Ph.D. thesis

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Application of nitrone compounds in medicinal research and development

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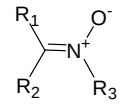
1. Introduction & aim

Nitrones of general structure **1** belong to a very exciting area of organic chemistry. Their special structure cause the capability for interesting reactions. Regarding biological behavior, certain nitrones possess free radical scavenging properties.



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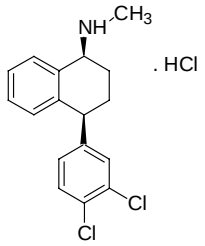
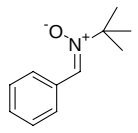
In the first part of my PhD work, a new patentable, industrially feasible process for the preparation of the antidepressant sertraline was my task. This could be achieved with the use of a nitrone intermediate.



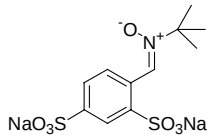
(+)-37
sertaline hidrochloride

Thereafter, we investigated the molecules having a nitrone moiety in medicinal aspects, and we have found, that certain nitrones , e.g. *N*-*tert*-butyl-α-phenyl-nitrone (PBN) or its 2,4-disulfonic acid disodium salt derivative (NXY-059)

developed in Phase III.clinical trials for the indication of stroke, provide the opportunity to treat neorodegenerative disorders like stroke, Alzheimer's disease, and other pathological problems.



PBN



NXY-059

Continuing my reasearch, I aimed at the synthesis of new nitrone compounds, whoose free radical scavenging and neuroprotective effects are more advantageous than that of similar reference compounds. First, we tried a small structural modification of PBN, the substitution of the *N-tert*-butyl group with a cyclopropyl moiety. Then, we combined the nitrone component with trolox, a known antioxidant compound.

2. Experimantal methods

During our research, we used preparative organic chemistry and separation methods. Reactions were followed by thin layer chromatography. Isolation of compounds was carried out by crystallization or liquid chromatographic technics. Structure of new compounds was elucidated by IR, ^{1}H and ^{13}C NMR, MS spectroscopic measurements. The biological evaluation of our new nitrone

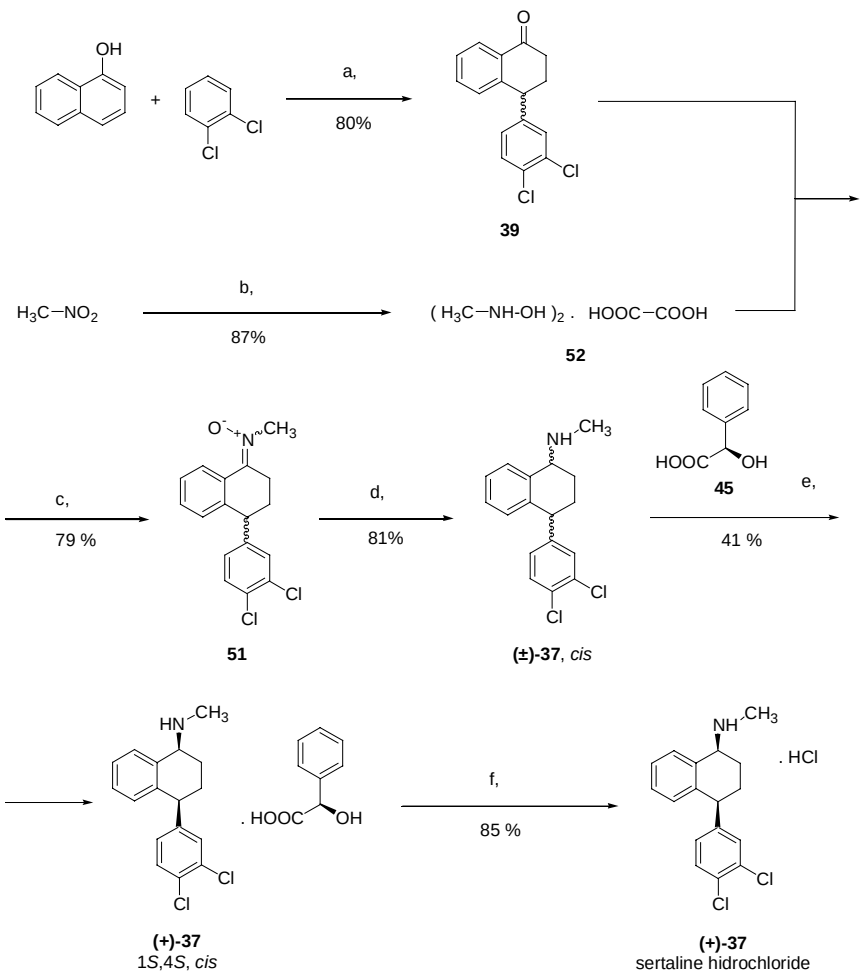
molecules was performed *in vitro* for scavenging different free radicals, inhibiting lipid peroxidation, and *in vivo* in a stroke model.

3. New scientific results

1. Novel industrial synthesis for the preparation of the excellent antidepressant agent, sertraline hydrochloride (+)-**37** has been elaborated, which involves the new intermediate *N*-[4-(3,4-dichlorophenyl)-3,4-dihydro-1(2*H*)-naphthalenylidene]-methanamine *N*-oxide **51**. Our procedure is in many respects more advantageous than processes reported before. The nitrone intermediate **51** is a stable compound in normal conditions. It can be obtained in a simple reaction from the corresponding tetralone in good yield, using acceptable reagents with regard to environmental and safety respects.

2. Reduction of the nitron intermediate of sertraline **51** to the desired *cis*-racemic amine (±)-**37** is stereoselective, thus after resolution, sertraline hydrochloride (+)-**37** can be obtained with a purity required for pharmaceutical ingredients.

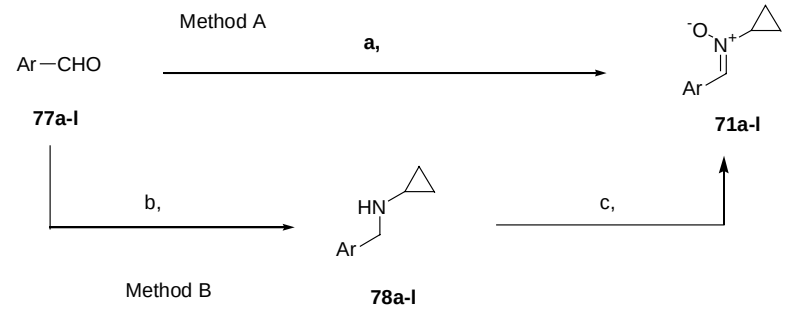
3. Reactions in the synthesis of sertraline were optimized modifying the solvent, the reagents, the temperature, the reaction time and the catalyst. Scale-up of our process has been done, and the procedure was feasible for production.



Scheme 1. Reagents and conditions: (a) $AlCl_3$, 100 ^oC, 1h; (b) H_2 / Pd / C, MeOH, HOOC-COOH . 2 H_2O, 23-34 ^oC, 4.5h; (c) KOAc, EtOH, 4h; (d) H_2 / Raney-Ni, MeOH, 25 ^oC, 6h; (e) EtOH, 25 ^oC; (f)1. NaOH, water, CH_2Cl_2; 2. HCl, EtOH.

4. Application of the nitron intermediate of sertraline **51** provides a new patented process for the preparation of sertraline. This allows Gedeon Richter Ltd. to produce the pharmaceutical ingredient sertraline.

5. Novel *N*-cyclopropyl-α-aryl and α-hetaryl nitrones (**71a-l**) have been synthetized. Preparations were performed by condensation of aromatic aldehydes and *N*-cyclopropyl-hydroxylamine or by oxidation of secondary amines prepared from aromatic aldehydes and N-cyclopropylamine via the corresponding Schiff base.



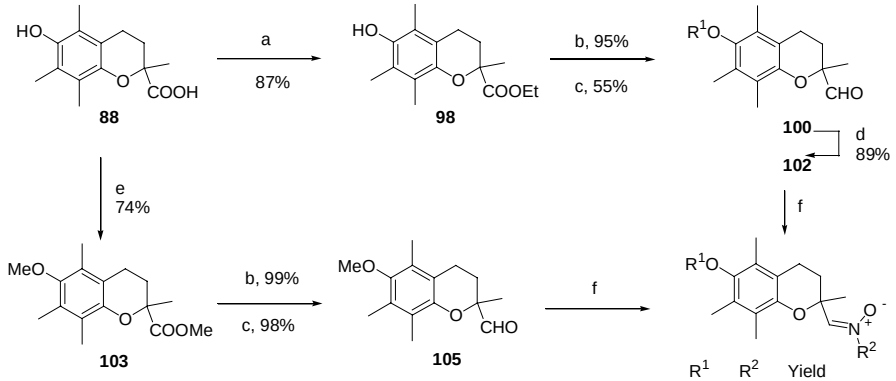
Scheme 2. Reagents and conditions: (a) cPr-NH-OH, MeOH, reflux, 3h; (b) 1. cPr-NH_2, $NaHCO_3$, MeOH, reflux, 4h; 2. $NaBH_4$, 1h at 0 ^oC, 12h at 20 ^oC; (c) Na_2WO_4, 30% H_2O_2, methanol, 1h at 0 ^oC, 12h at 20 ^oC.

Table 1. Preparation of *N*-cyclopropyl amines (**78a-l**) and *N*-cyclopropyl nitrones (**71a-l**) ([a] Method A, [b] Method B)

R	Amines	Yield (%)	Nitrones	Yield (%)
Phenyl	**78a**	93	**71a**	43[a] 63[b]
4-Fluoro-phenyl	**78b**	95	**71b**	50[b]
4-Cl-phenyl	**78c**	99	**71c**	53[b]
4-Br-phenyl	**78d**	97	**71d**	66[b]
2-Hidroxy- phenyl	**78e**	99	**71e**	52[b]
3,4-Methylenedioxy- phenyl	**78f**	95	**71f**	62[b]
3,4-Dimethoxy-phenyl	**78g**	97	**71g**	59[b]
4-Ethoxycarbonyl- phenyl	**78h**	88	**71h**	39[b]
3,5-Di-*tert*-butyl-4-hidroxy-phenyl	**78i**	62 (HCl-só)	**71i**	38[b]
4-Trifluoro-methyl- phenyl	**78j**	93	**71j**	44[b]
2-pirrol	**78k**	95	**71k**	41[b]
3-Piridyl	**78l**	96	**71l**	65[b]

6. Biological investigations on the new *N*-cyclopropyl-α-aryl and α-hetaryl nitrones (**71a-l**) proved the advantage of substituting the *N-tert*-butyl group, that is widely used among nitrone type free radical scavenging molecules, with an *N*-cyclopropyl moiety. *N*-cyclopropyl nitrones were more effective in *in vitro* free radical scavenging assays than the corresponding *N-tert*-butyl derivatives.

7. New derivatives of the known antioxidant trolox possessing a nitrone moiety (**93a,b**) have been synthetized starting from trolox in 4 steps.



	R^1	R^2	Yield
93a	H	tBu	84%
93b	H	cPr	60%
93c	Ac	tBu	67%
93d	Ac	cPr	29%
93e	Me	tBu	61%
93f	Me	cPr	80%

Scheme 3. Reagents and conditions: (a) EtOH, pTsOH, reflux, 5h; (b) DIBAL-H, toluene, -70 ^{o}C; (c) 1., oxalyl chloride, DMSO, CH_2Cl_2, -70 ^{o}C, 2., Et_3N; (d) acetyl chloride, Et_3N, CH_2Cl_2, 0 ^{o}C, 1.5h; (e) MeI, K_2CO_3, DMF, 40 ^{o}C; (f) R_2NHOH, EtOH, reflux, 3 h.

8. We expected from this combination that combining two different types of free radical scavenging abilities would provide our new molecules with beneficial effect. This was proved by i*n vitro* and *in vivo* biological experiments. New compounds exerted excellent free radical scavenging and lipid peroxidation inhibiting properties. Furthermore, they significantly decreased the cerebral infarct in an *in vivo* stroke model.

9. *O*-protected nitrone derivatives of trolox **93c-f** have also been synthetized. Their biological investigations confirmed the active contribution of the nitrone moiety in the free radical scavenging activity.

4. Publications related to the thesis:

Publications:

1. Vukics, Krisztina; Fodor, Tamás; Fischer, János; Fellegvári, Irén; Lévai, Sándor: Improved industrial synthesis of antidepressant sertraline. *Organic Process Research & Development*, 2002, *6*(1), 82-85.

2. Vukics, Krisztina; Tárkányi, Gábor; Dravecz, Ferenc; Fischer, János: Synthesis of C-aryl-N-cyclopropylnitrones. *Synthetic Communications*, 2003, *33*(19), 3419-3425.

3. Balogh, Gy, T.; Vukics, K.; Könczöl, Á.; Kis-Varga, Á.; Gere, A.; Fischer, J.: Nitrone Derivatives of Trolox as Neuroprotective Agents *Bioorg. Med. Chem. Lett.* 2005, *15*, 3012.

Patent:

Vukics, Krisztina; Fodor, Tamás; Fischer, János; Fellegvári, Irén; Lévai, Sándor: Novel intermediates for preparation of sertraline. PCT Int. Appl. WO 98/27050 A1, **1998**; *Chem Abstr.* 129:81571.

Poster:

Vukics, K.; Balogh, Gy. T.; Gere, A.; Stadler, K.; Tárkányi, G.; Fischer, J. Synthesis and free radical scavenging activity of *C*-aryl-*N*-cyclopropyl-nitrones. *Abstracts of Papers,* XVIIth International Symposium on Medicinal Chemistry, Barcelona, Spain, Sept 1-5, 2002; P 531.

Other publications:

1. Vukics, Krisztina; Fischer, János; Lévai, Sándor; Erdélyi, Péter: Process for the synthesis of mosapride citrate dihydrate. PCT Int. Appl. WO 2003/106440 A2, **2003**; *Chem Abstr.* 140:42188.

2. Fischer, János; Vukics, Krisztina; Erdélyi, Péter; Hegedűs, Béla; Tihanyi, Károly; Vastag, Mónika; Lévai, Sándor; Bálint, Sándorné; Lánczos, Krisztina: Pharmaceutical compositions containing atorvastatin salts with amino acids. PCT Int. Appl. WO 2003/82816 A1, **2003**; *Chem Abstr.* 139:297034.

3. Vukics, Krisztina; Gere, Anikó; Kis-Varga, Ágnes; Fischer, János: 3,4-Diaryl-

1,5- dihydro-pyrrol-2-ones as cyclooxygenase-2 inhibitors. *Abstracts of Papers*, International Symposium on Advances in Synthetic, Combinatorial, and Medicinal Chemistry, Moszkva, May 5-8, 2004, P 203.

4. Vukics, K.; Csomor, K.; Hruby, Gy.; Fischer, J.: Synthesis and biological evaluation of compounds exerting both antithrombotic and vasodilatory activities. *Abstracts of Papers*, Hungarian-German-Italian-Polish Joint Meeting on Medicinal Chemistry, Budapest, Sept 2-6, 2001, P 161.