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In Vitro Antitumor Activities of 2,6-di-[2-(Heteroaryl)vinyl]pyridines and Pyridiniums

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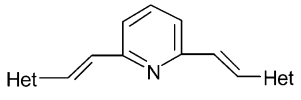
Abstract—The in vitro antitumor activities of 2,6-di-[2-(heteroaryl)vinyl]pyridines versus the standard National Cancer Institute 60 cell lines panel and of 2,6-di-[2-(heteroaryl)vinyl] pyridinium cations versus MCF7 (human mammary carcinoma) and LNCap (prostate carcinoma) cell lines are reported. Antiproliferative effects in both series are particularly evident for MCF7 mammary adenocarcinoma cells. Multivariate analysis of DNA microarray data for responsive tumor cell lines suggest a mechanistic pathway involving polyamine biosynthesis and prolactin signal transduction.

Introduction

We have recently reported on the synthesis and on the characterization of 2,6-di-[2-(heteroaryl)vinyl]pyridines (series **1**),[1] new potential antitumour agents where three heteroaromatic rings are linked by two ethylenic double bonds exerting a 'spacing' function. The above structural features are similar to those of antitumor drugs such as berenil, distamycin and pentamidine, including functionalised aromatic and heteroaromatic moieties linked by carbon and/or heteroatom linkers. The latter drugs are known to act as DNA binding agents and stable drug-DNA complexes characterised by crystal structural data[2] were obtained for pentamidine and berenil analogues where the central linkers were replaced with a furan ring. Evidence for the binding of the furyl and thiazolyl derivatives **1a** and **1c** to selected oligonucleotide sequences was provided by circular dichroism, and preliminary in vitro tests showed the furan derivative **1a** to be the most active in inhibiting the growth of MCF7 breast carcinoma cells[3] (Scheme 1).

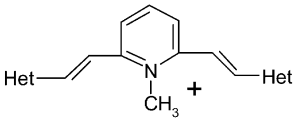
We here report the results of extensive antitumor in vitro tests for compounds **1a**–**1d** performed at the National Cancer Institute (NCI) for a standard 60 cell line panel. The above data, inserted into the prodigious amount of information already available in the NCI database,[4] allow comparisons with other drugs in the database and provide new opportunities for interpretation.

In order to improve water solubility, an important property in determining biological activity, we report also preliminary antitumor in vitro tests for recently synthesized 2,6-di-[2-(heteroaryl)vinyl] pyridinium cations (series **2**), some of which have been shown by



Series 1

1a-1d



Series 2

2a, 2c, 2e

Het

a) furan-2-yl
b) thiophen-2-yl
c) thiazol-2-yl
d) pyridin-2-yl
e) 1-methyl-pyrrol-2-yl

Scheme 1.

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spectroscopic methods (UV–visible, CD, NMR) to exhibit binding ability for a selected oligonucleotide sequence.[5]

Results and Discussion

Table 1 reports the $\log GI_{50}$ values for pyridine derivatives **1a**–**1d** tested in vitro at the National Cancer Institute against the standard 60 cell line panel.

The highest activity in Table 1 ($\log GI_{50} = -6.55$) is exhibited by the furan derivative **1a** towards MCF7 breast carcinoma cells, in agreement with preliminary in vitro tests for a few cell lines.[3] The interest of this finding is amplified by the fact that the above high activity is accompanied by an extreme selectivity of **1a** for a single cell line among the 60 considered in the NCI panel. The thiazole and terpyridine derivatives **1c** and **1d,** exhibiting terminal heteroaromatic moieties with a weak and strong electronwithdrawing character respectively, are less active ($\log GI_{50}$ in the range -4 to -5) than the furan and thiophene derivatives **1a** and **1b**, with terminal electrondonating five membered moieties. An interesting selectivity of **1d** for A498 renal cancer cells ($\log GI_{50} = -6.01$) can be also observed. The thiophene derivative **1b**, exhibiting an activity pattern in the range -4 to -5.81 with lower $\log GI_{50}$ values well distributed among cell lines from different tissues, is the only compound in Table 1 suitable for a multivariate statistical comparison with other drugs in the NCI database. Other compounds in Table 1 do not show sufficient activity variation among the 60 cell lines to warrant a reliable multivariate treatment. The sixty cell lines selected for the NCI panel are all derived from cancer tissues and we adopted such screening protocol for the evaluation of the potential antimitotic activity of novel drugs. Moreover the analysis of a large panel of cancer cell lines, widely different in several physiological and molecular aspects, revealed a selective activity on specific cell types and provided hints toward the possible mechanisms of action. Evaluation of the specificity of the drug effects on cancer cells and evaluation of their toxicity on normal cells (related or not-related to the antimitotic effects) would require specifically designed in vitro and in vivo experiments. Indeed, in the case of terminally differentiated cells results obtained in immortalized cell lines are not predictive of the drug effects on the normal cells in situ. Even in the case of proliferating cells the artificial conditions required for the in vitro survival and proliferation do not allow any definitive conclusions regarding the in vivo effects on normal cells.

The first multivariate insight into the NCI database, recently reported by our group,[6] pointed out that multivariate statistical methods are able to classify antitumor agents according to their mechanism of action, that is alkylating agents, RNA/DNA antimetabolites, DNA antimetabolites, antimitotic agents, topoisomerase II inhibitors. According to the SIMCA classification procedure,[6] fitting of a compound with unclassified mechanism of action into each class provides residual standard deviations (distances) with respect to each class: RSD values lower than the class critical distance indicate that the drug belongs to the considered class. Classification of **1b** into previously derived models for the above classes[6] appears to rule out the possibility that other known mechanisms may be operating (model distances higher than the corresponding critical distances). However, the possibility that **1b** acts through a peculiar new mechanism can not be excluded on the basis of the present statistical results and will be considered below (see comments of Table 2).

Multivariate statistical analysis provides other interesting opportunities for biological interpretation by means of the partial least squares projections to latent structures (PLS) procedure,[7] which allows to relate the expression level of thousands of different genes (called the 'descriptors' in the PLS procedure) to the in vitro therapeutical 'fingerprints' of a set of compounds (called the 'responses' in the PLS procedure) for the same cell lines (objects), and to evaluate the influence of each gene expression target in determining the therapeutical response(s) of known or newly discovered drugs.[8]

In this context, the gene expression targets were related to the in vitro activity of **1b** with the aim to find out which genes are highly relevant for its mechanism of action. PLS provides models characterised by statistical parameters such as the number of PLS components, the percentage of variance explained in the X matrix and in the dependent variable y, the predicting ability (Q^2) as well as the influence of each X variable in determining the y value, called variable importance for the projection (VIP). The latter parameter, representing a condensed summary of the importance of the X variables with respect to y, allows to rank all descriptors in order of decreasing importance.

In order to obtain a more reliable statistical result among the 60 cell lines considered in the NCI panel, we decided to consider only those for which **1b** exhibits log GI_{50} values lower than -4 (see Table 1). In fact, 31 cell lines with $\log GI_{50}$ above -4, representing the biological essay detection limit, provide no quantitative activity information and represent only an arbitrarily set plateau. A first PLS model included 9605 variables in the X matrix (gene expression targets from the NCI database) and one dependent variable (log GI_{50} values of **1b**) for the 28 cell lines taken into consideration. This model extracted two PLS components explaining already 96.4% variance of y, but had a rather low predicting ability ($Q^2 = 0.004$). Therefore a variable selection was operated and a second PLS model was derived by excluding gene expressions with very low systematic information, that is low predicting ability. This refined PLS model, including 6576 variables in the X matrix, extracted three PLS components explaining 99.1% of y variance and exhibited satisfactory Q^2 (0.589).

The VIP values of PLS models represent a proper statistical parameter to select the main gene expression targets involved in cancer chemotherapy for the selected drug. Table 2 reports the 'top 10' gene transcripts

(designated by the NCI database identification number) which are the same both for the complete (9605 variables) and the refined (6576 variables) models. The coefficients reported in the last column of Table 2 provide a useful indication on the correlation between molecular target level and biological response to the drug (log GI_{50}). A positive coefficient indicates that a higher level of the specific molecular target corresponds to a lower sensitivity to the drug.

Six transcripts reported in Table 2 are only partially sequenced and are not functionally annotated (ESTs). However, the top three and six positions in Table 2 are filled by two different NCI experiments (experiment identification: 2952 and 6506 respectively) both referred to the mRNA for a well-studied enzymatic protein: S-adenosylmethionine decarboxylase. This enzyme is essential for the biosynthesis of polyamines and provides an important target for the design of cancer

Table 1. In vitro antitumor activities, expressed as log GI_{50}, of **1a**–**1d** for the 60 NCI cell lines panel

NCI code		Panel	Cell line name	**1a**	**1b**	**1c**	**1d**
1	1	Non-small cell lung cancer	NCI-H23	> −4.30	> −4.00	> −4.30	−4.18
1	3	Non-small cell lung cancer	NCI-H522	> −4.30	−5.81	> −4.30	−4.87
1	4	Non-small cell lung cancer	A549/ATCC	> −4.30	−4.22	> −4.30	> −4.00
1	8	Non-small cell lung cancer	EKVX	> −4.30	> −4.00	−4.31	> −4.00
1	13	Non-small cell lung cancer	NCI-H226	> −4.30	> −4.00	> −4.30	> −4.00
1	17	Non-small cell lung cancer	NCI-H322M	> −4.30	−4.55	> −4.30	> −4.00
1	21	Non-small cell lung cancer	NCI-H460	> −4.30	−4.44	> −4.30	> −4.00
1	26	Non-small cell lung cancer	HOP-62	> −4.30	−4.08	> −4.30	> −4.00
1	29	Non-small cell lung cancer	HOP-92	> −4.30	> −4.00	> −4.30	> −4.00
4	1	Colon cancer	HT29	> −4.30	> −4.00	> −4.30	−4.25
4	2	Colon cancer	HCC-2998	> −4.30	−4.24	−4.56	−4.10
4	3	Colon cancer	HCT-116	> −4.30	> −4.00	> −4.30	−4.16
4	9	Colon cancer	SW-620	> −4.30	−4.61	> −4.30	−4.23
4	10	Colon cancer	COLO 205	> −4.30	−4.49	> −4.30	−4.40
4	15	Colon cancer	HCT-15	> −4.30	> −4.00	> −4.30	> −4.00
4	17	Colon cancer	KM12	> −4.30	−5.37	> −4.30	−4.33
5	1	Breast cancer	MCF7	−6.55	−5.45	−4.73	−4.58
5	2	Breast cancer	NCI/ADR-RES	> −4.30	> −4.00	> −4.30	−4.41
5	5	Breast cancer	MDA-MB-231/ATCC		> −4.00	> −4.30	> −4.00
5	6	Breast cancer	HS 578T	> −4.30	−5.59	> −4.30	−4.71
5	11	Breast cancer	MDA-MB-435	> −4.30	> −4.00	> −4.30	−4.08
5	12	Breast cancer	MDA-N	> −4.30	> −4.00	> −4.30	−4.17
5	13	Breast cancer	BT−549	> −4.30	−4.66	> −4.30	−4.73
5	14	Breast cancer	T−47D		−5.21	−4.51	−4.35
6	1	Ovarian cancer	OVCAR−3	> −4.30	−4.29	> −4.30	−4.10
6	2	Ovarian cancer	OVCAR−4	> −4.30	−4.91	> −4.30	−4.22
6	3	Ovarian cancer	OVCAR−5	> −4.30	> −4.00	> −4.30	> −4.00
6	5	Ovarian cancer	OVCAR−8	> −4.30	−4.22	−4.32	−4.44
6	10	Ovarian cancer	IGROV1	> −4.30	−4.30	−4.58	−4.21
6	11	Ovarian cancer	SK−OV−3	> −4.30	> −4.00	> −4.30	> −4.00
7	3	Leukemia	CCRF−CEM	> −4.30	> −4.00	> −4.30	−4.43
7	5	Leukemia	K−562	> −4.30	> −4.00	> −4.30	−4.32
7	6	Leukemia	MOLT−4	> −4.30	> −4.00	−4.42	−4.50
7	8	Leukemia	HL−60(TB)	> −4.30	> −4.00	> −4.30	−4.14
7	10	Leukemia	RPMI−8226	> −4.30	> −4.00	> −4.30	−4.36
7	19	Leukemia	SR	−4.47	−4.49	> −4.30	−4.21
9	4	Renal cancer	UO−31	> −4.30	−4.52	> −4.30	−4.58
9	8	Renal cancer	SN12C	> −4.30	−4.21	> −4.30	−4.22
9	13	Renal cancer	A498	> −4.30		> −4.30	−6.01
9	15	Renal cancer	CAKI−1	> −4.30	> −4.00	> −4.30	−4.29
9	16	Renal cancer	RXF 393	> −4.30	> −4.00	> −4.30	−4.21
9	18	Renal cancer	786−0	> −4.30	−4.45	> −4.30	> −4.00
9	23	Renal cancer	ACHN	> −4.30	−5.09	> −4.30	−4.45
9	24	Renal cancer	TK−10	> −4.30	−4.46	> −4.30	> −4.00
10	1	Melanoma	LOX IMVI	> −4.30	> −4.00	> −4.30	−4.18
10	2	Melanoma	MALME−3M	> −4.30	−5.76	> −4.30	−4.76
10	5	Melanoma	SK−MEL−2		> −4.00	> −4.30	> −4.00
10	7	Melanoma	SK−MEL−5	> −4.30	> −4.00	> −4.30	−4.31
10	8	Melanoma	SK−MEL−28	> −4.30	> −4.00	> −4.30	−4.09
10	14	Melanoma	M14	> −4.30	> −4.00	> −4.30	−4.36
10	20	Melanoma	UACC−62	> −4.30	> −4.00	> −4.30	−4.57
10	21	Melanoma	UACC−257	> −4.30	> −4.00	> −4.30	> −4.00
11	1	Prostate cancer	PC−3		> −4.00	> −4.30	−4.23
11	3	Prostate cancer	DU−145	> −4.30	> −4.00	> −4.30	> −4.00
12	2	CNS cancer	SNB−19	> −4.30	−4.10	> −4.30	> −4.00
12	5	CNS cancer	SNB−75	−4.88	−4.71	−4.66	−4.43
12	9	CNS cancer	U251		−4.26	−4.46	> −4.00
12	14	CNS cancer	SF−268	> −4.30	−5.58	> −4.30	−4.69
12	15	CNS cancer	SF−295	> −4.30	> −4.00	> −4.30	−4.18
12	16	CNS cancer	SF−539	> −4.30	> −4.00	> −4.30	> −4.00

chemotherapeutic agents.[9] Polyamines spermidine and spermine, and their diamine precursor putrescine, are ubiquitous polycations with many metabolic functions and their involvement in cell proliferation has been evidenced in healthy and tumour tissues.[10,11] The polyamine biosynthesis involves a concerted action of four separate enzymes, but the overall rate is predominantly regulated by changes in the activities of ornithine and adenosylmethionine decarboxylases, because spermidine and spermine synthases are expressed constitutively and largely regulated by the availability of their common substrate, decarboxylated *S*-adenosylmethionine.[12] An analysis of the *S*-adenosylmethionine decarboxylase mRNA levels in the 28 cell lines used for the SIMCA analysis revealed an inverse correlation between transcript level and sensitivity to the antiproliferative activity of compound **1b**. The above finding, however, does not allow to establish if the polyamine pathway is directly involved in the the antimitotic activity of compound **1b** or it is only functionally correlated to the real molecular target of the drug. Moreover, the presence of a certain degree of specificity for the class of compounds reported in this study can be inferred by the fact that the level of *S*-adenosylmethionine decarboxylase transcript was not found to be a crucial factor in determining sensitivity or resistance to several others antiproliferative drugs.[8] Interestingly, among the top 10 gene transcripts, ranked according to the VIP value, there is also the mRNA for the prolactin receptor (position 7, Table 2). The neuroendocrine hormone prolactin stimulates breast growth and differentiation during puberty, pregnancy, and lactation. Despite extensive and convincing data indicating that prolactin significantly contributes to the pathogenesis and progression of rodent mammary carcinoma, parallel observations for human breast cancer have not been concordant. In particular, the therapeutic alteration of somatolactogenic hormone levels has not consistently altered the course of human breast cancer. Within the last few years, however, several studies reported that prolactin is also locally synthesized both in the normal mammary gland and in the neoplastic tissue, and that it exerts its proliferative action in an autocrine/paracrine manner.[13–15] The growth regulatory effects of prolactin on human breast are mediated by its receptor, a member of the cytokine receptor family. The prolactin receptor is expressed in a wide variety of tissues and in human breast carcinoma[16] and it has been recently demonstrated that the mRNA encoding the prolactin receptor is up-regulated in six of 11 breast cancer cell lines when compared with normal breast tissue.[17] An analysis of these quantitative data[17] confirms a direct correlation between the mRNA levels for the prolactin receptor and the sensitivity to the antimitotic activity of compound **1b** in the examined breast cell lines. A possible link between the polyamine pathway and the prolactin receptor is suggested by the observation that the

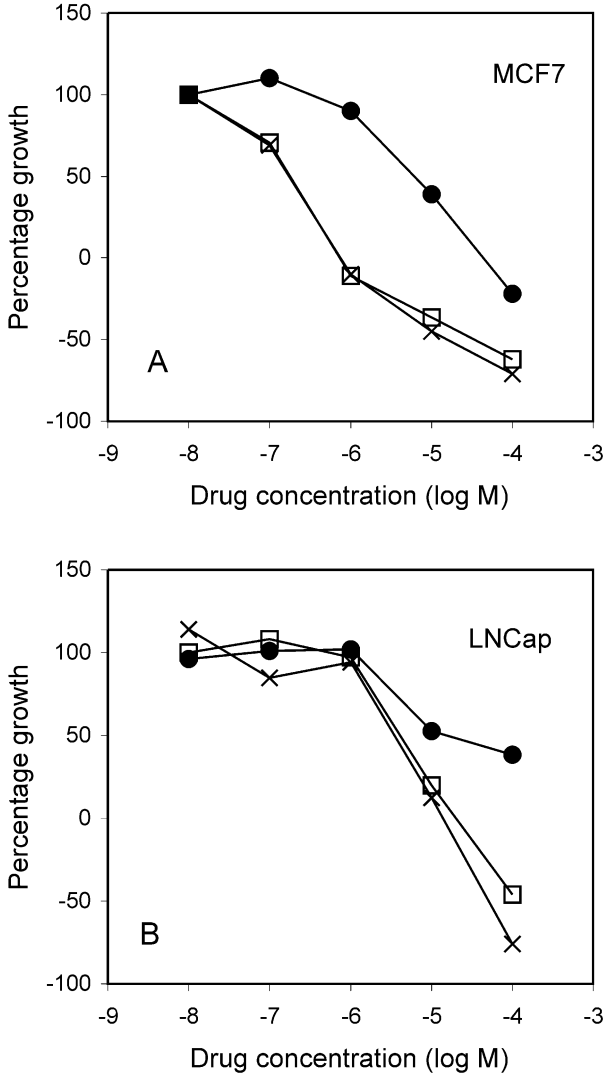

Figure 1. Dose—response curves of the antiproliferative activities of compounds **2a** (□), **2c** (●) and **2e** (×) on MCF7 (A) and LNCap (B) cell lines.

Table 2. Top 10 gene transcripts in the PLS model for the thiophene derivative **1b**

Rank	NCI code	Gene product[a]	Description	Coefficient
1	GC16501	EST	—	Negative
2	GC13878	EST	Similar TORF2	Negative
3	GC12354	AMD1	*S*-Adenosylmethionine decarboxilase 1	Positive
4	GC11493	EST	—	Negative
5	GC10892	EST	—	Negative
6	GC15908	AMD1	*S*-Adenosylmethionine decarboxilase 1	Positive
7	GC12336	PRLR	Prolactin receptor	Negative
8	GC12757	Unknown	—	Negative
9	GC13818	EST	—	Negative
10	GC16754	PDX1	Pyruvate dehydrogenase protein *X* component	Positive

[a]Nomenclature according to ref 22. EST, expressed sequence tag.

Table 3. In vitro antitumor activities, expressed as log GI_{50}, of **2a**, **2c** and **2e** for MCF7 and LNCap cell lines

Cell line	2a	2c	2e
MCF7 (breast)	−6.75	−5.2	−6.75
LNCap (prostate)	−5.38	−4.81	−5.47

prolactin stimulation of most lactational processes requires an earlier stimulating effect on the synthesis of the polyamines.[18]

Some of us have recently reported[5] the synthesis of water soluble 2,6-di-[2-(heteroayl)vinyl] pyridinium cations **2a** and **2e** and a study of their interactions with the decamer $d(CGTACGTACG)_2$, providing clear spectroscopic evidences for the DNA binding ability of compounds **2a** and **2e**. These results strongly suggest to test the anti-proliferative activity of the above water soluble compounds in cell cultures. Unfortunately compounds **2a**, **2c** and **2e** were not accepted in the standard 60 cell lines anti-tumor screening program of the National Cancer Institute. Therefore we decided to analyse their anti-proliferative activity in our laboratory. Two tumor cell lines, breast carcinoma (MCF7) and prostate carcinoma (LNCap), were selected, the former due to its sensitivity exhibited towards non cationic analogues of series **1**. The percent of growth and the inhibition exerted by different doses (0.01–100 μM) are shown in Figure 1 and the log GI_{50} values, calculated as described in the experimental section, are reported in Table 3. All three compounds were more active against the breast cell line (MCF7) with the furan and pyrrole derivatives **2a** and **2e** being the most potent. The same compounds were also active on the LNCap cell line, although log GI_{50} values were one order of magnitude lower. The observed activities for the MCF7 breast cell line, resembling those of the corresponding pyridines in series **1**, suggest the possibility that pyridiniums **2** may act through the same mechanism.

Conclusions

In conclusion, bisheteroaroarylvinyl pyridines and pyridiniums in both series **1** and **2** exhibit in vitro antitumor antiproliferative effects, particularly evident for MCF7 mammary adenocarcinoma cells. Multivariate comparisons with the 60 cell line NCI standard database suggest that the antitumor activity of pyridines **1** might be exerted through a novel mechanism. PLS shows that the top 10 genes include gene transcripts related to the polyamine biosynthetic pathway and to the prolactin signal transduction.

Experimental

Compounds

The synthesis and spectroscopic characterization of the tested compounds has been reported: 2,6-di-[2-(furan-2-yl)vinyl]pyridine (**1a**), mp 104–105 °C.[1]

2,6-di-[2-(Thien-2-yl)vinyl]pyridine (**1b**), mp 165–167 °C.[1]

2,6-di-[2-(Thiazol-2-yl)vinyl]pyridine (**1c**), mp 198–200 °C.[1]

2,6-di-[2-(Pyridin-2-yl)vinyl]pyridine (**1d**), mp 152–154 °C.[1]

2,6-di-[2-(Furan-2-yl)vinyl]pyridinium iodide (**2a**), mp 214–215 °C.[5]

2,6-di-[2-(Thiazol-2-yl)vinyl]pyridinium iodide (**2c**), mp 210–212 °C.[5]

2,6-di-[2-(1-Methyl-pyrrol-2-yl)vinyl]pyridinium iodide (**2e**), mp >210 °C.[5]

Biological essays

Human cell lines (LNCap and MCF7). Human prostate adenocarcinoma cells (LNCap) were grown in RPMI 1640. Human mammary adenocarcinoma (MCF7) were grown in Dulbecco's MEM (DMEM), 1.0 g/l D-glucose. Each medium was supplemented with 10% (vol/vol) heat-inactivated fetal bovine serum, 2 mM L-alanyl-L-glutamine, penicillin-streptomycin (50 units–50 μg for mL) and incubated at 37 °C in humidified atmosphere of 5% CO2, 95% air. The culture medium was changed twice a week.

Treatment with antitumor agents and MTT colorimetric assay. Each human cancer cell line (5×10^3 cells/0.33 cm^2) were plated in 96-well plates 'Nunclon™ Microwell™' (Nunc) and were incubated at 37 °C. After 24 h, cells were treated with the following compounds **2a**, **2c** and **2e** (final concentration 0.01–100 μM). Untreated cells were used as controls. Microplates were incubated at 37 °C in humidified atmosphere of 5% CO_2, 95% air for 2 days and then cytotoxicity was measured with colorimetric assay based on the use of tetrazolium salt MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide).[19] The results were read on a multiwell scanning spectrophotometer (Multiscan reader), using a wavelength of 570 nm. Each value was the average of eight wells (standard deviations were less than 20%). The GI_{50} value was calculated according to NCI: thus, GI_{50} is the concentration of test compound where $100\times(T-T_0)/(C-T_0)=50$ (T is the optical density of the test well after a 48-h period of exposure to test drug; T_0 is the optical density at time zero; C is the control optical density).

Multivariate methods

PLS[7] was carried out by means of SIMCA sofware package[20] using an X data matrix containing 576,300 (9605×60) elements x_{ik}, where index k is used for the gene expression profiles (variables) and index i for the cell lines (objects) and the antitumour activities, expressed as log GI_{50}, as the dependent variable (y). The PLS model describes the X matrix by a principal component-like model (eq 1) and the y values as a predictive relationship with the X matrix principal components, under

the constraint of maximizing the correlation between y and t (eq 2), where b_a is a proportionality coefficient for each dimension a. The number of significant dimension, A, was determined by the SIMCA program by using the cross validation technique.[21]

$$x_{ik} = \bar{x}_k + \sum_{a=1}^{a=A} t_{ia} p_{ak} + e_{ik} \quad (1)$$

$$y_{ia} = \sum b_a t_{ia} + h_{ia} \quad (2)$$

The statistical results obtained by the PLS method are able to detect what variables in the X block are relevant to determine the dependent variable (y) by means of the VIP values. The VIP values reflect, in fact, the importance of terms in the model both with respect to y, that is its correlation to the biological response, and with respect to X. SIMCA computes VIP values[7,20] by summing over all model dimensions the contributions VIN (variable influence). For a given PLS dimension, a, $(VIN)^2_{ak}$ is equal to the squared PLS weight $(w_{ak})^2$ of that term, multiplied by the percent explained of residual sum of squares by that PLS dimension. The accumulated (over all PLS dimensions) value, $VIP_k = \sum_a (VIN)^2_k$ is then divided by the total percent explained of residual sum of squares by the PLS model and multiplied by the number of terms in the model.

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References and Notes

1. Ballistreri, A.; Gregoli, L.; Musumarra, G.; Spalletti, A. *Tetrahedron* **1998**, *54*, 9721.
2. Trent, J. O.; Clark, G. R.; Kumar, A.; Wilson, W. D.; Boykin, D. W.; Hall, J. E.; Tidwell, R. R. *J. Med. Chem.* **1996**, *39*, 4554.
3. Fichera, M.; Gregoli, L.; Musumarra, G. *J. Phys. Org. Chem.* **2000**, *13*, 344.
4. Database resources currently available on the World Wide Web URL: http://www.dtp.nci.nih.gov
5. Fichera, M.; Fortuna, C. G.; Impallomeni, G.; Musumarra, G. *Eur. J. Org. Chem* **2002**, 145.
6. Musumarra, G.; Condorelli, D. F.; Costa, A. S.; Fichera, M. *J. Comp. Aided Mol. Design* **2001**, *15*, 219.
7. Wold, S. PLS in Chemistry. In *The Encyclopedia of Computational Chemistry*; Schleyer P. v. R.; Schreiner, P. R.; Allinger, N. L.; Clark, T.; Gasteiger, J.; Kollman, P.; Schaefer III, H. F., Eds.; John Wiley and Sons: Chichester, 1998; p 2006.
8. Musumarra, G.; Condorelli, D. F.; Scire, S.; Costa, A. S. *Biochem. Pharmacol.* **2001**, *62*, 547.
9. Tolbert, W. D.; Ekstrom, J. L.; Mathews, I. I.; Secrist, J. A., III; Kapoor, P.; Pegg, A. E.; Ealick, S. E. *Biochemistry* **2001**, *40*, 9484.
10. Jänne, J.; Alhonen, L.; Leinonen, P. *Ann. Med.* **1991**, *23*, 241.
11. Tabor, C. W.; Tabor, H. *Annu. Rev. Biochem.* **1984**, *53*, 749.
12. Pegg, A. E. *Biochem. J.* **1986**, *234*, 249.
13. Clevenger, C. V.; Plank, T. L. *J. Mammary Gland Biol. Neoplasia* **1997**, *2*, 59.
14. Goffin, V.; Touraine, P.; Pichard, C.; Bernichtein, S.; Kelly, P. A. *Mol. Cell. Endocrinol.* **1999**, *151*, 79.
15. Wennbo, H.; Tornell, J. *Oncogene* **2000**, *19*, 1072.
16. Reynolds, C.; Montone, K. T.; Powell, C. M.; Tomaszewski, J. E.; Clevenger, C. V. *Endocrinology* **1997**, *138*, 5555.
17. Peirce, S. K.; Chen, W. Y.; Chen, W. Y. *J. Endocrinol.* **2001**, *171*, R1.
18. Rillema, J. A.; Collins, S.; Williams, C. H. *Proc. Soc. Exp. Biol. Med.* **2000**, *224*, 41.
19. Mosmann, T. *J. Immunol. Meth.* **1983**, *65*, 55.
20. *SIMCA-P 8.0, User Guide and Tutorial*; Umetri AB: Umeå, Sweden, 1999.
21. Wold, S. *Technometrics* **1978**, *20*, 397.
22. Rebhan, M.; Chalifa-Caspi, V.; Prilusky, J.; Lancet, D. GeneCards: *Encyclopedia for Genes, Proteins and Diseases*; Weizmann Institute of Science: Bioinformatics Unit and Genome Center, Rehovot (Israel), 1997 (World Wide Web URL: http://bioinfo.weizmann.ac.il/cards).