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Synthesis of new 5-bromo derivatives of indole and spiroindole phytoalexins

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Electrophilic aromatic substitution is one of the most thoroughly studied reactions in organic chemistry. In the present paper, the 5-brominated spirobrassinol methyl ethers *VII*, *VIII* were obtained by electrophilic substitution of the aromatic core of indoline at the C-5 position in the presence of various brominating agents. The same products were also prepared from 5-bromoindole (*IX*) following the sequence for the synthesis 1-methoxyspirobrassinol methyl ether (*V*) from indoline. In addition, the new related 5-bromospiroindoline derivatives *XX–XXIII* were synthesised and their biological activity on human tumour cell lines was examined. The presence of bromine in the indole or indoline skeleton at the C-5 position resulted in the partial increase in anticancer activity on leukaemia cell lines (Jurkat, CEM). The structures of the newly prepared products were determined by ^{1}H and ^{13}C NMR spectroscopy, including HSQC, HMBC, COSY, NOESY and DEPT measurements.

Keywords: 5-bromobrassinin, spiroindole phytoalexins, bromination, spirocyclisation, anti-proliferative activity

Introduction

The indole skeleton is present in a wide number of natural products and pharmaceuticals and is one of the most important structural subunits for the discovery of new drug candidates (Boyd & Sperry, 2011). In recent decades, the number of new isolated indole compounds, many of which contain halogen on the aromatic ring, has increased significantly as a consequence of the increase in their biological activity (Pauletti et al., 2010).

Brassinin (*I*, Fig. 1) is an essential indole phytoalexin, a class of natural metabolites produced de novo by plants of the family *Brassicaceae* (syn. *Cruciferae*) in response to fungal attack and other forms of stress (pathogen infection, UV radiation) (Pedras et al., 2011). Brassinin (*I*) has been demonstrated as exhibiting chemo-preventative activity in preclinical models and this phytoalexin, together with a synthetic derivative, 5-bromobrassinin (*II*), have been classified as bioavailable inhibitors of indoleamine 2,3-dioxygenase (IDO), a tryptophan-catabolising enzyme that drives immune escape in cancer (Banerjee et al., 2008).

Since these compounds can serve as lead compounds for the development of new biologically active agents, research into their synthesis is desirable; this article reports on the synthesis and anti-proliferative

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†Dr. Peter Kutschy passed away on 11 June 2012.

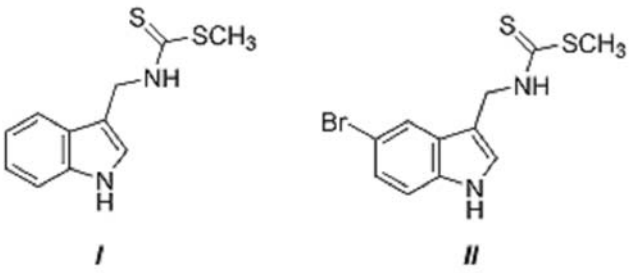


Fig. 1. Brassinin (*I*) and its synthetic derivative 5-bromobrassinin (*II*).

activity of new 5-bromo derivatives of the 1-methoxy and 1-Boc-spirobrassinol methyl ether *III* and the related 5-bromospiroindoline analogues. The retrosynthesis of the target compounds was based on the three synthetic routes (A, B, C) outlined in Fig. 2.

Experimental

All commercially available reagents were purchased at the highest available purity from Aldrich, Merck or Acros Organics (all from Slovakia) and were used without further purification. Solvents were dried and purified prior to use following standard procedures. ^{1}H and ^{13}C NMR spectra were recorded for compounds dissolved in $CDCl_3$ and $(CD_3)_2CO$ at ambient temperature on a Varian Mercury Plus 400 (USA) FT-NMR (400.13 MHz for ^{1}H and 100.6 MHz for ^{13}C) spectrometer using tetramethylsilane (TMS) as the internal reference. Chemical shifts are given in δ relative to TMS. Coupling constants (J) were obtained by first-order analysis and measured in Hertz (Hz). IR spectra were recorded on an Avatar FT-IR 6700 spectrometer using the attenuated total reflectance (ATR) method in the range of 4000–400 cm^{-1}. The EI mass spectra were recorded on a GC-MS Trio 1000 (Fisons Instruments, UK) spectrometer at ionisation energy of 70 eV. Microanalyses were performed using a Perkin–Elmer, Model 2400 analyser (USA). The progress of chemical reactions was monitored by thin layer chromatography, using Macherey-Nagel plates Alugram® Sil G/UV254 (Slovakia). Detection was carried out with ultraviolet light (254 nm). Preparative column chromatography was performed on a Kieselgel 60 Merck Type 9385 (Slovakia) (0.040–0.063 mm). Melting points were determined on a Koffler micro melting point apparatus and are uncorrected. The properties and composition of the corresponding products are summarized in Tables 1 and 2.

Cell culture

Jurkat and CEM (acute T-lymphoblastic leukaemia), A-549 (non-small cell lung adenocarcinoma), MCF-7 and MDA-MB-231 (mammary gland adenocarcinoma) and HeLa (cervical adenocarcinoma) cells were maintained in RPMI 1640 medium with Glutamax-I supplemented or D-MEM medium with Glutamax-I and glucose supplemented with 10 mass % foetal calf serum, penicillin (100 IU mL^{-1}) and streptomycin (100 mg mL^{-1}; all from Invitrogen, UK), in

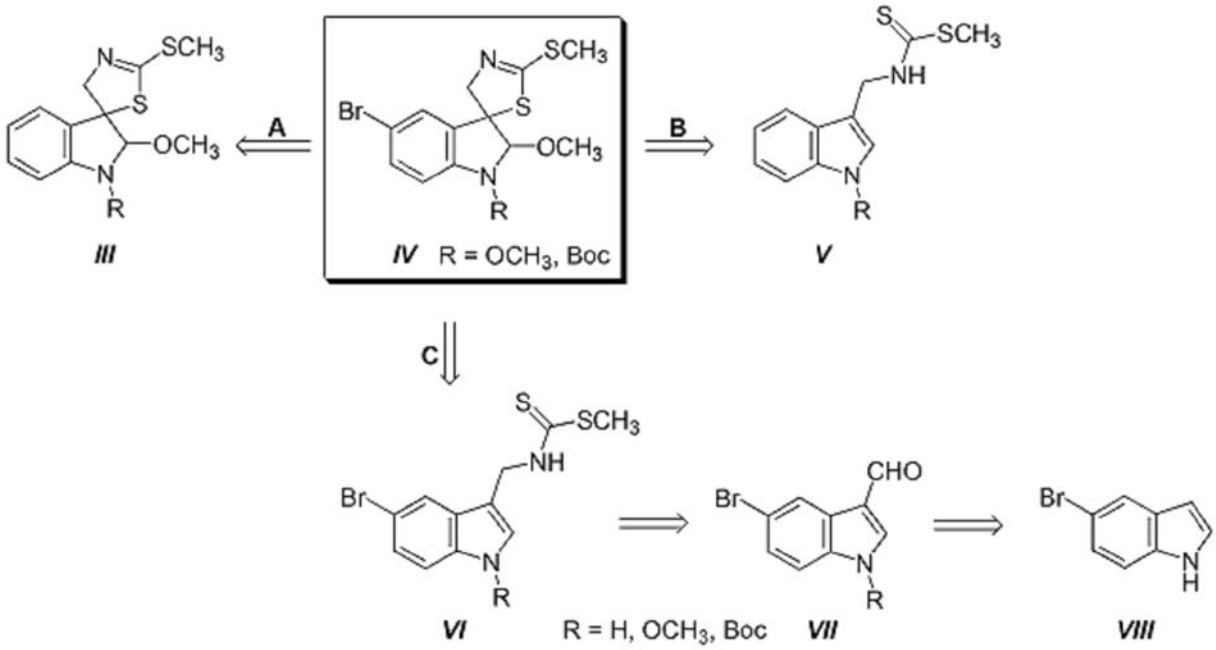


Fig. 2. Retrosynthetic analysis.

Table 1. Spectral data of newly prepared compounds

Compound	Spectral data
II	IR, $\tilde{\nu}$/cm^{-1}: 3349, 2925, 2849, 1446, 1371, 1087 ^{1}H NMR (400 MHz, $(CD_3)_2CO$)), δ: 10.41 (s, 1H, NH), 9.20 (s, 1H, NH—CH_2), 7.86 (d, 1H, J = 1.9 Hz, H-4), 7.48 (d, 1H, J = 2.2 Hz, H-2), 7.39 (d, 1H, J = 8.6 Hz, H-7), 7.24 (dd, 1H, J = 8.6 Hz, J = 1.9 Hz, H-6), 5.09 (d, 2H, J = 5.2 Hz, CH_2), 2.58 (s, 3H, SCH_3) ^{13}C NMR (100 MHz, $(CD_3)_2CO$), δ: 196.3 (CS), 133.9 (C-7a), 127.4 (C-3a), 125.1 (C-2), 122.8 (C-6), 120.0 (C-4), 111.9 (C-7), 110.6 (C-5), 109.3 (C-3), 40.4 (CH_2), 15.6 (SCH_3) MS, m/z (I_r/%): 316 (27) $(M + H)^+$, 208 (100), 129 (72), 102 (37), 43 (28)
XIIIa	IR, $\tilde{\nu}$/cm^{-1}: 2917, 2904, 2848, 1560, 1460, 1136 ^{1}H NMR (400 MHz, $CDCl_3$), δ: 7.36 (dd, 1H, J = 8.3 Hz, J = 1.9 Hz, H-6), 7.33 (d, 1H, J = 1.9 Hz, H-4), 6.82 (d, 1H, J = 8.3 Hz, H-7), 4.62 (s, 1H, H-2), 4.46 (d, 1H, J = 15.4 Hz, H-4$_a$'), 4.31 (d, 1H, J = 15.4 Hz, H-4$_b$'), 3.93 (s, 3H, CH_3O—N), 3.71 (s, 3H, CH_3O—C), 2.57 (s, 3H, SCH_3) ^{13}C NMR (100 MHz, $CDCl_3$), δ: 166.3 (C-2'), 146.4 (C-7a), 132.8 (C-6), 131.4 (C-3a), 126.1 (C-4), 116.1 (C-5), 114.3 (C-7), 105.0 (C-2), 73.1 (C-4'), 69.8 (C-3), 63.8 (CH_3O—N), 59.7 (CH_3O—C), 15.2 (SCH_3) MS, m/z (I_r/%): 376 (9) $(M + H)^+$, 345 (55), 313 (32), 72 (30), 43 (100)
XIIIb	IR, $\tilde{\nu}$/cm^{-1}: 2926, 2892, 2848, 1576, 1461, 1137 ^{1}H NMR (400 MHz, $CDCl_3$), δ: 7.39 (d, 1H, J = 1.9 Hz, H-4), 7.35 (dd, 1H, J = 8.3 Hz, J = 1.9 Hz, H-6), 6.81 (d, 1H, J = 8.3 Hz, H-7), 4.95 (d, 1H, J = 15.4 Hz, H-4$_b$'), 4.92 (s, 1H, H-2), 3.92 (s, 3H, CH_3O—N), 3.89 (d, 1H, J = 15.4 Hz, H-4$_a$'), 3.69 (s, 3H, CH_3O—C), 2.58 (s, 3H, SCH_3) ^{13}C NMR (100 MHz, $CDCl_3$), δ: 163.5 (C-2'), 147.0 (C-7a), 132.6 (C-6), 130.3 (C-3a), 126.9 (C-4), 116.0 (C-5), 114.5 (C-7), 108.6 (C-2), 69.7 (C-4'), 68.1 (C-3), 63.9 (CH_3O—N), 59.8 (CH_3O—C), 15.1 (SCH_3) MS, m/z (I_r/%): 376 (17) $(M + H)^+$, 345 (77), 313 (50), 149 (100), 72 (82)
XIVa	IR, $\tilde{\nu}$/cm^{-1}: 2955, 2926, 2869, 1741, 1677, 1550, 1493, 1265, 1093 ^{1}H NMR (400 MHz, $CDCl_3$), δ: 7.71 (d, 1H, J = 9.0 Hz, H-7), 7.53 (d, 1H, J = 9.0 Hz, H-6), 7.44 (s, 1H, H-4), 5.14 (s, 1H, H-2), 4.35 (d, 1H, J = 15.1 Hz, H-4$_a$'), 3.91 (d, 1H, J = 15.1 Hz, H-4$_b$'), 3.53 (s, 3H, OCH_3), 2.59 (s, 3H, SCH_3), 1.59 (s, 9H, $C(CH_3)_3$) ^{13}C NMR (100 MHz, $CDCl_3$), δ: 167.7 (C-2'), 145.6 (CO), 132.4 (C-7a), 132.3 (C-3a), 130.8 (C-6), 128.8 (C-7), 126.8 (C-4), 116.0 (C-5), 96.0 (C-2), 82.5 ($C(CH_3)_3$), 74.8 (C-4'), 70.0 (C-3), 58.5 (OCH_3), 28.3 ($C(CH_3)_3$), 15.2 (SCH_3)
XIVb	IR, $\tilde{\nu}$/cm^{-1}: 2957, 2917, 2849, 1713, 1680, 1552, 1472, 1261, 1093 ^{1}H NMR (400 MHz, $CDCl_3$), δ: 7.43 (d, 1H, J = 1.8 Hz, H-4), 7.40–7.31 (m, 2H, H-6, H-7), 5.46 (s, 1H, H-2), 4.83 (d, 1H, J = 15.8 Hz, H-4$_b$'), 4.29 (d, 1H, J = 15.8 Hz, H-4$_a$'), 3.50 (s, 3H, OCH_3), 2.58 (s, 3H, SCH_3), 1.59 (s, 9H, $C(CH_3)_3$) ^{13}C NMR (100 MHz, $CDCl_3$), δ: 165.0 (C-2'), 148.8 (CO), 140.1 (C-7a), 132.6 (C-6), 132.3 (C-3a), 128.6 (C-7), 126.6 (C-4), 115.6 (C-5), 99.0 (C-2), 82.7 ($C(CH_3)_3$), 71.4 (C-3), 66.6 (C-4'), 58.0 (OCH_3), 28.3 ($C(CH_3)_3$), 15.2 (SCH_3)
XVIII	IR, $\tilde{\nu}$/cm^{-1}: 2987, 2940, 2829, 1541, 1434, 1075 ^{1}H NMR (400 MHz, $CDCl_3$), δ: 7.71 (d, 1H, J = 0.8 Hz, H-4), 7.31 (m, 2H, H-6, H-7), 7.24 (d, 1H, J = 3.4 Hz, H-2), 6.29 (d, 1H, J = 3.4 Hz, H-3), 4.06 (s, 3H, OCH_3) ^{13}C NMR (100 MHz, $CDCl_3$), δ: 130.4 (C-7a), 125.8 (C-3a), 125.2 (C-6), 124. 0 (C-2), 123.6 (C-4), 113.1 (C-5), 109.6 (C-7), 97.6 (C-3), 66.1 (OCH_3) MS, m/z (I_r/%): 227 (15) $(M + H)^+$, 149 (43), 115 (29), 58 (27), 43 (100)
XIX	IR, $\tilde{\nu}$/cm^{-1}: 3099, 2942, 2819, 1652, 1449, 1363, 1050 ^{1}H NMR (400 MHz, $CDCl_3$), δ: 9.92 (s, 1H, CHO), 8.47 (d, 1H, J = 1.8 Hz, H-4), 7.88 (s, 1H, H-2), 7.47 (dd, 1H, J = 8.6 Hz, J = 1.8 Hz, H-6), 7.35 (d, 1H, J = 8.6 Hz, H-7), 4.19 (s, 3H, OCH_3) ^{13}C NMR (100 MHz, $CDCl_3$), δ: 183.8 (CHO), 132.3 (C-2), 131.3 (C-7a), 127.7 (C-6), 124.8 (C-4), 123.0 (C-3a), 117.1 (C-5), 113.4 (C-3), 110.2 (C-7), 67.1 (OCH_3) MS, m/z (I_r/%): 255 (47) $(M + H)^+$, 115 (52), 88 (22), 58 (29), 43 (100)
XX	IR, $\tilde{\nu}$/cm^{-1}: 3314, 3166, 2994, 1510, 1453, 1229, 1112 ^{1}H NMR (400 MHz, $CDCl_3$), δ: 11.06 (s, 0.8H, OH major), 10.83 (s, 0.2H OH minor), 8.33 (s, 0.8H, CH═N major), 8.28 (s, 0.2H, CH═N minor), 7.87 (d, 1H, J = 1.8 Hz, H-4), 7.66 (s, 1H, H-2), 7.35 (d, 1H, J = 8.6 Hz, H-7), 7.27 (dd, 1H, J = 8.6 Hz, J = 1.8 Hz, H-6) ^{13}C NMR (100 MHz, $CDCl_3$), δ: 144.3 (CH═N minor), 138.3 (C-2), 133.6 (C-7a), 131.7 (CH═N major), 128.1 (C-3a major), 127.9 (C-3a minor), 124.5 (C-6), 120.3 (C-4), 113.1 (C-7), 112.9 (C-5), 109.5 (C-3 minor), 105.9 (C-3 major) MS, m/z (I_r/%): 240 (30) $(M + H)^+$, 142 (30), 114 (21), 58 (30), 43 (100)
XXI	IR, $\tilde{\nu}$/cm^{-1}: 3227, 3126, 2937, 1513, 1439, 1226, 1050 ^{1}H NMR (400 MHz, $CDCl_3$), δ: 8.41 (s, 0.5H, CH═N), 8.25 (s, 0.5H, H-4), 8.23 (s, 0.5H, CH═N), 7.91 (s, 0.5H, H-4), 7.75 (s, 0.5H, H-2), 7.45 (s, 0.5H, H-2), 7.44–7.24 (m, 2H, H-6, H-7), 4.15 (s, 1.5H, OCH_3), 4.11 (s, 1.5H, OCH_3) ^{13}C NMR (100 MHz, $CDCl_3$), δ: 144.8 (CH═N), 139.0 (C-2), 131.3 (C-7a), 129.5 (CH═N), 129.4 (C-7a), 126.7 (C-6), 126.3 (C-6), 125.6 (C-2), 125.0 (C-4), 124.7 (C-3a), 122.6 (C-3a), 121.2 (C-4), 114.9 (C-5), 114.8 (C-5), 110.0 (C-7), 109.8 (C-7), 105.3 (C-3), 101.4 (C-3), 66.6 (OCH_3), 66.5 (OCH_3) MS, m/z (I_r/%): 270 (4) $(M + H)^+$, 114 (2), 58 (26), 43 (100)

Table 1. (continued

Compound	Spectral data
XXII	IR, $\tilde{\nu}$/cm^{-1}: 3456, 2980, 1720, 1450, 1368, 1155 ^{1}H NMR (400 MHz, $CDCl_3$), δ: 8.63 (s, 0.4H, CH═N minor), 8.28 (d, 0.6H, J = 1.9 Hz, H-4 major), 8.25 (s, 0.6H, CH═N major), 8.08 (d, 0.4H, J = 8.8 Hz, H-7 minor), 8.02 (d, 0.6H, J = 8.4 Hz, H-7 major), 7.84 (d, 0.4H, J = 1.7 Hz, H-4 minor), 7.74 (s, 0.6H, H-2 major), 7.70 (s, 0.4H, H-2 minor), 7.46 (dd, 1H, J = 8.8 Hz, J = 2.0 Hz, H-6), 1.69 (s, 3.6H, $C(CH_3)_3$ minor), 1.68 (s, 5.4H, $C(CH_3)_3$ major) ^{13}C NMR (100 MHz, $CDCl_3$), δ: 149.0 (CO minor), 148.9 (CO major), 144.6 (CH═N major), 138.4 (C-2 minor), 134.6 (C-7a major), 133.2 (C-7a minor), 132.4 (CH═N minor), 130.3 (C-3a minor), 128.7 (C-2 major), 128.6 (C-3a major), 128.3 (C-6), 125.3 (C-4 major), 121.2 (C-4 minor), 117.0 (C-5 minor), 116.9 (C-5 major), 116.5 (C-7), 113.6 (C-3 major), 108.9 (C-3 minor), 85.1 ($C(CH_3)_3$), 28.1 ($C(CH_3)_3$)
XXIII	IR, $\tilde{\nu}$/cm^{-1}: 3308, 2918, 2850, 1497, 1387, 1087 ^{1}H NMR (400 MHz, $CDCl_3$), δ: 7.73 (s, 1H, H-4), 7.38–7.29 (m, 3H, H-7, H-6, H-2), 7.02 (s, 1H, NH), 4.96 (d, 2H, J = 4.3 Hz, CH_2), 4.08 (s, 3H, OCH_3), 2.65 (s, 3H, SCH_3) ^{13}C NMR (100 MHz, $CDCl_3$), δ: 198.6 (CS), 130.8 (C-7a), 126.0 (C-6), 124.5 (C-3a), 123.8 (C-7), 121.6 (C-4), 113.7 (C-5), 110.0 (C-2), 106.0 (C-3), 66.3 (OCH_3), 42.4 (CH_2), 18.2 (SCH_3)
XXIV	IR, $\tilde{\nu}$/cm^{-1}: 3260, 2918, 1740, 1447, 1366, 1149, 1054 ^{1}H NMR (400 MHz, $CDCl_3$), δ: 7.99 (d, 1H, J = 8.8 Hz, H-7), 7.68 (s, 1H, H-4), 7.61 (s, 1H, H-2), 7.42 (dd, 1H, J = 8.8 Hz, J = 1.9 Hz, H-6), 7.11 (s, 1H, NH), 4.97 (d, 2H, J = 4.4 Hz, CH_2), 2.65 (s, 3H, SCH_3), 1.66 (s, 9H, $C(CH_3)_3$) ^{13}C NMR (100 MHz, $CDCl_3$), δ: 199.2 (CS), 149.1 (CO), 134.3 (C-7a), 130.8 (C-3a), 127.8 (C-6), 126.2 (C-2), 121.7 (C-4), 116.9 (C-7), 116.4 (C-5), 114.8 (C-3), 84.6 ($C(CH_3)_3$), 42.1 (CH_2), 28.2 ($C(CH_3)_3$), 18.3 (SCH_3)
XXV	IR, $\tilde{\nu}$/cm^{-1}: 3194, 2922, 1711, 1574, 1463, 1290, 1149 ^{1}H NMR ($CDCl_3$), δ: 8.64 (s, 1H, NH), 7.46 (d, 1H, J = 1.7 Hz, H-4), 7.39 (dd, 1H, J = 8.3 Hz, J = 1.7 Hz, H-6), 6.81 (d, 1H, J = 8.3 Hz, H-7), 4.66 (d, 1H, J = 15.2 Hz, H-4$_b$'), 4.50 (d, 1H, J = 15.2 Hz, H-4$_a$'), 2.63 (s, 3H, SCH_3) ^{13}C NMR (100 MHz, $CDCl_3$), δ: 177.7 (C-2), 164.0 (C-2'), 138.3 (C-7a), 133.3 (C-3a), 132.6 (C-6), 127.6 (C-4), 116.2 (C-5), 111.9 (C-7), 75.1 (C-4'), 64.5 (C-3), 15.8 (SCH_3)
XXVI	IR, $\tilde{\nu}$/cm^{-1}: 2915, 1729, 1579, 1462, 1289, 1148 ^{1}H NMR (400 MHz, $CDCl_3$), δ: 7.47 (d, 1H, J = 1.7 Hz, H-4), 7.35 (dd, 1H, J = 8.7 Hz, J = 1.7 Hz, H-6), 6.87 (d, 1H, J = 8.7 Hz, H-7), 4.66 (d, 1H, J = 15.2 Hz, H-4$_b$'), 4.47 (d, 1H, J = 15.2 Hz, H-4$_a$'), 4.07 (s, 3H, OCH_3), 2.63 (s, 3H, SCH_3) ^{13}C NMR (100 MHz, $CDCl_3$), δ: 170.2 (C-2), 163.8 (C-2'), 137.9 (C-7a), 132.7 (C-6), 130.9 (C-3a), 127.5 (C-4), 116.7 (C-5), 109.3 (C-7), 74.7 (C-4'), 64.0 (OCH_3), 62.6 (C-3), 15.8 (SCH_3)
XVIIa	IR, $\tilde{\nu}$/cm^{-1}: 3414, 2975, 2929, 1701, 1561, 1471, 1367, 1143, 1092 ^{1}H NMR (400 MHz, $CDCl_3$), δ: 7.46–7.70 (m, 1H, H-7), 7.47 (d, 1H, J = 2.0 Hz, H-4), 7.37 (dd, 1H, J = 8.6 Hz, J = 2.0 Hz, H-6), 5.60 (s, 1H, H-2), 4.38 (d, 1H, J = 15.1 Hz, H-4$_b$'), 4.03 (d, 1H, J = 15.1 Hz, H-4$_a$'), 2.59 (s, 3H, SCH_3), 1.60 (s, 9H, $C(CH_3)_3$) ^{13}C NMR (100 MHz, $CDCl_3$), δ: 166.0 (C-2'), 152.0 (CO), 138.5 (C-7a), 132.6 (C-6), 129.7 (C-3a), 127.1 (C-4), 116.3 (C-7), 115.8 (C-5), 88.5 (C-2), 83.3 ($C(CH_3)_3$), 75.5 (C-4'), 72.4 (C-3), 28.3 ($C(CH_3)_3$), 15.0 (SCH_3)
XVIIb	IR, $\tilde{\nu}$/cm^{-1}: 3402, 2975, 2928, 1701, 1561, 1474, 1367, 1159, 1063 ^{1}H NMR (400 MHz, $CDCl_3$), δ: 7.50–7.90 (m, 1H, H-7), 7.47 (s, 1H, H-4), 7.37 (d, 1H, J = 8.4 Hz, H-6), 5.90 (s, 1H, H-2), 5.04 (d, 1H, J = 15.7 Hz, H-4$_b$'), 4.26 (d, 1H, J = 15.6 Hz, H-4$_a$'), 2.58 (s, 3H, SCH_3), 1.60 (s, 9H, $C(CH_3)_3$) ^{13}C NMR (100 MHz, $CDCl_3$), δ: 164.1 (C-2'), 153.9 (CO), 138.5 (C-7a), 132.7 (C-6), 129.9 (C-3a), 127.0 (C-4), 116.2 (C-7), 115.7 (C-5), 91.8 (C-2), 83.3 ($C(CH_3)_3$), 69.6 (C-3), 67.9 (C-4'), 28.4 ($C(CH_3)_3$), 15.2 (SCH_3)
XXVIII	IR, $\tilde{\nu}$/cm^{-1}: 2918, 2849, 1762, 1727, 1591, 1463, 1288, 1148 ^{1}H NMR (400 MHz, $CDCl_3$), δ: 7.76 (d, 1H, J = 8.6 Hz, H-7), 7.50 (d, 1H, J = 2.1 Hz, H-4), 7.47 (dd, 1H, J = 8.6 Hz, J = 2.1 Hz, H-6), 4.71 (d, 1H, J = 15.3 Hz, H-4$_b$'), 4.49 (d, 1H, J = 15.3 Hz, H-4$_a$'), 2.63 (s, 3H, SCH_3), 1.63 (s, 9H, $C(CH_3)_3$) ^{13}C NMR (100 MHz, $CDCl_3$), δ: 173.8 (C-2), 163.6 (C-2'), 148.6 (CO), 137.4 (C-7a), 132.9 (C-6), 131.4 (C-3a), 127.1 (C-4), 118.2 (C-5), 116.9 (C-7), 85.4 ($C(CH_3)_3$), 76.1 (C-4'), 64.6 (C-3), 28.0 ($C(CH_3)_3$), 15.7 (SCH_3)

an atmosphere of 5 vol. % of CO_2 in humidified air at 37 °C. Cell viability, estimated by trypan blue exclusion, was greater than 95 % prior to each experiment.

Assessment of cytotoxicity by MTT assay

The cytotoxic effect of the tested compounds was studied using a colorimetric microculture assay with the MTT (thiazolyl blue tetrazolium bromide) endpoint. The amount of MTT reduced to formazan was proportional to the number of viable cells (Mosmann, 1983). In brief, 5×10^3 cells were plated per well in 96-well polystyrene microplates (Sarstedt, Germany) in the culture medium containing the tested chemicals at final concentrations of 10^{-4}–10^{-6} mol L^{-1}. After 72 h, 10 mL of MTT (5 mg mL^{-1}) was added to each well. After an additional 4 h at 37 °C, during which insoluble formazan was produced, 100 mL of 10 mass % of sodium dodecylsulphate were added to each well and a further 12 h were allowed for the formazan to

dissolve. The absorbance was measured at 540 nm using the automated MRX microplate reader (Dynatech laboratories UK). The absorbance of the control wells was taken as 100 %, and the results were expressed as a percentage of control.

General procedures for synthesis of 5-brominated spirobrassinol methyl ether XIIIa–XIVb

Procedure A: to a solution of a mixture of the corresponding diastereoisomers *XIa* and *XIb* (Kutschy et al., 2002) or *XIIa* and *XIIb* (Budovská et al., unpublished results; 0.101 mmol) in dry dichloromethane (1.8 mL) was added; *a*) a freshly prepared solution of bromine (0.006 mL, 0.111 mmol) and pyridine (0.042 mL, 0.505 mmol) in anhydrous dichloromethane (0.26 mL); *b*) *N*-bromosuccinimide (0.020 g, 0.111 mmol); *c*) a solution of bromine (0.257 mL, 0.111 mmol) in anhydrous dioxane (0.26 mL); *d*) phenyltrimethylammonium tribromide (0.228 g, 0.606 mmol). The reaction mixture was stirred at ambient temperature and, after dilution of the reaction mixture with dichloromethane (5 mL), treatment of the individual mixtures and drying of the organic layer over Na_2SO_4, the residue obtained after evaporation of the solvent was subjected to chromatography on silica gel to afford products *XIIIa–XIVb*.

Procedure B: to a stirred solution of the corresponding brassinin *XI* (Kutschy et al., 2009), *X* (Kutschy, 1998) (0.188 mmol) in a mixture of anhydrous dichloromethane–methanol (2.3 mL : 0.3 mL) at ambient temperature a freshly prepared solution of bromine (stock solution prepared by dissolving 0.04 mL of bromine in 1.76 mL of anhydrous dichloromethane) was added. After stirring for 1 h, triethylamine was added. After stirring for 5 min, the reaction mixture was diluted with dichloromethane (20 mL), washed with brine (2 × 10 mL) and dried over Na_2SO_4. The residue obtained after evaporation of the solvent was subjected to chromatography on silica gel to afford *XIIIa–XIVb*.

Procedure C: to a stirred solution of the corresponding 5-bromo-1-substituted brassinin *XXIII*, *XXIV* (0.145 mmol) in a mixture of anhydrous dichloromethane–methanol (1.4 mL : 0.1 mL) at ambient temperature a freshly prepared solution of bromine (0.369 mL, 0.159 mmol; stock solution obtained by dissolving 0.04 mL of bromine in 1.76 mL anhydrous dichloromethane) was added. After stirring for 30 min, triethylamine (0.044 mL, 0.032 g, 0.319 mmol) was added. After stirring for 5 min, the reaction mixture was diluted with dichloromethane (20 mL), washed with brine (2 × 10 mL) and dried over Na_2SO_4. The residue obtained after evaporation of the solvent was subjected to chromatography on silica gel to afford *XIIIa–XIVb*.

cis-(±)- and trans-(±)-5-Bromo-1-methoxyspirobrassinol methyl ether cis-(±)-XIIIa and trans-(±)-XIIIb

Following general procedure A, products (±)-*XIIIa* and (±)-*XIIIb* were obtained using the mixture of diastereoisomer *cis*-(±)-*XIa* and *trans*-(±)-*XIb* (0.030 g, 0.101 mmol) and by method; *a*) after stirring the reaction mixture for 40 min, washing with 1 M HCl (2 × 5 mL) and brine (5 mL) and isolated on silica gel (hexane–diethyl ether, $\varphi_r = 1 : 1$). Yield: (±)-*XIIIa* (0.019 g, 50 %), (±)-*XIIIb* (0.0185 g, 49 %); *b*) after stirring the reaction mixture for 40 min, adding triethylamine (0.030 mL, 0.022 g, 0.222 mmol), washing with brine (2 × 5 mL) and isolated on silica gel (hexane–diethyl ether, $\varphi_r = 1 : 1$). Yield: (±)-*XIIIa* (0.019 g, 50 %), (±)-*XIIIb* (0.0185 g, 49 %); *c*) after stirring the reaction mixture for 40 min, adding triethylamine (0.030 mL, 0.022 g, 0.222 mmol), washing with 1 M HCl (5 mL) and brine (5 mL), and isolated on silica gel (hexane–diethyl ether, $\varphi_r = 1 : 1$). Yield: (±)-*XIIIa* (0.019 g, 50 %), (±)-*XIIIb* (0.017 g, 45 %); *d*) after stirring the reaction mixture for 60 min, adding triethylamine (0.099 mL, 0.072 g, 0.707 mmol), and isolated on silica gel (hexane–diethyl ether, $\varphi_r = 1 : 1$). Yield: (±)-*XIIIa* (0.018 g, 47 %), (±)-*XIIIb* (0.0184 g, 48 %).

Following general procedure B, products (±)-*XIIIa* and (±)-*XIIIb* were obtained using brassinin *IX* (0.050 g, 0.188 mmol), a solution of bromine (1.307 mL, 0.564 mmol) and triethylamine (0.105 mL, 0.076 g, 0.752 mmol) and isolated on silica gel (hexane–diethyl ether, $\varphi_r = 1 : 1$). Yield: (±)-*XIIIa* (0.031 g, 44 %), (±)-*XIIIb* (0.034 g, 49 %).

Following general procedure C, products (±)-*XIIIa* and (±)-*XIIIb* were obtained using brassinin *XXIII* (0.050 g, 0.145 mmol) and isolated on silica gel (hexane–diethyl ether, $\varphi_r = 1 : 1$). Yield: (±)-*XIIIa* (0.021 g, 39 %), (±)-*XIIIb* (0.022 g, 41 %).

cis-(±)- and trans-(±)-5-Bromo-1-(tert-butoxycarbonyl)spirobrassinol methyl ether cis-(±)-XIVa and trans-(±)-XIVb

Following general procedure A, products (±)-*XIVa* and (±)-*XIVb* were obtained using a mixture of diastereoisomer *cis*-(±)-*XIIa* and *trans*-(±)-*XIIb* (0.037 g, 0.101 mmol) and by method *b*) after stirring for 8 h, adding triethylamine (0.056 mL, 0.041 g, 0.404 mmol), washing the reaction mixture with brine (2 × 5 mL), and isolated on silica gel (dichloromethane). Yield: (±)-*XIVa* (0.0175 g, 39 %), (±)-*XIVb* (0.0185 g, 41 %).

Following general procedure B, products (±)-*XIVa* and (±)-*XIVb* were obtained using brassinin *X* (0.063 g, 0.188 mmol), a solution of bromine (4.358 mL, 1.88 mmol) and triethylamine (0.288 mL, 0.209 g, 2.068 mmol) and isolated on silica gel (petrol

Table 2. Characterisation data of newly prepared compounds

Compound	Formula	M_r	w_i(calc.)/% w_i(found)/%			Yield
			C	H	N	%
II	$C_{11}H_{11}BrN_2S_2$	315.25	41.91	3.52	8.89	50
			41.86	3.43	8.67	
XIIIa	$C_{13}H_{15}BrN_2O_2S_2$	375.30	41.60	4.03	7.46	50
			41.51	3.97	7.39	
XIIIb	$C_{13}H_{15}BrN_2O_2S_2$	375.30	41.60	4.03	7.46	49
			41.39	3.91	7.22	
XIVa	$C_{17}H_{21}BrN_2O_3S_2$	445.39	45.84	4.75	6.29	40
			45.63	4.67	6.18	
XIVb	$C_{17}H_{21}BrN_2O_3S_2$	445.39	45.84	4.75	6.29	51
			45.79	4.81	6.22	
XVIII	C_9H_8BrNO	226.07	47.82	3.57	6.20	81
			47.69	3.61	6.07	
XIX	$C_{10}H_8BrNO_2$	254.08	47.27	3.17	5.51	77
			47.17	3.11	5.32	
XX	$C_9H_7BrN_2O$	239.07	45.22	2.95	11.72	94
			44.98	2.84	11.58	
XXI	$C_{10}H_9BrN_2O_2$	269.09	44.63	3.37	10.41	93
			44,56	3.24	10.48	
XXII	$C_{14}H_{15}BrN_2O_3$	339.18	49.57	4.46	8.26	90
			49.48	4.36	8.21	
XXIII	$C_{12}H_{13}BrN_2OS_2$	345.28	41.74	3.79	8.11	75
			41.66	3.82	8.01	
XXIV	$C_{16}H_{19}BrN_2O_2S_2$	415.37	46.27	4.61	6.74	66
			46.19	4.49	6.67	
XXV	$C_{11}H_9BrN_2OS_2$	329.24	40.13	2.76	8.51	62
			40.26	2.93	8.67	
XXVI	$C_{12}H_{11}BrN_2O_2S_2$	359.26	40.12	3.09	7.80	29
			40.22	3.14	7.94	
XXVIIa	$C_{16}H_{19}BrN_2O_3S_2$	431.37	44.55	4.44	6.49	25
			44.63	4.37	6.38	
XXVIIb	$C_{16}H_{19}BrN_2O_3S_2$	431.37	44.55	4.44	6.49	40
			44.51	4.45	6.39	
XXVIII	$C_{16}H_{17}BrN_2O_3S_2$	429.35	44.76	3.99	6.52	75
			44.75	3.93	6.48	

ether–ethyl acetate, $\varphi_r = 5 : 1$) to afford a mixture of products *XIVa* and *XIVb*. Individual isomers *XIVa* (0.024 g, 29 %) and *XIVb* (0.043 g, 51 %) were obtained by a further separation using chromatography on silica gel (dichloromethane).

Following general procedure C, products (±)-*XIVa* and (±)-*XIVb* were obtained using brassinin *XXIV* (0.060 g, 0.145 mmol) and isolated on silica gel (hexane–acetone, $\varphi_r = 5 : 1$) to afford a mixture of isomers *XIVa* and *XIVb*. The individual *cis*-isomer *XIVa* (0.026 g, 40 %) and *trans*-isomer *XIVb* (0.031 g, 49 %) were obtained by a further separation using chromatography on silica gel (dichloromethane).

5-Bromo-1-methoxy-1H-indole XVIII

Sodium tungstate dihydrate (0.084 g, 0.273 mmol) in water (0.555 mL) was added to a solution of 5-bromoindoline (*XVII*; 0.3 g, 1.515 mmol) in methanol (6 mL) and the mixture was stirred under cooling at –20 °C (Chandra & Brown, 2005). An aqueous solution of hydrogen peroxide (30 mass %; 1.44 mL, 13.1 mmol) in methanol (1.5 mL) was added drop-wise over 30 min at –20 °C and the stirring was continued at –10–0 °C for 30 min. Potassium carbonate (1.67 g, 12.08 mmol) and dimethyl sulphate (0.375 mL, 0.497 g, 3.939 mmol) were added and the reaction mixture was stirred at –10–0 °C for 1 h, then the mixture was poured into water (15 mL). After extraction with diethyl ether (1 × 50 mL and 2 × 30 mL), the combined organic phase was washed with brine (50 mL), dried with anhydrous Na_2SO_4, filtered and concentrated under vacuum. The residue was purified by chromatography on silica gel (hexane–dichloromethane–ethyl acetate, $\varphi_r = 5 : 2 : 1$) to afford indole *XVIII* (0.276 g, 81 %) as a pale yellow oil.

5-Bromo-1-methoxy-1H-indole-3-carboxaldehyde XIX

To DMF (0.318 mL), phosphorus oxychloride (0.312 mL, 0.524 g, 3.415 mmol) was added drop-wise at 0 ℃. The mixture was stirred for 10 min and then a solution of 5-bromo-1-methoxy-1*H*-indole (*XVIII*; 0.234 g, 1.035 mmol) in DMF (0.43 mL) was added. After the mixture was stirred at ambient temperature for 2 h, ice was added followed by a 16 mass % solution of sodium hydroxide (3.27 mL). The precipitated product was extracted with diethyl ether (1 × 30 mL and 2 × 15 mL), the combined organic layers were washed with brine (20 mL), dried with Na_2SO_4, filtered and concentrated under vacuum. The residue was allowed to crystallise from diethyl ether–hexane to yield aldehyde *XIX* (0.202 g, 77 %) as a crystalline.

General procedure for synthesis of oximes XX–XXII

To a stirred solution of the corresponding aldehyde *XV* (Wang et al., 2001), *XVI* (Ishiyama et al., 2012) and *XIX* (3.085 mmol) in ethanol (5.799 mL) a solution of hydroxylammonium chloride (0.321 g, 4.627 mmol) and sodium carbonate (0.228 g, 2.159 mmol) in water (0.438 mL) was added. The mixture was stirred for 40 min at 0 ℃ (*XX* and *XXI*) or heated at 50 ℃ for 15 min (*XXII*). After evaporation of the ethanol and the addition of water (15.6 mL), the product was extracted with ethyl acetate (*XX* and *XXI*) or diethyl ether (*XXII*) (1 × 30 mL and 1 × 15 mL) and after drying with anhydrous Na_2SO_4 the solvent was evaporated. The residue was allowed to crystallise from ethyl acetate–hexane (*XX*, *XXI*) and dichloromethane–hexane (*XXII*) to yield the corresponding oxime.

5-Bromo-1H-indole-3-carboxaldehyde oxime XX

Following the general procedure, product *XX* (0.693 g, 94 %) was obtained using aldehyde *XV* (0.691 g, 3.085 mmol) as a mixture of (*E*)- and (*Z*)-isomers at a 80 : 20 ratio.

5-Bromo-1-methoxy-1H-indole-3-carboxaldehyde oxime XXI

Following the general procedure, product *XXI* (0.772 g, 93 %) was obtained using aldehyde *XIX* (0.784 g, 3.085 mmol) as a mixture of (*E*)- and (*Z*)-isomers at a 50 : 50 ratio.

5-Bromo-1-(tert-butoxycarbonyl)indole-3-carboxaldehyde oxime XXII

Following the general procedure, product *XXII* (0.942 g, 90 %) was obtained using aldehyde *XVI* (1.0 g, 3.085 mmol) as a mixture of (*E*)- and (*Z*)-isomers at a 60 : 40 ratio.

General procedure for synthesis of brassinins II, XXIII and XXIV

To a solution of $NiCl_2 \cdot 6H_2O$ (0.349 g, 1.468 mmol) in methanol (20 mL) was added the corresponding oxime *XX*, *XXII* (1.474 mmol) in methanol (15 mL) followed by $NaBH_4$ (0.556 g, 14.74 mmol) in a single portion with stirring and cooling under flowing cold water. After 5 min, the methanol in the reaction mixture was evaporated to 1/4 of its original volume and the mixture was poured into a saturated solution of NH_4OH (37 mL). In the case of brassinin *XXIII*, sodium cyanoborohydride (0.926 g, 14.74 mmol) and NH_4OAc (1.25 g, 16.21 mmol) were added to a cooled solution at 0 ℃ of oxime *XXI* (1.474) in methanol (8.75 mL). To this mixture, a solution (4.083 mL) of $TiCl_3$ (30 mass % in 2 M HCl) neutralised with 2 M NaOH (2.916 mL) was added. After stirring for 15 min at 0 ℃, the reaction mixture was poured into water (23 mL) containing 26 mass % NH_4OH (4.43 mL). The black precipitate was collected by suction filtration and washed with water (30 mL), methanol (30 mL) and ethyl acetate (30 mL). After extraction of the products with ethyl acetate (1 × 50 mL and 2 × 30 mL), drying the extract with Na_2SO_4 and evaporation of the solvent, the crude amines obtained were immediately dissolved in methanol (12 mL) and triethylamine (0.616 mL, 0.447 g; 4.422 mmol) and carbon disulphide (0.267 mL, 0.337 g; 4.422 mmol) were added. After stirring for 5 min, methyl iodide (0.276 mL, 0.627 g, 4.422 mmol) was added and stirring was continued for 20 min at ambient temperature. The solvent was evaporated and the residue obtained after evaporation of the solvent was subjected to chromatography on silica gel (hexane–acetone, φ_r = 2 : 1) (*II*), (hexane–dichloromethane–ethyl acetate, φ_r = 5 : 2 : 1) (*XXIII*) and (hexane–acetone, φ_r = 3 : 1) (*XXIV*).

5-Bromobrassinin II

Following the general procedure, product *II* (0.232 g, 50 %) was obtained using oxime *XX* (0.352 g, 1.474 mmol) as a crystalline. According to the literature, 5-bromobrassinin was previously synthesised by the Advanced Synthesis Group, Newark, DE, USA (Banerjee et al., 2008), but its synthesis and spectral data have not yet been published.

5-Bromo-1-methoxybrassinin XXIII

Following the general procedure, product *XXIII* (0.382 g, 75 %) was obtained as a colourless oil using oxime *XXI* (0.397 g, 1.474 mmol).

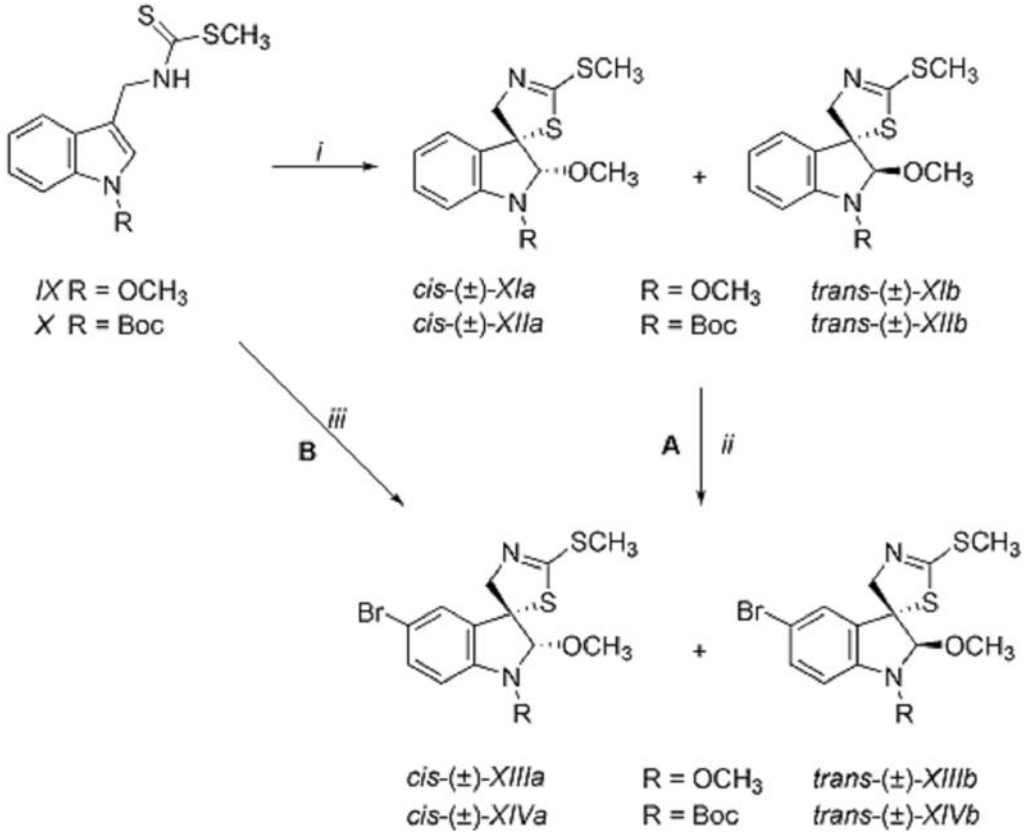


Fig. 3. Synthesis of target compounds *XIII* and *XIV* by synthetic routes A and B. Reagents and conditions: *i*) Br_2, CH_2Cl_2/MeOH (φ_r = 9 : 1), Et_3N, ambient temperature, 20 min, *XI*, 89 %, *XII*, 72 %; *ii*) bromination agent (Table 1); *iii*) Br_2, CH_2Cl_2/MeOH (φ_r = 9 : 1), Et_3N, ambient temperature, 60 min, *XIII*, 93 %, *XIV*, 80 %.

5-Bromo-1-(tert-butoxycarbonyl)brassinin XXIV

Following the general procedure, product *XXIV* (0.404 g, 66 %) was obtained as a crystalline using oxime *XXII* (0.5 g, 1.474 mmol).

General procedure for synthesis of spirobrassinins (±)-XXV and (±)-XXVI

To a stirred solution of the corresponding brassinin *II*, *XXIII* (0.159 mmol) in dichloromethane (2.69 mL) at ambient temperature was added pyridinium chlorochromate (0.239 g, 1.113 mmol) and the reaction mixture was stirred vigorously for 1 h (*XXV*) and for 2 h (*XXVI*). After diluting the mixture with dichloromethane (5 mL) and adding a small amount of silica gel, the solvent was evaporated and the residue obtained subjected to chromatography on silica gel (hexane–ethyl acetate, φ_r = 1 : 1) (*XXV*) and (hexane–ethyl acetate, φ_r = 1 : 1) (*XXVI*).

(±)-5-Bromospirobrassinin (±)-XXV

Following the general procedure, product (±)-*XXV* (0.032 g, 62 %) was obtained as a pale yellow oil using brassinin *II* (0.050 g, 0.159 mmol).

(±)-5-Bromo-1-methoxyspirobrassinin (±)-XXVI

Following the general procedure, product (±)-*XXVI* (0.013 g, 29 %) was obtained as a pale yellow oil using brassinin *XXIII* (0.055 g, 0.159 mmol).

cis-(±)- and trans-(±)-5-Bromo-1-(tert-butoxycarbonyl)spirobrassinol cis-(±)-XXVIIa and trans-(±)-XXVIIb

To a solution of brassinin *XXIV* (0.084 g, 0.202 mmol) in a mixture of dichloromethane–water (2.25 mL : 0.25 mL) at ambient temperature was added a freshly prepared stock solution of Br_2 (0.515 mL, 0.222 mmol), obtained by dissolving bromine (0.04 mL) in dichloromethane (1.76 mL). The reaction mixture was stirred for 30 min at ambient temperature and triethylamine (0.062 mL, 0.045 g, 0.0444 mmol) was added. Stirring continued for 5 min, then the reaction mixture was diluted with dichloromethane (15 mL) and washed with brine (2 × 15 mL). The organic layer was dried over Na_2SO_4 and the residue obtained after evaporation

Table 3. Electrophilic aromatic substitution of compounds *IX–XII* in the presence of various brominating agents produced by Fig. 3

Starting material	Brominating agent/eq.	Time/min	Products	Yield/%
(±)-*XIa*, (±)-*XIb*	Br_2 (1.1)/pyridine (5)	40	(±)-*XIIIa*, (±)-*XIIIb*	99
(±)-*XIa*, (±)-*XIb*	NBS (1.1)	40	(±)-*XIIIa*, (±)-*XIIIb*	99
(±)-*XIa*, (±)-*XIb*	DDB (1.1)	40	(±)-*XIIIa*, (±)-*XIIIb*	95
(±)-*XIa*, (±)-*XIb*	Phenyltrimethylammonium tribromide (6)	60	(±)-*XIIIa*, (±)-*XIIIb*	95
(±)-*XIIa*, (±)-*XIIb*	NBS (3)	480	(±)-*XIVa*, (±)-*XIVb*	80
IX	Br_2 (3)	60	(±)-*XIIIa*, (±)-*XIIIb*	93
X	Br_2 (10)	60	(±)-*XIVa*, (±)-*XIVb*	80

of the solvent subjected to chromatography on silica gel (dichloromethane), affording a mixture of diastereoisomers *XXVIIa* and *XXVIIb*. The individual isomers *XXVIIa* (0.022 g, 25 %) and *XXVIIa* (0.035 g, 40 %) were obtained by a further separation using chromatography on silica gel (dichloromethane–acetone, $\varphi_r = 30 : 1$).

(±)-5-Bromo-1-(tert-butoxycarbonyl) spirobrassinin (±)-XXVIII

To a solution of a mixture of diastereoisomers *XXVIIa* and *XXVIIb* (0.037 g, 0.086 mmol) in acetic acid (2.6 mL) was added CrO_3 (0.043 g, 0.43 mmol) and the reaction mixture was vigorously stirred at ambient temperature for 1 h. After pouring the mixture into water (11 mL), the product was extracted with diethyl ether (2 × 5 mL), washed with 10 mass % NaOH (2 × 5 mL), dried with anhydrous Na_2SO_4, filtered and concentrated under vacuum. The residue was purified by chromatography on silica gel (hexane–acetone, $\varphi_r = 3 : 1$) and allowed to crystallise from dichloromethane–hexane to afford spirobrassinin *XXVIII* (0.027 g, 75 %) as a crystalline.

Results and discussion

The known mixture of *cis*-(±) and *trans*-(±)-diastereoisomers of 1-methoxyspirobrassinol methyl ether (*XIa*, *XIb*) (Kutschy et al. (2002) and 1-(*tert*-butoxycarbonyl)spirobrassinol methyl ether (*XIIa*, *XIIb*; Budovská et al., unpublished results), as the starting materials for the first synthetic route A, was prepared via bromine-mediated spirocyclisation of the respective 1-substituted brassinins *IX* (Kutschy et al, 2009), *X* (Kutschy et al., 1998) in the presence of methanol (10 vol. %) as nucleophile following the procedure previously reported.

The new 5-bromo-1-methoxyspirobrassinol methyl ethers (*XIIIa*, *XIIIb*) and 5-bromo-1-(*tert*-butoxycarbonyl)spirobrassinol methyl ethers (*XIVa*, *XIVb*) were synthesised via electrophilic aromatic substitution of the indoline skeleton at the C-5 position in the presence of various brominating agents (bromine/pyridine, *N*-bromosuccinimide (NBS), dioxane dibromide (DDB), phenyltrimethylammonium tribromide; Fig. 3). Table 3 shows that the bromination of mixtures *XIa* and *XIb* in the first three cases was carried out using 1.1 eq. of reagent for 40 min while up to 6 eq. were required when phenyltrimethylammonium tribromide was used; the bromination was incomplete with a lower quantity of this reagent. The compounds *XIVa* and *XIVb* were synthesised by bromination of mixtures *XIIa* and *XIIb* in the presence of 3 eq. of NBS in anhydrous dichloromethane for 8 h. Interestingly, the amount of the brominating agent is probably dependent on the character of the group bonding onto the indoline nitrogen. The bulky *tert*-butoxycarbonyl group displays electron-acceptor properties towards the spiroindoline skeleton, unlike the methoxy group which exhibits electron-donor characteristics. The brominated diastereoisomers (±)-*XIIIa* and (±)-*XIIIb* thus prepared were separated by chromatography on silica gel using hexane–diethyl ether, $\varphi_r = 1 : 1$ as eluent and diastereoisomers (±)-*XIVa* and (±)-*XIVb* using dichloromethane.

The target products (±)-*XIIIa*, (±)-*XIIIb*, (±)-*XIVa*, (±)-*XIVb* were also prepared by the direct spirocyclisation reaction (synthetic route B, Fig. 3) of 1-methoxybrassinin (*IX*) (Kutschy et al. (2009) or 1-(*tert*-butoxycarbonyl)brassinin (*X*), (Kutschy et al. (1998) in dry dichloromethane using bromine (3 eq. for compound *IX* and 10 eq. for compound *X*) in the presence of methanol (10 vol. %). The reaction mixture was stirred for 60 min at ambient temperature and triethylamine was added subsequently to trap the hydrogen bromide liberated during the reaction. The ratio of diastereoisomers was determined by ^{1}H NMR spectra of the crude reaction products obtained after dilution with dichloromethane, washing with 1 M solution of hydrochloric acid and brine, drying and evaporation of the solvent. The ratios of the products were confirmed by the integration of separated signals corresponding to the H-2, H-$4_a'$, H-$4_b'$ protons. Diastereoisomers *cis*-(±)-*XIIIa* and *trans*-(±)-*XIIIb* were isolated by column chromatography with a 93 % yield in a 47 : 53 ratio, and isomers (±)-*XIVa* and (±)-*XIVb* were obtained with an 80 % yield (Table 3) after two chromatographic separations in a 37 : 63 ratio favouring the *trans*-isomer.

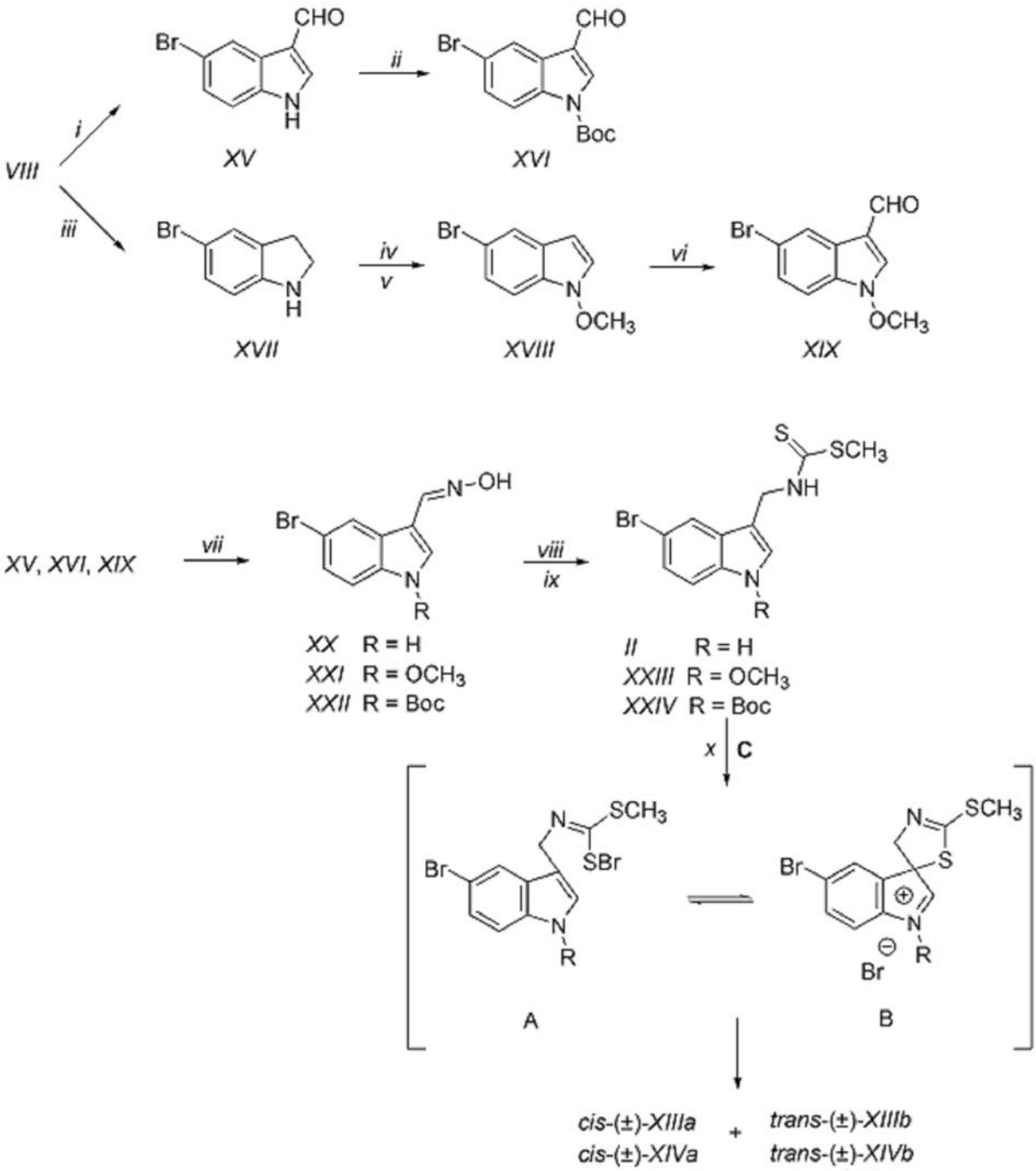


Fig. 4. Synthesis of target compounds *XIII* and *XIV* by synthetic route C. Reagents and conditions: *i*) $POCl_3$, DMF, 0 °C, 1 h, 87 %; *ii*) $(Boc)_2O$, THF, DMAP, 0 °C, 1 h, 85 %; *iii*) $NaBH_3CN$, AcOH, 15 °C, 3 h, 91 %; *iv*) $Na_2WO_4 \cdot 2H_2O$, 30 mass % H_2O_2, MeOH/H_2O, –20 °C, 1 h; *v*) K_2CO_3, $(CH_3O)_2SO_2$, 0 °C, 1 h, 81 %; *vi*) $POCl_3$, DMF, 0 °C, 2 h, 77 %; *vii*) $NH_2OH \cdot HCl$, Na_2CO_3, EtOH/H_2O, *XX*, 0 °C, 40 min, 94 %, *XXI*, 0 °C, 40 min, 93 %, *XXII*, 50 °C, 15 min, 90 %; *viii*) *II*, *XXIV*, $NiCl_2 \cdot 6H_2O$, $NaBH_4$, MeOH/H_2O, *XXIII*, $NaBH_3CN$, $AcONH_4$, 30 % $TiCl_3$, MeOH/H_2O, 0 °C, 15 min; *ix*) Et_3N, CS_2, MeOH, CH_3I, at, 20 min, *II*, 50 %, *XXIII*, 75 %, *XXIV*, 66 %; *x*) Br_2, CH_2Cl_2–MeOH (φ_r = 9 : 1), Et_3N, at, 20 min, *XIII*, 80 %, *XIV*, 89 %.

5-Bromo-1*H*-indole (*VIII*), which was used as a starting compound of the third synthetic route C, was reduced by a reaction with sodium cyanoborohydride in acetic acid to afford 5-bromoindoline (*XVII*) as previously described (Chandra & Brown, 2005). Indoline *XVII* was first oxygenated to 5-bromo-1-hydroxyindole by the Somei's tungstate method with a 30 mass % solution of hydrogen peroxide in the presence of sodium tungstate as a catalyst and 5-bromo-1-methoxyindole (*XVIII*) was then obtained by the methylation of 5-bromo-1-hydroxyindole with dimethyl sulphate using potassium carbonate

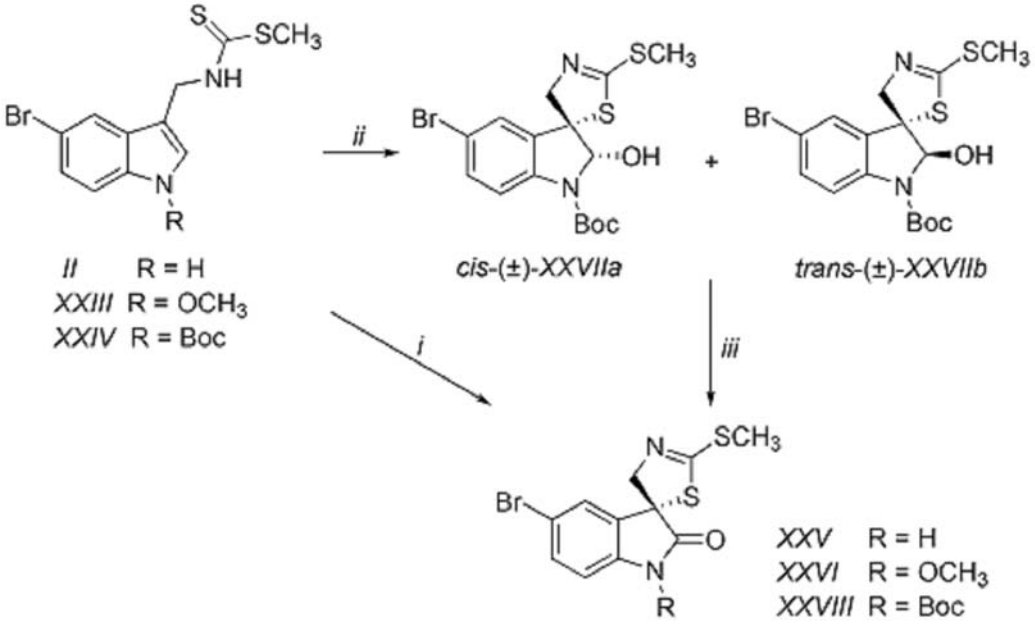


Fig. 5. Synthesis of 5-brominated spirobrassinins *XXV*, *XXVI* and *XXVIII*. Reagents and conditions: *i*) PCC, CH_2Cl_2, at, XXV, 1 h, 62 %, *XXVI*, 2 h, 29 %; *ii*) Br_2, CH_2Cl_2–H_2O ($\varphi_r = 9 : 1$), Et_3N, at, 20 min, 65 %; *iii*) CrO_3, CH_3COOH, at, 1 h, 75 %.

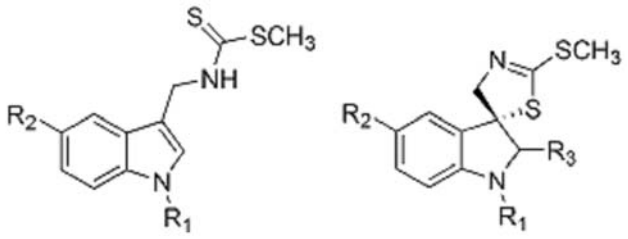
as a base (Somei & Kawasaki, 1989). Aldehydes *XV* (Wang et al., 2001) and *XIX* were synthesised with good yields by the Vilsmeier–Haack reaction (Acheson et al., 1978) of the respective indoles *VIII* and *XVIII* with phosphorus oxychloride in dimethylformamide. 5-Bromo-1-(*tert*-butoxycarbonyl)indole-3-carboxaldehyde (*XVI*) was prepared by the procedure previously described (Wang et al., 2001). The treatment of aldehydes *XV*, *XVI* and *XIX* with hydroxylamine hydrochloride provided the corresponding oximes *XX*–*XXII*, which were obtained as mixtures of (*Z*)- and (*E*)-isomers with high yields (Hanley et al., 1990). The reduction of oximes *XX* and *XXII* with sodium borohydride using catalytic nickel boride (Kutschy et al., 1998) or the reduction of 5-bromo-1-methoxyindole-3-carboxaldehyde oxime (*XXI*) in the presence of sodium cyanoborohydride and titanium catalyst (Pedras & Zaharia, 2000) provided the unstable oily amines which were immediately used as crude products in the next reaction. The treatment of amines with carbon disulphide and iodomethane in methanol in the presence of triethylamine afforded 5-bromobrassinins *II*, *XXIII* and *XXIV* in sufficient amounts (Fig. 4).

Having prepared brassinins *II*, *XXIII* and *XXIV*, the key spirocyclisation was performed with 1.1 eq. of bromine in dichloromethane in the presence of methanol as a nucleophile. As previously suggested, the reaction probably started at the thiocarbamoyl functionality with the formation of sulphenyl bromide A, in which the electrophilic sulphur attacked the 3-position of the indole skeleton to afford a spiroindoleninium intermediate B (Kutschy et al., 2002). Subsequently, the iminium ion B (R = OCH_3, Boc) reacted with the nucleophile (methanol) to afford the target products *XIIIa*, *XIIIb* with an 80 % yield in a 44 : 56 ratio and products *XIVa*, *XIVb* with an 89 % yield in a 29 : 71 ratio (Fig. 4). Due to the low reactivity of the spiroindoleninium intermediate B (R = H) with the nucleophile (Kutschy et al., 2002), 5-bromospirobrassinol methyl ethers were not prepared.

In order to obtain the novel 5-bromo-substituted spirocyclic compounds, 5-bromobrasinnin (*II*) and 5-bromo-1-methoxybrassinin (*XXIII*) were oxidised in a reaction with 7 eq. pyridinium chlorochromate in dichloromethane to afford the racemic products *XXV* and *XXVI* with 62 % and 29 % yields, respectively (Pedras et al., 2006) (Fig. 5). However, the treatment of 5-bromo-1-Boc-brassinin (*XXIV*) with PCC led to the oxidation of sulphur to form *S*-oxid. With the aim of obtaining 5-bromo-1-Boc-spirobrassinin (*XXVIII*), the spirocyclisation of brassinin *XXIV* using bromine in the presence of water at ambient temperature was first performed to afford the diastereoisomers of 5-bromo-1-Boc-spirobrassinol *cis*-(±)-*XXVIIa* and *trans*-(±)-*XXVIIb* with a 65 % yield in a 23 : 77 ratio. The target compound *XXVIII* was subsequently prepared by the oxidation of diastereoisomers *XXVIIa* and *XXVIIb* with CrO_3 in acetic acid with a 75 % yield (Fig. 5).

The structure of the individual target products was unequivocally confirmed by NMR studies, including 2D HSQC, HMBC, COSY and NOESY experiments. In the *cis*-diastereoisomer, the sulphur of the thiazoline ring and the 2-methoxy or 2-hydroxy

Table 4. Anti-proliferative activity of target compounds



Compound	R_1	R_2	R_3	Cell line, $IC_{50}/(\mu mol\ L^{-1})$					
				Jurkat	A-549	MCF-7	MDA	HeLa	CEM
I	H	H	–	> 100	> 100	> 100	> 100	> 100	90.2
II		Br		67.5	86.6	85.6	90.5	> 100	29.4
IX	OCH_3	H	–	37.5	100	100	100	100	63.5
XXIII		Br		59.8	85.0	85.6	79.7	> 100	36.1
X	Boc	H	–	17.8	21.4	23.0	21.4	16.9	19.6
XXIV		Br		NT	NT	> 100	47.9	75.7	NT
cis-(±)-*Xia*	OCH_3	H	OCH_3	57.4	100	100	100	53.2	100
cis-(±)-*XIIIa*		Br		97.1	> 100	> 100	> 100	> 100	69.0
trans-(±)-*XIb*		H		30.2	100	100	100	48.9	100
trans-(±)-*XIIIb*		Br		72.5	> 100	94.5	> 100	> 100	51.1
cis-(±)-*XIIa*	Boc	H	OCH_3	43.4	96.3	100	97.7	77.6	41.9
cis-(±)-*XIVa*		Br		48.0	> 100	41.1	> 100	> 100	34.2
trans-(±)-*XIIb*		H		37.3	70.5	70.2	87.0	74.3	37.9
trans-(±)-*XIVb*		Br		57.0	> 100	> 100	> 100	89.3	33.2
cis-(±)-*XXIXa*	Boc	H	OH	29.8	100	100	95.0	93.6	27.1
cis-(±)-*XXVIIa*		Br		29.2	NT	31.0	70.0	65.7	NT
trans-(±)-*XXIXb*		H		34.0	100	100	82.8	78.0	30.6
trans-(±)-*XXVIIb*		Br		32.1	NT	42.9	75.6	42.2	NT
(±)-*XXX*	H	H	O	63.4	> 100	> 100	> 100	> 100	> 100
(±)-*XXV*		Br		> 100	> 100	> 100	> 100	> 100	> 100
(±)-*XXXI*	OCH_3	H		41.1	> 100	> 100	> 100	> 100	> 100
(±)-*XXVI*		Br		> 100	> 100	> 100	> 100	> 100	> 100
(±)-*XXVIII*	Boc	Br		> 100	> 100	> 100	> 100	> 100	NT
Cisplatin				12.0	12.2	11.4	14.7	7.7	4.4
VP-16 (Etoposide)				1.2	14.3	10.9	21.2	3.9	1.1

The potency of the compounds was determined using the MTT (thiazolyl blue tetrazolium bromide) assay after 72 h incubation of cells and presented as IC_{50} values (concentration of a given compound that decreased the amount of viable cells to 50 % relative to untreated control cells). NT – not tested.

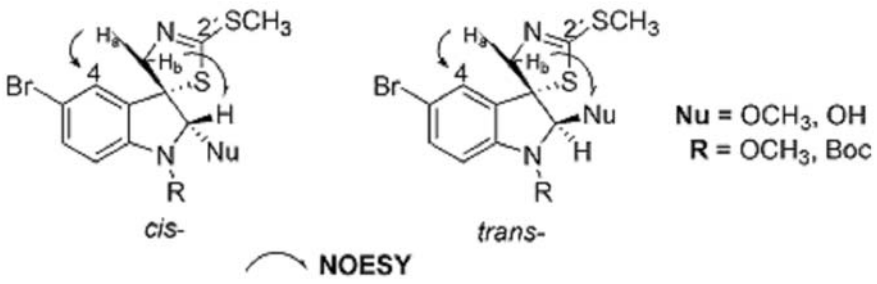


Fig. 6. NOESY interactions confirming *cis*- and *trans*-diastereoisomeric structure.

groups are located on the same sides of the indoline ring, while, in the *trans*-diastereoisomer, the sulphur and 2-methoxy or 2-hydroxy groups are on the opposite sides. In the NOESY spectra, the interaction between H-$4_b'$ and H-2 protons confirms the *cis*-configuration, whereas the interactions between H-$4_b'$ and methoxy or hydroxy protons correspond to the *trans*-diastereoisomers structures (Fig. 6).

The newly synthesised compounds were provided for testing the biological activity on a panel of six human tumour cell lines Jurkat (acute T-lymphoblastic leukaemia), A-549 (non-small cell lung cancer), MCF-7 (mammary gland adenocarcinoma), MDA-MB-231 (mammary gland adenocarcinoma), HeLa (cervical adenocarcinoma) and CEM (acute T-lymphoblastic leukaemia). The potency of these compounds was determined using the MTT (thiazolyl blue tetrazolium bromide) assay after 72 h incubation of cells and is presented in Table 4 as IC_{50} values (concentration of a given compound that decreased the amount of viable cells to 50 % relative to untreated control cells (Mosmann, 1983)). For comparison, Table 4 also includes IC_{50} values for non-brominated brassinins *I*, *X* (Kutschy et al., 1998), *IX* (Kutschy et al., 2009), diastereoisomers of 1-methoxyspirobrassinol methyl ether *cis*-(±)-*XIa*, *trans*-(±)-*XIb* (Kutschy et al., 2002), 1-Boc-spirobrassinol methyl ether *cis*-(±)-*XIIa*, *trans*-(±)-*XIIb* (Budovská et al., unpublished results) and 1-Boc-spirobrassinol *cis*-(±)-*XXIXa*, *trans*-(±)-*XXIXb* (Kutschy et al., 2002), and spirobrassinins *XXX* (Pedras et al., 2006), *XXXI* (Monde et al., 2005) whose syntheses were published previously, except for the synthesis of products *XII*. Next, the biological activities of the standard chemotherapeutic agents, cisplatin and etoposide are reported.

The present study sought to compare the relationship between the structure and biological activity of the new 5-bromo-substituted compounds on the aromatic ring of indole or indoline with the non-brominated derivatives previously discussed. The tested *cis*- and *trans*-diastereoisomers of 5-bromo-1-methoxyspirobrassinol methyl ether (±)-*XIIIa* and (±)-*XIIIb* exhibited a 50 % higher potency of antiproliferative activity over the non-brominated diastereoisomers (±)-*XIa* and (±)-*XIb* on CEM cell line. Unfortunately, these diastereoisomers (±)-*XIIIa* and (±)-*XIIIb* did not demonstrate any activity on the other cancer cell lines. The *cis*- and *trans*-diastereoisomers of 5-bromo-1-(*tert*-butoxycarbonyl) spirobrassinol methyl ether (±)-*XIVa* and (±)-*XIVb* exhibited approximately the same potency as non-brominated diastereoisomers (±)-*XIIa* and (±)-*XIIb* on the CEM cell line. Of all the compounds tested in this report, *cis*-(±)-5-bromo-1-(*tert*-butoxycarbonyl) spirobrassinol (±)-*XXVIIa* exhibited the highest antiproliferative activity on Jurkat cells with an IC_{50} value of 29.2 μmol L^{-1} (Table 4).

The presence of bromine appears to be important for the anticancer activity of brassinins *II* and *XXIII*. 5-Bromobrassinin (*II*) displayed the highest potency of anti-proliferative/cytotoxic activity especially on the CEM cancer cell line with an IC_{50} value of 29.4 μmol L^{-1} relative to brassinin (*I*), where the IC_{50} value was 90.2 μmol L^{-1}. Further, 5-bromo-1-methoxybrassinin (*XXIII*) demonstrated a higher activity with an IC_{50} value of 36.1 μmol L^{-1} than the non-brominated brassinin *IX* with an IC_{50} = 63.5 μmol L^{-1} on the CEM cell line. The 5-bromo-1-substituted spirobrassinins *XXV*, *XXVI* and *XXVIII* tested did not exhibit any anti-proliferative/cytotoxic activity. Ultimately, the newly prepared compounds were not as effective as the standard chemotherapeutic agents, cisplatin and etoposide, respectively.

Conclusions

New 5-brominated spirobrassinol methyl ethers and related 5-bromo derivatives of indole and spiroindoline phytoalexins were designed and synthesised. Their synthesis was based on either the electrophilic substitution of the aromatic core of indoline in the presence of various brominating agents or the products were obtained from 5-bromoindole following the sequence for the preparation of 1-methoxyspirobrassinol methyl ether from indoline. The anti-proliferative activity of the synthesised compounds against selected human tumour cell lines was examined. Substance *XXVIIa* displayed the highest anti-proliferative activity on Jurkat cells and compound *II* on the CEM cancer cell line. The presence of bromine on the aromatic ring of indole or indoline at the C-5 position resulted in the partial increase in anticancer activity relative to non-brominated compounds on leukaemia cell lines.

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