in chloroform, the wavenumbers are given in cm^{-1}. ^1H NMR spectra were measured on a Bruker Avance-DPX FT (300 MHz) in deuteriochloroform. ^{13}C NMR spectra of compounds **6**–**14** were measured on a Bruker Avance-DPX FT (75 MHz) in deuteriochloroform. Chemical shifts (δ-scale) are reported in ppm downfield from tetramethylsilane. Microanalyses were performed with a Perkin–Elmer, Model 2400 analyzer. The mass spectra of compounds **6**–**14** were recorded on a Finigan SSQ 700 spectrometer using the electron impact method at ionization energy 70 eV. The reaction course was monitored by thin layer chromatography, using Silufol plates (Kavalier). Preparative column chromatography was performed on silica gel Kieselgel Merck 60 F25 and on Kavalier 40/100 μm silica gel. Melting points were measured on a Kofler hot stage apparatus and are uncorrected.

Indole-3-carboxylic Acid (**4**)

To a solution of NaOH (5.25 g, 130 mmol) in water (125 ml), propan-2-ol (25 ml) and methyl indole-3-carboxylate (5 g, 28.5 mmol) were added and the reaction mixture was stirred for 1 h at 85–90 °C until complete dissolution of the ester. After evaporation of propan-2-ol, the aqueous solution was acidified with HCl (1:1) under ice cooling. The precipitated crude product was filtered off and washed with water. Yield 61%, m.p. 208–210 °C (acetone–water). For $C_9H_7NO_2$ (161.2) calculated: 67.08% C, 4.38% H, 8.69% N; found: 67.20% C, 5.00% H, 8.80% N. IR spectral data of the product were identical with those previously published[13].

N^3-(4-Chlorophenyl)-1*H*-indole-3-carboxamide (**6**)

To a solution of **4** (200 mg, 1.24 mmol) in a mixture of dry toluene (10 ml) and dry acetonitrile (5 ml), PCl_3 (0.12 ml, 1.38 mmol) was added. The reaction mixture was stirred for 25 min at room temperature until complete dissolution of the acid. The resulting solution was decanted from phosphorous acid deposited on the flask walls. The flask was washed with toluene (10 ml) and the combined toluene solution was evaporated to quarter of its original volume. The obtained solution was cooled to 0 °C and 4-chloroaniline (230 mg, 1.80 mmol) was added. The reaction mixture was stirred for 3 h at room temperature, the solvent was evaporated and the residue was purified by chromatography on silica gel (dichloromethane–methanol, 90:1). Yield 88%, m.p. 225–226 °C. For $C_{15}H_{11}ClN_2O$ (270.7) calculated: 66.55% C, 4.10% H, 10.35% N; found: 66.60% C, 4.50% H, 10.50% N. IR: 3410 (indole N–H); 3120 (N–H); 1640 (C=O). ^{1}H NMR: 7.00–8.63 m, 9 H (arom. H); 9.45 s, 1 H (indole N-H); 11.20 s, 1 H (CO-NH). ^{13}C NMR: 110.41 (q); 112.16, 120.92, 121.21, 121.30, 122.39, 126.24 (q); 126.53, 128.59, 129.03 (q); 136.39 (q); 139.00 (q) (arom. C); 163.44 (C=O). MS, *m/z* (%): 270 (M^+, 100), 144 (70), 126 (50).

N^3-(4-Methoxyphenyl)-1*H*-indole-3-carboxamide (**7**)

To a solution of **4** (2 g, 12.4 mmol) in chloroform (65 ml), dicyclohexylcarbodiimide (2.81 g, 13.6 mmol) and 4-methoxyaniline (1.68 g, 13.6 mmol) dissolved in chloroform (10 ml) were added. The reaction mixture was refluxed for 3 h. After cooling, the crystalline precipitate was filtered off with suction and triturated with dioxane (15 ml). The mixture was stirred for 15 min at room temperature and the crystalline precipitate (dicyclohexylurea) was filtered off. The filtrate was evaporated and the obtained residue was crystallized from a mixture of diethyl ether–hexane (1:1). Yield 80%, m.p. 200–202 °C. For $C_{16}H_{14}N_2O_2$ (266.2) calculated: 72.17% C, 5.30% H, 10.52% N; found: 72.45% C, 5.56% H, 11.10% N. IR: 3398 (indole N–H); 3217 (N–H); 1631 (C=O); 1236 (C–O–C). ^{1}H NMR: 3.76 s, 3 H (OCH_3); 6.90–8.25 m, 9 H (arom. H); 9.61 s, 1 H (indole N-H); 11.96 s, 1 H (N-H). ^{13}C NMR: 55.13 (CH_3); 110.60 (q); 111.89, 113.68, 120.53, 121.11, 121.36, 122.04, 126.40 (q); 128.28, 132.87 (q); 136.17 (q); 154.87 (q) (arom. C); 162.99 (C=O). MS, *m/z* (%): 266 (M^+, 50), 144 (65), 123 (100).

N^3-(2,4-Dimethylphenyl)-1*H*-indole-3-carboxamide (**8**)

To a solution of **4** (2 g, 12.4 mmol) in chloroform (70 ml), dicyclohexylcarbodimide (2.76 g, 13.4 mmol) and 2,4-dimethylaniline (1.64 g, 13.5 mmol) dissolved in chloroform (8 ml) were added. The reaction mixture was refluxed for 3 h, the crystalline precipitate separated after cooling was filtered off with suction and triturated with dioxane (15 ml). The mixture was stirred for 15 min at room temperature. The crystalline precipitate (dicyclohexylurea) was filtered off. The filtrate was evaporated and the obtained residue was crystallized from a mixture of diethyl ether–hexane (1:1). Yield 80%, m.p. 170 °C. For $C_{17}H_{16}N_2O$ (264.3) calculated: 77.25% C, 6.10% H, 10.60% N; found: 78.01% C, 6.38% H, 11.00% N. IR: 3390 (indole N–H); 3250 (N–H); 1640 (C=O). ^{1}H NMR: 2.53 s, 3 H (CH_3); 2.59 s, 3 H (CH_3); 6.13–7.36 m, 8 H (arom. H); 8.36 s, 1 H (indole N-H); 10.82 s, 1 H (N-H). ^{13}C NMR: 18.13 (CH_3); 20.70 (CH_3); 111.60 (q); 112.04 (q); 120.65, 121.21, 122.16, 126.54, 128.53, 130.91 (q); 133.22, 134.36 (q); 136.35 (q); 136.85 (q) (arom. C); 163.12 (C=O). MS, *m/z* (%): 264 (M^+, 20), 144 (34), 120 (100).

N^3-(4-Chlorophenyl)-1*H*-indole-3-carbothioamide (**9**)

To a suspension of **6** (0.13 g, 0.48 mmol) in dry toluene (4 ml), Lawesson's reagent (0.11 g, 0.27 mmol) was added. The reaction mixture was refluxed for 15 min. After cooling, the crystalline precipitate was filtered off with suction and crystallized from a mixture of dichloromethane and hexane. Yield 86%, m.p. 202–205 °C. For $C_{15}H_{11}ClN_2S$ (286.8) calculated: 62.82% C, 3.87% H, 9.77% N; found: 63.00% C, 3.75% H, 9.81% N. IR: 1050 (C=S); 1656 (C=C); 3410 (indole N–H); 3210 (N–H). ^{1}H NMR: 6.55–7.44 m, 9 H (arom. H); 9.80 s, 2 H (N-H). ^{13}C NMR: 112.29, 118.91, 121.00, 121.87, 122.52, 126.07 (q); 126.41 (q); 128.03, 128.37, 129.25 (q); 132.58 (q); 136.99 (q); 139.14 (arom. C); 191.14 (C=S). MS, *m/z* (%): 286 (M^+, 45), 160 (75), 126 (100).

N^3-(4-Methoxyphenyl)-1*H*-indole-3-carbothioamide (**10**)

To a suspension of **7** (1 g, 3.76 mmol) in dry toluene (15 ml), Lawesson's reagent (0.96 g, 2.37 mmol) was added and the reaction mixture was refluxed for 15 min. After cooling, the formed crystalline precipitate was filtered off with suction and crystallized from a mixture of dichloromethane and hexane. Yield 78%, m.p. 193 °C. For $C_{16}H_{14}N_2OS$ (282.4) calculated: 68.06% C, 5.00% H, 9.92% N; found: 68.40% C, 5.10% H, 10.00% N. IR: 1075 (C=S); 1650 (C=C); 3210 (N–H); 3400 (N–H). ^{1}H NMR: 3.78 s, 3 H (OCH_3); 6.96–8.53 m, 9 H (arom. H); 10.96 s, 1 H (indole N-H); 11.82 s, 1 H (N-H). ^{13}C NMR: 55.31 (OCH_3); 112.11, 113.51, 118.62 (q); 120.72, 121.86, 122.31 (q); 126.02, 126.48, 127.33 (q); 133.01, 136.83 (q); 156.93 (q) (arom. C); 190.51 (C=S). MS, *m/z* (%): 282 (M^+, 50), 249 (40), 160 (80), 123 (100).

N^3-(2,4-Dimethylphenyl)-1*H*-indole-3-carbothioamide (**11**)

To a suspension of **8** (1 g, 3.78 mmol) in dry toluene (30 ml), Lawesson's reagent (0.77 g, 1.90 mmol) was added and the reaction mixture was refluxed for 50 min. After cooling, the crystalline precipitate was filtered off with suction and crystallized from a mixture of dichloromethane and hexane. Yield 90%, m.p. 188 °C. For $C_{17}H_{16}N_2S$ (280.4) calculated: 72.82% C, 5.64% H, 9.99% N; found: 73.00% C, 5.75% H, 10.00% N. IR: 1100 (C=S); 1650 (C=C); 3200 (N–H); 3450 (indole N–H). ^{1}H NMR: 2.23 s, 3 H (CH_3); 2.33 s, 3 H (CH_3); 7.05–8.59 m, 8 H (arom. H); 10.69 s, 1 H (indole N-H); 11.74 s, 1 H (N-H). ^{13}C NMR: 17.88 (CH_3); 20.82 (CH_3); 112.16, 117.82 (q); 120.82, 122.02, 122.36, 126.22 (q); 126.88, 127.38, 128.18 (q); 131.03, 135.10 (q); 136.27, 136.44 (q); 136.93 (q) (arom. C); 191.88 (C=S). MS, *m/z* (%): 280 (M^+, 90), 160 (75), 120 (100).

2-(1*H*-Indol-3-yl)-6-chloro-1,3-benzothiazole (**12**)

To a suspension of **9** (515 mg, 1.80 mmol) in chloroform (18 ml), bromine (290 mg, 1.81 mmol) was added dropwise. The reaction mixture was stirred for 1 h at room temperature, then neutralized with triethylamine (0.46 ml, 3.3 mmol), poured into water (150 ml) and extracted with diethyl ether (2 × 50 ml). The extract was dried with anhydrous sodium sulfate, evaporated and purified by chromatography on silica gel (cyclohexane–acetone, 2:1). Yield 45%, m.p. 143–145 °C (methanol–water). For $C_{15}H_9ClN_2S$ (284.8) calculated: 63.27% C, 3.19% H, 9.84% N; found: 63.10% C, 3.20% H, 9.91% N. IR: 1173 (N–C); 1630 (C=N); 1640 (C–S); 3020 (C–H). ^{1}H NMR: 6.75–8.55 m, 9 H (arom. H); 9.25 s, 1 H (N-H). ^{13}C NMR: 104.67 (q); 111.63, 121.21, 122.18, 123.02 (q); 123.75, 124.08, 124.16, 125.16, 125.46 (q);

131.36 (q); 133.18, 136.58 (q); 148.20 (q); 154.44 (q) (arom. C). MS, *m/z* (%): 284 (M^+, 50), 143 (100), 116 (60).

2-(1*H*-Indol-3-yl)-6-methoxy-1,3-benzothiazole (**13**)

To a suspension of **10** (282 mg, 1 mmol) in chloroform (10 ml), bromine (160 mg, 1 mmol) was added dropwise. The reaction mixture was stirred for 1.5 h at room temperature, neutralized with triethylamine (0.25 ml, 1.8 mmol), poured into water (120 ml) and extracted with diethyl ether (2 × 40 ml). The extract was dried with anhydrous sodium sulfate, evaporated and the obtained residue was purified by chromatography on silica gel (cyclohexane–acetone, 2:1). Yield 32%, m.p. 168–171 °C (diethyl ether–hexane). For $C_{16}H_{12}N_2OS$ (280.4) calculated: 68.55% C, 4.31% H, 9.99% N; found: 68.70% C, 4.25% H, 10.00% N. IR: 1165 (N–C); 1239 (C–O–C); 1625 (C=N); 1640 (C–S); 3000 (C–H). ^{1}H NMR: 3.73 s, 1 H (OCH_3); 6.597.88 m, 8 H (arom. H); 11.46 s, 1 H (N-H). ^{13}C NMR: 55.11 (OCH_3); 61.00 (q); 105.44, 111.84, 113.68, 114.67, 120.53, 121.44 (q); 121.60, 122.11, 125.56, 129.55 (q); 134.96 (q); 142.88 (q); 154.86 (q); 156.62 (q) (arom. C). MS, *m/z* (%): 280 (M^+, 50), 143 (100), 116 (65), 32 (40).

2-(1*H*-Indol-3-yl)-4,6-dimethyl-1,3-benzothiazole (**14**)

To a suspension of **11** (110 mg, 0.39 mmol) in chloroform (4 ml), bromine (61 mg, 0.38 mmol) was added dropwise. The reaction mixture was stirred for 2 h at room temperature, neutralized with triethylamine (0.10 ml, 0.72 mmol), poured into water (80 ml) and extracted with diethyl ether (2 × 20 ml). The extract was dried with anhydrous sodium sulfate and evaporated. Yield 64%, m.p. 130–133 °C (ethanol–water). For $C_{17}H_{14}N_2S$ (278.4) calculated: 73.29% C, 5.07% H, 10.00% N; found: 73.35% C, 5.05% H, 10.06% N. IR: 1170 (N–C); 1635 (C=N); 1645 (C–S); 3025 (C–H). ^{1}H NMR: 2.10 s, 3 H (CH_3); 2.39 s, 3 H (CH_3); 6.55–7.50 m, 7 H (arom. H); 8.25 s, 1 H (N-H). ^{13}C NMR: 17.81 (CH_3); 20.93 (CH_3); 107.10 (q); 111.46, 121.15, 121.63, 122.25 (q); 123.06, 124.78, 125.84, 127.71 (q); 129.22 (q); 131.61 (q); 132.78, 135.12 (q); 144.37 (q); 157.18 (q) (arom. C). MS, *m/z* (%): 278 (M^+, 15).

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