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마자인의 카나비노이드가 인체피부흑색종세포에 미치는 억제효과

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The Inhibitory Effects of Cannabinoids of Cannabis Against Human Skin Melanoma Cells

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요 약

제라니올 (1), 올리비톨 (2), 카나비노이드 (3 과 4)와 5-플로르우라실 (5)을 MTT 정량분석법과 SRB 정량분석법으로 인체 피부흑색종세포에 대하여 성장 억제효과를 평가 하였다. 이들 화합물(1, 2, 3, 4와 5)은 마이크로 몰 농도의 범위에 대하여 억제 활성을 나타내었다. 일반적으로 이들 화합물은 1 μM - 100 μM 농도범위에서는 투여량에 따라 항암활성을 나타내었다. 인체 피부흑색종세포에 대한 이들 화합물의 50 %억제 농도 효과에 대한 비교는 다음과 같은 순서로 항암활성이 감소하였다. MTT 정량분석법 ; OLVTL >CBG > CBD > 5-FU >GRNL, SRB 정량분석법 ; CBG > OLVTL > CBD > GRNL > 5-FU. 카나비노이드 (3 과 4)와 5-플로르우라실 (5)을 MTT정량분석법과 SRB정량분석법으로 인체정상세포에 대하여 독성효과를 측정하였다. 이들 화합물은 마이크로몰농도의 농도범위에서는 투여량에 따라 세포독성을 보였다. 인체정상세포에 대한 이들 화합물의 50 % 독성효과에 대한 비교는 다음과 같은 순서로 세포독성이 감소하였다. MTT정량분석법과 SRB정량분석법 ; CBD > 5-FU > CBG. 따라서, 카나비지놀 (3)은 인체정상세포에 대하여 가장 낮은 세포독성을 나타내었다. 따라서 카나비지놀 (3)은 인체 피부흑색종세포에 대하여 가장 강한 성장억제활성을 보였다.

※ 본 논문은 일부 원광대학교 연구지원에 의하여 수행되었음.

I . Introduction

Cannabis is the material derived from the herbaceous plant Cannabis sativa, which grows vigorously many regions of the world. It occurs in male and female forms, with both sexes having large leaves which consist of five to 11 leaflets with serrated margins. A sticky resin which covers the flowering tops and upper leaves is secreted most abundantly by the female plant and this resin contains the active agents of the plant. While the cannabis plant contains more than 60 cannabinoid compounds, such as cannabidiol and cannabinol, the primary psychoactive constituent is delta-9- tetrahydrocannabinol or THC,[1)] the concentration of which largely determines the potency of the cannabis preparation. Most of the other cannabinoids are either inactive or only weakly active, although they may increase or decrease potency by interacting with THC.[2)]

Cannabis has veen erroneously classified as a narcotic, as a sedative and most recently as an hallucinogen. While the cannabinoids do possess hallucinogenic properties, together with stimulant and sedative effects, they in fact represent a unique pharmacological class of compounds. Unlike many other drugs of abuse, cannabis acts upon specific receptors in the brain and periphery. The discovery of the receptors and the naturally occurring substances in the brain that bind to these receptors is of great importance, in that it signifies an entirely new pathway system in the brain. Cannabis has had a long history of medical and therapeutic use in India and the Middle East3) where it has been variously used as an analgesic, anti-convulsant, anti-spamodic, anti-emetic and hypnotic.

The cannabinoids, the active constituents of the herbaceous plant Cannabis sativa L. (marihuana, hashish, bhang), have been known to affect many biological systems.4) Delta-9-tetrahydrocannabinol (delta-9-THC) belongs to a class of compounds known as cannabinoids which are responsible for the psychoactive properties of marijuana. The cannabinoids produce in man and animals a complex pattern of pharmacological effects, some of which are unique to this class of compounds.5) Delta-9-tetrahydrocannabinol is considered to be the predominant compound in preparation of Cannabis sativa L. responsible for the CNS effects in humans.1) The recognized CNS responses to these preparations include alterations in cognition and memory, euphoria, and sedation. Potential therapeutic applications of cannabis preparations that are of either historical or contemporary interest include analgesia, attenuation of the nausea and vomiting of cancer chemotheraphy, appetite stimulation, decreased intestinal motility of diarrhea, decreased bronchial constriction of asthma, decreased intraocular pressure of glaucoma, antirheumatic and antipyretic actions, and treatment of convulsant disorders. These effects have been reviewed recently.1,6)

Dronabinol (Marinol, Roxane Laboratories, Columbus, OH) is delta-9-tetra-hydrocannabinol formulated in sesame oil. It was approved in the U.S. in 1986 for treatment of cancer chemotherapy-induced nausea and vomiting refractory to other agents.7) Cannabidiol (4) is

a non-psychoactive constituent of Cannabis sativa L. Both hashish and marijuana are derived from cannabis and have been used for centuries for their medicinal and psychotomimetic properties.8) Cannabidiol (4) has been reported to possess potentially important pharmacological properties as an anti-epileptic,9) anxiolytic,10) and as an antidyskinetic agent.11) Watson and his colleagues12) reported that cannabigerol (3) and cannabinol methyl ether were not sensitizers. Most of the cannabinoids were found to be allergenically cross-reactive. In addition, it was shown that the presence of a free 1′-hydroxyl group was required for sensitization, but not to elicit a response in sensitive animals. Eisohly et al.13) have reported that cannabigerol type compounds having a methyl side chain in most cases exhibit an increased antifungal and antibacterial activities.

In a recent paper14), we reported the significant antitumor efficacy of cannabigerol (3) in a IC50 value of 31.30 μM in vitro MTT assay.

In hopes of finding better therapeutic agents for antitumor activity, we report here on cannabinoids (3 and 4) and their antitumor activities against human skin melanoma cell lines.

Fig. 1. *Cannabis sativa L.*

Ⅱ. Experimental

Ⅱ-1. Materials and Instruments

Ⅱ-1-1. Instruments

IR spectra were recorded on a Perkin-Elmer 457 grating infrared spectrophotometer. ^{1}H-NMR spectra were obtained on a Bruker WH-200 and WH-300 pulsed FT spectrometers. Chemical shifts are given in parts per million downfield from Me_4Si internal standard. Mass spectra were recorded on a Varian Mat CH-5 mass spectrometer. Analytical TLC was performed by using commercially available silica plates (polygram sil N-HR/UV_{254}), and the plates were visualized with fast blue phenol reagent. Medium pressure liquid chromatography was performed on an ALTEX glass column, 1 meter long, diameter 9 mm internal using an FMI pump and silica gel 60 (230-400 mesh) purchased from Merck. Fractions were collected with LKB 2070 or LKB 7000 fraction collectors at a rate of 2-10 ml/min.

Ⅱ-1-2. Chemicals

5-Fluorouracil, 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyl-2*H*-tetrazolium bromide, Fetal bovine serum, Sulforhodamine B protein was obtained from Sigma Chemical Co., Ltd. (St. Louis, USA). Olivetol and geraniol were purchased from Aldrich Chemical Co., Ltd. (Milwaukee, U.S.A.).

Cannabidiol (CBD)[15] was kindly provided by Prof. R. Mechoulam, in the Department of Natural Products, at School of Pharmacy, Hebrew University, Israel. All other chemicals were of reagent grade.

Ⅱ-1-3. Tumor cell lines and culture conditions

Tumor cells for the experiments were obtained from Korean Cell Line Bank in the Seoul National University. In vitro RPMI-1640 medium supplemented with 10 % FBS, streptomycin 0.1 % mg/ml and penicillin 100 units/ml at 37 ℃ in 5 % carbon dioxide, Cells were dissociated with 0.25 % trypsin just before transferring for experiment and were counted by Hemcytomer. The cytotoxic activities of cannabinoids (3 and 4) and 5-fluorouracil (5) against SK-MEL-3 cells and NIH 3T3 fibroblast were measured by the MTT and SRB methods.

Ⅱ-2. Experimental Methods

Ⅱ-2-1. Preperation of cannabigerol

Cannabigerol (3) obtained was identified by comparison of its spectral data (TLC, MS,

NMR and IR) with those published or by direct comparison with an authentic sample.[14]

Ⅱ-2-2. Evaluation of antitumor activity

The antitumor activity of cannabinoids (3 and 4) and 5-fluorouracil (5) was determined by the modification of the literature methods.[16-18] Percentage of antitumor activity was estimated by the following equation;

(mean A 540, 520 with sample) / (mean A 540, 520 without sample) X 100

All experimental data were expressed as the mean S.D. of three experiments. Student's t-test was used for statistical analysis.

II-2-2-1. 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyl-2H- tetrazolium bromide (MTT) assay

The assay is dependent on the cellular reduction of water-soluble MTT (Sigma Chemical Co. St. Louis, M.O.) by mitochondrial dehydrogenase of vial cells to a blue water-insoluble formazan crystal product which can be measured spectrophotometrically.[16,18] SK-MEL-3 cell lines were cultured in RPMI-1640 medium (Gubco Laboratories) containing 10 % fetal bovine serum. Exponentially growing tumor cells (5×10^5) were cultured for 48 hrs at 37 ℃ in a humidified 5 % CO_2 incubator in the presence or absence of cannabinoids (3 and 4) and 5-fluorouracil (5). MTT was added to each well and incubated at 37℃ for further 3hrs. After 3 hrs incubation at 37 ℃, the supernatant was removed from each well and 100 ㎕ of 100 % DMSO was added to solubilize the formazan crystals which were formed by the cellular reduction of MTT. After thorough mixing with a mechanical plate mixer, absorbance spectra were read on ELISA Reader (SPECTRAmax 250, Molecular Devices, USA) at a wavelength of 540 nm and a reference wavelength of 650 nm (absorbance peak for DMSO). All measurements were carried out in triplicates. There was good reproducibility between replicate wells with standard errors below-10 % (Fig. 1).

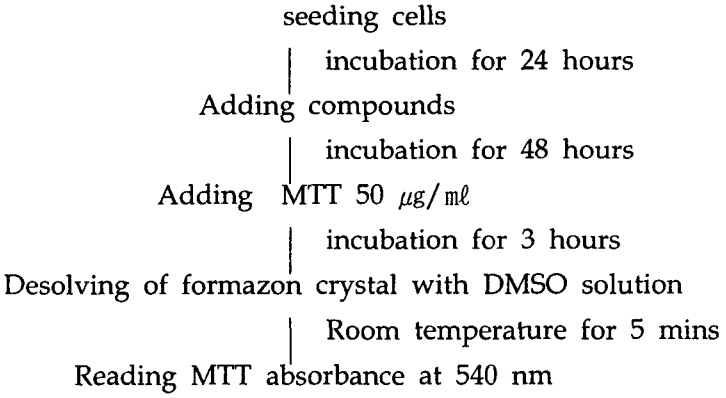


Fig. 1. Flow scheme of MTT assay.

Ⅱ-2-2-2. Sulforhodamine B protein (SRB) assay

The SRB assay was performed essentially according to the method of Skehan et al.[19] The methods of plating and incubation of cells were identical to those cells of the MTT assay.

TCA-fixed cells were stained for 30 minutes with 0.4 % (wt/vol) SRB dissolved in 1 % acetic acid. At the end of the staining period, SRB was removed and cultures were quickly rinsed four times with 1 % acetic acid to remove unbound dye. The acetic acid was poured directly into the culture wells from a beaker. This procedure permitted rinsing to be performed quickly so that desorption of protein-bound dye did not occur. Residual wash solution was removed by sharply flicking plates over a sink, which ensured the complete removal of rinsing solution. Because of the strong capillary action in 96-well plates, draining by gravity alone often failed to remove the rinse solution when plates were simply inverted. After being rinsed, the cultures were air dried until no standing moisture was visible. Bound dye was solubilized with 10 mM unbuffered Tris base (pH 10.5) for 5 minutes on a gyratory shaker.

Asorbance was read on ELISA reader (SPECTRAmax 250, Molecular Devices, USA). For maximum sensitivity, Asorbance was measured at 564 nm. Because readings were linear with dye concentrations only below 1.8 Asorbance units, however, suboptimal wavelengths were generally used, so that all samples in an experiment remained within the linear Asorbance range. With most cell lines, wavelengths of approximately 490-530 nm worked well for this purpose (Fig. 2).

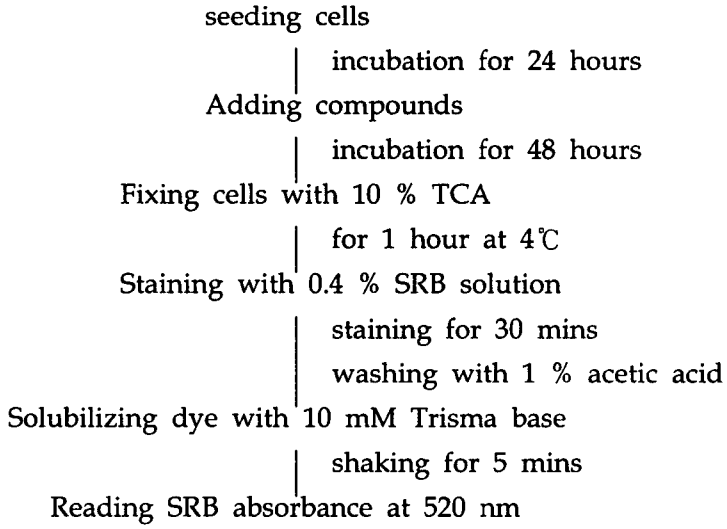


Fig. 2. Flow scheme of SRB assay.

Ⅱ-3. Evaluation of toxicity : Cytotoxicity assay.

In order to determine the cytotoxicity mediated by compounds (1, 2, 3, 4 and 5) the colorimetric assay was used. These compounds were serially diluted in EMEM (Eagle's

minimum essential medium) with 10 % FBS and mixed with equal volume of NIH 3T3 fibroblast (5 x 10^4 cells/ml). After one hour, fresh culture medium was supplied to a total volume of 1-100 μM. On the third day of incubation at 37 ℃ an incubator MTT terazolium dye (5 mg/ml ; 20 $\mu\ell$/well ; Polyscience, Inc. Warrigton, PA) was added to the cells. After 3h, the absorbance was measured at 540 nm (ELISA reader, SPECTRAmax 250, Molecular Devices, USA). Percentage of cytotoxicity was calculated using the following formula;

(mean A 540, 520 with sample) / (mean A 540, 520 without sample) X 100

All experimental data were expressed as the mean S.D. of three experiments. Student's t-test was used for statistical analysis. The 50 % cytotoxic dose (CD_{50}) was calculated using the computer program.

Ⅱ-4. Morphology

Changes in the morphology of SK-MEL-3 cells cultured in a medium with compounds (1, 2, 3, 4 and 5) were documented by microphotography.

Ⅱ-5. Statistical Analysis

All values, expressed as mean ± S. D., were statistically analyzed through analysis of Student's t-test. The P value less than 0.05 was considered as significant.

Ⅲ. Results

In vitro antitumor activity

Tables 1-6 show the antitumor activities of compounds (1, 2, 3, 4 and 5) against SK-MEL-3 cells. In general, the antitumor activities of these compounds in a were dose-dependent, and the susceptibility of the cancer cell lines to cannabigerol (3)(Table 1) was quite sensitive. The value of IC_{50} of cannabigerol (3) showed that cannabigerol (3) exerts the most potent antitumor activity (Table 6).

Toxicity

A colorimetric assay was used to detect the in vitro cytotoxicity mediated by cannabigerol (3). As shown in Fig. 3, cannabigerol (3)-mediated cytotoxicity was rapidly increased in the MTT assay when its concentration was raised from 50 μM to 100 μM. However, cannabigerol (3) was not changeable in MTT assay and SRB assay when its concentration was increased from 1 μM to 50 μM (Fig. 4). The values of MTT_{50} and SRB_{50} determined were at 60.46 μM and 82.98 μM, respectively(Table 12).

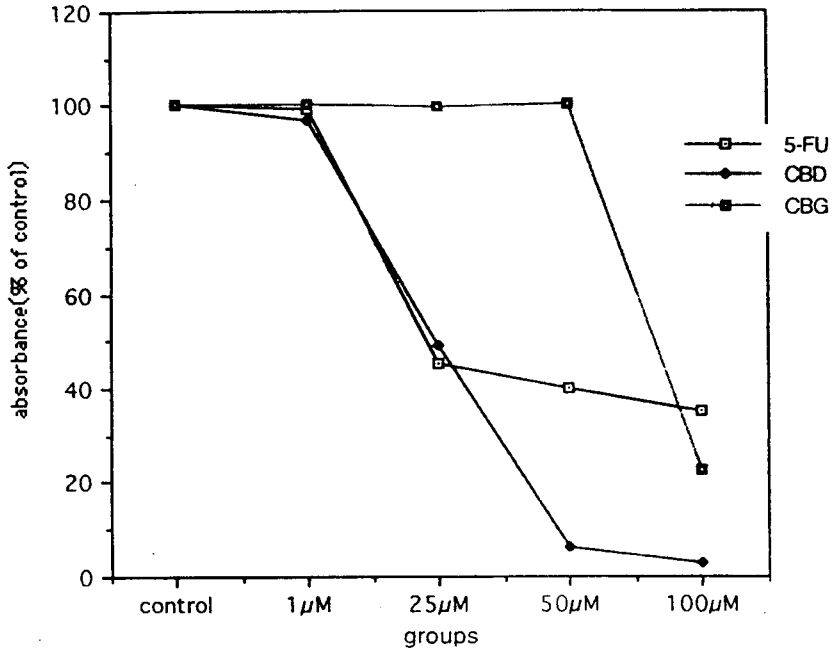


Fig. 3. In vitro cytotoxicities of cannabinoids (3 and 4) and 5-fluorouracil (5) by MTT assay. These compounds were serially diluted in RPMI-1640 with 10 % FBS and mixed with equal volume of NIH 3T3 fibroblasts (5 x 104 cells). The colorimetric assay was performed as described in Materials and Methods. Data are mean values of results obtained from 3 sets of experiments.

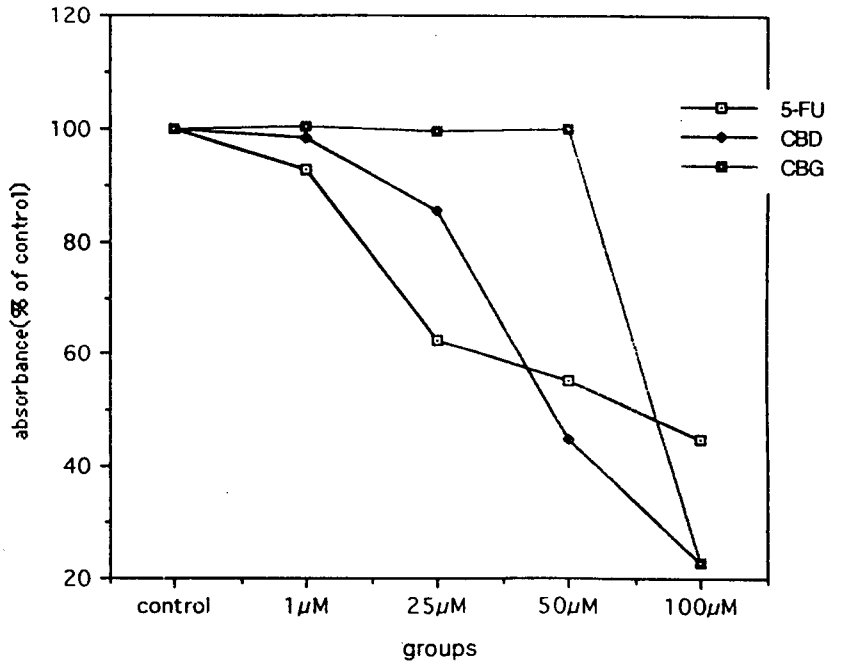


Fig. 4. In vitro cytotoxicities of cannabinoids (3 and 4) and 5-fluorouracil (5) by SRB assay. These compounds were serially diluted in RPMI-1640 with 10 % FBS and mixed with equal volume of NIH 3T3 fibroblasts (5 x 104 cells). The colorimetric assay was performed as described in Materials and Methods. Data are mean values of results obtained from 3 sets of experiments.

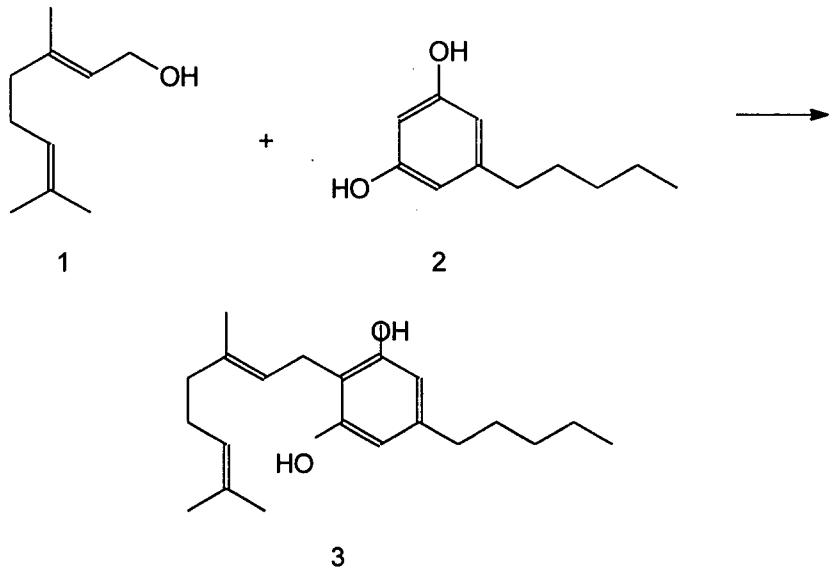
Ⅳ. Discussion

The present study shows the in vitro growth inhibitory activities of cannabinoids (3 and 4) and 5-fluorouracil (5) as a reference compound against SK-MEL-3 cell lines (Fig. 5 and Fig. 6). 5-Fluorouracil (5-FU) is commonly used as a therapeutic agent to treat cancers of the large bowel. However, therapy with 5-fluorouracil (5) as a single agent has only limited success. Additive agents or modulators for the 5-fluorouracil (5) effect are needed for more effective treatment of this highly resistant malignancy.[20)]

Table 1, 2 and 3 show the anti-tumor activities of cannabinoids (3 and 4) and 5-fluorouracil (5) against SK-MEL-3 cells. In general, the antitumor activities of these compounds (1, 2, 3, 4 and 5) were dose-dependent over the micromolar concentration range 1 to 100 μM, and the susceptibility of SK-MEL-3 cells to these compounds was quite different (Table 1-5). The comparison of IC_{50} values of these compounds in tumour cell lines shows that their susceptibility to these compounds decreses in the following order: OLVTL > CBG > CBD > 5-FU > GRNL in MTT assay, CBG > OVTL > CBD > GRNL> 5-FU in SRB assay (Table 6). Cannabigerol (3) was evaluated for antitumor efficacy against SK-MEL-3 cells. The antitumor activity of cannbigerol (3) against the tested cancer cell lines is given in Table 1. However, cannabigerol (3)[21)] was the most effective growth inhibitor of SK-MEL-3 cell lines, producing an IC_{50} of about 40 μM in MTT assay and 46 μM in SRB assay. The sulforhodamine B protein stain assay was compared with the tetrazolium (MTT) colorimetric assay for in vitro chemosensitivity testing of SK-MEL-3 cells. The SRB assay appeared to be more sensitive than the MTT assay, with a better linearity with cell number and higher reproducibility. Olivetol (OLVTL, 2), the simplest compound of this series of phenolics, was more potent than 5-fluorouracil (5) as a reference compound. This compound is structurally related to cannabinoids (3 and 4). However, the antitumor activity of cannabidiol (4) exhibit less active than that of olivetol on SK-MEL-3 cells(Tables 2 and 4).

Tables 7, 8 and 9 show the cytotoxic activities of cannabinoids (3 and 4) and 5-fluorouracil (5) against NIH 3T3 fibroblasts. In general, the cytotoxic activities of these compounds (3, 4 and 5) were in a dose-dependent manner over the concentration range 1 to 100 μM, and the susceptibility of NIH 3T3 fibroblasts to these compounds was quite different (Tables 7-11). The comparison of CD_{50} values of these compounds in NIH 3T3 fibroblasts shows that their susceptibility to these compounds decrease in the following order ; CBD > 5-FU > CBG in MTT assay and SRB assay (Table 12). However, cannabigerol (3) was the least cytotoxic effect of NIH 3T3 fibroblast, producing a CD_{50} of about 60 μM in MTT assay and 83 μM in SRB assay. Cannabigerol (3) was more potent than 5-flurouracil (5) as a reference compound. The activity of cannabidiol (4) exhibits more activity than 5-fluorouracil (5) on NIH 3T3 fibroblasts (Table 7). Cannabigerol (3) is structually related to geraniol with, a known inhibitory effect,[22)] inhibited the least effective

growth-inhibitory activity against the tested cancer cell lines. The compounds used are known to inhibit the activity of several enzymes of the arachidonate cascade including cyclo- oxygenase and lipoxygenase[23] and to both stimulate and inhibit phospholipase A_2 activity.[24] The actions of the cannabinoids on membrane associated enzymes are complex and dose-dependent. Although these actions are possibly associated with the ability of the cannabinoids to act as anticancer agents,[7] specific structural alterations may be critical in determining enzyme targets. Cannabigerol (3) has been selected as lead compounds for further examinations.



1. Silica,BF_3-OEt_2 in CH_2Cl_2,RT,2days

Fig. 5. Synthesis of cannabigerol (3)

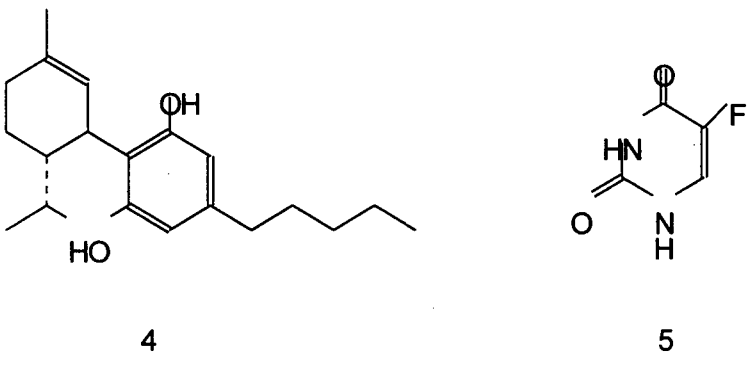


Fig. 6. The structures of cannabidiol (4) and 5-fluorouracil (5)

V. Summary

Geraniol (1), olivetol (2), cannabinoids (3 and 4) and 5-fluorouracil (5). were tested for their growth inhibitory effects against SK-MEL-3 cell lines using two different 3-(4,5-dimethylthiazol- 2yl)-2,5-diphenyl-2*H*-tetrazolium bromide (MTT) assay and sulforhodamine B protein (SRB) assay. These compounds showed inhibitory activity in vitro in the micromolar range against SK-MEL-3 cell lines. In general, the antitumor activity of these compounds (1, 2, 3, 4 and 5) was in a dose-dependent over the micromolar concentration range 1 to 100 μM. The comparison of IC_{50} values of these compounds in tumor cell lines shows that their susceptibility to these compounds decrease in the following order : OLVTL > CBG > CBD > 5-FU > GRNL in MTT assay, CBG > OLVTL > CBD > GRNL > 5-FU in SRB assay. Cannabinoids (3 and 4) and 5-fluorouracil (5) were tested for their cytotoxic effects on NIH 3T3 fibroblasts using two differnt MTT assay and SRB assay. These compounds exhibited petent cytotoxic activities in vitro in the micromolar range against NIH 3T3 fibroblasts. In general, the cytotoxic acivities of these compounds (3, 4 and 5) were in a dose-dependent over the micromolar concentraion range 1 to 100 μM. The comparison of CD_{50} values of these compounds on NIH 3T3 fibroblasts shows that their susceptibility to these compounds decrease on the following order ; CBD > 5-FU > CBG in MTT assay and SRB assay. Cannabigerol (3) was shown the least cytotoxic activity on NIH 3T3 fibroblasts. Cannabigerol (3) exhibited the most growth-inhibitory activity against SK-MEL-3 cell lines.

VI. REFERENCES

1. Gaoni, Y., and Mechoulam, R.: Isolation, structure and partial synthesis of an active constituent of hashish. *J. Am. Chem. Soc.* 86, 1646-1647 (1964).
2. Abood, M. E. and Martin, B. R. Neurobiology of marijuana abuse. Trends in Pharmacol. Sci. 13, 201-206 (1992).
3. a) Mechoulam, R.: The pharmacohistory of cannabis sativa. In Cannabinoid as therapeutic Agents, Boca Raton, Florida, CRC Press Inc., (1986). b) Nahas, G. G.: The medical use of cannabis. In Marihuana in science and Medicine. New York, Raven Press (1984).
4. Martin, B.: Cellular effects of cannabinoids. *Pharmacol. Rev.* 38, 45-74 (1986).
5. Dewey, W. L.: Cannabinoid pharmacology. *Pharmacol. Rev.* 38, 151-178 (1986).
6. Hollister, L. E. : Health aspects of cannabis. *Pharmacol. Rev.* 38, 1-20 (1986).
7. Plasse, T. F., Gorter, R, W., Krasnow, S. H., Lane, M., Shepard, K. V., and Wadleig H, R. G.: Recent clinical experience with dronabinol. *Pharmacol. Biochem. Behav.* 40, 695-700 (1991).
8. Mechoulam, R., Shani, A., Edery, H. and Grunfeld, Y.: Chemical basis of hashish activity. Science, 169, 611-612(1970)
9. Consroe, P., Martin, A. and Singh, V.: Antiepileptic potential cannabidiol analogs. *J. Clin. Pharmacol.* 21. 428s-436s(1981).
10. Guimaraes, F. S., Chiaretti, T. M., Graeff, F. G. and Tuard, A. W.: *Psychopharmacol.* 100, 558-559(1990).
11. Conti, L. H., Johannesen, J., Musty, R. and Consroe, P.: Marihuana : An International Research Report, Chesher, G., Consroe, P. and Musty, R. eds. 153-156, Australian Government Puhl. Service, Australia (1988).
12. Watson, E. S., Murphy, J. C., and Turner, C. E.: Allergenic properties of naturally occurring cannabinoids. *Pharmacol. Biochem. Behav.* 72, 954-955 (1983).
13. Eisohly, H. N., Turner, C. E., Clark, A. M., and Eisohly, M. A.; Synthesis and antimicrobial activities of certain cannabichromene and cannabigerol related compounds. *J. Pharm. Sci.* 71, 1319-1323 (1982).
14. a) Baek, S. H., Han, D. S., Yook, C. N., Kim, Y. C., and Kwak, J. S.: Synthesis and antitumor activity of cannabigerol. *Arch. Pharm. Res.* 19, 228-230 (1996). b) Baek, S. H.,

Yook, C. N., and Han, D. S.: Boron trifluoride etherate on alumina-A modified Lewis acid reagent (V). A convenient single step synthesis of cannabinoids. *Bull. Kor. Chem. Soc.* 16, 293-296 (1995).

15. We thank Porf. R. Mechoulam, in the Department of Natural Products, at School of Pharmacy, Hebrew Univesity for kindly providing us with sample of authentic cannabidiol.
16. Mosmann, T. J.: Rapid colorimetric assays for cellular growth and survival : application to proliferation and cytotoxicity assays. *Immunol. Methods,* 65, 55-63 (1983).
17. Carmichael, J., Degraff, W. G., Gazdar, A. F., Minna, J. D., and Mitchell, J. B.: Evaluation of a tetrazolium-based semiautomated colorimetric assay: Assessment of chemosensitivity testing. *Cancer Res.* 47, 936-942 (1987).
18. Keepers, Y. P., Pozao, P. E., Peters, G. J., Otte, J. A., Winograd, B. and Pinedo, H. M.: Comparison of the suforhoda,ine B protein and tetrazolium (MTT) assays for in vitro chemosensitivity testing. *Eur. J. Cancer.* 27. 897-900 (1991)
19. Skehan, P., Storeng, S., Studiero, D., Monke, A., McMahon, J., Vistica, D., Warren, J. T., Bodesh, H., Kenny, S. and Boyd, M. R.: New colorimetric cytotoxicity Assay for Anticancer-drug screening National cancer institute, 82, 1107-1112, 1990.
20. Boersma, H. H., Woerdenbag, H. J., Kampinga, H. H., Konings, A. W. T., Bauer J. and Scheithauer, W. : Modulation of 5-fluorouracil-induced cytotoxity by quercetin in two human colorectal cancer cell lines, *Planta Med.* 59. A682 (1993).
21. Formukong, E. A., Evans, A. T. and Evans, F. J.: The inhibitory effects of cannabinoids, The active constituents of *Cannabis sativa L.* on human and rabbit platelet aggregation. *J. Pharm. Pharmacol.* 41, 305-309 (1989).
22. Baik, K. U., and Ahn, B. Z.: Cytotoxic activities of some geranylated flavones against L 1210 cell. *J. Pharm. Soc. Korea,* 32, 125-128, (1988).
23. Evans, A. T., Formukong, E. A., and Evans, F. J.; Actions of cannabis constituents on enzymes of arachidonate metabolism. Antiinflammatory potential. *Biochem. Pharmacol.* 36, 2037-2040 (1987).
24. Evans, A. T., Formukong, E. A., and Evans, F. J.; Activation of phospholipase A_2 by cannabinoids: lack of correlation with CNS effects. *FEB. Lett.* 211, 119-122 (1987).

Table 1. The Antitumor Activity of Cannabigerol(3) by MTT Assay and SRB assay on SK-MEL-3 Cells.

Concentration (μM)	MTT assy		SRB assay	
Control	0.93±0.06	(100.0)	1.86±0.02	(100.0)
1	0.88±0.03	(94.7)	1.85±0.02	(99.6)
25	0.88±0.03***	(94.7)	1.85±0.03	(99.8)
50	0.14±0.02***	(14.8)	1.85±0.02	(99.5)
100	0.04±0.00***	(4.8)	1.64±0.04**	(87.9)

Cells were incubated for 48 hr. The cells were harvested with Trypsin-EDTA. The values represent the mea Standard deviations for triplicate experiments. Significantly different from the control value: *P<0.01, ***P<0.001 (Student's t-test).

Table 2. The Antitumor Activity of Cannabidiol(4) by MTT Assay and SRB assay on SK-MEL-3 Cells.

Concentration (μM)	MTT assy		SRB assay	
control	3.87±0.12	(100.0)	2.68±0.02	(100.0)
1	3.85±0.11	(99.6)	2.67±0.02	(99.6)
25	3.81±0.03	(98.4)	2.65±0.02*	(98.8)
50	3.79±0.02	(97.8)	2.60±0.02***	(97.2)
100	0.23±0.04***	(58.9)	1.56±0.24***	(58.2)

Cells were incubated for 48 hr. The cells were harvested with Trypsin-EDTA. The values represent the mean±Standard deviations for triplicate experiments. Significantly different from the control value: *P<0.05, ***P<0.001 (Student's t-test).

Table 3. The Antitumor Activity of 5-Fluouracil(5) by MTT Assay and SRB assay on SK-MEL-3 Cells.

Concentration (μM)	MTT assy		SRB assay	
control	3.10±0.71	(100.0)	2.87±0.05	(100.0)
1	2.42±0.50	(78.2)	2.83±0.04	(98.4)
25	2.00±0.13**	(64.6)	2.79±0.01*	(97.1)
50	1.94±0.20**	(62.7)	2.68±0.07**	(93.5)
100	1.53±0.37**	(49.2)	2.55±0.04***	(88.9)

Cells were incubated for 48 hr. The cells were harvested with Trypsin-EDTA. The values reprent the mean±Standard deviations for triplicate experiments. Significantly different from the control value: *P<0.05, **P<0.01, ***P<0.001 (Student's t-test).

Table 4. The Antitumor Activity of Olivetol(2) by MTT Assay and SRB assay on SK-MEL-3 Cells.

Concentration (μM)	MTT assy		SRB assay	
control	4.07±0.11	(100.0)	2.88±0.03	(100.0)
1	3.03±0.56*	(74.4)	2.75±0.21*	(95.4)
25	1.68±0.50***	(41.3)	2.31±0.31*	(80.1)
50	1.10±0.45***	(27.1)	1.79±0.47**	(62.2)
100	0.45±0.11***	(10.9)	1.42±0.31***	(49.4)

Cells were incubated for 48 hr. The cells were harvested with Trypsin-EDTA. The values represent the mean±Standard deviations for triplicate experiments. Significantly different from the control value: *P<0.05, **P<0.01, ***P<0.001 (Student's t-test).

Table 5. The Antitumor Activity of Geraniol(1) by MTT Assay and SRB assay on SK-MEL-3 Cells.

Concentration (μM)	MTT assy		SRB assay	
control	3.87±0.45	(100.0)	2.66±0.08	(100.0)
1	2.92±0.22*	(75.4)	2.46±0.07*	(92.5)
25	2.79±0.54**	(72.1)	2.22±0.17*	(87.2)
50	2.72±0.43**	(70.5)	2.20±0.23**	(82.8)
100	2.76±0.14**	(71.3)	2.02±0.16**	(76.0)

Cells were incubated for 48 hr. The cells were harvested with Trypsin-EDTA. The values represent the mean±Standard deviations for triplicate experiments. Significantly different from the control value: *P<0.05, **P<0.01(Student's t-test).

Table 6. The antitumor activities of cannabinoids (3 and 4), 5-fluorouracil (5),olivetol (2) and geraniol (1) on SK-MEL-3 cell lines. Comparison of IC50 for cannabinoids (3 and 4), 5-fluorouracil (5), olivetol (2) and geraniol (1) - SRB assay and MTT assay.

Compoundsa	IC50 (μM)b	
	MTT assay	SRB assay
CBG	40.35	46.19
CBD	69.59	138.98
5-FU	88.15	465.08
OLVTL	36.17	89.80
GRNL	199.96	219.17

a) Each compound was examined in four concentrations in triplicate experiments.

b) IC_{50} represents the concentration of a compound required for 50 % inhibition of cell growth

Table 7. The cytotoxicity of cannabigerol (3) by MTT assay and SRB assay against NIH 3T3 fibroblasts.

Concentration (μM)	MTT assay	SRB assay
Control	1.797±0.045 (100)	1.191±0.039 (100)
1	1.771±0.016 (98.55)	1.925±0.057 (100.3)
25	1.464±0.15 (81.47)	1.911±0.016 (99.58)
50	1.242±0.051 (69.12)**	1.920±0.014 (100.05)
100	0.19 ±0.052 (10.57)***	0.436±0.619 (22.72)**

Cells were incubated for 48 hr. The cells were harvested with Trypsin-EDTA. The values represent the mean ± standard deviations for triplicate experiments. Significantly different from the control value: **P<0.01, ***P<0.001 (Student's t-test).

Table 8. The cyotoxicity of cannabidiol (4) by MTT assay and SRB assay against NIH 3T3 fibroblasts.

Concentration (μM)	MTT assay	SRB assay
Control	4.02±0.12 (100.0)	2.94±0.11 (100.0)
1	3.90±0.24 (96.9)	2.89±0.09 (98.3)
25	1.98±0.36*** (49.3)	2.52±0.25** (85.5)
50	0.26±0.11*** (6.4)	1.33±0.75** (44.8)
100	0.11±0.01*** (2.8)	0.67±0.07*** (22.8)

Cells were incubated for 48 hr. The cells were harvested with Trypsin-EDTA. The values represent the mean ± standard deviations for triplicate experiments. Significantly different from the control value: **P<0.01, ***P<0.001 (Student's t-test).

Table 9. The cytotoxicity of 5-fluoruracil (5) by MTT assay and SRB assay against NIH 3T3 fibroblasts.

Concentration (μM)	MTT assay	SRB assay
Control	3.76±0.03 (100.0)	3.12±0.14 (100.0)
1	3.74±0.04 (99.4)	2.90±0.04** (92.8)
25	1.70±0.24** (45.1)	1.96±0.07*** (62.7)
50	1.51±0.17*** (40.0)	1.73±0.13*** (55.3)
7100	1.32±0.06*** (35.1)	1.40±0.05*** (44.9)

Cells were incubated for 48 hr. The cells were harvested with Trypsin-EDTA. The values represent the mean ± standard deviations for triplicate experiments. Significantly different from the control value: **P<0.01, ***P<0.001 (Student's t-test).

Table 10. The cytotoxicities of cannabinoids (3 and 4) and 5-fluorouracil (5) on NIH 3T3 fibroblasts. Comparison of CD_{50} for cannabinoids (3 and 4) and 5-fluorouracil (5) - SRB assay and MTT assay.

Compoundsa	CD50(μM)b	
	MTT assay	SRB assay
CBG	60.46	82.98
CBD	36.27	60.25
5-FU	41.27	75.90

a) Each compound was examined in four concentrations in triplicate experiments.

b) CD_{50} represents the concentration of a compound required for 50 % cytotoxic dose of cell growth.

Legend of Photos

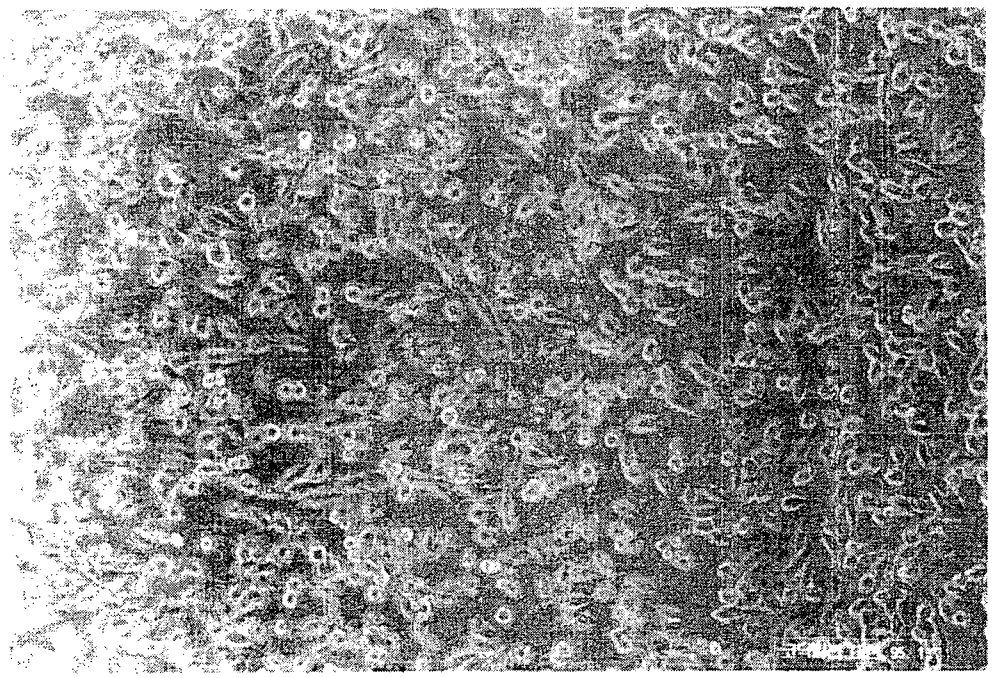


Photo 1. Inverted photomicrograph of SK-MEL-3 cells after incubation in unmodified medium (control) for 2 days x 200 Most cells had abundant cytoplasm and cytoplasmic process.

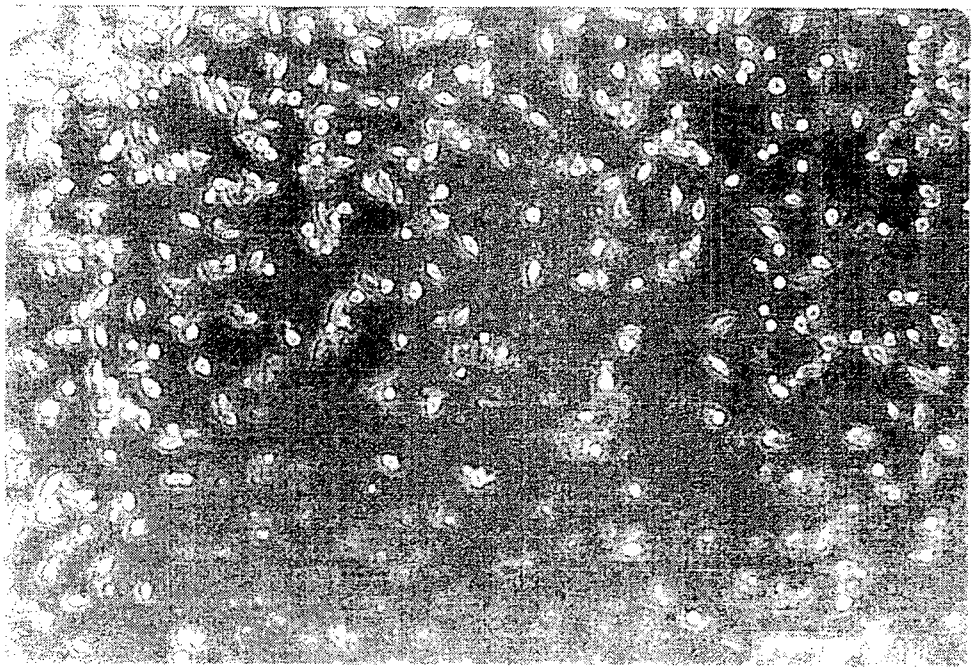


Photo 2. Inverted photomicrograph of SK-MEL-3 cells after incubation in the medium containing 100 μM cannabigerol (3) for 2 days x 200 Some cells showed degeneration and formed cell cluster.