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A new class of 1-aryl-5,6-dihydropyrrolo[2,1-*a*]isoquinoline derivatives as reversers of P-glycoprotein-mediated multidrug resistance in tumor cells

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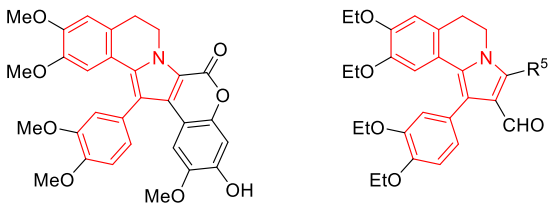
Supporting Information (chemical stability tests of compounds **14** and **15**) for this article is given via a link at the end of the document.

Abstract: A number of aza-heterocyclic compounds, which share with members of the lamellarin alkaloids' family the 5,6-dihydropyrrolo[2,1-*a*]isoquinoline (DHPIQ) scaffold, were synthesized and evaluated for their ability to reverse *in vitro* multidrug resistance (MDR) in cancer cells, through inhibition of P-glycoprotein (P-gp) and/or multidrug-resistance-associated protein-1 (MRP-1). Most of the investigated DHPIQs proved to be selective P-gp modulators, and the most potent one, 8,9-diethoxy-1-(3,4-diethoxyphenyl)-3-(furan-2-yl)-5,6-dihydropyrrolo[2,1-*a*]isoquinoline-2-carbaldehyde (**4**), attained submicromolar inhibition potencies (IC_{50} 0.19 μM). Schiff bases prepared by condensation of some 1-aryl-DHPIQ aldehydes with *p*-aminophenol also proved to be of some interest, and one of them, 4-((1-(4-fluorophenyl)-5,6-dihydro-8,9-dimethoxypyrrolo[2,1-*a*]isoquinolin-2-yl)methyleneamino)phenol (**15**), displayed IC_{50} of 1.01 μM. In drug combination assays in multidrug-resistant cells, some DHPIQ compounds, at non-toxic doses, significantly increased the cytotoxicity of doxorubicin in a concentration-dependent manner. Structure-activity relationship studies and investigation of the chemical stability of the Schiff bases provided physicochemical information useful for molecular optimization of lamellarin-like cytotoxic drugs active toward chemoresistant tumors as well as non-toxic reversers of P-gp-mediated MDR in tumor cells.

Introduction

The heterocyclic system 5,6-dihydropyrrolo[2,1-*a*]isoquinoline (DHPIQ), bearing an aryl group at 1-position, is the aza-heterocyclic scaffold of a group of marine alkaloids, i.e., lamellarins, which is a family of more than thirty polyaromatic compounds endowed with several biological activities, including anticancer activity.[1] Some members of the lamellarin family showed inhibition of HIV-1 integrase and human topoisomerase I, along with other effects on nuclear proteins. Some of these alkaloids showed cytotoxicity against tumor cells *in vitro*, whereas other members, at non-toxic doses, proved to be efficacious in reversing MDR, thereby increasing the antiproliferative activity of conventional anti-tumor chemotherapeutic agents in multidrug-resistant cells. In particular, lamellarin I (Figure 1) proved to significantly increase in dose-dependent manner the cytotoxicity of doxorubicin (DXR), daunorubicin and vinblastine in multidrug-resistant cells, with a potency as MDR modulator higher than that of verapamil (VRP), a first-generation P-glycoprotein (P-gp) inhibitor.[2] Besides lamellarins, numerous natural alkaloids have been discovered as potent inhibitors of P-gp efflux pump and other related pumps responsible for the development of MDR.[3-5]

Nevertheless, intrinsic or acquired MDR still remains a major hurdle to achieve success with the conventional chemotherapy in cancer patients.[6] Several mechanisms underlie MDR, which include enhanced drug efflux, increased DNA repair, reduced apoptosis and altered drug metabolism.[7-9] MDR in human tumor tissues is mostly related to the overexpression of the ATP-binding cassette (ABC) transporters,[10] which are encoded in humans by 49 genes.



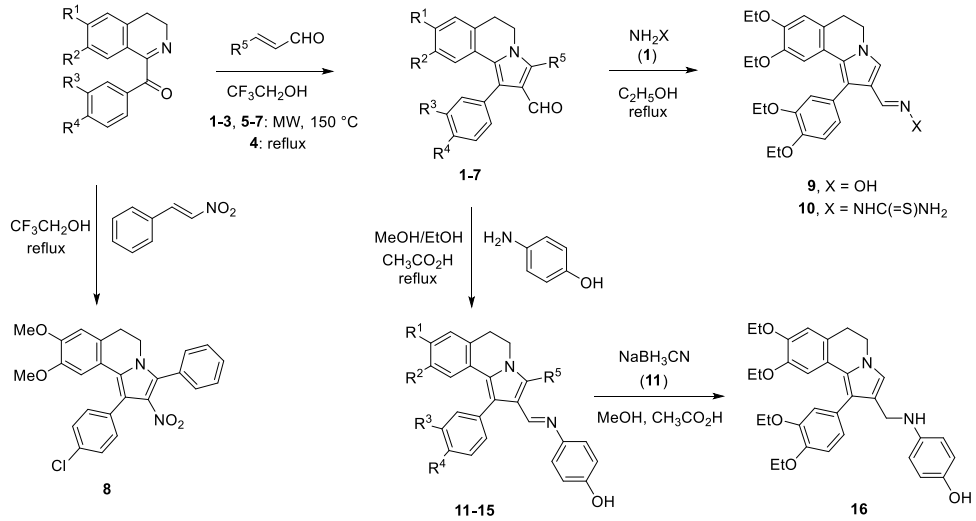
Lamellarin I

1, R^5 = H
2, R^5 = Me
3, R^5 = Ph

Figure 1. Structures of natural (lamellarin I) and synthetic (**1**-**3**) compounds containing 1-aryl-5,6-dihydropyrrolo[2,1-*a*]isoquinoline as scaffold.

Among the ABC transporters, three are mainly associated with MDR, namely P-gp (*ABCB1*), the multidrug-resistance-associated protein-1 (MRP1, *ABCC1*), and the breast cancer resistance protein (BCRP, *ABCG2*).[11-14] P-gp is overexpressed in many cancer cells under chemotherapeutic treatment; it exports a variety of chemotherapeutic agents outside of the cancer cells, decreasing intracellular drug accumulation.[15] The overexpression of the efflux pump MRP1 is responsible for MDR to many chemotherapeutics (e.g., doxorubicin, vincristine, cisplatin, methotrexate).[16] BCRP, the most recently identified

ABC transporter, is expressed in several hematological and solid tumors together with P-gp.[17,18]



Scheme 1. Synthesis of 1-aryl-DHPIQ congeners and adducts

The P-gp-mediated MDR reversal activity shown by lamellarin I prompted us to investigate the activity as P-gp modulators of recently synthesized 2-carbaldehyde of 1-aryl-DHPIQ (**1**-**3**, Figure 1).[19] These compounds were efficiently synthesized through a domino reaction between 1-aroyl-substituted 3,4-dihydroisoquinolines and α,β-unsaturated aldehydes, in the absence of catalyst under microwave irradiation. In the same study, some of the synthesized compounds were screened for the antiproliferative/cytotoxic activity on a panel of human cancer cell lines, including HCT116 (colon carcinoma cells), showing low growth inhibition potencies. The group at 3-position of the DHPIQ nucleus was the main structural variant explored, and compound **1** (R^5 = H), with IC_{50} of about 12 μM, proved to be a more potent cytotoxic than **2** (R^5 = Me) and **3** (R^5 = Ph), which showed IC_{50}s of about 67 and 183 μM, respectively.

In this study, a number of previously and newly synthesized DHPIQ derivatives were tested for their ability of modulating the activity of P-gp and MRP1 efflux pumps, through Calcein-AM transport assays in MDCK-MDR1 and MDCK-MRP1 cells. With the aim of investigating the ability to sensitize MDCK-MDR1 cells, some DHPIQ derivatives were screened for their cytotoxic effects in drug combination assays with DXR. Structure-activity relationships were examined and the stability of some imino derivatives of 1-aryl-DHPIQ toward chemical hydrolysis was evaluated.

Results and Discussion

Chemistry

The synthesis of compounds **1**-**3** and **5**-**7** has been reported earlier.[19] Most of them (**2**, **3**, **4** and **6**) were screened in this study in order to explore the effects on the biological activity of the substituents R^1 and R^2 (MeO, EtO), R^3 and R^4 (OEt, F), and R^5 (Me, Ph). To extend the structure-activity relationship (SAR) study on these lamellarin-like compounds, new analogs and derivatives were synthesized according to Scheme 1, focusing on some group replacements (**4**, **8**) and on a number of carbonyl adducts, namely oxime (**9**), thiosemicarbazone (**10**), and Schiff bases (**11**-**15**) prepared by condensation of a number of DHPIQ aldehydes with *para*-aminophenol (PAP).

The synthesis of 2-CHO DHPIQ compounds (**1**-**7)** was accomplished through a domino-reaction of 1-aroyl substituted 3,4-dihydroisoquinolines with *α*,*β*-unsaturated aldehydes, such as acrolein, crotonaldehyde, cinnamaldehyde and 3-(furan-2-yl)acrylaldehyde. The 2-NO_2 derivative **8** was prepared through a similar reaction between drotaveraldine and 2-nitrovinylbenzene. The aldehyde adducts, namely oxime **9** and thiosemicarbazone **10**, were prepared by condensation of compound **1** with hydroxylamine and thiosemicarbazide, respectively. The aldehyde compounds **1**, **3**, **5**-**7** were condensed with PAP to afford the Schiff bases **11**-**15**. The secondary amino derivative **16** was prepared through hydrogenation of the C=N double bond of **11**, using $NaBH_3CN$ as the reducing agent.

Biological studies

The P-gp inhibition potency of the DHPIQ compounds was assessed by measuring the transport inhibition of Calcein-AM, as a profluorescent P-gp substrate, in MDCK-MDR1 cell line overexpressing P-gp.[20] The activity of the same compounds was evaluated in MDCK-MRP1 cells overexpressing MRP1. MC18[21] and verapamil (VRP)[21], as selective inhibitors of P-gp and MRP1 efflux pumps, respectively, were used as positive controls (Figure 2).

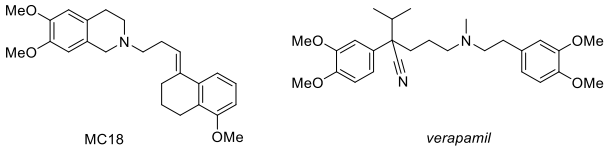


Figure 2. Structures of the selective inhibitors of the efflux pumps P-gp (MC18) and MRP1 (verapamil) used as positive controls.

The inhibition potencies (IC_{50}s) are reported in Table 1. With the only exception of two less soluble compounds **8** and **13**, which showed low activity at the maximum test concentration (50 μM), the DHPIQ derivatives showed high-to-moderate inhibitory potency on P-gp (IC_{50} from 0.19 to 11.2 μM). Most of the test compounds proved to be quite selective towards P-gp compared to MRP1; in two cases, namely **6** and the respective Schiff base **14**, a reversed or nil selectivity was observed. Compared to the reference compound MC18, several DHPIQ derivatives proved to be two-to-six times more potent as P-gp inhibitors. As for MRP1, compounds **6** and **15** proved to be slightly more potent than VRP.

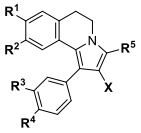
Compounds **3**, **4**, **6**, **12**, **15** and **16** were also evaluated for their ability to restore the cytotoxicity of DXR in MDCK-MDR1 cells, as a consequence of the P-gp inhibition. Figure 3 shows the effects of the test compounds at three concentrations (1, 10 and 25 μM) on the cytotoxicity of 10 μM DXR in multidrug-resistant cells. As shown in Figure 3, MDCK-MDR1 cells displayed resistance to DXR, but they were sensitized when co-incubated with the test DHPIQ compounds; their potentiating effects appeared related to the P-gp inhibition potencies. Indeed, compounds **3** and **4**, which inhibited P-gp with a submicromolar potency (IC_{50}s 0.24 and 0.19 μM, respectively), significantly reversed the resistance of tumor cells to DXR in a concentration-dependent manner, showing significant potentiating effects (**3** > **4**) at concentration as low as 1 μM with no own cytotoxicity even at 10 and 25 μM concentrations. Compound **6** did not show any own cytotoxicity up to 25 μM concentration, but did not even show any significant reversal of the DXR resistance (a low potentiating effect on DXR was observed only at 25 μM), which reflects the lower P-gp inhibition potency (IC_{50} = 10.7 μM).

Table 2. *In vitro* inhibition potency (IC_{50}) toward tumor cell growth.

Cmpd	IC_{50} ± SEM (μM)[a]	
	HepG2	HCT116
3	> 100	ND
4	> 100	ND
6	55.2 ± 7.2	41.3 ± 6.8
11	> 100	24.7 ± 1.5
12	> 100	ND
14	51.7 ± 5.8	26.9 ± 2.8
15	5.74 ± 1.06	ND
16	> 100	17.5 ± 0.3
PAP[b]	92.0 ± 11	17.2 ± 0.5
DXR[c]	5.68 ± 1.30	0.30 ± 0.08

[a] Each IC_{50} value is the mean ± SEM of two independent experiments performed in triplicate; HepG2: Human liver cancer cell line; HCT116: Human colon carcinoma cell line; ND = not determined. [b] *Para*-aminophenol. [c] Doxorubicin.

Table 1. Inhibition potency (IC_{50}) of 1-aryl-DHPIQ derivatives toward P-gp and MPR1 drug efflux pumps.



Cmpd	R^1/R^2	R^3	R^4	R^5	X	IC_{50} ± SEM (μM)[a]	
						P-gp	MRP1
2	OEt	OEt	OEt	Me	CHO	0.43 ± 0.07	9.34 ± 1.0
3	OEt	OEt	OEt	Ph	CHO	0.24 ± 0.05	>100
4	OEt	OEt	OEt	2-Furyl	CHO	0.19 ± 0.02	22.8 ± 3.4
6	OMe	H	F	H	CHO	10.7 ± 1.0	4.24 ± 0.7
8	OMe	H	Cl	Ph	NO_2	40%[b]	ND
9	OEt	OEt	OEt	H	CHNOH	1.28 ± 0.05	8.96 ± 0.90
10	OEt	OEt	OEt	H	$CHNNHC(S)NH_2$	0.24 ± 0.03	> 100
11	OEt	OEt	OEt	H	$CHNC_6H_4$-4-OH	11.2 ± 1.3	51.4 ± 4.3
12	OEt	OEt	OEt	Ph	$CHNC_6H_4$-4-OH	1.91 ± 0.7	6.68 ± 1.0
13	OMe	H	Cl	H	$CHNC_6H_4$-4-OH	29%[b]	ND
14	OMe	H	F	H	$CHNC_6H_4$-4-OH	10.2 ± 1.1	9.93 ± 1.2
15	OMe	H	F	Ph	$CHNC_6H_4$-4-OH	1.01 ± 0.2	2.23 ± 0.4
16	OEt	OEt	OEt	H	$CH_2NC_6H_4$-4-OH	0.71 ± 0.09	7.37 ± 0.8
MC18[c]						1.20 ± 0.30	
VRP[d]							4.53 ± 0.5

[a] Each IC_{50} value is the mean ± SEM of two independent experiments performed in triplicate; ND = not determined. [b] Average % inhibition at 50 μM concentration (maximum concentration tested; solubility limit). [c] MC18, P-gp-selective positive control. [d] Verapamil, MRP1-selective positive control.

The Schiff base **12** (IC_{50} = 1.91 μM) was *per se* not cytotoxic, but reversed the resistance to DXR in a concentration-dependent manner, showing potentiating effects slightly lower than those observed with **3**. In contrast, **15** (IC_{50} = 1.01 μM) showed either own cytotoxicity and sensitizing effects toward DXR at 10 and 25 μM. Finally, the amino derivative **16** (IC_{50} = 0.71 μM) showed a low but significant cytotoxicity at the highest concentration, but at non-toxic concentrations (1 and 10 μM) potentiated the effects of DXR in a concentration-dependent manner.

The cytotoxicity of these and two other compounds (**11** and **14**), as well as PAP as a possible toxic product [23] of the Schiff bases' hydrolysis, was evaluated on two human tumor cell lines, namely HepG2 (human liver cancer cells) and HCT116 (human colon carcinoma cells). The IC_{50} values are summarized in Table 2. HepG2 cell lines are widely used, not only to evaluate toxic effects of a variety of chemicals and drugs, but also in genotoxicity testing, as these cells express metabolizing enzymes required for activation of DNA-reactive carcinogens.[24] The HCT116 cells have been chosen for this screening because they express high levels of glutathione *S*-transferases (GST), which catalyze glutathione (GSH) conjugation of many different cytotoxic agents, making the compounds easily eliminable, with consequent MDR.[25]

As shown in Table 2, both tumor cell lines showed low sensitivity toward the test compounds. The Schiff base **15** (IC_{50} = 5.74 μM) turned out to be that with the highest cytotoxicity on the HepG2 cells, whereas the other compounds displayed IC_{50}s > 50 μM. The cytotoxicity of **15** does not appear to depend upon the hydrolytic release of PAP, which in turn inhibited the HepG2 cell growth with IC_{50} value of 92 μM. However, the Schiff base **15** proved to be the most cytotoxic toward MDCK-MDR1 (Figure 3) and HepG2 (Table 2) cell lines.

The HCT116 cell line showed a sensitivity toward the test DHPIQ compounds slightly higher than the HepG2 cell line, spanning a range of IC_{50} values from 17 to 40 μM. The aldehyde **6** was found to be about 1.5-fold less toxic than the corresponding Schiff base **14**, which in turn showed a potency comparable to PAP in inhibiting the growth of human colon carcinoma cells. Moreover, the Schiff base **11** (IC_{50} = 24.7 μM) resulted about 1.5-fold less toxic than the corresponding amino derivative **16**, which in turn showed a potency superimposable to that of PAP (IC_{50}s 17.5 and 17.2 μM, respectively). This was more evident with HCT116 cells than with HepG2 cells. It remains to prove that, at least in colon carcinoma cells, the amino derivative **16** may undergo oxidation (dehydrogenation) and subsequent hydrolysis of the imine derivative to release PAP in cell.

Structure-activity relationships

Within the limits of the examined molecular space, some clues in SARs can be deduced from the P-gp inhibition data, which may have utility in optimization studies. Lipophilicity of the R^1-R^5 substituents do increase the P-gp inhibition potency; indeed: (i) EtO groups proved to be more favorable than OMe, F, Cl as R^1-R^4 substituents; (ii) the lipophilic phenyl group (**3**) and its bioisosteric replacement 2-furyl (**4**), as R^5 substituents, improved the P-gp interaction of the DHPIQ derivatives compared to the methyl-substituted (**2**) or unsubstituted compounds (e.g., **12** > **11**; **15** > **14**); (iii) the amino derivative (**16**) turned out to be a more potent inhibitor than the parent Schiff base (**11**), likely because of different nitrogen basicity and lipophilicity. The 2-NO_2 DHPIQ analog (**8**) showed lower aqueous solubility and activity compared to the aldehyde derivatives. For P-gp modulation, no noteworthy advantage came from the aldehyde adducts. However, three derivatives (**10**, **15** and **16**) showed potency higher than the positive control MC18. The MDR reversal effects of the examined compounds, as assessed in co-incubation assays with DXR in a multidrug resistance cell model (Figure 3), appeared to be reasonably related to their P-gp inhibition potency, whereas the Schiff base **15** showed moderate cytotoxicity in both MDCK-MDR1 and HepG2 cell lines.

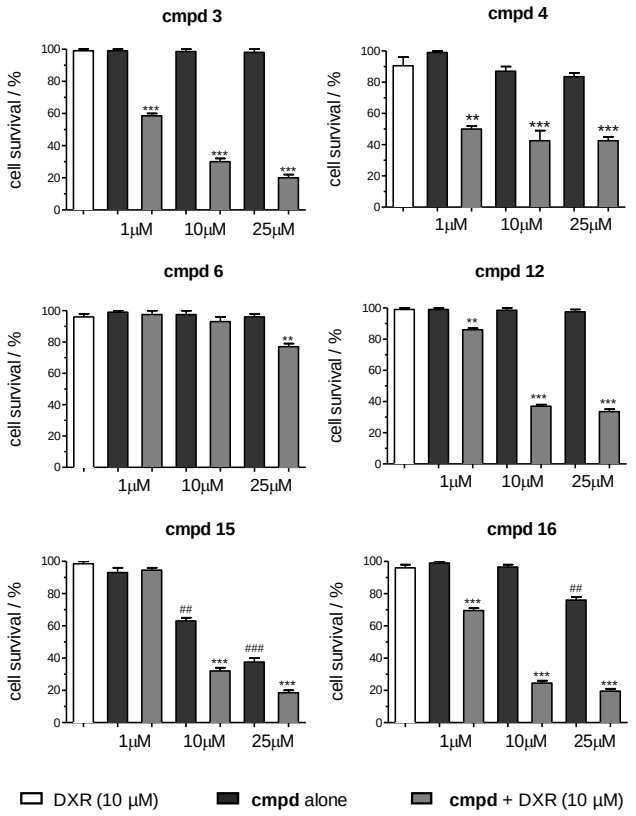


Figure 3. Dose-dependent effects on the in vitro growth of multidrug-resistant MDCK-MDR1 cells by 10 μM doxorubicin (DXR) alone or in the presence of 1, 10 and 25 μM concentrations of compounds **3**, **4**, **6**, **12**, **15** and **16**. Cell survival is represented as % of control cell growth in cultures containing no drug and test compounds. Each bar represents the mean ± SEM of two experiments in triplicate; one-way ANOVA followed by Bonferroni's post-hoc comparison test: ** $P < 0.01$, *** $P < 0.001$ vs. DXR alone; ## $P < 0.01$, ### $P < 0.001$ vs. respective test compound at the lowest concentration (1 μM).

Hydrolytic stability studies

Compounds **11**-**15** are Schiff bases with PAP, which is known for being cytotoxic in some tissues, due to its ability to trigger formation of reactive oxygen species (ROS).[23] To understand if the activity of these Schiff bases might be related to the release of PAP, we carried out a hydrolytic stability study on compounds **14** and **15**. These Schiff bases differ one from each other for R^5 (H and Ph in **14** and **15**, respectively), which could affect the rate of hydrolysis of the imine linkage.

The reversed phase (RP) HPLC proved to be unsuitable as analytical method for monitoring their hydrolytic stability. Compounds **14** and **15**, which are hydrophobic weak bases (with

calculated pK_a of the imine group about 4.5), could be reliably analyzed using aqueous mobile phases at acidic pH values (3 ÷ 4.5) to which the imino derivatives are not enough stable during the chromatographic analysis. Indeed, as shown by ^{1}H NMR data (Supporting Information), the Schiff bases **14** (*ca*. 80% hydrolyzed after 30 min) and **15** (*ca*. 40% hydrolyzed after 15 min) underwent rapid degradation in DCl/DMSO-d_6 solution. In this study, also UV spectrophotometry proved to be of limited applicability (Supporting Information).

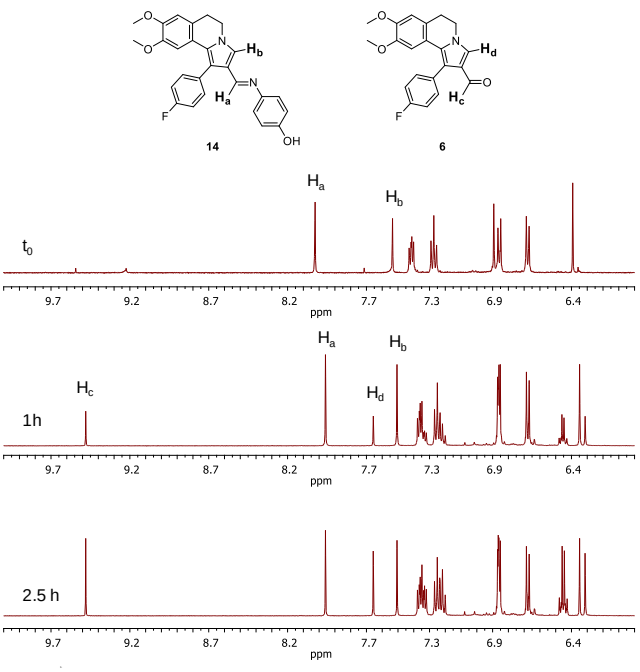


Figure 4. ^{1}H NMR spectra at 500 MHz of the Schiff base **14** and the aldehyde product **6** in D_2O/DMSO-d_6 solutions. The proton peaks labeled as H_a, H_b, H_c and H_d were monitored during the solvolysis reaction. Signals indicating PAP appeared in the range of 6.42-6.47 ppm (dd, 4H_{ar}).

In contrast, ^{1}H NMR (500 MHz) provided a means for monitoring the course of hydrolytic reaction of the examined Schiff bases. Characteristic proton peaks of the starting compounds (**14** and **15**) and the respective reaction products (**6** and **7**, respectively, and PAP) appeared in distinct regions of the spectra and the signals could be quantitatively related to each other by integration. The stability of **14** and **15** was monitored in D_2O/DMSO-d_6 solution at room temperature. Typical ^{1}H NMR spectra of the Schiff base **14** and hydrolyzed samples at three times (0, 1 and 2.5 h) in D_2O/DMSO-d_6 solution (1:15, v/v) are shown in Figure 4. Variations in the AUCs of the proton peaks (singlets) at 8.03 (H_a) and 7.53 ppm (H_b) in the Schiff base **14**, and 9.48 (H_c) and 7.66 ppm (H_d) in the aldehyde product **6**, as well as the appearance of a double doublet (dd) related to the aromatic protons of PAP (6.42-6.47 ppm), were monitored during the reaction course. After 30 min, two new peaks appeared as singlets at 9.48 (H_c) and 7.66 (H_d) ppm, clearly indicating the formation of the hydrolysis product **6**. After about 1 h, new peaks (6.47-6.42 ppm, dd, 4H) assigned to the aromatic protons of PAP appeared in solution. Calculation of area-under-the-curve (AUC) ratios related to the above proton peaks proved the occurrence of hydrolytic conversion of **14** into **6**, which progresses from about 28% at 1 h to 48% at 2.5 h and 64% at 22 h.

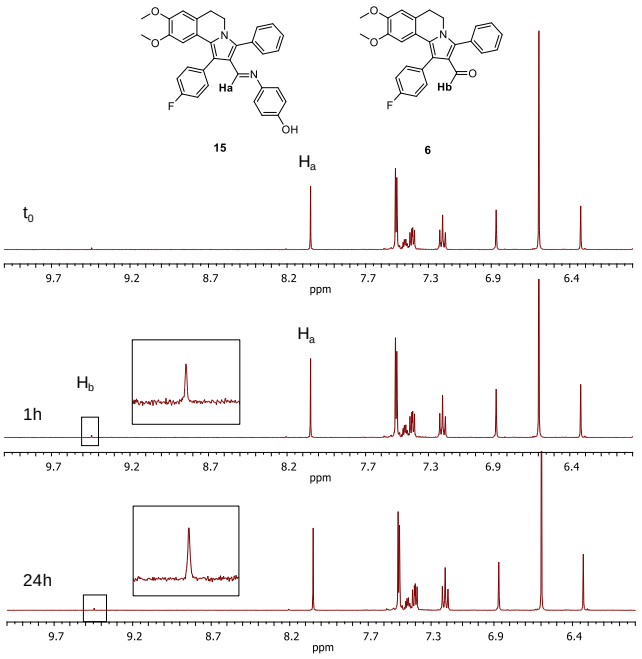


Figure 5. ^{1}H NMR spectra at 500 MHz of the Schiff base **15** and its hydrolysis product **7** in D_2O/DMSO-d_6 solutions. The proton peaks labeled as H_a and H_b were monitored during the solvolysis reaction.

As shown in Figure 6, the hydrolysis rate of compound **14** in D_2O/DMSO-d_6 solution is much greater than that of **15**. The higher stability of **15** ($t_{1/2}$ >> 24 h) is clearly due to the steric shield of the 3-phenyl group to the nucleophilic addition of water to the imine linkage. According to the ^{1}H NMR data, the hydrolysis of **14** in D_2O/DMSO-d_6 solution followed a pseudo-first-order kinetics with an experimental apparent rate constant (k_{obs}) of 0.207 h^{-1} and $t_{1/2}$ of 3.34 h.

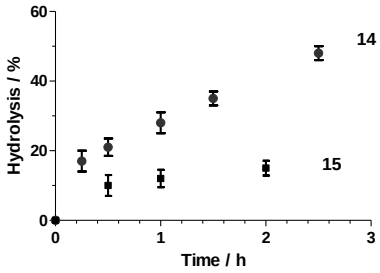


Figure 6. Solvolysis reaction course of the Schiff bases **14** and **15** in D_2O/DMSO-d_6 solution at room temperature, as determined by ^{1}H NMR spectrometry (500 MHz), during the first 3 hs of observation. Data points represent average values ± SEMs from two experiments in triplicate.

Similar results were obtained by simultaneously monitoring the disappearance of the Schiff base **14** and the appearance of the two hydrolysis products **6** and PAP in a buffered solution at pH 7.4 and fixed ionic strength (50 mM PBS, 0.15 M KCl, and 0.5% DMSO as the co-solvent) at room temperature, through

UV spectrophotometric analysis of the ternary mixture (Supporting Information). As shown in Figure 7, the Schiff base **14** underwent a pseudo first-order kinetics hydrolysis (r^2 = 0.9953) with k_{obs} of 0.214 h^{-1} and $t_{1/2}$ of 3.21 h.

Although carried out in remote conditions compared to those recurring in tumor cells, the results from these stability studies combined with the above biological findings may help in understanding the behavior of the examined Schiff bases.

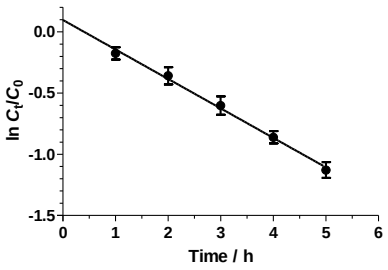


Figure 7. Pseudo-first-order plot for hydrolysis of compound **14** at 50 μM concentration in phosphate buffer (pH 7.4, 0.15 M KCl) and room temperature. The progress of the hydrolytic reaction was monitored by UV spectrophotometry. Data points represent the average values ± SEMs of two experimental measurements in triplicate.

The activity of compound **15**, which turned out to be highly stable at neutral (cytosolic) pH, may be attributed to the intact molecule. High stability toward the hydrolytic reaction at neutral pH could be predicted for compound **12** (R^5 = Ph). The potent P-gp inhibition, as well as the intrinsic cytotoxicity against MDCK-MDR1 cells and HepG2 cells, could be predominantly ascribed to the whole molecule **15**, and not to its hydrolytic products. In contrast, the Schiff base **14** (a less potent P-gp inhibitor) proved to be quite unstable in buffered solution at pH 7.4 ($t_{1/2}$ about 3.2 h), which suggests that its low cytotoxicity against HepG2 and HCT116 cells may be due to the combined effects of itself and the hydrolytic products **6** and PAP.

Conclusions

In this study, some synthetic lamellarin-like compounds were identified as potent P-gp inhibitors having potential for the treatment of multidrug-resistant tumors. A number of previously and newly synthesized 1-aryl-5,6-dihydropyrrolo[2,1-*a*]isoquinoline (DHPIQ) derivatives were prepared and assayed for their ability to modulate P-gp and MRP1-mediated MDR in suitable tumor cell models. The majority of them proved to be *in vitro* inhibitors of P-gp with selectivity over the MRP1 efflux pump, and some compounds attained submicromolar P-gp inhibition potency. Studies of the MDR-reversal activity of differently substituted 1-aryl-DHPIQ compounds, carried out in cells exhibiting MDR (MDCK-MDR1 cell line), provided *in vitro* proof that, at non-toxic concentrations, in case of 2-CHO derivatives (e.g., **3**), the cytotoxicity of DXR increased significantly. Among the aldehyde adducts, the Schiff base **15**, which should be hydrolytically stable at the cell pH, turned out to be itself moderately cytotoxic toward human HCT116 cells (IC_{50} 5.7 μM) and MDCK-MDR1 cells, as well as able to potentiate the cytotoxic activity of DXR. Structure-activity relationship analysis highlighted the role of lipophilicity of the different substituents in increasing the biological potency of the studied lamellarin-like compounds.

Overall, this work provides evidence in support of the DHPIQ heterocyclic nucleus as a molecular scaffold for building up promising non-cytotoxic modulators of the MDR phenotype, able to potentiate the cytotoxic activity of other antitumor drugs, as well as new anticancer agents active on resistant cells. The synthesis of additional well-designed DHPIQ derivatives, their biological testing and further SAR studies may help disclosing of novel agents combining higher cytotoxic activity on tumor cells and more potent modulating activity on MDR tumor cells.

Experimental Section

Chemistry

Materials and general procedures: All reagents and solvents were purchased from Merck, J.T. Baker, or Sigma-Aldrich Chemical Co. and used without further purification. ^{1}H and ^{13}C NMR spectra were recorded in $CDCl_3$ or [D_6]DMSO solutions at 25 °C, with a 600 MHz NMR spectrometer; peak positions are given in parts per million (δ) referenced to the appropriate solvent residual peak. Mass spectra were recorded with an LCMS-8040 Triple quadrupole liquid chromatograph-mass spectrometer from Shimadzu.

The synthesis procedures of compounds **1-3** and **5-7** were recently described;[19] compounds **4** and **8-16** were synthesized according to the following procedures.

1-(3,4-Diethoxyphenyl)-8,9-diethoxy-3-(furan-2-yl)-5,6-dihydropyrrolo[2,1-*a*]isoquinoline-2-carbaldehyde (4): 3-(Furan-2-yl)acrylaldehyde (71 mg, 0.58 mmol) was added to a solution of (6,7-diethoxy-1,2,3,4-tetrahydroisoquinolin-1-yl)(3,4-diethoxyphenyl) methanone (197 mg, 0.48 mmol) in trifluoroethanol (6 mL). The mixture was refluxed for 16 h, and TLC (sorbfil, EtOAc/hexane 2:1) monitored the reaction progress. The solvent was removed under vacuum; the residue was crystallized from EtOH to afford compound **4** as a beige powder (76 mg, 31%): mp 125-128 °C; ^{1}H NMR (600 MHz, $CDCl_3$,): δ = 1.17 (t, 3H, *J* = 7.0 Hz, O-CH_2-$\underline{CH_3}$), 1.39 (t, 3H, *J* = 7.0 Hz, O-CH_2-$\underline{CH_3}$), 1.42 (t, 3H, *J* = 7.0 Hz, O-CH_2-$\underline{CH_3}$), 1.46 (t, 3H, *J* = 7.0 Hz, O-CH_2-$\underline{CH_3}$), 2.98 (t, 2H, *J* = 6.6 Hz, 6-$\underline{CH_2}$), 3.57 (q, 2H, *J* = 7.0 Hz, O-$\underline{CH_2}$-CH_3), 4.02-4.08 (m, 4H, O-$\underline{CH_2}$-CH_3), 4.13 (q, 2H, *J* = 7.0 Hz, O-$\underline{CH_2}$-CH_3), 4.21 (t, 2H, *J* = 6.6 Hz, 5-CH_2), 6.58 (dd, 1H, *J* = 1.6, 3.3 Hz, CH-fur), 6.59 (s, 1H, 7-H), 6.68 (s, 1H, 10-H), 6.90 (d, 1H, *J* = 3.3 Hz, CH-fur), 6.92-6.94 (m, 3H, CH-Ar), 7.60 (br.s., 1H, CH-fur), 9.77 (s, 1H, CHO); ^{13}C NMR (150 MHz, DMSO-d_6): δ = 14.6, 14.9 (4C), 29.0, 43.2, 64.7, 64.6, 64.7 (2C), 109.9, 111.6, 112.8, 113.8, 114.0, 116.2, 120.9, 122.0, 123.2, 124.9, 127.1, 128.3, 128.6, 143.5, 143.7, 147.3, 147.6, 148.2, 148.9, 186.3; MS (LCMS) *m/z* = 516 $[M+H]^+$; Anal. calcd for $C_{31}H_{33}NO_6$: C, 72.21; H, 6.45; N, 2.72, found: C 73.0, H 6.58, N 2.90.

1-(4-Chlorophenyl)-8,9-dimethoxy-2-nitro-3-phenyl-5,6-dihydropyrrolo[2,1-*a*]isoquinoline (8): 2-Nitrovinylbenzene (173 mg, 1.2 mmol) was added to a solution of (4-chlorophenyl)(6,7-dimethoxy-1,2,3,4-tetrahydroisoquinolin-1-yl)methanone (383 mg, 1.2 mmol) in trifluoroethanol (10 mL). The mixture was refluxed for 24 h, and the reaction progress was monitored by TLC (sorbfil, EtOAc/hexane 2:1). The solvent was removed under vacuum, and the residue was crystallized from ethyl acetate to afford compound **8** as a yellow powder (300 mg, 56%): mp 225-226 °C; ^{1}H NMR (600 MHz, $CDCl_3$): δ = 2.96 (t, 2H, *J* = 6.1 Hz, 6-CH_2), 3.37 (s, 3H, OCH_3), 3.84-3.91 (m, 5H, OCH_3, 5-CH_2), 6.35 (s, 1H, 7-H), 6.69 (s, 1H, 10-H), 7.40-7.47 (m, 6H, CH-Ar), 7.49-7.54 (m, 3H, CH-Ar); ^{13}C NMR (150 MHz, DMSO-d_6): δ = 28.7, 42.5, 55.0, 55.9, 56.0, 107.4, 111.0, 114.6, 119.9, 125.1, 126.2, 128.6 (2C), 128.8, 129.1, 129.3, 130.2 (2C), 132.1 (2C), 132.5, 132.8, 133.0, 133.6, 147.7, 148.2; MS (LCMS): *m/z* = 461 $[M+H]^+$; Anal. calcd for $C_{26}H_{21}ClN_2O_4$: C 67.75, H 4.59, N 6.08; found: C 67.88, H 4.71, N 6.25.

1-(3,4-Diethoxyphenyl)-8,9-diethoxy-5,6-dihydropyrrolo[2,1-*a*]isoquinoline-2-carbaldehyde oxime (9): Hydroxylamine hydrochloride (10 mg, 0.22 mmol) was added to a solution of 1-(3,4-diethoxyphenyl)-8,9-diethoxy-5,6-dihydropyrrolo[2,1-*a*]isoquinoline-2-carbaldehyde (**1**, 70 mg, 0.16 mmol) in EtOH (4 mL). The mixture was refluxed for 32 h, and the reaction progress was monitored by TLC (sorbfil, EtOAc/hexane 2:3). The solvent was removed under vacuum, and water (3 mL) was added to the resulting residue and extracted with Et_2O (3×8 mL). The organic layers were combined and dried over $MgSO_4$. The solvent was removed under vacuum, and the residue was recrystallized from EtOAc–hexane to afford compound **9** as a yellow solid (24 mg, 33%): mp 156-158 ºC; ^{1}H NMR (600 MHz, DMSO-d_6): δ = 1.17 (m, 3H, *J* = 7.0 Hz, O-CH_2-CH_3), 1.35-1.45 (m, 6H, O-CH_2-CH_3), 1.47 (m, 3H, *J* = 7.0 Hz, O-CH_2-CH_3), 3.02 (m, 2H, *J* = 6.6 Hz, 6-CH_2), 3.59 (q, 2H, *J* = 7.0 Hz, O-CH_2-CH_3), 3.98 - 4.07 (m, 4H, O-CH_2-CH_3, 5-CH_2), 4.08 - 4.19 (m, 4H, O-CH_2-CH_3), 6.61 (s, 1H, 7-H), 6.68 (s, 1H, 10-H), 6.85 (s, 1H, CH-Ar), 6.86 (d, 1H, *J* = 8.3 Hz, CH-Ar), 6.95 (d, 1H, *J* = 8.3 Hz, CH-Ar), 7.24 (s, 1H, 3-H), 7.92 (s, 1H, CH=NOH); ^{13}C NMR (150 MHz, DMSO-d_6): δ = 14.6, 14.9 (3C), 29.2, 45.1, 63.8, 64.6, 64.7, 65.0, 109.2, 113.3, 114.0, 115.9, 117.4, 119.7, 121.5, 123.1 , 124.0, 127.5, 128.7, 139.5, 147.1, 147.3, 147.8, 149.1, 177; MS (LCMS) *m/z* = 465 $[M+H]^+$; Anal. calcd for $C_{27}H_{32}N_2O_5$: C 69.81, H 6.94, N 6.03, found: C 69.70, H 6.81, N 6.22.

2-((1-(3,4-Diethoxyphenyl)-8,9-diethoxy-5,6-dihydropyrrolo[2,1-*a*]isoquinolin-2-yl)methylene)hydrazinecarbothioamide (10): Thiosemicarbazide (17 mg, 0.19 mmol) was added to a solution of compound **1** (70 mg, 0.16 mmol) in EtOH (4 mL). The mixture was refluxed for 8 h, and the reaction progress was monitored by TLC (sorbfil, EtOAc/hexane 2:3). The solvent was removed under vacuum, and the residue was crystallized from EtOH to afford compound **10** as a yellow solid (206 mg, 88%): mp 174-176 ºC; ^{1}H NMR (600 MHz, $CDCl_3$): δ = 1.15 (t, 3H, *J* = 6.9 Hz, O-CH_2-CH_3), 1.36-1.43 (m, 6H, O-CH_2-CH_3), 1.46 (t, 3H, *J* = 6.9 Hz, O-CH_2-CH_3), 3.00 (t, 2H, *J* = 6.2 Hz, 6-CH_2), 3.57 (q, 2H, *J* = 6.9 Hz, O-CH_2-CH_3), 3.97-4.03 (m, 2H, O-CH_2-CH_3), 4.03-4.09 (m, 4H, O-CH_2-CH_3, 5-CH_2), 4,12 (q, 2H, *J* = 6.9 Hz, O-CH_2-CH_3), 5.99 (br.s, 1H, NH), 6.60 (s, 1H, 10-H), 6.61 (br.s., 1H, NH), 6.67 (s, 1H, 7-H), 6.84-6.89 (m, 2H, CH-Ar), 6.92 (d, 1H, *J* = 8.1 Hz, CH-Ar), 7.06 (s, 1H, 3-H), 7.60 (s, 1H, 10-H), 7.97 (s, 1H, CH=N); ^{13}C NMR (150 MHz, DMSO-d_6): δ = 14.6, 14.9 (2C), 15.0, 29.2, 29.3, 45.2 (2C), 63.9, 64.6, 64.7, 64.9 (2C), 109.2, 116.1, 118.7, 123.4 (2C), 124.1 (2C), 126.0, 126.9, 127.3, 147.2, 147.3, 148.1, 149.1 (2C); MS (LCMS) *m/z* = 523 $[M+H]^+$; Anal. calcd for $C_{28}H_{34}N_4O_4S$: 64.34%, H 6.56%, N 10.72%, found: C 64.1, H 6.36, N 10.54.

Synthesis of Shiff bases with *para*-aminophenol (11-15)

Para-aminophenol (PAP, 1.0 mmol) was added in a flask with a solution of the corresponding aldehyde derivative **1**, **3**, **5**-**7** (1.0 mmol) in absolute alcohol (MeOH for synthesis of **11** from **1** and **12** from **3**, EtOH for **13**-**15** from **5**-**7**). The reaction was carried out in the presence of glacial acetic acid (0.01 mmol) and $MgSO_4$ as a water-removal agent (2.0 mmol). The mixture was stirred and heated under reflux; the reaction progress was monitored by TLC (alufol, EtOAc/hexane 2:1). After cooling, the residue was filtered off and washed once with MeOH (2 mL) to afford compounds **11** and **12**. Isolation of **13**-**15** was obtained by removing solvent under vacuum and recrystallizing the residues from EtOAc/hexane.

4-(((1-(3,4-Diethoxyphenyl)-8,9-diethoxy-5,6-dihydropyrrolo[2,1-*a*]isoquinolin-2-yl)methylene)amino)phenol (11): White powder (250 mg, 53%): mp 216-218 ºC; ^{1}H NMR (600 MHz, DMSO-d_6): δ = 1.03 (t, 3H, *J* = 7.0 Hz, O-CH_2-CH_3), 1.23 (t, 3H, *J* = 7.0 Hz, O-CH_2-CH_3), 1.26 (t, 3H, *J* = 7.0 Hz, O-CH_2-CH_3), 1.30 (t, 3H, *J* = 7.0 Hz, O-CH_2-CH_3), 2.93 (t, 2H, *J* = 6.4 Hz, 6-CH_2), 3.48 (q, 2H, *J* = 7.0 Hz, O-CH_2-CH_3), 3.92-3.96 (m, 4H, O-CH_2-CH_3), 4.03 (q, 2H, *J* = 7.0 Hz, O-CH_2-CH_3), 4.08 (t, 2H, *J* = 6.4 Hz, 5-CH_2), 6.49 (s, 1H, 7-H), 6.66 (d, 2H, *J* = 8.5 Hz, C_6H_4-OH), 6.82-6.84 (m, 3H, CH-Ar, 10-H), 6.88 (d, 1H, *J* = 2.1 Hz, CH-Ar), 7.00 (d, 2H, *J* = 8.5 Hz, C_6H_4-OH), 7.48 (s, 1H, 3-H), 8.00 (s, 1H, CH=N), 9.23 (s, 1H, OH); ^{13}C NMR (150 MHz, DMSO-d_6): δ = 15.0, 15.2, 15.3 (2C), 28.9, 44.8, 63.7, 64.3 (2C), 64.4, 64.5, 109.2, 114.0, 114.4, 116.2 (2C), 116.4, 121.3 (2C), 121.6, 122.0 (3C), 123.5, 125.3, 126.1, 127.9, 144.9, 146.8, 147.9, 148.7, 152.3, 155.6; MS (LCMS) *m/z* = 541 $[M+H]^+$; Anal. calcd for $C_{33}H_{36}N_2O_5$: C, 73.31, H, 6.71, N, 5.18, found: C, 73.42, H, 6.90, N, 4.86.

4-(((1-(3,4-Diethoxyphenyl)-8,9-diethoxy-3-phenyl-5,6-dihydropyrrolo[2,1-*a*]isoquinolin-2-yl)methylene)amino)phenol (12): White solid (210 mg, 52%): mp 202-204 ºC; ^{1}H NMR (600 MHz, DMSO-d_6): δ = 1.03 (t, 3H, *J* = 7.0 Hz, O-CH_2-CH_3), 1.23 (t, 3H, *J* = 7.0 Hz, O-CH_2-CH_3), 1.26 (t, 3H, *J* = 7.0 Hz, O-CH_2-CH_3), 1.31 (t, 3H, *J* = 7.0 Hz, O-CH_2-CH_3), 2.89 (t, 2H, *J* = 6.1 Hz, 6-CH_2), 3.45 (q, 2H, *J* = 7.0 Hz, O-CH_2-CH_3), 3.89 (t, 2H, *J* = 6.1 Hz, 5-CH_2), 3.94-3.97 (m, 4H, O-CH_2-CH_3), 4.02 (q, 2H, *J* = 7.0 Hz, O-CH_2-CH_3), 6.45 (s, 1H, 7-H), 6.55-6.61 (m, 4H, CH-Ar), 6.82 (s, 1H, 10-H), 6.83-6.84 (m, 1H, CH-Ar), 6.94-6.95 (m, 2H, CH-Ar), 7.39-7.42 (m, 1H, CH-Ar), 7.47 (t, 2H, *J* = 7.2 Hz, CH-Ar), 7.51 (t, 2H, *J* = 7.2 Hz, CH-Ar), 8.04 (s, 1H, CH=N), 9.15 (s, 1H, OH); ^{13}C NMR (150 MHz, DMSO-d_6): δ = 14.9 (4C), 28.6, 31.0, 64.9, 65.0, 65.09, 65.8, 112.1, 113.0, 113.7, 115.1 (2C), 116.2, 117.5, 118.9 (2C), 119.3, 120.4, 122.2 (3C), 124.3, 124.5, 124.8, 126.7, 128.0 (2C), 131.0, 133.5, 137.6 (2C), 142.0, 146.8, 149.0, 151.9, 165.7; MS (LCMS) *m/z* = 617 $[M+H]^+$; Anal. calcd for $C_{39}H_{40}N_2O_5$: C, 75.95; H, 6.54; N, 4.54, found: C, 76.13, H 6.70, N 4.63.

4-(((1-(4-Chlorophenyl)-8,9-dimethoxy-5,6-dihydropyrrolo[2,1-*a*]isoquinolin-2-yl)methylene)amino)phenol (13): Beige solid (110 mg, 43 %): mp 281-283 ºC; ^{1}H NMR (600 MHz, DMSO-d_6): δ = 3.05 (t, 2H, *J* = 6.3 Hz, 6-CH_2), 3.41 (s, 3H, O-CH_3), 3.87 (s, 3H, O-CH_3), 4.13 (t, 2H, *J* = 6.3, 5-CH_2), 6.51 (s, 1H, 7-H), 6.71 (s, 1H, 10-H), 6.77 (d, 2H, *J* = 8.6 Hz, C_6H_4-OH), 7.02 (d, 2H, *J* = 8.6 Hz, C_6H_4-OH), 7.42 (dd, 4H, *J* = 5.7, 8.5 Hz, C_6H_4-4-Cl), 7.58 (s, 1H, 3-H), 8.11 (s, 1H, CH=N), 9.15 (s, 1H, OH); ^{13}C NMR (150 MHz, DMSO-d_6): δ = 21.7, 28.9, 40.6, 44.7, 55.1, 56.1 (2C), 107.7, 112.8, 116.14, 119.5, 121.1, 121.3, 122.1 (3C), 122.5, 125.6, 126.5, 129.0, 132.2, 133.2, 134.5, 144.7, 147.6, 147.8, 151.7; MS (LCMS) *m/z* = 457 $[M+H]^+$; Anal. calcd for $C_{27}H_{23}ClN_2O_3$: C, 70.66; H, 5.05; Cl, 7.73; N, 6.10, found: C 70.52, H 5.25, N 6.21.

4-(((1-(4-Fluorophenyl)-8,9-dimethoxy-5,6-dihydropyrrolo[2,1-*a*]isoquinolin-2-yl)methylene)amino)phenol (14): Beige solid (290 mg, 65 %): mp 168-170 ºC; ^{1}H NMR (600 MHz, DMSO-d_6): δ = 2.99 (t, 2H, *J* = 5.8 Hz, 6-CH_2), 3.26 (s, 3H, OCH_3), 3.74 (s, 3H, OCH_3), 4.13 (t, 2H, *J* = 5.8 Hz, 5-CH_2), 6.41 (s, 1H, 7-H), 6.70 (d, 2H, *J* = 8.7 Hz, C_6H_4-OH), 6.87 (d, 2H, *J* = 8.7 Hz, C_6H_4-OH), 6.91 (s, 1H, 10-H), 7.29 (d, 2H, *J* = 8.7 Hz, C_6H_4-4-F), 7.43 (d, 2H, *J* = 8.7 Hz, C_6H_4-4-F), 7.56 (s, 1H, 3-H), 8.04 (s, 1H, CH=N), 9.28 (s, 1H, OH); ^{13}C NMR (150 MHz, DMSO-d_6): δ = 28.9, 44.8, 55.1, 56.07, 107.5, 112.7, 115.9 (d, *J* = 21.7, 2C), 116.0, 116.1, 119.8, 121.3, 121.4, 122.1 (2C), 125.4, 126.4, 131.8 (d, *J* = 131.8, 1C), 133.3 (d, *J* = 7.2, 2C), 144.7, 147.5, 147.6, 147.7, 151.8, 155.7, 161.9 (d, *J* = 244.2, 1C); MS (LCMS) *m/z* = 443 $[M+H]^+$; Anal. calcd for $C_{27}H_{23}FN_2O_3$: C, 73.29; H, 5.24; N, 6.33, found: C 73.61, H 5.00, N 6.11.

4-(((1-(4-Fluorophenyl)-8,9-dimethoxy-3-phenyl-5,6-dihydropyrrolo[2,1-*a*]isoquinolin-2-yl)methylene)amino)phenol (15): Beige solid (250 mg, 69 %): mp 309-311 ºC; ^{1}H NMR (600 MHz, DMSO-d_6): δ = 2.92 (t, 2H, *J* = 6.2 Hz, 6-CH_2), 3.20 (s, 3H, O-CH_3), 3.70 (s, 3H, OCH_3), 3.92 (t, 2H, *J* = 6.2 Hz, 5-CH_2), 6.33 (s, 1H, 7-H), 6.59-6.60 (m, 4H, CH-Ar), 6.87 (s, 1H, 10-H), 7.20 (t, 2H, *J* = 8.7 Hz, CH-Ar), 7.41 (dd, 2H, *J* = 6.0, 8.7 Hz, CH-Ar), 7.43-7.45 (m, 1H, CH-Ar), 7.48 - 7.50 (m, 2H, CH-Ar), 7.51-7.52 (m, 2H, CH-Ar), 8.07 (s, 1H, CH=N), 9.15 (s, 1H, OH); ^{13}C NMR (150 MHz, DMSO-d_6): δ = 14.6, 21.3, 28.9, 42.58, 54.9, 56.0, 60.3, 108.2, 112,4, 115.3 (d, *J*=21.7, 1C), 116.0, 118.6, 118.9, 121.2, 121.82, 126.27, 127.2, 128.8, 128.9, 130.4, 131.5 (d, *J* = 7.2, 2C), 133.1 (d, *J* = 2.9, 2C), 133.58, 136.5, 145.0, 147.5, 152.0, 155.5, 161.7 (d, *J* = 242.8, 1C), 162.5, 170.9; MS (LCMS) *m/z* = 519 $[M+H]^+$; Anal. calcd for $C_{33}H_{27}FN_2O_3$: C 76.43, H 5.25, N 5.40, found: C 76.63, H 5.35, N 5.58.

4-(((1-(3,4-Diethoxyphenyl)-8,9-diethoxy-5,6-dihydropyrrolo[2,1-*a*]isoquinolin-2-yl)methyl)amino)phenol (16): $NaBH_3CN$ (70 mg, 1.11 mmol) was added to a solution of the compound **11** (200 mg, 0.37 mmol) in MeOH (15 mL). The reaction was carried out in the presence of glacial acetic acid (1 drop). The resulting solution was stirred at room temperature for 5 h; the reaction progress was monitored by TLC (sorbfil,

EtOAc/hexane 1:1). The solvent was removed under vacuum, and glacial acetic acid was added to the residue up to pH 7. The resulting solution was extracted with EtOAc (4×9 mL). The organic layers were combined and dried over $MgSO_4$. The solvent was removed under vacuum, and the residue was recrystallized from EtOAc/hexane to afford the amine product **16** as a beige solid (122 mg, 61%): mp 194-196 °C; ^{1}H NMR (600 MHz, DMSO-d_6): δ = 1.17 (t, 3H, *J* = 6.8 Hz, O-CH_2-CH_3), 1.32 (t, 3H, *J* = 6.8 Hz, O-CH_2-CH_3), 1.41 (t, 3H, *J* = 6.8 Hz, O-CH_2-CH_3), 1.44 (t, 3H, *J* = 6.8 Hz, O-CH_2-CH_3), 2.97 (t, 2H, *J* = 6.1 Hz, 6-CH_2), 3.59 (q, 2H, *J* = 6.8 Hz, O-CH_2-CH_3), 3.92 (q, 2H, *J* = 6.8 Hz O-CH_2-CH_3), 3.98-3.99 (m, 4H, 5-CH_2, CH_2-NH), 4.05 (q, 2H, *J* = 6.8 Hz, O-CH_2-CH_3), 4.10 (q, 2H, *J* = 6.8 Hz, O-CH_2-CH_3), 6.47 (d, 2H, *J* = 8.6 Hz, C_6H_4-OH), 6.64 (d, 2H, *J* = 8.6 Hz, C_6H_4-OH), 6.65-6.70 (m, 3H, CH-Ar, 7-H, 10-H), 6.88 (d, 1H, *J* = 8.1 Hz, CH-Ar), 6.92 (d, 1H, *J* = 8.1 Hz, CH-Ar), 6.98 (s, 1H, 3-H); ^{13}C NMR (150 MHz, DMSO-d_6): δ = 15.7, 19.1, 29.0, 55.0, 56.1 (2C), 56.6, 65.4, 108.3, 112.4, 115.2, 115.3, 116.0, 118.6, 118.9, 121.3, 121.8, 126.3, 127.2, 128.8, 130.4, 131.5, 133.0, 133.5, 133.6, 136.5, 145.0, 147.5, 147.8, 151.9, 155.5, 160.9, 162.5; MS (LCMS) *m/z* = 543 $[M+H]^+$; Anal. calcd for $C_{33}H_{38}N_2O_5$: C 73.04, H 7.06, N 5.16, found C 73.28, H 7.24, N 5.33.

Biology

Materials: CulturePlate 96/wells plates were purchased from PerkinElmer Life and Analytical Sciences (Boston, MA, USA). Calcein-AM, doxorubicin and MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazoliumbromide) were purchased from Sigma-Aldrich-RBI s.r.l. (Milan, Italy). Cell culture medium and reagents were purchased from EuroClone (Milan, Italy) and Sigma-Aldrich.

Cell cultures: MDCK-MDR1 and MDCK-MRP1 are a gift of Prof. P. Borst, NKI-AVL Institute, Amsterdam, Netherlands. HepG2 tumor cell line was purchased from ICLC (Genova, Italy). The HCT-116 tumor cell line was obtained from the National Cancer Institute, Biological testing Branch (Frederick, MD, USA). MDCK-MDR1, MDCK-MRP1 were grown in DMEM high glucose supplemented with 10% fetal bovine serum (FBS), 2 mM glutamine, 100 U/mL penicillin, 100 μg/mL streptomycin, in a humidified incubator at 37 °C with a 5 % CO_2 atmosphere. HepG2 was grown in MEM supplemented with 10% FBS, 2 mM glutamine, 100 U/mL penicillin, 100 μg/mL streptomycin, 1% NEAA, in a humidified incubator at 37 °C with a 5 % CO_2 atmosphere. The HCT-116 tumor cell line was maintained in the logarithmic phase at 37 °C in 5% CO_2 humidified air in RPMI 1640 medium supplemented with 10% FBS, 2 mM glutamine, penicillin (100 U/mL), and streptomycin (0.1 mg/mL).

Calcein-AM assays: These experiments were carried out according to a previously reported procedure.[25] MDCK-MDR1 or MDCK-MRP1 cell line (50,000 cells per well) was seeded into black CulturePlate 96/wells plate with 100 μL medium and allowed to become confluent overnight. Test compounds (100 μL), at different concentrations ranging from 0.1 to 100 μM, were solubilized in culture medium and added to each well. The 96/wells plate was incubated at 37 °C for 30 min. Calcein-AM in PBS (100 μL) was added to each well to yield a final concentration of 2.5 μM, and the plate was incubated for 30 min. The plate was washed 3 times with 100 mL ice cold PBS. Saline buffer (100 μL) was added to each well and the plate was read by a PerkinElmer Victor3 spectrofluorimeter at excitation and emission wavelengths of 485 nm and 535 nm, respectively. Under these conditions, Calcein cell accumulation in the absence and in the presence of tested compounds was evaluated, and a fluorescence basal level was estimated by untreated cells. In treated wells, the increase of fluorescence with respect to the basal level was measured. IC_{50} values were determined by fitting the fluorescence increase percentage versus log[dose].

Antiproliferative assay in MDCK-MDR1 cells: The co-administration assay with doxorubicin was performed in MDCK-MDR1 cells at 72 h.[26,27] On day 1, 10,000 cells/well were seeded into 96-well plates in a volume of 100 μL. On day 2, the test compounds, each in three concentrations (1, 10, and 25 μM), were added. On day 3, the medium was removed and the test compounds, each in three concentrations, were added alone and in combination with 10 μM doxorubicin. In all the experiments, the solvents (EtOH, DMSO) were added in each control to evaluate a possible solvent cytotoxicity. After the established incubation time, 0.5 mg/mL MTT was added to each well and after 3 h incubation at 37 °C the supernatant was removed. The formazan crystals were solubilized using 100 μL of DMSO and the absorbance values at 570 and 630 nm were determined on the microplate reader Victor 3.

Antiproliferative assay in HepG2 cells: The antiproliferative assay was performed in HepG2 cells at 48 h.[28,29] On day 1, 10,000 cells/well were seeded into 96-well plates in a volume of 100 μL. On day 2, the test compounds, each at different concentrations ranging from 0.1 to 100 μM), were added. In all the experiments, the solvents (EtOH, DMSO) were added in each control to evaluate a possible solvent cytotoxicity. After the established incubation time with test compound (48 h), 0.5 mg/mL MTT was added to each well and after 3 h incubation at 37 °C the supernatant was removed. The formazan crystals were solubilized using 100 μL of DMSO and the absorbance values at 570 and 630 nm were measured on the microplate reader Victor 3.

Antiproliferative assay in HCT116 cells: The growth inhibitory activities of the test compounds were evaluated by using the sulforhodamine-B (SRB) assay.[30] Cells were seeded into 96-well microtiter plates in 100 μL of the appropriate culture medium at plating densities of 50,000 cell/mL. After seeding, microtiter plates were incubated at 37 °C for 24 h prior to addition of the test compounds. After 24 h, several samples of each cell line were fixed *in situ* with cold trichloroacetic acid (TCA), to obtain a measure of the cell population at the time of compound addition. The test compounds were freshly dissolved in culture medium and stepwise diluted to the desired final concentrations. After the addition of different compound concentrations, the plates were further incubated at 37 °C for 72 h. Cells were fixed *in situ* by the gentle addition of 50 μL of cold 50% (w/v) TCA (final concentration, 10%) and incubated for 1 h at 4 °C. The supernatant was discarded, and the plates were washed four times with tap water and air-dried. Sulforhodamine-B solution (100 μL) at 0.4% (w/v) in 1% acetic acid was added to each well, and the plates were incubated for 30 min at room temperature. After staining, unbound dye was removed by washing five times with 1% acetic acid and the plates were air-dried. Bound stain was then solubilized with 10 mM trizma base and the absorbance was read on an automatic plate reader at 515 nm. The compound concentration able to inhibit cell growth by 50% (IC50) was then calculated from semi-logarithmic dose–response plots.

Statistical analysis: Data were analyzed by one-way ANOVA for repeated measures followed by post-hoc Bonferroni's multiple comparison test. Results are expressed as mean ± SD of at 2-3 independent experiments in triplicates. Statistical significance was accepted at a level of $P < 0.05$.

Stability tests by ^{1}H NMR

Deuterium oxide (D_2O), deuterium chloride (DCl), DMSO-d_6, NaH_2PO_4, Na_2HPO_4 and KCl were all purchased from Sigma-Aldrich (Milan, Italy). The Schiff base derivatives **14** and **15** were monitored during one day for chemical stability by ^{1}H-NMR at 500 MHz. Spectra were recorded on Agilent Spectrometer Technologies (Agilent Technologies Italia S.pA., Cernusco sul Naviglio, Milan, Italy). Each compound was studied at one concentration at room temperature in two different mixtures of deuterated solvents: a) 50 μL of D_2O in 750 μL DMSO-d_6, and b) 15 μL of DCl in 750 μL DMSO-d_6. The ^{1}H-NMR spectra for the starting materials and the decomposition products were compared to subsequent spectra at various time points. The formation of new proton peaks over time indicates instability of the starting Schiff base derivative. Each stability test was performed in duplicate.

Hydrolysis kinetics of compound **14** in 50 mM phosphate buffer solution (0.15 M KCl, pH 7.4) was monitored by UV spectrophotometry (Supporting Information).

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Conflict of interest

The authors declare no conflict of interest.

Keywords: efflux transporters – multidrug resistance – P-glycoprotein - pyrrolo[2,1-*a*]isoquinoline - structure-activity relationships

References:

[1] C. Bailly, *Curr. Med. Chem. Anticancer Agents* **2004**, *4*, 363-378.
[2] A.R. Quesada, M.D. García Grávalos, J.L. Fernández Puentes, *Br. J. Cancer* **1996**, *74*, 677-682.
[3] P. Joshi, R.A. Vishwakarma, S.B. Bharate, *Eur. J. Med. Chem.* **2017**, *138*, 273-292.
[4] S.P.C. Cole, *Annu. Rev. Pharmacol. Toxicol.* **2014**, *54*, 95-117.
[5] J.F. Lu, D. Pokharel, M. Bebawy, *Drug Metab. Rev.* **2015**, *47*, 406-419.
[6] J. Wang, N. Seebacher, H. Shi, Q. Kan, Z. Duan, *Oncotarget* **2017**, *8*, 84559-84571.
[7] G. Szakacs, J.K. Paterson, J.A. Ludwig, C. Booth-Genthe, M.M. Gottesman, *Nat. Rev. Drug. Discov.* **2006**, *5*, 219-234.
[8] S. Kachalaki, M. Ebrahimi, L. Mohamed Khosroshahi, S. Mohammadinejad, B. Baradaran, *Eur. J. Pharm. Sci.* **2016**, *89*, 20-30.
[9] M.R. Salehan, H.R. Morse, *Br. J. Biomed. Sci.* **2013**, *70*, 31-40.
[10] H. Glavinas, P. Krajcsi, J. Cserepes, B. Sarkadi, *Curr. Drug Deliv.* **2004**, *1*, 27-42.
[11] F. Staud, P. Pavek, *Int. J. Biochem. Cell Biol.* **2005**, *37*, 720-725.
[12] R.W. Robey, K.K. To, O. Polgar, M. Dohse, P. Fetsch, M. Dean, S. E. Bates, *Adv. Drug Delivery Rev.* **2009**, *61*, 3-13.
[13] S. Choudhuri, C.D. Klaassen, *Int. J. Toxicol.* **2006**, *25*, 231-259.
[14] F.J. Sharom, *Pharmacogenomics* **2008**, *9*, 105-127.
[15] M.M. Gottesman, T. Fojo, S.E. Bates, *Nat. Rev. Cancer* **2002**, *2*, 48-58.
[16] E. Baiceanu, K.A. Nguyen, L. Gonzalez-Lobato, R. Nasr, H. Baubichon-Cortay, F. Loghin, M. Le Borgne, L. Chow, A. Boumendjel, M. Peuchmaur, P. Falson, *Eur. J. Med. Chem.* **2016**, *122*, 408–418.
[17] K.K. To, D.C. Poon, Y. Wei, F. Wang, G. Lin, L.W. Fu, *Biochem. Pharmacol.* **2015**, *97*, 27-37.
[18] P. Zhang, M.C. de Gooijer, L.C. Buil, J.H. Beijnen, G. Li, O. van Tellingen, *Int. J. Cancer* **2015**, *137*, 2007-2018.
[19] M.D. Matveeva, T.N. Borisova, A.A. Titov, L.V. Anikina, S.V. Dyachenko, G.S. Astakhov, A.V. Varlamov, L.G. Voskressensky, *Synthesis* **2017**, *49*, 52-57.
[19] J.W.Polli, S.A. Wring, J.E. Humphreys, L. Huang, J.B. Morgan, L.O. Webster, C.S. Serabjit-Singh, *J. Pharmacol. Exp. Ther.* **2001**, *299*, 620-628.
[20] N.A. Colabufo, V. Pagliarulo, F. Berardi, M. Contino, C. Inglese, M. Niso, P. Ancona, G. Albo, A. Pagliarulo, R. Perrone, *Eur. J. Pharmacol.* **2008**, *601*, 38-42.
[21] D. Trompier, X.B. Chang, R. Barattin, A. du Moulinet D'Hardemare, A. Di Pietro, H. Baubichon-Cortay, *Cancer Res.* **2004**, *64*, 4950-4956.
[22] W. Chang, S. Barve, T.S. Chen, *J. Biochem. Mol. Toxicol.* **2012**, *26*, 71-78.
[23] V. Mersch-Sundermann, S. Knasmüller, X.J. Wu, F. Darroudi, F. Kassie, *Toxicology* **2004**, *198*, 329-340.
[24] K.D. Tew, S. Dutta, M. Schultz, *Adv. Drug Del. Rev.* **1997**, *26*, 91-104.
[25] M. Niso, M.L. Pati, F. Berardi, C. Abate, *RSC Adv.* **2016**, *6*, 88508-88518.
[26] P. Tardia, A. Stefanachi, M. Niso, D.A. Stolfa, G.F. Mangiatordi, D. Alberga, O. Nicolotti, G. Lattanzi, A. Carotti, F. Leonetti, R. Perrone, F. Berardi, A. Azzariti, N.A. Colabufo, S. Cellamare, *J. Med. Chem.* **2014**, *57*, 6403-6418.
[27] M. Niso, C. Abate, M. Contino, S. Ferorelli, A. Azzariti, R. Perrone, N.A. Colabufo, F. Berardi, *ChemMedChem* **2013**, *8*, 2026-2035.
[28] M.L. Pati, M. Niso, D. Spitzer, F. Berardi, M. Contino, C. Riganti, W.G. Hawkins, C. Abate, *Eur. J. Med. Chem.* **2018**, *144*, 359-371.
[29] M. Niso, C. Abate, M. Contino, S. Ferorelli, A. Azzariti, R. Perrone, N.A. Colabufo, F. Berardi, *ChemMedChem* **2013**, *8*, 2026-2035.
[30] K.T. Papazisis, G.D. Geromichalos, K.A. Dimitriadis, A.H. Kortsaris, Optimization of the sulforhodamine B colorimetric assay. *Immunol. Methods* **1997**, *208*, 151-158.

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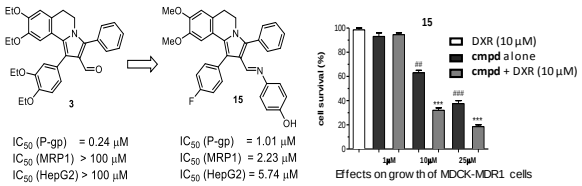

Fighting against multidrug resistance: Newly synthesized analogs of marine alkaloids were discovered as reversers of multidrug resistance (MDR) in cancer cells. Among them, some aldehyde derivatives (3) and Schiff bases (15) proved to be in vitro potent inhibitors P-glycoprotein (P-gp) and/or multidrug-resistance-associated protein-1 (MRP-1), showing effectiveness in increasing the activity of doxorubicin (DXR) in chemoresistant cancer cells.