Final project report on the K-105871 project titled „Preparation and stereochemical studies of pharmacologically active natural and synthetic derivatives”

principal investigator: Tibor Kurtán

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In the frame of the K-105871 project, the following research goals and activities were supported by the OTKA:

I. Configurational assignment of synthetic and natural products with conformational flexibility by the solid-state TDDFT-ECD method and comparison of solution and solid-state conformations.

II. Stereochemical studies of synthetic and natural products using solution TDDFT-ECD and -VCD approach, HPLC-ECD analysis of stereoisomeric mixture and racemic derivatives, study of axial chirality in biaryl natural products having both axial and central chirality.

III. Synthesis and stereochemical studies of chiral O-heterocycles such as phthalan, 1,3-dihydroisoindole, hexacyclic chromane, 2-pyrone and 4-hydroxycoumarin, 3-nitro or 3-aminoflavanone and isochroman derivatives

In the four-year period of the OTKA project, 45 SCI papers came out, 1 is accepted for publication and 1 is under revision in the research topics specified above and with reference to the financial support of OTKA. The scientific achievements are detailed below.

I. Configurational assignment by the solid-state TDDFT-ECD method

The solid-state TDDFT-ECD method was applied to optically active natural and synthetic derivatives having X-ray diffraction geometry with the relative configuration in order to determine the absolute configuration and compare the geometry of the solid-state and solution conformers.

The solid-state TDDFT-ECD method was applied to determine the absolute configuration of a synthetic TGR5 agonists having a chiral 3,4,5-trisubstituted 4,5-dihydro-1,2,4-oxadiazole ring, which was summarized in the ChemMedChem (**2013**, *8*, 1210-1223).

The stereochemistry of dendrodolides A-M, 13 new 12-membered macrolides isolated from the fungus *Dendrodochium sp.*, was determined by the modified Mosher’s method, excitoncoupled circular dichroism (ECCD), electronic solution and solid-state circular dichroism supported by calculations, and X-ray analysis. The scope and limitation of the applied methods were published in the Journal of Organic Chemistry (**2013**, 78, 7030− 7047).

The absolute configuration of xyloccensin Q, a phragmalin orthoester isolated from the twigs and leaves of the Chinese mangrove *Xylocarpus granatum*, was revised as its enantiomer by the solid-state TDDFT ECD method and X-ray diffraction analysis. Consequently, the absolute configurations of xyloccensins O-S and V were all corrected as their corresponding enantiomers. The result were summarized in a Planta Medica paper [**2014**, 80(11), 949-954].

The stereochemistry of xylogranatopyridines A, a new pyridine-containing limonoid from the twigs and leaves of the Chinese mangrove *Xylocarpus granatum* having significant inhibitory activity against protein tyrosine phosphatase 1B (PTP1B), was deduced by means of the solid-state TDDFT ECD method. The results were published in Tetrahedron (**2014**, 70, 6444-6449).

The absolute configuration of the natural product psammaplysin A, a member of bioactive spirooxepinisoxazolin alkaloids from *Verongiid sponges*, was assigned as (6*R*,7*R*) using ECD data recorded in MeOH, supported by TDDFT ECD calculations *in vacuo* and with PCM solvent model, and for the solid-state geometry. The correlation of the ECD data with the stereochemistry of psammaplysin A was based on a detailed conformational analysis of the truncated spirooxepine model compound and it permits configurational assignment of related psammaplysin analogues on the basis of their ECD spectra. The results were published in the *J. Nat Prod*. (**2015**, *78*, 2051-2056).

Conformational analysis and TDDFT-ECD calculations of three resorcylic acid derivatives with antioxidant activity, identified from the marine mangrove-derived endophytic fungus *Penicillium brocae*, were carried out to confirm the (14*R*) absolute configuration and identify the solution conformers and factors that govern the correlation between stereochemistry and signs of the characteristic ECD bands. For two derivatives, single crystal X-ray analysis allowed applying the solid-state TDDFT-ECD approach to compare the structures of solution and solid-state conformers and correlate the n–π* ECD transition with the helicity and absolute configuration of the dihydroisocoumarin moiety. Results were published in *RSC Advances* (**2015**, *5*, 39870-39877).

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II. Stereochemical studies in solution by ECD and VCD

a) Axial chirality or preferred helicity of bioactive biaryl natural products

Depending on the substitution pattern and hence the rotational energy barrier, biaryl natural products can bear axial chirality, an additional stereogenic element besides the central chirality elements, the assignment of which is often challenging and it requires the combination of different methods. The rotational energy barriers and transition-states for the inversion of the atropo-diastereomers were also regularly calculated for the studied biaryls.

The ECD study of homo- and heterodimeric biaryls with different substitution patterns, chromophores with or without axial chirality enabled us to collect versatile and instructive examples for a future review paper on the axial chirality of biaryl natural products.

The axial chirality of atropodiastereomeric bisdihydroanthracenones and mixed dihydro-anthracenone/anthraquinone dimers, isolated from *Talaromyces wortmannii*, an endophyte of *Aloe vera*, were determined by means of TDDFT ECD and VCD calculations. They exhibited antibacterial activity against *Staphylococcus aureus*, including (multi)drug-resistant clinical isolates. Results were published in the Journal of Medicinal Chemistry (**2013**, *56*, 3257-3272).

The absolute configuration of the antibacterial neosartorin, a biaryl natural product of the endophytic fungus *Aspergillus fumigatiaffinis* having both central and axial chirality elements, was established as (a*R*,5*S*,10*R*,5'*S*,6'*S*,10'*R*) for the first time on the basis of its ECD spectra aided with TDDFT-ECD calculations. The results were published in the Tetrahedron Letters (**2014**, *55*, 1020-1023).

The absolute configuration of the dimeric anthracene biaryl derivative torrubiellin B, isolated from the endophytic fungus *Acremonium sp.* and exhibiting strong *in vitro* anti-tumor activity (IC_{50} 0.2–2.6 μM) against several cisplatin-resistant solid cancer cell lines, was established on the basis of its electronic circular dichroism (ECD) spectra aided with TDDFT-ECD calculations. The result were summarized in a *Tetrahedron Lett.* paper (**2015**, *56*, 4430-4433).

The axial chirality of the atropisomeric cytotoxic natural products acetylalterporriol D and acetylalterporriol E, isolated from the endophytic fungus *Stemphylium globuliferum*, were elucidated on the basis of electronic circular dichroism (ECD) spectroscopy, which was published in *Eur. J. Org. Chem*. (**2015**, 2646-2653).

The central and axial chirality elements or preferred helicities of six cytotoxic xanthone-chromanone dimers of the mangrove-derived fungus *Aspergillus versicolor*, versixanthones A−F, featuring different formal linkages of tetrahydroxanthone and 2,2-disubstituted chroman-4-one monomers, were established by a combination of X-ray diffraction analysis, chemical conversions, and TDDFT-ECD calculations (*J. Nat. Prod*. **2015**, *78*, 2691-2698).

The absolute configuration of two new tetrahydroanthraquinone derivatives, altersolanol Q and 10-methylaltersolanol Q, and the new dimeric biaryl, alterporriol X, having axial chirality, were determined by TDDFT-ECD calculations or by comparing ECD data with those of known analogues (*Tetrahedron Lett*., **2016**, *57*, 4074-4078).

b) HPLC-ECD analysis for the stereochemical study of stereoisomeric mixtures

Chiral HPLC separations aided by HPLC-ECD measurements and ECD calculations were proven a powerful method to analyze partially racemized metabolites with low enantiomeric excess or the enantiomers of chiral racemic derivatives to determine the relationship between the characteristic ECD transitions and the absolute configuration.

The absolute configuration of enantiomeric dihydroisocoumarin derivatives, embeurekol A and B and the acetophthalidin derivative embeurekol C, isolated from the fungus *Embellisia eureka,* were determined by TDDFT ECD calculations of solution conformers, online HPLC-ECD analysis, and the modified Mosher method. Results were summarized in a Chirality paper (**2013**, *25*, 250-256).

Online HPLC-ECD analysis of natural and synthetic cryptocapsin-5,6- and 5,8-epoxides allowed configurational assignment of the separated stereoisomers, which were published in the Journal of Natural Products (**2013**, 76, 607-614).

Eleganketal A, a naturally occurring aromatic polyketide possessing a rare highly oxygenated spiro[isobenzofuran-1,3′-isochroman] ring system, was isolated from the fungus *Spicaria elegans* KLA03. Its structure including the absolute configuration was determined by combining spectroscopic analysis, synthesis of the racemic permethylated analogue, chiral-phase HPLC separation, and TDDFT-ECD analysis. The results were published in the Journal of Natural Products [**2014**, *77(7)*, 1718-1723].

The carotinoid components of red mamey (*Pouteria sapota*), β-cryptoxanthin-5,6-epoxide, β-cryptoxanthin-5′,6′-epoxide, 3′-deoxycapsanthin, and cryptocapsin were characterized by UV−vis spectroscopy, electronic circular dichroism (ECD), nuclear magnetic resonance (NMR) spectroscopy, and mass spectrometry (MS). Epoxidation of β-cryptoxanthin afforded the β-(5′*R*,6′*S*)- and (5′*S*,6′*R*)-cryptoxanthin-5′,6′-epoxides, which were identified by HPLC-ECD analysis. The results were published in the *J. Agr. Food Chem*. (**2015**, *63*, 6059-6065).

The absolute configuration of the separated enantiomers of synthetic (±)-trans-2,6-diortho-tolyl-3,4,5-trimethylpyridine-N-oxide having axially chirality, has been determined by ECD analyses supported by TDDFT calculations at different levels of theory, which was published in the journal of *Tetrahedron: Asymm*. (**2015**, *56*, 4430-4433).

The stereochemistry of the enantiomers of *rac*-arugosin J and (+)-xylarugosin, new phenolic natural products of the endophytic fungus *Xylaria sp*. isolated from healthy leaves of *Curcuma xanthorrhiza*, was determined by means of chiral HPLC-ECD analysis and solution TDDFT-ECD approach, which was summarized in *Tetrahedron Lett*. (**2015**, *56*, 1193-1197).

The absolute configuration of a 2-benzofuran-1(3*H*)-one derivative having radical scavenging activity was determined by means of online high-performance liquid chromatography, electronic circular dichroism (HPLC-ECD) measurements and time-dependent Density Functional Theory electronic circular dichroism (TDDFT-ECD) calculations (*Chirality*, **2016**, *28(8)*, 581-584.

c) Stereochemical studies by the solution TDDFT-ECD calculation method

The solution conformers of natural products with varying conformational flexibility were computed with different methods and subsequent ECD calculations of the conformers were used to determine the absolute configuration by comparison with the experimental ECD curves. Different combination of functionals and basis sets were tested in order to reach a solid assignment, which could lead to the revision of stereochemistry in the case of some related work.

The absolute configuration and conformation of methyl sarcotroates A, a diterpenoid from the Hainan soft coral *Sarcophyton trocheliophorum* possessing a tetradecahydro-cyclopenta[3′,4′]cyclobuta[1′,2′:4,5]-cyclonona[1,2-b]oxirene ring system, were determined by TDDFT ECD calculations of its solution conformers. Its hydroperoxy derivative exhibited significant inhibitory activity against protein tyrosine phosphatase 1B (PTP1B), being similar to that of positive control oleanolic acid. Results were published in Organic Letters (**2013**, *15(2)*, 274-277).

The absolute configurations of bislatumlides C and E were determined by TDDFT calculations of their solution ECD spectra, allowing the configurational assignment of the related bislatumlides A, B, D and F, which was published in the Journal of Organic Chemistry (**2013**, *78*, 3113-3119).

The scope of conformational analysis and ECD calculations was tested on the stereochemical studies of α-pyrone derivatives, pestalotiopyrones J–L isolated from the mangrove-derived endophytic fungus *Pestalotiopsis virgatula*. Results were published in Tetrahedron Letters (**2013**, 54 3256–3259).

The absolute configuration of tricycloalternarene A and bicycloalternarene A, meroterpenoids from the sponge-associated fungus *Alternaria* sp. JJY-32, was determined by ECD calculations, which could serve as reference for the configurational assignment of analogue derivatives. Results were published in the Journal of Natural Products (**2013**, *76*, 1946-1957).

The absolute configurations of dihydrosarsolenone and sarsolilides B, isolated from the Hainan soft coral *Sarcophyton trocheliophorum* Marenzeller, were determined by TDDFT ECD calculations, and they could be used as ECD reference compounds in the determination of the absolute configuration of their related derivatives. On the basis of the stereochemical studies, the reported relative configuration of sarsolenone had to be revised and its absolute configuration could be assigned. The results were published in the Eur. J. Org. Chem. (**2014**, 1841-1847).

The structure and stereochemistry of dendrodochols A−D, cytotoxic polihydroxy cyclohexanols from a *Dendrodochium* sp. fungus associated with the sea cucumber *Holothuria nobilis* Selenka, were elucidated by spectroscopic analysis including solution TDDFT ECD calculation approach and the modified Mosher's method. The results were published in the Journal of Natural Products (**2014**, *77*, 1179-1184).

The conformation, relative and absolute configuration of brocaeloids A, a 2,3-dihydroquinoline-4(1H)-one alkaloid isolated from the endophytic fungus *Penicillium brocae* MA-192, were studied by the combination of NMR methods, gas-phase and solution conformational analysis, TDDFT-ECD calculations and X-ray analysis. Based on ECD calculations, the helicity of the 2,3-dihydroquinoline-4(1H)-one chromophore was correlated with the characteristic ECD transitions, and the resultant helicity rule was found to coincide with that of the chroman-4-one chromophore. The studies were reported in the European Journal of Organic Chemistry (**2014**, 4029-4036).

A chiral tetralin norsesquiterpenoid, ligukangtinol, was isolated from the plant Ligularia kangtingensis. Its planar structure was determined by extensive analysis of spectroscopic data (MS, IR, and NMR), and (1*S*,3*R*) absolute configuration of the tetralin ring was established by TDDFT-ECD calculations of the solution conformers. Conformational analysis and ECD calculations proved that the semiempirical helicity rules of 6-hydroxytetralins correlating the ^1L_b Cotton effect and P/M helicity of the fused carbocyclic ring correctly predicts the absolute configuration and thus can be used for the configurational assignment of related substituted tetralin derivatives. The results were disclosed in the Chirality journal [**2014**, *26(9),* 574-579].

A novel pyrrolizidine alkaloid, penibruguieramine A, characterized by an unprecedented 1-alkenyl-2-methyl-8-hydroxymethylpyrrolizidin-3-one skeleton, was isolated from the endophytic fungus *Penicillium sp*. GD6, associated with the Chinese mangrove *Bruguiera gymnorrhiza*. The absolute configuration of penibruguieramine A was established by TDDFT ECD calculations of the vacuum and solution conformers, exploiting the transitions of the lactam chromophore. The results were published in the Organic Letters (**2014**, 16, 1390-1393).

A new 3*H*-oxepine-containing antifungal alkaloid, varioxepine A, characterized by a structurally unprecedented condensed 3,6,8-trioxabicyclo[3.2.1]octane motif, was isolated from the marine algal-derived endophytic fungus *Paecilomyces variotii*. Its absolute configuration was established by DFT conformational analysis and TDDFT-ECD calculations. The results were published in the Organic Letters (**2014**, 16, 4834-4837).

The complementarity of electronic circular dichroism (ECD) and vibrational circular dichroism (VCD) spectroscopic techniques is demonstrated by showing that each technique reveals the structure of a different molecular segment. Using conformationally flexible carbohydrate disulfides, it was demonstrated that the synergetic use of ECD and VCD yields more complete structural characterization as it provides improved and more reliable conformer resolution. The results were published in the Chirality journal [**2014**, *26(9),* 525-531].

The assignment of the relative and absolute configuration of the antifungal peniciadametizine A, a dithiodiketopiperazine derivative possessing a unique spiro[furan-2,7′-pyrazino[1,2-*b*][1,2]oxazine] skeleton, and the highly oxygenated analogue, peniciadametizine B, isolated from the marine fungus *Penicillium adametzioide*s was achieced by the combination of NMR and ECD measurements with Density Functional Theory (DFT) conformational analysis and Time-Dependent Density Functional Theory-Electronic Circular Dichroism (TDDFT-ECD) calculations. Results were published in *Marine Drugs* (**2015**, *13*, 3640-3652).

The planar structure and absolute configuration of a new fusarielin analogue, isolated from a marine-derived fungus *Penicillium sp*. derived from the Mediterranean sponge *Ircinia oros,* was unambiguously elucidated by comprehensive spectroscopic analysis (1D and 2D NMR, and mass spectrometry) and TDDFT ECD calculations. The studies were reported in the *Tetrahedron Lett.* (**2015**, *56*, 5317-5320).

In order to support the study of the enzymatic [4+2] cycloaddition cascade that has a central role in the biosynthesis of natural tetracyclic pyrroindomycins, the absolute configuration and solution conformers of the pyrroindomycin aglycon was determined by the solution TDDFT-ECD approach. The results were published in the *Nature Chem. Biol*. (**2015**, *11*, 259-268).

Four new diketopiperazines including spirobrocazines A-C and brocazine G were characterized from the mangrove-derived Penicillium brocae MA-231. Spirobrocazines A and B had a 6/5/6/5/6 cyclic system with a rare spirocyclic center at C-2 and their structures and absolute configurations were determined by spectroscopic analysis, TDDFT-ECD calculations, and X-ray diffraction (*Org. Lett*. **2016**, *18*, 5304-5307).

The absolute configurations of 8 new α-pyrones including 4-deoxyphomapyrone C, 4-deoxy-11-hydroxyphomapyrone C, 4-deoxy-(7*R*)-hydroxyphomapyrone C, and phomapyrone C were determined by TDDFT-ECD calculations for the solution conformers, which revealed that the conformation of the side chain was decisive for the sign of the characteristic high-wavelength ECD transition. (−)-4-Deoxy-8-hydroxyphomapyrone C was deduced as a diastereomeric mixture containing (8*S*)- and (8*R*)-4-deoxy-8-hydroxyphomapyrone C in a ratio of 2.6:1 (8*R*:8*S*) by chiral phase HPLC analysis and Mosher's ester analysis (*J. Nat. Prod.* **2016**, *79*, 1610-1618).

Six new pyridone alkaloids, named penipyridones A−F, were isolated from the fermentation broth of an Antarctic moss-derived fungus, *Penicillium funiculosum*. Although they possess the same major chromophore and some of them presented almost mirror ECD spectra, their absolute configurations were found to be uniformly (*S*), as evidenced by X-ray single-crystal diffraction analysis, stereocontrolled total synthesis, and chemical conversions. TDDFT-ECD calculations of two compounds revealed that subtle conformational changes are responsible for the significantly different ECD curves (*J. Nat. Prod.* **2016**, *79*, 1783-1790).

Two new biscembranoid-like compounds, bissubvilides A and B, were isolated together with sarsolilide B, the proposed biogenetic precursor, from the soft coral *Sarcophyton subviride*. The structures and absolute configurations were solved by spectroscopic analysis and TDDFT/ECD and DFT/NMR calculations. The bissubvilides represent a novel biscembranoid-like skeleton presumed to derive from a cembrane-type diene and a capnosane-type dienophile via a Diels-Alder reaction (*J. Nat. Prod.*, **2016**, *79(10)*, 2552-2558).

III. Synthesis and stereochemical study of chiral O-heterocycles with potential pharmacological activities

a) Preparation of phtalan and 1,3-dihydroisoindole derivatives

1-Arylphthalans and 1-aryl-1,3-dihydroisoindoles were prepared by oxa-Pictet-Spengler and Pictet-Spengler cyclization from substituted benzyl-alcohols and benzyl-amines. Chiral HPLC and online HPLC-ECD analysis of three 1-arylphtalans were carried out to study the effect of substitution pattern on the chiroptical properties of the 1-arylphthalan chromophore. The absolute configuration of the separated enantiomers were determined by ECD calculations and antioxidant activities were also measured. The Lewis-acid-mediated cyclization of 3,4,5-trimethoxybenzil-alcohol resulted in a tribenzocyclononene derivative showing separable chiral and achiral conformers, which were studied by ECD.

b) Preparation of hexacyclic derivatives with chroman and tetrahydroquinoline rings

Tetrahydro-2,6-methano-1-benzoxocin-4-one and -4-ol derivatives with different substitution pattern on the aromatic ring were synthesized in a domino reaction. The kinetic resolutions of tetrahydro-2,6-methano-1-benzoxocin-4-ols were tested in enzyme-catalyzed transesterification reactions. The absolute configuration of the tetrahydro-2,6-methano-1-benzoxocin-4-one and -4-ol derivatives were studied by the combination of chiral HPLC-ECD, TDDFT ECD calculations and the zinc porphyrin tweezer method to check the effect of different aromatic substitutions on the characteristic ECD transitions.

Starting from a tetrahydro-2,6-methano-1-benzoxocin-4-one derivative, hexacyclic condensed O,N-heterocycles containing both chroman and tetrahydroquinoline rings were synthesized by a Schmidt reaction–reduction–S_NAr–C-H activation–cyclization sequence. The regio- and diastereoselectivity of the final cyclization step were studied by 2D NMR methods, single crystal X-ray diffraction of three derivatives, HPLC-ECD analysis and ECD calculations.

In the frame of collaboration, the enzyme catalyzed Bayer-Villiger oxidations of two tetrahydro-2,6-methano-1-benzoxocin-4-ones were carried out and the regio- and enantioselective formation of lactones were examined by NMR measurements, chiral HPLC analysis, ECD measurements and calculations. The above results are the basis of a Ph.D. dissertation and they are the subject of three manuscripts in preparation.

3c) Enantioselective transformations of 2-pyrone and 4-hydroxycoumarin derivatives

Starting from 4-hydroxy-6-methyl-2-pyrone or substituted 4-hydroxycoumarins, a series of warfarin analogues was prepared in enantioselective organocatalytic reactions with α,β-unsaturated ketones. The domino Knoevenagel-hetero-Diels-Alder reaction of 4-hydroxy-6-methyl-2-pyrone and substituted 4-hydroxycoumarin derivatives with unsaturated aldehydes such (*S*)-citronellal, citral, 2,6-dimethyl-5-heptenal was carried out and the stereochemistry of the cyclic products was studied by X-ray diffraction, solution and solid-state ECD, TDDFT-ECD calculations and NMR analysis. A publication is under preparation on the synthesis, structural elucidation and chiroptical studies of these derivatives.

3d) Preparation and further transformations of 2,3-diarylchromanones and 3-nitroflavanones

Preparation of substituted 3-nitroflavanone derivatives was achieved in an organocatalytic enantioselective domino Knoevenagel-oxa-Michael ring-closure sequence. 3-Aminoflavanones were prepared in Neber rearrangement reactions and the amino group was utilized to built a condensed heterocyclic ring affording derivatives with a new skeleton for neuroprotective and antimicrobial studies. The chiral derivatives were studied by HPLC-ECD and TDDFT-ECD calculations and they imply a contribution to a Ph.D. thesis.

A new high-yield method has been described for the preparation of isoflavone by oxidation of enol acetate of flavanone with thallium(III) nitrate or phenyliodonium diacetate. Some key intermediates of these oxidations were investigated with quantum chemical (HF and DFT) methods, the results of which were disclosed in the Monatschefte Chemie (2014, *145*, 849-855).

Starting from racemic naringenin, a mixture of dracocephin A stereoisomers {6-(2”-pyrrolidinone-5”-yl)naringenin and its regioisomer, dracocephins B {8-(2”-pyrrolidinone-5”-yl)naringenin isolated from *Dracocephalum rupestre*, have been synthesized in a one-pot reaction. The separation of the isomers was achieved by preparative HPLC and the four stereoisomers of each natural product were separated by analytical chiral HPLC and their absolute configuration was studied by the combination of HPLC-ECD measurements and TDDFT-ECD calculations (under revision at the *Beilstein J. Org. Chem.*).

3e) Preparation of isochroman-4-one derivatives

A series of chiral isochroman-4-one derivatives were prepared from substituted benzaldehydes using the umpolung reaction, which were studied by chiral HPLC-ECD measurements and ECD calculations and pharmacological studies are to be carried out. The isochroman derivatives were tested for neuroprotective activities against neurotoxicity induced by A$\beta_{25\text{-}35}$ amyloid fragment and some of them showed significant activity.