2-[(3-Methoxyphenylethyl)phenoxy]-Based ABCB1 Inhibitors: Effect of Different Basic Side-Chains on Their Biological Properties

Nicola Antonio Colabufo,*[,†] Francesco Berardi,[†] Roberto Perrone,[†] Simona Rapposelli,*[,‡] Maria Digiacomo,[‡] Michael Vanni,[‡] and Aldo Balsamo[‡]

Dipartimento Farmacochimico, Universitá degli Studi di Bari, Via Orabona, 4, 70125 Bari, Italy, Dipartimento di Scienze Farmaceutiche, Università di Pisa, Via Bonanno, 6, 56126 Pisa, Italy

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Recently, 2-[(3-methoxyphenylethyl)phenoxy]-moiety has been selected for the design and synthesis of new small ABCB1 inhibitors. In the present paper, this moiety has been linked through a spacer of 2−5 carbon atoms to the nitrogen of three different basic nuclei such as: (i) *N*-4-arylpiperazine, (ii) *N*-4-methylpiperazine, and (iii) 6,7-dimethoxytetrahydroisoquinoline. The results demonstrated that all the selected basic nuclei were well tolerated and that, globally, the best inhibitory activity for each series was obtained when the spacer between the 2-[(3-methoxyphenylethyl)phenoxy] moiety and the basic nucleus consisted of a four-carbon chain. Among the synthesized compounds, *N*-4-methylpiperazine- **10c** (IC_{50} = 0.15 μM) and tetrahydroisoquinoline-derivatives **11c** (IC_{50} = 0.08 μM) with the spacer n = 4 for both series, displayed the best potency to inhibit ABCB1 activity. Moreover, for each compound, the ABCB1 interacting mechanism has been evaluated by three combined biological assays. *N*-4-methylpiperazine- (**10a**−**d**) and tetrahydroisoquinoline- (**11a**−**d**) derivatives were Cyclosporin A-like ABCB1 nontransported substrates.

Introduction

Multidrug resistance (MDR[a]) is the most ascertained cause of failure in cancer therapy. MDR is due to the contribution of several factors among which pharmacokinetic and tumor microenvironmental and cancer-cell-specific factors such as the overexpression of several ATP-dependent efflux pumps, known as ATP binding cassette (ABC) family.[1] The ABC transporters involved in MDR are: multiresistant proteins (MRPs), P-glycoprotein (ABCB1), and breast cancer resistant protein (BCRP/ABCG2).[2,3] Among these transporters, ABCB1 is the most studied pump, and several modulating ligands have been designed as potential MDR reverting agents.

The first attempts to modulate ABCB1 have been carried out by testing compounds displaying known pharmacological activity such as the calcium channel blocker verapamil and the antisteroid tamoxifen.[4,5] Unfortunately, these compounds interfered with several enzyme systems, resulting in unpredictable pharmacokinetic interaction.

Starting from these limits, compounds such as valspodar and biricodar[6,7] have been developed to reduce the toxic effects related to the specific pharmacologic activities of compounds mentioned above. Both valspodar and biricodar, although displaying high potency and low toxicity, significantly inhibited the metabolism and excretion of cytotoxic agents. On the basis of these pharmacokinetic limits, a new generation of ABCB1 modulators (zosuquidar, elacridar, and tariquidar, ONT-093, MS-209) has been designed (Chart 1).[8−11] These inhibitors did not display any pharmacokinetic interaction with the metabolism of chemotherapeutic drugs and showed high potency to inhibit ABCB1. However, these inhibitors failed in clinical trials, showing only a moderate in vivo efficacy to block ABCB1 pump.[12] In addition, one of the most interesting compounds, elacridar, displayed similar potency to also block ABCG2 transporter, thus indicating a lack of selectivity toward ABCB1 pump.[13]

Recently, we described arylmethyloxy- and arylmethylamino-phenyl derivatives (**1**−**4**) depicted in Figure 1, as potent ABCB1 modulating agents.[14] Among them, the arylmethylamino-phenyl derivatives (**3**, **4**) exhibited both high ABCB1 and ABCG2 inhibition activity, while the corresponding oxygen-analogues (**1**, **2**), although displaying reduced potency with respect to compounds **3**, **4**, they turned out to be inactive against ABCG2 pump. These results led us to consider the 2-[(3-methoxyphenylethyl)phenoxy] moiety, present in the more selective compounds previously synthesized, the pivotal molecular basis to design new and potentially selective ABCB1 inhibitors.[15−18]

Many computational approaches to modeling the ABCB1 transporter suggested a general pharmacophore in which both a planar aromatic domain and a basic nitrogen atom within an extended side chain constitute general features of a substrate/inhibitor of this transporter.[19,20] Because in compounds **1**, **2** is lacking the basic nitrogen that is present in almost all the known ABCB1 inhibitors, we designed and synthesized new compounds in which the 2-[(3-methoxyphenylethyl)phenoxy] moiety of **1**, **2** is joined by an aliphatic spacers of 2−5 methylene carbons to the nitrogen of different basic nucleus such as *N*-4-aryl- (**5**−**8**), *N*-4-heteroaryl- (**9a**−**d**) or *N*-4-methyl-substituted

* To whom correspondence should be addressed. For N.A.C.: phone, +39-080-5442727; fax, +39-0805442231; E-mail, colabufo@farmchim.uniba.it. For S.R.: Phone, +39-050-2219582; fax, +39-050-2219577; E-mail, rappsi@farm.unipi.it.

† Dipartimento Farmacochimico, Universitá degli Studi di Bari.

‡ Dipartimento di Scienze Farmaceutiche, Università di Pisa.

[a] Abbreviations: ABCB1, ATP binding cassette B1 family; ABCG2, ATP binding cassette G2 family; MDR: multidrug resistance; MRPs, multidrug resistant proteins; 5-HT_{1A}, serotonergic receptors 1A-subtype; D_2, dopaminergic receptor 2-subtype; P_{app}, apparent permeability; Caco-2, human colon adenocarcinoma cells; IC_{50}, half-maximal inhibitory concentration; r^2, statistical parameter; MDCK-ABCB1, Madin Darby canine kidney cells overexpressing ABCB1 protein; BCRP, breast cancer resistant protein; K_i, inhibition constant to define affinity value; MCF-7/Adr, human breast adenocarcinoma cells resistant to adriamycin; RPMI, Roswell Park Memorial Institute; DMEM, Dulbecco's minimum essential medium; Calcein-AM, calcein-acethoxy methyl ester; TEER, transepithelial electrical resistance; HBSS, Hank's balanced salt solution; BL, basolateral; AP, apical; PBS, phosphate buffered saline; HeLa, Henrietta Lacks cervical cancer cells; C6, rat glioma cells; K_d, inhibition constant of radioligand at the equilibrium.

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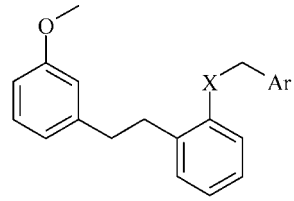
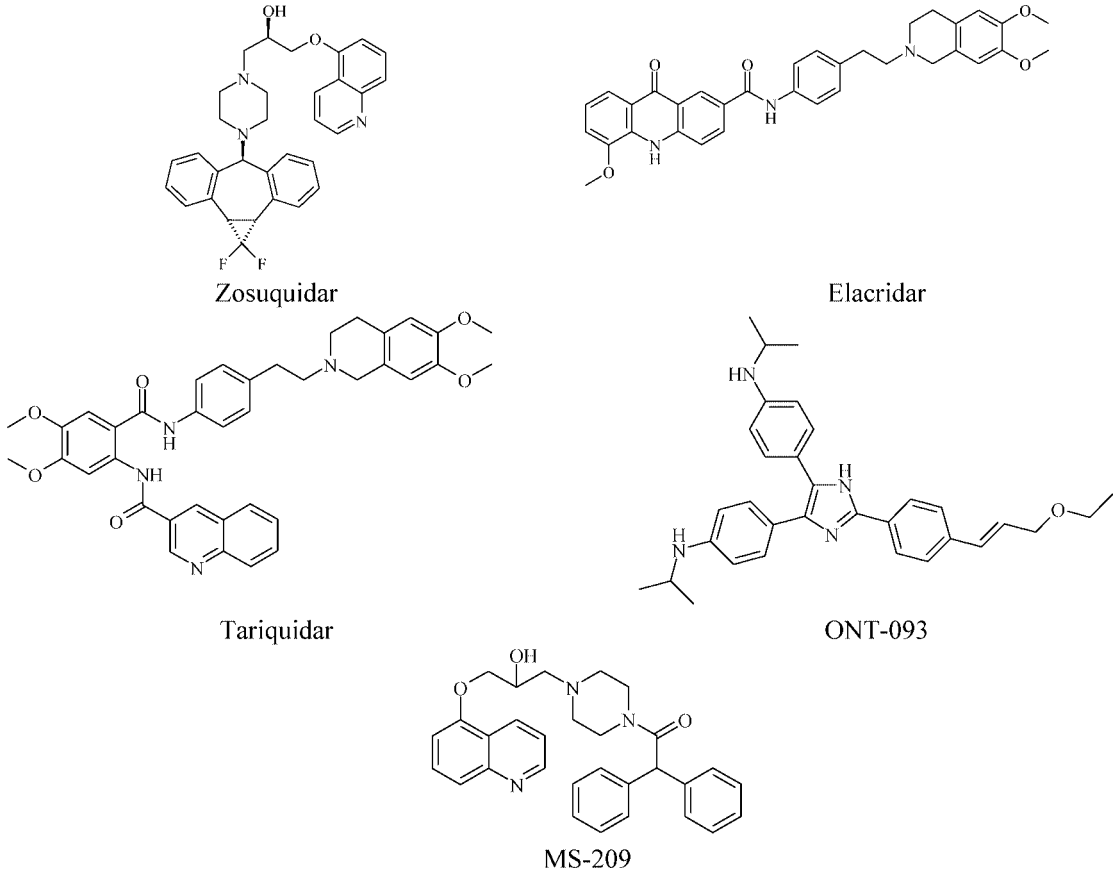


compound	X	Ar	ABCB1 inhibition	ABCG2 inhibition
			IC_{50} (μM)	
1	O	2,3-dimethoxyphenyl	0.25	120
2	O	3,4-dimethoxyphenyl	0.65	150
3	NH	2,3-dimethoxyphenyl	0.085	1.5
4	NH	3,4-dimethoxyphenyl	0.18	0.50

Figure 1. Structures and ABCB1 and ABCG2 inhibition activities of arylmethyloxy- and arylmethylamine-phenyl derivatives.

Chart 1. Third Generation ABCB1 Inhibitors



(**10a**−**d**) piperazine, or to an unsubstituted- (**11a**−**d**) or 6,7-dimethoxy-substituted-tetrahydroisoquinoline (**12a**−**d**) (Figure 2). The choice of tetrahydroisoquinoline nucleus has been made on the basis of the data reported in literature for compounds such as tariquidar and other related derivatives that possess this kind of pharmacophoric moiety. Regarding the other *N*-containing moieties, also in this case, on the basis of some published theoretical studies on the pharmacophoric group pivotal for P-gp activity, we planned to link some *N*-aryl-piperazine-nucleus to the 2-[(3-methoxyphenylethyl)phenoxy] moiety. Among the piperazine derivatives as *N*-4-substituents were selected the 3-Cl-Ph, 3-CF_3-Ph, 2-OCH_3-Ph or 4-F-Ph, and 2-pyrimidine groups.

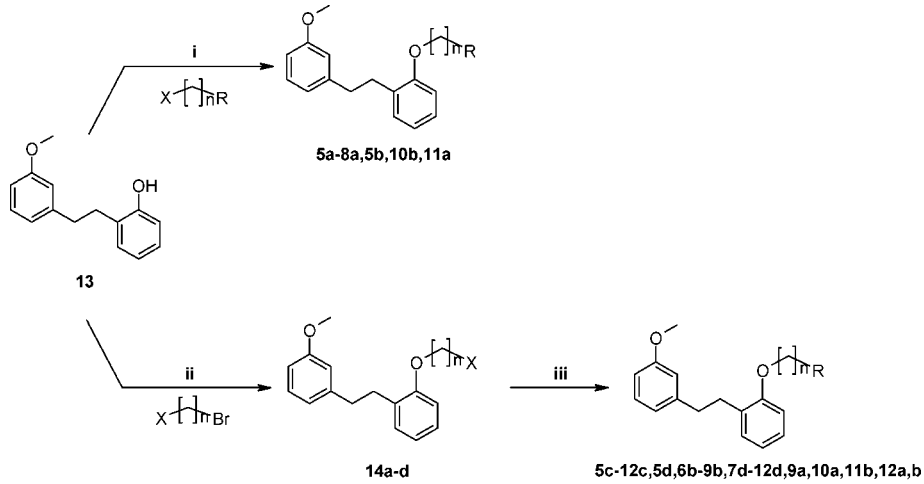
The new compounds were evaluated in vitro for their inhibitory activity toward ABCB1 and for elucidating their interacting mechanism. In addition, because of the presence in the new compounds of molecular features able to interact with serotonergic (5-HT_{1A}) and dopaminergic (D_2) receptors, the in vitro affinity for these receptors was also determined.[21,22]

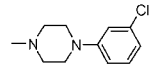
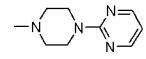
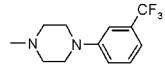
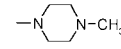
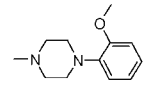
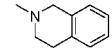
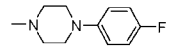
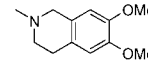
Moreover, on the basis of the marked overlap in the spectrum of activity of several compounds[3] to inhibit both ABCB1 and ABCG2 pumps, we investigated the affinity of the new compounds displaying the best ABCB1 inhibition effect also toward this latter transporter.

Chemistry

Final compounds **5**−**12** were synthesized by following the procedures described in Scheme 1. The reaction of phenol **13**[23] with the appropriate *N*-1-(2-chloroethyl)-*N*-4-arylpiperazine and *N*-1-(3-bromopropyl)-*N*-4-(3′-chlorophenyl)piperazine in the presence of KOH afforded the compounds **5a**−**8a**, and **5b**

Scheme 1[a]



compd	n	R	compd	n	R
5a	2		**9a**	2	
5b	3		**9b**	3	
5c	4		**9c**	4	
5d	5		**9d**	5	
6a	2		**10a**	2	
6b	3		**10b**	3	
6c	4		**10c**	4	
			10d	5	
7a	2		**11a**	2	
7b	3		**11b**	3	
7c	4		**11c**	4	
7d	5		**11d**	5	
8a	2		**12a**	2	
8b	3		**12b**	3	
8c	4		**12c**	4	
8d	5		**12d**	5	

[a] Reagents and conditions: (i) KOH, DMSO, reflux; (ii) (a) KOH, t-BuOH, mw 10 min, 120 °C,150 W; (b) KOH, t-BuOH, rt; (iii) K_2CO_3, CH_3CN, 80 °C.

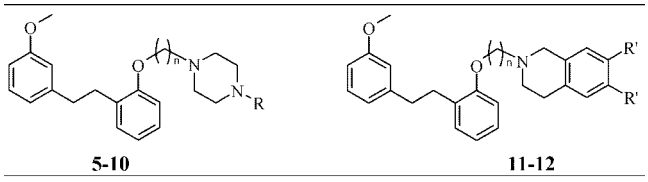


Figure 2. General formulas of compounds **5**−**12**.

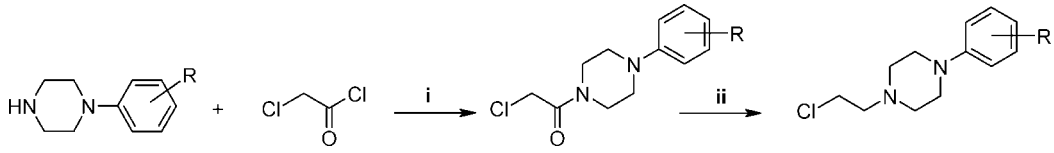
respectively. The same type of reaction carried out using 3-(chloropropyl)-*N*-4-methylpiperazine or *N*-(2-chloroethyl)-tetrahydroisoquinoline gave compounds **10b** and **11a**, respectively. The reaction of the phenol **13** with the appropriate 1,2-1,3- 1,4-, and 1,5-alkyl-dihalide in the presence of KOH afforded the intermediates **14a**−**d**, which by reaction with the appropriate arylpiperazine yielded the final compounds **5**−**9**. The reaction of **14** with the *N*-methylpiperazine afforded compound **10**, while the same reaction with 1,2,3,4,-tetrahydroisoquinoline or with 6,7-dimethoxytetrahydroisoquinoline led to final products **11** and **12**, respectively. The intermediate *N*-1-(2-chloroethyl)-arylpiperazines **23**−**26** used in the synthesis of **5a**−**8a** were prepared, as described in Scheme 2, by acylation of the appropriate arylpiperazine (**15**−**18**) with chloroacetylchloride, and subsequent reduction of the amide-derivative (**19**−**22**) with $LiAlH_4$.

Results and Discussion

ABCB1 Inhibition Activity. The ABCB1 inhibition activity and the interacting mechanism of the synthesized compounds were investigated as previously described.[23,24] This study was highlighted combining the results of the [^{3}H]vinblastine transport inhibition with those obtained by the evaluation of ATPase activation and of the apparent permeability (P_{app}) in Caco-2 cell monolayer. This latter parameter (P_{app}) was determined through the ratio between the basolateral to apical versus flux (B → A) and the apical to basolateral versus flux (A → B).

In the [^{3}H]vinblastine transport inhibition test (Table 1), all *N*-4-aryl- and *N*-4-heteroaromatic-piperazine derivatives displayed from moderate to appreciable ABCB1 inhibitory activity,

Scheme 2[a]

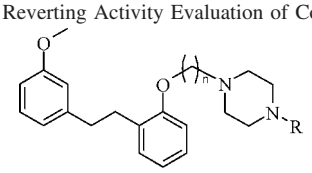


15: R = 3-Cl
16: R = 3-CF_3
17: R = 4-F
18: R = 2-OMe

19-22 **23-26**

[a] Reagents and conditions: (i) KOH, $CH_2Cl_2-H_2O$, rt; (ii) $LiAlH_4$, Et_2O, 40 °C.

Table 1. ABCB1 Reverting Activity Evaluation of Compounds **5**−**10**



5-10

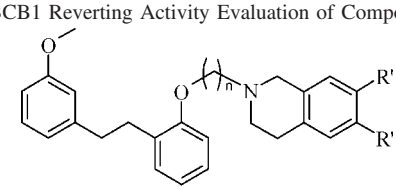
compd	n	R	P_{app} (BA)[a] P_{app} (AB)[a]	ATP-ase activation[b]	[^{3}H]vinblastine transport inhibition IC_{50} ± SEM (μM)[c]
5a	2	3-Cl-Ph	3.6	N	3.2 ± 0.3
5b	3	3-Cl-Ph	6.8	N	2.5 ± 0.3
5c	4	3-Cl-Ph	5.1	N	1.8 ± 0.3
5d	5	3-Cl-Ph	6.2	N	2.65 ± 0.5
6a	2	3-CF_3−Ph	2.6	N	40 ± 2.5
6b	3	3-CF_3−Ph	4.4	N	6.5 ± 0.3
6c	4	3-CF_3−Ph	4.4	N	0.75 ± 0.05
7a	2	2-OMe-Ph	6.8	Y (10%)	25 ± 2.5
7b	3	2-OMe-Ph	8.1	Y (20%)	14 ± 1.2
7c	4	2-OMe-Ph	3.9	Y (54%)	5.8 ± 0.80
7d	5	2-OMe-Ph	5.2	Y (15%)	1.27 ± 0.02
8a	2	4-F-Ph	2.2	Y (10%)	10 ± 1.2
8b	3	4-F-Ph	2.5	Y (38%)	5.3 ± 0.80
8c	4	4-F-Ph	3.5	Y (25%)	1.2 ± 0.40
8d	5	4-F-Ph	5.1	Y (40%)	2.12 ± 0.30
9a	2	2-Pym	6.6	N	2.0 ± 0.30
9b	3	2-Pym	131	N	1.2 ± 0.40
9c	4	2-Pym	8.2	N	0.2 ± 0.05
9d	5	2-Pym	7.1	N	1.72 ± 0.08
10a	2	Me	6.8	N	0.80 ± 0.10
10b	3	Me	4.7	N	2.5 ± 0.50
10c	4	Me	6.3	N	0.15 ± 0.05
10d	5	Me	2.5	N	6.82 ± 1.2
CyclosporinA			9.6[d]	N[d]	80 ± 7.5[d]
Verapamil			1.2[d]	Y[d]	20 ± 1.0[d]
Elacridar			<2[e]	N[e]	2.0[e]

[a] The experimental conditions (solvent and spectroscopic methods) to detect the concentration of each tested compound are reported in Experimental Section. [b] All compounds were tested at 100 μM and for positive response are reported the percentage value given in parentheses. [c] The values are the mean of three independent experiments, samples in triplicate. [d] See ref 14. [e] See ref 19.

with IC_{50} values ranging from 40 μM for **6a** to 0.20 μM for **9c**, indicating a competition for the ABCB1 binding site between the radiolabeled vinblastine (known ABCB1 substrate) and the tested compounds. The similar results obtained among both the *meta*-chlorophenyl (**5**) and the pyrimidyl- (**9**) derivatives seem to indicate that for these series of compounds the spacer length has not a marked influence on the ABCB1 activity; indeed, in the other series of compounds, the spacer length seems to play an inhomogeneous role: the best affinity toward ABCB1 was observed for a spacer length of four carbon atoms ($n = 4$) in derivatives **6** (**6c**: $IC_{50} = 0.75$ μM) and **8** (**8c**: $IC_{50} = 1.2$ μM), and for $n = 5$ in compounds of type **7** (**7d**: $IC_{50} = 1.27$ μM). Anyway, in all the series, the linker of two carbon atom ($n = 2$) seems to be detrimental.

Also the *N*-4-methyl-piperazine derivatives (**10a**−**d**) showed a good degree of [^{3}H]vinblastine transport inhibition. In this series, the compound having a linker of four carbon atoms (**10c**), displayed the highest ABCB1 inhibition activity with IC_{50} in the submicromolar range (0.15 μM).

Table 2. ABCB1 Reverting Activity Evaluation of Compounds **11**−**12**



11-12

compd	n	R′	P_{app} (BA)[a] P_{app} (AB)[a]	ATP-ase activation[b]	[^{3}H]vinblastine transport inhibition IC_{50} ± SEM (μM)[c]
11a	2	H	7.2	N	12 ± 1.5
11b	3	H	6.3	N	0.50 ± 0.02
11c	4	H	6.9	N	0.08 ± 0.01
11d	5	H	7.3	N	3.17 ± 0.2
12a	2	OMe	6.4	Y (55%)	0.75 ± 0.02
12b	3	OMe	4.3	N	0.14 ± 0.03
12c	4	OMe	5.8	N	0.19 ± 0.05
12d	5	OMe	4.9	Y (20%)	0.35 ± 0.04
CyclosporinA			9.6[d]	N[d]	80 ± 7.5[d]
Verapamil			1.2[d]	Y[d]	20 ± 1.0[d]
Elacridar			<2[e]	N[e]	2.0[e]

[a] The experimental conditions (solvent and spectroscopic methods) to detect the concentration of each tested compound are reported in Experimental Section. [b] All compounds were tested at 100 μM and for positive response are reported the percentage value given in parentheses. [c] The values are the mean of three independent experiments, samples in triplicate. [d] See ref 14. [e] See ref 19.

For compounds of type **11** and **12**, the *N*-4-substituted-piperazine moiety of **5**−**10** has been replaced by a tetrahydroisoquinoline nucleus also present in the chemical structures of other classes of ABCB1 inhibitors such as elacridar and tariquidar (Table 2). Analogously to the *N*-4-arylpiperazine derivatives, also within the tetrahydroisoquinoline derivatives (**11a**−**d**), the spacer elongation improved ABCB1 inhibitory activity with the best result obtained for compound **11c** ($n = 4$, $IC_{50} = 0.08$ μM); on the contrary, the inhibition properties of the 6,7-dimethoxytetrahydroisoquinoline-derivatives (**12a**−**d**) appeared only slightly influenced by the elongation of the spacer (Table 2).

The evaluation of the correlation between the pIC_{50} values of compounds **5** and **7**−**12** and the spacer length (Figure 3) indicates that globally the linker of four-methylene groups confers the best ABCB1 inhibition activity. For each set of compounds bearing from 2 to 4 methylene groups r^2 has been found from 0.90 to 0.99 excepting for *N*-methylpiperazine and for 6,7-dimethoxytetrahydroisoquinoline derivatives displaying $r^2 = 0.34$ and 0.59, respectively.

From the study on the ATPase activation, it appears that only the *N*-4-substituted-piperazine derivatives **7a**−**d** and **8a**−**d** and

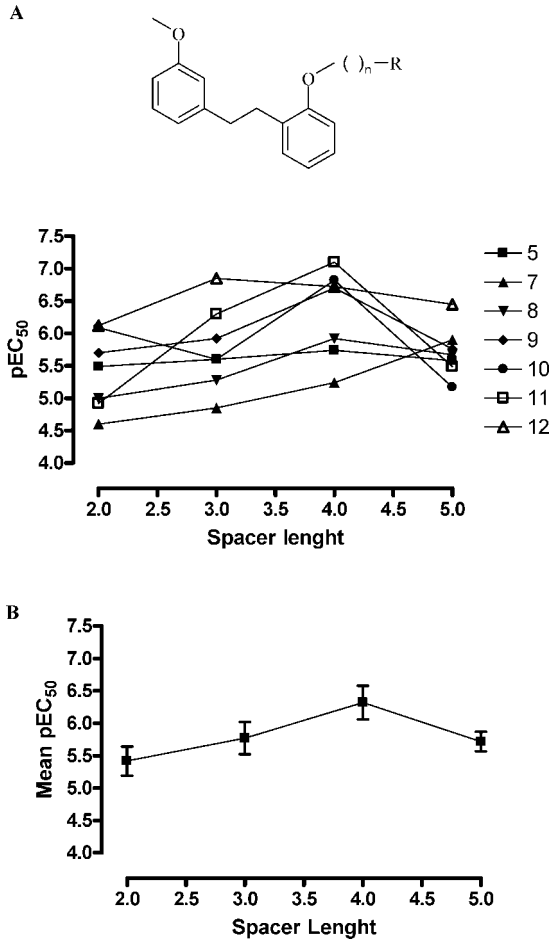


Figure 3. (A) Spacer elongation effect ($n = 2-5$) vs ABCB1 inhibition activity (pIC_{50}). (B) Graphic of pIC_{50} mean related to the spacer carbon numbers.

the 6,7-dimethoxytetrahydroisoquinoline compounds **12a**,**d** were able to activate this enzyme, thus indicating their nature as ABCB1 substrates. On the other hands, the BA/AB ratio, which defines the intrinsic biological properties of a modulator, permits the classification of these compounds as unambiguous ABCB1 substrates. As regards all the other compounds, they were unable to activate the ATPase but exhibited a BA/AB ratio >2, thus allowing us to consider them as ABCB1 Cyclosporin A-like transported substrates.

Cell Calcein Accumulation. Taking into account that Caco-2 cell lines express also other drug efflux proteins such as MRP2[24] and BCRP/ABCG2,[25] making interpretation of results difficult, the compounds displaying submicromolar activity (**6c**, **9c**, **10a,c**, **11b**,**c**, **12a**−**d**) in the [^{3}H]vinblastine transport inhibition were also tested in transfected MDCK-ABCB1 cell lines. In this assay, the calcein cell accumulation depends on ABCB1 inhibition so that it may be considered as a rapid and direct biological method to evaluate the inhibition activity of compounds. Regarding the tetrahydroisoquinoline derivatives **11b**,**c** and **12a**−**d**, the results listed in Table 4 overlapped those obtained in [^{3}H]vinblastine transport inhibition assay, excepting for compound **11c**, which was found almost 20-fold less active ($IC_{50} = 1.78$ μM vs 0.08 μM). On the contrary, among the piperazine derivatives (**6c**, **9c**, **10a**,**c**), only compound **6c** showed a result ($IC_{50} = 1.79$ μM) almost superimposed to that obtained on Caco-2 cell lines ($IC_{50} = 0.75$ μM), while the other three compounds resulted in being 10-fold less active ($IC_{50} = 1.17$ μM vs 0.2 μM, $IC_{50} = 7.76$ μM vs 0.80 μM, $IC_{50} = 4.78$ μM vs 0.15 μM, respectively). These apparent discrepancies between the results obtained in Caco-2 cell lines and in MDCK cell lines for compounds **6c**, **9c**, **10a**,**c**, and **11c** could be due to the expression of several different efflux proteins in both type of cell lines and to the different sensitivity of the two method used.[26]

Table 3. Affinity Binding Values of Compounds **5**−**12**

	$K_i \pm$ SEM[c]	
	D_2[a]	5-HT_{1A}[b]
5a	>1000 (43%)[d]	686 ± 50
5b	298 ± 20	1130 ± 200
5c	>1000 (35%)	1308 ± 150
5d	>1000 (25%)	>5000 (14%)
6a	>1000 (31%)	1409 ± 155
6b	1412 ± 100	2874 ± 150
6c	>1000 (27%)	>5000 (36%)
7a	427 ± 12	1241 ± 250
7b	6.3 ± 0.8	756 ± 60
7c	67 ± 1.5	1092 ± 90
7d	>1000 (35%)	>5000 (14%)
8a	>1000 (38%)	783 ± 15
8b	655 ± 30	800 ± 25
8c	1324 ± 250	>5000 (47%)
8d	847 ± 20	>5000 (27%)
9a	629 ± 80	656 ± 100
9b	20 ± 3.0	331 ± 12
9c	854 ± 10	558 ± 25
9d	>1000 (24%)	>5000 (38%)
10a	178	>5000 (48%)
10b	565	>5000 (40%)
10c	>1000 (14%)	>5000 (38%)
10d	>1000 (26%)	>5000 (24%)
11a	>1000 (35%)	1310 ± 120
11b	114 ± 15	1124 ± 180
11c	>1000 (48%)	1331 ± 150
11d	>1000 (16%)	>5000 (15%)
12a	>1000 (12%)	>5000 (26%)
12b	>1000 (35%)	>5000 (36%)
12c	>1000 (40%)	>5000 (18%)
12d	>1000 (18%)	>5000 (22%)
Haloperidol	6.4 ± 0.8	
8-OH-DPAT		3.3 ± 0.5

[a] Human cloned D_2 receptors in C6 rat glioma cells; [^{3}H]spiroperidol, $K_d = 0.2$ nM; nonspecific binding determined in the presence of 10 μM haloperidol. [b] Human cloned 5-HT_{1A} receptors in HeLa cells; [^{3}H]-8-OH-DPAT, $K_d = 8.8$ nM; nonspecific binding determined in the presence of 10 μM 8-OH-DPAT. [c] The value is the mean of three independent experiments, samples in triplicate. [d] K_i not obtained, the inhibition percentage at 10 μM given in parentheses.

Table 4. Calcein Cell Accumulation in MDCK-MDR1 Cells

compd	$IC_{50} \pm$ SEM (μM)[a]
6c	1.79 ± 0.45
9c	1.17 ± 0.20
10a	7.76 ± 0.90
10c	4.78 ± 0.20
11b	1.14 ± 0.60
11c	1.78 ± 0.80
12a)	0.78 ± 0.08
12b	0.09 ± 0.001
12c	0.16 ± 0.04
12d	0.11 ± 0.02

[a] The value is the mean of three independent experiments, samples in triplicate.

ABCG2 Inhibition Activity. Compounds displaying ABCB1 inhibition activity ≤1 μM (**6c**, **9c**, **10a**, **10c**, **11b**,**c**, and **12a**−**d**) were also evaluated for their ABCG2 inhibition properties using [^{3}H]mithoxantrone as a specific substrate. All these compounds at 100 μM were unable to interfere with [^{3}H]mithoxantrone efflux, with the only exception of compounds **6c** and **9c**, which slightly reduced the [^{3}H]mithoxantrone efflux by ABCG2 pump (24% and 16% of inhibition effect at 100 μM, respectively).

Serotonergic and Dopaminergic Receptor Affinity. All compounds were tested for their affinity toward serotonergic 5-HT_{1A} and dopaminergic D_2 receptors (Table 3). The results displayed that all compounds toward serotonergic 5-HT_{1A} receptors showed poor affinity with K_i values in the micromolar range. Regarding dopaminergic D_2 receptors affinity, 2-methoxyphenyl derivative **7b** ($n = 3$) and 1-pyrimidyl derivative **9b** ($n = 3$) displayed nanomolar receptor affinities ($K_i = 6.3$ nM and $K_i = 20$ nM, respectively). All the other compounds displayed a modest dopaminergic D_2 receptor affinity.

These findings indicated that these new compounds, although contain molecular features typical of serotonergic and dopaminergic agents (i.e., *N*-4-arylpiperazine nucleus), showed negligible affinity toward 5-HT_{1A} and D_2 receptors, with the only exception of derivatives **7b** and **9b** on D_2 receptor.

Conclusions

The aim of the present work was to verify the influence of the basic nucleus linked to the 2-[(3-methoxyphenylethyl)phenoxy] moiety on the activity toward two efflux pump proteins: ABCB1 and ABCG2. Globally, the results indicate that this scaffold appears able to maintain a satisfactory degree of ABCB1 inhibition activity and that the addition of a basic nitrogen to this moiety caused only in a few cases a slight improvement of ABCB1 inhibition activity. In the meantime, this chemical manipulation leads to more selective compounds endowed with reduced affinity toward ABCG2 and possessing negligible or modest affinity for 5-HT_{1A} and D_2 receptors.

Moreover, the results obtained in the two different types of ABCB1 inhibition tests seem to indicate that the tetrahydroisoquinoline derivatives are the more active and selective compounds toward ABCB1 protein.

Finally, the effect of the elongation of the linker between the 2-[(3-methoxyphenylethyl)phenoxy] moiety and the basic nucleus, it seems not to markedly influence the ABCB1 inhibition activity: it appeared to be detrimental only when the chain is two carbon atoms length, while globally, it appeared to furnish the best results when the spacer length is of four carbon atoms.

Experimental Section

Melting points were determined on a Kofler hot-stage apparatus and are uncorrected. NMR spectra were obtained with a Varian Gemini 200 MHz spectrometer. Chemical shifts (δ) are reported in parts per million downfield from tetramethylsilane and referenced from solvent references. Mass spectra were obtained on a Hewlett-Packard 5988 A spectrometer using a direct injection probe and an electron beam energy of 70 eV. The elemental compositions of the compounds agreed to within ±0.4% of the calculated value. Chromatographic separation was performed on silica gel columns by flash (Kieselgel 40, 0.040−0.063 mm; Merck) or gravity column (Kieselgel 60, 0.063−0.200 mm; Merck) chromatography. Reactions were followed by thin-layer chromatography (TLC) on Merck aluminum silica gel (60 F_{254}) sheets that were visualized under a UV lamp. Evaporation was performed in vacuo (rotating evaporator). Sodium sulfate was always used as the drying agent. The microwave-assisted procedures were carried out with a CEM Discover LabMate Microwave. Commercially available chemicals were purchased from Sigma-Aldrich. The UV−vis spectra of the final compounds and the corresponding calibration curves were recorded with LAMBDA BIO-20 spectrophotometer Perkin-Elmer.

2-{2-[2-(3-Methoxyphenyl)ethyl]phenoxy}ethyl-4-(3′-chlorophenyl)piperazine (5a). A solution of 2-[2-(3-methoxyphenyl)ethyl]phenol **13** (103 mg, 0.45 mmol) in a small amount of DMSO (2 mL) was added to a solution of KOH (88 mg, 1.60 mmol) in DMSO (2.5 mL). Stirring was continued over 15 min at room temperature, then a solution 1-(2-chloroethyl)-4-(3-chlorophenyl)piperazine **23** (116 mg, 0.45 mmol) in DMSO (2 mL) was added dropwise to the solution of potassium 2-[2-(3-methoxyphenyl)ethyl]benzenolate. The suspension was stirred at 40 °C for 1 h, then it was diluted with AcOEt and washed with water and brine. The organic layer was dried and concentrated. The crude product was transformed into the hydrochloride salt and crystallized from i-PrOH/i-Pr_2O to give **5a** (125 mg, 0.27 mmol, 57% yield) as a white solid: mp 85−87 °C. MS *m/z* 450 (M^+, 5). ^{1}H NMR ($CDCl_3$): δ 2.74−2.82 (m, 4H, *piperazine*), 2.85−2.93 (m, 6H, CH_2CH_2, CH_2N), 3.17−3.25 (m, 4H, *piperazine*), 3.78 (s, 3H, OMe), 4.16 (t, 2H, $J = 5.6$ Hz, OCH_2), 6.75−6.90 (m, 7H, Ar), 7.16−7.25 (m, 5H, Ar) ppm. ^{13}C NMR (CD_3OD): δ 160.98, 156.86, 152.02, 144.76, 136.05, 131.50, 131.36, 131.28, 130.28, 128.46, 122.82, 121.91, 121.78, 117,61, 115.97, 115.35, 112.86, 112.02, 64.15, 57.30, 55.59, 53.88, 47.36, 37.46, 32.41. Anal. $C_{27}H_{31}N_2O_2Cl\cdot HCl$. UV−vis (solvent: PBS) $\lambda = 254$ nm, $\varepsilon = 12030$.

3-{2-[2-(3-Methoxyphenyl)ethyl]phenoxy}propyl-4-(3′-chlorophenyl)piperazine (5b). A stirred solution of KOH (440 mg, 3.19 mmol) in DMSO (5 mL) was heated to 80 °C until the complete dissolution of KOH. After being cooled at room temperature, a solution of 2-[2-(3-methoxyphenyl)]ethyl]phenol **13** (500 mg, 2.19 mmol) in DMSO (3 mL) was added. The mixture was vigorously stirred for 20 min and then it was added to a solution of 1-(3-chlorophenyl)-4-(3-chloropropil)piperazine (598 mg, 2.19 mmol) dissolved in DMSO. The resulting mixture was stirred and refluxed for 20 h. Then it was diluted with AcOEt and washed with water, a solution 1N of NaOH and brine. The organic layer was evaporated to give a crude oil, which was purified by transformation into the hydrochloride salt and crystallization from i-PrOH to give **5b** (610 mg, 1.31 mmol, 60% yield) as a white solid: mp 135−137 °C; MS *m/z* 464 (M^+, 40). ^{1}H NMR ($CDCl_3$): δ 2.02−2.12 (m, 2H, CH_2), 2.59−2.70 (m, 6H, *piperazine*, CH_2N), 2.85−2.93 (m, 4H, CH_2CH_2), 3.20 (t, 4H, $J = 4.9$ Hz, *piperazine*), 3.78 (s, 3H, OMe), 4.05 (t, 2H, $J = 6.0$ Hz, OCH_2), 6.73−6.91 (m, 8H, Ar), 7.11−7.25 (m, 4H, Ar) ppm. ^{13}C NMR ($CDCl_3$): δ 165.34, 159.04, 154.27, 149.72, 147.81, 130.56, 130.53, 130.15, 129.49, 127.58, 121.64, 121.42, 120.90, 117.45, 115.10, 114.81, 111.50, 110.86, 65.01, 55.62, 55.40, 52.00, 46.53, 36.68, 31.97, 24.34. Anal. ($C_{28}H_{33}N_2O_2Cl\cdot HCl$) C, H, N. UV−vis (solvent: PBS) $\lambda = 230$ nm, $\varepsilon = 18850$.

4-{2-[2-(3-Methoxyphenyl)ethyl]phenoxy}butyl-4-(3′-trifluoromethylphenyl)piperazine (5c). A solution of 1-(4-chlorobutoxy)-2-[2-(3-methoxyphenyl)ethyl]benzene **14c** (200 mg, 0.63 mmol) in a small amount of acetonitrile (3 mL) heated at 80 °C was added to a solution of K_2CO_3 (105 mg, 0.76 mmol) in acetonitrile (15 mL). Stirring was continued over 15 min at room temperature, then a solution of 1-(3-chlorophenyl)piperazine hydrochloride **15** (124 mg, 0.63 mmol) in acetonitrile (2 mL) was added dropwise to the solution of potassium 2-(3-methoxyphenylethyl)benzenolate. The suspension was stirred at 80 °C for 4 h, then the potassium carbonate was filtered off and the solvent removed. The crude product was purified by column chromatography eluting with *n*-hexane/AcOEt (3:7) and transformation into the hydrochloride salt to give **5c** (90 mg, 0.19 mmol, 30% yield) as a white solid: mp 65−67 °C. MS *m/z* 478 (M^+, 34). ^{1}H NMR ($CDCl_3$): δ 1.90−2.20 (m, 4H, CH_2CH_2), 2.74−2.98 (m, 4H, CH_2CH_2), 3.10−3.30 (m, 2H, CH_2N), 3.41−3.59 (m, 4H, *piperazine*), 3.78 (s, 3H, OMe), 3.85−4.09 (m, 4H, *piperazine*), 4.39−4.59 (m, 2H, OCH_2), 6.50−6.96 (m, 5H, Ar), 7.12−7.23 (m, 3H, Ar), 7.34−7.45 (m, 2H, Ar), 7.55−7.65 (m, 1H, Ar), 7.69−7.73 (m, 1H, Ar) ppm. ^{13}C NMR ($CDCl_3$): δ 159.84, 156.47, 146.82, 144.15, 135.54, 130.64, 130.13, 129.93, 129.54, 127.52, 122.33, 121.06, 120.89, 117.82, 115.41, 114.70, 111.43, 110.99, 67.00, 57.51, 55.44, 51.34, 46.83, 36.34, 32.22, 27.12, 21.17. Anal. ($C_{29}H_{35}N_2O_2Cl\cdot HCl$) C, H, N. UV−vis (solvent: PBS) $\lambda = 230$ nm, $\varepsilon = 18870$.

5-{2-[2-(3-Methoxyphenyl)ethyl]phenoxy}pentyl-4-(3′-chlorophenyl)piperazine (5d). Compound **5d** was synthesized from 1-[(5-bromopentyloxy]-2-[2-(3-methoxyphenyl)ethyl]benzene **14d** (150 mg, 0.40 mmol) and from 1-(3-chlorophenyl)piperazine

hydrochloride **15** (93 mg, 0.40 mmol) following the same procedure described above for the preparation of **5c**. The crude product was purified by transformation into the hydrochloride to give **5d** (88 mg, 0.17 mmol, 41% yield) as a white solid: mp 113−115 °C. MS *m/z* 493 (M^+, 23). ^{1}H NMR ($CDCl_3$): δ 1.60−1.99 (m, 6H, $CH_2CH_2CH_2$), 2.88−3.02 (m, 8H, CH_2CH_2, *piperazine*), 3.23−3.62 (s, 6H, CH_2N, *piperazine*), 3.77 (s, 3H, OMe), 3.99 (t, 2H, J = 5.8 Hz, OCH_2), 6.75−6.94 (m, 7H, Ar), 7.11−7.24 (m, 5H, Ar) ppm. ^{13}C NMR ($CDCl_3$): δ 159.84, 156.82, 150.67, 144.27, 135.48, 130.55, 130.38, 130.05, 129.44, 127.43, 121.66, 121.06, 120.79, 117.51, 115.14, 114.76, 111.55, 111.10, 67.40, 57.59, 55.44, 51.67, 46.53, 36.59, 32.49, 29.09, 24.06, 23.54. Anal. ($C_{30}H_{37}N_2O_2Cl\cdot HCl$) C, H, N. UV−vis (solvent: PBS) λ = 226 nm, ε = 41854.

2-{2-[2-(3-Methoxyphenyl)ethyl]phenoxy}ethyl-4-(3′-trifluoromethyl)piperazine (6a). Compound **6a** was synthesized from 2-[2-(3-methoxyphenyl)ethyl]phenol **13** (91 mg, 0.40 mmol) and from 1-(2-chloroethyl)-4-(3-trifluoromethylphenyl)piperazine **24** (118 mg, 0.40 mmol) following the same procedure described above for the preparation of **5a**. The crude residue was subjected to a column chromatography eluting with hexane/AcOEt (1:1) and then it was transformed into the hydrochloride salt to give **6a** (73 mg, 0.15 mmol, 37% yield) as a white solid: mp 55−57 °C. MS *m/z* 484 (M^+, 5); 243 (100). ^{1}H NMR ($CDCl_3$): δ 2.78 (t, 4H, J = 4.7 Hz, *piperazine*), 2.85−2.94 (m, 6H, CH_2CH_2, CH_2N), 3.24 (t, 4H, J = 4.7 Hz, *piperazine*), 3.78 (s, 3H, OMe), 4.17 (t, 2H, J = 5.4 Hz, OCH_2), 6.73−6.94 (m, 5H, Ar), 7.03−7.24 (m, 6H, Ar), 7.34 (t, 1H, J = 7.8 Hz, Ar) ppm. ^{13}C NMR ($CDCl_3$): δ 159.90, 155.31, 149.92, 148.19, 146.80, 143.62, 130.40, 130.09, 129.47, 127.78, 122.22, 120.86, 120.15, 118.31, 114.67, 113.94, 111.92, 111.25, 63.16, 56.82, 55.36, 52.71, 46.70, 36.74, 31.60. Anal. ($C_{28}H_{31}N_2O_2F_3\cdot HCl$) C, H, N. UV−vis (solvent: PBS) λ = 230 nm, ε = 21090.

3-{2-[2-(3-Methoxyphenyl)ethyl]phenoxy}propyl-4-(3′-trifluoromethylphenyl)piperazine (6b). Compound **6b** was synthesized from 1-(3-bromopropoxy)-2-[2-(3-methoxyphenyl)ethyl]benzene **14b** (200 mg, 0.57 mmol) and from 1-[3-(trifluoromethyl)phenyl]piperazine **16** (131 mg, 0.57 mmol) following the same procedure described above for the preparation of **5c**. The crude product was transformed into the hydrochloride salt and crystallized from i-PrOH to afford **6b** (104 mg, 0.19 mmol, 34% yield) as a white solid: mp 130−132 °C. MS *m/z* 498 (M^+, 18). ^{1}H NMR ($CDCl_3$): δ 2.40−2.51 (m, 2H, CH_2), 2.80−2.90 (m, 4H, CH_2CH_2), 3.15−3.31 (m, 4H, *piperazine*), 3.35−3.50 (m, 2H, CH_2N), 3.51−3.70 (m, 4H, *piperazine*), 3.73 (s, 3H, OMe), 4.01−4.12 (m, 2H, OCH_2), 6.70−6.87 (m, 4H, Ar), 6.93 (t, 1H, J = 7.3 Hz, Ar), 7.12−7.24 (m, 5H, Ar), 7.35−7.48 (m, 2H, Ar) ppm. ^{13}C NMR ($CDCl_3$): δ 159.73, 156.04, 149.83, 147.86, 130.13, 130.00, 129.47, 127.56, 121.41, 120.90, 120.04, 118.20, 118.10, 114.78, 113.85, 113.78, 111.48, 110.83, 64.89, 55.35, 52.09, 46.53, 43.31, 36.63, 31.93, 24.32. Anal. ($C_{29}H_{33}F_3N_2O_2\cdot HCl$) C, H, N. UV−vis (solvent: PBS) λ = 245 nm, ε = 14090.

4{2-[2-(3-Methoxyphenyl)ethyl]phenoxy}butyl-4-(3′-trifluoromethylphenyl)piperazine (6c). Compound **6c** was synthesized from 1-(4-chlorobutoxy)-2-[2-(3-methoxyphenyl)ethyl]benzene **14c** (200 mg, 0.63 mmol) and from 1-(3-trifluoromethylphenyl)piperazine **16** (145 mg, 0.63 mmol) following the same procedure described above for the preparation of **5c**. The crude product was purified by column chromatography eluting with *n*-hexane/AcOEt (3:7) and transformed into the hydrochloride salt to give **6c** (122 mg, 0.24 mmol, 38% yield) as a white solid: mp 78−80 °C. MS *m/z* 512 (M^+, 6). ^{1}H NMR ($CDCl_3$): δ 1.75−1.95 (m, 4H, CH_2CH_2), 2.49 (t, 2H, J = 7.0 Hz, CH_2N), 2.58 (t, 4H, J = 4.9 Hz, *piperazine*), 2.85−2.95 (m, 4H, CH_2CH_2), 3.21 (t, 4H, J = 4.9 Hz, *piperazine*), 3.77 (s, 3H, OMe), 4.01 (t, 2H, J = 5.8 Hz, OCH_2), 6.72−6.90 (m, 6H, Ar), 7.02−7.38 (m, 6H, Ar) ppm. ^{13}C NMR ($CDCl_3$): δ 162.00, 154.25, 149.89, 148.35, 131.69, 130.05, 129.74, 128.49, 124.12, 122.00, 121.71, 120.33, 120.04, 117.56, 114.57, 114.30, 111.82, 111.51, 67.89, 56.94, 54.10, 52.65, 47.00, 37.11, 32.75, 27.41,21.70. Anal. ($C_{30}H_{35}N_2O_2F_3\cdot HCl$) C, H, N. UV−vis (solvent: PBS) λ = 230 nm, ε = 18950.

2{2-[2-(3-Methoxyphenyl)ethyl]phenoxy}ethyl-4-(2′-methoxyphenyl)piperazine (7a). Compound **7a** was synthesized from 2-[2-(3-methoxyphenyl)ethyl]phenol **13** (107 mg, 0.47 mmol) and from 1-(2-chloroethyl)-4-(2-methoxyphenyl)piperazine **26** (120 mg, 0.47 mmol) following the same procedure described above for the preparation of **5a**. The crude residue was transformed into the hydrochloride salt and crystallized from i-PrOH to give **7a** (163 mg, 0.36 mmol, 77% yield) as a white solid: mp 93−95 °C. MS *m/z* 446 (M^+, 6); 123 (45). ^{1}H NMR ($CDCl_3$): δ 2.69−2.87 (m, 4H, *piperazine*), 2.88−3.00 (m, 4H, CH_2CH_2), 3.05−3.19 (m, 4H, *piperazine*), 3.68 (t, 2H, J = 5.4 Hz, CH_2N), 3.77 (s, 3H, OMe), 3.86 (s, 3H, OMe), 4.17 (t, 2H, J = 5.8 Hz, CH_2O), 6.73−6.98 (m, 7H, Ar), 7.05−7.21 (m, 5H, Ar) ppm. ^{13}C NMR ($CDCl_3$): δ 160.01, 156.20, 152.71, 144.32, 131.56, 130.80, 129.77, 128.94, 126.68, 123.95, 122.00, 120.73, 120.22, 115.89, 114.32, 113.13, 111.81, 111.55, 66.24, 57.31, 56.20, 55.90, 52.63, 48.37, 37.11, 30.96. Anal. ($C_{28}H_{34}N_2O_3\cdot HCl$) C, H, N. UV−vis (solvent: PBS) λ = 230 nm, ε = 15420.

3-{2-[2-(3-Methoxyphenyl)ethyl]phenoxy}propyl-4-(2′-methoxyphenyl)piperazine (7b). Compound **7b** was synthesized from 1-(3-bromopropoxy)-2-[2-(3-methoxyphenyl)ethyl]benzene **14b** (200 mg, 0.57 mmol) and from 1-[2-(methoxyphenyl)]piperazine hydrochloride **18** (110 mg, 0.57 mmol) following the same procedure described above for the preparation of **5c**. The crude residue was purified by transformation into hydrochloride and crystallization from i-PrOH to give **7b** (136 mg, 0.30 mmol, 52% yield) as a white solid: mp 114−116 °C. MS (EI, 70 eV) *m/z*: M^+ 460 (66). ^{1}H NMR ($CDCl_3$): δ 2.39−2.49 (m, 2H, CH_2), 2.82−2.94 (m, 4H, CH_2CH_2), 3.21−3.35 (m, 2H, CH_2N), 3.49−3.61 (m, 4H, *piperazine*), 3.76 (s, 3H, OMe), 4.01−4.10 (m, 5H, OCH_2, OMe), 4.29−4.44 (m, 2H, *piperazine*), 4.94−5.04 (m, 2H, *piperazine*), 6.67−6.83 (m, 4H, Ar), 6.92 (t, 1H, J = 7.0 Hz, Ar), 7.02−7.21 (m, 5H, Ar), 7.46 (t, 1H, J = 8.5 Hz, Ar), 8.19 (d, 1H, J = 7.9 Hz, Ar). ^{13}C NMR (CD_3OD) δ: 160.00, 157.59, 156.19, 153.64, 145.03, 143.23, 131.32, 131.08, 130.24, 130.06, 128.31, 122.58, 121.96, 121.90, 115.44, 114.28, 112.71, 112.15, 66.02, 56.72, 55.97, 55.68, 54.88, 51.79, 37.58, 32.96, 25.40. Anal. ($C_{29}H_{36}N_2O_3$ HCl) C, H, N. UV−vis (solvent: PBS) λ = 230 nm, ε = 16540.

4{2-[2-(3-Methoxyphenyl)ethyl]phenoxy}butyl-4-(2′-methoxyphenyl)piperazine (7c). Compound **7c** was synthesized from 1-(4-chlorobutoxy)-2-[2-(3-methoxyphenyl)ethyl]benzene **14c** (200 mg, 0.63 mmol) and from 1-(2-methoxyphenyl)piperazine **18** (121 mg, 0.63 mmol) following the same procedure described above for the preparation of **5c**. The crude product was purified by column chromatography eluting with *n*-hexane/AcOEt (3:7) and transformation into the hydrochloride to give **7c** (109 mg, 0.23 mmol, 36% yield) as a white solid: mp 105−107 °C. MS *m/z* 474 (M^+, 73). ^{1}H NMR ($CDCl_3$): δ 1.65−1.95 (m, 4H, CH_2CH_2), 2.50 (t, 2H, J = 7.2 Hz, CH_2N), 2.6−2.71 (m, 4H, *piperazine*), 2.73−2.95 (m, 4H, CH_2CH_2), 3.01−3.15 (m, 4H, *piperazine*), 3.78 (s, 3H, OMe), 3.86 (s, 3H, OMe), 4.01 (t, 2H, J = 5.8 Hz, CH_2O), 6.71−7.00 (m, 9H, Ar), 7.09−7.20 (m, 3H, Ar) ppm. ^{13}C NMR ($CDCl_3$): δ 159.72, 156.44, 152.76, 143.98, 130.86, 130.24, 129.95, 129.40, 127.32, 123.35, 121.79, 121.00, 120.84, 114.47, 113.36, 113.39, 111.46, 111.23, 66.89, 57.33, 56.18, 55.36, 49.10, 48.74, 36.32, 32.06, 27.01, 21.06. Anal. ($C_{30}H_{38}N_2O_3\cdot HCl$) C, H, N. UV−vis (solvent: PBS) λ = 230 nm, ε = 18240.

5-{2-[2-(3-Methoxyphenyl)ethyl]phenoxy}pentyl-4-(2′-methoxyphenyl)piperazine (7d). Compound **7d** was synthesized from 1-[(5-bromopentyloxy]-2-[2-(3-methoxyphenyl)ethyl]benzene **14d** (150 mg, 0.40 mmol) and from 1-(2-methoxyphenyl)piperazine **18** (77 mg, 0.40 mmol) following the same procedure described above for the preparation of **5c**. The crude product was purified by transformation into the hydrochloride to give **7d** (160 mg, 0.30 mmol, 76% yield) as a white solid: mp 100−102 °C. MS *m/z* 488 (M^+, 53). ^{1}H NMR (CD_3OD): δ 1.65−1.73 (m, 2H, CH_2), 1.83−1.99 (m, 4H, CH_2CH_2), 2.82−2.92 (m, 4H, CH_2CH_2), 3.45−3.54 (m, 8H, *piperazine*), 3.70−3.76 (m, 2H, CH_2N), 3.89 (s, 3H, OMe), 3.93 (s, 3H, OMe), 4.03 (t, 2H, J = 5.9 Hz, CH_2O), 6.71−7.27 (m, 12H, Ar) ppm. ^{13}C NMR (CD_3OD): δ 161.00, 158.10, 153.73, 145.18, 130.94, 130.19, 129.53, 128.31, 128.24,

122.42, 121.87, 121.70, 121.43, 115.33, 114.15, 113.86, 112.53, 112.13, 68.48, 58.09, 56.68, 56.50, 55.68, 52.28, 43.76, 37.56, 33.54, 30.01, 24.73. Anal. ($C_{31}H_{40}N_2O_3$ HCl) C, H, N. UV−vis (solvent: PBS) $\lambda = 271$ nm, $\varepsilon = 4696$.

2{2-[2-(3-Methoxyphenyl)ethyl]phenoxy}ethyl-4-(4′-fluorophenyl)piperazine (8a). Compound **8a** was synthesized from 2-[2-(3-methoxyphenyl)ethyl]phenol **13** (186 mg, 0.82 mmol) and from 1-(2-chloroethyl)-4-(4-fluorophenyl)piperazine **25** (198 mg, 0.82 mmol) following the same procedure described above for the preparation of **5a.** The crude residue was subjected to a column chromatography eluting with hexane/AcOEt (1:1) and then it was transformed into the hydrochloride salt to give **8a** (143 mg, 0.33 mmol, 70% yield) as a white solid: mp 72−75 °C. MS *m/z* 434 (M^+, 6); 193 (100). ^{1}H NMR ($CDCl_3$): δ 2.75−2.81 (m, 4H, *piperazine*), 2.88−2.94 (m, 6H, CH_2N, CH_2CH_2), 3.09−3.14 (m, 4H, *piperazine*), 3.77 (s, 3H, OMe), 4.16 (t, 2H, $J = 5.6$ Hz, OCH_2), 6.73−7.00 (m, 9H, Ar), 7.12−7.22 (m, 3H, Ar) ppm. ^{13}C NMR ($CDCl_3$) δ: 163.90, 159.92, 155.29, 149.66, 143.67, 130.46, 130.36, 129.47, 127.76, 122.17, 120.84, 119.78, 119.62, 116.33, 115.89, 114.65, 111.88, 111.23, 63.07, 56.69, 55.35, 52.87, 48.03, 36.74, 31.64; Anal. ($C_{27}H_{31}N_2O_2F\cdot$HCl) C, H, N. UV−vis (solvent: PBS) $\lambda = 230$ nm, $\varepsilon = 13780$.

3-{2-[2-(3-Methoxyphenyl)ethyl]phenoxy}propyl-4-(4′-fluorophenyl)piperazine (8b). Compound **8b** was synthesized from 1-(3-bromopropoxy)-2-[2-(3-methoxyphenyl)ethyl]benzene **14b** (200 mg, 0.57 mmol) and from 1-[4-(fluorophenyl)]piperazine hydrochloride **17** (144 mg, 0.57 mmol) following the same procedure described above for the preparation of **5c**. The crude residue was purified by transformation into the hydrochloride salt and crystallization from i-PrOH to give **8b** (77 mg, 0.17 mmol, 30% yield) as a white solid: mp 123−125 °C. MS *m/z*: M^+ 448 (M^+, 89). ^{1}H NMR ($CDCl_3$): δ 2.32−2.50 (m, 2H, CH_2),2.81−2.91 (m, 4H, CH_2CH_2), 3.23−3.70 (m, 2H, CH_2N), 3.50−3.70 (m, 4H, *piperazine*), 3.75 (s, 3H, OMe), 3.96−4.20 (m, 4H, *piperazine*), 4.50−4.64 (m, 2H, OCH_2), 6.68−6.97 (m, 5H, Ar), 7.15−7.24 (m, 5H, Ar), 7.77−7.84 (m, 2H, Ar). ^{13}C NMR (CD_3OD): δ 162.36, 161.02, 157.57, 146.58, 145.03, 131.32, 131.05, 130.24, 128.31, 122.05, 121.91, 120.78, 120.58, 117.06, 116.61, 115.48, 112.67, 112.07, 66.04, 55.09, 55.68, 53.04, 49.29, 37.55, 32.90, 25.46. Anal. ($C_{28}H_{33}N_2O_2F\cdot$HCl) C, H, N. UV−vis (solvent: PBS) $\lambda = 230$ nm, $\varepsilon = 18350$.

4{2-[2-(3-Methoxyphenyl)ethyl]phenoxy}butyl-4-(4′-fluorophenyl)piperazine (8c). Compound **8c** was synthesized from 1-(4-chlorobutoxy)-2-[2-(3-methoxyphenyl)ethyl]benzene **14c** (200 mg, 0.63 mmol) and from 1-(4-fluorophenyl)piperazine hydrochloride **17** (160 mg, 0.63 mmol) following the same procedure described above for the preparation of **5c**. The crude product was purified by column chromatography eluting with *n*-hexane/AcOEt (3:7) and transformation into the hydrochloride salt to give **8c** (64 mg, 0.16 mmol, 26% yield) as an white solid: mp 88−90 °C. MS *m/z* 462 (M^+, 13). ^{1}H NMR ($CDCl_3$): δ 1.90−2.20 (m, 4H, CH_2CH_2), 2.81−2.95 (m, 4H, CH_2CH_2), 3.15−3.32 (m, 2H, CH_2N), 3.42−3.59 (m, 4H, *piperazine*), 3.78 (s, 3H, OMe), 3.98−4.25 (m, 4H, *piperazine*), 4.73 (m, 2H, OCH_2), 6.65−6.96 (m, 5H, Ar), 7.11−7.23 (m, 6H, Ar), 7.88−8.00 (m, 1H, Ar) ppm. ^{13}C NMR ($CDCl_3$): δ 160.86, 158.62, 154.40, 149.11, 145.23, 130.57, 129.70, 128.44, 126.68, 122.99, 121.34, 120.30, 120.16, 116.49, 115.95, 114.30, 111.87, 111.58, 64.00, 55.97, 54.91, 52.66, 49.13, 37.07, 31.76, 31.15, 24.73. Anal. ($C_{29}H_{35}N_2O_2F\cdot$HCl) C, H, N. UV−vis (solvent: PBS) $\lambda = 230$ nm, $\varepsilon = 16480$.

5-{2-[2-(3-Methoxyphenyl)ethyl]phenoxy}pentyl-4-(4′-fluorophenyl)piperazine (8d). Compound **8d** was synthesized from 1-[(5-bromopentyloxy]-2-[2-(3-methoxyphenyl)ethyl]benzene **14d** (150 mg, 0.40 mmol) and from 1-(4-fluorophenyl)piperazine hydrochloride **17** (101 mg, 0.40 mmol) following the same procedure described above for the preparation of **5c**. The crude product was purified by transformation into the hydrochloride to give **8d** (125 mg, 0.24 mmol, 61% yield) as a white solid: mp 123−125 °C. MS *m/z* 476 (M^+, 15). ^{1}H NMR ($CDCl_3$): δ 1.56−1.70 (m, 2H, CH_2), 1.82−1.92 (m, 4H, CH_2CH_2), 2.79−2.92 (m, 4H, CH_2CH_2), 3.03−3.21 (m, 4H, *piperazine*), 3.41−3.66 (m, 6H, *piperazine*, CH_2N), 3.79 (s, 3H, OMe), 3.99 (t, 2H, $J = 5.5$ Hz, CH_2O), 6.68−6.90 (m, 5H, Ar), 7.06−7.28 (m, 7H, Ar) ppm. ^{13}C NMR ($CDCl_3$): δ 159.86, 156.80, 144.24, 130.70, 130.45, 130.09, 129.47, 127.40, 122.28, 121.06, 120.84, 117.55, 117.04, 114.74, 111.61, 111.23, 67.40, 57.59, 55.49, 50.19, 50.16, 36.59, 32.40, 29.13, 24.01, 23.75. Anal. ($C_{30}H_{37}N_2O_2F\cdot$HCl) C, H, N. UV−vis (solvent: PBS) $\lambda = 228$ nm, $\varepsilon = 24918$.

2-{2-[2-(3-Methoxyphenyl)ethyl]phenoxy}ethyl-4-(2′-pyrimidil)piperazine (9a). To a solution of 1-(2-chloroethoxy)-2-[2-(3-methoxyphenyl)ethyl]benzene **14a** (106 mg, 0.37 mmol) in a small amount of acetonitrile (3 mL) was added K_2CO_3 (112 mg, 0.81 mmol), KI (7 mg, 0.04 mmol), and 2-piperazinylpyrimidine dihydrochloride (87 mg, 0.37 mmol). The resulting suspension was heated for 10 min by microwave irradiation at 160 °C and with a power of 120 W. Then the potassium carbonate was filtered off and the solvent removed. The crude product was transformed into the hydrochloride to give **9a** (104 mg, 0.19 mmol, 34% yield) as a white solid: mp 164−166 °C. MS *m/z* 418 (M^+, 42). ^{1}H NMR ($CDCl_3$): δ 2.67 (t, 4H, $J = 5.1$ Hz, *piperazine*), 2.86−2.94 (m, 6H, CH_2CH_2, CH_2N), 3.77 (s, 3H, OMe), 3.81−3.87 (m, 4H, *piperazine*), 4.15 (t, 2H, $J = 5.7$ Hz, CH_2O), 6.47 (t, 1H, $J = 4.8$ Hz, Pyr), 6.72−6.91 (m, 5H, Ar), 7.10−7.24 (m, 3H, Ar), 8.30 (d, 2H, $J = 4.6$ Hz, Pyr) ppm. ^{13}C NMR (CD_3OD): δ 161.03, 158.63, 156.90, 150.46, 144.76, 131.48, 131.37, 130.21, 128.44, 122.92, 121.92, 115.40, 113.20, 112.51, 112.29, 64.11, 57.59, 55.68, 53.21, 42.66, 37.55, 32.59. Anal. ($C_{25}H_{30}N_4O_2\cdot$HCl) C, H, N. UV−vis (solvent: PBS) $\lambda = 250$ nm, $\varepsilon = 15820$.

3-{2-[2-(3-Methoxyphenyl)ethyl]phenoxy}propyl-4-(2′-pyrimidyl)piperazine (9b). Compound **9b** was synthesized from 1-(3-bromopropoxy)-2-[2-(3-methoxyphenyl)ethyl]benzene **14b** (246 mg, 0.70 mmol) and from 2-piperazinylpyrimidine dihydrochloride (273 mg, 0.70 mmol) following the same procedure described above for the preparation of **5c**. The crude residue was purified by transformation into the hydrochloride to give **9b** (107 mg, 0.25 mmol, 35% yield) as a white solid: mp 143−145 °C. MS *m/z* 432 (M^+, 35); 324 (100). ^{1}H NMR ($CDCl_3$): δ 2.05−2.17 (m, 2H, CH_2), 2.54−2.71 (m, 6H, *piperazine*, CH_2N), 2.81−2.90 (m, 4H, CH_2CH_2), 3.77 (s, 3H, OMe), 3.87−3.92 (m, 4H, *piperazine*), 4.03−4.14 (m, 2H, OCH_2), 6.50 (t, 1H, $J = 4.8$ Hz, Pyr), 6.73−6.91 (m, 5H, Ar), 7.10−7.21 (m, 3H, Ar), 8.31 (d, 2H, $J = 4.8$ Hz, Pyr) ppm. ^{13}C NMR (CD_3OD): δ 161.00, 158.45, 156.82, 148.12, 145.01, 131.32, 130.60, 130.23, 128.31, 122.03, 121.92, 115.46, 112.71, 112.36, 112.11, 65.99, 56.03, 55.72, 52.26, 46.92, 37.55, 32.92, 25.40. Anal. ($C_{26}H_{32}N_4O_2\cdot$HCl) C, H, N.

4-{2-[2-(3-Methoxyphenyl)ethyl]phenoxy}butyl-4-(2′-pyrimidyl)piperazine (9c). Compound **9c** was synthesized from 1-(4-chlorobutoxy)-2-[2-(3-methoxyphenyl)ethyl]benzene **14c** (180 mg, 0.57 mmol) and from 2-piperazinylpyrimidine dihydrochloride (134 mg, 0.57 mmol) following the same procedure described above for the preparation of **5c**. The crude residue was purified by transformation into the hydrochloride and crystallization from i-PrOH/i-Pr_2O to give **9c** (83 mg, 0.17 mmol, 30% yield) as a white solid: mp 130−132 °C. MS *m/z* 446 (M^+, 31). ^{1}H NMR ($CDCl_3$): δ 1.72−1.87 (m, 4H, CH_2CH_2), 2.43−2.51 (m, 6H, CH_2CH_2, CH_2N), 2.86−2.94 (m, 4H, *piperazine*), 3.77−3.83 (m, 7H, OMe, *piperazine*), 4.00 (t, 2H, $J = 5.5$ Hz, CH_2O), 6.47 (t, 1H, $J = 4.7$ Hz, Pyr), 6.71−6.89 (m, 5H, Ar), 7.09−7.20 (m, 3H, Ar), 8.30 (d, 2H, $J = 4.8$ Hz, Pyr) ppm. ^{13}C NMR (CD_3OD): δ 160.98, 158.69, 157.87, 145.10, 131.17, 130.90, 130.24, 128.27, 127.13, 121.85, 121.61, 115.37, 112.71, 112.47, 112.00, 68.17, 58.29, 55.74, 52.42, 42.77, 37.44, 33.36, 27.79,22.38. Anal. ($C_{27}H_{34}N_4O_2\cdot$HCl) C, H, N. UV−vis (solvent: PBS) $\lambda = 230$ nm, $\varepsilon = 17440$.

5-{2-[2-(3-Methoxyphenyl)ethyl]phenoxy}pentyl-4-(2′-pyrimidyl)piperazine (9d). Compound **9d** was synthesized from 1-[(5-bromopentyloxy]-2-[2-(3-methoxyphenyl)ethyl]benzene **14d** (400 mg, 1.06 mmol) and from 2-piperazinylpyrimidine dihydrochloride (250 mg, 1.06 mmol) following the same procedure described above for the preparation of **5c**. The crude product was transformed into the hydrochloride to give **9d** (146 mg, 0.32 mmol, 30% yield) as a white solid: mp 135−137 °C. ^{1}H NMR ($CDCl_3$): δ 1.56−1.66 (m, 2H, CH_2), 1.82−1.94 (m, 4H, CH_2CH_2), 2.85−3.05 (m, 8H,

CH_2CH_2, *piperazine*), 3.63−3.76 (m, 6H, *piperazine*, CH_2N), 3.77 (s, 3H, OMe), 3.97 (t, 2H, $J = 5.7$ Hz, CH_2O), 6.71−6.90 (m, 6H, Ar), 7.09−7.25 (m, 3H, Ar), 8.55 (d, 2H, $J = 4.8$ Hz, Ar) ppm. ^{13}C NMR ($CDCl_3$): δ 159.81, 157.62, 157.60, 156.80, 144.24, 130.40, 130.04, 129.42, 127.40, 121.04, 120.75, 114.70, 111.57, 111.34, 111.14, 67.38, 57.66, 55.42, 51.65, 41.73, 36.59, 32.48, 29.11, 23.97, 23.54. Anal. ($C_{28}H_{36}N_4O_2 \cdot HCl$) C, H, N. UV−vis (solvent: PBS) $\lambda = 227$ nm, $\varepsilon = 32206$.

2-{2-[2-(3-Methoxyphenyl)ethyl]phenoxy}ethyl-4-(*N*-methyl)piperazine (10a). Compound **10a** was synthesized from 1-(2-chloroethoxy)-2-[2-(3-methoxyphenyl)ethyl]benzene **14a** (200 mg, 0.68 mmol) and from *N*-methylpiperazine (70 mg, 0.68 mmol) following the same procedure described above for the preparation of **9a**. The crude residue was transformed into the hydrochloride and crystallized from i-PrOH to give **10a** (54 mg, 0.15 mmol, 22% yield) as a white solid: mp 165−167 °C. ^1H NMR ($CDCl_3$): δ 2.28 (s, 3H, Me), 2.30−2.48 (m, 4H, *piperazine*), 2.50−2.71 (m, 4H, *piperazine*), 2.81−2.89 (m, 6H, CH_2CH_2, CH_2N), 3.78 (s, 3H, OMe), 4.11 (t, 2H, $J = 5.6$ Hz, OCH_2), 6.73−6.90 (m, 5H, Ar),7.09−7.25 (m, 3H, Ar) ppm. ^{13}C NMR (CD_3OD): δ 161.50, 156.94, 146.56, 131.60, 131.37, 130.24, 128.41, 122.92, 122.00, 115.51, 113.27, 112.25, 64.44, 57.43, 55.70, 51.48, 50.77, 43.45, 37.49, 32.63. Anal. ($C_{22}H_{30}N_2O_2 \cdot HCl$) C, H, N. UV−vis (solvent: PBS) $\lambda = 271$ nm, $\varepsilon = 2620$.

3-{2-[2-(3-Methoxyphenyl)ethyl]phenoxy}propyl-4-(3′-methyl)piperazine (10b). Compound **10b** was synthesized from 2-[2-(3-methoxyphenyl)]ethyl]phenol **13** (500 mg, 2.19 mmol) and 1-(3-chloropropyl)-4-methylpiperazine (390 mg, 2.19 mmol) following the same procedure described above for the preparation of **5b**. The crude residue was purified by transformation into the hydrochloride and crystallization from EtOH to give **10b** (380 mg, 1.03 mmol, 47% yield) as a white solid: mp 194−196 °C. MS *m/z* 368 (M^+, 22). ^1H NMR (DMSO): δ 2.10−2.32 (m, 2H, CH_2), 2.51−2.95 (m, 7H, CH_2CH_2, Me), 3.10−3.67 (m, 10H, *piperazine*, CH_2N), 3.71 (s, 3H, OMe), 4.06 (t, 2H, $J = 5.9$ Hz, OCH_2), 6.70−7.05 (m, 5H, Ar), 7.09−7.35 (m, 3H, Ar) ppm. ^{13}C NMR ($CDCl_3$): δ 159.91, 156.25, 149.53, 130.76, 130.41, 129.75, 128.40, 121.93, 120.51, 114.38, 111.87, 111.54, 66.44, 55.89, 55.70, 55.20, 52.67, 46.94, 43.21, 36.77, 29.27, 24.65. Anal. ($C_{23}H_{32}N_2O_2 \cdot HCl$) C, H, N. UV−vis (solvent: PBS) $\lambda = 278$ nm, $\varepsilon = 4110$.

4-{2-[2-(3-Methoxyphenyl)ethyl]phenoxy}butyl-4-(*N*-methyl)piperazine (10c). Compound **10c** was synthesized from 1-(4-chlorobutoxy)-2-[2-(3-methoxyphenyl)ethyl]benzene **14c** (180 mg, 0.57 mmol) and from *N*-methylpiperazine (57 mg, 0.57 mmol) following the same procedure described above for the preparation of **5c**. The crude residue was purified by transformation into hydrochloride and crystallized from i-PrOH/i-Pr_2O to give **10c** (47 mg, 0.11 mmol, 20% yield) as a white solid: mp 168−170 °C. MS *m/z* 382 (M^+, 30). ^1H NMR ($CDCl_3$): δ 1.66−1.87 (m, 8H), 2.28 (s, 3H, Me), 2.39−2.46 (m, 6H, *piperazine*, CH_2N), 2.81−2.94 (m, 4H, CH_2CH_2), 3.78 (s, 3H, OMe), 3.98 (t, 2H, $J = 5.8$ Hz, CH_2O), 6.72−6.89 (m, 5H, Ar), 7.09−7.24 (m, 3H, Ar) ppm. ^{13}C NMR (CD_3OD): δ 158.00, 145.02, 131.82, 130.90, 130.97, 130.19, 128.24, 121.91, 121.63, 115.37, 112.47, 112.11, 68.03, 57.40, 55.66, 51.24, 43.70, 40.70, 37.78, 37.46, 33.34, 27.64, 22.23. Anal. ($C_{24}H_{34}N_2O_2 \cdot HCl$) C, H, N. UV−vis (solvent: PBS) $\lambda = 271$ nm, $\varepsilon = 2460$.

5-{2-[2-(3-Methoxyphenyl)ethyl]phenoxy}pentyl-4-(*N*-methyl)piperazine (10d). Compound **10d** was synthesized from 1-[(5-bromopentyloxy]-2-[2-(3-methoxyphenyl)ethyl]benzene **14d** (400 mg, 1.06 mmol) and from *N*-methylpiperazine (106 mg, 1.06 mmol) following the same procedure described above for the preparation of **5c**. The crude residue was purified by transformation into the hydrochloride to give **10d** (115 mg, 0.26 mmol, 25% yield) as a white solid: mp 217−219 °C. MS *m/z* 396 (M^+, 28). ^1H NMR ($CDCl_3$): δ 1.52−1.65 (m, 2H, CH_2), 1.81−2.01 (m, 4H, CH_2CH_2), 2.86−2.90 (m, 7H, CH_2CH_2, Me), 3.03−3.11 (m, 4H, *piperazine*), 3.48−3.60 (m, 6H, *piperazine*, CH_2N), 3.79 (s, 3H, OMe), 3.88−4.06 (m, 2H, CH_2O), 6.72−6.91 (m, 5H, Ar), 7.10−7.23 (m, 3H, Ar) ppm. ^{13}C NMR ($CDCl_3$): δ 159.75, 156.71, 144.11, 130.36, 130.04, 139.42, 127.30, 121.00, 120.75, 114.60, 111.50, 111.26, 67.29, 57.22, 55.46, 50.10, 48.50, 43.20, 36.50, 32.35, 29.02, 23.72, 23.65. Anal. ($C_{25}H_{36}N_2O_2 \cdot HCl$) C, H, N. UV−vis (solvent: PBS) $\lambda = 271$ nm, $\varepsilon = 3056$.

2-{2-[2-(3-Methoxyphenyl)ethyl]phenoxy}ethyl-(3,4-dihydroisoquinoline) (11a). A stirred solution of KOH (360 mg, 6.40 mmol) in DMSO (2 mL) was heated to 80 °C until the complete dissolution of KOH. The solution was treated with a solution of 2-[2-(3-methoxyphenyl)]ethyl]phenol **13** (420 mg, 1.83 mmol) in DMSO (3 mL) and the resulting suspension was vigorously stirred for 20 min. Then a solution of 2-(2-chloroethyl)-1,2,3,4-tetrahydroisoquinoline (360 mg, 1.83 mmol) dissolved in DMSO (2 mL) was added and the stirring was continued for 24 h at 40 °C. The mixture was eluting with AcOEt and washed with water, 1N NaOH, and brine. The organic layer was evaporated to give a crude oil which was purified by transformation into the hydrochloride and crystallization from i-PrOH to give **11a** (194 mg, 0.46 mmol, 25% yield) as a white solid: mp: 156.158 °C. MS *m/z* 387 (M^+, 27); 146 (100). ^1H NMR ($CDCl_3$): δ 2.86−2.95 (m, 8H), 3.02 (t, 2H, $J = 5.9$ Hz, CH_2N), 3.76 (s, 3H, OMe), 3.82 (s, 2H, CH_2), 4.22 (t, 2H, $J = 5.9$ Hz, CH_2O), 6.72−6.92 (m, 5H, Ar), 7.04−7.22 (m, 7H, Ar) ppm. ^{13}C NMR ($CDCl_3$): δ 159.68, 155.33, 143.49, 130.42, 130.11, 129.80, 129.29, 128.91, 128.45, 127.61, 127.47, 126.94, 126.60, 121.90, 120.80, 114.41, 111.77, 111.26, 63.43, 55.26, 54.24, 53.25, 49.70, 36.52, 31.71,24.45. Anal. ($C_{26}H_{29}NO_2 \cdot HCl$) C, H, N. UV−vis (solvent: PBS) $\lambda = 230$ nm, $\varepsilon = 16040$.

3-{2-[2-(3-Methoxyphenyl)ethyl]phenoxy}propyl-(3,4-dihydroisoquinoline) (11b). A solution of K_2CO_3 (142 mg, 1.03 mmol) in THF (5 mL) was heated at 80 °C until the complete dissolution of K_2CO_3. Then a solution of 1,2,3,4-tetrahydroisoquinoline (172 mg, 1.29 mmol) in THF (7 mL) was added, and the resulting solution, cooled at 0 °C, was treated with a solution of 1-(3-bromopropoxy)-2-[2-(3-methoxyphenyl)ethyl]benzene **14b** (300 mg, 0.86 mmol) in THF (3 mL). The suspension was stirred at room temperature for 7 h, then KI (35 mg, 0.21 mmol) and DMF (1 mL) were added and the stirring was continued for 40 h. After this period, the solvent was evaporated, water was added, and the solution was extracted with AcOEt. The organic layer was dried and evaporated. The crude product was purified by column chromatography eluting with $CHCl_3/Et_2O$ (8:2) to give **11b** (233 mg, 0.58 mmol, 45% yield) as an white solid: mp 199−201 °C. MS *m/z* 401 (M^+, 27). ^1H NMR ($CDCl_3$): δ 2.12−2.19 (m, 2H, CH_2), 2.75−2.94 (m, 10H), 3.71 (s, 2H, CH_2), 3.77 (s, 3H, OMe), 4.07 (t, 2H, $J = 6.0$ Hz, CH_2O), 6.72−6.89 (m, 4H, Ar), 7.07−7.21 (m, 8H, Ar) ppm. ^{13}C NMR ($CDCl_3$): δ 156.09, 143.87, 141.84, 130.75, 130.20, 130.09, 129.40, 129.38, 128.91, 128.52, 127.52, 126.96, 126.61, 121.31, 120.88, 114.69, 111.99, 110.95, 65.05, 55.29, 53.23, 52.61, 49.26, 36.56, 31.86, 24.74, 24.56. Anal. ($C_{27}H_{31}NO_2$) C, H, N. UV−vis (solvent: PBS) $\lambda = 230$ nm, $\varepsilon = 14790$.

4{2-[2-(3-Methoxyphenyl)ethyl]phenoxy}butyl-(3,4-dihydroisoquinoline) (11c). Compound **11c** was synthesized from 1-(4-chlorobutoxy)-2-[2-(3-methoxyphenyl)ethyl]benzene **14c** (200 mg, 0.63 mmol) and from 1,2,3,4-tetrahydroisoquinoline (84 mg, 0.63 mmol) following the same procedure described above for the preparation of **5c**. The crude product was purified by column chromatography eluting with *n*-hexane/AcOEt (3:7) and transformation into the hydrochloride to give **11c** (60 mg, 0.13 mmol, 21% yield) as a white solid: mp: 115−117 °C. MS *m/z* 415 (M^+, 8); 146 (M^+, 100). ^1H NMR ($CDCl_3$): δ 1.80−1.92 (m, 4H, CH_2CH_2), 2.60 (t, 2H, $J = 6.8$ Hz, CH_2), 2.69−2.75 (m, 2H, CH_2), 2.83−2.98 (m, 6H, CH_2CH_2, CH_2N), 3.63 (s, 2H, CH_2), 3.77 (s, 3H, OMe), 4.02 (t, 2H, $J = 5.6$ Hz, CH_2O), 6.71−6.90 (m, 5H, Ar),6.96−7.24 (m, 7H, Ar) ppm. ^{13}C NMR ($CDCl_3$): δ 159.90, 156.58, 144.13, 130.82, 130.22, 130.00, 129.45, 128.96, 128.58, 127.60, 127.51, 127.05, 126.69, 121.02, 120.93, 114.67, 111.54, 111.12, 67.16, 55.36, 55.07, 52.27, 48.85, 36.45, 32.35, 27.16, 24.46, 21.66. Anal. ($C_{28}H_{33}NO_2 \cdot HCl$) C, H, N. UV−vis (solvent: PBS) $\lambda = 226$ nm, $\varepsilon = 42580$.

5-{2-[2-(3-Methoxyphenyl)ethyl]phenoxy}pentyl-(3,4-dihydroisoquinoline) (11d). Compound **11d** was synthesized from 1-[(5-bromopentyloxy]-2-[2-(3-methoxyphenyl)ethyl]benzene **14d**

(400 mg, 1.06 mmol) and from 1,2,3,4-tetrahydroisoquinoline (141 mg, 1.06 mmol) following the same procedure described above for the preparation of **5c**. The crude product was purified by transformation into the hydrochloride and crystallization from EtOH/i-PrO_2 to give **11d** (160 mg, 0.34 mmol, 32% yield) as a white solid: mp 129−131 °C. MS *m/z* 429 (M^+, 11). ^{1}H NMR ($CDCl_3$): δ 1.52−1.70 (m, 4H, CH_2CH_2), 1.85−1.92 (m, 2H, CH_2), 1.98−2.19 (m, 2H, CH_2), 2.80−2.92 (m, 4H, CH_2CH_2), 2.85−3.17 (m, 4H, CH_2, CH_2N), 3.43−3.63 (m, 2H, CH_2), 3.74 (s, 3H, OMe), 3.98 (t, 2H, J = 5.8 Hz, CH_2O), 6.68−6.90 (m, 5H, Ar), 7.06−7.28 (m, 7H, Ar) ppm. ^{13}C NMR ($CDCl_3$): δ 159.79, 156.82, 144.22, 130.82, 130.38, 130.02, 129.36, 129.00, 128.60, 127.61, 127.38, 127.03, 126.72, 121.01, 120.71, 114.63, 111.54, 111.14, 67.42, 55.38, 55.11, 52.29, 48.97, 36.57, 32.51, 29.13, 24.50, 24.08, 24.03. Anal. ($C_{29}H_{35}NO_2$·HCl) C, H, N. UV−vis (solvent: PBS) λ = 227 nm, ε = 32206.

2-{2-[2-(3-Methoxyphenyl)ethyl]phenoxy}ethyl-(6,7-dimethoxy-3,4-dihydroisoquinoline) (12a). Compound **12a** was synthesized from 1-(2-chloroethoxy)-2-[2-(3-methoxyphenyl)ethyl]benzene **14a** (150 mg, 0.44 mmol) and from 6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline (100 mg, 0.44 mmol) following the same procedure described above for the preparation of **5c**. The crude product was purified by transformation into the hydrochloride and crystallization from EtOH to give **12a** (89 mg, 0.18 mmol, 42% yield) as a white solid: mp 184−186 °C. MS *m/z* 447 (M^+, 16). ^{1}H NMR (DMSO): δ 2.83−2.99 (m, 6H, CH_2CH_2, CH_2N), 3.10−3.45 (m, 4H, CH_2), 3.63−3.73 (m, 11H, CH_2, OMe), 4.06−4.15 (m, 2H, CH_2O), 6.65−7.04 (m, 7H, Ar), 7.13−7.25 (m, 3H, Ar) ppm. ^{13}C NMR (DMSO): δ 159.00, 155.29, 143.09, 129.65, 129.43, 128.90, 127.03, 123.73, 123.04, 120.84, 120.35, 120.25, 113.90, 111.90, 111.61, 110.99, 110.08, 109.62, 62.64, 55.56, 55.49, 54.72, 54.01, 52.12, 49.28, 35.48, 31.18, 24.06. Anal. ($C_{28}H_{33}NO_4$·HCl) C, H, N. UV−vis (solvent: PBS) λ = 279 nm, ε = 2426.

3-{2-[2-(3-Methoxyphenyl)ethyl]phenoxy}propyl-(6,7-dimethoxy-3,4-dihydroisoquinoline) (12b). Compound **12b** was synthesized from 1-(3-bromopropoxy)-2-[2-(3-methoxyphenyl)ethyl]benzene **14b** (150 mg, 0.42 mmol) and from 6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline (96 mg, 0.42 mmol) following the same procedure described above for the preparation of **5c**. The crude product was purified by transformation into the hydrochloride to give **12b** (158 mg, 0.32 mmol, 76% yield) as a white solid: mp 138−140 °C. MS *m/z* 461 (M^+, 27). ^{1}H NMR ($CDCl_3$): δ 2.04−2.16 (m, 2H, CH_2), 2.44−2.58 (m, 2H, CH_2), 2.75−2.89 (m, 4H, CH_2CH_2), 3.18−3.39 (m, 4H, CH_2, CH_2N), 3.60−3.74 (m, 2H, CH_2), 3.70 (s, 3H, OMe), 3.81 (s, 3H, OMe), 3.82 (s, 3H, OMe), 4.07 (t, 2H, J = 5.1, CH_2O), 6.52 (s, 1H, Ar), 6.58 (s, 1H, Ar), 6.67−6.72 (m, 3H, Ar), 6.79−6.93 (m, 2H, Ar), 7.10−7.21 (m, 3H, Ar) ppm. ^{13}C NMR ($CDCl_3$): δ 159.81, 156.13, 149.68, 149.03, 143.93, 130.24, 130.18, 129.40, 127.56, 122.88, 121.37, 120.93, 118.44, 114.74, 111.76, 111.66, 110.03, 109.90, 65.12, 56.35, 56.26, 55.36, 53.07, 52.49, 49.41, 36.65, 32.00, 24.83, 24.21. Anal. ($C_{29}H_{35}NO_4$·HCl) C, H, N. UV−vis (solvent: PBS) λ = 278 nm, ε = 4112.

4-{2-[2-(3-Methoxyphenyl)ethyl]phenoxy}butyl-(6,7-dimethoxy-3,4-dihydroisoquinoline) (12c). Compound **12c** was synthesized from 1-(4-chlorobutoxy)-2-[2-(3-methoxyphenyl)ethyl]benzene **14c** (200 mg, 0.55 mmol) and from 6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline (126 mg, 0.55 mmol) following the same procedure described above for the preparation of **5c**. The crude product was purified by transformation into the hydrochloride and crystallized from EtOH/i-Pr_2O to give **12c** (144 mg, 0.28 mmol, 51% yield) as a white solid: mp 149−151 °C. MS *m/z* 475 (M^+, 44). ^{1}H NMR ($CDCl_3$): δ 1.63−1.72 (m, 2H, CH_2), 1.89−2.00 (m, 2H, CH_2), 2.15−2.28 (m, 2H, CH_2), 2.75−2.92 (m, 4H, CH_2CH_2), 3.05−3.38 (m, 4H, CH_2, CH_2N), 3.46−3.64 (m, 2H, CH_2), 3.74 (s, 3H, OMe), 3.79 (s, 3H, OMe), 3.84 (s, 3H, OMe), 3.98−4.09 (m, 2H, CH_2O), 6.41 (s, 1H, Ar), 6.58 (s, 1H, Ar), 6.70−6.93 (m, 5H, Ar), 7.11−7.23 (m, 3H, Ar) ppm. ^{13}C NMR ($CDCl_3$): δ 159.88, 156.10, 150.68, 149.00, 144.15, 130.15, 130.02, 129.20, 127.51, 122.50, 121.11, 120.93, 118.42, 114.67, 111.25, 111.14, 110.05, 109.76, 65.30, 56.29, 56.27, 54.91, 52.70, 52.14, 49.01, 36.52, 32.48, 24.75, 24.12, 21.75. Anal. ($C_{30}H_{37}NO_4$·HCl) C, H, N. UV−vis (solvent: PBS) λ = 228 nm, ε = 26690.

5-{2-[2-(3-Methoxyphenyl)ethyl]phenoxy}propyl-(6,7-dimethoxy-3,4-dihydroisoquinoline) (12d). Compound **12d** was synthesized from 1-(4-chlorobutoxy)-2-[2-(3-methoxyphenyl)ethyl]benzene **14d** (150 mg, 0.40 mmol) and from 6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline (92 mg, 0.40 mmol) following the same procedure described above for the preparation of **5c**. The crude product was purified by transformation into the hydrochloride to give **12d** (67 mg, 0.13 mmol, 32% yield) as a white solid: mp 136−138 °C. MS *m/z* 489 (M^+, 21). ^{1}H NMR ($CDCl_3$): δ 1.55−1.70 (m, 2H, CH_2), 1.74−1.82 (m, 2H, CH_2), 1.84−1.99 (m, 2H, CH_2), 2.00−2.18 (m, 2H, CH_2), 2.80−2.94 (m, 4H, CH_2CH_2), 3.05−3.28 (m, 4H, CH_2), 3.30−3.68 (m, 2H, CH_2N), 3.75 (s, 3H, OMe), 3.85 (s, 6H, OMe), 3.93−4.05 (m, 2H, CH_2O), 6.41 (s, 1H, Ar), 6.58 (s, 1H, Ar), 6.70−6.93 (m, 5H, Ar), 7.11−7.23 (m, 3H, Ar) ppm. ^{13}C NMR ($CDCl_3$): δ 159.88, 156.10, 150.68, 149.00, 144.15, 130.15, 130.02, 129.20, 127.51, 122.50, 121.11, 120.93, 118.42, 114.67, 111.25, 111.14, 110.05, 109.76, 65.30, 56.29, 56.27, 54.91, 52.70, 52.14, 49.01, 36.52, 32.48, 24.75, 24.12, 21.75. Anal. ($C_{31}H_{39}NO_4$·HCl) C, H, N. UV−vis (solvent: PBS) λ = 228 nm, ε = 21840.

Biological Method. Cell lines. The breast cancer cell line of human origin, MCF-7/Adr (resistant to adriamycin or doxorubicin), was routinely cultured in RPMI 1640 supplemented with 10% fetal bovine serum, 2 mM glutamine, 100 U/mL penicillin, and 100 μg/mL streptomycin in a humidified incubator at 37 °C with a 5% CO_2 atmosphere. Caco-2 cells were grown in DMEM medium with 10% heat-inactivated fetal bovine serum, 100 U/mL penicillin, 100 μg/mL streptomycin, and 2 mM L-glutamine. CulturePlate 96-well plates were purchased from Perkin-Elmer Life Science and Calcein-AM from Molecular Probes (Eugene, OR). Verapamil was purchased from Tocris Bioscience (Bristol, United Kingdom).

Permeability Experiments. Preparation of Caco-2 Monolayer. This procedure has been previously reported by Colabufo et al.[27] Briefly, Caco-2 cells were harvested with trypsin-EDTA and seeded onto MultiScreen Caco-2 assay system at a density of 10000 cells/well. The culture medium was replaced every 48 h for the first 6 days and every 24 h thereafter, and after 21 days in culture, the Caco-2 monolayer was utilized for the permeability experiments. The transepithelial electrical resistance (TEER) of the monolayers was measured daily before and after the experiment using a epithelial voltohommeter (Millicell -ERS; Millipore, Billerica, MA). Generally, TEER values obtained are greater than 1000 Ω for a 21 day culture.

Drug Transport Experiment. Apical to basolateral (A → B) and basolateral to apical (B → A) permeability of drugs were measured at 120 min and at various drugs concentrations (1−100 μM).[19] Drugs were dissolved in Hank's balanced salt solution (HBSS, pH 7.4) and sterile filtered. After 21 days of cell growth, the medium was removed from filter wells and from the receiver plate. The filter wells were filled with 75 μL of fresh HBSS buffer and the receiver plate with 250 μL per well of the same buffer. This procedure was repeated twice, and the plates were incubated at 37 °C for 30 min. After incubation time, the HBSS buffer was removed and drug solutions added to the filter well (75 μL). HBSS without the drug was added to the receiver plate (250 μL). The plates were incubated at 37 °C for 120 min. After incubation time, samples were removed from the apical (filter well) and basolateral (receiver plate) side of the monolayer and then were stored in a freezer (−20 °C) pending analysis. The concentration of compounds were analyzed using UV−vis spectroscopy. The apparent permeability (P_{app}), in units of nm/second, was calculated using the following equation:

$$P_{app} = \left(\frac{V_A}{\text{area} \times \text{time}}\right) \times \left(\frac{[\text{drug}]_{\text{acceptor}}}{[\text{drug}]_{\text{initial}}}\right) \quad (1)$$

Where V_A is the volume (in mL) in the acceptor well, area is the surface area of the membrane (0.11 cm^2 of the well), time is the total transport time in seconds (7200 s), $[\text{drug}]_{\text{acceptor}}$ is the concent-

ration of the drug measured by UV spectroscopy, and $[drug]_{initial}$ is the initial drug concentration (1×10^{-4} M) in the apical or basolateral wells.

Cell ATP Availability Assay. This experiment was performed as reported in technical sheet of ATPlite Kit for luminescence ATP detection using Victor3, from PerkinElmer Life Sciences.[28] Caco-2 cells were seeded into 96-well microplate in 100 μL of complete medium at a density 2×10^4 cells/well. The plate was incubated overnight in a humidified atmosphere 5% CO_2 at 37 °C. The medium was removed and 100 μL of complete medium in the presence or the absence of different concentrations (from 1 to 100 μM) of tested compounds was added. The plate was incubated for 2 h in a humidified atmosphere 5% CO_2 at 37 °C. Then, 50 μL of mammalian cell lysis solution was added to all wells and the plate stirred for 5 min in an orbital shaker. In all wells, 50 μL of substrate solution was added and the plate stirred for 5 min as above-reported. The plate was dark adapted for 10 min, and the luminescence was measured in Victor3, from PerkinElmer Life Sciences.

[^{3}H] Substrate Transport Inhibition. Caco-2 cells were seeded onto multiscreen plates, 10000 cells/well, for 21 days measuring the integrity of the cell monolayers by transepithelial electrical resistance (TEER, $\Omega \times cm^2$) with an epithelial voltohommeter. Mature Caco-2 cell monolayer exhibited a TEER > 800 $\Omega \times cm^2$ prior to use in transport experiments. Transport experiments for tested compounds were carried out as described by Taub et al.[26]

In each well to basolateral (BL) compartment in the absence and in the presence of ABCB1 inhibitors (from 200 nM to 400 μM) was added 20 nM [^{3}H]vinblastine for 120 min at 37 °C and its appearance in the apical (AP) compartment was monitored. At 120 min, a 20 μL sample was taken from donor compartment to determine the concentration of radioligand remaining in the donor chamber at the end of the experiment. Samples were analyzed using LS6500 Beckman Counter. For each compound, [^{3}H]vinblastine transport inhibition was calculated as radioactivity difference between radioligand in the presence and the absence of compound. These differences were expressed as inhibition percentage at single drug concentration.

[^{3}H] Mithoxantrone Transport Inhibition. Caco-2 cells were seeded onto multiscreen plates, 10000 cells/well, for 21 days measuring the integrity of the cell monolayers by transepithelial electrical resistance (TEER, $\Omega \times cm^2$) with an epithelial voltohommeter. Mature Caco-2 cell monolayer exhibited a TEER > 800 $\Omega \times cm^2$ prior to use in transport experiments. Transport experiments for tested compounds were carried out as previously described.[14,26]

In each well to basolateral (BL) compartment in the absence and in the presence of ABCB1 inhibitors (100 μM) was added 20 nM [^{3}H]mithoxantrone for 120 min at 37 °C and its appearance in the apical (AP) compartment was monitored. At 120 min, a 20 μL sample was taken from donor compartment to determine the concentration of radioligand remaining in the donor chamber at the end of the experiment. Samples were analyzed using LS6500 Beckman Counter. For each compound, [^{3}H]mithoxantrone transport inhibition was calculated as radioactivity difference between radioligand in the presence and absence of compound. These differences were expressed as percentage of inhibition effect for each single drug concentration.

Calcein-AM Assay in MDCK-ABCB1 Cells. MDCK-ABCB1 cells (a gift of Prof. P. Borst, NKI-AVL Institute, Amsterdam, Netherlands) were grown in DMEM high glucose supplemented with 10% fetal bovine serum, 2 mM glutamine, 100 U/mL penicillin, and 100 μg/mL streptomycin in a humidified incubator at 37 °C with a 5% CO_2 atmosphere. In this assay calcein accumulation in transfected MDCK cells has been monitored. Intact cells are incubated with acetoxymethyl (AM) esters of calcein (Calcein-AM), a lipophilic ABCB1 substrate that diffuses across the plasma membrane into the cell, where it is hydrolyzed by endogenous cytoplasmic esterases into highly fluorescent calcein. The hydrolyzed compound is not a MDR proteins substrate, and because it is hydrophilic, it cannot cross the cell membrane via passive diffusion. Thus, a rapid increase in the fluorescence of cytoplasmic calcein can be monitored. MDR pump, present in the plasma membrane, rapidly effluxes the calcein-AM before its entrance into the cytosol, determining a reduction in the fluorescent signal due to the accumulation of calcein. Evaluation of ABCB1 activity in the presence of pump inhibitors can be performed in a competitive manner. Compounds that block ABCB1 pump inhibit Calcein-AM efflux, increasing fluorescent calcein accumulation. This experiment was carried out as described by Feng et al.[29] with minor modifications. The cells (50000 cells per well) were seeded into black CulturePlate 96-well plate with 100 μL medium and allowed to become confluent overnight. Test compounds were solubilized in 100 μL of culture medium and added to monolayers. The 96-wells plate was incubated at 37 °C for 30 min. Calcein-AM was added in 100 μL of phosphate buffered saline (PBS) to yield a final concentration of 2.5 μM and the plate was incubated for 30 min. Each well was washed 3 times with ice cold PBS. Saline buffer was added to each well, and the plate was read to Victor3 fluorometer (PerkinElmer) at excitation and emission wavelengths of 485 and 535 nm, respectively. In these experimental conditions, calcein cell accumulation in the absence and in the presence of tested compounds was evaluated and fluorescence basal level was estimated by untreated cells. In treated wells, the increase of fluorescence with respect to basal level was measured. IC_{50} values were determined by fitting[30] the fluorescence increase percentage versus log[dose].

Radioligand Binding Assay at Rat Human Cloned 5-HT$_{1A}$ Receptor. Human 5-HT$_{1A}$ serotonin receptors stably expressed in HeLa cells were radiolabeled with 1.0 nM [^{3}H]-8-OH-DPAT (K_d = 8.8 nM).[21] Samples containing 40 μg of membrane protein, different concentrations of each compound ranging from 0.1 nM to 10 μM were incubated in a final volume of 500 μL of 50 mM Tris-HCl, pH 7.4, 5 mM $MgSO_4$ for 120 min at 37 °C. After this incubation time, samples were filtered through GF/C presoaked in polyethylenimine 0.5% for at least 30 min prior to use. The filters were washed twice with 1 mL of ice-cold buffer (50 mM Tris-HCl, pH 7.4). Nonspecific binding was determined in the presence of 10 μM 8-OH-DPAT.

Radioligand Binding Assay at Human Cloned D$_2$ Dopaminergic Receptors in C6 Rat Glioma. Binding of [^{3}H]spiroperidol at rat cloned D_2 receptor was performed according to Leopoldo et al.[22] with minor modifications. The reaction buffer consisted of 50 mM Tris, 10 mM MgCl2, 1 mM EDTA (pH 7.4), including 200 μg of dopamine D_2 receptor diluted membranes, 0.40 nM [^{3}H]spiroperidol (K_d = 0.20 nM), and 100 μL of the drug solution (six to nine concentrations) for a total volume of 1 mL. Samples were incubated at 27 °C for 60 min, then the incubation was stopped by rapid filtration through Whatman GF/C glass fiber filters (presoaked in 0.3% polyethylenimine). The filters were washed twice with 1 mL of ice-cold buffer (50 mM Tris, pH 7.4). Nonspecific binding was defined in the presence of 10 μM haloperidol.

Statistical Analysis. The IC_{50} values of the compounds reported in Tables 1, 2, and 4, and K_i values reported in Table 3 were determined by nonlinear curve fitting utilizing the GraphPad Prism program.[30]

The values r^2 have been determined by linear regression plotting the length of the spacer (from 2 to 4 methylene groups) vs pIC_{50} value for each series calculating the 95% confidence interval of the regression line (GraphPad Prism program).[30]

Supporting Information Available: Experimental procedures for the intermediate compounds, a table reporting the combustion analysis data of the final products and a table reporting the affinity binding values toward 5-HT$_{1a}$ and D_2 receptors. Flow CytoMetry (FCM) procedure and the plots for visualizing ABCB1 and ABCG2 in Caco-2 cells and ABCB1 in MDCK-ABCB1 cells. This material is available free of charge via the Internet at http://pubs.acs.org.

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