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A facile method for the synthesis of indole phytoalexin rutalexin

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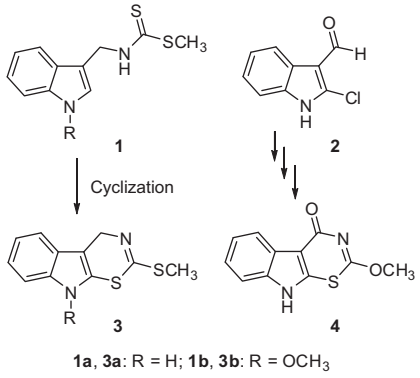
ABSTRACT

Novel methods for the preparation of the indole phytoalexin rutalexin (**8**) in high yields are presented. The synthesis of rutalexin (**8**) was achieved from previously synthesized 9-*tert*-butoxycarbonyl-2-methoxy-4-oxo-[1,3]thiazino[6,5-*b*]indole (**5**) by its hydrolysis, methylation, and deprotection. A second method starting from 9-Boc-cyclobrassinin (**9**) involved oxidation, methylation, and deprotection.

Phytoalexins are defined as antimicrobial low molecular weight secondary metabolites, that are produced by plants upon exposure to physical, biological, or chemical stress.[1] About 40 indole phytoalexins have been isolated from economically and dietary important plants of the family *Cruciferae*, which are cultivated worldwide.[2] Among them, several 1,3-thiazino[6,5-*b*]indole derivatives such as cyclobrassinin (**3a**) from the Chinese cabbage (*Brassica campestris* L. ssp. *pekinensis*),[3a] and sinalbin B (**3b**) from the white mustard (*Sinapis alba*)[3b] have been described. Indole phytoalexins demonstrate in vitro cytotoxic/cytostatic activities against human solid tumor and leukemia cell lines.[2c,e] Indole phytoalexins have also been shown to exhibit cancer chemopreventive activity in 7,12-dimethylbenz[*a*]anthracene (DMBA)-induced precancerous lesions in mouse mammary gland organ cultures.[2f] Compounds **3a** and **3b** have been prepared by oxidative bromocyclization of the corresponding indole phytoalexins brassinin (**1a**) or 1-methoxybrassinin (**1b**) using pyridinium tribromide (**3a**, 34%),[3a] NBS (**3a**, 35%;[3c] **3b**, 41%[3b]), dioxane dibromide (**3a**, 45%)[3d] or phenyltrimethylammonium tribromide (**3a**, 59%, Scheme 1).[3e]

In 1994 Gross et al. described the isolation and characterization of a new phytoalexin from kohlrabi (*Brassica oleracea* var. *gongylodes*) which was assigned as structure **4** and named cyclobrassinon.[4a] However only the ^{1}H NMR ($CDCl_3$) and mass spectrum were published and a melting point was not given.[4a]

We have previously studied the synthesis of thiazino[6,5-*b*]indole derivative **4** and its analogs (Scheme 1).[4b–d] Although our synthetic product **4** showed an identical mass spectrum to the natural product, it was poorly soluble in $CDCl_3$ and its ^{1}H NMR spectrum in DMSO-d_6 exhibited differences in the chemical shifts for OMe (δ 4.18[4b] vs δ 3.55[4a] in $CDCl_3$) and NH (δ 12.69[4b] vs δ 8.56[4a] in $CDCl_3$) which we attributed to the different solvent used. Although an original sample of the natural product isolated from kohlrabi was not available for comparison, we correctly assigned the structure of synthetic product **4** by ^{1}H NMR, ^{13}C NMR, and IR spectra. This assignment was also based on the experience we had gained from previous syntheses of analogous 2-substituted-4*H*-benzo[4,5]thieno[2,3-*e*]-1,3-thiazin-4-ones.[4e,f] It was later shown that the structure of the natural product cyclobrassinon had originally been misassigned, thus accounting



Scheme 1.

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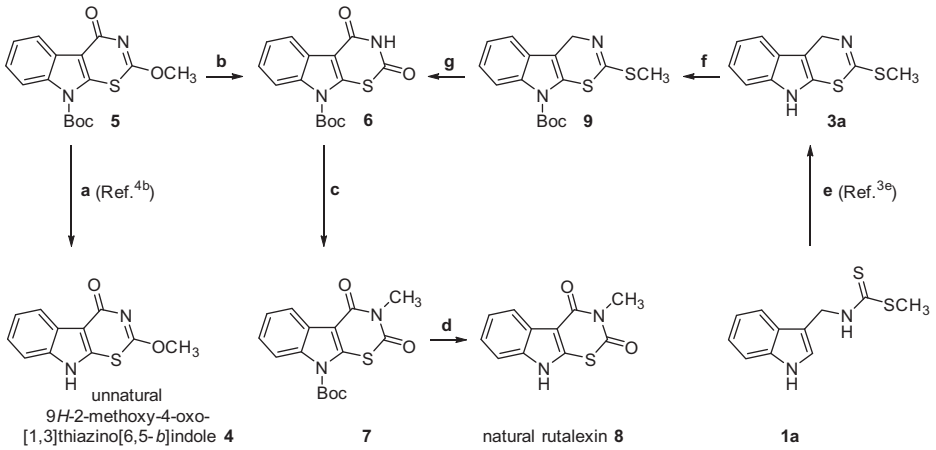

Scheme 2. Reagents and conditions: (a) 165–170 °C, 40 min, 70%; (b) HCl/H_2O (1:1, cat.), acetone, rt, overnight, 80%; (c) DBU, CH_3I, THF, under N_2, rt, 2 h, 95%; (d) 165–170 °C, 30 min., quant.; (e) $PhMe_3NBr_3$, Et_3N, CH_2Cl_2, rt, 1 min, 59%; (f) Boc-anhydride, DMAP, THF, 5 °C, 1 h, 88%; (g) PCC, CH_2Cl_2, 24 h, 66%.

Table 1
Antiproliferative activities of cyclobrassinin (**3a**), rutalexin (**8**), 9*H*-2-methoxy-4-oxo-[1,3]thiazino[6,5-*b*]indole (**4**), and 9-Boc-cyclobrassinin (**9**)

Compound	Cell line, IC_{50} (μmol × L^{-1})					
	Jurkat	MCF-7	MDA-MB-231	HeLa	CCRF-CEM	A-549
Cyclobrassinin (**3a**)	29.5	72.0	48.3	57.2	26.2	70.3
9*H*-2-Methoxy-4-oxo-[1,3]thiazino[6,5-*b*]indole (**4**)	50	>100	>100	47	63	>100
Rutalexin (**8**)	>100	37	>100	>100	>100	>100
9-Boc-cyclobrassinin (**9**)	54.0	100	100	100	47.2	100
Cisplatin	12	11.4	14.7	7.7	4.4	12.2
VP-16 (Etoposide)	1.2	10.9	21.2	3.9	1.1	14.3

The potency of compounds was determined using the MTT (Thiazolyl Blue Tetrazolium Bromide) assay after 72 h incubation of cells and presented as IC_{50}.[7]

for the spectroscopic discrepancies between the synthetic and natural products (see below).

In 2004 Pedras et al. isolated the phytoalexin rutalexin (**8**) from rutabaga tubers (*Brassica napus* L. ssp. *rapifera*).[5] Because the ^1H NMR spectroscopic data were similar to those reported for phytoalexin **4**, they also investigated phytoalexins produced in kohlrabi, again isolating rutalexin (**8**). To unambiguously prove the structures, they synthesized the proposed structures of rutalexin (**8**) and cyclobrassinon (**4**).[5] Comparison of the spectroscopic data (^1H NMR in $CDCl_3$, ^1H and ^{13}C NMR in DMSO-d_6) of the isolated phytoalexins (from kohlrabi and rutabaga) with synthetic rutalexin (**8**) and synthetic cyclobrassinon (**4**) showed that the structure of the natural product first isolated from kohlrabi and named cyclobrassinon was identical to rutalexin isolated from rutabaga. Therefore thiazino[6,5-*b*]indole derivative **4** is not a natural product and phytoalexin **8**, produced by kohlrabi and rutabaga, named rutalexin is the correct structure.[5]

In the present Letter we report the synthetic relationship between synthetic thiazino[6,5-*b*]indole derivative **4** and the natural product rutalexin (**8**) by removing the methyl group from the oxygen and introducing it to the imide-type nitrogen atom of the 1,3-thiazine ring. In the first step 9-Boc-derivative **5**, which was prepared according to the previously reported procedure,[4b] was selectively hydrolyzed to 9-Boc-1,3-thiazino[6,5-*b*]indole-2,4-dione (**6**, Scheme 2).[8] Next, the methylation of nitrogen was examined using literature conditions for related compounds. Methyl iodide in alcoholic sodium hydroxide,[6a,b] dimethyl sulfate in aqueous[6c] or methanolic[6e] sodium hydroxide and diazomethane in diethyl ether[6a,d] were all not suitable for methylation of **6** due to its low solubility and hydrolysis in strongly basic media.

It was found, that when using DBU (1,8-diazabicyclo[5.4.0]undec-7-ene) as a base and methyl iodide as an electrophile, the methylation of **6** proceeded smoothly in high yield (Scheme 2).[9] Finally the Boc group was removed from **7** by heating without solvent to yield rutalexin (**8**),[10] whose data were identical with those of the described natural product.[5] Interestingly the melting points of compounds **7** and **8** are identical, which is rationalized during heating of **7**, deprotection occurred before melting. This caused problems during the measurement of the ^{13}C NMR spectrum of **7**, where due to its insolubility, heating to 60 °C was required. Deprotection also occurred at this time and the ^{13}C spectrum of **7** could not be measured. A second method for the preparation of rutalexin (**8**) based on the biomimetic synthesis from the indole phytoalexin brassinin (**1a**) and proceeding via phytoalexin cyclobrassinin (**3a**) was examined. Cyclobrassinin (**3a**) was obtained by the bromocyclization of brassinin (**1a**) using phenyltrimethylammonium tribromide.[3e] Next, 9-Boc-cyclobrassinin (**9**)[11] was prepared by the reaction of cyclobrassinin (**3a**) with Boc anhydride in 88% yield. Oxidation of compound **9** using PCC gave of 9-Boc-1,3-thiazino[6,5-*b*]indole-2,4-dione (**6**) in moderate yields (66%, Scheme 2).

The antiproliferative activity of natural phytoalexins rutalexin (**8**) and cyclobrassinin (**3a**) as well as 9*H*-2-methoxy-4-oxo-[1,3]thiazino[6,5-*b*]indole (**4**) and 9-Boc-cyclobrassinin (**9**) was tested on selected human cancer cell lines; Jurkat (acute T-lymphoblastic leukemia), MCF-7 and MDA-MB-231 (mammary gland adenocarcinomas), HeLa (cervical adenocarcinoma), CCRF-CEM (acute T-lymphoblastic leukemia) and A-549 (non-small cell lung cancer). Results of this investigation are shown in Table 1, which also includes IC_{50} values for conventional anticancer agents etoposide and cisplatin for comparison. The highest antiproliferative effect was noted with cyclobrassinin (**3a**) where measured IC_{50} values of 26.2–72 μmol × L^{-1} were obtained depending on the cell line, with leukemic cells being the most sensitive. Rutalexin (**8**)

exhibited selective antiproliferative activity against the MCF-7 breast cancer cell line and synthetic 9*H*-2-methoxy-4-oxo-[1,3]thiazino[6,5-*b*]indole (**4**) and 9-Boc-cyclobrassinin (**9**) demonstrated moderate antiproliferative activity on Jurkat, CEM, and HeLa cancer cell lines. Based on our results we postulate that the presence of keto groups in both positions 2 and 4 and the methyl group on the thiazine-2,4-dione nitrogen is responsible for the selective effect of rutalexin (**8**) on MCF cells.

In summary, we have developed two novel, efficient methods for the preparation of the indole phytoalexin rutalexin (**8**) starting from 9-*tert*-butoxycarbonyl-2-methoxy-4-oxo-[1,3]thiazino[6,5-*b*]indole (**5**) and 9-Boc-cyclobrassinin (**9**). The advantages of the present methods in terms of facile manipulation, high yields, simple methodology, and simple work-up procedures, should make this protocol a valuable alternative to the existing method.

Acknowledgements

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Supplementary data

Supplementary data (experimental procedures and characterization data for compounds **6**–**9** and copies of ^{1}H and ^{13}C NMR spectra for compounds **4** and **8**) associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.tetlet.2015.05.001.

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8. *Data for* **6**: mp 283–285 °C (THF/*n*-hexane); Found: C, 56.44; H, 4.61; N, 8.55. $C_{15}H_{14}N_2O_4S$ requires C, 56.59; H, 4.43; N, 8.80; ν_{max} (KBr) 1723, 1680, 1647, 1440, 1347, 1213, 1140 cm^{-1}; δ_H (400 MHz, DMSO-d_6) 12.43 (1H, s, NH-D_2O exchangeable), 8.19 (1H, dd, J = 2.2, 6.9 Hz), 8.06 (1H, dd, J = 7.26, 1.5 Hz), 7.45 (1H, ddd, J = 7.4, 7.2, 1.8 Hz), 7.41 (1H, ddd, J = 7.3, 6.9, 1.3 Hz), 1.70 (9H, s, $C(CH_3)_3$); δ_C (100 MHz, DMSO-d_6) 164.2, 160.9, 148.9, 138.9, 135.5, 125.9, 125.4, 124.6, 120.0, 114.9, 106.3, 87.3, 27.5; EIMS: m/z (%): 318 (M^+, 5), 262 (26), 218 (30), 175 (100), 146 (40), 120 (42), 57 (60).
9. *Data for* **7**: mp 310–311 °C; Found: C, 58.01; H, 4.62; N, 8.62. $C_{16}H_{16}N_2O_4S$ requires C, 57.82; H, 4.85; N, 8.43; ν_{max} (KBr) 1720, 1647, 1567, 1347, 1140 cm^{-1}; δ_H (400 MHz, DMSO-d_6) 8.26 (1H, d, J = 7.6 Hz), 8.09 (1H, d, J = 7.9 Hz), 7.53-7.44 (2H, m), 3.38 (3H, s, CH_3), 1.71 (9H, s, $C(CH_3)_3$); EIMS: m/z (%): 332 (M^+, 5), 276 (26), 232 (30), 175 (100), 146 (40), 120 (35), 57 (67).
10. *Data for* **8**: mp 310–311 °C; Found: C, 56.84; H, 3.45; 12.03. $C_{11}H_8N_2O_2S$ requires C, 56.88; H, 3.47; N, 12.06; ν_{max} (KBr) 3215, 2925, 1728, 1668, 1633, 1463, 1232 cm^{-1}; δ_H (400 MHz, DMSO-d_6) 12.54 (1H, br s, NH-D_2O exchangeable), 8.09 (1H, m), 7.53 (1H, m), 7.28 (2H, m), 3.37 (3H, s, CH_3); δ_C (100 MHz, DMSO-d_6) 162.8, 159.9, 136.9, 135.0, 125.1, 123.6, 122.1, 119.5, 111.8, 101.4, 28.2; EIMS: m/z (%): 232 (M^+, 26), 175 (100), 147 (18), 120 (21). δ_H (400 MHz, $CDCl_3$) 8.57 (1H, br s, NH-D_2O exchangeable), 8.31 (1H, m), 7.38 (3H, m), 3.55 (3H, s, CH_3); Data published for **8** (Ref. 5) δ_H (500 MHz, DMSO-d_6) 12.57 (1H, br s, NH-D_2O exchangeable), 8.09 (1H, m), 7.54 (1H, m), 7.28 (2H, m), 3.37 (3H, s, CH_3); δ_C (125 MHz, DMSO-d_6) 162.8, 160.0, 136.9, 135.0, 125.1, 123.6, 122.1, 119.5, 111.8, 101.5, 28.2; δ_H (500 MHz, $CDCl_3$) 8.52 (1H, br s, NH-D_2O exchangeable), 8.28 (1H, m), 7.40 (3H, m), 3.57 (3H, s, CH_3); Data published for **8** (Ref. 4a) δ_H ($CDCl_3$) 8.56 (1H, s), 8.31 (1H, m), 7.41 (1H, m), 7.37–7.32 (2H, m), 3.55 (3H, s, CH_3).
11. *Data for* **9**: mp 92–93 °C (dichloromethane/*n*-hexane); Found: C, 57.33; H, 5.41; N, 8.42. $C_{16}H_{18}N_2O_2S_2$ requires C, 57.46; H, 5.42; N, 8.38; ν_{max} (KBr) 3049, 2978, 2840, 1716, 1626, 1448, 1364, 1249, 1109 cm^{-1}; δ_H (400 MHz, $CDCl_3$) 8.07 (1H, d, J = 7.5 Hz, H-8), 7.41–7.38 (1H, m, H-5), 7.29–7.22 (2H, m, H-7, H-6), 5.01 (2H, s, CH_2), 2.53 (3H, s, SCH_3), 1.70 (9H, s, $C(CH_3)_3$); δ_C (100 MHz, $CDCl_3$) 155.5, 149.7, 136.4, 127.6, 124.3, 124.0, 123.1, 117.0, 115.0, 108.4, 85.5, 47.8, 28.2, 15.1; EIMS: m/z (%): 334 (M^+, 24), 161 (100).