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In search of potent and selective inhibitors of neuronal nitric oxide synthase with more simple structures

Qing Jing [a,b], Huiying Li [c,d,e], Jianguo Fang [a,b], Linda J. Roman [f], Pavel Martásek [f,†], Thomas L. Poulos [c,d,e,*], Richard B. Silverman [a,b,*]

[a] Department of Chemistry, Chemistry of Life Processes Institute, Center for Molecular Innovation and Drug Discovery, Northwestern University, Evanston, IL 60208-3113, USA
[b] Department of Molecular Biosciences, Northwestern University, Evanston, IL 60208, USA
[c] Department of Molecular Biology and Biochemistry, University of California, Irvine, CA 92697-3900, USA
[d] Department of Pharmaceutical Sciences, University of California, Irvine, CA 92697, USA
[e] Department of Chemistry, University of California, Irvine, CA 92697-2025, USA
[f] Department of Biochemistry, The University of Texas Health Science Center, San Antonio, TX 78384-7760, USA

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ABSTRACT

In certain neurodegenerative diseases damaging levels of nitric oxide (NO) are produced by neuronal nitric oxide synthase (nNOS). It, therefore, is important to develop inhibitors selective for nNOS that do not interfere with other NOS isoforms, especially endothelial NOS (eNOS), which is critical for proper functioning of the cardiovascular system. While we have been successful in developing potent and isoform-selective inhibitors, such as lead compounds **1** and **2**, the ease of synthesis and bioavailability have been problematic. Here we describe a new series of compounds including crystal structures of NOS-inhibitor complexes that integrate the advantages of easy synthesis and good biological properties compared to the lead compounds. These results provide the basis for additional structure–activity relationship (SAR) studies to guide further improvement of isozyme selective inhibitors.

1. Introduction

Nitric oxide (NO) is an important second-messenger molecule that plays many fundamental physiological roles. It is produced from L-arginine by the nitric oxide synthase (NOS) family of enzymes, which includes neuronal NOS (nNOS), endothelial NOS (eNOS), and inducible NOS (iNOS). However, overproduction of NO by the nNOS in the brain is closely associated with many neurodegenerative diseases, including chronic pathologies such as Parkinson's,[1] Alzheimer's,[2] Huntington's,[3] headaches,[4] and neuronal damage in stroke.[5] Therefore, it has become a promising strategy to inhibit nNOS to block the excess generation of NO for the treatment of neurodegeneration.[6–8] A large number of nNOS inhibitors have been reported[9] but none of these inhibitors has entered clinical trials because of low potency or poor isoform selectivity.[9] The isozymes of NOS share ~50% sequence homology and show a highly similar heme active site structure, which is why most inhibitors directed at the substrate binding site show limited isoform selectivity. While we have succeeded in developing highly selective nNOS inhibitors,[10] combining ease of synthesis, bioavailability, and selectivity remains a challenging task.

We have previously developed two lead compounds (**1** and **2**) that exhibit excellent potency against nNOS. Compound **1** provides an excellent dual-selectivity of nNOS over the other two isoforms (K_i = 7 nM, e/n = 2667, i/n = 806),[11] but, unfortunately, the tedious synthesis limits its structure/activity optimization to improve bioavailability. The synthesis of **2** (three synthetic steps) is much easier than **1** (15 synthetic steps plus a chiral resolution), but its selectivity needs to be improved considerably.[12] On the basis of the current results, we attempted a new strategy to integrate both the advantages of easy synthesis and good activity for the next generation of inhibitors. The new compounds were designed with short synthetic routes, and their structures are ready for further optimization.

2. Chemistry

The synthesis of inhibitors **5** began with a condensation reaction to protect 4,6-dimethylpyridine, and the product, compound **3**, was deprotonated with *n*-BuLi then treated with a dibromide to obtain compound **4**. After removal of the protecting groups in the presence NH_2OH–HCl, inhibitors **5a–c** were obtained, as described in Scheme 1.

* Corresponding authors. Tel.: +1 949 824 7020 (T.L.P.), +1 847 491 5653 (R.B.S.).
E-mail addresses: poulos@uci.edu (T.L. Poulos), Agman@chem.northwestern.edu (R.B. Silverman).
† Present address: Department of Pediatrics and Center for Applied Genomics, 1st School of Medicine, Charles University, Prague, Czech Republic.

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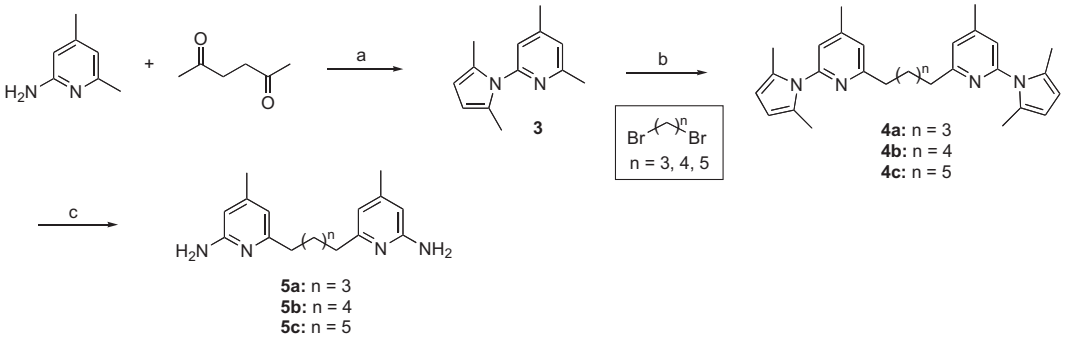
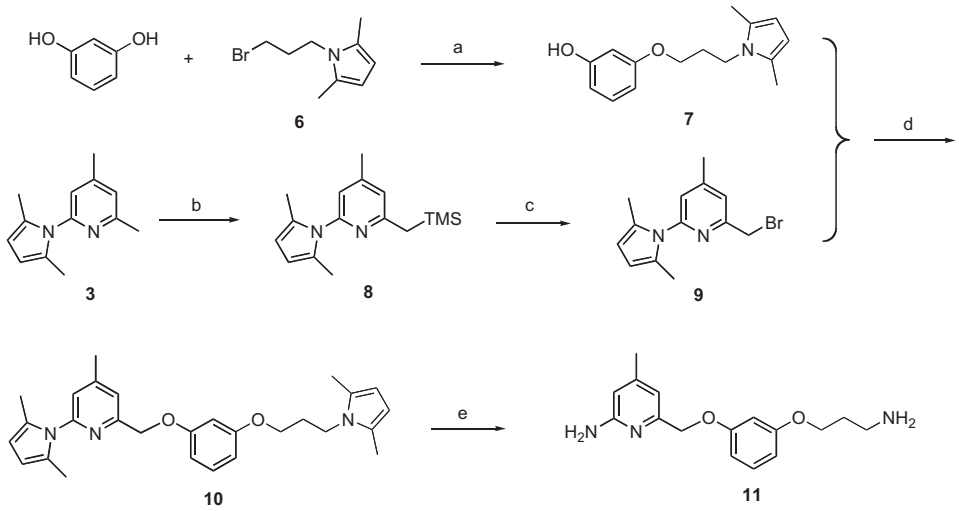


Scheme 1. Synthesis of **5a**–**c**. Reagents and conditions: (a) *p*-TsOH, toluene, reflux, 89%; (b) (i) *n*-BuLi, THF, −78 to 0 °C; (ii) dibromide, 51–55%; (c) NH_2OH–HCl, EtOH/H_2O (2/1), 100 °C, 20 h, 79–82%.



Scheme 2. Synthesis of **11**. Reagents and conditions: (a) K_2CO_3, acetone, reflux, 71%; (b) (i) *n*-BuLi, THF, −78 to 0 °C; (ii) TMSCl, 0 °C, 95%; (c) BrF_2CCF_2Br, CsF, DMF, 91%; (d) K_2CO_3, acetone, reflux, 79%; (e) NH_2OH–HCl, EtOH/H_2O (2/1), 20 h, 100 °C.

Compound **6** was prepared according to the general method for amine protection (see details in Supplementary Data). Addition of a three-fold excess of 1,3-dihydroxybenzene over **6** provided mono-substituted **7**. Compound **9** was prepared according to a literature report[13] as shown in Scheme 2. A standard ether synthesis linked fragments **7** and **9** together to produce **10** followed by deprotection of the amine to afford inhibitors **11**.

The synthesis of **14** and **15** followed a similar method as that for **11** but using NaH to deprotect 1,3-dihydroxybenzene without a large excess over 4-(bromomethyl)pyridine (Scheme 3). A second ether synthesis connected the 4-methyl aminopyridine head to generate **13** followed by deprotection of the amino group to yield **14**. Compound **15** followed the same pathway starting from 2,2′-biphenol.

In the synthesis of **18** and **19** (Scheme 4), a Mitsunobu reaction was used to add the *N*-Boc-piperidinemethyl fragment to dihydroxybenzene, and mono-substituted **16** was isolated in a 53% yield. An ether synthesis connected the 4-methylaminopyridine to generate **17**, followed by deprotection of the amino and Boc groups to yield **18**. Compound **19** followed the same pathway but started with 2,2′-biphenol.

The synthesis of inhibitor **24** started from 3-hydroxyaniline; the first step was protection of the amino group with Boc (Scheme 5),. A standard ether synthesis linked the 4-methylpyridine head successfully in the presence of K_2CO_3 in acetone. The Boc group was removed in 1.5 M HCl methanol after reaction for four hours. The 3-fluorophenethyl aldehyde was prepared from 3-fluorophenethanol via Dess–Martin oxidation. Reductive amination connected **22** and 3-fluorophenethyl aldehyde to provide **23**. After deprotection of the amino group, **24** was isolated in a 92% yield.

The synthesis of inhibitor **27** began with 3-hydroxybenzaldehyde, and the 4-methylpyridine head was added in the presence of **9** and K_2CO_3 in acetone (Scheme 6). Reductive amination connected **25** and 3-fluorophenethyl amine together to provide **26**. Compound **27** was obtained after deprotection of the amino group with NH_2OH–HCl.

2,6-Di(hydroxymethyl)pyridine was used as the starting material for the synthesis of **32** (Scheme 7). It was oxidized to mono aldehyde **28** with Dess–Martin periodinane (DMP) followed by reductive amination with 3-fluorophenethyl amine. The resulting secondary amine (**29**) was Boc-protected and then treated with **9** in the presence of NaH in DMF to construct **31**. Inhibitor **32** was obtained after deprotection of dimethylpyrrole and Boc, respectively, in a yield of 71% for the two steps.

The synthesis of **36** is illustrated in Scheme 8. First, 5-hydroxy-3-pyridinecarboxylic acid methyl ester was linked to the 4-methyl-2-aminopyridine head, and then the methyl ester was reduced to aldehyde **34** with Dibal-H at −78 °C. Reductive amination successfully connected the second head, 3-fluorophenethyl amine. Compound **36** was afforded after removing the protecting group.

3. Results and discussion

All of the inhibitors were assayed against three different isoforms of NOS, rat nNOS, bovine eNOS, and murine macrophage