Short thesis for the degree of doctor of philosophy (PhD)

Synthesis of bioactive *O*- and *O,N*-heterocycles and their structural analysis

Ádám Szappanos

Supervisor: Tibor Prof. Dr. Kurtán

UNIVERSITY OF DEBRECEN

Doctoral School of Chemistry

Debrecen, 2021

1. Introduction and objectives

Among the condensed *O*- and *O, N*-heterocycles containing a chroman or 2*H*-chromene core, there are several compounds that show remarkable pharmacological activity. These compounds were often prepared by the formation of appropriate functional groups on the flavonoid skeleton and their cyclization reactions resulted in the fused heterocyclic moiety. *O,N*-heterocyclic derivatives containing a chroman or 2*H*-chromene ring condensed with morpholine, pyrrole and thiazole subunits show a variety of bioactivity, including the tau protein kinase 1 (TPK1) inhibitor **1**, the ion channel modulator **2**, and the antibacterial derivative **3** (Figure 1).

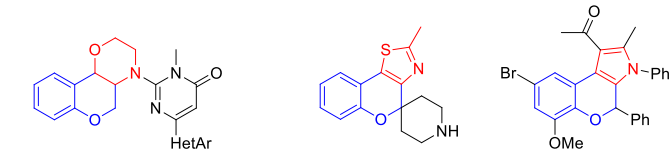


Figure 1. Structure and activity of bioactive chroman and 2*H*-chromene derivatives condensed with morpholine, thiazole and pyrrole units at the C-3–C-4 bond.

Isoflavonoids represent a significant subgroup of flavonoids. After isoflavones, pterocarpans represent the second largest group of natural isoflavonoids, which are known as phytoalexins (plant defensive agents), acting as potent inhibitors of pathogens (Figure 2).

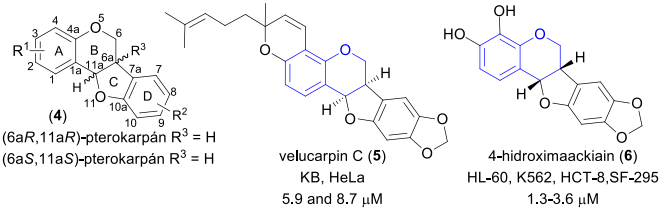
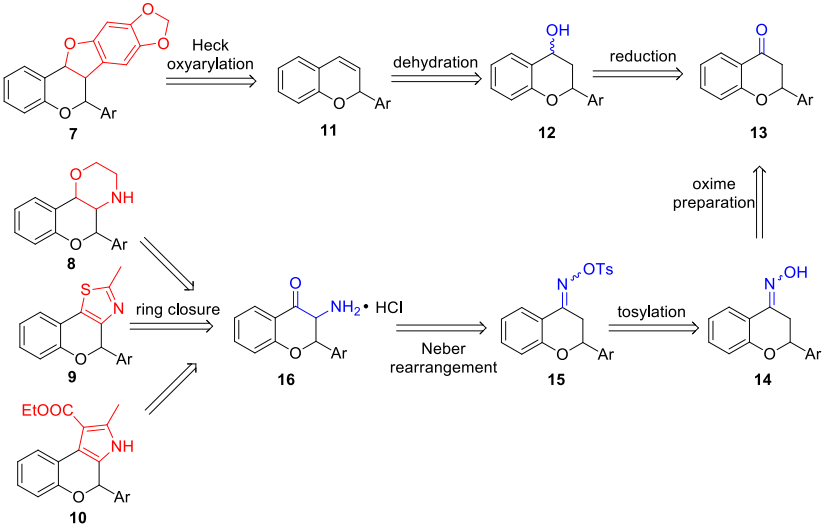


Figure 2. The pterocarpan skeleton with numbering and structures of naturally occurring cytotoxic pterocarpans containing 8,9-methylenedioxy substituent.

Recently, they gained considerable attention because of their antiproliferative activities in various cancer cell lines with low micromolar IC_{50} values (Figure 2.).

The remarkable bioactivity of these compounds prompted me in my doctoral research to aim at the synthesis of pterocarpans **7** substituted at the C-6 position with a naphthyl group, as well as chroman and 2*H*-chromene derivatives condensed with morpholine **8**, thiazole **9** and pyrrole **10** ring and investigate their antiproliferative activity on human cancer cell lines (A2780 and WM35) (Scheme 1.). The synthesis of *O*- and *O,N*-heterocyclic target compounds **7–10** can be traced back to the flavanone intermediate **13** (Scheme 1.), which represents the common flavonoid intermediate in the two synthetic pathways. Furthermore, we planned the separation of stereoisomers of the prepared chiral racemic compounds and the assignment of the absolute configuration by chiral HPLC-ECD studies.



Scheme 1. Retrosynthetic scheme for the preparation of condensed *O,N*-heterocycles *via* flavonoids, as planned in the frame of my doctoral research work**.**

2. Applied methods

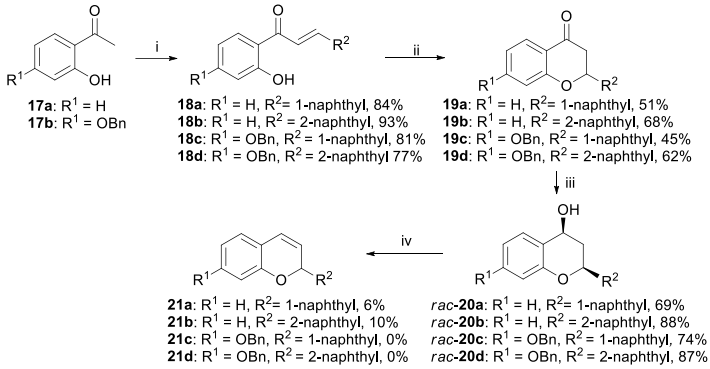
During the synthetic work, we used the macro-, semi-micro- and micro-methods of modern preparative organic chemistry Thin layer chromatography was used to monitor the reaction and to check the purity of the products. For the purification of the reaction mixture, crystallization, column chromatography and preparative thin layer chromatography were

utilized. The prepared compounds were characterized by classical analytical methods (elemental analysis, melting point), as well as by mass spectrometry, ^{1}H- and ^{13}C-NMR spectroscopic methods and single-crystal X-ray diffraction studies. In the NMR studies, the total ^{1}H- and ^{13}C-NMR assignments of the products were performed using two-dimensional techniques (^{1}H-^{1}H COSY, NOESY and ^{13}C-^{1}H HSQC, HMBC), and the homo- and heteronuclear three-bond coupling constants and NOE effects were used to determine the relative configuration. ECD spectra were measured in solution by online HPLC-ECD technique. The cytotoxic effect of the molecules was performed on A2780 (cervical cancer) and WM35 (melanoma) cell lines with MTT test.

3. New scientific results of the dissertation

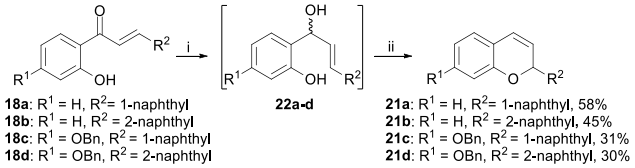
3.1. For the Heck-type oxyarylation reaction, four 2-naphthyl-2*H*-chromene derivatives with different substitution patterns were prepared by two synthetic routes *via* chalcone derivatives.

The synthesis of 2*H*-chromenes **21a-d**, starting materials of the Heck oxyarylation, was planned *via* ***rac*-20a-d** flavan-4-ol derivatives. In the first step, the appropriate acetophenone starting materials (**17a,b**) were reacted with naphthyl carbaldehydes in 50% aqueous KOH solution. The *Claisen-Schmidt* condensation yielded the expected **18a-d** chalcone analogues with high yield. The racemic flavanone analogues **19a-d** were prepared by intramolecular oxa-*Michael* addition, when chalcone derivatives **18a-d** were refluxed in ethanol in the presence of a mild base. The method provided the expected derivatives with moderate yields (45-68%), which is acceptable considering that the transformation was not complete due to the reversible reaction. After the cyclization, the carbonyl group of racemic flavanone analogues **19a-d** was reduced in methanol at room temperature using sodium tetrahydroborate ($NaBH_4$) as a reducing agent. The reduction was diastereoselective and the *cis*-flavan-4-ol analogues *rac*-**20a-d** were isolated with good yield (69-88%). The acid-catalyzed water elimination of *rac*-**20a-d** alcohols resulted in the expected **21a-d** 2*H*-chromene compounds just in traces or not at all when using various mild acidic conditions disclosed in the literature. (1 % HCl solution/acetone/Δ; 10 % HCl solution/acetone; or i-PrOH/Δ, PTSA/abs. toluene/Δ) (Scheme 2.). The low yield of the reaction can be explained by the acid-catalyzed dimerization of the 2-phenyl-2*H*-chromene derivative as described in the literature, which modified further our target compounds in the acidic reaction mixture.



Scheme 2. Synthesis of 2*H*-chromenes (method A): i: R^2-CHO, 50% KOH, MeOH, rt (77-93%) ii: NaOAc, EtOH, reflux (45-68%) iii: $NaBH_4$, MeOH, rt (69-88%) iv: 1% HCl/acetone, reflux (0-10%).

The solution for the low yield of the dehydration step of flavan-4-ol derivatives *rac*-**20a-d** was provided by a side-reaction described in the literature when the chalcone derivative was reduced with sodium tetrahydroborate in the presence of cerium(III)chloride affording the 2*H*-chromene derivative after acid treatment.



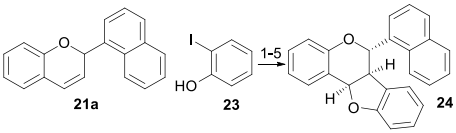
Scheme 3. Preparation of 2*H*-chromene derivatives *via* chalcone analogues in one-step. i: $NaBH_4$, $CeCl_3 \cdot 7H_2O$, THF/EtOH, 0°C ii: citric acid (30-58%).

By using this one-pot reaction on our **18a-d** chalcone analogues, we successfully prepared **21a-d** 2*H*-chromene derivatives in one step and with acceptable yields (Scheme 3.). The advantage of the process is the shorter synthetic route; two instead of four, while the overall yield from acetophenone increased from 2-6% to 23-49% and the dimerization did not prevail.

3.2. The synthesis of four 6-naphthyl-8,9-methylenedioxypterocarpan derivatives was performed by the Heck-oxyarylation reaction of 2*H*-

chromenes and 2-chloromercury-4,5-methylenedioxyphenol and the regioselectivity of the coupling was investigated.

To test catalytic Heck oxyarylation, the 2-naphthyl-2*H*-chromene derivative **21a** was reacted with *o*-iodophenol (**23**) using the catalytic conditions described in the literature (Entry 1: 20% $Pd(OAc)_2$, PPh_3, Ag_2CO_3), which resulted in the **24** *cis*,*cis*-6-(1-naphthyl) pterocarpane with low 16% yield (Table 1).



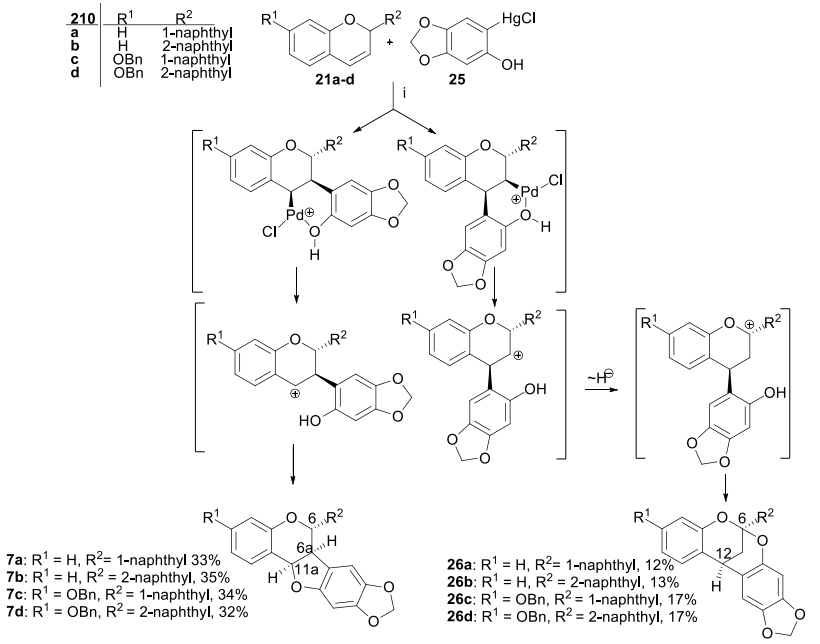
Entry	Reagent	Solvent	Time (h)	Temperature (°C)	Yield (%)
1	PPh_3, Ag_2CO_3 20 mol% $Pd(OAc)_2$	acetone	24	56	16
2	PPh_3, Ag_2CO_3 50 mol% $Pd(OAc)_2$	acetone	48	56	16
3	10 mol% Xantphos 1.2 ekv. DEA 5 mol% $Pd(OAc)_2$	DMF	48	160	-
4	20 mol% Xantphos 1.2 ekv. DEA 5 mol% $Pd(OAc)_2$	DMF	48	160	-
5	10 mol% Pd(C) NaOAc	NMP	24	140	-

Table 1. The catalytic Heck oxarylation of **21a** 2*H*-chromene derivative with *o*-iodophenol (**23**) utilizing different reaction conditions.

In order to improve the yield, the amount of catalyst was increased (entry 2), the phosphine ligand was replaced (entry 3 and 4), and Pd(C) was used in *N*-methyl-2-pyrrolidone (NMP) (entry 5) but the yield could not be increased.

There are many examples in the literature that catalytic Heck oxyarylation reactions are often unsuccessful or resulted in the product with low yield if the *o*-iodophenols were rich in electron-donating groups. These results, as well as our own experiments, justified the use of the much more reactive *o*-chloromercuryphenols instead of *o*-iodophenol derivatives.

Thus, the Heck-type oxyarylation reaction of 2*H*-chromenes **21a-d** was performed with 2-chloromercury-3,4-methylenedioxyphenol (**25**) using palladium chloride and lithium chloride in anhydrous acetone.



Scheme 4. Heck oxyarylation mechanism of 2-naphthyl-2*H*-chromene derivatives and 2-chloromercury-4,5-methylenedioxyphenol. i: $Li_2[PdCl]_4$, acetone, rt.

The coupling reaction was not regioselective, since it yielded two constitutional isomers, the **7a-d** pterocarpan derivatives as the major product (32-35%) and the **26a-d** dioxocine derivatives (12-17%). Although the cross-coupling step is not regioselective, the new chirality centers formed diastereoselectively, which also determines the (6*S**, 6a*R**, 11a*R**) and (6*R**, 12*R**) relative configuration of the resulting products **7a-d** and **26a-d**, respectively.

From the previous results of our research group, we concluded that the substituent at the C-2 position of 2*H*-chromene derivatives has an effect on the ratio of the resultant constitutional isomers. If there is no substituent at this position, the bridged 6,12-methanodibenzo[d,g][1,3]dioxocine derivative formed in a very small amount. When the starting 2*H*-chromene

derivative contained a C-2 phenyl group, the ratio of the two isomers was 4: 1 favoring the pterocarpan. In our case, where the C-2 substituent was a 1-naphthyl or 2-naphthyl group, we observed that the ratio of isomers changed to 2.75:1 and 2.69:1, respectively (entry 1 and 2, Table 2), and the formation of the bridged derivative was enhanced. If the 2*H*-chromene derivative contained an electron-donating group on ring A, such as a benzyloxy group at the C-7 position for our molecules **21c** and **21d**, the amount of dioxocine products increased further (entry 3 and 4, Table 2).
From the ratio of the isomers, it could be concluded that the proportion of the bridged dioxocine by-product in the Heck reaction increased with the growing steric bulk of the C-2 substituent of the 2*H*-chromene derivative. The electron-donating benzyloxy group at C-7 promoted the coupling of the aryl unit to C-4 through its electronic effect and hence the formation of the dioxocine product.

Entry	Chromene + ArHgCl	Products (yield %)	Ratio of products
1	**21a** + **25**	**7a** (33), **26a** (12)	**7a**:**26a** = 2,75:1
2	**21b** + **25**	**7b** (35), **26b** (13)	**7b**:**26b** = 2,69:1
3	**21c** + **25**	**7c** (34), **26c** (17)	**7c**:**26c** = 2,00:1
4	**21d** + **25**	**7d** (32), **26d** (17)	**7d**:**26d** = 1,88:1

Table 2. The results of Heck reaction.

3.3. The antoproliferative activity of our pterocarpan target compounds was evaluated on human cancer cell lines (A2780 and WM35) and the low micromolar IC_{50} values of the active derivatives provided the best activity in the family of pterocarpans.

In collaboration with the Department of Physiology, the antiproliferative activity of the synthesized **7a-d**, **24** pterocarpans were evaluated against A2780 cervical cancer and WM35 melanoma cell lines. The pterocarpans **7a** and **7b** showed promising activity when applied at 50 μM concentration, as they completely prevented cellular proliferation. The other three derivatives were found to have much weaker activity under the same conditions (Table 3).

The IC_{50} values were determined for the most active compounds, from which the 1-naphthyl derivative **7a** showed the best antiproliferative activity on both cell lines, which represents the best reported activities of pterocarpan derivatives (Table 3). There is only a single reported example on the antiproliferative activity of pterocarpans against A2780 cell line,

which consists in the natural pterocarpan glucosides, trifolirhizin, having 8,9-methylenedioxy substitution and moderate antiproliferative activity (50 % growth inhibition with concentration up to 100 μM). There is no literature data available on the antiproliferative activity of pterocarpans against WM35 melanoma cell line.

Compound	Cell line/IC_{50} (μM)	
	A2780	WM35
7a	0.80 ± 0.41	3.51 ± 1.84
7b	4.16 ± 1.06	6.15 ± 1.29
Doxorubicin[a]	0.07	0.14

[a] positive control

Table 3. *In vitro* antiproliferative activity of pterocarpans **7a** and **7b** against the A2780 and WM35 cell lines determined by MTT assay

The weak activity of **24** suggested that the 8,9-methylenedioxy substitution of the pterocarpan scaffold is required for the activity. The substitution pattern of the aromatic ring A had also considerable influence, since activity diminished in the presence of a C-3 benzyloxy substituent.

3.4. For the separation of enantiomers of chiral racemic pterocarpan and dioxocine derivatives and their flavanone and 2*H*-chromene precursors, we developed chiral HPLC methods, recorded the HPLC-ECD spectra of the separated enantiomers and their absolute configuration was determined by TDDFT-ECD calculations.

The antiproliferative activity of our pterocarpans prompted us to separate their enantiomers with HPLC using chiral stationary phase, record the online HPLC-ECD spectra of the enantiomers and assign the absolute configuration by means of TDDFT-ECD calculations.

The HPLC-ECD spectra of the first-eluting enantiomers of **7a** and **7b** were markedly different, which suggested that the 1- and 2-naphthyl chromophores have dominant contribution to the ECD features. Therefore, the ECD helicity rule of pterocarpan or comparison with the ECD spectra of natural pterocarpans cannot be used to assign the absolute configuration of these compounds.

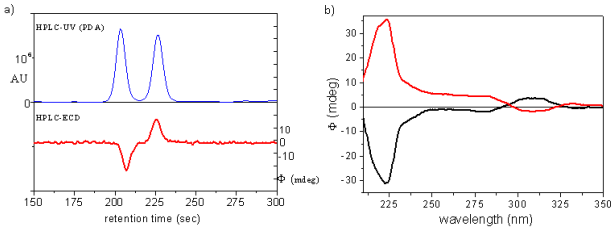


Figure 3. a) HPLC-UV and -ECD traces of **9a** on Chiralpak IA column with hexane/2-propanol 80:20 eluent monitored at 250 nm. b) HPLC-ECD spectra of the first- [black, (6*S*,6a*R*,11a*R*)] and second-eluting [red, (6*R*,6a*S*,11a*S*)] enantiomers of **7a**.

Thus, the solution TDDFT-ECD calculation protocol was utilized to determine the absolute configuration of the separated enantiomers, which resulted in two low-energy conformers that differed only the orientation of the naphthyl groups. The calculated ECD spectra of the conformers were in good agreement with the measured spectra of the second eluting enantiomer, and thus its absolute configuration could be assigned (Figure 3).

Using this procedure on our racemic molecules **19a-d**, **21a-d**, and **26a-d**, we observed that the calculated ECD spectra of the separated enantiomers reproduced the measured spectra well in each case, and thus their absolute configuration could be assigned.

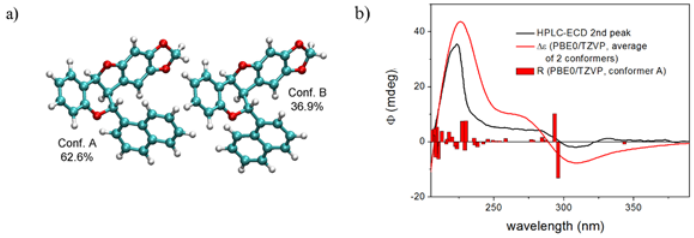
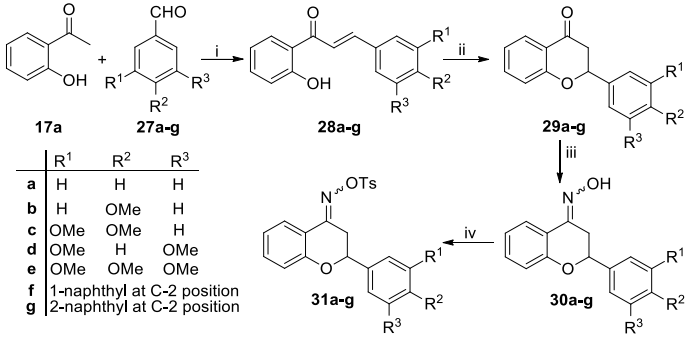


Figure 4. a) Structure and population of the low-energy (≥ 1%) B3LYP/TZVP PCM/$CHCl_3$ conformers of (6*R*,6a*S*,11a*S*)-**7a**, b) Experimental HPLC-ECD spectrum of the second-eluting enantiomer of **7a** (black curve) compared with the Boltzmann-weighted PBE0/TZVP PCM/$CHCl_3$ ECD spectrum (red curve) of (6*R*,6a*S*,11a*S*)-**7a** computed for the B3LYP/TZVP PCM/$CHCl_3$ conformers. Bars represent the rotational strength values of the lowest-energy conformer.

3.5. We developed a synthetic route for the preparation of 3-aminoflavanone from 2'-hydroxyacetophenone *via* Neber rearrangement of tosyloxime derivatives.

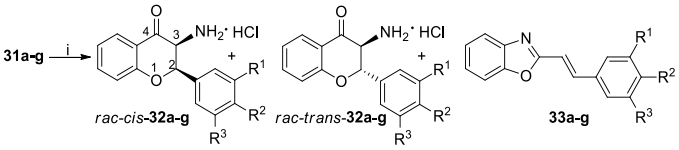
The **31a-g** tosyloxime starting materials of the Neber rearrangement for the synthesis of 3-aminoflavanone derivatives *rac*-**32a-g** were synthesized from 2'-hydroxyacetophenone (**17a**) in four steps with seven different substitution patterns at the C-2 aryl unit (Scheme 5). The first two steps were performed biomimetically as described for the synthesis of 2*H*-chromenes **21a-d**. The 2'-hydroxyacetophenone (**17a**) was reacted with seven different arylcarbaldehydes in a *Claisen-Schmidt* condensation reaction to give chalcone derivatives **28a-g** with good yield (65-98%), which were transformed to racemic flavanone analogues **29a-g** in a biomimetic intramolecular oxa-*Michael* addition. The reaction gave the expected products with acceptable yields (54-75%). In the next step, the synthesis of oximes **30a-g** was carried out with hydroxylamine hydrochloride refluxing in ethanol in the presence of a strong base to give the desired products with excellent yield (81-98%). The hydroxyl group of oximes was tosylated with tosyl chloride in anhydrous dichloromethane in the presence of triethylamine (**30a-g** → **31a-g**).



Scheme 5. Synthesis of tosyl oxime derivatives; i: NaOH, EtOH, rt, 1 d (65-98%). ii: NaOAc, EtOH, reflux, 3 h (54-75%). iii: $NH_2OH{\cdot}HCl$, NaOH, EtOH, reflux, 6 h (81-98%). iv: TsCl, dry CH_2Cl_2, Et_3N, reflux, 3 h (80-93%).

The *Neber* rearrangement of the tosyl oxime derivatives **31a-g** was performed in the presence of NaOEt base in anhydrous toluene stirred for one day at room temperature, followed by acidic hydrolysis of the concentrated filtrate with 3 N HCl in dichloromethane for two hours. Under the utilized conditions, both *cis*- (*rac*-*cis*-**32a-e,g**) and *trans*-3-aminoflavanone derivatives (*rac*-*trans*-**32a-g**) were obtained, which could be easily separated and isolated individually by our work-up procedure (Table 4.). If the acidic treatment was maintained for a long time, all the *cis*-

3-aminoflavanones were transformed to the lower-energy *trans* isomer by enolization at C-2 as reported in literature examples. The *cis*- (*rac*-*cis*-**32a-e,g**) and *trans*-3-aminoflavanone derivatives (*rac*-*trans*-**32a-g**) were isolated in 1:1 ratio when there was a phenyl group at the C-2 position (**32a**). Approximately twofold excess of the *trans* isomer was obtained with other C-2 aryl groups except for the 1-naphthyl group (**32f**), when only the *rac*-*trans*-**32f** diastereomer could be isolated (Table 4.). After the filtration of the *trans* isomer, the filtrate was concentrated and purification by column chromatography provided the 2-styrylbenzoxazole side-products **33a-g** with 10-20% yield, which were obtained by ring-opening of the γ-pyrone ring and intramolecular cyclization of the phenolic hydroxyl group on the intermediate of the Beckmann rearrangement.



Entry	Substrate	R^1	R^2	R^3	Overall yield **32**[a] (%)	Yield **33**[b] (%)	dr[c]
1	**31a**	H	H	H	*cis*-**32a**+*trans*-**32a** (60)	**33a** (15)	1:1
2	**31b**	H	OMe	H	*cis*-**32b**+*trans*-**32b** (46)	**33b** (20)	1:2.3
3	**31c**	OMe	OMe	H	*cis*-**32c**+*trans*-**32c** (62)	**33c** (16)	1:1.7
4	**31d**	OMe	H	OMe	*cis*-**32d**+*trans*-**32d** (66)	**33d** (n.d)[d]	1:2.2
5	**31e**	OMe	OMe	OMe	*cis*-**32e**+*trans*-**32e** (65)	**33e** (14)	1:2.8
6	**31f**	1-naphthyl			*trans*-**32f** (64)	**33f** (10)	0:1
7	**31g**	2-naphthyl			*cis*-**32g**+*trans*-**32g** (69)	**33g** (11)	1:2.2[e]

[a] sum isolated yield of diastereomers *cis*- and *trans*-**1a-g**,[b] isolated yield of the benzoxazole side-products **17a-g**, [c] ratio of diastereomers *cis*- and *trans*-**1** as determined from the isolated yields, [d] not determined, since it could not be isolated as a single component by column chromatography, [e] determined by NMR

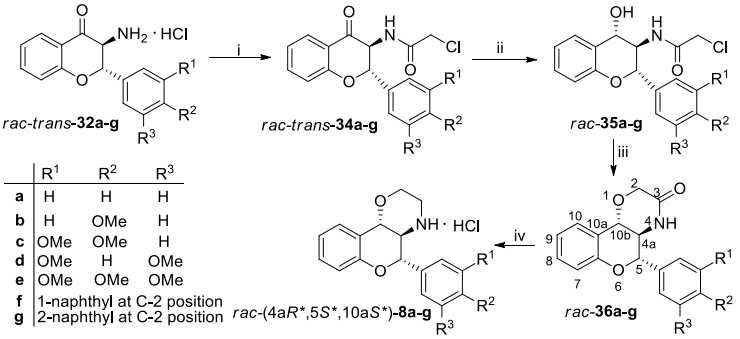
Table 4. Yields of the products obtained in the *Neber* reaction of **31a-g**. i: 1) NaOEt, dry toluene, rt, 1 d. 2) CH_2Cl_2, 3N HCl, rt, 2 h.

Our finding suggested that the *cis*-3-aminoflavanone derivatives formed initially through the corresponding 2*H*-azirine either diastereoselectively or together with the *trans* isomer and then the acidic hydrolysis promoted the

conversion of the *cis* isomer to the *trans* one by enolization-induced epimerization of the α-aminoketones.

3.6. *Via* 3-aminoflavanone, we carried out the synthesis of 19 2-arylchroman derivatives condensed with morpholine ring bearing various substitution pattern at the C-2 position.

Since the diastereomeric 3-aminoflavanone derivatives *rac-cis*-**32a-g** and *rac-trans*-**32a-g** could be obtained in pure form by simple filtration and trituration, we could use this asset to synthesize different stereoisomers of morpholine-condensed target molecules **8a-g** in a four-step sequence. (Scheme 6 and 7.).

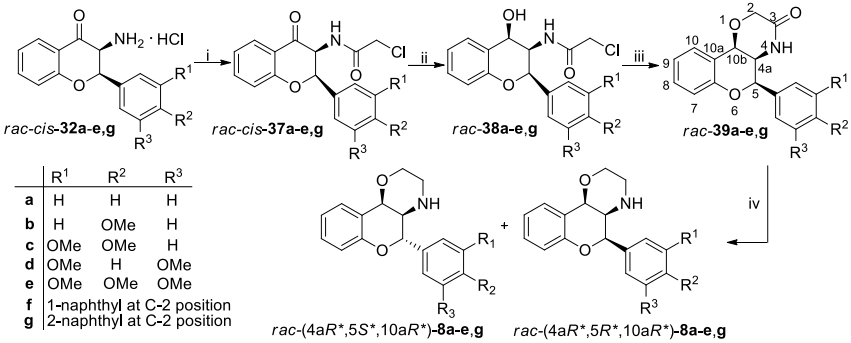


Scheme 6. Transformation of *rac-trans*-**32a-g** to the morpholine-condensed derivatives *rac*-(4a*R**,5*S**,10a*S**)-**8a-g**. i: $ClCH_2COCl$, Et_3N, dry CH_2Cl_2, rt, 5 min (71-82%). ii: $NaBH_4$, MeOH, rt, 15 min (88-98%). iii: NaH, dry THF, rt (71-82%). iv: 1) $LiAlH_4$, dry dioxane, Δ, 10 min 2) 3N HCl, rt, 1 h (46-84%).

In the first step, *rac-trans*-**32a-g** were acylated with chloroacetyl chloride, which provided the expected acetamide product *rac*-**34a-g** with good yield (71-82%). Then the reduction of C-4 carbonyl group with sodium tetrahydroborate in methanol was performed affording diastereoselectively the secondary alcohols *rac*-**35a-g** with excellent yield (88-98%) and *transz,transz* relative configuration. The ring closure of the obtained *rac*-**35a-g** alcohol derivatives with sodium hydride resulted in the lactam derivatives *rac*-**36a-g** with good yield (80-91%). In the last step, the reduction of the lactam ring with $LiAlH_4$ was performed by the optimized condition, which gave the expected *rac*-(4a*R**,5*S**,10a*S**)-**8a-g** morpholine final product with medium and good yields (46-84%) (Scheme 6). The *trans,trans* relative configuration was preserved during the cyclization with

sodium hydride (*rac*-**35a-g** → *rac*-**36a-g**) and the reduction of lactams with lithium tetrahydroaluminate.

The same synthetic scheme was also utilized for the preparation of stereisomeric *rac*-(4a*R**,5*R**,10a*R**)-**8a-e**, **g** target molecules starting from the *rac-cis*-**32a-e**, **g**. (Scheme 7). When the acylation reaction (*rac-cis*-**32a-e**,**g** → *rac-cis*-**37a-e**,**g**) was performed at room temperature, partial epimerization occurred at C-3 and the thermodynamically more stable *rac-trans*-**34a-e**, **g** formed as the main product. In order to avoid the epimerization, the acylation was carried out at 0 °C and the crude product of *rac-cis*-**37a-e**, **g** was reduced directly with $NaBH_4$ (*rac-cis*-**37a-e**, **g** → *rac*-**38a-e**, **g**). The reduction of the ketone carbonyl group occurred diastereoselectively (dr ≥ 95:5) and it provided the *cis*,*cis* stereoisomer of the alcohols *rac*-**38a-e**, **g** with 54-70% overall yield for two steps.

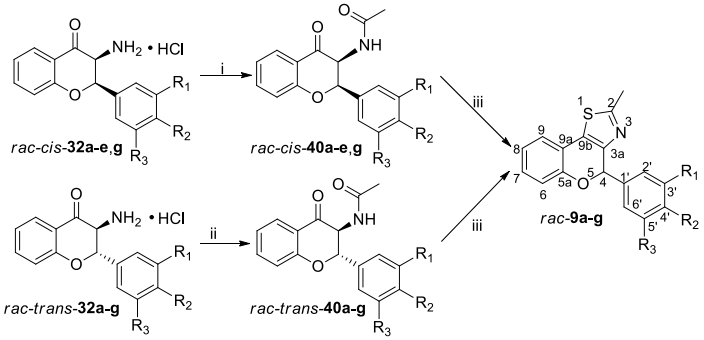


Scheme 7. Transformation of *rac-cis*-**32a-e**, **g** to the morpholine-condensed derivatives *rac*-(4a*R**,5*R**,10a*R**)- and *rac*-(4a*R**,5*S**,10a*R**)-**8a-e**, **g**. i: $ClCH_2COCl$, Et_3N, dry CH_2Cl_2, 0 °C, 15 min. ii: $NaBH_4$, MeOH, rt, 15 min (54-68% for two steps). iii: NaH, dry THF, rt (69-96%). iv: 1) $LiAlH_4$, dry dioxane, Δ, 10 min [33-60% for *rac*-(4a*R**,5*S**,10a*R**)-**8a-e**, **g** and 5-20% for *rac*-(4a*R**,5*R**,10a*R**)-**8a-e**, **g**].

Cyclization of the alcohol *rac*-**38a-e**,**g** with NaH afforded the lactam derivatives *rac*-**39a-e**,**g** with good yield (69-96%), which were reduced with $LiAlH_4$ to produce surprisingly the *rac*-(4a*R**,5*S**,10b*R**)-**8a-e**,**g** as the major product (33-60%) and *rac*-(4a*R**,5*R**,10b*R**)-**8a-e**,**g** (5-20%) as the minor one. In the major products *rac*-(4a*R**,5*S**,10a*R**)-**8a-e**,**g**, the benzylic C-5 chirality center was inverted to decrease the steric crowding of the *cis* substituents. During the synthesis of chromeno[4,3-*b*][1,4]oxazines **8a-e**,**g** substituted at C-5, three of the four possible diastereomers were synthesized, which enabled to study stereochemistry-activity relationships.

3.7. Fourteen thiazole and pyrrole condensed 2-aryl-2*H*-chromene compounds were prepared *via* 3-aminoflavanone derivatives.

The key intermediates for the formation of the thiazole ring are the α-aminoketone derivatives obtained during the above-mentioned *Neber* rearrangement (Table 4). These amines were transformed to the corresponding *rac-cis*-**32a-e,g** and *rac-trans*-**32a-g** acetamides with acetyl chloride. The epimerization, observed during the acylation step of the morpholine ring formation, also occurred during the acylation of *rac-cis*-**32a-e,g** at room temperature. Thus the reaction was carried out as above at low temperature with a short reaction time to preserve the *cis* relative configuration. After the successful synthesis of acetamides, thiazoles were prepared by heating with Lawesson's reagent in anhydrous toluene as described in the literature. The method resulted in the thiazole derivatives *rac*-**9a-g** with good yield (55-82%, Scheme 8.).

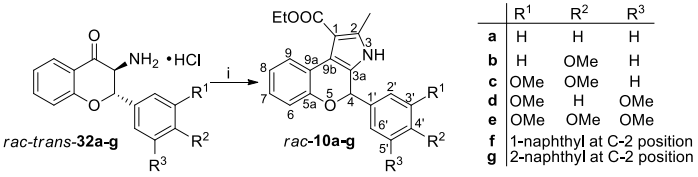


Scheme 8. Synthesis of thiazole-condensed derivatives *rac*-**9a-g** from 3-aminoflavanones *rac-cis*-**32a-e**, **g** and *rac-trans*-**32a-g**. i: acetyl chloride, Et_3N, dry THF, 0 °C, 15 min (64-75%, dr 95:5 in favor of *cis*). ii: acetyl chloride, Et_3N, dry THF, rt, 1 h (71-82%). iii: Lawesson's reagent, dry toluene, 70 °C, 4 h (55-82%).

The synthesis of thiazole derivatives was carried out with both diastereomers of the acylated amine derivatives, and we observed that the cyclization of the *trans*-*N*-acetyl derivatives (*rac-trans*-**40a-g**) provided consistently higher yields than those of the corresponding *cis* ones (*rac-cis*-**40a-e**,**g**).

The synthesis of the condensed *rac*-**10a-g** pyrrole derivatives was performed by the *Knorr* reaction of 3-aminoflavanones. During the reaction, the α-aminoketone derivatives *rac-trans*-**32a-g** were reacted with ethyl

acetoacetate using mild basic condition (Scheme 9.). The reaction resulted in the expected *rac*-**14a-g** derivatives with moderate yield (32-60%).



Scheme 9. Synthesis of pyrrole-condensed derivatives from 3-aminoflavanones. i: ethyl acetoacetate, NaOAc, EtOH/H_2O, Δ, 3 h (32-60%).

3.8. We investigated the antiproloferative activity of the condensed morpholine, thiazole, pyrrole derivatives and their precursors on human cancer cell lines (A2780 and WM35) and several compounds showed low micromolar IC_{50} values.

In collaboration with the Department of Physiology, the antiproliferative activity of *rac*-**8a-g**, *rac*-**9a-g**, *rac*-**10a-g** derivatives containing condensed morpholine, thiazole, pyrrole subunits and their precursors *rac-trans*-**34a-g**, *rac*-**35a-g**, *rac*-**36a-g**, *rac*-**38a-e**,**g**, *rac*-**39a-e,g** and *rac*-**40a-g** were evaluated initially against A2780 ovarian and WM35 melanoma cancer cell lines at 50 μM concentration, and the IC_{50} values were determined for the most active derivatives (Table 5).

Compound	Cell line/IC_{50} (μM)		
	A2780	WM35	HaCaT
rac-(4a*S**,5*R**,10b*R**)-**8b**	10.40 ± 2.71	33.66 ± 4.42	17.13 ± 7.95
rac-(4a*S**,5*R**,10b*S**)-**8d**	30.51 ± 6.75	27.49 ± 4.70	30.38 ± 49.83
rac-**35g**	0.15 ± 0.14	3.50 ±1.94	6.06 ± 3.30
rac-**9e**	2.72 ± 0.48	2.14 ± 1.85	6.23 ± 1.25
rac-**10b**	4.84 ± 1.38	5.83 ± 1.78	9.57 ± 8.77
rac-**10c**	5.34 ± 0.88	8.21 ± 4.38	1.97 ± 0.29
rac-**10g**	2.95 ± 1.37	9.37 ± 3.82	11.52 ± 3.37
Doxorubicin[a]	0.07	0.14	0.03

[a] Positive control

Table 5. *In vitro* antiproliferative activity of the condensed *O,N*-heterocycles and the **35g** precursor against A2780 and WM35 cell lines determined by MTT assay.

The best values were measured for the *rac*-**35g** alcohol and the *rac*-**9e** thiazole derivatives on both cell lines. The related *N*-acetyl derivatives *rac*-*cis*-**40a-e**,**g** or *rac*-*trans*-**40a-g** were inactive or they had much weaker activity. This suggested that the *N*-chloroacetyl derivatives act as an alkylating agent and the chloroacetyl moiety is essential for the activity. The *N*-chloroacetyl-3-amino-flavanone derivatives *rac*-*trans*-**34a-g** had usually weaker activity than the corresponding flavan-4-ol derivatives, which suggested that the reduction of the C-4 carbonyl group to hydroxyl improved the antiproliferative activity. 6.06 μM IC_{50} value was measured against the non-cancerous HaCaT human keratinocytes, which implies a remarkable 50 fold selectivity against A2780 cell line in case of the *rac*-**35g** alcohol, while for the thiazole derivative *rac*-**16e**, it was only a twofold selectivity. All the pyrrole-condensed derivatives *rac*-**10a-g** had distinct antiproliferative activity at 50 μM concentration and low micromolar IC_{50} values were measured for **10b**, **10c** and **10g** in the range of 2.95-9.37 μM.

3.9. We developed chiral HPLC methods for the separation of enantiomers of racemic condensed morpholine, thiazole and pyrrole derivatives and their precursors, and the absolute configuration of the separated enantiomers was determined by chiral HPLC-ECD measurements and TDDFT-ECD calculations.

The antiproliferative activity of our condensed chiral *O,N*-heterocycles prompted us to separate the enantiomers of thiazoles *rac*-**9a-g**, pyrroles *rac*-**10a-g**, and lactams *rac*-**36a-g** and *rac*-**39a-e,g** with HPLC using chiral stationary phase, measure the online HPLC-ECD spectra and determine the absolute configuration by TDDFT-ECD calculations.

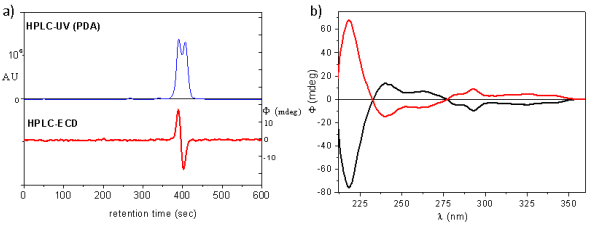


Figure 5. (a) HPLC-UV (blue) and –ECD (red) traces of *rac*-**9e** on Chiralpak IA column with hexane/2-propanol 80:20 eluent monitored at 240 nm. (b) HPLC-ECD spectra of the first-[(4*R*), black] and second-eluting [(4*S*), red] enantiomers of **9e** (b).

Baseline separation could be achieved in most cases but even partial separation of the enantiomers was sufficient to record mirror-image online HPLC-ECD spectra (Figure 5a. and 5b). The absolute configurations of the separated enantiomers were deduced by the solution TDDFT-ECD protocol, which also revealed the low-energy solution conformers of the studied molecules. The calculated and measured ECD spectra of the conformers were in good agreement for all cases, and thus the absolute configuration could be determined.

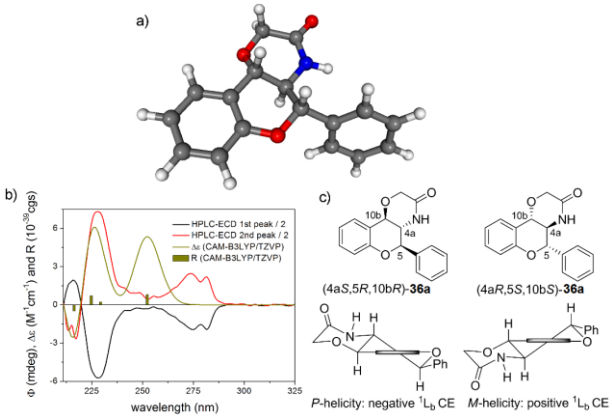


Figure 6. a) Single low-energy CAM-B3LYP/TZVP PCM/$CHCl_3$ conformer of (4a*R*,5*S*,10b*S*)-**36a** containing a flavan chromophore with *M*-helicity. b) HPLC-ECD spectra of the first (black line) and the second-eluting (red line) enantiomers of **36a** compared with the CAM-B3LYP/TZVP PCM/$CHCl_3$ // CAM-B3LYP/TZVP PCM/$CHCl_3$ spectrum of (4a*R*,5*S*,10b*S*)-**36a** (olive line). The bars represent rotational strength values for the single low-energy solution conformer. c) Structure and helicity of the separated enantiomers of rac-**36a**. Horizontal thick line represents the plane of the condensed benzene ring.

For the lactam derivatives *rac*-**36a-g** and *rac*-**39a-e,g**, we found that the semiempirical flavan helicity rule can be applied to our conformationally rigid condensed flavan derivatives containing three chirality centers to assign the absolute configuration (Figure 6c.). According to the flavan helicity rule, *M*-helicity of the benzene-condensed heterocycle produces a positive ^1L_b transition, while the *P*-helicity a negative one.

4. Possible applications of the results

In my doctoral research, I developed methods for the synthesis of substituted pterocarpans and substituted chroman and 2*H*-chromene

derivatives condensed with morpholine, thiazole and pyrrole rings. Several of the final products and their precursors showed antiproliferative activity with low micromolar IC_{50} values against A2780 (cervical cancer) and WM35 (melanoma) human cancer cell lines. These results may be useful in finding an even more active target molecule in the future against the mentioned cell lines, and may induce further research to test our molecules or similar structures against other cancer cell lines. The *rac*-**35g** flavanol derivative containing an *N*-chloroacetyl group has a structural analogy with acyclic acid ceramidase inhibitors (SACLAC) and act likely as an alkylating agent. The measured good activity and cell line selectivity has already initiated a new research project in our group to synthesize acid ceramidase inhibitor analogues.

In the course of the HPLC-ECD studies of our chiral racemic target compounds and their intermediates, the separation of enantiomers and assignment of their configuration by TDDFT-ECD calculations may assist future enantioselective synthesis of our molecules and the study of stereochemistry-activity relationship.

UNIVERSITY of DEBRECEN

UNIVERSITY AND NATIONAL LIBRARY
UNIVERSITY OF DEBRECEN
H-4002 Egyetem tér 1, Debrecen
Phone: +3652/410-443, email: publikaciok@lib.unideb.hu

Registry number: DEENK/480/2021.PL
Subject: PhD Publication List

Candidate: Ádám Szappanos
Doctoral School: Doctoral School of Chemistry
MTMT ID: 10038101

List of publications related to the dissertation

Foreign language scientific articles in international journals (2)

1. **Szappanos, Á.**, Mándi, A., Gulácsi, K., Lisztes, E., Tóth, I. B., Bíró, T., Antus, S., Kurtán, T.: Synthesis and antiproliferative activity of 6-naphthylpterocarpans.
Org. Biomol. Chem. 18 (11), 2148-2162, 2020. ISSN: 1477-0520.
DOI: http://dx.doi.org/10.1039/D0OB00110D
IF: 3.876

2. **Szappanos, Á.**, Mándi, A., Gulácsi, K., Lisztes, E., Tóth, I. B., Bíró, T., Ábrahám, A., Kiss-Szikszai, A., Bényei, A., Antus, S., Kurtán, T.: Synthesis and HPLC-ECD Study of Cytostatic Condensed O,N-Heterocycles Obtained from 3-Aminoflavanones.
Biomolecules. 10 (10), 1-43, 2020. EISSN: 2218-273X.
DOI: http://dx.doi.org/10.3390/biom10101462
IF: 4.879

List of other publications

Foreign language scientific articles in international journals (6)

3. Agócs, A., Murillo, E., Turcsi, E., Béni, S., Darcsi, A., **Szappanos, Á.**, Kurtán, T., Deli, J.: Isolation of allene carotenoids from mamey.
J. Food Compos. Anal. 65, 1-5, 2018. ISSN: 0889-1575.
DOI: http://dx.doi.org/10.1016/j.jfca.2017.04.004
IF: 2.994

4. Tóth, B., Chang, F. R., Hwang, T. L., **Szappanos, Á.**, Mándi, A., Hunyadi, A., Kurtán, T., Jakab, G., Hohmann, J., Vasas, A.: Screening of Luzula species native to the Carpathian Basin for anti-inflammatory activity and bioactivity-guided isolation of compounds from Luzula luzuloides (Lam.) Dandy & Wilmott.
Fitoterapia. 116, 131-138, 2017. ISSN: 0367-326X.
DOI: http://dx.doi.org/10.1016/j.fitote.2016.12.004
IF: 2.642

UNIVERSITY of DEBRECEN

UNIVERSITY AND NATIONAL LIBRARY
UNIVERSITY OF DEBRECEN
H-4002 Egyetem tér 1, Debrecen
Phone: +3652/410-443, email: publikaciok@lib.unideb.hu

5. Ilkei, V., Spaits, A., Prechl, A., Szigetvári, Á., Béni, Z., Dékány, M., Szántay, C., Müller, J., Könczöl, Á., **Szappanos, Á.**, Mándi, A., Antus, S., Martins, A., Hunyadi, A., Balogh, G. T., Kalaus, G., Bölcskei, H., Hazai, L., Kurtán, T.: Biomimetic synthesis and HPLC-ECD analysis of the isomers of dracocephins A and B.
Beilstein J. Org. Chem. 12, 2523-2534, 2016. EISSN: 1860-5397.
DOI: http://dx.doi.org/10.3762/bjoc.12.247
IF: 2.337

6. Tóth, B., Liktor-Busa, E., Kúsz, N., **Szappanos, Á.**, Mándi, A., Kurtán, T., Urbán, E., Hohmann, J., Chang, F. R., Vasas, A.: Phenanthrenes from Juncus inflexus with Antimicrobial Activity against Methicillin-Resistant Staphylococcus aureus.
J. Nat. Prod. 79 (11), 2814-2823, 2016. ISSN: 0163-3864.
DOI: http://dx.doi.org/10.1021/acs.jnatprod.6b00581
IF: 3.281

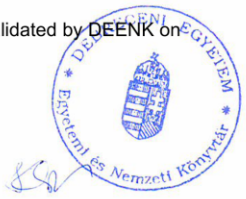
7. Turcsi, E., Murillo, E., Kurtán, T., **Szappanos, Á.**, Illyés, T. Z., Gulyás-Fekete, G., Agócs, A., Avar, P., Deli, J.: Isolation of β-Cryptoxanthin-epoxides, Precursors of Cryptocapsin and 3·-Deoxycapsanthin, from Red Mamey (Pouteria sapota).
J. Agric. Food Chem. 63 (26), 6059-6065, 2015. ISSN: 0021-8561.
DOI: http://dx.doi.org/10.1021/acs.jafc.5b01936
IF: 2.857

8. Gulácsi, K., Németh, I., **Szappanos, Á.**, Csillag, K., Illyés, T. Z., Kurtán, T., Antus, S.: Heck-oxyarylation of 2-phenyl-2H-chromenes and 1,2-dihydronaphthalenes.
Croat. Chem. Acta. 86 (2), 137-141, 2013. ISSN: 0011-1643.
DOI: http://dx.doi.org/10.5562/cca2103
IF: 0.556

Total IF of journals (all publications): 23,422
Total IF of journals (publications related to the dissertation): 8,755

The Candidate's publication data submitted to the iDEa Tudóstér have been validated by DEENK on the basis of the Journal Citation Report (Impact Factor) database.

27 October, 2021



5. List of publications

Publications in the subject of the thesis

1. Á. Szappanos, A. Mándi, K. Gulácsi, E. Lisztes, B-I. Tóth, T. Bíró, S. Antus, T. Kurtán: Synthesis and antiproliferative activity of 6-naphthylpterocarpans, *Organic & Biomolecular Chemistry*, **2020**, *18*, 2148-2162. IF: 3,876.

2. Á. Szappanos, A. Mándi, K. Gulácsi, E. Lisztes, B-I. Tóth, T. Bíró, A. Kónya-Ábrahám, A. Kiss-Szikszai, A. Bényei, S. Antus, T. Kurtán: Synthesis and HPLC-ECD Study of Cytostatic Condensed O,N-Heterocycles Obtained from 3-Aminoflavanones, *Biomolecules*, **2020**, *10*, 1462. IF: 4,879.

Publications in other subjects

1. K. Gulácsi, I. Németh, Á. Szappanos, K. Csillag, T-Z. Illyés, T. Kurtán, S. Antus: Heck-oxyarylation of 2-phenyl-2H-chromenes and 1,2-dihydronaphtalenes, *Croat. Chem. Acta*, **2013**, *86*, 137-141. IF: 0,556.

2. E. Turcsi, E. Murillo, T. Kurtán, Á. Szappanos, T-Z. Illyés, G. Gulyás-Fekete, A. Agócs, P. Avar, J. Deli: Isolation of β-Cryptoxanthin-epoxides, Precursors of Cryptocapsin and 3′-Deoxycapsanthin, from Red Mamey (Pouteria sapota), *J. Agric. Food Chem.* **2015**, *63*, 6059−6065. IF: 2,857.

3. V. Ilkei, A. Spaits, L. Hazai, H. Bölcskei, Gy. Kalaus, Á. Szappanos, A. Mándi, S. Antus, T. Kurtán, Gy.-T. Balogh, A. Prechl, Á. Szigetvári, Z. Béni, M. Dékány, Cs. Szántay Jr., J. Müller, Á. Könczöl, A. Hunyadi, A. Martins: Biomimetic synthesis and HPLC-ECD analysis of the isomers of dracocephins A and B, *Belstein J. Org. Chem*, **2016**, *12*, 2523-2534. IF: 2,337.

4. B. Tóth, E. Liktor-Busa, N. Kúsz, Á. Szappanos, A. Mándi, T. Kurtán, E. Urbán, J. Hohmann, F-R. Chang, A. Vasas: Phenanthrenes from Juncus inflexus with Antimicrobial Activity against Methicillin-Resistent Staphylococcus aureus, *J. Nat. Prod*., **2016**, *79*, 2814-2823. IF: 3,281.

5. B. Tóth, F-R. Chang, T-L. Hwang, Á. Szappanos, A. Mándi, A. Hunyadi, T. Kurtán, G. Jakab, J. Hohmann, A. Vasas: Screening of Luzula species native to the Carpathian Basin for anti-inflammatory activity and bioactivity-guided isolation of compounds from Luzula luzuloides, *Fitoterapia*, **2017**, *116*, 131-138. IF: 2,698.

6. A. Agócs, E. Murillo, E. Turcsi, Sz. Béni, A. Dercsi, Á. Szappanos, T. Kurtán, J. Deli: Isolation of Allene carotenoids from Mamey, *J. Food Compos. Anal*., **2018**, *65,* 1-5 IF: 2,994.

Lectures in the subject

1. Gulácsi K., Szappanos Á., Kurtán T., Mándi A., Papp T., Tóth E.: Synthesis of 6-substituted pterocarpanes and their hplc-cd studies, *Temesvári Akadémiai napok*, Temesvár 2011 május 26-27

2. Szappanos Á., Gulácsi K., Kurtán T., Papp T., Antus S.: Szubsztituált 3-nitroflavanonok és 2H-kromének előállítása, *XXXIV Kémiai Előadói Napok,* Szeged 2011 november 2-4

3. Szappanos Á., Papp T., Mándi A., Kurtán T., Antus S., Gulácsi K.: 3-Nitroflavanonok és 2H-kromének előállítása, *MTA Alkaloid és Flavanoidkémiai Munkabizottsági Ülés*, Balatonalmádi 2012 május 14-15

4. Szappanos Á., Mándi A., Papp T., Kurtán T., Antus S., Gulácsi K.: 6-Naftil pterokarpánok és 3-nitroflavanonok előállítása, *Heterociklusos és Elemorganikus Kémiai Munkabizottság ülése*, Balatonszemes 2012 június 6-8

5. Szappanos Á., Mándi A., Gulácsi K., Antus S., Kurtán T.: Naftilpterokarpánok és nitroflavanonok előállítása, *MTA Alkaloid és Flavanoidkémiai Munkabizottsági Ülés*, Balatonalmádi 2013 május 13-14

6. Szappanos Á., Mándi A., Gulácsi K., Kurtán T., Antus S.: Aminoflavanonok és 2Hkromének reakciói, *Heterociklusos és Elemorganikus Kémiai Munkabizottság ülése*, Balatonszemes 2013 június 5-7

7. Szappanos Á., Gulácsi K., Kurtán T., Mándi A., Antus S.: Aminoflavanonok és 2H-kromének reakciói, *XXXVI Kémiai Előadói Napok,* Szeged 2013 október 28-30

8. Szappanos Á., Mándi A., Czenke Z., Antus S., Kurtán T.: 2-Aril és 2-heteroaril krománok és kromonok szintézise és további átalakításaik, *MTA Alkaloidkémiai és Flavanoidkémiai Munkabizottsági Ülés*, Balatonalmádi 2014 május 12-13

9. Szappanos Á., Mándi A., Czenke Z., Antus S., Kurtán T.: 2-Aril és 2-heteroaril krománok és kromonok szintézise és további átalakításaik,

Heterociklusos és Elemorganikus Kémiai Munkabizottsági Ülés, Balatonszemes 2014 május 21-23

10. Szappanos Á., Gulácsi K., Antus S., Kurtán T.: 3-Aminokromán-4-on származékok előállítása Neber-reakcióval és alakítási lehetőségeik, *MTA Alkaloidkémiai és Flavanoidkémiai Munkabizottsági Ülés*, Balatonalmádi 2015 május 18-19

11. Szappanos Á., Gulácsi K., Antus S., Kurtán T.: 3-Aminokromán-4-on származékok előállítása Neber-reakcióval és alakítási lehetőségeik, *Heterociklusos és Elemorganikus Kémiai Munkabizottsági Ülés*, Balatonszemes 2015 május 27-29

12. Szappanos Á., Balla V., Auksz F., Gulácsi K., Antus S., Kurtán T.: 3-Aminokromán-4-on származékok átalakítási lehetőségei, *MTA Alkaloidkémiai és Flavanoidkémiai Munkabizottsági Ülés*, Mátrafüred 2016 április 14-15

13. Szappanos Á., Balla V., Auksz F., Gulácsi K., Antus S., Kurtán T.: 3-Aminokromán-4-on származékok átalakítási lehetőségei, *Heterociklusos és Elemorganikus Kémiai Munkabizottsági Ülés*, Balatonszemes 2016 május 18-20

Posters in the subject

1. Gulácsi K., Szappanos Á., Antus S.: Szubsztituált 2H-kromének előállítása, *MKE 1. Nemzeti Konferencia*, Sopron 2011 május 22-25

2. T. Kurtán, Á. Szappanos, T. Papp, K. Gulácsi, I. Szepesi, N. Rashid, S. Antus: Catalysis in Organic Synthesis, *ICCOS*, Moszkva 2012 szeptember 15-20

3. Á. Szappanos, A. Mándi, Z. Czenke, Zs. Fogarasi, H. Y. Zhang, S. Antus, T. Kurtán: Optically active flavanone and isochroman derivatives, *Chirality*, Prága 2014 július 27-30