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*Research Article*

# Thiadiazoline- and Pyrazoline-Based Carboxamides and Carbothioamides: Synthesis and Inhibition against Nitric Oxide Synthase

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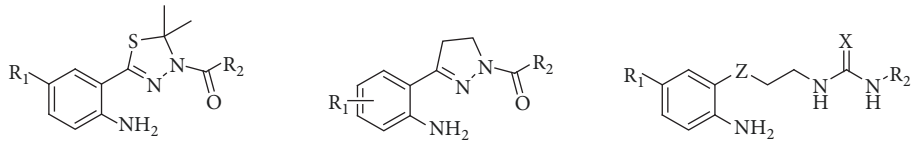
Two new families of pyrazoline and thiadiazoline heterocycles have been developed. Their inhibitory activities against two different isoforms of nitric oxide synthase (inducible and neuronal NOS) are reported. The novel derivatives were synthesized combining the arylthiadiazoline or arylpyrazoline skeleton and a carboxamide or carbothioamide moiety, used as starting material ethyl 2-nitrobenzoates or substituted nitrobenzaldehydes, respectively. The structure-activity relationships of final molecules are discussed in terms of the $R_1$ radical effects in the aromatic ring, the Y atom in the heterocyclic system, the X heteroatom in the main chain, and the $R_2$ substituent in the carboxamide or carbothioamide rest. In general, thiadiazolines **(5a–e)** inhibit preferentially the neuronal isoform; among them, **5a** is the best nNOS inhibitor (74.11% at 1 mM, $IC_{50}$ = 420 $\mu$M). In contrast, pyrazolines **(6a–r)** behave better as iNOS than nNOS inhibitors, **6m** being the best molecule of this series (76.86% at 1 mM of iNOS inhibition, $IC_{50}$ = 130 $\mu$M) and the most potent of all tested compounds.

## 1. Introduction

Heterocyclic rings having two or three heteroatoms in their skeleton have been widely described as part of pharmacological agents with interesting therapeutic applications. In this way, thiadiazoline system is part of structures with antileishmanial activity [1], as well as antimicrobial [2], anti-inflammatory [2, 3], anticonvulsant [4], or antitumoral [5, 6]. In addition, heterocyclic pyrazoline shows a wide spectrum of pharmacological properties such as anticancer [7–9], anti-inflammatory [10], anticonvulsant [11, 12], antimicrobial [13], antibacterial, antifungal, and antiparasitic [14]. These two types of heterocycles have also been recognized as nitric oxide synthase (NOS) inhibitors that could be useful in neurodegenerative diseases and inflammatory arthritis [15–19].

Nowadays, NOS inhibitors represent an important pharmacological group with diverse medical applications since the overproduction of nitric oxide ($NO^{\bullet}$) is involved in several pathological processes.

The enzyme NOS catalyzes the conversion of L-arginine (L-arg) in L-citrulline and $NO^{\bullet}$, an important molecular messenger involved in several physiological actions in mammals, such as neurotransmission [20], immune function [21], or blood flow [22]. Three isoforms of NOS have been identified; two of them are constitutively expressed: neuronal (nNOS), which takes part in neural signaling, and endothelial (eNOS), involved in the systemic blood pressure control and plateled aggregation inhibition; the third isoform, inducible NOS (iNOS), is expressed during immune activation and plays an important role in inflammatory response [23].



**1**

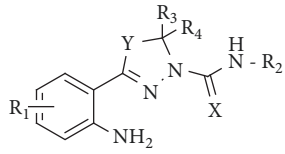
$R_1$ = H, Cl, OMe
$R_2$ = Me, Et, Pr, Bu, *c*-$C_3H_5$, *c*-$C_4H_7$, *c*-$C_5H_9$, *c*-$C_6H_{11}$, Ph

**2**

$R_1$ = H, 5-Cl, 4,5-$Cl_2$, 5-OMe, 4,5-$(OMe)_2$, 2,3,4-$(OMe)_3$
$R_2$ = Me, Et, Pr, *c*-$C_3H_5$, *c*-$C_4H_7$, *c*-$C_5H_9$, *c*-$C_6H_{11}$, Ph, $CH_2Ph$, $CH_2CH_2Ph$

**3**, Z = CO
**4**, Z = CHOH
$R_1$ = H, Cl, OMe
$R_2$ = Me, Et, Pr, Ph
X = O, S

Figure 1: Structure of thiadiazolines **1**, pyrazolines **2**, and *N*,*N*′-disubstituted urea and thiourea derivatives **3**-**4** with NOS inhibitory activity.



**5a–e** (Y = S)
**6a–r** (Y = $CH_2$)

X = O, S
Y = $CH_2$, S
$R_1$ = H, 5-Cl, 5-OMe, 4,5-$(OMe)_2$, 2,3,4-$(OMe)_3$
$R_2$ = Me, Et, Pr, Ph
$R_3$ = $R_4$ = H, Me

Figure 2: General structure of new derivatives **(5a–e)** and **(6a–r)**.

Due to its implication in many biological processes, the endogenous synthesis of NO• is tightly regulated. An overproduction of NO• by iNOS and nNOS is associated with diverse disorders, such as stroke [24], migraine [25], inflammatory arthritis [26], and various neurodegenerative processes like Parkinson's, Alzheimer's, or Huntington's diseases [27–29]. Moreover, NO• produced by stimulation of eNOS plays a physiological role in blood pressure and flow, but its underproduction can cause hypertension [30]. Therefore, the search of selective inhibitors for nNOS or iNOS, but not eNOS, represents an important therapeutic goal since it could help the treatment of diseases in which the first two isoforms are involved.

With this purpose, we have previously described the synthesis and biological evaluation of diverse NOS inhibitors with thiadiazoline **1** [31] and pyrazoline **2** [16, 32] scaffolds, bearing different acyl substituents in the heterocyclic ring. In addition, we have synthesized and evaluated the NOS inhibition of compounds with general structures **3** [33] and **4** [34] (Figure 1). These derivatives had more flexible structure containing *N*,*N*′-disubstituted thiourea and urea rests, isosterics to the terminal guanidine moiety of L-Arg, responsible for the inhibitors binding to the enzyme substrate region; thus it plays an essential role in the enzymatic inhibition [35, 36].

According to this background, two new families of derivatives containing arylthiadiazoline-based carboxamides **5** and arylpyrazoline-based carboxamides and carbothioamides **6** have been designed and synthesized in this study (Figure 2). Compounds **5** proceed by mix of the aryl-thiadiazoline fragment present in **1**, and a residue of carboxamide included in **3** and **4** molecules. Additionally, the derivatives **6** have been designed by combination of an aryl-pyrazoline moiety contained in **2** and a residue of carboxamide or carbothioamide, the terminal rest of the previously described derivatives **3** and **4** (Figure 3). All of them have different substituents in the aromatic ring and in the carboxamide or carbothioamide residues in order to perform structure-activity relationship studies. These compounds could be an interesting starting point for possible new alternatives in neurodegenerative or inflammatory disorders.

## 2. Experimental

### 2.1. Chemistry

*2.1.1. Materials and Methods.* Melting points were determined using an Electrothermal-1A-6301 apparatus and are uncorrected. $^1$H NMR and $^{13}$C NMR spectra were recorded on a Varian Inova Unity 300 spectrometer operating at 300.20 for $^1$H and 75.49 MHz for $^{13}$C, on a Varian Direct Drive 400 spectrometer operating at 400.17 MHz for $^1$H and 100.73 MHz for $^{13}$C, and on a Varian Direct Drive 500 spectrometer operating at 499.79 MHz for $^1$H and 125.69 MHz for $^{13}$C, in $CDCl_3$ (at concentration of ca 27 mg mL$^{-1}$ in all cases). The center of each peak of $CDCl_3$ [7.26 ppm ($^1$H) and 77.0 ppm ($^{13}$C)] was used as internal reference in a 5 mm $^{13}$C/$^1$H dual probe (Wilmad, No. 528-PP). The temperature of the sample was maintained at 297 K. The peaks are reported in ppm ($\delta$). High-resolution mass spectroscopy (HRMS) was carried out on a VG AutoSpec Q high-resolution mass spectrometer (Fision Instruments). Small scale microwave-assisted reactions were performed using an Initiator 2.0 single-mode microwave instrument producing controlled irradiation at 2.450 GHz (Biotage AB, Uppsala). Reaction time refers to holding time at 80°C, not to total irradiation time. An IR sensor outside the reaction vessel was used to control the temperature. Flash chromatography was carried

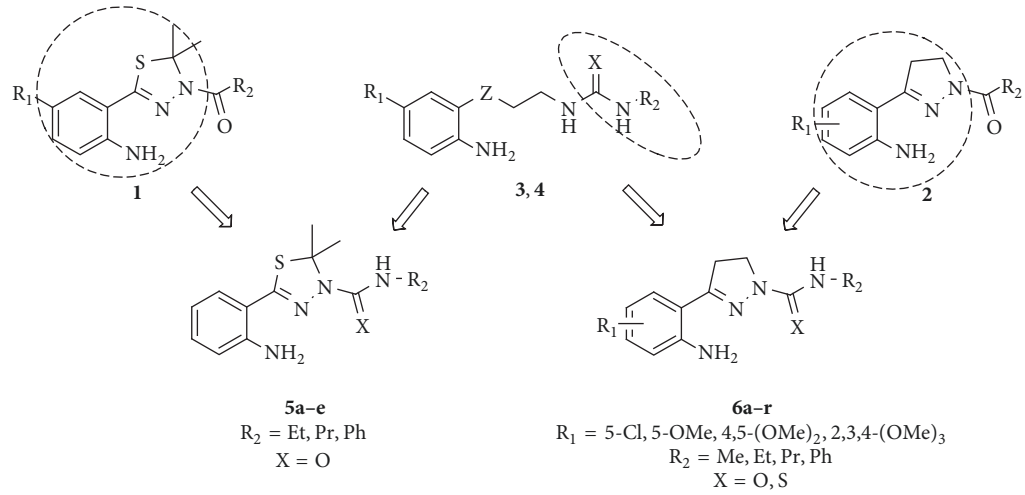



Figure 3: Design strategy and structure of compounds **(5a–e)** and **(6a–r)**.

out using silica gel 60, 230–240 mesh (Merk), and the solvent mixture reported within parentheses was used as eluent.

*2.1.2. General Procedure for the Synthesis of 2,2-Dimethyl-N-substituted-5-(2-nitro-5-phenylsubstituted)-1,3,4-thiadiazole-3(3H)-carboxamide Derivatives (16a–e).* To a solution of the previously described thiadiazole intermediates **14**-**15** (2 mmol) [31] in dry $CH_2Cl_2$, the corresponding isocyanate (3 mmol) and $Et_3N$ (2 mmol) were added under argon [37]. The reaction mixture was irradiated under microwave conditions at 80°C for 20 min. The residue was purified by flash chromatography (EtOAc/hexane, 1 : 10).

*2,2-Dimethyl-N-ethyl-5-(2-nitrophenyl)-1,3,4-thiadiazole-3(2H)-carboxamide **(16a)**.* Yellow oil, yield 407 mg (1.320 mmol) (66%). $^1$H NMR (499.79 MHz, $CDCl_3$): $\delta$/ppm 7.72 (dd, 1H, H-3′, $J_{3'\text{-}4'}$ = 7.9 Hz, $J_{3'\text{-}5'}$ = 1.3 Hz), 7.60 (ddd, 1H, H-5′, $J_{5'\text{-}4'}$ = $J_{5'\text{-}6'}$ = 7.6 Hz, $J_{5'\text{-}3'}$ = 1.3 Hz), 7.55 (ddd, 1H, H-4′, $J_{4'\text{-}3'}$ = $J_{4'\text{-}5'}$ = 7.8 Hz, $J_{4'\text{-}6'}$ = 1.6 Hz), 7.52 (dd, H-6′, $J_{6'\text{-}5'}$ = 7.6 Hz, $J_{6'\text{-}4'}$ = 1.5 Hz), 5.82 (bs, 1H, -CONH-), 3.26 (m, 2H, -C$\underline{H}_2$-$CH_3$), 2.07 (s, 6H, 2x-$CH_3$), 1.16 (t, 3H, -$CH_2$-C$\underline{H}_3$, $J$ = 7.3 Hz). $^{13}$C NMR (125.69 MHz, $CDCl_3$): $\delta$/ppm 154.6 (-CONH-), 148.6 (C-2′), 140.1 (C-5), 132.1 (C-5′), 130.7, 130.6 (C-6′, C-4′), 124.4 (C-1′), 123.9 (C-3′), 82.5 (C-2), 35.1 (-$\underline{C}H_2$-$CH_3$), 29.8 (2x-$CH_3$), 15.3 (-$CH_2$-$\underline{C}H_3$). MS (LSIMS): $m/z$ 331.0830 [M + Na]$^+$, Calcd. Mass for $C_{13}H_{16}N_4O_3NaS$ 331.0841.

*2,2-Dimethyl-5-(2-nitrophenyl)-N-propyl-1,3,4-thiadiazole-3(2H)-carboxamide **(16b)**.* Yellow oil, yield 387 mg (1.200 mmol) (60%). $^1$H NMR (499.79 MHz, $CDCl_3$): $\delta$/ppm 7.69 (dd, 1H, H-3′, $J_{3'\text{-}4'}$ = 7.8 Hz, $J_{3'\text{-}5'}$ = 1.3 Hz), 7.59 (ddd, 1H, H-5′, $J_{5'\text{-}4'}$ = $J_{5'\text{-}6'}$ = 7.6 Hz, $J_{5'\text{-}3'}$ = 1.3 Hz), 7.53 (ddd, 1H, H-4′, $J_{4'\text{-}3'}$ = $J_{4'\text{-}5'}$ = 7.8 Hz, $J_{4'\text{-}6'}$ = 1.6 Hz), 7.49 (dd, H-6′, $J_{6'\text{-}5'}$ = 7.6 Hz, $J_{6'\text{-}4'}$ = 1.5 Hz), 5.88 (bs, 1H, -CONH-), 3.18 (m, 2H, -C$\underline{H}_2$-$CH_2$-$CH_3$), 2.05 (s, 6H, 2x-$CH_3$), 1.53 (m, 2H, -$CH_2$-C$\underline{H}_2$-$CH_3$), 0.93 (t, 3H, -$CH_2$-$CH_2$-C$\underline{H}_3$ $J$ = 7.4 Hz). $^{13}$C NMR (125.69 MHz, $CDCl_3$): $\delta$/ppm 154.7 (-CONH-), 148.6 (C-2′), 139.9 (C-5), 132.0 (C-5′), 130.6 (C-6′, C-4′), 124.2 (C-1′), 123.8 (C-3′), 82.5 (C-2), 42.0 (-$\underline{C}H_2$-$CH_2$-$CH_3$), 29.8 (2x-$CH_3$), 23.3 (-$CH_2$-$\underline{C}H_2$-$CH_3$), 11.4 (-$CH_2$-$CH_2$-$\underline{C}H_3$). MS (LSIMS): $m/z$ 323.1169 [M + H]$^+$, Calcd. Mass for $C_{14}H_{19}N_4O_3S$ 323.1178.

*2,2-Dimethyl-5-(2-nitrophenyl)-N-phenyl-1,3,4-thiadiazole-3(2H)-carboxamide **(16c)**.* Yellow oil, yield 449 mg (1.260 mmol) (63%). $^1$H NMR (499.79 MHz, $CDCl_3$): $\delta$/ppm 7.91 (bs, 1H, -NH-), 7.74 (dd, 1H, H-3′, $J_{3'\text{-}4'}$ = 7.8 Hz, $J_{3'\text{-}5'}$ = 1.3 Hz), 7.64 (ddd, 1H, H-5′, $J_{5'\text{-}4'}$ = $J_{5'\text{-}6'}$ = 7.6 Hz, $J_{5'\text{-}3'}$ = 1.4 Hz), 7.59 (ddd, 1H, H-4′, $J_{4'\text{-}3'}$ = $J_{4'\text{-}5'}$ = 7.8 Hz, $J_{4'\text{-}6'}$ = 1.6 Hz), 7.54 (dd, H-6′, $J_{6'\text{-}5'}$ = 7.6 Hz, $J_{6'\text{-}4'}$ = 1.5 Hz), 7.49 (dd, 2H, H-2″, H-6″, $J_{2''\text{-}3''}$ = $J_{6''\text{-}5''}$ = 8.6 Hz, $J_{2''\text{-}4''}$ = $J_{6''\text{-}4''}$ = 1.1 Hz), 7.31 (m, 2H, H-3″, H-5″), 7.06 (td, 1H, H-4″, $J_{4''\text{-}3''}$ = $J_{4''\text{-}5''}$ = 7.4 Hz, $J_{4''\text{-}2''}$ = $J_{4''\text{-}6''}$ = 1.1 Hz), 2.11 (s, 6H, 2x-$CH_3$). $^{13}$C NMR (125.69 MHz, $CDCl_3$): $\delta$/ppm 151.7 (-CONH-), 148.6 (C-5), 141.0 (C-2′), 138.3 (C-1″), 132.1 (C-6′), 131.0 (C-5′), 130.6 (C-4′), 129.1 (C-3″, C-5″), 123.8 (C-4″), 123.7 (C-1′), 123.4 (C-3′), 119.4 (C-2″, C-6″), 82.4 (C-2), 29.8 (2x-$CH_3$). MS (LSIMS): $m/z$ 379.0837 [M + Na]$^+$, Calcd. Mass for $C_{17}H_{16}N_4O_3NaS$ 379.0841.

*2,2-Dimethyl-N-ethyl-5-(5-methoxy-2-nitrophenyl)-1,3,4-thiadiazole-3(2H)-carboxamide **(16d)**.* White solid, yield 419 mg (1.240 mmol) (62%). Mp: 164–167°C. $^1$H NMR (400.17 MHz, $CDCl_3$): $\delta$/ppm 7.94 (d, 1H, H-3′, $J_{3'\text{-}4'}$ = 8.9 Hz), 7.01 (d, 1H, H-4′, $J$ = 2.7 Hz), 6.99 (s, 1H, H-6′), 5.88 (bs, 1H, -CONH-), 3.92 (s, 3H, -$OCH_3$), 3.32–3.22 (m, 2H, -C$\underline{H}_2$-$CH_3$), 2.10 (s, 6H, 2x-$CH_3$), 1.16 (t, 3H, -$CH_2$-C$\underline{H}_3$, $J$ = 7.2 Hz). $^{13}$C NMR (125.69 MHz, $CDCl_3$): $\delta$/ppm 162.7 (C-5′), 154.8 (-CONH-), 141.7, 141.2 (C-2′, C-5), 127.9 (C-1′), 127.1 (C-3′), 116.2 (C-6′), 115.3 (C-4′), 82.8 (C-2), 56.3 (-$OCH_3$), 35.1 (-$\underline{C}H_2$-$CH_3$), 29.7 (2x-$CH_3$), 15.4 (-$CH_2$-$\underline{C}H_3$). MS (LSIMS): $m/z$ 361.0954 [M + Na]$^+$, Calcd. Mass for $C_{14}H_{18}N_4O_4NaS$ 361.0946.

*2,2-Dimethyl-5-(5-methoxy-2-nitrophenyl)-N-propyl-1,3,4-thiadiazole-3(2H)-carboxamide **(16e)**.* Yelow oil, yield 458 mg (1.300 mmol) (65%). $^1$H NMR (499.79 MHz, $CDCl_3$)

$\delta$/ppm 7.91 (d, 1H, H-3′, $J$ = 9.0 Hz), 7.00 (d, 1H, H-4′, $J_{4'\text{-}6'}$ = 2.8 Hz), 6.98 (d, 1H, H-6′, $J_{6'\text{-}4'}$ = 2.9 Hz), 5.93 (bs, 1H, -CONH-), 3.91 (s, 3H, -$OCH_3$), 3.19 (dd, 2H, -C$\underline{H}_2$-$CH_2$-$CH_3$, $J$ = 13.2 Hz, $J$ = 6.8 Hz), 2.09 (s, 6H, 2x-$CH_3$), 1.54 (dd, 2H, -C$\underline{H}_2$-$CH_3$, $J$ = 14.5 Hz, $J$ = 7.3 Hz), 0.92 (t, 3H, -$CH_2$-C$\underline{H}_3$, $J$ = 7.4 Hz). $^{13}$C NMR (125.69 MHz, $CDCl_3$): $\delta$/ppm 162.6 (C-5′), 154.9 (-CONH-), 141.7, 141.1 (C-2′, C-5), 127.8 (C-1′), 126.9 (C-3′), 116.1 (C-6′), 115.3 (C-4′), 82.7 (C-2), 56.3 (-$OCH_3$), 42.0 (-$\underline{C}H_2$-$CH_2$-$CH_3$), 29.7 (2x-$CH_3$), 23.4 (-$CH_2$-$\underline{C}H_2$-$CH_3$), 11.5 (-$CH_2$-$CH_2$-$\underline{C}H_3$). MS (LSIMS): $m/z$ 353.1309 $[M + H]^+$, Calcd. Mass for $C_{15}H_{21}N_4O_4S$ 353.1284.

*2.1.3. General Procedure for the Synthesis of 3-(2-nitro- or 6-nitrophenyl-substituted)-N-substituted-4,5-dihydro-1H- pyrazole-1-carboxamide or Carbothioamide Derivatives* ***(34a–r)***. Thioisocyanate or isocyanate (3 mmol) was added, under argon, to a solution of previously described pyrazole derivatives**30–33** (2 mmol) [16] in dry $CH_2Cl_2$ and $Et_3N$ (an equimolar amount) [37]. The reaction mixture was irradiated under microwave conditions at 80°C for 20 min. The crude mixture was purified by flash chromatography (EtOAc/hexane, 1 : 1).

*3-(5-Chlorophenyl-2-nitro-)-N-ethyl-4,5-dihydro-1H-pyrazole-1-carboxamide* ***(34a)***. White solid, yield 368 mg (1.240 mmol) (62%). Mp: 123–125°C. $^1$H NMR (400.17 MHz, $CDCl_3$): $\delta$/ppm 7.78 (d, 1H, H-3′, $J_{3'\text{-}4'}$ = 8.6 Hz), 7.51 (m, 2H, H-4′, H-6′), 5.78 (bs, 1H, -CONH-), 4.05 (t, 2H, H-5, $J_{5\text{-}4}$ = 10.2 Hz), 3.34 (m, 2H, -C$\underline{H}_2$-$CH_3$), 3.12 (t, 2H, H-4, $J_{4\text{-}5}$ = 10.2 Hz), 1.19 (t, 3H, -$CH_2$-C$\underline{H}_3$, $J$ = 7.2 Hz). $^{13}$C NMR (125.69 MHz, $CDCl_3$): $\delta$/ppm 154.8 (-CONH-), 148.0 (C-3), 146.7 (C-2′), 138.7 (C-5′), 129.9, 129.8 (C-4′, C-6′), 128.2 (C-1′), 125.5 (C-3′), 45.4 (C-5), 35.0 (-$\underline{C}H_2$-$CH_3$), 33.5 (C-4), 15.5 (-$CH_2$-$\underline{C}H_3$). MS (LSIMS): $m/z$ 297.0757 $[M + H]^+$, Calcd. Mass for $C_{12}H_{14}ClN_4O_3$ 297.0754.

*3-(5-Chlorophenyl-2-nitro)-N-propyl-4,5-dihydro-1H-pyrazole-1-carboxamide* ***(34b)***. White solid, yield 462 mg (1.220 mmol) (61%). Mp: 110–112°C. $^1$H NMR (300.20 MHz, $CDCl_3$): $\delta$/ppm 7.69 (d, 1H, H-3′, $J$ = 8.6 Hz), 7.43 (m, 2H, H-4′, H-6′), 5.76 (bs, 1H, -CONH-), 3.98 (t, 2H, H-5, $J$ = 10.4 Hz), 3.19 (m, 2H, 2H, -C$\underline{H}_2$-$CH_2$-$CH_3$), 3.05 (t, 2H, H-4, $J$ = 10.4 Hz), 1.50 (m, 2H, -$CH_2$-C$\underline{H}_2$-$CH_3$), 0.88 (t, 3H, -$CH_2$-$CH_2$-C$\underline{H}_3$, $J$ = 7.4 Hz). $^{13}$C NMR (125.69 MHz, $CDCl_3$): $\delta$/pp 154.9 (-CO-), 147.9 (C-3), 146.6 (C-2′), 138.7 (C-5′), 129.9, 129.8 (C-4′, C-6′), 128.1 (C-1′), 125.5 (C-3′), 45.4 (C-5), 41.8 (-$\underline{C}H_2$-$CH_2$-$CH_3$), 33.4 (C-4), 23.4 (-$CH_2$-$\underline{C}H_2$-$CH_3$), 11.3 (-$CH_2$-$CH_2$-$\underline{C}H_3$). MS (LSIMS): $m/z$ 311.0917 $[M + H]^+$, Calcd. Mass for $C_{13}H_{16}ClN_4O_3$ 311.0911.

*3-(5-Chlorophenyl-2-nitro)-N-phenyl-4,5-dihydro-1H-pyrazole-1-carboxamide* ***(34c)***. Yellow solid, yield 414 mg (1.200 mmol) (60%). Mp: 119–121°C. $^1$H NMR (400.17 MHz, $CDCl_3$): $\delta$/ppm 7.82 (d, 1H, H-3′, $J_{3'\text{-}4'}$ = 8.7 Hz), 7.53 (m, 3H, H-4′, H-2″, H-6″), 7.29 (m, 3H, H-3″, H-5″, H-6′), 7.05 (t, 1H, H-4″, $J_{4''\text{-}5''}$ = $J_{4''\text{-}3''}$ = 7.3 Hz), 4.14 (t, 2H, H-5, $J_{5\text{-}4}$ = 10.5 Hz), 3.21 (t, 2H, H-4, $J_{4\text{-}5}$ = 10.5 Hz). $^{13}$C NMR (125.69 MHz, $CDCl_3$): $\delta$/ppm 151.8 (-CONH-), 149.1 (C-3), 146.6 (C-2′), 138.9 (C-5′), 138.1 (C-1″), 130.2, 130.1 (C-4′, C-6′), 129.0 (C-3″, C-5″), 127.8 (C-1′), 125.6 (C-4″), 123.2 (C-3′), 119.1 (C-2″, C-6″), 45.1 (C-5), 33.9 (C-4). MS (LSIMS): $m/z$ 367.0569 $[M + Na]^+$, Calcd. Mass for $C_{16}H_{13}ClN_4O_3Na$ 367.0574.

*3-N-Ethyl-(5-methoxyphenyl-2-nitro)-4,5-dihydro-1H-pyrazole-1-carboxamide* ***(34d)***. Yellow solid, yield 520 mg (1.780 mmol) (89%). Mp: 128–130°C. $^1$H NMR (499.79 MHz, $CDCl_3$): $\delta$/ppm 8.03 (d, 1H, H-3′, $J_{3'\text{-}4'}$ = 8.9 Hz), 6.99 (dd, 1H, H-4′, $J_{4'\text{-}3'}$ = 8.9, $J_{4'\text{-}6'}$ = 2.7 Hz), 6.97 (d, 1H, H-6′, $J_{6'\text{-}4'}$ = 2.7 Hz), 5.81 (bs, 1H, -CO-) 4.05 (t, 2H, H-5, $J_{5\text{-}4}$ = 10.4 Hz), 3.92 (s, 3H, -$OCH_3$), 3.33 (m, 2H, -C$\underline{H}_2$-$CH_3$), 3.10 (t, 2H, H-4, $J_{4\text{-}5}$ = 10.4 Hz), 1.17 (t, 3H, -$CH_2$-C$\underline{H}_3$, $J$ = 7.2 Hz). $^{13}$C NMR (125.69 MHz, $CDCl_3$): $\delta$/ppm 163.2 (-CONH-), 155.5 (C-3), 151.4 (C-5′), 141.4 (C-2′), 130.5 (C-1′), 127.4 (C-3′), 115.8 (C-6′), 114.7 (C-4′), 56.3 (-$OCH_3$), 45.5 (C-5), 35.1 (-$\underline{C}H_2$-$CH_3$), 34.9 (C-4), 15.7 (-$CH_2$-$\underline{C}H_3$). MS (LSIMS): $m/z$ 293.1232 $[M + H]^+$, Calcd. Mass for $C_{13}H_{17}N_4O_4$ 293.1250.

*3-(5-Methoxyphenyl-2-nitro)-N-propyl-4,5-dihydro-1H-pyrazole-1-carboxamide* ***(34e)***. Yellow solid, yield 441 mg (1.440 mmol) (72%). Mp: 89–92°C. $^1$H NMR (499.79 MHz, $CDCl_3$): $\delta$/ppm 8.02 (d, 1H, H-3′, $J_{3'\text{-}4'}$ = 8.9 Hz), 6.99 (dd, 1H, H-4′, $J_{4'\text{-}3'}$ = 9.0, $J_{4'\text{-}6'}$ = 2.7 Hz), 6.97 (d, 1H, H-6′, $J_{6'\text{-}4'}$ = 2.7 Hz), 5.86 (bs, 1H, -CO-), 4.05 (t, 2H, $J_{5\text{-}4}$ = 10.4 Hz), 3.92 (s, 3H, -$OCH_3$), 3.25 (m, 2H, -C$\underline{H}_2$-$CH_2$-$CH_3$), 3.11 (t, 2H, H-4, $J_{4\text{-}5}$ = 10.4 Hz), 1.55 (m, 2H, -$CH_2$-C$\underline{H}_2$-$CH_3$), 0.93 (t, 3H, -$CH_2$-$CH_2$-C$\underline{H}_3$, $J$ = 7.4 Hz). $^{13}$C NMR (125.69 MHz, $CDCl_3$): $\delta$/ppm 163.2 (-CONH-), 155.5 (C-3), 151.4 (C-5′), 141.4 (C-2′), 130.4 (C-1′), 127.3 (C-3′), 115.8 (C-6′), 114.7 (C-4′), 56.3 (-$OCH_3$), 45.5 (C-5), 42.0 (-$\underline{C}H_2$-$CH_2$-$CH_3$), 34.8 (C-4), 23.6 (-$CH_2$-$\underline{C}H_2$-$CH_3$), 11.5 (-$CH_2$-$CH_2$-$\underline{C}H_3$). MS (LSIMS): $m/z$ 307.1422 $[M + H]^+$, Calcd. Mass for $C_{14}H_{19}N_4O_4$ 307.1406.

*3-(5-Methoxyphenyl-2-nitro)-N-phenyl-4,5-dihydro-1H-pyrazole-1-carboxamide* ***(34f)***. Yellow solid, yield 476 mg (1.400 mmol) (70%). Mp: 150–152°C. $^1$H NMR (400.17 MHz, $CDCl_3$): $\delta$/ppm 8.01 (d, 1H, H-3′, $J_{3'\text{-}4'}$ = 8.8 Hz), 7.80 (s, 1H, -CO-), 7.43 (d, 2H, H-2″, H-6″, $J$ = 7.6 Hz), 7.23 (m, 2H, H-3″, H-5″), 6.97 (m, 3H, H-4′, H-6′, H-4″), 4.08 (t, 2H, H-5, $J_{5\text{-}4}$ = 10.3 Hz), 3.88 (s, 3H, -$OCH_3$), 3.13 (t, 2H, H-4, $J_{4\text{-}5}$ = 10.3 Hz). $^{13}$C NMR (100.73 MHz, $CDCl_3$): $\delta$/ppm 163.3 (-CONH-), 152.6 (C-3), 152.3 (C-5′), 141.3 (C-2′), 138.5 (C-1″), 130.1 (C-1′), 129.1 (C-2″, C-6″), 127.5 (C-3′), 123.1 (C-4″), 119.1 (C-3″, C-5″), 116.0 (C-6′), 114.9 (C-4′), 56.3 (-$OCH_3$), 45.2 (C-5), 35.2 (C-4). MS (LSIMS): $m/z$ 341.1249 $[M + H]^+$, Calcd. Mass for $C_{17}H_{17}N_4O_4$ 341.1250.

*3-(4,5-Dimethoxy-2-nitrophenyl)-N-ethyl-4,5-dihydro-1H-pyrazole-1-carboxamide* ***(34g)***. Brown solid, yield 464 mg (1.440 mmol) (72%). Mp: 146-147°C. $^1$H NMR (499.79 MHz, $CDCl_3$): $\delta$/ppm 7.57 (s, 1H, H-3′), 6.91 (s, 1H, H-6′), 5.82 (m, 1H, -NH-), 4.04 (t, 2H, H-5, $J$ = 10.3 Hz), 4.00 (s, 3H, 5′-$OCH_3$), 3.97 (s, 3H, 4′-$OCH_3$), 3.33 (m, 2H, -C$\underline{H}_2$-$CH_3$), 3.07 (t, 2H, H-4, $J$ = 10.3 Hz), 1.17 (t, 3H, -$CH_2$-C$\underline{H}_3$, $J$ = 7.2 Hz). $^{13}$C NMR (125.69 MHz, $CDCl_3$): $\delta$/ppm 155.44

(-CONH-), 153.1 (C-3); 152.1 (C-5′), 149.7 (C-4′), 141.1 (C-2′), 122.4 (C-1′), 112.0 (C-6′), 108.0 (C-3′), 56.8 (5′-$OCH_3$), 56.7 (4′-$OCH_3$), 45.5 (C-5), 35.1 (C-4), 34.9 (-$\underline{C}H_2$-$CH_3$), 15.7 (-$CH_2$-$\underline{C}H_3$). MS (LSIMS): $m/z$ 323.1348 $[M + H]^+$, Calcd. Mass for $C_{14}H_{19}N_4O_5$ 323.1355.

*3-(4,5-Dimethoxy-2-nitrophenyl)-N-propyl-4,5-dihydro-1H-pyrazole-1-carboxamide* ***(34h)***. White solid, yield 464 mg (1.380 mmol) (69%). Mp: 144-145°C. $^1$H NMR (499.79 MHz, $CDCl_3$): $\delta$/ppm 7.39 (s, 1H, H-3′), 6.80 (s, 1H, H-6′), 5.81 (m, 1H, -NH-), 3.87 (t, 2H, H-5, $J$ = 10.3 Hz), 3.86 (s, 3H, 5′-$OCH_3$), 3.82 (s, 3H, 4′-$OCH_3$), 3.08 (m, 2H, -C$\underline{H}_2$-$CH_2$-$CH_3$), 2.93 (t, 2H, H-4, $J$ = 10.3 Hz), 1.40 (m, 2H, -$CH_2$-C$\underline{H}_2$-$CH_3$), 0.78 (t, 3H, -$CH_2$-$CH_2$-C$\underline{H}_3$, $J$ = 7.4 Hz). $^{13}$C NMR (125.69 MHz, $CDCl_3$): $\delta$/ppm 155.2 (-CONH-), 152.6 (C-3), 151.6 (C-5′), 149.3 (C-4′), 140.7 (C-2′), 121.8 (C-1′), 111.7 (C-6′), 107.5 (C-3′), 56.4 (5′-$OCH_3$), 56.3 (4′-$OCH_3$), 45.1 (C-5), 41.6 (-$\underline{C}H_2$-$CH_2$-$CH_3$), 34.4 (C-4), 23.3 (-$CH_2$-$\underline{C}H_2$-$CH_3$), 11.1 (-$CH_2$-$CH_2$-$\underline{C}H_3$). MS (LSIMS): $m/z$ 337.1527 $[M + H]^+$, Calcd. Mass for $C_{15}H_{21}N_4O_5$ 337.1512.

*3-(4,5-Dimethoxy-2-nitrophenyl)-N-methyl-4,5-dihydro-1H-pyrazole-1-carbothioamide* ***(34i)***. Brown solid, yield 441 mg (1.360 mmol) (68%). Mp: 179–181°C. $^1$H NMR (499.79 MHz, $CDCl_3$): $\delta$/ppm 7.59 (s, 1H, H-3′), 7.17 (bs, 1H, -NH-), 6.89 (s, 1H, H-6′), 4.40 (t, 2H, H-5, $J$ = 10.0 Hz), 3.99 (s, 3H, 5′-$OCH_3$), 3.98 (s, 3H, 4′-$OCH_3$), 3.16 (d, 3H, -$CH_3$, $J$ = 4.8 Hz), 3.13 (t, 2H, H-4, $J$ = 10.0 Hz). $^{13}$C NMR (125.69 MHz, $CDCl_3$): $\delta$/ppm 177.50 (-CSNH-), 155.8 (C-3), 153.1 (C-5′), 150.0 (C-4′), 141.0 (C-2′), 121.6 (C-1′), 111.8 (C-6′), 108.0 (C-3′), 56.8 (5′-$OCH_3$), 56.6 (4′-$OCH_3$), 49.2 (C-5), 34.7 (C-4), 31.6 (-$CH_3$). MS (LSIMS): $m/z$ 347.0800 $[M + Na]^+$, Calcd. Mass for $C_{13}H_{16}N_4O_4NaS$ 347.0790.

*3-(4,5-Dimethoxy-2-nitrophenyl)-N-ethyl-4,5-dihydro-1H-pyrazole-1-carbothioamide* ***(34j)***. Yellow solid, yield 474 mg (1.400 mmol) (70%). Mp: 194-195°C. $^1$H NMR (499.79 MHz, $CDCl_3$): $\delta$/ppm 7.60 (s, 1H, H-3′), 7.12 (m, 1H, -NH-), 6.89 (s, 1H, H-6′), 4.40 (t, 2H, H-5, $J$ = 10.2 Hz), 4.00 (s, 3H, 5′-$OCH_3$), 3.98 (s, 3H, 4′-$OCH_3$), 3.69 (m, 2H, -C$\underline{H}_2$-$CH_3$), 3.13 (t, 2H, H-4, $J$ = 10.2 Hz), 1.24 (t, 3H, -$CH_2$-C$\underline{H}_3$, $J$ = 7.2 Hz). $^{13}$C NMR (125.69 MHz, $CDCl_3$): $\delta$/ppm 176.4 (-CSNH-), 155.8 (C-3), 153.2 (C-5′), 150.0 (C-4′), 141.0 (C-2′), 121.7 (C-1′), 111.9 (C-6′), 108.0 (C-3′), 56.9 (5′-$OCH_3$), 56.7 (4′-$OCH_3$), 49.1 (C-5), 39.7 (-$\underline{C}H_2$-$CH_3$), 34.7 (C-4), 14.7 (-$CH_2$-$\underline{C}H_3$). MS (LSIMS): $m/z$ 339.1141 $[M + H]^+$, Calcd. Mass for $C_{14}H_{19}N_4O_4S$ 339.1127.

*3-(4,5-Dimethoxy-2-nitrophenyl)-N-propyl-4,5-dihydro-1H-pyrazole-1-carbothioamide* ***(34k)***. Yellow solid, yield 458 mg (1.300 mmol) (65%). Mp: 155-156°C. $^1$H NMR (499.79 MHz, $CDCl_3$): $\delta$/ppm 7.54 (s, 1H, H-3′), 7.16 (m, 1H, -NH-), 6.88 (s, 1H, H-6′), 4.35 (t, 2H, H-5, $J$ = 10.0 Hz), 3.97 (s, 3H, 5′-$OCH_3$), 3.95 (s, 3H, 4′-$OCH_3$), 3.55 (m, 2H, -C$\underline{H}_2$-$CH_2$-$CH_3$), 3.10 (t, 2H, H-4, $J$ = 10.0 Hz), 1.61 (m, 2H, -C$\underline{H}_2$-$CH_3$), 0.92 (t, 3H, $CH_3$, $J$ = 7.4 Hz). $^{13}$C NMR (125.69 MHz, $CDCl_3$): $\delta$/ppm 176.3 (-CSNH-), 155.5 (C-3), 153.0 (C-5′), 149.9 (C-4′), 140.9 (C-2′), 121.4 (C-1′), 111.8 (C-6′), 107.8 (C-3′), 56.7 (5′-$OCH_3$), 56.6 (4′-$OCH_3$), 48.9 (C-5), 46.5 (-$\underline{C}H_2$-$CH_2$-$CH_3$), 34.5 (C-4), 22.5 (-$CH_2$-$\underline{C}H_2CH_3$), 11.4 (-$CH_2$-$CH_2$-$\underline{C}H_3$). MS (LSIMS): $m/z$ 353.1274 $[M + H]^+$, Calcd. Mass for $C_{15}H_{21}N_4O_4S$ 353.1284.

*N-Ethyl-3-(6-nitro-2,3,4-trimethoxyphenyl)-4,5-dihydro-1H-pyrazole-1-carboxamide,* ***(34l)***. Yellow solid, yield 472 mg (1.340 mmol) (67%). Mp: 126–128°C. $^1$H NMR (499.79 MHz, $CDCl_3$): $\delta$/ppm 7.40 (s, 1H, H-5′), 5.69 (bs, 1H, -CO-), 4.05 (t, 2H, H-5, $J$ = 10.3 Hz), 3.98, 3.96, 3.89 (3s, 9H, 2′-$OCH_3$, 3′-$OCH_3$, 4′-$OCH_3$), 3.30 (m, 2H, -C$\underline{H}_2$-$CH_3$), 3.18 (t, 2H, H-4, $J$ = 10.3 Hz), 1.14 (t, 3H, -$CH_2$-C$\underline{H}_3$, $J$ = 7.2 Hz). $^{13}$C NMR (125.69 MHz, $CDCl_3$): $\delta$/ppm 155.25 (C-3), 153.7 (-CONH-), 152.4 (C-4′), 149.1 (C-3′), 146.5 (C-6′), 143.6 (C-2′), 116.6 (C-1′), 104.1 (C-5′), 62.0, 61.0 (2′-$OCH_3$, 3′-$OCH_3$), 56.5 (4′-$OCH_3$), 45.1 (C-5), 36.4 (-$\underline{C}H_2$-$CH_3$), 34.7 (C-4), 15.3 (-$CH_2$-$\underline{C}H_3$). MS (LSIMS): $m/z$ 353.1456 $[M + H]^+$, Calcd. Mass for $C_{15}H_{21}N_4O_6$ 353.1461.

*3-(6-Nitro-2,3,4-trimethoxyphenyl)-N-propyl-4,5-dihydro-1H-pyrazole-1-carboxamide* ***(34m)***. Yellow solid, yield 506 mg (1.380 mmol) (69%). Mp: 132–135°C. $^1$H NMR (400.17 MHz, $CDCl_3$): $\delta$/ppm 7.39 (s, 1H, H-5′), 5.74 (bs, 1H, -CO-), 4.03 (t, H-5, $J$ = 10.3 Hz), 3.96, 3.95, 3.88 (3s, 9H, 2′-$OCH_3$, 3′-$OCH_3$, 4′-$OCH_3$), 3.20 (t, H-4, $J$ = 10.3 Hz), 3.11 (m, 2H, -C$\underline{H}_2$-$CH_2$-$CH_3$), 1.50 (m, 2H, -$CH_2$-C$\underline{H}_2$-$CH_3$), 0.91 (t, 3H, -$CH_2$-$CH_2$-C$\underline{H}_3$, $J$ = 7.4 Hz). $^{13}$C NMR (125.69 MHz, $CDCl_3$): $\delta$/ppm 155.9 (C-3), 153.6 (-CONH-), 152.3 (C-4′), 149.4 (C-3′), 146.5 (C-6′), 143.6 (C-2′), 116.4 (C-1′), 104.1 (C-5′), 62.1, 61.1 (2′-$OCH_3$, 3′-$OCH_3$), 56.8 (4′-$OCH_3$), 44.9 (C-5), 41.8 (-$\underline{C}H_2$-$CH_2$-$CH_3$), 36.2 (C-4); 23.2 (-$CH_2$-$\underline{C}H_2$-$CH_3$), 11.2 (-$CH_2$-$CH_2$-$\underline{C}H_3$). MS (LSIMS): $m/z$ 389.1436 $[M + Na]^+$, Calcd. Mass for $C_{16}H_{22}N_4O_6Na$ 389.1437.

*3-(6-Nitro-2,3,4-trimethoxyphenyl)-N-phenyl-4,5-dihydro-1H-pyrazole-1-carboxamide* ***(34n)***. Yellow solid, yield 536 mg (1.340 mmol) (67%). Mp: 140–142°C. $^1$H NMR (400.17 MHz, $CDCl_3$): $\delta$/ppm 7.73 (bs, 1H, -CONH-), 7.44 (d, 2H, H-2″, H-6″, $J$ = 7.7 Hz), 7.42 (s, 1H, H-5′), 7.26 (t, 2H, H-3″, H-5″, $J$ = 7.9 Hz), 6.99 (t, 1H, H-4″, $J$ = 7.4 Hz), 4.12 (t, 2H, H-5, $J_{5\text{-}4}$ = 10.3 Hz), 3.97, 3.96, 3.91 (3s, 9H, 2′-$OCH_3$, 3′-$OCH_3$, 4′-$OCH_3$), 3.25 (t, 2H, H-4, $J_{4\text{-}5}$ = 10.3 Hz). $^{13}$C NMR (100.73 MHz, $CDCl_3$): $\delta$/ppm 153.9 (C-3), 152.3 (-CONH-); 152.2 (C-4′); 150.3 (C-3′); 146.7 (C-6′); 143.5 (C-2′); 138.5 (C-1″); 128.8 (C-3″, C-5″), 122.8 (C-4″), 118.9 (C-2″, C-6″), 116.2 (C-1′), 104.2 (C-5′), 62.1, 61.2 (2′-$OCH_3$, 3′-$OCH_3$), 56.6 (4′-$OCH_3$), 44.6 (C-5), 36.5 (C-4). MS (LSIMS): $m/z$ 401.1464 $[M + H]^+$, Calcd. Mass for $C_{15}H_{21}N_4O_6$ 401.1461.

*N-Methyl-3-(6-nitro-2,3,4-trimethoxyphenyl)-4,5-dihydro-1H-pyrazole-1-carbothioamide* ***(34o)***. Yellow solid, yield 482 mg (1.360 mmol) (68%). Mp: 122–125°C. $^1$H NMR (400.17 MHz, $CDCl_3$): $\delta$/ppm 7.44 (s, 1H, H-5′), 7.04 (s, 1H, -CSNH-), 4.43 (t, 2H, H-5, $J_{5\text{-}4}$ = 9.9 Hz), 3.98, 3.98, 3.90 (3s, 9H, 2′-$OCH_3$, 3′-$OCH_3$, 4′-$OCH_3$), 3.23 (t, 2H, H-4, $J_{4\text{-}5}$ = 9.9 Hz), 3.14 (d, 3H, -$CH_3$, $J$ = 4.7 Hz). $^{13}$C NMR (100.73 MHz, $CDCl_3$): $\delta$/ppm 177.1 (-CSNH-), 154.0 (C-3), 153.6 (C-4′), 152.2 (C-3′), 146.8 (C-6′), 143.2 (C-2′), 115.9 (C-1′), 104.2 (C-5′), 62.2, 61.2

(2′-$OCH_3$, 3′-$OCH_3$), 56.6 (4′-$OCH_3$), 48.7 (C-5), 36.0 (C-4), 31.4 (-$CH_3$). MS (LSIMS): $m/z$ 355.1075 $[M + H]^+$, Calcd. Mass for $C_{14}H_{19}N_4O_5S$ 355.1076.

*N-Ethyl-3-(6-nitro-2,3,4-trimethoxyphenyl)-4,5-dihydro-1H-pyrazole-1-carbothioamide **(34p)***. Yellow solid, yield 472 mg (1.280 mmol) (64%). Mp: 111–113°C. $^1$H NMR (400.17 MHz, $CDCl_3$): $\delta$/ppm 7.40 (bs, 1H, H-5′), 6.94 (s, 1H, -CSNH-), 4.38 (t, 2H, H-5, $J_{5\text{-}4}$ = 9.9 Hz), 3.95, 3.94, 3.86 (3s, 9H, 2′-$OCH_3$, 3′-$OCH_3$, 4′-$OCH_3$), 3.59 (m, 2H, -C$\underline{H}_2$-$CH_3$), 3.18 (t, 2H, H-4, $J_{4\text{-}5}$ = 9.9 Hz), 1.18 (t, 3H, -$CH_2$-C$\underline{H}_3$, $J$ = 7.3 Hz). $^{13}$C NMR (100.73 MHz, $CDCl_3$): $\delta$/ppm 175.92 (-CSNH-), 154.0 (C-3), 153.5 (C-4′), 152.2 (C-3′), 146.8 (C-6′), 143.2 (C-2′), 115.9 (C-1′), 104.2 (C-5′), 62.1, 61.2 (2′-$OCH_3$, 3′-$OCH_3$), 56.6 (4′-$OCH_3$), 48.6 (C-5), 39.5 (-$\underline{C}H_2$-$CH_3$), 35.9 (C-4), 14.5 (-$CH_2$-$\underline{C}H_3$). MS (LSIMS): $m/z$ 369.1234 $[M + H]^+$, Calcd. Mass for $C_{15}H_{21}N_4O_5S$ 369.1233.

*3-(6-Nitro-2,3,4-trimethoxyphenyl)-N-propyl-4,5-dihydro-1H-pyrazole-1-carbothioamide **(34q)***. Yellow solid, yield 505 mg (1.320 mmol) (66%). Mp: 94–96°C. $^1$H NMR (400.17 MHz, $CDCl_3$): $\delta$/ppm 7.40 (bs, 1H, H-5′), 7.02 (s, 1H, -CSNH-), 4.39 (t, 2H, H-5, $J_{5\text{-}4}$ = 10.0 Hz), 3.95, 3.94, 3.86 (3s, 9H, 2′-$OCH_3$, 3′-$OCH_3$, 4′-$OCH_3$), 3.55 (m, 2H, -C$\underline{H}_2$-$CH_2$-$CH_3$), 3.19 (t, 2H, H-4, $J_{4\text{-}5}$ = 10.0 Hz), 1.59 (m, 2H, -$CH_2$-C$\underline{H}_2$-$CH_3$), 0.91 (t, 3H, -$CH_2$-$CH_2$-C$\underline{H}_3$, $J$ = 7.4 Hz). $^{13}$C NMR (100.73 MHz, $CDCl_3$): $\delta$/ppm 176.07 (-CSNH-), 154.0 (C-3), 153.4 (C-4′), 152.2 (C-3′), 146.7 (C-6′), 143.2 (C-2′), 115.8 (C-1′), 104.2 (C-5′), 62.1, 61.2 (2′-$OCH_3$, 3′-$OCH_3$), 56.6 (4′-$OCH_3$), 48.6 (C-5), 46.4 (-$\underline{C}H_2$-$CH_2$-$CH_3$), 35.9 (C-4), 22.4 (-$CH_2$-$\underline{C}H_2$-$CH_3$), 11.3 (-$CH_2$-$CH_2$-$\underline{C}H_3$). MS (LSIMS): $m/z$ 383.1388 $[M + H]^+$, Calcd. Mass for $C_{16}H_{23}N_4O_5S$ 383.13893.

*3-(6-Nitro-2,3,4-trimethoxyphenyl)-N-phenyl-4,5-dihydro-1H-pyrazole-1-carbothioamide **(34r)***. Yellow oil, yield 533 mg (1.280 mmol) (64%). $^1$H NMR (400.17 MHz, $CDCl_3$): $\delta$/ppm 8.80 (bs, 1H, -CSNH-), 7.56 (d, 2H, H-2″, H-6″, $J$ = 7.4 Hz), 7.44 (s, 1H, H-5′), 7.32 (t, 2H, H-3″, H-5″, $J$ = 7.4 Hz), 7.15 (t, 1H, H-4″, $J$ = 7.4 Hz), 4.49 (t, 2H, H-5, $J_{5\text{-}4}$ = 9.9 Hz), 3.97, 3.96, 3.92 (3s, 9H, 2′-$OCH_3$, 3′-$OCH_3$, 4′-$OCH_3$), 3.28 (t, 2H, H-4, $J_{4\text{-}5}$ = 9.9 Hz). $^{13}$C NMR (125.69 MHz, $CDCl_3$): $\delta$/ppm (125.68 MHz, $CDCl_3$), $\delta$ 174.1 (-CSNH-), 154.4 (C-3), 154.2 (C-4′), 152.2 (C-3′), 146.8 (C-6′), 143.4 (C-2′), 138.6 (C-1″), 128.6 (C-3″, C-5″), 125.4 (C-4″), 124.2 (C-2″, C-6″), 115.6 (C-1′), 104.3 (C-5′), 62.2, 61.3 (2′-$OCH_3$, 3′-$OCH_3$), 56.6 (4′-$OCH_3$), 48.6 (C-5), 36.2 (C-4). MS (LSIMS): $m/z$ 417.1229 $[M + H]^+$, Calcd. Mass for $C_{19}H_{21}N_4O_5S$ 417.1246.

*2.1.4. General Procedure for the Synthesis of 5-(2-Amino-5-phenylsubstituted)-2,2-dimethyl-N-substituted-1,3,4-thiadiazole-3(3H)-carboxamide Derivatives **(5a–e)***. To a solution of each nitroarene **16a–e** (0.400 mmol) in ethanol, 453 mg of $SnCl_2$ was added. The resulting mixture was refluxed for 2 h. After this period, the reaction was neutralized with $NaHCO_3$ aqueous solution, extracted with AcOEt (2 × 15 ml), and dried with sodium sulfate [38]. Finally, the solvent was evaporated ad the residue was purified by flash chromatography (EtOAc/hexane, 1 : 10) to give the title derivatives **5a–e**.

*5-(2-Aminophenyl)-2,2-dimethyl-N-ethyl-1,3,4-thiadiazole-3(2H)-carboxamide **(5a)***. Brown oil, yield 94 mg (0.328 mmol) (84%). $^1$H NMR (400.17 MHz, $CDCl_3$): $\delta$/ppm 7.16 (t, 2H, H-6′, H-4′, $J$ = 7.6 Hz), 6.71 (t, 2H, H-5′, H-3′, $J$ = 8.2 Hz), 5.69 (bs, 1H, -CONH-), 4.90 (bs, 2H, -$NH_2$), 3.31 (m, 2H, -C$\underline{H}_2$-$CH_3$), 2.02 (s, 6H, 2x-$CH_3$), 1.16 (t, 3H, -$CH_2$-C$\underline{H}_3$, $J$ = 7.3 Hz). $^{13}$C NMR (100.73 MHz, $CDCl_3$): $\delta$/ppm 154.8 (-CONH-), 147.7 (C-2′), 145.2 (C-5), 131.0, 130.7 (C-6′, C-4′), 117.6, 116.4 (C-5′, C-3′), 113.5 (C-1′), 78.2 (C-2), 35.0 (-$\underline{C}H_2$-$CH_3$), 29.5 (2x-$CH_3$), 15.6 (-$CH_2$-$\underline{C}H_3$). MS (LSIMS): $m/z$ 301.1087 $[M + Na]^+$, Calcd. Mass for $C_{13}H_{18}N_4ONaS$ 301.1099.

*5-(2-Aminophenyl)-2,2-dimethyl-N-propyl-1,3,4-thiadiazole-3(2H)-carboxamide **(5b)***. Brown oil, yield 108 mg (0.372 mmol) (93%). $^1$H NMR (499.79 MHz, $CDCl_3$): $\delta$/ppm 7.17 (m, 2H, H-6′, H-4′), 6.72 (m, 2H, H-5′, H-3′), 5.77 (bs, 1H, -CONH-), 5.16 (bs, 2H, -$NH_2$), 3.24 (m, 2H, -C$\underline{H}_2$-$CH_2$-$CH_3$), 2.03 (s, 6H, 2x-$CH_3$), 1.56 (m, 2H, -$CH_2$-C$\underline{H}_2$-$CH_3$), 0.94 (t, 3H, -$CH_2$-$CH_2$-C$\underline{H}_3$, $J$ = 7.4 Hz). $^{13}$C NMR (125.69 MHz, $CDCl_3$): $\delta$/ppm 154.9 (-CONH-), 147.7 (C-2′), 145.3 (C-5), 131.0, 130.7 (C-4′, C-6′), 117.6, 116.3 (C-5′, C-3′), 113.5 (C-1′), 78.2 (C-2), 42.0 (-$\underline{C}H_2$-$CH_2$-$CH_3$), 29.5 (2x-$CH_3$), 23.5 (-$CH_2$-$\underline{C}H_2$-$CH_3$), 11.5 (-$CH_2$-$CH_2$-$\underline{C}H_3$). MS (LSIMS): $m/z$ 293.1440 $[M + H]^+$, Calcd. Mass for $C_{14}H_{21}N_4OS$ 293.1436.

*5-(2-Aminophenyl)-2,2-dimethyl-N-phenyl-1,3,4-thiadiazole-3(2H)-carboxamide **(5c)***. White solid, yield 114 mg (0.348 mmol) (87%). Mp: 113–116°C. $^1$H NMR (400.17 MHz, $CDCl_3$): $\delta$/ppm 7.79 (s, 1H, -CONH-), 7.49 (d, 2H, H-2″, H-6″, $J_{2''\text{-}3''}$ = $J_{6''\text{-}5''}$ = 8.6 Hz), 7.36 (dd, 2H, H-3″, H-5″, $J_{3''\text{-}2''}$ = $J_{5''\text{-}6''}$ = 8.6 Hz, $J_{3''\text{-}4''}$ = $J_{5''\text{-}4''}$ = 7.4 Hz), 7.27 (m, 2H, H-6′, H-4′), 7.12 (t, H-4″, $J_{4''\text{-}5''}$ = $J_{4''\text{-}3''}$ = 7.4 Hz), 6.80 (m, 2H, H-5′, H-3′), 5.30 (bs, 2H, $NH_2$), 2.15 (s, 6H, 2x-$CH_3$). $^{13}$C NMR (100.73 MHz, $CDCl_3$): $\delta$/ppm 151.9 (-CONH-), 148.9 (C-2′), 145.3 (C-5), 138.2 (C-1″), 131.4, 130.8 (C-4′, C-6′), 129.1 (C-3″, C-5″), 123.6 (C-4″), 119.8 (C-2″, C-6″), 117.8, 116.5 (C-3′,C-5′), 113.3 (C-1′), 78.2 (C-2), 29.6 (2x-$CH_3$). MS (LSIMS): $m/z$ 327.1269 $[M + H]^+$, Calcd. Mass for $C_{17}H_{19}N_4OS$ 327.1280.

*5-(2-Amino-5-methoxyphenyl)-2,2-dimethyl-N-ethyl-1,3,4-thiadiazole-3(2H)-carboxamide **(5d)***. Yelow oil, yield 86 mg (0.280 mmol) (70%). $^1$H NMR (499.79 MHz, $CDCl_3$): $\delta$/ppm 6.82 (dd, 1H, H-4′, $J_{4'\text{-}3'}$ = 8.8 Hz, $J_{4'\text{-}6'}$ = 2.7 Hz), 6.72 (d, 1H, H-6′, $J_{6'\text{-}4'}$ = 2.7 Hz), 6.70 (d, 1H, H-3′, $J_{3'\text{-}4'}$ = 8.8 Hz), 5.74 (bs, 1H, -CONH-), 3.75 (s, 3H, -$OCH_3$), 3.32 (dd, 2H, -C$\underline{H}_2$-$CH_3$, $J$ = 13.1 Hz, $J$ = 7.1 Hz), 2.03 (s, 6H, 2x$CH_3$), 1.17 (t, 3H, -$CH_2$-C$\underline{H}_3$, $J$ = 7.2 Hz). $^{13}$C NMR (125.69 MHz, $CDCl_3$): $\delta$/ppm 154.8 (-CONH-), 151.7 (C-5), 147.2 (C-5′), 139.4 (C-2′), 118.5 (C-4′), 117.9 (C-3′), 114.4 (C-1′), 114.1 (C-6′), 78.5 (C-2), 56.1 (-$OCH_3$), 35.2 (-$\underline{C}H_2$-$CH_3$), 29.6 (2x-$CH_3$), 15.6 (-$CH_2$-$\underline{C}H_3$). MS (LSIMS): $m/z$ 309.1382 $[M + H]^+$, Calcd. Mass for $C_{14}H_{21}N_4O_2S$ 309.1385.

*5-(2-Amino-5-methoxyphenyl)-2,2-dimethyl-N-propyl-1,3,4-thiadiazole-3(2H)-carboxamide **(5e)***. Yelow oil, yield 88 mg

(0.272 mmol) (68%). $^1$H NMR (499.79 MHz, $CDCl_3$): $\delta$/ppm 6.83 (dd, 1H, H-4′, $J_{4'\text{-}3'}$ = 8.8 Hz, $J_{4'\text{-}6'}$ = 2.8 Hz), 6.73 (d, 1H, H-6′, $J_{6'\text{-}4'}$ = 2.8 Hz), 6.70 (d, 1H, H-3′, $J_{3'\text{-}4'}$ = 8.8 Hz), 5.80 (bs, 1H, -CONH-), 3.75 (s, 3H, $-OCH_3$), 3.24 (dd, 2H, $-C\underline{H}_2$-$CH_2$-$CH_3$, $J$ = 13.6 Hz, $J$ = 6.5 Hz), 2.03 (s, 6H, 2x$CH_3$), 1.56 (dd, 2H, $-C\underline{H}_2$-$CH_3$, $J$ = 14.5 Hz, $J$ = 7.3 Hz), 0.94 (t, 3H, $-CH_2$-$C\underline{H}_3$, $J$ = 7.4 Hz). $^{13}$C NMR (125.69 MHz, $CDCl_3$): $\delta$/ppm 155.1 (-CONH-), 152.0 (C-5), 147.4 (C-5′), 139.7 (C-2′), 118.8 (C-4′), 118.2 (C-3′), 114.7 (C-1′), 114.4 (C-6′), 78.8 (C-2), 56.3 ($-OCH_3$), 42.3 ($-\underline{C}H_2$-$CH_2$-$CH_3$), 29.8 (2x-$CH_3$), 23.8 ($-CH_2$-$\underline{C}H_2$-$CH_3$), 11.7 ($-CH_2$-$CH_2$-$\underline{C}H_3$). MS (LSIMS): $m/z$ 323.1563 $[M + H]^+$, Calcd. Mass for $C_{15}H_{23}N_4O_2S$ 323.1542.

*2.1.5. General Procedure for the Synthesis of 3-(2-Amino or 6-aminophenyl-substituted)-N-substituted-4,5-dihydro-1H-pyrazole-1-carboxamide or Carbothioamide Derivatives* ***(6a–r)***. Amino-phenyl pyrazolines **6a–r** were prepared according to the same synthetic procedure described before for the thiazodiazoline derivatives **5a–e**. Purification of the final compounds was made using (EtOAc/hexane, 1 : 2) as eluent in the flash chromatography.

*3-(2-Amino-5-chlorophenyl)-N-ethyl-4,5-dihydro-1H-pyrazole-1-carboxamide* ***(6a)***. White solid, yield 86 mg (0.324 mmol) (81%). Mp: 215–217°C. $^1$H NMR (400.17 MHz, $CDCl_3$): $\delta$/ppm 7.14 (d, 1H, H-6′, $J$ = 2.4 Hz), 7.12 (dd, 1H, H-4′, $J$ = 8.6, $J$ = 2.4 Hz), 6.68 (d, 1H, H-3′, $J$ = 8.6 Hz), 5.54 (bs, 3H, $-NH_2$, -CONH-), 3.96 (t, 2H, H-5, $J$ = 10.2 Hz), 3.37 (m, 2H, $-C\underline{H}_2$-$CH_3$), 3.27 (t, 2H, H-4, $J$ = 10.2 Hz), 1.20 (t, 3H, $-CH_2$-$C\underline{H}_3$, $J$ = 10.2 Hz). $^{13}$C NMR (125.69 MHz, $CDCl_3$): $\delta$/ppm 154.6 (-CONH-), 153.3 (C-3), 144.4 (C-2′), 130.0 (C-4′), 128.3 (C-6′), 121.3 (C-5′), 116.9 (C-3′), 114.9 (C-1′), 43.1 (C-5), 34.8 ($-\underline{C}H_2$-$CH_3$), 33.3 (C-4), 15.5 ($-CH_2$-$\underline{C}H_3$). MS (LSIMS): $m/z$ 267.1021 $[M + H]^+$, Calcd. Mass for $C_{12}H_{16}ClN_4O$ 267.1013.

*3-(2-Amino-5-chlorophenyl)-N-propyl-4,5-dihydro-1H-pyrazole-1-carboxamide* ***(6b)***. White solid, yield 94 mg (0.336 mmol) (84%). Mp: 212–214°C. $^1$H NMR (400.17 MHz, $CDCl_3$): $\delta$/ppm 7.15 (d, 1H, H-6′, $J$ = 2.3 Hz), 7.11 (dd, 1H, H-4′, $J$ = 8.6 Hz, $J$ = 2.3 Hz), 6.68 (d, 1H, H-3′, $J$ = 8.6 Hz), 5.59 (bs, 3H, $-NH_2$, -CONH-), 3.95 (t, 2H, H-5, $J$ = 10.4 Hz), 3.27 (m, 4H, H-4, $-C\underline{H}_2$-$CH_2$-$CH_3$), 1.59 (m, 2H, $-CH_2$-$C\underline{H}_2$-$CH_3$), 0.95 (t, 3H, $-CH_2$-$CH_2$-$C\underline{H}_3$, $J$ = 7.4 Hz). $^{13}$C NMR (125.69 MHz, $CDCl_3$): $\delta$/ppm 154.9 (-CONH-), 153.5 (C-3), 144.6 (C-2′), 130.8 (C-4′), 128.5 (C-6′), 121.6 (C-5′), 117.2 (C-3′), 115.2 (C-1′), 43.4 (C-5), 41.9 ($-\underline{C}H_2$-$CH_2$-$CH_3$), 33.5 (C-4), 23.6 ($-CH_2$-$\underline{C}H_2$-$CH_3$), 11.3 ($-CH_2$-$CH_2$-$\underline{C}H_3$). MS (LSIMS): $m/z$ 281.1163 $[M + H]^+$, Calcd. Mass for $C_{13}H_{18}ClN_4O$ 281.1169.

*3-(2-Amino-5-chlorophenyl)-N-phenyl-4,5-dihydro-1H-pyrazole-1-carboxamide* ***(6c)***. Yellow solid, yield 98 mg (0.312 mmol) (78%). Mp: 112–115°C. $^1$H NMR (400.17 MHz, $CDCl_3$): $\delta$/ppm 7.47 (d, 2H, H-2″, H-6″, $J$ = 7.7 Hz), 7.32 (t, 2H, H-3″, H-5″, $J$ = 7.7 Hz), 7.17 (m, 2H, H-6′, H-4′), 7.07 (t, 1H, H-4″, $J_{4''\text{-}5''}$ = $J_{4''\text{-}3''}$ = 7.7 Hz), 6.72 (d, 1H, H-3′, $J_{3'\text{-}4'}$ = 8.6 Hz), 5.56 (s, 2H, $-NH_2$), 4.04 (t, 2H, H-5, $J_{5\text{-}4}$ = 10.3 Hz), 3.35 (t, 2H, H-4, $J_{4\text{-}5}$ = 10.3 Hz). $^{13}$C NMR (125.69 MHz, $CDCl_3$): $\delta$/ppm 154.5 (-CONH-), 151.7 (C-3), 144.8 (C-2′), 138.2 (C-1″), 130.6 (C-4′), 129.0 (C-3″, C-5″), 128.7 (C-6′), 123.3 (C-4″), 121.7 (C-5′), 119.4 (C-2″, C-6″), 117.4 (C-3′), 114.8 (C-1′), 43.1 (C-5), 33.9 (C-4). MS (LSIMS): $m/z$ 315.1013 $[M + H]^+$, Calcd. Mass for $C_{16}H_{16}ClN_4O$ 315.1013.

*3-(2-Amino-5-methoxyphenyl)-N-ethyl-4,5-dihydro-1H-pyrazole-1-carboxamide* ***(6d)***. White solid, yield 102 mg (0.388 mmol) (97%). Mp: 194-195°C. $^1$H NMR (499.79 MHz, $CDCl_3$): $\delta$/ppm 6.83 (dd, 1H, H-4′, $J_{4'\text{-}3'}$ = 8.8, $J_{4'\text{-}6'}$ = 2.8 Hz), 6.74 (d, 1H, H-6′, $J_{6'\text{-}4'}$ = 2.8 Hz), 6.71 (d, 1H, H-3′, $J_{3'\text{-}4'}$ = 8.8 Hz), 5.62 (bs, 1H, -CONH-), 5.20 (bs, 2H, $-NH_2$), 3.93 (t, 2H, H-5, $J_{5\text{-}4}$ = 10.1 Hz), 3.76 (s, 3H, $-OCH_3$), 3.37 (m, 2H, $-C\underline{H}_2$-$CH_3$), 3.27 (t, 2H, H-4, $J_{4\text{-}5}$ = 10.1 Hz), 1.19 (t, 3H, $-CH_2$-$C\underline{H}_3$, $J$ = 7.2 Hz). $^{13}$C NMR (125.69 MHz, $CDCl_3$): $\delta$/ppm 155.1 (-CONH-), 154.5 (C-3), 151.5 (C-5′), 140.6 (C-2′), 117.4 (C-4′), 117.4 (C-3′), 114.9 (C-1′), 114.2 (C-6′), 56.1 ($-OCH_3$), 43.4 (C-5), 35.2 ($-\underline{C}H_2$-$CH_3$), 33.9 (C-4), 15.9 ($-CH_2$-$\underline{C}H_3$). MS (LSIMS): $m/z$ 263.1490 $[M + H]^+$, Calcd. Mass for $C_{13}H_{19}N_4O_2$ 263.1508.

*3-(2-Amino-5-methoxyphenyl)-N-propyl-4,5-dihydro-1H-pyrazole-1-carboxamide* ***(6e)***. White solid, yield 100 mg (0.364 mmol) (91%). Mp: 187–190°C. $^1$H NMR (499.79 MHz, $CDCl_3$): $\delta$/ppm 6.82 (dd, 1H, H-4′, $J_{4'\text{-}3'}$ = 8.8 Hz, $J_{4'\text{-}6'}$ = 2.8 Hz), 6.74 (d, 1H, H-6′, $J_{6'\text{-}4'}$ = 2.8 Hz), 6.71 (d, 1H, H-3′, $J_{3'\text{-}4'}$ = 8.8 Hz), 5.66 (bs, 1H, -CONH-), 5.22 (bs, 2H, $-NH_2$), 3.93 (t, 2H, H-5, $J_{5\text{-}4}$ = 10.1 Hz), 3.76 (s, 3H, $-OCH_3$), 3.28 (m, 4H, $-C\underline{H}_2$-$CH_2$-$CH_3$, H-4), 1.57 (m, 2H, $-CH_2$-$C\underline{H}_2$-$CH_3$), 0.94 (t, 3H, $-CH_2$-$CH_2$-$C\underline{H}_3$, $J$ = 7.4 Hz). $^{13}$C NMR (125.69 MHz, $CDCl_3$): $\delta$/ppm 155.2 (-CONH-), 154.4 (C-3), 151.5 (C-5′), 140.5 (C-2′), 117.4 (C-4′), 117.3 (C-3′), 115.0 (C-1′), 114.2 (C-6′), 56.1 ($-OCH_3$), 43.5 (C-5), 42.0 ($-\underline{C}H_2$-$CH_2$-$CH_3$), 33.9 (C-4), 23.8 ($-CH_2$-$\underline{C}H_2$-$CH_3$), 11.5 ($-CH_2$-$CH_2$-$\underline{C}H_3$). MS (LSIMS): $m/z$ 277.1675 $[M + H]^+$, Calcd. Mass for $C_{14}H_{21}N_4O_2$ 277.1665.

*3-(2-Amino-5-methoxyphenyl)-N-phenyl-4,5-dihydro-1H-pyrazole-1-carboxamide* ***(6f)***. Yellow solid, yield 120 mg (0.384 mmol) (96%). Mp: 168–170°C. $^1$H NMR (499.79 MHz, $CDCl_3$): $\delta$/ppm 7.64 (s, 1H, -CONH-), 7.49 (dt, 2H, H-2″, H-6″, $J_{6''\text{-}5''}$ = $J_{2''\text{-}3''}$ = 8.1 Hz, $J_{6''\text{-}4''}$ = $J_{2''\text{-}4''}$ = 1.1 Hz), 7.31 (dd, 2H, H-3″, H-5″, $J_{3''\text{-}2''}$ = $J_{5''\text{-}6''}$ = 8.1 Hz, $J_{3''\text{-}4''}$ = $J_{5''\text{-}4''}$ = 7.5 Hz), 7.05 (tt, 1H, H-4″, $J_{4''\text{-}3''}$ = $J_{4''\text{-}5''}$ = 7.5 Hz, $J_{4''\text{-}6''}$ = $J_{4''\text{-}2''}$ = 1.1 Hz,), 6.87 (dd, 1H, H-4′, $J_{4'\text{-}3'}$ = 8.8 Hz, $J_{4'\text{-}6'}$ = 2.8 Hz), 6.78 (d, 1H, H-6′, $J_{6'\text{-}4'}$ = 2.8 Hz), 6.75 (d, 1H, H-3′, $J_{3'\text{-}4'}$ = 8.8 Hz), 5.27 (bs, 2H, $-NH_2$), 4.02 (t, 2H, H-5, $J_{5\text{-}4}$ = 10.3 Hz), 3.78 (s, 3H, $-OCH_3$), 3.36 (t, 2H, H-4, $J_{4\text{-}5}$ = 10.3 Hz). $^{13}$C NMR (125.69 MHz, $CDCl_3$): $\delta$/ppm 155.4 (-CONH-), 152.0 (C-3), 151.6 (C-5′), 140.6 (C-2′), 138.5 (C-1″), 129.1 (C-2″, C-6″), 123.3 (C-4″), 119.4 (C-3″, C-5″), 117.9 (C-4′), 117.6 (C-3′), 114.6 (C-1′), 114.28 (C-6′), 56.2 ($-OCH_3$), 43.2 (C-5), 34.2 (C-4). MS (LSIMS): $m/z$ 311.1516 $[M + H]^+$, Calcd. Mass for $C_{17}H_{19}N_4O_2$ 311.1508.

*3-(2-Amino-4,5-dimethoxyphenyl)-N-ethyl-4,5-dihydro-1H-pyrazole-1-carboxamide* ***(6g)***. Brown solid, yield 98 mg (0.336 mmol) (84%). Mp: 161–163°C. $^1$H NMR (499.79 MHz,

$CDCl_3$): $\delta$/ppm 6.67 (s, 1H, H-6′), 6.28 (s, 1H, H-3′), 5.58 (m, 1H, -NH-), 3.91 (m, 2H, H-5), 3.86 (s, 3H, 4′-$OCH_3$), 3.80 (s, 3H, 5′-$OCH_3$), 3.36 (m, 2H, -C$\underline{H}_2$-$CH_3$), 3.25 (t, 2H, H-4, $J$ = 10.0 Hz), 1.18 (t, 3H, $CH_3$, $J$ = 7.2 Hz). $^{13}$C NMR (125.69 MHz, $CDCl_3$): $\delta$/ppm 155.3 (-CONH-), 154.6 (C-3), 151.9 (C-4′), 142.3 (C-5′), 141.2 (C-2′), 113.1 (C-6′), 106.0 (C-1′), 99.9 (C-3′), 57.2 (5′-$OCH_3$), 55.9 (4′-$OCH_3$), 43.2 (C-5), 34.1 (C-4), 35.1 (-$\underline{C}H_2$-$CH_3$), 15.9 (-$CH_2$-$\underline{C}H_3$). MS (LSIMS): $m/z$ 293.1620 $[M + H]^+$, Calcd. Mass for $C_{14}H_{21}N_4O_3$ 293.1614.

*3-(2-Amino-4,5-dimethoxyphenyl)-N-propyl-4,5-dihydro-1H-pyrazole-1-carboxamide* ***(6h)***. Brown solid, yield 118 mg (0.388 mmol) (97%). Mp: 197–199°C. $^1$H NMR (499.79 MHz, $CDCl_3$): $\delta$/ppm 6.67 (s, 1H, H-6′), 6.28 (s, 1H, H-3′), 5.63 (bs, 1H, -NH-), 5.41 (bs, 2H, -$NH_2$), 3.91 (t, 2H, H-5, $J$ = 10.1 Hz), 3.86 (s, 3H, 4′-$OCH_3$), 3.81 (s, 3H, 5′-$OCH_3$), 3.27 (m, 4H, -C$\underline{H}_2$-$CH_2$-$CH_3$, H-4), 1.57 (m, 2H, -$CH_2$-C$\underline{H}_2$-$CH_3$), 0.94 (t, 3H, -$CH_2$-$CH_2$-C$\underline{H}_3$, $J$ = 7.4 Hz). $^{13}$C NMR (125.69 MHz, $CDCl_3$): $\delta$/ppm 155.4 (-CONH-), 154.6 (C-3), 151.9 (C-4′), 142.2 (C-5′), 141.2 (C-2′), 113.1 (C-6′), 106.0 (C-1′), 99.9 (C-3′), 57.2 (5′-$OCH_3$), 55.9 (4′-$OCH_3$), 43.2 (C-5), 42.0 (-$\underline{C}H_2$-$CH_2$-$CH_3$), 34.1 (C-4), 23.8 (-$CH_2$-$\underline{C}H_2$-$CH_3$), 11.5 (-$CH_2$-$CH_2$-$\underline{C}H_3$). MS (LSIMS): $m/z$ 307.1775 $[M + H]^+$, Calcd. Mass for $C_{15}H_{23}N_4O_3$ 307.1770.

*3-(2-Amino-4,5-dimethoxyphenyl)-N-methyl-4,5-dihydro-1H-pyrazole-1-carbothioamide* ***(6i)***. Brown solid, yield 106 mg (0.360 mmol) (90%). Mp: 180–182°C. $^1$H NMR (499.79 MHz, $CDCl_3$): $\delta$/ppm 6.79 (bs, 1H, -NH-), 6.67 (s, 1H, H-6′), 6.28 (s, 1H, H-3′), 4.28 (t, 2H, H-5, $J$ = 10.0 Hz), 3.87 (s, 3H, 4′-$OCH_3$), 3.81 (s, 3H, 5′-$OCH_3$), 3.29 (t, 2H, H-4, $J$ = 10.0 Hz), 3.21 (d, 3H, $CH_3$, $J$ = 4.7 Hz). $^{13}$C NMR (125.69 MHz, $CDCl_3$): $\delta$/ppm 175.7 (-CSNH-), 158.1 (C-3), 152.8 (C-4′), 142.8 (C-5′), 141.5 (C-2′), 113.0 (C-6′), 105.1 (C-1′), 99.8 (C-3′), 57.1 (5′-$OCH_3$), 56.0 (4′-$OCH_3$), 47.3 (C-5), 33.7 (C-4), 31.7 (-$CH_3$). MS (LSIMS): $m/z$ 295.1219 $[M + H]^+$, Calcd. Mass for $C_{13}H_{19}N_4O_2S$ 295.1229.

*3-(2-Amino-4,5-dimethoxyphenyl)-N-ethyl-4,5-dihydro-1H-pyrazole-1-carbothioamide* ***(6j)***. Yelow solid, yield 98 mg (0.316 mmol) (79%). Mp: 180–182°C. $^1$H NMR (499.79 MHz, $CDCl_3$): $\delta$/ppm 6.73 (m, 1H, -NH-), 6.67 (s, 1H, H-6′), 6.27 (s, 1H, H-3′), 5.38 (bs, 2H, -$NH_2$), 4.28 (t, 2H, H-5, $J$ = 10.0 Hz), 3.87 (s, 3H, 4′-$OCH_3$), 3.81 (s, 3H, 5′-$OCH_3$), 3.72 (m, 2H, -C$\underline{H}_2$-$CH_3$), 3.29 (t, 2H, H-4, $J$ = 10.0 Hz), 1.26 (t, 3H, -$CH_2$-C$\underline{H}_3$, $J$ = 7.2 Hz). $^{13}$C NMR (125.69 MHz, $CDCl_3$): $\delta$/ppm 174.65 (-CSNH-), 158.0 (C-3), 152.8 (C-4′), 142.8 (C-5′), 141.4 (C-2′), 113.0 (C-6′), 105.1 (C-1′), 99.8 (C-3′), 57.1 (5′-$OCH_3$), 56.0 (4′-$OCH_3$), 47.2 (C-5), 39.7 (-$\underline{C}H_2$-$CH_3$), 39.6 (C-4), 15.0 (-$CH_2$-$\underline{C}H_3$). MS (LSIMS): $m/z$ 309.1364 $[M + H]^+$, Calcd. Mass for $C_{14}H_{21}N_4O_2S$ 309.1385.

*3-(2-Amino-4,5-dimethoxyphenyl)-N-propyl-4,5-dihydro-1H-pyrazole-1-carbothioamide* ***(6k)***. Yellow solid, yield 106 mg (0.328 mmol) (82%). Mp: 193–195°C. $^1$H NMR (499.79 MHz, $CDCl_3$): $\delta$/ppm 6.83 (m, 1H, -NH-), 6.67 (s, 1H, H-6′), 6.29 (s, 1H, H-3′), 4.28 (t, 2H, H-5, $J$ = 10.0 Hz), 3.87 (s, 3H, 4′-$OCH_3$), 3.81 (s, 3H, 5′-$OCH_3$), 3.64 (m, 2H, -C$\underline{H}_2$-$CH_2$-$CH_3$), 3.29 (t, 2H, H-4, $J$ = 10.0 Hz), 1.67 (m, 2H, -$CH_2$-C$\underline{H}_2$-$CH_3$), 0.97 (t, 3H, -$CH_2$-$CH_2$-C$\underline{H}_3$, $J$ = 7.4 Hz). $^{13}$C NMR (125.69 MHz, $CDCl_3$): $\delta$/ppm 174.8 (-CSNH-), 158.0 (C-3), 152.7 (C-4′), 142.3 (C-5′), 141.5 (C-2′), 112.9 (C-6′), 105.3 (C-1′), 99.9 (C-3′), 57.1 (5′-$OCH_3$), 56.0 (4′-$OCH_3$), 47.2 (C-5), 46.5 (-$\underline{C}H_2$-$CH_2$-$CH_3$), 33.6 (C-4), 22.9 (-$CH_2$-$\underline{C}H_2$-$CH_3$), 11.5 (-$CH_2$-$CH_2$-$\underline{C}H_3$). MS (LSIMS): $m/z$ 323.1540 $[M + H]^+$, Calcd. Mass for $C_{15}H_{23}N_4O_2S$ 323.1542.

*3-(6-Amino-2,3,4-trimethoxyphenyl)-N-ethyl-4,5-dihydro-1H-pyrazole-1-carboxamide* ***(6l)***. Brown solid, yield 104 mg (0.322 mmol) (80%). Mp: 150–153°C. $^1$H NMR (400.17 MHz, $CDCl_3$): $\delta$/ppm 6.06 (s, H-5′), 5.62 (bs, 3H, -$NH_2$, -CONH-), 3.87 (m, 8H, H-5, 2′-$OCH_3$, 3′-$OCH_3$), 3.76 (s, 3H, 4′-$OCH_3$), 3.35 (m, 4H, H-4, -C$\underline{H}_2$-$CH_3$), 1.17 (t, 3H, -$CH_2$-C$\underline{H}_3$, $J$ = 7.2 Hz). $^{13}$C NMR (125.69 MHz, $CDCl_3$): $\delta$/ppm 155.3, 155.1 (-CONH-, C-3), 154.0 (C-4′), 153.6 (C-2′), 142.9 (C-6′), 134.2 (C-3′), 102.8 (C-1′), 95.1 (C-5′), 61.2, 61.0 (2′-$OCH_3$, 3′-$OCH_3$), 55.7 (4′-$OCH_3$), 43.6 (C-5), 36.4 (-$\underline{C}H_2$-$CH_3$), 34.9 (C-4), 15.8 (-$CH_2$-$\underline{C}H_3$). MS (LSIMS): $m/z$ 323.1720 $[M + H]^+$, Calcd. Mass for $C_{15}H_{23}N_4O_4$ 323.1710.

*3-(6-Amino-2,3,4-trimethoxyphenyl)-N-propyl-4,5-dihydro-1H-pyrazole-1-carboxamide* ***(6m)***. Brown solid, yield 112 mg (0.332 mmol) (83%). Mp: 156–158°C. $^1$H NMR (400.17 MHz, $CDCl_3$): $\delta$/ppm 6.06 (s, 1H, H-6′), 5.69 (bs, 3H, -$NH_2$, -CONH-), 3.88 (m, 8H, H-5, 2′-$OCH_3$, 3′-$OCH_3$), 3.76 (s, 3H, 4′-$OCH_3$), 3.35 (t, 2H, H-4, $J$ = 10.2 Hz), 3.26 (m, 2H, -C$\underline{H}_2$-$CH_2$-$CH_3$), 1.55 (m, 2H, -$CH_2$-C$\underline{H}_2$-$CH_3$), 0.93 (t, 3H, -$CH_2$-$CH_2$-C$\underline{H}_3$, $J$ = 7.40 Hz). $^{13}$C NMR (125.69 MHz, $CDCl_3$): $\delta$/ppm 155.4, 155.1 (-CONH-, C-3), 154.0 (C-4′), 153.6 (C-2′), 142.8 (C-6′), 134.3 (C-3′), 102.9 (C-1′), 95.2 (C-5′), 61.2, 61.0 (2′-$OCH_3$, 3′-$OCH_3$), 55.7 (4′-$OCH_3$), 43.7 (C-5), 41.8 (-$\underline{C}H_2$-$CH_2$-$CH_3$), 36.4 (C-4), 23.6 (-$CH_2$-$\underline{C}H_2$-$CH_3$), 11.3 (-$CH_2$-$CH_2$-$\underline{C}H_3$). MS (LSIMS): $m/z$ 337.1870 $[M + H]^+$, Calcd. Mass for $C_{16}H_{25}N_4O_4$ 337.1876.

*3-(6-Amino-2,3,4-trimethoxyphenyl)-N-phenyl-4,5-dihydro-1H-pyrazole-1-carboxamide* ***(6n)***. Brown solid, yield 110 mg (0.298 mmol) (74%). Mp: 163–165°C. $^1$H NMR (499.79 MHz, $CDCl_3$): $\delta$/ppm 7.69 (bs, 1H, -CONH-), 7.49 (d, 2H, H-2″, H-6″, $J$ = 7.7 Hz), 7.31 (t, 2H, H-3″, H-5″, $J$ = 7.7 Hz), 7.04 (t, 1H, H-4″, $J$ = 7.7 Hz), 6.08 (s, 1H, H-5′), 3.97 (t, 2H, H-5, $J_{5\text{-}4}$ = 10.3 Hz), 3.90, 3.86, 3.79 (3s, 9H, 2′-$OCH_3$, 3′-$OCH_3$, 4′-$OCH_3$), 3.44 (t, 2H, H-4, $J_{4\text{-}5}$ = 10.3 Hz). $^{13}$C NMR (125.69 MHz, $CDCl_3$): $\delta$/ppm 155.5 (C-3), 154.6 (-CONH-), 154.1 (C-4′), 152.1 (C-2′), 143.3 (C-6′), 138.5 (C-1″), 134.1 (C-3′), 128.9 (C-3″, C-5″), 122.9 (C-4″), 119.1 (C-2″, C-6″), 102.4 (C-1′), 95.0 (C-5′), 61.2, 61.1 (2′-$OCH_3$, 3′-$OCH_3$), 55.8 (4′-$OCH_3$), 43.4 (C-5), 36.7 (C-4). MS (LSIMS): $m/z$ 371.1706 $[M + H]^+$, Calcd. Mass for $C_{19}H_{23}N_4O_4$ 371.1719.

*3-(6-Amino-2,3,4-trimethoxyphenyl)-N-methyl-4,5-dihydro-1H-pyrazole-1-carbothioamide* ***(6o)***. Brown solid, yield

106 mg (0.326 mmol) (82%). Mp: 110–112°C. $^1$H NMR (400.17 MHz, $CDCl_3$): $\delta$/ppm 6.83 (bs, 1H, -CSNH-), 6.01 (s, 1H, H-5′), 4.21 (t, 2H, H-5, $J_{5\text{-}4}$ = 9.9 Hz), 3.85, 3.82, 3.74 (3s, 9H, 2′-$OCH_3$, 3′-$OCH_3$, 4′-$OCH_3$), 3.39 (t, 2H, H-4, $J_{4\text{-}5}$ = 9.9 Hz), 3.17 (d, 3H, -$CH_3$, $J$ = 4.8 Hz). $^{13}$C NMR (100.73 MHz, $CDCl_3$): $\delta$/ppm 175.9 (-CSNH-), 157.6 (C-3), 155.9 (C-4′), 154.3 (C-3′), 143.8 (C-6′), 134.1 (C-2′), 101.8 (C-1′), 94.8 (C-5′), 61.2, 61.0 (2′-$OCH_3$, 3′-$OCH_3$), 55.7 (4′-$OCH_3$), 47.6 (C-5), 36.2 (C-4), 31.5 (-$CH_3$). MS (LSIMS): $m/z$ 347.1156 $[M + Na]^+$, Calcd. Mass for $C_{14}H_{20}N_4O_3NaS$ 347.1154.

*3-(6-Amino-2,3,4-trimethoxyphenyl)-N-ethyl-4,5-dihydro-1H-pyrazole-1-carbothioamide* ***(6p)***. Brown solid, yield 110 mg (0.324 mmol) (81%). Mp: 131–133°C. $^1$H NMR (400.17 MHz, $CDCl_3$): $\delta$/ppm 6.79 (bs, 1H, -CSNH-), 6.01 (s, 1H, H-5′), 4.21 (t, 2H, H-5, $J_{5\text{-}4}$ = 9.9 Hz), 3.85, 3.82, 3.74 (3s, 9H, 2′-$OCH_3$, 3′-$OCH_3$, 4′-$OCH_3$), 3.68 (m, 2H, -C$\underline{H}_2$-$CH_3$), 3.38 (t, 2H, H-4, $J_{4\text{-}5}$ = 9.9 Hz), 1.23 (t, 3H, -$CH_2$C$\underline{H}_3$, $J$ = 7.2 Hz). $^{13}$C NMR (125.69 MHz, $CDCl_3$): $\delta$/ppm 174.83 (-CSNH-), 157.6 (C-3), 155.9 (C-4′), 154.3 (C-3′), 143.7 (C-6′), 134.1 (C-2′), 101.9 (C-1′), 94.9 (C-5′), 61.2, 61.0 (2′-$OCH_3$, 3′-$OCH_3$), 55.8 (4′-$OCH_3$), 47.6 (C-5), 39.5 (C-4), 36.2 (-C$\underline{H}_2$-$CH_3$), 14.8 (-$CH_2$-C$\underline{H}_3$). MS (LSIMS): $m/z$ 339.1482 $[M + H]^+$, Calcd. Mass for $C_{15}H_{23}N_4O_3S$ 339.1477.

*3-(6-Amino-2,3,4-trimethoxyphenyl)-N-propyl-4,5-dihydro-1H-pyrazole-1-carbothioamide* ***(6q)***. Brown solid, yield 118 mg (0.334 mmol) (83%). Mp: 144–146°C. $^1$H NMR (400.17 MHz, $CDCl_3$): $\delta$/ppm 6.86 (bs, 1H, -CSNH-), 6.01 (s, 1H, H-5′), 4.21 (t, 2H, H-5, $J_{5\text{-}4}$ = 9.9 Hz), 3.85, 3.82, 3.74 (3s, 9H, 2′-$OCH_3$, 3′-$OCH_3$, 4′-$OCH_3$), 3.61 (m, 2H, -C$\underline{H}_2$-$CH_2$-$CH_3$), 3.38 (t, 2H, H-4, $J_{4\text{-}5}$ = 9.9 Hz), 1.64 (m, 2H, -$CH_2$-C$\underline{H}_2$-$CH_3$), 0.94 (t, 3H, -$CH_2$-$CH_2$-C$\underline{H}_3$, $J$ = 7.4 Hz). $^{13}$C NMR (100.73 MHz, $CDCl_3$): $\delta$/ppm 174.94 (-CSNH-), 157.5 (C-3), 155.9 (C-4′), 154.3 (C-3′), 143.7 (C-6′), 134.1 (C-2′), 101.9 (C-1′), 94.8 (C-5′), 61.2, 61.0 (2′-$OCH_3$, 3′-$OCH_3$), 55.7 (4′-$OCH_3$), 47.6 (C-5), 46.4 (-C$\underline{H}_2$-$CH_2$-$CH_3$), 36.2 (C-4), 22.7 (-$CH_2$-C$\underline{H}_2$-$CH_3$), 11.4 (-$CH_2$-$CH_2$-C$\underline{H}_3$). MS (LSIMS): $m/z$ 353.1642 $[M + H]^+$, Calcd. Mass for $C_{16}H_{25}N_4O_3S$ 353.1647.

*3-(6-Amino-2,3,4-trimethoxyphenyl)-N-phenyl-4,5-dihydro-1H-pyrazole-1-carbothioamide* ***(6r)***. Brown solid, yield 138 mg (0.358 mmol) (89%). Mp: 150–152°C. $^1$H NMR (499.79 MHz, $CDCl_3$): $\delta$/ppm 8.62 (bs, 1H, -CSNH-), 7.58 (d, 2H, H-2″, H-6″, $J$ = 8.1 Hz), 7.38 (t, 2H, H-3″, H-5″, $J$ = 7.9 Hz), 7.21 (dd, 1H, H-4″, $J$ = 10.6 Hz, $J$ = 4.2 Hz), 6.05 (s, 1H, H-5′), 4.33 (t, 2H, H-5, $J_{5\text{-}4}$ = 9.8 Hz), 3.92, 3.87, 3.79 (3s, 9H, 2′-$OCH_3$, 3′-$OCH_3$, 4′-$OCH_3$), 3.50 (t, 2H, H-4, $J_{4\text{-}5}$ = 9.8 Hz). $^{13}$C NMR (125.69 MHz, $CDCl_3$): $\delta$/ppm 172.9 (-CSNH-), 158.5 (C-3), 156.2 (C-4′), 154.5 (C-3′), 144.0 (C-6′), 138.8 (C-1″), 134.2 (C-2′), 128.6 (C-3″, C-5″), 125.5 (C-4″), 124.6 (C-2″, C-6″), 101.6 (C-1′), 94.9 (C-5), 61.2, 61.1 (2′-$OCH_3$, 3′-$OCH_3$), 55.8 (4′-$OCH_3$), 47.5 (C-5), 36.4 (C-4). MS (LSIMS): $m/z$ 387.1490 $[M + H]^+$, Calcd. Mass for $C_{19}H_{23}N_4O_3S$ 387.1491.

### 2.2. Biology

*2.2.1. nNOS and iNOS Activity Determination.* All the experiments were performed *in vitro*, using reagents obtained mainly from Sigma-Aldrich Química and Merk (Spain). The radioactive L-[$H^3$]-arginine was obtained from Amersham Biosciences (Perkin Elmer) (Spain) and Calmodulin along with nNOS and iNOS recombinant enzymes from Enzo Life Sciences, Group Taper, Seville (Spain).

The NOS activity was measured by controlling the L-[$^3$H]-arginine conversion to L-[$^3$H]-citrulline in three experiments performed in triplicate, following the Bredt and Snyder method [39].

The reaction took place in a final incubation volume of 100 $\mu$L which includes 10 $\mu$L of an aliquot of recombinant enzyme added to 50 $\mu$L of a buffer with a final concentration of 25 mM tris-(hydroxymethyl)-aminometane hydrochloride (Tris-HCl), 1 mM DL-dithiothreitol (DTT), 4 $\mu$M 5,6,7,8-tetrahydro-L-biopterin dihydrocloride ($H_4$-biopterin), 10 $\mu$M flavin-adenine dinucleotide (FAD), 0,5 mM hypoxantine-9-$\beta$-D-ribofuranoside (inosine), 0,5 mg/mL bovine serum albumin (BSA), 0,1 mM $CaCl_2$, 10 $\mu$M L-arginine, 10 $\mu$g $mL^{-1}$ calmodulin (only for nNOS), and 0,05 $\mu$M L-[$^3$H]-arginine, at pH 7.6, 10 $\mu$L of a 10 mM in ethanol solution of each thiadiazoline **5a–e** or pyrazoline **6a–r** derivative, and enough water to reach 100 $\mu$L. In order to start the reaction, 10 $\mu$L of a 7.5 mM NADPH was added. NADPH was omitted in control incubations. All the samples were stirred and incubated for 30 minutes at 37°C. At the end of the incubation, 400 $\mu$L of a cold solution 0.1 M *N*-(2-hydroxymethyl)piperazine-*N*′-(2-ethanesulfonic acid) HEPES, 10 mM ethylene glycol-bis-(2-aminoethylether)-*N*,*N*,*N*′,*N*′-tetraacetic acid (EGTA), and 0.175 mg/mL L-citrulline at pH 5.5 stopped the reaction. This mixture goes through a column packed with Dowex-50W ion-exchange resin ($Na^+$ form) and was eluted with 1.2 mL of water losing around 98% of radioactivity. Finally, 50 $\mu$L of each sample was diluted with scintillation liquid and the measurements were taken. The nNOS activity was expressed in picomoles of L-[$^3$H]-citrulline produced/mg of protein/min.

*2.2.2. eNOS Inhibition.* Experiments were conducted in male Wistar rats obtained from Harlan Laboratories (Barcelona, Spain) weighing 200–250 g. The descending thoracic aortic rings were dissected and cut into rings which were mounted in organ chambers by means of two L-shaped stainless-steel wires inserted into the lumen and attached to the chamber and to an isometric force-displacement transducer coupled to a signal amplifier (Dynamometer UF1, Cibertec, Madrid) and connected to a computer via an A/D interface. Contractile tension was recorded by a Power-Lab 800 (AD Instruments, Cibertec, Madrid), as previously described [40]. The chamber was filled with Krebs solution (composition in mmol/L: NaCl, 118; KCl, 4.75; $NaHCO_3$, 25; $MgSO_4$, 1.2; $CaCl_2$, 2; $KH_2PO_4$, 1.2; and glucose, 11) at 37°C and gassed with 95% $O_2$ and 5% $CO_2$. Rings were stretched to 2 g of tension and equilibrated for 90–120 min. After equilibration, aortic rings with a functional endothelium were contracted

with phenylephrine (1 $\mu$mol/L) and a concentration-response curve was constructed by cumulative addition of acetylcholine (basal curve). Then, to evaluate the inhibition of eNOS activity, the rings were washed and incubated with vehicle (DMSO), **6m** (0.1, 0.5, 1 mmol/L), or L-NAME ($N^w$-nitro-L-arginine methyl ester hydrochloride) (1 mmol/L) for 30 min, and again a concentration-response curve to acetylcholine was constructed in phenylephrine-contracted rings. Results are expressed as percentage of phenylephrine-evoked contraction and area under curve (AUC) of the relaxant response was calculated as an index of eNOS activity. Data are expressed as the mean ± SEM and $n$ reflects the number of aortic rings from different rats.

*2.3. Statistical Analysis.* Data are expressed as the mean ± SEM. Statistically significant differences between groups were calculated by Students' $t$-test for unpaired observations or for multiple comparisons. ANOVA, followed by the Newmane-Keuls multiple range test, was used. A $P < 0.05$ was considered statistically significant.

## 3. Results and Discussion

*3.1. Synthetic Chemistry.* The general synthetic pathways followed to obtain the new compounds **5a–e** and **6a–r** are represented in Schemes 1 and 2, respectively.

Scheme 1 shows the general synthetic pathway of the final 5-(2-amino-5-phenylsubstituted)-2,2-dimethyl-*N*-substituted-1,3,4-thiadiazole-3(3*H*)-carboxamide derivatives **5a–e** described in the present report. We have introduced two principal modifications in these derivatives in order to obtain structure-activity relationships: (i) introduction of an electron-donating group at the H-5′ place of the benzene ring and (ii) insertion of an alkyl or aryl carboxamide moiety in the 3-position of the heterocyclic. The synthetic pathway begins with the esterification of the commercial 5-methoxy-2-nitrobenzoic acid **7** which leads to the ethyl benzoate **9** [31]. This one and the ethyl 2-nitrobenzoate **8** were transformed into the 2-nitro-5-substituted-benzohydrazides **10**-**11** in the presence of hydrazine and ethanol [31]. These two molecules were turned into the corresponding 2-nitro-5-substituted-*N*′-(1-isopropylidene)benzohydrazides **12**-**13** in presence of acetone and ethanol at room temperature. The subsequent addition of Lawesson's Reagent to the hydrazides led to the cyclation, obtaining the 2,3-dihydro-1,3,4-thiadiazole derivatives **14**-**15** [31]. Nucleophilic addition of alkyl or aryl-isocyanate to these last compounds using microwave gave the nitrophenyl thiadiazole-carboxamides **16a–e** [37], which were subjected to reduction in the presence of stannous chloride in refluxing ethanol [38] to give the desired aminoaryl thiadiazole-carboxamides **5a–e**.

On the other hand, Scheme 2 represents the synthetic pathway of the final 3-(2-amino or 6-aminophenyl-substituted)-*N*-substituted-4,5-dihydro-1*H*-pyrazole-1-carboxamide or carbothioamide derivatives. In these molecules, three major modifications have been made: (i) substitution of one, two, or three hydrogen atoms in the aromatic ring by electron-withdrawing or electron-donating groups; (ii) introduction of a carboxamide or carbothioamide moiety in the 1-position of pyrazoline heterocycle; and (iii) insertion of an alkyl or aryl substituent in the carboxamide or carbothioamide rest. In the synthesis of these compounds we have carried out the transformation of the commercially available 5-chloro-2-nitrobenzaldehyde **18**, 5-methoxy-2-nitrobenzaldehyde **19**, 4,5-dimethoxy-2-nitrobenzaldehyde **20**, and the synthesized 2,3,4-trimethoxy-6-nitrobenzaldehyde **21** (by nitration of 2,3,4-trimethoxybenzaldehyde **17** with a mixture of $HNO_3$ and AcOH), into the corresponding allylic alcohols **22–25**, by treatment with vinyl-magnesium bromide [16, 32]. These intermediates were further oxidized (Jones reagent) obtaining the enone derivatives **26–29** [16, 32]. The reaction of the enones with hydrazine in ethanol produced the 4,5-dihydro-1*H*-pyrazoles **30–33** [16, 32] which, in situ, were converted into the intermediate nitroderivatives **34a–r** by reaction with the adequate alkyl or aryl-isocyanates and -isothiocyanates [37]. Finally, reduction of the nitro group with $SnCl_2$ [38] in ethanol gave the final carboxamides and carbothioamides **6a–r**.

*3.2. Biological Assays*

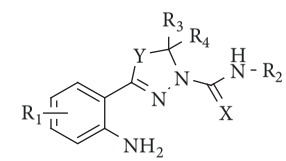
*3.2.1. nNOS and iNOS Inhibition.* The *in vitro* biological evaluation of the new heterocycles **5a–e** and **6a–r** as inhibitors of both inducible and neuronal NOS isoforms has been made in the presence of recombinant isoenzymes. We have used a 1 mM concentration for each final compound. The results of this assessment are shown in Table 1. The pyrazoline **2a** ($R_1$ = 2,3,4-$(OMe)_3$; $R_2$ = Ph) previously published [16] has been used as a control.

The nNOS inhibition shows that the tested molecules display a moderate inhibition percentage versus this isoform, excepting the thiadiazolines **5a–c** which exhibit considerable inhibition results. On the whole, thiadiazolines are better inhibitors than pyrazolines, being thiadiazolines with $R_1$ = H more potent that those with $R_1$ = OMe. In this way, the thiadiazole-carboxamides **5a** (X = O, Y = S, $R_2$ = Et, 74.11%) and **5c** (X = O, Y = S, $R_2$ = Ph, 67.02%) are the best nNOS inhibitors in this series as well as the best ones of all synthesized compounds. In addition, among pyrazoline derivatives, carbothioamides are better nNOS inhibitors than carboxamides. Within the carbothioamides, molecules with three methoxy electron-donating groups in the phenyl moiety (**6o–r**) are those with the best inhibition values, **6r** ($R_2$ = Ph, 47.12%) being the best inhibitor of this group. In this last series, the inhibition percentage increases when the volume of the substituent in the carbothioamide moiety rises, in the Me < Et < Pr < Ph order.

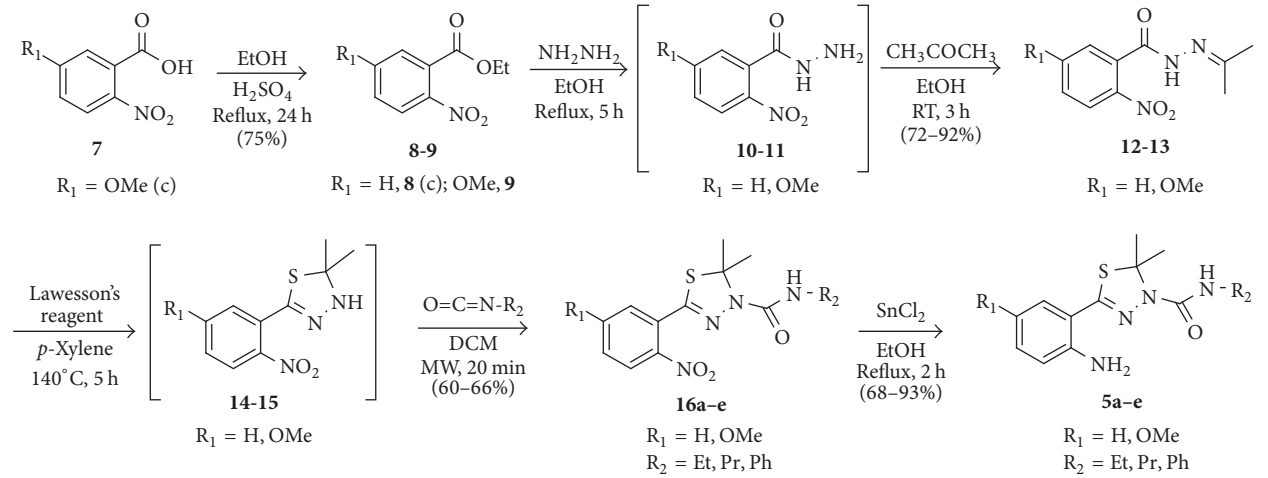
Concernig the iNOS inhibition, the assayed derivatives show better results versus this inducible isoenzyme, because half of the tested molecules have more than 50% of inhibition percentage. As a rule, aryl-pyrazolines exhibit better percentages than aryl-thiadiazolines, showing inhibition percentages in the range of 50.92% and 76.86%. Among molecules with a thiadiazoline moiety, compounds with a methoxy substituent in the aromatic ring ($R_1$) show better percentages of inhibition than the unsubstituted ones, because the only two synthesized derivatives **5d** and **5e** have more than

Table 1: Structure and *in vitro* iNOS and nNOS inhibition (%) observed in the presence of 1 mM concentration of compounds **(5a–e)** and **(6a–r)**. Pyrazoline **2a** has been included as a control.

| Compound | X | Y | $R_1$ | $R_2$ | $R_3 = R_4$ | % iNOS inhibition[a] | % nNOS inhibition[a] |
|---|---|---|---|---|---|---|---|
| **2a**[b] | - | - | 2,3,4-$(OMe)_3$ | Ph | H | 8.14 ± 1.56 | 62.87 ± 3.25 |
| **5a** | O | S | H | Et | Me | 28.09 ± 0.61 | 74.11 ± 1.87 |
| **5b** | O | S | H | Pr | Me | 58.07 ± 1.39 | 64.60 ± 1.50 |
| **5c** | O | S | H | Ph | Me | 42.06 ± 0.62 | 67.02 ± 0.17 |
| **5d** | O | S | 5-OMe | Et | Me | 53.54 ± 0.23 | 33.3 ± 1.05 |
| **5e** | O | S | 5-OMe | Pr | Me | 57.61 ± 1.33 | 26.8 ± 2.68 |
| **6a** | O | $CH_2$ | 5-Cl | Et | H | 13.93 ± 1.92 | 2.27 ± 1.27 |
| **6b** | O | $CH_2$ | 5-Cl | Pr | H | 61.97 ± 1.20 | 30.48 ± 2.21 |
| **6c** | O | $CH_2$ | 5-Cl | Ph | H | 53.78 ± 2.22 | 16.88 ± 0.33 |
| **6d** | O | $CH_2$ | 5-OMe | Et | H | 38.42 ± 1.61 | 12.79 ± 1.24 |
| **6e** | O | $CH_2$ | 5-OMe | Pr | H | 40.68 ± 2.48 | 17.69 ± 1.93 |
| **6f** | O | $CH_2$ | 5-OMe | Ph | H | 11.34 ± 1.39 | 22.70 ± 2.78 |
| **6g** | O | $CH_2$ | 4,5-$(OMe)_2$ | Et | H | 44.12 ± 0.65 | 7.83 ± 0.90 |
| **6h** | O | $CH_2$ | 4,5-$(OMe)_2$ | Pr | H | 47.94 ± 0.23 | 9.86 ± 2.60 |
| **6i** | S | $CH_2$ | 4,5-$(OMe)_2$ | Me | H | 36.51 ± 2.15 | 21.61 ± 1.82 |
| **6j** | S | $CH_2$ | 4,5-$(OMe)_2$ | Et | H | 43.56 ± 0.91 | 21.32 ± 1.91 |
| **6k** | S | $CH_2$ | 4,5-$(OMe)_2$ | Pr | H | 50.92 ± 0.53 | 7.42 ± 1.12 |
| **6l** | O | $CH_2$ | 2,3,4-$(OMe)_3$ | Et | H | 32.16 ± 2.03 | 19.97 ± 0.21 |
| **6m** | O | $CH_2$ | 2,3,4-$(OMe)_3$ | Pr | H | 76.86 ± 0.21 | 31.77 ± 0.82 |
| **6n** | O | $CH_2$ | 2,3,4-$(OMe)_3$ | Ph | H | 54.12 ± 0.23 | 25.88 ± 0.81 |
| **6o** | S | $CH_2$ | 2,3,4-$(OMe)_3$ | Me | H | 57.38 ± 0.23 | 31.2 ± 0.42 |
| **6p** | S | $CH_2$ | 2,3,4-$(OMe)_3$ | Et | H | 52.27 ± 0.72 | 38.73 ± 2.61 |
| **6q** | S | $CH_2$ | 2,3,4-$(OMe)_3$ | Pr | H | 71.29 ± 1.53 | 38.82 ± 1.61 |
| **6r** | S | $CH_2$ | 2,3,4-$(OMe)_3$ | Ph | H | 68.16 ± 1.34 | 47.12 ± 1.54 |

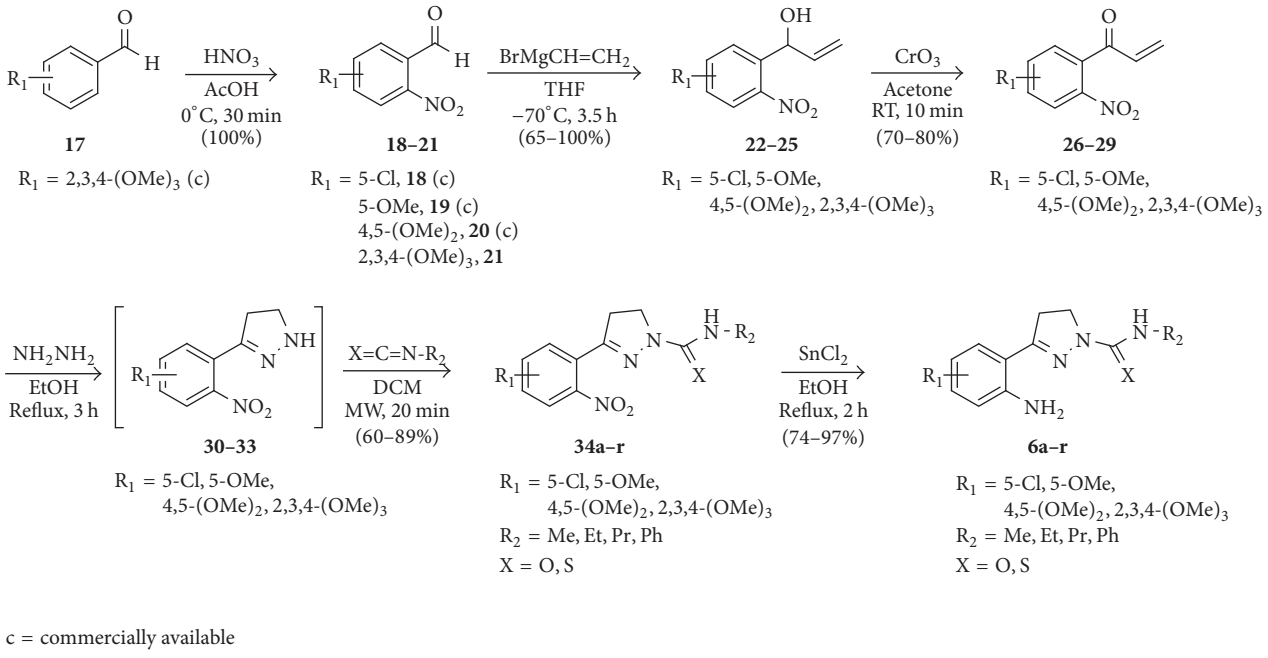



[a]Data represent the percentage of iNOS and nNOS inhibition produced by 1 mM concentration of each compound. Each value is the mean ± SEM of three experiments performed by triplicate using recombinant iNOS and nNOS enzymes. [b]See [16].




c = commercially available

Scheme 1: Synthetic route of the final compounds **(5a–e)**.




c = commercially available

Scheme 2: Synthetic pathway of the final compounds **(6a–r)**.

50% of inhibition; and, regarding the $R_2$ substituent in the carboxamide residue, propyl is the best one, derivative **5b** being the best iNOS inhibitor of this group (58.07%). With respect to the pyrazoline derivatives, about the aromatic ring substitution, the monosubstituted derivatives with an electron-withdrawing group show better iNOS inhibition values than the ones with an electron-donating group. Besides, disubstituted compounds with two donating groups produce lower inhibition than the trisubstituted derivatives. Moreover, there is not much difference between carboxamides and carbothioamides; but for the $R_1$ and $R_2$ substituents, we can see that compounds with the best inhibition values carry three electron-donating groups on the benzene ring and a propyl substituent in the carbothioamide (**6q**, 71.29%) or carboxamide (**6m**, 76.86%) residues, this last molecule being the best inhibitor of this series and the most powerful of all tested compounds. In addition, these compounds show a moderate selectivity iNOS/nNOS.

If we compare derivatives **5** and **6** with the previously synthesized **1–4** molecules (Figure 3), we can see that

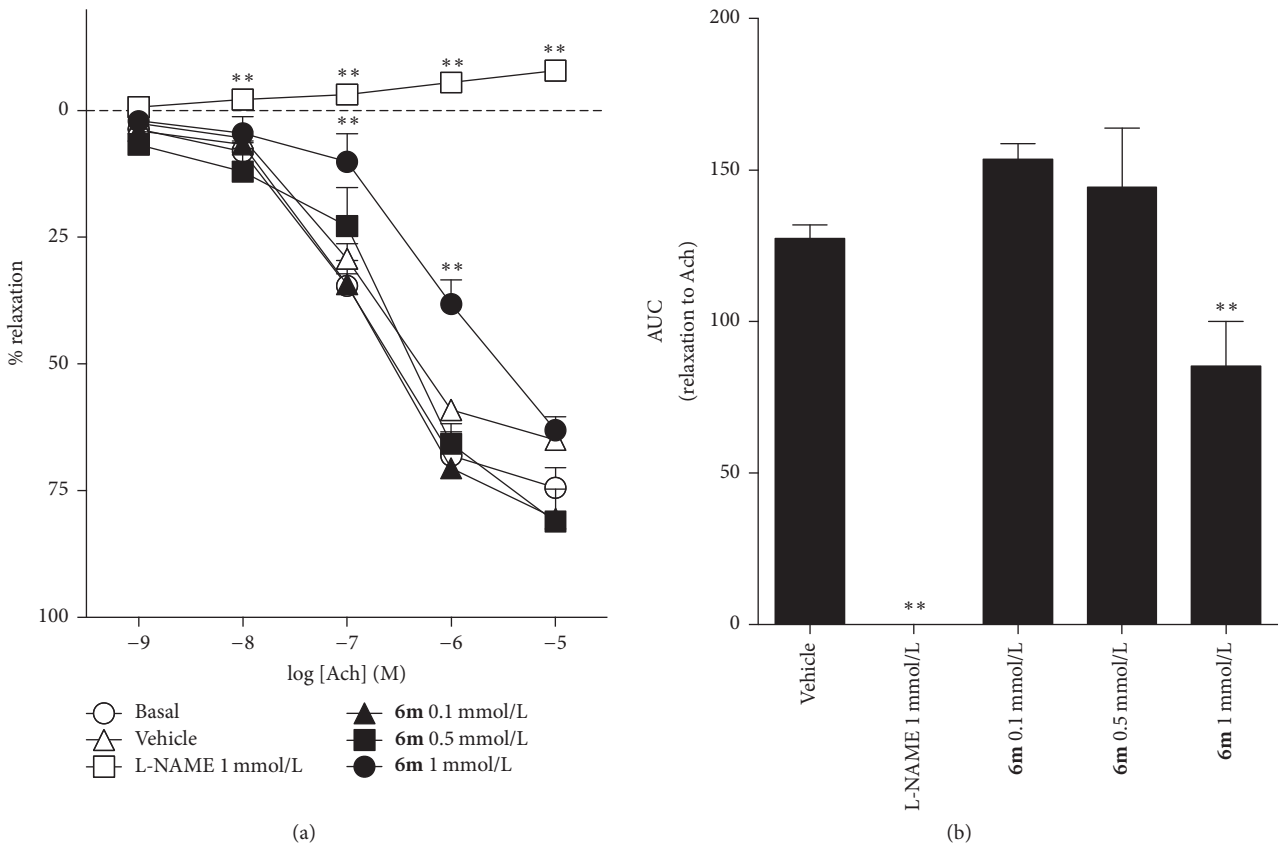



Figure 4: Effects of **6m** on eNOS activity. (a) Acetylcholine- (Ach-) evoked relaxation in aortic rings with endothelium contracted with 1 $\mu$mol/L phenylephrine under basal conditions and after incubation with DMSO, **6m** (0.1, 0.5, 1 mmol/L), or L-NAME (1 mmol/L) for 30 min ($n = 5$). (b) Area under relaxant-response curve (AUC) to Ach from experiments to (a). Data are expressed as the mean ± SEM of $n$ experiments. $^{**}P < 0.01$ versus vehicle group.

thiadiazoline-carboxamides **5** are better nNOS inhibitors than acyl-thiadiazolines **1** which had better iNOS inhibition values, although a general rule cannot be established. In addition, pyrazoline-carboxamides and carbothioamides **6** inhibit better iNOS than nNOS unlike the acyl-pyrazolines **2**. As a consequence, the type of substituent in the heterocyclic ring seems to be important in order to inhibit one isoform or another. Regarding the more flexible derivatives **3** and **4**, the new molecules **6** behave like the 3-oxopropyl-alkylureas and thioureas **3** due to the fact that they are also better iNOS inhibitors, unlike the derivatives with a hydroxyl group **4** (which exhibit better nNOS inhibition values).

Table 2 exhibits the $IC_{50}$ data for the nNOS inhibition of the most interesting thiadiazolines **5a–c**, and the $IC_{50}$ data for the iNOS inhibition of the best pyrazolines **6b**, **6m**, **6q,** and **6r**, and the $IC_{50}$ value of nNOS for the control **2a** [16]. Compound **5a** behaves as the best nNOS inhibitor (420 $\mu$M), and **6m** had the best result versus iNOS (130 $\mu$M).

*3.2.2. eNOS Inhibition.* In addition, the best iNOS inhibitor derivative **6m** was tested as an eNOS inhibitor in order to check the absence of side effects that could derive from the inhibition of this last isoform. In this way, acetylcholine-induced endothelium-dependent relaxation has been analyzed using endothelium intact rat aortic rings. This classic cholinergic agonist activates eNOS using a calcium-dependent mechanism [41]. Vehicle of **6m** (DMSO) was unable to alter the relaxant responses to acetylcholine as compared to basal. Similarly, **6m** at 0.1 and 0.5 mmol/L did not modify this response. However, **6m** at 1 mmol/L significantly inhibited the relaxation induced by acetylcholine, whereas the nonselective NOS inhibitor L-NAME [42] suppressed this relaxant response (Figure 4(a)). When we measured the area under curve of relaxation to acetylcholine, considered as an indirect index of eNOS activity, we found that **6m** inhibited by 33.1% this activity at 1 mmol/L (Figure 4(b)), confirming that this compound did not inhibit eNOS in the range 100–500 $\mu$M, although it displayed a poor eNOS inhibitory effect to higher concentration.

## 4. Conclusions

In summary, the synthesis of five new thiadiazoline- and eighteen pyrazoline-based carboxamides and carbothioamides **5** and **6**, each one with different substituents in the aromatic ring and in the carboxamide or carbothioamide moiety, is described. Furthermore, we evaluate the nNOS and iNOS

Table 2: $IC_{50}$ values (mM) for the inhibition of nNOS activity of compounds **5a–c** and iNOS activity of **6b**, **6m**, **6q,** and **6r**. Pyrazoline **2a** has been included as a control [16].

| $IC_{50}$ [a] | **2a** | **5a** | **5b** | **5c** | **6b** | **6m** | **6q** | **6r** |
|---|---|---|---|---|---|---|---|---|
| nNOS | 0.53 ± 0.12 | 0.42 ± 0.17 | 0.72 ± 0.25 | 0.77 ± 0.63 | b | b | b | b |
| iNOS | b | b | b | b | 0.74 ± 0.55 | 0.13 ± 0.14 | 0.53 ± 0.18 | 0.67 ± 0.34 |

[a]Data obtained by measuring percentage of inhibition with at least five concentrations of inhibitor. [b]Not tested.

inhibitory activity of all these new derivatives and the eNOS activity for the best iNOS inhibitor **6m**. In general, thiadiazolines are better nNOS inhibitors and pyrazolines present better inhibition against iNOS versus nNOS, carbothioamides being with three donating substituents in the aromatic ring the best ones. Thiadiazoline-carboxamide **5a** is the most powerful nNOS inhibitor tested, and pyrazoline-carbothioamide **6m** is the best iNOS of all them all. In addition, this last compound is approximately 2.5 times more selective to inhibit NOS in inflammatory processes than the constitutive nNOS. Moreover, **6m** does not inhibit eNOS at the concentration values necessary to inhibit the other isoforms, which is convenient in order to avoid hypertension as a side effect. Consequently, these novel derivatives could be an interesting starting point to find possible new therapeutic alternatives for neurodegenerative and inflammatory diseases.

## Conflicts of Interest

The authors declare that they have no conflicts of interest.

## Acknowledgments

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