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Synthesis and biological evaluation of 7-azaindole derivatives, synthetic cytokinin analogues

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Abstract—Cytokinins, N6-substituted adenine derivatives, are plant hormones playing important roles in various processes in plant development. Furthermore, cytokinins and their derivatives are able to control mammalian cell apoptosis and differentiation. The aim of our study was the synthesis of 7-azaindole derivatives as cytokinin analogues with the Hartwig–Buchwald coupling reaction in order to evaluate their biological properties on human myeloblastic leukaemia cells (HL-60 cell line). All these compounds presented a cytotoxic activity on HL-60 cells especially the 4-phenylaminopyrrolo[2,3-*b*]pyridine and the 4-phenethylaminopyrrolo [2,3-*b*]pyridine.

Cytokinins are one of the known classes of plant growth regulators, also called phytohormones. They play a major role in many different processes in plant development[1] including cell growth and division control, and the plant's cell differentiation with auxins.[2] Moreover, they regulate the storage of various metabolites as alkaloids. All naturally occurring cytokinins are N6-substituted adenine derivates which contain an aromatic ring or an isoprenic chain in N6 position (Fig. 1).

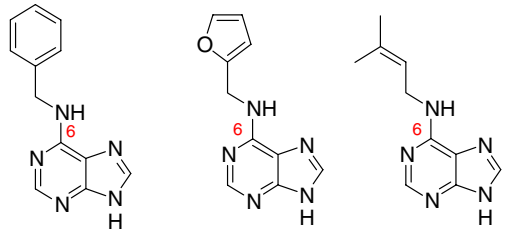

Figure 1. Structures of three natural cytokinins.

Due to their important role in plant development, natural cytokinins have been tested for their activity on animal cells. Cytokinins and cytokinin nucleosides control differentiation and apoptosis of human myeloid leukaemia cells.[3] These results were likewise obtained on various human cancer cell lines with certain cytokinin analogues.[4]

Cytokinins and their derivatives exert their biological effects on several levels. Certain 6-alkynyl- and 6-alkenylpurines have a profound inhibiting effect on 15-lipoxygenase which is implicated in the development of atherosclerosis.[5] Their antiproliferative and pro-apoptotic effects are related to their capacity to inhibit the cyclin-dependent kinase proteins (CDK) especially CDK1 and CDK2.[6]

As we are interested in heterocyclic compounds with potential pharmacological value, we decided to synthesise new cytokinin analogues where the purine skeleton was replaced by 7-azaindole. Our objective was to determine whether the nitrogen atom of the purine was required for cytokinin-like activity. Here, we describe the biological activity of eight 4-substituted pyrrolo[2,3-*b*] pyridine compounds prepared via Hartwig–Buchwald coupling.[7–9]

Keywords: Cytokinin; 7-Azaindole; N-Arylamination; HL-60 cell line.
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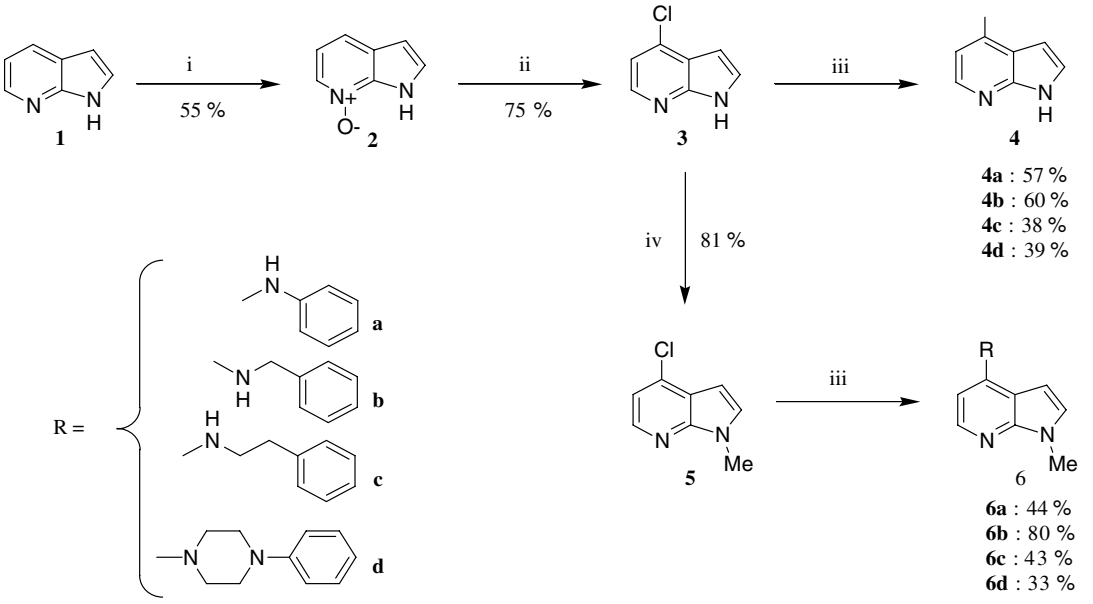

Scheme 1. Synthesis of 4-arylamino-substituted 7-azaindoles. Reagents and conditions: (i) *m*-CPBA, AcOEt, rt; (ii) MsCl, DMF, 70 °C; (iii) corresponding amine RH, $Pd_2(dba)_3$, *t*-BuONa, Xantphos, Dioxane, sealed tube; (iv) NaH, CH_3I, DMF, rt.

All compounds were synthesised from 4-chloro-7-azaindole **3** (Scheme 1). The compound **3** was prepared in two steps from 7-azaindole **1**.[10,11] The first step involved an N-oxidation with *m*-CPBA in ethyl acetate to provide **2**. This latter was then treated with methanesulfonyl chloride at 50 °C in *N*,*N*-dimethylformamide to give 4-chloro-1*H*-pyrrolo[2,3-*b*]pyridine **3** in 75% yield. Treatment of **3** with methyl iodide in the presence of sodium hydride led to product **5**. Then compounds **4a–d** and **6a–d** were obtained with moderate to good yields (33–80%), by the condensation of N-substituted 4-chloropyrrolo[2,3-*b*]pyridine **3** or **5** with arylamines in the presence of 2% $Pd_2(dba)_3$ and Xantphos as the ligand and *t*-BuONa as base in these reactions.[12]

Compounds **4a–d** and **6a–d** were evaluated in vitro for their antiproliferative activity using the human myeloblastic leukaemia HL-60 cell line.[13,14] The results expressed as IC_{50} (concentration reducing cell proliferation by 50%) are reported in Table 1. Cell cycle perturbations are shown in Figure 2.

Table 1. Characteristics and pharmacological activity of synthesised compounds

Compound	Formula	IC_{50} (μM)
Kinetin	$C_{10}H_9N_5O$	21.9
BAP	$C_{12}H_{11}N_5$	>100
4a	$C_{13}H_{11}N_3$ HCl	23.5
4b	$C_{14}H_{12}N_3$ HCl	12.6
4c	$C_{15}H_{15}N_3$ HCl	23.7
4d	$C_{17}H_{18}N_4$ HCl	23.6
6a	$C_{14}H_{13}N_3$ HCl	>100
6b	$C_{15}H_{15}N_3$ HCl	>100
6c	$C_{16}H_{17}N_3$ HCl	>100
6d	$C_{18}H_{20}N_4$ HCl	>100

Briefly, the cells were maintained in RPMI 1640 medium supplemented with 10% foetal bovine serum, 1% penicillin G/streptomycin, 1% L-glutamine and 0.2% β-mercaptoethanol at 37 °C in a humidified atmosphere of 5% CO_2 in air. Cell lines were incubated at 37 °C with different concentrations of 7-azaindole derivatives.

After 72-h incubation, viable cell number was determined by Trypan blue exclusion, and the concentrations lethal for 50% of the tumour cells (IC_{50}) were estimated from dose–response curves.

The IC_{50} values show interesting cytotoxic properties of compound **4b**, in contrast to derivatives **6a–d** which displayed no activity in this assay. On the other hand, the activities of **4a**, **4c**, **4d** and kinetin were similar. Overall the results show that the replacement of the purine ring by 7-azaindole led to an increased inhibitory activity compared to BAP.[3,4]

After 72 h of culture, the cellular cycle was analyzed with flow cytometry (Facscalibur®, Becton–Dickinson, Le Pont de Claix, France) after cell incubation with propidium iodide for 30 min at 37 °C. The apoptosis was evaluated by pre-G1 hypoploidy and the cellular proliferation by S/G2/M fraction.

Figure 2 presents a representative experiment studying the effects of three different concentrations of analogues on the cell cycle of HL-60 cells. The cells were cultured in 25-cm^2 tissue culture flasks at 37 °C in 5% CO_2 humidified air. HL-60 cells were cultured at a density of 5×10^5 cells/mL in RPMI-1640 medium with 20 μmol/L glutamine (Life Technologies, Cergy-Pontoise, France) supplemented with 15% heat-inactivated foetal calf serum (Life Technologies), 100 U/mL of penicillin G and 100 μg/mL of streptomycin (Boehringer–

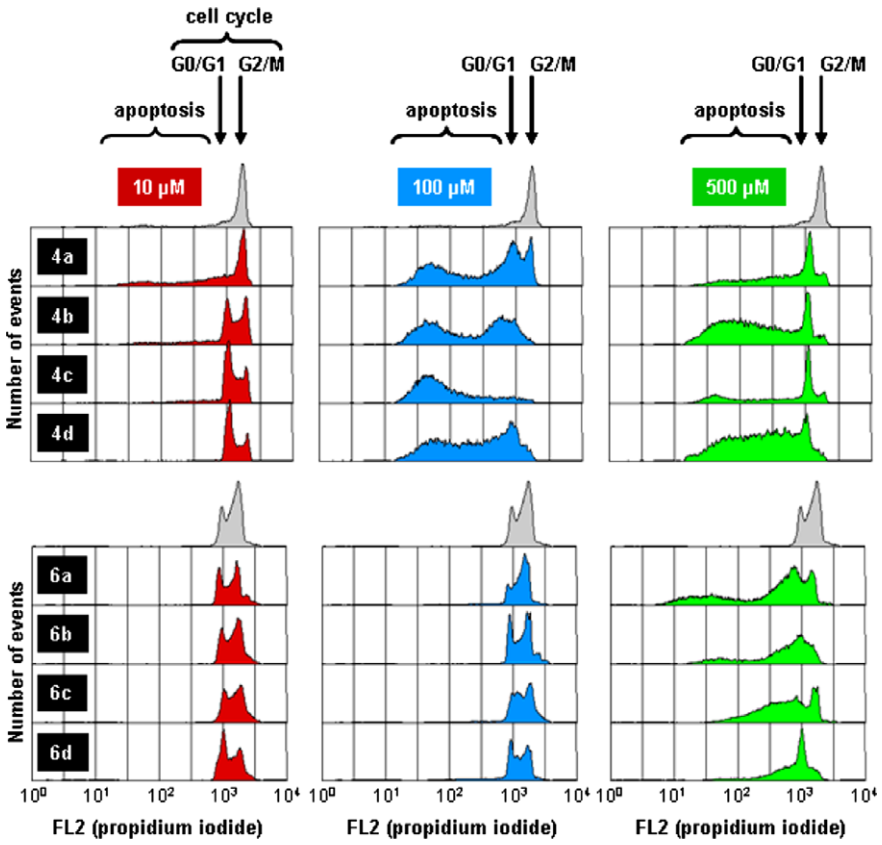

Figure 2. Effects of compounds **4a**–**d** and **6a**–**d** on HL-60 cell apoptosis and cellular cycle.

Mannheim, Mannheim, Germany). The cells were incubated with three different concentrations (10, 100 and 500 µM) of synthesised analogues. The cell cycle status and apoptosis was studied using propidium iodide (PI) for nuclear staining. PI is a fluorogenic compound that binds stoichiometrically to nucleic acids so that fluorescence emission is proportional to the DNA content of a cell. When apoptotic cells are stained with PI and analyzed with a flow cytometer, they display a broad hypodiploid (sub-G1) peak, which can be easily discriminated from the narrow peak of cells with normal (diploid) DNA content in the red fluorescence channels.[15] Propidium iodide staining was realized using Cycle Test™ kit (BD-Biosciences, Le Pont de Claix, France) according to the manufacturer's instructions. All cytometric analyses were performed with a 488-nm laser flow cytometer. In comparison to untreated cells (grey histogram), the three different concentrations used in these experiments provided an evaluation of the biological effects of the synthesised analogues.

All of these compounds were anti-proliferative as shown by the decrease of the S/G2/M fraction. *N*-Methyl-substituted analogues showed a pro-apoptotic effect at concentrations of 500 µM, whereas NH analogues had this effect at 10 µM. The 1*H*-4-(phenylamino)-pyrrolo[2,3-*b*]pyridine **4a** increased the pre-G1 fraction by 39.9% versus 19.9% without cytokinin.

In summary, we have prepared eight new cytokinin analogues where the purine ring of benzylaminopurine (BAP) was replaced with 7-azaindole. The biological tests showed that the NH group is essential for potent activity and the benzylamino substitution was the optimal structure. At the present time these structures are being submitted to further biological tests in order to determine their precise action on the cell cycle.

Acknowledgment

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References and notes

1. Mok, D. W.; Mok, M. C. *Annu. Rev. Plant. Physiol. Plant. Mol. Biol.* **2001**, *52*, 89.
2. Yahia, A.; Kevers, C.; Gaspar, T.; Chenieux, J. C.; Rideau, M.; Creche, J. *Plant Sci.* **1998**, *133*, 9.
3. Ishii, Y.; Hori, Y.; Sakai, S.; Honma, Y. *Cell Growth Differ.* **2002**, *13*, 19.
4. Dolezal, K.; Popa, I.; Kristof, V.; Spichal, L.; Fojtikova, M.; Holub, J. *Bioorg. Med. Chem.* **2006**, *14*, 875.
5. Bråthe, A.; Andresen, G.; Gundersen, L. L.; Malterud, K. E.; Rise, F. *Bioorg. Med. Chem.* **2002**, *10*, 1581.
6. Vermeulun, K.; Strnad, M.; Krystof, V.; Havlicek, L.; van der Aa, A.; Lenjou, M. *Leukemia* **2002**, *16*, 299.
7. Hartwig, J. K. *Angew. Chem., Int. Ed.* **1998**, *37*, 2046.
8. Wolfe, J. P.; Tomori, H.; Sadighi, J. P.; Yin, J.; Buchwald, S. L. . *J. Org. Chem.* **2000**, *65*, 1158.

9. Garnier, E.; Audoux, J.; Pasquinet, E.; Suzenet, F.; Poullain, D.; Lebret, B.; Guillaumet, G. *J. Org. Chem.* **2004**, *69*, 7809.
10. Benoit, S.; Gingras, S.; Soundararajan, N. PCT WO 03082289, 2003.
11. Cheng, C.-C.; Chang, C.-P.; Yu, W.-S.; Hung, F.-T.; Liu, Y.-I.; Wu, G.-R.; Chou, P.-T. *J. Phys. Chem.* **2003**, *107*, 1459.
12. *Typical procedure for Pd-catalysis*. A 100-mL oven-dried resealable flask capped with a rubber septum was evacuated and backfilled with Argon. The flask was charged with 4-chloro-1*H*-pyrrolo[2,3-*b*]pyridine (**3**) (0.131 mmol), sodium *tert*-butoxide (0.367 mmol), Pd_2dba_3 (0.00262 mmol), Xantphos (0.00524 mmol), and evacuated and backfilled with Argon. 1,4-Dioxane (10 mL) and *N*-alkylamine (0.393 mmol) were added and Argon was bubbled through the mixture for 20 min. The septum was replaced with a Teflon screwcap, the flask was sealed and the mixture was heated at 100 °C for 24 h. The mixture was cooled to room temperature, diluted with ethyl acetate (50 mL), filtered through Celite and concentrated in vacuo. The resulting oil was dissolved in dichloromethane (25 mL), washed twice with water, dried over Na_2SO_4, filtered and concentrated in vacuo. The crude material was purified by flash column chromatography (ethyl acetate/light petroleum) on silica gel to give the desired 4-substituted pyrrolo[2,3-*b*]pyridine (**4a–d**) and (**6a–d**) as solid. After, the HCl salts were prepared from the corresponding compound in hydrogen chloride 2.0 M solution in diethyl ether for 2 h at rt.
13. Gallagher, R.; Collins, S.; Trujillo, J.; McCredie, K.; Ahearn, M.; Tsai, S.; Metzgar, R.; Aulakh, G.; Ting, R.; Ruscetti, F.; Gallo, R. *Blood* **1979**, *54*, 713.
14. Dalton, W. T., Jr.; Ahearn, M. J.; McCredie, K. B.; Freireich, E. J.; Stass, S. A.; Trujillo, J. M. *Blood* **1988**, *71*, 242.
15. Nicoletti, I.; Migliorati, G.; Pagliacci, M. C.; Grignani, F.; Riccardi, C. *J. Immunol. Methods* **1991**, *139*, 271.