Isolation and structure elucidation of bioactive compounds from *Euphorbia* species

Summary of the Ph.D. Thesis

Norbert Kúsz

University of Szeged

Szeged, Hungary

2020
University of Szeged
Doctoral School of Pharmaceutical Sciences
Pharmacognosy Program
Head: Prof. Judit Hohmann DSc.

Supervisors:
Prof. Hohmann Judit DSc.
Dóra Rédei Ph.D.

Isolation and structure elucidation of bioactive compounds
from Euphorbia species

Summary of the Ph.D. Thesis

Norbert Kúsz

Final Exam Committee:
Head: Prof. Imre Máthé DSc.
Members: Prof. György Dombi DSc., Prof. József Deli DSc.

Reviewer Committee:
Head: Prof. Ferenc Fülöp DSc.
Reviewers: Szabolcs Béni Ph.D., Györgyi Horváth Ph.D.
Members: Szilvia Berkó Ph.D., Sándor Gonda Ph.D.

Szeged, Hungary
2020
INTRODUCTION

The genus *Euphorbia* is among the largest genera of flowering plants with approximately 1900 recognized species. Members of this nearly cosmopolitan genus can be characterized by the frequent occurrence of white, milky latex. Previously, several hypotheses have been proposed to explain the function of latex, like storage of plant nutrients, deposit of waste products, or water reserve, but nowadays it is widely considered as a key part of the plant self-defence mechanisms. This hypothesis has been supported by the fact that latices often contain specialized defensive metabolites (e.g. terpenoids, proteins) at much higher concentrations compared to other plant organs. *Euphorbia* species are collectively known as “spurges”. Their name has been derived from the Medieval French word “epurger” (“expurgare” in Latin), referring to the purgative properties of the latex and seeds. Spurges are commonly used in folk medicine for the treatment of microbial (e.g. gonorrhoea, syphilis) and parasitic infections, obstipation, asthma, coughs, rheumatism, snakebites, wounds and haemorrhages, eczema, sores, warts, and other skin disorders. In many cases, traditional applications of the plants were supported by modern pharmacological evidence.

*Euphorbia* species are prolific producers of macrocyclic diterpenoids. Due to their structural diversity and promising bioactivities, macrocyclic diterpenoids represent an intriguing group of secondary metabolites from the point of view of natural product discovery. In the last few decades macrocyclic diterpenoids have been the subject of extensive phytochemical investigations leading to the isolation of hundreds of new diterpene derivatives with more than 20 different skeletons. Furthermore, cyclic derivatives of the macrocyclic cembrane class are of chemotaxonomic significance because their distribution in the plant kingdom is limited to the Euphorbiaceae and Thymeleaceae families.
AIMS OF THE STUDY

In 1995, Hohmann et al. (Department of Pharmacognosy, University of Szeged) initiated a research project with the aim of investigating the secondary metabolites of Euphorbia species. As a part of ongoing research, the goals of my work were the isolation and structure determination of new diterpenoids from four selected spurge species (Euphorbia dulcis L., E. taurinensis All., E. guyoniana Boiss. & Reut., E. davidii Subils.), followed by the investigation of their bioactivity, executed in collaboration with cooperative partners. In order to meet these objectives, the main tasks were as follows:

- Screening of the plant materials for diterpenoid contents.
- Extraction of the plant materials.
- Chromatographic purification and isolation of diterpenoids.
- Structure elucidation of the isolated compounds.
- Assessment of the chemotaxonomic importance of the isolated compounds.
- Evaluation of the pharmacological properties of diterpenoids.

MATERIALS AND METHODS

The whole plants of E. dulcis were gathered in the flowering period at Homoródalmás, Romania. The whole plants of E. taurinensis were collected in Budapest, Hungary. The aerial parts of E. guyoniana were harvested in the droughty region Grand Erg Oriental of southern Tunisia. The whole plants of E. davidii were collected near Igar, Hungary.

The screening of plant materials (excepting E. guyoniana) for diterpenoid content started with the extraction of plant samples with MeOH. Diterpenoids have previously been described from E. guyoniana, thus screening of this Saharan species was unnecessary. The concentrated extracts were diluted with H₂O, and then partitioned with CHCl₃. The CHCl₃-soluble phases were separated on polyamide columns by using gradient solvent systems of MeOH–H₂O. The collected fractions
were investigated by thin-layer chromatography (TLC) under UV light (254 nm), then the chromatograms were visualized by \textit{cc} H$_2$SO$_4$ followed by heating the plates (110 °C).

The plant materials of \textit{E. dulcis}, \textit{E. taurinensis}, and \textit{E. davidii} were percolated with MeOH, while the air-dried powder of \textit{E. guyoniana} was extracted with CHCl$_3$. The extracts were subjected to liquid-liquid extraction, then the compounds of interest were further purified by multistep chromatographic methods including open-column chromatography (OCC), vacuum-liquid chromatography (VLC), preparative layer chromatography (PLC), and high-performance liquid chromatography (HPLC). Polyamide for OCC, and normal- (NP) and reversed-phase (RP) silica gel for VLC, PLC, and HPLC were used as stationary phase.

The structures of the isolated compounds were elucidated by means of 1D ($^1$H, JMOD) and 2D (HSQC, HMBC, $^1$H-$^1$H COSY, NOESY) NMR experiments coupled with HRESIMS measurements. The absolute configuration of one diterpenoid (compound 1, see later) was determined by single-crystal X-ray diffraction.

Plant extracts from \textit{E. davidii} were prepared for pharmacological screening. The MeOH extract of the plant was partitioned with \textit{n}-hexane, CHCl$_3$, and EtOAc. The dried plant material was subsequently extracted with boiling water.

The isolated diterpenoids were investigated on stable transfected HEK-GIRK1/4 (Kir3.1/3.4) and HEK-hERG (Kv11.1) cell lines. The ion currents were measured by an automated patch-clamp equipment. The MDR-modulating and cytotoxic effects of the diterpenoids were studied on L5178 mouse T-lymphoma cell line using rhodamine 123 accumulation and MTT cell viability assays. The antiproliferative activities of plant extracts of \textit{E. davidii} were evaluated on HeLa (cervix epithelial adenocarcinoma), MCF7 (breast epithelial adenocarcinoma), A2780 (ovarian carcinoma), and A431 (skin epidermoid carcinoma) cell lines by MTT assay.
RESULTS AND DISCUSSION

Screening of the plant materials for diterpenoid content

The MeOH extracts of *E. dulcis* and *E. taurinensis* were separated by OCC. TLC chromatograms of the collected fractions revealed that the plants contain complex mixtures of diterpenoids. The diterpenoids accumulated in the fractions eluted with 60% aqueous MeOH, as suggested by the dense brown, grey, and black spots on the plates with R$_f$ values of 0.15–0.70. TLC chromatogram of the extract of *E. davidii* did not exhibit characteristic spots attributable to diterpenoids.

Isolation of diterpenoids from *E. dulcis*

The crude MeOH extract of *E. dulcis* was partitioned with CHCl$_3$ (Figure 1). The CHCl$_3$-soluble phase was fractionated by OCC with mixtures of MeOH–H$_2$O (3:2, 4:1, 1:0). The diterpenoid-rich 60% MeOH fraction was separated by VLC using gradient
cyclohexane–EtOAc–EtOH mobile phases. Further purifications of the combined subfractions A21-32 and A38-47 by various VLC, PLC, and HPLC methods allowed the isolation of 11 diterpenoids (EUD-2–4, 6, 8, 10, 16, 19, 21–23).

**Isolation of diterpenoids from *E. taurinensis***

The MeOH extract of *E. taurinensis* was partitioned with CHCl₃, then the CHCl₃-soluble phase was submitted to polyamide OCC and eluted with mixtures of MeOH and H₂O (3:2, 4:1, 1:0) (**Figure 2**). TLC chromatograms indicated that diterpenoids presented in both 60% and 80% MeOH fractions. The fractions were first separated by VLC using stepwise gradient elution with cyclohexane–EtOAc–EtOH, which yielded 80 (B1-80) and 70 (A1-70) subfractions, respectively. Further purifications of the compounds by means of VLC, PLC, and HPLC resulted in the isolation of 5 diterpenoids (ETA-1, 2, 5–7) from the 80% MeOH fraction, and 2 diterpenoids (ETA-8, 9) from the 60% MeOH portion.

**Figure 2.** Isolation of diterpenoids from *E. taurinensis*
Isolation of diterpenoids from *E. guyoniana*

The powdered raw material of *E. guyoniana* was extracted with CHCl₃ in an ultrasonic bath. Initial fractionation of the lipophilic extract was performed by polyamide OCC using MeOH–H₂O mixtures (3:2, 4:1, 1:0) as mobile phases (Figure 3). According to the TLC chromatograms, diterpenoids were selectively enriched in the 60% MeOH fraction. VLC separation of the 60% MeOH portion was achieved with gradient cyclohexane–EtOAc–EtOH solvent systems. Purifications of the combined subfractions 17-21 and 22-26 were carried out by NP-HPLC and RP-HPLC methods, and yielded 2 diterpenoids (EGU-3, 4).

![Figure 3. Isolation of diterpenoids from *E. guyoniana*](image)

Isolation of flavonoid glycosides from *E. davidii*

The MeOH extract of *E. davidii* was subjected to repetitive liquid-liquid extraction using CHCl₃ and EtOAc (Figure 4). As the CHCl₃-soluble fraction did not contain any compound worthy of an isolation attempt, the latter and more promising EtOAc phase was chosen for further separation. The isolation procedure started with a VLC step, and mixtures of EtOAc–EtOH–H₂O were applied to elute the compounds. The collected fractions 4, 5, and 6 with high contents of flavonoids were further
separated by different chromatographic techniques (PLC, RP-HPLC). Finally, the plant material afforded 3 flavonoid glycosides (EDI-17–19).

Structure determination of the isolated compounds

The flavonoids were identified by comparison of their $^1$H and $^{13}$C NMR data with literature values. The molecular formulas of diterpenoids were determined by means of HRESIMS measurements. The structures of diterpenoids were established by 1D and 2D NMR spectroscopy. The $^1$H, JMOD, $^1$H-$^1$H COSY, HSQC, and HMBC spectra revealed the constitutions of the terpenoid scaffolds, then the relative configurations of stereogenic carbons were deduced by relevant NOESY correlations. As a result of the NMR studies, complete $^1$H and $^{13}$C NMR assignments were made for the new natural products. In addition, the absolute configuration of compound 1 was determined by single-crystal X-ray diffraction.
Diterpenoids of *E. dulcis*

The MeOH extract of *E. dulcis* yielded 9 novel (1–9) and 2 known (10, 11) jatrophone diterpenoids. Compounds 1–11 contain a $\Delta^{(5,6)}$ olefinic bond instead of the regular $\Delta^{(6,17)}$ double bond, and are esterified with acetyl, benzoyl, and tigloyl groups. The diterpenoids of *E. dulcis* differ only in their substitution patterns at C-7, C-8, C-9, C-14, and C-15. The jatrophanes contain a conserved benzoyl moiety at C-3, and interestingly tigloyl groups are found exclusively on C-7 and C-14. Compound 9, an interesting structural isomer of euphomelliferene B (10), have C-14 hydroxy and C-15 acetyl groups. To date just a few jatrophanes have been reported with the same arrangement of substituents.

![Diterpenoid Structure](image)

<table>
<thead>
<tr>
<th></th>
<th>$R_1$</th>
<th>$R_2$</th>
<th>$R_3$</th>
<th>$R_4$</th>
<th>$R_5$</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>1</strong> (EUD-8)</td>
<td>Tig</td>
<td>H</td>
<td>Ac</td>
<td>Ac</td>
<td>H</td>
</tr>
<tr>
<td><strong>2</strong> (EUD-4)</td>
<td>Tig</td>
<td>OAc</td>
<td>Ac</td>
<td>Ac</td>
<td>H</td>
</tr>
<tr>
<td><strong>3</strong> (EUD-19)</td>
<td>Tig</td>
<td>OAc</td>
<td>Ac</td>
<td>Tig</td>
<td>H</td>
</tr>
<tr>
<td><strong>4</strong> (EUD-16)</td>
<td>H</td>
<td>OAc</td>
<td>Ac</td>
<td>Tig</td>
<td>H</td>
</tr>
<tr>
<td><strong>5</strong> (EUD-10)</td>
<td>H</td>
<td>OAc</td>
<td>Ac</td>
<td>Ac</td>
<td>H</td>
</tr>
<tr>
<td><strong>6</strong> (EUD-23)</td>
<td>Ac</td>
<td>OH</td>
<td>Ac</td>
<td>Ac</td>
<td>H</td>
</tr>
<tr>
<td><strong>7</strong> (EUD-21)</td>
<td>Ac</td>
<td>OH</td>
<td>H</td>
<td>Ac</td>
<td>H</td>
</tr>
<tr>
<td><strong>8</strong> (EUD-22)</td>
<td>H</td>
<td>OH</td>
<td>H</td>
<td>Ac</td>
<td>H</td>
</tr>
<tr>
<td><strong>9</strong> (EUD-3)</td>
<td>Ac</td>
<td>OAc</td>
<td>Ac</td>
<td>H</td>
<td>Ac</td>
</tr>
<tr>
<td><strong>10</strong> (EUD-2)</td>
<td>Ac</td>
<td>OAc</td>
<td>Ac</td>
<td>Ac</td>
<td>H</td>
</tr>
<tr>
<td><strong>11</strong> (EUD-6)</td>
<td>Ac</td>
<td>H</td>
<td>Ac</td>
<td>Ac</td>
<td>H</td>
</tr>
</tbody>
</table>
The EUD-series is stereochemically homogeneous, as all jatrophanes are based on a highly oxygenated trans-bicyclo[10.3.0]pentadecane core, which comprises 2β and 13β-methyls, and 7β, 8β, 9α, 14β, and 15β acyl or hydroxy functions. Compounds 1 and 11 are not substituted at C-8. Considering the coupling constant pattern and diagnostic NOE correlations, it was deduced that these jatrophanes adopted an endo-type conformation, in which the H3-17 methyl group is perpendicular to the mean plane of macrocycle, and the adjacent protons H-4 and H-5 are antiperiplanar. Furthermore, the absolute configuration of compound 1 was established by single-crystal X-ray diffraction. Compound 1 crystallized in the monoclinic chiral space group $P2_1$ with three crystallographically independent but chemically identical conformers in the asymmetric unit. For all three conformers of 1, the assignment is most likely $(2S,3S,4S,7R,9R,13S,14S,15R)-9\alpha,14\beta$-diacetoxy-3β-benzoyloxy-15β-hydroxy-7β-tigloyloxyjatropha-5E,11E-diene.

Diterpenoids of *E. taurinensis*

NMR spectroscopic analysis of the compounds obtained from *E. taurinensis* led to the identification of a novel and a known segetane (12 and 14, respectively), a novel
and a known jatropane (13 and 15, respectively), and 3 known ingenane diterpenoids (16–18). The segetanes represent a unique class of diterpenoids: only 12 compounds had been isolated from *E. segetalis*, *E. paralias*, *E. portlandica*, and *E. peplus* prior to our work. Unlike most of the previously described segetanes, compound 12 contains a β-oriented acetyl group at C-14, and the C-17 bridge is not substituted. In compounds 12 and 14 a rare acetoxyacetoxymoiety is attached to C-5. The novel jatropane 13 bears an α-acetyl group at C-9, while the structurally related diterpenoid (15) possesses a C-9 β-cinnamoyl group. Compounds 13 and 15 were determined as endo-conformers based on relevant coupling constant values and NOE interactions.

**Diterpenoids of *E. guyoniana***

The CHCl₃ extract of *E. guyoniana* yielded 2 new (19, 20) jatropane diterpenoids. The compounds are highly esterified with acetyl, benzoxy, and isobutyryl groups, and differ from each other in only one substituent: 19 contains a C-7 isobutyryl moiety, while a C-7 acetyl group is found in 20. Similarly to other jatrophanes previously reported from the plant, 19 and 20 are esterified at C-2, and a keto group is located at C-14. The coupling constant values and important NOE correlations revealed the exo-conformation of the diterpenoids, in which the exomethylene points outward, and is parallel with the mean plane of the twelve-membered ring.
Flavonoid glycosides of *E. davidii*

The EtOAc phase of the MeOH extract of *E. davidii* afforded 3 flavonoid glycosides (21–23). The flavonoids were identified as myricetin-3-O-rhamnoside (21), quercetin-3-O-rhamnoside (22) and kaempferol-3-O-rhamnoside (23).

![Structures of Flavonoids](image)

**Chemotaxonomical aspects**

Macroyclic diterpenoids are considered to be important taxonomic biomarkers because of their structural diversity and limited distribution in the plant kingdom. *E. dulcis* afforded a series of jatrophane diterpenoids bearing a $\Delta^{(5,6)}$ olefinic bond instead of the more frequent 6(17)-exomethylene group. This structural feature has only been found in a minority of jatrophanes and, interestingly, most of those diterpenoids were reported from spurge species of subgenus *Esula*. In light of this, the presence of such compounds might be a useful chemotaxonomic marker for the characterization of members of the subgenus.

Our finding that *E. taurinensis* produces segetanes strongly supports the new taxonomic classification of *E. taurinensis*. The close intra-generic relationships between *E. taurinensis*, *E. segetalis*, and *E. paralias* have also been demonstrated by similarities in their diterpenoid compositions: compounds 14, 16, and 17 have earlier been identified in both *E. segetalis* and *E. paralias*, while 15 and 18 were described from *E. segetalis*.

Regarding *E. guyoniana*, in previous studies plant materials of Algerian origin were investigated, while we received the plant sample from Tunisia. The two isolated diterpenoids (19 and 20) are not identical with the previously reported jatrophanes.
This finding suggests a great variation in the diterpenoid compositions of populations of *E. guyoniana* grown at different geographical locations.

The latest classification suggests that *E. davidii* belongs to section *Poinsettia* of subgenus *Chamaesyce*. So far diterpenoids have only been detected in species of section *Anisophyllum*, and this fact could provide a reasonable explanation to why we failed to obtain any diterpenoids from *E. davidii* in spite of our best efforts.

The chemical constituents of *E. taurinensis* and *E. davidii* have not been studied previously. Furthermore, all isolated diterpenoids and flavonoid glycosides are described for the first time in the investigated spurges.

**Bioactivity of the isolated compounds and plant extracts**

**Ion channel blocking activity of the diterpenoids**

Diterpenoids are characteristic secondary metabolites of the *Euphorbia* species, however, limited data are available on their cardiac effects. Vasas *et al.* reported that myrsinane, premyrsinane, and cyclomyrsinane diterpenoids of *E. falcata* exert a selective GIRK blocking activity, and this finding served as a motivation to explore the electrophysiological effects of the isolated diterpenoids. Compounds 1–12 and 15–20 were investigated on a HEK-GIRK1/4 (Kir3.1/3.4) cell line. Majority of the tested compounds were found to exert a significant inhibitory effect on the GIRK proteins at 10 μm, and some of them displayed a notable blocking activity even at 1 μm concentrations.

The IC₅₀ values of the most potent diterpenoids were determined from the dose-response curves as follows: 1: 1.3 ± 0.2 μM; 2:1.6 ± 0.2 μM; 9: 3.4 ± 0.1 μM; 10: 1.7 ± 0.2 μM; 11: 2.6 ± 0.5 μM; 16: 12.2 ± 0.5 μM; 17:1.5 ± 0.1 μM. These jatrophane and ingenane diterpenoids were tested for their hERG-related cardiotoxicity on HEK-hERG (Kv11.1) cells. The results of the experiment demonstrated that none of the jatrophanes interfered with the function of the hERG proteins, however, the outward K⁺ flow was strongly hampered by compound 17.
To the best of our knowledge, our group is the first one to evaluate the electrophysiological effects of jatrophanes, segetanes, and ingenanes on GIRK and hERG proteins. Our investigations focusing on the GIRK channels showed no clear correlations between the inhibitory effects and the substitution patterns, so unfortunately, we could not establish any structure-activity relationships for the EUD-series. Nevertheless, jatropane and ingenane diterpenoids were proven to be potent inhibitors of the atrial GIRK proteins. Considering the selective activities of jatrophanes on GIRK channels, they may represent a group of potential lead compounds for the development of novel therapeutic agents against atrial fibrillation.

**MDR-reversing and cytotoxic activities of diterpenoids of E. taurinensis**

*Euphorbia* diterpenoids are best known for their strong MDR-reversing activity. Therefore, we examined the P-gp modulating and cytotoxic properties of compounds 12 and 15–18 on an L5178 mouse lymphoma cell line (Figure 5).

![Figure 5. Efflux pump modulating activities of the tested diterpenoids](image)

Compared to the positive control verapamil, all compounds were found to inhibit the P-gp efflux pump on the resistant mouse T-lymphoma cells. Compounds 12, 17, and 18 were shown to be the most powerful modulators at a concentration of 20 μM, with an efficacy of 7-9-fold higher compared to verapamil.
Based on our findings, neither the segetane, nor the jatrophane diterpenoids tested exert any cytotoxic activity on the sensitive parent and on the resistant MDR cells in the MTT assay. In contrast, the most active ingenane diterpenoids 17 and 18 displayed a cytotoxic effect on both cell lines. The IC$_{50}$ values of the most active compound 17 on the two cell lines were almost equal, indicating that it has no selectivity towards the resistant cell line, while compound 18 was more potent on the resistant cell line. The presence of a larger ester function at C-17 might enhance the cytotoxicity of the ingenanes, however, further data are needed to confirm this speculation.

Antiproliferative activities of the extracts of E. davidii

The prepared extracts (n-hexane, CHCl$_3$, EtOAc, 50% MeOH residue, H$_2$O) were screened in vitro for their antiproliferative activity against HeLa, MCF7, A2780, and A431 cell lines. The n-hexane and CHCl$_3$ extracts were found to exhibit a dose-dependent cell growth inhibitory activity on all cell lines. The flavonoid-rich EtOAc extract did not inhibit the proliferation of any cancer cell lines.
ACKNOWLEDGEMENTS

I would like to express my deepest appreciation to my supervisors, Dr. Dóra Rédei and Prof. Judit Hohmann for their guidance and constant support. Writing this thesis would not have been possible without their persistent help, warm encouragement, and constructive criticism.

I would like to express my sincere thanks to Dr. Gusztáv Jakab, Dr. Zoltán Barina, Prof. Mohamed Chaieb, and Prof. Gyula Pinke for the identification and collection of the plant materials.

I also wish to thank Dr. Péter Forgó for the NMR measurements; Attila Csorba for the HRESIMS measurements; Dr. Petra Bombicz, Dr. Krisztina Bereczki and Dr. Pierre Fertey for the X-ray data; Dr. Péter Orvos for the investigations of diterpenoids on GIRK and hERG channels; Dr. Annamária Kincses, Dr. Gabriella Spengler, and Dr. Katalin Burián for testing the compounds for their MDR-modulating and cytotoxic activities; and Dr. István Zupkó for measuring the antiproliferative activities of plant extracts.

I am indebted to Dr. Balázs Dankó for teaching me how to use the NMR instrument, and how to process NMR data. My special thanks is due to the staff members and laboratory personnel, especially to Erzsébet Berta and Anna Nagy who helped me in my laboratory research work. I would also like to thank my colleagues, Dr. Andrea Vasas, Dr. Katalin Veres, and Dr. Dezső Csupor, who have always provided me with help, advice, and positive reassurance. I would like to pay my special regards to Dr. Dóra Bokor for proofreading my thesis.

I am extremely grateful to my family and my friends, and to my love of life, Dr. Fanni Fekete for giving me so much emotional support, and for standing by me through thick and thin.

This work was supported by the project GINOP-2.3.2-15-2016-00012 (New ways in the natural product-based drug discovery—system metabolomic approaches to discover biologically active terpenoids of herbal and microbial origin).
THE THESIS IS BASED ON THE FOLLOWING PUBLICATIONS

   Diterpenoids from *Euphorbia dulcis* with potassium ion channel inhibitory activity with selective G protein-activated inwardly rectifying ion channel (GIRK) blocking effect

   Bioactive segetane, ingenane, and jatrophane diterpenes from *Euphorbia taurinensis*
   *Planta Medica* **2018**, 84: 729-735. If: 2.746

   Jatrophane diterpenes from *Euphorbia guyoniana* are new potent inhibitors of atrial GIRK channels

   First phytochemical investigation of secondary metabolites of *Euphorbia davidii* Subils. and antiproliferative activity of its extracts
PRESENTATIONS HELD IN THE SAME THEME OF THE THESIS

1. **Kúsz N**, Rédei D, Dankó B, Hohmann J.
   *Euphorbia dulcis*, az ígéretes diterpénforrás – Egy szűrővizsgálat eredményei
   Fiatal Gyógynövénykutatók Fóruma
   Budakalász, Hungary, 14 February 2014.

2. **Kúsz N**, Dankó B, Pinke Gy, Jakab G, Rédei D, Hohmann J.
   Phytochemical investigation of *Euphorbia dulcis* and *Euphorbia davidii*
   Trends in Natural Products Research 2014 : Young Scientists Meeting
   Olomouc, Czech Republic, 23-25 June 2014.

3. Rédei D, **Kúsz N**, Forgó P, Dankó B, Hohmann J.
   New jatrophane diterpenes from *Euphorbia dulcis*
   22nd Conference on Isoprenoids
   Prague, Czech Republic, 07-10 September 2014.

4. **Kúsz N**, Forgó P, Hohmann J, Rédei D.
   *Az Euphorbia dulcis* diterpénjeinek izolálása és szerkezet-meghatározása
   Az MTA Szteroid és Terpenoidkémiai Munkabizottság és az MTA Szegedi
   Akadémiai Bizottság Szerves és Gyógyszerkémiai Munkabizottság előadóülése
   Szeged, Hungary, 31 October 2014.

5. Rédei D, **Kúsz N**, Chaieb M, Hohmann J.
   Two new jatrophane diterpenes from *Euphorbia guyoniana*
   63rd International Congress and Annual Meeting of the Society for Medicinal
   Plant and Natural Product Research
   Budapest, Hungary, 23-27 August 2015.

   Isolation and structure determination of novel jatrophane diterpenes from
   *Euphorbia dulcis* and their GIRK-channel-inhibitory activity
   63rd International Congress and Annual Meeting of the Society for Medicinal
   Plant and Natural Product Research
   Budapest, Hungary, 23-27 August 2015.
7. **Kúsz N**, Orvos P.
   Az *Euphorbia dulcis* ból izolált jatrofánvázas diterpének szerkezetmeghatározása és GIRK-csatorna gátló hatásának vizsgálata
   Magyar Gyógyszerésztudományi Társaság, XII. Clauder Ottó Emlékverseny

   Novel MDR-modulating diterpenes from *Euphorbia taurinensis*
   65th International Congress and Annual Meeting of the Society for Medicinal Plant and Natural Product Research
   Basel, Switzerland, 03-07 September 2017.

   Az *Euphorbia taurinensis* diterpénjeinek szerkezetmeghatározása NMR spektroszkópiával
   Az MKE NMR Szakcsoport és MTA NMR Munkabizottság együttes ülése
   Balatonszemes, Hungary, 16-17 October 2017.

    Anticancer activity of diterpenes isolated from *Euphorbia taurinensis*
    5th Central European Forum for Microbiology

11. **Kúsz N**, Rédei D, Csorba A, Hohmann J.
    Változatos szerkezetű diterpének izolálása *Euphorbia* fajokból
    Az MTA Szteroid és Terpenoidkémiai Munkabizottságának előadóülése
    Szeged, Hungary, 27 November 2017