Received: 3 March 2022 | Revised: 30 March 2022 | Accepted: 2 April 2022

DOI: 10.1002/mrc.5273

LETTER - SPECTRAL ASSIGNMENT

WILEY

NMR spectroscopy study of new imidamide derivatives as nitric oxide synthase inhibitors

INTRODUCTION

Nitric oxide (NO) is a heterodiatomic molecule with an unpaired electron that behaves as a free radical. At the physiological level, it is involved in smooth muscle relaxation, blood pressure control, inflammation, immune system activation, and neurotransmission. [1] The isoenzymes of nitric oxide synthase (NOS) biologically catalyze NO formation from L-arginine. In mammalians, there are three NOS isoforms: two constitutive isoforms (neuronal or nNOS [2] and endothelial or eNOS), [3] whose activities depend on the intramolecular calcium concentration, and an inducible isoform, iNOS. [4] The latter is only expressed after transcriptional induction and its activity do not depend on calcium concentration, since the Ca/Calmodulin complex is part of its structure. [5]

NO produced by nNOS is mainly involved in neuronal signaling, synaptic plasticity, pain expression, and neurotoxicity. [6] NO synthesized by eNOS participates, among other actions, in vascular function regulation, as it promotes vasodilation, modulates blood pressure, and reduces the harmful effects of atherosclerosis. [7] Eventually, NO catalyzed by iNOS is part of nonspecific immunity, exerting cytostatic or cytotoxic actions against microorganisms and tumor cells. [8] It acts as a modulator of the immune system and as a pro-inflammatory mediator. [9]

An uncontrolled production of NO by nNOS is observed in several neurodegenerative disorders, such as Alzheimer's, Parkinson's, and Huntington's disease. [10] Overexpression of iNOS could cause pathological processes, such as asthma, arthritis, multiple sclerosis, colitis, tumor development, [11] and septic shock. [12] Finally, NO underproduction by eNOS can cause hypertension and atherosclerosis. [13] In this sense, the search for nNOS or iNOS inhibitors, but not eNOS inhibitors, can be beneficial for diverse pathologies such as those mentioned above.

In the research on new NOS inhibitor agents, we have recently designed and synthesized a novel set of aryl- and heteroaryl-imidamide derivatives **10**–**22**, aiming to find potent and selective molecules that could be useful as nNOS or iNOS inhibitors. [14, 15]

The structural elucidation of these compounds has been performed on the basis of different standard techniques by analyzing their spectroscopic (^{1}H and ^{13}C 1D NMR) and spectrometric (MS) data; nevertheless, a detailed NMR study, including 2D NMR [heteronuclear single quantum correlation (HSQC) and heteronuclear multiple bond correlation (HMBC)] techniques, has been carried out in some derivatives, to confirm their structures unambiguously.

In the present article, we discuss the unequivocal assignment results of the ^{1}H and ^{13}C NMR spectra signals for nine *N*-[3-(2-amino-5-substitutedphenyl)-3-hydroxypropyl]acetimidamides or benzimidamides (**10**–**18**) and four *N*-[(3-hydroxy-3-pyridin-3-yl)propyl] acetimidamides or benzimidamides (**19**–**22**), as well as those of *N*-[3-hydroxy-3-(2-nitro-5-substitutedphenyl)propyl]acetimidamides or benzimidamides **1**–**9**, the immediate synthetic precursors of the aminophenyl imidamides **10**–**18**.

EXPERIMENTAL

NMR spectra

NMR data were collected on Bruker Avance NEO spectrometers equipped with a Smart Probe BBFO, operating at 400.57 MHz for ^{1}H and 100.73 MHz for ^{13}C, at 499.79 MHz for ^{1}H and 125.68 MHz for ^{13}C, or 600.25 MHz for ^{1}H and 150.95 MHz for ^{13}C, in the deuterated solvents. The NMR data were recorded at 298 K, with chemical shifts δ reported in parts per million (ppm), and they were related to the residual solvent peak: CD_3OD, $\delta = 4.78$ ppm (^{1}H), $\delta = 49.15$ ppm (^{13}C); DMSO-d_6, $\delta = 2.50$ ppm (^{1}H), $\delta = 39.51$ ppm (^{13}C). Coupling constants J are indicated in Hertz. The ^{1}H NMR spectra were acquired with eight scans at 6.41 kHz of spectral width and 32 k data points, giving

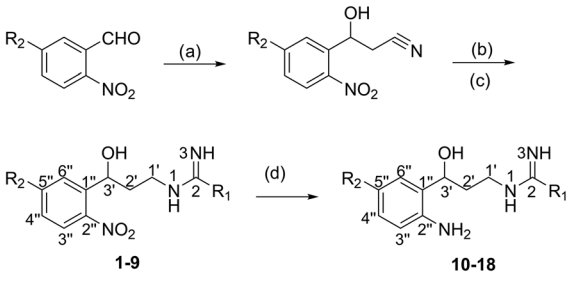
María Encarnación Camacho and María Dora Carrión contributed equally.

approximately 0.195 Hz of digital resolution (pulse width = 10 μs). For ^{13}C NMR spectra, 2000 scans were performed, the spectral width was 31.2 kHz with 65 k data points, providing around 0.476 Hz of digital resolution (pulse width = 10 μs). The DEPT experiments were run using the following parameters: pulse width (135°), 9.0 ms; recycle time, 1 s; ½ J (CH) = 4 ms; 65 K data points acquired and transformed from 1024 scans; spectral width, 15 KHz; and line broadening, 1.3 Hz. Spin multiplicities are specified as follows: s (singlet), bs (broad singlet), d (doublet), dd (double doublet), ddd (double double doublet), dddd (double double double doublet), dt (double triplet), t (triplet), td (triple doublet), tt (triple triplet), q (quadruplet), and m (multiplet). The 2D HSQC and HMBC experiments were run using the pulse sequences hsqcedetgpsp.3 and hmbccetgpl3nd, respectively (Standard sequence, Avance NEO) and pulse width 10 μs. ^{1}H/^{15}N 2D NMR (HSQC, HMBC) spectra were recorded on a 400 MHz Avance NEO spectrometer using a High-Field Triple Resonance Probe (HCN). Ammonia was used as an external standard set at 0.0 ppm, and DMSO-d_6 was used as a solvent in each sample. Nuclear Overhauser spectra were carried out on an Avance NEO spectrometer, operating at 400 MHz, with a pulse width of 10 μs. The mixing time in the NOESY experiments was 0.5 s.

RESULTS AND DISCUSSION

Aminophenyl imidamides **10**–**18** were prepared following the previously reported methodology described in Scheme 1, [14] and pyridine imidamides **19**–**22** were synthesized as shown in Scheme 2. [15] In addition, the structural data of all the nitrophenyl intermediates **1**–**9** and final compounds are collected in Table 1.

The complete structural characterization of the synthesized compounds was achieved by ^{1}H and ^{13}C NMR routine techniques. However, the unequivocal assignments for all NMR signals were performed through the combined information of one- and two-dimensional NMR experiments such as DEPT, HSQC, and HMBC, in order to determine the number of protons assigned to each carbon atom, the ^{13}C resonances of the tertiary, secondary and primary carbons, and the signals of quaternary carbons via two- and three-bond interactions, respectively.



SCHEME 1 Synthesis of *N*-substituted imidamide derivatives **10–18**. (a) CH_3CN, BuLi, THF, −78°C, then RT; (b) BH_3-THF, 0°C, then 4 h RT; (c) MeC(=NH)OEt or $PhCH_2SC(=NH)R_2$, EtOH

The unequivocal assignments of ^{1}H and ^{13}C signals (experimental data) for compounds **1**–**22** are compiled in Tables 2–7. The NMR spectra of all the compounds were performed on CD_3OD, except for intermediate derivatives **2** and **4** which were carried out in DMSO-d_6. Therefore, slight variations can be noticed in the chemical shifts depending on the solvent.

Regarding the ^{1}H and ^{13}C 1D NMR spectra, we can see a great similarity between the hydrogen chemical shifts H-1′, H-2′, and H-3′ and the carbon signals C-2 and C-1′ in the three series of compounds: *N*-[3-hydroxy-3-(2-nitro-5-substitutedphenyl)propyl]acetimidamides or benzimidamides **1**–**9**, *N*-[3-(2-amino-5-substitutedphenyl)-3-hydroxypropyl]acetimidamides or benzimidamides **10**–**18**, and *N*-[(3-hydroxy-3-pyridin-3-yl)propyl]acetimidamides or benzimidamides **19**–**22**. In addition, the C-4″ and C-6″ aromatic signals are also similar in the nitro intermediates **1**–**9** and the final amino derivatives **10**–**18**. Nevertheless, the rest of the chemical shifts are influenced by the amine group activating impact or by the deactivating effects of the nitro group and the pyridine heterocycle. Lastly, H-1 and H-3 signals linked to nitrogen atoms are undetectable, except for derivative **4** where the solvent was DMSO-d_6.

Compounds **4**, **8**, **13**, **17**, and **22** are characterized by the presence of a trifluoromethyl group in the *para*-position of the remaining benzimidamide. This group was confirmed by three quadruplets in their ^{13}C NMR 1D spectra, which became visible at δ 135.75–132.72 (q, $J_{C4\text{-}CF}$ = 32.5–33.4), δ 127.19–125.77 (q, $J_{C3,C5\text{-}CF}$ = 3.7–3.8), and δ 124.92–123.56 (q, $J_{C\text{-}F}$ = 271.6–272.89), respectively.

The HSQC experiments were carried out on some compounds of each series, and the results of these experiments were extrapolated to the rest of molecules. Table 8 shows the HSQC correlations for compounds **1**, **5**, **13**, **16**, and **21**.

The HSQC spectra obtained on derivatives **1** and **5** enabled us to identify the secondary carbon atom signals C-1′ and C-2′, as well as the assignment of the tertiary carbon signals C-3′, C-3″, C-4″, C-6″, and C-5″ (only for compounds **1**–**4**) in the nitro intermediates **1**–**9**. The ranges of chemical shifts of these carbon atoms are as follows: 41.60–36.02 (C-1′), 37.29–30.70 (C-2′), 67.80–65.40 (C-3′), 130.57–123.89 (C-3″), 134.81–114.16 (C-4″), 129.51–113.65 (C-6″), and 129.50–128.37 (C-5″) (except in derivatives **5**–**9** where carbons are quaternary).

SCHEME 2 Synthesis of *N*-substituted imidamide derivatives **19–22**. (a) CH_3CN, BuLi, THF, −78°C, then RT; (b) BH_3-THF, 0°C, then 4 h RT; (c) MeC(=NH)OEt or $PhCH_2SC(=NH)R$, EtOH

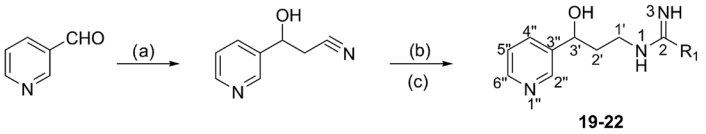


TABLE 1 Structural data of the intermediate (**1–9**) and final (**10–22**) compounds

Compound	R_1	R_2	Compound	R_1
1, **10**	Me	H	**19**	Me
2, **11**	C_6H_5	H	**20**	C_6H_5
3, **12**	*p*-Cl-C_6H_4	H	**21**	*p*-Cl-C_6H_4
4, **13**	*p*-CF_3-C_6H_4	H	**22**	*p*-CF_3-C_6H_4
5, **14**	Me	OMe		
6, **15**	C_6H_5	OMe		
7, **16**	*p*-Cl-C_6H_4	OMe		
8, **17**	*p*-CF_3-C_6H_4	OMe		
9, **18**	Me	Cl		

In the same way, the HSQC correlation spectra experiments performed on molecules **13** and **16** allowed assigning the tertiary and secondary signals of the aminophenyl derivatives **10–18**. These atoms exhibit chemical shifts in the range of 41.97–40.82 (C-1′), 35.01–34.24 (C-2′), 71.53–69.82 (C-3′), 120.65–118.07 (C-3″), 129.38–114.80 (C-4″), 129.12–113.73 (C-6″), and 119.36–119.27 (C-5″) (only for compounds **10–13** whose carbons are tertiary).

Finally, the secondary (C-1′, C-2′) and tertiary (C-3′, C-2″, C-4″-C-6″) atom chemical shifts of the imidamides with pyridine ring **19–22**, which were assigned with the HSQC tool, showed their signals in the following ranges: 41.73–38.89 (C-1′), 37.43–36.80 (C-2′), 69.99–67.28 (C-3′), 148.00–147.46 (C-2″), 135.92–133.35 (C-4″), 125.29–123.30 (C-5″), and 149.12–148.04 (C-6″).

The HMBC experiments were performed on the nitro-derivatives **1** and **5**, final amines **13** and **16**, and the pyridine **21** to establish the full structures of these molecules. Figure 1 exhibits the more important connections found in the 2D $^1H/^{13}C$ HMBC spectra for derivatives **5**, **16**, and **21**, as representative samples, in order to assign the quaternary carbon chemical shifts.

In the nitro-derivative **1**, the correlation between Me (R_2) (δ 2.26) and H-1′ (δ 3.51) and the ^{13}C signal at 166.08 ppm enabled the assignment of this shift to C-2; the correspondence between H-3″ and H-6″ (δ 7.90) and the peak at 148.74 ppm enabled the unequivocal assignation of C-1″; finally, the relationship between the peak at δ 7.71 ppm (H-4″) and the signal at 141.51 ppm allowed us to assign this peak to C-2″. In addition, the HMBC experiment of nitrophenyl-imidamide **5** showed the presence of key correlations between H-1′ (δ 3.55) and Me (R_2) (δ 2.28), with a ^{13}C signal at δ 166.14; thus, it could be assigned to C-2; a new correlation between H-3″ (δ 8.08) and the signal at 145.66 ppm enabled the assignment of C-1″, H-4″ (δ 7.00), and H-6″ (δ 7.44) connected with the signal of C-2″ (δ 141.24 ppm); finally, C-5″ was assigned to the peak at 165.50 ppm due to the observed correlation with the shifts at δ 3.93 ppm (OMe), δ 8.08 ppm (H-3″), and δ 7.44 (H-6″).

The HMBC experiment performed on the final amine **13** shows that the peak at δ 3.64 (H-1′) is connected to the ^{13}C signal at 164.70 ppm, which enabled the assignation of C-2, while H-2′ signals (δ 2.31 and δ 2.24) were correlated with the ^{13}C shift at δ 129.13 ppm, consequently, this peak corresponds to C-1″. Besides, the shift at δ 4.93 (H-3′) was correlated with C-2″ (146.14 ppm). Moreover, in the amine **16**, H-1′ (δ 3.65) and the *p*-ClPh signals H-2 and H-6 (δ 7.74) were associated with C-2 (164.75); another correlation between the peaks at δ 2.27 ppm (H-2′) and δ 4.95 ppm (H-3′) and the signal appearing at 131.27 ppm, allowed this signal to be assigned to C-1″; moreover, C-2″ corresponds to the signal at δ 138.87 ppm, due to the long-range correlation with H-4″ (δ 6.71) and H-6″ (δ 6.88); C-5″ is the peak at 154.44 ppm due to the correlations between H-3″ (δ 6.77), H-6″(δ 6.88), and OMe (δ 3.75); finally, we observed connections between H-2 and H-6 (δ 7.44) of the *p*-ClPh with the shift at 140.74; thus, this signal was assigned to the C-4 of this ring, and the correlation between H-3 and H-5 (δ 7.65 ppm) was assigned to the peak at 129.14 ppm, which allowed identifying the C-1 of this ring.

TABLE 2 ^{1}H NMR signal assignments of compounds **1–9**

Comp	H-1′	H-2′a	H-2′b	H-3′	H-3″	H-4″	H-5″	H-6″	H-1, H-3	OH	OCH_3
1	3.51 (m)	2.13 (m)	1.89 (m)	5.24 (d, 9.2)	7.90 (dd, 7.5, 4.7)	7.71 (t, 7.5)	7.48 (t, 7.5)	7.90 (dd, 7.5, 4.7)	n.o.	n.o.	-
2[a]	3.61 (m)	2.10 (m)	1.92 (m)	5.14 (d, 9.2)	7.87 (dd, 7.9, 1.3)	7.71 (t, 7.5)	7.53 (dt, 8.3, 1.3)	7.91 (dd, 8.3, 1.1)	9.77 (bs), 9.48, 9.05 (bs)	5.78 (d, 4.5)	-
3	3.87, 3.77 (2 m)	2.34 (m)	2.08 (m)	5.42 (dd, 9.6, 2.4)	8.02 (ddd, 8.2, 2.9, 1.2)	7.82 (dt, 7.4, 1.2)	7.58 (dt, 7.5, 1.5)	8.02 (ddd, 8.2, 2.9, 1.2)	n.o.	n.o.	-
4[a]	3.62 (m)	2.10 (m)	1.93 (m)	5.13 (dd, 9.2, 2.1)	7.86 (d, 7.5)	7.76 (t, 7.5)	7.52 (t, 7.5)	7.91 (d, 8.4)	9.98 (bs), 9.60, 9.35 (bs)	5.82 (bs)	-
5	3.55 (m)	2.14 (m)	1.83 (m)	5.42 (dd, 9.3, 2.0)	8.08 (d, 9.1)	7.00 (dd, 9.1, 2.7)	-	7.44 (d, 2.7)	n.o.	n.o.	3.93 (s)
6	3.90, 3.80 (2 m)	2.35 (m)	2.02 (m)	5.59 (dd, 9.4, 2.1)	8.14 (d, 9.1)	7.05 (dd, 9.1, 2.8)	-	7.53 (d, 2.8)	n.o.	n.o.	3.98 (s)
7	3.87, 3.76 (2 m)	2.31 (m)	1.98 (m)	5.55 (dd, 10.4, 2.1)	8.14 (d, 9.1)	7.04 (dd, 9.1, 2.8)	-	7.51 (d, 2.8)	n.o.	n.o.	3.97 (s)
8	3.87, 3.76 (2 m)	2.32 (m)	1.97 (m)	5.54 (dd, 9.4, 2.1)	8.14 (d, 9.1)	7.04 (dd, 9.1, 2.8)	-	7.51 (d, 2.8)	n.o.	n.o.	3.97 (s)
9	3.52 (m)	2.35 (m)	1.87 (m)	5.27 (dd, 9.5, 2.3)	8.00 (d, 8.7)	7.51 (dd, 8.7, 2.3)	-	7.91 (d, 2.3)	n.o.	n.o.	-

Note: Chemical shifts (in CD_3OD) are reported in δ (in ppm) relative to CD_3OD; multiplicities and coupling constants (Hz) are given in parentheses. ^{1}H signals for the R_2 substituent: **1**, Me: 2.26 (s); **2**, Ph: C2, C4, C6: 7.74 (m); C3, C5: 7.61 (t, 7.6); **3**, *p*-ClPh: C3, C5: 7.71 (d, 8.7); C2, C6: 7.87 (d, 8.7); **4**, *p*-CF_3Ph: C2, C3, C5, C6: 7.98 (dd, 26.1, 8.3); **5**, Me: 2.28 (s); **6**, Ph: C2, C6: 7.88 (d, 7.3), C4: 7.78 (t, 7.5); C3, C5: 7.68 (t, 7.8); **7**, *p*-ClPh: C2, C6: 7.85 (d, 8.7); C3, C5: 7.68 (d, 8.7); **8**, *p*-CF_3Ph: C2, C6: 8.02 (d, 8.3); C3, C5: 7.97 (d, 8.3); **9**, Me: 2.26 (s).

Abbreviations: bs, broad singlet; d, doublet; dd, double doublet; ddd, double double doublet; dt, double triplet; m, multiplet; n.o., not observable; s, singlet; t, triplet.

[a]Solvent used DMSO-d_6.

TABLE 3 ^{1}H NMR signal assignments of compounds **10–18**

Compound	H-1′	H-2′a	H-2′b	H-3′	H-3″	H-4″	H-5″	H-6″	H-1, H-3	OH	OCH_3
10	3.39 (m)	2.17 (m)	2.08 (m)	4.62 (m)	6.74 (d, 7.9)	7.03 (td, 7.9, 1.1)	6.69 (td, 7.9, 0.8)	7.14 (dd, 7.9, 1.1)	n.o.	n.o.	-
11	3.67 (m)	2.31 (m)	2.20 (m)	4.91 (dd, 8.4, 5.0)	6.76 (dd, 8.0, 1.0)	7.05 (td, 8.0, 1.5)	6.71 (td, 7.4, 1.0)	7.18 (d, 7.4)	n.o.	n.o.	-
12	3.61 (t, 7.0)	2.32 (m)	2.22 (m)	4.91 (m)	6.77 (dd, 7.8, 1.1)	7.06 (td, 7.8, 1.4)	6.72 (td, 7.4, 1.1)	7.18 (dd, 7.4, 1.4)	n.o.	n.o.	-
13	3.64 (t, 6.9)	2.31 (m)	2.24 (m)	4.93 (dd, 8.4, 5.2)	6.76 (dd, 7.9, 1.1)	7.04 (td, 7.9, 1.5)	6.71 (td, 7.5, 1.1)	7.19 (dd, 7.5, 1.5)	n.o.	n.o.	-
14	3.37 (m)	2.08 (m)	2.08 (m)	4.96 (d, 3.4)	6.79 (d, 8.7)	6.70 (dd, 8.7, 2.8)	-	6.81 (d, 2.8)	n.o.	n.o.	3.71 (s)
15	3.65 (t, 7.0)	2.26 (m)	2.26 (m)	4.95 (dd, 8.0, 5.3)	6.78 (d, 8.6)	6.71 (dd, 8.6, 2.8)	-	6.88 (d, 2.8)	n.o.	n.o.	3.74 (s)
16	3.65 (t, 6.9)	2.27 (m)	2.27 (m)	4.95 (dd, 7.8, 5.4)	6.77 (d, 8.6)	6.71 (dd, 8.6, 2.8)	-	6.88 (d, 2.8)	n.o.	n.o.	3.75 (s)
17	3.67 (t, 6.9)	2.28 (m)	2.28 (m)	4.95 (dd, 8.0, 5.3)	6.78 (d, 8.6)	6.72 (dd, 8.6, 2.9)	-	6.89 (d, 2.9)	n.o.	n.o.	3.75 (s)
18	3.32 (m)	1.99 (m)	1.99 (m)	4.73 (t, 7.0)	6.61 (d, 8.5)	6.89 (dd, 8.5, 2.5)	-	7.06 (d, 2.5)	n.o.	n.o.	-

Note: Chemical shifts (in CD_3OD) are reported in δ (in ppm) relative to CD_3OD; multiplicities and coupling constants (Hz) are given in parentheses. ^{1}H signals for the R_2 substituent: **10**, Me: 2.22 (s); **11**, Ph: C4: 7.73 (m); C2, C6: 7.71 (d, 7.8); C3, C5: 7.60 (t, 7.8); **12**, *p*-ClPh: C2, C6: 7.70 (dt, 8.7, 2.1); C3, C5: 7.64 (dt, 8.7, 2.4); **13**, *p*-CF_3Ph: C2, C3, C5, C6: 7.91 (m); **14**, Me: 2.19 (s); **15**, Ph: C2, C4, C6: 7.74 (m); C3, C5: 7.62 (t, 7.8); **16**, *p*-ClPh: C2, C6: 7.74 (d, 8.6): C3, C5: 7.65 (d, 8.6); **17**, *p*-CF_3Ph: C2, C3, C5, C6: 7.94 (m); **18**, Me: 2.14 (s).
Abbreviations: d, doublet; dd, double doublet; m, multiplet; n.o., not observable; s, singlet; t, triplet; td, triple doublet.

TABLE 4 ^{1}H NMR signal assignments of compounds **19–22**

Compound	H-1′	H-2′	H-3′	H-2″	H-4″	H-5″	H-6″	H-1, H-3	OH
19	3.38 (m)	1.97 (m)	4.78 (dd, 8.6, 4.6)	8.51 (d, 2.0)	7.83 (dt, 8.0, 2.0)	7.32 (ddd, 8.0, 4.9, 0.9)	8.40 (dd, 4.9, 2.0)	n.o.	n.o.
20	3.72 (m)	2.21 (m)	5.00 (dd, 9.1, 4.1)	8.65 (d, 2.0)	7.98 (dt, 7.9, 2.0)	7.48 (ddd, 7.9, 4.9. 0.9)	8.48 (dd, 4.9, 2.0)	n.o.	n.o.
21	3.68 (m)	2.18 (m)	4.98 (dd, 9.1, 4.0)	8.61 (d, 2.2)	7.94 (dddd, 7.9, 2.2, 1.6. 0.6)	7.42 (ddd, 8.0, 4.9. 0.6)	8.41 (dd, 4.9, 1.6)	n.o.	n.o.
22	3.74 (m)	2.23 (m)	5.00 (dd, 9.0, 4.1)	8.65 (d, 2.2)	7.99 (m)	7.49 (ddd, 7.8, 4.9, 0.8)	8.49 (dd, 4.9, 1.6)	n.o.	n.o.

Note: Chemical shifts (in CD_3OD) are reported in δ (in ppm) relative to CD_3OD; multiplicities and coupling constants (Hz) are given in parentheses. ^{1}H signals for the R_2 substituent: **19**, Me: 2.13 (s); **20**, Ph: C2, C6: 7.79 (m); C4: 7.75 (tt, 7.5, 7.5, 1.3, 1.3); C3, C5: 7.63 (m); **21**, *p*-ClPh: C2, C6: 7.77 (d, 8.9); C3, C5: 7.59 (d, 8.9); **22**, *p*-CF_3Ph: C2, C3, C5, C6: 7.97 (q, 8.2).
Abbreviations: d, doublet; dd, double doublet; ddd, double double doublet; dddd, double double double doublet; dt, double triplet; m, multiplet; n.o., not observable; s, singlet; tt, triple triplet; q, quadruplet.

TABLE 5 ^{13}C NMR chemical shifts of compounds **1–9**

Compound	C-2	C-1′	C-2′	C-3′	C-1″	C-2″	C-3″	C-4″	C-5″	C-6″	OCH_3
1	166.08	40.75	37.29	67.34	148.74	141.51	125.22	134.70	129.40[b]	129.12[b]	-
2[a]	163.17	36.02	30.70	65.40	147.36	140.19	123.89	133.49	128.34[b]	128.07[b]	-
3	165.01	41.50	37.29	67.50	148.81	140.74	125.30	134.81	129.50[b]	129.19[b]	-
4[a]	162.20	40.02	35.94	65.45	147.36	140.19	123.92	133.52	128.37[b]	128.22[b]	-
5	166.14	40.71	37.29	67.68	145.66	141.24	128.65	114.16	165.50	113.65	56.14
6	165.84	41.43	37.24	67.80	145.67	141.13	130.57	114.21	165.51	113.70	56.13
7	165.58	41.50	37.22	67.79	145.74	141.21	128.73	114.21	165.02	113.71	56.13
8	165.66	41.60	37.20	67.80	145.80	141.29	128.78	114.20	165.07	113.74	56.13
9	166.20	40.55	37.15	67.24	146.99	144.35	127.46	129.18	141.08	129.51	-

Note: [b]^{13}C signals that can be exchanged. δ (ppm) relative to CD_3OD.^{13}C signals for the R_2 substituent: **1**, Me: 19.08; **2**, Ph: C1: 133.22; C4: 129.14; C2, C6: 128.88; C3, C5: 128.15; **3**, *p*-ClPh: C4: 141.67; C2, C6: 130.83, 130.80; C3, C5: 130.58; C1: 129.23; **4**, *p*-CF_3Ph: C1: 133.14 (d, 1.0); C4: 132.72 (q, 32.5); C2, C6: 129.40; C3, C5: 125.77 (q, 3.7); **5**, Me: 19.07; **6**, Ph: C1: 134.61; C2, C6: 130.36; C3, C5: 128.96; C4: 128.70; **7**, *p*-ClPh: C4: 140.76; C2, C6: 130.78; C3, C5: 130.57; C1: 129.27; **8**, *p*-CF_3Ph: C4: 135.75 (q, 33.0); C1: 134.56 (d, 1.2); C2, C6: 130.09; C3, C5: 127,28 (q, 3.8); **9**, Me: 19.04. ^{13}C signals for the CF_3 substituent: **4**: 123.56 (q, 272.8); **8**:124.92 (q, 272.1).
[a]Solvent used DMSO-d_6.

TABLE 6 ^{13}C NMR chemical shifts of compounds **10–18**

Compound	C-2	C-1′	C-2′	C-3′	C-1″	C-2″	C-3″	C-4″	C-5″	C-6″	OCH_3
10	166.02	40.94	34.49	70.82	128.01	146.12	118.07	129.25	119.27	129.12	-
11	165.79	41.79	34.36	71.44	128.61	146.19	118.14	129.34	119.36	128.07	-
12	164.89	41.84	34.24	71.53	129.03	146.34	118.11	129.38	119.30	128.09	-
13	164.70	41.97	34.29	71.32	129.13	146.14	118.13	129.32	119.36	128.08	-
14	166.07	40.83	35.01	70.57	132.46	136.55	120.65	114.80	155.42	113.89	56.14
15	165.71	41.71	34.71	70.80	131.41	138.64	119.78	114.81	154.55	113.73	56.13
16	164.75	41.79	34.64	70.74	131.27	138.87	119.67	114.80	154.44	113.73	56.13
17	164.73	41.89	34.61	70.81	131.40	138.67	119.79	114.81	154.58	113.80	56.13
18	166.04	40.82	34.38	69.82	130.81	145.08	118.90	128.75	123.30	127.46	-

Note: δ (ppm) relative to CD_3OD.^{13}C signals for the R_2 substituent: **10**, Me: 18.99; **11**, Ph: C4: 134.62; C2, C6: 130.38; C1: 129.14; C3, C5: 128.90; **12**, *p*-ClPh: C4: 140.82; C2, C6: 130.69; C3, C5: 130.56; C1: 129.24; **13**, *p*-CF_3Ph: C4: 135.66 (q, 33.4); C1: 134.33 (d, 1.4); C2, C6: 130.07; C3, C5: 127.19 (q, 3.8); **14**, Me: 18.95; **15**, Ph: C4: 134.61; C1: 130.56; C2, C6: 130.36; C3, C5: 128.90; **16**, *p*-ClPh: C4: 140.74, C2, C6: 130.74, C3, C5: 130.53, C1: 129.14; **17**, *p*-CF_3Ph: C4: 135.70 (q, 33.0); C1: 134.37; C2, C6: 130.08; C3, C5: 127.21 (q, 3.7); **18**, Me: 19.03. ^{13}C signals for the CF_3 substituent: **13**: 124.88 (q, 271.6); **17**:124.90 (q, 272.4).

TABLE 7 ^{13}C NMR chemical shifts of compounds **19–22**

Compound	C-2	C-1′	C-2′	C-3′	C-2″	C-3″	C-4″	C-5″	C-6″
19	163.79	38.89	36.80	67.28	147.46	140.92	133.35	123.30	148.04
20	165.86	41.54	37.43	69.99	148.00	142.18	135.90	125.26	149.06
21	164.70	41.64	37.34	69.80	147.92	142.06	135.92	125.21	148.90
22	164.93	41.73	37.36	69.96	148.03	142.21	135.92	125.29	149.12

Note: δ (ppm) relative to CD_3OD.^{13}C signals for the R_2 substituent: **19**, Me: 18.47; **20**, Ph: C4: 134.66; C1: 130.50; C2, C6: 130.39; C3, C5: 128.95; **21**, *p*-ClPh: C4: 140.63; C2, C6: 130.84; C3, C5: 130.49; C1: 128.85; **22**, *p*-CF_3Ph: C4: 135.74 (q, 33.0); C1: 134.33; C2, C6: 130.14; C3, C5: 127.26 (q, 3.8). ^{13}C signals for the CF_3 substituent: **22**: 124.90 (q, 272.1).

Lastly, the quaternary carbon signals of pyridine derivative **21** confirmed by HMBC spectrum show correlations between H-1′ (δ 3.68), H-2, and H-6 (δ 7.77 ppm) of the *p*-ClPh and with C-2 (δ 164.70 ppm). Another connection between H-3′ (δ 4.98 ppm) and H-5″ (δ 7.42 ppm) and the signal at 142.06 ppm allowed us to assign this peak to C-3″. Similarly, the correspondence within the peaks H-3 and H-5 (δ 7.59 ppm) of the *p*-ClPh

TABLE 8 HSQC correlations found for compounds **1**, **5**, **13**, **16,** and **21**

$^{1}H/^{13}C$	1	5	13	16	21
H-1′	3.51	3.55	3.64	3.65	3.68
C-1′	40.75	40.71	41.97	41.79	41.64
H-2′	2.13, 1.89	2.14, 1.83	2.31, 2.24	2.27	2.18
C-2′	37.29	37.29	34.29	34.64	37.34
H-3′	5.24	5.42	4.93	4.95	4.98
C-3′	67.34	67.68	71.32	70.74	69.80
H-2″	-	-	-	-	8.61
C-2″	-	-	-	-	147.92
H-3″	7.90	8.08	6.76	6.77	-
C-3″	125.22	128.24	118.13	119.67	-
H-4″	7.71	7.00	7.04	6.71	7.94
C-4″	134.70	114.16	129.32	114.80	135.92
H-5″	7.48	-	6.71	-	7.42
C-5″	129.40	-	119.36	-	125.21
H-6″	7.90	7.44	7.19	6.88	8.41
C-6″	129.12	113.65	128.08	113.73	148.90

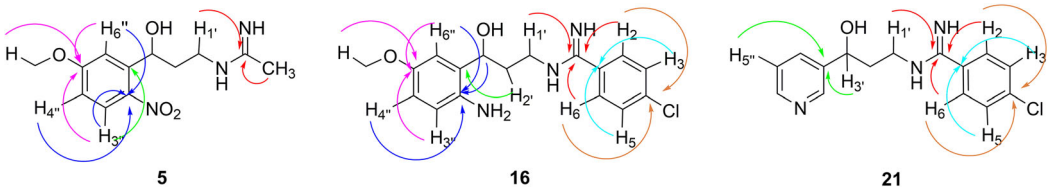


FIGURE 1 Selected $^{1}H/^{13}C$ HMBC correlations in **5**, **16**, and **21**

ring and the signal at 128.85 ppm allowed identifying this peak as C-1 of this cycle. Finally, the relationship between H-2 and H-6 (δ 7.77 ppm) was correlated with C-4 (140.63 ppm) of the same cycle.

The 2d-NOESY experiment performed on derivative **18** ($R_1 = Me$, $R_2 = Cl$) showed some NOE effects between the aromatic proton H-6″ and H-3′ of the lineal chain, and vice versa. Furthermore, other NOE effect was observed between H-6″ and H-2′. These NOE effects are compatible with the presence of an intramolecular hydrogen bond between the amino group in C-2″ of the aromatic ring and the hydroxyl group in the 3′ position of the side chain (Figure 2).

In order to complete the spectroscopic information of these compounds, an intensive study of derivative **18** was carried out, using DMSO-d_6 to fix the labile protons bound to O and N. Two-dimensional $^{1}H/^{15}N$ correlation experiments were carried out to unequivocally assign all ^{1}H and ^{15}N signals. The $^{1}H/^{15}N$ HSQC (Figure 3) allowed us to locate nitrogens N-1 at $\delta = 123.25$ ppm and N-3 at $\delta = 111.50$ ppm. Unfortunately, the nitrogen of the amino group did not show correlation with its protons. In addition, the $^{1}H/^{15}N$ HMBC (Figure 4) showed the correlation of methyl protons ($\delta = 2.14$ ppm) with both nitrogen atoms. Finally, the $^{1}H/^{13}C$ HMBC correlation of compound **18** (Figure 5) allowed, on the one hand, to assign the ^{1}H-NMR signal of the hydroxyl group ($\delta = 5.51$ ppm) due to the couplings with C-2′ ($\delta = 34.38$ ppm), C-3′ ($\delta = 69.82$ ppm) and C-1″ ($\delta = 130.81$ ppm) and, on the other hand, to distinguish between the two geometric isomers (Z/E) of the NH-3 proton, with H-3E being assigned to the signal at 8.72 ppm and H-3Z to the signal at 9.16 ppm, due to the coupling between the methyl group and H-3E but not with H-3Z. Moreover, an additional coupling between the methyl group and H-1 ($\delta = 9.73$ ppm) was also observed.

To design the aminoderivatives **10**–**18**, the 1-(3-(2-amino-5-substitutedphenyl)-3-hydroxypropyl)-3-alkylthioureas and the ureas described previously by our research group [16, 17] were taken as a model, replacing the urea or remaining thiourea with a

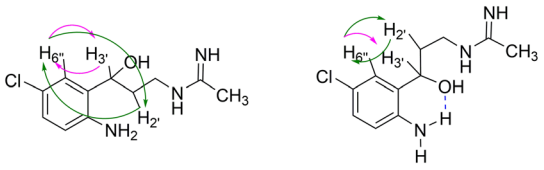


FIGURE 2 Found NOESY correlations for compound **18**

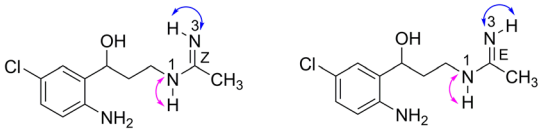


FIGURE 3 $^{1}H/^{15}N$ HSQC correlations of molecule 18, highlighting N-1 (pink) and N-3 (blue)

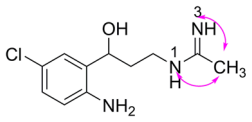


FIGURE 4 $^{1}H/^{15}N$ HMBC correlations of 18 highlighting the CH_3 interactions (pink)

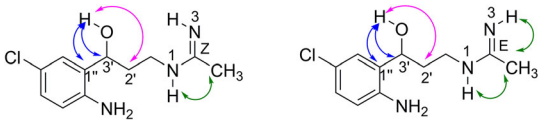


FIGURE 5 HMBC $^{1}H/^{13}C$ correlations of **18**, highlighting the CH_3 (green) and OH (blue and pink) interactions

carboximidamide residue. The most important differences in the ^{13}C signals among the three types of structures are present both in the C-2 of the main function and in the hydroxypropyl chain (C-1′, C-2′, and C-3′). The C-2 atoms of the imidamides show chemical shifts in the range of 166.07–164.70 ppm; thus, it is included between the range of thioureas (182.70–181.17 ppm) and that of ureas (161.53–157.13 ppm). Furthermore, with respect to the C-1′ signals, the imidamides fluctuate in the range of 41.97–40.82 ppm; therefore, they appear in a slightly higher field than thioureas and ureas, whose chemical shifts are 42.21–38.38 ppm and 40.65–36.89 ppm, respectively. Regarding the C-2′ signals, their ranges oscillate from 39.40 to 35.65 ppm in ureas, 37.64 to 34.69 ppm in thioureas, and 35.01 to 34.24 ppm in imidamides, with the latter showing less chemical shifts. Finally, C-3′ is similar in all three series, with the signals being included in the ranges of 74.02–69.22 for ureas, 72.55–69.08 for thioureas, and 71.53–70.57 for imidamides.

Lastly, the H-1′, H-2′, and H-3′ chemical shift differences of the hydroxypropyl chain in the three series of compounds are less significant than those of the corresponding carbons, with H-2′ and H-3′ signals being very similar in the three types of derivatives, and showing greater deviations in the H-1′ signals, which appear in a higher field for the thioureas (4.45–3.36 ppm) followed by the imidamides (3.67–3.32 ppm), and, finally, the ureas with a lower chemical shift (3.38–3.21 ppm).

ACKNOWLEDGMENTS

This work was partially supported by grants from the Vicerrectorado de Política Científica e Investigación de la Universidad de Granada (GREIB.PYR and GENIL/GREIB).

PEER REVIEW

The peer review history for this article is available at https://publons.com/publon/10.1002/mrc.5273.

Fabio Arias
María Encarnación Camacho
María Dora Carrión

Departamento de Química Farmacéutica y Orgánica, Facultad de Farmacia, Universidad de Granada, Granada, Spain

Correspondence
María Encarnación Camacho and María Dora Carrión, Departamento de Química Farmacéutica y Orgánica, Facultad de Farmacia, Universidad de Granada, 18071 Granada, Spain.
Email: dcarrion@ugr.es; ecamacho@ugr.es

María Encarnación Camacho and María Dora Carrión contributed equally.

ORCID

María Encarnación Camacho https://orcid.org/0000-0003-1214-5103
María Dora Carrión https://orcid.org/0000-0002-6794-3949

REFERENCES

[1] D. D. Thomas, A. L, J. S. I. Ridnour, W. Flores-Santana, C. H. Switzer, S. Doncelli, P. Hussain, C. Vecoli, N. Paolocci, S. Ambs, C. A. Colton, C. C. Harris, D. D. Roberts, D. A. Wink, *Free Radical Biol. Med.* **2008**, *45*(1), 18.
[2] L. Zhou, D. Y. Zhu, *Nitric Oxide - Biol. Chem.* **2009**, *20*(4), 223.
[3] R. Rafikov, F. V. Fonseca, S. Kumar, D. Pardo, C. Darragh, S. Elms, D. Fulton, S. M. Black, *J. Endocrinol.* **2011**, *210*(3), 271.

[4] J. S. Krumenacker, K. A. Hanafy, F. Murad, *Brain Res. Bull.* **2004**, *62*, 505.
[5] A. Conti, M. Miscusi, S. Cardali, A. Germano, H. Suzuki, S. Cuzzocrea, F. Tomasello, *Brain Res. Rev.* **2007**, *54*(1), 205.
[6] G. C. Brown, *Nitric Oxide – Biol. Chem* **2010**, *23*(3), 153.
[7] A. Daiber, N. Xia, S. Steven, M. Oelze, A. Hanf, S. Kröller-Schön, T. Münzel, H. Li, *Int. J. Mol. Sci.* **2019**, *20*(1), 187.
[8] P. Jayaraman, F. Parikh, E. Lopez-Rivera, Y. Hailemichael, A. Clark, G. Ma, D. Cannan, M. Ramacher, M. Kato, W. W. Overwijk, S.-H. Chen, V. Y. Umansky, A. G. Sikora, *J. Immunol.* **2012**, *188*, 5365.
[9] F. Aktan, *Life Sci.* **2004**, *75*, 639.
[10] N. P. Whitney, T. M. Fidem, H. Peng, Y. Huang, J. C. Zheng, *J. Neurochem.* **2009**, *108*, 1343.
[11] C. H. Switzer, S. A. Glynn, L. A. Ridnour, R. Y. Cheng, M. P. Vitek, S. Ambs, D. A. Wink, *Trends Pharmacol. Sci.* **2011**, *32*, 644.
[12] S. F. Liu, A. B. Malik, *Am. J. Physiol. Lung. Cell. Mol. Physiol.* **2006**, *290*, L622.
[13] C. Napoli, L. J. Ignarro, *Arch. Pharmacal Res.* **2009**, *32*(8), 1103.
[14] F. Arias, F. Franco-Montalbán, M. Romero, D. Carrión, M. E. Camacho, *Bioorg. Med. Chem.* **2021**, *44*, 116294.
[15] F. Arias, F. Franco-Montalbán, M. Romero, J. Duarte, M. D. Carrión, M. E. Camacho, *Bioorg. Chem.* **2022**, *120*, 105637.
[16] M. Chayah, M. E. Camacho, M. D. Carrión, M. A. Gallo, *Mag. Reson. Chem.* **2016**, *54*, 793.
[17] M. Chayah, M. E. Camacho, M. D. Carrión, M. A. Gallo, M. Romero, J. Duarte, *MedChemComm* **2016**, *7*, 667.

SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.