ELSEVIER

Il Farmaco 54 (1999) 101–111

IL FARMACO

3-(2-Carbamoylvinyl)-4,5-dimethylpyrrole-2-carboxylic acids as ligands at the NMDA glycine-binding site: a study on the 2-carbamoylvinyl chain modification

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Received 29 July 1998; accepted 7 December 1998

Abstract

Twenty 4,5-dimethylpyrrole-2-carboxylic acids (**5a**–**t**) with different 2-carbamoylvinyl chains in position 3 were prepared to further investigate the relationships between structure and in vitro affinity for the strychnine-insensitive glycine-binding site. None of these compounds was superior to (*E*)-3-(*N*-phenyl-2-carbamoylvinyl)-4,5-dimethylpyrrole-2-carboxylic acid **III** ($pK_i = 6.70$), which was taken as a reference standard, but overall the results obtained indicate that the *N*-phenyl-2-carbamoylvinyl substituent of **III** may be replaced with the *N*-(1-adamantyl)-2-carbamoylvinyl group as in **5h** ($pK_i = 6.20$) without considerable loss of affinity. This finding adds to previous knowledge.

Keywords: 3-(2-Carbamoylvinyl)-4,5-dimethylpyrrole-2-carboxylic acid; Strychnine-insensitive glycine site; NMDA receptor

1. Introduction

Overstimulation of the *N*-methyl-D-aspartate (NMDA) receptor by excessive release of endogenous L-glutamate causes abnormal influx of Ca^{2+} into the post-synaptic neurons, ultimately leading to cell death [1]. Excessive glutamatergic activity has been associated with many ischemia- and hypoxia-related disorders such as stroke, hypoglycemia and traumatic head and spinal cord injuries [2–6] and may be involved in some neurodegenerative diseases of the CNS, including Huntington's chorea and Alzheimer's dementia [7–10]. Among the several binding sites on the NMDA receptor complex, the strychnine-insensitive glycine-binding site plays a key role in the activation of the NMDA receptor [11–14] and therefore, during the last decade, many glycine antagonists have been prepared because of their therapeutic potential [15–19]. These compounds led to a model [15] of the glycine-binding site, the essential features of which are: (a) a NH hydrogen-bond donor, (b) a Coulombic interaction of an ionizable acidic group positioned in alpha to the NH, (c) a size-limited hydrophobic pocket, and (d) a hydrogen bond acceptor linked to a lipophilic chain. Several compounds can be fitted into this model and among them the glycine antagonist GV150526 [20] and a series of 4,5-disubstituted-3-(2-carbamoylvinyl)pyrrole-2-carboxylic acids [21]. As to point (d), it is worth noticing that compounds suitably modified in the lipophilic chain led, in the case of GV150526, to products with better affinity than the parent molecule [20].

We recently reported the synthesis and preliminary biological evaluation of a series of (*E*)-3-(*N*-phenyl-2-carbamoylvinyl)pyrrole-2-carboxylic acid derivatives, bearing various substituents at the 4- and 5-positions [21]. These compounds were studied in vitro as antagonists at the strychnine-insensitive glycine-binding site of the NMDA receptor complex, showing good potency and selectivity. The most active compounds of the series i.e. (*E*)-4,5-dibromo-3-(*N*-phenyl-2-carbamoylvinyl)pyrrole-2-carboxylic acid (**I**) ($pK_i = 7.95$) and its 4-bromo-5-methyl (**II**) ($pK_i = 7.24$) and 4,5-dimethyl (**III**) ($pK_i = 6.70$) analogs (Fig. 1), blocked

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PII: S0014-827X(98)00110-4

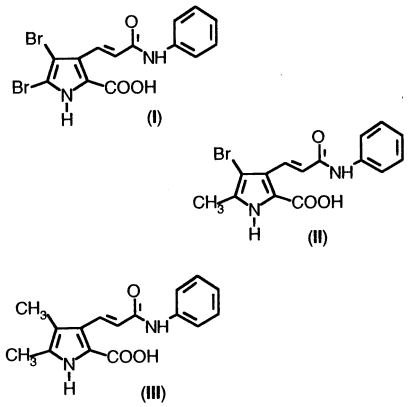


Fig. 1. Structures of analogs **I–III**.

NMDA-induced convulsions in mice when tested in vivo [21]. These results indicated that the 4,5-disubstituted pyrrole-2-carboxylates represent novel templates for the design of glycine antagonists.

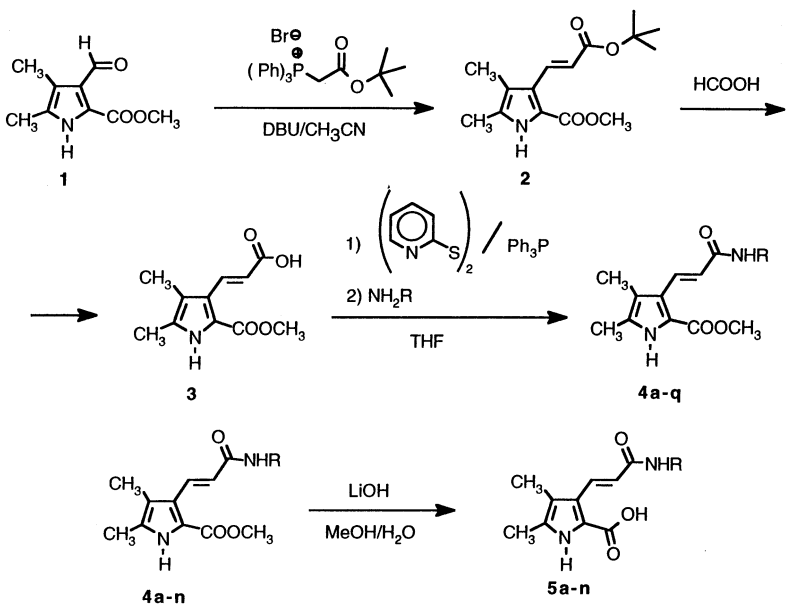
We had not explored however, the effects of the structural modification of the *N*-phenyl-2-carbamoylvinyl side chain on the affinity of the compounds, and to investigate this point we decided to synthesize a series of 4,5-disubstituted pyrrole-2-carboxylic acids bearing different chains in position 3. In the present study, (*E*)-3-(*N*-phenyl-2-carbamoylvinyl)-4,5-dimethyl-pyrrole-2-carboxylic acid (**III**) was chosen as a template, instead of the more active 4,5-dibromo derivative (**I**), because of its easier synthesis and of the putative equivalence of the structural information obtainable by modification of these two related compounds. Initially, investigation of the effects caused by aromatic substituents was carried out according to the Topliss decisional scheme [22] (**5b**,**c**,**e**,**f**,**o**,**p**) and subsequently, after ascertaining the ineffectiveness of the approach, by selecting additional compounds (**5a**,**d**,**g**,**j**,**k**,**l**,**n**,**q**,**r**) so that the resulting set (**5a–g**,**j–l**,**n–r**) would range over five out of the eight octants of the 3D space spanned by the scaled first three principal properties ($*t_1$, $*t_2$ and $*t_3$)[1] [23]. Non-aromatic substitution of the carbamoyl nitrogen was also considered (**5h**,**i**,**m**) as well as major modifications such as the retro compound **5s** and the des-carbamoyl derivative **5t**.

2. Chemistry

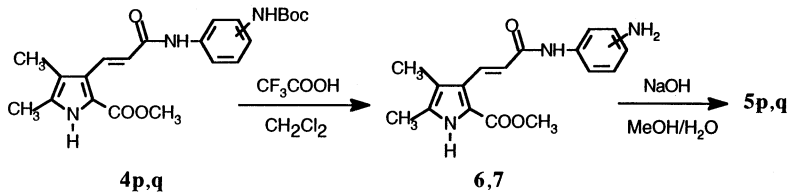
The synthesis of intermediate methyl 4,5-dimethyl-pyrrole-2-carboxylates (**4a–q**) was achieved as in Scheme 1. Wittig reaction of methyl 4,5-dimethyl-3-formyl-pyrrole-2-carboxylate (**1**) [24] with (*t*-butoxycarbonyl-methyl)-triphenylphosphonium bromide gave the *t*-butyl ester derivative **2**, which afforded the methyl (*E*)-3-(2-carboxyethenyl)-4,5-dimethylpyrrole-2-carboxylate (**3**) via selective HCOOH-mediated hydrolysis. Compounds **4a–q** were obtained by reaction of **3** with 2,2′-dipyridyl disulfide/triphenylphosphine and subsequent condensation with the suitable amine (*N*-Boc-1,4-diaminobenzene and *N*-Boc-1,3-diaminobenzene [25] for **4p** and **4q**, respectively). Hydrolysis of the intermediate esters **4a–n** carried out in basic media gave acids **5a–n**.

Product **5o** was obtained as sodium salt after hydrolysis with NaOH in aqueous MeOH. The *p*- and *m*-

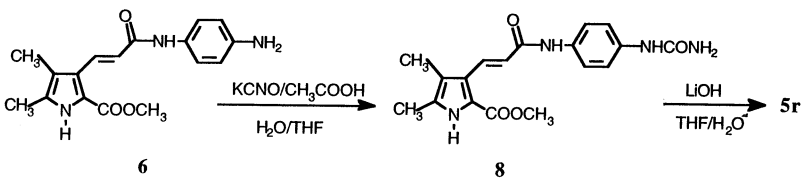
[1] $*t_1$ is mainly related to bulk and hydrophobicity; $*t_2$ is related to σ_m and σ_p; $*t_3$ is related to hydrophobicity and shape.



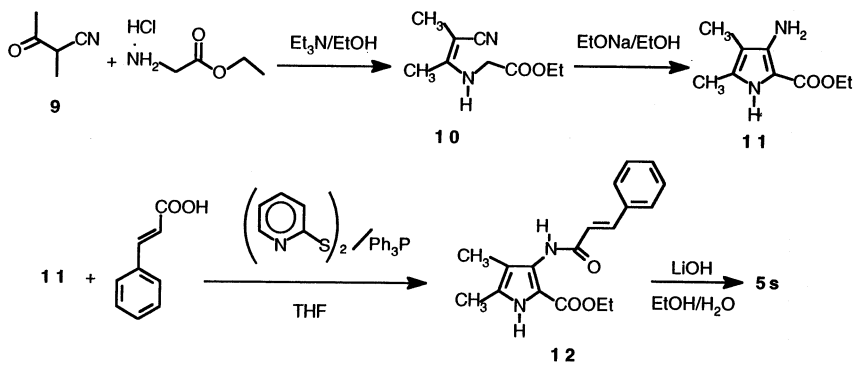
Scheme 1.



Scheme 2.



Scheme 3.



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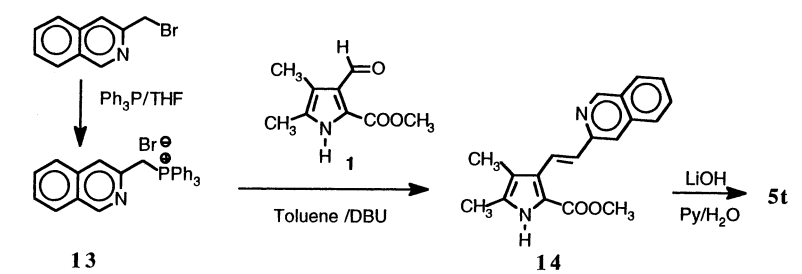


Scheme 5.

aminophenyl-substituted compounds **5p**,**q** were obtained from **4p**,**q**, which were first de-protected to **6**,**7** (trifluoroacetic acid) and then hydrolyzed with NaOH to their sodium salts (Scheme 2).

The ureido derivative **5r** was prepared by reaction of **6** with potassium isocyanate and subsequent hydrolysis of the ester **8** with LiOH (Scheme 3).

(*E*)-3-(cinnamoylamino)-4,5-dimethylpyrrole-2-carboxylic acid (**5s**) was prepared as shown in Scheme 4. The unknown 3-aminopyrrole derivative **11** was prepared by cyclization of the enamine **10** obtained by the condensation of glycine ethyl ester hydrochloride with 2-methyl-3-oxobutyronitrile (**9**) [26]. The condensation of 3-aminopyrrole (**11**) with cinnamic acid in the presence of 2,2′-dipyridyl disulfide/triphenylphosphine gave **12** which afforded **5s** upon basic hydrolysis.

The methyl (*E*)-3-[(3-isoquinolyl)-2-vinyl)]-4,5-dimethylpyrrole-2-carboxylate (**14**) was prepared by Wittig reaction between **1** and 3-isoquinolylmethyl-triphenylphosphonium bromide (**13**) in the presence of DBU. Hydrolysis of **14** afforded the lithium salt of the relative acid **5t** (Scheme 5).

3. Pharmacology

The affinity of **5a**–**t** for the glycine-binding site associated with the NMDA receptor channel complex was determined by the inhibition they induced of the binding of [^{3}H]glycine to crude synaptic membranes prepared from adult rat cerebral cortex (Section 5 and Table 1).

4. Results and discussion

The several aromatic substituents that were used to modify the 3-(*N*-phenyl-2-carbamoylvinyl) moiety of the lead compound **III** ($pK_i = 6.70$) all failed to give compounds endowed with a higher affinity, the highest pK_i values being those relative to **5r** ($pK_i = 6.4$), **5p** ($pK_i = 6.32$), **5k** ($pK_i = 6.25$) and **5g** ($pK_i = 6.23$). This is somewhat surprising in view of what had been reported for the analogues of GV 150526 [20], for which an increase in pK_i of 0.4 units is obtained when the phenyl ring is substituted with a *para*-amino group. The reversal of the order of the structural elements in the chain (**5s**), or its lengthening (**5n**) resulted in reduction of affinity, whereas removal of the carboxamido group and introduction of a heterocyclic moiety (**5t**) yielded a compound retaining a modest binding affinity. The affinity of the adamantyl derivative **5h** is essentially the same as those of **5g**,**k**, indicating that an *N*-aryl group is not an absolute requirement for efficient binding and that shape and bulk effects are probably as relevant as the electronic ones, as is shown by the pK_i of **5k** versus **5l,j** and of **5h** versus **5i,m**. This observation adds to previous knowledge of the structure–activity relationships in this class of compounds.

The results of this study confirm our previous report that (*E*)-3-(*N*-phenyl-2-carbamoylvinyl)-4,5-disubstitu-
ted pyrrole-2-carboxylic acids are a class of ligands of the glycine-binding site of the NMDA receptor, and indicate that these compounds deviate, to some extent, from the structure–activity relationships established in the indole series [20,27–30].

5. Experimental

5.1. Chemistry

Solvents and reagents were of the highest commercial grade and were used without additional purification. Melting points were determined on a Büchi SMP-510 capillary melting point apparatus, and are uncorrected. ^{1}H NMR spectra were recorded on Varian Unity 400, Varian Unity plus 500, Varian VXRS 5000 or Varian EM 60 spectrometer using TMS as internal standard; chemical shifts are reported in ppm (δ scale). EI-MS spectra (70 eV) were taken on a Fisons-Trio 1000 spectrometer (only molecular ions M^+ are given); FAB-MS were obtained on a VG-4 triple quadrupole on a 4-nitrobenzoic acid matrix and only $(M+H)^+$ are given. IR spectra were recorded on a Bruker IFS 48 spectrometer; absorbances are reported in ν (cm^{-1}). Elemental analyses were performed on a Carlo Erba analyzer and, when reported (C, H, N), were within ± 0.4 from the theoretical value.

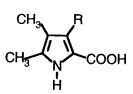
5.1.1. Methyl (E)-3-[2-(t-butoxycarbonyl)vinyl]-4,5-dimethylpyrrole-2-carboxylate (2)

DBU (0.225 ml, 1.5 mmol) was added to a suspension of (*t*-butoxycarbonylmethyl)triphenylphosphonium bromide (690 mg, 1.5 mmol) in 5 ml of CH_3CN and the resulting mixture stirred for 15 min at room temperature (r.t.); after this time 181 mg (1 mmol) of **1** [24] were added and the resulting solution was refluxed for 3 h. The solvent was evaporated to dryness and the residue purified by flash chromatography on silica gel (cyclohexane–EtOAc 7:3) and crystallization. 78% yield; m.p. 171°C (MeOH). ^{1}H NMR ($CDCl_3$) δ: 8.85 (s, 1H), 8.23 (d, $J = 16.5$ Hz, 1H), 6.24 (d, $J = 16.5$ Hz, 1H), 3.88 (s, 3H), 2.21 (s, 3H), 2.13 (s, 3H), 1.53 (s, 9H). IR ($CDCl_3$): 1688, 1632 cm^{-1}. MS (FAB): m/z 280 $(M+H)^+$.

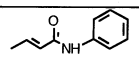
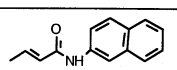
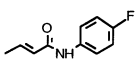
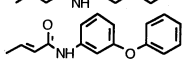
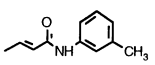
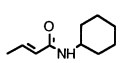
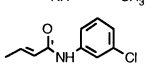
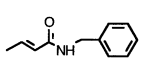
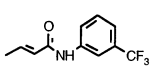
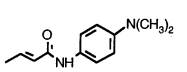
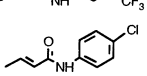
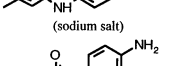
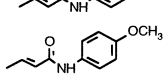
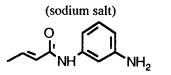
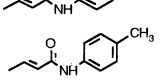
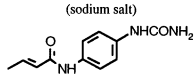
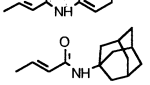
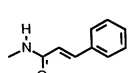
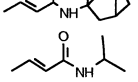
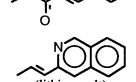
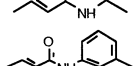
5.1.2. (E)-3-[2-(Methoxycarbonyl)-4,5-dimethyl-1H-pyrrol-3-yl]prop-2-enoic acid (3)

A 280 mg (1 mmol) sample of **2** was suspended in 12 ml of HCOOH and stirred at r.t. for 2 h.

Table 1
Affinities at the glycine-binding site of compounds **5a–t**[a]



5a-t

Comp	R	pK_i[a]	Comp	R	pK_i[a]
III		6.70	**5k**		6.25
5a		6.0	**5l**		5.6
5b		6.1	**5m**		5.38
5c		5.99	**5n**		4.55
5d		5.45	**5o**	 (sodium salt)	5.66
5e		6.09	**5p**	 (sodium salt)	6.32
5f		6.1	**5q**	 (sodium salt)	5.81
5g		6.23	**5r**		6.4
5h		6.2	**5s**		4.22
5i		4.83	**5t**	 (lithium salt)	5.74
5j		5.31			

[a] Inhibition of binding of [^{3}H]Gly (Refs. [28,29]).

HCOOH was evaporated under reduced pressure and the crude material purified by crystallization. 88% yield; m.p. 260°C (dec.) (MeOH). ^{1}H NMR (DMSO-d_6) δ: 12.2 (br s, 1H), 11.8 (s, 1H), 8.21 (d, $J = 16.4$ Hz, 1H), 6.13 (d, $J = 16.4$ Hz, 1H), 3.76 (s, 3H), 2.13 (s, 3H), 2.04 (s, 3H). IR (Nujol): 3298, 1668, 1612 cm^{-1}. MS (FAB): m/z 224 $(M + H)^+$.

*5.1.3. General procedure for the synthesis of methyl (E)-3-(N-substituted-2-carbamoylvinyl)-4,5-dimethyl-pyrrole-2-carboxylates (**4a–q**)*

Triphenylphosphine (524 mg, 2 mmol) and 2,2′-dipyridyldisulfide (440 mg, 2 mmol) were added to a solution of 223 mg (1 mmol) of **3** dissolved in 20 ml of anhydrous THF under N_2. The resulting mixture was stirred at r.t. for 2 h, after which 1.2–2.4 mmol of the suitable amine were added and stirring continued under reflux (at r.t. for **4f–i** and **4m–o**) for 16–60 h (4 days for **4c**). In the case of the isopropyl derivative a solution obtained from isopropylamine hydrochloride in CH_2Cl_2 and a stoichiometric amount of DBU, was added to the reaction mixture.

The solvent was evaporated to dryness and the residue dissolved in diethyl ether. The desired product crystallized spontaneously.

*5.1.3.1. Methyl (E)-3-[N-(4-fluorophenyl)-2-carbamoyl-vinyl]-4,5-dimethylpyrrole-2-carboxylate (**4a**).* 93% yield; m.p. 267–8°C (MeOH). ^{1}H NMR (DMSO-d_6) δ: 11.8 (br s, 1H), 10.2 (br s, 1H), 8.23 (d, $J = 16.1$ Hz, 1H), 7.71 (dd, 2H), 7.17 (t, 2H), 6.59 (d, $J = 16.1$ Hz, 1H), 3.8 (s, 3H), 2.15 (ds, 6H). IR (Nujol): 3294, 1672, 1661, 1622 cm^{-1}. MS (EI): m/z 316 (M^+).

*5.1.3.2. Methyl (E)-3-[N-(3-methylphenyl)-2-carbamoyl-vinyl]-4,5-dimethylpyrrole-2-carboxylate (**4b**).* 77% yield; m.p. 239°C (Et_2O). ^{1}H NMR (DMSO-d_6) δ: 11.7 (s, 1H), 10.0 (s, 1H), 8.2 (d, $J = 16.1$ Hz, 1H), 7.5 (s, 1H), 7.45 (m, 1H), 7.2 (t, 1H), 6.75 (d, 1H), 6.6 (d, $J = 16.1$ Hz, 1H), 3.8 (s, 3H), 2.3 (s, 3H), 2.2 (s, 3H), 2.1 (s, 3H). IR (Nujol): 3292, 1672, 1655, 1616 cm^{-1}. MS (EI): m/z 312 (M^+).

*5.1.3.3. Methyl (E)-3-[N-(3-chlorophenyl)-2-carbamoyl-vinyl]-4,5-dimethylpyrrole-2-carboxylate (**4c**).* 79% yield; m.p. 242°C (MeOH). ^{1}H NMR (DMSO-d_6) δ: 11.76 (br s, 1H), 10.25 (s, 1H), 8.24 (d, $J = 16$ Hz, 1H), 7.95 (t, 1H), 7.47 (dd, 1H), 7.33 (t, 1H), 7.08 (dd, 1H), 6.58 (d, $J = 16$ Hz, 1H), 3.78 (s, 3H), 2.15 (s, 3H), 2.12 (s, 3H). IR (Nujol): 3304, 1680, 1622 cm^{-1}. MS (FAB): m/z 333 $(M + H)^+$.

*5.1.3.4. Methyl (E)-3-[N-(3-trifluoromethylphenyl)-2-carbamoylvinyl]-4,5-dimethylpyrrole-2-carboxylate (**4d**).* 36% yield; m.p. 214°C (MeOH–Et_2O–hexane). ^{1}H NMR (acetone-d_6, 60 MHz) δ: 11.6 (br s, 1H), 9.6 (br s, 1H), 8.6 (s, 1H), 8.3 (s, 1H), 7.9–7.3 (m, 4H), 6.6 (d, 1H), 3.8 (s, 3H), 2.2 (s, 3H), 2.1 (s, 3H). IR (Nujol): 3302, 1672, 1664, 1612 cm^{-1}. MS (EI): m/z 366 (M^+).

*5.1.3.5. Methyl (E)-3-[N-(4-chlorophenyl)-2-carbamoyl-vinyl]-4,5-dimethylpyrrole-2-carboxylate (**4e**).* 70% yield; m.p. 275°C (MeOH). ^{1}H NMR (DMSO-d_6) δ: 11.76 (s, 1H), 10.21 (s, 1H), 8.24 (d, $J = 16.4$ Hz, 1H), 7.71 (d, 2H), 7.36 (d, 2H), 6.59 (d, $J = 16.4$ Hz, 1H), 3.78 (s, 3H), 2.15 (s, 3H), 2.12 (s, 3H). IR (Nujol): 3304, 1672, 1626 cm^{-1}. MS (FAB): m/z 333 $(M + H)^+$.

*5.1.3.6. Methyl (E)-3-[N-(4-methoxyphenyl)-2-carbamoylvinyl]-4,5-dimethylpyrrole-2-carboxylate(**4f**).* 77% yield; m.p. 276°C (Et_2O). ^{1}H NMR (DMSO-d_6) δ: 11.71 (br s, 1H), 9.94 (s, 1H), 8.17 (d, $J = 16.2$ Hz, 1H), 7.59 (d, 2H), 6.88 (d, 2H), 6.57 (d, $J = 16.2$ Hz, 1H), 3.77 (s, 3H), 3.71 (s, 3H), 2.15 (s, 3H), 2.11 (s, 3H). IR (Nujol): 3302, 1674, 1651, 1616 cm^{-1}. MS (FAB): m/z 329 $(M + H)^+$.

*5.1.3.7. Methyl (E)-3-[N-(4-methylphenyl)-2-carbamoyl-vinyl]-4,5-dimethylpyrrole-2-carboxylate (**4g**).* 60% yield; m.p. 268–70°C (Et_2O). ^{1}H NMR (DMSO-d_6) δ: 11.72 (s, 1H), 9.97 (s, 1H), 8.19 (d, $J = 16.4$ Hz, 1H), 7.55 (d, 2H), 7.10 (d, 2H), 6.58 (d, $J = 16.4$ Hz, 1H), 3.77 (s, 3H), 2.24 (s, 3H), 2.15 (s, 3H), 2.11 (s, 3H). IR (Nujol): 3304, 1676, 1657, 1618 cm^{-1}. MS (FAB): m/z 313 $(M + H)^+$.

*5.1.3.8. Methyl (E)-3-[N-(1-adamantyl)-2-carbamoyl-vinyl]-4,5-dimethylpyrrole-2-carboxylate (**4h**).* 90% yield; m.p. 218°C ($CHCl_3$–hexane). ^{1}H NMR (DMSO-d_6) δ: 11.59 (s, 1H), 7.95 (d, $J = 15.9$ Hz, 1H), 7.50 (s, 1H), 6.40 (d, $J = 15.9$ Hz, 1H), 3.74 (s, 3H), 2.12 (s, 3H), 2.04 (s, 3H), 2.01–1.62 (m, 15H). IR (Nujol): 3306, 1672, 1655, 1616 cm^{-1}. MS (EI): m/z 356 (M^+).

*5.1.3.9. Methyl (E)-3-(N-isopropyl-2-carbamoylvinyl)-4,5-dimethylpyrrole-2-carboxylate (**4i**).* 83% yield; m.p. 249–50°C (dec.) (Et_2O). ^{1}H NMR (DMSO-d_6) δ: 11.62 (s, 1H), 8.01 (d, $J = 16$ Hz, 1H), 7.86 (d, 1H), 6.36 (d, $J = 16$ Hz, 1H), 3.92 (m, 1H), 3.75 (s, 3H), 2.13 (s, 3H), 2.05 (s, 3H), 1.08 (d, 6H). IR (Nujol): 3302, 1668, 1651 cm^{-1}. MS (EI): m/z 264 (M^+).

*5.1.3.10. Methyl (E)-3-[N-(α-naphthyl)-2-carbamoyl-vinyl]-4,5-dimethylpyrrole-2-carboxylate (**4j**).* 69% yield; m.p. 275–6°C (Et_2O). ^{1}H NMR (DMSO-d_6) δ: 11.75 (s, 1H), 10.06 (s, 1H), 8.31 (d, $J = 16.5$ Hz, 1H), 8.17 (d, 1H), 7.96–7.93 (dd, 2H), 7.76 (d, 1H), 7.61–7.51 (m, 3H), 6.93 (d, $J = 16.5$ Hz, 1H), 3.81 (s,

3H), 2.20 (s, 6H). IR (Nujol): 3314, 3271, 1676, 1651, 1616 cm^{-1}. MS (FAB): m/z 349 $(M+H)^+$.

*5.1.3.11. Methyl (E)-3-[N-(β-naphthyl)-2-carbamoylvinyl]-4,5-dimethylpyrrole-2-carboxylate (**4k**).* 78% yield; m.p. 262°C (Et_2O). ^1H NMR (DMSO-d_6) δ: 11.75 (bs, 1H), 10.28 (s, 1H), 8.43 (d, 1H), 8.28 (d, $J=16.4$ Hz, 1H), 7.86 (d, 1H), 7.81 (dd, 2H), 7.64 (dd, 1H), 7.48–7.36 (m, 2H), 6.67 (d, $J=16.4$ Hz, 1H), 3.79 (s, 3H), 2.16 (s, 3H), 2.15 (s, 3H). IR (Nujol): 3312, 1676, 1655, 1616 cm^{-1}. MS (EI): m/z 348 (M^+).

*5.1.3.12. Methyl (E)-3-[N-(3-phenoxyphenyl)-2-carbamoylvinyl]-4,5-dimethylpyrrole-2-carboxylate (**4l**).* 84% yield; m.p. 194°C (MeOH). ^1H NMR (acetone-d_6, 60 MHz) δ: 10.6 (br s, 1H), 9.3 (br s, 1H), 8.4 (d, $J=16$ Hz, 1H), 7.6–6.9 (m, 9H), 6.6 (d, $J=16$ Hz, 1H), 3.8 (s, 3H), 2.2 (s, 3H), 2.1 (s, 3H). IR (Nujol): 3297, 1669, 1590 cm^{-1}. MS (EI): m/z 390 (M^+).

*5.1.3.13. Methyl (E)-3-(N-cyclohexyl-2-carbamoylvinyl)-4,5-dimethylpyrrole-2-carboxylate (**4m**).* 86% yield; m.p. 272°C (Et_2O). ^1H NMR (DMSO-d_6) δ: 11.61 (s, 1H), 8.01 (d, $J=16$ Hz, 1H), 7.85 (d, 1H), 6.38 (d, $J=16$ Hz, 1H), 3.75 (s, 3H), 3.60 (m, 1H), 2.13 (s, 3H), 2.05 (s, 3H), 1.79–1.1 (m, 10H). IR (Nujol): 3312, 1672, 1651, 1616 cm^{-1}. MS (FAB): m/z 305 $(M+H)^+$.

*5.1.3.14. Methyl (E)-3-(N-benzyl-2-carbamoylvinyl)-4,5-dimethylpyrrole-2-carboxylate (**4n**).* 79% yield; m.p. 247°C (Et_2O). ^1H NMR (DMSO-d_6) δ: 11.65 (s, 1H), 8.49 (t, 1H), 8.08 (d, $J=16$ Hz, 1H), 7.35–7.21 (m, 5H), 6.46 (d, $J=16$ Hz, 1H), 4.39 (d, 2H), 3.75 (s, 3H), 2.13 (s, 3H), 2.05 (s, 3H). IR (Nujol): 3312, 3267, 1670, 1651, 1614 cm^{-1}. MS (FAB): m/z 313 $(M+H)^+$.

*5.1.3.15. Methyl (E)-3-[N-(4-dimethylaminophenyl)-2-carbamoylvinyl]-4,5-dimethylpyrrole-2-carboxylate (**4o**).* 73% yield; m.p. 270–3°C (Et_2O). ^1H NMR (DMSO-d_6) δ: 11.69 (br s, 1H), 9.79 (br s, 1H), 8.14 (d, $J=15.9$ Hz, 1H), 7.50 (d, 2H), 6.69 (d, 2H), 6.56 (d, $J=15.9$ Hz, 1H), 3.77 (s, 3H), 2.83 (s, 6H), 2.15 (s, 3H), 2.11 (s, 3H). IR (Nujol): 3302, 1674, 1651,1616 cm^{-1}. MS (FAB): m/z 342 $(M+H)^+$.

*5.1.3.16. Methyl (E)-3-[N-(4-Boc-aminophenyl)-2-carbamoylvinyl]-4,5-dimethylpyrrole-2-carboxylate (**4p**).* 4% yield; m.p. 245°C (dec.) (Et_2O). ^1H NMR (DMSO-d_6) δ: 11.7 (s, 1H), 9.9 (s, 1H), 9.2 (s, 1H), 8.18 (d, $J=16$ Hz, 1H), 7.50 (d, 2H), 7.36 (d, 2H), 6.58 (d, $J=16$ Hz, 1H), 3.77 (s, 3H), 2.15 (s, 3H), 2.11 (s, 3H), 1.45 (s, 9H). IR (Nujol): 3342, 3302, 1693, 1678 cm^{-1}. MS (EI): m/z 413 (M^+).

*5.1.3.17. Methyl (E)-3-[N-(3-Boc-aminophenyl)-2-carbamoylvinyl]-4,5-dimethylpyrrole-2-carboxylate (**4q**).* 94% yield; m.p. 130°C (Et_2O). ^1H NMR (DMSO-d_6) δ: 11.73 (s, 1H), 10.03 (s, 1H), 9.37 (s, 1H), 8.23 (d, $J=16$ Hz, 1H), 7.86 (m, 1H), 7.49 (m, 1H), 7.17 (t, 1H), 7.02 (m, 1H), 6.65 (d, $J=16$ Hz, 1H), 3.81 (s, 3H), 2.18 (s, 3H), 2.14 (s, 3H), 1.49 (s, 9H). IR (Nujol): 3296, 1717, 1678, 1610 cm^{-1}. MS (FAB): m/z 414 $(M+H)^+$.

*5.1.4. Methyl (E)-3-[N-(4-aminophenyl)-2-carbamoylvinyl]-4,5-dimethylpyrrole-2-carboxylate (**6**) and methyl (E)-3-[N-(3-aminophenyl)-2-carbamoylvinyl]-4,5-dimethylpyrrole-2-carboxylate (**7**)*

A total of 0.8 ml of CF_3COOH was added to a suspension of 413 mg (1 mmol) of **4p** or **4q** in 11 ml of CH_2Cl_2. The mixture was stirred at r.t. for 24 h, after which the solvent was evaporated and the residue treated with EtOAc. The organic phase was washed once with 2 N Na_2CO_3 solution, twice with water and then dried (Na_2SO_4). The solvent was evaporated and the crude material was purified by crystallization.

Compound **6**: quantitative yield; m.p. 242°C (dec.) (MeOH). ^1H NMR (DMSO-d_6) δ: 11.7 (s, 1H), 9.7 (s, 1H), 8.1 (d, $J=16$ Hz, 1H), 7.3 (d, 2H), 6.6 (d, $J=16$ Hz, 1H), 6.5 (d, 2H), 4.9 (s, 2H), 3.8 (s, 3H), 2.2 (s, 3H), 2.1 (s, 3H). IR (Nujol): 3290, 1664, 1616 cm^{-1}. MS (EI): m/z 313 (M^+).

Compound **7:** 93% yield; m.p. 238°C (MeOH). ^1H NMR (DMSO-d_6) δ: 11.71 (s, 1H), 9.75 (s, 1H), 8.20 (d, $J=16$ Hz, 1H), 7.03 (m, 1H), 6.93 (t, 1H), 6.79 (m, 1H), 6.62 (d, $J=16$ Hz, 1H), 6.25 (m, 1H), 5.06 (s, 2H), 3.80 (s, 3H), 2.18 (s, 3H), 2.14 (s, 3H). IR (Nujol): 3296, 1680, 1657, 1620 cm^{-1}. MS (FAB): m/z 314 $(M+H)^+$.

*5.1.5. Methyl (E)-3-[N-(4-ureidophenyl)-2-carbamoylvinyl]-4,5-dimethylpyrrole-2-carboxylate (**8**)*

A 200 mg (2.4 mmol) sample of KCNO, dissolved in 1 ml of water, was added portion-wise to a solution of 312 mg (1 mmol) of **6** in 1 ml of water, 0.5 ml of glacial CH_3COOH and THF until complete solubilization. The mixture was stirred for 4 h at r.t. and the desired product separated as a white solid upon concentration of the solvent at reduced pressure. 94% yield; m.p. >300°C. ^1H NMR (DMSO-d_6) δ: 11.72 (s, 1H), 9.93 (s, 1H), 8.43 (s, 1H), 8.19 (d, $J=16$ Hz, 1H), 7.56 (m, 2H), 7.33 (m, 2H), 6.61 (d, $J=16$ Hz, 1H), 5.78 (s, 2H), 3.80 (s, 3H), 2.18 (s, 3H), 2.14 (s, 3H). IR (Nujol): 3308, 1674, 1616 cm^{-1}. MS (FAB): m/z 357 $(M+H)^+$.

*5.1.6. General procedure for the synthesis of (E)-3-(N-substituted-2-carbamoylvinyl)-4,5-dimethylpyrrole-2-carboxylic acids (**5a–r**)*

A total of 1 mmol of the suitable ester was suspended in 1 ml of MeOH–20% water (THF–20%

Table 2
Elemental analyses of new target compounds

5a	For $C_{16}H_{15}FN_2O_3 \cdot 1H_2O$	(M_W 320.32)	Calc.: C, 60.00; H, 5.35; N, 8.75 Found: C, 60.28; H, 5.37; N, 8.39
5b	For $C_{17}H_{18}N_2O_3$	(M_W 298.34)	Calc.: C, 68.44; H, 6.08; N, 9.39 Found: C, 68.39; H, 6.24; N, 9.27
5c	For $C_{16}H_{15}ClN_2O_3 \cdot 0.2H_2O$	(M_W 322.36)	Calc.: C, 59.61; H, 4.82; N, 8.69 Found: C, 59.57; H, 4.81; N, 8.39
5d	For $C_{17}H_{15}F_3N_2O_3 \cdot 1H_2O$	(M_W 370.33)	Calc.: C, 55.14; H, 4.63; N, 7.56 Found: C, 55.29; H, 4.73; N, 7.44
5e	For $C_{16}H_{15}ClN_2O_3 \cdot 1.2H_2O$	(M_W 340.38)	Calc.: C, 56.46; H, 5.15; N, 8.23 Found: C, 56.61; H, 5.22; N, 7.83
5f	For $C_{17}H_{18}N_2O_4 \cdot 1CH_3OH$	(M_W 346.38)	Calc.: C, 62.42; H, 6.40; N, 8.09 Found: C, 62.37; H, 6.00; N, 7.73
5g	For $C_{17}H_{18}N_2O_3 \cdot 1.7CH_3OH$	(M_W 352.81)	Calc.: C, 63.66; H, 7.09; N, 7.94 Found: C, 63.62; H, 6.73; N, 7.54
5h	For $C_{20}H_{26}N_2O_3 \cdot 1.2H_2O$	(M_W 364.06)	Calc.: C, 65.98; H, 7.86; N, 7.69 Found: C, 65.74; H, 7.90; N, 7.29
5i	For $C_{13}H_{18}N_2O_3 \cdot 1H_2O$	(M_W 268.31)	Calc.: C, 58.19; H, 7.51; N, 10.44 Found: C, 58.59; H, 7.52; N, 10.22
5j	For $C_{20}H_{18}N_2O_2 \cdot 1H_2O$	(M_W 352.39)	Calc.: C, 68.17; H, 5.72; N, 7.95 Found: C, 68.28; H, 5.97; N, 7.57
5k	For $C_{20}H_{18}N_2O_2 \cdot 0.8H_2O$	(M_W 348.79)	Calc.: C, 68.87; H, 5.66; N, 8.03 Found: C, 68.81; H, 5.42; N, 8.08
5l	For $C_{22}H_{20}N_2O_4 \cdot 0.2H_2O$	(M_W 380.01)	Calc.: C, 69.53; H, 5.41; N, 7.37 Found: C, 69.55; H, 5.56; N, 7.36
5m	For $C_{16}H_{22}N_2O_3 \cdot 1.5CH_3OH$	(M_W 338.43)	Calc.: C, 62.11; H, 8.34; N, 8.28 Found: C, 62.08; H, 7.96; N, 8.38
5n	For $C_{17}H_{18}N_2O_3 \cdot 1.2CH_3OH$	(M_W 336.79)	Calc.: C, 64.91; H, 6.82; N, 8.32 Found: C, 64.88; H, 6.46; N, 8.32
5o	For $C_{18}H_{20}N_3O_3Na \cdot 3H_2O$	(M_W 403.41)	Calc.: C, 53.59; H, 6.50; N, 10.42 Found: C, 53.49; H, 6.22; N, 10.11
5p	For $C_{16}H_{16}N_3O_3Na \cdot 3.5H_2O$	(M_W 384.36)	Calc.: C, 50.00; H, 6.03; N, 10.93 Found: C, 50.01; H, 5.71; N, 10.61
5q	For $C_{16}H_{16}N_3O_3Na \cdot 4.5H_2O$	(M_W 402.38)	Calc.: C, 47.76; H, 6.26; N, 10.44 Found: C, 47.35; H, 6.06; N, 10.13
5r	For $C_{17}H_{18}N_4O_4 \cdot 1H_2O$	(M_W 360.37)	Calc.: C, 56.66; H, 5.59; N, 15.55 Found: C, 56.54; H, 5.68; N, 15.16
5s	For $C_{16}H_{16}N_2O_3 \cdot 0.25H_2O \cdot 0.5THF$	(M_W 360.37)	Calc.: C, 66.55; H, 6.36; N, 8.62 Found: C, 66.47; H, 6.23; N, 8.74
5t	For $C_{18}H_{15}LiN_2O_2 \cdot 2.5H_2O$	(M_W 343.31)	Calc.: C, 62.97; H, 5.87; N, 8.16. Found: C, 63.29; H, 5.48; N, 7.94

water for **8**); $LiOH \cdot H_2O$ (NaOH for **4o**, **6** and **7**) (4 mmol) was added and the resulting mixture was stirred under reflux for 2–24 h. The solvent was evaporated to dryness to give a residue which was treated with water and EtOAc; 2 N HCl was added until acidic pH was reached. The organic phase was washed three times with water and dried (Na_2SO_4); the solvent was then evaporated to give a crude material which was purified by crystallization. In the case of **4o**, **6** and **7**, the residue obtained from the evaporation of the solvent was purified by crystallization from water and the products were obtained as sodium salts. In the case of **5r** the desired product separated, upon acidification, as a highly insoluble white solid, which was washed well with hot MeOH and used without any further purification. Elemental analysis results for compounds **5a–t** are listed in Table 2.

*5.1.6.1. (E)-3-[N-(4-Fluorophenyl)-2-carbamoylvinyl]-4,5-dimethylpyrrole-2-carboxylic acid (**5a**).* 73% yield; m.p. 204°C (dec.) (MeOH). ^{1}H NMR (DMSO-d_6) δ: 12.50 (s, 1H), 11.56 (s, 1H), 10.08 (s, 1H), 8.30 (d, $J = 15.9$ Hz, 1H), 7.69 (m, 2H), 7.14 (t, 2H), 6.53 (d, $J = 15.9$ Hz, 1H), 2.13 (s, 3H), 2.11 (s, 3H). IR (Nujol): 3290, 3213, 1659, 1647 cm^{-1}. MS (FAB): m/z 303 $(M + H)^+$. *Anal.* ($C_{16}H_{15}FN_2O_3 \cdot 1H_2O$) C, H, N.

*5.1.6.2. (E)-3-[N-(3-Methylphenyl)-2-carbamoylvinyl]-4,5-dimethylpyrrole-2-carboxylic acid (**5b**).* 80% yield; m.p. 208°C (dec.) (MeOH). ^{1}H NMR (DMSO-d_6) δ: 12.48 (s, 1H), 11.55 (s, 1H), 9.95 (s, 1H), 8.28 (d, $J = 15.9$ Hz, 1H), 7.48 (m, 2H), 7.17 (t, 1H), 6.84 (d, $J = 15.9$ Hz, 1H), 6.55 (d, 1H), 2.27 (s, 3H), 2.14 (s, 3H), 2.11 (s, 3H). IR (Nujol): 3312, 1672, 1618 cm^{-1}. MS (FAB): m/z 299 $(M+H)^+$. *Anal.* ($C_{17}H_{18}N_2O_3$) C, H, N.

*5.1.6.3. (E)-3-[N-(3-Chlorophenyl)-2-carbamoylvinyl]-4,5-dimethylpyrrole-2-carboxylic acid (**5c**).* 63% yield; m.p. 209°C (dec.) (MeOH). ^{1}H NMR (DMSO-d_6) δ: 12.53 (br s, 1H), 11.61 (s, 1H), 10.22 (s, 1H), 8.31 (d, $J = 16.2$ Hz, 1H), 7.94 (t, 1H), 7.47 (dd, 1H), 7.33 (t, 1H), 7.07 (dd, 1H), 6.53 (d, $J = 16.2$ Hz, 1H), 2.14 (s, 3H), 2.12 (s, 3H). IR (Nujol): 3300, 1668, 1612 cm^{-1}. MS (FAB): m/z 319 $(M+H)^+$. *Anal.* ($C_{16}H_{15}ClN_2O_3 \cdot 0.2H_2O$) C, H, N.

*5.1.6.4. (E)-3-[N-(3-Trifluoromethylphenyl)-2-carbamoylvinyl]-4,5-dimethylpyrrole-2-carboxylic acid (**5d**).* 72% yield; m.p. 155–8°C (dec.) (MeOH–Et_2O–hexane). ^{1}H NMR (DMSO-d_6) δ: 12.52 (br s, 1H), 11.64 (s, 1H), 10.39 (s, 1H), 8.37 (d, $J = 16$ Hz, 1H), 8.23 (s, 1H), 7.85 (d, 1H), 7.57 (t, 1H), 7.40 (d, 1H), 6.58 (d, $J = 16$ Hz, 1H), 2.17 (s, 3H), 2.16 (s, 3H). IR (Nujol): 3466, 1670, 1643 cm^{-1}. MS (FAB): m/z 353 $(M+H)^+$. *Anal.* ($C_{17}H_{15}F_3N_2O_3 \cdot 1H_2O$) C, H, N.

*5.1.6.5. (E)-3-[N-(4-Chlorophenyl)-2-carbamoylvinyl]-4,5-dimethylpyrrole-2-carboxylic acid (**5e**).* 43% yield; m.p. 175–8°C (EtOH). ^{1}H NMR (DMSO-d_6) δ: 12.5 (br s, 1H), 11.59 (s, 1H), 10.17 (s, 1H), 8.30 (d, $J = 16$ Hz, 1H), 7.70 (d, 2H), 7.36 (d, 2H), 6.54 (d, $J = 16$ Hz, 1H), 2.14 (s, 3H), 2.12 (s, 3H). MS (FAB): m/z 319 $(M+H)^+$. *Anal.* ($C_{16}H_{15}ClN_2O_3 \cdot 1.2H_2O$) C, H, N.

*5.1.6.6. (E)-3-[N-(4-Methoxyphenyl)-2-carbamoylvinyl]-4,5-dimethylpyrrole-2-carboxylic acid (**5f**).* 82% yield; m.p. 199°C (dec.) (MeOH). ^{1}H NMR (DMSO-d_6) δ: 12.44 (br s, 1H), 11.56 (s, 1H), 9.90 (s, 1H), 8.24 (d, $J = 16.2$ Hz, 1H), 7.59 (d, 2H), 6.87 (d, 2H), 6.53 (d, $J = 16.2$ Hz, 1H), 3.71 (s, 3H), 2.13 (s, 3H), 2.11 (s, 3H). IR (Nujol): 3261, 1653, 1618 cm^{-1}. MS (FAB): m/z 315 $(M+H)^+$. *Anal.* ($C_{17}H_{18}N_2O_4 \cdot 1CH_3OH$) C, H, N.

*5.1.6.7. (E)-3-[N-(4-Methylphenyl)-2-carbamoylvinyl]-4,5-dimethylpyrrole-2-carboxylic acid (**5g**).* 75% yield; m.p. 195–6°C (MeOH). ^{1}H NMR (DMSO-d_6) δ: 12.46 (br s, 1H), 11.57 (s, 1H), 9.94 (s, 1H), 8.26 (d, $J = 16.2$ Hz, 1H), 7.55 (d, 2H), 7.10 (d, 2H), 6.54 (d, $J = 16.2$ Hz, 1H), 2.24 (s, 3H), 2.14 (s, 3H), 2.11 (s, 3H). IR (Nujol): 3279, 3190, 1653, 1616 cm^{-1}. MS (FAB): m/z 299 $(M+H)^+$. *Anal.* ($C_{17}H_{18}N_2O_3 \cdot 1.7CH_3OH$) C, H, N.

*5.1.6.8. (E)-3-[N-(1-Adamantyl)-2-carbamoylvinyl]-4,5-dimethylpyrrole-2-carboxylic acid (**5h**).* 80% yield; m.p. 150°C (dec.) (MeOH). ^{1}H NMR (DMSO-d_6) δ: 12.3 (br s, 1H), 11.41 (s, 1H), 8.03 (d, $J = 16.2$ Hz, 1H), 7.45 (s, 1H), 6.35 (d, $J = 16.2$ Hz, 1H), 2.10 (s, 3H), 2.03 (s, 3H), 1.98 (m, 9H), 1.62 (m, 6H). IR (Nujol): 3270, 3196, 1645 cm^{-1}. MS (EI): m/z 342 (M^+). *Anal.* ($C_{20}H_{26}N_2O_3 \cdot 1.2H_2O$) C, H, N.

*5.1.6.9. (E)-3-(N-Isopropyl-2-carbamoylvinyl)-4,5-dimethylpyrrole-2-carboxylic acid (**5i**).* 71% yield; m.p. 184°C (MeOH–*i*-Pr_2O–hexane). ^{1}H NMR (DMSO-d_6) δ: 12.35 (br s, 1H), 11.46 (s, 1H), 8.06 (d, $J = 16$ Hz, 1H), 7.82 (d, 1H), 6.31 (d, $J = 16$ Hz, 1H), 3.90 (m, 1H), 2.11 (s, 3H), 2.04 (s, 3H), 1.07 (d, 6H). IR (Nujol): 3346, 3267, 1639, 1599 cm^{-1}. MS (FAB): m/z 251 $(M+H)^+$. *Anal.* ($C_{13}H_{18}N_2O_3 \cdot 1H_2O$) C, H, N.

*5.1.6.10. (E)-3-[N-(α-Naphthyl)-2-carbamoylvinyl]-4,5-dimethylpyrrole-2-carboxylic acid (**5j**).* 77% yield; m.p. 210°C (dec.) (EtOH). ^{1}H NMR (DMSO-d_6) δ: 12.46 (br s, 1H), 11.58 (s, 1H), 10.01 (s, 1H), 8.35 (d, $J = 16.4$ Hz, 1H), 8.14 (dd, 1H), 7.93 (dd, 1H), 7.88 (d, 1H), 7.73 (d, 1H), 7.56 (m, 1H), 7.50 (m, 2H), 6.86 (d, $J = 16.4$ Hz, 1H), 2.18 (s, 3H), 2.16 (s, 3H). IR (Nujol): 3242, 1651, 1618 cm^{-1}. MS (FAB): m/z 335 $(M+H)^+$. *Anal.* ($C_{20}H_{18}N_2O_2 \cdot 1H_2O$) C, H, N.

*5.1.6.11. (E)-3-[N-(β-Naphthyl)-2-carbamoylvinyl]-4,5-dimethylpyrrole-2-carboxylic acid (**5k**).* 42% yield; m.p. 191°C (dec.) (MeOH). ^{1}H NMR (DMSO-d_6) δ: 12.49 (br s, 1H), 11.59 (s, 1H), 10.24 (s, 1H), 8.40 (d, 1H), 8.35 (d, $J = 16.4$ Hz, 1H), 7.85 (d, 1H), 7.82 (d, 1H), 7.78 (d, 1H), 7.65 (dd, 1H), 7.45 (td, 1H), 7.38 (td, 1H), 6.63 (d, $J = 16.4$ Hz, 1H), 2.15 (s, 3H), 2.14 (s, 3H). IR (Nujol): 3265, 1651 cm^{-1}. MS (FAB): m/z 335 $(M+H)^+$. *Anal.* ($C_{20}H_{18}N_2O_2 \cdot 0.8H_2O$) C, H, N.

*5.1.6.12. (E)-3-[N-(3-Phenoxyphenyl)-2-carbamoylvinyl] 4,5-dimethylpyrrole-2-carboxylic acid (**5l**).* 55% yield; m.p. 208°C (dec.) (MeOH). ^{1}H NMR (DMSO-d_6) δ: 12.48 (s, 1H), 11.59 (s, 1H), 10.09 (s, 1H), 8.27 (d, $J = 16$ Hz, 1H), 7.44–7.38 (m, 4H), 7.30 (t, 1H), 7.15 (m, 1H), 7.04 m, 2H), 6.69 (m, 1H), 6.52 (d, $J = 16$ Hz, 1H), 2.13 (s, 3H), 2.10 (s, 3H). IR (Nujol): 3433, 1659, 1620 cm^{-1}. MS (FAB): m/z 377 $(M+H)^+$. *Anal.* ($C_{22}H_{20}N_2O_4 \cdot 0.2H_2O$) C, H, N.

*5.1.6.13. (E)-3-(N-Cyclohexyl-2-carbamoylvinyl)-4,5-dimethylpyrrole-2-carboxylic acid (**5m**).* 82% yield; m.p. 179–80°C (dec.) (MeOH–*i*-Pr_2O–hexane). ^{1}H NMR (DMSO-d_6) δ: 12.33 (br s, 1H), 11.46 (br s, 1H), 8.06 (d, $J = 16.4$ Hz, 1H), 7.81 (d, 1H), 6.33 (d, $J = 16.4$ Hz, 1H), 3.60 (m, 1H), 2.11 (s, 3H), 2.04 (s, 3H), 1.78–1.06 (m, 10H). IR (Nujol): 3287, 1645, 1593 cm^{-1}. MS (FAB): m/z 291 $(M+H)^+$. *Anal.* ($C_{16}H_{22}N_2O_3 \cdot 1.5CH_3OH$) C, H, N.

*5.1.6.14. (E)-3-(N-Benzyl-2-carbamoylvinyl)-4,5-dimethylpyrrole-2-carboxylic acid (**5n**).* 71% yield: m.p. 195–6°C (dec.) (MeOH–*i*-Pr_2O–hexane). ^{1}H NMR (DMSO-d_6) δ: 12.37 (br s, 1H), 11.49 (br s, 1H), 8.45 (t, 1H), 8.14 (d, $J = 16$ Hz, 1H), 7.33–7.2 (m, 5H), 6.41 (d, $J = 16$ Hz, 1H), 4.35 (d, 2H), 2.11 (s, 3H), 2.04 (s, 3H). IR (Nujol): 3258, 1647, 1601 cm^{-1}. MS (FAB): m/z 299 $(M + H)^+$. *Anal.* ($C_{17}H_{18}N_2O_3$ 1.2 CH_3OH) C, H, N.

*5.1.6.15. (E)-3-[N-(4-Dimethylaminophenyl)-2-carbamoylvinyl]-4,5-dimethylpyrrole-2-carboxylic acid sodium salt (**5o**).* 90% yield; m.p. 217°C (dec.) (H_2O). ^{1}H NMR (DMSO-d_6) δ: 10.45 (s, 1H), 9.48 (s, 1H), 8.69 (d, $J = 16.2$ Hz, 1H), 7.49 (d, 2H), 6.68 (d, 2H), 6.27 (d, $J = 16.2$ Hz, 1H), 2.82 (s, 6H), 2.06 (s, 3H), 2.05 (s, 3H). IR (Nujol): 3258, 1647, 1603 cm^{-1}. MS (FAB): m/z 350 $(M + H)^+$. *Anal.* ($C_{18}H_{20}N_3O_3Na \cdot 3H_2O$) C, H, N.

*5.1.6.16. (E)-3-[N-(4-Aminophenyl)-2-carbamoylvinyl]-4,5-dimethylpyrrole-2-carboxylic acid sodium salt (**5p**).* 60% yield; m.p. >300°C (dec.) (H_2O). ^{1}H NMR (DMSO-d_6) δ: 10.44 (s, 1H), 9.36 (s, 1H), 8.66 (d, $J = 16.5$ Hz, 1H), 7.30 (m, 2H), 6.48 (m, 2H), 6.26 (d, $J = 16.5$ Hz, 1H), 4.77 (s, 2H), 2.06 (s, 3H), 2.04 (s, 3H). IR (Nujol): 3288, 3190, 1670, 1612 cm^{-1}. MS (FAB): m/z 322 $(M + H)^+$. *Anal.* ($C_{16}H_{16}N_3O_3Na \cdot 3.5H_2O$) C, H, N.

*5.1.6.17. (E)-3-[N-(3-Aminophenyl)-2-carbamoylvinyl]-4,5-dimethylpyrrole-2-carboxylic acid sodium salt (**5q**).* 82% yield; m.p. 215–20°C (dec.) (H_2O). ^{1}H NMR (DMSO-d_6) δ: 10.48 (s, 1H), 9.44 (s, 1H), 8.70 (d, $J = 16.4$ Hz, 1H), 6.98 (t, 1H), 6.87 (t, 1H), 6.77 (m, 1H), 6.31 (d, $J = 16.4$ Hz, 1H), 6.19 (m, 1H), 4.98 (s, 2H), 2.07 (s, 3H), 2.05 (s, 3H). IR (Nujol): 3335, 3196, 1618 cm^{-1}. MS (FAB): m/z 322 $(M + H)^+$. *Anal.* ($C_{16}H_{16}N_3O_3Na \cdot 4.5H_2O$) C, H, N.

*5.1.6.18. (E)-3-[N-(4-Ureidophenyl)-2-carbamoylvinyl]-4,5-dimethylpyrrole-2-carboxylic acid (**5r**).* 72% yield; m.p. 174–8°C (dec.) (MeOH). ^{1}H NMR (DMSO-d_6) δ: 12.43 (s, 1H), 11.54 (s, 1H), 9.88 (s, 1H), 8.41 (s, 1H), 8.23 (d, $J = 16.4$ Hz, 1H), 7.52 (d, 2H), 7.30 (d, 2H), 6.53 (d, $J = 16.4$ Hz, 1H), 5.76 (s, 2H), 2.14 (s, 3H), 2.11 (s, 3H). IR (Nujol): 3500, 3344, 3252, 1655, 1619 cm^{-1}. MS (FAB): m/z 343 $(M + H)^+$. *Anal.* ($C_{17}H_{18}N_4O_4 \cdot 1H_2O$) C, H, N.

*5.1.7. Ethyl N-(3-cyanobutyl)glycinate (**10**)*

A total of 140 mg (1 mmol) of ethyl glycinate hydrochloride and 101 mg (1 mmol) of triethylamine were added to a solution of 2-methyl-3-oxobutyronitrile (**9**) [26] (97 mg, 1 mmol) in EtOH (2 ml). The mixture was stirred at r.t. for 16 h. The solvent was evaporated to dryness and the residue treated with water and Et_2O. The layers were separated and the organic phase was washed with water, dried (Na_2SO_4) and evaporated to give 170 mg of a residue which was purified by crystallization. 71% yield; m.p. 101–2°C ($CHCl_3$–light pet.). ^{1}H NMR ($CDCl_3$, 60 MHz) δ: 4.6 (br s, 1H), 4.23 (q, 2H), 3.90 (d, 2H), 2.06 (s, 3H), 1.69 (s, 3H), 1.25 (t, 3H). IR (Nujol): 3378, 2176, 1739, 1601 cm^{-1}. MS (EI): m/z 182 (M^+).

*5.1.8. Ethyl 3-amino-4,5-dimethylpyrrole-2-carboxylate (**11**)*

A 364 mg sample of **10** (2 mmol) was added to a solution of 2.4 mmol of EtONa in 5 ml of absolute EtOH. The mixture was heated at 70°C for 6 h, then cooled to r.t. The solvent was evaporated under reduced pressure and the residue treated with water and EtOAc. The water phase was extracted twice with EtOAc and the organic phases were combined, washed with water, dried (Na_2SO_4), and evaporated to dryness. The residue was further purified by filtration on silica gel (cyclohexane–EtOAc 1:1) and crystallization. 69% yield; m.p. 111–2°C (*i*-Pr_2O). ^{1}H NMR (DMSO-d_6) δ: 10.10 (s, 1H), 4.75 (s, 2H), 4.12 (q, 2H), 2.00 (s, 3H), 1.72 (s, 3H), 1.22 (t, 3H). IR (Nujol): 3449, 3285, 1659, 1605 cm^{-1}. MS (EI): m/z 182 (M^+).

*5.1.9. Ethyl (E)-3-(cinnamoylamino)-4,5-dimethylpyrrole-2-carboxylate (**12**)*

A mixture of cinnamic acid (148 mg, 1 mmol), 2,2′-dipyridyl disulfide (397 mg, 1.8 mmol) and $(Ph)_3P$ (472 mg, 1.8 mmol) were dissolved in 25 ml of anhydrous THF. The solution was stirred under N_2 at r.t. for 3 h. Then 200 mg (1.1 mmol) of **11** were added and the stirring continued for 14 h. The white solid which appeared was filtered and washed with diethyl ether and further purified by crystallization. 50% yield; m.p. 250–1°C (THF–hexane). ^{1}H NMR (DMSO-d_6) δ: 11.3 (s, 1H), 9.30 (s, 1H), 7.58–7.40 (m, 6H), 6.9 (d, 1H), 4.14 (q, 2H), 2.13 (s, 3H), 1.77 (s, 3H), 1.21 (t, 3H). IR (Nujol): 3290, 1674, 1659 cm^{-1}. MS (EI): m/z 312 (M^+).

*5.1.10. (E)-3-(cinnamoylamino)-4,5-dimethylpyrrole-2-carboxylic acid (**5s**)*

Hydrolysis of ethyl (*E*)-3-(cinnamoylamino)-4,5-dimethylpyrrole-2-carboxylate (**12**) was performed according to the general procedure reported for **4a**–**o** using EtOH as solvent with a reaction time of 2 h. 84% yield; m.p. 163–5°C (dec.) (THF–hexane). ^{1}H NMR (DMSO-d_6) δ: 12.0 (s, 1H), 11.2 (s, 1H), 9.27 (s, 1H), 7.60 (d, 2H), 7.48 (d, $J = 15.6$ Hz, 1H), 7.42 (t, 2H), 7.38 (m, 1H), 6.93 (d, $J = 15.6$ Hz, 1H), 2.11 (s, 3H), 1.76 (s, 3H). IR (Nujol): 3277, 1654, 1628 cm^{-1}. MS (FAB): m/z 285 $(M + H)^+$. *Anal.* ($C_{16}H_{16}N_2O_3 \cdot 0.25H_2O \cdot 0.5THF$) C, H, N.

*5.1.11. 3-Isoquinolylmethyl-triphenylphosphonium bromide (**13**)*

A total of 222 mg (1 mmol) of 3-(bromomethyl)-isoquinoline [31] was dissolved in 3 ml of anhydrous THF,

to which 262 mg (1 mmol) of triphenylphosphine were added. The mixture was refluxed for 24 h. The solid was filtered, washed well with THF and dried. 91% yield; m.p. 205°C. ^{1}H NMR (DMSO-d_6) δ: 9.10 (s, 1H), 8.04 (dd, 1H), 7.85–7.60 (m, 19H), 5.55 (d, 2H). IR (Nujol): 1626 cm^{-1}.

*5.1.12. Methyl (E)-3-[(3-isoquinolyl)-2-vinyl]-4,5-dimethylpyrrole-2-carboxylate (**14**)*

A 181 mg (1 mmol) sample of 3-formylpyrrole **1** was suspended in toluene (16 ml); DBU (0.22 ml, 1.48 mmol) and 726 mg (1.5 mmol) of the phosphonium bromide **13** were added, after which the mixture was stirred under reflux for 3 h. The solvent was evaporated under reduced pressure and the residue was purified by flash chromatography on silica gel (cyclohexane–EtOAc 1:1). 70% yield; m.p. 246°C (MeOH). ^{1}H NMR (DMSO-d_6) δ: 11.51 (s, 1H), 9.28 (s, 1H), 8.40 (d, $J = 16.4$ Hz, 1H), 8.07 (d, 1H), 7.89 (d, 1H), 7.76 (s, 1H), 7.72 (dt, 1H), 7.58 (dt, 1H), 7.13 (d, $J = 16.4$ Hz, 1H), 3.78 (s, 3H), 2.18 (s, 3H), 2.16 (s, 3H). IR (Nujol): 3288, 1674, 1666 cm^{-1}. MS (FAB): m/z 307 $(M+H)^+$.

*5.1.13. (E)-3-(3-isoquinolyl-2-vinyl)-4,5-dimethyl-pyrrole-2-carboxylic acid lithium salt (**5t**)*

A total of 307 mg (1 mmol) of **14** was suspended in 1 ml of aqueous pyridine (20% water); $LiOH \cdot H_2O$ (167 mg, 4 mmol) was added and the resulting mixture stirred under reflux for 6 h. The solvent was evaporated to dryness and the residue treated with water, giving a white solid that was filtered and washed with water and EtOAc, thus providing the pure product as lithium salt. 63% yield; m.p. 250°C (dec.). ^{1}H NMR (DMSO-d_6) δ: 10.46 (s, 1H), 9.21 (s, 1H), 8.85 (d, $J = 16.8$ Hz, 1H), 8.01 (d, 1H), 7.83 (d, 1H), 7.66 (m, 2H), 7.50 (m, 1H), 6.91 (d, $J = 16.8$ Hz, 1H), 2.15 (s, 3H), 2.10 (s, 3H). IR (Nujol): 3414, 1622 cm^{-1}. MS (FAB): m/z 299 $(M+H)^+$. *Anal.* ($C_{18}H_{15}LiN_2O_2 \cdot 2.5H_2O$) C, H, N.

5.2. Pharmacology

The test compounds were dissolved at a 5 mM concentration in 100% DMSO and tested in duplicate at seven concentrations (0.1–100 μM) in the [^{3}H]glycine displacement experiments. A reference compound was always included as a control.

Affinity for the glycine-binding site was measured by inhibition of the binding of [^{3}H]glycine to crude synaptic membranes prepared from adult rat cerebral cortex as described by Kishimoto et al. [32]. Incubation (20 min, 4°C) was carried out in 50 mM Tris/citrate (pH 7.10) using 20 nM [^{3}H]glycine. Data from displacement experiments, performed to determine the inhibition constants (K_i) of displacer ligands, were analyzed using the nonlinear curve fitting program LIGAND [33]. K_i values were measured from at least six-point inhibition curves and are the geometric mean of at least three different experiments. The standard error associated with the mean was < 0.05.

Acknowledgements

The authors of the group of the University of Urbino gratefully acknowledge financial support from Glaxo Wellcome; they also wish to thank Francesco Palazzi for technical assistance, Dr C. Marchioro and her colleagues (GW, Verona) for providing part of spectroscopic characterization, Dr M. Mugnaini (GW, Verona) for binding assays and Dr M. Hamdan for providing part of spectrometric analysis.

References

[1] D.W. Choi, S.M. Rothman, The role of glutamate neurotoxicity in hypoxic–ischemic neuronal death, Annu. Rev. Neurosci. 13 (1990) 171.
[2] D.W. Choi, Cerebral hypoxia: some new approaches and unanswered questions, J. Neurosci. 10 (1990) 2493.
[3] B. Meldrum, Protection against ischaemic neuronal damage by drugs acting on excitatory neurotransmission, Cerebrovasc. Brain Metab. Rev. 2 (1990) 27.
[4] J. McCulloch, Excitatory amino acid antagonists and their potential for the treatment of ischaemic brain damage in man, Br. J. Clin. Pharmacol. 34 (1992) 106.
[5] C.W. Cotman, A. Foster, T. Lanthorn, An overview of glutamate as a neurotransmitter, Adv. Biochem. Psycopharmacol. 27 (1981) 1.
[6] J.C. Watkins, R.H. Evans, Excitatory amino acid transmitters, Annu. Rev. Pharmacol. Toxicol. 21 (1981) 165.
[7] J.W. Olney, Excitotoxic amino acids and neuropsychiatric disorders, Annu. Rev. Pharmacol. Toxicol. 30 (1990) 47.
[8] S.M. Rothman, J.W. Olney, Excitotoxicity and the NMDA receptor, Trends NeuroSci. 10 (1987) 299.
[9] B. Meldrum, J. Garthwaite, Excitatory amino acid neurotoxicity and neurodegenerative disease, Trends Pharmacol. Sci. 11 (1990) 379.
[10] S.A. Lipton, P.A. Rosenberg, Excitatory amino acids as a final common pathway for neurologic disorders, New Engl. J. Med. 330 (1994) 613.
[11] J.W. Johnson, P. Ascher, Glycine potentiates the NMDA response in cultured mouse brain neurons, Nature 325 (1987) 529.
[12] N.W. Kleckner, R. Dingledine, Requirement for glycine in activation of NMDA-receptors expressed in *Xenopus* oocytes, Science 241 (1988) 835.
[13] A.M. Thomson, Glycine is a coagonist at the NMDA receptor/channel complex, Progr. Neurobiol. (Oxford) 35 (1990) 53.
[14] J.E. Huettner, Competitive antagonism of glycine at the *N*-methyl-D-aspartate (NMDA) receptor, Biochem. Pharmacol 41 (1991) 9.
[15] P.D. Leeson, L.L. Iversen, The glycine site on the NMDA receptor: structure–activity relationships and therapeutic potential, J. Med. Chem. 37 (1994) 4053.
[16] L.L. Iversen, J.A. Kemp, Non competitive NMDA antagonists as drugs, in: G.L. Collingridge, J.C. Watkins (Eds.), The NMDA Receptor, 2nd ed., IRL Press, Oxford, UK, 1994, pp. 469–486.
[17] J.J. Kulagowski, P.D. Leeson, Glycine-site NMDA receptor antagonists, Expert Opin. Ther. Pat. 5 (1995) 1061.

[18] J.J. Kulagowski, Glycine-site NMDA receptor antagonists: an update, Expert Opin. Ther. Pat. 6 (1996) 1069.
[19] R. Di Fabio, G. Gaviraghi, A. Reggiani, Strychnine-insensitive glycine binding site and the NMDA receptor, Chim. Ind. 78 (1996) 283.
[20] R. Di Fabio, A.M. Capelli, N. Conti, A. Cugola, D. Donati, A. Feriani, P. Gastaldi, G. Gaviraghi, C.T. Hewkin, F. Micheli, A. Missio, M. Mugnaini, A. Pecunioso, A.M. Quaglia, E. Ratti, L. Rossi, G. Tedesco, D.G. Trist, A. Reggiani, Substituted indole-2-carboxylates as in vivo potent antagonists acting at the strychnine-insensitive glycine binding site, J. Med. Chem. 40 (1997) 841.
[21] C. Balsamini, A. Bedini, G. Diamantini, G. Spadoni, A. Tontini, G. Tarzia, R. Di Fabio, A. Feriani, A. Reggiani, G. Tedesco, R. Valigi, (*E*)-3-(2-(*N*-Phenylcarbamoyl)vinyl)pyrrole-2-carboxylic acid derivatives: a novel class of glycine site antagonists, J. Med. Chem. 41 (1998) 808.
[22] Y. Connolly Martin, Topliss schemes for analog synthesis, in: Quantitative Drug Design. A Critical Introduction, Marcel Dekker, New York, 1978, pp. 255–258.
[23] B. Skagerberg, D. Bonelli, S. Clementi, G. Cruciani, C. Ebert, Principal properties for aromatic substituents: a multivariate approach for design in QSAR, Quant. Struct. Act. Relat. 8 (1989) 32.
[24] P.J. Crook, A.H. Jackson, G.W. Kenner, Synthese von α-Oxymesoporphyrin—IX, Liebigs Ann. Chem. 748 (1971) 26.
[25] C.T. Seto, G.M. Whitesides, Molecular self-assembly through hydrogen bonding: supramolecular aggregates based on the cyanuric acid–melamine lattice, J. Am. Chem. Soc. 115 (1993) 905.
[26] J.K. Rasmussen, A. Hassner, The electrophilic addition of chlorosulfonyl isocyanate to ketones: a general synthesis of β-keto-nitriles, Synthesis (1973) 682.
[27] A. Cugola, G. Gaviraghi, F. Micheli, Preparation of indolecarboxylate derivatives as antagonists of excitatory amino acids, WO 94/20465 to Glaxo S.p.A., C.A. 121 (1994) 300763y.
[28] A. Cugola, G. Gaviraghi, S. Giacobbe, Indole-2-carboxylic acid derivatives as NMDA receptor antagonists, EPO 568136A1 to Glaxo S.p.A., C.A. 120 (1994) 217702m.
[29] A. Cugola, Preparation of 2-indolecarboxylic acid NMDA receptor antagonists, WO 95/07887 to Glaxo S.p.A., C.A. 123 (1995) 32957 A.
[30] N. Conti, R. Di Fabio, E. De Magistris, A. Feriani, Indole derivatives as excitatory amino acid (EAA) antagonists, WO 96/27588 to Glaxo S.p.A., C.A. 125 (1996) 275646k.
[31] H. Erlenmeyer, H. Baumann, E. Sorkin, Comparison of the structures of thiazole and isoquinoline, Helv. Chim. Acta. 31 (1978) 1948.
[32] H. Kishimoto, J.R. Simon, M.H. Aprison, Determination of the equilibrium dissociation constants and number of glycine binding sites in several areas of the rat central nervous system using a sodium-independent system, J. Neurochem. 37 (1981) 1015.
[33] P.J. Munson, D. Rodbard, LIGAND: a versatile computerized approach for characterization of ligand-binding systems, Anal. Biochem. 107 (1980) 220.