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Design, synthesis and biological evaluation of novel 5-bromo derivatives of indole phytoalexins

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Keywords: 5-bromobrassinin; indole; phytoalexins; cyclization reactions; antiproliferative activity

Abstract

The increasing diversity of small molecule libraries is a major source for the discovery of new drug candidates. In term of this trend, we report the synthesis five series 5-bromosubstituted derivatives of indole phytoalexins **Type A-E** using straightforward synthetic approach. Novel compounds were screened in vitro for antiproliferative/cytotoxic activity against seven human cancer cell lines by MTT assay. Evaluation of their antiproliferative potency showed that the activity of some analogues was better or comparable to that of cisplatin and at the same time the toxicity of these compounds on 3T3 cells was lower than that of cisplatin. We found that all 5-bromosubstituted analogues of indole phytoalexins **Type A-E** exhibited lower or approximately the same activities as a previously studied correnspondingnon-brominated compounds.

Introduction

Cruciferous vegetables (such as broccoli, mustard greens, cabbage, cauliflower and turnip) are prolific producers of indole–sulfur substances when are exposed to physical, chemical (heavy metals, UV radiation) or biological stress (pathogen infection). These compounds, termed

indole phytoalexins, are a hallmark of the family Brassicaceae and serve as an important defense mechanism for the plants. The majority of indole phytoalexins are rather simple compounds. The basic structure of these compounds is an indole, oxindole or indoline nucleus with a linear chain or annexed heterocycle (**1,2,6**, Fig. 1.) Some of the indole phytoalexins carry unique structural features with spiro attached thiazoline ring (**3-5**, Fig. 1) [1].

The literature survey showed that indole phytoalexins possess a broad spectrum of biological activities. Indole phytoalexins exhibit a wide range of antifungal activities, moderate antibacterial effect [2-6] and antiprotozoal activity [7]. The anti-aggregation effect of spirobrassinin [(±)-**3**] was demonstrated in the cerebrospinal fluid of patients with multiple sclerosis [8]. Indole phytoalexins have been shown to also have cancer chemopreventive properties [9] and it was proven that high consumption of cruciferous vegetables may decrease human cancer risk [10]. Indole phytoalexins display anticancer and antiproliferative effects against human cancer cell lines [11-16].

In addition, a naturally occurring brassinin (**1**), is one of the most biologically active indole phytoalexins and exhibits various pharmacological effects. Its activities is partly a result of its dithiocarbamate group. Brassinin (**1**) acts as a mitochondrial inhibitor and antioxidant [17]. Brassinin (**1**) induces cell cycle arrest in G_1 phase through inhibition of the PI3K signaling pathway in HT-29 human colon cancer cells [18]. Another study showed that brassinin (**1**) is bioavailable indoleamine 2,3-dioxygenase inhibitor (IDO - enzyme that promotes tumor escape via mechnisms of immune tolerance) [19]. In several studies, brassinin has been the subject of combination therapy. Brassinin (**1**) in combination with capsaicin has synergistic anticancer effect on PC-3 human prostate cancer cells [20]. Lee et al. have revealed that a combination of brassinin (**1**) and paclitaxel synergistically inhibited A549 lung cancer cell growth [21]. It has also recently been found that the combination of brassinin-imatinib synergistically induces cytotoxicity and apoptosis in SW480 colon cancer cells. The combined treatment of brassinin (**1**) and imatinib have also revealed the anti-metastatic potencial of treatment [22]. Three novel biological activities of brassinin (**1**) were recently described. Brassinin (**1**) inhibits TNF-α-induced vascular inflammation in human umbilical vein endothelial cells (HUVECs) and may serve as a potencial therapeutic agent for atherosclerosis [23]. Brassinin (**1**) also effectively suppresses lipid accumulation in 3T3-L1 adipocytes and obesity-induced inflammatory responses through the Nrf2-HO-1 signaling pathway in an adipocyte-macrophage co-culture [24].

The indole phytoalexins are class of natural products displaying unique promising properties for the development of new drugs leads, and they are a wonderful challenge to synthetic chemists. Various synthetic structural and positional modifications of indole phytoalexins have been made to evaluate their antiproliferative activities for development of novel anticancer agents. 1-Boc substituted derivative of brassinin (**Type I**, Fig. 1) and thiourea derivatives of brassinins (**Type III**) display higher potencies of antiproliferative activity than the lead compounds **1** and **2** [25-27]. Homobrassinin (**Type II**) is more active than brassinin (**1**) and has been shown to cause ROS dependent apoptosis in Caco-2 colorectal cancer cells [28]. Likewise, the introduction of a substituted phenyl amino group to the compounds **3** and **4** (**Type V**) resulted in enhanced antiproliferative effect against human cancer cell lines [27,29]. Structural modification of phytoalexin (2*R*,3*R*)-(-)-1-methoxyspirobrassinol methyl ether [(2*R*,3*R*)-(-)-**6a**] - synthetic 2-aminoanalogues (**Type VII**), 2'-aminoanalogues (**Type VIII**) or 2,2'-diaminoanalogues (**Type IX**) exhibited remarkable anticancer activity [25,26,29-32]. Synthetic analogues of cyclobrassinin (**Type XI**, Fig. 1) with phenyl amino group instead of methylthio group have shown extraordinary anticancer properties [25,33].

According to the literature reports, the halogenation of natural products is a one of the most popular modification that allows optimalization of the biological activity of molecules. The majority of halogenated metabolites contain bromine. An interesting fact was observed in all types of indole scaffolds that halogenations generally occur at C-5, sometimes at C-6 or at both C-5 and C-6 of the indole ring. The bromination of many natural compounds is associated with increased biological activity [34].

From the group of synthetic derivatives of indole phytoalexins, 5-bromobrassinin (**Type IV**, Fig. 1) contains another bromine atom in the indole nucleus C-5. 5-Bromobrassinin has a better pharmacologic profile than brassinin with slower clearance [35]. 5-Bromobrassinin induced tumour regressions of mammary gland tumors in MMTV-*Neu* mice in combination with paclitaxel [36]. 5-Bromobrassinin belongs to the class of compounds having IDO inhibitor activity. 5-Bromobrassinin suppressed growth of highly aggressive B16-F10 melanoma isograft tumor [19]. The presence of bromine at the C-5 position of the indole nucleus of spiroindoline phytoalexins (**Type VI**, **Type X**) led to a partial increase of antiproliferative activities on leukaemic cells compared to natural non-brominated indole phytoalexins [37].

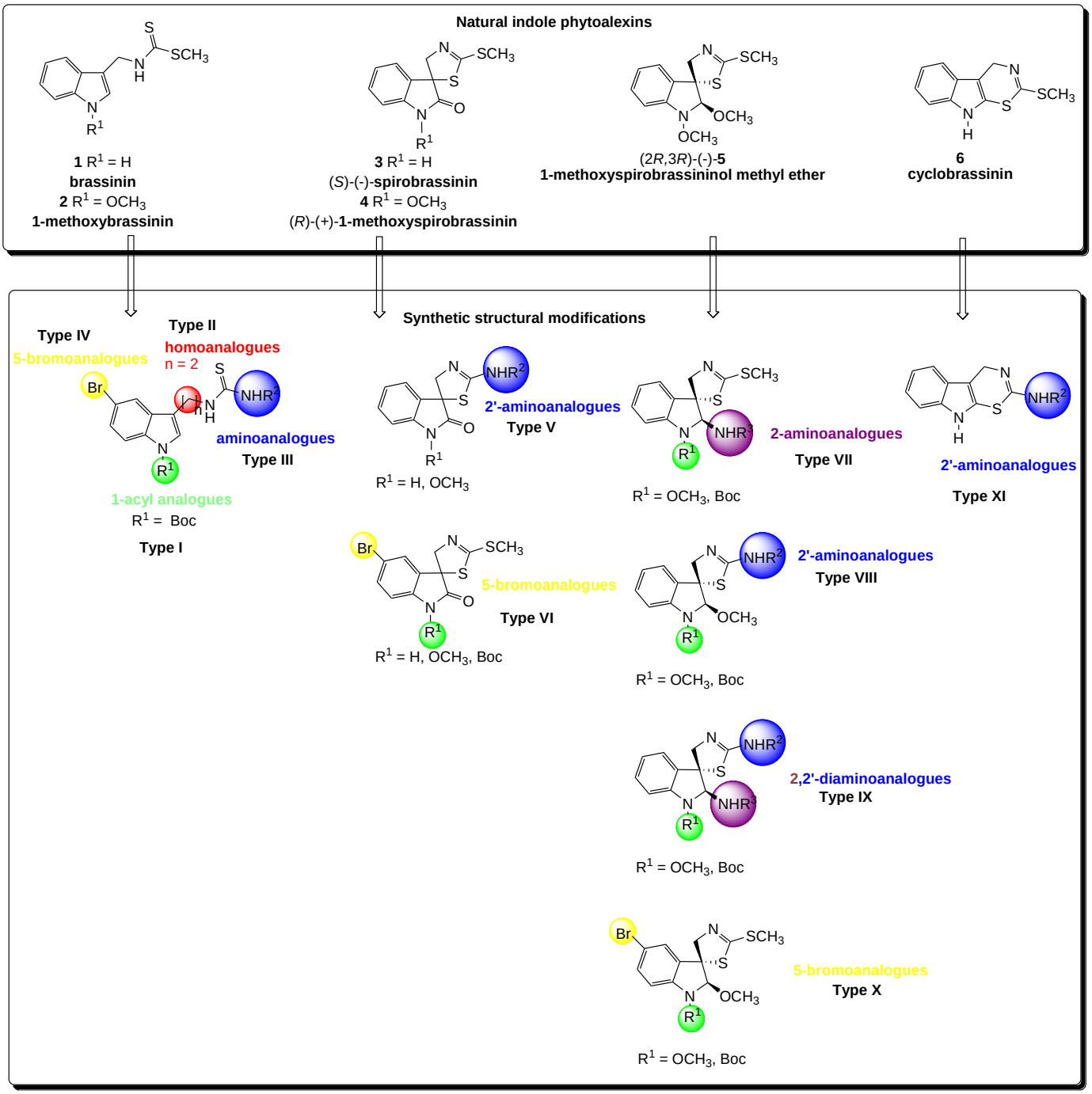


Figure 1: Chemical structures of selected natural indole phytoalexins and their synthetic structural modifications.

In view of the foregoing considerations, our work has been focused on the design and syntheses of a novel series of aminoanalogues of 5-bromobrassinin (**Type A** target compounds) and their cyclization products (**Type B-E** target compounds, Fig. 2) as a new and potent anticancer agents which can improve cancer treatments.

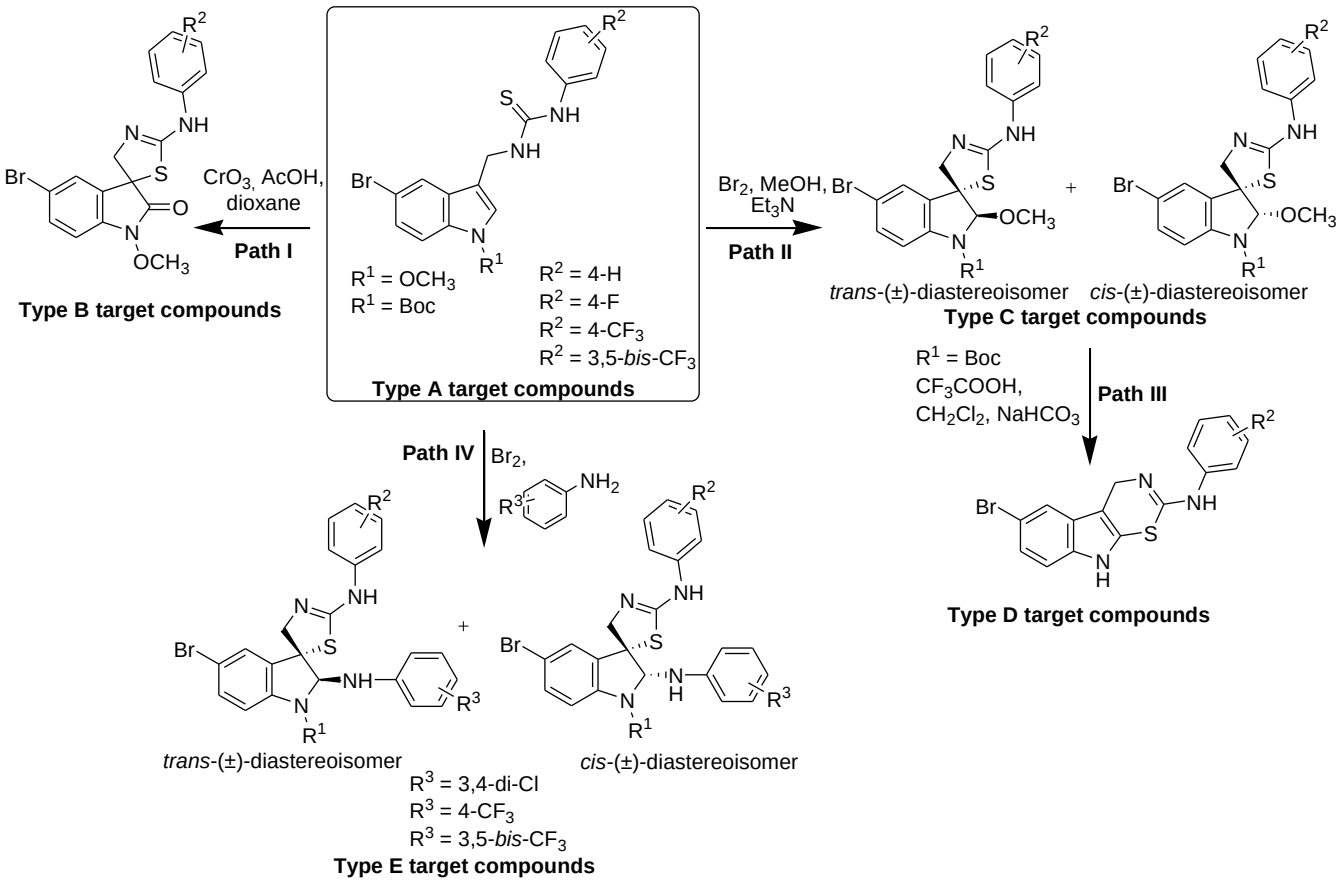


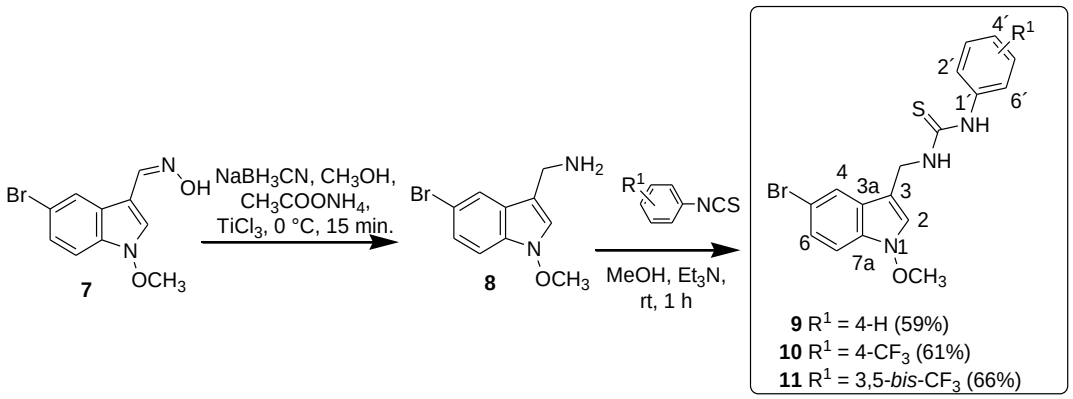
Figure 2: Strategic plan for the conversion of aminoanalogues of 5-bromobrassinin (**Type A**) into **Types B-E** target compounds

Results and discussion

Synthesis

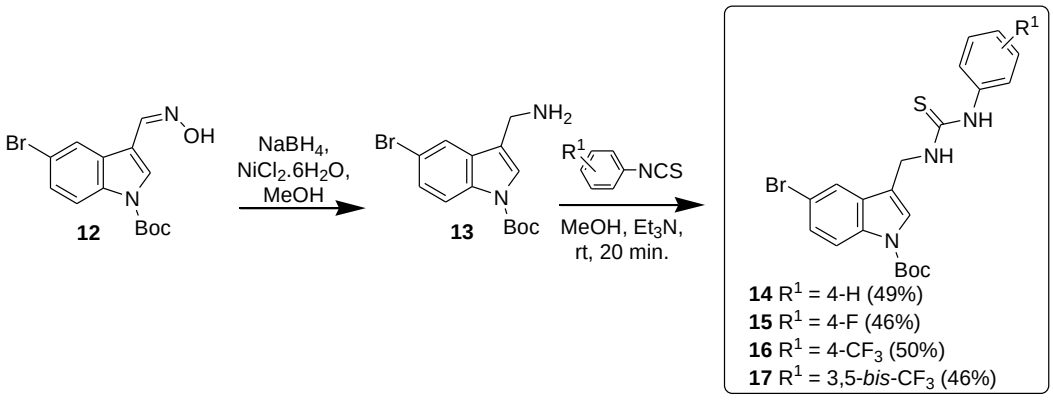
The synthetic ease and diversity of derivatives of indole phytoalexins as well as the reported potency of phytoalexins prompt us to investigated the effect of 5-bromo substitution on antiproliferative activity.

The synthesis of the **Type A** target compounds **9-11** starts from oxime **7**. Oxime **7** was reduced to a labile amine **8** by sodium cyanoborohydride and titanium trichloride catalysis using the methodology optimised in our group´s previuos work [37]. The key step of the preparation of thioureas **9-11** was the reaction of crude amine **8** with the appropriate isothiocyanate and triethylamine in methanol. Target thioureas **9-11** were prepared in 59-66% yield after two reaction steps starting from oxime **7** (Scheme 1).



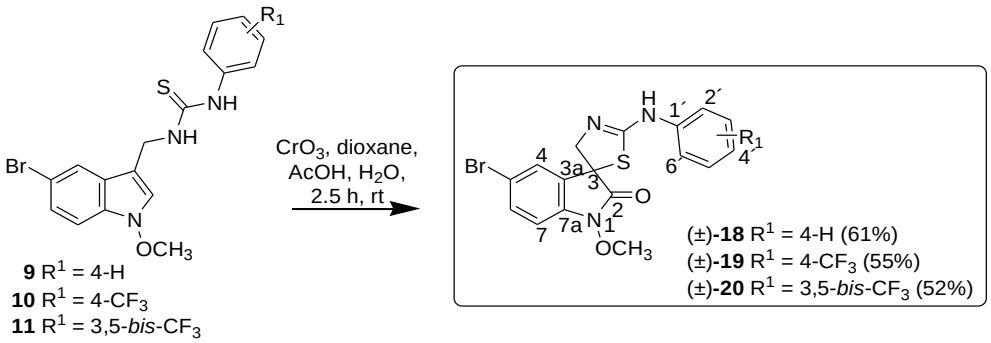
Scheme 1: Synthesis of **Type A** target compounds **9-11**.

Synthesis of target compounds **14-17** was achieved in a similar fashion than for molecules **9-11**. In this manner, reduction of the oxime **12** with $NaBH_4$ using $NiCl_2.6H_2O$ as a catalyst produced unstable amine **13** [37]. Amine **13** was reacted with appropriate isothiocyanate and triethylamine in methanol to give the consequent thioureas **14-17** in 46-50% after two reaction steps (Scheme 2).



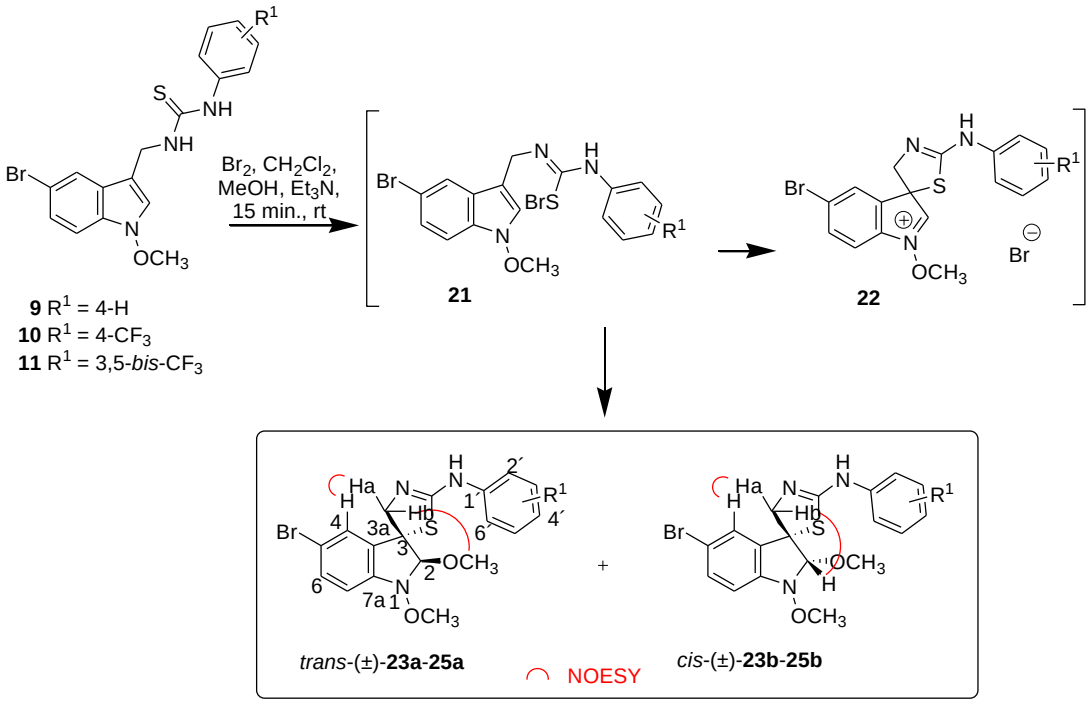
Scheme 2: Synthesis of **Type A** target compounds **14-17**.

Aminoanalogues of 5-bromobrassinin **9-11** were the basic substrates on which we tested oxidative spirocyclization reactions. Following a procedure that mimics the process used to produce 1-methoxyspirobrassinin [(±)-(**4**)], **Type B** target compounds (±)-**18-20** were prepared by cyclization with CrO_3 (5 eq.) in acetic acid and dioxane in reasonable yields (52-61%, Scheme 3). It should be noted that numerous attempts to synthesize spirocompounds (±)-**18-20** using pyridinium chlorochromate (according to published report 38) were unsuccessful.



Scheme 3: Synthesis of **Type B** target compounds (±)-**18-20**.

Then we tested the substrate scope **9-11, 14-17** with bromine as a cyclization reagent. Bromocyclization is an obvious route to produce target compounds of **Type C**. The prepared key thioureas **9-11** were further subjected to electrophilic cyclization using bromine (1.1 eq.) in a solvent mixture of anhydrous dichloromethane/methanol (9: 1), with methanol acting as a nucleophilic reagent. The reaction likely begins at the thiocarbamoyl group to form the sulfenyl bromide **21**, whose electrophilic sulfur attacks the position 3 of 5-bromoindole and yields the spiroindolinium intermediate **22**. Spiroindolinium intermediate **22** by reaction with methanol provides the desired diastereoisomers *trans*-(±)-**23a-25a** and *cis*-(±)-**23b-25b** (Scheme 4). In all cases, both diastereoisomers were isolated. The yields and ratios of the prepared 2'-amino analogues of 5-bromo-1-methoxyspirobrassinol methyl ether *trans*-(±)-**23a-25a** and *cis*-(±)-**23b-25b** are shown in Scheme 4. All diastereoisomeric pairs were prepared in a ratio of approximately 50:50. The ratios of the prepared diastereoisomers were determined by integration well resolved signals for the protons H-2, Ha and Hb in the ^{1}H NMR spectra of the crude reaction mixtures obtained after processing the reactions. The diastereoisomeric structures *trans*- and *cis*- of prepared compounds *trans*-(±)-**23a-25a** and *cis*-(±)-**23b-25b** were determined using a NOESY experiment.

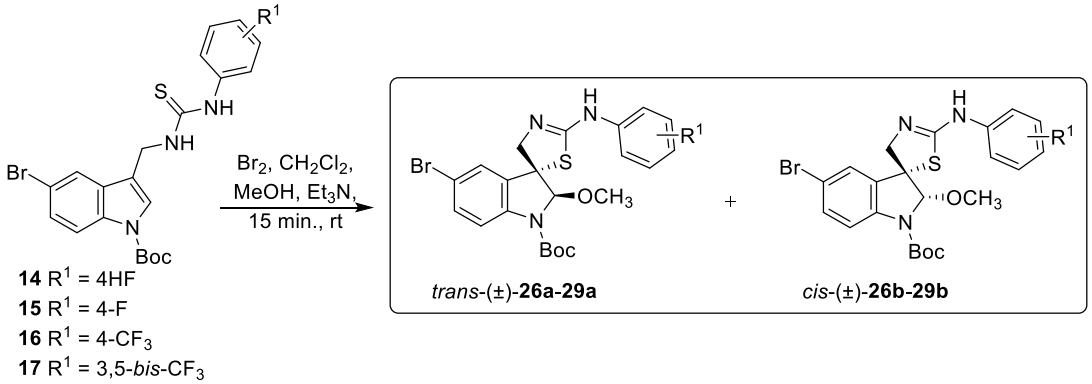


Compound	R^1	Yield	Ratio *trans*:*cis*
trans-(±)-**23a**, *cis*-(±)-**23b**	4-H	46% : 39%	57:43
trans-(±)-**24a**, *cis*-(±)-**24b**	4-CF_3	30% : 26%	54:46
trans-(±)-**25a**, *cis*-(±)-**25b**	3,5-*bis*-CF_3	34% : 18%	58:42

Scheme 4: Synthesis of **Type C** target compounds *trans*-(±)-**23a**-**25a** and *cis*-(±)-**23b**-**25b**.

Subsequent cyclization of the obtained thioureas **14**-**17** with bromine as a spirocyclizing agent in dichloromethane/methanol (9:1) afforded diastereoisomers of 2'-amino analogues of 1-Boc-spirobrasinol methyl ether *trans*-(±)-**26a**-**29a** and *cis*-(±)-**26b**-**129b** (Scheme 5). Unfortunately, in either case we could not separate *trans*- (±) - and *cis*- (±)-diastereoisomers. The pairs of diastereoisomers *trans*-(±)-**26a**-**29a** and *cis*-(±)-**26b**-**29b** showed very close Rf values in the various eluents, which hindered their chromatographic isolation. We have tried many combinations of solvents to find a suitable phase. No solvent has been shown to be a suitable eluent for the separation of diastereoisomers. The reaction with thiourea **14** provides mixture of products *trans*-(±)-**26a** and *cis*-(±)-**26b** in a 85% yield. Using thiourea **15**, a mixture of diastereoisomers of *trans*-(±)-**27a** and *cis*-(±)-**27b** was obtained in a 68% yield. Cyclization of thiourea **16** yielded a pair of diastereoisomers *trans*-(±)-**28a** and *cis*-(±)-**28b** in a 75% yield. In the case of thiourea **17**, cyclization yielded a mixture of diastereoisomers *trans*-(±)-**29a** and *cis*-(±)-**29b** in a 78% yield. The ratios of diastereoisomers were determined from the integrated

intensities of well resolved signals for the H-2, Ha and Hb protons in the ^{1}H NMR spectrum and in all cases the preference for *trans*-(±)-isomer *trans*-(±)-**26a-29a** was observed.

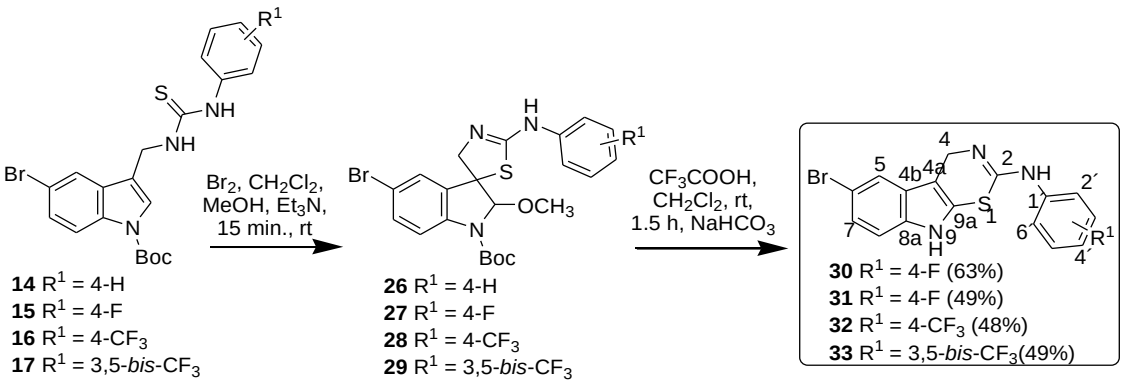


14 R^1 = 4HF
15 R^1 = 4-F
16 R^1 = 4-CF_3
17 R^1 = 3,5-*bis*-CF_3

Compound	R^1	Yield	Ratio *trans*:*cis*
trans-(±)-**26a**, *cis*-(±)-**26b**	4-H	85%	76:24
trans-(±)-**27a**, *cis*-(±)-**27b**	4-F	68%	79:21
trans-(±)-**28a**, *cis*-(±)-**28b**	4-CF_3	75%	78:22
trans-(±)-**29a**, *cis*-(±)-**29b**	3,5-*bis*-CF_3	78%	72:28

Scheme 5: Synthesis of **Type C** target compounds *trans*-(±)-**26a**-**29a** and *cis*-(±)-**26b**-**29b**.

The spiroindoline products *trans*-(±)-**26a**-**29a** and *cis*-(±)-**26b**-**29b** served as key intermediates for the synthesis of **Type D** target compounds **30-33**. Starting with a mixture of diastereoisomers *trans*-(±)-**26a**-**29a** and *cis*-(±)-**26b**-**29b**, reactions were carried out using 20 equivalents of trifluoroacetic acid in anhydrous dichloromethane. Trifluoroacetic acid initiated a cascade of reactions. Methanol was eliminated, followed by removal of the Boc-group and finally a rearrangement to derivatives **30-33** (Scheme 6). Thiazino[6,5-*b*]indole derivatives **30-33** were prepared in yields of 48-63% relative to thioureas **14-17**.



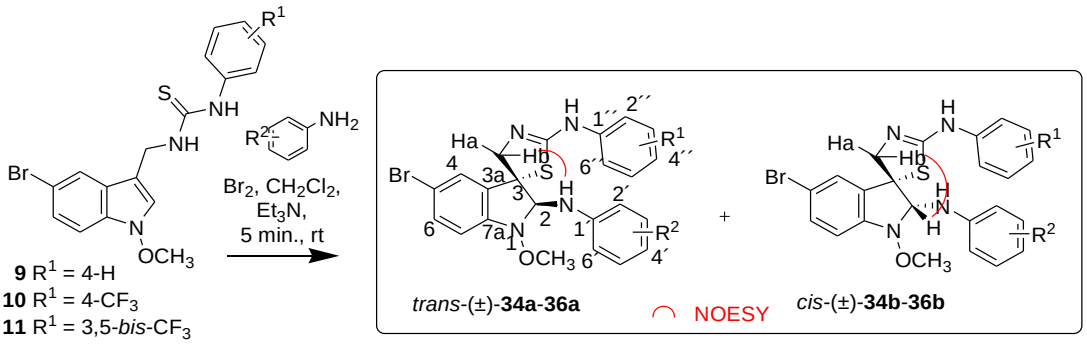
14 R^1 = 4-H
15 R^1 = 4-F
16 R^1 = 4-CF_3
17 R^1 = 3,5-*bis*-CF_3

26 R^1 = 4-H
27 R^1 = 4-F
28 R^1 = 4-CF_3
29 R^1 = 3,5-*bis*-CF_3

30 R^1 = 4-F (63%)
31 R^1 = 4-F (49%)
32 R^1 = 4-CF_3 (48%)
33 R^1 = 3,5-*bis*-CF_3(49%)

Scheme 6: Synthesis of **Type D** target compounds **30-33**.

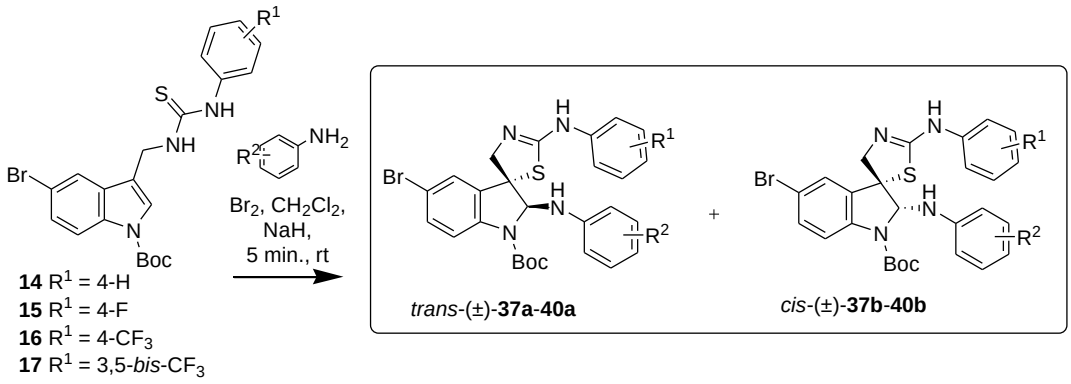
Bromine-initiated spirocyclization of thioureas **9-11** opened a new route for the synthesis of 2,2´-diaminoanalogues of 5-bromo-1-methoxyspirobrasinol methyl ether (±)-**34a**-(±)-**36b**. Anilines, in the role of nucleophile, captured intermediate **22**, resulting in the formation **Type E** target compounds (±)-**34a**-(±)-**36b** (Scheme 7). For the selection of anilines, we followed the Topliss scheme [39]. Substrates **9-11** were subjected to the spirocyclization conditions: 1.1 equiv. of bromine, 2 equiv. of corresponding aniline and 19 equiv. of triethylamine as base. The bromine-mediated cyclizations were performed in anhydrous dichloromethane. Application of these conditions resulted in the formation of a diastereoisomeric mixture *trans*-(±)-**34a** and *cis*-(±)-**34b** in the ratio 68:32. The use of thiourea **10** provided a 44:56 mixture of diastereoisomers *trans*-(±)-**35a** and *cis*-(±)-**35b**. The result of bromocyclization of thiourea **11** was a diastereoisomeric mixture *trans*-(±)-**36a** and *cis*-(±)-**36b** in the ratio 47:53. Yields of prepared diastereoisomers *trans*-(±)-**34a**-*cis*-(±)-**36b** are mentioned in Scheme 7. In all cases both diastereoisomers were obtained in lower yields. Unidentified side products and excess of used anilines caused complications with chromatography. It was necessary to repeat chromatography to receive pure diastereisomers. All of these factors affected yields. The ratios of *trans*-(±)-diastereoisomer and *cis*-(±)-diastereoisomer were established by integration of non-overlapping doublets of the H-2, Ha and Hb protons in the ^{1}H NMR spectra of crude reaction mixtures. *cis*-Diastereoisomeric structures were confirmed by interaction between Hb proton and H-2 proton in the NOESY spectra. *trans*-Diastereoisomers had the cross peaks between H-2 proton and NH proton in the NOESY spectra.



Compound	R^1	R^2	Yield	Ratio *trans*:*cis*
trans-(±)-**34a**, *cis*-(±)-**34b**	4-H	3,4-di-Cl	28% : 22%	68:32
trans-(±)-**35a**, *cis*-(±)-**35b**	4-CF_3	4-CF_3	18% : 31%	44:56
trans-(±)-**36a**, *cis*-(±)-**36b**	3,5-*bis*-CF_3	3,5-*bis*-CF_3	21% : 30%	47:53

Scheme 7: Synthesis of **Type E** target compounds *trans*-(±)-**34a-36a** and *cis*-(±)-**34b-36b**.

The thioureas **14-17** were also subjected to bromospirocyclization with substituted anilines in the standard manner as for thioureas **9-11**. However, the method was not successful for substrates **14-17**. Corresponding diaminoanalogues *trans*-(±)-**37a**-**40a** and *cis*-(±)-**37b**-**40b** could not be prepared under analogous conditions. The desired products (±)-**37a**-(±)-**40b** were not observed in the reaction mixture by either changing the amount of nucleophile and the order of reagents or changing the reaction time. The repeatedly unsuccessful results of the preparation of diaminoanalogues *trans*-(±)-**37a**-**40a** and *cis*-(±)-**37b**-**40b** forced us to consider the use of sodium hydride. Therefore we employed a sodium hydride (3 eq.) and corresponding aniline for generation sodium salt which was used in subsequent spirocyclization reaction. With optimized conditions in hand, thioureas **14-17** gave corresponding 2,2´-diaminoanalogues *trans*-(±)-**37a**-**40a** and *cis*-(±)-**37b**-**40b** in yields 20%-27%. In all cases, *trans*-(±)-*cis*-(±) diastereoisomer pairs arose in the ratio 50:50 (Scheme 8).



Compound	R^1	R^2	Yield	Ratio *trans*:*cis*
trans-(±)-**37a**, *cis*-(±)-**37b**	4-H	3,4-di-Cl	26% : 24%	50:50
trans-(±)-**38a**, *cis*-(±)-**38b**	4-F	4-CF_3	24% : 27%	50:50
trans-(±)-**39a**, *cis*-(±)-**39b**	4-CF_3	4-CF_3	26% : 27%	50:50
trans-(±)-**40a**, *cis*-(±)-**40b**	3,5-*bis*-CF_3	3,5-*bis*-CF_3	25% : 20%	50:50

Scheme 8: Synthesis of **Type E** target compounds *trans*-(±)-**37a**-**40a** and *cis*-(±)-**37b**-**40b**.

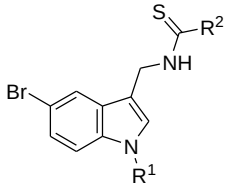
Antiproliferative activity

The novel 5-bromo derivatives of indole phytoalexins were tested for antiproliferative/cytotoxic activities on the panel of the seven human cancer cell lines: Jurkat (acute T-lymphoblastic leukemia), MCF-7 (mammary gland adenocarcinoma), MDA-MB-231 (mammary gland adenocarcinoma), A-549 (non-small cell lung cancer), HeLa (cervical

adenocarcinoma), HCT116 and CaCo-2 (colorectal carcinoma) and a non-malignant cell line NIH 3T3 (murine fibroblasts) using the MTT (Thiazolyl Blue Tetrazolium Bromide) assay [40]. Obtained IC_{50} values of prepared aminoanalogues of 5-bromo-1-methoxybrasinin **9-11** and 5-bromo-1-Boc-brasinin **14-17** are shown in Table 1. Table 1 includes the IC_{50} values of 5-bromo-1-methoxybrasinin (**41**) and 5-bromo-1-Boc-brasinin (**42**) and conventional antitumor agent cisplatin for comparison. The prepared set of thioureas **9-11** showed the highest activities on Jurkat, HCT116 and CaCo-2 cell lines. For thioureas **9** and **10**, a two-times higher effect on Jurkat cell line can be seen in comparison with the 5-bromo-1-methoxybrassinin (**41**). N-(5-Bromo-1-methoxyindol-3-yl)methyl-N'-(4-trifluoromethylphenyl) thiourea (**10**) displayed approximately the same potencies as a previously studied corrensponding non-brominated 4-trifluoromethylphenyl thiourea (see reference 26).

From the second set of thioureas, thioureas **16** and **17** were the most effective, showing activities with IC_{50} values <10 μmol.l^{-1} on Jurkat and MCF-7 cell lines. Prepared thioureas **16,17** are approximately 10-times more potent compared to the 5-bromo-1-Boc-brasinin (**42**) on the tested MCF-7 cell line. Lower IC_{50} values were also observed for these compounds **16,17** on the CaCo-2 colorectal carcinoma cell line and for substance **15** on the HCT116 cell line with an IC_{50} = 14.4 μM. Notably, thioureas **16,17** showed better or comparable activities with that of cisplatin. At the same time the toxicity of these compounds on 3T3 cells was lower than that of cisplatin. All four aminoanalogues of 5-bromo-1-Boc-brasinin **14-17** were found in this study to be a less potent inhibitor of proliferation of cancer cells than a previously studied corrensponding non-brominated thioureas (see reference 25).

Table 1: Antiproliferative activities of aminoanalogues of 5-bromo-1-methoxybrassinin **9-11** and 5-bromo-1-Boc-brassinin **14-17**



Comp.	R^1	R^2	Cell line IC_{50} (μmo l× L^{-1})						
			Jurkat	MCF-7	A-549	HeLa	HCT116	CaCo-2	3T3
41 [37]	OCH_3	SCH_3	59.8	85.6	85.0	>100	nt	nt	nt
9		C_6H_5NH	30.1	40.3	68.8	52.6	31.2	37.5	56.0
10		4-CF_3-C_6H_4NH	28.4	32.9	nt	31.7	33.6	30.7	41.1
11		3,5-*bis*-CF_3-C_6H_3NH	41.2	89.9	nt	81.2	77.1	76.3	39,8

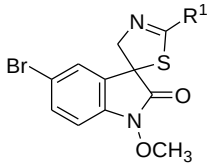
42 [37]		SCH_3	nt	>100	nt	75.7	nt	nt	nt
14		C_6H_5NH	27.2	39.4	>100	44.1	>100	32.2	45.2
15	Boc	4-F-C_6H_4NH	24.6	30.1	34.6	26.9	14.4	24.2	31.8
16		4-CF_3-C_6H_4NH	5.1	8.1	28.5	30.6	28.2	18.6	27.8
17		3,5-*bis*-CF_3-C_6H_3NH	5.9	8.7	35.3	29.1	23.8	17.2	30.1
Cisplatin [26]	-	-	16.2	15.6	9.5	13.1	15.3	15.2	20.9

nt – not tested

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} (concentration of a given compound that decreased amount of viable cells to 50% relative to untreated control cells).

The potencies of **Type B** target compounds (±)-**18-20** are presented in Table 2 as IC_{50} values. The spiroproduct **20** was proved to be the most effective, with good activity on the Jurkat cell line (IC_{50} = 28.2 µmol.l^{-1}). Substance **18** showed low activity on all lines. Compound **19** was only active on Jurkat cell. Compounds (±)-**18-20** are more active in comparison with the 5-bromo-1-methoxyspirobrassinin (**43**), but less active than corrensponding non-brominated 2´-aminoanalogues of 1-methoxyspirobrassinin (see reference 29).

Table 2: Antiproliferative activities of 2´-aminoanalogues of 5-bromo-1-methoxyspirobrassinin (±)-**18-20**



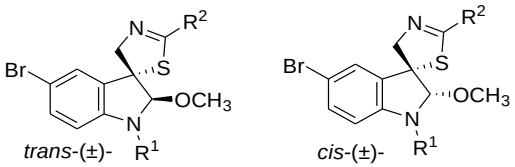
Comp.	**R^1**	**Cell line IC_{50} (µmo l× L^{-1})**						
		Jurkat	**MCF-7**	**A-549**	**HeLa**	**HCT116**	**CaCo-2**	**3T3**
43 [37]	SCH_3	>100	>100	>100	>100	nt	nt	nt
18	C_6H_5NH	73.2	91.4	94.2	83.6	83.9	78.3	84.3
19	4-CF_3-C_6H_4NH	31.3	80.7	nt	76.2	78.6	73.2	44.6
20	3,5-*bis*-CF_3-C_6H_3NH	28.2	37.1	55.4	50.9	34.8	34.2	36.9

nt – not tested

The highest inhibitory effects of 2´-aminoanalogues of 5-bromo-1-methoxyspirobrassinol methyl ether *trans*-(±)-**23a**-**25a**, *cis*-(±)-**23b**-**25b** and 2´-aminoanalogues of 5-bromo-1-Boc-spirobrassinol methyl ether *trans*-(±)-**26a**-**29a**, *cis*-(±)-**26b**-**29b** were notes with leukemic cells Jurkat (Table 3). As seen in the Table 3, 2´-aminoanalogue with 3,5-bis-(trifluoromethyl)phenyl group *cis*-(±)-**25b** was found to be the most active with IC_{50} from 29.4 to 33.2 µmol.l^{-1}. Compound *trans*-(±)-**23a** is inactive against all cell lines. All prepared 2´-aminoanalogues

trans-(±)-**23a**-**29a** and *cis*-(±)-**23b**-**29b** are more potent on all cell lines than 5-bromo-1-methoxyspirobrassinol methyl ether and 5-bromo-1-Boc-spirobrassinol methyl ether *trans*-(±)-**44a**-**45a** and *cis*-(±)-**44b**-**45b**. The corresponding non-brominated 2´-aminoanalogues of 1-methoxyspirobrassinol methyl ether and 1-Boc-spirobrassinol methyl ether were more active again (see reference 25, 26).

Table 3: Antiproliferative activities of 2´-aminoanalogues *trans*-(±)-**23a**-**29a**, *cis*-(±)-**23b**-**29b**

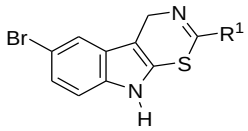


Comp.	R^1	R^2	Cell line IC_{50} (µmo l× L^{-1})						
			Jurkat	MCF-7	MDA-MB-231	HeLa	HCT116	CaCo-2	3T3
trans-(±)-**44a** [37]	OCH_3	SCH_3	72.5	94.5	>100	>100	nt	nt	nt
cis-(±)-**44b** [37]			97.1	>100	>100	>100	nt	nt	nt
trans-(±)-**23a**		C_6H_5NH	>100	>100	nt	>100	>100	>100	>100
cis-(±)-**23b**			27.8	57.0	nt	67.9	51.2	27.0	70.9
trans-(±)-**24a**		4-CF_3-C_6H_4NH	27.9	83.8	77.1	82.9	71.8	62.1	55.6
cis-(±)-**24b**			26.5	81.9	77.8	75.1	84.9	66.8	46.7
trans-(±)-**25a**		3,5-*bis*-CF_3-C_6H_3NH	40.7	44.2	74.9	54.1	59.8	61.0	40.1
cis-(±)-**25b**			29.9	29.4	29.8	31.2	33.2	30.7	43.2
trans-(±)-**45a** [37]	Boc	SCH_3	57.0	>100	>100	89.3	nt	nt	nt
cis-(±)-**45b** [37]			48.0	41.1	>100	>100	nt	nt	nt
trans-(±)-**26a** + *cis*-(±)-**26b**		C_6H_5NH	36.8	>100	87.6	>100	>100	>100	>100
trans-(±)-**27a** + *cis*-(±)-**27b**		4-F-C_6H_4NH	33.0	>100	36.6	56.9	70.9	47.1	59.9
trans-(±)-**28a** + *cis*-(±)-**28b**		4-CF_3-C_6H_4NH	41.6	81.2	47.6	74.3	89.9	46.9	73.0
trans-(±)-**29a** + *cis*-(±)-**29b**		3,5-*bis*-CF_3-C_6H_3NH	35.6	>100	48.5	75.9	88.0	88.3	>100

nt – not tested

In the case of 6-bromocyclobrassinin derivatives **30**-**33**, derivative **31** exhibited the highest activity on the CaCo-2 cell line (IC_{50} = 27.9 µmol.l^{-1}) and derivative **32** on the Jurkat cell line at (IC_{50} = 31.3 µmol.l^{-1}, Table 4). Derivatives **30**,**33** showed weak or no antiproliferative activity. Compared to their non-brominated analogues (see reference 25), it can be seen that all these derivatives **30**-**33** show lower activities on all cell lines.

Table 4: Antiproliferative activities of 2-aminoanalogues of 6-bromocyclobrassinin **30-33**

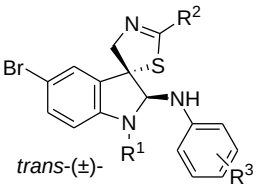


Comp.	R^1	Cell line IC_{50} (μmo l× L^{-1})						
		Jurkat	**MCF-7**	**A-549**	**HeLa**	**HCT116**	**CaCo-2**	**3T3**
30	C_6H_5NH	75.6	>100	>100	84.9	>100	54.7	87.7
31	4-F-C_6H_4NH	34.4	68.3	72.5	53.6	63.3	27.9	59.6
32	4-CF_3-C_6H_4NH	31.3	>100	>100	58.2	66.7	54.1	89.5
33	3,5-*bis*-CF_3-C_6H_3NH	88.8	>100	>100	>100	>100	>100	>100

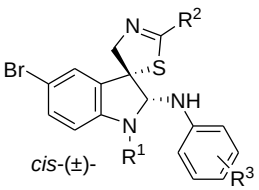
nt – not tested

2,2´-diaminoanalogues *trans*-(±)-**34a-40a**, *cis*-(±)-**34b-40b** showed lower antiproliferative activity against cancer cell lines (Table 4). Compounds *trans*-(±)-**36a** and *trans*-(±)-**37a** were the most active with IC_{50} in the range of 28.6 to 36.9 μmol.l^{-1}.

Table 5: Antiproliferative activities of 2,2´-diaminoanalogues *trans*-(±)-**34a-40a**, *cis*-(±)-**34b-40b**



trans-(±)-



cis-(±)-

Comp.	R_1	R_2	R_3	Cell line IC_{50} (μmo l× L^{-1})						
				Jurkat	**MCF-7**	**MDA-MB-231**	**HeLa**	**HCT116**	**CaCo-2**	**3T3**
trans-(±)-**34a**	OCH_3	C_6H_5NH	3,4-di-Cl	27.9	>100	nt	77.6	72.6	85.7	>100
cis-(±)-**34b**				>100	>100	nt	>100	>100	>100	>100
trans-(±)-**35a**		4-CF_3-C_6H_4NH	4-CF_3	51.1	>100	>100	>100	>100	>100	>100
cis-(±)-**35b**				68.9	>100	>100	>100	>100	>100	>100
trans-(±)-**36a**		3,5-*bis*-CF_3-C_6H_3NH	3,5-*bis*-CF_3	28.6	33.3	nt	>100	57.7	66.2	54.8
cis-(±)-**36b**				67.1	52.4	nt	>100	64.6	67.8	74.3
trans-(±)-**37a**	Boc	C_6H_5NH	3,4-di-Cl	72.4	40.7	32.8	44.9	>100	36.9	34.7
cis-(±)-**37b**				63.5	94.6	54.7	45.5	>100	34.1	51.2
trans-(±)-**38a**		4-F-C_6H_4NH	4-CF_3	>100	>100	>100	>100	>100	>100	>100
cis-(±)-**38b**				>100	>100	>100	>100	>100	>100	>100
trans-(±)-**39a**		4-CF_3-C_6H_4NH	4-CF_3	>100	>100	65.4	>100	>100	>100	>100
cis-(±)-**39b**				52.7	>100	81.5	>100	>100	>100	>100
trans-(±)-**40a**		3,5-*bis*-CF_3-C_6H_3NH	3,5-*bis*-CF_3	>100	>100	>100	>100	>100	>100	>100
cis-(±)-**40b**				>100	>100	>100	>100	>100	>100	>100

nt – not tested

Conclusion

We have prepared a library of 5-bromosubstituted analogues of indole phytoalexins **Type A-E**. **Type A** target compounds - thioureas served as key intermediates for synthesis other interesting series. Synthesis of 2´-aminoanalogues of 1-methoxyspirobrassinin (**Type B** target compounds) was achieved via oxidative cyclization of thioureas. The spirocyclization reaction of thioureas with bromine has been employed to prepare the 2´-aminoanalogues and 2,2´-diaminoanalogues **Type C** and **E**. 2-Aminoanalogues of 6-bromocyclobrassinin (**Type D** target compounds) were obtained from 2´-aminoanalogues of 1-Boc-spirobrassinol methyl ether (**Type C**) through trifluoroacetic acid initiated cascade reactions. Syntheses of new indole heterocycles were carried out for the purpose of evaluation of their antiproliferative activities. In this study, we found that all 5-bromosubstituted analogues of indole phytoalexins **Type A-E** are approximately the same or less potent inhibitors of cancer cell proliferation as the previously studied corresponding non-brominated compounds. Further work is also planned with aim of synthesise different kind of analogues with modified indole unit.

Supporting Information

Supporting Information File

Experimental procedures and characterization of new compounds.

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