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Multi-Drug-Resistance-Reverting Agents: 2-Aryloxazole and 2-Arylthiazole Derivatives as Potent BCRP or MRP1 Inhibitors

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2-Aryloxazole and 2-arylthiazole derivatives were evaluated for their inhibitory activity toward P-glycoprotein (P-gp) as well as their selectivity toward other ABC transporters involved in multidrug resistance such as BCRP and MRP1. These derivatives have 6,7-dimethoxytetrahydroisoquinoline or cyclohexylpiperazine moieties, which are the same basic nuclei of the potent P-gp inhibitors MC70 (EC_{50} = 0.05 μM) and PB28 (EC_{50} = 0.55 μM), respectively. The results demonstrate that 2-aryloxazole and 2-arylthiazole derivatives, planned as cycloisosteres of MC70, were found to be less potent than the reference compound in inhibiting P-gp. These compounds were evaluated for their BCRP and MRP1 inhibitory activities. In particular, 6,7-dimethoxytetrahydroisoquinoline derivatives, unsubstituted, 3-Br, 3-Cl, and 3-OCH_3 2-aryloxalzole derivatives showed the best BCRP inhibitory activity (EC_{50} range: 0.24–0.46 μM). In contrast, all cyclohexylpiperazine derivatives except one (EC_{50} = 0.56 μM), showed decreased BCRP inhibitory activity. All compounds tested were unable to inhibit the MRP1 pump, with the exception of the 2-OCH_3 and 4-OCH_3 derivatives of the 6,7-dimethoxytetrahydroisoquinoline series, which displayed high MRP1 inhibitory activity (EC_{50} = 0.84 and 0.90 μM, respectively).

Introduction

Multi-drug resistance (MDR) is a major cause of chemotherapeutic treatment failure in cancer therapy.[1,2] Among the studied mechanisms involved in MDR, increased drug efflux from tumor cells by some ATP binding cassette (ABC) transporters such as P-glycoprotein (P-gp), breast cancer resistant protein (BCRP), and multi-resistant proteins (MRPs) has been reported.[3–5]

The pharmacological strategy suggested to overcome MDR has been to improve the bioavailability of chemotherapeutic agents by co-administration of these agents with ABC transporter inhibitors.[6–11] The first reported P-gp inhibitors were the "L-type" calcium channel blocker verapamil and the immunosuppressant cyclosporin A, which, when administered at high doses, caused severe side effects through associated toxicity.[12] A more specific generation of P-gp inhibitors was subsequently developed, including analogues of the calcium channel blockers dexverapamil, dexniguldipine, an indolizilsulfone derivative (SR33557),[2] a triazinoaminopiperidine derivative (S9788, a non-immunosuppressive derivative of cyclosporin A), valspodar (PSC833),[13] and biricodar (VX-710, a derivative of FK-506).[14] These agents are more potent and less toxic owing to the removal of their non-MDR pharmacological actions; however, they interfere with anti-neoplastic metabolism by inhibiting cytochrome P450. The third generation of P-gp modulators, aimed to overcome the limitations of the second group, are tariquidar,[15] zosuquidar,[16] laniquidar, ONT-093,[17] and elacridar[18] (Figure 1), which, although in different phases of clinical trials, did not display satisfactory preliminary results.[19] Recently, we studied two potent P-gp inhibitors such as 1-cyclohexyl-4-[3-(5-methoxy-1,2,3,4-tetrahydronaphthalen-1-yl)-*n*-propyl]piperazine (PB28)[20,21] and 4′-(6,7-dimethoxy-3,4-dihydro-1*H*-isoquinolin-2-ylmethyl)biphenyl-4-ol (MC70), shown in Figure 2.[22] PB28, developed in our research group, is the most potent σ-2 receptor agonist reported.[20,21,23–25]

Previous SAR studies have demonstrated that starting from PB28, the progressive conformational restriction of the spacer and the replacement of its basic moiety with an isoquinoline moiety are important structural requirements for improving P-gp inhibitory activity.[26] These studies led to 6,7-dimethoxy-2-{3-[4-methoxy-3,4-dihydro-2*H*-naphthalen-(1*E*)-ylidene]propyl}-1,2,3,4-tetrahydroisoquinoline (MC18; Figure 2), which has high P-gp inhibitory activity similar to the reference compound PB28, but lacks σ receptor affinity.[26] The progressive decrease in conformational flexibility led to the design of MC70, which displays P-gp inhibitory activity in the nanomolar range (Figure 2). These three compounds were pharmacologically characterized for their inhibitory activity toward other ABC transporters involved in MDR such as BCRP and MRP1, by using tumor cell lines overexpressing each transporter (MDCK-BCRP and MDCK-MRP1, respectively).[27] As listed in Table 1, these compounds are moderate inhibitors of the BCRP pump (EC_{50} range: 20–90 μM), whereas only MC18 and MC70 inhibit

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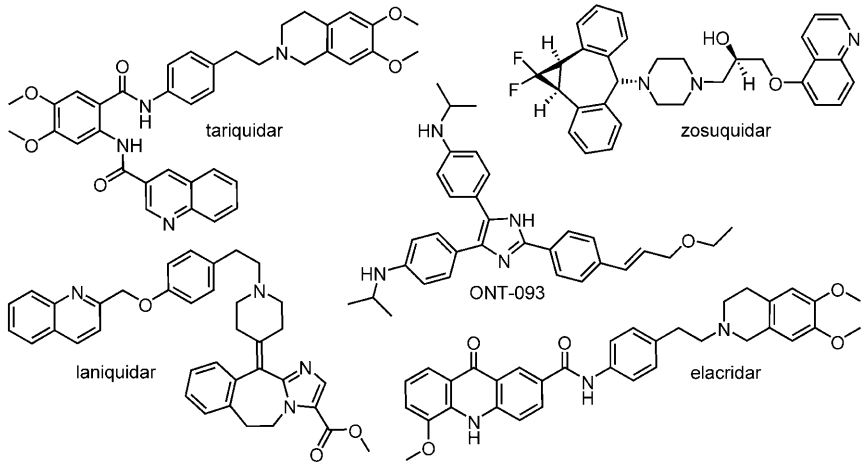


Figure 1. Third-generation P-gp inhibitors.

in its lack of activity, was linked to 2-aryloxazole (compounds **6a–e, 6j–m**) and to 2-arylthiazole (compound **7a**) fragments for obtaining compounds with high selectivity toward the MRP1 pump (Figure 2 and Scheme 1). In addition, all final compounds were tested for P-gp inhibitory activity in both Caco-2 and MDCK-MDR1 cells using different P-gp substrates ([^{3}H]vinblastine and calcein-AM, respectively) in order to establish a correlation between these methods.

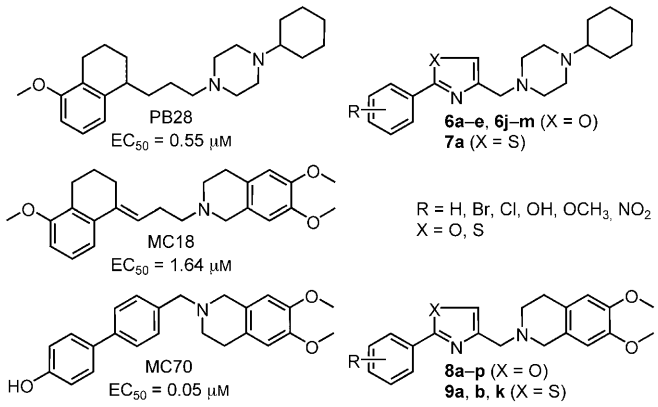
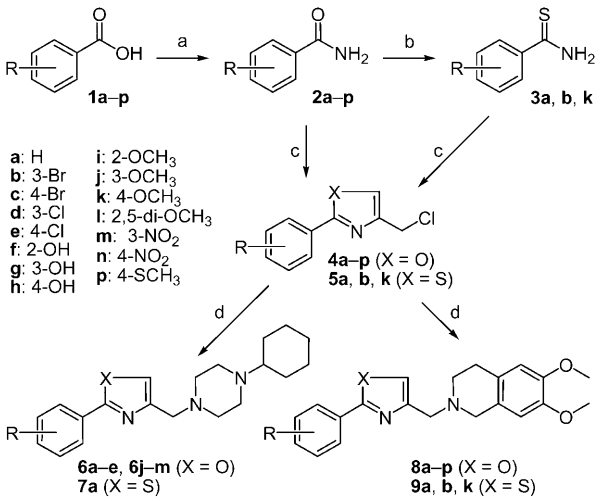


Figure 2. PB28, MC18 and MC70: potent P-gp inhibitors.



Scheme 1. Reagents and conditions: a) $SOCl_2$, Et_3N, NH_4OH/CH_2Cl_2; b) Lawesson's reagent, EtOH; c) 1,3-dichloroacetone, inhomogeneous for oxazoles, EtOH for thiazoles; d) cyclohexylpiperazine or 6,7-dimethoxytetrahydroisoquinoline, Na_2CO_3, CH_3CN.

Table 1. Biological characterization of PB28, MC18, and MC70 toward some ABC transporters involved in MDR.[27]

Compd	EC_{50} [μM]		
	Caco-2[a]	MDCK-BCRP[b]	MDCK-MRP1[c]
PB28	0.55[21]	20	>100 (15%)
MC18	1.64[26]	90	2.80
MC70[22]	0.05	73	9.30

[a] [^{3}H]Vinblastine transport inhibition assay. [b] Rhodamine-123 assay. [c] Calcein-AM assay.

the MRP1 pump (EC_{50} = 2.80 and 9.30 μM, respectively), and PB28 is inactive.[27]

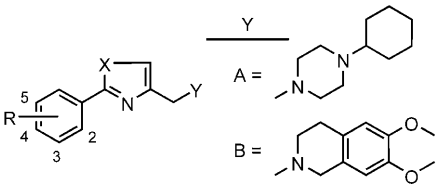
Because MC70 is about fivefold less active than MC18, and as PB28 is inactive against MRP1, we designed 2-aryloxazoles **8a–p** and 2-arylthiazoles **9a, b, k** by starting from MC70, to determine if the cycloisosterism could improve P-gp inhibitory activity and selectivity. Moreover, as PB28 is inactive toward the MRP1 pump, its basic moiety, which is probably involved

Results

Calcein accumulation in MDCK-MDR1 cells

As listed in Table 2, lead compound MC70 displays high MDR1 inhibitory activity (EC_{50} = 0.69 μM). In this assay all 2-aryloxazoles and 2-arylthiazoles bearing the 6,7-dimethoxytetrahydroisoquinoline group were found to be less active than MC70 (EC_{50} range: 1.94–39.2 μM). These results indicate that both the 2-aryloxazole and 2-arylthiazole nucleus as well as the presence and the position of selected substituents contributed negligibly in improving P-gp inhibitory activity. The series of compounds bearing cyclohexylpiperazine as the basic moiety displayed a similar trend (EC_{50} range: 3.14–32.3 μM). Moreover, PB28 also displayed moderate P-gp inhibitory activity in the calcein-AM assay (EC_{50} = 3.0 μM).

Table 2. Biological evaluation of 2-aryloxazole and 2-arylthiazole derivatives.



Compd	R	X	Y	$EC_{50} \pm SEM$ [μM][a] MDR1[c]	BCRP[c]	MRP1[d]	Caco-2[e]	B→A/A→B[b]	ATPase[b]
6a	H	O	A	32.3±5.0	0.56±0.02	>100	0.50±0.02	9.5	N
7a	H	S	A	14.9±3.0	55±3.0	>100	5.2±1.5	12.5	N
8a	H	O	B	3.74±2.1	0.31±0.02	15.0±1.0	1.5±0.5	8.1	N
9a	H	S	B	3.62±3.0	1.2±0.4	11.7±1.2	3.15±0.50	22.5	Y (30%)
6b	3-Br	O	A	7.13±0.5	25±2.8	>100	5.2±0.8	12	N
8b	3-Br	O	B	10.9±0.5	0.34±0.05	15±2.4	15±2.1	16	Y (82%)
9b	3-Br	S	B	3.66±0.5	2.3±0.8	32.7±2.4	25±4.5	5.7	Y (20%)
6c	4-Br	O	A	3.14±0.5	30±2.1	>100	1.80±0.20	9.5	N
8c	4-Br	O	B	15.9±4.8	7.0±0.65	>100	15±0.2	2.8	N
6d	3-Cl	O	A	8.49±5.0	15±1.1	>100	9.5±0.4	9.4	N
8d	3-Cl	O	B	8.37±2.2	0.24±0.07	75.6±5.0	30±2.5	10.4	Y (68%)
6e	4-Cl	O	A	5.66±2.0	73±3.0	>100	5.80±0.6	9.6	N
8e	4-Cl	O	B	20±3.5	3.9±0.6	46.7±2.5	6.2±0.5	10.7	Y (90%)
8f	2-OH	O	B	32.8±3.2	15±1.2	70±2.5	8.5±2.1	6.6	N
8g	3-OH	O	B	16.7±3.5	2.5±0.6	>100	42±6.0	7.8	N
8h	4-OH	O	B	12.7±4.0	90±4.2	86±6.5	50±2.5	8.5	N
8i	2-OCH_3	O	B	7.5±0.5	52±2.5	0.84±0.02	8.2±0.4	10.8	N
6j	3-OCH_3	O	A	11.8±0.2	22±1.5	>100	2.50±0.30	11.1	N
8j	3-OCH_3	O	B	6.53±2.5	0.46±0.07	95.3±7.5	8.30±0.5	8.2	N
6k	4-OCH_3	O	A	8.79±2.0	25±3.0	>100	2.25±0.2	9.7	N
8k	4-OCH_3	O	B	3.50±2.0	75±1.5	0.90±0.06	4.0±0.8	6.9	Y (39%)
9k	4-OCH_3	S	B	1.94±0.8	30±4.0	2.7±0.3	NT[f]	6.1	N
6l	2,5-di-OCH_3	O	A	4.39±0.80	12.7±2.1	>100	5.4±0.5	11.7	N
8l	2,5-di-OCH_3	O	B	3.10±0.5	2.0±0.8	53±1.5	6.0±0.8	10.8	N
6m	3-NO_2	O	A	7.83±4.0	29±1.2	>100	11.5±0.8	13.5	N
8m	3-NO_2	O	B	3.37±0.7	12±1.2	12.6±0.6	6.0±0.5	8.3	Y (78%)
8n	4-NO_2	O	B	39.2±4.5	5.4±0.40	86±3.0	6.4±08	17.2	Y (58%)
8p	4-SCH_3	O	B	2.9±0.4	17±0.8	1.7±0.5	NT[f]	11.7	N
PB28				3.0±0.25	20[27]	>100[27]	0.55[21]		
MC70				0.69±0.05	73[27]	9.30[27]	0.05[22]		
MC18				1.50±0.20	90[27]	2.80[27]	1.64[26]		
MK571						2.85[27]			

[a] Values are the means ±SEM of three independent experiments carried out in triplicate. [b] Determined in the Caco-2 cell line. [c] MDCK-MDR1 cells, with calcein-AM (2.5 μM) as probe. [d] MDCK-MRP1 cells, with calcein-AM (2.5 μM) as probe. [e] [^{3}H]Vinblastine (20 nM) as probe. [f] Not tested because of poor solubility under the experimental conditions.

Calcein accumulation in MDCK-BCRP cells

The lead compound MC70 displayed poor BCRP inhibitory activity (EC_{50} = 73 μM). Surprisingly, the corresponding unsubstituted 2-aryloxazole **8a** displayed high BCRP inhibitory activity (EC_{50} = 0.31 μM). The corresponding 2-arylthiazole derivative **9a** was found to be less active (EC_{50} = 1.2 μM) than **8a** but more active than MC70 as well. The presence of substituents has been evaluated at several positions of 2-aryloxazoles, and the results show that the 3-position is pivotal for BCRP inhibitory activity. Indeed, 3-bromo **8b**, 3-chloro **8d**, and 3-methoxy **8j** 2-aryloxazole derivatives displayed high BCRP inhibitory activity (EC_{50} = 0.34, 0.24, and 0.46 μM, respectively). As for unsubstituted compounds, 3-bromo-2-phenylthiazole derivative **9b** was found to be ~10-fold less active than the corresponding 2-aryloxazole derivative **8b** (EC_{50} = 2.3 vs. 0.34 μM). The shift of the substituents from the 3- to 4-position dramatically decreases BCRP inhibitory activity, as is the case with 4-bromo **8c**, 4-chloro **8e**, and 4-methoxy **8k** 2-aryloxazoles (EC_{50} = 7.0, 3.9, and 75 μM, respectively).

The importance of the 3-position was investigated in the methoxy derivative series by shifting the substituent to the 2-position (compound **8i**: EC_{50} = 52 μM) or by evaluating 2,5-dimethoxy substitution (compound **8l**: EC_{50} = 2.0 μM). Moreover, the presence of a hydroxy group was evaluated at several positions: compounds **8f–h** (OH substituent at the 2- to 4-positions, respectively) displayed low inhibitory activity (EC_{50} range: 2.5–90 μM). Derivatives **8m** and **8n**, with nitro groups respectively at the 3- and 4-position, displayed poor BCRP inhibitory activity (EC_{50} = 12 and 5.4 μM).

The unsubstituted 2-aryloxazole derivative bearing cyclohexylpiperazine as basic moiety (compound **6a**) displayed high BCRP inhibitory activity ($EC_{50}=0.56$ μM), while the corresponding 2-arylthiazole **7a** was found to be ~100-fold less active ($EC_{50}=55$ μM) than **6a**. Differing from the 6,7-dimethoxytetrahydroisoquinoline series, the presence and positions of substituents on the cyclohexylpiperazine derivatives was detrimental for BCRP inhibitory activity. 3-Bromo **6b**, 4-bromo **6c**, 3-chloro **6d**, 4-chloro **6e**, and 3-methoxy **6j** 2-aryloxazole derivatives were found to be ~100-fold less potent (EC_{50} range: 15–73 μM) than the corresponding 6,7-dimethoxytetrahydroisoquinoline derivatives. Moreover, 4-methoxy **6k**, 2,5-dimethoxy **6l**, and 3-nitro **6m** 2-aryloxazoles were found to have poor activity, as in the 6,7-dimethoxytetrahydroisoquinoline series (EC_{50} range: 12.7–29 μM). Moreover, reference compound PB28 also displayed poor BCRP inhibitory activity ($EC_{50}=20$ μM).

Calcein accumulation in MDCK-MRP1 cells

In this assay all compounds bearing cyclohexylpiperazine as the basic moiety, including PB28, were found to be inactive toward the MRP1 pump ($EC_{50}>100$ μM). In the 6,7-dimethoxytetrahydroisoquinoline series the best results were observed for 2-methoxy **8i** and 4-methoxy **8k** 2-aryloxazoles ($EC_{50}=0.84$ and 0.90 μM, respectively). In this series 3-methoxy **8j** and 2,5-dimethoxy **8l** derivatives displayed decreased MRP1 inhibitory activity ($EC_{50}=95.3$ and 53 μM, respectively). To ascertain the importance of a methoxy substituent at the 2- or 4-position, 4-thiomethyl derivative **8p** was prepared. The results show that **8p** has similar potency ($EC_{50}=1.7$ μM) to the corresponding 4-methoxy-2-phenyloxazole derivative **8k** with respect to MRP1 inhibition. Moreover, 2-arylthiazole derivative **9k** bearing a 4-methoxy group had slightly less MRP1 inhibitory activity ($EC_{50}=2.7$ μM) relative to compound **8k**.

The results obtained for compounds **8i** and **8k** were encouraging considering that the reference compound MK571, the most potent MRP1 inhibitor reported,[28] displays an EC_{50} value (2.85 μM) under the same experimental conditions. Moreover, relative to compounds **8i** and **8k**, all the other compounds belonging to the 6,7-dimethoxytetrahydroisoquinoline series were found to be poorly active in inhibiting MRP1. These findings demonstrate that with the exception of 2- and 4-methoxy-2-aryloxazole derivatives, the absence or presence of other studied substituents in both the 2-aryloxazole and 2-arylthiazole series decreased inhibitory activity toward MRP1 (EC_{50} range: 11.7–>100 μM).

BCRP inhibitors were obtained by using both basic nuclei. Moreover, as expected, compounds bearing the cyclohexylpiperazine moiety, as in PB28, were found to be inactive toward the MRP1 pump. In contrast, potent MRP1 inhibitors (compounds **8i** and **8k**) were obtained with the 6,7-dimethoxytetrahydroisoquinoline moiety. SAR studies show that the presence of 2- or 4-methoxy substituents on the 2-aryloxazole fragment is an important requirement for MRP1 inhibition ($EC_{50}=$ 0.84 and 0.90 μM, respectively). The shifting of the methoxy substituent to other positions, its absence, or the presence of other substituents strongly decreased MRP1 inhibitory activity. To determine if the methoxy substituent is involved in H-bond interactions, a 4-thiomethyl-substituted 2-aryloxazole was studied. This compound has MRP1 inhibitory potency ($EC_{50}=$ 1.7 μM) similar to that of the corresponding 4-methoxy-2-phenyloxazole derivative ($EC_{50}=0.90$ μM), demonstrating that the methoxy group at the 2- or 4-positions of the 2-aryloxazole moiety is important more for its electronic properties than as an H-bond acceptor. SAR studies are summarized in Figure 3,

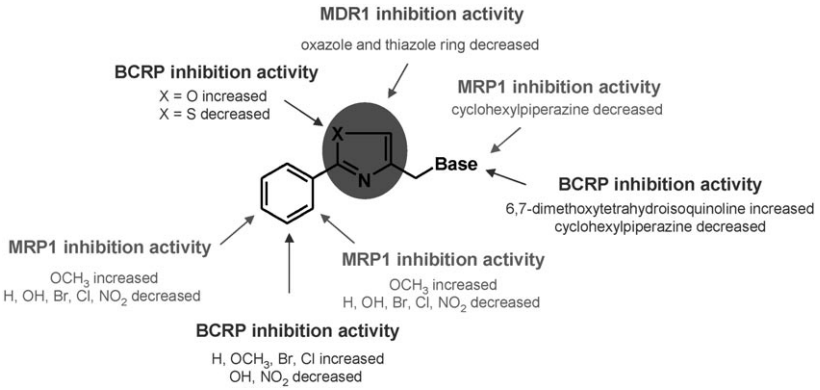


Figure 3. Structural features for MDR1, BCRP, and MRP1 inhibitors.

where the structural aspects for each transporter are listed. Moreover, through combined biological assays in Caco-2 cells ([^{3}H]vinblastine transport inhibition, BA/AB ratio, ATPase activation), all compounds tested were identified as either MDR1-unambiguous substrates or as MDR1-transported substrates.

Conclusions

The 2-aryloxazole moiety is a versatile scaffold for obtaining BCRP or MRP1 inhibitors. The presence and position of various substituents (3-methoxy, 3-bromo, and 3-chloro for BCRP inhibition, and 2-methoxy and 4-methoxy for MRP1 inhibition) proved to be informative structural features in this work. The 2-arylthiazole derivatives were found to be consistently less potent than the corresponding 2-aryloxazoles. Finally, replacement of the 4-biphenyl fragment with the corresponding cycloisosteres led to compounds with decreased P-gp inhibitory activity relative to the lead compound MC70. These are important findings that will contribute to improve SAR studies for the development of BCRP and MRP1 inhibitors, considering that only a few molecules have been developed toward these transporters so far.

Experimental Section

Chemistry

General methods. Column chromatography was performed with 1:30 Merck silica gel 60 Å (63–200 μm) as the stationary phase. Melting points were determined in open capillaries on a Gallenkamp electrothermal apparatus. Elemental analyses (C, H, and N) were performed on a Eurovector Euro EA 3000 analyzer; the results fell within ±0.4% of the theoretical values for the formula given. ^{1}H NMR spectra were recorded in $CDCl_3$ at 300 MHz on a Varian Mercury-VX spectrometer. All spectra were recorded on the free bases. All chemical shift values (δ) are reported in ppm. MS data were collected on an HP 6890-5973 MSD GC–MS instrument; only significant *m/z* peaks, with percent relative intensity in parentheses, are reported. All spectra were in accordance with the assigned structures. ESIMS analyses were performed on an Agilent 1100 LC/MSD trap system VL. UV/Vis spectra of tested compounds and their corresponding calibration curves were recorded with a Lambda BIO20 spectrophotometer (PerkinElmer). Commercially available chemicals were purchased from Sigma–Aldrich.

General synthetic procedure for final compounds 6a–e, 6j–m, 7a, 8a–p, and 9a,b,k. A solution containing 4-chloromethyl-2-aryloxazole **4a–p** or 4-chloromethyl-2-arythiazole **5a,b,k** (0.50 mmol), 6,7-dimethoxytetrahydroisoquinoline or 1-cyclohexylpiperazine (1.2 mmol) and Na_2CO_3 (0.50 mmol) in CH_3CN (20 mL) was stirred overnight. The solvent was evaporated, and the crude product was washed with H_2O (2×20 mL) and extracted with $CHCl_3$ (30 mL). The organic layer was dried (Na_2SO_4) and concentrated in vacuo. The crude product was purified by chromatography on a silica gel column, and the experimental conditions for each compound are reported below.

1-Cyclohexyl-4-(2-phenyloxazol-4-ylmethyl)piperazine (6a). Eluted with $CHCl_3$/MeOH (19:1); brown oil, recrystallized from MeOH/Et_2O (82% yield); mp: 301–303 °C; ^{1}H NMR ($CDCl_3$): δ = 1.14–1.94 (m, 10H, $(CH_2)_5$ cyclohexyl), 2.2–2.45 (m, 1H, CH_2CHCH_2), 2.50–2.80 (m, 8H, piperazine), 3.55 (s, 2H, NCH_2), 7.42–8.05 ppm (m, 6H, aromatic); UV/Vis (solvent PBS): λ_{max} = 266 nm, ε = 15010 $M^{-1}cm^{-1}$; MS *m/z*: 326 $[M+1]^+$ (4), 325 $[M]^+$ (17), 282 (15), 140 (78), 126 (100); Anal. C, H, N [$C_{20}H_{27}N_3O{\cdot}HCl{\cdot}H_2O$].

1-[2-(3-Bromophenyl)oxazol-4-ylmethyl]-4-cyclohexylpiperazine (6b). Eluted with $CHCl_3$/MeOH (19:1); brown oil, recrystallized from MeOH/Et_2O (24% yield); mp: 302–303 °C; ^{1}H NMR ($CDCl_3$): δ = 1.14–1.93 (m, 10H, $(CH_2)_5$ cyclohexyl), 2.00–2.16 (m, 1H, CH_2CHCH_2), 2.33–2.77 (m, 8H, piperazine), 3.54 (s, 2H, NCH_2), 7.25–8.22 ppm (m, 5H, aromatic); UV/Vis (solvent PBS): λ_{max} = 269 nm, ε = 15330 $M^{-1}cm^{-1}$; MS *m/z*: 405 $[M+2]^+$ (6), 403 $[M]^+$ (6), 138 (88), 126 (100); Anal. C, H, N [$C_{20}H_{26}BrN_3O{\cdot}2HCl$].

1-[2-(4-Bromophenyl)oxazol-4-ylmethyl]-4-cyclohexylpiperazine (6c). Eluted with $CHCl_3$/MeOH (19:1); brown oil, recrystallized from MeOH/Et_2O (quantitative); mp: 309–310 °C; ^{1}H NMR ($CDCl_3$): 1.04–2.00 (m, 10H, $(CH_2)_5$ cyclohexyl), 2.18–2.34 (m, 1H, CH_2CHCH_2), 2.44–2.86 (m, 8H, piperazine), 3.52 (s, 2H, NCH_2), 7.54–7.92 ppm (m, 5H, aromatic); UV/Vis (solvent PBS): λ_{max} = 274 nm, ε = 18890 $M^{-1}cm^{-1}$; MS *m/z*: 405 $[M+2]^+$ (12), 403 $[M]^+$ (14), 138 (92), 126 (100); Anal. C, H, N [$C_{20}H_{26}N_3OBr{\cdot}2.5HCl$].

1-[2-(3-Chlorophenyl)oxazol-4-ylmethyl]-4-cyclohexylpiperazine (6d). Eluted with $CHCl_3$/MeOH (19:1); brown oil, recrystallized from MeOH/Et_2O (80% yield); mp: 247 °C (dec); ^{1}H NMR ($CDCl_3$): 1.04–2.03 (m, 10H, $(CH_2)_5$ cyclohexyl), 2.16–2.26 (m, 1H, CH_2CHCH_2), 2.30–2.62 (m, 8H, piperazine), 3.53 (s, 2H, NCH_2), 7.25–8.05 ppm (m, 5H, aromatic); UV/Vis (solvent PBS): λ_{max} = 269 nm, ε = 16310 $M^{-1}cm^{-1}$; MS *m/z*: 361 $[M+2]^+$ (5), 359 $[M]^+$ (15), 138 (100), 126 (92); Anal. C, H, N [$C_{20}H_{26}N_3OCl{\cdot}2HCl{\cdot}0.5H_2O$].

1-[2-(4-Chlorophenyl)oxazol-4-ylmethyl]-4-cyclohexylpiperazine (6e). Eluted with $CHCl_3$/MeOH (19:1); brown oil, recrystallized from MeOH/Et_2O (67% yield); mp: 309–310 °C; ^{1}H NMR ($CDCl_3$): 1.05–1.88 (m, 10H, $(CH_2)_5$ cyclohexyl), 2.16–2.19 (m, 1H, CH_2CHCH_2), 2.21–2.62 (m, 8H, piperazine), 3.52 (s, 2H, NCH_2), 7.25–7.99 ppm (m, 5H, aromatic); UV/Vis (solvent PBS): λ_{max} = 272 nm, ε = 9770 $M^{-1}cm^{-1}$; MS *m/z*: 361 $[M+2]^+$ (5), 359 $[M]^+$ (13), 138 (100), 126 (82); Anal. C, H, N [$C_{20}H_{26}N_3OCl{\cdot}HCl{\cdot}2H_2O$].

1-Cyclohexyl-4-[2-(3-methoxyphenyl)oxazol-4-ylmethyl]piperazine (6j). Eluted with $CHCl_3$/MeOH (19:1); brown oil, recrystallized from MeOH/Et_2O (67% yield); mp: 379–383 °C; ^{1}H NMR ($CDCl_3$): δ = 1.10–2.45 (m, 11H, $(CH_2)_5$ cyclohexyl, CH_2CHCH_2), 2.59–2.80 (m, 8H, piperazine), 3.58 (s, 2H, NCH_2), 3.87 (s, 3H, CH_3), 6.97–7.60 ppm (m, 5H, aromatic); UV/Vis (solvent PBS): λ_{max} = 268 nm, ε = 14190 $M^{-1}cm^{-1}$; MS *m/z*: 356 $[M+1]^+$ (3), 355 $[M]^+$ (12), 188 (53), 140 (77), 126 (100); Anal. C, H, N [$C_{21}H_{29}N_3O_2{\cdot}2HCl{\cdot}H_2O$].

1-Cyclohexyl-4-[2-(4-methoxyphenyl)oxazol-4-ylmethyl]piperazine (6k). Eluted with $CHCl_3$/MeOH (19:1); brown oil, recrystallized from MeOH/Et_2O (59% yield); mp: 279–283 °C; ^{1}H NMR ($CDCl_3$): δ = 1.05–1.79 (m, 10H, $(CH_2)_5$ cyclohexyl), 2.16–2.25 (m, 1H, CH_2CHCH_2 cyclohexyl), 2.64–2.78 (m, 8H, piperazine), 3.52 (s, 2H, NCH_2), 3.84 (s, 3H, CH_3), 6.90–7.90 ppm (m, 5H, aromatic); UV/Vis (solvent PBS): λ_{max} = 276 nm, ε = 20260 $M^{-1}cm^{-1}$; MS *m/z*: 357 $[M+2]^+$ (1), 356 $[M+1]^+$ (2), 355 $[M]^+$ (11), 188 (80), 140 (100), 126 (100); Anal. C, H, N [$C_{21}H_{29}N_3O_2{\cdot}2HCl$].

1-Cyclohexyl-4-[2-(2,5-dimethoxyphenyl)oxazol-4-ylmethyl]piperazine (6l). Eluted with $CHCl_3$/MeOH (19:1). Colorless oil, recrystallized from MeOH/Et_2O (93% yield); mp: 222 °C (dec); ^{1}H NMR ($CDCl_3$): δ = 1.06–2.38 (m, 11H, $(CH_2)_5$ cyclohexyl, CH_2CHCH_2 cyclohexyl) 2.40–2.80 (m, 8H, piperazine), 3.64 (s, 2H, NCH_2), 3.81 and 3.88 (2 s, 6H, CH_3), 6.92–7.61 ppm (m, 4H, aromatic); UV/Vis (solvent PBS): λ_{max} = 320 nm, ε = 6640 $M^{-1}cm^{-1}$; MS *m/z*: 385 $[M]^+$ (6), 218 (79), 152 (100), 140 (91), 126 (69); Anal. C, H, N [$C_{22}H_{31}N_3O_3{\cdot}2HCl{\cdot}H_2O$].

1-Cyclohexyl-4-[2-(3-nitrophenyl)oxazol-4-ylmethyl]piperazine (6m). Eluted with $CHCl_3$/MeOH (19:1); brown oil, recrystallized from MeOH/Et_2O (93% yield). Mp > 250 °C; ^{1}H NMR ($CDCl_3$): δ = 1.02–2.38 (m, 11H, $(CH_2)_5$ cyclohexyl, CH_2CHCH_2 cyclohexyl) 2.40–2.80 (m, 8H, piperazine), 3.56 (s, 2H, NCH_2 phenyloxazole), 7.60–8.89 ppm (m, 5H, aromatic); UV/Vis (solvent PBS): λ_{max} = 264 nm, ε = 22760 $M^{-1}cm^{-1}$; MS *m/z*: 370 $[M]^+$ (31), 327 (69), 138 (100), 126 (87); Anal. C, H, N [$C_{20}H_{26}N_4O_3{\cdot}2HCl$].

1-Cyclohexyl-4-(2-phenylthiazol-4-ylmethyl)piperazine (7a). Eluted with $CHCl_3$/MeOH (19:1); brown oil, recrystallized from MeOH/Et_2O (76% yield); mp: 282–286 °C; ^{1}H NMR ($CDCl_3$): δ = 1.14–1.92 (m, 10H, $(CH_2)_5$ cyclohexyl), 2.2–2.44 (m, 1H, CH_2CHCH_2), 2.50–2.80 (m, 8H, piperazine), 3.75 (s, 2H, NCH_2), 7.38–7.95 ppm (m, 6H, aromatic); UV/Vis (solvent PBS): λ_{max} = 288 nm, ε = 10610 $M^{-1}cm^{-1}$; MS *m/z*: 341 $[M]^+$ (3), 203 (47), 174 (100), 152 (81); Anal. C, H, N [$C_{20}H_{27}N_3S{\cdot}2HCl.\ 0.5H_2O$].

6,7-Dimethoxy-2-(2-phenyl-oxazol-4-ylmethyl)-1,2,3,4-tetrahydro-isoquinoline (8a). Eluted with $CHCl_3$/MeOH (19:1); brown oil, recrystallized from MeOH/Et_2O (40% yield); mp: 218–220 °C; ^{1}H NMR ($CDCl_3$): δ = 2.85 (s, 4H, CH_2NCH_2Ar), 3.68–3.72 (m, 4H, NCH_2CH_2), 3.81 and 3.83 (2 s, 6H, CH_3), 6.50–8.07 ppm (m, 8H, aromatic); UV/Vis (solvent PBS): λ_{max} = 268 nm, ε = 17340 $M^{-1}cm^{-1}$; MS

m/z: 350 $[M]^+$ (1), 103 (13), 159 (8), 192 (100); Anal. C, H, N [$C_{21}H_{22}N_2O_3 \cdot 2HCl$].

2-[2-(3-Bromophenyl)oxazol-4-ylmethyl]-6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline (8b). Eluted with $CHCl_3$/AcOEt (1:1); brown oil, recrystallized from MeOH/Et_2O (51% yield); mp: 216–217 °C; ^{1}H NMR ($CDCl_3$): $\delta = 2.81$–2.85 (m, 4H, CH_2NCH_2Ar), 3.69–3.73 (m, 4H, NCH_2CH_2), 3.82 and 3.84 (2 s, 6H, CH_3), 6.51–8.23 ppm (m, 7H, aromatic); UV/Vis (solvent PBS): $\lambda_{max} = 276$ nm, $\varepsilon = 6020\ M^{-1}cm^{-1}$; MS *m/z*: 430 $[M+2]^+$ (1), 428 $[M]^+$ (1) 192 (100); Anal. C, H, N [$C_{21}H_{21}N_2O_3Br \cdot 1.5HCl$].

2-[2-(4-Bromophenyl)oxazol-4-ylmethyl]-6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline (8c). Eluted with $CHCl_3$/MeOH (19:1); brown oil, recrystallized from MeOH/Et_2O (83% yield); mp: 220–222 °C; ^{1}H NMR ($CDCl_3$): $\delta = 2.80$–2.92 (m, 4H, CH_2NCH_2Ar), 3.69–3.73 (m, 4H, NCH_2CH_2), 3.81 and 3.83 (2 s, 6H, CH_3), 6.50–7.94 ppm (m, 7H, aromatic); UV/Vis (solvent PBS): $\lambda_{max} = 276$ nm, $\varepsilon = 13340\ M^{-1}cm^{-1}$; MS *m/z*: 430 $[M+2]^+$ (1), 428 $[M]^+$ (1), 192 (100); Anal. C, H, N [$C_{21}H_{21}N_2O_3Br \cdot HCl$].

2-[2-(3-Chlorophenyl)oxazol-4-ylmethyl]-6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline (8d). Eluted with $CHCl_3$/MeOH (19:1); brown oil, recrystallized from MeOH/Et_2O (78% yield); mp: 214–218 °C; ^{1}H NMR ($CDCl_3$): $\delta = 2.80$–2.90 (m, 4H, CH_2NCH_2Ar), 3.67–3.71 (m, 4H, NCH_2CH_2), 3.81 and 3.83 (2 s, 6H, CH_3), 6.50–8.07 ppm (m, 7H, aromatic); UV/Vis (solvent PBS): $\lambda_{max} = 270$ nm, $\varepsilon = 6620\ M^{-1}cm^{-1}$; MS *m/z*: 387 $[M+2]^+$ (3), 385 $[M]^+$ (1), 192 (100); Anal. C, H, N [$C_{21}H_{21}N_2O_3Cl \cdot HCl$].

2-[2-(4-Chlorophenyl)oxazol-4-ylmethyl]-6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline (8e). Eluted with $CHCl_3$/MeOH (19:1); brown oil, recrystallized from MeOH/Et_2O (78% yield); mp: 225–227 °C; ^{1}H NMR ($CDCl_3$): $\delta = 2.81$–2.84 (m, 4H, CH_2NCH_2Ar), 3.67–3.71 (m, 4H, NCH_2CH_2), 3.81 and 3.83 (2 s, 6H, CH_3), 7.25–8.01 ppm (m, 7H, aromatic); UV/Vis (solvent PBS): $\lambda_{max} = 275$ nm, $\varepsilon = 12060\ M^{-1}cm^{-1}$; MS *m/z*: 384 $[M]^+$ (1), 192 (100); Anal. C, H, N [$C_{21}H_{21}N_2O_3Cl \cdot HCl$].

2-[4-(6,7-Dimethoxy-3,4-dihydro-1*H*-isoquinolin-2-ylmethyl)oxazol-2-yl]phenol (8f). Eluted with $CHCl_3$/AcOEt (7:3); brown oil, recrystallized from MeOH/Et_2O (65% yield); mp: 221–223 °C; ^{1}H NMR ($CDCl_3$): $\delta = 1.4$ (br s, 1H, OH, D_2O exchanged), 2.80–3.00 (m, 4H, CH_2NCH_2Ar), 3.75–3.76 (m, 4H, NCH_2CH_2), 3.82 and 3.83 (2 s, 6H, CH_3), 6.50–7.83 ppm (m, 7H, aromatic); UV/Vis (solvent PBS): $\lambda_{max} = 263$ nm, $\varepsilon = 10440\ M^{-1}cm^{-1}$; MS (ESI+) *m/z*: 367 $[M+1]^+$ (100), 174 (8); Anal. C, H, N [$C_{21}H_{22}N_2O_4 \cdot HCl \cdot 0.5H_2O$].

3-[4-(6,7-Dimethoxy-3,4-dihydro-1*H*-isoquinolin-2-ylmethyl)oxazol-2-yl]phenol (8g). Eluted with $CHCl_3$/MeOH (19:1); brown oil, recrystallized from MeOH/Et_2O (80% yield); mp: 225–227 °C; ^{1}H NMR ($CDCl_3$): $\delta = 1.4$ (br s, 1H, OH, D_2O exchanged) 2.89–2.91 (m, 4H, CH_2NCH_2Ar), 3.74–3.78 (m, 4H, NCH_2CH_2), 3.80 and 3.83 (2 s, 6H, CH_3), 6.53–7.62 ppm (m, 7H, aromatic); UV/Vis (solvent PBS): $\lambda_{max} = 269$ nm, $\varepsilon = 15340\ M^{-1}cm^{-1}$; MS (ESI+) *m/z*: 367 $[M+1]^+$ (100), 174 (4); Anal. C, H, N [$C_{21}H_{22}N_2O_4 \cdot HCl \cdot 0.5H_2O$].

4-[4-(6,7-Dimethoxy-3,4-dihydro-1*H*-isoquinolin-2-ylmethyl)oxazol-2-yl]phenol (8h). Eluted with $CHCl_3$/MeOH (19:1); brown oil, recrystallized from MeOH/Et_2O (80% yield); mp: 220–222 °C; ^{1}H NMR ($CDCl_3$): $\delta = 1.4$ (br s, 1H, OH, D_2O exchanged), 2.80–2.90 (m, 4H, CH_2NCH_2Ar), 3.74–3.81 (m, 4H, NCH_2CH_2), 3.81 and 3.83 (2 s, 6H, CH_3), 6.50–7.87 ppm (m, 7H, aromatic); UV/Vis (solvent PBS): $\lambda_{max} = 279$ nm, $\varepsilon = 20530\ M^{-1}cm^{-1}$; MS (ESI+) *m/z*: 367 $[M+1]^+$ (100), 174 (6); Anal. C, H, N [$C_{21}H_{22}N_2O_4 \cdot HCl \cdot 0.5H_2O$].

6,7-Dimethoxy-2-[2-(2-methoxyphenyl)oxazol-4-ylmethyl]-1,2,3,4-tetrahydroisoquinoline (8i). Eluted with $CHCl_3$/MeOH (19:1); brown oil, recrystallized from MeOH/Et_2O (75% yield); mp: 224–227 °C; ^{1}H NMR ($CDCl_3$): $\delta = 2.78$–2.85 (m, 4H, CH_2NCH_2Ar), 3.64–3.68 (m, 4H, NCH_2CH_2), 3.81, 3.82 and 3.87 (3 s, 9H, CH_3), 6.48–7.65 ppm (m, 7H, aromatic); UV/Vis (solvent PBS): $\lambda_{max} = 264$ nm, $\varepsilon = 11960\ M^{-1}cm^{-1}$; MS (ESI+) *m/z*: 367 $[M+1]^+$ (100), 174 (6); Anal. C, H, N [$C_{22}H_{24}N_2O_4 \cdot 2.5HCl$].

6,7-Dimethoxy-2-[2-(3-methoxyphenyl)oxazol-4-ylmethyl]-1,2,3,4-tetrahydroisoquinoline (8j). Eluted with $CHCl_3$/MeOH (19:1); brown oil, recrystallized from MeOH/Et_2O (75% yield); mp: 170–177 °C; ^{1}H NMR ($CDCl_3$): $\delta = 2.78$–2.85 (m, 4H, CH_2NCH_2Ar), 3.48–3.87 (m, 13H, $3CH_3$, NCH_2CH_2), 6.48–7.65 ppm (m, 7H, aromatic); UV/Vis (solvent PBS): $\lambda_{max} = 270$ nm, $\varepsilon = 13100\ M^{-1}cm^{-1}$; MS (ESI+) *m/z*: 381 $[M+1]^+$ (100), 188 (6); Anal. C, H, N [$C_{22}H_{24}N_2O_4 \cdot 2HCl$].

6,7-Dimethoxy-2-[2-(4-methoxyphenyl)oxazol-4-ylmethyl]-1,2,3,4-tetrahydroisoquinoline (8k). Eluted with $CHCl_3$/MeOH (19:1); brown oil, recrystallized from MeOH/Et_2O (51% yield); mp: 232–233 °C; ^{1}H NMR ($CDCl_3$): $\delta = 2.75$–2.85 (m, 4H, CH_2NCH_2Ar), 3.70–3.72 (m, 4H, NCH_2CH_2), 3.81, 3.83 and 3.87 (3 s, 9H, CH_3), 6.50–6.80 ppm (m, 7H, aromatic); UV/Vis (solvent PBS): $\lambda_{max} = 280$ nm, $\varepsilon = 18210\ M^{-1}cm^{-1}$; MS *m/z*: 380 $[M]^+$ (1), 192 (100), 133 (10); Anal. C, H, N [$C_{22}H_{24}N_2O_4 \cdot 1.5HCl$].

2-[2-(2,5-Dimethoxyphenyl)oxazol-4-ylmethyl]-6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline (8l). Eluted with $CHCl_3$/MeOH (19:1); brown oil, recrystallized from MeOH/Et_2O (57% yield); mp: 156 °C (dec); ^{1}H NMR ($CDCl_3$): $\delta = 2.79$–2.86 (m, 4H, CH_2NCH_2Ar), 365–3.75 (m, 4H, NCH_2CH_2), 3.81, 3.82, 3.83 and 3.90 (4 s, 12H, CH_3), 6.50–7.70 ppm (m, 6H, aromatic); UV/Vis (solvent PBS): $\lambda_{max} = 322$ nm, $\varepsilon = 5230\ M^{-1}cm^{-1}$; MS *m/z*: 410 $[M]^+$ (1), 219 (20), 192 (100); Anal. C, H, N [$C_{23}H_{26}N_2O_5 \cdot 2.5HCl$].

6,7-Dimethoxy-2-[2-(3-nitrophenyl)oxazol-4-ylmethyl]-1,2,3,4-tetrahydroisoquinoline (8m). Eluted with $CHCl_3$/AcOEt (1:1); brown oil, recrystallized from MeOH/Et_2O (78% yield); mp: 218–220 °C; ^{1}H NMR ($CDCl_3$): $\delta = 2.78$–2.90 (m, 4H, CH_2NCH_2Ar), 3.69–3.74 (m, 4H, NCH_2CH_2), 3.81 and 3.83 (2 s, 6H, CH_3), 6.50–8.91 ppm (m, 7H, aromatic); UV/Vis (solvent PBS): $\lambda_{max} = 266$ nm, $\varepsilon = 23510\ M^{-1}cm^{-1}$; MS *m/z*: 395 $[M]^+$ (1), 192 (100); Anal. C, H, N [$C_{21}H_{21}N_3O_5 \cdot HCl \cdot 0.5H_2O$].

6,7-Dimethoxy-2-[2-(4-nitrophenyl)oxazol-4-ylmethyl]-1,2,3,4-tetrahydroisoquinoline (8n). Eluted with $CHCl_3$/AcOEt (1:1); brown oil, recrystallized from MeOH/Et_2O (42% yield); mp: 215–216 °C; ^{1}H NMR ($CDCl_3$): $\delta = 2.80$–2.90 (m, 4H, CH_2NCH_2Ar), 3.69–3.74 (m, 4H, NCH_2CH_2), 3.81 and 3.84 (2 s, 6H, CH_3), 6.50–8.33 ppm (m, 7H, aromatic); UV/Vis (solvent PBS): $\lambda_{max} = 315$ nm, $\varepsilon = 11450\ M^{-1}cm^{-1}$; MS (ESI+) *m/z*: 396 $[M+1]^+$ (100), 165 (10); Anal. C, H, N [$C_{21}H_{21}N_3O_5 \cdot 1.5HCl$].

6,7-Dimethoxy-2-[2-(4-methylsulfanylphenyl)oxazol-4-ylmethyl]-1,2,3,4-tetrahydroisoquinoline (8p). Eluted with $CHCl_3$/AcOEt (1:1); brown oil, recrystallized from MeOH/Et_2O (73% yield); mp: 232–233 °C; ^{1}H NMR ($CDCl_3$): $\delta = 2.52$ (s, 3H, CH_3), 2.76–3.00 (m, 4H, CH_2NCH_2Ar), 3.72–3.74 (m, 4H, NCH_2CH_2), 3.81 and 3.84 (2 s, 6H, CH_3), 6.50–7.97 ppm (m, 7H, aromatic); UV/Vis (solvent PBS): $\lambda_{max} = 266$ nm, $\varepsilon = 23510\ M^{-1}cm^{-1}$; MS (ESI+) *m/z*: 397 $[M+1]^+$ (100), 204 (4); Anal. C, H, N [$C_{22}H_{24}N_2O_3S \cdot HCl \cdot 0.5H_2O$].

6,7-Dimethoxy-2-(2-phenylthiazol-4-ylmethyl)-1,2,3,4-tetrahydroisoquinoline (9a). Eluted with $CHCl_3$/MeOH (19:1); brown oil, recrystallized from MeOH/Et_2O (76% yield); mp: 199–201 °C; ^{1}H NMR ($CDCl_3$): $\delta = 2.81$–2.85 (m, 4H, CH_2NCH_2Ar), 3.70–3.81 (m,

4H, $2NCH_2CH_2$), 3.84 and 3.93 (2 s, 6H, CH_3), 6.51–7.97 ppm (m, 8H, aromatic); UV/Vis (solvent PBS): $\lambda_{max}=285$ nm, $\varepsilon=11\,660\ M^{-1}cm^{-1}$; MS *m/z*: 366 $[M]^+$ (1), 192 (100), 175 (18); Anal. C, H, N [$C_{21}H_{22}N_2O_2S\cdot HCl\cdot H_2O$].

2-[2-(3-Bromophenyl)thiazol-4-ylmethyl]-6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline (9b). Eluted with $CHCl_3$/MeOH (19:1); brown oil, recrystallized from MeOH/Et_2O; mp: 187–188 °C; ^1H NMR ($CDCl_3$): $\delta=2.80$–2.88 (m, 4H, CH_2NCH_2Ar), 3.60–3.99 (m, 10H, NCH_2CH_2 and $2CH_3$), 6.51–8.15 ppm (m, 7H, aromatic); UV/Vis (solvent PBS): $\lambda_{max}=227$ nm, $\varepsilon=36\,220\ M^{-1}cm^{-1}$; MS *m/z*: 446 $[M+2]^+$ (1), 444 $[M]^+$ (1) 192 (100); Anal. C, H, N [$C_{21}H_{21}N_2O_2SBr\cdot 1.5\,HCl$].

6,7-Dimethoxy-2-[2-(4-methoxyphenyl)thiazol-4-ylmethyl]-1,2,3,4-tetrahydroisoquinoline (9k). Eluted with $CHCl_3$/MeOH (19:1); brown solid, recrystallized from MeOH/Et_2O (58% yield); mp: 195–197 °C; ^1H NMR ($CDCl_3$): $\delta=2.80$–2.92 (m, 4H, CH_2NCH_2Ar), 3.60–3.72 (m, 4H, NCH_2CH_2), 3.81, 3.83 and 3.85 (3 s, 9H, CH_3) 6.50–7.91 ppm (m, 7H, aromatic); UV/Vis (solvent PBS): $\lambda_{max}=290$ nm, $\varepsilon=11\,640\ M^{-1}cm^{-1}$; MS *m/z*: 396 $[M]^+$ (1), 205 (20), 192 (100); Anal. C, H, N [$C_{22}H_{24}N_2O_3S\cdot 2\,HCl$].

Biology

Cell culture reagents were purchased from Celbio s.r.l. (Milan, Italy). CulturePlate 96-well plates were purchased from PerkinElmer Life Science, and calcein-AM was purchased from Fluka (Milan, Italy). Verapamil was purchased from Tocris Bioscience (Bristol, UK). MK571 was purchased from Calbiochem (San Diego, USA). Rhodamine-123 was purchased from Sigma–Aldrich (Milan, Italy). MDCK-MDR1 and MDCK-MRP1 were a gift of Professor P. Borst, NKI-AVL Institute, Amsterdam (The Netherlands); MDCK-BCRP cells were a gift of Dr. A. Schinkel, NKI-AVL Institute, Amsterdam (The Netherlands); 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). Cells were grown in Dulbecco's modified Eagle's medium (DMEM) high glucose supplemented with fetal bovine serum (10%), L-glutamine (2 mM), penicillin (100 U mL^{-1}), and streptomycin (100 μg mL^{-1}) in a humidified incubator at 37 °C under a 5% CO_2 atmosphere.

Calcein-AM experiment. These experiments were carried out as described by Feng et al. with minor modifications.[29] Each cell line (50000 cells per well) was seeded into black CulturePlate 96-well plates with 100 μL medium and allowed to become confluent overnight. Test compounds were solubilized in 100 μL culture medium and were added to the cell monolayers. The plates were then incubated at 37 °C for 30 min. Calcein-AM was added in 100 μL phosphate-buffered saline (PBS) to yield a final concentration of 2.5 μM, and plate incubation was continued for 30 min. Each well was washed three times with ice-cold PBS. Saline buffer was added to each well, and the plates were read with a Victor3 fluorimeter (PerkinElmer) at excitation and emission wavelengths of 485 nm and 535 nm, respectively. Under these experimental conditions, calcein cell accumulation in the absence and presence of tested compounds was evaluated, and basal-level fluorescence was estimated by untreated cell fluorescence. In treated wells, the increase in fluorescence was measured relative to the basal level. EC_{50} values were determined by fitting the percent fluorescence increase versus log[dose].[30]

Rhodamine-123 experiment. These experiments were carried out as described by Colabufo and co-workers with minor modifications.[21] Each cell line (50000 cells per well) was seeded into black CulturePlate 96-well plates with 100 μL medium and allowed to become confluent overnight. Test compounds were solubilized in 100 μL culture medium were and added to the cell monolayers. The plates were then incubated at 37 °C for 30 min. Rhodamine-123 was added in 100 μL PBS to yield a final concentration of 10 μM, and plates incubation was continued for 30 min. The medium was removed, PBS (100 μL) was added to each well, and the plates were read with a Victor3 fluorimeter (PerkinElmer) at excitation and emission wavelengths of 485 nm and 535 nm, respectively. Under these experimental conditions, Rhodamine-123 cell accumulation in the absence presence of tested compounds was evaluated, and basal-level fluorescence was estimated by the fluorescence of untreated cells. In treated wells, the increase in fluorescence relative to the basal level was measured. EC_{50} values were determined by fitting the percent fluorescence increase versus log[dose].[30]

Caco-2 cell line experiments. [^3H]Vinblastine transport inhibition, apparent permeability (P_{app}) both from apical to basolateral (A→B) and from basolateral to apical (B→A), and ATP-ase activation[31–34] were reported previously.[35,36]

Keywords: 2-aryloxazoles · 2-arylthiazoles · BCRP · multi-drug resistance · P-glycoprotein

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