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DEVELOPMENT OF A THERAPEUTIC AGENT FOR WOUND-HEALING ENHANCEMENT

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SCIENTIFIC PROGRESS DURING THE LAST QUARTER

PEPTIDE SYNTHESIS

During this quarter we synthesized and purified three peptides, one from PDGF A-chain sequence and two from PDGF B-chain sequence. These peptides are listed below.

| | |
|------------------|--|
| PDGF A (101-125) | Peptide #3, Table 9, p. 23 in the original proposal. |
| PDGF B (104-116) | Peptide #7, Table 4, p. 18 in the original proposal. |
| PDGF B (115-128) | Peptide #8, Table 4, p. 18 in the original proposal. |

BIOLOGICAL ASSAYS

During this quarter we conducted cell binding experiments and assays for mitogenesis. These assays are described below.

CELL BINDING ASSAYS (COMPETITION FOR RECEPTOR BINDING)

Competitive receptor binding assays were performed on four peptides using NIH 3T3 cells. The results are shown in Table 1 and Figures 1 through 5. As seen in Table 1, none of the peptides tested so far showed any binding. We tested two peptides at three doses and two at four doses. In the future, we will test peptides initially at three doses. Active peptides will be tested at four or more doses.

Statement A per telecon
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Table 1
RESULTS OF COMPETITIVE BINDING ASSAYS

| Peptide | Concentration (ng or μ g well) | CPM | Average \pm SD | % Maximum Binding | % Inhibition |
|------------------------------------|---------------------------------------|--|-------------------|----------------------|--------------|
| Control (Empty wells, no cells) | | 280.746 314.922 282.753 287.973 | 290 \pm 11 | | |
| 125 I PDGF B | 5 ng | 25291.9 26844.3 26228.4 | 26,121 \pm 781 | 100 | 0 |
| PDGF B | 100 ng | 7605.88 7693.76 6841.80 | 7,381 \pm 469 | 28 | 72 |
| | 50 ng | 9548.28 9074.44 8906.09 | 9,176 \pm 333 | 35 | 65 |
| | 25 ng | 11,152.7 11,619.7 10,649.4 | 11,141 \pm 486 | 43 | 57 |
| | 12.5 ng | 13,065.2 13,860.2 | 13,462 \pm 562 | 51 | 49 |
| | 6.25 ng | 15,780.8 17,614.3 | 16,698 \pm 1296 | 64 | 36 |
| PDGF A (12-28) | 500 μ g | 23,587.2 23,163.3 25,874.6 | 24,208 \pm 145 | 93 | 7 |
| | 250 μ g | 26,241.3 25,861.5 23,451.4 | 25,184 \pm 1513 | 96 | 4 |
| | 125 μ g | 23,002.4 24,337.9 24,966.6 | 24,102 \pm 1003 | 92 | 8 |
| PDGF B (22-36) | 500 μ g | 23,036.7 23,927.8 22,716.3 | 23,225 \pm 625 | 89 | 11 |
| | 100 μ g | 23,637.7 21,963.2 23,276.3 | 22,959 \pm 881 | 88 | 12 |
| | 50 μ g | 24,241.9 24,307.9 24,731.0 | 24,427 \pm 265 | 94 | 6 |
| | 10 μ g | 25,460.8 27,353.8 26,969.7 | 26,928 \pm 448 | 103 | -3 |

Table 1 (concluded)

| Peptide | Concentration (ng or μg well) | CPM | Average \pm SD | % Maximum Binding | % Inhibition |
|------------------|---|------------------|------------------------------------|------------------------------|---------------------|
| PDGF B (101-117) | 500 μ g | 24,978.2 | 24,046.3 | 92 | 8 |
| | | 24,618.9 | | | |
| | | 22,541.8 | | | |
| 250 μ g | 24,000.0 | 23,501 \pm 535 | 90 | 10 | |
| | 23,567.7 | | | | |
| | 22,936.9 | | | | |
| 125 μ g | 23,997.8 | 23,602 \pm 902 | 90 | 10 | |
| | 24,238.9 | | | | |
| | 22,570.4 | | | | |
| PDGF B (104-116) | 500 μ g | 26,246.9 | 26,468 \pm 423 | 101 | -1 |
| | | 26,200.9 | | | |
| | | 26,955.5 | | | |
| | 100 μ g | 25,191.7 | 26,678 \pm 1915 | 102 | -2 |
| 26,001.8 | | | | | |
| 28,839.2 | | | | | |
| 50 μ g | 26,491.3 | 27,164 \pm 904 | 104 | -4 | |
| | 28,192.3 | | | | |
| | 26,807.4 | | | | |
| 10 μ g | 26,560.7 | 26,585 \pm 438 | 102 | -2 | |
| | 26,159.8 | | | | |
| | 27,034.1 | | | | |

**Competitive Inhibition for Receptor
Binding on 3T3 Cells by PDGF A (12-28)**

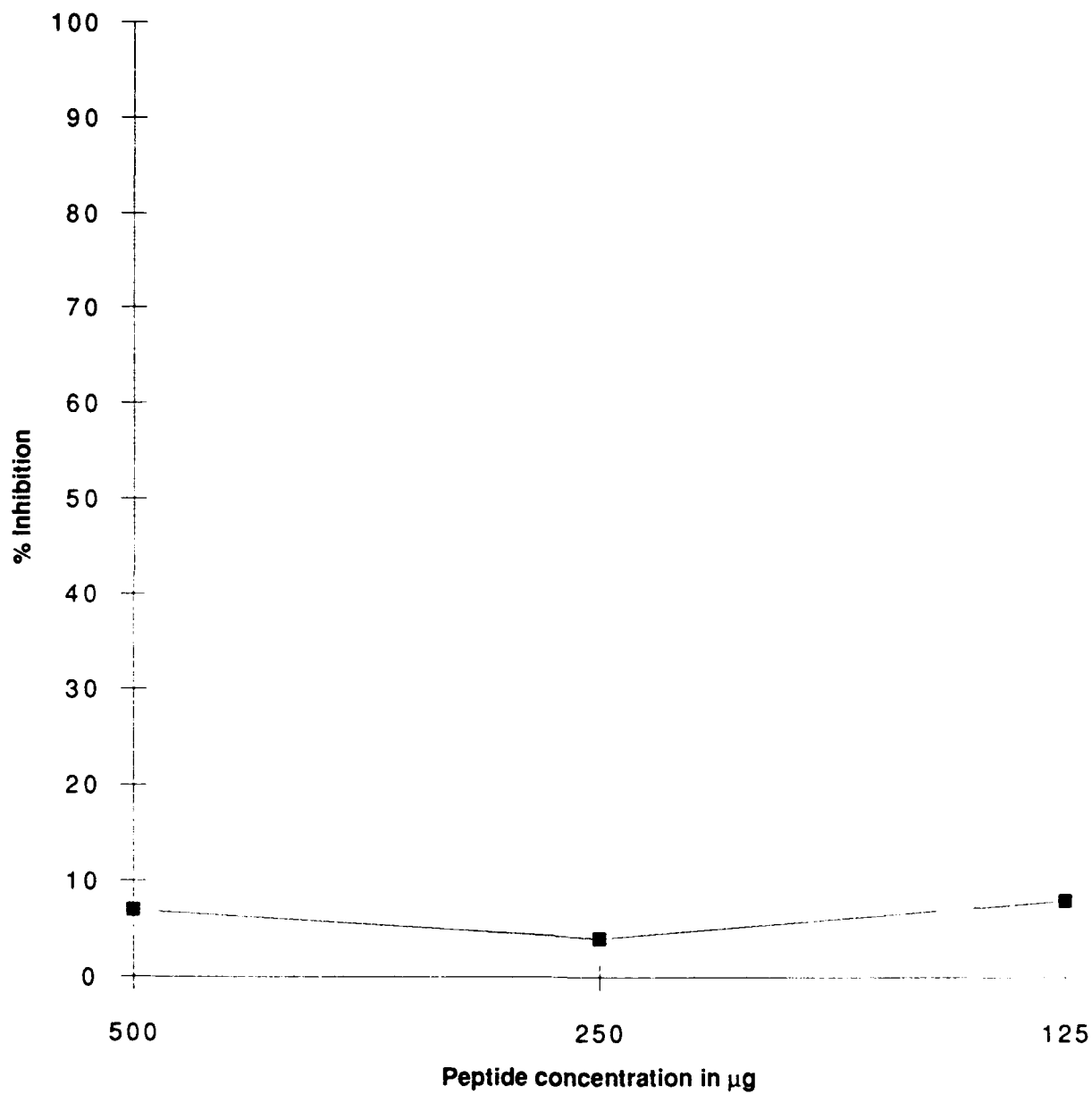


Figure 1

**Competitive inhibition for Receptor Binding
on 3T3 cells by PDGF B (22-36)**

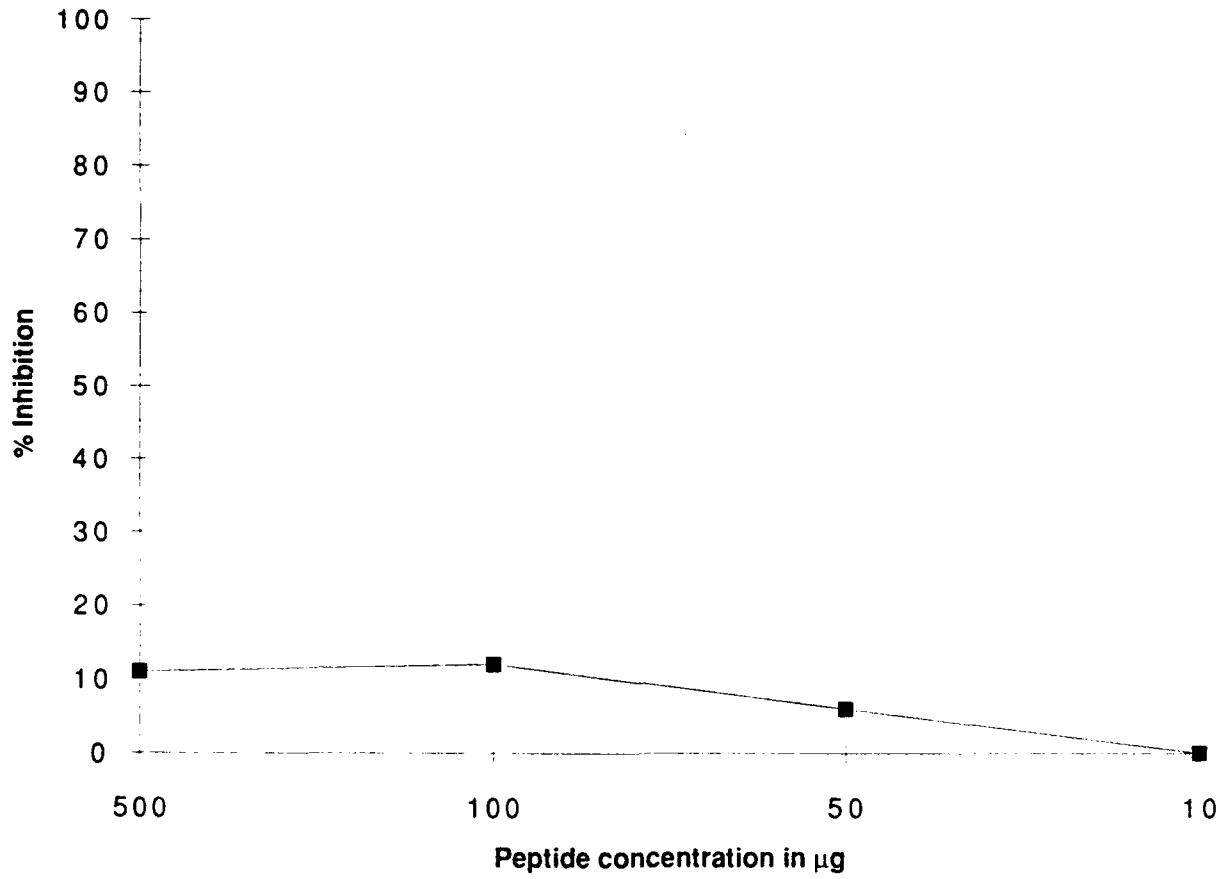


Figure 2

**Competitive Inhibition for Receptor
Binding on 3T3 Cells by PDGF B (101-117)**

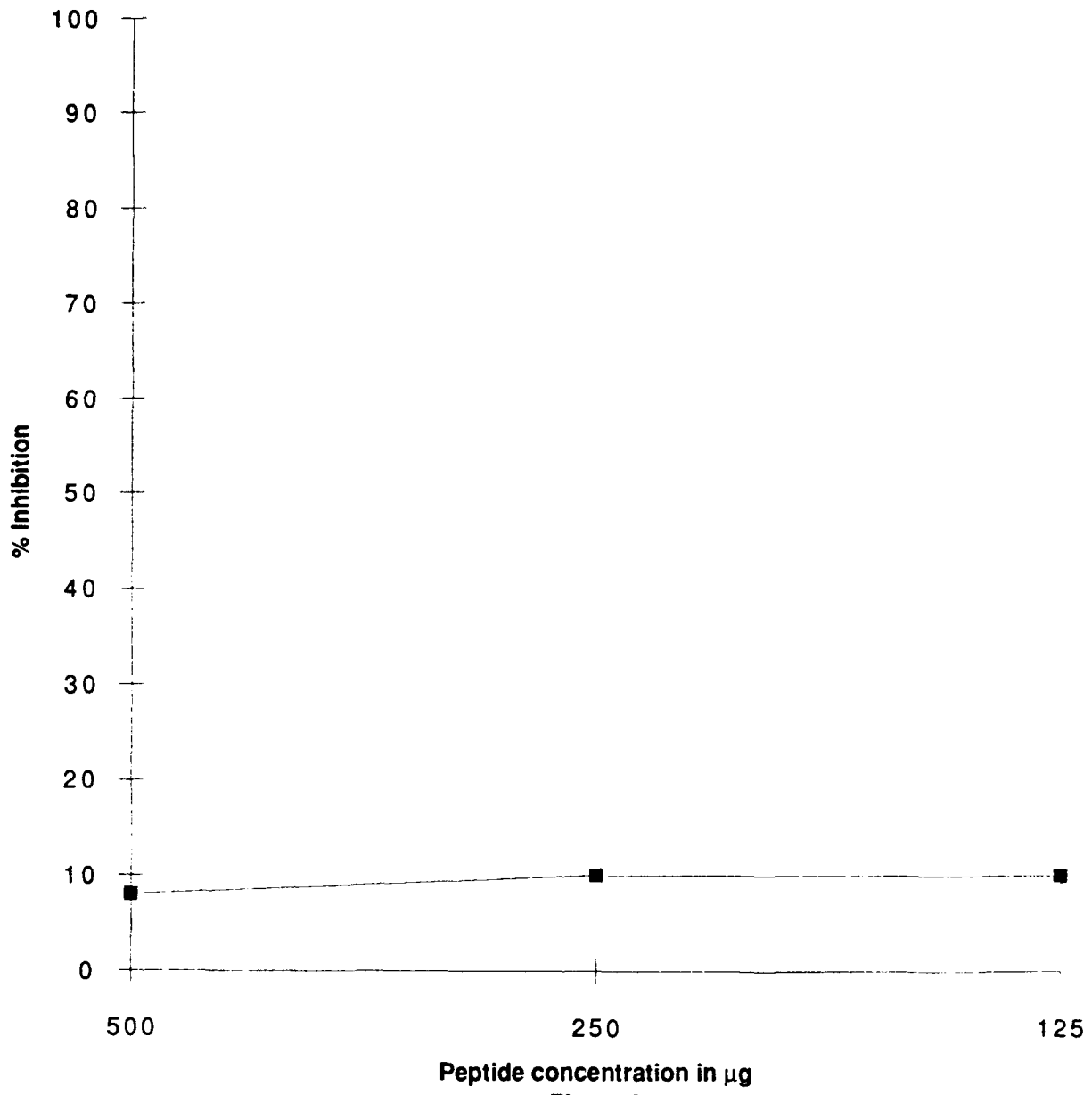


Figure 3

**Competitive inhibition for Receptor Binding
on 3T3 cells by PDGF B (104-116)**

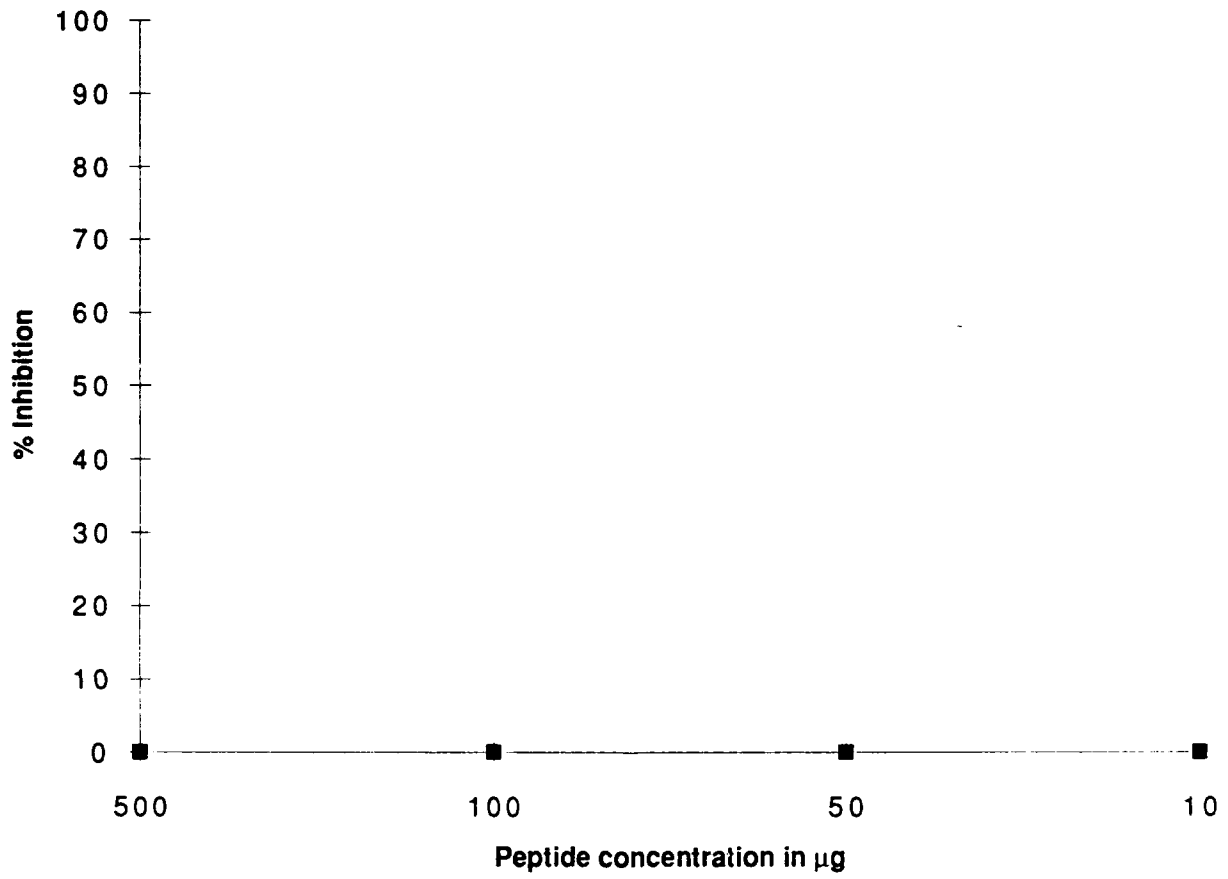
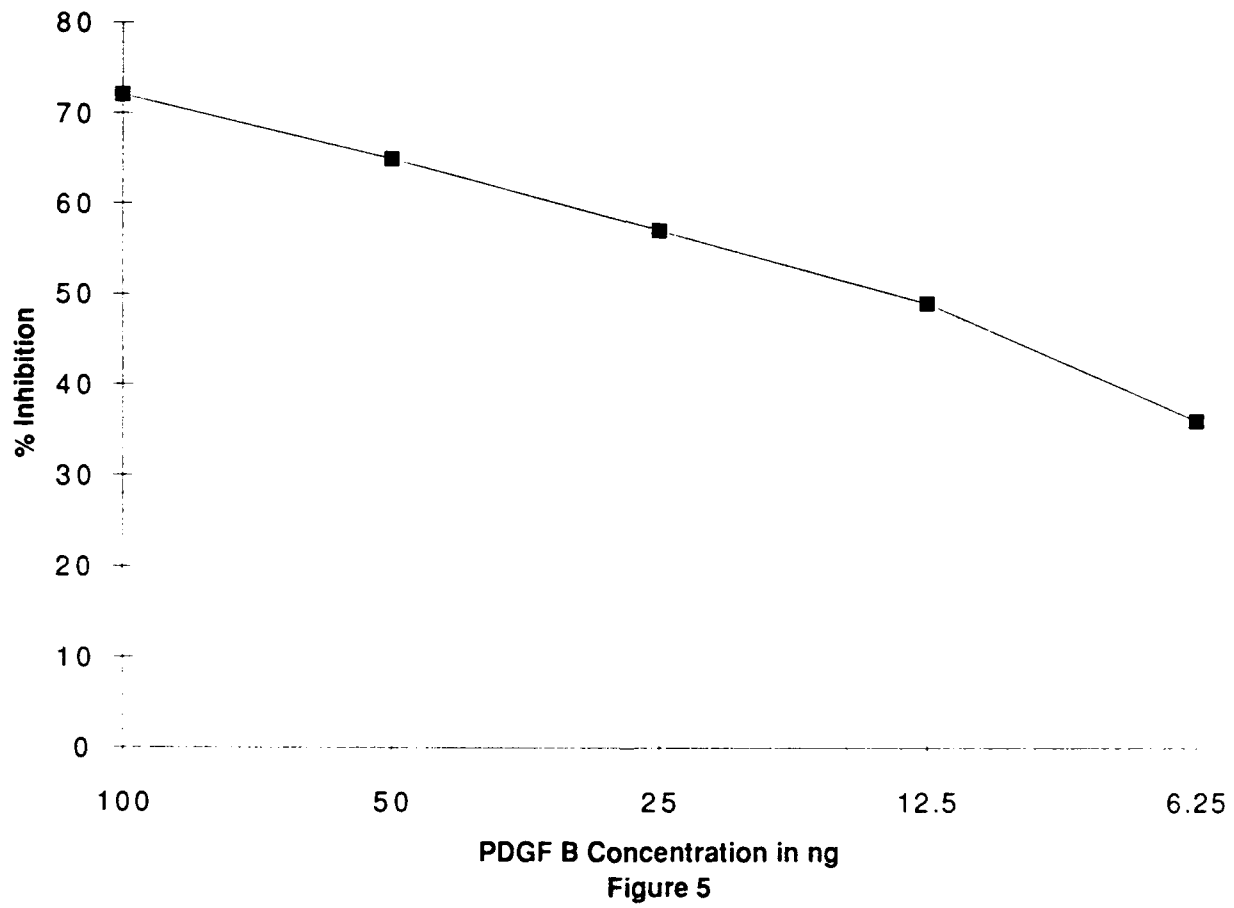


Figure 4

**Competitive Inhibition for Receptor
Binding on 3T3 Cells by PDGF B**



MITOGENESIS ASSAY

The mitogenesis assay was performed on 3T3 cells by measuring the thymidine incorporation. So far we have completed the assay with PDGF. The results are shown in Figure 6. Assays with peptides are in progress.

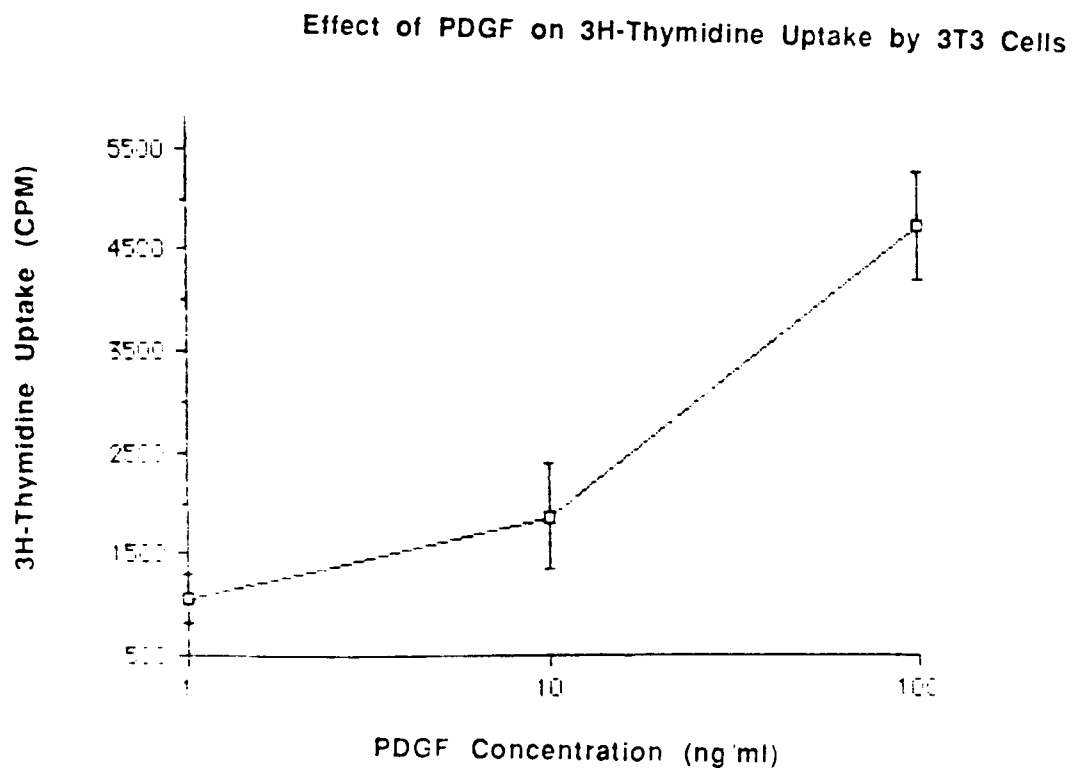


Figure 6

PLANS FOR NEXT QUARTER

During the next quarter we plan to synthesize more peptides; cell binding and mitogenesis assays will continue. Furthermore, we plan to optimize the conditions for chemotaxis assay.