N-3-Substituted Pyrimidinones as Potent, Orally Active, AT_1 Selective Angiotensin II Receptor Antagonists[†]

Aldo Salimbeni,*[,‡] Renato Canevotti,[‡] Fabio Paleari,[‡] Davide Poma,[‡] Saturnino Caliari,[‡] Francesco Fici,[‡] Rocco Cirillo,[§] Anna R. Renzetti,[§] Alessandro Subissi,[§] Laura Belvisi,[‖] Gianpaolo Bravi,[‖] Carlo Scolastico,[‖] and Antonio Giachetti[⊥]

Medicinal Chemistry and Pharmacology Departments, Istituto Lusofarmaco,[#] 20132 Milano, Pharmacology Department, Laboratori Guidotti,[#] 56010 Pisa, Organic and Industrial Chemistry Department, University of Milano, 20133 Milano, and A. Menarini Industrie Farmaceutiche Riunite, 50131 Firenze, Italy

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A novel series of nonpeptide angiotensin II (A II) antagonists containing a pyrimidinone ring which carries a C-linked biphenyltetrazole moiety and a carboxyheteroaryl group on the 3-position have been prepared. Their affinity for the AT_1 receptor was determined in a binding assay on rat adrenal cortical membranes. The *in vivo* antihypertensive properties were tested by evaluating the inhibition of the pressor response to A II followed by iv and id administration. Extensive molecular modeling studies, including comparison of molecular electrostatic potential distributions, conformational analysis, and overlays on a computational pharmacophore model of A II, were used to evaluate structural parameters of the new compounds, in comparison to other known A II antagonists (e.g., DUP-753 and SK&F 108566). According to the modeling studies, the introduction of a (carboxyheteroaryl)methyl moiety at the 3-position of the pyrimidinone ring led to derivatives with increased potency. Methyl 2-[[4-butyl-2-methyl-6-oxo-5-[[2′-(1*H*-tetrazol-5-yl)[1,1′-biphenyl]-4-yl]methyl]-1-(6*H*)-pyrimidinyl]methyl]-3-thiophenecarboxylate (**3k**, LR-B/081), one of the most potent compounds in the series (K_i = 1.4 nM), exhibited a marked antihypertensive activity on oral administration to conscious renal hypertensive rats, with long duration of action. It was selected for clinical evaluation in the treatment of hypertension in man.

Introduction

Over the last few years, angiotensin II (A II) receptor antagonists have been considered a reliable alternative to inhibitors of angiotensin-converting enzyme (ACE) and renin in order to influence the renin angiotensin system, which plays a pivotal role in the regulation of blood pressure and fluid balance.[1] Especially the discovery by the DuPont group of a series of (biphenylmethyl)imidazoles as nonpeptide, potent, and orally active A II receptor (AT_1 subtype) antagonists has provided an important advance in the area and stimulated a profusion of research. One of the most promising structures within this series is DUP-753 (Losartan)[2,3] (Figure 1), which has already been registered in some countries.

Considerable efforts in the design and synthesis of novel analogues of this lead compound have been performed by several pharmaceutical laboratories. As a result, a variety of nonpeptide A II receptor antagonists have been reported. With the exception of SK&F 108566[4a–c] (Figure 2) and related compounds, which were directly developed from the earlier nonpeptide Takeda lead,[4d] the most interesting classes of antagonists contain the (1*H*-tetrazol-5-yl)biphenyl moiety or a slight modification. As already outlined in a previous

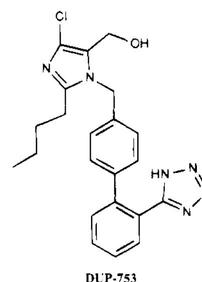

Figure 1

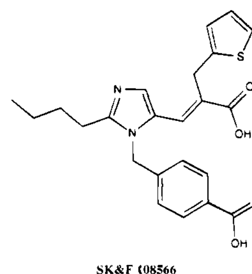

Figure 2

paper,[5] reports on efficient biphenyltetrazole replacements are scarce, especially in the imidazole series.

By contrast, a number of studies have appeared in which the imidazole moiety of DUP-753 is successfully replaced by other five- or six-membered ring heterocycles (one or more fused), indicating that the A II receptor is quite permissive in accepting this region of the nonpeptide antagonists. Examples of these imidazole-mimic groups that maintain high affinity for the AT_1 receptor include imidazopyridine,[6] benzimidazole,[7] quinoline,[8] pyrazole,[9] triazole,[10] pyrimidine,[11] and pyridine[12] as well as others. Efficient replacement of the

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* Author to whom correspondence should be addressed.
[‡] Istituto Lusofarmaco.
[§] Laboratori Guidotti.
[‖] University of Milano.
[⊥] A. Menarini Industrie Farmaceutiche Riunite.
[#] Companies related to A. Menarini Industrie Farmaceutiche Riunite.
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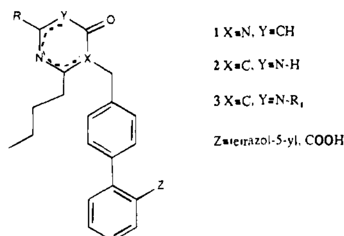


Figure 3. Compounds of general formula 1–**3**.

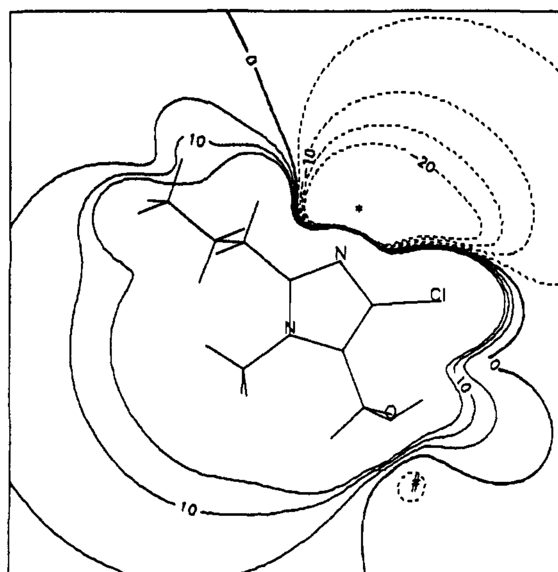
imidazole has also been realized with suitable acyclic structural units: for example, the amide group O=C—N was found to be a good substitute of the N=C—N imidazole region.[13]

On the basis of a preliminary comparative analysis of the molecular electrostatic potential (MEP) distributions, we focused our studies on the replacement of the tetra-substituted imidazole of DUP-753 with the pyrimidinone ring while preserving the other key structural features to exhibit efficient A II antagonism. An analogous modification has been described in patents by Ciba[14] and, more recently, UPSA[15] and Synthelabo.[16] Compounds of general formula **1** and **2** (Figure 3) were found to be potent A II receptor antagonists, the C-linked derivatives being more potent than the N-linked ones. As reported for imidazole-based A II antagonists,[2] the tetrazolyl analogues were found to be more potent than the corresponding carboxylic acids. Moreover, the introduction of an aromatic moiety on the N-3 atom of the pyrimidinone ring in the C-linked derivatives, following the suggestions of overlay studies employing a model of the octapeptide hormone derived from theoretical calculations, resulted in compounds **3** of enhanced potency (Figure 3 and Table 1). In this paper we describe the molecular modeling studies, the synthesis, and the biological activity of the new pyrimidinone derivatives.

Molecular Modeling

Molecular Electrostatic Potential. The recent discovery of novel classes of nonpeptide A II receptor antagonists has shown that the imidazolic ring in DUP-753 can be advantageously replaced by other heterocycles. By means of a comparative analysis of the MEP distributions of several simplified antagonists, characterized by different binding affinities, we found that the electrostatic behavior of the region surrounding the heterocycle is relevant for receptor interaction.[17] The following electrostatic potential characteristics (shown in Figure 4 for a DuPont imidazole-containing fragment) appear to be required for the recognition process: (a) a positive long-range MEP in the region of space surrounding the lipophilic C-2 side chain and (b) strongly electrophile attracting regions bulging out of the heterocycle —N= type nitrogen atom and the hydrogen bond acceptor moiety placed at the side of the heterocyclic ring.

The pyrimidinone ring in compounds of general formula **1** and **2** displays all the topological MEP characteristics indicated as necessary for A II receptor recognition (Figure 5): a positive potential region around the aliphatic chain and very deep negative zones around the —N= type ring nitrogen atom and the lactamic oxygen atom. Therefore the pyrimidinone ring represents a good candidate to replace the DuPont imidazole



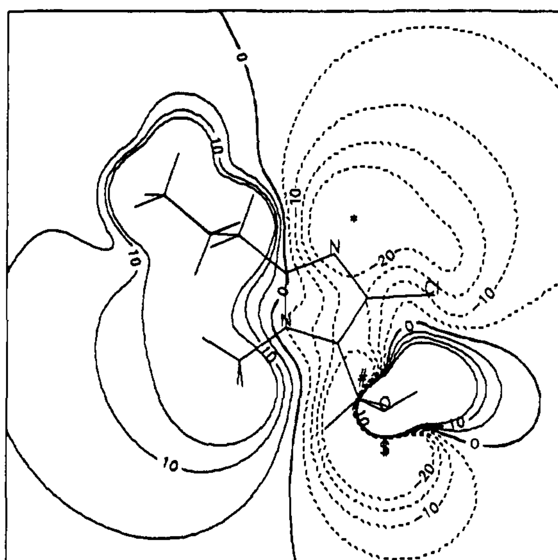
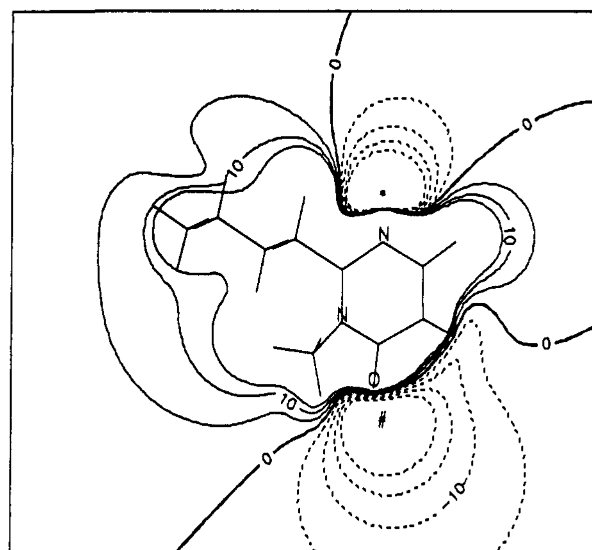


Figure 4. Isopotential maps (2D) for an imidazole-containing fragment (a) in the plane of the heterocycle (plane A) and (b) in the parallel plane 1.7 Å below plane A (plane B) obtained with *ab initio* calculations with a 3-21G basis set. The 0.0 isopotential contour level (bold line), the negative contours (broken lines), and positive contours (solid lines), and the MEP minima location (*, #, $) are shown. The contour values and the MEP minima values are given in kcal/mol.

ring; as already suggested for related dihydropyrimidines,[11] the new six-membered ring system has an additional atom which makes it possible to explore areas of the A II receptor that are not readily accessible by the use of imidazole-based A II antagonists.

As previously performed on simplified DuPont antagonists,[17] MEP distributions have been computed from an *ab initio* wave function with a 3-21G basis set on simplified structures derived from the MM2* [18] global minima of the corresponding A II antagonists, using the GAUSSIAN 90[19] software package; 2D isopotential maps in the plane containing the heterocycle (plane A) and in the parallel plane 1.7 Å below plane A (plane B, not shown) have been constructed by means of an interpolation technique with the SURFER[20] program.



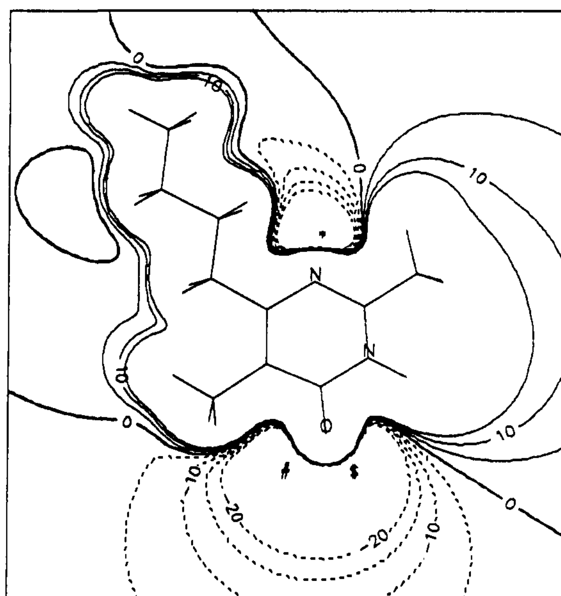


Figure 5. Isopotential maps (2D) for (a) N-linked and (b) C-linked pyrimidinone-containing fragments in plane A. Details are as in Figure 4.

Conformational Analysis. Molecular modeling studies have been performed on compound **2c**[14] (Figure 3, R = CH_3, X = C, Y = NH, Z = tetrazol-5-yl) obtained from DUP-753 by replacing the imidazole moiety with the C-linked pyrimidinonic system and preserving all the other key structural features known to maintain high receptor affinity. The conformational properties of compound **2c** have been determined, the aim being to verify if the new compound also responds to the geometrical activity model derived in a previously performed 3D quantitative structure–activity relationship (QSAR) study.[21] In fact, by the combined use of conformational analysis and chemometrics, we realized a comparative analysis of the 3D conformational features of 13 nonpeptide A II receptor antagonists and defined the spatial functionality relationships necessary for receptor binding. Interatomic distances between atoms belonging to relevant functional groups present in all the training set molecules have been adopted as 3D geometrical molecular descriptors.

Like for the training set A II antagonists, conformational analysis was carried out for compound **2c** performing *in vacuo* molecular mechanics calculations, randomly varying torsional space (minimum and maximum angular increments = 0.0° and 180.0°). The usage-directed Monte Carlo conformational search,[22] the MM2* force field,[23] and the TNCG energy minimization algorithm[24] implemented in the MacroModel Version 3.1X and the BatchMin Version 3.1 programs[18] were used to find the conformational minima within 8 kcal/mol of the global minimum. These conformers were then classified by the geometrical activity model on the basis of the values assumed by the selected interatomic distances in each of them. All the low-energy conformers of compound **2c** closely agreed with the interatomic distance 3D QSAR model.

Biological behavior of compound **2c** has predicted it to be fully active, indicating its ability to place in the 3D space the relevant functional groups exactly as in the more active training set compounds. The finding, as well as the electrostatic potential characteristics, was supported by the binding affinity in a nanomolar range (Table 1). Compound **2c** has been further developed on the basis of the SK&F inhibitors and according to molecular superimpositions with an A II model derived from computational studies.

Overlay Hypothesis. Several groups have attempted to overlay A II with nonpeptide antagonists to design new compounds and rationalize their biological behavior.[4a–c,25] The implicit assumption in these works is that functional groups of small nonpeptide molecules mimic critical elements of the octapeptide hormone at the receptor. Recent mutational data on the AT_1 receptor[26–28] question the validity of this hypothesis, showing that the binding mode for peptide and nonpeptide ligands on the AT_1 receptor is rather different, at least in the regions of the receptor examined. Only a few, positively charged residues have been located that could represent points of actual overlap between binding sites for peptides and nonpeptides. This fact agrees well with the finding that acid moieties of A II or its analogues are essential for binding.

It is possible that peptide and nonpeptide ligands share some receptor binding sites especially in the transmembrane domains, even though not all the interactions present in one class would also be present in the other.[25,26] This assumption has been adopted in the present work as well as in other studies[25] to design new compounds and explain their activities by developing overlays of A II and its nonpeptide antagonists. The principal problems related to this approach are the search for a pharmacophore conformational model of A II and the development of an overlay hypothesis. However, it should be kept in mind that the rationale suggested in these studies is purely speculative in the absence of the proper crystallographic data or ligand–receptor analyses that could prove one model or the other.

Starting from the Takeda lead, the SK&F 108566 compound (Figure 2) and its congeners have been designed by the SmithKline Beecham (SKB) group to closely approximate the C-terminus of A II on the basis of overlay studies employing a postulated bioactive

Table 1. Physical Properties and Biological Activity of Pyrimidinonic Compounds 1−3

1 — 2 and 3

Compd.	R	R_1	Z	Formula[a]	mp(°C)	AII binding[b] Ki(nM)		AII infused rat[c] AII pressure response iv %inhib.[d] or(ED_{50})	Duration[e](min)
1a	H	-	COOH	$C_{22}H_{22}N_2O_3$.HCl	202-205	1097		42	35
1b	CH_3	-	COOH	$C_{23}H_{24}N_2O_3$	186-188	263		34	15
2a	H	H	COOH	$C_{22}H_{22}N_2O_3.H_2O$	197-200	148		50	35
2b	CH_3	H	COOH	$C_{23}H_{24}N_2O_3$	208-210	113		53	35
2c	CH_3	H	tetrazol-5-yl	$C_{23}H_{24}N_6O$	246-248 (dec.)	3		87	55
3a	H	CH_2-phenyl	COOH	$C_{29}H_{28}N_2O_3$	198-200	40		39	25
3b	H	CH_2-thienyl	COOH	$C_{27}H_{26}N_2O_3S$	205-208	60		23	15
3c	H	-CH_2-phenyl-COOH	COOH	$C_{30}H_{28}N_2O_5.H_2O$	219-220	5137		46	25
3d	H	-CH_2-phenyl (COOH)	COOH	$C_{30}H_{28}N_2O_5$	185-187	379	(98)[f]	43	25
3e	CH_3	-CH_2-phenyl (COOH)	COOH	$C_{31}H_{30}N_2O_5$	150-155 (dec.)	401	(85)[f]	46	15
3f	CH_3	-CH_2-thienyl (COOH)	COOH	$C_{29}H_{28}N_2O_5S$	175-178 (dec.)	181	(40)[f]	33	15
3g	CH_3	-CH_2-furyl (COOH)	COOH	$C_{29}H_{28}N_2O_6$	143-148	264	(32)[f]	(0.85)	25
3h	CH_3	-CH_2-phenyl (COOH)	tetrazol-5-yl	$C_{31}H_{32}N_6O_3$	193-196	18	(3)[f]	(0.31)	65
3i	CH_3	-CH_2-thienyl (COOH)	tetrazol-5-yl	$C_{29}H_{28}N_6O_3S{\cdot}0.75H_2O$	215-219 (dec.)	14	(1.1)[f]	(0.45)	55
3j	CH_3	-CH_2-furyl (COOH)	tetrazol-5-yl	$C_{29}H_{28}N_6O_4{\cdot}0.75H_2O$	220-223 (dec.)	16	(0.62)[f]	(0.43)	45
3k	CH_3	-CH_2-thienyl ($COOCH_3$)	tetrazol-5-yl	$C_{30}H_{30}N_6O_3S$	178-180	1.4	(0.9)[f]	(0.27)	65
3l	CH_3	-CH_2-furyl ($COOCH_3$)	tetrazol-5-yl	$C_{30}H_{30}N_6O_4$	112-117 (dec.)	0.58	(0.33)[f]	(0.30)	65
3m	CH_3	-CH_2-thienyl (CH_3OOC)	tetrazol-5-yl	$C_{30}H_{30}N_6O_3S$	170-173	2.1		(0.54)	65
3n	CH_3	CH_2-phenyl	tetrazol-5-yl	$C_{30}H_{30}N_6O$	100-102 (dec.)	3		(1.07)	45
3o	CH_3	CH_2-thienyl	tetrazol-5-yl	$C_{28}H_{28}N_6OS$	162-164	1.27		(0.60)	55
DUP-753						11		(0.32)	55
EXP-7711						99		48	25
EXP-3174						6.8	(0.9)[f]		

[a] Satisfactory C, H, and N elemental analyses (±0.4%) were obtained. [b] Inhibition of specific binding of $[^3H]$A II to rat adrenal cortical membranes (AT_1 receptors) ($n \geq 3$). All new compounds were found inactive ($K_i > 4\ \mu M$) on bovine cerebellar cortical membranes (AT_2 receptors).[52] [c] Anesthetized, ganglion-blocked, A II-infused rat (see the Experimental Section for further details). [d] At 1 mg/kg. [e] Time from onset of action at a dose of 1 mg/kg until significant inhibition of pressure response is no longer observed. [f] No BSA was included in the assay mixture.

conformation of A II.[4,29] The acrylic carboxylic acid and thienyl groups aligned with the corresponding elements of the terminal Phe[8], the carboxybenzyl fragment directed to Tyr[4], and the N=C−N imidazole region- and the acrylic acid double bond-mimicking peptide amide bonds have been hypothesized in these studies. Moreover, the 2-butyl group is believed to fit into a lipophilic pocket that accommodates the Ile[5] side chain of A II.

The determination of the receptor-bound conformation of A II could significantly advance the rational design of potent A II antagonists. Garcia and others have recently proposed a receptor-bound conformation of A II developed from crystallographic data of the complex between A II and a high-affinity monoclonal antibody (MAb 131).[30] The crystallographic structure of the complex reveals an A II structure that is compatible with predicted bioactive conformations of A II derived from structure−activity studies and theoretical calculations.[31] In the complex, the deeply bound hormone is folded into a compact structure in which two turns bring the amino and carboxy termini close together: the first turn involves Ile[5], His[6], and Pro[7] residues, and the second one involves Asp[1] and Arg[2]. The structure of A II in the X-ray complex exhibits a close spatial disposition of Tyr[4], Pro[7], and Phe[8] consistent with the folded model and the overlay hypothesis employed by SKB in developing their class of A II antagonists. Manual alignment of SK&F 108566 with A II conformation in the complex showed in fact that the (thienylmethyl)-acrylic acid group overlapped with the Phe[8] and the Tyr[4] needed to be rotated by 80° around the $C\alpha-C\beta$ bond toward the center of the turn to overlap with the benzoate ring of the drug.[30] The X-ray crystal structure of A II bound to a high-affinity monoclonal antibody provides a reliable pharmacophore model, although no conclusive physical evidence exists to confirm that nonpeptides and the peptide interact with the same surface region of the receptor. Such a model, even if it does not represent an actual bioactive conformation of A II, is very useful to analyze the similarities between A II and the peptidomimetic receptor antagonists of A II.

Since the X-ray coordinates were not available to us, by means of molecular mechanics calculations (AMBER* force field),[18] we found a conformer of A II characterized by the same conformational features described in the X-ray complex structure.

The general overlay hypothesis between SK&F 108566 and A II proposed by SKB,[4c] previously described, has been adopted in this study. For overlay comparison between SK&F 108566 and DUP-753, and therefore for superimposition of biphenylyltetrazole-containing antagonists to A II, we have preferred to adopt the overlay hypothesis generated by aligning the butylimidazole portions common to the two structurally distinct classes of A II antagonists. In this overlay the benzoic acid of SK&F 108566 and the tetrazole of DUP-753 can be superimposed and aligned with the Tyr[4] phenol, while the acrylic acid of the former and the hydroxymethyl of DUP-753 point in the same general direction toward the Phe[8] carboxylic acid but at a slight distance from each other. Although in this overlay comparison DUP-753 lacks functionality in the vicinity of the thiophene ring of SK&F 108566 and SK&F 108566 likewise does not overlay the terminal phenyl ring of DUP-753, we retain

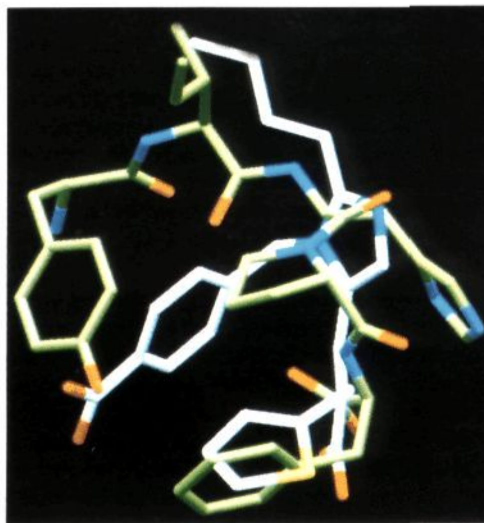


Figure 6. Overlay of an energy-minimized conformation of SK&F 108566 (white) on our computational model of A II active conformation (green). Only the Tyr[4]-Ile[5]-His[6]-Pro[7]-Phe[8] residues of the hormone are illustrated.

this hypothesis more reliable than the other one formulated by SKB chemists.[4c]

In the second SKB overlay comparison, the biphenyltetrazole moiety is overlapped with the thienylmethyl-substituted acrylic acid and the imidazole ring of DUP-753 can be superimposed on the benzoic acid portion of SK&F 108566, the hydroxymethyl group serving as a latent carboxylic acid. However, we have already shown that the substitution of the terminal phenyl ring in DUP-753 with a heterocyclic one gave practically inactive molecules.[5]

For these reasons, we agree only in part with the first overlay comparison (superimposition of SK&F 108566 benzoic acid with the tetrazole of DUP-753) and retain the second hypothesis as likely unreliable.

The superimpositions of a MM2* minimum energy conformation of SK&F 108566, DUP-753, and **2c** with our computational model of an A II active conformation are represented in Figures 6−8, respectively. Nonpeptide antagonists and octapeptide hormone have been superimposed by means of the rigid least squares superimposition task available within the MacroModel Version 3.1X.[18] In Figure 6 the superimposition points are −N= of the imidazole−CO His[6], liphophilic chain−Ile[5], benzoic acid CO_2H−OH Tyr[4], thiophene ring−Phe[8], and acrylic CO_2H−terminal Phe[8] CO_2H. In Figure 7 the biphenyltetrazole is directed toward Tyr[4], the butyl chain extends into the hydrophobic region of Ile[5], and the −N= type nitrogen atom of the imidazole ring and the hydroxymethyl group overlay respectively with the carbonyl groups of His[6] and Pro[7]. In Figure 8 the −N= type nitrogen atom and the carbonyl group of pyrimidinone are now overlapped to the carbonyl groups of His[6] and Pro[7], respectively, the other functionalities possessing the same alignment partners described in Figure 7. This last superimposition suggested that compound **2c** could be advantageously modified following the hypothesis of SKB chemists in order to mimic the C-terminal region of A II, which has been identified as crucial for both receptor recognition and activation. Starting from the structure of **2c**, a series of N-3-substituted pyrimidinones of general formula **3** (Figure 3 and Table 1) have been designed and synthesized.

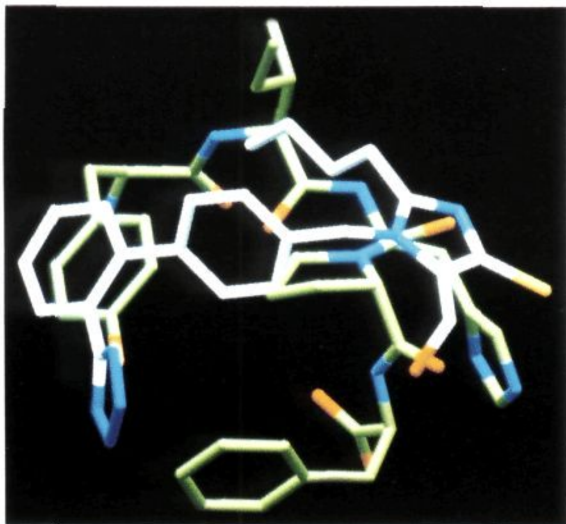


Figure 7. Overlay of an energy-minimized conformation of DUP-753 (white) on our computational model of A II active conformation (green). Details are as in Figure 6.

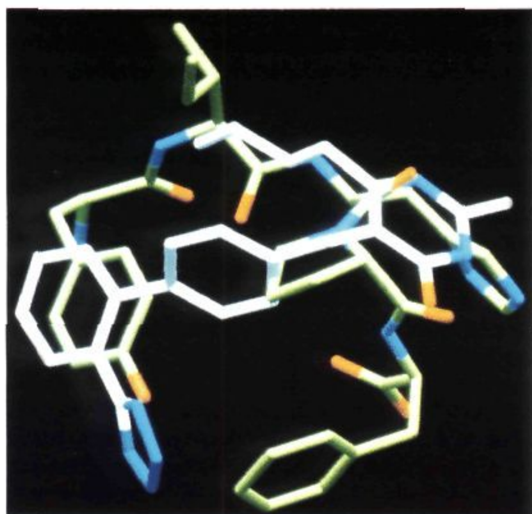


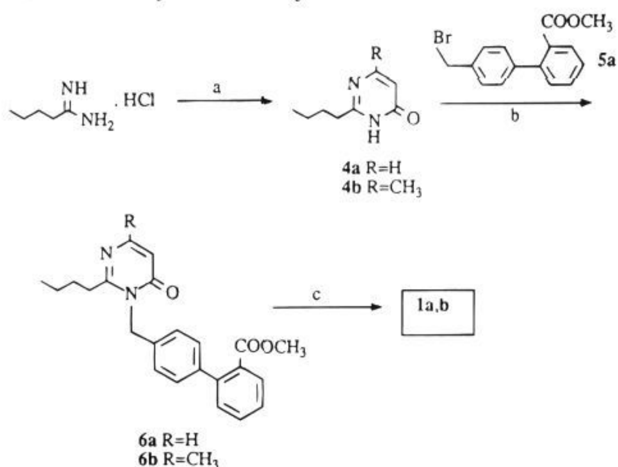
Figure 8. Overlay of an energy-minimized conformation of **2c** (white) on our computational model of A II active conformation (green). Details are as in Figure 6.

Chemistry

The synthesis of *N*-(biphenylmethyl)pyrimidinones **1** is described in Scheme 1. The intermediates **4a,b** were obtained by condensation of valeramidine with either ethyl propiolate or ethyl acetoacetate, respectively. The reaction between pyrimidinones **4** and the 4-(bromomethyl)biphenyl derivative **5a**[2] in the presence of K_2CO_3 in DMF led approximately to a 1:1 mixture of N- and O-substituted compounds, which were separated by chromatographic methods and characterized by NMR and IR analyses as reported for a structurally related series of pyrimidinones.[11] Subsequent hydrolysis of the N-alkylated regioisomers **6** furnished the corresponding acids **1**.

The synthesis of C-linked compounds **2** and **3** is outlined in Scheme 2. The substituted 3-oxoheptanoates **8**, obtained by the reaction between **7**[32] and (bromomethyl)biphenyl derivatives **5**[2] in THF in the presence of NaH, were condensed with the appropriate amidines in a mixture of MeOH/dioxane in the presence

Scheme 1. Synthesis of Pyrimidinones **1**[a]



[a] (a) Ethyl propiolate or ethyl acetoacetate, KOH, EtOH, reflux or room temperature; (b) K_2CO_3, DMF, 80 °C; (c) NaOH, MeOH, reflux.

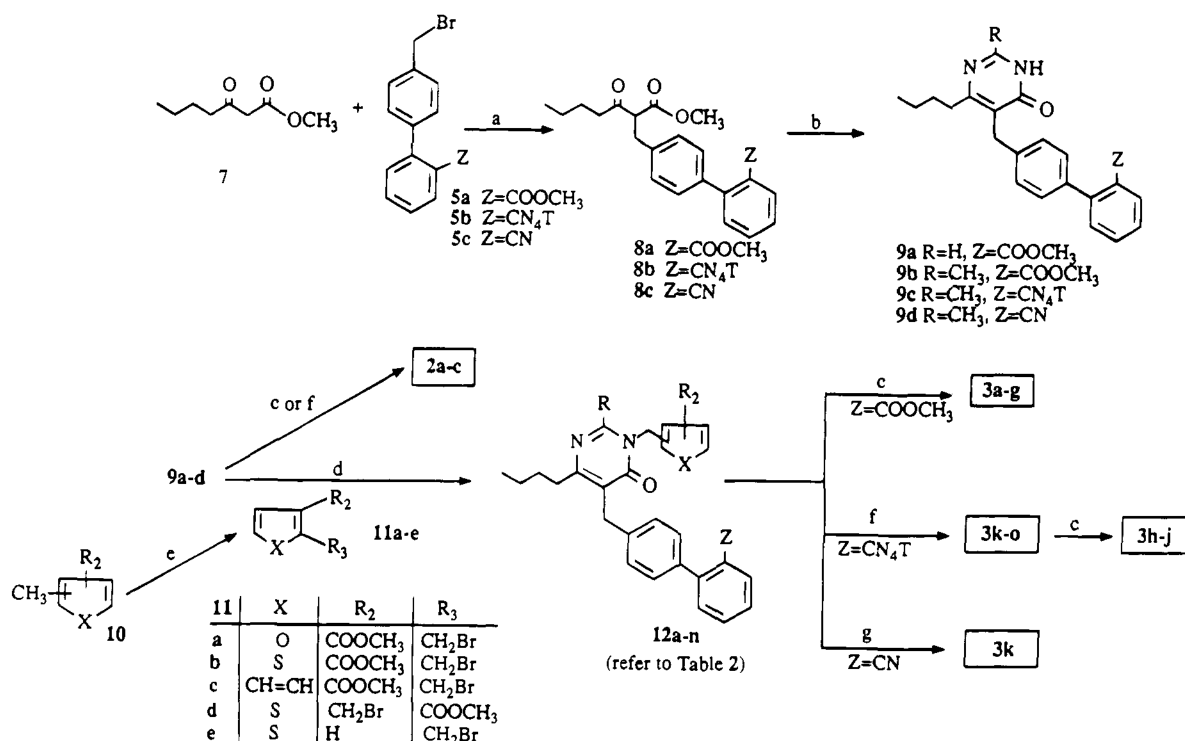
of CH_3ONa to give the corresponding pyrimidinone intermediates **9** in 50–80% yield. Alkaline hydrolysis of the esters **9a,b** furnished the target acids **2a,b**, while removal of triphenylmethyl protecting group from **9c** led to **2c**.

The alkylation of **9** by (bromomethyl)aryl or -heteroaryl intermediates **11** was initially performed in DMF in the presence of NaH (method A) and gave the desired N-3-substituted pyrimidinones **12** (Table 2), as well as the corresponding O-alkylated regioisomers, which could be separated, either by chromatography or crystallization. N-Alkylated pyrimidinones **12** were easily differentiated from the respective *O*-alkylated isomers by IR analysis, which showed a diagnostic amide carbonyl absorption near 1650 cm^{-1} that did not appear in the IR spectra of O-alkylated compounds. Furthermore, on silica gel plates with hexane–EtOAc solvent systems, all the N-alkylated pyrimidinones **12** reported in Table 2 always had R_f values lower than the corresponding O-alkylated isomers (see the Experimental Section).

NOE experiments were performed on compound **12i** to confirm the regiochemistry of N-3 vs N-1 alkylation. Irradiation of the thienylmethylic protons singlet at 5.87 ppm influenced the pyrimidinonic methyl singlet at 2.46 ppm, whereas neither the butylic chain proton signals nor the benzylic and aromatic ones were affected. This fact indicated that the alkylation took place on the N-3 nitrogen atom. The N-3 selectivity was probably due to the steric hindrance created by the butylic chain adjacent to the N-1 nitrogen atom. Finally, the X-ray crystal structure of the deprotected tetrazole derivative **3k** obtained by hydrolysis of **12i** further confirmed the structural assignement.[33]

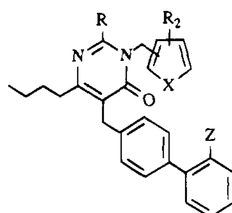
The ratio of N/O products was found to be strongly influenced by the nature of the R and R_2 substituents. When R was hydrogen, the N-alkylated derivatives **12a–d** were obtained in almost quantitative yield (ratio N/O > 9:1). When R was methyl, the N/O ratio decreased to 2.5:1 (R_2 = H) and to 1:1 (R_2 = $COOCH_3$). The problem of the low N regioselectivity was circumvented by performing the alkylation in the presence of excess lithium bromide (method B). This was highlighted in the preparation of compounds **12i,j,l–n**. In

Scheme 2. Synthesis of Pyrimidinone Derivatives **2** and **3**[a]



[a] (a) NaH, THF, room temperature; (b) R—C(=NH)—NH_2·HCl, CH_3ONa, CH_3OH, dioxane, room temperature; (c) NaOH, CH_3OH, reflux; (d) NaH, DMF, room temperature (method A), adding LiBr (method B); (e) NBS, AIBN, CCl_4, reflux; (f) CH_3OH, reflux; (g) NaN_3, Bu_3SnCl, toluene, reflux and then HCl, CH_3OH, room temperature. CN_4T = 1-(triphenylmethyl)tetrazol-5-yl.

Table 2. N-3-Substituted Pyrimidinonic Intermediates **12**



compd	R	R_2	Z	X	yield (%) (method)	mp (°C)
12a	H	H	$COOCH_3$	CH=CH	81 (A)	foam
12b	H	H	$COOCH_3$	S	73 (A)	foam
12c	H	4-$COOCH_3$	$COOCH_3$	CH=CH	84 (A)	oil
12d	H	2-$COOCH_3$	$COOCH_3$	CH=CH	90 (A)	oil
12e	CH_3	2-$COOCH_3$	$COOCH_3$	CH=CH	37 (A)	oil
12f	CH_3	3-$COOCH_3$	$COOCH_3$	S	48 (A)	oil
12g	CH_3	3-$COOCH_3$	$COOCH_3$	O	30 (A)	oil
12h	CH_3	2-$COOCH_3$	CN_4T[a]	CH=CH	49 (A)	foam
12i	CH_3	3-$COOCH_3$	CN_4T	S	62 (B)[b]	150–152[c]
12j	CH_3	3-$COOCH_3$	CN_4T	O	53 (B)[d]	140–142[c]
12k	CH_3	2-$COOCH_3$	CN_4T	S	26 (A)	foam
12l	CH_3	H	CN_4T	CH=CH	71 (B)	foam
12m	CH_3	H	CN_4T	S	75 (B)	foam
12n	CH_3	3-$COOCH_3$	CN	S	52 (B)	121–123[e]

[a] 1-(Triphenylmethyl)tetrazol-5-yl. [b] Yield from method A is 41%. [c] From EtOAc. [d] Yield from method A is 32%. [e] Trituration with Et_2O.

fact, following this procedure, the N/O ratio ranged from 5:1 (R_2 = H) to 2:1 (R_2 = $COOCH_3$). Alkaline hydrolysis or removal of the triphenylmethyl protecting group followed, if needed, by alkaline hydrolysis gave the final compounds **2** and **3**.

Compound **3k** was also synthesized by an alternative route: The cyano derivative **9d**[34] was alkylated by **11b** to give **12n** and then transformed into the tetrazolic compound employing NaN_3 and tributyltin chloride in toluene. Final hydrolysis with a methanolic solution of HCl gave compound **3k**.

Results and Discussion

The pyrimidinone compounds **1**–**3** were firstly examined *in vitro* as A II antagonists by evaluating their ability to displace [^{3}H]A II from rat adrenal cortical membranes (RACM) as a source of AT_1 receptor.[35] In some cases, as the binding affinity of compounds carrying a carboxyl group on the N-3 substituent was negatively influenced by bovine serum albumin (BSA), the test was also performed in the absence of BSA. The relative potencies of the antagonists are expressed as K_i values and are presented in Table 1. All the compounds were also examined *in vivo*, at a dose of 1 mg/kg, in anesthetized, ganglion-blocked, A II-infused rat, for effects on blood pressure (BP) after intravenous (iv) administration. The maximum percent inhibition of the pressor response to A II and the time from onset of action until significant inhibition of pressure response is no longer observed are reported for each compound in Table 1. The ED_{50} value was calculated for most of the compounds which showed a percentage inhibition higher than 60.

Generally, the compounds possessed remarkable affinity for AT_1 receptor,[35] equivalent or superior to that of the corresponding imidazole derivatives EXP-7711[2] or DUP-753. These results are in accordance with MEP studies, which have indicated that the pyrimidinone ring possessed all the topological MEP characteristics necessary for A II receptor recognition. Compounds carrying a free carboxyl group on the N-3 substituent displayed significatively lower affinity in the presence than in the absence of BSA. K_i values more consistent with *in vivo* data were obtained in the absence of BSA.

A free carboxylic function is known to bind easily to this protein, thus influencing the interaction with the receptors. No statistically significant difference was seen with the other investigated antagonists, i.e., those without a free carboxylic function. Analogous BSA effects have been reported for other diacidic antagonists.[36]

Considering the receptor affinity of the initially prepared compounds with a carboxylic acid group on the biphenyl portion, the biphenyl C-linked derivatives **2a,b** were found to be more potent than the corresponding N-linked derivatives **1a,b**. Due to the higher potency and potentially greater metabolic stability of a C–C bond than a C–N bond,[37] only pyrimidinones **2a,b** were considered for the subsequent chemical modifications.

The introduction of an arylmethyl substituent on the N-3 atom of the pyrimidinone ring influenced favorably (3–4 times) the affinity (compare, for example, **2a** vs **3a,b**), in accordance with our overlay hypothesis which suggested that the N-3 position could be advantageously modified. An analogous improvement of the activity was obtained by the Merck group[10b] by introducing a benzyl substituent on the N-2 atom of a series of 1,2,4-triazolinones. This result was attributed to the interaction of the new substituent with a hydrophobic area of the receptor, which is more distant from the heterocycle binding site than previously hypothesized for the AT_1 receptor–ligand model advanced by the DuPont group.[2] The presence of a carboxyl group on the N-3 arylmethyl substituents had different effects depending on its position and the type of heterocycle. The *para*-substituted **3c** is 50 times less active than the *ortho* **3d**; thus, it is quite evident that the carboxyl group had to be present in the *ortho* position. As regards to the type of heterocycle, the carboxy-substituted furan **3g** and thiophene **3f** were more active than the phenyl derivative **3e**. This finding was more evident in the tetrazole series, where the increment of the activity was about 3–5 times (**3h** vs **3i,j**).

The fact that this trend is common to both the SKB series[4a–c] and our biphenyltetrazole pyrimidinone series supports the reliability of the overlay hypothesis adopted. Furthermore, within the framework of this overlay scheme, agreement between the results reported here and the reduced binding affinities following the heteroaryl substitution of the biphenyl element of DUP-753 can be obtained.[5]

As already observed, the substitution of the carboxyl group on the biphenyl moiety with a tetrazole ring induced a strong increase in the activity (30–40 times for compounds **3e–g** vs **3h–j**). In comparison to DUP-753, most of the new tetrazole derivatives (**3h–o**) were found more potent (up to 20 times). The compounds **3k–o** compare favorably with EXP-3174, the imidazole-5-carboxylic metabolite of DUP-753 (see Table 1), to which most of the activity of the parent compound is attributed.[38]

On the basis of these findings, in order to confirm that N-3-substituted pyrimidinones agreed with the geometrical requirements for binding, the new molecule **3i** was superimposed with our conformational model of A II. The overlay is shown in Figure 9. It can be observed that the thiophene ring is well aligned to the phenyl of Phe[8] and the carboxyl group to the terminal CO_2H of the Phe[8], while all the other functionality correspondences remain fixed as in the previous overlay between A II and **2c** (Figure 8).

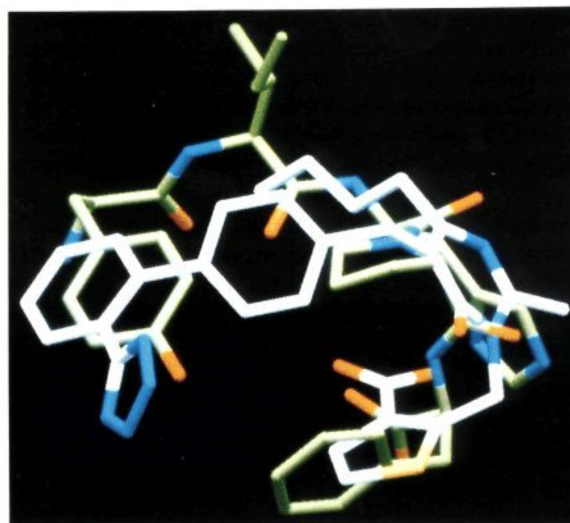


Figure 9. Overlay of an energy-minimized conformation of **3i** (white) on our computational model of A II active conformation (green). Details are as in Figure 6.

Compound **3i** has been subjected to a final examination, the aim being to verify if the 2-(carboxyaryl)methyl moiety modified the global conformational behavior of the molecule. The procedure already adopted for **2c**, in testing its agreement with the geometrical activity model, has been now performed on **3i**. A confirmation of activity has been obtained, indicating that the 3D geometric requirements for binding continue to be satisfied also by the new N-3-substituted pyrimidinones.

From Table 1 it can be observed that the presence of a carboxy substituent on the newly introduced arylmethyl moiety does not significantly affect the binding affinity. This fact could weaken our overlay hypothesis, where this group could mimic the phenylalanine carboxy terminal region of A II. Otherwise it is possible that the carboxy function has a role in a later phase of the formation of the antagonist–receptor complex (*vide infra*) and therefore does not affect the binding data. It is still possible that in nonpeptide antagonists this binding site contributes little to the total binding affinity. Similar conclusions could also be derived from different overlay schemes, for the alignment partners of other residues essential for A II binding. For example, in the mapping between peptides and nonpeptides which equates C-terminal Phe[8] with the acid moiety of the biphenyl terminal ring,[25] the presence of a group in the correspondence of Tyr[4] OH does not significantly alter the binding affinity in the imidazopyridine series.[25]

The inhibition of the pressor response to A II challenge via the iv route in the anesthetized rat model correlated well with the *in vitro* activity, with some discrepancies in the non-tetrazole derivatives. For example, the *in vitro* inactive compounds **1a** and **3c** (K_i = 1097 and 5137 nM, respectively) displayed the same inhibition of compounds with K_i lower than 100 nM. In contrast, fairly active compounds **3a,b** exhibited a low *in vivo* activity. As in the *in vitro* experiments, the replacement of the carboxyl group on the biphenyl moiety with a tetrazole ring resulted in a significant increase of the activity (**3e–g** vs **3h–j**). This substitu-

Table 3. Pressure Response of Compounds 3 in A II-Infused Rat by id Route[c]

compd	ED_{50}	duration (min)[d]
3h	4.2	>120
3i	7.24	>120
3j	14.5	75
3k	1.40	>120
3l	1.44	>120
3m	2.67	>120
3n	>10	60
3o	10	60
DUP-753	3.7	120

[c,d] See the corresponding footnotes of Table 1.

tion influenced favorably the duration of action (from 15–25 to 45–65 min). With regard to the influence of a carboxyl or a methoxycarbonyl group on the N-3 arylmethyl appendage, both substituents were found to be effective in enhancing the inhibition of the A II pressor response, the latter being more active than the former.

Potent compounds were finally evaluated intraduodenally (id) in the same rat model for effect on BP. ED_{50} values and duration of action are summarized in Table 3. The most striking result was the scarce potency and the inferior duration of action of compounds which lack a carboxylic or a methoxycarbonyl function on the benzyl or thienylmethyl substituent (**3n** vs **3h**, **3o** vs **3i**,**k**). The role of the carboxylic function in antihypertensive activity is not clear. As suggested by others authors,[7b] it could cause a secondary conformational change in the A II receptor to strengthen the antagonist–receptor complex after the initial binding. Studies are in progress to evaluate the effects on antihypertensive activity of possible bioavailability modifications.

Compounds **3k–m** bearing a [(methoxycarbonyl)-heteroaryl]methyl substituent on the N-3 atom resulted to possess the highest and most durature id activity of the series, and their potency was found to be superior to that of DUP-753. Comparison between esters and acids indicated that the esters were consistently more active than the acids (5–10 times). This fact is not surprising; the diacidic compounds are generally poorly absorbed after oral administration.[39] Preliminary *in vivo* metabolic studies (data not published) performed on compound **3k** showed that the ester function was minimally affected by hepatic enzymes. This suggested that the methyl ester **3k** did not act as a prodrug of the corresponding acid. Similar conclusions were reported by other authors for analogous alkoxycarbonylic compounds.[39,40]

On the basis of the initial pharmacological profile, compounds **3k** (LR-B/081) and **3l** were selected for further studies. When orally administered, they dose dependently antagonized A II-induced pressor response in normotensive rats (Figure 10a) and lowered BP in conscious renal hypertensive rats (Figure 11a). Their relative potency was similar to that of Losartan (Figures 10b and 11b).[41] The duration of the antihypertensive effect in renal hypertensive rats was greater for compound **3k** compared to that of compound **3l**. On the basis of these findings as well as further *in vivo* and *in vitro* biological studies,[41] compound **3k** was selected as a candidate for development.

Conclusions

On the basis of a comparative analysis of the MEP distributions and overlay studies employing a model of A II derived from theoretical calculations, we have prepared a new series of N-3-substituted pyrimidinones. The data presented here indicate that the pyrimidinone ring successfully replaces the tetrasubstituted imidazole of DUP-753 (Losartan). The introduction of an arylmethyl group on the nitrogen atom adjacent to the carbonyl group of the pyrimidinone ring favorably influences the activity, particularly *in vivo*. According to the adopted overlay comparison, the new structural element has been introduced to mimic the C-terminal region of the octapeptide and the thienylmethyl-substituted acrylic acid of SK&F 108566.

Compound **3k** (LR-B/081),[41] one of the most potent compounds *in vitro* and *in vivo*, was selected for development. It is now undergoing clinical investigation for the treatment of hypertension.

Experimental Section

General. Thin-layer chromatography was performed on silica gel plates (60 F_{254}, Merck). Flash chromatography was performed on silica gel (Kieselgel 60, 230–400 mesh) using the indicated solvent mixture. Melting points were measured with a Büchi 535 apparatus and are uncorrected. ^{1}H NMR spectra were obtained with a Bruker AC-200 spectrometer and are reported as parts per million (ppm) downfield from Me_4Si, with multiplicity, number of protons, and coupling constant in hertz (Hz). The infrared spectra were recorded on a Perkin-Elmer FTIR 1600 spectrometer. The mass spectra were obtained with a VG 7070 EQ-HF mass spectrometer. Where elemental analyses are indicated for C, H, and N, analytical values are within 0.4% of calculated values. Where solvation is indicated, the presence of solvent was verified by NMR. Gas chromatography (GC) was performed on a DANI 3600 gas chromatograph equipped with a flame ionization detector using a glass column filled with Chromosorb WHP.

2-Butylpyrimidin-4(3*H*)-one (4a). To a hot (60 °C) solution of valeramidine (3.88 g, 38.7 mmol), freshly prepared from the corresponding hydrochloride, and ethyl propiolate (4.3 mL, 42.6 mmol) in 30 mL of absolute EtOH was added 80% KOH (2.5 g, 38.7 mmol) dissolved in 10 mL of absolute EtOH. After heating at reflux for 3 h, the solvent was evaporated in vacuo and the residue was dissolved in water, acidified to pH 5 with 5 N HCl, and extracted with CH_2Cl_2. The combined organic phases were washed with brine, dried over Na_2SO_4, and evaporated at reduced pressure. The residue, purified by flash chromatography (CH_2Cl_2–MeOH, 96:4), gave compound **4a** as a foam (3.15 g, 53%): ^{1}H NMR ($CDCl_3$) δ 0.93 (t, 3H), 1.28–1.52 (m, 2H), 1.65–1.82 (m, 2H), 2.69 (t, 2H), 6.31 (d, 1H), 7.97 (d, 1H).

2-Butyl-6-methylpyrimidin-4(3*H*)-one (4b). A mixture of valeramidine hydrochloride (0.8 g, 5.85 mmol), ethyl acetoacetate (0.74 mL, 5.85 mmol), and 88% KOH (0.37 g, 5.85 mmol) in 0.5 mL of absolute EtOH was stirred for 3 h at room temperature and placed in a vacuum dessicator over P_2O_5 for 24 h. The residue, obtained after evaporation in vacuo, was purified by flash chromatography (CH_2Cl_2–MeOH, 96:4) affording compound **4b** as a white solid (0.65 g, 67%): mp 115–117 °C; ^{1}H NMR ($CDCl_3$) δ 0.93 (t, 3H), 1.28–1.50 (m, 2H), 1.65–1.84 (m, 2H), 2.29 (s, 3H), 2.65 (t, 2H), 6.15 (s, 1H).

Methyl 4′-[(2-Butyl-6-oxo-1(6*H*)-pyrimidinyl)methyl]-[1,1′-biphenyl]-2-carboxylate (6a). To a stirred solution of **4a** (0.4 g, 2.65 mmol) in 8 mL of dry DMF were added 0.7 g (5.30 mmol) of anhydrous K_2CO_3 and, dropwise, a solution of **5a**[2] (0.8 g, 2.65 mmol) in 2 mL of dry DMF. After heating at 80 °C for 10 h, the mixture was evaporated in vacuo, and the residue was taken up with water (20 mL), neutralized with HCl (1 N), and extracted with EtOAc (3 × 10 mL). The organic phase, dried over Na_2SO_4, was concentrated in vacuo, and the residue was chromatographed (hexane–EtOAc, 6:4) to afford **6a** as an oil (0.35 g, 35%): ^{1}H NMR ($CDCl_3$) δ 0.90 (t, 3H), 1.20–1.45 (m, 2H), 1.55–1.72 (m, 2H), 2.71 (t, 2H), 3.63 (s, 3H), 5.37 (s, 2H), 6.44 (d, 1H), 7.08–7.54 (m, 7H), 7.83 (dd, 1H), 7.88 (d, 1H).

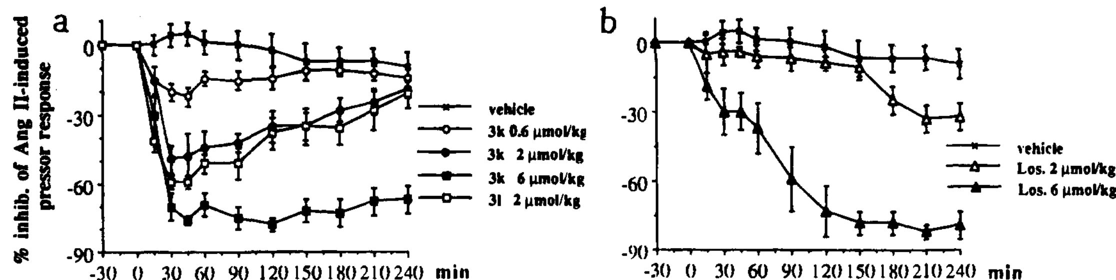


Figure 10. Effect of orally administered vehicle and (a) compounds **3k,l** or (b) Losartan (Los) on A II-induced pressor response in conscious, normotensive rats. Values represent the means ± SEM.

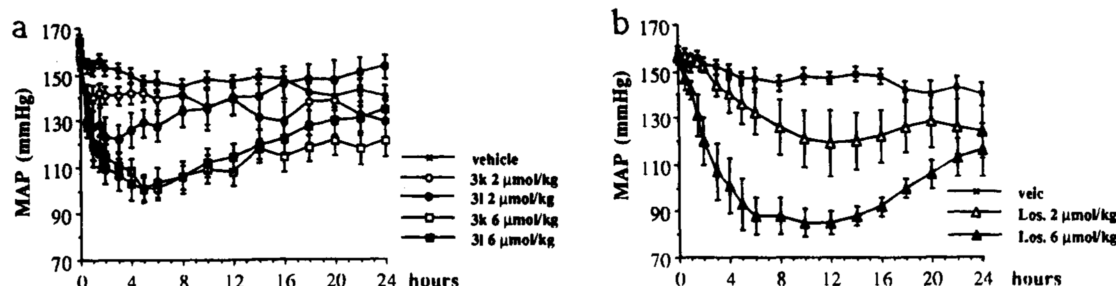


Figure 11. Effect of orally administered vehicle and (a) compounds **3k,l** or (b) Losartan (Los) on mean arterial pressure in conscious, renal hypertensive rats. Values represent the means ± SEM.

The following compound was analogously prepared.

Methyl 4'-[(2-Butyl-4-methyl-6-oxo-1(6*H*)-pyrimidinyl)-methyl][1,1'-biphenyl]-2-carboxylate (6b). Starting from **4b** (0.44 g, 2.65 mmol), compound **6b** was obtained as an oil (0.41 g, 40%): ^{1}H NMR ($CDCl_3$) δ 0.95 (t, 3H), 1.26–1.52 (m, 2H), 1.67–1.89 (m, 2H), 2.41 (s, 3H), 2.83 (t, 2H), 3.65 (s, 3H), 5.46 (s, 2H), 6.44 (s, 1H), 7.24–7.58 (m, 7H), 7.84 (dd, 1H).

Methyl 2-(Bromomethyl)-3-furancarboxylate (11a). To a stirred solution of methyl 2-methyl-3-furancarboxylate (**10a**; obtained by esterification of the corresponding acid[42] with MeOH in the presence of H_2SO_4; 28 g, 184 mmol) in 800 mL of CCl_4 were added *N*-bromosuccinimide (35.6 g, 200 mmol) and α,α'-azoisobutyronitrile (0.6 g, 4 mmol). The solution was stirred at 70 °C for 3 h. Then the reaction mixture was cooled to room temperature, and the floating succinimide was filtered off. The resulting solution was washed four times with water (40 mL), dried over Na_2SO_4, and evaporated at reduced pressure. The crude product was crystallized from hexane to obtain **11a** as a white solid (30 g, 74.5%): mp 44–46 °C; ^{1}H NMR ($CDCl_3$) δ 3.88 (s, 3H), 4.81 (s, 2H), 6.70 (d, 1H), 7.39 (d, 1H).

Similarly, the following compounds were prepared.

Methyl 2-(Bromomethyl)-3-thiophenecarboxylate (11b). Starting from methyl 2-methyl-3-thiophenecarboxylate (**10b**; obtained by esterification of the corresponding acid[43] with MeOH in the presence of H_2SO_4; 8.8 g, 55.2 mmol), compound **11b** (12.9 g, GC 65%, yield 75%) was obtained as a yellow oil and used without further purification: ^{1}H NMR ($CDCl_3$) δ 3.87 (s, 3H), 5.10 (s, 2H), 7.22 (d, 1H), 7.41 (d, 1H).

Methyl 3-(Bromomethyl)-2-thiophenecarboxylate (11d). Starting from methyl 3-methyl-2-thiophenecarboxylate (**10d**; obtained by esterification of the corresponding acid with MeOH in the presence of H_2SO_4; 4.8 g, 30.7 mmol), compound **11d** (7 g, GC 67%, yield 65%) was obtained as a yellow oil and used without further purification: ^{1}H NMR ($CDCl_3$) δ 3.89 (s, 3H), 4.91 (s, 2H), 7.17 (d, 1H), 7.45 (d, 1H).

The bromo derivatives **11c,e** were prepared according to the literature (refs 44 and 45, respectively) from methyl 2-methylbenzoate (**10c**) and 2-methylthiophene (**10e**).

Methyl 2'-(Methoxycarbonyl)-α-(1-oxopentyl)[1,1'-biphenyl]-4-propanoate (8a). A solution of **7**[32] (2.3 g, 14.5 mmol) in 10 mL of dry THF was slowly dropped into a stirred suspension of 80% NaH (0.22 g, 9.2 mmol) in 30 mL of dry THF, under N_2 atmosphere. After the effervescence was over, a solution of **5a**[2] (2.22 g, 7.3 mmol) in 10 mL of dry THF was added dropwise and stirring was continued during 10 h. Then water (80 mL) was added, and the pH was adjusted to 7 with 2 N HCl. The resulting solution was extracted twice with CH_2Cl_2 (40 mL); the organic layers were separated, dried over Na_2SO_4, and evaporated at reduced pressure. The crude product was purified by flash chromatography (EtOAc–hexane, 20:80) to afford **8a** as a clear oil (2.6 g, 93%): ^{1}H NMR ($CDCl_3$) δ 0.86 (t, 3H), 1.15–1.62 (m, 4H), 2.25–2.65 (m, 2H), 3.20 (d, 2H), 3.63 (s, 3H), 3.71 (s, 3H), 3.84 (t, 1H), 7.12–7.58 (m, 7H), 7.78 (dd, 1H).

Similarly, the following compounds were prepared.

Methyl α-(1-Oxopentyl)-2'-[1-(triphenylmethyl)-1*H*-tetrazol-5-yl][1,1'-biphenyl]-4-propanoate (8b). Starting from **7**[32] (4.1 g, 25.8 mmol) and **5b**[2] (8.3 g, 15 mmol), compound **8b** (7.5 g, 79%) was obtained as a white foam: ^{1}H NMR ($CDCl_3$) δ 0.84 (t, 3H), 1.12–1.61 (m, 4H), 2.18–2.6 (m, 2H), 3.06 (d, 2H), 3.66 (s, 3H), 3.69 (t, 1H), 6.81–7.52 (m, 22H), 7.90 (dd, 1H).

Methyl 2'-Cyano-α-(1-oxopentyl)[1,1'-biphenyl]-4-propanoate (8c). Starting from **7**[32] (6.4 g, 40.2 mmol) and **5c**[2] (7.3 g, 26.8 mmol), compound **8c** (5.6 g, 60%) was obtained as a yellow oil: ^{1}H NMR ($CDCl_3$) δ 0.85 (t, 3H), 1.12–1.33 (m, 2H), 1.38–1.58 (m, 2H), 2.24–2.68 (m, 2H), 3.22 (d, 2H), 3.71 (s, 3H), 3.85 (t, 1H), 7.22–7.82 (m, 8H).

6-Butyl-2-methyl-5-[[2'-[1-(triphenylmethyl)-1*H*-tetrazol-5-yl][1,1'-biphenyl]-4-yl]methyl]pyrimidin-4(1*H*)-one (9c). A solution of 30% CH_3ONa (20.0 g, 111 mmol) in MeOH was added, under N_2 atmosphere, to a stirred solution of 95% acetamidine hydrochloride (7.04 g, 74 mmol) in 25 mL of MeOH cooled at 4 °C. The white suspension was added with a solution of **8b** (23.5 g, 37 mmol) in 100 mL of dry MeOH and 25 mL of dry dioxane maintaining the temperature at 5–10 °C. Stirring was continued for 20 h at room temperature, and then solvents were evaporated at reduced pressure. The crude product was taken up with water, and pH was adjusted to 6 by adding 50% aqueous CH_3COOH. The resulting precipitate was filtered off and washed with water. After purification by flash chromatography (CH_2Cl_2–MeOH, 95:5), compound **9c** was obtained as a white powder (16.6 g, 70%): mp 176–179 °C (acetone); ^{1}H NMR ($CDCl_3$) δ 0.88 (t, 3H), 1.25–1.65 (m, 4H), 2.34 (s, 3H), 2.52 (t, 2H), 3.82 (s, 2H), 6.82–7.12 (m, 10H), 7.20–7.52 (m, 12H), 7.88 (m, 1H).

The following compounds were analogously prepared.

Methyl 4'-[(6-Butyl-1,4-dihydro-2-methyl-4-oxo-5-pyrimidinyl)methyl][1,1'-biphenyl]-2-carboxylate (9b). Starting from **8a** (0.9 g, 2.35 mmol), compound **9b** was obtained as a white powder (0.45 g, 50%): mp 144–147 °C (Et_2O); ^{1}H NMR ($CDCl_3$) δ 0.89 (t, 3H), 1.15–1.65 (m, 4H), 2.39 (s, 3H), 3.62 (s, 3H), 3.94 (s, 2H), 7.10–7.55 (m, 7H), 7.78 (dd, 1H).

4'-[(6-Butyl-1,4-dihydro-2-methyl-4-oxo-5-pyrimidinyl)-methyl][1,1'-biphenyl]-2-carbonitrile (9d). Starting from 8c (4 g, 11.4 mmol), compound **9d** was obtained as a white powder (3.2 g, 80%): mp 167–169 °C (H_2O) (lit.[34] mp 173 °C); ^{1}H NMR ($CDCl_3$) δ 0.89 (t, 3H), 1.20–1.65 (m, 4H), 2.40 (s, 3H), 2.61 (t, 2H), 3.96 (s, 2H), 7.30–7.75 (m, 8H).

Methyl 4'-[(6-Butyl-1,4-dihydro-4-oxo-5-pyrimidinyl)-methyl][1,1'-biphenyl]-2-carboxylate (9a). Starting from 8a (5 g, 13.1 mmol) and formamidine hydrochloride (1.05 g, 13.1 mmol), compound **9a** was obtained as a white powder (2.97 g, 60%): mp 133–135 °C (Et_2O); ^{1}H NMR (DMSO-d_6) δ 0.82 (t, 3H), 1.15–1.55 (m, 4H), 2.45–2.55 (m, 2H), 3.57 (s, 3H), 3.84 (s, 2H), 7.10–7.75 (m, 8H), 8.05 (s, 1H).

Methyl 2-[[4-Butyl-2-methyl-6-oxo-5-[[2'-[1-(triphenyl-methyl)-1*H*-tetrazol-5-yl][1,1'-biphenyl]-4-yl]methyl]-1(6*H*)-pyrimidinyl]methyl]benzoate (12h). Method A. A solution of **9c** (3.3 g, 5.13 mmol) in 10 mL of dry DMF was added, under N_2 atmosphere, to a stirred suspension of 80% NaH (0.18 g, 6.15 mmol) in 20 mL of dry DMF, maintaining the temperature at 15–20 °C. When effervescence stopped, **11c** (1.68 g, GC 85%, 6.15 mmol) dissolved in 10 mL of dry DMF was added to the mixture. After stirring at room temperature for about 2 h, the mixture was cautiously poured onto ice–water (200 mL), under vigorous stirring, and the pH was adjusted to 7 with 50% CH_3COOH. The resulting precipitate was filtered off, washed with water, and chromatographed (hexane–EtOAc, 60:40) to give compound **12h** with $R_f = 0.28$ as a white foam (2.0 g, 49%): ^{1}H NMR ($CDCl_3$) δ 0.91 (t, 3H), 1.15–1.65 (m, 4H), 2.36 (s, 3H), 2.53 (t, 2H), 3.85 (s, 2H), 3.93 (s, 3H), 5.73 (s, 2H), 6.75 (dd, 1H), 6.82–7.10 (m, 8H), 7.18–7.48 (m, 16H), 7.78 (dd, 1H), 8.08 (dd, 1H); IR (Nujol) 1719 ($COOCH_3$), 1657 (amide C=O) cm^{-1}.

The O-alkylated regioisomer with $R_f = 0.37$ was also recovered as a foam (1.9 g, 47%): ^{1}H NMR ($CDCl_3$) δ 0.88 (t, 3H), 1.25–1.55 (m, 4H), 2.55 (s, 3H), 2.57 (m, 2H), 3–84 (s, 3H), 3–91 (s, 2H), 5.80 (s, 2H), 6.85–7.50 (m, 25H), 7.83–7.99 (m, 2H); IR (Nujol) 1717 ($COOCH_3$) cm^{-1}.

Compounds **12a–g,k** were prepared according to this procedure and are reported in Table 2.

Methyl 2-[[4-Butyl-2-methyl-6-oxo-5-[[2'-[1-(triphenyl-methyl)-1*H*-tetrazol-5-yl][1,1'-biphenyl]-4-yl]methyl]-1(6*H*)-pyrimidinyl]methyl]-3-thiophenecarboxylate (12i). Method B. A solution of **9c** (12 g, 18.7 mmol) in 80 mL of dry DMF was added, under N_2 atmosphere, to a stirred suspension of 80% NaH (0.73 g, 24.3 mmol) in 20 mL of dry DMF, maintaining the temperature at 15–20 °C. When effervescence was over, anhydrous LiBr (3.73 g, 42.9 mmol) and, after a few minutes, **11b** (7.9 g, GC 61%, 20.5 mmol) dissolved in 20 mL of dry DMF were added to the mixture maintaining the temperature at 25 °C. After stirring at room temperature for about 2 h, the mixture was cautiously poured onto ice–water (500 mL) under vigorous stirring, and the pH was adjusted to 7 with 50% CH_3COOH. The resulting precipitate was filtered off, washed with water, and chromatographed (hexane–EtOAc, 60:40) to give compound **12i** with $R_f = 0.32$ as a white powder (9.2 g, 62%): mp 150–152 °C (EtOAc); ^{1}H NMR ($CDCl_3$) δ 0.88 (t, 3H), 1.20–1.62 (m, 4H), 2.46 (s, 3H), 2.49 (t, 2H), 3.87 (s, 2H), 3.90 (s, 3H), 5.87 (s, 2H), 6.82–7.10 (m, 10H), 7.10 (d, 1H), 7.15–7.50 (m, 13H), 7.88 (m, 1H); IR (Nujol) 1713 ($COOCH_3$), 1657 (amide C=O) cm^{-1}.

The O-alkylated regioisomer with $R_f = 0.41$ was also recovered as an oil (4.6 g, 31%): ^{1}H NMR ($CDCl_3$) δ 0.85 (t, 3H), 1.20–1.55 (m, 4H), 2.53 (s, 3H), 2.55 (m, 2H), 3.84 (s, 3H), 3.92 (s, 2H), 5.92 (s, 2H), 6.80–7.95 (m, 25H); IR (Nujol) 1713 ($COOCH_3$) cm^{-1}.

Compounds **12j,l–n** were prepared according to this procedure and are reported in Table 2.

Methyl 2-[[4-Butyl-2-methyl-6-oxo-5-[[2'-(1*H*-tetrazol-5-yl)[1,1'-biphenyl]-4-yl]methyl]-1(6*H*)-pyrimidinyl]-methyl]-3-thiophenecarboxylate (3k). A solution of **12i** (6.4 g, 8 mmol) in 100 mL of MeOH was refluxed until the reaction was completed. The solvent was evaporated under vacuum, and the residue was taken up with Et_2O (40 mL). The resulting precipitate was filtered off and crystallized from acetone to give compound **3k** as a white powder (3.55 g, 80%): mp 178–180 °C; ^{1}H NMR ($CDCl_3$) δ 0.91 (t, 3H), 1.25–1.75 (m, 4H), 2.43 (s, 3H), 2.59 (t, 2H), 3.89 (s, 3H), 3.91 (s, 2H), 5.80 (s, 2H), 7.05–7.22 (q, 4H), 7.23 (d, 1H, J = 5.4 Hz), 7.39 (d, 1H, J = 5.4 Hz), 7.43–7.61 (m, 3H), 8.01 (dd, 1H); MS (EI) *m/e* 554 (M). Anal. ($C_{30}H_{30}N_6O_3S$) C, H, N.

The following compounds were analogously prepared.

6-Butyl-2-methyl-5-[[2'-(1*H*-tetrazol-5-yl)[1,1'-biphenyl]-4-yl]methyl]pyrimidin-4(3*H*)-one (2c). Starting from **9c** (0.68 g, 1.06 mmol), compound **2c** was obtained as a white powder after crystallization with EtOH (0.36 g, 85%): mp 246–248 °C dec; ^{1}H NMR (DMSO-d_6) δ 0.81 (t, 3H), 1.10–1.46 (m, 4H), 2.23 (s, 3H), 2.42 (t, 2H), 3.34 (s, 2H), 7.04 (q, 4H), 7.42–7.68 (m, 4H), 12.31 (br s, 1H). Anal. ($C_{23}H_{24}N_6O$) C, H, N.

Methyl 2-[[4-Butyl-2-methyl-6-oxo-5-[[2'-(1*H*-tetrazol-5-yl)[1,1'-biphenyl]-4-yl]methyl]-1(6*H*)-pyrimidinyl]-methyl]-3-furancarboxylate (3l). Starting from **12j** (5 g, 6.4 mmol), compound **3l** was obtained as a white powder after crystallization from EtOAc (2.51 g, 73%): mp 112–117 °C dec; ^{1}H NMR ($CDCl_3$) δ 0.91 (t, 3H), 1.25–1.58 (m, 4H), 2.44 (s, 3H), 2.57 (t, 2H), 3.86 (s, 3H), 3.89 (s, 2H), 5.57 (s, 2H), 6.66 (d, 1H, J = 2 Hz), 7.11 (q, 4H), 7.35 (d, 1H, J = 2 Hz), 7.36–7.58 (m, 3H), 8.03 (dd, 1H). Anal. ($C_{30}H_{30}N_6O_4$) C, H, N.

Methyl 3-[[4-Butyl-2-methyl-6-oxo-5-[[2'-(1*H*-tetrazol-5-yl)[1,1'-biphenyl]-4-yl]methyl]-1(6*H*)-pyrimidinyl]-methyl]-2-thiophenecarboxylate (3m). Starting from **12k** (0.4 g, 0.5 mmol), compound **3m** was obtained as an ivory powder after flash chromatography (CH_2Cl_2–MeOH, 95:5) (0.12 g, 44%): mp 170–173 °C; ^{1}H NMR ($CDCl_3$) δ 0.92 (t, 3H), 1.22–1.72 (m, 4H), 2.38 (s, 3H), 2.62 (t, 2H), 3.90 (s, 3H), 5.61 (s, 2H), 6.54 (d, 1H, J = 5 Hz), 7.13 (q, 4H), 7.48–7.62 (m, 4H), 8.02 (dd, 1H); MS (FAB$^+$) *m/e* 555 (MH). Anal. ($C_{30}H_{30}N_6O_3S$) C, H, N.

6-Butyl-2-methyl-3-(phenylmethyl)-5-[[2'-(1*H*-tetrazol-5-yl)[1,1'-biphenyl]-4-yl]methyl]pyrimidin-4(3*H*)-one (3n). Starting from **12l** (0.7 g, 0.87 mmol), compound **3n** was obtained as a white powder (2.6 g, 60%) after flash chromatography (CH_2Cl_2–MeOH, 90:10): mp 100–102 °C dec; ^{1}H NMR ($CDCl_3$) δ 0.92 (t, 3H), 1.31–1.61 (m, 4H), 2.44 (s, 3H), 2.61 (t, 2H), 3.94 (s, 2H), 5.24 (s, 2H), 7.09–7.58 (m, 12H), 8.15 (m, 1H). Anal. ($C_{30}H_{30}N_6O$) C, H, N.

6-Butyl-2-methyl-5-[[2'-(1*H*-tetrazol-5-yl)[1,1'-biphenyl]-4-yl]methyl]-3-(2-thienylmethyl)pyrimidin-4(3*H*)-one (3o). Starting from **12m** (1.7 g, 2.3 mmol), compound **3o** was obtained as a white powder (1 g, 88%): mp 162–164 °C (Et_2O); ^{1}H NMR (DMSO-d_6) δ 0.81 (t, 3H), 1.18–1.47 (m, 4H), 2.43 (t, 2H), 2.53 (s, 3H), 3.83 (s, 2H), 5.37 (s, 2H), 6.95–7.03 (m, 3H), 7.11–7.18 (m, 3H), 7.45–7.72 (m, 5H). Anal. ($C_{28}H_{28}N_6OS$) C, H, N.

Methyl 2-[[4-Butyl-2-methyl-6-oxo-5-[[2'-(1*H*-tetrazol-5-yl)[1,1'-biphenyl]-4-yl]methyl]-1(6*H*)-pyrimidinyl]-methyl]-3-thiophenecarboxylate (3k). To a solution of **12n** (0.3 g, 0.59 mmol) in 3 mL of toluene, under N_2 atmosphere, were added NaN_3 (42 mg, 0.65 mmol) and tributyltin chloride (0.21 g, 0.65 mmol). After stirring at reflux for 60 h, the mixture was evaporated at reduced pressure and taken up with MeOH and a few drops of 37% HCl. The mixture was stirred at room temperature for 4 h and evaporated under vacuum. The residue was triturated with Et_2O. The resulting solid was filtered off and chromatographed (CH_2Cl_2–MeOH, 90:10) to give compound **3k** (0.12 g, 37%).

4'-[(2-Butyl-4-methyl-6-oxo-1(6*H*)-pyrimidinyl)methyl]-[1,1'-biphenyl]-2-carboxylic Acid (1b). A solution of **6b** (0.38 g, 0.97 mmol) in 10 mL of MeOH and 1.5 mL of 2 N NaOH was refluxed until the reaction was completed. The solvent was evaporated in vacuo, and the residue was taken up with water, washed with Et_2O, and acidified with aqueous HCl to pH 4. The resulting precipitate was filtered off and washed with water affording compound **1b** as a white powder (0.34 g, 90%): mp 186–188 °C; ^{1}H NMR (DMSO-d_6) δ 0.81 (t, 3H), 1.18–1.40 (m, 2H), 1.46–1.64 (m, 2H), 2.20 (s, 3H), 2.67 (t, 2H), 5.31 (s, 2H), 6.26 (s, 1H), 7.08–7.78 (m, 8H), 12.73 (s, 1H). Anal. ($C_{23}H_{24}N_2O_3$) C, H, N.

The following compounds were analogously prepared.

4'-[(2-Butyl-6-oxo-1(6*H*)-pyrimidinyl)methyl][1,1'-biphenyl]-2-carboxylic Acid (1a). Starting from **6a** (0.33 g, 0.88 mmol), compound **1a** was obtained as a white powder

(0.25 g, 80%). It was transformed into the corresponding hydrochloride by treatment with HCl–Et_2O: mp 202–205 °C; ^{1}H NMR (DMSO-d_6) δ 0.79 (t, 3H), 1.18–1.38 (m, 2H), 1.48–1.62 (m, 2H), 2.78 (t, 2H), 5.36 (s, 2H), 6.49 (d, 1H, J = 6.7 Hz), 7.12–7.60 (m, 7H), 7.73 (dd, 1H), 7.98 (d, 1H, J = 6.7 Hz). Anal. ($C_{22}H_{22}N_2O_3$·HCl) C, H, N.

4'-[(6-Butyl-1,4-dihydro-4-oxo-5-pyrimidinyl)methyl]-[1,1'-biphenyl]-2-carboxylic Acid (2a). Starting from **9a** (0.42 g, 1.11 mmol), compound **2a** was obtained as a white powder (0.35 g, 88%): mp 197–200 °C; ^{1}H NMR (DMSO-d_6) δ 0.83 (t, 3H), 1.12–1.56 (m, 4H), 2.45–2.58 (m, 2H), 3.84 (s, 2H), 7.12–7.71 (m, 8H), 8.05 (s, 1H). Anal. ($C_{22}H_{22}N_2O_3$·H_2O) C, H, N.

4'-[(6-Butyl-1,4-dihydro-2-methyl-4-oxo-5-pyrimidinyl)-methyl][1,1'-biphenyl]-2-carboxylic Acid (2b). Starting from **9b** (0.42 g, 1.07 mmol), compound **2b** was obtained as a white powder (0.34 g, 85%): mp 208–210 °C; ^{1}H NMR (DMSO-d_6) δ 0.84 (t, 3H), 1.12–1.52 (m, 4H), 2.27 (s, 3H), 2.45–2.55 (m, 2H), 3.81 (s, 2H), 7.08–7.72 (m, 8H). Anal. ($C_{23}H_{24}N_2O_3$) C, H, N.

4'-[[4-Butyl-1,6-dihydro-6-oxo-1-(phenylmethyl)-5-pyrimidinyl]methyl][1,1'-biphenyl]-2-carboxylic Acid (3a). Starting from **12a** (0.14 g, 0.3 mmol), compound **3a** was obtained as a white powder (0.1 g, 74%): mp 198–200 °C; ^{1}H NMR (DMSO-d_6) δ 0.83 (t, 3H), 1.15–1.55 (m, 4H), 2.50–2.60 (m, 2H), 3.87 (s, 2H), 5.12 (s, 2H), 7.12–7.72 (m, 13H), 8.55 (s, 1H). Anal. ($C_{29}H_{28}N_2O_3$) C, H, N.

4'-[[4-Butyl-1-[(4-carboxyphenyl)methyl]-1,6-dihydro-6-oxo-5-pyrimidinyl]methyl][1,1'-biphenyl]-2-carboxylic Acid (3c). Starting from **12c** (0.7 g, 1.33 mmol), compound **3c** was obtained as a white powder using 6 equiv of NaOH (0.59 g, 89%): mp 219–220 °C; ^{1}H NMR (DMSO-d_6) δ 0.84 (t, 3H), 1.18–1.58 (m, 4H), 2.54 (t, 2H), 3.87 (s, 2H), 5.19 (s, 2H), 7.12–7.72 (m, 10H), 7.93 (d, 2H), 8.57 (s, 1H); MS (EI) *m/e* 496 (M). Anal. ($C_{30}H_{28}N_2O_5$·H_2O) C, H, N.

4'-[[4-Butyl-1,6-dihydro-6-oxo-1-(2-thienylmethyl)-5-pyrimidinyl]methyl][1,1'-biphenyl]-2-carboxylic Acid (3b). Starting from **12b** (0.2 g, 0.42 mmol), compound **3b** was obtained as a white powder (0.17 g, 89%): mp 205–208 °C; ^{1}H NMR (DMSO-d_6) δ 0.82 (t, 3H), 1.12–1.52 (m, 4H), 2.50–2.60 (m, 2H), 3.89 (s, 2H), 5.29 (s, 2H), 6.95–7.70 (m, 11H), 8.54 (s, 1H). Anal. ($C_{27}H_{26}N_2O_3S$) C, H, N.

4'-[[4-Butyl-1-[(2-carboxyphenyl)methyl]-1,6-dihydro-6-oxo-5-pyrimidinyl]methyl][1,1'-biphenyl]-2-carboxylic Acid (3d). Starting from **12d** (0.5 g, 0.95 mmol), compound **3d** was obtained as a white powder after flash chromatography (CH_2Cl_2–MeOH–CH_3COOH, 88:10:2) and trituration with water (0.29 g, 61%): mp 185–187 °C; ^{1}H NMR (DMSO-d_6) δ 0.85 (t, 3H), 1.20–1.60 (m, 4H), 2.56 (t, 2H), 3.88 (s, 2H), 5.49 (s, 2H), 6.92 (d, 1H), 7.12–7.72 (m, 10H), 7.93 (d, 1H), 8.48 (s, 1H). Anal. ($C_{30}H_{28}N_2O_5$) C, H, N.

4'-[[4-Butyl-1-[(2-carboxyphenyl)methyl]-1,6-dihydro-2-methyl-6-oxo-5-pyrimidinyl]methyl][1,1'-biphenyl]-2-carboxylic Acid (3e). Starting from **12e** (0.13 g, 0.24 mmol), compound **3e** was obtained as a white powder (0.11 g, 92%): mp 150–155 °C dec; ^{1}H NMR (DMSO-d_6) δ 0.87 (t, 3H), 1.18–1.52 (m, 4H), 2.49 (s, 3H), 2.59 (t, 2H), 3.90 (s, 2H), 5.63 (s, 2H), 6.85 (d, 1H), 7.12–7.60 (m, 9H), 7.68 (dd, 1H), 8.01 (dd, 1H). Anal. ($C_{31}H_{30}N_2O_5$) C, H, N.

2-[[4-Butyl-2-methyl-6-oxo-5-[(2'-carboxy[1,1'-biphenyl]-4-yl)methyl]-1(6*H*)-pyrimidinyl]methyl]-3-thiophenecarboxylic Acid (3f). Starting from **12f** (0.25 g, 0.46 mmol), compound **3f** was obtained as a white powder using 6 equiv of NaOH (0.19 g, 80%): mp 175–178 °C dec; ^{1}H NMR (DMSO-d_6) δ 0.84 (t, 3H), 1.10–1.52 (m, 4H), 2.44 (s, 3H), 2.50–2.60 (m, 2H), 3.90 (s, 2H), 5.75 (s, 2H), 7.02–7.65 (m, 10H). Anal. ($C_{29}H_{28}N_2O_5S$) C, H, N.

2-[[4-Butyl-2-methyl-6-oxo-5-[(2'-carboxy[1,1'-biphenyl]-4-yl)methyl]-1(6*H*)-pyrimidinyl]methyl]-3-furancarboxylic Acid (3g). Starting from **12g** (0.15 g, 0.29 mmol), compound **3g** was obtained as a white powder using 6 equiv of NaOH (0.11 g, 78%): mp 143–148 °C; ^{1}H NMR (DMSO-d_6) δ 0.85 (t, 3H), 1.12–1.55 (m, 4H), 2.45 (s, 3H), 2.50–2.60 (m, 2H), 3.85 (s, 2H), 5.58 (s, 2H), 6.71 (d, 1H), 7.05–7.68 (m, 9H). Anal. ($C_{29}H_{28}N_2O_6$) C, H, N.

2-[[4-Butyl-2-methyl-6-oxo-5-[[2'-(1*H*-tetrazol-5-yl)[1,1'-biphenyl]-4-yl]methyl]-1(6*H*)-pyrimidinyl]methyl]-3-thiophenecarboxylic Acid (3i). Starting from **3k** (2 g, 3.61 mmol), compound **3i** was obtained as a white powder (1.8 g, 92%): mp 215–219 °C dec (acetone); ^{1}H NMR (DMSO-d_6) δ 0.83 (t, 3H), 1.15–1.55 (m, 4H), 2.42 (s, 3H), 2.45–2.52 (m, 2H), 3.84 (s, 2H), 5.73 (s, 2H), 7.05 (q, 4H), 7.40 (q, 2H), 7.46–7.70 (m, 4H). Anal. ($C_{29}H_{28}N_6O_3S$·0.75H_2O) C, H, N.

2-[[4-Butyl-2-methyl-6-oxo-5-[[2'-(1*H*-tetrazol-5-yl)[1,1'-biphenyl]-4-yl]methyl]-1(6*H*)-pyrimidinyl]methyl]benzoic Acid (3h). Starting directly from **12h** (2.5 g, 3.16 mmol), compound **3h** was obtained as a white powder, after flash chromatography (CH_2Cl_2–MeOH–CH_3COOH, 89.9:10:0.1) and trituration with water (1.23 g, 73%): mp 193–196 °C; ^{1}H NMR (DMSO-d_6) δ 0.85 (t, 3H), 1.18–1.60 (m, 4H), 2.32 (s, 3H), 2.45–2.55 (m, 2H), 3.83 (s, 2H), 5.60 (s, 2H), 6.65 (d, 2H), 7.04 (q, 4H), 7.35–7.60 (m, 6H), 8.00 (d, 1H). Anal. ($C_{31}H_{32}N_6O_3$) C, H, N.

2-[[4-Butyl-2-methyl-6-oxo-5-[[2'-(1*H*-tetrazol-5-yl)[1,1'-biphenyl]-4-yl]methyl]-1(6*H*)-pyrimidinyl]methyl]-3-furancarboxylic Acid (3j). Starting from **3l** (0.5 g, 0.93 mmol), compound **3j** was obtained as a white powder (0.44 g, 90%): mp 220–223 °C dec (EtOAc); ^{1}H NMR (DMSO-d_6) δ 0.83 (t, 3H), 1.12–1.52 (m, 4H), 2.44 (s, 3H), 2.42–2.54 (m, 2H), 3.80 (s, 2H), 5.56 (s, 2H), 6.71 (d, 1H, J = 2 Hz), 7.04 (2, 4H), 7.46–7.70 (m, 5H). Anal. ($C_{29}H_{28}N_6O_4$·0.75H_2O) C, H, N.

[^{3}H]Angiotensin II Binding to Rat Adrenal Cortex Membranes. Rat adrenal cortices were obtained from male Crl:CD BR rats (250–300 g; Charles River Italia, Calco, Italy) and membranes prepared as described[46] with minor modifications. Briefly, rat adrenal cortices were homogenized in 50 mM Tris HCl (pH 7.4) and centrifuged at 50000g at 4 °C for 15 min. The resulting pellet was washed twice in 100 mM NaCl, 5 mM Na_2EDTA, 10 mM Na_2HPO_4 (pH 7.4), and 0.1 mM phenylmethanesulfonyl fluoride (PMSF) by resuspension and centrifugation. For binding assays, the pellet was resuspended in a binding assay buffer of the following composition: 100 mM NaCl, 10 mM Na_2HPO_4, 5 mM Na_2EDTA, 0.1 mM PMSF, 0.2 mg/mL soybean trypsin inhibitor, 0.018 mg/mL *o*-phenanthroline, 2 mg/mL protease free BSA unless otherwise specified, and 0.14 mg/mL bacitracin, pH 7.4, to a final protein concentration of ca. 0.5–1 mg/mL. Protein concentration was determined using bovine γ-globulin as a standard.[47] Binding assays were performed in poly(propylene) tubes containing, in a final volume of 0.5 mL, binding assay buffer, [^{3}H]A II (0.8–1.2 nM), membranes (0.05–0.08 mg of protein), and varying concentrations of test compounds. After a 60 min 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% BSA solution), using a Brandell cell harvester, and the mixture was washed rapidly three times with 4 mL of ice-cold Tris HCl (50 mM, pH 7.4). The radioactivity trapped was determined by liquid scintillation counting using a Packard 2200 CA scintillation counter. Nonspecific binding was determined in the presence of 1 μM A II. The K_i values were calculated using the nonlinear, iterative fitting program EBDA/LIGAND.[48]

Angiotensin II-Induced Pressor Response in Anesthetized, Ganglion-Blocked Rats (iv or id Route). Male Sprague–Dawley rats (Nossan, Correzzana, Italy; 300–400 g) were anesthetized with ethyl urethane (1.25 g/kg im), and the trachea was cannulated. The left femoral and jugular veins were cannulated for the administration of A II and pentolinium tartrate (0.1 mg/kg/min throughout the experiment), respectively. A poly(ethylene) catheter was inserted into the left carotid artery and connected to a Statham pressure transducer. Blood pressure was recorded by means of a Gemini 7070 instrument (U. Basile, Comerio, Italy). Body temperature was maintained at 37 °C by means of an homeothermic blanket. When compounds were evaluate by iv route, following a 30 min stabilization period, A II (100 ng/kg) was given iv at 10 min intervals until three reproducible responses were obtained; then compounds were injected iv 5 min before the next A II challenge. A II challenging was continued throughout the experiment until pressor response returned near the basal value. Compounds were administered also by the id

route, and A II-induced pressor challenges were performed every 15 min. The test compounds were dissolved in 1% DMSO (water solution) with the addition of a stoichiometric amount of 0.01 N NaOH or suspended in methocel (0.5%). A single dose of test compound was tested in each animal. ED_{50}'s (95% confidence limits) of test compounds were determined using the least squares linear regression considering the inhibitory effect 5 or 30 min from the iv or id administration, respectively.

Angiotensin II-Induced Pressor Response in Conscious, Normotensive Rats. Male Sprague–Dawley rats (Charles River, Calco, Italy), weighing 280–340 g, were premedicated with ip fentanyl (0.024 mg/kg) plus fluanisone (1.2 mg/kg; Hypnorm) and anesthetized with sodium pentobarbital (30–35 mg/kg iv). Poly(ethylene) catheters were inserted into the left carotid artery and right jugular vein and exteriorized behind the head through a single channel swivel (U. Danuso, Milan, Italy). The carotid artery was connected to a Transpac II transducer (Abbott, Campoverde, Italy) connected to a 8805 D preamplifier (Hewlett-Packard, Milan, Italy), and blood pressure was recorded by a 7758 D polygraph (Hewlett-Packard). Data samples were taken using a IDAS BM 9000 instrument (Biomedica Mangoni, Pisa, Italy) coupled to a Compaq 386/20e computer. Eighteen hours after surgery following an overnight fast with water ad libitum, a submaximal pressor dose of A II (110 ng/kg iv) was injected three times at 20 min intervals to establish the base line response. Then, vehicle, **3k** (0.6–2–6 μmol/kg), **3l** (2 μmol/kg), or Losartan (2–6 μmol/kg) was administered orally, and A II bolus injections were performed every 15 min for the first hour and then every 30 min.

Conscious, Renal Hypertensive Rats. Normotensive rats were made hypertensive by unilateral ligation of the left renal artery as previously described.[49] Male Sprague–Dawley rats (Charles River, Calco, Italy) weighing 270–330 g were anesthetized with sodium pentobarbital (45 mg/kg ip), and a midline abdominal incision was made to expose the left kidney. The renal artery was closed with steril 4-0 silk, and the incision was closed by suturing of the muscle layer and skin. Five days later, the rats were anesthetized and instrumented as described above. Eighteen hours after surgery, only animals with a mean arterial pressure (MAP) value >150 mmHg were included in the study. To evaluate the antihypertensive effect vehicle, **3k,l** or Losartan (2–6 μmol/kg) was administered orally and the effects on MAP were monitored for the following 24 h.

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