Isolation and diversity-oriented semisynthetic modification of biologically active phenanthrenes from *Juncus* species

Summary of Ph.D. Thesis

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INTRODUCTION

Family Juncaceae represents a rather unique position among the angiosperms, consists of eight genera with about 500 species worldwide, of which *Juncus* (more than 300 species) is the most important one. Various *Juncus* species are used in traditional Chinese medicine or have economic significance, e.g. the whole herb of *J. effusus* and its medulla are used for the treatment of aphta, bleeding or pharyngitis. In Egypt, the seeds of *J. rigidus* is applied to treat diarrhoea and diuretic disorders.

The most characteristic secondary metabolites of *Juncus* species are phenanthrenes. Phenanthrenes form a rather uncommon class of aromatic secondary metabolites which are presumably formed during oxidative coupling of aromatic rings of stilbene precursors. The three major groups of these metabolites are mono-, di-, and triphenanthrenes. Monophenanthrenes are further divided according to the saturation of bond between C-9 and C-10 (phenanthrenes and dihydrophenanthrenes). These compounds have a limited occurrence; to date approx. 500 compounds were identified from numerous plant species. Among them, the main sources of phenanthrenes are the species of Orchidaceae and Juncaceae families. According to their rarity, phenanthrenes are considered to be important taxonomic markers: vinyl-substituted phenanthrenes have been isolated only from Juncaceae, while prenylated compounds were reported mainly from Orchidaceae species.

Juncaceae species are important sources of naturally occuring phenanthrenes; to date, more than one hundred phenanthrenes have been isolated from the family Juncaceae, but only from eight *Juncus* (*Juncus acutus, J. atratus*, *J. effusus, J. inflexus, J. maritimus, J. roemerianus, J. setchuensis,* and *J. subulatus*) and two *Luzula* (*Luzula luzuloides* and *L. sylvatica*) species. The metabolite profiles of *Luzula* species showed similarities to *Juncus* species.

Phenanthrenes have become the objects of numerous research programs, because of their structural diversity and wide range of pharmacological activities, including antiproliferative, antimicrobial (antibacterial, antiviral, antifungal), antioxidant, anti-inflammatory, spasmolytic and anxiolytic effects.

Numerous natural and synthetic, quinone substructure containing phenanthrenoids were described to have considerable antiproliferative activities. A fast and easy method for the preparation of quinones is the application of hypervalent iodine reagents, e.g. [bis(trifluoroacetoxy)iodo]benzene (PIFA). This reagent is highly reactive, has low toxicity, and reactions can be easily progressed at room temperature in common solvents under mild conditions in a short time.

The present thesis summarizes the results of the isolation of phenanthrenes from two *Juncus* species, *J. compressus* and *J. tenuis*, and the preparation of semisynthetic derivatives of three naturally occuring phenanthrenes, namely juncuenin B, juncusol and effusol.

AIMS OF THE STUDY

Juncaceae species are considerable sources of bioactive secondary metabolites, including phenanthrenes. Synthetic and semisynthetic phenanthrenes have also been described with considerable pharmacological effects. According to these aspects, the objects of this PhD-work were:

- Isolation and structure determination of phenanthrenes from *Juncus compressus* Jacq. and *Juncus tenuis* Willd.
- *In vitro* pharmacological evaluation of the isolated compounds, and according to the experimental data determination of structure-activity-relationships.
- According to the abovementioned characteristics of quinoidal molecules, preparing oxidised semisynthetic derivatives from the isolated phenanthrenes.
- Antiproliferative investigation of the prepared compounds comparing with the starting materials.
- Determination of structure-activity relationships based on the results of pharmacological evaluations and structure informations.

MATERIALS AND METHODS

The plant material (whole plant) of *Juncus compressus* Jacq. was collected in June 2014, near Gyula (GPS coordinates are 46°35′46.19′′N, 21°10′18.97′′E), while the aerial parts of *J. tenuis* Willd. were collected in the flowering period in the Botanical Garden of the University of Szeged in June 2019. The corresponding voucher specimens (No. 876 and 889) have been deposited at the Herbarium of the Department of Pharmacognosy, University of Szeged. The plant materials were dried at room temperature.

For the reaction processes juncuenin B was isolated previously from the methanolic extract of *Juncus inflexus* using chromatographic methods (VLC on silica gel, and gel chromatography on Sephadex LH-20) and was characterised by our group. *J. inflexus* contains this compound in approximately in 0.043% amount.

For the reaction processes hypervalent iodine reagents, [bis(trifluoroacetoxy)iodo]benzene (PIFA) and (diacetoxyiodo)benzene (PIDA) were used as oxidizing agents.

The compounds were isolated by combined chromatographic techniques, including column chromatography (CC), vacuum-liquid chromatography (VLC), medium pressure liquid chromatography (MPLC), rotation planar chromatography (RPC), and high-performance liquid chromatography (HPLC). Normal (NP) or reversed phase (RP) silica gel, polyamide and Sephadex LH-20 gel were applied as stationary phases. Racemic mixtures were separated to pure enantiomers on a Lux amylose-1 column. The isolated compounds were characterized, and their structures were elucidated by means of

different spectroscopic methods (1D and 2D NMR, HR-MS). The absolute configuration of the chiral compounds was determined by chiral HPLC analysis and ECD calculations.

The antiproliferative properties of the isolated compounds and their semisynthetic derivatives was determined by using the standard MTT assay. The compounds were tested on human breast (MCF-7, T47D, KCR, HTB-26), cervical (HeLa, SiHa, C33A), and ovarian (A2780, A2780 cis) cancer cell lines and on the non-tumoral NIH/3T3 (mouse embryonic fibroblast), and MCR-5 (human embryonic lung fibroblast) cell lines, using cisplatin as the positive control.

RESULTS AND DISCUSSION

Isolation of the phenanthrenes of *J. compressus*

The air-dried, whole plant of *J. compressus* (2.2 kg) was ground and percolated with methanol at room temperature (**Fig. 1**). The crude methanol extract was concentrated under reduced pressure. The residue was dissolved in 50% methanol and subjected to solvent–solvent partitioning with dichloromethane (CH_2Cl_2) and ethyl acetate (EtOAc), respectively. After evaporation, the CH_2Cl_2 fraction was chromatographed on a polyamide column with mixtures of MeOH and H_2O (1:1, 4:1, respectively), each eluent was collected as a fraction). The fraction obtained from the polyamide column with MeOH–H_2O 4:1 was further chromatographed by VLC on silica gel with a gradient system of cyclohexane-EtOAc–MeOH (from 98:2:0 to 5:5:1) to yield 28 major fractions (I–XXVIII). These fractions were further processed by using combined chromatographic techniques (VLC, RPC, MPLC, gel filtration, HPLC) to afford 11 compounds (**1**-**11**) (**Fig. 1**).

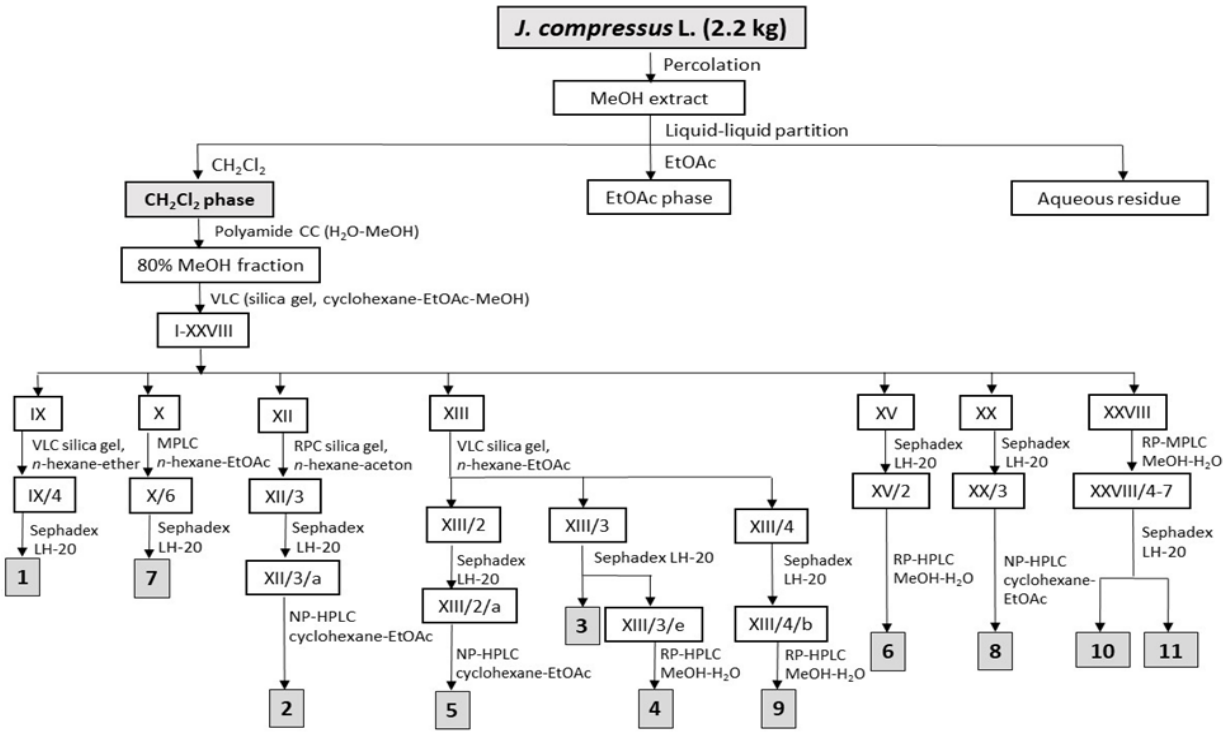


Figure 1. Isolation of the compounds of *J. compressus*

Isolation of the phenanthrenes of *J. tenuis*

The air-dried, whole plant of *J. tenuis* (1.68 kg) was ground and percolated with methanol at room temperature (**Fig.2**). The crude MeOH extract was concentrated under vacuo, the residue was dissolved in 50% methanol, and partitioned with *n*-hexane and CH_2Cl_2, respectively. After evaporation, the CH_2Cl_2 fraction was rechromatographed by VLC on silica gel with a gradient system of cyclohexane–EtOAc–MeOH (from 98:2:0 to 5:5:1) to yield 3 major fractions (I–III). These fractions were further separated by using combined chromatographic techniques (RPC, MPLC, HPLC) to afford 3 compounds (**3**, **5** and **12**) (**Fig.2**).

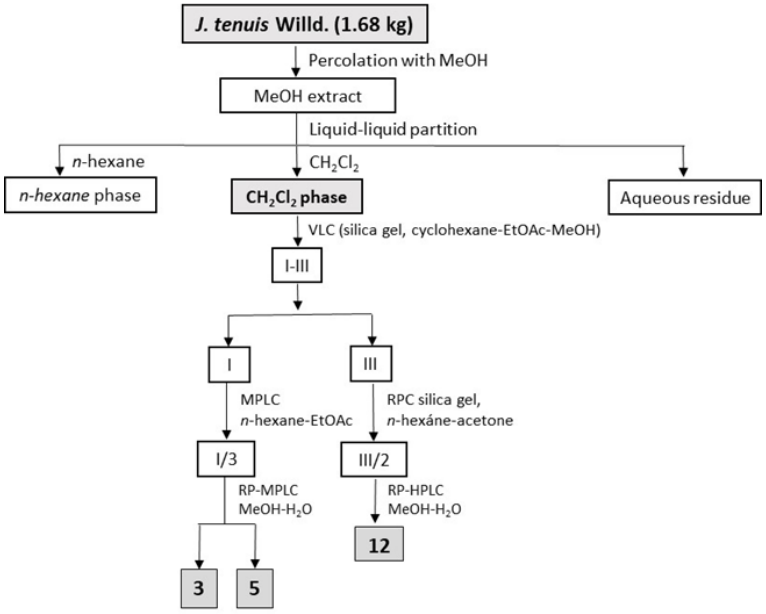


Figure 2. Isolation of the compounds of *J. tenuis*

Preparation of semisynthetic derivatives of juncuenin B

Eleven racemic and enantiomerically pure chiral semisynthetic derivatives (**13**-**23**) have been prepared from juncuenin B in five transformations (I-V) by hypervalent iodine(III) reagents using a diversity-oriented approach (**Fig. 3**). PIFA and PIDA were used as oxidants (PIFA in processes I-IV, PIDA in process V), under different conditions, in MeCN–MeOH (process I), MeOH (II), EtOH (III), *n*-BuOH (IV, V). For processing the reactions 50 or 100 mg starting material was dissolved at a concentration of 1 mg/mL, and 2 eq reagent was added. Each reaction mixture was stirred for 30 min at room temperature (reaction process I at 0 °C). Following the oxidation, the mixtures of products were subjected to solid-phase extraction on silica to remove the remaining oxidizing agent and the oxidation sideproducts. The purification process was processed by MPLC and HPLC.

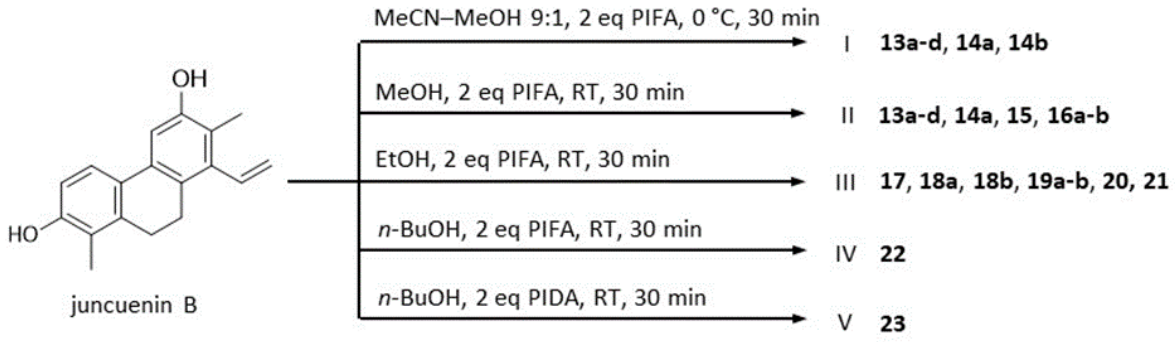


Figure 3. Preparation of the semisynthetic derivatives of juncuenin B

Preparation of the semisynthetic derivatives of juncusol and effusol

For processing the reactions of juncusol and effusol, PIFA was used as oxidizing agent in different reaction media (**Fig. 4**). 50 mg starting material was dissolved at a concentration of 1 mg/mL, and 2 eq reagent was added. Each reaction mixture was stirred for 30 min at room temperature. MeCN–MeOH 9:1 (VI and VII) and MeCN–EtOH 9:1 (VIII and IX) were chosen as solvents. The reaction mixtures were fractionated by MPLC, the final purification steps were processed on HPLC.

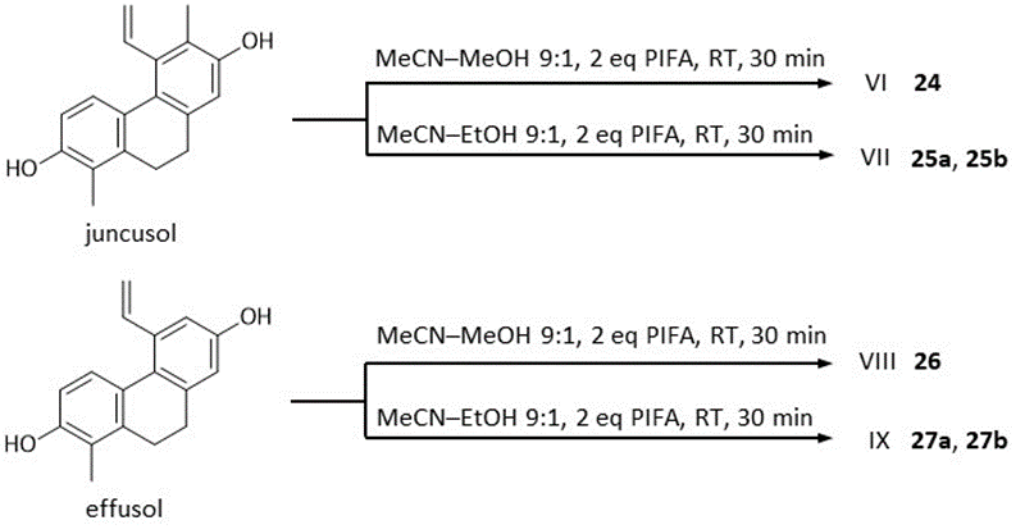
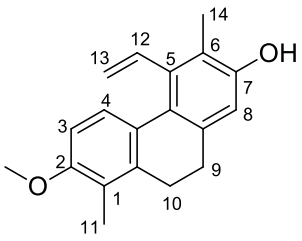


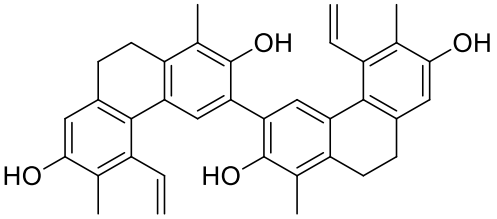
Figure 4. Preparation of the semisynthetic derivatives of juncusol and effusol

Compounds from *J. compressus* and *J. tenuis*

From the methanolic extract of *J. compressus* eleven compounds [nine phenanthrenes (**1**–**9**) and two flavonoids (**10**, **11**)] were isolated (**Fig. 5**). Purification of the methanolic extract of *J. tenuis* resulted in three phenanthrenes (**3**, **5**, and **12**). The structure elucidation of the compounds was carried out by using HR-MS measurements, 1D (^{1}H and ^{13}C) and 2D NMR (^{1}H-^{1}H COSY, HSQC, HMBC, NOESY) spectroscopic methods, and comparison of NMR spectra with literature data.



1



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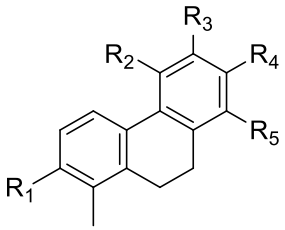
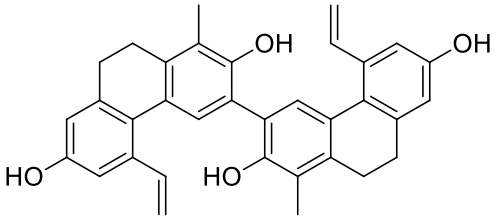


	R_1	R_2	R_3	R_4	R_5
3	OH	$CH=CH_2$	H	OH	H
4	OH	$CH(CH_3)OCH_3$	H	OH	H
5	OH	$CH=CH_2$	CH_3	OH	H
6	OH	$CH=CH_2$	H	CH_2OH	H
7	OCH_3	$CH=CH_2$	H	OH	H
12	OH	$CH=CH_2$	H	OH	CH_3



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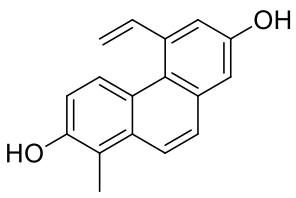

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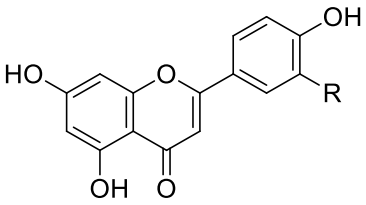


	R
10	H
11	OH

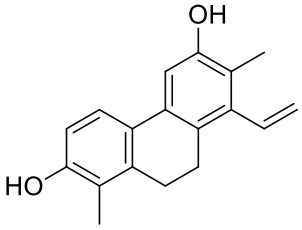
Figure 5. Structures of the compounds (**1**–**12**) isolated from *J. compressus* and *J. tenuis*

Compounds **1** and **2** were determined to be new natural metabolites, and were named as compressins A and B. Besides new compounds five dihydrophenanthrenes [effusol (**3**), effususol (**4**), juncusol (**5**), 2-hydroxy-1-methyl-7-oxymethylene-5-vinyl-9,10-dihydrophenanthrene (**6**), 7-hydroxy-1-methyl-2-methoxy-5-vinyl-9,10-dihidrophenanthrene (**7**)], one phenanthrene [dehydroeffusol (**9**)], one phenanthrene dimer [effususin A (**8**)], and two flavonoids [apigenin and luteolin (**10**, **11**)] were also isolated from *J. compressus*.

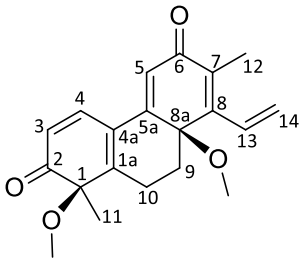
From the methanolic extract of *J. tenuis*, effusol (**3**), juncusol (**5**), and 2,7-dihydroxy-1,8-dimethyl-5-vinyl-9,10-dihydrophenanthrene (**12**) were isolated.

Semisynthetic derivatives of juncuenin B

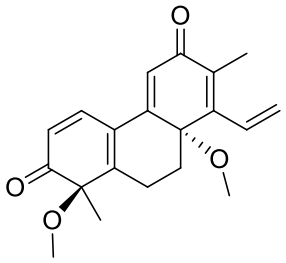
Eleven semisynthetic derivatives (**13**–**23**) have been prepared from juncuenin B, isolated previously from the roots of *J. inflexus*, during five reaction processes with different reagents, solvent systems, and temperature (**Fig. 6**).



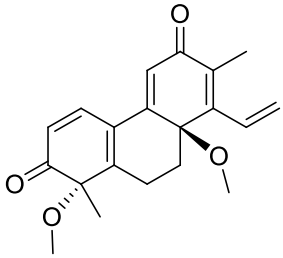
juncuenin B



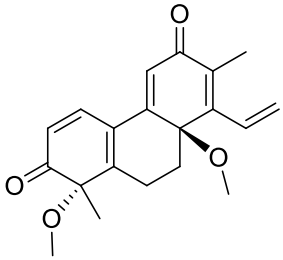
(1*R*,8a*R*)-**13a**



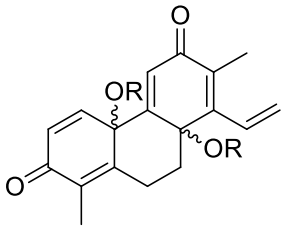
(1*R*,8a*S*)-**13b**



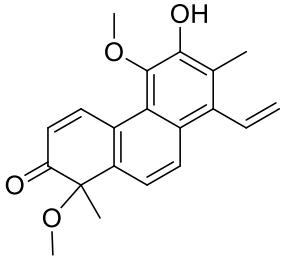
(1*S*,8a*R*)-**13c**



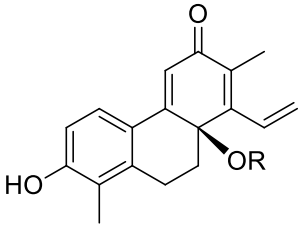
(1*S*,8a*S*)-**13d**



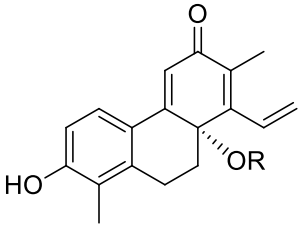
R=CH_3 *ent*-**14a**, *ent*-**14b**
R=C_2H_5 *rac*-**17**



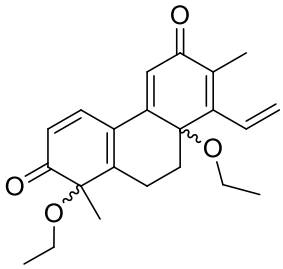
rac-**15**



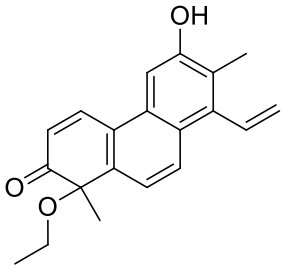
R=CH_3 (*R*)-**16a**
R=C_2H_5 (*R*)-**19a**



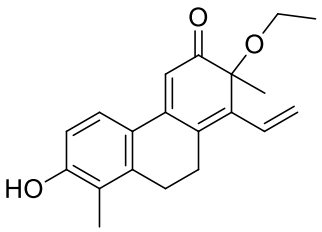
R=CH_3 (*S*)-**16b**
R=C_2H_5 (*S*)-**19b**



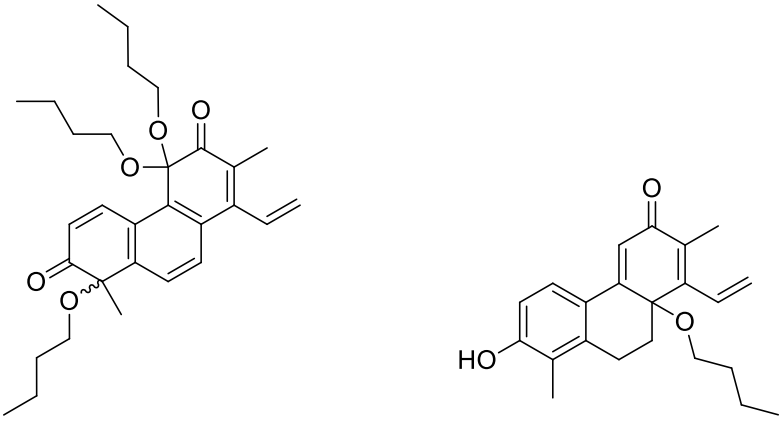
ent-**18a**, *ent*-**18b**



rac-**20**



rac-**21**



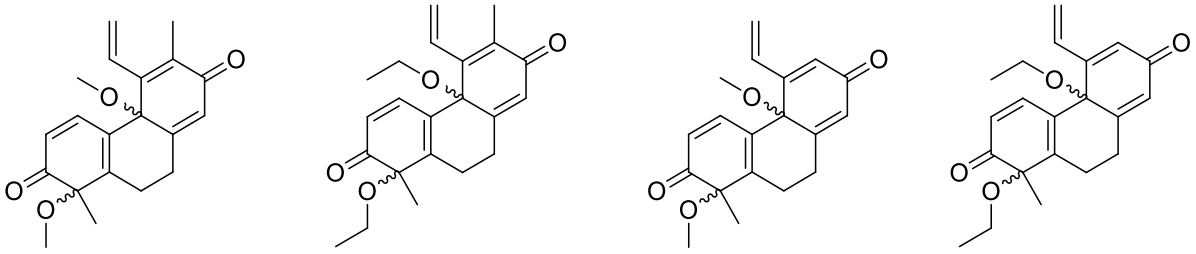
(1*R*,*S*;5*R*,*S*)-**22** *rac*-**23**

Figure 6. Structure of the semisynthetic derivatives of juncuenin B (**13**–**23**)

All the prepared compounds are chiral, most of them are bearing an *o*-, or *p*-quinol ring, and are substituted with methoxy-, ethoxy-, and butoxy groups, according to the solvent was used. According to the results of the MTT-assay investigation of these racemic compounds, compounds **13a-d**, **16a-b** and **19a-b** were divided to pure enantiomers for further investigation.

Semisynthetic derivatives of juncusol and effusol

Using effusol (**3**) and juncusol (**5**) as starting materials, isolated from *J. tenuis*, four semisynthetic derivatives (**24**-**27**) (**Fig. 7**) have been prepared from four reaction processeses under different reaction conditions. Compounds **24** and **25** are derived from juncusol, and **26** and **27** from effusol. The isolation and purification of compounds were performed by MPLC, and HPLC techniques.



rac-**24** *ent*-**25a**, *ent*-**25b** *rac*-**26** *ent*-**27a**, *ent*-**27b**

Figure 7. Structures of semisynthetic derivatives of juncusol and effusol

The isolated compounds are racemic mixtures, all of them contain a *p*-quinol ring, and are substituted with methoxy-, and ethoxy-groups. The most considerable structural attributes of these derivatives is the presence of a side chain at C-5a. Comparing this structure with natural compounds, it may be mentioned as a special case, as neither the naturally occuring phenanthrenes, nor the derivatives of juncuenin B are substitued in this position. This phenomenom may provide further structural informations reflected on antiproliferative activities.

Antiproliferative activity of the phenanthrenes isolated from *J. compressus*

As a continuation of pharmacological evaluation of the isolated phenanthrenes, their antiproliferative activity was measured using the standard MTT assay. The phenanthrenes, isolated from *J. compressus*, were tested against three human tumor cell lines [HeLa and SiHa (cervix adenocarcinoma) and A2780 (ovarian carcinoma)] using cisplatin as a positive control (**Table 1**).

Table 1. IC_{50} values of the compounds isolated from *J. compressus*

Compound	Concentration (μM)	Growth inhibition (%) ± SEM [calculated IC_{50} value (μM)]		
		HeLa	SiHa	A2780
1	10	41.7 ± 1.1	–	47.6 ± 1.6
	30	93.7 ± 0.4	38.6 ± 0.8	73.0 ± 0.5
		11.3		**13.2**
2	10	92.0 ± 0.4	–	–
	30	92.4 ± 0.2	32.8 ± 0.9	64.9 ± 2.0
		1.9		
3	10	96.4 ± 0.3	–	–
	30	97.8 ± 0.3	–	50.5 ± 1.6
		3.7		
4	10	–	–	18.3 ± 0.4
	30	30.4 ± 1.9	14.2 ± 2.8	72.1 ± 1.1
5	10	97.7 ± 0.4	17.1 ± 2.4	18.1 ± 1.3
	30	97.8 ± 0.2	29.3 ± 2.0	63.4 ± 1.0
		1.3		
6	10	96.8 ± 0.3		–
	30	98.8 ± 0.2	10.3 ± 1.5	–
		4.2		
7	10	45.4 ± 1.6	14.2 ± 1.0	–
	30	92.4 ± 0.2	29.5 ± 3.4	63.2 ± 2.5
		10.7		
8	10	–	–	–
	30	10.5 ± 0.5	14.4 ± 1.5	72.7 ± 1.8
9	10	75.2 ± 2.5	–	–
	30	96.5 ± 0.3	28.7 ± 2.0	57.3 ± 2.6
		7.8		
cisplatin	10	42.6 ± 2.3	88.6 ± 0.5	83.6 ± 1.2
	30	99.9 ± 0.3	90.2 ± 7.8	95.0 ± 0.3
		12.4	**7.8**	**1.3**

Some structural evidences reflected to the antiproliferative activity obtained from the bioactivity of the isolated compounds. Since **4** is markedly less effective, a bulky substituent instead of vinyl group seems to be disadvantageous. Compound bearing free hydroxy groups (in case of **3**) exerted more pronounced activity than its methyl ether (**7**) or its oxymethylene derivative (**6**) on HeLa cell line. The substantial difference in the activities of the isolated dimers (**2** and **8**) could be attributed to the presence of a methyl group at C-6, which is favoured in the monomers, too. Both of other cell

lines were substantially less sensitive. Although **1**, **4** and **8** exhibited some considerable activity against A2780 cells, none of them were comparable to cisplatin. Similarly, none of the presented agents elicited relevant growth inhibition (>40%) against SiHa cells, and therefore, IC_{50} values were not determined.

Antiproliferative activity of the semisynthetic derivatives of juncuenin B

The first step of the comprehensive evaluation of the antiproliferative activity was the investigation of all the obtained diastereomers. The compounds were measured on human breast (MCF-7, T47D), cervical (HeLa, SiHa, C33a) and ovarian (A2780) gynecological cell lines with cisplatin as positive control. In order to get some insights into their tumor selectivity, the compounds have also been tested on NIH/3T3 cell line. According to the structural points of view, it is evident that compounds without *p*-quinol ring (**15**, **20**, **21**) and compounds with two *p*-quinol moieties (**14**, **17**) did not exert antiproliferative activity. These compounds are substituted with an alkoxy group at C-4a. Comparing with the starting material juncuenin B, the most effective racemic compounds, **13a**-**d**, **16a**-**b** and **19a**-**b** were separated into pure enantiomers for further investigations. In the second step, the enantiomerically pure compounds (**13a**-**13d**, **16a**, **16b**, **19a** and **19b**) were evaluated for their antiproliferative activity against the same cell lines (**Table 2**).

Table 2 The IC_{50} values of the enantiopure semisynthetic juncuenin B analogues

Compound	Calculated IC_{50} values (μM ± SEM)						
	MCF-7	T47D	HeLa	SiHa	C33a	A2780	NIH/3T3
(1*R*,8a*R*)-**13a**	>30	>30	>30	>30	14.8±0.7	13.3±1.5	>30
(1*R*,8a*S*)-**13b**	>30	>30	>30	>30	10.2±1.7	9.0±1.0	>30
(1*S*,8a*R*)-**13c**	>30	12.5±0.9	>30	28.1±0.1	5.3±0.9	7.7±0.6	21.6±0.4
(1*S*,8a*S*)-**13d**	>30	>30	>30	>30	13.0±0.2	11.0±0.4	>30
(8a*R*)-**16a**	6.2±1.6	>30	0.9±0.4	>30	5.3±0.6	8.7±0.8	25.8±4.6
(8a*S*)-**16b**	>30	>30	>30	>30	>30	>30	>30
(8a*R*)-**19a**	20.6±0.3	8.8±1.1	3.7±0.9	>30	4.9±0.3	2.8±0.3	>30
(8a*S*)-**19b**	>30	>30	>30	>30	>30	>30	>30

IC_{50} values (μM, mean ± SEM) were determined by MTT assay by treating the cells with each compound (0.1–30 μM) for 72 hours. Data are based on two independent experiments.

The IC_{50} value of juncuenin B on MCF-7, C33a, and SiHa cell lines was determined for the first time. Among the investigated compounds, the highest activities were recorded for **13c**, **16a** and **19a**, reaching or exceeding that of the positive control cisplatin against HeLa and/or T47D cell lines. (8a*R*)-**16a** was found to be the most promising compound with substantial antiproliferative effects against all tested cell lines except for T47D and SiHa, but it was inactive against the non-tumoral NIH/3T3 cells.

Compounds **16a**, **16b**, **19a**, **19b**, and **23** contain a nonoxidized ring A, while ring C has a *p*-quinol structure substituted with an alkyl ether moiety. In the case of these compounds, the effect of the

length of the ether chain at C-8a is undefined: on the T47D, C33A, and A2780 cell lines, compound **19a** with an ethoxy group showed the highest inhibition. In case of HeLa cell line, the antiproliferative activity was inversely proportional to the chain length of the alkoxy groups (IC_{50} values **23** > **19a** > **16a**).

Compounds **16a** and **19a** showed an antiproliferative effect on HeLa, C33A, MCF-7, and A2780 cells, which was comparable to or stronger than that of juncuenin B, while their stereoisomers **16b** and **19b** had no relevant influence on the cell proliferation, indicating that (8a*R*) is the preferred absolute configuration of these compounds.

Regarding the pure enantiomers **13a**-**d**, the (1*S*,8a*R*)-configuration (**13c**) was the most beneficial for the antiproliferative effect. The activities of (1*S*,8a*R*)-**13c**, (*R*)-**16a**, and (*R*)-**19a** suggest the preference of the (*R*) configuration for the antiproliferative activity. Since **13a** is less active than **13c**, the (1*S*) configuration seems to be also essential in the case of quinoidal compounds.

Antiproliferative activity of the semisynthetic juncusol and effusol derivatives

The compounds isolated from *J. tenuis* (**3**, **5**, and **12**) and their semisynthesized analogues (**24**–**27**) were measured on human breast (MCF-7, KCR, T47D, and HTB-26), cervical (HeLa), and ovarian (A2780 and A2780cis) cancer cells, and on MRC-5 (human embryonic lung fibroblast) cell lines (**Table 3**).

Table 3. Antiproliferative activity of semisynthetic derivatives of juncusol and effusol

Compound	Calculated IC_{50} values (μM ± SEM)							
	A2780	A2780cis	KCR	MCF-7	HeLa	HTB-26	T47D	MRC-5
12	23.8±1.3	37.1±2.8	35.8±1.7	37.1±1.1	0.5±0.0	41.7±3.5	25.0±0.4	40.9±2.0
5	33.1±3.1	30.4±0.4	39.3±1.6	48.6±3.4	2.3±0.7	57.0±2.7	24.6±1.9	60.1±5.1
3	22.3±2.7	16.9±4.7	24.2±2.1	12.9±0.2	24.7±0.3	22.8±0.2	14.2±1.1	18.9±4.0
rac-**24**	80.3±3.0	88.2±3.1	>100	52.1±4.8	>100	74.3±3.6	36.5±1	>100
ent-**25a**	66.0±4.4	62.9±1.7	>100	80.5±3.5	94.7±3.4	94.3±2.0	56.3±3.4	>100
ent-**25b**	39.4±3.1	38.0±4.6	44.2±2.6	41.0±0.9	61.9±0.3	45.8±3.2	30.3±2.5	57.7±0.3
rac-**26**	8.6±0.5	10.9±2.2	18.9±1.4	5.8±0.2	12.9±0.4	10.9±0.9	7.0±1.0	12.2±0.2
ent-**27a**	25.2±1.8	22.5±0.2	23.5±0.8	11.7±0.7	24.4±0.8	16.1±0.2	11.6±0.3	14.3±0.5
ent-**27b**	22.0±2.0	22.1±1.6	29.4±0.9	10.2±0.1	35.0±1.5	20.1±1.1	14.2±0.6	23.4±1.3
cisplatin	3.6±0.3	7.3±0.2	6.7±0.4	1.4±1.1	2.3±0.1	20.1±0.2	5.9±0.1	0.6±0.1

Determinations were performed by MTT assay, by treating the cells with compounds (100–0.19 μM) for 72 h. Data are based on two independent experiments.

In this investigation, among the natural phenanthrenes, 2,7-dihydroxy-1,8-dimethyl-5-vinyl-9,10-dihydrophenanthrene (**12**) was the most active on all tested cell lines, with the exception of HeLa cell line (**Table 3**). The only difference between juncusol (**5**) and effusol (**3**) is the presence of a methyl group at C-6 in juncusol. Juncusol (**5**) and effusol (**3**) possessed significant antiproliferative activity on HeLa cells (IC_{50} values 0.5 μM for **5**, and 2.3 μM for **3**, respectively).

Among the derivatives, compound **26** was found to be the most promising semisynthetic component with substantial antiproliferative effects against all tested cell lines, except for KCR, which

was comparable to that of the positive control cisplatin. Unfortunately, **26** had antiproliferative activity (IC_{50} = 12.2 µM) against the non-tumoral MRC-5 cells. Compounds **27a** and **27b** showed marked antiproliferative activity against MCF-7 cells (IC_{50} values 11.7 µM for **27a**, and 10.2 µM for **27b**, respectively). In the case of 2,7-dihydroxy-1,8-dimethyl-5-vinyl-9,10-dihydrophenanthrene (**12**), IC_{50} value 12.9 µM was detected on MCF-7 cells. None of the juncusol derivatives exceeded the antiproliferative effects of the parent compound. Although compounds **24** and **25a** did not show an antiproliferative effect against normal (MRC-5) cells at tested concentrations, they possessed very weak activity against the investigated tumor cell lines. Comparing the data of effusol and juncusol derivatives (**24** and **26**), it can be stated that the presence of a methyl group at C-6 in the semisynthetic compounds resulted in a decrease of the toxic effect.

SUMMARY

The present PhD thesis includes the results of phytochemical investigation of two *Juncus* species, *J. compressus* and *J. tenuis*, and the preparation and characterization of oxidized derivatives of three phenanthrenes, juncuenin B, juncusol and effusol. The structure determination was carried out by spectroscopic analysis, and the antiproliferative activities of the compounds were determined by the MTT assay.

From the dried plant materials using combined chromatographic techniques 12 compounds (**1**–**12**) have been isolated, among them 10 phenanthrenes and 2 flavones. Two components, compressins A (**1**) and B (**2**), are new natural products. All of the isolated compounds were detected for the first time from the investigated plants.

Compressin A (**1**) showed inhibition against A2780 cell line with IC_{50} 13.19 µM, while compressin B (**2**) proved to be active against HeLa cells with IC_{50} value 1.86 µM, respectively. Based on our results and literature data, dimerization may increase the antiproliferative activity of phenanthrenes.

15 Semisynthetic phenanthrenoid derivatives of juncuenin B, juncusol (**5**), and effusol (**3**) with wide-range structural diversity were identified. The pure enantiomers of the most active products of juncuenin B were investigated. Compounds **13c**, **16a** and **19a** were found to be the most effective ones with IC_{50} values 5.3 µM on C33a (**13c**), 0.9 µM on HeLa (**16a**), and 2.8 µM on A2780 (**19a**) cell lines, respectively. According to the pure enantiomers of **16** and **19**, it was evident that (8a*R*) is the preferred configuration for anticancer effects. Moreover, these compounds did not have considerable activity against the non-tumoral NIH/3T3 cell line compared to the positive control cisplatin.

The semisynthetic derivatives of juncusol (**5**) and effusol (**3**) possessed an irregular structural attribute as these compounds are bearing substituents at C-5a. Their antiproliferative activities indicate that the presence of this structural moiety does not increase the antiproliferative effect of these quinoidal phenanthrenoid derivatives, as only one derivative, **26** showed considerable IC_{50} values on the tested cell lines compared with its parent compound, effusol (**3**).

Our results reveal that Juncaceae species are promising sources of biologically active compounds. Moreover, secondary metabolites of Juncaceae species, especially phenanthrenes, can be regarded as promising starting materials in the search for new pharmaceutical agents. Semisynthetic modification of phenanthrenes not only expanded the chemical space, but also resulted in more effective compounds.

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