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Short communication

Phenylpyrrole derivatives as neural and inducible nitric oxide synthase (nNOS and iNOS) inhibitors

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ABSTRACT

We have previously described a series of 3-phenyl-4,5-dihydro-1*H*-pyrazole derivatives as moderately potent nNOS inhibitors. As a follow up of these studies, several new 5-phenyl-1*H*-pyrrole-2-carboxamide derivatives have been synthesized, and their biological evaluation as *in vitro* inhibitors of both neural and inducible Nitric Oxide Synthase (nNOS and iNOS) is described. Some of these compounds show good iNOS/nNOS selectivity and the more potent compounds 5-(2-aminophenyl)-1*H*-pyrrole-2-carboxilic acid methylamide (QFF205) and cyclopentylamide (QFF212) have been tested as regulators of the *in vivo* nNOS and iNOS activity. Both compounds prevented the increment of the inducible NOS activity in both cytosol (iNOS) and mitochondria (i-mtNOS) observed in the MPTP model of Parkinson's disease.

1. Introduction

Nitric Oxide (NO) is an important bioregulator and an ubiquitous biomessenger involved in several physiological and pathological processes such as vasodilatation [1], non-specific host defense [2], ischemia reperfusion injury [3] chronic or acute inflammation [4], and neurological disorders like Alzheimer's disease [5] amyotrophic lateral sclerosis [6] and Huntington's disease [7].

In mammals, NO is synthesized from L-arginine in various cell types (neurons [8], endothelial cells [9] and macrophages [10]) by a family of nitric oxide synthase (NOS) [11] isoenzymes, with consumption of molecular oxygen, NADPH and other cofactors [12].

Based on its endogenous regulation, NOSs have been structurally classified as constitutive NOS (cNOS), that requires Ca^{2+}/Calmodulin (CaCAM) for its activation [13] and inducible NOS (iNOS), which is CaCAM independent [14]. cNOS has been subdivided into endothelial (eNOS) and neuronal (nNOS) attending to its localization in the vascular endothelium and in the brain, respectively. The inducible isoform (iNOS) is present in macrophages activated by inflammatory cytokines or by lipopolysaccharide (LPS).

More recently, a mitochondrial-localized NOS isoform situated in the internal membrane of the mitochondria (mtNOS) was discovered [15]. Even there was a controversy among the type(s) of mtNOS in terms of their classification as constitutive or inducible [16], in a recent paper the existence of both constitutive and inducible mitochondrial NOS has been proven (c-mtNOS and i-mtNOS, respectively) [17].

eNOS is involved in the regulation of smooth muscle relaxation and blood pressure, and in the inhibition of platelet aggregation [18] while nNOS has been shown to regulate neuronal transmission and cerebral blood flow [19]. The major function of iNOS is thought to serve in host defense mechanism [20]. Both mitochondrial c-mtNOS and i-mtNOS are involved in the NO production in the mitochondria that in turn controls the bioenergetic processes inside this organelle [21].

It has also been reported that an uncontrolled NO production by iNOS causes diseases such shock condition [22], inflammatory arthritis [23] chronic ileitis and colitis [24]. NO overproduction by nNOS produces neurotoxicity, and this fact has been associated with several neurological disorders such as Alzheimer's disease [5], amyotrophic lateral sclerosis [6] and Huntington's disease [7]. Recent reports showed iNOS activation and inflammatory reaction in neurodegenerative processes such as Parkinson's disease and Alzheimer's disease [25].

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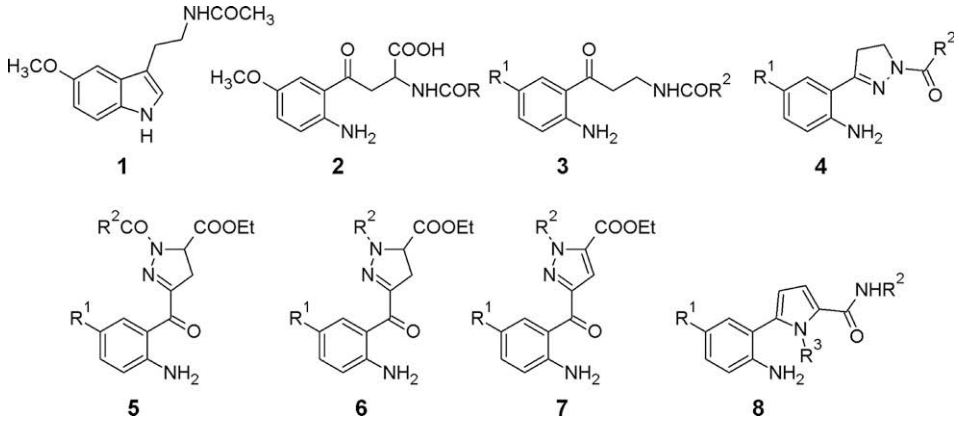
Thus nNOS and iNOS represent a therapeutic target since inhibition of these enzymes can help in the treatment of several disorders, and a selective inhibition of one of these isoforms would be desired. On the other hand, selective inhibitors may also constitute useful pharmacological tools in the research of NO biological functions.

Melatonin **1** is a hormone synthesized by many organs and tissues of the organisms including the pineal gland [26] that shows inhibitory effects in rat [27] and human [28] central nervous system (CNS), being this the reason for its anticonvulsant and neuroprotective properties [29]. Diverse experiments have suggested that melatonin attenuates glutamate-mediated responses in the rat striatum [30] and this inhibitory effect takes place through the inhibition of nNOS [31–33]. nNOS inhibition by melatonin has demonstrated to be dose-dependent and calmodulin-dependent [34].

Our search group has reported several nNOS inhibitors with a kynurenine structure **2**, showing a significant nNOS inhibition activity [34,35]. A second type of nNOS inhibitors described by our research group show a kinurenamine structure **3** [36] among them the main melatonin brain metabolite (**AMK**: **3**, $R^1 = MeO$, $R^2 = Me$). All these compounds inhibit nNOS in a dose-dependent manner, and it has been found that **AMK** rather than melatonin is the active metabolite against nNOS in rat striatum [37]. We have also published a new relatively potent and less flexible nNOS inhibitors of general formula **4**, bearing a 4,5-dihydro-1*H*-pyrazole moiety [38]. Finally, 3-benzoyl-4,5-dihydro-1*H*-pyrazole derivatives **5** [39], **6** [40], and 3-benzoyl-1*H*-pyrazoles **7** [40], show moderated both nNOS and iNOS inhibition, but in some cases a iNOS selectivity is observed.



In this paper we describe a new type of NOS inhibitors with general structure **8**, bearing a pyrrole moiety. These new molecules show moderate *in vitro* nNOS and iNOS inhibition and in some cases iNOS selectivity. A preliminary *in vivo* study of the more active compounds is also presented. Two compounds reduce the *in vivo* NOS activity in cytosol and mitochondria in the MPTP model Parkinson's disease.

2. Results and discussion

2.1. Drug design

Phenylpyrazolines **4** have been proven to be good nNOS inhibitors [38], and compounds **8** have been designed from phenylpyrazolines **4**, substituting the pyrazole moiety by a pyrrole ring. In order to test the potentiality as NOS inhibitors of these new molecules, docking studies of compounds **8a–v** inside both nNOS and iNOS binding sites have been tackled using Schrödinger software [41]. Phenylpyrazoles **4** have been also studied for comparison with the new molecules.

Potential maps needed for docking experiments were generated using Glide program, from the heme oxygenase domain of both isoenzymes, obtained from the crystal structures of $N^{(\omega)}$-propil-L-arginine co-crystallized with both nNOS (PDB id: 1QW6) and iNOS (PDB id: 1QW4) [42]. 3D structures of compounds **8a–v** were generated from fragment libraries, and optimized using the Macromodel module. Glide program was used for docking the ligands using the XP option.

Two different poses have been found for phenylpyrazolines **4** inside nNOS binding site, depending on the presence or the absence of 5′-substituent in these molecules. In all cases, the benzene ring lies almost parallel to the heme group, and the pyrazoline ring is situated between Gln478 and Glu592. This last residue is crucial for the interaction between NOS and L-arginine, since it forms an electrostatic reinforced hydrogen bond with the substrated, and also interacts with almost all NOS inhibitors.

When compound **4** has a non-substituted benzene ring ($R^1 = H$), the 2′-NH_2 group points toward Glu592, forming a hydrogen bond with this residue. Fig. 1A shows the best pose for compound **4m** ($R^1 = H$, $R^2 = c\text{-}C_3H_5$). Nevertheless, when compound **4** bears a 5′-substituent ($R^1 = OMe$ or Cl), this group will interact with Phe584 in a pose similar to that of Fig. 1A. For this reason, the benzene ring rotates, and the best pose for 5′-substituted pyrazoline derivatives shows the 2′-NH_2 group pointing to the heme side chains. Fig. 1B shows as an example the best pose found for compound 3-(2-amino-5-methoxyphenyl)-2,3-dihydro-1*H*-pyrazole **4f** ($R^1 = OMe$, $R^2 = c\text{-}C_4H_7$). In this case, no hydrogen bond is observed between the nNOS binding site and the inhibitor.

Pyrrole derivatives **8a–v** behave similarly to pyrazolines **4**, and two different poses have been found inside nNOS binding site for these derivatives (Fig. 1C, D). Compounds **8a–k** bear a 5′-substitued benzene ring, and the main pose obtained for these molecules is similar to that of 5′-substituted pyrazolines **4**. Fig. 1C shows the pose for compound **8d** inside nNOS. Nevertheless, it can be observed that in this case two hydrogen bonds are formed between both the pyrrole NH and the amide NH bonds, and one Glu592 carboxylate O atom.

In compounds **8l–v** ($R^1 = H$) the benzene ring is not substituted, and the main pose found for these molecules is similar to that of unsubstituted pyrazolines. Fig. 1D shows, the **8s**/nNOS complex, and it can be observed that the 2′-NH_2 group is pointing toward Glu592. In this complex, one hydrogen bond is formed between one

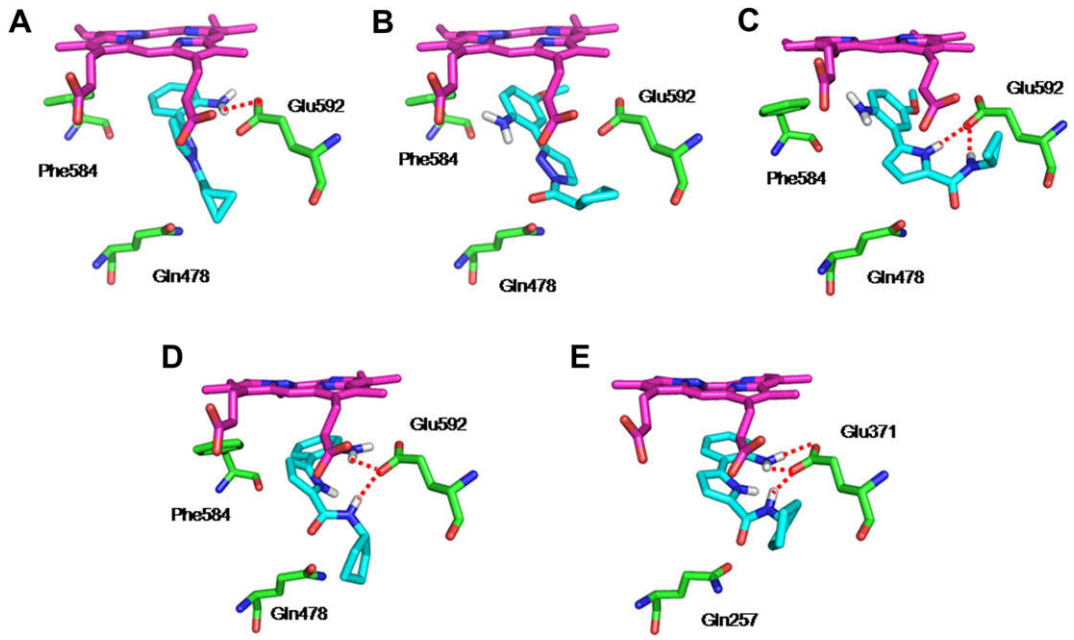


Fig. 1. (A, B) The preferred poses found for 3-(2-aminophenyl)-2,3-dihydro-1*H*-pyrazole (**4m**, $R^1 = H$, $R^2 = c\text{-}C_3H_5$), and 3-(2-amino-5-methoxyphenyl)-2,3-dihydro-1*H*-pyrazole (**4f**, $R^1 = OMe$, $R^2 = c\text{-}C_4H_7$) inside the nNOS binding site. The preferred pose found for compounds **8d** (C) and **8s** (D) in nNOS. These molecules interact with Glu592 by means of two hydrogen bonds (red dotted lines). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.). (E) The preferred pose obtained for compound **8s** inside iNOS, in this case three hydrogen bonds are formed with Glu371.

O atom of Glu592 carboxylate, and the 2′-NH_2 group. An additional hydrogen bond is formed between the same O atom of Glu592 carboxylate and the ligand amide NH bond.

Similar poses have been obtained inside the iNOS binding site for compounds **8a–v**. Nevertheless, in this isoenzyme, Gln257 is rotated in relation to its equivalent residue in nNOS (Gln478). For this reason, the *c*-C_5H_9 substituent in compound **8s** (Fig. 1E) is shifted in relation to Fig. 1D, and this slight modification of the ligand geometry allows the formation of a third hydrogen bond between the 2′NH_2 and the Glu371 carboxylate.

Docking studies indicate that compounds **8a–v** can fit inside nNOS binding site with reasonable geometries and that they can interact with the enzyme by means of several hydrogen bonds that are not present in the complexes formed by pyrazolines **4**. For this reason, it can be expected that these new molecules should be better nNOS inhibitors than pyrazolines **4**. Docking studies also indicate that compounds **8a–v** can bind correctly inside the iNOS binding site. These reasons prompted us to the synthesis and biological evaluation of these molecules.

2.2. Chemistry

Scheme 1 represents the general synthetic pathway followed in the preparation of the final 5-phenyl-1*H*-pyrrole-2-carboxamide derivatives described in this paper. Two main structural modifications were performed in these molecules: i) modification of the amide chain; and, ii) substitution of the benzene H-5′ atom. The pyrrolic ring has been constructed by means of the Hemetsberger reaction [43]. The synthetic pathway begins with the reaction of 2-nitro-cinnamaldehyde derivatives **10a–c** with ethyl azidoacetate to yield the corresponding 2-azido-5-(2-nitro-5-substituted-phenyl)-penta-2,4-dienoic acid ethyl ester **11a–c** [44]. While 2-nitrocinnamaldehyde **10c** is commercially available, compounds **10a** and **10b** have been prepared from the corresponding 2-nitro-5-substituted-benzaldehyde **9a–b**, by reaction with $Ph_3P{=}CHCHO$ according to the Wittig reaction [45]. 5-Chloro-2-nitrobenzaldehyde **9b** is also commercially available, and 5-methoxy-2-nitro-benzaldehyde **9a** was prepared by *O*-methylation of 5-hidroxy-2-nitrobenzaldehyde (CH_3I/K_2CO_3 in THF) [39].

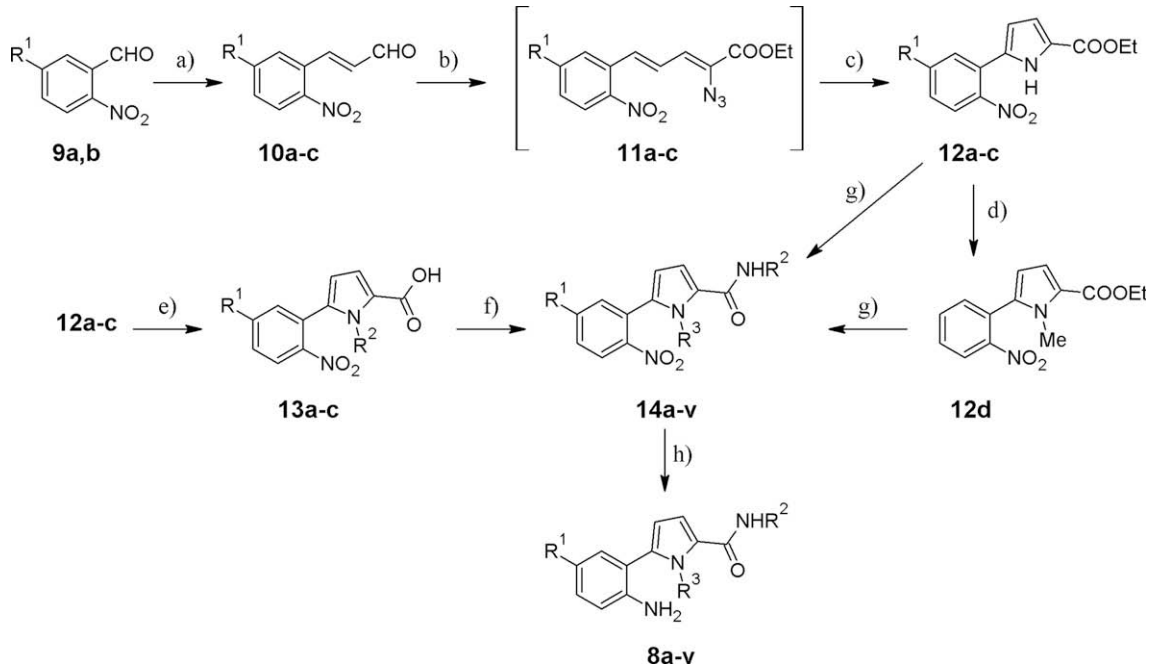
Azides derivatives **11a–c** cyclizise and yield the corresponding nitrophenylpyrrole derivatives **12a–c** when heated in *p*-xylene. Compound **12c** was treated with CH_3I under basic conditions and in the presence of 18-crown-6 ether to yield compound **12d**.

Two alternative procedures were employed in the synthesis of nitrocarboxamide derivatives **14a–v** from compounds **12a–d**. Compounds **14a**, **14g**, **14l** and **14v** were directly obtained from **12a–d**, respectively, by treatment with NH_4Cl/NH_4OH [46]. In all other cases, the ester moiety of compounds **12a–c** was previously hydrolyzed (NaOH, then AcOH) [47] to yield the carboxylic acid derivatives **13a–c**, which in turn were transformed into the acyl chloride ($SOCl_2$) and treated with the appropriated amine (R^3NH_2/TEA) to yield the corresponding *N*-substituted carboxamide **14** [48]. Finally, compounds **8a–v** were obtained by reduction of the nitro group in the corresponding derivative **14a–v**, performed by treatment with $Fe/FeSO_4$ [49].

2.3. In vitro NOS inhibition

Table 1 shows the *in vitro* inhibition percentage of nNOS and iNOS isoforms produced by a 1 mM concentration of each compound **8a–v**, compared with the control assays. In general, compounds **8a–v** 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.

Compounds **8a–f** bear a 5′-methoxy substituent ($R^1 = OMe$) in the benzene ring. Among them, **8a** and **8b** ($R^2 = H$, Me) do not inhibit nNOS. An increment in the volume of *N*-caboxamide substituent increases the inhibition percentage, compounds **8c** ($R^2 = Pr$, 43%) and **8d** ($R^2 = c\text{-}C_3H_5$, 32%) being the best inhibitors in this series of compounds. In compounds **8e** and **8f** ($R^1 = OMe$,



Scheme 1. General synthetic pathway followed in the preparation of compounds **8a–v**. a): $Ph_3P{=}CHCHO$; b): $N_3CH_2CO_2Et$, OH^-; c): thermolysis, *p*-xylene; d): MeI/OH^-, 18-crown-6; e): i) NaOH 1 N; ii) AcOH; f): i) $SOCl_2$; ii) R^3NH_2/TEA, CH_2Cl_2; g) NH_4Cl/NH_4OH; h): $Fe/FeSO_4$.

$R^2 = c\text{-}C_5H_9$, CH_2Ph), a decreasing of the nNOS inhibition can be observed again.

Compounds **8g–k** bear a 5′-chloro substituent in the benzene ring ($R^1 = Cl$) and show higher nNOS inhibition percentage, indicating that this substituent is the better one for the inhibition of this enzyme. The importance of the 5′-chloro substituent for the nNOS inhibition has been previously described in compounds **4** [38], **5** [39], and **6–7** [40], since the better nNOS inhibitors belonging to these families of compounds have such substituent in its benzene moiety. Compound **8k** with an *N*-cyclopropyl substituent is the best inhibitor in this series (48%). Compounds **8i** ($R^2 = Pr$, 33%) and **8j** ($R^2 = Bu$, 36%) also show good inhibition percentage, indicating that a R^3 must be a group with a moderate volume.

Finally, compounds **8l–v** show a low inhibition percentage and some of them produce an activation of the enzyme. This fact indicates that a non-substituted benzene moiety is detrimental for the nNOS inhibition activity.

Table 1 also shows the iNOS inhibition observed in the presence of 1 mM concentration of compounds **8**. Unfortunately, no clear relationship between the structure and the activity can be observed. Since compounds **8g–k** do not inhibit iNOS, it seems that

Table 1
In vitro nNOs and iNOS inhibition (%) observed in the presence of 1 mM concentration of compounds **8a–v**.

Compound	Code[a]	R^1	R^2	R^3	% nNOS inhibition[b]	% iNOS inhibition[b]
8a	QFF193	OMe	H	H	−18.14 ± 2.01	8.95 ± 0.50
8b	QFF194	OMe	Me	H	−47.97 ± 2.6	26.17 ± 6.85
8c	QFF195	OMe	Pr	H	43.45 ± 3.38	6.78 ± 3.93
8d	QFF196	OMe	*c*-C_3H_5	H	32.54 ± 2.63	21.36 ± 4.68
8e	QFF197	OMe	*c*-C_5H_9	H	15.33 ± 2.42	26.25 ± 3.22
8f	QFF198	OMe	CH_2Ph	H	8.89 ± 1.13	22.41 ± 1.94
8g	QFF199	Cl	H	H	34.48 ± 0.99	5.34 ± 2.34
8h	QFF200	Cl	Me	H	17.11 ± 0.74	1.27 ± 3.39
8i	QFF201	Cl	Et	H	33.40 ± 2.46	2.8 ± 1.75
8j	QFF202	Cl	Bu	H	36.46 ± 4.13	3.33 ± 1.27
8k	QFF203	Cl	*c*-C_3H_5	H	48.07 ± 1.30	7.20 ± 0.31
8l	QFF204	H	H	H	15.01 ± 2.66	3.07 ± 1.79
8m	QFF205	H	Me	H	−12.79 ± 0.21	32.68 ± 2.78
8n	QFF206	H	Et	H	4.15 ± 0.97	20.49 ± 5.19
8o	QFF207	H	Pr	H	−12.93 ± 2.17	13.17 ± 5.2
8p	QFF208	H	Bu	H	−0.01 ± 1.80	7.53 ± 2.76
8q	QFF210	H	*c*-C_3H_5	H	2.92 ± 0.87	−1.1 ± 1.75
8r	QFF211	H	*c*-C_4H_7	H	8.44 ± 1.87	20.1 ± 4.78
8s	QFF212	H	*c*-C_5H_9	H	5.36 ± 3.19	52.79 ± 1.7
8t	QFF213	H	*c*-C_6H_{11}	H	13.40 ± 0.46	17.20 ± 7.54
8u	QFF209	H	CH_2Ph	H	7.52 ± 2.50	28.11 ± 2.39
8v	QFF214	H	H	Me	9.56 ± 1.19	15.97 ± 1.62

[a] Internal code used in the identification of each compound.
[b] Data represent the mean ± SEM of the percentage of nNOS and iNOS inhibition produced by 1 mM concentration of each compound. Each value is the mean of three experiments performed by triplicate in homogenates of four rat striata in each one.

a 5′-Cl substituent is detrimental for the activity and consequently a 5′-MeO substituent or an unsubstituted benzene ring are preferable.

Regarding the influence of the *N*-substituent over the activity, the available information is also confused: compounds **8b** and **8m** ($R^2 = Me$) or **8n** ($R^2 = Et$) show moderate inhibition percentages; compound **8d** ($R^1 = MeO$, $R^2 = c\text{-}C_3H_5$) also show a moderate inhibition, but compound **8q** ($R^1 = Cl$, $R^2 = c\text{-}C_3H_5$) does not inhibit iNOS. On the other hand, a further increase in the R^2 volume gives place to an increment in the inhibition activity. Compounds **8e** (26%, $R^1 = MeO$, $R^2 = c\text{–}C_5H_9$), **8f** (22%, $R^1 = MeO$, $R^2 = CH_2Ph$), and **8u** (28%, $R^1 = H$, $R^2 = CH_2Ph$) show moderate inhibition, and compound **8s** (52%, $R^1 = H$, $R^2 = c\text{-}C_5H_9$) is the best inhibitor in all the series.

In contrast with what can be expected from the docking studies, compounds **8a–v** behave as weak NOS inhibitors, and this can be due to two different reasons. The first one is related with the fact that the scoring function used in Glide could not properly evaluate the interaction between the iron atom of the heme group and the benzene ring of the ligand, giving poor predictions for the score of each complex. The second one is that these molecules could act as non-competitive inhibitors.

Regarding the second possibility, we have found that melatonin **1** [34], kynurerine **2** (R = Me) [34], and AMK (**3**, $R^1 = OMe$, $R^2 = Me$) [37] behave as non-competitive nNOS inhibitors. We have also described that the incorporation of increasing amounts of calmodulin (CaM) in the incubation medium resulted in a progressive loss of the efficiency to inhibit nNOS. On the other hand, these molecules bound Ca–CaM complex, indicating that this interaction is responsible of nNOS inhibition.

Compounds **8a–v** do not bind calmodulin (data not shown) and this mechanism seem to be not applicable to the inhibition of nNOS by these molecules.

iNOS is not regulated by CaM, since CaM is bound to it with high affinity and functions as a permanent enzyme subunit. For this reason, interaction of compounds **8a–v** with CaM is not a suitable mechanism for iNOS inhibition.

Nevertheless, the contrast between docking studies and *in vitro* experimental values seems to suggest another type of mechanism, probably due to the interaction with other part of the enzyme.

2.4. In vivo *assays*

It has been described that melatonin **1** reduces the iNOS activity and expression in several inflammatory models [50]. Melatonin also inhibits the mitochondrial i-mtNOS activity and expression [21,51] and the nNOS activity [52] It has also found that melatonin shows neuroprotection properties in different models, including MPTP-induced Parkinsonism [28a,53].

Table 2
Total NOS activities (pmol L-[^{3}H]-citrulline × (mg of protein)$^{-1}$ × min^{-1}) measured in both the cytosol and mitochondria cell fractions isolated from the substantia nigra (SN) in mice treated with MPTP, MPTP/melatonin (aMT), MPTP/QFF-205 (**8m**) and MPTP/QFF-212 (**8s**).

Compound	Cytosol		Mitochondria	
	nNOS	iNOS	c-mtNOS	i-mtNOS
Control	95.44 ± 0.86	2.82 ± 0.09	28.64 ± 0.62	22.08 ± 0.12
MPTP	83.65 ± 0.37	29.37 ± 0.68	25.86 ± 0.54	43.75 ± 0.97
aMT	19.91 ± 1.93	19.02 ± 1.73	13.75 ± 0.32	23.60 ± 2.18
8m	65.53 ± 1.84	5.78 ± 0.84	9.95 ± 0.98	7.46 ± 0.98
8s	74.35 ± 1.93	6.53 ± 0.29	14.1 ± 1.83	9.45 ± 0.81

For these reasons, the more active iNOS inhibitors **8m** and **8s** have been selected to test their ability to reduce the *in vivo* NOS activity in both the cytosol and the mitochondria in the substantia nigra (SN) of the MPTP Parkinson's disease. Table 2 shows the total experimental NOS activities found in each cell fraction, and Fig. 2 shows the NOS relative activities in both cell fractions considering the NOS activities in control animals as 100%.

MPTP administration (see Section 4 for details) slightly decreases the nNOS (83.6 ± 0.4 vs. 95.4 ± 0.9 pmol/min/mg prot) and c-mtNOS (25.9 ± 0.5 vs. 28.6 ± 0.6 pmol/min/mg prot) activities in cytosol and mitochondria, respectively. Melatonin administration before the MPTP treatment significantly reduced nNOS (19.9 ± 1.9 vs. 83.6 ± 0.4 pmol/min/mg prot) and c-mtNOS (13.7 ± 0.3 vs. 25.9 ± 0.5 pmol/min/mg prot) activities in MPTP-treated mice (Table 2). Administration of compounds **8m** and **8s** reduced the nNOS activity in a lesser extent than melatonin, but they provoke a stronger reduction in c-mtNOS activity than that produced by melatonin (Table 2).

The iNOS activity present in the cytosol of control animals is almost undetectable, while MPTP administration increased it up to 10 times (29.4 ± 0.7 vs. 2.8 ± 0.1 pmol/min/mg prot). Melatonin administration partially counteracted the effect of MPTP on iNOS activity (19.0 ± 1.7 pmol/min/mg prot), while administration of compounds **8m** and **8s** significantly reduces the MPTP-induced iNOS activity to control values (5.8 ± 0.8 and 6.5 ± 0.3 pmol/min/mg, respectively). On the other hand, MPTP increased 2 times the i-mtNOS activity (43.7 ± 1.0 vs. 22.1 ± 0.1 pmol/min/mg prot), whereas melatonin absolutely prevented this effect of MPTP

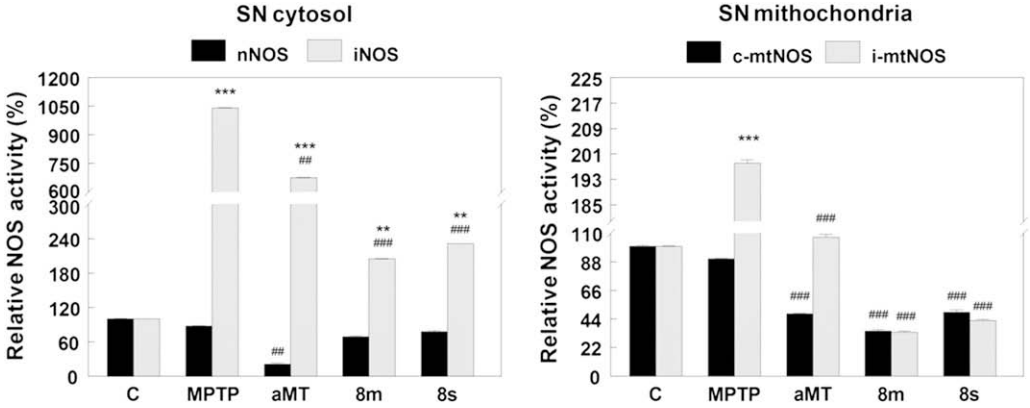


Fig. 2. Relative NOS activities (%) measured in both the cytosol (up) and mitochondria (down) cell fractions isolated from the substantia nigra (SN) in mice treated with MPTP, MPTP/melatonin (aMT), MPTP/QFF-205 (**8m**) and MPTP/QFF-212 (**8s**). C represents control animals treated with the vehicle (ethanol/saline). Data represents means ± SEM of seven experiments performed by triplicate in homogenates of four SN in each one. $^*P < 0.05$, $^{**}P < 0.01$ and $^{***}P < 0.001$ vs. control.

(23.6 ± 2.2 pmol/min/mg prot). Interestingly, compounds **8m** and **8s** were much more efficient than melatonin in reducing i-mtNOS activity in MPTP-treated animals (7.5 ± 1.0 and 9.4 ± 0.8 pmol/min/mg prot, respectively) (Table 2).

Since compounds **8m** and **8s** are not potent iNOS *in vitro* inhibitors, it seems that a direct interaction with this enzyme is not the molecular mechanism for the observed *in vivo* activities. More in deep studies are needed in order to clarify the biological mechanism by which these molecules diminish the NOS activities *in vivo*. At present we are testing two possibilities: i) Compounds **8m** and **8s** can be metabolized so that their *in vivo* activity can be due to a common metabolite that interacts with iNOS, and ii) These molecules, instead of a direct blockade of the NOS activity could modify the genomic expression of iNOS (or i-mtNOS), diminishing by this indirect route the activity of these enzymes. The last possibility seems to be more probable. In fact, melatonin exerts some of its functions through its interaction with ROR/RZR nuclear receptors [28a,54] and compounds **8a**–**v** could behave in a similar way.

Even if the molecular mechanism is still unknown, compounds **8m** and **8s** selectively decrease the NOS activity due to the inducible isoforms of this enzyme in both cytosol and mitochondria. Since i-NOS and i-mtNOS are those that suffer higher alteration in several physiological disorders, the potentiality of these molecules in the development of compounds with interesting pharmacological properties is clear.

3. Conclusions

In summary, the more interesting findings in this paper are two: i) compounds **8m** and **8s** show selectivity in the *in vitro* inhibition of the iNOS isoform; and ii) both compounds produce a strong reduction of both the iNOS (cytosol) and i-mtNOS (mitochondria) *in vivo* activities induced by the toxin administration in the MPTP Parkinson model.

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 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 $CDCl_3$, CD_3OD, $(CD_3)_2CO$ or DMSO-d_6 (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 an 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 (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 an eluent.

4.1. Preparation of 2-nitro-5-substituted-cinnamadehyde ***10a–b***. General method

2-Nitro-5-substituted-benzaldehyde **9a**,**b** (8.29 mmol) was added to a solution of 8.29 mmol of (triphenylphosphoranylidene)acetaldehyde in dichloromethane (25 mL). The mixture was stirred at room temperature between 8 and 24 h, under argon atmosphere. After this period, the mixture was concentrated to dryness, and the obtained solid was purified by flash chromatography (ethyl acetate:hexane 1:50).

4.1.1. 5-Methoxy-2-nitrocinnamaldehyde, ***10a***

(87%); mp. 123–125 °C; MS (LSIMS) *m*/*z* 230.0427 $(M + Na)^+$, Calcd. Mass for $C_{10}H_9NO_4Na$ 230.0429.

4.1.2. 5-Chloro-2-nitrocinnamaldehyde, ***10b***

(84%); mp. 180–181 °C; MS (LSIMS) *m*/*z* 233.9942 $(M + Na)^+$, Calcd. Mass for $C_9H_6NO_3ClNa$ 233.9933.

4.2. Preparation of α-azido-5-(2-nitro-5-substituted-phenyl)-2,4-pentadienoic acid ethyl esther ***11a–c***. General method

To stirred solution of ethyl azidoacetate (69.12 mmol) and the appropriate 2-nitro-5-substituted-cinnamadehyde **10a–c** (13.43 mmol) in dry ethanol, a solution of sodium ethanolate (70 mmol of Na in 60 mL of dry ethanol) was added dropwise. The reaction mixture was stirred under argon atmosphere at −20 °C for 4.5 h, and then poured into water. The aqueous mixture was extracted with ethyl acetate (3 × 50 mL), and the combined organic layers were dried (Na_2SO_4), filtered, and concentrated to yield a crude material which was recrystallized from methanol/diethyl ether or ethyl acetate/hexane. Compounds **11a–c** are unstable and spontaneously tend to cyclize to the 2-arylpyrroles derivatives, and these compounds were not isolated for this reason.

4.3. Preparation of 5-(2-nitro-5-substituted-phenyl)-1H-pyrrole-2-carboxylic *acid ethyl ester* ***12a–c***. General method

The appropriated azides **11a–c** were suspended in *p*-xilene and heated (60–125 °C) for 7–24 h. Evaporation of the solvent allows to obtain a solid that was recrystallized or purified by flash chromatography.

4.3.1. 5-(5-Methoxy-2-nitrophenyl)-1H-pyrrole-2-carboxylic *acid ethyl ester* ***12a***

(80 °C, 12 h, ethyl acetate:hexane 1:5, 95%); mp 146–148 °C; ^{1}H-NMR ($(CD_3)_2CO$): δ 11.15 (bs, 1H), 7.99 (d,1H), 7.17 (d, 1H), 7.08 (dd, 1H), 6.89 (dd, 1H), 6.34 (dd, 1H), 3.96 (s, 1H); ^{13}C-NMR ($(CD_3)_2CO$): δ 162.70, 161.22, 141.78, 132.31, 129.55, 127.04, 124.48, 116.56, 115.78, 114.10, 110.59, and 55.79; MS (LSIMS) *m*/*z* 313.0803 $(M + Na)^+$, Calcd. Mass for $C_{14}H_{14}N_2O_5Na$ 313.0800.

4.3.2. 5-(5-Chloro-2-nitrophenyl)-1H-pyrrole-2-carboxylic *acid ethyl ester* ***12b***

(60 °C, 8 h, recrystallized from methanol/diethyl ether, 96%); mp 121–123 °C; ^{1}H-NMR ($(CD_3)_2CO$): δ 11.30 (bs, 1H), 7.99 (d, 1H), 7.82 (d, 1H), 7.65 (dd, 1H), 6.92 (dd, 1H, dd), 6.41 (dd, 1H); ^{13}C-NMR (CD_3OD): δ 161.67, 147.97, 138.52, 131.98, 130.83, 130.72, 129.50, 128.89, 126.83, 116.70, and 111.82; MS (LSIMS) *m*/*z* 317.0298 $(M + Na)^+$, Calcd. Mass for $C_{13}H_{11}N_2O_4ClNa$ 317.0305.

4.3.3. 5-(2-Nitrophenyl)-1H-pyrrole-2-carboxylic *acid ethyl ester* ***12c***

(125 °C, 24 h recrystallized from dichloromethane/hexane, 91%); mp 150–151 °C; ^{1}H-NMR ($(CD_3)_2CO$): δ 11.35 (bs, 1H), 7.87 (d, 1H), 7.66 (m, 2H), 7.53 (pt, 1H), 6.90 (dd, 1H), 6.25 (dd, 1H); ^{13}C-NMR ($(CD_3)_2CO$): δ 164.14, 150.22, 133.37, 132.95, 132.50, 129.84, 127.74, 125.78, 125.09, 117.39, and 111.31; MS (LSIMS) *m*/*z* 261.0879 $(M + H)^+$, Calcd. Mass for $C_{13}H_{13}N_2O_4$ 261.0875.

4.4. Preparation of 1-methyl-5-(2-nitrophenyl)-1H-pyrrole-2-carboxylic *acid ethyl ester* ***12d***

Potassium *tert*-butoxide (11.6 mmol) was added to a solution of 18-crown-6 (1 mmol) in 20 mL of dry ether, and the mixture was stirred 15 min. After that, 0.5 mmol of **12c** was added, and reaction

mixture was stirred for another 15 min. Then, a solution of CH_3I (0.139 mmol) in 5 mL of ethyl ether was added dropwise at 0 °C, and the reaction mixture was stirred for 18 h at room temperature. Finally, the reaction mixture was washed with water (3 × 10 mL) and the aqueous layers extracted with ethyl acetate (3 × 10 mL). The combined organic layer was dried (Na_2SO_4), filtered and concentrated to obtain brown oil that was identified as compound **12d**.

4.5. 1-Methyl-5-(2-nitrophenyl)-1H-pyrrole-2-carboxylic acid ethyl ester **12d**

(92%); ^{1}H-NMR ($CDCl_3$): δ 8.06 (dd, 1H), 7.70 (pt, 1H,), 7.62 (pt, 1H), 7.46 (dd, 1H), 7.04 (d, 1H), 6.13 (d, 1H), 4.33 (q, 2H), 3.72 (s, 1H), 1.39 (t, 3H); ^{13}C-NMR ($CDCl_3$): δ 161.42, 149.85, 135.72, 133.41, 132.77, 129.88, 127.20, 124.45, 124.03, 117.39, 109.19, 59.98, 33.96, and 14.51; MS (LSIMS) *m*/*z* 297.0853 $(M + Na)^+$, Calcd. Mass for $C_{14}H_{14}N_2O_4Na$ 297.0851.

4.6. Preparation of 5-(2-nitro-5-substituted-phenyl)-1H-pyrrole-2-carboxylic acid. **13a–c**. General method

The appropriated ethyl 5-(2-nitro-5-substituted-phenyl)-1*H*-pyrrole-2-carboxylate **12a–c** (2.04 mmol) was stirred and dissolved in 1 N NaOH solution (4.08 mmol) at 100 °C, glacial AcOH (4.08 mmol) was then added, and the solution was stirred at room temperature for 1 h. The solution was extracted with ethyl acetate (3 × 50 mL), and the combined organic layers were washed with water, dried (Na_2SO_4), filtered, and concentrated to yield a crude material that was recrystallized from ethyl acetate/hexane.

4.6.1. 5-(5-Methoxy-2-nitrophenyl)-1H-pyrrole-2-carboxylic acid **13a**

(82%); mp 201–203 °C; ^{1}H-NMR ($(CD_3)_2CO$): δ 11.15 (bs, 1H); 7.99 (d, 1H); 7.17 (d, 1H); 7.08 (dd, 1H) 6.89 (dd, 1H); 6.34 (dd, 1H); 3.96 (bs, 1H); ^{13}C-NMR ($(CD_3)_2CO$): δ 162.70, 161.22, 141.78, 132.31, 129.55, 127.04, 124.48, 116.56, 115.78, 114.10, 110.59, and 55.79; MS (LSIMS) *m*/*z* 285.0486 $(M + Na)^+$, Calcd. Mass for $C_{12}H_{10}N_2O_5Na$ 285.0487.

4.6.2. 5-(5-Chloro-2-nitrophenyl)-1H-pyrrole-2-carboxylic acid **13b**

(93%); mp 197–199 °C; ^{1}H-NMR ($(CD_3)_2CO$): δ 11.30 (bs, 1H), 7.99 (d, 1H), 7.82 (d, 1H), 7.65 (dd, 1H), 6.92 (dd, 1H), 6.41 (dd, 1H); ^{13}C-NMR (CD_3OD): δ 161.67, 147.97, 138.52, 131.98, 130.83, 130.72, 129.50, and 128.89; MS (LSIMS) *m*/*z* 288.998 $(M + Na)^+$, Calcd. Mass for $C_{11}H_7N_2O_4ClNa$ 288.9992.

4.6.3. 5-(2-Nitrophenyl)-1H-pyrrole-2-carboxylic acid **13c**

(93%); mp 221–222 °C; ^{1}H-NMR ($(CD_3)_2CO$): δ 11.35 (bs, 1H), 7.87 (d, 1H), 7.66 (m, 2H), 7.53 (pt, 1H), 6.90 (dd, 1H), 6.25 (dd, 1H); ^{13}C-NMR ($(CD_3)_2CO$): δ 164.14, 150.22, 133.37, 132.95, 132.50, 129.84, 127.74, 125.78, 125.09, 117.39, and 111.31; MS (LSIMS) *m*/*z* 255.0379 $(M + Na)^+$, Calcd. Mass for $C_{11}H_8N_2O_4Na$ 255.0381.

4.7. Preparation of 5-(2-nitro-5-substituted-phenyl)-1H-pyrrole-2-carboxylic acid alkylamide **14b–f**, **h–k**, **m–u**. General method

$SOCl_2$ (11 mmol) was added to a solution of 5-(2-nitro-5-substituted-phenyl)-1*H*-pyrrole-2-carboxylic acid **13a–c** (1 mmol) in dry CH_3CN (30 mL), and the reaction mixture was stirred at 65–80 °C for 5 h. After this period, the mixture was concentrated to dryness, yielding a brown solid (the acyl chloride) that was solved in CH_2Cl_2 (10 mL), and a solution of the appropriated amine (R^3NH_2, 2 mmol) and TEA (3 mmol) in CH_2Cl_2 (3 mL) was added dropwise. The reaction mixture was stirred for 3 h at room temperature, washed with H_2O several times and the combined aqueous layers extracted with CH_2Cl_2 (3 × 50 mL). The combined organic layers were dried (Na_2SO_4), filtered, concentrated and the residue recrystallized or purified by flash chromatography.

4.7.1. 5-(5-Methoxy-2-nitrophenyl)-1H-pyrrole-2-carboxylic acid methylamide **14b**

(91%); mp 137–139 °C; ^{1}H-NMR ($CDCl_3$): δ 10.60 (bs, 1H), 7.90 (d, 1H), 6.99 (d, 1H), 6.87 (dd, 1H), 6.54 (pt, 1H), 6.30 (pt, 1H), 6.02 (m, 1H), 3.86 (s, 3H), 2.85 (d, 3H). ^{13}C-NMR ($CDCl_3$): δ 162.50, 161.72, 141.68, 130.37, 129.55, 127.47, 127.26, 116.39, 113.55, 111.12, 109.71, 56.05, and 26.21; MS (LSIMS) *m*/*z* 298.0801 $(M + Na)^+$, Calcd. Mass for $C_{13}H_{13}N_3O_4Na$ 298.0803.

4.7.2. 5-(5-Methoxy-2-nitrophenyl)-1H-pyrrole-2-carboxylic acid propylamide **14c**

(82%); mp 141–143 °C; ^{1}H-NMR ($CDCl_3$): δ 10.45 (bs, 1H), 7.90 (d, 1H), 6.99 (d, 1H), 6.88 (dd, 1H), 6.55 (d, 1H), 6.32 (d, 1H), 5.94 (bs, 1H), 3.87 (s, 3H), 3.27 (m, 2H), 1.54 (m, 2H), 0.91 (t, 3H); ^{13}C-NMR ($CDCl_3$): δ 162.51, 160.99, 141.73, 130.29, 129.49, 127.57, 127.27, 116.23, 113.63, 111.16, 109.49, 56.05, 41.23, 23.08, and 11.42; MS (LSIMS) *m*/*z* 326.1114 $(M + Na)^+$, Calcd. Mass for $C_{15}H_{17}N_3O_4Na$ 326.1116.

4.7.3. 5-(5-Methoxy-2-nitrophenyl)-1H-pyrrole-2-carboxylic acid cyclopropylamide **14d**

(71%); mp 158–159 °C; ^{1}H-NMR ($CDCl_3$): δ 10.40 (bs, 1H), 7.90 (d, 1H), 6.98 (d, 1H), 6.87 (dd, 1H), 6.52 (bs, 1H), 6.30 (pt, 1H), 6.13 (bs, 1H), 3.87 (s, 3H), 2.75 (m, 1H); 0.75 (m, 2H), 0.55 (m. 2H); ^{13}C-NMR ($CDCl_3$): δ 162.54, 141.68, 130.49, 129.41, 127.32, 116.29, 113.63, 111.26, 109.98, 56.07, 22.72, and 6.86; MS (LSIMS) *m*/*z* 324.0959 $(M + Na)^+$, Calcd. Mass for $C_{15}H_{15}N_3O_4Na$ 324.0960.

4.7.4. 5-(5-Methoxy-2-nitrophenyl)-1H-pyrrole-2-carboxylic acid cyclopentylamide **14e**

(90%); mp 181–183 °C; ^{1}H-NMR ($CDCl_3$): δ 10.70 (bs, 1H), 7.89 (d, 1H), 6.98 (d, 1H), 6.87 (dd, 1H), 6.52 (bs, 1H), 6.30 (bs, 1H), 5.87 (d, 1H), 4.17 (m, 1H), 3.86 (s, 3H), 1.95–1.36 (m, 8H); ^{13}C-NMR (CDCl3): δ 162.48, 160.69, 141.66, 130.40, 129.55, 127.59, 127.20, 116.18, 113.63, 111.03, 109.62, 56.04, 51.27, 33.25, and 23.81; MS (LSIMS) *m*/*z* 352.1273 $(M + Na)^+$, Calcd. Mass for $C_{17}H_{19}N_3O_4Na$ 352.1273.

4.7.5. 5-(5-Methoxy-2-nitrophenyl)-1H-pyrrole-2-carboxylic acid benzylamide **14f**

(90%); mp 181–182 °C; ^{1}H-NMR ($(CD_3)_2CO$): δ 11.27 (bs, 1H), 7.96 (bs, 1H); 7.92 (d, 1H), 7.25 (m, 5H), 7.18 (d, 1H), 7.00 (dd, 1H), 6.88 (dd, 1H), 6.28 (dd, 1H), 4.49 (d, 2H), 3.95 (s, 3H); ^{13}C-NMR ($(CD_3)_2CO$): δ 162.49, 160.72, 141.82, 139.90, 130.49, 129.56, 128.34, 128.23, 127.57, 126.86, 116.30, 113.60, 110.42, 110.15, 55.71, and 42.72; MS (LSIMS) *m*/*z* 374.1117 $(M + Na)^+$, Calcd. Mass for $C_{19}H_{17}N_3O_4Na$ 374.1116.

4.7.6. 5-(5-Chloro-2-nitrophenyl)-1H-pyrrole-2-carboxylic acid methylamide **14h**

(79%); mp 191–193 °C; ^{1}H-NMR ($(CD_3)_2CO$): δ 11.45 (bs, 1H), 7.92 (d, 1H), 7.79 (d, 1H), 7.57 (dd, 1H), 7.52 (bs, 1H), 6.78 (dd, 1H), 6.32 (dd, 1H), 2.78 (d, 3H); ^{13}C-NMR ($(CD_3)_2CO$): δ 162.47, 148.51, 138.77, 132.17, 130.70, 129.69, 129.37, 129.28, 127.12, 111.96, 111.60, and 26.55; MS (LSIMS) *m*/*z* 280.0485 $(M + H)^+$, Calcd. Mass for $C_{12}H_{11}N_3O_3Cl$ 280.0489.

4.7.7. 5-(5-Chloro-2-nitrophenyl)-1H-pyrrole-2-carboxylic acid ethylamide **14i**

(64%); mp 143–145 °C; ^{1}H-NMR ($CDCl_3$): δ 10.78 (bs, 1H), 7.75 (d, 1H), 7.56 (d, 1H), 7.36 (dd, 1H), 6.55 (dd, 1H), 6.35 (dd, 1H), 3.91 (bs,

1H), 3.35 (m, 2H), 1.16 (t, 3H); ^{13}C-NMR ($CDCl_3$): δ 160.82, 146.74, 138.47, 131.04, 128.46, 128.36, 128.30, 128.10, 125.83, 111.67, 109.92, 34.51, and 15.04; MS (LSIMS) *m/z* 316.0472 $(M + Na)^+$, Calcd. Mass for $C_{13}H_{12}N_3O_3ClNa$ 316.04648.

*4.7.8. 5-(5-Chloro-2-nitrophenyl)-1*H*-pyrrole-2-*carboxylic *acid butylamide* ***14j***

(74%); mp 105–107 °C; ^{1}H-NMR ($CDCl_3$): δ 10.40 (bs, 1H), 7.78 (d, 1H), 7.60 (d, 1H), 7.41 (dd, 1H), 6.58 (d, 1H), 6.40 (d, 1H), 5.93 (bs, 1H), 3.39 (m, 2H), 1.57 (m, 2H), 1.34 (m, 2H), 0.96 (t, 3H); ^{13}C-NMR ($CDCl_3$): δ 160.72, 146.69, 138.57, 130.88, 130.48, 128.36, 128.22, 128.17, 125.90, 111.83, 109.73, 39.36, 31.36, 20.17, and 13.84; MS (LSIMS) *m/z* 344.0777 $(M + Na)^+$, Calcd. Mass for $C_{15}H_{16}N_3O_3ClNa$ 344.0778.

*4.7.9. 5-(5-Chloro-2-nitrophenyl)-1*H*-pyrrole-2-*carboxylic *acid cyclopropylamide* ***14k***

(80%); mp 164–166 °C; ^{1}H-NMR ($CDCl_3$): δ 10.50 (bs, 1H), 7.74 (d, 1H), 7.58 (ps, 1H), 7.37 (dd, 1H), 6.52 (bs, 1H), 6.34 (bs, 1H), 6.09 (bs, 1H), 2.78 (m, 1H), 0.78–0.57 (m, 4H); ^{13}C-NMR ($CDCl_3$): δ 163.53, 148.07, 139.86, 132.25, 129.82, 129.51, 129.42, 127.18, 113.17, 111.53, 24.07, and 8.24; MS (LSIMS) *m/z* 328.0465 $(M + Na)^+$, Calcd. Mass for $C_{14}H_{12}N_3O_3ClNa$ 328.0465.

*4.7.10. 5-(2-Nitrophenyl)-1*H*-pyrrole-2-*carboxylic *acid methylamide* ***14m***

(76%); mp 163–164 °C; ^{1}H-NMR (DMSO-d_6): δ 11.86 (bs, 1H), 8.07 (q, 1H), 7.90 (d, 1H), 7.83 (m, 2H), 7.51 (ddd, 1H), 6.77 (dd, 1H), 6.16 (dd, 1H), 2.74 (d, 3H); ^{13}C-NMR (DMSO-d_6): δ 160.72, 147.83, 132.29, 131.22, 128.83, 128.48, 128.15, 126.00, 123.84, 110.66, 109.15, and 25.46; MS (LSIMS) *m/z* 268.0699 $(M + Na)^+$, Calcd. Mass for $C_{12}H_{11}N_3O_3Na$ 268.0698.

*4.7.11. 5-(2-Nitrophenyl)-1*H*-pyrrole-2-*carboxylic *acid ethylamide* ***14n***

(48%); mp 207–208 °C; ^{1}H-NMR (DMSO-d_6): δ 11.86 (bs, 1H), 8.10 (t, 1H), 7.91 (d, 1H), 7.66 (m, 2H), 7.51 (pt, 1H), 6.80 (pt, 1H), 6.15 (pt, 1H), 3.24 (m, 2H), 1.10 (t, 3H); ^{13}C-NMR (DMSO-d_6): δ 160.01, 147.86, 132.31, 131.22, 128.85, 128.57, 128.17, 126.02, 123.85, 110.77, 109.13, 33.28, and 14.98; MS (LSIMS) *m/z* 282.0855 $(M + Na)^+$, Calcd. Mass for $C_{13}H_{13}N_3O_3Na$ 282.0854.

*4.7.12. 5-(2-Nitrophenyl)-1*H*-pyrrole-2-*carboxylic *acid propylamide* ***14o***

(65%); mp 187–189 °C; ^{1}H-NMR (CD_3OD): δ 7.83 (d, 1H), 7.65 (m, 2H), 7.52 (ddd, 1H), 6.82 (d, 1H), 6.25 (d, 1H), 3.31 (m, 2H), 1.62 (m, 2H), 0.98 (t, 3H); ^{13}C-NMR ($CDCl_3$): δ 160.79, 148.73, 132.34, 130.84, 129.33, 128.41, 127.83, 126.27, 124.34, 111.14, 109.59, 41.27, 23.15, and 11.49; MS (LSIMS) *m/z* 296.1013 $(M + Na)^+$, Calcd. Mass for $C_{14}H_{15}N_3O_3Na$ 296.1011.

*4.7.13. 5-(2-Nitrophenyl)-1*H*-pyrrole-2-*carboxylic *acid butylamide* ***14p***

(82%); mp 169–170 °C; ^{1}H-NMR ($(CD_3)_2CO$): δ 11.29 (bs, 1H), 7.86 (dd, 1H), 7.77 (dd, 1H), 7.69 (pt, 1H), 7.55 (pt, 1H), 7.49 (bs, 1H), 6.79 (dd, 1H), 6.24 (dd, 1H), 3.27 (m, 2H), 1.49 (m, 2H), 1.32 (m, 2H), 0.87 (t, 3H); ^{13}C-NMR ($(CD_3)_2CO$): δ 160.55, 148.90, 132.18, 131.32, 129.30, 128.94, 128.34, 126.48, 123.89, 110.18, 109.88, 38.66, 31.89, 19.92, and 13.30; MS (LSIMS) *m/z* 310.1168 $(M + Na)^+$, Calcd. Mass for $C_{15}H_{17}N_3O_3Na$ 310.1167.

*4.7.14. 5-(2-Nitrophenyl)-1*H*-pyrrole-2-*carboxylic *acid cyclopropylamide* ***14q***

(80%); mp 214–215 °C; ^{1}H-NMR ($(CD_3)_2CO$): δ 11.50 (bs, 1H), 7.86 (d, 1H), 7.77 (d, 1H), 7.69 (t, 1H), 7.54 (m, 2H), 6.76 (d, 1H), 6.24 (d, 1H), 2.86 (m, 1H), 0.65–0.52 (m, 4H); ^{13}C-NMR ($(CD_3)_2CO$): δ 162.49, 149.65, 132.92, 132.03, 130.16, 129.49, 129.12, 127.17, 124.63, 111.24, 110.65, 23.21, and 6.40; MS (LSIMS) *m/z* 294.0855 $(M + Na)^+$, Calcd. Mass for $C_{14}H_{13}N_3O_3Na$ 294.0854.

*4.7.15. 5-(2-Nitrophenyl)-1*H*-pyrrole-2-*carboxylic *acid cyclobutylamide* ***14r***

(61%); mp 170–173 °C; ^{1}H-NMR ($CDCl_3$): δ 10.53 (bs, 1H), 7.77 (d, 1H), 7.58 (m, 2H), 7.44 (pt, 1H), 6.56 (pt, 1H), 6.32 (pt, 1H); 6.06 (d, 1H), 4.40 (m, 1H), 2.27 (m, 2H), 1.89 (m, 2H), 1.67 (m, 2H); ^{13}C-NMR ($CDCl_3$): δ 159.90, 148.73, 132.18, 131.08, 129.73, 128.27, 127.64, 126.44, 124.21, 110.98, 109.91, 44.69, 31.45, and 15.18; MS (LSIMS) *m/z* 286.1192 $(M + H)^+$, Calcd. Mass for $C_{15}H_{16}N_3O_3$ 286.1191.

*4.7.16. 5-(2-Nitrophenyl)-1*H*-pyrrole-2-*carboxylic *acid cyclopentylamide* ***14s***

(68%), mp 174–175 °C; ^{1}H-NMR ($CDCl_3$): δ 10.52 (bs, 1H), 7.75 (d, 1H), 7.57 (m, 2H), 7.41 (pt, 1H), 6.53 (d, 1H), 6.31 (pt, 1H), 5.86 (d, 1H), 4.20 (m, 1H), 1.99–1.35 (m, 8H); ^{13}C-NMR ($CDCl_3$): δ 160.56, 148.73, 132.16, 131.08, 129.56, 128.20, 127.94, 126.42, 124.18, 110.91, 109.74, 51.24, 33.27, and 23.80; MS (LSIMS) *m/z* 322.1168 $(M + Na)^+$, Calcd. Mass for $C_{16}H_{17}N_3O_3Na$ 322.1167.

*4.7.17. 5-(2-Nitrophenyl)-1*H*-pyrrole-2-*carboxylic *acid cyclohexylamide* ***14t***

(80%); mp 171–172 °C; ^{1}H-NMR ($CDCl_3$): δ 10.55 (bs, 1H), 7.75 (d, 1H), 7.56 (m, 2H), 7.41 (pt, 1H), 6.54 (d, 1H), 6.32 (d, 1H), 5.78 (d, 1H), 3.78 (m, 1H), 1.92–1.58 (m, 5H), 1.38–1.09 (m, 5H); ^{13}C-NMR ($CDCl_3$): δ 160.04, 148.70, 132.17, 131.01, 129.53, 128.19, 128.03, 126.46, 124.21, 110.90, 109.66, 48.22, 33.35, 25.57, and 24.96; MS (LSIMS) *m/z* 336.3434 $(M + Na)^+$, Calcd. Mass for $C_{17}H_{19}N_3O_3Na$ 336.3441.

*4.7.18. 5-(2-Nitrophenyl)-1*H*-pyrrole-2-*carboxylic *acid benzylamide* ***14u***

(70%); mp 137–138 °C; ^{1}H-NMR (CD_3OD): δ 7.82 (d, 1H), 7.65 (m, 2H), 7.51 (pt, 1H), 7.32 (m, 5H), 6.88 (d, 1H), 6.26 (d, 1H), 5.78 (bs, 1H), 4.54 (s, 2H); ^{13}C-NMR (CD_3OD): δ 160.20, 147.86, 139.77, 132.34, 131.26, 129.20, 128.26, 128.16, 128.16, 127.10, 126.63, 125.95, 123.87, 111.21, 109.24, and 41.84; MS (LSIMS) *m/z* 344.1020 $(M + Na)^+$, Calcd. Mass for $C_{18}H_{15}N_3O_3Na$ 344.1011.

*4.8. Preparation of 5-(2-nitro-5-substituted-phenyl)-1*H*-pyrrole-2-carboxamide* ***14a, g, l, v****. General method*

5-(2-Nitro-5-substituted-phenyl)-1*H*-pyrrole-2-*carboxylic* acid ethyl ester **12a–d** (1.92 mmol) and NH_4Cl (27.4 mmol) were added to a concentrated NH_4OH solution (60 mL), and the mixture was heated overnight at 100 °C in a pressure reactor. After cooling, the resulting precipitate was filtered off and the aqueous solution was extracted with AcOEt (3 × 30 mL). The combined organic layers were dried ($MgSO_4$), filtered and concentrated to yield a solid that was recrystallized ($AcOEt/C_6H_{12}$).

*4.8.1. 5-(5-Methoxy-2-nitrophenyl)-1*H*-pyrrole-2-carboxamide* ***14a***

(94%); mp 123–125 °C; ^{1}H-NMR ($(CD_3)_2CO$): δ 11.98 (bs, 1H), 7.95 (d, 1H), 7.20 (d, 1H), 7.05 (dd, 1H), 6.87 (dd, 1H), 6.30 (dd, 1H), 3.99 (s, 3H); ^{13}C-NMR ($(CD_3)_2CO$): δ 162.47, 162.09, 141.73, 130.23, 129.36, 128.16, 126.80, 115.98, 113.70, 111.07, 110.12, and 55.67; MS (LSIMS) *m/z* 284.0645 $(M + Na)^+$, Calcd. Mass for $C_{12}H_{11}N_3O_4Na$ 284.0647.

*4.8.2. 5-(5-Chloro-2-nitrophenyl)-1*H*-pyrrole-2-carboxamide* ***14g***

(81%); mp 220–222 °C; ^{1}H-NMR ($(CD_3)_2CO$): δ 11.22 (bs, 1H), 7.93 (d, 1H), 7.83 (d, 1H), 7.56 (dd, 1H), 7.30 (bs, 1H), 6.56 (bs, 1H), 6.90

(dd, 1H), 6.30 (dd, 1H); ^{13}C-NMR ($(CD_3)_2CO$): δ 162.69, 147.87, 138.28, 131.56, 129.62, 128.92, 126.62, 112.23, and 111.41; MS (LSIMS) *m/z* 288.0154 $(M + Na)^+$, Calcd. Mass for $C_{11}H_8N_3O_3ClNa$ 288.0152.

4.8.3. 5-(2-Nitrophenyl)-1H-pyrrole-2-carboxamide ***14l***

(90%); mp 137–138 °C; ^{1}H-NMR ($CDCl_3$): δ 9.90 (bs, 1H), 7.80 (d, 1H), 7.59 (m, 2H), 7.45 (pt, 1H), 6.64 (dd, 1H), 6.38 (pt, 1H), 5.68 (bs, 2H); ^{13}C-NMR (CD_3CD): δ 169.51, 149.99, 133.05, 132.92, 131.32, 128.72, 127.81, 124.80, 114.23, and 110.74; MS (LSIMS) *m/z* 254.0546 $(M + Na)^+$, Calcd. Mass for $C_{11}H_9N_3O_3Na$ 254.0542.

4.8.4. 1-Methyl-5-(2-nitrophenyl)-1H-pyrrole-2-carboxamide ***14v***

(55%); mp 138–140 °C; ^{1}H-NMR (CD_3COD): δ 8.05 (dd, 1H), 7.82 (pt, 1H), 7.73 (pt, 1H), 7.59 (dd, 1H), 6.88 (d, 1H), 6.05 (d, 1H), 3.71 (s, 3H); ^{13}C-NMR (CD_3COD): δ 164.13, 151.14, 134.39, 134.25, 133.55, 130.80, 127.73, 124.84, 113.37, 108.99, and 34.06; MS (LSIMS) *m/z* 268.0701 $(M + Na)^+$, Calcd. Mass for $C_{12}H_{11}N_3O_3Na$ 268.0698.

4.9. Preparation of 5-(2-amino-5-subtituted-phenyl)-1H-pyrrole-2-carboxylic acid alkylamide ***8a–v****. General method*

Fe (5.24 mmol) and $FeSO_4$ (0.524 mmol) were suspended in water and the corresponding nitroarene **14a–v** (0.524 mmol) was added over the reaction mixture and refluxed from 3 to 5 h. After cooling, the reaction mixture is filtered through Celite and washed thoroughly with dichloromethane. The aqueous phase was extracted with dichloromethane (3 × 15 ml) and ethyl acetate (3 × 15 ml). The combined organic layers were washed with brine, dried (Na_2SO_4), filtered and concentrated to yield a residue that was purified by recrystallization (CH_2Cl_2/C_6H_{12}) or by flash chromatography.

4.9.1. 5-(2-Amino-5-methoxyphenyl)-1H-pyrrole-2-carboxamide ***8a***

(72%); mp 123–125 °C; ^{1}H-NMR ($CDCl_3$): δ 10.21 (bs, 1H), 6.70 (ps, 1H), 6.90 (m, 2H), 6.67 (pt, 1H), 6.44 (pt, 1H), 5.83 (bs, 2H), 3.73 (s, 3H); ^{13}C-NMR ($CDCl_3$): δ 162.83, 153.36, 136.99, 134.33, 124.98, 119.68, 118.94, 115.05, 113.72, 111.86, 109.27, and 55.87; MS (LSIMS) *m/z* 254.0902 $(M + Na)^+$, Calcd. Mass for $C_{12}H_{13}N_3O_2Na$ 254.0905; Anal. $C_{12}H_{13}N_3O_2$ (C, H, N).

4.9.2. 5-(2-Amino-5-methoxyphenyl)-1H-pyrrole-2-carboxylic acid methylamide ***8b***

(48%); mp 202–204 °C; ^{1}H-NMR ($CDCl_3$): δ 10.05 (bs, 1H), 6.87 (d, 1H), 6.72 (m, 2H), 6.58 (dd, 1H), 6.42 (dd, 1H), 5.98 (bs, 1H), 3.75 (s, 3H), 2.95 (d, 3H); ^{13}C-NMR ($CDCl_3$): δ 161.79, 153.51, 136.77, 133.28, 126.07, 119.98, 118.96, 114.94, 113.64, 109.77, 109.03, 55.90, and 26.27; MS (LSIMS) *m/z* 268.1066 $(M + Na)^+$, Calcd. Mass for $C_{13}H_{15}N_3O_2Na$ 268.1062; Anal. $C_{13}H_{15}N_3O_2$ (C, H, N).

4.9.3. 5-(2-Amino-5-methoxyphenyl)-1H-pyrrole-2-carboxylic acid propylamide ***8c***

(62%); mp 183–184 °C; ^{1}H-NMR ($CDCl_3$): δ 10.00 (bs, 1H), 6.87 (d, 1H), 6.73 (m, 2H), 6.58 (pt, 1H), 6.43 (pt, 1H), 5.93 (bs, 1H), 3.73 (s, 3H), 3.56 (m, 2H), 1.60 (m, 2H), 0.96 (t, 3H); ^{13}C-NMR ($CDCl_3$): δ 161.13, 153.64, 136.00, 133.19, 126.24, 119.13, 114.91, 113.66, 109.73, 109.04, 55.87, 41.26, 23.17, and 11.45; MS (LSIMS) *m/z* 296.1377 $(M + Na)^+$, Calcd. Mass for $C_{15}H_{19}N_3O_2Na$ 296.1374; Anal. $C_{15}H_{19}N_3O_2$ (C, H, N).

4.9.4. 5-(2-Amino-5-methoxyphenyl)-1H-pyrrole-2-carboxylic acid cyclopropylamide ***8d***

(77%); mp 195–196 °C; ^{1}H-NMR ($CDCl_3$): δ 10.04 (bs, 1H), 6.86 (m, 1H), 6.72 (m, 2H), 6.56 (bs, 1H), 6.40 (pt, 1H), 6.10 (bs, 1H), 3.75 (s, 3H), 3.75 (bs, 2H), 2.81 (m, 1H), 0.82 (m, 2H), 0.60 (m, 2H); ^{13}C-NMR ($CDCl_3$): δ 158.85, 153.50, 136.85, 133.50, 125.87, 119.90, 118.99, 114.96, 113.69, 110.41, 109.49, 55.89, 22.71, and 7.01; MS (LSIMS) *m/z* 294.1219 $(M + Na)^+$, Calcd. Mass for $C_{15}H_{17}N_3O_2Na$ 294.1218; Anal. $C_{15}H_{17}N_3O_2$ (C, H, N).

4.9.5. 5-(2-Amino-5-methoxyphenyl)-1H-pyrrole-2-carboxylic acid cyclopentylamide ***8e***

(55%), mp 185–187 °C; ^{1}H-NMR (DMSO-d_6): δ 11.30 (bs, 1H), 7.77 (d, 1H), 6.89 (d, 1H), 6.83 (d, 1H), 6.70 (d, 1H), 6.62 (dd, 1H), 6.36 (d, 1H), 4.56 (bs, 2H), 4.17 (m, 1H), 3.67 (s, 3H), 1.93–1.38 (m, 8H); ^{13}C-NMR (DMSO-d_6): δ 160.47, 151.75, 139.23, 132.99, 127.26, 118.73, 117.96, 114.88, 113.84, 112.18, 108.56, 55.92, 50.89, 32.84, and 24.11; MS (LSIMS) *m/z* 322.1532 $(M + Na)^+$, Calcd. Mass for $C_{17}H_{21}N_3O_2Na$ 322.1531; Anal. $C_{17}H_{21}N_3O_2$ (C, H, N).

4.9.6. 5-(2-Amino-5-methoxyphenyl)-1H-pyrrole-2-carboxylic acid benzylamide ***8f***

(72%); mp 178–180 °C; ^{1}H-NMR (CD_3OD): δ 7.28 (m, 5H), 6.91 (m, 2H), 6.80 (d, 1H), 6.71 (dd, 1H), 6.43 (d, 1H), 4.53 (s, 2H), 3.74 (s, 3H); ^{13}C-NMR (CD_3OD): δ 163.58, 154.46, 140.50, 139.05, 135.03, 129.50, 128.47, 128.10, 127.09, 121.14, 119.60, 115.89, 114.70, 113.33, 109.92, 56.21, and 43.93; MS (LSIMS) *m/z* 344.1380 $(M + Na)^+$, Calcd. Mass for $C_{19}H_{19}N_3O_2Na$ 344.1375; Anal. $C_{19}H_{19}N_3O_2$ (C, H, N).

4.9.7. 5-(2-Amino-5-chlorophenyl)-1H-pyrrole-2-carboxamide ***8g***

(59%); mp 197–198 °C; ^{1}H-NMR ($CDCl_3$): δ 9.95 (bs, 1H), 7.31 (d, 1H), 7.08 (dd, 1H), 6.70 (d, 1H), 6.69 (pt, 1H), 6.46 (pt, 1H), 5.70 (bs, 2H), 3.98 (bs, 2H); ^{13}C-NMR ($(CD_3)_2CO$): δ 162.29, 144.19, 131.97, 128.24, 127.76, 127.07, 121.94, 119.29, 117.40, 111.56, and 108.86; MS (LSIMS) *m/z* 258.9987 $(M + Na)^+$, Calcd. Mass for $C_{11}H_{10}N_3OClNa$ 258.9992; Anal. $C_{11}H_{10}N_3OCl$ (C, H, N).

4.9.8. 5-(2-Amino-5-chlorophenyl)-1H-pyrrole-2-carboxylic acid methylamide ***8h***

(62%); mp 175–176 °C; ^{1}H-NMR ($CDCl_3$): δ 10.05 (bs, 1H), 7.24 (d, 1H), 7.05 (dd, 1H), 6.68 (d, 1H), 6.58 (dd, 1H), 6.40 (dd, 1H), 5.94 (bs, 1H), 4.00 (bs, 2H), 2.95 (d, 3H); ^{13}C-NMR ($(CD_3)_2CO$): δ 161.29, 144.20, 131.48, 128.28, 127.67, 127.51, 121.37, 119.37, 117.29, 110.15, 108.75, and 25.21; MS (LSIMS) *m/z* 272.0566 $(M + Na)^+$, Calcd. Mass for $C_{12}H_{12}N_3OClNa$ 272.0566; Anal. $C_{12}H_{12}N_3OCl$ (C, H, N).

4.9.9. 5-(2-Amino-5-chlorophenyl)-1H-pyrrole-2-carboxylic acid ethylamide ***8i***

(71%); mp 183–185 °C; ^{1}H-NMR ($(CD_3)_2CO$): δ 10.80 (bs, 1H), 7.42 (bs, 1H), 7.32 (d, 1H), 7.02 (dd, 1H), 6.82 (m, 2H), 6.43 (pt, 1H), 4.85 (bs, 2H), 3.34 (m, 2H), 1.12 (t, 3H); ^{13}C-NMR ($(CD_3)_2CO$): δ 160.60, 144.13, 130.57, 128.19, 127.60, 127.46, 121.28, 119.30, 117.20, 110.23, 108.66, 33.70, and 15.53; MS (LSIMS) *m/z* 286.0722 $(M + Na)^+$, Calcd. Mass for $C_{13}H_{14}N_3OClNa$ 286.0723; Anal. $C_{13}H_{14}N_3OCl$ (C, H, N).

4.9.10. 5-(2-Amino-5-chlorophenyl)-1H-pyrrole-2-carboxylic acid butylamide ***8j***

(68%); mp 161–163 °C; ^{1}H-NMR ($CDCl_3$): δ 10.09 (bs, 1H), 7.22 (d, 1H), 7.03 (dd, 1H), 6.66 (d, 1H), 6.58 (pt, 1H), 6.40 (pt, 1H), 5.92 (bs, 1H), 3.38 (m, 2H), 4.00 (bs, 2H), 1.55 (m, 2H), 1.37 (m, 2H), 0.93 (t, 3H); ^{13}C-NMR ($CDCl_3$): δ 160.98, 141.95, 131.80, 128.46, 126.63, 124.02, 119.93, 118.02, 109.66, 109.40, 39.32, 31.99, 20.19, and 13.87; MS (LSIMS) *m/z* 314.1037 $(M + Na)^+$, Calcd. Mass for $C_{15}H_{18}N_3OClNa$ 314.1036; Anal. $C_{15}H_{18}N_3OCl$ (C, H, N).

4.9.11. 5-(2-Amino-5-chlorophenyl)-1H-pyrrole-2-carboxylic acid cyclopropylamide ***8k***

(68%); mp 166–167 °C; ^{1}H-NMR ($(CD_3)_2CO$): δ 10.95 (bs, 1H), 7.47 (bs, 1H), 7.30 (d, 1H), 7.00 (dd, 1H), 6.82 (m, 2H), 6.41 (dd, 1H), 4.81 (bs, 2H), 2.82 (m, 1H), 0.68–0.49 (m, 4H); ^{13}C-NMR

($(CD_3)_2CO$): δ 161.91, 144.18, 131.68, 128.28, 127.70, 127.37, 121.47, 119.42, 117.32, 110.64, 108.75, 22.46, and 5.59; MS (LSIMS) *m/z* 298.0723 $(M+Na)^+$, Calcd. Mass for $C_{14}H_{14}N_3OClNa$ 298.0723; Anal. $C_{14}H_{14}N_3OCl$ (C, H, N).

4.9.12. 5-(2-Aminophenyl)-1H-pyrrole-2-carboxamide ***8l***

(64%); mp 104–105 °C; ^{1}H-NMR ($CDCl_3$): δ 9.75 (bs, 1H), 7.27 (dd, 1H), 7.11 (pt, 1H), 6.80 (pt, 1H), 6.74 (d, 1H), 6.69 (pt, 1H), 6.43 (pt, 1H), 5.80 (bs, 2H), 4.00 (bs, 2H); ^{13}C-NMR ($CDCl_3$): δ 157.35, 138.35, 128.79, 123.65, 123.54, 119.56, 113.84, 112.81, 111.41, 106.42, and 103.84; MS (LSIMS) *m/z* 224.0801 $(M+Na)^+$, Calcd. Mass for $C_{11}H_{11}N_3ONa$ 224.0799; Anal. $C_{11}H_{11}N_3O$ (C, H, N).

*4.9.13. 5-(2-Aminophenyl)-1H-pyrrole-2-*carboxylic *acid methylamide* ***8m***

(68%); mp 155–157 °C; ^{1}H-NMR ($CDCl_3$): δ 9.83 (bs, 1H), 7.28 (d, 1H), 7.12 (pt, 1H), 6.82 (t, 1H), 6.76 (d, 1H), 6.61 (pt, 1H), 6.42 (pt, 1H), 4.00 (bs, 2H), 5.97 (bs, 1H), 2.97 (d, 3H); ^{13}C-NMR ($CDCl_3$): δ 161.76, 143.67, 133.16, 128.86, 126.01, 119.22, 118.35, 116.72, 109.74, 108.95, and 26.27; MS (LSIMS) *m/z* 238.0959 $(M+Na)^+$, Calcd. Mass for $C_{12}H_{13}N_3ONa$ 238.0958; Anal. $C_{12}H_{13}N_3O$ (C, H, N).

*4.9.14. 5-(2-Aminophenyl)-1H-pyrrole-2-*carboxylic *acid ethylamide* ***8n***

(48%); mp 145–146 °C; ^{1}H-NMR ($CDCl_3$): δ 10.85 (bs, 1H), 7.27 (d, 1H), 7.11 (t, 1H), 6.80 (t, 1H), 6.75 (d, 1H), 6.61 (pt, 1H), 6.41 (pt, 1H), 5.95 (bs, 1H), 3.44 (m, 2H), 1.22 (t, 3H); ^{13}C-NMR ($CDCl_3$): δ 161.03, 143.70, 133.19, 128.87, 128.81, 126.10, 119.16, 118.39, 116.67, 109.68, 108.90, 34.40, and 15.18; MS (LSIMS) *m/z* 252.1113 $(M+Na)^+$, Calcd. Mass for $C_{13}H_{15}N_3ONa$ 252.1113; Anal. $C_{13}H_{15}N_3O$ (C, H, N).

*4.9.15. 5-(2-Aminophenyl)-1H-pyrrole-2-*carboxylic *acid propylamide* ***8o***

(51%); mp 115–116 °C; ^{1}H-NMR ($CDCl_3$): δ 10.00 (bs, 1H), 7.26 (dd, 1H), 7.09 (pt, 1H), 6.78 (pt, 1H), 6.73 (dd, 1H), 6.62 (dd, 1H), 6.40 (dd, 1H), 6.03 (bs, 1H), 3.98 (bs, 2H), 3.83 (m, 2H), 1.58 (m, 2H), 0.94 (t, 3H); ^{13}C-NMR ($CDCl_3$): δ 161.12, 143.73, 133.19, 128.82, 128.76, 126.11, 119.06, 118.35, 116.57, 109.69, 108.87, 41.17, 23.17, and 11.46; MS (LSIMS) *m/z* 266.1262 $(M+Na)^+$, Calcd. Mass for $C_{14}H_{17}N_3ONa$ 266.1269; Anal. $C_{14}H_{17}N_3O$ (C, H, N).

*4.9.16. 5-(2-Aminophenyl)-1H-pyrrole-2-*carboxylic *acid butylamide* ***8p***

(54%); mp 79–80 °C; ^{1}H-NMR ($CDCl_3$): δ 9.79 (bs, 1H), 7.27 (dd, 1H), 7.11 (pt, 1H), 6.80 (pt, 1H), 6.75 (d, 1H), 6.59 (pt, 1H), 6.41 (pt, 1H), 5.90 (bs, 1H), 4.10 (bs, 2H), 3.40 (m, 2H), 1.56 (m, 2H), 1.39 (m, 2H), 0.95 (t, 3H); ^{13}C-NMR ($CDCl_3$): δ 161.09, 143.78, 133.20, 128.89, 128.85, 126.18, 119.18, 118.42, 116.71, 109.52, 108.88, 39.29, 31.97, 20.18, and 13.85; MS (LSIMS) *m/z* 280.1428 $(M+Na)^+$, Calcd. Mass for $C_{15}H_{19}N_3ONa$ 280.1425; Anal. $C_{15}H_{19}N_3O$ (C, H, N).

*4.9.17. 5-(2-Aminophenyl)-1H-pyrrole-2-*carboxylic *acid cyclopropylamide* ***8q***

(49%); mp 137–138 °C; ^{1}H-NMR ($CDCl_3$): δ 9.90 (bs, 1H), 7.27 (dd, 1H), 7.09 (pt, 1H), 6.78 (pt, 1H), 6.73 (d, 1H), 6.60 (bs, 1H), 6.38 (pt, 1H), 6.22 (bs, 1H), 4.00 (bs), 2.81 (m, 1H), 0.81 (m, 2H), 0.59 (m, 2H); ^{13}C-NMR ($CDCl_3$): δ 162.55, 143.73, 133.46, 128.87, 125.81, 119.14, 118.31, 116.67, 110.25, 108.99, 22.67, and 6.94; MS (LSIMS) *m/z* 242.1287 $(M+H)^+$, Calcd. Mass for $C_{14}H_{16}N_3O$ 242.1293; Anal. $C_{14}H_{15}N_3O$ (C, H, N).

*4.9.18. 5-(2-Aminophenyl)-1H-pyrrole-2-*carboxylic *acid cyclobutylamide* ***8r***

(67%); mp 163–165 °C; ^{1}H-NMR ($CDCl_3$): δ 9.85 (bs, 1H), 7.26 (dd, 1H), 7.11 (pt, 1H), 6.80 (pt, 1H), 6.75 (d, 1H), 6.61 (dd, H-3), 6.40 (pt, 1H), 6.05 (d, 1H), 4.52 (m, 1H), 3.93 (bs, 2H), 2.37 (m, 2H), 1.92 (m, 2H), 1.73 (m, 2H); ^{13}C-NMR ($CDCl_3$): δ 160.12, 143.71, 133.36, 128.89, 128.84, 125.99, 119.17, 118.41, 116.69, 109.79, 108.94, 44.76, 31.76, and 15.20; MS (LSIMS) *m/z* 278.1269 $(M+Na)^+$, Calcd. Mass for $C_{15}H_{17}N_3ONa$ 278.1269; Anal. $C_{15}H_{17}N_3O$ (C, H, N).

*4.9.19. 5-(2-Aminophenyl)-1H-pyrrole-2-*carboxylic *acid cyclopentylamide* ***8s***

(69%); mp 163–165 °C; ^{1}H-NMR ($CDCl_3$): δ 9.85 (bs, 1H); 7.27 (d, 1H), 7.11 (pt, 1H), 6.80 (pt, 1H), 6.75 (dd, 1H), 6.58 (dd, 1H), 6.40 (pt, 1H), 5.83 (d, 1H), 4.33 (m, 1H), 4.00 (bs, 2H), 2.04 (m, 2H), 1.66 (m, 2H), 1.45 (m, 2H); ^{13}C-NMR ($CDCl_3$): δ 160.73, 143.72, 133.12, 128.85, 128.82, 126.24, 119.17, 118.41, 116.68, 109.52, 108.87, 51.25, 33.40, and 23.85; MS (LSIMS) *m/z* 270.1608 $(M+H)^+$, Calcd. Mass for $C_{16}H_{20}N_3O$ 270.1606; Anal. $C_{16}H_{19}N_3O$ (C, H, N).

*4.9.20. 5-(2-Aminophenyl)-1H-pyrrole-2-*carboxylic *acid cyclohexylamide* ***8t***

(59%); mp 152-154 °C; ^{1}H-NMR ($CDCl_3$): δ 9.98 (bs, 1H), 7.26 (d, 1H), 7.10 (pt, 1H), 6.78 (pt, 1H), 6.76 (dd, 1H), 6.60 (dd, 1H), 6.40 (dd, 1H), 5.83 (d, 1H), 3.85 (bs, 2H), 3.88 (m, 1H), 1.99–1.14 (m, 10H); ^{13}C-NMR ($CDCl_3$): δ 160.24, 143.36, 133.08, 128.92, 128.77, 126.36, 119.32, 118.62, 116.79, 109.63, 108.91, 48.23, 33.45, and 25.00; MS (LSIMS) *m/z* 306.1588 $(M+Na)^+$, Calcd. Mass for $C_{17}H_{21}N_3ONa$ 306.1582; Anal. $C_{17}H_{21}N_3O$ (C, H, N).

*4.9.21. 5-(2-Aminophenyl)-1H-pyrrole-2-*carboxylic *acid benzylamide* ***8u***

(52%); mp 178–180 °C; ^{1}H-NMR ($(CD_3)_2CO$): δ 11.40 (bs, 1H), 8.55 (t, 1H), 7.26 (m, 6H), 7.27 (pt, 1H), 6.89 (d, 1H), 6.82 (d, 1H), 6.68 (pt, 1H), 6.35 (d, 1H), 5.75 (bs, 2H), 4.38 (d, 2H); ^{13}C-NMR 100 MHz, ($(CD_3)_2CO$): δ 160.36, 140.00, 132.45, 129.06, 128.31, 128.00, 127.27, 126.76, 126.56, 118.43, 116.68, 111.88, 108.32, and 41.98; MS (LSIMS) *m/z* 314.1271 $(M+Na)^+$, Calcd. Mass for $C_{18}H_{17}N_3ONa$ 314.1269; Anal. $C_{18}H_{17}N_3O$ (C, H, N).

4.9.22. 5-(2-Aminophenyl)-1-methyl-1H-pyrrole-2-carboxamide ***8v***

(50%); mp 122–123 °C; ^{1}H-NMR (CD_3OD): δ 7.22 (pt, 1H), 7.10 (d, 1H), 7.00 (d, 1H), 6.92 (d, 1H), 6.85 (pt, 1H), 6.12 (d, 1H), 3.61 (s, 1H); ^{13}C-NMR (CD_3OD): δ 163.84, 145.56, 135.40, 132.07, 132.15, 126.91, 120.01, 118.08, 113.45, 108.82, and 33.92; MS (LSIMS) *m/z* 238.0961 $(M+Na)^+$, Calcd. Mass for $C_{12}H_{13}N_3ONa$ 238.0956; Anal. $C_{12}H_{13}N_3O$ (C, H, N).

4.10. In vitro *striatal nNOS activity determination*

L-Arginine, L-citrulline, *N*-(2-hydroxymethyl)piperazine-*N*′-(2-ethanesulfonic acid) (HEPES), D,L-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).

Male Wistar rats (2020–250 g) were used for the *in vitro* NOS determination. Animals 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.

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 [55] or used immediately to measure NOS activity. The nNOS activity was measured by the Bredt and Snyder method [56], monitoring the conversion of L-[^{3}H]-arginine to L-[^{3}H]-citrulline. 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 vortexed 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-citrulline, 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]-citrulline 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]-citrulline produced × (mg of protein)$^{-1}$ × min^{-1}.

4.11. In vitro *iNOS activity determination*

iNOS induction was achieved by intravenous injection of lipopolysaccharide (LPS) 20 mg/Kg. 8 h after its 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.

4.12. In vivo *experiments*

C57/BI6 mice (22–28 g) were employed in the *in vivo* experiments. Animals 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.

Mice were divided into the following groups: a) control group, injected with vehicle (ethanol/saline); b) MPTP group; c) MPTP + aMT group, and d) MPTP + each compound tested (compounds **8n** or **8s**). Seven doses of MPTP (15 mg/kg) were s. c. injected at intervals of 0, 2, 4, 6, 24, 26 and 28 hours. One dose of aMT or compounds **8m** (QFF-205) or **8s** (QFF-212) (20 mg/kg b.w.) was injected 1 hr before the first dose of MPTP. 32 h after treatments, the animals were sacrificed by cervical dislocation for biochemical analysis. Then, cytosol and mitochondria from substantia nigra (SN) were prepared as described elsewhere [57], and the nNOS and iNOS activities were measured as described above.

4.13. 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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References

[1] (a) W.C. Sessa, K. Pritchard, N. Seyedi, J. Wang, T.H. Hintze, Circ. Res. 74 (1994) 349–353;
(b) M.A. Awolesi, W.C. Sessa, B.E. Sumpio, J. Clin. Invest. 96 (1995) 1449–1454.
[2] L. Salermo, V. Sorrenti, C. Di Giacomo, G. Romeo, M.A. Siracusa, Curr. Pharm. Des. 8 (2000) 177–200.
[3] P. Kubes, Am. J. Physiol. 264 (1993) G143–G149.
[4] D. Salvemini, M.H. Marino, Expert Opin. Investig. Drugs 7 (1998) 65–75.
[5] (a) M.A. Smith, M. Vasak, M. Knipp, R.J. Castellani, G. Perry, Free Radic. Biol. Med. 25 (1998) 898–902.(b) D.T. Yew, H.W. Wong, W.P. Li, H.W. Lai, W.H. Yu, Neuroscience 89 (1999) 675–686.
[6] N.K. Wong, M.J. Strong, Eur. J. Cell Biol. 77 (1998) 338–343.
[7] P.J. Norris, H.J. Waldvogel, R.L. Faull, D.R. Love, P.C. Emson, Neuroscience 4 (1996) 1037–1047.
[8] B.M. Mayer, M. John, E. Bohme, FEBS Lett. 277 (1990) 215–219.
[9] J.S. Pollock, U. Förstermann, J.A. Mitchell, T.D. Warner, H.H. Schmidt, M. Nakane, F. Murad, Proc. Natl. Acad. Sci. U.S.A. 88 (1991) 10480–10484.
[10] (a) D.J. Stuehr, H.J. Cho, N.S. Kwon, M.F. Weise, C.F. Nathan, Proc. Natl. Acad. Sci. U.S.A. 88 (1991) 7773–7777;
(b) J.M. Hevel, K.A. White, M.A. Marletta, J. Biol. Chem. 266 (1991) 22789–22791.
[11] (a) R.G. Knowles, M. Palacios, M.R.J. Palmer, S. Moncada, Proc. Natl. Acad. Sci. U.S.A. 86 (1989) 5159–5162;
(b) T.B. McCall, N.K. Boughton-Smith, R.M.J. Palmer, B.J.R. Whittle, S. Moncada, Biochem. J. 261 (1989) 293–296.
[12] (a) D.J. Stuehr, O.W. Griffith, Adv. Enzymol. Relat. Areas Mol. Biol. 65 (1992) 287–346;
(b) O.W. Griffith, D.J. Stuehr, Annu. Rev. Physiol. 57 (1995) 707–736.
[13] (a) V. Schini, P. Vanhoutte, J. Pharmacol. Exp. Ther. 261 (1992) 553–559;
(b) U. Forstermann, J. Pollock, H. Schmidt, M. Heller, F. Murad, Proc. Natl. Acad. Sci. U.S.A. 88 (1991) 1788–1792.
[14] H.J. Cho, Q.W. Xie, J. Calaycay, R.A. Mumford, K.M. Swiderek, T.D. Lee, C. Natham, J. Exp. Med. 176 (1992) 599–604.
[15] (a) L. Kobzik, B. Stringer, J.L. Balligand, M.B. Reid, J.S. Stamler, Biochem. Biophys. Res. Commun. 211 (1995) 375–381;
(b) U. Frandsen, M. Lopez-Figueroa, Y. Hellsten, Biochem. Biophys. Res. Commun. 227 (1996) 88–93;
(c) T.E. Bates, A. Loesch, G. Burnstock, J.B. Clark, Biochem. Biophys. Res. Commun. 213 (1995) 896–900.
[16] (a) P.S. Brookes, Mitochondrion 3 (2004) 187–204;
(b) P. Ghafourifar, E. Cadenas, Trends Pharmacol. Sci. 26 (2005) 190–195.
[17] (a) G. Escames, J. León, M. Macías, H. Khaldy, D. Acuña-Castroviejo, FASEB J. 17 (2003) 932–934;
(b) G. Escames, L.C. López, F. Ortiz, A. López, J.A. García, E. Ros, D. Acuña-Castroviejo, FASEB J. 274 (2007) 2135–2147.
[18] D.D. Rees, R.M.J. Palmer, S. Moncada, Proc. Natl. Acad. Sci. U.S.A. 86 (1989) 3375–3378.
[19] G.A. Böhme, C. Bon, M. Lemaire, M. Reibaud, O. Piot, J.M. Stutzmann, A. Doble, J.C. Blanchard, Proc. Natl. Acad. Sci. U.S.A. 90 (1993) 9191–9194.
[20] G. Escames, D. Acuña-Castroviejo, L.C. López, D.X. Tan, M.D. Maldonado, M. Sánchez-Hidalgo, J. León, R.J. Reiter, J. Pharm. Pharmacol. 58 (2006) 1153–1165.
[21] L.C. López, G. Escames, V. Tapias, P. Utrilla, J. León, D. Acuña-Castroviejo, Int. J. Biochem. Cell. Biol. 38 (2006) 267–278.
[22] (a) P. Vallance, S. Moncada, New. Horiz. 1 (1993) 77–86;
C. Thiemermenn, Adv. Pharmacol. 28 (1994) 45–79;
(c) D.D. Rees, Biochem. Soc. Trans. 23 (1995) 1025–1029.
[23] P.S. Grabowski, P.K. Wright, R.J. Van't Holf, M.H. Helfrich, H. Ohshima, S.H. Ralston, Br. J. Rheumatol. 36 (1997) 651–655.
[24] H.G. Seo, I. Takata, M. Nakamura, H. Tatsumi, K. Suzuki, J. Fujii, N. Taniguchi, Arch. Biochem. Biophys. 324 (1995) 41–47.
[25] G.T. Liberatore, V. Jackson-Lewis, S. Vukosavic, A.S. Mandir, M. Vila, W.G. McAuliffe, V.L. Dawson, T.M. Dawson, S. Przedborski, Nat. Med. 5 (1999) 1354–1355.
[26] (a) R.J. Reiter, Endocr. Rev. 12 (1991) 151–180;
(b) R. Hardeland, S.R. Pandi-Perumal, D.P. Cardinali, Int. J. Biochem. Cell Biol. 38 (2006) 313–316.
[27] B. Rusak, G.D. Yu, Brain Res. 602 (1993) 200–204.

[28] (a) D. Acuña-Castroviejo, G. Escames, M. Macias, A. Muñoz-Hoyos, A. Molina-Carballo, M. Arauzo, R. Montes, F. Vives, J. Pineal Res. 19 (1995) 57–63.(b) J.D. Fauteck, J. Bockmann, T.M. Böckers, W. Wittkowski, R. Köling, A. Lücke, H. Straub, E.J. Speckmann, L. Tuxhorm, P. Wolf, H. Pannek, F. Oppel, Exp. Brain Res. 107 (1995) 321–325.
[29] M. Marco, P.P. Vicenzo, S. Gilberto, G. Tarzia, Curr. Med. Chem. 6 (1999) 501–518.
[30] G. Escames, D. Acuña-Castroviejo, F. Vives, NeuroReport 7 (1996) 597–600.
[31] D. Pozo, R.J. Reiter, J.R. Calvo, J.M. Guerrero, Life Sci. 55 (1994) 455–460.
[32] I. Bettahi, D. Pozo, C. Osuna, R.J. Reiter, D. Acuña-Castroviejo, J. Guerrero, J. Pineal Res. 20 (1996) 205–210.
[33] J. Leon, F. Vives, E. Crespo, E. Camacho, A. Espinosa, M.A. Gallo, G. Escames, D. Acuña-Castroviejo, J. Neuroendocrinol. 10 (1998) 297–302.
[34] J. León, M. Macías, G. Escames, E. Camacho, H. Khaldy, M. Martín, A. Espinosa, M.A. Gallo, D. Acuña-Castroviejo, Mol. Pharmacol. 58 (2000) 967–975.
[35] M.E. Camacho, J. León, M.D. Carrión, A. Entrena, G. Escames, H. Khaldy, D. Acuña-Castroviejo, M.A. Gallo, A. Espinosa, J. Med. Chem. 45 (2002) 263–274.
[36] A. Entrena, M.E. Camacho, M.D. Carrión, L.C. López-Cara, G. Velasco, J. León, G. Escames, D. Acuña-Castroviejo, V. Tapias, M.A. Gallo, A. Vivó, A. Espinosa, J. Med. Chem. 48 (2005) 8174–8181.
[37] J. León, G. Escames, M.I. Rodriguez, L.C. Lopez, V. Tapias, A. Entrena, E. Camacho, M.D. Carrión, M.A. Gallo, A. Espinosa, D.X. Tan, R.J. Reiter, D. Acuña-Castroviejo, J. Neurochem. 98 (2006) 2023–2033.
[38] M.E. Camacho, J. León, A. Entrena, G. Velasco, M.D. Carrión, G. Escames, A. Vivó, D. Acuña, M.A. Gallo, A. Espinosa, J. Med. Chem. 47 (2004) 5641–5650.
[39] M.D. Carrión, M.E. Camacho, J. León, G. Escames, V. Tapias, D. Acuña-Castroviejo, M.A. Gallo, A. Espinosa, Tetrahedron 60 (2004) 4051–4069.
[40] M.D. Carrión, L.C. López-Cara, M.E. Camacho, V. Tapias, G. Escames, D. Acuña-Castroviejo, A. Espinosa, M.A. Gallo, A. Entrena, Eur. J. Med. Chem. 43 (2008) 2579–2591.
[41] Schrödinger LLC, New York, NY, 2007. http://www.schrodinger.com/.
[42] R. Fedorov, E. Hartmann, D.K. Ghosh, I. Schlichting, J. Biol. Chem. 278 (2003) 45818–45825.
[43] (a) H. Hemetsberger, D. Knittel, H. Weidmann, Monatsh. Chem. 100 (1969) 1599–1603;
(b) H. Hemetsberger, D. Knittel, H. Weidmann, Monatsh. Chem. 101 (1970) 161–165;
(c) H. Hemetsberger, D. Knittel, Monatsh. Chem. 103 (1972) 194–204;
(d) J.P. Boukou-Poba, M. Farnier, R.A. Guilard, Tetrahedron Lett. 19 (1979) 1717–1720.
[44] (a) P. Molina, E. Aller, M.A. Lorenzo, Synthesis 12 (1993) 1239–1242;
(b) D.M.B. Hickey, C.J. Moody, C.W. Rees, J. Chem. Soc., Perkins Trans. 1 (1986) 1113–1117;
(c) M.J. Alves, T.L. Gilchrist, J. Chem. Soc., Perkin Trans. 1 (1998) 299–303.
[45] J.P. Marino, M.S. McClure, D.P. Holub, J.V. Comasseto, F.C. Tucci, J. Am. Chem. Soc. 124 (2002) 1664–1668.
[46] (a) D.D. Davey, J. Org. Chem. 52 (1987) 4379–4381;
(b) W.C. Guida, D.J. Mathre, J. Org. Chem. 45 (1980) 3172–3176.
[47] H. McKennis Jr., L.B. Turnbull, E.R. Bowman, E. Tamaki, J. Org. Chem. 28 (1963) 383–387.
[48] (a) G.H. Coleman, G. Wichols, C.M. Mc Closekey, H.D. Anspon, Org. Synth. Coll. 3 (1963) 712–713;
(b) P.J. Garratt, R. Jones, D.A. Tocher, J. Med. Chem. 38 (1995) 1132–1139.
[49] J. Zhu, R. Beugelmans, S. Bourdet, J. Chastanet, G.A. Roussi, J. Org. Chem. 60 (1995) 6389–6396.
[50] (a) E. Crespo, M. Macias, D. Pozo, G. Escames, M. Martin, F. Vives, J.M. Guerrero, D. Acuña-Castroviejo, FASEB J. 13 (1999) 1537–1546;
(b) D. Acuña-Castroviejo, M. Martin, M. Macias, G. Escames, J. Leon, H. Khaldy, R.J. Reiter, J. Pineal Res. 30 (2001) 65–74;
(c) J.C. Mayo, R.M. Sainz, D.X. Tan, I. Antolin, C. Rodriguez, R.J. Reiter, Endocrine 27 (2005) 169–178.
[51] G. Escames, L.C. Lopez, V. Tapias, P. Utrilla, R.J. Reiter, A.B. Hitos, J. Leon, M.I. Rodriguez, D. Acuña-Castroviejo, J. Pineal Res. 40 (2006) 1–8.
[52] G. Escames, J. Leon, L.C. Lopez, D. Acuña-Castroviejo, J. Neuroendocrinol. 16 (2004) 929–935.
[53] (a) D. Acuña-Castroviejo, A. Coto-Montes, M. Gaia Monti, G.G. Ortiz, R.J. Reiter, Life Sci. 60 (1997) 23–29;
(b) H. Khaldy, G. Escames, J. Leon, L. Bikjdaouene, D. Acuña-Castroviejo, Neurobiol. Aging. 24 (2003) 491–500;
(c) B. Thomas, K.P. Mohanakumar, J. Pineal Res. 36 (2004) 25–32.
[54] I. Wiesenberg, M. Missbach, J.P. Kahlen, M. Schrader, C. Carlberg, Nucleic Acids Res. 23 (1995) 327–333.
[55] O.H. Lowry, N.J. Rosebrough, A.S. Farr, R.J. Randall, J. Biol. Chem. 193 (1951) 265–267.
[56] D.S. Bredt, S.H. Snyder, Proc. Natl. Acad. Sci. U.S.A. 87 (1990) 682–685.
[57] J.E. Rice, J.G. Lindsay, in: J.M. Graham, D. Rickwood (Eds.), Subcellular Fractionation (1997).