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*Keith W. Crawford* June 30, 00

PI - Signature

Date

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## INTRODUCTION

During this phase of the project, I have focused on elucidating the mechanism of Sigma-2 receptor-mediated signal transduction in various breast and other tumor cell lines. I will outline the evidence which strongly implicate a role for sphingolipids in sigma-2 receptor-mediated signal transduction.

We hypothesize that sigma-2 receptors are expressed in high densities on tumor cells because of their involvement in cell-cycle regulation and proliferation. As we have already presented, chronic treatment of tumors with sigma-2 receptor agonists produces apoptosis, by a mechanism that we have shown to be independent of p53 tumor suppressor genotype and independent of known caspases (Crawford et al., 1999; Crawford and Bowen, 2000). The sphingolipid ceramide is known to be involved in pathways both leading to apoptosis and cell proliferation through the effects of Ceramide activated protein phosphatase (CAPP) and Ceramide activated Protein kinase (CAPK), respectively (**fig.1**). The work presented below represents the first evidence of the involvement of sphingolipids in the apoptotic signaling pathway of sigma-2 receptors in tumors.

## BODY

### **Ceramides, Ceramide analogues, and Inhibitors of Ceramide metabolism Mimic the cytotoxic effects of Sigma-2 agonists in Breast Tumor cell lines.**

Phenyl hexadecanoylamino morpholino propanol (PPMP), inhibits a glucosyl transferase that glycosylates ceramide for ganglioside synthesis, and hence, leads to increases in intracellular levels of ceramide. This compound produces cytotoxicity in breast tumor cells measured by LDH release into culture medium. When combined with Sigma-2 receptor agonists, PPMP produces additive toxicity by this assay (**fig. 2**), as opposed to the synergistic toxicity observed when sigma-2 receptor agonists are combined with anti-neoplastic agents working by different mechanisms.

If, according to our hypothesis, ceramide is involved in Sigma-2 receptor-induced apoptosis through the action of ceramide activated protein phosphatase (CAPP), then inhibitors of CAPP should abrogate sigma-2 mediated cytotoxicity. Okadaic acid, an inhibitor of Type 2 phosphatases including CAPP, abolished cytotoxicity from the sigma-2 receptor agonist CB-184 in MCF-7/Adr- breast tumors (mutant p53) but not MCF-7 cells (wild-type p53, **fig. 3**). The difference in response between the two cell lines may relate to p53 genotype since okadaic acid can produce cytotoxicity that is p53 dependent.

### **Sigma-2 receptor agonists Produce changes in intracellular Sphingolipids in Breast tumor and Neuroblastoma cell lines.**

To determine whether sphingolipids are involved in sigma-2 receptor signaling, MCF-7/Adr- and T47D breast cells were incubated in serum-free media in the presence of radiolabelled palmitic acid and/ or radiolabelled serine to label sphingolipids. Cells were treated with sigma-2 receptor ligands for 24 hr., lipids were extracted and analyzed by

thin-layer chromatography (TLC). Sphingolipids were identified using authentic standards visualized by iodine vapors (**fig. 4**) and the corresponding spots were scraped from the TLC plate and quantified by scintillation counting. The observed increases in ceramide and decreases in sphingomyelin (**fig.5**) suggested that Sigma-2 receptor either directly or indirectly modulated a sphingomyelinase (neutral, acidic, basic). These changes in sphingolipid levels could be partially abrogated by the sigma-2 receptor antagonist, AC-927 (**fig. 6**). These findings seemed to suggest that chronic sigma-2 receptor activation leads to apoptosis by increasing ceramide levels, presumably through sphingomyelin hydrolysis by a sphingomyelinase.

Atmospheric pressure chemical ionization liquid chromatography-mass spectrometry (APCI-LC/MS) is an extremely precise method for identifying and quantifying biochemicals. We have used this method to study changes in sphingolipid levels in cells treated with sigma-2 receptor agonists. A sample of authentic N-(palmitoyl)-sphingosine (C<sub>16</sub>-ceramide) was subjected to APCI-LC/MS. The peak at 520.5 m/z results from the removal of H<sub>2</sub>O from the sphingosine backbone of ceramide (**fig. 7**). In mixtures of sphingolipids, a fragment at m/z 520.5 may result from cleavage of other C<sub>16</sub>-ceramide derivatives such as sphingomyelin or gangliosides. Cells were treated in the absence or presence of sigma-2 agonist, extracted for total lipids and subjected to APCI-LC/MS. Two major peaks were found, peak 2 at RT=15.37 min, corresponded to authentic C<sub>16</sub>-ceramide; peak 1 at RT=13.67 min was not identified, but is consistent with a C<sub>16</sub>-sphingolipid, possibly C<sub>16</sub>-sphingomyelin or a ganglioside. The relative amount of ceramide (peak 2) was increased upon incubation with CB-184 in MCF-7/Adr- cells (**fig. 8**). When tumor cells are treated with sigma-2 receptor agonists and analyzed by APCI-LC/MS, the ratio of ceramide (area under peak 2) to other sphingolipids (area under peak 1) is increased (MCF-7/Adr- breast tumor cells, **fig. 9**) in a dose-dependent fashion (SK-N-SH neuroblastoma cells, **fig. 10**). Thus, our initial observations with TLC were confirmed by the more sensitive APCI-LC/MS.

We wanted to next determine what effect sigma-2 receptors may have of sphingomyelinase activity. Treatment of intact MCF-7 cells with the sigma-2 receptor agonist, CB-184 lead to a time-dependent decrease in sphingomyelinase activity (**fig. 11**). To determine if sigma-2 receptor might directly modulate sphingomyelinase activity, sigma-2 receptor agonists were examined in a detergent extract assay of sphingomyelinase activity. Modest concentrations of sigma-2 receptor agonists (1  $\mu$ M) were sometimes observed to increase or produce no change in sphingomyelinase activity. But frequently, decreased sphingomyelinase activity was observed (**figs. 12**), particularly at higher doses (100  $\mu$ M) of sigma-2 receptor agonists. These observations lead us to question whether the source of ceramide elevation was through activation of sphingomyelin hydrolysis. We had previously observed that fumonisin B1, an inhibitor of ceramide synthase, had no effect on sigma-2 receptor mediated cytotoxicity, implying that *de novo* ceramide synthesis was also not the source of ceramide in these tumor cells (**fig. 13**).

The enzyme sphingolipid ceramide N-deacylase (SCDase) can hydrolyze an acyl group from sphingomyelin (deacylase function). This enzyme also possesses an acylase function that can produce ceramide from sphingosine. Sigma-2 receptor modulation of this enzyme could account for decreases in sphingomyelin (producing

sphingosylphosphorylcholine) and also increases in ceramide . Thus, the decreases in sphingomyelinase activity oftentimes induced by sigma-2-receptor agonists in detergent extracts may result from a competition between sphingomyelinase and SCDase for substrate ( $[^3\text{H}]$ sphingomyelin).

A detergent extract of T47D cells was incubated with radiolabelled sphingomyelin, in the absence or presence of sigma-2 receptor agonists. Total lipids were extracted from the detergent preparation and analyzed by TLC to separate sphingolipids. In the presence of Sigma-2 receptor agonists (CB-184, BD-737), levels of sphingosylphosphorylcholine were increased 2-fold over control, and sphingomyelin levels were concomitantly reduced (reduction in sphingomyelin observed for CB-184, but not BD-737 treatment in a preliminary experiment, **fig. 14**). Similar findings were observed in an experiment using SKBr3 breast tumor cells. I am currently in the process of optimizing an assay to measure the acylation reaction and production of ceramide by SCDase in a detergent tumor cell extract.

## **Methods**

### **Cell Culture**

Human breast tumor cell lines (MCF-7, MCF-7/Adr-, T47D) and neuroblastoma (SK-N-SH) cells were cultured in DMEM containing 3.7 g/liter  $\text{Na}_2\text{CO}_3$ , fetal bovine serum (10%), insulin (10 mg/liter). For cytotoxicity assays, cells were transferred to DMEM + Ham's nutrient mixture F-12 (without phenol red) with 1.2 g/liter  $\text{Na}_2\text{HCO}_3$ . Cells were seeded 100,000 cells/well for cytotoxicity assays and 500,000-1,000,000 cells /well for sphingolipid measurements.

### **Cytotoxicity assay**

Cell death was assessed by release of lactate dehydrogenase (LDH) into the culture medium using the Cytotox 96 kit from Promega (Madison, WI). Method was as specified by manufacturer, with minor modifications. Cells were plated and cultured in 24-well plates for 1 to 2 days prior to experiment. Cells were treated as indicated. Supernatant (50  $\mu\text{l}$ ) from the treatment wells was transferred to a 96 well microtitre plate, assay substrate mix was added, followed by stop solution after a 30 min incubation. Formation of formazan was monitored 490 nm in a plate reader (Ceres 900). Values are expressed relative to lysis controls (Triton X-100 used to determine value for 100% cell kill) and drug-free controls.

### **Labeling cell membrane sphingomyelin**

MCF-7/Adr- cells and T47D cells were grown to a density of ~1,000,000 cells /well in DMEM with 10% FBS. After 24 h., cells were incubated for 48hrs. with [ $^3\text{H}$ ]palmitic acid (20  $\mu\text{Ci/ml}$ ; S.A. = 30-60 Ci/mmol) and [ $^{14}\text{C}$ ]serine (2  $\mu\text{Ci/ml}$ , S.A. = 50-60 mCi/mmol) in serum-free media to label sphingomyelin.

### **Lipid extraction**

Cells were treated with or without drugs for 24 hours in serum-free media. Media was removed and the cells washed with ice cold PBS. Cells were scraped from wells and extracted in chloroform:methanol: 0.1 N HCl (4:2:0.5). The organic layer was removed dried under a stream of  $\text{N}_2$  and the residue resuspended in 30  $\mu\text{l}$  of chloroform:methanol, 50:50.

### **Thin-layer chromatography**

Radiolabelled lipids resuspended from an extraction, or standards were spotted onto Silica G-60 (Whatman<sup>R</sup>) plates and run along with unlabelled standards. Lipids were chromatographed in a mobile phase consisting of chloroform:methanol: $\text{H}_2\text{O}$ :25% $\text{NH}_4\text{OH}$  (50:50:2:1) run to 70% of the plate length. The plate was removed from the chromatograph chamber, dried and then run in a mobile phase of chloroform:methanol: $\text{H}_2\text{O}$  :25%  $\text{NH}_4\text{OH}$  (90:10:0.5:0.5) for the entire length of the plate. The spots for the standards were visualized by placing the dried plate in a chamber with iodine vapors.

### LC/MS Analysis of Ceramide in Samples.

The levels of ceramide and ceramide derivatives were determined using atmospheric pressure chemical ionization liquid chromatography/mass spectrometry (APCI-LC/MS). Samples were chromatographed using a pair of 50 mm Zorbax Eclipse XDB C-18 reversed phase HPLC (4.6 mm i.d., 1 ml/min) columns with isocratic 95% methanol 0.05% acetic acid and subjected to MS using a Hewlett-Packard 1100 series system as follows: APCI mode with selected ion monitoring (SIM)  $m/z$  520.5; fragmentor voltage 70 V; vaporizer temp. 450 C; drying gas temp. 350 C; drying gas flow rate 7 L/min; corona current 7  $\mu$ A.

### Assay for sphingomyelinase activity/ SCDase activity in Tumor Cell Extracts

Cells were detached, pelleted and resuspended in buffer containing 100 mM-Tris-HCL (pH=7.4), either 7mM CHAPS or 1% Triton X-100, 1 mM EDTA, 1 mM AEBSF, 100  $\mu$ M leupeptin and mixed on ice for 45 min. Insoluble material was pelleted and the supernatant diluted to a protein concentration ranging from 0.5-0.8 mg/ml in bufer containing 50 mM TRIS-HCl, 20 mM MgCl<sub>2</sub>, 1 mM EDTA, 1mM AEBSF, and 100  $\mu$ M leupeptin.

Mmols of [<sup>3</sup>H]sphingomyelin were added per ml of extract and samples (250  $\mu$ l) were incubated in the presence or absence of sigma-2 receptor agonists for 60 min. at 37 C. The reaction was terminated by the addition of 1 ml of ice cold 10%TCA. Samples were centrifuged to remove precipitated protein and the supernatant was extracted in an equal volume of diethyl ether. **Sphingomyelinase activity** was assessed by measuring release of labeled phosphocholine from sphingomyelin, which was quantified by scintillation counting a fraction of the aequous phase. **Sphingolipid ceramide N-deacylase (SCDase)** activity was measured by drying the total organic phase, resuspending the sample in 30-40  $\mu$ l of ether, and subjecting the sample to thin-layer chromatography on Silica G-60 plates using chloroform:methanol:formate:water (60:30:7:3). Sphingosylphosphorylcholine and sphingomyelin were detected based on the migration of standards run with the samples.

# Biological Effects of Ceramides & Derivatives

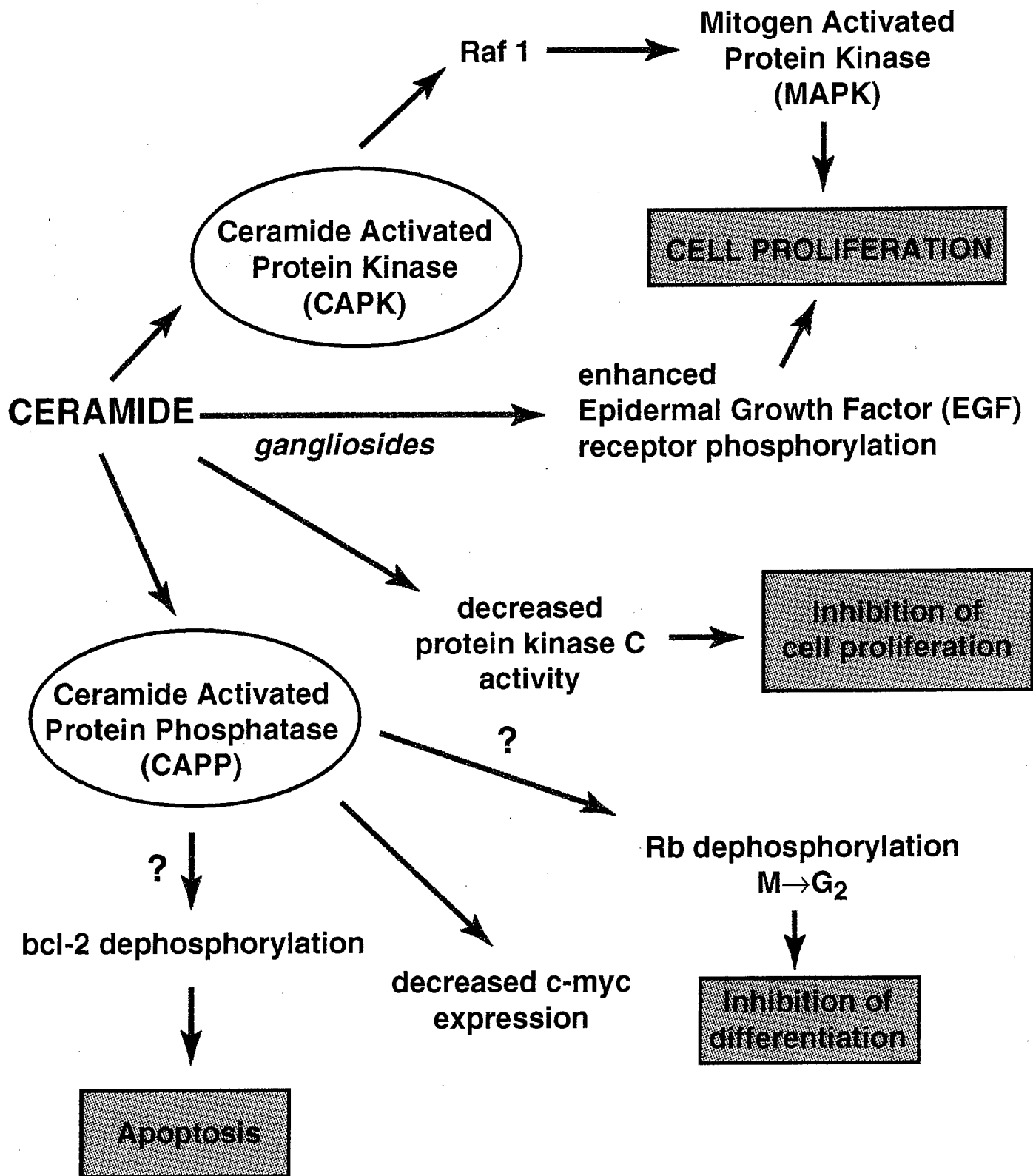
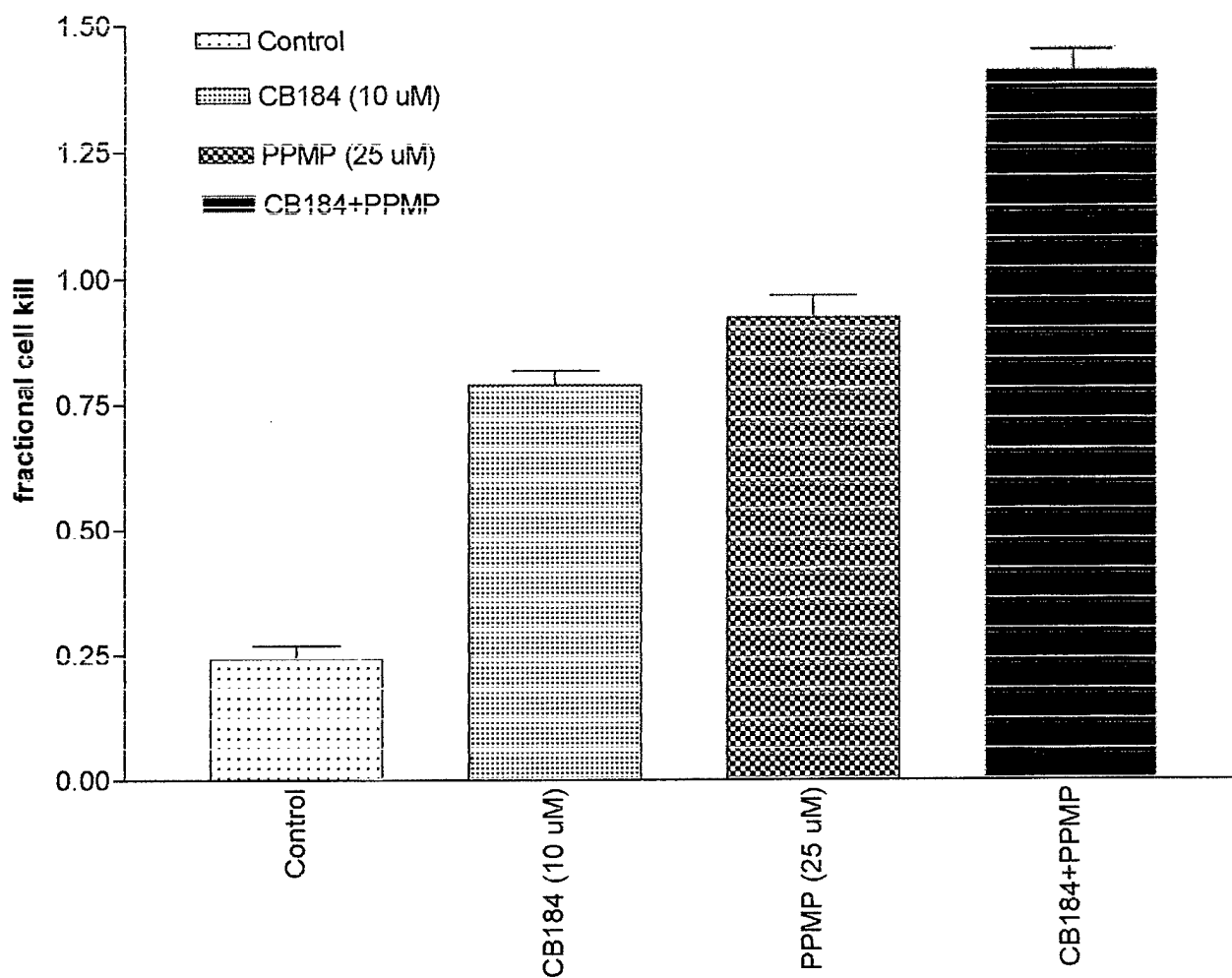


Figure 1. Biological Effects of Ceramides and Ceramide-Derivatives

Effect of Sigma Ligands and inhibitors of ceramide glycosylation on in MCF-7/Adr-cells (24hr)



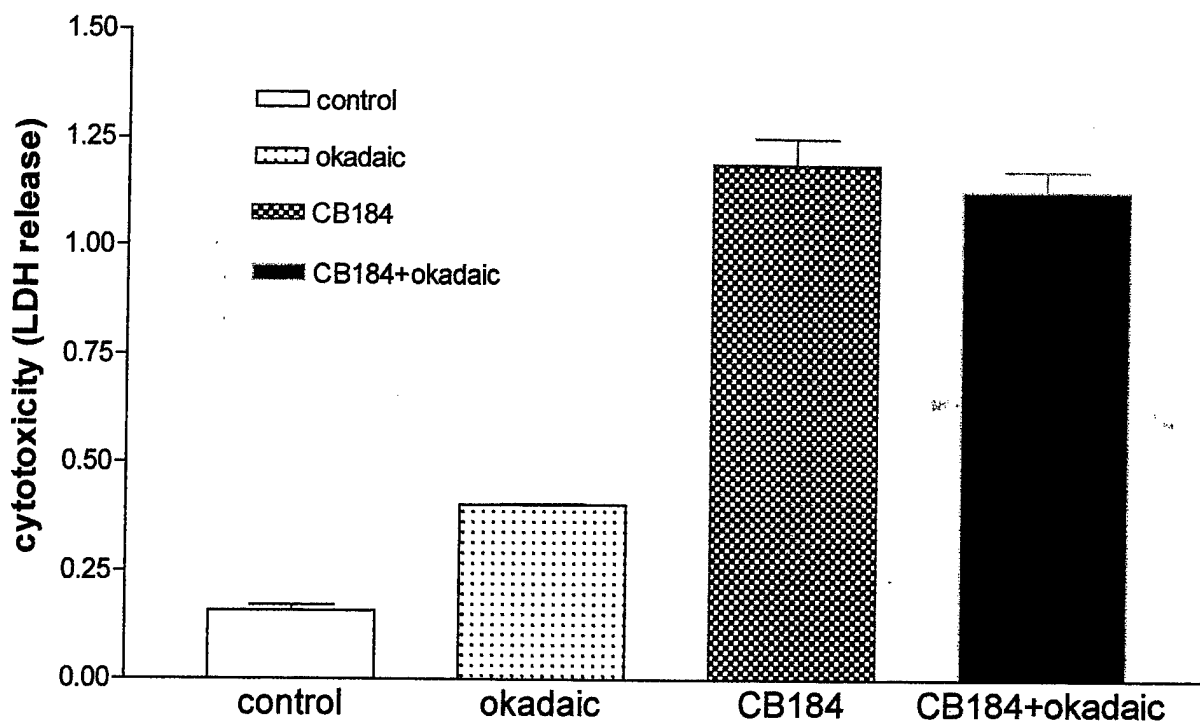
**Figure 2. Sigma-2 receptor agonists and inhibitors of ceramide glycosylation (PPMP) produce additive cytotoxicity in MCF-7/Adr- breast tumor cells.**

Cells were plated, incubated in the presence or absence of the designated compounds and cytotoxicity measured as described in *Methods*. Each bar represents the mean of quadruplicate samples (duplicate samples from two separate wells).

**Figure 3. Okadaic acid, inhibitor of Ceramide-Activated Protein Phosphatase, abolishes sigma-2 receptor-mediated cytotoxicity in MCF-7/Adr- cells (mutant p53) but not MCF-7 cells (wild-type p53).**

Cells were seeded at a density of 100,000 per well and grown to ~80% confluency. Cells were incubated with the indicated compounds and cytotoxicity was assessed by release of LDH into media. Okadaic acid is used at a concentration of 10 nM; CB184 at 10  $\mu$ M. Each bar represents quadruplicate samples (duplicate samples from two separate wells). The data presented above is representative of two separate experiments.

### Effect of Okadaic acid on Sigma -2-mediated Cytotoxicity in MCF-7 cells (24 hr)



### Effect of Okadaic acid on Sigma-2 mediated Cytotoxicity in MCF-7/Adr- cells (24 hr)

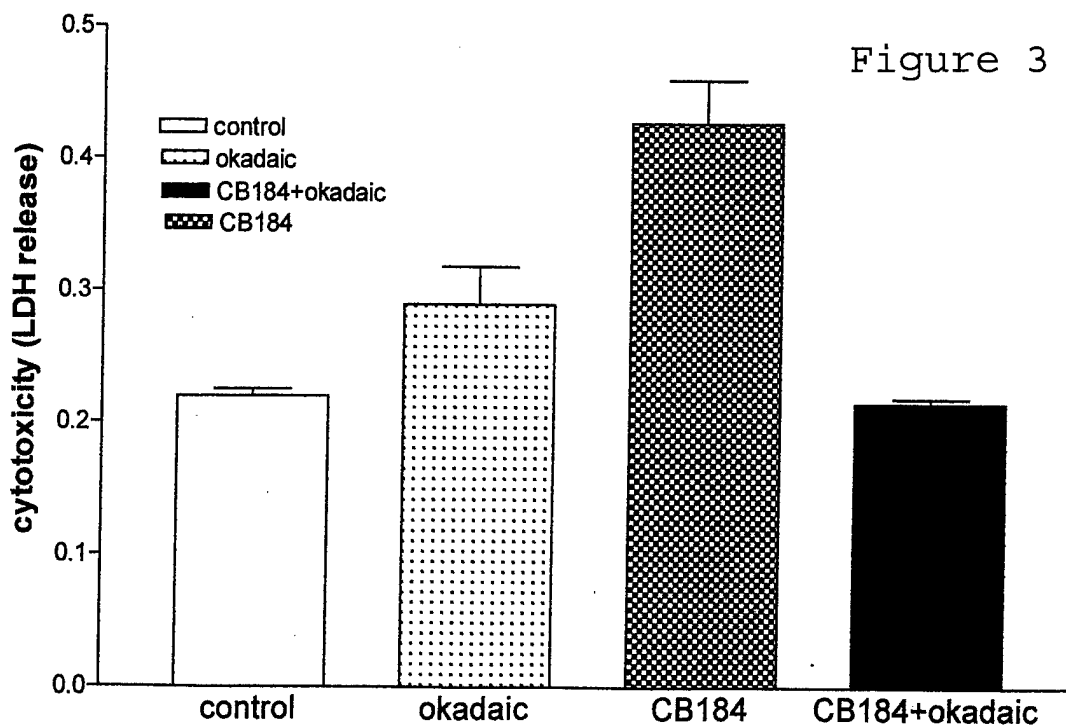
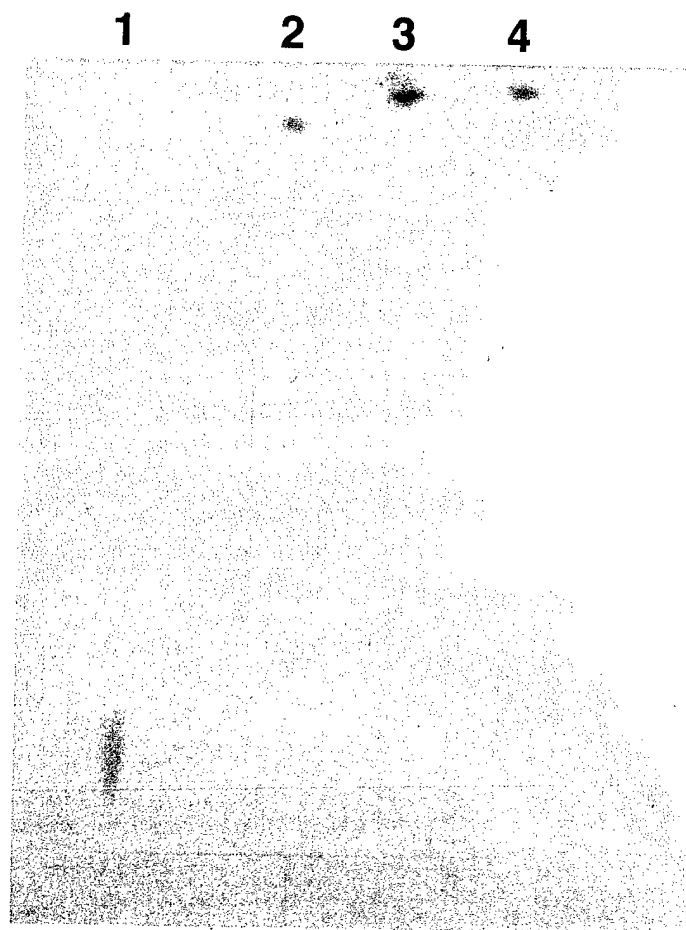


Figure 3 Continued

**Figure 4. Separation of Sphingolipids by thin-layer chromatography on Silica G-60 plates.**

Lipids were spotted on the plates in the designated amounts (bovine sphingomyelin 7  $\mu\text{g}/\mu\text{l}$ , 15  $\mu\text{l}$ ; ceramides, 5 $\mu\text{g}/\mu\text{l}$ , 20  $\mu\text{l}$ ). Lipids were separated by thin-layer chromatography as described in Methods.

**Separation of Sphingolipids by  
Thin-layer Chromatography (TLC)**



lane 1 = sphingomyelin, lane 2 = C2-ceramide,  
lane 3 = C8-ceramide, lane 4 = C16-ceramide

**Figure 5. Chronic exposure to a Sigma-2-selective agonist produce increases in ceramide levels and concomitant decreases in sphingomyelin levels.** MCF-7/Adr- or T47D breast tumor cells were labeled and treated for 24 hours with the indicated compound (100 $\mu$ M). Lipids were extracted and samples were analyzed by TLC as described in Methods. Lipids were identified according to their migration patterns as determined by standards (figure 4), scraped from the silica plate and radioactivity quantified by scintillation counting. Each bar represents the mean of triplicate samples. PPPP (phenyl hexodeconylamino pyrrolidino propanol, 25  $\mu$ M) inhibits glycosylation of ceramides leading to increased ceramide levels and cell death in cytotoxicity assays. CB-19 is a compound structurally similar to the agonist CB-184, but possessing no sigma-2 binding affinity.

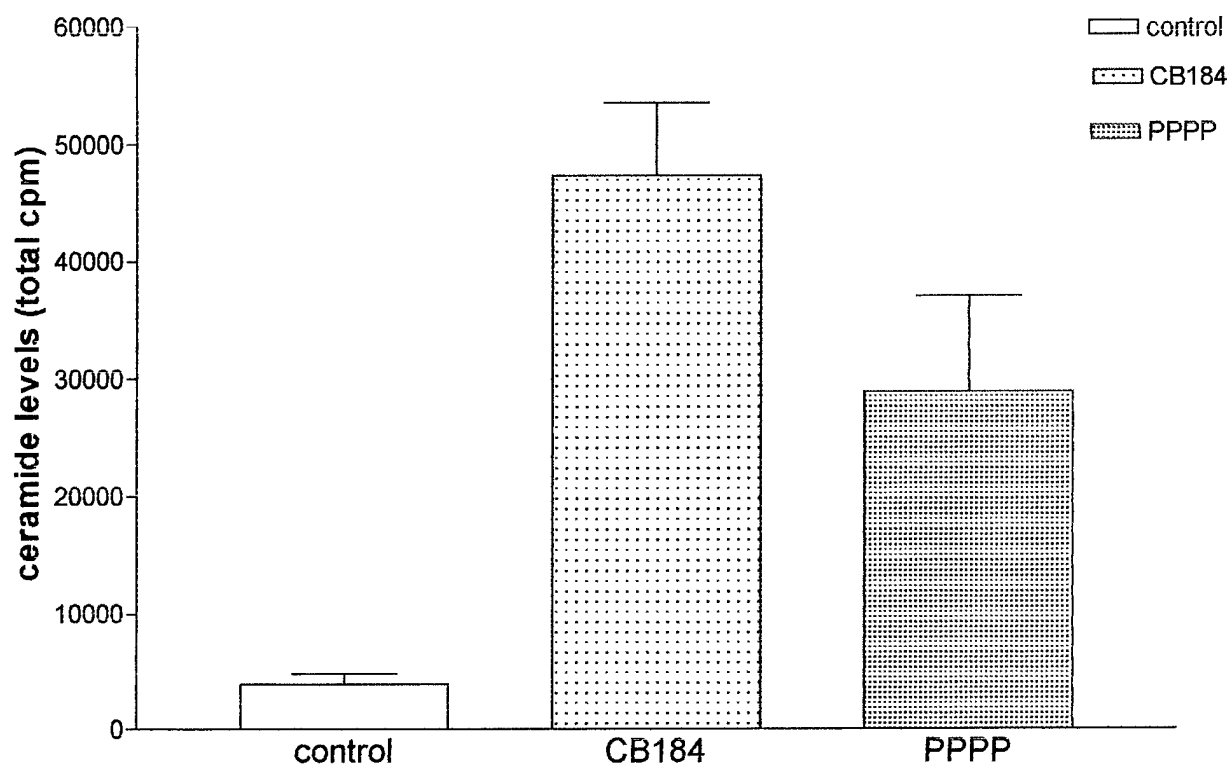
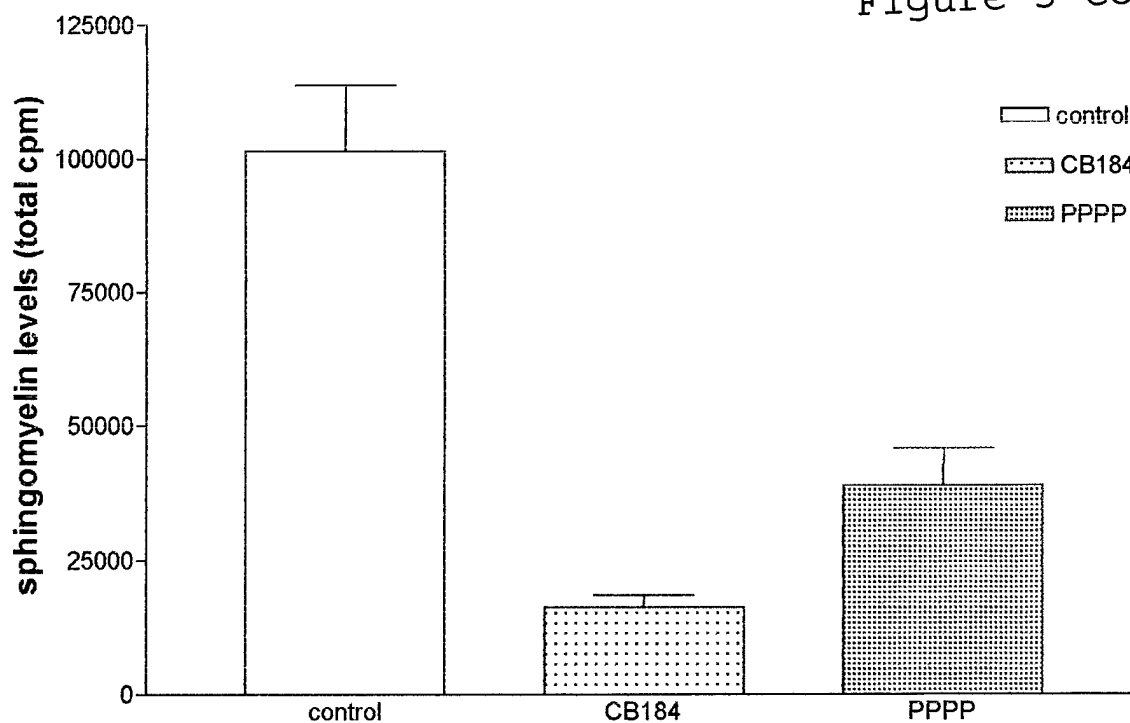
**Changes in ceramide levels in MCF-7/Adr- cells (24 hr)****Changes in sphingomyelin levels in MCF-7/ADR- cells (24hr)**

Figure 5 Continued

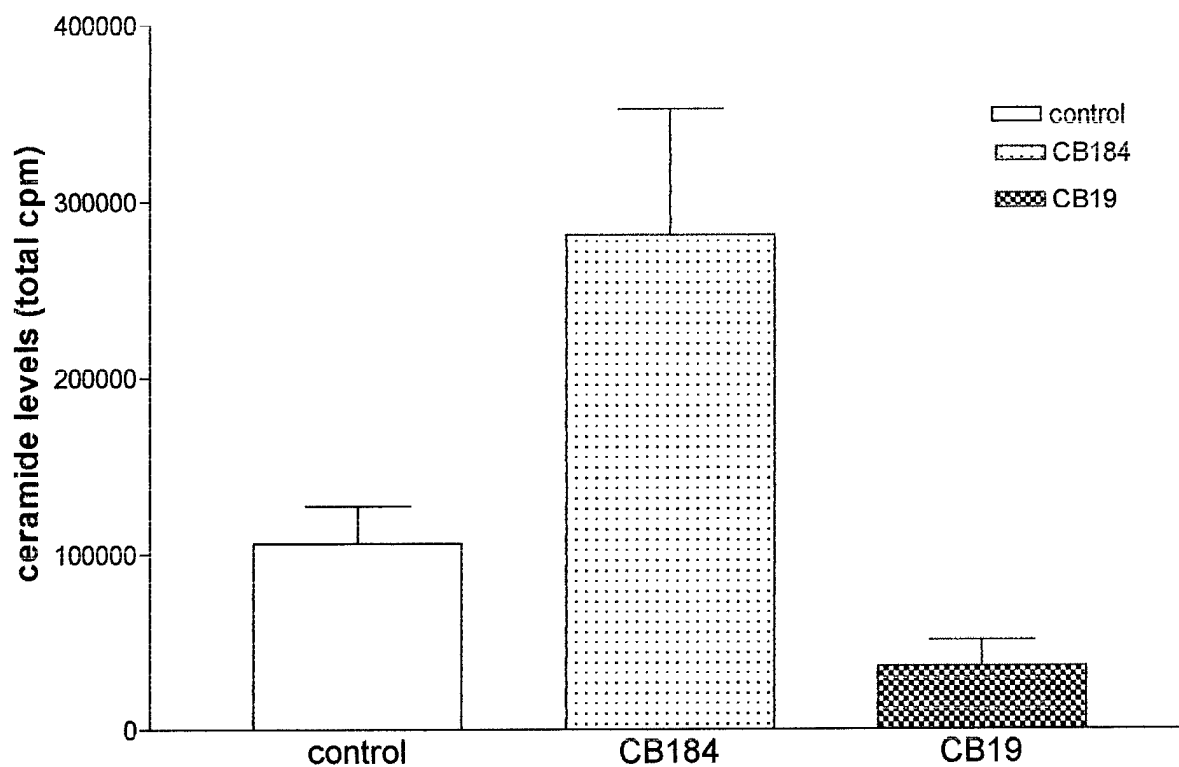
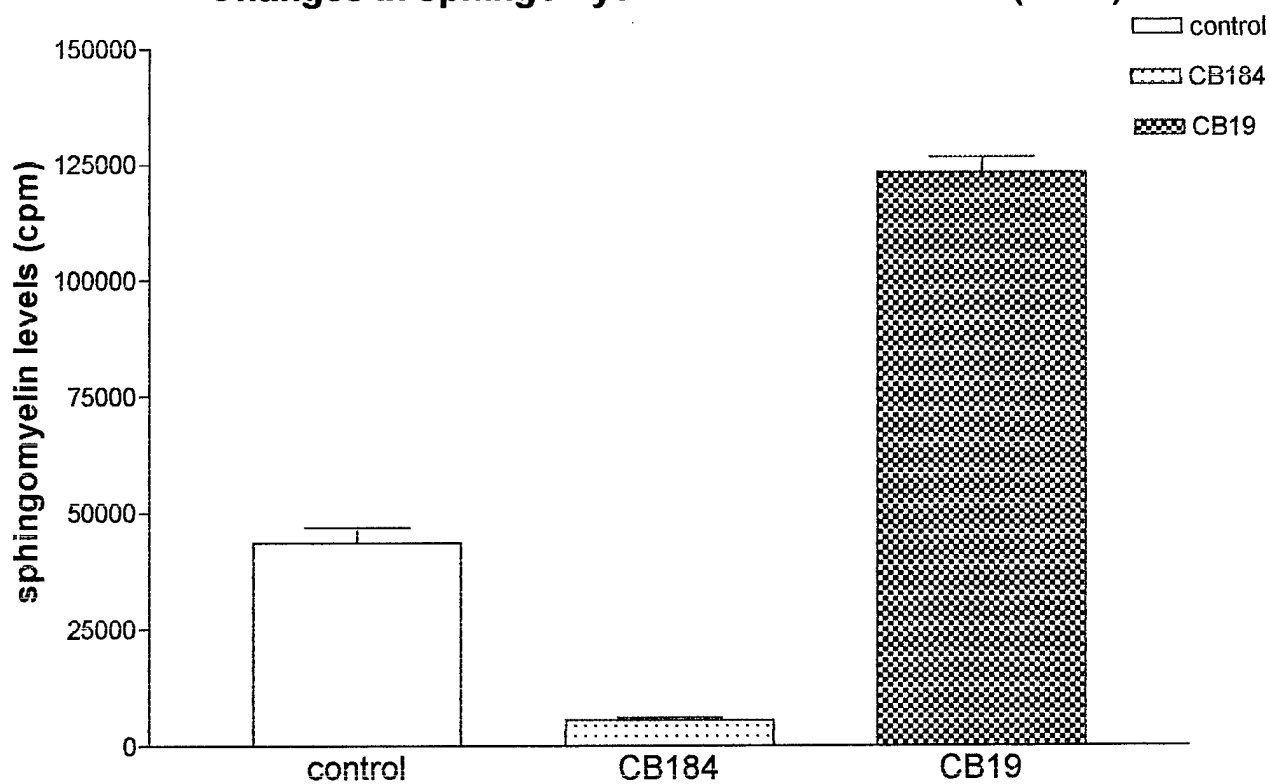
**Changes in ceramide concentration in T47D cells (24 hr)**

Figure 5 Continued

**Changes in sphingomyelin levels in T47D cell (24 hr)**

**Figure 6. The sigma-2 receptor selective antagonist, AC927, partially abrogates agonist-mediated changes in sphingolipids.**

MCF-7/Adr- cells were labeled, treated under conditions previously shown to enhance sigma-2 antagonism, extracted and chromatographed by TLC as described in Methods. Sigma-2 agonist (CB-184) and antagonist (AC927) were used at a concentration of 100  $\mu$ M.

**Effect of Sigma-2 receptor agonist/antagonist on ceramide levels  
(MCF-7/Adr-, 24 hrs.)**

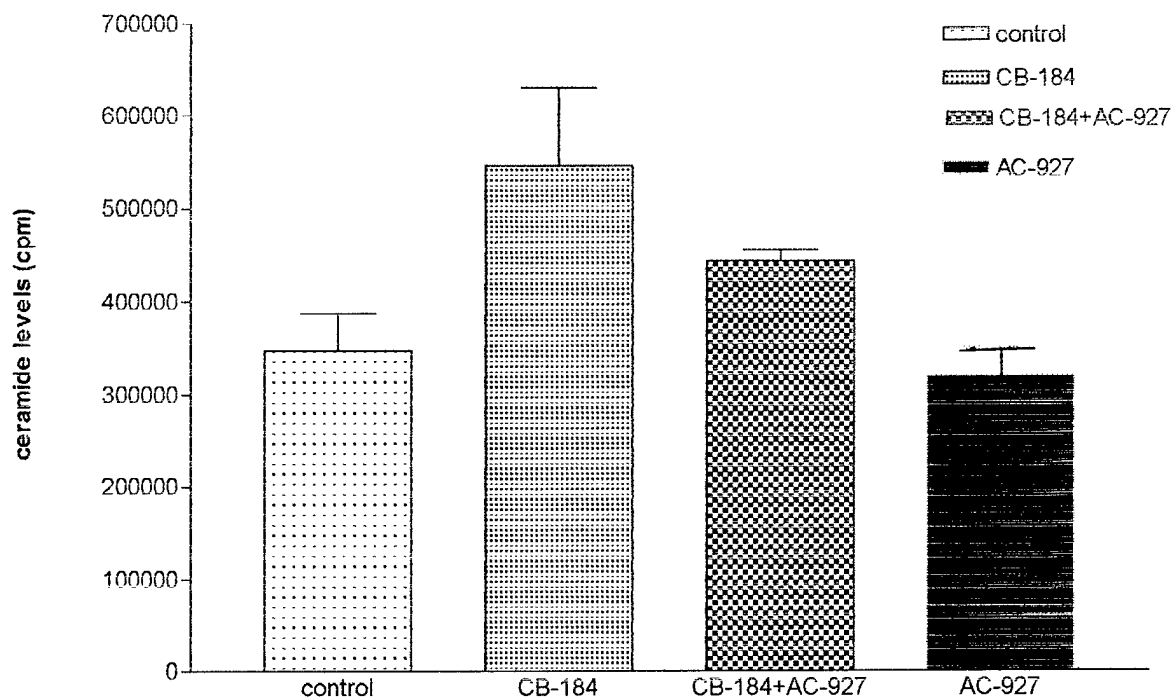
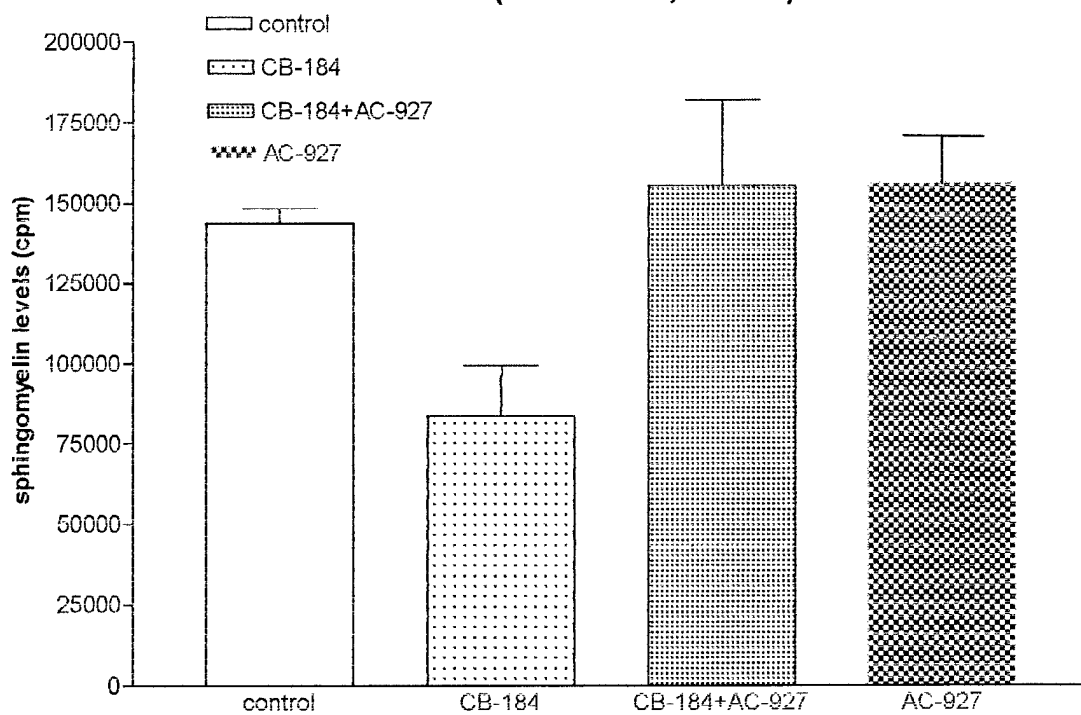
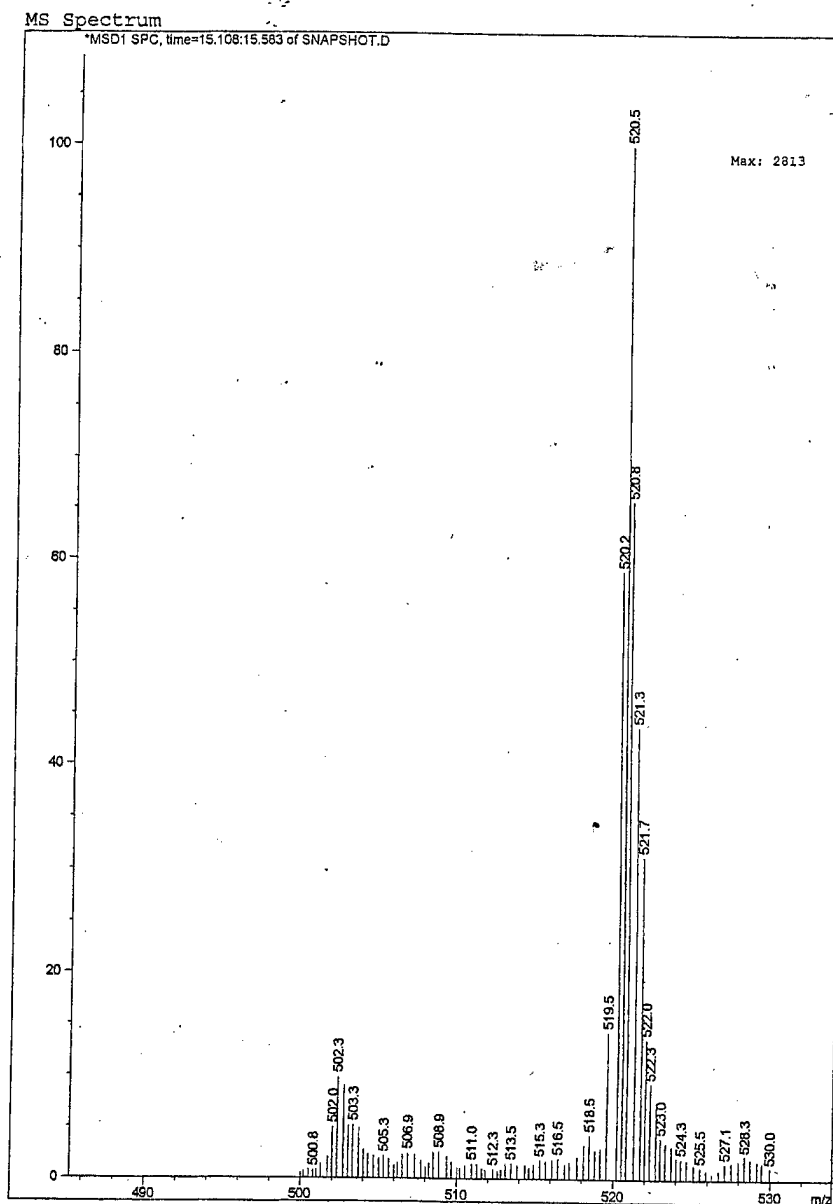


Figure 6 Continued

**Effect of sigma-2 receptor agonist/antagonist on sphingomyelin  
levels (MCF-7/Adr-, 24 hrs.)**





**Figure 7. Mass spectrum of ceramide.**

A sample of authentic N-(palmitoyl)-sphingosine (C<sub>16</sub>-ceramide) was subjected to APCI-LC/MS analysis as described in Methods, except that the full spectrum was taken. Mass region from 490 - 530 m/z is shown.

The peak at 520.5 m/z results from removal of a hydroxyl group in the form of H<sub>2</sub>O from the sphingosine backbone of ceramide (Couch et al., 1997). The ceramide molecule contains two hydroxyl groups, the loss of either of which as H<sub>2</sub>O could generate a fragment of 520.5 Da. In mixtures of sphingolipids, a fragment at m/z 520.5 may result from cleavage of other C<sub>16</sub>-ceramide derivatives such as sphingomyelin where one hydroxyl is involved in a phosphodiester bond or gangliosides such as glucosylceramide in which one hydroxyl group is involved in an ether linkage. Thus, selected ion monitoring at m/z 520.5 was used to detect ceramide and other putative sphingolipids.

**Figure 8. Mass spectral analysis of effect of CB-184 on sphingolipids in MCF-7/Adr-cells.**

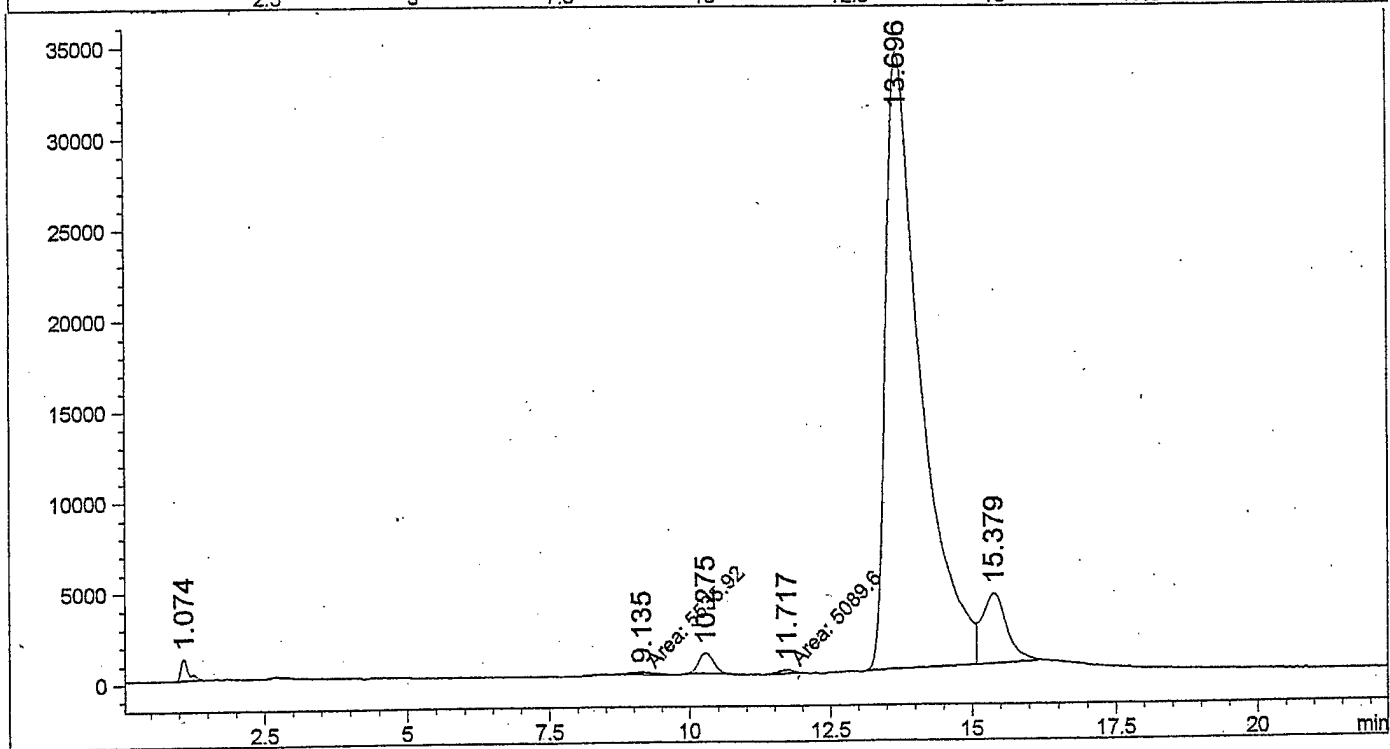
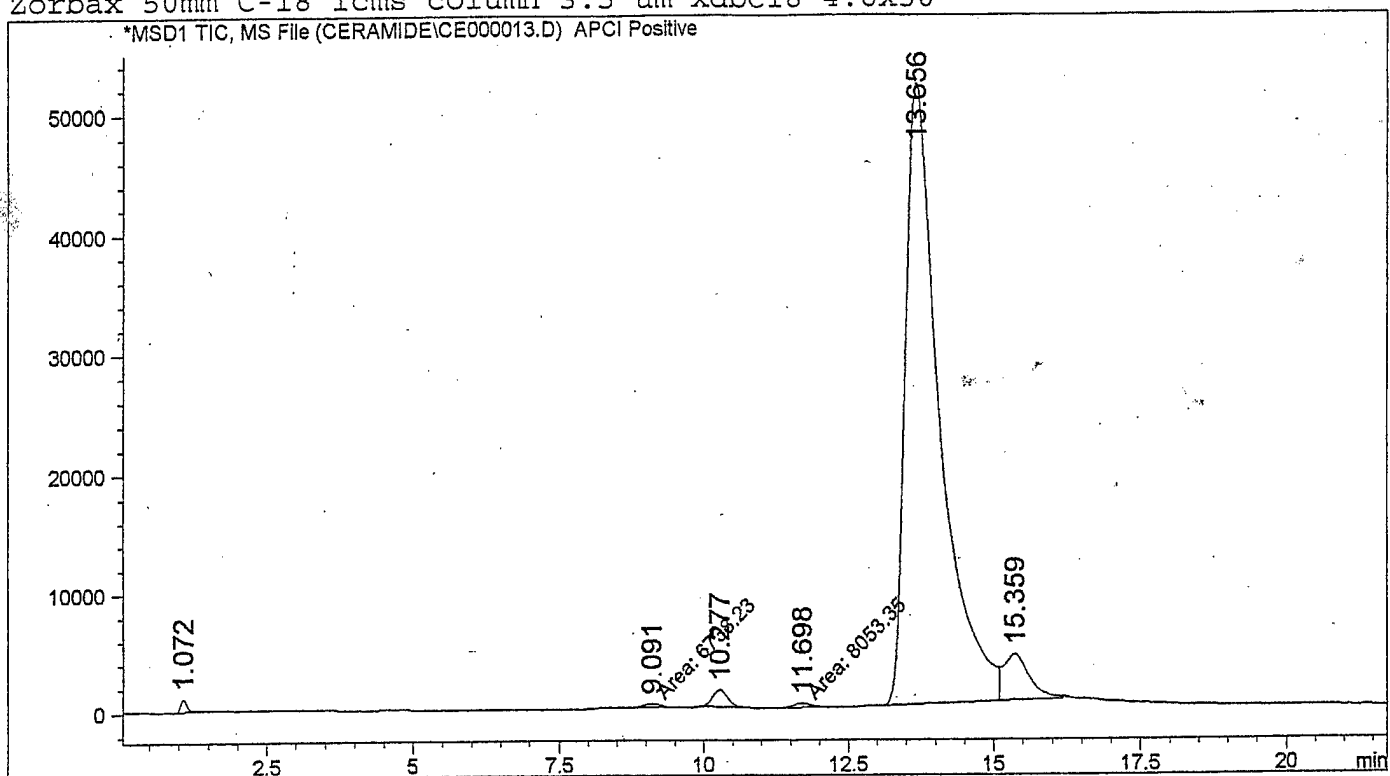
MCF-7/Adr- cells were treated for 24 hrs with 10  $\mu$ M CB-184 and extracted as described in Methods. The organic residue was evaporated under nitrogen and dissolved in absolute methanol. Samples were subjected to APCI-LC/MS analysis as described in Methods, with selected ion monitoring at 520.5 m/z to detect ceramide and other putative sphingolipids. **Panel A**, untreated control; **Panel B**, 10  $\mu$ M CB-184. Relative amount is shown on y-axis, retention time (RT) is shown on x-axis. Numbers over peaks are retention times in min.

Two major peaks were found in cell extracts. Peak 2 at RT = 15.37 min, corresponded to authentic C<sub>16</sub>-ceramide. Peak 1 at RT = 13.67 min was not identified, but is consistent with a C<sub>16</sub>-sphingolipid, possibly C<sub>16</sub>-sphingomyelin. The relative amount of ceramide (peak 2) was increased upon incubation with CB-184.

## Figure 8 Continued

Zorbax 50mm C-18 1cms column 3.5 um xdbc18 4.6x50

\*MSD1 TIC, MS File (CERAMIDEICE000013.D) APCI Positive



### Ceramide/Sphingolipid ratio in MCF-7/Adr- cells (24 hr)

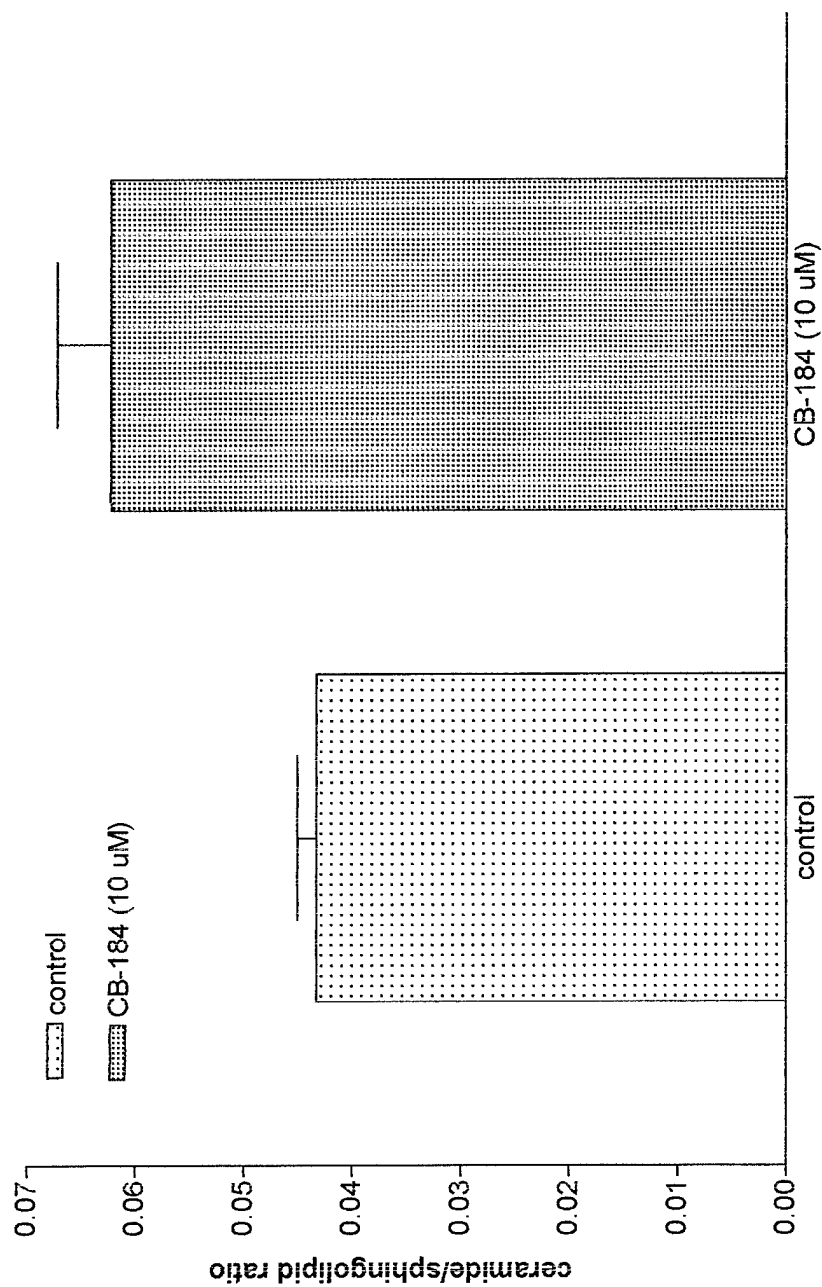


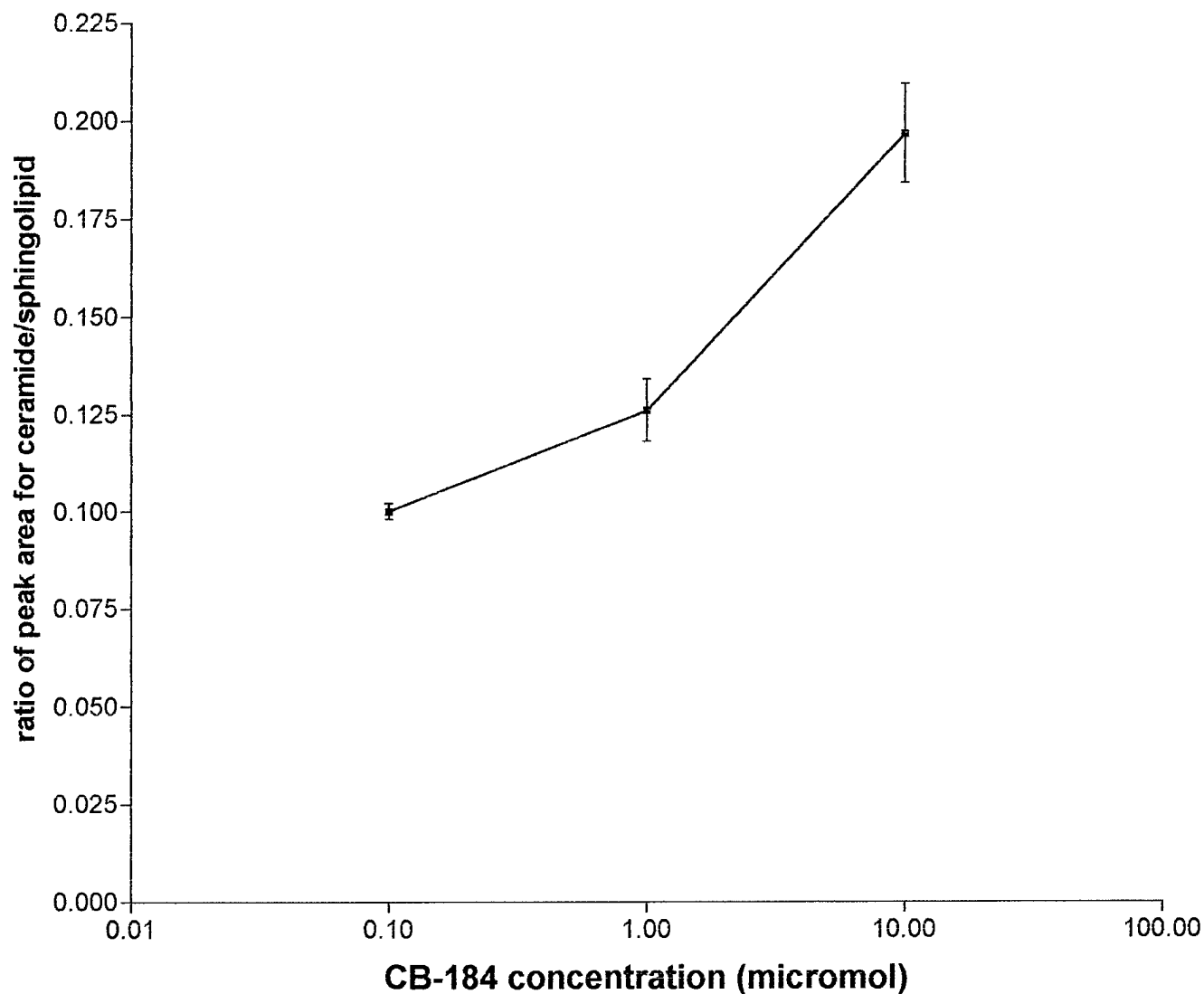
Figure 9. Effect of CB-184 on ceramide/sphingolipid ratio in MCF-7/Adr- cells.

**Figure 10. Dose response for changes in ceramide/sphingolipid ratio in SK-N-SH cells treated with CB-184.**

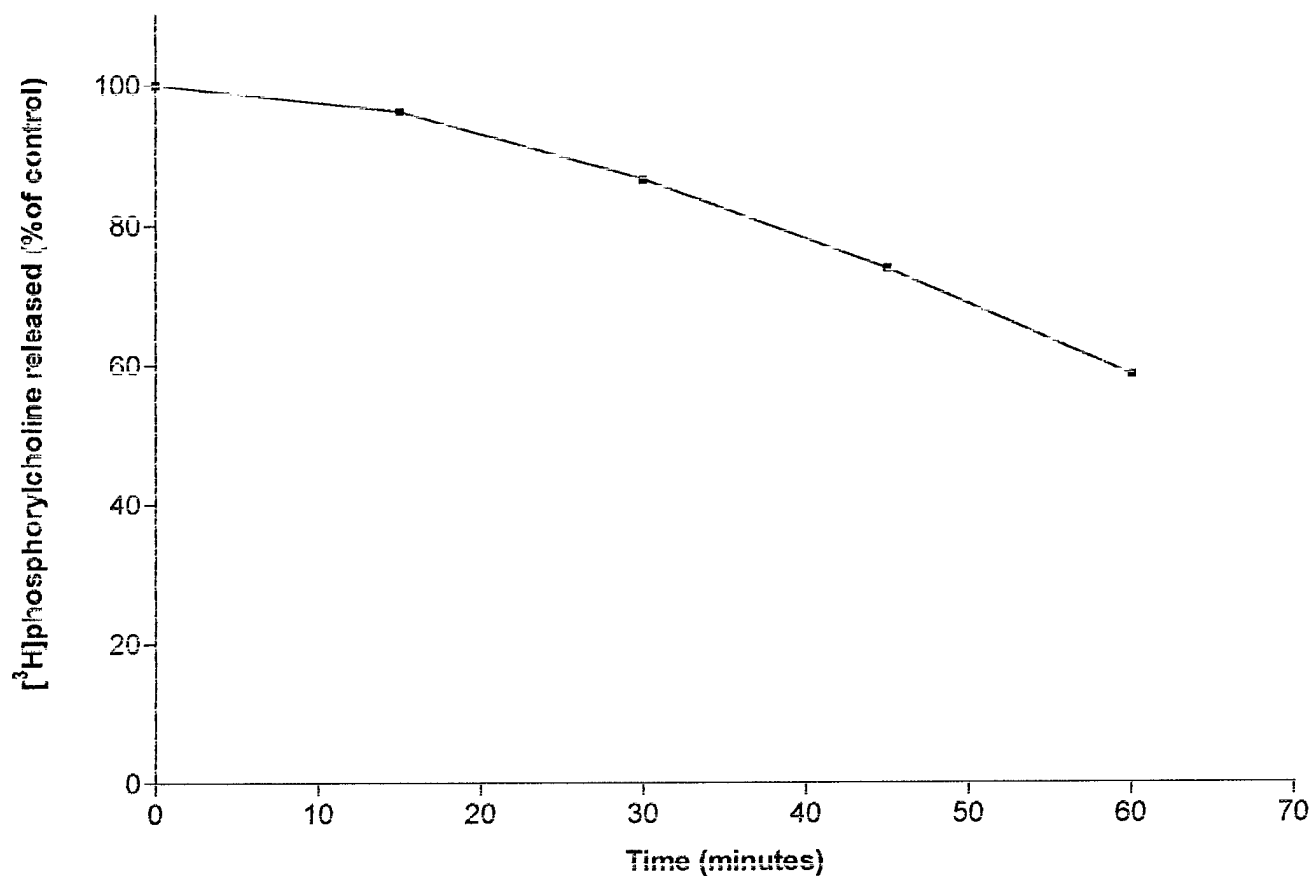
SK-N-SH cells were treated for 24 hr with various concentrations of CB-184. Cells were extracted and extracts subjected to APCI-LC/MS, with selected ion monitoring at 520.5 m/z. The areas under peak 1 (sphingolipid) and peak 2 (C<sub>16</sub>-ceramide) were calculated and the ratio of peak 2/peak 1 plotted vs. CB-184 concentration in order to determine the relative change in ceramide concentration.

CB-184 caused a dose-dependent increase in the ratio of ceramide/sphingolipid. This is consistent with a sigma-2 receptor-mediated relative increase in the cellular level of ceramide and confirms results obtained with thin layer chromatography using radiolabeled sphingolipids.

**CB-184 produces concentration-dependent changes in the ceramide/sphingolipid ratio in SK-N-SH cells**

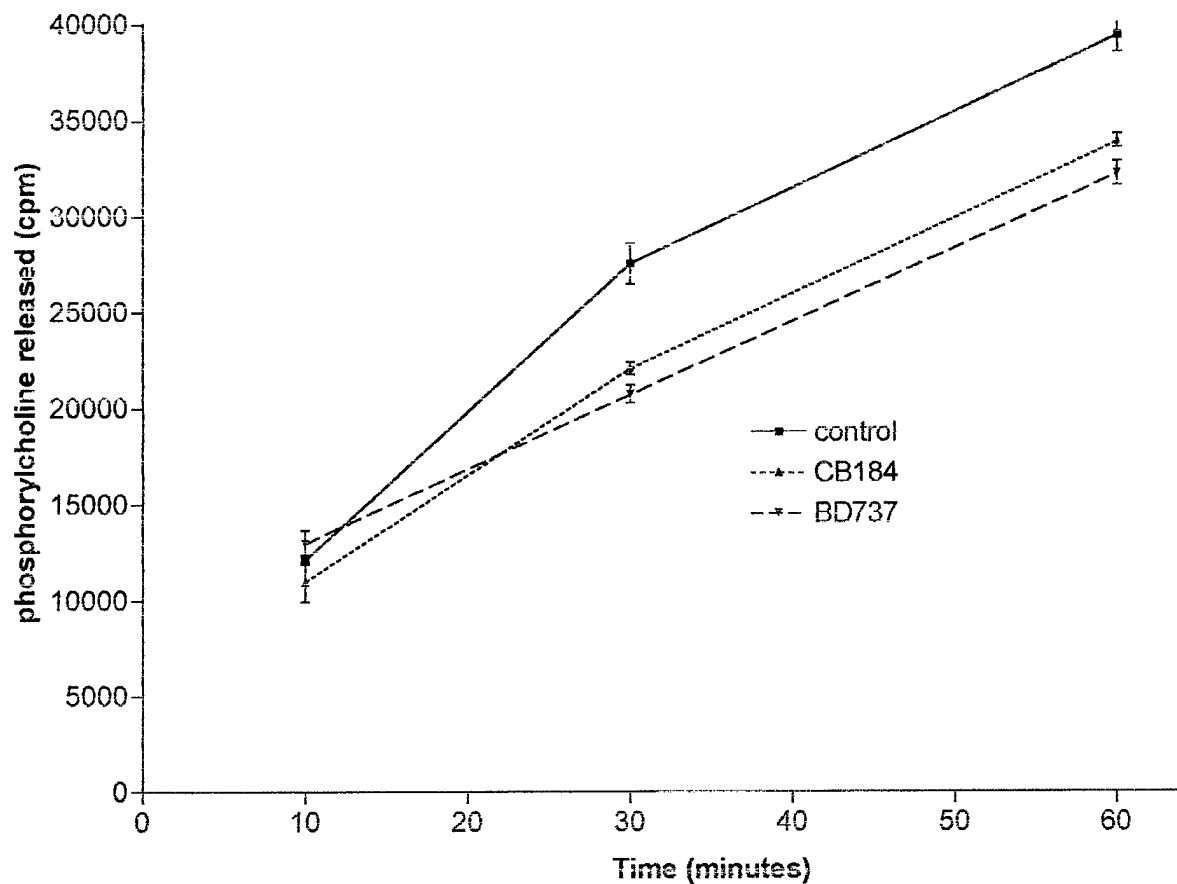


### Effect of CB-184 (100uM) on sphingomyelinase activity in MCF-7 cells



**Figure 11. Time-course for the effect of Sigma-2 receptor agonists on Neutral sphingomyelinase activity in intact MCF-7 cells.** MCF-7 cells were treated with 100  $\mu$ M CB-184 for the indicated times at 37 C (one 75 cm<sup>2</sup> flask/time point). Cells were then pelleted and extracted in buffer containing 7mM CHAPS and assayed for sphingomyelinase as described in *Methods*.

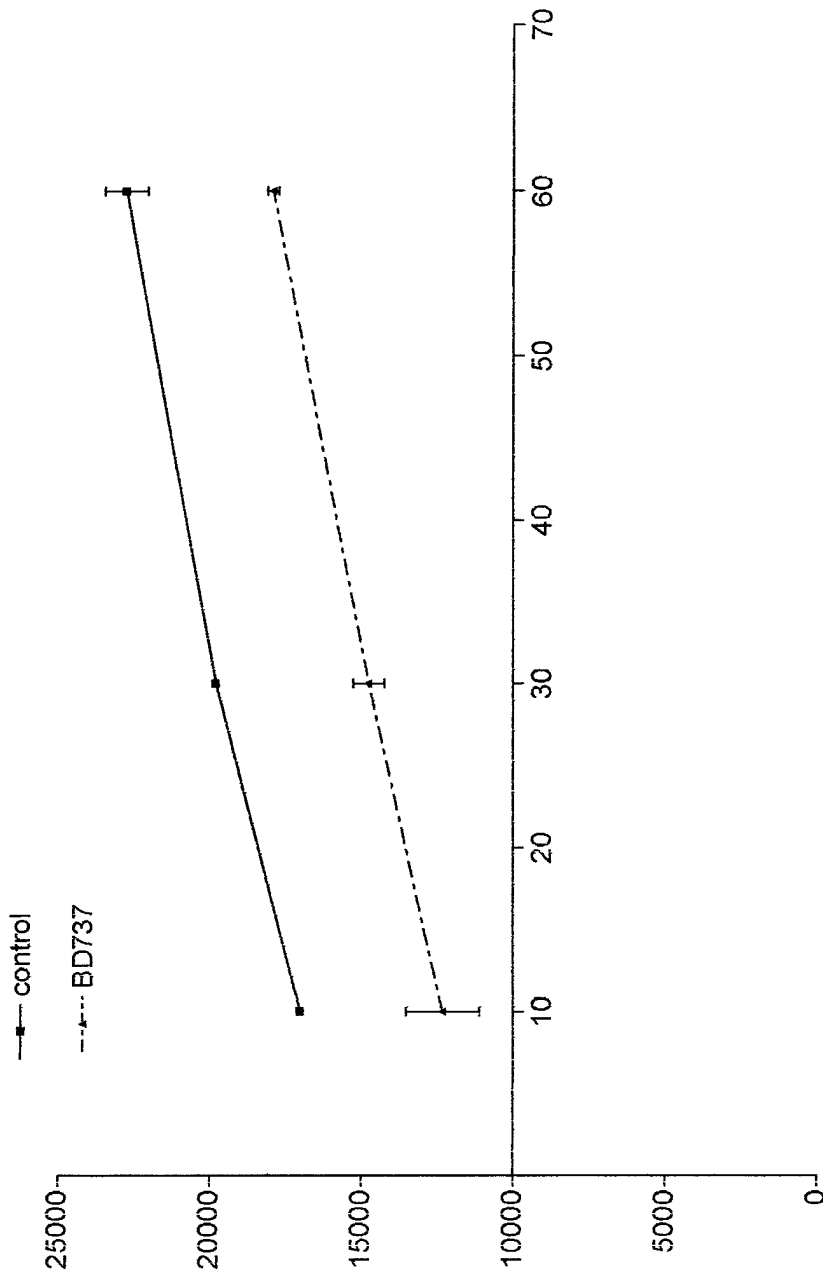
### Effect of sigma-2 receptor agonists (CB-184, BD-737) on sphingomyelinase activity



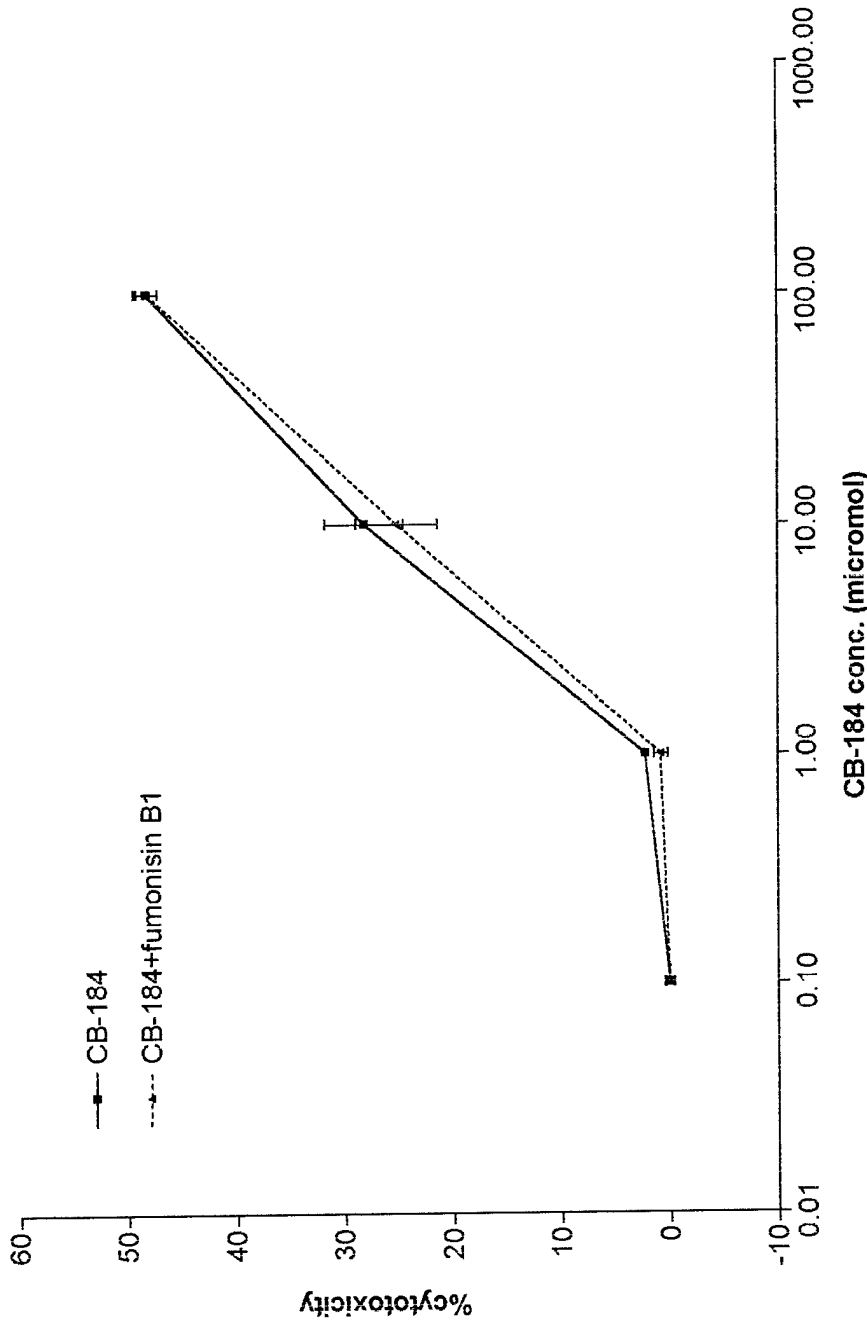
**Figure 12. Effect of Sigma-2 receptor agonists on Neutral sphingomyelinase activity in a tumor cell detergent extract.**

T47D cells were grown in 75 cm<sup>2</sup> vented culture flasks up to about 80% confluency using the conditions described in Methods. Assay for sphingomyelinase was performed as described in *Methods*.

Figure 12 Continued

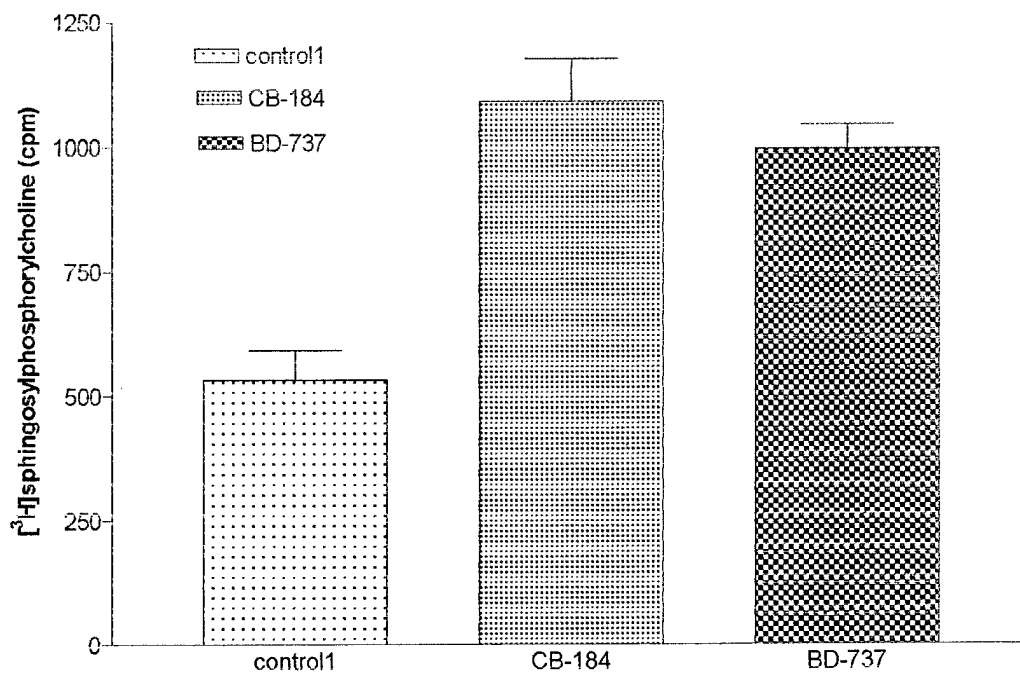


### Effect of ceramide synthase inhibition by fumonisin B1 (100 $\mu$ M) on sigma-2 receptor mediated cytotoxicity

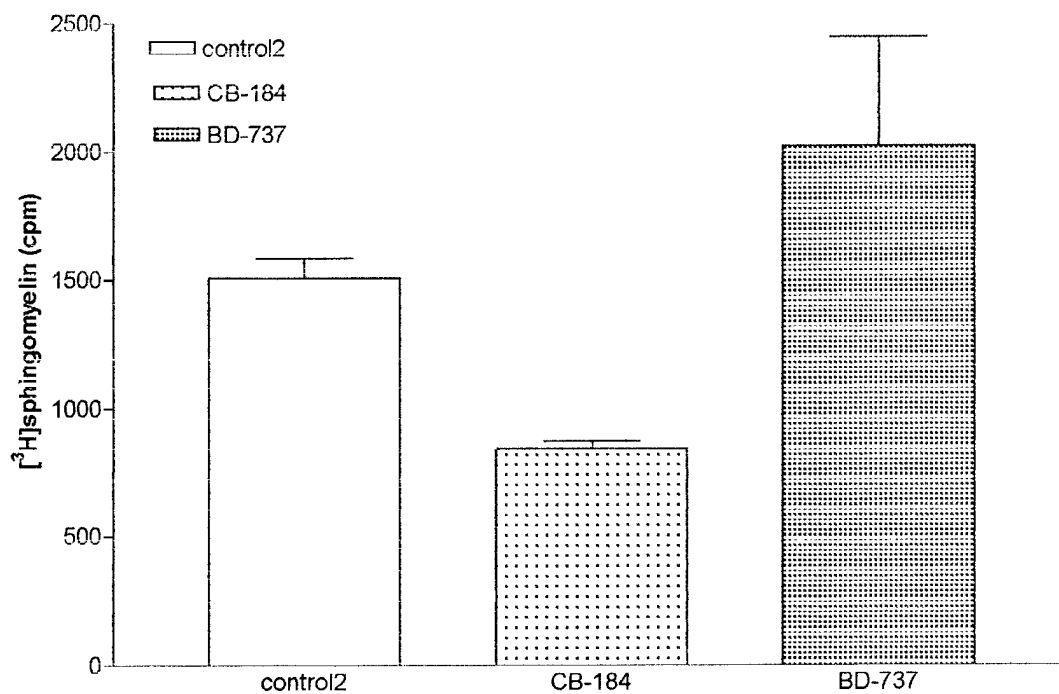


**Figure 13. Effect of Fumonisin B1 on sigma-2 receptor agonist mediated cytotoxicity in MCF-7/Adr- cells.** Cells were treated with the designated concentrations of CB-184 in the absence or presence of 100 $\mu$ M fumonisin B1 for 24 hr. Cytotoxicity was measured using the LDH assay as described in *Methods*.

**Effect of Sigma-2 receptor agonists on  
sphingosylphosphorylcholine production in a tumor cell extract  
(T47D)**



**Effect of Sigma-2 receptor agonists on sphingomyelin levels in  
a tumor cell extract (T47D)**



**Figure 14. Sigma-2 receptor agonist cause increased sphingosylphosphorylcholine production in a tumor cell detergent extract possibly through the hydrolysis of sphingomyelin by SCDase.**

Sphingomyelin ceramide deacylase was assayed as described in *Methods*.

## 7. Key Research Accomplishments

### Identification of Sphingolipids as Mediators in the Chronic Effects of Sigma-2 Receptor Agonists

- Assay for changes in Sphingomyelin/Ceramide in Cells using Thin-layer Chromatography
- Separation and quantification of Sphingolipids using atmospheric pressure chemical ionization -liquid chromatography /mass spectrometry (APCI-LC/MS).
- Developed assay for sphingomyelinase activity in tumor cell detergent extracts
- Developed assay for sphingolipid ceramide N-deacylase (SCDase) activity in tumor cell detergent extracts

## 8. Reportable Outcomes

### Abstracts

- Crawford, K., Huang, S., Walker, J.M., Bowen, W . Chronic Treatment with Sigma-2 Receptor Agonists Produces Changes in Sphingolipid Levels in Breast Tumor and Neuroblastoma Cells. Proceedings of The American Association of Cancer Research, #4145, 2000
- Crawford, K., Huang, S., Walker, J.M., Bowen, W. Sigma-2 Receptors: Role in Tumor Biology and Target for Chemotherapy. Department of Defense, *Era of Hope Meeting*, Atlanta, GA. #V-12, 2000

### Patents

New Application filed: *Potential of Antineoplastic Agents Using Sigma 2 Ligands*  
Ref. # E-165-99/O; W/O M&F 419-99

### Manuscript submitted

Crawford, KW. And Bowen, W.D.

### Funding Applied for Based on work supported by this Award

DoD Breast cancer Research Program, 2000  
HBCU/MI Collaborative Partnership Award

## 9. Conclusions

The work performed during this funding period provides the very first evidence of the involvement of sphingolipids in sigma-2 receptor mediated signal transduction. The results from experiments using TLC analysis are reinforced by the findings using APCIMS/LC, one of the most sensitive methods for definitive identification and quantification of biochemicals. The findings presented above have led us to formulate a hypothesis which we believe accounts for the high expression of sigma-2 receptors in tumors, and also the observation that chronic activation of these receptors leads to apoptosis in tumors. Ceramides and their derivatives (eg. sphingosine-1- $\text{PO}_4$ ) are known to be involved in cell cycle regulation and cell proliferation. Therefore, the high expression of sigma-2 receptors on tumors increases sphingolipid synthesis and increases cell proliferation. Ceramide also activates pathways involved in apoptosis, and we propose that chronic treatment with sigma-2 receptor agonists activates these pathways leading to death in tumors.

We are in the process of studying changes in protein levels that are consistent with ceramide-signaling pathways in breast tumors. We are also refining our studies of solubilized sigma-2 receptors and their ability to modulate effectors regulating sphingolipid concentrations. These studies may also lead to the purification and cloning of the sigma-2 receptor. These receptors may represent a tumor-specific target for chemotherapy.

## References

Crawford, KW., Vilver B.J., Bowen, W.D. Sigma-2 receptor activation induces apoptosis in breast, prostate and neuroblastoma tumor cells. #1104 Proceedings of the American Assoc. Cancer Res., 1999

Crawford, KW, Bowen, WD Sigma-2 receptors mediate a novel apoptotic pathway and potentiate anti-neoplastics in breast tumor cell lines. Submitted 2000