Pharm. Pharmacol. Commun. 1999, 5: 491–494
Received May 12, 1999
Accepted June 10, 1999

1-[(Aryloxy)alkyl]-1*H*-imidazoles as Inhibitors of Neuronal Nitric Oxide Synthase

L. SALERNO, V. SORRENTI*, F. GUERRERA, M. C. SARVÁ, M. A. SIRACUSA, C. DI GIACOMO* AND A. VANELLA*

*Department of Pharmaceutical Sciences and *Institute of Biological Chemistry, Viale Andrea Doria 6, 95125, University of Catania, Italy*

Abstract

A series of 1-[(aryloxy)alkyl]-1 *H*-imidazoles were synthesized from imidazole and various (aryloxy)alkyl bromides and tested for inhibitory activity against the three isoforms of nitric oxide synthase.

1-[2-(4-Bromophenoxy)ethyl]-1*H*-imidazole and 1-[2-[4-(trifluoromethyl)phenoxy] ethyl]-1*H*-imidazole showed inhibitory activity against the neuronal isoform but were less potent against the endothelial isoform. Thus they could be considered interesting for their selectivity. The remaining compounds had only modest activity.

Nitric oxide (NO) is involved in several biological functions (Kerwin et al 1995). The production of NO results from the oxidation of L-arginine to L-citrulline in a NADPH- and O_2-dependent pathway catalysed by the enzyme nitric oxide synthase (NOS). Three structurally distinct NOS isoforms have been identified (Marletta 1993): the constitutive endothelial NOS (eNOS) and neuronal NOS (nNOS), and the inducible NOS (iNOS). NO produced from eNOS is responsible for various biological effects such as the relaxation of vascular smooth muscle and the inhibition of platelet adhesion and aggregation. NO produced from nNOS is implicated in pain perception, mediation of long-term potentiation and memory, and control of cerebral blood flow, and NO synthesized by iNOS is implicated in the defensive response to infections.

Overproduction of NO by iNOS has been implicated in various pathological processes including septic shock, tissue damage after inflammation and rheumatoid arthritis. Overproduction of NO from nNOS has been associated with neurodegeneration involved in stroke, seizures, schizophrenia and Alzheimer's, Parkinson's and AIDS dementia. NO produced from eNOS usually has only physiological roles (Moncada et al 1991). Selective NOS inhibitors are therefore of interest as potential therapeutical agents (Marletta 1994).

Correspondence: L. Salerno, Dipartimento di Scienze Farmaceutiche, Viale Andrea Doria 6, Catania 95125, Italy.
E-Mail: siracusa@mbox.unict.it

To date, many compounds have been shown to inhibit NOS, including mono or disubstituted arginines, guanidines, isothioureas, amidines, indazoles and imidazoles (Kerwin et al 1995; Macdonald 1996; Southan & Szabò 1996). The majority of inhibitors described are non-selective or iNOS-selective, and only a few compounds, such as 7-nitro-indazole and 1-(2-trifluoromethyl-phenyl)-imidazole, selectively inhibit nNOS (Moore & Handy 1997).

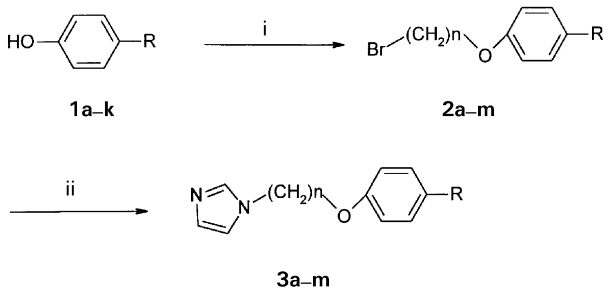
Imidazole and some of its derivatives are reported to be inhibitors of various isoforms of NOS (Wolff et al 1993; Chabin et al 1996) and various oxido-reductase enzymes (Rogerson et al 1976; Kato et al 1985). We recently described the synthesis of a series of *N*-phenacyl, *N*-phenethyl- and *N*-phenyl-hydroxyethyl-imidazoles and their inhibitory effects on NOS isoforms (Salerno et al 1999). Some of the compounds tested were considered selective inhibitors of nNOS because they showed a significant inhibitory effect against nNOS compared with eNOS. To explore other molecules containing the imidazole nucleus as pharmacophore, we describe here the synthesis of a series of 1-[(aryloxy)alkyl]-1*H*-imidazoles, **3a**–**m**, in which various phenoxy-ethyl, -propyl or -butyl chains were linked at the N-1 of the imidazole ring as potential selective inhibitors of nNOS. Some of these compounds have already been described in the literature as thromboxane synthetase inhibitors (**3a**, **3d**, **3i**; Cross et al 1985) or as anticonvulsants (**3c**; Robertson et al 1987).

In this study new and previously synthesized compounds were tested for inhibitory activity against neuronal rat recombinant NOS (nNOS), inducible mouse macrophage NOS (iNOS) and endothelial human platelet NOS (eNOS).

Materials and Methods

Chemistry

Melting points were determined on a Buchi 510 apparatus and are uncorrected. IR spectra were recorded on a Perkin-Elmer Model 281 spectrometer with KBr disk. ^{1}H NMR spectra were recorded on a Varian 200 MHz instrument in $CDCl_3$ solution. Chemical shifts are in ppm (δ) relative to tetramethylsilane as internal standard. Elemental analyses were performed on a C. Erba Model 1106 elemental analyser and data for C, H, N was within 0·4% of calculated values. Thin layer chromatography (TLC) was performed on Merck silica gel 60-F_{254} precoated aluminium plates. Flash chromatography using Merck Silica gel 60 (0·040–0·063 mm) was used for preparative chromatographic separations. Imidazole and phenols **1a–k** were purchased from Aldrich and (aryloxy)alkyl bromides **2a–m** were prepared according to the procedure described by Cross et al (1985). Analytical and spectral data were in agreement with data in the literature. The complete synthetic route for compounds **3a–m** is given in Figure 1.



a: R = H, n = 2
b: R = Br, n = 2
c: R = C_6H_5, n = 2
d: R = $COOC_2H_5$, n = 2
e: R = NO_2, n = 2
f: R = OC_3H_7, n = 2
g: R = COC_6H_5, n = 2
h: R = CH_3, n = 2
i: R = NH_2, n = 2
j: R = CF_3, n = 2
k: R = OCH_3, n = 2
l: R = H, n = 3
m: R = H, n = 4

Figure 1. Reagents: i. $Br(CH_2)nBr$, K_2CO_3, 2-butanone, reflux, 48 h; ii. imidazole, NaH, tetrahydrofuran, room temperature.

*General procedure for the synthesis of 1-[(aryloxy)-alkyl]-1*H*-imidazoles **3a–m***

The procedure described by Cross et al (1985) was adopted with slight modification. Sodium hydride 95% (7·3 mmol) was added to a solution of imidazole (7·3 mmol) in dry tetrahydrofuran and the mixture was stirred at room temperature for approximately 15 min. A solution of the appropriate (aryloxy)alkyl bromides **2a–m** (7·3 mmol) was then added and the mixture was stirred overnight. The solvent was evaporated and the residue was poured into water, alkalynized with 3 M sodium hydroxide, extracted twice with dichloromethane and dried. The organic solvent was removed under vacuum and the residue was purified by flash chromatography using silica gel and mixtures of ethyl acetate and methanol.

Proposed structures for all newly synthesized compounds were confirmed by elemental analyses and ^{1}H NMR. Melting points, analytical and spectral data for previously synthesized compounds were in agreement with data in literature.

*1-[2-(4-Bromophenoxy)ethyl]-1*H*-imidazole (**3b**).* Yield 65%; mp 78–79°C; ^{1}H NMR ($CDCl_3$): 4·14–4·21 (m, 2H, CH_2-O); 4·29–4·35 (m, 2H, N-CH_2); 6·70–6·78 (m, 2H, Ar); 7·02 (bs, 1H, H^5 Im); 7·07 (bs, 1H, H^4 Im); 7·33–7·41 (m, 2H, Ar); 7·59 (bs, 1H, H^2 Im). Anal. $C_{11}H_{11}BrN_2O$.

*1-[2-[4-(Propyloxy)phenoxy]ethyl]-1*H*-imidazole (**3f**).* Yield 40%; mp 45–46°C; ^{1}H NMR ($CDCl_3$): 1·01 (t, 3H, CH_3); 1·72–1·82 (m, 2H, CH_2-CH_2-CH_3); 3·85 (t, 2H, O-CH_2); 4·13–4·17 (m, 2H, CH_2-O); 4·18–4·32 (m, 2H, N-CH_2); 6·75–6·80 (m, 4H, Ar); 7·04–7·07 (m, 2H, H^5 and H^4 Im); 7·59 (s, 1H, H^2 Im). Anal. $C_{14}H_{18}N_2O_2$.

*1-[2-[4-(Benzoyl)phenoxy]ethyl]-1*H*-imidazole (**3g**).* Yield 45%; mp 117–118°C; ^{1}H NMR ($CDCl_3$): 4·27–4·32 (m, 2H, CH_2-O); 4·36–4·41 (m, 2H, N-CH_2); 6·91–7·84 (m, 12H, Ar, H^5, H^4 and H^2 Im). Anal. $C_{18}H_{16}N_2O_2$.

*1-[2-(4-Methylphenoxy)ethyl]-1*H*-imidazole (**3h**).* Yield 50%; mp 69–70°C; ^{1}H NMR ($CDCl_3$): 2·28 (s, 3H, CH_3); 4·15–4·21 (m, 2H, CH_2-O); 4·32–4·34 (m, 2H, N-CH_2); 6·73–6·78 (m, 2H, Ar); 7·03–7·10 (m, 4H, Ar, H^5 and H^4 Im); 7·60 (bs, 1H, H^2 Im). Anal. $C_{12}H_{14}N_2O$.

*1-[2-[4-(Trifluoromethyl)phenoxy]ethyl]-1*H*-imidazole (**3j**).* Yield 65%; oil; ^{1}H NMR ($CDCl_3$): 4·21–4·27 (m, 2H, CH_2-O); 4·33–4·39 (m, 2H, N-CH_2); 6·90–6·95 (m, 2H, Ar); 7·03–7·07 (m, 2H, H^5 and H^4 Im); 7·51–7·57 (m, 2H, Ar); 7·60 (bs, 1H, H^2 Im). Anal. $C_{12}H_{11}F_3N_2O$.

*1-[2-[4-(Methoxy)phenoxy]ethyl]-1*H*-imidazole* (***3k***). Yield 57%; mp 48–49°C; ^{1}H NMR ($CDCl_3$): 3·76 (s, 3H, CH_3); 4·13–4·19 (m, 2H, CH_2-O); 4·27–4·33 (m, 2H, N-CH_2); 6·80–6·81 (m, 4H, Ar); 7·03–7·07 (m, 2H, H^5 and H^4 Im); 7·59 (bs, 1H, H^2 Im). Anal. $C_{12}H_{14}N_2O_2$.

Biology

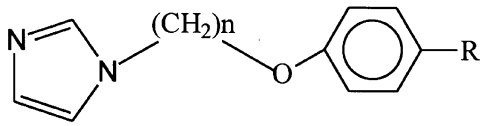
Enzymatic assay. iNOS isolated from immunostimulated mouse macrophage (RAW 264·7) cells and rat recombinant nNOS isolated from a Baculovirus over-expression system in SF9 cells, were purchased from Alexis. eNOS was prepared from washed platelets as described by Radomski et al (1990). The assay for NOS activity was carried out by measuring the rate of conversion of oxyhaemoglobin to methaemoglobin using an Hitachi UV 2000 spectrophotometer. A reference cuvette was charged with 5 μM oxyhaemoglobin (human A_0 ferrous purchased from Sigma) in 100 mM HEPES (pH 7·4) in a final volume of 500 μL. A typical sample contained 50 μM L-arginine, 1 mM Mg^{2+} (for iNOS only), 170 μM dithiothreitol, 100 μM NADPH, 12 μM BH_4, 1 mM Ca^{2+} (for nNOS and eNOS only), 20 units mL^{-1} calmodulin (for nNOS and eNOS only), 10 μL DMSO (or the same volume of DMSO solution of test compounds to a final concentration of 500, 100 and 50 μM), enzymatic extract (1·2 units mL^{-1} for nNOS and iNOS; cytosol derived from $1{\cdot}1 \times 10^{11}$ platelets for eNOS) and 5 μM oxyhaemoglobin in 100 μM HEPES pH 7·4 in a final volume of 500 μL. The HEPES buffer was pre-heated before use. NO formed reacts with oxyhaemoglobin yielding methaemoglobin which was measured at 401 nm.

Results were the mean (s.d. $\leq$10%) of at least three assays.

Results and Discussion

The inhibitory effect of 1-substituted imidazoles **3a**–**m** against nNOS, iNOS and eNOS was determined by monitoring the conversion of oxyhaemoglobin to methaemoglobin, according to Hevel &

Table 1. Inhibitory activity of compounds **3a**–**m** (500 μM) against neuronal, inducible and endothelial nitric oxide synthase.



Compound	n	R	Inhibition (%)		
			nNOS	iNOS	eNOS
3a[a]	2	-H	35·77	4	
3b	2	-Br	100 ($K_i = 125$ μM)	25	0 ($K_i = 4750$ μM)
3c[b]	2	$-C_6H_5$	30	15	
3d[a]	2	$-COOC_2H_5$	35·7	0	
3e[a]	2	$-NO_2$	30·6	10·34	
3f	2	$-OC_3H_7$	64	17·2	
3g	2	$-COC_6H_5$	85·7 ($K_i = 225$ μM)	17·9	0 (K_i ND)
3h	2	$-CH_3$	42·8	20	
3i[a]	2	$-NH_2$	22·9	22·69	
3j	2	$-CF_3$	100 ($K_i = 45$ μM)	4·6	0 (K_i = ND)
3k	2	$-OCH_3$	33		22
3l[c]	3	-H	56	0	
3m[d]	4	-H	45	9·6	
Imidazole			80 ($K_i = 175$ μM)	100 ($K_i = 60$ μM)	90 ($K_i = 185$ μM)
1-Phenyl-imidazole			60 ($K_i = 430$ μM)	100 ($K_i = 35$ μM)	40 ($K_i = 615$ μM)
Nitro-arginine			100 ($K_i = 0{\cdot}5$ μM)	100 ($K_i = 7{\cdot}6$ μM)	100 ($K_i = 0{\cdot}5$ μM)

[a]According to Cross et al (1985). [b]According to Robertson et al (1987) (percentage inhibition is expressed at 50 μM because of the very low solubility at higher concentrations). [c]According to Dou & Metzger (1976). [d]According to Shimazu et al (1996). K_i was not calculated for **3g** and **3j** because they are insoluble at concentrations greater than 500 μM.

Marletta (1994). Table 1 shows the percentage inhibition of NOS activity obtained at a dose of 500 μM. Inhibition constants were calculated for compounds showing a high degree of inhibition, by measuring the percentage inhibition of at least three concentrations of the inhibitor according to Dixon (1953). Inhibition of eNOS was determined for only the most active compounds. Imidazole, 1-phenyl-imidazole and nitro-arginine were used as references in all cases.

From the K_i values (Table 1), it is apparent that some compounds (**3b** and **3j**), showed a considerable degree of selectivity for nNOS. Although less potent than nitro-arginine, they were more selective. In addition, they had greater activity against nNOS than imidazole and 1-phenyl-imidazole, suggesting that the introduction of an aryloxyalkyl chain contributes to the inhibition of nNOS.

Structure–activity relationship studies on the influence on biological activity of the *para* substituent on the phenylic ring, suggest the importance of hydrophobic moieties. Compounds **3b** (4-Br) and **3j** (4-CF_3) were the most active. This differs from a previous study on the N-phenacyl series, where the most active compound was the derivative carrying a hydrophilic group (4-NO_2) (Salerno et al 1999).

All compounds tested were poor inhibitors of the inducible NOS.

In conclusion, **3b** and **3j** are interesting compounds which could be considered for their selectivity for nNOS over eNOS. Further investigation is necessary to identify the structural features of these imidazole derivatives and to elucidate their mechanism of action.

References

Chabin, R. M., McCauley, E., Calaycay, J. R., Kelly, T. M., Mac Nau, K. L., Wolfe, G. C., Hutchinson, N. I., Madhusudanaraja, S., Schmidt, J. A., Kozarich, J. W., Wong, K. K. (1996) Active-site structure analysis of recombinant human inducible nitric oxide synthase using imidazole. Biochemistry 35: 9567–9575

Cross, P., Dickinson, R., Parry, M., Randall, M. (1985) Selective thromboxane synthetase inhibitors. 1. 1-[(Aryloxy) alkyl)]-1*H*-imidazoles. J. Med. Chem. 28: 1427–1432

Dixon, M. (1953) The determination of enzyme inhibitor constants. Biochem. J. 55: 170–171

Dou, H. J. M., Metzger, J. (1976) Catalyse par transfert de phase en sèrie hètèrocyclique. N-alkylation des pyrazole et imidazole. Bull. Soc. Chim. Fr. 11–12: 1861–1864

Hevel, J. M., Marletta, M. A. (1994) Nitric-oxide synthase assays. In: Packer, L. (ed) Methods in Enzymology, Academic Press, San Diego, pp 250–258

Kato, K., Ohkawa, S., Terao, S., Terashita, Z., Nishikawa, K. (1985) Thromboxane synthetase inhibitors (TXSI). Design, synthesis, and evaluation of a novel series of ω-pyridylalkenoic acids. J. Med. Chem. 28: 287–294

Kerwin, J. F., Lancaster, J. R., Feldman, P. L. (1995) Nitric oxide: a new paradigm for second messengers. J. Med. Chem. 38: 4343–4362

Macdonald, J. E. (1996) Nitric oxide synthase inhibitors. Annu. Rep. Med. Chem. 31: 221–230

Marletta, M. A. (1993) Nitric oxide synthase structure and mechanism. J. Biol. Chem. 268: 12231–12234

Marletta, M. A. (1994) Approaches toward selective inhibition of nitric oxide synthase. J. Med. Chem. 37: 1899–1907

Moncada, S., Palmer, R. M. J., Higgs, E. A. (1991) Nitric oxide: Physiology, pathophysiology, and pharmacology. Pharmacol. Rev. 43: 109–142

Moore, P. K., Handy, R. L. C. (1997) Selective inhibitors of neuronal nitric oxide synthase – is no NOS really good NOS for the nervous system? Trends Pharmacol. Sci. 18: 204–211

Radomski, M. W., Palmer, R. M. J., Moncada, S. (1990) An L-arginine/nitric oxide pathway present in human platelets regulates aggregation. Proc. Natl Acad. Sci. USA 87: 5193–5197

Robertson, D. W., Beedle, E. E., Lawson, R., Leander, J. D. (1987) Imidazole anticonvulsants: stucture-activity relationships of [(biphenyloxy)alkyl]imidazoles. J. Med. Chem. 30: 939–943

Rogerson, T., Wilkinson, C., Hetarsky, K. (1976) Steric factors in the inhibitory interaction of imidazoles with microsomal enzymes. Biochem. Pharmacol. 26: 1039–1042

Salerno, L., Sorrenti, V., Guerrera, F., Sarvà, M. C., Siracusa, M. A., Di Giacomo, C., Vanella, A. (1999) N-substituted-imidazoles as inhibitors of nitric oxide synthase: a preliminary screening. Pharmazie: In press

Shimazu, K., Shimizu, M., Suzuki, K., Kuwano, E. (1996) Precocious methamorphosis-inducing activity of 1-substituted imidazoles. Nippon Noyaku Gakkaishi 21: 337–339

Southan, G. J., Szabò, C. (1996) Selective pharmacological inhibition of distinct nitric oxide synthase isoforms. Biochem. Pharmacol. 51: 383–394

Wolff, D. J., Datto, G. A., Samatovicz, R. A., Tempsick, R. A. (1993) Calmodulin-dependent nitric-oxide synthase. Mechanism of inhibition by imidazole and phenylimidazoles. J. Biol. Chem. 268: 9425–9429