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-bromospiroindolinone 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 1H and 13C NMR spectroscopy, including HSQC, HMBC, COSY, NOESY and DEPT measurements.

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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-catabalising 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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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$_3$)$_2$CO 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 $\delta$ 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