Synthesis of heterocycles linked with nitroxides and their precursors: synthesis of new, bioactive and sensor compounds

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Introduction

In the Institute of Organic and Medicinal Chemistry research is done on heterocyclic compounds, especially on the synthesis of probable bioactive compounds. Dr. Kálmán Hideg, Dr. Olga H. Hankovszky and their co-workers started to investigate the synthesis of paramagnetic pyrrolins, pyrrolidins and piperidins in the mid 70s. Several new spin traps, spin labelled bio molecules (amino acids, fatty acids etc.), double (spin and fluorescence) sensors and biologically active free radicals have been synthesised in the Institute since then. Based on our observation and also on that of other research groups it can concluded that the modification with 2,2,5,5-tetramethyl-pyrrol(id)ine, 2,2,6,6-tetramethyl-piperidine nitroxides or their precursors has beneficial influence on the basic drugs. This inspired our team to synthesise bioactive compounds with antioxidant activity.

During the past two decades many publications have discussed the use of nitroxides (cooxidant, contrast materials, auxiliary polymerisation, organic Ferromagnet) besides the classical use (spin labelling and spin trapping).

Though stable nitroxides have been known for about 50 years – because many reactions cannot be accomplished in the presence of free radical centre – their chemistry counts to be special and it imposes many problems and challenges on the chemist.

I joined the research team of the Institute in 2003, and I have been conducted my research as a PhD student since 2006. My thesis is based on the compounds synthesised and tested in the past six years.
Objective

1. Finding new methods for synthesizing nitroxides, which are condensed through carbon-carbon or carbon-heteroatom bonds to the heterocyclic ring.
2. Synthesizing dual (spin and fluorescence) sensors, which are excitable and emitting on different wavelengths.
3. Modifying class III. antiarrhythmic agent amiodarone, and class IV agent verapamil with 5- and 6-membered nitroxides and their precursors. We hoped that the new derivatives have antioxidant properties in addition to the original beneficial effect of the basic compounds.

Experimental procedures

Macro and half-micro methods of the modern, preparative organic chemistry were used for the synthesis of the compounds reported in the thesis. We used TLC to follow the reactions and flash column chromatography to purify the derivatives. The purity of the compounds was checked by TLC and melting point measurement. The structures were determined by IR, \(^1\)H NMR, \(^{13}\)C NMR, ESR and Mass spectroscopy. We used steady-state and optical spectroscopy, ESR, scanning-microscopy, in vitro and in vivo biological probes to test the new compounds. The biological experiments were carried out in the Department of Biochemistry and Medical Chemistry, University of Pécs Medical School and in Davis Heart and Lung Research Institute, Ohio State University, Columbus, USA.
Results

1.a. New quinolines (IV, IX, XII) were synthesised starting from aldehydes (I, II) and keton (III) with classical methods. Furthermore we synthesised porphyrin (V), oxazole (VI) and benzazoles (VII, VIII) starting from α,β-unsaturated-aldehyde (I) while thia diazole (X) and benzofurane (XI) anellated with nitrooxide starting from the ketone (III). These paramagnetic heterocycles can be utilized as enzyme (kinase) inhibitors or small antioxidant molecules.¹
1.b. Starting from 2-acetyl benzimidazole (XIII) pyrazino[1,2-a]benzimidazoles (XIV, XV, XVI) were synthesized. 2-substituted benzimidazoles (XIX, XX) were obtained by the lithiation of 1-hydroxymethyl-benzimidazole (XVII) and treatment with electrophiles. The ESR spectrum of compounds XIX and XX exhibited pH sensitivity in the range of pH 2 to 6.²

\[
\begin{align*}
\text{XIII} & \quad \text{XIV} \\
\text{XV} & \quad \text{XVI} \\
\text{XVII} & \quad \text{XVIII} \\
\text{XIX} & \quad \text{XX}
\end{align*}
\]

The alteration of the ESR coupling constant of nitroxides XIX and XX pH 2-6.
2. Nitroxides quench the fluorescence of fluorophores by dynamic mechanism. When the nitroxide is reduced to $N$-hydroxylamine or recombined with carbon radical, the intensity of fluorescence increases. This process can also be followed by fluorescence or EPR spectroscopy, therefore these compounds are called “dual sensors”.

Series of new donor-acceptor probes were synthesised in our laboratory earlier. Our objective was the synthesis of dual sensors emitting different wavelengths (370-780 nm), which enables us to follow the redox status in different applications. We synthesised several donor-acceptor compounds containing different fluorophores [pirene (XXI), coumarine (XXII), nitrobenzofurazan (XXIII), phenanthroline (XXIV), Nile red (XXV), BODIPY derivatives (XXVI, XXVII)] and nitroxides. Our experiments showed, that the shorter the donor-acceptor distance, the better the sensitivity of dual sensors is.\(^3\)

The compound (XXIV) exhibited fluorescence increase and EPR band broadening upon B-DNA addition, providing an evidence of binding of this complex to DNA.
Fluorescence emission spectra of 50 μM XXV (→) in a buffer (50 mM NaCl and 5 mM Tris), 50 μM XXV and 1.0 mM sodium ascorbate (−−) in a buffer, 50 μM XXV and 270 μM Calf thymus DNA (----) in a buffer, λ_{ex}: 453 nm.

EPR spectra of 50 μM XXV (→) in a buffer (50 mM NaCl and 5 mM Tris), 50 μM XXV and 270 μM Calf thymus DNA (----) in a buffer.
3. New amiodarone derivatives were synthesised starting from 2-methylbenzofurane. Primary biological experiments (MPT, Toxicity, recovery of organic phosphates) proved that compound XXVII is as effective as amiodarone, with lower toxicity.\textsuperscript{4,5} We synthesised new verapamil derivatives, modifying its nitrile group or amino group of nor-verapamil. The new compounds exhibited superoxide scavenging activity. In detailed \textit{in vitro} and \textit{in vivo} experiments hydroxilamines XXVIII and XXIX retained the Ca\textsuperscript{2+}-channel blocking activity; furthermore they improved the parameters of the Langendorff perfused heart during ischemia-reperfusion compared to the basic drug. Based on earlier and recent results, we can state, that the modification of drugs with nitroxides or their precursors is beneficial because these modified drugs inhibit the damages caused by ROS during ischemia developed \textit{“in statu nascendi”} and expand the spectrum biological activity.\textsuperscript{6}

![Chemical structures of amiodarone, verapamil, XXIII, XXVIII, XXIX, XXX](image)

\begin{figure}
\centering
\includegraphics[width=0.8\textwidth]{chart.png}
\caption{The effect of verapamil, amiodarone and their derivatives on the Rate-pressure Product measured on rat Langendorff perfused hearts.}
\end{figure}
List of publications:

   
   If. 2,333  cit. 2

   
   If. 2,247  cit. 0

   
   If. 0,922  cit. 3

   
   If. 2,286  cit. 4

   
   If. 5,44  cit. 6

   
   If. 3,643  cit. 1
List of lectures and posters:

1. *Synthesis and Study of Paramagnetic and Diamagnetic Amiodarone Derivatives*
   Balázs Bognár, Zita Bognár, Gábor Váršíró, Tamás Kálai, Balázs Sümmegi, Kálmán Hideg

2. *Synthesis and Study of Paramagnetic and Diamagnetic Amiodarone Derivatives*
   Tamás Kálai, Zita Bognár, Gábor Váršíró, Balázs Bognár, Anita Pálfi, Katalin Hántó, Balázs Sümmegi and Kálmán Hideg
   JMMC, Vienna, Austria, June 20-23, 2005.

3. *Modification of Amiodarone with Nitoxides and their Diamagnetic Precursors*
   Tamás Kálai, Zita Bognár, Gábor Váršíró, Balázs Bognár, Anita Pálfi, Katalin Hántó, Balázs Sümmegi and Kálmán Hideg

4. *Aminoetil-oldalláncban helyettesített amiodaron származékok szintézise és vizsgálata*
   Bognár Balázs, Radnai Balázs, Tucsek Zsuzsanna, Bognár Zita, Kálai Tamás, Sümmegi Balázs, Hideg Kálmán

5. *Nitroxidokkal és elővegyületeikkel módositott verapamil származékok szintézise és vizsgálata*
   Bognár Balázs, Rajarsi Mandal, Kálai Tamás, H. Hankovszky Olga, Periannan Kuppusamy, Hideg Kálmán

6. *Aminoetil-oldalláncban helyettesített amiodaron-származékok szintézise és vizsgálata*
   Bognár Balázs, Kálai Tamás, Sümmegi Balázs, Hideg Kálmán
7. *Synthesis and application of double (spin and fluorescent) sensor reagents*
Kálai, T.; Hideg, É.; Bognár, B.; Jekő, J.; Hideg, K.
1st Hungarian–Singaporean Workshop on Drug Discovery and Biomaterials (proceedings)

8. *HO-4038, A Pro-antioxidant Modification of Verapamil Protects the Heart against Ischemia-Reperfusion Injury through its Antioxidant and Pro-survival Akt Activity*
Iyyapu K Mohan, Mahmood Khan, Sheik Wisel, Karuppaiyah Selvendar, Arun Sridhar, Cynthia A. Carnes, Balazs Bognar, Tamás Kálai, Kálmán Hideg, and Periannan Kuppusamy

9. *Paramágneses benzimidazol-származékok szintézise*
Kálai Tamás, Bognár Balázs, Gulyás Gergely, Hideg Kálmán

10. *Nitrooxidokkal és elővegyületeikkel módosított verapamil-származékok szintézise és vizsgálata*
Bognár Balázs, Rajarsi Mandal, Kálai Tamás, H. Hankovszky Olga, Periannan Kuppusamy, Hideg Kálmán

11. *Vörös hullámhossz tartományban emittáló kettős (spin és fluoreszcens) szenzorok szintézise és vizsgálata*
Bognár Balázs, Kálai Tamás, Jekő József, Hideg Kálmán

12. *Synthesis and study of new paramagnetic and diamagnetic verapamil derivatives*
Balázs Bognár, Rajarsi Mandal, Tamás Kálai, Olga H. Hankovszky, Periannan Kuppusamy, Kálmán Hideg
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