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Structure−Activity Relationships Studies in a Series of *N*,*N*-Bis(alkanol)amine Aryl Esters as P-Glycoprotein (Pgp) Dependent Multidrug Resistance (MDR) Inhibitors†

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As a continuation of a previous research, a series of *N*,*N*-bis(alkanol)amine aryl esters, as Pgp-dependent MDR inhibitors, was designed and synthesized. The aromatic ester portions are suitably modulated, and new aryl rings (Ar_1 and Ar_2) were combined with *trans*-3-(3,4,5-trimethoxyphenyl)-vinyl, 3,4,5-trimethoxybenzyl and anthracene moieties that were present in the most potent previously studied compounds. The new compounds showed a wide range of potencies and efficacies on doxorubicin-resistant erythroleukemia K562 cells (K562/DOX) in the pirarubicin uptake assay. Selected compounds (**5**, **6**, **8**, **9**, and **21**) were further studied, evaluating their action on doxorubicin cytotoxicity potentiation on K562 cells; they significantly enhanced doxorubicin cytotoxicity on K562/DOX cells, confirming the results obtained with pirarubicin. Compound **9** shows the most promising properties as it was able to nearly completely reverse Pgp-dependent pirarubicin extrusion at nanomolar doses and increased the cytotoxicity of doxorubicin with a reversal fold (RF) of 19.1 at 3 μM dose.

Introduction

Drug transporter proteins are fundamental to the correct functioning of living organisms, being involved in the absorption, translocation, secretion, and excretion of a variety of endogenous and xenobiotic molecules. Because of their physiological role, these proteins can modulate pharmacokinetic and toxic properties of nutrients and drugs.[1]

The ABC superfamily of multidrug transporter proteins is one of the largest in living organisms, and its members are involved in several fundamental cellular processes.[2] ABCB1 (Pgp) is the most intensively studied member of the family since it has been found in several important tissues and blood−tissue barriers where, together with other family members, it seems to regulate the secretion of physiologically important lipophilic molecules[3] and the extrusion of xenobiotics that enter the organism.[4] Overexpression of Pgp and related proteins (mainly ABCC1 and ABCG2) that act as extrusion pumps, can induce classical multidrug resistance (MDR), a kind of acquired drug resistance of cancer cells and microorganisms to various chemotherapeutic drugs that usually are structurally and mechanistically unrelated.[5,6] Recently, the important role of ABC transporter proteins like Pgp in stem cells has been reported.[7]

In principle, modulation of the functions of Pgp and sister proteins would be one way of circumventing the appearance of MDR in cancer cells. This is the main reason prompting the design and synthesis of Pgp inhibitors,[8−12] although thus far, no drug of this class has been approved for cancer therapy.[13] However other potential uses of these agents are emerging such as that of enhancing drug penetration through biologically protective barriers, such as the blood−brain and blood−cerebrospinal fluid.[14,15] Recent evidence indicates that Pgp plays a role in the inhibition of viral infectivity of the human immunodeficiency virus (HIV).[16] Finally, the increasing interest in the functions and mechanism of action of Pgp and sister proteins requires the availability of new and potent molecules to be used as pharmacological tools.

Direct information on the structure of Pgp and related proteins is still incomplete,[17,18] but resolution of the structure of homologous bacterial transporters[19,20] has opened the way to the development of homology models[21−23] which provide useful details on the structure of the recognition site of ABC transporters. The information collected so far points to the existence of a large, polymorphous drug recognition domain where a variety of molecules can be accommodated in a plurality of binding modes throughout $\pi-\pi$, ion$-\pi$, hydrogen bond, and hydrophobic interactions.[24−26] These features have been generally confirmed by the recently described structure of mouse Pgp (ABCB1), which has 87% sequence identity to human Pgp.[27]

A few years ago, we described a new family of MDR reverters endowed with fairly good potency designed on the basis of a new concept. In brief, given the properties of the Pgp recognition site described above, we reasoned that flexible molecules carrying a basic nitrogen flanked, at suitable distances, by two aromatic moieties, would accommodate the recognition cavity choosing the most productive binding

†Dedicated to Prof. Fulvio Gualtieri on the occasion of his retirement.

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Chart 1. General Structures of Designed Compounds

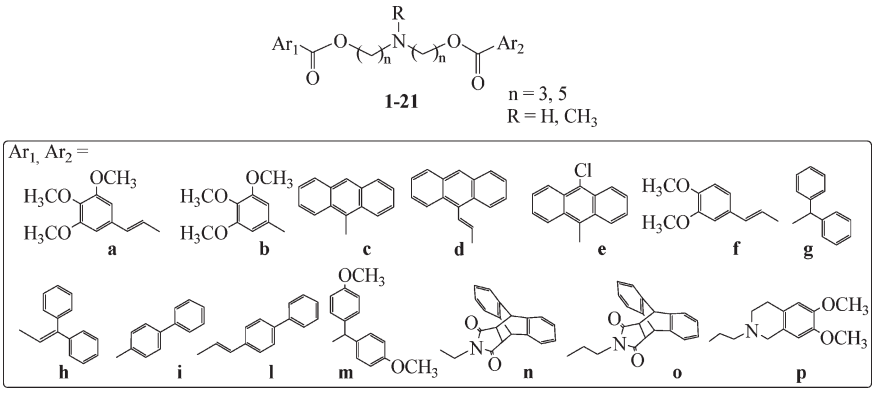
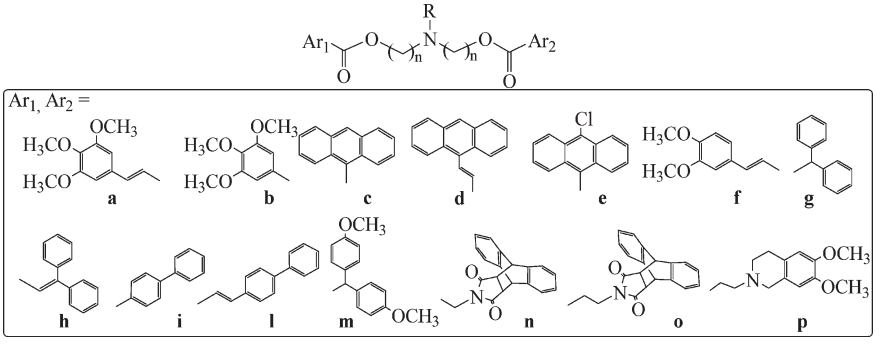


Table 1. MDR-Reverting Activity of Compounds **1**−**21**



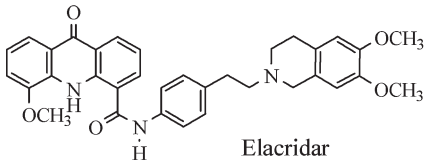
compd	R	*n*	Ar_1	Ar_2	$[I]_{0.5}$ μM[a]	α_{max}[b]
1[c]	CH_3	3	a	b	0.60 ± 0.15	0.90
2[c]	CH_3	3	a	c	0.18 ± 0.05	0.78
3	H	3	a	d	0.80 ± 0.10	0.92
4	CH_3	3	a	d	0.25 ± 0.04	0.93
5	CH_3	3	a	g	0.26 ± 0.06	0.77
6	CH_3	3	a	h	0.16 ± 0.03	0.66
7	CH_3	3	a	i	0.22 ± 0.02	0.77
8	CH_3	3	a	l	0.24 ± 0.05	0.85
9	CH_3	3	a	m	0.08 ± 0.01	0.79
10	CH_3	3	a	n	0.23 ± 0.03	0.73
11	CH_3	3	a	o	0.39 ± 0.10	0.63
12	CH_3	3	a	p	3.70 ± 1.40	0.63
13	CH_3	3	f	a	1.40 ± 0.30[d]	0.50
14	CH_3	3	f	b	0.70 ± 0.15[d]	0.34
15	CH_3	3	f	c	0.19 ± 0.03	0.57
16	CH_3	3	f	e	0.41 ± 0.08	0.98
17	CH_3	5	a	h	0.50 ± 0.09[d,e]	0.28
18	CH_3	5	a	l	0.36 ± 0.04[d,e]	0.40
19	CH_3	5	a	m	0.40 ± 0.10	0.50
20	CH_3	5	f	b	0.64 ± 0.13	0.70
21	CH_3	5	f	c	0.17 ± 0.02	1
MM36[f]					0.05 ± 0.01	0.70
verapamil					1.60 ± 0.30	0.70

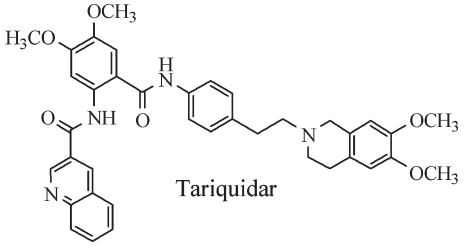
[a] Concentration of the inhibitor that causes a 50% increase in nuclear concentration of pirarubicin ($\alpha = 0.5$). [b] Efficacy of MDR-modulator and maximum increase that can be obtained in the nuclear concentration of pirarubicin in resistant cells. [c] See ref 28. [d] Concentration of the inhibitor that causes a 20% increase in nuclear concentration of pirarubicin ($\alpha = 0.2$). [e] This compound show a detergent-like effect at high concentrations causing permeabilization of the cell membrane. [f] See ref 30.

modes and therefore would interact with high affinity with protein. The good potencies of most of the compounds synthesized and studied confirmed our prediction that the entropy toll paid was compensated by the enthalpy gain due to the fact that these flexible molecules can optimize their interaction within the recognition site.[28]

Among the synthesized compounds, those containing a polymethylene chain linker displayed the best MDR-inhibitory activity. The length of the linker is important because MDR reverting potency generally increases with the number of methylenes, in most cases reaching the maximum for $n = 3$ and 5 (see Chart 1), as shown by compounds **1** and **2**[28]

Chart 2. Elacridar and Tariquidar Structures





reported for comparison in Table 1. In addition, the nature of the aromatic hydrophobic moieties is critical: as observed in previous works,[29−32] the presence of *trans*-3-(3,4,5-trimethoxyphenyl)vinyl or anthracene moieties is beneficial, as is the presence of the hydrogen-bond-acceptor methoxy groups on the aromatic rings.

In the present paper, we continue our structure−activity relationship studies in this series of molecules by describing several new analogues characterized by the *N*,*N*-bis(alkanol)-amine scaffold (n = 3, 5) with the aromatic ester portions suitably modulated (Chart 1). The new aryl rings (Ar_1 and Ar_2) were combined with *trans*-3-(3,4,5-trimethoxyphenyl)vinyl, 3,4,5-trimethoxybenzyl, and anthracene moieties (**a**, **b**, **c** structures of Chart 1) that were present in the most potent previously synthesized compounds.[28] We selected 10-chloroanthracene and *trans*-3-(3,4-dimethoxyphenyl)vinyl (**e**, **f**) to investigate the importance of the presence of chlorine and of the number of methoxy groups, respectively. Aromatic rings connected to the carbonyl function by a double bond (**d**, **h**, **l**) were chosen to exploit the previously found[28] positive influence on activity of the double bond present in the *trans*-3-(3,4,5-trimethoxyphenyl)vinyl group (**a**). We inserted aromatic groups with differently connected phenyl rings (**g**, **h**, **i**, **l**, **m**) to evaluate the effect of modulating flexibility also in the aromatic moieties. The two *cis*-9,10-dihydro-9,10-ethanoanthracene-11,12-dicarboximide derivative scaffolds (**n**, **o**) were chosen because they were present in compounds with reportedly potent MDR modulating activity,[33,34] as is the case of the 3-(6,7-dimethoxy-3,4-dihydroisoquinoline) propyl group (**p**) present in well-known Pgp blocking compounds such as Elacridar[35] and Tariquidar[36] (Chart 2).

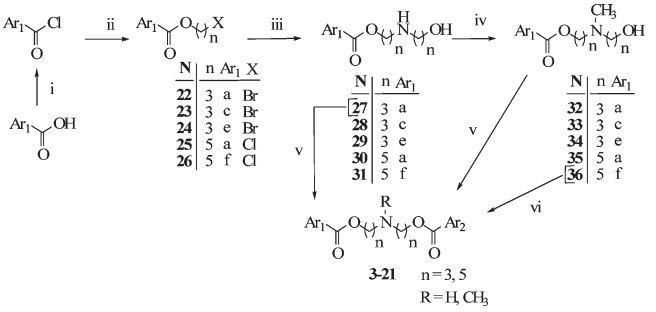
Chemistry

The reaction pathways used to synthesize the desired compounds (**3**−**21**) are described in Scheme 1 and their chemical and physical characteristics are reported in Table S1 (Supporting Information).

The haloesters **22**−**26**, obtained by esterification of the corresponding haloalkyl alcohols (3-bromopropan-1-ol or 5-chloropentan-1-ol) with *trans*-3-(3,4,5-trimethoxyphenyl)-acryloyl chloride, anthracene-9-carbonyl chloride, 10-chloroanthracene-9-carbonyl chloride, or *trans*-3-(3,4-dimethoxyphenyl)acryloyl chloride, were reacted with the commercially available aminoalcohols 3-aminopropan-1-ol and 5-aminopentan-1-ol to give **27**−**31**.

trans-3-(3,4,5-Trimethoxyphenyl)acryloyl chloride, anthracene-9-carbonyl chloride and *trans*-3-(3,4-dimethoxyphenyl)acryloyl chloride were obtained from the corresponding commercially available carboxylic acid by reaction with $SOCl_2$[a] in $CHCl_3$ (free of EtOH). 10-Chloroanthracene-9-carbonyl chloride was obtained by heating anthracene-9-carboxylic acid with an excess of $SOCl_2$. An approximately 40/60 mixture of 10-chloroanthracene-9-carbonyl chloride and anthracene-9-carbonyl chloride was obtained, which was used as such for the following reactions. Separation was performed on the final compound **16**.

Scheme 1. Synthesis of Compounds **3**−**21**[a]



[a] Reagents and conditions: (i) $SOCl_2$, $CHCl_3$ (free of EtOH); (ii) $CHCl_3$, $X(CH_2)_nOH$ (n = 3, 5; X = Br, Cl); (iii) K_2CO_3/CH_3CN, $H_2N(CH_2)_nOH$ (n = 3, 5); (iv) HCOOH/HCHO; (v) Ar_1COCl, $CHCl_3$; (vi) 3,4,5-trimethoxybenzoic acid, EDCI, DMAP; for the meaning of Ar_1 and Ar_2, see Table 1. Compounds **22**, **23**, **25**, **27**, **28**, **30**, **32**, **33**, **35** are described in ref 28.

The secondary amines **27**−**31** were alkylated by reductive methylation with HCOOH/HCHO to give the tertiary amines **32**−**36**. Compounds **4**−**19** and **21** were then obtained by reaction of **32**−**36** with the proper acyl chloride obtained from the corresponding carboxylic acid either commercially available (2,2-diphenylacetic acid, (*E*)-3-(biphenyl-4-yl)acrylic acid, biphenyl-4-carboxylic acid, 3,4,5-trimethoxybenzoic acid, anthracene-9-carboxylic acid, and *trans*-3-(3,4-dimethoxyphenyl)acrylic acid) or synthesized according to the literature (*trans*-(3-(anthracen-9-yl)acrylic acid,[37] 3,3-diphenylacrylic acid,[38] 3-(6,7-dimethoxy-3,4-dihydroisoquinolin-2(1*H*)-yl)propanoic acid,[39] 2,2-bis(4-methoxyphenyl)acetic acid,[40] *cis*-1,3,3a,4,9,9a-hexahydro-1,3-dioxo-4,9[1′,2′]-benzeno-2*H*-benz[*f*]isoindole-2-acetic acid,[41] *cis*-1,3,3a,4,9,9a-hexahydro-1,3-dioxo-4,9[1′,2′]-benzeno-2*H*-benz[*f*]isoindole-2-propanoic acid[41]).

Compound **3** was obtained by reaction of the secondary amine **27** with the acyl chloride obtained from *trans*-3-(anthracen-9-yl)acrylic acid.[37] Compounds **3** and **4** are a 50/50 mixture of *trans*-3-(3-(*trans*-3-(anthracen-9-yl)acryloyloxy)propylamino)propyl-3-(3,4,5-trimethoxyphenyl)acrylate and *trans*-3-(3-(*cis*-3-(anthracen-9-yl)acryloyloxy)propylamino)propyl-3-(3,4,5-trimethoxyphenyl)acrylate because, during reaction and purification of the final compounds, the 3-(anthracen-9-yl)acryloyl group isomerized.

[a] Abbreviations: $SOCl_2$, thionyl chloride; EDCI, 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride; DMAP, 4-dimethylaminopyridine; RF, reversal fold; MTT, 3-(4,5-dimethylthiazolyl-2)-2,5-diphenyltetrazolium bromide; abs EtOH, absolute ethanol; *t*-BOC, *t*-butylcarbamate; DOX, doxorubicin; DMSO, dimethylsulfoxide.

Compound **20** was obtained by reaction of **36** with 3,4,5-trimethoxybenzoic acid in the presence of 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDCl) and 4-dimethylaminopyridine (DMAP).

Pharmacological Studies

Modulation of Pirarubicin Uptake. The ability of compounds **3**−**21** to modulate Pgp action was evaluated on doxorubicin-resistant erythroleukemia K562 cells (K562/DOX) that, as reported in the literature, overexpress only Pgp.[42,43] K562 is a human leukemia cell line established from a patient with chronic myelogeneous leukemia in blast transformation.[44] K562/DOX cells resistant to doxorubicin express a unique membrane glycoprotein with a molecular mass of 180000 Da.[45] We measured the uptake of THP-adriamycin (pirarubicin) by 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.[30,31,46,47] Pgp-blocking activity is described by: (i) α, which represents the fold increase in the nuclear concentration of pirarubicin in the presence of the Pgp inhibitor 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 the Pgp inhibitor and is the maximum increase that can be obtained in the nuclear concentration of pirarubicin in resistant cells with a given compound, and (iii) $[I]_{0.5}$, which measures the potency of the inhibitor and represents the concentration that causes a half-maximal increase ($\alpha = 0.5$) in the nuclear concentration of pirarubicin (see Table 1). When it was impossible to measure $[I]_{0.5}$, the values $[I]_{<0.5}$ (for instance $[I]_{0.2}$) were reported.

We decided to make only functional tests excluding binding experiments that, in the case of transporter proteins like Pgp, provide limited information. In fact, given the properties of the recognition site discussed in the introduction, including the plurality of the binding modes, binding experiments will give information only on the binding site of the reference compound. The molecules studied lack any detectable cytotoxicity at the doses used in the test.

Cytotoxicity Test and MDR Reversal. The assessment of the ability of a compound to enhance the growth inhibitory effects of doxorubicin in tumor cell lines showing MDR due to Pgp overexpression, has also been proved useful in the quantification and characterization of MDR reversal by modulators of the MDR phenotype. The reversal effects of selected compounds (**5**, **6**, **8**, **9**, and **21**) on the MDR phenotype were investigated on doxorubicin-resistant erythroleukemia K562 cells (K562/DOX) overexpressing only Pgp.[42,43] Preliminarily, the compounds were evaluated for their intrinsic cytotoxicity on both K562 and K562/DOX cell lines by the MTT (3-(4,5-dimethylthiazolyl-2)-2,5-diphenyltetrazolium bromide) assay,[48] and then the MDR-reversal activity of studied compounds was tested using the same method in the presence of doxorubicin.

The IC_{50} doxorubicin concentrations resulting in 50% inhibition of cell growth compared to untreated control were calculated from plotted results. The reversal-fold (RF) values, a measure of MDR reversal, were calculated by dividing the doxorubicin IC_{50} values on K562/DOX cells in the absence of modulators by those on the same cells in the presence of modulators (see Table 2). Verapamil was used as reference compound.

Table 2. Effects of Studied MDR-Reversing Agents on Doxorubicin Cytotoxicity in K562 Cells and K562/DOX Cells

	K562	K562/DOX	
compd	IC_{50} (μM)[a]	IC_{50} (μM)[a]	RF[b]
DOX	0.049 ± 0.005	2.85 ± 0.26	
DOX + **5** (1 μM)	0.074	2.04 ± 0.11	1.4
DOX + **5** (3 μM)	0.085 ± 0.015	0.47 ± 0.16[c]	6.0
DOX + **6** (1 μM)	0.034	1.37 ± 0.15	2.1
DOX + **6** (3 μM)	0.032	0.34 ± 0.08[c]	8.3
DOX + **8** (1 μM)	0.028	1.79 ± 0.14	1.6
DOX + **8** (3 μM)	0.011	0.60 ± 0.01[c]	4.8
DOX + **9** (1 μM)	0.065 ± 0.001	0.69 ± 0.14[c]	4.2
DOX + **9** (3 μM)	0.040 ± 0.002	0.15 ± 0.026[c]	19.1
DOX + **21** (1 μM)	0.093	2.71 ± 0.09	1.1
DOX + **21** (3 μM)	0.055 ± 0.009	0.62 ± 0.04[c]	4.6
DOX + verapamil (1 μM)	0.035	2.21 ± 0.19	1.3
DOX + verapamil (3 μM)	0.029	0.74 ± 0.02[c]	3.9

[a] Mean ± SE of at least three determinations or mean of two determinations performed with quadruplicate cultures at each drug concentration tested and measured as described in Experimental Section. [b] Reversal fold of MDR was determined by dividing the doxorubicin IC_{50} values on K562/DOX cells in the absence of modulators by those in the presence of modulators. [c] $p < 0.001$ vs control doxorubicin-treated. Where not specified the IC_{50} value is not significant compared to control cells (K562 and K562/DOX cells treated with doxorubicin only).

Results

Modulation of Pirarubicin Uptake. The results obtained in doxorubicin-resistant erythroleukemia K562 cells are reported in Table 1, together with those of the two analogues **1**[28] and **2**,[28] MM36 (the most potent compound that we have found in previous studies[30]), and verapamil (the gold standard of Pgp inhibition) used as reference compounds.

All the new molecules studied are able to modulate the activity of Pgp and, with the exception of compound **12**, are more potent than the standard reference compound verapamil. Their potencies range from low nanomolar (compound **9**) to high nanomolar values (**6**, **15**, **21**), the remaining compounds being in the micromolar range. Some compounds (**3**, **4**, **16**, **21**) show nearly complete reversal of MDR (α_{max} close to 1), while a few show very low α_{max} (<0.5) and seem unable to achieve significant control of MDR. In these cases (**13**, **14**, **17**, **18**), the $[I]_{0.5}$ could not be evaluated and potency was measured at the lower value of α reported in Table 1. Compounds **17** and **18** had a detergent-like effect at high concentrations, causing permeabilization of the cell membrane that can be evidenced by trypan blue.

Effects of Compounds on Growth of K562/DOX and K562 Cells. The effects of compounds **5**, **6**, **8**, **9**, **21**, and verapamil on K562/DOX and K562 cell growth were evaluated. The compounds and verapamil had intrinsic toxicity in both cell lines, not exceeding 10% at the concentrations tested (1 and 3 μM) with the exception of compound **6**, which had 20% cell growth inhibition at both concentrations on sensitive cells and only at 3 μM on K562/DOX cells (data not shown).

Enhancement of Doxorubicin Potency. The effects of compounds **5**, **6**, **8**, **9**, **21**, and verapamil on doxorubicin-induced cytotoxicity on K562 and K562/DOX cells were examined after the cells were incubated at 37 °C for 72 h at 1 and 3 μM concentrations. When compounds **5**, **6**, **8**, **9**, and **21** were examined in combination with doxorubicin against doxorubicin-sensitive K562 cells at 1 and 3 μM, the sensitivity of cells to doxorubicin did not vary significantly.

As shown in Table 2, doxorubicin-induced cytotoxicity was not increased by compounds **5**, **8**, and **21** at 1 μM

concentration. In fact, the IC_{50} values of doxorubicin on K562/DOX were not significantly decreased ($p > 0.05$) in comparison to IC_{50} value of doxorubicin alone (RF = 1.4, 1.6, 1.1, respectively). On the other hand, compounds **6** and **9**, at the same concentration, were able to significantly reverse doxorubicin resistance in K562/DOX cells with a RF of 2.1 and 4.2, respectively. Compared to verapamil, all the compounds showed the poor reversal action of the standard, with the exception of compound **9**, which showed significantly higher reversal activity (RF = 4.2) than verapamil (RF = 1.3).

At the higher dose tested (3 μM) all compounds were able to significantly ($p < 0.001$) reverse doxorubicin resistance, their RF values ranging from 4.6 to 19.1, thus being more effective than verapamil (RF = 3.9). Compound **9** was the most potent at both doses and its RF at 3 μM (19.1) is definitely higher than that of verapamil at the same dose (3.9).

Our compounds appear to be chemically stable under the conditions of the biological tests. However we, at the moment, lack any information on their metabolic stability in the cell. This problem will be addressed in the future for the most potent and interesting compounds studied so far. In the meantime a research is in progress to find isosters of metabolically unstable ester function.

Discussion

As reported in the introduction, this new series of compounds was designed to further evaluate: (i) the role of the length of the tether, (ii) the role of the methoxy groups substituting the aromatic moieties, (iii) the role of the double bond present in the *trans*-3-(3,4,5-trimethoxyphenyl)acrylic acid, (iv) the effects of changing the connectivity and the conformational freedom of the two aromatic rings present in anthracene, and (v) the effect of introducing aromatic moieties that were present in some very potent Pgp inhibitors.

As regards the first point, we started studying the series of compounds with $n = 3$ and intended to go up to $n = 5$ or more, but the results obtained prompted us to change our plans. Unlike what was seen in a previous work,[28] going from $n = 3$ to $n = 5$ did not change activity too much while, in some cases, it reduced potency and efficacy (compare compounds **6**/**17**, **8**/**18**, and **9**/**19**). By also taking into consideration the previously reported compounds,[28] the length of the tether and the nature of the aryl moiety play a complex role that is not easy to explain. This seems to confirm that each molecule chooses the most productive binding mode in the recognition site of the Pgp protein.

Several potent Pgp inhibitors are characterized by methoxy-substituted aromatic rings.[8,9] In the present work, the positive influence of this substitution is confirmed, as can be seen by comparing compound **5** ($[I]_{0.5} = 0.26\ \mu M$; $\alpha_{max} = 0.77$) and the corresponding dimethoxyderivative **9** ($[I]_{0.5} = 0.08\ \mu M$; $\alpha_{max} = 0.79$) that is the most potent inhibitor of the series. On the other hand, the number of methoxy groups seems to be critical both for efficacy and potency, as can be seen by comparing compound **14** ($[I]_{0.2} = 0.70\ \mu M$; $\alpha_{max} = 0.34$) and compound **1** ($[I]_{0.5} = 0.60\ \mu M$; $\alpha_{max} = 0.90$).

Apparently, the presence of an arylvinyl moiety like that of cinnamic acid is beneficial for both potency and efficacy of the compounds of the series.[28] Here we have extended this feature to other aromatic groups such as the *trans*-(3-(anthracen-9-yl)acrylic (**d**) and (*E*)-3-(biphenyl-4-yl)acrylic (**l**) without much success, as can be seen by comparing the potency and efficacy of compounds **2** ($[I]_{0.5} = 0.18\ \mu M$; $\alpha_{max} = 0.78$) and **3** ($[I]_{0.5} = 0.80\ \mu M$; $\alpha_{max} = 0.92$), **7** ($[I]_{0.5} = 0.22\ \mu M$; $\alpha_{max} = 0.77$), and **8** ($[I]_{0.5} = 0.24\ \mu M$; $\alpha_{max} = 0.85$).

Changing the connectivity and the conformational freedom of the two phenyl rings of anthracene did not substantially modify activity, as shown by comparing compounds **2** ($[I]_{0.5} = 0.18\ \mu M$; $\alpha_{max} = 0.78$) and **5** ($[I]_{0.5} = 0.26\ \mu M$; $\alpha_{max} = 0.77$), **6** ($[I]_{0.5} = 0.16\ \mu M$; $\alpha_{max} = 0.66$), **7** ($[I]_{0.5} = 0.22\ \mu M$; $\alpha_{max} = 0.77$), and **8** ($[I]_{0.5} = 0.24\ \mu M$; $\alpha_{max} = 0.85$). However, combining this modification with the insertion of methoxy groups gave compound **9** that, as mentioned above, is the most potent compound of the series, including previously studied members.[28]

The insertion of aromatic moieties that are present in other potent Pgp inhibitors[33−36] gave mixed results, producing compounds with an activity comparable to that of the other members of the series such as **10** ($[I]_{0.5} = 0.23\ \mu M$; $\alpha_{max} = 0.73$) and **11** ($[I]_{0.5} = 0.39\ \mu M$; $\alpha_{max} = 0.63$), but also a compound such as **12** ($[I]_{0.5} = 3.70\ \mu M$; $\alpha_{max} = 0.63$), which is less potent than verapamil. Apparently, the contribution of these groups to binding at the recognition site cannot manifest completely when inserted in the general structure of the series. Once again, this seems to confirm our hypothesis that each single molecule chooses the most productive binding mode in the recognition site of the Pgp protein.

The results of enhancement of doxorubicin potency for compounds **5**, **6**, **8**, **9**, and **21**, confirmed the pharmacological results obtained with pirarubicin. All compounds significantly enhanced doxorubicin cytotoxicity on K562/DOX cells. Compounds **8** and **21**, at 3 μM, were slightly more active than verapamil (RF = 4.8, 4.6, and 3.9, respectively). At the same dose, compounds **5**, **6**, and **9** displayed definitely higher reversal folds than verapamil with an RF of 6.0, 8.3, and 19.1 respectively. Alone, all compounds weakly inhibited cell growth (10%) except compound **6** with 20% cell growth inhibition. These results indicate that compounds **5**, **8**, **9**, and **21** inhibit Pgp, the protein most thoroughly studied for its ability to confer the MDR phenotype in vitro and in vivo, but it is possible that the effects on doxorubicin cytotoxicity observed for compound **6** may be partially unspecific.

In conclusion, we have better defined the structure−activity relationships of this new group of Pgp inhibitors and identified a new compound, **9**, with nanomolar potency and good efficacy, which can be useful as a lead for the development of new inhibitors of 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 Spectrum RX I FT-IR spectrophotometer in Nujol mull for solids and neat for liquids. NMR spectra were recorded on 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. All reported compounds had a purity of at least 95%. 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 2000 (4.01.305)

or CA Index Name. When reactions were performed in anhydrous conditions, the mixtures were maintained under nitrogen.

General Procedure for the Synthesis of Haloesters (22−26). A 1 mmol portion of the appropriate carboxylic acid (*trans*-3-(3,4,5-trimethoxyphenyl)acrylic acid, anthracene-9-carboxylic acid, *trans*-3-(3,4-dimethoxyphenyl)acrylic acid) was transformed into the acyl chloride by reaction with $SOCl_2$ (2 mmol), in 5 mL of $CHCl_3$ (free of EtOH), at 60 °C for 4−8 h. 10-Chloroanthracene-9-carbonyl chloride was obtained by reaction of anthracene-9-carboxylic acid with $SOCl_2$ at 80 °C for 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 (3-bromopropan-1-ol or 5-chloropentan-1-ol) (1 equiv) was added. The reaction mixture was heated to 60 °C for 4−8 h and then cooled to room temperature, 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 3-bromopropyl ester **22** are reported as an example. IR (nujol) ν cm^{-1} 1714 (CO). ^{1}H NMR ($CDCl_3$) δ 2.21−2.23 (m, 2H, CH_2), 3.52 (t, 2H, CH_2Br, J = 6.8 Hz), 3.88 (s, 3H, OCH_3), 3.89 (s, 6H, 2 OCH_3), 4.34 (t, 2H, CH_2OCO, J = 6.0 Hz), 6.34 (d, 1H, CH=CH, J = 15.6 Hz), 6.75 (s, 2H, CH aromatics), 7.60 (d, 1H, CH=CH, J = 15.6 Hz) ppm.

General Procedure for the Synthesis of Hydroxyaminoesters (27−31). The appropriate haloester (**22−26**) (1 mmol) and the suitable aminoalkylalcohol (3-aminopropan-1-ol or 5-aminopentan-1-ol) (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, 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 CH_2Cl_2/abs EtOH/petroleum ether/NH_4OH (340:65:60:8) (compounds **27** and **31**), $CHCl_3$/MeOH (95:5) (compounds **28** and **29**), or CH_2Cl_2/MeOH/NH_4OH (98:2:0.2) (compound **30**) as 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 3-(3-hydroxypropylamino)propyl ester (**27**) are reported as an example. IR (neat) ν cm^{-1} 3300 (OH + NH), 1709 (CO). ^{1}H NMR ($CDCl_3$) δ 1.68−1.75 (m, 2H, CH_2), 1.84−1.95 (m, 2H, CH_2), 2.40 (bs, NH + OH), 2.75 (t, 2H, CH_2N, J = 7.0 Hz), 2.89 (t, 2H, CH_2N, J = 6.0 Hz), 3.81(t, 2H, CH_2OH, J = 5.2 Hz), 3.87 (s, 3H, OCH_3), 3.89 (s, 6H, 2 OCH_3), 4.27 (t, 2H, CH_2OCO, J = 6.4 Hz), 6.34 (d, 1H, CH=CH, J = 16.0 Hz), 6.75 (s, 2H, CH aromatics), 7.60 (d, 1H, CH=CH, J = 16.0 Hz) ppm.

General Procedure for the Synthesis of Methylhydroxyaminoesters (32−36). A 1 mmol portion of the appropriate hydroxyaminoester (**27−31**) was dissolved in 15 mL of anhydrous ethanol and HCOOH (17 mmol) and 37% HCHO solution (5 mmol) were added. The mixture was heated to 80 °C for 2−9 h and concentrated in vacuo. The residue was then dissolved in 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. Yields 95−100%. Their IR and ^{1}H NMR spectra were consistent with the proposed structures.

The spectra of *trans*-3-(3,4,5-trimethoxyphenyl)acrylic acid 3-[(3-hydroxypropyl)methylamino]-propyl ester (**32**) are reported as an example. IR (neat) ν cm^{-1} 1710 (CO). ^{1}H NMR ($CDCl_3$) δ 1.68−1.74 (m, 2H, CH_2), 1.87−1.94 (m, 2H, CH_2), 2.23 (s, 3H, NCH_3), 2.50 (t, 2H, CH_2N, J = 7.2 Hz), 2.61 (t, 2H, CH_2N, J = 6.0 Hz), 3.80 (t, 2H, CH_2OH, J = 5.2 Hz), 3.87 (s, 3H, OCH_3), 3.88 (s, 6H, 2 OCH_3), 4.24 (t, 2H, CH_2OCO, J = 6.4 Hz), 6.34 (d, 1H, CH=CH, J = 16.0 Hz), 6.75 (s, 2H, CH aromatics), 7.60 (d, 1H, CH=CH, J = 16.0 Hz) ppm.

***trans*-3-(3,4,5-Trimethoxyphenyl)acrylic Acid 3-[3-(3-Anthracen-9-yl-acryloyloxy)propylamino]-propyl Ester (3).** Following the general procedure described above for the general synthesis of esters, the acyl chloride, obtained from *trans*-3-(anthracen-9-yl)acrylic acid[37] (375 mg, 1.76 mmol), was allowed to react with **27** (300 mg, 0.88 mmol). The crude product was then purified by flash chromatography using $CHCl_3$/CH_3OH/NH_4OH (95:5:0.5) as eluting system. By ^{1}H NMR analysis, the product results a 50/50 mixture of *trans*/*trans* (a) and *trans*/*cis* (b) isomers.

The title compound (160 mg, 37% yield) was obtained as an oil. IR (neat) ν cm^{-1} 1714 (CO). ^{1}H NMR ($CDCl_3$) δ 1.00−1.10 (m, 2H, CH_2, b isomer), 1.67−1.76 (m, 4H, 2 CH_2N, b isomer), 1.90−2.15 (m, 4H, CH_2, a isomer), 2.35 (t, 2H, J = 6.8 Hz, b isomer); 2.75−2.85 (m, 4H, CH_2, a isomer), 3.71 (t, 2H, J = 6.0 Hz, b isomer), 3.80−3.84 (m, 18H, 6 OCH_3), 4.17 (t, 2H, CH_2O, J = 6.0 Hz, b isomer), 4.30 (t, 2H, CH_2O, J = 6.4 Hz, a isomer), 4.41 (t, 2H, CH_2O, J = 6.4 Hz, a isomer), 6.32 (d, 1H, CH=CH, J = 16.0 Hz, a isomer), 6.35 (d, 1H, CH=CH, J = 16.0 Hz, b isomer), 6.42 (d, 1H, CH=CH, J = 16.0 Hz, a isomer), 6.61 (d, 1H, CH=CH, J = 12.0 Hz, b isomer), 6.71 (s, 2H, CH aromatics, a isomer), 6.75 (s, 2H, CH aromatics, b isomer), 7.42−7.53 (m, 8H), 7.57 (d, 1H, CH=CH, J = 16.0 Hz, a isomer), 7.60 (d, 1H, CH=CH, J = 16.0 Hz, b isomer), 7.75 (d, 1H, CH=CH, J = 12.0 Hz, b isomer), 7.95−8.05 (m, 6H, CH aromatics), 8.22 (d, 2H, CH aromatics, J = 8.4 Hz, a isomer), 8.41 (s, 1H, CH aromatic, b isomer), 8.44 (s, 1H, CH aromatic, a isomer), 8.64 (d, 1H, CH=CH, J = 16.0 Hz, a isomer) ppm. Compound **3** was tested as a free base. Anal. ($C_{35}H_{37}NO_7$) C, H, N.

Compound **4** was obtained in the same way by reaction of **32** with *trans*-3-(anthracen-9-yl)acrylic acid.[37] The title compound (160 mg, 36% yield) was obtained as an oil and resulted in a 50/50 mixture of *trans*/*trans* (a) and *trans*/*cis* (b) isomers. IR (neat) ν cm^{-1} 1710 (CO). ^{1}H NMR ($CDCl_3$) δ 0.93−1.00 (m, 2H, CH_2, b isomer), 1.47 (t, 2H, CH_2N, J = 7.2 Hz, b isomer), 1.55−1.63 (m, 2H, CH_2, b isomer), 1.87 (s, 3H, NCH_3, b isomer), 1.89−1.98 (m, 4H, CH_2, a isomer), 2.07 (t, 2H, CH_2N, J = 7.2 Hz, b isomer), 2.28 (s, 3H, NCH_3, a isomer), 2.49−2.56 (m, 4H, CH_2N, a isomer), 3.68 (t, 2H, CH_2OCO, J = 6.4 Hz, b isomer), 3.83−3.88 (m, 18H, 6 OCH_3), 4.11 (t, 2H, CH_2OCO, J = 6.4 Hz, b isomer), 4.28 (t, 2H, CH_2OCO, J = 6.4 Hz, a isomer), 4.39 (t, 2H, CH_2OCO, J = 6.4 Hz, a isomer), 6.30 (d, 1H, CH=CH, J = 16.0 Hz, a isomer), 6.33 (d, 1H, CH=CH, J = 16.0 Hz, b isomer), 6.43 (d, 1H, CH=CH, J = 16.0 Hz, a isomer), 6.61 (d, 1H, CH=CH, J = 12.0 Hz, b isomer), 6.68 (s, 2H, CH aromatics, a isomer), 6.75 (s, 2H, CH aromatics, b isomer), 7.43−7.50 (m, 10H), 7.59 (d, 1H, CH=CH, J = 12.0 Hz, b isomer), 7.98−8.04 (m, 6H, CH aromatics), 8.22 (d, 2H, CH aromatics, J = 8.4 Hz, a isomer), 8.40 (s, 1H, CH aromatic, b isomer), 8.42 (s, 1H, CH aromatic, a isomer), 8.63 (d, 1H, CH=CH, J = 16.0 Hz, a isomer) ppm. Compound **4** was tested as a free base. Anal. ($C_{36}H_{39}NO_7$) C, H, N.

Compounds **5−12**, **15−19**, and **21** were obtained in the same way by reaction of the corresponding alcohol with the suitable acyl chloride. Compounds **5−12** and **21** were transformed into the oxalate and recrystallized from ethyl acetate, and compounds **13**, **14**, and **17−19** were transformed into the hydrochloride and recrystallized from the solvent reported in Table S1 (Supporting Information). Compounds **15** and **16** were tested as free bases. Their chemical and physical characteristics are reported in Table S1 (Supporting Information), and IR, ^{1}H NMR, and ^{13}C NMR spectra are reported in Tables S2 and S3 (Supporting Information).

3,4,5-Trimethoxybenzoic Acid 5-({5-[*trans*-3-(3,4-Dimethoxyphenyl)acryloyloxy]pentyl}-methylamino)pentyl ester (20). A solution of compound **36** (50 mg, 0.127 mmol) in 5 mL of CH_2Cl_2 was cooled at 0 °C, and 38 mg (0.178 mmol) of 3,4,5-trimethoxybenzoic acid, 5 mg (0.043 mmol) of 4-dimethylaminopiridine

(DMAP), and 49 mg (0.25 mmol) of 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDCl) were added. The reaction mixture was stirred for 1 h at 0 °C and 72 h at room temperature. Then CH_2Cl_2 was added, and the organic layer was washed twice with 0.1 N HCl and twice with a saturated solution of $NaHCO_3$. After drying with Na_2SO_4, the solvent was removed under reduced pressure. The crude product was then purified by flash chromatography using NH_4OH/abs EtOH/CH_2Cl_2/ethyl ether/petroleum ether (2.5:45:180:180:450) as eluting system.

The title compound (160 mg, 54% yield) was obtained as an oil. IR (neat) ν cm^{-1} 1713 (CO). ^1H NMR ($CDCl_3$) δ 1.66−1.81 (m, 12H, 6CH_2), 2.25 (s, 3H, NCH_3), 2.38−2.41 (m, 4H, 2 NCH_2), 3.87 (m, 15H, 5 OCH_3), 4.21 (t, 2H, CH_2O, J = 6.8 Hz), 4.33 (t, 2H, CH_2O, J = 6.8 Hz), 6.32 (d, 1H, CH═CH, J = 16.0 Hz), 6.87 (d, 1H, CH aromatic, J = 8.4 Hz), 7.07−7.15 (m, 2H, CH aromatics), 7.29 (s, 2H, CH aromatics), 7.64 (d, 1H, CH═CH, J = 16.0 Hz) ppm. ^{13}C NMR ($CDCl_3$) δ 23.98 (CH_2), 26.77 (CH_2), 26.80 (CH_2), 28.73 (CH_2), 42.09 (NCH_3), 55.90 (OCH_3), 55.96 (OCH_3), 56.26 (OCH_3), 57.60 (CH_2), 60.89 (OCH_3), 64.37 (CH_2), 65.09 (CH_2), 106.90 (CH), 109.71 (CH), 111.10 (CH), 115.91 (CH), 122.57 (CH), 125.47 (C), 127.46 (C), 144.55 (CH), 149.26 (C), 151.14 (C), 152.94 (C), 166.24 (C), 167.26 (C) ppm. Compound **20** was tested as a free base. Anal. ($C_{32}H_{47}NO_9$) C, H, N.

Compounds **13** and **14** were obtained in the same way by reaction of the corresponding alcohol with the suitable carboxylic acid, transformed into the hydrochloride, and recrystallized from the solvent reported in Table S1 (Supporting Information). Their chemical and physical characteristics are reported in Table S1, and IR, ^1H NMR, and ^{13}C NMR spectra are reported in Tables S2 and S3 (Supporting Information).

Pharmacology. Cell Lines and Cultures. The K562 cell line is a highly undifferentiated erythroleukemia originally derived from a patient with chronic myelogenous leukemia.[44] The K562 leukemia cells and the Pgp expressing K562/DOX cells were obtained from Prof. J. P. Marie (Hopital Hotel-Dieu, Paris, France). These cells were cultured in RPMI 1640 medium with GlutaMAX I (GIBCO) medium supplemented with 10% fetal calf serum (FCS) (GIBCO) at 37 °C in a humidified incubator with 5% CO_2. To maintain the resistance, every month, resistant cells were cultured for three days with 400 nM doxorubicin. The cell line was then used, one week later, during three weeks. Cultures initiated at a density of 10^5 cells/mL grew exponentially to about 10^6 cells/mL in 3 days. For the spectrofluorometric assays, in order 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. Cultured cells were counted with a Coulter counter before use. The viability of the cells, tested by Trypan Blue exclusion, was always greater than 95%.

Modulation of Pirarubucin Uptake. 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 ε_{480} = 11500 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 7.3.

Cellular Drug Accumulation. The uptake of pirarubicin in cells was followed by monitoring the decrease in the fluorescence signal at 590 nm (λ_{ex} = 480 nm) according to the previously described method.[49] 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.

Cytotoxicity Assay. Drugs and Chemicals. Doxorubicin hydrochloride (DOX) and verapamil were obtained from Sigma; dimethylsulfoxide (DMSO) and 3-(4,5-dimethylthiazolyl-2)-2,5-diphenyltetrazolium bromide (MTT) were purchased from Sigma. MTT stock solution was prepared as follows: 5 mg MTT/mL phosphate saline buffer (PBS) was filtered with 0.45 μm filter units (Nalgene) and stored at 4 °C for a maximum of 1 month. MTT working solution was prepared just before to culture application by diluting MTT stock solution 1:5 in prewarmed culture medium and 50 μL were added to each culture well, resulting in 0.25 mg MTT/200 μL total medium volume.

Test compounds and DOX stock solutions were prepared in DMSO at 10^{-2} M. Verapamil was prepared in water at 10^{-2} M. Drugs and test compounds were then diluted with complete medium to obtain the 10× desired final maximum test concentrations. Test compounds were evaluated for cytotoxicity and MDR reversal activity at 1 and 3 μM, and the corresponding doxorubicin concentrations tested were between 0.001 and 0.1 μM for K562 sensitive cells line and between 0.1 and 10 μM for K562/DOX resistant cells.

Verapamil was used as a standard chemomodulator and was evaluated at 1 and 3 μM. All experiments were carried out in quadruplicate.

MTT Assay. Cells, in exponential growth phase ($3-5 \times 10^5$ cells/mL), were seeded at 3000 cells/well, and either solutions of test compounds or solution of doxorubicin or combination of a solution of doxorubicin and test compounds were added to the wells and the plates were incubated at 37 °C for 72 h in 5% CO_2 incubator. Culture plates were centrifuged at low speed for 5 min, 50 μL of medium was removed from wells and replaced with 50 μL of MTT working solution, and plates were further incubated for 4 h. Following incubation, cells and formazan crystals were inspected microscopically. The supernatant was then carefully removed by slow aspiration and the formazan crystals were dissolved in 150 μL of DMSO; the absorbance of the solution was then read on an automated plate reader at a wavelength of 540 nm.

All results are presented as means ± SE and statistical analysis was performed using the one-way Anova test and Bonferroni's multiple comparison test (GraphPad Prism software, Inc. CA).

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Supporting Information Available: Chemical and physical characteristics, IR, ^1H NMR, and ^{13}C NMR spectra, and elemental analyses of compounds **3**−**21**. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Ito, K.; Suzuki, H.; Horie, T.; Sugiyama, Y. Apical/basolateral surface expression of drug transporters and its role in vectorial drug transport. *Pharm. Res.* **2005**, *22*, 1559–1577.
(2) Linton, K. J. Structure and function of ABC transporters. *Physiology* **2007**, *22*, 122–130.
(3) Kuhnke, D.; Jedlitschky, G.; Grube, M.; Krohn, M.; Juker, M.; Mosyagin, I.; Cascorbi, I.; Walker, L. C.; Kroemer, H. K.; Warzog, R. W.; Vogelgesang, S. MDR1-P-Glycoprotein (ABCB1) mediates transport of Alzheimer's amyloid-beta peptides—implications for the mechamism of Abeta clearance at the blood−brain barrier. *Brain Pathol.* **2007**, *17*, 347–353.
(4) Johnstone, R. W.; Ruefli, A. A.; Smyth, M. J. Multiple physiological functions for multidrug transporter P-glycoprotein? *Trends Biochem. Sci.* **2000**, *25*, 1–6.
(5) Mitscher, L. A.; Pillai, S. P.; Gentry, E. J.; Shankel, D. M. Multiple drug resistance. *Med. Res. Rev.* **1999**, *19*, 477–496. Volm, M.; Mattern, J. Resistance mechanisms and their regulation in lung cancer. *Crit. Rev. Oncog.* **1996**, *7*, 227–244.
(6) Aszalos, A.; Ross, D. D. Biochemical and clinical aspects of efflux pump related resistance to anti-cancer drugs. *Anticancer Res.* **1998**, *18*, 2937–2944.
(7) Dean, M. ABC Transporters, Drug Resistance, and Cancer Stem cells. *J. Mammary Gland Biol. Neoplasia* **2009**, *14*, 3–9.

(8) Teodori, E.; Dei, S.; Martelli, C.; Scapecchi, S.; Gualtieri, F. The functions and structure of ABC transporters: implications for the design of new inhibitors of Pgp and MRP1 to control multidrug resistance (MDR). *Curr. Drug Targets* **2006**, *7*, 893–909.

(9) Avendano, C.; Menendez, J. C. Recent advances in multidrug resistance modulators. *Med. Chem. Rev.−Online* **2004**, *1*, 419–444.

(10) Robert, J.; Jarry, C. Multidrug resistance reversal agents. *J. Med. Chem.* **2003**, *46*, 4805–4817.

(11) Nobili, S.; Landini, I.; Giglioni, B.; Mini, E. Pharmacological strategies for overcoming multidrug resistance. *Curr. Drug Targets* **2006**, *7*, 861–879.

(12) Fusi, F.; Saponara, S.; Valoti, M.; Dragoni, S.; D'Elia, P.; Sgaragli, T.; Alderighi, D.; Kawase, M.; Shah, A.; Motohashi, N.; Sgaragli, G. Cancer cell permeability-glycoprotein as a target of MDR reverters: possible role of novel dihydropyridine derivatives. *Curr. Drug Targets* **2006**, *8*, 949–959.

(13) Sorbera, L. A.; Castaner, J.; Silvestre, J. S.; Bayés, M. Zosuquidar trihydrochloride. *Drugs Future* **2003**, *28*, 125–136.

(14) Tan, B.; Piwnica-Worms, D.; Ratner, L. Multidrug resistance transporters and modulation. *Curr. Opin. Oncol.* **2000**, *12*, 450–458.

(15) Robey, R. W.; Lazarowski, A.; bates, S. E. P-Glycoprotein—a clinical target in drug-refractory epilepsy? *Mol. Pharmacol.* **2008**, *73*, 1343–1346.

(16) Owen, A.; Chandler, B.; Back, D. J. The implications of P-glycoprotein in HIV: friend or foe? *Fund. Clin. Pharmacol.* **2005**, *19*, 283–296.

(17) Rosenberg, M. F.; Kamis, A. B.; Callaghan, R.; Higgins, C. F.; Ford, R. C. Three dimensional structures of the mammalian multidrug-resistant P-glycoprotein demonstrate major conformational changes in the transmembrane domain upon nucleotide binding. *J. Biol. Chem.* **2003**, *278*, 8294–8299.

(18) Rosenberg, M. F.; Callaghan, R.; Modok, S.; Higgins, C. F.; Ford, R. C. Three-dimensional structure of P-glycoprotein. *J. Biol. Chem.* **2005**, *280*, 2857–2862.

(19) Ward, A.; Reyes, C. L.; Yu, J.; Roth, C. B.; Chang, G. Flexibility in the ABC transporter MsbA: alternating access with a twist. *Proc. Natl. Acad. Sci. U.S.A.* **2007**, *104*, 19005–19010.

(20) Dawson, R. J. P.; Locher, K. P. Structure of the multidrug ABC transporter Sav1866 from *Staphylococcus aureus* in complex with AMP-PNP. *FEBS Lett.* **2007**, *581*, 935–938.

(21) Zolnerciks, J. K.; Wooding, C.; Linton, K. J. Evidence for a Sav1866-like architecture for the human multidrug transporter P-glycoprotein. *FASEB J.* **2007**, *21*, 3937–3948.

(22) O'Mara, M. L.; Tieleman, D. P. P-Glycoprotein models of the apo and ATP-bound states based on homology with Sav1866 and Ma1K. *FEBS Lett.* **2007**, *581*, 4217–4222.

(23) Globish, C.; Pajeva, I. K.; Wiese, M. Identification of putative binding sits of P-glycoprotein based on its homology models. *ChemMedChem* **2008**, *3*, 280–295.

(24) Gottesman, M. M.; Fojo, T.; Bates, S. E. Multidrug resistance in cancer: role of ATP-dependent transporters. *Nature Rev. Cancer* **2002**, *2*, 48–58.

(25) Murray, D. S.; Schumacher, M. A.; Brennan, R. G. Crystal structure of QacR-diamidine complexes reveal additional multidrug-binding modes and a novel mechanism of drug charge neutralization. *J. Biol. Chem.* **2004**, *279*, 14365–14371.

(26) Schumacher, M. A.; Miller, M. C.; Brennan, R. G. Structural mechanism of the simultaneous binding of two drugs to a multidrug binding protein. *EMBO J.* **2004**, *23*, 2923–2930.

(27) Aller, S. G.; Yu, J.; Ward, A.; Weng, Y.; Chittaboina, S.; Zhuo, R.; Harrell, P. M.; Trinh, Y. T.; Zhang, Q.; Urbatsch, I. L.; Chang, G. Structure of P-glycoprotein reveals a molecular basis for poly-specific drug binding. *Science* **2009**, *323*, 1718–1722.

(28) Teodori, E.; Dei, S.; Garnier-Suillerot, A.; Gualtieri, F.; Manetti, D.; Martelli, C.; Romanelli, M. N.; Scapecchi, S.; Paiwan, S.; Salerno, M. Exploratory chemistry toward the identification of a new class of MDR reverters inspired by pervilleine and verapamil models. *J. Med. Chem.* **2005**, *48*, 7426–7436.

(29) Teodori, E.; Martelli, C.; Salerno, M.; Darghal, N.; Dei, S.; Garnier-Suillerot, A.; Gualtieri, F.; Manetti, D.; Scapecchi, S.; Romanelli, M. N. Isomeric *N*,*N*-bis(cyclohexanol)amine aryl esters: the discovery of a new class of highly potent Pgp-dependent multidrug resistance (MDR) inhibitors. *J. Med. Chem.* **2007**, *50*, 599–602.

(30) Teodori, E.; Dei, S.; Quidu, P.; Budriesi, R.; Chiarini, A.; Garnier-Suillerot, A.; Gualtieri, F.; Manetti, D.; Romanelli, M. N.; Scapecchi, S. 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. *J. Med. Chem.* **1999**, *42*, 1687–1697.

(31) Dei, S.; Teodori, E.; Garnier-Suillerot, A.; Gualtieri, F.; Scapecchi, S.; Budriesi, R.; Chiarini, A. Structure−activity relationships and optimization of the selective MDR modulator 2-(3,4-dimethoxyphenyl)-5-(9-fluorenylamino)-2-(methylethyl) pentanenitrile (SC11) and its *N*-methyl derivative (SC17). *Bioorg. Med. Chem.* **2001**, *9*, 2673–2682.

(32) Martelli, C.; Alderighi, D.; Coronnello, M.; Dei, S.; Frosini, M.; Le Bozec, B.; Manetti, D.; Neri, A.; Romanelli, M. N.; Salerno, M.; Scapecchi, S.; Mini, E.; Sgaragli, G.; Teodori, E. *N*,*N*-Bis-(cyclohexanol)amine Aryl Esters: A New Class of Highly Potent Transporter-Dependent Multidrug Resistance Inhibitors. *J. Med. Chem.* **2009**, *52*, 807–817.

(33) Alibert, S.; Santelli-Rouvier, C.; Castaing, M.; Berthelot, M.; Spengler, G.; Molnar, J.; Barbe, J. Effects of a Series of Dihydroanthracene Derivatives on Drug Efflux in Multidrug Resistant Cancer Cells. *Eur. J. Med. Chem.* **2003**, *38*, 253–263.

(34) Bisi, A.; Gobbi, S.; Rampa, A.; Velluti, F.; Piazzi, L.; Valenti, P.; Gyemant, N.; Molnár, J. New potent P-glycoprotein inhibitors carrying a polycyclic scaffold. *J. Med. Chem.* **2006**, *49*, 3049–3051.

(35) Dodic, N.; Dumaitre, B.; Daugan, A.; Pianetti, P. Synthesis and Activity against Multidrug Resistance in Chinese Hamster Ovary Cells of New Acridone-4-carboxamides. *J. Med. Chem.* **1995**, *38*, 2418–2426.

(36) Mistry, P.; Stewart, A. J.; Dangerfield, W.; Okiji, S.; Liddle, C.; Bootle, D.; Plumb, J. A.; Templeton, D.; Charlton, P. In Vitro and in Vivo Reversal of P-Glycoprotein-mediated Multidrug Resistance by a Novel Potent Modulator, XR9576. *Cancer Res.* **2001**, *61*, 749–758.

(37) Arjunan, P.; Shymasundar, N.; Berlin, K. D.; Najjar, D.; Rockley, M. G. Syntheses of selected ε-(2- or 9-anthryl)alkanoic acids and certain esters-carbon-13 spin-lattice relaxation time measurements of methyl 5-(2-anthryl)pentanoate and methyl 7-(2-anthryl)-heptanoate. *J. Org. Chem.* **1981**, *46*, 626–629.

(38) Collomb, D.; Chantegrel, B.; Deshayes, C. Chemoselectivity in the Rhodium(II) Acetate Catalysed Decomposition of α-Diazo-β-keto-γ,δ-alkenyl-δ-aryl Compounds: Aromatic C-H Insertion Reaction or Wolff Rearrangement-Electrocydization. *Tetrahedron* **1996**, *52*, 10455–10472.

(39) Wang, C.; Pan, X.; Liu, H.; Fu, Z.; Wei, X.; Yang, L. X. Synthesis and antitumor activity of 20-O-linked nitrogen-based camptothecin ester derivatives. *Bioorg. Med. Chem.* **2004**, *12*, 3657–3662.

(40) Baarschers, W. H.; Vukmanich, J. P. The chemistry of some methoxychlor derivatives. *Can. J. Chem.* **1986**, *64*, 932–935.

(41) Weber, E.; Finge, S.; Csoeregh, I. Modular Design of Hosts Involving a Rigid Succinimide Framework and N-Bonded Lateral Groups. Crystalline Inclusion Properties and Crystal Structures of Inclusion Compounds with Dioxane, MeOH, and DMF. *J. Org. Chem.* **1991**, *56*, 7281–7287.

(42) Vergote, J.; Moretti, J. L.; De Vries, E. G. E.; Garnier-Suillerot, A. Comparison of the kinetics of active efflux of ^{99m}Tc-MIBI in cells with P-glycoprotein-mediated and multidrug-resistance protein-associated multidrug-resistance phenotype. *Eur. J. Biochem.* **1998**, *252*, 140–146.

(43) Reungpatthanaphong, P.; Marbeuf-Gueye, C.; Le Moyec, L.; Salerno, M.; Garnier-Suillerot, A. Decrease of P-glycoprotein activity in K562/ADR cells by Mβ CD and filipin and lack of effect induced by cholesterol oxidase indicate that this transporter is not located in rafts. *J. Bioenerg. Biomembr.* **2004**, *36*, 533–543.

(44) Lozzio, C. B.; Lozzio, B. B. Human chronic myelogenous leukemia cell line positive philadelphia chromosome. *Blood* **1975**, *45*, 321–334.

(45) Tsuruo, T.; Ida, H.; Kawataba, H.; Oh-Hara, T.; Hamada, H.; Utakoji, T. Characteristics of resistance to adriamycin in human myelogeneous leukemia K562 resistant to adriamycin and in isolated clones. *Jpn. J. Cancer Res.* **1986**, *77*, 682–687.

(46) Dei, S.; Budriesi, R.; Paiwan, S.; Ferraroni, M.; Chiarini, A.; Garnier-Suillerot, A.; Manetti, D.; Martelli, C.; Scapecchi, S.; Teodori, E. Diphenylcyclohexylamine derivatives as new potent multidrug resistant (MDR) modulators. *Bioorg. Med. Chem.* **2005**, *13*, 985–998.

(47) Pereira, E.; Garnier-Suillerot, A. Correlation between the short-term measurements of drug accumulation in living cells and the long-term growth inhibition. *Biochem. Pharmacol.* **1994**, *47*, 1851–1857.

(48) Alley, M. C.; Scudiero, D. A.; Monks, A.; Hursey, M. L.; Czerwinski, M. J.; Fine, D. L.; Abbott, B. J.; Mayo, J. G.; Shoemaker, R. H.; Boyd, M. R. Feasibility of drug screening with panels of human tumour cell lines using a microculture tetrazolium assay. *Cancer Res.* **1988**, *48*, 589–601.

(49) Mankehetkorn, S.; Garnier-Suillerot, A. The ability of verapamil to restore intracellular accumulation of anthracyclines in multidrug resistant cells depends on the kinetics of their uptake. *Eur. J. Pharmacol.* **1998**, *343*, 313–321.