DOCTORAL (Ph.D.) THESIS

SYNTHESIS OF NEW CARBO- AND HETEROCYCLES MODIFIED WITH NITROXIDES

Győző Kulcsár

Head of the Program: József Deli, Ph.D, D.Sc

Project Leader: Kálmán Hideg, Ph.D, D.Sc

Head of the Department: Tamás Kálai, Ph.D, D.Sc

University of Pécs Medical School
Department of Organic and Medicinal Chemistry

Pécs
2013

I. Introduction

The tradition of research on heterocyclic organic compounds dates back several decades at the Department of Organic and Medicinal Chemistry of the University of Pécs. Dr. Kálmán Hideg and his coworkers were the first research team in Hungary that started to focus on the synthesis of stable nitroxide free radicals and their applications. Importance of the topic is well supported by the fact that research on free radicals developed into a new interdisciplinary field both in Hungary and in the world. Earlier, in the 70's and 80's, stable nitroxide free radicals were predominantly applied as spin labels for the study of protein structure and function. Biological application has been delayed by the belief that nitroxide radicals are just as harmful for the living organism as reactive oxygen species ($^{\cdot}OH$, $O_2^{\cdot -}$ etc.). During the last decades, results of our Department and those of other research groups have proven that these compounds and their diamagnetic amine precursors possess superoxide dismutase (SOD)-like and catalase activity. This finding is supported by the fact that the paramagnetic group may participate in certain single-electron-transfer processes (such as most of the oxido-reductive processes of the living organism). Compounds containing 2,2,5,5-tetramethyl-pyrrol(id)ine and 2,2,6,6-tetramethyl-piperidine rings exhibited antioxidant activity. Incorporation of these free radicals into biologically active substances, beyond maintaining the original therapeutic effects, the basic compound got antioxidant properties also. These studies showed that the stable nitroxide may contribute to pharmaceutical therapy in the future. Therefore synthesis of new 2,2,5,5-tetramethyl-pyrrol(id)ine and 2,2,6,6-tetramethyl-piperidine nitroxide derivatives is an interesting and exciting challenge.

I had the opportunity to join the research team as a student researcher and later as a Ph.D. student. In my Ph.D. thesis I summarize the synthesis of the compounds produced during that period, and results obtained with their application.

II. Objectives

1. Synthesis of new 5- and 6-membered carbo- and heterocycles anellated with pyrroline nitroxides.[1]

2. Synthesis of paramagnetic derivatives of biologically active flavonoids and polyphenolic compounds by the modification of the flavonoid B and C rings.[2] Synthesis of carbo- and heterocycles and flavonoid derivatives modified with pyrrol(id)ine nitroxides.[3]

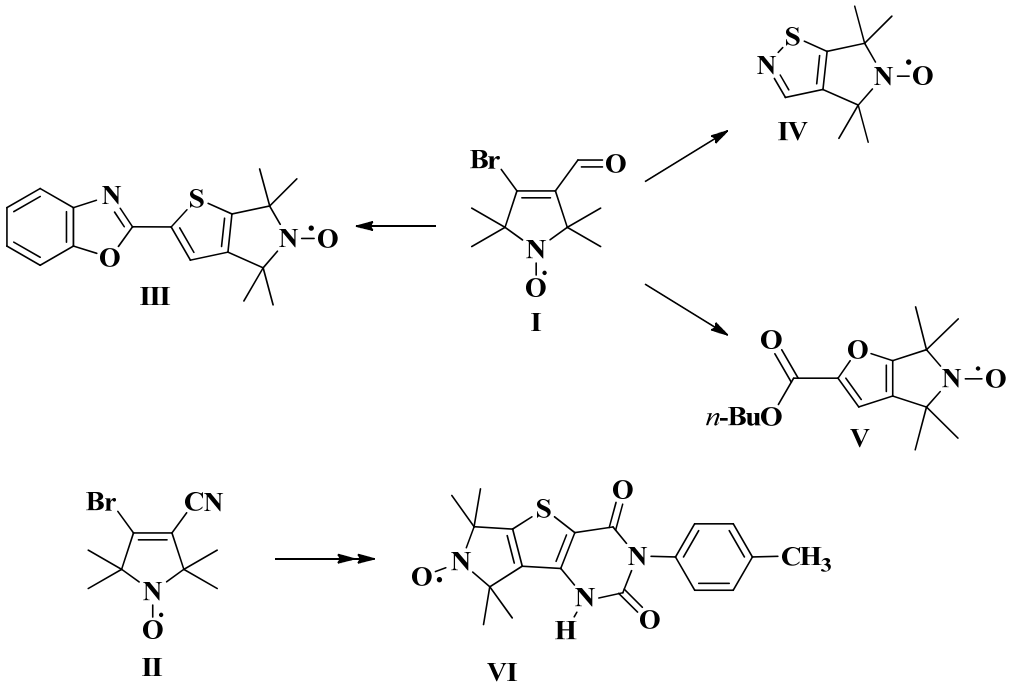
3. Synthesis and evaluation of new para- and diamagnetic quinazoline type poly(ADP-ribose)-polymerase inhibitor and cardio-protective compounds.[4]

III. Experimental procedures

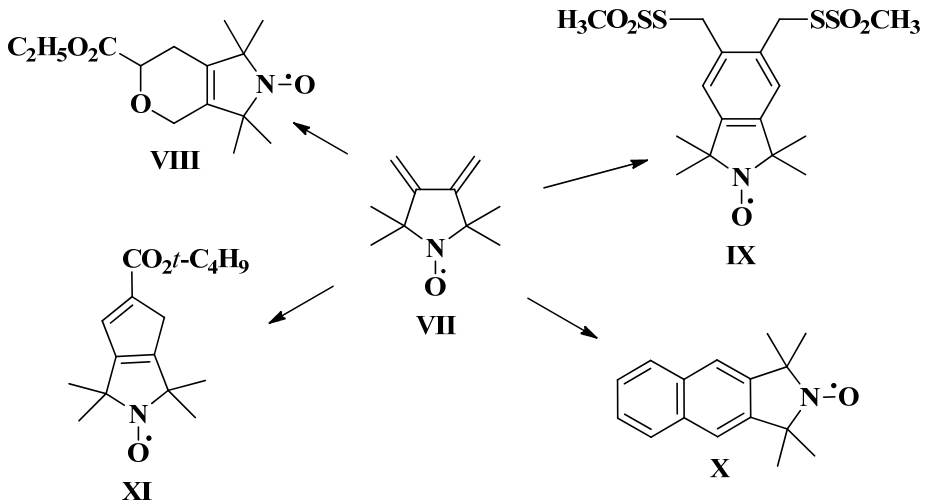
During our experiments we used macro and half-micro methods of the modern preparative organic chemistry. The compounds were purified on Merck Silica Gel 60 (0.040-0.063 mm) by flash column chromatography. The purity of the compounds was inspected by TLC on commercially available Merck Silica Gel 60 F_{254} plates. The melting points were determined with Boetius micro melting-point apparatus and uncorrected. Elementary analyses were performed on Fisons EA 1110 CHNS elemental analyzer. Mass spectra were recorded on Finnigan Automass-Multi and VG TRIO-2 instrument in EI mode. The NMR spectra were recorded with Varian Unity Inova 400 WB; chemical shifts were referenced to TMS. The infrared (IR) spectra recorded on Specord M85 instrument in KBr cuvettes, were in each case consistent with the assigned structure. The ESR spectra were obtained from a 10^{-4} molar solution ($CHCl_3$), using a Miniscope MS 200 spectrometer. All monoradicals exhibited three equally spaced lines with a_N=14.0-16.5 G. The biological results are based on *in vivo* and *in vitro* studies conducted at the Department of Biochemistry and Medical Chemistry and Ist Department of Internal Medicine, University of Pécs Medical School.

IV. New scientific findings

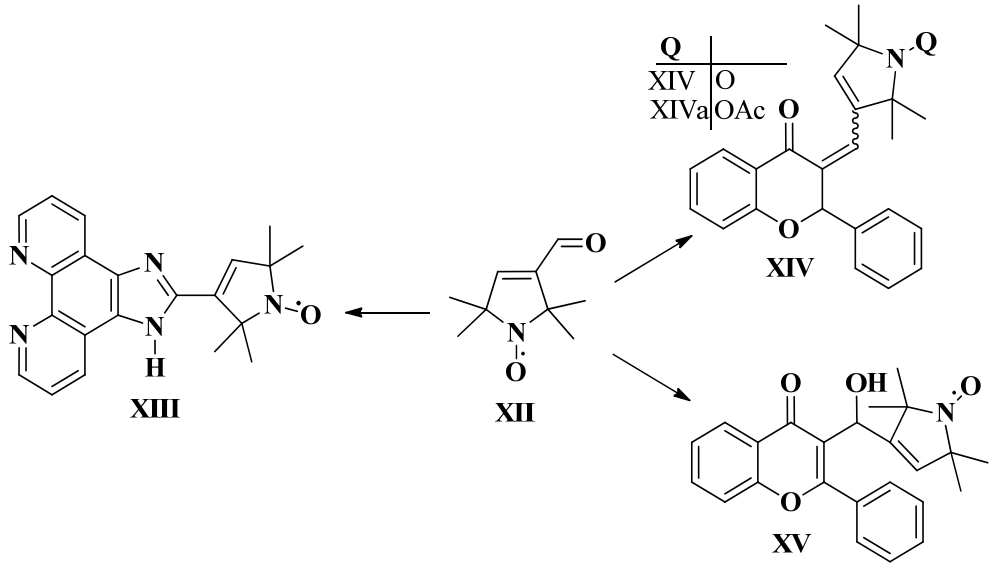
1. The scope and limitations of the reactions of aldehyde **I** were tested for synthesizing pyrroline nitroxides condensed heterocycles, without irreversible damage of the nitroxide moiety. The reaction of aldehyde **I** with 2-mercaptomethylbenzoxazole, ammonium thiocyanate, or *n*-butyl glycolate sodium salt afforded **III** 2'-thienyl-2-benzoxazole, **IV** isothiazole, **V** furan-*n*-buthyl-ester, respectively. Compound **II** with mercaptoacetic acid methyl ester and *p*-tolyl isocyanate gave compound **VI** thieno[3,2-d]pyrimidin-dione heterocycle.



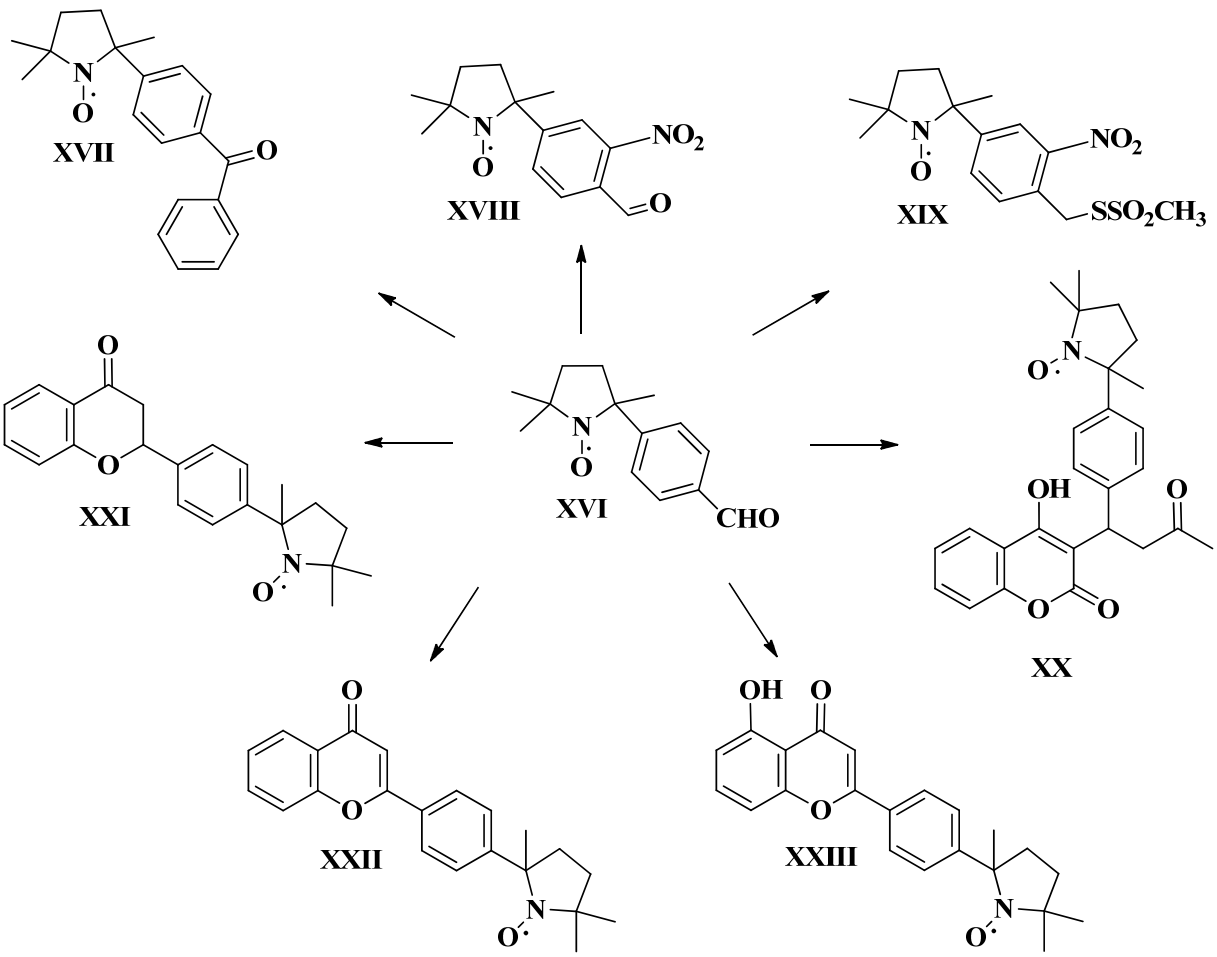
The Diels-Alder reaction of **VII** diene afforded **VIII** pyrano[3,4-c]pyrrole, **IX** crosslinking thiolspecific bismethanethiosulfonate, **X** naphthalene and **XI** cyclopentadiene derivatives.



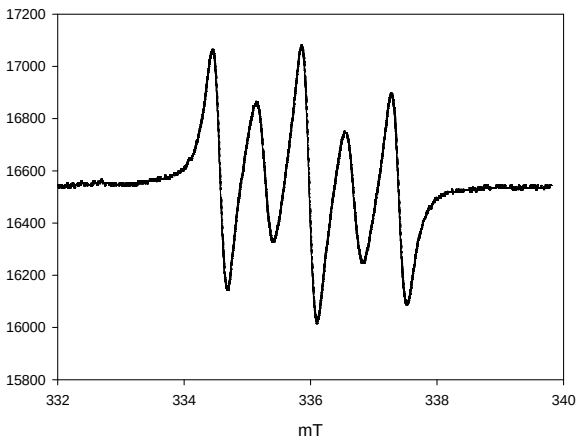
2. The paramagnetic aldehyde **XII** is an important reagent for synthesis of the **XIII** phenanthrimidazole and the **XIV** paramagnetic flavanon, **XV** paramagnetic flavon derivatives, with condensation and organometallic coupling reactions.

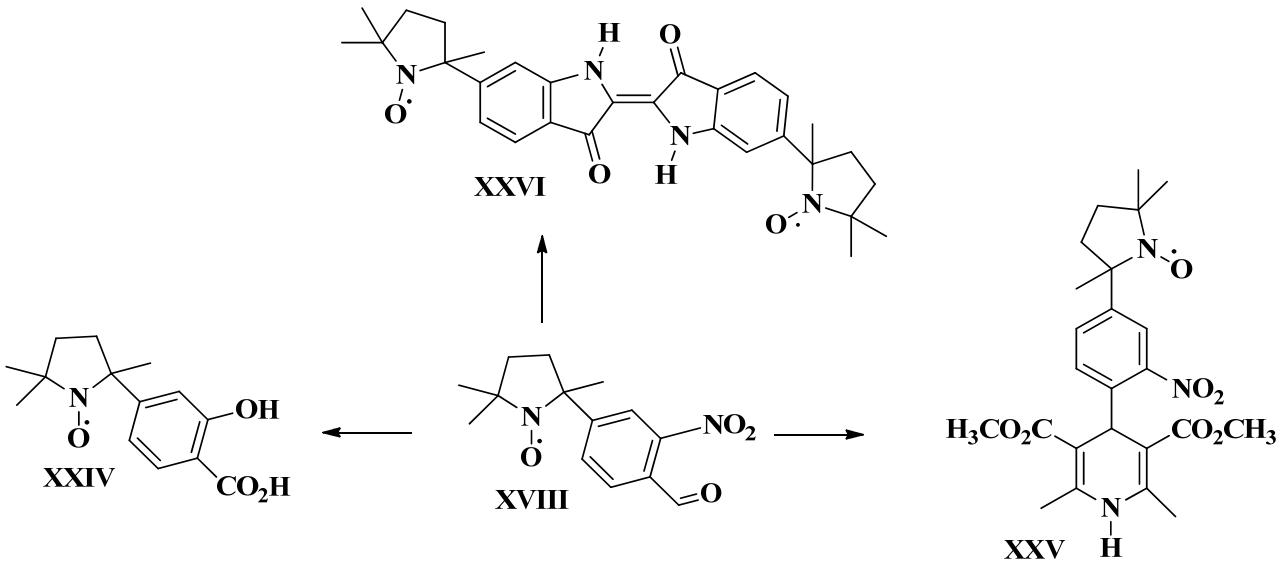


Reaction of 4-(1-oxyl-2,5,5,-trimethylpyrrolidine-2-yl)-benzaldehyde radical **XVI** was further investigated and extended to achieve photoactivable benzophenone (**XVII**), thiolspecific spin label **XIX**, paramagnetic flavon analogues (**XXI**-**XXIII**) and Warfarin analogue **XX**.

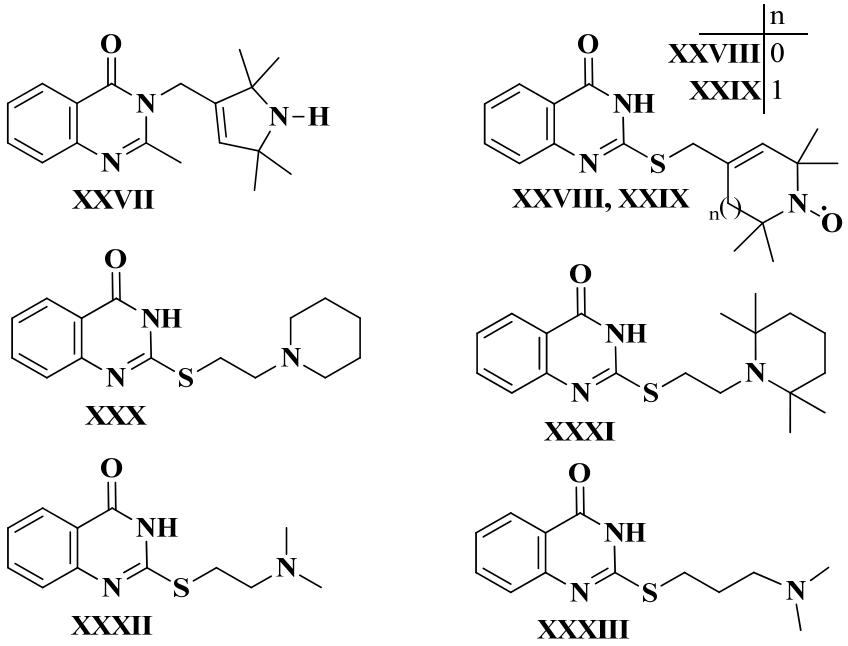


The 2-nitro-benzaldehyde bound to nitroxide **XVIII** was a valuable compound for synthesizing **XXIV** paramagnetic salicylic acid analogue and the **XXV** Nifedipine analogue, that is a 1,4-dihydropyridine-type calcium antagonist. The synthesis of the **XXVI** biradical indigo-derivative is a classical structure-proof the ortho-position of the nitro and the aldehyde groups in compound **XVIII.** The paramagnetic indigo (**XXVI**) formation is proven by quintet bands in its EPR spectrum.

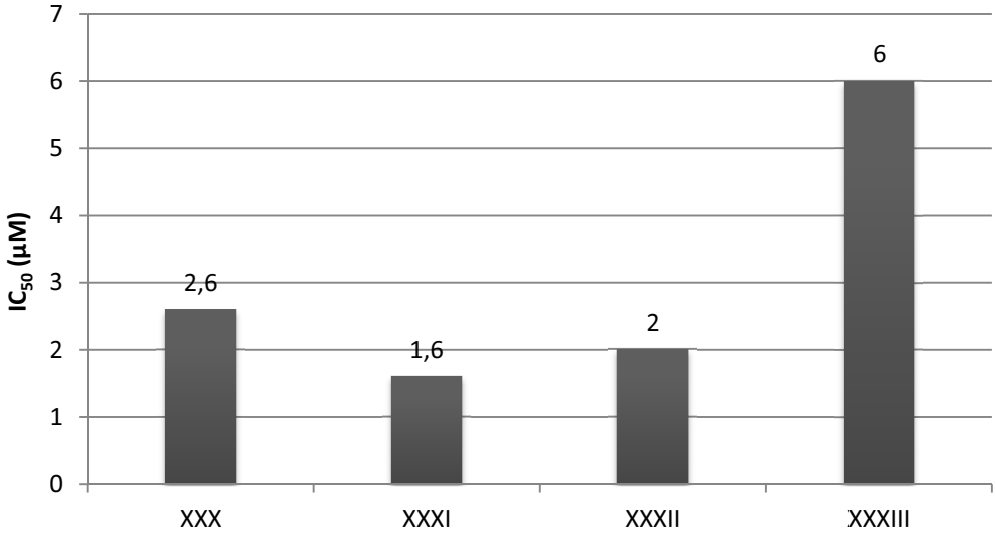




3. The 4-quinazolinone compounds possess several types of physiological effects. Due to their stability they are important starting compounds of pharmaceutical applications including PARP inhibition. We syntesized paramagnetically modified derivatives to study their PARP-inhibitory activity. Since alkylation of the NH-moiety lead to the loss of PARP inhibitory effects, therefore the 3-substitued-2-methylquinazoline-4(3*H*)-one (**XXVII)** derivative is inactive. Because of this all other modifications were carried out in position 2 of the quinazolin-4(3*H*)-one ring.



We synthesized the **XXVIII** and **XXIX** derivatives, but despite our expectations, these quinazolin-4(3*H*)-one derivatives also failed to show outstanding PARP-inhibition.



PARP inhibitory effect of the 4(3H)-quinazolinone derivatives on isolated rat liver enzymes

Unexpectedly, the non-radical type **XXX** *N*-ethyl-piperidine and **XXXI** *N*-ethyl-tetramethyl-piperidine derivatives, produced originally as reference compounds proved to be the most advantageous. For comparison, we also synthesized **XXXII** and **XXXIII** tertiary amine derivatives. Among these compounds, the easily obtainable derivative **XXX**, proved to be efficient in further biological tests. It was shown to restore systolic left ventricular function more efficiently in an animal model of infarction than enelapril.[5] Moreover, during infarction it influences other biochemical signal transduction pathways beneficially (it exerted beneficial effects on Akt and p38 MAPK signal system in *ex vivo* and *in vivo* models[6]) in addition to its PARP inhibition. Additionally, compound **XXX** inhibited protein kinase C (panPKC, PKC α/βII, PKC δ and PKC ε) phosphorylation, leading to activation of antihypertrophic factor GSK-3β.[7]

Based on results obtained previously in our Department and presented in my thesis, it has been proven that biologically active molecules modified by nitroxides or their precursors gain antioxidant properties while maintaining and even enhancing their original effects. The paradigm appears to be confirmed that harmful ROS and RNS must be eliminated immediately after formation, *in statu nascendi*.

V. List of publications

V.1. Publications that serve as basis for the present thesis

1. Kálai, T.; Kulcsár, G.; Jekő, J.; Ősz, E.; Hideg, K. Synthesis and reactions of paramagnetic aromatic aldehydes as useful synthetic building blocks, *Synthesis-Stuttgart* **2004**, 2115-2120. IF.: 2.203

2. Kálai, T.; Kulcsár, G.; Ősz, E.; Jekő, J.; Sümegi, B.; Hideg, K. Synthesis of paramagnetic and diamagnetic flavones and flavanones, *Arkivoc* **2004**, (VII) 266-276. IF.: 0.418

3. Kulcsár, G.; Kálai, T.; Jekő, J.; Hideg, K. Synthesis of paramagnetic carbo- and heterocycles, *Synthesis-Stuttgart* **2003**, 1361-1366. IF.: 2.074

4. Kulcsár, G.; Kálai, T.; Ősz, E.; Sár, C.; Jekő, J.; Sümegi, B.; Hideg, K. Synthesis and study of new 4-quinazolinone inhibitors of the DNA repair enzyme poly(ADP-ribose) polymerase (PARP), *Arkivoc* **2003**, (IV) 121-131. IF.: 0.392

V.2. Other publications

5. Bartha, É.; Kiss, G. N.; Kálmán, E.; Kulcsár, G.; Kálai, T.; Hideg, K.; Habon, T.; Sümegi, B.; Tóth, K.; Halmosi, R. *Journal of Cardiovascular Pharmacology* **2008,** *52* (3), 253-261. IF: 2.290

6. Pálfi, A.; Tóth, A.; Hantó, K.; Deres, P.; Szabados, E.; Szereday, Z.; Kulcsár, G.; Kálai, T.; Hideg, K.; Gallyas, F., Jr.; Sümegi, B.; Tóth, K.; Halmosi, R. *Journal of Molecular and Cellular Cardiology* **2006,** *41* (1), 149-159. IF: 4,859

7. Pálfi, A.; Tóth, A.; Kulcsár, G.; Hantó, K.; Deres, P.; Bartha, É.; Halmosi, R.; Szabados, E.; Czopf, L.; Kálai, T.; Hideg, K.; Sümegi, B.; Tóth, K. *Journal of Pharmacology and Experimental Therapeutics* **2005,** *315* (1), 273-282. IF: 4.335

V.3. Conference presentations

8. *Synthesis and study of cardiac drugs modified with nitroxides and their precursors*
   Kulcsár, G.; Kálai, T.; Fehér, G.; Koltai, K.; Tóth, K.; Sümegi, B.; Hideg, K.
   EPR 2005, a Joint Conference of 11th In Vivo EPR Spectroscopy and Imaging and 8th International EPR Spin Trapping, Columbus, OH, USA, 2005. szeptember 4-8.

9. *Új, heterociklusokhoz kapcsolt nitroxidok szintézise*
   Kulcsár, G.; Kálai, T.; Fehér, G.; Koltai, K.; Sümegi, B.; Tóth, K.; Hideg, K.
   MTA Gyógyszerkémia Bizottság, Pécs, 2005. június 9.

10. *Paramágneses benzaldehidszármazékok szintézise és reakciói*
    Kálai, T.; Kulcsár, G.; Ősz, E.; Jekő, J.; Hideg, K.
    MTA Heterociklusos Munkabizottság, Balatonszemes, 2004. május 20-21.

11. *Paramágneses policiklusos vegyületek szintézise*
    Kálai, T.; Kulcsár, G.; Balog, M.; Jekő, J.; Hideg, K.
    MKE Vegyészkonferencia 2003, Hajdúszoboszló, 2003. június 26-28.

12. *Effect of PARP-inhibitors and ACE-inhibitors on the progression of isoproterenol-induced heart*
    Bartha, E.; Halmosi, R.; Kulcsár, G.; Kiss, G. N.; Kálmán, E.; Sümegi, B.; Kálai, T.; Hideg, K.; Toth, K.
    ECS Congress 2007, Bécs, Ausztria, 2007. szeptember 1-5.

13. *Kettős hatású nitroxid gyökök és prekurzoraik vizsgálata*
    Kulcsár, G.; Kálai, T.; Fehér, G.; Koltai, K.; Sümegi, B.; Tóth, K.; Hideg, K.
    MKE Vegyészkonferencia 2005, Hajdúszoboszló, Magyarország, 2005. június 28-30.

14. *4-Hydroxy coumarine derivatives' dual action of platelet aggregation and red blood cell deformability*
    Fehér, G.; Koltai, K.; Késmarky, G.; Kulcsár, G.; Kálai, T.; Hideg, K.; Tóth, K.
    Haemophilia & Thrombophilia (Clinical and genetical aspects) 2nd International Symposium, Pécs, 2004. szeptember 23-25.

15. *Synthesis and reactions of paramagnetic aromatic aldehydes as useful synthetic building blocks*
    Kálai, T.; Kulcsár, G.; Jekő, J.; Ősz, E.; Hideg, K.
    XXIth European Colloquium on Heterocyclic Chemistry, Sopron, 2004. szeptember 12-15.

16. *Új, kinazolinon típusú PARP inhibitorok szintézise és vizsgálata*
    Kulcsár, G., Kálai, T.; Ősz, E.; P. Sár, C.; Jekő, J.; Sümegi, B.; Hideg, K.
    MKE Vegyészkonferencia 2003, Hajdúszoboszló, 2003. június 26-28.

VI. Acknowledgements

Experiments that form the basis of this doctoral work has been conducted in the Department of Organic and Medicinal Chemistry, Medical School, University of Pécs between 2001 and 2007. I am grateful for the generous financial support of OTKA K81123 and SROP 4.2.1.B-10/2/KONV-2010-0002.

I would like to thank Prof. Kálmán Hideg and Prof. Tamás Kálai for their continuous support of my scientific progress. I am especially grateful to dr. Cecília Sár for her ever helpful support in the laboratory experiments and in the preparation of publications from the very beginning. I thank dr. József Jekő for his help in the mass spectrometry measurements, and dr. Erzsébet Ősz† and dr. Zoltán Berente for their help in NMR spectroscopy studies.

I am grateful for professors Prof. Balázs Sümegi and Prof. Kálmán Tóth and their co-workers for their contribution to biological experiments.

I am thankful for the work and help of dr. Balázs Bognár associate professor, Mária Balog, Viola Csokona, Éva Lamperth, Noémi Lazsányi, Krisztina Kish chemical technicians, dr. Ivánné Deák secretary and Gábor Sajóvölgyi. I am also grateful for the supportive friendly milieu of the laboratory.

I am grateful for the members of the Department of Pathophysiology and Gerontology, Medical School, University of Pécs, for their support in the completion of my thesis. I also appreciate the opportunity to participate in the high level research activity of the Department.

I would like to express my gratitude for my family and friends for their support. They made it possible for me to concentrate on my work, freed of much of the daily burdens.