STUDY OF DILTIAZEM METABOLITES AND RATIONAL DESIGN OF NOVEL CALCIUM ANTAGONISTS

by

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TO YAN AND JERRY

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Abstract

This thesis includes the study of diltiazem (DTZ) metabolites and the rational design of novel class of dibenzotricyclic calcium antagonists.

DTZ is a calcium antagonist widely used in the treatment of cardiovascular disorders. DTZ is subject to significant first pass metabolism and is rapidly and extensively converted in humans to a variety of metabolites, several of which have potent pharmacological activities. Three newly discovered DTZ metabolites have recently been reported and tentatively identified as *N,O*-didemethylated DTZ (**4**), *O*-demethylated DTZ (**5**), and DTZ *N*-oxide (**6**). However, their identities were not confirmed since no authentic compounds were available.

Eight DTZ metabolites have been successfully synthesized, three of which were synthesized for the first time, some requiring 12 sequential chemical reactions. All eight synthetic DTZ metabolites were fully characterized. The three new DTZ metabolites (**4**, **5** and **6**) isolated from human urine are identical to the corresponding authentic compounds I synthesized as confirmed by spectroscopic and chromatographic methods. *In vitro* calcium antagonistic activities of all metabolites (except **6**) were evaluated on hamster aorta.

Attempts to find a new class of calcium antagonists with tissue selectivity have been made for more than a decade. Recently, a series of compounds with dibenzotricyclic systems (DBTs) were reported as a new class of calcium antagonists with tissue selectivity [Kurokawa, M. et al. *J. Med. Chem.* **1991**, *34*, 927—934]. These DBTs may confer antianginal activity without an effect on blood pressure. Structure-activity relationships showed that calcium antagonistic activity increased with a decrease of the angle between the planes of the two benzene rings in DBTs.

A number of dibenzothiazepinones and dibenzoxazepinones have been designed, assisted with molecular modeling. X-Ray and molecular mechanics calculations show that the DBTs in this study have smaller angles between the planes of the two benzene rings than the DBTs reported. As calcium antagonists, the DBTs synthesized in this study showed the potencies of their vasorelaxant activities to be comparable with that of DTZ at submicromolar concentration. Some dibenzothiazepinone derivatives also have anti-HIV activity.

Effects of all the DBTs and DTZ metabolites **4** and **5** on adenosine uptake by erythrocytes were also studied.

List of Abbreviations

The abbreviations used in this thesis are those detailed in the Notice to Authors in *J. Med. Chem.* **1991**, *31*, 11A—14A; except for those defined in the text.

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INTRODUCTION

1. 1 General Introduction

Calcium ions (Ca^{2+}) play a vital role in many biological processes including a variety of enzymatic reactions, activation of excitable cells, coupling of electrical activation and cellular secretion, hemostasis. The regulation of the intracellular concentration of this ion makes it possible to control such Ca^{2+}-dependent processes. The first drug shown to interfere with calcium function was verapamil.[1] At least five more different chemical families have been added according to the World Health Organization classification (Table 1 and Figure 1).[2] Calcium antagonists are a chemically heterogeneous group of

Table 1. Calcium Antagonist Classification

I. Phenylalkylamines	**II. Dihydropyridines**
Verapamil	Nifedipine
Gallopamil	Nitrendipine
Anipamil	Felodipine
III. Benzothiazepines	**IV. Diphenylpiperazines**
Diltiazem	Flunarizine
	Cinnarizine
V. Prenylamine Derivatives	**VI. Others**
Prenylamine	Bepridil
Fendiline	Caroverine
Terodiline	Perhexiline

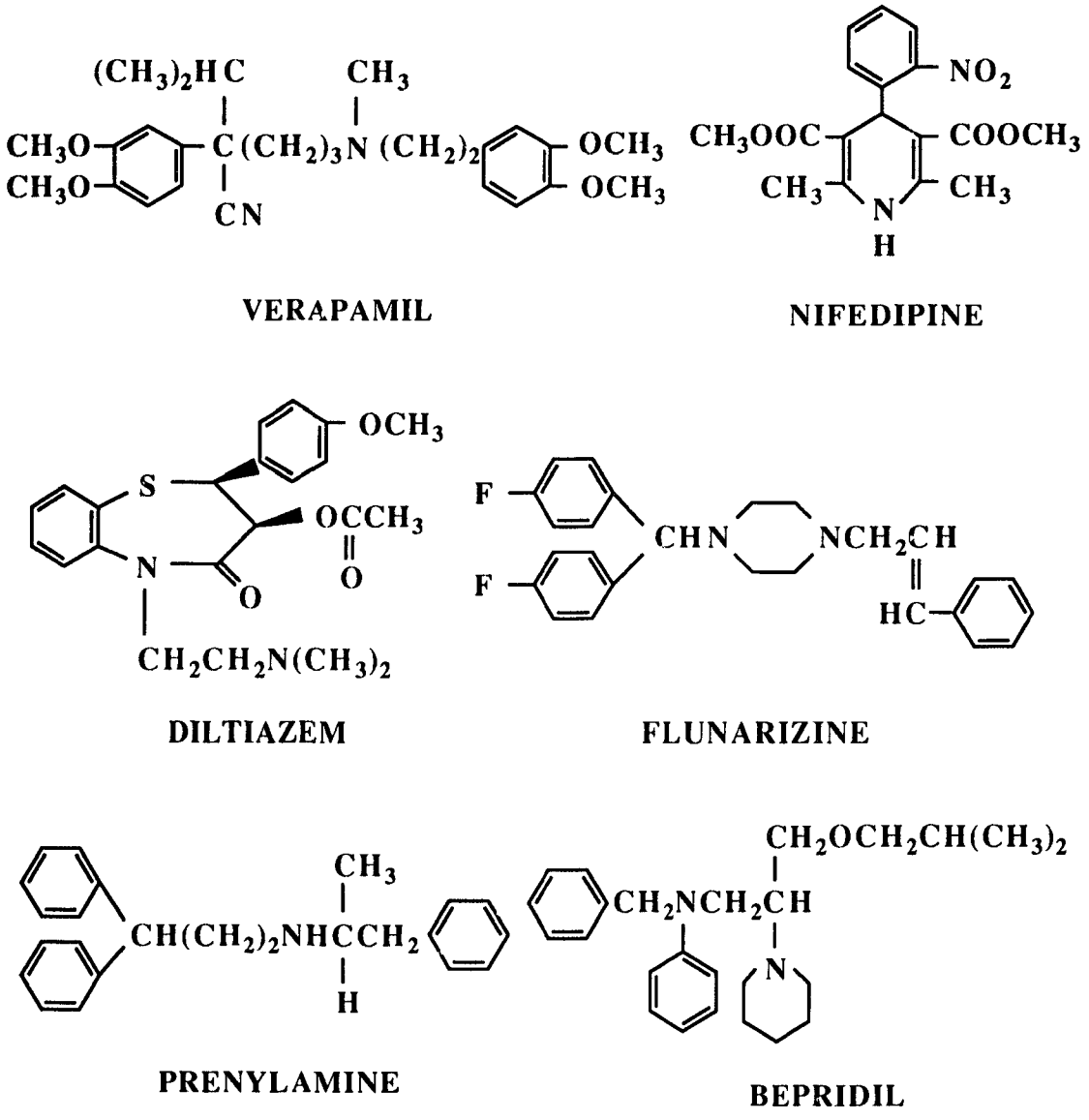


Figure 1. Representative chemical structures of different classes of calcium antagonists

compounds. Of the agents modifying Ca^{2+} mobilization or Ca^{2+} action, only the first three classes have achieved general therapeutic significance, although flunarizine[3] and lidoflazine[4] were marketed outside of North America for the treatment of cerebral and peripheral cardiovascular disorders several years ago and flunarizine was introduced into the Canadian market in 1991[5]. Verapamil,[6] diltiazem[7] and nifedipine,[8] representatives of first generation of calcium antagonists, are currently used for the treatment of angina and hypertension. In addition, verapamil is useful in correcting supraventricular arrhythmias.[9]

1. 1. 1 Mechanism of Action of Calcium Antagonists

1. 1. 1. 1 Role of Calcium

There are two principle contractile proteins in myocardium and vascular smooth muscle, actin and myosin. Contraction of all forms of muscle is an energy-requiring cyclic process involving interaction between myosin and actin. This process is calcium dependent.

In the specialized automatic and conducting cells in the heart, Ca^{2+} is involved in the genesis of the action potential, and in the myocardial contractile cells it links excitation to muscular contraction and controls energy storage and use. The lumina of systemic and coronary arteries are influenced by the movement of Ca^{2+} across the cell membranes of vascular smooth muscle.

In the relaxed state the concentration of Ca^{2+} —i.e., $[Ca^{2+}]$—in the myocardial cytoplasm (myoplasm) is several orders of magnitude lower than the extracellular concentration. During activation of the cardiac cell there is a marked rise in $[Ca^{2+}]$ in the myoplasm, where it binds to a specific subunit of the regulatory protein, troponin. In the

presence of ATP this process allows contraction to occur. A reduction of myoplasmic [Ca^{2+}] causes a dissociation of Ca^{2+} from troponin, which results in muscular relaxation.[10]

Although contraction of vascular smooth muscle, such as that in the walls of the coronary and systemic arterioles, also requires interaction between actin and myosin, there are important differences between the biochemical control of contraction in vascular smooth muscle and that in myocardium. The process that regulates contraction in vascular smooth muscle results from a cascade of reactions, the first of which involves a small Ca^{2+}-binding protein called calmodulin. When the [Ca^{2+}] in the vascular smooth muscle rises to approximately 10^{-6} M, Ca^{2+} binds to calmodulin, and the Ca^{2+}-calmodulin complex activates the enzyme myosin kinase; this in turn phosphorylates a light chain of myosin, which permits myosin to interact with actin, thereby leading to contraction of muscle cell and arteriolar constriction.[11] Although there are notable differences in the contractile mechanisms of myocardium and vascular smooth muscle, it is clear that the myoplasmic [Ca^{2+}] is critical to the contraction of both forms of muscle.

1. 1. 1. 2 Regulation of Myoplasmic [Ca^{2+}] through the Calcium Channels

Myoplasmic [Ca^{2+}] depends ultimately on the entry of Ca^{2+} into the cell. There are several mechanisms[12] that control the myoplasmic [Ca^{2+}], one of which is through the calcium channel.

The sarcolemma is an ion-impermeable lipid bilayer composed of phospholipid molecules separating aqueous compartments. Macromolecular proteins that traverse the

lipid bilayer and selectively permit ions to move from one side of this barrier to the other are referred to as ion channels.

Calcium channels are such proteins that serve the dual function of carrying current and chemical information and serve to link both electrical and chemical membrane stimulus-response coupling. Plasmalemmal calcium channels are usually categorized as receptor-operated (ROC) and potential-dependent (PDC) channels.[13] Sometimes PDC are also called voltage-dependent channels. The latter are also well known as receptor-mediated modulation of potential-dependent calcium channels.[14—17] The primary mode of action of calcium antagonists is at these PDC.

1. 1. 2 Current Status of Calcium Channels

1. 1. 2. 1 Classification of Calcium Channels

Plasmalemmal calcium channels are categorized as three distinct classes,[18,19] i.e., L, T and N-type. They are distinguishable by several characteristics including electrophysiological and pharmacological properties and by tissue and cellular localization and function. The calcium antagonists—nifedipine, verapamil and diltiazem—have played a major role in the pharmacological and biological characterization of potential-dependent calcium channels. The L channels inactivate slowly, are of large conductance, and are of importance in tissues, including the cardiovascular system, where relatively sustained and large amounts of Ca^{2+} are required. By contrast the T or transient channels, inactivate rapidly, and are likely to be involved in the generation of rhythmic or pacemaker activities, and to serve as a trigger for the initiation of other ionic currents. N channels have thus far been described only in neuronal cells, and this selective localization could explain, in part,

the insensitivity of many neuronal processes to the therapeutically available calcium antagonists.[19,20]

1. 1. 2. 2 Modulation of Calcium Channels

Voltage-dependent calcium channels are modulated both positively and negatively by a variety of mechanisms. Several messenger systems interact with ion channels in general to control channel availability.[14—16] These messenger systems, including cAMP, cGMP, protein kinase C, and calcium calmodulin, are frequently dually regulated by activating or inhibitory receptors. Additionally, the effect of a modulating process may differ according to the channel type and tissue localization. The enhancement of cardiac calcium channel function by cAMP-dependent protein phosphorylation has been particularly described.[21] Both stimulatory and inhibitory effects of protein kinase C activation have been observed for calcium channels in neurons and other preparations.[22—24] Regardless of the specific messenger, it is likely that channel phosphorylation is the key modulatory event, and that a variety of mechanisms may be involved in the same and different channel types. Calcium channels are also regulated through interaction with guanine nucleotide (G) binding proteins.[25—27] The actions of calcium channel ligands at the 1,4-dihydropyridine sensitive L-type channel in sensory neurons are modulated by G protein ligands, whereby the antagonists in the presence of GTPγS potentiate calcium current, an effect that is blocked by pertussis toxin.[28] This suggests that G protein state influences the mode of drug interactions at these channels. This channel G protein interaction may be an important determinant of channel function and drug affinity and mechanism.

1. 1. 2. 3 Structure of Calcium Channels

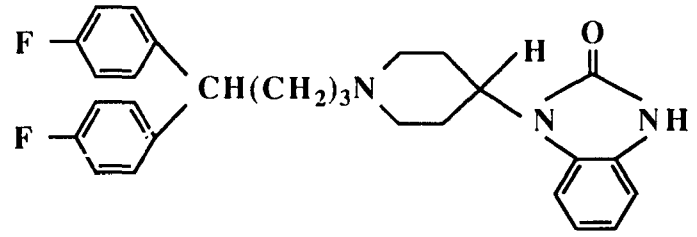
The 1,4-dihydropyridines (1,4-DHPs) have been used as high-affinity probes for the purification of the calcium channel / drug receptor complex from skeletal muscle. As many as five putative subunits have been identified and designated α_1, α_2, β, γ and δ [16, 29—32] The full length amino acid sequences of three of these putative skeletal muscle subunits (α_1, α_2 and β) have been deduced from cDNA clones [33—35] Studies indicate that only the α_1 subunit binds the calcium antagonists,[36] but it is likely that the other subunits are also concerned as an oligomeric assembly with calcium channel function [28,29]

Studies of calcium channels from different tissue sources show tissue-specific variation. Tissue-specific isoforms of DHP-sensitive calcium channels ("isochannels") have been shown to possess distinct electrophysiological, pharmacological, and structural properties. Molecular biological studies have revealed the primary sequences of both the skeletal muscle[33,34] and cardiac muscle[37,38] α_1 subunits The cardiac and skeletal muscle α_1 subunits are similar and share structural features with other voltage-dependent cation channels. These α_1 subunits, like the α subunits of Na^+ channels, are arranged in four repeating hydrophobic motifs, each consisting of six conserved transmembrane domains Partial amino acid sequences of tissue-specific α_1 subunits (skeletal, brain, cardiac and aorta) were compared. It suggests that differences among the species probably represent tissue specific variations of the α_1 subunits [39]

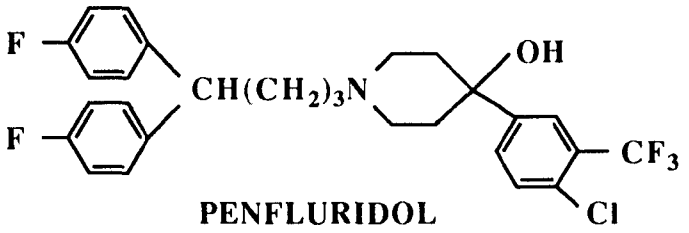
1. 1. 2. 4 A Number of Binding Sites for Calcium Antagonists

Over 70 compounds in various stages of clinical or preclinical development have been classified as calcium antagonists.[40] The major structural categories of ligands active at voltage-dependent calcium channels are the phenylalkylamine, 1,4-DHP and

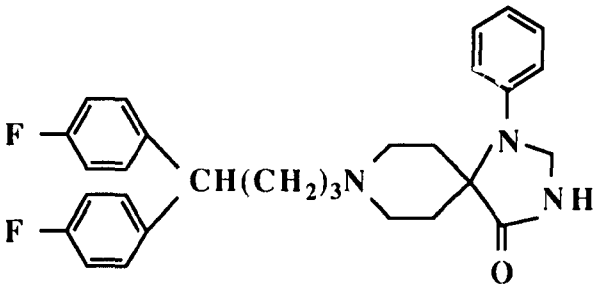
benzothiazepine classes depicted in Figure 1. These agents have been well characterized and act primarily at the L class of channel. There is good evidence that the phenylalkylamine, 1,4-DHP and benzothiazepine categories define three allosterically linked binding sites on a major protein component of the channel.[41] Additionally, discrete sites of action in brain and smooth cardiac and skeletal muscle are defined by the neuroleptic diphenylpiperidines including pimozide, penfluridol, and fluspirilene[42—44]



PIMOZIDE



PENFLURIDOL



FLUSPIRILENE

Figure 2. Structures of neuroleptic diphenylpiperidines

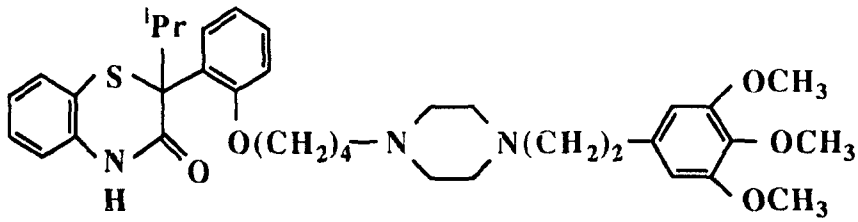
(Figure 2) and a fourth binding site for fluspirilene has been demonstrated.[45] A fifth and a sixth, allosterically linked binding sites on the L type of calcium channel have been also defined with HOE 166[46] (**I** in Figure 3) and SR 33557 (Figure 3),[47] respectively. It is clear that a very large number of structures possess some degree of calcium antagonistic activity.

1. 1. 3 Current Status of Calcium Antagonists

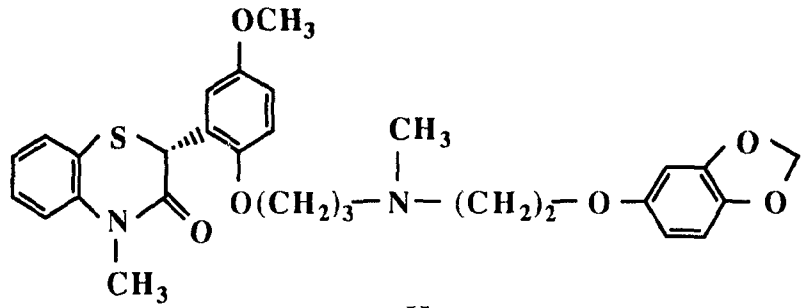
Besides currently used calcium antagonists, there are several new structural classes of ligands added,[48] some of which[46—50] are shown in Figure 3. These calcium antagonists appear to exert their effects primarily at the L type of channels. Compound **I** (HOE 166),[46] combining structural features from several classes, binds with high affinity, K_D 10^{-10} M, to skeletal muscle with a similar density to that defining other ligands. In smooth and cardiac muscle, **I** exhibits a potency intermediate between nifedipine and verapamil. Compound **II**,[49] possessing some structural similarity to **I**, showed the lowest cardioselectivity compared to verapamil and diltiazem. This suggests that compound **II** would exhibit fewer adverse effects due to cardiac inhibition than diltiazem and verapamil in therapeutic use. Compounds **III—VI**[50] represent a novel class of calcium antagonists. Compound **III** possessed the most potent calcium antagonistic activity *in vitro* in this series, being *ca.* 10 times more potent than that of diltiazem.

1. 2 Diltiazem and Its Metabolites

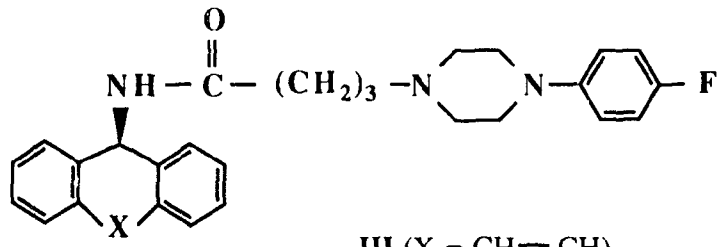
Diltiazem (DTZ) is a calcium antagonist widely used in the treatment of angina pectoris.[51,52] It probably acts by increasing myocardial oxygen supply and decreasing



I (HOE 166)



II

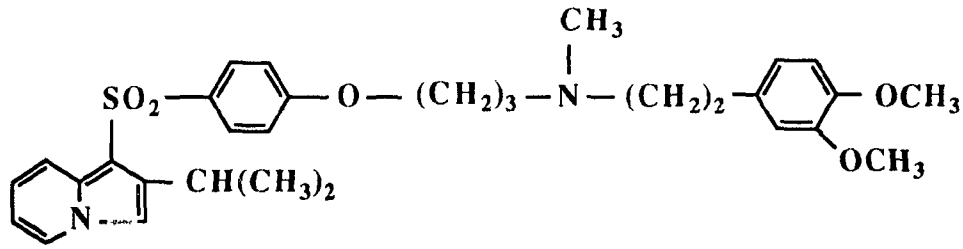


III (X = CH═CH)

IV (X = CH_2S)

V (X = CH_2O)

VI (X = CH_2CH_2)

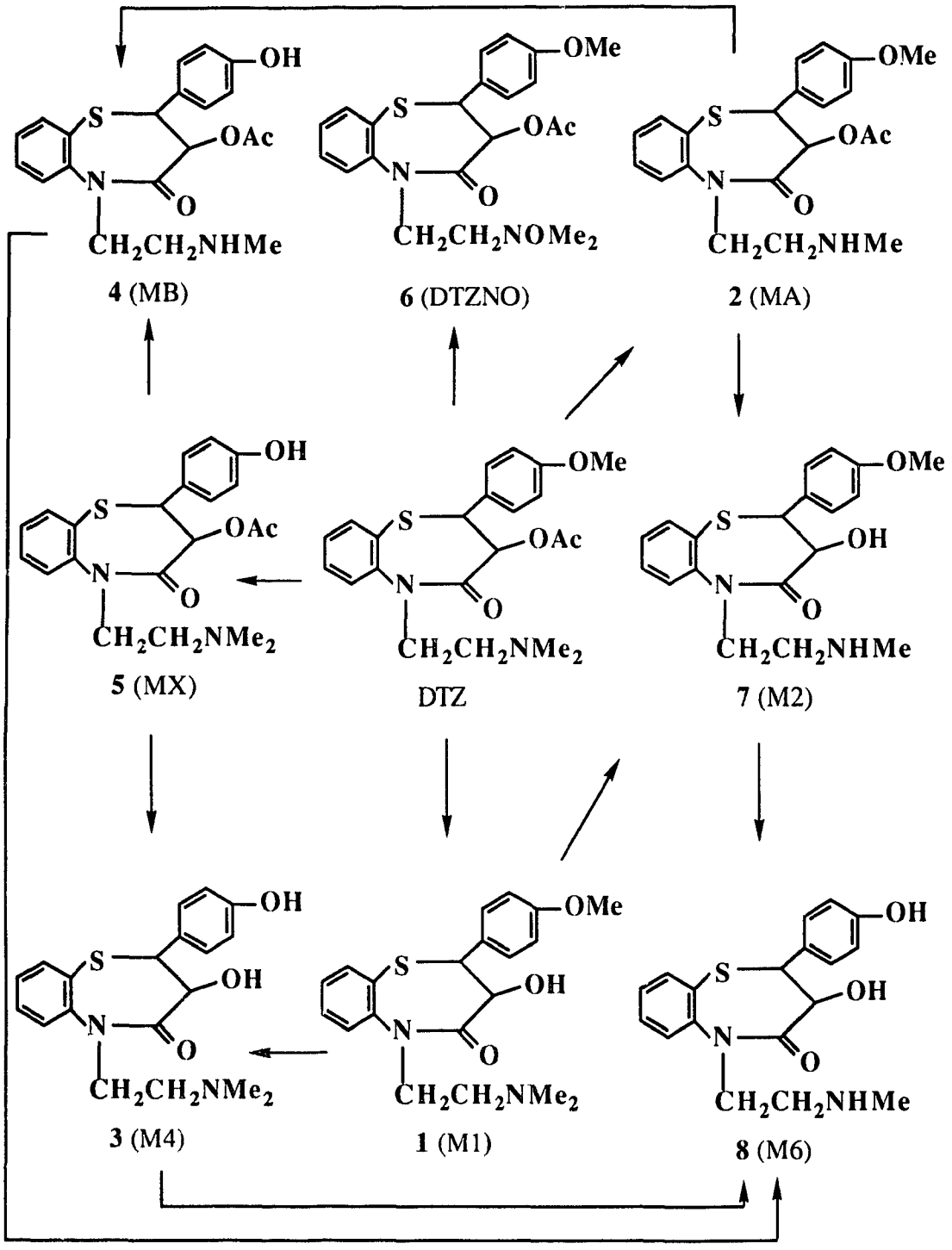


SR 33557

Figure 3. Some new structural classes of calcium antagonists

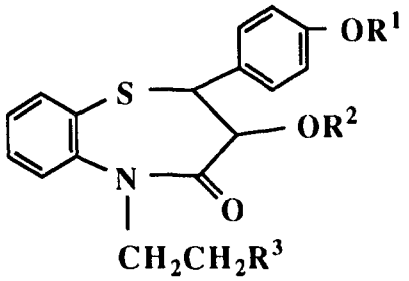
myocardial oxygen demand, mainly by coronary artery dilatation and / or via both direct and indirect hemodynamic alterations. In addition, it has also been shown to be effective as an antihypertensive[53,54] and anti-arrhythmic agent particularly in the control of supraventricular tachycardia.[55,56] Recently, some investigators reported that DTZ is effective in the prevention of reinfarction in patients with non-Q-wave infarction[57] and others have found it to be useful as an anti-platelet agent[58,59] and in the treatment of Raynaud's phenomenon[60] and tardive dyskinesia.[61,62] Thus DTZ is a therapeutic agent with a wide spectrum of activities.

DTZ is subject to significant first pass metabolism and is rapidly and extensively converted in humans to a variety of metabolites. Major metabolites are illustrated in Scheme I. Several of these have potent pharmacological activities. For instance, the coronary vasodilating potencies of deacetylated DTZ (**1**) and *N*-demethylated DTZ (**2**) are approximately 50% and 20%, respectively, of that of DTZ.[63] In contrast, **1** (deacetylated DTZ) and **3** (*O*-demethylated DTZ) are considerably more potent than DTZ in inhibiting platelet aggregation[64] and uptake of adenosine by erythrocytes.[65] In recent years, despite a rather safe record with its clinical use, many cases of serious adverse effects which might be linked to DTZ have been reported, including cutaneous vasculitis,[66] thrombocytopenia,[67] heart block,[68] parkinsonism,[69] and even fatal renal and hepatic toxicity.[70] The extent to which the metabolites contribute to both the therapeutic and adverse effects of DTZ needs to be investigated. Recently, three more new DTZ metabolites were detected from human urine and were tentatively identified as *N*,*O*-didemethylated DTZ (**4**), *O*-demethylated DTZ (**5**)[71] and diltiazem *N*-oxide (**6**). Their identities were not confirmed since no authentic compounds were available.



Scheme I. Major Basic DTZ Metabolites Found in Human Urine

Chart I. Composition of DTZ and Its Metabolites



Compd	Name[a]	R^1	R^2	R^3
DTZ		Me	Ac	NMe_2
1	M1	Me	H	NMe_2
2	MA	Me	Ac	NHMe
3	M4	H	H	NMe_2
4	MB	H	Ac	NHMe
5	MX	H	Ac	NMe_2
6	DTZNO	Me	Ac	$NOMe_2$
7	M2	Me	H	NHMe
8	M6	H	H	NHMe

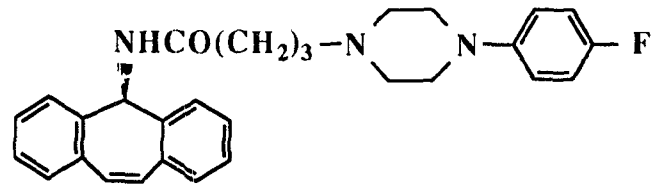
[a]For unsystematic names of diltiazem metabolites, see lit.[72] (M1, M2, M4, M6); lit.[73] (MA) and lit.[71] (MB, MX).

1. 3 A Novel Class of Calcium Antagonists, Dibenzotricyclic Systems (DBTs)

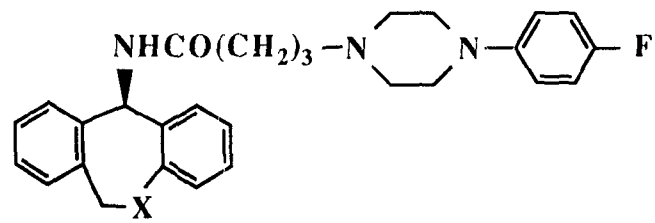
Calcium antagonists can influence cardiovascular hemodynamics by three principal actions: coronary arterial dilation, peripheral arterial dilation, and a negative inotropic effect. Nifedipine dilates the renal arteries, abolishing autoregulation in the experimental animal and, at least in humans, it also dilates the cerebral arteries. This widespread vasodilation reduces systemic vascular resistance both in animals and in humans,[74] increasing both contractility and heart rate. Nifedipine can also cause hypotension. Verapamil and diltiazem are less potent peripheral vasodilators than nifedipine.

Attempts to find tissue selective calcium antagonists have been made for more than a decade. Recently, a series of compounds with dibenzotricyclic structures (DBTs) were reported as a new class of calcium antagonists having selectivity for cardiac tissue over vascular tissue.[50] Compound **III** and its analogs (**IV—VI**) in Figure 4 represent this class of compounds. These DBTs may confer antianginal activity without an effect on blood pressure. Structure-activity relationships show that calcium antagonistic activity increases with a decrease of the angle between the planes of the two benzene rings in DBTs, at least within the range of 120° to 127° (Table 2) and the electrostatic potential of the bridge portion in the dibenzotricyclic system is crucial for activity.[50]

We thought that our dibenzothiazepinone derivatives (**9—13** in Figure 4 and Chart II) should have more acute angles of flexure than those of reference compounds **III—VI** due to the smaller bond angle of the S atom and the planar amide bond in the central ring of the tricyclic system. The X-ray diffraction and molecular modeling confirmed this prediction and showed the angle between the planes of the flanking aromatic rings of



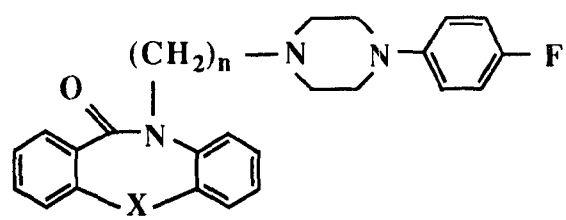
III



IV (X = S)

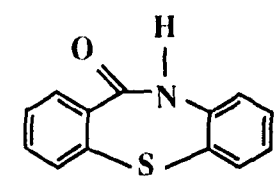
V (X = O)

VI (X = CH_2)



9—13 (X = S, n = 2—6)

15—18 (X = O, n = 3—6)



14

Figure 4. Structure comparison of the DBTs in this study with DBTs in the literature[50]

Table 2. Correlation of the Angles between the Planes of the Two Benzene Rings and the Calcium Antagonistic Activities of DBTs[50]

Compd[a]	Angle (deg)	Ca^{2+} antagonistic activity pA_2[b]
III	119.37	8.98±0.08
IV	123.64	8.71±0.08
VI	126.41	8.03±0.06
V	126.93	7.48±0.11
diltiazem	—	7.42±0.05
verapamil	—	8.16±0.15
nifedipine	—	9.83±0.08

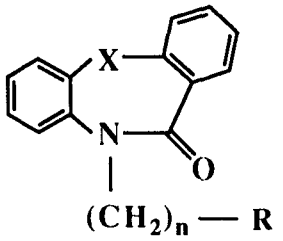
[a]Diltiazem, verapamil and nifedipine are listed here as reference for calcium antagonistic activity.

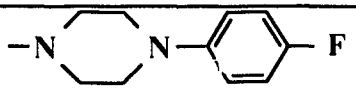
[b]Negative logarithm of the molar concentrations of the test compounds that cause a shift by a factor of 2 toward higher concentration in the calcium concentration-response curve.

unsubstituted dibenzothiazepinone, **14** (Figure 4), to be 108.4° (X-ray) and 101° (MMX calculation), *ca.* 13 degrees smaller than that obtained from X-ray crystallographic data (121°) of the tricyclic nucleus of the reference compound **III** (see Discussion section). Compounds **9—13** might be expected to have enhanced calcium antagonistic activity compared with the DBTs previously reported[50] providing the minimum angle requirement has been met. To test the effect of flexure angle in DBTs with this substitution pattern, compounds **15—18** were designed. These compounds may have an angle of flexure (*ca.* 120°) comparable to those of the reference compounds **III—VI**. To test whether the 4-[4-(fluorophenyl)]piperazinyl moiety with a carbon spacer of appropriate length is essential for

activity or not, the known compounds **19** and **20** were also synthesized. Dibenzothiazepinones with *N*-benzyl-*N*-methylamino replacing 4-[4-(fluorophenyl)]-piperazinyl (**21—24**) were also prepared on suggestion by an individual from the company which reported compounds **III—VI**, Dainippon Pharmaceutical Co., Ltd (Sato, F., personal communication).

Chart II. Composition of Dibenzotricyclic Systems



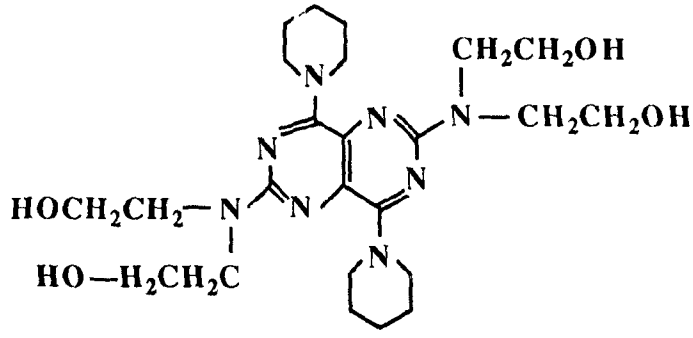
Compd	X	R	n
9	S		2
10	S	same as above	3
11	S	same as above	4
12	S	same as above	5
13	S	same as above	6
15	O	same as above	3
16	O	same as above	4
17	O	same as above	5
18	O	same as above	6
19	S	$-N(CH_3)_2$	2
20	S	same as above	3
21	S		3
22	S	same as above	4
23	S	same as above	5
24	S	same as above	6

1.4 Effects of Calcium Antagonists on *In Vitro* Adenosine Uptake by Erythrocyte

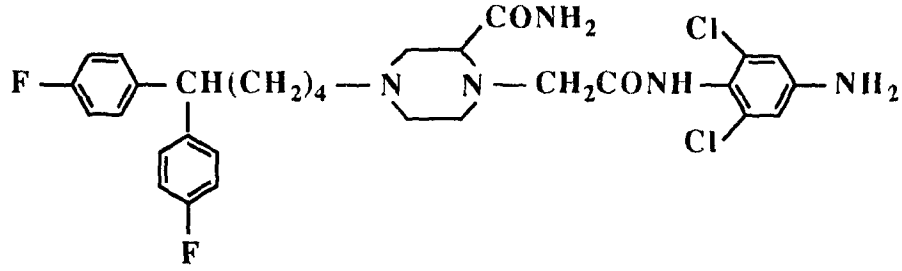
Adenosine is an endogenous purine nucleoside that plays a pivotal role in maintaining adequate oxygen and energy supply throughout the body.[75] Whenever there is a mismatch in the myocardium between energy supply and energy demand, the nucleotides (ATP, ADP, AMP) are rapidly degraded by consecutive dephosphorylation to form adenosine.[76] The actions of adenosine are mediated through specific cell-surface receptors,[77] of which at least two subtypes are known, A1 and A2. Due to its potent actions on many organs and systems, adenosine is an obvious target for the development of new drugs.

The growing evidence for a major role of adenosine as a "natural defence" for cardioprotection has been recognized.[78,79] It is known that adenosine is rapidly produced in response to myocardial ischemia.[80] During hypoxia, adenosine released into the coronary circulation dilates the coronary arteries to increase blood flow, and hence oxygen supply, to the myocardium. At the same time, it decreases cardiac workload and thus oxygen demand, thereby protecting the heart from ischemic injury.[81,82] The effect of adenosine is extremely short-lived because it is rapidly taken up by erythrocytes and metabolized mainly into inosine and hypoxanthine.[83]

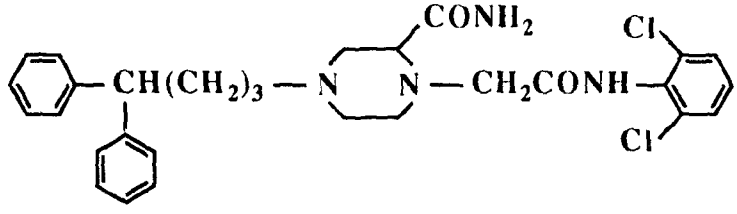
One mechanism involved in stimulating adenosine receptors indirectly, causing the actions of locally released adenosine to be prolonged, is believed to be through nucleoside transport inhibition.[78] There are several classes of nucleoside transport inhibitors. The structures of some representative compounds are shown in Figure 5. There is also evidence to suggest that the calcium antagonists such as verapamil, nifedipine, diltiazem



DIPYRIDAMOLE



R75231



MIOFLAZINE

Figure 5 Structures of some representative nucleoside transport inhibitors

and its metabolites may inhibit the uptake of adenosine, although they have considerably different potencies.[65, 84—87]

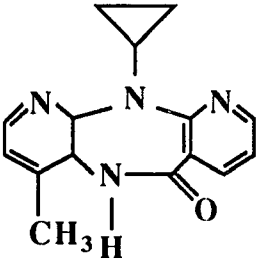
Nucleoside transport inhibitors prolong the actions of locally released adenosine by interfering with its uptake into the cell. A classic example of a transport inhibitor is dipyridamole (Figure 5), which exerts its coronary vasodilation and inhibition of platelet aggregation by inhibiting adenosine uptake, thus enhancing its actions on coronary artery or platelet A_2 receptors.[88] Newer adenosine uptake inhibitors include mioflazine, lidoflazine, and R75231 (Figure 5) developed by Janssen Pharmaceuticals.[89,90]

1.5 Anti-HIV Activity of Some Dibenzothiazepinone Derivatives

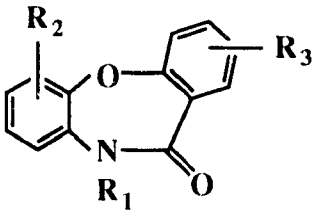
In the course of my synthesis of dibenzothiazepinone derivatives, I became aware that some pyridobenzo-, and dipyridodiazepinones,[91,92] and pyridobenz-, and dibenzoxazepinones as well as their derivatives[93] (Figure 6) are very potent non-nucleoside inhibitors of HIV-1 reverse transcriptase (HIV-1 RT) with IC_{50} values 20—80 nM.[91] These compounds were the results of intensive lead optimization following a high capacity screening program specifically designed to identity a non-nucleoside inhibitor of HIV-1 RT in Boehringer Ingelheim Pharmaceuticals, Inc. Since **25** (Figure 6), an analog of **VIII**, could be readily synthesized from **14**, it was prepared and submitted to National Institutes of Health (NIH), National Cancer Institute, Bethesda, Maryland, USA for evaluation of its anti-HIV activity. Compound **26** (Figure 6), which had been formed from a side reaction in the attempted acylation of **14** (see section 2. 2. 2), was also evaluated.

Since the discovery of a retroviral cause for acquired immunodeficiency syndrome (AIDS)[94—96] a decade ago, a tremendous effort has been made to search for and design

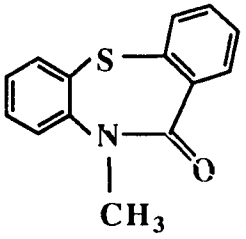
new anti-human immunodeficiency virus (HIV) agents. The majority of these agents act by (a) inhibiting enzymes such as RT, protease, or glycosidase, (b) arresting expression of



Nevirapine (VII)



VIII



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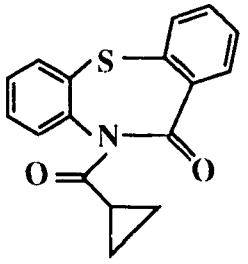


26

Figure 6. Composition of reference compounds and DBTs tested for anti-HIV activity

genes or gene products, (c) inhibiting viral processes such as giant cell formation or viral binding to the target cell. The nucleoside RT inhibitors are the most widely studied agents at both preclinical and clinical levels. Their inability to cure AIDS has stimulated the

discovery of several novel non-nucleoside RT inhibitors, possessing a variety of structures and demonstrating activity at nanomolar concentrations. Among these are nevirapine (**VII**) and tricyclic pyridobenzoxazepinones and dibenzoxazepinones.

Nevirapine has been found to be a potent (IC_{50} = 84 nM) and specific noncompetitive (K_i = 200 nM) inhibitor of HIV-1 RT which acts at an allosteric site of the enzyme.[91, 97] It blocks HIV-1 replication *in vitro* (IC_{50} =40 nM) in CD_4^+ human T-cells (c8166), but is noncytotoxic except at significantly higher doses (CC_{50} = 321,000 nM).[98] In comparision, AZT (3'-azido-3'-deoxythymidine, the first therapeutic agent to treat AIDS) is a more potent inhibitor of HIV-1 replication *in vitro* (IC_{50} = 6 nM), but is more cytotoxic (CC_{50} = 66,000 nM) than nevirapine in the same assay.[91] Like the diazepinone class of RT inhibitors, the oxazepinones are also inhibitors specific for HIV-1, exhibiting no inhibitory activity against HIV-2 RT or against mammalian DNA polymerases. However, as a class, oxazepinones are less potent inhibitors of HIV-1 RT than are diazepinones. In addition, oxazepinones suffer the disadvantage of being less soluble than diazepinones.

Unfortunately, the initial excitement of finding nevirapine and its congeners as non-nucleoside inhibitors of HIV-1 RT was dampened because resistance can develop in as little as a month.[99, 100] However, the contribution of nevirapine to the crystal structure of HIV-1 RT is still having a great deal of impact on the rational design of new HIV-1 RT inhibitors.[101] A crystal structure at 3.5 Å resolution of HIV-1 RT complexed with nevirapine was achieved. The structure of this complex suggests some mechanisms by which nevirapine inhibits HIV-1 RT and facilitates the design of new inhibitors that might prove to be effective drugs to control AIDS.

1.6 Objectives of the Project

The objectives of this project were: to synthesize the eight major DTZ metabolites shown in Chart I; to characterize the three unconfirmed DTZ metabolites (**4**—**6**) 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 DTZ metabolites; and to evaluate calcium antagonistic activity of DTZ metabolites and to study the effects of DTZ metabolites on the nucleoside transport, i.e., adenosine uptake by erythrocytes; assisted with molecular modeling to design and synthesize novel calcium antagonists, including dibenzothiazepinone and dibenzoxazepinone derivatives (**9**—**13**, **15**—**24**, Chart II); to evaluate their calcium antagonistic activities and effects on adenosine uptake by erythrocytes, and to evaluate anti-HIV activity of some dibenzothiazepinone derivatives (**9**—**14**, **25** and **26**).

DISCUSSION

2. 1 MOLECULAR MODELING

Molecular modeling is becoming an increasingly useful tool in determining the active conformation of known receptor ligands and in the prediction of novel, active compounds. In principle, when the structure of the target protein and the position of the active site are known, it is theoretically possible to rationally design compounds that will specifically interact with the receptor. When little is known about the structure of the receptor protein, much information can still be gleaned from studying the active conformations of receptor ligands.

Molecular mechanics is a valuable tool for rapid estimation of the structure and evaluation of the relative stabilities of conformers and diastereomers in σ-bonded organic compounds.[102] The Allinger MM2 program[103] is most often used because of the breadth of compounds treated and the care with which parameters were selected. The program treats molecules as a collection of deformable balls (atoms) whose size is related to the van der Waals radius and these atoms are held together by springs (bonds) whose natural lengths and angles, and harmonic stretching and bending force constants are chosen to reproduce the structure and strain energy of organic molecules. MMX[104] is an enhanced version of MM2 developed by the Department of Chemistry at Indiana University. This new program enhanced MM2 to give not only reasonable geometries for more exotic unsaturated hydrocarbons, but also heats of formation of conjugated π systems including radicals, cations, and anions. Some new parameters for many functional group types and a

new attractive term for hydrogen bonding were included in the program to overcome certain inadequacies of parameters and potential functions in MM2 for polar molecules.

It should be kept in mind that molecular mechanics calculates energies in a gas phase environment. For molecules containing polar functional groups in the presence of a solvent, especially water in real biological systems, the MMX minimized geometry might not reflect the real three-dimensional arrangment of atoms. In addition, none of the molecular mechanics programs can guarantee that the global minimum has been achieved. Any molecular mechanics program will minimize the potential energy of a molecule to a geometry in a local minimum, possibly in a global minimum, that closely resembles the input structure.

Molecular mechanics is one of the most conceptually straightforward of the techniques employed in computer-assisted drug design,[104—106] and also one of the most widely used because conformational minimizations for even quite large drug molecules can be solved within minutes on microcomputers. There are some software packages such as PCMODEL available for personal computers.[102,103,107,108]

2. 1. 1 Modeling of Reference Compound III and Dibenzothiazepinone 12

Compound **12** (Chart II) has been selected for molecular modeling for a better geometry comparison because it has the same numbers of atoms on the side chain as those of reference compound **III** (Figure 4 and Table 2), the most potent calcium antagonist in the series. Since compound **12** is an oil and its hydrochloride salt did not form a suitable crystal for X-ray crystallography, the X-ray crystallographic data of compound **14**, the tricyclic nucleus of **12**, were used as input for molecular mechanics calculations for **12**.

A single-crystal X-ray diffraction (Figure 7) reveals the crystal structure of **14** to adopt a "butterfly-like" conformation with a flexure angle of 108.4°, 13° smaller than that (121°) of the tricyclic nucleus of **III** from X-ray crystallographic data in the literature.[109] Due to electron delocalization effects, the amide moiety in the 7-membered ring adopts a virtually planar conformation, with a torsion angle (C12-C13-N1-C6) of -4.6°.

MMX was used to optimize the molecular geometries of compounds **III** and **12**. X-Ray crystallographic data for the tricyclic nuclei of both compounds were used as input for molecular mechanics calculations. The structure of the tricyclic nucleus of compound **III** with labeled atom numbers is shown in Figure 8. Optimized molecular geometries of **III** and **12** are shown in Figure 9. The angles between the planes of the flanking aromatic rings are 119° for **III** and 101° for **12**. The *para*-fluorophenyl group is almost perpendicular to the piperazine ring in both cases. Although the attachment point of the side chain of **12** is different from that of **III**, the five-carbon chain of **12** may allow the piperazine moiety to achieve the same relative position with respect to the tricyclic nucleus.

The flexure angles of DBTs reported here may have errors of *ca.* 1° or 2°, since they were measured manually by a protractor on a print-out after MMX calculations. The errors may come mainly from the orientation of the molecules achieved by rotation of the MMX minimized structures. Due to a slight twist of the benzene rings in the DBTs, it is difficult to rotate the molecule to an orientation in which the flexure angle can be measured accurately. However, many print-outs with different orientations of the MMX-minimized structures were generated and the flexure angles were measured as accurately as possible. The PCMODEL-generated structure from the X-ray crystallographic data was also used as a control to ensure measurement was appropriate and correct.

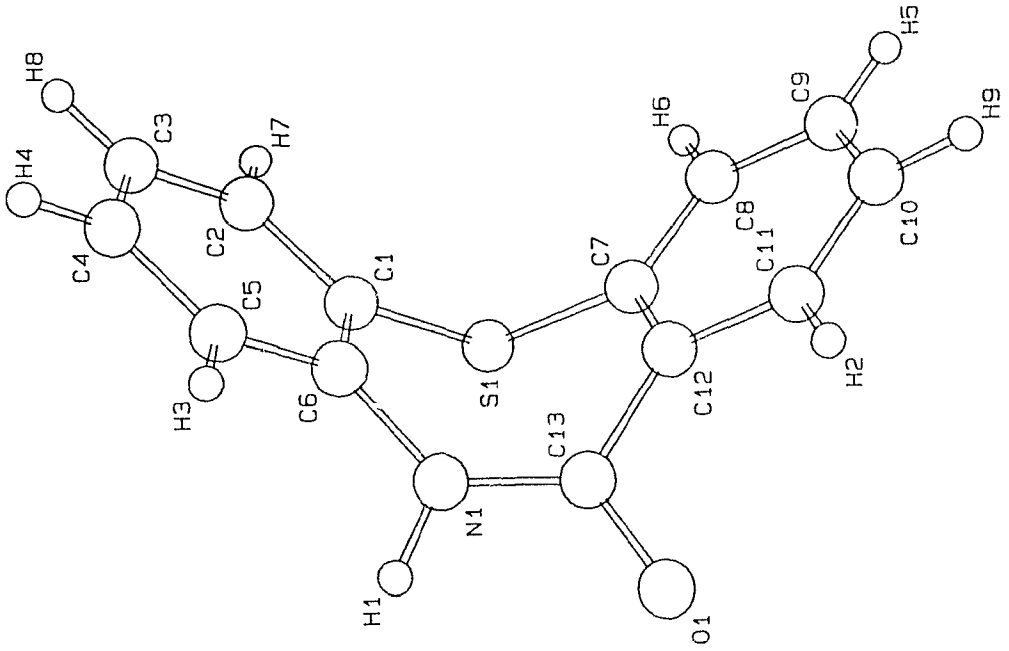


Figure 7. X-Ray crystal structure of **14**

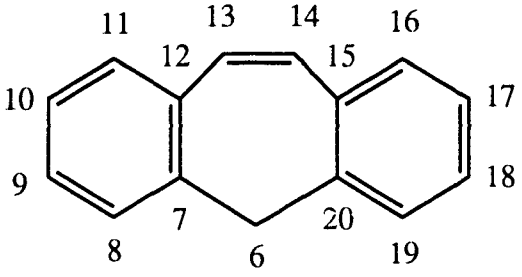
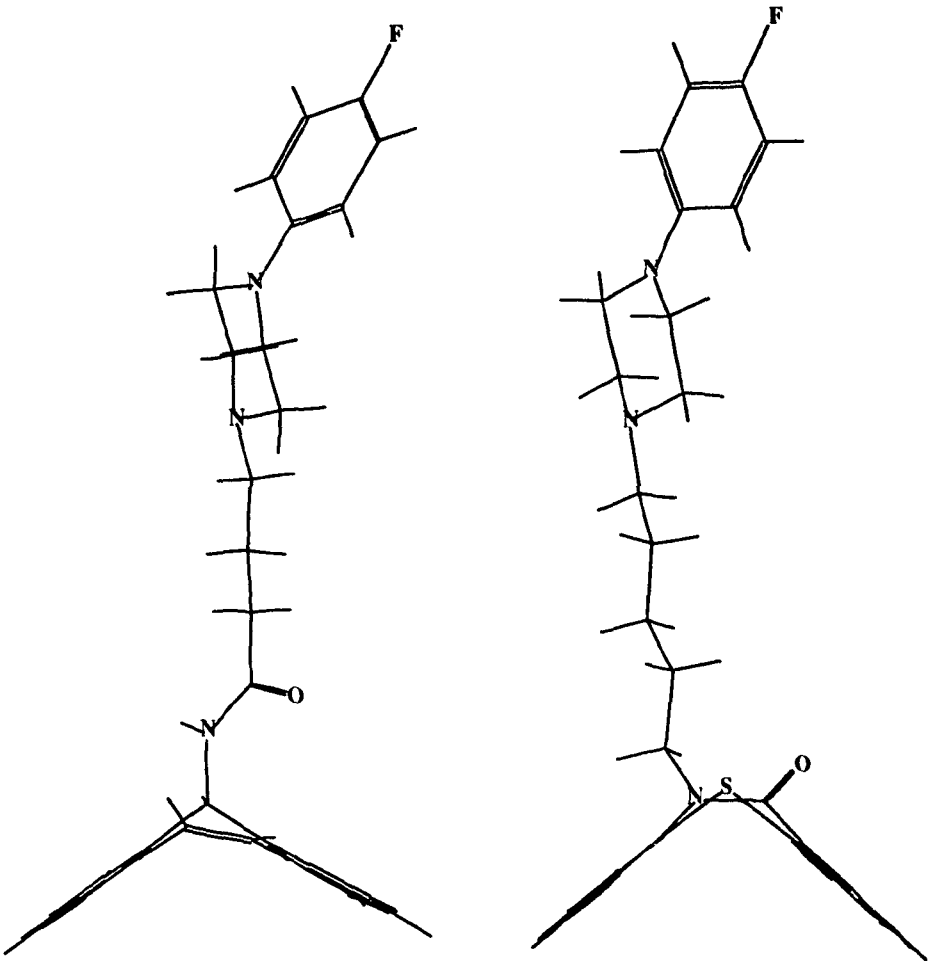


Figure 8. Structure of the tricyclic nucleus of compound **III**



III 12

Figure 9. Structure comparison of reference compound **III** with dibenzothiazepinone **12** after energy minimization with MMX

The angles between the planes of the flanking aromatic rings from molecular mechanics calculations in this study are smaller (2° and 7.4° for **III** and **12**, respectively) than those of X-ray crystal structures of these dibenzotricyclic systems. The value reported for **III** with AM1 calculations with geometry optimization from the literature[50] is also 1.6° lower than that of the X-ray structure.[109] The large discrepancy of the flexure angle (7.4°) of **12** between X-ray and MMX calculation, in this study, may be due to a poorly parameterized S atom in the molecular mechanics program, while no more than a 2° difference between X-ray and MMX calculation for compound **III** is expected since hydrocarbons are well parameterized for molecular mechanics. The X-ray crystallographic data of the tricyclic nucleus of **III** from literature[109] were used as input and the rest of the molecule was built using template fragment or standard bond length and angles available in PCMODEL for molecular mechanics calculations of reference compound **III**. The X-ray coordinates, parameters including intramolecular distances and bond angles of the tricyclic nucleus of **III** and the results of molecular mechanics calculations are listed in Tables 3—5. The molecular mechanics calculations for **14** using X-ray crystallographic data as input were performed. All parameters including intramolecular distances, bond angles and torsion angles are, in fact, very consistent with those from X-ray crystallographic data (Tables 6—9).

Table 3. Final X-Ray Fractional Coordinates for Non-hydrogen Atoms for the Tricyclic Nucleus[109] of Reference Compound **III**

Atom	X	Y	Z
C6	0.1517 (31)	0.1241 (4)	0.7326 (21)
C7	0.0720 (30)	0.1123 (4)	0.5525 (22)
C8	0.1991 (30)	0.1191 (4)	0.4433 (23)
C9	0.1206 (34)	0.1098 (4)	0.2767 (24)
C10	-0.0735 (38)	0.0961 (5)	0.2228 (26)
C11	-0.1993 (33)	0.0878 (5)	0.3268 (24)
C12	-0.1185 (29)	0.0934 (4)	0.4979 (23)
C13	-0.2418 (30)	0.0733 (4)	0.6002 (23)
C14	-0.1739 (29)	0.0561 (4)	0.7442 (24)
C15	0.0348 (32)	0.0526 (4)	0.8380 (23)
C16	0.0818 (33)	0.0156 (4)	0.9400 (24)
C17	0.2797 (35)	0.0068 (5)	1.0283 (23)
C18	0.4360 (32)	0.0341 (5)	1.0084 (22)
C19	0.3979 (30)	0.0720 (4)	0.9174 (21)
C20	0.1962 (28)	0.0819 (4)	0.8275 (19)

Estimated standard deviations in the least or second least significant figure are given in parentheses.

Table 4. Intramolecular Distances of the Tricyclic Nucleus of Reference Compound **III** from X-ray[109] and MMX Calculations

Atom	Atom	Distance	
		X-ray	MMX
C7	C6	1.55 (2)	1.509
C8	C7	1.39 (1)	1.406
C9	C8	1.43 (2)	1.397
C11	C10	1.36 (1)	1.395
C13	C12	1.46 (2)	1.480
C15	C14	1.45 (2)	1.479
C20	C15	1.42 (2)	1.407
C18	C17	1.38 (2)	1.401
C20	C19	1.43 (2)	1.407
C20	C6	1.53 (2)	1.509
C12	C7	1.38 (2)	1.407
C10	C9	1.34 (2)	1.401
C12	C11	1.45 (2)	1.409
C14	C13	1.32 (2)	1.351
C16	C15	1.43 (2)	1.410
C17	C16	1.39 (2)	1.395
C19	C18	1.39 (1)	1.397

Distances are in angstroms (Å). Estimated standard deviations in the least significant figure are given in parentheses.

Table 5. Intramolecular Bond Angles of the Tricyclic Nucleus of Reference Compound **III** Involving the Non-hydrogen Atoms from X-ray[109] and MMX Calculations.

Atom	Atom	Atom	Angle	
			X-ray	MMX
C20	C6	C7	107.8 (1)	109.26
C12	C7	C6	121.6 (1)	120.88
C9	C8	C7	118.8 (1)	121.06
C12	C11	C10	118.6 (2)	120.87
C13	C12	C7	125.0 (1)	122.58
C14	C13	C12	126.8 (1)	125.38
C16	C15	C14	116.9 (2)	117.89
C20	C15	C16	118.6 (1)	119.40
C18	C17	C16	117.7 (1)	119.68
C20	C19	C18	120.9 (1)	121.20
C19	C20	C6	121.2 (1)	119.75
C8	C7	C6	119.4 (1)	119.92
C12	C7	C8	118.9 (1)	119.20
C11	C10	C9	121.2 (2)	119.73
C11	C12	C7	120.3 (1)	119.42
C13	C12	C11	114.3 (1)	117.99
C15	C14	C13	129.5 (1)	125.51
C20	C15	C14	124.3 (1)	122.70
C17	C16	C15	122.7 (2)	120.97
C19	C18	C17	122.0 (2)	119.65
C15	C20	C6	120.9 (1)	121.16
C19	C20	C15	117.7 (1)	119.09

Angles are in degrees (°). Estimated standard deviations in the least significant figure are given in parentheses.

Table 6. Final X-Ray Fractional Coordinates for Non-hydrogen Atoms for **14**[a]

Atom	X	Y	Z
S1	0.1699 (2)	0.4762 (1)	0.1229 (1)
N1	0.1015 (4)	0.5457 (3)	0.3648 (3)
O1	0.1613 (4)	0.3954 (2)	0.4708 (2)
C1	0.1658 (5)	0.6102 (4)	0.1747 (3)
C13	0.1999 (6)	0.4543 (4)	0.3902 (3)
C11	0.5262 (6)	0.3869 (3)	0.3935 (3)
C12	0.3662 (5)	0.4246 (3)	0.3285 (3)
C5	0.1337 (6)	0.7353 (4)	0.3271 (4)
C6	0.1368 (5)	0.6302 (4)	0.2878 (3)
C4	0.1581 (6)	0.8216 (4)	0.2574 (4)
C9	0.6832 (6)	0.3583 (4)	0.2273 (4)
C8	0.5265 (6)	0.3947 (4)	0.1613 (4)
C2	0.1912 (6)	0.6989 (4)	0.1047 (4)
C3	0.1866 (6)	0.8042 (4)	0.1453 (4)
C7	0.3675 (5)	0.4288 (3)	0.2109 (3)
C10	0.6849 (6)	0.3553 (4)	0.3436 (4)

Estimated standard deviations in the least significant figure given in parentheses.

[a]Data obtained by Dr. T. S. Cameron, Department of Chemistry.

Table 7. Intramolecular Distances of **14** from X-ray[a] and MMX Calculations

Atom	Atom	Distance	
		X-ray	MMX
S1	C1	1.759 (5)	1.771
S1	C7	1.775 (4)	1.772
N1	C13	1.346 (5)	1.353
N1	C6	1.421 (5)	1.367
O1	C13	1.250 (5)	1.218
C1	C6	1.396 (6)	1.403
C1	C2	1.393 (6)	1.401
C13	C12	1.497 (5)	1.491
C11	C12	1.397 (5)	1.406
C11	C10	1.383 (6)	1.399
C12	C7	1.395 (5)	1.409
C5	C6	1.376 (6)	1.402
C5	C4	1.366 (6)	1.400
C4	C3	1.380 (6)	1.399
C9	C8	1.380 (6)	1.401
C9	C10	1.378 (6)	1.398
C8	C7	1.392 (5)	1.400
C2	C3	1.383 (6)	1.400

Distances are in angstroms (Å). Estimated standard deviations in the least significant figure are given in parentheses.

[a]X-Ray data obtained by Dr. T. S. Cameron, Department of Chemistry.

Table 8. Intramolecular Bond Angles of **14** Involving the Non-hydrogen Atoms from X-ray[a] and MMX Calculations.

Atom	Atom	Atom	Angle	
			X-ray	MMX
C1	S1	C7	98.1 (2)	97.15
C13	N1	C6	129.3 (3)	125.77
S1	C1	C6	120.5 (3)	120.65
S1	C1	C2	121.2 (3)	119.39
C6	C1	C2	118.2 (4)	119.96
N1	C13	O1	120.5 (4)	116.18
N1	C13	C12	121.1 (4)	125.39
O1	C13	C12	118.3 (4)	118.42
C12	C11	C10	121.3 (4)	120.91
C13	C12	C11	117.2 (4)	118.80
C13	C12	C7	123.9 (4)	122.18
C11	C12	C7	118.8 (4)	119.00
C6	C5	C4	121.3 (4)	120.63
N1	C6	C1	122.6 (4)	123.19
N1	C6	C5	117.4 (4)	117.42
C1	C6	C5	119.9 (4)	119.37
C5	C4	C3	120.0 (5)	119.83
C8	C9	C10	120.5 (4)	119.74
C9	C8	C7	120.6 (4)	120.64
C1	C2	C3	121.1 (4)	120.35
C4	C3	C2	119.4 (4)	119.82
S1	C7	C12	121.4 (3)	121.58
S1	C7	C8	119.1 (3)	118.51
C12	C7	C8	119.5 (4)	119.89
C11	C10	C9	119.2 (4)	119.81

Angles are in degrees (°). Estimated standard deviations in the least significant figure are given in parentheses.

[a]X-Ray data obtained by Dr. T. S. Cameron, Department of Chemistry.

Table 9. Torsion or Conformation Angles of **14** from X-ray[a] and MMX Calculations

(1)	(2)	(3)	(4)	Angle	
				X-ray	MMX
S1	C1	C6	N1	-2.8 (5)	-5.17
S1	C1	C6	C5	-179.9 (3)	176.48
S1	C1	C2	C3	179.5 (3)	-177.87
S1	C7	C12	C13	2.7 (6)	-1.57
S1	C7	C12	C11	-179.8 (3)	179.73
S1	C7	C8	C9	179.4 (4)	-178.78
N1	C13	C12	C11	136.1 (4)	136.64
N1	C13	C12	C7	46.4 (6)	-42.06
N1	C6	C1	C2	177.2 (4)	175.89
N1	C6	C5	C4	-177.1 (4)	-176.32
O1	C13	N1	C6	170.9 (4)	172.62
O1	C13	C12	C11	-39.6 (6)	-44.52
O1	C13	C12	C7	138.0 (4)	136.78
C1	S1	C7	C12	62.6 (4)	63.43
C1	S1	C7	C8	-117.7 (4)	-118.23
C1	C6	N1	C13	52.6 (6)	56.42
C1	C6	C5	C4	0.2 (6)	2.13
C1	C2	C3	C4	0.6 (7)	0.66
C13	N1	C6	C5	-130.2 (5)	-125.20
C13	C12	C11	C10	178.5 (4)	179.72
C13	C12	C7	C8	-177.0 (4)	-179.89
C11	C12	C7	C8	0.5 (6)	1.41
C11	C10	C9	C8	1.4 (7)	0.39
C12	C13	N1	C6	-4.6 (7)	-8.51
C12	C11	C10	C9	-1.7 (7)	0.64
C12	C7	C8	C9	-0.9 (7)	-0.42
C5	C6	C1	C2	0.1 (6)	-2.47
C5	C4	C3	C2	-0.3 (7)	-1.01
C6	C1	S1	C7	-62.2 (4)	-63.53
C6	C1	C2	C3	-0.5 (6)	1.09
C6	C5	C4	C3	-0.1 (7)	-0.39
C2	C1	S1	C7	117.8 (4)	116.42
C7	C12	C11	C10	0.8 (6)	-1.53
C7	C8	C9	C10	-0.1 (8)	-0.49

Angles are in degrees (°). Estimated standard deviations in the least significant figure are given in parentheses. The sign is positive if when looking from atom 2 to atom 3 a clockwise motion of atom 1 would superimpose it on atom 4.

[a]X-Ray data obtained by Dr. T. S. Cameron, Department of Chemistry.

2. 1. 2 Modeling of Dibenzothiazepinone 13 and Dibenzoxazepinone 18

Compounds **13** and **18** were selected for X-ray diffraction determination because they have suitable crystals for X-ray crystallography with the same chain length (6 carbon spacer) representative of dibenzothiazepinones and dibenzoxazepinones. X-Ray crystallographic data of **13** and **18** were used as input for molecular mechanics calculations. X-Ray coordinates, parameters including intramolecular distances, bond angles and torsion angles of **13** and **18** are shown in Tables 10—13 and Tables 14—17. The results of parameters after MMX calculations are listed in the corresponding X-ray crystallographic data tables. X-Ray structures of **13** is shown in Figures 10. The structure of **13** after geometry minimization with MMX (bond only) is shown in Figure 11. X-Ray structures of **18** is shown in Figure 12. The structure of **18** after geometry minimization with MMX (bond only) is shown in Figure 13. Structure comparisons of **13** with **18** after geometry minimization with MMX are shown in Figure 14.

Similarly to the crystal structure of **14**, an X-ray diffraction (Figure 10) shows the crystal structure of the dibenzotricyclic system of **13** to adopt a "butterfly-like" conformation with a flexure angle of 108°. The flexure angle after MMX geometry minimization is 101°, the same as that of **14**.

The parameters of **13** including intramolecular distances, bond angles after geometry minimization by MMX are very consistent with those of X-ray crystallographic data (Tables 11 and 12), while torsion angles involving N3 after MMX calculations are far

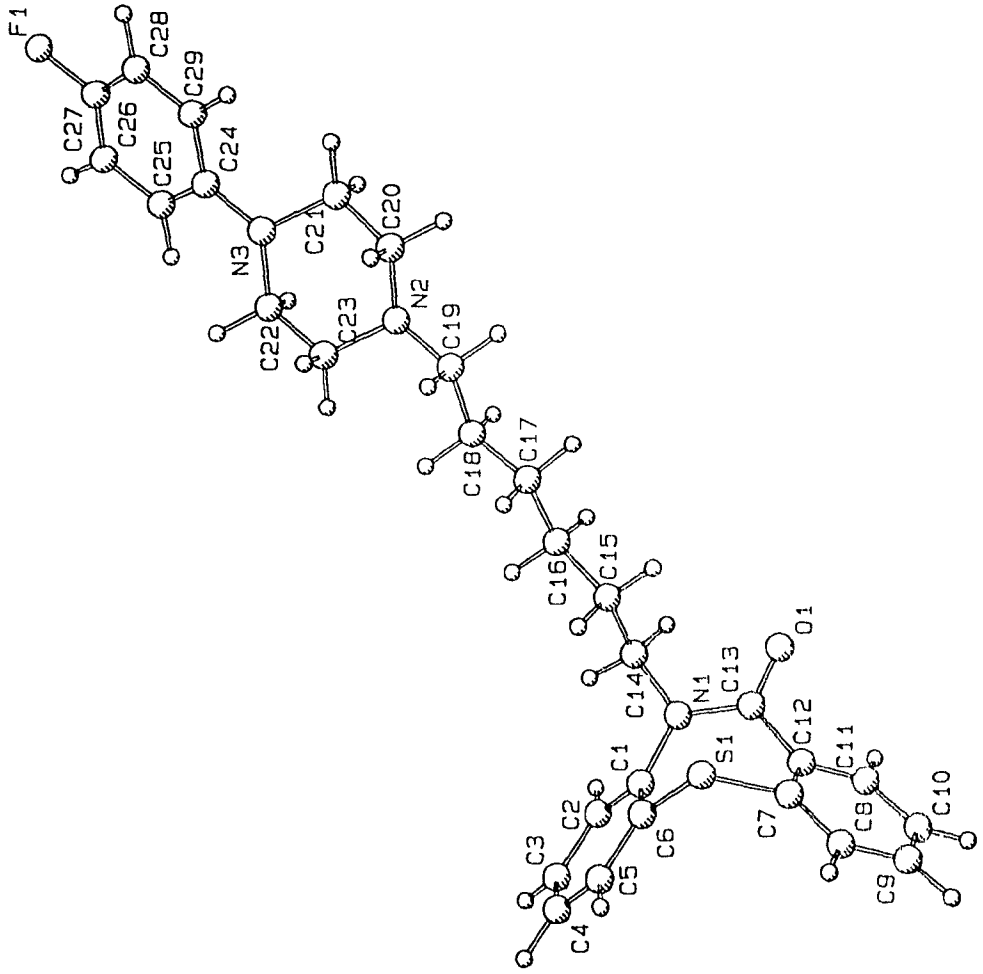


Figure 10. X-Ray crystal structure of **13**

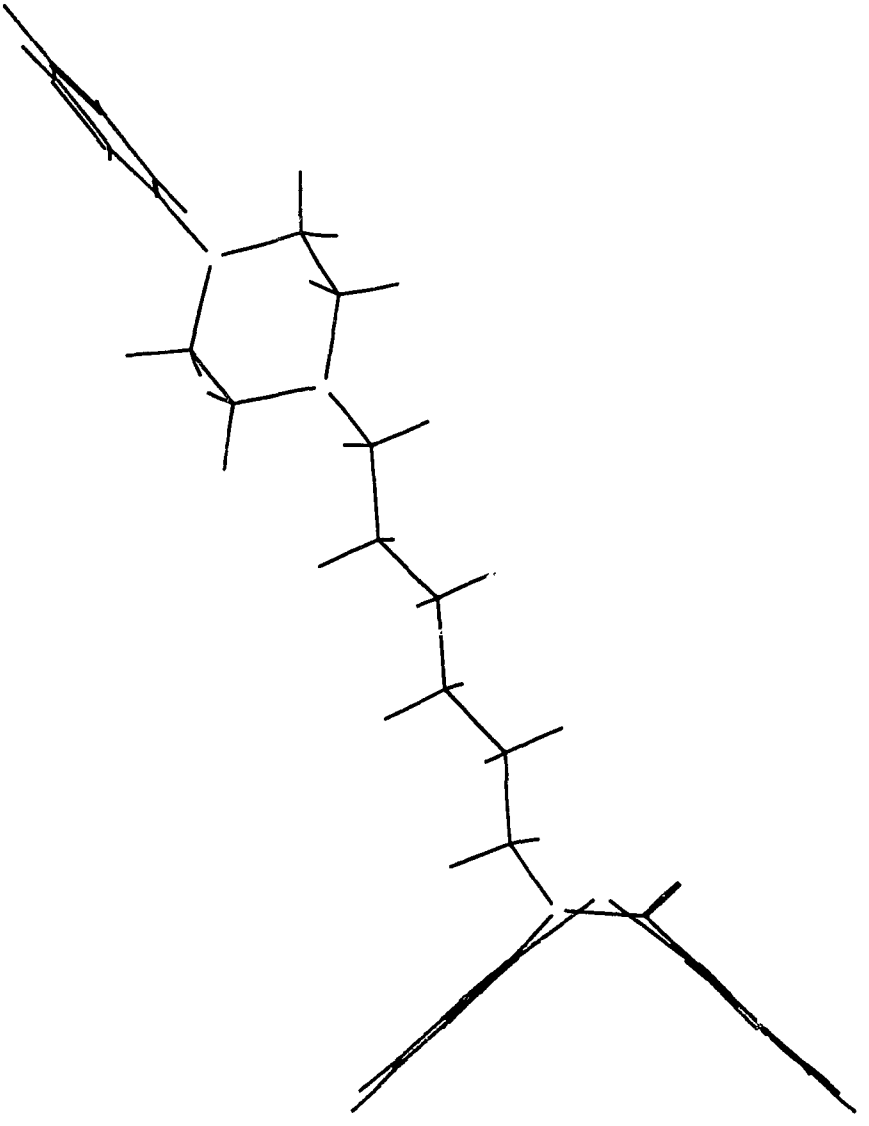


Figure 11. Structure 13 after geometry minimization with MMX

Table 10. Final X-Ray Fractional Coordinates for Non-hydrogen Atoms for **13**[a]

Atom	X	Y	Z
S1	0.6733 (3)	0.0075 (4)	0.0966 (2)
F1	0.6178 (8)	1.5962 (7)	0.8464 (5)
O1	0.8842 (8)	-0.1170 (9)	0.3805 (6)
N1	0.9097 (8)	0.060 (1)	0.2723 (6)
N2	0.6745 (8)	0.7757 (9)	0.6047 (6)
N3	0.6201 (8)	1.071 (1)	0.6579 (6)
C1	0.919 (1)	0.110 (1)	0.1746 (9)
C2	1.031 (1)	0.181 (1)	0.1705 (8)
C3	1.047 (1)	0.239 (1)	0.082 (1)
C4	0.945 (2)	0.228 (1)	-0.001 (1)
C5	0.823 (1)	0.160 (2)	0.004 (1)
C6	0.816 (1)	0.101 (1)	0.092 (1)
C7	0.745 (1)	-0.170 (1)	0.129 (1)
C8	0.706 (1)	-0.279 (2)	0.064 (1)
C9	0.760 (2)	-0.420 (2)	0.087 (1)
C10	0.855 (2)	-0.443 (1)	0.172 (1)
C11	0.894 (1)	-0.331 (2)	0.234 (1)
C12	0.841 (1)	-0.192 (1)	0.216 (1)
C13	0.879 (1)	-0.081 (1)	0.2940 (9)
C14	0.937 (1)	0.168 (1)	0.3524 (7)
C15	0.814 (1)	0.245 (1)	0.3616 (8)
C16	0.850 (1)	0.367 (1)	0.4381 (8)
C17	0.732 (1)	0.449 (1)	0.4556 (8)
C18	0.766 (1)	0.581 (1)	0.5190 (7)
C19	0.645 (1)	0.647 (1)	0.5420 (8)
C20	0.559 (1)	0.810 (1)	0.6397 (8)
C21	0.583 (1)	0.940 (1)	0.7086 (8)
C22	0.732 (1)	1.036 (1)	0.6150 (8)
C23	0.702 (1)	0.905 (1)	0.5494 (7)
C24	0.625 (1)	1.203 (1)	0.7081 (8)
C25	0.729 (1)	1.296 (1)	0.7234 (8)
C26	0.731 (1)	1.433 (1)	0.7712 (9)
C27	0.624 (2)	1.463 (1)	0.8014 (9)
C28	0.517 (1)	1.378 (2)	0.787 (1)
C29	0.561 (1)	1.245 (1)	0.7409 (9)

Estimated standard deviations in the least significant figure are given in parentheses.

[a]Data obtained by Dr. T. S. Cameron, Department of Chemistry.

Table 11. Intramolecular Distances of **13** from X-ray[a] and MMX Calculations

Atom	Atom	Distance	
		X-ray	MMX
S1	C6	1.77 (1)	1.771
S1	C7	1.80 (1)	1.775
F1	C27	1.40 (1)	1.352
O1	C13	1.24 (1)	1.218
N1	C1	1.46 (1)	1.377
N1	C13	1.38 (1)	1.363
N1	C14	1.49 (1)	1.473
N2	C19	1.48 (1)	1.466
N2	C20	1.48 (1)	1.460
N2	C23	1.47 (1)	1.460
N3	C21	1.49 (1)	1.458
N3	C22	1.51 (1)	1.458
N3	C24	1.41 (1)	1.369
C1	C2	1.39 (1)	1.403
C1	C6	1.38 (1)	1.405
C2	C3	1.39 (1)	1.400
C3	C4	1.38 (2)	1.398
C4	C5	1.38 (2)	1.400
C5	C6	1.39 (2)	1.401
C7	C8	1.36 (1)	1.399
C7	C12	1.38 (1)	1.410
C8	C9	1.41 (2)	1.402
C9	C10	1.36 (2)	1.397
C10	C11	1.36 (2)	1.401
C11	C12	1.38 (1)	1.404
C12	C13	1.50 (1)	1.498
C14	C15	1.52 (1)	1.539
C15	C16	1.54 (1)	1.539
C16	C17	1.54 (1)	1.541
C17	C18	1.51 (1)	1.540
C18	C19	1.53 (1)	1.544
C20	C21	1.53 (1)	1.534
C22	C23	1.51 (1)	1.535
C24	C25	1.38 (1)	1.402
C24	C29	1.41 (1)	1.400
C25	C26	1.43 (1)	1.398
C26	C27	1.35 (2)	1.401
C27	C28	1.36 (2)	1.398
C28	C29	1.39 (1)	1.400

Distances are in angstroms (Å). Estimated standard deviations in the least significant figure are given in parentheses.

[a]X-Ray data obtained by Dr. T. S. Cameron, Department of Chemistry.

Table 12. Intramolecular Bond Angles of **13** Involving the Non-hydrogen Atoms from X-ray[a] and MMX Calculations.

Atom	Atom	Atom	Angle		Atom	Atom	Atom	Angle	
			X-ray	MMX				X-ray	MMX
C6	S1	C7	97.6 (6)	94.97	C10	C11	C12	123 (1)	120.80
C1	N1	C13	123.7 (9)	121.40	C7	C12	C11	117 (1)	119.00
C1	N1	C14	117.7 (9)	117.74	C7	C12	C13	124 (1)	121.77
C13	N1	C14	118.6 (9)	120.83	C11	C12	C13	119 (1)	119.23
C19	N2	C20	108.1 (8)	112.32	O1	C13	N1	120 (1)	119.99
C19	N2	C23	111.8 (8)	113.26	O1	C13	C12	119 (1)	116.01
C20	N2	C23	107.4 (8)	109.11	N1	C13	C12	122 (1)	123.98
C21	N3	C22	110.3 (8)	119.53	N1	C14	C15	112.4 (8)	112.55
C21	N3	C24	115.7 (9)	119.88	C14	C15	C16	109.7 (8)	111.84
C22	N3	C24	117.8 (9)	120.16	C15	C16	C17	113.9 (9)	113.50
N1	C1	C2	116 (1)	119.41	C16	C17	C18	114.3 (9)	111.41
N1	C1	C6	122 (1)	121.77	C17	C18	C19	111.2 (9)	114.03
C2	C1	C6	121 (1)	118.77	N2	C19	C18	112.9 (9)	112.77
C1	C2	C3	121 (1)	120.83	N2	C20	C21	111.2 (9)	110.35
C2	C3	C4	118 (1)	119.92	N3	C21	C20	110.7 (9)	112.33
C3	C4	C5	121 (1)	119.67	N3	C22	C23	110.8 (8)	112.84
C4	C5	C6	121 (1)	120.24	N2	C23	C22	111.1 (9)	110.93
S1	C6	C1	121 (1)	120.28	N3	C24	C25	123 (1)	120.35
S1	C6	C5	121 (1)	119.30	N3	C24	C29	119 (1)	119.67
C1	C6	C5	118 (1)	120.38	C25	C24	C29	118 (1)	119.98
S1	C7	C8	118 (1)	119.14	C24	C25	C26	123 (1)	119.86
S1	C7	C12	120 (1)	120.83	C25	C26	C27	114 (1)	120.12
C8	C7	C12	122 (1)	120.02	F1	C27	C26	118 (1)	119.97
C7	C8	C9	119 (1)	120.47	F1	C27	C28	116 (1)	119.99
C8	C9	C10	120 (1)	119.78	C26	C27	C28	126 (1)	120.03
C9	C10	C11	120 (1)	119.87	C27	C28	C29	119 (1)	119.89
C24	C29	C28	119 (1)	120.10					

Angles are in degrees (°). Estimated standard deviations in the least significant figure are given in parentheses.

[a]X-Ray data obtained by Dr. T. S. Cameron, Department of Chemistry.

Table 13. Torsion or Conformation Angles of **13** from X-ray[a] and MMX Calculations

(1)	(2)	(3)	(4)	Angle	
				X-ray	MMX
S1	C6	C1	N1	-8 (1)	-9.74
S1	C6	C1	C2	178.9 (8)	172.78
S1	C6	C5	C4	-178 (1)	-175.22
S1	C7	C8	C9	-180 (1)	177.61
S1	C7	C12	C11	-178.5 (9)	-176.28
S1	C7	C12	C13	9 (2)	4.15
F1	C27	C26	C25	-178 (1)	-179.97
F1	C27	C28	C29	178 (1)	179.93
O1	C13	N1	C1	174 (1)	170.15
O1	C13	N1	C14	-5 (2)	-11.85
O1	C13	C12	C7	129 (1)	131.42
O1	C13	C12	C11	-44 (2)	-48.14
N1	C1	C2	C3	-176.8 (9)	-174.25
N1	C1	C6	C5	176 (1)	172.73
N1	C13	C12	C7	-51 (2)	-47.12
N1	C13	C12	C11	136 (1)	133.32
N1	C14	C15	C16	-174.0 (9)	-170.81
N2	C19	C18	C17	179.6 (9)	170.64
N2	C20	C21	N3	58 (1)	49.77
N2	C23	C22	N3	-59 (1)	-47.06
N3	C24	C25	C26	177 (1)	177.98
N3	C24	C29	C28	-178 (1)	-178.02
C1	N1	C13	C12	-6 (2)	-11.36
C1	N1	C14	C15	92 (1)	91.46
C1	C2	C3	C4	2 (2)	0.61
C1	C6	S1	C7	-62 (1)	-63.78
C18	C19	N2	C23	74 (1)	70.40
C19	N2	C23	C22	-179.8 (8)	-171.19
C20	C21	N3	C22	-53 (1)	-36.33
C21	N3	C22	C23	54 (1)	35.00
C21	N3	C24	C29	-52 (1)	90.18
C22	N3	C24	C25	-3 (2)	98.51
C23	C22	N3	C24	-170.4 (9)	-152.47
C24	C29	C28	C27	-1 (2)	-0.34
C25	C26	C27	C28	-2 (2)	0.41
C26	C27	C28	C29	2 (2)	-0.44

To be continued.

Table 13, continued.

(1)	(2)	(3)	(4)	Angle	
				X-ray	MMX
C1	C6	C5	C4	-2 (2)	2.34
C2	C1	N1	C13	-128 (1)	-118.05
C2	C1	N1	C14	51 (1)	63.90
C2	C1	C6	C5	3 (2)	-4.75
C2	C3	C4	C5	-1 (2)	-3.08
C3	C2	C1	C6	-3 (2)	3.29
C3	C4	C5	C6	1 (2)	1.62
C5	C6	S1	C7	114 (1)	113.77
C6	S1	C7	C8	-117 (1)	-116.63
C6	S1	C7	C12	61 (1)	62.48
C6	C1	N1	C13	59 (1)	64.48
C6	C1	N1	C14	-122 (1)	-113.57
C7	C8	C9	C10	-3 (2)	-0.58
C7	C12	C11	C10	0 (2)	-2.10
C8	C7	C12	C11	-1 (2)	2.81
C8	C7	C12	C13	-174 (1)	-176.75
C8	C9	C10	C11	2 (2)	1.30
C9	C8	C7	C12	3 (2)	-1.50
C9	C10	C11	C12	0 (2)	0.05
C10	C11	C12	C13	173 (1)	177.48
C12	C13	N1	C14	175 (1)	165.63
C13	N1	C14	C15	-88 (1)	-86.61
C14	C15	C16	C17	-178.1 (9)	-179.32
C15	C16	C17	C18	-172 (1)	-179.66
C16	C17	C18	C19	-174.0 (9)	-176.58
C18	C19	N2	C20	-167.9 (9)	-165.41
C19	N2	C20	C21	177.9 (9)	169.28
C20	N2	C23	C22	62 (1)	62.89
C20	C21	N3	C24	170.1 (9)	151.12
C21	N3	C24	C25	130 (1)	-88.99
C21	C20	N2	C23	-61 (1)	-64.27
C22	N3	C24	C29	174 (1)	-82.33
C24	C25	C26	C27	1 (2)	0.40
C25	C24	C29	C28	0 (2)	1.15
C26	C25	C24	C29	0 (2)	-1.18

Angles are in degrees (°). Estimated standard deviations in the least significant figure are given in parentheses. The sign is positive if when looking from atom 2 to atom 3 a clockwise motion of atom 1 would superimpose it on atom 4.

[a]X-Ray data obtained by Dr. T. S. Cameron, Department of Chemistry.

away from those of X-ray data (Table 13). MMX calculates torsion angles C21-N3-C24-C29 of 90.18°, C22-N3-C24-C25 of 98.51°, C21-N3-C24-C25 of -88.99°, and C22-N3-C24-C29 of -82.33°, while X-ray values are -52°, -3°, 130°, and 174°, respectively. The MMX calculation shows that the *para*-fluorophenyl group is almost perpendicular to the piperazine ring for minimized geometry as expected.

An X-ray diffraction (Figure 12) shows the crystal structure of **18** also to adopt a "butterfly-like" conformation with a flexure angle of 116.9°, 4° smaller than that of the tricyclic nucleus of **III** from X-ray crystallographic data.[109] The MMX minimized geometry shows the flexure angle of 115°, only 2° different from that of X-ray crystallographic data. This probably is due to a well parameterized O atom in the PCMODEL program.

The flexure angles of DBTs reported here by MMX calculations may be different from those in the real biological system, in which a solvent (water) effect is taken into account. Hydrogen-bonding between water and the lone pair electrons on oxygen or sulfur in the DBTs, **18** and **13**, respectively may sandwich water molecules between the two benzene rings and this will make the flexure angles bigger than those calculated.

The MMX calculations showed that the intramolecular distances, bond angles and torsion angles of **18** were virtually the same as those of **13** except for those involving the bridged heteroatoms. The minimized geometries of **13** and **18** are almost superimposable (Figure 14). The only major difference is the flexure angle of sulfur-bridged DBT and that of its oxygen congener.

The results of electrostatic potential calculations after geometry minimization with MMX showed **13** and **18** with a negative potential around the surface of the bridge portion, while the reference compound **III** had a positive potential. A study of compounds **III**, **IV** and **V** (Figure 4) in the literature[50] showed that the differences in charge density

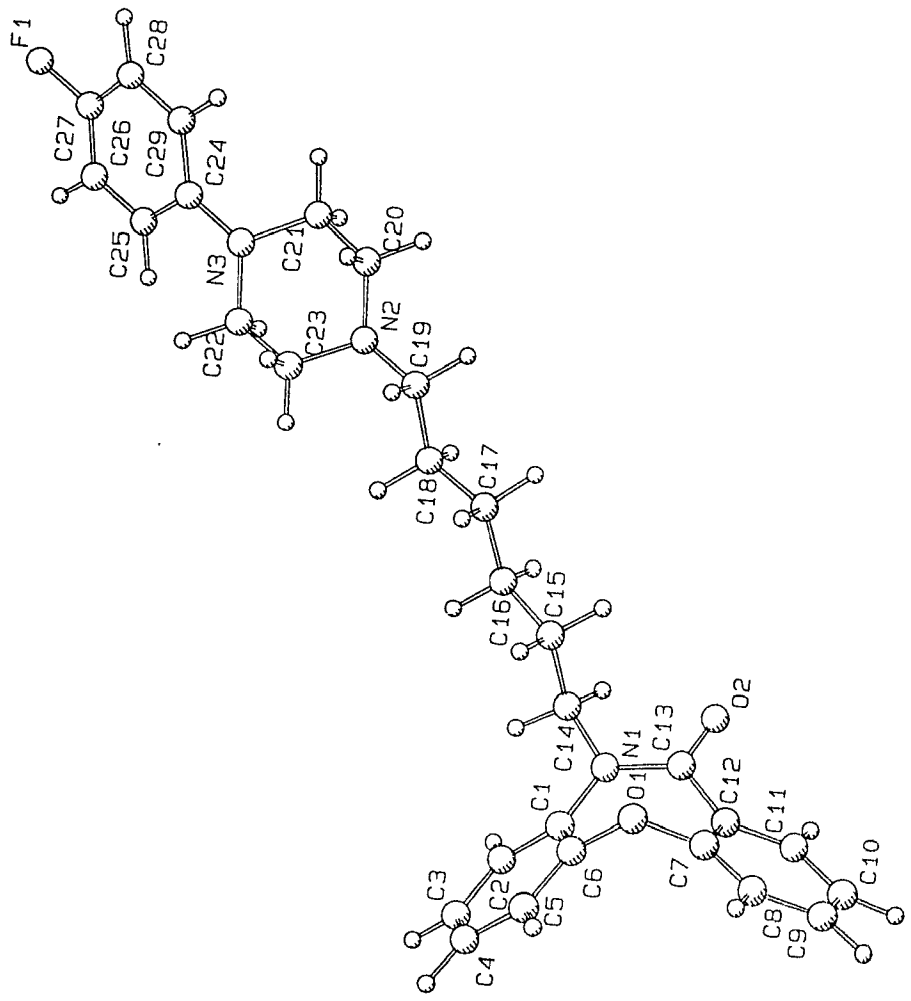


Figure 12. X-Ray crystal structure of **18**

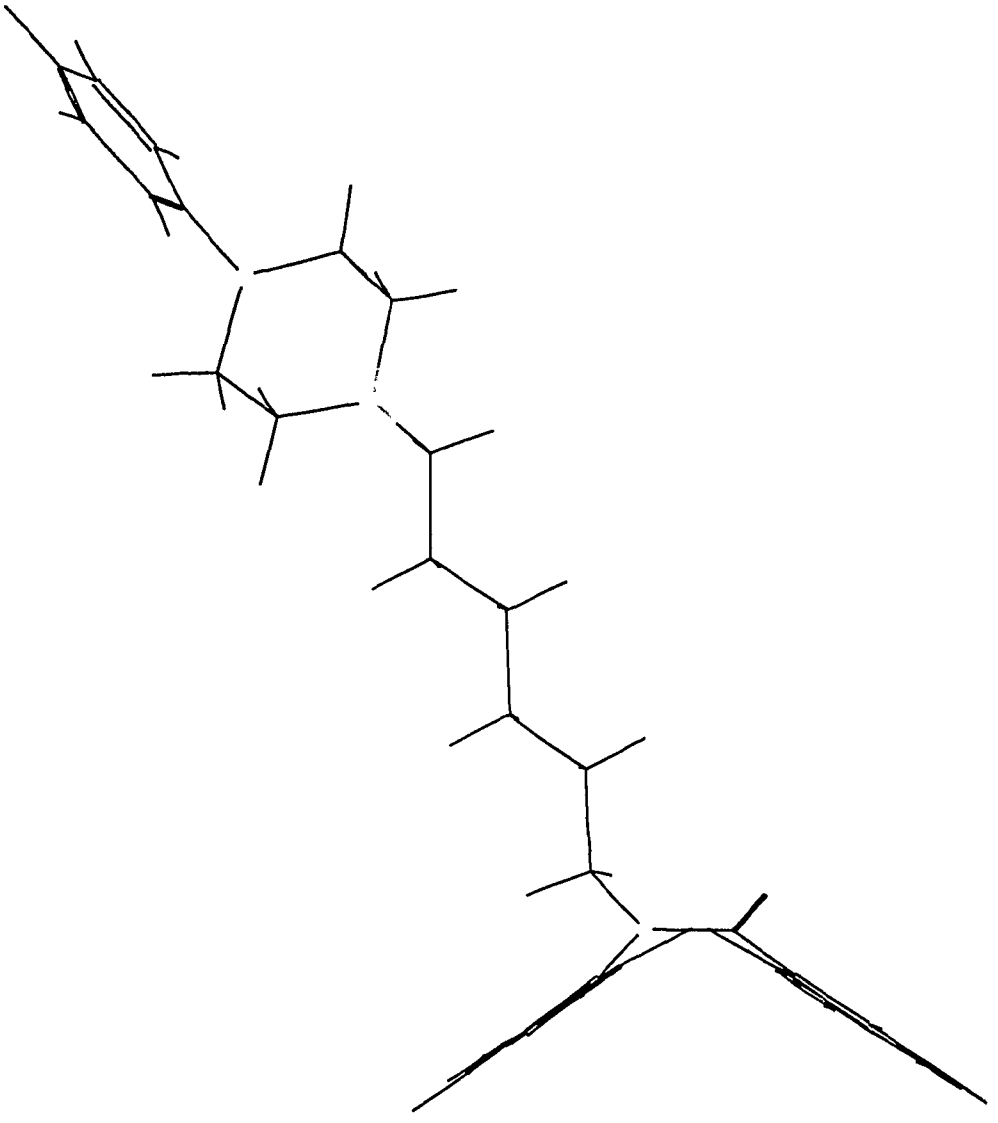


Figure 13. Structure **18** after geometry minimization with MMX

Table 14. Final X-Ray Fractional Coordinates for Non-hydrogen Atoms for **18**[a]

Atom	X	Y	Z
F1	0.5809 (3)	0.8084 (2)	0.0901 (2)
O1	-1.0060 (4)	0.8561 (2)	0.8441 (2)
O2	-1.1234 (3)	0.9448 (2)	0.5992 (2)
N1	-0.9377 (4)	0.9566 (2)	0.7081 (2)
N2	-0.2390 (4)	0.8239 (2)	0.3363 (2)
N3	0.0551 (4)	0.8017 (2)	0.2751 (2)
C1	-0.8827 (4)	0.9613 (3)	0.8036 (3)
C2	-0.7929 (5)	1.0176 (3)	0.8309 (3)
C3	-0.7366 (5)	1.0212 (3)	0.9224 (4)
C4	-0.7658 (6)	0.9706 (4)	0.9861 (4)
C5	-0.8555 (6)	0.9150 (3)	0.9604 (4)
C6	-0.9134 (5)	0.9121 (3)	0.8689 (4)
C7	-1.1527 (5)	0.8783 (3)	0.8285 (3)
C8	-1.2581 (6)	0.8527 (3)	0.8863 (3)
C9	-1.4026 (7)	0.8737 (4)	0.8706 (4)
C10	-1.4410 (6)	0.9182 (3)	0.7991 (5)
C11	-1.3362 (5)	0.9419 (3)	0.7394 (3)
C12	-1.1885 (5)	0.9220 (2)	0.7543 (3)
C13	-1.0808 (5)	0.9415 (3)	0.6823 (3)
C14	-0.8343 (5)	0.9688 (3)	0.6318 (3)
C15	-0.7636 (5)	0.9028 (2)	0.5988 (3)
C16	-0.6438 (4)	0.9192 (2)	0.5300 (3)
C17	-0.5691 (5)	0.8557 (3)	0.4915 (3)
C18	-0.4331 (5)	0.8732 (3)	0.4351 (3)
C19	-0.3676 (5)	0.8083 (3)	0.3926 (3)
C20	-0.2027 (5)	0.7634 (3)	0.2802 (3)
C21	-0.0757 (5)	0.7789 (2)	0.2190 (3)
C22	0.0197 (5)	0.8599 (2)	0.3355 (3)
C23	-0.1082 (5)	0.8410 (3)	0.3953 (3)
C24	0.1880 (5)	0.8059 (3)	0.2257 (3)
C25	0.2763 (6)	0.8652 (3)	0.2277 (3)
C26	0.4092 (6)	0.8661 (3)	0.1824 (4)
C27	0.4489 (5)	0.8084 (4)	0.1357 (4)
C28	0.3680 (6)	0.7489 (3)	0.1322 (3)
C29	0.2364 (5)	0.7480 (3)	0.1771 (3)

Estimated standard deviations in the least significant figure are given in parentheses.

[a]Data obtained by Dr. T. S. Cameron, Department of Chemistry.

Table 15. Intramolecular Distances of **18** from X-ray[a] and MMX Calculations

Atom	Atom	Distance	
		X-ray	MMX
F1	C27	1.387 (5)	1.352
O1	C6	1.408 (5)	1.357
O1	C7	1.408 (5)	1.359
O2	C13	1.236 (5)	1.220
N1	C1	1.438 (5)	1.378
N1	C13	1.367 (5)	1.364
N1	C14	1.487 (5)	1.474
N2	C19	1.476 (5)	1.465
N2	C20	1.476 (5)	1.460
N2	C23	1.466 (5)	1.460
N3	C21	1.473 (5)	1.458
N3	C22	1.461 (5)	1.458
N3	C24	1.425 (5)	1.369
C1	C2	1.406 (6)	1.403
C1	C6	1.369 (6)	1.403
C2	C3	1.387 (6)	1.400
C3	C4	1.370 (7)	1.399
C4	C5	1.389 (7)	1.399
C5	C6	1.391 (6)	1.400
C7	C8	1.381 (6)	1.398
C7	C12	1.385 (6)	1.409
C8	C9	1.383 (7)	1.401
C9	C10	1.371 (7)	1.399
C10	C11	1.382 (7)	1.400
C11	C12	1.403 (5)	1.407
C12	C13	1.496 (6)	1.491
C14	C15	1.512 (6)	1.539
C15	C16	1.529 (6)	1.539
C16	C17	1.517 (6)	1.541
C17	C18	1.538 (6)	1.540
C18	C19	1.524 (6)	1.544
C20	C21	1.506 (6)	1.534
C22	C23	1.514 (6)	1.534
C24	C25	1.399 (6)	1.401
C24	C29	1.397 (6)	1.401
C25	C26	1.392 (6)	1.399
C26	C27	1.355 (7)	1.400
C27	C28	1.365 (7)	1.399
C28	C29	1.379 (6)	1.399

Distances are in angstroms (Å). Estimated standard deviations in the least significant figure are given in parentheses.

[a]X-Ray data obtained by Dr. T. S. Cameron, Department of Chemistry.

Table 16. Intramolecular Bond Angles of **18** Involving the Non-hydrogen Atoms from X-ray[a] and MMX Calculations.

Atom	Atom	Atom	Angle		Atom	Atom	Atom	Angle	
			X-ray	MMX				X-ray	MMX
C6	O1	C7	110.9 (3)	117.40	C10	C11	C12	119.6 (5)	120.84
C1	N1	C13	124.1 (4)	121.74	C7	C12	C11	118.5 (4)	118.61
C1	N1	C14	118.8 (4)	117.67	C7	C12	C13	122.9 (5)	121.16
C13	N1	C14	117.2 (4)	120.51	C11	C12	C13	118.2 (5)	120.23
C19	N2	C20	109.7 (4)	112.41	O2	C13	N1	120.4 (4)	119.49
C19	N2	C23	111.8 (3)	113.24	O2	C13	C12	119.2 (5)	116.29
C20	N2	C23	107.5 (3)	109.07	N1	C13	C12	120.5 (4)	124.15
C21	N3	C22	111.1 (3)	119.52	N1	C14	C15	112.5 (4)	112.33
C21	N3	C24	115.3 (4)	119.99	C14	C15	C16	110.3 (4)	111.40
C22	N3	C24	117.4 (4)	120.10	C15	C16	C17	114.0 (4)	113.63
N1	C1	C2	119.2 (5)	120.65	C16	C17	C18	113.1 (4)	111.12
N1	C1	C6	122.1 (5)	120.97	C17	C18	C19	111.2 (4)	114.19
C2	C1	C6	118.7 (4)	120.74	N2	C19	C18	112.3 (4)	112.55
C1	C2	C3	119.2 (5)	120.78	N2	C20	C21	110.7 (4)	110.34
C2	C3	C4	121.2 (5)	120.08	N3	C21	C20	111.2 (4)	112.32
C3	C4	C5	120.3 (5)	119.49	N3	C22	C23	109.9 (4)	112.64
C4	C5	C6	118.3 (5)	120.10	N2	C23	C22	110.5 (4)	110.70
O1	C6	C1	119.9 (4)	119.99	N3	C24	C25	122.2 (5)	120.29
O1	C6	C5	117.8 (5)	119.20	N3	C24	C29	119.4 (5)	119.69
C1	C6	C5	122.3 (5)	120.74	C25	C24	C29	118.4 (4)	120.02
O1	C7	C8	118.0 (5)	119.09	C24	C25	C26	120.5 (5)	119.85
O1	C7	C12	119.9 (4)	120.48	C25	C26	C27	118.2 (5)	120.12
C8	C7	C12	122.1 (5)	120.42	F1	C27	C26	118.9 (6)	119.96
C7	C8	C9	118.2 (5)	120.40	F1	C27	C28	117.2 (6)	119.99
C8	C9	C10	121.1 (5)	119.63	C26	C27	C28	123.9 (5)	120.05
C9	C10	C11	120.5 (5)	120.02	C27	C28	C29	117.9 (5)	119.89
C24	C29	C28	121.1 (5)	120.08					

Angles are in degrees (°). Estimated standard deviations in the least significant figure are given in parentheses.

[a]X-Ray data obtained by Dr. T. S. Cameron, Department of Chemistry.

Table 17. Torsion or Conformation Angles of **18** from X-ray[a] and MMX Calculations

(1)	(2)	(3)	(4)	Angle	
				X-ray	MMX
F1	C27	C26	C25	179.9 (5)	179.93
F1	C27	C28	C29	-179.9 (4)	179.77
O1	C6	C1	N1	-2.4 (7)	-12.61
O1	C6	C1	C2	178.4 (4)	169.20
O1	C6	C5	C4	-179.0 (4)	-173.51
O1	C7	C8	C9	-179.3 (4)	177.00
O1	C7	C12	C11	178.6 (4)	-175.56
O1	C7	C12	C13	6.6 (7)	4.70
O2	C13	N1	C1	173.1 (5)	167.88
O2	C13	N1	C14	-6.7 (7)	-15.51
O2	C13	C12	C7	139.5 (5)	144.07
O2	C13	C12	C11	-32.5 (7)	-35.67
N1	C1	C2	C3	-178.3 (4)	-171.41
N1	C1	C6	C5	177.3 (4)	170.40
N1	C13	C12	C7	-41.3 (7)	-32.73
N1	C13	C12	C11	146.7 (5)	147.53
N1	C14	C15	C16	-172.5 (4)	-175.07
N2	C19	C18	C17	178.6 (4)	171.52
N2	C20	C21	N3	57.2 (5)	49.54
N2	C23	C22	N3	-60.0 (5)	-47.78
N3	C24	C25	C26	176.8 (5)	178.96
N3	C24	C29	C28	-176.9 (4)	-179.26
C1	N1	C13	C12	-6.0 (7)	-15.42
C1	N1	C14	C15	92.1 (5)	91.05
C1	C2	C3	C4	0.6 (8)	-1.59
C1	C6	O1	C7	-69.8 (5)	-62.58
C18	C19	N2	C23	74.1 (5)	70.70
C19	N2	C23	C22	-177.4 (4)	-170.69
C20	C21	N3	C22	-53.9 (5)	-36.51
C21	N3	C22	C23	54.7 (5)	35.63
C21	N3	C24	C29	-54.5 (6)	90.58
C22	N3	C24	C25	-5.4 (6)	98.22
C23	C22	N3	C24	-169.6 (4)	-151.62
C24	C29	C28	C27	-0.8 (8)	0.27
C25	C26	C27	C28	-1.6 (9)	0.33
C26	C27	C28	C29	1.5 (9)	-0.63

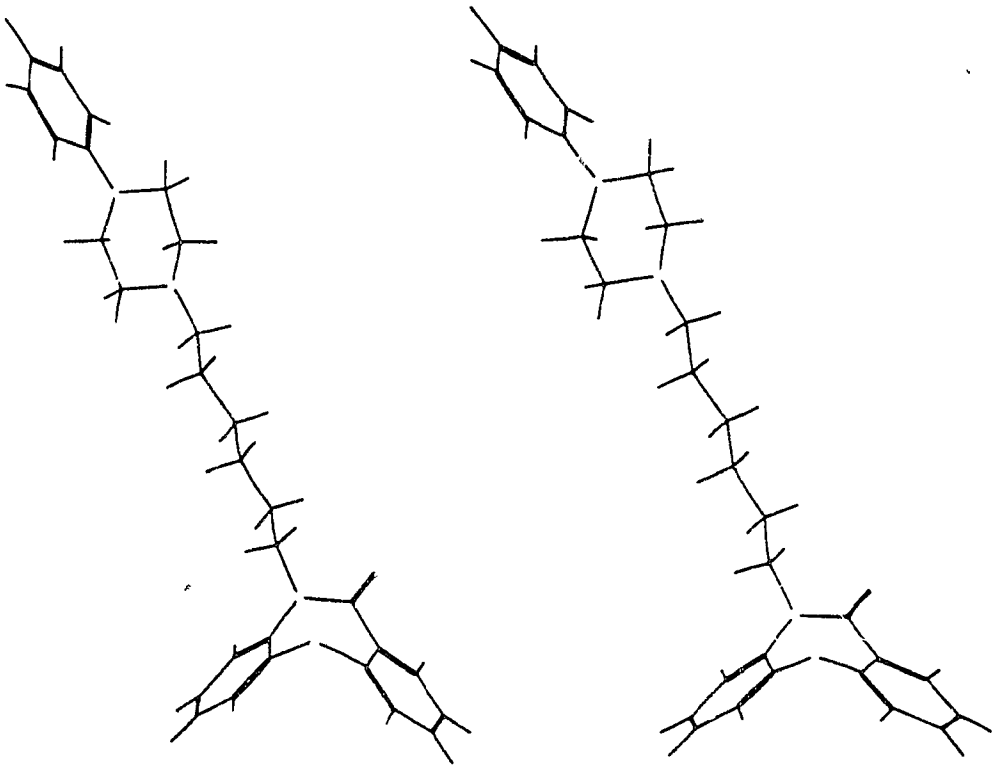
To be continued.

Table 17, continued.

(1)	(2)	(3)	(4)	Angle	
				X-ray	MMX
C1	C6	C5	C4	1.3 (8)	3.42
C2	C1	N1	C13	-133.7 (5)	-125.73
C2	C1	N1	C14	46.1 (6)	57.56
C2	C1	C6	C5	-1.9 (7)	-7.70
C2	C3	C4	C5	-1.2 (9)	-2.84
C3	C2	C1	C6	0.9 (7)	6.79
C3	C4	C5	C6	0.3 (8)	1.93
C5	C6	O1	C7	110.5 (5)	114.37
C6	O1	C7	C8	-117.3 (5)	-116.81
C6	O1	C7	C12	65.6 (5)	62.04
C6	C1	N1	C13	47.1 (7)	56.11
C6	C1	N1	C14	-133.1 (5)	-120.59
C7	C8	C9	C10	0.6 (9)	-0.49
C7	C12	C11	C10	0.6 (7)	-2.42
C8	C7	C12	C11	1.6 (7)	3.28
C8	C7	C12	C13	-170.3 (5)	-176.46
C8	C9	C10	C11	1.6 (9)	1.36
C9	C8	C7	C12	-2.3 (8)	-1.86
C9	C10	C11	C12	-2.2 (8)	0.11
C10	C11	C12	C13	173.0 (5)	177.33
C12	C13	N1	C14	174.2 (4)	161.20
C13	N1	C14	C15	-88.0 (5)	-85.70
C14	C15	C16	C17	-178.7 (4)	178.67
C15	C16	C17	C18	-170.8 (4)	-178.84
C16	C17	C18	C19	-176.4 (4)	-177.31
C18	C19	N2	C20	-166.8 (4)	-165.12
C19	N2	C20	C21	177.7 (4)	169.35
C20	N2	C23	C22	62.1 (5)	63.33
C20	C21	N3	C24	169.4 (4)	150.73
C21	N3	C24	C25	128.6 (5)	-89.06
C21	C20	N2	C23	-60.6 (5)	-64.19
C22	N3	C24	C29	171.6 (4)	-82.14
C24	C25	C26	C27	0.9 (8)	0.32
C25	C24	C29	C28	0.2 (8)	0.38
C26	C25	C24	C29	-0.2 (8)	- 0.68

Angles are in degrees (°). Estimated standard deviations in the least significant figure are given in parentheses. The sign is positive if when looking from atom 2 to atom 3 a clockwise motion of atom 1 would superimpose it on atom 4.

[a]X-Ray data obtained by Dr. T. S. Cameron, Department of Chemistry.



13 18

Figure 14. Structure comparison of **13** with **18** after geometry minimization with MMX (bond only)

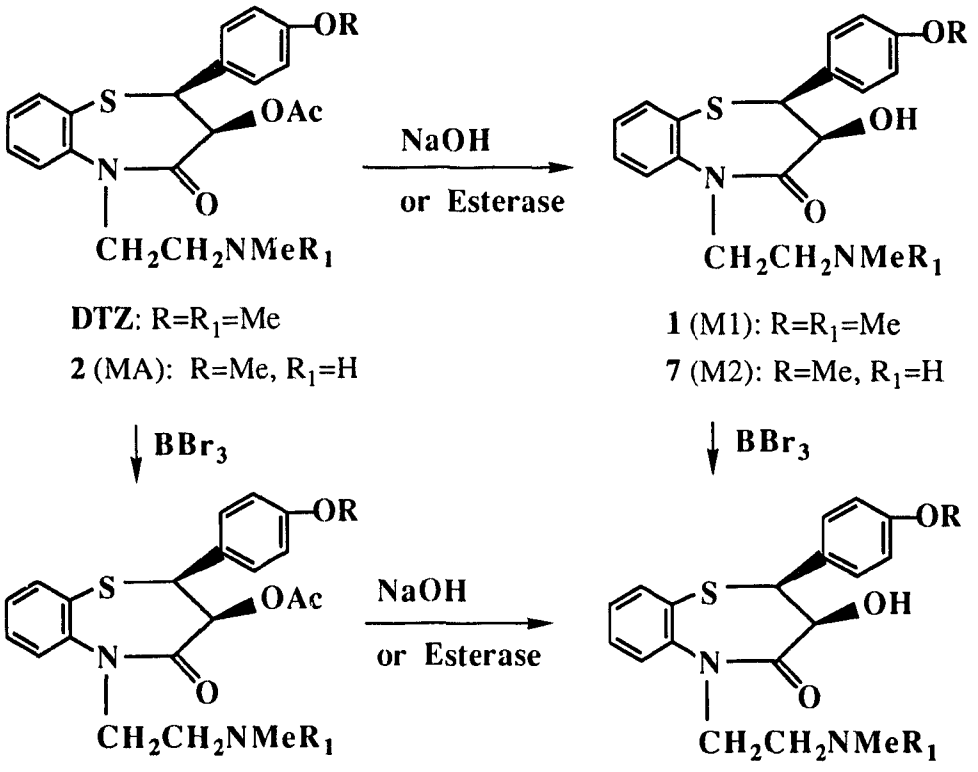
around the bridge portion of those tricyclic systems correlated with different potencies of calcium antagonistic activities. The electrostatic potentials on the Connolly's solvent accessible surface of the dibenzotricyclic systems of **III**, **IV** and **V** were calculated using AM1 net charge.[50] The results revealed that compounds **III** and **IV** with a positive potential around the surface of the bridge portion had potent activity, while compound **V** with a negative potential showed a lower activity. The researchers claimed that this opposite electronic feature might be responsible for the difference of calcium antagonistic activities between the oxygen-containing congener (**V**) and other compounds (**III** and **IV**) having activities.

2. 2 CHEMISTRY

2. 2. 1 PREPARATION OF DILTIAZEM METABOLITES

2. 2. 1. 1 Short Sequence Synthesis of Diltiazem Metabolites

Attempts were made initially to prepare **5** from commercial DTZ by *O*-demethylation with BBr_3, apparently the most economic route (see Scheme II). Boron



DTZ: R=R_1=Me
2 (MA): R=Me, R_1=H

1 (M1): R=R_1=Me
7 (M2): R=Me, R_1=H

5 (MX): R=H, R_1=Me
4 (MB): R=R_1=H

3 (M4): R=H, R_1=Me
8 (M6): R=R_1=H

Scheme II. Short Sequence Synthesis of DTZ Metabolites

tribromide[110] and boron trichloride[111] were reported to selectively demethylate methoxy groups and leave ester groups intact in aromatic molecules. However, in the case of DTZ, demethylation was also accompanied by extensive deacetylation, probably by HBr formed in the reaction, leading only to complex mixtures in which **3** was the major component.

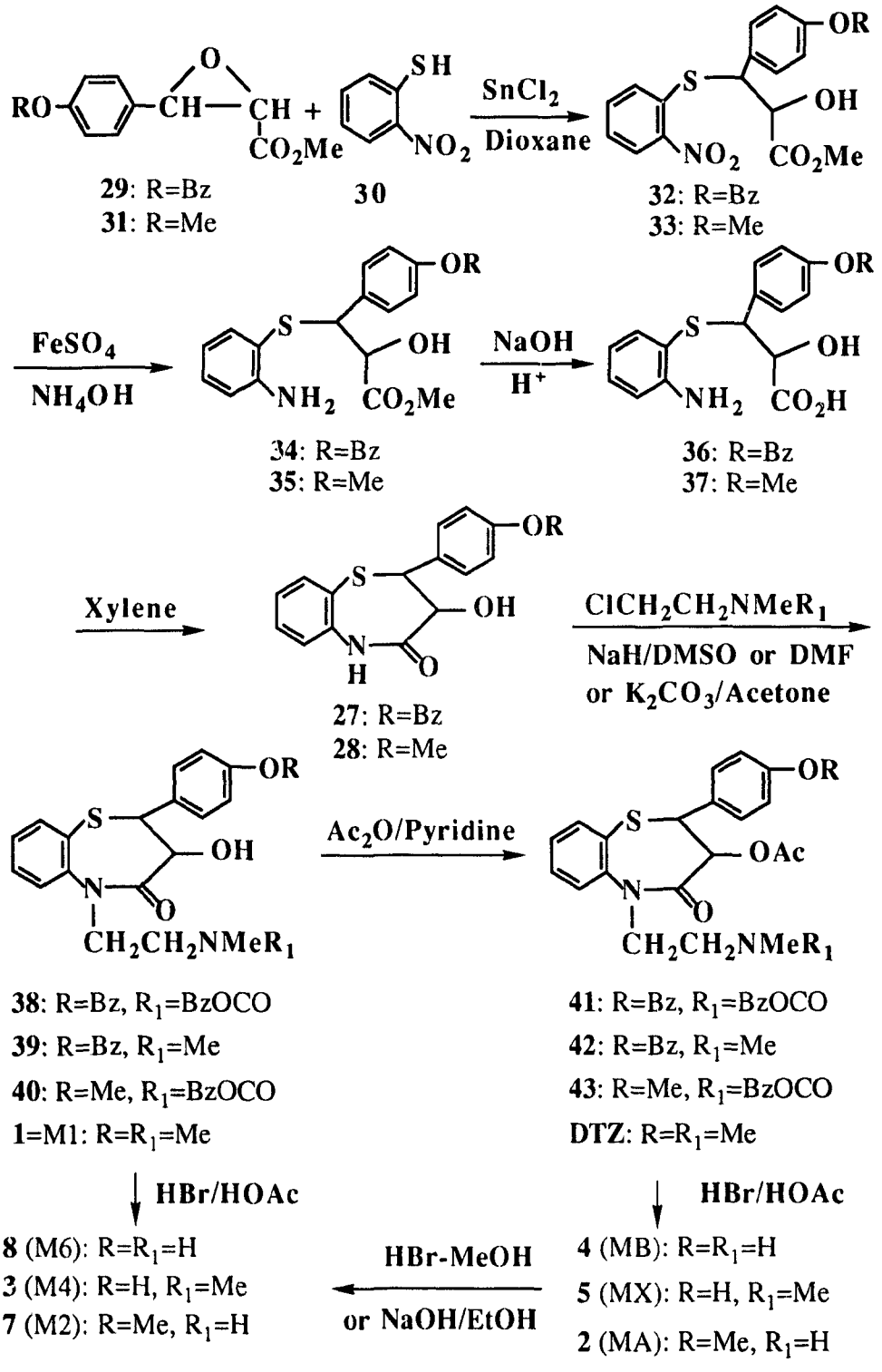
5-[2-(Dimethylamino)ethyl]-2,3-dihydro-3-hydroxy-2-(4-hydroxyphenyl)-1,5-benzothiazepin-4(5H)-one (3). Compound **3** was obtained by degradation of commercial DTZ with boron tribromide. Even though **3** is a side product from the failure of preparation of **5**, it still serves the purpose of confirming characterization of **3** in the multistep synthesis by comparison of their physicochemical, spectroscopic, chromatographic and optical rotatory properties.

For the same reason described above, **5-[2-(dimethylamino)ethyl]-2,3-dihydro-3-hydroxy-2-(4-methoxyphenyl)-1,5-benzothiazepin-4(5H)-one (1)** was obtained by direct ester hydrolysis of commercial DTZ hydrochloride

2. 2. 1. 2 Multistep Synthesis of Diltiazem Metabolites

Since the short sequence synthesis failed, all DTZ metabolites shown in Chart I were obtained by the multistep sequence depicted in Scheme III DTZ *N*-oxide was obtained by reaction of DTZ with 3-chloroperbenzoic acid

Published procedures[112—114] were modified to synthesize key intermediates (**27**, **28**). The glycidic ester **29** was accessible by a Darzens condensation on 4-benzyloxybenzaldehyde.[72,115,116] Heterogeneous conditions described in the literature, in my hands, gave only little product A modified procedure using an appropriate mixture of methanol and dioxane to make the reaction homogeneous gave satisfactory yields.

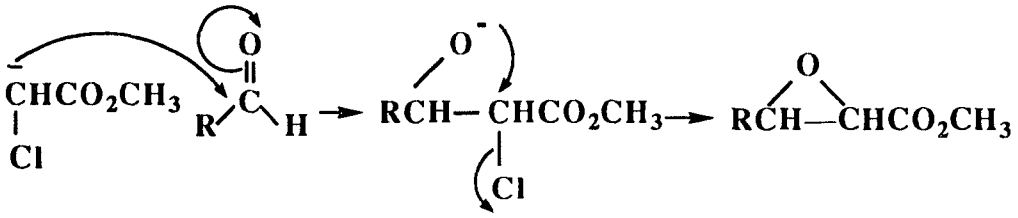


Scheme III. Multistep Synthesis of DTZ Metabolites

2-Nitrothiophenol (**30**) was obtained in quantitative yield by reduction of bis(2-nitrophenyl)disulfide with sodium borohydride in tetrahydrofuran (THF) [117] A direct coupling of freshly prepared **30** with **29** or **31** (Aldrich) in the presence of tin (II) chloride[119,120] gave **32** and **33**, respectively Reduction of nitro esters **32** and **33** to amino esters **34** and **35** with $FeSO_4$ / NH_4OH was achieved in yields of 65% and 86%, respectively, compared with reported[112] yields of 43% and 71% Ester hydrolysis with 5% NaOH[112] gave **36** and **37** in high yield. Cyclization of the amino carboxylic acids (**36** and **37**, respectively) in boiling xylene[112] gave the corresponding cyclic amides (**27** and **28**) in quantitative yields One of the alkylating agents, benzyl (2-chloroethyl)-*N*-methylcarbamate,[121] was prepared in quantitative yield by reacting 2-chloroethylamine hydrochloride with benzyl chloroformate in basic solution [122] The alkylation of **27** or **28** with 2-chloroethyldimethylamine hydrochloride or benzyl (2-chloroethyl)-*N*-methylcarbamate gave **1**, **38—40** in the presence of either NaH / DMSO or K_2CO_3 / acetone. Acetylation of **38—40** with acetic anhydride in the presence of pyridine furnished **41—43**. The final products (**2**, **4**, **5**) were obtained by deprotection with 25% (w/w) HBr-HOAc Deacetylated metabolites **8**, **3** and **7** can be obtained either from **38**, **39** and **40**, respectively, by deprotection or from **4**, **5** and **2**, respectively, by acid or base hydrolysis.

Methyl 3-(4-benzyloxyphenyl)glycidate (29). Darzens condensation was utilized to prepare **29** (Scheme IV) The reaction is considered as a nucleophilic addition at a carbonyl group followed by intramolecular nucleophilic displacement of a leaving group present in the nucleophile The first step in the reaction is addition of the carbanion of methyl chloroacetate to the carbonyl group of 4-benzyloxybenzaldehyde The alkoxide

oxygen formed in the addition then effects nucleophilic attack, displacing the chloride and forming an α, β-epoxy ester, i.e., **29**.



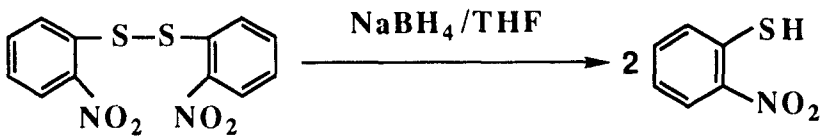
(**29**)

Scheme IV. Darzen's Condensation

This synthesis failed at the first trial when the general procedure[115,117] was followed exactly. Little product was formed when a mixture of 4-benzyloxybenzaldehyde and methyl chloroacetate in sodium methoxide / methanol was stirred overnight. Using freshly distilled methanol to ensure a reactive methoxide also gave the same result. In an effort to make the mixture homogeneous, three times more methanol than that used in the literature was added. However, the reaction still proceeded no further. It was suspected that the probable reason for failure was that the reaction mixtures were either not homogeneous or were too dilute. A search was made for a suitable solvent for 4-benzyloxybenzaldehyde; dioxane was found to be a good solvent for this reaction. A 50% yield was obtained after using dioxane as a co-solvent. Finally a 74% yield (see synthesis of **29** in Experimental section) was achieved when less methanol was used to make the reaction mixture more concentrated than that in the literature. The result indicates that reactants in homogeneous and concentrated enough solution are essential for the reaction to occur satisfactorily.

There was no stereochemical information available for **29** in the original literature.[72,115,116] An attempt was made to assign the stereochemistry of **29** by comparing proton NMR data of **29** with known glycidate derivatives. The chemical shifts and coupling constants of the two methine protons of **29** are identical with those of methyl *trans*-(±)-3-(4-methoxyphenyl)glycidate (**31**, from Aldrich), i.e., δ 3.51 (d, J = 1.7Hz, 1H, CH), δ 4.05 (d, J = 1.7Hz, 1H, CH). It has been well established that the coupling constant of methine protons of a *cis*-epoxide is usually larger than that of its *trans*-isomer. The coupling constant between α, β protons of *tert*-butyl *cis*-β-phenylglycidate is 5 Hz, while that of the *trans* isomer is 2 Hz.[123] The optical rotation of **29** is zero. All the pieces of evidence support the contention that **29** is methyl *trans*-(±)-3-(4-benzyloxyphenyl)-glycidate.

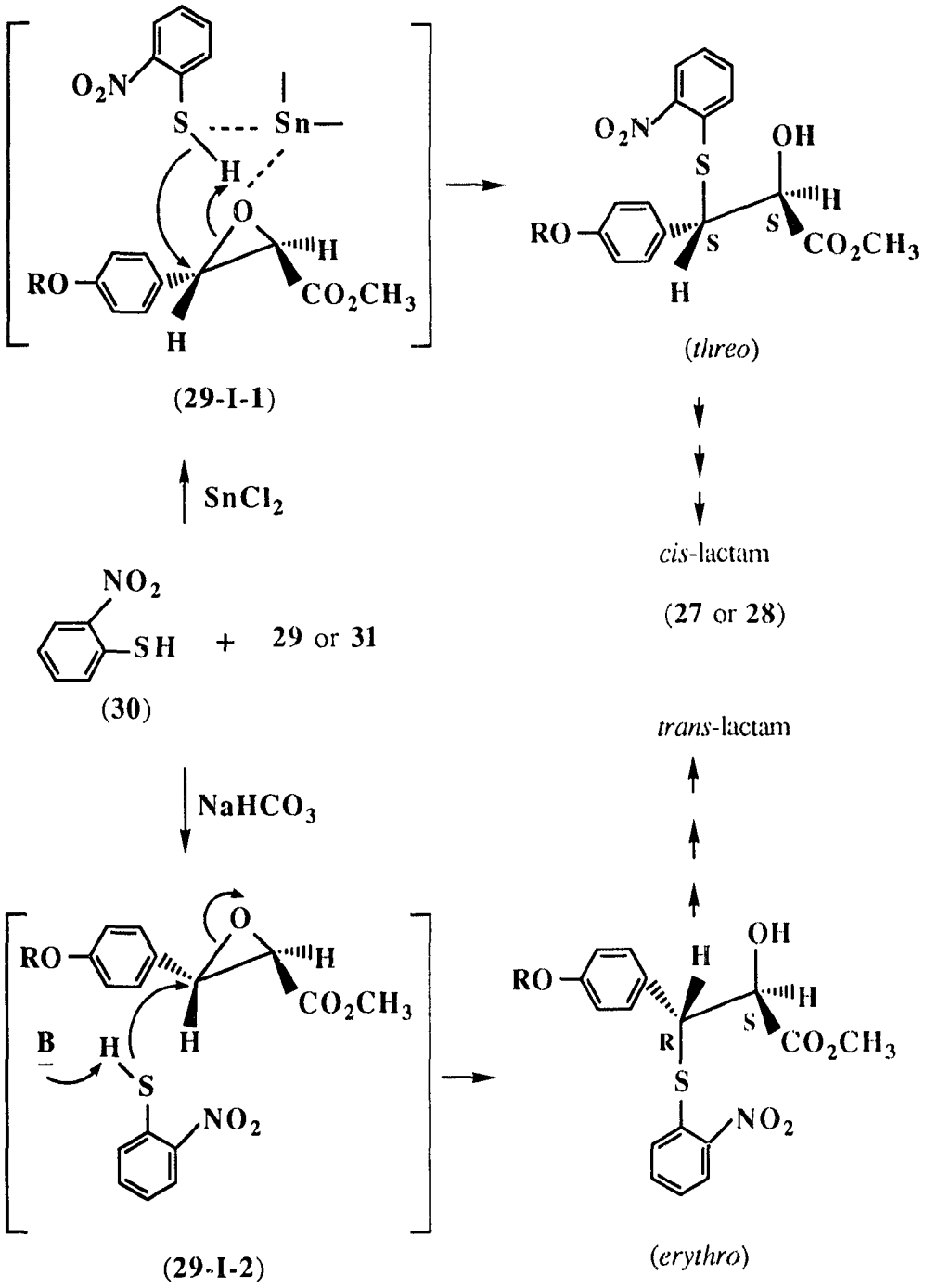
2-Nitrothiophenol (30). Normally, thiols may be stored as stable disulfides and the thiols regenerated as needed by reduction with any of several reducing reagents. The reagents utilized include lithium aluminum hydride,[124] sodium borohydride,[125] sodium borohydride/aluminum chloride,[126] zinc / acetic acid,[127] triphenylphosphine,[128] as well as other miscellaneous reagents.[118,129] For the purpose of the reduction of bis(2-nitrophenyl) disulfide to **30**, sodium borohydride[117] was used (Scheme V), because it is cheap and convenient, and it gives desirable results. The optimal reaction conditions include a three hour reaction time at 25°C, a ratio of disulfide to sodium borohydride of 1 to 3.5 giving a good yield and a solitary product. Since this reduction reaction is almost quantitative, there is no need to purify the product. Furthermore, since **30** can be readily oxidized, at least in part, back to the disulfide during the purification process, the crude product of **30** was used directly for the subsequent coupling reaction.



(30)

Scheme V. Reduction of Disulfide

Methyl 3-(4-benzyloxyphenyl)-2-hydroxy-3-(2-nitrophenylthio)-propionate (32) and methyl 2-hydroxy-3-(4-methoxyphenyl)-3-(2-nitrophenylthio)propionate (33). It was known[119] that in the reaction of **30** with *trans* 3-phenylglycidic esters carrying various substituents on the benzene ring, both reactivity and stereoselectivity of the oxirane ring-opening of the glycidates are markedly influenced by the electronic nature of the substituents. The presence of electron-donating groups is favorable for both reactivity and the preferential formation of *cis*-opening products, while the reverse is true for electron-withdrawing groups. A plausible mechanistic explanation is shown in Scheme VI (only one isomer of *trans*-**29** shown). As shown below, the nucleophilic attack of **29** by **30** may generate either *cis*- or *trans*- oxirane ring-opening products. It is anticipated that (±)-*trans*-**29** may produce four stereoisomers, two *threo*-isomers through intermediate **29-I-1** and two *erythro*-isomers through intermediate **29-I-2**. The intermediate **29-I-1** would provide suitable geometry in the reactants for selective *cis*-ring opening as well as electronic activation of the oxirane ring in the transition state, leading to ready and highly stereoselective formation of the *threo*- ester, while the intermediate **29-I-2** would be suitable for selective *trans*-ring opening and provide *erythro*-ester.[119] The *threo* isomer pair corresponds to the configuration of 2*S*, 3*S* and 2*R*, 3*R*, while the *erythro* pair to that of 2*R*, 3*S* and 2*S*, 3*R*.



Scheme VI. Ring Opening of Epoxide

It was reported[119] that the tin-catalysed reaction of *cis*-glycidate (*cis*-**31**) with **30** was also highly stereospecific and gave the *erythro*-isomer (>95%), the *cis*-opening product. This result ruled out another possible mechanistic explanation of the reaction, which involves formation of the benzylic carbocation followed by stereoselective attack of nucleophile. If this were the case, the *threo*-isomer would be obtained from either the *trans*- or *cis*-glycidates due to the characteristics of planar geometry of the benzylic carbocation.

Both **29** and **31** have electron-donating groups (benzyloxy- and methoxy-, respectively). The *cis*-opening resulting from attack of the thiol group of freshly prepared **30** from the side of the oxirane ring (**29** and **31**), in the presence of tin (II) chloride, gives **32** and **33**, respectively. The reaction must be carried out under an inert atmosphere since **30** is readily oxidized to disulfide when exposed to air. Stirring at 0°C for the first hour of reaction and gradually returning to room temperature afterwards is absolutely essential since this coupling reaction generates heat.

Methyl 2-acetyloxy-3-(4-benzyloxyphenyl)-3-(2-nitrophenylthio)-propionate (44) and methyl 2-acetyloxy-3-(4-methoxyphenyl)-3-(2-nitrophenylthio)propionate (45). To evaluate the stereochemistry of the oxirane ring opening, **44** and **45** were prepared by acetylation of **32** and **33**, respectively, with acetic anhydride in the presence of pyridine. Even though stereoselective *cis*-opening of glycidates is well established,[119] evaluation of the ratio of the isomeric nitro- esters (**32** and **33**) is still necessary. Synthesis of these acetylated derivatives serves the purpose of characterization of the *cis*- and *trans*-lactams later formed to ensure the right isomer has been obtained. ^{1}H NMR spectroscopy was used for the characterization.[119]

Methyl 3-(2-aminophenylthio)-3-(4-benzyloxyphenyl)-2-hydroxy-propionate (34) and methyl 3-(2-aminophenylthio)-2-hydroxy-3-(4-methoxyphenyl)propionate (35). Compounds **34** and **35** were prepared with the method developed by the Japanese researchers[112] except for the work-up procedure. There is no difficulty working on a few millimole scale; however, when the reaction was carried out on a large scale (20—30 times more than those in the literature[112]), the gel-like darkened products were very difficult to separate. Hence, co-solvents dichloromethane and methanol, in which the desired product is much more soluble than in ethyl acetate (used in the literature), were used to extract the product more efficiently. For such a large scale, mechanical stirring is necessary to ensure good mixing of the reactants. The yield obtained was 12% higher than that of the Japanese work in the synthesis of **35**, probably benefitting from better stirring and more efficient extraction.

3-(2-Aminophenylthio)-3-(4-benzyloxyphenyl)-2-hydroxypropionic acid (36) and 3-(2-aminophenylthio)-2-hydroxy-3-(4-methoxyphenyl)-propionic acid (37). Direct cyclization of amino ester (**34** or **35**) by heating with acid (20% sulfuric acid or acetic acid) in the literature[112] gave very poor yields, probably because the benzothiazepine ring is acid-labile when hot. Instead, a two-step synthesis from amino ester was utilized to get the cyclization product. Hydrolysis of amino esters (**34** and **35**) with 5% NaOH gave **36** and **37** in high yield.

2-(4-Benzyloxyphenyl)-2,3-dihydro-3-hydroxy-1,5-benzothiazepin-4(5H)-one (27) and 2,3-dihydro-3-hydroxy-2-(4-methoxyphenyl)-1,5-benzothiazepin-4(5H)-one (28). Cyclization of the amino carboxylic acids (**36** and **37**, respectively) in boiling xylene gave the corresponding cyclic amides, **27** and **28**,

respectively. It was initially found that the cyclization of the methoxy compound (**37**) was much easier than that of the benzyloxy congener (**36**). Benzothiazepinone **28** was obtained by refluxing **37** for only two hours in xylene with an almost quantitative yield, whereas **27** required longer time refluxing in the same solvent for a 54% yield. The major difference is probably the relative solubilities of the amino carboxylic acids in xylene. While **37** is readily soluble in boiling xylene, **36** is not. Another critical observation was made. The aqueous solutions of both amino carboxylic acids (**36** and **37**) must be adjusted to pH *ca.* 5 during the work-up in order to have a high yield in the successive cyclization reaction. At this pH, either the amino or carboxyl group is in the salt form. Infrared spectroscopy suggested that the zwitterionic forms of these amino acids predominate at pH 5. Nevertheless, sufficient undissociated amine was present for nucleophilic attack of the undissociated carboxyl. After optimal reaction conditions were worked out, a quantitative cyclization for both **36** and **37** was eventually achieved.

2-Chloroethylmethylamine hydrochloride (46). Compound **46** was easily prepared in high yield by reacting 2-methylaminoethanol with thionyl chloride by a standard procedure.[121]

Benzyl (2-chloroethyl)-*N*-methylcarbamate (47). Compound **47** was prepared in quantitative yield by reacting 2-chloroethylmethylamine hydrochloride with benzyl chloroformate in basic solution. It has been reported that the product, **47**, is subject to intermolecular cyclization[122] on attempted distillation at reduced pressure (20 mm Hg) at 170—180°C. Fortunately, compound **47** did not need to be purified. The crude product showed only one spot on TLC (CH_2Cl_2 / hexane, 1:1). The IR spectrum showed the expected functional groups. However, ^1H NMR in $CDCl_3$ showed extra signals: two pairs

of multiplets for the ethylene group (δ 3.54—3.60 and 3.71—3.76), a pair of broad singlets for the methyl group (δ 2.89—2.93) and a broad singlet for the benzylic methylene group (δ 5.09). These correspond to ^{13}C NMR at 25°C: a pair of equal intensity signals for methyl group (δ 34.38 and 34.60), three pairs of signals for methylenes (δ 41.65 and 41.98, δ 49.46 and 50.00, δ 66.14 and 66.33) and two equal intensity signals for carbonyl group (δ155.29 and 155.56). These were shown to be due to the presence of *cis*- and *trans*-isomers of **47** caused by π-bonding between nitrogen atom and carbonyl group at room temperature. Each pair of equal intensity ^{13}C signals was expected to coalesce into a singlet when decoupled ^{13}C NMR spectra were recorded at higher temperature. This was confirmed by ^{13}C NMR spectra at 60°C (see Experimental section).

5-[2-(*N*-Benzyloxycarbonyl-*N*-methylamino)ethyl]-2-(4-benzyloxyphenyl)-2,3-dihydro-3-hydroxy-1,5-benzothiazepin-4(5H)-one(38).

Attempts to prepare compound **38** were made by three methods. The first method was the alkylation in the presence of dimethylsulfinyl carbanion.[72] Although the procedure of alkylation of **27** with **47** in NaH / DMSO was followed exactly, the first trial was not very successful. Only a 14% yield of the presumed product was obtained after a separation of the reaction mixture by column liquid chromatography. The IR spectrum of the presumed product showed two carbonyl absorption bands at 1735 and 1665 cm^{-1} in chloroform corresponding to carbamate and amide, respectively. The second method was the alkylation in NaH / DMF. Since the first method involved the generation of dimethylsulfinyl carbanion, which might make the reaction more complicated, NaH / DMF was used to simplify the reaction conditions. However, the result was similar to that in method 1. The last method was the alkylation in K_2CO_3 / DMF. The result showed no difference from those from the methods mentioned above. An attempt was made to

determine whether the reagents, especially NaH, were of poor quality. Finally a 43% yield was achieved when the alkylation reaction was carried out in the presence of dimethylsulfinyl carbanion generated with newly purchased NaH and anhydrous DMSO under a dry nitrogen atmosphere. Recovery of an additional 32% of one of the starting materials (**27**) from the reaction was achieved with methanol.

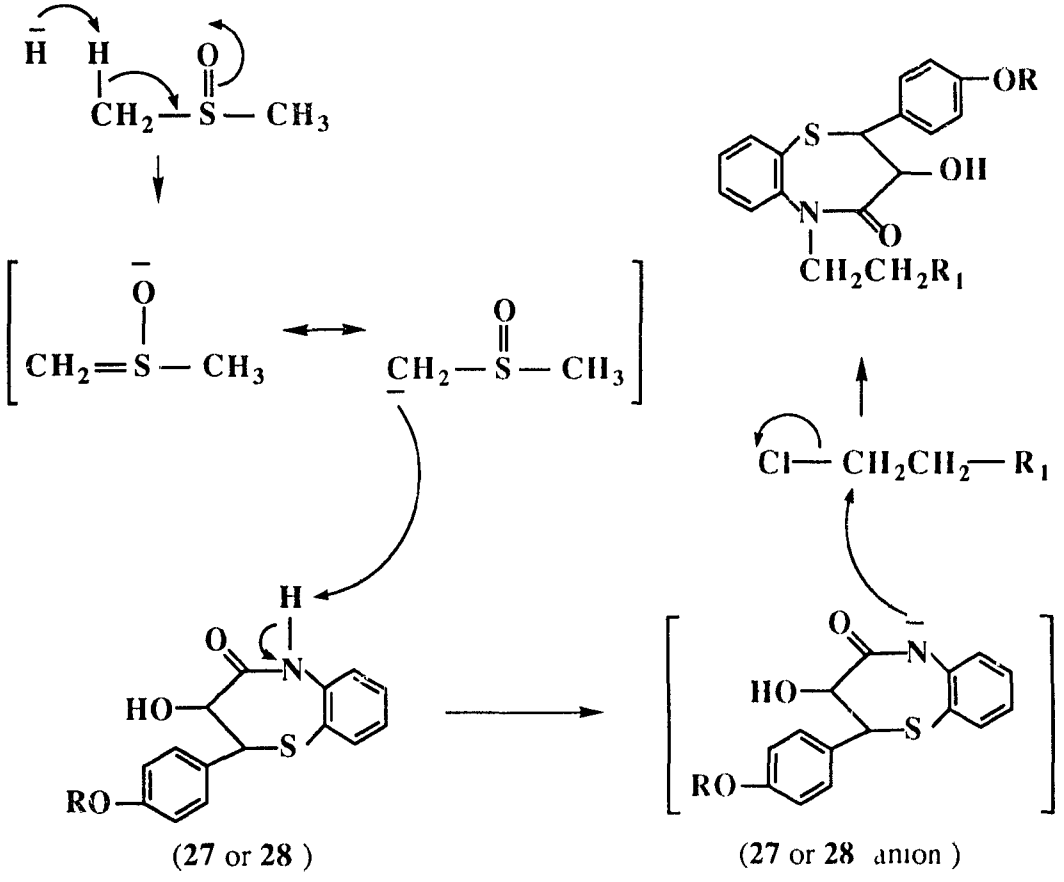
2-(4-Benzyloxyphenyl)-5-[2-(dimethylamino)ethyl]-2,3-dihydro-3-hydroxy-1,5-benzothiazepin-4(5H)-one (39). Compound **39** was prepared by two methods. The alkylation of **27** with 2-chloroethyldimethylamine hydrochloride in NaH (old) / DMSO gave results comparable to those in the the first trial of the preparation of **38**. The presumed product constituted only 20—30% of the reaction mixture according to TLC and was not separated. However, when switched from NaH (old) / DMSO to K_2CO_3 / DMF, the alkylation gave a satisfactory result (51% yield).

5-[2-(*N*-Benzyloxycarbonyl-*N*-methylamino)ethyl]-2,3-dihydro-3-hydroxy-2-(4-methoxyphenyl)-1,5-benzothiazepin-4(5H)-one (40). Compound **40** was prepared in 29% yield by the alkylation of **28** with **47** in the presence of NaH (new) / DMSO.

5-[2-(Dimethylamino)ethyl]-2,3-dihydro-3-hydroxy-2-(4-methoxyphenyl)-1,5-benzothiazepin-4(5H)-one (1). A 58% yield was achieved by refluxing **28** with 2-chloroethyldimethylamine hydrochloride in the presence of anhydrous K_2CO_3 in acetone under nitrogen.

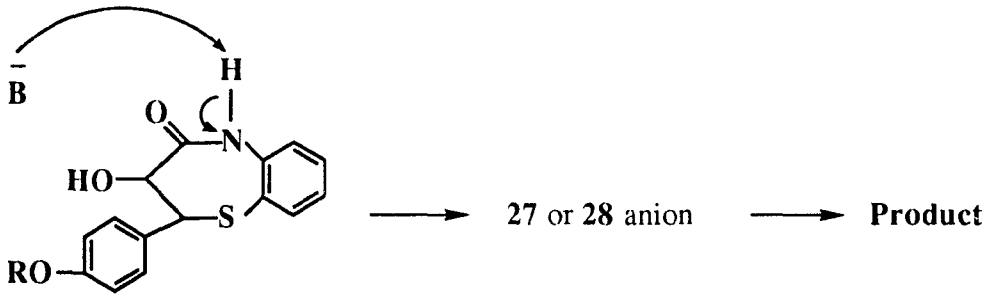
For the alkylation reactions described above, suitable solvents include DMSO, DMF and acetone depending upon the solubility of the cyclic amide For the benzyloxy cyclic amide, DMSO or DMF must be used in order to make the reaction mixture homogeneous. For the methoxy cyclic amide, acetone is preferred because it is easy to remove.

The alkylation reaction described above may be classified as indirect and direct in terms of generation of anions of **27** or **28** In an indirect alkylation (Scheme VII), the



Scheme VII. Alkylation in the Presence of Dimethylsulfinyl Carbanion

dimethylsulfinyl carbanion was generated by hydride first; then anion of **27** or **28** was formed by the dimethylsulfinyl carbanion under fairly mild conditions; finally the product was achieved by nucleophilic attack of alkylating agents. In the case of direct alkylation, anion of **27** or **28** was generated directly by hydride or carbonate as depicted in Scheme VIII. Using NaH / DMF, the reaction was homogeneous; when K_2CO_3 / DMF or acetone were used, the base gradually went into solution. Different yields obtained from these three methods may be related to the basicities of bases used, solvent effects, the nature of the substrates and the alkylating agents, and ambient conditions.



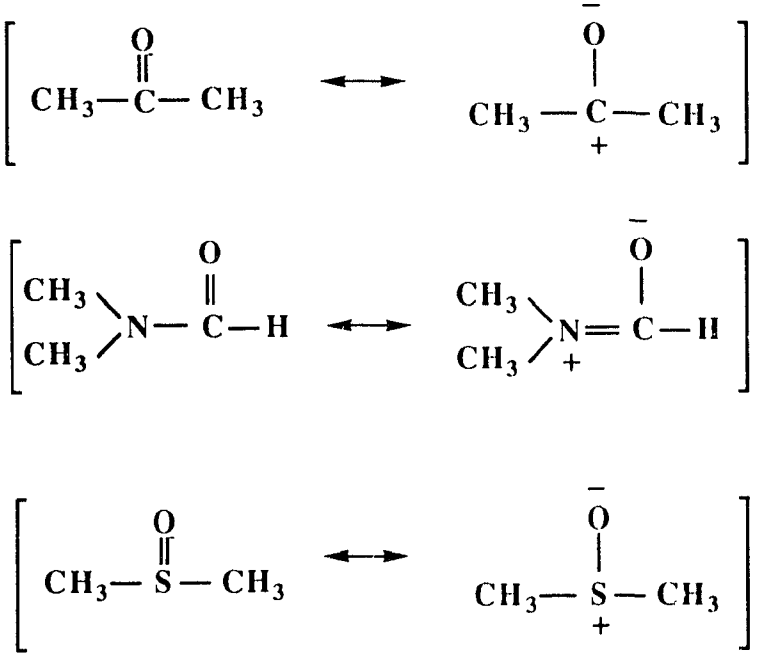
(**27** or **28**)

Scheme VIII. Alkylation in the Presence of Simple Base

(A). The basicity of bases utilized. Generally speaking, relative basicities of different bases can be estimated with relative acidities of their conjugate acids in a reversed order of magnitude. The acidity normally can be measured as the pK_a value, the negative logarithm of the dissociation constant of an acid, formally defined by equation 1. The base strength is normally solvent-dependent.

$$H-A \rightleftharpoons H^+ + A^- \qquad \text{(Eqn 1)}$$

(B). Solvent effects. It is very important to recognize that the pK_a values are solvent dependent.[130] The pK_a value depends upon the ability of solvent to solvate the proton, the anion, and the undissociated acid. Since solvation of the proton is constant in a given solvent and solvation of most neutral acids is small compared to that of their conjugate bases, differences in acidity brought about by structure variations or solvent changes are usually caused by changes in the energies of the anions. Carbonate is a weak base in a protic solvent like H_2O, but its basicity will be stronger in an aprotic solvent such as DMSO. In H_2O, carbonate anion can be stabilized with hydrogen bonding by H_2O. In aprotic solvents like acetone, DMF or DMSO, the situation is different. The resonance structures of those aprotic solvents are shown in Scheme IX. On the right hand of the



Scheme IX. Aprotic Solvent Effect

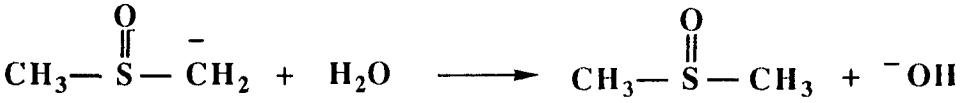
scheme, all these solvents show naked negative charges on oxygen and sterically hindered positive charges on carbon, nitrogen and sulfur atoms. These naked negative charges of the solvents can destabilize the already negatively charged carbonate species. This may contribute to the fact that carbonate is a strong enough base to pull the proton off the nitrogen of **27** or **28** in above aprotic solvents.

(C). The nature of the substrates. In structure **27** or **28**, there are three protons susceptible to deprotonation by a strong base, the one on nitrogen, the one on oxygen and the one at the C3 position. As mentioned previously, the acidities are solvent dependent. Let us consider DMSO as solvent. The proton on nitrogen is the most acidic (pK_a *ca.* 20)[130] of all because it is doubly activated by both the benzene ring and the carbonyl group; the anionic charge formed can be efficiently delocalized in the system. The methine proton at C3 position is less acidic (pK_a *ca.* 22—26).[130] The alcoholic proton is the least acidic (pK_a *ca.* 30 like methanol).[130] It is anticipated that the site of anion to be generated should be on nitrogen (anions of **27** or **28**).

(D). The nature of the alkylating agents. The alkylating agents used here are 2-chloroethyldimethylamine and benzyl (2-chloroethyl)-*N*-methylcarbamate (**47**). The intermediates **38** and **40** which result from alkylation by **47** always had lower yields whereas yields of **39** and **1** from alkylation by 2-chloroethyldimethylamine were higher (see Experimental section). Two possible considerations to take into account are the electronic and steric characters of the alkylating agents. The carbonyl group in the carbamate moiety has an electron-withdrawing feature. This makes chlorine in the side chain a worse leaving group than in the case of 2-chloroethyldimethylamine where the corresponding substituent is a methyl group with electron-donating character. Also, in the

case of **47**, the reactivity of anions of **27** or **28** may be masked by flipping-around of the side chain or hydrophobic interactions between the benzyl group of the side chain and the aromatic moieties of anions of **27** or **28**.

(E). Ambient conditions. The most sensitive external factor affecting this alkylation reaction is probably humidity during the reaction. In most cases, yields were higher when the reaction was done in drier weather. Theoretically, the presence of H_2O in the reaction should not hinder the alkylation reaction because H_2O (pK_a *ca.* 32)[130] is less acidic than **27** or **28** (pK_a *ca.* 20)[130] in DMSO However, H_2O is more acidic than DMSO (pK_a *ca.* 35)[130]. The following reaction (Scheme X) might have occurred and anions of **27** or **28** could not be generated for further alkylation reaction.



Scheme X. Effect of Humidity on Alkylation

3-Acetyloxy-5-[2-(*N*-benzyloxycarbonyl-*N*-methylamino)ethyl] 2-(4-benzyloxyphenyl)-2,3-dihydro-1,5-benzothiazepin-4-(5H)-one (41), 3-acetyloxy-2-(4-benzyloxyphenyl)-5-[2-(dimethylamino)ethyl]-2,3-dihydro-1,5-benzothiazepin-4(5H)-one (42) and **3-acetyloxy-5-[2-(*N*-benzyloxy-carbonyl-*N*-methylamino)ethyl]-2,3-dihydro-2-(4-methoxyphenyl)-1,5-benzothiazepin-4(5H)-one (43).** Compounds **41**, **42** and **43** were prepared by reacting the corresponding precursors with acetic anhydride in the presence of pyridine.

3-Acetyloxy-2,3-dihydro-2-(4-hydroxyphenyl)-5-[2-(methylamino)-ethyl]-1,5-benzothiazepin-4(5H)-one (4), 3-acetyloxy-5-[2-(dimethyl-amino)ethyl]-2,3-dihydro-2-(4-hydroxyphenyl)-1,5-benzothiazepin-4(5H)-one (5) and **3-acetyloxy-2,3-dihydro-2-(4-methoxyphenyl)-5-[(2-methyl-amino)ethyl]-1,5-benzothiazepin-4(5H)-one (2).** Compounds **4**, **5** and **2** were obtained by deprotection of the corresponding precursors (**41**, **42** and **43**, respectively) with 25% HBr-HOAc. This reagent efficiently hydrolyses the benzyl or benzyloxycarbonyl groups while keeping the ester moiety intact.

2,3-Dihydro-3-hydroxy-2-(4-hydroxyphenyl)-5-[2-(methylamino)-ethyl]-1,5-benzothiazepin-4(5H)-one (8) and **5-[2-(dimethylamino)ethyl]-2,3-dihydro-3-hydroxy-2-(4-hydroxyphenyl)-1,5-benzothiazepin-4(5H)-one (3).** Compounds **8** and **3** were prepared from **4** and **5**, respectively by ester hydrolysis in HBr-MeOH. The use of HBr-MeOH in these cases avoids the troublesome procedures used in the literature,[72] and simplifies the work-up procedure.

Both **8** and **3** have phenol functional groups. They are a secondary and a tertiary amine, respectively. The amphoteric properties of these compounds complicate their preparation from **38** and **39**. First of all, for instance, deprotection by 25% HBr-HOAc must be accompanied by acetylation of the hydroxyl group in **38** and **39**. The second step described[72] is hydrolysis of this acetate ester with NaOH-EtOH, followed by acidifiying the resultant reaction mixture with 10% HCl. In this step, mixtures of the HCl and HBr salts of **8** or **3** would be expected. Finally, treatment of the resultant salts with $NaHCO_3$ followed by extraction into EtOAc seemed to be inappropriate. Even though bicarbonate is

more acidic than phenol, carbonic acid ($pK_a = 6.37$)[131] is much more acidic than the HX salt of **8**. There is no pK_a value available for HX salt of **8**; however, the pK_a of the salt of another secondary amine, piperidine hydrochloride, is 11.123 at 25°C.[132]

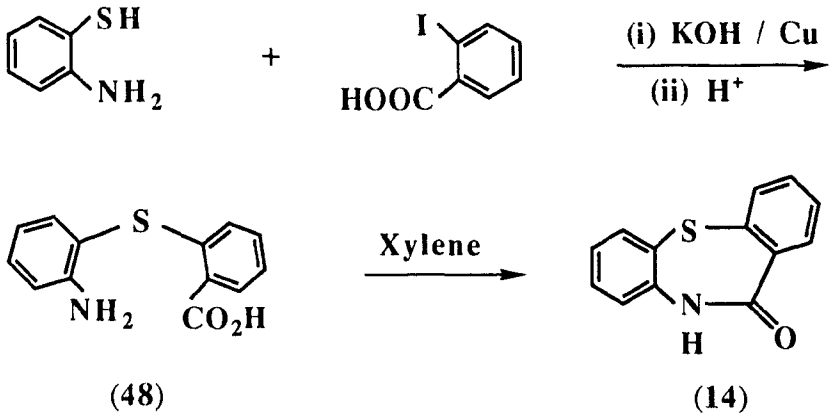
2,3-Dihydro-3-hydroxy-2-(4-methoxyphenyl)-5-[2-(methylamino)-ethyl]-1,5-benzothiazepin-4(5H)-one(7). Compound 7 can be obtained by either ester hydrolysis of **2** in alkaline conditions or deprotection of **40** with HBr-HOAc. However, **2** was obtained as a major product in the latter route. This probably resulted from the simultaneous acetylation of hydroxyl group in **40**. Compound **7** was obtained in 89% yield by saponification of **2**.

3-Acetyloxy-2,3-dihydro-5-[2-(dimethylamino)ethyl]-2-(4-methoxy-phenyl)-1,5-benzothiazepin-4(5H)-one-*N*-oxide(6). Compound **6** was prepared by oxidation of commercial DTZ hydrochloride with 3-chloroperbenzoic acid.[72]

2. 2. 2 Preparation of Dibenzotricyclic Systems (DBTs)

2. 2. 2. 1 Preparation of Dibenzo[*b,f*]-1,4-thiazepine Nucleus

The tricyclic dibenzothiazepine nucleus is accessible by published procedures.[133,134] Dibenzo[*b, f*]-1,4-thiazepin-11(10*H*)-one (**14**) was prepared by the two-step synthesis (**48** as intermediate) depicted in Scheme XI. The cyclization in xylene



(48) (14)

Scheme XI. Synthesis of Dibenzothiazepine Nucleus

gave a very good yield (83%), as expected since a similar reaction has been carried out in the synthesis of DTZ metabolites.

2. 2. 2. 2 Preparation of Target DBTs

10,11-Dihydro-10-methyldibenzo[*b,f*]-1,4-thiazepin-11-one (25) Compound **25** was prepared by methylation of **14** with methyl iodide in the presence of NaH.

10-(Cyclopropyl)carbonyl-10,11-dihydrodibenzo[*b,f*]-1,4-thiazepin-11-one (26) Compound **26** was obtained as side product in an attempt to prepare 10-acyl DBT derivatives.

10-(Dimethylaminoethyl)-10,11-dihydrodibenzo[*b,f*]-1,4-thiazepin-11-one (19) Compound **19** was prepared by reacting (2-chloroethyl)dimethylamine hydrochloride with **14** in the presence of excess of NaH.

10-(Dimethylaminopropyl)-10,11-dihydrodibenzo[*b,f*]-1,4-thiazepin-11-one (20) Compound **20** was obtained using a method analogous to that of **19**.

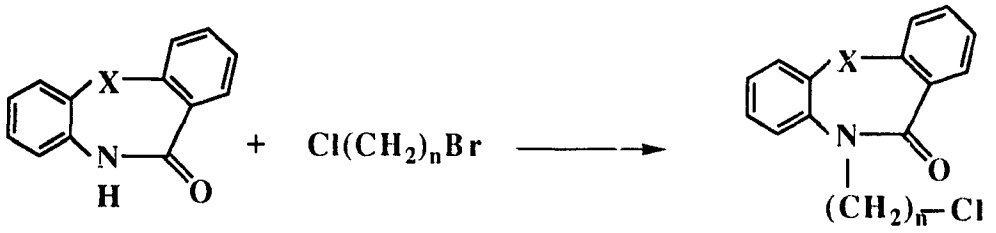
10-(ω-Chloroalkyl)-10,11-dihydrodibenzo[*b,f*]-1,4-thiazepin-11-one (49—53) Compounds **49—53** were obtained as principle components, mixed with their ω-bromo counterparts, by reaction of **14** and ω-bromochloroalkane.[135] These intermediates were characterized with FAB MS and used directly for the next step without further purification.

10-[ω-[4-(4-Fluorophenyl)-1-piperazinyl]alkyl]-10,11-dihydrodibenzo[*b,f*]-1,4-thiazepin-11-one (9—13) (n = 2—6) Conversion to **9—13** was effected by a Finkelstein halide exchange *in situ* in the presence of 2 equivalents of 4-fluorophenylpiperazine.

10-[ω-(*N*-Benzyl-*N*-methylamino)alkyl]-10,11-dihydrodibenzo-[*b,f*]-1,4-thiazepin-11-one (21—24) (n = 3—6) Compounds **21—24** were prepared using the same method as that of preparation of compounds **9—13**.

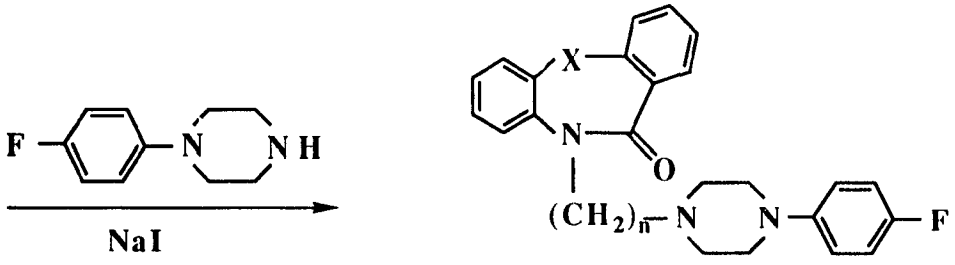
10-(ω-Chloroalkyl)-10,11-dihydrodibenz[*b,f*]-1,4-oxazepin-11-one (54—57) Alkylation of commercially available 10,11-dihydrodibenzo[*b,f*]-1,4-oxazepin-11-one with ω-bromochloroalkanes afforded compounds **54—57** using the same method as that of **49—53**, again used directly for the next step without further purification.

10-[ω-[4-[4-Fluorophenyl)-1-piperazinyl]alkyl]-10,11-dihydrodibenz[*b,f*]-1,4-oxazepin-11-one (15—18) (n = 3—6) Compounds **15—18** were obtained using the same method as preparation of compounds **9—13**. Scheme XII shows the general outline to achieve these dibenzotricyclic derivatives.



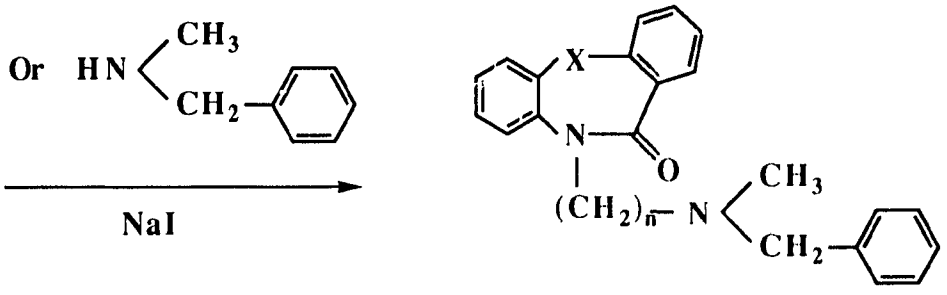
49—53 (X=S, n = 2—6)

54—57 (X=O, n = 3—6)



9—13 (X=S, n = 2—6)

15—18 (X=O, n = 3—6)



21—24 (X = S, n = 3—6)

Scheme XII. Synthesis of Target DBTs

2. 3 Characterization with Spectroscopic and Chromatographic Methods

2. 3. 1 Spectroscopy

Infrared (IR) and ^{1}H NMR spectroscopy were used for the characterization of every intermediate and target product throughout the synthesis of DTZ metabolites and DBTs. D_2O exchange experiments were performed whenever the molecule included exchangeable protons. The stereochemical characterization of some intermediates leading to synthetic DTZ metabolites was achieved by ^{1}H NMR. The chemical shifts of protons from stereoisomers and the coupling constants between the specific protons of DTZ metabolites provide reliable information about the stereochemistry of molecules investigated (see section 2.4. for details).

The ^{13}C NMR spectrum can provide a good deal of information about the carbon backbone of molecules. When ^{13}C NMR was used in combination with ^{1}H NMR as well as IR and MS, structural elucidation was made more easily. ^{13}C NMR spectroscopy was used successfully to characterize the intermediate benzyl (2-chloroethyl)-*N*-methyl carbamate (**47**) (see section 2. 2. 1. 2 for details).

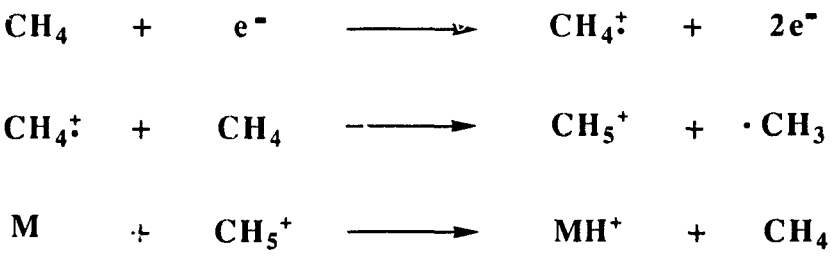
Distortionless Enhancement Polarization Transfer (DEPT) was used to determine multiplicities of ^{13}C resonances. For synthetic DTZ metabolites and DBTs, the ^{13}C NMR spectra of only key intermediates, **27** and **28**, and target products were taken to save spectrometer time.

DEPT is a special pulse sequence. It is very useful for the observation of low-γ nuclei which are J-coupled to ^{1}H, ^{19}F, or ^{31}P. It can generate separate carbon sub-spectra for methyl (up), methylene (down), and methine (up) signals. This is the same kind of

information traditionally obtained by off-resonance decoupling. Both sensitivity and resolution of DEPT spectra are far superior to those of the off-resonance decoupled spectra because the former can be acquired with broadband decoupling.

Mass spectrometry involves the formation, separation and detection of gas phase ions derived from the test molecule. Early ionization techniques such as electron impact (EI) and chemical ionization (CI) required the volatilization of compounds to the gas phase prior to ionization. These techniques were thus limited to thermally stable organic compounds which can be heated up to 200—300°C without significant decomposition. However, for DTZ metabolites, most of which possess polar functional groups (-OH, -NHR), special ionization techniques are required in order to produce an abundant molecular ion and to avoid thermal decomposition.

There are several ionization methods. The two methods commonly used to produce ions from thermally volatile materials are EI and CI. In the former technique, a 70 eV electron beam is used to ionize an organic molecule in the gas phase. This energy is also sufficient to cause extensive fragmentation, giving rise to a pattern of fragment ions which can help to characterize the compound. A disadvantage is the frequent absence of a molecular ion. This problem may be avoided by the use of CI mass spectrometry.[136] In this technique, a reagent gas (e.g., methane, isobutane, or ammonia) is allowed to pass into the ion chamber at a pressure of almost 10^{-4} mmHg. This gas is ionized by using electrons with energies up to 300 eV. For example, the generation of a CI spectrum using methane is depicted in Scheme XIII. If sample ions are volatilized into this mixture of ions, the CH_5^+ acts as a strong acid and protonates the sample. Thus in a positive CI spectrum, molecular weight information is obtained from protonation of sample molecules, and the observed m/z value for MH^+ is one unit greater than the true molecular weight.



Scheme XIII. Chemical Ionization Mechanism

Another method used to obtain molecular weight information for polar or thermolabile samples is fast atom bombardment (FAB).[137] This method is based upon giving a large pulse of energy to the sample. The effect of this is to put a relatively large amount of energy into translational modes involving sample molecules. Thus, intermolecular bonds involving the sample (e.g., hydrogen bonds) are broken in preference to covalent bonds, and the sample is desorbed from its environment into the gas phase. Thermal decomposition is reduced, or avoided, by avoiding an equilibrium distribution of the large amount of available energy. This is because the sample molecule leaves its solid or liquid environment within a time in the order of 10^{-12} s.

In FAB, the energy is provided by a beam of atoms or ions, respectively, of large translational energies (several keV). The sample may be bombarded in its solid state, but more commonly is first dissolved in a matrix of low volatility such as glycerol. FAB spectra frequently contain structurally useful fragment ions, but MH^+ is usually the most abundant ion, making molecular weight determination extremely easy by these techniques.

Ion-spray (IS) is an atmospheric pressure ionization technique.[138] A $[M + H]^+$ ion can be generated from very labile and high molecular weight compounds without thermal

degradation. In IS, an electric field is generated at the tip of the sprayer by applying a high voltage directly to the sprayer, with a counter electrode located a few milimeters away. Ions of one polarity are preferentially drawn to the surface of the drops by the electric field Each droplet contains many ions of both signs Only the excess ions of one polarity are available for evaporation.

2. 3. 2 CHROMATOGRAPHY

Chromatography encompasses a range of very powerful experimental techniques for separating mixtures of organic compounds Since chromatography was originally invented by the Russian scientist Tswett in 1903,[139] there has been enormous progress in both theory and application.

Thin layer chromatography (TLC) is one of the most widely used forms of chromatography, and is of enormous value for quick qualitative analysis of mixtures, for monitoring reactions and for determining the operating parameters to be used in preparative scale column chromatography

Throughout the synthesis, TLC was used to monitor reactions and check the purity of the products. TLC aluminum sheets (Silica gel 60 F_{254}) were used When illuminated with an UV lamp (254 nm) the adsorbent then glows a pale green color and the organic compounds show up usually as dark spots because they quench the fluorescence. An iodine chamber was used only occasionally as a visualization technique since almost all intermediates and target products can be seen under the UV lamp Under given conditions (adsorbent and solvent) the R_f value of a compound is characteristic Thus a match of the R_f value of a compound in a mixture with that of an 'authentic' sample of the compound provides a good indication that they are likely the same. Since the condition of the

adsorbent vary and solvent mixtures are difficult to reproduce accurately, it is necessary to demonstrate that the R_f values are the same by running the mixture and 'authentic' sample side by side and with co thin-layer by adding a little of the 'authentic' to a separate sample of the mixture to see whether the spots exactly coincide.

For checking the purity of the target products, their R_f values were determined on silica gel TLC with different solvents along with reversed phase HPLC. The purity of the compound may be confirmed if there is only one spot on the normal phase TLC and one peak besides the solvent peak on the reversed phase HPLC.

HPLC is a small scale form of column chromatography. It is an extraordinarily versatile technique, which can be used for the qualitative and quantitative analysis of mixtures of all kinds of compounds—volatile or non-volatile, thermally stable or not, polar and non-polar. Various detectors may be used with the UV absorbance detector being the most common. A retention time on HPLC is characteristic of a compound under a particular set of operating conditions. This can be used for provisional identification by comparison with an 'authentic' sample. The results may be subject to some degree of uncertainty. However, this can be overcome with the combination technique of LC-MS (see section 2. 5 for details). The pure synthetic DTZ metabolites were shown by HPLC along with LC-MS and LC-MS / MS to be identical to newly discovered DTZ metabolites in biological samples (see Experimental for details).

For preparative purposes, dry-column flash chromatography was used. This technique was developed by Dr. L.M. Harwood in the early 1980s.[140] It requires no special equipment and provides separating power that is at least as good as flash chromatography and that can be comparable with the resolution of an analytical TLC plate. The unusual advantage of this technique is its economy in terms of time and solvent compared with conventional or even flash column chromatography. Separation of two

grams (2 g) of reaction mixtures took only about an hour with *ca.* 90 mL of silica and *ca.* 200 mL of solvents.

2. 3. 3 Polarimetry

Many transparent substances that are characterized by a lack of symmetry in their molecular or crystalline structure have the ability to rotate the plane of polarized radiation. Materials possessing this property are said to be optically active. The angle through which the plane is rotated varies widely from one compound to another. The rotation is said to be *dextro* (+) if it is clockwise to an observer looking toward the light source, and *levo* (-) if counterclockwise. The reading of the rotation in degrees is given directly from a Perkin-Elmer model 141 polarimeter. For any given compound, the extent of rotation depends on the number of molecules in the path of the solution, determined by the concentration and the length of the cell containing it. It is also dependent on the wavelength of the light and on the temperature. The specific rotation, represented by the symbol $[\alpha]^t$, is defined by the formula

$$[\alpha]_\lambda^{\,t} = \frac{100\,\alpha}{l\ \ c}$$

where α is the angle (measured in degrees) through which the plane is rotated by a solution of concentration c grams of solute per 100 mL of solution, contained in a cell l decimeters in length; t designates the temperature and λ the wavelength. The latter is commonly specified as 589.3 nm, the "D-line" of a sodium vapor lamp, so $[\alpha]_\lambda^{\,t}$ then becomes $[\alpha]_D^{\,t}$.

2. 4 Stereochemistry of Intermediates and Synthetic DTZ Metabolites

3-Acetyloxy-5-[2-(dimethylamino)ethyl]-2,3-dihydro-2-(4-methoxyphenyl)-1,5-benzothiazepin-4(5H)-one has four diastereoisomers. It was initially reported[141] 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 d-*cis*-isomer (CRD-401, later called diltiazem), whose absolute configuration was assigned as (2S,3S)-*cis*,[142] was 1.5 to 2 times as active as the (±)-*cis*-isomers, while the potency of (2R,3R)-*cis*-isomer was very low in comparison with that of the (±)-*cis*-isomers.

It is very important to make certain that the appropriate isomer is obtained throughout the synthesis. Methyl (±)-*trans*-3-(4-methoxyphenyl)glycidate (**31**) is commercially available. No stereochemical information for methyl 3-(4-benzyloxyphenyl)glycidate (**29**) was available in the literature.[72,115,116] The stereochemistry of **29** was assigned by ^{1}H NMR in our study. It has been well established that the coupling constants of methine protons of *cis*-epoxides are usually larger (*ca.* 5Hz) than those of their *trans*-isomers (*ca.* 2Hz).[123] The chemical shifts and coupling constants of the two methine protons of **29** are identical to those of **31**, i.e., δ 3.51(d, J = 1.7Hz), and δ 4.05(d, J = 1.7Hz). Hence, **29** is assigned as methyl (±)-*trans*-3-(4-benzyloxyphenyl)glycidate.

The coupling of **31** with **30** is stereoselective.[119] 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 ^{1}H NMR signals in the acetylated derivatives as described by Hashiyama *et al.*[119] The *threo /erythro* ratios (peak integration) for **44** and **45** were found to be 95 / 5 and 97 / 3, respectively (Table 18).

Table 18. *Threo / Erythro* Ratios Determined by ^{1}H NMR Method.

Compds	δ (ppm) (*threo / erythro*)	Ratios (*threo / erythro*)
44	2.12 / 2.20	95 / 5
45	2.11 / 2.19	97 / 3

Finally, the *cis*-configuration was confirmed by ^{1}H NMR spectroscopy on the synthetic metabolites themselves. Coupling constants of the two methine protons (C2 and C3) of all synthetic metabolites measured from 7.24 to 7.70 Hz (Table 19), consistent with that of diltiazem (7.71 Hz).[143] The corresponding coupling constants of *trans*-diltiazem have a typical *antiperiplanar* value of 11.0 Hz.[143] All metabolites of diltiazem obtained by multistep synthesis were confirmed to be (±)-*cis* isomers by optical rotatory measurements (Table 19).

A commercial sample of diltiazem hydrochloride has a specific optical rotation of +115°.[144] Compounds **1** and **3** obtained from commercial DTZ by short sequence synthesis (Scheme II), and **6** directly from commercial DTZ proved to be optically active (Table 20). This is expected because the degradation or oxidation reactions do not change the stereochemistry.

Table 19. Specific Rotation and ^1H NMR Coupling Constants of DTZ and Metabolites Synthesized by Multistep Route

Compds	$[\alpha]_D^{21}$	$^3J_{HH}$(Hz)
DTZ*	+115.0°	7.68**
1	+0.5°	7.24
2	-4.6°	7.62
3	+1.1°	7.32
4	+0.7°	7.68
5	-0.5°	7.70
7	-1.2°	7.44
8	+1.3°	7.36

*Commercial; **Reference[143]: *cis*-DTZ, 7.71, *trans*-DTZ, 11.0 Hz.

Table 20. Specific Rotation and ^1H NMR Coupling Constants of DTZ and Metabolites Obtained by Degradation of Commercial DTZ

Compds	$[\alpha]_D^{21}$	$^3J_{HH}$(Hz)
DTZ*	+115.0°	7.68**
1	+134.5°	7.25
3	+163.8°	7.30
6	+114.0°	7.79

*Commercial; **Reference[143]: *cis*-DTZ, 7.71, *trans*-DTZ, 11.0 Hz.

2. 5 Identification of DTZ Metabolites

2. 5. 1 Purities of Synthetic DTZ Metabolites

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 21.

Table 21. Chromatographic Properties and Purities of Synthetic DTZ Metabolites

Compds	TLC[a] $R_f \pm$ SD,(n=3)	HPLC[b] t_r (min.)	Percent purity
DTZ	0.57±0.05	16.17	
1	0.50±0.04	8.53	≥ 99.5
2	0.36±0.04	14.87	≥ 99.6
3	0.47±0.04	4.55	≥ 95
4	0.32±0.03	6.37	≥ 97
5	0.46±0.03	6.74	≥ 98
6	0.25±0.02	17.51	≥ 99.6
7	0.23±0.02	8.03	≥ 98
8	0.22±0.02	4.34	≥ 96

[a]Silica gel 60 F_{254}, 0.2 mm, (Merck), $CHCl_3$ / MeOH(1:1); 22 ± 2°C, monitored at 254 nm.

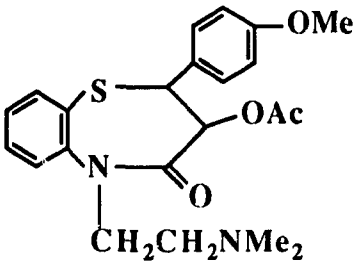
[b]Column: Zorbax Rx-C8, 4.6 x 250mm; flow rate: 1.0mL / min; isocratic, 30% CH_3CN + 0.1%TFA; column temperature: 40°C; wavelength of diode-array detector: 237nm.

2. 5. 2 Identity of Synthetic DTZ Metabolites with Biological Samples

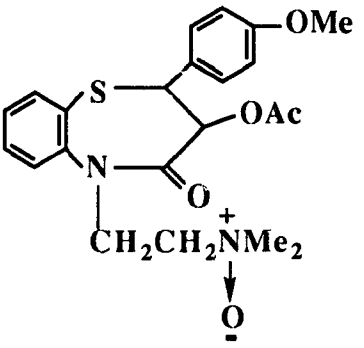
Improved chromatographic conditions were developed by Xie *et al.*[145,146] to reduce the peak tailing observed in the early methods.[147] The surface-deactivated Zorbax column gave outstanding performance with an acetonitrile / water mobile phase containing 0.1% trifluoroacetic acid (TFA) as both ion-pair and acidification reagent. The HPLC retention times (Table 21) of the synthetic metabolites were found to be identical with those of the metabolites from human urine (see Figure 15) as well as from other species such as rabbit, dog and rat. 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 provides very simple spectra dominated by the protonated molecule, $[M+H]^+$. The lack of structure information in such spectra may be overcome by the use of MS / MS to provide fragment ion spectra.[145] In this study, ion-spray LC-MS/MS has been used for the confirmation of all previously synthesized DTZ metabolites and for the identification of three unconfirmed metabolites (**4**, **5** and **6**). Ion-spray LC-MS and LC-MS / MS spectra for DTZ in human urine are shown in Figure 16. Ion-spray LC-MS / MS spectra for **4**, **5** and **6** are shown in Figures 17—19. The assignment of fragment ion structures is indicated in each of Figures 16—19. The match of retention times and MS / MS spectra of synthetic compounds with those from human urine supports the assigned structures. A detailed paper on the LC-MS / MS analysis of DTZ and its metabolites will be published elsewhere.[148]

No *S*-oxidation product was found in the DTZ *N*-oxide (**6**) isolated from the oxidation of DTZ with 3-chloroperbenzoic acid. The ^{1}H NMR of DTZ *N*-oxide demonstrated a tremendous down-field shift of the protons of the dimethylaminoethyl side chain due to the formation of *N*-oxide instead of *S*-oxide compared with the ^{1}H NMR of DTZ (Table 22). The two methyl groups on nitrogen became nonequivalent, and their proton signals were shifted down-field from δ 2.28 to δ 3.23 and 3.33. The four protons of the ethylene side chain are also shifted down-field. Therefore, there is no chemical shift change of the remainder of the protons, especially the two methine protons at C2 and C3 positions. The chemical shifts of these two methine protons should change for the *S*-oxide (either sulfoxide or sulfone). HPLC of synthetic DTZ *N*-oxide only showed one peak with the same retention time as that from biological sample and matched LC-MS / MS spectra (Figure 18). If there were sulfoxides formed, HPLC would be expected to have two peaks since the sulfoxide would be a diastereomeric pair.

Table 22. ^{1}H NMR Data of DTZ and DTZ *N*-Oxide



DTZ



DTZ-NO

DTZ ($CDCl_3$) δ	DTZ *N*-Oxide ($CDCl_3$) δ
1.90 (s, 3H, CH_3CO)	1.91 (s, 3H, CH_3CO)
2.28 (s, 6H, $N(CH_3)_2$)	3.23 (s, 3H, NCH_3), 3.33 (s, 3H, NCH_3)
3.82 (s, 3H, CH_3O)	3.83 (s, 3H, CH_3O)
2.42—2.53 (m, 1H, NCH)	3.42—3.61 (m, 1H, NCH)
2.67—2.77 (m, 1H, NCH)	3.80—3.93 (m, 1H, NCH)
3.68—3.80 (m, 1H, NCH)	4.36—4.53 (m, 1H, NCH)
4.40—4.50 (m, 1H, NCH)	4.56—4.75 (m, 1H, NCH)
5.02 (d, 1H, CH)	5.03 (d, 1H, CH)
5.14 (d, 1H, CH)	5.15 (d, 1H, CH)
6.88—7.72 (m, 8H, aromatic H)	6.90—7.70 (m, 8H, aromatic H)

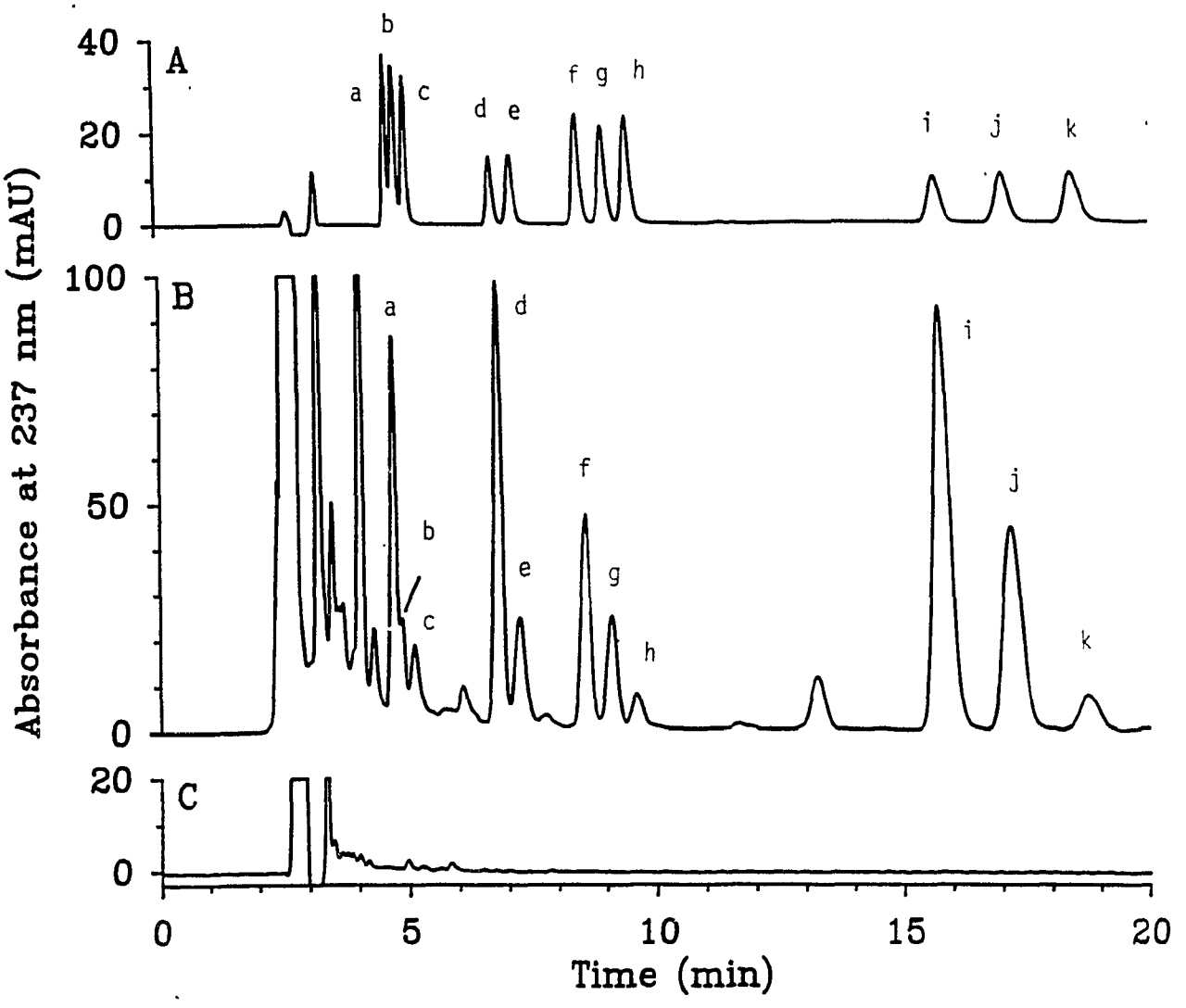


Figure 15. HPLC analysis of standard mixture of DTZ and its metabolites. Synthetic standard (A), a human urine extract (B) and human urine blank (C). a = **8**, b = **3**, c = **3** *N*-oxide, d = **4**, e = **5**, f = **7**, g = **1**, h = **1** *N*-oxide, i = **2**, j = diltiazem, k = **6**

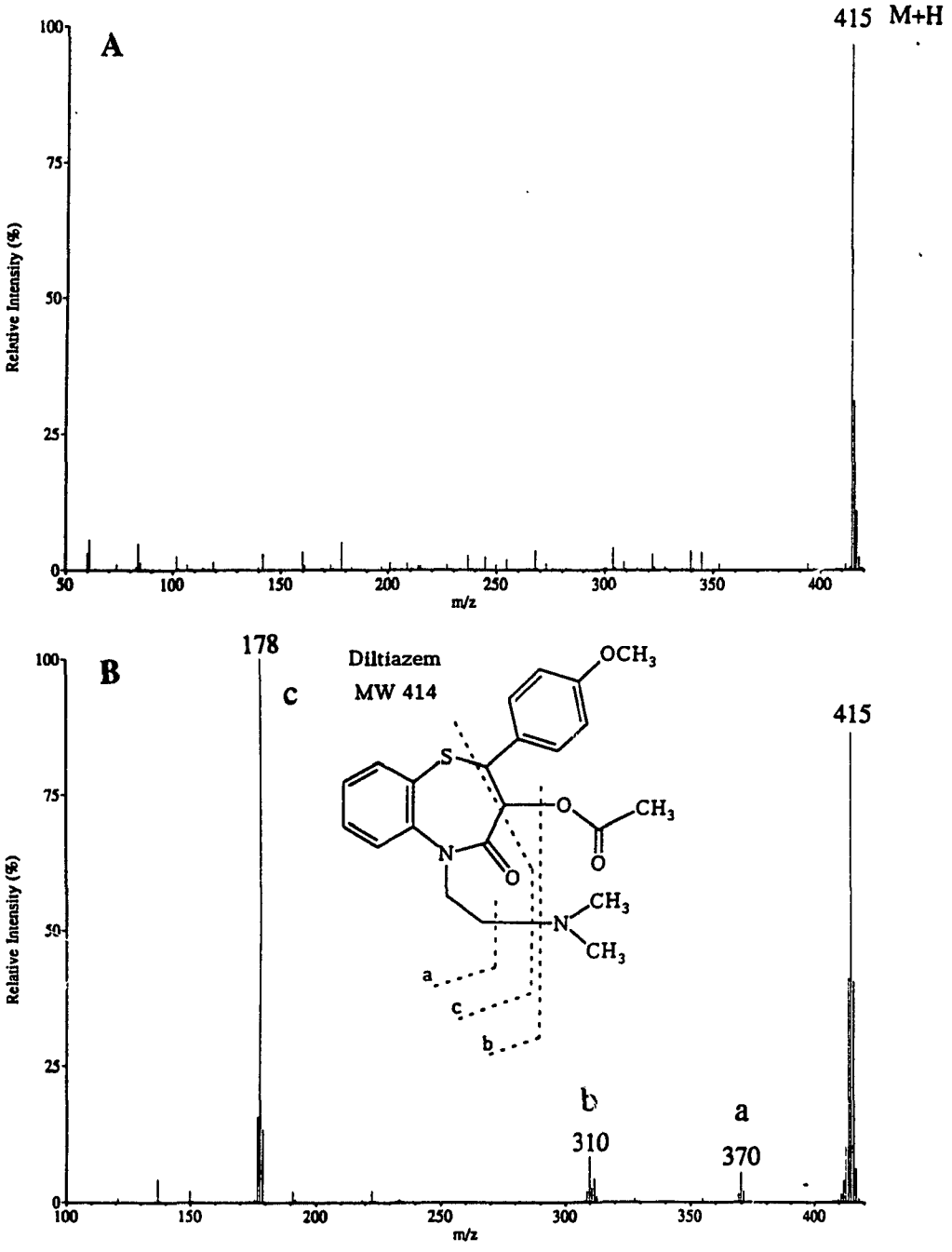


Figure 16 Ion-spray LC-MS (A) and LC-MS / MS (B) spectra for DTZ in human urine

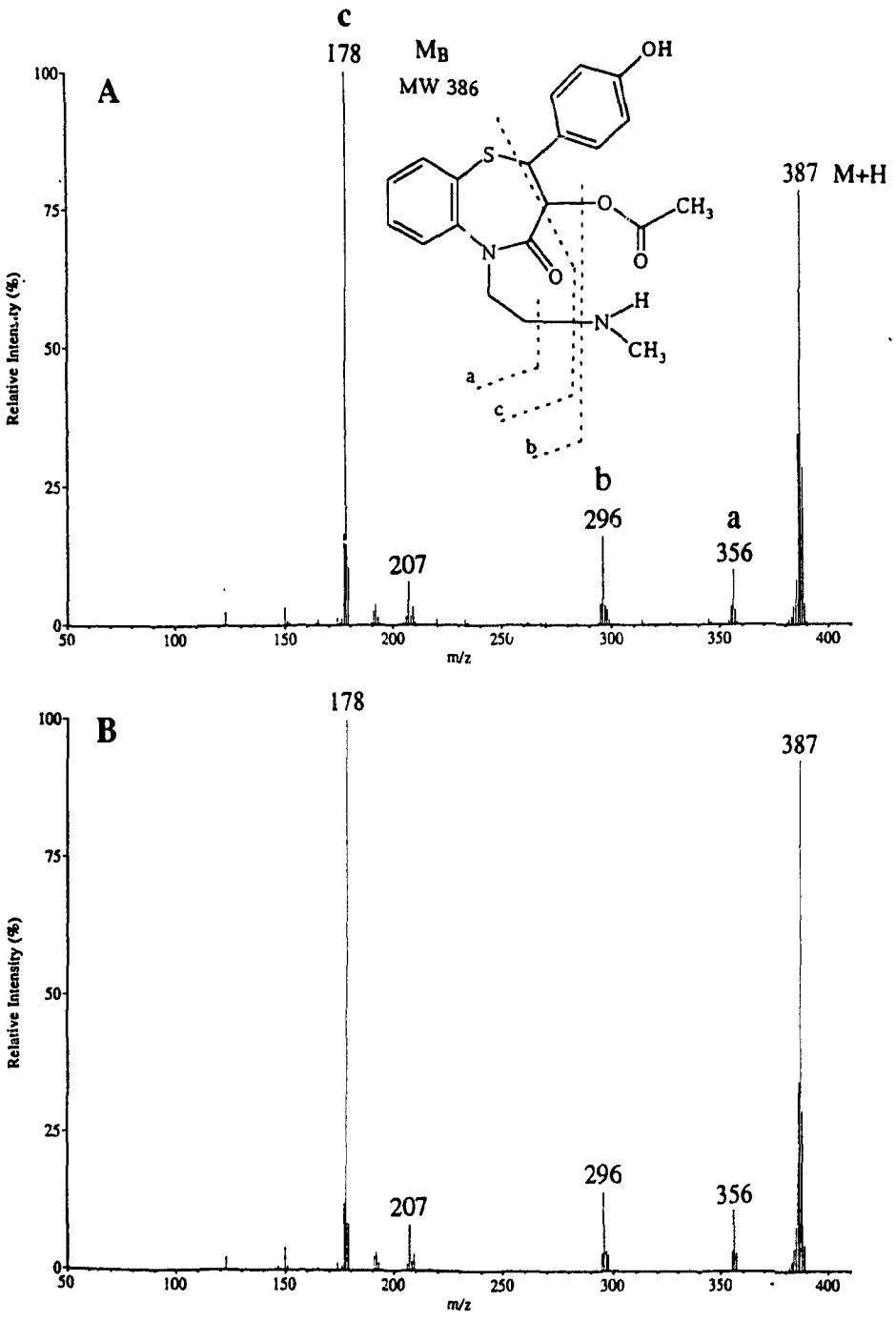


Figure 17. Ion-spray LC-MS / MS spectra for **4** (MB) from synthetic standard (A) and human urine extract (B)

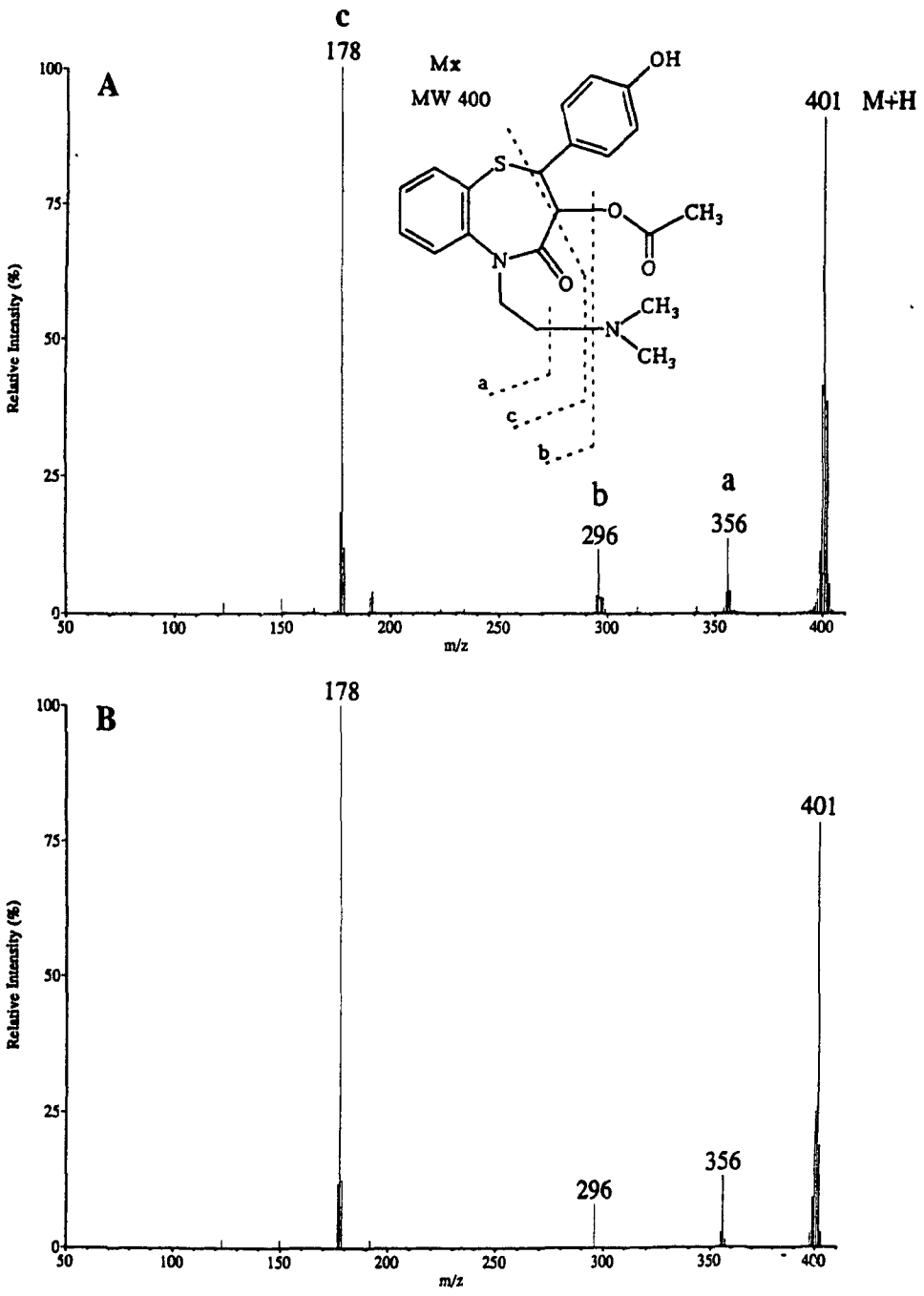


Figure 18. Ion-spray LC-MS / MS spectra for **5** (MX) from synthetic standard (A) and human urine extract (B)

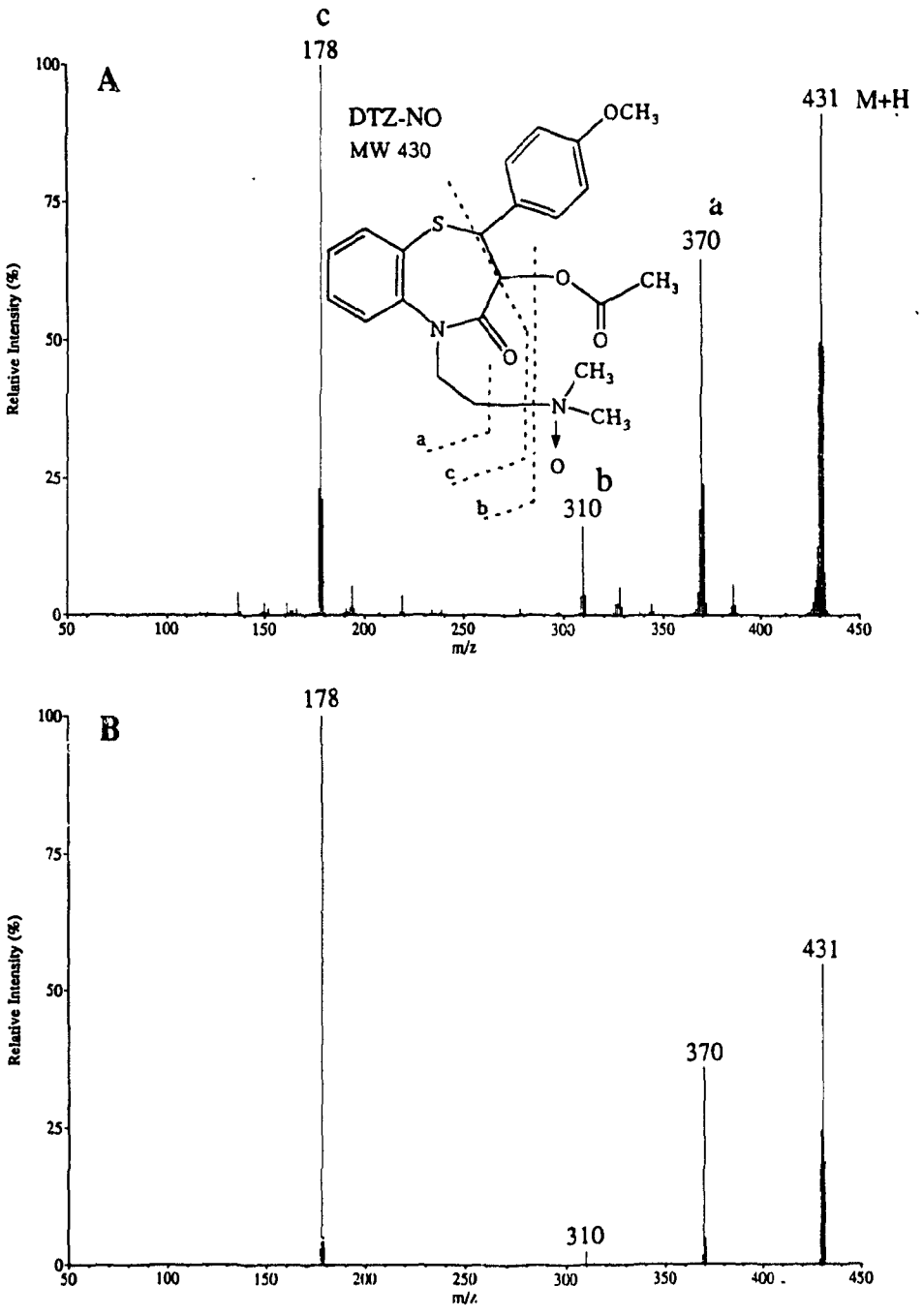


Figure 19. Ion-spray LC-MS / MS spectra for **6** (DTZNO) from synthetic standard (A) and human urine extract (B)

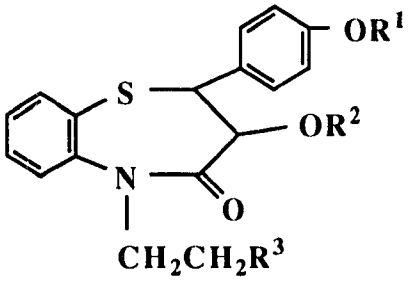
2. 6 Biological Evaluation

2. 6. 1 *In Vitro* Vasorelaxant Activity

2. 6. 1. 1 Vasorelaxant Activity of DTZ Metabolites on Hamster Aorta

In order to evaluate calcium antagonistic activity of synthetic DTZ metabolites, we used young hamster (72—85 days old, weighing 104—136 g) aorta ring preparations (*ca.* 3 mm rings) depolarized with potassium as discussed in the Experimental section. DTZ and its metabolites were dissolved in distilled water for the tests. The vasorelaxant activity recorded in this study is a reliable routine method for determining the activity of calcium channel antagonists.[149] For each experiment, DTZ was used as a control. The IC_{50} values (Table 23), reported for vasorelaxant activity, were calculated by a sigmoidal non-linear regression program according to the concentration response data. 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. Calcium antagonism measured as the contraction of hamster aorta preparations against the concentration of DTZ metabolites is shown in Figures 20 and 21.

Table 23. *In Vitro* Vasorelaxant Activity of DTZ and Synthetic Metabolites



Compd	R^1	R^2	R^3	IC_{50}[a]	n
DTZ	Me	Ac	NMe_2	0.98±0.17	8
1 (M1)	Me	H	NMe_2	2.5±0.2	3
2 (MA)	Me	Ac	NHMe	3.3±0.5	4
3 (M4)	H	H	NMe_2	46±9.0	4
4 (MB)	H	Ac	NHMe	112±17	4
5 (MX)	H	Ac	NMe_2	40±7.7	4
7 (M2)	Me	H	NHMe	20±5.3	4
8 (M6)	H	H	NHMe	126±14	3

[a]Micromolar concentration (μM) required to block Ca^{2+}-induced contraction of K^+-depolarized hamster aorta by 50%, $IC_{50} \pm$ SE (n = 3—8 rings for each compound).

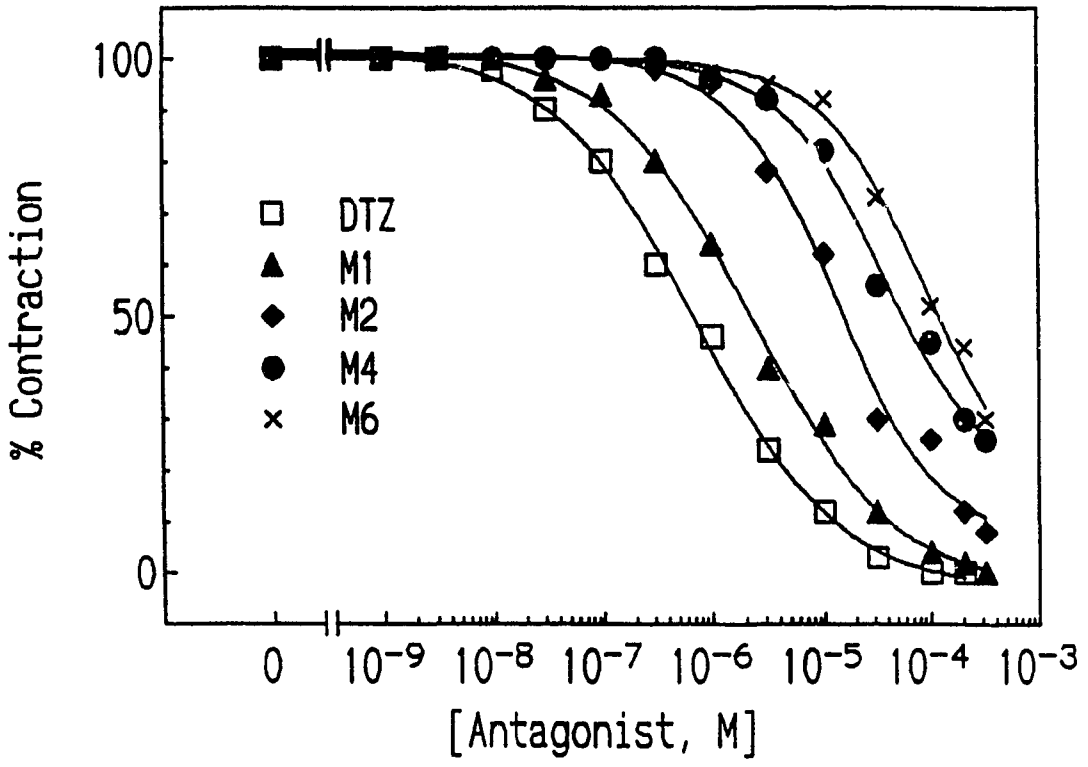


Figure 20. Vasorelaxant activity of DTZ metabolites (I)

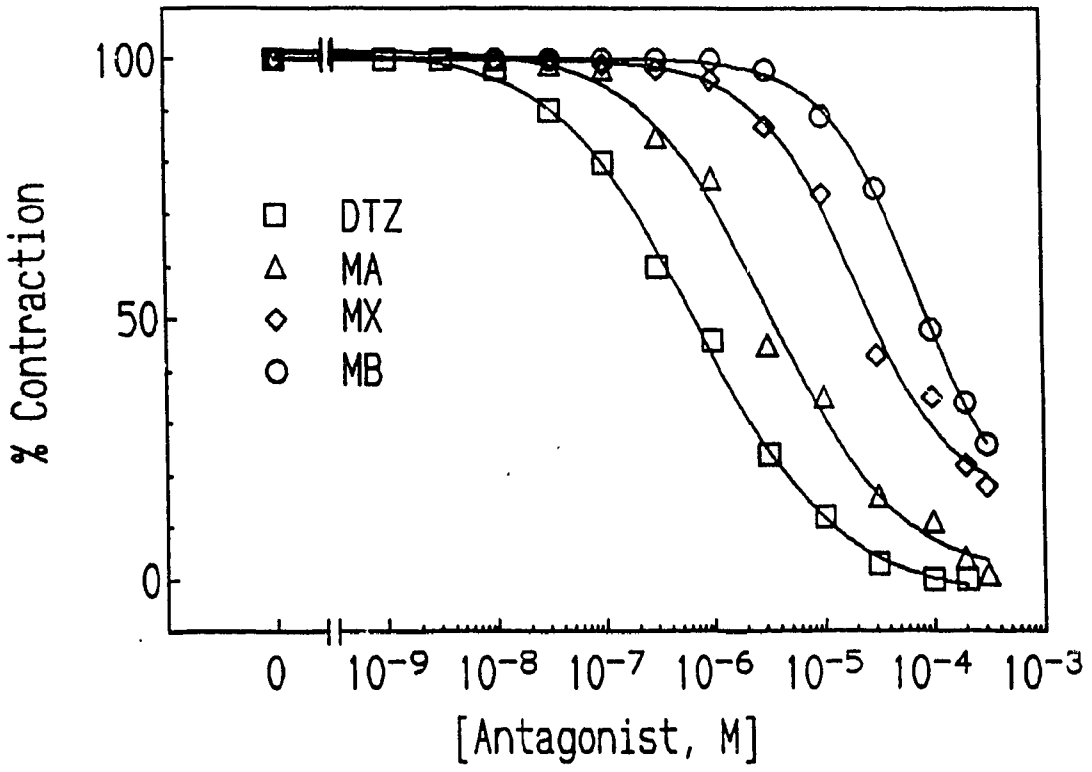
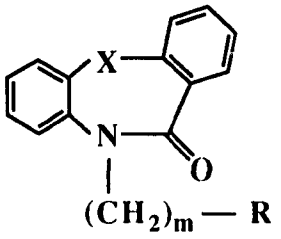


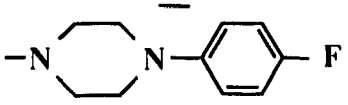
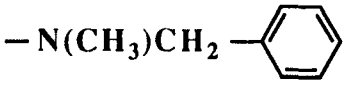
Figure 21. Vasorelaxant activity of DTZ metabolites (II)

2. 6. 1. 2 Vasorelaxant Activity of DBTs on Hamster Aorta

Isolated aorta ring preparations (about 3 mm rings) were used to evaluate vasorelaxant potencies of our dibenzotricyclic compounds. Several conditions were slightly different from those used for diltiazem metabolites. First of all, organ bath temperature was set at 37°C[150] (standard protocol) instead of 29°C (experiments done sharing a single thermal control). Secondly, the age and weight of hamsters used in this study covered a larger range (30—200 days old, weighing 75—164 g). Thirdly, 50% EtOH was used to make a stock solution (10^{-2} M) of each compound, and either 30% EtOH or distilled water was used to dilute this such that the final concentration of EtOH in the muscle bath was always less than 0.01%.[150] We found that concentrations of EtOH up to 0.05% had no effect on muscle contractile responses. Diltiazem was used as a control in these vasorelaxant tests. The IC_{50} values (Table 24), reported for vasorelaxant activity, were calculated by a sigmoidal non-linear regression program according to the concentration response data. For those compounds for which IC_{50} values could not be determined, values for % relaxation of hamster aorta at up to 1.0 μM of cumulative dose are reported. The results are shown in Table 24 and Figures 22—28.

Table 24. Vasorelaxation Properties of Dibenzotricyclic Compounds



Compd	X	R	m	IC_{50} (μM)[a]	% Relaxation[b]	n
DTZ	—		—	0.21 ± 0.11		8
9	S		2		16 ± 0.2	4
10	S	same as above	3	0.79 ± 0.11		4
11	S	same as above	4	0.88 ± 0.11		4
12	S	same as above	5	0.47 ± 0.13		4
13	S	same as above	6	0.92 ± 0.11		5
15	O	same as above	3	0.78 ± 0.12		4
16	O	same as above	4	0.70 ± 0.10		5
17	O	same as above	5	0.73 ± 0.11		4
18	O	same as above	6	0.52 ± 0.11		4
19	S	$-N(CH_3)_2$	2		0	4
20	S	same as above	3		0	4
21	S		3		22 ± 3.3	4
22	S	same as above	4		27 ± 8.2	4
23	S	same as above	5		32 ± 4.7	4
24	S	same as above	6		27 ± 9.1	4

[a]Micromolar (μM) concentration required to block Ca^{2+}-induced contraction of K^+-depolarized hamster aorta by 50%, IC_{50} ± SE (n = 4—8 rings).

[b]% Relaxation of hamster aorta at 1.0 μM of cumulative dose, % ± SE (n = 4 rings).

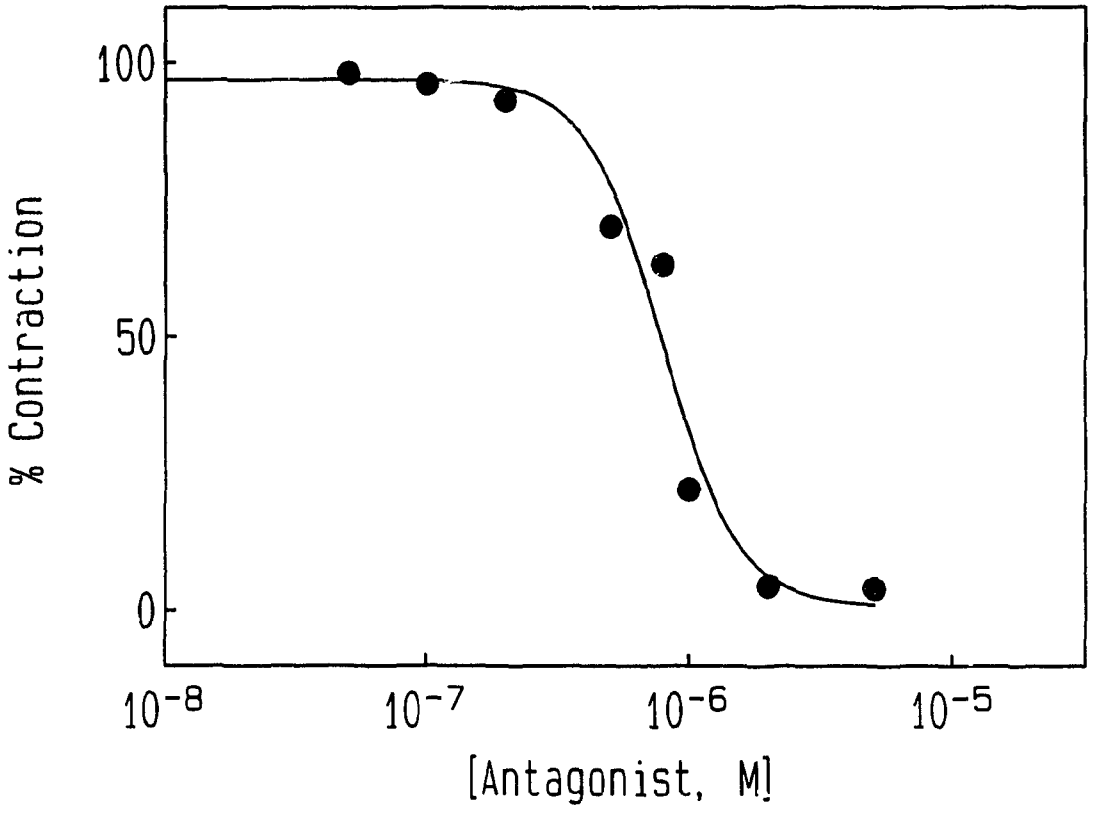


Figure 22. Vasorelaxant activity of **10**

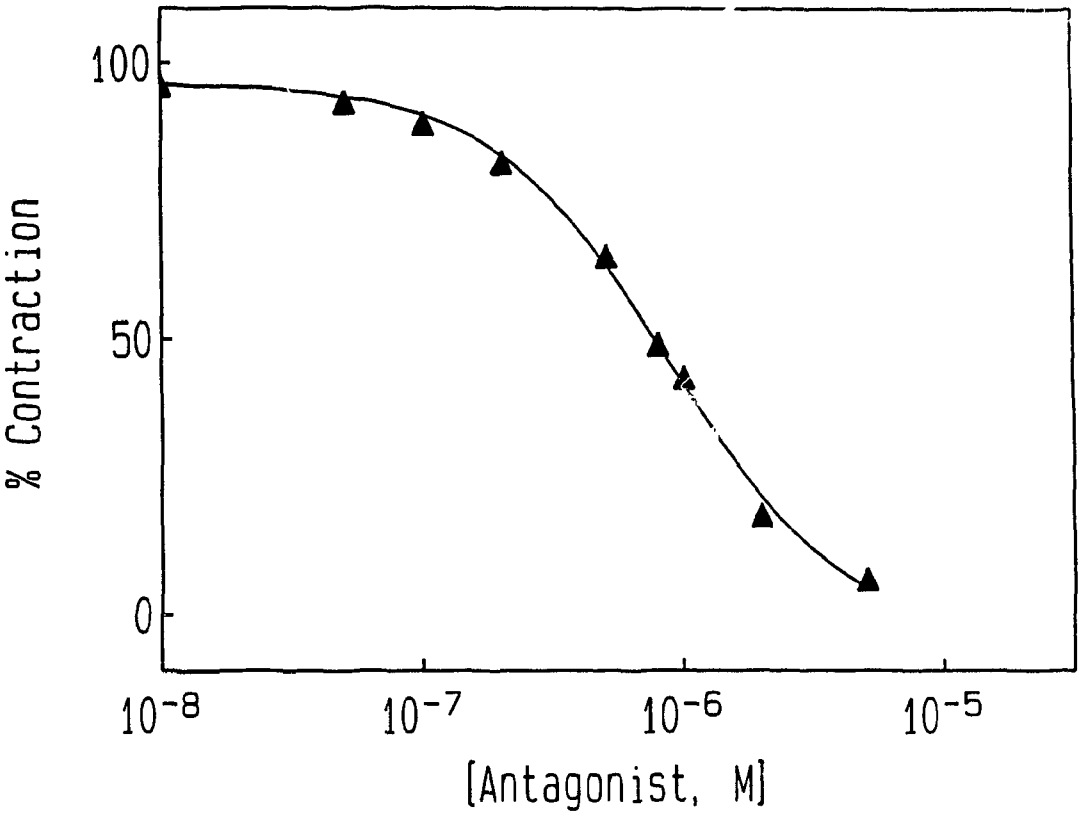


Figure 23. Vasorelaxant activity of **11**

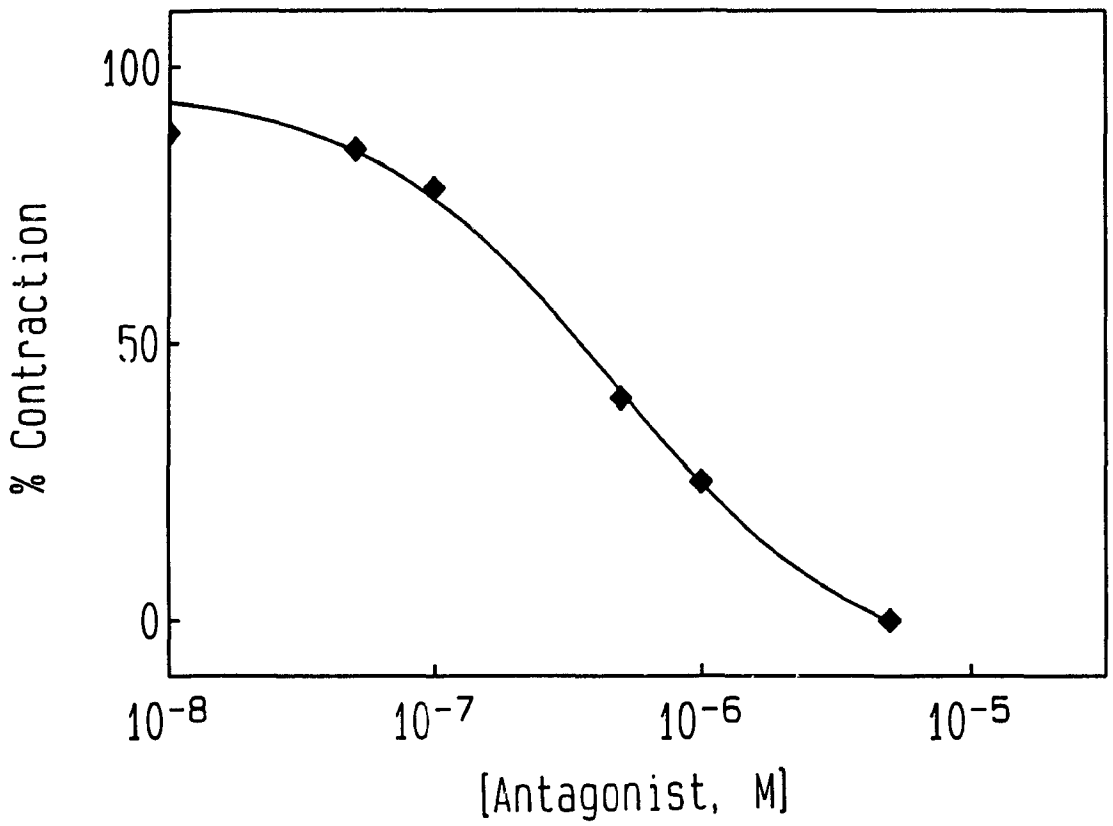


Figure 24. Vasorelaxant activity of **12**

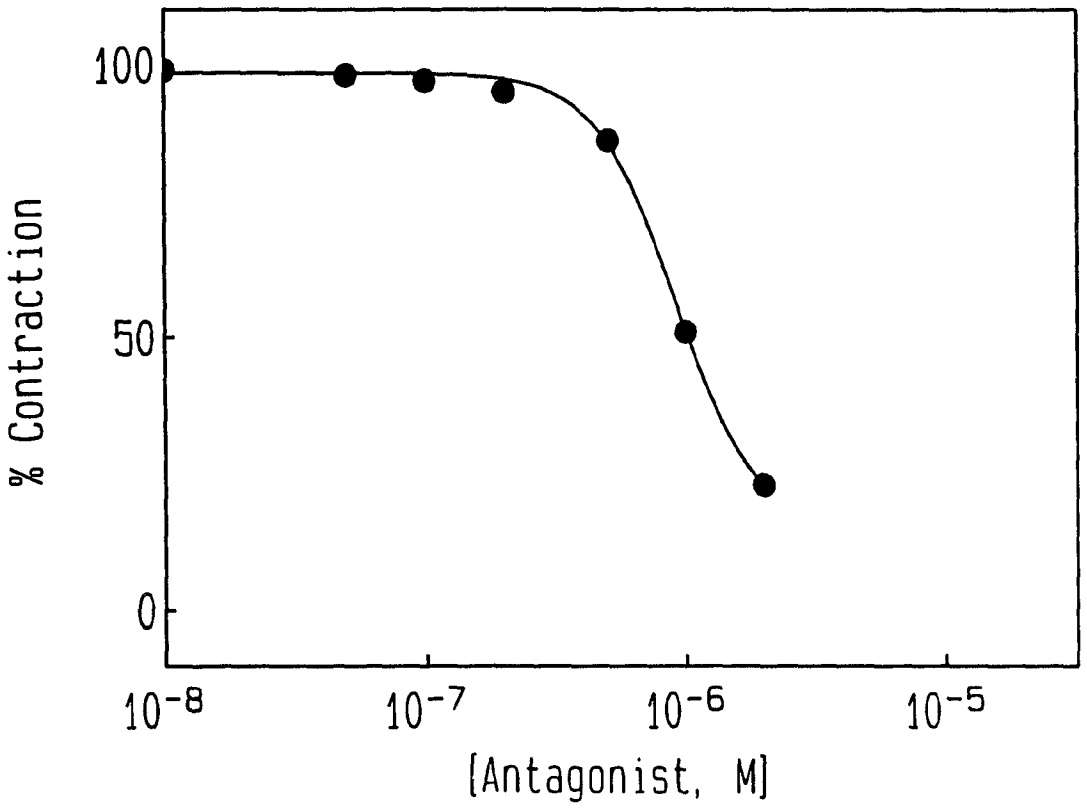


Figure 25. Vasorelaxant activity of **13**

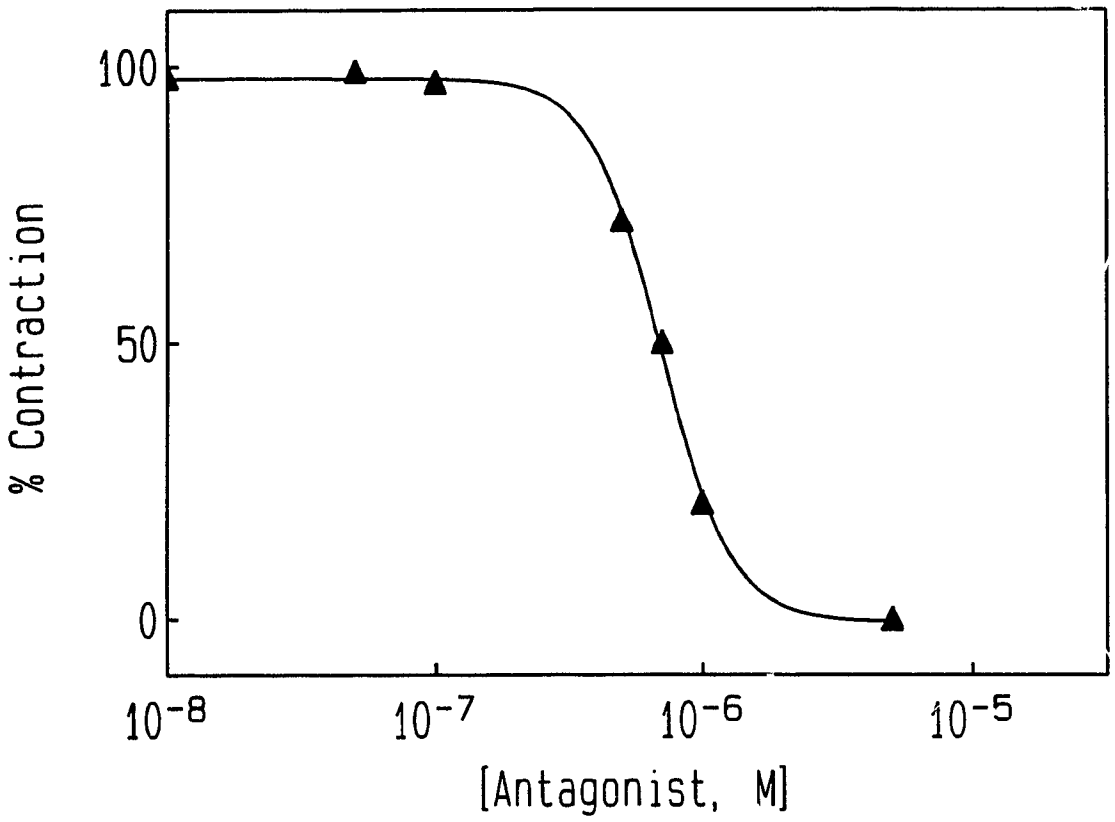


Figure 26. Vasorelaxant activity of **16**

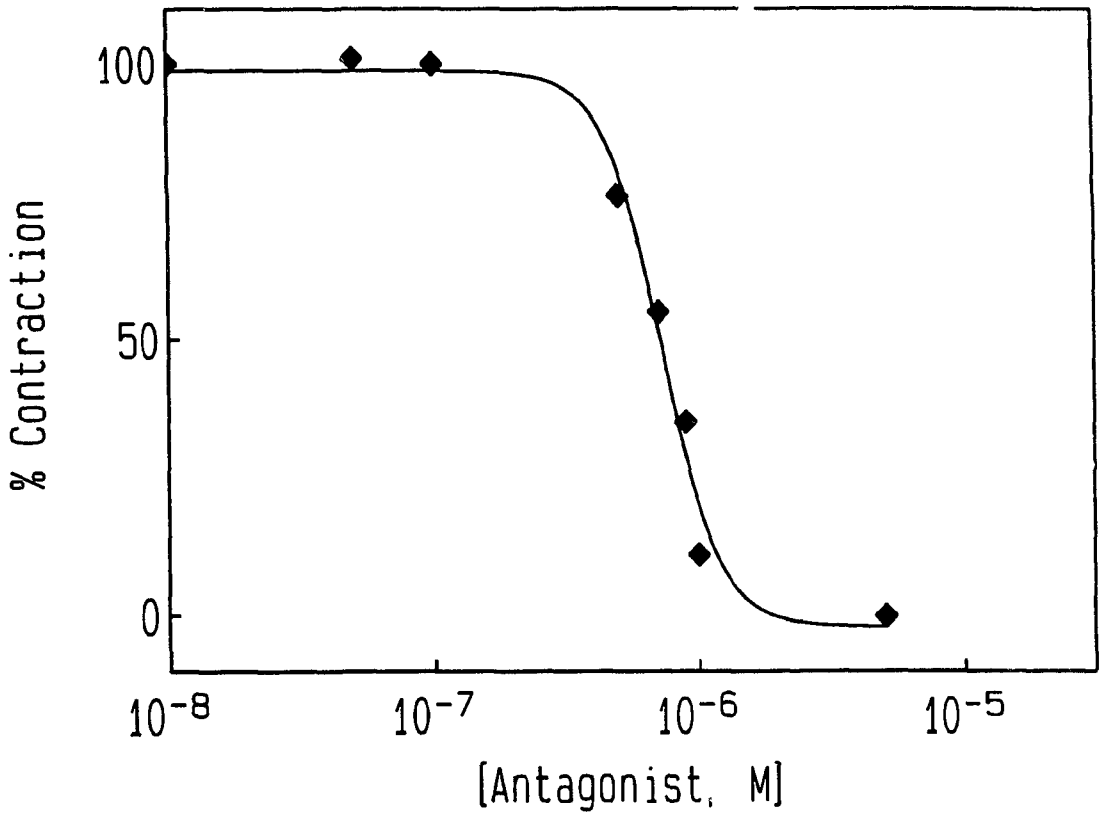


Figure 27. Vasorelaxant activity of **17**

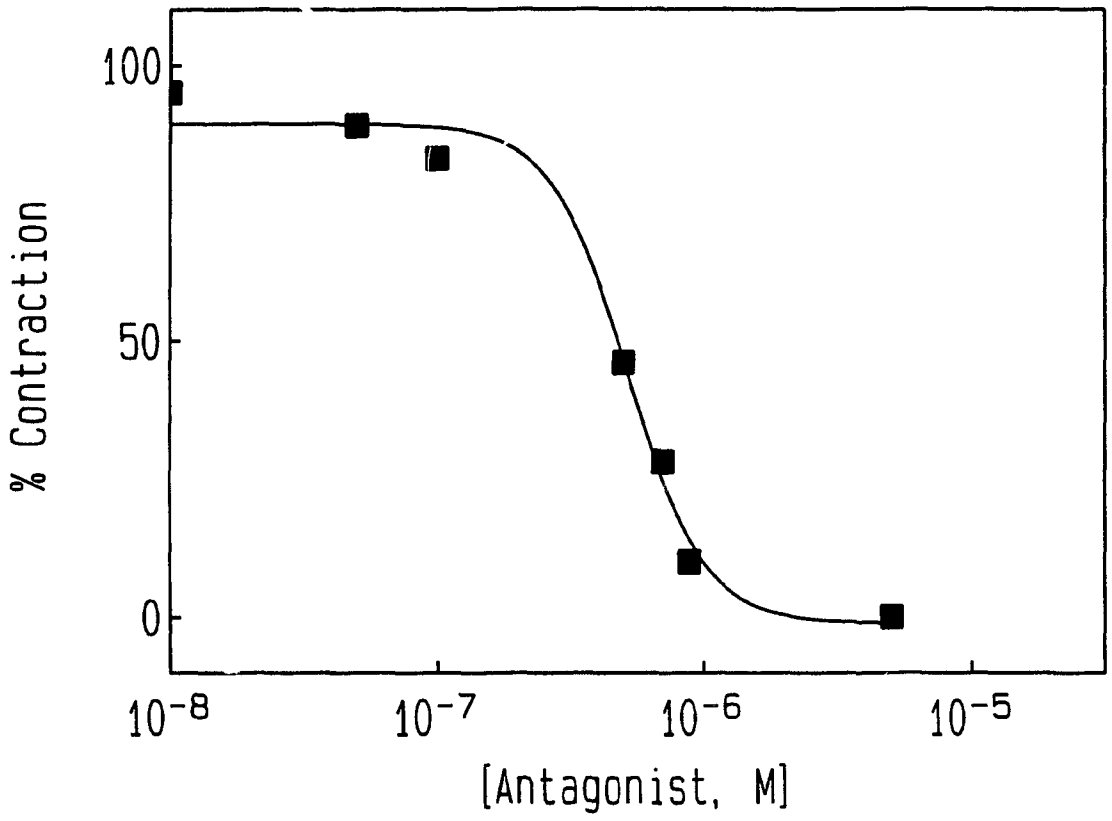


Figure 28. Vasorelaxant activity of **18**

2. 6. 2 Effects of Calcium Antagonists on *In Vitro* Adenosine Uptake by Erythrocytes

2. 6. 2. 1 Effects of DTZ Metabolites on *In Vitro* Adenosine Uptake by Erythrocytes

A published protocol[65,86,87] was used to evaluate effects of DTZ metabolites on *in vitro* adenosine uptake of human whole blood. The results[65,86,87] are shown in Table 25. Details of the uptake experiment are described in the Experimentai section.

Table 25. Effects of DTZ Metabolites on Adenosine Uptake[a]

Compd[b]	Name[c]	IC_{50} (μM)[d]
Dipyridamole		0.2
Diltiazem		301
(+)-**1**	M1	93
(+)-**2**	MA	538
(+)-**3**	M4	186
(±)-**4**	(±)-MB	118
(±)-**5**	(±)-MX	96
(+)-**7**	M2	> 1000
(+)-**8**	M6	> 1000

[a]Data were obtained from Dr. P. K. F. Yeung with permission.

[b]Only (±)-**4** and (±)-**5** are synthetic DTZ metabolites in this project; (+)-isomers are gifts from the Tanabe Seiyaku Co. (Osaka, Japan).

[c]For unsystematic names of DTZ metabolites, see lit.[72] (M1, M2, M4, M6); lit.[73] (MA) and lit.[71] (MB, MX).

[d]Micromolar concentration (μM) of inhibitors at which there was 50% inhibition of adenosine uptake.

Among DTZ and its metabolites, the order of potency is: (+)-**1** (M1) ≥ (±)-**5** (MX) > (±)-**4** (MB) > (+)-**3** (M4) > DTZ > (+)-**2** (MA) > (+)-**7** (M2) = (+)-**8** (M6).

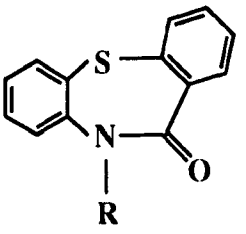
2. 6. 2. 2 Effects of DBTs on *In Vitro* Adenosine Uptake by Erythrocytes

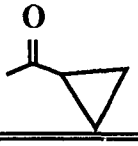
DBTs **9**—**13**, **21**—**24** and **15**—**18** were evaluated by the same protocol. Although DBTs are calcium antagonists, they are inactive on adenosine uptake.

2. 6. 3 Anti-HIV Activity of Dibenzothiazepinone Derivatives

In vitro anti-HIV activity of dibenzothiazepinone derivatives (**9**—**14**, **25** and **26**) was evaluated by the National Cancer Institute (NCI) *In Vitro* Anti-Aids Drug Discovery Program. A published procedure[151] was used in the NCI test for agents active against Human Immunodeficiency Virus (HIV). After T4 lymphocytes are treated with a small amount of HIV, with or without the addition of a test compound, the culture is allowed to go through a complete cycle of virus production and cytolysis is measured. Agents that interact with the virus / cell system to interfere with viral activities will protect cells from destruction. All tests are compared with at least one positive (e.g., AZT-treated) control done at the same time under identical conditions. Compounds **14**, **25**, **26** were shown to have moderate anti-HIV activity, while compounds with more elaborate side chains (**9**—**13**) were inactive. The results are shown in Table 26 and Figures 29—32.

Table 26. Anti-HIV Activity of Dibenzothiazepinones[a]



Compd	R	IC_{50}[b]	EC_{50}[c]	TI_{50} (IC/EC)
9	$(CH_2)_2$–N⟨ ⟩N–⟨ ⟩–F	> 200[d]	—	—
10	$(CH_2)_3$–N⟨ ⟩N–⟨ ⟩–F	35[d] (35, 34)	—	—
11	$(CH_2)_4$–N⟨ ⟩N–⟨ ⟩–F	> 200[d]	—	—
12	$(CH_2)_5$–N⟨ ⟩N–⟨ ⟩–F	9[d] (9.4, 8.3)	—	—
13	$(CH_2)_6$–N⟨ ⟩N–⟨ ⟩–F	11[d] (12, 9)	—	—
14	H	> 110[e]	2.8[d] (2.4, 3.1)	> 39
25	CH_3	83 ± 19[f]	5.1 ± 1.7[f]	16[f]
26		> 64[e]	27±6.1[g]	> 2.4

[a]Evaluated by National Cancer Institute, Bethesda, MD.

[b]Micromolar (μM) concentration of compounds killing 50% of HIV-uninfected cells, relative to uninfected, untreated controls.

[c]Micromolar (μM) concentration of compounds protecting 50% of HIV-infected cells from dying, relative to uninfected, untreated controls.

[d]2 determinations.

[e]4 determinations.

[f]6 determinations (IC_{50} or EC_{50} ± SE).

[g]3 determinations (EC_{50} ± SE).

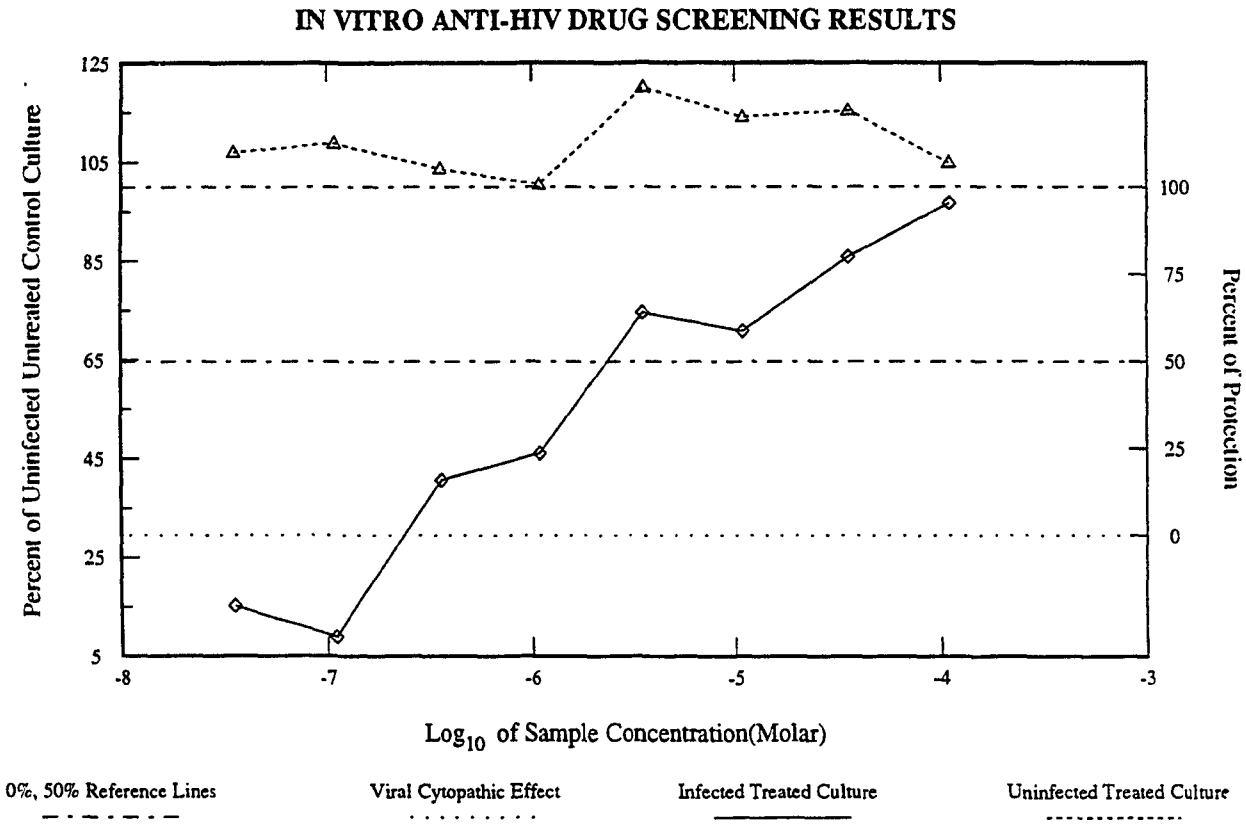


Figure 29. Anti-HIV activity of **14**

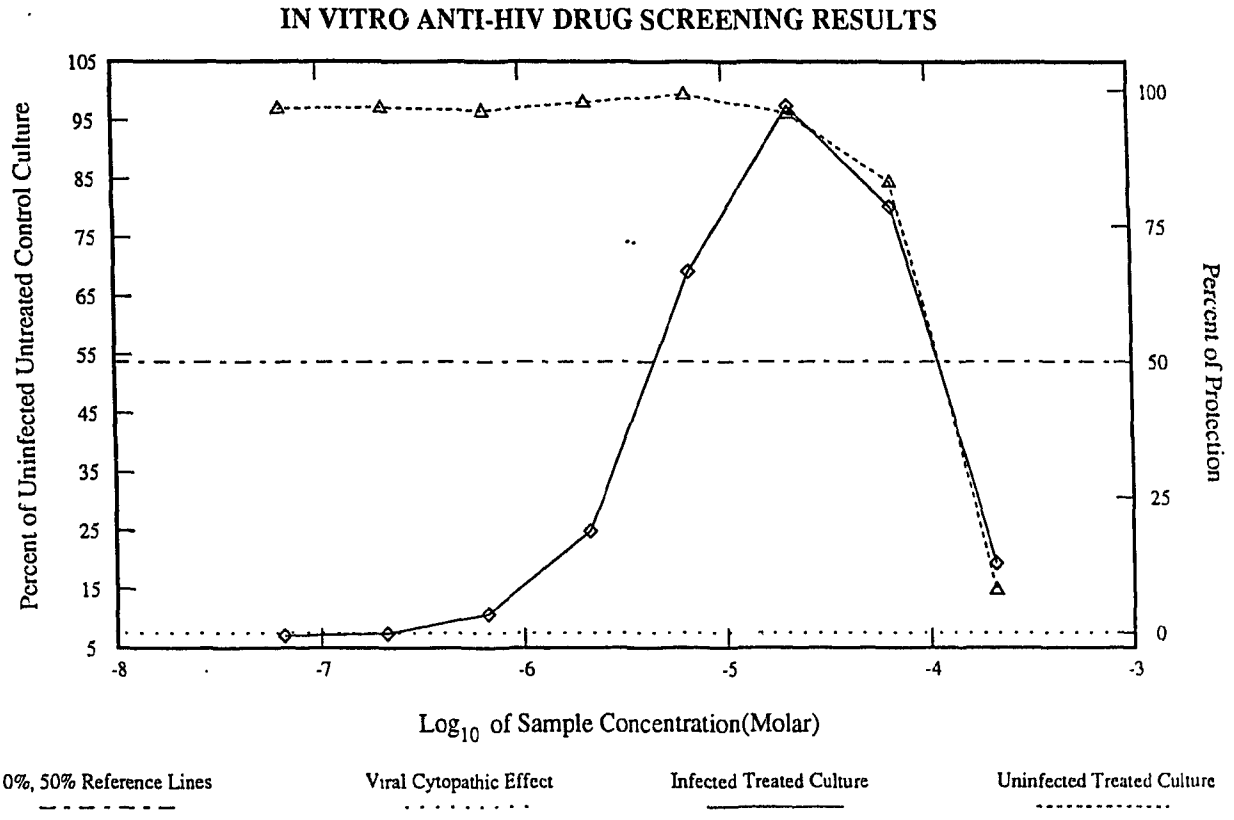


Figure 30. Anti-HIV activity of **25**

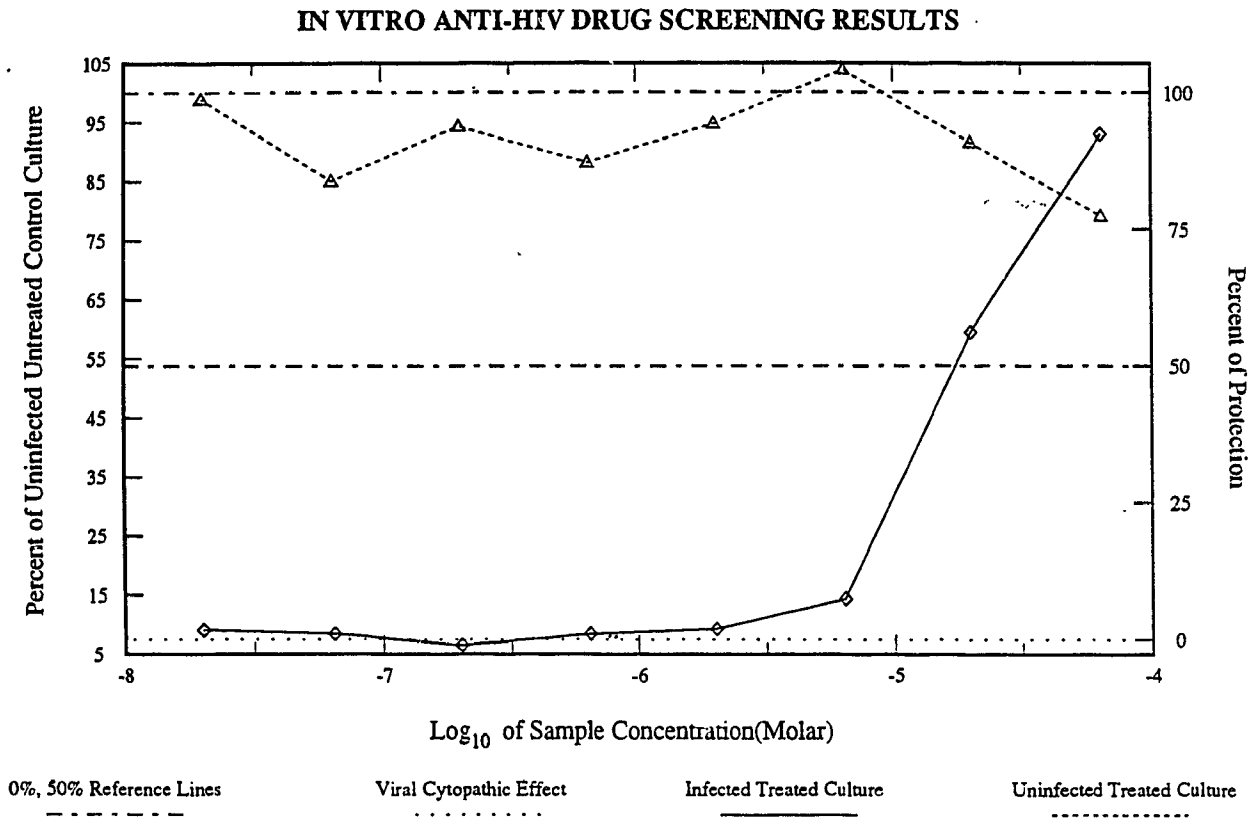


Figure 31. Anti-HIV activity of **26**

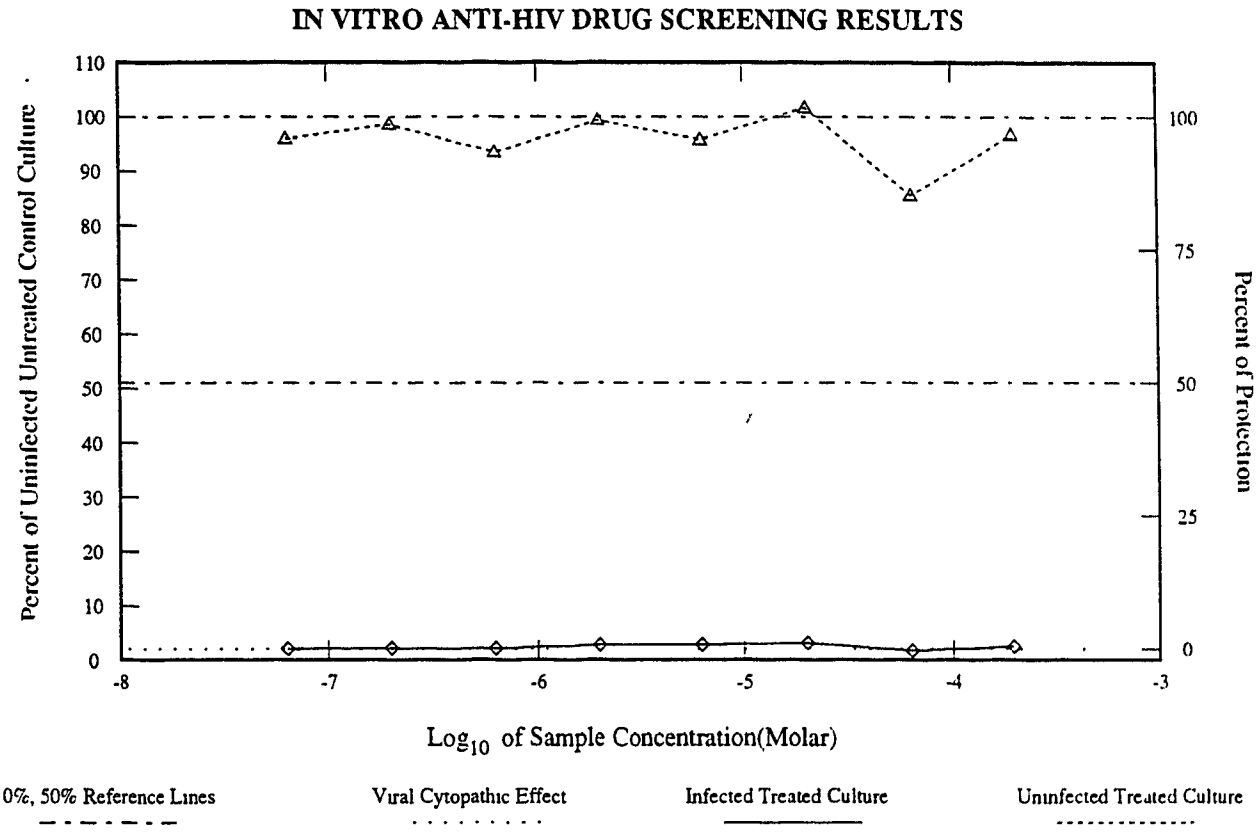


Figure 32. Anti-HIV activity of **9**

2. 7 Structure Activity Relationships

2. 7. 1 DTZ Metabolites

In our functional test for calcium antagonism, we found the vasorelaxant activities of DTZ metabolites on hamster aorta[152,153] to have the following rank order of potency: DTZ > **1** ≥ **2** > **7** > **5** ≥ **3** > **4** ≥ **8** (Table 23). This is consistent with the ranking of coronary-vasodilating activity of DTZ and its known metabolites,[63] and the following structure-activity relationships may be inferred. (a) The *para*-methoxy substituent on the 2-phenyl group seems to be most important for vasorelaxant activity among DTZ 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, **1**, **2** and **7** have dramatically greater potencies than **5**, **3**, **4** and **8**, respectively. (b) The *O*-acetyl group is not so important for activity, and **1** is the most potent metabolite studied. Similarly, **8** and **3** have comparable potencies with **4** and **5**, 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.[154] (c) *N*-Monodemethylation likewise has little effect on metabolite potency, with diltiazem, **5** and **3** all being only about three times as potent as **2**, **4** and **8**, respectively. (d) While loss of either acetyl or *N*-methyl had little effect on biological activity, loss of both decreased activity tenfold. Thus **7** is significantly less potent than either **1** and **2**.

Since **1** and **2** 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 **1** and **2** are formed from diltiazem and their

extent of diffusion to the sites of action. Diltiazem can be metabolized to **1** by esterases present in the liver. On the other hand, **2** 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.

2. 7. 2 Dibenzotricyclic Compounds

A number of DBTs[155—158] synthesized in this study have different vasorelaxation properties. Dibenzothiazepinones (**9**—**13**) and dibenzoxazepinones (**15**—**18**) have very similar *in vitro* vasorelaxation potencies (Table 24) regardless of different flexure angles (108.4° and 116.9°, respectively, from X-ray). This might be due to lack of critical angle requirements for the receptor in this flexure angle range, or flexure angle alterations by solvent participation involving H-bonding between water and heteroatoms in the central ring of the dibenzotricyclic compounds. *In vitro* vasorelaxation potencies of **10**—**13** and **15**—**18** range from 0.47 to 0.92 μM (Table 24) although the chain spacer changes from 3 to 6 carbons. A possibility to enable **13** (6 carbon spacer) to bind to the same site on the receptor as **10** (3 carbon spacer) is to bend the 6 carbon chain of **13**. This is readily achievable since a longer chain has more degrees of freedom.

A 4-(4-fluorophenyl)piperazinyl group attached to a carbon spacer is essential for vasorelaxant activity. Compounds **10**—**13** and **15**—**18** are calcium antagonists with IC_{50} in submicromolar concentrations, while compounds **19** and **20** with dimethylaminoethyl or dimethylaminopropyl side chains are inactive. Compounds **21**—**24** with *N*-benzyl-*N*-methylaminoalkyl groups are less active than their 4-(4-fluorophenyl)piperazinylalkyl congeners.

Reference compound **III** has previously been reported to be 10 times more potent than DTZ in rat aorta.[50] In the present study, the most potent compound in the series, **12**, was found to be almost half as potent as DTZ in hamster aorta. These findings raise a number of interesting possibilities. (a) These results suggest that, provided our DBTs (**9**—**13**, **15**—**18**) bind to receptors on the calcium channel in the same way as reference compounds **III**—**VI**, potencies of our DBTs are relatively insensitive to the flexure angle. This could be due to the flexure angles of our DBTs being below the optimal range. An optimal flexure angle may be required for optimal binding to receptors on the calcium channel. As reported by Kurokawa *et al.*,[50] too large an angle leads to diminished calcium antagonistic activity. Too small an angle of flexure may not facilitate the ligand binding to the receptor either. (b) Activity may also be affected by different orientations of the side chains. Even though free rotation of the carbon chain may allow the 4-fluorophenylpiperazine moiety in compounds **9**—**13** and **15**—**18** to bind at the same site on the calcium channel receptor as compound **III**, the chains are attached differently to the dibenzotricyclic nucleus. This may alter the orientation of the chain, thereby precluding optimal binding. (c) An acidic hydrogen and a carbonyl group in **III** are absent from the side chain in compounds **9**—**13** and **15**—**18**. Kurokawa *et al.*[50] inferred the acidic hydrogen and the carbonyl group might be prerequisite for interaction with the receptor. (d) A negative potential around the surface of the bridge portion of dibenzothiazepinones and dibenzoxazepinones (**13** and **18** as representatives) may reduce calcium antagonistic activity compared with the reference compound **III** and **IV**. Indeed, Kurokawa *et al.*[50] found a positive charge distribution in this portion of their molecules to correlate with higher activity, and a negative potential with lower activity. Our results are consistent with this suggestion.

EXPERIMENTAL

3. 1 Molecular Mechanics Calculations

Molecular mechanics calculations were performed on a PC 486 personal computer with Serena Software PCMODEL (version 4.0)[108] at the Institute for Marine Biosciences, National Research Council (NRC), Halifax, N. S. Atomic coordinates of X-ray diffraction data of compounds **14**, **13** and **18** were obtained by Dr. S. Cameron of the Department of Chemistry, Dalhousie University. The atomic coordinates of X-ray diffraction data of compound **III** tricyclic nucleus were obtained from the literature.[109]

An X-ray crystal structure input with WordPerfect 5.1 (WP51) was written in the following file format: Line 1: Title character string of up to 80 characters; Line 2: Cell parameters A,B,C,α,β,γ; Line 3 to end: X,Y,Z coordinates and atom symbol. This was saved as DOS. file and read by PCMODEL as an X-ray file. The MMX-Minimize command instructs the program to minimize the energy of the structure. First, the total energy of the structure will be computed and listed. Then iterative energy minimization is performed, the atomic movement (in 10^{-5} angstrom units) and the energy (in kcal / mole) is listed every 5 iterations. If energy starts to increase or if the energy stabilizes but with large atomic movement, the program will pause to check the effect of each atom's movement on the total energy. Atoms whose movement causes the energy to rise will became fixed until the next atom movement check. When the energy stabilizes, the minimized structure will be redrawn and the energy summary will be listed in the upper right-hand corner of the screen.

After a minimized structure is obtained, all parameters including intramolecular distances, intramolecular bond angles and torsion angles can be obtained using a Query

command under the Display mode. The angles between the planes of the two benzene rings of DBTs were measured manually with a protractor since the program has no such a function.

3. 2 Synthesis

All chemicals were purchased from Aldrich Chemical Company, Inc. All melting points were measured on a Thomas-Hoover Unimelt apparatus and are uncorrected. Purities of synthetic DTZ metabolites were confirmed with normal phase TLC (a single spot developed by up to three different solvent systems), reversed phase HPLC (a major peak with ≥ 95% purity) and microanalysis. Elemental analyses were performed by Canadian Microanalytical Service Ltd.(Delta, B.C) and were within 0.4% of the theoretical values. Chromatography and spectroscopy methods are described separately in Section 3. 3.

3. 2. 1 Synthesis of DTZ Metabolites

3. 2. 1. 1 Short Sequence Synthesis of DTZ Metabolites

2,3-Dihydro-5-[2-(dimethylamino)ethyl]-3-hydroxy-2-(4-hydroxyphenyl)-1,5-benzothiazepin-4(5*H*)-one (3). Commercial DTZ hydrochloride (4.0 g, 8.8 mmol) was dissolved in 1 N NaOH (*ca.* 8.8 mL). The resultant solution was extracted with CH_2Cl_2 (3 x 10 mL). The organic layer was washed with water and then dried over anhydrous $MgSO_4$. After solvent evaporation, diltiazem free base (3.6 g, 98%)

was obtained. A solution of DTZ free base (2.0 g, 4.8 mmol) in CH_2Cl_2 (18 mL) was placed in a two-neck 100 mL round bottomed flask fitted with a rubber septum and with a reflux condenser protected by a drying tube, and boron tribromide (1.21 g, 4.8 mmol) in 2 mL of CH_2Cl_2 was added by syringe at -80°C (cooled with dry ice-acetone bath). The reaction mixture turned yellow upon addition of boron tribromide and some white precipitate fell out. After stirring at -80°C for 1.5 h, 0°C for another 1.5 h and 20°C for two h, the reaction mixture (brown) was hydrolyzed by carefully shaking with 20 mL water, then extracted with diethyl ether (3 x 40 mL). The aqueous layer was neutralized with 5% Na_2CO_3, then extracted with CH_2Cl_2. The organic layer was dried over anhydrous $MgSO_4$. After solvent removal, the residue was converted to the hydrochloride salt, which was recrystallized from 70% EtOH to give HCl salt of **3** (0.76 g, 40%): mp 218—221°C; TLC R_f = 0.47 (CH_2Cl_2 / MeOH, 1:1); $[\alpha]^{21}_D$ = +163.8 (c = 0.334, H_2O); IR (KBr) 3500—2600 (br, NH^+), 3200 (OH), 1660 (C=O) cm^{-1}; ^1H NMR (d_6-DMSO) δ 2.81 (br s, 6 H, $N(CH_3)_2$), 3.00—3.20 and 3.25—3.60 (m, 2 H, CH_2N^+), 4.00—4.16 and 4.40—4.60 (m, 2 H, $CONCH_2$), 4.21 (dd, J = 6.8 Hz, 1 H, OCH), 4.79 (d, J = 6.9 Hz, 1 H, OH), 4.85 (d, J = 7.3 Hz, 1 H, SCH), 6.71—7.72 (m, 8 H, aromatic H), 9.53 (s, 1 H, phenolic OH), 10.40 (br s, 1 H, NH^+); ^{13}C NMR (d_6-DMSO) δ 42.24 (2 CH_3), 43.58 (CH_2), 52.88 (CH_2), 56.08 (CH), 69.73 (CH), 114.74—157.36 (m, aromatic C), 171.45 (CO); FAB MS m/z 359 (M+1). **3** was obtained by converting the HCl salt with NH_4OH, mp 138—140°C (lit.[73] mp 135—138°C).

2,3-Dihydro-5-[2-(dimethylamino)ethyl]-3-hydroxy-2-(4-methoxyphenyl)-1,5-benzothiazepin-4(5*H*)-one (1). Direct ester hydrolysis of diltiazem hydrochloride with diluted NaOH / EtOH gave **1**. HCl salt of DTZ (0.45 g, 1 mmol) was stirred in 5% NaOH / EtOH (10 / 90 mL) for two h at room temperature. The reaction

mixture was neutralized with 10% HCl. Solvents were removed under reduced pressure. The residue was treated with dilute NaOH and extracted with EtOAc. The organic layer was dried over anhydrous $MgSO_4$. After the solvent was removed, the residue was dissolved in HBr-MeOH (10%, w / w) and concentrated to give the crude hydrobromide salt. Recrystallization from EtOH afforded HBr salt of **1** (0.36 g, 80 %): mp 223—225 °C (lit.[72] mp 225—228°C); $[\alpha]^{21}_D$ = +134.5 (c = 0.460, H_2O); IR (KBr) 3400 (OH), 3300—2600 (br, NH^+), 1640 (C=O), 1250 (C—O) cm^{-1}; ^1H NMR (d_6-DMSO) δ 2.81(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 (dd, J = 7.1 Hz, 1 H, OCH), 4.39—4.58 (m, 1 H), 4.88—4.92 (two d, J = 6.6 and 7.2 Hz, 2 H, SCH, OH), 6.90—7.73 (m, 8 H, aromatic H), 9.64 (br s, 1 H, NH^+); ^1H NMR after D_2O exchange showed a doublet (J = 7.3 Hz, 1 H) at 4.23 ppm and disappearance of a doublet (J = 6.6 Hz) at 4.88—4.92 and of the broad singlet at 9.64 ppm.

3. 2. 1. 2. Multistep Synthesis of DTZ Metabolites

Methyl (±)-*trans*-3-(4-benzyloxyphenyl)glycidate (29). A modification of procedures by Falco *et al.*[115] was used to prepare **29**. Into a 500 mL three-neck round bottom flask equipped with drying tube and addition funnel, 100 mL of methanol (freshly distilled and dried with 4Å molecular sieves) were placed and sodium metal (8.3 g, 360 mmol) was added with stirring (cooled with ice-water bath). 4-Benzyloxybenzaldehyde (50.0 g, 240 mmol) and methyl chloroacetate (39.0 g, 360 mmol) were dissolved in dioxane (100 mL) and then added into the sodium methoxide solution during 30 min. This reaction mixture was stirred overnight at room temperature and checked with TLC (toluene

/ EtOAc, 9:1), R_f = 0.30 for product. The reaction mixture was poured onto ice (*ca.* 1000 g), neutralized with acetic acid and then extracted with CH_2Cl_2 (4 x 200 mL). The organic layer was dried over anhydrous $MgSO_4$. After the solvents were removed under reduced pressure, the residue was recrystallized from methanol to give white pellets of **29** (49.4 g, 74%): mp 107—108°C (lit.[115] 80%, mp 107—108°C); TLC R_f = 0.30 (toluene / EtOAc, 9:1); $[\alpha]^{23}_D$ = +1.3° (c = 1.0, $CHCl_3$); IR (KBr) 1750 (C=O), 1250 (C—O) cm^{-1}; ^1H 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, CO_2CH_2), 6.96—7.44 (m, 9 H, aromatic H). The coupling constant between the two methine protons is similar with that (1.7 Hz) of commercial methyl (±)-*trans*-3-(4-methoxyphenyl) glycidate (**31**).

2-Nitrothiophenol (30). Compound **30** was obtained using the method by Cashman and Liu[117]. Bis(2-nitrophenyl)disulfide (30.0 g, 97.3 mmol) was suspended in dry THF (145 mL) and a slurry of $NaBH_4$ (12.9 g, 341 mmol) in THF (50 mL) was added under nitrogen. The reaction mixture turned red-brown immediately upon addition of $NaBH_4$. The reaction was stirred at room temperature for three h and monitored by TLC (CH_2Cl_2), R_f values of disulfide and **30** being 0.61 and 0.56, respectively. The reaction was quenched with ice-water (200 mL), acidified with 15% sulfuric acid (color changed from red-brown to yellow) and then extracted with CH_2Cl_2 (4 x 200 mL). The organic layer was dried over anhydrous $MgSO_4$ for two h. The solvent was removed under reduced pressure to give 29 g (97%) of **30** as yellow solid, mp 49—53°C (lit[118] mp 58°C) which was used in the next step without further purification.

Methyl 3-(4-benzyloxyphenyl)-2-hydroxy-3-(2-nitrophenylthio)-propionate (32). To a solution of freshly prepared **30** (29.0 g, 180 mmol) in dioxane (220 mL) under N_2 were added compound **29** (50.5 g, 180 mmol) and tin (II) chloride

(2.2 g, 11.6 mmol) in an ice-water bath. The mixture was stirred for one h at 0°C and then 21 h at room temperature. The solvent was removed under reduced pressure. The residue was recrystallized from EtOAc (*ca.* 100 mL) to give **32** (52 g, 67%) as yellow needles: mp 142—143°C (lit.[73] 53%, mp 138—140°C); TLC R_f = 0.09 (CH_2Cl_2) and 0.6 (CH_2Cl_2 / EtOAc). IR (KBr) 3490 (OH), 1738 (C=O), 1515 and 1340 (NO_2), 1240 (C—O) cm^{-1}; ^1H NMR ($CDCl_3$) δ 3.25 (d, J = 5.5 Hz, 1 H, OH), 3.75 (s, 3 H, CO_2CH_3), 4.56—4.59 (dd, J = 3.3 and 5.4 Hz, 1 H, OCH), 4.73 (d, J = 3.2 Hz, 1 H, SCH), 5.04 (s, 2 H, OCH_2), 6.92—8.05 (m, 13 H, aromatic H). The ^1H NMR after D_2O exchange showed a doublet (J = 3.3 Hz, 1 H, OCH) at 4.56 ppm and a doublet (J = 3.2 Hz, 1 H, SCH) at 4.73 ppm and disappearance of a doublet (J = 5.5 Hz, 1 H, OH) at 3.25 ppm.

Methyl 2-acetyloxy-3-(4-benzyloxyphenyl)-3-(2-nitrophenylthio)-propionate (44). Compound **32** (200 mg, 0.45 mmol) was acetylated by heating with acetic anhydride (2.6 g, 25 mmol) and pyridine (5 drops) for 2 h on a boiling water bath. Acetic anhydride, acetic acid and pyridine were evaporated off completely under reduced pressure. **44** (160 mg, 74%) was obtained as a yellow oil: TLC R_f = 0.22 (CH_2Cl_2); IR ($CDCl_3$) 1750 (br, C=O), 1510 and 1340 (NO_2), 1220 (C—O) cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.11 (s, 3 H, CH_3CO), 3.63 (s, 3 H, CO_2CH_3), 4.88 (d, J = 5.3 Hz, 1 H, CH), 5.03 (s, 2 H, CH_2), 5.39 (d, J = 5.3 Hz, 1 H, CH), 6.93—8.05 (m, 13 H, aromatic H).

Methyl 2-hydroxy-3-(4-methoxyphenyl)-3-(2-nitrophenylthio)-propionate (33). A similar procedure was used to synthesize **33**. A solution of **31** (26.9 g, 130 mmol), freshly prepared **30** (20 g, 130 mmol) and tin (II) chloride (1.6 g, 8.4 mmol) in dioxane (100 mL) was stirred under N_2 at 0°C for one h and then at room temperature for 18 h. The solvent was removed under reduced pressure. The residue was

recrystallized from EtOAc to give **33** (24 g, 51 %) as yellow needles: mp 160—161°C (lit.[109], 56%, mp 157—158°C); TLC R_f = 0.07 (CH_2Cl_2); IR (KBr) 3490 (OH), 1720 (C=O), 1510 and 1340 (NO_2), 1240 (C—O) cm^{-1}; ^1H NMR ($CDCl_3$) δ 3.25 (d, J = 5.6 Hz, 1 H, OH), 3.76 (s, 3 H, CO_2CH_3), 3.80 (s, 3 H, OCH_3), 4.56—4.59 (dd, J = 3.3 and 5.5 Hz, 1 H, OCH), 4.73 (d, J = 3.3 Hz, 1 H, SCH), 6.87—8.03 (m, 8 H, aromatic H).

Methyl 2-acetyloxy-3-(4-methoxyphenyl)-3-(2-nitrophenylthio)-propionate (45). A procedure analogous to that used for preparation of **44** was followed and yellow crystals of **45** were obtained (75%): mp 100—102°C. TLC R_f = 0.23 (CH_2Cl_2); IR ($CHCl_3$) 1750 (br, C=O), 1510 and 1345 (NO_2), 1250 (C—O) cm^{-1}; ^1H NMR ($CHCl_3$) δ 2.10 (s, 3 H, CH_3CO), 3.64 (s, 3 H, CO_2CH_3), 3.78 (s, 3 H, OCH_3), 4.88 (d, J = 5.3 Hz, 1 H, CH), 5.40 (d, J = 5.3 Hz, 1 H, CH), 6.86—8.07 (m, 8 H, aromatic H).

Methyl 3-(2-aminophenylthio)-3-(4-benzyloxyphenyl)-2-hydroxy-propionate (34). Into a solution of **32** (35.4 g, 81 mmol) in MeOH (390 mL) was added a solution of ferrous sulfate ($FeSO_4 \cdot 7H_2O$) (168.0 g, 605 mmol) in H_2O (390 mL). The mixture was refluxed for 30 min with mechanical stirring. Though an addition funnel was added concentrated NH_4OH (28—30%, 106 mL) during 30 min. The reaction mixture darkened upon addition of NH_4OH. The mixture was refluxed for an additional 30 min 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 x 200 mL). The filtration residue was washed with three 200-mL portions of MeOH / CH_2Cl_2 (1:1). This organic phase was combined with the CH_2Cl_2

layer from the filtrate extract and dried over anhydrous $MgSO_4$. Solvents were removed under reduced pressure. The residues were recrystallized from methanol (*ca.* 110 mL) to give **34** as pale crystals (21.4 g, 65%): mp 121—122°C (lit.[73] 43%, mp 119—120°C); TLC R_f = 0.51 (CH_2Cl_2 / EtOAc, 1:1); IR (KBr) 3460 (br) and 3360 (NH_2 and OH), 1720 (C=O), 1240 (C—O) cm^{-1}; ^1H NMR ($CDCl_3$) δ 3.56 (s, 3 H, CO_2CH_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-aminophenylthio)-2-hydroxy-3-(4-methoxyphenyl)-propionate (35). A mixture of **33** (22.7 g, 62.5 mmol) in MeOH (350 mL) and $FeSO_4 \cdot 7H_2O$ (130.0 g, 469 mmol) in H_2O (350 mL) was refluxed for 30 min. To this mixture was added dropwise concentrated NH_4OH (28—30%, 83 mL) with mechanical stirring. The mixture was refluxed for an additional 30 min. Work-up in the same manner as that for **34** described above gave crude solid, recrystallization of which from 70% ethanol gave **35** (17.8 g, 86%) as pale crystals: mp 90—91°C (lit.[109] 71%, 93—94°C); TLC R_f = 0.55 (CH_2Cl_2 / EtOAc, 1:1); IR (KBr) 3530 (OH), 3450 and 3360 (NH_2), 1735 (C=O), 1250 (C—O) cm^{-1}; ^1H NMR ($CDCl_3$) δ 3.57 (s, 3 H, CO_2CH_3), 3.79 (s, 3 H, OCH_3), 3.90 (br s, 1 H, OH), 4.32 (br s, 2 H, NH_2), 4.50 (br s, 2 H, CHCH), 6.61—7.35 (m, 8 H, aromatic H).

3-(2-Aminophenylthio)-3-(4-benzyloxyphenyl)-2-hydroxypropionic acid (36). Compound **34** (27.0 g, 66 mmol) was heated with 5% NaOH (264 mL, 330 mmol) on a boiling water-bath until all the starting material dissolved (*ca.*10 min) and then allowed to cool. The solution was neutralized with 10% HCl to pH *ca.* 5. The resultant precipitate was collected by filtration and recrystallized from ethanol to give **36** (24.7 g,

95%) as colorless crystals: mp 174—175°C (dec.); TLC R_f = 0.35 ($CHCl_3$ / MeOH, 1:1); IR (KBr) 3300 (OH), 3100—2500 (br, NH^+), 1610 (CO_2^-), 1240 (C—O) cm^{-1}; ^1H NMR (d_6-DMSO) δ 4.27 (br d, J = 5.2 Hz, 1 H, CH), 4.34 (br d, J = 5.1 Hz, 1 H, CH), 5.03 (s, 2 H, OCH_2), 5.60—6.25 (br s, NH_2) 6.33—7.40 (m, 13 H, aromatic H); FAB MS m/z 396 (M+1).

3-(2-Aminophenylthio)-2-hydroxy-3-(4-methoxyphenyl)propionic acid (37). Compound **35** (13.4 g, 40.2 mmol) was heated with 5% NaOH (160 mL, 201 mmol) on a boiling water-bath for *ca.* five min and then allowed to cool. The resultant clear solution was diluted with H_2O (*ca.* 150 mL) and acidified with 10% HCl to pH *ca.* 5. The precipitated crystals were collected by suction filtration and recrystallized from ethanol (*ca.* 150 mL) to give **37** (12.3 g , 96%): mp 178—179°C (lit.[109] 86%, mp 167—170°C); TLC R_f = 0.36 ($CHCl_3$ / MeOH, 1:1); IR (KBr) 3320 (OH), 3100—2500 (br, NH^+), 1620 (CO_2^-), 1240 (C—O) cm^{-1}; ^1H NMR (d_6-DMSO) δ 3.69 (s, 3 H, OCH_3), 4.25 (br d, J = 5.5 Hz, 1 H, CH), 4.32 (br d, J = 5.5 Hz, 1 H, CH), 5.70—6.25 (br s, NH_2), 6.31—7.17 (m, 8 H, aromatic H).

2-(4-Benzyloxyphenyl)-2,3-dihydro-3-hydroxy-1,5-benzothiazepin-4(5*H*)-one (27). Compound **36** (1.75 g, 4.4 mmol) was refluxed for 7 h in xylene (60 mL) in a flask fitted with a Dean-Stark trap. After cooling, the crystalline product was collected on a filter to give **27** (1.57 g, 95%) as colorless prisms: mp 235—237°C (lit.[73] mp 230—232°C); TLC R_f = 0.52 (CH_2Cl_2 / EtOAc, 1:1); IR (KBr) 3320 (OH), 1681 C=O, amide I), 1640 (C=O, amide II), 1240 (C—O) cm^{-1}; ^1H NMR (d_6-DMSO) δ 4.27 (br dd, 1 H, OCH), 4.83 (br d, 1 H, OH), 5.03 (d, J = 6.7 Hz, 1 H, SCH), 5.09 (s, 2 H, CH_2), 6.98—8.41 (m, 13 H, aromatic H) , 10.30 (s, 1 H, NH). ^{13}C NMR (d_6-DMSO) δ

57.09 (CH), 69.62 (CH), 69.23 (CH_2), 113.3—158.1 (m, aromatic C), 172.49 (s, CO); FAB MS m/z 378 (M+1).

2,3-Dihydro-3-hydroxy-2-(4-methoxyphenyl)-1,5-benzothiazepin-4(5*H*)-one (28). Compound **37** (0.9 g, 2.8 mmol) was refluxed for two h in xylene (25 mL) in a flask fitted with a Dean-Stark trap. After cooling, the crystals were collected by filtration to give **28** (0.8 g, 94%) as light-grey needles: mp 168—169°C (lit.[109] 63%, mp 167—170°C); TLC R_f = 0.76 ($CHCl_3$ / MeOH, 1:1); IR (KBr) 3350 (br OH), 1680 (C=O, amide I), 1650 (C=O, amide II), 1250 (C—O) cm^{-1};. ^1H NMR (d_6-DMSO) δ 3.76 (s, 3 H, OCH_3), 4.29 (br dd, J = 6.6 Hz, 1 H, OCH), 4.77 (br d, J = 6.7 Hz, 1 H, OH), 5.05 (d, J = 6.7 Hz, 1 H, SCH), 6.88—7.60 (m, 8 H, aromatic), 10.32 (s, 1 H, NH).

(2-Chloroethyl)methylamine hydrochloride (46). 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 one 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 **46** (12.1 g, 93%) as needles: mp 115—117°C; ^1H NMR (D_2O) δ 2.57 (s, 3 H, CH_3N^+), 3.24 (t, J = 5.3 Hz, 2 H, CH_2N^+), 3.68 (t, J = 5.3 Hz, 2 H, $ClCH_2$).

Benzyl (2-chloroethyl)methylcarbamate (47). Compound **46** (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 simultanously benzyl chloroformate (15.7 g, 92 mmol) and 4 N NaOH (25 mL, 100 mmol) with stirring over 30 min and the stirring was continued for an additional 30 min. The reaction mixture was extracted with ether (3 x 30 mL). After

drying with Na_2SO_4, the ether was removed under reduced pressure. The residue (oil, quantitative yield) showed only one spot on TLC R_f = 0.14 (CH_2Cl_2 / hexane, 1:1); IR ($CHCl_3$) 1680 (C=O) cm^{-1}; ^1H NMR (d_6-DMSO) δ 2.89—2.93 (two br s, 3 H, NCH_3), 3.54—3.60 (m, 2 H, NCH_2), 3.74 (t, J = 6.3 Hz, 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 (two equal intensity CH_3), 41.65 and 41.98 (two equal intensity CH_2), 49.46 and 50.00 (two equal intensity CH_2), 66.14 and 66.33 (two equal intensity CH_2), 127.23—136.68 (aromatic C), 155.29 and 155.56 (two 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).

5-[2-(*N*-Benzyloxycarbonyl-*N*-methylamino)ethyl]-2-(4-benzyloxyphenyl)-2,3-dihydro-3-hydroxy-1,5-benzothiazepin-4(5*H*)-one (38). Compound **38** was prepared by alkylation of **27** with **47** in the presence of dimethylsulfinyl carbanion. To 80% NaH in mineral oil (0.43 g, 14.4 mmol) was added DMSO (39 mL) under N_2. The mixture was heated with stirring at 70°C for one h. After cooling to room temperature, the mixture was treated with **27** (4.5 g, 12 mmol). The resultant mixture was stirred for one h at room temperature. Compound **47** (2.73 g, 12 mmol) was added. The mixture was stirred overnight at 50—55°C, then poured into ice-water (*ca.* 250 mL). Solvent was removed under reduced pressure (70°C, 0.5 mmHg), and the residue was extracted with toluene (3 x 100 mL). Toluene extracts were combined and dried over anhydrous $MgSO_4$. Solvent was removed under reduced pressure and the residue was recrystallized from EtOAc to give **38** (2.95 g, 43%): mp 122—123°C (lit.[73] 68%, mp 122—123°C); TLC R_f = 0.60 (CH_2Cl_2 / EtOAc, 1:1); IR (KBr) 3400 (OH), 1702 (C=O), 1660 (C=O) cm^{-1}; ^1H NMR (d_6-DMSO) δ 2.74—2.88 (m, 1 H, OH), 3.05

(s, 3 H, NCH_3), 3.62—3.77 (m, 2 H, NCH_2), 4.04—4.40 (m, 2 H, NCH_2), 4.40—4.50 (m, 1 H, OCH), 4.88—4.92 (m, 1 H, SCH), 5.17 (br s, 4 H, 2 OCH_2), 6.95—7.69 (m, 18 H, aromatic H); ^{13}C NMR (d_6-DMSO) δ 35.16 (CH_3), 46.52 (CH_2), 47.25 (CH_2), 56.65 (CH), 67.20 (CH_2), 69.48 (CH), 70.06 (CH_2), 114.64—159.25 (m, aromatic C), 171.86 (CON). Starting material, **27**, was recovered in 32% yield.

2-(4-Benzyloxyphenyl)-2,3-dihydro-5-[2-(dimethylamino)-ethyl]-3-hydroxy-1,5-benzothiazepin-4(5*H*)-one (39). Compound **39** was obtained by alkylation with (2-chloroethyl)dimethylamine hydrochloride in the presence of K_2CO_3 / DMF. A mixture of **27** (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 **39** (2.21 g, 38%): mp 145.5—146.5°C; TLC R_f = 0.52 ($CHCl_3$ / MeOH, 1:1); IR (KBr) 3400 (OH), 1670 (C=O), 1240 (C—O) cm^{-1}; ^1H 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 dd, 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).

5-[2-(*N*-Benzyloxycarbonyl-*N*-methylamino)ethyl]-2,3-dihydro-3-hydroxy-2-(4-methoxyphenyl)-1,5-benzothiazepin-4(5*H*)-one (40). Compound **40** was obtained in a similar manner as **38**. NaH (0.6 g, 20 mmol, 80% oil dispersion) in DMSO (54 mL) was heated at 70°C for one h under N_2. To this solution was added **28** (5.0 g, 16.6 mmol) at room temperature. After one h stirring, **47** (3.78 g,

16.6 mmol) was added and the mixture was kept at 50—55°C for 24 h. The reaction mixture was poured onto ice-water (*ca.* 250 mL) and extracted with toluene (3 x 100 mL). Toluene extracts were combined, treated with Norite and dried over Na_2SO_4. Solvent removal gave a yellow oil (7.23 g) which was treated with MeOH (10 mL) to precipitate one of the starting materials (25% of **28** was recovered). After MeOH was evaporated, the resulting oil was treated with ether (3 x15 mL) to remove **47**. Compound **40** (2.37 g, 29%) was obtained as gel-like oil and was used in the next step without further purification: TLC R_f = 0.50 (CH_2Cl_2 /EtOAc, 1:1); IR ($CHCl_3$) 3500 (OH), 1700 (C=O), 1670 (C=O) cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.82—2.87 (m, 1 H, OH), 3.01 (s, 3 H, NCH_3), 3.50—3.75 (m, 2 H, NCH_2), 3.80 (s, 3 H, OCH_3), 3.90—4.40 (m, 2 H, NCH_2), 4.40—4.45 (m, 1 H, OCH), 4.85—4.90 (m, 1 H, SCH), 5.10 (br s, 2 H, OCH_2), 6.88—7.69 (m, 13 H, aromatic H).

2,3-Dihydro-5-[2-(dimethylamino)ethyl]-3-hydroxy-2-(4-methoxyphenyl)-1,5-benzothiazepin-4(5*H*)-one (1). A mixture of **28** (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 3 days. Inorganic compounds were removed by filtration. The filtrate was reduced to about 5 mL and converted into HCl salt with HCl-EtOH (10%, w / w). After solvent was removed under reduced pressure, the residue was recrystallized from EtOH to give the HCl salt of **1** (260 mg, 58%) as white crystals: mp 224—225.5°; TLC 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}; ^1H 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 (two d, J = 6.6 and 7.2 Hz, 2 H, SCH, OH),

6.90—7.73 (m, 8 H, aromatic H), 10.46 (br s, 1 H, NH^+); the ^1H 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 (CO); FAB MS m/z 373 (M+1). The product was converted to its HBr salt, mp 224—226°C (lit.[73] 12%, mp 225—228°C).

3-Acetyloxy-5-[2-(*N*-benzyloxycarbonyl-*N*-methylamino)ethyl]-2-(4-benzyloxyphenyl)-2,3-dihydro-1,5-benzothiazepin-4-(5*H*)-one (41). A mixture of **38** (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 two h on a boiling water bath. The reaction mixture was allowed to cool to ambient temperature and then poured into ice-water (*ca.* 60 mL). The mixture was extracted with EtOAc and the extract was washed with 5% HCl, brine and water. The separated EtOAc layer was dried over anhydrous $MgSO_4$. Compound **41** (2.77 g, quantitative yield) was obtained as colorless crystals after the solvent was removed under reduced pressure: mp 51—53°C; TLC 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) 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 (m, aromatic C), 156.03—156.31 (2 s, NCO_2), 167.36 (CO), 169.85 (NCO).

3-Acetyloxy-2-(4-benzyloxyphenyl)-2,3-dihydro-5-[2-(dimethyl-amino)ethyl]-1,5-benzothiazepin-4(5*H*)-one (42). A mixture of **39** (1.5 g, 3.3 mmol), acetic anhydride (2.8 g, 27.6 mmol) and pyridine (13.6 g, 172 mmol) was stirred for 24 h at room temperature and then poured into ice-water (*ca.* 25 mL) and stirred for an additional 30 min. The same work-up as **41** gave compound **42** (1.6 g, quantitative yield) as needles: mp 225—227°C; TLC R_f = 0.50 ($CHCl_3$ / MeOH, 1:1); IR (KBr) 2700—2300 (br, NH^+), 1745 (C=O, ester), 1680 (C=O, amide) 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 (43). A mixture of **40** (0.8 g, 1.6 mmol), acetic anhydride (1.3 g, 13 mmol) and pyridine (6.4 g, 81 mmol) was stirred for 24 h at room temperature and then poured into ice-water (*ca.* 30 mL). The same work-up as **41** gave compound **43** (0.84 g , quantitative yield) as oil; TLC 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) 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 (4). After **41** (2.0 g, 3.3 mmol) was stirred

in freshly prepared 25% (w / w) of HBr-AcOH (8 mL) for four h at room temperature, the reaction mixture was poured into ice-water (*ca.* 50 mL) and shaken with ether. The aqueous phase was separated and neutralized with powdered $NaHCO_3$ and then extracted with EtOAc. The organic layer was separated and dried over anhydrous $MgSO_4$. The solvent was removed under reduced pressure, the residue was titrated to pH *ca.* 4.5 with 9% (w / w) HBr-MeOH. Recrystallization of the residue from ethanol gave HBr salt of **4** (0.4 g, 27%): mp 260—262°C (dec.); TLC 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 (m, aromatic C), 166.89 (CO), 168.53 (CO); accurate mass by high-resolution FAB MS 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 (5). Compound **42** (0.8 g, 1.65 mmol) was stirred in freshly prepared 25% (w / w) of HBr-AcOH (2 mL) at room temperature. After four h stirring, the mixture was poured into ice-water (*ca.* 25 mL) and shaken with ether. The aqueous phase was separated and neutralized with powdered $NaHCO_3$ and then extracted with EtOAc (5 x 20 mL). The organic layer was separated and dried over anhydrous $MgSO_4$. The solvent was removed under reduced pressure, the residue was

titrated to pH *ca.* 4.5 with 9% (w / w) HBr-MeOH. After the solvent was removed under reduced pressure, the residue was recrystallized from EtOH to give the HBr salt of **5** (0.4 g, 51%): mp 233—235°C (dec.); TLC 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, 1H, 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 (m, aromatic C), 167.08 (CO), 169.17 (CO); accurate mass by high-resolution FAB MS 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 (2). After compound **43** (0.8 g, 1.5 mmol) was stirred in freshly prepared 25% (w / w) HBr-AcOH (3 mL) for 7.5 h at room temperature, the reaction mixture was poured into ice-water (*ca.* 15 mL) and shaken with ether. The aqueous phase was separated and made alkaline with powdered Na_2CO_3. The resultant mixture was extracted with EtOAc and the organic extract was dried over anhydrous $MgSO_4$. After the solvent was removed under reduced pressure, the residue was treated with HCl-MeOH. The HCl salt of **2** (0.24 g, 37%) was obtained as crystals after the recrystallization from EtOH: mp 217—220°C; TLC 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, 2 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 (m, aromatic C), 166.88 (CO), 169.13 (CO); CI MS m/z 401 (M+1). Treatment of the HCl salt with NH_4OH gave free **2**, mp 133—135°C. Anal. calcd for $C_{21}H_{24}N_2O_4S \cdot 1/2H_2O$: C, 61.59; H, 5.91; N, 6.84. Found: C, 61.81; H, 5.94; N, 6.70.

2,3-Dihydro-3-hydroxy-2-(4-hydroxyphenyl)-5-[2-(methylamino)-ethyl]-1,5-benzothiazepin-4(5*H*)-one (8). Compound **8** was obtained by hydrolysis of **4**. A mixture of the HBr salt of **4** (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 the HBr salt of **8** (0.28 g, 67%): mp 170—172°C; TLC 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); ^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 (m, aromatic C), 171.38 (CO);

CI MS m/z 345 (M+1). The HBr salt of **8** was converted to the HCl salt with NH_4OH followed by treatment with HCl / MeOH (10%), mp 240—244°C (lit.[73] mp > 235°C).

2,3-Dihydro-5-[2-(dimethylamino)ethyl]-3-hydroxy-2-(4-hydroxyphenyl)-1,5-benzothiazepin-4(5*H*)-one (3). A mixture of **5•HBr** (0.2 g, 0.4 mmol) and 10% HBr-MeOH (6 mL) was refluxed for 15 min. After cooling, the solvent was removed under reduced pressure. The residue was recrystallized from EtOH to give the HBr salt of **3** (0.1 g, 57%): mp 217—220°C (dec.); TLC 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 (m,aromatic C), 171.45 (CO); FAB MS m/z 359 (M+1). Treatment of the HBr salt with NH_4OH gave free **25**, mp 136—139°C (lit.[73] mp 135—138°C).

2,3-Dihydro-3-hydroxy-2-(4-methoxyphenyl)-5-[2-(methylamino)-ethyl]-1,5-benzothiazepin-4(5*H*)-one (7). After the HCl salt of **2** (0.5 g, 1.1 mmol) was stirred in 5% NaOH-EtOH (10 / 150 mL, v / v) for seven 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 EtOAc. The organic layer was separated and dried over anhydrous $MgSO_4$. 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 **7** (0.4 g, 89%): mp 200—202°C (lit.[73] 203—207°C); TLC 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 (m, aromatic C), 171.36 (CO); CI MS 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 (6). 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 chloroform (15 mL) was stirred at room temperature (20°C) for two 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 H_2O (20 x 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 **6** (0.25 g, 27%): mp 65—68°C; TLC 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), 4.36—4.53 and 4.56—4.75 (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 (m, aromatic C), 168.29 (CO), 169.85 (CO); accurate mass of **6** by high-resolution FAB MS m/z (M+1) for $C_{22}H_{27}N_2O_5S$, calcd 431.1641, found 431.1658. Anal. calcd for $C_{22}H_{26}N_2O_5S \cdot 2H_2O$: C, 56.64; H, 5.62; N, 6.00. Found: C, 56.83; H, 5.97; N, 5.91.

3. 2. 2 Synthesis of Dibenzotricyclic Systems (DBTs)

3. 2. 2. 1 Dibenzo[*b*,*f*]-1,4-thiazepine Derivatives

2-(2-Aminophenylthio)benzoic acid (48). A mixture of 5-iodobenzoic acid (99.0 g, 0.4 mol), 2-aminothiophenol (55.0 g, 0.4 mol), KOH (78.0 g, 1.4 mol) and Cu powder (5.0 g, 0.08 mol) in H_2O (900 mL) was stirred and refluxed for 6.5 h. It was filtered while hot and the filtrate was acidified with acetic acid (*ca.* 150 mL). The color of the filtrate changed from purple to blue. The precipitated product was collected by filtration and crystallized from aqueous acetic acid (HOAc / H_2O=*ca.* 450 / 100 mL). Compound **48** (92.0 g, 94%) was obtained as grey crystals: mp 162—164°C (lit.[159] mp 157—158°C); TLC R_f = 0.32 ($CHCl_3$ / MeOH, 9:1); IR (KBr) 3480 and 3380 (NH_2), 3200—2500, 1680 (C=O) cm^{-1}; ^1H NMR ($CDCl_3$) δ 5.50—6.65 (br s, 2 H, NH_2), 6.75—8.17 (m, 8 H aromatic H); EI MS m/z 245 (98), 227 (100), 199 (32), 195 (35), 183 (20), 167 (40), 80 (?) and 64 (52).

Dibenzo[*b*,*f*]-1,4-thiazepin-11(10*H*)-one (14). 2-(2-Aminophenylthio)-benzoic acid (87.0 g, 0.35 mol) was refluxed for 12 h in xylene (450 mL) in a flask fitted with a Dean-Stark trap. After cooling, the product was collected on a filter to give off-white crystals (67.0 g, 83%): mp 258—260°C (lit.[160] 258—260°C); TLC R_f = 0.64 ($CHCl_3$ / MeOH, 9:1); IR (KBr) 3180 (N-H), 1650 (C=O) cm^{-1}; ^1H NMR (d_6-DMSO) δ 7.12—7.68 (m, 8 H, aromatic H), 10.72 (s, 1 H, NH); EI MS m/z 227 (100), 209 (15), 195 (28), 183 (13), 167 (17), 83 (80) and 65 (100).

10-Methyldibenzo[*b,f*]-1,4-thiazepin-11(10*H*)-one (25). To a solution of **14** (1.0 g, 4.4 mmol) in DMF (25 mL) at 55°C under N_2 was added NaH (0.1320 g, 4.4 mmol, 80% oil dispersion). This mixture was stirred at the same temperature for an additional 30 min and then cooled in an ice bath. Methyl iodide (0.7 g, 4.8 mmol) was added dropwise over a period of 5 min through a syringe. The mixture was stirred for one h in an ice-water bath. TLC showed the reaction was complete. The reaction mixture was poured into H_2O (*ca.* 30 mL) and extracted with CH_2Cl_2 (3 x 30 mL). The organic layer was successively washed with H_2O and brine and then dried over anhydrous $MgSO_4$. Crystalline product (0.94 g, 88%) was obtained after the solvent was evaporated. TLC R_f = 0.74 ($CHCl_3$ / MeOH, 9:1). Crystallization from ether gave cubical diamonds: mp 102—104°C; IR (KBr) 1630 (C=O) cm^{-1}; ^1H NMR ($CDCl_3$) δ 3.62 (s, 3 H, NCH_3), 7.09—7.77 (m, 8 H, aromatic H); ^{13}C NMR ($CDCl_3$) δ 38.89 (CH_3), 124.83, 126.13, 128.48, 129.50, 130.89, 130.95, 131.80, 133.07 (8 CH), 134.83, 137.93, 138.98, 144.90 (4 quarternary C), 168.94 (CO); FAB MS m/z 242 (M+H). Anal. calcd for $C_{14}H_{11}NOS \cdot 1/4H_2O$: C, 68.40; H, 4.72; N, 5.70. Found: C, 68.56; H, 4.45; N, 5.74.

10-Cyclopropylcarbonyldibenzo[*b,f*]-1,4-thiazepin-11(10*H*)-one (26). A solution of compound **14** (0.76 g, 3.3 mmol) and NaH (0.1 g, 3.3 mmol, 80% oil dispersion) in DMF (10 mL) was stirred under N_2 at room temperature for 0.5 h. To this mixture was added 1-bromo-3-chloropropane (0.62 g, 3.3 mmol) through a syringe. The reaction mixture was stirred at room temperature for 22 h. After the solvent was removed under reduced pressure, the residue was treated with H_2O (10 mL) and extracted with a mixture of CH_2Cl_2 (20 mL) and EtOAc (60 mL). The organic layer was dried over anhydrous $MgSO_4$. After the solvents were evaporated, the residue was treated with MeOH (6 mL) to precipitate out unreacted **14**. Compound **26** (60 mg) was obtained after

separation by dry-column flash chromatography with a gradient from 50 to 60% of CH_2Cl_2 in hexane: mp 145—147°C; IR (KBr) 1700 (C=O), 1670 (C=O) cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.80—1.61 (m, 4 H, CH_2CH_2), 2.51—2.64 (m, 1 H, COCH), 7.18—8.09 (m, 8 H, aromatic H); ^{13}C NMR ($CDCl_3$) δ 10.87 (CH_2), 11.21 (CH_2), 15.93 (CH), 128.27, 128.47, 129.00, 131.50, 131.94, 132.46, 132.76, 133.80 (8 CH), 135.02, 136.61, 138.21, 140.69 (4 quarternary C), 169.34 (CO), 176.21 (CO); FAB MS m/z 296 (M+H). Anal. calcd for $C_{17}H_{13}NO_2S$: C, 69.13; H, 4.44; N, 4.74. Found: C, 68.70; H, 4.35; N, 4.93.

10-[2-(Dimethylamino)ethyl]dibenzo[*b,f*]-1,4-thiazepin-11(10*H*)-one (19). A mixture of **14** (2.0 g, 8.8 mmol), (2-chloroethyl)dimethylamine hydrochloride (1.65 g, 11.4 mmol) and NaH (0.74 g, 24.6 mmol, 80% oil dispersion) in DMF (25 mL) was stirred at *ca.* 55°C for 48 h. The reaction mixture was filtered through a glass fiber filter. After the filtrate was evaporated to dryness, the residue was treated with 5% NaOH (2 x 10 mL) and extracted with a mixture of CH_2Cl_2 (80 mL) and ether (20 mL). The organic layer was dried over anhydrous $MgSO_4$. The HCl salt of **19** (0.7 g, 24%) was obtained after treatment of the organic layer with HCl-ether and crystallization from abs. EtOH / ether (*ca.* 12 mL): mp 235—237°C; IR (KBr) 2600—2200 (br, NH^+) and 1630 (C=O) cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.82 (br s, 3 H, CH_3), 2.94 (br s, 3 H, CH_3), 3.30—3.35 and 3.35—3.46 (m, 2 H, CH_2N^+), 4.32—4.38 and 4.95—5.00 (m, 2 H, $CONCH_2$), 7.15—7.68 (m, 8 H, aromatic H), 12.86 (br s, 1 H, NH^+); ^{13}C NMR ($CDCl_3$) δ 42.62 (CH_3), 43.90 (CH_3), 46.56 (CH_2), 54.88 (CH_2), 125.85, 127.38, 128.90, 130.68, 131.20, 131.26, 131.38, 133.31 (8 CH), 135.56, 137.09, 138.77, 142.52 (4 quarternary C), 169.23 (CO). The free base **19** was obtained by treating the HCl salt with dilute NaOH, mp 138—140°C (lit.[161], mp 141—142°C).

10-[3-(Dimethylamino)propyl]dibenzo[*b,f*]-1,4-thiazepin-11(10*H*)-one (20). A mixture of **14** (2.0 g, 8.8 mmol), (3-chloropropyl)-dimethylamine hydrochloride (1.8 g, 11.4 mmol) and NaH (0.74 g, 24.6 mmol, 80% oil dispersion) in DMF (25 mL) was heated under N_2 at 55°C for 48 h. The reaction mixture was filtered through a glass fiber filter. After the solvent was removed under reduced pressure, the residue was recrystallized from Et_2O to give **20** (1.55 g, 56%); mp 115—116° (lit.[161], mp 114—115°C); ^1H NMR ($CDCl_3$) δ 1.78—1.84 (m, 2 H, CH_2), 2.15 (s, 6 H, $N(CH_3)_2$), 2.29—2.35 and 2.37—2.42 (m, 2 H, NCH_2), 3.62—3.67 and 4.73—4.79 (2 dt, J = 7.4 and 13.9 Hz, 2 H, $CONCH_2$), 7.09—7.70 (m, 8 H, aromatic H); ^{13}C NMR ($CDCl_3$) δ 26.36 (CH_2), 45.46 (2 CH_3), 49.23 (CH_2), 57.00 (CH_2), 125.96, 126.29, 128.51, 129.57, 130.59, 130.89, 131.40, 133.20 (8 CH), 136.75, 138.31, 139.18, 143.40 (4 quarternary C), 168.75 (CO). The maleate salt was obtained by treating **20** with maleic acid in Et_2O followed by recrystallization from EtOH / Et_2O (1:2): mp 143.5—145°C; IR (KBr) 2750—2250 (br) and 1640 (C=O) cm^{-1}.

General methods for preparation of compounds 49—53. A mixture of **14** (2.0 g, 8.8 mmol), the appropriate bromochloroalkane (1.3 eq) and NaH (0.32 g, 10.6 mmol as 80% oil dispersion) in DMF (25 mL) was stirred under N_2 at *ca.* 60°C for 24 h (**49**), 18 h (**50**), 2 h (**51** and **52**) and at room temperature for 1.5 h (**53**). The reaction mixture was filtered through a glass fiber filter while hot. The filtrate was removed under reduced pressure. The crude products were used directly for the next reaction without further purification. All crude products were characterized with TLC ($CHCl_3$ / EtOAc, 1:1) and FAB MS.

10-(2-Chloroethyl)dibenzo[*b,f*]-1,4-thiazepin-11(10*H*)-one (49): R_f = 0.62; FAB MS m/z: 290 (M+H).

10-(3-Chloropropyl)dibenzo[*b,f*]-1,4-thiazepin-11(10*H*)-one (50): R_f = 0.63; FAB MS m/z: 304 (M+H).

10-(4-Chlorobutyl)dibenzo[*b,f*]-1,4-thiazepin-11(10*H*)-one (51): R_f = 0.62; FAB MS m/z: 318 (M+H).

10-(5-Chloropentyl)dibenzo[*b,f*]-1,4-thiazepin-11(10*H*)-one (52): R_f = 0.65; FAB MS m/z: 332 (M+H).

10-(6-Chlorohexyl)dibenzo[*b,f*]-1,4-thiazepin-11(10*H*)-one (53): R_f = 0.66; FAB MS m/z: 346 (M+H).

General methods for preparation of compounds 9—13. A mixture of the respective crude product from the previous step, 4-fluorophenylpiperazine (2 eq) and NaI (1 eq) in DMF (25 mL) was stirred at *ca.* 80°C for *ca.* 24 h. After the solvent was removed under reduced pressure, the residue was treated with 5% NaOH and extracted with ether (**9**, **13**) or EtOAc (**10**—**12**). The organic layer was dried over anhydrous $MgSO_4$. After the solvent was removed, the residue was crystallized from an appropriate solvent. Hydrochloride salts of **9**—**13** were obtained by treatment of the crude product with HCl-Et_2O followed by recrystallization from appropriate solvents. Yields are reported from **14**.

10-[2-[4-(4-Fluorophenyl)-1-piperazinyl]ethyl]dibenzo[*b,f*]-1,4-thiazepin-11(10*H*)-one (9) hydrochloride. 8% Yield; mp 260—262°C (EtOH); IR (KBr) 2500—2000 (br, NH^+), 1635 (C=O) cm^{-1}; ^1H NMR (CD_3OD + $CDCl_3$) δ 3.00—4.00 (br m, 10 H, 5 NCH_2), 4.29 (dt, J = 7.3 and 14.0 Hz, 1 H, CONCH), 4.93 (dt, J = 6.3 and 13.8 Hz, 1 H, CONCH), 6.96—7.72 (m, 12 H, aromatic H); ^{13}C NMR (CD_3OD + $CDCl_3$) δ 47.00 (CH_2), 48.66 (2 CH_2), 53.28 (CH_2), 55.14 (2 CH_2), 116.26, 116.56, 119.83, 119.93, 126.45, 128.28, 129.62, 131.21, 131.76, 131.91, 132.32, 134.07 (12 CH), 136.89, 137.66, 139.70, 143.31, 146.93 (5 quarternary C), 159.06 (d, J = 239.3 Hz, C—F), 171.02 (CO); accurate mass of **9** by high-resolution FAB MS m/z (M+1) for $C_{25}H_{25}FN_3OS$, calcd 434.1702, found, 434.1711. Anal. calcd for $C_{25}H_{25}ClFN_3OS$: C, 63.89; H, 5.15; N, 8.94. Found: C, 63.65; H, 5.38; N, 8.91.

10-[3-[4-(4-Fluorophenyl)-1-piperazinyl]propyl]dibenzo[*b,f*]-1,4-thiazepin-11(10*H*)-one (10). 48% Yield; mp 130—132°C (abs. EtOH); IR (KBr) 1635 (C=O) cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.85—1.95 (m, 2 H, CH_2), 2.40—2.65 (m, 6 H, 3 NCH_2), 2.95—3.12 (m, 4 H, 2 NCH_2), 3.70 (dt, J = 6.7 and 13.4 Hz, 1 H, CONCH), 4.80 (dt, J = 7.1 and 13.5 Hz, 1 H, CONCH), 6.81—7.71 (m, 12 H, aromatic H); ^{13}C NMR ($CDCl_3$) δ 25.49 (CH_2), 49.25 (CH_2), 50.15 (2 CH_2), 53.09 (2 CH_2), 55.43 (CH_2), 115.31, 115.81, 117.69, 117.78, 125.96, 126.30, 128.59, 129.59, 130.67, 130.92, 131.44, 133.24 (12 CH), 136.68, 138.34, 139.14, 143.67, 148.07 (5 quarternary C), 157.13 (d, J = 238.5 Hz, C—F), 168.78 (CO); Accurate mass of **10** by high-resolution FAB MS m/z (M+1) for $C_{26}H_{27}FN_3OS$, calcd 448.1859, found 448.1862. Anal. calcd for $C_{26}H_{26}FN_3OS$: C, 69.77; H, 5.86; N, 9.39. Found: C, 69.85; H, 5.97; N, 9.40. The HCl salt of **10**: mp 238—241°C (EtOH / Et_2O, 3:2); IR (KBr) 2600—2100 (br, NH^+) and 1640 (C=O) cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.06—2.28 and

2.42—2.62 (m, 2 H, CH_2), 2.81—3.16 (m, 2 H, CH_2), 3.16—3.38 (m, 2 H, CH_2), 3.38—3.72 (m, 6 H, 3 CH_2), 3.72 —3.87 (m, 1 H, CONCH), 4.83 —5.00 (m, 1 H, CONCH), 6.93—7.75 (m, 12 H, aromatic H), 12.11 (br s, 1 H, NH^+); the ^1H NMR spectrum after D_2O exchange showed disappearance of signal at 12.11 ppm; ^{13}C NMR ($CDCl_3$) δ 22.95 (CH_2), 47.73 (2 CH_2), 48.21 (CH_2), 50.01 (CH_2), 52.30 (CH_2), 55.58 (CH_2), 115.84, 116.14, 119.53, 119.66, 126.04, 127.13, 128.94, 130.33, 131.08, 131.08, 131.29, 133.49 (12 CH), 136.38, 137.83, 138.73, 142.59, 146.16 (5 quarternary C), 158.35 (d, J = 241.7 Hz, C—F), 169.37 (CO).

10-[4-[4-(4-Fluorophenyl)-1-piperazinyl]butyl]dibenzo[*b,f*]-1,4-thiazepin-11(10*H*)-one (11). 65% Yield; mp 162—163°C (EtOAc), IR (KBr) 1635 (C=O) cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.52—1.84 (m, 4 H, 2 CH_2), 2.37 (t, J = 7.0 Hz, 2 H, NCH_2), 2.55 (t, J = 4.9 Hz, 4 H, 2 NCH_2), 3.09 (t, J = 5.0 Hz, 4 H, 2 NCH_2), 3.63 (dt, J = 7.1 and 13.5 Hz, 1 H, CONCH), 4.77 (dt, J = 7.4 and 13.6 Hz, 1 H, CONCH), 6.83—7.71 (m, 12 H, aromatic H); ^{13}C NMR ($CDCl_3$) δ 24.08 (CH_2), 26.03 (CH_2), 50.14 (2 CH_2), 50.66 (CH_2), 53.16 (2 CH_2), 58.04 (CH_2), 115.30, 115.59, 117.66, 117.76, 125.84, 126.27, 128.53, 129.56, 130.63, 130.89, 131.48, 133.26 (12 CH), 136.77, 138.37, 139.12, 143.45, 148.08 (5 quarternary C), 157.21 (d, J=239.1 Hz, C—F), 168.74 (CO); accurate mass of **11** by high-resolution FAB MS m/z (M+1) for $C_{27}H_{29}FN_3OS$, calcd 462.2015, found 462.2050. Anal. calcd for $C_{27}H_{28}FN_3OS$: C, 70.25; H, 6.11; N, 9.10. Found: C, 70.53; H, 6.15; N, 9.14. The HCl salt of **11**: mp 270—272°C (90% EtOH), IR (KBr) 2600—2100 (br, NH+) and 1630 (C=O) cm^{-1}; ^1H NMR (CD_3OD + $CDCl_3$) δ 1.60—1.86 (m, 2 H, CH_2), 1.86—2.16 (m, 2 H, CH_2), 2.80—3.70 (m, 10 H, 5 CH_2), 3.75 (dt, J = 6.0 and 13.9 Hz, 1 H, CONCH), 4.86 (dt, J = 6.7 and 14.4 Hz, 1 H, CONCH), 6.94—7.67 (m, 12 H, aromatic H); ^{13}C NMR

(CD_3OD + $CDCl_3$) δ 21.80 (CH_2), 25.48 (CH_2), 48.40 (CH_2), 49.82 (2 CH_2), 52.66 (CH_2), 57.15 (2 CH_2), 116.22, 116.51, 119.76, 119.87, 126.69, 127.74, 129.48, 130.82, 131.51, 131.69, 131.79, 133.99 (12 CH), 137.40, 138.54, 139.63, 143.06, 146.86 (5 quarternary C), 158.98 (d, J = 239.0 Hz, C—F), 170.73 (CO).

10-[5-[4-(4-Fluorophenyl)-1-piperazinyl]pentyl]dibenzo[*b,f*]-1,4-thiazepin-11(10*H*)-one (12). 47% Yield; mp 163—165°C (EtOH / Et_2O, 2:1), IR (KBr) 2700—2200 (br, NH^+), 1625 (C=O) cm^{-1}; ^1H NMR (d_6-DMSO) δ 1.24—1.44 (m, 2 H, CH_2), 1.44—1.64 (m, 2 H, CH_2), 1.64—1.89 (m, 2 H, CH_2), 2.90—3.22 (m, 6 H, 3 NCH_2), 3.43—3.60 (m, 2 H, NCH_2), 3.60—3.80 (m, 3H, NCH_2 + CONCH), 4.62 (dt, J = 7.5 and 13.7 Hz, 1 H, CONCH), 7.00—7.67 (m, 12 H, aromatic H), 10.94 (br s, 1 H NH^+); ^{13}C NMR (d_6-DMSO) δ 22.54 (CH_2), 23.42 (CH_2), 26.95 (CH_2), 45.94 (2 CH_2), 49.32 (CH_2), 50.40 (2 CH_2), 55.06 (CH_2), 115.23, 115.52, 117.66, 117.77, 126.30, 126.50, 128.73, 130.11, 130.69, 130.84, 131.03, 132.91 (12 CH), 135.56, 137.99, 138.19, 142.59, 146.36 (5 quarternary C), 156.47 (d, J = 236.4 Hz, C—F), 167.43 (CO); accurate mass of **12** by high-resolution FAB MS m/z (M+1) for $C_{28}H_{31}FN_3OS$, calcd 476.2172, found 476.2179. Anal. calcd for $C_{28}H_{31}ClFN_3OS \cdot 3/4H_2O$: C, 63.99; H, 6.33; N, 7.99. Found: C, 64.00; H, 6.15; N, 7.99.

10-[6-[4-(4-Fluorophenyl)-1-piperazinyl]hexyl]dibenzo[*b,f*]-1,4-thiazepin-11(10*H*)-one (13). 39% Yield; mp 133.5—135°C (abs. EtOH); IR (KBr) 1630 (C=O) cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.12—1.55 (m, 6 H, 3 CH_2), 1.55—1.72 (m, 2 H, CH_2), 2.34 (t, J = 7.2 Hz, 2 H, NCH_2), 2.57 (br s, 4 H, 2 NCH_2), 3.11 (br s, 4 H, 2 NCH_2), 3.57 (dt, J = 6.9 and 13.5 Hz, 1 H, CONCH), 4.73 (dt, J = 7.5 and 13.4 Hz, 1

H, CONCH), 6.85—7.71 (m, 12 H, aromatic H); ^{13}C NMR ($CDCl_3$) δ 26.78 (2 CH_2), 27.20 (CH_2), 28.00 (CH_2), 50.15 (2 CH_2), 50.85 (CH_2), 53.28 (2 CH_2), 58.60 (CH_2), 115.32, 115.61, 117.68, 117.78, 125.88, 126.23, 128.51, 129.51, 130.59, 130.88, 131.47 and 133.23 (12 CH), 136.81, 138.39, 139.18, 143.49 and 148.06 (5 quarternary C), 157.22 (d, J = 238.4 Hz, C—F) 168.89 (CO); accurate mass of **13** by high-resolution FAB MS m/z (M+1) for $C_{29}H_{33}FN_3OS$, calcd 490.2328, found 490.2367. Anal. calcd for $C_{29}H_{32}FN_3OS$: C, 71.14; H, 6.59; N, 8.58. Found: C, 70.89; H, 6.61; N, 8.53. The HCl salt of **13**: 210—211.5°C (EtOH / Et_2O, 3:1), IR (KBr) 2700—2100 (br, NH^+) and 1630 (C=O) cm^{-1}; ^1H NMR (d_6-DMSO) δ 1.11—1.42 (m, 4 H, 2 CH_2), 1.42—1.61 (m, 2 H, CH_2), 1.61—1.81 (m, 2 H, CH_2), 2.93—3.18 (m, 6 H, 3 CH_2), 3.42—3.59 (m, 3 H, CH_2 + CONCH), 4.62 (dt, J = 7.5 and 13.7 Hz, 1 H, CONCH), 6.99—7.67 (m, 12 H, aromatic H), 10.76 (br s, 1 H, NH^+); the ^1H NMR spectrum after D_2O exchange showed disappearance of signal at 10.76 ppm; ^{13}C NMR (d_6-DMSO) δ 22.81 (CH_2), 25,63 (2 CH_2), 27.10 (CH_2), 45.98 (2 CH_2), 49.30 (CH_2), 50.45 (2 CH_2), 55.17 (CH_2), 115.24, 115.53, 117.67, 117.77, 126.29, 126.46, 128.72, 130.08, 130.68, 130.81, 131.03, 132.89 (12 CH), 135.57, 138.03, 138.18, 142.60, 146.36 (5 quarternary C), 156.48 (d, J = 236.9 Hz, C—F), 167.40.

General method for preparation of compounds 21—24. A mixture of the crude respective chloroalkyl DBT (**50—53**, 8.8 mmol), *N*-benzyl-*N*-methylamine (2.1 g, 17.6 mmol) and NaI (1.3 g, 8.8 mmol) in DMF (25 mL) was stirred at *ca.* 80°C for 24 h (**21**, **22**) or at *ca.* 70°C overnight (**23**, **24**). In the case of **23** and **24**, the reaction mixtures were filtered through glass fiber filters. After the solvent was removed under reduced pressure, the residue was treated with 5% NaOH and extracted with ether (**21**),

EtOAc (**22**) or $CHCl_3$ (**23**, **24**). The organic layer was dried over anhydrous $MgSO_4$ to give crude **21**—**24**. Yields are reported from **14**.

10-[3-(*N*-Benzyl-*N*-methylamino)propyl]dibenzo-[*b,f*]-1,4-thiazepin-11(10*H*)-one (21). Compound **21** (2.3 g, 66%) was obtained as yellow oil after separation by dry column flash chromatography using a gradient from 50% to 100% of $CHCl_3$ in hexane. IR (neat) 1640 (C=O) cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.80—1.96 (m, 2 H, CH_2), 2.09 (s, 3 H, NCH_3), 2.36—2.59 (m, 2 H, NCH_2), 3.40 (s, 2 H, NCH_2Ar), 3.34—3.46 (dt, J = 7.2 and 13.5 Hz, 1 H, CONCH), 3.61—3.75 (dt, J = 6.7 and 13.6 Hz, 1 H, CONCH), 7.04—7.66 (m, 13 H, aromatic H); ^{13}C NMR ($CDCl_3$) δ 25.96 (CH_2), 41.90 (CH_3), 49.29 (CH_2), 54.72 (CH_2), 62.33 (CH_2), 125.92, 126.22, 126.81, 128.13, 128.13, 128.47, 128.93, 128.93, 129.53, 130.57, 130.86, 131.44, 133.18 (13 CH), 136.62, 138.32, 139.21, 139.21, 143.63 (5 quarternary C), 168.69 (CO). The trifluoroacetate salt was obtained by treating **21** with trifluoroacetic acid (TFA) in ether and crystallization from EtOH / Et_2O (1:3, 40 mL total): mp 135—136.5°C; TLC R_f = 0.43 ($CHCl_3$ / MeOH, 9:1); IR (KBr) 3430, 2800—2200 (br, NH^+), 1675 (CO_2^-) and 1620 (C=O); ^1H NMR (d_6-DMSO) δ 1.61—2.17 (br m, 2 H, CH_2), 2.66 (s, 3 H, CH_3N^+), 2.93—3.29 (br m, 2 H, CH_2N^+), 3.66—3.82 (dt, J = 6.6 and 14.1 Hz, 1 H, CONCH), 4.27 (br s, 2 H, $ArCH_2N^+$), 4.55—4.75 (dt, J = 6.8 and 14.0 Hz, 1 H, CONCH), 7.16—7.70 (m, 13 H, aromatic H), 9.77 (br s, 1 H NH^+). Anal. calcd for $C_{26}H_{25}F_3N_2O_3S \cdot 1/2H_2O$: C, 61.04; H, 5.12; N, 5.48. Found: C, 61.31; H, 5.01; N, 5.55.

10-[4-(*N*-Benzyl-*N*-methylamino)butyl]dibenzo[*b,f*]-1,4-thiazepin-11(10*H*)-one (22). The oxalate salt of **22** was obtained in a yield of 0.9 g (21%) by

treating the crude product in $CHCl_3$ / Et_2O (1:1, 40 mL total) with 1.0 M of oxalic acid in EtOH and by recrystallization from EtOH / Et_2O (1:1, 40 mL total): mp 181—183°C; TLC R_f = 0.5 ($CHCl_3$ / EtOAc, 1:1); IR (KBr) 2750—2550 (br, NH^+), 1640 (sh, C=O) and 1620 (C=O) cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.48—1.89 (m, 2 H, CH_2), 1.96—2.32 (m, 2 H, CH_2), 2.66 (s, 3 H, CH_3N^+), 2.83—3.35 (m, 2 H, CH_2N^+), 3.56—3.70 and 4.75—4.93 (m, 2 H, $CONCH_2$), 3.97—4.38 (br s, 2 H, $ArCH_2N^+$), 7.07—7.73 (m, 13 H, aromatic H), 10.42 (br s, 1 H, NH^+); ^{13}C NMR ($CDCl_3$) δ 20.60 (CH_2), 24.90 (CH_2), 39.45 (CH_3), 48.60 (CH_2), 54.80 (CH_2), 59.45 (CH_2), 125.86—142.29 (m, aromatic C), 169.30 (br, CO). Compound **22** was obtained by treatment of the oxalate salt with 5% NaOH and extracted with $CHCl_3$. Anal. calcd for $C_{25}H_{26}N_2OS \cdot 1/2H_2O$ for **22**: C, 72.96; H, 6.61; N, 6.80. Found: C, 72.77; H, 6.52; N, 6.49.

10-[5-(*N*-Benzyl-*N*-methylamino)pentyl]dibenzo[*b*,*f*]-1,4-thiazepin-11(10*H*)-one (23). Compound **23** (1.5 g, 42%) was obtained as a solid after separation by dry-column flash chromatography with a gradient from 0 to 100% of EtOAc in $CHCl_3$: mp 200—203°C; TLC R_f = 0.13 ($CHCl_3$ / EtOAc, 1:1); ^1H NMR ($CDCl_3$) δ 1.35—1.55 (m, 2 H, CH_2), 1.55—1.78 (m, 2 H, CH_2), 1.78—2.23 (br s, 2 H, CH_2), 2.67 (br s, 3 H, NCH_3), 2.78—3.22 (br s, 2 H, NCH_2), 3.59 (dt, J = 5.6 and 13.8 Hz, 1 H, CONCH), 4.19 (br s, 2 H, NCH_2Ar), 4.81 (dt, J = 7.3 and 13.7 Hz, 1 H, CONCH), 7.05—7.72 (m, 13 H, aromatic H); accurate mass of **23** by high-resolution FAB MS m/z (M+1) for $C_{26}H_{29}N_2OS$, calcd 417.2001, found 417.1980. The oxalate salt was obtained by treating compound **23** with 1.0 M of oxalic acid in abs. EtOH / $CHCl_3$ (5:3), followed by recrystallization from EtOH: mp 212—214°C; IR (KBr) 2700—2400 (br, NH^+), 1640 (sh, C=O) and 1630 (C=O) cm^{-1}.

10-[6-(*N*-Benzyl-*N*-methylamino)hexyl]dibenzo[*b,f*]-1,4-thiazepin-11(10*H*)-one (24). Compound **24** (1.4 g, 47%) was obtained as an oil after separation by dry-column flash chromatography with a gradient from 0 to 100% of EtOAc in $CHCl_3$: ^1H NMR ($CDCl_3$) δ 1.11—1.44 (m, 4 H, 2 CH_2), 1.44—1.57 (m, 2 H, CH_2), 1.57—1.72 (m, 2 H, CH_2), 2.20 (s, 3 H, NCH_3), 2.37(t, J = 7.3 Hz, 2 H, NCH_2), 3.52 (s, 2 H, NCH_2Ar), 3.52—3.64 (m, 1 H, CONCH), 4.71 (dt, J = 7.6 and 13.5 Hz, 1 H, CONCH), 7.02—7.74 (m, 13 H, aromatic H); accurate mass of **24** by high-resolution FAB MS m/z (M+1) for $C_{27}H_{31}N_2OS$, calcd 431.2157, found 431.2164. The HCl salt was obtained by treating **24** with 10% HCl-Et_2O in $CHCl_3$ / Et_2O (1:2) followed by recrystallization from EtOH / Et_2O (3:4): mp 231—233°C. IR (KBr) 2750—2300 (br, NH^+) and 1630 (C=O) cm^{-1}.

3. 2. 2. 2 Dibenz[*b,f*]-1,4-oxazepine Derivatives

General method for preparation of compounds 54—57. These compounds were synthesized by a method similar to that used for **49—53**. A mixture of 10,11-dihydrodibenz[*b,f*]-1,4-oxazepin-11-one (2.0 g, 9.5 mmol), the appropriate bromochloroalkane (12.3 mmol) and NaH (0.34 g, 11.4 mmol) in DMF (25 mL) was stirred under N_2 at *ca.* 60 °C for 48h (**54**) and 24 h (**55—57**). After the solvent was evaporated under reduced pressure, the residue was washed with H_2O and extracted with Et_2O / $CHCl_3$. The organic layer was dried over anhydrous $MgSO_4$. The crude products were used directly for the next reaction without further purification. TLC R_f values ($CHCl_3$ / EtOAc, 1:1) for **54—57** are listed below.

10-(3-Chloropropyl)dibenz[*b,f*]-1,4-oxazepin-11(10*H*)-one (54): R_f = 0.56.

10-(4-Chlorobutyl)dibenz[*b,f*]-1,4-oxazepin-11(10*H*)-one (55): R_f = 0.83.

10-(5-Chloropentyl)dibenz[*b,f*]-1,4-oxazepin-11(10*H*)-one (56): R_f = 0.85.

10-(6-Chlorohexyl)dibenz[*b,f*]-1,4-oxazepin-11(10*H*)-one (57): R_f = 0.82.

General methods for preparation of compounds 15—18. A mixture of the respective crude product (**54—57**) from the previous step, 4-fluorophenylpiperazine (3.75 g, 21 mmol) and NaI (1.42 g, 9.5 mmol) in DMF (25 mL) was stirred at room temperature for 17 h (**54**), at *ca.* 80°C for *ca.* 25 h (**55, 56**) or at *ca.* 80°C for *ca.* 42 h (**57**). After the solvent was removed under reduced pressure, the residue was treated with 5% NaOH and extracted with Et_2O / $CHCl_3$ (**55, 56**) or $CHCl_3$ (**54, 57**). After the organic layer was dried over anhydrous $MgSO_4$, the product was purified as described below. Yields are reported from 10,11-dihydrodibenz[*b,f*]-1,4-oxazepin-11-one.

10-[3-[4-(4-Fluorophenyl)-1-piperazinyl]propyl]dibenz[*b,f*]-1,4-oxazepin-11(10*H*)-one (15). The HCl salt (1.0 g, 23%) was obtained by treating crude **15** with 10% HCl-Et_2O in $CHCl_3$ / Et_2O (1:1): mp 210—212°C; IR (KBr) 2700—2200 (br, NH^+) and 1645 (C=O) cm^{-1}; ^1H NMR (d_6-DMSO) δ 2.01—2.26 (m, 2 H,

CH_2), 2.94—3.30 (m, 6 H, 3 NCH_2), 3.49—3.61 (m, 2 H, NCH_2), 3.61—3.75 (m, NCH_2, overlapped with solvent peak), 4.05—4.28 (m, 2 H, $CONCH_2$), 6.94—7.80 (m, 12 H, aromatic H), 10.92 (br s, 1 H, NH^+); ^{13}C NMR (d_6-DMSO) δ 22.17 (CH_2), 45.43 (CH_2), 45.99 (2 CH_2), 50.58 (2 CH_2), 52.86 (CH_2), 115.31, 115.48, 117.70, 117.76, 119.77, 121.44, 123.69, 125.51, 126.27, 126.96, 131.53, 133.86 (12 CH), 126.15, 133.70, 146.29, 154.02, 160.06 (5 quarternary C), 156.48 (d, J = 237.7 Hz, C—F), 165.29 (CO). Anal. calcd for $C_{26}H_{27}ClFN_3O_2 \cdot H_2O$: C, 64.26; H, 6.01; N, 8.65. Found: C, 64.66; H, 5.74; N, 8.68.

10-[4-[4-(4-Fluorophenyl)-1-piperazinyl]butyl]dibenz[*b,f*]-1,4-oxazepin-11(10*H*)-one (16). The crude product was treated with MeOH and the resultant precipitate was recrystallized from EtOH to give compound **16** (60% yield), mp 99—101°C; IR (KBr) 1642 (C=O) cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.49—1.89 (m, 4 H, 2 CH_2), 2.39 (t, J = 7.2 Hz, 2 H, NCH_2), 2.55 (t, J = 4.9 Hz, 4 H, 2 NCH_2), 3.09 (t, J = 4.8 Hz, 4 H, 2 NCH_2), 4.03—4.34 (m, 2 H, $CONCH_2$), 6.80—7.89 (m, 12 H, aromatic H); ^{13}C NMR ($CDCl_3$) δ 23.72 (CH_2), 25.71 (CH_2), 48.22 (CH_2), 50.12 (2 CH_2), 53.10 (2 CH_2), 57.82 (CH_2), 115.37, 115.55, 117.70, 117.76, 119.66, 121.66, 123.48, 125.28, 125.81, 126.51, 132.08, 133.29 (12 CH), 126.91, 134.61, 147.99, 155.01, 160.76 (5 quarternary C), 157.10 (d, J = 237.7 Hz, C—F), 166.39 (CO). Anal. calcd for $C_{27}H_{28}FN_3O_2 \cdot H_2O$: C, 69.95; H, 6.52; N, 9.06. Found: C, 70.18; H, 6.50; N, 9.08.

10-[5-[4-(4-Fluorophenyl)-1-piperazinyl]pentyl]dibenz[*b,f*]-1,4-oxazepin-11(10*H*)-one (17). Separation by dry-column flash chromatography with a gradient from 0 to 40% of EtOAc in CH_2Cl_2 gave **17** (1.3 g, 60%) as an oil: IR (neat) 1650 (C=O) cm^{-1}; accurate mass of **17** by high-resolution FAB MS m/z (M+1) for

$C_{28}H_{31}FN_3O_2$, calcd 460.2400, found 460.2405. This oil was treated with 10% HCl-Et_2O in $CHCl_3$ / Et_2O (1:2.5), followed by recrystallization from EtOH / Et_2O (1:2) to give white crystals: mp 196—198°C; ^1H NMR (d_6-DMSO) δ 1.23—1.43 (m, 2 H, CH_2), 1.51—1.68 (m, 2 H, CH_2), 1.68—1.85 (m, 2 H, CH_2), (m ,6 H, 3 NCH_2), 3.40—3.59 (m, 2 H, NCH_2), 3.59—3.80 (m, 2 H, NCH_2), 3.98—4.33 (m, 2 H, $CONCH_2$), 6.94—7.78 (m, 12 H, aromatic H), 10.72 (br s, 1 H, NH^+); ^{13}C NMR (d_6-DMSO) δ 22.50 (CH_2), 23.70 (CH_2), 26.73 (CH_2), 45.95 (2 CH_2), 50.38 (2 CH_2), 54.95 (CH_2), 115.30, 115.48, 117.69, 117.75, 119.69, 121.36, 123.96, 125.46, 126.19, 126.74, 131.51, 133.66 (12 CH), 126.41, 133.72, 146.31, 154.28, 160.06 (5 quarternary C), 156.47 (d, J = 236.5 Hz, C—F), 165.10 (CO). Anal. calcd for $C_{28}H_{31}ClFN_3O_2 \cdot 7/4H_2O$: C, 63.75; H, 6.59; N, 7.97. Found: C, 63.27; H, 6.02; N, 7.91.

10-[6-[4-(4-Fluorophenyl)-1-piperazinyl]hexyl]dibenz[*b*,*f*]-1,4-oxazepin-11(10*H*)-one (18). Treatment of the crude product **18** with MeOH and recrystallization from EtOH / MeOH (1:1) gave off-white crystals (1.3 g, 59%): mp 124—126°C; IR (KBr) 1640 (C=O) cm^{-1}. The HCl salt was obtained by treatment of **18** with 10% HCl-Et_2O and recrystallization from EtOH / Et_2O (3:2): mp 211—214°C; IR (KBr) 2600—2000 (br, NH^+) and 1645 (C=O) cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.33—1.56 (m, 4 H, 2 CH_2), 1.62—1.81 (m, 2 H, CH_2), 1.81—1.99 (m, 2 H, CH_2), 3.01—3.21 (m, 2 H, NCH_2), 3.55—3.70 (m, 4 H, 2 NCH_2), 3.94—4.37 (m, 4 H, 2 NCH_2), 4.69—4.91 (m, 2 H, $CONCH_2$), 7.11—8.07 (m, 12 H, aromatic H), 12.54 (br s, 1 H, NH^+); ^{13}C NMR ($CDCl_3$) δ 23.45 (CH_2), 25.51 (CH_2), 25.83 (CH_2), 27.14 (CH_2), 47.73 (CH_2), 48.74 (2 CH_2), 51.21 (2 CH_2), 57.22 (CH_2), 117.83, 118.01, 119.75, 121.76, 123.55, 123.65, 123.72, 125.39, 125.98, 126.73, 132.01, 133.41 (12 CH), 126.81, 134.43,

136.94, 155.06, 160.73 (5 quarternary C), 163.10 (d, J = 252.8 Hz, C—F), 166.45 (CO). Anal. calcd for $C_{29}H_{33}ClFN_3O_2 \cdot 2H_2O$: C, 63.78; H, 6.46; N, 7.69. Found: C, 64.05; H, 6.31; N, 7.73.

3. 3 Characterization with Spectroscopic and Chromatographic Methods

3. 3. 1 Spectroscopy

Thin-layer chromatography (TLC, silica gel 60 GF_{254}, 0.2 mm, Merck, Darmstadt) was used to monitor reactions and check product homogeneity. Optical rotatory measurements were made on a Perkin-Elmer 141 polarimeter (at NRC). 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 ^1H and 75.5 MHz for ^{13}C, were recorded on a Bruker MSL 300 spectrometer. 500 MHz (Bruker AMX 500) for ^1H and 125 MHz for ^{13}C, were also used when the Bruker MSL 300 spectrometer was not available (at NRC). Ion-spray LC-MS / MS spectra were recorded on a SCIEX API-III triple quadrupole mass spectrometer (at NRC). Ammonia chemical ionization mass spectra were acquired on a VG Analytical 20-250 quadrupole mass spectrometer. HPLC was performed on a HP1090 HPLC (at NRC) with a built-in diode array detector (DAD) and Chemstation. X-Ray diffraction data were obtained from a Rigaku AFC5R diffractometer at Laboratory of Crystallography, Department of Chemistry.

All the UV spectra of DTZ metabolites were obtained on an UV-diode array detector (UV-DAD) from qualitative analysis with HPLC. Samples of 1—3 μg were usually injected. The UV spectrum was recorded in the range from 210 to 400 nm. All DTZ

metabolites showed 237nm as an absorption maximum. IR spectroscopy was used for identification of all intermediates and target products.

About 10 mg of sample in an appropriate deuterated solvent were used for recording both ^{1}H and ^{13}C NMR spectra. For ^{1}H spectrum, normally 6 μs was used as a pulse length (90° pulse is 12 μs), and a pulse delay of 6—10 sec was utilized between each pulse. A Bruker Aspect 3000 computer was used to manipulate data. Normally 16—256 transients were acquired for each sample and 16K data points in the Free Induction Decay (FID) were normally collected. Digital resolution of 0.3 Hz was usually achieved.

For ^{13}C spectrum, the pulse length was 3 μs (6 μs is 90° pulse). A pulse delay of 1 sec was used. 16000—40000 transients were acquired for each sample and 32K data points in the FID were collected. Digital resolution of 2 Hz was achieved.

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=10,000 for **4**, **5** and **6**; 6,000 for **9**—**13**, **23**, **24** and **17**) were measured on a VG Analytical ZAB-EQ mass spectrometer with glycerol matrix. Ion-spray LC-MS / MS spectra were recorded on a SCIEX API-III triple quadrupole mass spectrometer. Mass spectra of only target products or key intermediates were recorded and each is reported after the synthesis of the compound.

3. 3. 2 Chromatography

HPLC was performed on HP1090 HPLC with a built-in diode array detector (DAD) and Chemstation fitted with a Zorbax Rx-C8 column (250 x 4.6 mm I.D., 5 μm). The chromatogram was developed at a flow rate of 1.0 mL/min with isocratic mobile phase

of CH_3CN / H_2O (30:70) with 0.1% TFA, pH 2. The column temperature was maintained at 40°C. The absorbance of the HPLC eluate was monitored at 237 nm.

Normal phase TLC for monitoring reactions and checking the purity of the products was normally performed at *ca.* 20—25°C on silica gel 60 F_{254} (Merck) with an appropriate solvent system. Absorbance of each component was visualized at 254 nm. The R_f value of each compound is reported after the synthesis. Each diltiazem metabolite showed only one spot on TLC.

Dry-column flash chromatography was used for purification of some less easily purified intermediates or products. The separations were carried out on TLC-grade silica gel (Merck Silica gel 60, 2—25μm). The silica was packed into a standard P3 sintered glass filter funnel and the eluting solvent was drawn through by suction using a water pump. The elution was carried out by adding the solvent in pre-measured portions, allowing the column to run 'dry' after each one before the next was added. For packing the column, the funnel was filled with dry TLC-grade silica up to the lip and tapped gently to settle the powder and remove any voids. The suction was gently applied at first and increased gradually to full water pump vacuum. This produced a head space for addition of the mixture and the solvent. For the selection of initial solvent, preliminary TLC experiments were carried out to find a solvent mixture that will give an R_f of *ca.* 0.1—0.2 for the fastest running component of interest. The initial solvent was used to pre-elute the packed column. The solvent front could be seen to descend in a horizontal line. After loading the sample mixture (in the initial solvent), a gradient of increasing solvent strength was used. A few portions of initial solvent were used and then in each successive portion the proportion of the stronger solvent was increased by *ca.* 2—5%, depending on how difficult each component in the mixture was to separate.

3. 3. 3 Polarimetry

Optical rotatory measurements were performed on a Perkin Elmer 141 polarimeter at 21°C with path length of 1.0000 ± 0.0002 decimeter at 589.3 nm. The instrument was calibrated with sucrose $\{[\alpha]^{21}_D = +66.5°\}$ before measurement of samples.

3. 3. 4 X-Ray Structure Determination of 13, 14 and 18

10-[6-[4-(4-Fluorophenyl)-1-piperazinyl]hexyl]dibenzo[*b,f*]-1,4-thiazepin-11(10*H*)-one (13). $C_{29}H_{32}FN_3OS$, M_r = 489.65, triclinic, P1, *a* = 10.67 (1) Å, *b* = 9.16 (2) Å, *c* = 14.06 (3) Å, α = 88.8 (2)°, β = 105.0 (1)°, γ = 89.8 (2)°, V = 1327 (4) Å^3, Z = 2, D_x = 1.225 g / cm^3, λ (Cu K_α) = 0.71069 Å, μ = 1.470 cm^{-1}, F (000) = 520, T = 291 ± 1 K, final R = 0.068 for 3705 observed reflections.

Dibenzo[*b,f*]-1,4-thiazepin-11(10*H*)-one (14). $C_{13}H_9NOS$, M_r = 227.28, monoclinic, $P2_1$, *a* = 7.145 (3) Å, *b* = 12.298 (4) Å, *c* = 11.853 (4) Å, β = 95.31 (3)°, V = 1037.1 Å^3, Z = 4, D_x = 1.456 g / cm^3, λ (Cu K_α) = 0.71069 Å, μ = 2.72 cm^{-1}, F (000) = 472, T = 291 ± 1 K, final R = 0.037 for 881 observed reflections.

10-[6-[4-(4-Fluorophenyl)-1-piperazinyl]hexyl]dibenz[*b,f*]-1,4-oxazepin-11(10*H*)-one (18). $C_{29}H_{32}N_3O_2$, M_r = 473.59, monolinic, $P2_1$, *a* = 9.074 (2) Å, *b* = 19.327 (7) Å, *c* = 14.302 (2) Å, β = 92.32 (2)°, V = 2506 (1) Å^3, Z = 4, D_x = 1.225 g / cm^3, λ (Cu K_α) = 0.71069 Å, μ = 0.79 cm^{-1}, F (000) = 1008, T = 291 ± 1 K, final R = 0.062 for 4565 observed reflections.

3. 4 Identity of Synthetic DTZ Metabolites with Biological Samples

The following work was done by Xie *et al.*[145]. Urine samples were collected from healthy human volunteers, dogs, rabbits and rats over a 24 h period after they were given a single oral dose of diltiazem. The dose for each species was: humans 90 mg tablet; dogs 30 mg tablet; rabbits 5 mg / kg solution; and rats 15 mg / kg solution. Details of the method used to isolate diltiazem and its basic metabolites from urine have been reported.[147] 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., NY, U.S.A) and the dry residue stored at -20°C until analysis. On the day of analysis, the residue was dissolved in MeOH and aliquots injected into the HPLC.

3. 5 Biological Evaluation

3. 5. 1 *In Vitro* Vasorelaxant Activity

Young hamsters (72—85 days old and 104—136 g, Golden Syrian) for evaluation of vasorelaxation of DTZ metabolites and hamsters (30—200 days old and weighing 75—164 g, Golden Syrian) for DBTs were purchased from Canadian Hybrid Farms (Halls Harbour, N.S.). Vasorelaxation experiments were performed in the Department of Pharmacology using a Grass Model 7D polygraph (Grass Instrument Co. Quincy, Mass., USA) and organ bath apparatus. DTZ metabolites and DBTs were tested in the form of HCl, HBr, TFA, oxalate or maleate salts.

3. 5. 1. 1 Vasorelaxant Activity of DTZ metabolites on Hamster Aorta

Isolated aorta ring preparations (about 3 mm ring) 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 through 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 non-linear regression program (GraphPAD INPLOT^{TM} version 3.0).

3. 5. 1. 2 Vasorelaxant Activity of DBTs on Hamster Aorta

The conditions for evaluation of vasorelaxant activity of DBTs on hamster aorta were the same as those for DTZ metabolites except for a bath temperature of 37°C for vascular smooth muscles. The stock solutions of DTZ (control) and DBTs (10^{-2} M) were prepared in 50% EtOH. The solutions of **9**—**13** and **15**—**18** were diluted to a concentration of 10^{-3} M with 30% EtOH and the rest with distilled water. A series dilution to 10^{-6} M with distilled water for vasorelaxaton tests.

3. 5. 2 *In Vitro* Adenosine Uptake Experiment

Dibasic sodium phosphate buffer (pH 9.1, 100 mL) prepared from Na_2HPO_4 (1.78 g) in distilled H_2O (100 mL) was titrated with the aid of pH meter to pH 7.4 with monobasic potassium phosphate buffer (pH 4.4, *ca.* 9 mL) prepared from KH_2PO_4 (2.18 g) in distilled H_2O (100 mL), to make an isotonic phosphate buffer solution (PBS).

Separate solutions of *erythro*-9 (2-hydroxy-3-nonyl)adenine (EHNA) HCl (10.0 mg) in distilled H_2O (10 mL) and dipyridamole (10.0 mg) in MeOH (10 mL) were prepared. Ethylenediaminetetraacetic acid (EDTA) disodium salt (134.5 mg) was placed in a 100-mL volumetric flask. To this were added aliquots of the EHNA solution (860 μL) and the dipyridamole solution (5.000 mL). Normal saline (0.9%) was added to make 100 mL of "stopping solution" with the final concentrations of EHNA (26 μM), dipyridamole (99 μM) and EDTA (4 mM).

Adenosine (10.0 mg) was dissolved in distilled H_2O (10 mL), and 160 μL of this was transferred to a 100-mL volumetric flask. Normal saline (*ca.* 50 mL) was added. To this was added 11 μL of a solution of [2-^{3}H]adenosine (Amersham, 27 Ci / μmole, 1 mCi /

mL), and the volume brought to 100 mL with saline to give "working tracer solution" with the final concentration of adenosine (6 μM). When added to 10 mL of scintillation fluid, 100 μL of this "working tracer solution" gave *ca.* 10,000 cpm.

Fresh whole blood obtained from healthy volunteers was anticoagulated with PBS-heparin (9 mL of blood + 1 mL of PBS containing 100 I. U. of sodium heparin). PBS (0.05 mL) containing various concentrations (0.01 μM—400 μM) of DTZ and its metabolites or PBS-EtOH (2:1, 0.05 mL) containing various concentrations (0.01 μM—50 μM) of DBTs was added to 200 μL of anticoagulated whole blood. The mixture was vortexed and allowed to stand at room temperature (25°C) for 15 min. To it was added 100 μL of "working tracer solution". Uptake of adenosine was terminated after 2 s of incubation by rapid addition of 0.5 mL of the "stopping solution". The mixture was vortexed, and centrifuged at 4°C for 10 min (1760 x g). An aliquot of the supernatant (0.2 mL) was mixed with 10 mL of scintillation fluid and the radioactivity was determined in a liquid scintallation counter (LS 5000 TA, Beckman Instrument, U.S.A.). Dipyridamole and diltiazem were used as control for every experiment.

3. 5. 3 Anti-HIV Activity of Dibenzothiazepinone Derivatives

Anti-HIV activities of dibenzothiazepinones were evaluated at National Cancer Institute, National Institutes of Health, Bethesda, MD. USA. Briefly, the dibenzothiazepinone was dissolved in dimethyl sulfoxide (DMSO), then diluted 1:100 in cell culture medium before preparing serial half-$\log_{10}$ dilutions. T4 lymphocytes (CEM cell line) were added and after a brief interval HIV-1 was added, resulting in a 1:200 final dilution of the compound. Uninfected cells with the compound served as a toxicity control, and infected and uninfected cells without the compound served as basic controls. Cultures

were incubated at 37°C in a 5% carbon dioxide atmosphere for 6 days. The tetrazolium salt, XTT, was added to all wells, and cultures were incubated to allow formazan color development by viable cells. Individual wells were analyzed spectrophotometrically to quantitate formazan production and, in addition, were viewed microscopically for detection of viable cells and confirmation of protective activity. Drug-treated virus-infected cells were compared with drug-treated noninfected cells and with other appropriate controls (untreated infected and untreated noninfected cells, drug-containing wells without cells, *etc.*) on the same plate. Data were reviewed in comparison with other tests done at the same time and a determination about activity was made.

CONCLUSION

Eight diltiazem metabolites have been successfully synthesized, three of which were synthesized for the first time, some requiring 12 sequential chemical reactions. All eight synthetic DTZ metabolites were fully characterized. That three new DTZ metabolites (**4**, **5** and **6**) isolated from human urine are identical to the corresponding authentic compounds I synthesized has been confirmed by spectroscopic and chromatographic methods. *In vitro* calcium antagonistic activities of all metabolites (except **6**) were evaluated on hamster aorta with the rank of order of potency: DTZ > **1** ≥ **2** > **7** > **5** ≥ **3** > **4** ≥ **8**. The contribution of DTZ metabolites to therapeutic and adverse effects of DTZ was proposed. The relationships between structure of DTZ metabolites and calcium antagonistic activity were also discussed. Effects of DTZ metabolites **4** and **5** on adenosine uptake by erythrocytes were also studied.

A number of dibenzothiazepinones and dibenzoxazepinones have been designed with the assistance of molecular modeling, using other dibenzotricyclics as leads. X-Ray and molecular mechanics calculations show that the DBTs in this study have smaller angles between the planes of the two benzene rings than the DBTs previously reported. As calcium antagonists, compounds **10**—**13** and **15**—**18** synthesized are slightly less potent than diltiazem, but are of the same order of magnitude of potency at submicromolar concentration. Dibenzotricyclic compounds (**9**—**13**, **15**—**24**) show no activity on inhibition of adenosine uptake by erythrocytes. Some dibenzothiazepinone derivatives (**14**, **25** and **26**) also have moderate anti-HIV activity.

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