yielding 1.82 g (70.0%) of **16a**: mp 237.9–239.6 °C dec; ^1H NMR (DMSO-d_6) δ (ppm) 2.88–3.14 (br m, 4 H), 7.20 (s, 1 H), 8.29 (br s, 3 H), 9.35 (br s, 2 H); mass spectrum *m/e* (rel inten) 144 (5), 143 (10), 114 (100), 113 (42), 86 (35), 30 (94); exact mass *m/e* 143.051, calcd for $C_5H_9N_3S$ 143.052. Identical to mass spectrum in ref 18.

5-(2-Aminoethyl)-4-methylthiazole Dihydrobromide* (16d). As for compound **7a** from 3.54 g (0.01 mol) of **14d**. The obtained hydrochloric salt which did not crystallize was, after liberation of the free base with $NaOCH_3$ in MeOH, converted to the hydrobromic salt, yielding after crystallization from hot EtOH/EtOAc 2.7 g (88.8%) of **16d**: mp >300 °C; ^1H NMR (DMSO-d_6) δ (ppm) 2.12 (s, 3 H), 2.74–3.14 (br m, 4 H), 7.92 (br s, 3 H), 8.36 (s, 0.5 H), 8.76 (br s, 2 H). ^1H NMR (D_2O) δ (ppm) 2.21 (s, 3 H), 3.04 (t, J = 6.6 Hz, 2 H), 3.28 (t, J = 6.6 Hz, 2 H), ^{13}C NMR (D_2O) δ (ppm) 14.01, 28.53, 25.98, 42.19, 115.29, 136.96, 171.35; mass spectrum, *m/e* (rel inten) 143 (12), 142 (9), 126 (7), 113 (33), 30 (63); exact mass M^+ = 142.054, calcd for $C_6H_{10}N_2S$ 142.056. *Mixed salt 1.64 HBr, 0.36 HCl. Anal. ($C_6H_{12}Br_{1.64}Cl_{0.36}N_2S$) C, H, N, S, Br, Cl.

General Procedure for the Preparation of 4- or 5-(2-Aminoethyl)thiazole Dihydrobromides (16b–d, 17). A solution of 0.01 mol of the appropriate 4- or 5-(2-phthalimidoethyl)thiazole hydrobromide (**14b–d, 15**) is refluxed for 5 h in 50.0 mL of 30% HBr solution. After cooling, the mixture is concentrated in vacuo (20 mmHg, 80 °C). Last traces of H_2O and HBr are removed by coevaporation with toluene, after which the remaining solid is crystallized from an appropriate solvent. By this method the following thiazoles were prepared.

5-(2-Aminoethyl)-2,4-dimethylthiazole Dihydrobromide Hemihydrate (16b). From 3.67 g (0.01 mol) of **14b**, yielding after crystallization from hot EtOH/EtOAc 2.6 g (81.8%) of **16b**, mp dec starting at 200 °C; ^1H NMR (DMSO-d_6) δ (ppm) 2.39 (s, 3 H), 2.82 (s, 3 H), 2.97–3.13 (br m, 4 H), 8.09 (br s, 3 H), 10.30 (br s, 1 H); mass spectrum *m/e* (rel inten) 156 (4), 127 (100), 126 (62), 82 (52), 30 (79); exact mass M^+ = 156.073, calcd for $C_7H_{12}N_2S$ 156.072. Anal. ($C_7H_{15}Br_2N_2SO_{0.5}$) C, H, N, S, Br.

2-Amino-5-(2-aminoethyl)-4-methylthiazole Dihydrobromide (16c). From 3.69 g (0.01 mol) of **14c**, yielding after crystallization from hot EtOH/Et_2O 2.4 g (75.2%) of **16c**: mp dec starting at 275 °C; ^1H NMR (DMSO-d_6) δ (ppm) 2.16 (s, 3 H), 2.84–3.12 (br m, 4 H), 8.08 (br s, 3 H), 9.32 (br s, 2 H); mass spectrum *m/e* (rel inten) 158 (5), 157 (7), 127 (18), 104 (31), 30 (2); exact mass M^+ = 157.065, calcd for $C_6H_{11}N_3S$ 157.067. Anal. ($C_6H_{13}Br_2N_3S$) C, H, N, S, Br.

2-Amino-4-(2-aminoethyl)thiazole Dihydrobromide Hemihydrate (17). From 3.55 g (0.01 mol) of **15**, yielding after crystallization from hot MeOH 1.95 g (63.9%) of **17**, mp dec starting at 110 °C; ^1H NMR (DMSO-d_6) δ (ppm) 2.96 (t, J = 6.3 Hz, 2 H), 3.12 (t, J = 6.3 Hz, 2 H), 6.72 (s, 1 H), 8.04 (br s, 3 H), 9.27 (br s, 2 H), 9.80 (br s, 1 H); mass spectrum *m/e* (rel inten) 144 (47), 143 (4), 115 (42), 114 (100), 30 (16); exact mass M^+ = 143.052, calcd for $C_5H_9N_3S$ 143.052. Anal. ($C_5H_{12}Br_2N_3SO_{0.5}$) C, H, N, S, Br.

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Registry No. **7a**, 142437-63-6; **8**, 24697-70-9; **9**, 3598-60-5; **10a**, 3197-25-9; **10b**, 3783-77-5; **11a**, 133118-36-2; **11b**, 112357-34-3; **12**, 51132-00-4; **13a**, 62-56-6; **13b**, 62-55-5; **13c**, 115-08-2; **14a**, 136604-50-7; **14b**, 136604-56-3; **14c**, 136604-53-0; **14d**, 136604-55-2; **15**, 95914-09-3; **16b**, 142437-64-7; **16b free base**, 142437-68-1; **16c**, 142457-00-9; **16c** free base, 142437-67-0; **16d**, 142437-65-8; **16d** free base, 58981-35-4; **17**, 142437-66-9; **17 free base**, 124458-10-2; phthalic anhydride, 85-44-9; 4-aminobutan-1-ol, 13325-10-5; 4-chloro-1-butanol, 928-51-8; phthalimide, 85-41-6; 5-chloro-2-pentanone, 5891-21-4; 3-buten-2-one, 78-94-4.

Synthesis, Characterization, and Ca^{2+} Antagonistic Activity of Diltiazem Metabolites[1]

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Diltiazem is a calcium antagonist widely used in the treatment of angina and hypertension. The contributions of metabolites of diltiazem to the vasorelaxant effects of diltiazem were investigated. The synthesis and spectroscopic characterization of eight major *cis*-diltiazem metabolites are described. Three of the compounds—N, O-didemethylated metabolite (**21**), O-demethylated metabolite (**22**), and diltiazem *N*-oxide (**27**)—have been recently reported and have not previously been synthesized. The identities of all eight synthetic metabolites have been verified with samples obtained from human urine using combined LC-MS/MS. The Ca^{2+} antagonistic activities of diltiazem and its metabolites (except **27**) were studied on hamster arota preparations depolarized with KCl. The order of potencies (IC_{50} ± SE, μM) is as follows: diltiazem (0.98 ± 0.47) > **17** (2.46 ± 0.38) ≥ **23** (3.27 ± 1.02) > **26** (20.2 ± 10.5) > **22** (40.4 ± 15.4) ≥ **25** (45.5 ± 18.1) > **21** (112.2 ± 33.2) ≥ **24** (126.7 ± 24.2). Structure–activity relationships are also discussed.

Introduction

Ca^{2+} antagonists, widely used in the treatment of angina pectoris, hypertension and certain cardiac arrhythmias, are classified structurally into three groups: dihydropyridines represented by nifedipine and nicardipine, phenylalkylamines represented by verapamil, and benzothaizepines represented by diltiazem.[2–4] Diltiazem is subject to sig-

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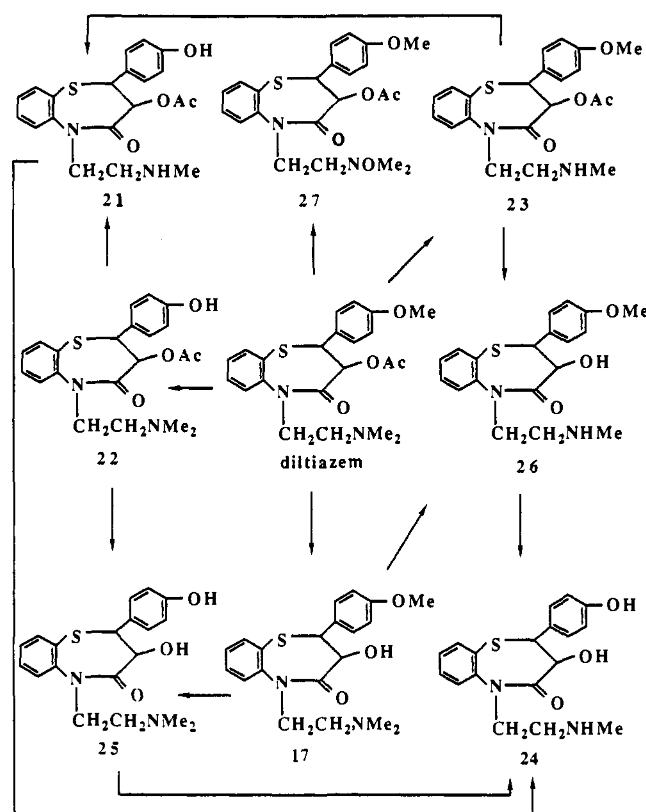
Scheme I. Major Basic Diltiazem Metabolites Found in Human Urine



nificant first pass metabolism and is rapidly and extensively converted in humans to a variety of metabolites (illustrated in Scheme I), several of which have potent pharmacological activities. For instance, the coronary vasodilating potencies of deacetylated diltiazem (**17**) and N-demethylated diltiazem (**23**) are approximately 50% and 20%, respectively, of that of diltiazem.[5] In contrast, **17** and **25** have 2.5 and 5 times the antiplatelet activity of diltiazem, respectively.[6] In recent years, many cases of serious adverse effects which might be linked to diltiazem have been reported, including cutaneous vasculitis,[7] thrombocytopenia,[8] heart block,[9] parkinsonism,[10] and even fatal renal hepatic toxicity.[11] The extent to which the metabolites contribute to both the therapeutic and adverse effects of diltiazem needs to be investigated. Recently, three new diltiazem metabolites were detected from human urine and were tentatively identified as the N,O-dideme-

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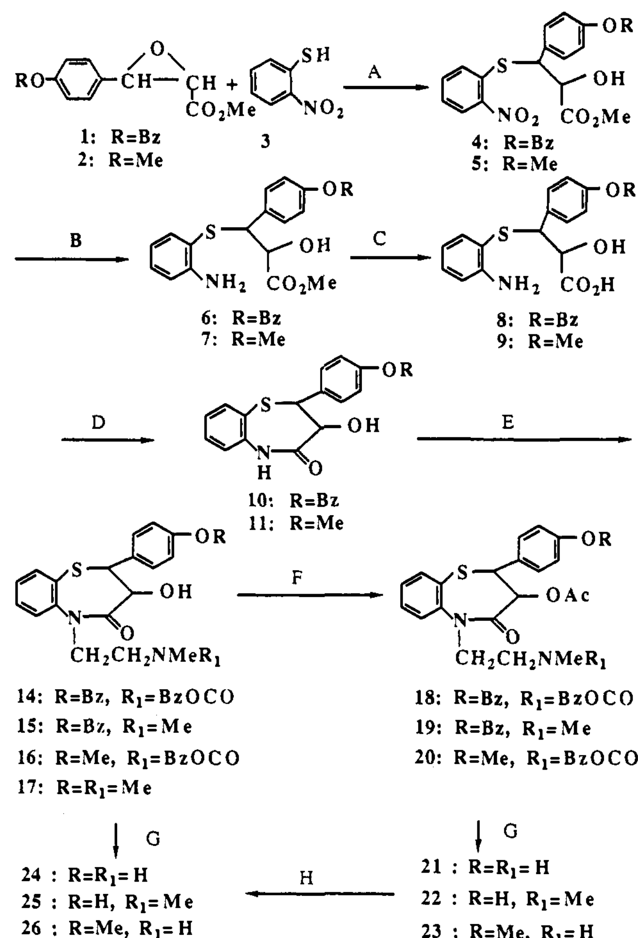
Scheme II. Overall Chemical Synthesis of DTZ Metabolites[a]



[a] Key: (A) $SnCl_2$, dioxane, N_2, room temperature; (B) $FeSO_4$, NH_4OH, H_2O, reflux, 30 min; (C) 5% NaOH, 100 °C, 10 min; (D) xylene, 140 °C, 12 h; (E) N_2, $ClCH_2CH_2NMeR_1$ (**13** if R_1 = BzOCO; (2-chloroethyl)dimethylamine hydrochloride if R_1 = Me), NaH/DMSO, or K_2CO_3/acetone, 50–55 °C overnight; (F) Ac_2O, pyridine, room temperature, 24 h; (G) 25% (w/w) HBr/HOAc, room temperature, 4 h; (H) 9% (w/w) HBr/MeOH, reflux, 10 min for **25**, 8 h for **24**, 5% NaOH in EtOH, room temperature, 7 h for **26**.

thylated metabolite (**21**),[12] the O-demethylated metabolite (**22**),[12] and diltiazem *N*-oxide (**27**). Their identities were not confirmed since no authentic compounds were available.

The purpose of this work was to synthesize the eight major diltiazem metabolites shown in Scheme I, to characterize the three unconfirmed diltiazem metabolites (**21**, **22**, **27**) isolated from biological samples by comparing their retention times on reversed-phase high-performance liquid chromatography (HPLC) and LC-MS/MS with those of synthetic diltiazem metabolites, and to evaluate Ca^{2+} antagonistic activities of diltiazem metabolites.

Results and Discussion

Synthesis of Diltiazem Metabolites. Attempts made initially to prepare **22** from diltiazem by O-demethylation with BBr_3[13,14] failed, leading only to complex mixtures in which **25** was the major component. Eventually **21** and

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22, together with other metabolites, were obtained by the multistep sequence depicted in Scheme II. 27 was obtained by reaction of diltiazem with 3-chloroperbenzoic acid.

Published procedures[15-17] were modified to synthesize key intermediates (10, 11). The glycidic ester 1 was accessible by a Darzens condensation on 4-(benzyloxy)-benzaldehyde.[18-20] Heterogeneous conditions described in the literature, in our hands, gave only low yields. A modified procedure using an appropriate mixture of methanol and dioxane to make the reaction homogeneous improved the yield by 50% compared with the literature.[20] 2-Nitrothiophenol (3) was obtained in quantitative yield by reduction of bis(2-nitrophenyl) disulfide with sodium borohydride in tetrahydrofuran (THF).[21] A direct coupling of freshly prepared 3 with 1 or 2 (from Aldrich) in the presence of tin(II) chloride[22,23] gave 4 or 5, respectively. Reduction of nitro esters 4 and 5 to amino esters 6 and 7 with $FeSO_4/NH_4OH$ was achieved in yields of 65% and 86%, respectively, compared with reported[15] yields of 43% and 71%. Ester hydrolysis with 5% NaOH[15] gave 8 and 9 in high yield. Cyclization of the amino carboxylic acids (8 and 9, respectively) in boiling xylene[15] gave the corresponding cyclic amides (10 and 11) in quantitative yields. One of the alkylating agents, benzyl (2-chloroethyl)-methylcarbamate (13), was prepared in quantitative yield by reacting (2-chloroethyl)methylamine hydrochloride (12) with benzyl chloroformate in basic solution.[24] The alkylation of 10 or 11 with (2-chloroethyl)dimethylamine hydrochloride or 13 gave 14–17[20] in the presence of either NaH/DMSO or K_2CO_3/acetone. Acetylation of 14–16 with acetic anhydride in the presence of pyridine furnished 18–20. The final products (21–23) were obtained by deprotection with 25% (w/w) HBr–HOAc. 24–26 can be obtained either from 14–16, respectively, by deprotection or from 21–23, respectively, by acid or base hydrolysis.

Table I. Chromatographic Properties, Purities of Synthetic Diltiazem Metabolites, and Their in Vitro Biological Data

compd[a]	TLC:[b] $R_f \pm$ SD (n = 3)	HPLC:[c] t_r (min)	% purity	Ca^{2+} IC_{50}[d]
diltiazem	0.57 ± 0.05	16.17		0.98 ± 0.47
17	0.50 ± 0.04	8.53	≥99.5	2.46 ± 0.38
21	0.32 ± 0.03	6.37	≥97	112.2 ± 33.2
22	0.46 ± 0.03	6.74	≥98	40.4 ± 15.4
23	0.36 ± 0.04	14.87	≥99.6	3.27 ± 1.02
24	0.22 ± 0.02	4.34	≥96	126.7 ± 24.2
25	0.47 ± 0.04	4.55	≥95	45.5 ± 18.1
26	0.23 ± 0.02	8.03	≥98	20.2 ± 10.5
27	0.25 ± 0.02	17.51	≥99.6	

[a] For unsystematic names (in parentheses) of diltiazem metabolites, see lit.[20] (17 = M1, 24 = M6, 25 = M4, 26 = M2); lit.[31] (23 = MA) and lit.[12] (21 = MB, 22 = MX). [b] Silica gel 60 F_{254} (Merck), $CHCl_3$/MeOH (1:1); 22 ± 2 °C, monitored at 254 nm. [c] HP1090 fitted with Zorbax Rx-C8 column (4.6 × 250 mm); flow rate, 1.0 mL/min; isocratic, 30% CH_3CN + 0.1% TFA; column temperature, 40 °C; wavelength of diode-array detector, 237 nm. [d] Molar concentration (10^{-6} M) required to block Ca^{2+}-induced contraction of K^+-depolarized hamster aorta by 50%, IC_{50} ± SE, n = 3–8.

Stereochemistry of Synthetic Diltiazem Metabolites. 3-(Acetyloxy)-2,3-dihydro-5-[2-(dimethylamino)-ethyl]-2-(4-methoxyphenyl)-1,5-benzothiazepin-4(5*H*)-one has four stereoisomers due to two asymmetric carbons at positions 2 and 3. It was initially reported[25] that when injected into the femoral vein of the anesthetized dog, the (±)-cis isomers produced a significant increase in the coronary sinus outflow, whereas the (±)-trans isomers were inactive. On the other hand, the (+)-cis isomer (CRD-401, later called diltiazem), whose absolute configuration was assigned as (2*S*,3*S*)-cis,[26] was 1.5–2 times as active as the (±)-cis isomers, while the potency of the (2*R*,3*R*)-cis isomer was very low in comparison with that of the cis racemate. It is very important to make certain that the right isomer is obtained throughout the synthesis. (±)-Methyl *trans*-3-(4-methoxyphenyl)glycidate (2) is commercially available. No stereochemical information for methyl 3-[4-(benzyl-oxy)phenyl]glycidate (1) was available in the literature.[18-20] The stereochemistry of 1 was assigned by ^1H NMR in our study. It has been well established that the coupling constants of methine protons of cis epoxides are usually larger (ca. 5 Hz) than those of their trans isomers (ca. 2 Hz).[27] We have found that chemical shifts and coupling constants of the two methine protons of 1 are identical to those of 2, i.e., δ 3.51 (d, J = 1.7 Hz) and δ 4.05 (d, J = 1.7 Hz). Hence, 1 is assigned as (±)-methyl *trans*-3-[4-(benzyloxy)phenyl]glycidate. The coupling of 1 or 2 with 3 is stereoselective.[22] The threo/erythro ratios in the products (corresponding to the cis-/trans-lactams, respectively) were determined by measuring the ratios of the intensities of methyl ^1H NMR signals in the acetylated derivatives of 4 and 5 as described by Hashiyama et al.[22] The threo/erythro ratios for 4 and 5 were found to be 95/5 and 97/3, respectively. Finally, the cis configuration was confirmed by ^1H NMR spectroscopy on the synthetic metabolites themselves. Coupling constants of the two methine pro-

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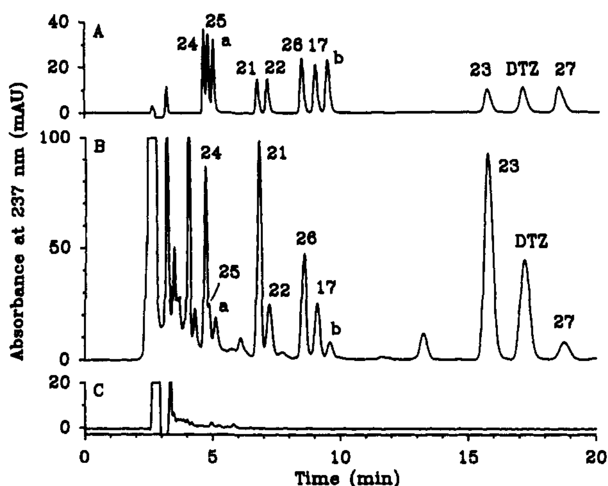

Figure 1. HPLC analysis of standard mixture of diltiazem and its metabolites (A), a human urine extract (B), and a human urine blank (C): (a) **25** *N*-oxide, (b) **17** *N*-oxide; DTZ = diltiazem.

tons (C2 and C3) of all synthetic metabolites measured from 7.24 to 7.79 Hz, consistent with that of (2*S*,3*S*)-*cis*-diltiazem (7.71 Hz).[28] The corresponding coupling constants of *trans*-diltiazem have a typical antiperiplanar value of 11.0 Hz.[28] All synthetic metabolites of diltiazem were confirmed to be (±)-cis racemate by optical rotatory measurements. A commerical sample of diltiazem hydrochloride has a specific optical rotation value of +115° as expected.[29] Diltiazem *N*-oxide obtained from commerical diltiazem has a value of +114°, while all other synthetic diltiazem metabolites have values between +1.3° and −4.6° (see Experimental Section for details).

Spectroscopic Characterization and Purities of Synthetic Diltiazem Metabolites. All intermediates and target products were characterized with infrared (IR) spectroscopy and proton nuclear magnetic resonance spectroscopy (^1H NMR). Some key intermediates such as **10** and **11** and all target products were also characterized with ^{13}C NMR. Multiplicities of ^{13}C resonances were determined by Distortionless Enhancement of Polarization Transfer 135 pulse sequence (DEPT-135). Ammonia chemical ionization or fast atom bombardment mass spectrometry (NH_3 CIMS or FABMS) were also utilized for characterization of all target products. To determine purities of all synthetic metabolites, both reversed-phase HPLC and normal-phase thin-layer chromatography (TLC) were used. Detailed HPLC and TLC conditions are described in the Experimental Section. Retention times on reversed-phase HPLC and R_f values on normal-phase TLC of all synthetic metabolites and their purities are tabulated in Table I.

Identity of Synthetic Diltiazem Metabolites with Biological Samples. Improved chromatographic conditions were developed to reduce the peak tailing observed in our early methods.[30] The surface-deactivated Zorbax column gave outstanding performance with an acetonitrile/water mobile phase containing 0.1% trifluoroacetic acid (TFA) as both ion-pair and acidification reagent. The HPLC retention times (Table I) of the synthetic metabolites were found to correlate with those of the metabolites from human urine (see Figure 1). The combination of HPLC with tandem mass spectrometry (LC-MS/MS) is a powerful tool for the detection, identification and quantitation of drugs and drug metabolites. The recent introduction of the ion-spray LC-MS interface has allowed the analysis of medium to highly polar compounds with high sensitivity (10–100-pg detection limits) and ease of use. Ion-spray is an atmospheric pressure ionization technique[32] that provides very simple spectra dominated by the protonated molecule, $[M + H]^+$. The lack of structural information in such spectra may be overcome by the use of MS/MS to provide fragment ion spectra.[33] In this study, ion-spray LC-MS/MS has been used for the confirmation of all previously synthesized diltiazem metabolites and for the identification of three unconfirmed metabolites (**21**, **22**, and **27**). Ion-spray LC-MS/MS spectra for **21**, **22**, and **27**, with assignments of fragment ion structures, are shown in Figures 2–4. The match of retention times and MS/MS spectra of synthetic compounds with those from human urine supports the assigned

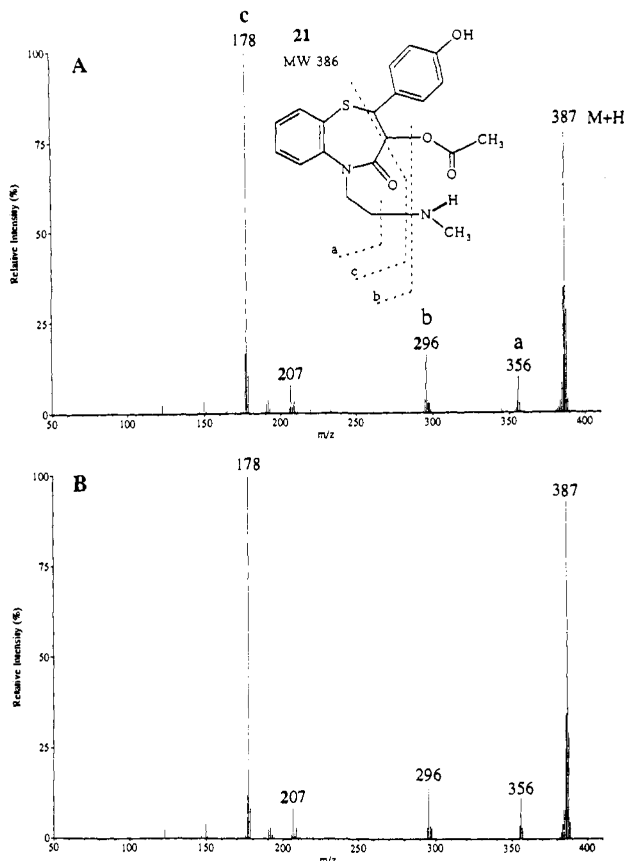

Figure 2. Ion-spray LC-MS/MS spectra for **21** from synthetic standard (A) and human urine extract (B).

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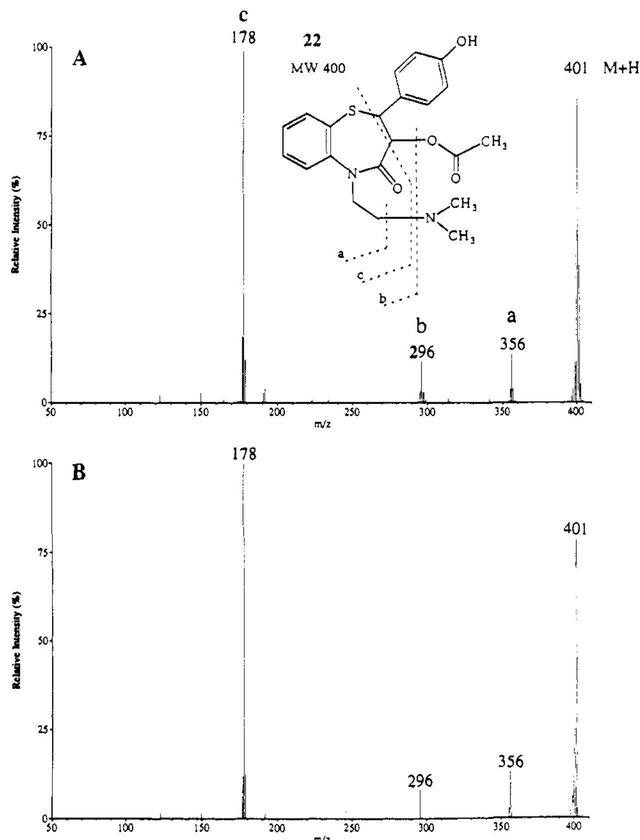

Figure 3. Ion-spray LC-MS/MS spectra for 22 from synthetic standard (A) and human urine extract (B).

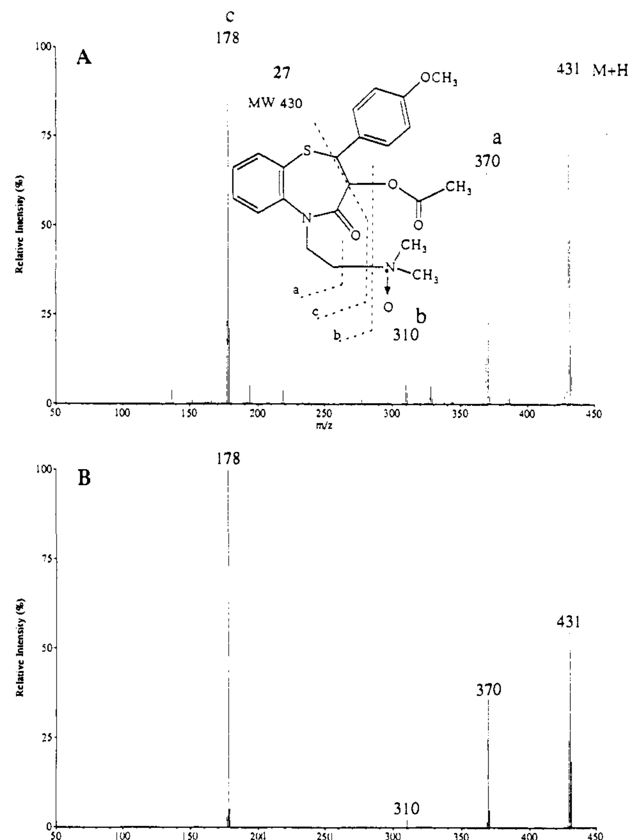

Figure 4. Ion-spray LC-MS/MS spectra for 27 from synthetic standard (A) and human urine extract (B).

structures. A detailed paper on the LC-MS/MS analysis of diltiazem and its metabolites will be published elsewhere.[34]

Ca^{2+} Antagonistic Activity of Diltiazem Metabolites. In order to evaluate Ca^{2+} antagonistic activity of synthetic diltiazem metabolites, we used young hamster aorta ring preparations depolarized with potassium as discussed in the Experimental Section. The vasorelaxant activity recorded in this study is a reliable routine method[35,36] for determining the activity of calcium channel antagonists. For each experiment, diltiazem was used as a control. The IC_{50} values, reported for calcium antagonistic activity, were calculated by a sigmoidal nonlinear regression program according to the concentration response data (Table I). Some of the IC_{50} values were obtained by extrapolation because complete relaxation could not be achieved at the concentrations of metabolites used without compromising accuracy due to changes of organ bath volume. Diltiazem *N*-oxide (**27**) was not tested in this study because of its insolubility in the Krebs solution.

It is believed that diltiazem exerts its vasodilation action through voltage operated calcium channels. There are at least three distinct calcium ligand binding sites, which are specific for 1,4-dihydropyridines, verapamil, and diltiazem.[37] In our functional test for calcium antagonism, we found the vasorelaxant activities of diltiazem metabolites on hamster aorta to have the following rank order of potency: diltiazem > **17** ≥ **23** > **26** > **22** ≥ **25** > **21** ≥ **24** (Table I). This is consistent with the ranking of coronary-vasodilating activity of diltiazem and its known metabolites,[5] and the following structure–activity relationships may be inferred. (a) The *p*-methoxy substituent on the 2-phenyl group seems to be most important for vasorelaxant activity among diltiazem metabolites, perhaps either by interacting with the diltiazem binding site on the receptor or by masking an unfavorable interaction of the phenolic hydroxyl. Thus diltiazem, **17**, **23**, and **26** have dramatically greater potencies than **22**, **25**, **21**, and **24**, respectively. (b) The *O*-acetyl group is not so important for activity, and **17** is the most potent metabolite studied. Similarly, **24** and **25** have comparable potencies with **21** and **22**, respectively. This finding is consistent with an earlier report that the *cis*-acetyloxy group at C3 of diltiazem is not a prerequisite for calcium channel blocking activity.[38] (c) N-Monodemethylation likewise has little effect on metabolite potency, with diltiazem, **22**, and **25** all being only about 3 times as potent as **23**, **21**, and **24**,

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respectively. (d) While loss of either acetyl or *N*-methyl had little effect on biological activity, loss of both decreased activity 10-fold. Thus **26** is significantly less potent than either **17** and **23**.

Since **17** and **23** are nearly equipotent with the parent drug, diltiazem, in calcium antagonistic activity, they may reinforce the therapeutic effect of diltiazem. The extent of this contribution depends upon how much **17** and **23** are formed from diltiazem and their extent of diffusion to the sites of action. Diltiazem can be metabolized to **17** by esterases present in the liver. On the other hand, **23** might be formed mainly by mixed function oxidase in the liver. Since most of the diltiazem metabolites possess hydroxyl, phenol, or secondary amine functional groups, they are further metabolized by conjugation. This may nullify their action as calcium antagonists.

In conclusion, eight *cis*-diltiazem metabolites were synthesized, of which **21**, **22**, and **27** were synthesized for the first time. Characterization of **21**, **22**, and **27** in human urine was made by matching LC-MS/MS retention times and spectra against those of the synthetic compounds.[33] Vasorelaxant activity of each diltiazem metabolite (except **27**) was evaluated using hamster aorta ring preparations.

Experimental Section

All the melting points were measured on a Thomas-Hoover Unimelt apparatus and are uncorrected. Thin-layer chromatography (TLC, silica gel 60 GF_{254}, Merck, Darmstadt) was used to monitor reactions and check product homogeneity. Optical rotatory measurements were made on a Perkin-Elmer 141 polarimeter. Infrared (IR) spectra were recorded with a Perkin-Elmer Model 197 infrared spectrophotometer. Nuclear magnetic resonance (NMR) spectra (tetramethylsilane as internal standard), 300 MHz for ^{1}H and 75.5 MHz for ^{13}C, were recorded on a Bruker MSL 300 spectrometer. Ion-spray LC-MS/MS spectra were recorded on a SCIEX API-III triple quadrupole mass spectrometer. Ammonia chemical ionization mass spectra were acquired on a VG Analytical 20-250 quadrupole mass spectrometer. Fast atom bombardment spectra and accurate mass measurements (resolution = 10000) were measured on a VG Analytical ZAB-EQ mass spectrometer with glycerol matrix. HPLC was performed on HP1090 HPLC with a built-in diode array detector (DAD) and Chemstation. Elemental analyses were performed by Canadian Microanalytical Service Ltd. (Delta, B.C.) and were within 0.4% of the theoretical values. Young hamsters (72–85 days old, Golden Syrian) were purchased from Canadian Hybrid Farms (Halls Harbour, N.S.). All chemicals were purchased from Aldrich Chemical Co., Inc.

Methyl 3-[4-(Benzyloxy)phenyl]glycidate (1). Into a solution of $NaOCH_3$ (Na metal, 8.3 g, 0.36 mol) in MeOH (100 mL) under anhydrous conditions was added a mixture of 4-(benzyloxy)benzaldehyde (50.0 g, 0.24 mol) and methyl chloroacetate (39.0 g, 0.36 mol) in dioxane (100 mL) during 30 min. This reaction mixture was stirred overnight at room temperature and then poured onto ice (ca. 1000 mL). The resultant mixture was neutralized with acetic acid and then extracted with CH_2Cl_2 (4 × 200 mL). The organic layer was dried over $MgSO_4$. After the solvents were removed under reduced pressure, the residue was recrystallized from MeOH to give white pellets of **1** (49.4 g, 74%): mp 107–108 °C (lit.[20] 48%, mp 107–108 °C); IR (KBr) 1750 (C=O), 1250 (C—O) cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 3.51 (d, J = 1.7 Hz, 1 H, CH), 3.82 (s, 3 H, OCH_3), 4.05 (d, J = 1.7 Hz, 1 H, CH), 5.07 (s, 2 H, OCH_2), 6.96–7.44 (m, 9 H, aromatic H).

Methyl 3-[(2-Aminophenyl)thio]-3-[4-(benzyloxy)-phenyl]-2-hydroxypropionate (6). A mixture of **4** (35.4 g, 81 mmol) in MeOH (390 mL) and $FeSO_4 \cdot 7H_2O$ (168.0 g, 605 mmol) in H_2O (390 mL) was refluxed for 30 min. To this mixture was added concentrated NH_4OH (28–30%, 106 mL) during 30 min with mechanical stirring. The mixture was refluxed for an additional 30 min and then allowed to cool. The solids were collected by suction filtration. MeOH was removed from the filtrate under reduced pressure. The resultant aqueous phase was extracted with CH_2Cl_2 (3 × 200 mL). The filtration residue was washed with three 200-mL portions of $MeOH/CH_2Cl_2$ (1:1). The organic extracts were combined and dried over $MgSO_4$. After solvents were removed under reduced pressure, the residue was recrystallized from MeOH (ca. 110 mL) to give **6** as pale yellow crystals (21.4 g, 65%): mp 121–122 °C (lit.[20] 43%, mp 119–120 °C); IR (KBr) 3460 (br) and 3360 (NH_2 and OH), 1720 (C=O), 1240 (C—O) cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 3.56 (s, 3 H, $COOCH_3$), 3.90 (br s, 1 H, OH), 4.31 (br s, 2 H, NH_2), 4.49 (br s, 2 H, CHCH), 5.05 (s, 2 H, OCH_2), 6.60–7.45 (m, 13 H, aromatic H).

Methyl 3-[(2-Aminophenyl)thio]-2-hydroxy-3-(4-methoxyphenyl)propionate (7). Similarly, **5** (22.7 g, 62.5 mmol) gave **7** (17.8 g, 86%) as pale crystals (70% EtOH): mp 90–91 °C (lit.[20] 71%, 93–94 °C); IR (KBr) 3530 (OH), 3450 and 3360 (NH_2), 1735 (C=O), 1250 (C—O) cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 3.57 (s, 3 H, $COOCH_3$), 3.79 (s, 3 H, OCH_3), 3.90 (br s, 1 H, OH), 4.50 (s, 2 H, CHCH), 6.61–7.35 (m, 8 H, aromatic H).

(2-Chloroethyl)methylamine Hydrochloride (12). Into 2-(methylamino)ethanol (7.5 g, 100 mmol) in $CHCl_3$ (10 mL) was added $SOCl_2$ (15.5 g, 130 mmol) in $CHCl_3$ (15 mL) during 1 h. The reaction mixture was stirred for an additional 10 min. Solvent and excess $SOCl_2$ were removed under reduced pressure at a bath temperature not exceeding 75 °C. The residue was recrystallized from acetone to give **12** (12.1 g, 93%) as needles: mp 115–117 °C; ^{1}H NMR (D_2O) δ 2.57 (s, 3 H, NCH_3), 3.24 (t, J = 5.3 Hz, 2 H, NCH_2), 3.68 (t, J = 5.3 Hz, 2 H, $ClCH_2$).

Benzyl (2-Chloroethyl)methylcarbamate (13). Compound **12** (12.0 g, 92 mmol) was dissolved in 4 N NaOH (50 mL) and cooled in an ice-cold water bath. To this solution were added benzyl chloroformate (15.7 g, 92 mmol) and 4 N NaOH (25 mL, 100 mmol) simultaneously with stirring over 30 min, and the stirring was continued for an additional 30 min. The reaction mixture was extracted with ether (3 × 30 mL). After drying with Na_2SO_4, the ether was removed under reduced pressure. The product (oil, quantitative yield) showed only one spot on TLC (CH_2Cl_2/hexane, 1:1) with R_f 0.14 and was used in the next step without further purification; IR ($CHCl_3$) 1680 (C=O) cm^{-1}; ^{1}H NMR (d_6-DMSO) δ 2.89–2.93 (2 br s, 3 H, NCH_3), 3.54–3.60 (m, 2 H, NCH_2), 3.71–3.76 (m, 2 H, $ClCH_2$), 5.09 (br s, 2 H, OCH_2), 7.28–7.46 (m, 5 H, aromatic H); ^{13}C NMR (d_6-DMSO) δ at 25 °C 34.38 and 34.60 (2 s, equal intensity, CH_3), 41.65 and 41.98 (2 s, equal intensity, CH_2), 49.46 and 50.00 (2 s, equal intensity, CH_2), 66.14 and 66.33 (2 s, equal intensity, CH_2), 127.23–136.68 (aromatic C), 155.29 and 155.56 (2 s, equal intensity, C=O); ^{13}C NMR (d_6-DMSO) δ at 60 °C 34.19 (CH_3), 41.36 (CH_2), 49.62 (CH_2), 66.00 (CH_2), 126.04–136.58 (aromatic C), 155.12 (C=O).

2-[4-(Benzyloxy)phenyl]-2,3-dihydro-5-[2-(dimethylamino)ethyl]-3-hydroxy-1,5-benzothiazepin-4(5*H*)-one (15). A mixture of **10** (4.5 g, 11.9 mmol), (2-chloroethyl)dimethylamine hydrochloride (1.89 g, 13.1 mmol), and K_2CO_3 (3.78 g, 27.4 mmol) in DMF (120 mL) was stirred at 50–55 °C under N_2 and kept at the same temperature for 20 h. Inorganic compounds were removed by filtration. The filtrate was evaporated to dryness under reduced pressure. The residue was recrystallized from $CHCl_3$/MeOH (1:2, 18 mL total) to give **15** (2.21 g, 38%): mp 145.5–146.5 °C; R_f = 0.52 ($CHCl_3$/MeOH, 1:1); IR (KBr) 3400 (br, OH), 1670 (C=O), 1240 (C—O) cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 2.28 (s, 6 H, N-$(CH_3)_2$), 2.43–2.51 and 2.66–2.75 (m, 2 H, NCH_2), 2.88 (br d, J = 7.2 Hz, 1 H, OH), 3.67–3.76 and 4.44–4.53 (m, 2 H, $CONCH_2$), 4.31 (br t, J = 7.2 Hz, 1 H, OCH), 4.90 (d, J = 7.2 Hz, 1 H, SCH), 5.07 (s, 2 H, OCH_2), 6.98–7.70 (m, 13 H, aromatic H).

2,3-Dihydro-5-[2-(dimethylamino)ethyl]-3-hydroxy-2-(4-methoxyphenyl)-1,5-benzothiazepin-4(5*H*)-one (17). A mixture of **11** (301.0 mg, 1 mmol), (2-chloroethyl)dimethylamine hydrochloride (158.5 mg, 1.1 mmol), and K_2CO_3 (318.0 mg, 2.3 mmol) in acetone (20 mL) was refluxed for 2 days. Inorganic compounds were removed by filtration. The filtrate was reduced to about 5 mL and treated with HCl/EtOH (10%, w/w). After solvent was removed under reduced pressure, the residue was recrystallized from EtOH to give HCl salt of **17** (260 mg, 58%) as white crystals: mp 224–225.5 °C; R_f = 0.50 ($CHCl_3$/MeOH, 1:1); $[\alpha]^{21}_D$ +0.5° (c = 0.407, H_2O); IR (KBr) 3400–2600 (br, OH and NH^+), 1640 (C=O), 1250 (C—O) cm^{-1}; ^{1}H NMR (d_6-DMSO) δ 2.80 (br s, 6 H, $N(CH_3)_2$), 3.12–3.14 and 3.36–3.44 (m, 2 H, CH_2N^+), 3.76 (s, 3 H, OCH_3), 3.97–4.17 and 4.39–4.58 (m, 2 H, $CONCH_2$), 4.24 (br t, J = 7.1 Hz, 1 H, OCH), 4.88–4.92 (2 d, J = 6.6 and 7.2 Hz, 2 H, SCH, OH), 6.90–7.73 (m, 8H, aromatic H), 10.46 (br s, 1 H, NH^+); the ^{1}H NMR spectrum after D_2O exchange

showed change of a broad triplet to a doublet at 4.24 ppm and disappearance of OH signal in 4.88–4.92 and of NH^+ signal at 10.46 ppm; ^{13}C NMR (d_6-DMSO) δ 42.22 (2 CH_3), 43.58 (CH_2), 52.88 (CH_2), 55.05 (CH_3), 55.85 (CH), 68.89 (CH), 113.30–159.07 (m, aromatic C), 171.38 (NCO); FABMS m/z 373 (M + 1). The product was converted to its HBr salt, mp 224–226 °C (lit.[17] 12%, mp 225–228 °C).

3-(Acetyloxy)-5-[2-[*N*-(benzyloxycarbonyl)-*N*-methylamino]ethyl]-2-[4-(benzyloxy)phenyl]-2,3-dihydro-1,5-benzothiazepin-4(5*H*)-one (18). A mixture of 14 (2.7 g, 4.7 mmol), acetic anhydride (3.9 g, 38 mmol) and pyridine (18.6 g, 235 mmol) was stirred for 24 h at room temperature and for 2 h on a boiling water bath. The reaction mixture was allowed to cool to ambient temperature and then poured into icewater (ca. 60 mL). The mixture was extracted with ethyl acetate and the extract was washed with 5% HCl, brine, and water. The separated ethyl acetate layer was dried over $MgSO_4$. Compound 18 (2.77 g, quantitative yield) was obtained as colorless crystals after the solvent was removed under reduced pressure: mp 51–53 °C; R_f = 0.24 ($CHCl_3$/toluene/MeOH, 8:8:1); IR (KBr) 1740 (C=O, ester), 1695 (C=O, carbamate), 1675 (C=O, amide), 1240 and 1220 (C—O) cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.90 (s, 3 H, $COCH_3$), 2.99–3.02 (2 s, equal intensity, 3 H, NCH_3), 3.50–3.55 (m, 2 H, CH_2NCO_2), 3.85–4.00 and 4.23–4.32 (m, 2 H, $CONCH_2$), 4.99–5.17 (m, 6 H, 2 OCH_2, 2 CH), 6.95–7.68 (m, 18 H, aromatic H); ^{13}C NMR ($CDCl_3$) δ 20.45 (CH_3), 35.16 (CH_3), 47.11 (2 CH_2), 54.43 (CH), 67.15 (CH_2), 70.01 (CH_2), 71.29 (CH), 114.66–159.06 (aromatic C), 156.03–156.31 (2 s, NCO_2), 167.36 (CO), 169.85 (NCO).

3-(Acetyloxy)-2-[4-(benzyloxy)phenyl]-2,3-dihydro-5-[2-(dimethylamino)ethyl]-1,5-benzothiazepin-4(5*H*)-one (19). Similarly, 15 (1.5 g, 3.3 mmol) gave 19 (1.6 g, quantitative yield) as the acetate salt: mp 225–227 °C; R_f = 0.50 ($CHCl_3$/MeOH, 1:1); IR (KBr) 2700–2300 (br, NH^+), 1745 (C=O, ester), 1680 (C=O, amide), 1250 and 1230 (C—O) cm^{-1}; ^1H NMR (d_6-DMSO) δ 1.90 (s, 3 H, $COCH_3$), 2.88 (s, 6 H, $N(CH_3)_2$), 3.23–3.27 and 3.46–3.49 (m, 2 H, CH_2N^+), 4.40–4.44 and 4.55–4.58 (m, 2 H, $CONCH_2$), 5.03 (d, J = 8.0 Hz, 1 H, CH), 5.08 (s, 2 H, OCH_2), 5.12 (d, J = 8.0 Hz, 1 H, CH), 6.98–7.72 (m, 13 H, aromatic H).

3-(Acetyloxy)-5-[2-[*N*-(benzyloxycarbonyl)-*N*-methylamino]ethyl]-2,3-dihydro-2-(4-methoxyphenyl)-1,5-benzothiazepin-4(5*H*)-one (20). Similarly, 16 (0.8 g, 1.6 mmol) gave 20 (0.84 g, quantitative yield) as an oil: R_f = 0.48 (CH_2Cl_2/EtOAc, 1:1); IR (liquid film) 1750 (C=O, ester), 1705 (C=O, carbamate), 1690 (C=O, amide), 1260 and 1230 (C—O) cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.90 (s, 3 H, $COCH_3$), 3.01–3.02 (2 s, equal intensity, 3 H, NCH_3), 3.40–3.60 and 3.62–3.78 (m, 2 H, CH_2NCO_2), 3.81 (s, 3 H, OCH_3), 3.85–4.05 and 4.16–4.38 (m, 2 H, $CONCH_2$), 4.99–5.18 (m, 4 H, OCH_2, CHCH), 6.88–7.67 (m, 13 H, aromatic H).

3-(Acetyloxy)-2,3-dihydro-2-(4-hydroxyphenyl)-5-[2-(methylamino)ethyl]-1,5-benzothiazepin-4(5*H*)-one (21). After 18 (2.0 g, 3.3 mmol) was stirred in freshly prepared 25% (w/w) HBr/AcOH (8 mL) for 4 h at room temperature, the reaction mixture was poured into icewater (ca. 50 mL) and skaken with ether. The aqueous phase was separated and neutralized with powdered $NaHCO_3$ and then extracted with ethyl acetate. The organic layer was separated and dried over $MgSO_4$. The solvent was removed under reduced pressure, and the residue was titrated to pH ca. 4.5 with 9% (w/w) HBr/MeOH. Recrystallization of the residue from ethanol gave the HBr salt of 21 (0.4 g, 27%): mp 260–262 °C dec; R_f = 0.32 ($CHCl_3$/MeOH, 1:1); $[\alpha]^{21}_D$ +0.7° (c = 0.303, H_2O); IR (KBr) 3500–2700 (br, OH, NH^+), 1750 (C=O, ester), 1675 (C=O, amide), 1240 (C—O) cm^{-1}; ^1H NMR (d_6-DMSO) δ 1.96 (s, 3 H, $COCH_3$), 2.78 (s, 3 H, NCH_3), 3.06–3.15 and 3.31–3.43 (m, 2 H, CH_2N^+), 4.10–4.19 and 4.34–4.44 (m, 2 H, $CONCH_2$), 5.00 (d, J = 7.7 Hz, 1 H, CH), 5.06 (d, J = 7.7 Hz, 1 H, CH), 6.86–7.91 (m, 8 H, aromatic H), 8.91 (br s, 2 H, NH_2^+), 9.24 (s, 1 H, phenolic OH); the ^1H NMR spectrum after D_2O exchange showed disappearance of two signals at 8.91 and 9.24 ppm; ^{13}C NMR (d_6-DMSO) δ 19.42 (CH_3), 32.25 (CH_3), 44.72 (CH_2), 45.37 (CH_2), 53.18 (CH), 70.04 (CH), 114.34–156.84 (aromatic C), 166.89 (CO), 168.53 (CO); accurate mass by high-resolution FABMS m/z (M + 1) for $C_{20}H_{23}N_2O_4S$ calcd 387.1379, found 387.1373.

3-(Acetyloxy)-2,3-dihydro-5-[2-(dimethylamino)ethyl]-2-(4-hydroxyphenyl)-1,5-benzothiazepin-4(5*H*)-one (22). Similar treatment of 19 (0.8 g, 1.65 mmol) gave 22, isolated as the HBr salt (0.4 g, 51%): mp 233–235 °C dec; R_f = 0.46 ($CHCl_3$/MeOH, 1:1); $[\alpha]^{21}_D$ −0.5° (c = 0.310, H_2O); IR (KBr) 3500–2700 (br, OH, NH^+), 1740 (C=O, ester), 1675 (C=O, amide), 1230 (C—O) cm^{-1}; ^1H NMR (d_6-DMSO) δ 1.84 (s, 3 H, $COCH_3$), 2.82 (s, 6 H, $N(CH_3)_2$), 3.00–3.22 and 3.30–3.60 (m, 2 H, CH_2N^+), 4.00–4.20 and 4.30–4.50 (m, 2 H, $CONCH_2$), 4.98 (d, J = 7.6 Hz, 1 H, CH), 5.11 (d, J = 7.7 Hz, 1 H, CH), 6.74–7.80 (m, 8 H, aromatic H), 9.58 (s, 1 H, phenolic OH), 9.61 (br s, 1 H, NH^+); the ^1H NMR spectrum after D_2O exchange showed disappearance of two signals at 9.58 and 9.61 ppm; ^{13}C NMR (d_6-DMSO) δ 20.14 (CH_3), 42.44 (2 CH_3), 43.76 (CH_2), 52.75 (CH_2), 53.22 (CH), 70.38 (CH), 114.64–157.42 (aromatic C), 167.08 (CO), 169.17 (CO); accurate mass by high-resolution FABMS m/z (M + 1) for $C_{21}H_{25}N_2O_4S$ calcd 401.1536, found 401.1531.

3-(Acetyloxy)-2,3-dihydro-2-(4-methoxyphenyl)-5-[(2-methylamino)ethyl]-1,5-benzothiazepin-4(5*H*)-one (23). Similarly, 20 (0.8 g, 1.5 mmol) gave 23, isolated initially as the HCl salt (0.24 g, 37%): mp 217–220 °C; R_f = 0.36 ($CHCl_3$/MeOH, 1:1); $[\alpha]^{21}_D$ −4.6° (c = 0.437, H_2O); IR (KBr) 3450–2650 (br, NH^+), 1750 (C=O, ester), 1680 (C=O, amide), 1240 (C—O) cm^{-1}; ^1H NMR (d_6-DMSO) δ 1.84 (s, 3 H, $COCH_3$), 2.59 (s, 3 H, NCH_3), 2.87–3.12 and 3.22–3.33 (m, 1 H, CH_2N^+), 3.78 (s, 3 H, OCH_3), 3.92–4.07 and 4.32–4.55 (m, 2 H, $CONCH_2$), 5.00 (d, J = 7.5 Hz, 1 H, CH), 5.16 (d, J = 7.6 Hz, 1 H, CH), 6.92–7.86 (m, 8 H, aromatic H), 8.96 (br s, 2 H, NH_2^+); the ^1H NMR spectrum after D_2O exchange showed disappearance of only one signal at 8.96 ppm; ^{13}C NMR (d_6-DMSO) δ 20.15 (CH_3), 32.34 (CH_3), 44.69 (CH_2), 44.78 (CH_2), 52.94 (CH), 54.98 (CH_3), 70.24 (CH), 113.46–159.17 (aromatic C), 166.88 (CO), 169.13 (CO); CIMS m/z 401 (M + 1). Treatment of the HCl salt with NH_4OH gave free 23, mp 133–135 °C. Anal. ($C_{21}H_{24}N_2O_4S \cdot {}^1/_2H_2O$) C, H, N.

2,3-Dihydro-3-hydroxy-2-(4-hydroxyphenyl)-5-[2-(methylamino)ethyl]-1,5-benzothiazepin-4(5*H*)-one (24). A mixture of the HBr salt of 21 (0.39 g, 1 mmol) and 9% HBr/MeOH (2 mL) in MeOH (50 mL) was refluxed for 8 h. After cooling, the solvent was removed under reduced pressure. The residue was recrystallized from EtOH to give HBr salt of 24 (0.28 g, 67%): mp 170–172 °C; R_f = 0.22 ($CHCl_3$/MeOH, 1:1); $[\alpha]^{21}_D$ +1.9° (c = 0.357, H_2O); IR (KBr) 3500–2600 (br, OH, NH^+), 1660 (C=O) cm^{-1}; ^1H NMR (d_6-DMSO) δ 2.62 (s, 3 H, NCH_3), 2.88–3.05 and 3.22–3.41 (m, 2 H, CH_2N^+), 3.83–3.99 and 4.30–4.45 (m, 2 H, $CONCH_2$), 4.21 (br t, J = 7.3 Hz, 1 H, OCH), 4.73 (d, J = 7.1 Hz, 1 H, OH), 4.85 (d, J = 7.5 Hz, 1 H, SCH), 6.71–7.72 (m, 8 H, aromatic H), 8.47 (br s, 2 H, NH_2^+), 9.49 (s, 1 H, phenolic OH); the ^1H NMR spectrum after D_2O exchange showed a doublet (J = 7.5 Hz, 1 H) at 4.21 ppm and disappearance of signals at 4.73, 8.47, and 9.49 ppm; ^{13}C NMR (d_6-DMSO) δ 32.66 (CH_3), 44.88 (CH_2), 45.26 (CH_2), 55.86 (CH), 68.57 (CH), 114.60–157.27 (aromatic C), 171.38 (CO); CIMS m/z 345 (M + 1). The HBr salt of 24 was converted to the HCl salt with NH_4OH followed by treatment with HCl/MeOH (10%), mp 240–244 °C (lit.[20] mp > 235 °C).

2,3-Dihydro-5-[2-(dimethylamino)ethyl]-3-hydroxy-2-(4-hydroxyphenyl)-1,5-benzothiazepin-4(5*H*)-one (25). Similar hydrolysis of the HBr salt of 22 (0.2 g, 0.4 mmol) gave the HBr salt of 25 (0.1 g, 57%): mp 217–220 °C dec; R_f = 0.47 ($CHCl_3$/MeOH, 1:1); $[\alpha]^{21}_D$ +1.1° (c = 0.355, H_2O); IR (KBr) 3500–2600 (br, OH, NH^+), 1660 (C=O) cm^{-1}; ^1H NMR (d_6-DMSO) δ 2.84 (s, 6 H, $N(CH_3)_2$), 2.96–3.22 and 3.22–3.40 (m, 2 H, CH_2N^+), 3.91–4.14 and 4.38–4.52 (m, 2 H, $CONCH_2$), 4.21 (br t, J = 7.1 Hz, 1 H, OCH), 4.77 (d, J = 7.0 Hz, 1 H, OH), 4.85 (d, J = 7.4 Hz, 1 H, SCH), 6.71–7.73 (m, 8 H, aromatic), 9.45 (br s, 1 H, NH^+), 9.49 (s, 1 H, phenolic OH); the ^1H NMR spectrum after D_2O exchange showed a doublet (J = 7.5 Hz) at 4.21 ppm and disappearance of signals at 4.77, 9.45, and 9.49 ppm; ^{13}C NMR (d_6-DMSO) δ 42.57 (CH_3), 43.75 (CH_2), 53.10 (CH_2), 56.03 (CH), 68.68 (CH), 114.67–157.29 (aromatic C), 171.45 (CO); FABMS m/z 359 (M + 1). Treatment of HBr salt with NH_4OH gave free 25, mp 136–139 °C (lit.[20] mp 135–138 °C).

2,3-Dihydro-3-hydroxy-2-(4-methoxyphenyl)-5-[2-(methylamino)ethyl]-1,5-benzothiazepin-4(5*H*)-one (26). After the HCl salt of 23 (0.5 g, 1.1 mmol) was stirred in 5% NaOH/EtOH (10/150 mL) for 7 h at room temperature, the reaction mixture was neutralized with 10% HCl. The solvents were removed under reduced pressure, and the residue was treated with 5% NaOH and extracted with ethyl acetate. The organic layer was separated

and dried over $MgSO_4$. After the solvent was removed, the residue was treated with HCl/MeOH. Recrystallization of the residue from MeOH/EtOAc (1:6) gave the HCl salt of **26** (0.4 g, 89%): mp 200–202 °C (lit.[20] mp 203–207 °C); R_f = 0.23 ($CHCl_3$/MeOH, 1:1); $[\alpha]^{21}_D$ -1.2° (*c* = 0.424, H_2O); IR (KBr) 3500–2600 (br, OH, NH^+), 1650 (C═O) cm^{-1}; ^1H NMR (d_6-DMSO) δ 2.59 (s, 3 H, NCH_3), 2.85–3.05 and 3.25–3.40 (m, 2 H, CH_2N^+), 3.76 (s, 3 H, OCH_3), 3.85–4.00 and 4.35–4.45 (m, 2 H, $CONCH_2$), 4.24 (t, *J* = 7.1 Hz, 1 H, OCH), 4.82 (d, *J* = 6.9 Hz, 1 H, OH), 4.91 (d, *J* = 7.4 Hz, 1 H, SCH), 6.89–7.73 (m, 8 H, aromatic H), 8.45 (br s, 2 H, NH_2^+); the ^1H NMR spectrum after D_2O exchange showed a doublet (*J* = 7.4 Hz) at 4.24 ppm and disappearance of signals at both 4.82 and 8.45 ppm; ^{13}C NMR (d_6-DMSO) δ 32.54 (CH_3), 44.88 (CH_2), 45.22 (CH_2), 54.99 (CH_3), 55.65 (CH), 68.58 (CH), 113.20–159.04 (aromatic C), 171.36 (CO); CIMS *m/z* 359 (M + 1).

3-(Acetyloxy)-2,3-dihydro-5-[2-(dimethylamino)ethyl]-2-(4-methoxyphenyl)-1,5-benzothiazepin-4(5*H*)-one *N*-Oxide (27). A solution of diltiazem free base (0.9 g, 2.2 mmol), converted from commercial diltiazem hydrochloride, and 3-chloroperbenzoic acid (0.7 g, 2.2 mmol as 50–60% purity) in 15 mL of chloroform was stirred at room temperature (20 °C) for 2 days. The solvent was removed under reduced pressure and the residue was passed through an alumina column using $CHCl_3$/MeOH (9:1) as an eluent. The fractions containing two spots on TLC ($CHCl_3$/MeOH, 1:1) were evaporated to dryness, the residue was dissolved in chloroform (50 mL). The resultant solution was shaken with water (20 × 5 mL) until the TLC of the organic layer showed only one spot. The organic solvent was removed and the residue was recrystallized from MeOH/ether to give **27** (0.25 g, 27%): mp 65–68 °C; R_f = 0.25 ($CHCl_3$/MeOH, 1:1); $[\alpha]^{21}_D$ +114° (*c* = 0.316, MeOH); IR (KBr) 1745 (C═O, ester), 1680 (C═O, amide), 1240 (C—O), 960 (N—O) cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.91 (s, 3 H, $COCH_3$), 3.23 (s, 3 H, NCH_3), 3.33 (s, 3 H, NCH_3), 3.42–3.61 and 3.80–3.93 (m, 2 H, CH_2N^+), 3.83 (s, 3 H, OCH_3), 4.56–4.75 and 4.99–5.08 (m, 2 H, $CONCH_2$), 5.03 (d, *J* = 7.8 Hz, 1 H, CH), 5.15 (d, *J* = 7.8 Hz, 1 H, CH), 6.90–7.70 (m, 8 H, aromatic H); ^{13}C NMR ($CDCl_3$) δ 20.42 (CH_3), 45.53 (CH_2), 54.53 (CH), 55.26 (CH_3), 59.49 (CH_3), 60.13 (CH_3), 66.92 (CH_2), 71.27 (CH), 113.87–159.84 (aromatic C), 168.29 (CO), 169.85 (CO). Accurate mass of **27** by high-resolution FABMS *m/z* (M + 1) for $C_{22}H_{27}N_2O_5S$ calcd 431.1641, found 431.1658. Anal. ($C_{22}H_{26}N_2O_5S \cdot 2H_2O$) C, H, N.

Identification of New Diltiazem Metabolites. Urine samples were collected from healthy human volunteers over a 24-h period after they were given a single 90-mg oral dose of diltiazem. Details of the method used to isolate diltiazem and its basic metabolites from urine have been reported.[30] Briefly, to 0.5–1.0 mL of urine was added 0.5 mL of 10% $(NH_4)_2CO_3$ solution. The mixture was extracted with 5 mL of methyl *tert*-butyl ether, followed by 5 mL of CH_2Cl_2. After centrifugation to separate the organic from the aqueous layer, the combined organic fraction was evaporated to dryness under a gentle stream of N_2 at 50 °C. The residue was reconstituted with 0.2 mL of 0.01 N HCl and washed with 2 mL of methyl *tert*-butyl ether. After separation by centrifugation, the aqueous layer was lyophilized (SpeedVac, Savant Corp.) and the dry residue stored at -20 °C until analysis. On the day of analysis, the residue was dissolved in MeOH and aliquots were analyzed by HPLC as described for the synthetic metabolites (Table I, footnote *c*).

Ca^{2+} Antagonistic Activity of Diltiazem Metabolites on Hamster Aorta. Isolated aorta ring preparations (about 3 mm ring, from hamsters weighing 104–136 g) were suspended in a 20-mL jacketed organ bath with normal Krebs ($CaCl_2$ 2.0 mM, $MgSO_4$ 1.2 mM, KH_2PO_4 1.0 mM, KCl 5.0 mM, $NaHCO_3$ 25 mM, NaCl 105 mM, D-glucose 11.5 mM) at about 29 °C. A mixture of 95% O_2 + 5% CO_2 was bubbled though the medium to yield pH of 7.4. After equilibration for at least 1 h, the muscle was washed with high-K^+ (110 mM) Krebs solution in which NaCl was replaced by an equimolar amount of KCl. After attainment of a steady plateau tension, the muscle was exposed to increasing concentrations of test compounds and relaxant responses were recorded and normalized with respect to the initial recorded tension. IC_{50} values were calculated by a sigmoidal nonlinear regression program (GraphPAD INPLOT version 3.0) and tabulated in Table I.

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