Synthesis and Pharmacological Evaluation of New Pyrazolidine-3,5-diones as AT_1 Angiotensin II Receptor Antagonists

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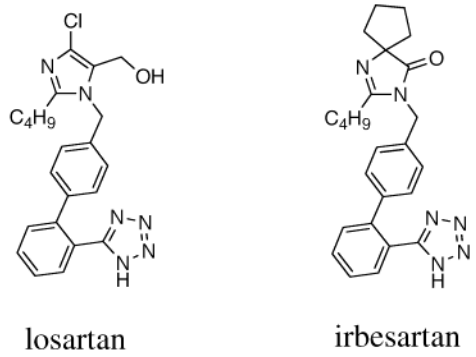
On the basis of the structure of the non-peptide receptor antagonist irbesartan, a new series of AT_1 ligands was designed. In these compounds the central imidazolone nucleus of irbesartan was replaced by a pyrazolidine-3,5-dione structure. The key intermediate *N*-alkylpyrazolidine-3,5-diones were synthesized according to a new and general method. The most active compounds possess a spirocyclopentane ring at position 4, a linear butyl chain at position 1, and the [2′-(5-tetrazolyl)biphenyl-4-yl]methyl or [2′-(benzoylaminosulfonyl)biphenyl-4-yl]methyl group at position 2. Affinity toward the AT_1 and AT_2 receptors was assessed by the ability of the compounds to competitively displace [^{3}H]AII from its specific binding sites. The most active compounds, **28** and **48**, displayed high affinity for the AT_1 receptor, good selectivity AT_1 versus AT_2, and potent in vitro antagonist activity.

Introduction

The renin−angiotensin system (RAS) plays a central role in blood pressure regulation and electrolyte homeostasis.[1] Angiotensin II (AII), an octapeptide that is formed within the RAS from angiotensin I by angiotensin-converting enzyme (ACE), is one of the most powerful vasoconstrictors known. Since the discovery that ACE inhibitors such as captopril or enalapril can be used successfully for the treatment of hypertension,[2] much interest has revolved around exploration of novel ways to interfere with the renin−angiotensin cascade. This was due to the fact that ACE is a nonspecific protease, which is also responsible for the degradation of bradykinin. Therefore, ACE inhibitors produce bradykinin potentiation and lead to side effects such as dry cough and angioedema.[3] To avoid these side effects, it seemed promising to look for more selective compounds at other targets in the RAS in order to suppress the activity of AII. The most direct and potentially the most specific approach to block the RAS is to antagonize AII at its receptor sites.[4] Two major receptor subtypes, designated AT_1 and AT_2, have been identified in a variety of human and animal tissues.[5] The AT_1 receptor is G-protein-coupled and mediates most of the known physiological effects of AII, including the maintenance of blood pressure.[6] The structure, the coding gene, and expression of the AT_2 subtype receptor of AII have been described;[7] however, less is known about the function and intracellular functional response coupling to this receptor. The AT_2 receptor is thought to be involved in fetal growth and adult tissue repair and remodeling, especially in the cardiovascular system. However, there are still conflicting results, in vitro and in vivo, as to whether AT_2 receptors limit and/or accelerate the growing processes in cardiovascular tissues.[8]

Chart 1. Structures of Losartan and Irbesartan.



The discovery by DuPont of the first potent and orally active non-peptide AII antagonist, losartan (Cozaar)[9−11] (Chart 1), has stimulated extensive research interest in this area. Numerous patents and publications on AII antagonists have appeared over the past few years.[12] Most of this published work has focused on variations of the losartan heterocyclic system, while retaining a [2′-(5-tetrazolyl)biphenyl-4-yl]methyl (or closely related) side chain that is linked to the heterocycle directly or through a heteroatom. Indeed, the diversity of platforms compatible with effective AII antagonism is remarkable, encompassing numerous *N*- or *C*-linked five- and six-membered nitrogen heterocycles, ring-fused congeners, and acyclic analogues.

A structure−activity relationship (SAR) analysis of the losartan type AT_1 antagonists has brought to light key elements implied in the drug−receptor interaction:[12] a bulky substituent (the chlorine atom of losartan and the tetramethylene group of irbesartan (Avapro)[13,14] (Chart 1)) which could occupy a large hydrophobic cavity in the AT_1 receptor; an alkyl chain (the butyl chain of losartan and irbesartan) which could fit into a second

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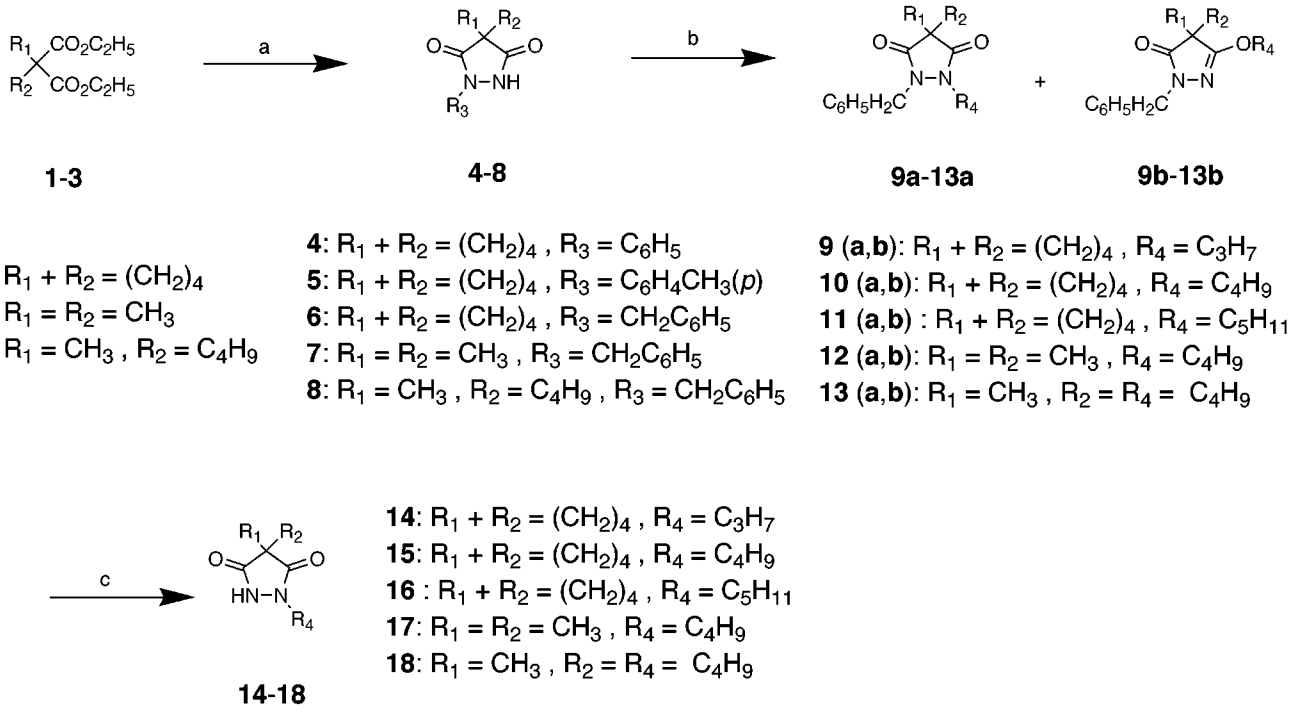
Scheme 1[a]



[a] Reagents and conditions: (a) R_3NHNH_2, C_2H_5ONa, C_6H_5Cl; (b) R_4Br, K_2CO_3, DMF; (c) Pd/C, HCO_2NH_4, MeOH.

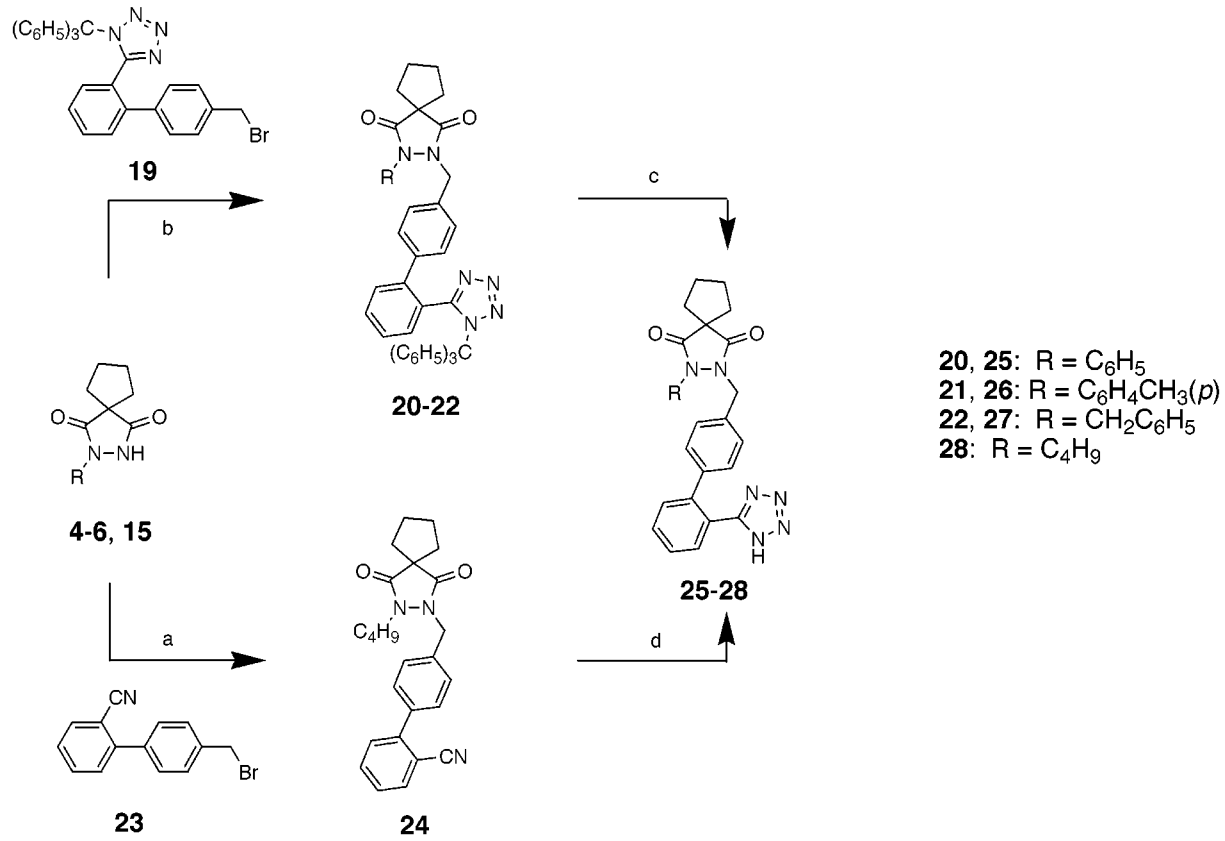
lipophilic pocket in the AT_1 receptor; a biaromatic structure (the biphenyl group of losartan and irbesartan) able to fit into a third hydrophobic pocket; an acidic group (the tetrazole group of losartan and irbesartan) able to interact with a basic residue of the AT_1 receptor; a moiety (hydroxymethyl group of losartan and carbonyl group of irbesartan) which is capable of hydrogen-bonding interaction with the AT_1 receptor; a heterocyclic nitrogen (at the 3-position of the imidazole ring of losartan and at the 3-position of the imidazolinone ring of irbesartan) acting as hydrogen bond acceptor.

On the basis of the structure of irbesartan, we replaced the basic nitrogen at the 3-position of the imidazolinone ring by a carbonyl group which maintains hydrogen bond acceptor characteristics. Thus, we explored a series of pyrazolidine-3,5-diones in which the two heterocyclic carbonyl groups are believed to interact by specific hydrogen bonding with the receptor. Herein, we report the SAR study that led to the discovery of new AT_1 selective antagonists, which are represented by compounds **28** and **48**.

Chemistry

Tables 1−5 list the target pyrazolidine-3,5-diones prepared in this investigation. The synthesis of the key intermediates **4**−**8** and **14**−**18** is outlined in Scheme 1. *N*-Benzyl- and *N*-arylpyrazolidine-3,5-diones **4**−**8** were obtained by condensation of the disubstituted malonates **1**,[15] **2**, and **3** with the desired monosubstituted hydrazines. When the cyclocondensation was performed in refluxing EtOH in the presence of sodium (method of Conrad and Zart),[16] the *N*-arylpyrazolidine-3,5-diones **4** and **5** were obtained in sufficient yields (≅50%). However, under these conditions, the *N*-benzyl derivatives **6**−**8** were isolated in about only 20% yield. Changing the solvent from EtOH to chlorobenzene allowed us to significantly increase the yields of compounds **6**−**8**. This latter method cannot be generalized for the synthesis of *N*-alkylpyrazolidine-3,5-diones since the desired monoalkylhydrazines are not commercially available. Therefore, we developed a reaction protocol enabling an efficient construction of the pyrazolidine-3,5-dione skeleton bearing various alkyl substituents on one of the nitrogen atoms of the heterocycle. Hence, the compounds **14**−**18** were synthesized in a two-step sequence from the previously described intermediates **6**−**8**. Alkylation of these *N*-benzyl derivatives with the appropriate alkyl bromide (R_4Br) gave a mixture of isomers **a** and **b**, respectively due to *N*- and *O*-alkylation. When alkylation was carried out at room temperature in DMF, in the presence of K_2CO_3, equal amounts of isomers **a** and **b** were obtained. The ratios were determined from the ^1H NMR spectra of the reaction mixture; for the isomers **9b**−**13b**, the protons $O-CH_2-C_nH_{2n+1}$ are shifted more downfield (300 MHz, $CDCl_3$, t, 4.50−4.75 ppm) as compared to the isomers **9a**−**13a** ($N-CH_2-C_nH_{2n+1}$, t, 3.45−3.50 ppm). Different reaction conditions were tested in order to increase the proportion of the desired isomers **a**, but ratios were similar to those previously reported (conditions investigated: DMF, NaH, rt; acetone, K_2CO_3, rt; acetone, K_2CO_3, 0 °C). Due to similar R_f values on TLC, attempts to separate these isomers by column chromatography were unsuccessful. Therefore, debenzylation was performed on the mixture of both isomers **a** and **b**. When this deprotection was carried out in methanol, in the presence of ammonium formate and a catalytic amount of Pd/C, only the isomers **a** underwent debenzylation. The remarkable resistance of isomers **b** to hydrogenolysis under catalytic transfer hydrogenation seems to be characteristic of the α,β-unsaturated *N*-benzyl system. Indeed, similar behavior has been noted previously for pyridines.[17] The desired compounds **14**−**18** were easily

Scheme 2[a]



[a] Reagents and conditions: (a) R = C_4H_9, K_2CO_3, DMF; (b) R = C_6H_5 or *p*-$C_6H_4CH_3$ or $CH_2C_6H_5$, K_2CO_3, DMF; (c) HCl (37%), water/THF; (d) $(C_4H_9)_3SnCl$, NaN_3, DMF.

separated from the reaction mixture due to their ability to form salts in aqueous NaOH. Furthermore, this workup procedure allowed us to isolate, at this step, the isomers **9b**−**13b** as pure compounds.

Coupling of the desired biphenylmethylene bromide derivatives with the *N*-substituted pyrazolidine-3,5-diones was generally performed at room temperature in the presence of K_2CO_3. The major product of the reaction was the desired *N*-alkylated isomer, mixed with a small amount of *O*-alkylated derivative. In all cases, the two isomers were separated by fractional crystallization. Compounds **25**−**28**, bearing a tetrazolyl group, were synthesized according to the procedures described in Scheme 2. *N*-Substituted pyrazolidine-3,5-diones **4**−**6** were alkylated with *N*-triphenylmethyl-5-[4′-(bromomethyl)biphenyl-2-yl]tetrazole (**19**)[10] according to the conditions described above. The trityl group of **20**−**22** was cleaved by treatment with HCl in water/THF to yield the final compounds **25**−**27** in good yields. Compound **28** was obtained from pyrazolidine-3,5-dione (**15**) by alkylation with 4-(bromomethyl)-2′-cyanobiphenyl (**23**)[10] followed by formation of the tetrazole ring with tributyltin azide in DMF.

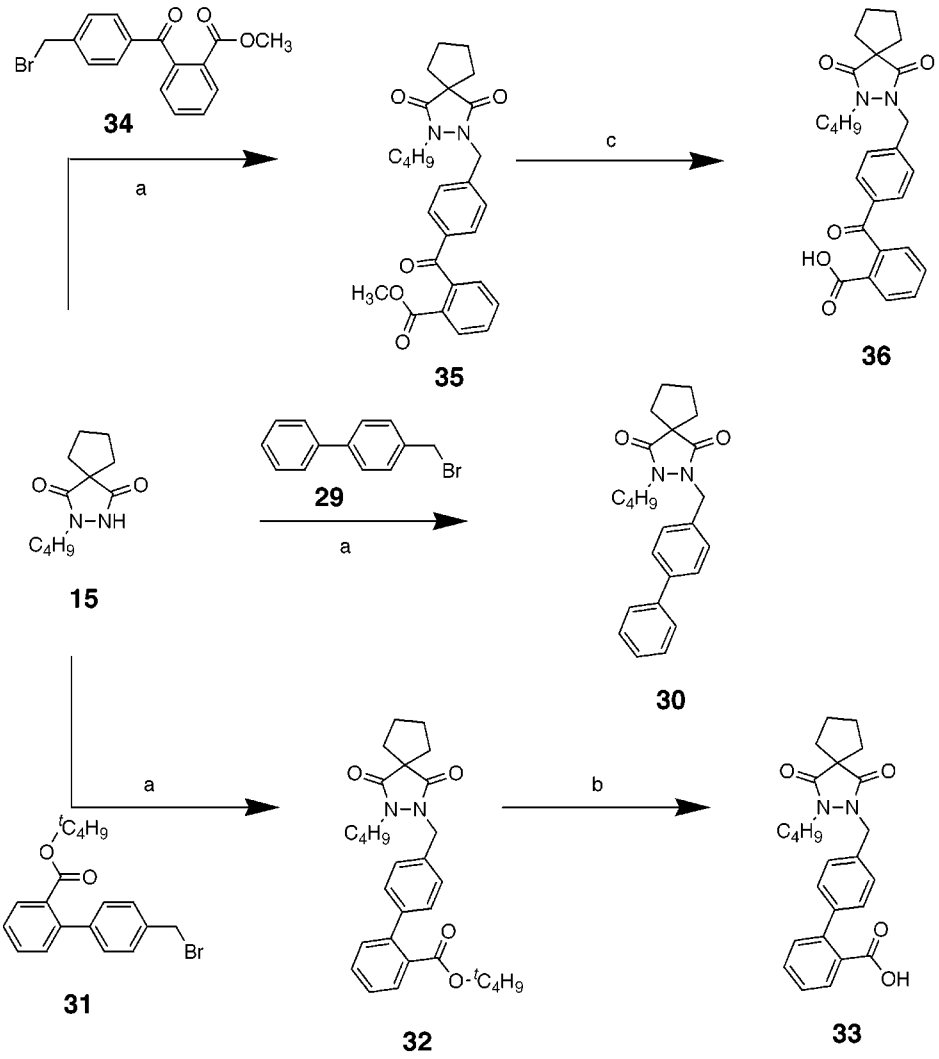
Carboxylic acid **33** was prepared as shown in Scheme 3 by coupling the pyrazolidine-3,5-dione (**15**) with the known bromomethylbiphenyl *tert*-butyl ester (**31**)[10] followed by acidic cleavage of the *tert*-butyl group. Compound **30** has been obtained by alkylation of **15** with commercially available 2-(bromomethyl)biphenyl. Alkylation of **15** with the bromomethylbenzophenone **34**[9b] afforded the ester **35** which was hydrolyzed under acidic conditions to afford the acid **36**.

Synthesis of pyrazolidine-3,5-diones bearing an acylsulfonamide group at the 2′-position of the biphenyl system is illustrated in Scheme 4. Alkylation of **14**−**18** with the (bromomethyl)biphenylsulfonamide intermediate **37**[18,19] afforded the desired *N*-alkylated products **38**−**42**. Upon removal of the *tert*-butyl protecting group with trifluoroacetic acid, the resulting sulfonamides **43**−**47** were converted to the acylsulfonamides **48**−**53** by acylation with the requisite anhydride in refluxing pyridine. Reaction of **43** with cyclohexyl isocyanate in the presence of K_2CO_3 gave the sulfonylurea **54** in 63% yield.

The synthesis of the phthalamic acid derivative **57** and its retroamide analogues **62** and **63** was achieved by the procedures shown in Schemes 5 and 6, respectively. Alkylation of pyrazolidine-3,5-dione (**15**) with 4-nitrobenzyl bromide in acetone in the presence of K_2CO_3 gave a mixture of the two isomers **55a** and **55b** (Scheme 5). When the coupling was carried out at room temperature, the desired isomer **55a** was only obtained in 42% yield. However, when the benzyl bromide derivative was added at 0 °C, the *N*-alkylation product **55a** was isolated in 75% yield. Reduction of the nitro group of **55a** using tin chloride gave the aniline derivative **56**, which reacted with phthalic anhydride in pyridine to produce the acid **57**.

Alkylation of **15** with methyl 4-(bromomethyl)benzoate afforded the two isomers **58a** and **58b**, which were separated by column chromatography (Scheme 6). When the coupling was carried out in DMF, the desired isomer **58a** was isolated in 40% yield. However, when this alkylation was achieved in acetone, the desired *N*-

Scheme 3[a]



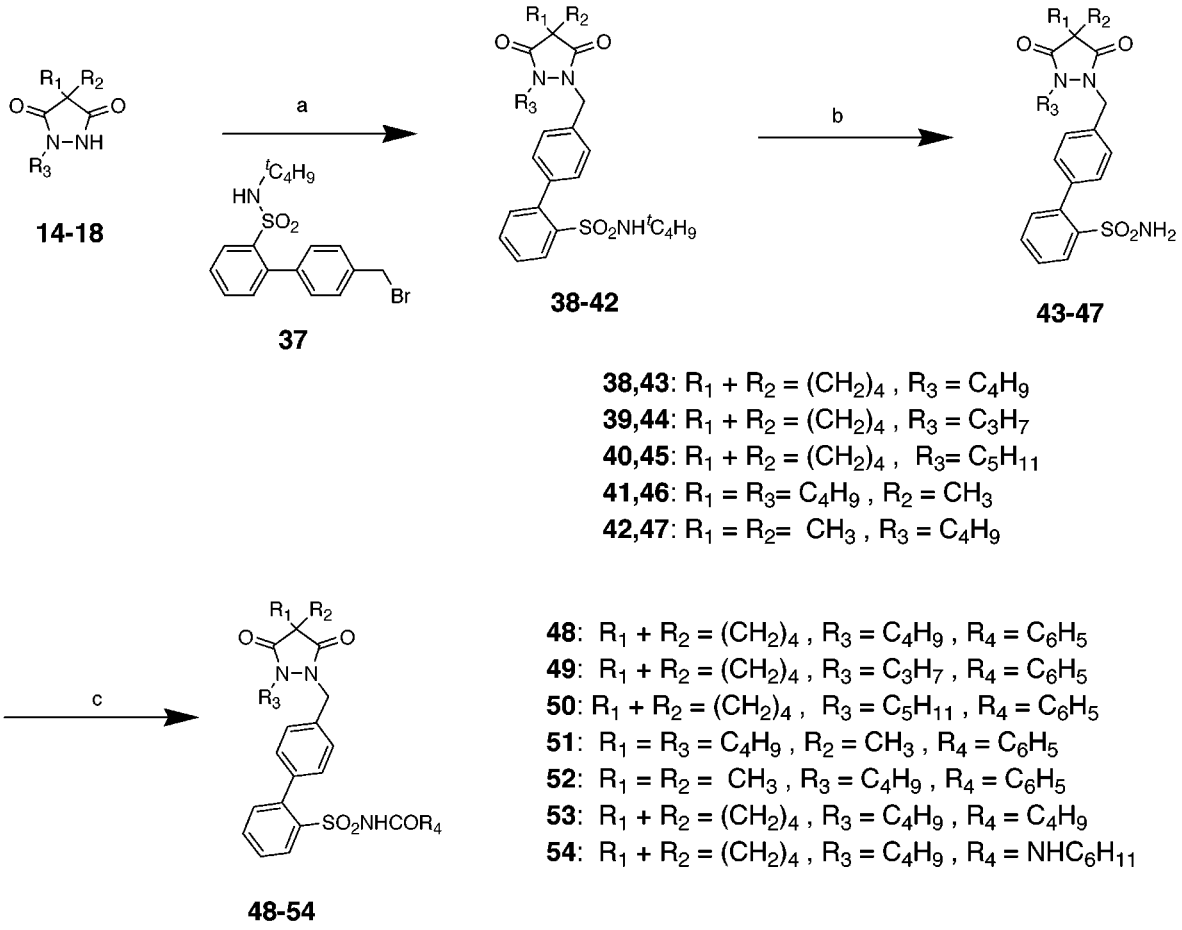
[a] Reagents and conditions: (a) K_2CO_3, DMF; (b) HCl(g), dioxane; (c) HCl (37%), AcOH.

alkylated product **58a** was obtained in 70% yield. Hydrolysis of **58a** in acetic acid in the presence of concentrated HCl afforded **59** in 89% yield. Coupling of the acid chloride of **59** with the desired aniline derivatives under Schotten−Baumann type conditions yielded the esters **60** and **61**. Saponification of **60** and **61** at room temperature in the presence of NaOH gave the corresponding carboxylic acids **62** and **63** in 22% and 40% yields, respectively. These low yields were explained by the formation of side products due to the high sensitivity (ring opening) of *N*,*N*′-disubstituted pyrazolidine-3,5-diones in aqueous NaOH solution.

Results and Discussion

In Vitro Binding Affinity. The title compounds were tested for their affinity toward the AT_1 and AT_2 receptors as measured by their ability to displace [^{3}H]-AII from its specific binding sites, in PLC-PRF-5 human hepatoma cell line[20] for AT_1 or calf cerebellum for AT_2.[21] The pyrazolidine-3,5-dione heterocycle was first functionalyzed with the biphenylmethylene moiety common to almost all the AII antagonists. Table 1 summarizes some structural variations at the 2′-biphenyl position. By analogy with the structure of irbesartan, all the compounds presented in Table 1 maintained a butyl chain at position 1 and a cyclobutyl moiety at position 4 of the pyrazolidine-3,5-dione ring. Almost all the losartan type non-peptide AII receptor antagonists possess an acidic moiety (carboxylate, tetrazole, acylsulfonamide) positioned at the 2′-biphenyl position.[12c] This group, which is ionized at physiological pH, is thought to interact with a positively charge residue of the receptor; involvement of the Lys[199] (AT_1: TMVII) as a couterion for this acidic moiety has been postulated in the literature.[12c,22,23] As expected, neutral compounds **30** and **24** displayed only weak affinity toward the AT_1 receptor. Surprisingly, replacement of the nitrile moiety (compound **24**) with a carboxylic acid group (compound **33**) did not result in a substantial increase of the affinity toward the AT_1 receptor. It was concluded that the carboxylic moiety might not be well positioned for an ionic interaction with the receptor. In this regard, we envisaged to replace the biphenyl structure in order to position this carboxylic group in a better orientation. Introduction of a carbonyl group (compound **36**) or an amide group (compound **62**) in the biphenyl structure did not result in an increase of the affinity (Table 2). However, the retroamide **57** displayed significantly better affinity than the biphenyl analogue **33**. Furthermore, the total loss of affinity observed by displacement

Scheme 4[a]



[a] Reagents and conditions: (a) K_2CO_3, DMF; (b) TFA, anisole; (c) $(R_4CO)_2O$, pyridine or $C_6H_{11}NCO$, K_2CO_3, acetone.

of the carboxylic acid moiety from an *ortho*-position (compound **62**) to a *meta*-position (compound **63**) indicates that the nature and orientation of this acidic moiety play a principal role in the affinity of these compounds toward the AT_1 receptor.

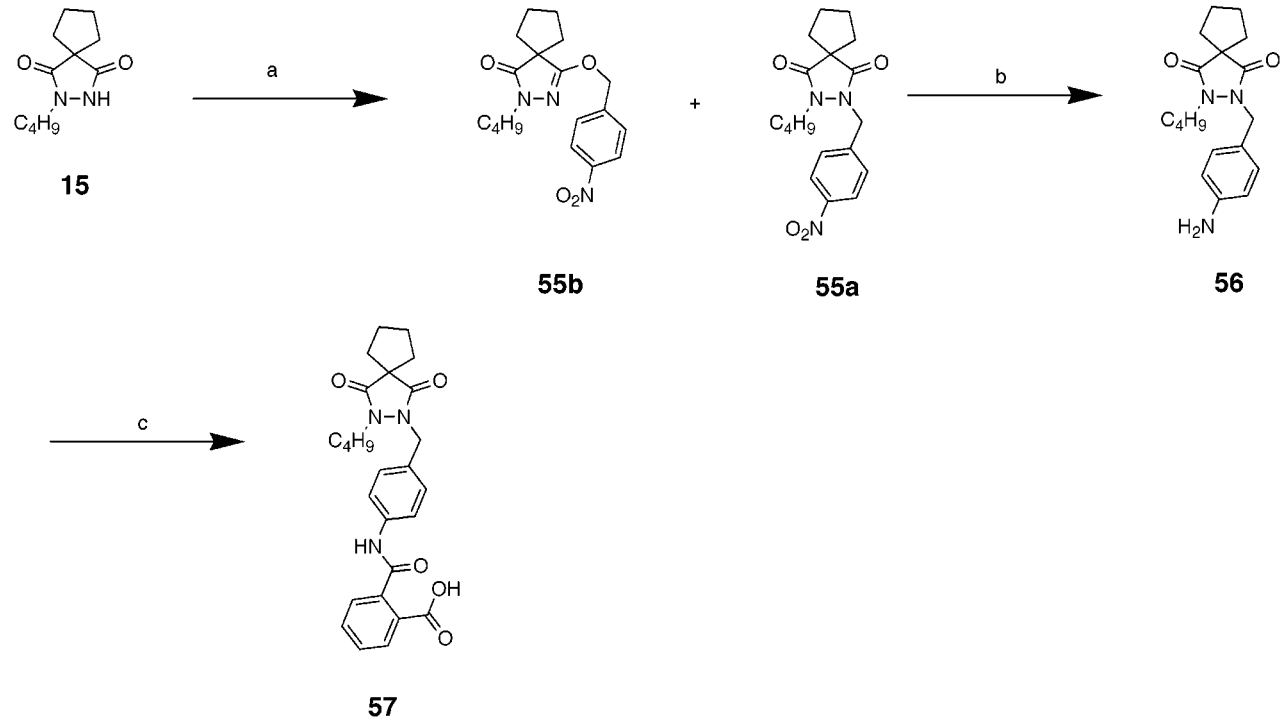
Replacement of the carboxylic acid group of compound **33** by a tetrazole moiety (compound **28**) resulted in a 100-fold greater binding affinity (Table 1). To the best of our knowledge, such a difference of affinity between a molecule substituted with a biphenylcarboxylic acid and its biphenyltetrazole homologue has never been reported. Introduction of an acylsulfonamide moiety in place of the tetrazole has been envisaged originally in order to increase the affinity for the AT_2 receptor.[24] Intermediates **43** and **38**, which could not be ionized under the assay conditions, had very weak receptor binding affinity. In contrast, the derivative **48**, rendered acidic by acylation of the sulfonylamine moiety, showed higher binding affinity toward the AT_1 receptor than its tetrazole analogue **28**. However, contrary to our beliefs, no enhancement of AT_2 binding affinity was observed for the benzoylsulfonamide versus the tetrazolyl derivative. This observation is in agreement with previously reported work in other series.[25,18] In fact, all the compounds reported in this study displayed only weak affinity toward the AT_2 receptor (% displacement of [^{3}H]AII at 10^{-5} M < 20%).

The *N*-pentanoylsulfonamide **53** and the sulfonylurea **54** bind the AT_1 receptor with good affinity (Table 1). These compounds are in the same range of affinity for the AT_1 receptor as the benzoylsulfonamide analogue **48**. The SARs at the pyrazolidine-3,5-dione 1-position are illustrated in Tables 3 and 4. Optimal activity occurred with the linear butyl chain (compound **28**). Indeed, introduction of an aromatic ring, i.e. phenyl (**25**), *p*-tolyl (**26**), or benzyl (**27**), gave rise to compounds of lower binding affinity (Table 3). Furthermore, shortening the *n*-butyl side chain at the N1 position of the lead compound **48** to *n*-propyl (compound **49**) decreased AT_1 affinity (Table 4). Similarly, replacement of the *n*-butyl moiety with the *n*-pentyl chain (compound **50**) in the benzoylsulfonamide series resulted in a slight decrease of affinity. The SAR at the pyrazolidine-3,5-dione 1-position is in agreement with those reported at the imidazolone 2-position of irbesartan[13] as well as at the imidazole 2-position of losartan.[10]

The AT_1 binding affinity of the pyrazolidine-3,5-dione derivatives with various substituents at position 4 is presented in Table 5. The receptor seems to accommodate various lipophilic substituents at this position as shown by the good affinity of compounds **51** and **52**. However, maximum affinity was obtained with the spirocyclopentyl derivative **48**.

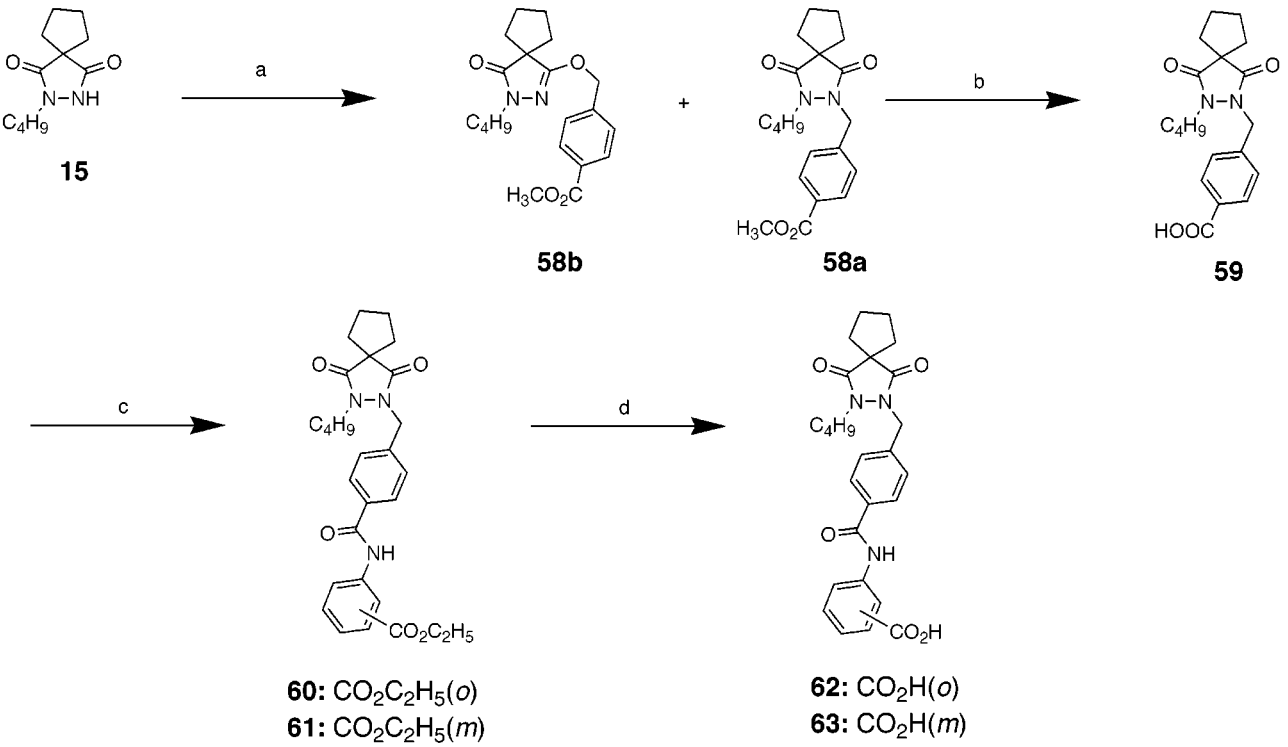
In Vitro Antagonist Activity. AII stimulates the phosphoinositide-turnover signaling system following binding to its specific cell surface receptor, leading to an elevation in intracellular cytosolic Ca^{2+} concentration. Our interest was to examine the effects of blocking the AT_1 receptor with selected compounds (K_i < 100 nM) on AII-mediated increase in cytosolic Ca^{2+} concentration

Scheme 5[a]



[a] Reagents and conditions: (a) *p*-$BrCH_2C_6H_4NO_2$, K_2CO_3, acetone; (b) $SnCl_2$, $EtOH/H_2O$; (c) (1) phthalic anhydride, pyridine, (2) HCl (37%).

Scheme 6[a]

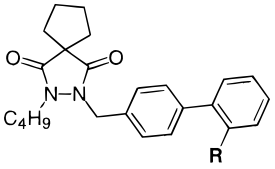


[a] Reagents and conditions: (a) *p*-$BrCH_2C_6H_4CO_2CH_3$, K_2CO_3, acetone; (b) HCl (37%), AcOH; (c) (1) **59**, $SOCl_2$, CH_2Cl_2, (2) $H_2NC_6H_4CO_2C_2H_5$ (*o* or *m*), NEt_3, CH_2Cl_2; (d) NaOH, H_2O/EtOH.

in PLC-PRF-5 cells. Furthermore, the in vitro antagonist activity and the in vitro binding affinity of selected compounds have been evaluated on the same cell preparation (PLC-PRF-5 cell line) in order to directly elaborate the affinity/activity correlation.

AII induced a maximal elevation of intracellular cytosolic Ca^{2+} concentration from 56 (±3) to 492 (±12) nM (mean ± SEM of 15 separate cell preparations). Preincubation of the cells with **25**, **28,** or **48**−**54** (10^{-6} M) led to an abolition of the AII-induced increase in intracellular cytosolic Ca^{2+} concentration. Standardization of three different independent experiments was performed by calculation of the percentage inhibition of the AII-induced increase in intracellular Ca^{2+} con-

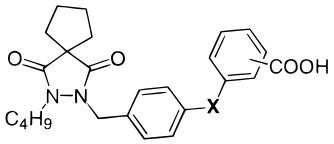
Table 1. AT_1 Binding Affinity and in Vitro Antagonist Activity of Pyrazolidine-3,5-diones: Variations at 2′-Biphenyl Position



compd	R	K_i[a] (nM)	IC_{50}[b] (nM)
30	H	>10000	nd
24	CN	>10000	nd
33	CO_2H	3162 ± 30	nd
28	CN_4H	25 ± 3	22 ± 3
43	SO_2NH_2	>10000	nd
38	SO_2NH^tBu	>10000	nd
48	$SO_2NHCOC_6H_5$	10 ± 2	12 ± 3
53	$SO_2NHCOC_4H_9$	13 ± 2	15 ± 3
54	$SO_2NHCONHC_6H_{11}$	32 ± 4	38 ± 4
irbesartan		2 ± 1	3 ± 1

[a] AT_1 binding, human hepatoma cell line, PLC-PRF-5. Each value represents the mean ± SD of three independent experiments. The K_d (dissociation constant), B_{max} (maximal number of binding sites), and Hill coefficient of [^{3}H]AII are given as follows: 1.1 nM, 700 (fmol/mg protein), 1.0 ± 0.2. [b] Inhibition of AII (50 nM)-induced intracellular $[Ca^{2+}]_i$ increase in PLC-PRF-5 cells; nd, not determined.

Table 2. AT_1 Binding Affinity and in Vitro Antagonist Activity of Pyrazolidine-3,5-diones: Replacement of Biphenyl Structure



compd	X	position	K_i[a] (nM)	IC_{50}[b] (nM)
33	/	*o*-COOH	3162 ± 30	nd
36	CO	*o*-COOH	3301 ± 40	nd
62	CONH	*o*-COOH	1995 ± 30	nd
63	CONH	*m*-COOH	>10000	nd
57	NHCO	*o*-COOH	398 ± 20	nd
irbesartan			2 ± 1	3 ± 1

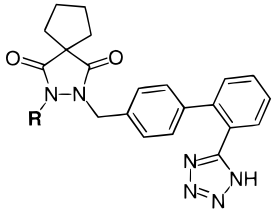
[a] AT_1 binding, human hepatoma cell line, PLC-PRF-5. Each value represents the mean ± SD of three independent experiments. The K_d (dissociation constant), B_{max} (maximal number of binding sites), and Hill coefficient of [^{3}H]AII are given as follows: 1.1 nM, 700 (fmol/mg protein), 1.0 ± 0.2. [b] Inhibition of AII (50 nM)-induced intracellular $[Ca^{2+}]_i$ increase in PLC-PRF-5 cells; nd, not determined.

centration in the absence (100%) and presence of compounds **25**, **28**, **48**−**54**, and irbesartan[26] (10^{-10}−10^{-6} M), yielding the IC_{50} indicated in Tables 1−5. As shown in these tables, compounds **25**, **28**, and **48**−**54** are highly specific inhibitors of the AII-induced stimulation of the phosphoinositide signaling system. Furthermore, for all selected ligands, the potent functional antagonist properties correlate well with the respective specific binding affinities. The best compounds, **28** and **48**, blocked the AII-induced efflux in PLC-PRF-5 cells with an IC_{50} of 22 and 12 nM, respectively.

Conclusion

In summary, this study shows that the new series of 5-(biphenyl-4-ylmethyl)pyrazolidine-3,5-diones provides ligands with good affinity, high selectivity toward the AT_1 receptor, and potent in vitro antagonist activity. Modification of the various substituents of the pyrazolidine-3,5-dione ring indicated that a *n*-butyl group at position 1 as well as a spirocyclopentyl group at position 4 are essential for high affinity. Among the acidic isosteres tested in the biphenyl moiety, the benzoylsulfonamide group proved to be the best. The pyrazolidine-3,5-dione structure is considered as a good template and an efficient replacement of the imidazolinone ring of irbesartan. However, the difference in affinity between irbesartan and **28** suggests that the second carbonyl function at the pyrazolidinedione 5-position is not positioned in the optimal direction to mimic the basic nitrogen of irbesartan.[27]

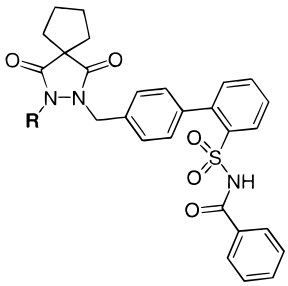
Table 3. AT_1 Binding Affinity and in Vitro Antagonist Activity of Pyrazolidine-3,5-diones: Variations at Position 1



compd	R	K_i[a] (nM)	IC_{50}[b] (nM)
28	C_4H_9	25 ± 3	22 ± 3
25	C_6H_5	158 ± 10	89 ± 10
27	$CH_2C_6H_5$	402 ± 20	nd
26	*p*-$C_6H_4CH_3$	381 ± 30	nd
irbesartan		2 ± 1	3 ± 1

[a] AT_1 binding, human hepatoma cell line, PLC-PRF-5. Each value represents the mean ± SD of three independent experiments. The K_d (dissociation constant), B_{max} (maximal number of binding sites), and Hill coefficient of [^{3}H]AII are given as follows: 1.1 nM, 700 (fmol/mg protein), 1.0 ± 0.2. [b] Inhibition of AII (50 nM)-induced intracellular $[Ca^{2+}]_i$ increase in PLC-PRF-5 cells; nd, not determined.

Table 4. AT_1 Binding Affinity and in Vitro Antagonist Activity of Pyrazolidine-3,5-diones: Variations at Position 1 (Aliphatic Substituents)



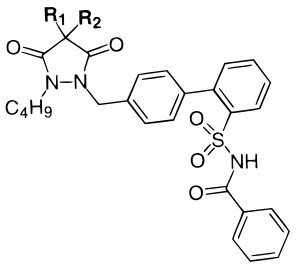
compd	R	K_i[a] (nM)	IC_{50}[b] (nM)
48	C_4H_9	10 ± 2	12 ± 3
49	C_3H_7	40 ± 5	52 ± 4
50	C_5H_{11}	25 ± 5	28 ± 4
irbesartan		2 ± 1	3 ± 1

[a] AT_1 binding, human hepatoma cell line, PLC-PRF-5. Each value represents the mean ± SD of three independent experiments. The K_d (dissociation constant), B_{max} (maximal number of binding sites), and Hill coefficient of [^{3}H]AII are given as follows: 1.1 nM, 700 (fmol/mg protein), 1.0 ± 0.2. [b] Inhibition of AII (50 nM)-induced intracellular $[Ca^{2+}]_i$ increase in PLC-PRF-5 cells; nd, not determined.

Experimental Section

A. Chemistry. General. Melting points were determined with a Büchi SMP-20 melting point apparatus and are uncorrected. Infrared spectra were recorded on a Perkin-Elmer

Table 5. AT_1 Binding Affinity and in Vitro Antagonist Activity of Pyrazolidine-3,5-diones: Variations at Position 4



compd	R_1	R_2	K_i[a] (nM)	IC_{50}[b] (nM)
48	$(CH_2)_4$		10 ± 2	12 ± 3
52	CH_3	CH_3	42 ± 3	26 ± 4
51	CH_3	C_4H_9	32 ± 3	33 ± 5
irbesartan			2 ± 1	3 ± 1

[a] AT_1 binding, human hepatoma cell line, PLC-PRF-5. Each value represents the mean ± SD of three independent experiments. The K_d (dissociation constant), B_{max} (maximal number of binding sites), and Hill coefficient of [^{3}H]AII are given as follows: 1.1 nM, 700 (fmol/mg protein), 1.0 ± 0.2. [b] Inhibition of AII (50 nM)-induced intracellular $[Ca^{2+}]_i$ increase in PLC-PRF-5 cells; nd, not determined.

297 spectrophotometer. ^{1}H (80 MHz) NMR spectra were recorded on a Brücker WP80SY spectrometer, and ^{1}H (300 MHz) NMR spectra were recorded on a Brücker AC300P spectrometer. They are reported in ppm on the δ scale, from the indicated reference. Electron-impact mass spectra (EI-MS) were obtained on a Finnigan TSQ700 instrument. Combustion analysis were carried out in the Elementar Analysis Department of the CNRS (F-69390 Vernaison). When analyses are indicated by the symbols of the elements, the results are within ±0.4% of theoretical values. Analytical thin-layer chromatography (TLC) was performed on silica gel 60 F_{254} plates from E. Merck reagents and visualized by UV irradiation and/or iodine. Flash chromatography was conducted with silica gel (230−430 mesh, E. Merck). Starting materials were purchased from Aldrich and were used as received. Irbesartan was synthesized according to the reported procedure.[13]

Ethyl 2-Butyl-2-methylmalonate (3). Ethyl 2-butylmalonate (10.17 mL, 46 mmol) was added to a solution of sodium (1.06 g, 0.046 atg) in absolute EtOH (100 mL). The solvent was evaporated under reduced pressure. To a suspension of the resulting residue in THF (100 mL) was added dropwise iodomethane (4.31 mL, 69 mmol). The mixture was allowed to stir at room temperature for 2 h. The solvent was removed and water was added. The suspension was extracted with Et_2O. The combined organic layers were dried ($MgSO_4$) and concentrated. Further purification by distillation afforded **3** as a colorless oil (6.25 g, 59%): bp 66 °C (0.42 mbar); R_f 0.62 (hexane/EtOAc = 8:2); IR (KBr) 1700 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.90 (t, J = 7.25 Hz, 3H), 1.21 (t, J = 7.11 Hz, 6H), 1.20−1.30 (m, 4H), 1.40 (s, 3H), 1.82 (m, 2H), 4.20 (q, J = 7.11 Hz, 4H).

General Procedure for Cyclocondensation of Hydrazines with Dialkylmalonates. The desired monosubstituted hydrazine (20 mmol) was added to a solution of sodium (0.084 atg) in absolute EtOH (50 mL). After stirring at room temperature for 1 h, the solvent was evaporated. To a suspension of the resulting solid in dry chlorobenzene (100 mL) was added the diester (24 mmol) and the mixture was heated at reflux for 12 h. After cooling, the solvent was removed under reduced pressure. Water (100 mL) was added and the solution was washed with Et_2O, acidified with 6 N aqueous HCl and extracted with EtOAc. The combined organic extracts were washed with water and dried ($MgSO_4$), and the solvent was removed to afford the compounds **4**−**8**, which were further purified by crystallization.

2-Phenyl-2,3-diazaspiro[4.4]nonane-1,4-dione (4): yield 52%; mp 178−180 °C (EtOH); R_f 0.70 (EtOAc); IR (KBr) 3170−3060, 1740, 1620 cm^{-1}; ^{1}H NMR (80 MHz, $CDCl_3$) δ 1.75−2.25 (m, 8H), 7.10−7.70 (m, 5H).

2-(*p*-Tolyl)-2,3-diazaspiro[4.4]nonane-1,4-dione (5): yield 48%; mp 152−155 °C (EtOH); R_f 0.71 (EtOAc); IR (KBr) 3150, 1720, 1680 cm^{-1}; ^{1}H NMR (80 MHz, $CDCl_3$) δ 1.75−2.15 (m, 8H), 2.30 (s, 3H), 6.25 (brs, 1H exchangeable in D_2O), 7.20 (d, J = 8.27 Hz, 2H), 7.50 (d, J = 8.27 Hz, 2H).

2-Benzyl-2,3-diazaspiro[4.4]nonane-1,4-dione (6): yield 63%; mp 95−98 °C (cyclohexane); R_f 0.54 (EtOAc); IR (KBr) 3100, 1715, 1640 cm^{-1}; ^{1}H NMR (80 MHz, DMSO-d_6) δ 1.70−2.10 (m, 8H), 4.70 (s, 2H), 7.10 (m, 5H).

1-Benzyl-4,4-dimethylpyrazolidine-3,5-dione (7): yield 61%; mp 154−157 °C (EtOH); R_f 0.42 (EtOAc); IR (KBr) 3090, 1740, 1655 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.28 (s, 6H), 4.71 (s, 2H), 7.30 (m, 5H).

1-Benzyl-4-butyl-4-methylpyrazolidine-3,5-dione (8): yield 35%; mp 135−137 °C (acetonitrile); R_f 0.58 (EtOAc); IR (KBr) 3100, 1740, 1640 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.85 (t, J = 7.20 Hz, 3H), 1.05−1.18 (m, 2H), 1.20−1.30 (m, 2H),1.30 (s, 3H), 1.62−1.70 (m, 2H), 4.70 (s, 2H), 7.30 (m, 5H).

General Procedure for Alkylation of Compounds 6−8 and Subsequent Debenzylation. K_2CO_3 (60 mmol) was added portionwise to a solution of compounds **6**−**8** (20 mmol) in DMF (15 mL). After stirring for 1 h, the desired alkyl bromide (25 mmol) was added to the reaction mixture, which was allowed to stir for 10 h at room temperature. Water (80 mL) was added and the resulting suspension was extracted with EtOAc. The combined organic extracts were washed with water and dried ($MgSO_4$), and the solvent was removed to afford the mixture of isomers (**9a,b**−**13a,b**) which was used in the next step without further purification. To a solution of these isomers (**9a,b**−**13a,b**) in MeOH (50 mL) were added successively Pd/C (1 g) and ammonium formate (100 mmol). The mixture was heated at 40 °C for 4 h, cooled, and filtered through Celite. The solvent was evaporated and 1 N aqueous NaOH (50 mL) was added. The mixture was washed with Et_2O. The combined organic extracts were washed with water and dried ($MgSO_4$), and the solvent was removed to afford the isomers (**9b**−**13b**). The basic aqueous solution was acidified with 6 N aqueous HCl, and the mixture was extracted with EtOAc. The combined organic extracts were washed with water and dried ($MgSO_4$), and the solvent was removed to afford compounds **14**−**18**.

2-Propyl-2,3-diazaspiro[4.4]nonane-1,4-dione (14): yield 87%; R_f 0.32 (EtOAc); IR (KBr) 3100, 1740, 1660 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.93 (t, J = 7.53 Hz, 3H), 1.67 (m, 2H), 1.91 (m, 2H), 2.03 (m, 6H), 3.56 (t, J = 7.23 Hz, 2H).

2-Butyl-2,3-diazaspiro[4.4]nonane-1,4-dione (15): yield 90%; R_f 0.51 (EtOAc); IR (KBr) 3100, 1720, 1650 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.90 (t, J = 7.50 Hz, 3H), 1.00−1.80 (m, 4H), 2.00 (m, 8H), 3.55 (t, J = 7.50 Hz, 2H).

2-Pentyl-2,3-diazaspiro[4.4]nonane-1,4-dione (16): yield 80%; R_f 0.63 (EtOAc); IR (KBr) 3100, 1735, 1660 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.93 (t, J = 7.44 Hz, 3H), 1.30 (m, 4H), 1.62 (m, 2H), 1.88 (m, 2H), 1.97 (m, 6H), 3.58 (t, J = 7.44 Hz, 2H), 10.13 (brs, 1H exchangeable in D_2O).

1-Butyl-4,4-dimethylpyrazolidine-3,5-dione (17): yield 83%; R_f 0.36 (hexane/EtOAc = 6:4); IR (KBr) 3140, 1740, 1660 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.97 (t, J = 7.29 Hz, 3H), 1.36 (m, 8H), 1.68 (m, 2H), 3.65 (t, J = 6.84 Hz, 2H).

1,4-Dibutyl-4-methylpyrazolidine-3,5-dione (18): yield 83%; R_f 0.26 (hexane/EtOAc = 6:4); IR (KBr) 3100, 1740, 1670 cm^{-1}; ^{1}H NMR (80 MHz, $CDCl_3$) δ 0.80 (t, J = 7.21 Hz, 3H), 0.83 (t, J = 7.36 Hz, 3H), 1.10−1.31 (m, 8H), 1.20 (m, 3H), 1.50−1.70 (m, 2H), 3.60 (t, J = 7.17 Hz, 2H).

2-Benzyl-4-propyloxy-2,3-diazaspiro[4.4]non-3-en-1-one (9b): R_f 0.75 (hexane/EtOAc = 6:4); IR (KBr) 1700, 1600 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.02 (t, 3H), 1.78 (m, 2H), 1.91 (m, 8H), 4.02 (t, J = 7.23 Hz, 2H), 4.72 (s, 2H), 7.40 (m, 5H).

2-Benzyl-4-butyloxy-2,3-diazaspiro[4.4]non-3-en-1-one (10b): R_f 0.71 (hexane/EtOAc = 6:4); IR (KBr) 1700, 1600 cm^{-1}; ^{1}H NMR (80 MHz, $CDCl_3$) δ 0.90 (t, J = 7.25 Hz, 3H),

1.20−1.70 (m, 4H), 2.00 (m, 8H), 4.10 (t, J = 7.25 Hz, 2H), 4.75 (s, 2H), 7.30 (m, 5H).

2-Benzyl-4-pentyloxy-2,3-diazaspiro[4.4]non-3-en-1-one (11b): R_f 0.79 (hexane/EtOAc = 6:4); IR (KBr) 1700, 1600 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.90 (t, J = 7.20 Hz, 3H), 1.30 (m, 4H), 1.65 (m, 2H), 1.95 (m, 8H), 4.10 (t, J = 7.23 Hz, 2H), 4.75 (s, 2H), 7.20 (m, 5H).

1-Benzyl-3-butyloxy-4,4-dimethylpyrazolidin-2-en-5-one (12b): R_f 0.52 (hexane/EtOAc = 6:4); IR (KBr) 1700, 1600 cm^{-1}; ^{1}H NMR (80 MHz, $CDCl_3$) δ 0.90 (t, J = 7.22 Hz, 3H), 1.30 (m, 8H), 1.67 (m, 2H), 4.10 (t, J = 7.22 Hz, 2H), 4.75 (s, 2H), 7.30 (m, 5H).

1-Benzyl-4-butyl-3-butyloxy-4-methylpyrazolidin-2-en-5-one (13b): R_f 0.78 (hexane/EtOAc = 6:4); IR (KBr) 1710, 1610 cm^{-1}; ^{1}H NMR (80 MHz, $CDCl_3$) δ 0.79 (m, 6H), 0.84−1.00 (m, 8H), 1.25 (s, 3H), 1.59 (m, 2H), 4.12 (t, J = 7.32 Hz, 2H), 4.75 (s, 2H), 7.31 (m, 5H).

General Procedure for Alkylation of Pyrazolidine-3,5-diones 4−6 and 14−18 with the Desired Alkyl Bromide Derivatives. K_2CO_3 (60 mmol) was added portionwise to a solution of compounds **4**−**6** and **14**−**18** (30 mmol) in DMF (10 mL). The desired alkyl bromide (45 mmol) was then added to the reaction mixture, which was allowed to stir for 20 h at room temperature. Water (80 mL) was added and the resulting suspension was extracted with Et_2O. The combined organic extracts were washed with water and dried ($MgSO_4$), and the solution was concentrated. The resulting precipitate was collected by filtration, washed with Et_2O and further purified by crystallization.

2-Phenyl-3-[[2′-[1-(triphenylmethyl)-*1H*-tetrazol-5-yl]-(1,1′-biphenyl)-4-yl]methyl]-2,3-diazaspiro[4.4]nonane-1,4-dione (20): yield 35%; mp 152−154 °C (EtOH); R_f 0.51 (hexane/EtOAc = 6:4); IR (KBr) 1740, 1650 cm^{-1}; ^{1}H NMR (80 MHz, $CDCl_3$) δ 1.80 (m, 8H), 4.60 (s, 2H), 6.60−7.90 (m, 28H).

2-(*p*-Tolyl)-3-[[2′-[1-(triphenylmethyl)-*1H*-tetrazol-5-yl]-(1,1′-biphenyl)-4-yl]methyl]-2,3-diazaspiro[4.4]nonane-1,4-dione (21): yield 30%; mp 165−168 °C (acetonitrile); R_f 0.83 (CH_2Cl_2); IR (KBr) 1720, 1690 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.91−1.97 (m, 8H), 2.40 (s, 3H), 4.56 (s, 2H), 6.75 (d, J = 6.87 Hz, 2H), 6.89 (m, 6H), 7.00 (d, J = 7.03 Hz, 2H), 7.10 (d, J = 7.10 Hz, 2H), 7.21 (m, 12H), 7.39 (t, J = 7.67 Hz, 1H), 7.50 (t, J = 7.67 Hz, 1H), 7.88 (d, J = 7.67 Hz, 1H).

2-Benzyl-3-[[2′-[1-(triphenylmethyl)-*1H*-tetrazol-5-yl]-(1,1′-biphenyl)-4-yl]methyl]-2,3-diazaspiro[4.4]nonane-1,4-dione (22): yield 42%; mp 198−200 °C (EtOAc); R_f 0.59 (hexane/EtOAc = 6:4); IR (KBr) 1725, 1690 cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.80 (m, 8H), 4.61 (s, 2H), 4.68 (s, 2H), 6.85 (s, 1H), 6.90 (m, 5H), 7.05 (d, J = 8.11 Hz, 2H), 7.11 (d, J = 8.11 Hz, 2H), 7.20 (d, J = 7.89 Hz, 2H), 7.30 (m, 12H), 7.50 (d, J = 7.89 Hz, 1H), 7.55 (t, J = 7.89 Hz, 1H), 7.64 (t, J = 7.89 Hz, 1H), 7.80 (d, J = 7.89 Hz, 1H).

2-Butyl-3-[[2′-cyano(1,1′-biphenyl)-4-yl]methyl]-2,3-diazaspiro[4.4]nonane-1,4-dione (24): yield 33%; mp 120−122 °C (toluene/cyclohexane = 1:5); R_f 0.80 (EtOAc); IR (KBr) 2210, 1715, 1675 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.88 (t, J = 7.27 Hz, 3H), 1.24 (m, 2H), 1.46 (m, 2H), 1.96−2.03 (m, 8H), 3.54 (t, J = 7.53 Hz, 2H), 4.85 (s, 2H), 7.35 (s, 1H), 7.38 (s, 1H), 7.48 (m, 3H), 7.51 (t, J = 8.20 Hz, 1H), 7.66 (t, J = 8.20 Hz, 1H), 7.80 (d, J = 8.20 Hz, 1H); EI-MS [M$^{\cdot+}$] 401. Anal. ($C_{25}H_{27}N_3O_2 \cdot 0.5H_2O$) C, H, N.

3-[[(1,1′-Biphenyl)-4-yl]methyl]-2-butyl-2,3-diazaspiro[4.4]nonane-1,4-dione (30): yield 60%; mp 104−107 °C (EtOH); R_f 0.62 (hexane/EtOAc = 6:4); IR (KBr) 1730, 1680 cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6) δ 0.90 (t, J = 7.17 Hz, 3H), 1.15 (sextet, J = 7.17 Hz, 2H), 1.40 (quintet, J = 7.15 Hz, 2H), 1.82 (m, 8H), 3.50 (t, J = 7.15 Hz, 2H), 4.80 (s, 2H), 7.30 (m, 3H), 7.43 (m, 2H), 7.67 (m, 4H); EI-MS [M$^{\cdot+}$] 376.5. Anal. ($C_{24}H_{28}N_2O_2$) C, H, N.

2-Butyl-3-[[2′-(*tert*-butyloxycarbonyl)(1,1′-biphenyl)-4-yl]methyl]-2,3-diazaspiro[4.4]nonane-1,4-dione (32): yield 35%; mp 96−97 °C (hexane); R_f 0.50 (hexane/EtOAc = 7:3); IR (KBr) 1710, 1670 cm^{-1}; ^{1}H NMR (80 MHz, $CDCl_3$) δ 0.90 (t, J = 7.20 Hz, 3H), 1.10−1.15 (m, 13H), 2.00 (m, 8H), 3.50 (t, J = 7.05 Hz, 2H), 4.80 (s, 2H), 7.32 (m, 5H), 7.48 (m, 2H), 7.75 (d, J = 8.05 Hz, 1H).

2-Butyl-3-[4-(2-carbomethoxybenzoyl)benzyl]-2,3-diazaspiro[4.4]nonane-1,4-dione (35): yield 39%; mp 81−82 °C (cyclohexane); R_f 0.25 (hexane/EtOAc = 7:3); IR (KBr) 1710, 1670 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.87 (t, J = 7.47 Hz, 3H), 1.22 (sextet, J = 7.12 Hz, 2H), 1.42 (quintet, J = 7.32 Hz, 2H), 1.95 (m, 8H), 3.47 (t, J = 7.47 Hz, 2H), 3.62(s, 3H), 4.80 (s, 2H), 7.28 (d, J = 8.00 Hz, 2H), 7.37 (d, J = 7.95 Hz, 1H), 7.57 (t, J = 7.50 Hz, 1H), 7.63 (t, J = 7.99 Hz, 1H), 7.73 (d, J = 8.30 Hz, 2H), 8.05 (d, J = 8.54 Hz, 1H).

2-Butyl-3-[[2′-(*N-tert*-butylaminosulfonyl)(1,1′-biphenyl)-4-yl]methyl]-2,3-diazaspiro[4.4]nonane-1,4-dione (38): yield 51%; mp 146−148 °C (EtOH); R_f 0.50 (hexane/EtOAc = 6:4); IR (KBr) 3280, 1715, 1675, 1295, 1140 cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6) δ 0.80 (t, J = 7.26 Hz, 3H), 0.95 (s, 9H), 1.17 (sextet, J = 7.37 Hz, 2H), 1.40 (quintet, J = 7.38 Hz, 2H), 1.80 (m, 8H), 3.50 (t, J = 6.75 Hz, 2H), 4.84 (s, 2H), 6.50 (s, 1H), 7.30 (m, 3H), 7.38 (s, 1H), 7.42 (d, J = 7.60 Hz, 1H), 7.50 (m, 2H), 8.20 (d, J = 7.37 Hz, 1H); EI-MS [M$^{\cdot+}$] 511. Anal. ($C_{28}H_{37}N_3O_4S$) C, H, N.

3-[[2′-(*N-tert*-Butylaminosulfonyl)(1,1′-biphenyl)-4-yl]-methyl]-2-propyl-2,3-diazaspiro[4.4]nonane-1,4-dione (39): yield 42%; mp 151−154 °C (EtOH); R_f 0.47 (hexane/EtOAc = 6:4); IR (KBr) 3300, 1725, 1680, 1300, 1145 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.83 (t, J = 7.30 Hz, 3H), 0.97 (s, 9H), 1.61 (m, 2H), 2.00 (m, 8H), 3.50 (t, J = 7.35 Hz, 2H), 4.87 (s, 2H), 7.34 (m, 4H), 7.55 (m, 4H), 8.16 (d, J = 8.02 Hz, 1H).

3-[[2′-(*N-tert*-Butylaminosulfonyl)(1,1′-biphenyl)-4-yl]-methyl]-2-pentyl-2,3-diazaspiro[4.4]nonane-1,4-dione (40): yield 47%; mp 144−145 °C (acetonitrile); R_f 0.32 (hexane/EtOAc = 7:3); IR (KBr) 3300, 1720, 1680, 1300, 1150 cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6) δ 0.80 (t, J = 7.25 Hz, 3H), 0.95 (s, 9H), 1.20 (m, 4H), 1.42 (m. 2H), 1.83 (m, 8H), 3.50 (t, J = 7.25 Hz, 2H), 4.85 (s, 2H), 6.57 (s, 1H), 7.30 (m, 5H), 7.58 (m, 2H), 8.02 (d, J = 8.05 Hz, 1H).

1,4-Dibutyl-2-[[2′-(*N-tert*-butylaminosulfonyl)(1,1′-biphenyl)-4-yl]methyl]-4-methylpyrazolidine-3,5-dione (41): yield 35%; mp 148−152 °C (EtOH); R_f 0.61 (hexane/EtOAc = 6:4); IR (KBr) 3300, 1730, 1690, 1300, 1140 cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6) δ 0.78 (t, J = 7.33 Hz, 3H), 0.82 (t, J = 7.17 Hz, 3H), 0.96 (s, 9H), 1.02 (m, 2H), 1.15 (s, 3H), 1.20 (m, 4H), 1.39 (m, 2H), 1.60 (m, 2H), 3.58 (m, 2H), 4.81 (d, J = 16.01 Hz, 1H), 4.96 (d, J = 16.01 Hz, 1H), 6.61 (s, 1H), 7.28 (d, J = 7.15 Hz, 1H), 7.35 (d, J = 8.15 Hz, 2H), 7.40 (d, J = 8.15 Hz, 2H), 7.60 (m, 2H), 8.03 (d, J = 7.78 Hz, 1H).

1-Butyl-2-[[2′-(*N-tert*-butylaminosulfonyl)(1,1′-biphenyl)-4-yl]methyl]-4,4-dimethylpyrazolidine-3,5-dione (42): yield 50%; mp 133−135 °C (EtOH); R_f 0.38 (hexane/EtOAc = 6:4); IR (KBr) 3300, 1730, 1690, 1300, 1140 cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6) δ 0.82 (t, J = 7.02 Hz, 3H), 0.96 (s, 9H), 1.20 (m, 8H), 1.50 (m, 2H), 3.53 (t, J = 7.02 Hz, 2H), 4.85 (s, 2H), 6.56 (s, 1H), 7.31 (m, 3H), 7.40 (d, J = 7.95 Hz, 2H), 7.59 (m, 2H), 8.05 (d, J = 7.95 Hz, 1H).

General Procedure for Deprotection of the *tert*-Butylsulfonamide Moiety. A few drops of anisole were added to a solution of compounds **38**−**42** (10 mmol) in TFA (400 mmol). The mixture was allowed to stir at room temperature for 12 h. After removal of the solvent under reduced pressure, water was added and the suspension was made basic with $NaHCO_3$. The resulting precipitate was collected by filtration, washed with water and Et_2O and further purified by crystallization.

3-[[2′-(Aminosulfonyl)(1,1′-biphenyl)-4-yl]methyl]-2-butyl-2,3-diazaspiro[4.4]nonane-1,4-dione (43): yield 83%; mp 98−100 °C (toluene); R_f 0.47 (hexane/EtOAc = 4:6); IR (KBr) 3350, 3200, 3060, 1720, 1670, 1335, 1160 cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6) δ 0.82 (t, J = 7.37 Hz, 3H), 1.17 (sextet, J = 7.23 Hz, 2H), 1.40 (quintet, J = 7.23 Hz, 2H), 1.80 (m, 8H), 3.52 (t, J = 7.37 Hz, 2H), 4.87 (s, 2H), 7.20 (s, 2H), 7.25 (m, 3H), 7.40 (d, J = 8.14 Hz, 2H), 7.60 (m, 2H), 8.02 (dd, J = 7.37 Hz, 1.47 Hz, 1H); EI-MS [M$^{\cdot+}$] 455. Anal. ($C_{24}H_{29}N_3O_4S$) C, H, N.

3-[[2′-(Aminosulfonyl)(1,1′-biphenyl)-4-yl]methyl]-2-propyl-2,3-diazaspiro[4.4]nonane-1,4-dione (44): yield 95%;

mp 157−159 °C (toluene); R_f 0.39 (hexane/EtOAc = 4:6); IR (KBr) 3320, 3220, 3070, 1720, 1660, 1330, 1160 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 0.85 (t, J = 7.26 Hz, 3H), 1.53 (sextet, J = 7.23 Hz, 2H), 2.00 (m, 8H), 3.52 (t, J = 7.26 Hz, 2H), 4.37 (s, 2H), 4.83 (s, 2H), 7.30 (m, 2H), 7.45 (m, 4H), 7.60 (m, 1H), 8.10 (d, J = 8.00 Hz, 1H).

3-[[2′-(Aminosulfonyl)(1,1′-biphenyl)-4-yl]methyl]-2-pentyl-2,3-diazaspiro[4.4]nonane-1,4-dione (45): yield 89%; mp 108−110 °C (toluene); R_f 0.56 (hexane/EtOAc = 4:6); IR (KBr) 3350, 3220, 3080, 1710, 1670, 1340, 1160 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6) δ 0.76 (t, J = 7.20 Hz, 3H), 1.15 (m, 4H), 1.40 (m, 2H), 1.82 (m, 8H), 3.50 (t, J = 7.20 Hz, 2H), 4.84 (s, 2H), 7.20 (s, 2H), 7.29 (m, 3H), 7.35 (m, 2H), 7.55 (m, 2H), 8.01 (d, J = 7.96 Hz, 1H).

2-[[2′-(Aminosulfonyl)(1,1′-biphenyl)-4-yl]methyl]-1-butyl-4,4-dimethylpyrazolidine-3,5-dione (47): yield 89%; mp 124−126 °C (toluene); R_f 0.29 (hexane/EtOAc = 4:6); IR (KBr) 3360, 3300, 3200, 1720, 1675, 1335, 1165 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6) δ 0.83 (t, J = 7.40 Hz, 3H), 1.17 (m, 8H), 1.40 (m, 2H), 3.54 (t, J = 7.40 Hz, 2H), 4.87 (s, 2H), 7.19 (s, 2H), 7.30 (m, 3H), 7.40 (m, 2H), 7.60 (m, 2H), 8.02 (d, J = 7.82 Hz, 1H).

General Procedure for Deprotection of the 1-(Triphenylmethyl)-*1H*-tetrazol-5-yl Moiety. HCl (37%) (40 mmol) was added dropwise to a solution of compounds **20**−**22** (10 mmol) in 20 mL of a mixture of water/THF (1:4). The mixture was allowed to stir at room temperature for 8 h. The THF was removed under reduced pressure and the solution was made basic with 2 N aqueous NaOH (10 mL), washed with Et_2O and acidified with 3 N aqueous HCl. The resulting precipitate was collected by filtration, washed with water and further purified by crystallization.

2-Phenyl-3-[[2′-(*1H*-tetrazol-5-yl)(1,1′-biphenyl)-4-yl]methyl]-2,3-diazaspiro[4.4]nonane-1,4-dione (25): yield 51%; mp 174−176 °C (EtOH/H_2O = 1:1); R_f 0.45 (EtOAc); IR (KBr) 1730, 1690 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6) δ 1.84−1.90 (m, 8H), 4.62 (s, 2H), 6.80 (d, J = 8.07 Hz, 2H), 7.00 (d, J = 8.07 Hz, 2H), 7.18 (d, J = 8.10 Hz, 2H), 7.36−7.70 (m, 7H); EI-MS [$M^{\cdot+}$] 465. Anal. ($C_{27}H_{24}N_6O_2$) C, H, N.

3-[[2′-(*1H*-Tetrazol-5-yl)(1,1′-biphenyl)-4-yl]methyl]-2-(*p*-tolyl)-2,3-diazaspiro[4.4]nonane-1,4-dione (26): yield 54%; mp 198−200 °C (EtOH); R_f 0.56 (CH_2Cl_2/EtOH = 9:1); IR (KBr) 1720, 1655 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6) δ 1.81−1.90 (m, 8H), 2.33 (s, 3H), 4.60 (s, 2H), 6.85 (d, J = 8.06 Hz, 2H), 7.00 (d, J = 8.06 Hz, 2H), 7.10 (d, J = 8.19 Hz, 2H), 7.37 (d, J = 8.19 Hz, 2H), 7.50−7.72 (m, 4H); EI-MS [$M^{\cdot+}$] 478. Anal. ($C_{28}H_{26}N_6O_2$) C, H, N.

2-Benzyl-3-[[2′-(*1H*-tetrazol-5-yl)(1,1′-biphenyl)-4-yl]methyl]-2,3-diazaspiro[4.4]nonane-1,4-dione (27): yield 27%; mp 186−187 °C (acetonitrile); R_f 0.56 (CH_2Cl_2/EtOH = 9:1); IR (KBr) 1720, 1650 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6) δ 1.80 (m, 8H), 4.70 (s, 2H), 4.72 (s, 2H), 7.07 (d, J = 8.09 Hz, 2H), 7.18 (d, J = 8.09 Hz, 2H), 7.23 (m, 2H), 7.42 (m, 3H), 7.58 (m, 2H), 7.67 (m, 2H); EI-MS [$M^{\cdot+}$] 478. Anal. ($C_{28}H_{26}N_6O_2$) C, H, N.

2-Butyl-3-[[2′-(*1H*-tetrazol-5-yl)(1,1′-biphenyl)-4-yl]methyl]-2,3-diazaspiro[4.4]nonane-1,4-dione (28). Tributyltin chloride (5.4 mL, 20 mmol) and sodium azide (1.3 g, 20 mmol) were added successively to a solution of compound **24** (2 g, 5 mmol) in DMF (10 mL). The mixture was heated at 130 °C under nitrogen atmosphere for 12 h. After cooling, the solution was poured into ice/water and was then acidified with 6 N aqueous HCl. The resulting precipitate was collected by filtration, washed with water and hexane, and further purified by crystallization to afford **28** as colorless crystals (1.04 g, 47%): mp 169−170 °C (EtOH); R_f 0.41 (EtOAc); IR (KBr) 1710, 1650 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6) δ 0.80 (t, J = 7.08 Hz, 3H), 1.13 (sextet, J = 7.08 Hz, 2H), 1.36 (quintet, J = 7.08 Hz, 2H), 1.81 (m, 8H), 3.50 (t, J = 6.56 Hz, 2H), 4.80 (s, 2H), 7.10 (d, J = 7.49 Hz, 2H), 7.21 (d, J = 7.49 Hz, 2H), 7.50−7.75 (m, 4H); EI-MS [$M^{\cdot+}$] 444. Anal. ($C_{25}H_{28}N_6O_2$) C, H, N.

2-Butyl-3-[4-(2-carboxybenzoyl)benzyl]-2,3-diazaspiro[4.4]nonane-1,4-dione (36). HCl (37%) (5 mL, 60 mmol) was added to a solution of compound **35** (0.88 g, 1.93 mmol) in acetic acid (100 mL). The mixture was heated under reflux for 10 h. The solvent was removed under reduced pressure and water (100 mL) was added. The resulting precipitate was collected by filtration, washed with water and Et_2O, and further purified by crystallization to afford **36** as colorless crystals (0.66 g, 77%): mp 144−145 °C (cyclohexane); R_f 0.50 (CH_2Cl_2/EtOH = 95:5); IR (KBr) 3300−2500, 1710, 1650 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6) δ 0.78 (t, J = 7.32 Hz, 3H), 1.14 (m, 2H), 1.35 (m, 2H), 1.95 (m, 8H), 3.45 (m, 2H), 4.87 (s, 2H), 7.36 (m, 3H), 7.61 (m, 4H), 7.97 (d, J = 7.85 Hz, 1H), 13.19 (s, 1H exchangeable in D_2O); EI-MS [$M^{\cdot+}$] 448. Anal. ($C_{26}H_{28}N_2O_5$) C, H, N.

4′-[[2-Butyl-1,4-dioxo-2,3-diazaspiro[4.4]nonan-3-yl]-methyl]-1,1′-biphenyl-2-carboxylic Acid (33). HCl(g) was bubbled for 1 min into a solution of compound **32** (1 g, 1.70 mmol) in dioxane (15 mL). The mixture was allowed to stir at room temperature for 12 h. The solution was poured into ice/water (150 mL), made basic with 2 N aqueous NaOH, washed with Et_2O and acidified with 3 N aqueous HCl. The resulting precipitate was collected by filtration, washed with water, dried under vacuum and further purified by crystallization to afford **33** as a colorless solid (0.33 g, 47%): mp 139−140 °C (EtOAc); R_f 0.58 (hexane/EtOAc = 9:1); IR (KBr) 1700, 1650 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6) δ 0.80 (t, J = 7.15 Hz, 3H), 1.20 (sextet, J = 7.15 Hz, 2H), 1.40 (quintet, J = 7.15 Hz, 2H), 1.80 (m, 8H), 3.50 (t, J = 7.15 Hz, 2H), 4.80 (s, 2H), 7.30 (m, 4H), 7.40 (d, J = 7.61 Hz, 1H), 7.45 (t, J = 7.61 Hz, 1H), 7.65 (t, J = 7.61 Hz, 1H), 7.75 (d, J = 7.61 Hz, 1H), 12.80 (s, 1H exchangeable in D_2O); EI-MS [$M^{\cdot+}$] 420. Anal. ($C_{25}H_{28}N_2O_4$) C, H, N.

General Procedure for Synthesis of Acylsulfonamides 48−53. The desired anhydride (40 mmol) was added dropwise to a solution of the sulfonylamine **43**−**47** (10 mmol) in pyridine (10 mL). The solution was heated under reflux for 48 h. After cooling, the mixture was poured into ice/water (100 mL) and acidified with 37% HCl. The resulting precipitate was collected by filtration, washed with water and Et_2O, and further purified by crystallization.

3-[[2′-(Benzoylaminosulfonyl)(1,1′-biphenyl)-4-yl]methyl]-2-butyl-2,3-diazaspiro[4.4]nonane-1,4-dione (48): yield 52%; mp 188−192 °C (EtOH); R_f 0.37 (hexane/EtOAc = 4:6); IR (KBr) 3150, 1720, 1660 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6) δ 0.84 (t, J = 7.19 Hz, 3H), 1.18 (sextet, J = 7.34 Hz, 2H), 1.43 (quintet, J = 7.38 Hz, 2H), 1.85 (m, 8H), 3.51 (t, J = 7.16 Hz, 2H), 4.90 (s, 2H), 7.22 (m, 5H), 7.42 (m, 2H), 7.63 (m, 5H), 8.19 (d, J = 7.78 Hz, 1H), 12.16 (s, 1H exchangeable in D_2O); EI-MS [$M^{\cdot+}$] 559. Anal. ($C_{31}H_{33}N_3O_5S$) C, H, N.

3-[[2′-(Benzoylaminosulfonyl)(1,1′-biphenyl)-4-yl]methyl]-2-propyl-2,3-diazaspiro[4.4]nonane-1,4-dione (49): yield 79%; mp 244−245 °C (EtOH); R_f 0.26 (hexane/EtOAc = 4:6); IR (KBr) 3130, 1715, 1680, 1650 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6) δ 0.71 (t, J = 7.36 Hz, 3H), 1.46 (m, 2H), 1.89 (m, 8H), 3.46 (t, J = 7.36 Hz, 2H), 4.86 (s, 2H), 7.21 (m, 5H), 7.40 (m, 2H), 7.62 (m, 5H), 8.16 (d, J = 7.74 Hz, 1H), 12.10 (s, 1H exchangeable in D_2O); EI-MS [$M^{\cdot+}$] 545. Anal. ($C_{30}H_{31}N_3O_5S$) C, H, N.

3-[[2′-(Benzoylaminosulfonyl)(1,1′-biphenyl)-4-yl]methyl]-2-pentyl-2,3-diazaspiro[4.4]nonane-1,4-dione (50): yield 73%; mp 188−190 °C (EtOH); R_f 0.26 (hexane/EtOAc = 4:6); IR (KBr) 3170, 1715, 1665 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6) δ 0.84 (t, J = 7.10 Hz, 3H), 1.22 (m, 4H), 1.46 (m, 2H), 1.87 (m, 8H), 3.52 (t, J = 7.10 Hz, 2H), 4.80 (s, 2H), 7.25 (m, 5H), 7.45 (m, 2H), 7.62 (m, 5H), 8.18 (d, J = 7.90 Hz, 1H), 12.16 (s, 1H exchangeable in D_2O); EI-MS [$M^{\cdot+}$] 573. Anal. ($C_{32}H_{35}N_3O_5S$) C, H, N.

3-[[2′-(Benzoylaminosulfonyl)(1,1′-biphenyl)-4-yl]methyl]-1,4-dibutyl-4-methylpyrazolidine-3,5-dione (51): yield 69%; mp 132−134 °C (cyclohexane); R_f 0.36 (hexane/EtOAc =4:6); IR (KBr) 3270, 1730, 1680 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6) δ 0.75 (t, J = 7.33 Hz, 3H), 0.81 (t, J = 7.17 Hz, 3H), 1.01 (m, 2H), 1.18 (s, 3H), 1.25 (m, 4H), 1.40 (m, 2H), 1.60 (m, 2H), 3.51 (m, 2H), 4.80 (d, J = 16.01 Hz, 1H), 5.00 (d, J = 16.01 Hz, 1H), 7.30 (m, 5H), 7.45 (m, 2H), 7.64 (m, 5H),

8.20 (d, J = 8.05 Hz, 1H), 12.12 (s, 1H exchangeable in D_2O); EI-MS [$M^{\cdot+}$] 575. Anal. ($C_{32}H_{37}N_3O_5S$) C, H, N.

3-[[2′-(Benzoylaminosulfonyl)(1,1′-biphenyl)-4-yl]methyl]-1-butyl-4,4-dimethylpyrazolidine-3,5-dione (52): yield 82%; mp 191−192 °C (EtOH); R_f 0.25 (hexane/EtOAc = 4:6); IR (KBr) 3240, 1730, 1685 cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6) δ 0.83 (t, J = 7.35 Hz, 3H), 1.22 (m, 8H), 1.42 (m, 2H), 3.53 (t, J = 7.35 Hz, 2H), 4.88 (s, 2H), 7.32 (m, 5H), 7.48 (m, 2H), 7.67 (m, 5H), 8.25 (d, J = 7.93 Hz, 1H), 12.10 (s, 1H exchangeable in D_2O); EI-MS [$M^{\cdot+}$] 533. Anal. ($C_{29}H_{31}N_3O_5S$) C, H, N.

2-Butyl-3-[[2′-(valerylaminosulfonyl)(1,1′-biphenyl)-4-yl]methyl]-2,3-diazaspiro[4.4]nonane-1,4-dione (53): yield 57%; mp 154−156 °C (EtOH); R_f 0.54 (hexane/EtOAc = 4:6); IR (KBr) 3150, 1720, 1660 cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6) δ 0.76 (t, J = 7.13 Hz, 3H), 1.18 (t, J = 7.24 Hz, 3H), 1.17 (m, 4H), 1.30 (m, 2H), 1.41 (m, 2H), 1.80 (m, 8H), 1.91 (m, 2H), 3.50 (t, J = 6.94 Hz, 2H), 4.88 (s, 2H), 7.29 (m, 5H), 8.07 (d, J = 7.74 Hz, 1H), 8.09 (t, J = 7.74 Hz, 1H), 8.10 (t, J = 7.74 Hz, 1H), 11.55 (s, 1H exchangeable in D_2O); EI-MS [$M^{\cdot+}$] 539. Anal. ($C_{29}H_{37}N_3O_5S$) C, H, N.

2-Butyl-3-[[2′-(cyclohexylaminocarbonylaminosulfonyl)-(1,1′-biphenyl)-4-yl]methyl]-2,3-diazaspiro[4.4]nonane-1,4-dione (54). K_2CO_3 (0.91 g, 6.58 mmol) was added portionwise to a solution of compound **43** (1 g, 2.19 mmol) in acetone (50 mL). The mixture was stirred for 1 h at room temperature. Cyclohexyl isocyanate (0.84 mL, 6.58 mmol) was then added dropwise to the solution which was refluxed for 12 h. After removal of the solvent under reduced pressure, water was added and the mixture was acidified with 3 N aqueous HCl. The resulting precipitate was collected by filtration and washed with acetone. The filtrate was concentrated to afford an oil which was triturated in Et_2O. The resulting precipitate was collected by filtration and purified by crystallization to give **54** as colorless crystals (0.80 g, 63%): mp 155−159 °C (acetonitrile); R_f 0.64 (hexane/EtOAc = 4:6); IR (KBr) 3380, 3200, 3060, 1725, 1680 cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6) δ 0.83 (t, J = 7.45 Hz, 3H), 1.00 (m, 2H), 1.12 (m, 5H), 1.30 (m, 3H), 1.57 (m, 4H), 1.77 (m, 8H), 3.20 (s, 1H), 3.47 (t, J = 7.45 Hz, 2H), 4.82 (s, 2H), 5.88 (s, 1H), 7.17 (m, 4H), 7.33 (d, J = 7.74 Hz, 1H), 7.47 (m, 2H), 8.00 (d, J = 7.74 Hz, 1H); EI-MS [$M^{\cdot+}$] 580. Anal. ($C_{31}H_{40}N_4O_5S$) C, H, N.

2-Butyl-3-(4-nitrobenzyl)-2,3-diazaspiro[4.4]nonane-1,4-dione (55a) and 2-Butyl-4-(4-nitrobenzyloxy)-2,3-diazaspiro[4.4]non-3-en-1-one (55b). 4-Nitrobenzyl bromide (9.72 g, 45 mmol) was added portionwise to a cold (0 °C) mixture of compound **15** (6.3 g, 30 mmol) and K_2CO_3 (8.29 g, 60 mmol) in acetone (50 mL). The reaction mixture was allowed to stir at 0 °C for 1 h. The solvent was evaporated, water (100 mL) was added, and the resulting suspension was extracted with EtOAc. The combined organic extracts were washed with water and dried ($MgSO_4$), and the solvent was removed to afford the mixture of isomers **55a** and **55b**. These isomers were separated by column chromatography on silica gel (hexanes−EtOAc mixtures of increasing polarity) and purified by crystallization.

55a: 7.77 g, 75%; mp 95−96 °C (EtOH); R_f 0.19 (hexane/EtOAc = 7:3); IR (KBr) 1720, 1675, 1500, 1330 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.85 (t, J = 7.44 Hz, 3H), 1.25 (sextet, J = 7.11 Hz, 2H), 1.46 (quintet, J = 7.11 Hz, 2H), 1.98 (m, 8H), 3.51 (t, J = 7.44 Hz, 2H), 4.87 (s, 2H), 7.43 (d, J = 8.49 Hz, 2H), 8.22 (d, J = 8.49 Hz, 2H).

55b: 1.55 g, 15%; mp 48−49 °C (EtOH); R_f 0.32 (hexane/EtOAc = 7:3); IR (KBr) 1720, 1600, 1515, 1345 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.90 (t, J = 7.10 Hz, 3H), 1.30 (m, 2H), 1.61 (m, 2H), 2.00 (m, 8H), 3.60 (t, J = 7.02 Hz, 2H), 5.30 (s, 2H), 7.55 (d, J = 8.52 Hz, 2H), 8.25 (d, J = 8.49 Hz, 2H).

3-(4-Aminobenzyl)-2-butyl-2,3-diazaspiro[4.4]nonane-1,4-dione (56). Tin chloride (10.84 g, 57.18 mmol) was added to a solution of compound **55a** (3.95 g, 11.43 mmol) in EtOH (70 mL) and water (2 mL). The mixture was refluxed for 2 h. The solvent was removed under reduced pressure, water (200 mL) was added, and the resulting suspension was extracted with EtOAc. The combined organic extracts were washed with water and 2 N aqueous NaOH and dried ($MgSO_4$), and the solvent was removed to afford **56** as a yellow powder (2.81 g, 78%): mp 95−96 °C (EtOH); R_f 0.55 (hexane/EtOAc = 4:6); IR (KBr) 3400, 3340, 3220, 1700, 1600 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.90 (t, J = 7.23 Hz, 3H), 1.25 (sextet, J = 7.15 Hz, 2H), 1.49 (quintet, J = 7.15 Hz, 2H), 1.92 (m, 8H), 3.51 (t, J = 7.23 Hz, 2H), 3.72 (brs, 2H), 4.65 (s, 2H), 6.60 (d, J = 8.40 Hz, 2H), 7.00 (d, J = 8.49 Hz, 2H).

2-Butyl-3-[4-(2-carboxybenzamido)benzyl]-2,3-diazaspiro[4.4]nonane-1,4-dione (57). Phthalic anhydride (0.47 g, 3.17 mmol) was added to a solution of compound **56** (1 g, 3.17 mmol) in pyridine (50 mL). The mixture was allowed to stir at room temperature for 1 h and was then poured into cold water (150 mL). The mixture was acidified with 6 N aqueous HCl. The resulting precipitate was collected by filtration, washed with water and purified by crystallization to afford **57** as white crystals (0.95 g, 65%): mp 140−143 °C (acetonitrile); R_f 0.49 (CH_2Cl_2/MeOH = 8:2); IR (KBr) 3430, 3260, 1710, 1670, 1640, 1590 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.84 (t, J = 7.35 Hz, 3H), 1.18 (sextet, J = 7.20 Hz, 2H), 1.42 (quintet, J = 7.20 Hz, 2H), 1.81 (m, 8H), 3.50 (t, J = 6.84 Hz, 2H), 4.76 (s, 2H), 7.23 (d, J = 7.89 Hz, 2H), 7.61 (m, 2H), 7.68 (m, 3H), 7.87 (d, J = 7.35 Hz, 1H), 10.4 (brs, 1H), 13.05 (brs, 1H exchangeable in D_2O); EI-MS [$M^{\cdot+}$] 463. Anal. ($C_{26}H_{29}N_3O_5 \cdot H_2O$) C, H, N.

2-Butyl-3-(4-carbomethoxybenzyl)-2,3-diazaspiro[4.4]-nonane-1,4-dione (58a) and 2-Butyl-4-[4-(carbomethoxybenzyl)oxy]-2,3-diazaspiro[4.4]non-4-en-1-one (58b). Methyl 4-(bromomethyl)benzoate (3.43 g, 15 mmol) was added portionwise to a mixture of compound **15** (2.10 g, 10 mmol) and K_2CO_3 (2.76 g, 20 mmol) in acetone (30 mL). The reaction mixture was allowed to stir at room temperature for 5 h. The solvent was evaporated, water (100 mL) was added, and the resulting suspension was extracted with EtOAc. The combined organic extracts were washed with water and dried ($MgSO_4$), and the solvent was removed to afford the mixture of isomers **58a** and **58b**. These isomers were separated by column chromatography on silica gel (hexanes−EtOAc mixtures of increasing polarity) and purified by crystallization.

58a: 2.5 g, 70%; mp 108−110 °C (EtOH); R_f 0.34 (hexane/EtOAc = 6:4); IR (KBr) 1735, 1710, 1690 cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6) δ 0.80 (t, J = 7.26 Hz, 3H), 1.12 (sextet, J = 7.26 Hz, 2H), 1.33 (quintet, J = 7.26 Hz, 2H), 1.75 (m, 8H), 3.45 (t, J = 6.95 Hz, 2H), 3.82 (s, 3H), 4.90 (s, 2H), 7.40 (d, J = 7.98 Hz, 2H), 7.96 (d, J = 7.98 Hz, 2H).

58b: 0.36 g, 10%; mp 59−63 °C (EtOH); R_f 0.46 (hexane/EtOAc = 6:4); IR (KBr) 1715, 1695, 1600 cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6) δ 0.86 (t, J = 7.35 Hz, 3H), 1.19 (sextet, J = 7.33 Hz, 2H), 1.31 (quintet, J = 7.26 Hz, 2H), 1.75 (m, 8H), 3.48 (t, J = 6.69 Hz, 2H), 3.85 (s, 3H), 5.28 (s, 2H), 7.55 (d, J = 8.13 Hz, 2H), 7.96 (d, J = 8.13 Hz, 2H).

2-Butyl-3-(4-carboxybenzyl)-2,3-diazaspiro[4.4]nonane-1,4-dione (59). HCl (37%) (5 mL, 60 mmol) was added to a solution of compound **58a** (2 g, 5.57 mmol) in acetic acid (100 mL). The mixture was heated under reflux for 10 h. The solvent was then removed under reduced pressure and water (100 mL) was added. The resulting precipitate was collected by filtration, washed with water and Et_2O, and further purified by crystallization to afford **59** as a white solid (1.70 g, 89%): mp 106−109 °C (EtOH/water = 1:1); R_f 0.48 (CH_2Cl_2/MeOH = 9:1); IR (KBr) 3300−2500, 1720, 1700, 1650 cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6) δ 0.80 (t, J = 7.37 Hz, 3H), 1.12 (sextet, J = 7.48 Hz, 2H), 1.25 (quintet, J = 7.50 Hz, 2H), 1.80 (m, 8H), 3.46 (t, J = 6.97 Hz, 2H), 4.87 (s, 2H), 7.40 (d, J = 8.15 Hz, 2H), 7.91 (d, J = 8.15 Hz, 2H), 13.02 (brs, 1H exchangeable with D_2O); EI-MS [$M^{\cdot+}$] 344.

2-Butyl-3-[4-(2-carbethoxyphenylaminocarbonyl)benzyl]-2,3-diazaspiro[4.4]nonane-1,4-dione (60). Thionyl chloride (1.27 mL, 17.4 mmol) was added dropwise to a cold solution (0 °C) of compound **59** (1.5 g, 4.35 mmol) in CH_2Cl_2 (100 mL). The mixture was allowed to stir at room temperature overnight. Evaporation of the solvent and excess of thionyl chloride afforded an oil which was triturated in petroleum ether. The resulting precipitate was collected by filtration and

dried under vacuum. A solution of this acyl chloride in CH_2Cl_2 (5 mL) was added dropwise to a cold (−20 °C) solution of ethyl 2-aminobenzoate (0.72 g, 4.35 mmol) and triethylamine (1.82 mL, 13.06 mmol) in CH_2Cl_2 (10 mL). The reaction mixture was allowed to warm to room temperature and stirring was continued for 2 h. The solvent was evaporated, water was added, and the resulting suspension was extracted with Et_2O. The combined organic extracts were washed with water and 1 N aqueous NaOH and dried ($MgSO_4$), and the solvent was removed to afford **60** as a white powder (1.92 g, 90%): mp 100−103 °C (cyclohexane); R_f 0.44 (hexane/EtOAc = 4:6); IR (KBr) 3270, 1720, 1680 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6) δ 0.81 (t, J = 7.05 Hz, 3H), 1.13 (sextet, J = 7.13 Hz, 2H), 1.30 (t, J = 7.20 Hz, 3H), 1.36 (quintet, J = 7.13 Hz, 2H), 1.82 (m, 8H), 3.51 (t, J = 6.57 Hz, 2H), 4.35 (q, J = 7.13 Hz, 2H), 4.91 (s, 2H), 7.25 (t, J = 7.50 Hz, 1H), 7.50 (d, J = 7.53 Hz, 2H), 7.68 (t, J = 7.53 Hz, 1H), 7.98 (d, J = 7.40 Hz, 2H), 8.02 (d, J = 7.40 Hz, 1H), 8.50 (d, J = 7.40 Hz, 1H), 11.54 (brs, 1H exchangeable in D_2O); EI-MS [$M^{\cdot+}$] 491.

2-Butyl-3-[4-(3-carbethoxyphenylaminocarbonyl)benzyl]-2,3-diazaspiro[4.4]nonane-1,4-dione (61). Obtained by the same procedure described for **60** (1.66 g, 78%): mp 141−142 °C (EtOAc); R_f 0.57 (hexane/EtOAc = 4:6); IR (KBr) 3350, 1710, 1670 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6) δ 0.81 (t, J = 7.33 Hz, 3H), 1.18 (sextet, J = 7.28 Hz, 2H), 1.31 (t, J = 7.13 Hz, 3H), 1.40 (quintet, J = 7.28 Hz, 2H), 1.80 (m, 8H), 3.50 (t, J = 7.12 Hz, 2H), 4.31 (q, J = 7.13 Hz, 2H), 4.90 (s, 2H), 7.42 (d, J = 8.14 Hz, 2H), 7.50 (t, J = 7.98 Hz, 1H), 7.70 (t, J = 7.87 Hz, 1H), 7.95 (d, J = 8.14 Hz, 2H), 8.05 (d, J = 8.14 Hz, 1H), 8.40 (s, 1H), 10.48 (brs, 1H); EI-MS [$M^{\cdot+}$] 491.

2-Butyl-3-[4-(2-carboxyphenylaminocarbonyl)benzyl]-2,3-diazaspiro[4.4]nonane-1,4-dione (62). NaOH (0.084 g, 2.11 mmol) was added to a solution of compound **60** (0.52 g, 1.05 mmol) in a mixture (15 mL) of EtOH/water (1:1). The mixture was allowed to stir for 10 h at room temperature. The solvent was evaporated, and water (100 mL) was added. The aqueous solution was washed with Et_2O and acidified with 3 N aqueous HCl. The resulting precipitate was collected by filtration, washed with water and Et_2O, and further purified by crystallization to afford **62** as a white solid (0.107 g, 22%): mp 202−203 °C (acetone); R_f 0.32 (CH_2Cl_2/MeOH = 9:1); IR (KBr) 3320, 3280−2500, 1720, 1675, 1660 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6) δ 0.81 (t, J = 7.40 Hz, 3H), 1.12 (sextet, J = 7.38 Hz, 2H), 1.35 (quintet, J = 7.38 Hz, 2H), 1.80 (m, 8H), 3.50 (t, J = 6.42 Hz, 2H), 4.90 (s, 2H), 7.20 (t, J = 7.38 Hz, 1H), 7.51 (d, J = 8.37 Hz, 2H), 7.65 (t, J = 8.30 Hz, 1H), 8.00 (d, J = 8.33 Hz, 2H), 8.10 (d, J = 8.25 Hz, 1H), 8.70 (d, J = 8.35 Hz, 1H), 12.00 (brs, 1H exchangeable with D_2O), 12.30 (brs, 1H exchangeable in D_2O); EI-MS [$M^{\cdot+}$] 463. Anal. ($C_{26}H_{29}N_3O_5$) C, H, N.

2-Butyl-3-[4-(3-carboxyphenylaminocarbonyl)benzyl]-2,3-diazaspiro[4.4]nonane-1,4-dione (63). Obtained by the same procedure described for **62** (0.194 g, 40%): mp 157−158 °C (EtOH); R_f 0.48 (EtOAc); IR (KBr) 3320, 3280−2500, 1720, 1670, 1650 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6) δ 0.90 (t, J = 7.06 Hz, 3H), 1.20 (sextet, J = 7.22 Hz, 2H), 1.40 (quintet, J = 7.22 Hz, 2H), 1.80 (m, 8H), 3.48 (t, J = 6.84 Hz, 2H), 4.89 (s, 2H), 7.40 (m, 3H), 7.67 (d, J = 7.51 Hz, 1H), 7.95 (d, J = 7.75 Hz, 2H), 8.01 (d, J = 7.89 Hz, 1H), 8.40 (s, 1H), 10.42 (brs, 1H), 13.00 (brs, 1H exchangeable in D_2O); EI-MS [$M^{\cdot+}$] 463. Anal. ($C_{26}H_{29}N_3O_5$) C, H, N.

B. Biological Methods. Membrane Preparation. Crude membranes from PLC-PRF (human hepatoma cell line) were prepared according to the protocol described by Maeda and co-workers.[28] The membranes are not highly purified but the authors showed that the results obtained by this method are similar to those reported with purified membrane preparations. Briefly, cells were lysed in ice-cold buffer (Tris/HCl (50 mM), $MgCl_2$ (2 mM), pH 7.4) with a Polytron (1100 rpm, maximal setting 10 s). The homogenate was centrifuged at 30000*g* for 15 min at 4 °C and the supernatant discarded. The pellet was resuspended in Tris/HCl (20 mM), sucrose (250 mM), pH 7.4, at a membrane concentration of 2 mM and stored at −70 °C until required. Crude membranes from calf cerebellum were obtained with the same protocol after dissection on dental wax over ice. Tissue was homogenized in 40 volumes of ice-cold Tris/HCl buffer as described above using a Polytron (setting = 5, 20 s) before the centrifugation step.

AT_1 Receptor Binding Assay (PLC-PRF Cells). Aliquots containing 100 μg of proteins were incubated at 25 °C for 1 h in the same Tris/HCl buffer used for membrane preparation. Incubation was initiated by the addition of 3 nM [^{3}H]AII in a total incubation volume of 500 μL. Nonspecific binding was measured by incubation in the presence of 1 μM losartan, a selective AT_1 ligand. Test compounds were studied in the range of concentrations 10^{-10}−10^{-5} M. Binding was terminated by rapid vaccum filtration onto glass fiber filter (GF/C Whatman preincubated in poly(ethylenimine) 0.1%, 2 h). Filters were washed four times with 1 mL of the ice-cold corresponding buffer. Dry filters were placed into vials containing 2 mL of scintillation fluid and the radioactivity was counted in a scintillation counter (model 1609, Wallac). The K_i values (concentration for 50% displacement of the specifically bound [^{3}H]AII) was calculated using the Cheng and Prussof equation introduced in a graphic software (Kaleidagraph for Macintosh). Interassay K_i values for a given test compound may vary by <15%.

AT_2 Receptor Binding Assay (Calf Cerebellum Cortex). The general protocol described above was modified for AT_2. A preincubation of membranes was performed without [^{3}H]AII in a sodium phosphate (100 mM) buffer, pH 7.0 at 37 °C for 30 min in the presence of DTT (1 mM), PMSF (0.1 mM), EDTA (5 mM), and BSA (0.5%) to abolish residual AT_1 receptor binding. Membranes were pelleted and resuspended in the same assay mixture. Membranes (300 μg) were incubated with [^{3}H]AII (5 nM) at 25 °C for 60 min. Filtrations, washing and calculation procedures were performed as described above. Nonspecific binding was measured by incubation in the presence of PD 123177 (1 μM), a selective AT_2 ligand.

Measurement of Intracellular Ca^{2+} Concentration. Cellular suspension (10 millions cells/mL) was incubated with the cell permeant fluorescent probe Fura 2-AM (5 mM) in Krebs-Ringer buffer (pH 7.4) consisting of NaCl (132 mM), KCl (4 mM), $CaCl_2$ (1 mM), $MgCl_2$ (0.5 mM), glucose (5 mM), HEPES (9.5 mM) at 37 °C for 30 min. Loaded cells were washed free of extracellular dye and centrifuged at 700 rpm during 10 min. After three washes, the cells were resuspended at 1 million/mL in a thermostated quartz cuvette (3 mL). Cells were maintained in suspension by a magnetic stirrer. A spectrofluorimeter equipped for dual excitation and mono emission wavelength (Fluorolog, Jobin-Yvon-SPEX) was used to record the ratio of fluorescence emission intensity at 510 nm resulting for alternative excitation of cells at 340 and 380 nm. The recorded ratios (6/s) were then automatically converted to cytosolic Ca^{2+} concentration using the established equation described by Grynkiewicz and collaborators.[29]

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