Articles

Design, Synthesis, and in Vitro Activity of Catamphiphilic Reverters of Multidrug Resistance: Discovery of a Selective, Highly Efficacious Chemosensitizer with Potency in the Nanomolar Range

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On the basis of the results obtained in previous research, three series of compounds (A−C), derived from verapamil, were designed and synthesized to obtain drugs able to revert multidrug resistance (MDR), an acquired resistance that frequently impairs cancer chemotherapy. The ability of the obtained compounds to revert MDR was evaluated on anthracycline-resistant erythroleukemia K 562 cells, measuring the uptake of THP-adriamycin (pirarubicin) by continuous spectrofluorometric monitoring of the decrease of the fluorescence signal of the anthracycline at 590 nm (λ_{ex} = 480 nm), after incubation with cells. Cardiovascular activity, which is responsible for unwanted side effects, was also evaluated. The results obtained show that many of the compounds studied are potent reverters of MDR and are endowed with reduced cardiovascular activity. One of the compounds (**7**, MM36) presents a pharmacological profile (unprecedented nanomolar potency, high reversal of MDR, low cardiovascular activity) that makes it a promising drug candidate to treat MDR and a useful tool for studying P-glycoprotein.

Introduction

Drug resistance is a phenomenon that frequently impairs proper treatment of infections and cancer with chemotherapeutics. There are two major manifestations of drug resistance: intrinsic drug resistance, which relates to the failure of many microorganisms and tumors to respond to initial chemotherapy; acquired drug resistance, which occurs when a microorganism or a tumor initially responds to chemotherapy but later relapses and appears to be strongly resistant to the original treatment. A number of specific resistance mechanisms against drugs have been described, for both infectious and cancer diseases.[1]

Multidrug resistance (MDR) is a particular case of acquired drug resistance observed in vivo and in vitro that describes the simultaneous emergence of cellular resistance to the toxic action of the chemotherapic drug originally used and to other chemicals, having different structure and different mechanism of action.[2]

Perhaps the best understood and most widely implicated mechanism is that concerned with altered membrane transport of resistant tumor cells, often referred to as typical or classical MDR.[3,4] In studying the biochemical mechanisms that induce acquired resistance, it became clear that cells exhibiting MDR accumulate a lower intracellular concentration of drug. This effect is associated with accelerated efflux of antitumor agents by an ATP-dependent process. A membrane glycoprotein termed P-glycoprotein (also known as Pg-170, PGP, P-170) was isolated and proposed as the transporter protein that pumps out the antitumor agent, whether from the cytoplasm or from the membrane is still a matter of debate.[3−5]

P-170 is overexpressed in cells showing MDR. In fact, while being present in many tissues, P-170 shows high levels in numerous malignant cells, and increasing amounts of it are found in patients with increasing resistance to chemotherapy. Thus, a significant correlation with MDR, both in vitro and in vivo, was detected.[6] More recently, a new 190-KDa protein called MRP (MDR-related protein) has been identified. It belongs to the same superfamily of the ATP-binding cassette (ABC) of transporter proteins and also operates as an extrusion pump, but its mechanism of action is different from that of P-170 and seems to involve glutathione.[7,8] It is interesting that analogous mechanisms of drug extrusion have been found in microorganisms,[9,10] making it more and more evident that the multidrug efflux systems encountered in prokaryotic cells are very similar to those observed in eukaryotic cells.[11] Moreover, besides their role in cancer cell resistance, these proteins seem to have a physiological function as well, since they are expressed also in nontumoral tissues: P-170 has been reported to be an important element of the blood−brain barrier (BBB),[12] of the intestinal epithelium,[13] and, together with other ATP-dependent transporters, in the hepatobiliary secretion.[14]

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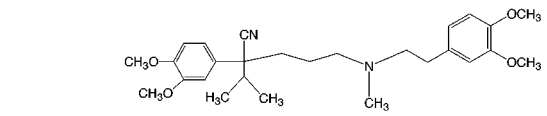
Since MDR is one of the main obstacles to successful chemotherapy of cancer, a number of biochemical, pharmacological, and clinical strategies have been devised to overcome it. A first approach, which however has obvious drawbacks, is that of using high concentrations of cytotoxic drug to overcome the effects of cell extrusion. Another approach is that of using drugs which are not good substrates for P-170. There are a number of drugs that are not transported by the carrier protein, such as cyclophosphamide, methotrexate, and *cis*-platin, while chemical modification of MDR-producing drugs could possibly result in active, but not P-170-transported, analogues.

However, once P-170's activity had been recognized as one of the major factors leading to MDR, blocking the efflux of drugs by inhibition of the functions of P-glycoprotein has become one of the most popular approaches to circumvent MDR. Recently, the name *chemosensitizers* for drugs that act by blocking the P-170 protein has been proposed. In the past few years many molecules that revert MDR have been identified.[15,18] They belong to quite different chemical classes, and besides cyclosporin A, which is a peptide, all share the feature of being highly lipophilic and possessing an amino group that is protonated under physiological conditions. Most of the molecules found active were, however, known drugs with a definite pharmacological action that induced unwanted side effects. This is perhaps the main reason for the disappointing results recorded in clinical trials with compounds active in vitro.[19,20] Only recently, the problem of the specific design of chemosensitizers has been approached, and a second generation of drugs able to revert MDR is appearing on the stage: some of these molecules are now undergoing clinical trials.[16,18,21]

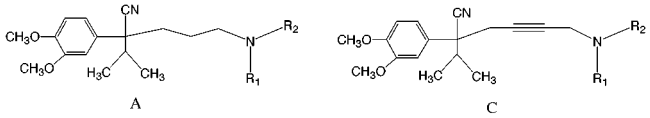
The fairly heterogeneous chemical structure of the compounds found active in reverting MDR has so far hampered structure−activity studies. The broad selectivity of P-170 suggests that there might be more than a single binding site, and it is not surprising that the efforts to establish structure−activity relationships in this class of compounds have been frustrating, with only qualitative, generic indications having been obtained.[22−24] All these studies (reviewed in ref 22) have indeed reached the conclusion that MDR-reverting activity is normally present in lipophilic compounds containing a hydrophilic *N*-alkyl group that is protonated at physiological pH: such molecules are often characterized by a two-ring structure linked by a single alkyl bridge to the amino group. Recently, Klopman and co-workers,[25] using computational methods, have tried to approach the problem from a quantitative point of view and identified additional substructural features for MDR-reverting activity; their work, however, has not added substantially new information to the small amount previously found. In this respect, the role of lipophilicity seems particularly elusive: while this property is generally considered important for MDR-reverting activity,[26−28] to the best of our knowledge, usually no significant quantitative relationships could be established between partition coefficients and activity,[29−31] and successful correlations were found only for highly homogeneous sets of molecules.[32,33]

Among the chemosensitizers of the first generation, verapamil has been one of the most studied, both in vitro and in vivo, and has been used as the lead molecule in several attempts to identify more potent and selective drugs.[29,34−36] In a previous study,[29] we identified some verapamil analogues that were, as chemosensitizers, slightly more potent than the lead and, at the same time, showed in some cases lower activity on the cardiovascular system. On the basis of these results and aiming to improve MDR reversal activity and to reduce unwanted cardiovascular effects, we have decided to synthesize and study the sets of compounds shown in Chart 1, having the general structures A−C and reported in Tables 1−3.

Chart 1

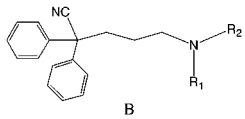


Verapamil



A

C



B

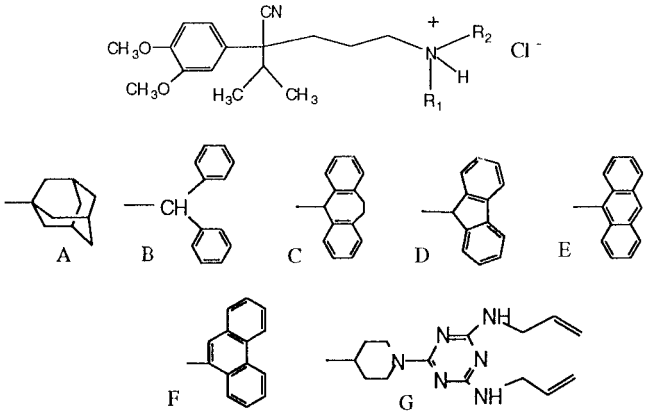
R_1 = H, CH_3
R_2 = the groups shown in Tables 1-3

The verapamil moiety of A was chosen since, although compounds containing this group are likely to show also cardiovascular properties, the group nevertheless confers high affinity toward the P-170 protein.[35,36] We hoped that the changes that we were going to introduce in the rest of the molecule would reduce cardiovascular activity, as indeed was the case in some of the compounds previously synthesized.[29] The corresponding moiety of set C was chosen since it was present in one of the compounds previously studied, showing slightly higher MDR-reverting activity with respect to verapamil, while being almost devoid of cardiovascular effects.[29] Finally, compounds of the B series were designed as analogues of verapamil with an additional aromatic ring in their structure.

There are indications that, besides the overall lipophilicity of the molecule, weak polar interactions such as those produced by the overlapping of π orbitals of aromatic rings can play an important role in stabilizing the binding of MDR-reverting agents to P-170 protein.[25] In this respect it is important to consider that the transmembrane segments of P-170 are reported to be rich in aromatic amino acid residues.[37] The contribution of aromatic rings to MDR-reverting activity seems confirmed by the recent report that some polyaromatic natural compounds are potent inhibitors of multidrug resistance caused by P-170.[38]

This rationale was also at the basis of our choice of the large substituent (R_2) on the nitrogen atom. The adamantane moiety was chosen to introduce a fairly lipophilic nonaromatic group and to compare its effects on chemosensitizing activity with those having two or three aromatic cycles. The highly significant correlation

Table 1. Chemical and Physical Characteristics of Compounds **1**−**14**



compd	R_1	R_2	mp (°C)	analysis[a]
1	H	A	100−104[b]	$C_{26}H_{39}ClN_2O_2$
2	H	B	104−106[c]	$C_{29}H_{35}ClN_2O_2$
3	H	CH_2-B	148−150[c]	$C_{30}H_{37}ClN_2O_2$
4	H	C	157−159[c]	$C_{31}H_{37}ClN_2O_2$
5	H	D	145−150[c]	$C_{29}H_{32}ClN_2O_2$
6	H	CH_2-D	128−130[c]	$C_{30}H_{35}ClN_2O_2$
7	H	CH_2-E	118−120[c]	$C_{31}H_{35}ClN_2O_2$
8	H	CH_2-F	109−110[c]	$C_{31}H_{35}ClN_2O_2$
9	H	G	191−193[c]	$C_{30}H_{46}ClN_8O_3 \cdot 3H_2O$
10	CH_3	A	175−178[c]	$C_{27}H_{41}ClN_2O_2$
11	CH_3	CH_2-A	71−75[c]	$C_{28}H_{43}ClN_2O_2$
12	CH_3	D	97−100[c]	$C_{30}H_{35}ClN_2O_2$
13	CH_3	CH_2-D	98−102[c]	$C_{31}H_{37}ClN_2O_2$
14	CH_3	CH_2-E	97−101[c]	$C_{32}H_{37}ClN_2O_2$

[a] All compounds have been analyzed for C, H, N after vacuum drying at a temperature below the melting point; the results obtained range within ±0.4 of the theoretical values. IR and ^{1}H NMR spectra are consistent with the proposed structures. [b] Recrystallization solvent: absolute ethanol. [c] Recrystallization solvent: absolute ethanol + anhydrous ether.

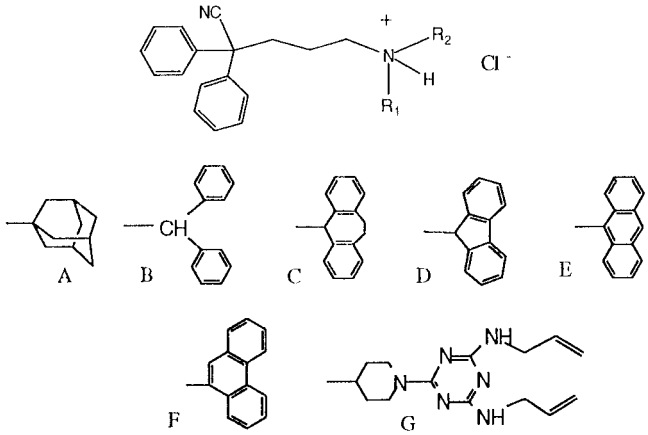
reported[30] between P-170 affinity and surface area of drugs belonging to different chemical classes was also considered. Finally, the 2,4,6-substituted triazine moiety (Tables 1−3, R = G) was chosen for its presence in S9788, one of the most studied second-generation chemosensitizers.[39]

Chemistry

The reaction pathways used to synthesize compounds **1**−**34** involved simple chemistry, and the standard methods reported in Schemes 1−4 were used. Whenever possible, the final products were obtained by alkylation of the suitable primary or secondary amine with 5-bromo-2-(3,4-dimethoxyphenyl)-2-isopropylpentanenitrile (**35**), 5-bromo-2,2-diphenylpentanenitrile[40] (**37**), and 6-chloro-2-(3,4-dimethoxyphenyl)-2-isopropyl-4-hexynenitrile (**38**) (Scheme 1).

This kind of reaction was unsuitable to obtain compounds **9** and **29** which were synthesized through the one-pot formation and reduction with $NaBH_3CN$ of the Schiff bases obtained from amines **36** and **39** and 1-[4,6-bis(allylamino)-1,3,5-triazin-2-yl]-4-piperidone[39] in the presence of activated molecular sieves (Scheme 2); amines **36** (already described,[41] but obtained in a different way) and **39** have been synthesized through the Gabriel synthesis, as reported in Scheme 4. The most useful way to obtain compounds **30**−**32** and **34** was the Mannich reaction of 2-(3,4-dimethoxyphenyl)-2-isopropyl-4-pentynenitrile (**40**)[42] with the suitable *N*-methylamino derivative; the reaction does not work with primary amines (Scheme 3).

Table 2. Chemical and Physical Characteristics of Compounds **15**−**23**



compd	R_1	R_2	mp (°C)	analysis[a]
15	H	A	190−192[b]	$C_{27}H_{33}ClN_2$
16	H	B	108−110[b]	$C_{30}H_{29}ClN_2$
17	H	CH_2-B	201−205[b]	$C_{31}H_{31}ClN_2$
18	H	C	189−192[b]	$C_{32}H_{31}ClN_2$
19	H	D	240−242[b]	$C_{30}H_{27}ClN_2$
20	H	CH_2-E	230−232[c]	$C_{32}H_{29}ClN_2$
21	H	CH_2-F	68−70[b]	$C_{32}H_{29}ClN_2$
22	CH_3	D	107−110[b]	$C_{31}H_{29}ClN_2$
23	CH_3	CH_2-E	208−210[b]	$C_{33}H_{31}ClN_2$

[a] All compounds have been analyzed for C, H, N after vacuum-drying at a temperature below the melting point; the results obtained range within ±0.4 of the theoretical values. IR and ^{1}H NMR spectra are consistent with the proposed structures. [b] Recrystallization solvent: absolute ethanol + anhydrous ether. [c] Recrystallization solvent: absolute ethanol.

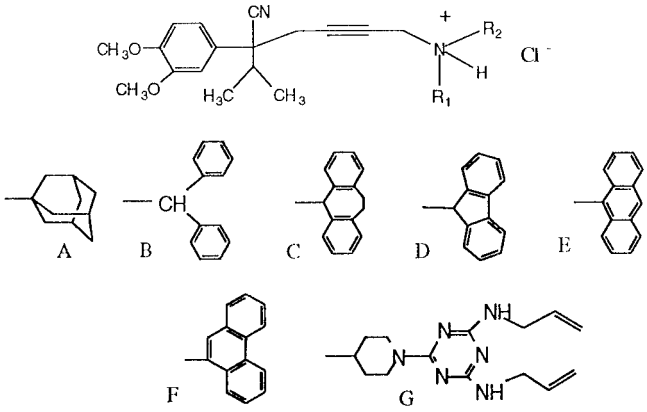
The amines used were either commercially available (1-aminoadamantane, aminodiphenylmethane, 2,2-diphenylethylamine, 9-aminofluorene, 9-(methylaminomethyl)anthracene) or synthesized according to the literature (9-(methylamino)phenanthrene,[43] 1-(methylaminomethyl)adamantane,[44] 1-(methylamino)adamantane,[45] 9-(aminomethyl)fluorene[46]) or already described but obtained with different procedures as described in Scheme 4 (5-amino-10,11-dihydro-5*H*-dibenzo[*a*,*c*]cycloheptene (**44**),[47] 9-(aminomethyl)anthracene (**46**),[48] 9-(methylamino)fluorene (**48**)).[49] *N*-Methyl-9-(aminomethyl)fluorene (**50**), which has not been reported before, was obtained as reported in Scheme 4.

The compounds of the A and C series possess a chiral center and therefore are racemic mixtures of two enantiomers. It is well-known that both enantiomers of verapamil are equally active in modulating MDR, while they differ markedly in cardiovascular activity.[19,50] Therefore, it would be interesting to test the enantiomers of our chiral compounds. However, at this stage of the research, we decided to neglect this problem to favor a search for structure−activity relationships, and all compounds have been studied as racemates. The synthesis and biological evaluation of the enantiomers of the most interesting compounds of the series, which are presently underway, will be presented in due time.

Pharmacology

MDR-Reverting Activity. The ability of the examined compounds to revert MDR was evaluated on anthracycline-resistant erythroleukemia K 562 cells,

Table 3. Chemical and Physical Characteristics of Compounds **24**−**34**



compd	R_1	R_2	mp (°C)[a]	analysis[b]
24	H	B	111−115	$C_{30}H_{33}ClN_2O_2$
25	H	CH_2-B	171−175	$C_{31}H_{35}ClN_2O_2$
26	H	D	148−150	$C_{30}H_{31}ClN_2O_2$
27	H	CH_2-E	228−230	$C_{32}H_{33}ClN_2O_2$
28	H	CH_2-F	127−130	$C_{32}H_{33}ClN_2O_2$
29	H	G	114−118	$C_{31}H_{44}Cl_2N_2O_2 \cdot 3H_2O$
30	CH_3	A	81−85	$C_{28}H_{39}ClN_2O_2$
31	CH_3	CH_2-A	77−80	$C_{29}H_{41}ClN_2O_2$
32	CH_3	D	94−96	$C_{31}H_{33}ClN_2O_2$
33	CH_3	CH_2-D	108−110	$C_{32}H_{35}ClN_2O_2$
34	CH_3	CH_2-E	117−120	$C_{33}H_{35}ClN_2O_2$

[a] All compounds were crystallized from absolute ethanol + anhydrous ether. [b] All compounds have been analyzed for C, H, N after vacuum drying at a temperature below the melting point; the results obtained range within ±0.4 of the theoretical values. IR and ^{1}H NMR spectra are consistent with the proposed structures.

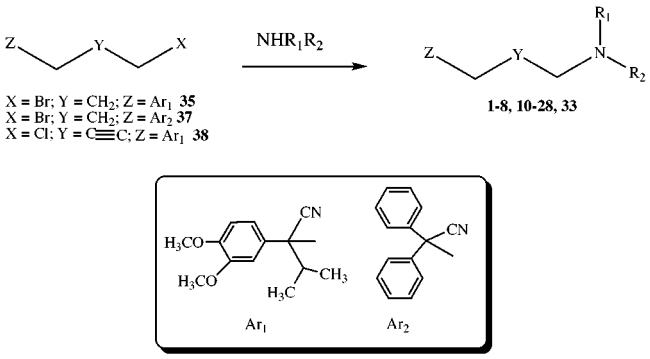
measuring the uptake of THP-adriamycin (pirarubicin) by continuous spectrofluorometric monitoring of the decrease of the fluorescence signal of the anthracycline at 590 nm (λ_{ex} = 480 nm) after incubation with cells. The decrease of fluorescence occurring during incubation with cells is due to quenching after intercalation of anthracycline between the base pair of DNA. We have previously shown that this methodology allows accurate measurement of the nuclear concentration of anthracyclines in the steady state, their initial rates of uptake, and kinetics of active efflux.[29,51−55]

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

$$\alpha = [(C_n)_{Ri} - (C_n)_{Ro}]/[(C_n)_S - (C_n)_{Ro}]$$

where $(C_n)_S$ is the overall nuclear concentration of pirarubicin in sensitive cells and $(C_n)_{Ro}$ and $(C_n)_{Ri}$ are the overall nuclear concentrations of pirarubicin in resistant cells, in the absence and presence of a concentration [i] of inhibitor, respectively. The value of α represents the fold increase in the nuclear concentration of pirarubicin in the presence of the MDR-reverting agent; α varies between 0 (in the absence of inhibitor) and 1 (when the amount of pirarubicin in resistant cells is the same as in sensitive cells). Figure 1 shows two typical experiments performed with verapamil and compound **7**, respectively. The potency of the compounds

Scheme 1



in reverting MDR is expressed by $[i]_{0.5}$ which represents the concentration of the inhibitor that causes a half-maximal increase in nuclear concentration of pirarubicin (α = 0.5), while its efficacy is expressed as α_{max}, which is the maximum increase that can be obtained in the nuclear concentration of pirarubicin in resistant cells with a given inhibitor (see Table 4).

Cardiovascular Activity. Inotropic and chronotropic activities were tested on guinea pig isolated atria preparations, and vasodilator activity was tested on guinea pig aortic strip preparations following standard procedures, details of which have been already reported.[56]

Potency of the drugs is defined as EC_{50} (negative inotropic activity), IC_{50} (vasodilator activity), and EC_{30} (negative chronotropic activity). Activity is defined as the percent decrease in developed tension on isolated left atrium (negative inotropic activity), percent decrease in atrial rate on spontaneously beating isolated right atrium (negative chronotropic activity), and percent inhibition of calcium-induced contraction on K^+-depolarized aortic strips (vasodilator activity) at the concentrations indicated in the footnotes of Table 4.

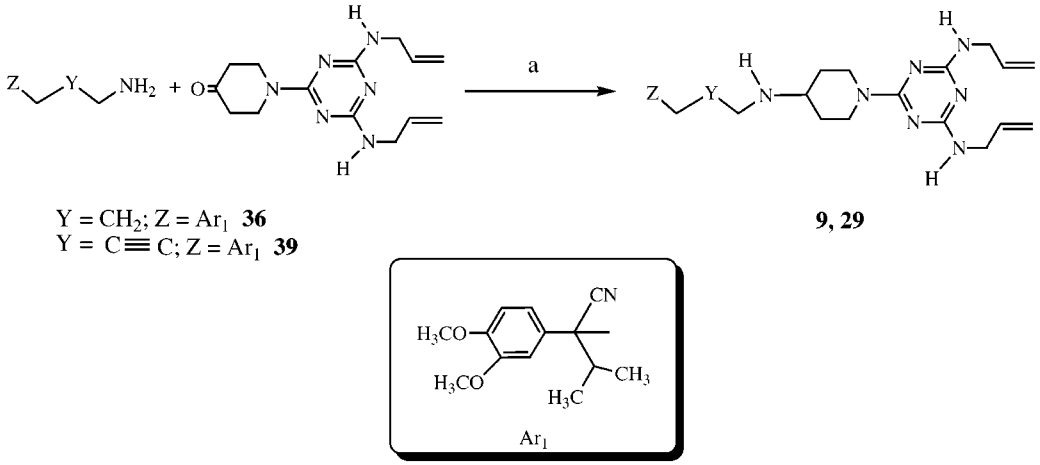
Results

The results of MDR-reverting activity and of cardiovascular activity are reported in Table 4. In general, all compounds show good MDR-reverting activity with a potency that is comparable to that of verapamil and in several cases higher. On the contrary, cardiovascular activity is, as expected,[29] always lower. Actually, some compounds show very low intrinsic activity and may be considered inactive, both on myocardial and vascular preparations. As a consequence, when the maximum effect, i.e., intrinsic activity (evaluated up to the 10^{-4} M concentration), was lower than 50%, the corresponding EC_{50} inotropic, EC_{30} chronotropic, and IC_{50} values were not calculated.

Considering each single series of compounds, those maintaining the verapamil moiety (A series) appear to be more potent in reversing MDR than the other ones (B and C series). Compounds **7**, **9, 12**, **19**, **25**, and **29** show the most interesting pharmacological profile in terms of MDR-reversing properties and cardiovascular activity.

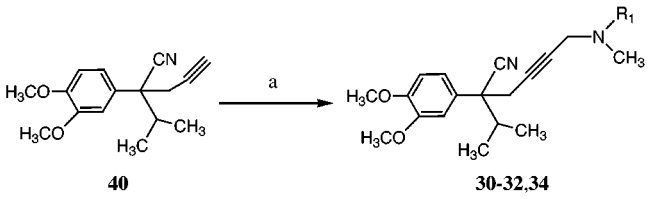
Among them, compound **7** (MM36) is the most potent MDR reverter, showing an $[i]_{0.5}$ in the nanomolar range (0.05 μM). To the best of our knowledge, also based on a recent survey reporting the potency of chemosensitizers of the first and second generation,[57] **7** is one of

Scheme 2[a]



[a] (a) MeOH, $NaBH_3CN$.

Scheme 3[a]



[a] (a) $CH_2O/NHCH_3R_1$.

the most potent and efficacious drugs so far described, its potency being approached only by the acridine derivative GF 120918[58] and its efficacy by a recently described series of taxane derivatives.[59]

A noteworthy feature of **7** is that it is highly selective as an MDR reverter, since it is inactive as vasodilator and its $[i]_{0.5}$ is definitely low with respect to the EC_{50} inotropic and the EC_{30} chronotropic, the potency ratios being 22 and 17, respectively. The corresponding values for verapamil are 0.4 and 0.04 (0.24 for vasodilator activity). In addition, it must be noted that the maximum negative inotropic and chronotropic effects of compound **7** are definitely lower with respect to those of verapamil, which elicits higher cardiac effects at lower concentrations. From this point of view, compound **12** seems even more specific, as it is inactive up to the concentration of 10^{-4} M on all the assays used to evaluate cardiovascular action. However, **12** is only slightly more potent than verapamil in reversing MDR ($[i]_{0.5} = 1$ against 1.6 μM).

Among the compounds of the B series, **19** has the most interesting pharmacological profile which, however, is less promising with respect to **7**, since, even if efficacy is slightly better ($\alpha_{max} = 0.8$), potency ($[i]_{0.5} = 0.3$ μM) and selectivity with regards to the cardiovascular system are lower (potency ratios of MDR-reverting activity with respect to negative inotropic, chronotropic activity = 6 and 1, respectively, inactive as vasodilator).

Compound **25**, the most interesting compound of the C series, is some 15 times more potent than verapamil and shows similar efficacy ($[i]_{0.5} = 0.1$ and 1.6 μM, respectively, and $\alpha_{max} = 0.7$ for both). However, it possess some toxicity (20% of cells dead at 1 μM), and although greater than that of verapamil, its selectivity is less impressive than that of **7** (potency ratios of MDR-reverting activity with respect to negative inotropic, chronotropic, and vasodilator activity = 11, 8, and 41, respectively).

Compounds **9** and **29**, which represent quite different structures since they carry the 2,4,6-substituted triazine moiety present in the second-generation MDR reverter S9788, do show chemosensitizing activity but maintain some cardiovascular action. Of them, **9** is equipotent to verapamil but shows higher reverting efficacy ($\alpha_{max} = 0.9$); it presents also a slightly better selectivity since it does not show negative inotropy, and the potency ratios for chronotropy and vasodilation are 11 and 2, respectively. Compound **29** is some 10 times more potent than verapamil as chemosensitizer ($[i]_{0.5} = 0.2$ against 1.6 μM), possesses higher reverting efficacy ($\alpha_{max} = 0.9$), but shows a selectivity similar to **9**, being inactive as vasodilator and showing potency ratios of MDR-reverting activity with respect to negative inotropic and chronotropic activity of 10 and 4, respectively.

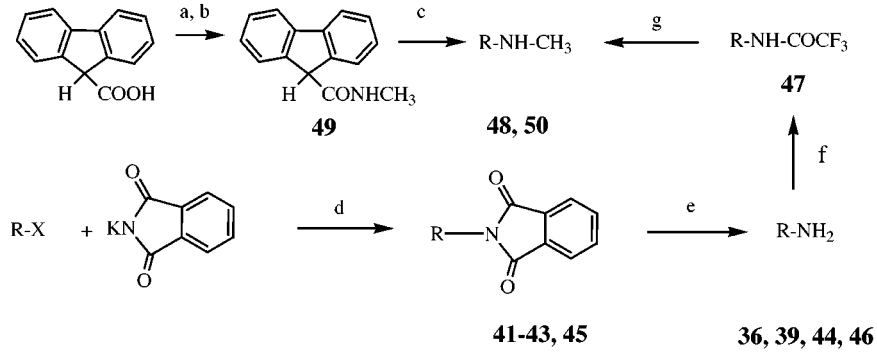
Discussion

Qualitative structure−activity relationships of the compounds synthesized in the present work give few univocal answers. The less questionable one seems to be that the 2-(3,4-dimethoxyphenyl)-2-isopropylpentanenitrile moiety of verapamil is, once again, the most useful to impart affinity for the P-170 protein.[25] As a matter of fact, the compounds of the A series appear to be generally more potent MDR reverters than those of the B and C series.

Another general feature of the compounds studied is that, confirming previous observations in the verapamil series,[29] *N*-methyl derivatives are generally less potent, as MDR reverters, than the *N*-desmethyl counterparts (compare, for instance, **6** with **13** and **7** with **14**); however, this is not always the case, as is exemplified by the potency of **26** with respect to **32**.

In our compounds the lipophilic group is linked to the nitrogen either directly or through a methylene group. This feature, which has obvious consequences on the conformational space achievable by each compound, does not seem to be relevant. When the comparison is possible, there are cases in which the compounds with the nitrogen directly linked to the lipophilic group are less potent (compare **10** and **11** or **24** and **25**) and cases in which they are more potent (compare **5** and **6** or **12** and **13**) with respect to their counterparts.

Scheme 4[a]



[a] (a) $SOCl_2$; (b) NH_2CH_3; (c) BH_3/THF; (d) 18-crown-6, dry toluene; (e) NH_2-NH_2 H_2O; (f) $(CF_3CO)_2O$; (g) CH_3I, KOH.

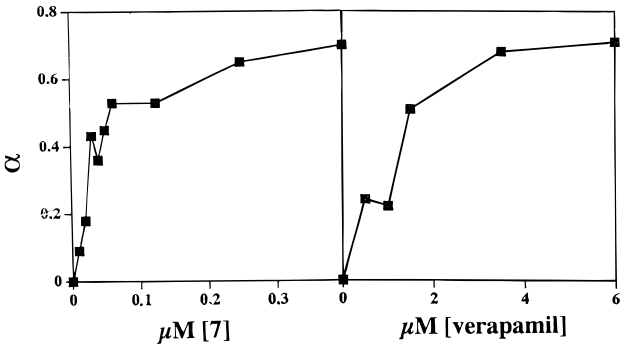


Figure 1. Effect of verapamil derivative **7** (left) and verapamil (right) on the pirarubicin accumulation in living resistant cells. α represents the fold increase in the nuclear concentration of pirarubicin in the presence of the MDR-reverting agent (α varies between 0 in the absence of inhibitor and 1 when the amount of pirarubicin is the same as in sensitive cells).

Mixed results are also observed when the influence of aromaticity is considered. Comparing the activity of the compounds carrying the nonaromatic, highly lipophilic adamantane moiety with compounds of similar lipophilicity, but with aromatic cycles, would suggest that aromaticity plays a role. As a matter of fact **1** is less potent than **2**, **4**, and **5**, and similarly **30** is less potent than **32**. However the trend is inverted when **11** is compared to **13** and **14**, which are less potent, even if they carry aromatic rings.

When the planarity of the aromatic moiety is taken into consideration, contrasting results are again obtained. There is no significant difference in the potency of **2**, **4**, and **5**. Although **3** is more potent than **6**, both are much less potent than **7**. Similarly, **11** is less potent than **13** and **14**, but **16** is more potent than **18** and **19**. Finally, compounds **9** and **29**, which carry a 2,4,6-substituted triazine moiety in place of the hydrocarbon groups of the other compounds and therefore have a quite different structure and lipophilicity, suggest that other N-substituents can provide the right affinity for the P-170 protein, in particular those containing nitrogen atoms.

In general, sound structure−activity relationships could not be deduced by simple qualitative analysis of the results reported in Table 4. As a consequence, we tried to obtain quantitative structure−activity relationships, evaluating the properties that were reported to be important in the interaction of both substrates and inhibitors with P-glycoprotein,[26−28,60,61] namely: molecular weight, hydrophobicity, molecular volume, and molecular surface. For each compound, molecular parameters describing such properties were calculated for the nonprotonated form of the molecule and processed for a possible correlation with $[i]_{0.5}$.

We were unable to establish any correlation of these parameters with the MDR inhibitory concentration at $\alpha = 0.5$ ($[i]_{0.5}$) considering either the whole set of compounds or any other, more homogeneous, subset such as series A−C of Tables 1−3. This is in contrast with the findings of other authors,[30,32,33] and at present, we do not know the reasons for this lack of correlation. We observed that very similar values of the parameter considered correspond to quite different $[i]_{0.5}$ suggesting that, beyond a threshold value for these parameters, other, yet unidentified, factors play a role in potent MDR-reverting molecules. As a matter of fact, the parameters for **7** ($[i]_{0.5} = 0.05$) and **27** ($[i]_{0.5} = 2$) are respectively: molecular volume, 392.39 and 393.71 Å^3; molecular surface, 527.56 and 542.03 Å^2; Connolly surface, 498.10 and 502.04 Å^2; log *P*, 6.35 and 6.77; molecular refractivity, 14.37 and 14.70; MW, 466.62 and 476.61. Interestingly, a recent CoMFA study,[62] performed on phenothiazines and related drugs endowed with MDR-reverting activity, reached quite similar conclusions.

In conclusion, although we were successful in obtaining several potent and selective inhibitors of MDR, among which **7** (MM36) possesses unprecedented potency and selectivity, it is fair to admit that the rationale of our design has not been confirmed by qualitative and quantitative structure−activity relationships of the compounds obtained. Designing chemosensitizers remains a fairly empirical job that relies on few, poorly understood structural elements, until better knowledge of the site(s) of interaction of P-glycoprotein is available. Toward this end, compound **7**, besides being a promising drug candidate to revert MDR, represents a powerful pharmacological tool for P-glycoprotein studies.

Experimental Section

Chemistry. All melting points were taken on a Büchi apparatus and are uncorrected. Infrared spectra were recorded with a Perkin-Elmer 681 spectrophotometer in Nujol mull for solids and neat for liquids. NMR spectra were recorded on a Gemini 200 spectrometer. Chromatographic separations were performed on a silica gel column by gravity chromatography (Kieselgel 40, 0.063−0.200 mm; Merck) or flash chromatography (Kieselgel 40, 0.040−0.063 mm; Merck). Yields are given after purification, unless otherwise stated. Where analyses are indicated by symbols, the analytical results are within ±0.4% of the theoretical values.

Table 4. MDR-Reverting Activity and Cardiovascular Activity of Compounds **1**−**34**

	MDR-reverting activity[a]			cardiovascular activity					
				negative inotropy		negative chronotropy		vasorelaxant activity	
compd	α_{max}	$[i]_{0.5}$ (μM) ($\alpha = 0.5$) (mean ± SEM)	% dead cells (1 μM)	activity (%)[b] (mean ± SEM)	EC_{50} (μM)[c] (95% cl)	activity (%)[d] (mean ± SEM)	EC_{30} (μM)[c] (95% cl)	activity (%)[e] (mean ± SEM)	IC_{50} (μM)[c] (95% cl)
1	0.7	2.0 ± 0.4	0	45 ± 1.8	*i*	80 ± 3.8	1.70 (1.37−2.06)	81 ± 2.1	7.15 (6.85−7.60)
2	0.7	0.25 ± 0.05	5	73 ± 3.5	0.66 (0.45−0.93)	81 ± 2.3	0.69 (0.62−0.78)	32 ± 2.3	*i*
3	0.7	0.25 ± 0.05	5	89 ± 4.8	0.83 (0.76−0.92)	74 ± 3.1[f]	0.54 (0.38−0.75)	37 ± 2.8	*i*
4	0.8	0.30 ± 0.06	10	70 ± 1.4	0.68 (0.49−0.98)	86 ± 4.3[g]	0.21 (0.18−0.24)	24 ± 1.4	*i*
5	0.8	0.25 ± 0.05	0	33 ± 1.2	*i*	74 ± 4.5	0.65 (0.57−0.72)	46 ± 2.1	*i*
6		toxic[h]	100	71 ± 5.2	0.47 (0.27−0.66)	68 ± 4.2	2.24 (1.89−2.64)	26 ± 1.1	*i*
7	0.7	0.05 ± 0.01	3	55 ± 0.2	1.11 (0.85−1.45)	65 ± 4.3	0.86 (0.76−1.00)	29 ± 2.3	*i*
8		toxic[h]	100	65 ± 3.4	1.13 (0.88−1.50)	43 ± 2.1[g]	*i*	19 ± 0.9	*i*
9	0.9	1.6 ± 0.3	0	39 ± 1.7	*i*	87 ± 4.4	1.75 (1.52−2.05)	62 ± 4.8	3.45 (3.02−3.97)
10		*i*		80 ± 0.3	3.99 (3.43−4.81)	81 ± 2.3[f]	0.91 (0.86−0.98)	65 ± 3.1	3.81 (3.12−4.60)
11	0.65	1.4 ± 0.3	1	68 ± 4.2[f]	1.17 (0.93−1.51)	72 ± 3.5	0.70 (0.64−0.81)	39 ± 1.4	*i*
12	0.8	1.0 ± 0.2	0	36 ± 2.6	*i*	26 ± 1.4	*i*	15 ± 0.9	*i*
13	0.5	2.2 ± 0.4	3	75 ± 4.3	1.01 (0.88−1.15)	31 ± 2.3	*i*	11 ± 0.9	*i*
14	0.5	2.5 ± 0.5	3	85 ± 2.3	0.65 (0.59−0.73)	19 ± 0.7	*i*	9 ± 0.4	*i*
15	0.8	0.5 ± 0.1	0	80 ± 1.2	1.03 (0.70−1.45)	69 ± 4.3[f]	1.26 (0.97−1.64)	55 ± 3.1	3.49 (2.95−4.12)
16	0.5	2.0 ± 0.4	2	94 ± 3.1	0.38 (0.29−0.49)	28 ± 1.4	*i*	15 ± 0.5	*i*
17		toxic[h]	40	75 ± 3.4	2.15 (1.85−2.52)	82 ± 4.3	1.16 (0.96−1.39)	19 ± 0.7	*i*
18	0.5	0.5 ± 0.1	3	80 ± 4.1	0.64 (0.47−0.90)	5 ± 0.4	*i*	5 ± 0.3	*i*
19	0.8	0.30 ± 0.06	0	67 ± 3.1	1.81 (1.31−2.46)	57 ± 1.5	0.31 (0.25−0.39)	37 ± 2.1	*i*
20	0.7	0.5 ± 0.1	20	48 ± 2.5	*i*	67 ± 5.5	0.66 (0.52−0.79)	6 ± 0.5	*i*
21		toxic[h]	100	61 ± 3.5	2.11 (1.75−2.55)	38 ± 1.4	*i*	3 ± 0.2	*i*
22	0.9	3.0 ± 0.6	3	55 ± 1.3[f]	0.90 (0.77−1.07)	9 ± 0.7	*i*	5 ± 0.4	*i*
23	0.8	2.1 ± 0.4	0	70 ± 4.8	0.89 (0.81−0.98)	22 ± 0.9	*i*	10 ± 0.3	*i*
24	0.6	0.5 ± 0.1	6	69 ± 3.2	1.54 (1.25−1.82)	24 ± 1.2	*i*	23 ± 1.9	*i*
25	0.7	0.10 ± 0.02	20	61 ± 5.3[f]	1.06 (0.92−1.18)	67 ± 0.8	0.83 (0.68−1.03)	54 ± 2.5	4.12 (2.85−5.35)
26	0.7	1.5 ± 0.3	10	77 ± 3.6	1.10 (0.87−1.35)	84 ± 3.8	8.25 (7.92−8.63)	42 ± 1.6	*i*
27	0.7	2.0 ± 0.4	15	74 ± 5.1	1.04 (0.88−1.32)	34 ± 2.5	*i*	12 ± 0.6	*i*
28	0.5	0.5 ± 0.1	20	88 ± 1.5[f]	1.29 (1.11−1.52)	31 ± 1.3	*i*	10 ± 0.7	*i*
29	0.9	0.20 ± 0.04	0	67 ± 3.4	1.89 (1.72−2.15)	81 ± 1.4[f]	0.80 (0.78−0.82)	16 ± 0.9	*i*
30	0.8	0.8 ± 0.1	0	66 ± 4.7	2.91 (2.33−3.62)	89 ± 2.6[g]	0.37 (0.29−0.48	33 ± 2.3	*i*
31	0.8	1.3 ± 0.3	0	77 ± 5.6	1.16 (0.91−1.48)	92 ± 4.7[f]	0.24 (0.18−0.31)	65 ± 3.4	3.42 (2.98−4.07)
32	0.9	0.5 ± 0.1	0	71 ± 5.4	0.35 (0.26−0.47)	24 ± 1.1	*i*	6 ± 0.3	*i*
33		toxic[h]	30	78 ± 0.5	1.11 (0.85−1.38)	25 ± 1.3	*i*	12 ± 0.6	*i*
34		toxic[h]	30	65 ± 3.4	0.63 (0.52−0.76)	39 ± 3.3	*i*	46 ± 3.8	*i*
verapamil	0.7	1.6 ± 0.3	0	84 ± 2.1[f]	0.61 (0.40−0.80)	94 ± 3.4[j]	0.07 (0.05−0.10)	95 ± 1.7[f]	0.38 (0.20−0.70)

[a] Evaluated on erythroleukaemia K 562 cell line (see Experimental Section). [b] Activity: decrease in developed tension in isolated guinea pig left atrium at 5×10^{-5} M, expressed as percent changes from the control ($n = 5-6$). The left atria were driven at 1 Hz. The 5×10^{-5} M concentration gave the maximum effect for most compounds. [c] Calculated from log concentration−response curves (probit analysis according to Litchfield and Wilcoxon,[67] with $n = 5-7$). [d] Activity: decrease in atrial rate on guinea pig spontaneously beating isolated right atrium at 5×10^{-5} M, expressed as percent changes from the control ($n = 7-8$). Pretreatment heart rate ranged from 165 to 195 beats/min. The 5×10^{-5} M concentration gave the maximum effect for most compounds. [e] Activity: percent inhibition of calcium-induced contraction on K^+-depolarized guinea pig aortic strip at 5×10^{-5} M ($n = 5-6$). The 5×10^{-5} M concentration gave the maximum effect for most compounds. [f] At 10^{-5} M. [g] At 5×10^{-6} M. [h] The compounds were considered toxic when the dead cells at 1 μM exceeded 20%. [i] When the maximum effect was <50%, the EC_{50} inotropic, EC_{30} chronotropic, and IC_{50} values were not calculated and the compounds are considered inactive. [j] At 10^{-6} M.

5-Bromo-2-(3,4-dimethoxyphenyl)-2-isopropylpentanenitrile (35). 2-(3,4-Dimethoxyphenyl)-3-methylbutanenitrile[63] (3 g, 13.7 mmol) was dissolved in 20 mL of anhydrous THF and cooled to −78 °C; 12 mL (19.2 mmol) of *n*-butyllithium (1.6 M in hexane) was added, and the mixture was left at −78 °C for 2 h. Then 2.8 mL (27.4 mmol) of 1,3-dibromopropane was added; the mixture was allowed to warm to room temperature, treated with a saturated solution of NH_4Cl, and extracted with diethyl ether. The organic layer was dried over Na_2SO_4, the solvent eliminated under reduced pressure, and the residue purified by flash chromatography using ethyl acetate/cyclohexane (7:3) as eluting system. Title compound (2.33 g, 50% yield) was obtained as an oil. IR (neat): ν 2240 (CN) cm^{-1}. ^1H NMR ($CDCl_3$): δ 0.75 (d, $J = 6.7$ Hz, 3H, CH_3), 1.15 (d, $J = 6.6$ Hz, 3H, CH_3), 1.35−1.45 (m, 1H, CH), 1.70−2.30 (m, 4H, CH_2CH_2), 3.20−3.30 (m, 2H, CH_2Br), 3.85 (s, 3H, OCH_3), 3.88 (s, 3H, OCH_3), 6.70−6.97 (m, 3H, aromatics). Anal. ($C_{16}H_{22}BrNO_2$) C, H, N.

6-Chloro-2-(3,4-dimethoxyphenyl)-2-isopropyl-4-hexynenitrile (38). Following the same procedure described for **35**, starting from 2-(3,4-dimethoxyphenyl)-3-methylbutanenitrile[63] (1.3 g, 5.9 mmol) and 1,4-dichloro-2-butyne (1.3 mL, 13.3 mmol), compound **38** (1.06 g, 58.4% yield) was obtained. IR (neat): ν 2230 (CN) cm^{-1}. ^1H NMR ($CDCl_3$): δ 0.84 (d, $J = 6.6$ Hz, 3H, CH_3), 1.17 (d, $J = 6.6$ Hz, 3H, CH_3), 2.30−2.45 (m, 1H, $CH(CH_3)_2$), 2.80−3.05 (m, 2H, CH_2), 3.86 (s, 3H, OCH_3), 3.89 (s, 3H, OCH_3), 4.00−4.07 (m, 2H, CH_2−Cl), 6.81−6.86 (m, 1H, aromatic), 6.98−7.02 (m, 2H, aromatics). Anal. ($C_{17}H_{20}ClNO_2$) C, H, N.

2-(3,4-Dimethoxyphenyl)-2-isopropyl-5-[(diphenylmethyl)amino]pentanenitrile (2). Compound **35** (300 mg, 0.88 mmol), aminodiphenylmethane (160 mg, 0.88 mmol), and 1 mL of triethylamine were heated at 60 °C for 15 h. The reaction mixture was cooled to room temperature and treated with $CHCl_3$, and the organic layer was washed with water. After drying with Na_2SO_4, the solvent was removed under reduced pressure and the residue purified by column chromatography using cyclohexane/ethyl acetate (7:3) as eluting system. Title compound (160 mg, 41% yield) was obtained as a thick oil. IR (neat): ν 2240 (CN) cm^{-1}. ^1H NMR ($CDCl_3$): δ 0.80 (d, $J = 6.8$ Hz, 3H, CH_3), 1.18 (d, $J = 6.6$ Hz, 3H, CH_3), 1.45−1.65 (m, 1H, $CH(CH_3)_2$), 1.78−2.30 (m, 4H, CH_2−CH_2), 2.44−2.61 (m, 2H, CH_2−N), 3.86 (s, 3H, OCH_3), 3.88 (s, 3H, OCH_3), 4.72 (s, 1H, $CH(Ph)_2$), 6.78−6.95 (m, 3H, aromatics), 7.12−7.38 (m, 10H, aromatics).

The oily product was transformed into the hydrochloride that was recrystallized from absolute ethanol and anhydrous ether. Mp: 104−106 °C. Anal. ($C_{29}H_{35}ClN_2O_2$) C, H, N.

Compounds **1**, **3**−**8**, and **10**−**14** were obtained in the same way; their chemical and physical characteristics are reported in Table 1, and their IR and ^1H NMR spectra are consistent with the proposed structures.

2,2-Diphenyl-5-[(diphenylmethyl)amino]pentanenitrile (16). Following the procedure described for **2**, starting from 5-bromo-2,2-diphenylpentanenitrile[40] (**37**) (300 mg, 0.95 mmol), compound **16** (160 mg, 40% yield) was obtained as a thick oil. IR (neat): ν 2240 (CN) cm^{-1}. ^1H NMR ($CDCl_3$): δ 1.45−1.55 (m, 1H, NH), 1.58−1.78 (m, 2H, CH_2), 2.40−2.56 (m, 2H, CH_2), 2.63 (t, $J = 6.7$ Hz, 2H, CH_2−N), 4.78 (s, 1H, $CH(Ph)_2$), 7.18−7.48 (m, 20H, aromatics).

The hydrochloride was recrystallized from absolute ethanol and anhydrous ether. Mp: 108−110 °C. Anal. ($C_{30}H_{29}ClN_2$) C, H, N.

Compounds **15** and **17**−**23** were obtained in the same way; their chemical and physical characteristics are reported in Table 2, and their IR and ^1H NMR spectra are consistent with the proposed structures.

2-(3,4-Dimethoxyphenyl)-2-isopropyl-6-[(diphenylmethyl)amino]-4-hexynenitrile (24). Following the procedure described for **2**, starting from compound **38** (300 mg, 0.98 mmol), compound **24** (290 mg, yield 65%) was obtained as a thick oil. IR (neat): ν 2240 (CN) cm^{-1}. ^1H NMR ($CDCl_3$): δ 0.88 (d, $J = 6.6$ Hz, 3H, CH_3), 1.20 (d, $J = 6.6$ Hz, 3H, CH_3), 2.20−2.35 (m, 1H, $CH(CH_3)_2$), 2.80−3.12 (m, 2H, CH_2), 3.29 (s, 2H, CH_2−N), 3.81 (s, 3H, OCH_3), 3.83 (s, 3H, OCH_3), 5.03 (s, 1H, CH), 6.75−6.85 (m, 1H, aromatic), 6.92−7.10 (m, 2H, aromatics), 7.14−7.45 (m, 10H, aromatics).

The hydrochloride was recrystallized from absolute ethanol and anhydrous ether. Mp: 113−115 °C. Anal. ($C_{30}H_{33}ClN_2O_2$) C, H, N.

Compounds **25**−**28** and **33** were obtained in the same way; their chemical and physical characteristics are reported in Table 3, and their IR and ^1H NMR spectra are consistent with the proposed structures.

2-(3,4-Dimethoxyphenyl)-2-isopropyl-5-[*N*-4-[1-[4,6-bis(allylamino)-1,3,5-triazin-2-yl]]piperidinylamino]pentanenitrile (9). 5-Amino-2-(3,4-dimethoxyphenyl)-2-isopropylpentanenitrile[42] (**36**) (150 mg, 0.48 mmol) and 1-[4,6-bis(allylamino)-1,3,5-triazin-2-yl]-4-piperidone hydrochloride[39] were dissolved in 7 mL of anhydrous MeOH. The pH of the solution was adjusted to 6 with HCl-saturated absolute ethanol and $NaBH_3CN$ (30 mg, 0.46 mmol), and 200 mg of activated molecular sieves (4 Å) was added. The reaction mixture was stirred for 24 h at room temperature and then filtered. The solvent was removed under reduced pressure and the residue dissolved in CH_2Cl_2 and washed with water and then with 10% solution of $NaHCO_3$. After drying with Na_2SO_4, the organic layer was removed under reduced pressure and the residue purified by column chromatography using ethyl acetate/cyclohexane (1:9) as eluting system. Compound **9** (130 mg, 33% yield) was obtained as a thick oil. IR (neat): ν 2230 (CN) cm^{-1}. ^1H NMR ($CDCl_3$): δ 0.76 (d, $J = 6.6$ Hz, 3H, CH_3), 1.14−1.25 (m, 7H, CH_3, CH_2−C−CH_2-), 1.50−1.60 (m, 1H, $CH(CH_3)_2$), 1.75−1.85 (m, 2H, CH_2), 2.00−2.22 (m, 2H, CH_2), 2.55−2.88 (m, 6H, 3 CH_2−N), 3.84 (s, 3H, OCH_3), 3.86 (s, 3H, OCH_3), 3.90−4.00 (m, 4H, $2CH_2$−NH), 4.55−4.62 (m, 2H, 2NH), 4.98−5.11 (m, 4H, $2CH_2$═C), 5.79−5.93 (m, 2H, 2CH═C), 6.78−6.87 (m, 3H, aromatics).

The oily product was transformed into the hydrochloride that recrystallized from absolute ethanol and anhydrous ether. Mp: 191−193 °C. Anal. ($C_{30}H_{45}ClN_8O_2 \cdot 3H_2O$) C, H, N.

Compound **29** was obtained in the same way starting from 6-amino-2-(3,4-dimethoxyphenyl)-2-isopropyl-4-hexynenitrile (**39**); its chemical and physical characteristics are reported in Table 3, and its IR and ^1H NMR spectra are consistent with the proposed structure.

6-[(1-Adamantyl)methylamino]-2-(3,4-dimethoxyphenyl)-2-isopropyl-4-hexynenitrile (30). A solution of formaldehyde (0.2 mL, 40% solution in water), 1-(methylaminomethyl)adamantane[44] (290 mg, 1.75 mmol), and $CuSO_4$ (30 mg) was added to a solution of 2-(3,4-dimethoxyphenyl)-2-isopropyl-4-pentynenitrile (**40**)[42] (460 mg, 1.78 mmol) in 4 mL of EtOH/H_2O (1:1).The pH of the solution was adjusted to 8 with 50% sulfuric acid. The mixture was heated to reflux for 16 h then 15 mL of NH_4OH was added, and the solution was extracted with chloroform. The organic layer was dried over Na_2SO_4 and removed under reduced pressure and the residue purified by column chromatography using CH_2Cl_2/MeOH (95:5) as eluting system. Compound **30** (100 mg, 13% yield) was obtained as a thick oil. IR (neat): ν 2230 (CN) cm^{-1}. ^1H NMR ($CDCl_3$): δ 0.84 (d, $J = 6.6$ Hz, 3H, CH_3), 1.16 (d, $J = 6.6$ Hz, 3H, CH_3), 1.47−1.66 (m, 11H, adamantyl H), 1.96−2.16 (m, 4H, adamantyl H), 2.21−2.30 (m, 4H, CH_3−N, CH), 2.78−3.05 (m, 2H, CH_2−C≡C), 3.46 (s, 2H, C≡C−CH_2), 3.87 (s, 3H, OCH_3), 3.89 (s, 3H, OCH_3), 6.82−6.86 (m, 1H, aromatic), 6.95−7.02 (m, 2H, aromatics).

The oily product was transformed into the hydrochloride that recrystallized from absolute ethanol and anhydrous ether. Mp: 80−85 °C. Anal. ($C_{28}H_{39}ClN_2O_2$) C, H, N.

Compounds **31**, **32**, and **34** were obtained in the same way; their chemical and physical characteristics are reported in Table 3, and their IR and ^1H NMR spectra are consistent with the proposed structures.

***N*-[4-Cyano-4-(3,4-dimethoxyphenyl)-5-methylhexyl]-phthalimide (41).** 18-Crown-6-ether (80 mg, 0.3 mmol) and 5-bromo-2-(3,4-dimethoxyphenyl)-2-isopropylpentanenitrile (**35**) (570 mg, 1.67 mmol) were dissolved in 3 mL of anhydrous toluene, then potassium phthalimide (340 mg, 1.83 mmol) was

added, and the mixture was heated to reflux for 6 h. The reaction mixture was cooled to room temperature, and 10 mL of H_2O was added; after separation, the organic layer was dried with Na_2SO_4 and removed under reduced pressure. Compound **41** (570 mg, 84% yield) was obtained as a thick oil. IR (neat): ν 1710 (CO) cm^{-1}. ^1H NMR ($CDCl_3$): δ 0.70 (d, J = 6.6 Hz, 3H, CH_3), 1.14 (d, J = 6.6 Hz, 3H, CH_3), 1.30−1.45 (m, 1H, CH), 1.70−2.40 (m, 4H, $2CH_2$), 3.50−3.65 (m, 2H, CH_2−N), 3.79 (s, 3H, OCH_3), 3.83 (s, 3H, OCH_3), 6.70−6.83 (m, 3H, aromatics), 7.65−7.81 (m, 4H, aromatics). Anal. ($C_{24}H_{26}N_2O_4$) C, H, N.

5-Amino-2-(3,4-dimethoxyphenyl)-2-isopropylpentanenitrile (36). Hydrazine hydrate (0.5 mL, 10.3 mmol) was added to a solution of compound **41** (570 mg, 1.40 mmol) in 3 mL of tetrahydrofuran and 2 mL of ethanol. The mixture was stirred for 3 h at room temperature and the solid filtered off and washed with tetrahydrofuran. The filtrate was evaporated under reduced pressure and the resulting material purified by column chromatography using $CHCl_3$/MeOH (9:1) as eluting system. Compound **36** (200 mg, 84% yield) was obtained as an oil. IR (neat): ν 2235 (CN) cm^{-1}. ^1H NMR ($CDCl_3$): δ 0.76 (d, J = 6.6 Hz, 3H, CH_3), 1.16 (d, J = 6.6 Hz, 3H, CH_3), 1.39−1.58 (m, 3H, CH and NH_2), 1.70−1.90 (m, 2H, CH_2), 1.99−2.20 (m, 2H, CH_2), 2.55−2.70 (m, 2H, CH_2−N), 3.85 (s, 3H, OCH_3), 3.83 (s, 3H, OCH_3), 6.79−6.92 (m, 3H, aromatics). Anal. ($C_{16}H_{24}N_2O_2$) C, H, N.

***N*-[5-Cyano-5-(3,4-dimethoxyphenyl)-6-methyl-2-heptynyl]phthalimide (42).** Following the procedure described for **41**, starting from 6-chloro-2-(3,4-dimethoxyphenyl)-2-isopropyl-4-hexynenitrile (**38**) (500 mg, 1.67 mmol), compound **42** (560 mg, 89% yield) was obtained as a thick oil. IR (neat): ν 1710 (CO) cm^{-1}. ^1H NMR ($CDCl_3$): δ 0.83 (d, J = 6.6 Hz, 3H, CH_3), 1.14 (d, J = 6.6 Hz, 3H, CH_3), 2.30−2.42 (m, 1H, CH), 2.80−2.90 (m, 2H, CH_2), 3.79 (s, 3H, OCH_3), 3.84 (s, 3H, OCH_3), 4.36 (s, 2H, CH_2−N), 6.71−6.76 (m, 1H, aromatic), 6.94−6.99 (m, 2H, aromatics), 7.72−7.78 (m, 2H, aromatics), 7.85−7.89 (m, 2H, aromatics). Anal. ($C_{25}H_{24}N_2O_2$) C, H, N.

6-Amino-2-(3,4-dimethoxyphenyl)-2-isopropyl-4-hexynenitrile (39). Following the procedure described for **36**, starting from **42** (570 mg, 1.45 mmol), compound **39** (280 mg, 85% yield) was obtained as an oil. IR (neat): ν 2230 (CN) cm^{-1}. ^1H NMR ($CDCl_3$): δ 0.82 (d, J = 6.6 Hz, 3H, CH_3), 1.14 (d, J = 6.6 Hz, 3H, CH_3), 1.70 (bs, 2H, NH_2), 2.26−2.38 (m, 1H, CH), 2.70−3.00 (m, 2H, CH_2), 3.29 (s, 2H, CH_2−N), 3.85 (s, 3H, OCH_3), 3.87 (s, 3H, OCH_3), 6.79−7.01 (m, 3H, aromatics). Anal. ($C_{17}H_{22}N_2O_2$) C, H, N.

***N*-(10,11-Dihydro-5*H*-dibenzo[*a*,*c*]cyclohepten-5-yl)-phthalimide (43).** Following the procedure described for **41**, starting from commercially available 5-chlorodibenzosuberane (460 mg, 2 mmol), compound **43** (670 mg, 99% yield) was obtained as a pink solid. Mp: 193−195 °C. IR (Nujol): ν 1710 (CO) cm^{-1}. ^1H NMR ($CDCl_3$): δ 2.95−3.15 (m, 2H, CH_2), 3.55−3.75 (m, 2H, CH_2), 6.75 (s, 1H, CH), 7.10−7.30 (m, 6H, aromatics), 7.32−7.43 (m, 2H, aromatics), 7.62−7.80 (m, 4H, aromatics). Anal. ($C_{23}H_{17}NO_2$) C, H, N.

5-Amino-10,11-dihydro-5*H*-dibenzo[*a*,*c*]cycloheptene (44). Following the procedure described for **36**, starting from **43** (670 mg, 1.97 mmol), compound **44** was obtained as a white solid. Recrystallizations from 2-propanol gave 90 mg (22% yield). Mp: 90−91 °C (lit.[47] mp 91−92 °C). IR (Nujol): ν 3400−3220 (NH) cm^{-1}. ^1H NMR ($CDCl_3$): δ 2.15 (bs, 2H, NH_2), 3.10−3.30 (m, 2H, CH_2), 3.30−3.50 (m, 2H, CH_2), 5.45 (s, 1H, CH), 7.08−7.23 (m, 6H, aromatics), 7.35−7.45 (m, 2H, aromatics).

***N*-(9-Anthranylmethyl)phthalimide (45).** Following the procedure described for **41**, starting from 9-(chloromethyl)-anthracene (500 mg, 2.2 mmol), compound **45** (730 mg, 98% yield) was obtained as a yellow solid. Mp: 240−242 °C. IR (Nujol): ν 1700 (CO) cm^{-1}. ^1H NMR ($CDCl_3$): δ 5.85 (s, 2H, CH_2−N), 7.40−7.80 (m, 8H, aromatics), 8.02 (d, J = 8.5 Hz, 2H, aromatics), 8.46 (s, 1H, aromatic), 8.65 (d, J = 8.5 Hz, 2H, aromatics). Anal. ($C_{23}H_{15}NO_2$) C, H, N.

9-(Aminomethyl)anthracene (46). Following the procedure described for **36**, starting from compound **45** (410 mg, 1.21 mmol), compound **46** (200 mg, 80% yield) was obtained as a yellow-brown solid. Mp: 38−40 °C. IR (Nujol): ν 3400 (NH_2) cm^{-1}. ^1H NMR ($CDCl_3$): δ 1.75 (bs, 2H, NH_2), 4.85 (s, 2H, CH_2−N), 7.04−7.62 (m, 4H, aromatics), 8.05 (d, 2H, aromatics), 8.25−8.48 (m, 3H, aromatics).

***N*-(9-Fluorenyl)trifluoroacetamide (47).** Trifluoroacetic anhydride (1.5 mL, 11.2 mmol) was added to a solution of 9-aminofluorene (1.4 g, 7.73 mmol) in 50 mL of anhydrous ethyl ether. The mixture was stirred for 1 h; then the solvent was evaporated under reduced pressure to give 2.09 g (97% yield) of a white solid. Mp: 250−252 °C. IR (Nujol): ν 1700 (CO) cm^{-1}. ^1H NMR ($CDCl_3$): δ 1.54 (s, 1H, NH), 6.21 (s, 1H, CH), 7.35−7.75 (m, 8H, aromatics). Anal. ($C_{15}H_{10}F_3NO$) C, H, N.

9-(Methylamino)fluorene (48). Methyl iodide (2.26 mL, 35.5 mmol) was added to a solution of **47** (2.02 g, 7.29 mmol) in 70 mL of anhydrous acetone. The mixture was stirred for 30 min, then the solvent was removed, and 25 mL of water and KOH (1.65 g, 29.4 mmol) were added. The mixture was heated to 80 °C for 8 h, then cooled, and extracted with chloroform. The organic layer was dried over Na_2SO_4 and evaporated under reduced pressure to give 750 mg (53% yield) of oily **48**. IR (neat): ν 3400−3220 (NH) cm^{-1}. ^1H NMR ($CDCl_3$): δ 2.10 (s, 1H, NH), 2.21 (s, 3H, N−CH_3), 4.92 (s, 1H, CH), 7.28−7.73 (m, 8H, aromatics).

***N*-Methylfluorene-9-carboxamide (49).** 9-Fluorenecarboxylic acid (5.4 g, 25 mmol) was converted into the corresponding acyl chloride using $SOCl_2$ (32 mL) in 50 mL of anhydrous benzene at 60 °C for 2 h. After removal of the solvent the crude acyl chloride was dissolved in 50 mL of anhydrous tetrahydrofuran and cooled to 0 °C; then methylamine (40% solution in water) (78.2 mL) was slowly added. The mixture was stirred for 30 min and then extracted with CH_2Cl_2. The organic layer was washed twice with water, dried over Na_2SO_4, and evaporated under reduced pressure to give a white solid purified by column chromatography using CH_2Cl_2/MeOH (97:3) as eluting system. Compound **49** was obtained as a white solid (2 g, 25% yield). Mp: 233−235 °C. IR (Nujol): ν 1648 (CO) cm^{-1}. ^1H NMR (DMSO): δ 2.10 (s, 1H, NH), 2.66−3.00 (d, 3H, NCH_3), 4.81 (s, 1H, CH), 7.29−7.80 (m, 8H, aromatics). Anal. ($C_{15}H_{13}NO$) C, H, N.

***N*-Methyl-9-(aminomethyl)fluorene (50).** Borane−tetrahydrofuran complex (1.0 M in tetrahydrofuran; 24 mL, 24 mmol) was added to a solution of **49** (2 g, 8.96 mmol) in 20 mL of anhydrous tetrahydrofuran cooled to −18 °C. The mixture was stirred for 30 min then heated to reflux for 1 h; the solvent was removed, and 6 mL of 6 N HCl was added. The mixture was heated to reflux for 5 h, then, after cooling, neutralized with 10% NaOH solution, and extracted with ethyl ether. The organic layer was dried over Na_2SO_4 and evaporated under reduced pressure to give a residue that was purified by flash chromatography using $CHCl_3$/MeOH/CH_3COOH (90:5:5) as eluting system. Compound **50** was obtained as the acetate salt which was treated with 10% solution of NaOH to give 600 mg of the oily free base (32% yield). IR (neat): ν 3400−3220 (NH) cm^{-1}. ^1H NMR ($CDCl_3$): δ 2.53 (s, 3H, NCH_3), 3.13 (d, J = 6.4 Hz, 2H, CH_2), 4.15 (t, J = 6.4 Hz, 1H, CH), 7.36−7.50 (m, 4H, aromatics), 7.64−7.68 (m, 2H, aromatics), 7.82−7.86 (m, 2H, aromatics). Anal. ($C_{15}H_{15}N$) C, H, N.

QSAR Calculations. Volume, van der Waals surface, and Connolly surface were calculated using the MSI package Insight II (v. 2.3.0/95.0) (MSI, San Diego, CA) implemented on a Risc IBM. The molecules were first generated using the Builder module and minimized with Discover (v. 2.9.7/95.0) using the cvff force field and the conjugate gradient algorithm. The log *P* and molecular refractivity values of the molecules were calculated using the software package ClogP 2.0 (Biobyte Corp., Claremont, CA) implemented on a Pentium 200. It is known that, in general, accuracy decreases as molecular mass and complexity increase; in this respect, log *P* values for **20**, **21**, and **23** ($\geq$ 7.00) are signaled by the software as very high and unrealistic. Molecular weight was calculated as the sum of the individual weights of the atoms comprising the molecule (values are exact). Statistical analysis was performed with the aid of the software package CA-Cricket Graph III (v. 1.0)

(Computer Associates) implemented on a Quadra 800 Macintosh. The calculated parameters are reported in Table 5, which is included as Supporting Information.

Pharmacology. 1. Drugs and Chemicals. Purified pirarubicin was provided by Laboratoire Roger Bellon (France). Concentrations were determined by diluting stock solutions to approximately 10^{-5} M and using $\epsilon_{480} = 11\,500\ M^{-1}\ cm^{-1}$. Stock solutions were prepared just before use. Unless otherwise stated, buffer solutions were HEPES buffer containing 5 mM HEPES, 132 mM NaCl, 3.5 mM KCl, 1 mM $CaCl_2$, 5 mM glucose, at pH 7.25.

2. Cell Lines and Cultures. K 562 is a human leukemia cell line, established from a patient with a chronic myelogeneous leukemia in blast transformation.[64] K 562 cells resistant to doxorubicin were obtained by continuous exposure to increasing doxorubicin concentrations and were maintained in medium containing doxorubicin (400 nM) until 1−4 weeks before experiments. This subline expresses a unique membrane glycoprotein with a molecular weight of 180 000 Da.[65] Doxorubicin-sensitive and -resistant erythroleukemia K 562 cells were grown in suspension, in RPMI 1640 (Sigma) medium supplemented with L-glutamine and 10% FCS at 37 °C in a humidified atmosphere of 95% air and 5% CO_2. Cultures, initiated at a density of 10^5 cells/mL, grew exponentially to $8-10 \times 10^5$ cells/mL in 3 days. For the spectrofluorometric assays, to have cells in the exponential growth phase, culture was initiated at 5×10^5 cells/mL, and cells were used 24 h later, when the culture had grown to about $8-10 \times 10^5$ cells/mL. Cell viability was assessed by trypan blue exclusion. The cell number was determined by Coulter counter analysis.

A "resistance factor" (RF) was defined as the IC_{50} of resistant cells divided by the IC_{50} of the corresponding sensitive cells. The resistance factors obtained were 30 and 8 for doxorubicin and pirarubicin, respectively.

3. Cellular Drug Accumulation. The uptake of THP-adriamycin in cells was followed by monitoring the decrease in the fluorescence signal at 590 nm ($\lambda_{ex} = 480$ nm) following the method previously described.[66] 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 °C. We checked that tested compounds did not affect the fluorescence of THP-adriamycin.

Supporting Information Available: Table 5 with the calculated chemical physical parameters of compounds **1**−**34**. This material is available free of charge via the Internet at http://pubs.acs.org.

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