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Structure-Activity Relationship Studies on 6,7-Dimethoxy-2-phenethyl-1,2,3,4-tetrahydroisoquinoline Derivatives as Multidrug Resistance (MDR) reversers

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Abstract

A series of derivatives were synthesized and studied with the aim to investigate the structure-activity relationships of the two P-glycoprotein (P-gp) modulators elacridar and tariquidar. Then different aryl substituted amides were inserted and to explore the effects of varying the amide function, the corresponding isosteric ester derivatives and some alkylamine analogues were synthesized. The new compounds were studied to evaluate their P-gp interaction profile and selectivity towards the two other ABC transporters, Multidrug-Resistance-associated Protein-1 (MRP-1) and Breast Cancer Resistance Protein (BCRP). The investigation on the chemical stability of amide and ester derivatives towards spontaneous or enzymatic hydrolysis, showed that these compounds resulted stable in phosphate buffer solution and human plasma. This study allowed us to evaluate the selectivity of the three series on the three efflux pumps and to propose the structural requirements that define the P-gp interaction profile. We identified two P-gp substrates and a P-gp inhibitor and three ester derivatives active on BCRP that opens a new scenario in the development of ligands active towards this pump.

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Introduction

The transmembrane proteins of the ABC (ATP Binding Cassette) transporter family are able to transport a variety of compounds across the cell membrane using ATP hydrolysis energy. These proteins are expressed in many tissues where they play important protective roles by regulating the permeability of biological membranes.[1] Several ABC transporters are overexpressed in cancer cells thus causing their chemoresistance, as these proteins can bind a large number of hydrophobic compounds including anticancer drugs.[2-4] Many structurally and mechanistically unrelated chemotherapeutic drugs are ABC proteins substrates and are actively pumped out by the transporters located at the plasma membrane of the tumor cells resulting in a decrease of their cellular concentration below the effective dose.[1] This cancer cells defence mechanism is called multidrug resistance (MDR) and represents the main obstacle to achieve success with chemotherapy; since even in cases when the tumor initially responds to chemotherapy, resistance can rapidly develop as a results of drug treatment.[5]

The ABC transporter family includes structurally related membrane proteins that share a common feature of being made of two domains: the nucleotide binding domain (NBD) and the transmembrane spanning domain (TMD).[6] The function of NBD is to hydrolyse ATP and the resulting energy is used by the TMD to translocate substances through the membrane by conformational changes.[7]

The human genome encodes 49 ABC transporters classified into seven subfamilies (ABC-A to ABC-G). Among the transporters involved in drug efflux from human cells, three are mainly associated with multidrug resistance (MDR): P-glycoprotein (P-gp, ABCB1), the Multidrug-Resistance-associated Protein-1 (MRP1, ABCC1), and the Breast Cancer Resistance Protein (BCRP, ABCG2).[8-11]

P-gp is the most studied ABC transporter[12] and is the first efflux transporter discovered to be involved in drug resistance.[13] In fact, P-gp is overexpressed in many cancer cells as a result of chemotherapy treatment causing an acquired resistance to a variety of anticancer drugs.[2-4] Moreover, in addition to the decrease in the intracellular concentration of chemotherapeutic agents, P-gp might display other mechanisms of conferring resistance. P-gp overexpressing cells are less sensitive to caspase-dependent apoptosis induced by a range of different stimuli, including Fas ligand, tumor necrosis factor, UV irradiation, and serum starvation. Thus the cells with high P-gp expression may have a higher survival rate or less apoptosis induction.[13]

MRP1 was discovered for the first time in not expressing P-gp cancer cells that showed resistance to chemotherapeutics treatment due to increased efflux of anticancer drugs.[14,15] This transporter has broad spectrum anticancer resistance activity and for this reason it belongs to the three main MDR proteins. Overexpression of this efflux pump was shown to be associated with resistance to many anticancer drugs such as cisplatin, etoposide, doxorubicin, vincristine, methotrexate and purine analogs.[16]

BCRP is the most recently ABC transporter identified to be involved in multidrug-resistance ~~BCRP~~. Differently from P-gp and MRP1, BCRP is a half-transporter which requires at least dimerization,[17] or even tetramerization,[18,19] to be functional. Many evidences suggest that BCRP transporter is expressed in several hematological and solid tumors, together with P-gp, compromising the therapeutic effectiveness of several substrate agents.[20,21] Moreover, BCRP is also expressed in cancer stem cells, thus causing direct protection against chemotherapeutics.[22,23]

Cancer stem cells are capable of long-term self-renewal and are probably responsible for long-term failure of many cancer chemotherapies.

BCRP and P-gp are the two dominant ABC transporters located at the blood-brain barrier (BBB). They are responsible of the extrusion of several drugs that are substrates of these two transporters including anticancer drugs.[24] For this reason many drugs have a limited capacity to cross the blood-brain barrier (BBB) and reach therapeutically meaningful concentrations.[25]

Since their discovery and elucidation of the mechanism of action, P-gp, MRP1 and BCRP have been considered suitable targets for circumventing MDR.[26] This is the main reason prompting the design and synthesis of transporters modulators to co-administrate with the antineoplastic drugs, that are substrates of these proteins, and that would restore their efficacy in resistant cancer cells.[27]

A large number of compounds showing P-gp modulating activity has been synthesized and studied (classified as first, second- or third-generation)[28-30] and several have reached clinical trials.[31] However, most of these trials have not been successful because of limitations due to the changes in the pharmacokinetic properties of the co-administered chemotherapeutic drug[32] since some P-gp modulators are also able of inhibiting CyP3A4 enzymes. Nevertheless the third generation of P-gp modulators have not shown an appreciable impact on CyP3A4 and do not require a reduction in the dose of anticancer drug.[33]

The third generation P-gp modulators elacridar and tariquidar are not specific for P-gp because they are also able to bind the BCRP transporter.[34] The common structural feature of the two compounds is the presence of a basic 6,7-dimethoxy-2-phenethyl-1,2,3,4-tetrahydroisoquinoline nucleus linked to an aryl substituted amide (Figure 1).

Structure-activity relationship studies on these compounds suggested that the 6,7-dimethoxy-2-phenethyl-1,2,3,4-tetrahydroisoquinoline nucleus is essential for the inhibition of the two transporter proteins P-gp and BCRP while changes at the aryl substituted amide nucleus cause variations in the selectivity.[24]

In this study, with the aim to better evaluate the features for selectivity towards these transporters we decided to deeper explore the structure-activity relationships of these compounds. As a matter of fact one of the problems of MDR modulators might be the lack of information on the structural requirements for selectivity against the large family of transporters. Toward this end we designed and synthesized new series of molecules characterized by the presence of a 6,7-dimethoxy-2-phenethyl-1,2,3,4-tetrahydroisoquinoline scaffold linked, as the two lead compounds, to an aryl substituted amide, on which different aryl nucleus have been inserted (compounds **1-6**, Figure 1). To explore the consequences of varying the amide function, we also synthesized the corresponding isosteric ester derivatives (**7-12**) and some analogue compounds containing an alkylamine function (**13-16**) (Figure 1). The inserted aryl moieties were chosen since they were present in compounds previously studied and proved to be very potent multidrug resistance reversers.[35-38]

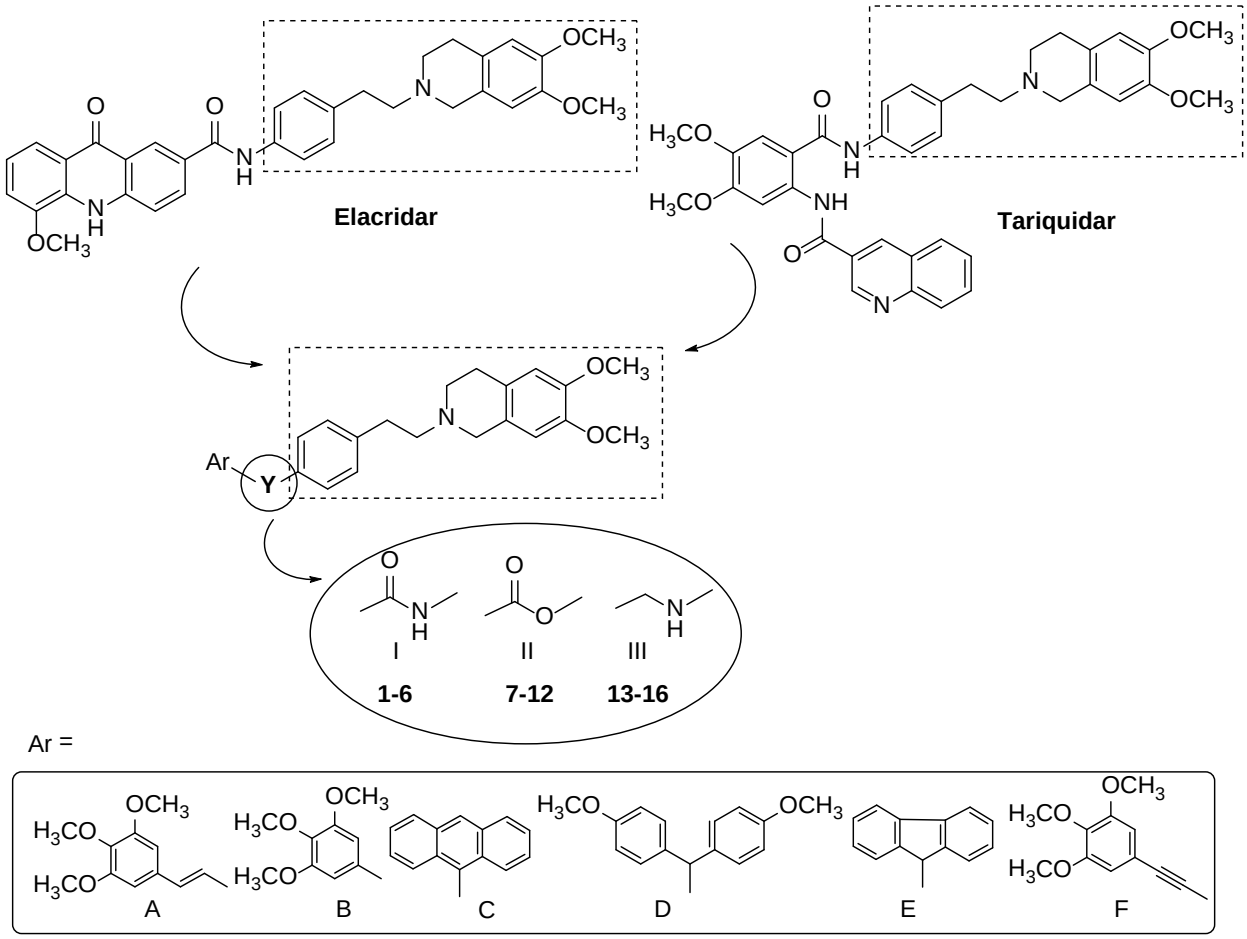


Figure 1: Structures of lead compounds and designed derivatives.

The synthesized compounds were studied to evaluate their P-gp interaction profile and selectivity towards the two other ABC transporters, MPR1 and BCRP.

The P-gp interacting-mechanism of each compound was investigated by three combined biological assays: *i)* Apparent Permeability (P_{app}) determination (BA/AB) in Caco-2 cell monolayer; *ii*) ATP cell depletion in cells overexpressing the transporter MDCK-MDR1 cells; *iii*) the inhibition of Calcein-AM transport in MDCK-MDR1 cells.

The inhibitory activity on MRP1 and BCRP, was evaluated on tumor cell lines overexpressing each transporter (MDCK-MRP1 and MDCK-BCRP cells). The assay is performed by measuring the inhibition of the efflux of the pro-fluorescent probe Calcein-AM in in MDCK-MRP1 cells or the fluorescent probe Hoechst 33342 in MDCK-BCRP cells.

Furthermore, the stability of amide and ester derivatives **1-12**, towards spontaneous or enzymatic hydrolysis was investigated in phosphate buffer solution (PBS) and human plasma, respectively, and the degradation profiles were evaluated.

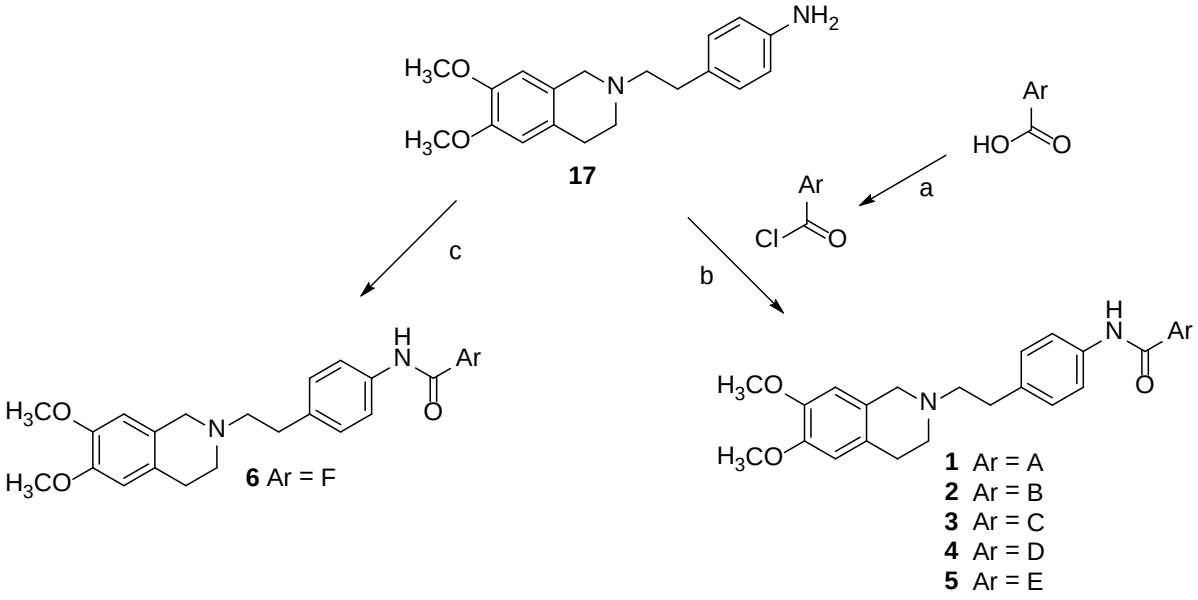
Results and Discussion

Chemistry

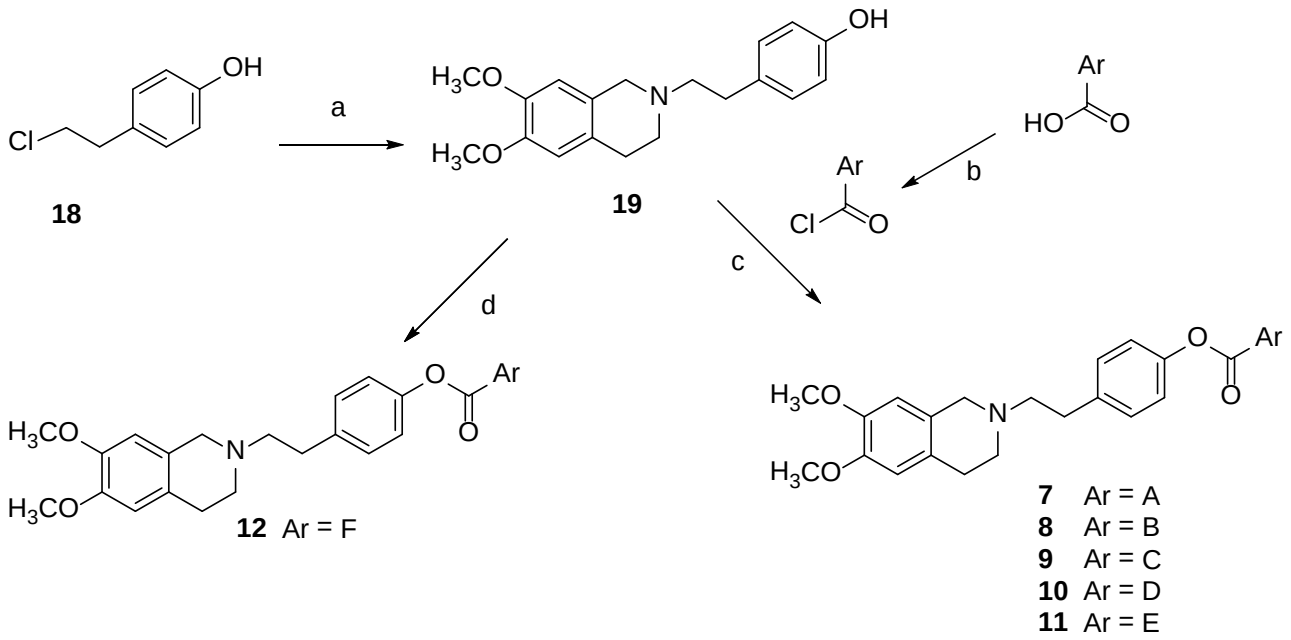
The reaction pathways used to synthesize derivatives **1-16** are reported in Schemes 1-4. Amide derivatives were synthesized starting from aniline **17**, which in turn was obtained by alkylation of 6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline hydrochloride with 1-(2-bromoethyl)-4 nitrobenzene

followed by catalytic reduction of the nitro group.[39] Amides **1-5** were then obtained by reaction of **17** with the appropriate carboxylic acid chloride (Scheme 1), while amide **6**, carrying a triple bond, was obtained by reaction of **17** with 3-(3,4,5-trimethoxyphenyl)propiolic acid using the activating agent EDCI (1-(3-dimethylaminopropyl)-3-ethylcarbodiimmide hydrochloride) in the presence of DMAP (4-dimethylaminopyridine) (Scheme 1). *(E)*-3-(3,4,5-trimethoxyphenyl)acrylic acid, 3,4,5-trimethoxybenzoic acid, anthracene-9-carboxylic acid and 9*H*-fluorene-9-carboxylic acid are commercially available, while 3-(3,4,5-trimethoxyphenyl)propiolic acid[40] and 2,2-bis(4-methoxyphenyl)acetic acid[41] were synthesized as described previously. Ester derivatives were obtained as reported in Scheme 2. The chloroethyl derivative **18** was obtained by the commercially available 4-(hydroxyethyl)phenol by treatment with $SOCl_2$ in ethanol-free $CHCl_3$ and anhydrous triethylamine, slightly modifying the procedure described by Smith;[42] **18** was then reacted with 6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline hydrochloride in anhydrous acetonitrile in the presence of K_2CO_3, leading to phenol **19**. As in the case of amides, esters **7-11** were obtained by reaction of **19** with the appropriate carboxylic acid chloride, while propiolic derivative **12** was obtained by reaction of **19** with 3-(3,4,5-trimethoxyphenyl)propiolic acid using EDCI and DMAP.

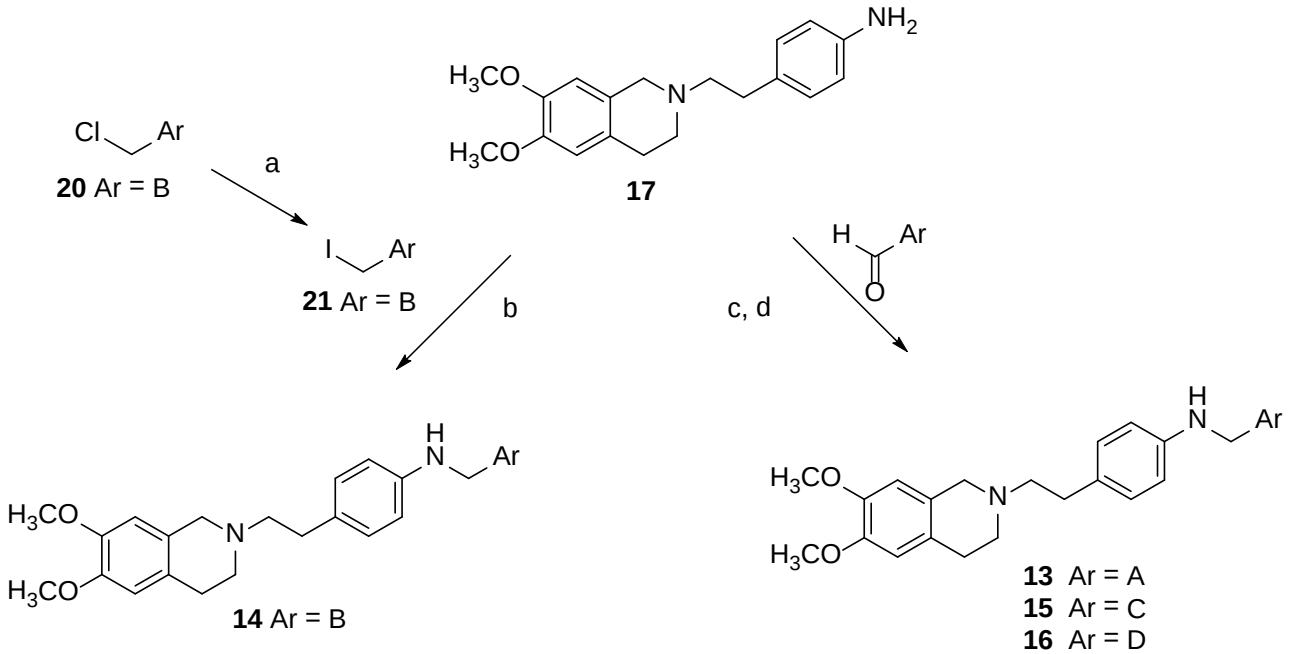
Alkylamine derivatives were obtained as reported in Scheme 3. Amine **14** was obtained, with a low yield (15%), by reaction of aniline **17** with 5-(iodomethyl)-1,2,3-trimethoxybenzene **21**, obtained by treatment of the chloro analog **20**[43] with NaI in acetone. Therefore, the other desired amines **13**, **15** and **16** were obtained, with better yields, according to the Mattson procedure[44] by reductive alkylation of the suitable aldehyde with amine **17** using titanium (IV) isopropoxide as Lewis catalyst and $NaBH_3CN$ as reducing agent. The aldehydes are commercially available (anthracene-9-carbaldehyde) or synthesized by us (Scheme 4). Reduction of (*E*)-methyl 3-(3,4,5-trimethoxyphenyl)acrylate, using 2 equiv of DIBAL-H in anhydrous CH_2Cl_2 at -78°C furnished the corresponding alcohol **22**,[45] which was oxidized to (*E*)-3-(3,4,5-trimethoxyphenyl)acrylaldehyde **23** by means of pyridinium chlorochromate and celite in anhydrous CH_2Cl_2. Finally, (2,2-bis(4-methoxyphenyl)acetaldehyde **24** was obtained by reduction of ethyl 2,2-bis(4-methoxyphenyl)acetate using a little excess of DIBAL-H in toluene at -78°C.



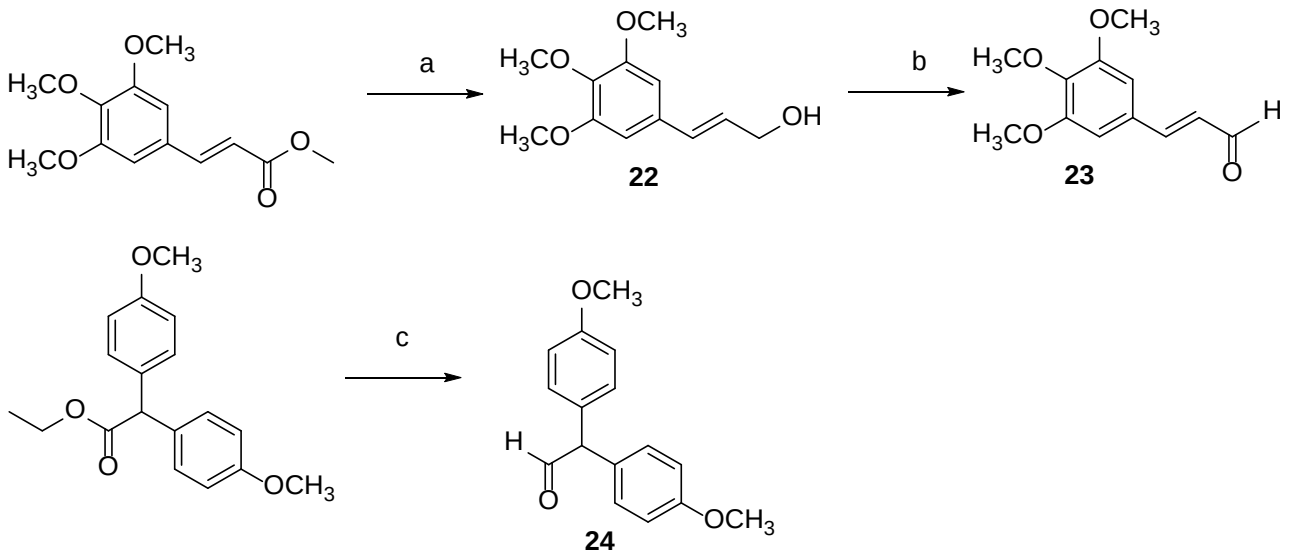
Scheme 1. *Reagents and conditions:* a) $SOCl_2$, ethanol-free $CHCl_3$; b) ethanol-free $CHCl_3$; c) EDCI, DMAP, an. CH_2Cl_2. For the meaning of Ar, see Table 1.



Scheme 2. *Reagents and conditions:* a) 6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline HCl, K_2CO_3, an. CH_3CN; b) $SOCl_2$, ethanol-free $CHCl_3$; c) ethanol-free $CHCl_3$; d) EDCI, DMAP, an. CH_2Cl_2. For the meaning of Ar, see Table 1.



Scheme 3. *Reagents and conditions:* a) NaI, acetone; b) **17**, K_2CO_3, an. CH_3CN; c) titanium(IV) isopropoxide, suitable aldehyde: anthracene-9-carbaldehyde or **23** or **24**; d) $NaBH_3CN$, abs. EtOH. For the meaning of Ar, see Table 1.



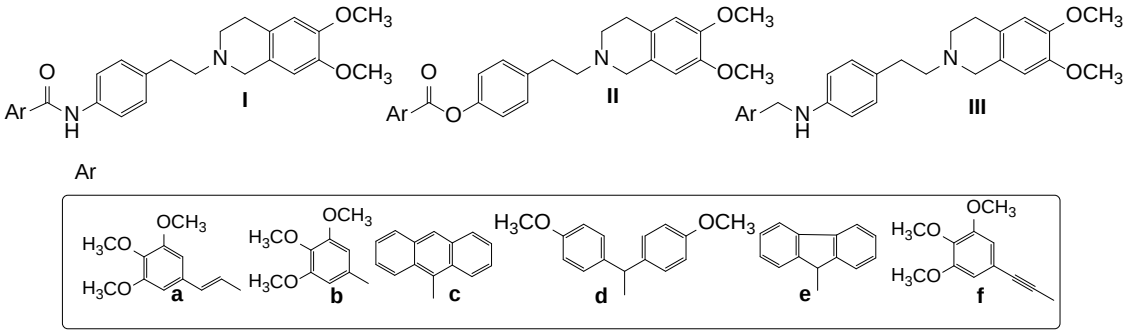
Scheme 4. *Reagents and conditions:* a) DIBAL-H (2 eq.), an. CH_2Cl_2, -78°C; b) PCC, celite, an. CH_2Cl_2; c) DIBAL-H, toluene, -78°C.

Biological studies

The P-gp interacting mechanism of the compounds was investigated by three combined assays, previously reported: *i)* Apparent Permeability (P_{app}) determination (BA/AB) in Caco-2 cell monolayer;[46] *ii*) ATP cell depletion in cells overexpressing the transporter MDCK-MDR1;[47] and *iii*) the inhibition of the transport of a pro-fluorescent probe (Calcein-AM) in MDCK-MDR1 cells.[48] The first assay measures the ratio between two fluxes: from the basolateral to apical compartments (BA, representative of passive diffusion) and from the apical to basolateral compartments (AB, representative of active transport). If (BA/AB) < 2, the compound can be considered an inhibitor, also taking into account the results of the ATPase assay and Calcein-AM modulation. In the same manner, if (BA/AB) > 2, the compound is classified as a substrate.[49,50] The second assay detects the consumption of ATP due to transport mediated by the pump; generally, a substrate, being transported by the pump, induces ATP cell depletion (unambiguous substrate, category I), while a P-gp inhibitor does not induce ATP consumption. However, there is a third substrate category (known as category IIB3) displaying a P_{app} value > 2 but not inducing an ATP cell depletion.[51] The third assay establishes the potency of the interaction between the compounds and the pump by measuring the transport inhibition of the pro-fluorescent probe Calcein-AM, that is a P-gp substrate, in a cell line overexpressing P-gp (MDCK-MDR1 cells). The selectivity of all compounds **1-16** *vs* P-gp was measured by detecting also the activity of the same compounds towards the other ABC transporters MRP1 and BCRP. For this purpose, the interaction with MRP1 was evaluated by measuring the inhibition of the efflux of Calcein-AM (that is also a MRP1 substrate) in cells overexpressing MRP1 (MDCK-MRP1 cells); the interaction with BCRP was evaluated by the measure of the inhibition of the efflux of the fluorescent probe Hoechst 33342 (that is a BCRP substrate) in cells overexpressing BCRP (MDCK-BCRP cells).

The results of the three assays on compounds **1-16** are reported in Table 1 together with those on tariquidar and elacridar used as reference compounds.

Table 1: Biological results of derivatives **1-16**.



Compd	Structure	Ar	EC_{50} μM[a] P-gp	MRP1	BCRP	ATP cell depletion	P_{app}[b]
1	I	A	0.78±0.12	NA	NA	no	18
2	I	B	1.04±0.20	NA	NA	no	5.1
3	I	C	0.66±0.13	NA	NA	no	10.6
4	I	D	0.30±0.06	NA	NA	no	6.6
5	I	E	0.70±0.11	NA	NA	no	5.3
6	I	F	0.70±0.14	NA	NA	no	>20
7	II	A	1.40±0.20	NA	NA	no	1.7
8	II	B	0.93±0.18	NA	17±2.3	no	5.5
9	II	C	1.23±0.24	NA	5.9±1.10	no	>20
10	II	D	0.33±0.07	NA	NA	no	4.7
11	II	E	10±1.80	NA	NA	no	7.9
12	II	F	2±0.40	NA	10±1.5	yes	4.8
13	III	A	0.68±0.13	NA	NA	no	5.6
14	III	B	0.73±0.15	NA	NA	no	5.6
15	III	C	1.01±0.20	NA	NA	no	16.4
16	III	D	0.57 ±0.11	NA	NA	no	4.7
Tar[c]			0.044±0.001	nd	0.010±0.005	yes[d]	22
Elacr			0.014±0.003	NA	10±2.0	yes[e]	>20

[a] Values are the mean ±SEM of two independent experiments, with samples in triplicate. **[**b] Apparent permeability estimation: values are from two independent experiments, with samples in duplicate. [c] See reference 52. [d] Percentage of the effect at a concentration of 50 μM (30%). [e] Percentage of the effect at a concentration of 10 μM (23%). NA = not active; nd = not determined.

As shown in Table 1, all the compounds displayed a high/moderate activity on P-gp (EC_{50} ranging from 0.30 to 10 μM). The amide derivatives of series I were more potent than the corresponding ester analogues (series II) (EC_{50} = 0.78 *vs* 1.40 μM for Ar = A (compounds **1** and **7**); EC_{50} = 0.66 *vs* 1.23 μM for Ar = C (compounds **3** and **9**); EC_{50} = 0.70 *vs* 10 μM for Ar = E (compounds **5** and **11**); EC_{50} = 0.70 *vs* 2 μM for Ar = F (compounds **6** and **12**), respectively) except for compounds **2** and **8**, bearing the 3,4,5-trimethoxy phenyl moiety (B), and compounds **4** and **10**, bearing the 2,2-bis(4-methoxyphenyl) moiety (D), that showed comparable activity (EC_{50} = 1.04 *vs* 0.93 μM, and EC_{50}= 0.30 *vs* 0.33 μM, respectively). Also the alkylamine derivatives (**13-16**, series III) were more potent than the corresponding ester derivatives (**7-10**) and showed activity values (EC_{50} ranging from 0.57 to 1.01 μM) comparable to those of compounds **1-4** of series I.

With respect to the reference compounds tariquidar (EC_{50} = 0.044 μM) and elacridar (EC_{50} = 0.014 μM), all compounds of the three series were less potent but more selective since they were inactive towards both MRP1 and BCRP except for compounds **8**, **9**, **12** belonging to series II. In fact, they displayed moderate activities on BCRP (EC_{50} ranging from 5.9 to 17 μM).

The Apparent Permeability determination P_{app} (BA/AB) in Caco-2 cell monolayer indicated that only compound **7** had a BA/AB ratio < 2 and therefore can be considered a P-gp inhibitor since it did not induce ATP cell depletion and inhibited Calcein-AM transport. Compound **12** showing a BA/AB ratio > 2 and displaying the ability to induce ATP cell depletion is defined as P-gp unambiguous substrate (category I). All the other compounds were not transported substrates (category IIB3), since they had a BA/AB ratio > 2 and were not able to induce ATP cell depletion.[51] Therefore, the esters derivatives **7** and **12** were the only ones displaying a different P-gp interacting mechanism (inhibitor and unambiguous substrate, respectively) compared to the other compounds.

These results suggest that in this series of compounds the presence of an amide function is not a prerequisite for the activity on the transporter proteins P-gp and BCRP. All amide and alkylamine derivatives appeared to be selective for P-gp efflux pump, whereas three ester derivatives were also able to bind BCRP. The nature of the aryl moieties does not influence the behaviour of these compounds within each series, even if, in all the three series, compounds with the 2,2-bis(4-methoxyphenyl) moiety (D) showed the best activity on P-gp.

Chemical stability tests

An investigation about the chemical stability of compounds **1-12** (series I and II) in phosphate buffer solution (PBS) and human plasma was performed since the amide and ester groups present in these compounds may be susceptible to spontaneous or enzymatic hydrolysis.

The degradation analyses were performed by LC-MS/MS method operating in Multiple Reaction Monitoring (MRM) mode. The half-life values were obtained by monitoring the variation of analyte concentration at different incubation times in phosphate buffer solution (PBS) or human plasma. Each set of samples was incubated at 37°C in triplicate at four different times, 0, 30, 60 and 120 min. The human plasma batch was collected from a pool of healthy volunteers and its hydrolytic activity was checked using ketoprofene ethylester (KEE) as reference compound (half-life < 2 h). The raw data processing and evaluation of calibration results are reported in Supporting Information.

Under these experimental conditions all the tested compounds resulted stable in phosphate buffer solution (PBS), whereas in human plasma were stable or showed a negligible degradation rate.

Conclusions

In these new series of compounds the presence of an amide, ester or alkylamine appears to be an important structural requirement for defining the P-gp selectivity and interacting mechanism. In fact, both amide and alkylamine derivatives (series I and III) were P-gp modulators resulting to be more selective than the reference compounds tariquidar and elacridar, which resulted active also on the BCRP transporter. The derivatives of these two series appeared to be substrates belonging to the category IIB3.

The ester derivatives were in general less potent on P-gp than the corresponding amide and alkylamine analogues but, interestingly, three of them were also able to bind the BCRP transporter.

Also in the series II most of the compounds appeared to be not transported substrates (category IIB3), with two interesting exceptions: compound **12** is a P-gp unambiguous substrate and compound **7**, bearing the (*E)*-3-(3,4,5-trimethoxyphenyl)vinyl fragment, is a P-gp inhibitor endowed with high selectivity.

The presence of amide or ester groups in the compounds of series I and II does not compromise their chemical stability to spontaneous or enzymatic hydrolysis. In fact, they resulted stable in PBS and also in human plasma allowing us to predict the *in vivo* bioavailability of these compounds.

In summary, the design of the reported three series I, II, III allowed the identification of two potent and selective P-gp substrates, compounds **4** and **10**, and a P-gp inhibitor endowed with high selectivity, compound **7**. It is noteworthy also the identification in the ester series II of new compounds **8**, **9**, **12** active on BCRP, a result that opens a new scenario in the development of ligands active towards this pump.

Experimental part

Chemistry

All melting points were taken on a Büchi apparatus and are uncorrected. NMR spectra were recorded on a Bruker Avance 400 spectrometer (400 MHz for ^{1}H NMR, 100 MHz for ^{13}C NMR) using residual solvent such as chloroform (δ=7.26) as internal standard. 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.

The semi-preparative LC-UV apparatus consisted of a Perkin-Elmer series 200 (Norwalk, CT) composed by quaternary pump, autosampler, column oven and UV-VIS detector coupled with a Biologic BioFrac fraction collector (from Bio-Rad, Milan, Italy). The LC-MS analysis was carried out using a Varian 1200L triple quadrupole system equipped by two Prostar 210 pumps, a Prostar 410 autosampler and a Elettrospray Source (ESI). The mass spectrometer acquired the positive ions in scan mode in the range between 150-700 m/z. Raw-Data were collected and processed by Varian Workstation Vers. 6.8 software. The expected ions species are composed by the molecular adducts with common positive ions present in solution such as $[H]^+$, $[NH_4]^+$, $[Na]^+$. In our conditions the most abundant ion species obtained were the molecular adducts $[M+H]^+$.

The compounds **1-16** were obtained in a purity of no less than 95%. Their combustion analyses are indicated by symbols, and the analytical results are within ±0.4% of the theoretical values. Compounds were named following IUPAC rules as applied by ChemBio-Draw Ultra 12.0 software. When reactions were performed in anhydrous conditions, the mixtures were maintained under nitrogen. Compounds **1-16** that were in the form of free bases, were transformed into the hydrochloride by treatment with acetyl chloride in anhydrous CH_3OH, or into the oxalate by treatment with oxalic acid in warm ethyl acetate. The salts were crystallized from abs. ethanol/petroleum ether.

(*E*)-*N*-(4-(2-(6,7-Dimethoxy-3,4-dihydroisoquinolin-2(1*H*)-yl)ethyl)phenyl)-3-(3,4,5-trimethoxyphenyl)acrylamide (1)

A 0.23 mmol (54.3 mg) portion of (*E*)-3-(3,4,5-trimethoxyphenyl)acrylic acid was transformed into the acyl chloride by reaction with 0.07 mL of $SOCl_2$ (0.91 mmol) in 4 mL of $CHCl_3$ (free of ethanol) at 60°C for 4 h. The reaction mixture was cooled to rt, and the solvent was removed under

reduced pressure; the mixture was then treated twice with cyclohexane and the solvent was removed under reduce pressure. The acyl chloride obtained was dissolved in $CHCl_3$ (free of ethanol), and to this solution 0.04 mL of Et_3N (0.27 mmol) and 0.23 mmol (68.0 mg) of **17**[39] were added. The mixture was kept at rt. After 24 h the organic layer was washed twice with Na_2CO_3 saturated solution. After drying with Na_2SO_4, the solvent was removed under reduced pressure and the residue was purified by flash chromatography using CH_2Cl_2/diethylether/abs. EtOH/petroleum ether/NH_4OH (180:180:90:450:5) as eluting system. The title compound (47.6 mg, 40%) was obtained as a pale yellow oil.
Anal. calcd for $C_{31}H_{36}NO_6$: C 69.90; H 6.81; N 5.26; found: C 70.17; H 7.03; N 5.11.
The oily product was transformed into the hydrochloride. Mp (HCl): 250-252°C.

Compounds **2**, **3**, **4** and **5** were obtained in the same way starting from 3,4,5-trimethoxybenzoic acid, anthracene-9-carboxylic acid, 2,2-bis(4-methoxyphenyl)acetic acid[41] and 9*H*-fluorene-carboxylic acid respectively.

_N_-(4-(2-(6,7-Dimethoxy-3,4-dihydroisoquinolin-2(1*H*)-yl)ethyl)phenyl)-3,4,5-trimethoxybenzamide (2)
Chromatographic eluent: CH_2Cl_2/diethylether/abs. EtOH/petroleum ether/NH_4OH (180:180:45:45:2.5). Yield: 65%.
Anal. calcd for $C_{29}H_{34}NO_6$: C 68.76; H 6.76; N 5.53; found: C 68.47; H 6.41; N 5.75.
The oily product was transformed into the hydrochloride. Mp (HCl): 140-142°C.

_N_-(4-(2-(6,7-Dimethoxy-3,4-dihydroisoquinolin-2(1*H*)-yl)ethyl)phenyl)anthracene-9-carboxamide (3)
Chromatographic eluent: CH_2Cl_2/MeOH/NH_4OH (97:3:0.3). Yield: 58%.
Anal. calcd for $C_{34}H_{32}N_2O_3$: C 79.04; H 6.24; N 5.42; found: C 78.79; H 6.51; N 5.28.
The oily product was transformed into the hydrochloride. Mp (HCl): 255-256°C.

_N_-(4-(2-(6,7-Dimethoxy-3,4-dihydroisoquinolin-2(1*H*)-yl)ethyl)phenyl)-2,2-bis(4-methoxyphenyl)acetamide (4)
Chromatographic eluent: CH_2Cl_2/MeOH/NH_4OH (98:2:0.2). Yield: 67%.
Anal. calcd for $C_{35}H_{38}N_2O_5$: C 74.18; H 6.76; N 4.94; found: C 73.97; H 6.47; N 5.12.
The oily product was transformed into the hydrochloride. Mp (HCl): 146-148°C.

_N_-(4-(2-(6,7-Dimethoxy-3,4-dihydroisoquinolin-2(1*H*)-yl)ethyl)phenyl)-9H-fluorene-9-carboxamide (5)
Chromatographic eluent: CH_2Cl_2/diethylether/abs. EtOH/petroleum ether/NH_4OH (180:180:45:45:2.5). Yield: 47%.
Anal. calcd for $C_{33}H_{32}N_2O_3$: C 78.55; H 6.39; N 5.55; found: C 78.27; H 6.60; N 5.31.
The oily product was transformed into the hydrochloride. Mp (HCl) 188-190°C.

_N_-(4-(2-(6,7-Dimethoxy-3,4-dihydroisoquinolin-2(1*H*)-yl)ethyl)phenyl)-3-(3,4,5-trimethoxyphenyl)propiolamide (6)

To a solution of **17**[39] (70.5 mg, 0.23 mmol) in 4 mL of anhydrous CH_2Cl_2 cooled to 0°C were added in this sequence: 3-(3,4,5-trimethoxyphenyl)propiolic acid[40] (80.0 mg, 0.34 mmol), DMAP (36.0 mg, 0.29 mmol) and EDCI (99.0 mg, 0.54 mmol). The reaction mixture was stirred at 0°C for 1 h and then at room temperature for 48 h. The mixture was treated with CH_2Cl_2 and the organic layer was washed with water and then with a saturated solution of $NaHCO_3$. After drying with Na_2SO_4, the solvent was removed under reduced pressure and the residue was purified by flash chromatography using CH_2Cl_2/MeOH/NH_4OH (98:2:0.2) as eluting system. The title compound (73.0 mg, 61% yield) was obtained as a pale yellow oil.
Anal. calcd for $C_{31}H_{34}N_2O_6$: C 70.17; H 6.46; N 5.28; found: C 70.45; H 6.23; N 5.41.
The oily product was transformed into the oxalate. Mp (oxalate): 188-190°C.

4-(2-Chloroethyl)phenol (18)[42]
To a solution of 200.0 mg (1.45 mmol) of 4-(2-hydroxyethyl)phenol and 0.5 mL (3.60 mmol) of an. Et_3N in $CHCl_3$ (free of ethanol), 0.2 mL (2.90 mmol) of $SOCl_2$ were added. After 24 h the solvent was removed under reduced pressure, the reaction mixture was treated with CH_2Cl_2, and the resulting organic layer was washed twice with a saturated solution of $NaHCO_3$, dried on Na_2SO_4, and the solvent was removed under reduced pressure. The crude compound was purified by flash chromatography using CH_2Cl_2/MeOH/NH_4OH (99:1:0.1) as eluting system. The title compound (169.7 mg, 75%) was obtained as a pale yellow oil.

4-(2-(6,7-Dimethoxy-3,4-dihydroisoquinolin-2(1*H*)-yl)ethyl)phenol (19)
To a solution of 169.7 mg (1.08 mmol) of **18** in an. CH_3CN, 297.7 mg (1.30 mmol) of 6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline hydrochloride and 373.2 mg (2.70 mmol) of K_2CO_3 were added. After 24 h at 80°C the solvent was removed under reduced pressure, the reaction mixture was treated with H_2O and the aqueous layer was extracted three times with CH_2Cl_2. The organic layer was dried over Na_2SO_4, and the solvent was removed under reduced pressure. The crude compound was purified by flash chromatography using CH_2Cl_2/MeOH/NH_4OH (95:5:0.5) as eluting system. The title compound (224.8 mg, 67%) was obtained as a pale yellow oil.

(*E*)-4-(2-(6,7-Dimethoxy-3,4-dihydroisoquinolin-2(1*H*)-yl)ethyl)phenyl 3-(3,4,5-trimethoxyphenyl)acrylate (7)
A 0.19 mmol (45.5 mg portion) of (*E*)-3-(3,4,5-trimethoxyphenyl)acrylic acid was transformed into the acyl chloride by reaction with 0.06 mL of $SOCl_2$ (0.78 mmol) in 5 mL of $CHCl_3$ (free of ethanol) at 60°C for 4 h. The reaction mixture was cooled to room temperature, and the solvent was removed under reduced pressure; the mixture was then treated twice with cyclohexane and the solvent removed under reduce pressure. The acyl chloride obtained was dissolved in $CHCl_3$ (free of ethanol), and a solution of 0.19 mmol (59.8 mg) of **19** in $CHCl_3$ (free of ethanol) was added. The mixture was kept at room temperature. After 24 h, the reaction mixture was treated with CH_2Cl_2 and the organic layer was washed twice with $NaHCO_3$ saturated solution. After drying with Na_2SO_4, the solvent was removed under reduced pressure and the residue was purified by flash chromatography using CH_2Cl_2/MeOH/NH_4OH (97:3:0.3) as eluting system. The title compound (39.3 mg, 40% yield) was obtained as a pale yellow oil.
Anal. calcd for $C_{31}H_{35}NO_7$: C 69.78; H 6.61; N 2.62; found: C 70.02; H 6.88; N 2.74.
The oily product was transformed into the hydrochloride. Mp (HCl): 215-217°C.

Compounds **8**, **9**, **10** and **11** were obtained in the same way starting from 3,4,5-trimethoxybenzoic acid, anthracene-9-carboxylic acid, 2,2-bis(4-methoxyphenyl)acetic acid[41] and 9*H*-fluorene-carboxylic acid respectively.

4-(2-(6,7-Dimethoxy-3,4-dihydroisoquinolin-2(1*H*)-yl)ethyl)phenyl 3,4,5-trimethoxybenzoate (8)

Chromatographic eluent: CH_2Cl_2/MeOH/NH_4OH (97:3:0.3). Yield: 56%.
Anal. calcd for $C_{29}H_{33}NO_7$: C 68.62; H 6.55; N 2.76; found: C 68.35; H 6.76; N 2.93.
The oily product was transformed into the hydrochloride. Mp (HCl): 207-208°C.

4-(2-(6,7-Dimethoxy-3,4-dihydroisoquinolin-2(1*H*)-yl)ethyl)phenyl anthracene-9-carboxylate (9)

Chromatographic eluent: CH_2Cl_2/MeOH/NH_4OH (97:3:0.3). Yield: 31%.
Anal. calcd for $C_{34}H_{31}NO_4$: C 78.89; H 6.04; N 2.71; found**:** C 79.05; H 5.91; N 2.60.
The oily product was transformed into the hydrochloride. Mp (HCl): 171-173°C (dec).

4-(2-(6,7-Dimethoxy-3,4-dihydroisoquinolin-2(1*H*)-yl)ethyl)phenyl 2,2-bis(4-methoxyphenyl)acetate (10)

Chromatographic eluent: CH_2Cl_2/MeOH/NH_4OH (98:2:0.2). Yield: 47%.
Anal. calcd for $C_{35}H_{37}NO_6$: C 74.05; H 6.57; N 2.47; found: C 73.88; H 6.39; N 2.62.
The oily product was transformed into the hydrochloride. Mp (HCl): 210-213°C.

4-(2-(6,7-Dimethoxy-3,4-dihydroisoquinolin-2(1*H*)-yl)ethyl)phenyl 9H-fluorene-9-carboxylate (11)

Chromatographic eluent: CH_2Cl_2/MeOH/NH_4OH (97:3:0.3). Yield: 39%.
Anal. calcd for $C_{33}H_{31}NO_4$: C 78.39; H 6.18; N 2.77; found: C 78.15; H 6.30; N 2.91.
The oily product was transformed into the hydrochloride. Mp (HCl): 180-183°C.

4-(2-(6,7-Dimethoxy-3,4-dihydroisoquinolin-2(1*H*)-yl)ethyl)phenyl 3-(3,4,5-trimethoxyphenyl)propiolate (12)

To a solution of **19** (96.0 mg, 0.31 mmol) in 4 mL of anhydrous CH_2Cl_2 cooled to 0°C were added in this sequence: 3-(3,4,5-trimethoxyphenyl)propiolic acid[40] (109.0 mg, 0.46 mmol), DMAP (30.0 mg, 0.25 mmol) and EDCI (106.0 mg, 0.55 mmol). The reaction mixture was stirred at 0°C for 1 h and then was kept at room temperature for 48 h. The mixture was treated with CH_2Cl_2 and the organic layer was washed with water and then with a saturated solution of $NaHCO_3$. After drying with Na_2SO_4, the solvent was removed under reduced pressure and the residue was purified by flash chromatography using CH_2Cl_2/MeOH/NH_4OH (98:2:0.2) as eluting system. The title compound (30.2 mg, 18%) was obtained as a pale yellow oil.
Anal. calcd for $C_{31}H_{33}NO_7$: C 70.04; H 6.26; N 2.63; found: C 70.29; H 6.05; N 2.44.
The oily product was transformed into the oxalate. Mp (oxalate): 161-164°C (dec).

5-(Iodomethyl)-1,2,3-trimethoxybenzene (21)

To a solution of 97.0 mg (0.45 mmol) of 5-(chloromethyl)-1,2,3-trimethoxybenzene (**21**)[43] in ethyl acetate, 268.0 g (1.80 mmol) of NaI were added. The mixture was maintained at reflux in the dark for 14 h. The organic layer was washed twice with H_2O and dried on Na_2SO_4, and the solvent was removed under reduced pressure in the dark. The title compound (124.0 mg, 90% yield) was obtained as a red oil.

(*E*)-3-(3,4,5-Trimethoxyphenyl)prop-2-en-1-ol (22)[45]

To a solution of (*E*)-methyl 3-(3,4,5-trimethoxyphenyl)acrylate (1.0 g, 3.96 mmol) in 25 mL of an. CH_2Cl_2 at -78 °C, DIBAL-H (5.82 ml, 8.72 mmol, 1.5 M in toluene) was added dropwise. The mixture was stirred for 1 h at the same temperature, then a solution of NaOH 10% (10 mL) was added. The solution was warmed to room temperature and was stirred for 90 min. The aqueous layer was extracted twice with ethyl acetate and the organic layers were washed with a saturated aqueous NaCl solution, dried over Na_2SO_4, and concentrated in vacuo. The residue was purified by flash chromatography using CH_2Cl_2/CH_3OH (99:1) as eluting system, to give the title compound (672.0 mg, 76% yield).

(*E*)-3-(3,4,5-Trimethoxyphenyl)acrylaldehyde (23)

To a suspension of 121.0 mg (0.562 mmol) of pyridinium chlorochromate and 96.0 mg of celite in 5 mL of an. CH_2Cl_2, a solution of **22** (84.0 g, 0.375 mmol) in 3 mL of an. CH_2Cl_2 was added. The mixture was stirred at room temperature for 2 h, then diethyl ether was added, and the mixture was filtered and concentrated in vacuo. The residue was purified by flash chromatography using CH_2Cl_2/*n*-hexane (90:10) as eluting system. The title compound was obtained in a 90% yield (75.0 mg).

2,2-bis(4-Methoxyphenyl)acetaldehyde (24)

To a solution of ethyl 2,2-bis(4-methoxyphenyl)acetate (223.0 mg, 0.732 mmol) in an. toluene maintained at -78°C, DIBAL-H (0.6 mL of a solution 1.5 M in toluene, 0.879 mmol) was added. After 1 h at the same temperature, 3 mL of a solution of NaOH 10% were added, and the mixture was left to reach rt, extracted with diethyl ether, dried on Na_2SO_4 and concentrated in vacuo. The residue was purified by flash chromatography using CH_2Cl_2/*n*-hexane (70:30) as eluting system yielding 78.0 mg (41% yield) of the title compound.

4-(2-(6,7-Dimethoxy-3,4-dihydroisoquinolin-2(1*H*)-yl)ethyl)-N-(3,4,5-trimethoxybenzyl)aniline (14)

To a solution of 110.0 mg of **21** (0.360 mmol) in an. CH_3CN, 158.0 mg (0.500 mmol) of **17**[39] were added. The reaction mixture was stirred at room temperature in the dark for 3 h, then was treated with CH_2Cl_2 and the organic layer was washed twice with a saturated solution of $NaHCO_3$and dried on Na_2SO_4. The solvent was removed under reduced pressure and the residue was purified by flash chromatography using $CH_2Cl_2/CH_3OH/NH_4OH$ (98:2:0.2) as eluting system. The title compound was further purified by semi-preparative LC-UV apparatus using a isocratic elution with 10 mM of formic acid in milliQ water:acetonitrile 70:30 (v/v). (20.0 mg, 9% yield) was obtained as a pale yellow oil.

Anal. calcd for $C_{29}H_{36}N_2O_5$: C 70.71; H 7.37; N 5.69; found: C 71.00; H 7.62; N 5.51.

The oily product was transformed into the hydrochloride. Mp (HCl): 103-105°C.

(*E*)-4-(2-(6,7-Dimethoxy-3,4-dihydroisoquinolin-2(1*H*)-yl)ethyl)-N-(3-(3,4,5-trimethoxyphenyl)allyl)aniline (13)

A mixture of aldehyde **23** (63.4 mg, 0.284 mmol), an equimolar amount of **17**[39] (88.8 mg) and an excess of titanium(IV) isopropoxide (0.1 mL, 0.360 mmol) in 1 mL of abs. EtOH was stirred with a drying tube at room temperature. After 20 h, the IR spectrum of the mixture showed no ketone band, and the viscous solution was diluted with abs ethanol (2 mL). Sodium cyanoborohydride (18.0 mg, 0.288 mmol) was added, and the solution was stirred for 6 h. Water (0.5 mL) was then added, and the mixture was concentrated in vacuo. The crude product was dissolved in CH_2Cl_2, filtered to remove the solids, washed with a solution of $NaHCO_3$ and water, dried over Na_2SO_4 and concentrated in vacuo. The crude substance was then purified by column chromatography using CH_2Cl_2/MeOH/NH_4OH (99:1:0.1) as eluting system. The title compound (23.0 mg, 20% yield) was obtained as a pale yellow oil.

Anal. calcd for $C_{31}H_{38}N_2O_5$: C 71.79; H 7.38; N 5.40; found: C 71.42; H 7.63; N 5.18.

The oily product was transformed into the hydrochloride. Mp (HCl): low melting solid.

Compounds **15** and **16** were obtained in the same way starting from anthracene-9-carbaldehyde and 2,2-bis(4-methoxyphenyl)acetaldehyde respectively.

***N*-(Anthracen-9-ylmethyl)-4-(2-(6,7-dimethoxy-3,4-dihydroisoquinolin-2(1*H*)-yl)ethyl)aniline (15)**

Chromatographic eluent: CH_2Cl_2/MeOH/NH_4OH (99:1:0.1). Yield: 38%.

Anal. calcd for $C_{34}H_{34}N_2O_2$: C 81.24; H 6.82; N 5.57; found: C 81.56; H 7.03; N 5.35.

The oily product was transformed into the oxalate. Mp (oxalate): 115-120°C (dec).

***N*-(2,2-bis(4-Methoxyphenyl)ethyl)-4-(2-(6,7-dimethoxy-3,4-dihydroisoquinolin-2(1*H*)-yl)ethyl)aniline (16)**

Chromatographic eluent: CH_2Cl_2/MeOH/NH_4OH (98:2:0.2). Yield: 41%.

Anal. calcd for $C_{35}H_{40}N_2O_4$: C 76.06; H 7.29; N 5.07; found: C 75.81; H 7.07; N 4.86.

The oily product was transformed into the hydrochloride. Mp (HCl): 110-112°C.

Biology

Materials. Cell culture reagents were purchased from Celbio s.r.l. (Milano, Italy). CulturePlate 96/wells plates were purchased from PerkinElmer Life Science; Calcein-AM, MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazoliumbromide) were obtained from Sigma-Aldrich (Milan, Italy).

Cell cultures. MDCK-MDR1, MDCK-MRP1 and MDCK-BCRP cells are a gift of Prof. P. Borst, NKI-AVL Institute, Amsterdam, Nederland. MDCK cells were grown in DMEM high glucose supplemented with 10% fetal bovine serum, 2 mM glutamine, 100 U/mL penicillin, 100 μg/mL streptomycin, in a humidified incubator at 37°C with a 5% CO_2 atmosphere. Caco-2 cells were a gift of Dr. Aldo Cavallini and Dr. Caterina Messa from the Laboratory of Biochemistry, National Institute for Digestive Diseases, “S. de Bellis”, Bari (Italy).

Calcein-AM experiment. These experiments were carried out as described by Guglielmo *et al*. with minor modifications.[53] Each cell line (30,000 cells per well) was seeded into black CulturePlate 96/wells plate with 100 µl medium and allowed to become confluent overnight. 100 µl of test compounds were solubilized in culture medium and added to monolayers. 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 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 (PerkinElmer) at excitation and emission wavelengths of 485 nm 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. EC_{50} values were determined by fitting the fluorescence increase percentage versus log[dose].

Hoechst 33342 experiment. These experiments were carried out as described by Guglielmo et al. with modifications.[53] Each cell line (30,000 cells per well) was seeded into black CulturePlate 96/wells plate with 100 µl medium and allowed to become confluent overnight. 100 µl of test compounds were solubilized in culture medium and added to monolayers. 96/Wells plate was incubated at 37°C for 30 min. Hoechst 33342 was added in 100 µl of Phosphate Buffered Saline (PBS) to yield a final concentration of 8 µM and plate was incubated for 30 min. The supernatants were drained and the cells were fixed for 20 min under light protection using 100 µL per well of a 4% PFA solution. Each well was washed 3 times with ice cold PBS. Saline buffer was added to each well and the plate was read to Victor3 (PerkinElmer) at excitation and emission wavelengths of 340/35 nm and 485/20 nm, respectively. In these experimental conditions Hoechst 33342 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. EC_{50} values were determined by fitting the fluorescence increase percentage versus log[dose].

ATPlite assay. The MDCK-MDR1 cells were seeded into 96-well microplate in 100 µl of complete medium at a density $2x10^4$ cells/well.[53] 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 absence of different concentrations of test compounds was added. The plate was incubated for 2h in a humidified atmosphere 5% CO_2 at 37°C. 50 µl of mammalian cell lysis solution was added to all wells and the plate shacked for five minutes in an orbital shaker. 50 µl of substrate solution was added to all wells and the plate shacked for five minutes in an orbital shaker. The plate was dark adapted for ten minutes and the luminescence was measured.

Permeability Experiments.

Preparation of Caco-2 monolayer. Caco-2 cells were seeded onto a Millicell® assay system (Millipore), where a cell monolayer is set in between a filter cell and a receiver plate, at a density of 20,000 cells/well. The culture medium was replaced every 48 h and the cells kept for 21 days in culture. The Trans Epithelial Electrical Resistance (TEER) of the monolayers was measured daily, before and after the experiment, using an epithelial voltohometer (Millicell® -ERS).[53] Generally, TEER values greater than 1000 Ω for a 21day culture, are considered optimal.

Drug transport experiment. After 21 days of Caco-2 cell growth, the medium was removed from filter wells and from the receiver plate, which were filled with fresh HBSS buffer (Invitrogen). 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 and reference compounds, were added to the filter well at the concentration of 100 μM, while fresh HBSS was added to the receiver plate. The plates were incubated at 37°C for 120 min. Afterwards, samples were removed from the apical (filter well) and basolateral (receiver plate) side of the monolayer to measure the permeability.
The apparent permeability (P_{app}), in units of nm/second, was calculated using the following equation:

$$P_{app} = \left(\frac{V_A}{\text{Area x time}} \right) \text{ x } \left(\frac{[\text{drug}]_{\text{acceptor}}}{[\text{drug}]_{\text{initial}}} \right)$$

V_A = the volume (in mL) in the acceptor well;
Area = the surface area of the membrane (0.11 cm^2 of the well);
time = the total transport time in seconds (7200 sec);
$[drug]_{acceptor}$ = the concentration of the drug measured by U.V. spectroscopy;
$[drug]_{initial}$ = the initial drug concentration (1 x 10^{-4} M) in the apical or basolateral wells.

Chemical stability tests

Chemicals

Acetonitrile, ethanol (Chromasolv), formic acid and ammonium formate (MS grade), NaCl, KCl, Na_2HPO_4 $2H_2O$, KH_2PO_4 (Reagent grade), verapamil hydrochloride (used as internal standard) and ketoprofen (analytical standard) were purchased by Sigma-Aldrich (Milan, Italy). Ketoprofen Ethyl Ester (KEE) were obtained by Fisher's reaction from ketoprofen and ethanol. MilliQ water 18 MΩ was obtained from Millipore's Simplicity system (Milan - Italy).
Phosphate buffer solution (PBS) was prepared by adding 8.01 g L^{-1} of NaCl, 0.2 g L^{-1} of KCl, 1.78 g L^{-1} of Na_2HPO_4 $2H_2O$ and 0.27 g L^{-1} of KH_2PO_4. Human plasma was collected from healthy volunteer and was kept at -80°C until use.

Instrumental

The LC-MS/MS analysis was carried out using a Varian 1200L triple quadrupole system (Palo Alto, CA, USA) equipped by two Prostar 210 pumps, a Prostar 410 autosampler and an Elettrospray Source (ESI) operating in positive ions. The ion sources and ion optics parameters were optimized during the calibration of the instrument introducing, via syringe pump at 10 μL min^{-1}, a 1 μg mL^{-1} tuning solution.
Raw-data were collected an processed by Varian Workstation Vers. 6.8 software.
G-Therm 015 thermostatic oven was used to maintained the samples at 37°C during the test of degradation.

Preparation of samples

Each sample was prepared adding 10 µl of Working solution 1 to 100 µL of PBS or human plasma. The obtained solutions correspond to 1 µM of analyte. Each set of samples was incubated in triplicate at four different times, 0, 30, 60 and 120 min. at 37°C. Therefore the degradation profile of each analyte was represented by a batch of 12 samples (4 incubation times x 3 replicates). After the incubation, the samples were added with 300 µL of ISTD solution and centrifuged. The supernatants were transferred in autosampler vials and dried under a gentle stream of nitrogen.
The dried samples were dissolved in 1.0 mL of mQ water:acetonitrile 80:20 added with 10 mM of formic acid. The obtained sample solutions were analyzed by LC-MS/MS method described in Supporting Information.

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Supporting Information.

Physical and chemical data for compounds **1-16**, **18-19**, **21-24** and chemical stability data of compounds **1-12**.

Keywords

6,7-Dimethoxytetrahydroisoquinoline derivatives; multidrug resistance reversers; P-gp modulators; tariquidar and elacridar.

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