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LACTAMIMIDES: A NOVEL CHEMICAL CLASS OF CALCIUM ANTAGONISTS WITH DILTIAZEM-LIKE PROPERTIES

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Abstract—The effects of a series of lactamimides on [^{3}H]*d-cis*-diltiazem binding to rat brain membranes, on [^{3}H]nitrendipine binding to cardiac membranes, and on calcium-induced contractions in depolarized guinea pig taenia and ileum preparations were examined. Several of the lactamimides examined displaced [^{3}H]*d-cis*-diltiazem binding and antagonized, in a competitive fashion, calcium-induced contractions. Over the series of lactamimides, there was a highly significant, positive linear correlation ($r = 0.87$, $P < 0.001$) between their potency to displace [^{3}H]*d-cis*-diltiazem and their potency to antagonize calcium-induced contractions in the depolarized taenia and ileum preparations. Of the lactamimides examined, MDL 16,582A [*N*-(2,2-diphenylpentyl)azacyclotridecan-2-imine · hydrochloride] had potency equivalent to *d-cis*-diltiazem with pA_2 values of 7.27 and 7.38, respectively, against calcium-induced contractions in the guinea pig ileum. These lactamimides are a novel chemical class displaying diltiazem-like calcium antagonist properties.

It has been shown that calcium antagonists of diverse chemical structures interact in an allosteric manner with the calcium ion-channel protein [1]. The chemical classes are exemplified by verapamil (a phenylalkylamine derivative), diltiazem (a benzothiazepine derivative) and the dihydropyridines, e.g. nitrendipine [2]. In binding experiments using [^{3}H]nitrendipine as the ligand, it has been shown that diltiazem enhances binding whereas verapamil displaces [^{3}H]nitrendipine in a non-competitive manner [3–9].

We have been examining the pharmacological effects of a series of lactamimides (Table 1), and one compound, MDL 12,330A [*N*-(*cis*-2-phenylcyclopentyl)azacyclotridecan-2-imine · HCl], has been studied in some detail. This compound was originally reported to inhibit stimulated adenylate cyclase activity in a number of tissues [10–13]. In addition, the compound has negative inotropic and chronotropic effects on isolated guinea pig hearts, and these effects are reversed by the administration of calcium [14]. In 1985, Lee *et al.* [15] found that MDL 12,330A enhances [^{3}H]nitrendipine binding in rat cerebral cortical and cardiac homogenates in a manner similar to diltiazem, and Rampe *et al.* [16] confirmed the finding. In addition, using patch clamp techniques, they showed that MDL 12,330A selectively reduces calcium currents with a potency similar to that of diltiazem [16].

From our studies, it became clear that certain lactamimide analogs belong to a novel chemical class displaying diltiazem-like calcium antagonism. In this report, we discuss the criteria used to establish diltiazem-like calcium antagonistic properties and describe the structural features of the lactamimide molecule that imparts potency to this class of compounds.

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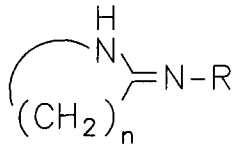
METHODS

[^{3}H]d-cis-*Diltiazem binding assays.* The whole brain from male Sprague–Dawley rats (200–300 g) was dissected and homogenized in cold 50 mM Tris–HCl buffer (pH 7.4) for two 30-sec periods by Polytron (Brinkmann, setting No. 5). The homogenate was washed two times (48,000 *g* for 10 min, 4°) with intermittent resuspension of the pellet in fresh buffer. The final pellet was resuspended to an original tissue concentration of 100 mg/ml in Tris–HCl buffer. The binding assay of [^{3}H]*d-cis*-diltiazem was performed as previously described [17, 18]. Briefly, a 100-μl aliquot of the homogenate was incubated with 2.5 nM [^{3}H]*d-cis*-diltiazem in a total volume of 1 ml in 50 mM Tris–HCl buffer (pH 7.4) for 60 min at 25° or at 37°. The reaction was terminated by rapid filtration under a vacuum through 0.05% polyethylenimine-pretreated Whatman GF/B glass fiber filters. Filters were immediately washed two times with 4 ml of cold Tris–HCl buffer. Pretreatment of the filters with 0.05% polyethylenimine served to eliminate [^{3}H]*d-cis*-diltiazem binding to the filter. The filters were dried, and the radioactivity in the filters was extracted overnight in scintillation fluid. Samples were counted by liquid scintillation spectrophotometry with an efficiency of 43%. Specific [^{3}H]*d-cis*-diltiazem binding was defined as the difference in binding determined in the absence and presence of 30 μM *d-cis*-diltiazem, and it represented 50–55% of

Table 1. A representative group of lactamimides selected for testing as calcium antagonists



	MDL No.*	n	R
1.	16,582A	11	2,2-diphenylpentyl
2.	12,011A	11	*O*-biphenyl
3.	12,330A	11	*cis*-2-phenylcyclopentyl
4.	13,858A	11	2-norbornyl
5.	13,254V	6	2,2-diphenylpentyl
6.	12,366A	5	2,2-diphenylpentyl
7.	13,172A	4	2,2-diphenylpentyl
8.	13,799A	6	2-(1-adamantanyl)benzyl
9.	13,116A	5	α-(*p*-butoxyphenyl)benzyl
10.	11,887A	5	*p*-chloro-α-(*p*-chlorophenyl)benzyl
11.	11,842A	5	*m*-trifluoro-α-(phenyl)benzyl
12.	11,894A	5	*cis*-2-cyclohexylcyclopentyl
13.	12,057A	5	2-adamantanyl
14.	12,334A	7	*cis*-2-phenylcyclopentyl
15.	11,615A	5	*cis*-2-phenylcyclopentyl
16.	15,123A	3	*cis*-2-phenylcyclopentyl

* A = HCl; V = fumarate.

Table 2. Calcium antagonists and [^{3}H]diltiazem displacing properties of the lactamimides

	MDL No.	pA_2*		pK_i† [^{3}H]Diltiazem binding
		Ileum	Taenia	
1.	16,582A	7.27 ± 0.19‡		6.49
2.	12,011A	6.59 ± 0.16‡		6.51
3.	12,330A	6.66 ± 0.21‡	6.10§	6.41
4.	13,858A	6.07 ± 0.02		5.92
5.	13,254V	6.63 ± 0.11		
6.	12,366A	6.31 ± 0.10	6.64§	
7.	13,172A	6.04 ± 0.08	6.15§	
8.	13,799A		6.63 ± 0.14	6.72
9.	13,116A		5.86 ± 0.14	6.17
10.	11,887A		6.33 ± 0.39	6.08
11.	11,842A		5.60 ± 0.15	5.92
12.	11,894A		5.54 ± 0.26	5.54
13.	12,057A		5.47 ± 0.31	4.07
14.	12,334A	4.84 ± 0.07		5.26
15.	11,615A			4.16
16.	15,123A			4.96
	Diltiazem			
17.	*d-cis*	7.38 ± 0.13‡	7.42 ± 0.10	7.42
18.	*l-cis*			5.39

* pA_2 values were calculated by the method of Arunlakshana and Schild [19] or by the equation, log (dose ratio −1) = log [B] − log K_B, where B and K_B are concentration and dissociation constant of the antagonist, respectively [21]. Values are means ± SE (N = 3–6) unless indicated otherwise.

† pK_i values were calculated by the method of Cheng and Prusoff [22] using displacement of [^{3}H]diltiazem from rat brain membranes at 25°. Values are means of triplicate determinations on the same membrane preparation.

‡ Standard errors were calculated by an approximation of the method described by Draper and Smith [23], in which the terms g and t were dropped to yield the equation: $SE = s/b_1[(pA_2 - X)^2/S_{xx}]^{1/2}$, where s = estimate of standard deviation, b_1 = slope of the regression line, X = mean of log [dose], and S_{xx} = corrected sum of square of log [dose].

§ N = 1.

the total amount of filter-retained radioactivity.

[^{3}H]*Nitrendipine binding assay*. Membranes were prepared from the hearts of male Sprague–Dawley rats, and [^{3}H]nitrendipine binding was conducted as described in detail by Lee *et al.* [15] as modified from Ehlert *et al.* [5].

K^+-*depolarized taenia and small intestine*. Strips of taenia from the caecum or pieces of ileum, 2 cm in length, from male guinea pigs (200–400 g) were set up in isolated organs baths in Ca^{2+}-free K^+ Tyrode's solution (NaCl, 137 mM; KCl, 40 mM, NaH_2PO_4, 0.4 mM; $NaHCO_3$, 11.9 mM; glucose, 5.5 mM) and gassed with 95% O_2, 5% CO_2 at 37°. Contractions were measured with isotonic transducers with a 1 g load. Cumulative concentration–response curves were obtained. Tissues were then washed and incubated with the test compounds for 20–25 min, and then Ca^{2+} concentration–response curves were re-established. The preparations were shown to be stable for at least 5 hr and to give reproducible concentration–response curves to Ca^{2+}. Dose ratios were determined graphically at ED_{50} values from the Ca^{2+} dose–response curve, and Schild plots [19] were constructed to determine the pA_2 value using inverse regression line analysis [20].

In experiments involving the determination of pA_2 values, test compounds were prepared in ethanol as a 1 mM solution, which was diluted to the desired concentration with ethanol before use. The amount of vehicle used (0.1%, v/v) in these experiments produced negligible effects.

Materials. [^{3}H]*d-cis*-Diltiazem (80.7 Ci/mmol) and [^{3}H]nitrendipine (85.9 Ci/mmol) were obtained from Dupont NEN. *d*- and *l-cis*-Diltiazem were gifts from Marion Laboratories, Kansas City, MO, and the lactamimides (MDL compounds) were synthesized by George Claxton and Martin Grisar at the Merrell Dow Research Institute, Cincinnati, OH.

RESULTS

[^{3}H]d-cis-*Diltiazem binding to cerebral cortical membranes*. The binding of [^{3}H]*d-cis*-diltiazem was displaced in a stereoselective fashion by *d*- and *l-cis*-diltiazem, as shown in Fig. 1A. *l-cis*-Diltiazem was fifty times less active than the *d*-isomer. The maximum inhibition of the total [^{3}H]*d-cis*-diltiazem binding in rat membranes by *d-cis*-diltiazem was 53% at 30 μM (the concentration used to define non-specific binding of [^{3}H]*d-cis*-diltiazem) (Fig. 1A). The displacement of [^{3}H]*d-cis*-diltiazem by a representative group of lactamimides is shown in Fig. 1B. The maximum inhibition of total binding by this group was also approximately 50%, and strongly suggests that both diltiazem and the lactamimides bind to the same site on the membrane. However, even the most potent lactamimide, MDL 13,799A, was a log unit less potent than *d-cis*-diltiazem with a pK_i value of 6.72 compared to a value of 7.42 for diltiazem (Table 2). As shown in Table 3, the displacement of [^{3}H]*d-cis*-diltazem by unlabeled *d-cis*-diltiazem was temperature dependent. Increasing the temperature from 25° to 37° increased the IC_{50} value 2.55 times. Similarly, increasing the temperature of incubation for the lactamimide MDL 12,330A increased the IC_{50} 1.44 times (Table 3).

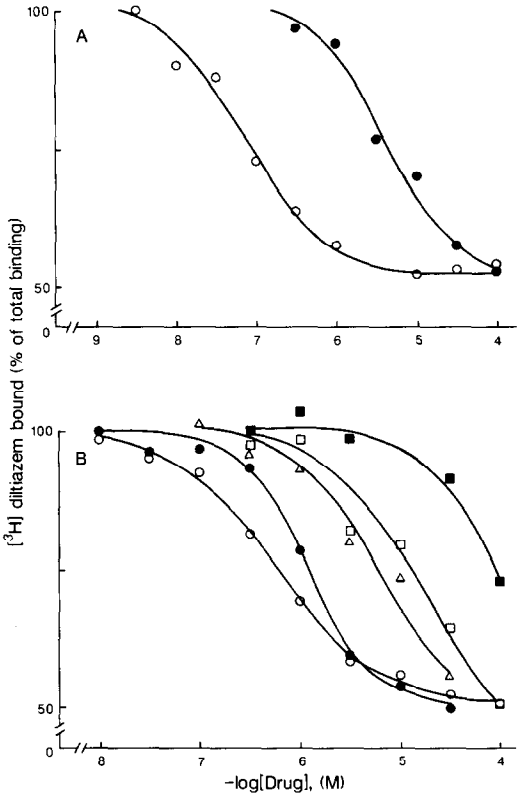


Fig. 1. Inhibition of [^{3}H]*d-cis*-diltiazem binding by *d*- and *l-cis*-diltiazem (A) and selected lactamimides (B). The inhibition of [^{3}H]*d-cis*-diltiazem binding was determined by incubating [^{3}H]*d-cis*-diltiazem (2.5 nM) at 25° with six to nine concentrations of the drugs [Panel A: (○) *d-cis*-diltiazem; (●) *l-cis*-diltiazem; Panel B: (○) MDL 12,330A; (●) MDL 13,858A, (△) MDL 12,334A; (□) MDL 15,123A; (■) MDL 11,615A]. All solutions were freshly made before use, and each point is the average of three separate experiments performed in duplicate.

Table 3. Temperature dependence of displacement of [^{3}H]*d-cis*-diltiazem by *d-cis*-diltiazem and lactamimides

Compound	IC_{50} (nM)	
	25°	37°
d-cis-Diltiazem	43 ± 7	110 ± 25
MDL 12,330A	280 ± 30	403 ± 77

The IC_{50} values were calculated from concentration–response curves at concentrations giving 50% inhibition of the specific [^{3}H]*d-cis*-diltiazem binding to rat whole brain membranes. Ligand concentration was 2.5 nM. Values are means ± SE, N = 3.

[^{3}H]*Nitrendipine binding to rat heart membranes*. Lee *et al.* [15] have shown that MDL 12,330A, like *d-cis*-diltiazem, is able to enhance the specific binding of [^{3}H]nitrendipine at 37° with EC_{50} values of 6.1×10^{-8} and 3.4×10^{-8} M in rat cerebral cortex and heart respectively. This enhancement by *d-cis*-diltiazem and MDL 12,330A is temperature depen-

Table 4. Enhancement of [^{3}H]nitrendipine binding to rat heart membranes by *d-cis*-diltiazem and lactamimides

Compound	[^{3}H]Nitrendipine binding (% of control)			
	25°		37°	
	10^{-7} M	10^{-6} M	10^{-7} M	10^{-6} M
MDL 16,582A	119 ± 6	123 ± 6	252 ± 13	224 ± 14
MDL 12,011A	117 ± 7	123 ± 10	152 ± 9	152 ± 15
MDL 12,330A	102 ± 1	112 ± 2	141 ± 8	165 ± 13
MDL 13,858A	105 ± 4	106 ± 4	108 ± 7	116 ± 8
d-cis-Diltiazem	96 ± 5	83 ± 8	218 ± 4	262 ± 10

Values are the means ± SE of single experiments run in triplicate at the concentrations of drugs indicated. Ligand concentration was 0.1 nM, and control specific binding was 49.6 ± 1.7 fmol/mg protein.

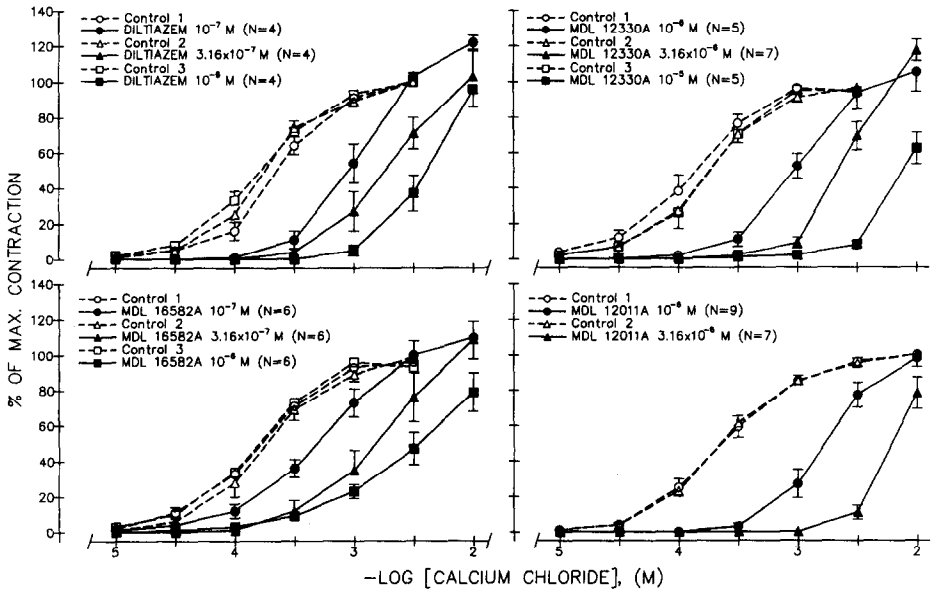


Fig. 2. Effects of diltiazem and the lactamimides MDL 12,330A, MDL 16,582A, and MDL 12,011A on Ca^{2+}-induced contractions of K^+-depolarized guinea pig ileum. Cumulative concentration–response curves to Ca^{2+} were obtained prior to, and after, preincubation with different concentrations of the antagonists. Vertical bars are ± SE.

dent and is due to a decrease in the dissociation rate constant of [^{3}H]nitrendipine for the dihydropyridine receptor/calcium channel protein complex. The results displayed in Table 4 show that the lactamimides related to MDL 12,330A also enhanced [^{3}H]nitrendipine binding to varying degrees. The enhancement was temperature dependent and correlated with their ability to displace [^{3}H]*d-cis*-diltiazem. MDL 16,582A produced an enhancement of [^{3}H]nitrendipine binding similiar to *d-cis*-diltiazem.

Calcium antagonism in guinea pig ileum and taenia. Figure 2 shows that the concentration effect–response curve for calcium was displaced to the right in a parallel fashion by increasing concentrations of diltiazem, MDL 16,582A, MDL 12,330A and MDL 12,011A in the guinea pig ileum. Schild plots, as shown in Fig. 3, gave pA_2 values of 6.59 ± 0.16, 6.66 ± 0.21, 7.27 ± 0.19 and 7.38 ± 0.13 for MDL 12,011A, MDL 12,330A, MDL 16,582A and diltiazem respectively. The slopes of the plots were close to unity, suggesting competitive antagonism (Fig. 3). Essentially similar results were obtained in the guinea pig taenia preparation for MDL 12,330A and diltiazem (pA_2 6.10 and 7.42, respectively) (Table 2). When the pK_i values against [^{3}H]*d-cis*-diltiazem binding for a number of lactamimides were plotted against their pA_2 value as calcium antagonists of Ca^{2+}-mediated contractions in the guinea pig

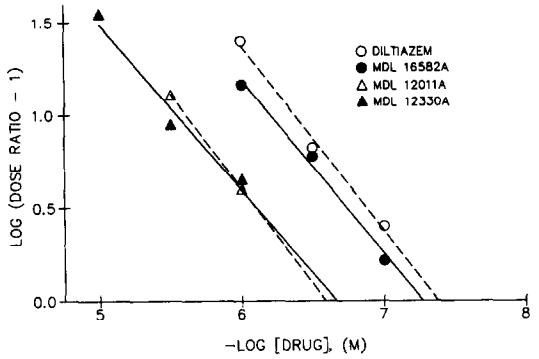


Fig. 3. Schild plot of the antagonistic effects of *d-cis*-diltiazem and lactamimides. Plots of log (dose ratio −1) against −log [M] gave straight lines which yielded mean pA_2 values and mean slopes (±SD) as follows: *d-cis*-diltiazem, 7.38 ± 0.13, 1.00 (0.15); MDL 16,582A, 7.27 ± 0.19, 0.94 (0.21); MDL 12,011A, 6.59 ± 0.16, 1.02 (0.20); MDL 12,330A, 6.66 ± 0.21, 0.89 (0.16). Data were obtained from calcium concentration–response curves obtained in 40 mM K^+-deploarized guinea pig ileum preparations.

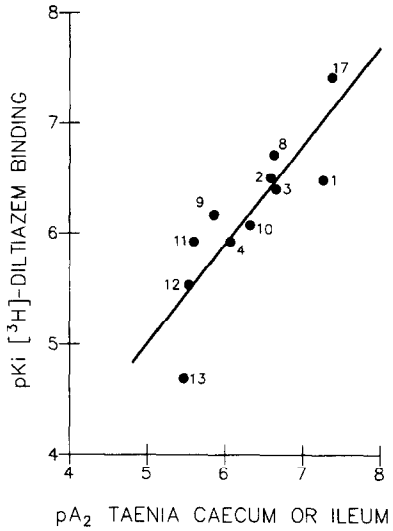


Fig. 4. Correlation between displacement of [^{3}H]*d-cis*-diltiazem and antagonism of Ca^{2+}-induced contractions. The negative logarithm of the inhibitory constant (pK_i, M) against [^{3}H]*d-cis*-diltiazem binding in rat brain membranes was derived from the IC_{50} value obtained by the method of Cheng and Prusoff [22] and is plotted against the pA_2 value (M) obtained by the methods described in the legend to Table 2 using antagonism of Ca^{2+}-induced contraction of the K^+-depolarized guinea pig taenia or ileum preparation. The correlation coefficient ($r = 0.87$, $p < 0.001$) and the slope (0.90) were calculated by linear regression analysis. The numbers correspond to compounds listed in Table 2.

taenia or ileum preparation, there was a straight-line relationship with a slope of 0.90 and a correlation coefficient of 0.87, $P < 0.001$ (Fig. 4).

DISCUSSION

There does not appear to be an structural similarity between the lactamimides and diltiazem, and yet there has been considerable evidence generated from patch clamp studies, the enhancement of [^{3}H]nitrendipine binding [15] and [^{3}H]PN 200–110 binding and the inhibition of $^{45}Ca^{2+}$ uptake [16] that the lactamimide, MDL 12,330A, acts as a calcium antagonist of the diltiazem type.

The displaceable [^{3}H]*d-cis*-diltiazem binding site probably represents the interaction of diltiazem with the calcium channel protein because, in accordance, with reported *in vitro* pharmacological potency [15, 24, 25], the *d*-isomer of *cis*-diltiazem (IC_{50} = 81 nM) was fifty times more potent than the *l*-isomer (IC_{50} = 4100 nM) at displacing the specific binding of [^{3}H]*d-cis*-diltiazem to rat brain membranes. Moreover, there was a close correlation between the potencies of a series of lactamimides to displace [^{3}H]*d-cis*-diltiazem and their potencies as calcium antagonists on the guinea pig taenia or ileum. Spedding [26] has argued cogently for the predictive value of antagonism of calcium-induced contractions in depolarized guinea pig taenia preparations as a means of assessing the relative potency of calcium antagonists of the different subgroups. It is clear from our data that the lactamimides and diltiazem are able to antagonize calcium-induced contractions in both the guinea pig taenia and the ileum preparations. Moreover, those compounds tested in both preparations (diltiazem, MDL 12,330A and MDL 12,366A) maintained equivalent potencies against calcium-induced contractions in these two preparations (Table 2). Therefore, it is reasonable to suggest that the guinea pig ileum preparation is also predictive of calcium antagonistic potency. From the data, it is clear that the lactamimide derivative, MDL 16,582A, is a calcium antagonist with almost equal potency to diltiazem (pA_2 of 7.27 and 7.38 for MDL 16,582A and diltiazem, respectively) on the K^+-depolarized guinea pig ileum.

Further evidence that the lactamimides are interacting with the calcium channel protein in a manner similar to diltiazem comes from the observation of an enhancement of [^{3}H]nitrendipine binding to heart membranes. Of the compounds tested, MDL 16,582A produced almost as great an enhancement of binding as diltiazem. Other 12 carbon-membered ring lactamimides, such as MDL 12,011A and MDL 12,330A, were also able to enhance [^{3}H]nitrendipine binding. However, it would appear that the enhancement of [^{3}H]nitrendipine binding was the least sensitive measure of calcium antagonistic potency. It appears that below a certain potency assessed by either [^{3}H]*d-cis*-diltiazem displacement or Ca^{2+} antagonism in the taenia or ileum preparation, no enhancement of [^{3}H]nitrendipine binding occurs.

The enhancement of [^{3}H]nitrendipine binding is temperature dependent [9, 27]. In contrast to the enhancement of [^{3}H]nitrendipine binding with increasing temperature, the displacement of [^{3}H]*d-cis*-diltiazem was enhanced at lower temperatures. These temperature phenomena were seen with both diltiazem and the lactamimides, further underscoring the similarity of these two chemical classes of calcium channel antagonists.

From the relative potencies of the lactamimides studied, several structure–activity features can be

concluded. It is clear that the ring size contributes an important element with the 12 carbon-membered ring compounds (MDL 12,011A; MDL 12,330A; MDL 13,858A; MDL 16,582A) being most potent at displacing [^{3}H]*d-cis*-diltiazem, enhancing [^{3}H]nitrendipine binding, and antagonizing Ca^{2+}-induced contractions. As the ring size was contracted (side chain kept constant), activity dropped. Comparing MDL 12,330A to MDL 12,334A, MDL 11,615A and MDL 15,123A, it was evident that rings with 8 or less carbons were relatively weak displacers of [^{3}H]*d-cis*-diltiazem. In the 12 carbon-membered ring series, the most effective side chain for enhanced activity was the 2,2-diphenylpentyl chain of MDL 16,582A. The only compound with a ring size less than 12 which had considerable potency as a Ca^{2+} antagonist was MDL 13,799A, which has only a 7 carbon-membered ring. However, the side chain, an α-(1-adamantanyl) benzyl group, is very large and hydrophobic. As a result, the large side chain may bind to the site where normally the 12 carbon-membered ring attaches itself, thus conferring potency to a compound with a smaller lactamimide ring.

In conclusion, it is clear that the lactamimides described here represent a new chemical class of calcium antagonists that have diltiazem-like properties in *in vitro* test systems. It remains to be seen if the different chemical nature of the lactamimides imparts additional properties to this class of calcium antagonists. In this regard, MDL 16,582A, which displays *in vitro* potency similar to diltiazem, may be worth considering as a suitable tool for such investigations.

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