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

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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 corresponding non-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 G1 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 mechanisms 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 (2R,3R)-(-)-1-methoxyspirobrassinin methyl ether [(2R,3R)-(-)-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].
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 1:** Chemical structures of selected natural indole phytoalexins and their synthetic structural modifications.
The synthesis of the antiproliferative activity.

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 previous 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 NaBH₄ using NiCl₂.6H₂O 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.

Amino analogues 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 CrO₃ (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 1H 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.
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

| Compound          | R¹      | Yield | Ratio trans/cis |
|-------------------|---------|-------|-----------------|
| trans-(±)-23a, cis-(±)-23b | 4-H     | 46% : 39% | 57:43 |
| trans-(±)-24a, cis-(±)-24b | 4-CF₃   | 30% : 26% | 54:46 |
| trans-(±)-25a, cis-(±)-25b | 3,5-bis-CF₃ | 34% : 18% | 58:42 |

Scheme 4: Synthesis of Type C target compounds trans-(±)-23a-25a and cis-(±)-23b-25b.
intensities of well resolved signals for the H-2, Ha and Hb protons in the ¹H NMR spectrum and in all cases the preference for trans-(±)-isomer trans-(±)-26a-29a was observed.

| Compound | R¹ | 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-CF₃ | 75% | 78:22 |
| trans-(±)-29a, cis-(±)-29b | 3,5-bis-CF₃ | 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.

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 diastereoisomers. 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 1H 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          | R¹   | R²         | Yield     | Ratio trans/cis |
|-------------------|------|------------|-----------|-----------------|
| trans-(±)-34a, cis-(±)-34b | 4-H  | 3,4-di-Cl  | 28% : 22% | 68:32           |
| trans-(±)-35a, cis-(±)-35b | 4-CF₃ | 4-CF₃     | 18% : 31% | 44:56           |
| trans-(±)-36a, cis-(±)-36b | 3,5-bis-CF₃ | 3,5-bis-CF₃ | 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).

The repeated results of the preparation of thioureas 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         | R¹   | R²           | 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-CF₃        | 24% : 27% | 50:50           |
| trans-(±)-39a, cis-(±)-39b | 4-CF₃ | 4-CF₃       | 26% : 27% | 50:50           |
| trans-(±)-40a, cis-(±)-40b | 3,5-bis-CF₃ | 3,5-bis-CF₃ | 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<sub>50</sub> values of prepared aminoanalogues of 5-bromo-1-methoxybrassinin 9-11 and 5-bromo-1-Boc-brassinin 14-17 are shown in Table 1. Table 1 includes the IC<sub>50</sub> values of 5-bromo-1-methoxybrassinin (41) and 5-bromo-1-Boc-brassinin (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 corresponding 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<sub>50</sub> values <10 µmol.l<sup>-1</sup> on Jurkat and MCF-7 cell lines. Prepared thioureas 16,17 are approximately 10-times more potent compared to the 5-bromo-1-Boc-brassinin (42) on the tested MCF-7 cell line. Lower IC<sub>50</sub> 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<sub>50</sub> = 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-brassinin 14-17 were found in this study to be a less potent inhibitor of proliferation of cancer cells than a previously studied corresponding 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<sup>1</sup> | R<sup>2</sup> | Cell line IC<sub>50</sub> (µmol l<sup>-1</sup>) | Jurkat | MCF-7 | A-549 | HeLa | HCT116 | CaCo-2 | 3T3 |
|-------|--------------|--------------|---------------------------------------------|--------|--------|-------|------|--------|--------|-----|
| 41 [37] | SCH<sub>3</sub> | 59.8 | 85.6 | 85.0 | >100 | nt | nt | nt | 56.0 |
| 9 | OCH<sub>3</sub> | C<sub>6</sub>H<sub>5</sub>NH | 30.1 | 40.3 | 68.8 | 52.6 | 31.2 | 37.5 | 56.0 |
| 10 | 4-CF<sub>3</sub>C<sub>6</sub>H<sub>4</sub>NH | 28.4 | 32.9 | nt | 31.7 | 33.6 | 30.7 | 41.1 |
| 11 | 3,5-bis-CF<sub>3</sub>C<sub>6</sub>H<sub>4</sub>NH | 41.2 | 89.9 | nt | 81.2 | 77.1 | 76.3 | 39.8 |
The potency of compounds was determined using the MTT (Thiazolyl Blue Tetrazolium Bromide) assay after 72 h incubation of cells and presented as IC\textsubscript{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\textsubscript{50} values. The spiroproduct 20 was proved to be the most effective, with good activity on the Jurkat cell line (IC\textsubscript{50} = 28.2 µmol.l\textsuperscript{-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 corresponding non-brominated 2ˊ-aminoanalogues of 1-methoxyspirobrassinin (see reference 29).

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

| Comp. | R\textsuperscript{1} | Jurkat IC\textsubscript{50} (µmol l\textsuperscript{-1}) | MCF-7 IC\textsubscript{50} (µmol l\textsuperscript{-1}) | A-549 IC\textsubscript{50} (µmol l\textsuperscript{-1}) | HeLa IC\textsubscript{50} (µmol l\textsuperscript{-1}) | HCT116 IC\textsubscript{50} (µmol l\textsuperscript{-1}) | CaCo-2 IC\textsubscript{50} (µmol l\textsuperscript{-1}) | 3T3 IC\textsubscript{50} (µmol l\textsuperscript{-1}) |
|-------|---------------------|--------------------------------|-------------------------------|-----------------------------|-----------------------------|-----------------------------|-----------------------------|-----------------------------|
| 43 [37] | SCH\textsubscript{2} | >100 | >100 | >100 | >100 | nt | nt | nt |
| 18 | C\textsubscript{6}H\textsubscript{4}NH | 73.2 | 91.4 | 94.2 | 83.6 | 83.9 | 78.3 | 84.3 |
| 19 | 4-CF\textsubscript{3}-C\textsubscript{6}H\textsubscript{4}NH | 31.3 | 80.7 | nt | 76.2 | 78.6 | 73.2 | 44.6 |
| 20 | 3,5-bis-CF\textsubscript{3}-C\textsubscript{6}H\textsubscript{4}NH | 28.2 | 37.1 | 55.4 | 50.9 | 34.8 | 34.2 | 36.9 |

The highest inhibitory effects of 2ˊ-aminoanalogues of 5-bromo-1-methoxyspirobrassinol methyl ether trans-(±)-23a-25a, cis-(±)-23b-25b and 2ˊ-aminoanalogues of 5-bromo-1-Boc-spirobrassinol methyl ether trans-(±)-26a-29a, cis-(±)-26b-29b were noted with leukemic cells Jurkat (Table 3). As seen in the Table 3, 2ˊ-aminoanalogue with 3,5-bis-(trifluoromethyl)phenyl group cis-(±)-25b was found to be the most active with IC\textsubscript{50} from 29.4 to 33.2 µmol.l\textsuperscript{-1}. Compound trans-(±)-23a is inactive against all cell lines. All prepared 2ˊ-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. | R¹ | R² | Cell line IC₅₀ (µmol L⁻¹) |
|-------|----|----|--------------------------|
|       |    |    | Jurkat | MCF-7 | MDA-MB-231 | HeLa | HCT116 | CaCo-2 | 3T3 |
| trans-(±)-44a [37] | SCH₃ |      | 72.5 | 94.5 | >100 | >100 | nt | nt | nt |
| cis-(±)-44b [37] |   |    | 97.1 | >100 | >100 | >100 | nt | nt | nt |
| trans-(±)-23a | OCH₃ | C₅H₅NH | >100 | >100 | nt | >100 | >100 | >100 | >100 | >100 |
| cis-(±)-23b |   |    | 27.8 | 57.0 | nt | 67.9 | 51.2 | 27.0 | 70.9 |
| trans-(±)-24a | 4-CF₃C₅H₅NH | | 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-CF₃C₅H₅NH | | 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] | SCH₃ | | 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 | Boc | C₅H₅NH | 36.8 | >100 | 87.6 | >100 | >100 | >100 | >100 | >100 |
| trans-(±)-27a + cis-(±)-27b | 4-F-C₅H₅NH | | 33.0 | >100 | 36.6 | 56.9 | 70.9 | 47.1 | 59.9 |
| trans-(±)-28a + cis-(±)-28b | 4-CF₃C₅H₅NH | | 41.6 | 81.2 | 47.6 | 74.3 | 89.9 | 46.9 | 73.0 |
| trans-(±)-29a + cis-(±)-29b | 3,5-bis-CF₃C₅H₅NH | 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 (IC₅₀ = 27.9 µmol.L⁻¹) and derivative 32 on the Jurkat cell line at (IC₅₀ = 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-bromocyclobassinin 30-33

| Comp. | R¹ | Cell line IC₅₀ (µmol L⁻¹) |
|-------|----|--------------------------|
|       |     | Jurkat | MCF-7 | A-549 | HeLa | HCT116 | CaCo-2 | 3T3 |
| 30    | C₂H₅NH | 75.6   | >100  | >100  | 84.9 | >100   | 54.7   | 87.7 |
| 31    | 4-F-C₂H₅NH | 34.4 | 68.3  | 72.5  | 53.6 | 63.3   | 27.9   | 59.6 |
| 32    | 4-CF₃-C₂H₅NH | 31.3 | >100  | >100  | 58.2 | 66.7   | 54.1   | 89.5 |
| 33    | 3,5-bis-CF₃-C₂H₅NH | 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 IC₅₀ 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

| Comp. | R₁ | R₂ | R₃ | Cell line IC₅₀ (µmol L⁻¹) |
|-------|----|----|----|--------------------------|
|       |     |     |     | Jurkat | MCF-7 | MDA-MB-231 | HeLa | HCT116 | CaCo-2 | 3T3 |
| trans-(±)-34a | C₂H₅NH | 3,4-di-Cl | 27.9 | >100 | nt | 77.6 | 72.6 | 85.7 | >100 |
| cis-(±)-34b | C₂H₅NH | 3,4-di-Cl | 27.9 | >100 | nt | 77.6 | 72.6 | 85.7 | >100 |
| trans-(±)-35a | OCH₃ | 4-CF₃-C₂H₅NH | 4-CF₃ | 51.1 | >100 | >100 | >100 | >100 | >100 |
| cis-(±)-35b | OCH₃ | 4-CF₃-C₂H₅NH | 4-CF₃ | 51.1 | >100 | >100 | >100 | >100 | >100 |
| trans-(±)-36a | 3,5-bis-CF₃-C₂H₅NH | 3,5-bis-CF₃ | 28.6 | 33.3 | nt | 100 | 57.7 | 66.2 | 54.8 |
| cis-(±)-36b | 3,5-bis-CF₃-C₂H₅NH | 3,5-bis-CF₃ | 28.6 | 33.3 | nt | 100 | 57.7 | 66.2 | 54.8 |
| trans-(±)-37a | C₂H₅NH | 3,4-di-Cl | 72.4 | 40.7 | 32.8 | 44.9 | >100 | 36.9 | 34.7 |
| cis-(±)-37b | C₂H₅NH | 3,4-di-Cl | 63.5 | 94.6 | 54.7 | 45.5 | >100 | 34.1 | 51.2 |
| trans-(±)-38a | 4-F-C₂H₅NH | 4-CF₃ | >100 | >100 | >100 | >100 | >100 | >100 | >100 |
| cis-(±)-38b | 4-F-C₂H₅NH | 4-CF₃ | >100 | >100 | >100 | >100 | >100 | >100 | >100 |
| trans-(±)-39a | 4-CF₂-C₂H₅NH | 4-CF₃ | >100 | >100 | 65.4 | >100 | >100 | >100 | >100 |
| cis-(±)-39b | 4-CF₂-C₂H₅NH | 4-CF₃ | >100 | >100 | 65.4 | >100 | >100 | >100 | >100 |
| trans-(±)-40a | 3,5-bis-CF₂-C₂H₅NH | 3,5-bis-CF₃ | >100 | >100 | >100 | >100 | >100 | >100 | >100 |
| cis-(±)-40b | 3,5-bis-CF₂-C₂H₅NH | 3,5-bis-CF₃ | >100 | >100 | >100 | >100 | >100 | >100 | >100 |
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-bromocyclobassinin (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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References
1. Pedras, M.S.C.; Abdoli, A. *RSC Adv.* **2017**, *7*, 23633–23646.
2. Takasugi, M.; Katsui, N.; Shirata, A. *J. Chem. Soc. Chem. Commun.* **1986**, *1077–1078.
3. Takasugi, M.; Monde, K.; Katsui, N.; Shirata, A. *Chem. Lett.* **1987**, *1631–1632.
4. Gross, D.; Porzel, A.; Schmidt, J. *Z. Naturforsch. C: Biosci.* **1994**, *49*, 281–285.
5. Monde, K.; Takasugi, M.; Shirata, A. *Phytochemistry* **1995**, *39*, 581–586.
6. Storck, M.; Sacristan, M.D. *Z. Naturforsch.* **1995**, *50c*, 15–20.
7. Mezencev, R.; Galizzi, M.; Kutschy, P.; Docampo, R. *Exp. Parasitol.* **2009**, *122*, 66–69.
8. Kristofikova, Z.; Gazova, Z.; Siposova, K.; Bartos, A.; Ricny, J.; Kotoucova, J.; Sirova, J.; Ripova, D. *Neurochem. Res.* **2014**, *39*, 1502–1510.
9. Mehta, R.G.; Naithani, R.; Huma, L.; Hawthorne, M.; Moriarty, R.M.; McCormick, D.L.; Steele, V.E.; Kopelovich, L. *Curr. Med. Chem.* **2008**, *15*, 2785–2825.
10. Higdon, J.V.; Delage, B.; Williams, D.E.; Dashwood, R.H. *Pharmacol. Res.* **2007**, *55*, 224–236.
11. Mezencev, R.; Mojžiš, J.; Pilátová, M.; Kutschy, P.; Čurillová, Z. *Int. J. Canc. Prev.* **2004**, *1*, 105–112.
12. Pilátová, M.; Šarišský, M.; Kutschy, P.; Miroššay, A.; Mezencev, R.; Čurillová, Z.; Suchý, M.; Monde, K.; Miroššay, L.; Mojžiš, J. *Leukemia Res.* **2005**, *29*, 415–421.
13. Chripkova, M.; Drutovic, D.; Pilatova, M.; Mikes, J.; Budovska, M.; Vaskova, J.; Broggini, M.; Mirossay, L.; Mojžiš, J. *Toxicol. In Vitro* **2014**, *28*, 909–915.
14. Mezencev, R.; Mojžiš, J.; Pilátová, M.; Kutschy, P.; Čurillová, Z. *Effects of phytoalexins on the growth of cancer cells*. In: Frank Columbus (ed.): *Trends in cancer prevention Research*, Nova Science Publishers, New York, **2007**, pp 81–91.
15. Kutschy, P.; Mezencev, R. *Indole Phytoalexins from Brassicaceae: Synthesis and Anticancer Activity*. In: *Targets in Heterocyclic Systems – Chemistry and Properties*; Attanas, O. A.; Spinelli, D., Eds.; Italian Society of Chemistry: Urbino, Italy, **2008**, Vol. 12, pp 120-148. ISBN: 978-88-86208-56-7 ISSN 1724–9449.
16. Chripkova, M.; Zigo, F.; Mojžiš, J. *Molecules* **2016**, *21*, 1626–1640.
17. Domico, L.M.; Zeevalk, G.D.; Bernard, L.P.; Cooper, K.R. *Neurotoxicology* **2006**, *27*, 816–825.
18. Izutani, Y.; Yogosawa, S.; Sowa, Y.; Sakai, T. *Int. J. Oncol.* **2012**, *40*, 816–824.
19. Banerjee, T.; Duhadaway, J.B.; Gaspari, P.; Sutanto-Ward, E.; Munn, D.H.; Mellor, A.L.; Malachowski, W.P.; Prendergast, G.C.; Muller, A.J. *Oncogene* **2008**, *27*, 2851–2857.
20. Kim, S.M.; Oh, E.Y.; Lee, J.H.; Nam, D.; Lee, S.G.; Lee, J.; Kim, S.H.; Shim, B.S.; Ahn, K.S. *Phytother. Res.* **2015**, *29*, 1828–1836.
21. Lee, J.H.; Kim, C.; Sethi, G.; Ahn, K.S. *Oncotarget* **2015**, *6*, 6386–6405.
22. Bakar-Ates, F.; Ozkan, E. *Phytother. Res.* **2019**, *33*, 397–402.
23. Han, B.H.; Yoon, J.J.; Choi, E.S.; Jeong, D.H.; Lee, Y.J.; Kang, D.G.; Lee, H.S. *Mol. Med. Rep.* **2017**, *16*, 6890–6895.
24. Kang, B.; Kim, C.Y.; Hwang, J.; Suh, H.J.; Choi, H.S. *Phytother. Res.* **2019**, *33*, 1426–1437.
25. Budovská, M.; Pilátová, M.; Varinská, L.; Mojžiš, J.; Mezencev, R. *Bioorg. Med. Chem.* **2013**, *21*, 6623–6633.
26. Budovská, M.; Baláž, M.; Mezencev, R.; Tischlerová, V.; Zigová, M.; Mojžiš, J. *J. Fluorine Chem.* **2018**, *216*, 24–32.
27. Budovská, M.; Tischlerová, V.; Mojžiš, J.; Kozlov, O.; Gondová, T. *Monatsh. Chem.* **2020**, *151*, 63–77.
28. Kello, M.; Drutovic, D.; Chripková, M.; Pilatová, M.; Budovská, M.; Kuliková, L.; Urdzik, P.; Mojžiš, J. *Molecules* **2014**, *19*, 10877–10897.
29. Budovská, M.; Tischlerová, V.; Možiš, J.; Harvanová, M.; Kozlov, O.; Gondová, T.; Tomášková, N. Tetrahedron 2017, 73, 6356–6371.
30. Mezencev, R.; Kutschy, P.; Salayova, A.; Curillová, Z.; Možis, J.; Pilatova, M.; McDonald, J. Chemotherapy 2008, 54, 372–378.
31. Kutschy, P.; Salayová, A.; Čurillová, Z.; Mezencev, R.; Možiš, J.; Pilátová, M.; Balentová, E.; Pazdera, P.; Sabol, M.; Zburová, M. Bioorg. Med. Chem. 2009, 17, 3698–3712.
32. Tischlerová, V.; Kello, M.; Budovská, M.; Možiš, J. World J. Gastroenterol. 2017, 23, 4341–4353.
33. Solárová, Z.; Kello, M.; Varinská, L.; Budovská, M.; Solár, P. Biomed. Pharmacother. 2017, 85, 463–471.
34. Pauletti, P.M.; Cintra, L.S.; Braguine, C.G.; da Silva Filho, A.A.; e Silva, M.L.A.; Cunha, W.R.; Januário, A.H. Mar. Drugs 2010, 8, 1526–1549.
35. Di Pucchio, T.; Danese, S.; De Cristofaro, R.; Rutella, S. Expert. Opin. Ther. Pat. 2010, 20, 229–250.
36. Hou, D.Y.; Muller, A.J.; Sharma, M.D.; DuHadaway, J.; Banerjee, T.; Johnson, M.; Mellor, A.L.; Prendergast, G.C.; Munn, D.H. Cancer Res. 2007, 67, 792–801.
37. Očenášová, L.; Kutschy, P.; Gonda, J.; Pilátová, M.; Gönciová, G.; Možiš, J.; Pazdera, P. Chem. Pap. 2016, 70, 635–648.
38. Pedras, M.S.C.; Suchý, M.; Ahiahonu, P.W.K. Org. Biomol. Chem. 2006, 4, 691–701.
39. Topliss, J.G. J. Med. Chem. 1972, 15, 1006–1011.
40. Mosmann, T. J. Immunol. Methods 1983, 65, 55–63.