acetate/hexanes). Anal. ($C_{20}H_{28}O_2$) C, H.

Pharmacology. Materials. Male ICR mice (22–30 g) and Sprague-Dawley rats (250–275 g) obtained from Dominion Laboratories (Dublin, VA) were maintained on a 14:10-h light:dark cycle and received food and water ad libitum. Δ^8-, Δ^9-, and $\Delta^{9,11}$-THC were obtained from the National Institute on Drug Abuse as the (-) enantiomers. ^{3}H-CP-55,940 was kindly provided by Dr. Kenner C. Rice (Lab. Med. Chem./NIDDK, NIH, Bethesda, MD).

Drug Preparation and Administration. The procedure of Olson et al.[28] was used to prepare micellular suspensions suitable for injection, resulting in a final vehicle composition of ethanol:emulphor:saline (1:1:18), which was administered via tail-vein injection (0.1 mL/10 g, iv) to mice or intraperitoneally (0.1 mL/100 g, ip) to rats.

Behavioral Evaluations. Locomotor activity (% inhibition), antinociception (via tail-flick latency; expressed as %MPE), hypothermia (Δ °C), and catalepsy (i.e. ring immobility; expressed as % immobility) were evaluated in mice by previously reported methods.[3,20,21,29]

To establish the drug discrimination model in rats, animals were trained to discriminate between vehicle and Δ^9-THC (3 mg/kg, ip) 30 min postinjection. The protocol design was a slight modification[30,31] of the standard two-level operant procedure for a FR-10 schedule of food reinforcement.[32,33]

Antagonist properties of the cannabinoids were determined as described previously.[3,20,21,29] Animals were pretreated with drug 10 min prior to administration of 3 mg/kg Δ^9-THC, and all pharmacological evaluations were performed as described above.

Statistical analysis was performed using ANOVA with Dunnett's t test for comparisons to control (agonist evaluations), and the Scheffe's F test for multiple comparisons (antagonist evaluations). Differences were considered significant at the $p < 0.05$ level (two-tailed). The ED_{50} value for agonist activity was determined by unweighted least-squares linear regression of the log dose-probit analysis.

In Vitro Binding Assays. The filtration procedure used for ^{3}H-CP-55,940 binding is a modification of the centrifugation method described by others.[4] Five rats were decapitated and their cortices rapidly dissected free and homogenized in 30 mL of 0.32 M sucrose which contained 2 mM EDTA and 5 mM $MgCl_2$. The homogenate was centrifuged at 1600g for 10 min, and the supernatant was removed. The pellet was washed twice by resuspending in 0.32 M sucrose/2 mM EDTA/5 mM $MgCl_2$ and centrifuging again as described above. The original supernatant was combined with the wash supernatants and centrifuged at 39000g for 15 min. The resulting P_2 pellet was suspended in 50 mL of buffer (50 mM Tris·HCl, pH 7.0, 2 mM EDTA, 5 mM $MgCl_2$) and incubated at 37 °C for 10 min before centrifugation at 23000g for 10 min. The P_2 pellet was resuspended in 50 mL of 50 mM Tris·HCl/2 mM EDTA/5 mM $MgCl_2$ and incubated at 30 °C for 10 min before centrifugation at 11000g for 15 min. The final pellet was resuspended in 10 mL of 50 mM Tris·HCl (pH 7.4) which contained 1 mM EDTA and 3 mM $MgCl_2$ and then stored at -40 °C.

The binding assay was performed in silanized glass tubes which contained 100 μL of radiolabeled ligand (final concentration 1 nM), 100 μL of competing unlabeled drug, 150 μg of membrane protein (75 μL), and sufficient buffer (50 mM Tris·HCl, pH 7.4, 1 mM EDTA, 3 mM $MgCl_2$ and 5 mg/mL bovine serum albumin [BSA]), to make a final volume of 1 mL. After a 1-h incubation at 30 °C, the reaction was terminated by the addition of 2 mL of ice-cold 50 mM Tris·HCl (pH 7.4) buffer containing 1 mg of BSA/mL and rapid filtration through polyethylenimine-treated Whatman GF/C glass-fiber filters. The reaction tube was washed with a 2-mL aliquot of buffer, which was then also filtered. The filters were washed with two 4-mL aliquots of ice-cold buffer. The filters were shaken for 60 min in 10 mL of scintillation fluid, and radioactivity was quantitated by liquid scintillation spectrometry. Specific binding was defined as the difference between the binding that occurred in the presence and absence of 10 μM unlabeled CP-55,940.

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Synthesis and Biological Activity of the Putative Metabolites of the Atypical Antipsychotic Agent Tiospirone

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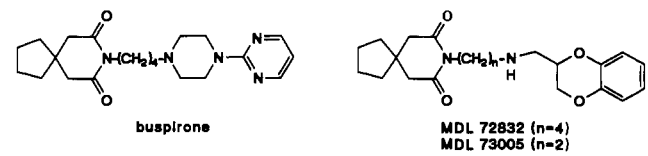
Putative oxidative metabolites of the lead antipsychotic agent tiospirone (1) were synthesized to assist in the identification of the authentic metabolic products found in human urine samples. Thus far, six authentic metabolites have been correlated to the synthetic species.[4a] The putative metabolites were further examined in vitro to assess their central nervous system therapeutic potential. SAR analysis of these derivatives indicates that hydroxyl substitution, particularly in the azaspirodecanedione region of the molecule, diminishes the dopamine D-2 affinity of the species without significantly altering the serotonin type-1A and type-2 interactions. In addition, an increase in α_1-adrenergic affinity appears to be linked to the attenuation of effects at the dopamine receptors. The biological profile of the 6-hydroxytiospirone metabolite 42 was exemplary in these respects and the in vivo actions of this compound suggest potent antipsychotic potential with a minimal liability for extrapyramidal side effects (EPS). While compound 42 has been unambiguously characterized as an actual human metabolite of tiospirone, the role of 42 in the observed antipsychotic activity of the parent drug, if any, has not yet been determined.

Introduction

On the basis of extensive preclinical studies and preliminary clinical evaluations, tiospirone (1, 8-[4-[4-(1,2-benzisothiazol-3-yl)-1-piperazinyl]butyl]-8-azaspiro[4.5]-decane-7,9-dione, a.k.a. tiaspirone or BMY 13859) is a lead compound from the azaspirodecanedione class of pharmaceuticals indicated for the treatment of psychotic dis-

Chart I



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‡ Analytical Research, Bristol-Myers Squibb Co., Evansville, IN 47721.

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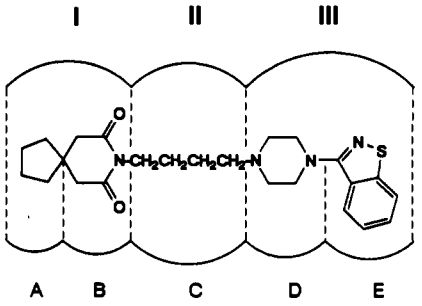
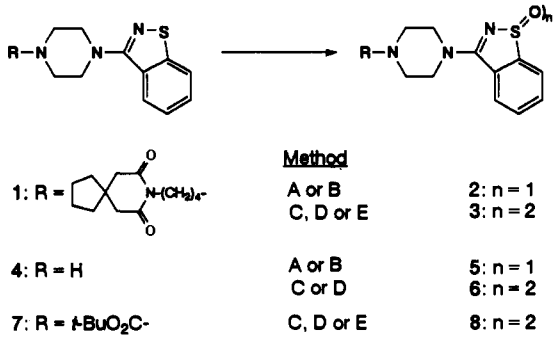
Chart II



orders.[1] Showing classical antipsychotic attributes, this compound demonstrates potent in vitro affinity for dopamine-D_2 receptors but, unlike prevailing treatments, does not cause dopamine receptor supersensitivity following chronic administration. Tiospirone also possesses strong in vitro interactions at the serotonin type-1A and type-2 receptors, a significant facet of its neuropharmacology as there is increasing evidence supporting the role of serotonin systems in the modulation of dopamine neurotransmission.[1d] Thus, while its unique blend of serotonergic and dopaminergic interactions make this compound truly novel versus currently marketed antipsychotic agents, it is also predicted to have low EPS (extrapyramidal side effects) liability based on the preclinical paradigms relating drug efficacy to the induction of neuroleptic behaviors.

Previous ADME investigations into the metabolic fate of buspirone,[2,1c] a currently marketed anxiolytic agent (Chart I), documented extensive metabolism of this compound, a fact pertinent to tiospirone as both compounds are substructurally homologous. Putative oxidative metabolites of tiospirone were synthesized to provide reference standards for the studies directed toward elucidating the structures of the authentic drug metabolites found in human urine samples. Pharmacological evaluation of this putative metabolite family was also performed to determine if any of these derivatives contribute, either positively or negatively, to the pharmacological actions of the parent compound.

Scheme I[a]



[a] Methods: (A) H_2SO_4, HNO_3, −10 °C; (B) MCPBA, CH_2Cl_2, −78 °C; (C) MCPBA, CH_2Cl_2, room temperature; (D) Oxone, CH_3OH; (E) $NaIO_4$, MeOH, H_2O.

Synthesis

Tiospirone is one member of the first generation of psychotropic agents fashioned from the unique *N*-(4-heteroaryl-1-piperazinyl)alkylimide structural class.[3] Specifically, tiospirone (Chart II) consists of an azaspirodecanedione (I) bridged by a tetramethylene chain (II) to a piperazinylbenzisothiazole (III).

Preliminary investigations into the metabolism of tiospirone in humans using ^{14}C-labeled drug showed that only a minor amount of unchanged tiospirone could be detected in the urine.[4a] Furthermore, none of the detectable metabolites appeared to be present as conjugates. Accordingly, it was reasoned that the relatively high aliphatic character of tiospirone required enzymatic oxidation to promote excretion. Attempts to target the most likely sites of such oxidative processes were done by systematic evaluation of each of the five subunits of tiospirone (A–E, Chart II). The preparation of putative oxidative metabolites based on this analysis is discussed below.

Subunit E. Enzymatic oxidation at sulfur could yield either the *S*-oxide or *S,S*-dioxide. The *S*-oxide of tiospirone (**2**) was originally prepared[5] by reaction of tiospirone (**1**) with a solution of concentrated sulfuric (H_2SO_4) and nitric (HNO_3) acids at −15 to −5 °C (Scheme I, method A). These stringent conditions were detrimental to the molecule at temperatures above 0 °C and resulted in complex mixtures of aromatic ring nitration products. A less severe and generally more applicable protocol for the oxidation of a benzisothiazole to its *S*-oxide utilizes *m*-chloroperoxybenzoic acid (*m*-CPBA) in methylene chloride at −78 °C (method B). The use of *m*-CPBA as oxidant yields the dioxide as well as the oxide unless the tem-

(1) (a) Yevich, J. P.; New, J. S.; Smith, D. W.; Lobeck, W. G.; Catt, J. D.; Minielli, J. L.; Eison, M. S.; Taylor, D. P.; Riblet, L. A.; Temple, D. L., Jr. Synthesis and Biological Evaluation of 1-(1,2-Benzisothiazol-3-yl)- and (1,2-Benzisoxazol-3-yl)piperazine Derivatives as Potential Antipsychotic Agents. *J. Med. Chem.* **1986**, *29*, 359–369. (b) Moore, N. C.; Meyendorff, E.; Yeragani, V.; Lewitt, P. A.; Gershon, S. Tiaspirone in Schizophrenia. *J. Clin. Psychopharmacol.* **1987**, *7*, 98–101. Jain, A. K.; Kelwala, S.; Moore, N. C.; Gershon, S. A Controlled Clinical Trial of Tiaspirone in Schizophrenia. *Int. Clin. Psychopharmacol.* **1987**, *2*, 129–133. (c) Other azaspirodecanedione-containing drugs include the anxiolytic agent Buspar (see: New, J. S. The Discovery and Development of Buspirone: A New Approach to the Treatment of Anxiety. *Med. Res. Rev.* **1990**, *10*, 283–326) and the Merrill-Dow agents MDL 72832 and MDL 73005 (see: Hibert, M. F.; Gittos, M. W.; Middlemiss, D. N.; Mir, A. K.; Fozard, J. R. Graphics Computer-Aided Receptor Mapping as a Predictive Tool for Drug Design: Development of Potent, Selective, and Stereospecific Ligands for the 5-HT_{1A} Receptor. *J. Med. Chem.* **1988**, *31*, 1087–1093). See also: Hrib, N. J.; Jurcak, J. G.; Huger, F. P.; Errico, C. L.; Dunn, R. W. Synthesis and Biological Evaluation of a Series of Substituted N-Alkoxyimides and -amides as Potential Atypical Antipsychotic Agents. *J. Med Chem.* **1991**, *34*, 1068–1072. (d) Abou-Gharbia, M.; Moyer, J. A. *Annual Reports in Medicinal Chemistry*; Academic Press, Inc.: San Diego, CA, 1990; Vol. 25, pp 1–10.

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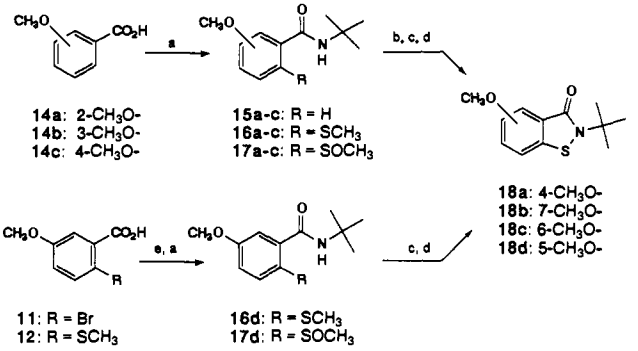
(3) Wu, Y.-H.; Rayburn, J. W. *N*-(Heteroarcyclic)Piperazinylalkyl-Azaspiroalkanediones. U.S. Patent 3 717 634, 1973. Temple, D. L.; Yevich, J. P.; Lobeck, W. G. N-[(4-[3-Cyano-Substituted Pyridyl]-Piperazino)Alkyl]-Azaspirodecanediones. U.S. Patent 4 305 944, 1981.

(4) (a) Mayol, R. F.; Jajoo, H. K.; Klunk, L. J.; Blair, I. A. Metabolism of the Antipsychotic Drug Tiospirone in Humans. *Drug Metab. Dispos.* **1991**, *19*, 394–399. (b) Jajoo, H. K.; Blair, I. A.; Klunk, L. J.; Mayol, R. F. Characterization of in vitro Metabolites of the Antipsychotic Drug Tiospirone by Mass Spectrometry. *Biomed. Environ. Mass Spectrom.* **1990**, *19*, 281–285.

(5) Yevich, J. P.; Lobeck, W. G., Jr. Antipsychotic Benzisothiazole S-Oxide Compound. U.S. Patent 4 656 173, 1987.

Scheme II[a]



[a] Methods: (a) i. $SOCl_2$, $CHCl_3$, reflux, ii. t-$BuNH_2$, CH_2Cl_2, 0 °C (method F); (b) BuLi, Me_2S_2, THF, −78 °C; (c) method E or B; (d) $Cl_3COCOCl$, $ClCH_2CH_2Cl$, 0 °C; (e) i. BuLi, THF, −78 °C, ii. t-BuLi, −90 °C, iii. Me_2S_2, −78 °C.

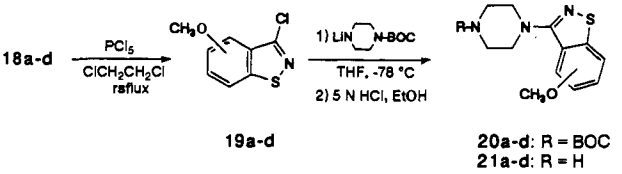
perature is vigilantly kept below −75 °C. The *S,S*-dioxide of tiospirone (3) could be prepared from commercially available saccharin and the requisite piperazine derivative by previously disclosed methodology.[5] Subsequent synthetic endeavors have revealed convenient, one-step methods for the preparation of *S,S*-dioxides from the corresponding benzisothiazoles by room temperature oxidation with either *m*-CPBA in methylene chloride, potassium peroxymonosulfate (Oxone) in methanol, or sodium metaperiodate ($NaIO_4$) in MeOH/H_2O (Scheme I, methods C, D, or E, respectively).

It was anticipated that tiospirone would generate the benzisothiazolylpiperazine (BITP) fragment (4) through an oxidative N-dealkylation pathway similar to that observed for the antidepressant agent trazodone[6] and the anxiolytic agent buspirone.[2a] Consequently, the *S*-oxides of BITP were also selected for synthesis. The preparation of 3-(1-piperazinyl)-1,2-benzisothiazole 1-oxide (5) was achieved in good yield from BITP (4) using either of the aforementioned protocols (methods A or B). Preparation of dioxide 6 could be achieved by either direct conversion from 4 using methods C or D or by acid hydrolysis of piperazinecarboxylate 8 (prepared from 7 by any of methods C, D, or E).

Enzymatic oxidation of the benzene ring portion of tiospirone was another anticipated route of metabolism for this region of the molecule. Methoxy-substituted benzisothiazole derivatives were prepared through an adaptation of the procedure reported by Uchida and Kozuka.[7] With standard ortho-lithiation techniques, the preparation of the requisite 2-(methylthio)benzamides (16) was achieved via treatment of the dianion of carboxamides **15a–c** with methyl disulfide (Scheme II). The methoxy-substituted carboxamides 15 were prepared from the corresponding commercially available benzoic acids 14 (method F). Oxidation of the methylthio-substituted benzamides 16 to the corresponding sulfoxides 17 was achieved by reaction with sodium metaperiodate ($NaIO_4$) in methanol/water at room temperature (method E) or with *m*-CPBA in methylene chloride at −78 °C (method B).

While Uchida reported good conversion of the sulfoxide to the benzisothiazolone using thionyl chloride ($SOCl_2$), all attempts to duplicate the reaction with the methoxy-substituted benzamides 17 yielded only the 2-(methylthio)benzamide precursors 16. Trichloromethyl chloroformate ($Cl_3COCOCl$, diphosgene) proved to be the reagent of choice in our laboratories, affording the *N-tert*-butyl-1,2-benzisothiazol-3(2*H*)-ones 18 in good yield under mild conditions. While this reaction scheme proved straightforward for the preparation of the 4-methoxy, 6-methoxy, and 7-methoxy analogues **18a–c**, the 5-methoxy-substituted analogue **18d** required alternate methodology.

Scheme III



N-tert-Butyl-3-methoxybenzamide (**15b**) did not yield any of the required 6-methylthio-substituted carboxamide as the ortho-directing effects of both the *tert*-butylamide and the methoxyl moiety directed metalation and subsequently substitution exclusively to the 2-position. Dilithiation of 2-bromo-5-methoxybenzoic acid (11)[8] at −90 °C followed by addition of methyl disulfide afforded the desired product (12, Scheme II) in 63% yield with a fair amount (32%) of desbromo starting material. Due to the difficulty in separating these carboxylic acid derivatives, the components of the mixture were converted to the respective *tert*-butylbenzamides by method F. As separation was still a problem, conversion of **16d** to its sulfoxide (**17d**) permitted facile separation from *N-tert*-butyl-3-methoxybenzamide by flash chromatography. Cyclization of sulfoxide **17d** to benzisothiazolone **18d** with diphosgene was achieved in the usual manner.

Conversion of benzisothiazolones **18a–d** to 3-chlorobenzisothiazole derivatives **19a–d** was achieved by reaction with phosphorus pentachloride (Scheme III). The original synthesis of 3-(1-piperazinyl)-1,2-benzisothiazole (4) required heating an ethanolic solution of 3-chloro-1,2-benzisothiazole with excess piperazine in a closed vessel (i.e., bomb).[1a] Analogous attempts with the methoxy-substituted 3-chlorobenzisothiazoles were disappointing. The poor conversions were compounded by the difficulty in purifying the piperazine products from the excess piperazine reagent. The use of *N*-carboalkoxypiperazine derivatives improved the yields and made the resultant products easier to isolate. Generation of the more reactive piperazine anion significantly reduced the severity of the requisite reaction conditions and hence became the method of choice for the synthesis of N-protected 3-(1-piperazinyl)benzisothiazoles. Reaction of methoxy-substituted 3-chlorobenzisothiazoles **19** with the anion of *N*-BOC-piperazine efficiently afforded the BOC-protected 3-piperazinylbenzisothiazoles **20a–d**. Deprotection to the methoxylated BITP fragments **21a–d** was effected by treatment with warm aqueous 5 N HCl in EtOH.

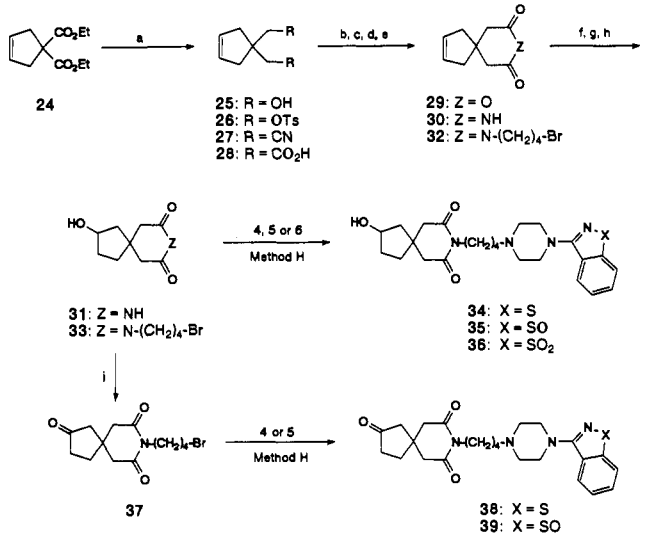
Subunit A. Perhaps the most lipophilic segment of tiospirone is the cyclopentane ring (subunit A, Chart I) of the azaspirodecanedione moiety (I). Enzymatic hydroxylation at C-1 or C-4 seemed unlikely due to the steric crowding inherent in their neopentyl nature. Therefore, attention was focused on the introduction of oxygen at the symmetrically equivalent C-2 or C-3 positions. Unfortu-

(6) Yamato, C.; Takahashi, T.; Fujita, T. Studies on Metabolism of Trazodone. III. Species Differences. *Xenobiotica* **1976**, *6*, 295–306.

(7) Uchida, Y.; Kozuka, S. A Novel Route to 2-Substituted 1,2-Benzisothiazol-3(2*H*)-ones. *J. Chem. Soc., Chem. Commun.* **1981**, 510–511.

(8) Bunton, C. A.; Kenner, G. W.; Robinson, M. J. T.; Webster, B. R. Experiments Related to the Biosynthesis of Novobiocin and Other Coumarins. *Tetrahedron* **1963**, 1001–1010.

Scheme IV[a]



[a] Methods: (a) LAH, THF, 0 °C; (b) *p*-TsCl, pyridine, 0 °C; (c) KCN, KI, DMF, 120 °C; (d) NaOH, 70% EtOH/H_2O, reflux; (e) Ac_2O, reflux; (f) NH_4OH, $PhCH_3$, reflux; (g) B_2H_6–THF, NaOH, H_2O_2 (method G); (h) K_2CO_3, CH_3CN or DMF, reflux (method H), $Br(CH_2)_4Br$; (i) PCC, CH_2Cl_2.

nately, the remote and unreactive nature of these sites made synthetic conversion from tiospirone or any of its precursors quite difficult. Diverse synthetic approaches finally enabled introduction of a hydroxyl substituent at the desired position through the methodology shown in Scheme IV.

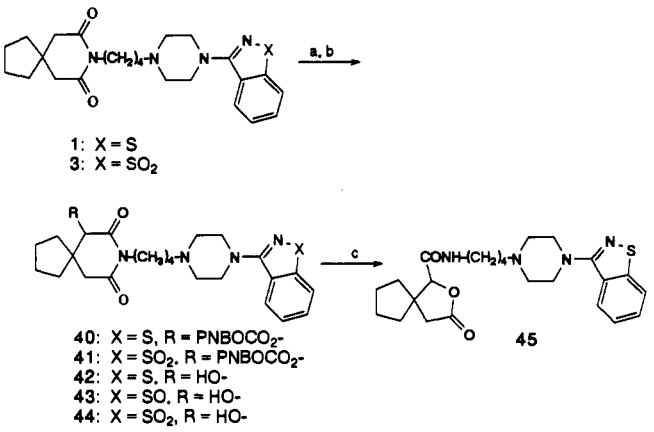
Starting with diethyl 3-cyclopentene-1,1-dicarboxylate (**24**),[9] a four-step chain homologation sequence was utilized to prepare diacid **28**. Conversion to anhydride **29** was effected by refluxing the diacid in excess acetic anhydride followed by in vacuo removal of the unreacted reagent. Imide **30** was prepared from reaction of the anhydride with concentrated ammonium hydroxide in refluxing toluene with azeotropic removal of water. Hydroboration–oxidation (method G) of imide **30** (Scheme IV) yielded hydroxy-substituted imide **31** in poor yield, perhaps due to interactions between the imide moiety and the reagent. Better yields were obtained after the imide was N-alkylated (e.g., 1,4-dibromobutane) to tertiary imide **32**. Standard hydroboration–oxidation conditions (method G) resulted in isolation of the hydroxylated imide **33** in very good yield. While allylic oxidation offers a route to the C-1 (or C-4) hydroxy analogues, this chemistry was not pursued.

Coupling of **33** with BITP **4** according to method H (K_2CO_3, CH_3CN or DMF, reflux) afforded putative metabolite **34** in excellent yield. With the realization that metabolic oxidation at sulfur may occur on this derivative as well, alkyl bromide **33** was coupled with BITP fragment **5** to afford the dioxidation product **35**. Preparation of **36** was similarly effected with BITP fragment **6**. Synthesis of the ketone analogue of **33** proceeded with PCC oxidation of this hydroxyimide derivative in methylene chloride to afford the oxo imide **37**. Condensations of **37** with BITP fragments **4** and **5** according to method H yielded the putative oxotiospirone metabolites **38** and **39**, respectively.

Subunit B. Although the neopentyl nature of the carbon atom adjacent to the imide carbonyl in tiospirone was thought to be a deterrent to enzymatic hydroxylation, it was also felt that this effect might be partially offset by the activating influence of the carbonyl group. Investigations revealed that the α-methylene position was sufficiently active to allow synthetic functionalization via metalation and subsequent reaction with electrophiles. Initial attempts to incorporate oxygen into the C-6 position utilized an adaptation of a literature precedent.[10] Lithiation of **1** with lithium bis(trimethylsilyl)amide (LHMDS) and subsequent reaction of the anion with dibenzyl peroxydicarbonate gave the desired benzyl carbonate. However, attempted cleavage of this intermediate to the desired alcohol using either catalytic or transfer hydrogenation over palladium on carbon (Pd/C) afforded only unreacted benzyl carbonate. Since the *p*-nitrocarbobenzoxy group is more readily removed by hydrogenolysis than the carbobenzoxy group,[11] we elected to prepare bis(4-nitrobenzyl) peroxydicarbonate [$(PNBOCO_2)_2$].[12]

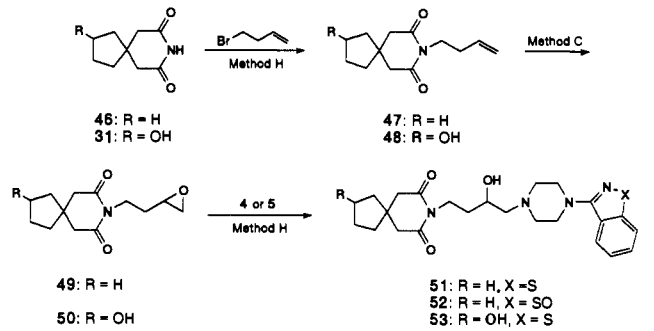
Scheme V shows the application of this methodology to the preparation of the 6-hydroxytiospirone metabolite **42**

Scheme V[a]



[a] Methods: (a) i. LHMDS, THF, −78 °C, ii. $(PNBOCO_2)_2$; (b) H_2, Pd/C, MeOH, THF; (c) KF-Al_2O_3, CH_3CN.

Scheme VI



Scheme VI

(9) Depres, J.-P.; Greene, A. E. Improved Selectivity in the Preparation of Some 1,1-Difunctionalized 3-Cyclopentenes. High Yield Synthesis of 3-Cyclopentenecarboxylic Acid. *J. Org. Chem.* **1984**, *49*, 928–931.

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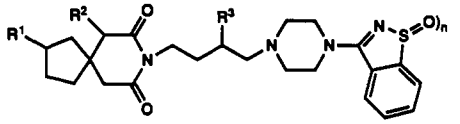
(11) Berse, C.; Baucher, R.; Piche, L. Preparation of L-Cystinyl and L-Cysteinyl Peptides through Catalytic Hydrogenation of Intermediates. *J. Org. Chem.* **1957**, *22*, 805–808.

(12) Strain, F.; Bissinger, W. E.; Dial, W. R.; Rudoff, H.; Dewitt, B. J.; Stevens, H. C.; Langston, J. H. Esters of Peroxycarbonic Acids. *J. Am. Chem. Soc.* **1950**, *72*, 1254–1263. Bis(4-nitrobenzyl) peroxydicarbonate was prepared as a pale yellow solid (mp 104 °C) by a variation of the literature procedure and was found to be a relatively stable material which decomposed at its melting point with slow gas evolution. In comparison, dibenzyl peroxydicarbonate decomposed with a sudden vigorous expulsion of material from the melting point capillary tube.

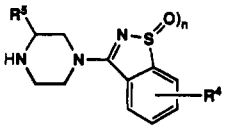
Table I. Physical Data for Putative Metabolites



compd	R^1	R^2	R^3	n	solvent	mp, °C	formula[a]
2	H	H	H	1	EtOH	150–2	$C_{24}H_{32}N_4O_3S·0.2H_2O$
3	H	H	H	2	CH_2Cl_2/Et_2O	185–7	$C_{24}H_{32}N_4O_4S$
34	HO	H	H	0	CH_3CN/EtOAc	223–7	$C_{24}H_{32}N_4O_3S·HCl$
35	HO	H	H	1	*i*-PrOH/*i*-Pr_2O	92–4	$C_{24}H_{32}N_4O_4S·C_4H_4O_4$
36	HO	H	H	2	CH_3CN	174–5	$C_{24}H_{32}N_4O_5S$
38	O	H	H	0	EtOH/*i*-Pr_2O	243–5	$C_{24}H_{30}N_4O_3S·HCl$
39	O	H	H	1	CH_3CN/EtOH	203–6	$C_{24}H_{30}N_4O_4S·HCl·0.2H_2O$
42	H	HO	H	0	EtOAc/hexane	151	$C_{24}H_{32}N_4O_3S$
43	H	HO	H	1	CH_2Cl_2/acetone	171–2	$C_{24}H_{32}N_4O_4S·0.2H_2O$
44	H	HO	H	2	CH_2Cl_2/Et_2O	170–2	$C_{24}H_{32}N_4O_5S$
45	H	HO[b]	H	0	acetone/Et_2O	194–7	$C_{24}H_{32}N_4O_3S·1.4HCl$
51	H	H	HO	0	EtOH	212–4	$C_{24}H_{32}N_4O_3S·HCl$
52	H	H	HO	1	CH_3CN/EtOH	235	$C_{24}H_{32}N_4O_4S·HCl·0.25H_2O$
53	HO	H	HO	0	CH_3CN/EtOH	167–9	$C_{24}H_{32}N_4O_4S·HCl·0.2H_2O$

[a] C, H, N analyses were within ±0.4% of the calculated values. [b] Rearrangement product of 42.

Table II. Physical Data for Putative Metabolic Fragments



compd	R^4	R^5	n	solvent	mp, °C	formula[a]
5	H	H	1	EtOH/H_2O	256 (dec)	$C_{11}H_{13}N_3OS·HCl$
6	H	H	2	EtOH	317–20 (dec)	$C_{11}H_{13}N_3O_2S·1.1HCl$
21a	4-MeO	H	0	EtOH	240–7	$C_{12}H_{15}N_3OS·1.1HCl$
21b	7-MeO	H	0	EtOH	284–7	$C_{12}H_{15}N_3OS·HCl$
21c	6-MeO	H	0	EtOH	280–1	$C_{12}H_{15}N_3OS·1.2HCl·0.1H_2O$
21d	5-MeO	H	0	EtOH	216–8	$C_{12}H_{15}N_3OS·1.1HCl·0.15H_2O$
55	H	O	2		>250	$C_{11}H_{11}N_3O_3S$

[a] C, H, N analyses were within ±0.4% of the calculated values.

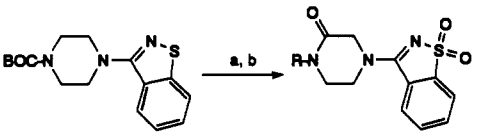
and its sulfur-oxidized derivatives *S*-oxide **43** and *S,S*-dioxide **44**. Reaction of the anion of **1** with $(PNBOCO_2)_2$ afforded carbonate **40**, which was hydrogenolyzed with Pd/C to α-hydroxy imide **42**. The *S*-oxide **43** was prepared from **42** by the aforementioned sulfur oxidation methodology (method B). A demonstration of the flexibility of the current methods is seen in the preparation of *S,S*-dioxide **44** from intermediate carbonate **41** starting from the previously prepared dioxide of tiospirone (**3**).

The earlier metabolite studies on buspirone[2] identified a lactone derivative structurally analogous to **45** (Scheme V). This unusual product may have arisen through either in vivo rearrangement of the α-hydroxy imide precursor (i.e., **42**) or artifactually during the procedural isolation of the metabolites. Synthetically, equilibrium-controlled intramolecular rearrangement of **42** effected by KF–Al_2O_3 in CH_3CN yielded the lactone amide **45** in 89% converted yield after five cycles.

Subunit C. Preparation of potential tiospirone metabolites with hydroxyl substitution in the tetramethylene bridge (subunit C, Chart I) produced three compounds: the primary monooxidation product **51** and the two dioxidation products **52** and **53**. Scheme VI presents the synthetic methodology by which these three putative metabolites were prepared.

Putative metabolite **51** was prepared in three steps from 8-azaspiro[4.5]decane-7,9-dione (**46**). Alkylation of **46** according to method H with 4-bromopropene yielded tertiary imide **47** in good yield. *m*-CPBA oxidation (method C) of **47** afforded epoxide **49** which, when reacted with

Scheme VII[a]



7 **54**: R = BOC; **55**: R = H

[a] Methods: (a) RuO_4, H_2O, CH_2Cl_2; (b) 200 °C.

either BITP (**4**) or BITP *S*-oxide **5**, provided putative metabolite **51** or the dioxidized product **52**, respectively. Preparation of the putative dihydroxylated metabolite **53** was similarly achieved.

Subunit D. On the basis of a similar observation in the metabolism studies on the antiarrhythmic agent encainide,[13] the chromatographic behavior of a tiospirone metabolite suggested a lactam-like derivative of the piperazinyl ring of BITP was probable. A priori considerations anticipated oxidation would occur adjacent to the more basic and reactive piperazinyl nitrogen atom. The synthesis of lactam **55** was achieved in two steps from previously prepared *N*-BOC-BITP **7** (Scheme VII). The BOC group is an efficient activator of the adjacent carbon atoms

(13) Jajoo, H. K.; Mayol, R. F.; LaBudde, J. A.; Blair, I. A. Structural Characterization of Urinary Metabolites of the Antiarrhythmic Drug Encainide in Human Subjects. *Drug Metab. Disp.* **1990**, *18*, 28–35.

Table III. Biological Activity of Putative Metabolites of Tiospirone

compd	IC_{50}, nM: DA binding[a]	5-HT_{1A} binding[b]	5-HT_2 binding[c]	α_1-adrenergic binding[d]	DA/α_1 ratio	ED_{50}, mg/kg: inhibn of CAR[e]	identified metabolite[f]
1	8.4	2.85	0.40	47	0.18	10.5 (5.0–22.1) po 0.35 (0.30–0.43) im	–
2	IA	IA	IA	IA		11.0 (7.8–15.5) po	
3	IA	IA	IA	IA		>100 po, >10 im	
4	IA	190	110	IA		>100 po	
5	IA	IA	IA	IA		>100 po	
6	IA	IA	IA	IA		>100 po	√
21a	IA	(121)	IA	IA			
21b	IA	IA	IA	IA			
21c	IA	IA	IA	IA			
21d	IA	(20.9)	IA	IA			
34	52.5	3.75	2.05	0.876	60	>100 po	√
35	IA	IA	IA	IA		>100 sc	
36	IA	IA	IA	IA		>100 sc	√
38	68.3	2.99	2.92	1.30	52	≥60.3 sc	
39	IA	IA	IA	IA		>100 sc	
42	39.2	5.06	1.10	1.63	24	8.4 (5.5–11.2) po, 1.0 (0.74–1.33) im	√
43	IA	IA	IA	IA			√
44	IA	IA	IA	IA			
45	150	98.7	4.50	2.50	60		
51	111	24.9	10.2	8.30	13	>80 po	
52	IA	IA	IA	IA		>50 po	
53	491	14.2	19.8	7.15	69		
55	IA	IA	IA	IA		>50 sc	√

[a] DA binding represents the inhibition of [^{3}H]spiperone binding in rat corpus striatal tissue. Binding values (IC_{50}) greater than 1000 nM are considered inactive (IA). [b] 5-HT_{1A} binding represents the inhibition of [^{3}H]8-OH-DPAT binding in rat hippocampal tissue. Binding values (IC_{50}) greater than 250 nM are considered inactive (IA). Values in parentheses indicate 5-HT_1 binding and represent inhibition of [^{3}H]5-HT binding in rat hippocampal tissue. [c] 5-HT_2 binding represents the inhibition of [^{3}H]spiperone binding in rat cerebral cortex tissue. Binding values (IC_{50}) greater than 200 nM are considered inactive (IA). [d] α_1-adrenergic binding represents the inhibition of [^{3}H]WB-4101 binding in rat cerebral cortex tissue. Binding values (IC_{50}) greater than 100 nM are considered inactive (IA). [e] Refer to ref 15. The values in parentheses represent 95% fiducial limits. [f] Checks (√) indicate that the compound has been unambiguously identified as an authentic human metabolite of tiospirone (refer to ref 4b).

and facilitates ruthenium tetroxide oxidation[14] to afford **54** in high yield; oxidation of sulfur could not be avoided. Heating **54** at 200 °C effects deprotection at nitrogen to provide the free piperazine **55** in quantitative yield.

The experimental methodologies and spectral data for all of the original compounds described are found in the Experimental Section. Elemental compositions of the putative metabolites of tiospirone are assembled in Table I. Compositions of the putative metabolic BITP fragments are presented in Table II. Each of these compounds was evaluated in vitro for central nervous system (CNS) biological activity (vide infra). In addition, each putative synthetic metabolite was utilized as a reference standard against which the LC spectrum of authentic tiospirone metabolites was compared. The procedures and findings of the metabolite identification studies have been reported in detail elsewhere[4a] and are briefly summarized below.

Pharmacology

The results from the pharmacological examination of the putative metabolites of tiospirone are presented in Table III. The compounds were evaluated for their in vitro binding affinity at dopamine D-2 receptors (DA), serotonin type-1A (5-HT_{1A}) and type-2 (5-HT_2) receptors, and α_1-adrenergic receptors. A majority of compounds were also examined in vivo for their ability to inhibit a conditioned-avoidance response in rats (CAR). This test is used to differentiate a compound's therapeutic tranquilizing effects from its nonspecific sedative–hypnotic properties and is a standard first tier screen for potential antipsychotic agents.[15]

For most of the putative metabolites of tiospirone, a considerable decrease in binding affinity at each of the receptor sites examined was observed as a result of oxygen substitution. In particular, oxidation at sulfur was clearly disruptive and caused a dramatic attenuation of the receptor binding affinities. While reasonable correlation can be found between the in vitro and in vivo activities for the majority of compounds based on this analysis, exceptions to the rule reveal some interesting pharmacological phenomena.

One particular anomaly is witnessed in consideration of the results for the *S*-oxide of tiospirone, **2**. Although this compound demonstrates poor in vitro binding affinity, it is observed to be as efficacious as tiospirone in the in vivo CAR model. Enzymatic interconversion between **2** and tiospirone (**1**) has been proposed to account for this observation. Interconversions of this type have been previously documented[16] and the conceivable prodrug formulations of tiospirone utilizing this observation may supply greater opportunities and flexibilities for dosing regimens.

Certain oxygenated derivatives did not experience an overall inactivation of their in vitro binding affinities. Without exception, these compounds (i.e., **34**, **38**, **42**, **45**, **51**, and **53**) are all less potent at the dopamine D-2 receptor than the parent agent (**1**) and all show a considerable increase in α_1-adrenergic binding affinity. The DA/α_1 receptor selectivity ratios clearly suggest that the α_1-ad-

(14) Sheehan, J. C.; Tulis, R. W. Oxidation of Cyclic Amines with Ruthenium Tetroxide. *J. Org. Chem.* **1974**, *39*, 2264–2267.

(15) New, J. S.; Yevich, J. P.; Eison, M. S.; Taylor, D. P.; Eison, A. S.; Riblet, L. A.; VanderMaelen, C. P.; Temple, D. L., Jr. Buspirone Analogues. 2. Structure-Activity Relationships of Aromatic Imide Derivatives. *J. Med. Chem.* **1986**, *29*, 1476–1482.

(16) Kwan, K. C.; Heimlich, K. R. The Role of Pharmacokinetics in Drug Product Design. *Int. J. Pharm.* **1980**, *6*, 225–241.

renergic affinity may be enhanced at the expense of the dopaminergic interactions through substitutions about the tiospirone skeleton. While there is some variance in the effects of oxygen substitution on the serotonergic interactions, it does appear that the 5-HT components are only minimally affected by substitutions about the azaspirodecanedione moiety (i.e., **34**, **38**, **42**). This is not surprising as it has long been felt that the serotonergic interactions of compounds of this type [i.e., *N*-[(4-aryl-1-piperazinyl)alkyl]imides] are mediated primarily through the arylpiperazine portion of the molecule. The ability to conserve the serotonergic component of these compounds while manipulating the DA/α_1 ratio may be a powerful tool in determining the roles of each of these receptor populations in the observed biological activity of the azaspirodecanedione-containing pharmaceuticals or, more generally, the class of glutarimide-based CNS agents. The development of second-generation species with conceivably greater indices of sedation (e.g., more robust adrenergic blockade) and lower incidence of EPS (e.g., less effective DA interactions) utilizing the parameters revealed in this analysis are under investigation.

The pronounced attenuation of dopamine D-2 receptor interactions may be a primary factor underlying the general lack of in vivo antipsychotic potential demonstrated by most of these compounds. Another explanation may reside in the increased hydrophilicities of these species. While the metabolism studies indicated a minimum of conjugate formation as detected in the urinary workup, the fact that only 39% of the actual dose was recovered in the urine does not preclude the possibility that conjugate formation may be occurring. An alternative interpretation of the in vivo results may therefore be based on the relative facility of conjugate formation. Evidence in support of these hypotheses may be found to some extent in the oxotiospirone analogue **38**, which demonstrates marginal in vivo effects. The corresponding 2-hydroxy analogue **34**, which has a very similar in vitro profile, does not demonstrate any in vivo antipsychotic potential. The relative differences in their hydrophilicity or susceptibility to conjugation may be sufficient to enable the detection of the CNS activity of compound **38**. The activity of 6-hydroxytiospirone (**42**) is quite surprising when compared to these other oxygenated derivatives. Whereas it is essentially equipotent to **34** and **38** in the in vitro analysis, compound **42** is considerably more efficacious than these compounds, and almost as potent as tiospirone, in the in vivo screens predictive of antipsychotic activity.[17] The steric impact of the adjacent quaternary carbon and/or the possibility of intramolecular hydrogen bonding stabilization between the hydroxyl proton and the carbonyl oxygen may act to preserve the lipophilicity of this molecule or diminish the propensity, relative to the isomeric carbinols, to form a readily excretable conjugate.

Positive aspects of the diminished dopaminergic interactions of **42** are possibly being expressed as the decreased ability of this compound to induce catalepsy.[18] This may translate to a wider window of efficacy for this compound versus tiospirone and the currently marketed neuroleptogenic antipsychotic agents. In light of the lower DA affinity, the conserved in vivo potency relative to tiospirone underscores the importance of the serotonergic interactions to the observed antipsychotic activity of this compound.

The last column of Table III indicates which of the synthetic putative oxygenated metabolites of tiospirone were actually found in human urine samples following a single oral dose of ^{14}C-labeled tiospirone. The amount of identified metabolites represented 50% of the total radioactivity in the urine and accounted for ca. 20% of the original dose.[4a] The major urinary metabolic species (9.3%) were determined to be the two BITP *S,S*-dioxide fragments, **6** and **55**. Contrary to what had been observed for buspirone,[2] the unoxidized aryl piperazine portion of the molecule (i.e., BITP fragment **4**) was not observed. 6-Hydroxytiospirone (**42**) and the corresponding *S,S*-dioxide **44** accounted for a considerable portion of the oral dose (5.1%). The *S*-oxide of this metabolite, **43**, was not observed. 2-Hydroxytiospirone (**34**) and its *S,S*-dioxide **36** accounted for the next largest portion of the identified urinary metabolites (2.6%). Unchanged tiospirone accounted for only 1.2% of the original dose. The *S,S*-dioxide of tiospirone was surprisingly absent from the samples. It was interesting to have observed no *S*-oxide products in the human urine samples, as previous studies with rat liver microsomes had indicated their formation to be the primary route of metabolism.[4b]

Conclusions

Numerous putative metabolites of tiospirone were synthesized and evaluated in biological screens predictive of antipsychotic potential. In comparison to tiospirone, all but a few of these compounds demonstrated reduced in vitro affinities for the receptor sites examined. The six target compounds that did exhibit significant in vitro interactions typically were found to be devoid of in vivo antipsychotic activity. Evidence suggests that this may have been due to the increased hydrophilicity as a result of hydroxyl substitution although an increased propensity for conjugate formation cannot be ruled out. It is also possible that the observed attenuation of dopamine receptor affinity may have significantly diminished any antipsychotic potential.

Two compounds (**2** and **42**) demonstrated significant in vivo antipsychotic activity. It has been proposed that **2**,

(17) (a) Additional in vivo testing of the compound's ability to block apomorphine-induced stereotyped behaviors (APO) also indicates **2** to be as potent as the parent drug. The APO test is a more mechanism-based paradigm measuring a drug's ability to antagonize a dopamine-mediated behavior in rats. Janssen, P. A. J.; Niemegeers, C. J. E.; Schellekens, K. H. L.; Lenaerts, F. M. Is it Possible to Predict the Clinical Effects of Neuroleptic Drugs (Major Tranquilizers) from Animal Data? *Arzneim.-Forsch.* **1967**, *17*, 841–854. (b) Besides the CAR data in Table III, compound **42** exhibits ED_{50} values of 26 mg/kg po (11.0–48.0) and 2.3 mg/kg im (0.0026–5.07) in the APO screen. This compares favorably with tiospirone, which exhibits ED_{50} values of 13.1 mg/kg po (9.3–18.4) and 0.6 mg/kg im (0.4–1.1) in the APO screen. The specific procedure used has been previously reported (refer to ref 21).

(18) (a) The induction of catalepsy screen measures the undesireable catalepsy-inducing properties of a drug's in vivo profile and is thought to correlate to the drug's propensity to produce adverse extrapyramidal side effects in man. Costall, B.; Naylor, R. J. On Catalepsy and Catatonia and the Predictability of the Catalepsy Test for Neuroleptic Activity. *Psychopharmacology* **1974**, *34*, 233–241. (b) Compound **42** produced inverted U dose–response curves with an absence of true neuroleptic-like catalepsy at the highest doses tested (100 mg/kg po, 40 mg/kg im). Muscle weakness and flacidity were observed, especially at the higher doses tested, although the animals were still capable of getting down from the side of the testing cage. Using doses on the rising portions of the dose–response curves, ED_{50} estimates for induction of catalepsy were calculated yielding values of 56 mg/kg po (0.54–184) and 5.7 mg/kg im (1.35–69.0) at the 3 h time point. In comparison, tiospirone exhibits an ED_{50} for catalepsy induction of 84.4 mg/kg po (55.6–128.2) and 2.8 mg/kg im (2.1–3.8). The specific procedure used here has been previously reported (refer to ref 21).

which possesses a nondescript binding profile, derives its pharmacology as a result of in vivo conversion to tiospirone through enzymatic reduction of its S-oxide moiety. Compound 42 is of special interest as the in vitro binding profile of this compound, particularly the comparatively low dopaminergic interactions, would not suggest a particularly noteworthy antipsychotic nature. However, 42 is as active as tiospirone even following oral administration. Furthermore, its diminished dopamine D-2 affinity appears to have been directly translated into a lower liability for EPS as evidenced by its inability to induce catalepsy. The actual role played by the strong in vitro serotonergic and α_1-adrenergic interactions in the observed antipsychotic activity of this compound remains to be delineated. However, one could argue an excellent case for their importance in the observed antipsychotic profile of this compound.

Experimental Section

Chemistry. Melting points were taken in Kimax soft-glass capillary tubes using a Thomas-Hoover Uni-melt capillary melting point apparatus (Model 6406 K) and are uncorrected. Infrared spectra were recorded on either a Mattson Instruments, Inc. Alpha Centauri or a Perkin-Elmer 1800 Fourier transform infrared spectrophotometer. NMR spectra were obtained on either a Varian EM-360A (60 MHz, ^1H), a Varian VXR-200 (200 MHz, ^1H), or a Bruker AC-300 spectrometer (300 MHz, ^1H), the latter two being equipped with computer switchable 5.0 mm $^1H/^{13}C$ probes. Chemical shifts are reported in parts per million (δ) downfield from tetramethylsilane. The deuterated NMR solvents contained 99.8–99.9% deuterium in the indicated positions and were obtained from MSD Isotopes (Montreal, Canada). ^1H NMR coupling constants (J values) are listed in hertz (Hz) and spin multiplicities are reported as singlet (s), doublet (d), triplet (t), quartet (q), multiplet (m), and broad (b). Electron impact mass spectra (EIMS) were acquired on a Kratos Analytical MS25RFA operated at 5 kV. Fast atom bombardment mass spectra (FABMS) were acquired on a Kratos Analytical MS25RFA equipped with an Ion Tech, LTD Xenon FAB source operated at 5 kV in either a glycerol or nitrobenzyl alcohol (NOBA) matrix. Chemical ionization mass spectral data (CIMS) were acquired on a Finnegan 4500 quadrupole mass spectrometer equipped with a Vacumetrics discharge chemical ionization probe using methane or isobutane reagent gas at 0.3 Torr source pressure. Microanalyses and Karl Fischer H_2O determinations were performed by the Analytical Department of Bristol-Myers Squibb Co. (Wallingford, CT). Analytical thin-layer chromatography (TLC) was performed on 0.25-mm EM silica gel 60 F-254 coated glass plates and preparative flash chromatography was performed on EM silica gel (32–62 μm). The solvent systems used are reported in each experimental. All solvents were Baker-Analyzed reagent grade and were used without further purification except tetrahydrofuran (THF), which was distilled from sodium/benzophenone ketyl. Butyllithium was purchased from Alfa Ventron and titrated prior to use against diphenylacetic acid (used as titrant and indicator) in THF at room temperature. All nonaqueous reactions were carried out in flame-dried glassware under a nitrogen atmosphere.

General Procedures for the Preparation of 1,2-Benzisothiazole 1-Oxides from 1,2-Benzisothiazoles. Method A. The benzisothiazole (BITZ) derivative was dissolved in concentrated H_2SO_4 (7 mL/g BITZ) and cooled to −15 °C. A 1:1 solution of concentrated H_2SO_4 and HNO_3 (total volume = 2 mL/g BITZ) was slowly added so that the internally monitored reaction temperature did not exceed −5 °C. Upon completion of addition, the reaction mixture was poured onto crushed ice (4 × total volume) and stirred. The solution was maintained below 0 °C while being made basic with solid Na_2CO_3. The solution was repeatedly extracted with CH_2Cl_2. The extracts were combined, dried over anhydrous Na_2SO_4, and filtered. The solvent was evaporated in vacuo to yield the crude product which was purified as indicated.

Method B. The substrate (e.g., benzisothiazole derivative) was dissolved in CH_2Cl_2 (6 mL/mmol BITP) and cooled to −78 °C. A solution of *m*-CPBA (80%, 1.1 equiv) in CH_2Cl_2 (3 mL/mmol) at −78 °C was slowly added and the resultant mixture stirred for 0.5 h. The reaction was diluted with CH_2Cl_2, washed with $NaHCO_3$, and dried over anhydrous Na_2SO_4. The dried organic phase was then filtered and evaporated in vacuo to afford the crude product which was purified as indicated.

General Procedures for the Preparation of 1,2-Benzisothiazole 1,1-Dioxides from 1,2-Benzisothiazoles. Method C. The substrate (e.g., benzisothiazole derivative) was dissolved in CH_2Cl_2 (6 mL/mmol BITP). A solution of *m*-CPBA (80%, 2.1 equiv) in CH_2Cl_2 (3 mL/mmol) was slowly added and the resultant mixture was stirred at room temperature until TLC examination indicated consumption of starting material. The reaction was diluted with CH_2Cl_2, washed with $NaHCO_3$, and dried over anhydrous Na_2SO_4. The dried organic phase was then filtered and evaporated in vacuo to afford the crude product which was purified as indicated.

Method D. To a 0 °C solution of substrate (1.0 equiv) in MeOH (7.5 mL/g) was added a solution of 49.5% potassium peroxymonosulfate (Oxone, 3.0 equiv) in water (volume equivalent to MeOH). The resulting mixture was stirred at room temperature for 2 h and then basified with $NaHCO_3$ and extracted twice with CH_2Cl_2. The organic phase was washed sequentially with saturated $NaHCO_3$ and brine, dried over Na_2SO_4, and evaporated to give a residue which was purified as indicated.

Method E. A 0.1 M solution of substrate (1.0 equiv) in MeOH was added dropwise to a stirring 0.1 M aqueous solution of $NaIO_4$ (7.0 equiv) at room temperature. The reaction was stirred until TLC examination indicated consumption of starting material (typically 48 h). The MeOH was removed in vacuo and the resulting aqueous solution was basified with 3 N NaOH and extracted repeatedly with CH_2Cl_2. The combined extracts were dried (Na_2SO_4), filtered, and stripped to dryness, and the crude product was purified as indicated.

8-[4-[4-(1,2-Benzisothiazol-3-yl)-1-piperazinyl]butyl]-8-azaspiro[4.5]decane-7,9-dione *S*-Oxide (2). This compound was prepared according to method A from tiospirone (1). The crude reaction product was dissolved in hot ethanol, filtered, and allowed to cool. After several days, the solid which had slowly precipitated was isolated by filtration and dried to afford a 57% yield of the title compound as a beige solid: IR (KBr) 1720, 1670, 1070 cm^{-1}; ^1H NMR (DMSO-d_6) δ 8.15 (m, 2 H), 7.75 (m, 2 H), 3.99 (m, 4 H), 3.70 (t, J = 7.0 Hz, 2 H), 2.65 (s, 4 H), 2.56 (m, 4 H), 2.37 (m, 2 H), 1.49 (m, 12 H).

8-[4-[4-(1,2-Benzisothiazol-3-yl)-1-piperazinyl]butyl]-8-azaspiro[4.5]decane-7,9-dione *S*,*S*-Dioxide (3). The title compound was prepared from tiospirone hydrochloride according to method D. Flash chromatography of the resulting solid residue in EtOAc/MeCN (1:1) gave the title compound in 72% yield as a light yellow solid. Recrystallization gave off-white crystals: IR (neat) 1723, 1670, 1354, 1304, 1165, 1137 cm^{-1}; ^1H NMR ($CDCl_3$) δ 8.07–7.94 (m, 1 H), 7.74–7.51 (m, 3 H), 4.06–4.02 (m, 4 H), 3.81–3.74 (m, 2 H), 2.64–2.58 (m, 4 H), 2.59 (s, 4 H), 2.45–2.39 (m, 2 H), 1.74–1.67 (m, 4 H), 1.65–1.50 (m, 8 H).

3-(1-Piperazinyl)-1,2-benzisothiazole 1-Oxide (5). This compound was prepared according to method A from BITP (4). The crude reaction product was flash chromatographed with 15% MeOH/CH_2Cl_2 to afford a 50% yield of the title compound as a light yellow solid: mp 162–4 °C; IR (KBr) 1535, 1042 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.91 (m, 2 H), 7.62 (m, 2 H), 3.99 (m, 4 H), 3.08 (m, 4 H), 2.01 (s, 1 H); EIMS m/z (relative intensity) 235 (M^+, 10), 218 (58), 177 (21), 167 (100), 151 (63), 85 (72), 56 (90). Conversion to the hydrochloride salt followed by recrystallization afforded 5 as an off-white powder.

3-(1-Piperazinyl)-1,2-benzisothiazole 1,1-Dioxide (6). The title compound was prepared from 8 (2.8 g) by gentle heating (70 °C) in 1 N HCl (100 mL) for 3 h. The reaction was allowed to cool to room temperature and basified with 3 N NaOH. After repeated extractions with CH_2Cl_2, the combined extracts were dried (Na_2SO_4) and filtered. In vacuo removal of the solvent afforded 6 as a white solid (1.31 g, 66%): IR (KBr) 1291, 1164, 1135 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.97–7.84 (m, 2 H), 7.74–7.64 (m, 2 H), 4.02 (t, J = 5.0 Hz, 4 H), 3.07 (t, J = 5.0 Hz, 4 H), 1.82 (bs, 1 H); CIMS m/z 252 $[M + H]^+$. Conversion to the hydrochloride salt and recrystallization yielded the analytically pure test compound.

1,1-Dimethylethyl 4-(1,2-Benzisothiazol-3-yl)-1-piperazinecarboxylate (7). A solution of di-*tert*-butyl dicarbonate (22.06 g, 1.00 equiv) in 125 mL of CH_2Cl_2 was cooled to 0 °C. BITP (4; 22.70 g, 1.02 equiv) in 50 mL of CH_2Cl_2 was slowly added via addition funnel. The reaction was monitored by TLC while the solution was allowed to warm to room temperature. Upon completion of reaction (ca. 3 h), the solvent was removed in vacuo and the resulting brown solid was recrystallized from CH_3CN to afford the title compound (28.7 g, 2 crops, 89%) as a white solid: mp 105–7 °C; IR (KBr) 1700 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.88 (m, 2 H), 7.42 (m, 2 H), 3.66 (m, 4 H), 3.49 (m, 4 H), 1.49 (s, 9 H); EIMS *m/z* (relative intensity) 319 (M^+, 35), 264 (100), 220 (31).

1,1-Dimethylethyl 4-(1,2-Benzisothiazol-3-yl)-1-piperazinecarboxylate *S,S*-Dioxide (8). The title compound was prepared according to method E from **7**. Recrystallization of the crude reaction product from EtOH afforded **8** in 92% yield: mp 192–4 °C; IR (KBr) 1702, 1305, 1125 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.96–7.62 (m, 4 H), 4.05 (t, J = 5.0 Hz, 4 H), 3.68 (t, J = 5.0 Hz, 4 H), 1.49 (s, 9 H); EIMS *m/z* (relative intensity) 351 (M^+, 10), 296 (100). Anal. ($C_{16}H_{21}N_3O_4S$) C, H, N.

5-Methoxy-2-(methylthio)benzoic Acid (12). 2-Bromo-5-methoxybenzoic acid[8] (11, 5.0 mmol) was dissolved in anhydrous THF (50 mL) and chilled to −78 °C. BuLi (1.1 equiv) was added dropwise and the temperature was kept below −70 °C. The anion was then chilled to −115 °C, *t*-BuLi (1.4 equiv) was added, and the temperature was kept below −85 °C. When addition was complete, the insoluble dianion was allowed to warm to −75 °C and freshly distilled methyl disulfide was added at a rate to keep the temperature below −70 °C. The reaction was allowed to warm and quenched at −40 °C with 15% aqueous NH_4Cl. The THF was removed in vacuo, and the aqueous phase was washed with CH_2Cl_2 (3 × 50 mL). The aqueous phase was acidified (6 N HCl), extracted (CH_2Cl_2, 3 × 75 mL), dried on $MgSO_4$, filtered, and concentrated in vacuo to a white solid. NMR integration indicated a 2:1 mixture of **12** and *m*-anisic acid.

Method F. General Procedure for the Preparation of Benzamides 15a–c. The appropriate benzoic acid was stirred in $CHCl_3$ (1 mL/g) and DMF (2 drops/mL $CHCl_3$). $SOCl_2$ (4 equiv) was added in a single volume at room temperature and the mixture was slowly warmed to reflux. The reaction was monitored by IR. Reflux times ranged from 2 to 4 h. Upon disappearance of the acid carbonyl stretch, the reaction was cooled to room temperature and the solvent and excess $SOCl_2$ were removed in vacuo. The acid chloride was immediately dissolved in CH_2Cl_2 (50 mL/0.01 mol acid chloride) and slowly added to a solution of *tert*-butylamine (4 equiv) in CH_2Cl_2 (80 mL/0.01 mol amine) at 0 °C. The reaction was permitted to warm to room temperature and stand ca. 18 h. The CH_2Cl_2 solution was washed sequentially with 1.5 N HCl (3×), 0.5 N NaOH (2×), and brine. The organic layer was dried over $MgSO_4$, filtered, and concentrated in vacuo to yield the crude amide which could be used without further purification.

General Procedure for the Preparation of Substituted 2-(Methylthio)benzamides 16a–c. Benzamide **15** was dissolved in anhydrous THF (0.05–0.08 mol/L) and cooled to −10 °C under N_2 with stirring. BuLi (2.5 equiv) was added dropwise and the temperature was kept at −10 °C. The resulting dianion was then cooled to −78 °C. After 15–30 min, methyl disulfide (3 equiv) was added dropwise to keep the temperature below −70 °C. The solution was stirred 1 h and then allowed to warm. At 0 °C, the reaction was quenched with 15% aqueous NH_4Cl. The mixture was concentrated in vacuo and partitioned between CH_2Cl_2 and H_2O, and the aqueous layer was further extracted with CH_2Cl_2 (3 × 50 mL). The organic extracts were combined, dried on $MgSO_4$, filtered, and concentrated to afford the crude derivatives **16a–c**, which could be taken on without further purification.

***N*-(1,1-Dimethylethyl)-5-methoxy-2-(methylthio)benzamide (16d).** The title compound was prepared according to method F from the mixture obtained from the preparation of **12**. The ratio of *S*-methyl benzamide to des-*S*-methyl material as judged by NMR integration was ca. 9:2. This mixture was used without further purification.

General Procedure for the Preparation of 2-(Methylsulfinyl)benzamides 17a–d. The title compounds could be prepared by method E or by a modification of method B. In the modification of method B, the −78 °C reaction solution was permitted to warm to room temperature and stirred overnight prior to workup.

***N*-(1,1-Dimethylethyl)-6-methoxy-2-(methylsulfinyl)benzamide (17a).** The title compound was prepared from **16a** (35.9 g, 1.0 equiv) and *m*-CPBA (34.6 g, 1.2 equiv) by the modified method B. Following flash chromatography in 3% MeOH/CH_2Cl_2, the white sticky solid was recrystallized from 10% EtOAc/hexane to afford 27.8 g (74%) of **17a** as a white solid: mp 125–9 °C; IR (KBr) 3400, 1665, 1025 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.94 (d, J = 7.2 Hz, 1 H), 7.61 (dd, J = 7.2, 7.2 Hz, 1 H), 7.40 (b s, 1 H), 7.10 (d, J = 7.2 Hz, 1 H), 3.98 (s, 3 H), 2.87 (s, 3 H), 1.47 (s, 9 H). Anal. ($C_{13}H_{19}NO_3S$) C, H, N.

***N*-(1,1-Dimethylethyl)-3-methoxy-2-(methylsulfinyl)benzamide (17b).** The title compound was prepared from **16b** (7.75 g, 1.0 equiv) and *m*-CPBA (6.34 g, 1.2 equiv) by the modified method B. The crude reaction material was flash chromatographed in 3% MeOH/CH_2Cl_2 to afford 5.14 g (62%) of **17b** as a white solid: mp 56–60 °C; IR (KBr) 3440, 1650, 1060 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.40 (dd, J = 8.0, 8.0 Hz, 1 H), 7.04 (d, J = 8.0 Hz, 1 H), 6.96 (d, J = 8.0 Hz, 1 H), 6.52 (b s, 1 H), 3.91 (s, 3 H), 3.04 (s, 3 H), 1.47 (s, 9 H). Anal. ($C_{13}H_{19}NO_3S \cdot 0.25H_2O$) C, H, N, H_2O.

***N*-(1,1-Dimethylethyl)-4-methoxy-2-(methylsulfinyl)benzamide (17c).** The title compound was prepared from **16c** (17.5 g, 1.0 equiv) and *m*-CPBA (16.9 g, 1.2 equiv) by the modified method B. Following flash chromatography in a 50% EtOAc/hexane to EtOAc gradient, the desired product (11.5 g, 61%) was obtained as a white solid: mp 150–3 °C; IR (KBr) 3450, 1640, 1025 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.65 (d, J = 3.0 Hz, 1 H), 7.52 (d, J = 9.0 Hz, 1 H), 6.88 (dd, J = 3.0, 9.0 Hz, 1 H), 6.54 (b s, 1 H), 3.99 (s, 3 H), 2.86 (s, 3 H), 1.48 (s, 9 H). Anal. ($C_{13}H_{19}NO_3S$) C, H, N.

***N*-(1,1-Dimethylethyl)-5-methoxy-2-(methylsulfinyl)benzamide (17d).** The title compound was prepared from the crude mixture obtained from the preparation of **16d** (7.40 g, 1.0 equiv) and $NaIO_4$ (4.57 g, 0.60 equiv) according to method E. TLC after 5 h suggested the reaction was complete. The reaction mixture was flash chromatographed in EtOAc and the desired product (3.5 g, 76%) was obtained as a yellow solid: mp 48 °C; IR (KBr) 3420, 1650, 1025 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.90 (d, J = 9.0 Hz, 1 H), 7.12–6.98 (m, 2 H), 6.82 (b s, 1 H), 3.87 (s, 3 H), 2.80 (s, 3 H), 1.48 (s, 9 H). Anal. ($C_{13}H_{19}NO_3S$) C, H, N.

General Procedure for the Preparation of 2-Substituted-1,2-benzisothiazolones (18a–d). Sulfoxide **17** (1.0 equiv) was dissolved in dichloroethane (0.25 mmol/mL) and chilled to 0 °C with stirring under N_2. Trichloromethyl chloroformate (1.0 equiv) was added dropwise. The solution was stirred, with warming to room temperature or slight heating, and monitored by TLC. The reactions were typically complete after 0.5–1 h. NaOH (10%, 5 equiv) was added to the reaction at room temperature and stirred vigorously for 2 h. The mixture was extracted with CH_2Cl_2 (3 × 50 mL), and the combined CH_2Cl_2 extracts were dried on $MgSO_4$, filtered, and concentrated in vacuo to the crude products which could be used without further purification.

2-(1,1-Dimethylethyl)-4-methoxy-1,2-benzisothiazol-3-(2*H*)-one (18a). The title compound was prepared from **17a** (5.38 g) according to the general procedure. The crude product was recrystallized from EtOAc/hexane to afford 3.3 g (69%, 2 crops); mp 141–3 °C. Anal. ($C_{12}H_{15}NO_2S$) C, H, N.

2-(1,1-Dimethylethyl)-7-methoxy-1,2-benzisothiazol-3-(2*H*)-one (18b). The title compound was prepared from **17b** (0.54 g) according to the general procedure as a white solid (0.44 g, 94%); mp 118–20 °C. Anal. ($C_{12}H_{15}NO_2S$) C, H, N; C: calcd, 60.73; found, 60.10.

2-(1,1-Dimethylethyl)-6-methoxy-1,2-benzisothiazol-3-(2*H*)-one (18c). The title compound was prepared from **17c** (9.6 g) according to the general procedure as a beige solid (7.8 g, 92%); mp 60–3 °C. Anal. ($C_{12}H_{15}NO_2S$) C, H, N; N: calcd, 5.90; found, 6.32.

2-(1,1-Dimethylethyl)-5-methoxy-1,2-benzisothiazol-3-(2*H*)-one (18d). The title compound was prepared from **17d** (3.0 g) according to the general procedure. The crude product was flash chromatographed in 25% EtOAc/hexane to afford 2.4 g (92%) of **18d** as a solid; mp 92–4 °C. Anal. ($C_{12}H_{15}NO_2S$) C, H, N.

General Procedure for the Preparation of Methoxy-Substituted 3-Chloro-1,2-benzisothiazoles (19a–d). Benzisothiazolone 18 was dissolved in dichloroethane (0.4 mol/L) and PCl_5 (1 equiv) was added. The mixture was refluxed and monitored by TLC. If the reaction was judged incomplete after 1 h at reflux, an additional 0.1–0.5 equiv of PCl_5 was added until the starting material was consumed. The reaction was cooled, and the solvent and excess reagent were removed under reduced pressure.

3-Chloro-4-methoxy-1,2-benzisothiazole (19a). The title compound was prepared in 98% yield from **18a** according to the general procedure and was purified by flash chromatography in 5% EtOAc/hexane. Anal. (C_8H_6ClNOS) C, H, N.

3-Chloro-7-methoxy-1,2-benzisothiazole (19b). The title compound was prepared as a mixture with 3,4-dichloro-7-methoxy-1,2-benzisothiazole from **18b** according to the general procedure. Attempts to resolve the mixture by flash chromatography were unsuccessful, and the material was used without further purification.

3-Chloro-6-methoxy-1,2-benzisothiazole (19c). The title compound was prepared in 94% yield from **18c** according to the general procedure and was purified by flash chromatography in 50% EtOAc/hexane to afford a white solid; mp 80–3 °C. Anal. (C_8H_6ClNOS) C, H, N.

3-Chloro-5-methoxy-1,2-benzisothiazole (19d). The title compound was prepared in quantitative yield from **18d** according to the general procedure and was purified by flash chromatography in 35% EtOAc/hexane to afford a white solid, mp 70–3 °C. Anal. (C_8H_6ClNOS) C, H, N.

General Procedure for the Preparation of 1,1-Dimethylethyl 4-(Methoxy-substituted-1,2-benzisothiazol-3-yl)-1-piperazinecarboxylates (20a–d). 1,1-Dimethylethyl 1-piperazinecarboxylate (2.5 equiv) was dissolved in anhydrous THF (1–10 mol/L) and chilled to −78 °C under N_2 with stirring. BuLi (2.5 equiv) was added and the temperature was kept below −70 °C. 3-Chloro-1,2-benzisothiazole (**19**) in THF (10 mol/L) was added dropwise to keep the temperature below −70 °C. The reaction was monitored by TLC and typically judged complete once the addition was made. The solution was allowed to warm to 0 °C before quenching with 15% aqueous NH_4Cl. The mixture was concentrated in vacuo and then extracted with CH_2Cl_2. The organic phase was washed with cold 0.5 N HCl (to remove excess piperazine reagent), dried on $MgSO_4$, filtered, and concentrated to dryness.

1,1-Dimethylethyl 4-(4-Methoxy-1,2-benzisothiazol-3-yl)-1-piperazinecarboxylate (20a). This compound was prepared by the general procedure from **19a** (7.42 g). Flash chromatography of the crude reaction mixture in 35% EtOAc/hexane afforded 3.04 g (54%) of title compound as an oil. ^1H NMR, IR, and MS were consistent with the assigned structure.

1,1-Dimethylethyl 4-(7-Methoxy-1,2-benzisothiazol-3-yl)-1-piperazinecarboxylate (20b). This compound was prepared by the general procedure from the mixture obtained in the preparation of **19b**. Flash chromatography of the crude reaction mixture in 30% EtOAc/hexane afforded the title compound as an oil. ^1H NMR, IR, and MS were consistent.

1,1-Dimethylethyl 4-(6-Methoxy-1,2-benzisothiazol-3-yl)-1-piperazinecarboxylate (20c). This compound was prepared by the general procedure from **19c** (4.86 g). Flash chromatography of the crude reaction mixture in 35% EtOAc/hexane afforded 2.82 g (80%) of title compound as a white solid. ^1H NMR, IR, and MS were consistent with the assigned structure.

1,1-Dimethylethyl 4-(5-Methoxy-1,2-benzisothiazol-3-yl)-1-piperazinecarboxylate (20d). This compound was prepared by the general procedure from **19d** (1.24 g). Flash chromatography of the crude reaction mixture in 35% EtOAc/hexane afforded 1.93 g (88%) of the title compound as a solid; mp 77–80 °C. Anal. ($C_{17}H_{23}N_3O_3S$) C, H, N.

General Procedure for BOC-piperazine Cleavage: Preparation of Piperazinylbenzisothiazoles 21a–d. Crude carbamates **20a–d** (1 equiv) were dissolved in a minimal amount of warm EtOH. Ethanolic HCl (5 N, 5 equiv) was added and the resultant solution was heated at 90 °C for ca. 15 min. The solution was allowed to cool to room temperature and the solvent was removed in vacuo. The crude materials were recrystallized from EtOH as the HCl salts.

1,1-Bis(hydroxymethyl)-3-cyclopentene (25). A mechanically stirred suspension of LAH (23.0 g, 0.606 mol) in 800 mL of anhydrous THF was cooled to 0 °C. Diester **24** (50.7 g, 0.239 mol) in 200 mL of anhydrous THF was slowly added via dropping funnel. Upon completion of addition, the mixture was allowed to stir at 0 °C for 4 h. The reaction was carefully quenched by sequential addition of 23 mL of H_2O, 23 mL of 3 N NaOH, and 69 mL of H_2O. The reaction was filtered through a bed of Celite and the resultant cake was repeatedly washed with hot THF. Evaporation of the solvent in vacuo afforded an off-white solid which was recrystallized from toluene to yield 26.0 g (85%) of **25** as white needles: mp 168–70 °C; IR (KBr) 3320–3280, 3065 cm^{-1}; ^1H NMR ($CDCl_3$) δ 5.53 (s, 2 H), 3.62 (s, 4 H), 2.15 (s, 4 H); CIMS m/z (relative intensity) 129 ($[M + H]^+$, 22), 111 (61), 93 (100).

1,1-Cyclopent-3-enedimethanol Bis(4-methylbenzenesulfonate) (26). Cyclopentene **25** (31.3 g, 0.245 mol) was dissolved in a minimal amount of pyridine (ca. 90 mL) and cooled to 0 °C. *p*-Toluenesulfonyl chloride (141 g, 0.737 mol), dissolved in a minimum of pyridine (ca. 200 mL), was added over the course of 2 h via a dropping funnel to the 0 °C solution of **25**. After 5 h, the reaction mixture was poured onto 1 L of crushed ice and allowed to warm to room temperature. The resulting white solid was collected by filtration, washed with cold H_2O, and allowed to air dry. Recrystallization from methanol yielded 98.0 g (92%) of **26** as white needles: mp 113–5 °C; ^1H NMR ($CDCl_3$) δ 7.72 (d, J = 9.1 Hz, 4 H), 7.30 (d, J = 9.1 Hz, 4 H), 5.47 (s, 2 H), 3.87 (s, 4 H), 2.45 (s, 6 H), 2.13 (s, 4 H); CIMS m/z (relative intensity) 437 ($[M + H]^+$, 10), 265 (3), 173 (22), 155 (6), 93 (100).

1,1-Bis(cyanomethyl)-3-cyclopentene (27). A mechanically stirred suspension of KCN (23.0 g, 0.353 mol) and KI (1.20 g, 7.23 mmol) in anhydrous DMF (300 mL) was heated to 120 °C. Ditosylate **26** (50.0 g, 0.115 mol) in anhydrous DMF (300 mL) was slowly added via dropping funnel. The reaction was followed by TLC until no starting material was evident (10–15 h). The reaction was allowed to cool to room temperature and then diluted with 1.8 L of H_2O. The solution was extracted repeatedly with 0.5-L portions of CH_2Cl_2. The organic extracts were combined and evaporated in vacuo to a brown liquid. Distillation under high vacuum afforded 15.8 g (94%) of **27** as a clear liquid: bp 118–21 °C (1.0–1.2 mm); IR (neat) 3060, 2260, 1625 cm^{-1}; ^1H NMR ($CDCl_3$) δ 5.61 (s, 2 H), 2.60 (s, 4 H), 2.42 (s, 4 H); EIMS m/z (relative intensity) 146 (M^+, 21), 119 (4), 106 (100).

3-Cyclopentene-1,1-diacetic Acid (28). A mechanically stirred solution of cyclopentene **27** (11.5 g, 78.6 mmol) in 200 mL of 70% EtOH/H_2O containing NaOH (35.0 g, 0.875 mol) was refluxed at 100 °C until a worked up aliquot indicated the absence of starting material by TLC (1–2 days). The reaction was allowed to cool to room temperature and made strongly acidic with concentrated HCl. Any insoluble salts were filtered and washed with hot EtOH. Addition of H_2O at this point precipitated any amide intermediates from incomplete hydrolysis as brown solids. The solution was evaporated to dryness and the resultant solid was recrystallized from H_2O to yield 12.0 g (two crops, 83%) of **28** as white crystals: mp 134–6 °C; IR (KBr) 3110–3040, 2960–2920, 1730–1710 cm^{-1}; ^1H NMR (DMSO-d_6) δ 5.60 (s, 2 H), 2.48 (s, 4 H), 2.30 (s, 4 H); CIMS m/z (relative intensity) 185 ($[M + H]^+$, 72), 167 (100), 139 (8), 125 (21).

8-Oxaspiro[4.5]dec-2-ene-7,9-dione (29). Diacid **28** (10.5 g, 57.0 mmol) was dissolved in acetic anhydride (40.0 g, 0.392 mol) and heated to reflux for 15 h. The excess acetic anhydride was distilled off and the resulting oil taken up in a minimum of hot benzene. Slow dilution with hot hexane afforded 8.90 g (two crops, 95%) of **29** as off-white needles: mp 64–5 °C; IR (KBr) 1800, 1770 cm^{-1}; ^1H NMR ($CDCl_3$) δ 5.68 (s, 2 H), 2.78 (s, 4 H), 2.34 (s, 4 H); CIMS m/z (relative intensity) 167 ($[M + H]^+$, 100), 149 (15), 139 (8), 121 (8), 80 (10).

8-Azaspiro[4.5]dec-2-ene-7,9-dione (30). Anhydride **29** (8.78 g, 52.8 mmol) was carefully dissolved in concentrated ammonium hydroxide solution (25.0 g, 0.411 mol) and heated at 100 °C for 2 h. The reaction vessel was fitted with a Dean–Stark trap, the reaction volume was doubled with toluene, and the solution was refluxed for 1–2 days until evolution of H_2O ceased. The toluene was evaporated in vacuo and the resulting solid was recrystallized from H_2O to yield 5.75 g (66%) of **30** as white plates: mp 150–3 °C; IR (KBr) 3220, 2860, 1740, 1700–1660, 1385, 1300–1270 cm^{-1};

^{1}H NMR ($CDCl_3$) δ 8.73 (b s, 1 H), 5.68 (s, 2 H), 2.63 (s, 4 H), 2.32 (s, 4 H); EIMS *m/z* (relative intensity) 165 (M^+, 83), 123 (32), 107 (51), 79 (100).

Method G. General Procedure for the Hydroboration–Oxidation of Cyclopentene Derivatives. To a stirring solution of the cyclopentene derivative in anhydrous THF (0.2 M solution) at 0 °C was added, over the course of 0.5 h, borane–THF complex (Aldrich, 1 M solution, 1.0 equiv). The solution was stirred until TLC examination indicated total consumption of starting material (ca. 3 h). The reaction was sequentially quenched with 3 N NaOH solution (0.4 equiv) and 30% hydrogen peroxide solution (1.2 equiv): the peroxide addition should take about 0.5 h. The reaction was stirred for 15 h and then extracted repeatedly with CH_2Cl_2. The combined extracts were combined, dried (Na_2SO_4), filtered, and evaporated in vacuo to afford the crude product, which was purified as specified below.

Method H. General Procedure for the N-Alkylation of Imides and Piperazines. The nitrogen nucleophile, the alkylating species, and K_2CO_3 were combined in CH_3CN or DMF and heated to reflux for 15–20 h until TLC indicated complete reaction. The reaction was allowed to cool to room temperature and then filtered through a bed of Celite. The solvent was evaporated in vacuo and flash chromatography of the resultant residue yielded the desired compounds as specified below.

2-Hydroxy-8-azaspiro[4.5]decane-7,9-dione (31). This compound was prepared from imide **30** according to method G as a white solid in 24% yield following recrystallization from EtOAc/hexane: mp 131–4 °C; IR (KBr) 3440–3160, 2960, 1740, 1680, 1390, 1280 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 4.60 (b s, 1 H), 4.17 (m, 1 H), 3.38 (s, 1 H), 2.57 (s, 2 H), 2.42 (s, 2 H), 1.61 (m, 6 H); EIMS *m/z* (relative intensity) 183 (M^+, 21), 165 (4), 155 (77), 154 (76), 140 (68), 126 (100).

8-(4-Bromobutyl)-8-azaspiro[4.5]dec-2-ene-7,9-dione (32). The title compound was prepared from imide **30** (2.50 g, 1.00 equiv), 1,4-dibromobutane (7.4 mL, 4.09 equiv), and K_2CO_3 (4.38 g, 2.09 equiv) in 200 mL of CH_3CN according to method H. Vacuum distillation of the resultant residue afforded **32** (4.10 g, 90%) as a clear liquid: bp 210 °C (0.5 mm); ^{1}H NMR ($CDCl_3$) δ 5.66 (s, 2 H), 3.80 (t, *J* = 7.0 Hz, 2 H), 3.41 (t, *J* = 7.0 Hz, 2 H), 2.68 (s, 4 H), 2.27 (s, 4 H), 1.94–1.58 (m, 4 H); EIMS *m/z* (relative intensity) 301 ($(M + 2)^+$, 16), 299 (M^+, 16), 220 (100), 192 (15), 178 (20).

8-(4-Bromobutyl)-2-hydroxy-8-azaspiro[4.5]decane-7,9-dione (33). This compound was prepared from **32** according to method G as a viscous liquid. Flash chromatography in EtOAc/hexane (3:2) afforded the title compound in 75% yield as a clear oil; IR (neat) 3450 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 5.32 (s, 1 H), 4.24 (b s, 1 H), 3.81 (t, *J* = 7.1 Hz, 2 H), 3.42 (t, *J* = 7.1 Hz, 2 H), 2.82 (s, 2 H), 2.61 (s, 2 H), 2.09–1.53 (m, 10 H); CIMS *m/z* (relative intensity) 320 ($[M + H + 2]^+$, 61), 318 ($[M + H]^+$, 68), 302 (25), 300 (25), 95 (100).

8-[4-[4-(1,2-Benzisothiazol-3-yl)-1-piperazinyl]butyl]-2-hydroxy-8-azaspiro[4.5]decane-7,9-dione (34). BITP (4; 0.170 g, 1.10 equiv), bromide **33** (0.221 g, 1.00 equiv), and K_2CO_3 (0.229 g, 3.10 equiv) were reacted in 30 mL of CH_3CN according to method H. Flash chromatography of the resultant liquid in 10% EtOH/$CHCl_3$ afforded the title compound (0.309 g, 97%) as a clear liquid: ^{1}H NMR ($CDCl_3$) δ 7.85 (m, 2 H), 7.41 (m, 2 H), 4.42 (m, 1 H), 3.79 (b s, 2 H), 3.52 (m, 4 H), 2.75 (s, 2 H), 2.68 (m, 4 H), 2.57 (s, 2 H), 2.45 (b s, 2 H), 2.07–1.73 (m, 6 H), 1.57 (m, 4 H); EIMS *m/z* (relative intensity) 456 (M^+, 40), 293 (100), 232 (52), 177 (41). Conversion to the hydrochloride salt followed by recrystallization afforded **34** as a white powder.

8-[4-[4-(1,2-Benzisothiazol-3-yl)-1-piperazinyl]butyl]-2-hydroxy-8-azaspiro[4.5]decane-7,9-dione *S*-Oxide (35). BITP *S*-oxide **5** (1.68 g, 1.10 equiv), bromide **33** (2.04 g, 1.00 equiv), and K_2CO_3 (2.70 g, 3.05 equiv) were reacted in 75 mL of CH_3CN according to method H. Flash chromatography of the resultant oil in a 5%–10% MeOH/CH_2Cl_2 gradient afforded the title compound (2.90 g, 96%) as an off-white foam: ^{1}H NMR ($CDCl_3$) δ 8.01 (m, 1 H), 7.86 (m, 1 H), 7.60 (m, 2 H), 4.44 (b s, 1 H), 4.09 (m, 4 H), 3.78 (b s, 2 H), 2.82 (s, 2 H), 2.70 (m, 4 H), 2.60 (s, 2 H), 2.43 (b s, 2 H), 2.04–1.72 (m, 6 H), 1.53 (m, 4 H); FABMS *m/z* 473 (M + H). Conversion to the maleate salt followed by recrystallization afforded **35** as granular yellow crystals.

S,S-Dioxide **36** was similarly prepared from BITP *S,S*-dioxide **6** and bromide **33**. Recrystallization of the chromatographed product afforded **36** as a yellow powder.

8-(4-Bromobutyl)-8-azaspiro[4.5]decane-2,7,9-trione (37). Bromide **33** (15.92 g, 1.00 equiv) was dissolved in 100 mL of CH_2Cl_2 and added to a solution of PCC (120 g, 11 equiv) in 500 mL of CH_2Cl_2 and stirred at room temperature for 2 h. The solution was filtered through a bed of Celite and the salts were washed with CH_2Cl_2. The solvent was evaporated in vacuo to give a red-brown sludge which was flash chromatographed in 20% EtOAc/hexane to afford the title compound (15.50 g, 98%) as a clear oil: IR (neat) 1744, 1727, 1675, 1357 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 3.82 (t, *J* = 7.1 Hz, 2 H), 3.41 (t, *J* = 7.1 Hz, 2 H), 2.69 (s, 4 H), 2.40 (t, *J* = 7.7 Hz, 2 H), 2.20 (s, 2 H), 2.01–1.61 (m, 6 H); EIMS *m/z* 317 ($(M + 2)^+$), 315 (M^+), 236, 222, 208, 194.

8-[4-[4-(1,2-Benzisothiazol-3-yl)-1-piperazinyl]butyl]-8-azaspiro[4.5]decane-2,7,9-trione (38). The title compound was prepared from BITP (4; 6.79 g, 1.30 equiv), bromide **37** (7.50 g, 1.00 equiv), and K_2CO_3 (10.04 g, 3.06 equiv) in 300 mL of CH_3CN according to method H. Flash chromatography of the resultant oil in 6% MeOH/CH_2Cl_2 afforded **38** (9.00 g, 83%) as an off-white foam: ^{1}H NMR ($CDCl_3$) δ 7.88 (m, 2 H), 7.44 (m, 2 H), 3.83 (b s, 2 H), 3.60 (m, 4 H), 2.73 (b s, 8 H), 2.41 (m, 4 H), 2.23 (s, 2 H), 1.97 (t, *J* = 7.8 Hz, 2 H), 1.60 (b s, 4 H); EIMS *m/z* (relative intensity) 454 (M^+, 56), 291 (100), 232 (48), 177 (45). Conversion to the hydrochloride salt followed by recrystallization afforded **38** as an off-white solid.

8-[4-[4-(1,2-Benzisothiazol-3-yl)-1-piperazinyl]butyl]-8-azaspiro[4.5]decane-2,7,9-trione *S*-Oxide (39). The title compound was prepared from BITP *S*-oxide **5** (1.76 g, 1.05 equiv), bromide **37** (2.24 g, 1.00 equiv), and K_2CO_3 (2.88 g, 2.95 equiv) in 75 mL of CH_3CN according to method H. Flash chromatography of the resultant oil in 5% MeOH/CH_2Cl_2 afforded **39** (2.81 g, 84%) as a yellow foam. Conversion to the hydrochloride salt followed by recrystallization afforded a white powder: ^{1}H NMR (DMSO-d_6) δ 11.65 (b s, 1 H), 8.29 (m, 1 H), 8.13 (m, 1 H), 7.79 (m, 2 H), 4.70 (m, 2 H), 3.90 (m, 2 H), 3.64 (m, 4 H), 3.51–3.04 (m, 4 H), 2.78 (s, 4 H), 2.32 (t, *J* = 7.0 Hz, 2 H), 2.18 (s, 2 H), 1.87 (t, *J* = 7.0 Hz, 2 H), 1.71 (b s, 2 H), 1.49 (b s, 2 H); FABMS *m/z* 471 (MH^+).

General Procedure for Functionalization at C-6 of 8-Azaspiro[4.5]decane-7,9-diones. To a solution of azaspirodecanedione (1.0 equiv) in THF (4 mL/mmol) was added a 1 M solution of $LiN(Me_3Si)_2$ (2.0 equiv) at −78 °C under Ar. The solution was stirred at −78 °C for 3 h and then a solution of electrophile (1.1 equiv) in THF (3 mL/mmol electrophile) was added over 10 min. Stirring was continued at −78 °C for 1.5 h and then acetic acid (1.1 equiv) was added. The cooling bath was removed and the reaction mixture poured into a solution of H_2O/EtOAc (2:3, 3 × reaction volume). The organic phase was separated and washed with H_2O and the combined aqueous phases were back-extracted with EtOAc. The combined organic layers were washed with brine, dried (Na_2SO_4), and evaporated.

8-[4-[4-(1,2-Benzisothiazol-3-yl)-1-piperazinyl]butyl]-6-[[[(4-nitrobenzyl)oxy]carbonyl]oxy]-8-azaspiro[4.5]decane-7,9-dione (40). The title compound was prepared according to the general procedure from tiospirone (1; 3.00 g, 6.82 mmol) and bis(4-nitrobenzyl) peroxydicarbonate (2.94 g, 7.50 mmol). Flash chromatography (EtOAc) of the resulting oil gave the title compound (2.05 g, 47%) as a light yellow foam: IR (neat) 1760, 1735, 1630 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 8.24 (d, *J* = 8.8 Hz, 2 H), 7.88 (dd, *J* = 8.0, 1.1 Hz, 1 H), 7.78 (dd, *J* = 8.0, 1.1 Hz, 1 H), 7.57 (d, *J* = 8.8 Hz, 2 H), 7.48–7.29 (m, 2 H), 5.37, 5.30 (q, *J* = 13.4 Hz, 2 H), 5.26 (s, 1 H), 3.80–3.74 (m, 2 H), 3.55–3.50 (m, 4 H), 2.84, 2.56 (q, *J* = 17.4 Hz, 2 H), 2.66–2.61 (m, 4 H), 2.45–2.38 (m, 2 H), 1.8–1.3 (b m, 12 H). Further elution gave unreacted starting material (0.31 g, 10%).

8-[4-[4-(1,2-Benzisothiazol-3-yl)-1-piperazinyl]butyl]-6-[[[(4-nitrobenzyl)oxy]carbonyl]oxy]-8-azaspiro[4.5]decane-7,9-dione *S,S*-Dioxide (41). The title compound was prepared from tiospirone *S,S*-dioxide (**3**; 2.73 g, 5.78 mmol) and bis(4-nitrobenzyl) peroxydicarbonate (2.49 g, 6.36 mmol) according to the general procedure. Flash chromatography of the resultant gum in EtOAc/CH_3CN (2:1) gave the title compound (1.05 g, 27%) as a bright yellow foam: IR (neat) 1760, 1747, 1683, 1525, 1350, 1165 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 8.23 (d, *J* = 8.7 Hz, 2 H), 7.96–7.91

(m, 1 H), 7.82–7.78 (m, 1 H), 7.72–7.57 (m, 2 H), 7.57 (d, J = 8.8 Hz, 2 H), 5.38 and 5.30 (AB q, J = 13.4 Hz, 2 H), 5.26 (s, 1 H), 4.1–4.0 (m, 4 H), 3.82–3.66 (m, 2 H), 2.84 and 2.62 (AB q, J = 17.5 Hz, 2 H), 2.65–2.56 (m, 4 H), 2.43–2.36 (m, 2 H), 1.82–1.32 (m, 12 H).

General Procedure for the Hydrogenolysis of [[(Nitrobenzyl)oxy]carbonyl]oxy Intermediates. A mixture of the [[(nitrobenzyl)oxy]carbonyl]oxy intermediate and 10% Pd/C (0.25 g/mol substrate) in MeOH/THF (2:1, 10 mL/mmol substrate) was hydrogenated in a Parr shaker at 55 psi for 1.5 h. Additional 10% Pd/C (2 × initial amount) was added and hydrogenation was continued at 55 psi for 1 h. The resulting mixture was filtered through a pad of Celite and the pad was washed with EtOAc. The filtrate was evaporated and the residue was purified by flash chromatography.

8-[4-[4-(1,2-Benzisothiazol-3-yl)-1-piperazinyl]butyl]-6-hydroxy-8-azaspiro[4.5]decane-7,9-dione (42). The title compound was prepared from intermediate **40** (1.98 g, 3.12 mmol). Purification by flash chromatography (EtOAc) gave the title compound (0.98 g, 69%) as a solid. Analytically pure material was crystallized as pale yellow crystals: IR (neat) 3430, 1728, 1673 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.90–7.76 (m, 2 H), 7.49–7.29 (m, 2 H), 4.18 (s, 1 H), 3.82–3.69 (m, 2 H), 3.59–3.54 (m, 5 H), 2.79, 2.52 (AB q, J = 17.4 Hz, 2 H), 2.75–2.66 (m, 4 H), 2.49–2.44 (m, 2 H), 2.11–1.98 (m, 1 H), 1.74–1.19 (b m, 11 H).

8-[4-[4-(1,2-Benzisothiazol-3-yl)-1-piperazinyl]butyl]-6-hydroxy-8-azaspiro[4.5]decane-7,9-dione *S*-Oxide (43). This compound was prepared according to method B from putative tiospirone metabolite **42**. Flash chromatography of the crude reaction product in EtOAc/MeOH (7:3) afforded the title compound in 97% yield. Recrystallization gave a white, microcrystalline solid: IR (neat) 3380, 1722, 1672 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.88–7.80 (m, 2 H), 7.52–7.32 (m, 2 H), 4.27–4.14 (m, 2 H), 4.12 (s, 1 H), 3.90–3.79 (m, 4 H), 3.51–3.41 (m, 2 H), 3.29–3.17 (m, 4 H), 2.85, 2.49 (AB q, J = 17.4 Hz, 2 H), 2.06–1.60 (b m, 11 H), 1.38–1.18 (m, 2 H).

8-[4-[4-(1,2-Benzisothiazol-3-yl)-1-piperazinyl]butyl]-6-hydroxy-8-azaspiro[4.5]decane-7,9-dione *S*,*S*-Dioxide (44). The title compound was prepared from [[(nitrobenzyl)oxy]carbonyl]oxy intermediate **41** (1.04 g, 1.56 mmol). Flash chromatography in EtOAc/MeCN (1:1) afforded the product (506 mg, 66%) as an off-white foam. Crystallization from CH_2Cl_2/ether at −20 °C gave cream-colored crystals: IR (neat) 3480, 1727, 1672, 1303, 1165 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.97–7.92 (m, 1 H), 7.83–7.79 (m, 1 H), 7.73–7.57 (m, 2 H), 4.19 (d, J = 1.7 Hz, 1 H), 4.05–4.00 (m, 4 H), 3.84–3.64 (m, 2 H), 3.49 (d, J = 1.7 Hz, 1 H, D_2O exchangeable), 2.79 and 2.52 (AB q, J = 17.5 Hz, 2 H), 2.60 (t, J = 5.1 Hz, 4 H), 2.40 (t, J = 6.8 Hz, 2 H), 2.09–1.99 (m, 1 H), 1.74–1.33 (m, 10 H), 1.26–1.15 (m, 1 H).

1-[*N*-[4-[4-(1,2-Benzisothiazol-3-yl)-1-piperazinyl]butyl]carbamoyl]-2-oxaspiro[4.4]nonan-3-one (45). A mixture of putative tiospirone metabolite **42** (1.14 g, 2.5 mmol) and KF-Al_2O_3 (0.36 g, 1 equiv) in CH_3CN (25 mL) was vigorously stirred at room temperature under Ar for 4 h. The supernatant was then decanted and replaced with fresh acetonitrile (25 mL), and stirring was continued for 4 h. This process was repeated until the residue contained no more starting material (five times in total). The combined supernatants were evaporated and the resulting gum was purified by flash chromatography (EtOAc, then EtOAc/acetone 1:1) to give first the title compound (0.75 g, 66%) as an off-white foam and then unreacted starting material (0.29 g, 26%) as a pale yellow foam. The title compound was dissolved in excess ethanolic HCl and the solution was evaporated to dryness. Recrystallization gave a cream-colored powder: IR (CH_2Cl_2) 3430, 1785, 1680 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.86–7.80 (m, 2 H), 7.54–7.36 (m, 2 H), 7.29 (b s, 1 H), 4.77 (s, 1 H), 4.12–4.08 (m, 4 H), 3.56–3.50 (m, 2 H), 3.47–3.33 (m, 2 H), 3.14–3.04 (m, 4 H), 2.62, 2.37 (q, J = 17.1 Hz, 2 H), 2.1–1.4 (b m, 13 H).

8-(3-Butenyl)-8-azaspiro[4.5]decane-7,9-dione (47). The title compound was prepared from imide **46** (13.85 g, 1.00 equiv), 4-bromobutene (12.19 g, 1.09 equiv), and K_2CO_3 (13.51 g, 1.18 equiv) in 170 mL of anhydrous DMF according to method H. Flash chromatography of the resultant oil in 20% EtOAc/hexane afforded **47** (13.51 g, 74%) as a yellow oil: IR (neat) 1727, 1676 cm^{-1}; ^1H NMR ($CDCl_3$) δ 5.75 (m, 1 H), 5.04 (m, 2 H), 3.88 (t, J = 7.2 Hz, 2 H), 2.57 (s, 4 H), 2.32 (m, 2 H), 1.71 (m, 4 H), 1.50 (m, 4 H); EIMS m/z (relative intensity) 221 (M^+, 72), 180 (70), 152 (69), 109 (100).

8-(3-Butenyl)-2-hydroxy-8-azaspiro[4.5]decane-7,9-dione (48). The title compound was prepared from imide **31** (2.39 g, 1.00 equiv), 4-bromobutene (1.46 mL, 1.10 equiv), and K_2CO_3 (3.62 g, 2.00 equiv) in 100 mL of CH_3CN according to method H. Flash chromatography of the resultant oil in 50% EtOAc/hexane afforded **48** (1.54 g, 50%) as a clear oil: IR (neat) 3452, 2949, 1723, 1667 cm^{-1}; ^1H NMR (DMSO-d_6) δ 5.71 (m, 1 H), 5.03 (d, J = 8.4 Hz, 1 H), 4.96 (b s, 1 H), 4.59 (d, J = 3.8 Hz, 1 H), 4.12 (m, 1 H), 3.71 (t, J = 7.2 Hz, 2 H), 2.72 (s, 2 H), 2.58 (s, 2 H), 2.18 (m, 2 H), 1.86–1.43 (m, 6 H); CIMS m/z (relative intensity) 238 ([M + H]$^+$, 100), 220 (8), 196 (13), 178 (12), 123 (28).

8-(3,4-Epoxybutyl)-8-azaspiro[4.5]decane-7,9-dione (49). The title compound was prepared from imide **47** (4.54 g) according to method C. Flash chromatography of the resulting residue in 25% EtOAc/hexane afforded the title compound (3.65 g, 75%) as a clear, viscous liquid: ^1H NMR ($CDCl_3$) δ 4.01 (m, 3 H), 2.72 (m, 1 H), 2.61 (s, 4 H), 2.42 (m, 1 H), 1.92–1.44 (m, 10 H); EIMS m/z 237 (M^+).

8-(3,4-Epoxybutyl)-2-hydroxy-8-azaspiro[4.5]decane-7,9-dione (50) was similarly prepared from **48** (1.54 g) according to method C. Flash chromatography in 10% hexane/EtOAc afforded the title compound (1.66 g, 87%) as a clear liquid. Spectral data were consistent with the desired compound.

8-[4-[4-(1,2-Benzisothiazol-3-yl)-1-piperazinyl]-3-hydroxybutyl]-8-azaspiro[4.5]decane-7,9-dione *S*-Oxide (52). Epoxide **49** (2.43 g, 1.00 equiv), BITP *S*-oxide **5** (3.10 g, 1.28 equiv) and K_2CO_3 (2.85 g, 1.01 equiv) were combined in 100 mL of CH_3CN according to method H. Flash chromatography in a 3%–5% MeOH/CH_2Cl_2 gradient afforded the title compound (2.48 g, 51%) as a yellow foam. Conversion to the hydrochloride salt followed by recrystallization afforded a white powder: IR (KBr) 3367, 3064, 2952, 1667, 1532 cm^{-1}; ^1H NMR (DMSO-d_6) δ 10.85 (b s, 1 H), 8.28 (m, 1 H), 8.11 (m, 1 H), 7.77 (m, 2 H), 5.62 (b s, 1 H), 4.68 (m, 2 H), 4.21–3.58 (m, 7 H), 3.51–3.03 (m, 4 H), 2.66 (s, 4 H), 1.72–1.32 (m, 10 H); FABMS m/z 473 (MH^+).

Compound **51** was similarly prepared from epoxide **49** and BITP **4**. Spectral data were consistent with the desired compound. Conversion to the hydrochloride salt followed by recrystallization afforded a white solid.

8-[4-[4-(1,2-Benzisothiazol-3-yl)-1-piperazinyl]-3-hydroxybutyl]-2-hydroxy-8-azaspiro[4.5]decane-7,9-dione (53). Epoxide **50** (1.66 g, 1.00 equiv), BITP (**4**; 1.59 g, 1.11 equiv), and K_2CO_3 (1.82 g, 2.00 equiv) were combined in 75 mL of CH_3CN according to method H. Flash chromatography in 5% MeOH/CH_2Cl_2 gave the title compound (1.45 g, 47%) as a white foam. Conversion to the hydrochloride salt and recrystallization afforded a white powder: IR (KBr) 3378, 2943, 1725, 1670, 1495 cm^{-1}; ^1H NMR (DMSO-d_6) δ 10.21 (b s, 1 H), 8.13 (m, 2 H), 7.60 (m, 1 H), 7.48 (m, 1 H), 5.61 (b s, 1 H), 4.62 (b s, 1 H), 4.22–3.95 (m, 4 H), 3.90–3.39 (m, 10 H), 3.31 (s, 4 H), 2.78–2.48 (m, 4 H), 1.90–1.35 (m, 8 H); FABMS m/z 473 (MH^+).

4-(1,2-Benzisothiazol-3-yl)-1-[[(1,1-Dimethylethyl)oxy]carbonyl]piperazin-2-one *S*,*S*-Dioxide (54). Ruthenium dioxide hydrate (60%, 0.320 g, 0.35 equiv) was added to a solution of $NaIO_4$ (8.92 g, 10.2 equiv) in 75 mL of H_2O. BOC-BITP **7** (1.31 g, 1.00 equiv) was slowly added via addition funnel as a solution in 90 mL of CH_2Cl_2. The biphasic solution proceeds from yellow to dark green to black in minutes. The solution was vigorously stirred for 20 h, after which the phases were separated and the aqueous layer was twice extracted with CH_2Cl_2. The combined organic fractions were filtered through a bed of Celite, dried over anhydrous Na_2SO_4, filtered, and evaporated in vacuo to a blackish solid. Flash chromatography in an EtOAc/hexane (1:1) to EtOAc gradient afforded the title compound (890 mg, 65%) as a white solid: mp 128–30 °C; IR (KBr) 1778, 1735 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.90 (m, 2 H), 7.71 (m, 2 H), 4.70 (b s, 2 H), 4.15 (b m, 4 H), 1.37 (s, 9 H); CIMS m/z (relative intensity) 266 (100).

4-(1,2-Benzisothiazol-3-yl)piperazin-2-one *S*,*S*-Dioxide (55). BOC-BITP derivative **54** (800 mg) was heated in a flask to 200 °C. The white solid was observed to rapidly melt and evolve gas. When the bubbling ceased, the flask was allowed to cool and the resultant solid was flash chromatographed in a 3%–7.5% MeOH/CH_2Cl_2 gradient to give the title compound (575 mg, 99%) as an off-white solid: IR (KBr) 3380, 3250, 1690 cm^{-1}; ^1H NMR

(DMSO-d_6) δ 8.43 (b s, 1 H), 8.19 (m, 1 H), 8.01 (m, 1 H), 7.82 (m, 2 H), 4.72 (b m, 2 H), 4.30 (b m, 4 H); CIMS m/z (relative intensity) 266 ($[M + H]^+$, 100).

Pharmacology. In Vitro Investigations. The methods used in the determination of in vitro binding affinities have been previously reported. Briefly, dopamine D-2 affinity was assayed against [^{3}H]spiperone in homogenized rat corpus striatum tissues.[15] The affinity for 5-HT_{1A} and 5-HT_1 receptors was determined in homogenized rat hippocampal tissues versus [^{3}H]8-OH-DPAT and [^{3}H]5-HT, respectively.[19] The remaining in vitro studies utilized homogenized rat cerebral cortex tissues. The reference ligand used in the determination of 5-HT_2 affinity[20] was [^{3}H]-spiperone; [^{3}H]WB-4101 was used to assay α_1-adrenergic receptor sites.[21] The calculation of the 50% inhibition of specific binding (IC_{50}) was performed in duplicate in the standard manner using a log-probit analysis with $n = 5$; where n is the number of different test ligand concentrations used to calculate the IC_{50}.

In Vivo Investigations. Procedures for the conditioned avoidance response (CAR) test, the inhibition of apomorphine-induced stereotypy (APO) test, and the induction of catalepsy test have all been previously described.[15,17,18]

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Design of Potent Linear PAF Antagonists

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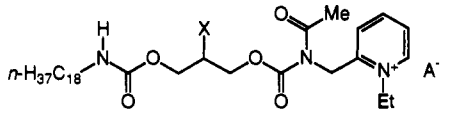
A new series of linear PAF antagonists have been prepared as simplified models of our previously described tetrahydrofuran and dioxolane derivatives.

Introduction

As part of the continuing effort of our laboratories in the area of PAF antagonists, we initiated a research program based on the design of simple, easy-to-make, achiral PAF analogues.[1] A preceding paper described and evaluated a number of PAF-related structures featuring a disubstituted tetrahydrofuran or dioxolane ring as the key modification of the PAF glycerol framework.[2] Those compounds, which were envisioned as conformationally restricted PAF analogues, showed excellent activities and surpassed in some cases that of the chemically related reference compound 2-[[*N*-acetyl-*N*-[[2-methoxy-3-[(octadecylcarbamoyl)oxy]propoxy]carbonyl]amino]-methyl]-1-ethylpyridinium chloride (CV-6209, **1**).[3] In general, we found that the activity of the compounds was maintained within the same order of magnitude independently of the nature of the five-membered ring pattern shown in Figure 1. Furthermore, the presence of a well-defined acyl carbamate pyridinium group (B) at one end and a lipophilic chain (A) at the other proved to be essential for attaining high levels of activity.

Prompted by these results and in search of more simplified compounds, we sought to examine new entities in which the ring would be replaced by a linear, achiral framework maintaining the nature of the substituents. Ideally, these compounds would have an expeditious synthesis and avoid the issue of manufacturing diastereomeric and enantiomeric mixtures. Finally, their evaluation would determine the putative influence upon bioactivity of the C-2 substituent present in several other known glycerol-derived PAF antagonists (e.g. **3** vs **1** and **2**).[3,4] In this paper we report the synthesis and biological evaluation of a series of linear PAF antagonists as substitutes of our previously reported five-membered ring systems.



1: X = OMe; A = Cl (Takeda; CV-6209)
2: X = 3-isoxazolyloxy; A = Cl (Sankyo)
3: X = H; A = I

Chemistry

The synthesis of the target compounds was accomplished by minor variations of known methodologies.[2,3b] The compounds were easily synthesized from inexpensive glycols according to the five-step sequence outlined for the sulfide series in Scheme I, which in general terms comprises (1) derivatization of one of the glycol hydroxyl groups by reaction with either an alkyl halide (ether series,

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