

Bowman-Birk Protease Inhibitor

(11-13)

가

(1,2)

40%

globulin

protease inhibitor

hemagglutinin, saponin, phytic

glycinin albumin

legumelin

acid

50 60%

glycinin

(14,15)

conglycinin cytochrome C, β - amylase, 1.8S

가

glubuline, hemagglutinin, lipoxy- genase, protease inhibitor (3,4)

가 , protease inhibitor

가

protease inhibitor

protease inhibitor

가

(5-7)

가

가

(8)

protease

protease inhibitor

가

inhibitor

Bowman-Birk protease inhibitor

.

protease inhibitor

(BBPI)

cysteine

(9)

BBPI가

protease

(10)

protease

inhibitor가

1. Protease inhibitor

6%
 (16-18) protease inhibitor Kunitz
 trypsin inhibitor(KTI), Bowman-Birk protease
 inhibitor(BBPI) isoinhibitor

1) Kunitz trypsin inhibitor

1946 Kunitz KTI (19-22)
 Fig. 1 181
 2 2 disulfide
 가 22,000
 (23,24) Tia, Tib, Tic 3
 isoinhibitor가
 (25,26)
 가 (27-29) Protease
 trypsin chymotrypsin
 motrypsin 가 8.3
 (30) 가 가

2) Bowman-Birk protease inhibitor

Bowman (30,31) BBPI
 Fig. 2 71
 14
 cysteine 가
 cysteine 2
 7 disulfide bond (32,33)
 KTI 가 (30,34) KTI
 8,000 (30,35,36) Protease

trypsin
 (Lys-Ser) chymotrypsin
 (Leu-Ser)가
 protease 가 1.0
 (36)

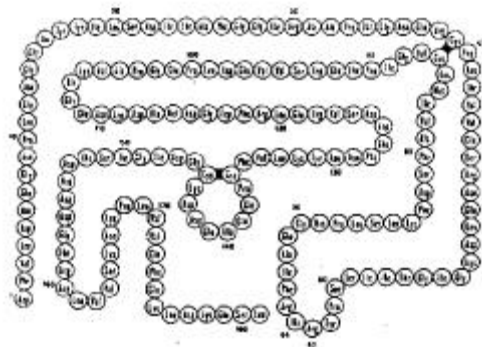


Fig. 1. Amino acid sequence of the soybean Kunitz trypsin inhibitor(From Koide, T. and Ikenaka, T., *Eur. J. Biochem.*, 32, 417 (1973))

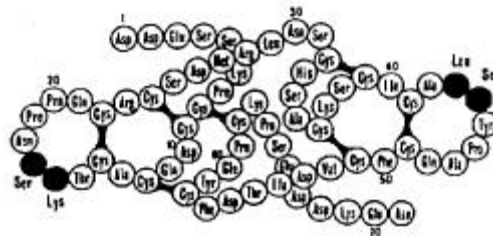


Fig. 2. Amino acid sequence of the soybean Bowman -Birk protease inhibitor (From Odani, S. and Ikenaka, T., *J. Biochem.*, 74, 697 (1973))

3) BBPI isoinhibitors

BBPI BBPI
 isoinhibitor가
 가 1977
 Odani (37) CM-cellulose

fury BBPI trypsin inhibitor-A B, C-II, D-II, E-1 4 isoinhibitor C-II trypsin chymotrypsin 1 elastase 가 double headed trypsin inhibitor , D-II 75 trypsin 가 isoinhibitor (38-39) Hwang (16) DEAE-cellulose (45)

Tracy

PI-I PI-V 5 protease inhibitor PI-V가 BBPI inhibitor 66 70 10 12 cysteine 가 6800 7000 isoinhibitor Stahlhut Hymowitz (40) Amsoy 71 BBPI 4 isoinhibitor Tan-Wilson (41) Amsoy DEAE-cellulose 10 BBPI isoinhibitor protease , BBPI group BBPI group I BBST-E가 A' BBPI 3 , group IV BBST-A cysteine glycine 가 isoinhibitor crude BBPI gel filtration 10 BBPI isoinhibitor (42) (43)

BBPI 7 iso- inhibitor

2. BBPI

protease inhibitor가 initiation, promotion progrssion 가 (44) protease inhibitor BBPI BBPI chymotrypsin

1) *in vitro* system BBPI

1993 Kennedy (46) BBPI, BBIC (Bowman-Birk inhibitor concentrate), PBBI(purified Bowman-Birk inhibitor) chymotrypsin inhibitor 가 hamster DMBA(7,12- dimethyl- benz [a]anthrancene) tumor (Fig. 3), BBIC 5 tumor PBBI 1% 가 BBIC PBBI tumor protease inhibitor DMBA 45 inhibitor tumor BBIC chymotrypsin

Table 1. Anticarcinogenesis *in vivo* system by soybean BBI and(or) BBIC

Researcher	cancer-inducing agents	target cancer	system	addition type	Ref.
Weed, H. et al.	dimethylhydrazine (DMH)	colon	mouse	dietary addition of soybean extract	51
St. Clair, W. et al.	dimethylhydrazine (DMH)	colon, liver	mice	dietary addition of BBPI	52
Billings, P.C. et al.	dimethylhydrazine (DMH)	colon, anal gland	mice	protease inhibitor	53
Messaff, P. et al.	7,12-dimethylbenz(a)anthracene	oral	hamster	protease inhibitor	54
Witschi, H. et al.	hamster cheek pouch	lung	mice	soybean derived BBPI	55
von Hofe et al.	nitrobenzylamine and potato chymotrypsin inhibitor. (From Kennedy, A.R., Billings, P.C., Maki, P.A. and Newberne, P., Nutr. Cancer, 19, 191 (1993))	esophageal neoplasms	rat	BBPI	56

Fig. 3. Prevention of hamster cheek pouch carcinogenesis by BBIC, BBPI and potato chymotrypsin inhibitor. (From Kennedy, A.R., Billings, P.C., Maki, P.A. and Newberne, P., Nutr. Cancer, 19, 191 (1993))

가 BBIC

Yavelow (45) protease inhibitor

Min mouse BBIC

0.5% BBPI

42 50%, 41% BBPI (47)

Table 2.

Fernandes Banerji (48) BP(benzopyrene) 3.

field bean protease inhibitor BBPI 가 BBPI

. Becke (49) 18 BBPI

Troll (50) protease inhibitor가 chymotrypsin (44,46,66,67) BBPI

74% (68-72) oncogene gene

BBPI (c-myc, c-fos) 가 (73-75) Yavelow

Table 1. cell (45) proteolytic enzyme

in vitro BBPI BBPI

(57) 1993 Kennedy BBPI C3H10T1/2 Bloom syndrome (chromosomal

Table 2. Anticarcinogenesis *in vitro* system by soybean BBI and(or) BBIC

Researcher	Cancer-inducing agents	Target cancer	System	Addition type	year
Kennedy, A.R. et.al.	radiation	transformation		protease inhibitor	58
Long, S. et.al.	TPA	plasminogen activator	mouse fibroblast	protease inhibitor antipain	59
Kennedy, A.R. et.al.	radiation	transformation		antipain	60
Baturay, N.Z. et.al.	pyrene(cocarcinogen) benzo(a)pyrene	malignant transformation	cultured mouse fibroblast	soybean extract containing BBPI	61
Billings, P.C. et.al.	radiation	transformation	C3H10T 1/2 cells	potato chymotrypsin inhibitor	62
Billings, P.C. et.al.	radiation	transformation	C3H/10T 1/2 cells	potato carboxypeptidaseI	63
Billings, P.C. et.al.	radiation	transformation	C3H10T 1/2 cells	specific protease substrate	64
St. Clair, W.H. et.al.	radiation	DNA	cellular and tissue	BBPI	65

abnormality)

. Kennedy (46)

1% BBIC 가

⁽⁷⁶⁾, DNA RNA

⁽⁷⁷⁾

가

(Fig. 4).

(Fig. 5) BBIC 가

^(78,79)

BBPI

BBIC

가

4. BBPI

BBPI

(80).

BBPI

BBPI

가

가

BBPI

protease inhibitor

trypsin inhibitor KTI

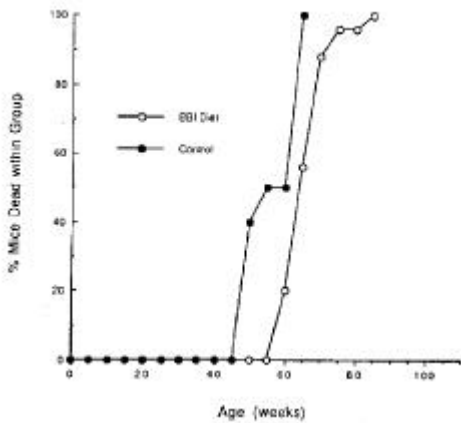


Fig. 4. Mortality curve for mice maintained on normal diets or those containing 1% BBIC. (From Kennedy, A.R., Szuhaj, B.F., Newberne, P.M. and Billings, P.C., *Nutr. Cancer*, 19, 281 (1993))

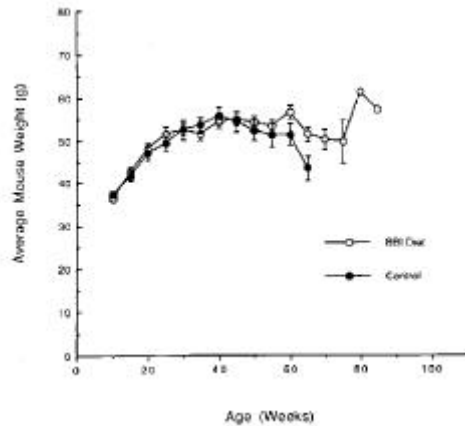


Fig. 5. Growth curves for mice maintained on normal diets or those containing 1% BBIC. (From Kennedy, A.R., Szuhaj, B.F., Newberne, P.M. and Billings, P.C., *Nutr. Cancer*, 19, 281 (1993))

가
 가 KT1
 가 BBPI BBPI가 chymotrypsin
 가 protease inhibitor
 가 BBPI protease inhibitor
 가

1. Rackis, J.J. and Gumbmann, M.R. (1981) Protease inhibitors : Physiological properties and nutritional significance. In "Antinutrients and natural toxicants in

foods." Ory, R.L., Ed., Food and Nutrition Press. Westport, CT, pp.203-237
 2. (1992), 1992.11., pp77-81
 3. Catasimpoalas, N., Roger, D.A., Circle, S.J. and Meyer, E.W. (1967) Purification and structural studies of the 11S component of soybean protein. *Cereal Chem.*, 44:631
 4. Hill, J.E. and Breidenbach, R.W. (1974) Proteins of soybean seeds. I. Isolation and characterization of major component. *Plant, Physiol.*, 53:744
 5. Lightner, J. and Pearce G. (1993) Isolation of signaling mutants of tomato (*Lycopersicon esculentum*). *Mol. Gen. Genet.*, 241:595
 6. Laskowski, M. Jr. and Kato, I. (1980) Protein inhibitors of proteinase. *Ann. Rev. Biochem.*, 49:593

7. Richardson, H. (1977) The Proteinase inhibitors of plant and microorganism. *Phytochem.*, 16:159
8. Birk, Y. (1976) Proteinase inhibitors from plant sources. *Methods Enzymol.*, 45:695
9. Tan-Wilson, A.L., Hartl, R.M., Delfel, N.F. and Wilson, K.A. (1985) Differential expression of Kunitz and Bowman-Birk inhibitor in plant and callus tissue. *Plant Physiol.*, 78:310
10. Baumgartner, B. and Chrispeels, M.J. (1976) Partial characterization of a proteinase inhibitor which inhibits the major endopeptidase present in the cotyledons of mug beans. *Plant Physiol.*, 58:1
11. Ryan, C.A. (1968) Synthesis of chymotrypsin inhibitor in potato leaflets induced by detachment. *Plant Physiol.*, 43:1859
12. Green, T.R. and Ryan, C.A. (1972) Wound-induced proteinase inhibitor in plant leaves.- A possible defence mechanism against insect. *Sci.*, 175:776
13. Graham, J.S., Pearce, G., Merryweather, J., Titani, K., Wriesson, L. and Ryan, C.A. (1985) Wound-induced proteinase inhibitor from tomato leaves. *J. Biol. Chem.*, 261:6555
14. Westfall, R.J. and Hague, S.M. (1948) The nutritive quality and the trypsin inhibitor of soybean flour heated at various temperature. *J. Nutr.*, 35:379
15. Lyman, R.L. and Lepkovsky, S. (1957) The effect of raw soybean meal and trypsin inhibitor diets on pancreatic enzyme secretion in the rat. *J. Nutr.*, 62:269
16. Hwang, D.L.R., David, L., Yang, W.K. and Foard, D.E. (1977) Purification, partial characterization and immunological relationships of multiple low molecular weight protease inhibitor of soybeans. *Biochim. Biophys. Acta.*, 495:369
17. Rackis, J.J. and Anderson R.L. (1964) Isolation of four soybean trypsin inhibitors by DEAE-cellulose chromatography. *Biochem. Biophys. Res. Comm.*, 15:230
18. Ryan, C.A. (1973) Proteolytic enzymes and their inhibitors in plants. *Ann. Rev. Plant Physiol.*, 24:173
19. Kunitz, M. (1945) Crystallization of trypsin inhibitor from soybean. *Sci.*, 101:668
20. Kunitz, M. (1946) Crystalline soybean trypsin inhibitor. *J. Gen. Physiol.*, 29:149
21. Kunitz, M. (1947) Crystalline soybean trypsin inhibitor. *J. Gen. Physiol.*, 30:291
22. Kunitz, M. (1947) Isolation of crystalline protein component of trypsin and soybean trypsin inhibitor. *J. Gen. Physiol.*, 30:311
23. Koide, T. and Ikenaka, T. (1973) Studies on trypsin inhibitor. III. Amino acid sequence of the carboxyl terminal region and the complete amino acid sequence of soybean trypsin inhibitor(Kunitz). *Eur. J. Biochem.*, 32:417
24. Yamamoto, M. and Ikenaka, T. (1967) Studies on soybean trypsin inhibitor. I. Purification and characterization of two soybean trypsin inhibitor. *J. Biochem.*, 62:141
25. Hymowitz, T. and Hadley, H.H. (1972) Inheritance of a trypsin inhibitor variants in seed protein of soybean. *Crop Sci.*, 12:197

26. Orf, J.H. and Hymowitz, T. (1979) Inheritance of the absence of the Kuintz trypsin inhibitor in seed protein of soybeans. *Crop Sci.*, 19:107
27. , (1985) Trypsin inhibitor . Trypsin inhibitors DEAE-Cellulose . , 10:107
28. , (1986) Trypsin inhibitor . Trypsin inhibitors , 11:51
29. , , (1988) Trypsin inhibitor . Trypsin inhibitors , 20:63
30. Bowman, D.E. (1948) Further differentiation of bean trypsin inhibiting factors. *Arch. Biochem. Biophys.*, 16:109
31. Bowman, D.E. (1946) Differentiation of soybean antitryptic factors. *Proc. Soc. Exp. Biol.*, 63:547
32. Birk, Y. (1985) The Bowman-Birk inhibitor : Trypsin and chymotrypsin inhibitor from soybeans. *Int. J. Peptide Protein Res.*, 25:113
33. Odani, S. and Ikenaka, T. (1973) Studies on soybean trypsin inhibitor. V . Disulfide bridges on soybean Bowman-Birk inhibitors. *J. Biochem.*, 74:697
34. Birk, Y. and Gertler, A. (1961) Effect of mild chemical and enzymatic treatments of soybean meal and soybean trypsin inhibitors on their biochemical properties. *J. Nutr.*, 75:379
35. Odani, S., Koide, T. and Ikenaka, T. (1972) Studies on soybean trypsin inhibitors. *J. Biochem.*, 71:831
36. Odani, S. and Ikenaka, T. (1972) Studies on soybean trypsin inhibitor . Complete amino acid sequence and anti-proteinase sites of Bowman-Birk soybean proteinase inhibitor. *J. Biochem.*, 71:839
37. Odani, S. and Ikenaka, T. (1977) Isolation and partial characterization of four soybean double headed proteinase inhibitor. *J. Biochem.*, 82:1513
38. Odani, S. and Ikenaka, T. (1977) Studies on soybean trypsin inhibitor. XI. Complete amino acid sequence of a soybean trypsin-chymotrypsin-elastase inhibitor C-II. *J. Biochem.*, 82:1523
39. Odani, S. and Ikenaka, T. (1978) Studies on soybean trypsin inhibitor. XII. Linear sequence of two soybean double headed trypsin inhibitor D-II and E- II. *J. Biochem.*, 83:737
40. Stahlhut, R.W. and Hymowitz, T. (1983) Variation in the low molecular weight proteinase inhibitors of soybean. *Crop Sci.*, 23:766
41. Tan-Wolson, A.L. Cosgratt, S.E., Duggan, M.C. Obach, R.S. and Wilson, K.A. (1985) Bowman-Birk proteinase isoinhibitors complements of soybean strain. *J. Agric. Food Chem.*, 133:389
42. , , (1989) Bowman-Birk protease inhibitor , 32:116
43. (1989) Isolation, characterization and immunological analysis of soybean

- Bowman-Birk trypsin inhibitor and iso inhibitors.
44. Kennedy, A.R. (1993) Cancer prevention by protease inhibitors. *Preventive Medicine*, 22:796
 45. Yavelow, J., Collins, M., Birk, Y., Troll, W. and Kennedy, A.R. (1985) Nanomolar concentrations of Bowman-Birk soybean protease inhibitor suppress X-ray induced transformation *in vitro*. *Proc. Natl. Acad. Sci.(USA)*, 82:5395
 46. Kennedy, A.R., Billings, P.C., Maki, P.A. and Newberne, P. (1993) Effects of various protease inhibitor preparations on oral carcinogenesis in hamsters induced by 7,12-dimethylbenz[a]anthracene. *Nutr. Cancer*, 19:191
 47. Kennedy, A.R., Beazer-Barclay, Y., Kinzler, K.W. and Newberne, P.M. (1996) Suppression of carcinogenesis on the intestines of min mice by the soybean-derived Bowman-Birk inhibitor. *Cancer Res.*, 56:679
 48. Fernandes, A.O. and Banerji, A. (1995) Inhibition of benzopyrene-induced forestomach tumors by field bean protease inhibitors. *Carcinogenesis*, 16:1843
 49. Becke, F.F. (1981) Inhibition of spontaneous hepatocarcinogenesis in C3H/HeN mice by edl Pro A, an isolated soy protein. *Carcinogenesis*, 11:1213
 50. Troll, W. and Wiesner R. (1980) Soybean diet lowers breast tumor incident in irradiated rats. *Carcinogenesis*, 1:469
 51. Weed H., McGandy R.B., Kennedy A.R. (1985) Protection against dimethylhydrazine induced adenomatous tumors of the mouse colon by dietary addition of an extract of soybeans containing the Bowman-Birk protease inhibitor. *Carcinogenesis*, 6:1239
 52. St. Clair W., Billings, P., Carew J., Keller-McGandy C., Newberne P., Kennedy A.R. (1990) Suppression of DMH-induced carcinogenesis in mice by dietary addition of the Bowman-Birk protease inhibitor. *Cancer Res.*, 50:580
 53. Billings P.C., Newberne P., Kennedy A.R. (1990) Protease inhibitor suppression of colon and anal gland carcinogenesis induced by dimethylhydrazine. *Carcinogenesis*, 11:1083
 54. Messadi P.V., Billings P., Shklar G., Kennedy A.R. (1986) Inhibition of oral carcinogenesis by a protease inhibitor. *J. Natl. Cancer Inst.*, 76:447
 55. Whischi, H., Kennedy, A.R. (1989) Modulation of lung development in mice with the soybean-derived Bowman-Birk protease inhibitor. *Carcinogenesis*, 10:2275
 56. von Hofe, Newberne P.M., Kennedy A.R. (1991) Inhibition of N-nitrosomethyl benzyleamine induced esophageal neoplasms by the Bowman-Birk protease inhibitor. *Carcinogenesis*, 12, 2147
 57. Kennedy, A.R., Szuhaj, B.F., Newberne, P.M. and Billings, P.C. (1993) Preparation and production of a cancer chemopreventive agent, Bowman-Birk inhibitor concentrate. *Nutr. Cancer*, 19:281
 58. Kennedy A.R. and Little J.B. (1981) Effects of protease inhibitors on radiation transformation *in vitro*. *Cancer Res.*, 41:2103
 59. Long S., Quigley J., Troll W. and

- Kennedy A.R. (1981) Protease inhibitor antipain suppresses TPA induction of plasminogen activator in transformable mouse embryo fibroblasts. *Carcinogenesis*, 2:933
60. Kennedy A.R. (1982) Antipain, but no cycloheximide, suppresses radiation transformation when present for only one day at five days post-irradiation. *Carcinogenesis*, 3:1093
61. Baturay N.Z. and Kennedy A.R. (1986) Pyrene acts as a cocarcinogen with the carcinogens, benzo(a)pyrene, -propiolactone and radiation in the induction of malignant transformation of cultured mouse fibroblasts : Soybean extract containing the Bowman-Birk inhibitor acts as an anticarcinogen. *Cell Biol. Toxicol.*, 2:21
62. Billings P.C., St. Clair W., Ryan C.A. and Kennedy R. (1987) Inhibition of radiation-induced transformation of C3H/10T 1/2 cells by chymotrypsin inhibitor I from potatoes. *Carcinogenesis*, 8:809
63. Billings P.C., Morrow A.R., Ryan C.A. and Kennedy A.R. (1989) Inhibition of radiation-induced transformation of C3H/10T 1/2 cells by carboxypeptidase Inhibitor I and Inhibitor II from potatoes. *Carcinogenesis*, 10:687
64. Billings P.C., Habres J.M. and Kennedy A.R. (1990) Inhibition of radiation-induced transformation of C3H10T 1/2 cells by specific protease substrates. *Carcinogenesis*, 11:329
65. St. Clair W.H. and Kennedy A.R. (1991) Comparison of the Bowman-Birk protease inhibitor to WR1065 for protection against radiation-induced DNA, cellular and tissue damage. *Cancer J.*, 4:278
66. Kennedy A.R. (1985) The conditions for the modification of radiation transformation in vitro by a tumor promoter and protease inhibitors. *Carcinogenesis*, 6:1441
67. St. Clair W.H. (1991) Suppression of 3-methylcholanthrene-induced cellular transformation by timed administration of the Bowman-Birk protease inhibitor. *Carcinogenesis*, 12:935
68. Billings P.C., Carew J.A., Keller-McGandy C.E., Goldberg A. and Kennedy A.R. (1987) A serine protease activity in C3H/10T 1/2 cells that is inhibited by anticarcinogenic protease inhibitors. *Proc. Natl. Acad. Sci.(USA)*, 84:4801
69. Billings C., St. Clair W., Owen A.J. and Kennedy A.R. (1988) Potential intracellular target proteins of the anticarcinogenic Bowman-Birk protease inhibitor identified by affinity chromatography. *Cancer Res.*, 48:1798
70. Billings, P.C. and Habres, J.M. (1992) A growth regulated protease activity which is inhibited by the anticarcinogenic Bowman-Birk protease inhibitor. *Proc. Natl. Acad. Sci. (USA)*, 89:3120
71. Billings, P.C., Habres, J.M., Liao, D.C. and Tuttle S.W. (1991) A protease activity in human fibroblasts which is inhibited by the anticarcinogenic Bowman-Birk protease inhibitor. *Cancer Res.*, 51:5539
72. Carew J.A. and Kennedy A.R. (1990) Identification of a proteolytic activity

- which responds to anticarcinogenic protease inhibitors in C3H10T 1/2 cells. *Cancer Lett.*, 49:153
73. Caggana M. and Kennedy A.R.(1989) C-fos mRNA levels are reduced in the presence of antipain and the Bowman-Birk inhibitor. *Carcinogenesis*, 10:2145
74. Chang J.D., Li J.H., Billings P.C. and Kennedy A.R. (1990) Effects of protease inhibitors on c-myc expression in normal and transformed C3H10T 1/2 cells. *Mol. Carcinogenesis*, 3:226
75. Chang J.D. and Kennedy, A.R. (1993) Suppression of c-myc by anticarcinogenic protease inhibitors. In : Troll Walter, Kennedy A.R., Eds., *Protease Inhibitors as Cancer Chemopreventive Agent.*, New York, Plenum, pp265- 280
76. Kennedy A.R., Radner B. and Nagasawa H. (1984) Protease inhibitors reduce the frequency of spontaneous chromosome abnormalities in cells from patients with Bloom syndrome. *Proc. Natl. Acad. Sci.(USA)*, 81:1827
77. Flick M.B. and Kennedy A.R. (1991) Effect of protease inhibitors on DNA amplification in SV40-transformed Chinese hamster embryo cells. *Cancer Lett.*, 56: 102
78. Goldstein, B.D., Witz G., Amoruso, M. and Troll, W. (1979) Protease inhibitors antagonize the activation of polymorphonuclear leukocyte oxygen consumption. *Biochem. Biophys. Res. Comm.*, 88:854
79. Witz, G., Goldstein, B.D., Amoruso, M., Stone D.S. and Troll, W. (1980) Retinoid inhibition of superoxide anion radical production by human polymorphonuclear leukocytes stimulated with tumor promoters. *Biochem. Biophys. Res Comm.*, 97:883
80. Von Hofe, E., Brent R. and Kennedy, A.R. (1990) Inhibition of C-ray induced exencephaly by protease inhibitors. *Radiat. Res.*, 123:108
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