Pergamon

Biochemical Pharmacology, Vol. 50, No. 4, pp. 451–457, 1995.

0006-2952/95 $9.50 + 0.00

0006-2952(95)00174-3

REVERSAL OF MULTIDRUG RESISTANCE BY VERAPAMIL ANALOGUES

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(*Received* 14 *December* 1994; *accepted* 10 *April* 1995)

Abstract—The basic distinguishing feature of multidrug resistant (MDR) cells is a decrease in steady-state drug levels as compared to drug-sensitive controls. It is well-known that verapamil increases the sensitivity of MDR cells to drugs, thus reverting drug resistance. A limiting factor for its clinical use is the pronounced cardiovascular effects of the calcium channel antagonist which occur at the high plasma concentrations required to block P-glycoprotein transport efficiently. From a clinical point of view, it is important to find verapamil derivatives with low calcium channel blocking activity and high reverting activity. This was the aim of the present study. In this context we have investigated the ability of 20 verapamil analogues with restricted molecular flexibility to increase cellular accumulation of anticancer drugs and overcome resistance, and their inotropic, chronotropic, and slow calcium channel antagonistic activity. In this study an anthracycline derivative 4′-*O*-tetrahydropyranyl adriamycin, and an erythroleukaemia K562 cell line were used. Three of the 20 derivatives checked were completely devoid of calcium channel blocking activity while exhibiting MDR reverting ability comparable to that of verapamil. These derivatives could be useful for the treatment of MDR in cancer patients and for the design and development of other verapamil derivatives.

Key words: multidrug resistance; verapamil analogues; restricted flexibility; adriamycin

Tumour cells having the MDR§ phenotype exhibit a reduced sensitivity to cytotoxic drugs when compared with the parental drug-sensitive cells. The cellular pharmacological basis for MDR *in vitro* appears to be a reduced steady-state accumulation of drugs in resistant cells compared to sensitive cells, caused by the overexpression of the *mdr1* gene which encodes for a P-gp of 170–180 kDa. P-gp is thought to function as an energy-dependent drug efflux pump [1–5].

Several agents, including well-known calcium channel blockers such as verapamil, have been shown to increase the sensitivity of MDR cells to drugs, thus reverting drug resistance [6–9]. These agents are therefore of potential therapeutic use. A limiting factor, however, is the pronounced cardiovascular effects of the calcium channel antagonist which occur at the high plasma concentrations required to block P-gp transport efficiently. It has been clearly demonstrated that the reversal of doxorubicin resistance by verapamil was not due to its effect on calcium channels [9]. In the past few years, several analogues of verapamil have been designed and studied which, with respect to the rather flexible parent molecule, possess fewer degrees of rotational freedom and can therefore provide information on the active conformation of verapamil at its binding site on the Ca^{2+} channel [10–14]. The verapamil analogues were tested for negative inotropic and chronotropic activity on guinea pig atria and for relaxant activity on guinea pig aorta strips, while Ca^{2+} channel binding was estimated by measuring the displacement of (−)-[^{3}H]-D888 from ventricle membrane homogenates. The results showed that reduction of the conformational freedom of the verapamil molecule has a profound influence on binding and on cardiovascular activity, leading in some cases to the disappearance of calcium antagonism [10–14].

These findings prompted us to evaluate the effect of such compounds as to their ability to increase cellular accumulation of anticancer drugs and overcome resistance in order to identify derivatives with low or null calcium blocking activity and high MDR reversion. As far as MDR activity is concerned, it was hoped to collect more information on structure–active relationships in this class of compounds, since it would be of great clinical interest to find agents lacking calcium antagonism but endowed with high reverting action. An anthracycline derivative, THP-adriamycin, and an erythroleukaemia K562 cell line were used. Our results have enabled us to identify three molecules that lack calcium antagonism and exhibit MDR reverting ability comparable to that of verapamil, suggesting a pathway by which more potent and safer verapamil analogues can be designed and developed.

MATERIALS AND METHODS

Drugs and chemicals. Purified THP-adriamycin (Scheme 1) was kindly provided by Laboratoire

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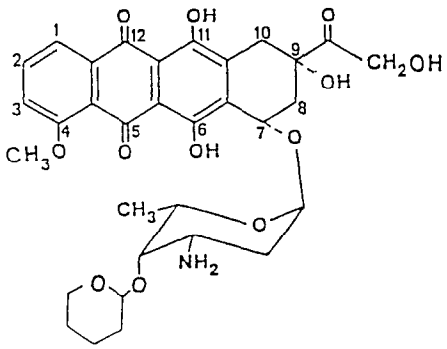
§ Abbreviations: THP, 4′-*O*-tetrahydropyranyl; MDR, multidrug resistance.



4'-*O*-tetrahydropyranyl-adriamycin

Scheme 1.

Roger Bellon (France). Concentrations were determined by diluting stock solutions to approximately 10^{-5} M and using $\varepsilon_{480} = 11500\ M^{-1}cm^{-1}$. Stock solutions were prepared just before use. The verapamil analogues were provided by the research group that originally synthesized them and are pure chemicals as stated in the original publications [10–14]: their structures are shown in Scheme 2. All other reagents were of the highest quality available and deionized double-distilled water was used throughout the experiments. Unless otherwise stated, buffer solutions were HEPES buffer containing 5 mM HEPES, 132 mM NaCl, 3.5 mM KCl, 1 mM $CaCl_2$, 0.5 mM $MgCl_2$, 5 mM glucose, at pH 7.25.

Absorption spectra of THP-adriamycin were recorded on a Cary 219 spectrophotometer and fluorescence spectra of THP-adriamycin on a Jobin Yvon JY 3CS spectrofluorometer.

Cell lines and cultures. Anthracycline-sensitive and anthracycline-resistant erythroleukaemia K562 cells were a gift of Dr Tapiero (Departement de Pharmacologie Cellulaire, ICIG, 94800 Villejuif, France). They were grown in RPMI (Flow) medium supplemented with L-glutamine and 10% FCS at 37° in a humidified atmosphere of 95% air and 5% CO_2. Cultures initiated at a density of 10^5 cells/mL grew exponentially to 8–10 × 10^5 cells/mL in 3 days. For the short-term measurements of drug accumulation, the culture was initiated at 5 × 10^5 cells/mL in order to have cells in the exponential growth phase. The cells were used 24 hr later when they were at approximately 8–10 × 10^5 cells/mL. Cell viability was assessed by trypan blue exclusion. The cell number was determined by Coulter counter analysis.

For long-term growth inhibition, cells were grown in culture as described above. The ability of a molecule to reverse MDR was assessed as follows: 1 × 10^5 cells/mL were cultured in the presence of various THP-adriamycin concentrations and in the simultaneous presence or absence of resistance modifying agent. We first verified that the resistance-modifying agents alone had no effect on cell proliferation at the concentrations used. The IC_{50} values were determined by plotting the percentage of cell growth inhibition versus the logarithm of antitumour drug concentration: IC_{50} is the drug concentration that inhibits cell division by 50% after 72 hr while the percentage of cell growth inhibition is defined as $[N_0 - N_x)/(N_0 - 1)] \times 100$, where N_0 and N_x are the numbers of cells in the absence and presence of THP-adriamycin at concentration x, respectively.

A "resistance factor" was obtained by dividing the IC_{50} of resistant cells by the IC_{50} of the corresponding sensitive cells. The resistance factors obtained were 28 and 8 for adriamycin and THP-adriamycin, respectively.

Total RNAs were prepared from frozen cells according to a CsCl–guanidinium isothiocyanate method proposed by Maniatis *et al.* [15] and adapted by Ferrandis *et al.* [16]. Transcript level of the MDR1 gene was measured comparatively to that of the KB-8–5 cell line which shows an arbitrary expression of 30 AU [17]. Our K562 resistant cells exhibited an MDR1 gene transcript level of 800 AU (Benard and Garnier-Suillerot, unpublished data).

Cellular drug accumulation. The uptake of THP-adriamycin in cells was followed by monitoring the decrease in the fluorescence signal at 590 nm (λ_{ex} = 480 nm). This spectrofluorometric method has been previously described [18–22]. Using this method it is possible to accurately quantify the kinetics of the drug uptake by the cells and the amount of anthracycline intercalated between the base pairs in the nucleus at the steady state, as incubation of the cells with the drug proceeds without compromising cell viability. All experiments were conducted in 1 cm quartz cuvettes containing 2 ml of buffer at 37°.

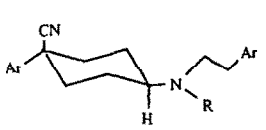
We checked that test verapamil derivatives did not affect the fluorescence of THP-adriamycin.

RESULTS

The overall concentration C_n of THP-adriamycin bound to the nucleus of drug-resistant cells was determined at the steady-state in the presence of verapamil derivatives at different concentrations. In all cases, C_n increased as the concentrations of inhibitor increased and this can be quantified using the following equation:

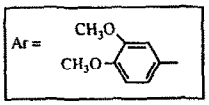
$$\alpha = [(C_n)_{R_i} - (C_n)_{R_0}]/[(C_n)_S - (C_n)_{R_0}] \qquad (1)$$

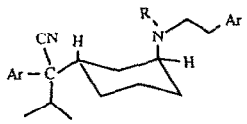
where $(C_n)_S$ is the overall concentration of drug bound to the nucleus of sensitive cells and $(C_n)_{R_0}$ and $(C_n)_{R_i}$ are the overall concentrations of drug bound to the nucleus of resistant cells in the absence and presence of a concentration $[i]$ of inhibitor respectively. α is the fold increase in the nuclear concentration of THP-adriamycin in the presence of reversing agent. α varies between 0 (in the absence of inhibitor) and 1 (when the amount of drug in resistant cells is the same as in sensitive cells). Figure 1 shows two typical experiments performed with compounds (**4**) and (**8**), respectively. The variation in the overall concentration of drug bound to the nuclei $(C_n)_{R_i}$ at the steady state after the addition of different concentrations of compound (**4**) or (**8**) has been plotted as a function of their concentrations.



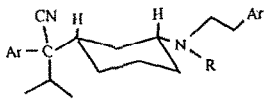
R=H (1)

R=CH_3 (2)

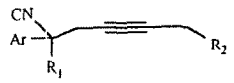


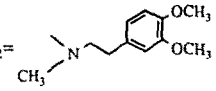


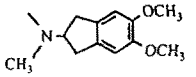
R=H (R*R*R*) (3)
R=H (R*R*S*) (4)
R=CH_3, (R*R*R*) (5)
R=CH_3, (R*R*S*) (6)

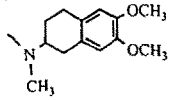


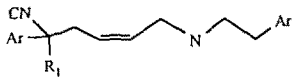
R=H (R*S*S*) (7)
R=H (R*S*R*) (8)
R=CH_3, (R*S*S*) (9)
R=CH_3, (R*S*R*) (10)



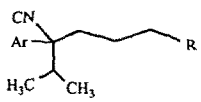
R_1= H, R_2=  (11)

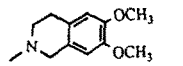
R_1=iPr, R_2=  (12)

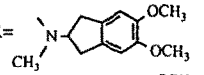
R_1=iPr, R_2=  (13)

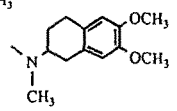


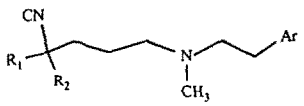
(14)



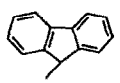
R=  (15)

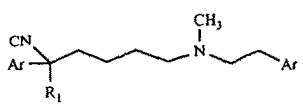
R=  (16)

R=  (17)



R_1=R_2= phenyl (18)

R_1R_2=  (19)



(20)

Scheme 2.

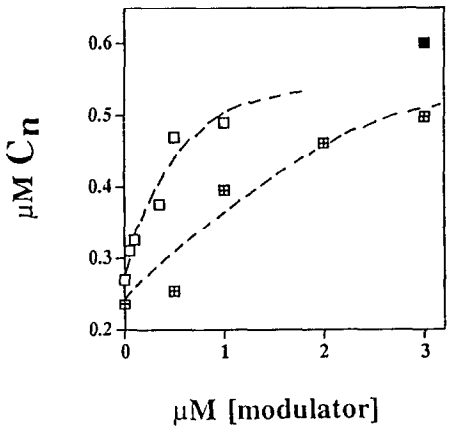


Fig. 1. Effect of verapamil derivatives (**4**) and (**8**) on THP-adriamycin accumulation in living cell nuclei. C_n, the overall concentration of THP-ADR bound to the nucleus at the steady state, has been plotted as a function of the modulator concentrations (□ (**4**), ⊞ (**8**)), the filled square corresponding to the overall concentration of THP-ADR bound to the nucleus after the addition of Triton X-100. It does not depend on the modulator concentration. The experimental conditions are described in the Materials and Methods section.

The inhibitor concentrations required to obtain $\alpha = 0.5$, i.e. to cause a half-maximal increase in cellular THP-adriamycin accumulation, are reported in Table 1.

The THP-adriamycin concentrations required to inhibit 50% of cell proliferation, IC_{50}, was determined in the presence of each inhibitor at the concentration close to that required to obtain $\alpha = 0.5$. $(IC_{50})_{R_i}$, the IC_{50} value of resistant cells obtained in the presence of a concentration $[i]$ of inhibitor, can be expressed as:

$$\beta = [(IC_{50})_{R_i} - (IC_{50})_{R_0}]/[(IC_{50})_S - (IC_{50})_{R_0} \quad (2)$$

where $(IC_{50})_{R_0}$ and $(IC_{50})_S$ are the THP-adriamycin concentrations required to inhibit 50% of resistant and sensitive cell proliferation in the absence of inhibitor. Under our experimental conditions, $(IC_{50})_{R_0} = 32$ nM and $(IC_{50})_S = 4$ nM. The inhibitor concentrations used, as well as the β value calculated using equation (2), are reported in Table 1.

Except for a few cases, the inhibitor concentrations required to obtain $\alpha = 0.5$ are also those required to obtain $\beta = 0.5$. This corroborates our previous observation showing a good correlation between the ability of molecules to reverse MDR and to increase drug accumulation measured at short term [23].

The negative inotropic, chronotropic and vaso-relaxant activities of these compounds as well as their affinity for calcium channels have been previously determined [10–14]. They are reported in Table 1 to allow a comparison with their anti MDR activity.

DISCUSSION

Structure–activity relationships

With the possible exception of (**11**), all compounds examined show MDR reversal activity comparable to that of verapamil; in a few cases the potency is higher than that of the parent compound.

It is not an easy task to rationalize the data collected in the present work into sound and consistent structure–activity relationships. Nevertheless some useful preliminary conclusions can be extracted from the limited series of compounds studied. They can be useful in the design of more potent molecules devoid of undesired side-effects.

The most interesting observation is that secondary amines seem more efficient in reverting MDR. Thus, compounds (**3**) and (**4**) are more effective than the corresponding *N*-methyl derivatives (**5**) and (**6**). However, this property seems strongly dependent on the overall stereochemistry of the molecules, since in the corresponding 3-equatorial isomers [7–10] the most potent compound is actually the *N*-methyl derivative (**10**). These results are in contrast with those observed when testing calcium antagonistic activity which is always higher for *N*-methyl derivatives with regard to their *N*-desmethyl counterpart [12, 24].

The flexibility of the molecule, which seems critical to inducing a selectivity toward cardiac tissues in the case of calcium antagonism [12–14], does not seem to play any relevant role in MDR reversion. In fact, potent MDR reversing compounds are found both in flexible and semirigid compounds (compare, for instance, (**3**), (**4**), (**18**) and (**19**)). In this respect the reduced flexibility of the homoveratryl moiety seems more important, as compound (**15**) is actually more effective than the less rigid (**16**) and (**17**) or than the fully flexible verapamil.

In general, the present results confirm the hypothesis, suggested by the lack of enantioselectivity of verapamil in MDR reversal [25], that these two pharmacological effects are not due to the same mechanism of action. Indeed the structure–activity relationships of calcium antagonism and MDR reversing activity are different as is easily verified by inspection of the results reported in Table 1. Thus, compound (**7**), which is more active as negative inotropic and chronotropic, is far less efficient in reversing MDR than (**3**), which has a poor cardiac activity.

In this respect compounds (**1**), (**3**) and (**13**) have remarkable properties in that they do not show any detectable calcium antagonistic activity while their potency as MDR reversing drug is comparable to that of verapamil. Accordingly, compounds (**15**), (**19**) and (**20**), which maintain some cardiac activity but show higher potency than verapamil as MDR reversers, seem quite interesting.

This is a remarkable result since it seems to make possible, by manipulation of the verapamil molecule, the development of a potent MDR reversing drug devoid of side effects due to calcium antagonistic activity.

As mentioned above, inhibition of the P-pg protein is not stereospecific since both enantiomers of verapamil show the same MDR-reversing activity [25–27], in contrast to the greater potency of S enantiomer as calcium channel antagonist [28].

In general there is enough evidence to suggest that the stereoisomers of calcium antagonists, which

Table 1. Effect of verapamil derivatives on the short-term measurements of THP-ADR accumulation in drug-resistant K562 cells and on their long-term growth inhibition*

Compounds	[*i*]μM† ($\alpha = 0.5$)	[*i*]μM‡	α	β	ED_{50} (μM), negative inotropic activity§	ED_{30} (μM), negative chronotropic activity§	IC_{50} (μM), vasorelaxant activity§	IC_{50} (μM) binding§	Σf_i¶	Σf_i‖
Verapamil	1.6	2	0.60	0.62	0.61	0.07	0.38	0.15	0	
1	1.6	2	0.56	0.43	NA	NA	NA	ND		0
2	2	1.2	0.53	0.64	NA	NA	NA	3.64		+0.5
3	0.3	0.35	0.51	0.14	0.90	2.40	17	0.48		+2.2
4	0.2	0.3	0.60	0.43	0.66	1.40	NA	0.54		+2.2
5	1.2	1.5	0.56	0.64	0.10	0.07	NA	0.39		+2.7
6	0.8	1.0	0.58	0.50	0.06	1.30	18	0.50		+2.7
7	2.2	2.5	0.60	0.52	0.11	0.06	NA	0.46		+2.2
8	1.3	1.5	0.53	0.68	1.10	0.37	3.8	0.41		+2.2
9	1.0	1.5	0.63	0.68	1.30	0.23	NA	0.27		+2.7
10	0.3	1.2	0.90	0.83	0.20	0.42	10	0.26		+2.7
11	6.8	10	0.65	0.69	49	4.90	NA	3.89	−1.7	
12	2.2	3	0.77	0.76	0.49	1.10	NA	2.57	−0.22	
13	0.5	0.7	0.63	0.72	NA	NA	NA	1.15	+0.31	
14	1.3	2	0.74	0.91	3.5	0.14	NA	0.13		
15	0.6	0.65	0.50	0.14	NA	0.52	NA	0.35		
16	1.0	1.5	0.65	0.61	0.68	0.56	NA	0.32		
17	1.6	1.6	0.53	0.79	0.70	0.19	NA	ND		
18	0.3	0.2	0.38	0.32	**	**	NA	0.20		
19	0.25	0.3	0.61	0.14	**	**	NA	0.30		
20	1.3	2	0.87	0.87	28	0.24	NA	0.08		

* A compound was considered not active when its ED_{50} or ED_{30} or IC_{50} was higher than 100 μM.

† [i] ($\alpha = 0.5$) are the inhibitory concentrations which cause a half-maximal increase in cellular THP-ADR accumulation.

‡ [*i*] are the inhibitory concentrations used for long-term growth inhibition. α and β are the parameters defined by equations (1) and (2), respectively (see results).

§ See references [10, 11, 13, 14].

¶ Variation of lipophilicity evaluated as sum of fragmental values f_i referred to verapamil.

‖ Variation of lipophilicity evaluated as sum of fragmental values f_i referred to compound **1**.

** The activity of these compounds has been tested with a different bioassay (see ref. [10]); they show inotropic and chronotropic activities comparable to that of verapamil

NA, not active.

ND, not determined.

differ markedly in their potencies as calcium blockers, are equally effective in modulating drug transport by the P-gp protein [29].

In contrast with this lack of enantioselectivity of the P-gp protein, there are indications that the spatial arrangement of active molecules may play a role. It has been reported that the MDR pump shows some stereoselectivity for quinidine as its diastereoisomer quinine is a relatively weak inhibitor: flux inhibition by quinidine is approximately two-fold greater than that of quinine [23, 30]. Accordingly, in the series studied here 3-axially substituted cyclohexanes (**3**) and (**4**) show higher MDR reversing activity than their diastereomeric 3-equatorial counterparts (**7**) and (**8**). The situation becomes somewhat less clear when the *N*-methylated analogues ((**5**), (**6**) and (**9**), (**10**)) are considered yet when taken together these data seem to support the view that steric factors might be important in MDR-reversing activity and suggest that their role in MDR reversion has so far been underestimated with respect to lipophilicity (*vide infra*).

Since the compounds examined are racemates it would be worthwhile to test the enantiomers of our compounds to examine the importance of stereochemistry in P-gp modulating activity in greater depth and verify if the differences observed among diastereoisomers also extend to enantiomers. We are currently attempting to synthesize and test the enantiomers of (**3**)–(**10**).

Influence of lipophilicity

Zamora *et al.* [31] and Ford *et al.* [32] have reported that lipophilicity is one of the common features of MDR reverting agents: a relationship between the lipophilicity of our compounds and their ability to reverse MDR could thus be expected.

A good estimation of the lipophilicity of a compound is given by log*P* where *P* is the partition coefficient in an *n*-octanol/water system. In a first approximation the log*P* value of a compound can be estimated by adding the f_i values of its fragments [33]. Thus, we have determined the variation of log*P* as compared to verapamil using the equation:

$$\log P = \log P_o + \Sigma f_i$$

where P_o stands for the verapamil in *n*-octanol/water partition coefficient and f_i is the *n*-octanol/water

fragmental constant of the fragments of every single compound that differentiate it from verapamil [34]. The results are reported in Table 1.

However, it has been observed [34] that cyclic compounds turn out to be less lipophilic than predicted when measured by these calculations and display log*P* values lower than values calculated by Rekker's fragment system. To overcome this problem we thought it sounder to calculate the Σf_i of cyclic compounds (**2**)–(**10**) by referring to the log*P* compound (**1**), which in this case assumes the reference value of zero. These values are also reported in Table 1.

The results show that even if a sound, meaningful correlation cannot be established, there is a positive correlation between lipophilicity and MDR-reversing activity. For example, the less potent compound (**11**) is also that with lower lipophilicity as compared to verapamil while compounds (**11**)–(**13**) show reversion of MDR increasing with an increase in lipophilicity.

As observed in the discussion of structure–activity relationships above, the cyclic compounds (**1**)–(**10**) are in sharp contrast with this trend as the less lipophilic desmethyl derivatives are more efficient in reversing MDR than their more lipophilic *N*-methyl counterparts.

These results confirm that besides lipophilicity, the spatial arrangement of the molecule and perhaps its possibility to establish hydrogen bonds play a major role in MDR reversion. Lipophilicity appears, at least in this group of compounds, a necessary but not unique condition for activity.

Acknowledgements—This investigation was supported by the ARC (Association pour la Recherche contre le Cancer), l'Université Paris Nord and le Centre National de la Recherche Scientifique. E. P. is grateful to CNPq (Brazil) for a fellowship.

REFERENCES

1. Bradley G, Juranka PF and Ling V, Mechanism of multidrug resistance. *Biochim Biophys Acta* **948**: 87–128, 1988.
2. Inaba H and Johnson RK, Uptake and retention of adriamycin and daunorubicin by sensitive and anthracycline-resistant sublines of P388 leukemia. *Biochem Pharmacol* **27**: 2123–2130, 1978.
3. Beck WT, Cellular pharmacology of vinca alkaloid resistance and its circumvention. *Adv Enzyme Reg* **22**: 207–227, 1984.
4. Beck WT, Drug accumulation and binding in P-glycoprotein-associated multidrug resistance. In: *Molecular and Cellular Biology of Multidrug Resistance in Tumor Cells* (Ed. Roninson IB), pp. 215–227. Plenum, New York, 1991.
5. Gottesman MM and Pastan I, Biochemistry of multidrug resistance mediated by the multidrug transporter. *Ann Rev Biochem* **62**: 385–427, 1993.
6. Tsuruo T, Iida H, Tsukagoshi S and Sakurai Y, Overcoming of vincristine resistance in P388 leukemia *in vivo* and *in vitro* through enhanced cytotoxicity of vincristine and vinblastine by verapamil. *Cancer Res* **41**: 1967–1972, 1981.
7. Tsuruo T, Tsukagoshi S and Sakurai Y, Potentiation of vincristine and adriamycin effects in human hemopoietic tumor cell line by calcium antagonists and calmodulin inhibitors. *Cancer Res* **43**: 2267–2272, 1983.
8. Tsuruo T, Iida H, Nojiri M, Tsukagoshi S and Sakurai Y, Circumvention of vincristine and adriamycin resistance in vito and in vivo by calcium influx blockers. *Cancer Res* **43**: 2905–2910, 1983.
9. Huet S and Robert J, The reversal of doxorubicin resistance by verapamil is not due to an effect on calcium channels. *Int J Cancer* **41**: 283–283, 1988.
10. Gualtieri F, Teodori E, Bellucci C, Pesce E and Piacenza G, SAR studies and in the field of calcium (II) antagonists. Effect of modifications at the tetrasubstituted carbon of verapamil-like compounds. *J Med Chem* **28**: 1621–1628, 1985.
11. Dei S, Romanelli MN, Scapecchi S, Teodori S, Chiarini A and Gualtieri F, Verapamil analogues with restricted molecular flexibility. *J Med Chem* **34**: 2219–2225, 1991.
12. Dei S, Romanelli MN, Scapecchi S, Teodori E, Gualtieri F, Chiarini A, Voigt W and Lemoine H, Verapamil analogues with restricted molecular flexibility: Synthesis and pharmacological evaluation of the four isomers of α[1-[3-[*N*-[1-[2-(3,4-dimethoxyphenyl)-ethyl]]- *N*- methylamino]cyclohexyl]]- α- isopropyl- 3,4-dimethoxybenzene-acetonitrile. *J Med Chem* **36**: 439–445, 1993.
13. Romanelli MN, Dei S, Scapecchi S, Teodori E, Gualtieri F, Budriesi R and Mannhold R, Semi-rigid analogues of the calcium antagonist verapamil: a molecular modelling study. *J Computer-Aided Molec Design* **8**: 123–134, 1994.
14. Teodori E, Dei S, Romanelli MN, Scapecchi S, Gualtieri F, Budriesi R, Chiarini A, Lemoine H and Mannhold R, Synthesis and pharmacological evaluation of verapamil analogs with restricted molecular flexibility. *Eur J Med Chem* **29**: 139–148, 1994.
15. Maniatis T, Fristch E and Sambrook J, *Molecular Cloning. A Laboratory Manual*. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, 1982.
16. Ferrandis E, Da Silva J, Riou G and Benard J, Co-activation of the MDR1 and MYCN genes in human neuroblastoma cells during the metastatic process in the nude mouse. *Cancer Res* **54**: 2256–2261, 1994.
17. Golstein LJ, Fojo AT, Crist W, Green A, Brodeur G, Pastan I and Gottesman MM, Expression of the multidrug resistance, MDR1, gene in neuroblatomas. *J Clin Oncol* **8**: 128–136, 1990.
18. Tarasiuk J, Frezard F, Garnier-Suillerot A and Gattegno L, Anthracycline incorporation in human lymphocytes. Kinetics of uptake and nuclear concentration. *Biochim Biophys Acta* **1013**: 109–117, 1989.
19. Frezard F and Garnier-Suillerot A, Determination of the osmotic active drug concentration in the cytoplasm of anthracycline-resistant and -sensitive K562 cells. *Biochim Biophys Acta* **1091**: 29–35, 1991.
20. Frezard F and Garnier-Suillerot A, Comparison of the membrane transport of anthracycline derivatives in drug-resistant and drug-sensitive K562 cells. *Eur J Biochem* **196**: 483–491, 1991.
21. Frezard F and Garnier-Suillerot A, DNA-containing liposomes as a model for the study of cell membrane permeation by anthracycline derivatives. *Biochemistry* **30**: 5038–5043, 1991.
22. Tarasiuk J, Foucrier J and Garnier-Suillerot A, Cell cycle dependent uptake and release of anthracycline by drug-resistant and drug-sensitive human leukaemic K562 cells, *Biochem Pharmacol* **45**: 1801–1808, 1993.
23. Pereira E and Garnier-Suillerot A, Correlation between the short-term measurements of drug accumulation in living cells and the long-term growth inhibition. *Biochem Pharmacol* **47**: 1851–1857, 1994.
24. Mannhold R, Steiner R, Haas W and Kaufmann R, Investigations on the structure–activity relationships of verapamil. *Naunyn-Schmiedeberg's Arch Pharmacol* **302**: 217, 1978.

25. Haüsserman K, Benz B, Gekeler V, Schumacher K and Eichelbaum M, Effects of verapamil enantiomers and major metabolites on the cytotoxicity of vincristine and daunomycin in human lymphoma cell lines. *Eur J Clin* **40**: 53–59, 1991.
26. Plumb JA, Milroy R and Kaye SB, The activity of verapamil as a resistance modifier *in vitro* in drug-resistant human tumour cell lines is not stereospecific. *Biochem Pharmacol* **39**: 787–792, 1990.
27. Gruber A, Peterson C and Reizenstein P, D-Verapamil and L-verapamil are equally effective in increasing vincristine accumulation in leukemic cells *in vitro*. *Int J Cancer* **41**: 224–226, 1988.
28. Goll A, Ferry DR and Glossmann H, Target size analysis and molecular properties of Ca^{2+} channels labelled with [^{3}H]verapamil. *Eur J Biochem* **141**: 177–186, 1984.
29. Höllt V, Kouba M, Dietel M and Vogt G, Stereoisomers of calcium antagonists which differ markedly in their potencies as calcium blockers are equally effective in modulating drug transport by P-glycoprotein. *Biochem Pharmacol* **43**: 2601–2608, 1992.
30. Wigler PW and Patterson FK, Reversal agent inhibition of the multidrug resistance pump in human leukemic lymphoblasts. *Biochim Biophys Acta* **1189**: 1–6, 1994.
31. Zamora JM, Pearce HL and Beck WT, Physical–chemical properties shared by compounds that modulate multidrug resistance in human leukemic cells. *Molec Pharmacol* **33**: 454–462, 1988.
32. Ford JM and Hait WN. Pharmacology of drugs that alter multidrug resistance in cancer. *Pharmacol Rev* **42**: 155–199, 1990.
33. Rekker RF, The hydrophobic fragmental constant. Its derivation and applications. A means of characterizing membrane systems. *Pharmacochemistry Library* (Vol. 1). Elsevier, Amsterdam, 1977.
34. Van de Waterbeemd H and Testa B, Parametrization of lipophilicity. In: *Advances in Drug Research* (Vol. 16), (Ed. Testa B), pp. 87–210, B. Testa Ed, Academic Press, London, 1987.