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A New Class of Selective and Potent Inhibitors of Neuronal Nitric Oxide Synthase

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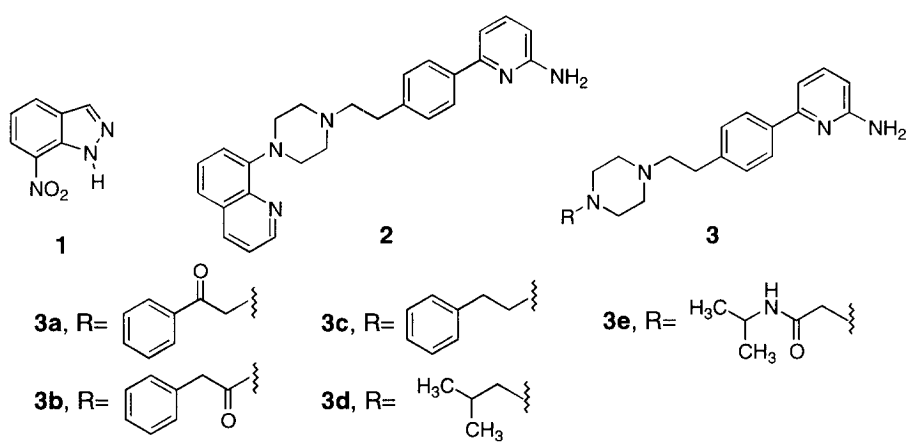
Abstract: The synthesis and SAR of a series of 6-(4-(substituted)phenyl)-2-aminopyridines as inhibitors of nitric oxide synthase are described. Compound **3a** from this series shows potent and selective inhibition of the human nNOS isoform, with pharmacokinetics sufficient to provide in vivo inhibition of nNOS activity.

The enzyme nitric oxide synthase occurs in three isoforms, an inducible form (iNOS) and two constitutive forms, neuronal (nNOS) and endothelial (eNOS).[1] Experiments using nNOS knockout mice and the 'selective' nNOS inhibitor 7-nitroindazole, **1**, indicate a role for nNOS in stroke,[2] Parkinson's disease,[3] and pain.[4] Pharmacological studies using compound **1**, however, are hampered by its poor in vitro selectivity for nNOS over eNOS (see below) and poor aqueous solubility. We therefore undertook a program to find improved nNOS inhibitors, beginning with a random file screening program. Herein, we report the results of an analog synthesis program around lead compound **2** from our screening effort, and its transformation to the potent and selective nNOS inhibitor **3a**. Recently, a series of N-phenylamidines with potent nNOS inhibitory activity were reported.[5]



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The major shortcoming of **2** is its binding to dopamine D_2 (human K_i = 11 nM) and serotonin (human 5-HT_{1A} K_i = 3 nM and human 5-HT_{2A} K_i = 14 nM) receptors, which would compromise its use in pharmacological studies aimed at studying the effects of nNOS inhibition in vivo. The aryl piperazine portion of **2** was assumed to be responsible for this D_2 and 5-HT receptor affinity , and so analogs without this pharmacophore were pursued. Their synthesis is outlined in Scheme 1.

SCHEME 1

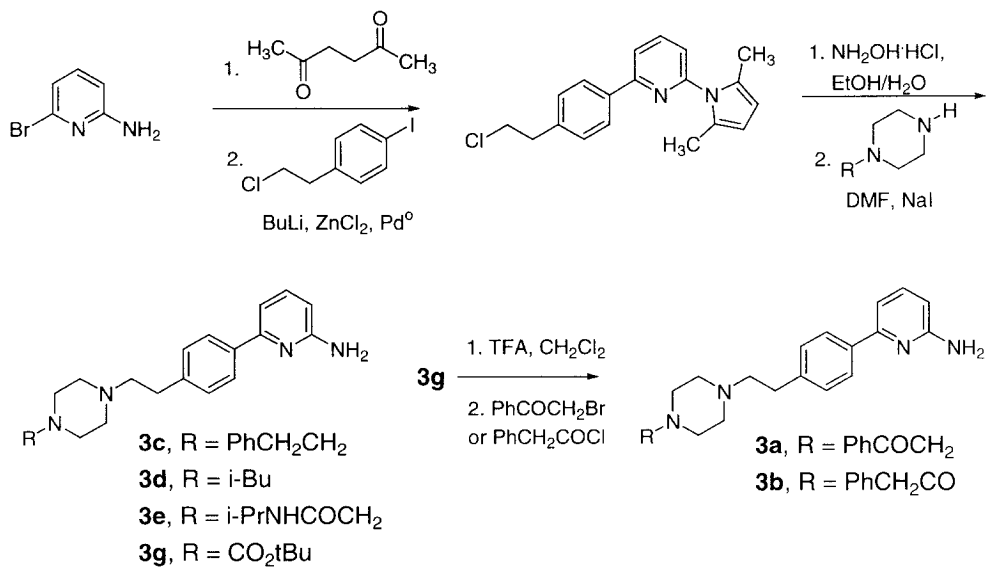

TABLE 1

CMPD	R	rat nNOS, IC_{50}	human nNOS, IC_{50}	human eNOS, IC_{50}*
1		2,120 ± 889 nM	2,870 ± 1,040 nM	1,890 ± 729 nM
2		130 ± 15 nM	212 ± 38 nM	825 ± 516 nM
3a	$PhCOCH_2$	127 ± 8.8 nM	140 ± 20 nM	887 ± 392 nM
3b	$PhCH_2CO$	108 ± 40 nM	137 ± 23 nM	685 ± 67 nM
3c	$PhCH_2CH_2$	247 ± 43 nM	264 ± 52 nM	448 ± 27 nM
3d	i-Bu	193 ± 24 nM	350 ± 76 nM	372 ± 32 nM
3e	i-PrNHCOCH$_2$	256 + 35 nM	330 + 35 nM	815 + 76 nM

*IC_{50} values for nitric oxide synthase inhibition, ± S.E.M.[6]

The key step in the synthesis of compounds in series **3** is the coupling of the pyrrolyl-protected derivative of 2-amino-6-bromopyridine via metalation with butyl lithium, followed by exchange for zinc, and finally palladium-mediated coupling with 4-(2-chloroethyl)iodobenzene. After removal of the pyrrolyl protecting group, the piperazine moeity is appended by alkylation and the terminal substituent added after removal of the t-BOC protecting group where necessary.

Reviewing the structure-activity relationships (SAR) from the table above, it appears than an sp^2 center proximal to the terminal piperazine nitrogen in **2** and **3** is important for selectively inhibiting nNOS over eNOS. This function is fulfilled by the quinoline group of **2** and the carbonyl groups in compounds **3a**, **3b**, and **3e**. Where this substituent is alkyl (**3c**) or aralkyl (**3d**), selectivity is lost. One hypothesis to explain the greater nNOS selectivity of sp^2-substituted side chain analogs relies on a recent report that the nNOS enzyme substrate-binding site is more open compared to eNOS, and therefore perhaps better able to accommodate more sterically demanding ligands.[7] Once X-ray crystallographic data for the nNOS and eNOS enzymes become available, the molecular basis for these SAR may become clearer.[8] Finally, **3a** was chosen for further profiling since it is one of the most potent human nNOS inhibitors and is slightly more selective than **3b**.

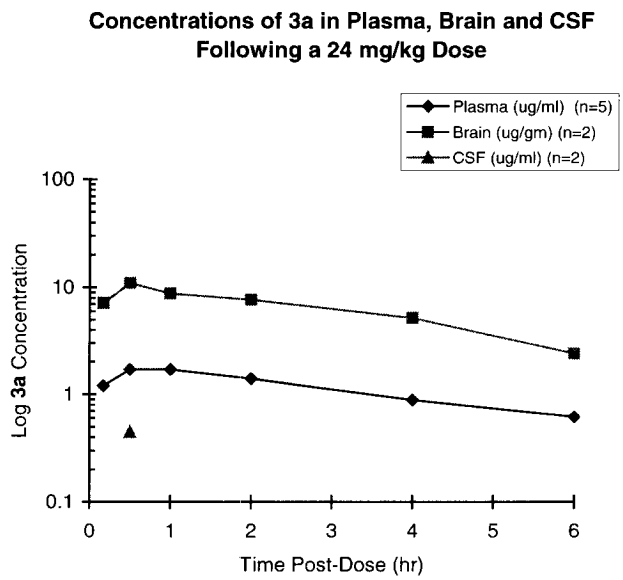

Figure 1

Gratifyingly, compound **3a** showed insignificant binding to the dopamine D_2 receptor (IC_{50} > 10,000 nM). In addition, further evaluation against an extensive panel of receptors showed significant affinity for only m2 (K_i = 0.32 uM) and m4 (K_i = 0.59 uM) muscarinic receptors. Pharmacokinetic studies showed that **3a** affords a Cmax of 1.7 ug/mL in plasma, 0.45 ug/mL in CSF, and 11 ug/gm in whole brain 30 min following a 24 mg/kg subcutaneous dose in rats (Figure 1), with an elimination half-life of 3.6 h. These levels should

provide greater than 50% inhibition of nNOS in the brain for at least 6 h. In addition, **3a** shows no effect on blood pressure or heart rate in rats when administered subcutaneously, even at doses up to 100 mg/kg, indicating weak in vivo effects against eNOS. Compound **3a**, then, represents a potent, selective inhibitor of the human nNOS enzyme with a pharmacokinetic profile providing drug levels above the nNOS IC_{50} value for at least 6 hours. Evaluation of **3a** in animal models involving nNOS is in progress, and will be reported in due course.

References and Notes

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