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Identification of Novel Antimitotic Agents Acting at the Tubulin Level by Computer-assisted Evaluation of Differential Cytotoxicity Data

Kenneth D. Paull, Chii M. Lin, Louis Malspeis, and Ernest Hamel[1]

Information Technology Branch [K. D. P.] and Laboratory of Molecular Pharmacology [C. M. L., E. H.], Developmental Therapeutics Program, Division of Cancer Treatment, National Cancer Institute, NIH, Bethesda, Maryland 20892, and Laboratory of Pharmaceutical Chemistry, Developmental Therapeutics Program, Division of Cancer Treatment, National Cancer Institute, Frederick Cancer Research and Development Center, Frederick, Maryland 21701 [L. M.]

ABSTRACT

Data generated in the new National Cancer Institute drug evaluation program, which are based on inhibition of cell growth in 60 human tumor cell lines, were probed with nine known antimitotic agents using the COMPARE algorithm. Cytotoxicity data were available on approximately 7000 compounds at the time of the analysis, and, based on the criteria used, 82 compounds were selected as positive by the computer search. Nine were the probe compounds themselves, and 41 were analogues of known antimitotic agents. Among the remaining 32 compounds there were 19 distinct chemical species. Agents in ten of these groups (containing 20 compounds) were effective inhibitors of *in vitro* tubulin polymerization and caused the mitotic arrest of cells grown in culture. Two compounds were related natural products binding in the Vinca domain of tubulin, and the others were synthetic agents which interfered with colchicine binding. The remaining 12 agents (one natural product, the remainder synthetic) fell into several groups: two compounds were weak inhibitors of tubulin polymerization, inhibited colchicine binding, and caused mitotic arrest; one compound weakly inhibited tubulin polymerization but did not cause an increase in the number of cells arrested in mitosis; two compounds caused mitotic arrest at micromolar concentrations, but thus far no *in vitro* interaction with tubulin has been observed; the remainder neither inhibited tubulin polymerization nor caused a rise in the number of cultured cells arrested in mitosis. Tubulin-dependent GTP hydrolysis was stimulated or inhibited by all agents which inhibited tubulin polymerization with the exception of one compound. The analysis of differential cytotoxicity data thus appears to have great promise for the identification of new antimitotic agents with antineoplastic potential.

INTRODUCTION

The National Cancer Institute has screened large numbers of compounds for many years in a continuing search for effective antineoplastic drugs. Recently the initial *in vivo* screen (murine P388 leukemia) has been replaced with a tissue culture screen of 60 human tumor cell lines, with a major goal being the development of tumor-specific therapeutic agents (1–3). Data obtained are entered into a database on a VAX 9000 computer. At the time the study presented here was initiated (late 1990) over 7000 compounds, including about 200 "standard" agents of known therapeutic utility and/or mechanism of action, had been processed through the screen.

Several analytical approaches to this growing database are being explored. We have developed an algorithm, COMPARE, which evaluates patterns of cytotoxicity among the cell lines in the screen (4). This algorithm permits the pattern of cytotoxicity against the 60 cell lines obtained with any agent to be compared with those obtained with all other agents in the database. The algorithm also provides a numerical evaluation (Pearson correlation coefficient) of the degree of similarity between the patterns obtained with two agents. We noted that when the probe compound (or "seed") was a known antimitotic agent, many of the compounds identified as having similar differential cytotoxicity patterns were known to interfere with tubulin function. We wondered whether other compounds of unknown mechanism of action identified in these screening probes would also interact with tubulin. Since such interactions are readily confirmed in biochemical assays, we initiated this systematic evaluation of the potential of the COMPARE algorithm to provide mechanistic information based on differential cytotoxicity data.

MATERIALS AND METHODS

Materials. Tubulin and heat-treated microtubule-associated proteins were prepared as described elsewhere (5). [8-^{14}C]GTP (repurified by anion exchange chromatography) and [^{3}H]colchicine were obtained from Moravek Biochemicals and Du Pont, respectively. All drugs were provided by the Drug Synthesis and Chemistry Branch, National Cancer Institute. They were dissolved in dimethyl sulfoxide, and equivalent amounts of the solvent were included in all control reaction mixtures. Monosodium glutamate (from Sigma) was repurified to remove Mg^{2+} (6). HL-60 human leukemia cells were a gift of Dr. T. Breitman (Laboratory of Biological Chemistry, National Cancer Institute).

The COMPARE Algorithm. Each time a compound is tested in the NCI[2] screen, the data obtained must pass quality control standards prior to entry in the database. These data are processed to yield delta values for each cell line, defined as the difference obtained when the $\log_{10}$ of its TGI is subtracted from the $\log_{10}$ of the mean of the TGIs of all successfully evaluated cell lines in the specific test with the compound.

When a compound is selected as a seed for a probe of the database, the delta value for each cell line successfully tested with the seed is compared to the delta values with the same cell lines (if successfully tested) for all compound entries in the database. For each compound in the database a set of pairs of delta values is obtained. The commercially available SAS statistical program was used to calculate a Pearson product moment correlation coefficient for each set of delta value pairs. All compounds in the data base are rank-ordered in comparison to the seed for similarity of pattern of differential cytotoxicity, with a correlation coefficient of 1.0 signifying identical patterns.

Methods. The tubulin polymerization assays have been described (7, 8). In brief, varying concentrations of drug were preincubated for 15 min with 1.0 mg/ml (10 μM) tubulin in 1.0 M monosodium glutamate plus $MgCl_2$ as indicated. GTP was added (0.4 mM), and polymerization was followed turbidimetrically at 350 nm for 20 min. The drug concentration required to inhibit the extent of polymerization by 50% was determined from the data. At least three experiments were performed with each agent. The binding of [^{3}H]colchicine to tubulin (triplicate samples) was determined by the DEAE-cellulose filter technique (9). For measurement of GTP hydrolysis (10), samples were applied to polyethyleneimine-cellulose thin-layer sheets. Following thin-layer chromatography, product [8-^{14}C]GDP and residual [8-^{14}C]GTP were

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[1] To whom requests for reprints should be addressed, at Building 37, Room 5C25, NIH, Bethesda, MD 20892.

[2] The abbreviations used are: NCI, National Cancer Institute; TGI, lowest drug concentration that completely inhibits growth; IC_{50}, concentration of drug required to inhibit the increase in cell number by 50% relative to the increase in control cultures.

located by autoradiography and quantitated by counting in a liquid scintillation spectrometer.

HL-60 cells were grown at 37°C in a 5% CO_2 atmosphere in 5-ml suspension cultures in RPMI 1640 containing 17% fetal calf serum (both from Gibco-BRL), 0.3% L-glutamine, 0.01 mg/ml gentamicin sulfate, varying drug concentrations, and 1% (v/v) dimethyl sulfoxide. For determination of IC_{50} values, cells were counted after 40 h. Since cell number did not decrease significantly with drug treatment during this period, the IC_{50} value is defined as the concentration of drug required to inhibit the increase in cell number by 50% relative to the increase in the control cultures. For the determination of the mitotic index, cells were grown for 18 h. They were collected by centrifugation at 1000 rpm for 5 min. The cells were first washed with phosphate-buffered saline (pH 7.2), recollected by centrifugation, swollen by resuspension for 10 min in 0.033 M phosphate buffer, and recollected by centrifugation. The cells were fixed by the addition of ice-cold 1.5% (v/v) ethanol-0.5% (v/v) acetic acid. After 15 min they were recollected by centrifugation, resuspended in 0.25 ml of 75% ethanol-25% acetic acid, and transferred to a microscope slide. After the solvent had evaporated, the cells were stained with Giemsa and examined by bright-field microscopy. At least 200 cells were examined for the determination of a mitotic index, with control values routinely less than 5%.

RESULTS

Initial Evaluation of Compounds the COMPARE Algorithm Indicated Were Antimitotic Agents. Substantial numbers of agents have now been evaluated in the new human cell cytotoxicity drug screen of the NCI. Most of the compounds evaluated have been newer submissions, but approximately 200 standard agents have also been examined. These standard agents included a number of antimitotic drugs. The COMPARE algorithm was developed to permit the rapid selection of compounds with similar patterns of cytotoxicity toward the tumor panel. The algorithm rank-orders all entries in the database for similarity of pattern of differential cytotoxicity relative to the seed compound. The database was initially probed with five antimitotic compounds, vincristine, vinblastine, colchicine, podophyllotoxin, and taxol. We arbitrarily selected the 100 compounds most similar in pattern to each of the seeds. There was considerable overlap among the five lists, and most compounds on these lists were analogues of known antimitotic agents. Compounds with novel structures on these lists were first evaluated for effects on *in vitro* tubulin polymerization. Positive and negative compounds in the tubulin polymerization assay were then evaluated in terms of their cytotoxicity with the human tumor cell lines and in terms of their similarity of differential cytotoxicity pattern toward the seeds. That is, the quantitative correlation coefficients were examined.

Fig. 1 (previously presented in Ref. 11) is an attempt to present a visual image of what the COMPARE algorithm evaluates, patterns of differential cytotoxicity. Even though there were wide differences in the cytotoxicity of maytansine, the halichondrins, and VM-26 (see figure legend for details), some cell lines were more sensitive and others less sensitive than average toward each drug. In terms of a quantitative comparison of patterns, with maytansine as seed, halichondrin B and homohalichondrin B had correlation coefficients of 0.8, while that of VM-26 was 0.3. In computing the correlation coefficient the algorithm does not consider the quantitative deviation from the average as much as the qualitative pattern of more resistant or more sensitive. The ovarian carcinoma cell line OVCAR-3 is about 1000-fold more sensitive than the average line to maytansine but only 12-fold more sensitive to the halichondrins.

The Computer Search to Evaluate the COMPARE Algorithm. Based on the initial evaluations, we determined that the COMPARE algorithm would yield optimal results with antimitotic agents by imposing two restrictions on the compounds selected with any seed. First, the correlation coefficient should be at least 0.6. Second, our initial results indicated that compounds with low cytotoxicity generally did not greatly affect tubulin polymerization. Therefore, we imposed the second criterion that selected compounds be toxic (50% growth inhibition) at 1 μM or less in the original screen with HL-60 (TB) human leukemia cells.

Originally nine seeds were used. These agents were taxol, vincristine, vinblastine, colchicine, podophyllotoxin, maytansine, rhizoxin, dolastatin 10, and combretastatin A-4. The most potent compounds indicated by these nine seeds to be potential antimitotic agents were homohalichondrin B (NSC 609394) and halichondrin B (NSC 609395). These are complex polyether natural products derived from sponges of the genera *Halichondria* (12) and *Axinella* (13). We confirmed that these two agents inhibited tubulin polymerization and that halichondrin B was a noncompetitive inhibitor of *Vinca* alkaloid binding to tubulin, as presented elsewhere (11). Since halichondrin B and homohalichondrin B may bind at a distinct site on tubulin, the database was probed again with halichondrin B as a tenth seed, but no additional compounds were obtained.

Results of the Computer Search. Eighty-two compounds (including halichondrin B and homohalichondrin B) met the criteria summarized above. Besides the 9 seeds themselves,[3] there were 13 analogues of podophyllotoxin, 3 of colchicine, 9 of dolastatin 10, 7 of combretastatin A-4, 3 of taxol, 3 carbamates, and 2 benzylbenzodioxole derivatives (14). In addition there were 32 structurally novel compounds (19 distinct chemical species) as summarized in Table 1 and Figs. 2-5 (4 compounds, indicated as Compounds A–D in Table 1, remain proprietary, and we are unable to present their structures).

These 82 compounds were generally identified with multiple seeds. Including the probe with halichondrin B, only 7 compounds were identified with 1 seed, 2 with 2 seeds, 1 with 4 seeds, and 1 with 5 seeds. In contrast, 3 compounds were identified with 6 seeds, 5 with 7 seeds, 25 with 8 seeds, 24 with 9 seeds, and 14 with 10 seeds.

Evaluation of the Novel Chemical Structures for *in Vitro* Inhibition of Tubulin Polymerization and as Antimitotic Agents. Since we had initially surveyed most of the compounds presented in Figs. 2–5 for effects on tubulin polymerization to validate the utility of the COMPARE algorithm, we knew that most of these agents inhibited the reaction. To obtain quantitative measures of these inhibitory effects we determined the IC_{50} values of these compounds after a 20-min incubation (Table 1).

First we examined all compounds under our "standard" assay condition. Tubulin (10 μM) was preincubated with drug in 1.0 M monosodium glutamate (commercial preparation) supplemented with 1 mM $MgCl_2$ for 15 min at 37°C.[4] The polymerization reaction was initiated at 37°C following the addition of GTP. The preincubation results in substantial reduction in IC_{50} values for drugs which bind slowly to tubulin, especially colchicinoids (15, 16).

[3] When the database is probed using the COMPARE algorithm, the seed compound always identifies itself.

[4] Most commercial preparations of monosodium glutamate that we have examined have been contaminated with "endogenous" Mg^{2+}, in amounts sufficient to yield concentrations up to 1 mM in 1 M glutamate.

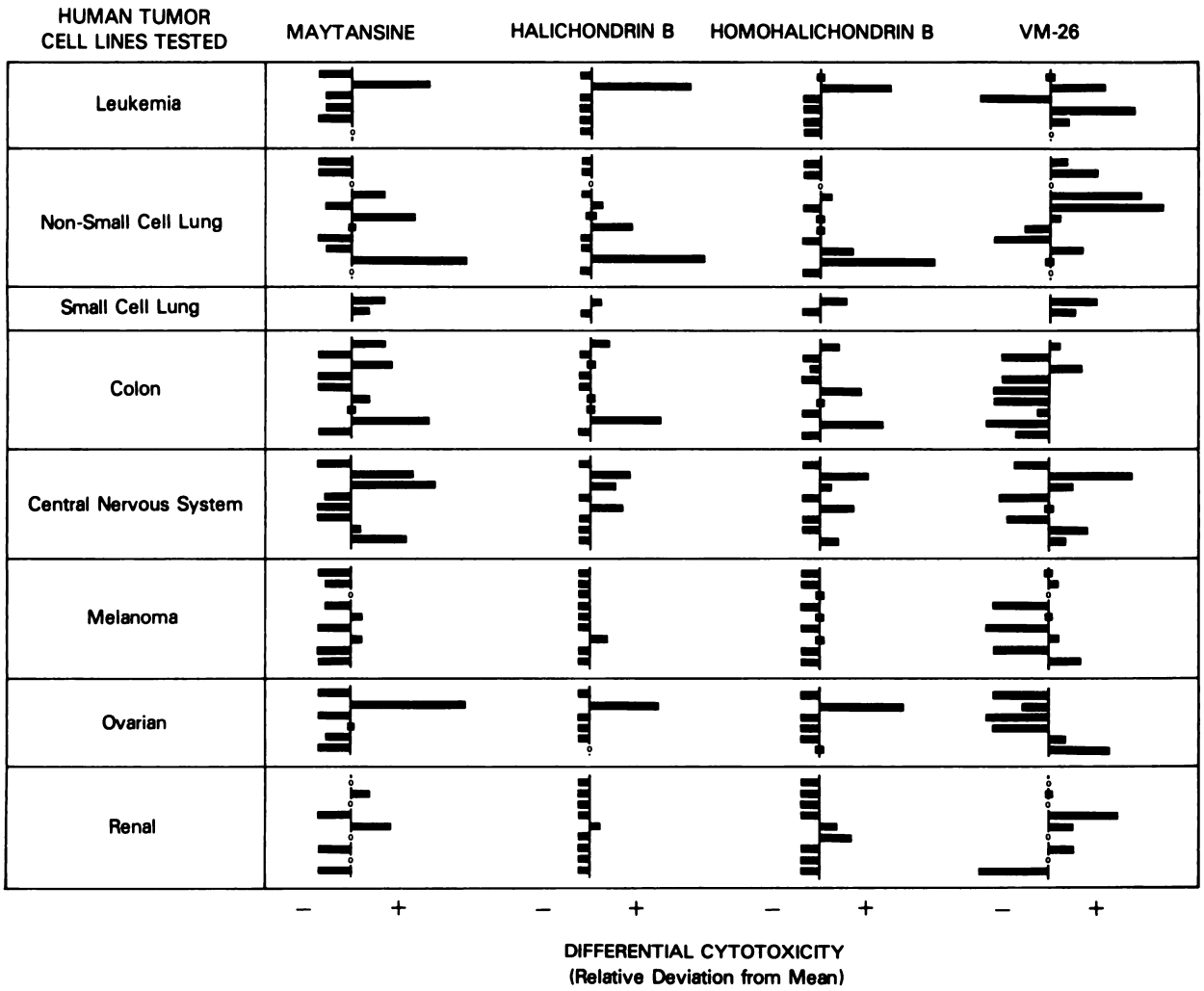


Fig. 1. Patterns of differential cytotoxicity toward human tumor cell lines. Drugs entered into the new National Cancer Institute screen are evaluated against 60 different human tumor cell lines. Their cytotoxic effects are evaluated and entered into a database on a VAX 9000 computer. The data summarized here are modified to present a visual image consistent with the COMPARE screening algorithm. For each cell line the TGI is obtained. For each agent a mean TGI log is determined, defined as the mean of the $\log_{10}$s of the individual TGI values. For each agent the difference between the $\log_{10}$ of each cell line and the mean TGI log for that agent is determined, to yield positive values for cell lines more sensitive than average (*bars projecting to the right*) and negative values for cell lines less sensitive than average (*bars projecting to the left*). The algorithm permits these values to be compared for all agents in the database, yielding Pearson correlation coefficients. For the figure, the data for each agent have been normalized against the maximally sensitive cell line for that agent, with the maximum deviation of equal length with all agents. The NCI screening data have yielded average TGI values of about 0.1 nM for maytansine, 7 nM for halichondrin B and homohalichondrin B, and 5 μM for VM-26. The maximally sensitive cell lines for each agent relative to the average TGI for that agent were as follows: about 1000-fold with maytansine; about 12-fold with halichondrin B; about 12-fold with homohalichondrin B; and about 8-fold with VM-26. A *circle on axis* indicates that a cell line was not successfully tested with the agent; a *square centered on an axis* indicates that the agent yielded a TGI value equivalent to the average value. The cell lines used in the NCI screen (order as in the figure): leukemia lines CCRF-CEM, HL-60 (TB), K-562, MOLT-4, RPMI-8226, and SR; non-small cell lung carcinoma lines A549/ATCC, EKVX, HOP-18, HOP-62, HOP-92, NCI-H226, NCI-H23, NCI-H322M, NCI-H460, NCI-H522, and LXFL-529L; small cell lung carcinoma lines DMS 114 and DMS 273; colon carcinoma lines COLO 205, DLD-1, HCC-2998, HCT-116, HCT-15, HT29, KM12, KM20L2, and SW-620; central nervous system cancer lines SF-268, SF-295, SF-539, SNB-19, SNB-75, SNB-78, U251, and XF 498; melanoma lines LOX IMVI, MALME-3M, M14, M19-MEL, SK-MEL-2, SK-MEL-28, SK-MEL-5, UACC-257, and UACC-62; ovarian carcinoma lines IGROV1, OVCAR-3, OVCAR-4, OVCAR-5, OVCAR-8, and SK-OV-3; and renal carcinoma lines 786–0, A498, ACHN, CAKI-1, RXF-393, RXF-631, SN12C, TK-10, and UO-31.

Recently, with benzylbenzodioxole analogues, we noted little progression of inhibition of turbidity development at higher drug concentrations (8). We attributed this to aberrant polymer formation analogous to that described with colchicine in the presence of higher concentrations of Mg^{2+} (17, 18). A similar problem was observed with at least two of the compounds examined here (NSC 624285 and NSC 635478). All apparently inactive and weakly active compounds were also examined in a second polymerization reaction condition (the "sensitive" assay) (8). The monosodium glutamate used in these experiments was repurified to remove Mg^{2+} (confirmed by atomic absorption spectroscopy), the reaction mixture was supplemented with 0.25 mM $MgCl_2$, and the preincubation and incubation took place at 30°C. This second reaction condition permitted ready quantitation of IC_{50} values for additional compounds which interfere with tubulin polymerization.[5]

[5] The compounds which yielded IC_{50} values only in the sensitive assay all had readily apparent effects on the standard polymerization reaction, as compared with the control reaction. In particular, there were significant reductions in the rate of turbidity development. These agents failed to adequately suppress the rise in turbidity in the standard assay to permit determination of IC_{50} values.

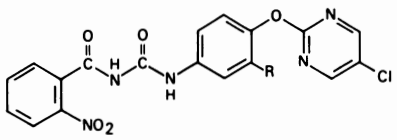
Table 1 *Effects of novel chemical structures predicted to be antitubulin agents by the COMPARE algorithm on tubulin polymerization and on mitosis in HL-60 human leukemia cells*

Compound (NSC no.)	No. of Seeds[a]	Inhibition of tubulin polymerization[b] (IC_{50}; μM)		Inhibition of HL-60 cell growth[c]	
		37°C/1 mM Mg^{2+} system	30°C/0.25 mM Mg^{2+} system	IC_{50} (μM)	% mitoses (μM drug)
56030	10	11±0.7[d]		0.2	26(2)
83292	9	>100	2.5±0.3	0.3	25(3)
609394[e]	8	~15[f]		0.001[g]	22(0.01)[g]
609395[h]	8	7.2±0.3		0.0003[g]	23(0.001)[g]
619859	1	>100	7.6±0.6	3	26(10)
622691	10	>100	2.5±0.5	0.5	14(2.5)
Compound A[i]	10	2.5±0.3	0.94±0.08		
624285	8	>100	3.0±0.6	0.4	29(4)
624544	9	2.4±0.6		0.07	27(0.7)
624545	8	4.2±0.6			
624546	8	2.3±0.1			
624547	6	2.1±0.5			
Compound B[i]	9	6.3±0.3		0.4	24(4)
Compound C[i]	9	8.7±0.2			
Compound D[i]	9	7.9±0.3			
630032	9	12±2		0.9	32(8)
631583	8	51±3	5.8±0.5	2	30(20)
635477	8	3.3±0.3	1.0±0.1	0.2	29(2)
635478	8	>100	1.6±0.2		
635479	8	5.5±0.5			
626391	9	90±8	15±1	7	14(60)
627777	7	>100	21±2	0.6	19(2)
625538	1	53±2	16±0.2	0.04	0(0.2)
83265	8	>100	>100	2	29(20)
622093	8	>100	>100	5	21(40)
76455	1	>100	>100	5	2(20)
376265	1	>100	>100	0.03	8(1)
628301	9	>100	>100	>30	2(100)
633268	1	>100	>100		
633270	2	>100	>100	0.3	3(2)
633272	1	>100	>100		
633274	1	>100	>100		

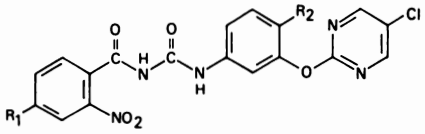
[a] Number of seeds which identified each agent as a potential antitubulin compound. The tenth probe with halichondrin B is included in this tabulation.
[b] Tubulin polymerization reactions: 1.0 mg/ml (10 μM) tubulin; 1 M monosodium glutamate (pH 6.6 with HCl); drugs with 4% (v/v) dimethyl sulfoxide; $MgCl_2$ as indicated; preincubation for 15 min at the indicated temperature; 0.4 mM GTP added; and 20 min incubation at the indicated temperature. IC_{50} value represents graphical determination of drug concentration which inhibits extent of polymerization by 50% (at least three independent determinations). Commercial monosodium glutamate was used in the 37°C/1 mM Mg^{2+} system; repurified glutamate was used in the 30°C/0.25 mM Mg^{2+} system.
[c] IC_{50} values were determined as described in the text, using multiple drug concentrations. The mitotic index for each drug was determined at a single concentration, as indicated for each agent in parentheses following the percentage of mitotic cells observed (see text for experimental detail).
[d] SDs presented for data from three independent determinations.
[e] Homohalichondrin B; data from Bai *et al.* (11).
[f] Supplies of homohalichondrin B were inadequate for an accurate determination of its IC_{50} value (11).
[g] Cytotoxicity data for homohalichondrin B and halichondrin B obtained with L1210 murine leukemia cells (11).
[h] Halichondrin B; data from Bai *et al.* (11).
[i] Proprietary compounds, the structures of which we are unable to reveal at the present time.

In at least one of these two assays we were able to demonstrate significant inhibition of tubulin polymerization for 23 of the 32 novel compounds the COMPARE algorithm had indicated were antitubulin agents. With 20 compounds, IC_{50} values substoichiometric to the tubulin concentration (10 μM) were obtained. For comparison with the agents summarized in Table 1, in previous studies (8, 19, 20) we have obtained IC_{50} values in the standard assay of 1.2 μM for dolastatin 10, 1.4 μM for thiocolchicine, 2.4 μM for colchicine, 2.5 μM for combretastatin A-4, 1.5 μM for vinblastine, 3.5 μM for maytansine, and 6.8 μM for rhizoxin. In the present studies a value of 2.6 μM was obtained for podophyllotoxin. In the sensitive assay a concurrent value of 0.86 μM was obtained for podophyllotoxin, and previously values of 0.64 and 1.1 μM were obtained for thiocolchicine and combretastatin A-4, respectively.

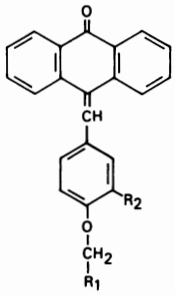
As in our previous study (8), IC_{50} values in the sensitive assay were always significantly lower than those obtained in the standard assay, when values could be obtained in both systems.



NSC 624285 : R = NO_2
NSC 624544 : R = CF_3
NSC 624545 : R = CH_3



NSC 624546 : $R_1 = NO_2$, $R_2 = CH_2CH_3$
NSC 624547 : $R_1 = H$, $R_2 = CH_3$



NSC 635477 : $R_1 = R_2 = H$
NSC 635478 : $R_1 = H$, $R_2 = OCH_3$
NSC 635479 : $R_1 = CH_3$, $R_2 = H$

Fig. 2. Structurally novel compounds selected by the COMPARE algorithm with antimitotic agents as seeds: the most potent inhibitors of tubulin polymerization.

Furthermore, there was generally a greater relative drop for less active agents. Thus, there was about a 3-fold reduction in the IC_{50} values for NSC 624285 (from 2.5 to 0.94 μM) and NSC 635477 (from 3.3 to 1.0 μM), but a 6–8-fold reduction for NSC 631583 (from 51 to 5.8 μM) and NSC 626391 (from 90 to 15 μM).[6]

Nine compounds which the COMPARE algorithm predicted would be antimitotic agents, however, did not affect tubulin polymerization *in vitro*. Moreover, these nine compounds also had no significant effect on polymerization dependent on microtubule-associated proteins.

Since the COMPARE algorithm uses data generated by the evaluation of drug effects on cell growth, as opposed to a cell-free biochemical assay, we evaluated the antimitotic effects of both the inactive compounds and those that inhibit tubulin polymerization (Table 1).

First, IC_{50} values were determined for effects on the growth of HL-60 cells in culture (cell number was measured as opposed to protein in the original screening studies). The IC_{50} values for most compounds fell within the expected range (0.01–1.0 μM). In six cases (see Table 1) the IC_{50} value we obtained fell in the range of 2–7 μM, representing both compounds which inhibited tubulin polymerization and compounds which were inactive as inhibitors. One agent (NSC 628301), which did not inhibit polymerization, was noncytotoxic in our hands, as opposed to the findings in screening studies. The reason for the discrepancy is unknown, but it may indicate that the compound is chemically unstable.

Next we evaluated each drug at one or more concentrations, generally at a concentration 3–10 times its IC_{50} value, for inhibition of mitosis (a mitotic cell defined morphologically as a cell with condensed chromosomes and no nuclear membrane). All agents examined which inhibited tubulin polymerization with IC_{50} values substoichiometric to the tubulin concentration (*i.e.*, less than 10 μM) caused a significant rise in the mitotic index (range, 14–32%). Furthermore, two of three superstoichiometric inhibitors also caused a significant increase in the mitotic index. The only inhibitor of tubulin polymerization which failed to increase the mitotic index was NSC 625538. It is perhaps of interest that this compound was selected by only a single seed.

In addition, two compounds which failed to inhibit tubulin polymerization, NSC 83265 and NSC 622093, caused the appearance of substantial numbers of cells arrested in mitosis. Both of these agents were selected by eight seeds. A borderline increase in mitotic cells was also observed with NSC 376265.

The remaining agents which failed to inhibit tubulin polymerization, although cytotoxic (with the exception of NSC 628301, see above), did not cause a significant change in the mitotic index. They thus seem to represent false positive predictions of the COMPARE algorithm.

Evaluation of the Novel Chemical Structures for Effects on Tubulin-dependent GTP Hydrolysis. We have previously proposed (21) that tubulin-dependent GTP hydrolysis (in 1 M glutamate) could be used as a simple *in vitro* screen to identify compounds which interact with tubulin, since antimitotic agents all seem either to stimulate or to inhibit this reaction (22, 23). The COMPARE algorithm has permitted us to identify a new group of antimitotic agents, and it was of interest to determine whether the GTP hydrolysis assay would have also permitted the identification of these compounds. In addition, the GTPase assay is technically more suitable for large-scale biochemical screening than the tubulin polymerization assay. It therefore might prove useful for indicating which compounds selected by the COMPARE algorithm represent false positives.

Accordingly, representatives of each class of new agent, both active and inactive, were examined for effects on tubulin-dependent GTP hydrolysis in 1.0 M glutamate (Table 2). [Halichondrin B (11) has previously been shown to inhibit the reaction.] If we use the criterion suggested previously (21) (that the hydrolytic reaction in the presence of the test compound deviating by more than 15% from the control reaction is to be considered positive), then all the substoichiometric polymerization inhibitors were positive. A borderline result was obtained only with NSC 83292, while unequivocal stimulation occurred with the others. Stimulation of GTP hydrolysis occurred as well with NSC 626391 and NSC 625538, two of the three superstoichiometric inhibitors, while the other agent in this group (NSC

[6] For NSC 625538, however, the reduction was about 3-fold (from 53 to 16 μM).

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