were assayed for radioactivity in a Micromedic gamma counter. Nonspecific binding was defined as binding in the presence of 10^{-6} M ANP-(103–126).[25] Specific binding was calculated as total binding minus nonspecific binding. Binding data was analyzed by using a nonlinear curve fitting program.[27]

Acknowledgment. We acknowledge R. I. Hecht, M. G. Jennings, and J. F. Zobel for sequencing and amino acid analysis and P. Toren and E. W. Kolodziej for mass spectroscopy. The technical assistance of D. Whipple and M. Zupec was greatly appreciated as were the useful discussions with F. S. Tjoeng. We also thank E. H. Blaine and R. E. Manning for support during this work.

Synthesis and D_2 Dopaminergic Activity of Pyrrolidinium, Tetrahydrothiophenium, and Tetrahydrothiophene Analogues of Sulpiride

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All of the existing dopamine receptor models recognize the amine nitrogen of agonist and antagonist drugs as playing a crucial role in receptor interactions. However, there has been some controversy as to which molecular form of the amine, charged or uncharged, is most important in these interactions. We have synthesized and examined the biological activity of permanently charged and permanently uncharged analogues of the dopaminergic antagonist, sulpiride. Sulpiride and the permanently charged pyrrolidinium (**6, 7**) and tetrahydrothiophenium (**9**) analogues were able to antagonize the inhibitory effect of apomorphine on the K^+-induced release of [^{3}H]acetylcholine from striatal slices. In contrast, the permanently uncharged tetrahydrothiophene analogue **8** was inactive at concentrations up to 100 μM. Additionally, both sulpiride and the tetrahydrothiophenium analogue were able to displace [^{3}H]spiperone from D_2 binding sites, while the tetrahydrothiophene analogue was unable to produce any significant displacement. These results are consistent with our previous observations on permanently charged chlorpromazine analogues and provide further evidence that dopaminergic antagonists bind in their charged molecular forms to anionic sites on the D_2 receptor.

Dopamine antagonists are an important class of therapeutic agents and are useful in a variety of pathological conditions. The design of new dopamine antagonists with improved safety and efficacy has been aided in the past few years by the development of several very useful dopamine receptor models.[1-7] To a certain extent, these models complement one another; however, several discrepancies do exist. One of the major discrepancies that needs to be resolved concerns the protonation state of the amine nitrogen during its interaction with the dopamine receptor.

A common feature of all dopamine antagonists is a basic nitrogen atom. At physiological pH, an equilibrium between a charged ammonium ion and an uncharged amine exists for these compounds. The amount of each species that is present depends upon the pK_a of the amine. Since the majority of dopamine antagonists have pK_a values between 8 and 9 and are thus substantially protonated at physiological pH, several investigators have suggested that the active form of dopamine antagonists is the charged, protonated form.[1,2,8] Based upon a reported pK_a of 5.9 for butaclamol, Philipp et al.[6] proposed that the uncharged amine is the important species and binds to the dopamine receptor via hydrogen bond formation. Recently, Chrzanowski et al.[8] independently determined the pK_a of butaclamol and found it to be in the range of 7.0–7.5. They concluded that since this pK_a allows for approximately 50% protonation at physiological pH, both charged and uncharged species need to be considered when describing possible ligand–receptor interactions. Obviously, since both charged and uncharged amine species can exist in solution at physiological pH, it is difficult to establish which of these two molecular species is actually responsible for dopamine antagonist activity.[9]

The question of which molecular species, charged or uncharged, is most important for the binding of dopamine agonists to the dopamine receptor has recently been addressed.[10,11] Studies with permanently charged and permanently uncharged sulfur and selenium analogues of dopamine suggested that the charged, protonated form of dopamine is the molecular species required for maximal activation of the dopamine D_2 receptor. In order to determine if dopamine antagonists also require a charged species for maximal activity, our group originally investigated a series of permanently charged and uncharged analogues of chlorpromazine (**1**).[12] Permanently charged ammonium (**2**) and sulfonium (**3**) analogues of chlorpromazine retained the ability to both bind and act as

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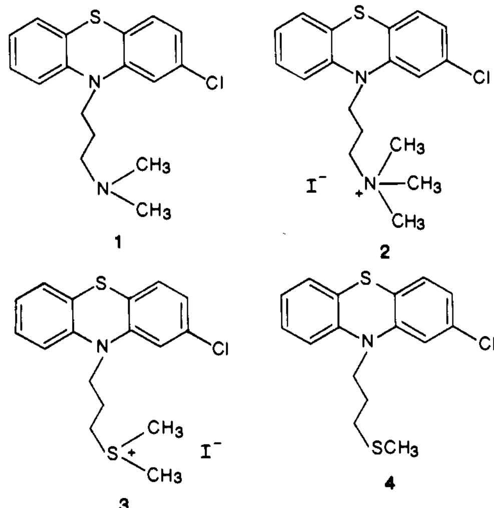
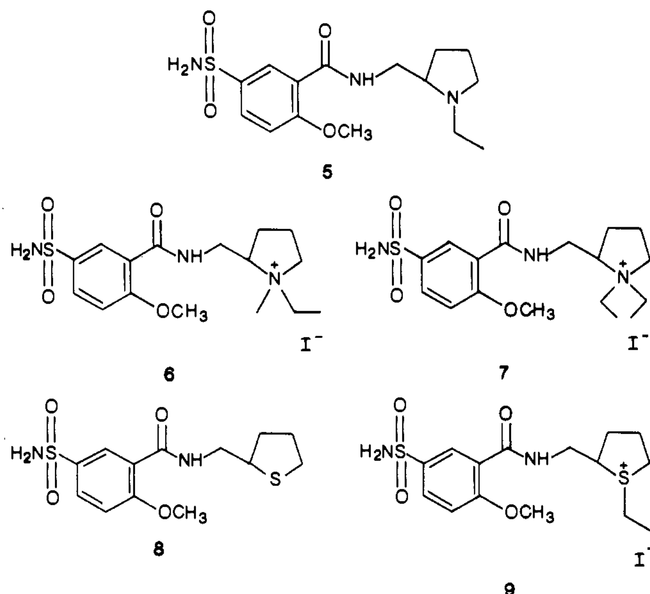
antagonists at the dopamine D_2 receptor. Unfortunately, the pharmacological activity of the permanently uncharged methyl sulfide analogue (**4**) could not be evaluated due to its lack of aqueous solubility. Thus, in contrast to the work with dopamine agonists, our chlorpromazine studies did not allow us to compare the relative importance of the charged and uncharged molecular species for dopamine antagonists.



In order to overcome the solubility problems encountered with the chlorpromazine analogues, we have extended our previously employed approach[10-12] to a series of sulpiride analogues. Sulpiride (**5**) is a D_2 selective dopamine antagonist with high aqueous solubility.[13] The sulfamoyl portion of the molecule permits adequate aqueous solubility even in the absence of the basic nitrogen atom. We now report the synthesis and biological activity of permanently charged pyrrolidinium (**6**, **7**) and tetrahydrothiophenium (**9**) salts of sulpiride as well as a permanently uncharged tetrahydrothiophene analogue (**8**).

Chemistry

The treatment of sulpiride with either iodomethane or iodoethane in refluxing acetonitrile provided pyrrolidinium iodides **6** and **7**, respectively. Compound **6** was actually



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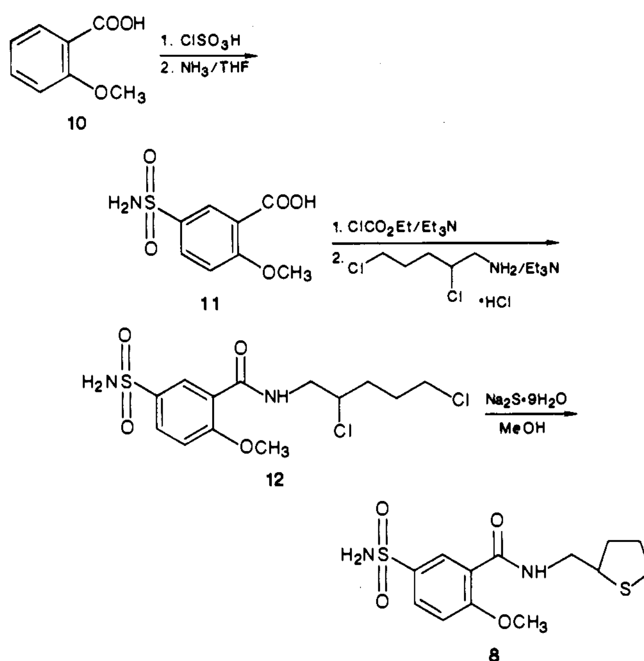
Scheme I



a mixture of diastereomers in a ratio of 4:1. Since numerous attempts using chromatography and recrystallization for separation of the diastereomers were unsuccessful, this compound was tested as a diastereomeric mixture.

The synthesis of the tetrahydrothiophene analogue of sulpiride was accomplished by three related, but independent, routes. Initially *o*-anisic acid (**10**) was converted to 2-methoxy-5-sulfamoylbenzoic acid (**11**) by sequential chlorosulfonation[14] and ammonium displacement (Scheme I). This acid was then activated with ethyl chloroformate,[15] and the resulting adduct was reacted in situ with 2,5-dichloroamylamine hydrochloride and triethylamine to afford benzamide **12**. Treatment of this dichloro benzamide with a 5:1 molar excess of sodium sulfide nonahydrate[16] resulted in the formation of tetrahydrothiophene **8** in a 74% yield. This ultimately proved to be the most efficient way to synthesize **8**, although some initial difficulty was encountered in finding the proper conditions for the cyclization reaction. The overall yield for this route, calculated from *o*∞anisic acid, was 48%. However, since the initial cyclization attempts were not encouraging, two other routes to tetrahydrothiophene **8** were simultaneously investigated.

It was felt that the initial failure of **12** to react with sodium sulfide might be due to the sulfamoyl group. The protons on the nitrogen atom are acidic and may react with the sulfide ion, leading to undesirable results. Therefore, an alternate approach to **8** sought to prepare the tetrahydrothiophene ring prior to introduction of the sulfamoyl group. This approach is shown in Scheme II and also uses *o*-anisic acid as the starting material. Activation of this acid followed by reaction with 2,5-dichloroamylamine hydrochloride and triethylamine[15] furnished benzamide **13**. Treatment of **13** with sodium sulfate nonahydrate[16] gave tetrahydrothiophene **14** in a 54% yield after purification by flash chromatography. Reaction of **14** with

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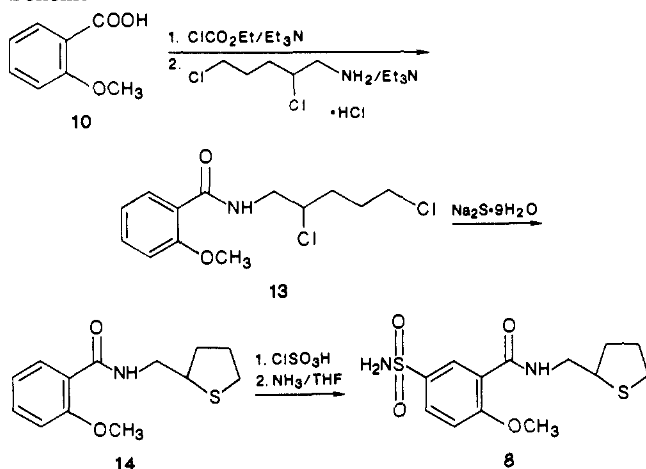
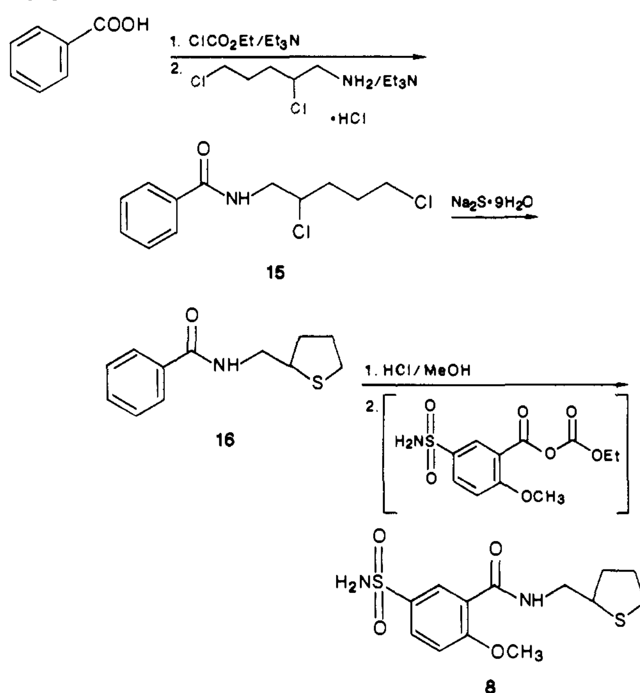
(16) Birch, S. F.; Dean, R. A.; Hunter, N. J.; Whitehead, E. V. *J. Org. Chem.* **1957**, *22*, 1590.

Scheme II



Scheme III



chlorosulfuric acid[14] provided the corresponding sulfonyl chloride. This product could not be easily purified; therefore, it was not isolated, but was instead reacted with a saturated solution of ammonia in THF to provide 8. This last two-step reaction sequence was extremely inefficient, providing 8 from 14 in only 17% yield. The major problem was the chlorosulfonation reaction. This reaction repeatedly gave inconsistent results and low yields. As a result, the overall yield of 8 from o-anisic acid via this route was only 6.8%.

The third route to tetrahydrothiophene 8 is shown in Scheme III. Benzamide 15 was synthesized from benzoic acid and 2,5-dichloroamylamine hydrochloride in a manner analogous to previous syntheses. Treatment of 15 with sodium sulfide nonahydrate[16] gave tetrahydrothiophene 16 in a 34% yield after purification by flash chromatography. N-(2-Pyrrolidinyl)methylbenzamides have been hydrolyzed to their corresponding amines by using potassium hydroxide and ethanol.[17] The use of these conditions for the hydrolysis of benzamide 16 led only to the recovery of starting material. Hydrolysis of benzamide 16 was accomplished by using concentrated hydrochloric acid and methanol (1:1 mixture). Attempts to isolate 2-tetrahydrothiophenemethylamine as its hydrochloride salt were not successful. The amine was therefore used directly in the next reaction with the activated adduct of benzoic acid 11. This two-step procedure provided 8 in a moderate yield of 36% from 16. Although these last two reactions were only moderately efficient, the major hindrance to this synthetic approach was the low yield achieved in the cyclization reaction. As a result, the overall yield of 8 from benzoic acid via this route was only 9.4%.

A comparison of the three approaches to tetrahydrothiophene 8 clearly shows that route A is the best approach to the desired product. Examination of these pathways also reveals the curious finding that the cyclization reaction with sodium sulfide is dependent upon the substitution of the aromatic ring. Benzamides 12, 13, and 15 were cyclized to tetrahydrothiophenes 8, 14, and 16 in yields of 74%, 54% and 34%, respectively. This was completely unexpected since the aromatic ring seems to be too far removed from the site of the reaction to exert any effect. Each of these reactions was repeated several times, and the yields obtained consistently fell into one of three ranges—67–74%, 48–54%, or 28–34%—depending upon the starting benzamide. An explanation for this curious behavior is currently being sought.

The synthesis of tetrahydrothiophenium iodide 9 was completed in a straightforward manner by the treatment of 8 with iodoethane. This compound was quite hygroscopic, which made its isolation difficult. Numerous crystallization attempts and filtrations were necessary to obtain a solid product.

Table I. Dissociation Constants (K_B) for the Inhibition of Agonist Effects on the K^+ Evoked Release of [^{3}H]Acetylcholine for Sulpiride and Analogues

compd		ED_{50}, nM (95% CI)	K_B, *mM*
5	Apo	75.9 (43.7–128)	
	Apo + 5 (10 μM)	2455 (676–8913)	0.319
6	Apo	25.1 (1.9–324)	
	Apo + 6 (100 μM)	2344 (1659–3236)	1.09
7	Apo	26.3 (9.1–64.6)	
	Apo + 7 (100 μM)	2188 (646–7413)	1.22
8	Apo	48.1 (15.1–154)	
	Apo + 8 (100 μM)	34.9 (14.8–82.5)	*a*
9	Apo	38.4 (19.1–77.1)	
	Apo + 9 (100 μM)	806 (378–1720)	5.00

[a] No statistically significant antagonism observed. Apo = Apomorphine.

Biological Results and Discussion

The pyrrolidinium (6, 7), tetrahydrothiophene (8), and the tetrahydrothiophenium (9) analogues of sulpiride were evaluated for their ability to antagonize apomorphine's effect on potassium-evoked [^{3}H]acetylcholine release. Apomorphine, a dopamine agonist, inhibits the potassium-induced release of [^{3}H]acetylcholine from striatal slices by stimulating D_2 receptors. The K_B values for the inhibition of agonist effects of these four analogues as well as for sulpiride (5) are summarized in Table I. As expected, sulpiride was found to antagonize the actions of apomorphine at the D_2 receptor. The permanently charged pyrrolidinium and tetrahydrothiophenium analogues of sulpiride were also found to antagonize the effect of apomorphine, although at higher concentrations than sulpiride. In contrast, the permanently uncharged analogue 8 was inactive at concentrations up to 100 μM in antagonizing the effects of apomorphine.

The permanently charged tetrahydrothiophenium analogue 9 and the permanently uncharged tetrahydro-

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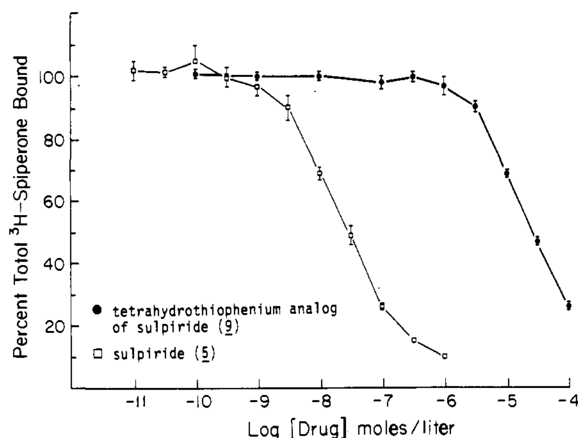

Figure 1. Inhibition of total [^{3}H]spiperone binding by various concentrations of sulpiride (**5**) and the tetrahydrothiophenium analogue of sulpiride (**9**). Each value is the mean ± SEM (N = 3).

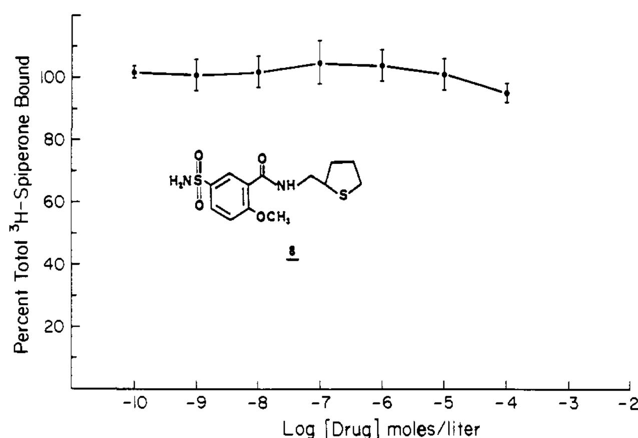

Figure 2. Inhibition of total [^{3}H]spiperone binding by various concentrations of the tetrahydrothiophene analogue of sulpiride (**8**). Each value is the mean ± SEM (N = 3).

thiophene analogue **8** were also evaluated for their ability to displace [^{3}H]spiperone from D_2 binding sites in rat striatal membranes. As shown in Figure 1, both sulpiride and compound **9** were able to displace [^{3}H]spiperone from its binding site with K_i values of (5.51 ± 0.60) × 10^{-9} M and (5.17 ± 0.22) × 10^{-6} M, respectively. In contrast, compound **8** was unable to cause any significant displacement of concentrations up to 100 μM (Figure 2).

The results of this study are consistent with the previous observations on the effects of permanently charged analogues of chlorpromazine (**2**, **3**).[12] The ability of permanently charged analogues of chlorpromazine and sulpiride to both bind and act as antagonists at the dopamine D_2 receptor, coupled with the inactivity of the permanently uncharged sulpiride analogue, suggests that dopaminergic antagonists bind in their charged molecular forms to anionic sites on the D_2 receptor. Additionally, these studies show that a basic nitrogen atom is not required for dopamine antagonist activity. The activity of sulfonium analogues **3** and **9** provides further evidence that sulfonium salts can be used as bioisosteres for amines. This replacement has been previously demonstrated for dopamine,[10,11] isolevorphanol,[18] and oxotremorine.[19] Although sulfonium salts and amines cannot be considered bioisosteres in the classical sense of the term, recent broadening of the concept to include functionalities that lead to similar biological effects and that possess some similarity in terms of steric and local electronic configuration allows for this designation.[20,21] Finally, taken together with previous studies involving permanently charged and uncharged sulfur and selenium analogues of dopamine,[11] these results support the concept that both dopamine agonists and dopamine antagonists bind to a similar recognition site on the D_2 dopamine receptor for the amine nitrogen.

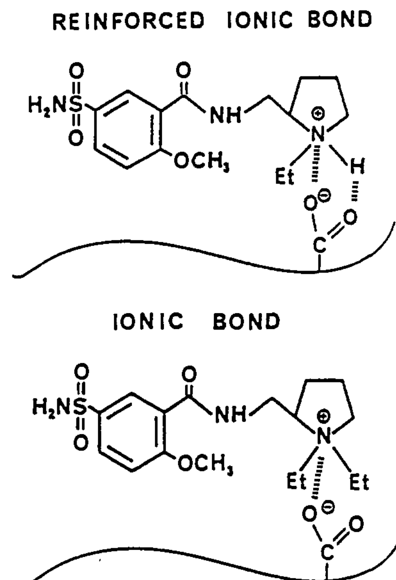

Figure 3. Comparison of the binding of sulpiride and a permanently charged analogue to the dopamine receptor: importance of a hydrogen atom at the cationic moiety.

Although several studies have now shown that permanently charged analogues of chlorpromazine and sulpiride are active as dopamine receptor antagonists, the activity of these compounds, in all cases, is less than that of the parent compounds. The lower activity of the permanently charged compounds may be due to their lack of a hydrogen atom at the charged center.[22,23] Without a hydrogen atom, these analogues are not capable of forming a reinforced ionic bond with the receptor, but are only capable of forming an ionic bond. This is demonstrated in Figure 3 for sulpiride and the permanently charged ethyl iodide salt of sulpiride (**7**). Since an ionic bond has approximately one-half the bond strength of a reinforced ionic bond (5 kcal/mol vs 10 kcal/mol)[22] and since the interaction of the amine nitrogen with the dopamine receptor is thought to be a major contributor to binding of dopamine antagonists to the dopamine receptor,[1,2,6] the lower activity of the permanently charged analogues may be due to the inability of these compounds to form a reinforced ionic bond with an anionic site on the dopamine D_2 receptor. Therefore, on the basis of this and previous studies, the predicted order of activity for a series of dopamine antagonists with structural modifications of the basic amine moiety is $[NR_2H]^+ > [NR_3]^+ = [SR_2]^+ >>> SR$.

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Experimental Section

Melting points (uncorrected) were determined on a Thomas-Hoover melting point apparatus. NMR spectral data was obtained with either a Bruker HX-90E NMR spectrometer (90 MHz), a Bruker WP-80DS NMR spectrometer (80 MHz), or an IBM AF/270 spectrometer (270 MHz) in the pulse mode. Chemical shifts are expressed in parts per million (ppm) on the δ scale relative to tetramethylsilane. IR spectral data were obtained with a Beckman 4230 infrared spectrophotometer. Mass spectra were obtained with a Kratos MS25RFA double focusing mass spectrometer or at The Ohio State University Chemical Instrumentation Center by use of a Kratos MS-30 mass spectrometer. The spectroscopic data for all new compounds were consistent with the assigned structures. Elemental analyses were performed by Galbraith Laboratories, Inc., Knoxville, TN, and were within ±0.4% of the theoretical values for the elements indicated. Prior to use, 2,5-dichloroamylamine hydrochloride obtained from Aldrich Chemical Company was dissolved in methanol, passed through activated charcoal, and recrystallized from ethanol.

2-[(2-Methoxy-5-sulfamoylbenzamido)methyl]-1-ethyl-1-methylpyrrolidinium Iodide (6). Sulpiride (5) (1.00 g, 2.93 mmol) was dissolved in warm acetonitrile (30 mL), and iodomethane (1.0 mL, 16.1 mmol) was added. The solution was refluxed for 2 h. During the course of the reaction, a white solid precipitated from the solution. After 2 h, the solvent and excess iodomethane were removed in vacuo, leaving 1.34 g (94.6%) of a white solid. As evidenced by the appearance of two methyl peaks in the ^{1}H NMR, the product was an 80:20 mixture of diastereomers: mp 180–183 °C; ^{1}H NMR (D_2O, 270 MHz) δ 8.01 (d, J = 2.50 Hz, 1 H, 6-ArH), 7.87 (dd, J = 2.54 Hz and 8.88 Hz, 1 H, 4-ArH), 7.15 (d, J = 8.97 Hz, 1 H, 3-ArH), 3.87–3.74 (m, 2 H, CONHCH_2), 3.83 (s, 3 H, OCH_3), 3.58–3.16 (m, 5 H, NCH_2 and NCHR), 2.99 (s, 3 H, NCH_3, 20.4 mol %), 2.83 (s, 3 H, NCH_3, 79.6 mol %), 2.36–1.85 (m, 4 H, CH_2CH_2), 1.24 (t, J = 7.23 Hz, 3 H, NCH_2CH_3); IR (KBr) cm^{-1} 3370 (NH), 3240 and 3180 (NH_2), 1640 (C=O), 1335 and 1170 (SO_2); mass spectrum (FAB), m/z 356 (M^+). Anal. ($C_{16}H_{26}IN_3O_4S$) C, H, N.

2-[(2-Methoxy-5-sulfamoylbenzamido)methyl]-1,1-diethylpyrrolidinium Iodide (7). Sulpiride (5) (341 mg, 1.00 mmol) was dissolved in warm acetonitrile (10 mL), and iodoethane (7.8 mL) was added. The solution was refluxed for 6.5 h. After approximately 1.5 h, a white solid started to precipitate from the solution. After 6.5 h, the hot reaction mixture was filtered to provide 411 mg (82.6%) of the desired quaternary pyrrolidinium salt: mp 193–195 °C; ^{1}H NMR (D_2O, 270 MHz) δ 8.05 (d, J = 1.71 Hz, 1 H, 6-ArH), 7.91 (dd, J = 1.85 Hz and 8.91 Hz, 1 H, 4-ArH), 7.19 (d, J = 8.95 Hz, 1 H, 3-ArH), 3.99–3.86 (m, 2 H, CONHCH_2), 3.88 (s, 3 H, OCH_3), 3.57–3.25 (m, 7 H, NCH_2 and NCHR), 2.35–1.90 (m, 4 H, CH_2CH_2), 1.25 (m, 6 H, NCH_2CH_3); IR (KBr) cm^{-1} 3380 (NH), 3220 and 3160 (NH_2), 1645 (C=O), 1340 and 1170 (SO_2); mass spectrum (FAB), m/z 370 (M^+). Anal. ($C_{17}H_{28}IN_3O_4S$) C, H, N.

2-Methoxy-5-sulfamoylbenzoic Acid (11). Chlorosulfuric acid (50 mL) was cooled to 0 °C in an ice bath, and *o*-anisic acid (10.0 g, 65.7 mmol) was slowly added in small increments. Upon addition, the solution turned yellow. The reaction was warmed to room temperature at which time all of the *o*-anisic acid dissolved. The reaction was slowly warmed to between 60 and 70 °C and was allowed to proceed for 40 min at this temperature. The reaction mixture was *slowly* poured into a large Erlenmeyer flask containing crushed ice. (*Caution: chlorosulfuric acid reacts violently with water!*) A white solid immediately precipitated. This solid was filtered, dried, and recrystallized from dichloromethane to yield 13.98 g (84.9%) of 2-methoxy-5-(chlorosulfonyl)benzoic acid, mp 147–149 °C (lit.[24] mp 147–148 °C).

This sulfonyl chloride (3.00 g, 12.0 mmol) was dissolved in THF (10 mL) and was added to a saturated NH_3/THF solution. A white solid immediately precipitated. The solution was stirred at room temperature for 0.5 h and then filtered. The white solid that was obtained was dried to remove any THF and the dissolved in water (50 mL). Concentrated HCl was added dropwise until the solution was acidic. The desired benzoic acid crystallized from the acidic aqueous solution to provide 2.62 g (94.6%) of 11 as white crystals: mp 222–224 °C (lit.[25] mp 220 °C); ^{1}H NMR (DMSO-d_6, 90 MHz) δ 8.08 (d, J = 2.54 Hz, 1 H, 6-ArH), 7.91 (dd, J = 2.54 Hz and 8.73 Hz, 4-ArH), 7.33 (s, 2 H, NH_2), 7.27 (d, J = 8.73 Hz, 1 H, 3-ArH), 3.88 (s, 3 H, OCH_3); IR (KBr) cm^{-1} 3370 and 3270 (NH_2), 2950 (br, OH), 1705 (C=O), 1330 and 1165 (SO_2); mass spectrum, m/z 231 (M^+, base), 215, 202, 151, 136.

***N*-(2,5-Dichloropentyl)-2-methoxy-5-sulfamoylbenzamide (12).** Benzoic acid 11 (1.90 g, 8.22 mmol) was suspended in acetone (50 mL), and triethylamine (1.17 mL, 8.22 mmol) was added. The resulting solution was cooled to 0 °C by use of an ice bath. Ethyl chloroformate (0.79 mL, 8.22 mmol) was added, and the reaction mixture was stirred for an additional 0.5 h at 0 °C. Over time, a white solid precipitated from the solution. 2,5-Dichloroamylamine hydrochloride (1.59 g, 8.22 mmol) was added to the reaction mixture along with a 20% excess of triethylamine (1.41 mL, 9.86 mmol). The reaction mixture was stirred for 0.5 h at 0 °C and was then allowed to warm to room temperature. The reaction mixture, which was actually a suspension at this point, was stirred for an additional 2.5 h at room temperature. The solvent was evaporated, leaving a white solid residue, which was washed with 10% HCl (25 mL), filtered, and dried. The white solid that was obtained was recrystallized from methanol to give 2.45 g (80.7%) of benzamide 12 as white crystals: mp 143–144 °C; ^{1}H NMR (MeOH-d_4, 270 MHz) δ 8.39 (d, J = 2.51 Hz, 1 H, 6-ArH), 8.01 (dd, J = 2.50 Hz and 8.80 Hz, 1 H, 4-ArH), 7.30 (d, J = 8.85 Hz, 1 H, 3-ArH), 4.20 (m, 1 H, CHCl), 3.77 (dd, J = 5.16 Hz and 13.93 Hz, 1 H, NCH_2), 3.66 (dd, J = 7.08 Hz and 13.93 Hz, 1 H, NCH_2), 3.62 (t, J = 6.12 Hz, 2 H, CH_2Cl), 2.09–1.87 (m, 4 H, CH_2CH_2); IR (KBr) cm^{-1} 3360, 3300 and 3230 (NH and NH_2), 1615 (C=O), 1335 and 1165 (SO_2); mass spectrum, m/z 334 (M^+ – HCl, ^{37}Cl), 332 (M^+ – HCl, ^{35}Cl), 318, 214, 200, 184, 118, 41 (base).

***N*-(2,5-Dichloropentyl)-2-methoxybenzamide (13).** Triethylamine (4.6 mL, 32.8 mmol) was added to a solution of *o*-anisic acid (5.00 g, 32.8 mmol) in dichloromethane (100 mL) and the solution was cooled to 0 °C by use of an ice bath. Ethyl chloroformate (3.15 mL, 32.8 mmol) was added, and the solution was stirred for 30 min at 0 °C. 2,5-Dichloroamylamine hydrochloride (6.30 g, 32.8 mmol) and a 20% excess of triethylamine (5.5 mL, 39.4 mmol) were then added to the reaction mixture. The reaction was stirred for 30 min at 0 °C at which time it was warmed to room temperature and stirred for an additional 2.5 h. The solvent was removed in vacuo, leaving a yellow oil, which was dissolved in dichloromethane (50 mL) and washed with 10% HCl (2 × 50 mL), saturated $NaHCO_3$ (2 × 50 mL), and water (1 × 50 mL). The chloroform layer was dried ($MgSO_4$) and evaporated to give a yellow oil. This oil was dissolved in a small amount of diethyl ether and induced to crystallization by cooling the solution of –78 °C via a dry ice/acetone bath and scratching the flask. The crystals obtained in this manner were washed with petroleum ether and dried to afford 6.93 g (72.7%) of benzamide 13: mp 34–35 °C; ^{1}H NMR ($CDCl_3$, 270 MHz) δ 8.38 (br, 1 H, NH), 8.20 (dd, J = 1.90 Hz and 7.80 Hz, 1 H, ArH ortho to amide), 7.47 (ddd, J = 1.97 Hz, 7.32 Hz and 8.30 Hz, 1 H, ArH para to amide), 7.09 (ddd, J = 7.80 Hz, 7.32 Hz, and 1.02 Hz, 1 H, ArH para to OCH_3), 7.00 (dd, J = 1.02 Hz and 8.32 Hz, 1 H, ArH ortho to OCH_3), 4.17 (m, 1 H, CHCl), 4.00 (m, 1 H, 1 × NCH_2), 4.00 (s, 3 H, OCH_3), 3.59 (m, 3 H, CH_2Cl and 1 × NCH_2), 2.11–1.85 (m, 4 H, CH_2CH_2); IR (KBr) cm^{-1} 3360 (NH), 1660 (C=O); mass spectrum, m/z 293 (M^+, $^{37}Cl_2$), 291 (M^+, ^{37}Cl and ^{35}Cl), 289 (M^+, $^{35}Cl_2$), 253, 135 (base), 105. Anal. ($C_{13}H_{17}Cl_2NO_2$) C, H, N.

***N*-[(2-Tetrahydrothienyl)methyl]-2-methoxybenzamide (14).** A mixture of benzamide 13 (2.5 g, 8.62 mmol) and sodium sulfide nonahydrate (6.2 g, 25.8 mmol) in methanol (35 mL) was refluxed for 24 h at which time the methanol was evaporated under vacuum. The residue was partitioned between dichloromethane (20 mL) and water (20 mL). The dichloromethane layer was collected, dried ($MgSO_4$), and evaporated. The resulting yellow oil was purified by flash chromatography using 0.5% methanol in dichloromethane as the eluting solvent. This provided 1.15 g (53.1%) of a yellow oil, which resisted all attempts at crystallization: ^{1}H NMR ($CDCl_3$, 270 MHz) δ 8.31 (br, 1 H, NH), 8.20 (dd, J = 1.86 Hz and 7.78 Hz, 1 H, ArH ortho to amide), 7.45 (ddd,

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J = 1.86 Hz, 7.34 Hz and 8.32 Hz, 1 H, ArH para to amide), 7.08 (ddd, J = 0.98 Hz, 7.78 Hz and 7.34 Hz, 1 H, ArH para to OCH_3), 6.98 (dd, J = 0.98 Hz and 8.32 Hz, 1 H, ArH ortho to OCH_3), 3.99 (s, 3 H, OCH_3), 3.82–3.70 (m, 2 H, SCHR and 1 × NCH_2), 3.46 (m, 1 H, 1 × NCH_2), 2.91 (m, 2 H, SCH_2), 2.11–1.78 (m, 4 H, CH_2CH_2); IR (neat) cm^{-1} 3390 (NH), 1660 (C=O); mass spectrum, m/z 251 (M^+), 152, 135 (base), 100. Anal. ($C_{13}H_{17}NO_2S$) C, H, N.

***N*-(2,5-Dichloropentyl)benzamide (15).** Triethylamine (5.70 mL, 40.9 mmol) was added to a solution of benzoic acid (5.00 g, 40.9 mmol) in dichloromethane (100 mL). The solution was cooled to 0 °C, and ethyl chloroformate (3.91 mL, 40.9 mmol) was added. The solution was stirred for 30 min at 0 °C at which time 2,5-dichloroamylamine hydrochloride (7.87 g, 40.9 mmol) and triethylamine (6.85 mL, 49.1 mmol) were added. The reaction was stirred at 0 °C for 30 min, warmed to room temperature, and stirred for an additional 2.5 h. The solvent was then evaporated, and the resulting oil was dissolved in chloroform (50 mL). The chloroform solution was washed with 10% HCl (2 × 50 mL), saturated $NaHCO_3$ (2 × 50 mL), and water (1 × 50 mL), dried ($MgSO_4$), and evaporated. The resulting oil was recrystallized from diethyl ether to give 8.11 g (76.2%) of benzamide 15 as white crystals: mp 67–68 °C (lit.[17] mp 56–57 °C); ^1H NMR ($CDCl_3$, 270 MHz) δ 7.80 (m, 2 H, ArH *ortho* to amide), 7.55–7.41 (m, 3 H, ArH), 6.60 (br, 1 H, NH), 4.17 (m, 1 H, CHCl), 4.02 (m, 1 H, 1 × NCH_2), 3.61–3.48 (m, 3 H, CH_2Cl and 1 × NCH_2), 2.18–1.83 (m, 4 H, CH_2CH_2); IR (KBr) cm^{-1} 3320 (NH), 1655 (C=O); mass spectrum, m/z 263 (M^+, $^{37}Cl_2$), 261 (M^+, ^{37}Cl and ^{35}Cl), 259 (M^+, $^{35}Cl_2$), 224, 134, 105 (base), 77.

***N*-[(2-Tetrahydrothienyl)methyl]benzamide (16).** A mixture of benzamide 15 (2.50 g, 9.61 mmol) and sodium sulfide nonahydrate (11.6 g, 48.0 mmol) in methanol (25 mL) was stirred at reflux temperature for 28 h. The reaction was then stopped and evaporated to dryness. The residue was partitioned between dichloromethane (20 mL) and water (20 mL). The dichloromethane layer was collected, dried ($MgSO_4$), and concentrated to an oil, which was purified by flash chromatography using dichloromethane as the eluting solvent. The clear liquid that was obtained spontaneously crystallized to give 649 mg (30.5%) of a white solid: mp 108–110 °C; ^1H NMR ($CDCl_3$, 270 MHz) δ 7.75 (m, 2 H, ArH ortho to amide), 7.44 (m, 3 H, ArH), 6.54 (br, 1 H, NH), 3.70 (m, 2 H, SCHR and 1 × NCH_2), 3.41 (m, 1 H, 1 × NCH_2), 2.87 (m, 2 H, SCH_2), 2.08–1.75 (m, 4 H, CH_2CH_2); IR (KBr) cm^{-1} 3350 (NH), 1640 (C=O); mass spectrum, m/z 221 (M^+), 105, 100 (base), 77. Anal. ($C_{12}H_{15}NOS$) C, H, N.

***N*-[(2-Tetrahydrothienyl)methyl]-2-methoxy-5-sulfamoylbenzamide (8). Route A.** A mixture of benzamide 12 (2.00 g, 5.4 mmol) and sodium sulfide nonahydrate (6.48 g, 27.0 mmol) in methanol (20 mL) was refluxed for 21 h. The reaction mixture was then evaporated to dryness, and the yellow residue was dissolved in water (20 mL) and transferred to an Erlenmeyer flask. Ethyl acetate (5 mL) was added, and the flask was shaken vigorously for several seconds. Within 5 min, the product began to crystallize in the aqueous layer. The solution was allowed to stand for 90 min to room temperature to assure complete crystallization. The crystals were then collected and dried. Recrystallization from acetonitrile afforded 1.32 g (74.0%) of the desired tetrahydrothiophene as white crystals: mp 225–228 °C dec; ^1H NMR (MeOH-d_4, 270 MHz) δ 8.38 (d, J = 2.48 Hz, 1 H, 6-ArH), 7.99 (dd, J = 2.56 Hz and 8.80 Hz, 1 H, 4-ArH), 7.28 (d, J = 8.82 Hz, 1 H, 3-ArH), 4.04 (s, 3 H, OCH_3), 3.66–3.43 (m, 3 H, NCH_2 and SCHR), 2.87 (m, 2 H, SCH_2), 2.08–1.79 (m, 4 H, CH_2CH_2); IR (KBr) cm^{-1} 3380 and 3280 (NH and NH_2), 1625 (C=O), 1345 and 1180 (SO_2); mass spectrum, m/z 330 (M^+), 231, 214, 100 (base), 87. Anal. ($C_{13}H_{18}N_2O_4S_2$) C, H, N.

Route B. Chlorosulfuric acid (6 mL) was cooled to 0 °C in an ice bath. Benzamide 14 (1.15 g, 4.57 mmol) was dissolved in dichloromethane (4 mL) and added dropwise to the chlorosulfuric acid. The resulting solution was allowed to warm to room temperature and was then gradually heated to between 60 °C and 70 °C by use of an oil bath. During heating, the dichloromethane evaporated, and the solution turned from a light yellow color to a dark brownish yellow color. The reaction was stirred for 40 min at 60–70 °C and then was *carefully* poured over crushed ice. (*Caution: chlorosulfuric acid reacts violently with water!*) A gum separated from the aqueous solution. Dichloromethane (50 mL) was added, and the two layers were separated. The organic layer was dried ($MgSO_4$), decolorized with activated charcoal, and evaporated to give a yellow oil. This oil was dried for 1 h on a vacuum pump, and after drying, a light yellow, fluffy solid was obtained (930 mg, 58.2% crude yield). The intermediate sulfonyl chloride was verified by proton NMR; however, the product obtained was not purified, but was immediately used in the next reaction.

The crude sulfonyl chloride (250 mg) was mixed with dichloromethane (10 mL), resulting in the formation of a milky solution and an insoluble gum. The solution was decanted to remove it from the insoluble gum. Ammonia gas was bubbled into the solution for 20 min at which time the reaction was stopped and evaporated to dryness. The residue obtained was crystallized from methanol and recrystallized from acetonitrile to give 150 mg (17.3% overall from 14) of benzamide 8 as light yellow crystals, mp 219–221 °C dec. Except for the slightly lower melting point, this product was in all respects identical with the product obtained via route A.

Route C. A solution of benzamide 16 (500 mg, 2.26 mmol) in methanol (7.5 mL) and concentrated HCl (7.5 mL) was refluxed for 24 h. The solution was then evaporated to dryness, and the residue was partitioned between dichloromethane (10 mL) and water (10 mL). The aqueous layer was collected, basified with 10% NaOH, and extracted with dichloromethane (10 mL). The dichloromethane layer was collected, dried ($MgSO_4$), and evaporated to provide 148 mg (55.8% crude yield) of a yellow liquid. A proton NMR of this liquid was consistent with the formation of 2-tetrahydrothiophenemethylamine; however, attempts to isolate and purify the product through formation of the hydrochloride salt were not successful. This product, therefore, was used in the next reaction without purification.

Benzoic acid 11 (292 mg, 1.26 mmol) was suspended in acetone (10 mL), triethylamine (0.18 mL, 1.26 mmol) was added, and the resulting solution was cooled to 0 °C in an ice bath. Ethyl chloroformate (0.12 mL, 1.26 mmol) was added, and the reaction mixture was stirred at 0 °C. Over time, a white solid precipitated from the solution. After 30 min, the crude preparation of 2-tetrahydrothiophenemethylamine (148 mg, 1.26 mmol) in acetone (1 mL) was added to the reaction. The reaction was stirred for 30 min at 0 °C, warmed to room temperature, and stirred for an additional 2.5 h. The acetone was then evaporated, leaving a white solid, which was washed with 10% HCl, filtered, and dried. The white solid so obtained was recrystallized from acetonitrile to give 269 mg (36.1% overall from 16) of benzamide 8, mp 224–227 °C dec. The product obtained in this manner was identical in all respects with the product obtained via route A.

2-[(2-Methoxy-5-sulfamoylbenzamido)methyl]-1-ethyltetrahydrothiophenium Iodide (9). A mixture of benzamide 8 (200 mg, 0.605 mmol) and iodoethane (1 mL) in methanol (10 mL) was refluxed for 3 days. An additional 1 mL of iodoethane was then added, and the reaction was stirred under reflux for 2 more days. The reaction was then stopped, and the methanol and excess iodoethane were evaporated. The residue was partitioned between ethyl acetate (10 mL) and water (10 mL). The aqueous layer was collected and washed twice more with ethyl acetate. The water was then evaporated, and the residue was washed once with methanol. The residue was then dissolved in a minimal amount of methanol and placed in the freezer. The crystals so obtained were collected and dried to yield 110 mg (37.4%) of a yellow solid, (Note: isolation of the desired product was sometimes difficult since the crystals tended to be quite hygroscopic. Numerous crystallization attempts and filtrations were sometimes necessary to obtain a solid product.): mp 142–143 °C; ^1H NMR (MeOH-d_4, 270 MHz) δ 8.41 (d, J = 2.47 Hz, 1 H, 6-ArH), 8.03 (dd, J = 2.45 Hz and 8.80 Hz, 1 H, 4-ArH), 7.33 (d, J = 8.86 Hz, 1 H, 3-ArH), 4.36 (m, 1 H, SCHR), 3.89 (m, 2 H, NCH_2), 3.75–3.52 (m, 2 H, SCH_2), 3.35 (q, J = 7.46 Hz, 2 H, SCH_2CH_3), 2.68–2.09 (m, 4 H, CH_2CH_2), 1.42 (t, J = 7.45 Hz, 3 H, SCH_2CH_3); IR (KBr) cm^{-1} 3320 and 3280 (NH and NH_2), 1635 (C=O), 1335 and 1170 (SO_2); mass spectrum (FAB), m/z 359 (M^+), 214 (base). Anal. ($C_{15}H_{23}IN_2O_4S_2$) C, H, N.

Biological Testing. The preparation of rat striatal homogenates and [^3H]spiperone binding assays along with the measurement of the K^+-induced release of [^3H]acetylcholine from

mouse striatal slices was carried out in an identical fashion as described in an earlier publication.[12]

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Registry No. **5**, 15676-16-1; *cis*-**6**, 118894-81-8; *trans*-**6**, 118894-82-9; **7**, 118894-83-0; **8**, 118894-84-1; **9**, 118894-85-2; **10**, 579-75-9; **11**, 22117-85-7; **12**, 67833-50-5; **13**, 118894-86-3; **14**, 118894-87-4; **14** (sulfonyl chloride deriv.), 118894-88-5; **15**, 67304-97-6; **16**, 118894-89-6; 2-methoxy-5-(chlorosulfonyl)benzoic acid, 51904-91-7; 2,5-dichloroamylamine hydrochloride, 62922-45-6; benzoic acid, 65-85-0; 2-tetrahydrothiophenemethylamine, 83171-40-8.

A Minor Modification of Residue 1 in Potent Vasopressin Antagonists Dramatically Reduces Agonist Activity

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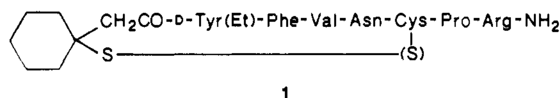
[1-(β,β-Pentamethylene-β-mercaptopropionic acid),2-(*O*-ethyl)-D-tyrosine,4-valine,9-desglycine]arginine-vasopressin (SK&F 101926, 1), a potent in vivo and in vitro vasopressin V_2 receptor antagonist, was recently tested in human volunteers and shown to be a full antidiuretic agonist. A new animal model for vasopressin activity has been developed in dogs that duplicates the clinical agonist findings exhibited with SK&F 101926. In this model we have discovered that substitution of a *cis*-4′-methyl group on the Pmp moiety at residue 1 of vasopressin antagonists results in substantially reduced agonist activity compared to the unsubstituted molecule (SK&F 101926). The corresponding analogue with a *trans*-4′-methyl group exhibits more agonist activity than the cis molecule. These findings can be explained by viewing the biological activities of compounds such as 1 as the interaction of the vasopressin receptor with a number of discrete molecular entities, conformers of 1, which present different pharmacophores. Models have been developed to assist in the understanding of these results.

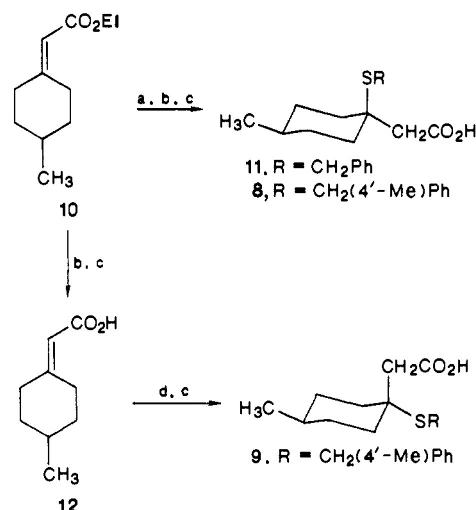
Evaluation of a potent vasopressin V_2 receptor antagonist [1-(β,β-pentamethylene-β-mercaptopropionic acid),2-(*O*-ethyl)-D-tyrosine,4-valine,9-desglycine]arginine-vasopressin (SK&F 101926,[1] 1)] in human volunteers



revealed that the molecule behaved as an antidiuretic agonist rather than an antagonist.[2] This result was unexpected since SK&F 101926 is (1) a potent in vivo antidiuretic hormone antagonist (aquaretic) in rat, dog, and squirrel monkey and (2) a potent in vitro V_2-receptor antagonist in rat, dog, squirrel monkey, and human renal medullary tissue preparations with no apparent agonist activity.[3] In an attempt to derive an animal model for the agonist activity observed in humans, we discovered that pretreatment of dogs with cyclooxygenase inhibitors such as indomethacin unmasks in vivo agonist activity of SK&F 101926.[4] We have subsequently applied the model to the determination of the structure–activity relationship (SAR) for partial agonism of V_2-receptor antagonists in dog. Of particular interest for study were those structural modifications that appeared to be essential for the initial conversion of agonists into antagonists.

One such modification was the pentamethylenemercaptopropionic acid (Pmp) residue substituted at position 1 of vasopressin.[5] Early in our work with vasopressin antagonists we developed a synthesis of Pmp[6] that allowed us to prepare a wide variety of Pmp-modified peptides to test design hypotheses with regard to steric bulk and electronic factors.[7] In preliminary V_2-receptor assays the biological activity of vasopressin analogues containing these Pmp modifications proved generally unexceptional. However, when these peptides were evaluated in the indomethacin-petreated dog model for

Scheme I. Synthesis of 4′-MePmp Intermediates[a]



[a] (a) 0.1 equivalent of NaH, excess mercaptan, toluene; (b) K_2CO_3, water/MeOH; (c) HCl; (d) mercaptan, excess piperidine, reflux.

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