Communications to the Editor

A Highly Potent, Orally Active Imidazo[4,5-*b*]pyridine Biphenylacylsulfonamide (MK-996; L-159,282): A New AT_1-Selective Angiotensin II Receptor Antagonist

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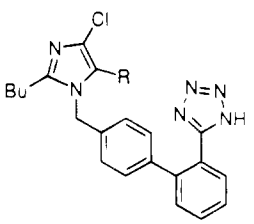
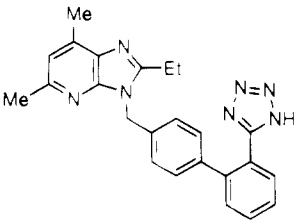
The renin–angiotensin system (RAS) is a major physiological mechanism that regulates the blood pressure and fluid/electrolyte balance in normal and pathophysiological conditions.[1] The clinical success of angiotensin-converting enzyme (ACE) inhibitors[2] in the treatment of hypertension and congestive heart failure has made the RAS a major target for drug discovery programs in the pharmaceutical industry.[3] Despite their wide clinical use, the ACE inhibitors suffer from occasional side effects, such as dry cough and angioneurotic edema.[4] These side effects are thought to be due to elevation of bradykinin and substance P levels, caused by inhibition of the degradation of these peptides.[5] Alternatively, the most direct mode of intervening in the RAS, possibly with minimal potential side effects, is to inhibit specifically interactions of the primary effector hormone angiotensin II (AII) at the receptor level.[6] AII receptors are present on the membranes of target tissues and organs, and two major subtypes of the receptors,[7] designated AT_1 and AT_2, have been identified in a variety of animal and human tissues.[8] At the present time, the major physiological functions of AII appear to be associated with the G-protein-coupled AT_1 receptors,[9] and antagonists directed toward this receptor subtype offer a promising approach to novel antihypertensive agents.[10] The functional role of the AT_2 receptor has yet to be clearly demonstrated.[11]

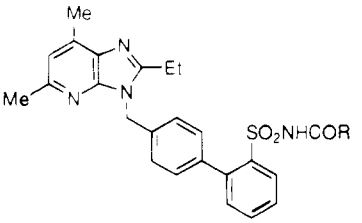
The discovery of AT_1-selective AII antagonist losartan (DuP 753; MK 954; **1a**)[12] and its high-affinity metabolite EXP3174 (**1b**)[13] has generated significant interest in the search for other nonpeptide AII antagonists bearing novel heterocyclic elements.[14] The tetrazole group is a common acidic function present in many of these antagonists, including the potent imidazopyridine antagonist L-158,809 (**2**)[15] reported from our laboratories. Despite its prolonged *in vivo* duration of action in rats, L-158,809 displayed a shorter duration of action in rhesus monkeys[15c] and dogs[16] after intravenous administration, a result which may be attributed to its rapid metabolism and clearance via glucuronidation of the tetrazole moiety in these species.[17] We envisaged that replacing the tetrazole moiety of L-158,809 with an acidic function resistant to glucuronidation might improve its pharmacological properties. Toward this goal, several acidic groups, including acidic heterocyles, were considered.[18] On the basis of the potential isosteric relationships and pK_a considerations, we selected the acylsulfonamide (SO_2NHCOR) group as a potential replacement for the tetrazole moiety.[21] Recently, we have shown that incorporation of acylsulfonamides as tetrazole equivalents led to several series of potent AII antagonists,[23] and several of these antagonists exhibited favorable pharmacological properties compared to their tetrazole counterparts.[23b,c] In this communication, we report the identification and pharmacological characterization of MK-996 (**3e**, L-159,282), a highly potent, AT_1-selective, and orally active imidazo[4,5-*b*]pyridine biphenylacylsulfonamide antagonist, which is currently in phase II clinical trials for the treatment of hypertension.[24]



1a (R = CH_2OH)
(Losartan, DuP 753, MK 954)
1b (R = COOH)
(EXP3174)



2
(L-158,809)



3
3e (R= Ph)
(L-159,282, MK-996)

The synthesis of imidazo[4,5-*b*]pyridine biphenylacylsulfonamides **3a–3e** is illustrated in Scheme 1. Alkylation of the sodium salt of 5,7-dimethyl-2-ethylimidazo[4,5-*b*]pyridine (**4**)[15a] with 4′-(bromomethyl)-1,1′-biphenyl-2-(*N*-*tert*-butyl)sulfonamide (**5**)[23a] gave after purification the desired N^3-alkylated product **6**,[25] which upon treatment with anhydrous trifluoroacetic acid (TFA) at ambient temperature afforded the sulfonamide **7**. Reaction of the sulfonamide **7** with acylimidazoles, obtained by treating carboxylic acids with 1,1′-carbonyldiimidazole (CDI), in the presence of 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU), provided the desired acylsulfonamides **3a–3e** (Table 1) in good yields.[26] Alternatively, acylation of **7** with acid chlorides in pyridine afforded the acylsulfonamides in fair yields.

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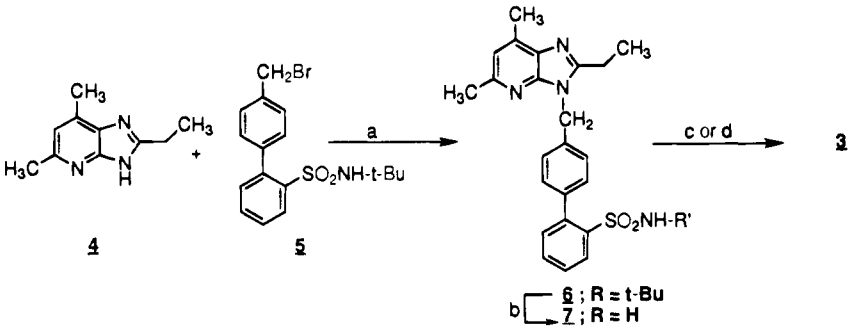
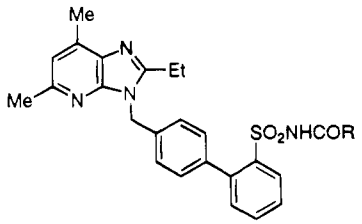
Scheme 1



[a] Reagents: (a) NaH, DMF, 50 °C, 3 h (76%); (b) TFA, 25 °C, 8 h (98%); (c) RCOOH, 1,1′-carbonyldiimidazole, THF, reflux; DBU, THF, 50 °C, 12 h (70–90%); (d) RCOCl, pyridine, 25 °C, 12 h (40–50%).

Table 1. Imidazo[4,5-*b*]pyridine Biphenylacylsulfonamides



		IC50 (nM)		conscious rats		
compd	R	AT_1[a]	AT_2[b]	dose (mg/kg)[c]	% peak inhibn of AII pressor response[d]	duration of action (h)
3a	Me	2.2	>10000	1.0, iv	100 ± 0	3.5
3b	*n*-Pr	0.27	3600	1.0, iv	99 ± 1	>6
3c	*i*-Pr	0.21	1800	0.1, iv	100 ± 0	5
3d	c-Pr	0.68	2400	0.1, iv	95 ± 5	>6
				0.3, po	99 ± 5	>6
3e (MK-996)	Ph	0.20	2900	0.03, iv	78 ± 5	6
				0.1, iv	94 ± 2	6
				1.0, po	99 ± 1	>6
				0.1, po	64 ± 4	>5
2	L-158,809[e]	0.3	50000	0.1, iv	85 ± 5	>6
				0.1, po	75 ± 6	>6
1a	Losartan[e]	50	50000			
1b	EXP3174[e]	6	50000			

[a] Rabbit aorta. [b] Rat midbrain. [c] iv = intravenously administered; po = orally administered. [d] The protocol used is described in ref 15c. [e] The data from ref 15.

The *in vitro* receptor binding affinities (IC_{50} values)[27a] were determined using rabbit aorta (AT_1)[15b] and rat midbrain (AT_2)[27b] receptor preparations. Table 1 indicates that imidazo[4,5-*b*]pyridine biphenylacylsulfonamides **3a–3e** are AT_1-selective antagonists with potency similar to the corresponding tetrazole compound L-158,809, although their AT_2 receptor binding affinities are increased somewhat.[28] From the binding data it is evident that both alkanoyl- and benzoylsulfonamide groups are well-accommodated by the AT_1 receptor.[29] The benzoylsulfonamide **3e** (MK-996) exhibited excellent AT_1 receptor binding affinity (IC_{50} = 0.2 nM)[30] and selectivity (Table 1). Scatchard analysis of specific binding of [^{125}I]Sar^1,Ile^8-AII to rabbit aorta (AT_1) in the presence and absence of the antagonist indicated competitive and reversible binding of MK-996 to the AT_1 receptor with an estimated K_i of 0.48 nM. In functional studies, the antagonist (5 nM) blocked AII-stimulated aldosterone release in rat adrenal cortical cells, shifted the AII concentration–response curve to the right without altering the maximal contractile response, and exhibited no agonist activity. The estimated pA_2 value of 10.3 confirmed that MK-996 is a high affinity antagonist, and is approximately 250-fold more potent than losartan (pA_2 = 7.9).[31] MK-996 exhibited high specificity for the AT_1 receptor versus other G-protein-coupled receptors.[32]

Table 2. *In Vivo* Data of MK-996

rhesus monkeys ($n \geq 2$)[a]			conscious dogs ($n \geq 2$)[b]		
dose (mg/kg)	% inhibn of AII response	duration (h)	dose (mg/kg)	% inhibn of AII response	duration (h)
0.03, iv	44 ± 9	>3			
0.1, iv	85 ± 2	>6	0.1, iv	80 ± 1	4
0.1, po	47 ± 5	~3	0.3, po	95 ± 5	>6
0.3, po	71 ± 2	>5	1.0, po	95 ± 5	>24

[a] Sodium/volume-depleted animals; for protocol see ref 15c. [b] Normotensive mongrel dogs.[35]

The *in vivo* activity of imidazopyridine acylsulfonamides **3a–3e** was evaluated by assessing the inhibition of the pressor responses to exogenously administered AII in conscious normotensive rats.[33] Several of these antagonists, including MK-996, displayed good duration of action (>5 h) following intravenous (iv) or oral (po) administration (Table 1). The benzoylsulfonamide MK-996 was selected for further in-depth evaluation in several animal species.[34] This antagonist blocked AII-induced pressor responses in conscious normotensive rats, sodium/volume-depleted rhesus monkeys[15c] and conscious normotensive dogs[35] in a dose-dependent manner following iv or po administration (Tables 1 and 2), without changing basal blood pressure, heart rate or the pressor response to methoxamine (rat and rhesus monkeys) or norepinephrine (dog). Both iv and po potencies (ED_{50}) of the antagonist in rats and monkeys were similar to those of L-158,809 but significantly greater than losartan (Table 3).[36] The po/iv ED_{50} ratios suggest good oral bioavailability for MK-996 in rats, rhesus monkeys, and dogs.[37] Figure 1 illustrates a comparison of the inhibition of the pressor responses to AII in rats, rhesus monkeys, and dogs after iv administration of MK-996. At a dose which produced approximately 80% inhibition (peak response) in each species, MK-996 exhibited similar duration in the three species. MK-996 (1.0 mg/kg, iv) blocked the AII-induced pressor response (100% peak response) in anesthetized chimpanzees[38] (Figure 2). In comparison to L-158,809 (1.0 mg/kg), MK-996 exhibited a duration of action exceeding 24 h, with approximately 69% and 52% inhibition of the AII pressor response at 10 and 24 h, respectively (Figure 2). Similar prolonged duration of action (>24 h) was also observed for MK-996 in conscious dogs after oral administration (1.0 mg/kg) (Table 2). The pharmacological data presented here demon-

Table 3. A Comparison of *In Vivo* Potencies

	rat			rhesus monkeys			dogs	
	MK-996	L-158,809	Losartan	MK-996	L-158,809	Losartan	MK-996	L-158,809
iv	0.014	0.029	0.28	0.036	0.011	0.32	0.017	<0.3[a]
po	0.067	0.023	0.66	0.10	0.10	10.32	0.035	ND[b]
po/iv	5	0.8	2.4	3	9	32	2	ND[b]

[a] 73% (peak) inhibition of AII pressor response. [b] Not determined.

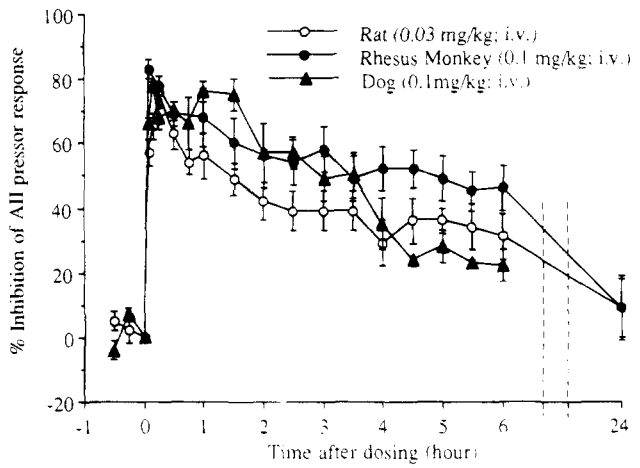


Figure 1. A comparison of the duration of action of MK-996 in conscious rats (n = 8), rhesus monkeys (n = 4), and normotensive dogs (n = 3). The pressor responses to AII was measured in each animal after receiving a single iv dose of MK-996. Data are expressed as mean ± SEM.

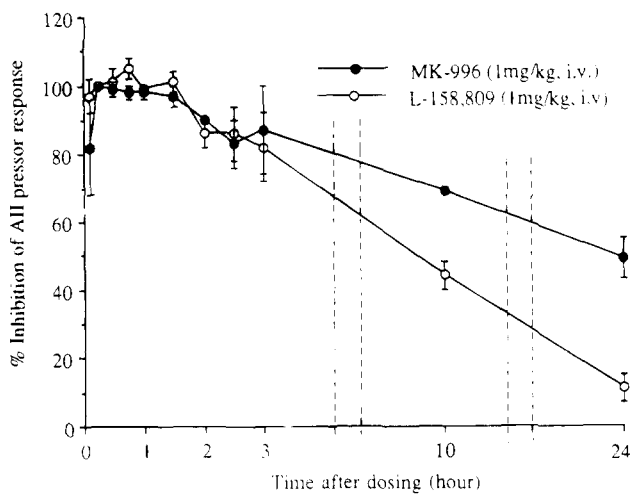


Figure 2. Inhibition of AII-induced pressor responses following iv administration of MK-996 or L-158,809 to anesthetized chimpanzees (**n** = 2). Data are expressed as mean ± SEM.

strate that, unlike L-158,809 and losartan, MK-996 possesses little interspecies variability in its duration of action. This lack of species variability displayed by MK-996 may partly be attributed to its structural novelty and the absence of glucuronidation[39] of the acylsulfonamide moiety. Furthermore, there is no evidence that MK-996 forms an active metabolite *in vivo*.[37]

The antihypertensive activity of MK-996 was evaluated in 7-day aortic coarcted conscious rats.[15c] MK-996 (3.0 mg/kg, po) reduced the mean arterial blood pressure to the normotensive levels with duration of action exceeding 6 h and was similar to the responses and duration exhibited by enalapril (3.0 mg/kg, po) in these animals (Figure 3).[40]

In summary, the imidazo[4,5-*b*]pyridine biphenyl-

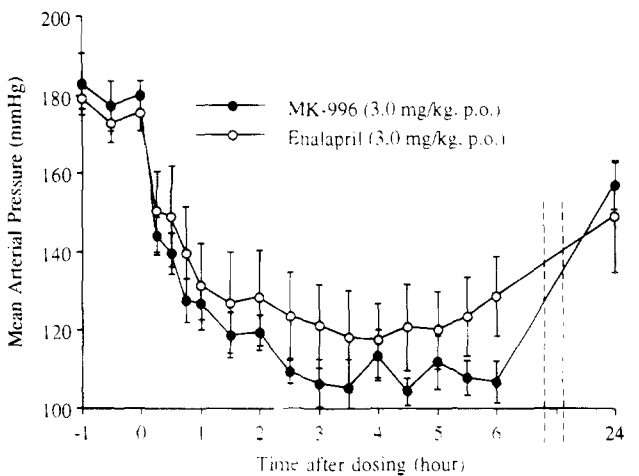


Figure 3. A comparison of antihypertensive effects of MK-996 and enalapril in aortic coarcted rats(n = 5). Data are expressed as mean ± SEM.

acylsulfonamide, MK-996, represents a potent new class of non-tetrazolyl angiotensin II receptor antagonists. MK-996 is a highly potent and orally active AT_1 selective antagonist with excellent *in vivo* potency and duration of action in rats, rhesus monkeys, dogs, and chimpanzees. The pharmacological profile of MK-996 demonstrates lack of species variability in its duration of action. *In vivo*, MK-996 is significantly more potent than losartan and does not appear to form any active metabolites. The structural novelty and these excellent pharmacological properties of MK-996 make it a valuable tool for investigating the physiological roles of AII and also a promising new agent for antihypertensive therapy.

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(16) The observed duration of blocking the pressor response (80% peak inhibition) of exogenous AII in nomotensive dogs[35] was approximately 1.5 h at a dose of 1.0 mg/kg, iv.

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(18) In general, the tetrazole compounds are significantly more potent AII antagonists compared to their carboxylic acid counterparts. Replacing the tetrazole in L-158,809 with triflamide ($NHSO_2CF_3$) and several acidic heterocycles including oxathiadiazole *S*-oxide[19] led to analogs with shorter duration of action in rats. Recently, AII antagonists incorporating squaric acid,[20a] triflamide,[20b] and oxadiazolones and related heterocyles[20c] have been reported.

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(21) (a) The application of acylsulfonamides as carboxylic acid equivalents is well-documented in the design of a number of therapeutic targets.[22] The acylsulfonamide (SO_2NHCOR) group can be viewed as an isostere of tetrazole.[23b] The pK_a value of the antibacterial agent sulfabenzamide (4.6) is similar to that of 5-aryltetrazoles (5–6). (b) The acylsulfonamides offer possibilities for a broad range of structural variations that could be useful in adjusting physicochemical properties, or for accessing potential receptor binding sites unavailable to the tetrazole. (c) Replacement of the tetrazole moiety of L-158,809 with the isomeric N-sulfonylcarboxamide ($CONHSO_2R$)[22b] group resulted in analogs with reduced *in vitro* and *in vivo* potencies,[24] although this group was also found to be resistant toward glucuronidation. These results will be the subject of a future publication.

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(25) Three regioisomers, N(3), N(1), and N(4), were obtained in a ratio of approximately 400:1:4 during the alkylation of **4**. The desired isomer **6** was purified by either crystallization or flash column (silica gel) chromatography. The regiochemistry of the products was assigned by examination of the nuclear Overhauser effects between the benzylic protons, the methylene protons of the ethyl group, and the two methyl group protons. The X-ray crystal stucture of the benzoylsulfonamide **3e** further confirmed the structural assignment of **6**.

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(27) (a) The standard error (expressed as percent of mean values) of IC_{50} values is estimated to be less than 30%. The reported IC_{50}s represent an avarage of two or more determinations. (b) Chang, R. S. L.; Lotti, V. J.; Chen, T. B.; Faust, K. A. Two Angiotensin II Binding Sites in Rat Brain Revealed Using [^{125}I]Sar^1,Ile^8-Angiotensin II and Selective Nonpeptide Antagonists. *Biochem. Biophys. Res. Commun.* **1990**, *171*, 813–817.

(28) In general, the acylsulfonamide group increased AT_2 receptor binding affinities of imidazopyridine-based AII antagonists.[24] The AT_2 potency enhancing properties of acylsulfonamide groups have also been demonstrated in several other series of AII antagonists.[23]

(29) Acyl groups bearing substituted alkyls and aryl functions are well-tolerated. A full account of the structure-activity relationships developed in this series will be the subject of a future publication.

(30) (a) The following tissues were used as sources for AII receptors: rabbit aorta (AT_1), heart (AT_1 and AT_2), kidney (AT_1), adrenal (AT_1), and brain (AT_1); rat adrenal (AT_1 and AT_2), rat brain (AT_2), and human adrenal (AT_1 and AT_2). The AT_1 potencies (IC_{50}) for MK-996 ranged from 0.11 to 0.43 nM in these receptor binding assays. (b) In various AT_1 receptor binding assays, MK-996 (K_i = 0.11–0.43 nM) was equipotent to L-158,809, and 80–200 times and 4–14 times more potent than losartan (K_i = 15–49 nM) and its metabolite EXP3174 (K_i = 0.76–6.2 nM), respectively.[31]

(31) Chang, R. S. L.; Bendensky, R. J.; Chen, T.-B.; Faust, K. A.; Kling, P. J.; O'Malley, S. A.; Naylor, E. M.; Chakravarty, P. K.; Patchett, A. A.; Greenlee, W. J.; Clineschmidt, B. V.; Lotti, V. J. In Vitro Pharmacology of MK-996, A New Potent and Selective Angiotensin II (AT_1) Receptor Antagonist. *Drug Dev. Res* **1994**, *32*, 161–171.

(32) MK-996 was inactive (IC_{50} >1 μM) in a number of receptor binding assays, including oxytocin, vasopressin, bradykinin, neurotensin, endothelin, and α-adrenergic receptors.

(33) The potassium salts of **3a–3e** were used for intravenous (iv) and oral (po) studies using the literature protocols.[15c]

(34) The selection of **3e** was made based on the further comparison of sulfonamides **3d** and **3e** in rhesus monkeys and dogs for their *in vivo* potency and duration of action.

(35) Female mongrel dogs, weighing 9.7–15 kg, were surgically implanted with chronic arterial catheters with access ports (Access Technologies) at least 2 weeks before the experiment. The access ports were flushed twice per week with 0.9% saline (20 mL) and filled with 2 mL of heparin (1000 units/mL). The dogs were maintained at on 60 mequiv of sodium per day with water *ad libitum*. Dogs were denied food 18 h prior to and during the experiment. On the day of the experiment, the dogs were placed on slings (Alice King Chatham), and routine aseptic procedures were employed. Sterile Huber point needles (22 gauge, without hubs) attached to Tygon tubing (24 in.) were inserted into ports. The arterial tubing was attached to a Statham blood pressure transducer (Spectramed) for the continuous monitoring of mean arterial pressure (MAP) and heart rate using the Buxco-IBM system. Two sterile catheters were inserted in the saphenous or brachial vein for the administration of AII and the antagonist. The dogs were initially challenged with norepinephrine (1.6 μg/kg, iv) to check the patency of the catheter. AII was then loaded into the catheter until a response was observed. Bolus intravenous injections of AII (0.1μg/kg, iv) were given at −45, −30 and −15 minutes. When the responses to AII were consistent, the antagonist or its vehicle was administered either intravenously, or orally by gavage at 0 min. AII was then given at 5, 10, 15 (iv) or 10, 20 (oral), 30, 45, and 60 min, and every 30 min thereafter for 6 h. The 24 h time point was also measured when deemed necessary.

(36) The iv potency of MK-996 is 8–20-fold higher than losartan in conscious rats and rhesus monkeys. MK-996 is approximately 10-fold and 100-fold more potent orally than losartan in rats and rhesus monkeys, respectively.

(37) Kivlighn, S. D.; Zingaro, G. J.; Gabel, R. A.; Broten, T. P.; Schorn, T. W.; Schaffer, L. W.; Naylor, E. M.; Chakravarty, P. K.; Patchett, A. A.; Greenlee, W. J.; Siegl, P. K. S. In Vivo Pharmacology of a Novel AT1 selective Angiotensin II Receptor Antagonist: MK-996. *Am. J. Hyptens.* In press.

(38) Two adult male chimpanzees (~60 kg), located at the New Iberia Research Facility, New Iberia Parish, LA, were used in this study. Each animal was fasted 12 h prior to the study, sedated with ketamine and acepromazine, and maintained on gas anesthesia (with 1.5% isoflurane) during the study. All procedures were conducted using sterile techniques. An intraarterial and three iv catheters were placed for direct arterial blood pressure measuremant and for administration of AII, AVP and MK-996, respectively. Mean arterial blood pressure was measured using Statham Gould pressure transducer and displayed on a Hewlett-Packard recorder. After establishing reproducible baseline pressor responses to AVP (50 ng/kg, iv) and AII (0.1 μg/kg, iv), MK-996 (1.0 mg/kg) was injected iv bolus. Subsequent challenges with AII and AVP were given at 5, 15, 30, 45, and 60 min and every 30 min there after for 3 h. At the end of this period, animals were returned to cages for recovery and reanesthetized, as described above, for collecting data at 10 and 24 h points.

(39) Incubation of MK-996 and its analogs with liver slices from rats, monkeys, and human have demonstrated that the acylsulfonamide group in these antagonists is resistant to glucuronidation. Extensive glucuronidation of the tetrazole moiety in L-158,809 has been observed following similar treatment with liver slices. (Stearns, R. A. unpublished results).

(40) The heart rate in these animals remained unaltered during the course of these experiments.