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Abstracts

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PG1

ALKALOIDS OF AMARYLLIDACEAE FAMILY USED FOR THE PREPARATION OF SEMI-SYNTHETIC DERIVATIVES

SUCHÁNKOVÁ, D.,[1] RITOMSKÁ, A.,[1] PEŘINOVÁ, R.,[1] CAHLÍKOVÁ, L.[1]

[1] Department of Pharmacognosy and pharmaceutical botany, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic

e-mail: hulcovd@faf.cuni.cz

Alkaloids are a very important group of secondary metabolites with a number of interesting biological effects (anticancer, analgesic, anticholinesterase, antimalarial, …). Amaryllidaceae family is source of structurally unique compounds, Amaryllidaceae alkaloids. For this reason, the Amaryllidaceae family is interesting goal in searching for active substances with various biological activities.[1]

Some Amaryllidaceae alkaloids that contain one hydroxy group are suitable for the preparation of simple semi-synthetic derivatives. The alkaloids vittatine, ambelline and haemanthamine were used in the studies, where simple esters were prepared. These alkaloids show no inhibitory activity against human cholinesterase. Inhibition of cholinesterases is one of goals in the therapy of Alzheimer´s disease. During the studies, it was shown that derivatives of these alkaloids are significantly more active and even some of them in nanomolar concentrations.[2, 3, 4] The inhibitory potentials against human acetyl and butyrylcholinesterase were determined using a modified method of Ellman.

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PG2

DERIVATIVES OF AMARYLLIDACEAE ALKALOID AMBELLINE AS SELECTIVE INHIBITORS OF HEPATIC STAGE OF *PLASMODIUM* INFECTION *IN VITRO*

HRADISKÁ BREITEROVÁ, K.,[1] RITOMSKÁ, A.,[1] FONTINHA, D.,[2] FRANCISCO, D.,[2]

[1] Secondary Metabolites of Plants as Potential Drugs Research Group, Department of Pharmacognosy and Pharmaceutical Botany, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic

[2] Prudêncio Lab, Instituto de Medicina Molecular João Lobo Antunes, Faculdade de Medicina, Universidade de Lisboa, Av. Prof. Egas Moniz, Edf. Egas Moniz, 1649-028 Lisboa, Portugal

e-mail: breiterk@faf.cuni.cz

The increasing incidence rate of malaria and the ensuing mortality prompts the development of novel antimalarial drugs. In this work, the activity of twenty-eight Amaryllidaceae alkaloids belonging to seven different structural types, as well as twenty semisynthetic derivatives of the β-crinane alkaloid ambelline and eleven derivatives of the α-crinane alkaloid haemanthamine against the hepatic stage of *Plasmodium* infection was assessed. The most active compounds, 11-*O*-(3,5-dimethoxybenzoyl)ambelline and 11-*O*-(3,4,5-trimethoxybenzoyl)ambelline, displayed IC_{50} values in the nanomolar range of 48 and 47 nM, while the IC_{50} of currently used standard primaquine is 5.74 ± 0.86 μM. Strikingly, the derivatives of haemanthamine with analogous substituents did not display any significant activity, even though their structures are noticeably similar. Interestingly, all active derivatives were strictly selective against the hepatic stage of infection, as they did not demonstrate any activity against its blood stage. As the hepatic stage is a bottleneck of the plasmodial infection, liver-selective compounds can be considered crucial for further development of the malaria prophylactics. Especially, when all evaluated derivatives showed very low or none toxicity against the liver-derived HepG2 cell line.[1]

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PG3

BIOLOGICAL ACTIVITIES OF SEMISYNTHETIC MONTANINE-TYPE DERIVATIVES

MAAFI, N.,[1] CAHLÍKOVÁ, L.,[1] SUCHÁNKOVÁ, D.,[1] KŘOUSTKOVÁ, J.,[1] HRADISKÁ BREITEROVÁ, K.,[1] KONEČNÁ, K.,[2] JANĎOUREK, O.,[2] DIEPOLTOVÁ, A.,[2]

[1] ADINACO Research Group, Department of Pharmacognosy and Pharmaceutical Botany, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic

[2] Department of Biological and Medical Sciences, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic

e-mail: negarm@faf.cuni.cz

Based on existing knowledge regarding the bioactivity of the montanine-type Amaryllidaceae alkaloids, they were selected to evaluate the impact of the scaffold chemical modification on their biological activity.[1] Utilizing the intramolecular rearrangement of haemanthamine to 3-*O*-methylpancracine and using previously isolated montanine as starting materials, a library of about 90 aromatic and aliphatic derivatives of montanine-type alkaloids was synthesized, possessing various substitution on either C2 or C3 of 5,11-methanomorphanthridine core.[2] These semisynthetic compounds were selectively screened against human acetylcholinesterase (*h*AChE) and butyrylcholinesterase (*h*BuChE) enzymes, three strains of non-pathogenic *Mycobacterium*, a panel of eight Gram-positive and Gram-negative bacteria, a panel of eight fungal strains, and a panel of eight cancer cell line. From this library, three derivatives with an IC_{50} value less than 5 μM against *h*AChE, one derivative with IC_{50} value of 1.73 μM against *h*BuChE, one derivative with significant activity against all tested strains of *Mycobacterium* with MIC value of 7.9 μM, one compound active against *Staphylococcus aureus* and *Klebsiella pneumoniae*, and two compounds active against cancer cell lines with IC_{50} values ranging between 1.18–8.06 μM were found. Further biological tests or in-silico studies were performed.

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PG4

CARLTONINE DERIVATIVES AS POTENTIAL LEAD DRUG CANDIDATES FOR THE TREATMENT OF ALZHEIMER'S DISEASE

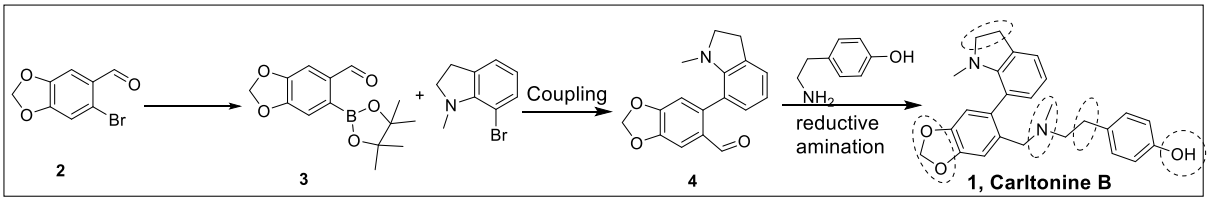
MAMUN, A. A., [1] KONEČNÝ, J., [2] MUCKOVA, L.,[2] PULKRABKOVA, L.,[2] SOUKUP, O., [2] CAHLÍKOVÁ, L., [1] KORÁBEČNÝ, J.,[2]

[1] Department of Pharmacognosy and Phramaceutical Botany, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic

[2] Department of Toxicology and Military Pharmacy, Trebesska 1575, 500 05 Hradec Králové, Czech Republic

e-mail: almamuna@faf.cuni.cz

The most important group of secondary metabolites in the plant family Amaryllidaceae are alkaloids. Novel alkaloids carltonine B has been isolated from the alkaloidal extract of *Narcissus pseudonarcissus* cv. Carlton, demonstrated highly selective *in-vitro* butyrylcholinesterase (BuChE) inhibition potency with IC_{50} value of 31 nM[1]. Unfortunately, the alkaloid is present in small amounts in the plant hampering its large-scale isolation and comprehensive biological evaluations with potential follow-up for commercial use. In our ongoing study, we determined a pharmacophore of the carltonine scaffold, namely (4-[2-(benzylamino)ethyl]phenol), responsible for the selective BuChE inhibition profile[2]. These preliminary data will allow us to generate a set of carltonine B related compounds, as well as to perform the total synthesis of carltonine B. Thus, we are intended to optimize the template scaffold from the physicochemical point of view as well as to achieve maximum therapeutic effect.



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PG5

NEGLECTED STEREOCHEMISTRY IN AMARYLLIDACEAE ALKALOIDS

KŘOUSTKOVÁ, J.,[1] AL MAMUN, A.,[1] CAHLÍKOVÁ, L.,[1] KUNEŠ, J.,[2]

[1]Department of Pharmacognosy and Pharmaceutical Botany, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic

[2]Department of Organic and Bioorganic Chemistry, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic

e-mail: marikoj2@faf.cuni.cz

Although the bulbs of the Amaryllidaceae family may seem to have been largely explored, our work on their alkaloids shows they still have a lot to offer. Carltonine, a new pharmacophore in Alzheimer's disease research, was discovered where galanthindole scaffold is condensed with tyramine.[1] Although galanthindole alkaloids have long been known, only Řezanka and co-workers[2] described their atropisomerism. Interestingly, this carltonine group is also characterized by axial chirality. Therefore, dynamic NMR analysis was performed with an increasing temperature as the determining technique. Moreover, atropisomerism was later identified in the narcikachnine-type of Amaryllidaceae alkaloids.[3,4] Unfortunately, no samples were obtained for X-ray analysis. NMR spectroscopy was the key in all identifications; of course, MS and optical methods were also used in the structural analysis.

Recently, more alkaloids with galanthindole core were discovered in our ongoing study, and this short talk focuses on their structure.

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PG6

THE EFFECT OF SMALL PHENOLIC METABOLITES OF FLAVONOIDS (BENZOIC ACIDS) ON METAL-TRIGGERED FENTON REACTION AND COPPER-INDUCED RED BLOOD CELL LYSIS

LOMOZOVÁ, Z.,[1] KUČERA, R.,[2] MLADĚNKA, P.,[3]

[1] Department of Pharmacognosy and Pharmaceutical Botany, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic

[2] Department of Pharmaceutical Chemistry and Pharmaceutical Analysis, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic

[3] Department of Pharmacology and Toxicology, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic

e-mail: lomozovz@faf.cuni.cz

Flavonoids, the secondary metabolites of plants, are a common part of our diet. In the human body, they are massively metabolized before entering the systemic circulation and are decomposed into simple polyphenolic compounds. One group of their metabolites are benzoic acid derivatives.[1] The positive effects of flavonoids have been mainly attributed to their antioxidant activity, which includes direct free radical scavenging, metal chelation, and interference with enzymes that form reactive oxygen species.[2] They may, however, exhibit pro-oxidative effects related to their metal reducing properties. The effects of flavonoid metabolites have been investigated to a lesser extent and therefore the aim of this work was to determine their impact *in vitro* on the iron and copper-triggered Fenton reaction and *ex vivo* on copper-induced red blood cell lysis. 2,4,6-trihydroxybenzoic acid enhanced both iron and copper-induced Fenton reaction and worsened red blood cell lysis. Dihydroxy-substituted benzoic acids had antioxidant activity *in vitro* and neutral effect *ex vivo*. 3-hydroxybenzoic acid had neutral effect on the production of free hydroxyl radicals, but increased red blood cell lysis. None of the metabolites was able to block copper-induced toxicity *ex vivo*.

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PG7

DESIGN OF SEMISYNTHETIC DERIVATIVES OF AMARYLLIDACEAE ALKALOID AMBELLINE AND EXPLORATION OF THEIR *IN VITRO* CYTOTOXIC ACTIVITIES

RITOMSKÁ, A.,[1] KOUTOVÁ, D.,[2] KŘOUSTKOVÁ, J.,[1] KRÁLOVEC, K.,[3], MUTHNÁ, D.,[2] KUNEŠ, J.,[4] NOVÁKOVÁ, L.,[5] HAVELEK, R.,[2] CAHLÍKOVÁ, L.[1]

[1] Department of Pharmacognosy and Pharmaceutical Botany, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic

[2] Department of Medical Biochemistry, Faculty of Medicine in Hradec Králové, Charles University, Czech Republic

[3] Department of Biological and Biochemical Sciences, Faculty of Chemical Technology, University of Pardubice, Czech Republic

[4] Department of Organic and Bioorganic Chemistry, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic

[5] Department of Analytical Chemistry, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic

e-mail: ritomska@faf.cuni.cz

Amaryllidaceae alkaloid ambelline belonging to the crinane-type group lacks any significant biological activity. However, its analogs prepared by derivatization of the C-11 hydroxyl group possess different interesting properties. Continuing our earlier work, twelve novel aromatic esters were developed (**10**, **14**, **16**, **17**, **22–25**, **30–33**) and studied, together with previously synthesized derivatives (**2–9**, **11–13**, **15**, **18–21**, **26–29**) in term of their cytotoxic activity. To describe and foresee the structure-activity relationship for further research, substances synthesized and described in our previous work were also included in this cytotoxicity study. Most of the active derivatives with the most pronounced cytotoxic activity contain a methyl group (**10**), methoxy group (**14–17**), or ethoxy group (**18**) on a substituted aromatic ring. However, 11-*O*-(4-chloro-3-nitrobenzoyl)ambelline (**32**) demonstrated the most promising IC_{50} values ranging from 0.6 ± 0.1 μM to 9.9 ± 0.2 μM. *In vitro* cytotoxicity studies indicated the strongest antiproliferative activity of **32** in a dose-dependent and time-dependent manner. Besides, **32** was found to be effective in decreasing viability and triggering apoptosis of MOLT-4 T-lymphoblastic leukemic cells.

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PG8

HIGHLY SELECTIVE BUTYRYLCHOLINESTERASE INHIBITORS RELATED TO AMARYLLIDACEAE ALKALOIDS – SYNTHESIS AND BIOLOGICAL EVALUATION

PIDANÝ, F.,[1] KORÁBEČNÝ, J.,[2] CAHLÍKOVÁ, L.,[1]

[1] Department of Pharmacognosy and Pharmaceutical Botany, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic

[2] Department of Toxicology and Military Pharmacy, Faculty of Military Health Sciences, University of Defence, Trebešská 1575, 500 01 Hradec Králove, Czech Republic

e-mail: pidanyf@faf.cuni.cz

Butyrylcholinesterase (BChE) and its inhibitors received particular attention in many fields of research. Indeed, the enzyme is implicated in many pathophysiological or toxicological processes and has been associated with xenobiotic detoxification, metabolic syndrome, and insulin resistance. At the behavioral level, it interferes with anxiety, aggression, and neurodegenerative disease such as Alzheimer's disease (AD). Although the exact etiology of AD has not been fully elucidated, among the common features of the disease is dysfunction of the cholinergic system. Intriguingly, in the late stages of AD, the levels of acetylcholinesterase are markedly reduced by more than 90 %, while the level of BChE gradually increases to 165 % of the normal level.[1] In our previous study, new Amaryllidaceae alkaloids isolated from *Narcissus pseudonarcissus* cv. Carlton, specifically carltonine A and B, demonstrated a very potent BChE inhibition profile in the nanomolar range.[2] We retained some structural fragments from carltonine A/B that are believed to be responsible for the high inhibition of BChE, namely the 4-[2-(benzylamino)ethyl]phenolic group, and proposed new structural modifications leading to new derivatives with higher activity and selectivity. Top-ranked compounds were subjected to test their ability to pass into the CNS using the *in vitro* PAMPA assay. The safety profile was verified by the ability of compounds to damage human neuroblastoma cell line.*The study was supported by project SVV 260 548.*

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PG9

CYTOTOXIC ALKALOIDS FROM *VINCA MINOR* L.

VRABEC, R.,[1] HAVELEK, R.,[2] KOUTOVA, D.,[2] KROUSTKOVA, J.,[3] KUNES, J.,[3] SUCHANKOVA, D.,[1] HOSTALKOVA, A.,[1] CHLEBEK, J.,[1] LOCAREK, M.,[1] SOUKUP, O.,[4] OPLETAL, L.[1]

[1] Department of Pharmacognosy and Pharmaceutical Botany, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic

[2] Department of Medical Biochemistry, Faculty of Medicine in Hradec Králové, Charles University, Czech Republic

[3] Department of Organic and Bioorganic Chemistry, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic

[4] Department of Toxicology and Military Pharmacy, Faculty of Military Health Sciences, University of Defence, Hradec Králové, Czech Republic

e-mail: vrabecr@faf.cuni.cz

A series of over 20 monoterpene indole alkaloids were isolated from *Vinca minor* L. (Apocynaceae), some of which were identified for the first time from this species. Within the focus of our research group, the alkaloids were initially tested against the key enzymes of the pathophysiology of Alzheimer's disease. Isolated compounds were shown to be weak inhibitors of acetylcholinesterase; however, most of them selectively inhibited butyrylcholinesterase (BuChE). The most active alkaloid in this regard was 2-ethyl-3[2-(3-ethylpiperidinyl)-ethyl]]-1*H*-indole, which inhibited BuChE with an IC_{50} of 650 nM as a reversible competitive inhibitor (K_i = 55 nM). Compounds obtained in sufficient amounts were screened for anti-proliferative potential on a panel of 10 different cell lines. Most of the tested alkaloids did not exhibit significant cytotoxic potential; however, alkaloid eburnamonine strongly inhibited the growth of Jurkat cell line (lymphocytic leukemia). Another cytotoxic compound, vincarubine, a dimeric indole alkaloid structurally similar to the well-known natural chemotherapeutics vincristine and vinblastine, practically inhibited the proliferation of all tested cell lines. For that reason, vincarubine was further studied to elucidate its mechanism of action. Using the A549 cell line (lung cancer), the compound was found to exhibit an increasing, dose-dependent influence on viability and proliferation after 24h of exposition and in real-time using the xCELLigence method. Its influence on the A549 cell cycle was studied as well.

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PG10

INTERACTIONS OF SELECTED ISOQUINOLINE ALKALOIDS WITH TRANSITION METALS

PARVIN, M. S.,[1] CHLEBEK, J.,[1] HOŠŤÁLKOVÁ, A.,[1] MLADĚNKA P.,[2]

[1]Department of Pharmacognosy and Pharmaceutical Botany, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic

[2]Department of Pharmacology and Toxicology, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic

e-mail: parvins@faf.cuni.cz

Transition metals (e.g. iron and copper) play a fundamental role in the human body and dyshomeostasis may cause harmful effects known for the development of some neurodegenerative and other diseases [1,2]. Alkaloids are the largest group of plant secondary metabolites, some of them possess interesting activity against various diseases whose progression may be related to transition metal imbalance, e.g. galanthamine is used to slow the progression of Alzheimer's disease [3]. We have tested 28 selected isoquinoline alkaloids for their interactions with two biogenic metals, iron, and copper ions by using competitive spectrophotometric methods. Further, ferrozine and BCS methods were also used for the determination of reduction activities of alkaloids. Interestingly, all alkaloids in the form of free bases were able to chelate both ferrous and ferric (total iron) ions to some extent. Galanthine and glaucine demonstrated iron chelation activity at all four pH's ranging from 4.5 to 7.5. In addition, significant reduction activities have also been observed by tested alkaloids. Copper was more significantly reduced by alkaloids than iron. The highest reduction in iron ions were achieved at pH 4.5, the most active substances were isocorydine, glaucine, sinoacutine, and scoulerine. All alkaloids except protopine alkaloids showed at least a slight copper reduction in all test conditions. The most significant reduction activity in a ratio of 1:1 (alkaloid: copper) was achieved by glaucine.

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