Cycloalkyl Indole-2-Carboxylates as Useful Tools for Mapping the "North-Eastern" Region of the Glycine Binding Site Associated with the NMDA Receptor

Fabrizio Micheli*, Romano Di Fabio*, Anna M. Capelli, Alfredo Cugola, Ornella Curcuruto, Aldo Feriani, Paola Gastaldi, Giovanni Gaviraghi, Carla Marchioro, Alessandra Orlandi, Alfonso Pozzan, Anna M. Quaglia, Angelo Reggiani, Frank van Amsterdam

Glaxo Wellcome S.p.A., Medicines Research Centre, Via Fleming 4, 37100 Verona, Italy

Key Words: *Neuroprotective agents; neurotransmitters; molecular modelling/mechanics*

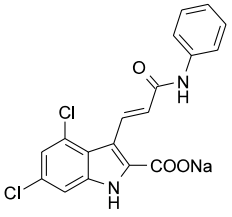
Summary

A novel series of indole-2-carboxylate analogues of GV150526 (**1**) in which the terminal phenyl ring belonging to the side chain present in the position C-3 has been replaced with a bridged cycloalkyl group was synthesized and evaluated for its pharmacological profile. Modelling studies on this class of novel glycine antagonist allowed us to identify an asymmetric lipophilic pocket present in the "North-Eastern" region of the pharmacophoric model of the glycine binding site associated to the NMDA receptor. Among the derivatives prepared, 3-[2-(1-adamantylaminocarbonyl)ethenyl]-4,6-dichloroindole-2-carboxylic acid **6b** and 3-[2-(norbornylaminocarbonyl)ethenyl]-4,6-dichloroindole-2-carboxylic acid **6l** were found to be antagonists acting at the strychnine-insensitive glycine binding site, showing nanomolar affinity for the glycine binding site (K_i = 63 and 19 nM, respectively), coupled with high glutamate receptor selectivity (IC_{50} >10^{-5} M at the NMDA, AMPA, KA binding sites) and high *in vivo* potency after systemic administration by inhibition of convulsion induced by NMDA in mice.

Introduction

Glutamate is the most abundant excitatory neurotransmitter present in the CNS. It is now widely recognized that in pathological conditions[1–4] such as stroke, an abnormal amount of glutamate is released into synaptic clefts. This is due to an increase of release of glutamate presynaptically and to the block of the re-uptake processes. This leads to the over-stimulation of *N*-methyl-D-aspartate (NMDA) receptor raising significantly the intracellular level of Ca^{++} in the post-synaptic neurons, causing the activation of several neurotoxic cascades responsible for irreversible neuronal damage [5].

Based on the considerable body of experience gained in animal models of stroke, therapeutic benefit can be obtained by modulating the conductance of Ca^{++} through the ion channel associated with the NMDA receptor[6] using competitive and non competitive NMDA antagonists[7,8]. Recently, the glycine binding site has become one of the most attractive targets for neuroprotection after stroke, in view of the role of glycine as a co-agonist of glutamate[9–12] in the activation of this ionotropic receptor complex and the favorable therapeutic index seen for glycine-antagonists[13–35].



1

Figure 1 **GV150526**

The indole-2-carboxylate derivative GV150526 **1** (Figure 1) was identified by GlaxoWellcome[22–27] as a potent and selective glycine antagonist endowed with nanomolar affinity *in vitro* and excellent *in vivo* activity in the MCAo model in rats both pre and post-ischemia. The present paper deals with the synthesis and the pharmacological characterization of a novel series of analogues of compound **1**, in which the aromatic phenylamido moiety was replaced with various cycloalkyl derivatives. This new series of indole-2-carboxylates showed nanomolar affinity for the glycine binding site coupled with high receptor selectivity; moreover, these molecules are endowed with a high *in vivo* potency in the NMDA-induced convulsion model in mice, after systemic administration.

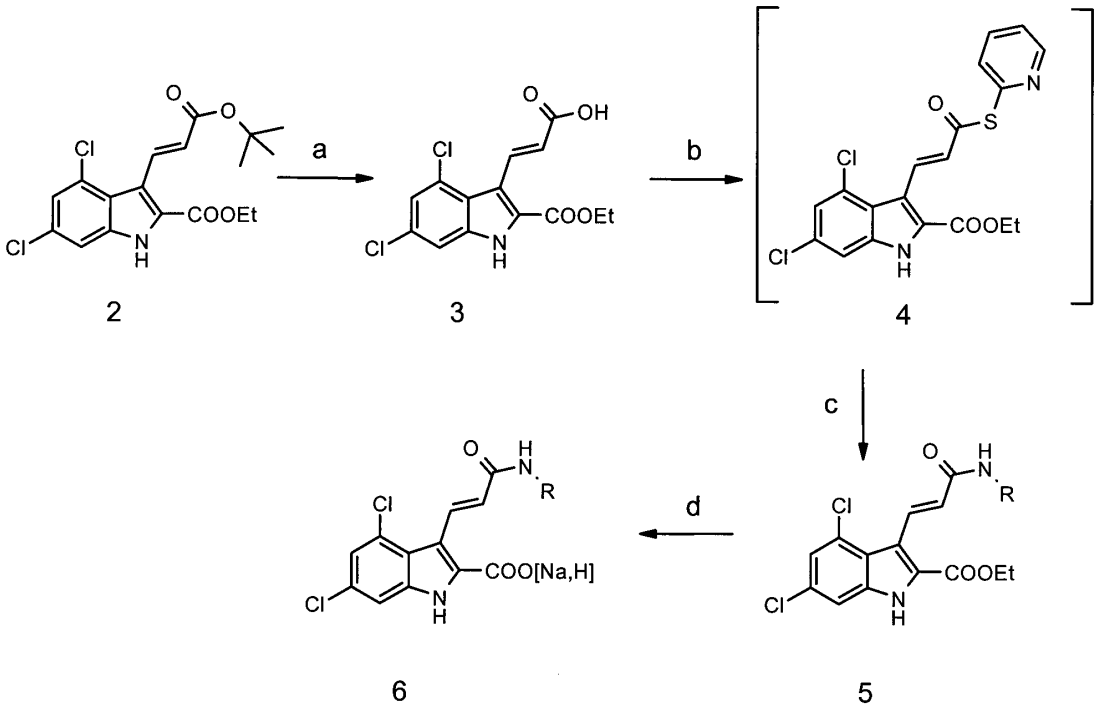
Results and Discussion

Synthesis

Compounds of general structure **6** have been prepared (Scheme 1) starting from the indole-2-carboxylate **2** as previously reported[22]. Chemoselective hydrolysis of the *tert*-butyl ester protecting group in quantitative yield was obtained using formic acid at room temperature. The key intermediate **3** was transformed into the corresponding alkylamido derivatives by activation of the carboxyl group *via* the formation of the corresponding 2-pyridyl thioester **4** using the well known "oxidation-reduction" procedure[36] in the presence of 2,2′-dipyridyldisulphide and triphenylphosphine. The derivative **4** was found to be stable enough to be purified by standard chromatographic methods and it was reacted with the desired alkyl amines. Alternatively, a "one-pot" procedure was used: the thiopyridyl ester was formed "in situ" and treated with the desired amines. Amides **5** were smoothly obtained in high

  0365-6233/99/0303/0073 $17.50 +.50/0

Scheme 1



a. formic acid; b. 2-Aldrithiol, PPh_3, THF or DMF; c. RNH_2, DMF;
d NaOH or LiOH followed by acid quenching

yield following both the procedures. Finally, the basic hydrolysis of the ethyl ester present in position C-2 gave the target compounds **6** in quantitative yields.

Biology

The biological evaluation of the new chemical entities (NCE) was performed using the following screening sequence previously described[22]: a) binding assay[1] to evaluate the affinity for the glycine site ; b) selectivity for the glutamate receptors (NMDA/AMPA/KA); c) *in vivo* anticonvulsant activity in the NMDA induced convulsions model in mice (*iv* and *po*)[2].

Analysis of Experimental Findings

After the identification of GV150526 and the exploration of the aromatic phenylamidic moiety present in the terminal position of the C-3 side chain, our objective was to acquire further SAR elements regarding the "North-Eastern" region of the receptor, enhnacing the precision of the 3D pharmacophoric model of the glycine binding site.

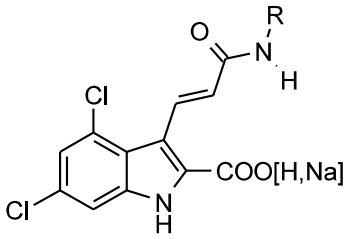
In the paper[22] dealing with the discovery process of GV150526 **1,** it was proved how the presence of the amidic carbonyl group belonging to the α,β-unsaturated C-3 side chain, in view of the suitable stereoelectronical features, was crucial to maximize the affinity at the glycine binding site of this series of ligands. Therefore, we decided to maintain this key "pharmacophoric point" and to map in detail the so-called "size-limited hydrophilic pocket" in order to gather information both in terms of allowed space to the terminal substituents on the C-3 side chain and on the recognition role of the aromatic moiety. This exploration should allow us to understand the structural requirements useful to design novel series of glycine antagonists.

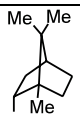
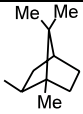
To perform this task, the terminal aromatic ring was replaced initially with the corresponding cyclohexyl derivative. As reported in Table 1, compound **6a** showed only a slight decrease in affinity at the glycine binding site associated with the NMDA-receptor with respect to compound **1** (K_i = 10 nM *vs.* K_i = 3 nM, respectively). In view of this result, it was realized that the "North-Eastern" region of the pharmacophore was worth being further analyzed. A first series of symmetric derivatives (**6b**–**6f**) bearing alkyl substituents with increasing steric bulk with respect to cyclohexyl derivative **6a** was synthesized and evaluated in terms of *in vitro* affinity at the glycine binding site. The results obtained are shown in Table 1. All these compounds were endowed with a reduced affinity for the glycine binding site with respect to both compounds **1** and **6a**, confirming the limited space available

[1] K_i values for the products **6a–o** were measured from at least six-point inhibition curves and they are the geometric means of at least three independent experiments. The standard error of the mean was less than 0.05 [22].

[2] The research complied with national legislation and with company policy on the Care and Use of Animals and with related codes of practice.

Table 1



No.	R	K_i (nM)[22]	No.	R	K_i (nM)[22]
1	**GV150526**	**3**	**6g**		**1000**
6a		**10**	**6h**		**25**
6b		**63**	**6i**		**10**
6c		**200**	**6l**		**20**
6d		**25**	**6m**		**40**
6e		**31**	**6n**		**200**
6f		**31**	**6o**		**186**

within this region of the receptor. In particular, both the adamantyl derivative **6b** and its homologated derivative **6c** showed a significant decrease in terms of affinity (K_i = 63 and 200 nM, respectively *vs.* 10 nM for **6a**). Conversely, a slight reduction of the steric bulk (from adamantyl **6b** to noradamantyl **6d**) caused a partial improvement of the affinity (63 *vs*. 25 nM, respectively). Finally the same affinity at the glycine binding site was observed for derivatives **6e** and **6f** (K_i = 31 nM): in view of this last result, the terminal alkyl substituents should lie in the same "allowed area" of the receptor.

After this preliminary exploration, a second series of unsymmetrical cycloalkyl substituents (**6g**–**6o**) were carefully chosen and synthesized to map this region of the receptor in more detail. The affinity for the glycine binding site of this subclass of derivatives is reported in Table 1. In particular, it is worth marking as the norbornyl derivative **6i**, despite the higher steric bulk, showed an affinity comparable to the cyclohexyl derivative **6a**. This result was explained by hypothesizing the presence of an asymmetric pocket of limited size able to accept lipophilic substituents with a defined molecular geometry[3)].

Computational Chemistry Studies

Based on the results described above, the different compounds prepared were used as tools to map the "North-Eastern" region of the pharmacophore model of the glycine binding site. Modelling studies were performed using both

[3)] It is worth noting that the introduction of a basic heteroatom into the the terminal lipophilic moiety seemed to be forbidden (derivative **6g**, K_i = 1 μM).

Sybyl (TRIPOS Ass.) and Catalyst[37] software (the methods used for these analyses are described in footnote[4] and[5], respectively).

"Excluded Volumes" Analysis

Indole derivatives **6a–o** were arbitrarily divided into two groups: the so called "higher affinity compounds" ($K_i \leq$ 10 nM) and the so called "lower affinity molecules" ($K_i \geq$ 100 nM).

As depicted in Figure 2A, subtracting the combined volumes occupied by the "higher affinity compounds" from the space occupied by the "lower affinity molecules" a partially forbidden region of the receptor was identified, based on the assumption that compound **1** and these cycloalkyl indole derivatives bind in the same way to the glycine site of the NMDA receptor. This forbidden region should be located nearby the *ortho* position of the aromatic ring of compound **1,** confirming what has been previously observed[6]. Conversely, the *para* position should be able to accept substituents with a limited steric bulk. Moreover, an additional size limited region located behind this phenyl ring has been identified. Finally, as can be seen in Figure 2 (A and B), this receptor pocket does not seem to possess a spherically shaped profile. As depicted in Figure 2B, compound **6i** (both the lowest energy conformers are shown), despite its increased bulk with respect to **1** or **6a**, fits perfectly into this pocket. This new receptor model could allow us to explain the reduced affinity observed for the less active derivatives with respect to the "higher affinity compounds".

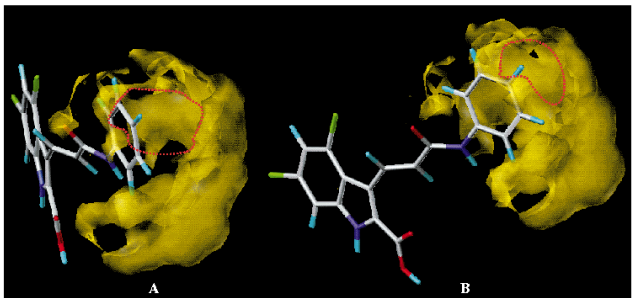


Figure 2. "Excluded Volumes" Analysis. (A) GV 150526 **1** is positioned within the unsymmetrical binding region identified . From this figure, it is evident that the *ortho* and *meta* positions of the terminal phenyl ring are located nearby a disallowed region of the receptor (yellow area). Conversely, the *para* position seems to be located in a region that could tolerate some substituents of limited steric bulk within the same receptor (red dotted area). (B) Both the lowest energy conformers of compound **6i** (atom-type colored structure E = 39.9 kcal/mol, magenta colored structure E = 40.7 kcal/mol) are represented within the unsymmetrical binding region identified. Despite the increased steric bulk with respect to compound **1**, this compound perfectly fits the allowed area in the described pocket.

Unbiased Pharmacophoric Evaluation

Based on the assumptions described above, the molecules shown in Table 1 were used to generate a 3D chemical function based hypothesis using the Catalyst software[37] . The aim of this approach was to further validate the pharmacophore model of the glycine binding site previously proposed[22] via an unbiased method for pharmacophore generation. A detailed description of the experimental procedure employed is reported in footnote [6].

Table 3: Catalyst's three best and null hypotheses for the indole-2-carboxylate analogues activity.

Hypo	HBA	HBD	LIP	NegIoniz	COST	RMS	*r*
1	1	1	2	1	32.85	1.30	0.94
2	1	1	2	1	50.36	2.10	0.70
3	1	1	2	1	53.22	2.32	0.61
null	–	–	–	–	67.10	2.95.	0.00

[4] Computational methods: Sybyl (TRIPOS Ass.): The pharmacophore conformer of compound 1 (GV150526) was used as reference structure of this study regarding the values of its side chain rotable bonds, which define the orientation of the side chain carbonyl group. As we aimed at keeping fixed the orientation of this primary pharmacophore feature, we forced the corresponding rotatable bonds of compounds **6a–n** (Table 1) to assume almost the same values of those of compound **1**. At the same time, these "artificial" conformations were relaxed by minimization to allow them to reach their closer local minimum. Then, the amidic rotable bond (NH-Cycloalkyl) of these structures were submitted to Systematic Search protocol implemented within Sybyl using 10 deg. as resolution. In addition, an energy window of 25 kcal/mol was applied so as to discard very high energy structures. All the conformations obtained were minimized using Powell algorithm, filtered for duplicates and superimposed using as reference points the primary pharmacophore features previously described [22]. After that, the volumes occupied by the conformers endowed with high affinity ($pK_i > 8$) and lower affinity compounds ($pK_i < 7$) were calculated and combined using the algorithms implemented within Sybyl. As far as the derivatives endowed with 10 nM $\leq K_i \leq$ 100 nM (**6b**, **6d**, **6e**, **6f**, and **6h**) are concerned, the envelop of their minimized conformations, being spherically shaped, did not add any further information in terms of geometry and allowed space in the pocket. Actually, the number of conformations which superimpose with the not allowed area obtained is comparable to that of the conformations which are completely enclosed in the allowed pocket. This fact should also explain the intermediate affinity of these molecules.

[5] Computational methods: Catalyst[37]: The molecules for which stereochemistry is known (**1**, **6a**, **6b**, **6c**, **6d**, **6e**, **6f**, **6h**, **6m**, and **6n**) were imported in Catalyst. The software then automatically generated conformational models[38] for each compound using the Poling Algorithm[39]. The models, containing a representative set of conformers covering a 20 kcal/mol energy range above the estimated global minimum, were submitted to Catalyst's hypothesis generation. The chemical functions used in this generation step included hydrogen bond donor, hydrogen bond acceptor, hydrophobic and negative ionizable. In generating a common features hypothesis, Catalyst attempts to find all the possible combinations of chemical functions that fit on all compounds and that share the same spatial position. The statistical relevance of various hypotheses so obtained is assessed on the basis of their cost relative to the null hypothesis and their correlation coefficients *r*. The three lowest cost hypotheses obtained are listed in Table 3. The total fixed cost is 28.98 and the cost of the null hypothesis is 67.10. The cost range over the generated hypothesis is 20.37, while the cost range between best and null hypothesis is 34.25. As expected, the small cost range and the small difference between the fixed cost and the best hypothesis suggest that molecules in the model are fairly rigid, and share a high degree of structural similarity. In order to test for chance correlation, 19 other hypotheses were generated by scrambling, in a random way, the experimental activities in the training set and then regenerating the set of hypotheses. None of the scrambled hypotheses had a lower cost than our best hypothesis, indicating that there is a 95% chance this hypothesis represents a true correlation in the data.

[6] The introduction of an isopropyl group in the *ortho* position on the terminal phenyl of compound **1** caused a significant reduction of the affinity as can be observed in Table 3 in ref.[22].

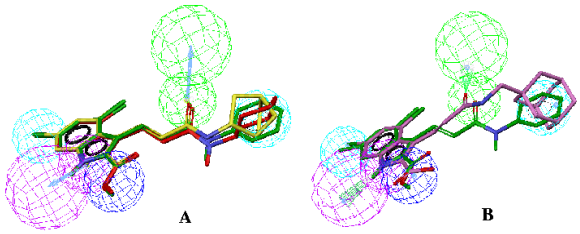


Figure 3. A) Superimposition of GV 150526A (**1**) with compounds **6a** and **6i** (red and yellow, respectively) As described in the main text, there is a good superimposition of the pharmacophoric points of these molecules with compound **1**. B) Superimposition of GV 150526A (**1**) with compound **6c** (pink). As described in the main text, it is clear from this figure that at least one pharmacophoric point is missed with respect to compound **1**; in the case represented here, when the adamantyl substituent of compound **6c** is forced to superimpose with the aromatic moiety of compound **1**, the crucial interaction with the carbonyl group is lost, causing a detrimental effect in terms of affinity for the glycine binding site.

As shown in Figure 3A, the most potent molecules (**1, 6a, 6i**) fit very well into the five pharmacophoric points of the receptor model hypothesis[22]. The terminal hydrophobic group of the side chain in the position C-3 and the chlorine atom in position C-6 overlap with two hydrophobic features; the indolic N-H maps with the hydrogen bond donor; the COOH with the negative ionizable, and the C=O group present within the C-3 side chain with the hydrogen bond acceptor feature. Conversely, a poorer fit is observed with the less potent molecules (**6c**, **6n**). In particular, as depicted in Figure 3B, when the compound **6c** is forced to occupy only the lipophilic "allowed" region of the receptor, the orientation of the carbonyl group of its side chain changes dramatically, leading to the disruption of a key hydrogen bond interaction; as a result, its affinity for the receptor is greatly reduced.

Racemic compounds (**6g**, **6i**, **6l**, **6o**) forced to fit this model show a better fitting for molecules possessing the (*R*) stereocenter with respect to those having the (*S*) one.

Figure 4 shows the correlation existing between the observed and estimated K_i values of the compounds listed in Table 1. As predicted by the model, the affinity of these compounds for the receptor decrease when increasing of the steric bulk of the cycloalkyl substituents in the C-3 side chain; compound **1,** however, is predicted by this model to be less

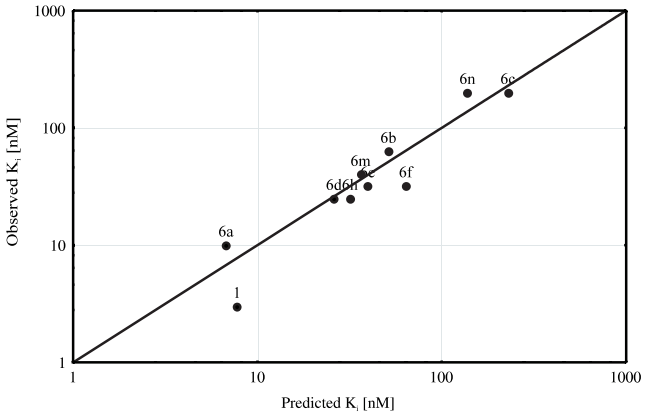


Figure 4. Correlation line (r = 0.94) displaying observed K_is vs. calculated values using the statistically most significative hypothesis [Hypothesis no. 1 described in Table 3] derived by the compounds used in the Catalyst model.

potent than was observed. The higher affinity of this derivative cannot be explained on the basis of the pharmacophoric features of this model only. Additional electronic features (not implemented in Catalyst) typical of a phenyl ring can be hypothesized. In particular the phenyl ring could be involved in π-stacking interactions and/or the presence of the phenylamidic moiety could modify considerably the stereoelectronic properties of the carbonyl group (H-bond acceptor). In conclusion, the results of this unbiased model do not only confirm the validity of the five points pharmacophoric model, but also shed new light on the role of the aromatic features of the C-3 side chain, explaining better the increase of pK_is obtained with the analogues of GV150526 **1** on introducing electron-donor substituents on the terminal phenyl ring[22]. Obviously, a further refinement of the pharmacophoric model described above will be possible once additional derivatives are made and characterized.

Biological Characterization

The most *in vitro* active compounds (K_i < 100 nM) were evaluated in terms of selectivity towards the glutamate receptors (NMDA/AMPA/KA). As observed for compound **1**[22–27], all the products tested were found to be highly selective (IC_{50} >10^{-5} M). Moreover, they were found to behave as competitive antagonists at the glycine binding site.

Finally, these compounds were tested for their ability to inhibit the convulsions caused by "in vivo" administration of NMDA[3], a surrogate for stroke models, starting from the basic assumption that NMDA receptor overactivation is the key event in neurodegeneration following cerebral ischemia. The ability of the new chemical entities to counteract NMDA-induced convulsions was used as the end point of the model. Some of the products described in Table 1 showed excellent ED_{50}s in inhibiting convulsions in mice. In particular, compound **6b** and **6l**, the 1-adamantyl and the norbornyl derivative, showed an outstanding anticonvulsant potency when tested in the range of doses between 0.01–3 mg/kg *iv* and 1–100 mg/kg *po* according to the general procedure described in ref. [8a]. The estimated ED_{50}s obtained by intravenous route were respectively 0.01 and 0.02 mg/kg (compared to 0.06 mg/kg for GV150526, **1**), while the ED_{50}s obtained with oral administration were 4.6 and 11 mg/kg respectively (compared to 6 mg/kg for GV150526, **1**).

Conclusions

A novel series of indole 2-carboxylates was explored with the aim of identifying potent and selective glycine antagonists both *in vitro* and *in vivo*. By replacing the terminal aromatic ring belonging to the α,β-unsaturated C-3 side chain of the indole nucleus of compound **1** with suitable cycloalkyl derivatives, the "North-Eastern" portion of the receptor was mapped, clarifying the key structural requirements necessary to design novel classes of glycine antagonists. In particular, an asymmetric region of limited size able to accept suitable cycloalkyl substituents was identified. Moreover, the presence of an aromatic moiety was proved to be crucial to maximize the affinity of this class of indole-2-carboxylates, confirming the previous findings obtained for substituted analogues of GV150526 **1**.

Acknowledgments

We would like to thank Dr. M. Mugnaini for the *in vitro* experimental part and Mr. S. Costa for the *in vivo* experiments. Moreover, we are indebted to Dr. A. Garofalo for the elemental analyses performed.

Experimental

Infrared spectra were recorded on a Bruker IFS 48 spectrometer. ^{1}H NMR spectra were recorded on a Varian Unity 400 (400 MHz); the data are reported as follows: chemical shift in ppm from the Me_4Si line as external standard, multiplicity (b = broad, s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet) and coupling constants.

Chromatography was carried out by use of Merck Silica Gel 60 (230–400 mesh) as described by Still *et al*. Mass spectra were performed on a Triple Quadrupole (VG-4 Fison Instrument, UK) equipped with Fast Atom Bombardment (FAB) ionization. Elemental analyses were determined by a EA 1108 Carlo Erba elemental analyzer; C, H, N analyses were within 0.4% of the theoretical values for the formulae given unless otherwise noted. Melting points were determined on a Büchi 530 apparatus (scale 0 °C–250 °C) and are uncorrected. All the reactions were carried out under a controlled atmosphere in flame dried glassware. Anhydrous DMF was purchased from Aldrich; THF was used after distillation over K/benzophenone; CH_2Cl_2 and CH_3CN were used after distillation over P_2O_5. Reactions were monitored by analytical thin-layer chromatography (TLC) using Merck Silica Gel 60 F-254 glass plates (0.25 mm).

*(E) 3-(2-tert-butylcarboxyethenyl)-4,6-dichloroindole-2-carboxylic Acid Ethyl Ester (***2***)*

An aliquot of (*tert*-butoxycarbonylmethylene)triphenylphosphorane (5.6 g, 15 mmol) and ethyl 3-formyl-4,6-dichloroindole-2-carboxylate (3.3 g, 11.6 mmol) were dissolved in a 1:1 mixture CH_3CN/dioxane (60 mL) . The resulting solution was heated at 70 °C for 7 h under an atmosphere of nitrogen. At the end of the reaction the solvent was evaporated under reduced pressure and the crude residue purified by flash chromatography (cyclohexane/AcOEt 1:1) to give 3.4 g of pure compound **2** (75%): mp 157–158 °C. IR (Nujol) ν = 3302 cm^{-1} (NH), 1703 and 1674 (C=O).– ^{1}H NMR ([D_6]DMSO): δ = 9.20 (bs, 1H), 8.32 (d, 1H, *J* = 16Hz), 7.33 (d, 1H), 7.19 (d, 1H), 6.48 (d, 1H, *J* = 16Hz), 4.43 (q, 2H), 1.56 (s, 9H), 1.42 (t, 3H).– MS *m/z* = 383 [M]$^+$.

*(E) 3-(2-carboxyethenyl)-4,6-dichloroindole-2-carboxylic Acid Ethyl Ester (***3***)*

(*E*) Ethyl 3-[2-(*tert*-butoxycarbonyl)ethenyl]-4,6-dichloroindole-2-carboxylate (0.5 g, 1.3 mmol) was suspended in HCOOH (60 mL) and the suspension was stirred at 23 °C for 2 h. The solvent was evaporated *in vacuo* to give 0.408 g of title compound as a white solid (95%): mp >250 °C. IR (Nujol) ν = 3246–3128 cm^{-1} (NH), 1699 and 1670, (C=O).– ^{1}H NMR ([D_6]DMSO) δ =12.6 (bs, 2H), 8.28 (d, 1H, *J* = 16.2 Hz), 7.51 (d, 1H), 7.32 (d, 1H), 6.44 (d, 1H, *J* = 16.2 Hz), 4.37 (q, 2H), 1.35 (t, 3H).– MS *m/z* 329 [M]$^+$.

*General Procedure for the Synthesis of Amides (***5a–o***)*

To an aliquot of 3-(2′-carboxyethenyl)-4,6-dichloroindole-2-carboxylic acid ethyl ester **3** (300 mg, 0.91 mmol) dissolved in dry THF (18 mL), 2,2′-dipyridyl disulfide (282 mg, 1.28 mmoles) and PPh_3 (336 mg, 1.28 mmol), were added at room temperature under an atmosphere of nitrogen. The solution was stirred for 1.5 h, then the chosen amine derivative (1.1 mmoles) was added. The reaction mixture was stirred at room temperature for 3 hours. The resultant precipitate was filtered to give pure title compounds (45-90%).

General Procedure for the Basic Hydrolysis of the Ethyl Esters

Procedure A. To an aliquot of indole-2-carboxylic acid ethyl ester derivative **5a–o** (312 mg, 0.68 mmol) suspended in isopropyl alcohol (20 mL), NaOH was added (108 mg, 2.7 mmol). The solution was heated at 60 °C for 1.5 h. At the end of the reaction the solution was diluted with water (30 mL) and then the solution was concentrated *in vacuo*. The precipitate was filtered and washed with cold water to give pure sodium salt derivative (65–95%).

Procedure B. To an aliquot of indole-2-carboxylic acid ethyl ester derivative **5a–o** (100 mg, 0.27 mmoles) suspended in EtOH (6 mL), $LiOH{\cdot}H_2O$ was added (34.3 mg, 0.82 mmol). The solution was heated at 60 °C for 1.5 h, then concentrated and diluted with water and acidified with 1N HCl. The resulting precipitate was filtered and washed with water to give the pure carboxylic acid derivative (85–95%).

*3-[2-(Cyclohexylaminocarbonyl)ethenyl]-4,6-dichloroindole-2-carboxylic Acid Ethyl Ester (***5a***)*

Prepared from **3** following the general procedure described above: IR (Nujol) ν = 3000 cm^{-1} (NH), 1674 (C=O).– ^{1}H NMR ([D6]DMSO): δ = 12.47 (bs, 1H), 8.05 (d, 1H, *J* = 15.9 Hz), 7.97 (d, 1H), 7.47 (d, 1H), 7.27 (d, 1H), 6.50 (d, 1H, *J* = 15.9 Hz), 4.34 (m, 2H), 3.56 (m,1H), 1.85–1.05 (m, 11H), 1.35 (t, 3H).– MS *m/z* 409 [M+1]$^+$.

*3-[2-(1-Adamantylaminocarbonyl)ethenyl]-4,6-dichloroindole-2-carboxylic Acid Ethyl Ester (***5b***)*

Prepared from **3** following the general procedure described above**:** mp = 151 °C. IR (Nujol) ν = 3335 cm^{-1} (NH), 1672 and 1657, (C=O).– ^{1}H NMR ([D6]DMSO): δ = 11.6 (bs, 1H), 7.98 (d, 1H, *J* = 15.6 Hz), 7.57 (bs, 1H), 7.47 (d, 1H), 7.24 (d, 1H), 6.52 (d, 1H, *J* = 15.6 Hz), 4.34 (q, 2H), 2.10–1.64 (m, 15H), 1.34 (t, 3H).– MS *m/z* 461 [M+1]$^+$.

*3-[2-(1-Adamantylmethylaminocarbonyl)ethenyl]-4,6-dichloroindole-2-carboxylic Acid Ethyl Ester (***5c***)*

Prepared from **3** following the general procedure described above. mp > 250 °C. IR (Nujol) ν = 3306 cm^{-1} (NH), 1680 (C=O).–^{1}H NMR ([D6]DMSO): δ = 12.6 (bs, 1H), 8.00 (d, 1H, *J* = 15.6 Hz), 7.94 (bt, 1H), 7.47 (d, 1H), 7.27 (d, 1H), 6.56 (d, 1H, *J* = 15.6 Hz), 4.34 (q, 2H), 2.87 (d, 2H), 1.92 (m, 3H), 1.6 (m, 12H), 1.32 (t, 3H).– MS *m/z* 475 [M+1]$^+$.

*3-[2-(Noradamantyl-3-aminocarbonyl)ethenyl]-4,6-dichloroindole-2-carboxylic Acid Ethyl Ester (***5d***)*

Prepared from **3** following the general procedure described above: mp 140 °C . IR (Nujol) ν = 3368–3167 cm^{-1} (NH), 1718 and 1657 (C=O).–^{1}H NMR ([D6]DMSO): δ = 12.49 (bs, 1H), 8.15 (bs, 1H), 8.02 (d, 1H, *J* = 15.9 Hz), 7.49 (d, 1H), 7.29 (d, 1H), 6.56 (d, 1H, *J* = 15.9 Hz), 4.36 (q, 2H), 2.42 (m, 1H), 2.22 (m, 2H), 2.06 (m, 2H), 1.96 (m, 2H), 1.84 (dd, 2H), 1.62–1.46 (m, 4H), 1.34 (t, 3H).– MS *m/z* 447 [M+1]$^+$.

*3-[2-(Cyclopropylaminocarbonyl)ethenyl]-4,6-dichloroindole-2-carboxylic Acid Ethyl Ester (***5e***)*

Prepared from **3** following the general procedure described above: mp > 250 °C. IR (Nujol) ν = 3314 and 3260 cm^{-1} (NH), 1678 and 1659 (C=O).–^{1}H NMR ([D6]DMSO): δ = 12.50 (bs, 1H), 8.17 (d,1H, , *J* = 16.0 Hz), 8.04 (d, 1H), 7.45 (d, 1H), 7.26 (d, 1H,), 6.44 (d, 1H, *J* = 16.0 Hz), 4.65 (q, 2H), 2.76 (m, 1H), 1.31 (t, 3H), 0.65 (m, 2H), 0.44 (m, 2H).– MS *m/z* 367 [M+1]$^+$.

*3-[2-(Cyclopropylmethylaminocarbonyl)ethenyl]-4,6-dichloroindole-2-carboxylic Acid Ethyl Ester (***5f***)*

Prepared from **3** following the general procedure described above: mp > 250 °C . IR (Nujol) ν = 3304 cm^{-1} (NH), 1676 and 1661(C=O).–^{1}H NMR ([D6]DMSO): δ = 12.48 (bs, 1H), 8.18 (t, 1H), 8.03 (d, 1H, *J* = 16.0 Hz),7.47 (d, 1H), 7.27 (d, 1H), 6.53 (d, 1H, *J* = 16.0 Hz), 4.34 (q, 2H), 3.04 (t, 2H), 1.32 (t, 3H), 0.95 (m, 1H), 0.40 (m, 2H), 0.16 (m, 2H).– MS *m/z* 381 [M]$^+$.

*3-[2-(3-Quinuclidineaminocarbonyl)ethenyl]-4,6-dichloroindole-2-carboxylic Acid Ethyl Ester (***5g***)*

Prepared from **3** following the general procedure described above: mp 160 °C. IR (Nujol) ν = 1718 and 1680 cm^{-1} (C=O).– ^{1}H NMR ([D6]DMSO): δ = 12.5 (bs, 1H), 8.2 (d, 1H), 8.00 (d, 1H, *J* = 15.7 Hz), 7.47 (d ,1H), 7.3(d, 1H), 6.6 (d, 1H, *J* = 15.7 Hz), 4.34 (q, 2H), 3.84 (m, 1H), 3.08 (m, 1H), 2.8–1.2(m, 10H), 1.32 (t, 3H).– MS *m/z* 436 [M+1]$^+$.

*3-[2-(2-Adamantylaminocarbonyl)ethenyl]-4,6-dichloroindole-2-carboxylic Acid Ethyl Ester (***5h***)*

Prepared from **3** following the general procedure described above: IR (Nujol) ν = 3371 cm^{-1} (NH), 1651 and 1607 (C=O).– ^{1}H NMR ([D6]DMSO): δ = 12.5 (bs, 1H), 8.01 (d, 1H, *J* = 15.8 Hz), 8.00 (bs, 1H), 7.47 (d, 1H), 7.3 (d, 1H), 6.7 (d, 1H, *J* = 15.8 Hz), 4.34 (q, 2H), 4.00 (m, 1H), 2.29 (m, 1H), 2.11 (m, 1H), 1.9–0.9 (m, 4H) ; 1.34 (t, 3H).– MS *m/z* 461 $[M+1]^+$.

*3-{2-[(±)-endo-2-Norbornylaminocarbonyl]ethenyl}-4,6-dichloroindole-2-carboxylic Acid Ethyl Ester (***5i***)*

Prepared from **3** following the general procedure described above: mp 240 °C. IR (Nujol) ν = 3312 cm^{-1} (NH), 1680 and 1657 (C=O).– ^{1}H NMR ([D6]DMSO): δ = 12.5 (bs, 1H), 8.06 (d, 1H, *J* = 15.8 Hz), 8.01 (d, 1H), 7.45 (d, 1H), 7.3 (d, 1H), 6.57 (d, 1H, *J* = 15.8 Hz), 4.34 (q, 2H),4.03 (m,1H), 2.31 (bs, 1H), 2.14 (bs, 1H), 1.88 (m, 1H), 1.6–0.9 (m, 8H),1.33 (t, 3H) .–MS *m/z* 421 $[M+1]^+$.

*3-{2-[(±)-exo-2-Norbornylaminocarbonyl]ethenyl}-4,6-dichloroindole-2-carboxylic acid Ethyl Ester (***5l***)*

Prepared from **3** following the general procedure described above: mp >250 °C. IR (Nujol) ν = 3302 cm^{-1} (NH), 1676 and 1659 (C=O).–^{1}H NMR ([D6]DMSO): δ = 11.9 (bs, 1H), 8.03 (d, 1H, *J* = 15.8 Hz), 7.95 (bd, 1H), 7.48 (d, 1H), 7.28 (d, 1H), 6.53 (d, 1H, *J* = 15.8 Hz), 4.35 (q, 2H), 3.62 (m, 1H), 2.30–2.10 (m, 2H), 1.62 (m, 1H), 1.50–1.30 (m, 3H), 1.30 (m, 1H), 1.20–1.00 (m, 3H), 1.33 (t, 3).– MS *m/z* 421 $[M+1]^+$.

*3-[2-(1R-Bornyl-2-aminocarbonyl)ethenyl]-4,6-dichloroindole-2-carboxylic Acid Ethyl Ester (***5m***)*

Prepared from **3** following the general procedure described above: mp 210 °C. IR (Nujol) ν = 3312 cm^{-1} (NH), 1682 and 1657 (C=O).– ^{1}H NMR ([D6]DMSO): δ = 12.51 (bs, 1H), 8.03 (d, 1H, *J* = 15.8 Hz), 7.96 (bd, 1H), 7.49 (d, 1H), 7.29 (d, 1H), 6.64 (d, 1H, *J* = 15.8 Hz), 4.36–4.24 (m, 3H), 2.18 (m, 1H), 1.70–1.60 (m, 3H), 1.40–0.92 (m, 3H), 1.33 (t, 3H), 0.93 (s, 3H), 0.84 (s, 3H), 0.73 (s, 3H).– MS *m/z* 463 $[M+1]^+$.

*3-[2-(1R-isobornyl-2-aminocarbonyl) ethenyl]-4,6-dichloroindole-2-carboxylic Acid Ethyl Ester (***5n***)*

Prepared from **3** following the general procedure described above: mp 210 °C· IR (Nujol) ν = 3400 cm^{-1} (NH), 1703 and 1682 (C=O),.– ^{1}H NMR ([D6]DMSO): d = δ 12.48 (bs, 1H), 7.98 (d, 1H, *J* = 16.0 Hz), 7.47 (d, 2H), 7.27 (d, 1H), 6.61 (d, 1H, *J* = 16.0 Hz), 4.4–4.25 (m, 3H), 3.86 (m,1H), 1.50–1.15 (m, 6H), 1.31 (t, 3H), 0.91 (s, 3H), 0.78 (s, 6H).– MS *m/z* 463 $[M+1]^+$.

*3-[2-(1,2,3,4-tetrahydro-1-naphthylaminocarbonyl)ethenyl]-4,6-dichloroindole-2-carboxylic Acid Ethyl Ester (***5o***)*

Prepared from **3** following the general procedure described above: IR (Nujol) ν = 3302 cm^{-1} (NH), 1676 (C=O).– ^{1}H NMR ([D6]DMSO): δ = 12.52 (bs, 1H), 8.53 (d, 1H), 8.14 (d, 1H, *J* = 16.0 Hz), 7.49 (d, 1H), 7.29 (d, 1H), 7.16 (m, 4H), 6.58 (d, 1H, *J* = 16.0 Hz), 5.13 (m, 1H), 4.35 (q, 2H), 2.75 (m,1H), 1.93–1.75 (m, 4H), 1.33 (t, 3H).– MS *m/z* 456 $[M+1]^+$.

*3-[2-(Cyclohexylaminocarbonyl)ethenyl]-4,6-dichloroindole-2-carboxylic Acid (***6a***)*

Prepared from **5a** following the general procedure B: mp > 250 °C. IR (Nujol) ν = 3418 and 3229 cm^{-1}(NH), 1695–1684 (C=O).– ^{1}H NMR ([D6]DMSO): δ = 13.64 (bs, 1H), 12.44 (s, 1H), 8.05 (d, 1H, *J* = 15.9 Hz), 7.99 (d, 1H), 7.46 (d, 1H), 7.27 (d, 1H), 6.53 (d, 1H, *J* = 15.9 Hz), 3.65 (dm, 1H), 1.82–1.55 (m, 5H), 1.38–1.10 (m, 5H).– MS *m/z* 381 $[M+1]^+$.

*3-[2-(1-Adamantylaminocarbonyl)ethenyl]-4,6-dichloroindole-2-carboxylic Acid (***6b***)*

Prepared from **5b** according to procedure B**:** mp > 250°C. IR (Nujol) ν = 3418 cm^{-1} (NH), 1647 (C=O).– ^{1}H NMR ([D6]DMSO): δ = 13.60 (bs, 1H), 12.40 (s, 1H), 8.02 (d, 1H, *J* = 15.6 Hz), 7.58 (bs, 1H), 7.45 (d, 1H), 7.25 (d, 1H), 6.55 (d, 1H, *J* = 15.6 Hz), 2.06–1.96 (m, 9H), 1.64 (m, 6H). MS *m/z* 455 $[M+1]^+$.

*3-[2-(1-Adamantylmethylaminocarbonyl)ethenyl]-4,6-dichloroindole-2-carboxylic Acid Sodium Salt (***6c***)*

Prepared from **5c** according to procedure A: mp > 250 °C . IR (Nujol) ν = 3429–3198 cm^{-1} (NH), 1653–1612 (C=O).– ^{1}H NMR ([D6]DMSO): δ = 11.7 (bs, 1H), 8.37 (d, 1H, *J* = 15.6 Hz), 7.72 (t, 1H), 7.38 (d, 1H), 7.07 (d, 1H), 6.94 (d, 1H, *J* = 15.6 Hz), 2.87 (d, 2H), 1.92 (m, 3H), 1.70–1.40 (m, 12H).– MS *m/z* 469 $[M+1]^+$.

*3-[2-(Noradamantyl-3-aminocarbonyl)ethenyl]-4,6-dichloroindole-2-carboxylic Acid Sodium Salt (***6d***)*

Prepared from **5d** according to procedure A: mp > 250 °C . IR (Nujol) ν = 1609 cm^{-1} (C=O).– ^{1}H NMR ([D6]DMSO): δ = 11.50 (bs, 1H), 8.30 (d, 1H, *J* = 15.9 Hz), 7.83 (bs, 1H), 7.34 (d, 1H), 7.04 (d, 1H), 6.87 (d, 1H, *J* = 15.9 Hz), 2.42 (t, 1H), 2.19 (bs, 2H), 2.06 (d, 2H), 1.94 (m, 2H), 1.80 (m, 2H), 1.50 (m, 4H).– MS *m/z* 441 $[M+1]^+$.

*3-[2-(Cyclopropylaminocarbonyl)ethenyl]-4,6-dichloroindole-2-carboxylic Acid (6e***)**

Prepared from **5e** according to procedure B: mp > 250 °C. IR (Nujol) ν = 2950 cm^{-1} (NH),1647 (C=O).– ^{1}H NMR ([D6]DMSO): δ = 13.62 (bs, 1H), 12.43 (s, 1H), 8.17 (d,1H, *J* = 16.0 Hz), 8.06 (d, 1H), 7.45 (d, 1H), 7.26 (d, 1H,), 6.46 (d, 1H, *J* = 16.0 Hz), 2.76 (m, 1H), 0.64 (m, 2H), 0.44 (m, 2H). MS *m/z* 339 $[M+1]^+$.

*3-[2-(Cyclopropylmethylaminocarbonyl)ethenyl]-4,6-dichloroindole-2-carboxylic Acid Sodium Salt (***6f***)*

Prepared from **5f** according to procedure A: mp > 250 °C. IR (Nujol) ν = 3437–3375 cm^{-1} (NH), 1655–1641 (C=O).– ^{1}H NMR ([D6]DMSO): δ = 11.7 (bs, 1H), 8.38 (d, 1H, *J* = 16.0 Hz), 7.96 (t, 1H),7.38 (d, 1H), 7.07 (d, 1H), 6.94 (d, 1H, *J* = 16.0 Hz), 3.02 (t, 2H), 0.95 (m, 1H), 0.40 (m, 2H), 0.18 (m, 2H). MS *m/z* 375 $[M]^+$.

*3-[2-((±)-3-Quinuclidineaminocarbonyl)ethenyl]-4,6-dichloroindole-2-carboxylic Acid (***6g***)*

Prepared from **5g** according to procedure B: mp > 250 °C. IR (Nujol) ν = 3366 cm^{-1} (NH), 1661 and 1618 (C=O).– ^{1}H NMR ([D6]DMSO): δ = 13.4–12.4 (bs, 1H), 11.96 (bs, 1H), 8.46 (d, 1H), 8.29 (d ,1H, *J* = 15.7 Hz), 7.41(d, 1H), 7.12 (d, 1H), 6.80 (d, 1H, *J* = 15.7 Hz), 4.14 (m, 1H), 3.6–3.0 (m, 6H), 2.2–1.6 (m, 5H).– MS *m/z* 408 $[M+1]^+$.

*3-[2-(2-Adamantylaminocarbonyl)ethenyl]-4,6-dichloroindole-2-carboxylic Acid Sodium Salt (***6h***)*

Prepared from **5h** according to procedure A**:** mp > 250 °C. IR (Nujol) ν = 1653 cm^{-1} (C=O). ^{1}H NMR ([D6]DMSO): δ = 11.3–11.6 (bs, 1H), 8.34 (d, 1H, *J* = 15.8 Hz), 7.74 (bd, 1H), 7.37 (d, 1H), 7.05 (d, 1H), 6.88 (d, 1H, *J* = 15.8 Hz), 3.95 (d, 1H), 2.03 (d, 2H), 1.8–1.6 (m, 10H), 1.46 (d, 1H).– MS *m/z* 455 $[M+1]^+$.

*3-{2-[(±)-endo-2-Norbornylaminocarbonyl]ethenyl}-4,6-dichloroindole-2-carboxylic Acid Sodium Salt (***6i***)*

Prepared from **5i** according to procedure A: mp > 250 °C. IR (Nujol) ν = 3420 cm^{-1} (NH), 1651 (C=O.– ^{1}H NMR ([D6]DMSO): δ = 11.69 (bs, 1H), 8.34 (d, 1H, *J* = 15.8 Hz), 7.84 (d, 1H), 7.37 (d, 1H), 7.05 (d, 1H), 6.87 (d, 1H, *J* = 15.8 Hz), 4.00 (m, 1H), 2.29 (m, 1H), 2.11 (m, 1H), 1.9–0.9 (m, 4H).– MS *m/z* 415 $[M+1]^+$.

*3-{2-[(±)-exo-2-Norbornylaminocarbonyl]ethenyl}-4,6-dichloroindole-2-carboxylic Acid Sodium Salt (**6l**)*

Prepared from **5l** according to procedure A: mp > 250 °C. IR (Nujol) ν = 3296–3161 cm^{-1} (NH), 1682 and 1663(C=O).– ^{1}H NMR ([D6]DMSO): δ = 13.62 (bs, 1H), 12.42 (s, 1H), 8.05 (d, 1H, *J* = 15.8 Hz), 7.95 (d, 1H), 7.45 (s, 1H), 7.25 (s, 1H), 6.53 (d, 1H, *J* = 15.8 Hz), 3.62 (m, 1H), 2.21–2.10 (m, 2H), 1.61 (m, 1H), 1.50–1.35 (m, 3H), 1.30 (m, 1H), 1.19–1.05 (m, 3H).– MS *m/z* 415 $[M+1]^+$.

*3-[2-(1R-Bornyl-2-aminocarbonyl)ethenyl]-4,6-dichloroindole-2-carboxylic Acid Sodium Salt (**6m**)*

Prepared from **5m** according to procedure A: mp > 250 °C. IR (Nujol) ν = 3431 and 3377 cm^{-1} (NH), 1647–1610 (C=O).– ^{1}H NMR ([D6]DMSO): δ = 11.69 (bs, 1H), 8.37 (d, 1H, *J* = 15.8 Hz), 7.75 (bd, 1H), 7.39 (d, 1H), 7.07 (d, 1H), 6.98 (d, 1H, *J* = 15.8 Hz), 4.24 (m, 1H), 2.12 (m, 1H), 1.80–1.55 (m, 3H), 1.40–1.10 (m, 2H), 0.99 (m, 1H), 0.93 (s, 3H), 0.84 (s, 3H), 0.73 (s, 3H).– MS *m/z* 456 $[M+1]^+$.

*3-[2-(1R-Isobornyl-2-aminocarbonyl)ethenyl]-4,6-dichloroindole-2-carboxylic Acid Sodium Salt (**6n**)*

Prepared from **5n** according to procedure A: mp >250 °C. IR (Nujol) ν = 3192 cm^{-1} (NH), 1609 (C=O).– ^{1}H NMR ([D6]DMSO): δ = 11.6 (bs, 1H), 8.34 (d, 1H, *J* = 16.0 Hz), 7.35 (d, 1H), 7.21 (bd, 1H), 7.05 (d, 1H), 6.89 (d, 1H, *J* = 16.0 Hz), 1.8–1.6 (m, 4H), 1.48 (m, 1H), 1.2–1.06 (m, 2H), 0.91 (s, 3H), 0.76 (ss, 6H). MS *m/z* 457 $[M]^+$.

*3-[2-((±)-1,2,3,4-Tetrahydro-1-naphthylaminocarbonyl)ethenyl]-4,6-dichloroindole-2-carboxylic Acid Sodium Salt (**6o**)*

Prepared from **5o** according to procedure A: mp >250 °C. IR (Nujol) ν = 1607 cm^{-1} (C=O).– ^{1}H NMR ([D6]DMSO): δ = 11.6 (bs, 1H), 8.47 (d,1H), 8.26 (d, 1H, *J* = 16.0 Hz), 7.36 (d, 1H), 7.05 (d, 1H), 7.22–7.04 (m, 4H), 6.93 (d, 1H, *J* = 16.0 Hz), 5.09 (m, 1H), 2.72 (m, 2H), 1.90 (m, 2H), 1.72 (m, 1H).– MS *m/z* 450 $[M]^+$.

References

[1] B. Meldrum, J. Garthwaite, *Trends Pharmacol*. **1990**, *11*, 379–387.

[2] D. W. Choi, *J. Neurosci.* **1990**, *10*, 2493–2501.

[3] B. Meldrum, *Cerebrovasc. Brain Metab. Rev*. **1990**, 2, 27–57.

[4] J. McCullogh, *Br. J. Clin. Pharmacol*. **1992**, *34*, 106–114.

[5] D. W.Choi, S. M. Rothman, *Ann. Rev. Pharmacol. Toxicol.* **1991**, *31*, 171–182.

[6] *The NMDA Receptor, 2nd Ed*. (Ed.: G. L. Collingridge, J. C., Watkins), IRL Press, Oxford (UK), **1994**.

[7] E. H. F. Wong, J. A. Kemp, *Ann. Rev. Pharmacol. Toxicol.* **1991**,*31*,401–425.

[8] *Excitatory Amino Acid Antagonists.* (Ed.: B. Meldrum), Blackwell, Oxford (UK), **1991**.

[9] J. W. Johnson, P. Asher, *Nature* **1987**, *325*, 529–531.

[10] N. W. Kleckner, R. Dingledine, *Science* **1988**, *241*, 835–837.

[11] J.E. Huettner, *Biochem. Pharmacol*. **1991**, *41*, 9–16.

[12] A. M. Thomson, *Prog. Neurobiol.* **1990**, *35*, 53–74.

[13] R. G. M. Morris, E. Anderson, G. S. Lynch, M. Baudry, *Nature* **1986**, *319*, 774–776.

[14] W. Koek, F. C. Colpaert, *J. Pharmacol. Exp. Ther*. **1990**, *252*, 349–357.

[15] C. Chiamulera, S. Costa, A. Reggiani, *Psycopharmacol.* **1990**, *112*, 551–552.

[16] M. G. Palefreyman, M. B. Baron in: *Excitatory Amino Acid Antagonists* (Ed.: B. Meldrum), Blackwell, Oxford (UK), **1991**. Chapter 6

[17] A. J. Carter, *Drugs Future* **1992**, *17*, 595–613.

[18] J. A. Kemp, P. D. Leeson, *TiPS* **1993**, *14*, 20–25.

[19] P.D. Leeson, L. L. Iversen, *J. Med. Chem.* **1994**, *37*, 4053–4067.

[20] L. L. Iversen, J.A. Kemp, in *The NMDA Receptor, 2nd Ed*. (Ed.: G. L. Collingridge, J. C., Watkins), IRL Press, Oxford (UK), **1994**, 469–486.

[21] R. Di Fabio, G. Gaviraghi, A. Reggiani, *La Chimica e l'Industria* **1996**, *78*, 283–289.

[22] 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, *J. Med. Chem.* **1997**, *40*, 841–850.

[23] R. Di Fabio, A. Cugola, D. Donati, A. Feriani, G. Gaviraghi, E. Ratti, D. G. Trist, *ACS Book of Abstracts*, 211th ACS National Meeting, New Orleans, LA, 24–28 March **1996**, Abstract 107.

[24] A. Cugola, G. Gaviraghi, F. Micheli (Glaxo S.p.A.), PCT Int. Appl., WO 9420465 A1 940915

[25] G. Gaviraghi, R. Di Fabio, A. Cugola, D. Donati, A. Feriani, E. Ratti, D.G. Trist, A. Reggiani, *Proceedings, XIVth International Symposium on Medicinal Chemistry*, (Ed.: F. Awouters), Maastricht, **1996**

[26] A. Reggiani, S. Costa, C. Pietra, E. Ratti, D. G. Trist, L. Ziviani, G. Gaviraghi, *Eur. J. Neurol*. **1995**, *2*, 6.

[27] C. Pietra, L. Ziviani, E. Valerio, G. Tarter, A. Reggiani, *Stroke*, **1996**, *27*, 26.

[28] J. E. Huettner, *Science*, **1989**, *243*, 1611–1613.

[29] F. G. Salituro, B. L. Harrison, B. M. Baron, P. L. Nyce, K. T. Stewart, I. A. McDonald, *J. Med. Chem.* **1990**, *33*, 2944–2946.

[30] N. M. Gray, M. S. Dappen, B. K. Cheng, A. A. Cordi, J. P. Biesterfeldt, W. F. Hood, J. B. Monahan, *J. Med. Chem.* **1991**, *34*, 1283–1292.

[31] F. G. Salituro, R. C. Tomlinson, B. M. Baron, D. A. Demeter, H. J. R. Weintraub, I. A. McDonald, *Bioorg. Med. Chem. Lett.* **1991**, *1*, 455–460.

[32] F. G. Salituro, B. L. Harrison, B. M. Baron, P. L. Nyce, K. T. Stewart, J. H. Kehne, H. S. White, I. A. McDonald, *J. Med. Chem.* **1992**, *35*, 1791–1799.

[33] T. S. Rao, N. M. Gray, M. S. Dappen, J. A. Cler, S. J. Mick, M. R. Emmett, S. Iyergan, J. B. Monahan, A. A. Cordi, P. L. Wood, *Neuropharmacol.* **1993**, 32, 139–147.

[34] E. C. R. Smith, L. A. McQuaid, D. O. Calligaro, P. J. O'Malley, *Bioorg. Med. Chem. Lett.* **1993**, 81–84.

[35] B.M. Baron, B.L. Harrison, J.H. Kehne, C.J. Schmodt, P.L.M. Van Giersbergen, H.S. White, B.W. Siegel, Y. Senyah, T.C. McCloskey, G.M. Fadayel, V.L. Taylor, M.K. Murawsky, P. Nyce, F.G. Salituro, *Eur. J. Pharm.* **1997**, *323*, 181–192.

[36] R. Di Fabio, V. Summa, T. Rossi, *Tetrahedron* **1993**, *49*, 2300–2306

[37] Catalyst rel. 3.1 (1997) Molecular Simulation Inc. San. Diego CA. USA

[38] A. Smellie, S. Teig, A.D. Kahn, L. J.Teig. *Chem.Inf.Comput.Sci*. **1995**, *35*, 285–294

[39] A. Smellie, S. Teig, A.D. Kahn, L. J.Teig. *Chem.Inf.Comput.Sci*. **1995**, *35*, 295–304

Received: October 16, 1998 [FP342]