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N-substituted-imidazoles as inhibitors of nitric oxide synthase: a preliminary screening

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Identification of potent and selective inhibitors of inducible or neuronal nitric oxide synthase (NOS) is of great interest because of their therapeutic potential for treatment of diseases mediated by overproduction of nitric oxide. Imidazole derivatives are described in the literature as inhibitors of various isoforms of NOS as well as inhibitors of various oxido-reductase enzymes. In this paper, we describe the synthesis and inhibitory activities towards neuronal rat recombinant NOS (nNOS), inducible mouse macrophage NOS (iNOS) and endothelial human platelet NOS (eNOS) of a series of 1-substituted imidazoles i.e. *N*-phenacyl, *N*-phenethyl- and *N*-phenyl-hydroxyethyl-imidazoles. The results show that the *N*-(4-nitrophenacyl)imidazole **2e** may be an interesting molecule. In fact, this substance, although active only in the micromolar range on nNOS, could be considered for ist selectivity for nNOS versus eNOS, in particular if compared with the reference substances (imidazole, 1-phenyl-imidazole and nitro-arginine). Thus **2e** represents a chemical structure which can be easily modified in order to improve the observed potency and selectivity.

1. Introduction

Nitric oxide synthase (NOS), the enzyme catalyzing the synthesis of the free radical gas nitric oxide (NO) and L-citrulline from the substrate L-arginine, has been identified as three isoforms. The neuronal (nNOS) and endothelial (eNOS) isoforms are constitutively expressed and are regulated by Ca^{++}/calmodulin, whereas the inducible (iNOS) isoform is only expressed under some conditions and is Ca^{++}/calmodulin independent, since it contains tightly bound calmodulin. All isoforms are protoporphyrin IX heme enzymes and require NADPH, tetrahydrobiopterin (BH_4), FMN and FAD as cofactors. Each isoform has been isolated, purified, characterized and cloned. Although NO produced by NOS is a biological messenger involved in different physiological processes including vasodilatation, platelet aggregation (eNOS), macrophage function (iNOS), neuronal communication (nNOS) etc., overproduction of NO can be detrimental. In fact, an overproduction of NO by iNOS is associated with ulcerative colitis, tissue damage following inflammation, rheumatoid arthritis and septic shock whereas an overproduction of NO by nNOS is associated with stroke, seizures, schizophrenia, migraine headaches, Alzheimer's and Parkinson's diseases and AIDS dementia [1–5].

The pathophysiological importance of NO derived from iNOS and nNOS suggests that inhibitors of these isoforms may be therapeutically useful, in particular those that do not affect the protective and physiological roles of eNOS. To date, many compounds have been shown to inhibit NOS with various degrees of potency and selectivity, including mono- or di-substituted arginines, guanidines, isothioureas, amidines, indazoles and imidazoles [6–8].

Many 1-substituted imidazoles are described in the literature as inhibitors of different enzymes belonging to the extensive family of cytochrome P_{450} oxido-reductase [9–12]. Since it has been demonstrated that NOS is a highly specialized cytochrome P_{450} monooxygenase which is sensitive to an inhibition by imidazole [13], some imidazole analogues have been studied as inhibitors [14, 15]. Among them, 1-phenyl imidazole and 1-(2-trifluoromethyl-phenyl)-imidazole (TRIM) are noteworthy with the first showing selectivity for inducible isoform [16] and the second for the neuronal isoform of NOS [17, 18].

With the aim of exploring the inhibitory activity on various isoforms of NOS of other molecules containing the imidazole nucleus as a pharmacophore, we describe in this paper the synthesis and the biological activity of a series of 1-substituted imidazoles, *N*-phenacyl, *N*-phenethyl- or *N*-phenyl-hydroxyethyl-imidazoles **2a–n**, **3a**, **e**, **i** and **4a**, **e**, **i**. Some of these substances are already described in the literature as anticonvulsant and antimycotic agents (**2a–e**, **2i**, **2k**, **4a**, **4e**, **4i**) [19, 20] or regarding their inhibitory effect on human placental aromatase and 17α-hydroxylase/17–20 lyase (**3a**, **3e**) [21]. In the present study newly and previously synthesized compounds were tested in order to evaluate their inhibitory activity towards rat recombinant nNOS, mouse macrophage iNOS and human platelet eNOS.

2. Investigations, results and discussion

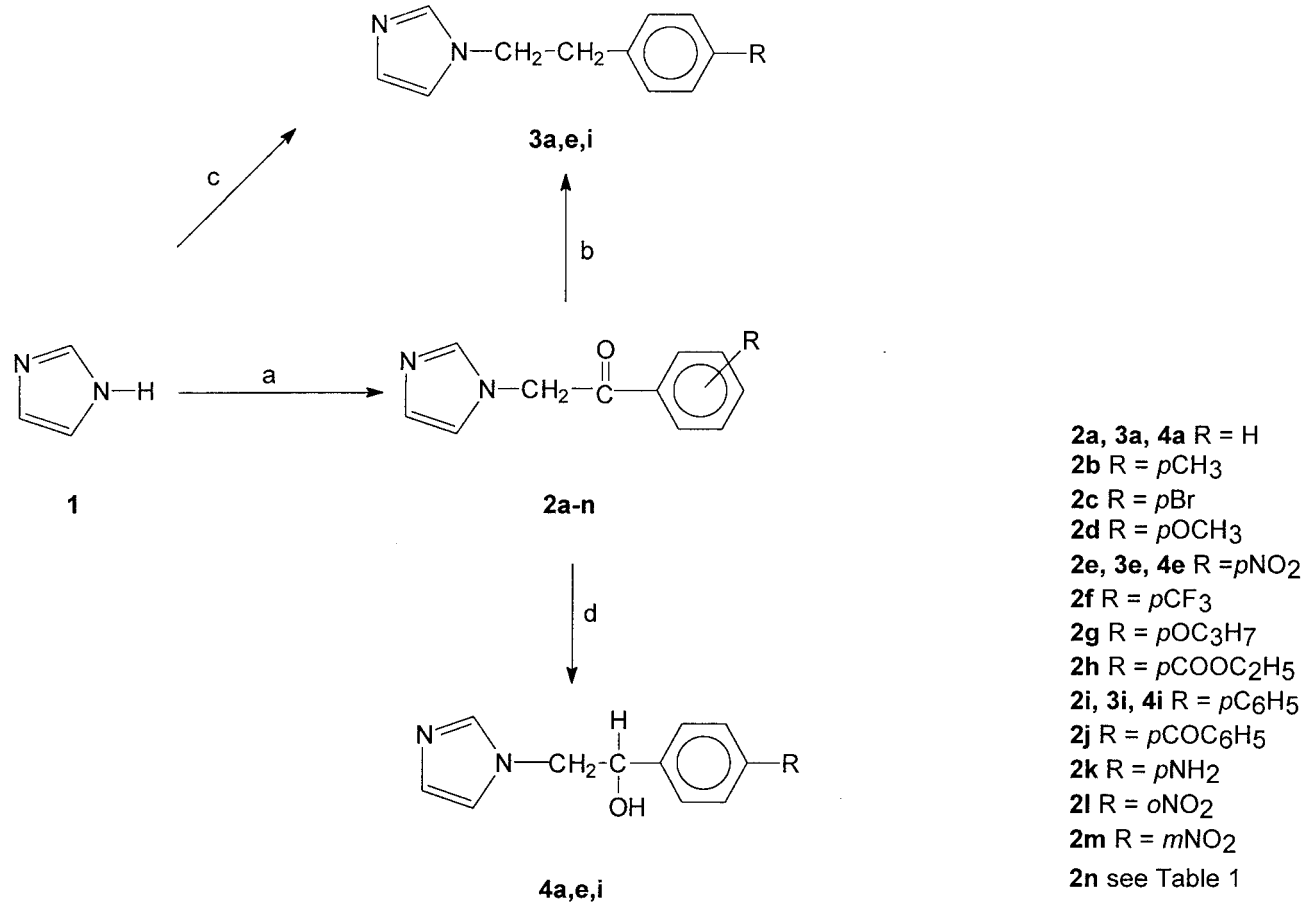
2.1. Chemistry

Synthetic pathways for the synthesis of title compounds are illustrated in the Scheme and are in agreement with standard protocols. In brief, N-phenacylimidazoles **2a–n** were prepared by reaction of the corresponding phenacyl bromide with imidazole (**1**) in dimethylformamide [19]. N-(phenyl)ethyl-imidazoles **3a**, **e**, **i** were obtained by alkylation of imidazole with appropriate phenyl-ethyl-bromides carried out in tetrahydrofurane solution using potassium carbonate as base [21] or by catalytic reduction of the corresponding N-phenacyl-imidazole in the case of **3i**. The sodium borohydride reduction of derivatives **2a**, **e**, **i** gave the enantiomeric mixture of the corresponding alcohols **4a**, **e**, **i** [20]. Purification of all the synthesized compounds was performed with flash chromatography. Physical properties are listed in Table 1. The proposed structures for all new synthesized compounds (**2g**, **2h**, **2j**, **2l–n**, **3i**) were confirmed by elemental analyses, IR and ^{1}H NMR data. ^{1}H NMR and IR spectra are also reported for previously synthesized compounds, where no spectroscopic data were available.

2.2. Pharmacological screening

The ability of the 1-substituted imidazoles **2a–n**, **3a**, **e**, **i** and **4a**, **e**, **i** to inhibit nNOS, iNOS and eNOS was determined by monitoring the conversion of oxyhemoglobin in methemoglobin, according to Hevel et al. [22]. The results reported in Table 2 represent the percentages of inhibition

Scheme



(a) Br-$CH_2COC_6H_4R$, DMF. (b) [H_2], Pd/C, CH_3COOH. (c) $BrCH_2CH_2C_6H_4R$, K_2CO_3, THF. (d) $NaBH_4$, CH_3OH.

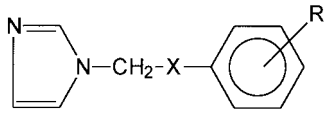
of NOS activity obtained at a dose of 500 μM; for compounds that showed a high degree of inhibition, results are also expressed as K_i. Inhibition towards eNOS was determined only for the most promising compounds. Selectivity for nNOS compared with iNOS and eNOS is defined as the ratio of K_i iNOS or K_i eNOS to K_i nNOS. Imidazole, 1-phenyl-imidazole and nitroarginine were always used as reference substances.

Starting from literature data [13–18] reporting that imidazole and 1-phenyl-imidazole possess inhibitory activity against various isoforms of NOS, we synthesized and tested a series of *N*-phenacyl-imidazoles **2a**–**n** i.e. derivatives in which an ethanonic chain was interposed between imidazole and variously substituted benzene rings.

Evaluation of the obtained results showed that some of the compounds under study, compared with imidazole and 1-phenyl-imidazole, were more potent against nNOS and less potent towards iNOS (K_i nNOS = 175 and 430 μM and K_i iNOS = 60 and 35 μM for imidazole and 1-phenyl-imidazole, respectively); all the tested compounds proved to be less active than the known unselective NOS inhibitor nitro arginine [23], which, as confirmed in this study, possesses the same potency towards all isoforms (K_i = 0.5, 7.6, 0.5 μM for nNOS, iNOS and eNOS, respectively). We thus focused our attention on neuronal NOS as a biological target; in fact, identification of potent and selective inhibitors of this isoform is clearly a very worthwhile goal in terms of providing tools for investigating various biological functions of NO in the nervous system; in addition, they could be therapeutically useful in clinical conditions associated with an excessive production of NO in the CNS [24, 25].

With regard to the influence of the *para*-substituents present at the phenylic rings of derivatives **2a**–**k** on the biological activity, it appears evident that the presence of a substituent in this position is critical; in fact, the unsubstituted derivative **2a** was devoid of activity at 500 μM. Regarding the introduced substituents, the results show that electron-releasing groups (-OCH_3, -OC_3H_7, -NH_2) generally gave a poor contribution to activity, whereas electron-withdrawing groups (-Br, -NO_2, -CF_3, -COC_6H_5), generally gave a good contribution to inhibitory potency. Indeed, derivative **2e** (*p*-NO_2) proved to be the most interesting compound of this series (K_i nNOS = 105 μM). Hydrophobic effects of the *para*-substituents were not clear since the most interesting compounds, **2e** and **2i** (% of inhibition of nNOS at 50 μM = 48), present hydrophilic (*p*-NO_2) and hydrophobic (*p*-C_6H_5) substituents, respectively. These data need further analyses in order to understand the structural requirement for inhibition of nNOS. However, among the derivatives **2a**–**k**, compound **2e** is interesting because it is active against nNOS in the micromolar range (K_i nNOS = 105 μM) but not against iNOS (K_i iNOS = 1100 μM) or eNOS (K_i eNOS = >5000 μM). The other compounds are not or only slightly active, with the exception for derivative **2i**, although it shows the same potency towards all the three isoforms (% of inhibition of nNOS, iNOS and eNOS at 50 μM = 45, 48 and 30, respectively).

Table 1: Physical properties of compounds 2a–n, 3a, e, i, 4a, e, i

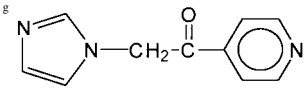


Compd.	X	R	M.p. (°C)	Yield (%)	Molecular formula[a]	IR (KBr cm^{-1})	^1H NMR data[b]
2a[c]	CO	H	116–117	65	$C_{11}H_{10}N_2O$		
2b[c]	CO	*p*-CH_3	135–136	75	$C_{12}H_{12}N_2O$	1690 (C=O)	2.45 (s, 3 H, CH_3); 5.37 (s, 2 H, CH_2); 6.94 (br s, 1 H, imidazole H^5); 7.13 (br s, 1 H, imidazole H^4); 7.30–7.34 (m, 2 H, arom.); 7.51 (br s, 1 H, imidazole H^2); 7.85–7.89 (m, 2 H, arom.).
2c[c]	CO	*p*-Br	165–167	80	$C_{11}H_9BrN_2O$	1700 (C=O)	5.37 (s, 2 H, CH_2); 6.93–6.94 (m, 1 H, imidazole H^5); 7.13–7.16 (m, 1 H, imidazole H^4); 7.50 (br s, 1 H, imidazole H^2); 7.65–7.71 (m, 2 H, arom.); 7.80–7.87 (m, 2 H, arom.).
2d[d]	CO	*p*-OCH_3	170–171	70	$C_{12}H_{12}N_2O$	1691 (C=O)	3.90 (s, 3 H, CH_3); 5.35 (s, 2 H, CH_2); 6.94–7.01 (m, 3 H, arom. and imidazole H^5); 7.13 (br s, 1 H, imidazole H^4); 7.51 (br s, 1 H, imidazole H^2); 7.93–7.97 (m, 2 H, arom.).
2e[e]	CO	*p*-NO_2	165–168 dec.	60	$C_{11}H_9N_3O_3$	1714 (C=O), 1526, 1354 (NO_2)	5.46 (s, 2 H, CH_2); 6.95–6.96 (m, 1 H, imidazole H^5); 7.16–7.20 (m, 1 H, imidazole H^4); 7.53 (br s, 1 H, imidazole H^2); 8.12–8.17 (m, 2 H, arom.); 8.36–8.41 (m, 2 H, arom.).
2f[f]	CO	*p*-CF_3	140–141	50	$C_{12}H_9F_3N_2O$	1700 (C=O)	5.45 (s, 2 H, CH_2); 6.96 (br s, 1 H, imidazole H^5); 7.16 (br s, 1 H, imidazole H^4); 7.54 (br s, 1 H, imidazole H^2); 7.79–7.83 (m, 2 H, arom.); 8.07–8.12 (m, 2 H, arom.).
2g	CO	*p*-OC_3H_7	105–106	35	$C_{14}H_{16}N_2O_2$	1691 (C=O)	1.06 (t, J = 7.4 Hz, 3 H, CH_3); 1.85 (m, 2 H, $CH_2CH_2CH_3$); 4.01 (t, J = 6.4 Hz, 2 H, $CH_2CH_2CH_3$); 5.35 (s, 2 H, CH_2); 6.96–7.01 (m, 3 H, imidazole H^5 and arom.); 7.14 (br s, 1 H, imidazole H^4); 7.53 (br s, 1 H, imidazole H^2); 7.9–7.97 (m, 2 H, arom.).
2h	CO	*p*-$COOC_2H_5$	89–90	50	$C_{14}H_{14}N_2O_3$	1710, 1690 (C=O)	1.43 (t, J = 7.2 Hz, 3 H, CH_3); 4.43 (q, J = 7.2 Hz, 2 H, CH_2CH_3); 5.45 (s, 2 H, CH_2); 6.96 (br s, 1 H, imidazole H^5); 7.15 (br s, 1 H, imidazole H^4); 7.54 (br s, 1 H, imidazole H^2); 8.01–8.21 (m, 4 H, arom.).
2i[e]	CO	*p*-C_6H_5	194–196	70	$C_{17}H_{14}N_2O$	1694 (C=O)	5.43 (s, 2 H, CH_2); 6.97–6.98 (m, 1 H, imidazole H^5); 7.15–7.19 (m, 1 H, imidazole H^4); 7.42–7.77 (m, 8 H, arom and imidazole H^2); 8.02–8.07 (m, 2 H, arom.).
2j	CO	*p*-COC_6H_5	141–143	50	$C_{18}H_{14}N_2O_3$	1705, 1649 (C=O)	5.47 (s, 2 H, CH_2); 6.98 (br s, 1 H, imidazole H^5); 7.17 (br 1 H, imidazole H^4); 7.47–8.11 (m, 10 H, arom. and imidazole H^2).
2k[e]	CO	*p*-NH_2	200–205 dec.	55	$C_{11}H_{11}N_3O$	3567, 3400, 3317 (NH_2), 1660 (C=O)	5.5 (s, 2 H, CH_2); 6.20 (br s, 2 H, NH_2); 6.58–6.62 (m, 2 H, arom.); 6.88 br s, 1 H, imidazole H^5); 7.06–7.07 (m, 1 H, imidazole H^4); 7.55 (s, 1 H, imidazole H^2); 7.71–7.75 (m, 2 H, arom.).
2l	CO	*o*-NO_2	142–143	45	$C_{11}H_9N_3O_3$	1729 (C=O), 1519, 1333 (NO_2)	5.11 (s, 2 H, CH_2); 6.96 (br s, 1 H, imidazole H^5); 7.07 (br s, 1 H, imidazole H^4); 7.39 (dd, Jo = 9 and Jm = 1.5 Hz, 1 H, arom. H^4); 7.49 (br s, 1 H, imidazole H^2); 7.63–7.82 (m, 2 H, arom. H^5 and H^6); 8.24 (dd, Jo = 9 and Jm = 1.4 Hz, 1 H, arom. H^3).
2m	CO	*m*-NO_2	120–121	60	$C_{11}H_9N_3O_3$	1711 (C=O), 1524, 1537 (NO_2)	5.50 (s, 1 H, CH_2); 6.97 (br s, 1 H, imidazole H^5); 7.17 (br s, 1 H, imidazole H^4); 7.55 (br s, 1 H, imidazole H^2); 7.90 (t, Jo = 8 Hz, arom. H^5); 8.3 (d, Jo = 7.8 Hz, 1 H, arom. H^6); 8.51 (d, Jo = 7.8 Hz, 1 H, arom. H^4); 8.79–8.80 (m, 1 H, arom. H^2).

Table 1: (Continued)

Compd.	X	R	M.p. (°C)	Yield (%)	Molecular formula[a]	IR (KBr cm^{-1})	^1H NMR data[b]
2n[g]	CO		163–164	55	$C_{10}H_9N_3O$	1717 (C=O)	5.42 (s, 2 H, CH_2); 6.94–6.96 (m, 1 H, imidazole H^5); 7.16–7.17 (m, 1 H, imidazole H^4); 7.53 (br s, 1 H, imidazole H^2); 7.74–7.77 (m, 2 H, arom); 8.89–8.92 (m, 2 H, arom.).
3a[h]	CH_2	H	wax	40	$C_{11}H_{12}N_2$		
3e[h]	CH_2	*p*-NO_2	102–104	60	$C_{11}H_{11}N_3O_2$		
3i	CH_2	*p*-C_6H_5	122–123	35	$C_{11}H_{16}N_2$	2924 (C–H)	3.08 (t, J = 7.2 Hz, 2 H, $CH_2C_6H_5$); 4.19 (t, J = 7.2 Hz, 2 H, NCH_2); 6.86 (br s, 1 H, imidazole H^5); 7.04–7.59 (m, 11 H, arom. and imidazole H^4 and H^2).
4a[c]	CHOH	H	155–157	60	$C_{11}H_{11}N_2O$		
4e[e]	CHOH	*p*-NO_2	187–189	65	$C_{11}H_{11}N_3O_3$	3116 (OH), 1517, 1346 (NO_2)	4.03–4.26 (m, 2 H, CH_2); 4.97–5.02 (m, 1 H, CH); 6.03–6.06 (m, 1 H, OH); 6.82 (s, 1 H, imidazole H^5); 7.10 (s, 1 H, imidazole H^4); 7.48 (s, 1 H, imidazole H^2); 7.58–7.63 (m, 2 H, arom.); 8.17–8.23 (m, 2 H, arom.).
4i[e]	CHOH	*p*-C_6H_5	182–183	80	$C_{17}H_{16}N_2O$	3188 (OH)	4.00–4.23 (m, 2 H, CH_2); 4.85–4.88 (m, 1 H, CH); 5.74–5.76 (m, 1 H, OH); 6.84 (s, 1 H, imidazole H^5); 7.16 (s, 1 H, imidazole H^4); 7.31–7.69 (m, 10 H, arom. and imidazole H^2).

[a] Elemental analyses were within ±0.4% of the theoretical values.
[b] For compounds **2k**, **4a**, **4e** and **4i** spectra were recorded in DMSO-d_6, for the other compounds in $CDCl_3$.
[c] According to literature [19].
[d] According to literature [31].
[e] According to literature [20].
[f] According to literature [32].

[g]


[h] According to literature [21].

We thus selected the *p*-NO_2 phenacyl derivative **2e** for further chemical modifications. To investigate the importance of the position of the substituent, we synthesized the *ortho*- and *meta*-NO_2 derivatives **2l** and **2m**. The data obtained confirmed that the *para*-substitution was better for activity although the *ortho*-derivative remained interesting for ist selectivity (K_i iNOS = 1750 μM and K_i eNOS = >5000 μM) but showed a decrease in its potency towards nNOS (K_i nNOS = 375 μM). The bioisosteric replacement of the *p*-NO_2-phenyl moiety with a 4-pyridyl ring results in a significant decrease in nNOS inhibition as shown by the analogue **2n** (% of inhibition of nNOS at 500 μM = 0) confirming the importance of this substitution.

Finally, in order to investigate the influence of the ethanonic chain, we selected derivatives **2a, e, i** (carrying H, NO_2 or C_6H_5 moiety at the *para* position of the phenyl ring) as substrates for total or partial reduction of the keto group, for synthesizing analogues **3a**, **e**, **i** and **4a**, **e**, **i**.

The unsubstituted analogues **3a** and **4a** were slightly more active than the corresponding ketonic **2a** (% of inhibition at 500 μM = 32.16, 12.4 and 0, respectively), the *p*-NO_2 analogues **3e** and **4e** showed a decrease in inhibitory activity of nNOS (% of inhibition at 500 μM of **2e**, **3e** and **4e** = 100, 50 and 29.8, respectively) and the biphenyl-derivatives **3i** and **4i**, althought active against nNOS (K_i nNOS = 175 and 125 μM, respectively), were less interesting due to their nonselectivity (% of inhibition of iNOS at 500 μM = 25 for **3i** and K_i iNOS = 1350 μM for **4i**, K_i eNOS = 150 and 110 μM for **3i** and **4i**, respectively). Then, in relation to the various chains, the contribution to the inhibitory activity of the *para*-substituents was different since the potency increased in the order H < NO_2 < C_6H_5 for derivatives **3** and **4** and in the order H < C_6H_5 < NO_2 for derivatives **2**.

Thus, among the synthesized and tested compounds, derivatives **2e** and **4i** showed the highest potency of these series, 2e being the most promising because ist selectivity for nNOS vs eNOS as shown by their ratios of K_i eNOS to K_i nNOS (> 47 for **2e** and 0.88 for **4i**).

In conclusion, although the examined compounds are not very potent inhibitors of NOS, this preliminary screening allowed us to state that *N*-(4-nitrophenacyl)imidazole (**2e**) may be an interesting molecule. In fact, this substance, although active only in the micromolar range on nNOS, could be considered for its selectivity for nNOS vs eNOS, in particular if compared with the reference substances imidazole, 1-phenyl-imidazole and nitro-arginine. Thus, **2e** represents a chemical structure which can be easily modified in order to improve the observed potency and selectivity.

3. Experimental

Melting points were determined using a Büchi 510 apparatus and are uncorrected. IR spectra were recorded on a Perkin-Elmer Model 281 spectrometer with KBr disk. ^1H NMR spectra were recorded on a Varian 200 MHz instrument in $CDCl_3$ or DMSO-d_6 solution. Chemical shifts are given in ppm (δ) relative to tetramethylsilane as internal standard and signals were characterized as s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), or br (broad signals). Elemental analyses were performed on a C. Erba Model 1106 elemental analyzer and the data of C, H, N, are within ±0.4% of calculated values. TLC separations were performed on Merck silica gel 60-F_{254} precoated aluminum plates. Preparative chromatographic separations were conducted by means of flash chromatography using Merck Silica gel 60 0.040–0.063 mm. Imidazole and a number of bromo-derivatives are commercially available and were used without further purification. The remaining phenacyl bromides were newly prepared following common methods [26–29] and used directly in the next step.

Table 2: Inhibitory activities on NOS of compounds 2a–n, 3a, e, i and 4a, e, i

Compd.	% of inhibition of nNOS at 500 μM (K_iμM)[b]	% of inhibition of iNOS at 500 μM (K_iμM)[b]	% of inhibition of eNOS at 500 μM (K_iμM)[b]	Selectivity[a]	
				iNOS/nNOS	eNOS/nNOS
2a	0	0			
2b	40	13			
2c	20	12			
2d	9.1	16			
2e	100 (105)	31.62 (1100)	0 (>5000)	10.47	>47.61
2f	36.7	27.3			
2g	7.8	24			
2h	13	0			
2i	45[c]	48[c]	30[c]	1.06[d]	0.66[d]
2j	28.79	0			
2k	0	2.4			
2l	87 (375)	11.7 (1750)	0 (>5000)	4.66	>13.33
2m	19	4			
2n	0	3			
3a	36.26	32.8			
3e	50	0			
3i	70.15 (175)	25 ([e])	100 (150)		0.85
4a	12.4	30.6			
4e	29.8	21.8			
4i	80.6 (125)	24 (1350)	100 (110)	10.08	0.88
Imidazole	80 (175)	100 (60)	90 (185)	0.34	1.05
1-Phenylimidazole	60 (430)	100 (35)	40 (615)	0.08	1.43
Nitroarginine	100 (0.5)	100 (7.6)	100 (0.5)	15.2	1

[a] Defined as the ratio of K_i iNOS or K_i eNOS to K_i nNOS
[b] Inhibition constants were obtained by measuring % of inhibition with at least three concentrations of inhibitor as described in literature [33]. Values had a standard deviation of $\leq 10\%$ ($n \geq 3$)
[c] % of inhibition is expressed at 50 μM owing to the very low solubility at higher concentrations
[d] Defined as the ratio of % of inhibition of iNOS or eNOS to nNOS at 50 μM
[e] K_i was not calculated because it is insoluble at concentrations higher than 500 μM

3.1. Chemistry

3.1.1. General procedure for the preparation of the N-phenacyl-imidazoles **2a–n**

A mixture of the appropriate phenacyl-bromide (5 mmol), imidazole (25 mmol) and 1.5 ml of DMF was stirred at 5–10 °C for 3 h then suspended in H_2O and the resulting precipitate was collected (or extracted) and washed with H_2O. The crude product was then purified from an excess of imidazole and from the corresponding 1–3-bis(phenacyl)imidazolium bromide, by flash chromatography eluting with mixtures of ethyl acetate/methanol. Melting points, yields, analytical and spectroscopic data for both previously [19] and newly synthesized compounds are given in Table 1.

3.1.2. General procedure for the preparation of the N-phenethyl-imidazoles **3a**, **e**

Imidazole (23 mmol) was added to a mixture of anh. K_2CO_3 (11.5 mmol) and dry THF (5 ml). The mixture was stirred at room temperature for 10 min before bromo-derivatives (23 mmol) were added, then stirred for additional 5 h. After filtration, THF was evaporated in *vacuo* and the residue was purified by flash chromatography performed with a mixture of ethyl acetate/methanol 8 : 2 v/v as eluent. Yields, melting points, analytical and spectroscopic data are reported in Table 1 and are in agreement with literature data [21].

3.1.3. N-[2-(Biphenyl)ethyl]imidazole (**3i**)

A mixture of the corresponding *N*-phenacyl derivative **2i** (0.4 g, 1.5 mmol), 0.1 g of 10% Pd/C as catalyst and 10 ml of glacial acetic acid was stirred under hydrogen at 70 °C for 10 h. After chilling, the mixture was filtered from the catalyst and neutralized with Na_2CO_3; the solid obtained was filtered, washed with H_2O and dried. The crude product was purified by flash chromatography eluting with a mixture of CH_2Cl_2/MeOH 9.75 : 0.25 to obtain pure **3i**. Meeting point, yield, analytical and spectroscopic data are reported in Table 1.

3.1.4. General procedure for the preparation of N-(phenyl-hydroxyethyl)-imidazoles **4a**, **e**, **i**

A mixture of the appropriate N-phenacyl imidazole **2a**, **e**, **i** (1.9 mmol), 1.9 mol of $NaBH_4$ and 5 ml of MeOH was refluxed for 2 h. After 5 ml of H_2O were added, the mixture was neutralized with dilute HCl and then refluxed for 30 min. After cooling, the solution was alkalinized with aqueous 5% NaOH and the precipitate was collected and crystallized by EtOH. Melting points, yields, analytical and spectroscopic data (Table 1) are in agreement with literature data [20].

3.2. Enzymatic assay

NOS isoenzymes: inducible nitric oxide synthase isolated from immunostimulated mouse macrophage (RAW 264.7) cells and neuronal rat recombinant nitric oxide synthase isolated from a Baculovirus overexpression system in SF9 cells, were purchased from Alexis. Endothelial NOS was prepared from washed platelets as described by Radomski et al. [30]. The assay for NO synthase activities was carried out measuring the rate of conversion of oxyhemoglobin to methemoglobin using a Hitachi UV 2000 spectrophotometer. A reference cuvette was charged with 5 μM oxyhemoglobin (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^{++} (required only for iNOS), 170 μM Dithiothreitol (DTT), 100 μM NADPH, 12 μM BH_4, 1 mM Ca^{++} (required only for nNOS and eNOS), 20 U/ml Calmodulin (required only for nNOS and eNOS), 10 μl of 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 U/ml for nNOS and iNOS, cytosol derived from 1.1×10^{11}

platelets for eNOS) and 5 μM oxyhemoglobin in 100 μM HEPES pH 7.4 in a final volume of 500 μl. The HEPES buffer was pre-heated prior to use. NO formed reacts with oxyhemoglobin to yield methemoglobin which can be measured at 401 nm.
All assays were the mean of at least three values with a standard deviation of $\leq$10%.

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