

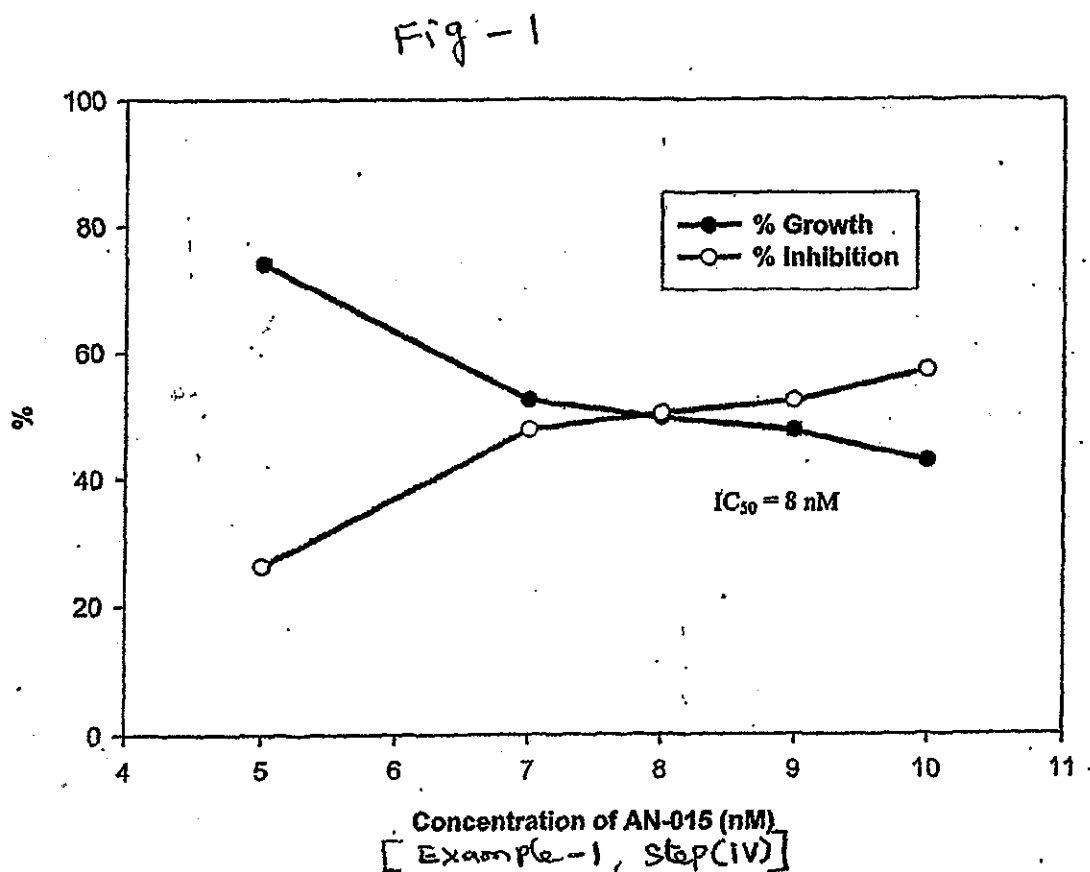


Fig. 1. Effect of Imatinib analog (AN-015) on proliferation of D32p210 cells.

D32p210 cells were grown in RPMI medium supplemented with 10% FBS in humidified air at 5% CO₂ and 37°C. For MTT assay, 5×10^3 cells / well were seeded in a 96-well plate and required drug (AN015) concentration ranging from 1nM to 10 μ M was added and incubated for 24 hrs. Cell proliferation was assessed by incubating the cells with 20 μ l of MTT (5mg/ml stock) for additional 3 hrs followed by addition of lysis buffer to dissolve the formazan crystals. After overnight incubation, the absorbance was recorded using ELISA reader at a dual wavelength of 570-630 nm. After a series of such experiments, a narrower range of concentrations (5 nM to 10 nM) was selected and MTT assay was repeated to determine the IC₅₀ value.

Fig-2

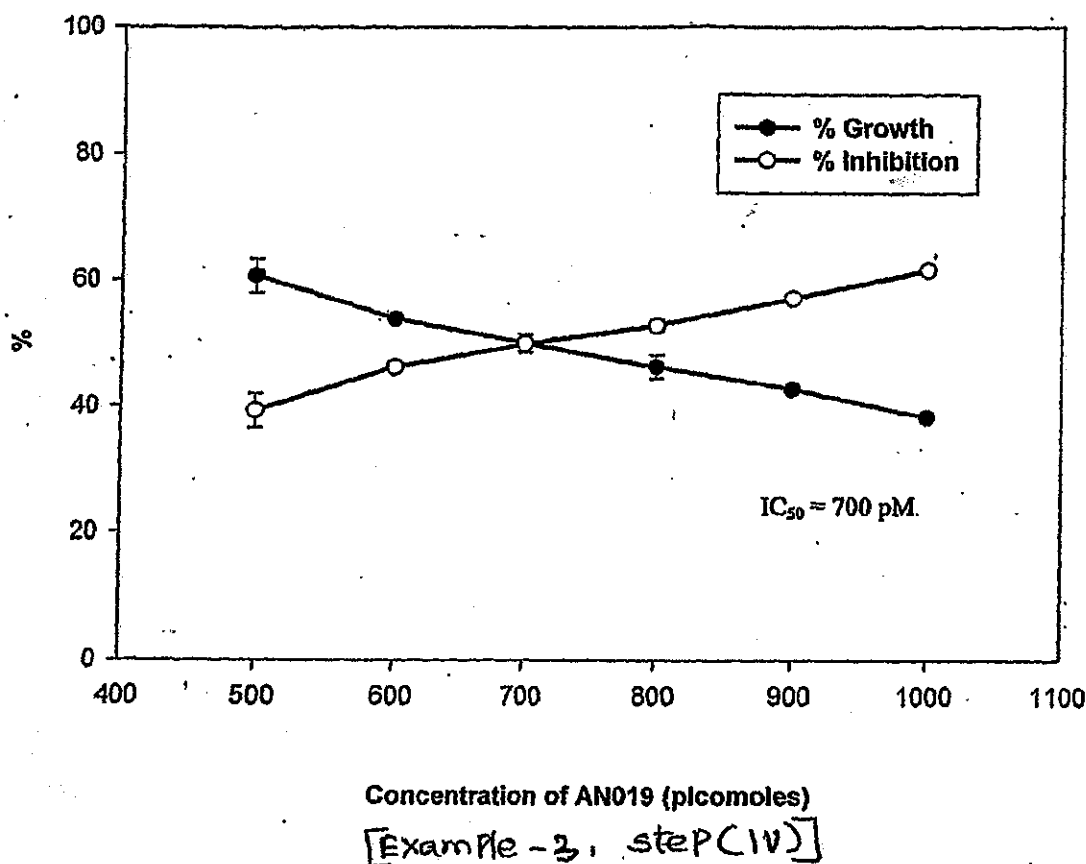


Fig. 2. Effect of Imatinib analog (AN-019) on proliferation of D32p210 cells.

D32p210 cells were grown in RPMI medium supplemented with 10% FBS in humidified air at 5% CO₂ and 37°C. For MTT assay, 5×10^3 cells / well were seeded in a 96-well plate and required drug (AN019) concentration ranging from 100pM to 10 μ M was added and incubated for 24 hrs. Cell proliferation was assessed by incubating the cells with 20 μ l of MTT (5mg/ml stock) for additional 3 hrs followed by addition of lysis buffer to dissolve the formazan crystals. After overnight incubation, the absorbance was recorded using ELISA reader at a dual wavelength of 570-630 nm. After a series of such experiments, a narrower range of concentrations (500 pM to 1 nM) was selected and MTT assay was repeated to determine the IC₅₀ value.

Fig-3



Fig. 3 Agarose gel electrophoresis of DNA extracted from D32p210 cells treated with AN-015 and AN-019

After treatment cells were lysed and total cellular DNA was extracted and electrophoresed on a 1% agarose gel containing 0.05 mg/ml ethidium bromide at 5 V/cm. The gels were then photographed under UV illumination. Lane 1: Control cells; lane 2: Cells treated with 10 nM, AN-015; lane 3: Cells treated with 700 pM, AN-019.

* AN-015 = [Example-1, step (IV)]
* * AN-019 = [Example-3, step (IV)]

Fig -4

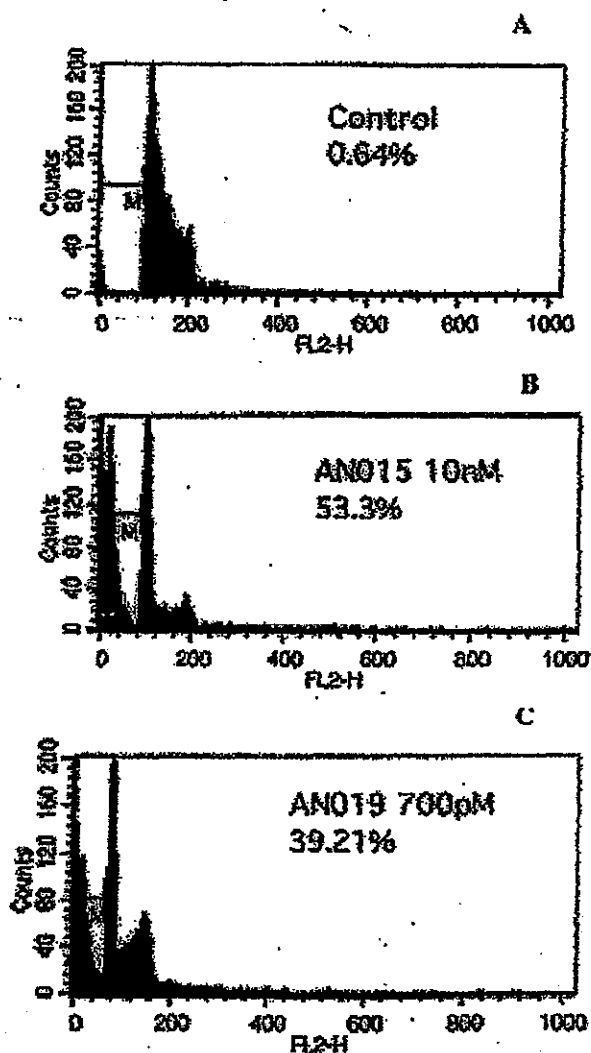


Fig. 4. Flow cytometric analysis of the control and AN-015 and AN-019 treated D32p210 cells.

Cells exposed to 10 nM of AN-015 and 700 pM of AN-019 for 24 h were fixed, and stained with propidium iodide and the DNA content was quantified by FACS. The number of hypodiploid (sub-G0/G1 phase) cells is expressed as a percentage of the total number of cells. (A) Control cells, (B) AN-015, 10 nM, and (C) AN-019, 700 pM.

AN-015 = [Example-1, step (IV)]
 AN-019 = [Example-3, step (IV)]

Fig-5

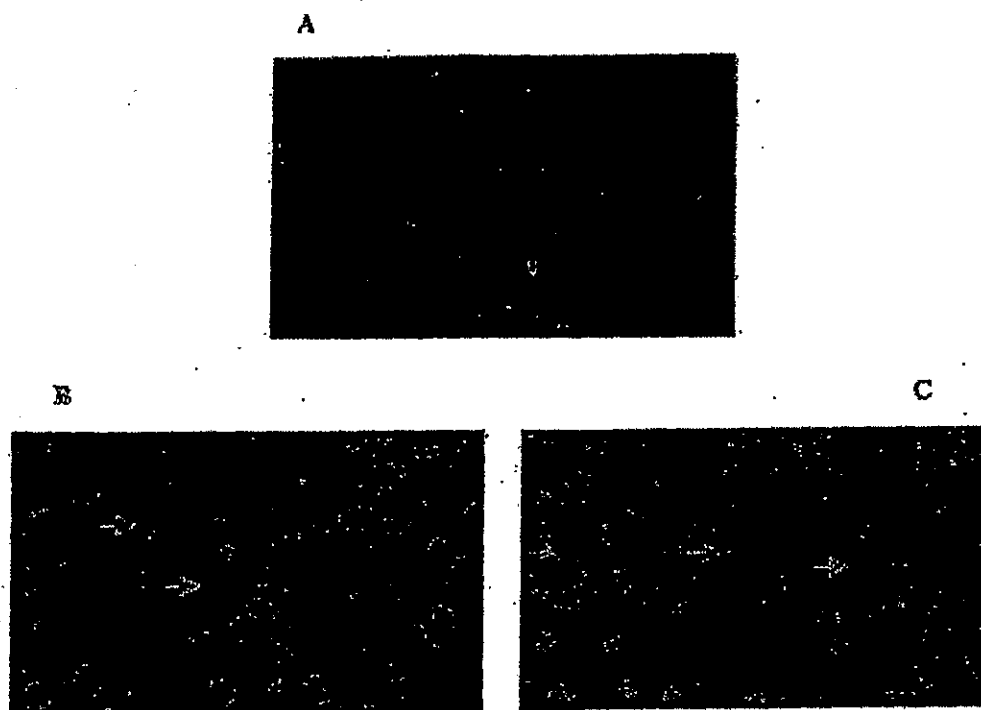


Fig. 5 Phase contrast microscopic studies of D32p210 cells treated with AN-015 (10 nM) and AN-019 (700 pM).

Cells were photographed under phase contrast microscopy (Magnification 400 X). Arrows indicate a typical apoptotic cell with apoptotic bodies. (A) Control cells, (B) AN-015, 10 nM, and (C) AN-019, 700 pM.

AN-015 = [Example-1, step (iv)]
AN-019 = [Example-3, step (iv)]

Sample	IACH/RL/040205/001	NIMS/YD/040205/002
<u>Imatinib</u>		
1nM	6.1%	1.3%
10nM	16.4%	1.9%
100nM	19.0%	5.1%
1μM	20.0%	20.8%
2μM	12.4%	19.2%
5μM	20.0%	22.9%
10μM	5.0%	19.2%
<u>AN015</u>		
1nM	9.0%	3.8%
10nM	10.0%	10.0%
100nM	20.7%	20.7%
200nM	7.5%	21.0%
500nM	10.0%	19.8%
1μM	11.6%	22.3%
<u>AN019</u>		
100pM	9.0%	4.5%
500pM	20.7%	8.3%
1nM	21.6%	9.5%
2nM	37.2%	15.6%
5nM	31.5%	15.5%
10nM	28.4%	15.4%

FIG - 6

AN015 = [Example-1, step (iv)]

AN019 = [Example-3, step (iv)]

