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Evaluation of action mechanisms of toxic chemicals using JFCR39, a panel of human cancer cell lines

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Running Title:

Evaluation of toxic chemicals using JFCR39

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Introduction	452
Discussion	790

Nonstandard abbreviations

GI50: 50% growth inhibition concentration

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Abstract

We previously established a panel of human cancer cell lines, JFCR39, coupled to an anti-cancer drug activity database; this panel is comparable to the NCI60 panel developed by the National Cancer Institute. The JFCR39 system can be used to predict the molecular targets or evaluate the action mechanisms of the test compounds by comparing their cell growth inhibition profiles (i.e., fingerprints) with those of the standard anti-cancer drugs using the COMPARE program. In this study, we used this drug activity database-coupled JFCR39 system to evaluate the action mechanisms of various chemical compounds including toxic chemicals, agricultural chemicals, drugs, and synthetic intermediates. Fingerprints of 130 chemicals were determined and stored in the database. Sixty nine of 130 chemicals (approx. 60%) satisfied our criteria for the further analysis, and were classified into the following three clusters by cluster analysis of the fingerprints of these chemicals and several standard anti-cancer drugs: 1) clusters consisted of only anti-cancer drugs, 2) clusters of chemicals which shared similar action mechanisms (for example, ouabain and digoxin), and 3) a cluster of chemicals whose action mechanisms were unknown. These results suggested that chemicals belonging to a cluster (i.e., a cluster of toxic chemicals, a cluster of anti-cancer drugs etc.) shared similar action mechanism. In summary, the JFCR39 system can classify chemicals based on their fingerprints, even when their action mechanisms are unknown, and it is highly probable that the chemicals within a cluster share common action mechanisms.

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Introduction

Determining the action mechanism or identifying the molecular target of a chemical with pharmacological activity or adverse side effects is highly desirable. Although various test methods are currently available for determining the action mechanisms of chemicals, such as methods based on animal models, methods based on cellular models, bacterial mutagenicity test, the uterotrophic assay (Kanno et al., 2002), Hershberger test (Hershberger et al., 1953) and the reporter assay for the nuclear receptor agonists, determination of the action mechanisms of pharmacologically active chemicals, including the toxic chemicals, is still a difficult and challenging task. Therefore, it is highly desirable to develop efficient test methods for evaluating toxicity of chemicals.

A number of screening methods are currently available for discovering new anti-cancer drugs. One very powerful and unique approach using multiple cancer cell lines was developed at NCI (Paull et al., 1989; Weinstein et al., 1992; Weinstein et al., 1997) and also in our laboratory (Akashi et al., 2007; Akashi and Yamori, 2007; Dan et al., 2003; Dan et al., 2002; Nakamura et al., 2007; Nakatsu et al., 2005; Yamori, 2003; Yamori et al., 1999). This bioinformatics-based approach enables mechanism-oriented evaluation of anti-cancer drugs. For example, we can evaluate the cell toxicity *in vitro* by determining the 50% growth inhibition (GI50), total growth inhibition (TGI), and 50% lethal concentration (LC50) across a panel of 39 human cancer cell lines (JFCR39). We can also predict the molecular targets or evaluate the action mechanisms of the test compounds by comparing the cell growth

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inhibition profiles (termed fingerprint) across the panel for these compounds with those of the standard anti-cancer drugs using the COMPARE algorithm (Yamori et al., 1999). We have used this system successfully, and demonstrated that the molecular targets of the novel chemicals MS-274, FJ5002, and ZSTK474 were topoisomerases I and II (Yamori et al., 1999), telomerase (Naasani et al., 1999), and PI 3-kinase (Yaguchi et al., 2006), respectively. Several other interesting studies, based on a panel of cancer cells, classified anti-cancer drugs according to their action mechanism or molecular targets by cluster analysis of their GI50 values (Dan et al., 2002; Weinstein et al., 1992; Weinstein et al., 1997). Correlation analysis has also been used to explore the genes associated with the sensitivity of the cells in the panel to anti-cancer drugs (Nakatsu et al., 2005; Okutsu et al., 2002; Scherf et al., 2000; Zembutsu et al., 2002).

In this study, we have examined the potential of the JFCR39 system in classifying various chemicals, and predicted their action mechanisms. For this purpose, we have determined the fingerprints of 130 different types of chemicals including toxic chemicals, pesticides, drugs and synthetic intermediates, and then classified these chemicals according to the cluster analysis of their fingerprints.

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Material and Methods

Cell Lines and Cell Cultures. The panel of human cancer cell lines has been described previously (Dan et al., 2002; Yamori et al., 1999) and consists of the following 39 human cancer cell lines: lung cancer, NCI-H23, NCI-H226, NCI-H522, NCI-H460, A549, DMS273, and DMS114; colorectal cancer, HCC-2998, KM-12, HT-29, HCT-15, and HCT-116; gastric cancer, MKN-1, MKN-7, MKN-28, MKN-45, MKN-74, and St-4; ovarian cancer, OVCAR-3, OVCAR-4, OVCAR-5, OVCAR-8, and SK-OV-3; breast cancer, BSY-1, HBC-4, HBC-5, MDA-MB-231, and MCF-7; renal cancer, RXF-631L and ACHN; melanoma, LOX-IMVI; glioma, U251, SF-295, SF-539, SF-268, SNB-75, and SNB-78; and prostate cancer, DU-145 and PC-3. All cell lines were cultured in RPMI 1640 (Nissui Pharmaceutical, Tokyo, Japan) with 5% fetal bovine serum, penicillin (100 units/mL), and streptomycin (100 μg/mL) at 37^{o}C under 5% CO_2.

Determination of cell growth inhibition profiles. Growth inhibition experiments were performed to assess the sensitivity of the cells to various chemicals as described before (Dan et al., 2002; Yamori et al., 1999). Growth inhibition was measured by determining the changes in the amounts of total cellular protein after 48 hours of chemical treatment using a sulforhodamine B assay. For each chemical, the growth assay was performed using a total of five different concentrations of the chemical (for example,

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10^{-4}, 10^{-5}, 10^{-6}, 10^{-7}, and 10^{-8} M) and one negative control. All assays were performed in duplicate. This GI50 calculation method is well established and reliable through anti-cancer drug screen using NCI60 as well as JFCR39 (Paull et al., 1989; Yamori, 2003; Yamori et al., 1999). At each test concentration, the percentage growth was calculated using the following seven absorbance measurements: growth at time zero (T0), growth of the control cells (C), and test growth in the presence of five different concentrations (T) of a drug. The percentage growth inhibition was calculated as: % growth = 100 x [(T–T0)/(C-T0)] when $T \geq T0$, and % growth = 100 x [(T–T0)/T] when $T < T0$. The GI50 values, which represent 50% growth inhibition concentration, were calculated as 100 x [(T-T0)/(C-T0)] = 50. When the GI50 of a chemical could not be calculated, the highest used concentration was assigned as its GI50 value. Absolute values of GI50 were then log transformed for further analysis. We certified the accuracy of measured GI50 data by using reference control chemicals, such as MMC, paclitaxel and SN-38, every experiment and by checking the dose response curves.

Chemicals. Spironolactone, para-aminoazobenzene, para-cresidine, neostigmin bromide, para-dichlorobenzene, phenytoin, ortho-toluidine, imipramine, cobalt chloride, atrazine, propylthiouracil, (d,l) thalidomide, carbon tetrachloride, hydroquinone, monocrotaline, vinyl chloride, tributyl-tin chloride, valproic acid, benzene, acrylamide, pentachlorophenol, aniline, 1,3-diphenylguanidine, polypropylene glycol, 10,10'-oxy-bis(phenoxyarsine), testosterone propionate, carbaryl, acephate,

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bisphenol A, 17-beta-estradiol, diethylstilbestrol, and alpha-bungarotoxin were purchased from Wako (Tokyo, Japan). Snake venoms from Agkistrodon halys blomhoffii, Trimeresurus flavoviridis, Crotalus atrox, Naja nigricollis, and Naja naja kaouthia were purchased from LATOXAN (Valence, France). 2-Aminomethylpyridine, 1H-1,2,4-triazole, 1H-1,2,3-triazole, 3,4,4'-trichlorocarbanilide, edifenphos, dichlorvos, O-ethyl O-4-nitrophenyl phenylphosphonothioate, 2,4-dinitrophenol, N-methylaniline, 1,2-dichloro-3-nitrobenzene, 4-ethylnitrobenzene, 2-vinylpyridine, 3-amino-1H-1,2,4-triazole, N-ethyl-N-nitrosourea, 5-aza-2'- deoxycytidine, ethynyl estradiol, 3-methylcholanthrene, phenobarbital, acetaminophen, isoniazid, capsaicin, colcemid, 2.4-dinitrochlorobenzene, dexamethasone were from Sigma Chemicals (St. Louis, MO). Methoprene acid, methoprene, all-trans retinoic acid and 9-cis retinoic acid were from BIOMOL International L.P. (Plymouth Meeting, PA). Levothyroxine was from ICN (Costa Mesa, CA); 3-iodo-2-propynyl butylcarbamate was from Olin Japan Inc. (Tokyo, Japan); p-chlorophenyl-3-iodopropargylformal was from Nagase ChemteX (Osaka, Japan); 2,3,3,3-2',3',3',3'-octachlorodipropylether was from Sankyo Chemical Industries, LTD. (Tokyo, Japan); 1,2-benzisothiazolin-3-one was from Riverson (Osaka, Japan); zinc butylxanthate was from Ouchishinko Chemical Industrial Co., Ltd. (Tokyo, Japan); and 4-amino-2,6-dichlorophenol was from Tokyo Kasei Kogyo Co. Ltd. (Tokyo, Japan).

Hierarchical Clustering. Hierarchical clustering analysis was carried out using the average linkage

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method and the ‘‘GeneSpring’’ software (Silicon Genetics, Inc., Redwood, CA). Pearson correlation coefficients were used to determine the degree of similarity.

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Result

Sensitivity of JFCR39 to chemicals. Sensitivity of the JFCR39 panel of cells to 130 chemicals was determined as described in Materials and Methods. Table 1 summarizes abbreviations, applications, targets, and known mechanisms of 130 chemicals and 21 anti-cancer drugs. Approximately 15% of the chemicals were assessed twice or more. Approximately 40% of the chemicals tested had little effect on the growth of cells in the JFCR39 panel. However, rest of the chemicals significantly inhibited the cell growth across the JFCR39 panel. For example, Figure 1 shows the dose response curves of the cells in the JFCR39 panel against digoxin. The concentration at which the cell growth is inhibited by 50% represents GI50. Figure 2 shows the fingerprints of four chemicals (digoxin, ouabain, snake venom from Naja nigricollis (SV-NN), and snake venom from Naja naja kaouthia (SV-NNK)), which differentially inhibited the growth of cells in the JFCR39 panel; these fingerprints were drawn based on a calculation using a set of GI50s, and clearly represented the GI50 pattern. These results were highly reproducible as the Pearson correlation coefficient of the duplicate experiments for digoxin was 0.839 ($p<0.001$) and that for ouabain was 0.864 ($p<0.001$). Interestingly, digoxin and ouabain, both of which are cardiac glycosides and inhibit Na-K ATPase, showed similar fingerprints. The fingerprints of snake venoms from the *Naja naja kaouthia* (SV-NNK) and *Naja nigricollis* (SV-NN), which belong to the elapidae, known as cobra, were also similar, but were different from the fingerprints of digoxin and ouabain. Table

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2 summarizes only a portion of the GI50 values from 160 experiments involving 130 chemicals and 42 experiments involving 21 anti-cancer drugs. GI50 values from all experiments were described in the Supplemental data (Table S1). All these data were stored in a chemosensitivity database and used for further analysis.

Classification of the chemicals by hierarchical clustering. Sixty nine chemicals were selected for further analysis based on the following criteria: a) GI50 values for the test chemical can be determined for at least 10 cell lines in the JFCR39 panel, and b) the range of log GI50 for the test chemical is over 0.6, suggesting differential growth inhibition. We analyzed the GI50 values of these 69 chemicals and 20 anti-cancer drugs by hierarchical clustering analysis (Figure 3). We roughly found 12 clusters (threshold: r = 0, Figure 3 clusters A-L), which were further divided into 49 sub-clusters (threshold: r=0.408, Figure 3 clusters A1-L6).

Analysis of clusters. Most anti-cancer drugs we have tested belonged either to cluster A or cluster H, depending on their modes of action (Dan et al., 2002). The targets of the anti-cancer drugs belonging to the cluster A were related to DNA (Topo I, antimetabolite of pyridine, DNA alkylator) and the target of the anti-cancer drugs belonging to the cluster H was tubulin. We presently found that cisplatin exceptionally belonged to cluster F2, not cluster A, although it is known to crosslink DNA strands

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(Jamieson and Lippard, 1999; Wong and Giandomenico, 1999). We were also able to precisely group the clusters into several sub-clusters having similar characteristics. For example, the cardiac glycosides digoxin and ouabain were grouped in one cluster (cluster F3). The snake venoms from the *Naja naja kaouthia* and *Naja nigricollis*, on the other hand, belonged to the cluster D2. These results are in accordance with the similar fingerprints shown in Figure 2. Interestingly, the snake venoms from the *Crotalus atrox* and *Trimeresurus flavoviridis*, species belonging to the viperidae snake family, formed another cluster (cluster D3), which was different from that of the elapidae family of snakes, *Naja naja kaouthia* and *Naja nigricollis*. 9-cis retinoic acid, 13-cis retinoic acid, and TTNPB, which are RAR agonist (Astrom et al., 1990), also formed a separate cluster (cluster D1). Similarly, agricultural chemicals paraquat, ziram, and thiram formed a single cluster (cluster F1).

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Discussion

The JFCR39 system coupled to a drug activity database is a good model for investigating the diversity of chemosensitivity in cancer cells. We have previously established panels of human cancer cell lines, JFCR39 (Yamori, 2003) and JFCR45 (Nakatsu et al., 2005), and used these panels of cells to demonstrate that they provide powerful means to predict the action mechanisms of drugs, and also used them to identify new target compounds. In this manuscript, we utilized the JFCR39 system to evaluate various chemicals (such as toxic chemicals, agricultural chemicals, and synthetic intermediates), which are not anti-cancer drugs, and classified them according to their molecular target or action mechanism. As a result, these chemicals were classified into a number of clusters. Our results also suggested that each cluster consisted of chemicals sharing a common action mechanism.

We determined the growth inhibition of cells in the JFCR39 panel by 130 chemicals and calculated their 50% growth inhibition concentration (GI50). Some of the chemicals were assessed twice or more to confirm the reproducibility of the assay. We had to exclude 61 chemicals from further analysis, as they did not inhibit the cells in the JFCR39 panel significantly. Rests of the chemicals (69 of 130, approx. 60%) met our selection criteria and were evaluated by cluster analysis.

First, we found that the chemicals tested in duplicate formed tight clusters, showing high reproducibility. Next, we investigated the difference between these 69 test chemicals and the anti-cancer

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drugs. Sixty-nine chemicals, which are not anti-cancer drugs, formed several clusters, which were different from the anti-cancer drug clusters. These results suggest that the action mechanisms of these chemicals are different from the action mechanisms of the anti-cancer drugs. Exceptionally, we found that cisplatin did not belong to the cluster A that consisted of DNA-targeting anti-cancer drugs. We do not understand the reason at present. However, there is a possibility that cisplatin has other action mechanisms, which may have made the fingerprint of cisplatin different from those of other DNA-targeting drugs. Indeed, it is known that cisplatin forms DNA-protein cross-links (Chvalova et al., 2007; Zwelling et al., 1979).

Our analysis also identified several interesting clusters. For example, the cluster F3 consisted of cardiac glycosides digoxin and ouabain, both of which inhibit Na-K ATPase (Reuter et al., 2002). The cluster D1 consisted of 9-cis retinoic acid, 13-cis retinoic acid, and TTNPB, which are RAR agonists. These results suggest that chemicals other than the anti-cancer drugs also form clusters when they share the same action mechanisms. Interestingly, the snake venoms from the *Naja naja kaouthia* and *Naja nigricollis*, which belonged to the ellapidae family, formed one cluster (cluster D2). In contrast, the snake venoms from the *Crotalus atrox* and *Trimeresurus flavoviridis*, which belonged to the viperidae family, formed a cluster (cluster D3) that was different from the elapidae cluster. These results are reasonable because it is known that the snake venoms from different snake families not only differ in compositions but also show different levels of toxicity and have different action mechanisms.

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Agricultural chemicals paraquat, ziram and thiram were also classified into a single cluster (cluster F1). Among these agricultural chemicals, the action mechanism of ziram is not known. However, it is known that both paraquat and thiram induce oxidative stress (Cereser et al., 2001; Suntres, 2002). Therefore, based on our observation, we could suggest that ziram also act by inducing oxidative stress. Other agricultural chemicals, methoprene (insect growth regulator) and carbaryl (chorine esterase inhibitor) formed cluster L3 although their common mechanism is unknown. Cluster D4 and D5 consist of the antibacterial agents or fungicides. Especially, IPBC and CPIP belonging cluster D4 are the iodo-type antibacterial agents.

Thus, cluster analysis of GI50 values of various chemicals, determined using the JFCR39 cell panel, suggests that the JFCR39 system could, at least partly, allow classification of chemical compounds on the basis of their action mechanisms. Our analysis also suggests that the chemicals belonging the same cluster share a common action mechanism. We are going to develop a larger library of reference chemicals with known action mechanisms, i.e. various inhibitors of biological pathways, and expand our database by integrating their GI50 measurements, which will make the cluster analysis as well as the COMPARE analysis more informative for predicting the mechanism of test chemicals.

In conclusion, to evaluate the potential of the JFCR39 system in predicting the action mechanisms of toxic chemicals, we investigated the fingerprints of 130 different types of chemical compounds including toxic chemicals, pesticides, drugs and synthetic intermediates. Using the hierarchical

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clustering analysis, we classified 69 chemicals, at least partly, on the basis of their action mechanisms. Thus, this approach using the JFCR39 cell panel is not only useful in predicting the action mechanisms of toxic chemicals but also in evaluating their toxicity**.**

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Footnotes

a) Unnumbered footnote

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Legends for figures

Figure 1. Dose response curves of digoxin against growth of JFCR-39 cells.

X-axis is the concentration of digoxin and Y-axis is the % growth. The GI50 represents the concentration required to inhibit cell growth by 50% compared with untreated controls.

Figure 2. Fingerprints of digoxin, ouabain, SV-NN, and SV-NNK.

Fingerprint shows the differential growth inhibition pattern of the cells in the JFCR-39 panel against the test chemical. X- axis represents relative value of GI50; (-1) x (log GI50- MG-MID); MG-MID is the mean value of the log GI50. Zero means the mean GI50 and one means the GI50 value is ten-fold more sensitive than the mean GI50. Exp-ID and JCI numbers are the ID for the experiment and ID for the chemical, respectively, in our database.

Figure 3. Hierarchical clustering of 69 test chemicals and 20 anti-cancer drugs based on their GI50 values.

Hierarchical clustering method was an “average linkage method” using the Pearson correlation as distance. We classified the chemicals into two kinds of clusters; their threshold values were r=0 and r=0.408 ($p<0.01$), respectively. Gradient color indicates relative level (log transformed) of GI50. Red,

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more sensitive than the mean GI50 (2.0); yellow, mean GI50 (0.0); and green, less sensitive than the mean GI50 (-2.0). On the color scale, red represents the GI50 value that is 100-fold higher the mean GI50.

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Table 1. List of chemicals tested. Chemical names, abbreviations, and applications/targets/ mechanisms of the test compounds are summarized.

JCI No	name	abbreviation	application / target / mechanism
-691	Trioctyltin	TOT	Organotin
-690	Triphenyltin	TPT	Organotin
-689	Dibutyltin		Organotin
-688	AM-580		RARalpha
-687	TTNPB		RAR
-686	13-cis Retinoic acid	13-cis	RAR
-607	Methoprene		agricultural chemical
-606	Methoprene acid		RXR
-605	5-aza-2'- deoxycytidine	5-AzaC	methylation
-604	Carbaryl		agricultural chemical
-603	Acephate		agricultural chemical
-602	Sodium Arsenite		agricultural chemical
-601	Testosterone propionate	TP	testosterone
-600	Ethynyl estradiol	EE	estrogenic
-599	Thiram		agricultural chemical
-598	Dimethylformamide	DMF	solvent
-568	alpha-Bungarotoxin	alphaBuTX	neurotoxin
-567	Snake Venom from Trimeresurus flavoviridis	SV-TF	snake venom
-566	Snake Venom from Crotalus atrox	SV-CA	snake venom
-565	Snake Venom from Agkistrodon halys blomhoffii	SV-AHB	snake venom
-564	Dexamethasone	DEX	steroid
-563	3-methylcholanthrene	3-MC	teratogenicity / carcinogenicity
-562	N-ethyl-N-nitrosourea	ENU	teratogenicity / carcinogenicity
-561	Diethylnitrosamine	DEN	teratogenicity / carcinogenicity
-560	All trans retinoic acid	ATRA	RAR + RXR
-559	9-cis retinoic acid	9-cis	RAR
-558	Levothyroxine	T4	thyroid hormone
-557	3-Amino-1H-1,2,4-triazole	3AST	agricultural chemical
-555	2-Vinylpyridine	2VP	synthetic intermediate
-553	Phenobarbital	PB	antiepileptic
-552	Acetaminophen	APAP	analgetic
-551	Isoniazid		phthisic
-549	4-Ethylnitrobenzene	4ENB	synthetic intermediate
-548	1,2-Dichloro-3-nitrobenzene	1,2DC3NB	pigment / synthetic intermediate
-546	N-Methylaniline	NMA	synthetic intermediate
-545	2-Aminomethylpyridine	2AMP	synthetic intermediate
-544	1H-1,2,4-Triazole		synthetic intermediate
-543	1H-1,2,3-Triazole		synthetic intermediate
-542	4-amino-2,6-dichlorophenol	4A2,6DCP	synthetic intermediate
-541	2,4-dinitrophenol	2,4 DNP	agricultural chemical
-513	Capsaicin		food constituent
-485	2-Methoxyestradiol		estrogenic
-466	Colcemid		spindle inhibitor
-465	2,4-Dinitrochlorobenzene	2,4DCB	pigment / mutagenesis
-464	Troglltazone		diabetic
-463	Clofibrate		antilipemic
-459	Bis(2-ethylhexyl)Phthalate	DEHP	plasticizer
-458	Thiourea		agricultural chemical
-447	Cacodylic acid		agricultural chemical
-446	Amitrole		agricultural chemical

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Table 1. List of chemicals tested. (Cont'd)

JCI No	name	abbreviation	application / target / mechanism
-445	4-Octylphenol	OP	reproductive effector
-444	2,6-Dimethylaniline	2,6-Xylidene	natural product
-443	1,2-Dibromo-3-chloropropane	DBCP	agricultural chemical
-442	1,1-Dimethylhydrazine	1,1DMH	reproductive effector
-441	Sulfanylamide		agricultural chemical
-440	Streptozotocin		agricultural chemical
-439	Spironolactone		aldosterone antagonist
-438	para-Aminoazobenzene	pAAB	pigment / mutagenicity / carcinogenicity
-437	para-Cresidine		pigment / carcinogenicity
-436	Neostigmin bromide		parasympathomimetics
-435	para-Dichlorobenzene	pDCB	pigment / agricultural chemical
-434	Phenytoin		antiepileptic
-433	ortho-Toluidine	oToluidine	pigment
-432	Imipramine		antidepressant
-431	Cobalt chloride		teratogenicity / mutagenicity
-428	Atrazine		agricultural chemical
-427	Propylthiouracil		teratogenicity / carcinogenicity
-426	Thalidomide(L+D)		teratogenicity
-425	Carbon tetrachloride	CCl4	teratogenicity / carcinogenicity
-424	Hydroquinone		oxidative stress
-423	Monocrotaline		mutagenicity / carcinogenicity
-422	Vinyl chloride		carcinogenicity
-421	Tributyltin chloride	TBT	ship bottom paint / organotin
-420	Valproic acid		antiepileptic
-419	Benzene		carcinogenicity
-418	Acrylamide		neurotoxin / carcinogenicity
-417	Hexachlorobenzene	BHC	agricultural chemical / carcinogenicity
-346	2-deoxy-glucose	2-DG	glycolytic pathway / glycosylation inhibitor
-325	Pentachlorophenol	PCP	agricultural chemical / teratogenicity / carcinogenicity
-324	Aniline		oxidative stress / methemoglobinemia / carcinogenicity
-323	Triazine		agricultural chemical
-322	Edifenphos	EDDP	agricultural chemical / antibiotics / choline esterase
-321	gamma-1,2,3,4,5,6-□Hexachlorocyclohexane	gamma-BHC	agricultural chemical / carcinogenicity
-320	Dichlorvos	DDVP	agricultural chemical / teratogenicity / carcinogenicity
-319	O-ethyl O-4-nitrophenyl phenylphosphonothioate	EPN	agricultural chemical
-318	Cadmium chloride	CdCl2	teratogenicity / carcinogenicity
-317	phenylmercury acetate	PMA	fungicides / mutagenicity
-316	Mercaptoacetic Acid		synthetic intermediate
-315	1,3-diphenylguanidine	DPG	vulcanizing agent
-314	3,4,4'-Trichlorocarbanilide	TCC	cosmetics / antibacterial agent
-313	3-Iodo-2-propynyl butylcarbamate	IPBC	antibacterial agent
-311	2,3,3,3-2',3',3',3'-□octachlorodipropylether	S-421	agricultural chemical / antibacterial agent
-310	1,2-Benzisothiazolin-3-one	BIT	antibacterial agent
-309	Isobornylthiocyanoacetate	IBTA	antibacterial agent
-308	p-Chlorophenyl-3-iodopropargylformal	CPIP	antibacterial agent
-307	Zinc butylxanthate	ZBX	vulcanizing agent
-306	Polypropylene glycol	PG	synthetic intermediate
-305	10,10'-Oxy-bis(phenoxyarsine)	OBPA	antibacterial agent
-296	Snake Venom□from Naja naja kaouthia	SV-NNK	snake venom
-295	Snake Venom from Naja nigricollis	SV-NN	snake venom

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Table 1.List of chemicals tested. (Cont'd)

JCI No	name	abbreviation	application / target / mechanism
-294	2,5-di(tert-butyl)-1,4-hydroquinone	DTBHQ	oxidative stress
-293	Ibotenic Acid		mushroom toxin / neurotoxin
-292	N-methy-4-phenyl-1,2,3,6-tetrahydropyridine	MPTP	neurotoxin
-289	Tetrodotoxine		natural product / Na+ channel inhibitor
-288	ICI 182,780		estrogen antagonist
-275	Benzophenone		agricultural chemical
-274	1,2-dibromo-3-chloropropane	DBCP	antibacterial agent / insecticide / carcinogenicity
-273	Zineb		agricultural chemical
-272	Dieldrin		insecticide
-271	Hexachlorobenzene	HCB	antibacterial agent / carcinogenicity
-270	Ziram		antibacterial agent / vulcanizing agent
-269	chlordane		insecticide / carcinogenicity
-268	4,4'-Dichlorodiphenyltrichloroethane	p,p'-DDT	insecticide / carcinogenicity / teratogenicity
-267	Bisphenol A	BPA	estrogenic
-266	17-beta-estradiol	E2	estrogenic
-265	Diethylstilbestrol	DES	estrogenic
-261	Paraquat		agricultural chemical / oxidative stress
-247	Ouabain		cardiac glycosides
-245	Okadaic acid		natural product / PP1, PP2A inhibitor
-242	Antimycin A1		agricultural chemical
-232	Digoxin		cardiac glycosides
-201	OH-Flutamide		Flutamide derivative / androgen antagonist
-200	Flutamide		anti-cancer drugs / androgen antagonist
-185	30%H2O2		oxidative stress
-182	N-Acetyl-L-cysteine	NAC	super oxyside scavenger
-181	L-Ascorbic acid		food constituent
-179	Dopamine		neurotransmitter
-177	Caffeine		food constituent
-168	Cycloheximide		protein synthesis inhibitor
-144	4-Hydroxy phenylretinamide	4-HPR	RAR
-137	Indomethacin		cox inhibitor
-99	SN-38		Irinotecan derivative / Topo I
-96	Toremifene		anti-cancer drugs / estrogen antagonist
-95	Tamoxifen		anti-cancer drugs / estrogen antagonist
-63	Cychlospolin A		anti-cancer drugs / helper T cell
-46	HCFU		anti-cancer drugs / antimetabolite(pyrimidine)
-36	Taxotere		anti-cancer drugs / tubulin
-35	Taxol		anti-cancer drugs / tubulin
-34	Colchicine		antipodagric / tubulin
-33	Cisplatin		anti-cancer drugs / DNA cross linker
-32	Carboplatin		anti-cancer drugs / DNA cross linker
-31	Irinotecan		anti-cancer drugs / Topo I
-30	Camptothecin	CPT	anti-cancer drugs / Topo I
-24	Methotrexate		anti-cancer drugs / DHFR
-19	Vincristine		anti-cancer drugs / tubulin
-18	Vinblastine		anti-cancer drugs / tubulin
-16	Mitomycin-C	MMC	anti-cancer drugs / DNA alkylator
-9	Tegafur		anti-cancer drugs / antimetabolite(pyrimidine)
-8	5-Fluorouracil	5-FU	anti-cancer drugs / antimetabolite(pyrimidine)
-5	Cytarabine		anti-cancer drugs / antimetabolite(pyrimidine)
-4	Nitrogen mustard		anti-cancer drugs / DNA alkylator

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Table 2. $\log_{10}$ GI50 values of chemicals for each cell line in the JFCR-39 panel.
Hi-conc means the highest concentration of the test chemical used. When the growth inhibition was over 50% at the Hi-Conc, GI50 was assigned the Hi-Conc value.

Exp-ID	S3416	S3415	S3413	S3245	S3117	S3414	S3118	S3246	S3125	S3124	S3123	S1636	S1635	S1634	S1718
JCI No	-687	-686	-559	-559	-559	-560	-560	-560	-567	-566	-565	-296	-295	-294	-294
Name or Abbr.	TTNPB	13-cis	9-cis	9-cis	9-cis	ATRA	ATRA	ATRA	SV-TF	SV-CA	SV-AHB	SV-NNK	SV-NN	DTBHQ	DTBHQ
Hi-Conc.	-4	-4	-4	-4	-4	-4	-4	-4	-4	-4	-4	-4	-4	-4	-4
HBC-4	-4.76	-4.00	-4.53	-4.40	-4.43	-4.42	-4.41	-4.41	-5.87	-5.80	-5.66	-7.25	-7.31	-4.72	-4.80
BSY-1	-4.78	-4.16	-4.60	-4.73	-4.73	-4.69	-4.70	-4.81	-6.31	-6.06	-5.76	-6.93	-7.34	-5.07	-4.93
HBC-5	-4.80	-4.41	-4.56	-4.57	-4.61	-4.61	-4.47	-4.51	-6.98	-6.45	-5.73	-7.64	-7.72	-4.89	-4.78
MCF-7	-4.73	-4.35	-4.40	-4.39	-4.48	-4.48	-4.54	-4.66	-5.87	-5.78	-5.68	-6.77	-7.08	-5.29	-5.25
MDA-MB-231	-4.75	-4.21	-4.70	-4.55	-4.69	-4.63	-4.53	-4.65	-5.90	-5.86	-5.84	-6.84	-7.39	-5.52	-5.30
U251	-4.77	-4.14	-4.61	-4.51	-4.61	-4.57	-4.45	-4.63	-6.45	-5.76	-5.70	-6.85	-7.44	-4.96	-5.11
SF-268	-4.75	-4.00	-4.24	-4.55	-4.40	-4.47	-4.48	-4.76	-5.90	-5.79	-5.70	-7.53	-7.67	-4.77	-4.81
SF-295	-4.80	-4.29	-4.54	-4.66	-4.60	-4.59	-4.48	-4.57	-6.19	-5.80	-5.74	-6.89	-6.97	-4.87	-4.97
SF-539	-4.95	-4.35	-4.75	-4.80	-4.79	-4.80	-4.71	-4.76	-6.39	-5.96	-5.81	-7.79	-7.75	-4.79	-4.86
SNB-75	-5.31	-5.28	-5.13	-5.19		-4.71	-4.69	-4.87	-6.41	-6.33	-5.93	-7.60	-7.70	-4.67	-4.80
SNB-78	-4.77	-4.25	-4.69	-4.78	-4.86	-4.49	-4.70	-4.68	-6.19	-6.00	-5.95	-6.97	-7.53	-4.75	-4.75
HCC 2998	-4.68	-4.00	-4.48	-4.61	-4.62	-4.55	-4.62	-4.76	-5.91	-5.75	-5.67	-6.47	-6.77	-4.82	-4.75
KM-12	-4.70	-4.00	-4.46	-4.51	-4.48	-4.51	-4.47	-4.58	-5.93	-5.80	-5.65	-6.77	-6.87	-4.74	-4.77
HT-29	-4.73	-4.00	-4.47	-4.53	-4.50	-4.60	-4.52	-4.56	-5.90	-5.80	-5.56	-5.89	-6.78	-4.80	-4.89
HCT-15	-4.72	-4.25	-4.45	-4.49	-4.48	-4.52	-4.57	-4.53	-5.88	-5.76	-5.57	-6.73	-6.82	-4.72	-4.77
HCT-116	-4.77	-4.07	-4.67	-4.59	-4.67	-4.71	-4.61	-4.64	-6.46	-6.10	-5.77	-6.58	-6.82	-4.98	-5.13
NCI-H23	-4.74	-4.00	-4.47	-4.60	-4.59	-4.61	-4.55	-4.63	-6.11	-5.75	-5.72	-6.86	-7.42	-4.76	-4.90
NCI-H226	-4.72	-4.00	-4.61	-4.68	-4.78	-4.80	-4.54	-5.48	-5.95	-5.81	-5.76	-6.73	-6.78	-4.89	-4.91
NCI-H522	-4.72	-4.45	-4.68	-4.82	-4.77	-4.71	-4.71	-4.68	-6.45	-5.99	-5.78	-7.62	-7.46	-5.37	-5.37
NCI-H460	-4.70	-4.00	-4.55	-4.63	-4.58	-4.68	-4.55	-4.49	-5.96	-5.82	-5.72	-7.44	-7.42	-4.84	-4.84
A549	-4.79	-4.00	-4.72	-4.77	-4.78	-4.70	-4.62	-4.53	-5.91	-5.79	-5.71	-6.80	-6.83	-4.83	-4.87
DMS273	-4.57	-4.21	-4.50	-4.62	-4.55	-4.57	-4.51	-4.49	-6.20	-5.81	-5.72	-7.43	-7.44	-4.91	-4.98
DMS114	-4.77	-4.16	-4.33	-4.62	-4.49	-4.51	-4.53	-4.61	-6.66	-6.33	-5.77	-6.83	-6.88	-5.12	-5.21
LOX-IMVI	-4.77	-4.69	-4.68	-4.66	-4.70	-4.77	-4.74	-4.74	-6.75	-6.59	-5.76	-6.86	-6.94	-5.05	-5.15
OVCAR-3	-4.77	-4.38	-4.56	-4.67	-4.72	-4.64	-4.62	-4.71	-6.61	-6.13	-5.89	-6.77	-6.79	-4.89	-4.86
OVCAR-4	-4.72	-4.05	-4.63	-4.64	-4.64	-4.58	-4.39	-4.54	-6.73	-6.23	-5.80	-6.82	-6.90	-5.13	-4.90
OVCAR-5	-4.75	-4.00	-4.33	-4.39	-4.42	-4.44	-4.34	-4.44	-5.92	-5.74	-5.67	-6.46	-6.71	-5.22	-5.26
OVCAR-8	-4.75	-4.23	-4.50	-4.53	-4.59	-4.66	-4.67	-4.70	-5.95	-5.77	-5.69	-6.82	-6.84	-4.64	-4.70
SK-OV-3	-4.79	-4.00	-4.49	-4.51	-4.81	-4.52	-4.54	-4.50	-5.76	-5.64	-4.91	-6.75	-6.76	-4.64	-4.74
RXF-631L	-4.77	-4.00	-4.54	-4.58	-4.60	-4.72	-4.63	-4.61	-5.91	-5.80	-5.59	-7.13	-7.46	-4.81	-4.84
ACHN	-4.73	-4.00	-4.56	-4.66	-4.56	-4.50	-4.40	-4.76	-5.90	-5.79	-5.73	-6.74	-6.80	-4.71	-4.83
St-4	-4.74	-4.00	-4.42	-4.54	-4.65	-4.53	-4.49	-4.57	-5.91	-5.81	-5.76	-7.65	-7.70	-4.68	-4.75
MKN1	-4.75	-4.33	-4.56	-4.63	-4.62	-4.56	-4.45	-4.48	-6.15	-5.81	-5.78	-7.67	-7.68	-4.59	-4.81
MKN7	-4.78	-4.40	-4.68	-4.59	-4.70	-4.73	-4.56	-4.65	-6.29	-5.85	-5.76	-6.70	-6.90	-4.79	-4.84
MKN28	-4.71	-4.28	-4.56	-4.59	-4.59	-4.65	-4.56	-4.60	-6.10	-5.93	-5.68	-6.51	-6.81	-4.72	-4.89
MKN45	-4.72	-4.00	-4.51	-4.41	-4.46	-4.73	-4.41	-4.43	-6.06	-5.90	-5.69	-6.71	-6.82	-4.71	-4.87
MKN74	-4.74	-4.40	-4.61	-4.63	-4.61	-4.73	-4.68	-4.67	-5.97	-5.92	-5.61	-6.92	-7.00	-5.10	-5.42
DU-145	-4.68	-4.00	-4.25	-4.78	-4.41	-4.42	-4.44	-4.54	-6.08	-5.82	-5.75	-7.43	-7.55	-4.59	-5.02
PC-3	-4.74	-4.00	-4.58	-4.65	-4.48	-4.74	-4.39	-4.51	-5.83	-5.77	-5.61	-6.67	-6.69	-4.89	-4.74

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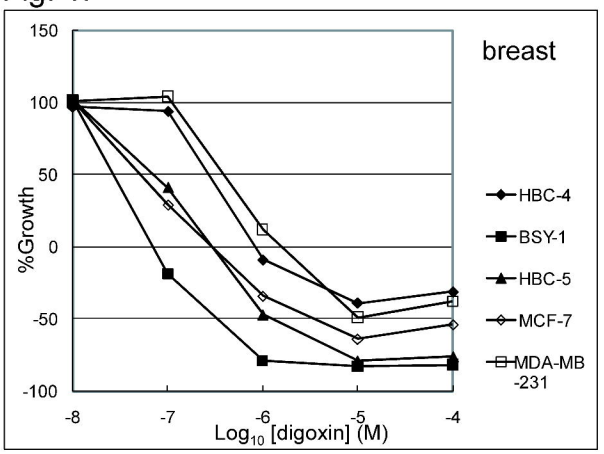
Table 2. $\log_{10}$GI50 values of chemicals for each cell line in the JFCR-39 panel (Cont'd)

Exp-ID	S3243	S3244	S1534	S3237	S3238	S1525	S3236	S1928	S1421	S1705	S1327	S1413	S1413	S3408	S3409	S2421
JCI No	-599	-599	-270	-270	-270	-261	-261	-261	-247	-247	-232	-232	-232	-421	-421	-421
Name or Abbr.	Thiram	Thiram	Ziram	Ziram	Ziram	Paraquat	Paraquat	Paraquat	Ouabain	Ouabain	Digoxin	Digoxin	Digoxin	TBT	TBT	TBT
Hi-Conc.	-4	-4	-4	-4	-4	-4	-3	-4	-4	-6	-4	-4	-4	-4	-4	-4
HBC-4	-4.71	-4.79	-5.80	-5.73	-5.70	-4.00	-3.61	-4.00	-7.54	-7.28	-6.57	-6.96	-6.96	-6.79	-6.77	-6.72
BSY-1	-6.97	-7.12	-6.85	-6.76	-6.60	-4.00	-4.45	-4.51	-8.00	-7.76	-7.58	-7.68	-7.68	-7.03	-7.01	-6.83
HBC-5	-7.41	-7.66	-7.18	-7.47	-7.47	-4.68	-4.70		-7.76	-7.51	-7.15	-7.44	-7.44	-6.76	-6.88	-6.83
MCF-7	-4.77	-4.80	-6.00	-5.84	-5.83	-4.06	-3.72	-4.00	-7.64	-7.51	-7.29	-7.39	-7.39	-6.86	-6.84	-6.79
MDA-MB-231	-4.66	-4.68	-5.64	-5.75	-5.63	-4.00	-3.57	-4.00	-7.40	-6.81	-6.41	-6.72	-6.72	-6.83	-6.81	-6.70
U251	-4.75	-4.78	-5.71	-5.79	-5.82	-4.00	-3.69	-4.00	-7.75	-7.16	-7.01	-7.40	-7.40	-6.79	-6.77	-6.72
SF-268	-4.86	-4.96	-5.74	-5.83	-7.01	-4.00	-4.08	-4.00	-8.00	-7.77	-7.42	-7.70	-7.70	-6.84	-6.85	-6.71
SF-295	-4.77	-4.89	-5.71	-5.70	-5.79	-4.47	-4.37	-4.20	-8.00	-7.64	-7.42	-7.55	-7.55	-6.75	-6.73	-6.76
SF-539	-4.75	-4.88	-5.73	-5.75	-5.77	-4.00	-4.03	-4.00	-8.00	-7.70	-7.46	-7.63	-7.63	-6.77	-6.72	-6.67
SNB-75	-4.71	-4.96	-5.79	-5.92	-5.80	-4.00	-3.94	-4.00	-7.70	-7.45	-6.86	-7.40	-7.40	-6.99	-6.95	-7.05
SNB-78	-4.70	-4.78	-5.69	-5.64	-5.69	-4.00	-3.78	-4.00	-7.98	-7.64	-7.45	-7.60	-7.60	-6.72	-6.79	-6.70
HCC 2998	-4.82	-4.69	-5.76	-5.79	-5.81	-4.00	-3.70	-4.00	-7.64	-6.77	-6.68	-7.25	-7.25	-6.77	-6.79	-6.72
KM-12	-4.80	-4.80	-5.43	-5.74	-5.73	-4.00	-3.58	-4.00	-7.67	-7.12	-6.69	-7.34	-7.34	-7.00	-6.98	-6.74
HT-29	-4.68	-4.85	-5.75	-5.77	-5.76	-4.10	-4.03	-4.07	-7.75	-7.31	-7.20	-7.34	-7.34	-6.89	-6.84	-6.66
HCT-15	-4.68	-4.75	-5.70	-5.72	-5.83	-4.00	-3.64	-4.00	-7.74	-7.63	-6.92	-7.54	-7.54	-6.88	-6.84	-6.70
HCT-116	-4.72	-4.72	-5.74	-5.68	-5.77	-4.00	-3.60	-4.00	-8.00	-7.57	-7.47	-7.62	-7.62	-6.90	-6.85	-6.74
NCI-H23	-4.69	-4.78	-5.96	-5.85	-5.84	-4.19	-4.18	-4.00	-8.00	-7.67	-7.50	-7.84	-7.84	-6.90	-6.85	-6.76
NCI-H226	-6.33	-6.74	-5.63	-5.96	-6.12	-4.41	-4.41	-4.00	-8.00	-7.37	-6.93	-7.61	-7.61	-6.99	-6.91	-6.74
NCI-H522	-7.49	-7.50	-7.44	-7.66	-8.00	-4.49	-4.71	-4.59	-8.00	-7.64	-7.59	-7.91	-7.91	-6.83	-6.80	-6.25
NCI-H460	-6.14	-6.16	-6.30	-6.10	-6.15	-4.30	-4.45	-4.37	-8.00	-7.74	-7.60	-7.77	-7.77	-6.98	-6.98	-6.56
A549	-4.84	-4.82	-5.97	-5.91	-5.91	-4.49	-4.49	-4.41	-8.00	-7.80	-7.66	-7.91	-7.91	-6.82	-6.87	-6.73
DMS273	-6.64	-6.58	-6.43	-6.84	-6.82	-4.25	-4.43	-4.30	-8.00	-7.71	-7.48	-7.72	-7.72	-6.74	-6.75	-6.70
DMS114	-7.18	-7.39	-7.37	-7.38	-7.43	-4.50	-4.63	-4.27	-8.00	-7.84	-7.73	-8.00	-8.00	-7.11	-7.12	-7.02
LOX-IMVI	-4.68	-4.71	-5.66	-5.71	-5.70	-4.00	-3.51	-4.00	-7.80	-7.80	-7.39	-7.46	-7.46	-6.93	-6.94	-6.76
OVCAR-3	-4.86	-6.25	-6.07	-6.35	-6.32	-4.00	-4.46	-4.28	-8.00	-7.66	-7.64	-7.59	-7.59	-6.80	-6.82	-6.74
OVCAR-4	-4.77	-6.67	-5.90	-5.91	-5.87	-4.00	-4.21	-4.48	-8.00	-7.71	-7.71	-7.72	-7.72	-6.91	-7.20	-6.80
OVCAR-5	-4.90	-6.00	-6.11	-6.91	-6.74	-4.00	-3.98	-4.00	-7.89	-7.62	-7.12	-7.42	-7.42	-6.90	-6.93	-6.75
OVCAR-8	-4.62	-4.74	-5.59	-5.68	-5.67	-4.00	-3.84	-4.00	-7.85	-7.44	-6.97	-7.29	-7.29	-6.73	-6.67	-6.57
SK-OV-3	-4.39	-4.38	-4.92	-5.49	-5.53	-4.00	-3.39	-4.00	-7.74	-7.67	-7.18	-7.38	-7.38	-6.80	-6.77	-6.68
RXF-631L	-4.75	-4.65	-5.57	-5.63	-5.60	-4.00	-3.60	-4.00	-7.10	-6.00	-6.42	-6.20	-6.20	-6.78	-6.76	-6.68
ACHN	-4.52	-4.60	-5.64	-5.68	-5.69	-4.00	-3.51	-4.00	-8.00	-7.72	-7.44	-7.59	-7.59	-6.78	-6.77	-6.76
St-4	-4.59	-4.72	-5.99	-5.73	-5.81	-4.00	-3.58	-4.00	-8.00	-7.65	-7.45	-7.60	-7.60	-6.80	-6.80	-6.72
MKN1	-4.80	-4.85	-6.82	-5.84	-5.92	-4.41	-4.61	-4.48	-8.00	-7.72	-7.68	-7.72	-7.72	-7.25	-7.15	-6.87
MKN7	-4.79	-4.82	-6.56	-5.84	-5.82	-4.08	-4.29	-4.32	-7.80	-7.48	-6.98	-7.47	-7.47	-7.34	-7.07	-6.86
MKN28	-7.18	-7.21	-5.82	-7.09	-7.13	-4.00	-4.40	-4.18	-7.70	-7.42	-6.77	-7.37	-7.37	-6.84	-6.82	-6.87
MKN45	-6.65	-6.71	-6.05	-7.18	-6.86	-4.00	-4.29	-4.35	-7.77	-7.25	-6.99	-7.52	-7.52	-6.96	-6.97	-6.87
MKN74	-7.05	-7.08	-6.35	-5.86	-7.05	-4.00	-4.47	-4.06	-7.91	-7.65	-7.55	-7.74	-7.74	-6.97	-7.37	-7.05
DU-145	-4.47	-4.70	-5.68	-5.68	-5.65	-4.00	-3.57	-4.00	-8.00	-7.64	-7.59	-7.71	-7.71	-6.90	-6.89	-6.70
PC-3	-4.42	-4.77	-5.61	-5.53	-5.56	-4.00	-3.64	-4.37	-8.00	-7.62	-7.41	-7.62	-7.62	-6.77	-6.78	-6.73

Fig. 1.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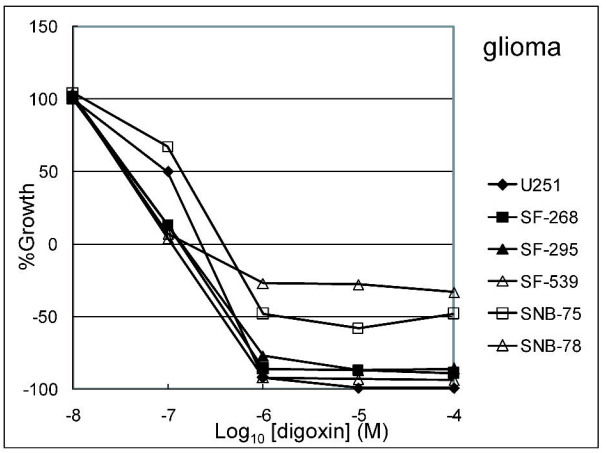
%Growth
Log10 [digoxin] (M)
HBC-4
BSY-1
HBC-5
MCF-7
MDA-MB -231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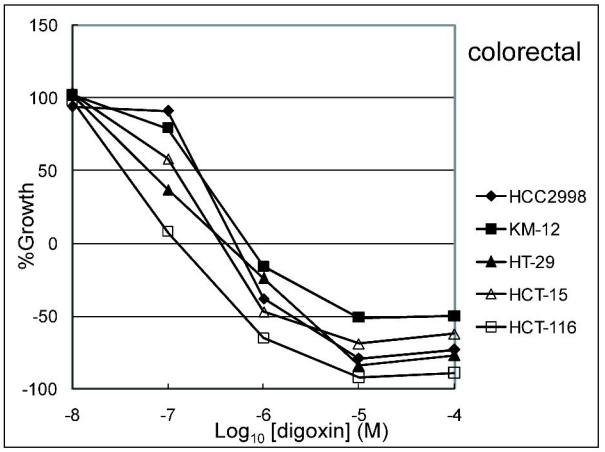
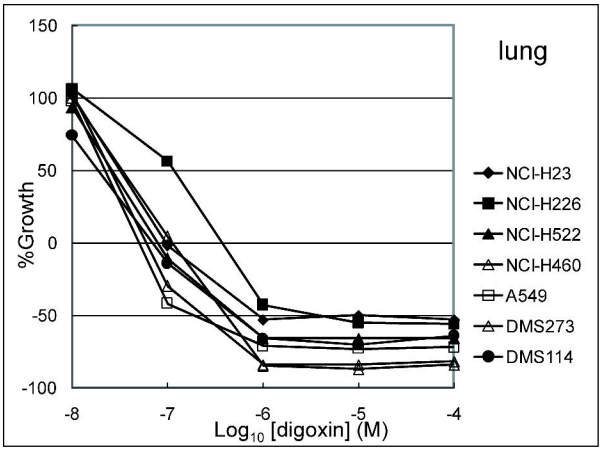
%Growth
Log10 [digoxin] (M)
U251
SF-268
SF-295
SF-539
SNB-75
SNB-78


colorectal
%Growth
Log10 [digoxin] (M)
HCC2998
KM-12
HT-29
HCT-15
HCT-116


lung
%Growth
Log10 [digoxin] (M)
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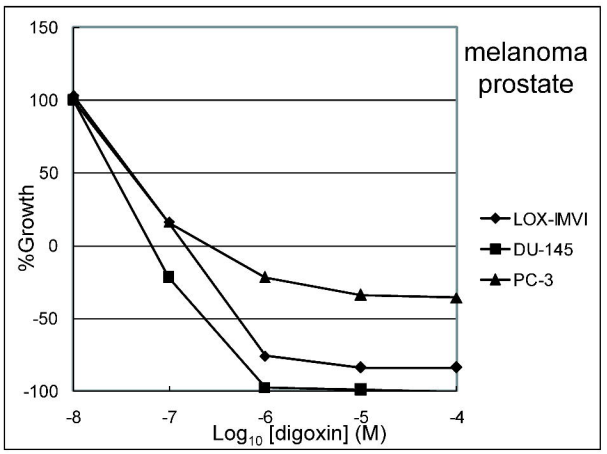
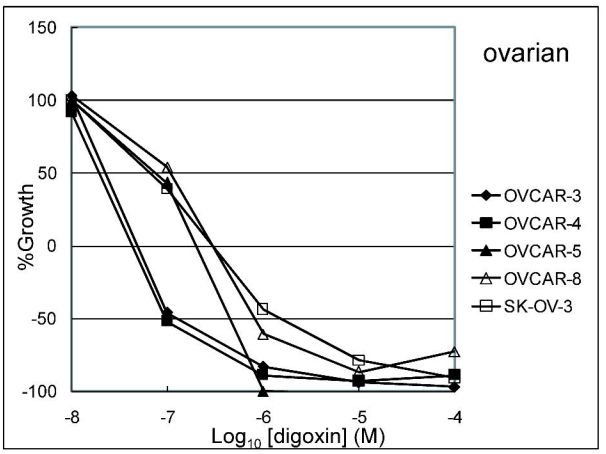
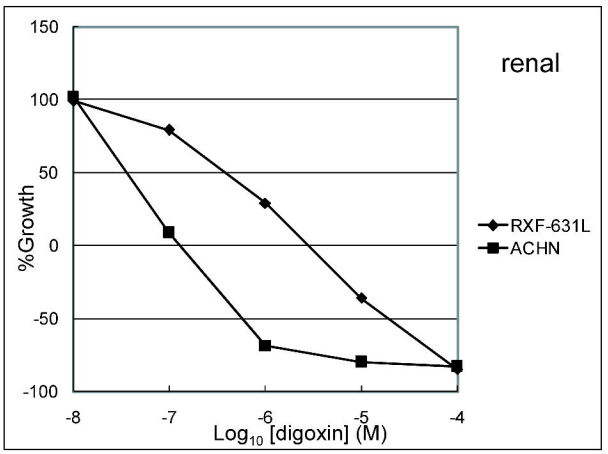
NCI-H226
NCI-H522
NCI-H460
A549
DMS273
DMS114


melanoma
prostate
%Growth
Log10 [digoxin] (M)
LOX-IMVI
DU-145
PC-3


ovarian
%Growth
Log10 [digoxin] (M)
OVCAR-3
OVCAR-4
OVCAR-5
OVCAR-8
SK-OV-3


renal
%Growth
Log10 [digoxin] (M)
RXF-631L
ACHN

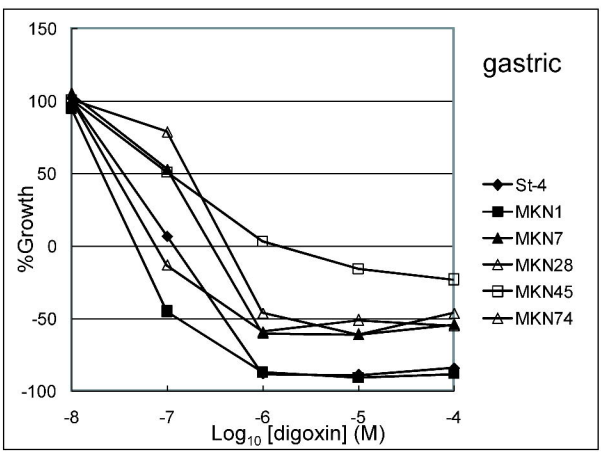
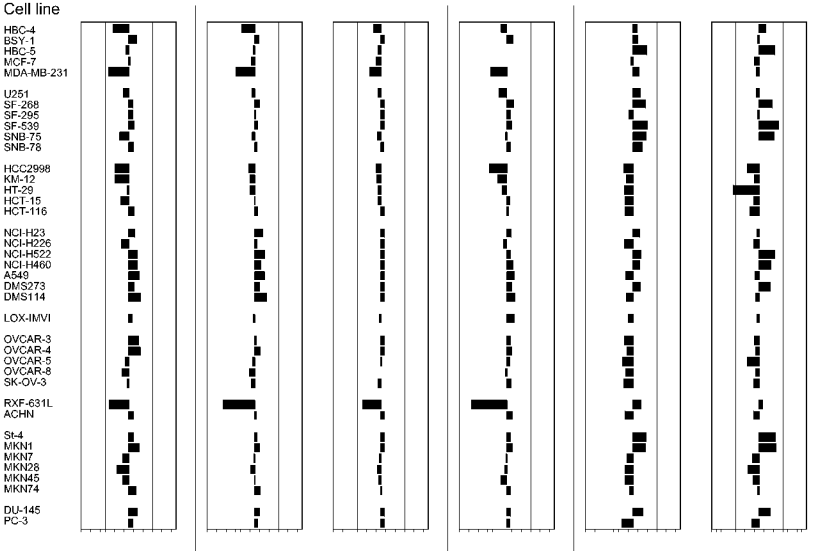

gastric
%Growth
Log10 [digoxin] (M)
St-4
MKN1
MKN7
MKN28
MKN45
MKN74

Fig.2.



Exp-ID	S1327	S1413	S1421	S1705	S1635	S1636
JCI No.	JCI: -232	JCI: -232	JCI: -247	JCI: -247	JCI: -295	JCI: -296
Chemical name	Digoxin	Digoxin	Ouabain	Ouabain	SV-NN	SV-NNK

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Fig.3.