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Biomolecular-chemical Screening

A Novel Screening Approach for the Discovery of Biologically Active Secondary Metabolites

II. Application Studies with Pure Metabolites†

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The novel screening strategy called "biomolecular-chemical screening" combines the advantages of the chemical screening approach—the analysis of the chromatographic and chemical behaviour of secondary metabolites on TLC plates—with binding studies of these molecules with bio-macromolecules like DNA. This approach was advantageously used to detect the interaction of pure compounds with DNA. In order to prove the reliability of the biomolecular-chemical screening and to examine DNA-binding properties, 470 pure secondary metabolites were analysed by this method. Besides the confirmation of already known binders with the TLC-based method, for a number of natural products DNA-binding properties were discovered for the first time. In consequence, binding of pure compounds can be measured by 1D TLC in a reliable and easy manner, in which DNA is applied together with the test compound at the starting spot. Analysis is performed *via* differences in Rf-values in comparison to a reference chromatogram without DNA.

It is generally accepted that nature provides a broad spectrum of structurally diverse metabolites which are expected to serve as new lead structures for pharmacological and agricultural applications[1]. For anticancer and antiinfective treatment, between 1989 and 1995 even 60% of the approved drugs and the pre-new drug application candidates (excluding biologics) have been of natural origin[2]. Beside topoisomerase inhibitors and tubulin interacting agents, DNA-binding compounds are in current use and as such play an important role in cancer therapy. Actinomycin D, bleomycin A_2, calicheamicin γ_1, doxorubicin, mitomycin C and rebeccamycin are low molecular weight microbial metabolites that interact with the DNA double helix, damage DNA or prevent correct replication. In addition, antisense strategies covering the field of therapeutic adjustment of gene expression or selective inhibition of a virus or microorganism also make use of DNA-binding compounds to stabilize nucleic acid triplex complexes *in vivo*[3]. Because of the ongoing interest, considerable research is underway to expand the spectrum of compounds with different modes of DNA-binding.

In the previous paper we described a novel screening approach called biomolecular-chemical screening in which the interaction of low molecular weight substances with DNA can be determined by means of thin-layer chromatography[4]. In this paper we report on DNA-binding properties of 470 pure natural compounds from our laboratory stock which were examined with the new one-dimensional TLC method. This investigation aimed at both, to prove the reliability of the method developed, and to discover natural products with hitherto unknown DNA affinity for additional biological profiling.

† Dedicated to Prof. Dr. AXEL ZEECK on the occasion of his 60th birthday.

Materials and Methods

General

To obtain random DNA, salmon sperm DNA (Sigma; 2 mg/ml in water) was homogenized by sonication for 6 minutes (Labsonic U, Braun). DNA-fragments were between 300 and 3,000 base pairs in size, as revealed by agarose gel analysis. Melting curve measurements were performed using the Cary 1E UV-visible spectrophotometer (Varian) as reported previously[4]. Interaction with DNA is indicated by a change in melting temperature (ΔT_m [°C]).

Thin-layer Chromatography

TLC binding studies were performed with the 1D-TLC method on silica gel plates (Merck, HPTLC-ready-to-use-plates, silica gel RP-18 WF_{254S} on glass)[4]. Pure substances (total amount per spot: 5 μg) were analyzed for DNA-binding properties in a solvent system consisting of methanol - 1 M aqueous ammonium acetate (4 : 1). Homogenized salmon sperm DNA (total amount per spot: 4 μg) was spotted above the sample spots prior to chromatography.

Detection was performed by UV extinction or fluorescence (254 and 366 nm) as well as by colorization with staining reagents (*e.g.* anisaldehyde-H_2SO_4, orcinol reagent, Ehrlich's reagent, and blue tetrazolium reagent). Changes in Rf-values indicate an interaction between ligand and DNA and are expressed by the Rf_2/Rf_1-ratio in which Rf_1 represents the Rf-value without, and Rf_2 with DNA.

Investigated Low Molecular Weight Natural Substances

All compounds tested are natural products and derivatives, either commercially available, published or unpublished, which are part of a collection of natural products, located at the Hans-Knöll-Institute for Natural Products Research, Jena, Germany. The compounds were dissolved in a suitable organic solvent (*e.g.* methanol, acetone, chloroform or water) resulting in a 1 mg/ml concentrated stock solution which then was spotted onto the TLC-plates.

Results

Detection of DNA-binding Compounds

According to the 1D-TLC method described in the previous paper 470 selected natural products were examined. 5 μg of each pure secondary metabolite dissolved in a suitable organic solvent was applied twice on a RP-18 WF_{254S}-TLC-plate: In the first lane without DNA and in a second lane together with a homogenized random-DNA sample (4 μg, dissolved in water). After chromatography using the solvent system methanol - 1 M aqueous ammonium acetate (4 : 1) the ratio of the Rf-values (Rf_2/Rf_1) of the natural product obtained with DNA (Rf_2) and without DNA (Rf_1) was determined. An interaction with DNA is indicated when the Rf_2/Rf_1-ratio decreases significantly below 1. Visualization of the metabolites was therefore realized by UV detection (254 and 366 nm) and/or staining with defined staining reagents. Because a chromatographic step on TLC is involved in the binding studies, it was possible to differentiate between DNA-binding properties of the natural product itself and impurities of the sample. The compounds examined were structurally classified according to the system introduced by BERDY[5] (see Table 1).

The results of the binding studies are summarized in Fig. 1 and Table 1. As expected among the quinones (hydroquinones, naphthoquinones, and anthraquinones; number of test compounds: n=15) a major percentage (66.6%) of compounds with DNA-binding properties was observed. Analogous results were obtained with the polyethers (71.4%; n=7), the macrolides (36.4%; n=11), and alkaloids with 19.3% (n=57). In contrast, with other *N*-heterocycles (3.1%; n=65), *O*-heterocycles (2.1%; n=47) and aromatic compounds (2.9%; n=34) only few compounds exhibited DNA-binding properties. None of the saccharides (mono-, di-, oligo- and polysaccharides; n=50) and amino acids (n=48) showed DNA affinity.

In order to give more detailed information, Table 2 provides an overview of the compounds found to interact with DNA, the obtained Rf_2/Rf_1-ratio, as well as known biological activities cited from literature. In order to prove the reliability of the method, about 5% of all compounds selected for our studies were known DNA binders. In all cases we established the DNA-binding properties independently by the 1D-TLC method, which *vice versa* proved the reliability of the biomolecular-chemical screening method. Other compounds identified as DNA-binders on TLC have been reported to exhibit antiviral (*e.g.* formycin B[42] or puromycin[43]) or antitumor activity (*e.g.* echitamine[11] or ivermectin[22]), or act as inhibitors of DNA synthesis (*e.g.* bacitracin[27] or calcimycin[30]). The TLC-based binding towards DNA of these type of natural compounds implicates a possible interaction with DNA being responsible for the biological activity.

For a number of natural products examined here, DNA-binding was detected for the first time. The TLC based

Table 1. DNA-binding behaviour of selected natural products analysed by 1D TLC-binding studies.

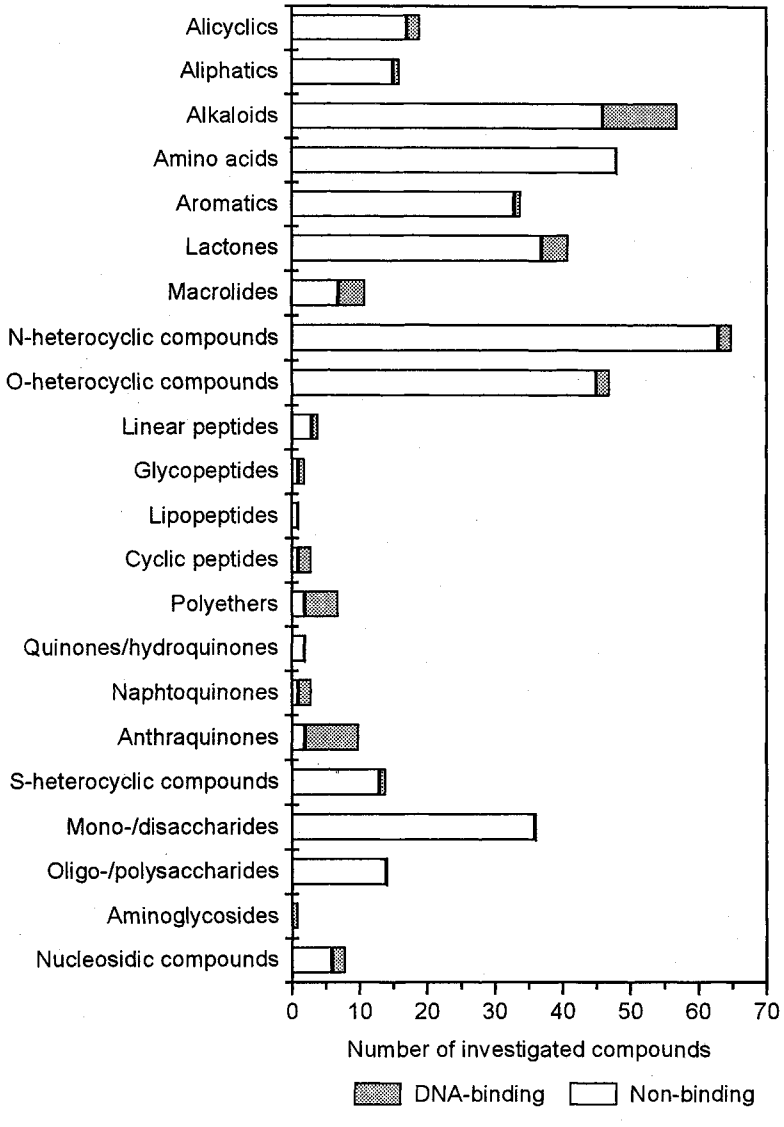
Chemical class	Number of tested compounds	DNA-binding
Alicyclics	19	2
Aliphatics	16	1
Alkaloids	57	11
Amino acids	48	0
Aromatics	34	1
Lactones	41	4
Macrolides	11	4
N-heterocyclic compounds	65	2
O-heterocyclic compounds	47	2
Peptides	10	4
Linear peptides	(4)	(1)
Glycopeptides	(2)	(1)
Lipopeptides	(1)	(0)
Cyclic peptides	(3)	(2)
Polyether	7	5
Quinones	15	10
Quinones/hydroquinones	(2)	(0)
Naphthoquinones	(3)	(2)
Anthraquinones	(10)	(8)
S-heterocyclic compounds	14	1
Sugars	59	3
Mono- and disaccharides	(36)	(0)
Oligo- and polysaccharides	(14)	(0)
Aminoglycosides	(1)	(1)
Nucleosidic compounds	(8)	(2)
Total	470	50

findings were proven by comparative DNA melting curve measurements. For example, the cytotoxic effect of culmorin[6] produced by *Fusarium graminearum* might be explained by DNA-binding as discovered with the 1D-TLC method ($Rf_2/Rf_1=0.27$; $\Delta T_m=1.0°C$). Surprisingly DNA-binding properties were found with compounds which have a defined biological activitiy seemingly unrelated to any DNA interaction. Examples are raubasine produced by *Rauwolfia* spp. ($Rf_2/Rf_1=0.87$; $\Delta T_m=1.0°C$) which shows affinity to the α-2C adrenergic receptor[13], megalomicin from *Micromonospora megalomicea* ($Rf_2/Rf_1=0.66$; $\Delta T_m=1.0°C$) an inhibitor of intra-golgi transport[23], and decarestrictine D from *Penicillium corylophilum* and *P. simplicissimum* ($Rf_2/Rf_1=0.91$; $\Delta T_m=0.8°C$), or deschlorothricin from *Streptomyces antibioticus* ($Rf_2/Rf_1=0.89$; $\Delta T_m=1.2°C$), both inhibitors of *de novo* cholesterol biosynthesis[17,21]. Furthermore, affinity to DNA was discovered for a number of compounds for which no biological effect has been described up to now. Examples are 1-*O*-(3-hydroxybenzoyl)-β-D-4,5-didesoxy-4-aminoribopyranose ($Rf_2/Rf_1=0.84$; $\Delta T_m=0.8°C$), 6-phenyl-3,5-diethyl-4-hydroxy-2*H*-pyran-2-on ($Rf_2/Rf_1=0.80$; $\Delta T_m=0.9°C$) or yohimbin carboxylic acid ($Rf_2/Rf_1=0.70$; $\Delta T_m=1.1°C$).

Discussion

By analysing a broad range of pure low molecular weight natural metabolites with the new TLC-based DNA binding

Fig. 1. Graphical overview on DNA-binding properties of selected natural products analyzed by 1D TLC-binding studies.



assay, the reliability of the screening approach has been proven. However, in a few cases the 1D-TLC method may fail to detect a defined DNA affinity for some compounds. If compounds exhibit a very low Rf-value ($Rf<0.1$) in the standard solvent system, it will be impossible to detect changes in the Rf-values. For these type of compounds a suitable solvent system should be developed. But within different solvent systems the values of the Rf_2/Rf_1-ratio are not comparable. Some compounds may be not detectable by the routinely applied UV-extinction/fluorescence (256 and 366 nm) or anisaldehyde-H_2SO_4. Here, more special staining reagents, *e.g.* orcinol reagent, Ehrlich's reagent or blue tetrazolium reagent, or densitometric read-outs have to be used to open the window to structural diversity.

DNA-binding properties of already known DNA-binders such as intercalators and groove-binders belonging to different chemical classes could be independently identified by the 1D-TLC method. Additional prove for the applicability of the method derived from DNA-melting curve analysis and CD-measurements of DNA applied under solvent conditions as on the TLC[4]. Thus, TLC-based DNA-binding detection constitutes a reliable and easy-to-handle method which can advantageously be applied in preliminary binding studies and, for pure compounds, allows a high throughput analysis due to parallelization of TLC. Future application will be directed towards the

Table 2. Survey of detected DNA-binding natural products, Rf_2/Rf_1-ratios as derived from the 1D-TLC method and their reported biological activities.

Chemical class Compound	Rf_2/Rf_1[a]	Biological activity
Alicyclics		
Culmorin	0.27	Cytotoxic[6]
Gabosin F	0.82	—[b]
Aliphatics		
Cyclopentan-1-on-3-methylen-1'-methylether-2-carboxylic acid	0.87	—[b]
Alkaloids		
Cephalotaxine	0.91	DNA-binding[7]
Chelerythrine	0	Inhibition of Protein Kinase C, DNA-binding[8,9]
Conodurine	0.80	Antimicrobial[10]
Echitamine	0.85	Antitumor[11]
Pheanthine	0.80	—[b]
Quebrachamine	0.80	α-2 Receptor antagonist[12]
Raubasine	0.87	α-2C Receptor antagonist[13]
Sanguinarine	0.80	DNA-binding[14]
Tetrandrine	0.87	Anti-inflammatory, antineoplastic[15]
Tomatidine	0.50	Cytotoxic[16]
Yohimbine carboxylic acid	0.70	—[b]
Aromatics		
2-Carbinol-3,6-methyl-5-methoxy-anisol	0.87	—[b]
Lactones		
Decarestrictine D	0.91	Inhibition of cholesterol biosynthesis[17]
5-Hexyldihydro-2(3H)-furanone	0.88	Antifungal[18]
Inamycin	0.88	DNA-binding[19]
Trioxsalen	0.70	DNA-binding[20]
Macrolides		
Deschlorothricin	0.89	Inhibition of cholesterol biosynthesis[21]
Ivermectin	0.90	Anthelmintic, antitumor[22]
Megalomicin	0.66	Inhibition of intra-golgi-transport[23]
Rutamycin A	0.91	ATPase inhibition[24]
N-heterocyclic compounds		
3-Ethyl-4-hydroxy-6,8-dimethyl-1,8-dihydro-1,2,6,8,9-pentaaza-cyclopentanaphtalen-5,7-dion	0.89	—[b]
8-Methyl-5-phenyl-3-[(8-phenyl-1,2,3,4-tetrahydro-isoquinolen-5-yl)-hydrazone]-1,3,6,7,8,9-hexa-hydropyrrolo-[3,2]-isoquinolin-2-on	0.81	—[b]

Table 2. (Continued)

Chemical class Compound	Rf_2/Rf_1[a]	Biological activity
O-heterocyclic compounds		
Cycloserine	0.91	DNA-binding[25]
6-Phenyl-3,5-diethyl-4-hydroxy-2*H*-pyran-2-on	0.80	—[b]
Peptides		
Actinomycin D	0	DNA-binding[26]
Bacitracin	0.80	Inhibition of DNA synthesis[27]
Distamycin	0.80	DNA-binding[28]
Phleomycin	0.70	DNA-binding[29]
Polyether		
Calcimycin	0.88	Inhibition of DNA synthesis[30]
Monensin A	0.50	Antiviral[31]
Nigericin	0.88	Antiviral[32]
Salinomycin	0.92	Antiviral[31]
X-206	0.75	Antiviral[33]
Quinones		
Aclacinomycin	0.66	DNA-binding[34]
Aklanonic acid	0.82	DNA-binding[35]
Aklavinone	0.77	DNA-binding[36]
2-Chloro-3(2-ethoxycarbonylic-3-benzylamino-β-phenylethylen)-[1,4]-naphtoquinone	0.88	—[b]
Daunomycin	0	DNA-binding[37]
Doxorubicin	0	DNA-binding[37]
Iremycin	0.73	DNA-binding[38]
Nogalamycin	0	DNA-binding[39]
Steffimycin	0.88	DNA-binding[40]
Vitamin K	0.92	DNA-binding[41]
S-heterocyclic compounds		
2-Methyl-3-ethoxycarbonylic-4-oxo-5-dimethylaminomethylen-thiophen	0.70	—[b]
Sugars		
Formycin B	0.90	Antiviral[42]
1-*O*-(3-Hydroxybenzoyl)-β-D-4,5-didesoxy-4-aminoribopyranose	0.84	—[b]
Puromycin	0.84	Antiviral, antitumor[43]

[a] Changes in Rf-values are expressed by the Rf_2/Rf_1-ratio in which Rf_1 represents the Rf-value without, and Rf_2 represents the Rf-value with DNA;

[b] No biological activity reported up to now.

examination of libraries from combinatorial synthesis approaches[44].

Applying the 1D-TLC binding studies it was possible to detect DNA-binding properties of a number of compounds that were not known before. As the identified compounds belong to different chemical classes, the results indicate the potential of the presented method not only to detect strong DNA-binding molecules of limited structural variety.

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References

1) VERPOORTE, R.: Exploration of nature's chemodiversity: The role of secondary metabolites as leads in drug development. Drug Discovery Today 3: 232~238, 1998
2) CRAGG, G. M.; D. J. NEWMAN & K. M. SNADER: Natural products in drug discovery and development. J. Nat. Prod. 60: 52~60, 1997
3) WILSON, W. D.; S. MIZAN, F. A. TANIOUS, S. YAO & G. ZON: The interaction of intercalators and groove-binding agents with DNA triple-helical structures: The influence of ligand structure, DNA backbone modifications and sequence. J. Mol. Recognit. 7: 89~98, 1994
4) MAIER, A.; C. MAUL, M. ZERLIN, I. SATTLER, S. GRABLEY & R. THIERICKE: Biomolecular-chemical screening. A novel screening approach for the discovery of biologically active secondary metabolites. I. Screening strategy and validation. J. Antibiotics 52: 945~951, 1999
5) BERDY, J. E.; A. ASZALOS, M. BOSTIAN & K. L. MCNITT: Handbook of antibiotic compounds, Vol. I~XIV. CRC Press, Florida, 1987
6) YOUNG, Y. & D. J. GAMES: Supercritical fluid chromatography of *Fusarium* mycotoxins. J. Chromatogr. 627: 247~254, 1992
7) HUANG, M. T.: Harringtonine, an inhibitor of initiation of protein biosynthesis. Mol. Pharmacol. 11: 511~519, 1975
8) HERBERT, J. M.; J. M. AUGEREAU, J. GLEYE & J. P. MAFFRAND: Chelerythrine is a potent and specific inhibitor of protein kinase C. Biochem. Biophys. Res. Commun. 172: 993~999, 1990
9) BAJAJ, N. P.; M. J. MCLEAN, M. J. WARING & E. SMEKAL: Sequence-selective, pH-dependent binding to DNA of benzophenanthridine alkaloids. J. Mol. Recognit. 3: 48~54, 1990
10) VAN BEEK, T. A.; R. VERPOORTE, A. B. SVENDSEN & R. FOKKENS: Antimicrobially active alkaloids from *Tabernaemontana chippii*. J. Nat. Prod. 48: 400~423, 1985
11) KAMARAJAN, P.; N. SEKAR, V. MATHURAM & S. GOVINDASAMY: Antitumor effect of echitamine chloride on methylcholonthrene induced fibrosarcoma in rats. Biochem. Int. 25: 491~498, 1991
12) DEUTSCH, H. F.; M. A. EVENSON, P. DRESCHER, C. SPARWASSER & P. O. MADSEN: Isolation and biological activity of aspidospermine and quebrachamine from an aspidosperma tree source. J. Pharm. Biomed. Anal. 12: 1283~1287, 1994
13) BLAXALL, H. S.; T. J. MURPHY, J. C. BAKER, C. RAY & D. B. BYLUND: Characterization of the α-2C adrenergic receptor subtype in the opossum kidney and in the OK cell line. J. Pharmacol. Exp. Ther. 259: 323~329, 1991
14) SIMANEK, V.: Benzophenanthridine alkaloids. The Alkaloids 26: 185~240, 1985
15) WANG, G. & J. R. LEMOS: Tetrandrine: a new ligand to block voltage-dependent Ca^+ and Ca^+-activated K^+ channels. Life Sciences 56: 295~306, 1995
16) FRIEDMAN, M.; P. R. HENIKA & B. E. MACKEY: Feeding of potato, tomato and eggplant alkaloids affects food consumption and body and liver weights in mice. J. Nutr. 126: 989~999, 1996
17) GRABLEY, S.; E. GRANZER, K. HUTTER, D. LUDWIG, M. MAYER, R. THIERICKE, G. TILL, J. WINK, S. PHILIPPS & A. ZEECK: Secondary metabolites by chemical screening: 8. Decarestrictines, a new family of inhibitors of cholesterol biosynthesis from *Penicillium*. I. Strain description, fermentation, isolation, properties. J. Antibiotics 45: 56~65, 1992
18) SAKURAI, K.; H. MATSUMOTO & J. ADACHI: Antifungal studies on drugs. I. Antifungal activity of five-membered lactone derivatives. Yakugaku Zasshi 88: 919~928, 1968
19) AHMAD, S. I.: A mutant of *Escherichia coli* hyper-resistant to a number of DNA-damaging agents: location of the mutational site. J. Photochem. Photobiol. B. 36: 47~53, 1996
20) GENCHEVA, M.; B. ANACHKOVA & G. RUSSEV: Mapping the sites of initiation of DNA replication in rat and human rRNA genes. J. Biol. Chem. 271: 2608~2614, 1996
21) KAWASHIMA, A.; Y. NAKAMURA, Y. OHTA, T. AKAMA, M. YAMAGISHI & K. HANADA: New cholesterol biosynthesis inhibitors MC-031 (*O*-demethylchlorothricin), -032 (*O*-demethylhydroxychlorothricin), -033 and -034. J. Antibiotics 45: 207~212, 1992
22) DIDIER, A. & F. LOOR: The abamectin derivative ivermectin is a potent P-glycoprotein inhibitor. Anticancer Drugs 7: 745~751, 1996
23) BONAY, P.; S. MUNRO, M. FRESNO & B. ALARCON: Intra-golgi transport inhibition by megalomicin. J. Biol. Chem. 271: 3719~3726, 1996
24) TONSGARD, J. H.; B. TUNG, K. S. KORNAFEL & G. S. GETZ: Environmentally induced differential amplification of mitochondrial populations. Biochem. J. 270: 511~518, 1990
25) HOTTA, K.; C. B. ZHU, P. PHOMSUWANSIRI, J. ISHIKAWA, S. MIZUNO, M. HATSU & S. IMAI: PCR inhibition assay for DNA-targeted antibiotics. J. Antibiotics 48: 1267~1272, 1995
26) SHINOMIYA, M.; W. CHU, R. G. CARLSON, R. F. WEAVER & F. TAKUSAGAWA: Structural, physical, and biological characteristics of RNA·DNA binding agent N8-actinomycin D. Biochemistry 34: 8481~8491, 1995
27) LAWRENCE, D. A.; R. SONG & P. WEBER: Surface thiols of human lymphocytes and their changes after *in vitro* and *in vivo* activation. J. Leukoc. Biol. 60: 611~618, 1996
28) TUITE, E.; U. SEHLSTEDT, P. HAGMAR, B. NORDEN & M.

TAKAHASHI: Effects of minor and major groove-binding drugs and intercalators on the DNA association of minor groove-binding proteins RecA and deoxyribonuclease I detected by flow linear dichroism. Eur. J. Biochem. 243: 482~492, 1997

29) MOORE, C. W.: Cleavage of cellular and extracellular *Saccharomyces cerevisiae* DNA by bleomycin and phleomycin. Cancer Res. 49: 6935~6940, 1989

30) LIU, H. L.; H. M. LU, D. G. LI, F. QI & Z. M. JIANG: Effects of egtazic acid and calcimycin on synthesis of DNA and collagen in cultured human lung fibroblasts. Chung Kuo Yao Li Hsueh Pao 17: 63~65, 1996

31) NAKAMURA, M.; S. KUNIMOTO, Y. TAKAHASHI, H. NAGANAWA, M. SAKAUE, S. INOUE, T. OHNO & T. TAKEUCHI: Inhibitory effects of polyethers on human immunodeficiency virus replication. Antimicrob. Agents Chemother. 36: 492~494, 1992

32) IRURZUN, A.; P. S. SANCHEZ, I. NOVOA & L. CARRASCO: Monensin and nigericin prevent the inhibition of host translation by poliovirus, without affecting p220 cleavage. J. Virol. 69: 7453~7460, 1995

33) MURRAY, R. D. H. & J. A. ROBINSON: Chemical and biochemical aspects of polyether-ionophore antibiotics biosynthesis. Fortschr. Chem. Org. Naturst. 58: 1~81, 1991

34) SHELTON, C. J.; M. M. HARDING & A. S. PRAKASH: Enzymatic and chemical footprinting of anthracycline antitumor antibiotics and related saccharide side chains. Biochemistry 35: 7974~7982, 1996

35) ECKARDT, K.; D. TRESSELT, G. SCHUMANN, W. IHN & C. WAGNER: Isolation and chemical structure of aklanonic acid, an early intermediate in the biosynthesis of anthracyclines. J. Antibiotics 38: 1034~1039, 1985

36) TOBE, H.; A. YOSHIMOTO & T. ISHIKURA: New anthracyclinone metabolites from two blocked mutants of *Streptomyces galilaeus* MA144-M1. J. Antibiotics 35: 1641~1645, 1982

37) WARING, M. J.: DNA modification and cancer. Ann. Rev. Biochem. 50: 159~192, 1981

38) FRITSCHE, H.; U. WAHNERT, J. B. CHAIRES, N. DATTAGUPTA, F. B. SCHLESSINGER & D. M. CROTHERS: Anthracycline antibiotics. Interaction with DNA and nucleosomes and inhibition of DNA synthesis. Biochemistry 26: 1996~2000, 1987

39) FOX, K. R. & Z. ALAM: Footprinting studies of DNA-sequence recognition by nogalamycin. Eur. J. Biochem. 209: 31~36, 1992

40) BRODASKY, T. F. & S. MIZSAK: Steffimycin C, a new member of the steffimycin anthracyclines. Isolation and structural characterization. J. Antibiotics 38: 849~855, 1985

41) HASSPIELER, B. M.; G. D. HAFFNER & K. ADELI: Influence of DT diaphorase on quinone-mediated genotoxicity in human and fish cell lines. Mutat. Res. 360: 43~49, 1996

42) WACLAWSKI, A. P. & P. J. SINKO: Oral absorption of anti-acquired immune deficiency syndrome nucleoside analogues. 2. Carrier-mediated intestinal transport of stavudine in rat and rabbit preparations. J. Pharm. Sci. 85: 478~485, 1996

43) MARUYAMA, T.; K. UTSUMI, H. TOMIOKA, M. KASAMOTO, Y. SATO, T. ANNE & E. DECLERCQ: Synthesis, antiviral, antibacterial and antitumor cell activities of 2′-deoxy-2′-fluoropuromycin. Chem. Pharm. Bull. 43: 955~959, 1995

44) PAULULAT, T.; Y.-Q. TANG, S. GRABLEY & R. THIERICKE: Combinatorial chemistry: The impact of natural products. Chimica Oggi, Chemistry Today 17: 52~56, 1999