1,6-Disubstituted indole derivatives as selective human neuronal nitric oxide synthase inhibitors

Shawn Maddaford[a], **Paul Renton**[a], **Joanne Speed**[a], **Subhash C. Annedi**[a,*], **Jailall Ramnauth**[a], **Suman Rakhit**[a], **John Andrews**[a], **Gabriela Mladenova**[a], **Lisa Majuta**[b], and **Frank Porreca**[b]

[a]NeurAxon Inc., 2395 Speakman Drive, Suite #1001, Mississauga, ON, Canada L5K 1B3

[b]Department of Pharmacology, University of Arizona, 1501 N. Campbell Ave., Tucson, AZ, USA

Abstract

A series of 1,6-disubstituted indole derivatives was designed, synthesized and evaluated as inhibitors of human nitric oxide synthase (NOS). By varying the basic amine side chain at the 1-position of the indole ring, several potent and selective inhibitors of human neuronal NOS were identified. In general compounds with bulkier side chains displayed increased selectivity for nNOS over eNOS and iNOS isoforms. One of the compounds, (*R*)-**8** was shown to reduce tactile hyperesthesia (allodynia) after oral administration (30 mg/kg) in an in vivo rat model of dural inflammation relevant to migraine pain.

Keywords

1,6-Disubstitued indole derivatives; Nitric oxide; Nitric oxide synthase; Nitric oxide synthase inhibitors; Selective neuronal nitric oxide synthase inhibitors

Nitric oxide (NO) is an inorganic free radical that has diverse roles both in normal and pathological processes, including the regulation of blood pressure, neurotransmission and macrophage defense systems.[1] NO is synthesized from the enzyme catalysis of L-arginine to L-citrulline by three isoforms of nitric oxide synthase (NOS): two constitutive forms in neuronal cells (nNOS) and endothelial cells (eNOS), and an inducible form in macrophage cells (iNOS). Overstimulation or overproduction of NO by nNOS and iNOS has been shown to play a key role in several disorders, including septic shock, arthritis, diabetes, ischemia-reperfusion injury, pain and various neurodegenerative diseases.[2] However, any inhibitors to treat these conditions must avoid eNOS inhibition as this will lead to unwanted effects such as enhanced white cell and platelet activation, hypertension and atherogenesis.[3] Therefore, the development of selective NOS inhibitors is of considerable interest, both from a therapeutic perspective and also as specific pharmacological tools.[4]

Although there is low homology among the three NOS primary sequences (~50%), the active sites of the enzymes appears to be relatively conserved with 16 out of 18 residues within 6 Å being identical, presumably explains the difficulty obtaining selective NOS

*Corresponding author. Tel.: +1 416 673 8457; fax: +1 905 823 5836. sannedi@neuraxon.com (S.C. Annedi).

inhibitors.[5] Investigation into the synthesis and chemistry of novel isoform-selective NOS inhibitors has been an ongoing challenge, even though the general pharmacophore requirements are well established.[6–12] Synthesis of substrate (L-arginine) based peptidomimetic non-selective as well as selective nNOS inhibitors have been extensively reported in the literature.[13] In an effort to improve PK/PD properties by decreasing their peptidic nature, various small molecule selective nNOS inhibitors have also been reported.[14]

The pharmacophore model we adopted for the arginine binding site of the NOS enzyme includes a guanidine isosteric group (amidine group) and a basic amine group, both attached to a central aryl scaffold (indole core) as shown in Figure 1.[4,15] The amidine group makes an important bidentate interaction with the conserved glutamic acid residue to achieve the necessary potency; whereas the basic amine is assumed to provide the nNOS isoform selectivity.[15] Our design strategy is based on an indole core as an aryl scaffold and exploring various basic amine side chains for achieving the NOS isoform selectivity. As part of our ongoing efforts to find small molecule selective nNOS inhibitors for treating CNS disorders, herein we report the synthesis and biological activity evaluations of a series of 1,6-disubstituted indole derivatives and in vivo activity of (*R*)-**8** in a rat model relevant to migraine pain.[15]

Two general approaches were undertaken for the preparation of 1,6-disubstituted indole derivatives as shown in Schemes 1–5. 6-Nitro-1*H*-indole (**1**) was alkylated with various 2-chloro-ethanamine derivatives in the presence of potassium carbonate to obtain the alkylated nitro-intermediates **2–4** (Scheme 1). The nitro group in compounds **2–4** was reduced to the corresponding amine in the presence of palladium on carbon under an atmosphere of hydrogen. These anilines were coupled to the thiophene-2-carbimidothioate **5**, resulting in the final compounds **6**, **7** and (±)-**8**, respectively.[16]

During the synthesis of compound (±)-**8**, rearrangement through a ring opening (quarternization) reaction was observed (Scheme 2) under basic conditions.[17] Two nitroindole derivatives, (±)-**4** and the rearranged product **9** were easily separated by silica gel column chromatography. Following the same synthetic protocol and coupling to the thiophene-2-carbimidothioate **5** or the furan-2-carbimidothioate **10** as outlined in Scheme 2 provided the target compounds **11** and **12**, respectively.[18] Compound (±)-**4** was separated into its enantiomers (*R*)-**4** and (*S*)-**4** by resolution with dibenzoyl-L-tartaric acid in ethanol (Scheme 2). The separated enantiomers were converted into the final compounds (*R*)-**8** and (*S*)-**8** as described above. The stereochemistry of (*S*)-**8** was determined by an independent chiral synthesis as shown in Scheme 3. (*S*)-2-(2-Chloroethyl)-1-methylpyrrolidine was reacted with 6-nitro-1*H*-indole (**1**), followed by reduction and coupling with the thiophene-2-carbimidothioate **5** under standard conditions described in Scheme 1, provided the pure enantiomer (*S*)-**8**.[19]

Compounds **22–29** were prepared as shown in Scheme 4. The 6-nitro-1*H*-indole (**1**) was alkylated in the presence of sodium hydride in DMF with appropriate 3 or 4-carbon chloroalkyl iodides to obtain intermediates **13** and **14**, respectively. The chloride in **13** and **14** was then substituted with various amines in the presence of potassium carbonate and potassium iodide in refluxing acetonitrile to obtain the substituted amine intermediates **15–**

19, **20** and **21**, respectively. Reduction of the nitro group and coupling with the thiophene-2-carbimidothioate **5** or the furan-2-carbimidothioate **10** was carried out as described above to obtain the target compounds **22–29**. Target compounds **32** and **33** were prepared under similar conditions described above reacting with the thiophene-3-carbimidothioate **30** and furan-3-carbimidothioate **31**, respectively, as shown in Scheme 5.[20]

All compounds were converted into their dihydrochloride salts to improve their water solubility,[21] and measured their inhibitory activities against all three human isoforms of NOS (Tables 1 and 2).[22] In general, compounds with bulkier side chains displayed moderate nNOS inhibitory activities and good selectivities against eNOS and iNOS. Most of the tested compounds displayed poor inhibitory activities for the iNOS isoform. The most selective nNOS inhibitor from the series was compound (*S*)-**8** with 120- and 360- fold selectivity for nNOS over eNOS and iNOS, respectively (Table 1). The most potent nNOS inhibitor was compound **22**, which also displayed very good selectivity (68-fold) over eNOS. In general compounds with a cyclic side chain ((±)-**8**, (*R*)-**8**, (*S*)-**8**, **11**, **23**, **24** and **29**) showed increased selectivity for nNOS over eNOS and iNOS isoforms compared to the compounds with an acyclic side chain (**6**, **25**, **26** and **28**). The diethyl amine group in compound **22** seems to fit the requirement of a bulky substituent. This is apparent when compared to dimethyl and monomethyl substituted amines (**25** and **26**) where reduction in nNOS selectivity was observed with the smaller substituents. Compound **29** has an imidazole group, which fits the requirement of a bulky substituent, however displayed poor activity for nNOS (IC$_{50}$ = 1.22 µM). This may be due to the reduced basicity of the imidazole nitrogen and is consistent with our previous observation with 2-aminobenzothiazole compounds. [15] Compound **28** with a 4-carbon linker showed better activity for nNOS compared to the corresponding compounds with 2 and 3-carbon linkers (**6** and **25**). Furanyl amidines (**12** and **27**) did not show any significant difference in potency or selectivity when compared to their corresponding thiophene amidine compounds **11** and **24**. At the same time, replacement of 2-thiophene and 2-furanyl amidine groups (**24** and **27**) with regioisomeric 3- thiophene and 3-furanyl amidines (**32** and **33**) did not show any significant differences in activity or selectivity among NOS isoforms (Table 2).

To investigate the potential therapeutic use of these selective nNOS inhibitors, compound (*R*)-**8** was evaluated in a rat model of migraine pain.[23] This model involves the application of an inflammatory soup (IS) onto the dura via a cannula with subsequent measurements in the development of cutaneous allodynia in the hind-paws, which peaks between 3 and 4 h. Oral administration of (*R*)-**8** (30 mg/kg), 15 min prior to the IS attenuated the development of allodynia between 2 and 6 h post-dose with maximum effect showing at 4 h (Fig. 2).

In summary, we have designed and synthesized a series of 1,6- disubstituted indole derivatives that are selective inhibitors of nNOS over both eNOS and iNOS isoforms. We have shown that by introducing a bulky amino-substituent at the 1-position of the indole core, the selectivity for nNOS over eNOS can increase over 100-fold. Replacement of 2-thiophene amidine group by 2-furanyl amidine or regioisomeric 3-thiophene and 3-furanyl amidine groups did not show any significant difference in potency or selectivity among NOS isoforms. Overall, compound (±)-**8** and its enantiomers (*R*)-**8** and (*S*)-**8** showed superior potency for nNOS and increased selectivity over eNOS and iNOS isoforms. The in vivo

results of compound (*R*)-**8** in a rodent migraine pain model, suggests that these compounds may be useful for further evaluation in potential therapeutic applications of disease states in which nNOS inhibitors are thought to play a key role.

Acknowledgments

References and notes

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21. The free base in anhydrous methanol was treated with HCl (1 M solution in diethyl ether) and stirred at room temperature for 10 min. The solvent was evaporated and dried under reduced pressure to obtain the dihydrochloride salt as a solid.

22. Recombinant human iNOS, eNOS and nNOS were produced in Baculovirus-infected Sf9 cells (ALEXIS). In a radiometric method, inhibitory activities were measured by the conversion of [^{3}H]-L-arginine into [^{3}H]-L-citrulline. The enzymatic reaction was carried out in the presence or absence of varying concentrations of the inhibitor in water. The negatively charged [^{3}H]-L-citrulline was separated from the positively charged [^{3}H]-L-arginine using resin beads. Inhibition of enzyme activity by the inhibitor is measured by dividing the enzymatic conversion in the presence of inhibitor divided by the enzymatic conversion in the absence of inhibitor. IC$_{50}$ value is the concentration of compound that gives rise to 50% inhibition. All assays were performed in duplicate. All assays were performed in duplicate.

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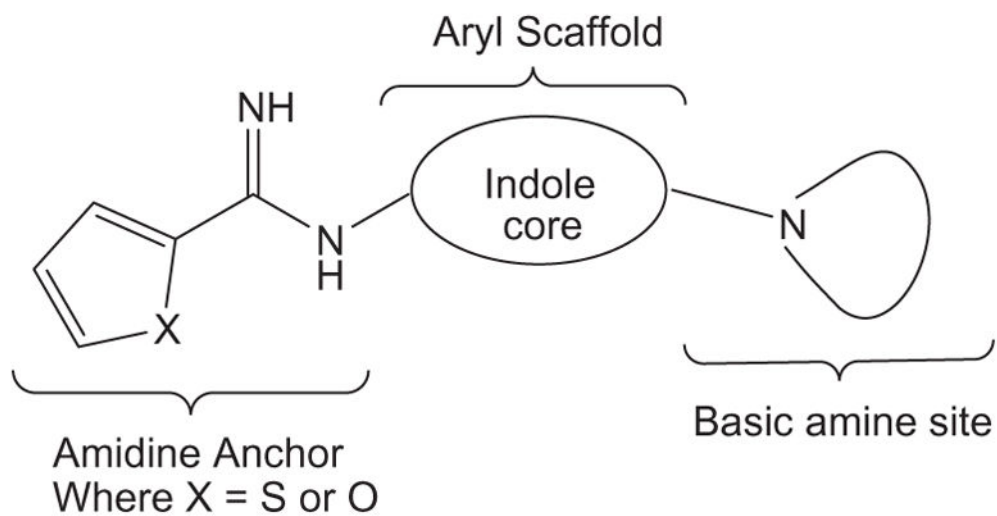
Figure 1.
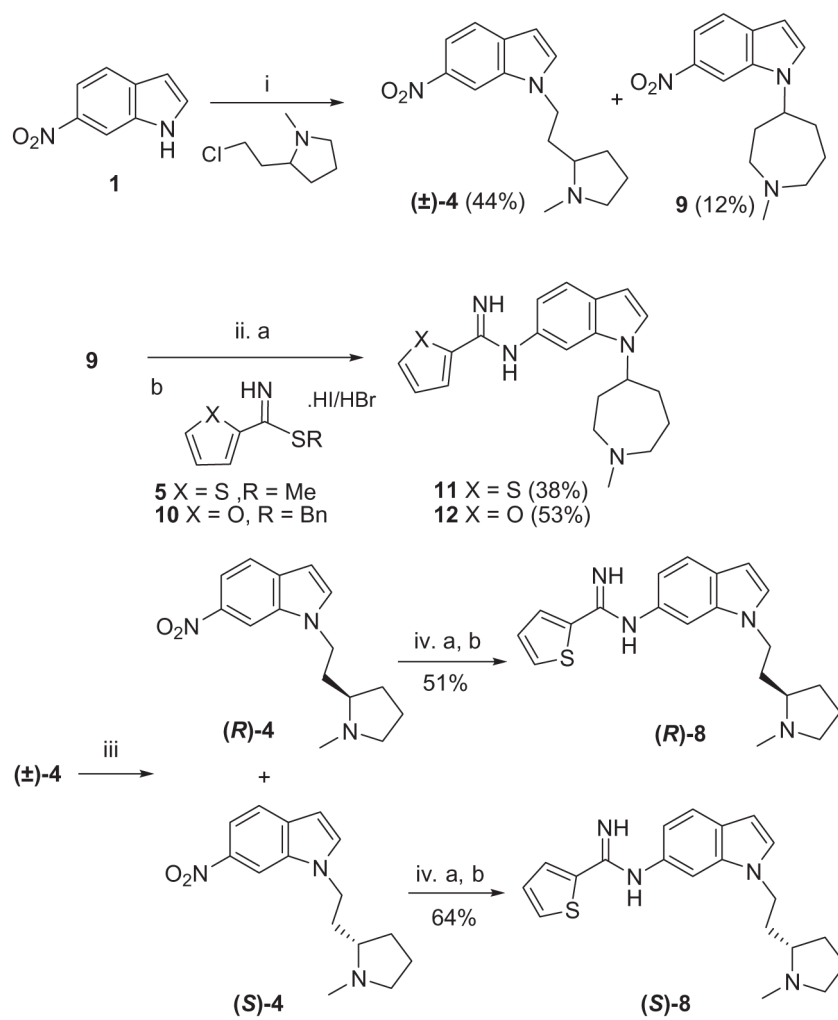Pharmacophore model for selective nNOS inhibitor design.
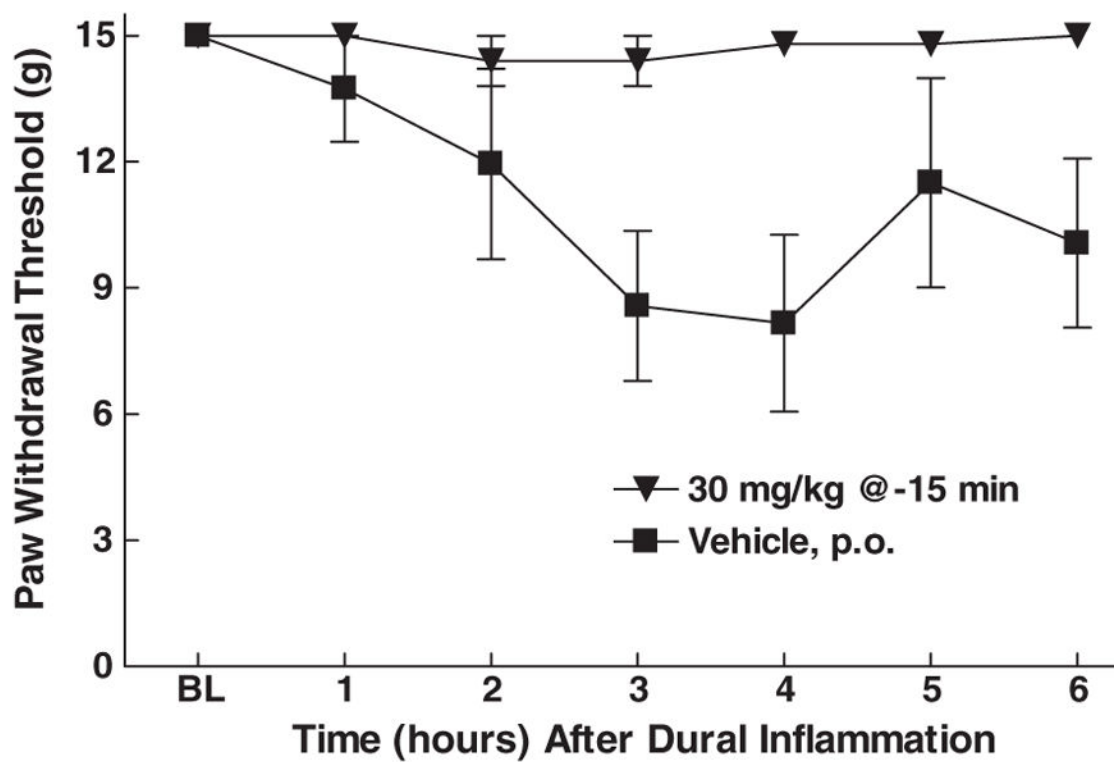
Figure 2.
Oral administration of compound (*R*)-**8** (30 mg/kg) attenuates the tactile hyperesthesia (allodynia) of the hindpaw in rats with inflammation of the dura in migraine pain model.
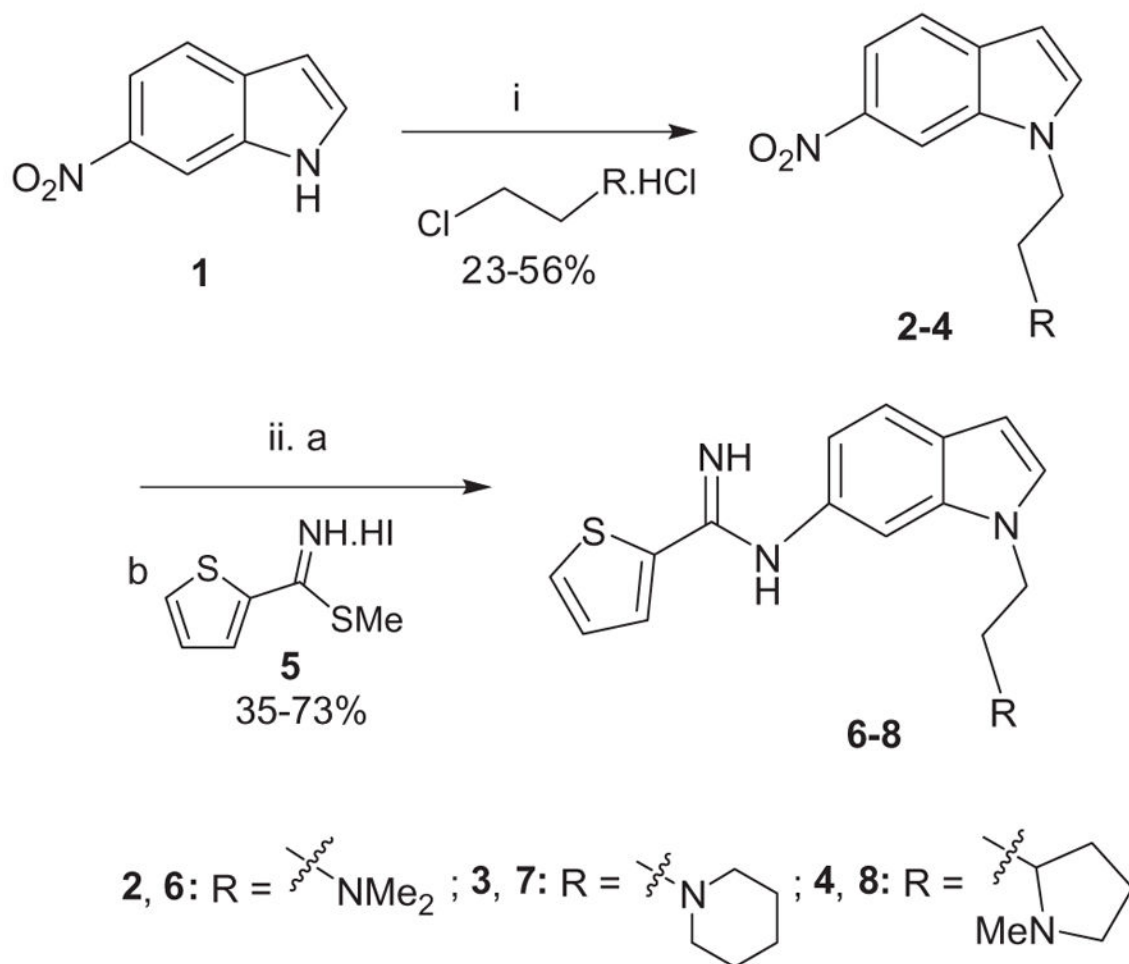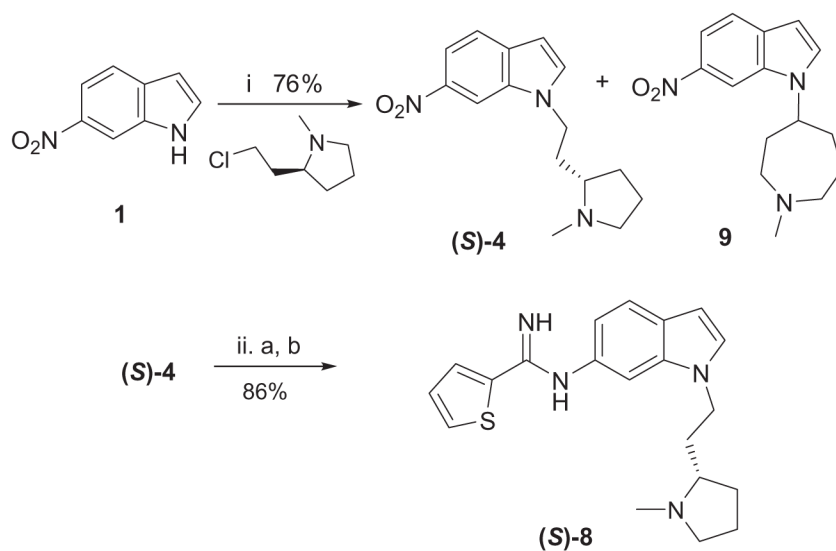
Scheme 1.

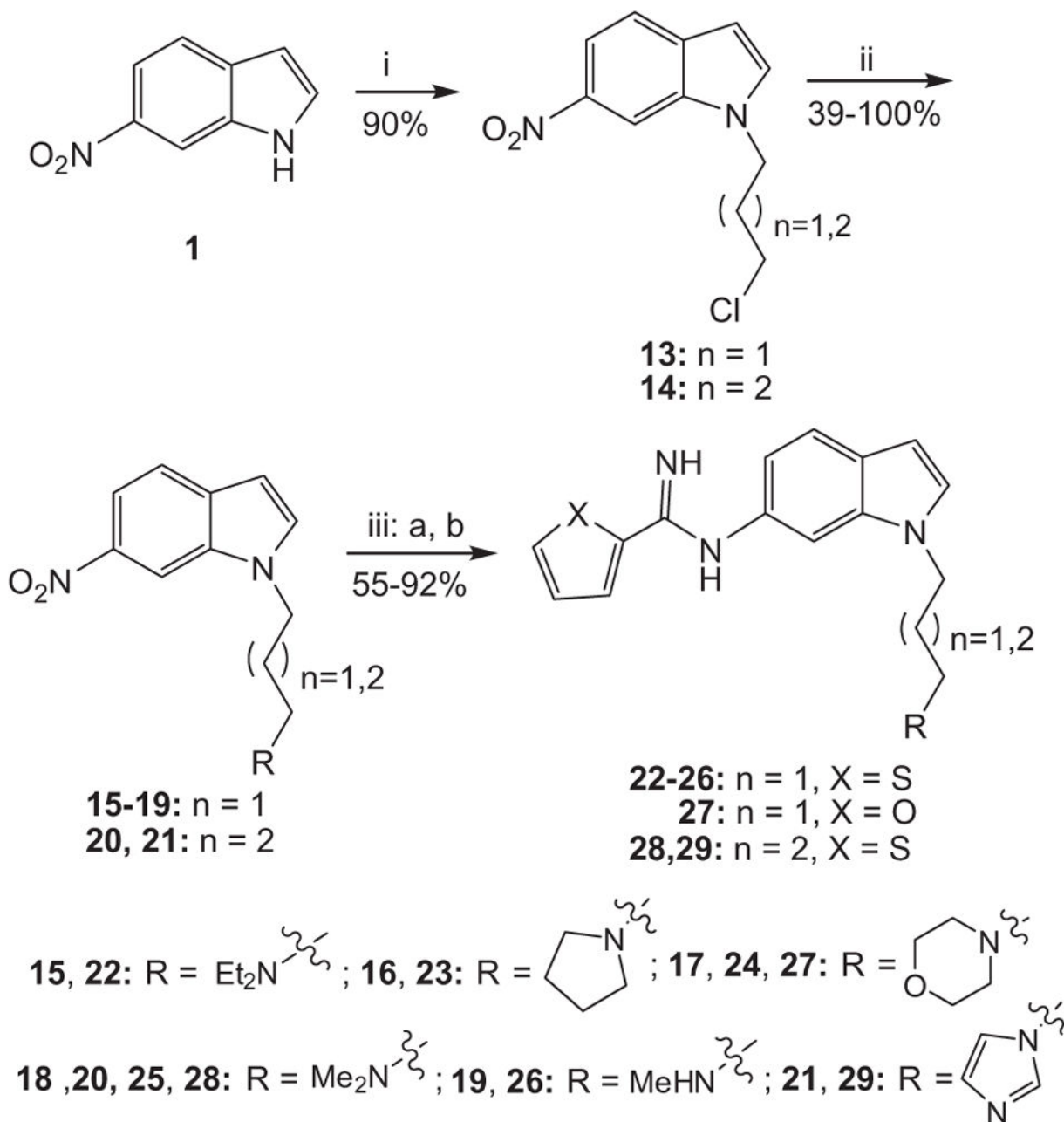Reagents and conditions: (i) K_2CO_3, DMF, 80 °C; (ii) (a) Pd–C/H_2, EtOH, rt, (b) **5**, EtOH, rt.

Scheme 2.
Reagents and conditions: (i) K_2CO_3, DMF, 80 °C; (ii) (a) Pd–C/H_2, EtOH, rt, (b) **5** or **10**, EtOH, rt; (iii) dibenzoyl-L-tartaric acid, EtOH; (iv) (a) Pd–C/H_2, EtOH, rt, (b) **5**, EtOH, rt.

Scheme 3.
Reagents and conditions: (i) K_2CO_3, DMF, 80 °C; (ii) (a) Pd–C/H_2, EtOH, rt, (b) **5**, EtOH, rt.

Scheme 4.
Reagents and conditions: (i) NaH, DMF, $Cl(CH_2)_nI$; (ii) K_2CO_3, CH_3CN, KI, R·HCl, reflux; (iii) (a) Pd–C/H_2, EtOH, rt, (b) **5** or **10**, EtOH, rt.
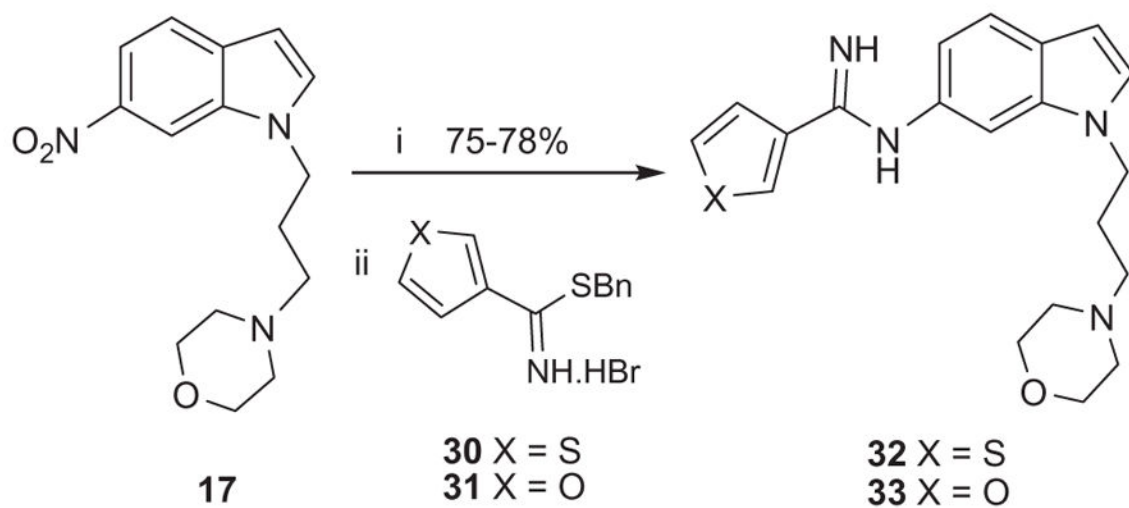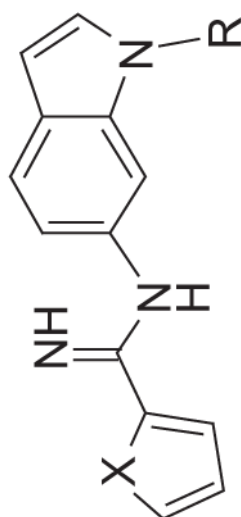
Scheme 5.
Reagents and conditions: (i) Pd–C/H$_2$, EtOH, rt; (ii) **30** or **31**, EtOH, rt.

Table 1

Inhibition of human NOS enzymes by 1,6-disubstituted indole derivatives

Compound	X	R	nNOS IC$_{50}$[a] (μM)	eNOS IC$_{50}$[a] (μM)	iNOS IC$_{50}$[a] (μM)	eNOS/nNOS	iNOS/nNOS
6	S	NMe$_2$	1.2 (0.99–1.3)	15 (8.4–28)	60 (37–98)	13	50
7	S	(piperidine)	0.49 (0.39–0.6)	3.8 (1.1–12.2)	21 (6.1–72)	8	43
(±)-**8**	S	MeN	0.22 (0.08–0.5)	19 (11–33.9)	68 (17–268)	86	309
(**R**)-**8**	S	(R) MeN	0.32 (0.12–0.7)	16 (6.6–38.2)	72 (20–255)	50	225
(**S**)-**8**	S	(S) MeN	0.2 (0.12–0.3)	24 (15–37.1)	72 (19.7–267)	120	360
11	S	NMe	0.87 (0.46–1.65)	37.7 (24.9–57.1)	NT[b]	43	NC[c]
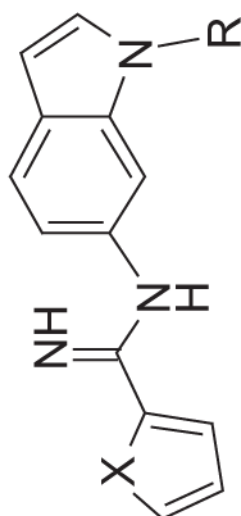
Compound	X	R	nNOS IC$_{50}$[a] (µM)	eNOS IC$_{50}$[a] (µM)	iNOS IC$_{50}$[a] (µM)	eNOS/nNOS	iNOS/nNOS
12	O	(azepane-NMe)	0.52 (0.36–0.77)	30.6 (24.0–39.1)	1.2 (0.83–1.9)	59	2
22	S	(NEt$_2$)	0.15 (0.08–0.24)	10.2 (5.6–18.3)	NT[b]	68	NC[c]
23	S	(pyrrolidine)	0.45 (0.29–0.69)	11.2 (4.4–27.9)	NT[b]	25	NC[c]
24	S	(morpholine)	0.7 (0.5–0.9)	28.3 (12.4–49.5)	82 (49.9–82.1)	40	117
25	S	(NMe$_2$)	2.73 (1.9–3.8)	23.3 (16.8–32.3)	NT[b]	8	NC[c]
26	S	(NHMe)	1.78 (1.5–2.1)	11.8 (9.2–15.2)	NT[b]	6	NC[c]
27	O	(morpholine)	2.4 (2.1–2.5)	34 (24.1–49.6)	36 (32–37)	14	15
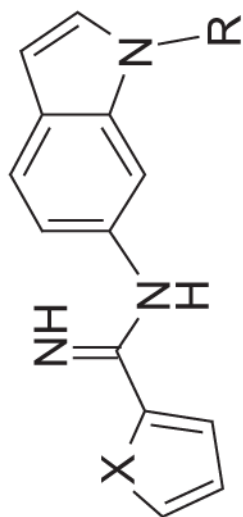
Compound	X	R	nNOS IC$_{50}$ [a] (µM)	eNOS IC$_{50}$ [a] (µM)	iNOS IC$_{50}$ [a] (µM)	eNOS/nNOS	iNOS/nNOS
28	S	(NMe$_2$ group)	0.26 (0.2–0.3)	2.53 (1.4–4.3)	66 (41–105)	10	254
29	S	(imidazole group)	1.22 (0.6–2.2)	23 (13–39)	>100[d]	19	NC[c]

Values reported in parentheses are 95% confidence intervals.

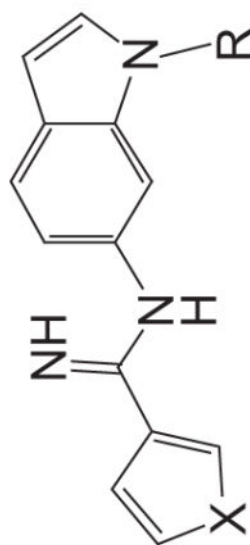[a] Inhibitory activities were measured by the conversion of [^{3}H]-L-arginine into [^{3}H]-L-citrulline.

[b] Not tested.

[c] Not calculable.

[d] Not active at the maximum test concentration of 100 µM.

Table 2

Inhibition of human NOS enzymes by 1,6-disubstituted indole derivatives

Compound	X	R	nNOS IC$_{50}$ [a] (μM)	eNOS IC$_{50}$ [a] (μM)	iNOS IC$_{50}$ [a] (μM)	eNOS/nNOS	iNOS/nNOS
32	S		1.4 (1.1–1.6)	17.1 (11.4–24.7)	70 (31.8–155)	12.1	50
33	O		1.0 (0.68–1.4)	19.1 (15.9–24.7)	>100[b]	19	NC[c]

Values reported in parentheses are 95% confidence intervals.

[a] Inhibitory activities were measured by the conversion of [^{3}H]-L-arginine into [^{3}H]-L-citrulline.

[b] Not active at the maximum test concentration of 100 μM.

[c] Not calculable.