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Isobrassinin and its analogues: Novel types of antiproliferative agents

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Abstract—Isobrassinin (2-(*S*-methyldithiocarbamoylaminomethyl)indole (**7a**), a regioisomer of the cruciferous phytoalexin brassinin (**1**), exerted marked antiproliferative effects on the HeLa, A431 and MCF7 cell lines (>78.6% inhibition at 30 μM). For structure–activity relationships, further analogues were synthesized. The highest cytotoxic effect was displayed by 2-phenylimino-1,3-thiazino[5,6-*b*]indole (**10**) (10 μM, 76.8%—HeLa and 46.3%—MCF7). The effect of the natural phytoalexin brassinin was also determined.

Substituted dithiocarbamic acid esters are a common class of organic molecules. The different derivatives in this group exhibit a wide range of biological effects (antibacterial,[1] antifungal,[2] antioxidant activity,[3] inhibition of cardiac hypertrophy,[4] etc.). A steadily increasing number of studies have been published on dithiocarbamates and their anticancer activity. 4-Methanesulfinylbutyl dithiocarbamic acid methyl ester (Sulforamate) has proved to be a potential cancer chemopreventive compound as a phase II enzyme inducer.[5] A series of alkyl/arylsulfonyl-*N*,*N*-diethyldithiocarbamates display moderate to powerful tumour growth-inhibitory properties against several cancer cell lines in vitro.[6] 4(3*H*)-Quinazolinone derivatives with a dithiocarbamate side chain exhibit antitumour activity against human myelogenous leukaemia K562 cells.[7,8] Pyrrolidine dithiocarbamate stimulates apoptosis by suppressing the activation of nuclear factor κB (NF-κB) in various cancer cells (e.g., acute myelogenous leukaemia[9] and pancreatic adenocarcinoma[10]). A variety of 4-substituted-piperazine-1-carbodithioic acid 3-cyano-3,3-diphenylpropyl esters have been found to be effective against the HL-60 and Bel-7402 cell lines.[11] Different metal [Pt(II), Pd(II), Au(III), Cu(II)] complexes of dithiocarbamate derivatives (methyl- and ethylsarcosinedithiocarbamate, *N*,*N*-dimethyldithiocarbamate, *S*-methyl-*N*,*N*-dimethyldithiocarbamate and diethyldithiocarbamate) have been prepared and their cytotoxicities were studied.[12–14] The Pt(II) complexes of these sulfur-containing molecules can act as chemoprotectants in platinum-based chemotherapy, modulating cisplatin nephrotoxicity.[15]

Besides the compounds mentioned above, probably the most interesting group of dithiocarbamates exhibiting antitumour activity are the phytoalexins from cruciferous plants. The phytoalexins are a group of structurally diverse, low molecular weight, generally lipophilic antimicrobial substances formed in plants. They are not present in healthy plant tissue, but are synthetized in response to pathogen attack or physical or chemical stress, probably as a result of the de novo synthesis of enzymes.[16] Some of the *cruciferae* species that have been examined accumulate a series of specific indole-sulfur compounds. The basic structures are characterized by an indole ring variably substituted at positions 2 and/or 3 with nitrogen- and sulfur-containing substituents.[17] Typical representatives of dithiocarbamate and

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thiazino[6,5-*b*]indole-type phytoalexins from cruciferous plants are brassinin (**1**), 1-methoxybrassinin (**2**), 4-methoxybrassinin (**3**), cyclobrassinin (**4**) and sinalbin B (**5**) (Fig. 1).

Among these compounds, brassinin (**1**) and cyclobrassinin (**4**) proved active in inhibiting the formation of preneoplastic mammary lesions in culture.[18] The former also exerts an antiproliferative effect in human acute T-lymphoblastic leukaemia cells.[19] Brassinin and its derivatives are inhibitors of indolamine 2,3-dioxygenase, a new cancer immunosuppression target.[20] These compounds can serve as lead compounds for the generation of more efficient analogues.[21]

In our work on the chemistry of sulfur- and nitrogen-containing condensed-skeleton heterocycles,[22–25] we earlier prepared brassinin (**1**) and cyclobrassinin (**4**) and some of their derivatives.[26] As a continuation, our present aim was the preparation of regioisomers of cruciferous phytoalexins (**1**, **4**), 2-(*S*-methyldithiocarbamoylaminomethyl)indole (**7a**, isobrassinin), 2-methylthio-1,3-thiazino[5,6-*b*]indole (**8a**, isocyclobrassinin) and their analogues, and investigation of their antiproliferative effects against human cell lines.

The key intermediate, 2-aminomethylindole (**6**) (Scheme 1), was prepared from commercial indole-2-carboxylic acid. In the first step, indole-2-carboxamide was obtained by the method of Larock et al.[27] The reduction of indole-2-carboxamide to 2-aminomethylindole was performed with lithium aluminium hydride in tetrahydrofuran.

Starting from amine **6**, 2-(*S*-methyldithiocarbamoylaminomethyl)indole (**7a**, isobrassinin[28]) was prepared with carbon disulfide, using chloroform as solvent and triethylamine and catalytic 4-dimethylaminopyridine as base, followed by treatment with methyl iodide.[29,30] When benzyl bromide was used instead of the latter alkylating agent, 2-(*S*-benzyldithiocarbamoylaminomethyl)indole (**7b**) was obtained in good yield.[30] The selective Hugerschoff ring-closure of **7a**,**b** with phenyltrimethylammonium tribromide gave 2-methylthio-1,3-thiazino[5,6-*b*]indole (**8a**) and its benzyl analogue **8b**.[31] Thiourea derivatives **9a**,**b** were synthesized from 2-aminomethylindole with methyl and phenyl isothiocyanates in refluxing chloroform.[32] The oxidative ring-closure of **9b** with phenyltrimethylammonium tribromide and basic treatment provided 2-phenylimino-1,3-thiazino[5,6-*b*]indole derivative **10** (Scheme 1).[31]

The cytotoxic activities of the synthetized compounds were determined by the MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] assay,[33] using the HeLa, MCF7 and A431 cell lines.[34] The results are summarized in Table 1.

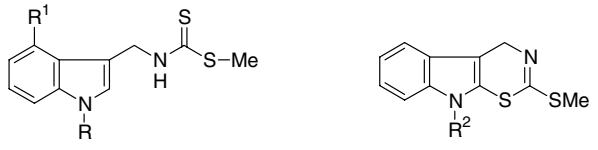
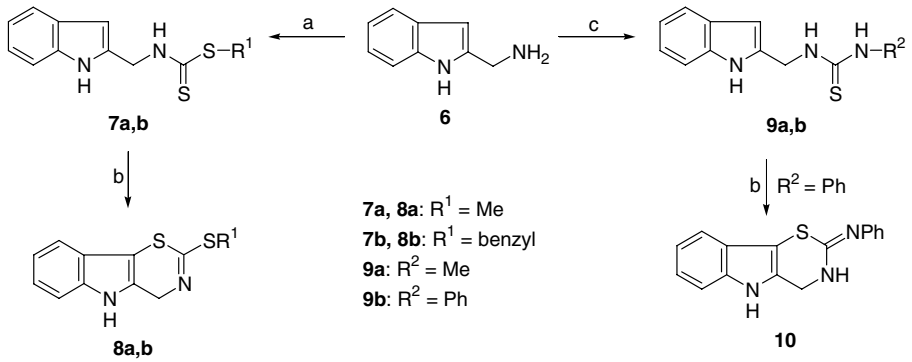


Figure 1. Dithiocarbamate and 1,3-thiazino[6,5-*b*]indole phytoalexins from cruciferous plants.

Table 1. In vitro tumour cell growth inhibition data (HeLa, MCF7 and A431 cell lines) for the compounds synthesized

Compound	Concentration (μM)	Inhibition (%) ± SEM[a]		
		HeLa	MCF7	A431
Cisplatin	10	42.6 ± 2.9	53.0 ± 2.3	88.6 ± 0.5
1	30	25.2 ± 5.5	21.5 ± 2.5	NT[b]
7a	30	83.6 ± 1.9	86.2 ± 1.8	78.6 ± 7.0
7b	30	76.1 ± 2.2	89.0 ± 0.9	70.7 ± 5.2
8a	30	44.5 ± 1.5	<10.0	<10.0
8b	30	45.8 ± 2.1	37.3 ± 3.2	NT[b]
9a	30	<10.0	<10.0	<10.0
9b	30	50.0 ± 1.8	57.4 ± 1.9	45.7 ± 2.2
10	30	99.8 ± 0.3	83.8 ± 1.9	NT[b]
10	10	76.8 ± 1.2	46.3 ± 4.4	NT[b]

[a] SEM—standard error of the mean.
[b] Not tested.



Scheme 1. Reagents and conditions: (a) $CHCl_3$, Et_3N, cat. DMAP, CS_2, 0 °C then rt 2 h, MeI (for **7a**) or benzyl bromide (for **7b**), rt 3 h, 85% (**7a**), 91% (**7b**); (b) CH_2Cl_2, $PhMe_3NBr_3$, rt 5 min, Et_3N, rt 10 min, 76% (**8a**), 82% (**8b**), 72% (**10**); (c) $CHCl_3$, dioxane MeNCS (for **9a**) or PhNCS (for **9b**), reflux, 2 h, 92% (**9a**), 95% (**9b**).

2-(*S*-Methyldithiocarbamoylaminomethyl)indole (**7a**) displayed marked antiproliferative effects on the HeLa, A431 and MCF7 cell lines: >78.6% inhibition was observed at 30 μM. Replacement of the methyl group of isobrassinin by a benzyl group resulted in similarly high activity: **7b** induced 70.7–89.0% inhibition. A comparison of the literature data on the in vitro antiproliferative activities of brassinin (**1**) and cyclobrassinin (**2**) reveals that the latter (containing a thiazine ring) has a lower cytotoxic effect.[35] Isocyclobrassinin (**8a**) also exerted less pronounced activity (<10.0–44.5%) than that of **7a**. The isosteric transformation of the dithiocarbamate moiety of isobrassinin to thiourea led to a loss of inhibitory activity. For the methyl thiourea derivative **9a**, no substantial inhibition was observed, but the phenyl analogue still exhibited >45.7% activity for all three cell lines. The most interesting results emerged from the oxidative ring-closure of phenyl thiourea **9b**. The highest cytotoxic effect was induced by **10**, which at 10 μM reduced the cell growth of HeLa and MCF7 cells by 76.8% and 46.3%, respectively. This is comparable to the activity of cisplatin (10 μM, 42.6%—HeLa, 53.0%—MCF7). The effects of natural phytoalexin brassinin[26] (**1**) were also determined (30 μM, 25.2%—HeLa, 21.5%—MCF7).

In summary, indolylmethyldithiocarbamates and some analogues were prepared, and were found to have noteworthy in vitro antiproliferative effects. Isobrassinin (**7a**) and its benzyl analogue (**7b**) are a new type of dithiocarbamate antiproliferative agent. The 2-phenylimino-1,3-thiazino[5,6-*b*]indole derivative **10**, a sulfur analogue of β-carboline proved to be a novel type of antitumour compound. Work on the preparation of further derivatives and the screening of their antiproliferative effects is in progress.

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30. General procedure for dithiocarbamates **7a**,**b**: To a stirred solution of 2-aminomethylindole (**6**) (0.5 g, 3.5 mmol) in chloroform (20 mL), triethylamine (0.48 ml, 3.5 mmol)

and 4-dimethylaminopyridine (0.06 g, 0.5 mmol) were added. Carbon disulfide (0.23 mL, 3.8 mmol) was next added dropwise under ice cooling and the mixture was stirred at the same temperature for 2 h. Methyl iodide or benzyl bromide (3.5 mmol) in chloroform (5 mL) was then added dropwise to the solution and it was stirred for 3 h at RT. The organic phase was extracted in turn with 3% hydrochloric acid (10 mL) and with water (10 mL), dried (Na_2SO_4) and evaporated. The residue was subjected to column chromatography using *n*-hexane/ethyl acetate 4:1. Compound **7a**: light pink crystalline powder; mp 95–97 °C (Lit.[28] mp 82–84 °C); ^{1}H NMR δ ($CDCl_3$): 8.90 (1H, bs, NH-1), 7.56 (1H, d, J = 7.8 Hz, H-4), 7.33 (1H, d, J = 7.8 Hz, H-7) 7.31 (1H, bs, N*H*–CH_2), 7,18 (1H, t, J = 7.8, H-6), 7.08 (1H, t, J = 7.8, H-5), 6.41 (1H, s, H-3), 5.08 (2H, d, J = 5.5 Hz, CH_2), 2.67 (3H, s, CH_3); ^{13}C NMR δ ($CDCl_3$): 201.7, 136.9, 135.0, 128.2, 123.2, 121,1, 120.7, 111,9, 102.7, 44.5, 19.1. Anal. Calcd for $C_{11}H_{12}N_2S_2$ (236.36): C, 55.90; H, 5.12; N, 11.85; S, 27.13. Found: C, 55.75; H, 4.88; N, 11.83; S, 27.38. Compound **7b**: light pink crystalline powder; mp 124–126 °C; ^{1}H NMR δ ($CDCl_3$): 8.83 (1H, bs, NH-1), 7.55 (1H, d, J = 7.8 Hz, H-4), 7.48–7.22 (7H, m, H-7, N*H*–CH_2 and C_6H_5, overlapping signals), 7,18 (1H, t, J = 7.8, H-6), 7.12 (1H, t, J = 7.8, H-5), 6.40 (1H, s, H-3), 5.06 (2H, d, J = 5.5 Hz, NH–C*H*$_2$), 4.56 (2H, s, S–CH_2); ^{13}C NMR δ ($CDCl_3$): 200.0, 136.9, 136.6, 134.7, 129.7 (2× C), 129.4 (2× C), 128.3, 128.2, 123.2, 121.1, 120.7, 111.9, 102.8, 44.5, 41.0. Anal. Calcd for $C_{17}H_{16}N_2S_2$ (312.45): C, 65.35; H, 5.16; N, 8.97; S, 20.53. Found: C, 65.11; H, 5.27; N, 8.92; S, 20.66.

31. General procedure for 1,3-thiazino[5,6-*b*]indoles **8a**,**b** and **10**: To an intensively stirred solution of **8a**,**b** or **10** (0.85 mmol) in dichloromethane (10 mL) at RT, phenyltrimethylammonium tribromide (0.32 g, 0.85 mmol) was added in one portion. After stirring for 5 min, triethylamine (0.36 mL, 2.6 mmol) was added in one portion. After 10 min the mixture was evaporated (water bath <50 °C) and the residue was purified by column chromatography, using *n*-hexane/ethyl acetate 3:2 as eluent. Compound **8a**: grey crystalline powder; mp 121–124 °C (decomp.); ^{1}H NMR δ ($CDCl_3$): 8.07 (1H, bs, NH-5), 7.42 (1H, d, J = 7.8 Hz, H-9), 7.33 (2H, d, J = 7.8 Hz H-6), 7,20 (1H, t, J = 7.8, H-7), 7.14 (1H, t, J = 7.8, H-8), 4.98 (2H, s, CH_2), 2.52 (3H, s, CH_3); ^{13}C NMR δ ($CDCl_3$): 158.7, 136.7, 126.3, 125.6, 123.3, 121.1, 118.4, 112.1, 99.7, 49.4, 16.1. Anal. Calcd for $C_{11}H_{10}N_2S_2$ (234.34): C, 56.38; H, 4.30; N, 11.95; S, 27.37. Found: C, 56.22; H, 4.51; N, 11.83; S, 27.45. Compound **8b**: light grey crystalline powder; mp 173–175 °C (dec); ^{1}H NMR δ ($CDCl_3$): 8.08 (1H, bs, NH-5), 7.41 (1H, d, J = 7.8 Hz, H-9), 7.40–7.20 (6H, m, H-6 and C_6H_5, overlapping signals), 7,26 (1H, t, J = 7.8, H-7), 7.18 (1H, t, J = 7.8, H-8), 5.00 (2H, s, NH–C*H*$_2$), 4.35 (2H, s, S–CH_2); ^{13}C NMR δ ($CDCl_3$): 157.5, 137.7, 136.7, 131.6, 129.7 (2× C), 129.2 (2× C), 128.0, 126.2, 123.4, 121.1, 118.5, 112.0, 100.0, 49.5, 37.6. Anal. Calcd for $C_{17}H_{14}N_2S_2$ (310.44): C, 65.77; H, 4.55; N, 9.02; S 20.66,. Found: C, 65.52; H, 4.71; N, 9.01; S, 20.91. Compound **10**: light brown crystalline powder; mp 200–204 °C (dec); ^{1}H NMR δ (DMF-d_7): 11.43 (1H, bs, CH_2–N*H*), 9.04 (1H, bs, NH-5), 7.78 (2H, d, H-2′,6′), 7.47 (1H, d, J = 7.8 Hz, H-9), 7.39 (1H, d, J = 7.8 Hz, H-6), 7.28 (2H, t, J = 7.7, H-3′,5′), 7.16 (1H, t, J = 7.7, H-4′), 7.07 (1H, t, J = 7.8, H-7), 7.00 (1H, t, J = 7.8, H-8), 4.83 (2H, s, CH_2); ^{13}C NMR δ (DMF-d_7): 163.1, 137.2, 129.1 (2× C), 128.4, 126.2, 122.3, 122.2, 122.1, 120.0, 119,4 (2× C), 117,4, 112,5, 98.4, 45,4. Anal. Calcd for $C_{16}H_{13}N_3S$ (279.36): C, 68.79; H, 4.69; N, 15.04; S, 11.48. Found: C, 68.62; H, 4.91; N, 15.10; S, 11.62.
32. General procedure for thioureas **9a**,**b**: To a stirred solution of 2-aminomethylindole (**6**) (0.29 g, 2 mmol) in chloroform (10 mL) and dioxane (10 mL), the corresponding isothiocyanate (2 mmol) was added in one portion. The reaction mixture was refluxed for 2 h. After evaporation the residue was triturated with *n*-hexane, decanted and purified by column chromatography using *n*-hexane/ethyl acetate 4:1 as eluent. Compound **9a**: white crystalline powder; mp 99–100 °C; ^{1}H NMR δ ($CDCl_3$): 9.51 (1H, bs, NH-1), 7.55 (1H, d, J = 7.8 Hz, H-4), 7.35 (1H, d, J = 8 Hz, H-7), 7,17 (1H, t, J = 7.8 Hz, H-6), 7,09 (1H, t, J = 7.8, H-5), 6.36 (1H, s, H-3), 6.05 (2H, bs, 2× NH–CS), 4.89 (1H, s, CH_2), 2.87 (3H, d, J = 4.4, CH_3); ^{13}C NMR δ ($CDCl_3$): 182.9, 136.9, 136.7, 128.1, 123.0, 121.0, 120.6, 112.0, 101.6, 42.4, 31.1. Anal. Calcd for $C_{11}H_{13}N_3S$ (219.31): C, 60.24; H, 5.97; N, 19.16; S, 14.62. Found: C, 60.10; H, 6.04; N, 19.01; S, 14.81. Compound **9b**: white crystalline powder; mp 168–169 °C; ^{1}H NMR δ ($CDCl_3$): 9.51 (1H, bs, NH–CS), 8.10 (1H, bs, NH-1), 7.52 (1H, d, J = 7.8 Hz, H-4), 7.48–7.18 (7H, m, H-7, H-6 and C_6H_5, overlapping signals), 7,12 (1H, t, J = 7.8 Hz, H-5), 6.45 (1H, bs, NH–CS), 6.29 (1H, s, H-3), 4.96 (2H, d, J = 6.0 Hz, CH_2); ^{13}C NMR δ ($CDCl_3$): 182.0, 136.9, 136.5, 136.2, 131.0 (2× C), 128.6 (2× C), 128.1, 126.3, 122.9, 121.0, 120.4, 112.0, 101.9, 42.9. Anal. Calcd for $C_{16}H_{15}N_3S$ (281.38): C, 68.30; H, 5.37; N, 14.93; S, 11.40. Found: C, 68.11; H, 5.53; N, 14.91; S, 11.67.
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34. Cervix adenocarcinoma (HeLa), breast adenocarcinoma (MCF7) and squamous skin carcinoma (A431) cells were maintained in minimal essential medium supplemented with 10% foetal bovine serum, antibiotic–antimycotic agents and non-essential amino acids. Briefly, human cancer cells were seeded onto a 96-well microplate. On the second day of the procedure, the original medium was removed and 200 μL new medium containing the test substances was added. The tested compounds were dissolved in DMSO, in a final concentration never exceeding 0.1%, which has no substantial effect on cell growth. After an incubation period of 72 h, living cells were assayed by the addition of 20 μL of 5 mg/mL MTT solution. MTT was converted by intact mitochondrial reductase and precipitated as blue crystals during a 4 h contact period. The medium was then removed and the precipitated crystals were dissolved in 100 mL DMSO during a 60 min period of shaking. Finally, the reduced MTT was assayed at 545 nm by using a microplate reader. All in vitro experiments were carried out on two microplates with at least 5 parallel wells.
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