Palacký University Olomouc

Faculty of Science

Laboratory of Growth Regulators & Chemical Biology and Genetics

Department

Martin Hönig

Summary of the Doctoral Thesis

Synthesis and study of mechanism of action of new cytokinin derivatives

P1527 Biology

1501V019 Experimental biology

Supervisor

Mgr. Lucie Plíhalová, Ph.D.

Olomouc

2019

This PhD. thesis was carried out in the Laboratory of Growth Regulators & Centre of the Region Haná for Biotechnological and Agricultural Research within the framework of internal Ph.D. Study of Experimental Biology, guaranteed by the Laboratory of Growth Regulators, Faculty of Science, Palacký University in Olomouc, between the years 2016-2019.

Ph.D. candidate: **Mgr. Martin Hönig**

Supervisor: **Mgr. Lucie Plíhalová, Ph.D.**
Laboratory of Growth Regulators, Centre of the Region Haná for Biotechnological and Agricultural Research, Palacký University & Institute of Experimental Botany ASCR, Olomouc, Czech Republic

Opponents: **RNDr. Radomíra Vaňková, CSc.**
Laboratory of Hormonal Regulations in Plants, Institute of Experimental Botany ASCR, Prague, Czech Republic

Suresh Rattan, Ph.D., Dr.scient
Department of Molecular Biology and Genetics,Aarhus University, Aarthus, Denmark

prof. RNDr. Vojtěch Adam, Ph.D.
Department of Chemistry and Biochemistry, Mendel University in Brno, Brno, Czech Republic

The evaluation of this Ph.D. thesis was written by **Prof. Ing. Miroslav Strnad, CSc. DSc.**, Laboratory of Growth Regulators, Faculty of Science, Palacky University in Olomouc.

The oral defence will take place on **26.8.2019** before the Commission for the Ph.D. thesis of the Study Program Experimental Biology, **room…….**, Šlechtitelů 27, Olomouc – Holice.

The PhD. thesis and expert reviews will be available 14 days before the defence in the Study Department of Faculty of Science (Mgr. M. Karásková), Palacký University, 17. listopadu 12, Olomouc.

After the defense, the Ph.D. thesis will be stored in the Library of the Biological Departments of Faculty of Science, Palacky University, Šlechtitelů 27, Olomouc – Holice.

Prof. Ing. Miroslav Strnad, CSc. DSc.
Chairman of the Commision for the Ph.D. thesis,
Study Program Experimental Biology,
Faculty of Science, Palacký University in Olomouc

Content

Introduction

Cytokinins (CKs) are a class of plant hormones that affect various aspects of plant growth and development, such as, cell division, leaf senescence, response to biotic and abiotic factors and many others (Kieber and Schaller, 2014). Since their discovery in the 1950s and synthesis of Kin by Miller *et al*. (1955), a wide range of CKs have been synthesized (Matsubara, 1980; Plíhalová et al., 2016; Skoog et al., 1967). Prepared compounds were usually tested for CK activity in various bioassays such as callus proliferation assays, pigment formation assays, leaf senescence delaying assays and many others, in various plant species (Gyulai and Heszky, 1994). Biological data from classical CK assays and later on from CK response (Romanov et al., 2002) and receptor binding bacterial (*E. coli*) assays (Spíchal et al., 2004) enabled researchers to start establishing complex structure activity relationships (SAR). From these, highly active CK derivatives were synthesized and utilized in agriculture (Koprna et al., 2016) or in tissue culture (Aremu et al., 2012a, 2012b; Madzikane-Mlungwana et al., 2017; Moyo et al., 2018).

In the 1990s CK 6-furfurylaminopurin (kinetin, Kin) was found to affect human fibroblasts (Rattan and Clark, 1994). Later on CKs, were shown to possess antioxidant properties in various animal cells and influence the activity of several antioxidant enzymes (Jabłońska-Trypuć et al., 2016; Olsen et al., 1999). A life-prolonging effect of CK was found in an *in-vivo* experiment with *Zaprionus fruitflies* (Sharma et al., 1997).

Kin derivative 6-furfurylamino-9-(2-tetrahydropyran-2-yl)purine was prepared by the introduction of a tetrahydropyranyl group to the N9 atom of Kin moiety (Szüčová et al., 2016). This compound possessed high activity in wheat leaf senescence assay (WLSA) and also delayed the senescence of human fibroblasts (Szüčová et al., 2016). Moreover, topical application to the human face improved the appearance of photodamaged skin (McCullough et al., 2008).

This work deals with the preparation and biological properties of Kin derivatives and/or analogues with a modified furfuryl ring including compounds accompanied by either THF (tetrahydrofuran-2-yl) or THP (tetrahydropyran-2-yl) protective groups. Prepared compounds were primarily studied for their antisenescent activity in plants and protective properties in human skin cells.

Besides CK conjugates with two subcomponents of hyaluronic acid, D-glucuronic acid and N-acetyl-D-glucosamine were also prepared to test their biological activity as the conjugation of CKs with different sugars in the N9 position of the purine moiety can dramatically change their activity including increased efficiency in antisenescent tests (Doležal et al., 2018, 2007; Holub et al., 1998).

Aims and scope

A large number of CK derivatives with various structural modifications of adenine moiety have been prepared to date. Even small structural change can lead to complete loss of biological activity. Hence well-described structure-activity relationship is necessary for the preparation of highly active compounds. New CK derivatives were prepared and their biological activity was studied to reveal highly active molecules with possible practical application.

The overall aims of this doctoral thesis are:

- Preparation of Kin derivatives and/or analogues with modification of the furfuryl ring.
- Preparation of N9-purine substituted kinetin-like compounds with THF and THP protection groups.
- Preparation of CK sugar conjugates.
- Formulation of structure-activity relationship and identification of key structural features.
- Evaluation of the biological activity of newly prepared compounds in both plants and animals.

Material and methods

General procedure

The chromatographic purity and mass spectra of the prepared compounds were analyzed using the HPLC-PDA-MS method. Samples (10 µl of 3.10^{-5} M in 1% methanol) were injected onto a reverse-phased column (Symmetry C18, 5 µm, 150 mm × 2.1 mm; Waters, Milford, MA, USA) incubated at 25 °C. Solvent (A) consisted of 15 mM ammonium formate adjusted to pH 4.0. The solvent (B) consisted of methanol. The flow-rate was set to 200 µL/min. A binary gradient was used: 0 min, 10 % of B; 24 min; 90 % of B; 34 min; 90 % of B; 45 min; 10 % of B using the *Waters* Alliance *2695* Separations *Module* (Waters, Manchester, UK). The effluent was then introduced to the Waters 2996 PDA detector (Waters, Manchester, UK) (scanning range 210–700 nm with 1.2 nm resolution) and a tandem mass analyser *Q-Tof micro Mass* Spectrometer (Waters, Manchester, UK) with an electrospray. The cone voltage was set to 20 V. Analyses were performed in positive mode (ESI+) or negative mode (ESI-) therefore molecular ions were recorded as $[M + H]^+$, $[M-H]^-$ or ESI adduct ions. ^{1}H NMR spectra were measured on a Jeol 500 SS spectrometer operating at a temperature of 300 K and a frequency of 500.13 MHz The samples were prepared by dissolving the compounds in DMSO-d_6. Tetramethylsilane (TMS) was used as an internal standard. Thin-layer chromatography (TLC) was carried out using silica gel 60 WF_{254} plates (Merck). $CHCl_3$/MeOH (9:1, v/v) or EtOAc/MeOH/NH_3 (34:4:2, v/v) were used as the mobile phase. Purification *via* column chromatography was carried out using silica gel Davisil R LC60A 40-63 micron.

Synthetic procedure I

6-chloro-9-(tetrahydrofuran-2-yl)purine (**A**) or 2,6-dichloro-9-(tetrahydrofuran-2-yl)purine (**B**) were prepared according to modified protocols from Szüčová et al. (2009) and Plíhalová (2016), respectively. Only 1.5 equivalents of 2,3-dihydrofurane were used in both reactions (instead of 2.5 eq.) which allowed subsequent crystallization in EtOH at -20 °C. Thanks to this modification, a pale yellow solid compound **A** was obtained..

In the next step, 6-chloropurine, **A** or **B** was refluxed with the appropriate amine (1.2 eq.) in the n-propanol with an excessive amount of triethylamine (3 eq) for 4-6 hours as summarized in the reaction scheme (Fig. 1). The crude reaction mixture was then concentrated *in vacuo* and the product was extracted into EtOAc using liquid-liquid extraction (water : EtOAc). The ethyl

acetate phase was then dried over $MgSO_4$ and concentrated under vacuum. The crystallization technique as well as column chromatography were used for final product purification and are described in detail in Hönig et al (2018, **I**)

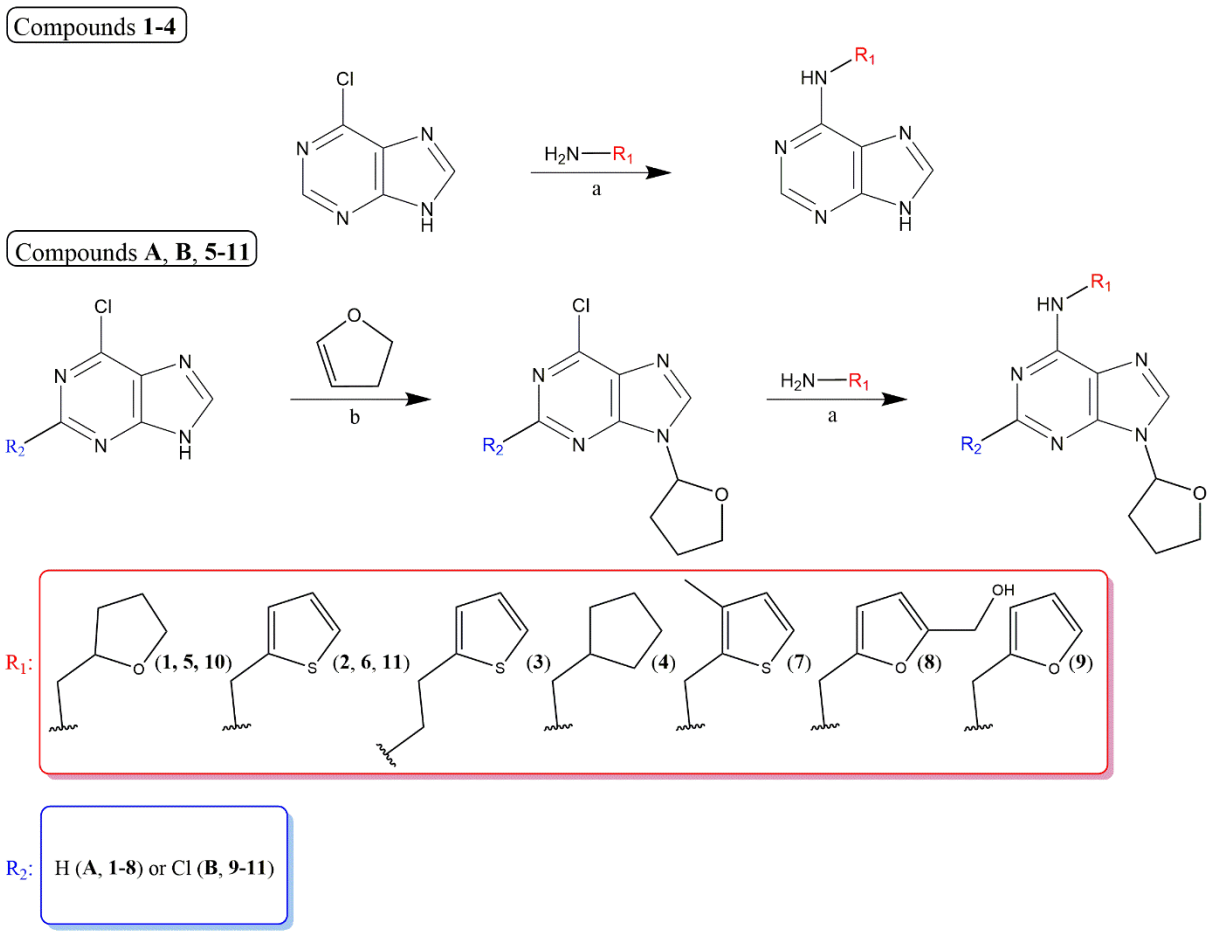

Figure 1 *Reaction scheme for the synthesis of prepared 6-substituted, 6,9-disubstituted and 2,6,9-trisubstituted purine derivatives with 9-THF protecting group published in Hönig et al., (2018,* ***I****). a) Et3N, propanol, 100 °C, 4 h (**1**, **2**, **5**, **9**, **10**), 5 h (**3**, **4**, **6**, **8**) and 6 h (**7**); b) EtOAc, CF3COOH, NH3, RT, 3.5 h.*

Synthetic procedure II

6-chloro-9-(tetrahydropyran-2-yl)purine (**C**) or 2,6-dichloro-9-(tetrahydropyran-2-yl)purine (**B**) were prepared according to modified protocols as published in Szücová et al. (2009) and Bíbová et al. (2018) respectively. A smaller equivalent of 3,4-dihydropyran (1.5 instead of 2.5) was used which allowed us to obtain **C** as a solid compound after crystallization in EtOH at -20 °C overnight.

In the following step, 6-chloropurine, 2,6-dichloropurine, **C** or **D** was refluxed with appropriate amine (1.2 eq.) and excessive amount of triethylamine (3 eq.) for 5 h in n-propanol. The crude reaction mixture was then concentrated *in vacuo* and the product was extracted into EtOAc using liquid-liquid extraction (water : EtOAc). The ethyl acetate phase was then dried over $MgSO_4$ and concentrated under vacuum. Crystallization in different solvents or solvent mixtures was preferably used as a last purification step. The reaction scheme (Fig. 2) and reaction conditions are described in detail in the section Unpublished results.

Preparation of 6-(thiophen-2-ylmethylamino)-9-(tetrahydropyran-2-yl)purine (**14**) has been described in the thesis of Ryšavá (2019). However, Ryšavá used a longer reaction time and utilized diethyl ether instead of ethanol for final crystallization to obtain a compound of similar purity and yield.

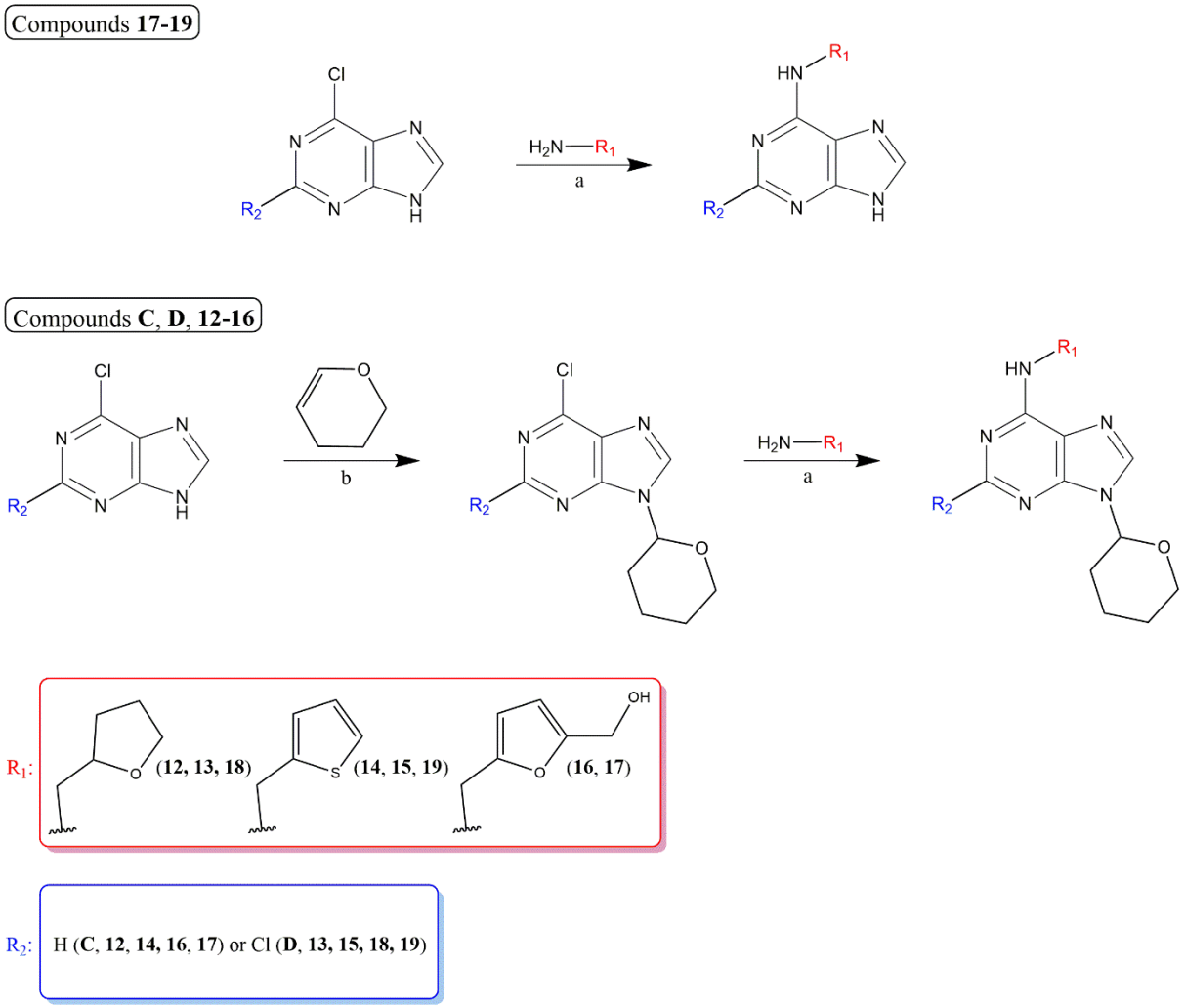

Figure 2 *Reaction scheme for the synthesis of prepared 6-substituted, 6,9-disubstituted and 2,6,9-trisubstituted purine derivatives with 9-THP protecting group. a) Et3N, propanol, 100 °C, 5 h; b) EtOAc, CF3COOH, NH3, RT, 3.5 h (**C**) overnight (**D**)*

Synthetic procedure III

Acetylation of N-Acetyl-D-glucosamine was performed as described in Kong et al. (2016) for the acetylation of N-Acetyl-D-mannosamine using acetic anhydride in pyridine. Peracetylated N-Acetyl-D-glucosamine was attached to the purine moiety as described in Ando et al. (2007) for reaction of 6-chloropurine with 1,2,3,4,6-penta-O-acetyl-β-D-glucose. However, crystallization was preferably used for product purification instead of silica gel column chromatography. The synthetic procedure is summarized in Fig. 3 and described in more detail in the section Unpublished results of the thesis.

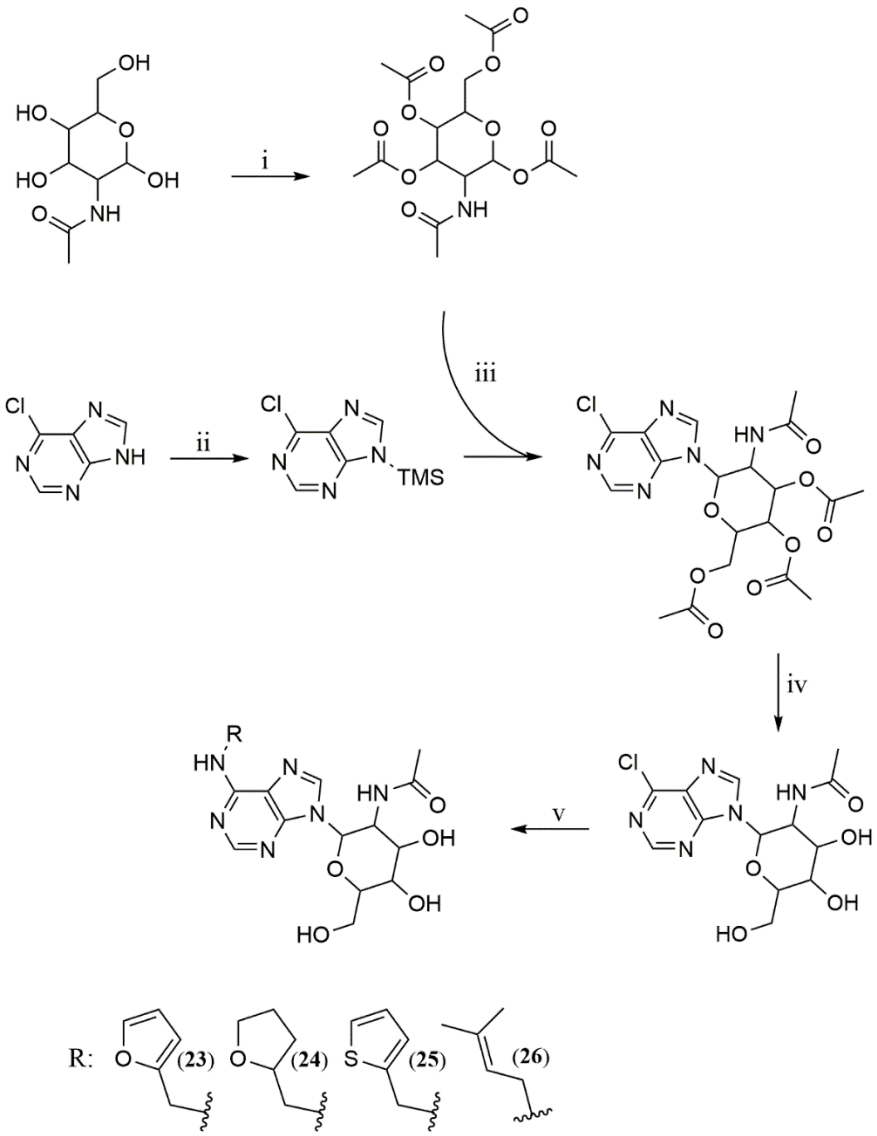

Figure 3 *Preparation of N-acetylglucosamine substituted cytokinins. i) Ac_2O, Py, 4h, rt.; ii) BSA, DCE, 0.5h, reflux; iii) TMSOTf, 5h, reflux; iv) 7N ammonia in MeOH, 3h; v) Et_3N, n-propanol, R-NH_2.*

Synthetic procedure IV

Protection of the D-glucuronic acid carboxyl group by the allyl group and its subsequent one-step removal was performed as described in Alaoui et al. (2006). Hydroxyl group protection and further reaction with 6-chlorpurine was performed as described in Synthetic procedure III. The synthetic route is summarized in Fig. 4 and described in more detail in section Unpublished results of the thesis.

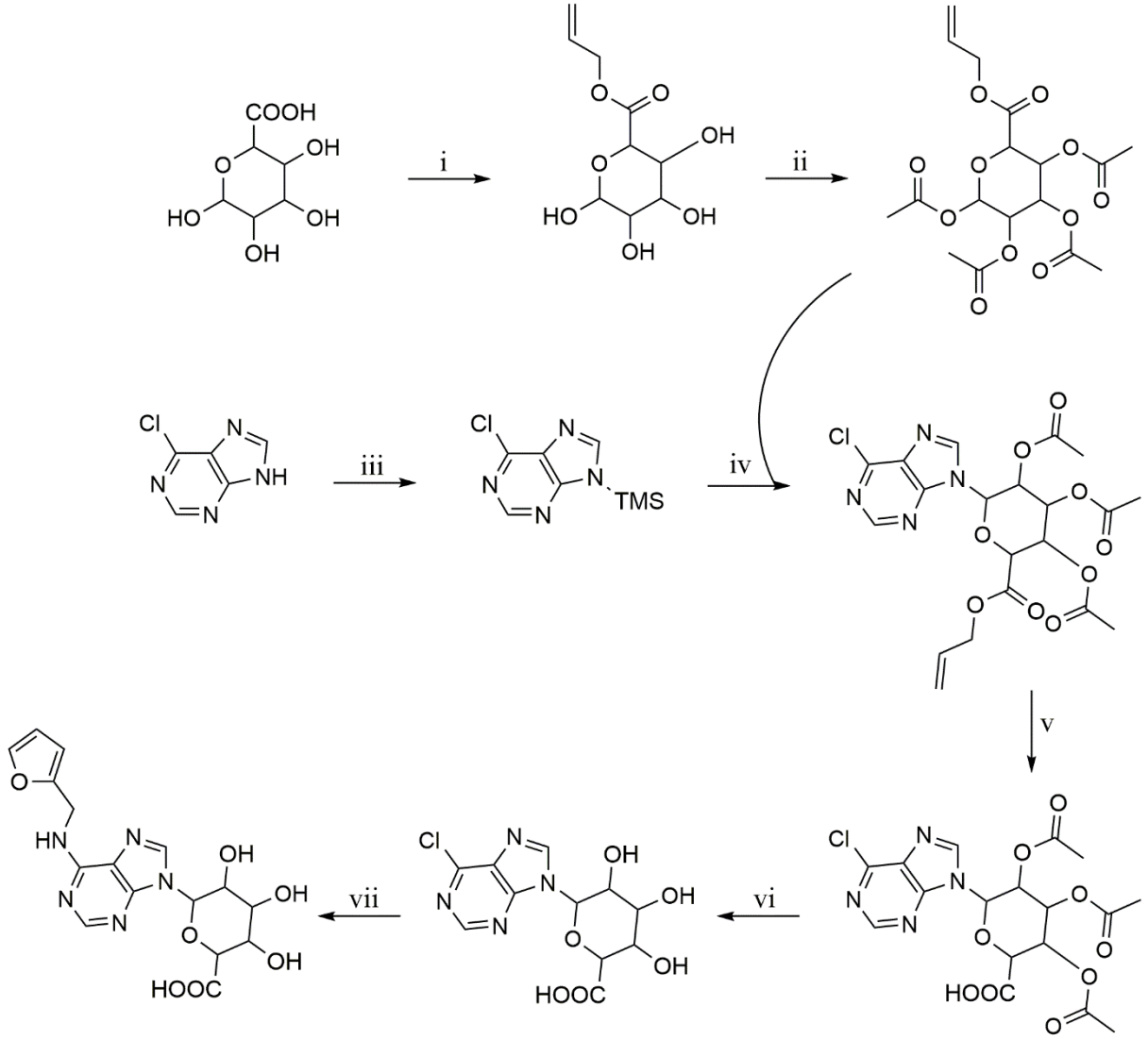

Figure 4 *Preparation of D-glucuronic acid substituted Kin. i) AllBr, DBU, DMF, overnight, rt.; ii) Ac_2O, Py, 5h, rt.; iii) BSA, DCE, 0.5h, reflux; iv) TMSOTf, 3h, reflux; v) $Pd(PPh_3)_4$, THF, 2h, ice; vi) 7N ammonia in MeOH, 3h; vii) furfurylamin, Et_3N, n-propanol, reflux. Abbreviations: AllBr, Allyl bromide; BSA, Bis(trimethylsilyl)acetamide; DCE, Dichloroethane; DBU, 1,8-Diazabicyclo[5.4.0]undec-7-ene; Pd(PPh3)4, Tetrakis(triphenylphosphine)palladium(0); Py, Pyridine; TMSOTf, Trimethylsilyl trifluoromethanesulfonate*

pH stability

In order to evaluate the stability of prepared compounds due to the pH lability of 9-substituted THF group, the pH stability test was slightly modified and performed according to the literature (Szüčová et al., 2009). The pH stability of 6-(tetrahydrofuran-2-ylmethylamino)-9-(tetrahydrofuran- 2-yl)purine (**5**), 6-(thiophen-2-ylmethylamino)-9-(tetrahydrofuran-2-yl) purine (**6**) and 2-chloro-6-furfurylamino-9-(tetrahydrofuran-2-yl)purine (**9**) was analysed by HPLC-PDA (System Gold; Beckman Instruments, Fullerton, CA, USA); analytes were detected at 270 nm using a PDA detector (Beckman System Gold 168). The solution of tested compound (10^{-2} M; DMSO) was prepared and diluted to 10^{-4} M using McIlvaine buffer solution for the appropriate pH (2, 3, 4, 5, 6 or 7). One hour after incubation at 25 °C, 5 μL of the solution was directly injected onto a reversed phase column (Symmetry C18; 5 μm, 150 x 2.1mm; Waters, Milford, USA). At a flow-rate of 0.3 mL/min, the following binary gradient was used: 0 min,10% B; 0-24 min; linear gradient to 90% B; 25-34 min; isocratic elution of 90% B; 35-45 min; linear gradient to 10% B, where A was 15mM formic acid adjusted to pH 4 with ammonium and B was 100% methanol The HPLC measurement of the solutions was repeated after a 24 h incubation at 25 °C.

Biological activity

Evaluation of CK activity in tobacco callus bioassay, amaranthus caudatus betacyanin bioassay and wheat leaf senescence bioassay (WLSA) was carried out according to Hönig et al. (2018, **I**). In the same study, are described the methods used for cytotoxicity measurement *via* resazurin reduction assay on human skin cells as well as methods for phototoxicity and photoprotection assessment. Oxygen radical absorbance capacity evaluation and protocols for oxidative stress bioassays in *Caenorhaditis elegans* are also presented in this study (Hönig et al., 2018; **I**).
Markers of damage caused by UVA and UVB irradiation including ROS production, GSH depletion and Caspase-3-activity were studied as described in (Hönig et al., 2017; **V**).

Survey of published results

Publication I

- Eleven Kin derivatives were prepared and properly characterized using ^{1}H NMR, mass spectrometry combined with HPLC purity determination and elemental C, H, N analyses. The biological activity of new compounds was studied in both plant and animal systems. Cytokinin-like activity was determined in three CK bioassays, such as tobacco callus, wheat leaf senescence assay and Amaranthus bioassay. It was found that despite the saturation of furfuryl substituent in 6-(tetrahydrofuran-2-ylmethylamino)-9-(tetrahydrofuran-2-yl)purine, this compound possessed the highest activity in WLSA. Selected compounds were subsequently tested on normal human dermal fibroblasts (NHDF) and keratinocyte cell lines (HaCaT) to exclude possible phototoxic effects and, on the other hand, to reveal possible UVA and UVB photoprotective activity. Protection against 5- hydroxy-1,4-naphthoquinone (juglone) induced oxidative stress was tested in *Caenorhabditis elegans in vivo*. Compounds 6-(thiophen-2-ylmethylamino)-9-(tetrahydrofuran-2-yl)purine, and 2-chloro-6-furfurylamino-9-(tetrahydrofuran-2-yl)purine, were found to be the most active in human skin cell protection against UVA/UVB irradiation and concurrently possessed highest activity in oxidative stress protection in *C. elegans*. Protection of *C. elegans* and NHDF from oxidative and UV stress indicate antioxidant properties. These compounds did not act as direct radical scavengers in the ORAC assay. The obtained data suggest that the mechanism of photo- and nematode protection against oxidative stress is indirect and triggers other mechanisms of oxidative protection. (Hönig et al., 2018, **I**)

Publication II

- Antisenescent activity of natural CKs and their derivatives together with antioxidant properties in plants were reviewed. The effect on chlorophyll content, photosystem II and other parts of the photosynthetic apparatus was summarized. The influence of CKs on activation of antioxidative enzymes in senescing tissues is described as well as changes in the levels of naturally occurring antioxidants, such as phenolic acids and flavonoids, in plant explants. Emphasis was placed on the structure-activity relationship, and key structural motives necessary for antisenescent activity of CKs were postulated.

The role of different CK receptors and downstream proteins that are involved in the antisenescent and antioxidant activity of CKs were discussed. (Hönig et al., 2018, **II**).

Publication III

- Several fluorescent derivatives of iP were designed and prepared and properly characterized to study the properties of the CK receptor. Several fluorescent labels were used and attached to the C2 or N9 atom of the adenine moiety *via* 2- or 6-carbon linker. All prepared compounds were screened for affinity for the *Arabidopsis thaliana* CK receptor (CRE1/AHK4). Most compounds did not interact with the receptor due to attached label. However two C2-labeled rhodamine B (with 2- and 6-carbon linker) iP and one N9- labeled 4-chloro-7- nitrobenzofurazan (NBD) iP interacted well. Arabidopsis seedlings were used for in planta staining experiments in Arabidopsis thaliana cell suspension culture using live cell confocal microscopy (Kubiasová et al., 2018, **III**).

Publication IV

- The photoprotective and antioxidant properties of CKs and other natural products was reviewed. The antioxidant properties of Kin and its THP derivative in animals especially in human and human skin cells were discussed. These two compounds are currently used in cosmetics for the treatment of photodamaged skin due to their ability to reduce symptoms of skin photoaging such as fine wrinkles, rough skin texture and mottled hyperpigmentation. (Plíhalová et al, 2018, **IV**).

Publications V and VI (patent and patent application)

- N9-THF substituted derivatives published in Hönig et al., (2018, **I**) are part of the granted Czech patent no. 307722 and PCT application WO/2017/036434 (PCT/CZ2016/050029) as substances possessing anti-senescent and UV-photoprotective properties that can be used in cosmetics preparations, plant protection preparations and in preparations for the treatement/application of tissue cultures (Hönig et al., 2017, **V**; Hönig et al., 2019, **VI**). 6-(Tetrahydrofuran-2-ylmethylamino)-9-(tetrahydrofuran-2-yl)purine protected NHDF cells against UVA induced ROS production as well as depletion of endogenous antioxidant GSH. Concurrently, in UVB irradiated NHDF cells treated with 6-(tetrahydrofuran-2-ylmethylamino)-9-(tetrahydrofuran-2-yl)purine, lower activity of protein caspase-3, which is activated during apoptosis was measured (Hönig et al., 2017, **V**; Hönig et al., 2019, **VI**).

Conclusions and perspectives

- A number of new CK derivatives was prepared, including kinetin derivatives and/or analogs with modified furfuryl ring, in combination with some other substitutions, such as THF or -THP groups attached to the N9 atom of the purine moiety. In addition, C2-chloro derivatives of these compounds were prepared as well. The majority of the compounds were prepared for the first time.

- New CK conjugates with N-acetylglucosamine and D-glucuronic acid were prepared for the first time.

- Fluorescently labeled iP and BAP were prepared with Cyanine5 dye attached to N9 atom by a two-carbon linker.

- The prepared compound helped us to establish the SAR of CK derivatives related to their anti-senescent properties.

- Newly prepared derivatives with significant anti-senescent properties are promising for use in agriculture and tissue culture.

- Photoprotective activity against both UVA and UVB radiation of selected prepared compounds as well as the lack of phototoxicity were determined in human dermal fibroblasts and keratinocytes.

- The newly designed and prepared compounds 6-(thiophen-2-ylmethylamino)-9-(tetrahydrofuran-2-yl)purine and 2-chloro-6-furfurylamino-9-(tetrahydrofuran-2-yl)purine were able to protect *Caenorhabditis elegans* against juglone induced oxidative stress. Furthermore, compound 6-(tetrahydrofuran-2-ylmethylamino)-9-(tetrahydrofuran-2-yl)purine protected NHDF cells against UVA induced ROS production and GSH depletion. In contrast, none of the tested compounds was able directly scavenge reactive oxygen species in the ORAC assay.

In summary, standard routes to prepare CK bases and their C2-chloro and/or N9-THF/THP derivatives were optimized to obtain compounds in decent yields and purity. New routes for CK conjugates with N-acetylglucosamine and D-glucuronic acid were implemented as well as the methods for the preparation of Cyanine5 fluorescently labeled CKs.

Photoprotective and antioxidant activities of CK derivatives were shown on human cells and *Caenorhabditis elegans*. It was suggested that the photoprotective activity of these derivatives might be connected with their antioxidant activity, as ROS production is one of the main causes of UVA radiation damage in human skin. However, to clarify the connection between photoprotective and antioxidant properties of prepared CKs, additional molecular and gene expression studies are required and carried out in the near future.

List of publications

This thesis is based on the following papers, which are referred to in the text by corresponding roman numerals. The papers are appended in the Supplement section.

I. **Hönig, M.**; Plíhalová, L.; Spíchal, L.; Grúz, J.; Kadlecová, A.; Voller, J.; Rajnochová Svobodová, A.; Vostálová, J.; Ulrichová, J.; Doležal, K.; Strnad, M. New cytokinin derivatives possess UVA and UVB photoprotective effect on human skin cells and prevent oxidative stress. *Eur. J. Med. Chem.* **2018**, *150*, 946–957, doi:10.1016/J.EJMECH.2018.03.043.

II. **Hönig, M.***; Plíhalová, L.*; Husičková, A.; Nisler, J.; Doležal, K. Role of Cytokinins in Senescence, Antioxidant Defense and Photosynthesis. *Int. J. Mol. Sci.* **2018**, *19*, 4045, doi:10.3390/ijms19124045. * These authors contributed equally to this work.

III. Kubiasová, K.; Mik, V.; Nisler, J.; **Hönig, M.**; Husičková, A.; Spíchal, L.; Pěkná, Z.; Šamajová, O.; Doležal, K.; Plíhal, O.; Benková, E.; Strnad, M.; Plíhalová, L. Design, synthesis and perception of fluorescently labeled isoprenoid cytokinins. *Phytochemistry* **2018**, *150*, 1–11, doi:10.1016/j.phytochem.2018.02.015.

IV. Plíhalová, L.; **Hönig, M.**; Rajnochová Svobodová, A.; Vostálová, J. Cytokininové deriváty jako možné modulátory předčasného stárnutí kůže. *Ref. výb. dermatovenerol*. **2018**, 3, 6-16, ISSN: 1213-9106

V. **Hönig, M.**; Plíhalová, L.; Doležal, K.; Voller, J.; Strnad, M.; Spíchal L.; Vostálová, J.; Rajnochová Svobodová, A.; Ulrichová J.; Kadlecová A.; Plíhal O. Adenine derivatives and their use as UV-photoprotective agens. PCT application WO2017036434A1, **2017**

VI. **Hönig, M.**; Plíhalová, L.; Doležal, K.; Voller, J.; Strnad, M.; Spíchal L.; Vostálová, J.; Rajnochová Svobodová, A.; Ulrichová J.; Kadlecová A.; Plíhal O. Adenine derivatives and their use. Czech Republic. Patent no. 307722, **2019**

Published abstracts

13. dny studentů experimentální biologie rostlin, 7. - 8. 9. 2015, Brno, Česká republika, *Preparation and biological activity of kinetin like aromatic cytokinins* (prezentace)

GR 2016, 3. - 5. 3. 2016, Malá Morávka, Česká republika, *NEW PHOTOPROTECTIVE CYTOKININ DERIVATIVES FOR COSMETICS* (prezentace)

IPGSA Meeting, 21 - 25. 6. 2016 Toronto, Canada, *PREPARATION AND BIOLOGICAL ACTIVITY OF KINETIN LIKE AROMATIC CYTOKININS* (poster)

CBPRS, 21. - 23. 5. 2017, Kouty nad Desnou, Česká republika - *New kinetin derivatives with UVA and UVB photoprotectivity defend Caenorhabditis elegans against oxidative stress* (prezentace)

G4G IV, 19. - 22. 6. 2017, Olomouc, Česká republika - *C2, C6 and N9 substituted kinetin derivatives with UV-photoprotective effects against UVA and UVB radiation* (poster)

CBPRS 2018, 24. - 26. 5. 2018, Luhačovice, Česká republika - *Design of new N9-substituted cytokinins* (prezentace)

PBE 2018, 17. - 21. 6. 2018 Copenhagen, Denmark - *New cytokinin derivatives possess UVA and UVB photoprotective effect on human skin cells and prevent oxidative stress* (poster)

Souhrn (in Czech)

Název disertační práce: Příprava a studium mechanismu účinku nových cytokininových derivátů.

Disertační práce je věnována přípravě nových cytokininových derivátů, studiu jejich biologických účinků a mechanismu jejich působení. Cytokininy jsou skupinou rostlinných hormonů ovlivňující různé aspekty rostlinného růstu a vývoje. Působí mimo jiné na buněčné dělení, ovlivňují senescenci listů, a odpověď rostliny na biotické i abiotické stresové faktory (Kieber and Schaller, 2014).

V rámci disertační práce byla připravena řada C2 a N9 substituovaných cytokininových derivátů, především derivátů kinetinu, s modifikovaným C6 substituentem. Tyto látky byly připraveny již dříve popsanou dvou krokovou syntézou, která byla optimalizována pro potřeby těchto konkrétních cytokininových derivátů. K C2 a N9 substituovaným derivátům byly pro účely studie struktury a aktivity připraveny také odpovídající volné báze.

Dále byly připraveny cytokininové deriváty s N9 substituovaným N-acetylglucosaminem a kyselinou glukuronovou, tedy jednotkami polysacharidu kyseliny hyaluronové.

Připravené látky byly následně otestovány ve standardních cytokininových biotestech (amarantový, kalusový, senescenční) pro určení cytokininové aktivity.

Vybrané deriváty kinetinu byly testovány na normálních lidských kožních fibroblastech (NHDF) a linii keratinocytů (HaCaT) aby bylo možné vyvrátit případné cytotoxické či fototoxické vlastnosti připravených látek a zároveň prostudovat potenciální ochranné účinky. Bylo zjištěno, že dva z připravených derivátů vykazovaly výraznou ochranu kožních buněk proti UVA i UVB záření. Dále byla u připravených derivátů popsána schopnost ochrany modelového organismu *Caenorhabditis elegans* proti uměle vyvolanému oxidativnímu stresu.

Následné studium buněk po ovlivnění UVA i UVB zářením naznačuje, že ochranné účinky těchto látek mohou souviset s jejich antioxidačními vlastnostmi. Mechanismus účinku těchto derivátů je však stále předmětem dalších experimentů, nicméně se již podařilo vyvrátit možnost, že by tyto látky byly přímo schopny interagovat s volnými kyslíkovými radikály.

References

Alaoui, A. El, Monneret, C., Florent, J., 2006. Protecting Groups for Glucuronic Acid : Application to the Synthesis of New Paclitaxel (Taxol) Derivatives 9628–9636.

Aremu, A.O., Bairu, M.W., Doležal, K., Finnie, J.F., Van Staden, J., 2012a. Topolins: A panacea to plant tissue culture challenges? Plant Cell. Tissue Organ Cult. 108, 1–16.

Aremu, A.O., Bairu, M.W., Szüčová, L., Doležal, K., Finnie, J.F., van Staden, J., 2012b. Shoot and root proliferation in "Williams" banana: Are the topolins better cytokinins? Plant Cell. Tissue Organ Cult. 111, 209–218.

Doležal, K., Plíhalová, L., Vylíčilová, H., Zatloukal, M., Plíhal, O., Voller, J., Strnad, M., Bryksová, M., Vostálová, J., Svobodová, A.R., Ulrichová, J., Spíchal, L., 2018. 6-aryl-9-glycosylpurines and use thereof. US 10,100,077.

Doležal, K., Popa, I., Hauserová, E., Spíchal, L., Chakrabarty, K., Novák, O., Kryštof, V., Voller, J., Holub, J., Strnad, M., 2007. Preparation, biological activity and endogenous occurrence of N6-benzyladenosines. Bioorganic Med. Chem. 15, 3737–3747.

Gyulai, G., Heszky, L.E., 1994. Cytokinin Bioassays: A Short Overview. Acta Agron. Hungarica 43, 185–197.

Holub, J., Hanuš, J., Hanke, D.E., Strnad, M., 1998. Biological activity of cytokinins derived from Ortho- and Meta-hydroxybenzyladenine. Plant Growth Regul. 26, 109–115.

Jabłońska-Trypuć, A., Matejczyk, M., Czerpak, R., 2016. N6-benzyladenine and kinetin influence antioxidative stress parameters in human skin fibroblasts. Mol. Cell. Biochem. 413, 97–107.

Kieber, J.J., Schaller, G.E., 2014. Cytokinins. Arab. B. 12, e0168.

Kong, H., Chen, W., Liu, T., Lu, H., Yang, Q., Dong, Y., 2016. Synthesis of NAM-thiazoline derivatives as novel O-GlcNAcase inhibitors. Carbohydr. Res. 429, 54–61.

Koprna, R., De Diego, N., Dundálková, L., Spíchal, L., 2016. Use of cytokinins as agrochemicals. Bioorg. Med. Chem. 24, 484–492.

Madzikane-Mlungwana, O., Moyo, M., Aremu, A.O., Plíhalová, L., Doležal, K., Van Staden, J., Finnie, J.F., 2017. Differential responses to isoprenoid, N 6 -substituted aromatic cytokinins and indole-3-butyric acid in direct plant regeneration of Eriocephalus africanus. Plant Growth Regul. 82, 103–110.

Matsubara, S., 1980. Structure-activity relationships of cytokinins. Phytochemistry 19, 2239–2253.

McCullough, J.L., Garcia, R.L., Reece, B., 2008. A clinical study of topical Pyratine 6 for improving the appearance of photodamaged skin. J. Drugs Dermatol. 7, 131–5.

Moyo, M., Aremu, A.O., Plačková, L., Plíhalová, L., Pěnčík, A., Novák, O., Holub, J., Doležal, K., Staden, J. Van, 2018. Deciphering the growth pattern and phytohormonal content in Saskatoon berry (Amelanchier alnifolia) in response to in vitro cytokinin application. N. Biotechnol. 42, 85–94.

Olsen, A., Siboska, G.E., Clark, B.F.C., Rattan, S.I.S., 1999. N6-Furfuryladenine, Kinetin, Protects against Fenton Reaction-Mediated Oxidative Damage to DNA. Biochem. Biophys. Res. Commun. 265, 499–502.

Plíhalová, L., Vylíčilová, H., Doležal, K., Zahajská, L., Zatloukal, M., Strnad, M., 2016. Synthesis of aromatic cytokinins for plant biotechnology. N. Biotechnol. 33, 614–624.

Rattan, S.I.S., Clark, B.F.C., 1994. Kinetin Delays the Onset of Aging Characteristics in Human Fibroblasts. Biochem. Biophys. Res. Commun. 201, 665–672.

Romanov, G.A., Kieber, J.J., Schmülling, T., 2002. A rapid cytokinin response assay in Arabidopsis indicates a role for phospholipase D in cytokinin signalling. FEBS Lett. 515, 39–43.

Sharma, S.P., Kaur, J., Rattan, S.I.S., 1997. Increased longevity of kinetin-fed Zaprionus fruitflies is accompanied by their reduced fecundity and enhanced catalase activity. Biochem. Mol. Biol. Int. 51, 869–875.

Skoog, F., Hamzi, H.Q., Szweykowska, A.M., Leonard, N.J., Carraway, K.L., Fujii, T., Helgeson, J.P., Loeppky, R.N., 1967. Cytokinins: Structure/activity relationships. Phytochemistry 6, 1169–1192.

Spíchal, L., Rakova, N.Y., Riefler, M., Mizuno, T., Romanov, G.A., Strnad, M., Schmülling, T., 2004. Two Cytokinin Receptors of Arabidopsis thaliana, CRE1/AHK4 and AHK3, Differ in their Ligand Specificity in a Bacterial Assay. Plant Cell Physiol. 45, 1299–1305.

Szüčová, L., Zatloukal, M., Spíchal, L., Fröhlich, L., Doležal, K., Strnad, M., Massino, F., 2016. 6,9-Disubstituted Purine Derivatives and their Use for Treating Skin. EP2043630.