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Synthesis and *in vitro* antiproliferative effect of isomeric analogs of cyclobrassinin phytoalexin possessing the 1,3-thiazino[5,6-*b*]indole-4-one skeleton

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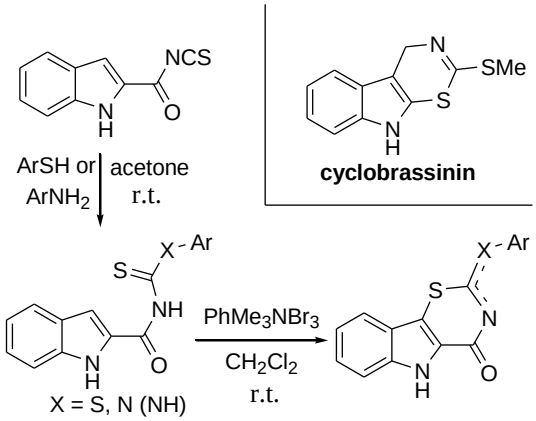
Abstract

Isomeric analogs of cyclobrassinin phytoalexin possessing the 1,3-thiazino[5,6-*b*]indole-4-one skeleton have been prepared. Starting from 1*H*-indole-2-carbonyl isothiocyanate, *N*-aryl-*N'*-(indole-2-carbonyl)-substituted thiourea or the corresponding *S*-aryl-*N*-(indole-2-carbonyl)dithiocarbamate intermediates were prepared and then transformed by ring-closure via Hugerschoff reaction with phenyltrimethylammonium tribromide. Attempted synthesis of *N*'-(indole-2-carbonyl)-*N*-(3,4,5-trimethoxyphenyl)thiourea unexpectedly delivered the 2-(indole-2-carbonylamino)-5,6,7-trimethoxy-benzothiazole ring system. The structures of the new ring systems were determined by means of IR and NMR spectroscopy. The new derivatives synthesized exert moderate *in vitro* antiproliferative effects on a panel of adherent human cell lines (HeLa, A2780, A431 and MCF7).



Keywords: 1,3-Thiazino[5,6-*b*]indole-4-ones, indole-2-carboxamide moiety, phytoalexin isomeric analogs, IR, ^{1}H and ^{13}C NMR

Introduction

Among the possible isomers of 1,3-thiazinoindoles condensed at bond *b* of the indole skeleton, the most interesting derivatives are 1,3-thiazino[6,5-*b*]indoles. Compounds possessing this ring system, such as cyclobrassinin and their biosynthetic or synthetic precursors (brassinin), belong to the family of natural phytoalexins and were isolated from cruciferous plants, first from *Chinese cabbage* (Figure 1).[1]

Phytoalexins are antimicrobial substances of low molecular weight produced by plants in response to infection or stress, which form part of their active defense mechanisms, probably as a result of the de novo synthesis of enzymes, as a part of the immune system of plants.[2] In addition to their antimicrobial activities, brassinin and cyclobrassinin have various other pharmacological effects. They inhibit the formation of preneoplastic mammary lesions in culture.[3] They also exerts in vitro antiproliferative effect on a number of different human cell lines.[4,5] As for the pharmacodynamic mechanism, brassinin and its derivatives are inhibitors of indolamine 2,3-dioxygenase, a new cancer immunosuppression target.[6] *Izutani et al.* revealed, that brassinin induces G1 phase arrest through increase of p21 and p27 by inhibition of the phosphatidylinositol 3-kinase signalling pathway in human colon cancer cells.[7] *Kello et al.* investegated ROS-dependent antiproliferative effect of homobrassinin, a brassinin derivative, in human colorectal cancer caco2 cells.[8] *Lee et al.* showed that brassinin inhibits STAT3 signaling pathway through modulation of PIAS-3 and SOCS-3 expression and sensitizes human lung cancer xenograft in nude mice to paclitaxel.[9]

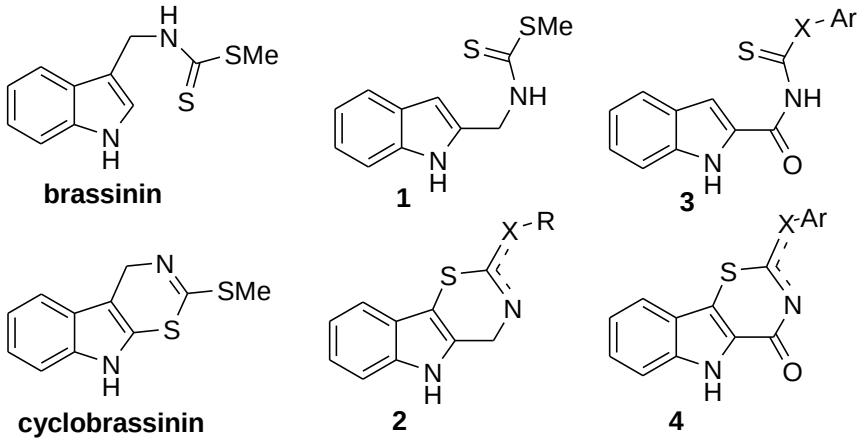


Figure 1

Extensive investigations have been focused on the natural members of *S*,*N*-phytoalexins from antiproliferative point of view. In contrast, relatively few isomeric bioisoster indole compounds of this type were sythesized and investigated from pharmacological aspects. A series of 2-alkyl- or 2-arylimino-1,3-thiazino[5,4-*b*]indol-4-one derivatives[10] inhibits human leukocyte elastase and α-chymotrypsin. The 4-oxo-1,3-thiazino[6,5-*b*]indole derivatives of cyclobrassinone were prepared *Kutschy et al*. It turned out later, however, that the original structure of this phytoalexin was most probably misinterpreted.[11]

In our previous work we prepared isobrassinin (**1**), 2-methylthio-1,3-thiazino[5,6-*b*]indole (isocyclobrassinin, **2**, X = S, R = Me, Figure 1) and its 2-benzylthio- analog **2** (X = S, R = Bn, Figure 1) and found them to exert good *in vitro* antiproliferative effects on cervical adenocarcinoma (HeLa), breast adenocarcinoma (MCF7) and squamous skin carcinoma (A431) cell lines.[5] For the investigation of structure–

activity relationships, further 2-aryl-1,3-thiazino[5,6-*b*]indole analogs were synthesized. The highest cytotoxic effect was displayed by 2-phenylimino-1,3-thiazino[5,6-*b*]indole **2** (X = N, R = Ph, Figure 1), which demonstrated inhibitory activity on the above three cell lines comparable to that of cisplatin.[5]

In order to improve the possible efficacy our attention next turned to the incorporation of indol-2-carboxamide moiety (**3**, **4**; Figure 1) into isobrassinin and isocyclobrassinin analogon compounds (**1**, **2**; Figure 1). Motifs containing the indole-2-carboxamide moiety have the increased ability to bind to different receptors and enzymes. Compounds containing this pharmacophore, therefore, can be found in varied biologically active compounds. Certain derivatives, which target allosteric modulation of cannabnoid receptor 1 (CB1),[12] glycogene phosphorylase inhibitors,[13] neurotensin (NT) (8–13) mimetics,[14] monoamine oxidase inhibitors,[15] agonists of nociceptin/orphanin FQ (N/OFQ receptors,[16] dopa D_3 receptor,[17] selective antagonists of NR2B subunit containing *N*-methyl-*D*-aspartate (NMDA) receptor,[18] can be useful in different mental and CNS disorders. Furthermore, other indole-2-carboxamide counterparts may act as antioxidants,[19] coagulation factor Xa inhibitors[20] and can have useful in different pathological conditions, such as heart failure conditions,[21] diabetes,[22] osteoporosis,[23] Chron's dishease,[24] and certain infections.[25] These are also inhibitors of different enzymes (Rho kinase,[26] endothelin-converting enzyme,[27] vascular endothelial growth factor FR2 VEGFR2 tyrosine kinase,[28] topoisomerase I,[29] type 5 17β-hydroxysteroid dehydrogenase,[30] tubulin polimerization[31]), may act as apoptosis inducers[32] and potential DNA-intercalating compounds[33] (like duocarmycin A). In addition, they exert promising and remarkable *in vitro* antiproliferative effects.

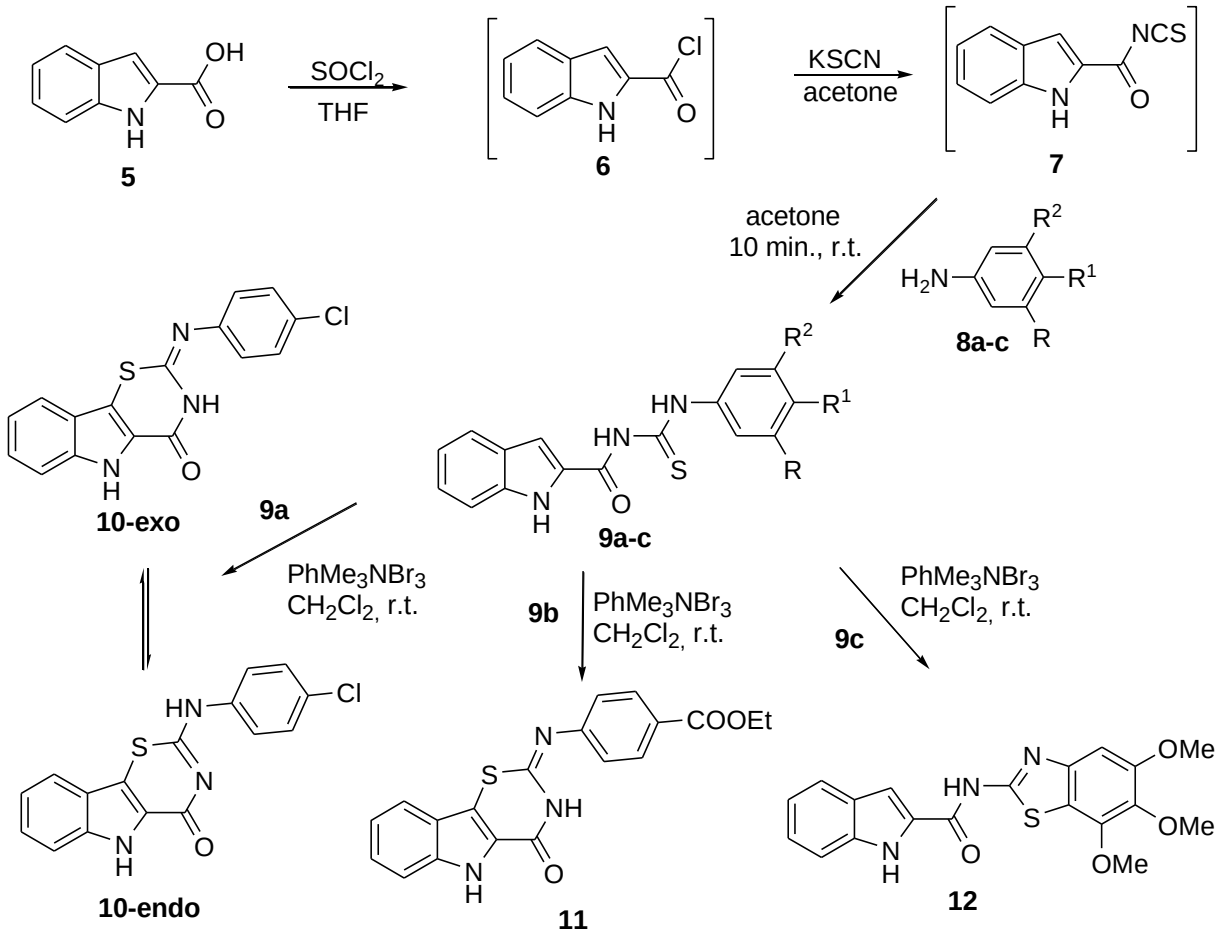
Subsequent to our recent investigations on 1,3-thiazinoindole derivatives[5,34-36] and the chemistry of sulfur- and nitrogen-containing heterocycles with condensed skeleton,[37] we set out to investigate the synthesis, structure and *in vitro* antiproliferative effect of new 1,3-thiazino[5,6-*b*]indole-4-one derivatives and their intermediates.

Results and Discussion

Our starting material 1*H*-indole-2-carbonyl isothiocyanate **7** was obtained from 1*H*-indole-2-carboxylic acid (**5**) (Scheme 1). Treatment of **5** with thionyl chloride in tetrahydrofuran provided 1*H*-indole-2-carbonyl chloride (**6**), which was reacted with potassium thiocyanate in acetone at room temperature. The reaction furnished isothiocyanate **7**, which was used in the next step without any further purification (Scheme 1).

The key intermediate *N*,*N*'-disubstituted thioureas **9a–c** were synthesized form acyl isothiocyanate **7** by its reaction with substituted aniline derivatives **8a–c** in acetone in good yields (Scheme 1). According to our previous results for the preparation of our target 1,3-thiazino[5,6-*b*]indole-4-one derivatives, the Hugerschoff reaction was applied. The oxidative cyclization of (4-chlorophenyl)thiourea derivative **9a** with phenyltrimethylammonium tribromide in dichloromethane yielded thiazinoindole **10**. It turned out, however, that this compound exists as an equilibrium mixture of tautomers **10-*endo*** and **10-*exo*** in hexadeuterodimethyl sulfoxide under NMR measuring conditions.

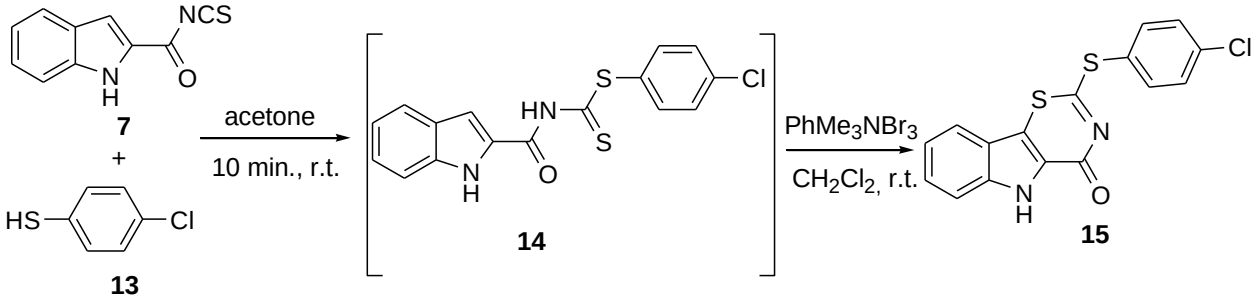
4-(Ethoxycarbonyl)phenyl-substituted thiourea **9b** gave the excepted 2-[4-(ethoxycarbonyl)phenylimino]-1,3-thiazino[5,6-*b*]indole-4-one (**11**) in good yield upon treatment with the bromide reagent applied above. For 3,4,5-trimethoxyphenyl-substituted **9c**, the preferable reaction path in the Hugerschoff reaction was the formation of thiazole ring system 2-(indol-2-carbonylamino)-5,6,7-trimethoxy-benzothiazole (**12**).



a: R = H, R^1 = Cl , R^2 = H; **b**: R = H, R^1 = COOEt, R^2 = H; **c**: R = R^1 = R^2 = OMe

Scheme 1

In order to obtain further analogs, 2-(4-chlorophenylthio)-1,3-thiazino[5,6-*b*]indole-4-one **15** was also prepared (Scheme 2). The reaction of isocyanate **7** with 4-chlorothiophenol provided dithiocarbamate derivative **14**, which underwent oxidative ring closure reaction providing thiazine **15** when treated with phenyltrimethylammonium tribromide.



Scheme 2

The structures of the synthesized compounds were verified by IR, ^{1}H and ^{13}C NMR spectroscopy including 2D-HMQC, DEPT, ^{1}H,^{13}C and ^{13}C,^{15}N-HMBC measurments. Spectral data are given in Tables 1 and 2.

These techniques provided unambiguous proof of the formation of the expected structures. The only exception is compound **12**, which formed instead of desired 2-(3,4,5-trimethoxyphenylimino)-1,3-thiazino[5,6-*b*]indole-4-one. The benzthiazole moiety in **12** is verified by the following spectral data.

- The benzene ring bearing the three methoxy substituents is penta-substituted (only a single aromatic carbon is protonated).
- The six benzene carbons have carbon lines with different chemical shifts.
- The substitution is asymmetric and the three methoxy groups have different ^{1}H and ^{13}C NMR signals.
- The C-2 atom in the indole moiety is unsubstituted (the appropriate ^{1}H NMR singlet appears at 7.74 ppm).
- The ring-chain tautomerism and the N-inversion around the exo *sp*^{2} N atom result in slow motion of the electron distribution in molecules **10** and **11**. Consequently, the carbon lines of S–C= and lactam C=O groups have broadened and thus in the ^{13}C NMR spectrum cannot be assigned in every cases. Similarly, the H-2',6' signals in the ^{1}H NMR are broadened. Such broadenings are not observed in the spectra of **12**.

It is to be noted that in compounds **9a–c** a chelate-type hydrogen bonding can form within the CO-NH-CS moiety. (A six-membered ring is formed by H-bonding between the carbonyl oxygen and the SH-hydrogen of the CO-N=C-SH tautomeric form). This assumption is supported by the following observations.

- The νNH IR band of this group is extremely broadened (around 2000 cm^{-1}) and thus hardly observable. Such a diffuse absorption band is characteristic of chelates.[38]
- The C=S line in ^{13}C NMR appears at 179.5**–**179.7 ppm for **9a–c**, outside the usual interval (173**–**176 ppm[39]).
- The electron-withdrawing or electron-releasing character of the substituents in the NH-aryl group does not have any influence on the IR frequencies and the NMR shifts of the CO-NH-CS group. This makes highly probable that these two parts of the molecule (NHAr and CONHCS) are isolated from one another. All these features are in good agreement with the presence of the stable chelate ring.

Table 1. Characteristic IR frequencies[a] and ^{1}H NMR data[b] of compounds **9a–c, 10**–**12** and **15**[c]

Com-	νNH	ν(N)C=O	γC$_{Ar}$H	γC$_{Ar}$H	H–3	H–4	H–5	H–6	H–7	H–2',6'	H–3',5'	NH	NH
pound	band	band	band [d]	band [e]	*s* [d]	~ *d* [d]	~ *t* [d]	~ *t* [d]	~ *d* [d]	*d* (2H)[e]	*d/t* (2H)[e]	amide	indole
9a	3358	1641	739, 733	844, 820	7.83	7.70	7.11	7.31	7.51	7.48	7.73	11.6, 12.0	12.6
9b	3346, 3294	1658	740	8.46	7.82	7.70	7.11	7.31	7.51	7.93	8.01	11.6, 12.0	12.8
9c	3390, 3216	1649	746	705	7.81	7.70	711	7.31	7.51	7.13	–	11.5, 12.0	12.6
10	3221	1616	740	828	–	7.67	7.15	7.40	7.53	~7.6	7.44	11.1	12.4
11	~3450	1649	754	716	–	7.68	7.18	7.42	7.56	~7.7	7.99	~11.1	12.3
12	3260	1648	749	810	7.74	7.70	7.10	7.28	7.51	7.17[f]	–	12.9	12.0
15	3230	1631	743	828	–	7.75	7.18	7.46	7.58	7.87	7.71	–	12.7

[a]In KBr discs (cm^{-1}). Further bands, Ester: νC=O: 1713 (**9b**), 1735 (**11**); νC–O: 1276, 1153 (**9b**), 1307, 1191 (**11**), νC–O (methoxy groups): 1222, 1125 (**9c**), 1278, 1110 (**12**); [b]In DMSO-d$_6$ solution at 500 MHz. Chemical shifts in ppm (δ_{TMS} = 0 ppm). Further signals: CH$_3$ and CH$_2$ (OEt, **9b, 11**): 1.34, *t*, *J*: 7.1 Hz and 4.34 *qa*; OCH$_3$, *s*: 3.69 (**9c**, 6H, *Pos 3',5'*), 3.80 (**9c**, 3H, Pos. *4'*), 3.80, 3.90, 4.03 (12, 3-3H, Pos. *7'*, *6'* and *5'*); [c]Assignments were supported by HMQC and ^{1}H,^{13}C HMBC measurements; [d]Indole ring; [e]Aryl group; [f] Pos. *4'*.

Table 2. ^{13}C NMR chemical shifts[a] of compounds **9a–c, 10**–**12** and **15**[b]

Com-	C=S	C=O	C–2	C–3	C–3a	C–4	C–5	C–6	C–7	C–7a	C–1'	C–2',6'	C–3',5'	C–4'
pound	ester	amide	indole ring								aryl group			
9a	179.7	161.9	128.8	109.2	127.3	123.0	120.9	125.8	113.1	138.4	130.8	129.0	126.8	137.5
9b	179.7	162.3	129.2[e]	109.7	127.7.	123.4	121.3	126.3	113.5	138.8	143.2	124.5	130.6	128.0
9c	179.5	162.4	129.3	109.6	127.7	123.4	121.3	126.2	113.5	138.8	134.6	103.05	153.5	136.5
10	?	162.2[c]	123.8	110.9	121.9	120.8	121.4	127.5	114.1	138.4	128.9	123.7	129.9	123.8
11	?	?	123.9	111.0	122.0	120.7	121.4[d]	127.5	114.2	138.6	126.2	121.5[d]	131.2	121.5[d]
12	158.9[e]	160.6	129.9	107.4	127.8	123.2	121.2	125.6	113.4	138.4	147.2[f]	100.2[g]	145.9[h]	154.4[i]
15	175.4[e]	160.4	121.5	115.3	123.2	121.3	121.9	128.4	114.3	138.4	123.5	139.3	131.4	137.9

[a]In ppm (δ_{TMS} = 0 ppm) at 125.7 MHz. Solvent: DMSO-d_6. Further signals, CH_3: 15.1 (**9b**, **11**), CH_2: 61.6 (**9b**), 61.3 (**11**), 21.8 (**11**); OCH_3 (Pos.. 3,5 and 4): 56.9 and 61.0 (**9c**), OCH_3 (Pos.. 5-7): 57.1, 61.3, 61.8(**12**); C=O (ester): 166.0 (**9b**), 166.2 (**11**). Due to slow motion of the NH-C=NAr ⇄ N=C-NHAr moiety (tautomerism) were not possible to identify the SC= (**10** and **11**) and C=O (amide) lines(**11**); [b]Assignments were supported by DEPT (except for **9a, 11**), 2D-HMQC, ^{1}H,^{13}C HMBC, for **12** also by ^{1}H,^{15}N HMBC measurements; [c]Broadened signal; [d]Reversed assignments is also possible; [e] SC=N line; [f/g/h/i]C-3a, 4, 5 and 6 lines, resp. in benzthiazole moiety.

Concerning the ring-chain tautomerism possible in **10–12**, the difference in the shape and shift of NH signal between **10–11** and **12**, respectively, is striking. In **12** the preferred structure of the aromatic benzothiazole part excludes the tautomerism and the amide NH signal is sharp and has higher shift at 12.0 ppm. In contrast, the tautomeric equilibrium of **10** and **11** results in a broad NH signal and a lower shift (11.1 ppm).

Table 3. *In vitro* antiproliferative effects of compounds prepared

Compound	Concentr.	Growth inhibition, % ± SEM[a]			
		HeLa	A2780	MCF7	A431
9a	10 μM	–	–	–	n.t.
	30 μM	47.5 ± 1.7	–	49.2 ± 0.7	n.t.
9b	10 μM	61.4 ± 0.5	–[a]	–	45.2 ± 0.2
	30 μM	58.8 ± 1.4	–	–	50.2 ± 0.3
9c	10 μM	35.5 ± 1.8	29.6 ± 1.0	–	–
	30 μM	48.2 ± 2.9	52.0 ± 1.4	31.2 ± 1.9	44.8 ± 0.8
10	10 μM	–	61.5 ± 1.0	44.8 ± 1.4	–
	30 μM	63.8 ± 0.7	79.1 ± 0.5	70.1 ± 0.8	70.6 ± 0.7
11	10 μM	61.2 ± 0.9	55.3 ± 1.5	36.0 ± 2.1	67.2 ± 0.8
	30 μM	81.7 ± 2.4	87.6 ± 1.8	82.3 ± 2.5	81.7 ± 0.6
12	10 μM	–	60.4 ± 1.2	43.3 ± 1.8	–
	30 μM	89.0 ± 0.9	94.7 ± 0.1	92.5 ± 0.2	68.4 ± 1.1
15	10 μM	47.1 ± 1.9	89.6 ± 2.1	69.6 ± 1.3	n.t.
	30 μM	93.0 ± 2.3	95.3 ± 0.4	89.8 ± 0.3	n.t.
Cisplatin	10 μM	42.6 ± 2.3	83.6 ± 1.2	53.0 ± 2.3	88.5 ± 0.5
	30 μM	99.9 ± 0.3	95.0 ± 0.3	86.9 ± 1.2	90.2 ± 1.8

[a]Substances eliciting less than 25% inhibition of cell proliferation were regarded as ineffective and the results are not presented; n.t.: not tested

The *in vitro* antiproliferative activities of the compounds prepared were examined on human tumor cell lines[40] HeLa (cervix adenocarcinoma), MCF7 (breast adenocarcinoma), A2780 (ovarian carcinoma) and A431 (squamous carcinoma) by means of MTT assay. The results are summarized in Table 3.

Modest growth inhibition effects were observed for isocyclobrassinin analogs **9a–c**. The ring-closed 4-arylimino-thiazines **10** and **11** performed moderate cell growth inhibition (up to 67.2%, 10 µM, compound **11**, A431 cell line). Interestingly, thiazole **12** also has relatively good inhibition effect on cell line A2780 (60.4% at 10 µM). Of our compounds prepared 2-(4-chlorophenylthio)-4,5-dihydro-1,3-thiazino[5,6-*b*]indole-4-one **15** showed the highest antiproliferative activity exhibiting 89.6 % (10 µM) growth inhibition on cell line A2780. This value is comparable to that of cisplatin.

Conclusions

In summary, isomeric analogs of cyclobrassinin phytoalexin possessing the 1,3-thiazino[5,6-*b*]indole-4-one skeleton have been prepared, and were found to have noteworthy *in vitro* antiproliferative effects. The 2-(4-chlorophenylthio)-4,5-dihydro-1,3-thiazino[5,6-*b*]indole-4-one derivative **15**, a sulfur analogue of β-carboline showed the highest antiproliferative activity. The structures of the novel compounds prepared were confirmed by means of IR and NMR spectroscopy and discussed in some detail.

Experimental Section

General. Melting points were determined on a Kofler micro melting apparatus and are uncorrected. Elemental analyses were performed with a Perkin-Elmer 2400 CHNS elemental analyser. Merck Kieselgel $60F_{254}$ plates were used for TLC, and Merck Silica gel 60 (0.063-0.100) for column chromatography. The ^{1}H and ^{13}C NMR spectra were recorded in $CDCl_3$ solution in 5 mm tubes at room temperature, on a Bruker DRX 500 spectrometer at 500 (^{1}H) and 126 (^{13}C) MHz, with the deuterium signal of the solvent as the lock and TMS as internal standard. DEPT spectra were run in a standard manner, using only the $\Theta = 135^{0}$ pulse to separate CH/CH_3 and CH_2 lines phased "up" and "down", respectively. The 2D-HSQC and -HMBC spectra were obtained by using the standard Bruker pulse programs. Indole-2-carboxylic acid was purchased from Fluka. Indole-2-carbonyl chloride was prepared by an earlier method.[35]

The antiproliferative properties of the prepared analogs were determined *in vitro* against a panel of human adherent cancer cell lines[40] including HeLa (cervix adenocarcinoma), MCF7 (breast adenocarcinoma), A2780 (ovarian carcinoma) and A431 (squamous carcinoma). All cell lines were purchased from the European Collection of Cell Cultures (ECCAC, Salisbury, UK). The cells were maintained in minimal essential medium (Lonza Ltd, Basel, Switzerland) supplemented with 10% foetal bovine serum, 1% non-essential amino acids and an antibiotic-antimycotic mixture. Near-confluent cancer cells were seeded onto a 96-well microplate at the density of 5000 cells/well and, after overnight standing, new medium (200 µL) containing the tested compound at 10 and 30 µM was added. After incubation for 72 h at 37 ºC in humidified air containing 5% CO_2, the viability of the cells was determined by the addition of 5 mg/mL 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) solution. During a 4-h contact period, the MTT was converted by intact mitochondrial reductase and the precipitated formazan crystals were dissolved in 100 µL DMSO. Finally, the reduced MTT was assayed at 545 nm, using a microplate reader; wells with untreated cells were utilized as controls. All *in vitro* experiments were carried out on two microplates with at least five parallel wells. Stock

solutions of the tested substances (10 mM) were prepared in DMSO. The highest DMSO content of the medium (0.3%) did not have any substantial effect on the cell proliferation. Cisplatin (Ebewe Pharma GmbH, Unterach, Austria) was used as the reference agent.

General procedure for the preparation of *N*-aryl-*N'*-(1*H*-indole-2-carbonyl)thioureas (9a–c). To a stirred solution of potassium thiocyanate (2.0 g, 20.6 mmol) in dry acetone (30 mL) a solution of freshly prepared indole-2-carbonyl chloride (3.4 g, 20,0 mmol) in acetone (10 ml) was added in one portion at room temperature. The reaction mixture was stirred for 1 h at room temperature and filtered through a sintered glass. After the evaporation of the solvent, the crystalline product was taken up in diethyl ether (2 x 10 mL) and filtered. Isothiocyanate (**7**) was used in the next step without any further purification.

To the stirred solution of 1*H*-indole-2-carbonyl isothiocyanate **7** (0.5 g, 2,4 mmol) in acetone (10 mL) the appropriately substituted aniline **8a–c** derivative was added portionwise. The reaction mixture was stirred at room temperature for 1 h (**9c** precipitated as white crystalline product) and then concentrated. The crystalline residues were triturated with acetone (**9a–c)**, filtered off and purified as indicated.

***N*-(4-chlorophenyl)-*N'*-(1*H*-indole-2-carbonyl)thiourea (9a).** A pale-yellow crystalline powder, mp 209–212 ºC (methanol) , yield 92%. Anal. Calcd. for $C_{16}H_{12}ClN_3OS$ (329.80): C, 58.27; H, 3.67; N, 12.74; S, 9.72. Found: C, 58.05; H, 3.89; N, 12.55; S, 9.95.

***N*-[4-(ethoxycarbonyl)phenyl]-*N'*-(1*H*-indole-2-carbonyl)thiourea (9b).** A pale-yellow crystalline powder, mp 212–215 ºC (methanol), yield 87%. Anal. Calcd. for $C_{19}H_{17}N_3O_3S$ (367.42): C, 62.11; H, 4.66; N, 11.44; S, 8.73. Found: C, 62.40; H, 4.81; N, 11.27; S, 8.95.

***N'*-(1*H*-indole-2-carbonyl)-*N*-(3,4,5-trimethoxyphenyl)thiourea (9c).** A pale-yellow crystalline powder, mp 208–210 ºC (methanol+chloroform), yield 93%. Anal. Calcd. for $C_{19}H_{19}N_3O_4S$ (385.44): C, 59.21; H, 4.97; N, 10.90; S, 8.32. Found: C, 59.07; H, 5.21; N, 10.67; S, 8.61.

General procedure for the preparation of 4,5-dihydro-1,3-thiazino[5,6-*b*]indole-4-one derivatives (10, 11) and benzothiazole (12). To an intensively stirred suspension of thiourea derivatives **9a–c** (0.7 mmol) in dichloromethane (20 mL), phenyltrimethylammonium tribromide (0.26 g, 0.69 mmol) was added in one portion at room temperature. After stirring for 5 min, triethylamine (0.39 mL, 2. 8 mmol) was added in one portion to the clear solution. The solvent was evaporated (water bath <50 °C) and the residue was purified by column chromatography, using *n*-hexane/ethyl acetate 3:2 as an eluent, to give **10–12** as crystalline powders.

2-(4-Chlorophenylimino)-4,5-dihydro-1,3-thiazino[5,6-*b*]indole-4-one (10). A yellow crystalline powder, mp 302–305 ºC, yield 78%. Anal. Calcd. for $C_{16}H_{10}ClN_3OS$ (327.79): C, 58.63; H, 3.08; N, 12.82; S, 9.78. Found: C, 58.38; H, 3.27; N, 12.57; S, 10.05.

2-[(4-(Ethoxycarbonyl)phenylimino]-4,5-dihydro-1,3-thiazino[5,6-*b*]indole-4-one (11). A yellow crystalline powder, mp 289–291 ºC, yield 81%. Anal. Calcd. for $C_{19}H_{15}N_3O_3S$ (365.41): C, 62.45; H, 4.14; N, 11.50; S, 8.78. Found: C, 62.28; H, 4.40; N, 11.33; S, 9.05.

2-(Indole-2-carbonylamino)-5,6,7-trimethoxy-benzothiazole (12). A pale-yellow crystalline powder, mp 258–261 ºC, yield 84%. Anal. Calcd. for $C_{19}H_{17}N_3O_4S$ (383.42): C, 59.52; H, 4.47; N, 10.96; S, 8.36. Found: C, 59.77; H, 4.67; N, 10.75; S, 8.24.

General procedure for the preparation of 2-(4-chlorophenylthio)-4,5-dihydro-1,3-thiazino[5,6-*b*]indole-4-one (15). To a stirred solution of acyl isothiocyanate **7** (0.5 g, 2,4 mmol) in acetone (10 mL) thiophenol **13** derivative was added portionwise. The reaction mixture was stirred at room temperature for 1 h and then concentrated.

The crystalline residue was triturated with diisopropylether+ethyl acetate. Product **14** was filtered off and used without further purification in the next ring-closing step.

To an intensively stirred suspension of thiourea derivative **14** (0.7 mmol) in dichloromethane (20 mL), phenyltrimethylammonium tribromide (0.26 g, 0.69 mmol) was added in one portion at room temperature. After stirring for 5 min, triethylamine (0.39 mL, 2. 8 mmol) was added in one portion to the clear solution. The solvent was evaporated (water bath <50 °C) and the residue was purified by column chromatography, using *n*-hexane/ethyl acetate 3:2 as an eluent, to give **15** as a yellow crystalline powder, mp 288–290 ºC, yield 67%. Anal. Calcd. for $C_{16}H_9ClN_2OS_2$ (344.84): C, 55.73; H, 2.63; N, 8.12; S, 18.60. Found: C, 55.93; H, 2.82; N, 8.33; S, 18.76

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