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Short communication

Pyrazoles and pyrazolines as neural and inducible nitric oxide synthase (nNOS and iNOS) potential inhibitors (III)☆

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Abstract

We have previously described a series of 4,5-dihydro-1*H*-pyrazole as moderately potent nNOS inhibitors. As a follow up of these studies, we report here the preparation and the preliminary evaluation of a series of 1-alkyl-3-benzoyl-4,5-dihydro-1*H*-pyrazole and 1-alkyl-3-benzoyl-1*H*-pyrazole as potential inhibitors of both neuronal and inducible nitric oxide synthases (nNOS and iNOS). None of the reported compounds exhibited significant iNOS or nNOS inhibition, although the 1-benzyl-3-(2-amino-5-chlorobenzoyl)-1*H*-pyrazole-5-carboxylic acid ethyl ester derivative (**10l**), which shows an inhibition of 50% versus iNOS at a 1 mM final concentration and no activity against nNOS, is potentially amenable of further optimization. The reasons for the inactivity of the reported series are discussed on the basis of docking studies.

Keywords: Neural nitric oxide synthase; Inducible nitric oxide synthase; Inhibition; Benzoyl-pyrazole; Benzoyl-pyrazoline

1. Introduction

Nitric oxide (NO) is an important bioregulator and ubiquitous messenger that mediates several normal and physiopathological processes [1,2]. NO biosynthesis is mediated by nitric oxide synthase (NOS), which catalyses the L-arginine oxidation. Classically, three isoforms of NOS have been identified, two of them are constitutively expressed and are known as neural NOS (nNOS) and endothelial cell NOS (eNOS), while the third one is induced by several factors, and hence is called inducible NOS (iNOS). nNOS is responsible of the NO production in the central nervous system where it acts as a neurotransmitter [3]. eNOS is found primarily in vascular endothelium and regulates the blood pressure and the vascular tone [4–6]. Finally, iNOS expression is induced in macrophages and other cell types by numerous inflammatory stimuli, including endotoxin (LPS) and cytokines (e.g. IL-1), and participates in host defense, and possibly in chronic inflammatory processes [7].

The NO action can be physiological or pathological. An excessive NO production by nNOS is involved in different neurological disorders such as Alzheimer's disease [8], the amyotrophic lateral sclerosis [9] and Huntington's disease [10]. The induction of iNOS produces a continuous and elevated NO production, and is involved in a variety of diseases, including septic shock [11], inflammatory arthritis [12], and inflammatory bowel disease [13]. Development of NOS inhibitors constitutes a current strategy in the research of new compounds showing interesting properties.

Recently, we have synthesized and evaluated a series of NOS inhibitors with general structures **1**–**4** [14–17]. Compounds **1** [14] and **2** [15] have a kynurenine and kynurenamine structures, respectively, and show good nNOS inhibition.

☆ For parts I and II, see Refs. [16,17].
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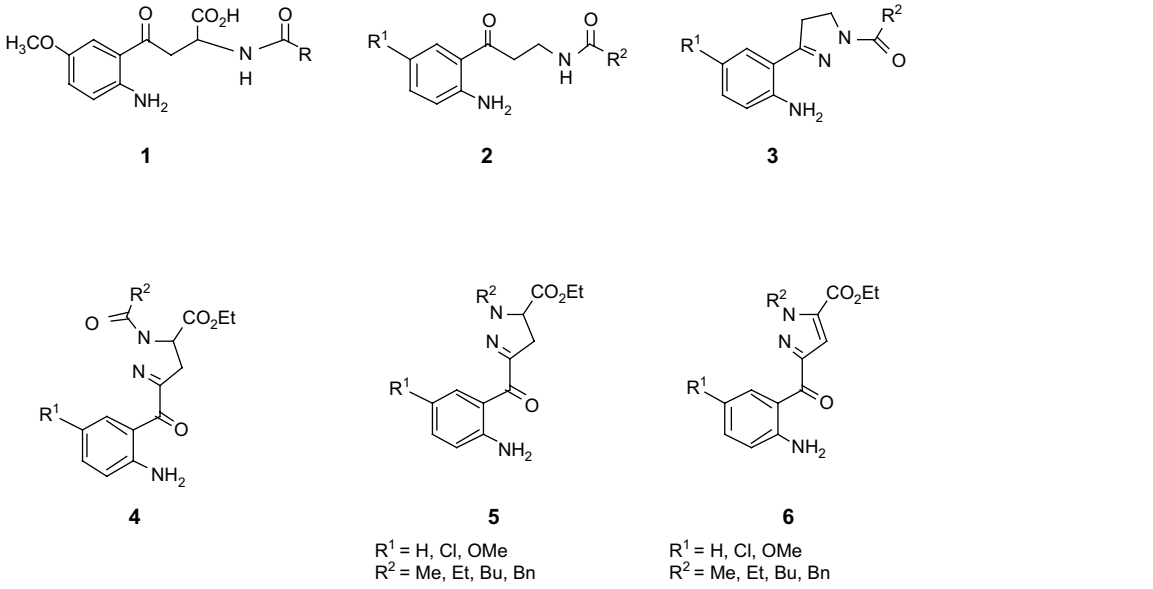
Pyrazoline derivatives **3** [16] constitute a new class of potent nNOS inhibitors, and 1-benzoyl-Δ^2-pyrazoline derivatives **4** have shown a moderate selectivity in the inhibition of the iNOS isoform [17].



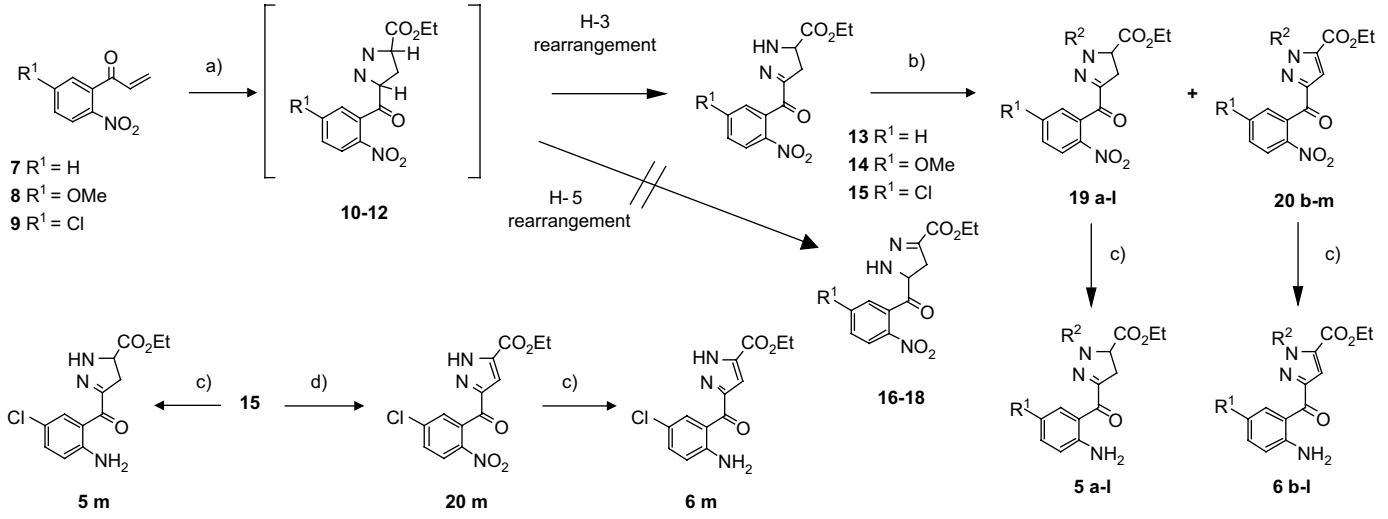
In this paper the synthesis and biological evaluation as NOS inhibitors of a series of benzoyl-pyrazoline **5** and benzoyl-pyrazole **6** derivatives are described. Both families of compounds are characterized by the presence of an N_1-alkyl group, and some of them show moderate inhibition and good iNOS selectivity.

2. Results and discussion

2.1. Chemistry

Scheme 1 shows the general synthetic pathway followed in the preparation of compounds **5** and **6**. Benzoyl-Δ^2-pyrazoline derivatives **13–15** were synthesized from the corresponding (2-nitro-5-substituted)phenyl vinyl ketones **7–9** by reaction with ethyl diazoacetate, as previously described [17]. In this reaction, a 1,3-dipolar cycloaddition takes place to yield the Δ^1-pyrazoline intermediates **10–12** which suffer the appropriated H-3 rearrangement to give the final product. The possibility of an H-5 rearrangement and the formation of the regioisomeic Δ^2-pyrazolines **16–18** has been previously discussed and rejected [18].

Compounds **13–15** were treated with the corresponding $(R^2O)_2SO_2$ or R^2Br in order to obtain the *N*-alkyl derivatives **19a–l**. During the *N*-alkylation reaction of compounds **13–15**, an unexpected aromatization produces the pyrazole derivatives **20b–m**. This aromatization can be explained by the participation of the 2′-NO_2 group [19], which acts as an oxidant, yielding smaller quantities of side products bearing



Scheme 1. General synthetic pathway followed in the preparation of compounds **5** and **6**. (a): N_2CHCO_2Et; (b) $(R^2O)_2SO_2$ or R^2Br, K_2CO_3, THF or 1,4-dioxane; (c) Fe/$FeSO_4$, H_2O; (d) *c*-$C_6H_{11}Br$, K_2CO_3, THF.

reduced nitrogen functions, which were not isolated in the preparation of compounds **19** and **20**.

Table 1 shows the reaction conditions used and the final yield obtained for each compounds **19a–l** and **20b–m**. It can be observed that both pyrazole and pyrazoline derivatives are obtained with variable yields depending on both the alkylating agent and the reaction conditions.

The more reactive alkyl sulphates allow the use of milder conditions in the *N*-alkylation reaction (THF, 70 °C, 15–22 h). The total amount of pyrazoline **19** depends on the reactivity of the alkylating agent, and higher yields are obtained with $(MeO)_2SO_2$ than with $(EtO)_2SO_2$ and $(BuO)_2SO_2$. No significant influence of the substrate R^1 substituent is observed since compounds **13–15** give the corresponding pyrazoline derivatives with similar yields.

The less reactive alkyl halides require the use of 1,4-dioxane as solvent and higher temperature (120 °C, 3 h). In this case, only the more reactive $PhCH_2Br$ gives place to small yields of pyrazoline **19d**, **19h**, or **19l**, while *c*-$C_6H_{11}Br$ does not react.

The aromatization seems to proceed better in an opposing manner: the worse the alkylating agent is the higher the yield of pyrazole derivative **20**. In fact, no *N*-alkylation is observed in the reaction of **15** with *c*-$C_6H_{11}Br$ and only the nitropyrazole **20m** ($R^2 = H$) is isolated. This behaviour is probably due to a kinetic control in both processes: when alkylation is slow, the aromatization can take place more easily.

Reduction (Fe/FeSO4) of nitro derivatives **19a–l** and **20b–m** yields the corresponding final products **5a–l** and **6b–m**, respectively. Finally, compound **5m** was obtained by reduction of compound **15**.

2.2. nNOS and iNOS inhibition

Compounds **5** and **6** have been tested for its inhibition of both nNOS and iNOS isoforms. Table 2 illustrates the inhibition of both isoenzymes in the presence of 1 mM concentration of each final compound. In general, compounds **5** and **6** behave as weak inhibitors against both isoenzyme, and for this reason additional biological assays are not recommended. Nevertheless some conclusions can be drawn from the experimental data.

Table 1
Reaction conditions and yields (in parenthesis) for each isolated product in the *N*-alkylation reaction of compounds **13–15**

Substrate	Reagent[a]	Compound **11** (yield)	Compound **12** (yield)	R^1	R^2
13	$(MeO)_2SO_2$[b]	**19a** (84)	[d]	H	Me
13	$(EtO)_2SO_2$[b]	**19b** (46)	**20b** (26)	H	Et
13	$(BuO)_2SO_2$[b]	**19c** (8)	**20c** (35)	H	Bu
13	$PhCH_2Br$[c]	**19d** (26)	**20d** (27)	H	$PhCH_2$
14	$(MeO)_2SO_2$[b]	**19e** (84)	[d]	OMe	Me
14	$(EtO)_2SO_2$[b]	**19f** (38)	**20f** (17)	OMe	Et
14	$(BuO)_2SO_2$[b]	**19g** (33)	**20g** (23)	OMe	Bu
14	$PhCH_2Br$[c]	**19h** (11)	**20h** (38)	OMe	$PhCH_2$
15	$(MeO)_2SO_2$[b]	**19i** (82)	**20i** (9)	Cl	Me
15	$(EtO)_2SO_2$[b]	**19j** (37)	**20j** (27)	Cl	Et
15	$(BuO)_2SO_2$[b]	**19k** (29)	**20k** (28)	Cl	Bu
15	$PhCH_2Br$[c]	**19l** (15)	**20l** (11)	Cl	$PhCH_2$
15	*c*-$C_6H_{11}Br$[b]	[d]	**20m** (55)[d,e]	Cl	H

[a] Anhydrous K_2CO_3 was used as a base in all reactions.
[b] THF, 70 °C.
[c] Dioxane, 120 °C.
[d] Not detected.
[e] No alkylation observed.

Table 2
nNOs and iNOS inhibition (%) observed in the presence of 1 mM concentration of compounds **5a–m** and **6b–m**

Compounds	R^1	R^2	% nNOS inhibition[a]	% iNOS inhibition[a]
5a	H	Me	−9.8 ± 3.0	12.7 ± 5.2
5b	H	Et	22.1 ± 4.4	−1.5 ± 1.7
5c	H	Bu	29.6 ± 2.1	12.2 ± 1.4
5d	H	$PhCH_2$	28.4 ± 5.8	9.4 ± 1.4
5e	OCH_3	Me	−7.8 ± 4.0	0.6 ± 4.0
5f	OCH_3	Et	1.7 ± 2.7	17.1 ± 0.7
5g	OCH_3	Bu	−10.0 ± 2.3	18.6 ± 1.2
5h	OCH_3	$PhCH_2$	9.3 ± 1.2	10.5 ± 2.6
5i	Cl	Me	1.3 ± 2.3	22.9 ± 0.9
5j	Cl	Et	41.5 ± 2.2	14.7 ± 3.2
5k	Cl	Bu	38.5 ± 3.5	10.5 ± 3.6
5l	Cl	$PhCH_2$	18.3 ± 0.4	17.3 ± 0.6
5m	Cl	H	32.1 ± 2.2	19.1 ± 0.7
6b	H	Et	39.5 ± 1.6	12.5 ± 2.1
6c	H	Bu	21.9 ± 3.1	5.4 ± 0.9
6d	H	$PhCH_2$	21.9 ± 0.4	13.5 ± 4.4
6f	OCH_3	Et	20.3 ± 0.5	24.6 ± 1.1
6g	OCH_3	Bu	26.2 ± 0.9	20.5 ± 0.4
6h	OCH_3	$PhCH_2$	19.4 ± 2.9	30.5 ± 0.8
6i	Cl	Me	14.5 ± 6.2	7.3 ± 3.9
6j	Cl	Et	35.5 ± 1.4	27.5 ± 2.1
6k	Cl	Bu	7.8 ± 0.5	18.7 ± 1.0
6l	Cl	$PhCH_2$	2.7 ± 1.4	50.4 ± 3.2
6m	Cl	H	40.2 ± 2.2	12.8 ± 4.6

[a] Data represent the mean ± SEM of the percentage of nNOS inhibition produced by 1 mM concentration of each compound. Each value is the mean of three experiments performed by triplicate.

Compound **5a** is the simplest one in the pyrazoline series and does not inhibit nNOS: even a small activation of the enzyme is observed. Changing the *N*-methyl group by a bigger one, the nNOS inhibition percentage grows slightly, but not enough to consider these compounds as good nNOS inhibitors. Compounds **5e–h** bear a 5′-OMe group and in all of them the nNOS inhibition decreases in relation to compounds **5a–d**, indicating that the substitution in this position of the benzene ring by a polar electron-donating group is detrimental for the inhibition activity.

Compounds **5i–m** bear a 5′-chloro substituent and all of them show better nNOS inhibition, indicating that an electron-withdrawing group in this position increases the nNOS inhibition activity. The influence the *N*-substituent is not so clear. Compounds **5m** ($R^2 = H$), **5i** ($R^2 = Me$), **5j** ($R^2 = Et$) and **5k** ($R^2 = Bu$) show inhibition percentage of 32%, 1.3%, 41% and 38%, respectively. An additional increment in the R^2 volume decreases inhibition activity, and compound **5l** ($R^2 = CH_2Ph$) inhibits the nNOS activity by 18%.

Pyrazole derivatives **6b–m** show higher nNOS inhibition in relation to the pyrazoline analogs **5a–m**. The benzene ring is not substituted in compounds **6b–d** and the *N*-ethyl group gives the better inhibition percentage. Compounds **6f–h** bear

a 5′-methoxy group and they are as potent as compounds **6b–d**, indicating that this type of substitution does not significantly affect the inhibition. Finally, in compounds **6i–m**, the benzene ring bears a 5′-chloro substituent and these compounds show better inhibition percentages in this series, like in pyrazoline derivatives **5i–m**. Similarly to compounds **5i–m**, the influence of the *N*-alkyl (R^2) group is not so clear. Compounds **6m** ($R^2 = H$) and **6j** ($R^2 = Et$) are the more potent inhibitors (40% and 35%, respectively), and an additional increase in the R^2 volume decreases the inhibition.

From the previous data, it can be concluded that the 5′-Cl substituent is the best one for the nNOS inhibition in both families of compounds **5** and **6**, and $R^2 = Et$ or $R^2 = Bu$ is the more appropriated substituents in the *N*-1 position of both pyrazole and pyrazoline moieties.

Some of these compounds also inhibit iNOS (Table 2). Among pyrazoline derivatives, the inhibition percentages are low; in fact only compound **5i** ($R^1 = Cl$, $R^2 = Me$) shows a 23% of inhibition, and changing the *N*-Me group by any other one diminishes the iNOS inhibition. On the other hand, compounds belonging to the pyrazole family **6** show higher inhibition percentages than the corresponding pyrazoline compounds **5**. Among them, compounds **6d**, **6h** and **6l** show 13%, 30% and 50% inhibition percentages, respectively, indicating that the *N*-CH_2Ph group is the best one for the activity. In both families of compounds, the 5′-Cl substitution is better for the inhibition than the 5′-OMe group, and this is better than a non-substituted benzene ring. 5′-Cl substituent is also preferred in the *N*-acyl benzoyl-pyrazolines **4** [17].

2.3. Docking studies

Several iNOS and nNOS crystal structures can be found in the Brookhaven protein databank. A recent paper [20] describes the crystal structure of N^{ω}-propyl-L-arginine (NPA), a nNOS selective inhibitor, co-crystallized with the heme oxygenase domain of both nNOS (PDB id: 1QW6) and iNOS (PDB id: 1QW4). NPA binds to nNOS (or iNOS) in the enzyme active site, similarly to the natural substrate L-arginine. The stronger interaction between the inhibitor and the enzyme is the reinforced hydrogen bond formed between the guanidinium moiety of the inhibitor and Glu592 (Glu371 in iNOS) carboxylate (Fig. 1). On the other hand, the NPA amine group also forms an hydrogen bond with one of the Glu592 carboxylate O atoms, while the NPA carboxylate interacts by means of hydrogen bonds with both the Tyr562 (Tyr341 in iNOS) hydroxyl and Gln478 $N^{\varepsilon 2}$-H atoms.

In the same paper, authors justify the nNOS selectivity of N^{ω}-propyl-L-arginine as a consequence of the mutation of Asn498 in nNOS by a Thr277 in iNOS. These residues are

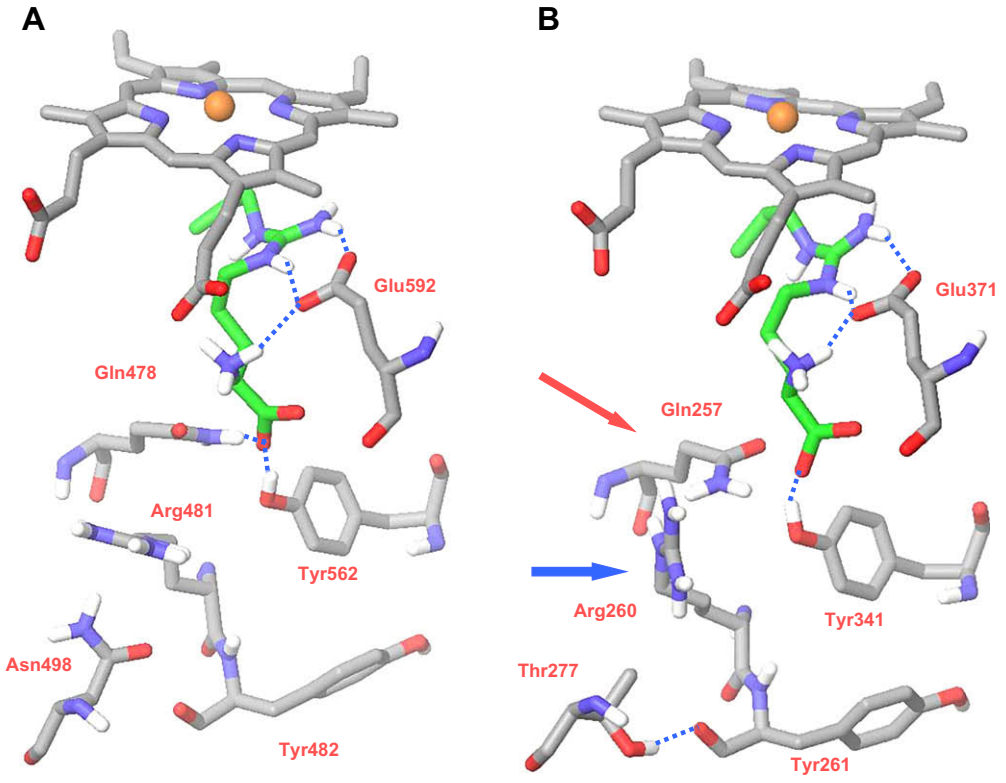


Fig. 1. Detailed views of N^{ω}-propyl-L-arginine inside the binding site of the heme oxygenase domain in both nNOS (A) and iNOS (B) complexes' crystal structures. Dotted blue lines represent hydrogen bonds between the inhibitor and the enzymes. A reinforced hydrogen bond between the positively charged ligand guanidinium moiety and Glu592 (nNOS) or Glu371 (iNOS) is the main interaction stabilizing the complex. Additional hydrogen bonds between the ligand amine group and Glu592 (or Glu371), and the ligand carboxylate and Tyr562 (or Tyr341) also contribute to the complex stability. In iNOS Thr277 (Asn498 in nNOS) modifies the Arg260 side-chain (blue arrow), which in turn alters the orientation of Gln257 side-chain (red arrow) and prevents the formation of the hydrogen bond between this residue and the ligand, being this the reason of the higher nNOS selectivity of this inhibitor (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.).

situated in the substrate access channel of both enzymes, relatively far away from the binding site (Fig. 1). In nNOS, the Asn498 side-chain interacts with Arg481 side-chain, which in turn stabilises the Gln478 side-chain, allowing the formation of the hydrogen bond between Gln478 $N^{\varepsilon 2}$-H and one of the NPA carboxylate O atoms. Instead of that, in iNOS the corresponding Thr277 side-chain forms a hydrogen bond with Tyr261, allowing the rotation of Arg260 side-chain (Fig. 1, blue arrow). This rotation modifies the Gln257 side-chain orientation (Fig. 1, red arrow) preventing the formation of the hydrogen bond with the NPA carboxylate. As a consequence, the iNOS/NPA complex will be less stable and this could be the reason for the observed selectivity of this inhibitor for nNOS.

Glide program [21] has been used for the docking studies. Three dimensional structures of the heme oxygenase domain of both nNOS and iNOS isoenzymes have been obtained from 1QW6 and 1QW4 crystal structures, respectively. Preparation of enzyme, the heme group and ligands has been performed following standard procedures included in the Glide program suite.

In general, Glide's Gscore values obtained for complexes of compounds **9b–m** and **10b–m** in both nNOS and iNOS binding sites are small (about −3.50 kcal/mol) indicating that these complexes are weak, and consequently both families of compounds are weak inhibitors. No linear relationships have been found between Gscore values and the inhibition percentage.

One pose has been obtained for compounds **6b–m** inside nNOS binding site. Fig. 2 shows such a pose for compound **6g**. It can be observed that the ligand CO_2Et group is situated almost parallel to the heme group, in a similar position to the NPA guanidinium moiety, probably due to a favourable interaction between the Fe atom and the electron-rich π-system of the ester group. The aminophenyl moiety of the ligand points towards Glu592, and only one hydrogen bond is formed between one of the Glu592 carboxylate O atom and the 2′-NH_2 group of the ligand. Finally, the ligand *N*-alkyl substituent is situated into a binding pocket near both heme side-chains (red arc). This binding pocket is big enough to accommodate even an *N*-CH_2Ph substituent, and for this reason, all compounds **6b–m** show the same preferred pose.

In compounds **5a–m**, the pyrazoline C-5 atom is chiral and two enantiomers must be considered. Nevertheless, the binding zone below the Fe atom is relatively big and the CO_2Et substituent of both *R* and *S* enantiomers can be accommodated easily in this zone. For this reason, the preferred pose of compounds **5a–m** inside nNOS is equivalent to that of their analogs **6b–m**, being very small the differences in Gscore values between both families of compounds and between *R* and *S* enantiomers of compounds **5a–m**. Compounds **5a–m** are slightly worse nNOS inhibitors than compounds **6b–m**, and the reason could be the existence of the two enantiomers which form two complexes, one of them slightly less stable.

Docking experiments performed with compounds **5a–m** and **6b–m** on iNOS indicate that both families behave in a similar way. Gscore values are small in all compounds, about of the same order than in nNOS experiments. Nevertheless, the main pose obtained for these compounds in iNOS is slightly different to that described for nNOS. Fig. 2 shows the pose obtained for compound **6g** in the iNOS binding site. It can be observed that the CO_2Et group of the ligand is situated near the Fe atom and almost parallel to the heme group, similarly to the nNOS/**6g** complex. On the other hand, in iNOS the Gln257 side-chain is rotated in relation to its homologous residue in nNOS (Gln478) (*vide supra*), and this side-chain prevents the ligand aminophenyl moiety to be situated near to Glu371. Consequently, the ligand *N*-butyl substituent is accommodated between Gln257 and Glu371 (red arrow), while the aminophenyl moiety is situated near the heme side-chains.

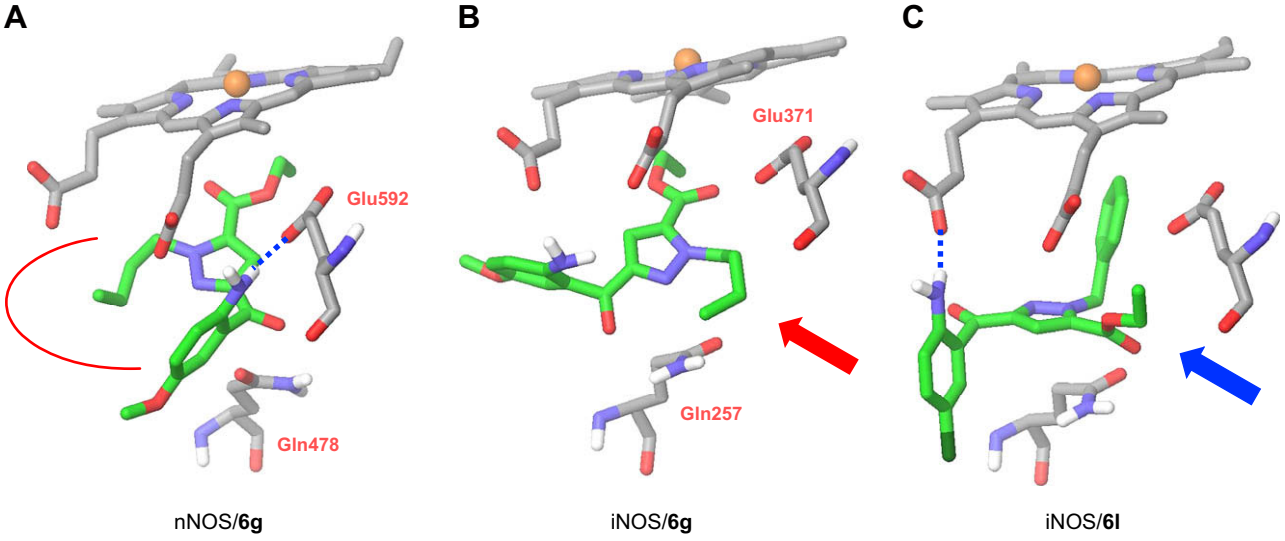


Fig. 2. The preferred poses obtained for compound **6g** in nNOS (A) and iNOS (B), and for compound **6l** in iNOS (C). Rotation of iNOS Gln257 side-chain in relation to the same nNOS residue (Gln478) changes the relative situation of **6g** aminophenyl and *N*-butyl groups inside both enzymes. The bigger *N*-CH_2Ph substituent of compound **6l** cannot be accommodated between Gln257 and Glu371 (right) and yields a different pose that could explain the higher activity and selectivity of this molecule (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.).

This pose is common to all compounds except **6l** and **6h**, which show a different pose. Fig. 2 also shows the preferred pose obtained for compound **6l**. The *N*-CH_2Ph substituent is too big to be accommodated between Gln257 and Glu371, and this part of the binding site is fulfilled by the CO_2Et group (blue arrow), while the *N*-CH_2Ph occupies the zone below the heme group. Finally, the aminophenyl moiety is situated near the heme side-chain, and a hydrogen bond between the ligand 2′-NH_2 group and one of the heme carboxylates is formed. This hydrogen bond can be formed in this complex due to the big volume of the *N*-CH_2Ph group, which shifts the molecule far away from the heme group and allows the rotation of the aminophenyl moiety in order to form the hydrogen bond.

In general, all complexes have been demonstrated to be weak, and this can be due to two different reasons. On the one hand, the reinforced hydrogen bond described for L-arginine and N^{ω}-propyl-L-arginine (which is also present in other known and potent NOS inhibitors) is not formed by our compounds, and this can be one reason for the small Gscore value and consequently the lack of potency of these compounds.

On the other hand, Fig. 2 shows that in all complexes the ligand phenyl ring and CO bond are not coplanar, and the expected conjugation among both moieties is broken. The additional energy necessary for the ligand to adopt such conformations in the complexes is detrimental for the stability of the complexes and hence this could be another reason for the small activity of these molecules.

Glide's Gscore values are the sum of several terms of energy, among them a term defining the internal torsional energy of the ligand conformer. In our ligands, this term partially reflects the lack of conjugation between the phenyl ring and the CO bond among other rotational components, and is always positive giving place to the observed small negative Gscore values.

Nevertheless, a real quantification of internal torsional energy inside the protein force field is difficult to assess and additional calculations are needed. For this reason, ab initio calculations have been performed using model compounds in order to estimate the rotational barrier around the Ph–CO bond as well as the possible influence of the lack of conjugation between both the CO bond and the phenyl ring on the stability of the complex.

In a first stage, a conformational search of both 3-(2-aminobenzoyl)-4,5-dihydro-1*H*-pyrazole **21** and 3-(2-aminobenzoyl)-1*H*-pyrazole **22** (considered as model compounds of **5** and **6**, respectively) using MacroModel [22] program has been tackled, in order to identify the more stable conformer of these molecules. Further, the preferred conformer has been optimized by means of Gaussian 98 program [23] using the Hartree–Fock Hamiltonian at a 6-31G* level. Fig. 3 shows such conformer for both compounds, and it can be observed that the CO bond and the benzene ring lie almost in the same plane, defining a dihedral angle of about 20°. An intramolecular hydrogen bond between the 2′-NH_2 and the CO groups also stabilized such conformation. Finally, the CO group and the pyrazole (or pyrazoline) C=N double bond are situated in an antiperiplanar disposition, probably with

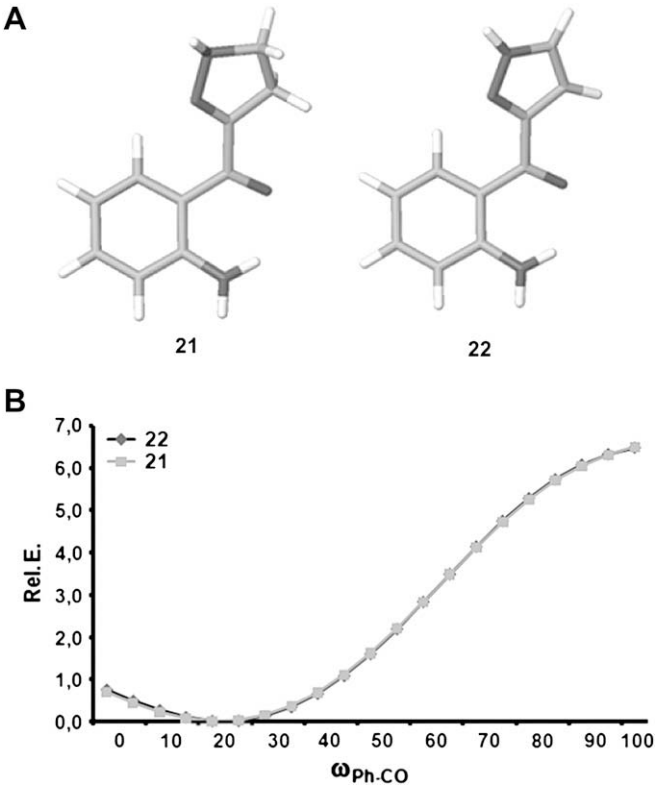


Fig. 3. (A) The preferred calculated conformation (Gaussian 98) of both 3-(2-aminobenzoyl)-2,3-dihydro-1*H*-pyrazole **21** and 3-(2-aminobenzoyl)-1*H*-pyrazole **22**, considered as model compounds of compounds **5** and **6**. (B) Variation of the relative energy (kcal/mol) in compounds **21** and **22** during the rotation around the Ph–CO bond.

the object of minimizing the electrostatic interactions between both heteroatoms.

This conformation has been used as starting point, and a relaxed scan around the Ph–CO bond has been performed by means of Gaussian program. Fig. 3 also shows the energy variation during the rotation. It can be observed that in both molecules energy grows continuously when rotation takes places from 20° to 100°, being the difference between them very small. A higher energy increment in compounds **6** would be expected since in these molecules conjugation is extended over the benzene ring, the CO, the pyrazole moiety and the CO_2Et group. Nevertheless, ab initio calculation on model compounds indicates a similar energy increment in both molecules.

In our complexes, the ligand Ph–CO torsional angle value has a value between 70° and 95°, and hence ligands need 4–6 kcal/mol to adopt such conformation. Such energy comes from the enzyme–ligand interaction and consequently the complexes are weaker.

3. Conclusions

In general, compounds **5a–m** and **6b–m** behave as weak inhibitors of both iNOS and nNOS. Docking studies indicate that the putative complexes formed by these molecules inside the nNOS and iNOS binding sites are not too stable, and this is

the reason for the small inhibition percentage observed. The small stability of these complexes seems to be mainly due to two factors: (i) the lack of the reinforced hydrogen bond formed between the ligand and Glu592 (nNOS) or Glu371 (iNOS) and observed in nNOS/N^{ω}-propyl-L-arginine complex; and (ii) an unfavorable ligand conformation that weakens the Ph–CO conjugation.

Compound **6l** shows the better iNOS inhibition percentage and the better iNOS/nNOS selectivity of all studied compound. These two facts could be due to the different binding mode of such compound observed in the iNOS complex. Further modifications of this molecule could serve to obtain more potent and selective iNOS inhibitors.

4. Experimental section

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 Bruker AMX 300 spectrometer operating at 75.479 MHz for ^{13}C and 300.160 MHz for ^{1}H and on a Bruker ARX 400 spectrometer operating at 400.132 MHz for ^{1}H and 100.623 MHz for ^{13}C, in $CDCl_3$ (at concentration of ca. 27 mg/mL 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 parts per million (δ). High-resolution mass spectroscopy (HRMS) was carried out on a VG AutoSpec Q high-resolution mass spectrometer (Fisons Instruments). Elemental analyses were performed on a Perkin Elmer 240 C and agreed with theoretical values within ±0.4%. Flash chromatography was carried out using silica gel 60, 230–240 mesh (Merck), and the solvent mixture reported within parentheses was used as eluent.

*4.1. Preparation of 1-alkyl-3-(2-nitro-5-substituted-benzoyl)-4,5-dihydro-1H-pyrazole-5-carboxylic acid ethyl esters (**19a–l**) and 1-alkyl-3-(2-nitro-5-substituted-benzoyl)-1H-pyrazole-5-carboxylic acid ethyl esters (**20b–m**)*

To a solution of the corresponding 3-(2-nitro-5-substituted-benzoyl)-4,5-dihydro-1*H*-pyrazole-5-carboxylic acid ethyl esters **13–15** (1 mmol) in THF (4 mL) or 1,4-dioxane (7 mL), 2.58 mmol of K_2CO_3 and 2.12 mmol of the alkylating agent were added. The mixture was refluxed and stirred, and after cooling the K_2CO_3 precipitated was filtered off and the filtrate was concentrated in vacuo. The residue was solved in water (100 mL) and extracted with ethyl acetate (3 × 30 mL), and the combined layers were dried (Na_2SO_4), filtered and concentrated to yield a residue that was purified by flash chromatography (ethyl acetate/hexane 1:9).

*4.1.1. 1-Methyl-3-(2-nitrobenzoyl)-4,5-dihydro-1H-pyrazole-5-carboxylic acid ethyl ester, **19a***

Yield 84%, ^{1}H NMR ($CDCl_3$): $\delta = 8.05$ (dd, $J = 8.1$, 1.1 Hz, 1H), 7.69 (dt, $J = 7.8$, 7.5, 1.1 Hz, 1H), 7.58 (dt, $J = 8.1$, 7.8, 1.4 Hz, 1H), 7.50 (dd, $J = 7.5$, 1.4 Hz, 1H), 4.25 (m, 3H), 3.46 (dd, $J = 17.3$, 13.1 Hz, 1H), 3.32 (dd, $J = 17.3$, 10.4 Hz, 1H), 3.10 (s, 3H), 1.32 ppm (t, $J = 7.1$ Hz, 3H); ^{13}C NMR ($CDCl_3$): $\delta = 186.28$, 169.44, 148.26, 144.97, 135.44, 133.62, 130.42, 129.46, 123.92, 68.29, 62.01, 39.79, 34.69, 14.22 ppm; MS (LSIMS) *m/z* 328.0909 $(M + Na)^+$, calcd mass for $C_{14}H_{15}N_3O_5Na$: 328.0909.

*4.1.2. 1-Ethyl-3-(2-nitrobenzoyl)-4,5-dihydro-1H-pyrazole-5-carboxylic acid ethyl ester, **19b***

Yield 46%, ^{1}H NMR ($CDCl_3$): $\delta = 8.02$ (dd, $J = 8.1$, 1.1 Hz, 1H), 7.67 (dt, $J = 7.7$, 7.5, 1.1 Hz, 1H), 7.56 (dt, $J = 8.1$, 7.7, 1.6 Hz, 1H), 7.50 (dd, $J = 7.5$, 1.6 Hz, 1H), 4.37 (dd, $J = 13.0$, 10.4 Hz, 1H), 4.23 (q, $J = 7.1$ Hz, 2H), 3.37 (m, 4H), 1.29 (t, $J = 7.1$ Hz, 3H), 1.12 ppm (t, $J = 7.2$ Hz, 3H); ^{13}C NMR ($CDCl_3$): $\delta = 186.08$, 169.81, 148.23, 144.58, 135.39, 133.48, 130.56, 129.55, 123.75, 66.05, 61.94, 46.95, 34.44, 14.18, 12.46; MS (LSIMS) *m/z* 342.106724 $(M + Na)^+$, calcd mass for $C_{15}H_{17}N_3O_5Na$: 342.106591.

*4.1.3. 1-Butyl-3-(2-nitrobenzoyl)-4,5-dihydro-1H-pyrazole-5-carboxylic acid ethyl ester, **19c***

Yield 8%, ^{1}H NMR ($CDCl_3$): $\delta = 8.02$ (dd, $J = 8.1$, 1.2 Hz, 1H), 7.67 (dt, $J = 7.7$, 7.5, 1.2 Hz, 1H), 7.56 (dt, $J = 8.1$, 7.7, 1.6 Hz, 1H), 7.50 (dd, $J = 7.5$, 1.6 Hz, 1H), 4.37 (dd, $J = 12.4$, 10.2 Hz, 1H), 4.24 (q, $J = 7.1$ Hz, 2H), 3.33 (m, 4H), 1.50 (m, 2H), 1.28 (m, 2H), 1.30 (t, $J = 7.1$ Hz, 3H), 0.86 ppm (t, $J = 7.2$ Hz, 3H); ^{13}C NMR ($CDCl_3$): $\delta = 186.10$, 169.86, 148.15, 143.99, 135.47, 133.56, 130.36, 129.57, 123.82, 66.39, 62.01, 51.92, 34.38, 29.52, 19.97, 14.23, 13.75 ppm; MS (LSIMS) *m/z* 370.1355 $(M + Na)^+$, calcd mass for $C_{17}H_{21}N_3O_5Na$: 370.1378.

*4.1.4. 1-Benzyl-3-(2-nitrobenzoyl)-4,5-dihydro-1H-pyrazole-5-carboxylic acid ethyl ester, **19d***

Yield 26%, ^{1}H NMR ($CDCl_3$): $\delta = 8.09$ (dd, $J = 8.1$, 1.1 Hz, 1H), 7.70 (dt, $J = 7.7$, 7.5, 1.1 Hz, 1H), 7.59 (dt, $J = 8.1$, 7.7, 1.5 Hz, 1H), 7.50 (dd, $J = 7.5$, 1.5 Hz, 1H), 7.30 (m, 3H), 7.13 (m, 2H), 4.68 (d, $J = 14.8$ Hz, 1H), 4.46 (d, $J = 14.8$ Hz, 1H), 4.16 (m, 3H), 3.37 (dd, $J = 17.4$, 12.9 Hz, 1H), 3.29 (dd, $J = 17.4$, 11.4 Hz, 1H), 1.26 ppm (t, $J = 7.1$ Hz, 3H); ^{13}C NMR ($CDCl_3$): $\delta = 186.51$, 169.60, 147.82, 145.10, 135.57, 134.87, 133.69, 130.42, 129.41, 128.81, 128.77, 128.18, 123.96, 64.71, 61.93, 56.33, 34.40, 14.16 ppm; MS (LSIMS) *m/z* 404.1220 $(M + Na)^+$, calcd mass for $C_{20}H_{19}N_3O_5Na$: 404.1222.

*4.1.5. 3-(5-Methoxy-2-nitrobenzoyl)-1-methyl-4,5-dihydro-1H-pyrazole-5-carboxylic acid ethyl ester, **19e***

Yield 84%, ^{1}H NMR ($CDCl_3$): $\delta = 8.01$ (d, $J = 9.1$ Hz, 1H), 6.93 (dd, $J = 9.1$, 2.8 Hz, 1H), 6.81 (d, $J = 2.8$ Hz, 1H), 4.20 (q, $J = 7.1$ Hz, 2H), 3.83 (s, 3H), 3.39 (dd, $J = 17.3$, 13.0 Hz, 1H), 3.25 (dd, $J = 17.3$, 10.7 Hz, 1H), 3.03 (s, 3H), 1.25 ppm (t, $J = 7.1$ Hz, 3H); ^{13}C NMR ($CDCl_3$): $\delta = 186.17$, 169.32, 163.57, 144.76, 140.24, 137.98, 126.38, 114.97, 113.74, 68.08, 61.08, 56.05, 39.65, 34.50,

14.04 ppm; MS (LSIMS) *m/z* 358.1013 $(M+Na)^+$, calcd mass for $C_{15}H_{17}N_3O_6Na$: 358.1015.

4.1.6. 1-Ethyl-3-(5-methoxy-2-nitrobenzoyl)-4,5-dihydro-1H-pyrazole-5-carboxylic acid ethyl ester, **19f**

Yield 38%, ^{1}H NMR ($CDCl_3$): $\delta = 8.03$ (d, $J = 9.1$ Hz, 1H), 6.96 (dd, $J = 9.1$, 2.8 Hz, 1H), 6.86 (d, $J = 2.8$ Hz, 1H), 4.35 (dd, $J = 13.1$, 10.5 Hz, 1H), 4.21 (q, $J = 7.1$ Hz, 2H), 3.87 (s, 3H), 3.34 (m, 4H), 1.27 (t, $J = 7.1$ Hz, 3H), 1.08 ppm (t, $J = 7.2$ Hz, 3H); ^{13}C NMR ($CDCl_3$): $\delta = 186.19$, 169.87, 163.63, 144.59, 140.88, 138.09, 126.35, 115.22, 113.90, 65.95, 61.92, 56.16, 46.30, 34.47, 14.17, 12.43 ppm; MS (LSIMS) *m/z* 350.1355 $(M+H)^+$, calcd mass for $C_{16}H_{20}N_3O_6$: 350.1352.

4.1.7. 1-Butyl-3-(5-methoxy-2-nitrobenzoyl)-4,5-dihydro-1H-pyrazole-5-carboxylic acid ethyl ester, **19g**

Yield 33%, ^{1}H NMR ($CDCl_3$): $\delta = 8.05$ (d, $J = 9.1$ Hz, 1H), 6.97 (dd, 1H, $J = 9.1$, 2.8 Hz, 1H), 6.87 (d, 1H, $J = 2.8$ Hz, 1H), 4.36 (dd, $J = 13.2$, 10.3 Hz, 1H), 4.22 (q, $J = 7.1$ Hz, 2H), 3.88 (s, 3H), 3.32 (m, 4H), 1.48 (m, 2H), 1.29 (t, $J = 7.1$ Hz, 3H), 1.23 (m, 2H), 0.84 ppm (t, $J = 7.2$ Hz, 3H); ^{13}C NMR ($CDCl_3$): $\delta = 186.14$, 169.87, 163.61, 143.98, 140.85, 138.20, 126.36, 115.24, 113.83, 66.31, 61.93, 56.87, 51.87, 34.41, 29.49, 19.93, 14.19, 13.68 ppm; MS (LSIMS) *m/z* 400.1488 $(M+Na)^+$, calcd mass for $C_{18}H_{23}N_3O_6Na$: 400.1484.

4.1.8. 1-Benzyl-3-(5-methoxy-2-nitrobenzoyl)-4,5-dihydro-1H-pyrazole-5-carboxylic acid ethyl ester, **19h**

Yield 15%, ^{1}H NMR ($CDCl_3$): $\delta = 8.12$ (d, $J = 9.1$ Hz, 1H), 7.30 (m, 3H), 7.12 (m, 2H), 7.00 (dd, $J = 9.1$, 2.8 Hz, 1H), 6.88 (d, $J = 2.8$ Hz, 1H), 4.69 (d, $J = 14.8$ Hz, 2H), 4.45 (d, $J = 14.8$ Hz, 1H), 4.17 (m, 3H), 3.91 (s, 3H), 3.36 (dd, $J = 17.3$ Hz, 12.8 Hz, 1H), 3.29 (dd, $J = 17.3$, 11.7 Hz, 1H), 1.26 ppm (t, $J = 7.1$ Hz, 3H); ^{13}C NMR ($CDCl_3$): $\delta = 186.59$, 169.67, 163.79, 145.21, 140.52, 138.27, 134.86, 128.82, 128.75, 128.17, 126.56, 115.32, 113.75, 64.67, 61.92, 56.32, 56.20, 34.42, 14.16 ppm; MS (LSIMS) *m/z* 434.1322 $(M+Na)^+$, calcd mass for $C_{21}H_{21}N_3O_6Na$: 434.1328.

4.1.9. 3-(5-Chloro-2-nitrobenzoyl)-1-methyl-4,5-dihydro-1H-pyrazole-5-carboxylic acid ethyl ester, **19i**

Yield 82%, mp 83–85 °C; ^{1}H NMR ($CDCl_3$): $\delta = 8.01$ (d, $J = 8.7$ Hz, 1H), 7.52 (dd, $J = 8.7$, 2.3 Hz, 1H), 7.44 (d, $J = 2.3$ Hz, 1H), 4.27 (m, 3H), 3.44 (dd, $J = 17.4$, 13.3 Hz, 1H), 3.30 (dd, $J = 17.4$, 10.3 Hz, 1H), 3.12 (s, 3H), 1.31 ppm (t, $J = 7.1$ Hz, 3H); ^{13}C NMR ($CDCl_3$): $\delta = 184.47$, 169.24, 146.33, 144.28, 140.31, 136.99, 130.27, 129.49, 125.41, 68.18, 62.07, 39.64, 34.44, 14.20 ppm; MS (LSIMS) *m/z* 362.0515 $(M+Na)^+$, calcd mass for $C_{14}H_{14}ClN_3O_5Na$: 362.0519.

4.1.10. 3-(5-Chloro-2-nitrobenzoyl)-1-ethyl-4,5-dihydro-1H-pyrazole-5-carboxylic acid ethyl ester, **19j**

Yield 40%, ^{1}H NMR ($CDCl_3$): $\delta = 7.98$ (d, $J = 8.7$ Hz, 1H), 7.53 (dd, $J = 8.7$, 2.2 Hz, 1H), 7.49 (d, $J = 2.2$ Hz, 1H), 4.38 (dd, $J = 13.3$, 10.0 Hz, 1H), 4.25 (q, $J = 7.1$ Hz, 2H), 3.38 (m, 4H), 1.32 (t, $J = 7.1$ Hz, 3H), 1.13 ppm ($J = 7.2$ Hz, 3H); ^{13}C NMR ($CDCl_3$): $\delta = 184.29$, 169.60, 146.35, 143.96, 140.18, 136.96, 130.23, 129.63, 125.25, 66.06, 62.04, 46.89, 34.22, 14.18, 12.53 ppm; MS (LSIMS) *m/z* 376.0673 $(M+Na)^+$, calcd mass for $C_{15}H_{16}N_3O_5ClNa$: 376.0676.

4.1.11. 1-Butyl-3-(5-chloro-2-nitrobenzoyl)-4,5-dihydro-1H-pyrazole-5-carboxylic acid ethyl ester, **19k**

Yield 29%, ^{1}H NMR ($CDCl_3$): $\delta = 7.98$ (d, $J = 8.7$ Hz, 1H), 7.50 (dd, $J = 8.7$, 2.3 Hz, 1H), 7.45 (d, $J = 2.3$ Hz, 1H), 4.46 (dd, $J = 13.3$, 10.3 Hz, 1H), 4.23 (q, $J = 7.1$ Hz, 2H), 3.33 (m, 4H), 1.50 (m, 2H), 1.29 (t, $J = 7.1$ Hz, 3H), 1.26 (m, 2H), 0.86 ppm (t, $J = 7.2$ Hz, 3H); ^{13}C NMR ($CDCl_3$): $\delta = 184.20$, 169.56, 146.28, 143.71, 140.14, 137.04, 130.19, 129.62, 125.27, 66.36, 62.03, 51.81, 34.13, 29.54, 19.92, 14.17, 13.66 ppm; MS (LSIMS) *m/z* 404.0990 $(M+Na)^+$, calcd mass for $C_{17}H_{20}N_3O_5ClNa$: 404.0989.

4.1.12. 1-Benzyl-3-(5-chloro-2-nitrobenzoyl)-4,5-dihydro-1H-pyrazole-5-carboxylic acid ethyl ester, **19l**

Yield 12%, ^{1}H NMR ($CDCl_3$): $\delta = 8.04$ (d, $J = 8.7$ Hz, 1H), 7.54 (dd, $J = 8.7$, 2.3 Hz, 1H), 7.46 (d, $J = 2.3$ Hz, 1H), 7.31 (m, 3H), 7.13 (m, 2H), 4.70 (d, $J = 15.1$ Hz, 1H), 4.48 (d, $J = 15.1$ Hz, 1H), 4.17 (m, 3H), 3.37 (dd, $J = 17.3$, 12.5 Hz, 1H), 3.27 (dd, $J = 17.3$, 11.5 Hz, 1H), 1.27 ppm (t, $J = 7.1$ Hz, 3H); ^{13}C NMR ($CDCl_3$): $\delta = 184.70$, 169.41, 145.98, 144.56, 140.37, 137.09, 134.71, 130.32, 129.52, 128.83, 128.78, 128.27, 125.47, 64.77, 62.02, 56.28, 34.17, 14.15 ppm; MS (LSIMS) *m/z* 438.0835 $(M+Na)^+$, calcd mass for $C_{20}H_{18}N_3O_5ClNa$: 438.0834.

4.1.13. 1-Ethyl-3-(2-nitrobenzoyl)-1H-pyrazole-5-carboxylic acid ethyl ester, **20b**

Yield 26%, mp 91–93 °C; ^{1}H NMR ($CDCl_3$): $\delta = 8.12$ (dd, $J = 8.1$, 1.1 Hz, 1H), 7.75 (dt, $J = 7.7$, 7.5, 1.1 Hz, 1H), 7.65 (dt, $J = 8.1$, 7.7, 1.6 Hz, 1H), 7.59 (dd, $J = 7.5$, 1.6 Hz, 1H), 7.45 (s, 1H), 4.51 (q, $J = 7.2$ Hz, 2H), 4.35 (q, $J = 7.1$ Hz, 2H), 1.37 (t, $J = 7.1$ Hz, 3H), 1.34 ppm (t, $J = 7.2$ Hz, 3H); ^{13}C NMR ($CDCl_3$): $\delta = 186.74$, 159.14, 148.38, 135.27, 133.84, 130.93, 129.61, 123.91, 112.73, 61.54, 48.10, 15.26, 14.22 ppm; MS (LSIMS) *m/z* 340.0909 $(M+Na)^+$, calcd mass for $C_{15}H_{15}N_3O_5Na$: 340.0909.

4.1.14. 1-Butyl-3-(2-nitrobenzoyl)-1H-pyrazole-5-carboxylic acid ethyl ester, **20c**

Yield 35%, ^{1}H NMR ($CDCl_3$): $\delta = 8.12$ (dd, $J = 8.1$, 1.2 Hz, 1H), 7.75 (dt, $J = 7.7$, 7.4, 1.2 Hz, 1H), 7.65 (dt, $J = 8.1$, 7.7, 1.5 Hz, 1H), 7.58 (dd, $J = 7.4$, 1.5 Hz, 1H), 7.46 (s, 1H), 4.46 (t, $J = 7.2$ Hz, 2H), 4.34 (q, $J = 7.1$ Hz, 2H), 1.71 (m, 2H), 1.37 (t, $J = 7.1$ Hz, 3H), 1.23 (m, 2H), 0.87 ppm (t, $J = 7.2$ Hz, 3H); ^{13}C NMR ($CDCl_3$): $\delta = 186.82$, 159.19, 148.29, 148.17, 135.35, 134.14, 133.88, 130.90, 129.57, 123.94, 112.69, 61.51, 52.54, 32.20, 19.61, 14.22, 13.56 ppm; MS (LSIMS) *m/z* 346.1406 $(M+H)^+$, calcd mass for $C_{17}H_{20}N_3O_5$: 346.1402.

4.1.15. 1-Benzyl-3-(2-nitrobenzoyl)-1H-pyrazole-5-carboxylic acid ethyl ester, ***20d***

Yield 27%, mp 106–108 °C; ^{1}H NMR ($CDCl_3$): $\delta = 8.15$ (dd, $J = 8.1$, 1.0 Hz, 1H), 7.76 (dt, $J = 7.7$, 7.5, 1.0 Hz, 1H), 7.66 (dt, $J = 8.1$, 7.7, 1.5 Hz, 1H), 7.60 (dd, $J = 7.5$, 1.5 Hz, 1H), 7.51 (s, 1H), 7.26 (m, 3H), 7.12 (m, 2H), 5.66 (s, 2H), 4.30 (q, $J = 7.1$ Hz, 2H), 1.32 ppm (t, $J = 7.1$ Hz, 3H); ^{13}C NMR ($CDCl_3$): $\delta = 186.84$, 159.02, 148.76, 148.08, 135.90, 135.33, 134.29, 133.96, 130.96, 129.58, 128.64, 128.05, 127.59, 124.02, 113.05, 61.61, 55.95, 14.17 ppm; MS (LSIMS) *m/z* 402.1064 $(M + Na)^+$, calcd mass for $C_{20}H_{17}N_3O_5Na$: 402.1065.

4.1.16. 1-Ethyl-3-(5-methoxy-2-nitrobenzoyl)-1H-pyrazole-5-carboxylic acid ethyl ester, ***20f***

Yield 17%, mp 104–106 °C; ^{1}H NMR ($CDCl_3$): $\delta = 8.13$ (d, $J = 9.1$ Hz, 1H), 7.43 (s, 1H), 7.05 (dd, $J = 9.1$, 2.8 Hz, 1H), 6.99 (d, $J = 2.8$ Hz, 1H), 4.51 (q, $J = 7.2$ Hz, 2H), 4.34 (q, $J = 7.1$ Hz, 2H), 3.91 (s, 3H), 1.31 ppm (t, $J = 7.2$ Hz, 6H); ^{13}C NMR ($CDCl_3$): $\delta = 186.72$, 163.92, 159.14, 148.34, 140.94, 137.95, 133.81, 126.50, 115.57, 114.07, 112.72, 61.52, 56.24, 48.11, 15.53, 14.22 ppm; MS (LSIMS) *m/z* 340.0909 $(M + H)^+$, calcd mass for $C_{16}H_{18}N_3O_6$: 348.1195.

4.1.17. 1-Butyl-3-(5-methoxy-2-nitrobenzoyl)-1H-pyrazole-5-carboxylic acid ethyl ester, ***20g***

Yield 23%, ^{1}H NMR ($CDCl_3$): $\delta = 8.15$ (d, $J = 9.1$ Hz, 1H), 7.45 (s, 1H), 7.06 (dd, $J = 9.1$, 2.8 Hz, 1H), 6.97 (d, $J = 2.8$ Hz, 1H), 4.47 (t, $J = 7.3$ Hz, 2H), 4.35 (q, $J = 7.1$ Hz, 2H), 3.92 (s, 3H), 1.72 (m, 2H), 1.37 (t, $J = 7.1$ Hz, 3H), 1.23 (m, 2H), 0.88 ppm (t, $J = 7.3$ Hz, 3H); ^{13}C NMR ($CDCl_3$): $\delta = 186.79$, 163.98, 159.14, 148.26, 140.90, 138.03, 134.07, 126.51, 115.60, 113.99, 112.69, 61.49, 56.23, 52.54, 32.26, 19.91, 14.26, 13.57 ppm; MS (LSIMS) *m/z* 398.1323 $(M + Na)^+$, calcd mass for $C_{18}H_{21}N_3O_6Na$: 398.1328.

4.1.18. 1-Benzyl-3-(5-methoxy-2-nitrobenzoyl)-1H-pyrazole-5-carboxylic acid ethyl ester, ***20h***

Yield 38%, ^{1}H NMR ($CDCl_3$): $\delta = 8.16$ (d, $J = 9.1$ Hz, 1H), 7.49 (s, 1H), 7.26 (m, 3H), 7.12 (m, 2H), 7.06 (dd, $J = 9.1$, 2.7 Hz, 1H), 6.98 (d, $J = 2.7$ Hz, 1H), 5.66 (s, 2H), 4.29 (q, $J = 7.1$ Hz, 2H), 3.91 (s, 3H), 1.31 ppm (t, $J = 7.1$ Hz, 3H); ^{13}C NMR ($CDCl_3$): $\delta = 186.78$, 163.99, 159.01, 148.72, 140.82, 137.96, 135.94, 134.23, 128.64, 128.02, 127.55, 126.59, 115.64, 114.06, 113.02, 61.59, 56.25, 55.91, 14.16 ppm; MS (LSIMS) *m/z* 432.1174 $(M + Na)^+$, calcd mass for $C_{21}H_{19}N_3O_6Na$: 432.1171.

4.1.19. 3-(5-Chloro-2-nitrobenzoyl)-1-methyl-1H-pyrazole-5-carboxylic acid ethyl ester, ***20i***

Yield 9%, mp 128–130 °C; ^{1}H NMR ($CDCl_3$): $\delta = 8.11$ (d, $J = 8.7$ Hz, 1H), 7.60 (dd, $J = 8.7$, 2.3 Hz, 1H), 7.52 (d, $J = 2.3$ Hz, 1H), 7.48 (s, 1H), 4.36 (q, $J = 7.1$ Hz, 2H), 4.10 (s, 3H), 1.38 ppm (t, $J = 7.1$ Hz, 3H); ^{13}C NMR ($CDCl_3$): $\delta = 185.21$, 159.21, 147.94, 145.99, 140.79, 136.92, 134.85, 130.78, 129.47, 125.51, 112.57, 61.68, 40.58, 14.24 ppm; MS (LSIMS) *m/z* 360.0361 $(M + Na)^+$, calcd mass for $C_{14}H_{12}N_3O_5ClNa$: 360.0363.

4.1.20. 3-(5-Chloro-2-nitrobenzoyl)-1-ethyl-1H-pyrazole-5-carboxylic acid ethyl ester, ***20j***

Yield 27%, mp 100–102 °C; ^{1}H NMR ($CDCl_3$): $\delta = 8.07$ (d, $J = 8.7$ Hz, 1H), 7.59 (dd, $J = 8.7$, 2.3 Hz, 1H), 7.53 (d, $J = 2.3$ Hz, 1H), 7.45 (s, 1H), 4.50 (q, $J = 7.2$ Hz, 2H), 4.34 (q, $J = 7.1$ Hz, 2H), 1.33 (t, $J = 7.2$ Hz, 3H), 1.31 ppm (t, $J = 7.1$ Hz, 3H); ^{13}C NMR ($CDCl_3$): $\delta = 185.17$, 159.04, 147.97, 146.43, 140.64, 136.74, 134.05, 130.83, 129.64, 125.40, 112.75, 61.62, 48.19, 15.26, 14.22 ppm; MS (LSIMS) *m/z* 374.0522 $(M + Na)^+$, calcd mass for $C_{15}H_{14}N_3O_5ClNa$: 374.051968.

4.1.21. 1-Butyl-3-(5-chloro-2-nitrobenzoyl)-1H-pyrazole-5-carboxylic acid ethyl ester, ***20k***

Yield 28%, mp 58–60 °C; ^{1}H NMR ($CDCl_3$): $\delta = 8.08$ (d, $J = 8.7$ Hz, 1H), 7.60 (dd, $J = 8.7$, 2.2 Hz, 1H), 7.53 (d, $J = 2.2$ Hz, 1H), 7.46 (s, 1H), 4.46 (t, $J = 7.3$ Hz, 2H), 4.35 (q, $J = 7.1$ Hz, 2H), 1.69 (m, 2H), 1.37 (t, $J = 7.1$ Hz, 3H), 1.23 (m, 2H), 0.87 ppm (t, $J = 7.3$ Hz, 3H); ^{13}C NMR ($CDCl_3$): $\delta = 185.22$, 159.07, 147.88, 146.32, 140.64, 136.82, 134.32, 130.79, 129.61, 125.42, 112.69, 61.59, 52.59, 32.18, 19.59, 14.21, 13.53 ppm; MS (LSIMS) *m/z* 402.0827 $(M + Na)^+$, calcd mass for $C_{17}H_{18}N_3O_5ClNa$: 402.0832.

4.1.22. 1-Benzyl-3-(5-chloro-2-nitrobenzoyl)-1H-pyrazole-5-carboxylic acid ethyl ester, ***20l***

Yield 11%, mp 135–136 °C; ^{1}H NMR ($CDCl_3$): $\delta = 8.16$ (d, $J = 8.7$ Hz, 1H), 7.61 (dd, $J = 8.7$, 2.3 Hz, 1H), 7.56 (d, $J = 2.3$ Hz, 1H), 7.51 (s, 1H), 7.26 (m, 3H), 7.12 (m, 2H), 5.66 (s, 2H), 4.31 (q, $J = 7.1$ Hz, 1H), 1.32 ppm (t, $J = 7.1$ Hz, 3H); ^{13}C NMR ($CDCl_3$): $\delta = 185.30$, 158.95, 148.33, 146.25, 140.80, 136.76, 135.76, 134.45, 130.92, 129.67, 128.70, 128.14, 127.61, 125.35, 113.10, 61.73, 56.01, 14.20 ppm; MS (LSIMS) *m/z* 436.0670 $(M + Na)^+$, calcd mass for $C_{20}H_{16}N_3O_5ClNa$: 436.0676.

4.1.23. 3-(5-Chloro-2-nitrobenzoyl)-1H-pyrazole-5-carboxylic acid ethyl ester, ***20m***

Yield 55%, mp 199–201 °C; ^{1}H NMR ($(CD_3)_2CO$): $\delta = 13.71$ (br s, 1H), 8.24 (d, $J = 8.8$ Hz, 1H), 7.81 (dd, $J = 8.8$, 2.3 Hz, 1H), 7.75 (br s, 1H), 7.38 (s, 1H), 4.36 (q, $J = 7.1$ Hz, 2H), 1.33 ppm (t, $J = 7.1$ Hz, 3H); ^{13}C NMR ($(CD_3)_2CO$): $\delta = 185.22$, 158.37, 151.14, 146.39, 140.05, 137.05, 136.30, 131.17, 129.37, 126.02, 109.10, 61.49, 13.62 ppm; MS (LSIMS) *m/z* 324.0390 $(M + H)^+$, calcd mass for $C_{13}H_{11}N_3O_5Cl$: 324.0387.

4.2. General procedure for the preparation of compounds ***5a–m*** *and* ***6b–m***

To a suspension of the corresponding nitroarenes **15**, **19a–l**, **20b–m** (0.524 mmol) in water, a mixture of Fe (5.24 mmol) and $FeSO_4$ (0.524 mmol) was added. The reaction mixture was refluxing from 3 h, filtered through Celite and extracted with dichloromethane (3 × 15 mL) and ethyl acetate (3 × 15 mL).

The combined organic layers' phase was washed with brine, dried (Na_2SO_4), and evaporated. The residue was recrystallized from dichloromethane/hexane and/or purified by flash chromatography using the appropriate solvents in each case.

4.2.1. 3-(2-Aminobenzoyl)-1-methyl-4,5-dihydro-1H-pyrazole-5-carboxylic acid ethyl ester, ***5a***

Yield 90%, mp 80–82 °C; ^{1}H NMR ($CDCl_3$): $\delta = 8.33$ (dd, $J = 8.5$, 1.5 Hz, 1H), 7.24 (ddd, $J = 8.4$, 7.2, 1.5 Hz, 1H), 6.65 (m, 1H), 6.64 (d, $J = 8.4$ Hz, 1H), 5.96 (br s, 2H), 4.27 (q, $J = 7.1$ Hz, 2H), 4.04 (t, $J = 12.5$, 11.7 Hz, 1H), 3.51 (dd, $J = 17.3$, 12.5 Hz, 1H), 3.31 (dd, $J = 17.3$, 11.7 Hz, 1H), 3.19 (s, 3H), 1.32 ppm (t, $J = 7.1$ Hz, 3H); ^{13}C NMR ($CDCl_3$): $\delta = 188.10$, 170.24, 150.52, 147.42, 133.93, 133.83, 118.23, 116.85, 115.93, 67.95, 61.77, 40.84, 37.08, 14.26 ppm; MS (LSIMS) *m/z* 298.1170 $(M + Na)^+$, calcd mass for $C_{14}H_{17}N_3O_3Na$: 298.1167. Anal. $C_{14}H_{17}N_3O_3$ (C, H, N).

4.2.2. 3-(2-Aminobenzoyl)-1-ethyl-4,5-dihydro-1H-pyrazole-5-carboxylic acid ethyl ester, ***5b***

Yield 84%, ^{1}H NMR ($CDCl_3$): $\delta = 8.35$ (dd, $J = 8.2$, 1.6 Hz, 1H), 7.23 (ddd, $J = 8.4$, 7.2, 1.6 Hz, H-4′), 6.64 (m, 2H), 5.95 (br s, 2H), 4.25 (q, $J = 7.1$ Hz, 2H), 4.18 (dd, $J = 12.5$, 11.5 Hz, 1H), 3.41 (m, 4H), 1.31 (t, $J = 7.1$ Hz, 3H), 1.27 ppm (t, $J = 7.3$ Hz, 3H); ^{13}C NMR ($CDCl_3$): $\delta = 188.13$, 170.78, 150.51, 146.93, 133.90, 133.84, 118.35, 116.87, 115.93, 65.39, 61.78, 47.72, 36.91, 14.28, 12.77 ppm; MS (LSIMS) *m/z* 312.1317 $(M + Na)^+$, calcd mass for $C_{15}H_{19}N_3O_3Na$: 312.1324. Anal. $C_{15}H_{19}N_3O_3$ (C, H, N).

4.2.3. 3-(2-Aminobenzoyl)-1-butyl-4,5-dihydro-1H-pyrazole-5-carboxylic acid ethyl ester, ***5c***

Yield 53%, ^{1}H NMR ($CDCl_3$): $\delta = 8.34$ (dd, $J = 8.4$, 1.6 Hz, 1H), 7.22 (dt, $J = 8.4$, 7.3, 1.6 Hz, 1H), 6.64 (m, 2H), 5.94 (br s, 2H), 4.21 (m, 3H), 3.37 (m, 4H), 1.66 (m, 2H), 1.40 (m, 2H), 1.30 (t, $J = 7.1$ Hz, 3H), 0.93 ppm (t, $J = 7.3$ Hz, 3H); ^{13}C NMR ($CDCl_3$): $\delta = 188.06$, 170.69, 150.44, 146.26, 133.85, 133.68, 118.48, 116.83, 115.88, 65.79, 61.70, 52.78, 36.80, 29.81, 20.19, 14.24, 13.86 ppm; MS (LSIMS) *m/z* 318.1814 $(M + H)^+$, calcd mass for $C_{17}H_{24}N_3O_3$: 318.1817. Anal. $C_{17}H_{23}N_3O_3$ (C, H, N).

4.2.4. 3-(2-Aminobenzoyl)-1-benzyl-4,5-dihydro-1H-pyrazole-5-carboxylic acid ethyl ester, ***5d***

Yield 63%, ^{1}H NMR ($CDCl_3$): $\delta = 8.40$ (dd, $J = 8.1$, 1.5 Hz, 1H), 7.30 (m, 6H), 6.67 (m, 2H), 4.82 (d, $J = 14.4$ Hz, 1H), 4.57 (d, $J = 14.4$ Hz, 1H), 4.17 (q, $J = 7.1$ Hz, 2H), 4.05 (t, $J = 12.3$, 12.2 Hz, 1H), 3.38 (dd, $J = 17.3$, 12.3 Hz, 1H), 3.29 (dd, $J = 17.3$, 12.2 Hz, 1H), 1.27 ppm (t, $J = 7.2$ Hz, 3H); ^{13}C NMR ($CDCl_3$): $\delta = 188.19$, 170.42, 150.58, 147.25, 135.53, 133.93, 129.31, 128.68, 128.04, 118.29, 116.90, 115.90, 64.11, 61.71, 56.97, 36.87, 14.21 ppm; MS (LSIMS) *m/z* 374.1485 $(M + Na)^+$, calcd mass for $C_{20}H_{21}N_3O_3Na$: 374.1480. Anal. $C_{20}H_{21}N_3O_3$ (C, H, N).

4.2.5. 3-(2-Amino-5-methoxybenzoyl)-1-methyl-4,5-dihydro-1H-pyrazole-5-carboxylic acid ethyl ester, ***5e***

Yield 90%, mp 86–88 °C; ^{1}H NMR ($CDCl_3$): $\delta = 7.98$ (d, $J = 3.0$ Hz, 1H), 6.92 (dd, $J = 8.9$, 3.0 Hz, 1H), 6.59 (d, $J = 8.9$ Hz, 1H), 5.69 (br s, 2H), 4.25 (q, $J = 7.1$ Hz, 2H), 4.03 (t, $J = 12.4$, 11.8 Hz, 1H), 4.07 (s, 3H), 3.50 (dd, $J = 17.3$, 12.4 Hz, 1H), 3.29 (dd, $J = 17.3$, 11.8 Hz, 1H), 3.17 (s, 3H), 1.30 ppm (t, $J = 7.1$ Hz, 3H); ^{13}C NMR ($CDCl_3$): $\delta = 187.28$, 170.17, 150.17, 147.55, 145.26, 122.86, 118.14, 118.12, 116.19, 67.85, 61.74, 55.86, 40.80, 37.18, 14.23 ppm; MS (LSIMS) *m/z* 328.1271 $(M + Na)^+$, calcd mass for $C_{15}H_{19}N_3O_4Na$: 328.1273. Anal. $C_{15}H_{19}N_3O_4$ (C, H, N).

4.2.6. 3-(2-Amino-5-methoxybenzoyl)-1-ethyl-4,5-dihydro-1H-pyrazole-5-carboxylic acid ethyl ester, ***5f***

Yield 71%, ^{1}H NMR ($CDCl_3$): $\delta = 8.06$ (d, $J = 3.0$ Hz, 1H), 6.93 (dd, $J = 8.9$, 3.0 Hz, 1H), 6.61 (d, $J = 8.9$ Hz, 1H), 5.72 (br s, 2H), 4.25 (q, $J = 7.1$ Hz, 2H), 4.17 (t, $J = 12.2$, 12.0 Hz, 1H), 3.75 (s, 3H), 3.40 (m, 4H), 1.31 (t, $J = 7.1$ Hz, 3H), 1.30 ppm (t, $J = 7.2$ Hz, 3H); ^{13}C NMR ($CDCl_3$): $\delta = 187.17$, 170.65, 150.15, 147.22, 145.34, 123.11, 118.22, 118.12, 115.81, 65.59, 61.74, 56.80, 46.73, 36.90, 14.24, 12.82 ppm; MS (LSIMS) *m/z* 320.1603 $(M + H)^+$, calcd mass for $C_{16}H_{22}N_3O_4$: 320.1610. Anal. $C_{16}H_{21}N_3O_4$ (C, H, N).

4.2.7. 3-(2-Amino-5-methoxybenzoyl)-1-butyl-4,5-dihydro-1H-pyrazole-5-carboxylic acid ethyl ester, ***5g***

Yield 91%, mp 64–66 °C; ^{1}H NMR ($CDCl_3$): $\delta = 8.05$ (d, $J = 2.9$ Hz, 1H), 6.92 (dd, $J = 8.9$, 2.9 Hz, 1H), 6.60 (d, $J = 8.9$ Hz, 1H), 5.60 (br s, 2H), 4.25 (q, $J = 7.1$ Hz, 2H), 4.20 (t, $J = 12.3$, 11.6 Hz, 1H), 3.74 (s, 3H), 3.38 (m, 4H), 1.70 (m, 2H), 1.40 (m, 2H), 1.30 (t, $J = 7.1$ Hz, 3H), 0.92 ppm (t, $J = 7.3$ Hz, 3H); ^{13}C NMR ($CDCl_3$): $\delta = 187.14$, 170.62, 150.17, 146.64, 145.22, 123.08, 118.26, 115.75, 65.96, 61.73, 55.76, 52.83, 36.79, 29.92, 20.19, 14.25, 13.87 ppm; MS (LSIMS) *m/z* 370.1743 $(M + Na)^+$, calcd mass for $C_{18}H_{25}N_3O_4Na$: 370.1742. Anal. $C_{18}H_{25}N_3O_4$ (C, H, N).

4.2.8. 3-(2-Amino-5-methoxybenzoyl)-1-benzyl-4,5-dihydro-1H-pyrazole-5-carboxylic acid ethyl ester, ***5h***

Yield 75%, ^{1}H NMR ($CDCl_3$): $\delta = 8.06$ (d, $J = 3.0$ Hz, 1H), 7.31 (m, 5H), 6.93 (dd, $J = 8.9$, 3.0 Hz, 1H), 6.60 (d, $J = 8.9$ Hz, 1H), 4.78 (d, $J = 14.4$ Hz, 1H), 4.50 (d, $J = 14.4$ Hz, 1H), 4.19 (q, $J = 7.2$ Hz, 2H), 4.08 (t, $J = 12.3$, 11.5 Hz, 1H), 3.64 (s, 3H), 3.43 (dd, $J = 17.4$, 12.3 Hz, 1H), 3.31 (dd, $J = 17.4$, 11.5 Hz, 1H), 1.28 ppm (t, $J = 7.2$ Hz, 3H); ^{13}C NMR ($CDCl_3$): $\delta = 187.15$, 170.37, 150.23, 147.73, 145.37, 135.85, 129.11, 128.11, 127.95, 123.23, 118.43, 118.07, 115.87, 64.49, 61.74, 57.07, 55.79, 36.87, 14.21 ppm; MS (LSIMS) *m/z* 404.1590 $(M + Na)^+$, calcd mass for $C_{21}H_{23}N_3O_4Na$: 404.1586. Anal. $C_{21}H_{23}N_3O_4$ (C, H, N).

4.2.9. 3-(2-Amino-5-chlorobenzoyl)-1-methyl-4,5-dihydro-1H-pyrazole-5-carboxylic acid ethyl ester, ***5i***

Yield 92%, mp 82–84 °C; ^{1}H NMR ($CDCl_3$): $\delta = 8.35$ (d, $J = 2.4$ Hz, 1H), 7.15 (dd, $J = 8.8$, 2.4 Hz, 1H), 6.57 (d,

$J = 8.8$ Hz, 1H), 5.97 (br s, 2H), 4.25 (q, $J = 7.1$ Hz, 2H), 4.08 (t, $J = 12.7$, 11.4 Hz, 1H), 3.47 (dd, $J = 17.3$, 12.7 Hz, 1H), 3.28 (dd, $J = 17.3$, 11.4 Hz, 1H), 3.21 (s, 3H), 1.31 ppm (t, $J = 7.1$ Hz, 3H); ^{13}C NMR ($CDCl_3$): $\delta = 186.60$, 169.99, 148.90, 146.50, 133.61, 132.72, 120.23, 118.64, 118.12, 67.63, 61.80, 40.55, 36.71, 14.19 ppm; MS (LSIMS) *m/z* 332.0777 $(M + Na)^+$, calcd mass for $C_{14}H_{16}ClN_3O_3Na$: 332.0777. Anal. $C_{14}H_{16}ClN_3O_3$ (C, H, N).

4.2.10. 3-(2-Amino-5-chlorobenzoyl)-1-ethyl-4,5-dihydro-1H-pyrazole-5-carboxylic acid ethyl ester, ***5j***

Yield 50%, ^{1}H NMR ($CDCl_3$): $\delta = 8.41$ (d, $J = 2.5$ Hz, 1H), 7.16 (dd, $J = 8.8$, 2.5 Hz, 1H), 6.58 (d, $J = 8.8$ Hz, 1H), 5.96 (br s, 2H), 4.25 (q, $J = 7.1$ Hz, 2H), 4.22 (t, $J = 12.8$, 11.2 Hz, 1H), 3.75 (m, 4H), 1.31 (t, $J = 7.1$ Hz, 3H), 1.29 ppm (t, $J = 7.2$ Hz, 3H); ^{13}C NMR ($CDCl_3$): $\delta = 186.69$, 170.49, 148.91, 146.11, 133.53, 133.00, 120.36, 118.86, 118.13, 65.39, 61.82, 47.57, 36.55, 14.24, 12.79 ppm; MS (LSIMS) *m/z* 346.0932 $(M + Na)^+$, calcd mass for $C_{15}H_{18}ClN_3O_3Na$: 346.0934. Anal. $C_{15}H_{18}ClN_3O_3$ (C, H, N).

4.2.11. 3-(2-Amino-5-chlorobenzoyl)-1-butyl-4,5-dihydro-1H-pyrazole-5-carboxylic acid ethyl ester, ***5k***

Yield 54%, mp 90–92 °C; ^{1}H NMR ($CDCl_3$): $\delta = 8.40$ (d, $J = 2.6$ Hz, 1H), 7.16 (dd, $J = 8.8$, 2.6 Hz, 1H), 6.58 (d, $J = 8.8$ Hz, 1H), 5.93 (br s, 2H), 4.25 (q, $J = 7.1$ Hz, 2H), 4.23 (t, $J = 12.8$, 11.0 Hz, 1H), 3.39 (m, 4H), 1.70 (m, 2H), 1.42 (m, 2H), 1.31 (t, $J = 7.1$ Hz, 3H), 0.96 ppm (t, $J = 7.3$ Hz, 3H); ^{13}C NMR ($CDCl_3$): $\delta = 186.63$, 170.48, 148.82, 145.44, 133.42, 133.01, 120.37, 118.98, 118.11, 65.76, 61.81, 52.50, 36.42, 29.86, 20.19, 14.24, 13.85 ppm; MS (LSIMS) *m/z* 374.1249 $(M + Na)^+$, calcd mass for $C_{17}H_{22}ClN_3O_3Na$: 374.12473. Anal. $C_{17}H_{22}ClN_3O_3$ (C, H, N).

4.2.12. 3-(2-Amino-5-chlorobenzoyl)-1-benzyl-4,5-dihydro-1H-pyrazole-5-carboxylic acid ethyl ester, ***5l***

Yield 68%, ^{1}H NMR ($CDCl_3$): $\delta = 8.39$ (d, $J = 2.5$ Hz, 1H), 7.35 (m, 5H), 7.19 (dd, $J = 8.8$, 2.5 Hz, 1H), 6.59 (d, $J = 8.8$ Hz, 1H), 5.95 (br s, 2H), 4.85 (d, $J = 14.5$ Hz, 1H), 4.53 (d, $J = 14.5$ Hz, 1H), 4.21 (q, $J = 7.1$ Hz, 2H), 4.08 (t, $J = 12.5$ Hz, 1H), 3.30 (d, $J = 12.5$ Hz, 2H), 1.29 ppm (t, $J = 7.1$ Hz, 3H); ^{13}C NMR ($CDCl_3$): $\delta = 187.03$, 170.15, 148.93, 146.81, 135.19, 133.70, 133.02, 129.47, 128.81, 128.14, 120.42, 118.74, 118.15, 64.11, 61.82, 56.87, 36.39, 14.21 ppm; MS (LSIMS) *m/z* 408.1087 $(M + Na)^+$, calcd mass for $C_{20}H_{20}ClN_3O_3Na$: 408.1090. Anal. $C_{20}H_{20}ClN_3O_3$ (C, H, N).

4.2.13. 3-(2-Amino-5-chlorobenzoyl)-4,5-dihydro-1H-pyrazole-5-carboxylic acid ethyl ester, ***5m***

Yield 76%, mp 122–124 °C; ^{1}H NMR ($CDCl_3$): $\delta = 8.41$ (d, $J = 2.3$ Hz, 1H), 7.17 (dd, $J = 8.8$, 2.3 Hz, 1H), 6.90 (br s, 1H), 6.58 (d, $J = 8.8$ Hz, 1H), 6.05 (br s, 2H), 4.42 (dd, $J = 12.4$, 5.4 Hz, 1H), 4.22 (q, $J = 7.1$ Hz, 2H), 3.44 (dd, $J = 17.6$, 5.4 Hz, 1H), 3.29 (dd, $J = 17.6$, 12.4 Hz, 1H), 1.27 ppm (t, $J = 7.1$ Hz, 3H); ^{13}C NMR ($CDCl_3$): $\delta = 187.25$, 171.86, 150.70, 149.30, 134.16, 132.90, 120.42, 118.21, 62.15, 60.52, 35.44, 14.19 ppm; MS (LSIMS) *m/z* 318.0618 $(M + Na)^+$, calcd mass for $C_{13}H_{14}ClN_3O_3Na$: 318.0621. Anal. $C_{13}H_{14}Cl\ N_3O_3$ (C, H, N).

4.2.14. 3-(2-Aminobenzoyl)-1-ethyl-1H-pyrazole-5-carboxylic acid ethyl ester, ***6b***

Yield 70%, mp 60–62 °C; ^{1}H NMR ($CDCl_3$): $\delta = 8.27$ (dd, $J = 1.6$, 8.6 Hz, 1H), 7.35 (s, 1H), 7.28 (dd, $J = 7.6$, 1.6 Hz, 1H), 6.68 (m, 2H), 6.17 (br s, 2H), 4.68 (q, $J = 7.2$ Hz, 2H), 4.36 (q, $J = 7.1$ Hz, 2H), 1.49 (t, $J = 7.2$ Hz, 3H), 1.38 ppm (t, $J = 7.1$ Hz, 3H); ^{13}C NMR ($CDCl_3$): $\delta = 188.89$, 159.46, 151.36, 150.09, 134.45, 134.38, 132.61, 118.10, 116.97, 115.86, 114.42, 61.35, 47.90, 15.79, 14.26 ppm; MS (LSIMS) *m/z* 310.1165 $(M + Na)^+$, calcd mass for $C_{15}H_{17}N_3O_3Na$: 310.1167. Anal. $C_{15}H_{17}N_3O_3$ (C, H, N).

4.2.15. 3-(2-Aminobenzoyl)-1-butyl-1H-pyrazole-5-carboxylic acid ethyl ester, ***6c***

Yield 57%, mp 63–64 °C; ^{1}H NMR ($CDCl_3$): $\delta = 8.27$ (dd, $J = 8.6$, 1.4 Hz, 1H), 7.35 (s, 1H), 7.29 (dd, $J = 7.3$, 1.4 Hz, 1H), 6.68 (m, 2H), 6.18 (br s, 2H), 4.64 (t, $J = 7.3$ Hz, 2H), 4.36 (q, $J = 7.1$ Hz, 2H), 1.87 (m, 2H), 1.37 (m, 2H), 1.37 (t, $J = 7.1$ Hz, 3H), 0.95 ppm (t, $J = 7.3$ Hz, 3H); ^{13}C NMR ($CDCl_3$): $\delta = 188.89$, 159.51, 151.36, 149.99, 134.43, 132.89, 118.00, 116.97, 115.84, 114.36, 61.34, 52.40, 32.67, 19.83, 14.27, 13.69 ppm; MS (LSIMS) *m/z* 338.1479 $(M + Na)^+$, calcd mass for $C_{17}H_{21}N_3O_3Na$: 338.1480. Anal. $C_{17}H_{21}N_3O_3$ (C, H, N).

4.2.16. 3-(2-Aminobenzoyl)-1-benzyl-1H-pyrazole-5-carboxylic acid ethyl ester, ***6d***

Yield 45%, mp 116–118 °C; ^{1}H NMR ($CDCl_3$): $\delta = 8.32$ (dd, $J = 8.1$, 1.5 Hz, 1H), 7.40 (s, 1H), 7.29 (m, 6H), 6.69 (m, 2H), 5.84 (s, 2H), 4.32 (q, $J = 7.1$ Hz, 2H), 1.33 ppm (t, $J = 7.1$ Hz, 3H); ^{13}C NMR ($CDCl_3$): $\delta = 188.79$, 159.40, 151.42, 150.53, 136.56, 134.57, 134.53, 128.69, 128.02, 127.82, 118.02, 117.00, 115.89, 114.74, 61.46, 55.72, 14.23 ppm; MS (LSIMS) *m/z* 372.1315 $(M + Na)^+$, calcd mass for $C_{20}H_{19}N_3O_3Na$: 372.1324. Anal. $C_{20}H_{19}N_3O_3$ (C, H, N).

4.2.17. 3-(2-Amino-5-methoxybenzoyl)-1-ethyl-1H-pyrazole-5-carboxylic acid ethyl ester, ***6f***

Yield 75%, mp 78–80 °C; ^{1}H NMR ($CDCl_3$): $\delta = 7.97$ (d, $J = 2.9$ Hz, 1H), 7.40 (s, 1H), 6.99 (dd, $J = 8.9$, 2.9 Hz, 1H), 6.66 (d, $J = 8.9$ Hz, 1H), 5.90 (br s, 2H), 4.69 (q, $J = 7.2$ Hz, 2H), 4.36 (q, $J = 7.1$ Hz, 2H), 3.76 (s, 3H), 1.50 (t, $J = 7.2$ Hz, 3H), 1.38 (t, $J = 7.1$ Hz, 3H); ^{13}C NMR ($CDCl_3$): $\delta = 188.10$, 159.48, 150.26, 150.07, 146.20, 132.77, 123.70, 118.33, 117.84, 116.52, 114.53, 61.39, 55.88, 48.87, 15.70, 14.24 ppm; MS (LSIMS) *m/z* 340.1271 $(M + Na)^+$, calcd mass for $C_{16}H_{19}N_3O_4Na$: 340.1273. Anal. $C_{16}H_{19}N_3O_4$ (C, H, N).

4.2.18. 3-(2-Amino-5-methoxybenzoyl)-1-butyl-1H-pyrazole-5-carboxylic acid ethyl ester, ***6g***

Yield 82%, mp 82–84 °C; ^{1}H NMR ($CDCl_3$): $\delta = 7.97$ (d, $J = 3.0$ Hz, 1H), 7.40 (s, 1H), 6.99 (dd, $J = 8.9$, 3.0 Hz, 1H), 6.66 (d, $J = 8.9$ Hz, 1H), 5.91 (br s, 2H), 4.64 (t, $J = 7.3$ Hz,

2H), 4.36 (q, $J = 7.1$ Hz, 2H), 3.76 (s, 3H), 1.88 (m, 2H), 1.38 (t, $J = 7.1$ Hz, 3H), 1.36 (m, 2H), 0.94 ppm (t, $J = 7.3$ Hz, 3H); ^{13}C NMR ($CDCl_3$): $\delta = 188.11$, 159.52, 150.21, 150.09, 146.23, 133.04, 123.78, 118.33, 117.85, 116.47, 114.50, 61.36, 55.87, 52.39, 32.65, 19.87, 14.26, 13.71 ppm; MS (LSIMS) *m/z* 368.1587 $(M + Na)^+$, calcd mass for $C_{18}H_{23}N_3O_4Na$: 368.1586. Anal. $C_{18}H_{23}N_3O_4$ (C, H, N).

4.2.19. 3-(2-Amino-5-methoxybenzoyl)-1-benzyl-1H-pyrazole-5-carboxylic acid ethyl ester, **6h**

Yield 75%, mp 116–118 °C; ^{1}H NMR ($CDCl_3$): $\delta = 7.95$ (d, $J = 3.0$ Hz, 1H), 7.44 (1H, s), 7.28 (m, 5H), 6.98 (dd, $J = 9.0$, 3.0 Hz, 1H), 6.65 (d, $J = 9.0$ Hz, 1H), 5.84 (s, 2H), 4.34 (q, $J = 7.1$ Hz, 2H), 3.62 (s, 3H), 1.35 ppm (t, $J = 7.1$ Hz, 3H); ^{13}C NMR ($CDCl_3$): $\delta = 187.93$, 159.47, 150.85, 150.08, 146.34, 136.58, 133.28, 128.70, 128.05, 127.89, 124.13, 118.39, 117.60, 116.14, 114.75, 61.50, 55.76, 55.58, 14.22 ppm; MS (LSIMS) *m/z* 402.1423 $(M + Na)^+$, calcd mass for $C_{21}H_{21}N_3O_4Na$: 402.1429. Anal. $C_{21}H_{21}N_3O_4$ (C, H, N).

4.2.20. 3-(2-Amino-5-chlorobenzoyl)-1-methyl-1H-pyrazole-5-carboxylic acid ethyl ester, **6i**

Yield 78%, mp 112–114 °C; ^{1}H NMR ($CDCl_3$): $\delta = 8.36$ (d, $J = 2.5$ Hz, 1H), 7.37 (s, 1H), 7.22 (dd, $J = 8.9$, 2.5 Hz, 1H), 6.64 (d, $J = 8.9$ Hz, 1H), 6.20 (br s, 2H), 4.36 (q, $J = 7.1$ Hz, 2H), 4.28 (s, 3H), 1.38 ppm (t, $J = 7.1$ Hz, 3H); ^{13}C NMR ($CDCl_3$): $\delta = 187.50$, 159.52, 149.85, 149.63, 134.43, 133.71, 133.32, 120.29, 118.43, 118.36, 114.28, 61.49, 40.49, 14.27 ppm; MS (LSIMS) *m/z* 330.0623 $(M + Na)^+$, calcd mass for $C_{14}H_{14}ClN_3O_3Na$: 330.0621. Anal. $C_{14}H_{14}ClN_3O_3$ (C, H, N).

4.2.21. 3-(2-Amino-5-chlorobenzoyl)-1-ethyl-1H-pyrazole-5-carboxylic acid ethyl ester, **6j**

Yield 80%, mp 111–113 °C; ^{1}H NMR ($CDCl_3$): $\delta = 8.38$ (d, $J = 2.4$ Hz, 1H), 7.37 (s, 1H), 7.23 (dd, $J = 8.9$, 2.4 Hz, 1H), 6.64 (d, $J = 8.9$ Hz, 1H), 6.19 (br s, 2H), 4.69 (q, $J = 7.2$ Hz, 2H), 4.37 (q, $J = 7.1$ Hz, 2H), 1.51 (t, $J = 7.2$ Hz, 3H), 1.38 ppm (t, $J = 7.1$ Hz, 3H); ^{13}C NMR ($CDCl_3$): $\delta = 187.65$, 159.36, 149.87, 149.69, 134.38, 133.52, 132.91, 120.27, 118.48, 118.33, 114.46, 61.44, 47.99, 15.71, 14.26 ppm; MS (LSIMS) *m/z* 321.0876 $(M)^+$, calcd mass for $C_{15}H_{16}ClN_3O_3$: 321.0880. Anal. $C_{15}H_{16}ClN_3O_3$ (C, H, N).

4.2.22. 3-(2-Amino-5-chlorobenzoyl)-1-butyl-1H-pyrazole-5-carboxylic acid ethyl ester, **6k**

Yield 77%, mp 87–88 °C; ^{1}H NMR ($CDCl_3$): $\delta = 8.38$ (d, $J = 2.5$ Hz, 1H), 7.38 (s, 1H), 7.23 (dd, $J = 8.9$, 2.5 Hz, 1H), 6.64 (d, $J = 8.9$ Hz, 1H), 6.19 (br s, 2H), 4.65 (t, $J = 7.3$ Hz, 2H), 4.37 (q, $J = 7.1$ Hz, 2H), 1.89 (m, 2H), 1.40 (m, 2H), 1.38 (t, $J = 7.1$ Hz, 3H), 0.97 ppm (t, $J = 7.3$ Hz, 3H); ^{13}C NMR ($CDCl_3$): $\delta = 187.76$, 159.43, 149.88, 149.55, 134.43, 133.61, 133.18, 120.28, 118.45, 118.35, 114.43, 61.47, 52.47, 32.60, 19.84, 14.30, 13.73 ppm; MS (LSIMS) *m/z* 372.1090 $(M + Na)^+$, calcd mass for $C_{17}H_{20}ClN_3O_3Na$: 372.1090. Anal. $C_{17}H_{20}ClN_3O_3$ (C, H, N).

4.2.23. 3-(2-Amino-5-chlorobenzoyl)-1-benzyl-1H-pyrazole-5-carboxylic acid ethyl ester, **6l**

Yield 54%, mp 135–136 °C; ^{1}H NMR ($CDCl_3$): $\delta = 8.42$ (d, $J = 2.4$ Hz, 1H), 7.35 (m, 6H), 7.24 (dd, $J = 8.8$, 2.4 Hz, 1H), 6.65 (d, $J = 8.8$ Hz, 1H), 6.20 (br s, 2H), 5.85 (s, 2H), 4.34 (q, $J = 7.1$ Hz, 2H), 1.35 ppm (t, $J = 7.1$ Hz, 3H); ^{13}C NMR ($CDCl_3$): $\delta = 187.63$, 159.38, 150.07, 149.92, 136.30, 134.49, 133.75, 133.26, 128.82, 128.26, 128.23, 120.30, 118.84, 114.65, 61.56, 55.71, 14.25 ppm; MS (LSIMS) *m/z* 406.0933 $(M + Na)^+$, calcd mass for $C_{20}H_{18}ClN_3O_3Na$: 406.0934. Anal. $C_{20}H_{18}ClN_3O_3$ (C, H, N).

4.2.24. 3-(2-Amino-5-chlorobenzoyl)-1H-pyrazole-5-carboxilic acid ethyl ester, **6m**

Yield 78%, mp 136–138 °C; ^{1}H NMR ($CDCl_3$): $\delta = 11.62$ (br s, 1H), 8.11 (s, 1H), 7.33 (s, 1H), 7.26 (dd, $J = 8.9$, 2.5 Hz, 1H), 6.67 (d, $J = 8.9$ Hz, 1H), 6.17 (br s, 2H), 4.44 (q, $J = 7.1$ Hz, 2H), 1.41 ppm (t, $J = 7.1$ Hz, 3H); ^{13}C NMR ($CDCl_3$): $\delta = 185.70$, 160.46, 149.80, 135.03, 132.13, 120.67, 118.66, 117.90, 111.78, 61.81, 14.34 ppm; MS (LSIMS) *m/z* 316.0469 $(M + Na)^+$, calcd mass for $C_{13}H_{12}ClN_3O_3Na$: 316.0464. Anal. $C_{13}H_{12}ClN_3O_3$ (C, H, N).

4.3. Striatal nNOS activity determination

L-Arginine, L-citruline, *N*-(2-hydroxymethyl)piperazine-*N*′-(2-ethanesulfonic acid) (HEPES), DL-dithiothreitol (DTT), leupeptin, aprotinin, pepstatin, phenylmethyl-sulfonylfluoride (PMSF), hypoxantine-9-β-D-ribofuranosid (inosine), ethylene glycol-bis-(2-aminoethylether)-*N*,*N*,*N*′,*N*′-tetraacetic acid (EGTA), bovine serum albumin (BSA), Dowex-50W (50 × 8-200), FAD, NADPH and 5,6,7,8-tetrahydro-L-biopterin dihydrochloride (H4-biopterin) were obtained from Sigma–Aldrich Química (Spain). L-[^{3}H]-arginine (58 Ci/mmol) was obtained from Amersham (Amersham Biosciences, Spain). Tris-(hydroxymethyl)-aminomethane (Tris-HCl) and calcium chloride were obtained from Merck (Spain).

Rats male Wistar (200–250 g) were maintained in the University's facility in a 12 h/12 h light/dark cycle at 22 ± 2 °C and with free access to food and tap water. All experiments were performed according to the Spanish Government Guide and the European Community Guide for animal care.

Rats were killed by cervical dislocation, and the striata were quickly collected and immediately used to measure NOS activity. Upon removal, the tissues were cooled in ice-cold homogenizing buffer (25 mM Tris-HCl, 0.5 mM DTT, 10 μg/mL leupeptin, 10 μg/mL pepstatine, 10 μg/mL aprotinine, 1 mM PMSF, pH 7.6). Two striata were placed in 1.25 mL of the same buffer and homogenized in a Polytron (10 s × 6). The crude homogenate was centrifuged for 5 min at 1000 g, and aliquots of the supernatant were either stored at −20 °C for total protein determination or used immediately to measure NOS activity. The nNOS activity was measured by the Bredt and Snyder [24] method, monitoring the conversion of L-[^{3}H]-arginine to L-[^{3}H]-citruline. The final incubation volume was 100 μL and consisted of 10 μL crude homogenate

added to a buffer to give a final concentration of 25 mM Tris-HCl, 1 mM DTT, 30 μM H4-biopterin, 10 μM FAD, 0.5 mM inosine, 0.5 mg/mL BSA, 0.1 mM $CaCl_2$, 10 μM L-arginine, and 50 nM L-[^{3}H]-arginine, at pH 7.6. The reaction was started by the addition of 10 μL of NADPH (0.75 mM final) and 10 μL of each pyrazole derivative in DMSO to give a final concentration of 1 mM. The tubes were vortex and incubated at 37 °C for 30 min. Control incubations were performed by the omission of NADPH. The reaction was halted by the addition of 400 μL of cold 0.1 M HEPES, 10 mM EGTA, and 0.175 mg/mL L-citruline, pH 5.5. The reaction mixture was decanted into a 2 mL column packet with Dowex-50W ion-exchange resin (Na^+ form) and eluted with 1.2 mL of water. L-[^{3}H]-citruline was quantified by liquid scintillation spectroscopy. The retention of L-[^{3}H]-arginine in this process was greater than 98%. Specific enzyme activity was determined by subtracting the control value, which usually amounted to less than 1% of the radioactivity added. The nNOS activity was expressed as picomoles of L-[^{3}H]-citruline produced (mg of protein)$^{-1}$ min^{-1}.

4.4. iNOS activity determination

iNOS induction was achieved by intravenous injection of lipopolysaccharide (LPS) 20 mg/kg. Eight hours after the administration, rats were killed by cervical dislocation, and lungs were quickly collected homogenized in ice-cold homogenizing buffer (1 mg tissue/15 mL buffer, 25 mM Tris-HCl, 0.5 mM DTT, 10 μg/mL leupeptin, 10 μg/mL pepstatine, 10 μg/mL aprotinine, 1 mM PMSF, pH 7.6). The crude homogenate was incubated in the presence of EDTA 10 mM to eliminate the nNOS activity that could exist. iNOs activity was measured using the same procedure described above for the determination of nNOS activity.

4.5. Statistical analysis

Data are expressed as the mean ± SEM. One-way analysis of variance, followed by the Newman–Keuls multiple range test was used. A $P < 0.05$ value was considered statistically significant.

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