ABSTRACT

Charles University, Faculty of Pharmacy in Hradec Králové

Department of Pharmaceutical Botany

Candidate: **Mgr. Kateřina Breiterová**

Supervisor: **Assoc. prof. Ing. Lucie Cahlíková PhD.**

Title of Doctoral Thesis: **Alkaloids of the Amaryllidaceae family as potential drugs in therapy of diseases of affluence**

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Narcissus cv. Professor Einstein was chosen based on results of previous screening studies for detailed phytochemical work for the purpose of isolation of the widest range of AmA. From 34,3 kg of fresh bulbs was obtained 31,7 g of purified alkaloidal extract, which was processed using column chromatography with stepwise elution by light petrol, chloroform and ethanol in different ratios to almost 500 fractions. These fractions were fused into 27 subfractions, which were processed by preparative TLC, vacuum column chromatography and crystallization. Finally, 25 pure alkaloids were isolated. All compounds were identified by GC-MS, ESI-MS, NMR, optical rotation and literature. One compound was identified as a new unpublished alkaloid of lycorine structure type.

All alkaloids isolated in sufficient amount were tested for their biological activities associated with Alzheimer‘s disease (inhibition of *h*AChE, *h*BuChE, POP, GSK-3β), cytotoxicity, AKR1C3 inhibition and activity against the liver stage malaria *in vitro*.

Inhibition of erytrocytic *h*AChE and serum *h*BuChE was determined by modified Ellman‘s method. Except the galanthamine activity against *h*AChE were all other tested compounds considered inactive. Determination of POP inhibition was performed by spectrophotometric method and four compounds showed activity comparable to used standard berberine (IC_{50} = 142 ± 21 μM): homolycorine (IC_{50} = 173 ± 41 μM), norlycoramine (IC_{50} = 209 ± 14 μM), eugenine (IC_{50} = 130 ± 8 μM) and norpluviine (IC_{50} = 148 ± 10 μM). These compounds belong to the most potent POP inhibitors from the currently tested Amaryllidaceae alkaloids. Alkaloids masonine, caranine and 9-*O*-demethylhomolycorine showed interesting inhibition activity against GSK-3β in the determination by luminescent method.

Majority of isolated alkaloids went through the screening of cytotoxic activity on nine cancer (Jurkat, MOLT-4, A549, HT-29, PANC-1, A2780, HeLa, MCF-7 a SAOS-2) and two

healthy (MRC-5 a NHDF) cell lines. From this screening haemanthamine, lycorine and pancracine advanced to the IC_{50} determination in chosen cell lines. Haemanthamine and lycorine are well known because of their cytotoxic effect but in pancracine it was not previously described. This compound was chosen for detailed study because of elucidation of its mechanism of action. Hence, pancracine went through study of the cell cycle and apoptosis induction interference.

Some of isolated compounds were screened for inhibition activity against AKR1C3 enzyme, but none of them showed any significant activity.

Alkaloids were also tested for their activity against liver stage malaria *in vitro*. Here, none of the alkaloids showed notable activity. That was showed by semisynthetic derivatives of ambelline – the most active compound was the one labeled as LC-104 (IC_{50} = 0,048 ± 0,014 μM).