Journal of Medicinal Chemistry

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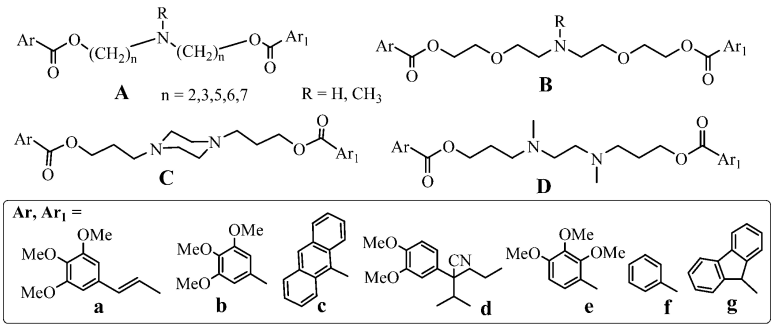
J. Med. Chem., **2005**, 48 (23), 7426-7436• DOI: 10.1021/jm050542x • Publication Date (Web): 14 October 2005



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Exploratory Chemistry toward the Identification of a New Class of Multidrug Resistance Reverters Inspired by Pervilleine and Verapamil Models

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Received June 8, 2005

On the basis of the present knowledge of the substrate recognition site of ABC transporter proteins and inspired by the structures of verapamil and pervilleine A, a new class of Pgp-mediated multidrug resistance (MDR) reverters has been designed and synthesized. The new compounds are flexible molecules carrying one or two basic nitrogen atoms flanked, at properly modulated distance, by two aromatic moieties. Most of the molecules studied possess MDR inhibitory activity on anthracycline-resistant erythroleukemia K 562 cells, showing a potency that is higher than that of the reference compound verapamil and, in a few cases (**7**, **12**, **13**, **17**, **20**, **22**, **28**), is in the high nanomolar range. These compounds may be useful leads to develop new MDR reverting agents. In fact, the chemical structure of the class is fairly simple and can be implemented in a variety of ways that will allow the synthesis of new compounds that might be useful leads for the development of drugs to control Pgp-dependent MDR.

Introduction

Drug resistance is the major cause of failure of chemotherapy. Multidrug resistance (MDR) is a kind of acquired drug resistance of cancer cells and microorganisms to a variety of chemotherapic drugs that usually are structurally and mechanistically unrelated.[1,2] MDR can be the result of several biochemical mechanisms among which the most widely implicated and studied is that resulting from altered cell membrane transport.[3] This kind of resistance is often referred to as classical MDR[4] and is due to a lower cell concentration of cytotoxic drugs associated with accelerated efflux of the chemotherapic, as a consequence of the overexpression of a number of proteins that act as extrusion pumps. Several families of pumps, using a variety of energy sources, are present in mammals and microorganisms.[2,5,6] At the moment, the best-known extrusion pumps are P-glycoprotein (Pgp) and MRP1 proteins[7] that belong to the ABC superfamily of transporters that use ATP as their energy source. In mammals, besides cancer cells, these proteins have been found in several important tissues, such as the blood−brain barrier (BBB), intestinal epithelium, and hepatic cells, where they apparently regulate the secretion of lipophilic molecules and the extrusion of xenobiotics that have entered the organism,[8−10] processing a large variety of substrates.

The mechanism of action of these extruding proteins is still controversial. As a matter of fact, a number of models have been proposed to explain their involvement in MDR:[11] the dominant drug pump concept[12] has given origin to three main models, such as the "aqueous pore", the "hydrophobic vacuum cleaner", and the "flippase" models.[12] Very recently, a model that is a hybrid of the first two mentioned above has been proposed by Loo and co-workers.[13]

The structure of Pgp has so far been resolved only at low resolution,[14,15] whereas the structure of some pumps of bacterial origin showing homology with Pgp, such as MsbA, has been obtained at 4.5 (EC-MsbA) and 3.8 Å resolution (VC-MsbA).[16−18] This achievement has cast new light on their structures and mechanism of action and has opened the way to the development of homology models for Pgp[19,20] and MRP1.[21] From these and many other reports on the structure of extruding pumps, it appears that the recognition sites are large, flexible and rich in amino acids able to give electrostatic, hydrophobic, and π-stacking interactions, such as aromatic amino acids.[22−26] For instance, it has been reported that the volume of the recognition site of *Escherichia coli* AcbR pump (that does not belong to the ABC but to the RND superfamily) is some 5000 Å^3 in size and can bind several ligands simultaneously.[25] Moreover, it has been shown that ligands with very close structures bind in quite different modes to the recognition site of the *Staphylococcus aureus* QacR repressor protein, which is considered a model for multidrug recognition.[26] At present, it appears realistic to think that the interacting sites of these transport proteins are large, polymorphous drug recognition domains, where a variety of substrates can be accommodated in a plurality of binding modes.[25,27−29]

Inhibition of the functions of Pgp and sister proteins is considered a realistic approach to circumvent MDR, and drugs possessing inhibitory properties have been and are actively being sought.[30−33] However, even if several molecules are being evaluated in clinical trials, so far, no drug has been approved for therapy.[34,35] The main problem associated with the development of effective Pgp-mediated MDR inhibitors seems due to poor specificity, low affinity for the binding site, interference

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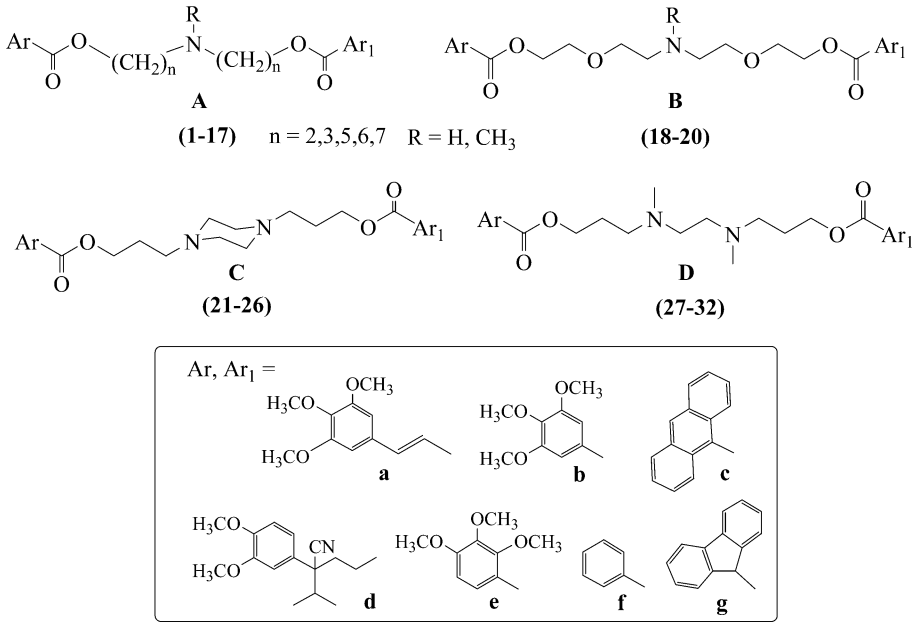
10.1021/jm050542x CCC: $30.25
Published on Web 10/14/2005

Chart 1



with the physiological role of Pgp and sister proteins, and, last but not least, interference with the pharmacokinetics of the associated chemotherapic.

Nature is a generous source of molecules able to modulate extruding pumps.[31,32] Very recently, the MDR modulating activity of a new family of tropane alkaloids named pervilleines has been reported.[36−38] To us, since we have for a long time been engaged in the design and study of MDR modulators,[39−42] the structural similarity of pervilleines and verapamil was immediately apparent. In fact, the two compounds may be reconciled to a unique structural scaffold, where a basic linker (L) tethers two aromatic moieties (H1, H2), as shown in Figure 1. Building on this idea, we designed a new series

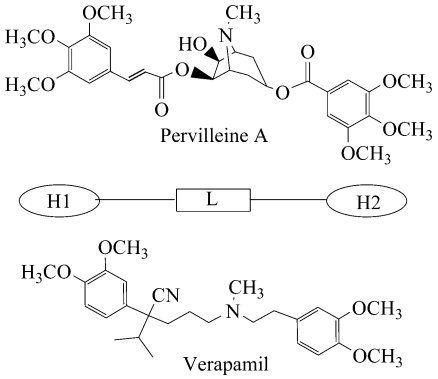


Figure 1.

of molecules with the structures shown in Chart 1. We reasoned that, given the properties of the Pgp recognition site described above, flexible molecules carrying a basic nitrogen flanked, at properly modulated distance, by two (or three) aromatic moieties would bind with high affinity as they will be able to find the best accommodation within the site (Figure 2). Multiple aromatic rings and at least one protonable nitrogen atom are the main features of most of the pharmacophores proposed for Pgp interaction with substrates and inhibitors;[43−47] here we add, as a further critical property, molecular flexibility. Of course, we were aware that, upon binding,

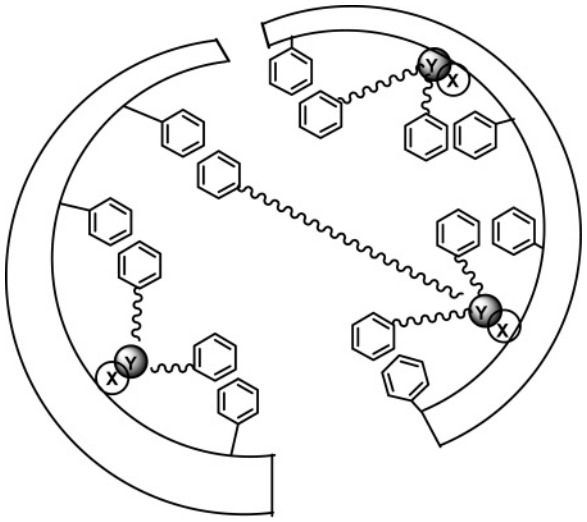
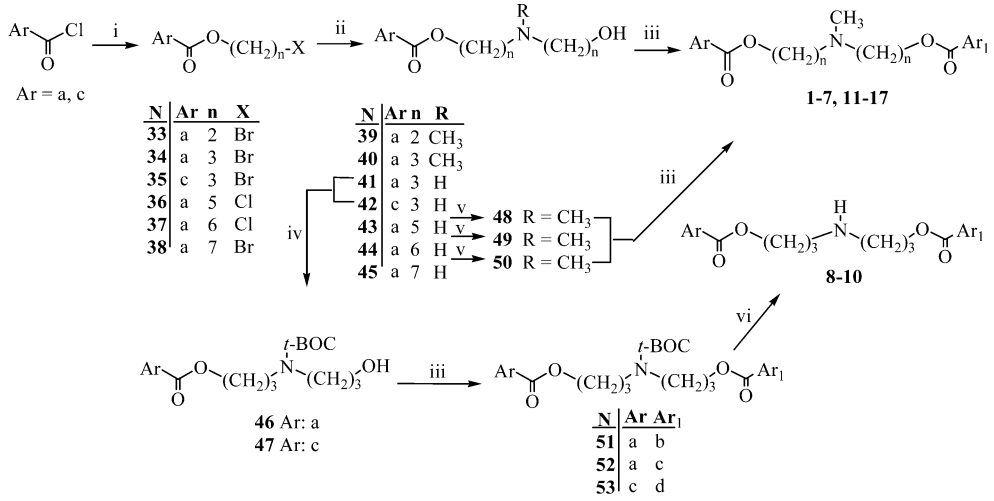


Figure 2. Flexible molecules such as bis- or tris(arylalkyl)-amines carrying aromatic moieties at different distances can adapt to the recognition site of Pgp binding with high affinity, in a variety of binding modes. X and Y represent atoms or groups of atoms able to establish electrostatic interactions.

such flexible molecules would pay a toll in terms of entropy; however, we were confident that loss in binding energy would be compensated by the enthalpic gain deriving from the fact that flexible molecules can choose, within the recognition site, the most productive binding mode. Interestingly, a similar approach, labeled as "polyvalency", has recently been used successfully by Sauna and co-workers,[48,49] who synthesized and studied several homodimers of the natural MDR inhibitor stipiamide.

In the starting phase of the research, as a proof of concept, we decided to limit our efforts to molecules carrying one nitrogen atom tethered by very simple linkers, such as polyethylene chains, to aromatic moieties. We also explored the potential of ethoxyethylene linkers, mainly to face the expected problems of solubility for long hydrocarbon chains, and that of linkers carrying two nitrogen atoms, such as ethylendiamine

Scheme 1[a]



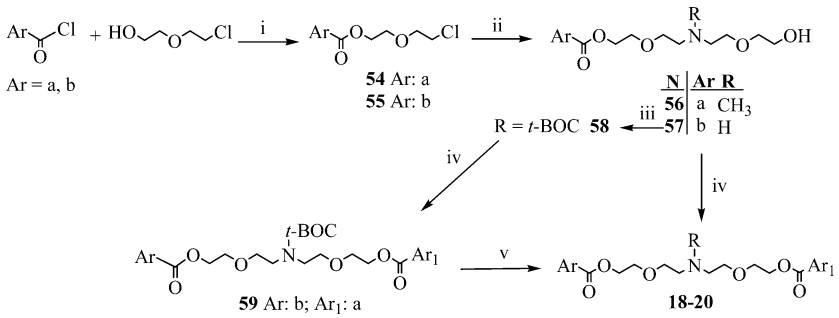
N	Ar	n	X
33	a	2	Br
34	a	3	Br
35	c	3	Br
36	a	5	Cl
37	a	6	Cl
38	a	7	Br

N	Ar	n	R
39	a	2	CH_3
40	a	3	CH_3
41	a	3	H
42	c	3	H
43	a	5	H
44	a	6	H
45	a	7	H

N	Ar	Ar_1
51	a	b
52	a	c
53	c	d

[a] Reagents and conditions: (i) $CHCl_3$, X−$(CH_2)_n$−OH (n = 2−7; X = Br, Cl); (ii) K_2CO_3/CH_3CN, RNH−$(CH_2)_n$−OH (n = 2−7; R = H, CH_3); (iii) Ar_1COCl, $CHCl_3$; (iv) (t-ButOCO)$_2$O/Et_3N; (v) HCOOH/HCHO; (vi) CF_3COOH, CH_2Cl_2. For the meaning of Ar and Ar_1, see Table 1.

Scheme 2[a]



N	Ar	R
56	a	CH_3
57	b	H

[a] Reagents and conditions: (i) $CHCl_3$; (ii) K_2CO_3/CH_3CN, $NHCH_3(CH_2)_2O(CH_2)_2OH$[52] or $NH_2(CH_2)_2O(CH_2)_2OH$; (iii) (t-ButOCO)$_2$O/Et_3N; (iv) Ar_1COCl, $CHCl_3$; (v) CF_3COOH, CH_2Cl_2. For the meaning of Ar, Ar_1, and R, see Table 1.

and piperazine. The aromatic moieties where chosen among those present in pervilleines or those that, in our previous work on verapamil-derived MDR reverters, gave the best results, such as the anthracene and fluorene moieties present in compounds MM36[41] and SC11,[39] which were active at nanomolar doses. It must be remarked that, for each series, we did not synthesize all the compounds that can be obtained by combining the aromatic fragments shown in Chart 1, as we could have easily done using a combinatorial chemistry approach. As a matter of fact, since HTS facilities were not available, we followed the classic medicinal chemistry approach, modulating the choice of the compounds to synthesize by following biological results.

Chemistry

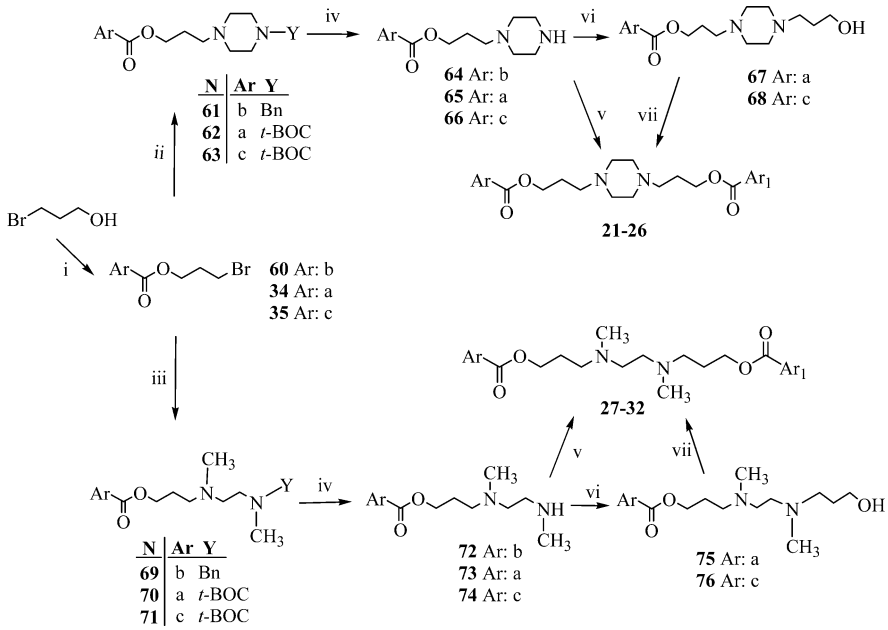
The reaction pathways used to synthesize the desired compounds (**1**−**32**) are described in Schemes 1−3 and their chemical and physical characteristics are reported in Table 1.

Compounds **1**−**17** were synthesized as reported in Scheme 1. The halo esters **33**−**38**, obtained by esterification of the corresponding haloalkyl alcohol (2-bromoethanol, 3-bromopropan-1-ol, 5-chloropentan-1-ol, 6-chlorohexan-1-ol, or 7-bromoheptan-1-ol) with *trans*-3-(3,4,5-trimethoxyphenyl)acryloyl chloride or anthracene-9-carbonyl chloride, were reacted with the commercially available amino alcohols 2-(methylamino)ethanol, 3-aminopropan-1-ol, 5-aminopentan-1-ol, 6-aminohexan-1-ol or 3-methylaminopropan-1-ol,[50] and 7-aminoheptan-1-ol[51] to give **39**−**45**.

The secondary amines **43**−**45** were alkylated by reductive methylation with HCOOH/HCHO to give the tertiary amines **48**−**50**. The amino function of the secondary amines **41** and **42** was protected by transformation into the *tert*-butyl carbamate (*t*-BOC) to give compounds **46** and **47**. Compounds **1**−**7**, **11**−**17**, **51**−**53** were then obtained by reaction of **39**, **40**, and **46**−**50** with the proper acyl chloride. Finally the secondary amines **8**−**10** were obtained after acidic cleavage of **51**−**53** with CF_3COOH.

A similar pathway allowed the synthesis of the ethoxyethylamino derivatives **18**−**20** (Scheme 2). By reaction of 2-(2-chloroethoxy)ethanol with *trans*-3-(3,4,5-trimethoxyphenyl)acryloyl chloride or 3,4,5-trimethoxybenzoyl chloride, compounds **54** and **55** were obtained, respectively. The tertiary amines **18** and **20** were obtained by reaction of **54** with 2-(2-methylaminoethoxy)ethanol[52] followed by esterification with the appropriate acyl chloride. The secondary amine **19** was obtained by reaction of **55** with 2-(2-aminoethoxy)-ethanol (commercially available) to give **57**, which in turn was reacted with di-*tert*-butyl dicarbonate to give **58**. The protected compound was reacted with *trans*-3-(3,4,5-trimethoxyphenyl)acryloyl chloride to give **59** that

Scheme 3[a]



[a] Reagents and conditions: (i) ArCOCl, $CHCl_3$; (ii) 1-benzylpiperazine or *N*-*t*-BOC-piperazine,[53] K_2CO_3/CH_3CN; (iii) *N*-benzyl-*N*,*N*′-dimethylethane-1,2-diamine[54] or *N*-*t*-BOC-N,N′-dimethylethane-1,2-diammine,[55] K_2CO_3/CH_3CN; (iv) Pd/C, $HCOONH_4$, MeOH or CF_3COOH, CH_2Cl_2; (v) $Ar_1COOCH_2CH_2Br$ (**34**, **35**), K_2CO_3/ CH_3CN; (vi) 3-bromopropan-1-ol, K_2CO_3/CH_3CN; (vii) Ar_1COCl, $CHCl_3$. For the meaning of Ar and Ar_1, see Table 1.

after cleavage of the protective group with CF_3COOH gave the desired compound.

Piperazine and dimethylaminoethane derivatives **21**−**32** were synthesized as shown in Scheme 3. Compounds **60**, **34**, and **35**, obtained by esterification of 3-bromopropan-1-ol with the suitable acyl chloride, were reacted with 1-benzylpiperazine or *N*-*t*-BOC-piperazine[53] to obtain compounds **61**−**63**. Cleavage of the protective group was performed by hydrogenation with $HCOONH_4$, Pd/C 10% or with CF_3COOH to give compounds **64**−**66**, which were alkylated with the bromo esters **34** or **35** to obtain the desired compounds **21**−**24**. In the case of **25** and **26**, this procedure, gave very poor yields and therefore a different pathway was followed. Compounds **65** and **66** were alkylated with 3-bromopropan-1-ol to give **67** and **68** that in turn were esterified with 4-cyano-4-(3,4-dimethoxyphenyl)-5-methylhexanoyl chloride or fluorene-9-carbonyl chloride to give **25** and **26**, respectively.

The synthesis of compounds **27**−**32** was performed using a similar reaction pathway by reaction of **60**, **34**, and **35** with *N*-benzyl-*N*,*N*′-dimethylethane-1,2-diamine[54] or *N*-*t*-BOC-*N*,*N*′-dimethylethane-1,2-diamine,[55] as shown in Scheme 3.

Pharmacological studies

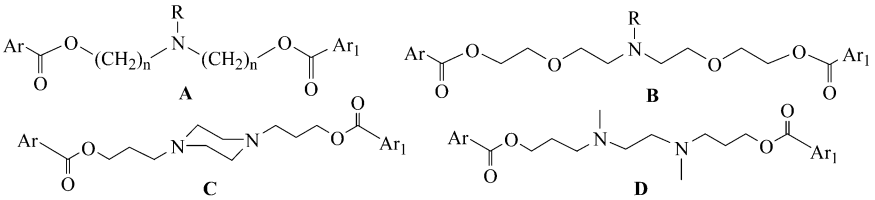
MDR-Reverting Activity. The ability of the examined compounds to revert MDR was evaluated on anthracycline-resistant erythroleukemia K 562 cells, measuring the uptake of THP-adriamycin (pirarubicin) by the continuous spectrofluorometric signal of the anthracycline at 590 nm ($\lambda_{ex} = 480$ nm) after incubation of the cells, following the protocols reported in previous papers.[39,41,56] MDR-reverting activity is described by (i) α, which represents the fold increase in the nuclear concentration of pirarubicin in the presence of the MDR-reverting agent and varies between 0 (in the absence of the inhibitor) and 1 (when the amount of pirarubicin in resistant cells is the same as in sensitive cells), (ii) α_{max}, which expresses the efficacy of MDR-modulator and is the maximum increase that can be obtained in the nuclear concentration of pirarubicin in resistant cells with a given inhibitor; and (iii) $[I]_{0.5}$, which measures the potency of MDR-reverting agent and represents the concentration of the inhibitor that causes a half-maximal increase ($\alpha = 0.5$) in nuclear concentration of pirarubicin (see Table 2).

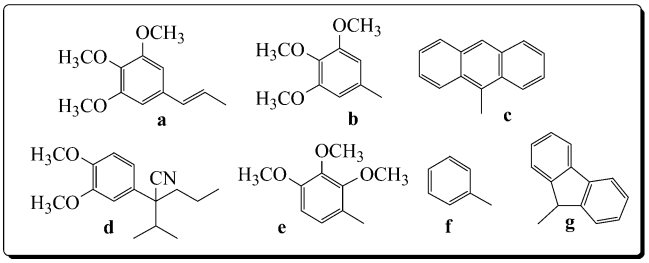
Even if binding data with Pgp are not available at the moment, this test indicates that our compounds inhibit the Pgp-operated extrusion of the reporter molecule pyrarubicin, as does the reference molecule verapamil. The molecules studied lack any detectable cytotoxicity at the doses used in the test.

Results and Discussion

The results of the MDR-reverting activity of the compounds synthesized are reported in Table 2. Inspection of the table indicates that most of the molecules studied possess MDR-inhibitory activity and show a potency that is higher than that of the reference compound verapamil and, in a few cases (**7**, **12**, **13**, **17**, **20**, **22**, **28**), is in the same high nanomolar range of MM36, the most potent compound that we have found in previous studies.[41] Overall, the results obtained seem to confirm that the rationale upon which the present work is based was sound and that the entropic toll paid by molecules while binding is compensated by the enthalpic gain deriving from the fact that such flexible molecules can chose, within the recognition site, the most productive binding mode. Therefore, the molecules of this new class can be taken as leads to design new potent and selective MDR-reverting agents. In fact, since the new structures are only loosely related to verapamil, it can be expected that their members lack

Table 1. Chemical and Physical Characteristics of Compounds **1**−**32**





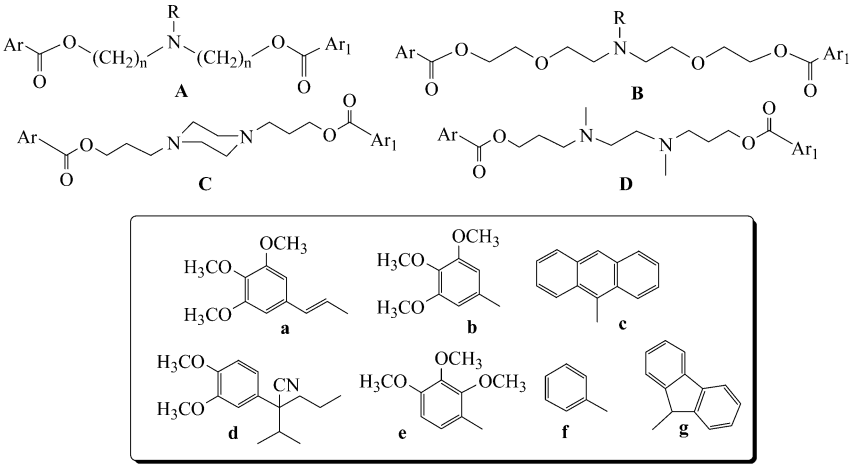
no.	structure	R	n	Ar	Ar_1	% yield	salt/mp (°C)	analysis[a]
1	**A**	CH_3	2	**a**	**e**	34	oxa[b]/50−54[c]	$C_{29}H_{37}NO_{14}$
2	**A**	CH_3	2	**a**	**c**	52	oxa/97−99[d]	$C_{34}H_{35}NO_{11}$
3	**A**	CH_3	2	**a**	**b**	74	oxa/82−85[d]	$C_{29}H_{37}NO_{14}$
4	**A**	CH_3	2	**a**	**f**	58	oxa/103−105[d]	$C_{26}H_{31}NO_{11}$
5	**A**	CH_3	2	**a**	**g**	96	oxa/134−136[d]	$C_{33}H_{35}NO_{11}$
6	**A**	CH_3	3	**a**	**b**	56	oxa/96−98[c]	$C_{31}H_{41}NO_{14}$
7	**A**	CH_3	3	**a**	**c**	90	oxa/85−87[c]	$C_{36}H_{39}NO_{11}$
8	**A**	H	3	**a**	**b**	64	oxa/172−174[c]	$C_{30}H_{39}NO_{14}$
9	**A**	H	3	**a**	**c**	36	oxa/156−158[c]	$C_{35}H_{37}NO_{11}$
10	**A**	H	3	**c**	**d**	60	HCl/50−54[e]	$C_{37}H_{43}ClN_2O_6$
11	**A**	CH_3	5	**a**	**b**	65	oxa/50−54[c]	$C_{35}H_{49}NO_{14}$
12	**A**	CH_3	5	**a**	**c**	40	oxa/60−65[c]	$C_{40}H_{47}NO_{11}$
13	**A**	CH_3	5	**a**	**g**	57	oxa/66−70[c]	$C_{39}H_{47}NO_{11}$
14	**A**	CH_3	6	**a**	**b**	36	liquid free base	$C_{35}H_{51}NO_{10}$
15	**A**	CH_3	6	**a**	**c**	46	oxa/171−173[c]	$C_{42}H_{51}NO_{11}$
16	**A**	CH_3	7	**a**	**b**	57	oxa/58−60[c]	$C_{39}H_{57}NO_{14}$
17	**A**	CH_3	7	**a**	**c**	43	oxa/79−81[c]	$C_{44}H_{55}NO_{11}$
18	**B**	CH_3	−	**a**	**b**	72	oxa/58−60[c]	$C_{33}H_{45}NO_{16}$
19	**B**	H	−	**a**	**b**	43	oxa/121−123[c]	$C_{32}H_{43}NO_{16}$
20	**B**	CH_3	−	**a**	**c**	61	oxa/88−90[c]	$C_{38}H_{43}NO_{13}$
21	**C**	−	−	**a**	**b**	85	oxa/215−220[c]	$C_{34}H_{46}N_2O_{14}$
22	**C**	−	−	**a**	**c**	59	oxa/214−217[c]	$C_{39}H_{44}N_2O_{11}$
23	**C**	−	−	**c**	**c**	31	free base/160−163	$C_{40}H_{38}N_2O_4$
24	**C**	−	−	**a**	**a**	61	free base/159−161	$C_{34}H_{46}N_2O_{10}$
25	**C**	−	−	**c**	**d**	33	oxa/223−226[c]	$C_{43}H_{51}N_3O_{10}$
26	**C**	−	−	**a**	**g**	59	oxa/207−210[c]	$C_{38}H_{44}N_2O_{11}$
27	**D**	−	−	**a**	**b**	50	oxa/50−55[c]	$C_{34}H_{48}N_2O_{14}$
28	**D**	−	−	**a**	**c**	69	oxa/160−163[c]	$C_{39}H_{46}N_2O_{11}$
29	**D**	−	−	**c**	**c**	69	free base/120−122	$C_{40}H_{40}N_2O_4$
30	**D**	−	−	**a**	**a**	69	oxa/159−164[c]	$C_{36}H_{50}N_2O_{14}$
31	**D**	−	−	**c**	**d**	22	oxa/160−163[c]	$C_{43}H_{53}N_3O_{10}$
32	**D**	−	−	**a**	**g**	59	oxa/156−160[c]	$C_{38}H_{46}N_2O_{11}$

[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. [b] Oxa = oxalate. [c] Recrystallization solvent: ethyl acetate. [d] Recrystallization solvent: absolute ethanol. [e] Recrystallization solvent: absolute ethanol/anhydrous diethyl ether.

any cardiovascular activity. Indeed, this has been confirmed in the case of **2** and **24**, which did not show any detectable cardiovascular action on heart and aorta models in the assays that we have previously described[41] (data not shown).

In detail, some clear-cut structure−activity relationships can be found by inspection of the results reported in Table 2. Comparing the compounds of the **A** series that have the same aromatic moieties at the ends of the molecule (**2**, **7**, **12**, **15**, **17**), it is apparent that the length of the linker is critical: MDR-reverting potency increases with the number of methylenes, reaching the maximum for $n = 5$ (**12**; $[I]_{0.5} = 0.10$ μM). For $n = 6$ there is a drop in potency (**15**; $[I]_{0.5} = 0.61$ μM) that, however, is nearly completely recovered for $n = 7$ (**17**; $[I]_{0.5} = 0.15$ μM). Recently, Sauna and co-workers,[48] studying stipiamide homodimers, reported that increasing the length of the spacer between the two monomers from 11 up to 35 Å, significantly enhanced the capacity of the dimers to inhibit drug efflux. Our series of compounds seem to behave much in the same way. In fact, the extended conformation distance between the two aromatic moieties of the compounds that carry the same aromatic moieties ranges from 16.43 to 27.53 Å (Table 3). Reasonably, in both cases, the distance of the bound conformations might change considerably, since the molecules are rather flexible, a property that is at the base of our design and that Sauna and co-workers consider critical also for stipiamide dimers.[48] Indeed, an expected consequence of flexibility is that any molecule of either set may bind in its own way, according to the length of the linker, much like what has been observed

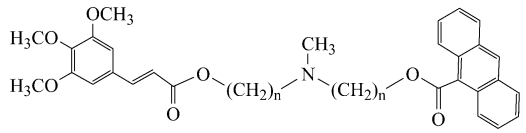
Table 2. MDR-Reverting Activity of Compounds **1**−**32**



no.	structure	R	*n*	Ar	Ar_1	$[I]_{0.5}$, μM	α_{max}
1	**A**	CH_3	2	**a**	**e**	6.40 ± 1.6	0.80
2	**A**	CH_3	2	**a**	**c**	1.00 ± 0.2	0.84
3	**A**	CH_3	2	**a**	**b**	3.10 ± 0.8	0.86
4	**A**	CH_3	2	**a**	**f**	3.70 ± 0.9	0.86
5	**A**	CH_3	2	**a**	**g**	0.80 ± 0.2	0.70
6	**A**	CH_3	3	**a**	**b**	0.60 ± 0.15	0.90
7	**A**	CH_3	3	**a**	**c**	0.18 ± 0.05	0.78
8	**A**	H	3	**a**	**b**	0.50 ± 0.1	1
9	**A**	H	3	**a**	**c**	0.21 ± 0.05	0.74
10	**A**	H	3	**c**	**d**	2.10 ± 0.5	0.90
11	**A**	CH_3	5	**a**	**b**	0.80 ± 0.2	0.84
12	**A**	CH_3	5	**a**	**c**	0.10 ± 0.02	0.80
13	**A**	CH_3	5	**a**	**g**	0.11 ± 0.03	0.83
14	**A**	CH_3	6	**a**	**b**	2.0 ± 0.4	0.6
15	**A**	CH_3	6	**a**	**c**	0.61 ± 0.15	0.68
16	**A**	CH_3	7	**a**	**b**	1.0 ± 0.4	0.55
17	**A**	CH_3	7	**a**	**c**	0.15 ± 0.05	0.55
18	**B**	CH_3	−	**a**	**b**	4.20 ± 1.0	0.90
19	**B**	H	−	**a**	**b**	7.0 ± 2.0	1
20	**B**	CH_3	−	**a**	**c**	0.17 ± 0.04	0.84
21	**C**	−	−	**a**	**b**	0.38 ± 0.10	0.61
22	**C**	−	−	**a**	**c**	0.16 ± 0.04	0.90
23	**C**	−	−	**c**	**c**	2.50 ± 0.5[a]	0.50
24	**C**	−	−	**a**	**a**	0.37 ± 0.10	0.80
25	**C**	−	−	**c**	**d**	0.62 ± 0.15	0.65
26	**C**	−	−	**a**	**g**	0.23 ± 0.06	0.70
27	**D**	−	−	**a**	**b**	1.30 ± 0.3	0.78
28	**D**	−	−	**a**	**c**	0.19 ± 0.04	0.70
29	**D**	−	−	**c**	**c**	0.80 ± 0.2[a]	0.50
30	**D**	−	−	**a**	**a**	2.60 ± 0.5	0.80
31	**D**	−	−	**c**	**d**	0.40 ± 0.1	0.74
32	**D**	−	−	**a**	**g**	0.57 ± 0.15	0.64
verapamil						1.60 ± 0.3	0.70
MM36						0.05 ± 0.01	0.70

[a] Concentration of the inhibitor that causes a 40% increase in nuclear concentration of pirarubicin ($\alpha = 0.4$).

Table 3. Calculated Extended Conformation Distance (*d*) between the Centers of the Aromatic Moieties[a]



no.	*n*	*d* (Å)	no.	*n*	*d* (Å)
2	2	16.43	**15**	6	25.85
7	3	17.49	**17**	7	27.53
12	5	22.35			

[a] The molecules have been drawn in the all-trans configuration and minimized with Discover (cvff force field) ($d < 0.01$). A pseudoatom has been created at the center of aromatic rings.

for the already mentioned *S. aureus* QacR repressor protein.[26] If this is true, the difference in potency of **15** with respect to **12** and **17** can be rationalized by admitting that **15** cannot find, inside the recognition site, a binding mode as productive as those of **12** and **17**. It is interesting that, a few months ago, Cianchetta and co-workers[57] have proposed that Pgp substrates interact, among others, with two hydrophobic areas 16.5 Å apart, which seems compatible with the length of inhibitors such as our compounds and stipiamide homodimers.

A second important feature of the **A** series is that also the nature of the aromatic hydrophobic moieties is critical. As observed in our previous works,[39,41] the presence of antracene (**c**) or fluorene (**g**) moieties is beneficial with respect to the benzene (**f**) one (**2**, $[I]_{0.5}$ = 1.0 μM, and **5**, $[I]_{0.5}$ = 0.80 μM, versus **4**, $[I]_{0.5}$ = 3.70 μM), as is the presence of the hydrogen-bond-acceptor methoxy groups. As a matter of fact, within the **A** series, the best combination of aryl moieties is that presenting antracene (**c**) [or fluorene (**g**)] and (3,4,5-trimethoxyphenyl)vinyl moieties (**a**) (**2**, $[I]_{0.5}$ = 1.0 μM; **7**, $[I]_{0.5}$ = 0.18 μM; **9**, $[I]_{0.5}$ = 0.21 μM; **12**, $[I]_{0.5}$ = 0.10 μM; **13**, $[I]_{0.5}$ = 0.11 μM; **17**, $[I]_{0.5}$ = 0.15 μM).

A third interesting finding in the **A** series is that secondary and tertiary amines present more or less the same activity (compare **6**, $[I]_{0.5} = 0.60$ μM, and **8**, $[I]_{0.5} = 0.50$ μM, or **7**, $[I]_{0.5} = 0.18$ μM, and **9**, $[I]_{0.5} = 0.21$ μM).

All these SARs are essentially maintained in the **B**, **C**, and **D** series. Thus, in the **B** series, the compound carrying the antracene (**c**) and (3,4,5-trimethoxyphenyl)-vinyl moieties (**a**) and having a length similar to compound **12**, is the most potent (**20**, $[I]_{0.5} = 0.17$ μM), whereas secondary and tertiary amines show similar activity (**18**, $[I]_{0.5} = 4.2$ μM; **19**, $[I]_{0.5} = 7.0$ μM). Series **C** and **D** are characterized by two ionizable nitrogen atoms, but SARs found in the one nitrogen series hold also in this case: the most potent compounds are **22** and **28**, both containing the antracene (**c**) and (3,4,5-trimethoxyphenyl)vinyl moieties (**a**) (**22**, $[I]_{0.5} = 0.16$ μM; **28**, $[I]_{0.5} = 0.19$ μM) and showing a length close to that of **12** (22.28 and 22.71 Å vs 22.35 Å). The results obtained in all series indicate that the nature of the linker is not critical, provided it contains at least a protonable nitrogen atom.

In conclusion, we have designed and studied a new class of MDR reverters, among which potent and selective molecules have been found. Even if suggested by the structure of a drug used for other purposes (verapamil) and of a compound of natural origin (pervilleine A), the class was mainly designed on the basis of the information that has recently been collected on the nature of the recognition site of Pgp and sister proteins. The general structure of the compounds of the class is fairly simple and can be implemented in several ways that will allow the synthesis of a variety of new compounds that might be useful leads for the development of drugs to control Pgp-dependent MDR.

Experimental Section

Chemistry. All melting points were taken on a Büchi apparatus and are uncorrected. Infrared spectra were recorded with a Perkin-Elmer 681 or a Perkin-Elmer Spectrum RX I FT-IR spectrophotometer in Nujol mull for solids and neat for liquids. NMR spectra were recorded on a Gemini 200 or a Bruker Avance 400 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. We have chosen to perform and report only the combustion analyses of final compounds. The identity and purity of the intermediates was ascertained through IR, NMR, and TLC chromatography. Compounds were named following IUPAC rules as applied by Beilstein-Institut AutoNom (version 2.1), a software for systematic names in organic chemistry. When reactions were performed in anhydrous conditions, the mixtures were maintained under nitrogen.

General Procedure for the Synthesis of Esters (33−38, 54, 55, 60). A 1-mmol portion of the appropriate carboxylic acid (commercially available) was transformed into the acyl chloride by reaction with $SOCl_2$ (2 mL), in 5 mL of anhydrous C_6H_6, at 60 °C for 4−8 h. The reaction mixture was cooled to room temperature and the solvent was removed under reduced pressure. The acyl chloride obtained was dissolved in $CHCl_3$ (free of EtOH) and the suitable alcohol (1 equiv) was added. The reaction mixture was heated to 60 °C for 4−8 h, cooled to room temperature, and treated with CH_2Cl_2, and the organic layer washed with 10% NaOH solution. After drying with Na_2SO_4, the solvent was removed under reduced pressure. The substances obtained were almost pure and used as such for the next reaction. Their IR and ^{1}H NMR spectra are consistent with the proposed structures.

The spectra of *trans*-3-(3,4,5-trimethoxyphenyl)acrylic acid 2-bromoethyl ester **33** are reported as an example. IR (Nujol): ν cm^{-1} 1711 (CO). ^{1}H NMR ($CDCl_3$): δ 7.65 (d, $J = 15.9$ Hz, 1H, CH=*CH*), 6.76 (s, 2H, aromatics), 6.38 (d, $J = 15.9$ Hz, 1H, *CH*=CH), 4.52 (t, $J = 6.0$ Hz, 2H, CH_2O), 3.89 (s, 9H, $3OCH_3$), 3.65 (t, $J = 6.0$ Hz, 2H, CH_2Br) ppm.

General Procedure for the Synthesis of Hydroxyamino Esters (39−45, 56, 57). The appropriate halo ester (1 mmol) and the suitable aminoalkyl alcohol (1.2 mmol) were dissolved in 1 mL of anhydrous CH_3CN, and K_2CO_3 (1 mmol) was added. The mixture was heated at 60 °C for 5−10 h. The reaction mixture was cooled to room temperature and treated with CH_2Cl_2, and the organic layer was washed with 10% NaOH solution. After drying with Na_2SO_4, the solvent was removed under reduced pressure and the residue purified by flash chromatography using $CHCl_3$/MeOH (95:5) (compounds **39**, **40**, **42**, **45**, **56**) or CH_2Cl_2/abs EtOH/petroleum ether/NH_4-OH (340:65:60:8) (compounds **41**, **43**, **44**, **57**) as the eluting system. Yields 50−70%. Their IR and ^{1}H NMR spectra are consistent with the proposed structures.

The spectra of *trans*-3-(3,4,5-trimethoxyphenyl)acrylic acid 2-[(2-hydroxyethyl)methylamino]ethyl ester (**39**) are reported as an example. IR (neat): ν cm^{-1} 3410 (OH), 1709 (CO). ^{1}H NMR ($CDCl_3$): δ 7.63 (d, $J = 16.1$ Hz, 1H, CH=*CH*), 6.78 (s, 2H, aromatics), 6.36 (d, $J = 16.1$ Hz, 1H, *CH*=CH), 4.33 (t, $J = 5.5$ Hz, 2H, CH_2O), 3.90 (s, 6H, $2OCH_3$), 3.88 (s, 3H, OCH_3), 3.61 (t, $J = 5.1$ Hz, 2H, CH_2OH), 2.80 (t, $J = 5.1$ Hz, 2H, CH_2N), 2.65 (t, $J = 5.5$ Hz, 2H, CH_2N), 2.37 (s, 3H, NCH_3) ppm.

***trans*-3-(3,4,5-Trimethoxyphenyl)acrylic Acid 3-(*tert*-Butoxycarbonylbutylamino)propyl Ester (46).** To a solution of 0.1 g (0.28 mmol) of **41** in 5 mL of THF cooled to 0 °C were added 80 mg (0.36 mmol) of di-*tert*-butyl dicarbonate and 0.08 mL of triethylamine. The mixture was maintained at room temperature for 6 h and then concentrated in vacuo. The residue was dissolved in CH_2Cl_2 and the organic layer was washed with water. After drying with Na_2SO_4, the solvent was removed under reduced pressure. Compound **46** was obtained as a light yellow oil (120 mg, 95% yield). IR (neat): ν cm^{-1} 3448 (OH), 1708 (CO). ^{1}H NMR ($CDCl_3$): δ 7.60 (d, $J = 16.1$ Hz, 1H, CH=*CH*), 6.75 (s, 2H, aromatics), 6.33 (d, $J = 16.1$ Hz, 1H, *CH*=CH), 4.23 (t, $J = 5.8$ Hz, 2H, CH_2O), 3.88 (s, 6H, $2OCH_3$), 3.87 (s, 3H, OCH_3), 3.57−3.25 (m, 6H, $2CH_2N$, CH_2-OH), 1.98−1.52 (m, 5H, $2CH_2$ and OH), 1.46 (s, 9H, $3CH_3$) ppm.

Compounds **47** and **58** were obtained in the same way from the corresponding secondary amines **42** and **57**, respectively. Their IR and ^{1}H NMR spectra are consistent with the proposed structures.

***trans*-3-(3,4,5-Trimethoxyphenyl)acrylic Acid 5-[(5-Hydroxypentyl)methylamino]pentyl Ester (48).** To a solution of 0.8 g (1.95 mmol) of **43** in 25 mL of anhydrous ethanol were added 24 mL of HCOOH and 24 mL of HCHO. The mixture was heated to 80 °C for 7 h and concentrated in vacuo. The residue was then dissolved in CH_2Cl_2 and the organic layer was washed with a saturated solution of Na_2CO_3 and with water. After drying with Na_2SO_4, the solvent was removed under reduced pressure. The crude product was then purified by flash chromatography using CH_2Cl_2/abs EtOH/ethyl ether/petroleum ether/NH_4OH 360:180:360:900:9.9 as the eluting system. Compound **48** was obtained as an oil (0.53 g, 64% yield). IR (neat): ν cm^{-1} 3404 (OH), 1710 (CO). ^{1}H NMR ($CDCl_3$): δ 7.55 (d, $J = 16.1$ Hz, 1H, CH=*CH*), 6.72 (s, 2H, aromatics), 6.31 (d, $J = 16.1$ Hz, 1H, *CH*=CH), 4.16 (t, $J = 6.2$ Hz, 2H, CH_2O), 3.85 (s, 6H, $2OCH_3$), 3.83 (s, 3H, OCH_3), 3.59 (t, $J = 6.1$ Hz, 2H, CH_2OH), 2.62 (s, 1H, OH), 2.31−2.27 (m, 4H, $2CH_2N$), 2.16 (s, 3H, NCH_3), 1.76−1.22 (m, 12H, $6CH_2$) ppm.

Compounds **49** and **50** were obtained in the same way starting from **44** and **45**, respectively. Their IR and ^{1}H NMR spectra are consistent with the proposed structures.

2,3,4-Trimethoxybenzoic Acid 2-(*N*-Methyl-[2-[*trans*-3-(3,4,5-trimethoxyphenyl)acryloyloxy]ethyl]amino)ethyl Ester (1). Following the general procedure described above for the general synthesis of esters, the acyl chloride, obtained from 2,3,4-trimethoxybenzoic acid (375 mg, 1.76 mmol), was allowed to react with **39** (300 mg, 0.88 mmol). The crude product was then purified by flash chromatography using CH_2Cl_2/abs EtOH/ethyl ether/petroleum ether/NH_4OH 360:180:360:900:9.9 as the eluting system.

The title compound (160 mg, 34% yield) was obtained as an oil. IR (neat): ν cm^{-1} 1714 (CO). ^1H NMR ($CDCl_3$): δ 7.63−7.55 (m, 2H, CH=*CH* and aromatics), 6.73 (s, 2H, aromatics), 6.69 (d, J = 10.9 Hz, 1H, aromatics), 6.37 (d, J = 16.1 Hz, 1H, *CH*=CH), 4.37 (t, J = 5.6 Hz, 2H, CH_2O), 4.31 (t, J = 5.8 Hz, 2H, CH_2O), 3.92 (s, 3H, OCH_3), 3.90−3.84 (m, 15H, $5OCH_3$), 2.87−2.80 (m, 4H, 2 CH_2N), 2.43 (s, 3H, NCH_3) ppm. The oily product was transformed into the oxalate that was recrystallized from ethyl acetate. Mp: 50−54 °C. Anal. ($C_{29}H_{37}NO_{14}$) C, H, N.

Compounds **2**−**7**, **11**−**18**, and **20** were obtained in the same way by reaction of the corresponding alcohol with the suitable acyl chloride. Compounds **2**−**7**, **11**−**13**, **15**−**18**, and **20** were transformed into the oxalate and recrystallized from the solvent reported in Table 1. Their chemical and physical characteristics are reported in Table 1. IR and ^1H NMR spectra are reported in the Supporting Information.

3,4,5-Trimethoxybenzoic Acid 3-(*N*-*tert*-Butoxycarbonyl[3-[*trans*-3-(3,4,5-trimethoxyphenyl)acryloyloxy]propyl]-amino)propyl Ester (51). Following the procedure described for compound **1**, starting from compound **46** (120 mg, 0.26 mmol) and 3,4,5-trimethoxybenzoyl chloride (120 mg, 0.52 mmol), compound **51** (180 mg, 36% yield) was obtained as a light yellow oil. IR (neat): ν cm^{-1} 1714 (CO), 1693 (CO). ^1H NMR ($CDCl_3$): δ 7.60 (d, J = 16.0 Hz, 1H, CH=*CH*), 7.29 (s, 2H, aromatics), 6.75 (s, 2H, aromatics), 6.34 (d, J = 16.0 Hz, 1H, *CH*=CH), 4.34 (t, J = 6.2 Hz, 2H, CH_2O), 4.22 (t, J = 6.2 Hz, 2H, CH_2O), 3.90 (s, 12H, $4OCH_3$), 3.88 (s, 6H, $2OCH_3$), 3.46−3.31 (m, 4H, $2CH_2N$), 2.16−1.94 (m, 4H, $2CH_2$), 1.45 (s, 9H, $3CH_3$) ppm.

Compounds **52**, **53**, and **59** were obtained in the same way from the corresponding alcohol with the suitable acyl chloride. Their IR and ^1H NMR spectra are consistent with the proposed structures.

3,4,5-Trimethoxybenzoic Acid 3-[3-[*trans*-3-(3,4,5-Trimethoxyphenyl)acryloyloxy]propyl]amino)propyl Ester (8). To a solution of 120 mg (0.18 mmol) of **51** in 2 mL of CH_2Cl_2 was added 0.4 mL of CF_3COOH under vigorous stirring. After 3 h at room temperature, the solution was concentrated in vacuo. The residue was then dissolved in ethyl acetate and the organic layer was washed with a saturated solution of $NaHCO_3$. After drying with Na_2SO_4, the solvent was removed under reduced pressure. Then 60 mg (64% yield) of the pure title compound was obtained. IR (neat): ν cm^{-1} 3580 (NH), 1711 (CO). ^1H NMR ($CDCl_3$): δ 7.60 (d, J = 16.0 Hz, 1H, CH=*CH*), 7.29 (s, 2H, aromatics), 6.75 (s, 2H, aromatics), 6.33 (d, J = 16.0 Hz 1H, *CH*=CH), 4.40 (t, J = 6.6 Hz, 2H, CH_2O), 4.29 (t, J = 6.2 Hz, 2H, CH_2O), 3.90 (s, 12H, $4OCH_3$), 3.88 (s, 6H, $2OCH_3$), 2.82−2.73 (m, 4H, $2CH_2N$), 2.01−1.87 (m, 4H, $2CH_2$), 1.65 (bs, 1H, NH) ppm. The oily product was transformed into the oxalate that was recrystallized from ethyl acetate. Mp: 172−174 °C. Anal. ($C_{30}H_{39}NO_{14}$) C, H, N.

Compounds **9**, **10**, and **19** were obtained in the same way by reaction of the corresponding *N*-t*ert*-butoxycarbonylamino ester. Compounds **9** and **19** were transformed into the oxalate and recrystallized from ethyl acetate, and compound **10** was transformed into the hydrochloride by treating the free base with HCl/abs EtOH and recrystallizing from EtOH/anhydrous diethyl ether. Their chemical and physical characteristics are reported in Table 1. IR and ^1H NMR spectra are reported in the Supporting Information.

3,4,5-Trimethoxybenzoic Acid 3-(4-Benzylpiperazin-1-yl)propyl Ester (61). To a solution of 0.5 g (1.5 mmol) of compound **60** in 1 mL of anhydrous CH_3CN were added 1-benzylpiperazine (0.41 mL, 2.3 mmol) and K_2CO_3 (0.2 g, 1.5 mmol). The mixture was heated at 60 °C for 6 h, cooled to room temperature, and treated with CH_2Cl_2. The organic layer was washed with 10% NaOH solution, and after drying with Na_2SO_4, the solvent was removed under reduced pressure and the residue purified by flash chromatography using CH_2Cl_2/abs EtOH/ethyl ether/petroleum ether/NH_4OH 360:180:360:900:9.9 as the eluting system. Title compound (0.45 g, 70% yield) was obtained as an oil. IR (neat): ν cm^{-1} 1714 (CO). ^1H NMR ($CDCl_3$): δ 7.29−7.25 (m, 7H, aromatics), 4.33 (t, J = 6.6 Hz, 2H, CH_2O), 3.87 (s, 9H, $3OCH_3$), 3.48 (s, 2H, CH_2Ph), 2.55−2.43 (m, 10H, $5CH_2N$), 2.03−1.89 (m, 2H, CH_2) ppm.

4-[3-[*trans*-3-(3,4,5-Trimethoxyphenyl)acryloyloxy]propy]piperazine-1-carboxylic Acid *tert*-Butyl Ester (62). Following the procedure described for compound **61**, starting from **34** (1.16 g, 3.2 mmol) and 1-BOC-piperazine[53] (0.74 g, 4.0 mmol), compound **62** (1.41 g, 94% yield) was obtained as a yellow oil. IR (neat): ν cm^{-1} 1714 (CO), 1693 (CO). ^1H NMR ($CDCl_3$): δ 7.49 (d, J = 15.9 Hz, 1H, CH=*CH*), 6.65 (s, 2H, aromatics), 6.24 (d, J = 15.9 Hz, 1H, *CH*=CH), 4.16 (t, J = 6.4 Hz, 2H, CH_2O), 3.78 (s, 6H, $2OCH_3$), 3.76 (s, 3H, OCH_3), 3.33 (t, J = 4.9 Hz, 4H, $2CH_2N$), 2.37 (t, J = 7.0 Hz, 2H, CH_2N), 2.32−2.29 (m, 4H, $2CH_2N$), 1.82−1.77 (m, 2H, CH_2), 1.35 (s, 9H, $3CH_3$) ppm.

Compound **63** was obtained in the same way from **35**. Its IR and ^1H NMR spectra are consistent with the proposed structure.

3,4,5-Trimethoxybenzoic Acid 3-[[2-(Benzylmethylamino)ethyl]methylamino]propyl Ester (69). Following the procedure described for compound **61**, starting from **60** (0.29 g, 0.87 mmol) and *N*-benzyl-*N*,*N*′-dimethylethane-1,2-diamine[54] (0.18 g, 1.0 mmol), compound **69** (0.19 g, 51% yield) was obtained as an oil. IR (neat) ν cm^{-1} 1714 (CO). ^1H NMR ($CDCl_3$): δ 7.29−7.24 (m, 7H, aromatics), 4.33 (t, J = 6.1 Hz, 2H, CH_2O), 3.90 (s, 9H, $3OCH_3$), 3.50 (s, 2H, CH_2Ph), 2.53−2.46 (m, 6H, $3CH_2N$), 2.23 (s, 3H, NCH_3), 2.20 (s, 3H, NCH_3), 1.96−1.89 (m, 2H, CH_2) ppm.

***trans*-3-(3,4,5-Trimethoxyphenyl)acrylic Acid 3-[[2-(*tert*-Butoxycarbonylmethylamino)ethyl]methylamino]-propyl Ester (70).** Following the procedure described for compound **61**, starting from **34** (1.5 g, 4.2 mmol) and 1-BOC-*N*,*N*′-dimethylethane-1,2-diamine[55] (0.74 mg, 4.2 mmol), compound **70** (1.0 g, 53% yield) was obtained as a brown oil. IR (neat): ν cm^{-1} 1714 (CO), 1693 (CO). ^1H NMR ($CDCl_3$): δ 7.50 (d, J = 15.9 Hz, 1H, CH=*CH*), 6.67 (s, 2H, aromatics), 6.26 (d, J = 15.9 Hz, 1H, *CH*=CH), 4.15 (t, J = 6.4 Hz, 2H, CH_2O), 3.79 (s, 6H, $2OCH_3$), 3.77 (s, 3H, OCH_3), 3.28−3.15 (m, 2H, CH_2N), 2.78 (s, 3H, NCH_3), 2.43−2.40 (m, 4H, $2CH_2N$), 2.18 (s, 3H, NCH_3), 1.80−1.74 (m, 2H, CH_2), 1.36 (s, 9H, $3CH_3$) ppm.

Compound **71** was obtained in the same way from **35**. Its IR and ^1H NMR spectra are consistent with the proposed structure.

3,4,5-Trimethoxybenzoic Acid 3-(piperazin-1-yl)propyl Ester (64). To 0.42 g (1.0 mmol) of **61** dissolved in 5 mL of anhydrous MeOH were added 0.21 g of Pd/C 10% and 0.31 g (4.9 mmol) of $HCOONH_4$. The mixture was refluxed for 5 h and then filtered, and the solvent was removed under reduced pressure. The residue was made alkaline with $NaHCO_3$, extracted with CH_2Cl_2, and dried. After removal of the solvent under reduced pressure, the residue was purified by flash chromatography using CH_2Cl_2/abs EtOH/petroleum ether/NH_4OH (340:65:60:8). Compound **64** (160 mg, 48% yield) was obtained as an oil. IR (neat): ν cm^{-1} 3340 (NH), 1713 (CO). ^1H NMR ($CDCl_3$): δ 7.24 (s, 2H, aromatics), 4.33 (t, J = 6.1 Hz, 2H, CH_2O), 3.86 (s, 9H, $3OCH_3$), 2.88−2.84 (m, 4H, $2CH_2N$), 2.48−2.40 (m, 6H, $3CH_2N$), 2.00−1.89 (m, 3H, CH_2 and NH) ppm.

Compound **72** was obtained in the same way from **69**. Its IR and ^1H NMR spectra are consistent with the proposed structure.

***trans* 3-(3,4,5-Trimethoxyphenyl)acrylic Acid 3-(piperazin-1-yl)propyl Ester (65).** To 1.41 g (3.03 mmol) of **62** dissolved in 5 mL of CH_2Cl_2 was added 6.06 mL of trifluoroacetic acid. After 2 h at room temperature, the solvent and the acid in excess were removed under reduced pressure. The

dark green oil obtained was dissolved in CH_2Cl_2 and washed with 10% NaOH solution. After drying of the organic phase with Na_2SO_4, the solvent was removed under reduced pressure. Compound **65** (1.0 g, 91% yield) was obtained as a yellow oil. IR (neat): ν cm^{-1} 3340 (NH), 1731 (CO). ^1H NMR ($CDCl_3$): δ 7.55 (d, J = 15.9 Hz, 1H, CH=*CH*), 6.71 (s, 2H, aromatics), 6.30 (d, J = 15.9 Hz, 1H, *CH*=CH), 4.21 (t, J = 6.4 Hz, 2H, CH_2O), 3.84 (s, 6H, $2OCH_3$), 3.83 (s, 3H, OCH_3), 2.87−2.83 (m, 4H, $2CH_2N$), 2.43−2.39 (m, 6H, $3CH_2N$), 2.09 (bs, 1H, NH), 1.88−1.84 (m, 2H, CH_2) ppm.

Compounds **66**, **73**, **74**, were obtained in the same way from **63**, **70**, and **71**, respectively. Their IR and ^1H NMR spectra are consistent with the proposed structures.

3,4,5-Trimethoxybenzoic Acid 3-(4-{3-[*trans*-3-(3,4,5-Trimethoxyphenyl)acryloyloxy]propyl}piperazin-1-yl)-propyl Ester (21). Following the procedure described for **61**, starting from **64** (135 mg, 0.40 mmol) and **34** (140 mg, 0.40 mmol), compound **21** (210 mg, 85% yield) was obtained as a light yellow oil. IR (neat): ν cm^{-1} 1713 (CO). ^1H NMR ($CDCl_3$): δ 7.61 (d, J = 15.7 Hz, 1H, CH=*CH*), 7.30 (s, 2H, aromatics), 6.76 (s, 2H, aromatics), 6.35 (d, J = 15.7 Hz, 1H, *CH*=CH), 4.38 (t, J = 6.1 Hz, 2H, CH_2O), 4.27 (t, J = 6.1 Hz, 2H, CH_2O), 3.90 (s, 12H, $4OCH_3$), 3.88 (s, 6H, $2OCH_3$), 2.65−2.38 (m, 12H, $6CH_2N$), 2.18−1.88 (m, 4H, $2CH_2$) ppm. The oily product was transformed into the oxalate that was recrystallized from ethyl acetate. Mp: 215−220 °C. Anal. ($C_{34}H_{46}N_2O_{14}$) C, H, N.

Compounds **22**−**24** and **27**−**30** were obtained in the same way. In particular, compound **22** was generated from **65** and **35**, **23** from **66** and **35**, **24** from **65** and **34**, **27** from **72** and **34**, **28** from **74** and **34**, **29** from **74** and **35**, and **30** from **73** and **34**. Compounds **22**, **27**, **28**, and **30** were transformed into the oxalate and recrystallized from ethyl acetate. Their chemical and physical characteristics are reported in Table 1. IR and ^1H NMR spectra are reported in the Supporting Information.

***trans*-3-(3,4,5-Trimethoxyphenyl)acrylic Acid 3-[4-(3-hydroxypropyl)piperazin-1-yl]propyl Ester (67).** To a solution of 0.4 g (1.1 mmol) of compound **65** in 1 mL of anhydrous CH_3CN were added 3-bromo-1-propanol (0.096 mL, 1.1 mmol) and K_2CO_3 (0.15 g, 1.1 mmol). The mixture was heated at 60°C for 4 h. The reaction mixture was cooled to room temperature and treated with CH_2Cl_2, and the organic layer was washed with 10% NaOH solution. After drying with Na_2SO_4, the solvent was removed under reduced pressure and the residue was purified by flash chromatography using CH_2Cl_2/abs EtOH/ethyl ether/petroleum ether/NH_4OH (360:180:360:900:9.9) as eluting system. Title compound (70 mg, 15% yield) was obtained as an oil. IR (neat): ν cm^{-1} 3340 (NH), 1731 (CO). ^1H NMR ($CDCl_3$): δ 7.59 (d, J = 15.8 Hz, 1H, CH=*CH*), 6.75 (s, 2H, aromatics), 6.33 (d, J = 15.8 Hz, 1H, *CH*=CH), 4.25 (t, J = 6.4 Hz, 2H, CH_2O), 3.89 (s, 6H, $2OCH_3$), 3.88 (s, 3H, OCH_3), 3.80 (t, J = 5.0 Hz, 2H, CH_2OH), 2.62−2.47 (m, 13H, $6CH_2N$ and OH), 1.93−1.86 (m, 2H, CH_2), 1.74−1.70 (m, 2H, CH_2) ppm.

Compounds **68**, **75**, and **76** were obtained in the same way from **66**, **73**, and **74**, respectively. Their IR and ^1H NMR spectra are consistent with the proposed structures.

Anthracene-9-carboxylic Acid 3-(4-{3-[4-Cyano-4-(3,4-dimethoxyphenyl)-5-methylhexanoyloxy]propyl}piperazin-1-yl)propyl Ester (25). 4-Cyano-4-(3,4-dimethoxyphenyl)-5-methylhexanoic acid[58] (110 mg, 0.36 mmol) was transformed into the acyl chloride by reaction with oxalyl chloride (0.06 mL, 0.72 mmol) in 5 mL of anhydrous C_6H_6, at 60 °C for 12 h. The reaction mixture was cooled to room temperature and the solvent removed under reduced pressure. The acyl chloride obtained was dissolved in $CHCl_3$ (EtOH-free), and compound **68** (150 mg, 0.36 mmol) was added. The reaction mixture was heated at 60 °C for 12 h, cooled to room temperature, and treated with CH_2Cl_2, and the organic layer was washed with 10% NaOH solution. After drying with Na_2SO_4, the solvent was removed under reduced pressure and the residue purified by flash chromatography using $CHCl_3$/MeOH/AcOH (85:10:0.5) as the eluting system. Title compound (80 mg, 33% yield) was obtained as an oil. IR (neat): ν cm^{-1} 2230 (CN), 1715 (CO). ^1H NMR ($CDCl_3$): δ 8.55 (s, 1H, aromatics), 8.05−8.03 (m, 4H, aromatics), 7.57−7.49 (m, 4H, aromatics), 6.94−6.79 (m, 3H, aromatics), 4.66 (t, J = 6.0 Hz, 2H, CH_2O), 4.08−3.93 (m, 2H, CH_2O), 3.90 (s, 3H, OCH_3), 3.88 (s, 3H, OCH_3), 2.84−2.45 (m, 12H, $6CH_2N$), 2.22−1.95 (m, 5H, $2CH_2$, *CH*(CH_3)$_2$), 1.88−1.65 (m, 4H, $2CH_2$) 1.22 (d, J = 6.2 Hz, 3H, CH*CH*$_3$), 0.80 (d, J = 6.2 Hz, 3H, CH*CH*$_3$) ppm. The oily product was transformed into the oxalate that was recrystallized from ethyl acetate. Mp: 223−226 °C. Anal. ($C_{43}H_{51}N_3O_{10}$) C, H, N.

Compound **31** was obtained in the same way from **76** and transformed into the oxalate that was recrystallized from ethyl acetate. Its chemical and physical characteristics are reported in Table 1 and IR and ^1H NMR spectra are reported in the Supporting Information.

9*H*-Fluorene-9-carboxylic Acid 3-(4-{3-[*trans*-3-(3,4,5-Trimethoxyphenyl)acryloyloxy]propyl}piperazin-1-yl)-propyl Ester (26). Following the procedure described for **25**, starting from **67** (70 mg, 0.16 mmol) and the acyl chloride obtained from 70 mg (0.33 mmol) of fluorencarboxylic acid and 0.5 mL (0.66 mmol) of $SOCl_2$ in dry C_6H_6, compound **26** (60 mg, 59% yield) was obtained as an oil. IR (neat): ν cm^{-1} 1713 (CO). ^1H NMR ($CDCl_3$): δ 7.76 (d, J = 7.5 Hz, 2H, aromatics), 7.66 (d, J = 7.5 Hz, 2H, aromatics), 7.61 (d, J = 15.8 Hz, 1H, CH=*CH*), 7.43 (t, J = 7.5 Hz, 2H, aromatics), 7.34 (t, J = 7.5 Hz, 2H, aromatics), 6.77 (s, 2H, aromatics), 6.36 (d, J = 15.8 Hz, 1H, *CH*=CH), 4.87 (s, 1H, CH), 4.27 (t, J = 6.4 Hz, 2H, CH_2O), 4.21 (t, J = 6.4 Hz, 2H, CH_2O), 3.89 (s, 6H, $2OCH_3$), 3.88 (s, 3H, OCH_3), 2.50−2.46 (m, 8H, $4CH_2N$), 2.38−2.34 (m, 4H, $2CH_2N$), 1.95−1.88 (m, 2H, CH_2), 1.87−1.80 (m, 2H, CH_2) ppm. The oily product was transformed into the oxalate that was recrystallized from ethyl acetate. Mp: 207−210 °C. Anal. ($C_{38}H_{44}N_2O_{11}$) C, H, N.

Compound **32** was obtained in the same way from **75** and transformed into the oxalate that was recrystallized from ethyl acetate. Its chemical and physical characteristics are reported in Table 1 and IR and ^1H NMR spectra are reported in the Supporting Information.

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

Cell Lines and Cultures. K 562 is a human leukemia cell line.[59] 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 mass of 180 000 Da.[60] 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 × 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 × 10^5 cells/mL. Cell viability was assessed by trypan blue exclusion. The cell number was determined by Coulter counter analysis.

Cellular Drug Accumulation. The uptake of pirarubicin cells was followed by monitoring the decrease in the fluorescence signal at 590 nm (λ_{ex} = 480 nm) according to the previously described method.[61] 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 pirarubicin.

Supporting Information Available: IR and ^1H NMR spectra and elemental analysis results of compounds **1**−**32.**

This material is available free of charge via the Internet at http://pubs.acs.org.

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JM050542X