Synthesis and Structure−Activity Relationship of a New Series of Potent AT_1 Selective Angiotensin II Receptor Antagonists: 5-(Biphenyl-4-ylmethyl)pyrazoles

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The synthesis and pharmacological activity of a new series of 5-(biphenyl-4-ylmethyl)pyrazoles as potent angiotensin II antagonists both *in vitro* (binding of [^{3}H]AII) and *in vivo* (iv, inhibition of AII-induced increase in blood pressure, pithed rats; po, furosemide-treated sodium-depleted rats) are reported. The various substituents of the pyrazole ring have been modified taking into account the receptor's requirements derived from related structure−activity relationship studies. A propyl or butyl group at position 1 as well as a carboxylic acid group at position 4 were shown to be essential for high affinity. Different groups at position 3 (H, small alkyl, phenyl, benzyl) provided good binding affinity, but oral activity was highly discriminating: bulky alkyl groups provided the highest potencies. Among the acidic isosteres tested in the biphenyl moiety, the tetrazole group proved to be the best. Compound **14n** (3-*tert*-butyl-1-propyl-5-[[2′-(1*H*-tetrazol-5-yl)-1,1′-biphenyl-4-yl]methyl]-1*H*-pyrazole-4-carboxylic acid, UR-7280) shows high potency both *in vitro* (IC_{50} = 3 nM) and *in vivo* (iv, 61.2 ± 10% decrease in blood pressure at 0.3 mg/kg; po, 30 mmHg fall in blood pressure at 0.3 mg/kg), in comparison to losartan (IC_{50} = 59 nM; iv, 62.5 ± 8.9% decrease in blood pressure at 1 mg/kg; po, 13 mmHg fall in blood pressure at 3 mg/kg). These data, together with the good pharmacokinetic profile of **14n** in different species, have led to its selection for clinical evaluation as an antihypertensive agent.

The renin−angiotensin system (RAS) plays a key role in regulating cardiovascular homeostasis and electrolyte/fluid balance in normotensive and hypertensive subjects.[1] Activation of the renin−angiotensin cascade begins with renin secretion from the juxtaglomerular apparatus of the kidney and culminates in the formation of the octapeptide angiotensin II (AII), which then interacts with specific receptors present in different tissues.[2] Two basic types of receptors, both having a broad distribution, have been characterized so far: the AT_1 receptor, responsible for the majority of effects attributed to this peptide, and the AT_2 receptor, with a functional role yet uncertain.[3]

The main effects of AII are the regulation of blood pressure through vasoconstriction, thereby effecting an increase in vascular resistance, the regulation of volemia through the stimulated release of vasopressin and aldosterone, which induces saline retention, and the regulation of the adrenocorticotropic hormone (ACTH). Thus, reducing the levels of AII by inhibition of one of the RAS enzymes or directly blocking the AII receptors is in theory a good approach for treating hypertension, confirmed by the success of angiotensin-converting enzyme (ACE) inhibitors as antihypertensives.[4] Substantial effort has been made to find renin inhibitors, although orally active agents have only recently been reported.[5] No less effort has been devoted to finding AII antagonists, which besides being the most direct way of controlling the RAS could have the additional advantage of lacking the side effects, such as cough and angioedema, observed with ACE inhibitors, as these are probably caused by partial inhibition of the cleavage of bradykinin and substance P.[6]

Starting from the initial leads reported by Takeda,[7] researchers at DuPont[8] discovered losartan, **I**, the first orally active AT_1 selective nonpeptide AII antagonist that reached the market for the treatment of hypertension (1994, Cozaar). Whereas reports on effective replacements of the biphenyltetrazole "tail" of losartan are scarce, the imidazolic "head" of the molecule, postulated to act mainly to link the required functionalities, has been successfully replaced by a wide variety of cyclic and acyclic structures, leading to a number of compounds currently in clinical trials.[9]

The simple oxidation of the hydroxymethyl group of losartan to a carboxylic acid yielded its active metabolite EXP3174 (**II**), which showed a substantial increase of *in vitro* potency but diminished oral antihypertensive activity due to poor bioavailability. The introduction of alkyl or pentafluoroethyl groups in place of the chlorine atom of **I**, while maintaining a carboxylic acid group in position 5, increased intrinsic activity, but low bioavailability (12−15%) was again observed.[10] This was attributed to poor absorption from the gastrointestinal (GI) tract rather than to other factors such as extensive first-pass metabolism.[11] The presence of two acid functionalities was put forth as a possible cause for poor absorption in a related series of compounds, in which the imidazolic carboxylic acid group was replaced by a carboxamide, leading to a compound of high oral bioavailability.[12] A series of pyrazole derivatives **III**, explored independently by Glaxo[13] and Merck,[14] also provided highly potent compounds. With the exception of a few concrete compounds (i.e., R_2 = Bu, R_1 = cyclopropylmethyl), oral bioavailability of **III** was again of concern.[13]

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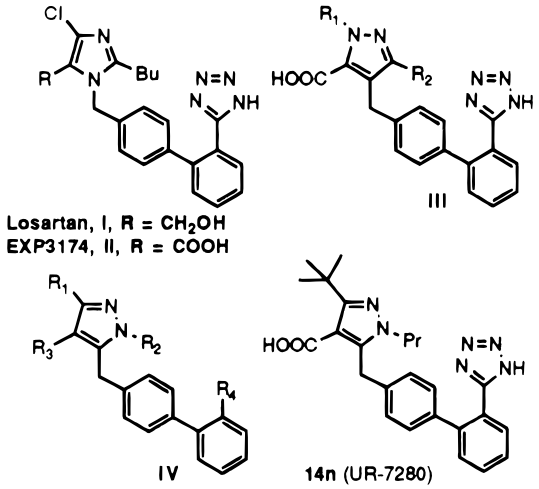
Chart 1. Angiotensin II Antagonists



We became interested in exploring a series of 5-(biphenyl-4-ylmethyl)pyrazoles **IV**, in which the disposition of the ring nitrogen atoms of **I** and **III** was altered, giving rise to a different charge distribution that might confer useful physicochemical properties. Herein we report the structure−activity relationship (SAR) study which led to the finding of new highly potent AT_1 selective AII antagonists, which are represented by compound **14n** (UR-7280).[15]

Chemistry

The synthesis of pyrazoles **IV** involves a Suzuki[16] cross-coupling reaction of the corresponding aromatic boronic acids with the key intermediates **5**, which are obtained following the route outlined in Scheme 1.

Reaction of β-keto esters **1** with 4-bromophenylacetyl chloride (**2**) was generally carried out with sodium in ether,[17] which provided diketoesters **3** in around 50% yield, accompanied in most cases by diacylated or O-acylated products. A wide range of reported conditions were tested in order to improve the yield of 45% obtained for **3n**. The acylation in the presence of magnesium chloride and pyridine[18] was initially the most promising in preventing the formation of secondary products. Starting materials were recovered in 20−30% yield. Lowering the reaction temperature to −20 °C, however, gave **3n** in quantitative yield.

N-Alkylpyrazoles **5** were obtained from **3** either directly by reaction with the corresponding *N*-alkylhydrazine (method A) or by N-alkylation of the previously formed pyrazole ring (method B). Thus, method A involved annulation with substituted hydrazines to afford mixtures of regioisomers **5** and **6** in the proportions and yields indicated in Table 1. Unsymmetrical 1,3-diketones generally give, on reaction with alkylhydrazines, mixtures of regioisomers[19] whose proportion depends on the nature of the substituents as well as on the solvent and reaction conditions employed.[20] The conditions described here (refluxing EtOH) were selected among a wide set tested for the obtention of **5b**. Other solvents (MeOH, EtOH−H_2O, dioxane, CH_2Cl_2, DMF) gave a greater amount of **6b** or lower yields due either to low conversion or to increased formation of pyrazolinones. These secondary products, considerable in the cases of **5e,h**, resulted from annulation of the ester carbonyl group and one of the remaining ketones.[21] Phenylhydrazine gave a sole isomer (**5f**) as expected.[22] The 3-unsubstituted pyrazole was obtained by cyclization of **8** with butylhydrazine, which gave a 9:1 mixture of **5a** and **6a** as predicted from the higher reactive nature of the aldehyde vs the ketone group. Reaction of **2** with ethyl hydrogenmalonate and butyllithium[23] followed by treatment with dimethylformamide dimethyl acetal[20] gave the β-keto aldehyde equivalent **8.**

Method B involved reaction with hydrazine monohydrate followed by alkylation with propyl iodide in the presence of NaH. In this case, the **5**:**6** ratio increased with the sterical hindrance of the R_1 substituent. Different bases, solvents, and reaction temperatures were assayed in order to increase the percentage of **5l,n**, but results were similar to that reported in Table 1. The proportions of the desired regioisomers were substantially improved, however, by effecting the reaction *without base* in an excess of refluxing iodopropane, to provide a 85:15 mixture of **5l**:**6l** and a 99.2:0.8 mixture of **5n**:**6n**. These results may reflect the predominance of the 5-alkyl-3-(4-bromobenzyl) tautomer in compounds **4** or only the difference in sterical hindrance of substituents at positions 3 and 5.

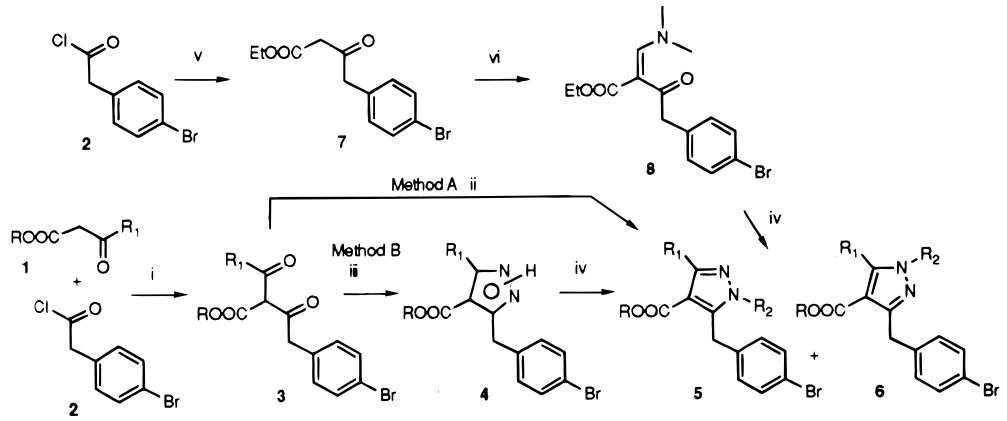
Only **5l**−**n** could be separated from their isomers **6** at this stage, either by flash chromatography or by recrystallization from acetonitrile. In other cases separation was effected later in the synthesis at the stages indicated in Table 1.

Generally (Scheme 2), the coupling of pyrazoles **5** with the trityl-protected boronic acid **9**[24] was effected under the usual conditions[16] ($Pd(PPh_3)_4$, Na_2CO_3, toluene−H_2O) to give compounds **10** in 40−60% yield, which could be increased to 80−90% using the milder conditions recently reported ($Pd(PPh_3)_4$, CsF, DME).[25] Deprotection of the tetrazole ring afforded esters **12**, which were hydrolyzed to acids **14** with KOH in EtOH (or 2-methoxyethanol in the case of the most hindered **14n**). Under these strong basic conditions the trityl group was also labile allowing the direct conversion of **10** to **14**. The regioisomers **15** were obtained from **6** or **11** using the same reaction sequence or, in some instances (see Table 1), were separated from **14** by recrystallization in the last step. Amides **16** were prepared using a DCC-mediated coupling of acids **14** and ammonia.[26]

Structural assignment was made on the basis of ^{1}H-NMR−NOESY experiments over compounds **14b** and **15b**. In **14b** significant NOE cross-peaks were seen between the benzylic methylene protons and the butyl chain methylene α to the pyrazole nitrogen, while the methyl group did not show NOE effects with any signal. The same experiment over **15b** demonstrated the proximity of the pyrazole methyl group to the butyl chain methylene, whereas the benzylic methylene showed NOE cross-peaks only with the adjacent phenyl protons. The structural assignment of the other members of this series was made on the basis of ^{1}H-NMR displacements of the benzylic methylene protons, which were always around 0.2 ppm higher in the 3-R_1 regioisomers (**5**, **10**, **12**, **14**, **17**, **19**, **23**, **30**, **32**) than in the corresponding 5-R_1 ones.

The obtention of the hydroxymethyl (**25**), aldehyde (**26**), and acetyl (**27**) derivatives (see Table 3) was effected following an analogous procedure to that described in Scheme 2 from **17**, **19**, and **23**, respectively. The preparation of these intermediates (Scheme 3) involved reduction of a mixture of **5b** and **6b** (LAH,

Scheme 1[a]



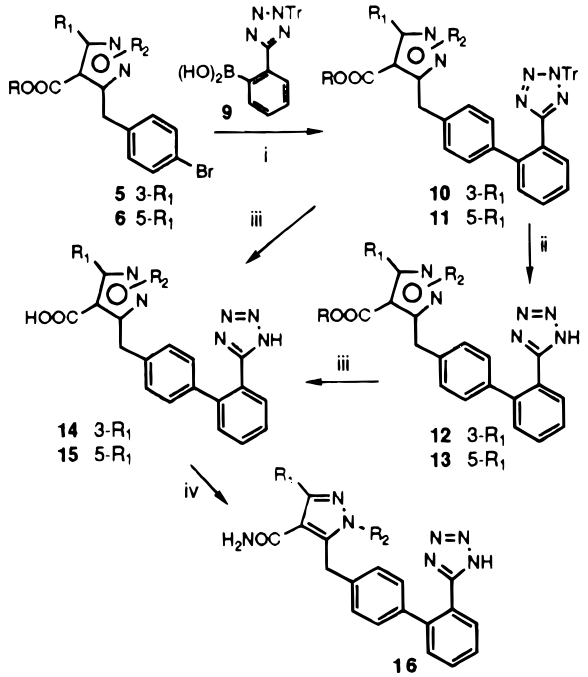
[a] (i) Na, Et_2O, 20 °C, 18 h or $MgCl_2$, Pyr, −30 °C, 3 h; (ii) H_2NNHR_2, EtOH, reflux, 5 h; (iii) $N_2H_4 \cdot H_2O$, AcOH, 20 °C, 18 h; (iv) PrI, NaH, DMF, 20 °C, 18 h or PrI (10 equiv), reflux, 72 h; (v) $EtOOCCH_2COOH$, BuLi, THF, −65 °C, 1 h; (vi) $(Me)_2NCH(OMe)_2$, 0 °C, 10 min.

Table 1. Obtention of Alkylated Pyrazoles **5**

	compound														
	a	**b**	**c**	**d**	**e**	**f**	**g**	**h**	**i**	**j**	**k**	**l**	**m**	**n**	**o**
R	Et	Me	Me	Me	Me	Me	Me	Et	Et	Et	Me	Et	Et	Me	Et
R_1	H	Me	Me	Me	Me	Me	Et	*i*-Pr	Pr	Ph	Et	*i*-Pr	*c*-Pr	*t*-Bu	Bn
R_2	Bu	Bu	Pr	Et	CH_2CF_3	Ph	Bu	Bu	Bu	Bu	Pr	Pr	Pr	Pr	Pr
method	A	A	B	A	A	A	A	A	A	A	B	B	B	B	B
% **5**[a]	90	50	50	50	65	100	35	65	35	20	50	70[b]	80	94[c]	50
yield (%)[d]	49	63	77	65	20	49	66	30	65	48	80	90	84	90	70
sep[e]	10	14	14	f	14		10	10	f	14	10	5	5	5	f
	(R)	(R)	(R)	(R)		(FC)	(FC)		(R)	(FC)	(R)	(FC)	(R)		

[a] Percentage of **5** in a purified mixture of **5** + **6**, determined by ^{1}H-NMR, GC, or HPLC. [b] With an optimized procedure from **4** (10 equiv of PrI, reflux, 72 h), a 85:15 mixture of **5l**:**6l** was obtained in 85% yield. [c] With an optimized procedure from **4** (10 equiv of PrI, reflux, 72 h), a 99.2:0.8 mixture of **5n**:**6n** was obtained in 90% yield. [d] Yield of **5** + **6**. [e] Figures indicate the compound number at which stage isomer separation was carried out, either by flash chromatography (FC) or recrystallization (R). [f] Final product obtained as a 1:1 mixture of regioisomers.

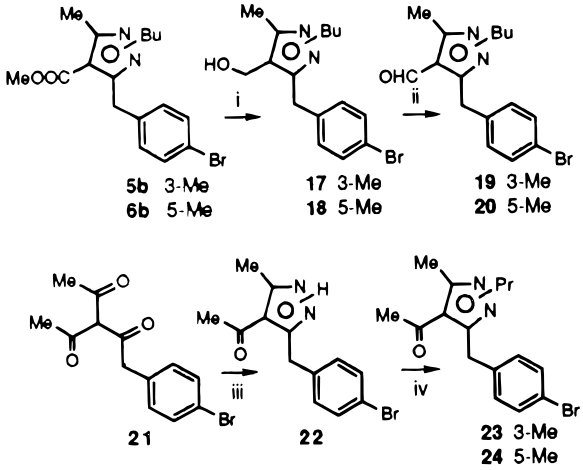
Scheme 2[a]



[a] (i) $Pd(PPh_3)_4$, Na_2CO_3, toluene−H_2O, 80 °C (or CsF, DME, reflux), 18 h; (ii) HCl, EtOH, 20 °C, 2 h; (iii) KOH, EtOH−H_2O or $MeOCH_2CH_2OH$−H_2O, reflux, 2−48 h; (iv) NH_3, DCC, HOBT, DMF, CH_2Cl_2, 20 °C, 5 h.

ether) to give **17** and **18**, which were separated by flash chromatography and subsequently oxidized (MnO_2, CH_2Cl_2) to aldehydes **19** and **20**. The acetyl derivatives **23** and **24** were obtained as a 1:1 mixture after reaction of

Scheme 3[a]

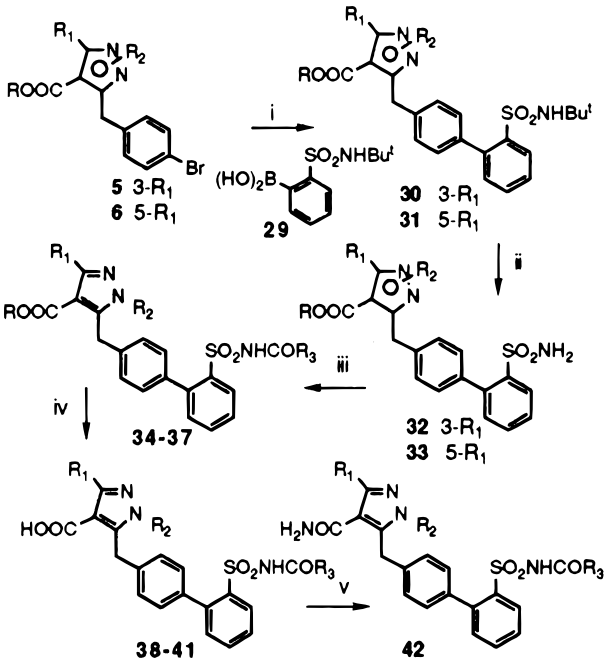


[a] (i) $LiAlH_4$, Et_2O, 20 °C, 2 h; (ii) MnO_2, CH_2Cl_2, 40 °C, 18 h; (iii) $N_2H_4 \cdot H_2O$, AcOH, 20 °C, 18 h; (iv) PrI, NaH, DMF, 20 °C, 18 h.

2,4-pentanodione with **2**, annulation to pyrazole **22**, and alkylation with iodopropane. The active regioisomer of the final product, **27**, was obtained by recrystallization in the last step of the synthesis. Decarboxylated derivative **28** (Table 3) was obtained after refluxing the parent acid **14n** with 1 N HCl/CH_3CN.

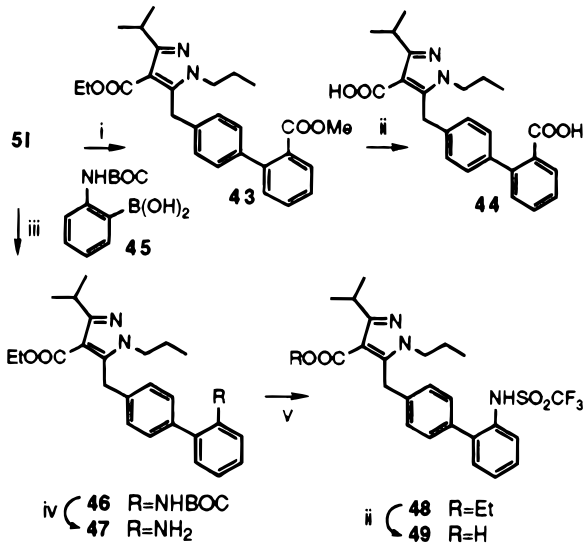
Sulfonamides **38**−**42** were prepared from **5** as depicted in Scheme 4. Palladium-catalyzed cross-coupling of **5** with 2-[(*N*-*tert*-butylamino)sulfonyl]phenylboronic acid (**29**) afforded compounds **30**, which were deprotected with TFA to give aminosulfonyl derivatives **32**.[27]

Scheme 4[a]



[a] (i) $Pd(PPh_3)_4$, CsF, DME, reflux, 18 h; (ii) TFA, anisole, 20 °C, 18 h; (iii) ClCOPh, pyridine, 20 °C, 12 h or $O(COO\text{-}t\text{-}Bu)_2$ (or ClCOO-*i*-Bu), K_2CO_3, DME, reflux, 2 h; (iv) KOH, EtOH−H_2O, reflux, 2−48 h or NaOH, EtOH−H_2O, 20 °C, 6 days; (v) a. DCI, THF, 20 °C, 3 h, b. NH_3, EtOH, 20 °C, 18 h.

Scheme 5[a]



[a] (i) Methyl 2-chlorobenzoate, Zn, $NiCl_2$, PPh_3, pyridine, 80 °C, 5 h; (ii) KOH, EtOH−H_2O, reflux, 24 h; (iii) **45**, $Pd(PPh_3)_4$, CsF, DME, reflux, 18 h; (iv) TFA, CH_2Cl_2, 20 °C, 2 h; (v) Tf_2O, TEA, CH_2Cl_2, −70 °C, 1 h.

Acylation of **32** with benzoyl chloride in pyridine or alkoxycarbonylation with di-*tert*-butyl dicarbonate or isobutyl chloroformate gave compounds **34**−**37**, which were hydrolyzed to the corresponding carboxylic acids **38**−**41**. Formation of amide **42** was achieved by DCI-mediated coupling of **41** with ammonia.

Other tetrazole replacements were prepared as shown in Scheme 5. Diacid **44** was obtained using a nickel-catalyzed cross-coupling reaction[28] with methyl 2-chlorobenzoate followed by basic hydrolysis. This type of coupling provided an alternative synthesis to compounds **12**, involving reaction of compounds **5** with 2-chlorobenzonitrile ($NiCl_2$, Zn, PPh_3, pyridine) followed by tetrazole formation with N_3SnBu_3/toluene.[8] Using this procedure a 40% overall yield of **12n** from **5n** was obtained. Trifluoromethylsulfonamide **49** was prepared from **5l** by subsequent coupling with 2-[(*tert*-butoxycarbonyl)amino]phenylboronic acid (**45**),[29] deprotection (TFA, CH_2Cl_2), trifluoroacetylation (Tf_2O, TEA, CH_2Cl_2), and hydrolysis (KOH, EtOH).

Results and Discussion

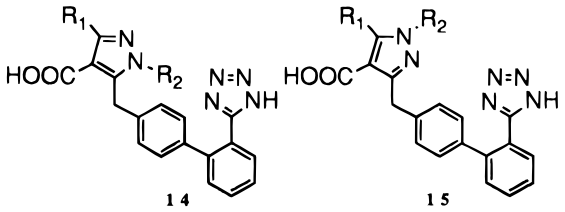
***In Vitro* AII Antagonism.** The new compounds were tested for their *in vitro* binding affinity to AT_1 (rat liver) and AT_2 (rat adrenal) receptors.[30] The IC_{50} values (concentration for 50% displacement of the specifically bound [^{3}H]AII) were determined in order to compare the relative potencies of the antagonists (Tables 2−4).

The different positions R_1−R_4 of pyrazoles **IV** were modified taking into account SAR studies of related series. It has been established in imidazole derivatives related to losartan that small alkyl groups at position 4 enhance overall activity over halogen, and propyl or butyl groups at position 2 are optimal for activity.[8−10] Similar results are observed in the pyrazole series **III**, where a wider range of substituents (aryl, benzyl) have been introduced with good results at position 1.[13,14] Groups capable of establishing a hydrogen bond acceptor interaction with the receptor, such as a carboxylic acid, seem to be needed for good activity in position 5 of imidazoles and pyrazoles **III**.[8,9,13] Finally, the biphenylic tetrazole has been replaced by other acidic isosteres, such as a sulfonamide group. This has provided different results depending on the series of compounds considered; in the (biphenylylmethyl)imidazo[4,5-*b*]pyridine derivatives reported by Merck,[31] it was shown to provide higher metabolic stability than its parent tetrazole derivative. However, in the more closely related imidazole series, the acylsulfonamide compounds maintained binding affinity but displayed shorter duration of action compared to their tetrazole analogues.[27] A carboxylic acid and a trifluoromethanesulfonamide group have been introduced with different results.[8,12]

The effect of substitution at the 1- and 3-positions of the pyrazoles **IV** was investigated using the carboxylic acids **14** (Table 2). Maintaining a methyl group in the 3-position, the N_1 substituents were modified (**14b**−**f**). Alkyl groups such as butyl or propyl provided the best results, with binding affinities for the AT_1 receptor in the nanomolar range. Shortening the chain length to ethyl or trifluoroethyl and introducing a phenyl group resulted in a substantial decrease in potency. Thus, interchanging the N_1−C_2 atoms of imidazoles such as **II** maintains *in vitro* potency, a result which contrasts with the observed in a related series of triazoles,[32] where such a change provides a substantial decrease in potency.

The nature of the substituents at the 3-position was modified in the 1-butyl or 1-propyl derivatives. All the substituents tested (hydrogen, small alkyl, phenyl, or benzyl) provided binding affinities in the nanomolar range with optimal results for the 3-methyl-1-butyl (**14b**) and 3-*tert*-butyl-1-propyl (**14n**) derivatives. However, major differences in activity were seen on *in vivo* evaluation (see below). The activity of some of the acids **15** was evaluated and shown to be 2 orders of magnitude lower than that of the corresponding regioisomers **14**, a result consistent with that described for the related series of pyrazoles **III**.[13]

Table 2. Carboxylic Acids **14** and **15**

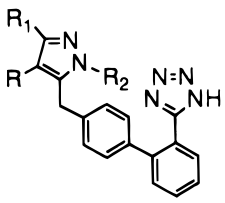


14 **15**

compd no.	R_1	R_2	mp,[a] °C	formula	anal.[b]	AT_1 binding rat liver IC_{50},[c] nM	peak inhibition AII pressor response, pithed rats (iv), % (dose, mg/kg)[d]	max fall in BP ± SEM (po), mmHg (dose, mg/kg)[e]
14a	H	Bu	216−217	$C_{22}H_{22}N_6O_2 \cdot 0.25H_2O$	C, H, N	9.1	84.2 ± 6.8 (0.3)	40 ± 5 (10)
							50.6 ± 5.4 (0.1)	10 ± 3 (3)
14b	Me	Bu	220−221	$C_{23}H_{24}N_6O_2 \cdot H_2O$	C, H, N	2.2	62.6 ± 4.8 (0.3)	20 ± 4 (3)
14c	Me	Pr	240−242	$C_{22}H_{22}N_6O_2$	C, H, N	9.3	77.8 ± 1.5 (1)	0 ± 3 (3)
14d[f]	Me	Et	200	$C_{21}H_{20}N_6O_2 \cdot 0.25H_2O$	C, H, N	120	61.2 ± 8.8 (1)	NT[g]
14e	Me	CH_2CF_3	236	$C_{21}H_{17}F_3N_6O_2 \cdot 0.25H_2O$	C, H, N	770	NA[h] (3)	NT
14f	Me	Ph	154−156	$C_{25}H_{20}N_6O_2 \cdot 2H_2O$	C, H, N	78	57.4 ± 5.6 (3)	NT
14g	Et	Bu	191−192	$C_{24}H_{26}N_6O_2 \cdot 0.25H_2O$	C, H, N	8.7	65.2 ± 10.9 (0.3)	20 ± 4 (3)
14h	*i*-Pr	Bu	174−176	$C_{25}H_{28}N_6O_2$	C, H, N	7.3	42.7 ± 9.6 (0.3)	35 ± 5 (3)
14i[f]	Pr	Bu		$C_{25}H_{28}N_6O_2 \cdot 0.5H_2O$	C, H, N	10.0	29.7 ± 16.3 (3)	NT
14j	Ph	Bu	164−168	$C_{28}H_{26}N_6O_2 \cdot 0.25H_2O$	C, H, N	9.0	44.1 ± 10.8 (3)	NT
14k	Et	Pr	229	$C_{23}H_{24}N_6O_2$	C, H, N	3.7	82.9 ± 1.0 (0.3)	28 ± 3 (1)
							35.7 ± 3.9 (0.1)	10 ± 4 (0.3)
14l	*i*-Pr	Pr	213	$C_{24}H_{26}N_6O_2$	C, H, N	6.7	80.4 ± 3.7 (0.3)	30 ± 5 (0.3)
							57.7 ± 10.8 (0.1)	15 ± 3 (0.1)
14m	*c*-Pr	Pr	186−188	$C_{24}H_{24}N_6O_2 \cdot H_2O$	C, H, N	4.4	54.7 ± 9.6 (0.3)	0 ± 2 (0.3)
14n	Bu^t	Pr	195−196	$C_{25}H_{28}N_6O_2$	C, H, N	3.0	61.2 ± 10.0 (0.3)	30 ± 3 (0.3)
							12.0 ± 2.7 (0.1)	15 ± 4 (0.1)
14o[f]	CH_2Ph	Pr		$C_{28}H_{26}N_6O_2 \cdot H_2O$	C, H, N	4.6	37.7 ± 4.6 (1)	NT
15b	Me	Bu	191−195	$C_{23}H_{24}N_6O_2 \cdot H_2O$	C, H, N	290	NT	NT
15g	Et	Bu	191−195	$C_{24}H_{26}N_6O_2 \cdot 0.25H_2O$	C, H, N	110	22.9 ± 6.2 (3)	NT
15l	*i*-Pr	Pr	158−161	$C_{24}H_{26}N_6O_2$	C, H, N	490	NT	NT
losartan, **I**						59	93.5 ± 1.2 (3)	39 ± 2 (10)
							62.5 ± 8.9 (1)	13 ± 5 (3)
EXP3174, **II**						2.7	76.9 ± 3.3 (1)	NT

[a] All compounds recrystallized from EtOAc or MeOH−EtOAc mixtures. [b] Analyses for the elements indicated were within 0.4% of the theoretical values. [c] Displacement of specifically bound [^{3}H]AII from rat liver AT_1 receptor preparation. Assays were performed in duplicate. [d] Percent peak inhibition (% ± SEM) of pressor response induced by exogenously administered AII (submaximal dose of 3 μg/kg, iv) in groups of two or more pithed rats, after iv administration of test compounds at the indicated dose. [e] Maximum fall in blood pressure (mmHg) in groups of four sodium-depleted rats, after administration of test compounds at the indicated dose (mg/kg, po). [f] Mixture of regioisomers (1:1). [g] Not tested. [h] Not active (<10% inhibition at the dose indicated).

Table 3. Compounds **12**, **16**, and **25**−**28**



comp no.[a]	R	R_1	R_2	AT_1 IC_{50},[b] nM	peak inhibition AII pressor response, pithed rats (iv), % (dose, mg/kg)[c]
12b	COOMe	Me	Bu	55	52.5 ± 8.7 (3)
12n	COOMe	*t*-Bu	Pr	19	47.3 ± 6.3 (1)
16b	$CONH_2$	Me	Bu	70	74.9 ± 4.7 (3)
16m	$CONH_2$	*c*-Pr	Pr	9.9	80.6 ± 2.5 (1)[d]
25	CH_2OH	Me	Bu	350	11.5 ± 1.3 (3)
26	CHO	Me	Bu	74	40.8 ± 9.9 (1)
27	COMe	Me	Bu	65	11.8 ± 2.4 (1)
28	H	*t*-Bu	Pr	94	NT[e]

[a] See experimental part. [b,c] See footnotes c and d of Table 2. [d] Maximum fall in blood pressure of 20 ± 3 mmHg at 1 mg/kg, po (conditions described in footnote e of Table 2). [e] Not tested.

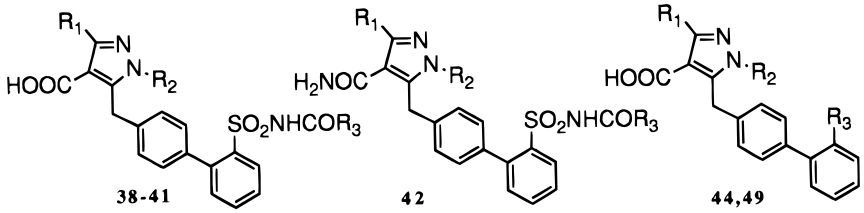
The carboxylic acid at the pyrazole 4-position was replaced by other groups. As shown in Table 3, the potencies of all the compounds tested were less than those of the corresponding acids. Introducing alkyl esters provided a loss of activity of 1 order of magnitude (**12b,n** vs **14b,n**), and also a decrease in binding affinity was observed for amides **16**. The hydroxymethyl (**25**), carboxaldehyde (**26**), and acetyl (**27**) as well as the unsubstituted derivative **28** also showed substantially less potency than did their parent acids **14b**,**n**.

Finally, the tetrazole group was replaced by other acidic functionalities (Table 4). The substituted sulfonamides **38**−**41** displayed somewhat less *in vitro* activity than did the corresponding tetrazole derivatives. Only amide **42** maintained activity in relation to its tetrazole amide counterpart **16m**. Other tetrazole surrogates, such as a carboxylic acid group and a trifluoromethylsulfonamide, provided a substantial decrease in potency (**44** and **49** vs **14l**).

The binding affinities for the AT_2 receptor were in all cases superior to 10 μM, except for **16m**, which showed values of 9.8 μM. Thus, no clear enhancement of AT_2 binding affinity was observed for the sulfonyl vs the tetrazolyl derivatives, as reported in other series.[27]

***In Vivo* Pharmacology**. The new compounds were evaluated for inhibition of AII-induced increase in mean arterial blood pressure in pithed rats. The percentage decrease in arterial blood pressure at a submaximal dose of 3 μg/kg AII was calculated for each test compound given at a dose of 3 mg/kg iv and decreasing doses until less than 80% response was observed (Tables 2−4).

Table 4. Compounds **38**−**42**, **44**, and **49**



comp no.	R_1	R_2	R_3	mp,[a] °C	formula	anal.[b]	AT_1 binding rat liver IC_{50},[c] nM	peak inhibition AII pressor response, pithed rats (iv), % (dose, mg/kg)[d]
38	Me	Bu	Ph	121−124	$C_{29}H_{29}N_3O_5S·0.5H_2O$	C, H, N	12	76.0 ± 2.7 (3)
39	Me	Bu	OBu^t	92−96	$C_{27}H_{33}N_3O_6S·0.5H_2O$	C, H, N	13	77.2 ± 1.8 (1)[e]
40	Me	Bu	OBu^i	79−82	$C_{27}H_{33}N_3O_6S$	C, H, N	30	76.3 ± 4.1 (1)[e]
41	*c*-Pr	Pr	Ph	230−232	$C_{30}H_{29}N_3O_5S·1H_2O$	C, H, N	18	18.5 ± 5.4 (1)
42	*c*-Pr	Pr	Ph	187−191	$C_{30}H_{30}N_4O_4S$	C, H, N	7.4	63.8 ± 9.2 (0.3)[f]
44	*i*-Pr	Pr	COOH	226−230	$C_{28}H_{25}KN_6O_2·1H_2O$	C, H, N	130	16.3 ± 4.3 (1)
49	*i*-Pr	Pr	$NHSO_2CF_3$	75−78	$C_{24}H_{26}F_3N_3O_4S$	C, H, N	74	NA[g] (1)

[a−d] See footnotes a−d of Table 2. [e] These compounds were also tested for oral activity in sodium-depleted rats as indicated in Table 2. Maximum fall in blood pressure for **39** and **40** at a 3 mg/kg dose was respectively: 25 ± 2 and 25 ± 5 mmHg. [f]Not active when tested orally (3 mg/kg) in pithed rats. [g] Not active (< 10% inhibition).

The most active compounds by iv route were also evaluated for oral activity in a furosemide-treated sodium-depleted rat model. The maximum fall in blood pressure (mmHg) was calculated for each test compound at a dose of 10 mg/kg po or less (Table 2).[30]

Generally speaking, the iv results correlated quite well with the *in vitro* binding affinities previously discussed. The carboxylic acids **14**, which exhibited the highest binding affinities of this series, were also the most potent compounds *in vivo*. However, there was no clear relationship between binding affinity and intravenous potency in a concrete series of compounds. For the 1-butyl derivatives, the iv potency varied in the order H > Et > Me > *i*-Pr > Ph > Pr. The 3-unsubstituted acid **14a** was around 30 times more potent than the 3-phenyl (**14j**) or 3-propyl (**14i**) derivatives, while their binding affinities were on the same order of magnitude. The reason for this discrepancy remains unclear, although it could be due to differences in affinity for plasmatic proteins or metabolism. These results contrast with those described by Merck[14] for the pyrazole series **III**, in which the phenyl-substituted compound shows good iv activity at 1 mg/kg. However, Glaxo[13] reports the same compound to be completely inactive following oral administration to renal hypertensive rats. In view of the lack of iv activity of compound **14j** and its difficult synthesis (a 1:1 mixture of **5j**:**6j** was obtained), no other aromatic substituents were introduced in position 3.

Similar results were obtained in the 1-propyl series: the iv potency followed the order Et > *i*-Pr > *t*-Bu > *c*-Pr > Me > CH_2Ph, the ethyl derivative **14k** being more than 10 times more potent than the benzyl **14o**. These results again contrast with those observed in the pyrazole series **III**, where the benzyl derivative showed high potency both *in vitro* and *in vivo*.[14] In our case, small alkyl substituents are optimal for intravenous activity, with **14a**,**k**−**n** as the most interesting compounds.

In contrast to the results observed *in vitro* and by iv route, the oral activity of the carboxylic acids **14** showed a clear dependence on the substituent at the 3-position. The activity order *i*-Pr > Me > Et > H in the 1-butyl and *t*-Bu ≥ *i*-Pr > Et > *c*-Pr > Me in the 1-propyl series indicates an increase in oral activity with the sterical bulk of the 3 substituent, probably caused by an improvement in bioavailability. Presumably, the highly polar character of the carboxylic acid group at the 4-position is increasingly masked as the volume of the 3-substituent increases, whereas the hydrogen-bonding acceptor properties of that function are retained. Other factors, such as an increase in overall lipophilicity caused by the bulky 3-substituents, could also contribute to increased oral absorption (in calculation of log *P* of aromatic rings, a *tert*-butyl group contributes with a factor of 1.98, an isopropyl group with 1.53, and an ethyl group with 1.02).[33]

The poor *in vitro* activity of the regioisomeric acids **15** and the unsubstituted derivative **28** was also confirmed *in vivo*. Analogously, the alkyl esters **12** were much less active *in vivo* than their parent acids, indicating that they are not metabolically hydrolyzed, at least not at a useful rate. The hydroxymethyl (**25**) and aldehyde (**26**) derivatives showed poor iv potency, in agreement with their binding affinities. These results indicate that none of them is metabolized by oxidation to the parent acid *in vivo*, in contrast to what happens in the imidazole series, where the hydroxymethyl derivative (i.e., losartan, **I**) is converted to the more active metabolite (EXP3174, **II**). In the pyrazole type of compounds **III** the hydroxymethyl and ester derivatives showed also substantially less potency than the corresponding acids.[13b]

A carboxamide group was introduced in position 4 with the aim of increasing absorption in relation to the parent carboxylic acid, as reported in the case of Glaxo's bromobenzofurans.[12] However, among the amides **16**, only **16m** showed similar iv potency but diminished oral activity in relation to its parent acid **14m**.

The *in vivo* results of the compounds arising from tetrazole replacement agreed quite well with the binding affinities: the carboxylic acid **44** and trifluoromethylsulfonylamino **49** were poorly active, whereas the substituted sulfonamides showed intermediate potency. Only in the case of amide **42** was the activity of the phenylsulfonamide somewhat superior to that of the

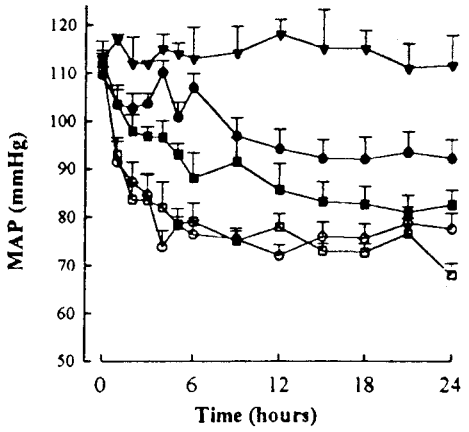


Figure 1. Oral antihypertensive effects of compound **14n** and losartan (**I**) in furosemide-treated sodium-depleted rats. The fall in mean arterial pressure (MAP, mmHg) was monitored using a telemetry device for up to 24 h. SEM are indicated for each point value; *n* is the number of animals treated: (▼) vehicle, 10 mL/kg po, *n* = 5; (□) **14n**, 1 mg/kg po, *n* = 7; (■) **14n**, 0.3 mg/kg po, *n* = 7; (○) losartan, 10 mg/kg po, *n* = 13; (●) losartan, 3 mg/kg po, *n* = 10.

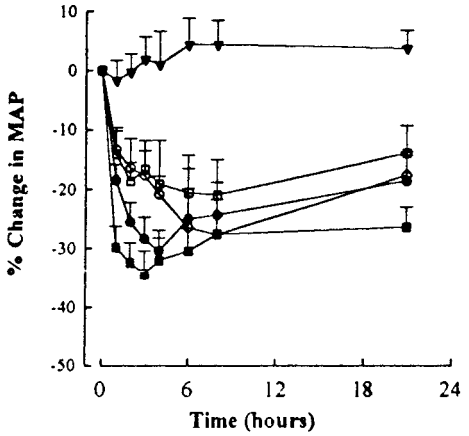


Figure 2. Oral antihypertensive effects of compound **14n** and losartan (**I**) in renal hypertensive rats (RHR). The fall in mean arterial pressure (% MAP) was measured with a tail-cuff method for up to 24 h. Basal blood pressure values were in the range of 124−143 mmHg, without significant differences among groups. SEM are indicated for each point value; *n* is the number of animals treated: (▼) vehicle, 10 mL/kg po, *n* = 11; (□) **14n**, 0.1 mg/kg po, *n* = 8; (■) **14n**, 0.3 mg/kg po, *n* = 8; (○) losartan, 10 mg/kg po, *n* = 10; (●) losartan, 3 mg/kg po, *n* = 13.

tetrazole analogue **16m**. It is interesting to note that **42** is the only amide to have greater activity both *in vitro* and iv than its parent acid **41**, although it was only poorly active when administered orally.

Compound **14n** was selected for in-depth pharmacological evaluation, since it exhibited the highest oral potency of this series. *In vitro* it behaved as a competitive, slowly reversible, selective AT_1 antagonist, demonstrated by binding studies in rat liver membranes and functional antagonism in rabbit aorta (pA_2 = 10.2, 9.84−10.55).[34] *In vivo* it showed long duration of action and produced a dose-dependent decrease in blood pressure when administered orally to furosemide-treated sodium-depleted rats (Figure 1) or renal hypertensive rats[35] (Figure 2). In both tests, compound **14n** is 10-fold more potent than the reference compound losartan (**I**), which was also 10-fold less potent *in vitro*.

In accordance with the *in vivo* results, compound **14n** showed a good pharmacokinetic profile in rats, with a

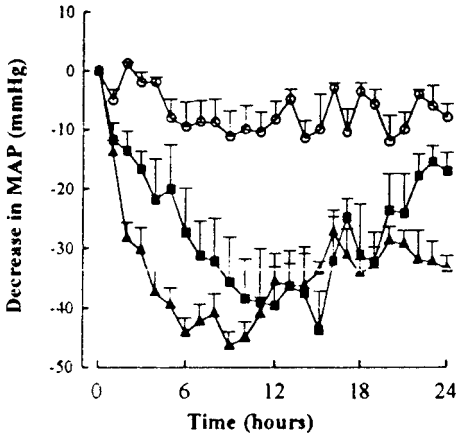


Figure 3. Oral antihypertensive effects of compound **14n** in furosemide-treated sodium-depleted dogs. The fall in mean arterial pressure (ΔMAP, mmHg) was monitored using a telemetry device for up to 24 h. Basal blood pressure values were in the range of 95−103 mmHg, without significant differences among groups. SEM are indicated for each point value; *n* is the number of animals treated: (▼) vehicle, 10 mL/kg po, *n* = 4; (▲) **14n**, 10 mg/kg po, *n* = 4; (■) **14n**, 3 mg/kg po, *n* = 4.

half-life of 7.4 h and 45% bioavailability.[36] This result may be due mainly to the presence of the bulky *tert*-butyl group at position 3, which masks the highly polar character of the carboxylic acid group at position 4. In fact, compounds **14k**−**n** showed increased oral bioavailability after oral administration with the sterical bulk of the substituent at the 3-position, with a minimum of 20% for **14k**.[36]

In addition, compound **14n** also showed good features in other species, since it produced a long-lasting dose-dependent decrease in blood pressure in conscious furosemide-treated sodium-depleted dogs (Figure 3) and displayed a good pharmacokinetic profile in rhesus monkeys (half-life of 15 h and 49% bioavailability).[37]

In summary, this study shows that the new series of 5-(biphenyl-4-ylmethyl)pyrazoles provide potent angiotensin II antagonists both *in vitro* and *in vivo*. Modification of the various substituents of the pyrazole ring indicated that a propyl or butyl group at position 1 as well as a carboxylic acid group at position 4 are essential for high affinity. Different groups at position 3 (H, small alkyl, phenyl, benzyl) provided good binding affinity, but oral activity was highly discriminating: bulky alkyl groups provided the highest potencies. Among the acidic isosteres tested in the biphenyl moiety, the tetrazole group proved to be the best. The high potency and good pharmacokinetic parameters of 3-*tert*-butyl-1-propyl-5-[[2′-(1*H*-tetrazol-5-yl)-1,1′-biphenyl-4-yl]methyl]-1*H*-pyrazole-4-carboxylic acid (**14n**, UR-7280) in different species have resulted in its selection for clinical evaluation as an antihypertensive agent.

Experimental Section

A. Chemistry. Melting points were determined with a Mettler FP 80 central processor melting point apparatus and are uncorrected. Infrared spectra were recorded on a Perkin-Elmer 983 spectrophotometer. ^{1}H- (80 MHz) and ^{13}C- (20.1 MHz) NMR spectra were recorded on a Brücker AC 80 spectrometer, and ^{1}H- (300 MHz) NMR spectra were recorded on a Brucker Avance DPX-300 spectrometer. They are reported in ppm on the δ scale, from the indicated reference. Combustion analyses were performed with a Carlo Erba 1106 analyzer. Liquid chromatography was performed with a forced

flow (flash chromatography) of the indicated solvent system on SDS silica gel Chromagel 60 ACC- (230−400 mesh). Analytical thin-layer chromatography (TLC) was performed with Macherey-Nagel 0.25 mm silica gel SIL G-25 plates. Compound **I** was kindly provided by the DuPont Merck Pharmaceutical Co. Compounds **1** were commercially available or prepared as shown for **1m**.

Ethyl 3-Cyclopropyl-3-oxopropanoate (1m). To a cooled (−70 °C) solution of monoethyl malonate (33.97 g, 257 mmol) in THF (639 mL) was added *n*-BuLi (1.6 M in hexanes, 319 mL, 514 mmol), and the mixture was stirred under an argon atmosphere for 15 min. The resulting solution was cooled to −65 °C, and cyclopropanecarbonyl chloride (15.55 g, 149 mmol) was added. The reaction mixture was stirred for 1 h at this temperature and then allowed to warm up to room temperature. Some drops of water were added, and THF was removed. The residue was taken up in 1 N HCl/Et_2O and extracted with Et_2O. The organic phase was washed with saturated $NaHCO_3$, dried, and concentrated to a residue. This was purified by chromatography on silica gel (hexane−EtOAc mixtures of increasing polarity) to afford **1m** as a yellow oil (14.70 g, 63%): ^{1}H-NMR (80 MHz, $CDCl_3$) δ (TMS) 1.1 (m, 4H), 1.28 (t, J = 7.2 Hz, 3H), 2.04 (m, 1H), 3.55 (s, 2H), 4.21 (q, J = 7.2 Hz, 2H).

4-Bromophenylacetyl Chloride (2). 4-Bromophenylacetic acid (20.0 g, 93 mmol) in thionyl chloride (29.5 mL, 0.4 mol) was heated under an argon atmosphere at 60 °C for 2 h. Removal of excess thionyl chloride *in vacuo* yielded **2** as a colorless oil (21.5 g), which was directly used in the next step as obtained: ^{1}H-NMR (80 MHz, $CDCl_3$) δ (TMS) 4.07 (s, 2H), 7.08 (d, J = 8 Hz, 2H), 7.48 (d, J = 8 Hz, 2H).

Methyl 2-[(4-Bromophenyl)acetyl]-3-oxopentanoate (3g). Sodium (30% dispersion in toluene, 8.2 mL, 94 mmol) was placed in a flask under an argon atmosphere. After washing with Et_2O, anhydrous Et_2O (108 mL) was added, and the mixture was cooled to 0 °C. A solution of methyl 3-oxopentanoate (**1g**; 11.6 mL, 93 mmol) in Et_2O (65 mL) was added, and the resulting white suspension was stirred at room temperature overnight. The reaction mixture was cooled to 0 °C, and a solution of **2** (21.5 g, 93 mmol) in anhydrous Et_2O (43 mL) was added over 30 min. The solution was stirred at room temperature overnight and then refluxed for 1 h and allowed to cool. A solution of concentrated H_2SO_4 (6.5 mL) in H_2O (65 mL) was added dropwise, the layers were separated, and the aqueous phase was extracted with Et_2O. The combined organic layers were dried and concentrated. The residue was chromatographed on silica gel (hexane−EtOAc mixtures of increasing polarity) to afford **3g** as a white solid (18.0 g, 59%): mp 34−35 °C; ^{1}H-NMR (80 MHz, $CDCl_3$) δ (TMS) 1.13 (t, J = 7.2 Hz, 3H), 2.67 (q, J = 7.2 Hz, 2H), 3.77 (s, 3H), 3.90 (s, 2H), 7.12 (d, J = 8 Hz, 2H), 7.41 (d, J = 8 Hz, 2H), 17.65 (s, 1H). Anal. ($C_{14}H_{15}BrO_4$) C, H, N.

Methyl 2-[(4-Bromophenyl)acetyl]-4,4-dimethyl-3-oxopentanoate (3n). To a suspension of $MgCl_2$ (22.48 g, 240 mmol) in CH_3CN (240 mL) was added, under nitrogen, methyl 4,4-dimethyl-3-oxopropanoate (39.34 mL, 240 mmol), and the mixture was cooled to −30 °C. Pyridine (38.8 mL, 480 mmol) was added, and the mixture was stirred for 15 min at −30 °C. A solution of **2** (56.20 g, 240 mmol) in CH_3CN (10 mL) was added dropwise for 1.5 h, and the resulting white suspension was stirred for 2 h at −30 °C. The reaction was quenched by addition of 6 N HCl (161 mL), and the two phases were separated. The aqueous phase was extracted with Et_2O, and the combined organic layers were dried over $MgSO_4$ and concentrated to afford **3n** as a yellowish solid which was used in the next step without further purification (85.0 g, quantitative). A sample was recrystallized from Et_2O−hexane to give **3n** as a white solid: mp 69 °C; ^{1}H-NMR (80 MHz, $CDCl_3$) δ (TMS) 1.12 (s, 9H), 3.76 (s, 3H), 3.86 (s, 2H), 5.06 (s, 1H), 7.06 (d, J = 8 Hz, 2H), 7.45 (d, J = 8 Hz, 2H). Anal. ($C_{16}H_{19}BrO_4 \cdot 0.25H_2O$) C, H, N.

Methyl 5(3)-[(4-Bromophenyl)acetyl]-3(5)-*tert*-butyl-1*H*-pyrazole-4-carboxylate (4n). To a solution of **3n** (86.0 g, 240 mmol) in AcOH (805 mL) was added $NH_2NH_2 \cdot H_2O$ (17.4 mL, 360 mmol), and the mixture was stirred at room temperature for 18 h. The solvent was concentrated to ca. 250 mL, and saturated aqueous $NaHCO_3$ solution (100 mL) was added followed by the addition of more $NaHCO_3$ (solid) until pH = 7. The aqueous phase was extracted with EtOAc, and the combined organic phases were washed with 1 N NaOH, dried over $MgSO_4$, and concentrated. The crude product was obtained as a white solid, which was used in the next step without further purification (77.0 g, 91%). A sample was recrystallized from CH_3CN to give **4n** as a white solid: mp 105 °C; ^{1}H-NMR (80 MHz, $CDCl_3$) δ (TMS) 1.43 (s, 9H), 3,75 (s, 3H), 4.14 (s, 2H), 7.06 (d, J = 8 Hz, 2H), 7.38 (d, J = 8 Hz, 2H), 10.3 (s, 1H); ^{13}C-NMR (20.1 MHz, $CDCl_3$) δ (TMS) 23.5 (q), 32.9 (q), 33.79 (s), 50.85 (d), 107.9 (s), 120.2 (s), 130.4 (d), 131.4 (d), 138.15 (s), 153.1 (s), 157.2 (s), 164.4 (s). Anal. ($C_{16}H_{19}BrN_2O_2$) C, H, N.

Methyl 5-[(4-Bromophenyl)acetyl]-1-butyl-3-ethyl-1*H*-pyrazole-4-carboxylate (5g) and Methyl 3-[(4-Bromophenyl)acetyl]-1-butyl-5-ethyl-1*H*-pyrazole-4-carboxylate (6g). Method A. A solution of **3g** (5 g, 0.015 mol), butylhydrazine oxalate (2.73 g, 15 mmol), and NEt_3 (2.13 mL, 15 mmol) in EtOH (127 mL) was refluxed for 5 h under an argon atmosphere. The solvent was removed, and the resulting residue was dissolved in EtOAc and H_2O. The layers were separated, and the aqueous phase was extracted with EtOAc. The combined organic layers were dried and concentrated. The residue was chromatographed on silica gel (hexane−EtOAc mixtures of increasing polarity) to afford a 35:65 mixture of the two regioisomers **5g** and **6g**, as a colorless oil (3.80 g, 66%): ^{1}H-NMR (80 MHz, $CDCl_3$) δ (TMS) 0.7−2.0 (m, 10H), 2.89 (q, J = 7.2 Hz, 2H), 3.70 (s, 3H), 3.98 (t, J = 8 Hz, 2H), 4.11 (s, 0.65 × 2H), 4.30 (s, 0.35 × 2H), 7.0−7.5 (m, 4H).

Methyl 5-[(4-Bromophenyl)acetyl]-3-*tert*-butyl-1-propyl-1*H*-pyrazole-4-carboxylate (5n) and Methyl 3-[(4-Bromophenyl)acetyl]-5-*tert*-butyl-1-propyl-1*H*-pyrazole-4-carboxylate (6n). Method B. To a cooled (0 °C) mixture of 55% NaH (11.04 g, 253 mmol) and DMF (450 mL) was added **4n** (77.0 g, 219 mmol) under an argon atmosphere. The mixture was stirred for 10 min, and propyl iodide (22.3 mL, 219 mmol) was added. The reaction mixture was stirred at room temperature overnight. The solvent was removed, the residue was taken up in EtOAc−H_2O, and the aqueous phase was extracted with EtOAc. The combined organic layers were dried and concentrated. The residue consisted of a 94:6 mixture of regioisomers (GC), from which **5n** was obtained by recrystallization from acetonitrile (73.0 g, 85%, 77% from the first step). The mother liquors were chromatographed on silica gel (hexane−EtOAc mixtures of increasing polarity) to afford **5n** (3.5 g, 4%) and **6n** (2.0 g, 2%).

5n: mp 82−83 °C; ^{1}H-NMR (80 MHz, $CDCl_3$) δ (TMS) 0.81 (t, J = 7.2 Hz, 3H), 1.41 (s, 9H), 1.68 (m, 2H), 3.74 (s, 3H), 3.83 (t, J = 7.2 Hz, 2H), 4.22 (s, 2H), 6.95 (d, J = 8 Hz, 2H), 7.40 (d, J = 8 Hz, 2H). Anal. ($C_{19}H_{25}BrN_2O_2$) C, H, N.

6n: mp 76 °C; ^{1}H-NMR (80 MHz, $CDCl_3$) δ (TMS) 0.95 (t, J = 7.2 Hz, 3H), 1.42 (s, 9H), 1.90 (m, 2H), 3.62 (s, 3H), 3.89 (s, 2H), 4.15 (t, J = 7.2 Hz, 2H), 7.05 (d, J = 8 Hz, 2H), 7.35 (d, J = 8 Hz, 2H). Anal. ($C_{19}H_{25}BrN_2O_2$) C, H, N.

A more favorable mixture of isomers was obtained using the following procedure: A solution of **4n** (12.5 g, 35 mmol) in propyl iodide (36 mL, 0.35 mol) was heated at reflux for 72 h. The solvent was removed, the residue was taken up in EtOAc−H_2O, and the aqueous phase was extracted with EtOAc. The combined organic layers were washed with $Na_2S_2O_3$ and NaOH, dried, and concentrated to afford a 99.2:0.8 mixture of **5n**:**6n** (12.6 g, 90%), from which pure **5n** was obtained after recrystallization from acetonitrile (11.0 g, 78%).

Ethyl 2-[(4-Bromophenyl)acetyl]-2-[(*N*,*N*-dimethylamino)methylidene]acetate (8). To compound **7** (obtained from **2** as described for **1m**, 17.6 g, 62 mmol) was added, at 0 °C under an argon atmosphere, dimethylformamide dimethyl acetal (9.9 mL, 74 mmol), and the mixture was stirred for 10 min. The solvent was then concentrated and the residue chromatographed on silica gel (hexane−EtOAc mixtures of increasing polarity) to afford **8** as a colorless oil (6.0 g, 29%): ^{1}H-NMR (80 MHz, $CDCl_3$) δ (TMS) 1.29 (t, J = 7.2 Hz, 3H), 2.94 (s, 6H), 3.98 (s, 2H), 4.21 (q, J = 7.2 Hz, 2H), 7.10 (d, J = 8 Hz, 2H), 7.38 (d, J = 8 Hz, 2H), 7.54 (s, 1H).

Methyl 1-Butyl-3-ethyl-5-[[2′-[2-(triphenylmethyl)-2*H*-tetrazol-5-yl]-1,1′-biphenyl-4-yl]methyl]-1*H*-pyrazole-4-carboxylate (10g) and Methyl 1-Butyl-5-ethyl-3-[[2′-[2-(triphenylmethyl)-2*H*-tetrazol-5-yl]-1,1′-biphenyl-4-yl]methyl]-1*H*-pyrazole-4-carboxylate (11g). In a flask under an argon atmosphere were placed a 35:65 mixture of **5g** and **6g** (2.0 g, 5.3 mmol), 2-[2′-(triphenylmethyl)-2′*H*-tetrazol-5-yl]phenylboronic acid (**9**; 2.96 g, 6.8 mmol), Na_2CO_3 (1.03 g, 9.8 mmol), toluene (15.6 mL), and H_2O (5 mL). The system was purged and then refilled with argon (3×). Next, $Pd(PPh_3)_4$ (0.16 g, 0.14 mmol) was added, and then it was purged and refilled with argon again. The reaction mixture was heated at 80 °C overnight and allowed to cool, and the two layers formed were separated. The aqueous phase was extracted with EtOAc, and the combined organic phases were dried and concentrated. The residue was chromatographed on silica gel (hexane−EtOAc mixtures of increasing polarity) to afford the title compounds.

10g: foamy solid (0.51 g, 14%); mp 55−60 °C; ^{1}H-NMR (80 MHz, $CDCl_3$) δ (TMS) 0.7−2.0 (m, 10H), 2.90 (q, J = 7.2 Hz, 2H), 3.74 (s, 3H), 3.74 (t, J = 8 Hz, 2H), 4.30 (s, 2H), 6.7−8.0 (m, 23H).

11g: white solid (1.00 g, 28%); mp 112−114 °C; ^{1}H-NMR (80 MHz, $CDCl_3$) δ (TMS) 0.7−2.0 (m, 10H), 2.92 (q, J = 7.2 Hz, 2H), 3.68 (s, 3H), 4.00 (t, J = 8 Hz, 2H), 4.12 (s, 2H), 6.7−8.0 (m, 23H).

Methyl 3-*tert*-Butyl-1-propyl-5-[[2′-[2-(triphenylmethyl)-2*H*-tetrazol-5-yl]-1,1′-biphenyl-4-yl]methyl]-1*H*-pyrazole-4-carboxylate (10n). In a flask under argon were placed **5n** (13.0 g, 33.05 mmol), **9** (16.16 g, 37.45 mmol), CsF (11.43 g, 74.87 mmol), and dimethoxyethane (119.6 mL). The system was purged, $Pd(PPh_3)_4$ (2.59 g, 1.13 mmol) was added, and then it was purged and refilled with argon again. The reaction mixture was heated at 100 °C overnight and then allowed to cool to room temperature and poured into H_2O−EtOAc. The two layers were separated, and the aqueous phase was extracted with EtOAc. The organic phase was washed with H_2O and brine, and the combined organic phases were dried and concentrated. The residue was chromatographed on silica gel (hexane−EtOAc mixtures of increasing polarity) to afford **10n** as a white solid (19.75 g, 85%): mp 158−160 °C; ^{1}H-NMR (80 MHz, $CDCl_3$) δ (TMS) 0.75 (t, J = 7.2 Hz, 3H), 1.43 (s, 9H), 1.62 (m, 2H), 3.67 (s, 3H), 3.69 (t, J = 7.2 Hz, 2H), 4.21 (s, 2H), 6.7−7.5 (m, 22H), 7.8 (m, 1H). Anal. ($C_{44}H_{44}N_6O_2$) C, H, N.

Methyl 3-*tert*-Butyl-1-propyl-5-[[2′-(1*H*-tetrazol-5-yl)-1,1′-biphenyl-4-yl]methyl]-1*H*-pyrazole-4-carboxylate (12n). A mixture of **10n** (1.0 g, 1.4 mmol), EtOH (100 mL), THF (4 mL), and concentrated HCl (0.78 mL) was stirred at room temperature for 2 h. The mixture was then poured into H_2O−Et_2O, and the layers were separated. The aqueous phase was extracted with Et_2O, and the combined organic phases were dried and concentrated. The residue was chromatographed on silica gel (hexane−EtOAc mixtures of increasing polarity) to afford **12n** as a white solid (0.46 g, 72%): mp 167−171 °C; ^{1}H-NMR (80 MHz, $CDCl_3$) δ (TMS) 0.85 (t, J = 7.2 Hz, 3H), 1.40 (s, 9H), 1.73 (m, 2H), 3.75 (s, 3H), 3.90 (t, J = 7.2 Hz, 2H), 4.31 (s, 2H), 7.15 (s, 4H), 7.5 (m, 4H), 8.15 (m, 1H). Anal. ($C_{26}H_{30}N_6O_2$) C, H, N.

The same procedure was used for the obtention of **12g**: mp 182−184 °C. Anal. ($C_{25}H_{28}N_6O_2$) C, H, N.

3-*tert*-Butyl-1-propyl-5-[[2′-(1*H*-tetrazol-5-yl)-1,1′-biphenyl-4-yl]methyl]-1*H*-pyrazole-4-carboxylic Acid (14n). A mixture of **10n** (4.9 g, 7 mmol), KOH (3.6 g, 55 mmol), 2-methoxyethanol (51 mL), and H_2O (20 mL) was refluxed for 24 h. The solvent was evaporated and the residue taken up in H_2O-EtOAc. The layers were separated, and the aqueous phase was washed with EtOAc and acidified to pH = 2 with 1 N HCl, whereupon a white solid precipitated. The solid was filtered off to afford a crude product which was recrystallized from EtOH/EtOAc to give **14n** as a white solid (2.6 g, 83%): mp 197 °C; ^{1}H-NMR (300 MHz, CD_3OD) δ (TMS) 0.78 (t, J = 7.2 Hz, 3H), 1.42 (s, 9H), 1.56 (m, 2H), 3.86 (t, J = 7.2 Hz, 2H), 4.39 (s, 2H), 4.86 (s, 2H + H_2O), 7.07 (m, 4H), 7.54 (m, 2H), 7.64 (m, 2H). Anal. ($C_{25}H_{28}N_6O_2$) C, H, N.

3-Cyclopropyl-1-propyl-5-[[2′-(1*H*-tetrazol-5-yl)-1,1′-biphenyl-4-yl]methyl]-1*H*-pyrazole-4-carboxamide (16m). To a solution of **14m** (0.3 g, 0.69 mmol) in anhydrous CH_2Cl_2 (2.8 mL) and DMF (0.6 mL) were added DCC (0.14 g, 0.71 mmol) and 1-hydroxybenzotriazole (0.10 g, 0.74 mmol), and the mixture was stirred under an argon atmosphere for 30 min. Next, NH_3 (30% aqueous solution, 0.07 mL) was added, and the reaction mixture was stirred at room temperature for 5 h. Then, further CH_2Cl_2 was added, and the insoluble material was filtered off. The organic phase was washed with saturated $NaHCO_3$ solution and H_2O, dried, and concentrated. The residue was chromatographed on silica gel (hexane−EtOAc mixtures of increasing polarity) to afford **16m** as a white solid (0.13 g, 42%): mp 179−182 °C; ^{1}H-NMR (80 MHz, $CDCl_3$ + CD_3OD) δ (TMS) 0.81 (t, J = 7.2 Hz, 3H), 1.25 (m, 4H), 1.63 (m, 2H), 2.89 (m, 1H), 3.86 (t, J = 7.2 Hz, 2H), 4.41 (s, 2H), 4.50 (s, 3H + H_2O), 7.07 (s, 4H), 7.5 (m, 4H). Anal. ($C_{24}H_{25}N_7O\cdot0.25H_2O$) C, H, N.

The same experimental procedure was used for the obtention of **16b**: mp 204−208 °C. Anal. ($C_{23}H_{25}N_7O\cdot H_2O$) C, H, N.

5-[(4-Bromophenyl)methyl]-1-butyl-4-(hydroxymethyl)-3-methyl-1*H*-pyrazole (17) and 3-[(4-Bromophenyl)methyl]-1-butyl-4-(hydroxymethyl)-5-methyl-1*H*-pyrazole (18). A solution of a 1:1 mixture of **5b** and **6b** (1.38 g, 3.6 mmol) in anhydrous Et_2O (26 mL) was added dropwise to a solution of $LiAlH_4$ (0.276 g, 7.3 mmol) in anhydrous Et_2O (40 mL), and the mixture was stirred at room temperature under an argon atmosphere for 2 h. The solution was cooled to 0 °C and then treated successively with a mixture of H_2O (0.45 mL) and THF (1 mL), 15% NaOH (0.45 mL), and H_2O (1.25 mL). The mixture was stirred for 10 min, dried, and filtered. The solid formed was washed several times with EtOAc, and the filtrate was concentrated to afford a residue that was purified by chromatography on silica gel (hexane−EtOAc mixtures of increasing polarity) to give the two regioisomers as colorless oils.

17 (0.45 g, 36%): ^{1}H-NMR (80 MHz, $CDCl_3$) δ (TMS) 0.81 (t, J = 6.4 Hz, 3H), 1.1−2.0 (m, 5H), 2.23 (s, 3H), 3.77 (t, J = 8 Hz, 2H), 3.95 (s, 2H), 4.43 (s, 2H), 6.97 (d, J = 8 Hz, 2H), 7.37 (d, J = 8 Hz, 2H).

18 (0.47 g, 37%): ^{1}H-NMR (80 MHz, $CDCl_3$) δ (TMS) 0.93 (t, J = 6.4 Hz, 3H), 1.1−2.0 (m, 5H), 2.20 (s, 3H), 3.90 (s, 2H), 3.96 (t, J = 8 Hz, 2H), 4.30 (s, 2H), 7.05 (d, J = 8 Hz, 2H), 7.32 (d, J = 8 Hz, 2H).

5-[(4-Bromophenyl)methyl]-1-butyl-3-methyl-1*H*-pyrazole-4-carboxaldehyde (19). To a solution of **17** (0.276 g, 0.82 mmol) in CH_2Cl_2 (2.5 mL), under argon, was added MnO_2 (0.50 g, 5.74 mmol), and the reaction mixture was heated at 40 °C overnight. More CH_2Cl_2 was added, the resulting mixture was filtered through Celite, and the filtrate was concentrated to afford 0.24 g of a crude product. This was purified by chromatography on silica gel (hexane−EtOAc mixtures of increasing polarity) to yield **19** as a colorless oil (0.15 g, 54%): ^{1}H-NMR (80 MHz, $CDCl_3$) δ (TMS) 0.95 (t, J = 6.4 Hz, 3H), 1.1−2.0 (m, 4H), 2.48 (s, 3H), 4.01 (t, J = 8 Hz, 2H), 4.12 (s, 2H), 7.15 (d, J = 8 Hz, 2H), 7.39 (d, J = 8 Hz, 2H), 9.83 (s, 1H).

1-Butyl-4-(hydroxymethyl)-3-methyl-5-[[2′-(1*H*-tetrazol-5-yl)-1,1′-biphenyl-4-yl]methyl]-1*H*-pyrazole (25): obtained by cross-coupling of **17** with **9** followed by tetrazole deprotection, as described above for **12n**; mp 154−156 °C; ^{1}H-NMR (80 MHz, $CDCl_3$) δ (TMS) 0.7−1.8 (m, 8H), 2.15 (s, 3H), 3.76 (t, J = 8 Hz, 2H), 3.98 (s, 2H), 4.26 (s, 2H), 6.8−8.0 (m, 9H). Anal. ($C_{23}H_{26}N_6O\cdot0.5Et_2O$) C, H, N.

1-Butyl-3-methyl-5-[[2′-(1*H*-tetrazol-5-yl)-1,1′-biphenyl-4-yl]methyl]-1*H*-pyrazole-4-carboxaldehyde (26): obtained by cross-coupling of **19** with **9** followed by tetrazole deprotection, as described above for **12n;** mp 81−85 °C; ^{1}H-NMR (80 MHz, $CDCl_3$) δ (TMS) 0.86 (t, J = 6.4 Hz, 3H), 1.1−2.0 (m, 4H), 2.41 (s, 3H), 3.91 (t, J = 8 Hz, 2H), 4.31 (s, 2H), 7.09 (s, 4H), 7.5 (m, 4H), 8.2 (m, 1H), 9.81 (s, 1H). Anal. ($C_{23}H_{24}N_6O\cdot0.25H_2O$) C, H, N.

4-Acetyl-1-butyl-3-methyl-5-[[2′-(1*H*-tetrazol-5-yl)-1,1′-biphenyl-4-yl]methyl]-1*H*-pyrazole (27): obtained by cross-coupling of a 1:1 mixture of **23** and **24** with **9** followed by

tetrazole deprotection and recrystallization, as described above for **12n**; ^{1}H-NMR (80 MHz, $CDCl_3$) δ (TMS) 0.7−2.0 (m, 7H), 2.55 (s, 3H), 2.65 (s, 3H), 4.16 (t, J = 7.2 Hz, 2H), 4.49 (s, 2H), 6.9−7.6 (m, 8H), 8.2 (m, 1H). Anal. ($C_{24}H_{26}N_6O\cdot0.5H_2O$) C, H, N.

3-*tert*-Butyl-1-propyl-5-[[2′-(1*H*-tetrazol-5-yl)-1,1′-biphenyl-4-yl]methyl]-1*H*-pyrazole (28). A solution of **14n** (0.30 g, 0.67 mmol) in CH_3CN (50 mL) was treated with 1 N HCl (50 mL), and the reaction mixture was refluxed for 48 h. The mixture was allowed to cool, and the solvent was concentrated. The residue thus obtained was dissolved in a mixture of EtOAc and H_2O, and the two phases were separated. The aqueous phase was extracted with EtOAc, and the combined organic phases were dried and concentrated to a crude product. Purification by chromatography on silica gel (hexane−EtOAc, 10%) afforded **28** as a white solid (0.18 g, 67%): mp 165−166 °C; ^{1}H-NMR (80 MHz, $CDCl_3$) δ (TMS) 0.82 (t, J = 7.2 Hz, 3H), 1.23 (s, 9H), 1.66 (m, 2H), 3.80 (t, J = 7.2 Hz, 2H), 3.97 (s, 2H), 5.84 (s, 1H), 7.06 (s, 4H), 7.55 (m, 4H), 8.10 (m, 1H). Anal. ($C_{24}H_{28}N_6\cdot H_2O$) C, H, N.

2-[(*tert*-Butylamino)sulfonyl]phenylboronic Acid (29). To a solution of *N*-*tert*-butylbenzenesulfonamide (37.30 g, 175 mmol) in THF (298 mL), at −78 °C under argon, was added *n*-BuLi (1.6 M in hexanes, 273.3 mL, 437 mmol). The mixture was allowed to warm to room temperature, while stirring for 4 h, and then it was stirred at that temperature for 30 min more. The reaction mixture was cooled to −60 °C, triisopropyl borate (60.6 mL, 262 mmol) was added, and the resulting mixture was stirred at room temperature overnight; 2 N HCl (21 mL) was added, and the mixture was stirred at room temperature for 30 min. The solvent was removed and the residue taken up in EtOAc and washed with H_2O and 1 N NaOH. The aqueous phase was made acid with HCl and extracted with EtOAc. The combined organic extracts were dried and concentrated to a crude product, which was purified by recrystallization from Et_2O/hexane to yield **29** as a white solid (28.90 g, 64%): mp 120−122 °C; ^{1}H-NMR (80 MHz, $CDCl_3$) δ (TMS) 1.22 (s, 9H), 4.97 (s, 1H), 5.99 (s, 2H), 7.5 (m, 2H), 8.0 (m, 2H).

Methyl 5-[[2′-(Aminosulfonyl)-1,1′-biphenyl-4-yl]methyl]-1-butyl-3-methyl-1*H*-pyrazole-4-carboxylate (32b). To a solution of **31b** (mp 163−164 °C; obtained from **5b** and **29** as described for **10g** followed by two recrystallizations, 2.65 g, 5.3 mmol) in trifluoroacetic acid (58 mL) was added anisole (1 mL), and the mixture was stirred under an argon atmosphere for 18 h. The solvent was concentrated and the residue purified by chromatography on silica gel (hexane−EtOAc mixtures) to give **32b** as a white solid (2.32 g, 98%): mp 49−54 °C; ^{1}H-NMR (80 MHz, $CDCl_3$) δ (TMS) 0.7−2.0 (m, 7H), 2.44 (s, 3H), 2.46 (s, 3H), 3.72 (s, 3H), 3.92 (t, J = 7.2 Hz, 2H), 4.39 (s, 2H), 4.42 (s, 2H), 7.5 (m, 7H), 8.2 (m, 1H).

Methyl 1-Butyl-3-methyl-5-[[2′-[[(phenylcarbonyl)amino]sulfonyl]-1,1′-biphenyl-4-yl]methyl]-1*H*-pyrazole-4-carboxylate (34). To a solution of **32b** (0.50 g, 1.13 mmol) in pyridine (10.8 mL) was added benzoyl chloride (0.13 mL), and the reaction mixture was stirred at room temperature under an argon atmosphere for 12 h. Then, saturated aqueous KH_2PO_4 (33 mL) was added, and it was extracted with EtOAc. The organic phase was washed with 1 N HCl, dried, and concentrated to a crude product. This was chromatographed on silica gel (hexane−EtOAc, 1:1) to afford **34** as a foam (0.53 g, 83%): ^{1}H-NMR (80 MHz, $CDCl_3$) δ (TMS) 0.7−2.0 (m, 7H), 2.44 (s, 3H), 3.78 (s, 3H), 3.96 (m, 3H), 4.38 (s, 2H), 7.5 (m, 12H), 8.2 (m, 1H).

Methyl 1-Butyl-3-methyl-5-[[2′-[[(*tert*-butoxycarbonyl)amino]sulfonyl]-1,1′-biphenyl-4-yl]methyl]-1*H*-pyrazole-4-carboxylate (35). A mixture of **32b** (0.50 g, 1.13 mmol), di-*tert*-butyl dicarbonate (0.49 g, 2.26 mmol), K_2CO_3 (0.31 g, 2.26 mmol), and anhydrous DME (23 mL) was refluxed under an argon atmosphere for 2 h. The resulting solution was allowed to cool, poured into 10% $NaHSO_4$ (99.7 mL), and extracted with EtOAc. The organic phase was dried and concentrated to a crude product, which was chromatographed on silica gel (hexane−EtOAc) to afford **35** as an oil (0.43 g, 70%): ^{1}H-NMR (80 MHz, $CDCl_3$) δ (TMS) 0.7−2.0 (m, 7H), 1.29 (s, 9H), 2.44 (s, 3H), 3.46 (s, 1H), 3.80 (s, 3H), 3.96 (t, J =8 Hz, 2H), 4.45 (s, 2H), 7.0−7.8 (m, 7H), 8.4 (m, 1H).

1-Butyl-3-methyl-5-[[2′-[[(*tert*-butoxycarbonyl)amino]-sulfonyl]-1,1′-biphenyl-4-yl]methyl]-1*H*-pyrazole-4-carboxylic acid (39). A mixture of **35** (0.26 g, 0.5 mmol), 2 N NaOH (2.5 mL), and EtOH (25 mL) was stirred at room temperature for 6 days. The solvent was removed and the residue taken up in EtOAc−H_2O. The aqueous phase was acidified and extracted with EtOAc, dried, and concentrated to afford **39** as a white solid (0.10 g, 38%): mp 92−96 °C; ^{1}H-NMR (80 MHz, $CDCl_3$) δ (TMS) 0.7−2.0 (m, 7H), 1.29 (s, 9H), 2.45 (s, 3H), 3.96 (t, J = 8 Hz, 2H), 4.45 (s, 2H), 7.0−7.6 (m, 9H), 8.2 (m, 1H). Anal. ($C_{27}H_{33}N_3O_6S\cdot0.5H_2O$) C, H, N.

3-Cyclopropyl-5-[[2′-[[(phenylcarbonyl)amino]sulfonyl]-1,1′-biphenyl-4-yl]methyl]-1-propyl-1*H*-pyrazole-4-carboxamide (42). To a solution of **41** (0.15 g, 0.28 mmol) in THF (10 mL) was added 1,1′-carbonyldiimidazole (0.057 g, 0.35 mmol), and the mixture was stirred at room temperature under an argon atmosphere for 3 h. Next, NH_3 (32% aqueous solution, 0.33 mL) and EtOH (0.7 mL) were added, and the reaction mixture was stirred at room temperature overnight and then at reflux for 1 h. The solvent was removed, and the residue was taken up in CH_2Cl_2 and H_2O. The aqueous phase was acidified to pH = 6 and extracted with EtOAc. The combined organic extracts were dried and concentrated to a crude product. This was chromatographed on silica gel (hexane−EtOAc mixtures of increasing polarity) to afford **42** as a white solid (0.06 g, 40%): mp 187−191 °C; ^{1}H-NMR (80 MHz, $CDCl_3$) δ (TMS) 0.82 (t, J = 7.2 Hz, 3H), 0.91 (d, 4H), 1.68 (m, 2H), 2.59 (m, 1H), 3.86 (t, J = 7.2 Hz, 2H), 4.32 (s, 2H), 6.6 (br s, 2H), 7.0−7.5 (m, 13H), 8.2 (m, 1H). Anal. ($C_{30}H_{30}N_4O_4S$) C, H, N.

Ethyl 3-Isopropyl-5-[[2′-(methoxycarbonyl)-1,1′-biphenyl-4-yl]methyl]-1-propyl-1*H*-pyrazole-4-carboxylate (43). A mixture of **5l** (0.50 g, 1.3 mmol), methyl 2-chlorobenzoate (0.22 g, 1.3 mmol), $NiCl_2$ (0.017 g, 0.13 mmol), triphenylphosphine (0.07 g, 0.26 mmol), and pyridine (1.3 mL) was heated at 80 °C under an argon atmosphere. Next, powdered zinc (0.18 g, 2.73 mmol) was added, and the mixture was heated at 80 °C for 5 h. It was then allowed to cool, filtered through celite, and washed with toluene. The filtrate was concentrated, taken up in toluene, filtered, and washed again with toluene. The new filtrate was washed with 1 N HCl, saturated $NaHCO_3$ solution, and H_2O. The organic phase was dried and concentrated. The aqueous phase was extracted with EtOAc, dried, and concentrated. The combined residues were chromatographed on silica gel (hexane−EtOAc mixtures of increasing polarity) to afford **43** as a white foam (0.30 g, 53%): ^{1}H-NMR (80 MHz, $CDCl_3$) δ (TMS) 0.82 (t, J = 7.2 Hz, 3H), 1.29 (t, J = 7.2 Hz, 3H), 1.30 (d, J = 6.4 Hz, 6H), 1.70 (m, 2H), 3.57 (q, J = 6.4 Hz, 1H), 3.60 (s, 3H), 3.90 (t, J = 7.2 Hz, 2H), 4.28 (q, J = 7.2 Hz, 2H), 4.40 (s, 2H), 7.0−8.0 (m, 8H).

Ethyl 5-[(2′-Amino-1,1′-biphenyl-4-yl)methyl]-3-isopropyl-1-propyl-1*H*-pyrazole-4-carboxylate (47). A cooled (0 °C) solution of compound **46** (obtained by cross-coupling of **5l** and **45** as described for **10n**, 0.34 g, 0.67 mmol) in CH_2Cl_2 (5.5 mL) was treated with trifluoroacetic acid (0.62 mL, 8.1 mmol) and the reaction mixture stirred at room temperature under an argon atmosphere overnight. The volatiles were removed *in vacuo*, and the residue was taken up in CH_2Cl_2 and washed with aqueous 10% $NaHCO_3$. The organic phase was dried and concentrated to a crude product. Purification by chromatography on silica gel (hexane−EtOAc, 10%) afforded **47** as a yellow oil (0.20 g, 74%): ^{1}H-NMR (80 MHz, $CDCl_3$ + CD_3OD) δ (TMS) 0.81 (t, J = 7.2 Hz, 3H), 1.31 (d, J = 6.4 Hz, 6H), 1.2−1.8 (m, 5H), 3.57 (q, J = 6.4 Hz, 1H), 3.71 (s, 2H), 3.91 (t, J = 7.2 Hz, 2H), 4.27 (q, J = 7.2 Hz, 2H), 4.41 (s, 2H), 6.8−7.5 (m, 8H).

Ethyl 3-Isopropyl-1-propyl-5-[[2′-[[(trifluoromethyl)-sulfonyl]amino]-1,1′-biphenyl-4-yl]methyl]-1*H*-pyrazole-4-carboxylate (48). To a cooled (−70 °C) solution of **47** (0.20 g, 0.5 mmol) and NEt_3 (0.09 mL, 0.64 mmol) in CH_2Cl_2 (3.2 mL) was added dropwise 1 M triflic anhydride (0.51 mL) in CH_2Cl_2, and the resulting mixture was stirred under an argon atmosphere for 45 min. A further portion of triflic anhydride (0.06 mL) was added, and stirring continued for 15 min. The

reaction mixture was allowed to warm up to 0 °C, and H_2O (0.64 mL) was added. The reaction mixture was then allowed to warm up to room temperature, and more H_2O (6.4 mL) was added. The layers were separated, and the organic phase was dried and concentrated. The residue was purified by chromatography on silica gel (hexane−EtOAc mixtures of increasing polarity) to give **48** as an oil (0.13 g, 50%): ^{1}H-NMR (80 MHz, $CDCl_3$) δ (TMS) 0.81 (t, J = 7.2 Hz, 3H), 1.31 (d, J = 6.4 Hz, 6H), 1.2−1.8 (m, 5H), 3.56 (q, J = 6.4 Hz, 1H), 3.91 (t, J = 7.2 Hz, 2H), 4.27 (q, J = 7.2 Hz, 2H), 4.44 (s, 2H), 5.91 (br s, 1H), 6.8−7.5 (m, 8H).

B. Biological Methods. Angiotensin II Receptor Binding Assay. AII receptors from rat liver microsomes were prepared using a previously described method.[30] Livers were obtained after cervical dislocation, collected in 50 mM Tris-HCl buffer, pH 7.5, so that the concentration was 20% (w/v), and homogenized at 1000 rpm. The homogenate was centrifuged at 1000*g* for 10 min and the supernatant further centrifuged at 100000*g* for 1 h. The resultant membrane pellet was then resuspended in the above buffer at a concentration of 1 g of wet wt/mL; 700 μL aliquots of the membrane suspension were stored frozen at −70 °C until used.

Aliquots containing 15 mg of protein were incubated at 25 °C for 1 h in incubation buffer containing (final concentrations): NaCl (120 mM), $MgCl_2$ (5 mM), 0.006% bovine serum albumin, and Tris (50 mM), adjusted to pH 7.5. Incubation was initiated by the addition of 2 nM [^{3}H]AII. Total incubation volume was 250 μL. Nonspecific binding was measured by incubation in the presence of 0.1 mM Sar^1,Ile^8-AII. Test compounds were studied in the range of concentrations 10^{-10}−10^{-5} M. Binding was terminated by rapid filtration using a Millipore multiscreen device. Filters were washed three times with 250 μL of the corresponding buffer. Dry filters were placed into vials containing 3 mL of scintillation fluid, and the radioactivity was counted in a scintillation counter. The IC_{50} value (concentration for 50% displacement of the specifically bound [^{3}H]AII) was estimated from the linear portion of the displacement curve. Assays were performed in duplicate. Interassay IC_{50} values for a given test compound may vary by <20%.

Inhibition of Angiotensin II-Induced Pressor Response in Pithed Rats.[30] Male Sprague−Dawley rats (body wt 250 g) were anesthetized with sodium pentobarbital (50 mg/kg, ip). The trachea was cannulated, and the rats were pithed through the orbit with a stainless steel pithing rod. The rats were immediately placed on a rodent ventilator (vol, 1 mL/100 g of body wt; rate, 74 strokes/min). The carotid artery was cannulated and connected to a pressure transducer for arterial pressure measurement. A dose−pressor response curve for AII was obtained as follows: AII (0.01−100 mg/kg) was administered intravenously in a cumulative manner, and each succesive injection was given immediately after the maximal effect of the preceding dose was obtained. The effect of a submaximal dose of AII (3 μg/kg, iv) was calculated in no-treated animals. Test compounds (or vehicle) were given to animals 15 min before injection of AII. The inhibition (%) of the effect induced by AII (3 μg/kg, iv) was calculated for each test compound in relation to the one obtained in no-treated animals. Experiments were done in quintuplicate.

Oral Activity in Furosemide-Treated Sodium-Depleted Rats.[30] Male Sprague−Dawley rats (250 g) were surgically instrumented with a telemetry device (TA11PA-C40, Data Sciences Inc.) for continuous recording of blood pressure and heart rate. After 1 week, rats were fed with a sodium deficient diet (ICN, sodium-deficient diet, rat, modified, 902902, 4% sodium free salt mixture) and given furosemide (5 mg/kg, sc) 48, 24, and 1 h before oral administration of test compounds. Blood pressure and heart rate were monitored for up to 48 h postdose. Experiments were done by quadruplicate.

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