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Il Farmaco 54 (1999) 64–76

IL FARMACO

Synthesis of biphenylyltetrazole derivatives of 1-aminopyrroles as angiotensin II antagonists

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Received 1 September 1998; accepted 30 November 1998

Abstract

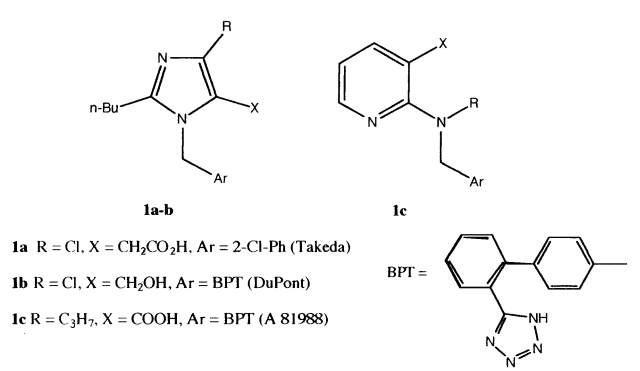
Based on preliminary molecular modelling study, the synthesis of two different classes of biphenylyltetrazole derivatives of 1-aminopyrroles, as potentially active non-peptide angiotensin II (AII) antagonists, is reported. Some *NH*-Boc protected 1-aminopyrroles were deprotected, *N*-acylated, *N*-alkylated with 5-[4′-bromomethyl-1,1′-biphenyl-2-yl]-1-triphenylmethyl-1*H*-tetrazole, and then detritylated to give the first class of title compounds. Other 1-*NH*-Boc protected 1,2-diaminopyrroles were regioselectively subjected to the 1-alkylation with 5-[4′-bromomethyl-1,1′-biphenyl-2-yl]-1-triphenylmethyl-1*H*-tetrazole, to the acylation of the amino group at 2-position of the pyrrole ring, and then to the detritylation process to yield the second class of title compounds. © 1999 Elsevier Science S.A. All rights reserved.

Keywords: Pyrrole; Biphenylyltetrazole; Deprotection; *N*-Acylation; *N*-Alkylation

1. Introduction

The renin–angiotensin system (RAS) is known to play an important role in the regulation of blood pressure and electrolyte balance [1,2]. The blocking of the RAS with angiotensin-converting enzyme (ACE) inhibitors has been shown to be effective in the control of hypertension and congestive heart failure also if there are some side effects such as dry cough and angioedema caused by the non specific action of ACE [3,4].

On the basis of the effectiveness of ACE inhibitors in cardiovascular control, there has been intense activity regarding the discovery of oral angiotensin II (AII) antagonists as a means of inhibiting the RAS, with the hope of obtaining greater pharmacological selectivity than observed with ACE inhibitors. Starting from the initial lead reported by Takeda **1a** (see Fig. 1) [5], researchers at DuPont discovered Losartan **1b** (see Fig. 1) [6], the first orally active nonpeptide AII antagonist which was marketed for the treatment of hypertension (1994, Cozaar) [7]. Weinstock et al. [8] showed that the replacement, in Losartan, of the biphenylyltetrazole (BPT) moiety, which is linked to an imidazole ring via



1a-b **1c**

1a R = Cl, X = CH_2CO_2H, Ar = 2-Cl-Ph (Takeda)

1b R = Cl, X = CH_2OH, Ar = BPT (DuPont)

1c R = C_3H_7, X = COOH, Ar = BPT (A 81988)

Fig. 1.

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PII: S0014-827X(98)00100-1

a methylene group, implies poor activity while the imidazole moiety can be successfully replaced by other cyclic and acyclic structures **1c** (see Fig. 1).

Therefore, the imidazole heterocycle has been postulated as useful mainly to link the required functionalities. In fact, Alvarez-Builla and co-workers have confirmed recently that imidazole ring in Losartan reference can be replaced by 2-*n*-butylbenzimidazole or 1*H*-naphthol[2,3-d][1,2,3]triazole conferring promising activities to the molecules prepared. Indeed they have shown that even an arylthienyl bridge can be used instead of a biphenyl without a significant decrease in activity [9].

Ellingboe et al. [10b] prepared a series of pyrido-[2,3-d]pyrimidine by using the common structural similarities among some of the reported AII antagonists.

They demonstrated the importance of the electronic and steric character of the substituents on the pyrido[2,3-d]pyrimidine ring system.

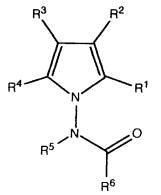
A substituent capable of participation in a hydrogen bond was not beneficial at positions near the nitrogen atoms of the pyrimidine ring (positions 2, 4) while the introduction of alkyl groups in these positions increased binding affinity; on the other hand a hydrogen-bond acceptor near the nitrogen atom of the saturated ring (position 7) appeared important for binding to the AT_1 receptor.

The alkyl groups may fit into a lipophilic pocket in the AT_1 receptor and hydrogen-bond acceptor adjacent to the point of attachment of the BPT group in the heterocyclic portion may increase drug–receptor interactions and binding affinity.

Thus, a target compound should incorporate a carboxylic acid, an alkyl group and a tetrazole substituted biphenyl moiety, each attached to a central heterocycle. However, the exact location of these groups and the optimum heterocycle ring still had to be determined exactly [10a].

In a previous work [2], we modified the imidazole system with the substitution of the chlorine in DUP 753 with some electron-deficient heteroaromatic moieties leading to the conjugated bis-heteroaryl system. This replacement produced compounds with potent AII antagonist activity.

We verified, by molecular modelling studies, that the substituents in heterocycle rings are able to modify the charge distribution in such a way as to influence the activity. In order to correlate the results of the binding data with molecular structure we examined the molecular electrostatic potential (MEP) of our compounds. The MEP is a very important index of reactivity and recognition in that its magnitude and shape can give useful information on the site and geometry of likely attack. The MEP is a measurable quantity that depends on the three-dimensional (3D) structure and charge distribution of the molecule.



Compound	R^1	R^2	R^3	R^4	R^5	R^6
5a	$CH_2CO_2C_2H_5$	H	$COCH_3$	CH_3	CH_2BPT	C_4H_9
5b	$CH_2CO_2C_2H_5$	H	COC_6H_5	CH_3	CH_2BPT	C_4H_9
5e	CH_3	CH_3	$COCH_3$	$CO_2C_2H_5$	CH_2BPT	C_4H_9
9a	CH_3	$CO_2C_2H_5$		$NHCOC_4H_9$	CH_2BPT	$OC(CH_3)_3$

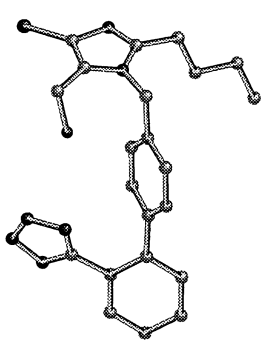
Fig. 2.

Based on our previous results, we were interested in exploring a series of biphenylyltetrazole derivatives of 1-aminopyrroles (see Fig. 2). In fact, in the previous heteroaromatic rings and in Winn et al.'s report [10a] the presence of a nitrogen atom outside the ring could favour an increased binding affinity of the molecules with the receptor because of an enlargement of the zone of negative potential around these nitrogen atoms, increasing the potency of the AII antagonists.

2. Molecular modelling

We chose to compare the MEP distributions and structures of compounds **5a**, **5b**, **5e** and **9a** (see Fig. 2) with those of DUP 753 (Losartan) [11] (see Fig. 3) and A 81988 [10a] (see Fig. 4) considered as reference systems.

Since the X-ray coordinates of DUP 753 and A 81988 were not available to us, several minimum energy structures have been generated using the SYBYL molecular modelling software [12] starting from model built arrangements with randomly chosen but reasonable dihedral angles. The partial charges for the electrostatic contribution in SYBYL have been computed with the Gasteiger–Hückel (GH) method [13–15] and the



DUP 753

Fig. 3. Minimum energy structure of DUP 753 chosen as reference geometry.

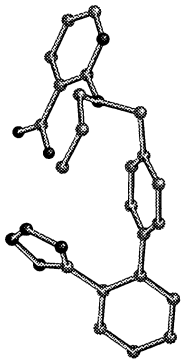


Fig. 4. Minimum energy structure of A 81988 chosen as reference geometry.

dielectric constant has been set equal to the value of R, the separation between a pair of atoms ($\varepsilon = R$).

In order to reduce the number of possible conformations we compared the structures obtained with simplified models optimised with ab initio calculations carried out at the SCF level with a 3-21G basis set [2,16], and with the X-ray resolved structures of some other AII receptor antagonists [17–21] contained in the Cambridge Structural Database [22].

By following this procedure, we found suitable reference geometries of the two compounds DUP 753 and A 81988 (see Fig. 5).

In order to determine the 3D structure of the *N*-acyl-1-aminopyrrole, a model system, in which the R_1, R_2, R_3, R_4, R_6 chains were replaced by hydrogen atoms and the R_5 chain was replaced by a methyl group (see Fig. 2), was optimised by ab initio calculations [23] carried out at the SCF level with a 6-31G* basis set [24,25].

The final structure showed a perpendicular arrangement of the pyrrole ring with respect to the NCO plane. The optimised value of the N–N distance (1.37 Å) was slightly longer than a standard double bond between N atoms but decidedly shorter than standard single bonds.

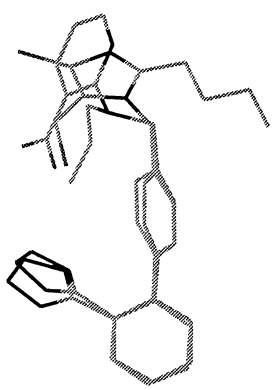


Fig. 5. Superimposition between DUP 753 and A 81988.

The SYBYL force field with the inclusion of the electrostatic contribution in the calculation (GH charges, $\varepsilon = R$) was able to satisfactorily reproduce our ab initio model structure, thus conformational searches for compounds **5a**, **5b**, **5e** and **9a** have been performed with SYBYL. The threshold used was a maximum energy difference of 10 kcal/mol with respect to the most stable conformer.

We compared the MEP produced by the GH charges of some low energy conformers of the molecules **5a**, **5b**, **5e** and **9a** with the MEP of the molecules DUP 753 (better reference) and A 81988 (see Figs. 6 and 7).

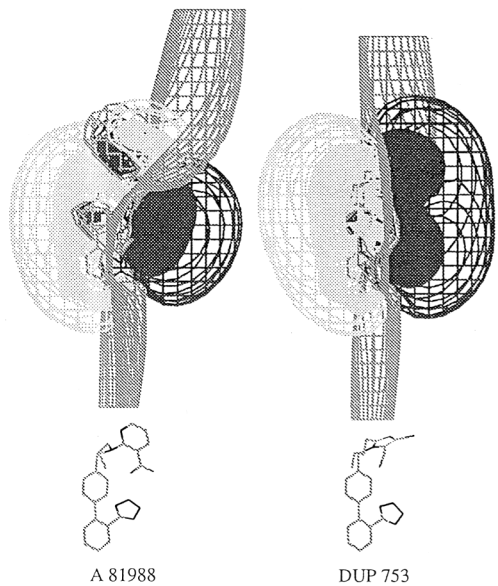


Fig. 6. MEP of A 81988 and DUP 753 produced by Gasteiger–Hückel partial charges (black, light grey solid surfaces = −5 and +5 kcal/mol, respectively; black, grey, light grey grid surfaces = −1 and +1 kcal/mol, respectively).

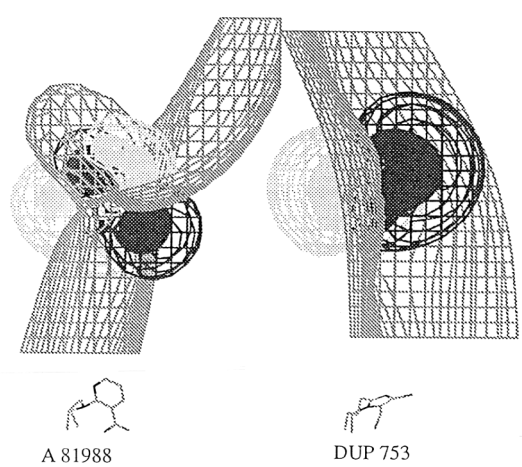


Fig. 7. MEP of simplified models of A 81988 and DUP 753, in which the BPT segment has been replaced by a methyl group, produced by Gasteiger–Hückel partial charges (black, light grey solid surfaces = −5 and +5 kcal/mol, respectively; black, grey, light grey grid surfaces = −1 and +1 kcal/mol, respectively).

The isopotential surfaces at +5 and −5 kcal/mol and at +1 and −1 kcal/mol of the complete structures and of the simplified models, in which the BPT segment has been replaced by a methyl group (see Figs. 7, 9, 11, 13 and 15) were examined. By comparison of the isopotential surfaces of the complete structures with the partial structures we noted that the BPT segment gave a positive contribution in the positive part and a negative contribution in the negative part, increasing the extension of both zones (see Figs. 6, 8, 10, 12 and 14).

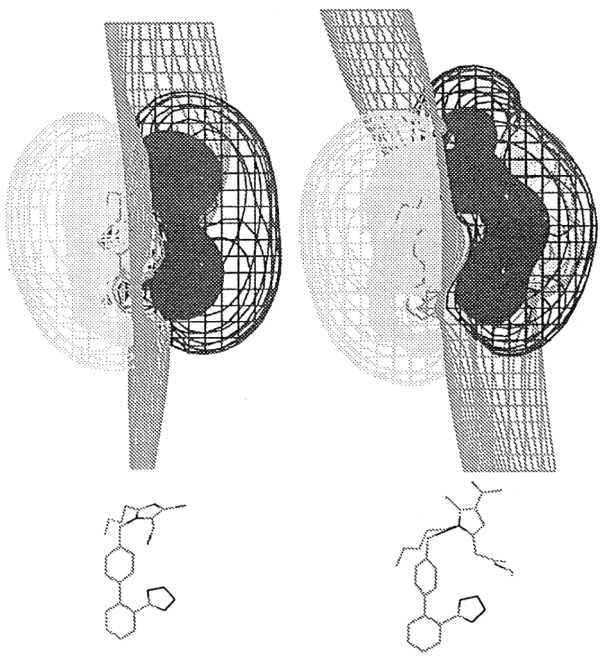


Fig. 8. MEP of DUP 753 and **5a** produced by Gasteiger–Hückel partial charges (black, light grey solid surfaces = −5 and +5 kcal/mol, respectively; black, grey, light grey grid surfaces = −1 and +1 kcal/mol, respectively).

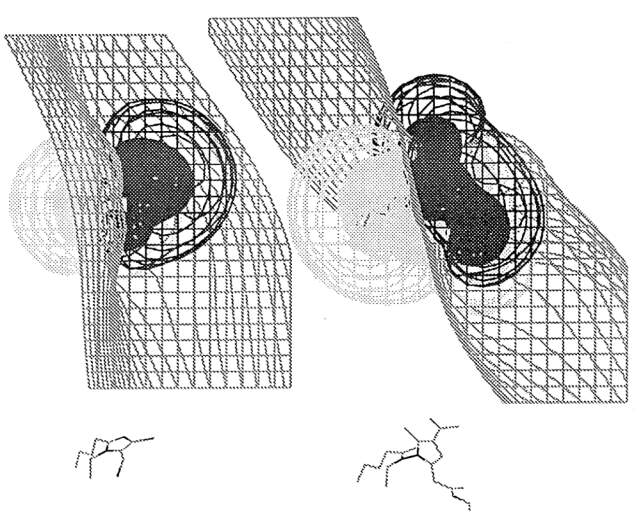


Fig. 9. MEP of simplified models of DUP 753 and **5a** produced by Gasteiger–Hückel partial charges (black, light grey solid surfaces = −5 and +5 kcal/mol, respectively; black, grey, light grey grid surfaces = −1 and +1 kcal/mol, respectively).

The overall shape of the isopotential surfaces at +1 and −1 kcal/mol of some low energy conformers of the new molecules were similar to that of the reference structures, even if greatly enlarged.

The molecules examined showed the negative and positive isopotential surfaces oriented in a similar way to that of the reference molecules.

Molecular mechanics (SYBYL) and quantum mechanics (Gaussian94) [23] calculations, geometries and MEP visualisations have been performed on the IRIS/4D-420-GTXB workstation.

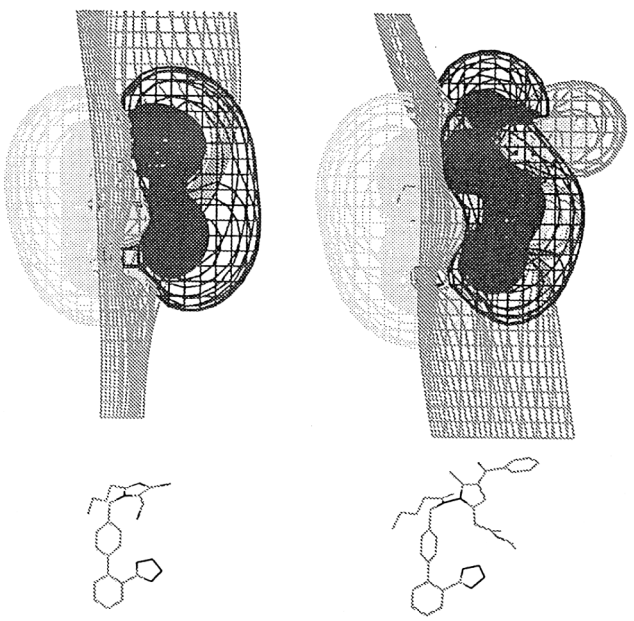


Fig. 10. MEP of DUP 753 and **5b** produced by Gasteiger–Hückel partial charges (black, light grey solid surfaces = −5 and +5 kcal/mol, respectively; black, grey, light grey grid surfaces = −1 and +1 kcal/mol, respectively).

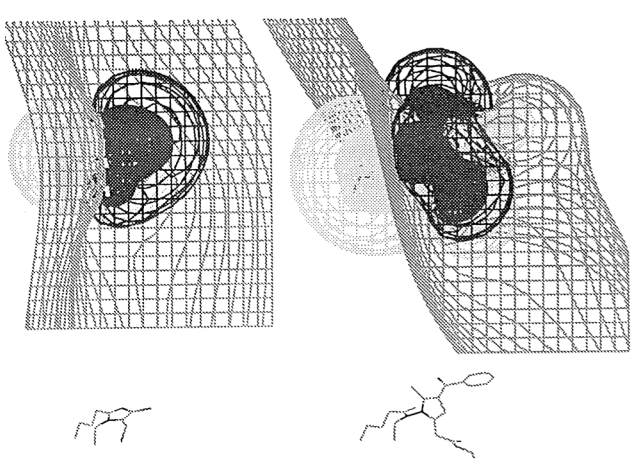


Fig. 11. MEP of simplified models of DUP 753 and **5b** produced by Gasteiger–Hückel partial charges (black, light grey solid surfaces = −5 and +5 kcal/mol, respectively; black, grey, light grey grid surfaces = −1 and +1 kcal/mol, respectively).

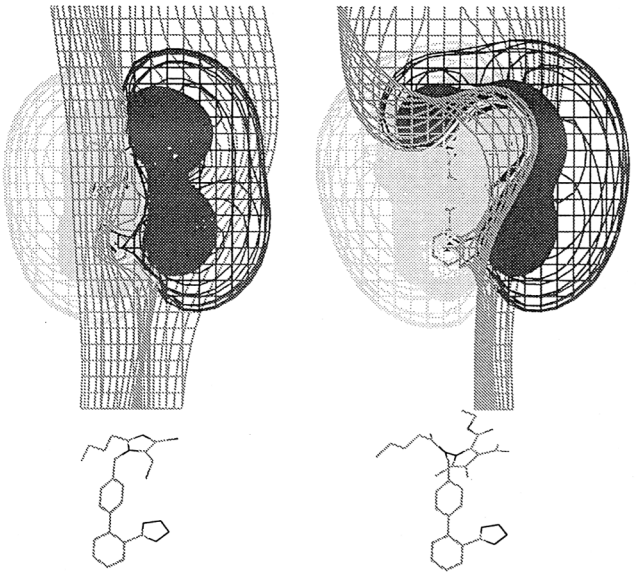


Fig. 12. MEP of DUP 753 and **5e** produced by Gasteiger–Hückel partial charges (black, light grey solid surfaces = −5 and +5 kcal/mol, respectively; black, grey, light grey grid surfaces = −1 and +1 kcal/mol, respectively).

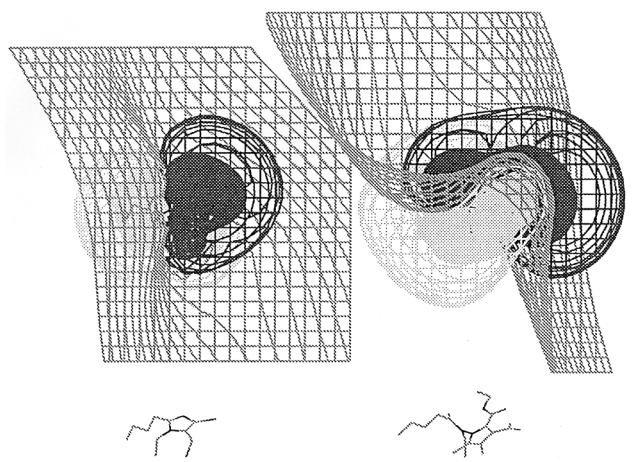


Fig. 13. MEP of simplified models of DUP 753 and **5e** produced by Gasteiger–Hückel partial charges (black, light grey solid surfaces = −5 and +5 kcal/mol, respectively; black, grey, light grey grid surfaces = −1 and +1 kcal/mol, respectively).

3. Chemistry

A series of *N*-acyl-1-aminopyrrole compounds [26] containing the BPT moiety linked to the *N*-amino group by a methylene group (**5a–e**), was obtained as shown in Scheme 1. The removal of the Boc protecting group (CO_2CMe_3) from *NH*-Boc protected 1-aminopyrrole derivatives **1a–e** was carried out by heating at 170°C. The resulting deprotected 1-aminopyrrole derivatives **2a–e** (69.5–91.3%), dissolved in anhydrous THF, were at first treated with valeryl chloride in the presence of anhydrous pyridine, to yield the *NH*-acylated compounds **3a–e** (80.4–94.5%). The subsequent *N*-alkylation of the derivatives **3a–e** with 5-[4′-bromomethyl-1,1′-biphenyl-2-yl]-1-triphenylmethyl-1*H*-tetrazole was achieved in dichloromethane at room temperature (r.t.) in the presence of sodium hydroxide providing the derivatives **4a–e** (59.2–88.7%). Finally, the removal of the trityl protecting group (Ph_3C) from the tetrazole ring was performed by treatment of the compounds **4a–e** under

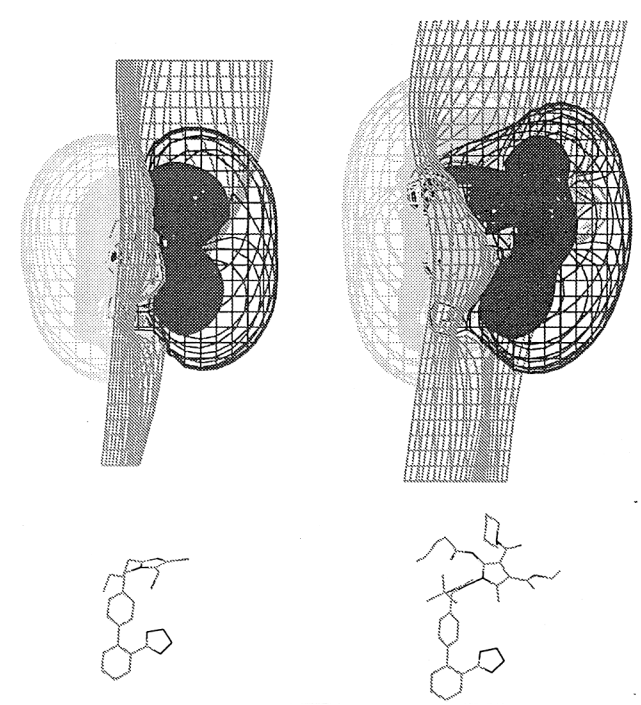


Fig. 14. MEP of DUP 753 and **9a** produced by Gasteiger–Hückel partial charges (black, light grey solid surfaces = −5 and +5 kcal/mol, respectively; black, grey, light grey grid surfaces = −1 and +1 kcal/mol, respectively).

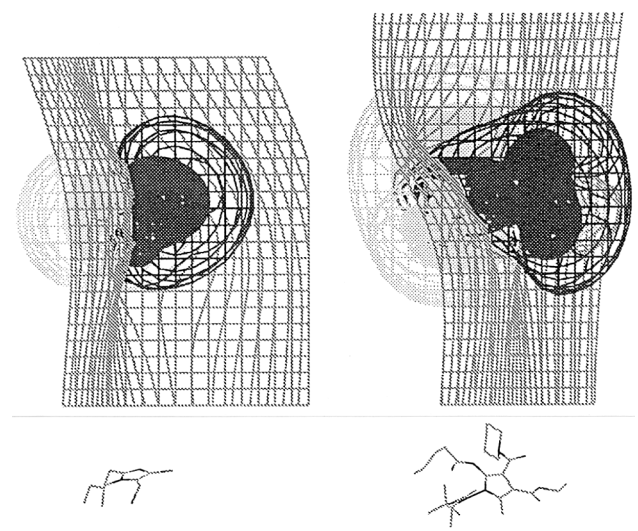
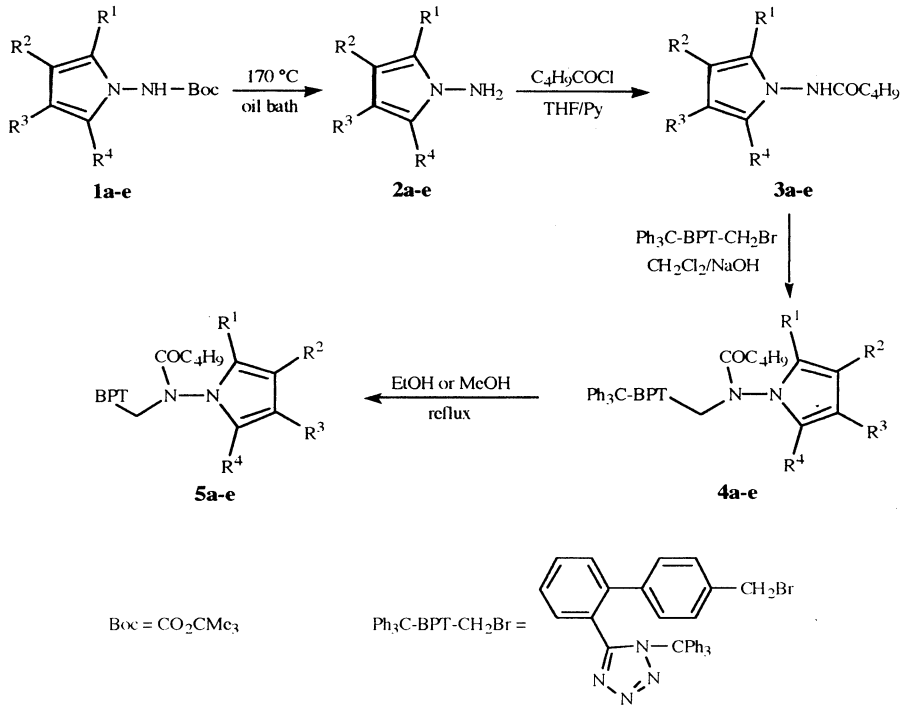


Fig. 15. MEP of simplified models of DUP 753 and **9a** produced by Gasteiger–Hückel partial charges (black, light grey solid surfaces = −5 and +5 kcal/mol, respectively; black, grey, light grey grid surfaces = −1 and +1 kcal/mol, respectively).



Compound **1**-**5**	R^1	R^2	R^3	R^4
a	CH_2CO_2Et	H	COMe	Me
b	CH_2CO_2Et	H	COPh	Me
c	CH_2CO_2Me	H	COMe	Me
d	CH_2CO_2Me	H	COPh	Me
e	Me	Me	COMe	CO_2Et

Scheme 1.

reflux with methyl or ethyl alcohol, giving the final products **5a–e** in good yields (86.2–93.4%).

The reaction of 1-*NH*-Boc protected 1,2-diaminopyrroles **6a–b** (see Scheme 2) with 5-[4′-bromomethyl-1,1′-biphenyl-2-yl]-1-triphenylmethyl-1*H*-tetrazole in dichloromethane at r.t. in the presence of sodium hydroxide led exclusively to the *N*-alkylation of the amino group linked to the nitrogen heteroatom, producing the compounds **7a–b** (57–74.8%). Acylation of the amino group at 2-position of the pyrrole ring of these compounds with valeryl chloride at r.t. furnished the derivatives **8a–b** (63.8–64%). The target products **9a–b** (79.9–95.1%) were obtained by detritylation of the compounds **8a–b** with methyl or ethyl alcohol under reflux.

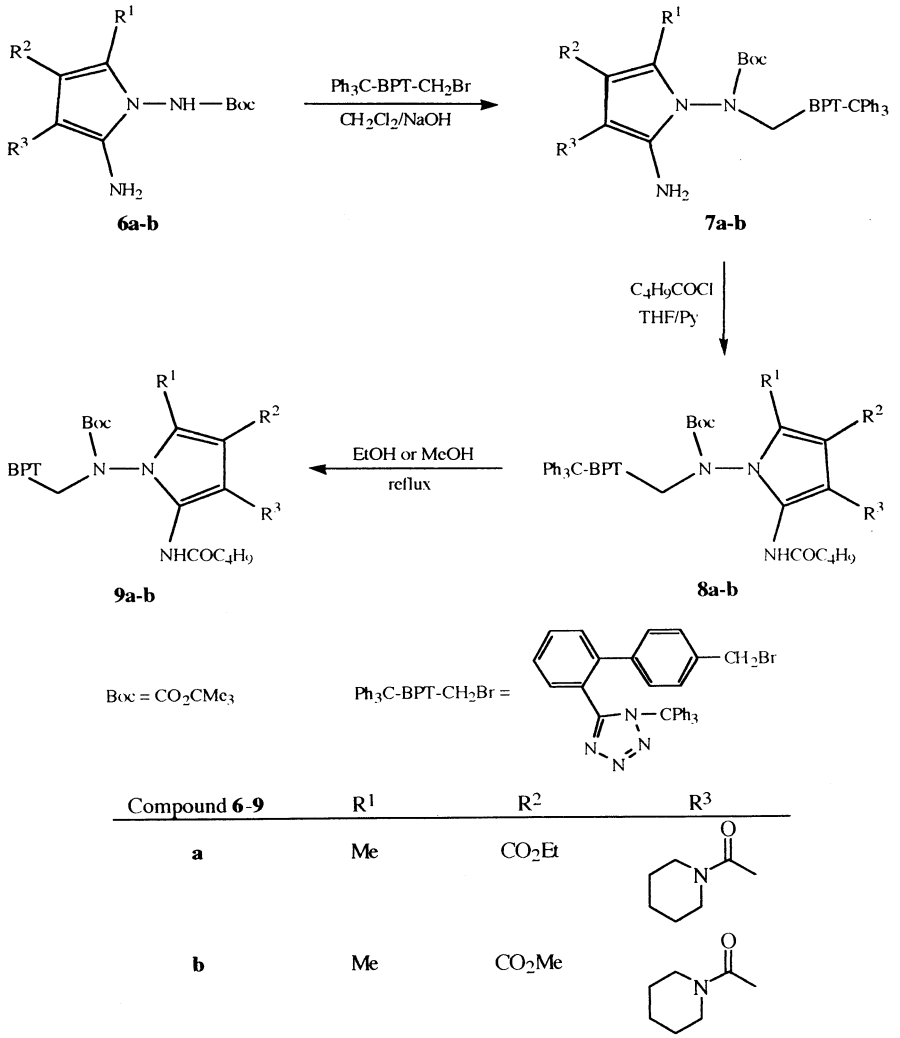
4. Pharmacology

The series of compounds (**5a**, **5b**, **5e**, and **9a**) were measured for their interaction with the receptor by way of their inhibition of [^{3}H]AII binding to rat adrenal cortex membrane preparations (AT_1 receptors). In spite of the promising chemical and theoretical assumptions, these new compounds unfortunately were found inactive ($pK_i \leq 5$).

5. Conclusions

Unfortunately the examined compounds did not possess remarkable affinity for AT_1 receptor. The reduced binding affinity shown by these central 1-aminopyrrole compounds might be due to the fact that the conformational behaviour of these molecules is not in agreement with the geometrical activity model derived from published data and theoretical calculations. The almost planar disposition of the 1-aminopyrrole moiety could allow the structure to exist in conformations not optimal for receptor binding. Nevertheless, there is a conceptual difference between the computational results and the way the compounds act in the biological experiments.

The calculations deal with molecules in the gas phase, no outside influences are present at all, whereas compounds in a biological environment are surrounded by a complicated liquid phase where they interact with many other molecules in polar and apolar circum-



Scheme 2.

stances. This might cause discrepancies in any comparison with experimental results and does not facilitate our search for a relationship between antagonist activity and calculated properties.

The energy differences among the rotational isomers of our compounds are small enough to allow the substituents large rotational freedom. Interactions with solvent molecules might determine the actual conformation of the compounds rather than the intramolecular forces that dictate the molecular mechanic equilibrium geometries.

More populated low-energy conformers, which are incompatible with the hypothesised receptor-bound structure, might be present. Thus the important functional groups could assume spatial positions that give rise to unfavourable interactions inside the receptor.

However, it should be emphasised that this obviously is pure speculation and is based on possible interpretations of the negative results and that this is not a model directly supported by the presented data.

6. Experimental

6.1. Chemistry

Commercially available solvents were used without further purification, except for THF, which was distilled on sodium hydroxide and stored under 4Å molecular sieves. Valeryl chloride was commercial material (Janssen Chimica) and was used without further purification. 5-[4′-Bromomethyl-1,1′-biphenyl-2-yl]-1-triphenyl-methyl-1*H*-tetrazole was prepared as reported [27]. 1-*NH*-Boc protected aminopyrroles **1a–b** are known [28], **1c–e** are new compounds and were prepared by analogous methodology; **6a** was previously obtained [29], **6b** was synthesised according to the same procedure. Characterisation and spectroscopic data of **1c–e** and **6b** are reported below. Melting points were determined in open capillary tubes with a Gallenkamp apparatus and are uncorrected. All yields referred to pure isolated products. All FT-IR spectra were per-

formed in KBr for Nujol mulls and were obtained using a Nicolet Impact 400 spectrophotometer. MS spectra were performed with a Hewlett–Packard 5995C spectrometer. All ^{1}H NMR spectra were measured using a Bruker AC-200 (200 MHz) Fourier transform spectrometer equipped with cryomagnet, in DMSO-d_6 solution unless otherwise stated. All the spectra were measured at 298 K. Chemical shifts (δ_H) are reported in ppm downfield from internal Me_4Si. *J*-Values are given in Hz. The abbreviations used are as follows: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; br s, broad singlet. The assignments of NH and NH_2 signals were confirmed by the disappearance of the signals after addition of deuterium oxide. Macherey–Nagel precoated silica gel SIL G-25UV$_{254}$ plates (0.25 mm thick) were used for analytical TLC and silica gel Amicon LC 60Å (35–70 μ) for column chromatography.

1c: 55.4% yield as colourless crystals from ether, m.p. 103–104°C. ^{1}H NMR δ: 1.45 (s, 9H, OBu^t), 2.29 (s, 6H, $2CH_3$), 3.50–3.63 (m, 5H, $CH_2CO_2CH_3$ and OCH_3), 6.39 (s, 1H, CH), 10.30 (br s, 1H, NH). IR (KBr) cm^{-1}: 3270, 1740, 1660, 1640, 1580. MS *m*/*z* (rel. int.%): 310 (M^+, 29), 254 (44), 195 (100). *Anal.* Calc. for $C_{15}H_{22}N_2O_5$: C, 58.05; H, 7.15; N, 9.03. Found: C, 57.98; H, 7.24; N, 8.93%.

1d: 72.2% yield as colourless crystals from ether, m.p. 152–154°C (dec.). ^{1}H NMR δ: 1.46 (s, 9H, OBu^t), 2.29 (s, 3H, CH_3), 3.52–3.67 (m, 5H, $CH_2CO_2CH_3$ and OCH_3), 6.16 (s, 1H, CH), 7.47–7.68 (m, 5H, arom.), 10.38 (br s, 1H, NH). IR (KBr) cm^{-1}: 3195, 1740, 1730, 1620, 1600, 1570. MS *m*/*z* (rel. int.%): 372 (M^+, 91), 316 (100), 271 (45), 256 (59). *Anal.* Calc. for $C_{20}H_{24}N_2O_5$: C, 64.50; H, 6.50; N, 7.52. Found: C, 64.36; H, 6.62; N, 7.36%.

1e: 62% yield as white powder from dichloromethane–light petroleum (b.p. 40–60°C), m.p. 137–139°C. ^{1}H NMR δ: 1.28 (t, 3H, $J = 7.1$ Hz, OCH_2CH_3), 1.45 (s, 9H, OBu^t), 1.94 (s, 3H, CH_3), 2.01 (s, 3H, CH_3), 2.17 (s, 3H, CH_3), 4.30 (q, 2H, $J = 7.1$ Hz, OCH_2CH_3), 10.36 (br s, 1H, NH). IR (KBr) cm^{-1}: 3316, 1742, 1716, 1638, 1601. MS *m*/*z* (rel. int.%): 324 (M^+, 51), 251 (69), 151 (100). *Anal.* Calc. for $C_{16}H_{24}N_2O_5$: C, 59.24; H, 7.46; N, 8.64. Found: C, 59.36; H, 7.32; N, 8.78%.

6b: 60.02% yield as beige powder from ether–light petroleum (b.p. 40–60°C), m.p. 165–168°C (dec.). ^{1}H NMR δ: 1.43–1.54 (m, 15H, cyclic CH_2 and OBu^t), 2.16 (s, 3H, CH_3), 3.62 (s, 3H, OCH_3), 3.32–3.40 (m, 5H, cyclic CH_2), 4.71 (s, 2H, NH_2), 9.90 (s, 1H, NH). IR (KBr) cm^{-1}: 3440, 3360, 3220, 1725, 1700, 1670. MS *m*/*z* (rel. int.%): 380 (M^+, 44), 324 (57), 280 (56), 195 (61), 84 (100). *Anal.* Calc. for $C_{18}H_{28}N_4O_5$: C, 56.83; H, 7.42; N, 14.73. Found: C, 56.95; H, 7.42; N, 14.60%.

*6.1.1. Synthesis of the 1-aminopyrrole derivatives (**2a–e**)*

The *NH*-Boc protected 1-aminopyrrole derivatives **1a–e** (1 mmol) were heated in an oil bath at 170°C until the starting compounds disappeared (20–40 min, monitored by TLC). The resulting dark residues were purified by chromatography on a silica gel column by elution with cyclohexane–ethyl acetate mixtures to give the relevant 1-aminopyrrole derivatives **2a–e**.

2a: 69.5% yield as yellowish powder from dichloromethane–light petroleum (b.p. 40–60°C), m.p. 104–106°C. ^{1}H NMR δ: 1.20 (t, 3H, $J = 6.6$ Hz, OCH_2CH_3), 2.24 (s, 3H, CH_3), 2.43 (s, 3H, CH_3), 3.65 (s, 2H, $CH_2CO_2CH_2CH_3$), 4.09 (q, 2H, $J = 6.6$ Hz, OCH_2CH_3), 5.50 (s, 2H, NH_2), 6.26 (s, 1H, CH). IR (KBr) cm^{-1}: 3335, 1707, 1657. MS *m*/*z* (rel. int.%): 224 (M^+, 30), 151 (100). *Anal.* Calc. for $C_{11}H_{16}N_2O_3$: C, 58.91; H, 7.19; N, 12.49. Found: C, 58.79; H, 7.30; N, 12.65%.

2b: 84.7% yield as white powder from ethyl ether, m.p. 79.5–81.5°C. ^{1}H NMR δ: 1.19 (t, 3H, $J = 7.1$ Hz, OCH_2CH_3), 2.48 (s, 3H, CH_3), 3.68 (s, 2H, $CH_2CO_2CH_2CH_3$), 4.12 (q, 2H, $J = 7.1$ Hz, OCH_2CH_3), 5.61 (s, 2H, NH_2), 6.04 (s, 1H, CH), 7.47–7.55 (m, 3H, arom.), 7.62–7.66 (m, 2H, arom.). IR (KBr) cm^{-1}: 3352, 3272, 1722, 1612, 1564. MS *m*/*z* (rel. int.%): 286 (M^+, 80), 213 (100). *Anal.* Calc. for $C_{16}H_{18}N_2O_3$: C, 67.12; H, 6.34; N, 9.78. Found: C, 67.00; H, 6.45; N, 9.66%.

2c: 75.9% yield as yellowish powder from dichloromethane–light petroleum (b.p. 40–60°C), m.p. 93–93.5°C. ^{1}H NMR δ: 2.23 (s, 3H, CH_3), 2.43 (s, 3H, CH_3), 3.62 (s, 3H, OCH_3), 3.66 (s, 2H, $CH_2CO_2CH_3$), 5.50 (s, 2H, NH_2), 6.26 (s, 1H, CH). IR (KBr) cm^{-1}: 3340, 3280, 1710, 1660. MS *m*/*z* (rel. int.%): 210 (M^+, 55), 195 (22), 151 (100). *Anal.* Calc. for $C_{10}H_{14}N_2O_3$: C, 58.05; H, 7.15; N, 9.03. Found: C, 57.96; H, 7.23; N, 9.03%.

2d: 83.1% yield as yellowish crystals from dichloromethane–light petroleum (b.p. 40–60°C), m.p. 103–104°C. ^{1}H NMR δ: 2.47 (s, 3H, CH_3), 3.62 (s, 3H, OCH_3), 3.70 (s, 2H, $CH_2CO_2CH_3$), 5.60 (s, 2H, NH_2), 6.03 (s, 1H, CH), 7.47–7.65 (m, 5H, arom.). IR (KBr) cm^{-1}: 3359, 3270, 1733, 1720, 1613, 1563. MS *m*/*z* (rel. int.%): 272 (M^+, 95), 213 (100). *Anal.* Calc. for $C_{15}H_{16}N_2O_3$: C, 66.16; H, 5.92; N, 10.29. Found: C, 66.31; H, 5.78; N, 10.43%.

2e: 91.3% yield as yellow oil. ^{1}H NMR ($CDCl_3$) δ: 1.37 (t, 3H, $J = 7.1$ Hz, OCH_2CH_3), 2.08 (s, 3H, CH_3), 2.12 (s, 3H, CH_3), 2.43 (s, 3H, CH_3), 4.30–4.41 (m, 4H, NH_2 and OCH_2CH_3). IR (KBr) cm^{-1}: 3354, 3220, 1735, 1627. MS *m*/*z* (rel. int.%): 224 (M^+, 18), 151 (100). *Anal.* Calc. for $C_{11}H_{16}N_2O_3$: C, 58.91; H, 7.19; N, 12.49. Found: C, 58.92; H, 7.31; N, 12.34%.

*6.1.2. Synthesis of the N-acyl-1-aminopyrrole derivatives (**3a–e**)*

Valeryl chloride (181 mg, 1.501 mmol) dissolved in anhydrous THF (1 ml) was slowly added to a stirred solution of the 1-aminopyrrole derivatives **2a–e** (1 mmol) and anhydrous pyridine (127 mg, 1.605 mmol) in anhydrous THF (4 ml). After the formation of a white solid, the reaction mixture was refluxed until 1-aminopyrrole derivatives disappeared (2–5 h, monitored by TLC). After the evaporation of the solvent under reduced pressure, the residue was solved in ethyl ether, poured into a separatory funnel and washed twice with water. The organic layer was dried over anhydrous sodium sulfate and evaporated under reduced pressure to give the residue that afforded the *N*-acyl derivatives **3a–e** by crystallisation from ethyl ether–light petroleum (b.p. 40–60°C) or by chromatography on a silica gel column by elution with cyclohexane–ethyl acetate mixtures.

3a: 85.6% yield as colourless plates from ethyl ether–light petroleum (b.p. 40–60°C), m.p. 73–75°C. ^{1}H NMR δ: 0.90 (t, 3H, $J = 7.2$ Hz, $COCH_2CH_2CH_2CH_3$), 1.19 (t, 3H, $J = 7.1$ Hz, OCH_2CH_3), 1.27–1.38 (m, 2H, $COCH_2CH_2CH_2CH_3$), 1.49–1.61 (m, 2H, $COCH_2CH_2CH_2CH_3$), 2.22–2.32 (m, 8H, $2CH_3$ and $COCH_2CH_2CH_2CH_3$), 3.33 (d, 1H, $J = 16.8$ Hz, $CH_2CO_2CH_2CH_3$), 3.58 (d, 1H, $J = 16.8$ Hz, $CH_2CO_2CH_2CH_3$), 4.07 (q, 2H, $J = 7.1$ Hz, OCH_2CH_3), 6.40 (s, 1H, CH), 10.96 (s, 1H, NH). IR (KBr) cm^{-1}: 3301, 1719, 1690, 1665. MS *m*/*z* (rel. int.%): 308 (M^+, 47), 235 (41), 208 (100). *Anal.* Calc. for $C_{16}H_{24}N_2O_4$: C, 62.32; H, 7.84; N, 9.08. Found: C, 62.44; H, 7.71; N, 9.21%.

3b: 80.4% yield as orange oil. ^{1}H NMR δ: 0.91 (t, 3H, $J = 7.4$ Hz, $COCH_2CH_2CH_2CH_3$), 1.19 (t, 3H, $J = 7.3$ Hz, OCH_2CH_3), 1.28–1.40 (m, 2H, $COCH_2CH_2CH_2CH_3$), 1.51–1.64 (m, 2H, $COCH_2CH_2CH_2CH_3$), 2.28–2.36 (m, 5H, CH_3 and $COCH_2CH_2CH_2CH_3$), 3.37 (d, 1H, $J = 17.0$ Hz, $CH_2CO_2CH_2CH_3$), 3.63 (d, 1H, $J = 17.0$ Hz, $CH_2CO_2CH_2CH_3$), 4.07 (q, 2H, $J = 7.3$ Hz, OCH_2CH_3), 6.19 (s, 1H, CH), 7.51–7.69 (m, 5H, arom.), 11.05 (s, 1H, NH). IR (KBr) cm^{-1}: 3247, 3190, 1746, 1713, 1684, 1641, 1570. MS *m*/*z* (rel. int.%): 370 (M^+, 61), 270 (76), 196 (100). *Anal.* Calc. for $C_{21}H_{26}N_2O_4$: C, 68.09; H, 7.07; N, 7.56. Found: C, 68.21; H, 7.17; N, 7.43%.

3c: 94.5% yield as white powder from ethyl ether, m.p. 77–78°C. ^{1}H NMR δ: 0.90 (t, 3H, $J = 7.0$ Hz, $COCH_2CH_2CH_2CH_3$), 1.23–1.41 (m, 2H, $COCH_2CH_2CH_2CH_3$), 1.49–1.69 (m, 2H, $COCH_2CH_2CH_2CH_3$), 2.22–2.32 (m, 8H, $2CH_3$ and $COCH_2CH_2CH_2CH_3$), 3.55–3.64 (m, 5H, OCH_3 and $CH_2CO_2CH_3$), 6.41 (s, 1H, CH), 10.99 (s, 1H, NH). IR (KBr) cm^{-1}: 3309, 1726, 1689, 1664. MS *m*/*z* (rel. int.%): 294 (M^+, 43), 194 (100). *Anal.* Calc. for $C_{15}H_{22}N_2O_4$: C, 58.05; H, 7.15; N, 9.03. Found: C, 57.97; H, 7.27; N, 9.17%.

3d: 91.7% yield as yellow oil. ^{1}H NMR ($CDCl_3$) δ: 0.94 (t, 3H, $J = 7.2$ Hz, $COCH_2CH_2CH_2CH_3$), 1.31–1.49 (m, 2H, $COCH_2CH_2CH_2CH_3$), 1.63–1.78 (m, 2H, $COCH_2CH_2CH_2CH_3$), 2.23 (s, 3H, CH_3), 2.40 (t, 2H, $J = 7.3$ Hz, $COCH_2CH_2CH_2CH_3$), 3.42 (d, 1H, $J = 16.0$ Hz, $CH_2CO_2CH_3$), 3.64 (d, 1H, $J = 16.0$ Hz, $CH_2CO_2CH_3$), 3.68 (s, 3H, OCH_3), 6.26 (s, 1H, CH), 7.42–7.52 (m, 3H, arom.), 7.74–7.79 (m, 2H, arom.), 9.34 (s, 1H, NH). IR (KBr) cm^{-1}: 3241, 1744, 1716, 1637, 1577. MS *m*/*z* (rel. int.%): 356 (M^+, 83), 256 (78), 196 (100). *Anal.* Calc. for $C_{20}H_{24}N_2O_4$: C, 67.40; H, 6.79; N, 7.86. Found: C, 67.40; H, 6.64; N, 7.99%.

3e: 93.1% yield as yellow oil. ^{1}H NMR ($CDCl_3$) δ: 0.88 (t, 3H, $J = 7.1$ Hz, $COCH_2CH_2CH_2CH_3$), 1.26–1.39 (m, 5H, OCH_2CH_3 and $COCH_2CH_2CH_2CH_3$), 1.54–1.67 (m, 2H, $COCH_2CH_2CH_2CH_3$), 1.83 (s, 3H, CH_3), 1.95 (s, 3H, CH_3), 2.08 (s, 3H, CH_3), 2.30 (t, 2H, $J = 7.1$ Hz, $COCH_2CH_2CH_2CH_3$), 4.29 (q, 2H, $J = 7.1$ Hz, OCH_2CH_3), 9.60 (s, 1H, NH). IR (KBr) cm^{-1}: 3243, 1738, 1683, 1645. MS *m*/*z* (rel. int.%): 308 (M^+, 11), 235 (100). *Anal.* Calc. for $C_{16}H_{24}N_2O_4$: C, 62.32; H, 7.84; N, 9.08. Found: C, 62.42; H, 7.72; N, 9.21%.

*6.1.3. Synthesis of the compounds **4a–e***

Sodium hydroxide as tritured pellets (80 mg, 2 mmol) was added to a stirred solution of the *N*-acyl derivatives **3a–e** (1 mmol) and 5-[4′-bromomethyl-1,1′-biphenyl-2-yl]-1-triphenylmethyl-1*H*-tetrazole (835 mg, 1.5 mmol) in dichloromethane (4 ml). The reaction mixture was allowed to stand at r.t. until the *N*-acyl derivatives disappeared (7–9 days, monitored by TLC). The solvent was removed under reduced pressure and the residue was solved in ethyl acetate, poured into a separatory funnel and washed with water until neutrality of the aqueous phase. The organic layer was dried over anhydrous sodium sulfate and evaporated under reduced pressure. The crude product was purified by chromatography on a silica gel column by elution with cyclohexane–ethyl acetate mixtures to obtain the respective products **4a–e**.

4a: 88.7% yield as white foam. ^{1}H NMR δ: 0.81 (t, 3H, $J = 7.2$ Hz, $COCH_2CH_2CH_2CH_3$), 1.10–1.22 (m, 5H, OCH_2CH_3 and $COCH_2CH_2CH_2CH_3$), 1.36–1.82 (m, 7H, CH_3 and $COCH_2CH_2CH_2CH_3$), 2.28 (s, 3H, CH_3), 3.31 (s, 2H, $CH_2CO_2CH_2CH_3$), 4.04 (q, 2H, $J = 7.0$ Hz, OCH_2CH_3), 4.62 (d, 1H, $J = 14.4$ Hz, CH_2–Ar), 4.99 (d, 1H, $J = 14.4$ Hz, CH_2–Ar), 6.51 (s, 1H, CH), 6.90–7.77 (m, 23H, arom.). IR (KBr) cm^{-1}: 1741, 1692, 1669. *Anal.* Calc. for $C_{49}H_{48}N_6O_4$: C, 74.98; H, 6.16; N, 10.71. Found: C, 74.86; H, 6.28; N, 10.60%.

4b: 73.1% yield as white foam. ^{1}H NMR δ: 0.80 (t, 3H, $J = 7.2$ Hz, $COCH_2CH_2CH_2CH_3$), 1.08–1.24 (m, 5H, OCH_2CH_3 and $COCH_2CH_2CH_2CH_3$), 1.39–1.49 (m, 2H, $COCH_2CH_2CH_2CH_3$), 1.72–1.82 (m, 5H, CH_3

and $COCH_2CH_2CH_2CH_3$), 3.28 (s, 2H, $CH_2CO_2CH_2$-CH_3), 3.99 (q, 2H, $J = 7.1$ Hz, OCH_2CH_3), 4.65 (d, 1H, $J = 14.4$ Hz, CH_2–Ar), 4.97 (d, 1H, $J = 14.4$ Hz, CH_2–Ar), 6.25 (s, 1H, CH), 6.86–7.79 (m, 28H, arom.). IR (KBr) cm^{-1}: 1738, 1689, 1643, 1601. *Anal*. Calc. for $C_{54}H_{50}N_6O_4$: C, 76.57; H, 5.95; N, 9.92. Found: C, 76.44; H, 6.07; N, 9.78%.

4c: 59.2% yield as white foam. ^{1}H NMR δ: 0.78 (t, 3H, $J = 7.1$ Hz, $COCH_2CH_2CH_2CH_3$), 1.07–1.22 (m, 2H, $COCH_2CH_2CH_2CH_3$), 1.38–1.49 (m, 2H, $COCH_2CH_2CH_2CH_3$), 1.68–1.83 (m, 5H, CH_3 and $COCH_2CH_2CH_2CH_3$), 2.26 (s, 3H, CH_3), 3.30 (s, 2H, $CH_2CO_2CH_3$), 3.55 (s, 3H, OCH_3), 4.64 (d, 1H, $J =$ 14.3 Hz, CH_2–Ar), 4.95 (d, 1H, $J = 14.3$ Hz, CH_2–Ar), 6.49 (s, 1H, CH), 6.89–7.79 (m, 23H, arom.). IR (KBr) cm^{-1}: 1742, 1687, 1662, 1578. *Anal*. Calc. for $C_{48}H_{46}N_6O_4$: C, 74.78; H, 6.01; N, 10.90. Found: C, 74.90; H, 6.15; N, 10.76%.

4d: 68.9% yield as white foam. ^{1}H NMR ($CDCl_3$) δ: 0.88 (t, 3H, $J = 7.1$ Hz, $COCH_2CH_2CH_2CH_3$), 1.22–1.33 (m, 2H, $COCH_2CH_2CH_2CH_3$), 1.58–1.65 (m, 2H, $COCH_2CH_2CH_2CH_3$), 1.83–1.95 (m, 2H, $COCH_2CH_2$-CH_2CH_3), 2.10 (s, 3H, CH_3), 3.04 (s, 2H, CH_2CO_2-CH_3), 3.59 (s, 3H, OCH_3), 4.65 (d, 1H, $J = 14.0$ Hz, CH_2–Ar), 4.78 (d, 1H, $J = 14.0$ Hz, CH_2–Ar), 6.33 (s, 1H, CH), 6.90–7.78 (m, 28H, arom.). IR (KBr) cm^{-1}: 1742, 1687, 1644, 1585. *Anal*. Calc. for $C_{53}H_{48}N_6O_4$: C, 76.42; H, 5.81; N, 10.09. Found: C, 76.27; H, 5.81; N, 10.22%.

4e: 71.0% yield as white foam. ^{1}H NMR δ: 0.75 (t, 3H, $J = 7.1$ Hz, $COCH_2CH_2CH_2CH_3$), 1.10–1.22 (m, 5H, OCH_2CH_3 and $COCH_2CH_2CH_2CH_3$), 1.40–1.54 (m, 2H, $COCH_2CH_2CH_2CH_3$), 1.61–1.77 (m, 8H, $2CH_3$ and $COCH_2CH_2CH_2CH_3$), 1.95 (s, 3H, CH_3), 4.22 (q, 2H, $J = 7.1$ Hz, OCH_2CH_3), 4.70 (d, 1H, $J = 14.3$ Hz, CH_2–Ar), 4.90 (d, 1H, $J = 14.3$ Hz, CH_2–Ar), 6.78–7.76 (m, 23H, arom.). IR (KBr) cm^{-1}: 1737, 1685, 1646, 1600. *Anal*. Calc. for $C_{49}H_{48}N_6O_4$: C, 74.98; H, 6.16; N, 10.71. Found: C, 74.85; H, 6.28; N, 10.56%.

6.1.4. Synthesis of the compounds **7a–b**

Sodium hydroxide as trituted pellets (80 mg, 2 mmol) was added to a stirred solution of the 1-*NH*-Boc protected 1,2-diaminopyrroles **6a–b** (1 mmol) and 5-[4′-bromomethyl-1,1′-biphenyl-2-yl]-1-triphenylmethyl-1*H*-tetrazole (835 mg, 1.5 mmol) in dichloromethane (4 ml). The reaction mixture was allowed to stand at r.t. until the pyrrole derivatives disappeared (5–7 days, monitored by TLC). The reaction mixture was poured into a separatory funnel and washed with brine until neutrality of the aqueous phase. The organic layer was dried over anhydrous sodium sulfate and the extraction solvent was removed under reduced pressure. The residue was chromatographed on a silica gel column by elution with cyclohexane–ethyl acetate mixtures to afford the pertinent pure products **7a–b**.

7a: 74.8% yield as white powder from chloroform–light petroleum (b.p. 40–60°C), m.p. 119–126°C (dec.). ^{1}H NMR δ: 1.13 (t, 3H, $J = 7.1$ Hz, OCH_2CH_3), 1.32–1.50 (m, 15H, cyclic CH_2 and OBu^t), 1.64 (s, 3H, CH_3), 3.30–3.42 (m, 4H, cyclic CH_2), 4.00 (q, 2H, $J = 7.1$ Hz, OCH_2CH_3), 4.38 (d, 1H, $J = 14.7$ Hz, CH_2–Ar), 4.59 (br s, 2H, NH_2), 5.02 (d, 1H, $J = 14.7$ Hz, CH_2–Ar), 6.86–7.79 (m, 23H, arom.). IR (KBr) cm^{-1}: 3420, 3320, 1690, 1642, 1601. *Anal*. Calc. for $C_{52}H_{54}N_8O_5$: C, 71.70; H, 6.25; N, 12.86. Found: C, 71.56; H, 6.37; N, 12.72%.

7b: 57.0% yield as white powder from chloroform–light petroleum (b.p. 40–60°C), m.p. 118–125°C (dec.). ^{1}H NMR δ: 1.32–1.62 (m, 18H, cyclic CH_2, OBu^t and CH_3), 3.32–3.43 (m, 4H, cyclic CH_2), 3.55 (s, 3H, OCH_3), 4.37 (d, 1H, $J = 14.7$ Hz, CH_2–Ar), 4.73 (br s, 2H, NH_2), 5.06 (d, 1H, $J = 14.7$ Hz, CH_2–Ar), 6.88–7.81 (m, 23H, arom.). IR (KBr) cm^{-1}: 3411, 3314, 1707, 1691, 1604. *Anal*. Calc. for $C_{51}H_{52}N_8O_5$: C, 71.48; H, 6.12; N, 13.07. Found: C, 71.36; H, 6.26; N, 13.07%.

6.1.5. Synthesis of the N-acyl derivatives (**8a–b**)

Valeryl chloride (181 mg, 1.501 mmol) dissolved in anhydrous THF (1 ml) was slowly added to a stirred solution of the 1-aminopyrrole derivatives **7a–b** (1 mmol) and anhydrous pyridine (127 mg, 1.605 mmol) in anhydrous THF (4 ml). After the formation of a white solid, the reaction mixture was stirred at r.t. until compounds **7a–b** disappeared (17–24 h, monitored by TLC). After the evaporation of the solvent under reduced pressure, the residue was solved in dichloromethane, poured into a separatory funnel and washed twice with water. The organic layer was dried over anhydrous sodium sulfate and evaporated under reduced pressure to give a residue that directly provided the *N*-acyl derivatives **8a–b** by crystallisation from ethyl ether.

8a: 63.8% yield as whitish powder from ethyl ether, m.p. 105–110°C (dec.). ^{1}H NMR δ: 0.90 (t, 3H, $J = 7.1$ Hz, $COCH_2CH_2CH_2CH_3$), 1.16 (t, 3H, $J = 7.1$ Hz, OCH_2CH_3), 1.32–1.59 (m, 22H, OBu^t, cyclic CH_2, CH_3 and $COCH_2CH_2CH_2CH_3$), 2.24–2.30 (m, 2H, $COCH_2CH_2CH_2CH_3$), 3.18–3.23 (m, 2H, cyclic CH_2), 3.68–3.74 (m, 2H, cyclic CH_2), 4.04 (q, 2H, $J = 7.1$ Hz, OCH_2CH_3), 4.25–4.36 (m, 1H, CH_2–Ar), 4.92–5.23 (m, 1H, CH_2–Ar), 6.90–7.81 (m, 23H, arom.), 9.56 (br s, 1H, NH). IR (KBr) cm^{-1}: 3165, 1710, 1680, 1599. *Anal*. Calc. for $C_{57}H_{62}N_8O_6$: C, 71.68; H, 6.54; N, 11.73. Found: C, 71.81; H, 6.42; N, 11.73%.

8b: 64.0% yield as light yellow powder from chloroform–ethyl ether–light petroleum (b.p. 40–60°C), m.p. 157–158°C (dec.). ^{1}H NMR δ: 0.89 (t, 3H, $J = 7.1$ Hz, $COCH_2CH_2CH_2CH_3$), 1.30–1.56 (m, 22H, OBu^t, cyclic CH_2, CH_3 and $COCH_2CH_2CH_2CH_3$), 2.25 (t, 2H, $J =$ 6.6 Hz, $COCH_2CH_2CH_2CH_3$), 3.17–3.22 (m, 2H, cyclic CH_2), 3.47–3.51 (m, 2H, cyclic CH_2), 3.58 (s, 3H,

OCH_3), 4.23–4.34 (m, 1H, CH_2–Ar), 4.91–5.19 (m, 1H, CH_2–Ar), 6.87–7.79 (m, 23H, arom.), 9.48 (br s, 1H, NH). IR (KBr) cm^{-1}: 3165, 1710, 1670, 1600. *Anal.* Calc. for $C_{56}H_{60}N_8O_6$: C, 71.47; H, 6.43; N, 11.91. Found: C, 71.61; H, 6.31; N, 12.04%.

6.1.6. Synthesis of the biphenyltetrazole derivatives (5a–e) and (9a–b)

The compounds **4a–e** and **8a–b** were heated in EtOH or MeOH under reflux until complete deprotection (1.5–3 h, monitored by TLC). The solvent was eliminated under reduced pressure and the crude product was chromatographed on a silica gel column by elution with cyclohexane–ethyl acetate mixtures to yield the biphenyltetrazole derivatives **5a–e** and **9a–b**.

5a: 89.4% yield as yellowish foam. ^1H NMR δ: 0.79 (t, 3H, $J = 7.2$ Hz, $COCH_2CH_2CH_2CH_3$), 1.10–1.23 (m, 5H, OCH_2CH_3 and $COCH_2CH_2CH_2CH_3$), 1.37–1.49 (m, 2H, $COCH_2CH_2CH_2CH_3$), 1.72–1.91 (m, 5H, CH_3 and $COCH_2CH_2CH_2CH_3$), 2.32 (s, 3H, CH_3), 3.21 (d, 1H, $J = 17.1$ Hz, $CH_2CO_2CH_2CH_3$), 3.34 (d, 1H, $J = 17.1$ Hz, $CH_2CO_2CH_2CH_3$), 4.04 (q, 2H, $J = 7.1$ Hz, OCH_2CH_3), 4.65 (d, 1H, $J = 14.3$ Hz, CH_2–Ar), 4.98 (d, 1H, $J = 14.3$ Hz, CH_2–Ar), 6.51 (s, 1H, CH), 7.01–7.12 (m, 4H, arom.), 7.49–7.69 (m, 4H, arom.), 16.21 (br s, 1H, NH). IR (KBr) cm^{-1}: 3471, 1738, 1692, 1666, 1579. *Anal.* Calc. for $C_{30}H_{34}N_6O_4$: C, 66.40; H, 6.32; N, 15.49. Found: C, 66.54; H, 6.20; N, 15.37%.

5b: 93.4% yield as yellow foam. ^1H NMR δ: 0.79 (t, 3H, $J = 7.1$ Hz, $COCH_2CH_2CH_2CH_3$), 1.09–1.22 (m, 5H, OCH_2CH_3 and $COCH_2CH_2CH_2CH_3$), 1.39–1.51 (m, 2H, $COCH_2CH_2CH_2CH_3$), 1.76–1.91 (m, 5H, CH_3 and $COCH_2CH_2CH_2CH_3$), 3.22 (d, 1H, $J = 17.3$ Hz, $CH_2CO_2CH_2CH_3$), 3.34 (d, 1H, $J = 17.3$ Hz, $CH_2CO_2CH_2CH_3$), 4.01 (q, 2H, $J = 7.1$ Hz, OCH_2CH_3), 4.68 (d, 1H, $J = 14.3$ Hz, CH_2–Ar), 4.98 (d, 1H, $J =$ 14.3 Hz, CH_2–Ar), 6.27 (s, 1H, CH), 7.03–7.66 (m, 13H, arom.), 16.23 (br s, 1H, NH). IR (KBr) cm^{-1}: 3431, 1733, 1685, 1638, 1597, 1569. *Anal.* Calc. for $C_{35}H_{36}N_6O_4$: C, 69.52; H, 6.00; N, 13.90. Found: C, 69.40; H, 6.12; N, 14.05%.

5c: 86.2% yield as white foam. ^1H NMR δ: 0.78 (t, 3H, $J = 7.2$ Hz, $COCH_2CH_2CH_2CH_3$), 1.09–1.22 (m, 2H, $COCH_2CH_2CH_2CH_3$), 1.38–1.52 (m, 2H, $COCH_2CH_2CH_2CH_3$), 1.69–1.79 (m, 2H, $COCH_2CH_2CH_2CH_3$), 1.89 (s, 3H, CH_3), 2.31 (s, 3H, CH_3), 3.18–3.30 (m, 2H, $CH_2CO_2CH_3$), 3.58 (s, 3H, OCH_3), 4.66 (d, 1H, $J = 14.3$ Hz, CH_2–Ar), 4.95 (d, 1H, $J = 14.3$ Hz, CH_2–Ar), 6.50 (s, 1H, CH), 7.01–7.07 (m, 4H, arom.), 7.48–7.68 (m, 4H, arom.), 16.43 (br s, 1H, NH). IR (KBr) cm^{-1}: 3470, 1742, 1686, 1577. *Anal.* Calc. for $C_{29}H_{32}N_6O_4$: C, 65.89; H, 6.10; N, 15.90. Found: C, 66.02; H, 6.10; N, 15.78%.

5d: 92.7% yield as yellow foam. ^1H NMR δ: 0.81 (t, 3H, $J = 7.1$ Hz, $COCH_2CH_2CH_2CH_3$), 1.14–1.25 (m, 2H, $COCH_2CH_2CH_2CH_3$), 1.43–1.50 (m, 2H, $COCH_2CH_2CH_2CH_3$), 1.79–1.88 (m, 2H, $COCH_2CH_2CH_2CH_3$), 1.92 (s, 3H, CH_3), 3.34–3.62 (m, 5H, $CH_2CO_2CH_3$ and OCH_3), 4.76 (d, 1H, $J = 14.3$ Hz, CH_2–Ar), 4.97 (d, 1H, $J = 14.3$ Hz, CH_2–Ar), 6.28 (s, 1H, CH), 7.04–7.16 (m, 4H, arom.), 7.47–7.73 (m, 9H, arom.), 16.25 (br s, 1H, NH). IR (KBr) cm^{-1}: 3151, 1743, 1690, 1643, 1572. *Anal.* Calc. for $C_{34}H_{34}N_6O_4$: C, 69.14; H, 5.80; N, 14.23. Found: C, 69.28; H, 5.68; N, 14.38%.

5e: 86.2% yield as light yellow oil. ^1H NMR δ: 0.79 (t, 3H, $J = 7.1$ Hz, $COCH_2CH_2CH_2CH_3$), 1.09–1.29 (m, 5H, OCH_2CH_3 and $COCH_2CH_2CH_2CH_3$), 1.40–1.61 (m, 2H, $COCH_2CH_2CH_2CH_3$), 1.67–1.78 (m, 5H, CH_3 and $COCH_2CH_2CH_2CH_3$), 1.87 (s, 3H, CH_3), 2.02 (s, 3H, CH_3), 4.30 (q, 2H, $J = 7.1$ Hz, OCH_2CH_3), 4.76 (d, 1H, $J = 14.3$ Hz, CH_2–Ar), 4.90 (d, 1H, $J = 14.3$ Hz, CH_2–Ar), 7.03–7.16 (m, 4H, arom.), 7.50–7.72 (m, 4H, arom.), 16.20 (br s, 1H, NH). IR (KBr) cm^{-1}: 3060, 1737, 1686, 1644, 1597. *Anal.* Calc. for $C_{30}H_{34}N_6O_4$: C, 66.40; H, 6.32; N, 15.49. Found: C, 66.26; H, 6.32; N, 15.60%.

9a: 79.9% yield as white powder from ethyl ether, m.p. 195–197°C (dec.). ^1H NMR δ: 0.88 (t, 3H, $J = 7.1$ Hz, $COCH_2CH_2CH_2CH_3$), 1.14–1.65 (m, 25H, OCH_2CH_3, $COCH_2CH_2CH_2CH_3$, OBu^t, CH_3 and cyclic CH_2), 2.19–2.25 (m, 2H, $COCH_2CH_2CH_2CH_3$), 3.08–3.71 (m, 4H, cyclic CH_2), 4.09 (q, 2H, $J = 7.1$ Hz, OCH_2CH_3), 4.25–4.37 (m, 1H, CH_2–Ar), 4.94–5.24 (m, 1H, CH_2–Ar), 7.05–7.18 (m, 4H, arom.), 7.50–7.89 (m, 4H, arom.), 9.28 and 9.63 (2 br s, 1H, NH), 16.31 (br s, 1H, NH). IR (KBr) cm^{-1}: 3360, 1712, 1695, 1585. *Anal.* Calc. for $C_{38}H_{48}N_8O_6$: C, 64.03; H, 6.79; N, 15.72. Found: C, 64.17; H, 6.66; N, 15.77%.

9b: 95.1% yield as white powder from ethyl ether, m.p. 179–182°C (dec.). ^1H NMR δ: 0.88 (t, 3H, $J = 7.1$ Hz, $COCH_2CH_2CH_2CH_3$), 1.17–1.64 (m, 22H, OBu^t, cyclic CH_2, CH_3, and $COCH_2CH_2CH_2CH_3$), 2.18–2.24 (m, 2H, $COCH_2CH_2CH_2CH_3$), 3.12–3.60 (m, 4H, cyclic CH_2), 3.64 (s, 3H, OCH_3), 4.24–4.36 (m, 1H, CH_2–Ar), 4.95–5.23 (m, 1H, CH_2–Ar), 7.09–7.18 (m, 4H, arom.), 7.50–7.69 (m, 4H, arom.), 9.31 and 9.61 (2 br s, 1H, NH), 16.31 (br s, 1H, NH). IR (KBr) cm^{-1}: 3339, 1718, 1709, 1602, 1575. *Anal.* Calc. for $C_{37}H_{46}N_8O_6$: C, 63.59; H, 6.63; N, 16.03. Found: C, 63.48; H, 6.69; N, 16.17%.

6.2. Pharmacology

6.2.1. Pharmacology [^{3}H]AII binding assay

Rat adrenal cortex membranes were prepared according to Chang et al. [30]. AII (1 μM) was used for the determination of non specific binding. Losartan (10^{-11}–10^{-4} M) was tested as a reference standard. The test compounds were dissolved in 100% DMSO at the concentration of 10^{-2} M and then diluted with

assay buffer [31] and used in the assay in range of concentrations from 10^{-11} to 10^{-4} M. For the assay, 50 μl of test compound were added into test tubes containing 100 μl of membrane suspension (0.05 mg of protein), 50 μl of [^{3}H]AII (Amersham, UK, 1.2 nM, final concentration), and assay buffer in a final volume of 0.5 ml. After 60 min. of incubation at 25°C, the reaction was terminated by filtration under reduced pressure through glass fiber GF/B filters (presoaked for 3–5 h in 0.5% bovine serum albumin solution) using a Brandel cell harvester and washed rapidly three times with ice-cold Tris–HCl (50 mM, pH 7.4). The radioactivity was determined by scintillation counting using a Packard 2200 CA scintillation counter. The K_i values were determined using EBDA/LIGAND, a non linear iterative fitting program [32].

Acknowledgements

This work was supported by financial assistance from the Ministero dell'Università e della Ricerca Scientifica e Tecnologica (MURST, Rome), the Consiglio Nazionale delle Ricerche (CNR, Rome) and the Università degli Studi di Urbino.

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